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E. S. D.

COD:01

SOLICITUD Nº 01012068
FECHA SOLICITUD: Febrero 15 de 2.001
PATENTE: INDOLINONAS SUSTITUIDAS CON PIRROLES INHIBIDORAS DE PROTEINQUINASAS
SOLICITANTE: SUGEN, INC.

ASUNTO: PRESENTACIÓN PRIORIDADES

DILIA RODRIGUEZ D'ALEMAN, en mi condición de apoderada de la sociedad solicitante, en esta oportunidad por el presente escrito me permito presentar:

- Copia certificada de la prioridad Estadounidense reivindicada Nº 60/182.710 de Febrero 15 de 2.000.
- Copia certificada de la prioridad Estadounidense reivindicada Nº. 60/216,422 de Julio 6 de 2.000.
- Copia certificada de la prioridad Estadounidense reivindicada Nº. 60/243.532 de Octubre 27 de 2.000.
- Traducción al Español de las mencionadas prioridades

Estas prioridades están siendo presentadas dentro del término de 3 meses que exige el Convenio de Paris, toda vez que la solicitud fué radicada 15 de Febrero de 2.001.

Ruego a usted continuar con el presente trámite.

Señor Jefe,

DILIA RODRIGUEZ D'ALEMAN
LCD/sit
P- 410

P. 410

PTO 304151

THE UNITED STATES OF AMERICA

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March 30, 2001

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APPLICATION NUMBER: ***60/216,422***
FILING DATE: ***July 06, 2000***

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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR §1.53(c).

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[] Additional inventors are being named on the 0 separately numbered sheets attached hereto.

TITLE OF THE INVENTION (280 characters max)

PYRROLE SUBSTITUTED 2-INDOLINONE PROTEIN KINASE INHIBITORS

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ENCLOSED APPLICATION PARTS *(check all that apply)*

[X] Specification *Number of Pages* 174 [] Small Entity Statement

[] Drawing(s) *Number of Sheets* [X] Other (specify) Return Postcard

METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT *(check one)*

[X] A check or money order is enclosed to cover the filing fees. $150.00

[X] The Commissioner is hereby authorized to charge filing fees or credit any overpayment to Deposit Account Number: 50-1273

FILING FEE AMOUNT ($) $150.00

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

[X] No.

[] Yes, the name of the U.S. Government agency and the Government contract number are:

Respectfully submitted,

Signature

Name For Richard J. Warburg Reg. No. 32,327, Michael A. Whittaker Reg. No. 46,230

Date July 6, 2000

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Docket No. 030597.0178.PRV2

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JC540 U.S. PTO 07/06/00

JC406 U.S. PTO 60/210477 07/06/00

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PROVISIONAL APPLICATION

UNDER 37 CFR § 1.53(C)

TITLE: PYRROLE SUBSTITUTED 2-INDOLINONE PROTEIN KINASE INHIBITORS

APPLICANT(S): Peng Cho Tang, Todd Miller, Xiaoyuan Li, Connie Sun, Chung Chen Wei, Sheri Shirazian

Correspondence Enclosed:

Provisional Application Cover Sheet (1 pg); Cover Sheet (1pg); Spec (174 pgs); Claims (12 pgs); Abstract (1 pg); Check No. 502529 in the amount of $150.00; and Return Postcard

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DESCRIPTION

PYRROLE SUBSTITUTED 2-INDOLINONE PROTEIN KINASE INHIBITORS

INTRODUCTION

The present invention relates generally to organic chemistry, biochemistry, pharmacology and medicine. More particularly, it relates to pyrrole substituted 2-indolinone compounds, and their physiologically acceptable salts and prodrugs, which modulate the activity of protein kinases ("PKs") and are expected to exhibit a salutary effect against disorders related to abnormal PK activity.

BACKGROUND OF THE INVENTION

The following is offered as background information only and is not admitted to be prior art to the present invention.

PKs are enzymes that catalyze the phosphorylation of hydroxy groups on tyrosine, serine and threonine residues of proteins. The consequences of this seemingly simple activity are staggering; cell growth, differentiation and proliferation, i.e., virtually all aspects of cell life in one way or another depend on PK activity. Furthermore, abnormal PK activity has been related to a host of disorders, ranging from relatively non-life threatening diseases such as psoriasis to extremely virulent diseases such as glioblastoma (brain cancer).

The PKs can be conveniently broken down into two classes, the protein tyrosine kinases (PTKs) and the serine-threonine kinases (STKs).

One of the prime aspects of PTK activity is their involvement with growth factor receptors. Growth factor receptors are cell-surface proteins. When bound by a growth factor ligand, growth factor receptors are converted to an active form which

interacts with proteins on the inner surface of a cell membrane. This leads to phosphorylation on tyrosine residues of the receptor and other proteins and to the formation inside the cell of complexes with a variety of cytoplasmic signaling molecules that, in turn, effect numerous cellular responses such as cell division (proliferation), cell differentiation, cell growth, expression of metabolic effects to the extracellular microenvironment, etc. For a more complete discussion, see Schlessinger and Ullrich, Neuron, 9:303-391 (1992) which is incorporated by reference, including any drawings, as if fully set forth herein.

Growth factor receptors with PTK activity are known as receptor tyrosine kinases ("RTKs"). They comprise a large family of transmembrane receptors with diverse biological activity. At present, at least nineteen (19) distinct subfamilies of RTKs have been identified. An example of these is the subfamily designated the "HER" RTKs, which include EGFR (epithelial growth factor receptor), HER2, HER3 and HER4. These RTKs consist of an extracellular glycosylated ligand binding domain, a transmembrane domain and an intracellular cytoplasmic catalytic domain that can phosphorylate tyrosine residues on proteins.

Another RTK subfamily consists of insulin receptor (IR), insulin-like growth factor I receptor (IGF-1R) and insulin receptor related receptor (IRR). IR and IGF-1R interact with insulin, IGP-I and IGF-II to form a heterotetramer of two entirely extracellular glycosylated α subunits and two β subunits which cross the cell membrane and which contain the tyrosine kinase domain.

A third RTK subfamily is referred to as the platelet derived growth factor receptor ("PDGFR") group, which includes PDGFRα, PDGFRβ, CSFIR, c-kit and c-fms. These receptors consist of glycosylated extracellular domains composed of variable numbers of immunoglobin-like loops and an intracellular domain wherein

the tyrosine kinase domain is interrupted by unrelated amino acid sequences.

Another group which, because of its similarity to the PDGFR subfamily, is sometimes subsumed into the later group is the fetus liver kinase ("flk") receptor subfamily. This group is believed to be made up of kinase insert domain-receptor fetal liver kinase-1 (KDR/FLK-1, VEGF-R2), flk-1R, flk-4 and fms-like tyrosine kinase 1 (flt-1).

A further member of the tyrosine kinase growth factor receptor family is the fibroblast growth factor ("FGF") receptor subgroup. This group consists of four receptors, FGFR1-4, and seven ligands, FGF1-7. While not yet well defined, it appears that the receptors consist of a glycosylated extracellular domain containing a variable number of immunoglobin-like loops and an intracellular domain in which the tyrosine kinase sequence is interrupted by regions of unrelated amino acid sequences.

Still another member of the tyrosine kinase growth factor receptor family is the vascular endothelial growth factor (VEGF") receptor subgroup. VEGF is a dimeric glycoprotein similar to PDGF but has different biological functions and target cell specificity in vivo. In particular, VEGF is presently thought to play an essential role is vasculogenesis and angiogenesis.

A more complete listing of the known RTK subfamilies is described in Plowman et al., DN&P, 7(6):334-339 (1994) which is incorporated by reference, including any drawings, as if fully set forth herein.

In addition to the RTKs, there also exists a family of entirely intracellular PTKs called "non-receptor tyrosine kinases" or "cellular tyrosine kinases." This latter designation, abbreviated "CTK," will be used herein. CTKs do not contain extracellular and transmembrane domains. At present, over 24 CTKs in 11 subfamilies (Src, Frk, Btk, Csk, Abl, Zap70, Fes, Fps, Fak, Jak and Ack) have been identified. The Src

subfamily appear so far to be the largest group of CTKs and includes Src, Yes, Fyn, Lyn, Lck, Blk, Hck, Fgr and Yrk. For a more detailed discussion of CTKs, see Bolen, Oncogene, 8:2025-2031 (1993), which is incorporated by reference, including any drawings, as if fully set forth herein.

The serine/threonine kinases, STKs, like the CTKs, are predominantly intracellular although there are a few receptor kinases of the STK type. STKs are the most common of the cytosolic kinases; i.e., kinases that perform their function in that part of the cytoplasm other than the cytoplasmic organelles and cytoskelton. The cytosol is the region within the cell where much of the cell's intermediary metabolic and biosynthetic activity occurs; e.g., it is in the cytosol that proteins are synthesized on ribosomes.

RTKs, CTKs and STKs have all been implicated in a host of pathogenic conditions including, significantly, cancer. Other pathogenic conditions which have been associated with PTKs include, without limitation, psoriasis, hepatic cirrhosis, diabetes, angiogenesis, restenosis, ocular diseases, rheumatoid arthritis and other inflammatory disorders, immunological disorders such as autoimmune disease, cardiovascular disease such as atherosclerosis and a variety of renal disorders.

With regard to cancer, two of the major hypotheses advanced to explain the excessive cellular proliferation that drives tumor development relate to functions known to be PK regulated. That is, it has been suggested that malignant cell growth results from a breakdown in the mechanisms that control cell division and/or differentiation. It has been shown that the protein products of a number of proto-oncogenes are involved in the signal transduction pathways that regulate cell growth and differentiation. These protein products of proto-oncogenes include the extracellular growth factors, transmembrane growth

factor PTK receptors (RTKs), cytoplasmic PTKs (CTKs) and cytosolic STKs, discussed above.

In view of the apparent link between PK-related cellular activities and wide variety of human disorders, it is no surprise that a great deal of effort is being expended in an attempt to identify ways to modulate PK activity. Some of this effort has involved biomimetic approaches using large molecules patterned on those involved in the actual cellular processes (e.g., mutant ligands (U.S. App. No. 4,966,849); soluble receptors and antibodies (App. No. WO 94/10202, Kendall and Thomas, Proc. Nat'l Acad. Sci., 90:10705-09 (1994), Kim, et al., Nature, 362:841-844 (1993)); RNA ligands (Jelinek, et al., Biochemistry, 33:10450-56); Takano, et al., Mol. Bio. Cell 4:358A (1993); Kinsella, et al., Exp. Cell Res. 199:56-62 (1992); Wright, et al., J. Cellular Phys., 152:448-57) and tyrosine kinase inhibitors (WO 94/03427; WO 92/21660; WO 91/15495; WO 94/14808; U.S. Pat. No. 5,330,992; Mariani, et al., Proc. Am. Assoc. Cancer Res., 35:2268 (1994)).

In addition to the above, attempts have been made to identify small molecules which act as PK inhibitors. For example, bis- monocylic, bicyclic and heterocyclic aryl compounds (PCT WO 92/20642), vinylene-azaindole derivatives (PCT WO 94/14808) and 1-cyclopropyl-4-pyridylquinolones (U.S. Pat. No. 5,330,992) have been described as tyrosine kinase inhibitors. Styryl compounds (U.S. Pat. No. 5,217,999), styryl-substituted pyridyl compounds (U.S. Pat. No. 5,302,606), quinazoline derivatives (EP App. No.0 566 266 A1), selenaindoles and selenides (PCT WO 94/03427), tricyclic polyhydroxylic compounds (PCT WO 92/21660) and benzylphosphonic acid compounds (PCT WO 91/15495) have all been described as PTK inhibitors useful in the treatment of cancer.

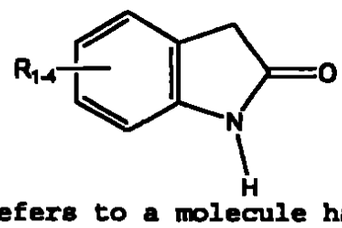
SUMMARY OF THE INVENTION

Our own efforts to identify small organic molecules which modulate PK activity and which, therefore, are expected to be

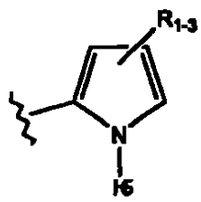
useful in the treatment and prevention of disorders involving abnormal PK activity, has led us to the discovery of a family of pyrrole substituted 2-indolinone compounds, and their prodrugs and physiologically acceptable salts, which exhibit PK modulating ability and are thereby expected to have a salutary effect against disorders related to abnormal PK activity.

Thus, the present invention relates generally to pyrrole substituted 2-indolinones and their prodrugs and physiologically acceptable salts, which modulate the activity of receptor tyrosine kinases (RTKs), non-receptor protein tyrosine kinases (CTKs) and serine/threonine protein kinases (STKs). In addition, the present invention relates to the preparation and use of pharmaceutical compositions of the disclosed compounds and their physiologically acceptable salts and prodrugs in the treatment or prevention of PK driven disorders such as, by way of example and not limitation, cancer, diabetes, hepatic cirrhosis, cardiovasacular disease such as atherosclerosis, angiogenesis, immunological disease such as autoimmune disease and renal disease.

The terms "2-indolinone," indolin-2-one and "2-oxindole" are used interchangeably herein to refer to a molecule having the chemical structure:



A "pyrrole" refers to a molecule having the chemical structure:



"Pyrrole substituted 2-indolinone" and "3-pyrrolidenyl-2-indolinone" are used interchangeably herein to refer to a chemical compound having the general structure shown in Formula 1.

A "pharmaceutical composition" refers to a mixture of one or more of the compounds described herein, or physiologically acceptable salts or prodrugs thereof, with other chemical components, such as physiologically acceptable carriers and excipients. The purpose of a pharmaceutical composition is to facilitate administration of a compound to an organism.

A "prodrug" refers to an agent which is converted into the parent drug in vivo. Prodrugs are often useful because, in some situations, they may be easier to administer than the parent drug. They may, for instance, be bioavailable by oral administration whereas the parent drug is not. The prodrug may also have improved solubility in pharmaceutical compositions over the parent drug. An example, without limitation, of a prodrug would be a compound of the present invention which is administered as an ester (the "prodrug") to facilitate transmittal across a cell membrane where water solubility is detrimental to mobility but then is metabolically hydrolyzed to the carboxylic acid, the active entity, once inside the cell where water solubility is beneficial.

A further example of a prodrug might be a short polypeptide, for example, without limitation, a 2 - 10 amino acid polypeptide, bonded through a terminal amino group to a carboxy group of a compound of this invention wherein the polypeptide is hydrolyzed or metabolized in vivo to release the active molecule.

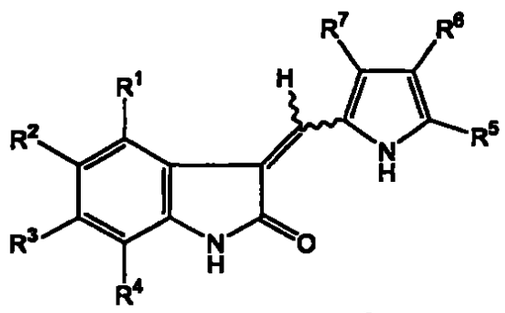
As used herein, a "physiologically acceptable carrier" refers to a carrier or diluent that does not cause significant irritation to an organism and does not abrogate the biological activity and properties of the administered compound.

An "excipient" refers to an inert substance added to a pharmaceutical composition to further facilitate administration of a compound. Examples, without limitation, of excipients include calcium carbonate, calcium phosphate, various sugars and types of starch, cellulose derivatives, gelatin, vegetable oils and polyethylene glycols.

1. **CHEMISTRY**

A. General Structural Features.

In one aspect, the present invention relates to pyrrole substituted 2-indolinones having the following chemical structure:



1

wherein:

R^1 is selected from the group consisting of hydrogen, halo, alkyl, cyclkoalkyl, aryl, heteroaryl, heteroalicyclic, hydroxy, alkoxy, $-C(O)R^{15}$, $-NR^{13}R^{14}$, $-(CH_2)_rR^{16}$ and $-C(O)NR^8R^9$;

R^2 is selected from the group consisting of hydrogen, halo, alkyl, trihalomethyl, hydroxy, alkoxy, $-NR^{13}R^{14}$, $-NR^{13}C(O)R^{14}$, $-C(O)R^{15}$, aryl, heteroaryl and $-S(O)_2NR^{13}R^{14}$;

R^3 is selected from the group consisting of hydrogen, halogen, alkyl, trihalomethyl, hydroxy, alkoxy, $-C(O)R^{15}$, $-NR^{13}R^{14}$, aryl, heteroaryl, $-NR^{13}S(O)_2R^{14}$, $-S(O)_2NR^{13}R^{14}$, $-NR^{13}C(O)R^{14}$, and $-NR^{13}C(O)OR^{14}$;

R^4 is selected from the group consisting of hydrogen, halogen, alkyl, hydroxy, alkoxy and $-NR^{13}R^{14}$;

is selected from the group consisting of hydrogen, alkyl and $-C(O)R^{10}$;

R^6 is selected from the group consisting of hydrogen, alkyl and $-C(O)R^{10}$;

R^7 is selected from the group consisting of hydrogen, alkyl, aryl, heteroaryl, $-C(O)R^{17}$ and $-C(O)R^{10}$;

R^6 and R^7 may combine to form a group selected from the group consisting of $-(CH_2)_4-$, $-(CH_2)_5-$ and $-(CH_2)_6-$;

with the proviso that at least one of R^5, R^6 or R^7 must be $-C(O)R^{10}$;

R^8 and R^9 are independently selected from the group consisting of hydrogen, alkyl and aryl;

R^{10} is selected from the group consisting of hydroxy, alkoxy, aryloxy, $-N(R^{11})(CH_2)_nR^{12}$ and $-NR^{13}R^{14}$;

R^{11} is selected from the group consisting of hydrogen and alkyl;

R^{12} is selected from the group consisting of $-NR^{13}R^{14}$, hydroxy, $-C(O)R^{15}$, aryl and heteroaryl;

R^{13} and R^{14} are independently selected from the group consisting of hydrogen, alkyl, cycloalkyl, aryl and heteroaryl;

R^{13} and R^{14} may combine to form a group selected from the group consisting of $-(CH_2)_4-$, $-(CH_2)_5-$, $-(CH_2)_2O(CH_2)_2-$, and $-(CH_2)_2N(CH_3)(CH_2)_2-$;

R^{15} is selected from the group consisting of hydrogen, hydroxy, alkoxy and aryloxy;

R^{16} is selected from the group consisting of hydroxy, $-C(O)R^{15}$, $-NR^{13}R^{14}$ and $C(O)NR^{13}R^{14}$;

R^{17} is selected from the group consisting of alkyl, cycloalkyl, aryl and heteroaryl; and,

n and r are independently 1, 2, 3, or 4.

An additional aspect of this invention is a pharmaceutically acceptable salt of a compound hereof.

As used herein, the term "alkyl" refers to a saturated aliphatic hydrocarbon including straight chain and branched chain

groups. Preferably, the alkyl group has 1 to 20 carbon atoms (whenever a numerical range; e.g. "1-20", is stated herein, it means that the group, in this case the alkyl group, may contain 1 carbon atom, 2 carbon atoms, 3 carbon atoms, etc. up to and including 20 carbon atoms). More preferably, it is a medium size alkyl having 1 to 10 carbon atoms. Most preferably, it is a lower alkyl having 1 to 4 carbon atoms. The alkyl group may be substituted or unsubstituted. When substituted, the substituent group(s) is preferably one or more independently selected from the group consisting of halo, hydroxy, unsubstituted lower alkoxy, aryl optionally substituted with one or more halo, hydroxy, unsubstituted lower alkyl or unsubstituted lower alkoxy groups, aryloxy optionally substituted with one or more halo, hydroxy, unsubstituted lower alkyl or unsubstituted lower alkoxy groups, 6-member heteroaryl having from 1 to 3 nitrogen atoms in the ring, the carbons in the ring being optionally substituted with one or more halo, hydroxy, unsubstituted lower alkyl or unsubstituted lower alkoxy groups, 5-member heteroaryl having from 1 to 3 heteroatoms selected from the group consisting of nitrogen, oxygen and sulfur, the carbon atoms of the group being optionally substituted with one or more halo, hydroxy, unsubstituted lower alkyl or unsubstituted lower alkoxy groups, 5- or 6-member heteroalicyclic group having from 1 to 3 heteroatoms selected from the group consisting of nitrogen, oxygen and sulfur, the carbon and nitrogen (if present) atoms in the group being optionally substituted with one or more halo, hydroxy, unsubstituted lower alkyl or unsubstituted lower alkoxy groups, mercapto, (unsubstituted lower alkyl)thio, arylthio optionally substituted with one or more halo, hydroxy, unsubstituted lower alkyl or unsubstituted lower alkoxy groups, cyano, acyl, thioacyl, O-carbamyl, N-carbamyl, O-thiocarbamyl, N-thiocarbamyl, C-amido, N-amido, nitro, N-sulfonamido, S-sulfonamido, $R^{18}S(O)$-, $R^{18}S(O)_2$-, $-C(O)OR^{18}$, $R^{18}C(O)O$-, and $-NR^{18}R^{19}$,

wherein R^{18} and R^{19} are independently selected from the group consisting of hydrogen, unsubstituted lower alkyl, trihalomethyl, unsubstituted (C_3-C_6)cycloalkyl, unsubstituted lower alkenyl, unsubstituted lower alkynyl and aryl optionally optionally substituted with one or more halo, hydroxy, unsubstituted lower alkyl or unsubstituted lower alkoxy groups.

A "cycloalkyl" group refers to a 3 to 8 member all-carbon monocyclic ring, an all-carbon 5-member/6-member or 6-member/6-member fused bicyclic ring or a multicyclic fused ring (a "fused" ring system means that each ring in the system shares an adjacent pair of carbon atoms with each other ring in the system) group wherein one or more of the rings may contain one or more double bonds but none of the rings has a completely conjugated pi-electron system. Examples, without limitation, of cycloalkyl groups are cyclopropane, cyclobutane, cyclopentane, cyclopentene, cyclohexane, cyclohexadiene, adamantane, cycloheptane and, cycloheptatriene. A cycloalkyl group may be substituted or unsubstituted. When substituted, the substituent group(s) is preferably one or more independently selected from the group consisting of unsubstituted lower alkyl, trihaloalkyl, halo, hydroxy, unsubstituted lower alkoxy, aryl optionally substituted with one or more halo, hydroxy, unsubstituted lower alkyl or unsubstituted lower alkoxy groups, aryloxy optionally substituted with one or more halo, hydroxy, unsubstituted lower alkyl or unsubstituted lower alkoxy groups, 6-member heteroaryl having from 1 to 3 nitrogen atoms in the ring, the carbons in the ring being optionally substituted with one or more halo, hydroxy, unsubstituted lower alkyl or unsubstituted lower alkoxy groups, 5-member heteroaryl having from 1 to 3 heteroatoms selected from the group consisting of nitrogen, oxygen and sulfur, the carbon atoms of the group being optionally substituted with one or more halo, hydroxy, unsubstituted lower alkyl or unsubstituted lower alkoxy groups, 5- or 6-member heteroalicyclic group having from 1

to 3 heteroatoms selected from the group consisting of nitrogen, oxygen and sulfur, the carbon and nitogen (if present)atoms in the group being optionally substituted with one or more halo, hydroxy, unsubstituted lower alkyl or unsubstituted lower alkoxy groups, mercapto,(unsubstituted lower alkyl)thio, arylthio optionally substituted with one or more halo, hydroxy, unsubstituted lower alkyl or unsubstituted lower alkoxy groups, cyano, acyl, thioacyl, O-carbamyl, N-carbamyl, O-thiocarbamyl, N-thiocarbamyl, C-amido, N-amido, nitro, N-sulfonamido, S-sulfonamido, $R^{18}S(O)$-, $R^{18}S(O)_2$-, $-C(O)OR^{18}$, $R^{18}C(O)O$-, and $-NR^{18}R^{19}$ are as defined above.

An "alkenyl" group refers to an alkyl group, as defined herein, consisting of at least two carbon atoms and at least one carbon-carbon double bond.

An "alkynyl" group refers to an alkyl group, as defined herein, consisting of at least two carbon atoms and at least one carbon-carbon triple bond.

An "aryl" group refers to an all-carbon monocyclic or fused-ring polycyclic (i.e., rings which share adjacent pairs of carbon atoms) groups having a completely conjugated pi-electron system. Examples, without limitation, of aryl groups are phenyl, naphthalenyl and anthracenyl. The aryl group may be substituted or unsubstituted. When substituted, the substituted group(s) is preferably one or more independently selected from the group consisting of unsubstituted lower alkyl, trihaloalkyl, halo, hydroxy, unsubstituted lower alkoxy, mercapto,(unsubstituted lower alkyl)thio, cyano, acyl, thioacyl, O-carbamyl, N-carbamyl, O-thiocarbamyl, N-thiocarbamyl, C-amido, N-amido, nitro, N-sulfonamido, S-sulfonamido, $R^{18}S(O)$-, $R^{18}S(O)_2$-, $-C(O)OR^{18}$, $R^{18}C(O)O$-, and $-NR^{18}R^{19}$, with R^{18} and R^{19} as defined above.

As used herein, a "heteroaryl" group refers to a monocyclic or fused ring (i.e., rings which share an adjacent pair of atoms) group having in the ring(s) one or more atoms selected from the

group consisting of nitrogen, oxygen and sulfur and, in addition, having a completely conjugated pi-electron system. Examples, without limitation, of heteroaryl groups are pyrrole, furan, thiophene, imidazole, oxazole, thiazole, pyrazole, pyridine, pyrimidine, quinoline, isoquinoline, purine and carbazole. The heteroaryl group may be substituted or unsubstituted. When substituted, the substituted group(s) is preferably one or more independently selected from the group consisting of unsubstituted lower alkyl, trihaloalkyl, halo, hydroxy, unsubstituted lower alkoxy, mercapto, (unsubstituted lower alkyl)thio, cyano, acyl, thioacyl, O-carbamyl, N-carbamyl, O-thiocarbamyl, N-thiocarbamyl, C-amido, N-amido, nitro, N-sulfonamido, S-sulfonamido, $R^{18}S(O)$-, $R^{18}O)_2$-, -C(O)OR^{18}, $R^{18}C(O)O$-, and -$NR^{18}R^{19}$, with R^{18} and R^{19} as defined above.

A "heteroalicyclic" group refers to a monocyclic or fused ring group having in the ring(s) one or more atoms selected from the group consisting of nitrogen, oxygen and sulfur. The rings may also have one or more double bonds. However, the rings do not have a completely conjugated pi-electron system. The heteroalicyclic ring may be substituted or unsubstituted. When substituted, the substituted group(s) is preferably one or more independently selected from the group consisting of unsubstituted lower alkyl, trihaloalkyl, halo, hydroxy, unsubstituted lower alkoxy, mercapto, (unsubstituted lower alkyl)thio, cyano, acyl, thioacyl, O-carbamyl, N-carbamyl, O-thiocarbamyl, N-thiocarbamyl, C-amido, N-amido, nitro, N-sulfonamido, S-sulfonamido, $R^{18}S(O)$-, $R^{18}S(O)_2$-, -C(O)OR^{18}, $R^{18}C(O)O$-, and -$NR^{18}R^{19}$, with R^{18} and R^{19} as defined above.

A "hydroxy" group refers to an -OH group.

An "alkoxy" group refers to both an -O-(unsubstituted alkyl) and an -O-(unsubstituted cycloalkyl) group.

An "aryloxy" group refers to both an -O-aryl and an -O-heteroaryl group, as defined herein.

A "mercapto" group refers to an -SH group.

A "alkylthio" group refers to both an S-(unsubstituted alkyl) and an -S-(unsubstituted cycloalkyl) group.

A "arylthio" group refers to both an -S-aryl and an -S-heteroaryl group, as defined herein.

An "acyl" group refers to a -C(O)-R" group, where R" is selected from the group consisting of hydrogen, unsubstituted lower alkyl, trihalomethyl, unsubstituted cycloalkyl, aryl optionally substituted with one or more unsubstituted lower alkyl, trihalomethyl, unsubstituted lower alkoxy, halo and $-NR^{18}R^{19}$ groups, heteroaryl (bonded through a ring carbon) optionally substituted with one or more unsubstituted lower alkyl, trihaloalkyl, unsubstituted lower alkoxy, halo and $-NR^{18}R^{19}$ groups and heteroalicyclic (bonded through a ring carbon) optionally substituted with one or more unsubstituted lower alkyl, trihaloalkyl, unsubstituted lower alkoxy, halo and $-NR^{18}R^{19}$ groups.

An "aldehyde" group refers to an acyl group in which R" is hydrogen.

A "thioacyl" group refers to a -C(S)-R" group, with R" as defined herein.

An "ester" group refers to a -C(O)O-R" group with R" as defined herein except that R" cannot be hydrogen.

An "acetyl" group refers to a $-C(O)CH_3$ group.

A "halo" group refers to fluorine, chlorine, bromine or iodine.

A "trihalomethyl" group refers to a $-CX_3$ group wherein X is a halo group as defined herein.

A "trihalomethanesulfonyl" group refers to a $X_3CS(=O)_2-$ groups with X as defined above.

A "cyano" group refers to a -C≡N group.

A "methylenedioxy" group refers to a $-OCH_2O-$ group where the two oxygen atoms are bonded to adjacent carbon atoms.

An "ethylenedioxy" group refers to a $-OCH_2CH_2O-$ where the two oxygen atoms are bonded to adjacent carbon atoms.

An "S-sulfonamido" group refers to a $-S(O)_2NR^{18}R^{19}$ group, with R^{18} and R^{19} as defined herein.

An "N-sulfonamido" group refers to a $-NR^{18}S(O)_2R^{19}$ group, with R^{18} and R^{19} as defined herein.

An "O-carbamyl" group refers to a $-OC(O)NR^{18}R^{19}$ group with R^{18} and R^{19} as defined herein.

An "N-carbamyl" group refers to an $R^{18}OC(O)NR^{19}-$ group, with R^{18} and R^{19} as defined herein.

An "O-thiocarbamyl" group refers to a $-OC(S)NR^{18}R^{19}$ group with R^{18} and R^{19} as defined herein.

An "N-thiocarbamyl" group refers to a $R^{18}OC(S)NR^{19}-$ group, with R^{18} and R^{19} as defined herein.

An "amino" group refers to an $-NR^{18}R^{19}$ group, wherein R^{18} and R^{19} are both hydrogen.

A "C-amido" group refers to a $-C(O)NR^{18}R^{19}$ group with R^{18} and R^{19} as defined herein.

An "N-amido" group refers to a $R^{18}C(O)NR^{19}-$ group, with R^{18} and R^{19} as defined herein.

A "nitro" group refers to a $-NO_2$ group.

B. Preferred Structural Features.

A presently preferred embodiment of this invention is a compound in which:

R^1 and R^4 are hydrogen;

R^2 is selected from the group consisting of hydrogen, halo, lower alkoxy, $-C(O)R^{15}$ and $-S(O)_2NR^{13}R^{14}$;

R^3 is selected from the group consisting of hydrogen, lower alkoxy, $-C(O)R^{15}$, $-S(O)_2NR^{13}R^{14}$, aryl and heteroaryl;

R^5 is $-C(O)R^{10}$;

R^6 is selected from the group consisting of hydrogen and lower alkyl; and,

R^7 is selected from the group consisting of hydrogen, lower alkyl

and $-C(O)R^{17}$.

It is another presently preferred embodiment of this invention that, in a compound having a structure as described in the previous paragraph:

R^{10} is selected from the group consisting of hydroxy, lower alkoxy and $-NR^{11}(CH_2)_nR^{12}$, wherein

n is 2 or 3;

R^{11} is selected from the group consisting of hydrogen and lower alkyl; and,

R^{12} is selected from the group consisting of aryl and $-NR^{13}R^{14}$.

It is a further presently preferred embodiment of this invention that, in a compound having a structure as described in the previous two paragraphs, R^{13} and R^{14} are independently selected from the group consisting of hydrogen, lower alkyl, and, combined, $-(CH_2)_4-$, $-(CH_2)_5-$, $-(CH_2)_2O(CH_2)_2-$ or $-(CH_2)_2N(CH_3)(CH_2)_2-$.

A presently preferred embodiment of this invention is a compound in which:

R^1 is selected from the group consisting of hydrogen, lower alkyl, $(CH_2)_rR^{16}$ and $-C(O)NR^8R^9$;

R^2 is selected from the group consisting of hydrogen, halogen, aryl and $-S(O)_2NR^{13}R^{14}$;

R^3 is selected from the group consisting of hydrogen, lower alkyl, lower alkoxy, aryl, heteroaryl and $-C(O)R^{15}$;

R^4 is hydrogen;

R^5 is selected from the group consisting of hydrogen and lower alkyl;

R^6 is $-C(O)R^{10}$;

R^7 is selected from the group consisting of hydorgen, lower alkyl and aryl;

R^{16} is selected from the group consisting of hydroxy and $-C(O)R^{15}$; and,

r is 2 or 3.

A presently preferred embodiment of this invention is a

compound having as structure described in the paragraph just above in which R^3 is aryl optionally substituted with one or more groups selected from the group consisting of lower alkyl, lower alkoxy and halo.

Likewise, it is a presently preferred embodiment of this invention that, in a compound in which:

R^1 is selected from the group consisting of hydrogen, lower alkyl, $(CH_2)_rR^{16}$ and $-C(O)NR^8R^9$;

R^2 is selected from the group consisting of hydrogen, halogen, aryl and $-S(O)_2NR^{13}R^{14}$;

R^3 is selected from the group consisting of hydrogen, lower alkyl, lower alkoxy, aryl, heteroaryl and $-C(O)R^{15}$;

R^4 is hydrogen;

R^5 is selected from the group consisting of hydrogen and lower alkyl;

R^6 is $-C(O)R^{10}$;

R^7 is selected from the group consisting of hydorgen, lower alkyl and aryl;

R^{16} is selected from the group consisting of hydroxy and $-C(O)R^{15}$; and,

r is 2 or 3,

R^{10} is selected from the group consisting of hydroxy, lower alkoxy, $-NR^{13}R^{14}$ and $-NR^{11}(CH_2)_nR^{12}$, wherein n is 1, 2 or 3, R^{11} is hydrogen and R^{12} is selected from the group consisting of hydroxy, lower alkoxy, $-C(O)R^{15}$, heteroaryl and $-NR^{13}R^{14}$.

A further presently preferred embodiment of this invention is a compound having a structure as described in the paragraph immediately above in which R^{13} and R^{14} are independently selected from the group consisting of hydrogen, lower alkyl, heteroaryl and, combined, $-(CH_2)_4-$, $-(CH_2)_5-$, $-(CH_2)_2O(CH_2)_2-$, or $-(CH_2)_2N(CH_3)(CH_2)_2-$.

Another presently preferred embodiment of this invention is a compound in which:

R^1 is $-C(O)NR^8R^9$, wherein R^8 is hydrogen and R^9 is aryl optionally substituted with one or more groups selected from the group consisting of halo, hydroxy and lower alkoxy;

R^2 is selected from the group consisting of hydrogen, halogen, aryl and $-S(O)_2NR^{13}R^{14}$;

R^3 is selected from the group consisting of hydrogen, lower alkyl, lower alkoxy, aryl, heteroaryl and $-C(O)R^{15}$;

R^4 is hydrogen;

R^5 is selected from the group consisting of hydrogen and lower alkyl;

R^6 is $-C(O)R^{10}$;

R^7 is selected from the group consisting of hydorgen, lower alkyl and aryl;

R^{16} is selected from the group consisting of hydroxy and $-C(O)R^{15}$; and,

r is 2 or 3,

A still further presently preferred embodiment of this invention is a compound in which:

R^1 is selected from the group consisting of hydrogen and lower alkyl;

R^2 is selected from the group consisting of hydrogen, halo, lower alkoxy, aryl, $-C(O)R^{15}$ and $-S(O)_2NR^{13}R^{14}$;

R^3 is selected from the group consisting of hydrogen, halo, aryl, heteroaryl and $-C(O)R^{15}$;

R^4 is hydrogen;

R^5 is $-C(O)R^{10}$; and,

R^6 and R^7 combine to form a $-(CH_2)_4-$ group.

In a compound having a structure as described in the paragraph immediately above, it is a presently preferred embodiment that R^{10} is selected from the group consisting of hydroxy, alkoxy, $-NR^{13}R^{14}$ and $-NH(CH_2)_nNR^{13}R^{14}$ wherein n is 2 or 3.

It is a presently preferred embodiment of this invention

that, in a compound having a structure as described in the two paragraphs immediately above, R^{13} and R^{14} are independently selected from the group consisting of hydrogen, lower alkyl, and, combined, $-(CH_2)_4-$, $-(CH_2)_5-$, $-(CH_2)_2O(CH_2)_2-$ or $-(CH_2)_2N(CH_3)(CH_2)_2-$.

A compound in which:
R^1 and R^4 are selected from the group consisting of hydrogen, lower alkyl, lower alkoxy and halogen;
R^2 is selected from the group consisting of hydrogen, lower alkyl, lower alkoxy, halo, and $-S(O)_2NR^{13}R^{14}$;
R^3 is selected from the group consisting of hydrogen, lower alkyl, lower alkoxy, halo, aryl and $-C(O)R^{15}$;
R^5 and R^6 are selected from the group consisting of hydrogen and lower alkyl; and,
R^7 is $-C(O)R^{10}$ is also a presently preferred embodiment of this invention.

Finally, with regard to chemical structure, in a compound having a structure as described in the paragraph immediately above, R^{10} is selected from the group consisting of hydroxy, alkoxy, $-NR^{13}R^{14}$ and $-NR^{11}(CH_2)_nR^{12}$, wherein n is 2 or 3, R^{11} is selected from the group consisting of hydrogen and lower alkyl; and, R^{12} is $-NR^{13}R^{14}$, wherein R^{13} and R^{14} are independently selected from the group consisting of hydrogen, lower alkyl, and, combined, $-(CH_2)_4-$, $-(CH_2)_5-$, $-(CH_2)_2O(CH_2)_2-$ or $-(CH_2)_2N(CH_3)(CH_2)_2-$.

The chemical formulae referred to herein may exhibit the phenomena of tautomerism and structural isomerism. For example, the compounds described herein may adopt an E or a Z configuration about the double bond connecting the 2-indolinone moiety to the pyrrole moiety or they may be a mixture of E and Z. This invention encompasses any tautomeric or structural isomeric form and mixtures thereof which possess the ability to modulate RTK, CTK and/or STK activity and is not limited to any one

tautomeric or structural isomeric form.

2. **BIOCHEMISTRY/PHARMACOTHERAPY**

Another aspect of this invention relates to a method for the modulation of the catalytic activity of a PK by contacting a PK with a compound of this invention or a physiologically acceptable salt or prodrug thereof.

As used herein, "PK" refers to receptor protein tyrosine kinase (RTKs), non-receptor or "cellular" tyrosine kinase (CTKs) and serine-threonine kinases (STKs).

The term "method" refers to manners, means, techniques and procedures for accomplishing a given task including, but not limited to, those manners, means, techniques and procedures either known to, or readily developed from known manners, means, techniques and procedures by, practitioners of the chemical, pharmaceutical, biological, biochemical and medical arts.

As used herein, the term "modulation" or "modulating" refers to the alteration of the catalytic activity of RTKs, CTKs and STKs. In particular, modulating refers to the activation of the catalytic activity of RTKs, CTKs and STKs, preferably the activation or inhibition of the catalytic activity of RTKs, CTKs and STKs, depending on the concentration of the compound or salt to which the RTK, CTK or STK is exposed or, more preferably, the inhibition of the catalytic activity of RTKs, CTKs and STKs.

The term "catalytic activity" as used herein refers to the rate of phosphorylation of tyrosine under the influence, direct or indirect, of RTKs and/or CTKs or the phosphorylation of serine and threonine under the influence, direct or indirect, of STKs.

The term "contacting" as used herein refers to bringing a compound of this invention and a target PK together in such a manner that the compound can affect the catalytic activity of the PK, either directly, i.e., by interacting with the kinase itself, or indirectly, i.e., by interacting with another molecule on which the catalytic activity of the kinase is dependent. Such

"contacting" can be accomplished "in vitro," i.e., in a test tube, a petri dish or the like. In a test tube, contacting may involve only a compound and a PK of interest or it may involve whole cells. Cells may also be maintained or grown in cell culture dishes and contacted with a compound in that environment. In this context, the ability of a particular compound to affect a PK related disorder, i.e., the IC_{50} of the compound, defined below, can be determined before use of the compounds in vivo with more complex living organisms is attempted. For cells outside the organism, multiple methods exist, and are well-known to those skilled in the art, to get the PKs in contact with the compounds including, but not limited to, direct cell microinjection and numerous transmembrane carrier techniques.

A further aspect of this invention is that the modulation of the catalytic activity of PKs using a compound of this invention may be carried out in vitro or in vivo.

"In vitro" refers to procedures performed in an artificial environment such as, e.g., without limitation, in a test tube or culture medium.

As used herein, "in vivo" refers to procedures performed within a living organism such as, without limitation, a mouse, rat or rabbit.

A still further aspect of this invention is that the protein kinase whose catalytic activity is being modulated by a compound of this invention is selected from the group consisting of receptor protein tyrosine kinases, cellular (or non-receptor) tyrosine kinases and serine-threonine kinases.

It is an aspect of this invention that the receptor protein kinase whose catalytic activity is modulated by a compound of this invention is selected from the group consisting of EGF, HER2, HER3, HER4, IR, IGF-1R, IRR, PDGFRα, PDGFRβ, CSFIR, C-Kit, C-fms, Flk-1R, Flk4, KDR/Flk-1, Flt-1, FGFR-1R, FGFR-2R, FGFR-3R and FGFR-4R.

In addition, it is an aspect of this invention that the cellular tyrosine kinase whose catalytic activity is modulated by a compound of this invention is selected from the group consisting of Src, Frk, Btk, Csk, Abl, ZAP70, Fes/Fps, Fak, Jak, Ack, Yes, Fyn, Lyn, Lck, Blk, Hck, Fgr and Yrk.

Another aspect of this invention is that the serine-threonine protein kinase whose catalytic activity is modulated by a compound of this invention is selected from the group consisting of CDK2 and Raf.

A pharmaceutical composition of a compound of this invention with a pharmaceutically acceptable carrier or excipient is yet another aspect of this invention. Such pharmaceutical composition may contain both carriers and excipients as well as other components generally known to those skilled in the formulation of pharmaceutical compositions.

A method for treating or preventing a protein kinase related disorder in an organism comprising administering a therapeutically effective amount of a compound, salt or prodrug of this invention to an organism in need of such treatment is another aspect of this invention.

As used herein, "PK related disorder," "PK driven disorder," and "abnormal PK activity" all refer to a condition characterized by inappropriate, i.e., under or, more commonly, over, PK catalytic activity, where the particular PK can be an RTK, a CTK or an STK. Inappropriate catalytic activity can arise as the result of either: (1) PK expression in cells which normally do not express PKs, (2) increased PK expression leading to unwanted cell proliferation, differentiation and/or growth, or, (3) decreased PK expression leading to unwanted reductions in cell proliferation, differentiation and/or growth. Over-activity of a PK refers to either amplification of the gene encoding a particular PK or production of a level of PK activity which can correlate with a cell proliferation, differentiation and/or

growth disorder (that is, as the level of the PK increases, the severity of one or more of the symptoms of the cellular disorder increases). Under-activity is, of course, the converse, wherein the severity of one or more symptoms of a cellular disorder increase as the level of the PK activity decreases.

As used herein, the terms "prevent", "preventing" and "prevention" refer to a method for barring an organism from acquiring a PK related disorder in the first place.

As used herein, the terms "treat", "treating" and "treatment" refer to a method of alleviating or abrogating a PK mediated cellular disorder and/or its attendant symptoms. With regard particularly to cancer, these terms simply mean that the life expectancy of an individual affected with a cancer will be increased or that one or more of the symptoms of the disease will be reduced.

The term "organism" refers to any living entity comprised of at least one cell. A living organism can be as simple as, for example, a single eukariotic cell or as complex as a mammal, including a human being.

The term "therapeutically effective amount" as used herein refers to that amount of the compound being administered which will relieve to some extent one or more of the symptoms of the disorder being treated. In reference to the treatment of cancer, a therapeutically effective amount refers to that amount which has the effect of (1) reducing the size of the tumor, (2) inhibiting (that is, slowing to some extent, preferably stopping) tumor metastasis, (3) inhibiting to some extent (that is, slowing to some extent, preferably stopping) tumor growth, and/or, (4) relieving to some extent (or, preferably, eliminating) one or more symptoms associated with the cancer.

It is an aspect of this invention that the above-referenced protein kinase related disorder is selected from the group consisting of a receptor protein tyrosine kinase related

disorder, a cellular tyrosine kinase disorder and a serine-threonine kinase related disorder.

In yet another aspect of this invention, the above referenced protein kinase related disorder is selected from the group consisting of an EGFR related disorder, a PDGFR related disorder, an IGFR related disorder and a flk related disorder.

The above referenced protein kinase related disorder is a cancer selected from the group consisting of squamous cell carcinoma, sarcomas such as Kaposi's sarcoma, astrocytoma, glioblastoma, lung cancer, bladder cancer, colorectal cancer, gastrointestinal cancer, head and neck cancer, melanoma, ovarian cancer, prostate cancer, breast cancer, small-cell lung cancer and glioma in a further aspect of this invention.

The above referenced protein kinase related disorder is selected from the group consisting of diabetes, an autoimmune disorder, a hyperproliferation disorder, von Hippel-Lindau disease, restenosis, fibrosis, psoriasis, osteoarthritis, rheumatoid arthritis, an inflammatory disorder and angiogenesis in yet another aspect of this invention.

Additional disorders which may be treated or prevented using the compounds of this invention are immunological disorders such as autoimmune disease, AIDS and cardiovasular disorders such as atherosclerosis.

It is as aspect of this invention that a chemical compound that modulates the catalytic activity of a protein kinase may be identified by contacting cells expressing said protein kinase with a compound, salt or prodrug of the present invention and then monitoring said cells for an effect.

By "monitoring" is meant observing or detecting the effect of contacting a compound with a cell expressing a particular PK. The observed or detected effect can be a change in cell phenotype, in the catalytic activity of a PK or a change in the interaction of a PK with a natural binding partner. Techniques

for observing or detecting such effects are well-known in the art.

The above-referenced effect is selected from a change or an absence of change in a cell phenotype, a change or absence of change in the catalytic activity of said protein kinase or a change or absence of change in the interaction of said protein kinase with a natural binding partner in a final aspect of this invention.

"Cell phenotype" refers to the outward appearance of a cell or tissue or the biological function of the cell or tissue. Examples, without limitation, of a cell phenotype are cell size, cell growth, cell proliferation, cell differentiation, cell survival, apoptosis, and nutrient uptake and use. Such phenotypic characteristics are measurable by techniques well-known in the art.

A "natural binding partner" refers to a polypeptide that binds to a particular PK in a cell. Natural binding partners can play a role in propagating a signal in a PK-mediated signal transduction process. A change in the interaction of the natural binding partner with the PK can manifest itself as an increased or decreased concentration of the PK/natural binding partner complex and, as a result, in an observable change in the ability of the PK to mediate signal transduction.

It is also an aspect of this invention that a compound described herein, or its salt or prodrug, might be combined with other chemotherapeutic agents for the treatment of the diseases and disorders discussed above. For instance, a compound, salt or prodrug of this invention might be combined with alkylating agents such as fluorouracil (5-FU) alone or in further combination with leukovorin; or other alkylating agents such as, without limitation, other pyrimidine analogs such as UFT, capecitabine, gemcitabine and cytarabine, the alkyl sulfonates, e.g., busulfan (used in the treatment of chronic granulocytic

leukemia), improsulfan and piposulfan; aziridines, e.g., benzodepa, carboquone, meturedepa and uredepa; ethyleneimines and methylmelamines, e.g., altretamine, triethylenemelamine, triethylenephosphoramide, triethylenethiophosphoramide and trimethylolmelamine; and the nitrogen mustards, e.g., chlorambucil (used in the treatment of chronic lymphocytic leukemia, primary macroglobulinemia and non-Hodgkin's lymphoma), cyclophosphamide (used in the treatment of Hodgkin's disease, multiple myeloma, neuroblastoma, breast cancer, ovarian cancer, lung cancer, Wilm's tumor and rhabdomyosarcoma), estramustine, ifosfamide, novembrichin, prednimustine and uracil mustard (used in the treatment of primary thrombocytosis, non-Hodgkin's lymphoma, Hodgkin's disease and ovarian cancer); and triazines, e.g., dacarbazine (used in the treatment of soft tissue sarcoma).

Likewise a compound, salt or prodrug of this invention might be expected to have a beneficial effect in combination with other antimetabolite chemotherapeutic agents such as, without limitation, folic acid analogs, e.g. methotrexate (used in the treatment of acute lymphocytic leukemia, choriocarcinoma, mycosis fungiodes breast cancer, head and neck cancer and osteogenic sarcoma) and pteropterin; and the purine analogs such as mercaptopurine and thioguanine which find use in the treatment of acute granulocytic, acute lymphocytic and chronic granulocytic leukemias.

A compound, salt or prodrug of this invention might also be expected to prove efficacious in combination with natural product based chemotherapeutic agents such as, without limitation, the vinca alkaloids, e.g., vinblastin (used in the treatment of breast and testicular cancer), vincristine and vindesine; the epipodophylotoxins, e.g., etoposide and teniposide, both of which are useful in the treatment of testicular cancer and Kaposi's sarcoma; the antibiotic chemotherapeutic agents, e.g., daunorubicin, doxorubicin, epirubicin, mitomycin (used to treat

stomach, cervix, colon, breast, bladder and pancreatic cancer), dactinomycin, temozolomide, plicamycin, bleomycin (used in the treatment of skin, esophagus and genitourinary tract cancer); and the enzymatic chemotherapeutic agents such as L-asparaginase.

In addition to the above, a compound, salt or prodrug of this invention might be expected to have a beneficial effect used in combination with the platinum coordination complexes (cisplatin, etc.); substituted ureas such as hydroxyurea; methylhydrazine derivatives, e.g., procarbazine; adrenocortical suppressants, e.g., mitotane, aminoglutethimide; and hormone and hormone antagonists such as the adrenocorticosteriods (e.g., prednisone), progestins (e.g., hydroxyprogesterone caproate); estrogens (e.g., diethylstilbesterol); antiestrogens such as tamoxifen; androgens, e.g., testosterone propionate; and aromatase inhibitors such as anastrozole.

Finally, the combination of a compound of this invention might be expected to be particularly effective in combination with mitoxantrone or paclitaxel for the treatment of solid tumor cancers or leukemias such as, without limitation, acute myelogenous (non-lymphocytic) leukemia.

DETAILED DESCRIPTION OF THE INVENTION

1. BRIEF DESCRIPTION OF THE TABLES

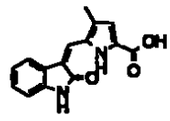
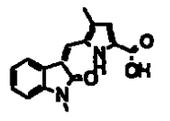
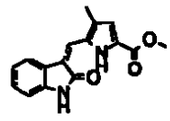
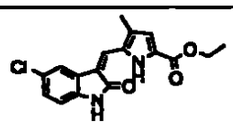
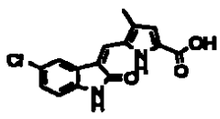
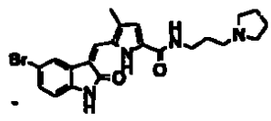
TABLE 1 shows the chemical structures of some exemplary compounds of this invention. The compound numbers correspond to the Example numbers in the Examples section. That is, the synthesis of Compound 1 in Table 1 is described in Example 1. The compounds presented in Table 1 are exemplary only and are not to be construed as limiting the scope of this invention in any manner.

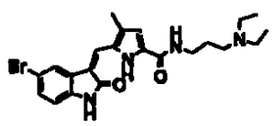
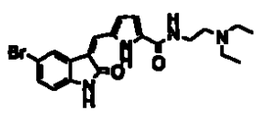
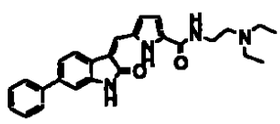
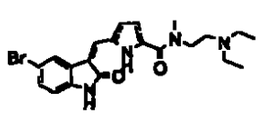
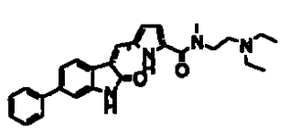
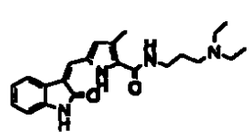
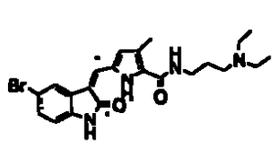
TABLE 2 shows the results of biological testing of some exemplary compounds of this invention. The results are reported in terms of IC_{50}, the micromolar (μM) concentration of the compound being tested which causes a 50% change in the activity of the target PKT compared to the activity of the PTK in a control to

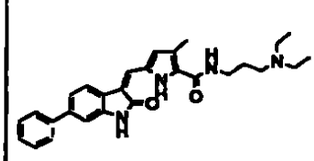
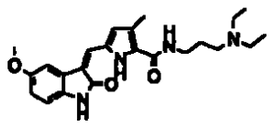
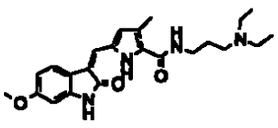
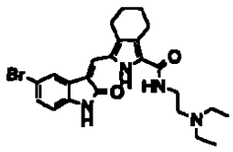
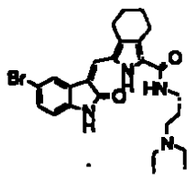
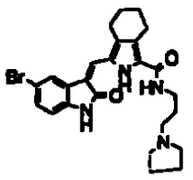
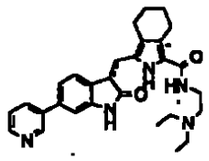
which no test compound has been added. Specifically, the results shown indicate the concentration of a test compound needed to cause a 50% reduction of the activity of the target PTK. Bioassays which have been or mau be used to evaluate compounds are described in detail below.

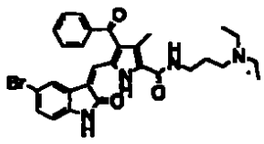
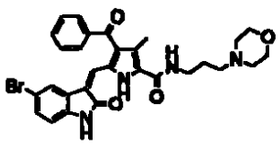
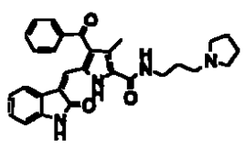
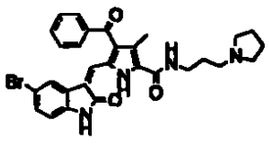
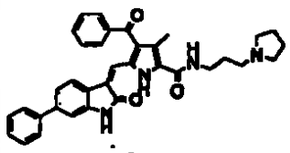
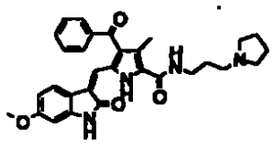
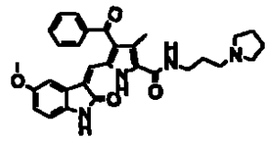
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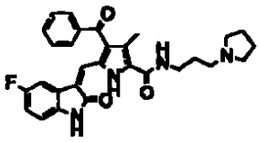
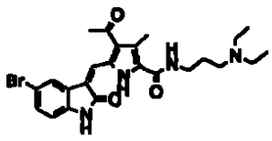
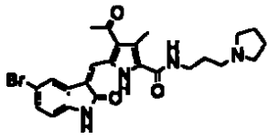
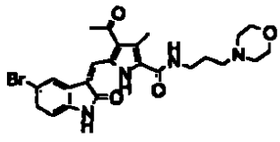
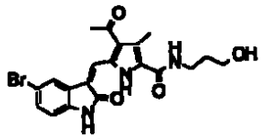
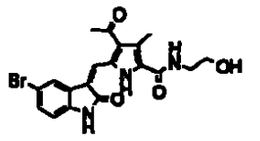
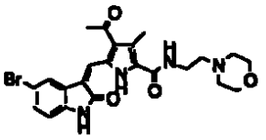
Table 1

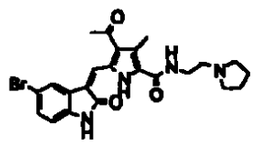
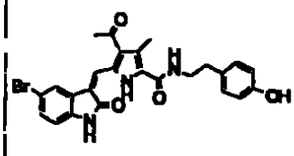
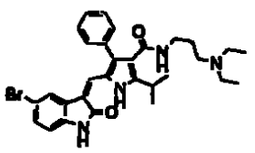
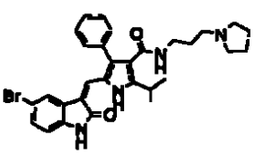
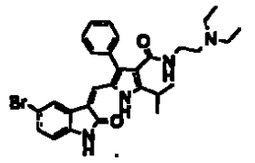
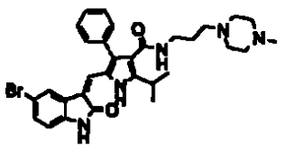
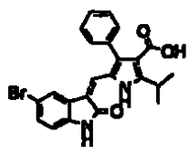
Example	Structure	Name
1		4-Methyl-5-(2-oxo-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrole-2-carboxylic acid
2		4-Methyl-5-(1-methyl-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrole-2-carboxylic acid
3		4-Methyl-5-(2-oxo-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrole-2-carboxylic acid methyl ester
4		5-(5-Chloro-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-4-methyl-1H-pyrrole-2-carboxylic acid ethyl ester
5		5-(5-Chloro-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-4-methyl-1H-pyrrole-2-carboxylic acid
6		5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-4-methyl-1H-pyrrole-2-carboxylic acid (3-pyrrolidin-1-ylpropyl)amide

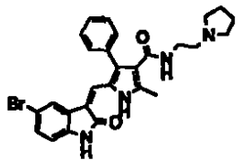
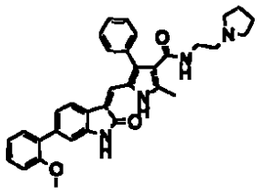
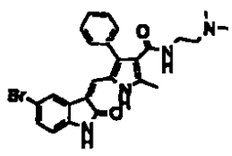
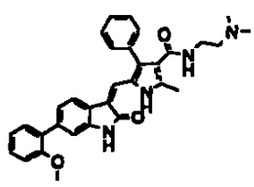
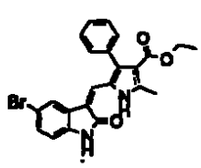
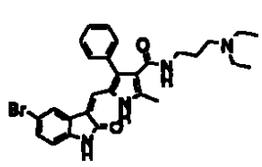
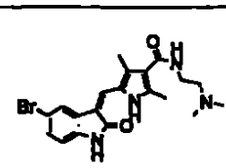
7		5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-4-methyl-1H-pyrrole-2-carboxylic acid (3-diethylaminopropyl)amide
8		5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrole-2-carboxylic acid (2-diethylaminoethyl)amide
9		5-(2-Oxo-6-phenyl-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrole-2-carboxylic acid (2-diethylaminoethyl)amide
10		5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrole-2-carboxylic acid (2-diethylaminoethyl)methylamide
11		5-(2-Oxo-6-phenyl-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrole-2-carboxylic acid (2-diethylaminoethyl)methylamide
12		3-Methyl-5-(2-oxo-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrole-2-carboxylic acid (3-diethylaminopropyl)amide
13		5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethiyl)-3-methyl-1H-pyrrole-2-carboxylic acid (3-diethylaminopropyl)amide

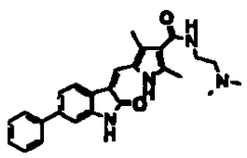
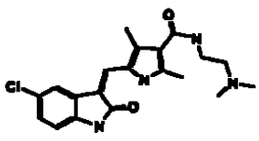
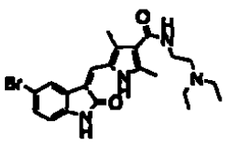
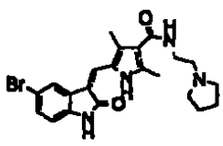
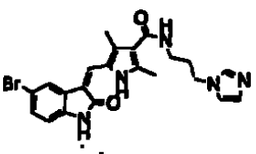
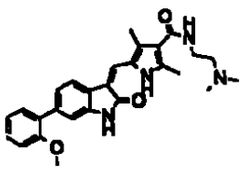
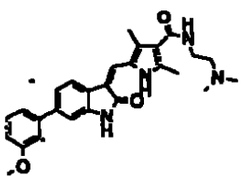
14		3-Methyl-5-(2-oxo-6-phenyl-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrole-2-carboxylic acid (3-diethylaminopropyl)amide
15		5-(5-Methoxy-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-3-methyl-1H-pyrrole-2-carboxylic acid (3-diethylaminopropyl)amide
16		5-(6-Methoxy-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-3-methyl-1H-pyrrole-2-carboxylic acid (3-diethylaminopropyl)amide
17		3-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-4,5,6,7-tetrahydro-2H-isoindole-1-carboxylic acid (2-diethylaminoethyl)amide
18		3-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-4,5,6,7-tetrahydro-2H-isoindole-1-carboxylic acid (3-diethylaminopropyl)amide
19		3-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-4,5,6,7-tetrahydro-2H-isoindole-1-carboxylic acid (3-pyrrolidin-1-ylpropyl)amide
20		3-(2-Oxo-6-pyridin-3-yl-1,2-dihydroindol-3-ylidenemethyl)-4,5,6,7-tetrahydro-2H-isoindole-1-carboxylic acid (2-diethylaminoethyl)amide

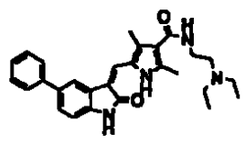
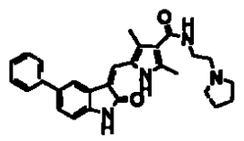
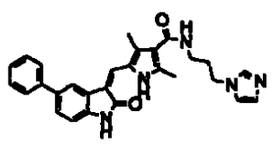
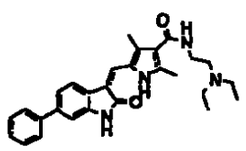
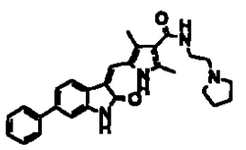
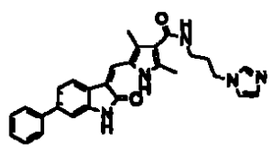
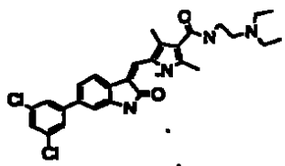
21		4-Benzoyl-5-(5-bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-3-methyl-1H-pyrrole-2-carboxylic acid (3-diethylaminopropyl)amide
22		4-Benzoyl-5-(5-bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-3-methyl-1H-pyrrole-2-carboxylic acid (3-morpholin-4-ylpropyl)amide
23		4-Benzoyl-3-methyl-5-(2-oxo-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrole-2-carboxylic acid (3-pyrrolidin-1-ylpropyl)amide
24		4-Benzoyl-5-(5-bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-3-methyl-1H-pyrrole-2-carboxylic acid (3-pyrrolidin-1-ylpropyl)amide
25		4-Benzoyl-3-methyl-5-(2-oxo-6-phenyl-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrole-2-carboxylic acid (3-pyrrolidin-1-ylpropyl)amide
26		4-Benzoyl-5-(6-methoxy-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-3-methyl-1H-pyrrole-2-carboxylic acid (3-pyrrolidin-1-ylpropyl)amide
27		4-Benzoyl-5-(5-methoxy-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-3-methyl-1H-pyrrole-2-carboxylic acid (3-pyrrolidin-1-ylpropyl)amide

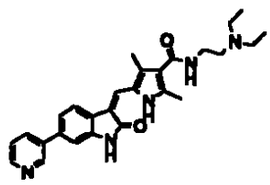
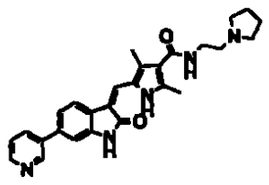
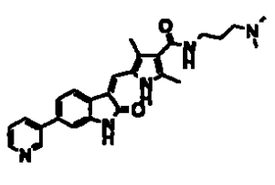
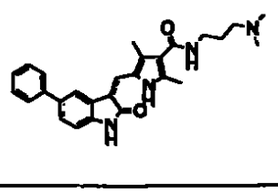
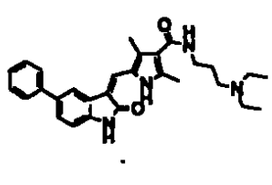
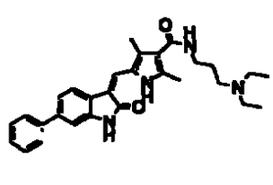
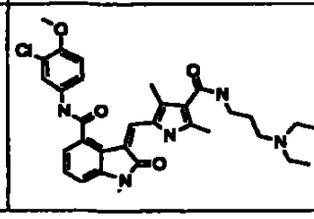
28		4-Benzoyl-5-(5-fluoro-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-3-methyl-1H-pyrrole-2-carboxylic acid (3-pyrrolidin-1-ylpropyl)amide
29		4-Acetyl-5-(5-bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-3-methyl-1H-pyrrole-2-carboxylic acid (3-diethylaminopropyl)amide
30		4-Acetyl-5-(5-bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-3-methyl-1H-pyrrole-2-carboxylic acid (3-pyrrolidin-1-ylpropyl)amide
31		4-Acetyl-5-(5-bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-3-methyl-1H-pyrrole-2-carboxylic acid (3-morpholin-4-ylpropyl)amide
32		4-Acetyl-5-(5-bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-3-methyl-1H-pyrrole-2-carboxylic acid (3-hydroxy-propyl)amide
33		4-Acetyl-5-(5-bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-3-methyl-1H-pyrrole-2-carboxylic acid (2-hydroxy-ethyl)amide
34		4-Acetyl-5-(5-bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-3-methyl-1H-pyrrole-2-carboxylic acid (2-morpholin-4-yl-ethyl)amide

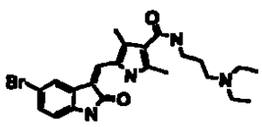
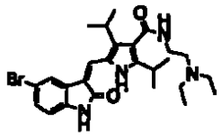
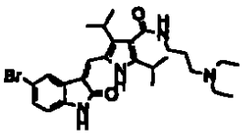
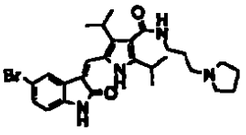
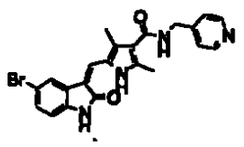
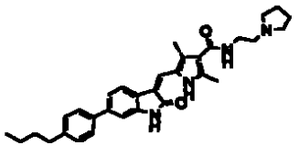
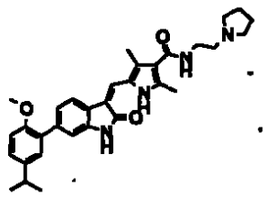
35		4-Acetyl-5-(5-bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-3-methyl-1H-pyrrole-2-carboxylic acid (2-pyrrolidin-1-yl-ethyl)amide
36		4-Acetyl-5-(5-bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-3-methyl-1H-pyrrole-2-carboxylic acid [2-(4-hydroxy-phenyl)-ethyl]amide
37		5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2-isopropyl-4-phenyl-1H-pyrrole-3-carboxylic acid (3-diethylaminopropyl)amide
38		5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2-isopropyl-4-phenyl-1H-pyrrole-3-carboxylic acid (3-pyrrolidin-1-ylpropyl)amide
39		5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2-isopropyl-4-phenyl-1H-pyrrole-3-carboxylic acid (2-diethylaminoethyl)amide
40		5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2-isopropyl-4-phenyl-1H-pyrrole-3-carboxylic acid [3-(4-methyl-piperazin-1-yl)-propyl]amide
41		5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2-isopropyl-4-phenyl-1H-pyrrole-3-carboxylic acid

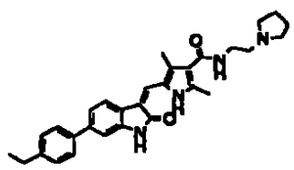
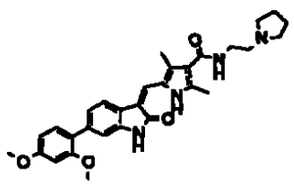
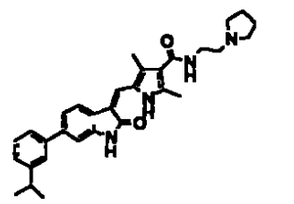
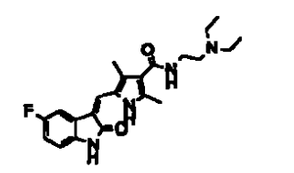
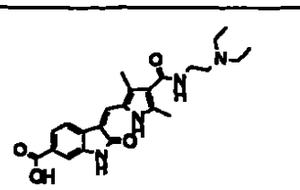
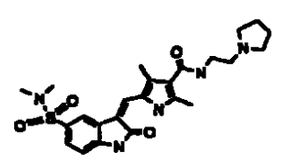
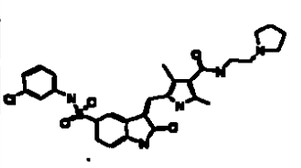
42		5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2-methyl-4-phenyl-1H-pyrrole-3-carboxylic acid (2-pyrrolidin-1-yl-ethyl)amide
43		5-[6-(2-Methoxy-phenyl)-2-oxo-1,2-dihydroindol-3-ylidenemethyl]-2-methyl-4-phenyl-1H-pyrrole-3-carboxylic acid (2-pyrrolidin-1-yl-ethyl)amide
44		5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2-methyl-4-phenyl-1H-pyrrole-3-carboxylic acid (2-dimethylamino-ethyl)amide
45		5-[6-(2-Methoxy-phenyl)-2-oxo-1,2-dihydroindol-3-ylidenemethyl]-2-methyl-4-phenyl-1H-pyrrole-3-carboxylic acid (2-dimethylamino-ethyl)amide
46		5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2-methyl-4-phenyl-1H-pyrrole-3-carboxylic acid ethyl ester
47		5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2-methyl-4-phenyl-1H-pyrrole-3-carboxylic acid (3-diethylaminopropyl)amide
48		5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (2-dimethylamino-ethyl)amide

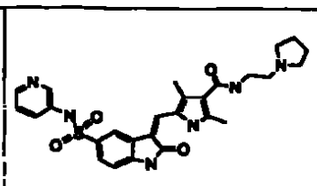
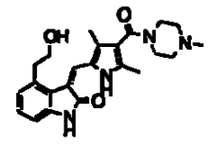
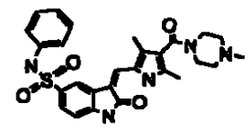
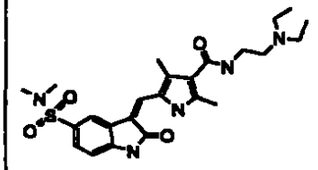
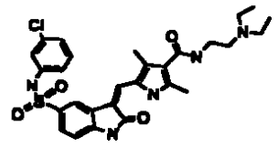
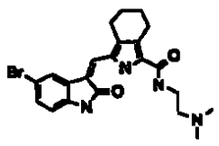
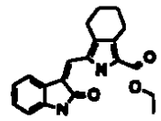
49		2,4-Dimethyl-5-(2-oxo-6-phenyl-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrole-3-carboxylic acid (2-dimethylamino-ethyl)amide
50		5-(5-Chloro-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (2-dimethylamino-ethyl)amide
51		5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (2-diethylaminoethyl)amide
52		5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (2-pyrrolidin-1-yl-ethyl)amide
53		5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (3-imidazol-1-ylpropyl)amide
54		5-[6-(2-Methoxy-phenyl)-2-oxo-1,2-dihydroindol-3-ylidenemethyl]-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (2-dimethylamino-ethyl)amide
55		5-[6-(3-Methoxy-phenyl)-2-oxo-1,2-dihydroindol-3-ylidenemethyl]-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (2-dimethylamino-ethyl)amide

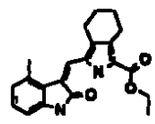
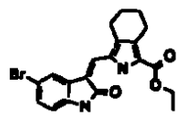
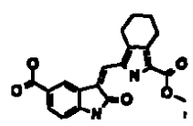
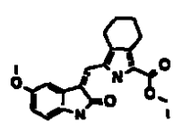
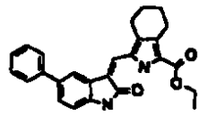
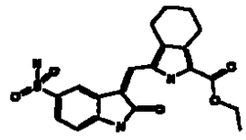
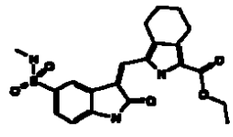
56		2,4-Dimethyl-5-(2-oxo-5-phenyl-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrole-3-carboxylic acid (2-diethylaminoethyl)amide
57		2,4-Dimethyl-5-(2-oxo-5-phenyl-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrole-3-carboxylic acid (2-pyrrolidin-1-yl-ethyl)amide
58		2,4-Dimethyl-5-(2-oxo-5-phenyl-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrole-3-carboxylic acid (3-imidazol-1-ylpropyl)amide
59		2,4-Dimethyl-5-(2-oxo-6-phenyl-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrole-3-carboxylic acid (2-diethylaminoethyl)amide
60		2,4-Dimethyl-5-(2-oxo-6-phenyl-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrole-3-carboxylic acid (2-pyrrolidin-1-yl-ethyl)amide
61		2,4-Dimethyl-5-(2-oxo-6-phenyl-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrole-3-carboxylic acid (3-imidazol-1-ylpropyl)amide
62		5-[6-(3,5-Dichloro-phenyl)-2-oxo-1,2-dihydroindol-3-ylidenemethyl]-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (2-diethylaminoethyl)amide

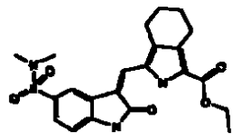
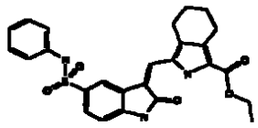
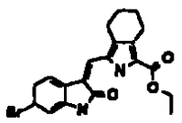
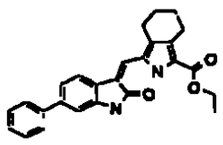
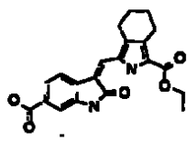
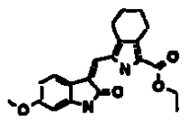
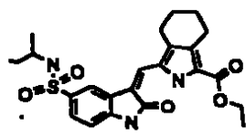
63		2,4-Dimethyl-5-(2-oxo-6-pyridin-3-yl-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrole-3-carboxylic acid (2-diethylaminoethyl)amide
64		2,4-Dimethyl-5-(2-oxo-6-pyridin-3-yl-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrole-3-carboxylic acid (2-pyrrolidin-1-yl-ethyl)amide
65		2,4-Dimethyl-5-(2-oxo-6-pyridin-3-yl-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrole-3-carboxylic acid (3-dimethylamino-propyl)amide
66		2,4-Dimethyl-5-(2-oxo-5-phenyl-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrole-3-carboxylic acid (3-dimethylamino-propyl)amide
67		2,4-Dimethyl-5-(2-oxo-5-phenyl-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrole-3-carboxylic acid (3-diethylaminopropyl)amide
68		2,4-Dimethyl-5-(2-oxo-6-phenyl-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrole-3-carboxylic acid (3-diethylaminopropyl)amide
69		3-[4-(3-Diethylamino-propylcarbamoyl)-3,5-dimethyl-1H-pyrrol-2-ylmethylene]-2-oxo-2,3-dihydro-1H-indole-4-carboxylic acid (3-chloro-4-methoxy-phenyl)amide

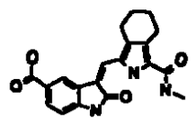
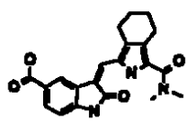
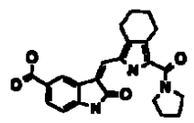
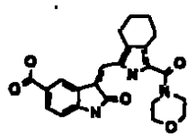
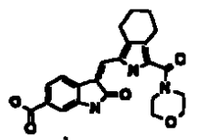
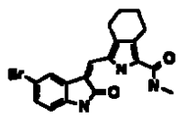
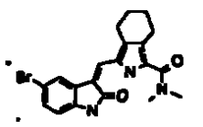
70		5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (3-diethylaminopropyl)amide
71		5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2,4-diisopropyl-1H-pyrrole-3-carboxylic acid (2-diethylaminoethyl)amide
72		5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2,4-diisopropyl-1H-pyrrole-3-carboxylic acid (3-diethylaminopropyl)amide
73		5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2,4-diisopropyl-1H-pyrrole-3-carboxylic acid (3-pyrrolidin-1-ylpropyl)amide
74		5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (pyridin-4-ylmethyl)amide
75		5-[6-(4-Butyl-phenyl)-2-oxo-1,2-dihydroindol-3-ylidenemethyl]-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (2-pyrrolidin-1-yl-ethyl)amide
76		5-[6-(5-Isopropyl-2-methoxy-phenyl)-2-oxo-1,2-dihydroindol-3-ylidenemethyl]-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (2-pyrrolidin-1-yl-ethyl)amide

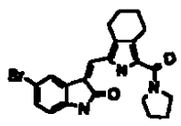
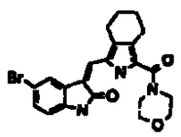
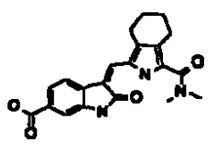
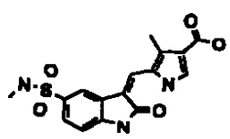
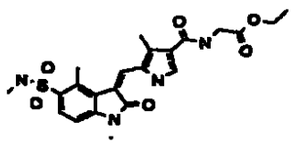
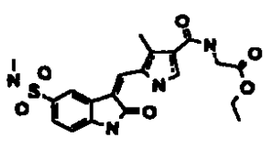
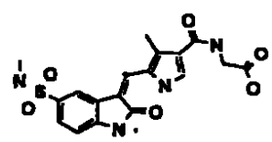
77		5-[6-(4-Ethyl-phenyl)-2-oxo-1,2-dihydroindol-3-ylidenemethyl]-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (2-pyrrolidin-1-yl-ethyl)amide
78		5-[6-(2,4-Dimethoxy-phenyl)-2-oxo-1,2-dihydroindol-3-ylidenemethyl]-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (2-pyrrolidin-1-yl-ethyl)amide
79		5-[5-(3-Isopropyl-phenyl)-2-oxo-1,2-dihydroindol-3-ylidenemethyl]-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (2-pyrrolidin-1-yl-ethyl)amide
80		5-(5-Fluoro-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (2-diethylaminoethyl)amide
81		3-[4-(2-diethylaminoethylcarbamoyl)-3,5-dimethyl-1H-pyrrol-2-ylmethylene]-2-oxo-2,3-dihydro-1H-indole-6-carboxylic acid
82		5-(5-Dimethylsulfamoyl-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (2-pyrrolidin-1-yl-ethyl)amide
83		5-[5-(3-Chloro-phenylsulfamoyl)-2-oxo-1,2-dihydroindol-3-ylidenemethyl]-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (2-pyrrolidin-1-yl-ethyl)amide

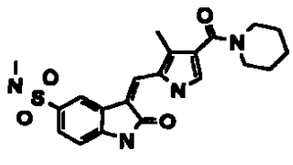
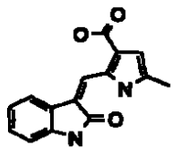
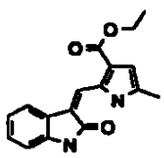
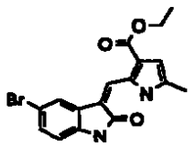
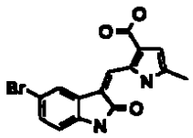
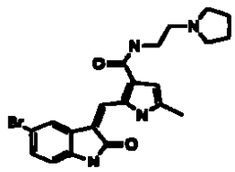
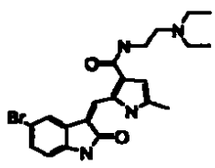
84		2,4-Dimethyl-5-[2-oxo-5-(pyridin-3-ylsulfamoyl)-1,2-dihydroindol-3-ylidenemethyl]-1H-pyrrole-3-carboxylic acid (2-pyrrolidin-1-yl-ethyl)amide
85		3-[3,5-Dimethyl-4-(4-methyl-piperazine-1-carbonyl)-1H-pyrrol-2-ylmethylene]-4-(2-hydroxy-ethyl)-1,3-dihydroindol-2-one
86		3-[3,5-Dimethyl-4-(4-methyl-piperazine-1-carbonyl)-1*H*-pyrrol-2-ylmethylene]-2-oxo-2,3-dihydro-1*H*-indole-5-sulfonic acid phenylamide
87		5-(5-Dimethylsulfamoyl-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (2-diethylaminoethyl)amide
88		5-[5-(3-Chloro-phenylsulfamoyl)-2-oxo-1,2-dihydroindol-3-ylidenemethyl]-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (2-diethylaminoethyl)amide
89		3-(5-Bromo-2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-4,5,6,7-tetrahydro-2*H*-isoindole-1-carboxylic acid (2-dimethylamino-ethyl)-amide
90		3-(2-Oxo-1,2-dihydro-indol-3-ylidenemethyl)-4,5,6,7-tetrahydro-2*H*-isoindole-1-carboxylic acid ethyl ester

91		3-(4-Methyl-2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-4,5,6,7-tetrahydro-2*H*-isoindole-1-carboxylic acid ethyl ester
92		3-(5-Bromo-2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-4,5,6,7-tetrahydro-2*H*-isoindole-1-carboxylic acid ethyl ester
93		3-(3-Ethoxycarbonyl-4,5,6,7-tetrahydro-2*H*-isoindol-1-ylmethylene)-2-oxo-2,3-dihydro-1*H*-indole-5-carboxylic acid
94		3-(5-Methoxy-2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-4,5,6,7-tetrahydro-2*H*-isoindole-1-carboxylic acid ethyl ester
95		3-(2-Oxo-5-phenyl-1,2-dihydro-indol-3-ylidenemethyl)-4,5,6,7-tetrahydro-2*H*-isoindole-1-carboxylic acid ethyl ester
96		3-(2-Oxo-5-sulfamoyl-1,2-dihydro-indol-3-ylidenemethyl)-4,5,6,7-tetrahydro-2*H*-isoindole-1-carboxylic acid ethyl ester
97		3-(5-Methylsulfamoyl-2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-4,5,6,7-tetrahydro-2*H*-isoindole-1-carboxylic acid ethyl ester

98		3-(5-Dimethylsulfamoyl-2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-4,5,6,7-tetrahydro-2*H*-isoindole-1-carboxylic acid ethyl ester
99		3-(2-Oxo-5-phenylsulfamoyl-1,2-dihydro-indol-3-ylidenemethyl)-4,5,6,7-tetrahydro-2*H*-isoindole-1-carboxylic acid ethyl ester
100		3-(6-Bromo-2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-4,5,6,7-tetrahydro-2*H*-isoindole-1-carboxylic acid ethyl ester
101		3-(2-Oxo-6-phenyl-1,2-dihydro-indol-3-ylidenemethyl)-4,5,6,7-tetrahydro-2*H*-isoindole-1-carboxylic acid ethyl ester
102		3-(3-Ethoxycarbonyl-4,5,6,7-tetrahydro-2*H*-isoindol-1-ylmethylene)-2-oxo-2,3-dihydro-1*H*-indole-6-carboxylic acid
103		3-(6-Methoxy-2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-4,5,6,7-tetrahydro-2*H*-isoindole-1-carboxylic acid ethyl ester
104		3-(5-Isopropylsulfamoyl-2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-4,5,6,7-tetrahydro-2*H*-isoindole-1-carboxylic acid ethyl ester

105		3-(3-Methylcarbamoyl-4,5,6,7-tetrahydro-2*H*-isoindol-1-ylmethylene)-2-oxo-2,3-dihydro-1*H*-indole-5-carboxylic acid
106		3-(3-Dimethylcarbamoyl-4,5,6,7-tetrahydro-2*H*-isoindol-1-ylmethylene)-2-oxo-2,3-dihydro-1*H*-indole-5-carboxylic acid
107		2-Oxo-3-[3-(pyrrolidine-1-carbonyl)-4,5,6,7-tetrahydro-2*H*-isoindol-1-ylmethylene]-2,3-dihydro-1*H*-indole-5-carboxylic acid
108		3-[3-(Morpholine-4-carbonyl)-4,5,6,7-tetrahydro-2*H*-isoindol-1-ylmethylene]-2-oxo-2,3-dihydro-1*H*-indole-5-carboxylic acid
109		3-[3-(Morpholine-4-carbonyl)-4,5,6,7-tetrahydro-2*H*-isoindol-1-ylmethylene]-2-oxo-2,3-dihydro-1*H*-indole-6-carboxylic acid
110		3-(5-Bromo-2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-4,5,6,7-tetrahydro-2*H*-isoindole-1-carboxylic acid methylamide
111		3-(5-Bromo-2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-4,5,6,7-tetrahydro-2*H*-isoindole-1-carboxylic acid dimethylamide

112		5-Bromo-3-[3-(pyrrolidine-1-carbonyl)-4,5,6,7-tetrahydro-2*H*-isoindol-1-ylmethylene]-1,3-dihydro-indol-2-one
113		5-Bromo-3-[3-(morpholine-4-carbonyl)-4,5,6,7-tetrahydro-2*H*-isoindol-1-ylmethylene]-1,3-dihydro-indol-2-one
114		3-(3-Dimethylcarbamoyl-4,5,6,7-tetrahydro-2*H*-isoindol-1-ylmethylene)-2-oxo-2,3-dihydro-1*H*-indole-6-carboxylic acid
115		4-Methyl-5-(5-methylsulfamoyl-2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-1*H*-pyrrole-3-carboxylic acid
116		([4-Methyl-5-(4-methyl-5-methylsulfamoyl-2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-1*H*-pyrrole-3-carbonyl]-amino)-acetic acid ethyl ester
117		([4-Methyl-5-(5-methylsulfamoyl-2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-1*H*-pyrrole-3-carbonyl]-amino)-acetic acid ethyl ester
118		([4-Methyl-5-(5-methylsulfamoyl-2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-1*H*-pyrrole-3-carbonyl]-amino)-acetic acid

119		3-[3-Methyl-4-(piperidine-1-carbonyl)-1*H*-pyrrol-2-ylmethylene]-2-oxo-2,3-dihydro-1*H*-indole-5-sulfonic acid methylamide
120		5-Methyl-2-(2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-1*H*-pyrrole-3-carboxylic acid
121		5-Methyl-2-(2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-1*H*-pyrrole-3-carboxylic acid ethyl ester
122		2-(5-Bromo-2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-5-methyl-1*H*-pyrrole-3-carboxylic acid ethyl ester
123		2-(5-Bromo-2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-5-methyl-1*H*-pyrrole-3-carboxylic acid
124		2-(5-Bromo-2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-5-methyl-1H-pyrrole-3-carboxylic acid (2-pyrrolidin-1-yl-ethyl)-amide
125		2-(5-Bromo-2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-5-methyl-1H-pyrrole-3-carboxylic acid (2-diethylamino-ethyl)-amide

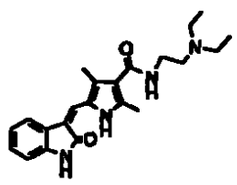
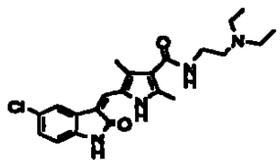
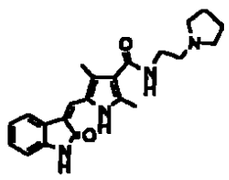
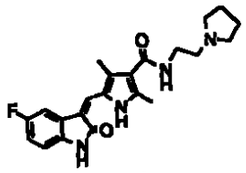
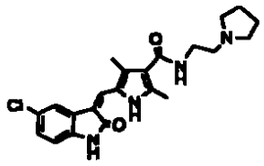
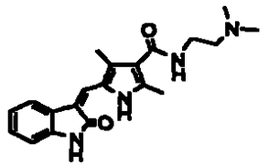
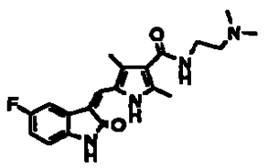
126		2,4-dimethyl-5-(2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-1H-pyrrole-3-carboxylic acid (2-diethylaminoethyl)amide
127		5-(5-Chloro-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (2-diethylaminoethyl)amide
128		2,4-dimethyl-5-(2-oxo-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrole-3-carboxylic acid (2-pyrrolidin-1-yl-ethyl)amide
129		5-(5-Fluoro-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (2-pyrrolidin-1-yl-ethyl)amide
130		5-(5-Chloro-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (2-pyrrolidin-1-yl-ethyl)amide
131		2,4-dimethyl-5-(2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-1H-pyrrole-3-carboxylic acid (2-dimethylaminoethyl)amide
132		5-(5-Fluoro-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (2-dimethylaminoethyl)amide

Table 2

Example	bio flkGST IC50 (nM)	bio FGFR1 IC50 (nM)	bio PDGF IC50 (nM)	bio EGF IC50 (nM)	cell EGF IC50 (nM)	Her2 IC50 (nM)	cdk2aps IC50 (nM)	bio pyk2 IC50 (nM)
1	57.66	15.16	>100	>100	>100			>100
2	>100		>100	>100	>100			
3	9.85	9.62	>100	>100	>100			>100
4	3.57	>20	>100	>100	>100	>100		
5	8.3	16.06	>100	>100	>100	>100		
6	4.04		>100	3.26	7.82	2.43		
7	7.74		>100	5.07	9.8	4.24		
8	12.1		>100	51.34	20.06	5.5		
9	0.66		>100	>100	>100	16.36		
10	5.72		>100	94.04	15.86	8.06		
11	9.77		>100	>100	>100	>100		
12	>20		21.46	>100		27.73		
13	>20		81.92	8.17		2.66		
14	13.01		42.41	>100		66.02		
15	>20		>100	>100		98.61		
16	>20		98.06	>100		23.32		
17	8.25	2.47	94.35	0.83	11.47	15.94	>10	
18	2.67			2.57	9.23	4.99		
19	7.5			6.86	34.18	8.37		
20	11.53			>100	41.16	8		
21	7.16		>100	40.34		27.69		
22	>20		>100	>100		87.67		
23	>20		>100	36.64		4.05		
24			>100	16.64		5.31		
25	12.55		>100	23.48		7.9		
26	16.03		66.87	34.67		10.04		
27			>100	29.6		3.91		
28	4.5		71.27	53.66		2.67		
29	10.12		>100	26.72		3.98		
30	9.4		>100	16.59		4.1		
31	>50		>100	9.83		47.19		
32	45.74		5.94	>100		>100		
34	>50		>100	>100		>100		

Example	bio flt3GST IC50 (mM)	bio FGFR1 IC50 (mM)	bio PDGF IC50 (mM)	bio EGF IC50 (mM)	cell EGF IC50 (mM)	Her2 IC50 (mM)	cdk2aps IC50 (mM)	bio pyk2 IC50 (mM)
35	>20		>100	80.4		54.14		
36	>20		>100	>100		>100		
37	0.22		3.06	10.78	9.84	1.4		
38	4.17		3.06	6.04	8.87	2.16		
39	3.36		4.89	3.67	14.54	3.53		
40	4.5		7.9	6.52		6.27		
42	0.1		0.12	11.95	74.55	20.43		
43	1.12		8.38	>100	37.33	53.37		
44	<0.05		0.02	20.73	67.46	6.99		
45	1.71		>100	>100	29.95	>100		
46	30.62		6.18	>100	>100	>100		
47	0.06	1.56	0.06	11.42	41.54	8.4	>20	1.05
48	0.006	0.3	<0.78	17.88	21.58	7.93		0.09
49			<0.78	>100	43.88	>100		
50			<0.78	>100	20.34	>100		
51	0.006	1.66	0.01	18.1	21.61	23.24	18.89	0.35
52	0.08	1.26	<0.78	12.53	>100	>100	10.66	0.45
53			<0.78	>100	>100	>100		
54	1.98		<0.78	23.88	9.78	7.02		
55	0.27		0.53	6.03	35.99	77.82		
56	2.32		3.19	>100	10.03	7.11		
57	0.06		7.98	>100	9.97	6.94		
58			21.14	>100	>100	>100		
59			<0.78	>100	>100	>100		
60			<0.78	>100	>100	>100		
61			<0.78	>100	>100	>100		
62	8.00		8.32	>100	>100	>100		
63	0.21		<0.78	8.59	>100	>100		
64	0.55		<0.78	30.49	>100	>100		
65	0.37		<0.05	>100	74.36	15.97		
66	<0.05			>100	11.84	2.76		
67	0.39		24.77	31.38	19.79	2.58		
68	1.16		0.03	>100	23.52	34.13		
69	0.3		56.55	>100	97.54	>100		

Example	bio flt3ST IC50 (mM)	bio FGFR1 IC50 (mM)	bio PDGF IC50 (mM)	bio EGF IC50 (mM)	cell EGF IC50 (mM)	Her2 IC50 (mM)	cdk2epa IC50 (mM)	bio pyk2 IC50 (mM)
70	0.09	1.50	0.0030	10.57	8.42	7.88	12.62	0.83
71	15.21		22.5	>100		9.91		
72	6.06		10.54	>100	39.94	9.85		
73	5.95		14.12	>100	39.5	8.59		
74	1.2		0.09	46.75		>100		
75	2.7		61.55	>100		>100		
76	3.33		19.18	5.11		3.01		
77	0.49		25.01	>100		>100		
78	1.94		70.82	9.33		4.25		
79	1.49		>100	27.39		>100		
80	0.08	1.1	0.002	>100		50.9		
	0.13	4.29	0.001	>100		50.19	17.19	0.28
81	0.21		0.16	>100		>100		
82	2.03	7.69	6.86	>100		>100		0.31
83	0.34	0.41	9.48	2.16		66.9		0.008
84	1.36		12.51	57.2		6.86		0.008
85	0.2	0.8	2.59	>100		3.76		
86	1.45	1.3	19.8	41.8		>100		3.58
87	3.27	7.58	5.48	>100		9.1		0.17
88	0.35	1.16	8.06	2.36		>100		0.09
89	7.84		47.58	8.53	9.67	15.97		
115	7.3		7.48	>100		>100	0.006	
116	>20		>100	>100		>100	<0.0005	
117	0.91		12.9	>100		>100	0.006	
118	1.93		1.2	>100		>100	0.002	
119	1.36		61.63	>100		>100	<0.0005	
126								
127								
128	4.09	4.55	0.017	>20				
129	0.54	3.68	0.0014	>20				
130	0.1	0.34	0.087	>20				
131	0.09	1.51	0.11	>20				
132	0.013	1.14	0.0003	>20				

2. INDICATIONS/TARGET DISEASES

The PKs whose catalytic activity is modulated by the compounds of this invention include protein tyrosine kinases of which there are two types, receptor tyrosine kinases (RTKs) and cellular tyrosine kinases (CTKs), and serine-threonine kinases (STKs). RTK mediated signal transduction is initiated by extracellular interaction with a specific growth factor (ligand), followed by receptor dimerization, transient stimulation of the intrinsic protein tyrosine kinase activity and phosphorylation. Binding sites are thereby created for intracellular signal transduction molecules and lead to the formation of complexes with a spectrum of cytoplasmic signaling molecules that facilitate the appropriate cellular response (e.g., cell division, metabolic effects on the extracellular microenvironment, etc.). See, Schlessinger and Ullrich, 1992, Neuron 9:303-391.

It has been shown that tyrosine phosphorylation sites on growth factor receptors function as high-affinity binding sites for SH2 (src homology) domains of signaling molecules. Fantl et al., 1992, Cell 69:413-423, Songyang et al., 1994, Mol. Cell. Biol. 14:2777-2785), Songyang et al., 1993, Cell 72:767-778, and Koch et al., 1991, Science 252:668-678. Several intracellular substrate proteins that associate with RTKs have been identified. They may be divided into two principal groups: (1) substrates that have a catalytic domain, and (2) substrates which lack such domain but which serve as adapters and associate with catalytically active molecules. Songyang et al., 1993, Cell 72:767-778. The specificity of the interactions between receptors and SH2 domains of their substrates is determined by the amino acid residues immediately surrounding the phosphorylated tyrosine residue. Differences in the binding affinities between SH2 domains and the amino acid sequences surrounding the phosphotyrosine residues on particular receptors

are consistent with the observed differences in their substrate phosphorylation profiles. Songyang et al., 1993, Cell 72:767-778. These observations suggest that the function of each RTK is determined not only by its pattern of expression and ligand availability but also by the array of downstream signal transduction pathways that are activated by a particular receptor. Thus, phosphorylation provides an important regulatory step which determines the selectivity of signaling pathways recruited by specific growth factor receptors, as well as differentiation factor receptors.

STKs, being primarily cytosolic, affect the internal biochemistry of the cell, often as a down-line response to a PTK event. STKs have been implicated in the signaling process which initiates DNA synthesis and subsequent mitosis leading to cell proliferation.

Thus, PK signal transduction results in, among other responses, cell proliferation, differentiation, growth and metabolism. Abnormal cell proliferation may result in a wide array of disorders and diseases, including the development of neoplasia such as carcinoma, sarcoma, glioblastoma and hemangioma, disorders such as leukemia, psoriasis, arteriosclerosis, arthritis and diabetic retinopathy and other disorders related to uncontrolled angiogenesis and/or vasculogenesis.

A precise understanding of the mechanism by which the compounds of this invention inhibit PKs is not required in order to practice the present invention. However, while not hereby being bound to any particular mechanism or theory, it is believed that the compounds interact with the amino acids in the catalytic region of PKs. PKs typically possess a bi-lobate structure wherein ATP appears to bind in the cleft between the two lobes in a region where the amino acids are conserved among PKs. Inhibitors of PKs are believed to bind by non-covalent

interactions such as hydrogen bonding, van der Waals forces and ionic interactions in the same general region where the aforesaid ATP binds to the PKs. More specifically, it is thought that the 2-indolinone component of the compounds of this invention binds in the general space normally occupied by the adenine ring of ATP. Specificity of a particular molecule for a particular PK may then arise as the result of additional interactions between the various substituents on the 2-indolinone core and the amino acid domains specific to particular PKs. Thus, different indolinone substituents may contribute to preferential binding to particular PKs. The ability to select compounds active at different ATP (or other nucleotide) binding sites makes the compounds of this invention useful for targeting any protein with such a site. The compounds disclosed herein may thus have utility as in vitro assays for such proteins as well as exhibiting in vivo therapeutic effects through interaction with such proteins.

In another aspect, the protein kinase, the catalytic activity of which is modulated by contact with a compound of this invention, is a protein tyrosine kinase, more particularly, a receptor protein tyrosine kinase. Among the receptor protein tyrosine kinases whose catalytic activity can be modulated with a compound of this invention, or salt thereof, are, without limitation, EGF, HER2, HER3, HER4, IR, IGF-1R, IRR, PDGFRα, PDGFRβ, CSFIR, C-Kit, C-fms, Flk-1R, Flk4, KDR/Flk-1, Flt-1, FGFR-1R, FGFR-2R, FGFR-3R and FGFR-4R.

The protein tyrosine kinase whose catalytic activity is modulated by contact with a compound of this invention, or a salt or a prodrug thereof, can also be a non-receptor or cellular protein tyrosine kinase (CTK). Thus, the catalytic activity of CTKs such as, without limitation, Src, Frk, Btk, Csk, Abl, ZAP70, Fes, Fps, Fak, Jak, Ack, Yes, Fyn, Lyn, Lck, Blk, Hck, Fgr and Yrk, may be modulated by contact with a compound or salt of this invention.

Still another group of PKs which may have their catalytic activity modulated by contact with a compound of this invention are the serine-threonine protein kinases such as, without limitation, CDK2 and Raf.

In another aspect, this invention relates to a method for treating or preventing a PK related disorder by administering a therapeutically effective amount of a compound of this invention, or a salt or a prodrug thereof, to an organism.

It is also an aspect of this invention that a pharmaceutical composition containing a compound of this invention or a salt or prodrug thereof is administered to an organism for the purpose of preventing or treating a PK related disorder.

This invention is therefore directed to compounds that modulate PK signal transduction by affecting the enzymatic activity of RTKs, CTKs and/or STKs, thereby interfering with the signals transduced by such proteins. More particularly, the present invention is directed to compounds which modulate RTK, CTK and/or STK mediated signal transduction pathways as a therapeutic approach to the treatment of many kinds of solid tumors, including but not limited to carcinomas, sarcomas including Kaposi's sarcoma, erythroblastoma, glioblastoma, meningioma, astrocytoma, melanoma and myoblastoma. Treatment or prevention of non-solid tumor cancers such as leukemia are also contemplated by this invention. Indications may include, but are not limited to brain cancers, bladder cancers, ovarian cancers, gastric cancers, pancreas cancers, colon cancers, blood cancers, lung cancers and bone cancers.

Further examples, without limitation, of the types of disorders related to inappropriate PK activity that the compounds described herein may be useful in preventing, treating and studying, are cell proliferative disorders, fibrotic disorders and metabolic disorders.

Cell proliferative disorders, which may be prevented, treated or further studied by the present invention include cancer, blood vessel proliferative disorders and mesangial cell proliferative disorders.

Blood vessel proliferative disorders refer to disorders related to abnormal vasculogenesis (blood vessel formation) and angiogenesis (spreading of blood vessels). While vasculogenesis and angiogenesis play important roles in a variety of normal physiological processes such as embryonic development, corpus luteum formation, wound healing and organ regeneration, they also play a pivotal role in cancer development where they result in the formation of new capillaries needed to keep a tumor alive. Other examples of blood vessel proliferation disorders include arthritis, where new capillary blood vessels invade the joint and destroy cartilage, and ocular diseases, like diabetic retinopathy, where new capillaries in the retina invade the vitreous, bleed and cause blindness.

Two structurally related RTKs have been identified to bind VEGF with high affinity: the fms-like tyrosine 1 (fit-1) receptor (Shibuya et al., 1990, Oncogene,5:519-524; De Vries et al., 1992, Science, 255:989-991) and the KDR/FLK-1 receptor, also known as VEGF-R2. Vascular endothelial growth factor (VEGF) has been reported to be an endothelial cell specific mitogen with in vitro endothelial cell growth promoting activity. Ferrara & Henzel, 1989, Biochein. Biophys. Res. Comm., 161:851-858; Vaisman et al., 1990, J. Biol. Chem., 265:19461-19566. Information set forth in U.S. application Ser. Nos. 08/193,829, 08/038,596 and 07/975,750, strongly suggest that VEGF is not only responsible for endothelial cell proliferation, but also is the prime regulator of normal and pathological angiogenesis. See generally, Klagsburn & Soker, 1993, Current Biology, 3(10)699-702; Houck, et al., 1992, J. Biol. Chem.,' 267:26031-26037.

Normal vasculogenesis and angiogenesis play important roles

in a variety of physiological processes such as embryonic development, wound healing, organ regeneration and female reproductive processes such as follicle development in the corpus luteum during ovulation and placental growth after pregnancy. Folkman & Shing, 1992, J. Biological Chem., 267(16):10931-34. Uncontrolled vasculogenesis and/or angiogenesis has been associated with diseases such as diabetes as well as with malignant solid tumors that rely on vascularization for growth. Klagsburn & Soker, 1993, Current Biology, 3(10):699-702; Folkham, 1991, J. Natl. Cancer Inst., 82:4-6; Weidner, et al., 1991, New Engl. J. Med., 324:1-5.

The surmised role of VEGF in endothelial cell proliferation and migration during angiogenesis and vasculogenesis indicates an important role for the KDR/FLK-1 receptor in these processes. Diseases such as diabetes mellitus (Folkman, 198, in XIth Congress of Thrombosis and Haemostasis (Verstraeta, et al., eds.), pp. 583-596, Leuven University Press, Leuven) and arthritis, as well as malignant tumor growth may result from uncontrolled angiogenesis. See e.g., Folkman, 1971, N. Engl. J. Med., 285:1182-1186. The receptors to which VEGF specifically binds are an important and powerful therapeutic target for the regulation and modulation of vasculogenesis and/or angiogenesis and a variety of severe diseases which involve abnormal cellular growth caused by such processes. Plowman, et al., 1994, DN&P, 7(6):334-339. More particularly, the KDR/FLK-1 receptor's highly specific role in neovascularization make it a choice target for therapeutic approaches to the treatment of cancer and other diseases which involve the uncontrolled formation of blood vessels.

Thus, one aspect of the present invention relates to compounds capable of regulating and/or modulating tyrosine kinase signal transduction including KDR/FLK-1 receptor signal transduction in order to inhibit or promote angiogenesis and/or

vasculogenesis, that is, compounds that inhibit, prevent, or interfere with the signal transduced by KDR/FLK-1 when activated by ligands such as VEGF. Although it is believed that the compounds of the present invention act on a receptor or other component along the tyrosine kinase signal transduction pathway, they may also act directly on the tumor cells that result from uncontrolled angiogenesis.

Although the nomenclature of the human and murine counterparts of the generic "flk-I" receptor differ, they are, in many respects, interchangeable. The murine receptor, Flk-1, and its human counterpart, KDR, share a sequence homology of 93.4% within the intracellular domain. Likewise, murine FLK-I binds human VEGF with the same affinity as mouse VEGF, and accordingly, is activated by the ligand derived from either species. Millauer et al., 1993, Cell, 72:835-846; Quinn et al., 1993, Proc. Natl. Acad. Sci. USA, 90:7533-7537. FLK-1 also associates with and subsequently tyrosine phosphorylates human RTK substrates (e.g., PLC-γ or p85) when co-expressed in 293 cells (human embryonal kidney fibroblasts).

Models which rely upon the FLK-1 receptor therefore are directly applicable to understanding the KDR receptor. For example, use of the murine FLK-1 receptor in methods which identify compounds that regulate the murine signal transduction pathway are directly applicable to the identification of compounds which may be used to regulate the human signal transduction pathway, that is, which regulate activity related to the KDR receptor. Thus, chemical compounds identified as inhibitors of KDR/FLK-1 in vitro, can be confirmed in suitable in vivo models. Both in vivo mouse and rat animal models have been demonstrated to be of excellent value for the examination of the clinical potential of agents acting on the KDR/FLK-1 induced signal transduction pathway.

Thus, in one aspect, this invention is directed to compounds

that regulate, modulate and/or inhibit vasculogenesis and/or angiogenesis by affecting the enzymatic activity of the KDR/FLK-1 receptor and interfering with the signal transduced by KDR/FLK-1. In another aspect, the present invention is directed to compounds which regulate, modulate and/or inhibit the KDR/FLK-1 mediated signal transduction pathway as a therapeutic approach to the treatment of many kinds of solid tumors including, but not limited to, glioblastoma, melanoma and Kaposi's sarcoma, and ovarian, lung, mammary, prostate, pancreatic, colon and epidermoid carcinoma. In addition, data suggest the administration of compounds which inhibit the KDR/Flk-1 mediated signal transduction pathway may also be used in the treatment of hemangioma, restenois and diabetic retinopathy.

A further aspect of this invention relates to the inhibition of vasculogenesis and angiogenesis by other receptor-mediated pathways, including the pathway comprising the flt-1 receptor.

Receptor tyrosine kinase mediated signal transduction is initiated by extracellular interaction with a specific growth factor (ligand), followed by receptor dimerization, transient stimulation of the intrinsic protein tyrosine kinase activity and autophosphorylation. Binding sites are thereby created for intracellular signal transduction molecules which leads to the formation of complexes with a spectrum of cytoplasmic signalling molecules that facilitate the appropriate cellular response, e.g., cell division and metabolic effects to the extracellular microenvironment. See, Schlessinger and Ullrich, 1992, Neuron, 9:1-20.

The close homology of the intracellular regions of KDR/FLK-1 with that of the PDGF-β receptor (50.3% homology) and/or the related flt-1 receptor indicates the induction of overlapping signal transduction pathways. For example, for the PDGF-β receptor, members of the src family (Twamley et al., 1993, Proc. Natl. Acad. Sci. USA, 90:7696-7700), phosphatidylinositol-3'-

kinase (Hu et al., 1992, Mol. Cell. Biol., 12:981-990), phospholipase cγ (Kashishian & Cooper, 1993, Mol. Cell. Biol., 4:49-51), ras-GTPase-activating protein, (Kashishian et al., 1992, EMBO J., 11:1373-1382), PTP-ID/syp (Kazlauskas et al., 1993, Proc. Natl. Acad. Sci. USA, 10 90:6939-6943), Grb2 (Arvidsson et al., 1994, Mol. Cell. Biol., 14:6715-6726), and the adapter molecules Shc and Nck (Nishimura et al., 1993, Mol. Cell. Biol., 13:6889-6896), have been shown to bind to regions involving different autophosphorylation sites. See generally, Claesson-Welsh, 1994, Prog. Growth Factor Res., 5:37-54. Thus, it is likely that signal transduction pathways activated by KDR/FLK-1 include the ras pathway (Rozakis et al., 1992, Nature, 360:689-692), the PI-3'-kinase, the src-mediated and the plcγ-mediated pathways. Each of these pathways may play a critical role in the angiogenic and/or vasculogenic effect of KDR/FLK-1 in endothelial cells. Consequently, a still further aspect of this invention relates to the use of the organic compounds described herein to modulate angiogenesis and vasculogenesis as such processes are controlled by these pathways.

Conversely, disorders related to the shrinkage, contraction or closing of blood vessels, such as restenosis, are also implicated and may be treated or prevented by the methods of this invention.

Fibrotic disorders refer to the abnormal formation of extracellular matrices. Examples of fibrotic disorders include hepatic cirrhosis and mesangial cell proliferative disorders. Hepatic cirrhosis is characterized by the increase in extracellular matrix constituents resulting in the formation of a hepatic scar. An increased extracellular matrix resulting in a hepatic scar can also be caused by a viral infection such as hepatitis. Lipocytes appear to play a major role in hepatic cirrhosis. Other fibrotic disorders implicated include atherosclerosis.

Mesangial cell proliferative disorders refer to disorders brought about by abnormal proliferation of mesangial cells. Mesangial proliferative disorders include various human renal diseases such as glomerulonephritis, diabetic nephropathy and malignant nephrosclerosis as well as such disorders as thrombotic microangiopathy syndromes, transplant rejection, and glomerulopathies. The RTK PDGFR has been implicated in the maintenance of mesangial cell proliferation. Floege et al., 1993, Kidney International 43:47S-54S.

Many cancers are cell proliferative disorders and, as noted previously, PKs have been associated with cell proliferative disorders. Thus, it is not surprising that PKs such as, for example, members of the RTK family have been associated with the development of cancer. Some of these receptors, like EGFR (Tuzi et al., 1991, Br. J. Cancer 63:227-233, Torp et al., 1992, APMIS 100:713-719) HER2/neu (Slamon et al., 1989, Science 244:707-712) and PDGF-R (Kumabe et al., 1992, Oncogene, 7:627-633) are over-expressed in many tumors and/or persistently activated by autocrine loops. In fact, in the most common and severe cancers these receptor over-expressions (Akbasak and Suner-Akbasak et al., 1992, J. Neurol. Sci., 111:119-133, Dickson et al., 1992, Cancer Treatment Res. 61:249-273, Korc et al., 1992, J. Clin. Invest. 90:1352-1360) and autocrine loops (Lee and Donoghue, 1992, J. Cell. Biol., 118:1057-1070, Korc et al., supra, Akbasak and Suner-Akbasak et al., supra) have been demonstrated. For example, EGFR has been associated with squamous cell carcinoma, astrocytoma, glioblastoma, head and neck cancer, lung cancer and bladder cancer. HER2 has been associated with breast, ovarian, gastric, lung, pancreas and bladder cancer. PDGFR has been associated with glioblastoma and melanoma as well as lung, ovarian and prostate cancer. The RTK c-met has also been associated with malignant tumor formation. For example, c-met has been associated with, among other cancers, colorectal,

thyroid, pancreatic, gastric and hepatocellular carcinomas and lymphomas. Additionally c-met has been linked to leukemia. Over-expression of the c-met gene has also been detected in patients with Hodgkins disease and Burkitts disease.

IGF-IR, in addition to being implicated in nutritional support and in type-II diabetes, has also been associated with several types of cancers. For example, IGF-I has been implicated as an autocrine growth stimulator for several tumor types, e.g. human breast cancer carcinoma cells (Arteaga et al., 1989, J. Clin. Invest. 84:1418-1423) and small lung tumor cells (Macauley et al., 1990, Cancer Res., 50:2511-2517). In addition, IGF-I, while integrally involved in the normal growth and differentiation of the nervous system, also appears to be an autocrine stimulator of human gliomas. Sandberg-Nordqvist et al., 1993, Cancer Res. 53:2475-2478. The importance of IGF-IR and its ligands in cell proliferation is further supported by the fact that many cell types in culture (fibroblasts, epithelial cells, smooth muscle cells, T-lymphocytes, myeloid cells, chondrocytes and osteoblasts (the stem cells of the bone marrow)) are stimulated to grow by IGF-I. Goldring and Goldring, 1991, Eukaryotic Gene Expression,1:301-326. Baserga and Coppola suggest that IGF-IR plays a central role in the mechanism of transformation and, as such, could be a preferred target for therapeutic interventions for a broad spectrum of human malignancies. Baserga, 1995, Cancer Res., 55:249-252, Baserga, 1994, Cell 79:927-930, Coppola et al., 1994, Mol. Cell. Biol., 14:4588-4595.

STKs have been implicated in many types of cancer including, notably, breast cancer (Cance, et al., Int. J. Cancer, 54:571-77 (1993)).

The association between abnormal PK activity and disease is not restricted to cancer. For example, RTKs have been associated with diseases such as psoriasis, diabetes mellitus, endometriosis, angiogenesis, atheromatous plaque development,

Alzheimer's disease, restenosis, von Hippel-Lindau disease, epidermal hyperproliferation, neurodegenerative diseases, age-related macular degeneration and hemangiomas. For example, EGFR has been indicated in corneal and dermal wound healing. Defects in Insulin-R and IGF-1R are indicated in type-II diabetes mellitus. A more complete correlation between specific RTKs and their therapeutic indications is set forth in Plowman et al., 1994, DN&P 7:334-339.

As noted previously, not only RTKs but CTKs including, but not limited to, src, abl, fps, yes, fyn, lyn, lck, blk, hck, fgr and yrk (reviewed by Bolen et al., 1992, FASEB J., 6:3403-3409) are involved in the proliferative and metabolic signal transduction pathway and thus could be expected, and have been shown, to be involved in many PTK-mediated disorders to which the present invention is directed. For example, mutated src (v-src) has been shown to be an oncoprotein ($pp60^{v\text{-}src}$) in chicken. Moreover, its cellular homolog, the proto-oncogene $pp60^{c\text{-}src}$ transmits oncogenic signals of many receptors. Over-expression of EGFR or HER2/neu in tumors leads to the constitutive activation of $pp60^{c\ src}$, which is characteristic of malignant cells but absent in normal cells. On the other hand, mice deficient in the expression of c-src exhibit an osteopetrotic phenotype, indicating a key participation of c-src in osteoclast function and a possible involvement in related disorders.

Similarly, Zap70 has been implicated in T-cell signaling which may relate to autoimmune disorders.

STKs have been associated with inflamation, autoimmune disease, immunoresponses, and hyperproliferation disorders such as restenosis, fibrosis, psoriasis, osteoarthritis and rheumatoid arthritis.

PKs have also been implicated in embryo implantation. Thus, the compounds of this invention may provide an effective method of preventing such embryo implantation and thereby be useful as birth control agents.

Finally, both RTKs and CTKs are currently suspected as being involved in hyperimmune disorders.

A method for identifying a chemical compound that modulates the catalytic activity of one or more of the above discussed protein kinases is another aspect of this invention. The method involves, for example, contacting cells expressing the desired protein kinase with a compound of this invention (or its salt or prodrug) and monitoring the cells for any effect that the compound has on them. The effect may be any observable, either to the naked eye or through the use of instrumentation, change or absence of change in a cell phenotype. The change or absence of change in the cell phenotype monitored may be, for example, without limitation, a change or absence of change in the catalytic activity of the protein kinase in the cells or a change or absence of change in the interaction of the protein kinase with a natural binding partner.

Examples of the effect of a number of exemplary compounds of this invention on several PTKs are shown in Table 2. The compounds and data presented are not to be construed as limiting the scope of this invention in any manner whatsoever.

In yet another aspect, the compounds of the instant invention can also be used as anti-infective agents. For example, indolinone compounds are known to exhibit antibacterial and antifungal activities. *See, e.g.*, Singh and Jha (1989), "Indolinone derivatives as potential antimicrobial agents," *Zentralblatt fur Mikrobiologie* 144(2):105-9. In addition, indolinone compounds have been reported to exhibit significant antiviral activity. *See, e.g.*, Maass *et al.* (1993), "Viral resistance to the thiazolo-iso-indolinones, a new class of nonnucleoside inhibitors of human immunodeficiency virus type 1 reverse transcriptase," *Antimicrobial Agents and Chemotherapy* 37(12):2612-7.

3. **PHARMACEUTICAL COMPOSITIONS AND USE**

A compound of the present invention, a prodrug thereof or a physiologically acceptable salt of either the compound or its prodrug, can be administered as such to a human patient or can be administered in pharmaceutical compositions in which the foregoing materials are mixed with suitable carriers or excipient(s). Techniques for formulation and administration of drugs may be found in "Remington's Pharmacological Sciences," Mack Publishing Co., Easton, PA, latest edition.

Routes of Administration.

As used herein, "administer" or "administration" refers to the delivery of a compound, salt or prodrug of the present invention or of a pharmaceutical composition containing a compound, salt or prodrug of this invention to an organism for the purpose of prevention or treatment of a PK-related disorder.

Suitable routes of administration may include, without limitation, oral, rectal, transmucosal or intestinal administration or intramuscular, subcutaneous, intramedullary, intrathecal, direct intraventricular, intravenous, intravitreal, intraperitoneal, intranasal, or intraocular injections. The preferred routes of administration are oral and parenteral.

Alternatively, one may administer the compound in a local rather than systemic manner, for example, via injection of the compound directly into a solid tumor, often in a depot or sustained release formulation.

Furthermore, one may administer the drug in a targeted drug delivery system, for example, in a liposome coated with tumor-specific antibody. The liposomes will be targeted to and taken up selectively by the tumor.

Composition/Formulation.

Pharmaceutical compositions of the present invention may be manufactured by processes well known in the art, e.g., by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping or lyophilizing processes.

Pharmaceutical compositions for use in accordance with the present invention may be formulated in conventional manner using one or more physiologically acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. Proper formulation is dependent upon the route of administration chosen.

For injection, the compounds of the invention may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks' solution, Ringer's solution, or physiological saline buffer. For transmucosal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

For oral administration, the compounds can be formulated by combining the active compounds with pharmaceutically acceptable carriers well known in the art. Such carriers enable the compounds of the invention to be formulated as tablets, pills, lozenges, dragees, capsules, liquids, gels, syrups, slurries, suspensions and the like, for oral ingestion by a patient. Pharmaceutical preparations for oral use can be made using a solid excipient, optionally grinding the resulting mixture, and processing the mixture of granules, after adding other suitable auxiliaries if desired, to obtain tablets or dragee cores. Useful excipients are, in particular, fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol, cellulose preparations such as, for example, maize starch, wheat starch, rice starch and potato starch and other materials such as gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-

cellulose, sodium carboxymethylcellulose, and/or polyvinyl-pyrrolidone (PVP). If desired, disintegrating agents may be added, such as cross-linked polyvinyl pyrrolidone, agar, or alginic acid. A salt such as sodium alginate may also be used.

Dragee cores are provided with suitable coatings. For this purpose, concentrated sugar solutions may be used which may optionally contain gum arabic, talc, polyvinyl pyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for identification or to characterize different combinations of active compound doses.

Pharmaceutical compositions which can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a plasticizer, such as glycerol or sorbitol. The push-fit capsules can contain the active ingredients in admixture with a filler such as lactose, a binder such as starch, and/or a lubricant such as talc or magnesium stearate and, optionally, stabilizers. In soft capsules, the active compounds may be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols. Stabilizers may be added in these formulations, also.

For administration by inhalation, the compounds for use according to the present invention are conveniently delivered in the form of an aerosol spray using a pressurized pack or a nebulizer and a suitable propellant, e.g., without limitation, dichlorodifluoromethane, trichlorofluoromethane, dichlorotetra-fluoroethane or carbon dioxide. In the case of a pressurized aerosol, the dosage unit may be controlled by providing a valve to deliver a metered amount. Capsules and cartridges of, for example, gelatin for use in an inhaler or insufflator may be

formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

The compounds may also be formulated for parenteral administration, e.g., by bolus injection or continuous infusion. Formulations for injection may be presented in unit dosage form, e.g., in ampoules or in multi-dose containers, with an added preservative. The compositions may take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulating materials such as suspending, stabilizing and/or dispersing agents.

Pharmaceutical compositions for parenteral administration include aqueous solutions of a water soluble form, such as, without limitation, a salt, of the active compound. Additionally, suspensions of the active compounds may be prepared in a lipophilic vehicle. Suitable lipophilic vehicles include fatty oils such as sesame oil, synthetic fatty acid esters such as ethyl oleate and triglycerides, or materials such as liposomes. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Optionally, the suspension may also contain suitable stabilizers and/or agents that increase the solubility of the compounds to allow for the preparation of highly concentrated solutions.

Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, e.g., sterile, pyrogen-free water, before use.

The compounds may also be formulated in rectal compositions such as suppositories or retention enemas, using, e.g., conventional suppository bases such as cocoa butter or other glycerides.

In addition to the fomulations described previously, the compounds may also be formulated as depot preparations. Such long acting formulations may be administered by implantation (for

example, subcutaneously or intramuscularly) or by intramuscular injection. A compound of this invention may be formulated for this route of administration with suitable polymeric or hydrophobic materials (for instance, in an emulsion with a pharamcologically acceptable oil), with ion exchange resins, or as a sparingly soluble derivative such as, without limitation, a sparingly soluble salt.

A non-limiting example of a pharmaceutical carrier for the hydrophobic compounds of the invention is a cosolvent system comprising benzyl alcohol, a nonpolar surfactant, a water-miscible organic polymer and an aqueous phase such as the VPD co-solvent system. VPD is a solution of 3% w/v benzyl alcohol, 8% w/v of the nonpolar surfactant Polysorbate 80, and 65% w/v polyethylene glycol 300, made up to volume in absolute ethanol. The VPD co-solvent system (VPD:D5W) consists of VPD diluted 1:1 with a 5% dextrose in water solution. This co-solvent system dissolves hydrophobic compounds well, and itself produces low toxicity upon systemic administration. Naturally, the proportions of such a co-solvent system may be varied considerably without destroying its solubility and toxicity characteristics. Furthermore, the identity of the co-solvent components may be varied: for example, other low-toxicity nonpolar surfactants may be used instead of Polysorbate 80, the fraction size of polyethylene glycol may be varied, other biocompatible polymers may replace polyethylene glycol, *e.g.*, polyvinyl pyrrolidone, and other sugars or polysaccharides may substitute for dextrose.

Alternatively, other delivery systems for hydrophobic pharmaceutical compounds may be employed. Liposomes and emulsions are well known examples of delivery vehicles or carriers for hydrophobic drugs. In addtion, certain organic solvents such as dimethylsulfoxide also may be employed, although often at the cost of greater toxicity.

Additionally, the compounds may be delivered using a sustained-release system, such as semipermeable matrices of solid hydrophobic polymers containing the therapeutic agent. Various sustained-release materials have been established and are well known by those skilled in the art. Sustained-release capsules may, depending on their chemical nature, release the compounds for a few weeks up to over 100 days. Depending on the chemical nature and the biological stability of the therapeutic reagent, additional strategies for protein stabilization may be employed.

The pharmaceutical compositions herein also may comprise suitable solid or gel phase carriers or excipients. Examples of such carriers or excipients include, but are not limited to, calcium carbonate, calcium phosphate, various sugars, starches, cellulose derivatives, gelatin, and polymers such as polyethylene glycols.

Many of the PK modulating compounds of the invention may be provided as physiologically acceptable salts wherein the claimed compound may form the negatively or the positively charged species. Examples of salts in which the compound forms the positively charged moiety include, without limitation, quaternary ammonium (defined elsewhere herein), salts such as the hydrochloride, sulfate, carbonate, lactate, tartrate, maleate, succinate wherein the nitrogen atom of the quaternary ammonium group is a nitrogen of the selected compound of this invention which has reacted with the appropriate acid. Salts in which a compound of this invention forms the negatively charged species include, without limitation, the sodium, potassium, calcium and magnesium salts formed by the reaction of a carboxylic acid group in the compound with an appropriate base (e.g. sodium hydroxide (NaOH), potassium hydroxide (KOH), Calcium hydroxide ($Ca(OH)_2$), etc.).

Dosage.

Pharmaceutical compositions suitable for use in the present invention include compositions wherein the active ingredients are contained in an amount sufficient to achieve the intended purpose, e.g., the modulation of PK activity or the treatment or prevention of a PK-related disorder.

More specifically, a therapeutically effective amount means an amount of compound effective to prevent, alleviate or ameliorate symptoms of disease or prolong the survival of the subject being treated.

Determination of a therapeutically effective amount is well within the capability of those skilled in the art, especially in light of the detailed disclosure provided herein.

For any compound used in the methods of the invention, the therapeutically effective amount or dose can be estimated initially from cell culture assays. Then, the dosage can be formulated for use in animal models so as to achieve a circulating concentration range that includes the IC_{33} as determined in cell culture (i.e., the concentration of the test compound which achieves a half-maximal inhibition of the PK activity). Such information can then be used to more accurately determine useful doses in humans.

Toxicity and therapeutic efficacy of the compounds described herein can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., by determining the IC_{50} and the LD_{50} (both of which are discussed elsewhere herein) for a subject compound. The data obtained from these cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage may vary depending upon the dosage form employed and the route of administration utilized. The exact formulation, route of administration and dosage can be chosen by the individual physician in view of the patient's condition. (See e.g., Fingl, et al., 1975, in "The Pharmacological Basis of Therapeutics", Ch. 1 p.1).

Dosage amount and interval may be adjusted individually to provide plasma levels of the active species which are sufficient to maintain the kinase modulating effects. These plasma levels are referred to as minimal effective concentrations (MECs). The MEC will vary for each compound but can be estimated from in vitro data, e.g., the concentration necessary to achieve 50-90% inhibition of a kinase may be ascertained using the assays described herein. Dosages necessary to achieve the MEC will depend on individual characteristics and route of administration. HPLC assays or bioassays can be used to determine plasma concentrations.

Dosage intervals can also be determined using MEC value. Compounds should be administered using a regimen that maintains plasma levels above the MEC for 10-90% of the time, preferably between 30-90% and most preferably between 50-90%.

In cases of local administration or selective uptake, the effective local concentration of the drug may not be related to plasma concentration and other procedures known in the art may be employed to determine the correct dosage amount and interval.

The amount of a composition administered will, of course, be dependent on the subject being treated, the severity of the affliction, the manner of administration, the judgment of the prescribing physician, etc.

Packaging.

The compositions may, if desired, be presented in a pack or dispenser device, such as an FDA approved kit, which may contain one or more unit dosage forms containing the active ingredient. The pack may for example comprise metal or plastic foil, such as a blister pack. The pack or dispenser device may be accompanied by instructions for administration. The pack or dispenser may also be accompanied by a notice associated with the container in a form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals, which notice is

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reflective of approval by the agency of the form of the compositions or of human or veterinary administration. Such notice, for example, may be of the labeling approved by the U.S. Food and Drug Administration for prescription drugs or of an approved product insert. Compositions comprising a compound of the invention formulated in a compatible pharmaceutical carrier may also be prepared, placed in an appropriate container, and labeled for treatment of an indicated condition. Suitable conditions indicated on the label may include treatment of a tumor, inhibition of angiogenesis, treatment of fibrosis, diabetes, and the like.

4. SYNTHESIS

The compounds of this invention, as well as the precursor 2-oxindoles and aldehydes, may be readily synthesized using techniques well known in the chemical arts. It will be appreciated by those skilled in the art that other synthetic pathways for forming the compounds of the invention are available and that the following is offered by way of example and not limitation.

A. General synthetic procedure.

The following general methodology may be employed to prepare the compounds of this invention:

The appropriately substituted 2-oxindole (1 equiv.), the appropriately substituted aldehyde (1.2 equiv.) and a base (0.1 equiv.) are mixed in a solvent (1-2 ml/mmol 2-oxindole) and the mixture is then heated for from about 2 to about 12 hours. After cooling, the precipitate that forms is filtered, washed with cold ethanol or ether and vacuum dried to give the solid product. If no precipitate forms, the reaction mixture is concentrated and the residue is triturated with dichloromethane/ether, the resulting solid is collected by filtration and then dried. The

product may optionally be further purified by chromatography.

The base may be an organic or an inorganic base. If an organic base is used, preferably it is a nitrogen base. Examples of organic nitrogen bases include, but are not limited to, diisopropylamine, trimethylamine, triethylamine, aniline, pyridine, 1,8-diazabicyclo[5.4.1]undec-7-ene, pyrrolidine and piperidine.

Examples of inorganic bases are, without limitation, ammonia, alkali metal or alkaline earth hydroxides, phosphates, carbonates, bicarbonates, bisulfates and amides. The alkali metals include, lithium, sodium and potassium while the alkaline earths include calcium, magnesium and barium.

In a presently preferred embodiment of this invention, when the solvent is a protic solvent, such as water or alcohol, the base is an alkali metal or an alkaline earth inorganic base, preferably, a alkali metal or an alkaline earth hydroxide.

It will be clear to those skilled in the art, based both on known general principles of organic synthesis and on the disclosures herein which base would be most appropriate for the reaction contemplated.

The solvent in which the reaction is carried out may be a protic or an aprotic solvent, preferably it is a protic solvent. A "protic solvent" is a solvent which has hydrogen atom(s) covalently bonded to oxygen or nitrogen atoms which renders the hydrogen atoms appreciably acidic and thus capable of being "shared" with a solute through hydrogen bonding. Examples of protic solvents include, without limitation, water and alcohols.

An "aprotic solvent" may be polar or non-polar but, in either case, does not contain acidic hydrogens and therefore is not capable of hydrogen bonding with solutes. Examples, without limitation, of non-polar aprotic solvents, are pentane, hexane, benzene, toluene, methylene chloride and carbon tetrachloride.

Examples of polar aprotic solvents are chloroform, tetrahydrofuran, dimethylsulfoxide and dimethylformamide.

In a presently preferred embodiment of this invention, the solvent is a protic solvent, preferably water or an alcohol such as ethanol.

The reaction is carried out at temperatures greater than room temperature. The temperature is generally from about 30° C to about 150° C, preferably about 80°C to about 100° C, most preferable about 75° C to about 85° C, which is about the boiling point of ethanol. By "about" is meant that the temperature range is preferably within 10 degrees Celcius of the indicated temperature, more preferably within 5 degrees Celcius of the indicated temperature and, most preferably, within 2 degrees Celcius of the indicated temperature. Thus, for example, by "about 75° C" is meant 75° C ± 10° C, preferably 75° C ± 5° C and most preferably, 75° C ± 2° C.

B. Synthetic methods used in the examples which follow:

Method A: Formylation of pyrroles

$POCl_3$ (1.1 equiv.) is added dropwise to dimethylformamide (3 equiv.)at -10° C followed by addition of the appropriate pyrrole dissolved in dimethylformamide. After stirring for two hours, the reaction mixture is diluted with H_2O and basified to pH 11 with 10 N KOH. The precipitate which forms is collected by filtration, washed with H_2O and dried in a vacuum oven to give the desired aldehyde.

Method B: Saponification of pyrrolecarboxylic acid esters

A mixture of a pyrrolecarboxylic acid ester and KOH (2 - 4 equiv.) in EtOH is refluxed until reaction completion is indicated by thin layer chromatography (TLC). The cooled reaction mixtrue is acidified to pH 3 with 1 N HCl. The precipitate which forms is collected by filtration, washed with H_2O and dried in a vacuum oven to give the desired pyrrolecarboxylic acid.

Method C: Amidation

To a stirred solution of a pyrrolecarboxylic acid dissolved in dimethylformamide(0.3M) is added 1-ethyl-3-(3-dimethylamino-propyl)carbodiimide (1.2 equiv.), 1-hydroxybenzotriazole (1.2 equiv.), and triethylamine (2 equiv.). The appropriate amine is added (1 equiv.) and the reaction stirred until completion is indicated by TLC. Ethyl acetate is then added to the reaction mixture and the solution washed with saturated $NaHCO_3$ and brine (with extra salt), dried over anhydrous $MgSO_4$ and concentrated to afford the desired amide.

Method D: Condensation of aldehydes and oxindoles containing carboxylic acid substituents

A mixture of the oxindole (1 equivalent), 1 equivalent of the aldehyde and 1 - 3 equivalents of piperidine (or pyrrolidine) in ethanol (0.4 M) is stirred at 90-100° C until reaction completion is indicated by TLC. The mixture is then concentrated and the residue acidified with 2N HCl. The precipitate that forms is washed with H_2O and EtOH and then dried in a vacuum oven to give the product.

Method E: Condensation of aldehydes and oxindoles not containing carboxylic acid substituents

A mixture of the oxindole (1 equivalent), 1 equivalent of the aldehyde and 1 -.3 equivalents of piperidine (or pyrrolidine) in ethanol (0.4 M) is stirred at 90-100° C until reaction completion is indicated by TLC. The mixture is cooled to room temperature and the solid which forms is collected by vacuum

filtration, washed with ethanol and dried to give the product. If a precipitate does not form upon cooling of the reaction mixture, the mixture is concentrated and purified by column chromatography.

C. Examples of oxindole syntheses

The following examples of the synthesis of representative oxindoles is not to be construed as limiting the scope of this invention in any manner whatsoever. Alternate routes to the oxindoles shown as well as other oxindoles to be used to make the compounds of this invention will become apparent to those skilled in the art based on the following disclosures. Such syntheses and oxindoles are within the scope and spirit of this invention.

5-Amino-2-oxindole

5-Nitro-2-oxindole (6.3 g) was hydrogenated in methanol over 10% palladium on carbon to give 3.0 g (60% yield) of the title compound as a white solid.

5-Bromo-2-oxindole

2-Oxindole (1.3 g) in 20 mL acetonitrile was cooled to -10 °C and 2.0 g N-bromosuccinimide was slowly added with stirring. The reaction was stirred for 1 hour at -10 °C and 2 hours at 0 °C. The precipitate was collected, washed with water and dried to give 1.9 g (90 % yield) of the title compound.

4-Methyl-2-oxindole

Diethyl oxalate (30 mL) in 20 mL of dry ether was added with stirring to 19 g of potassium ethoxide suspended in 50 mL of dry ether. The mixture was cooled in an ice bath and 20 mL of 3-nitro-o-xylene in 20 mL of dry ether was slowly added. The thick dark red mixture was heated to reflux for 0.5 hr, concentrated to a dark red solid, and treated with 10% sodium hydroxide until almost all of the solid dissolved. The dark red mixture was treated with 30% hydrogen.peroxide until the red color changed to yellow. The mixture was treated alternately with 10% sodium

hydroxide and 30% hydrogen peroxide until the dark red color was no longer present. The solid was filtered off and the filtrate acidified with 6N hydrochloric acid. The resulting precipitate was collected by vacuum filtration, washed with water, and dried under vacuum to give 9.8 g (45% yield) of 2-methyl-6-nitrophenylacetic acid as an off-white solid. The solid was hydrogenated in methanol over 10 % palladium on carbon to give 9.04 g of the title compound as a white solid.

7-Bromo-5-chloro-2-oxindole

5-Chloro-2-oxindole (16.8 g) and 19.6 g of N-bromosuccinimide were suspended in 140 mL of acetonitrile and refluxed for 3 hours. Thin layer chromatography (silica, ethyl acetate) at 2 hours of reflux showed 5-chloro-2-oxindole or N-bromosuccinimide (Rf 0.8), product (Rf 0.85) and a second product (Rf 0.9) whose proportions did not change after another hour of reflux. The mixture was cooled to 10 °C, the precipitate was collected by vacuum filtration, washed with 25 mL of ethanol and sucked dry for 20 minutes in the funnel to give 14.1 g of wet product (56 % yield). The solid was suspended in 200 mL of denatured ethanol and slurry-washed by stirring and refluxing for 10 minutes. The mixture was cooled in an ice bath to 10 °C. The solid product was collected by vacuum filtration, washed with 25 mL of ethanol and dried under vacuum at 40 °C to give 12.7 g (51% yield) of 7-bromo-5-chloro-2-oxindole.

5-Fluoro-2-oxindole

5-Fluoroisatin (8.2 g) was dissolved in 50 mL of hydrazine hydrate and refluxed for 1.0 hr. The reaction mixtures were then poured in ice water. The precipitate was then filtered, washed with water and dried in a vacuum oven to afford the title compound.

5-Nitro-2-oxindole

2-Oxindole (6.5 g) was dissolved in 25 mL concentrated

sulfuric acid and the mixture maintained at -10 to -15 °C while 2.1 mL of fuming nitric acid was added dropwise. After the addition of the nitric acid the reaction mixture was stirred at 0 °C for 0.5 hr and poured into ice-water. The precipitate was collected by filtration, washed with water and crystallized from 50% acetic acid. The crystalline product was then filtered, washed with water and dried under vacuum to give 6.3 g (70%) of 5-nitro-2-oxindole.

5-Aminosulfonyl-2-oxindole

To a 100 mL flask charged with 27 mL of chlorosulfonic acid was added slowly 13.3 g of 2-oxindole. The reaction temperature was maintained below 30 °C during the addition. After the addition, the reaction mixture was stirred at room temperature for 1.5 hr, heated to 68 °C for 1 hr, cooled, and poured into water. The precipitate was washed with water and dried in a vacuum oven to give 11.0 g of 5-chlorosulfonyl-2-oxindole (50% yield) which was used without further purification.

5-Chlorosulfonyl-2-oxindole (2.1 g) was added to 10 mL of ammonium hydroxide in 10 mL of ethanol and stirred at room temperature overnight. The mixture was concentrated and the solid collected by vacuum filtration to give 0.4 g (20% yield) of the title compound as an off-white solid.

5-Isopropylaminosulfonyl-2-oxindole

To a 100 mL flask charged with 27 mL chlorosulfonic acid was slowly added 13.3 g 2-oxindole. The reaction temperature was maintained below 30° C during the addition. The reaction mixture was stirred at room temperature for 1.5 hour, heated to 68° C for 1 hour, cooled, and poured into water. The precipitate which formed was filtered, washed with water and dried in a vacuum oven to give 11.0 g (50%) of 5-chlorosulfonyl-2-oxindole which was used without further purification.

A suspension of 3 g 5-chlorosulfonyl-2-oxindole, 1.15 g isopropylamine and 1.2 mL of pyridine in 50 mL of dichloromethane was stirred at room temperature for 4 hours during which time a white solid formed. The solid was collected by vacuum filtration, slurry-washed with hot ethanol, cooled, collected by vacuum filtration and dried under vacuum at 40° C overnight to give 1.5 g (45%) of 5-isopropylaminosulfonyl-2-oxindole.

^{1}HNMR (360 MHz, DMSO-d6) δ 10.69 (s, br, 1H, NH), 7.63 (dd, *J* = 2 and 8 Hz, 1H), 7.59 (d, *J* = 2 Hz, 1H), 7.32 (d, *J* = 7 Hz, 1H, N*H*-SO_2-), 6.93 (d, *J* = 8 Hz, 1H), 3.57 (s, 2H), 3.14-3.23 (m, 1H, C*H*-$(CH_3)_2$), 0.94 (d, *J* = 7 Hz, 6H, 2xCH_3).

5-Phenylaminosulfonyl-2-oxindole

A suspension of 5-chlorosulfonyl-2-oxindole (1.62 g, 7 mmol), aniline (0.782 mL, 8.4 mmol) and pyridine (1 mL) in dichloromethane (20 ml) was stirred at room temperature for 4 hours. The reaction mixture was diluted with ethyl acetate (300 mL) and acidified with 1N hydrochloric acid (16 mL). The organic layer was washed with sodium bicarbonate and brine, dried and concentrated. The residue was washed with ethanol (3 mL) and then chromatographed on silica gel eluting with methanol/ dichloromethane 1:9 to give of 5-phenylaminosulfonyl-2-oxindole.

^{1}HNMR (360 MHz, DMSO-d6) δ 10.71 (s, br, 1H, NH), 10.10 (s, br, 1H, NH), 7.57-7.61 (m, 2H), 7.17-7.22 (m, 2H), 7.06-7.09 (m, 2H), 6.97-7.0 (m, 1H), 6.88 (d, *J* = 8.4 Hz, 1H), 3.52 (s, 2H).

2-Oxo-2,3-dihydro-1H-indole-5-sulfonic acid pyridin-3-ylamide

A solution of 5-chlorosufonyl-2-oxindole (3 g) and 3-aminopyridine (1.46g) in pyridine (15 mL) was stirred at room temperature overnight at which time a brown solid was present. The solid was filtered, washed with ethanol and dried under vacuum to yield 1.4 g (38%) of 2-oxo-2,3-dihydro-1H-indole-5-sulfonic acid pyridin-3-ylamide.

^{1}HNMR (360 MHz, DMSO-d6) δ 10.74 (s, 1H, NH), 10.39 (s, 1H,

SO_2NH), 8.27-8.28 (d, 1H), 8.21-8.23 (m, 1H), 7.59-7.62 (m, 2H), 7.44-7.68 (m, 1H), 7.24-7.28 (m, 1H), 6.69-6.71 (d, 1H), 3.54 (s, 2H).

MS *m/z* (APCI+) 290.2.

5-Phenyloxindole

5-Bromo-2-oxindole (5 g, 23.5 mmol) was dissolved in 110 mL toluene and 110 mL ethanol with stirring and a little heat. Tetrakis(triphenylphosphine)palladium(0) (1.9 g, 1.6 mmol) was added followed by 40 mL (80 mmol) 2M aqueous sodium carbonate. To this mixture was added benzene boronic acid (3.7 g, 30.6 mmol) and the mixture was heated in a 100° C oil bath for 12 hours. The reaction was cooled, diluted with ethyl acetate (500 mL), washed with saturated sodium bicarbonate (200 mL), water (200 mL), 1N HCl (200 mL) and brine (200 mL). The organic layer was dried over magnesium sulfate and concentrated to afford a brown solid. Trituration with dichloromethane afforded 3.8 g (77%) of 5-phenyl-2-oxindole as a tan solid.

^{1}H NMR (360 MHz, DMSO-d6) δ 10.4 (br s, 1H, NH), 7.57 (dd, *J* = 1.8 and 7.2 Hz, 1H), 7.5 to 7.35 (m, 5H), 7.29 (m, 1H), 6.89 (d, *J* = 8.2 Hz, 1H), 3.51 (s, 2H, CH_2CO).

MS *m/z* 209 [M^+].

In similar fashion, the following oxindoles can be prepared:

6-(3,5-Dichlorophenyl)-1,3-dihydroindol-2-one

^{1}H NMR (360 MHz, DMSO-d6) δ 10.46 (br, 1H, NH), 7.64 (d, *J* = 1.8 Hz, 2H), 7.57 (m, 1H), 7.27 (m, 2H), 7.05 (d, *J* = 1.1 Hz, 1H), 3.5 (s, 2H).

MS-EI *m/z* 277/279 $[M]^+$.

6-(4-Butylphenyl)-1,3-dihydroindol-2-one

^{1}H NMR (360 MHz, DMSO-d6) δ 10.39 (s, 1H, NH), 7.49 (d, *J* = 8.0 Hz, 2H), 7.25 (d, *J* = 8 Hz, 3H), 7.17 (dd, *J* = 1.5 and 7.8 Hz, 1H), 6.99 (d, *J* = 1.5 Hz, 1H), 3.48 (s, 2H, CH_2CO), 2.60 (t, *J* = 7.5 Hz,. 2Hz, CH_2CH_3), 1.57 (m, 2H, CH_2), 1.32 (m, 2H, CH_2),

0.9 (t, J = 7.5 Hz, 3H, CH_3).

6-(5-Isopropyl-2-methoxyphenyl)-1,3-dihydroindol-2-one

^{1}H NMR (360 MHz, DMSO-d6) δ 10.29 (br s, 1H, NH), 7.16-7.21 (m, 2H), 7.08 (d, J = 2.4 Hz, 1H), 6.97-7.01 (m, 2H), 6.89 (d, J = 0.8 Hz, 1H), 3.71 (s, 3H, OCH_3), 3.47 (s, 2H, CH_2CO), 2.86 (m, 1H, $CH(CH_3)_2$), 1.19 (d, J = 6.8 Hz, 6H, $CH(CH_3)_2$).

MS-EI m/z 281 $[M]^+$.

6-(4-Ethylphenyl)-1,3-dihydroindol-2-one

^{1}H NMR (360 MHz, DMSO-d6) δ 10.39 (br s, 1H, NH), 7.50 (d, J = 8.2 Hz, 2H), 7.28 (d, J = 8.2 Hz, 2H), 7.25 (d, J = 7.5 Hz, 1H), 7.17 (dd, J = 1.6 & 7.5 Hz, 1H), 6.99 (d, J = 1.6 Hz, 1H), 3.48 (s, 2H, CH_2CO), 2.63 (q, J = 7.6 Hz, 2H, CH_2CH_3), 1.20 (t, J = 7.6 Hz, 3H, CH_2CH_3).

MS-EI m/z 237 $[M]^+$.

6-(3-Isopropylphenyl)-1,3-dihydroindol-2-one

^{1}H NMR (360 MHz, DMSO-d6) δ 10.37 (br s, 1H, NH), 7.43 (m, 1H), 7.35-7.39 (m, 1H), 7.17-7.27 (m, 3H), 7.01 (d, J = 1.8 Hz, 1H), 3.49 (s, 2H, CH_2CO), 2.95 (m, 1H, $CH(CH_3)_2$), 1.24 (d, J = 6.8 Hz, 6H, $CH(CH_3)_2$).

MS-EI m/z 251 $[M]^+$.

6-(2,4-Dimethoxyphenyl)-1,3-dihydroindol-2-one

^{1}H NMR (360 MHz, DMSO-d6) δ 10.28 (br s, 1H, NH), 7.17 (m, 2H), 6.93 (dd, J = 1.6 & 7.6 Hz, 1H), 6.86 (d, J = 1.6 Hz, 1H), 6.63 (d, J = 2.4 Hz, 1H), 6.58 (dd, J = 2.4 & 8.5 Hz, 1H), 3.79 (s, 3H, OCH_3), 3.74 (s, 3H, OCH_3), 3.45 (s, 2H, CH_2CO).

MS-EI m/z 269 $[M]^+$.

6-Pyridin-3-yl-1,3-dihydroindol-2-one

^{1}H NMR (360 MHz, DMSO-d6) δ 10.51 (s, 1H, NH), 8.81 (d, J = 2.5 Hz, 1H), 8.55 (dd, J = 1.8 and 5.7 Hz, 1H), 8 (m, 1H), 7.45 (dd, J = 5.7 and 9.3 Hz, 1H), 7.3 (m, 2H), 7.05 (s, 1H), 3.51 (s, 2H, CH_2CO).

MS *m/z* 210 $[M]^+$.

2-Oxo-2,3-dihydro-1H-indole-4-carboxylic acid (3-chloro-4-ethoxyphenyl)-amide

To a solution of 4-carboxy-2-oxindole (200 mg, 1.13 mmol) and 3-chloro-4-methoxyphenylamine (178 mg, 1.13 mmol) in dimethylformamide (15 mL) at room temperature was added benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (BOP reagent, 997 mg, 2.26 mmol) followed by 4-dimethylaminopyridine (206 mg, 1.69 mmol). The mixture was stirred at room temperature for 72 hours. The reaction was then diluted with ethyl acetate (300 mL), washed with saturated sodium bicarbonate (100 mL), water, 2N hydrochloric acid (100 mL), water (3x200 mL) and brine. It was then dried over magnesium sulfate and concentrated. The residue was triturated with ethyl acetate to give 2-oxo-2,3-dihydro-1H-indole-4-carboxylic acid (3-chloro-4-methoxyphenyl)-amide as a pink solid.

^{1}HNMR (360 MHz, DMSO-d6) δ 10.50 (s, br, 1H, NH), 10.12 (s, br, 1H, NH), 7.9 (s, *J* = 2.5 Hz, 1H), 7.62 (dd, *J* = 2.5 & 9 Hz, 1H), 7.38 (d, *J* = 7.6 Hz, 1H), 7.32 (t, *J* = 7.6 Hz, 1H), 7.13 (d, *J* = 9 Hz, 1H), 6.98 (d, *J* = 7.6 Hz, 1H), 3.83 (s, 3H, OCH_3), 3.69 (s, 2H, CH_2).

MS-EI *m/z* 316 $[M]^+$.

4-Carboxy-2-oxindole

A solution of trimethylsilyldiazomethane in hexane (2 M) was added dropwise to a solution of 2.01 g 2-chloro-3-carboxy-nitrobenzene in 20 mL methanol at room temperature until no further gas evolution occurred. Acetic acid was then added to quench excess trimethylsilyldiazomethane. The reaction mixture was evaporated under vacuum and the residue was dried in an oven overnight. The 2-chloro-3-methoxycarbonylnitrobenzene obtained was pure enough for the following reaction.

Dimethyl malonate (6.0 mL) was added to an ice-cold suspension of 2.1 g sodium hydride in 15 mL DMSO. The reaction

mixture was stirred at 100° C for 1 hour and then cooled to room temperature. 2-Chloro-3-methoxycarbonylnitrobenzene (2.15 g) was added in one portion and the mixture was heated to 100° C for 1.5 hours. The reaction mixture was then cooled to room temperature, poured into ice water, acidified to pH 5 and extracted with ethyl acetate. The organic layer was washed with brine, dried over anhydrous sodium sulfate and concentrated to give 3.0 g of the dimethyl 2-methoxycarbonyl-6-nitrophenyl- malonate.

Dimethyl 2-methoxycarbonyl-6-nitrophenylmalonate (3.0 g) was refluxed in 50 mL of 6 N hydrochloric acid overnight. The mixture was concentrated to dryness, 20 mL ethanol and 1.1 g of tin(II) chloride were added and the mixture was refluxed for 2 hours. The mixture was filtered through Celite, concentrated and chromatographed on silica gel using ethyl acetate:hexane:acetic acid as eluent to give 0.65 g (37%) of 4-carboxy-2-oxindole as a white solid.

^{1}HNMR (360 MHz, DMSO-d6) δ 12.96 (s, br, 1H, COOH), 10.74 (s, br, 1H, NH), 7.53 (d, *J* = 8Hz, 1H), 7.39 (t, *J* = 8Hz, 1H), 7.12 (d, *J* = 8Hz, 1H), 3.67 (s, 2H).

D. Examples - Synthesis of pyrrole substituted 2-indolinones.

The following syntheses of representative compounds of this invention are shown by way of example only and are not to be construed as limiting the scope of this invention as to synthetic approach or as to the compounds which comprises this invention.

Example 1

4-Methyl-5-(2-oxo-1,2-dihydroindol-3-ylidenemethyl)-1*H*-pyrrole-2-carboxylic acid

4-Methyl-2-pyrrolecarboxylic acid ethyl ester (commercially available) was formylated using method A to give (73%) of 5-formyl-4-methyl-2-pyrrolecarboxylic acid ethyl ester. It was then hydrolysed using method B to give 5-formyl-4-methyl-1*H*-pyrrole-2-

carboxylic acid (58%).

Oxindole (133 mg, 1 mmol) was condensed with 5-formyl-4-methyl-1*H*-pyrrole-2-carboxylic acid (153 mg) using method D to give 268 mg (100%) of the title compound as an orange-red solid.

^{1}HNMR (360 MHz, DMSO-d6) δ 13.84 (s, br, 1H, N*H*), 12.84 (s, br, 1H, COO*H*), 10.98 (s, br, 1H, NH), 7.82 (d, *J* = 7.5 Hz, 1H), 7.67 (s, 1H, H-vinyl), 7.18 (t, *J* = 7.5 Hz, 1H), 7.01 (t, *J* = 7.5 Hz, 1H), 6.88 (d, *J* = 7.5 Hz, 1H), 6.71 (d, *J* = 2.2 Hz, 1H), 2.32 (s, 3H, CH_3).

MS (negative mode) 266.8 $[M-1]^+$.

Example 2

4-Methyl-5-(1-methyl-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-1*H*-pyrrole-2-carboxylic acid

1-Methyl-1,3-dihydroindol-2-one (147 mg, 1 mmol) was condensed with 5-formyl-4-methyl-1*H*-pyrrole-2-carboxylic acid (153 mg) using method D to give 250 mg (86%) of the title compound.

^{1}HNMR (360 MHz, DMSO-d6) δ 13.82 (s, br, 1H, N*H*), 12.88 (s, br, 1H, 7.83 (d, *J* = 7.5 Hz, 1H), 7.65 (s, 1H, H-vinyl), 7.26 (t, *J* = 7.5 Hz, 1H), 7.02-7.09 (m, 2H), 6.70 (d, *J* = 2.2 Hz, 1H), 2.32 (s, 3H, CH_3).

MS *m/z* 283.0 $[M+1]^+$.

Example 3

4-Methyl-5-(2-oxo-1,2-dihydroindol-3-ylidenemethyl)-1*H*-pyrrole-2-carboxylic acid methyl ester

Oxindole (105 mg, 0.79 mmol) was condensed with 5-formyl-4-methyl-1*H*-pyrrole-2-carboxylic acid methyl ester (110 mg, 0.67 mmol) using method E to give 153.2 mg (81%) of the title compound.

^{1}HNMR (360 MHz, DMSO-d6) δ 13.98 (s, br, 1H, N*H*), 10.97 (s, br, 1H, N*H*), 7.82 (d, *J* = 7.6 Hz, 1H), 7.67 (s, 1H, H-vinyl), 7.2 (dt, *J*= 1.2 & 7.7 Hz, 1H), 7.01 (dt, *J* = 1.2, 7.7 Hz, 1H), 6.90

(d, J = 7.6 Hz, 1H), 6.77 (d, J = 2 Hz, 1H).

MS (ES) m/z 283 [M^++1].

Example 4

5-(5-Chloro-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-4-methyl-1*H*-pyrrole-2-carboxylic acid ethyl ester

5-Chloro-1,3-dihydroindol-2-one (2.22 g, 13.2 mmol) was condensed with 5-formyl-4-methyl-1*H*-pyrrole-2-carboxylic acid ethyl ester (2.43 g) using method E to give 4.1 g (94%) of the title compound as an orange solid.

^{1}HNMR (360 MHz, DMSO-d6) δ 13.95 (s, br, 1H, N*H*), 7.98 (d, J = 2.2 Hz, 1H, H-4), 7.78 (s, 1H, H-vinyl), 7.18 (dd, J= 2.2 & 8.3 Hz, 1H, H-6), 6.87 (d, J = 8.3 Hz, 1H, H-7), 7.34 (d, J = 1.8 Hz, 1H, H-3'), 4.27 (q, J = 7.2 Hz, 2H, OCH_2CH_3), 2.33 (s, 3H, CH_3), 1.29 (t, J = 7.2 Hz, 3H, OCH_2CH_3).

MS-EI m/z 330 [M^+].

Example 5

5-(5-Chloro-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-4-methyl-1*H*-pyrrole-2-carboxylic acid

A mixture of 5-(5-chloro-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-4-methyl-1*H*-pyrrole-2-carboxylic acid ethyl ester (1.3 g, 4 mmol) and potassium hydroxide in methanol (25 mL) and ethanol (25 mL) was heated to reflux for overnight. Insoluble materials were removed by filtration and the mixture was neutralized with 6N hydrochloric acid to give 0.876 g (70%) of the title compound.

^{1}HNMR (360 MHz, DMSO-d6) δ 13.80 (s, br, 1H, N*H*), 12.90 (s, br, 1H, COO*H*), 11.06 (s, br, 1H, N*H*), 8.02 (d, J = 1.8 Hz, 1H, H-4), 7.81 (s, 1H, H-vinyl), 7.20 (dd, J= 1.8 & 8.3 Hz, 1H, H-6), 6.89 (d, J = 8.3 Hz, 1H, H-7), 6.72 (d, J = 1.8 Hz, 1H, H-3'), 2.35 (s, 3H, CH_3).

MS-EI m/z 302 [M^+].

Example 6

5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-4-methyl-1*H*-pyrrole-2-carboxylic acid (3-pyrrolidin-1-yl-propyl)amide

5-Bromo-1,3-dihydroindol-2-one (0.16 g, 0.76 mmol) was condensed with 5-formyl-4-methyl-1*H*-pyrrole-2-carboxylic acid (3-pyrrolidin-1-ylpropyl)amide (0.2 g, prepared by method C) to give 60 mg (17%) of the title compound as an orange solid.

^{1}HNMR (300 MHz, DMSO-d6) δ 13.61 (s, br, 1H, *NH*), 11.02 (s, br, 1H, *NH*), 8.42 (t, *J* = 5.8 Hz, 1H, CON*H*CH$_2$), 8.12 (d, *J* = 1.8 Hz, 1H, H-4), 7.78 (s, 1H, H-vinyl), 7.30 (dd, *J* = 1.8 & 8.4 Hz, 1H, H-6), 6.82 (d, *J* = 8.4 Hz, 1H, H-7), 6.77 (d, *J* = 2.4 Hz, 1H, H-3'), 3.22-3.31 (m, 2H, CH$_2$), 2.38-2.43 (m, 6H, 3xC*H*$_2$), 2.35 (s, 3H, CH$_3$), 1.62-1.71 (m, 6H, 3xCH$_2$).

MS-EI *m/z* 456 and 458 [M$^+$-1 and M$^+$+2].

Example 7

5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-4-methyl-1*H*-pyrrole-2-carboxylic acid (3-diethylamino-propyl)amide

5-Bromo-1,3-dihydroindol-2-one (0.16 g, 0.75 mmol) was condensed with 5-formyl-4-methyl-1*H*-pyrrole-2-carboxylic acid (3-diethylaminopropyl)amide (0.2 g, prepared by method C) to give 30 mg (8%) of the title compound as an orange solid.

^{1}HNMR (300 MHz, DMSO-d6) δ 13.61 (s, br, 1H, *NH*), 11.02 (s, br, 1H, *NH*), 8.40 (m, 1H, CON*H*CH$_2$), 8.12 (d, *J* = 1.5 Hz, 1H, H-4), 7.78 (s, 1H, H-vinyl), 7.30 (dd, *J* = 1.5 & 8.2 Hz, 1H, H-6), 6.82 (d, *J* = 8.2 Hz, 1H, H-7), 6.78 (d, *J* = 2.4 Hz, 1H, H-3'), 3.23 (m, 2H, CH$_2$), 2.38-2.45 (m, 6H, CH$_2$ & N(CH$_2$CH$_3$)$_2$), 2.35 (s, 3H, CH$_3$), 1.61 (m, 2H, CH$_2$), 0.93 (t, *J* = 7.1 Hz, 6H, N(CH$_2$C*H*$_3$)$_2$).

MS-EI *m/z* 458 and 460 [M$^+$-1 and M$^+$+2].

Example 8

5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-1*H*-pyrrole-2-carboxylic acid (2-diethylaminoethyl)amide

5-Bromo-1,3-dihydroindol-2-one (212 mg, 1 mmol) was

condensed with 5-formyl-1*H*-pyrrole-2-carboxylic acid (2-diethylaminoethyl)amide (prepared from ethyl pyrrole-2-carboxylate by method A, B and then C) to give 162 mg (38%) of the title compound.

^{1}H NMR (300 MHz, DMSO-d6) δ 13.53 (s, br, 1H, NH), 11.06 (s, br, 1H, NH), 8.37 (t, 1H, CONHCH$_2$), 7.89 (m, 2H), 7.32 (dd, *J* = 2.0 Hz, 1H), 6.96 (s, 1H), 6.80-6.84 (m, 2H), 3.3 (m, 2H, CH$_2$), 2.45-2.55 (m, 6H, N(CH$_2$CH$_3$)$_2$ & CH$_2$), 0.95 (t, *J* = 7.2 Hz, 6H, N(CH$_2$CH$_3$)$_2$).

MS-EI *m/z* 430 and 432 [M$^+$-1 and M$^+$+ 1].

Example 9

5-(2-Oxo-6-phenyl-1,2-dihydroindol-3-ylidenemethyl)-1*H*-pyrrole-2-carboxylic acid (2-diethylaminoethyl)amide

6-Phenyl-1,3-dihydroindol-2-one (209 mg, 1mmol) was condensed with 5-formyl-1*H*-pyrrole-2-carboxylic acid (2-diethylaminoethyl)amide to give 182 mg (42%) of the title compound.

^{1}H NMR (300 MHz, DMSO-d6) δ 13.56 (s, br, 1H, NH), 11.06 (s, br, 1H, NH), 8.36 (t, 1H, CONHCH$_2$), 7.77 (s, 1H, H-vinyl), 7.73 (d, *J* = 7.8 Hz, 1H), 7.64 (d, *J* = 7.2 Hz, 2H), 7.46 (m, 2H), 7.32 (m, 2H), 7.11 (s, 1H), 6.96 (m, 1H), 6.80 (m, 1H), 3.31-3.32 (m, 2H, CH$_2$), 2.46-2.53 (m, 6H, N(CH$_2$CH$_3$)$_2$ & CH$_2$), 0.96 (t, *J* = 6.9 Hz, 6H, N(CH$_2$CH$_3$)$_2$).

MS-EI *m/z* 428 [M$^+$].

Example 10

5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-1*H*-pyrrole-2-carboxylic acid (2-diethylaminoethyl)-methyl-amide

5-Bromo-1,3-dihydroindol-2-one (212 mg, 1 mmol) was condensed with 5-formyl-1*H*-pyrrole-2-carboxylic acid (2-diethylaminoethyl)methylamide to give 246 mg (55%) of the title compound.

^{1}H NMR (360 MHz, DMSO-d6) δ 13.54 (s, br, 1H, NH), 11.06 (s,

br, 1H, NH), 7.90 (m, 2H), 7.33 (dd, J = 1.8 & 8.4 Hz, 1H), 6.82-6.85 (m, 3H), 3.55 (s, br, 2H, CH_2), 3.25 (s, br, 3H, NCH_3), 2.57 (t, J = 6.5 Hz, 2H, CH_2), 2.45 (m, 4H, $N(CH_2CH_3)_2$), 0.91 (m, 6H, $N(CH_2CH_3)_2$).

MS-EI m/z 444 and 446 [M^+-1 and M^+ +1].

Example 11

5-(2-Oxo-6-phenyl-1,2-dihydroindol-3-ylidenemethyl)-1*H*-pyrrole-2-carboxylic acid (2-diethylaminoethyl)methylamide

6-Phenyl-1,3-dihydroindol-2-one (209 mg, 1 mmol) was condensed with 5-formyl-1*H*-pyrrole-2-carboxylic acid (2-diethylaminoethyl)methylamide to give 277 mg (63%) of the title compound.

^{1}H NMR (360 MHz, DMSO-d6) δ 13.58 (s, br, 1H, NH), 11.04 (s, br, 1H, NH), 7.78 (s, 1H, H-vinyl), 7.73 (d, J = 7.8 Hz, 1H), 7.64 (d, J = 7.5 Hz, 2H), 7.46 (m, 2H), 7.33-7.36 (m, 2H), 7.11 (s, 1H), 6.84 (m, 1H), 6.78 (m, 1H), 3.55 (s, br, 2H, CH_2), 3.25 (s, br, 3H, NCH_3), 2.58 (t, 2H, CH_2), 2.44 (m, 4H, $N(CH_2CH_3)_2$), 0.92 (m, 6H, $N(CH_2CH_3)_2$).

Example 12

3-Methyl-5-(2-oxo-1,2-dihydroindol-3-ylidenemethyl)-1*H*-pyrrole-2-carboxylic acid (3-diethylaminopropyl)amide

Oxindole (66.5 mg, 0.5 mmol) was condensed with 5-formyl-3-methyl-1H-pyrrole-2-carboxylic acid (3-diethylaminopropyl)amide (prepared from 3-formyl-3-methyl-1H-pyrrole-2-carboxylic acid ethyl ester by method B then C) to give 39 mg (21%) of the title compound.

^{1}H NMR (300 MHz, DMSO-d6) δ 13.34 (s, br, 1H, NH), 10.88 (s, br, 1H, NH), 7.62-7.67 (m, 3H), 7.17 (m, 1H), 6.99 (m, 1H), 6.87 (d, J = 7.6 Hz, 1H), 6.63 (d, J = 1 Hz, 1H), 3.26-3.32 (m, 2H, CH_2), 2.41-2.48 (m, 6H, CH_2 & $N(CH_2CH_3)_2$), 2.29 (s, 3H, CH_3), 1.63 (m, 2H, CH_2), 0.93 (t, J = 7.2 Hz, 6H, $N(CH_2CH_3)_2$).

MS-EI *m/z* 380 [M^+].

Example 13

5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-3-methyl-1*H*-pyrrole-2-carboxylic acid (3-diethylamino-propyl)amide

5-Bromo-1,3-dihydroindol-2-one (106 mg, 0.5 mmol) was condensed with 5-formyl-3-methyl-1H-pyrrole-2-carboxylic acid (3-diethylaminopropyl)amide to give 35 mg (15%) of the title compound.

^{1}H NMR (300 MHz, DMSO-d6) δ 13.35 (s, br, 1H, NH), 11.00 (s, br, 1H, NH), 7.89 (d, *J* = 1.9 Hz, 1H, H-4), 7.80 (s, 1H, H-vinyl), 7.74 (t, *J* = 5.3 Hz, 1H, $CONHCH_2$), 7.31 (dd, *J* = 1.9 & 8.4 Hz, 1H, H-6), 6.83 (d, *J* = 8.4 Hz, 1H, H-7), 6.63 (s,1H, H-3'), 3.26 (m, 2H, CH_2), 2.41-2.48 (m, 6H, CH_2 & $N(CH_2CH_3)_2$), 2.29 (s, 3H, CH_3), 1.63 (m, 2H, CH_2), 0.93 (t, *J* = 7.1 Hz, 6H, $N(CH_2CH_3)_2$).

MS-EI *m/z* 458 and 460 [M^+-1 and M^++1].

Example 14

3-Methyl-5-(2-oxo-6-phenyl-1,2-dihydroindol-3-ylidenemethyl)-1*H*-pyrrole-2-carboxylic acid (3-diethylaminopropyl)amide

6-Phenyl-1,3-dihydroindol-2-one (105 mg, 0.5 mmol) was condensed with 5-formyl-3-methyl-1H-pyrrole-2-carboxylic acid (3-diethylaminopropyl)amide to give 67.8 (30%) of the title compound.

^{1}H NMR (300 MHz, DMSO-d6) δ 13.37 (s, br, 1H, NH), 11.02 (s, br, 1H, NH), 7.23-7.73 (m, 11H), 3.29 (m, 2H, CH_2), 2.41-2.48 (m, 6H, CH_2 & $N(CH_2CH_3)_2$), 2.29 (s, 3H, CH_3), 1.64 (m, 2H, CH_2), 0.94 (t, *J* = 7.0 Hz, 6H, $N(CH_2CH_3)_2$).

MS-EI *m/z* 456 [M^+].

Example 15

5-(5-Methoxy-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-3-methyl-1*H*-pyrrole-2-carboxylic acid (3-diethylamino-propyl)amide

5-Methoxy-1,3-dihydroindol-2-one (82.5 mg, 0.5 mmol) was

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condensed with 5-formyl-3-methyl-1H-pyrrole-2-carboxylic acid (3-diethylaminopropyl)amide to give 80 mg (39%) of the title compound.

^{1}H NMR (300 MHz, DMSO-d6) δ 13.45 (s, br, 1H, NH), 10.70 (s, br, 1H, NH), 7.68-7.70 (m, 2H), 7.32 (d, *J* = 1.8 Hz, 1H), 6.72-6.79 (m, 2H), 6.60 (s, 1H), 3.73 (s, 3H, OCH_3), 3.28 (m, 2H, CH_2), 2.41-2.48 (m, 6H, CH_2 & $N(CH_2CH_3)_2$), 2.29 (s, 3H, CH_3), 1.63 (m, 2H, CH_2), 0.93 (t, *J* = 7.0 Hz, 6H, $N(CH_2CH_3)_2$).

MS *m/z* 410 [M$^+$].

Example 16

5-(6-Methoxy-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-3-methyl-1*H*-pyrrole-2-carboxylic acid (3-diethylamino-propyl)amide

6-Methoxy-1,3-dihydroindol-2-one (82.5 mg, 0.5 mmol) was condensed with 5-formyl-3-methyl-1H-pyrrole-2-carboxylic acid (3-diethylaminopropyl)amide to give 63 mg (31%) of the title compound.

^{1}H NMR (300 MHz, DMSO-d6) δ 13.22 (s, br, 1H, NH), 10.86 (s, br, 1H, NH), 7.39-7.63 and 6.37-6.55 (m, 6H), 3.73 (s, 3H, OCH_3), 3.3 (m, 2H, CH_2), 2.45 (m, 6H, CH_2 & $N(CH_2CH_3)_2$), 2.28 (s, 3H, CH_3), 1.63 (m, 2H, CH_2), 0.93 (m, 6H, $N(CH_2CH_3)_2$).

MS *m/z* 410 [M$^+$].

Example 17

3-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-4,5,6,7-tetrahydro-2*H*-isoindole-1-carboxylic acid (2-diethylamino-ethyl)amide

4,5,6,7-Tetrahydro-2*H*-isoindole-1-carboxylic acid ethyl ester (May, Donald A.; Lash, Timothy D.; J. Org. Chem., 1992, 57:18, 4820-4828) was formylated using method A then B to give 3-formyl-4,5,6,7-tetrahydro-2*H*-isoindole-1-carboxylic acid.

5-Bromo-1,3-dihydroindol-2-one (1.43 g, 6.8 mmol) was condensed with 3-formyl-4,5,6,7-tetrahydro-2*H*-isoindole-1-carboxylic acid (2-diethylaminoethyl)amide (1.97 g) to give 2.2

g (67%) of the title compound as a yellow-orange solid.

^{1}H NMR (360 MHz, DMSO-d6) δ 13.47 (s, 1H, NH), 11.0 (s, 1H, NH), 8.0 (d, 1H, NH), 7.70 (s, 1H, CH), 7.28 (dd, J = 2.1 and 8.2 Hz, 1H, ArH), 7.16 (m, 1H, ArH), 6.8 (d, J = 8.3Hz, 1H, ArH), 3.3 (s, 2H, CONH), 2.5 (m, 6H, 3xNCH_2), 2.78 (br m, 2H, pyrrole CH_2), 2.72 (br m, 2H, pyrroleCH_2), 1.7 (br m, 4H, N(CH_2CH_3)$_2$), 1.74 (br s, 4H, $CH_2CH_2CH_2CH_2$), 0.96 (t, J = 7.4 Hz, 6H, N(CH_2CH_3)$_2$).

MS-EI m/z 484 and 486 [M^+-1 and M^++1].

Example 18

3-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-4,5,6,7-tetrahydro-2H-isoindole-1-carboxylic acid (3-diethylaminopropyl)amide

5-Bromo-1,3-dihydroindol-2-one (20 mg, 0.1 mmol) was condensed with 3-formyl-4,5,6,7-tetrahydro-2H-isoindole-1-carboxylic acid (3-diethylaminopropyl)amide (30 mg) to give 33 mg (46%) of the title compound as an orange solid.

^{1}H NMR (360 MHz, DMSO-d6) δ 10.9 (s, 1H, NH), 8.0 (m, 1H, NH), 7.68 (m, 1H, ArH), 7.4 (m, 1H, ArH), 7.29 (d, J = 1.9 and 8.5Hz, 1H, ArH), 6.8 (d, J = 8 Hz, 1H, ArH), 2.7 (br m, 4H, 2xNCH_2), 2.4 (m, 8H, 4xNCH_2), 1.7 (br m, 4H, N(CH_2CH_3)$_2$), 1.6 (br m, 2H, $CH_2CH_2CH_2$), 0.93 (t, J = 7.4 Hz, 6H, N(CH_2CH_3)$_2$).

MS-EI m/z 499 and 501 [M^+ and M^++2].

Example 19

3-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-4,5,6,7-tetrahydro-2H-isoindole-1-carboxylic acid (3-pyrrolidin-1-ylpropyl)amide

5-Bromo-1,3-dihydroindol-2-one (80 mg, 0.4 mmol) was condensed with 3-formyl-4,5,6,7-tetrahydro-2H-isoindole-1-carboxylic acid (3-pyrrolidin-1-ylpropyl)amide (120 mg) to give 43 mg (22%) of the title compound as a tan-orange solid.

^{1}H NMR (360 MHz, DMSO-d6) δ 13.4 (s, 1H, NH), 10.9 (s, 1H, NH), 8.0 (m, 1H, NH), 7.69 (m, 1H, ArH), 7.49 (m, 1H, ArH), 7.28

(d, J = 1.7 and 7.8 Hz, 1H, Ar*H*), 6.8 (d, J = 8 Hz, 1H, Ar*H*), 3.3 (br m, 2H, 2xNCH_2), 2.8 (m, 4H, 2xpyrroleCH_2), 2.5 (br m, 4H, N(CH_2CH$_3$)$_2$), 1.6 (br m, 8H, 2xpyrroleCH$_2$CH_2, CH$_2$CH_2CH$_2$ and CONHCH_2).

MS-EI m/z 497 and 499 [M^+ and M^++2].

Example 20

3-(2-Oxo-6-pyridin-3-yl-1,2-dihydroindol-3-ylidenemethyl)-4,5,6,7-tetrahydro-2*H*-isoindole-1-carboxylic acid (2-diethylaminoethyl)amide

6-Pyridin-3-yl-1,3-dihydroindol-2-one (60 mg, 0.4 mmol) was condensed with 3-formyl-4,5,6,7-tetrahydro-2*H*-isoindole-1-carboxylic acid (2-diethylaminoethyl)amide (80 mg) to give 50 mg (38%) of the title compound as a reddish solid.

^{1}H NMR (360 MHz, DMSO-d6) δ 13.4 (s, 1H, NH), 11 (s, 1H, NH), 8.9 (d, 1H, N*H*), 8.7 (dd, 1H, Ar*H*), 8.1 (dd, 1H, Ar*H*), 7.9 (d, 1H, Ar*H*), 7.6 (s, 1H, C*H*), 7.5 (dd, 1H, Ar*H*), 7.3 (dd, 1H, Ar*H*), 7.1 (m, 2H, Ar*H*), 3.35 (m, 2H, CONHCH_2), 2.8 (m, 4H, 2xpyrroleCH_2), 2.5 (br m, 6H, N(CH_2CH$_3$)$_2$ and NCH_2), 1.75 (br s, 4H, 2xpyrroleCH$_2$CH_2), 0.9 (t, 6H, N(CH$_2$CH_3)$_2$).

MS-EI m/z 484 [M^+].

Example 21

4-Benzoyl-5-(5-bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-3-methyl-1*H*-pyrrole-2-carboxylic acid (3-diethylaminopropyl)amide

To a mixture of benzoyl chloride (1 equiv.) and aluminum chloride (1 equiv.) in dichloroethane at 0° C was added ethyl 3,5-dimethyl-2-pyrrolecarboxylate (1 equiv.). The mixture was stirred at 80° C for 4 hr. The mixture was then extracted with ethyl acetate (EtOAc) and H_2O. The combined organic extracts were washed with saturated sodium bicarbonate and brine, dried and concentrated to give (51%) of 4-benzoyl-3,5-dimethyl-1*H*-pyrrole-2-carboxylic acid.

A mixture of 4-benzoyl-3,5-dimethyl-1*H*-pyrrole-2-carboxylic acid ethyl ester (4.13 g, 15.2 mmol) and ceric ammonium nitrate

(33 g, 4equiv.) in 50 mL of tetrahydrofuran (THF):acetic acid (HOAc):H_2O 1:1:1 was refluxed overnight. The reaction mixture was then cooled, extracted with EtOAc and then basified to pH 9 with sodium carbonate. The organic layer was then washed with brine, dried ($MgSO_4$) and concentrated followed by column chromatography to give 3.25 g (75%) of 4-benzoyl-5-formyl-3-methyl-1*H*-pyrrole-2-carboxylic acid ethyl ester as a yellow solid.

5-Bromo-1,3-dihydro-indol-2-one was condensed with 4-benzoyl-5-formyl-3-methyl-1*H*-pyrrole-2-carboxylic acid using method D to give 4-benzoyl-5-(5-bromo-2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-3-methyl-1*H*-pyrrole-2-carboxylic acid.

The above carboxylic acid was then coupled with N,N-diethyl-1,3-propanediamine using method C to give the title compound.

^{1}H NMR (360 MHz, DMSO-d6) δ 7.96 (m, 1H, CONHCH$_2$), 7.76 (d, *J* = 7.0 Hz, 2H), 7.68 (t, 1H), 7.56 (m, 2H), 7.40 (s, 2H) 7.33 (dd, *J* = 1.6 & 8.3 Hz, 1H, H-6), 6.84 (d, *J* = 8.3 Hz, 1H, H-7), 3.33 (m, 2H, CH$_2$), 2.42-2.46 (m, 6H, 3xCH$_2$), 2.10 (s, 3H, CH$_3$), 1.65 (m, 2H, CH$_2$), 0.94 (t, *J* = 7.0 Hz, 6H, N(CH$_2$CH$_3$)$_2$).

MS Electron Impact *m/z* 564 [M$^+$+1].

Example 22

4-Benzoyl-5-(5-bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-3-methyl-1*H*-pyrrole-2-carboxylic acid (3-morpholin-4-ylpropyl)amide

^{1}H NMR (360 MHz, DMSO-d6) δ 14.10 (s, 1H, NH), 11.14 (br s, 1H, NH), 7.92 (m, 1H, CONHCH$_2$), 7.75 (m, 2H), 7.69 (t, 1H), 7.56 (m, 2H), 7.42 (m, 2H), 7.33 (dd, *J* = 1.9 & 8.3 Hz, 1H, H-6), 6.85 (d, *J* = 8.3 Hz, 1H, H-7), 3.56 (m, 4H, 2xCH$_2$), 3.33 (m, 2H, CH$_2$), 2.35 (m, 6H, 3xCH$_2$), 2.10 (s, 3H, CH$_3$), 1.70 (m, 2H, CH$_2$).

Example 23

4-Benzoyl-3-methyl-5-(2-oxo-1,2-dihydroindol-3-ylidenemethyl)-1*H*-pyrrole-2-carboxylic acid (3-pyrrolidin-1-ylpropyl)amide

^{1}H NMR (300 MHz, DMSO-d6) δ 14.18 (s, 1H, NH), 11.14 (br s, 1H, NH), 8.01 (m, 1H, CONHCH$_2$), 7.74 (m, 1H), 7.67 (m, 1H), 7.55

(m, 1H), 7.32 (s, 1H, H-vinyl), 7.17 (m, 1H), 6.92 (m, 1H), 3.36 (m, 2H, CH_2), 2.44 (m, 6 H, 3xCH_2), 2.11 (s, 3H, CH_3), 1.65-1.75 (m, 6H, 3xCH_2).

MS Electron Impact m/z 482 [M^+].

Example 24

4-Benzoyl-5-(5-bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-3-methyl-1*H*-pyrrole-2-carboxylic acid (3-pyrrolidin-1-ylpropyl)amide

^{1}H NMR (360 MHz, DMSO-d6) δ 14.01 (s, 1H, *NH*), 11.18 (br s, 1H, *NH*), 7.98 (m, 1H, CONHCH_2), 7.75 (m, 2H), 7.68 (m, 1H), 7.55 (m, 2H), 7.40 (m, 2H), 7.33 (dd, *J* = 2.0 & 8.2 Hz, 1H, H-6), 6.84 (d, *J* = 8.2 Hz, 1H, H-7), 3.34 (m, 2H, CH_2), 2.42-2.47 (m, 6 H, 3xCH_2), 2.09 (s, 3H, CH_3), 1.70 (m, 2H, CH_2), 1.64 (m, 4H, 2xCH_2).

Example 25

4-Benzoyl-3-methyl-5-(2-oxo-6-phenyl-1,2-dihydroindol-3-ylidenemethyl)-1*H*-pyrrole-2-carboxylic acid (3-pyrrolidin-1-ylpropyl)amide

^{1}H NMR (300 MHz, DMSO-d6) δ 14.15 (s, 1H, *NH*), 11.16 (br s, 1H, *NH*), 7.98 (m, 1H, CONHCH_2), 7.77 (d, *J* = 7.7 Hz, 2H), 7.69 (m, 1H), 7.53-7.63 (m, 4H), 7.44 (m, 2H), 7.33-7.37 (m, 2H), 7.24 (s, 2H), 7.12 (s, 1H), 3.36 (m, 2H, CH_2), 2.43-2.48 (m, 6 H, 3xCH_2), 2.12 (s, 3H, CH_3), 1.74 (m, 2H, CH_2), 1.69 (m, 4H, 2xCH_2).

MS Electron Impact m/z 558 [M^+].

Example 26

4-Benzoyl-5-(6-methoxy-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-3-methyl-1*H*-pyrrole-2-carboxylic acid (3-pyrrolidin-1-ylpropyl)amide

^{1}H NMR (300 MHz, DMSO-d6) δ 13.99 (s, 1H, *NH*), 11.05 (br s, 1H, *NH*), 7.93 (m, 1H, CONHCH_2), 7.72 (m, 2H), 7.65 (m, 1H), 7.54 (m, 2H), 7.15 (s, 1H, H-vinyl), 7.04 (d, *J* = 8.4 Hz, 1H, H-4), 6.51 (dd, *J* = 2.3 & 8.4 Hz, 1H, H-5) , 6.44 (d, *J* = 2.3 Hz, 1H,

H-7), 3.74 (s, 3H, OCH_3), 3.35 (m, 2H, CH_2), 2.42-2.46 (m, 6 H, 3xCH_2), 2.10 (s, 3H, CH_3), 1.72 (m, 2H, CH_2), 1.65 (m, 4H, 2xCH_2).

MS Electron Impact *m/z* 512 [M^+].

Example 27

4-Benzoyl-5-(5-methoxy-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-3-methyl-1*H*-pyrrole-2-carboxylic acid (3-pyrrolidin-1-ylpropyl)amide

^{1}H NMR (360 MHz, DMSO-d6) δ 14.24 (s, 1H, N*H*), 10.90 (br s, 1H, N*H*), 7.97 (m, 1H, CONHCH_2), 7.75 (d, *J* = 7.2 Hz, 2H), 7.69 (m, 1H), 7.56 (m, 2H), 7.24 (s, 1H, H-vinyl), 6.79 (m, 2H), 6.66 (m, 1H), 3.67 (s, 3H, OCH_3), 3.34 (m, 2H, CH_2), 2.43-2.48 (m, 6 H, 3xCH_2), 2.14 (s, 3H, CH_3), 1.71 (m, 2H, CH_2), 1.66 (m, 4H, 2xCH_2).

MS Electron Impact *m/z* 512 [M^+].

Example 28

4-Benzoyl-5-(5-fluoro-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-3-methyl-1*H*-pyrrole-2-carboxylic acid (3-pyrrolidin-1-ylpropyl)amide

^{1}H NMR (300 MHz, DMSO-d6) δ 14.20 (s, 1H, N*H*), 11.14 (br s, 1H, N*H*), 8.03 (m, 1H, CONHCH_2), 7.75 (m, 2H), 7.68 (m, 1H), 7.55 (m, 2H), 7.38 (s, 1H, H-vinyl), 7.08 (m, 1H), 7.01 (m, 1H), 6.87 (m, 1H), 3.34 (m, 2H, CH_2), 2.42-2.48 (m, 6 H, 3xCH_2), 2.09 (s, 3H, CH_3), 1.70 (m, 2H, CH_2), 1.65 (m, 4H, 2xCH_2).

MS Electron Impact *m/z* 500 [M^+].

Example 29

4-Acetyl-5-(5-bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-3-methyl-1*H*-pyrrole-2-carboxylic acid (3-diethylaminopropyl)amide

5-Bromo-1,3-dihydro-indol-2-one was condensed with 4-acetyl-5-formyl-3-methyl-1*H*-pyrrole-2-carboxylic acid (3-diethylaminopropyl)amide (prepared from 4-acetyl-5-formyl-3-methyl-1*H*-pyrrole-2-carboxylic acid ethyl ester by method B then C) to give the title compound.

^{1}H NMR (300 MHz, DMSO-d6) δ 14.19 (s, 1H, NH), 11.19 (br s, 1H, NH), 8.15 (m, 1H, CONHCH$_2$), 8.11 (s, 1H, H-vinyl), 7.72 (d, J = 1.8 Hz, 1H, H-4), 7.38 (dd, J = 1.8 & 8.2 Hz, 1H, H-6), 6.87 (d, J = 8.2 Hz, 1H, H-7), 3.27 (m, 2H, CH$_2$), 2.57 (s, 3H, CH$_3$CO), 2.46 (m, 9 H, CH$_3$ & 3xCH$_2$), 1.64 (m, 2H, CH$_2$), 0.93 (t, J = 7.1 Hz, 6H, N(CH$_2$CH$_3$)$_2$).

Example 30

4-Acetyl-5-(5-bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-3-methyl-1H-pyrrole-2-carboxylic acid (3-pyrrolidin-1-ylpropyl)amide

^{1}H NMR (300 MHz, DMSO-d6) δ 8.14 (m, 1H, CONHCH$_2$), 8.10 (s, 1H, H-vinyl), 7.70 (d, 1H, H-4), 7.36 (dd, J = 1.6 & 8.1 Hz, 1H, H-6), 6.85 (d, J = 8.1 Hz, 1H, H-7), 3.32 (m, 2H, CH$_2$), 2.57 (s, 3H, CH$_3$CO), 2.44 (s, 3H, CH$_3$), 2.35-2.48 (m, 6H, 3xCH$_3$), 1.65-1.71 (m, 6H, 3xCH$_2$).

MS m/z 499 & 501 [M$^+$] & [M$^+$+2].

Example 31

4-Acetyl-5-(5-bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-3-methyl-1H-pyrrole-2-carboxylic acid (3-morpholin-4-ylpropyl)amide

^{1}H NMR (300 MHz, DMSO-d6) δ 14.20 (s, 1H, NH), 11.26 (br s, 1H, NH), 8.09 (m, 2H, H-vinyl & CONHCH$_2$), 7.73 (d, J = 1.5 Hz, 1H, H-4), 7.38 (dd, J = 1.5 & 8.3 Hz, 1H, H-6), 6.87 (d, J = 8.3 Hz, 1H, H-7), 3.55 (m, 4H, 2xCH$_2$), 3.26 (m, 2H, CH$_2$), 2.57 (s, 3H, CH$_3$CO), 2.44 (s, 3H, CH$_3$), 2.35 (m, 6H, 3xCH$_3$), 1.68 (m, 2H, CH$_2$).

MS-EI m/z 514 & 516 [M$^+$-1] & [M$^+$+1].

Example 32

4-Acetyl-5-(5-bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-3-methyl-1H-pyrrole-2-carboxylic acid (3-hydroxypropyl)amide

^{1}H NMR (360 MHz, DMSO-d6) δ 14.17 (s, 1H, NH), 11.25 (br s, 1H, NH), 8.10 (s, 1H, H-vinyl), 8.03 (m, 1H, CONHCH$_2$), 7.71 (br s, 1H, H-4)., 7.37 (br d, J = 8.4 Hz, 1H, H-6), 6.87 (d, J = 8.4

Hz, 1H, H-7), 4.51 (br s, 1H, OH), 3.51 (br s, 2H, CH_2), 3.36 (m, 2H, CH_2), 2.57 (s, 3H, CH_3CO), 2.43 (s, 3H, CH_3), 1.70 (m, 2H, CH_2).

MS-EI *m/z* 445 & 447 [M^+-1] & [M^++1].

Example 34

4-Acetyl-5-(5-bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-3-methyl-1*H*-pyrrole-2-carboxylic acid (2-morpholin-4-ylethyl)amide

^{1}H NMR (360 MHz, DMSO-d6) δ 14.19 (s, 1H, NH), 11.14 (br s, 1H, NH), 8.10 (s, 1H, H-vinyl), 7.84 (m, 1H, $CONHCH_2$), 7.71 (d, *J* = 1.8 Hz, 1H, H-4), 7.38 (dd, *J* = 1.8 & 8.2 Hz, 1H, H-6), 6.87 (d, *J* = 8.2 Hz, 1H, H-7), 3.58 (m, 4H, 2xCH_2), 3.40 (m, 2H, CH_2), 2.57 (s, 3H, CH_3CO), 2.49 (m, 4H, 2xCH_2), 2.45 (m, CH_3 & CH_2).

MS-EI *m/z* 500 & 502 [M^+-1] & [M^++1].

Example 35

4-Acetyl-5-(5-bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-3-methyl-1*H*-pyrrole-2-carboxylic acid (2-pyrrolidin-1-ylethyl)amide

^{1}H NMR (360 MHz, DMSO-d6) δ 14.17 (s, 1H, NH), 11.23 (s, 1H, NH), 8.11 (s, 1H, H-vinyl), 7.91 (m, 1H, $CONHCH_2$), 7.73 (d, *J* = 1.9 Hz, 1H, H-4), 7.39 (dd, *J* = 1.9 & 8.3 Hz, 1H, H-6), 6.88 (d, *J* = 8.3 Hz, 1H, H-7), 3.40 (m, 2H, CH_2), 2.62 (m, 2H, CH_2), 2.57 (s, 3H, CH_3CO), 2.49 (m, 4H, 2xCH_2), 2.44 (s, 3H, CH_3), 1.69 (m, 4H, 2xCH_2).

Example 36

4-Acetyl-5-(5-bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-3-methyl-1*H*-pyrrole-2-carboxylic acid [2-(4-hydroxyphenyl)ethyl]amide

^{1}H NMR (300 MHz, DMSO-d6) δ 14.21 (s, 1H, NH), 11.18 (s, 1H,OH), 9.09 (s, 1H, NH), 8.06-8.10 (m, 2H), 7.73 (s, 1H), 7.38 (d, *J* = 7.8 Hz, 1H), 7.04 (d, *J* = 7.1 Hz, 2H), 6.83 (d, *J* = 7.8 Hz, 1H), 6.67 (d, *J* = 7.1 Hz, 2H), 3.42 (m, 2H, CH_2), 2.72 (m, 2H, CH_2), 2.56 (s, 3H, CH_3CO), 2.37 (s, 3H, CH_3).

MS-EI *m/z* 507 & 509 [M^+-1] & [M^++1].

Example 37

5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2-isopropyl-4-phenyl-1*H*-pyrrole-3-carboxylic acid (3-diethylaminopropyl)amide

A mixture of 2-aminoacetophenone hydrochloride (1 equiv.), ethyl isobutyrylacetate (1.2 equiv.) and sodium acetate (2.4 equiv.) in H_2O was stirred at 100° C for 18 hours and then cooled to room temperature. The aqueous layer was decanted off and the oil was dissolved in ethyl acetate. It was then washed with water and brine and then dried to give (93%) of 2-isopropyl-4-phenyl-1*H*-pyrrole-3-carboxylic acid ethyl ester as a red brown oil.

^{1}HNMR (300 MHz, DMSO-d6) δ 11.21 (s, br, 1H, N*H*), 7.14-7.27 (m, 5H), 6.70 (d, *J* = 2.7 Hz, 1H), 4.02 (q, *J* = 7.1 Hz, 2H, OCH_2CH_3), 3.65 (m, 1H, $CH(CH_3)_2$), 1.22 (d, *J* = 7.5 Hz, 6H, $CH(CH_3)_2$), 1.04 (t, *J* = 7.1 Hz, 3H, OCH_2CH_3).

MS-EI *m/z* 257 [M$^+$].

The above pyrrole was formylated using method A to give (41%) 5-formyl-2-isopropyl-4-phenyl-1*H*-pyrrole-3-carboxylic acid ethyl ester as a reddish solid.

^{1}HNMR (300 MHz, DMSO-d6) δ 12.35 (s, br, 1H, N*H*), 9.14 (s, 1H, CHO), 7.36 (s, 5H), 3.96 (q, *J* = 7.1 Hz, 2H, OCH_2CH_3), 3.74 (m, 1H, $CH(CH_3)_2$), 1.29 (d, *J* = 6.9 Hz, 6H, $CH(CH_3)_2$), 0.90 (t, *J* = 7.1 Hz, 3H, OCH_2CH_3).

MS-EI *m/z* 285 [M$^+$].

The pyrrolecarboxylic acid ester was hydrolysed using method B to give (57%) of 5-formyl-2-isopropyl-4-phenyl-1*H*-pyrrole-3-carboxylic acid as a beige solid.

^{1}HNMR (300 MHz, DMSO-d6) δ 12.28 (s, br, 1H, COO*H*), 12.02 (s, br, 1H, N*H*), 9.10 (s, 1H, CHO), 7.35 (s, 5H), 3.81 (m, 1H, $CH(CH_3)_2$), 1.28 (d, *J* = 6.9 Hz, 6H, $CH(CH_3)_2$).

MS-EI *m/z* 257 [M$^+$].

5-Bromo-1,3-dihydroindol-2-one (120 mg, 0.31 mmol) was condensed with 5-formyl-2-isopropyl-4-phenyl-1*H*-pyrrole-3-

carboxylic acid (3-diethylaminopropyl)amide (prepared by method C) to give 120 mg (71%) of the title compound.

^{1}HNMR (300 MHz, DMSO-d6) δ 14.23 (s, br, 1H, NH), 11.08 (s, br, 1H, NH), 7.38-7.55 (m, 7H, Ar-H & CONHCH$_2$), 7.30 (s, 1H, H-vinyl), 7.26 (dd, J = 1.8 & 7.8 Hz, 1H), 6.85 (d, J = 8.7 Hz, 1H), 3.36 (m, 1H, CH(CH$_3$)$_2$), 3.07 (m, 2H, CH$_2$), 2.34 (q, J = 7.1 Hz, 4H, N(CH$_2$CH$_3$)$_2$), 2.22 (t, J = 6.9 Hz, 2H, CH$_2$), 1.40 (m, 2H, CH$_2$), 1.31 (d, J = 6.9 Hz, 6H, CH(CH$_3$)$_2$), 0.86 (t, J = 7.1 Hz, 6H, N(CH$_2$CH$_3$)$_2$).

MS m/z 565.1 [M$^+$+1].

Example 38

5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2-isopropyl-4-phenyl-1H-pyrrole-3-carboxylic acid (3-pyrrolidin-1-ylpropyl)amide

5-(5-bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2-isopropyl-4-phenyl-1H-pyrrole-3-carboxylic acid (127 mg, 0.28 mmol) was condensed with 3-pyrrolidin-1-yl-propylamine (43 mg, 0.336 mmol) to give 140 mg (66%) of the title compound.

^{1}HNMR (300 MHz, DMSO-d6) δ 14.40 (s, br, 1H, NH), 7.38-7.47 (m, 7H), 7.23-7.27 (m, 2H), 6.84 (d, J = 8.1 Hz, 1H), 3.36 (m, 1H, CH(CH$_3$)$_2$), 3.08 (m, 2H, CH$_2$), 2.30 (m, 4H, 2xCH$_2$), 2.20 (t, J = 7.0 Hz, 2H, CH$_2$), 1.62 (m, 4H, 2xCH$_2$), 1.42 (t, J = 7.0 Hz, 2H, CH$_2$), 1.31 (d, J = 7.2 Hz, 6H, CH(CH$_3$)$_2$).

MS-EI m/z 560 and 562 [M$^+$ -1 and M$^+$+1].

Example 39

5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2-isopropyl-4-phenyl-1H-pyrrole-3-carboxylic acid (2-diethylaminoethyl)amide

5-Bromo-1,3-dihydroindol-2-one (57 ,g. 0.27 mmol) was condensed with 5-formyl-2-isopropyl-4-phenyl-1H-pyrrole-3-carboxylic acid (2-diethylaminoethyl)amide (120 mg) to give 78 mg (53%) of the title compound as a yellow solid.

^{1}HNMR (300 MHz, DMSO-d6) δ 14.23 (s, br, 1H, NH), 11.09 (s,

br, 1H, NH), 7.38-7.51 (m, 6H), 7.25-7.28 (m, 2H), 7.19 (t, 1H, CONHCH$_2$), 6.85 (d, J = 7.8 Hz,1H), 3.43 (m, 1H, CH(CH$_3$)$_2$), 3.11 (m, 2H, CH$_2$), 2.28-2.39 (m, 6H, N(CH$_2$CH$_3$)$_2$ & CH$_2$, 1.31 (d, J = 6.9 Hz, CH(CH$_3$)$_2$), 0.85 (t, J = 7.0 Hz, 6H, N(CH$_2$CH$_3$)$_2$.

MS-EI m/z 548 and 550 [M$^+$ -1 and M$^+$+1].

Example 40

5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2-isopropyl-4-phenyl-1*H*-pyrrole-3-carboxylic acid [3-(4-methylpiperazin-1-yl)propyl]amide

5-Bromo-1,3-dihydroindol-2-one (53 mg, 0.25 mmol) was condensed with 5-formyl-2-isopropyl-4-phenyl-1*H*-pyrrole-3-carboxylic acid [3-(4-methylpiperazin-1-yl)propyl]amide (300 mg) to give 65 mg of the title compound.

^{1}HNMR (300 MHz, DMSO-d6) δ 14.22 (s, br, 1H, NH), 11.08 (s, br, 1H, NH), 7.23-7.50 (m, 9H), 6.85 (d, J = 8.7 Hz, 1H), 3.37 (m, 1H, CH(CH$_3$)$_2$), 3.05 (m, 2H, CH$_2$), 2.24 (m, 8H, 4xCH$_2$), 2.11 (m, 5H, CH$_2$ & CH$_3$),1.42 (m, 2H, CH$_2$), 1.31 (d, J = 7.2 Hz, 6H, CH(CH$_3$)$_2$).

MS-EI m/z 589 and 591 [M$^+$ -1 and M$^+$+1].

Example 41

5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2-isopropyl-4-phenyl-1*H*-pyrrole-3-carboxylic acid

5-Bromo-1,3-dihydroindol-2-one (170 mg, 0.8 mmol) was condensed with 5-formyl-2-isopropyl-4-phenyl-1*H*-pyrrole-3-carboxylic acid (205 mg) using method D to give 210 mg (58%) of the title compound as a yellow solid.

^{1}HNMR (300 MHz, DMSO-d6) δ 14.31 (s, br, 1H, NH), 11.16 (s, br, 1H, NH), 7.26-7.44 (m, 7H), 7.11 (s, 1H, H-vinyl), 6.85 (d, J = 7.8 Hz, 1H), 3.78 (m, 1H, CH(CH$_3$)$_2$), 1.34 (d, J = 6.9 Hz, 6H, CH(CH$_3$)$_2$).

MS-EI m/z 452 [M$^+$+1].

Example 42

5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2-methyl-4-phenyl-1*H*-pyrrole-3-carboxylic acid (2-pyrrolidin-1-ylethyl)amide

5-Bromo-1,3-dihydroindol-2-one (44 mg, 0.21 mmol) was condensed with 5-formyl-2-methyl-4-phenyl-1*H*-pyrrole-3-carboxylic acid (2-pyrrolidin-1-ylethyl)amide (70 mg, prepared in the same manner as the isopropyl analog, above) to give 0.03 g (27%) of the title compound as a yellow solid.

^{1}HNMR (300 MHz, DMSO-d6) δ 13.87 (s, br, 1H, N*H*), 11.11 (s, br, 1H, N*H*), 7.36-7.51 (m, 6H), 7.26 (dd, *J* = 1.8 & 8.1 Hz, 1H), 7.2 (s, 1H, H-vinyl), 7.09 (m, 1H, CONHCH$_2$), 6.83 (d, *J* = 8.1 Hz, 1H), 3.17 (m, 2H, NC*H*$_2$), 2.48 (m, C*H*$_3$), 2.29-2.35 (m, 6H, 3xNC*H*$_2$), 1.59 (m, 4H, 2xC*H*$_2$).

MS-EI *m/z* 518 and 520 [M$^+$ -1 and M$^+$+1].

Example 43

5-[6-(2-Methoxyphenyl)-2-oxo-1,2-dihydroindol-3-ylidenemethyl]-2-methyl-4-phenyl-1*H*-pyrrole-3-carboxylic acid (2-pyrrolidin-1-ylethyl)amide

6-(2-Methoxyphenyl)-1,3-dihydroindol-2-one (50 mg, 0.21 mmol) was condensed with 5-formyl-2-methyl-4-phenyl-1*H*-pyrrole-3-carboxylic acid (2-pyrrolidin-1-ylethyl)amide (70 mg) to give 0.04 g (35%) of the title compound as a yellow-orange solid.

^{1}HNMR (300 MHz, DMSO-d6) δ 13.82 (s, br, 1H, N*H*), 11.02 (s, br, 1H, N*H*), 7.48 (m, 2H), 7.43 (m, 1H), 7.38 (m, 2H), 7.32 (m, 1H), 7.24 (m, 2H), 7.16 (s, 1H, H-vinyl), 7.08 (m, 2H), 7.03 (m, 1H), 7.0 (m, 2H), 3.74 (s, 3H, OC*H*$_3$), 3.19 (m, 2H, NC*H*$_2$), 2.49 (m, C*H*$_3$), 2.32-2.38 (m, 6H, 3xNC*H*$_2$), 1.59 (m, 4H, 2xC*H*$_2$).

MS-EI *m/z* 546 [M$^+$].

Example 44

5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2-methyl-4-phenyl-1*H*-pyrrole-3-carboxylic acid (2-dimethylaminoethyl)amide

5-Bromo-1,3-dihydroindol-2-one (46 mg, 0.22 mmol) was condensed with 5-formyl-2-methyl-4-phenyl-1*H*-pyrrole-3-carboxylic acid (2-dimethylaminoethyl)amide (65 mg) to give 60 mg (55%) of the title compound as a yellow solid.

^{1}HNMR (360 MHz, DMSO-d6) δ 13.86 (s, br, 1H, N*H*), 11.09 (s, br, 1H, N*H*), 7.47-7.49 (m, 2H), 7.38-7.41 (m, 4H), 7.26 (dd, *J* = 2.2 & 8.3 Hz, 1H), 7.21 (s, 1H, H-vinyl), 7.04 (m, 1H, CONHCH$_2$), 6.77 (d, *J* = 8.3 Hz, 1H), 3.15 (m, 2H, NC*H*$_2$), 2.48 (m, C*H*$_3$), 2.16 (t, *J* = 6.8 Hz, 2H, 3xNCH$_2$), 2.02 (s, 6H, 2xNC*H*$_3$).

MS *m/z* 493 and 494.8 [M$^+$ and M$^+$+2].

Example 45

5-[6-(2-Methoxyphenyl)-2-oxo-1,2-dihydroindol-3-ylidenemethyl]-2-methyl-4-phenyl-1*H*-pyrrole-3-carboxylic acid (2-dimethylaminoethyl)amide

6-(2-Methoxyphenyl)-1,3-dihydroindol-2-one (53 mg, 0.22 mmol) was condensed with 5-formyl-2-methyl-4-phenyl-1*H*-pyrrole-3-carboxylic acid (2-dimethylaminoethyl)amide (65 mg) to give 0.05 g (44%) of the title compound as an orange gum.

^{1}HNMR (300 MHz, DMSO-d6) δ 13.82 (s, br, 1H, N*H*), 11.02 (s, br, 1H, N*H*), 7.37-7.52 (m, 5H), 7.32 (m, 1H), 7.22-7.27 (m, 2H), 7.16 (s, 1H), 7.08 (m, 2H), 7.03 (m, 1H), 7.0 (m, 2H), 3.74 (s, 3H, OC*H*$_3$), 3.15 (m, 2H, NC*H*$_2$), 2.49 (m, C*H*$_3$), 2.16 (t, *J* = 6.5 Hz, 2H, NC*H*$_2$), 2.02 (s, 6H, 2xNC*H*$_3$).

MS *m/z* 521 [M$^+$+1].

Example 46

5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2-methyl-4-phenyl-1*H*-pyrrole-3-carboxylic acid ethyl ester

5-Bromo-1,3-dihydroindol-2-one (60 mg, 0.29 mmol) was condensed with 5-formyl-2-methyl-4-phenyl-1*H*-pyrrole-3-carboxylic acid ethyl ester (75 mg) to give 78 mg (60%) of the title compound as an orange solid.

^{1}HNMR (360 MHz, DMSO-d6) δ 14.01 (s, br, 1H, N*H*), 11.13 (s,

br, 1H, NH), 7.42-7.46 (m, 3H), 7.27-7.34 (m, 4H), 7.12 (s, 1H), 6.84 (dd, J = 2.2 & 8.3 Hz, 1H), 3.99-4.03 (m, 2H, OCH_2CH_3), 2.61 (s, 3H, CH_3), 0.98-1.03 (m, 3H, OCH_2CH_3).

MS-EI m/z 450 and 452 [M^+ -1 and M^++1].

Example 47

5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2-methyl-4-phenyl-1*H*-pyrrole-3-carboxylic acid (3-diethylaminopropyl)amide

5-bromo-1,3-dihydroindol-2-one (0.47 g, 2.2 mmol) was condensed with 5-formyl-2-methyl-4-phenyl-1*H*-pyrrole-3-carboxylic acid (3-diethylaminopropyl)amide (0.75 g) to give 0.11 g (42%) of the title compound as an orange solid.

^{1}HNMR (300 MHz, DMSO-d6) δ 13.86 (s, br, 1H, NH), 7.42-7.46 (m, 3H), 7.37-7.50 (m, 7H), 7.24-7.28 (m, 2H), 6.83 (d, J = 8.1 Hz, 1H), 3.09 (m, 2H, NCH_2), 2.45 (s, 3H, CH_3), 2.38 (q, J = 7.1 Hz, 4H, 2xNCH_2CH_3), 2.26 (t, J = 6.9 Hz, 2H, NCH_2), 1.42 (m, 2H, NCH_2), 0.87 (t, J = 7.1 Hz, 6H, 2xNCH_2CH_3).

MS-EI m/z 535.0 and 537 [M^+ and M^++2].

Example 48

5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-dimethylamino-ethyl)amide

A mixture of tert-butyl 3-oxobutyrate and sodium nitrite (1 equiv.) in acetic acid was stirred at room temperature to give tert-butyl-2-hydroximino-3-oxobutyrate.

Ethyl-3-oxobutyrate (1 equiv.), zinc dust (3.8 equiv.) and the crude tert-butyl-2-hydroximino-3-oxobutyrate in acetic acid was stirred at 60° C for 1 hr. The reaction mixture was poured into H_2O and the filtrate was collected to give (65%) 2-tert-butyloxycarbonyl-3,5-dimethyl-4-ethoxycarbonylpyrrole.

A mixture of 2-tert-butyloxycarbonyl-3,5-dimethyl-4-ethoxycarbonylpyrrole and triethyl orthoformate (1.5 equiv.) in trifluoroacetic acid was stirred at 15°C for 1 hour. The reaction was concentrated and the residue was purified to give

(64%) 2,4-dimethyl-3-ethoxycarbonyl-5-formylpyrrole as yellow needles.

2,4-Dimethyl-3-ethoxycarbonyl-5-formylpyrrole was hydrolyzed using method B to give (90%) 5-formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid.

^{1}H NMR (360 MHz, DMSO-d6) δ 12 (br s, 2H, N*H* and CO_2H), 9.58 (s, 1H, CHO), 2.44 (s, 3H, CH_3), 2.40 (s, 3H, CH_3).

MS *m/z* 267 [M^+].

5-Bromo-1,3-dihydroindol-2-one (0.17 g, 0.8 mmol) was condensed with 5-formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-dimethylaminoethyl)amide (0.2 g, prepared by method C) using method B to give 0.3 g (83%) of the title compound as a yellow solid.

^{1}HNMR (360 MHz, DMSO-d6) δ 13.60 (s, br, 1H, N*H*), 10.94 (s, br, 1H, N*H*), 8.07 (d, *J* = 1.8 Hz, 1H, H-4), 7.75 (s, 1H, H-vinyl), 7.44 (t, *J* = 5.2 Hz, 1H, CONHCH_2), 7.24 (dd, *J* = 1.8 & 8.4 Hz, 1H, H-6), 6.82 (d, *J* = 8.4 Hz, 1H, H-7), 3.26-3.33 (m, 2H, NCH_2), 2.42 (s, 3H, CH_3), 2.41 (s, 3H, CH_3), 2.38 (t, *J* = 6.7 Hz, 2H, NCH_2), 2.18 (s, 6H, $N(CH_3)_2$).

MS-EI *m/z* 430 and 432 [M^+-1 and M^++1].

Example 49

2,4-Dimethyl-5-(2-oxo-6-phenyl-1,2-dihydroindol-3-ylidenemethyl)-1*H*-pyrrole-3-carboxylic acid (2-dimethylaminoethyl)amide

6-Phenyl-1,3-dihydroindol-2-one (0.17 g, 0.8 mmol) was condensed with 5-formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-dimethylaminoethyl)amide (0.2 g) to give 0.13 g (36%) of the title compound as a yellow-orange solid.

^{1}HNMR (360 MHz, DMSO-d6) δ 13.59 (s, br, 1H, N*H*), 10.93 (, br, 1H, N*H*), 7.85 (d, *J* = 7.92 Hz, 1H, H-4), 7.63-7.65 (m, 3H), 7.40-7.47 (m, 3H,), 7.32-7.36 (m, 1H, Ar-*H*), 7.30 (dd, *J* = 1.6 & 7.9 Hz, 1H, H-5), 7.11 (d, *J* = 1.6 Hz, 1H, H-7), 3.28-3.34 (m, 2H, NCH_2), 2.43 (s, 3H, CH_3), 2.41 (s, 3H, CH_3), 2.38 (t, *J* = 6.8

Hz, 2H, NCH_2), 2.18 (s, 6H, $N(CH_3)_2$).

MS-EI *m/z* 428 [M^+].

Example 50

5-(5-Chloro-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-dimethylamino-ethyl)amide

5-Chloro-1,3-dihydroindol-2-one (0.1 g, 0.6 mmol) was condensed with 5-formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-dimethylaminoethyl)amide (0.15 g) to give 0.22 g (90%) of the title compound as a yellow solid.

^{1}HNMR (300 MHz, DMSO-d6) δ 13.61 (s, br, 1H, *NH*), 10.98 (, br, 1H, *NH*), 7.96 (d, *J* = 2.0 Hz, 1H, H-4), 7.75 (s, 1H, H-vinyl), 7.50 (t, *J* = 5.5 Hz, 1H, $CONHCH_2$), 7.12 (dd, *J* = 2.0 & 8.3 Hz, 1H, H-6), 6.86 (d, *J* = 8.3 Hz, 1H, H-7), 3.26-3.31 (m, 2H, NCH_2), 2.42 (s, 3H, CH_3), 2.40 (s, 3H, CH_3), 2.36 (t, *J* = 6.6 Hz, 2H, NCH_2), 2.17 (s, 6H, $N(CH_3)_2$).

MS-EI *m/z* 386 [M^+].

Example 51

5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-diethylamino-ethyl)amide

5-Bromo-1,3-dihydroindol-2-one (0.17 g, 0.8 mmol) was condensed with 5-formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-diethylaminoethyl)amide (0.2 g) to give 0.09 g (26%) of the title compound as a yellow solid.

^{1}HNMR (360 MHz, DMSO-d6) δ 13.61 (s, br, 1H, *NH*), 10.98 (, br, 1H, *NH*), 8.09 (d, *J* = 1.7 Hz, 1H, H-4), 7.76 (s, 1H, H-vinyl), 7.42 (t, *J* = 5.5 Hz, 1H, $CONHCH_2$), 7.24 (dd, *J* = 1.7 & 8.0 Hz, 1H, H-6), 6.82 (d, *J* = 8.0 Hz, 1H, H-7), 3.23-3.32 (m, 2H, NCH_2), 2.46-2.55 (m, 6H, $3xNCH_2$), 2.43 (s, 3H, CH_3), 2.42 (s, 3H, CH_3), 0.96 (t, *J* = 7.2 Hz, 6H, $2xNCH_2CH_3$).

MS-EI *m/z* 458 and 460 [M^+-1 and M^++1].

Example 52

5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2,4-dimethyl-

1H-pyrrole-3-carboxylic acid (2-pyrrolidin-1-yl-ethyl)amide

5-Bromo-1,3-dihydroindol-2-one (0.09 g, 0.4 mmol) was condensed with 5-formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-pyrrolidin-1-ylethyl)amide (0.1 g) to give 0.14 g (81%) of the title compound as a yellow-orange solid.

^{1}HNMR (300 MHz, DMSO-d6) δ 13.61 (s, br, 1H, N*H*), 10.98 (, br, 1H, N*H*), 8.09 (d, *J* = 1.9 Hz, 1H, H-4), 7.76 (s, 1H, H-vinyl), 7.53 (t, *J* = 5.5 Hz, 1H, CONHCH$_2$), 7.24 (dd, *J* = 1.9 & 8.5 Hz, 1H, H-6), 6.81 (d, *J* = 8.5 Hz, 1H, H-7), 3.29-3.35 (m, 2H, NCH$_2$), 2.54 (t, *J* = 6.9 Hz, 2H, NCH$_2$), 2.47 (m, under DMSO), 2.42 (s, 3H, CH$_3$), 2.40 (s, 3H, CH$_3$), 1.66-1.69 (m, 4H, 2xCH$_2$).

MS-EI *m/z* 456 and 458 [M$^+$-1 and M$^+$+1].

Example 53

5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (3-imidazol-1-yl-propyl)amide

5-Bromo-1,3-dihydroindol-2-one (0.09 g, 0.4 mmol) was condensed with 5-formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (3-imidazol-1-ylpropyl)amide (0.1 g) to give 0.1 g (59%) of the title compound as an orange solid.

^{1}HNMR (300 MHz, DMSO-d6) δ 13.63 (s, br, 1H, N*H*), 10.99 (, br, 1H, N*H*), 8.09 (d, *J* = 2.2 Hz, 1H, H-4), 7.77 (s, 1H, H-vinyl), 7.71 (t, *J* = 5.7 Hz, 1H, CONHCH$_2$), 7.65 (s, 1H, Ar-H), 7.25 (dd, *J* = 2.2 & 8.4 Hz, 1H, H-6), 7.20 (s, 1H, Ar-H), 6.89 (s, 1H, Ar-H), 6.81 (d, *J* = 8.4 Hz, 1H, H-7), 4.02 (t, *J* = 6.7 Hz, 2H, NCH$_2$), 3.18 (q, *J* = 6.7 Hz, 2H, NCH$_2$), 2.43 (s, 3H, CH$_3$), 2.41 (s, 3H, CH$_3$), 1.93 (m, 2H, CH$_2$).

MS-EI *m/z* 467 and 469 [M$^+$-1 and M$^+$+1].

Example 54

5-[6-(2-Methoxyphenyl)-2-oxo-1,2-dihydroindol-3-ylidenemethyl]-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-dimethylaminoethyl)amide

6-(2-Methoxyphenyl)-1,3-dihydroindol-2-one (30 mg, 0.13

mmol) was condensed with 5-formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-dimethylaminoethyl)amide (30 mg) to give 0.06 g (100%) of the title compound as a yellow-orange gum.

^{1}HNMR (300 MHz, DMSO-d6) δ 13.60 (s, br, 1H, *NH*), 10.89 (s, br, 1H, *NH*), 7.79 (d, *J* = 8.4 Hz, 1H), 7.63 (s, 1H, H-vinyl), 7.46 (t, *J* = 5.5 Hz, 1H, CONHCH$_2$), 7.28-7.35 (m, 2H), 6.99-7.11 (m, 4H), 3.76 (s, 3H, OC*H*$_3$), 3.27-3.31 (m, 2H, NC*H*$_2$), 2.43 (s, 3H, C*H*$_3$), 2.39 (s, 3H, C*H*$_3$), 2.37 (m, 2H, NC*H*$_2$), 2.18 (s, 6H, N(C*H*$_3$)$_2$).

MS-EI *m/z* 458 [M$^+$].

Example 55

5-[6-(3-Methoxyphenyl)-2-oxo-1,2-dihydroindol-3-ylidenemethyl]-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-dimethylaminoethyl)amide

6-(3-Methoxyphenyl)-1,3-dihydroindol-2-one (30 mg, 0.13 mmol) was condensed with 5-formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-dimethylaminoethyl)amide (30 mg) to give 8 mg (14%) of the title compound as a yellow-orange solid.

^{1}HNMR (360 MHz, DMSO-d6) δ 13.59 (s, br, 1H, *NH*), 10.92 (s, br, 1H, *NH*), 7.84 (d, *J* = 7.6 Hz, 1H), 7.65 (s, 1H, H-vinyl), 7.42 (m, 1H, CONHCH$_2$), 7.36 (d, *J* = 7.8 Hz, 1H), 7.29 (dd, *J* = 1.6 & 7.6 Hz, 1H), 7.20 (d, *J* = 7.8 Hz, 1H), 7.14 (d, *J* = 2.8 Hz, 1H), 7.11 (d, *J* = 1.6 Hz, 1H), 6.91 (dd, *J* = 2.8 & 7.8 Hz, 1H), 3.82 (s, 3H, OC*H*$_3$), 3.21-3.33 (m, 2H, NC*H*$_2$), 2.43 (s, 3H, C*H*$_3$), 2.40 (s, 3H, C*H*$_3$), 2.36-2.40 (m, 2H, NC*H*$_2$), 2.18 (s, 6H, N(C*H*$_3$)$_2$).

MS-EI *m/z* 458 [M$^+$].

Example 56

2,4-Dimethyl-5-(2-oxo-5-phenyl-1,2-dihydroindol-3-ylidenemethyl)-1*H*-pyrrole-3-carboxylic acid (2-diethylaminoethyl)amide

5-Phenyl-1,3-dihydroindol-2-one (80 mg, 0.4 mmol) was condensed with 5-formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-diethylaminoethyl)amide (0.1 g) using method B to give 79 mg

(46%) of the title compound.

^{1}HNMR (300 MHz, DMSO-d6) δ 13.66 (s, br, 1H, NH), 10.95 (, br, 1H, NH), 8.15 (d, J = 1.2 Hz, 1H), 7.81 (s, 1H, H-vinyl), 7.71 (d, J = 7.5 Hz, 1H), 7.40-7.47 (m, 4H), 7.31 (m, 1H), 6.95 (d, J = 8.1 Hz, 1H), 3.2-3.31 (m, 2H, NCH_2), 2.46-2.55 (m, 6H, 3xNCH_2), 2.44 (s, 6H, 2xCH_3), 0.96 (t, J = 7.4 Hz, 6H, 2xNCH_2CH_3).

MS-EI m/z 456 [M^+].

Example 57

2,4-Dimethyl-5-(2-oxo-5-phenyl-1,2-dihydroindol-3-ylidenemethyl)-1*H*-pyrrole-3-carboxylic acid (2-pyrrolidin-1-ylethyl)amide

5-Phenyl-1,3-dihydroindol-2-one (0.04 g, 0.2 mmol) was condensed with 5-formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-pyrrolidin-1-ylethyl)amide (0.04 g) to give the title compound as a yellow-orange solid.

^{1}HNMR (300 MHz, DMSO-d6) δ 13.65 (s, br, 1H, NH), 10.96 (, br, 1H, NH), 8.15 (d, J = 1.0 Hz, 1H), 7.80 (s, 1H, H-vinyl), 7.71 (d, J = 7.2 Hz, 2H), 7.49 (t, J = 6.3 Hz, 1H, CONHCH_2), 7.41-7.46 (m, 3H), 7.31 (m, 1H), 6.95 (d, J = 7.8 Hz, 1H), 4.08 (m, 4H, 2x NCH_2), 3.32 (m, 2H, NCH_2), 2.55 (t, J = 7.1 Hz, 2H, NCH_2), 2.47 (m, under DMSO), 2.43 (s, 6H, 2xCH_3), 1.66 (m, 4H, 2xCH_2).

MS-EI m/z 454 [M^+].

Example 58

2,4-Dimethyl-5-(2-oxo-5-phenyl-1,2-dihydroindol-3-ylidenemethyl)-1*H*-pyrrole-3-carboxylic acid (3-imidazol-1-ylpropyl)amide

5-Phenyl-1,3-dihydroindol-2-one (8 mg, 0.04 mmol) was condensed with 5-formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (3-imidazol-1-ylpropyl)amide (10 mg) to give 10 mg (59%) of the title compound as an orange solid.

^{1}HNMR (300 MHz, DMSO-d6) δ 13.67 (s, br, 1H, NH), 10.96 (, br, 1H, NH), 8.16 (d, J = 1.2 Hz, 1H), 7.81 (s, 1H, H-vinyl), 7.65-7.72 (m, 4H), 7.44 (m, 3H), 7.31 (m, 1H, CONHCH_2), 7.21 (s,

1H, Ar-H), 4.02 (t, J = 6.5 Hz, 2H, NCH_2), 3.19 (q, J = 6.5 Hz, 2H, $CONHCH_2$), 2.44 (s, 6H, 2xCH_3), 1.93 (m, 2H, CH_2CH_2 CH_2).

MS-EI m/z 465 [M^+].

Example 59

2,4-Dimethyl-5-(2-oxo-6-phenyl-1,2-dihydroindol-3-ylidenemethyl)-1*H*-pyrrole-3-carboxylic acid (2-diethylaminoethyl)amide

6-Phenyl-1,3-dihydroindol-2-one (0.08 g, 0.4 mmol) was condensed with 5-formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-diethylaminoethyl)amide (0.1 g) to give 65 mg (38%) of the title compound as a yellow solid.

^{1}HNMR (300 MHz, DMSO-d6) δ 13.61 (s, br, 1H, *NH*), 10.99 (, br, 1H, *NH*), 7.86 (d, J = 7.8 Hz, 1H), 7.62-7.66 (m, 3H), 7.40-7.47 (m, 3H), 7.28-7.36 (m, 2H), 7.10 (d, J = 1.2 Hz, 1H), 3.26 (m, 2H, NCH_2), 2.46-2.55 (m, 6H, 3xNCH_2), 2.44 (s, 3H, CH_3), 2.41 (s, 3H, CH_3), 0.97 (t, J = 7.2 Hz, 6H, 2xNCH_2CH_3).

MS-EI m/z 456 [M^+].

Example 60

2,4-Dimethyl-5-(2-oxo-6-phenyl-1,2-dihydroindol-3-ylidenemethyl)-1*H*-pyrrole-3-carboxylic acid (2-pyrrolidin-1-ylethyl)amide

6-Phenyl-1,3-dihydroindol-2-one (30 mg, 0.15 mmol) was condensed with 5-formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-pyrrolidin-1-ylethyl)amide (40 mg) to give 5.9 mg (8.5%) of the title compound as a yellow-orange solid.

^{1}HNMR (300 MHz, DMSO-d6) δ 13.60 (s, br, 1H, *NH*), 10.99 (, br, 1H, *NH*), 7.86 (d, J = 7.8 Hz, 1H), 7.63-7.66 (m, 3H), 7.51 (m, 1H, $CONHCH_2$), 7.45 (m, 2H), 7.28-7.36 (m, 2H), 7.10 (d, J = 1.5 Hz, 1H), 3.31 (m, 6H, 3xNCH_2), 2.55 (t, J = 6.6 Hz, 2H, NCH_2), 2.43 (s, 3H, CH_3), 2.40 (s, 3H, CH_3), 1.67 (m, 4H, 2xCH_2).

MS-EI m/z 454 [M^+].

Example 61

2,4-Dimethyl-5-(2-oxo-6-phenyl-1,2-dihydroindol-3-ylidenemethyl)-1*H*-pyrrole-3-carboxylic acid (3-imidazol-1-ylpropyl)amide

6-Phenyl-1,3-dihydroindol-2-one (8 mg, 0.04 mmol) was condensed with 5-formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (3-imidazol-1-ylpropyl)amide (10 mg) to give 7.3 mg (43%) of the title compound as an orange solid.

^{1}HNMR (300 MHz, DMSO-d6) δ 13.62 (s, br, 1H, *NH*), 10.99 (, br, 1H, *NH*), 7.86 (d, *J* = 8.2 Hz, 1H), 7.62-7.70 (m, 5H), 7.45 (m, 2H), 7.35 (m, 1H), 7.30 (dd, *J* = 1.4 & 8.2 Hz, 1H), 7.21 (s, 1H), 7.10 (d, *J* = 1.4 Hz, 1H), 6.89 (s, 1H), 4.02 (t, *J* = 6.9 Hz, 2H, CH_2), 3.19 (m, 2H, NCH_2 CH_2), 2.43 (s, 3H, CH_3), 2.41 (s, 3H, CH_3), 1.93 (t, *J* = 6.9 Hz, 2H, NCH_2).

MS-EI *m/z* 465 [M^+].

Example 62

5-[6-(3,5-Dichlorophenyl)-2-oxo-1,2-dihydroindol-3-ylidenemethyl]-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-diethylaminoethyl)amide

6-(3,5-Dichlorophenyl)-1,3-dihydroindol-2-one (64 mg, 0.23 mmol) was condensed with 5-formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-diethylaminoethyl)amide (60 mg) to give 53 mg (44%) of the title compound as a light brown solid.

^{1}HNMR (360 MHz, DMSO-d6) δ 13.62 (s, br, 1H, *NH*), 10.99 (s, 1H, *NH*), 7.89 (d, *J* = 7.9 Hz, 1H, H-4), 7.69-7.71 (m, 3H), 7.55 (m, 1H, $CONHCH_2$), 7.37 (m, 2H), 7.14 (d, *J* = 1.4 Hz, 1H, H-7), 3.27 (m, 2H, NCH_2), 2.48-2.58 (m, 6H, 3xNCH_2), 2.45 (s, 3H, CH_3), 2.42 (s, 3H, CH_3), 0.97 (t, *J* = 6.8 Hz, 6H, 3xNCH_2CH_3).

MS *m/z* 526.9 [M^++1].

Example 63

2,4-Dimethyl-5-(2-oxo-6-pyridin-3-yl-1,2-dihydroindol-3-ylidenemethyl)-1*H*-pyrrole-3-carboxylic acid (2-diethylaminoethyl)amide

6-Pyridin-3-yl-1,3-dihydroindol-2-one (40 mg, 0.19 mmol) was condensed with 5-formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-diethylaminoethyl)amide (50 mg) give 29 mg (33%) of the title

compound as a light orange solid.

^{1}HNMR (300 MHz, DMSO-d6) δ 13.62 (s, br, 1H, N*H*), 11.05 (s, br, 1H, N*H*), 8.86 (s, br, 1H), 8.53 (d, *J* = 5.8 Hz, 1H), 8.04 (m, 1H), 7.91 (d, *J* = 8.1 Hz, 1H), 7.70 (s, 1H, H-vinyl), 7.40-7.48 (m, 2H), 7.35 (d, *J* = 7.5 Hz, 1H), 7.14 (s, 1H), 3.26 (m, 2H, NC*H*$_2$), 2.48-2.55 (m, 3xNC*H*$_2$), 2.42 (s, 3H, C*H*$_3$), 2.38 (s, 3H, C*H*$_3$), 0.96 (t, *J* = 6.9 Hz, 6H, 2xNCH$_2$C*H*$_3$).

MS-EI *m/z* 457 [M$^+$].

Example 64

2,4-Dimethyl-5-(2-oxo-6-pyridin-3-yl-1,2-dihydroindol-3-ylidenemethyl)-1*H*-pyrrole-3-carboxylic acid (2-pyrrolidin-1-ylethyl)amide

6-Pyridin-3-yl-1,3-dihydroindol-2-one (60 mg, 0.28 mmol) was condensed with 5-formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-pyrrolidin-1-ylethyl)amide (75 mg) to give 90 mg (71%) of the title compound as a light orange solid.

^{1}HNMR (300 MHz, DMSO-d6) δ 13.61 (s, br, 1H, N*H*), 11.05 (s, br, 1H, N*H*), 8.86 (d, *J* = 1.5 Hz, 1H), 8.54 (dd, *J* = 1.5 & 4.8 Hz, 1H), 8.05 (m, 1H), 7.91 (d, *J* = 7.8 Hz, 1H), 7.70 (s, 1H, H-vinyl), 7.44-7.53 (m, 2H), 7.36 (dd, *J* = 1.5 & 8.1 Hz, 1H), 7.15 (d, *J* = 1.2 Hz, 1H), 3.33 (m, 2H, NC*H*$_2$), 2.47-2.57 (m, 6H, 3xNC*H*$_2$), 2.43 (s, 3H, C*H*$_3$), 2.41 (s, 3H, C*H*$_3$), 1.67 (m, 4H, 2xC*H*$_2$).

MS-EI *m/z* 455 [M$^+$].

Example 65

2,4-Dimethyl-5-(2-oxo-6-pyridin-3-yl-1,2-dihydroindol-3-ylidenemethyl)-1*H*-pyrrole-3-carboxylic acid (3-dimethylaminopropyl)amide

6-Pyridin-3-yl-1,3-dihydroindol-2-one (42 mg, 0.2 mmol) was condensed with 5-formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (3-dimethylaminopropyl)amide (50 mg) to give 67 mg (75%) of the title compound as yellow-brown solid.

^{1}HNMR (360 MHz, DMSO-d6) δ 13.61 (s, br, 1H, N*H*), 11.00 (s, br, 1H, N*H*), 8.86 (s, br, 1H), 8.54 (s, br, 1H), 8.04 (m, 1H), 7.90 (d, *J* = 8.0 Hz, 1H), 7.69 (s, 1H, H-vinyl), 7.63 (m, 1H), 7.45-7.48 (m, 1H), 7.35 (dd, *J* = 1.7 & 8.0 Hz, 1H), 7.15 (d, *J* = 1.7 Hz, 1H), 3.21-3.27 (m, 2H, NC*H*$_2$), 2.43 (s, 3H, C*H*$_3$), 2.41 (s, 3H, C*H*$_3$), 2.28 (m, 2H, NC*H*$_2$), 2.14 (s, 6H, 2xNC*H*$_3$), 1.64 (m, 2H, C*H*$_2$).

MS-EI *m/z* 443 [M$^+$].

Example 66

2,4-Dimethyl-5-(2-oxo-5-phenyl-1,2-dihydroindol-3-ylidenemethyl)-1*H*-pyrrole-3-carboxylic acid (3-dimethylaminopropyl)amide

5-Phenyl-1,3-dihydroindol-2-one (67 mg, 0.32 mmol) was condensed with 5-formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (3-dimethylaminopropyl)amide (81 mg) to give 40 mg (28%) of the title compound as an orange solid.

^{1}HNMR (360 MHz, DMSO-d6) δ 13.66 (s, br, 1H, NH), 10.92 (s, br, 1H, NH), 8.14 (s, 1H), 7.79 (s, 1H), 7.71 (m, 2H), 7.62 (m, 1H), 7.44 (m, 3H), 7.32 (m, 1H), 6.95 (m, 1H), 3.33 (m, 2H, NC*H*$_2$), 2.43 (s, 6H, 2xC*H*$_3$), 2.27 (m, 2H, NC*H*$_2$), 2.13 (s, 6H, 2xNC*H*$_3$), 1.63 (m, 2H, C*H*$_2$).

MS-EI *m/z* 442 [M$^+$].

Example 67

2,4-Dimethyl-5-(2-oxo-5-phenyl-1,2-dihydroindol-3-ylidenemethyl)-1*H*-pyrrole-3-carboxylic acid (3-diethylaminopropyl)amide

5-Phenyl-1,3-dihydroindol-2-one (1.5 g, 7.16 mmol) was condensed with 5-formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (3-diethylaminopropyl)amide (2 g) to give 1.3 g (40%) of the title compound as a yellow-orange solid.

^{1}HNMR (360 MHz, DMSO-d6) δ 13.64 (s, 1H, NH), 10.91 (s, 1H, NH), 8.14 (d, *J* = 1.4 Hz, 1H, Ar*H*), 7.8 (s, 1H, Ar*H*), 7.7 (dd, *J* = 1.2 and 8.5 Hz, 2H, Ar*H*), 7.6 (t, *J* = 5.3 Hz, 1H, CONHCH$_2$), 7.4 (m, 3H, Ar*H*), 7.3 (t, *J* = 7.4 Hz, 1H, Ar*H*), 6.9 (d, *J* = 8.0

(3-diethylaminopropyl)amide (0.51 g) to give 0.84 g of the title compound as a red-orange solid.

^{1}HNMR (360 MHz, DMSO-d6) δ 13.61 (s, 1H, NH), 10.99 (s, 1H, NH), 8.09 (d, *J* = 1.8 Hz, 1H, Ar*H*), 7.7 (m, 4H), 7.2 (dd, *J* = 1.8 and 8.3 Hz, 2H, Ar*H*), 6.8 (d, *J* = 7.8 Hz, 1H, Ar*H*), 3.3 (br s, 4H, 2xNCH_2), 3.2 (m, 2H, $CONHCH_2$), 2.6 (br s, 2H, NCH_2 and 2xCH_3), 2.4 (s, 6H, 2xCH_3), 1.66 (m, 2H, $CH_2CH_2CH_2$), 0.98 (t, *J* = 7.1 Hz, 6H, NCH_2CH_3).

MS-EI *m/z* 472 and 474 [M^+ -1 and M^++1].

Example 71

5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2,4-diisopropyl-1*H*-pyrrole-3-carboxylic acid (2-diethylamino-ethyl)amide

5-Bromo-1,3-dihydroindol-2-one (100 mg, 0.47 mmol) was condensed with 5-formyl-2,4-diisopropyl-1*H*-pyrrole-3-carboxylic acid (2-diethylaminoethyl)amide (150 mg) to give 0.15 g (62%) of the title compound as a yellow-orange solid.

^{1}HNMR (300 MHz, DMSO-d6) δ 13.97 (s, 1H, NH), 10.95 (s, 1H, NH), 8.09 (d, *J* = 1.3 Hz, 1H, Ar*H*), 7.84 (m, 1H), 7.79 (s, 1H), 7.23 (dd, *J* = 1.3 and 8.1 Hz, 1H, Ar*H*), 6.8 (d, *J* = 8.1 Hz, 1H, Ar*H*), 3.5 (m, 1H, C*H*), 3.3 (m, 3H, C*H* and $NHCH_2$), 2.5 (br m, 6H, 3xNCH_2), 1.28 (d, *J* = 6.9 Hz, 6H, 2xCH_3), 1.23 (d, *J* = 6.6 Hz, 6H, 2xCH_3), 0.96 (m, 6H, 2xCH_2CH_3).

MS-EI *m/z* 514 and 516 [M^+-1 and M^++1].

Example 72

5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2,4-diisopropyl-1*H*-pyrrole-3-carboxylic acid (3-diethylamino-propyl)amide

5-Bromo-1,3-dihydroindol-2-one (90 mg, 0.42 mmol) was condensed with 5-formyl-2,4-diisopropyl-1*H*-pyrrole-3-carboxylic acid (3-diethylaminopropyl)amide (140 mg) to give 54 mg (25%) of the title compound as red-brown solid.

^{1}HNMR (300 MHz, DMSO-d6) δ 13.98 (s, 1H, NH), 10.96 (s, 1H, NH), 8.09 (d, *J* = 1.7 Hz, 2H), 7.78 (s, 1H, H-vinyl), 7.23 (dd, *J* = 1.7 and 8.1 Hz, 1H, Ar*H*), 6.82 (d, *J* = 8.1 Hz, 1H, Ar*H*), 3.5 (m, 1H, C*H*), 3.25 (m, 2H, NHCH_2), 3.15 (m, 1H, C*H*), 2.7 (br s, 6H, 3xNCH_2), 1.7 (br m, 2H, $CH_2CH_2CH_2$), 1.28 (d, *J* = 6.9 Hz, 6H, 2xCH_3), 1.24 (d, *J* = 5.9 Hz, 6H, 2xCH_3), 1.06 (m, 6H, 2xCH_2CH_3).

MS-EI *m/z* 528 and 530 [M^+-1 and M^++1].

Example 73

5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2,4-diisopropyl-1*H*-pyrrole-3-carboxylic acid (3-pyrrolidin-1-ylpropyl)amide

5-Bromo-1,3-dihydroindol-2-one (130 mg, 0.6 mmol) was condensed with 5-formyl-2,4-diisopropyl-1*H*-pyrrole-3-carboxylic acid (3-pyrrolidin-1-ylpropyl)amide (150 mg, 0.45 mmol) to give 36 mg (15%) of the title compound as a tan-orange solid.

^{1}HNMR (300 MHz, DMSO-d6) δ 13.98 (s, 1H, NH), 10.97 (s, 1H, NH), 8.10 (d, *J* = 1.6 Hz, 2H), 7.78 (s, 1H, H-vinyl), 7.23 (dd, *J* = 1.6 and 7.6 Hz, 1H, Ar*H*), 6.82 (d, *J* = 7.6 Hz, 1H, Ar*H*), 3.5 (m, 1H, C*H*), 3.25 (m, 2H, NHCH_2), 3.15 (m, 1H, C*H*), 2.7 (br s, 6H, 3xNCH_2), 1.7 (br m, 6H, 3xNCH_2CH_2), 1.28 (d, *J* = 5.6 Hz, 6H, 2xCH_3), 1.24 (d, *J* = 5.7 Hz, 6H, 2xCH_3).

MS-EI *m/z* 526 and 528 [M^+-1 and M^++1].

Example 74

5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (pyridin-4-ylmethyl)-amide

5-Bromo-1,3-dihydroindol-2-one (170 mg, 0.8 mmol) was condensed with 5-formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (pyridin-4-ylmethyl)amide (200 mg) to give 14 mg (4%) of the title compound as a yellow solid.

^{1}HNMR (300 MHz, DMSO-d6) δ 13.67 (s, 1H, NH), 11.01 (s, br, 1H, NH), 8.51 (dd, *J* = 1.6 & 4.3 Hz, 2H), 8.23 (t, *J* = 6.0 Hz, 1H, CONHCH_2), 8.11 (d, *J* = 1.9 Hz, 1H), 7.78 (s, 1H, H-vinyl),

7.31 (d, J = 6.0 Hz, 2H), 7.25 (dd, J = 1.9 & 8.1 Hz, 1H), 6.82 (d, J = 8.1 Hz, 1H), 4.45 (d, J = 6.0 Hz, 2H, NCH_2), 2.46 (s, 6H, $2xCH_3$).

MS-EI m/z 450 and 452 [M^+-1 and M^++1].

Example 75

5-[6-(4-Butylphenyl)-2-oxo-1,2-dihydroindol-3-ylidenemethyl]-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-pyrrolidin-1-ylethyl)amide

5-[6-(4-Butylphenyl)]-1,3-dihydroindol-2-one (50 mg, 0.19 mmol) was condensed with 5-formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-pyrrolidin-1-ylethyl)amide (50 mg) to give 74 mg (76%) of the title compound as an orange solid.

^{1}HNMR (360 MHz, DMSO-d6) δ 13.58 (s, 1H, NH), 10.93 (s, br, 1H, NH), 7.82 (d, J = 7.9 Hz, 1H), 7.63 (s, 1H, H-vinyl), 7.54 (d, J = 7.9 Hz, 2H), 7.46 (m, 1H, CONH), 7.26 (m, 3H), 7.09 (s, 1H), 3.30 (m, 2H, CH_2), 2.52-2.63 (m, 4H, $2xCH_2$), 2.49 (m, 4H, $2xCH_2$), 2.43 (s, 3H, CH_3), 2.40 (s, 3H, CH_3), 1.68 (m, 4H, $2xCH_2$), 1.58 (m, 2H, CH_2), 1.34 (m, 2H, CH_2), 0.91 (t, J= 7.2 Hz, 3H, CH_2CH_3).

MS-EI m/z 510 [M^+].

Example 76

5-[6-(5-Isopropyl-2-methoxyphenyl)-2-oxo-1,2-dihydroindol-3-ylidenemethyl]-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-pyrrolidin-1-ylethyl)amide

6-(5-Isopropyl-2-methoxyphenyl)-1,3-dihydroindol-2-one (50 mg, 0.17 mmol) was condensed with 5-formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-pyrrolidin-1-yl-ethyl)amide (45 mg) to give 67 mg (75%) of the title compound as an orange solid.

^{1}HNMR (360 MHz, DMSO-d6) δ 13.60 (s, 1H, NH), 10.82 (s, br, 1H, NH), 7.77 (d, J = 7.9 Hz, 1H), 7.61 (s, 1H, H-vinyl), 7.45 (m, 1H, CONH), 7.0-7.19 (m, 5H), 3.73 (s, 3H, OCH_3), 3.32 (m, 2H, CH_2), 2.87 (m, 1H, $CH(CH_3)_2$), 2.56 (m, 2H, CH_2), 2.48 (m, 4H,

2xCH_2), 2.43 (s, 3H, CH_3), 2.40 (s, 3H, CH_3), 1.68 (m, 4H, 2xCH_2), 1.21 (d, J = 6.8 Hz, 6H, $CH(CH_3)_2$).

MS m/z 527.2 [M^++1].

Example 77

5-[6-(4-Ethylphenyl)-2-oxo-1,2-dihydroindol-3-ylidenemethyl]-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-pyrrolidin-1-ylethyl)amide

6-(4-Ethylphenyl)-1,3-dihydroindol-2-one (45 mg, 0.19 mmol) was condensed 5-formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-pyrrolidin-1-ylethyl)amide (50 mg) to give 60 mg (65%) of the title compound as a yellow-orange solid.

^{1}HNMR (300 MHz, DMSO-d6) δ 13.59 (s, 1H, NH), 10.96 (s, br, 1H, NH), 7.83 (d, J = 8.4 Hz, 1H), 7.64 (s, 1H, H-vinyl), 7.51-7.56 (m, 3H), 7.25-7.30 (m, 3H), 7.08 (d, J = 1 Hz, 1H), 3.31 (m, 2H, CH_2), 2.63 (m, 2H, CH_2CH_3), 2.55 (m, 2H, CH_2), 2.49 (m, 4H, 2xCH_2), 2.42 (s, 3H, CH_3), 2.40 (s, 3H, CH_3), 1.67 (m, 4H, 2xCH_2), 1.20 (t, J= 7.5 Hz, 3H, CH_2CH_3).

MS-EI m/z 482 [M^+].

Example 78

5-[6-(2,4-Dimethoxyphenyl)-2-oxo-1,2-dihydroindol-3-ylidenemethyl]-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-pyrrolidin-1-ylethyl)amide

6-(2,4-Dimethoxyphenyl)-1,3-dihydroindol-2-one (51 mg, 0.19 mmol) was condensed with 5-formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-pyrrolidin-1-ylethyl)amide (50 mg) to give 30 mg (31%) of the title compound as an orange solid.

^{1}HNMR (300 MHz, DMSO-d6) δ 13.59 (s, 1H, NH), 10.86 (s, br, 1H, NH), 7.75 (d, J = 7.8 Hz, 1H), 7.60 (s, 1H, H-vinyl), 749 (m, 1H, CONH), 7.22 (d, J = 8.4 Hz, 1H), 7.03 (m, 1H), 6.97 (s, 1H), 6.58-6.65 (m, 2H), 3.79 (s, 3H, OCH_3), 3.76 (s, 3H, OCH_3), 3.33 (m, 2H, CH_2), 2.55 (m, 2H, CH_2), 2.50 (m, 4H, 2xCH_2), 2.42 (s, 3H, CH_3), 2.39 (s, 3H, CH_3), 1.67 (m, 4H, 2xCH_2).

MS-EI *m/z* 514 [M^+].

Example 79

5-[6-(3-Isopropylphenyl)-2-oxo-1,2-dihydroindol-3-ylidenemethyl]-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-pyrrolidin-1-ylethyl)amide

6-(3-Isopropylphenyl)-1,3-dihydroindol-2-one (48 mg, 0.19 mmol) was condensed with 5-formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-pyrrolidin-1-ylethyl)amide (50 mg) to give 59 mg (63%) of the title compound as an orange solid.

^{1}HNMR (300 MHz, DMSO-d6) δ 13.63 (s, 1H, N*H*), 10.97 (s, br, 1H, N*H*), 7.87 (d, *J* = 7.8 Hz, 1H), 7.68 (s, 1H, H-vinyl), 7.24-7.55 (m, 6H), 7.13 (s, 1H), 3.34 (m, 2H, CH_2), 3.30 (m, 1H, CH(CH_3)$_2$), 2.60 (m, 2H, CH_2), 2.50 (m, 4H, 2xCH_2), 2.45 (s, 3H, CH_3), 2.43 (s, 3H, CH_3), 1.70 (m, 4H, 2xCH_2), 1.27 (d, *J* = 6.9 Hz, 6H, CH(CH_3)$_2$).

MS-EI *m/z* 496 [M^+].

Example 80

5-(5-Fluoro-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-diethylamino-ethyl)amide

5-Fluoro-1,3-dihydroindol-2-one (0.54 g, 3.8 mmol) was condensed with 5-formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-diethylaminoethyl)amide to give 0.83 g (55%) of the title compound as a yellow green solid.

^{1}HNMR (360 MHz, DMSO-d6) δ 13.66 (s, 1H, N*H*), 10.83 (s, br, 1H, N*H*), 7.73 (dd, *J* = 2.5 & 9.4 Hz, 1H), 7.69 (s, 1H, H-vinyl), 7.37 (t, 1H, CON*H*CH_2CH_2), 6.91 (m, 1H), 6.81-6.85 (m, 1H), 3.27 (m, 2H, CH_2), 2.51 (m, 6H, 3xCH_2), 2.43 (s, 3H, CH_3), 2.41 (s, 3H, CH_3), 0.96 (t, *J* = 6.9 Hz, 6H, N(CH_2CH_3)$_2$).

MS-EI *m/z* 398 [M^+].

Example 81

3-[4-(2-Diethylaminoethylcarbamoyl)-3,5-dimethyl-1*H*-pyrrol-2-

ylmethylene]-2-oxo-2,3-dihydro-1*H*-indole-6-carboxylic acid

2-Oxo-2,3-dihydro-1*H*-indole-6-carboxylic acid (80 mg, 0.45 mmol) was condensed with 5-formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-diethylaminoethyl)amide to give 210 mg (92%) of the title compound as a yellow orange solid.

^{1}HNMR (360 MHz, DMSO-d6) δ 13.6 (s, 1H, N*H*), 7.76 (d, *J* = 8.0 Hz, 1H), 7.66 (s, 1H, H-vinyl), 7.57 (dd, *J* = 1.5 & 8.0 Hz, 1H), 7.40-7.42 (m, 2H), 3.28 (m, 2H, C*H*$_2$), 2.88 (m, H-piperidine), 2.54 (m, 6H, 3xCH$_2$), 2.44 (s, 3H, C*H*$_3$), 2.40 (s, 3H, C*H*$_3$), 1.56 (m, H-piperidine), 0.97 (t, *J* = 6.98 Hz, 6H, N(CH$_2$C*H*$_3$)$_2$).

MS *m/z* 424 [M$^+$].

Example 82

5-(5-Dimethylsulfamoyl-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-pyrrolidin-1-ylethyl)amide

2-Oxo-2,3-dihydro-1*H*-indole-5-sulfonic acid dimethylamide (90 mg, 0.38 mmol) was condensed with 5-formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-pyrrolidin-1-ylethyl)amide (100 mg) to give 100 mg (54%) of the title compound as a yellow solid.

^{1}HNMR (360 MHz, DMSO-d6) δ 13.65 (s, 1H, N*H*), 11.30 (s, br, 1H, N*H*), 8.25 (d, 1H), 7.92 (s, 1H, H-vinyl), 7.48-7.53 (m, 2H), 7.07 (d, *J* = 8.2 Hz, 1H), 3.33 (m, 2H, C*H*$_2$), 2.61 (s, 6H, N(CH$_3$)$_2$), 2.56 (t, 2H, C*H*$_2$), 2.49 (m, 4H, 2xCH$_2$), 2.45 (s, 3H, C*H*$_3$), 2.44 (s, 3H, C*H*$_3$), 1.67 (m, 4H, 2xC*H*$_2$).

MS-EI *m/z* 485 [M$^+$].

Example 83

5-[5-(3-Chlorophenylsulfamoyl)-2-oxo-1,2-dihydroindol-3-ylidenemethyl]-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-pyrrolidin-1-ylethyl)amide

2-Oxo-2,3-dihydro-1*H*-indole-5-sulfonic acid (3-chlorophenyl)amide (120 mg, 0.38 mmol) was condensed with 5-formyl-2,4-

dimethyl-1*H*-pyrrole-3-carboxylic acid (2-pyrrolidin-1-ylethyl)amide (100 mg) to give 150 mg (69%) of the title compound as a yellow orange solid.

^{1}HNMR (360 MHz, DMSO-d6) δ 13.55 (s, 1H, N*H*), 11.26 (br s, 1H, N*H*), 10.30 (br s,1H, N*H*), 8.26 (d, 1H), 7.79 (s, 1H, H-vinyl), 7.51-7.57 (m, 2H), 7.22 (t, *J* = 8.1 Hz, 1H), 7.15 (m, 1H), 7.07 (m, 1H), 7.0 (m, 2H), 3.44 (m, 2H, C*H*$_2$), 2.57 (t, *J* = 7.0 Hz, 2H, C*H*$_2$), 2.49 (m, 4H, 2xCH$_2$), 2.44 (s, 3H, C*H*$_3$), 2.43 (s, 3H, C*H*$_3$), 1.68 (m, 4H, 2xC*H*$_2$).

MS *m/z* 568 [M$^+$].

Example 84

2,4-Dimethyl-5-[2-oxo-5-(pyridin-3-ylsulfamoyl)-1,2-dihydroindol-3-ylidenemethyl]-1*H*-pyrrole-3-carboxylic acid (2-pyrrolidin-1-ylethyl)amide

2-Oxo-2,3-dihydro-1*H*-indole-5-sulfonic acid pyridin-3-ylamide (110 mg, 0.38 mmol) was condensed with 5-formyl-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (2-pyrrolidin-1-ylethyl)amide (100 mg) to give 150 mg (74%) of the title compound as an orange solid.

^{1}HNMR (360 MHz, DMSO-d6) δ 13.58 (s, 1H, N*H*), 8.21 (d, *J* = 2.0 Hz, 2H), 8.04 (m, 1H), 7.76 (s, 1H, H-vinyl), 7.49-7.54 (m, 2H), 7.41 (m, 1H), 7.14 (m, 1H), 6.94 (d, *J* = 8.5 Hz, 1H), 3.33 (m, 2H, C*H*$_2$), 2.56 (t, *J* = 7.06 Hz, 2H, C*H*$_2$), 2.49 (m, 4H, 2xCH$_2$), 2.43 (s, 6H, 2xCH$_3$), 1.68 (m, 4H, 2xC*H*$_2$).

MS *m/z* 535 [M$^+$].

Example 85

3-[3,5-Dimethyl-4-(4-methylpiperazine-1-carbonyl)-1*H*-pyrrol-2-ylmethylene]-4-(2-hydroxyethyl)-1,3-dihydroindol-2-one

4-(2-Hydroxyethyl)-1,3-dihydroindol-2-one (71 mg, 0.4 mmol) was condensed with 3,5-dimethyl-4-(4-methyl-piperazine-1-carbonyl)-1*H*-pyrrole-2-carbaldehyde to give 90 mg (55%) of the title compound as an orange solid.

^{1}HNMR (300 MHz, DMSO-d6) δ 14.25(s, 1H, NH), 10.88 (s, 1H, NH), 7.57 (s, 1H, H-vinyl), 7.03 (m, 1H), 6.75-6.82 (m, 2H), 4.86 (m, 1H, OH), 3.70 (m, 2H, CH_2), 3.04 (m, 2H, CH_2), 2.48 (m, 4H, 2xCH_2), 2.28 (br s, 7H), 2.19 (s, 3H, CH_3), 2.18 (s, 3H, CH_3).

MS *m/z* (+ve) 4.09.3 [M^+].

Example 86

3-[3,5-Dimethyl-4-(4-methylpiperazine-1-carbonyl)-1*H*-pyrrol-2-ylmethylene]-2-oxo-2,3-dihydro-1*H*-indole-5-sulfonic acid phenylamide

2-Oxo-2,3-dihydro-1*H*-indole-5-sulfonic acid phenylamide (110 mg, 0.4 mmol) was condensed with 3,5-dimethyl-4-(4-methylpiperazine-1-carbonyl)-1*H*-pyrrole-2-carbaldehyde (100 mg) to give 50 mg (24%) of the title compound as a yellow solid.

^{1}HNMR (300 MHz, DMSO-d6) δ 13.52(s, 1H, NH), 11.26 (s, 1H, NH), 10.08 (s, 1H, NH), 8.21 (d, *J* = 1.6 Hz, 1H), 7.75 (s, 1H, H-vinyl), 7.50 (dd, *J* = 1.6 & 8.3 Hz, 1H), 7.19 (m, 2H), 7.10 (m, 2H), 6.97 (m, 2H), 2.49 (m, 4H, 2xCH_2), 2.28 (m, 10H, 2xCH_3 & 2xCH_2), 2.18 (s, 3H, CH_3).

MS-EI *m/z* 519 [M^+].

Example 87

5-(5-Dimethylsulfamoyl-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-diethylaminoethyl)amide

2-Oxo-2,3-dihydro-1*H*-indole-5-sulfonic acid dimethylamide (90 mg, 0.38 mmol) was condensed with 5-formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-diethylaminoethyl)amide (100 mg) to give 80 mg (43%) of the title compound as a yellow solid.

^{1}HNMR (300 MHz, DMSO-d6) δ 11.30 (s, 1H, NH), 8.27 (d, *J* = 1.7 Hz, 1H), 7.94 (s, 1H, H-vinyl), 7.49 (dd, *J* = 1.7 & 8.0 Hz, 1H), 7.44 (m, 1H, CONHCH_2CH_2), 7.07 (d, *J* = 8.0 Hz, 1H), 3.26 (m, 2H, CH_2), 2.60 (s, 6H, N(CH_3)$_2$), 2.53 (m, 2H, CH_2), 2.45-2.50 (m, 10H, 2xCH_3 & N(CH_2CH_3)$_2$, 0.96 (t, *J* = 7.2 Hz, 6H, N(CH_2CH_3)$_2$).

MS-EI *m/z* 487 [M$^+$].

Example 88

5-[5-(3-Chlorophenylsulfamoyl)-2-oxo-1,2-dihydroindol-3-ylidenemethyl]-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-diethylaminoethyl)amide

2-Oxo-2,3-dihydro-1*H*-indole-5-sulfonic acid (3-chlorophenyl)amide (120 mg, 3.8 mmol) was condensed with 5-formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-diethylaminoethyl)amide (100 mg) to give 80 mg (37%) of the title compound as a yellow solid.

^{1}HNMR (360 MHz, DMSO-d6) δ 13.55 (s, 1H, NH), 11.24 (s, 1H, NH), 10.29 (s, 1H, NH), 8.25 (d, *J* = 1.87 Hz, 1H), 7.79 (s, 1H, H-vinyl), 7.52 (dd, *J* = 1.87 & 8.3 Hz, 1H), 7.42 (m, 1H, CONHCH$_2$CH$_2$), 7.22 (t, *J* = 8.02 Hz, 1H), 7.15 (t, *J* = 2 Hz, 1H), 7.08 (m, 1H), 7.0 (m, 2H), 3.27 (m, 2H, CH$_2$), 2.48-2.57 (m, 6H, 3xCH$_2$), 2.45 (s, 3H, CH$_3$), 2.44 (s, 3H, CH$_3$), 0.97 (t, *J* = 7.0 Hz, 6H, N(CH$_2$CH$_3$)$_2$).

MS *m/z* 570.1 [M$^+$].

Example 95

3-(2-Oxo-5-phenyl-1,2-dihydroindol-3-ylidenemethyl)-4,5,6,7-tetrahydro-2*H*-isoindole-1-carboxylic acid ethyl ester

^{1}HNMR (360 MHz, DMSO-d6) δ 13.74 (s, 1H, NH), 11.00 (s, 1H, NH), 8.13 (d, *J* = 1.7 Hz, 1H), 7.74 (s, 1H, H-vinyl), 7.70 (d, *J* = 7.7 Hz, 2H), 7.49 (dd, *J* = 1.7 & 8.0 Hz, 1H), 7.44 (t, *J* = 7.7 Hz, 2H), 7.32 (m, 1H), 6.96 (d, *J* = 8.0 Hz, 1H), 4.26 (q, *J* = 7.0 Hz, 2H, OCH$_2$CH$_3$), 2.79 (m, 2H, CH$_2$), 2.72 (m, 2H, CH$_2$), 1.73 (m, 4H, 2xCH$_2$), 1.30 (t, *J* = 7.0 Hz, 3H, OCH$_2$CH$_3$).

MS-EI *m/z* 412 [M$^+$].

Example 99

3-(2-Oxo-5-phenylsulfamoyl-1,2-dihydroindol-3-ylidenemethyl)-4,5,6,7-tetrahydro-2*H*-isoindole-1-carboxylic acid ethyl ester

^{1}HNMR (360 MHz, DMSO-d6) δ 13.64 (s, 1H, NH), 11.33 (s, 1H, NH), 10.07 (s, 1H, NH), 8.24 (d, *J* = 1.8 Hz, 1H), 7.74 (s, 1H, H-

vinyl), 7.57 (dd, J = 1.8 & 8.0 Hz, 1H), 7.21 (t, J = 7.6 Hz, 2H), 7.11 (d, J = 7.6 Hz, 2H), 6.99 (d, J = 8.0 Hz, 1H), 6.98 (d, J = 7.6 Hz, 1H), 4.27 (q, J = 7.0 Hz, 2H, OCH_2CH_3), 2.80 (m, 2H, CH_2), 2.73 (m, 2H, CH_2), 1.73 (m, 4H, 2xCH_2), 1.30 (t, J = 7.0 Hz, 3H, OCH_2CH_3).

MS-EI m/z 491 [M^+].

Example 109

3-[3-(Morpholine-4-carbonyl)-4,5,6,7-tetrahydro-2*H*-isoindol-1-ylmethylene]-2-oxo-2,3-dihydro-1*H*-indole-6-carboxylic acid

^{1}HNMR (360 MHz, DMSO-d6) δ 13.60 (s, 1H, NH), 12.75 (br s, 1H, COOH), 11.08 (s, 1H, NH), 7.85 (d, J = 7.8 Hz, 1H), 7.71 (s, 1H, H-vinyl), 7.62 (dd, J = 1.4 & 7.8 Hz, 1H), 7.41 (d, J = 1.4 Hz, 1H), 3.65 (m, 4H, 2xCH_2), 3.55 (m, 4H, 2xCH_2), 2.81 (m, 2H, CH_2), 2.54 (m, 2H, CH_2).1.73 (m, 4H, 2xCH_2).

MS-EI m/z 421 [M^+].

Example 112

5-Bromo-3-[3-(pyrrolidine-1-carbonyl)-4,5,6,7-tetrahydro-2H-isoindol-1-ylmethylene]-1,3-dihydro-indol-2-one

^{1}HNMR (360 MHz, DMSO-d6) δ 13.56 (s, 1H, NH), 11.00 (s, 1H, NH), 8.05 (d, J = 1.8 Hz, 1H), 7.74 (s, 1H, H-vinyl), 7.28 (dd, J = 1.3 & 8.3 Hz, 1H), 6.83 (d, J = 8.3 Hz, 1H), 3.57 (m, 4H, 2xCH_2), 2.79 (m, 2H, CH_2), 2.65 (m, 2H, CH_2), 1.88 (m, 4H, 2xCH_2), 1.71 (m, 4H, 2xCH_2).

MS-EI m/z 439 & 441 [M^+-1] & [M^++1].

Example 114

3-(3-Dimethylcarbamoyl-4,5,6,7-tetrahydro-2*H*-isoindol-1-ylmethylene)-2-oxo-2,3-dihydro-1*H*-indole-6-carboxylic acid

^{1}HNMR (360 MHz, DMSO-d6) δ 13.60 (s, 1H, NH), 12.72 (br s, 1H, COOH), 11.05 (s, 1H, NH), 7.85 (d, J = 7.9 Hz, 1H), 7.72 (s, 1H, H-vinyl), 7.62 (dd, J = 1.3 & 7.9 Hz, 1H), 7.42 (d, J = 1.3 Hz, 1H), 3.03 (s, 6H, $N(CH_3)_2$), 2.81 (m, 2H, CH_2), 2.55 (m, 2H, CH_2), 1.73 (m, 4H, 2xCH_2).

MS-EI m/z 379 [M^+].

Example 115
4-Methyl-5-(5-methylsulfamoyl-2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-1H-pyrrole-3-carboxylic acid

^{1}HNMR (300 MHz, DMSO-d6) 13.56 (br s, 1H, NH), 8.24 (d, *J* = 1.5 Hz, 1H), 7.86 (s, 1H, H-vinyl), 7.74 (d, *J* = 2.96 Hz, 1H), 7.56 (dd, *J* = 1.5 & 8.1 Hz, 1H), 7.20 (br m, 1H, $NHCH_3$), 7.03 (d, *J* = 8.1 Hz, 1H), 2.57 (s, 3H, CH_3), 2.41 (s, 3H, CH_3).

MS-EI *m/z* 361 [M^+].

Example 116
{[4-Methyl-5-(4-methyl-5-methylsulfamoyl-2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-1H-pyrrole-3-carbonyl]-amino}-acetic acid ethyl ester

4-Methyl-1*H*-pyrrole-3-carboxylic acid ethyl ester (lit. ref. D.O. Cheng, T. L. Bowman and E. LeGoff; J. Heterocyclic Chem.; 1976; 13; 1145-1147) was formylated using method A, hydrolysed using method B followed by amidation (method C) to give [(5-formyl-4-methyl-1*H*-pyrrole-3-carbonyl)-amino]-acetic acid ethyl ester.

4-Methyl-5-methylaminosulfonyl-2-oxindole (50 mg, 0.21 mmol) was condensed with [(5-formyl-4-methyl-1*H*-pyrrole-3-carbonyl)-amino]-acetic acid ethyl ester (100 mg, 0.42 mmol) and piperidine (0.1 mL) in ethanol (2 mL) to give 50 mg (52%) of the title compound.

^{1}HNMR (360 MHz, DMSO-d6) δ 13.59 (s, 1H, NH), 11.29 (v.br. s, 1H, N*H*-CO), 8.33 (t, *J* = 5.8 Hz, 1H, CO$NHCH_2$), 7.83 (d, *J* = 3.11 Hz, 1H), 7.80 (s, 1H, H-vinyl), 7.71 (d, *J* = 8.5 Hz, 1H), 7.34 (br m, 1H, $NHCH_3$), 6.89 (d, *J* = 8.5 Hz, 1H), 4.11 (q, *J* = 7.1 Hz, 2H, OCH_2CH_3), 3.92 (d, *J* = 5.8 Hz, 2H, GlyCH_2), 2.86 (s, 3H, CH_3), 2.48 (s, 3H, CH_3), 2.42 (d, *J* = 4.71 Hz, 3H, $HNCH_3$), 1.20 (t, *J* = 7.1 Hz, 3H, OCH_2CH_3).

MS-EI *m/z* 460 [M^+].

Example 117
{[4-Methyl-5-(5-methylsulfamoyl-2-oxo-1,2-dihydro-indol-3-

ylidenemethyl)-1H-pyrrole-3-carbonyl]-amino}-acetic acid ethyl ester

A mixture of 5-methylaminosulfonyl-2-oxindole (0.06 g, 0.22 mmol), [(5-formyl-4-methyl-1*H*-pyrrole-3-carbonyl)-amino]-acetic acid ethyl ester (0.075 g, 0.27 mmol) and piperidine (2 drops) in ethanol (5 mL) was heated in a sealed tube at 90° C for 12 hrs. After cooling, the precipitate was collected by vacuum filtration, washed with ethanol, triturated with dichloromethane/ether and dried to give 0.035 g (36%) of the title compound as a yellowish brown solid.

^{1}H NMR (360 MHz, DMSO-d6) δ 13.6 (s, 1H, NH), 11 (v.br. s, 1H, NH-CO), 8.30 (t, *J* = 5.7 Hz, 1H, CONHCH$_2$), 8.25 (d, *J* = 1.2 Hz, 1H), 7.88 (s, 1H, H-vinyl), 7.84 (d, *J* = 3.3 Hz, 1H), 7.57 (dd, *J* = 1.9 & 8.5 Hz, 1H), 7.14 (br m, 1H, NHCH$_3$), 7.04 (d, *J* = 8.5 Hz, 1H), 4.11 (q, *J* = 6.7 Hz, 2H, OCH$_2$CH$_3$), 3.92 (d, *J* = 5.7 Hz, 2H, GlyCH$_2$), 2.55 (s, 3H, CH$_3$), 2.41 (m, 3H, NCH$_3$), 1.20 (t, *J* = 6.7 Hz, 3H, OCH$_2$CH$_3$).

MS *m/z* 446 [M$^+$].

Example 118

{[4-Methyl-5-(5-methylsulfamoyl-2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-1H-pyrrole-3-carbonyl]-amino}-acetic acid

A mixture of [(5-formyl-4-methyl-1*H*-pyrrole-3-carbonyl)-amino]-acetic acid ethyl ester (0.142 g, 0.59 mmol) and 1N NaOH (1.2 mL) in methanol (10 mL) was stirred at room temperature for 1 hr. The reaction was concentrated and the residue was condensed with 5-methylaminosulfonyl-2-oxindole (0.13 g, 0.48 mmol) and piperidine (0.12 mL) in ethanol (12 mL) to give 0.11 g (52%) of the title compound.

^{1}HNMR (300 MHz, DMSO-d6) δ 13.98 (br s, 1H, NH), 8.17 (s, 1H), 7.80 (s, 1H), 7.75 (d, *J* = 3.1Hz, 1H), 7.51 (dd, *J* = 2 & 8.2 Hz, 1H), 7.21 (m on br s, 2H), 6.97 (d, *J* = 8.1 Hz, 1H), 3.41 (d, *J* = 4.2 Hz, 2H, CH$_2$NH), 2.54 (s, 3H, pyrrole-CH$_3$), 2.39 (s, 3H, ArCH$_3$).

MS *m/z* 417 $[M-1]^+$.

Example 120

5-Methyl-2-(2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-1H-pyrrole-3-carboxylic acid

^{1}HNMR (300 MHz, DMSO-d6) δ 13.77 (br s, 1H, NH), 12.49 (s, 1H, COOH), 11.07 (s, 1H, NH), 8.39 (s, 1H, H-vinyl), 7.43 (d, *J* = 7.47 Hz, 1H), 7.20 (t, *J* = 7.47 Hz, 1H), 7.03 (t, *J* = 7.47 Hz, 1H), 6.91 (d, *J* = 7.47 Hz, 1H), 6.49 (d, *J* = 1.53 Hz, 1H), 2.34 (s, 3H, CH_3).

MS *m/z* 269 $[M+H]^+$.

Example 121

5-Methyl-2-(2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-1H-pyrrole-3-carboxylic acid ethyl ester

^{1}HNMR (300 MHz, DMSO-d6) δ 13.79 (s, 1H, NH), 11.08 (s, 1H, NH), 8.31 (s, 1H, H-vinyl), 7.45 (d, *J* = 7.52 Hz, 1H), 7.20 (t, *J* = 7.52 Hz, 1H), 7.03 (t, *J* = 7.52 Hz, 1H), 6.91 (d, *J* = 7.52 Hz, 1H), 6.50 (d, *J* = 2.1 Hz, 1H), 4.26 (q, *J* = 7.2 Hz, 2H, OCH_2CH_3), 2.33 (s, 3H, CH_3), 1.32 (t, *J* = 7.2 Hz, 3H, OCH_2CH_3).

MS *m/z* 297.1 $[M+H]^+$.

Example 122

2-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-5-methyl-1*H*-pyrrole-3-carboxylic acid ethyl ester

^{1}HNMR (360 MHz, DMSO-d6) δ 13.72(s, 1H, NH), 11.16 (s, 1H, NH), 8.29 (s, 1H, H-vinyl), 7.53 (d, *J* = 2.0 Hz, 1H), 7.35 (dd, *J* = 2.0 & 8.05 Hz, 1H), 6.87 (t, *J* = 8.05 Hz, 1H), 6.53 (d, *J* = 2.4 Hz, 1H), 4.28 (q, *J* = 7.03 Hz, 2H, OCH_2CH_3), 2.35 (s, 3H, CH_3), 1.33 (t, *J* = 7.03 Hz, 3H, OCH_2CH_3).

MS *m/z* 375 & 377 $[M+H]^+$.

Example 123

2-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-5-methyl-1*H*-pyrrole-3-carboxylic acid

^{1}HNMR (300 MHz, DMSO-d6) δ 13.72(s, 1H, NH), 12.57 (s, 1H, COOH), 11.19 (s, 1H, NH), 8.36 (s, 1H, H-vinyl), 7.51 (d, *J* = 1.4

Hz, 1H), 7.34 (dd, *J* = 1.4 & 8.17 Hz, 1H), 6.87 (t, *J* = 8.17 Hz, 1H), 6.52 (d, *J* = 2.5 Hz, 1H), 2.35 (s, 3H, CH_3).

MS *m/z* 347 & 349 $[M+H]^+$.

Example 124

2-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-5-methyl-1*H*-pyrrole-3-carboxylic acid (2-pyrrolidin-1-ylethyl)-amide

To a solution of 2-formyl-5-methyl-1*H*-pyrrole-3-carboxylic acid (250 mg, 1.63 mmol) in dimethylformamide (3 mL) was added 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (376 mg, 1.2 equiv.), 1-hydroxybenzotriazole (265 mg, 1.2 equiv.), triethylamine (0.45 mL, 2 equiv.) and 1-(2-aminoethyl)pyrrolidine (0.23 mL. 1.1 equiv.). After stirring at room temperature overnight, the reaction was diluted with saturated sodium bicarbonate and brine (with extra salt) and extracted with 10% methanol in dichloromethane. The combined organic layers were washed with brine, dried over anhydrous sodium sulfate and concentrated to give 130 mg of 2-formyl-5-methyl-1*H*-pyrrole-3-carboxylic acid (2-pyrrolidin-1-yl-ethyl)-amide.

A mixture of 5-bromo-2-oxindole (106 mg, 0.5 mmol), 2-formyl-5-methyl-1*H*-pyrrole-3-carboxylic acid (2-pyrrolidin-1-yl-ethyl)-amide (125 mg, 1 equiv.) and piperidine (0.2 mL) in ethanol (2 mL) was heated in a sealed tube at 80°C for 1 hr and then cooled. The precipitate which formed was collected by vacuum filtration, washed with ethanol and ethyl acetate and dried to give the title compound as an orange solid.

^{1}HNMR (300 MHz, DMSO-d6) δ 13.62 (s, 1H, N*H*), 11.06 (br s, 1H, N*H*), 8.56 (s, 1H, H-vinyl), 8.15 (m, 1H, CON*H*CH_2), 7.48 (d, *J* = 1.8 Hz, 1H), 7.31 (dd, *J* = 1.8 & 7.9 Hz, 1H), 6.86 (d, *J* = 7.9 Hz, 1H), 6.60 (d, *J* = 2.3 Hz, 1H), 3.35 (m, 2H, HN$CH_2$$CH_2$), 2.56 (t, *J* = 6.91 Hz, 2H, HN$CH_2$$CH_2$), 2.35 (s, 3H, CH_3), 1.67 (m, 4H, 2xCH_2).

MS *m/z* 443/ 445 [M^+ and M^++2].

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Example 125

2-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-5-methyl-1*H*-pyrrole-3-carboxylic acid (2-diethylaminoethyl)-amide

To a solution of 2-formyl-5-methyl-1*H*-pyrrole-3-carboxylic acid (320 mg, 2.1 mmol) in dimethylformamide (3 mL) was added 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (483 mg, 1.2 equiv.), 1-hydroxybenzotriazole (340 mg, 1.2 equiv.), triethylamine (0.59 mL, 2 equiv.) and N,N-diethylethylenediamine (0.32 mL, 1.1 equiv.). After stirring at room temperature overnight, the reaction was diluted with saturated sodium bicarbonate and brine (with extra salt) and extracted with 10% methanol in dichloromethane. The combined organic layers were washed with brine, dried over anhydrous sodium sulfate and concentrated to give 2-formyl-5-methyl-1*H*-pyrrole-3-carboxylic acid (2-diethylaminoethyl)-amide.

A mixture of 5-bromo-2-oxindole (106 mg, 0.5 mmol), 2-formyl-5-methyl-1*H*-pyrrole-3-carboxylic acid (2-diethylamino-ethyl)-amide (126 mg, 1 equiv.) and piperidine (0.2 mL) in ethanol (2 mL) was heated in a sealed tube at 80°C for 1 hr and then cooled. The precipitate was collected by vacuum filtration, washed with ethanol and ethyl acetate and dried to give the title compound as an orange solid.

^{1}HNMR (360 MHz, DMSO-d6) δ 13.62 (s, 1H, N*H*), 11.11 (br s, 1H, N*H*), 8.54 (s, 1H, H-vinyl), 8.1 (m, 1H, CONHCH$_2$), 7.49 (d, *J* = 2.2 Hz, 1H), 7.31 (dd, *J* = 2.2 & 8.3 Hz, 1H), 6.86 (d, *J* = 8.3 Hz, 1H), 6.58 (d, *J* = 2.24 Hz, 1H), 3.31 (m, 2H, HNC*H*$_2$CH$_2$), 2.59 (m, 6H, 3xC*H*$_2$), 2.36 (s, 3H, C*H*$_3$), 0.99 (t, *J* = 6.8 Hz, 6H, N(CH$_2$C*H*$_3$)$_2$).

MS *m/z* 445/ 447 [M$^+$ and M$^+$+2].

Example 126

2,4-Dimethyl-5-(2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-1H-pyrrole-3-carboxylic acid (2-diethylamino-ethyl)-amide

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A mixture of 1,3-dihydro-indol-2-one (266 mg, 2 mmol), 5-formyl-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (2-diethylamino-ethyl)-amide (530 mg, 2 mmol) and piperidine (1 drop) in ethanol was heated at 90°C for 2 hours. The reaction was cooled to room temperature, the resulting precipitate was collected by vacuum filtration, washed with ethanol and dried to give 422 mg (55%) of the title compound as a light yellow solid.

^{1}H NMR (400 MHz, DMSO-d6) δ 13.7 (s, 1H, NH), 10.9 (s, 1H, NH), 7.88 (d, *J* = 7.6 Hz, 1H), 7.64 (s, 1H, H-vinyl), 7.41 (t, *J* = 5.4 Hz, 1H, NH), 7.13 (dt, *J* = 1.2 & 7.6 Hz, 1H), 6.99 (dt, *J* = 1.2 & 7.6 Hz, 1H), 6.88 (d, *J* = 7.6 Hz, 1H), 3.28 (m, 2H), 2.48-2.55 (m, 6H), 2.44 (s, 3H, CH_3), 2.41 (s, 3H, CH_3), 0.97 (t, *J* = 7.2 Hz, 6H, $N(CH_2CH_3)_2$).
MS + ve APCI 381 [M^+ + 1].

Example 127

5-(5-Chloro-2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (2-diethylamino-ethyl)-amide

A mixture of 5-Chloro-1,3-dihydro-indol-2-one (335 mg, 2 mmol), 5-formyl-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (2-diethylamino-ethyl)-amide (530 mg, 2 mmol) and piperidine (1 drop) in ethanol was heated at 90°C for 2 hours. The reaction was cooled to room temperature, the resulting precipitate was collected by vacuum filtration, washed with ethanol and dried to give 565 mg (68%) of the title compound as an orange solid.

^{1}H NMR (400 MHz, DMSO-d6) δ 13.65 (s, 1H, NH), 11.0 (s, 1H, NH), 7.98 (d, *J* = 2.1 Hz, 1H) 7.77 (s, 1H H-vinyl), 7.44 (t, NH), 7.13 (dd, *J* = 2,1 & 8.4 Hz, 1H) 6.87 (d, *J* = 8.4 Hz, 1H), 3.28 (g, 2H), 2.48-2.53 (m, 6H), 2.44 (s, 3H, CH_3), 2.43 (s, 3H, CH_3), 0.97 (t, *J* = 7.0 Hz, 6H, $N(CH_2CH_3)_2$)
MS + ve APCI 415 [M^+ + 1].

Example 128

2,4-Dimethyl-5-(2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-1H-pyrrole-3-carboxylic acid (2-pyrrolidin-1-ethyl)-amide

1,3-Dihydro-indol-2-one was condensed with 5-formyl-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (2-pyrrolidin-1-yl-ethyl)-amide to give the title compound.
MS + ve APCI 379 [M^+ + 1].

Example 129

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5-(5-Fluoro-2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (2-pyrrolidin-1-yl-ethyl)-amide

5-Fluoro-1,3-dihydro-indol-2-one was condensed with 5-formyl-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (2-pyrrolidin-1-yl-ethyl)-amide to give the title compound.
MS + ve APCI 397 [M^+ + 1].

Example 130

5-(5-Chloro-2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (2-pyrrolidin-1-yl-ethyl)-amide

5-Chloro-1,3-dihydro-indol-2-one was condensed with 5-formyl-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (2-pyrrolidin-1-yl-ethyl)-amide to give the title compound.
MS + ve APCI 413 [M^+ + 1].

Example 131

2,4-Dimethyl-5-(2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-1H-pyrrole-3-carboxylic acid (2-dimethylaminoethyl)-amide

1,3-Dihydro-indol-2-one was condensed with 5-formyl-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (2-dimethylaminoethyl)amide to give the title compound.

^{1}H NMR (400 MHz, DMSO-d6) δ 13.63 (s, 1H, NH), 10.90 (s, 1H, NH), 7.78 (d, J = 7.8 Hz, 1H), 7.63 (s, 1H H-vinyl), 7.48 (t, 1H, NH), 7.13 (dt, 1H), 6.98 (dt, 1H), 6.88 (d, J=7.7 Hz, 1H), 3.31 (q, J=6.6 Hz, 2H), 2.43 (s, 3H, CH_3), 2.40 (s, 3H, CH_3), 2.38 (t, J=6.6 Hz, 2H), 2.19 (s, 6H, $N(CH_2CH_3)_2$)
MS + ve APCI 353 [M^+ + 1].

Example 132

5-(5-Fluoro-2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (2-dimethylaminoethyl)-amide

5-Fluoro-1,3-dihydro-indol-2-one was condensed with 5-formyl-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (2-dimethylaminoethyl)amide to give the title compound.

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^{1}H NMR (400 MHz, DMSO-d6) δ 13.68 (s, 1H, NH), 10.90 (s, 1H, NH), 7.76 (dd, *J* = 2.4 & 9.4 Hz, 1H), 7.71 (s, 1H H-vinyl), 7.51 (t, 1H, NH), 6.93 (m, 1H), 6.84 (dd, J=4.6 & 8.4 Hz, 1H), 3.31 (q, J=6.6 Hz, 2H), 2.43 (s, 3H, CH_3), 2.41 (s, 3H, CH_3), 2.38 (t, *J*=6.6 Hz, 2H), 2.19 (s, 6H, $N(CH_2CH_3)_2$)
MS + ve APCI 371 [M^+ + 1].

5. BIOLOGICAL EVALUATION

It will be appreciated that, in any given series of compounds, a spectrum of biological activity will be afforded. In its presently preferred embodiments, this invention relates to novel pyrrole substituted 2-indolinones demonstrating the ability to modulate RTK, CTK, and STK activity. The following assays are employed to find those compounds demonstrating the optimal degree of the desired activity.

A. Assay Procedures.

The following assays may be used to determine the level of activity and effect of the different compounds of the present invention on one or more of the PKs. Similar assays can be designed along the same lines for any PK using techniques well known in the art.

Several of the assays described herein are performed in an ELISA (Enzyme-Linked Immunosorbent Sandwich Assay) format (Voller, et al., 1980, "Enzyme-Linked Immunosorbent Assay," Manual of Clinical Immunology, 2d ed., Rose and Friedman, Am. Soc. Of Microbiology, Washington, D.C., pp. 359-371). The general procedure is as follows: a compound is introduced to cells expressing the test kinase, either naturally or recombinantly, for a selected period of time after which, if the test kinase is a receptor, a ligand known to activate the receptor is added. The cells are lysed and the lysate is transferred to the wells of an ELISA plate previously coated with a specific antibody recognizing the substrate of the enzymatic phosphorylation reaction. Non-substrate components of the cell lysate are washed away and the amount of

phosphorylation on the substrate is detected with an antibody specifically recognizing phosphotyrosine compared with control cells that were not contacted with a test compound.

The presently preferred protocols for conducting the ELISA experiments for specific PKs is provided below. However, adaptation of these protocols for determining the activity of compounds against other RTKs, as well as for CTKs and STKs, is well within the scope of knowledge of those skilled in the art.

Other assays described herein measure the amount of DNA made in response to activation of a test kinase, which is a general measure of a proliferative response. The general procedure for this assay is as follows: a compound is introduced to cells expressing the test kinase, either naturally or recombinantly, for a selected period of time after which, if the test kinase is a receptor, a ligand known to activate the receptor is added. After incubation at least overnight, a DNA labeling reagent such as 5-bromodeoxyuridine (BrdU) or H^3-thymidine is added. The amount of labeled DNA is detected with either an anti-BrdU antibody or by measuring radioactivity and is compared to control cells not contacted with a test compound.

GST-FLK-1 BIOASSAY

This assay analyzes the tyrosine kinase activity of GST-Flk1 on poly(glu,tyr) peptides.

Materials and Reagents:

1. Corning 96-well ELISA plates (Corning Catalog No. 25805-96).
2. poly(glu,tyr) 4:1, lyophilizate (Sigma Catalog # P0275).
3. Preparation of poly(glu,tyr)(pEY) coated assay plates: Coat 2 ug/well of poly(glu,tyr)(pEY) in 100 ul PBS, hold at room temperature for 2 hours or at 4°C overnight. Cover

plates well to prevent evaporation.

4. PBS Buffer

To make 1 liter of a 1x working solution, mix .02 g KH_2PO_4, 1.15 g Na_2HPO_4, 0.2 g KCl and 8 g NaCl in approx. 900ml dH_2O. When all reagents have dissolved, adjust the pH to 7.2 with HCl. Bring total volume to 1 L with dH_2O.

5. PBST Buffer

To 1 L of PBS Buffer, add 1.0 ml Tween-20. Stir until dissolved.

6. TBB - Blocking Buffer

To make one liter of a 1x working solution, mix 1.21 g TRIS, 8.77 g NaCl, 1 ml TWEEN-20 in approximately 900 ml dH_2O. Adjust pH to 7.2 with HCl. Add 10 g BSA, stir to dissolve. Bring total volume to 1 L with dH_2O. Filter to remove particulate matter.

7. 1% BSA in PBS

To make a 1x working solution, add 10 g BSA to approx. 990 ml PBS buffer, stir to dissolve. Adjust total volume to 1 L with PBS buffer, filter tc remove particulate matter.

8. 50 mM Hepes pH 7.5.

9. GST-Plk1cd purified from sf9 recombinant baculovirus transformation (SUGEN, Inc.).

10. 4% DMSO in dH_2O.

11. 10 mM ATP in dH_2O.

12. 40 mM $MnCl_2$

13. Kinase Dilution Buffer (KDB)

Mix 10 ml Hepes (pH 7.5), 1 ml of 5M NaCl, 40 µL of 100 mM $NaVO_4$ and 0.4 ml of 5% BSA in dH_2O with 88.56 ml of dH_2O.

14. NUNC 96-well V bottom polypropylene plates, Applied Scientific Catalog # AS-72092

15. EDTA

Mix 14.12 g ethylenediaminetetraacetic acid (EDTA) to

approx. 70 ml dH_2O. Add 10 N NaOH until EDTA dissolves. Adjust pH to 8.0. Adjust total volume to 100 ml with dH_2O.

16. 1^0 Antibody Dilution Buffer.
    Mix 10 ml of 5% BSA in PBS buffer with 89.5 ml TBSTw.
17. Anti-phosphotyrosine monoclonal antibody conjugated to horseradish peroxidase (PY99 HRP, Santa Cruz Biotech).
18. 2,2'-Azinobis(3-ethylbenzthiazoline-6-sulfonic acid (ABTS, Moss, Cat. No. ABST).
19. 10% SDS.

Procedure:

1. Coat Corning 96-well ELISA plates with 2 µg of polyEY peptide in sterile PBS as described in step 3 of Materials and Reagents.
2. Remove unbound liquid from wells by inverting plate. Wash once with TBSTw. Pat the plate on a paper towel to remove excess liquid.
3. Add 100 µl of 1% BSA in PBS to each well. Incubate, with shaking, for 1 hr. at room temperature.
4. Repeat step 2.
5. Soak wells with 50 mM Hepes pH7.5 (150 µl/well).
6. Dilute test compound with dH_2O/4% DMSO to 4 times the desired final assay concentration in 96-well polypropylene plates.
7. Add 25 µl diluted test compound to ELISA plate. In control wells, place 25 µl of dH_2O/4% DMSO.
8. Add 25 µl of 40 mM $MnCl_2$ with 4x ATP (2 µM) to each well.
9. Add 25 µl 0.5M EDTA to negative control wells.
10. Dilute GST-Flk1 to 0.005 µg(5 ng)/well with KDB.
11. Add 50 µl of diluted enzyme to each well.
12. Incubate, with shaking, for 15 minutes at room

temperature.

13. Stop reaction by adding 50 µl of 250 mM EDTA (pH 8.0).
14. Wash 3X with TBST and pat plate on paper towel to remove excess liquid.
15. Add 100 µl per well anti-phosphotyrosine HRP conjugate, 1:5,000 dilution in antibody dilution buffer. Incubate, with shaking, for 90 min. at room temperature.
16. Wash as in step 14.
17. Add 100 µl of room temperature ABTS solution to each well.
18. Incubate, with shaking, for 10 to 15 minutes. Remove any bubbles.
19. Stop reaction by adding 20 µl of 10% SDS to each well.
20. Read results on Dynatech MR7000 ELISA reader with test filter at 410 nM and reference filter at 630 nM.

PYK2 BIOASSAY

This assay is used to measure the <u>in vitro</u> kinase activity of HA epitope-tagged full length pyk2 (FL.pyk2-HA) in an ELISA assay.

<u>Materials and reagents</u>:

1. Corning 96-well Elisa plates.
2. 12CA5 monoclonal anti-HA antibody (SUGEN, Inc.)
3. PBS (Dulbecco's Phosphate-Buffered Saline (Gibco Catalog # 450-1300EB)
4. TBST Buffer
   Mix 8.766 g NaCl, 6.057 g TRIS and 1 ml of 0.1% Triton X-100 in approx. 900 ml dH_2O. Adjust pH to 7.2, bring volume to 1 L.
5. Blocking Buffer
   Mix 100 g of 10% BSA, 12.1 g of 100 mM TRIS, 58.44 g of 1M NaCl and 10 mL of 1% TWEEN-20.

6. FL.pyk2-HA from sf9 cell lysates (SUGEN, Inc.).
7. 4% DMSO in MilliQue H_2O.
8. 10 mM ATP in dH_2O.
9. 1M $MnCl_2$.
10. 1M $MgCl_2$.
11. 1M Dithiothreitol (DTT).
12. 10X Kinase buffer phosphorylation mix
    Mix 5.0 ml 1M Hepes (pH 7.5), 0.2 ml 1M $MnCl_2$, 1.0 ml $MgCl_2$, 1.0 ml 10% Triton X-100 in 2.8 ml dH_2O. Just prior to use, add o.1 ml 1M DTT.
13. NUNC 96-well V bottom polypropylene plates.
14. EDTA
15. Biotin conjugated anti-phosphotyrosine mab (Upstate Biotechnology Inc., clone 4G10, cat.# 16-103, ser. # 14495).
16. Vectastain Elite ABC reagent (Avidin peroxidase conjugate, Vector Laboratories Cat. No. PK-6100).
17. ABTS Solution
    Mix 19.21 g citric acid and 35.49 g Na_2HPO_4 in approx. 900 ml dH_2O. Adjust pH to 4.0 with phosphoric acid. Add 5 or 10 g ABST. When all dissolved, filter.
18. Hydrogen peroxide 30% solution.
19. ABTS/H_2O_2
    Mix 15 ml ABTS solution with 3 ul 30% H_2O_2 5 min. before use.
20. 0.2 M HCl.

Procedure:

1. Coat Corning 96 well ELISA plates with 0.5 µg per well 12CA5 anti-HA antibody in 100 µl PBS. Store overnight at 4°C.
2. Remove unbound HA antibody from wells by inverting plate. Pat the plate on a paper towel to remove excess

liquid.

3. Add 150 µl Blocking Buffer to each well. Incubate, with shaking, for 1 hr at room temperature.
4. Wash plates with TBS-T.
5. Dilute lysate in PBS (1.5 µg lysate/100 µl PBS).
6. Add 100 µl of diluted lysate to each well. Shake at room temperature for 1 hr.
7. Wash as in step 4.
8. Add 50 µl of 2X kinase Buffer to ELISA plate containing captured pyk2-HA.
9. Add 25 µL of 40 µM test compound in 4% DMSO to each well. For control wells use 4% DMSO alone.
10. Add 25 µL of 0.5 M EDTA to negative control wells.
11. Add 25 µl of 20 µM ATP to all wells. Incubate, with shaking, for 10 minutes.
12. Stop reaction by adding 25 µl 500 mM EDTA (pH 8.0) to all wells.
13. Wash as in step 4.
14. Add 100 µl biotin conjugated anti-phosphotyrosine mab (1:5000 dilution in Blocking Buffer) to each well. Incubate, with shaking for 30 min. at room temperature.
15. Make up Vectastain ABC reagent. Allow 30 min. for complete coupling of the avidin with the biotinylated HRP. Add 1 drop (or 50 uL) reagent A to 15 ml Blocking Buffer. Mix by inverting tube several times. Add 1 drop (or 50 uL) reagent B and mix again. Allow ABC reagent to mix at room temperature while the biotin-4G10 anti-phosphotyrosine is incubating in the assay plate.
16. Wash as in step 4.
17. Add 100 µl per well of prepared Vectastain peroxidase conjugate. Incubate, with shaking, for 30 min. at room temperature.

18. Wash as in step 4, then was once with PBS.
19. Add 100 µl of ABTS/H_2O_2 solution to each well.
20. Incubate, with shaking, for 10 to 15 minutes. Remove any bubbles.
21. Stop reaction with the addition of 100 µl of 0.2M HCl per well.
22. Read assay on Dynatech MR7000 ELISA reader with test filter at 410 nM and reference filter at 630 nM.

FGFR1 BIOASSAY

This assay is used to measure the <u>in vitro</u> kinase activity of FGF1-R in an ELISA assay.

<u>Materials and Reagents</u>:

1. Costar 96-well Elisa plates (Corning Catalog # 3369).
2. Poly(Glu-Tyr) (Sigma Catalog # P0275).
3. PBS (Gibco Catalog # 450-1300EB)
4. 50 mM Hepes Buffer Solution.
5. Blocking Buffer (5% BSA/PBS).
6. Purified GST-FGFR1 (SUGEN, Inc.)
7. Kinase Dilution Buffer.
   Mix 500 µl 1M Hepes (GIBCO), 20 µl 5% BSA/PBS, 10 µl 100mM sodium orthovanadate and 50 µl 5M NaCl.
8. 10mM ATP
9. 1M $MnCl_2$
10. ATP/$MnCl_2$ phosphorylation mix
    Mix 20 µL ATP, 400 µL $MnCl_2$ and 9.56 ml dH_2O.
11. NUNC 96-well V bottom polypropylene plates (Applied Scientific Catalog # AS-72092).
12. 0.5M EDTA.
13. 0.05% TBST
    Add 500 µL TWEEN to 1 liter TBS.
14. Rabbit polyclonal anti-phosphotyrosine serum (SUGEN,

Inc.).

15. Goat anti-rabbit IgG peroxidase conjugate (Biosource, Catalog # ALI0404).
16. ABTS Solution
17. 30% Hydrogen peroxide.
18. ABTS/H_2O_2

Procedure:

1. Coat Costar 96 well ELISA plates with 1 µg per well Poly(Glu,Tyr) in 100µl PBS. Store overnight at 4° C.
2. Wash coated plates once with PBS.
3. Add 150 µL of 5%BSA/PBS Blocking Buffer to each well. Incubate, with shaking, for 1 hr.room temperature.
4. Wash plate 2x with PBS, then once with 50mM Hepes. Pat plates on a paper towel to remove excess liquid and bubbles.
5. Add 25µL of 0.4 mM test compound in 4% DMSO or 4% DMSO alone (controls) to plate.
6. Dilute purified GST-FGFR1 in Kinase Dilution Buffer (5 ng kinase/50ul KDB/well).
7. Add 50µL of diluted kinase to each well.
8. Start kinase reaction by adding 25µl/well of freshly prepared ATP/Mn++ (0.4 ml 1M $MnCl_2$, 40 µL 10 mM ATP, 9.56 ml dH_2O), freshly prepared).
9. This is a fast kinase reaction and must be stopped with 25µL of 0.5M EDTA in a manner similar to the addition of ATP.
10. Wash plate 4x with fresh TBST.
11. Make up Antibody Dilution Buffer: Per 50 ml:
    Mix 5 ml of 5% BSA, 250 µl of 5% milk and 50 µl of 100mM sodium vanadate, bring to final volume with with 0.05% TBST.
12. Add 100 µl per well of anti-phosphotyrosine (1:10000

dilution in ADB). Incubate, with shaking for 1 hr. at room temperature.

13. Wash as in step 10.
14. Add 100 µl per well of Biosource Goat anti-rabbit IgG peroxidase conjugate (1:6000 dilution in ADB). Incubate, with shaking for 1 hr. at room temperature.
15. Wash as in step 10 and then with PBS to remove bubbles and excess TWEEN.
16. Add 100 µl of ABTS/H_2O_2 solution to each well.
17. Incubate, with shaking, for 10 to 20 minutes. Remove any bubbles.
18. Read assay on Dynatech MR7000 elisa reader: test filter at 410 nM, reference filterat 630 nM.

EGFR BIOASSAY

This assay is used to the <u>in vitro</u> kinase activity of FGF1-R in an ELISA assay.

<u>Materials and Reagents</u>:

1. Corning 96-well Elisa plates.
2. SUMO1 monoclonal anti-EGFR antibody (SUGEN, Inc.).
3. PBS
4. TBST Buffer
5. Blocking Buffer: for 100 ml, mix 5.0 g Carnation Instant Non-fat Milk® with 100 ml of PBS.
6. A431 cell lysate (SUGEN, Inc.).
7. TBS Buffer:
8. TBS + 10% DMSO: for 1L, mix 1.514 g TRIS, 2.192 g NaCl and 25 ml DMSO; bring to 1 liter total volume with dH_2O.
9. ATP (Adenosine-5'-triphosphate, from Equine muscle, Sigma Cat. No. A-5394), 1.0 mM solution in dH_2O. This reagent should be made up immediately prior to use and kept on ice.
10. 1.0 mM $MnCl_2$.

11. ATP/$MnCl_2$ phosphorylation mix: to make 10 ml, mix 300 µl of 1 mM ATP, 500 µl $MnCl_2$ and 9.2 ml dH_2O. Prepare just prior to use, keep on ice.
12. NUNC 96-well V bottom polypropylene plates.
13. EDTA.
14. Rabbit polyclonal anti-phosphotyrosine serum (SUGEN, Inc.).
15. Goat anti-rabbit IgG peroxidase conjugate (Biosource Cat. No. ALI0404).
16. ABTS.
17. 30% Hydrogen peroxide.
18. ABTS/H_2O_2.
19. 0.2 M HCl.

Procedure:

1. Coat Corning 96 well ELISA plates with 0.5 µg SUMO1 in 100 µl PBS per well, store overnight at 4° C.
2. Remove unbound SUMO1 from wells by inverting plate to remove liquid. Wash 1x with dH_2O. Pat the plate on a paper towel to remove excess liquid.
3. Add 150 µl of Blocking Buffer to each well. Incubate, with shaking, for 30 min. at room temperature.
4. Wash plate 3x with deionized water, then once with TBST. Pat plate on a paper towel to remove excess liquid and bubbles.
5. Dilute lysate in PBS (7 µg lysate/100 µl PBS).
6. Add 100 µl of diluted lysate to each well. Shake at room temperature for 1 hr.
7. Wash plates as in 4, above.
8. Add 120 µl TBS to ELISA plate containing captured EGFR.
9. Dilute test compound 1:10 in TBS, place in well
10. Add 13.5 µl diluted test compound to ELISA plate. To

control wells, add 13.5 µl TBS in 10% DMSO.

11. Incubate, with shaking, for 30 minutes at room temperature.
12. Add 15 µl phosphorylation mix to all wells except negative control well. Final well volume should be approximately 150 µl with 3 µM ATP/5 mM $MnCl_2$ final concentration in each well. Incubate with shaking for 5 minutes.
13. Stop reaction by adding 16.5 µl of EDTA solution while shaking. Shake for additional 1 min.
14. Wash 4x with deionized water, 2x with TBST.
15. Add 100 µl anti-phosphotyrosine (1:3000 dilution in TBST) per well. Incubate, with shaking, for 30-45 min. at room temperature.
16. Wash as in 4, above.
17. Add 100 µl Biosource Goat anti-rabbit IgG peroxidase conjugate (1:2000 dilution in TBST) to each well. Incubate with shaking for 30 min. at room temperature.
18. Wash as in 4, above.
19. Add 100 µl of ABTS/H_2O_2 solution to each well.
20. Incubate 5 to 10 minutes with shaking. Remove any bubbles.
21. If necessary, stop reaction by adding 100 µl 0.2 M HCl per well.
22. Read assay on Dynatech MR7000 ELISA reader: test filter at 410 nM, reference filter at 630 nM.

PDGFR BIOASSAY

This assay is used to the <u>in vitro</u> kinase activity of FGF1-R in an ELISA assay.

<u>Materials and Reagents</u>:

1. Corning 96-well Elisa plates
2. 28D4C10 monoclonal anti-PDGFR antibody (SUGEN, Inc.).

3. PBS.
4. TBST Buffer.
5. Blocking Buffer (same as for EGFR bioassay).
6. PDGFR-β expressing NIH 3T3 cell lysate (SUGEN, Inc.).
7. TBS Buffer.
8. TBS + 10% DMSO.
9. ATP.
10. $MnCl_2$.
11. Kinase buffer phosphorylation mix: for 10 ml, mix 250 µl 1M TRIS, 200 µl 5M NaCl, 100 µl 1M $MnCl_2$ and 50µl 100 mM Triton X-100 in enough dH_2O to make 10 ml.
12. NUNC 96-well V bottom polypropylene plates.
13. EDTA.
14. Rabbit polyclonal anti-phosphotyrosine serum (SUGEN, Inc.).
15. Goat anti-rabbit IgG peroxidase conjugate (Biosource Cat. No. ALI0404).
16. ABTS.
17. Hydrogen peroxide, 30% solution.
18. ABTS/H_2O_2.
19. 0.2 M HCl.

Procedure:

1. Coat Corning 96 well ELISA plates with 0.5 µg 28D4C10 in 100 µl PBS per well, store overnight at 4° C.
2. Remove unbound 28D4C10 from wells by inverting plate to remove liquid. Wash 1x with dH_2O. Pat the plate on a paper towel to remove excess liquid.
3. Add 150 µl of Blocking Buffer to each well. Incubate for 30 min. at room temperature with shaking.
4. Wash plate 3x with deionized water, then once with TBST. Pat plate on a paper towel to remove excess liquid and bubbles.

5. Dilute lysate in HNTG (10 µg lysate/100 µl HNTG)
6. Add 100 µl of diluted lysate to each well. Shake at room temperature for 60 min.
7. Wash plates as described in Step 4.
8. Add 80 µl working kinase buffer mix to ELISA plate containing captured PDGFR.
9. Dilute test compound 1:10 in TBS in 96-well polypropylene plates.
10. Add 10 µl diluted test compound to ELISA plate. To control wells, add 10 µl TBS + 10% DMSO. Incubate with shaking for 30 minutes at room temperature.
11. Add 10 µl ATP directly to all wells except negative control well (final well volume should be approximately 100 µl with 20 µM ATP in each well.) Incubate 30 minutes with shaking.
12. Stop reaction by adding 10 µl of EDTA solution to each well.
13. Wash 4x with deionized water, twice with TBST.
14. Add 100 µl anti-phosphotyrosine (1:3000 dilution in TBST) per well. Incubate with shaking for 30-45 min. at room temperature.
15. Wash as in Step 4.
16. Add 100 µl Biosource Goat anti-rabbit IgG peroxidase conjugate (1:2000 dilution in TBST) to each well. Incubate with shaking for 30 min. at room temperature.
17. Wash as in Step 4.
18. Add 100 µl of ABTS/H_2O_2 solution to each well.
19. Incubate 10 to 30 minutes with shaking. Remove any bubbles.
20. If necessary stop reaction with the addition of 100 µl 0.2 M HCl per well.

21. Read assay on Dynatech MR7000 ELISA reader with test filter at 410 nM and reference filter at 630 nM.

CELLULAR HER-2 KINASE ASSAY

This assay is used to measure HER-2 kinase activity in whole cells in an ELISA format.

Materials and Reagents:

1. DMEM (GIBCO Catalog #11965-092).
2. Fetal Bovine Serum (FBS, GIBCO Catalog #16000-044), heat inactivated in a water bath for 30 min. at 56° C.
3. Trypsin (GIBCO Catalog #25200-056).
4. L-Glutamine (GIBCO Catalog #25030-081)
5. HEPES (GIBCO Catalog #15630-080).
6. Growth Media
   Mix 500 ml DMEM, 55 ml heat inactivated FBS, 10 ml HEPES and 5.5 ml L-Glutamine.
7. Starve Media
   Mix 500 ml DMEM, 2.5 ml heat inactivated FBS, 10 ml HEPES and 5.5 ml L-Glutamine.
8. PBS.
9. Flat Bottom 96-well Tissue Culture Micro Titer Plates (Corning Catalog # 25860).
10. 15 cm Tissue Culture Dishes (Corning Catalog #08757148).
11. Corning 96-well ELISA Plates.
12. NUNC 96-well V bottom polypropylene plates.
13. Costar Transfer Cartridges for the Transtar 96 (Costar Catalog #7610).
14. SUMO 1: monoclonal anti-EGFR antibody (SUGEN, Inc.).
15. TBST Buffer.
16. Blocking Buffer : 5% Carnation Instant Milk® in PBS.
17. EGF Ligand: EGF-201, Shinko American, Japan.
    Suspend powder in 100 uL of 10mM HCl. Add 100uL 10mM NaOH. Add 800 uL PBS and transfer to an Eppendorf tube,

store at -20°C until ready to use.

18. HNTG Lysis Buffer
For Stock 5X HNTG, mix 23.83 g Hepes, 43.83 g NaCl, 500 ml glycerol and 100 ml Triton X-100 and enough dH_2O to make 1 L of total solution.
For 1X HNTG*, mix 2 ml HNTG, 100 µL 0.1M Na_3VO_4, 250 µL 0.2M $Na_4P_2O_7$ and 100 µL EDTA.
19. EDTA.
20. Na_3VO_4. To make stock solution, mix 1.84 g Na_3VO_4 with 90 ml dH_2O. Adjust pH to 10. Boil in microwave for one minute (solution becomes clear). Cool to room temperature. Adjust pH to 10. Repeat heating/cooling cycle until pH remains at 10.
21. 200 mM $Na_4P_2O_7$.
22. Rabbit polyclonal antiserum specific for phosphotyrosine (anti-Ptyr antibody, SUGEN, Inc.).
23. Affinity purified antiserum, goat anti-rabbit IgG antibody, peroxidase conjugate (Biosource Cat # ALI0404).
24. ABTS Solution.
25. 30 % Hydrogen peroxide solution.
26. ABTS/H_2O_2.
27. 0.2 M HCl.

Procedure:

1. Coat Corning 96 well ELISA plates with SUMO1 at 1.0 ug per well in PBS, 100 ul final volume/well. Store overnight at 4°C.
2. On day of use, remove coating buffer and wash plate 3 times with dH_2O and once with TBST buffer. All washes in this assay should be done in this manner, unless otherwise specified.
3. Add 100 ul of Blocking Buffer to each well. Incubate

plate, with shaking, for 30 min. at room temperature. Just prior to use, wash plate.

4. Use EGFr/HER-2 chimera/3T3-C7 cell line for this assay.
5. Choose dishes having 80-90 % confluence. Collect cells by trypsinization and centrifuge at 1000 rpm at room temperature for 5 min.
6. Resuspend cells in starve medium and count with trypan blue. Viability above 90% is required. Seed cells in starve medium at a density of 2,500 cells per well, 90 ul per well, in a 96 well microtiter plate. Incubate seeded cells overnight at 37° under 5% CO_2.
7. Start the assay two days after seeding.
8. Test compounds are dissolved in 4% DMSO. Samples are then further diluted directly on plates with starve-DMEM. Typically, this dilution will be 1:10 or greater. All wells are then transferred to the cell plate at a further 1:10 dilution (10µl sample and media into 90 µl of starve media. The final DMSO concentration should be 1% or lower. A standard serial dilution may also be used.
9. Incubate under 5% CO_2 at 37°C for 2 hours.
10. Prepare EGF ligand by diluting stock EGF (16.5 uM) in warm DMEM to 150 nM.
11. Prepare fresh HNTG* sufficient for 100 ul per well; place on ice.
12. After 2 hour incubation with test compound, add prepared EGF ligand to cells, 50 ul per well, for a final concentration of 50 nM. Positive control wells receive the same amount of EGF. Negative controls do not receive EGF. Incubate at 37° C for 10 min.
13. Remove test compound, EGF, and DMEM. Wash cells once with PBS.

14. Transfer HNTG* to cells, 100 ul per well. Place on ice for 5 minutes. Meanwhile, remove blocking buffer from ELISA plate and wash.

15. Scrape cells from plate with a micropipettor and homogenize cell material by repeatedly aspirating and dispensing the HNTG* lysis buffer. Transfer lysate to a coated, blocked, washed ELISA plate. Or, use a Costar transfer cartridge to transfer lysate to the plate.

16. Incubate, with shaking, at room temperature for 1 hr.

17. Remove lysate, wash. Transfer freshly diluted anti-Ptyr antibody (1:3000 in TBST) to ELISA plate, 100 ul per well.

18. Incubate, with shaking, at room temperature, for 30 min.

19. Remove anti-Ptyr antibody, wash. Transfer freshly diluted BIOSOURCE antibody to ELISA plate(1:8000 in TBST, 100 ul per well).

20. Incubate, with shaking, at room temperature for 30 min.

21. Remove BIOSOURCE antibody, wash. Transfer freshly prepared ABTS/H_2O_2 solution to ELISA plate, 100 ul per well.

22. Incubate, with shaking, for 5-10 minutes. Remove any bubbles.

23. Stop reaction with the addition of 100ul of 0.2M HCl per well.

24. Read assay on Dynatech MR7000 ELISA reader woth test filter set at 410 nM and reference filter at 630 nM.

CDK2/CYCLIN A ASSAY

This assay is used to measure the <u>in vitro</u> serine/threonine kinase activity of human cdk2/cyclin A in a Scintillation Proximity Assay (SPA).

<u>Materials and Reagents</u>.

1. Wallac 96-well polyethylene terephthalate (flexi)

plates (Wallac Catalog # 1450-401).

2. Amersham Redivue [γ^{33}P] ATP (Amersham catalog #AH 9968).
3. Amersham streptavidin coated polyvinyltoluene SPA beads (Amersham catalog #RPNQ0007). The beads should be reconstituted in PBS without magnesium or calcium, at 20 mg/ml.
4. Activated cdk2/cyclin A enzyme complex purified from Sf9 cells (SUGEN, Inc.).
5. Biotinylated peptide substrate (Debtide). Peptide biotin-X-PKTPKKAKKL is dissolved in dH_2O at a concentration of 5 mg/ml.
6. Peptide/ATP Mixture: for 10 ml, mix 9.979 ml dH_2O, 0.00125 ml "cold" ATP, 0.010 ml Debtide and 0.010 ml γ^{33}P ATP. The ultimate concentration per well will be 0.5 µM "cold" ATP, 0.1 µg Debtide and 0.2 µCi γ^{33}P ATP.
7. Kinase buffer: for 10 ml, mix 8.85 ml dH_2O, 0.625 ml TRIS(pH 7.4), 0.25 ml 1M $MgCl_2$, 0.25 ml 10% NP40 and 0.025 ml 1M DTT, added fresh just prior to use.
8. 10 mM ATP in dH_2O.
9. 1M Tris, pH adjusted to 7.4 with HCl.
10. 1M $MgCl_2$.
11. 1M DTT.
12. PBS (Gibco Catalog # 14190-144).
13. 0.5M EDTA.
14. Stop solution: For 10 ml, mix 9.25 ml PBS, 0.005 ml 100 mM ATP, 0.1 ml 0.5 M EDTA, 0.1 ml 10% Triton X-100 and 1.25 ml of 20 mg/ml SPA beads.

Procedure:

1. Prepare solutions of test compounds at 5x the desired final concentration in 5% DMSO. Add 10 ul to each well. For negative controls, use 10 ul 5% DMSO alone in wells.

2. Dilute 5 µl of cdk2/cyclin A solution with 2.1 ml 2x kinase buffer.
3. Add 20 ul enzyme to each well.
4. Add 10 µL of 0.5 M EDTA to the negative control wells.
5. To start kinase reaction, add 20 µL of peptide/ATP mixture to each well. Incubate for 1 hr. without shaking.
6. Add 200 µl stop solution to each well.
7. Hold at least 10 min.
8. Spin plate at approx. 2300 rpm for 3-5 min.
9. Count plate using Trilux or similar reader.

BRDU INCORPORATION ASSAYS

The following assays use cells engineered to express a selected receptor and then evaluate the effect of a compound of interest on the activity of ligand-induced DNA synthesis by determining BrdU incorporation into the DNA.

The following materials, reagents and procedure are general to each of the following BrdU incorporation assays. Variances in specific assays are noted.

Materials and Reagents:

1. The appropriate ligand.
2. The appropriate engineered cells.
3. BrdU Labeling Reagent: 10 mM, in PBS (pH7.4) (Boehringer Mannheim, Germany).
4. FixDenat: fixation solution (ready to use) (Boehringer Mannheim, Germany).
5. Anti-BrdU-POD: mouse monoclonal antibody conjugated with peroxidase (Boehringer Mannheim, Germany).
6. TMB Substrate Solution: tetramethylbenzidine (TMB, Boehringer Mannheim, Germany).
7. PBS Washing Solution : 1X PBS, pH 7.4.
8. Albumin, Bovine (BSA), fraction V powder (Sigma Chemical Co., USA).

General Procedure:

1. Cells are seeded at 8000 cells/well in 10% CS, 2mM Gln in DMEM, in a 96 well plate. Cells are incubated overnight at 37°C in 5% CO_2.
2. After 24 hours, the cells are washed with PBS, and then are serum-starved in serum free medium (0%CS DMEM with 0.1% BSA) for 24 hours.
3. On day 3, the appropriate ligand and the test compound are added to the cells simultaneously. The negative control wells receive serum free DMEM with 0.1% BSA only; the positive control cells receive the ligand but no test compound. Test compounds are prepared in serum free DMEM with ligand in a 96 well plate, and serially diluted for 7 test concentrations.
4. After 18 hours of ligand activation, diluted BrdU labeling reagent (1:100 in DMEM, 0.1% BSA) is added and the cells are incubated with BrdU (final concentration=10 μM) for 1.5 hours.
5. After incubation with labeling reagent, the medium is removed by decanting and tapping the inverted plate on a paper towel. FixDenat solution is added (50 μl/well) and the plates are incubated at room temperature for 45 minutes on a plate shaker.
6. The FixDenat solution is thoroughly removed by decanting and tapping the inverted plate on a paper towel. Milk is added (5% dehydrated milk in PBS, 200 μl/well) as a blocking solution and the plate is incubated for 30 minutes at room temperature on a plate shaker.
7. The blocking solution is removed by decanting and the wells are washed once with PBS. Anti-BrdU-POD solution (1:200 dilution in PBS, 1% BSA) is added (50 μl/well) and the plate is incubated for 90 minutes at room

temperature on a plate shaker.

8. The antibody conjugate is thoroughly removed by decanting and rinsing the wells 5 times with PBS, and the plate is dried by inverting and tapping on a paper towel.

9. TMB substrate solution is added (100 µl/well) and incubated for 20 minutes at room temperature on a plate shaker until color development is sufficient for photometric detection.

10. The absorbance of the samples are measured at 410 nm (in "dual wavelength" mode with a filter reading at 490 nm, as a reference wavelength) on a Dynatech ELISA plate reader.

EGF-Induced BrdU Incorporation Assay

Materials and Reagents:

1. Mouse EGF, 201 (Toyobo Co., Ltd., Japan).
2. 3T3/EGFRc7.

EGF-Induced Her-2-driven BrdU Incorporation Assay

Materials and Reagents:

1. Mouse EGF, 201 (Toyobo Co., Ltd., Japan).
2. 3T3/EGFr/Her2/EGFr (EGFr with a Her-2 kinase domain).

EGF-Induced Her-4-driven BrdU Incorporation Assay

Materials and Reagents:

1. Mouse EGF, 201 (Toyobo Co., Ltd., Japan).
2. 3T3/EGFr/Her4/EGFr (EGFr with a Her-4 kinase domain).

PDGF-Induced BrdU Incorporation Assay

Materials and Reagents:

1. Human PDGF B/B (Boehringer Mannheim, Germany).
2. 3T3/EGFRc7.

FGF-Induced BrdU Incorporation Assay

Materials and Reagents:

1. Human FGF2/bFGF (Gibco BRL, USA).
2. 3T3c7/EGFr

IGF1-Induced BrdU Incorporation Assay

Materials and Reagents:

1. Human, recombinant (G511, Promega Corp., USA)
2. 3T3/IGF1r.

Insulin-Induced BrdU Incorporation Assay

Materials and Reagents:

1. Insulin, crystalline, bovine, Zinc (13007, Gibco BRL, USA).
2. 3T3/H25.

HGF-Induced BrdU Incorporation Assay

Materials and Reagents:

1. Recombinant human HGF (Cat. No. 249-HG, R&D Systems, Inc. USA).
2. BxPC-3 cells (ATCC CRL-1687).

Procedure:

1. Cells are seeded at 9000 cells/well in RPMI 10% FBS in a 96 well plate. Cells are incubated overnight at 37°C in 5% CO_2.
2. After 24 hours, the cells are washed with PBS, and then are serum starved in 100 µl serum-free medium (RPMI with 0.1% BSA) for 24 hours.
3. On day 3, 25 µl containing ligand (prepared at 1 µg/ml in RPMI with 0.1% BSA; final HGF conc. is 200 ng/ml) and test compounds are added to the cells. The negative control wells receive 25 µl serum-free RPMI with 0.1% BSA only; the positive control cells receive the ligand (HGF) but no test compound. Test compounds are prepared at 5 times their final concentration in serum-free RPMI with ligand in a 96 well plate, and serially diluted to give 7 test concentrations. Typically, the highest final concentration of test compound is 100 µM, and 1:3 dilutions are used (i.e.

418 449 455

final test compound concentration range is 0.137-100 µM).

4. After 18 hours of ligand activation, 12.5 µl of diluted BrdU labeling reagent (1:100 in RPMI, 0.1% BSA) is added to each well and the cells are incubated with BrdU (final concentration is 10 µM) for 1 hour.

5. Same as General Procedure.

6. Same as General Procedure.

7. The blocking solution is removed by decanting and the wells are washed once with PBS. Anti-BrdU-POD solution (1:100 dilution in PBS, 1% BSA) is added (100 µl/well) and the plate is incubated for 90 minutes at room temperature on a plate shaker.

8. Same as General Procedure.

9. Same as General Procedure.

10. Same as General Procedure.

HUV-EC-C Assay

This assay is used to measure a compound's activity against PDGF-R, FGF-R, VEGF, aFGF or Flk-1/KDR, all of which are naturally expressed by HUV-EC cells.

DAY 0

1. Wash and trypsinize HUV-EC-C cells (human umbilical vein endothelial cells, (American Type Culture Collection, catalogue no. 1730 CRL). Wash with Dulbecco's phosphate-buffered saline (D-PBS, obtained from Gibco BRL, catalogue no. 14190-029) 2 times at about 1 ml/10 cm^2 of tissue culture flask. Trypsinize with 0.05% trypsin-EDTA in non-enzymatic cell dissociation solution (Sigma Chemical Company, catalogue no. C-1544). The 0.05% trypsin is made by diluting 0.25% trypsin/1 mM EDTA (Gibco, catalogue no. 25200-049) in the cell dissociation solution. Trypsinize with about 1 ml/25-30 cm^2 of tissue culture flask for about 5 minutes at 37°C. After cells have detached from the

flask, add an equal volume of assay medium and transfer to a 50 ml sterile centrifuge tube (Fisher Scientific, catalogue no. 05-539-6).

2. Wash the cells with about 35 ml assay medium in the 50 ml sterile centrifuge tube by adding the assay medium, centrifuge for 10 minutes at approximately 200x g, aspirate the supernatant, and resuspend with 35 ml D-PBS. Repeat the wash two more times with D-PBS, resuspend the cells in about 1 ml assay medium/15 cm^2 of tissue culture flask. Assay medium consists of F12K medium (Gibco BRL, catalogue no. 21127-014) and 0.5% heat-inactivated fetal bovine serum. Count the cells with a Coulter Counter® (Coulter Electronics, Inc.) and add assay medium to the cells to obtain a concentration of 0.8-1.0 x 10^5 cells/ml.

3. Add cells to 96-well flat-bottom plates at 100 µl/well or 0.8-1.0 x 10^4 cells/well, incubate ~24h at 37°C, 5% CO_2.

<u>DAY 1</u>

1. Make up two-fold test compound titrations in separate 96-well plates, generally 50 µM on down to 0 µM. Use the same assay medium as mentioned in day 0, step 2 above. Titrations are made by adding 90 µl/well of test compound at 200 µM (4X the final well concentration) to the top well of a particular plate column. Since the stock test compound is usually 20 mM in DMSO, the 200 µM drug concentration contains 2% DMSO.

A diluent made up to 2% DMSO in assay medium (F12K + 0.5% fetal bovine serum) is used as diluent for the test compound titrations in order to dilute the test compound but keep the DMSO concentration constant. Add this diluent to the remaining wells in the column at 60 µl/well. Take 60 µl from the 120 µl of 200 µM test compound dilution in the top well of the column and mix with the 60 µl in the second well of the column. Take 60 µl from this well and mix with the 60 µl in the third well of the column,

and so on until two-fold titrations are completed. When the next-to-the-last well is mixed, take 60 µl of the 120 µl in this well and discard it. Leave the last well with 60 µl of DMSO/media diluent as a non-test compound-containing control. Make 9 columns of titrated test compound, enough for triplicate wells each for: (1) VEGF (obtained from Pepro Tech Inc., catalogue no. 100-200, (2) endothelial cell growth factor (ECGF) (also known as acidic fibroblast growth factor, or aFGF) (obtained from Boehringer Mannheim Biochemica, catalogue no. 1439 600), or, (3) human PDGF B/B (1276-956, Boehringer Mannheim, Germany) and assay media control. ECGF comes as a preparation with sodium heparin.

2. Transfer 50 µl/well of the test compound dilutions to the 96-well assay plates containing the 0.8-1.0x10^4 cells/100 µl/well of the HUV-EC-C cells from day 0 and incubate ~2 h at 37° C, 5% CO_2.

3. In triplicate, add 50 µl/well of 80 µg/ml VEGF, 20 ng/ml ECGF, or media control to each test compound condition. As with the test compounds, the growth factor concentrations are 4X the desired final concentration. Use the assay media from day 0 step 2 to make the concentrations of growth factors. Incubate approximately 24 hours at 37°C, 5% CO_2. Each well will have 50 µl test compound dilution, 50 µl growth factor or media, and 100 µl cells, which calculates to 200 µl/well total. Thus the 4X concentrations of test compound and growth factors become 1X once everything has been added to the wells.

<u>DAY 2</u>

1. Add ^{3}H-thymidine (Amersham, catalogue no. TRK-686) at 1 µCi/well (10 µl/well of 100 µCi/ml solution made up in RPMI media + 10% heat-inactivated fetal bovine serum) and incubate ~24 h at 37°C, 5% CO_2. RPMI is obtained from Gibco BRL, catalogue no. 11875-051.

DAY 3

1. Freeze plates overnight at -20°C.

DAY 4

Thaw plates and harvest with a 96-well plate harvester (Tomtec Harvester 96®) onto filter mats (Wallac, catalogue no. 1205-401), read counts on a Wallac Betaplate™ liquid scintillation counter.

In Vivo Animal Models

XENOGRAFT ANIMAL MODELS

The ability of human tumors to grow as xenografts in athymic mice (*e.g.*, Balb/c, nu/nu) provides a useful in vivo model for studying the biological response to therapies for human tumors. Since the first successful xenotransplantation of human tumors into athymic mice, (Rygaard and Povlsen, 1969, Acta Pathol. Microbial. Scand. 77:758-760), many different human tumor cell lines (*e.g.*, mammary, lung, genitourinary, gastro-intestinal, head and neck, glioblastoma, bone, and malignant melanomas) have been transplanted and successfully grown in nude mice. The following assays may be used to determine the level of activity, specificity and effect of the different compounds of the present invention. Three general types of assays are useful for evaluating compounds: cellular/catalytic, cellular/biological and in vivo. The object of the cellular/catalytic assays is to determine the effect of a compound on the ability of a TK to phosphorylate tyrosines on a known substrate in a cell. The object of the cellular/biological assays is to determine the effect of a compound on the biological response stimulated by a TK in a cell. The object of the in vivo assays is to determine the effect of a compound in an animal model of a particular disorder such as cancer.

Suitable cell lines for subcutaneous xenograft experiments include C6 cells (glioma, ATCC # CCL 107), A375 cells (melanoma, ATCC # CRL 1619), A431 cells (epidermoid carcinoma, ATCC # CRL

1555), Calu 6 cells (lung, ATCC # HTB 56), PC3 cells (prostate, ATCC # CRL 1435), SKOV3TP5 cells and NIH 3T3 fibroblasts genetically engineered to overexpress EGFR, PDGFR, IGF-1R or any other test kinase. The following protocol can be used to perform xenograft experiments:

Female athymic mice (BALB/c, nu/nu) are obtained from Simonsen Laboratories (Gilroy, CA). All animals are maintained under clean-room conditions in Micro-isolator cages with Alpha-dri bedding. They receive sterile rodent chow and water ad libitum.

Cell lines are grown in appropriate medium (for example, MEM, DMEM, Ham's F10, or Ham's F12 plus 5% - 10% fetal bovine serum (FBS) and 2 mM glutamine (GLN)). All cell culture media, glutamine, and fetal bovine serum are purchased from Gibco Life Technologies (Grand Island, NY) unless otherwise specified. All cells are grown in a humid atmosphere of 90-95% air and 5-10% CO_2 at 37°C. All cell lines are routinely subcultured twice a week and are negative for mycoplasma as determined by the Mycotect method (Gibco).

Cells are harvested at or near confluency with 0.05% Trypsin-EDTA and pelleted at 450 x g for 10 min. Pellets are resuspended in sterile PBS or media (without FBS) to a particular concentration and the cells are implanted into the hindflank of the mice (8 - 10 mice per group, 2 - 10 x 10^6 cells/animal). Tumor growth is measured over 3 to 6 weeks using venier calipers. Tumor volumes are calculated as a product of length x width x height unless otherwise indicated. P values are calculated using the Students t-test. Test compounds in 50 - 100 µL excipient (DMSO, or VPD:D5W) can be delivered by IP injection at different concentrations generally starting at day one after implantation.

TUMOR INVASION MODEL

The following tumor invasion model has been developed and may be used for the evaluation of therapeutic value and

efficacy of the compounds identified to selectively inhibit KDR/FLK-1 receptor.

Procedure

8 week old nude mice (female) (Simonsen Inc.) are used as experimental animals. Implantation of tumor cells can be performed in a laminar flow hood. For anesthesia, Xylazine/Ketamine Cocktail (100 mg/kg ketamine and 5 mg/kg Xylazine) are administered intraperitoneally. A midline incision is done to expose the abdominal cavity (approximately 1.5 cm in length) to inject 10^7 tumor cells in a volume of 100 µl medium. The cells are injected either into the duodenal lobe of the pancreas or under the serosa of the colon. The peritoneum and muscles are closed with a 6-0 silk continuous suture and the skin is closed by using wound clips. Animals are observed daily.

Analysis

After 2-6 weeks, depending on gross observations of the animals, the mice are sacrificed, and the local tumor metastases to various organs (lung, liver, brain, stomach, spleen, heart, muscle) are excised and analyzed (measurement of tumor size, grade of invasion, immunochemistry, in situ hybridization determination, etc.).

Measurement Of Cell Toxicity

Therapeutic compounds should be more potent in inhibiting receptor tyrosine kinase activity than in exerting a cytotoxic effect. A measure of the effectiveness and cell toxicity of a compound can be obtained by determining the therapeutic index, i.e., IC_{50}/LD_{50}. IC_{50}, the dose required to achieve 50% inhibition, can be measured using standard techniques such as those described herein. LD_{50}, the dosage which results in 50% toxicity, can also be measured by standard techniques as well (Mossman, 1983, J. Immunol. Methods, 65:55-63), by measuring the amount of LDH released (Korzeniewski and Callewaert, 1983, J. Immunol. Methods, 64:313,

Decker and Lohmann-Matthes, 1988, J. Immunol. Methods, 115:61), or by measuring the lethal dose in animal models. Compounds with a large therapeutic index are preferred. The therapeutic index should be greater than 2, preferably at least 10, more preferably at least 50.

B. Examples - Biological Activity.

Examples of the in vitro potency of compounds of this invention are shown in Table 2.

CONCLUSION

It will be appreciated that the compounds, methods and pharmaceutical compositions of the present invention are effective in modulating PK activity and therefore are expected to be effective as therapeutic agents against RTK, CTK-, and STK-related disorders.

One skilled in the art would also readily appreciate that the present invention is well adapted to carry out the objects and obtain the ends and advantages mentioned, as well as those inherent herein. The molecular complexes and the methods, procedures, treatments, molecules, specific compounds described herein are presently representative of preferred embodiments, are exemplary, and are not intended as limitations on the scope of the invention. Changes therein and other uses will occur to those skilled in the art which are encompassed within the spirit of the invention are defined by the scope of the claims.

It will be readily apparent to one skilled in the art that varying substitutions and modifications may be made to the invention disclosed herein without departing from the scope and spirit of the invention.

All patents and publications mentioned in the specification are indicative of the levels of those skilled in the art to which the invention pertains. All patents and publications are herein incorporated by reference to the same extent as if each

individual publication was specifically and individually indicated to be incorporated by reference.

The invention illustratively described herein suitably may be practiced in the absence of any element or elements, limitation or limitations which is not specifically disclosed herein. Thus, for example, in each instance herein any of the terms "comprising", "consisting essentially of" and "consisting of" may be replaced with either of the other two terms. The terms and expressions which have been employed are used as terms of description and not of limitation, and there is no intention that in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the invention claimed. Thus, it should be understood that although the present invention has been specifically disclosed by preferred embodiments and optional features, modification and variation of the concepts herein disclosed may be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this invention as defined by the appended claims.

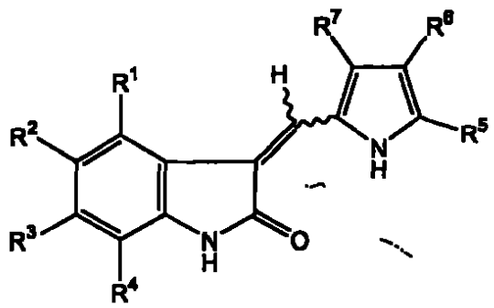
In addition, where features or aspects of the invention are described in terms of Markush groups, those skilled in the art will recognize that the invention is also thereby described in terms of any individual member or subgroup of members of the Markush group. For example, if X is described as selected from the group consisting of bromine, chlorine, and iodine, claims for X being bromine and claims for X being bromine and chlorine are fully described.

Other embodiments are within the following claims.

CLAIMS

WHAT IS CLAIMED:

1. A compound having the chemical structure:



wherein,

R^1 is selected from the group consisting of hydrogen, halo, alkyl, cyclkoalkyl, aryl, heteroaryl, heteroalicyclic, hydroxy, alkoxy, $-C(O)R^{15}$, $-NR^{13}R^{14}$, $-(CH_2)_rR^{16}$ and $-C(O)NR^8R^9$;

R^2 is selected from the group consisting of hydrogen, halo, alkyl, trihalomethyl, hydroxy, alkoxy, $-NR^{13}R^{14}$, N-amido, $C(O)R^{15}$, aryl, heteroaryl and $-S(O)_2NR^{13}R^{14}$;

R^3 is selected from the group consisting of hydrogen, halo, alkyl, trihalomethyl, hydroxy, alkoxy, $-C(O)R^{15}$, $-NR^{13}R^{14}$, aryl, heteroaryl, N-sulfonamido, S-sulfonamido, N-amido and N-carbamyl;

R^4 is selected from the group consisting of hydrogen, halogen, alkyl, hydroxy, alkoxy and $-NR^{13}R^{14}$;

R^5 is selected from the group consisting of hydrogen, alkyl and

$-C(O)R^{10}$;

R^6 is selected from the group consisting of hydrogen, alkyl and $-C(O)R^{10}$;

R^7 is selected from the group consisting of hydrogen, alkyl, aryl, heteroaryl, $-C(O)R^{17}$ and $-C(O)R^{10}$;

R^6 and R^7 may combine to form a group selected from the group consisting of $-(CH_2)_4-$, $-(CH_2)_5-$ and $-(CH_2)_6-$;

with the proviso that at least one of R^5, R^6 or R^7 must be $-C(O)R^{10}$;

R^8 and R^9 are independently selected from the group consisting of hydrogen, alkyl and aryl;

R^{10} is selected from the group consisting of hydroxy, alkoxy, aryloxy, $-N(R^{11})(CH_2)_nR^{12}$ and $-NR^{13}R^{14}$;

R^{11} is selected from the group consisting of hydrogen and alkyl;

R^{12} is selected from the group consisting of $-NR^{13}R^{14}$, hydroxy, $-C(O)R^{15}$, aryl and heteroaryl;

R^{13} and R^{14} are independently selected from the group consisting of hydrogen, alkyl, cycloalkyl, aryl and heteroaryl;

R^{13} and R^{14} may combine to form a group selected from the group consisting of $-(CH_2)_4-$, $-(CH_2)_5-$, $-(CH_2)_2O(CH_2)_2-$, and $-(CH_2)_2N(CH_3)(CH_2)_2-$;

R^{15} is selected from the group consisting of hydrogen, hydroxy, alkoxy and aryloxy;

R^{16} is selected from the group consisting of hydroxy, $-C(O)R^{15}$, $-NR^{13}R^{14}$ and $C(O)NR^{13}R^{14}$;

R^{17} is selected from the group consisting of alkyl, cycloalkyl, aryl and heteroaryl;

n and r are independently 1, 2, 3, or 4; or,

a pharmaceutically acceptable salt of said compound.

2. The compound or salt of claim 1, wherein

R^1 and R^4 are hydrogen;

R^2 is selected from the group consisting of hydrogen, halo, lower alkoxy, $-C(O)R^{15}$ and $-S(O)_2NR^{13}R^{14}$;

R^3 is selected from the group consisting of hydrogen, lower alkoxy, $-C(O)R^{15}$, $-S(O)_2NR^{13}R^{14}$, aryl and heteroaryl;

R^5 is $-C(O)R^{10}$;

R^6 is selected from the group consisting of hydrogen and lower alkyl; and,

R^7 is selected from the group consisting of hydrogen, lower alkyl and $-C(O)R^{17}$.

3. The compound or salt of claim 2, wherein:
R^{10} is selected from the group consisting of hydroxy, lower alkoxy and $-NR^{11}(CH_2)_nR^{12}$, wherein:

n is 2 or 3;

R^{11} is selected from the group consisting of hydrogen and lower alkyl; and,

R^{12} is selected from the group consisting of aryl and $-NR^{13}R^{14}$.

4. The compound of claim 3, wherein R^{13} and R^{14} are independently selected from the group consisting of hydrogen, lower alkyl, and, combined, $-(CH_2)_4-$, $-(CH_2)_5-$, $-(CH_2)_2O(CH_2)_2-$ or $-(CH_2)_2N(CH_3)(CH_2)_2-$.

5. The compound or salt of claim 1, wherein,

R^1 is selected from the group consisting of hydrogen, lower alkyl, $(CH_2)_rR^{16}$ and $-C(O)NR^8R^9$;

R^2 is selected from the group consisting of hydrogen, halogen, aryl and $-S(O)_2NR^{13}R^{14}$;

R^3 is selected from the group consisting of hydrogen, lower alkyl, lower alkoxy, aryl, heteroaryl and $-C(O)R^{15}$;

R^4 is hydrogen;

R^5 is selected from the group consisting of hydrogen and lower alkyl;

R^6 is $-C(O)R^{10}$;

R^7 is selected from the group consisting of hydorgen, lower alkyl and aryl;

R^{16} is selected from the group consisting of hydroxy and $-C(O)R^{15}$; and,

r is 2 or 3.

6. The compound or salt of claim 5 wherein R^3 is aryl optionally substituted with one or more groups selected from the group consisting of lower alkyl, lower alkoxy and halo.

7. The compound or salt of claim 5 wherein R^{10} is selected from the group consisting of hydroxy, lower alkoxy, $-NR^{13}R^{14}$ and $-NR^{11}(CH_2)_nR^{12}$, wherein

n is 1, 2 or 3;

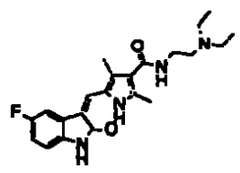
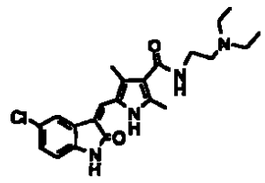
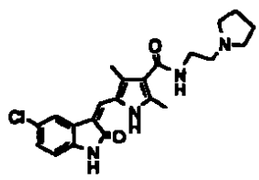
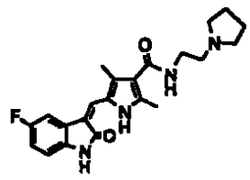
R^{11} is hydrogen; and,

R^{12} is selected from the group consisting of hydroxy, lower alkoxy, $-C(O)R^{15}$, heteroaryl and $-NR^{13}R^{14}$.

8. The compound of claim 7 wherein R^{13} and R^{14} are independently selected from the group consisting of hydrogen, lower alkyl, heteroaryl and, combined, $-(CH_2)_4-$, $-(CH_2)_5-$, -

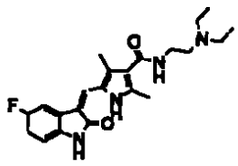
$(CH_2)_2O(CH_2)_2$-, or -$(CH_2)_2N(CH_3)(CH_2)_2$-.

9. The compound or salt of claim 8, wherein the compound is selected from the group consisting of

and

10. The compound or salt of claim 9, wherein the compound is



11. A pharmaceutical composition, comprising

a compound or salt of claim 10; and,

a pharmaceutically acceptable carrier or excipient.

12. A method for the modulation of the catalytic activity of a protein kinase comprising contacting said protein kinase with a compound or salt of claim 10.

13. The method of claim 12 wherein said protein kinase is selected from the group consisting of a receptor tyrosine kinase, a non-receptor tyrosine kinase and a serine-threonine kinase.

14. A method for treating or preventing a protein kinase related disorder in an organism comprising administering a therapeutically effective amount of a compound or salt of claim 10 to said organism.

15. The method of claim 14 wherein said protein kinase related disorder is selected from the group consisting of a receptor tyrosine kinase related disorder, a non-receptor tyrosine kinase related disorder and a serine-threonine kinase related disorder.

16. The method of claim 14 wherein said protein kinase related disorder is selected from the group consisting of an EGFR related disorder, a PDGFR related disorder, an IGFR related disorder and a flk related disorder.

17. The method of claim 14 wherein said protein kinase related disorder is a cancer selected from the group consisting of squamous cell carcinoma, astrocytoma, Kaposi's sarcoma, glioblastoma, lung cancer, bladder cancer, head and neck cancer, melanoma, ovarian cancer, prostate cancer, breast

cancer, small-cell lung cancer, glioma, colorectal cancer, genitourinary cancer and gastrointestinal cancer.

18. The method of claim 14 wherein said protein kinase related disorder is selected from the group consisting of diabetes, an autoimmune disorder, a hyperproliferation disorder, restenosis, fibrosis, psoriasis, von Heppel-Lindau disease, osteoarthritis, rheumatoid arthritis, angiogenesis, an inflammatory disorder, an immunological disorder and a cardiovascular disorder.

19. The method of claim 14 wherein said organism is a human.

20. The compound or salt of claim 5, wherein R^1 is $-C(O)NR^8R^9$, wherein:

R^8 is hydrogen; and,

R^9 is aryl optionally substituted with one or more groups selected from the group consisting of halo, hydroxy and lower alkoxy.

21. The compound of claim 1, wherein:

R^1 is selected from the group consisting of hydrogen and lower alkyl;

R^2 is selected from the group consisting of hydrogen, halo, lower alkoxy, aryl, $-C(O)R^{15}$ and $-S(O)_2NR^{13}R^{14}$;

R^3 is selected from the group consisting of hydrogen, halo, aryl, heteroaryl and $-C(O)R^{15}$;

R^4 is hydrogen;

R^5 is $-C(O)R^{10}$; and,

R^6 and R^7 combine to form a $-(CH_2)_4-$ group.

22. The compound of claim 21, wherein:
R^{10} is selected from the group consisting of hydroxy, alkoxy, $-NR^{13}R^{14}$ and $-NH(CH_2)_nNR^{13}R^{14}$; and

n is 2 or 3.

23. The compound of claim 22 wherein R^{13} and R^{14} are independently selected from the group consisting of hydrogen, lower alkyl, and, combined, $-(CH_2)_4-$, $-(CH_2)_5-$, $-(CH_2)_2O(CH_2)_2-$ or $-(CH_2)_2N(CH_3)(CH_2)_2-$.

24. The compound of claim 1, wherein:

R^1 and R^4 are selected from the group consisting of hydrogen, lower alkyl, lower alkoxy and halogen;

R^2 is selected from the group consisting of hydrogen, lower alkyl, lower alkoxy, halo, and $-S(O)_2NR^{13}R^{14}$;

R^3 is selected from the group consisting of hydrogen, lower alkyl, lower alkoxy, halo, aryl and $-C(O)R^{15}$;

R^5 and R^6 are selected from the group consisting of hydrogen and lower alkyl; and,

R^7 is $-C(O)R^{10}$.

25. The compound of claim 24, wherein:

R^{10} is selected from the group consisting of hydroxy, alkoxy, $-NR^{13}R^{14}$ and $-NR^{11}(CH_2)_nR^{12}$, wherein

n is 2 or 3;

R^{11} is selected from the group consisting of hydrogen and lower alkyl; and,

R^{12} is $-NR^{13}R^{14}$, wherein

R^{13} and R^{14} are independently selected from the group consisting of hydrogen, lower alkyl, and, combined, $-(CH_2)_4-$, $-(CH_2)_5-$, $-(CH_2)_2O(CH_2)_2-$ or $-(CH_2)_2N(CH_3)(CH_2)_2-$

26. A pharmaceutical composition, comprising

a compound or salt of claim 1; and,

a pharmaceutically acceptable carrier or excipient.

27. A method for the modulation of the catalytic activity of a protein kinase comprising contacting said protein kinase with a compound or salt of claim 1.

28. The method of claim 27 wherein said protein kinase is selected from the group consisting of a receptor tyrosine kinase, a non-receptor tyrosine kinase and a serine-threonine kinase.

29. A method for treating or preventing a protein kinase related disorder in an organism comprising administering a therapeutically effective amount of a compound or salt of claim 1 to said organism.

30. The method of claim 29 wherein said protein kinase related disorder is selected from the group consisting of a receptor tyrosine kinase related disorder, a non-receptor tyrosine kinase related disorder and a serine-threonine kinase related disorder.

31. The method of claim 29 wherein said protein kinase related disorder is selected from the group consisting of an EGFR related disorder, a PDGFR related disorder, an IGFR related disorder and a flk related disorder.

32. The method of claim 29 wherein said protein kinase related disorder is a cancer selected from the group consisting of squamous cell carcinoma, astrocytoma, Kaposi's sarcoma, glioblastoma, lung cancer, bladder cancer, head and neck cancer, melanoma, ovarian cancer, prostate cancer, breast cancer, small-cell lung cancer, glioma, colorectal cancer, genitourinary cancer and gastrointestinal cancer.

33. The method of claim 29 wherein said protein kinase

related disorder is selected from the group consisting of diabetes, an autoimmune disorder, a hyperproliferation disorder, restenosis, fibrosis, psoriasis, von Heppel-Lindau disease, osteoarthritis, rheumatoid arthritis, angiogenesis, an inflammatory disorder, an immunological disorder and a cardiovascular disorder.

34. The method of claim 29 wherein said organism is a human.

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ABSTRACT OF THE DISCLOSURE

The present invention relates to pyrrole substituted 2-indolinone compounds and their physiologically acceptable salts which modulate the activity of protein kinases and therefore are expected to be useful in the prevention and treatment of protein kinase related cellular disorders such as cancer.

09570'22L9T209

TRADUCCIÓN

ESTADOS UNIDOS DE AMÉRICA

A quien corresponda:

DEPARTAMENTO DE COMERCIO - OFICINA DE PATENTES Y MARCAS

29 de marzo del 2001

CERTIFICO QUE ADJUNTA A LA PRESENTE SE ENCUENTRA UNA COPIA FIEL DE LOS REGISTROS EN LA OFICINA DE PATENTES Y MARCAS DE LOS ESTADOS UNIDOS DE LOS DOCUMENTOS DE LA SOLICITUD DE PATENTE ABAJO IDENTIFICADA QUE REUNIAN LOS REQUISITOS PARA LA ASIGNACIÓN DE UNA FECHA DE PRESENTACIÓN EN VIRTUD DE 35 USC 111.

SOLICITUD NUMERO: 60/182.710

FECHA DE DEPÓSITO: 15 de febrero del 2000

.En nombre del COMISARIO DE PATENTES Y MARCAS.

(Firmado) E. Bornett Certificaciones.

(La presente certificación se encuentra unida en forma inviolable a sus adjuntos y lleva una oblea dorada que dice): OFICINA DE PATENTES Y MARCAS - ESTADOS UNIDOS DE NORTEAMÉRICA.

Carátula para Presentación Provisional de Patente

Esta solicitud es para presentación de una SOLICITUD DE PATENTE PROVISIONAL bajo el CRF 37, Art. 1.53 (c)

INVENTOR/ES SOLICITANTE/S

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Título de la invención : 2- INDOLINONAS SUSTITUIDAS CON PIRROLES INHIBIDORAS DE PROTEINQUINASAS

Dirección Postal: Nro. de cliente: 22249

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Adjuntos:

Memoria descriptiva: 176 páginas

Forma de pago:

Se autoriza al Comisario a cobrar los aranceles de presentación a través de la cuenta número : 12-2475. Arancel : $150,00

La invención no fue hecha por una agencia del Gobierno de los Estados Unidos ni por contrato con una agencia del Gobierno de los Estados Unidos.

Atentamente, firmado Bernard F. Rose Fecha: 15/02/2000

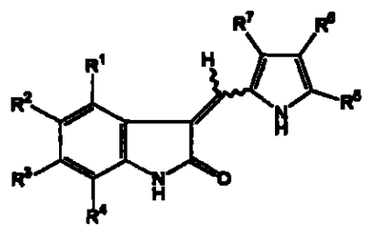
Nro. de registro: 42.112

Teléfono: (408)993-1555

Nro. de expediente: 02421772

REIVINDICACIONES

1. Un compuesto que tiene la estructura química:



donde:

R^1 se selecciona del grupo formado por hidrógeno, halo, alquilo, cicloalquilo, arilo, heteroarilo, heteroalicíclico, hidroxi, alcoxi, $-(CO)R^{15}$, $-NR^{13}R^{14}$, $-(CH_2)_rR^{16}$ y $-C(O)NR^9R^9$;

R^2 se selecciona del grupo formado por hidrógeno, halo, alquilo, trihalometilo, hidroxi, alcoxi, $-NR^{13}R^{14}$, $-NR^{13}C(O)R^{14}$, $-C(O)R^{15}$, arilo, heteroarilo, y $-S(O)_2NR^{13}R^{14}$;

R^3 se selecciona del grupo formado por hidrógeno, halógeno, alquilo, trihalometilo, hidroxi, alcoxi, $-(CO)R^{15}$, $-NR^{13}R^{14}$, arilo, heteroarilo, $-NR^{13}S(O)_2R^{14}$, $-S(O)_2NR^{13}R^{14}$, $-NR^{13}C(O)R^{14}$, y $-NR^{13}C(O)OR^{14}$;

R^4 se selecciona del grupo formado por hidrógeno, halógeno, alquilo, hidroxi, alcoxi y $-NR^{13}R^{14}$;

R^5 se selecciona del grupo formado por hidrógeno, alquilo y $-C(O)R^{10}$;

R^6 se selecciona del grupo formado por hidrógeno, alquilo y $-C(O)R^{10}$;

R^7 se selecciona del grupo formado por hidrógeno, alquilo, arilo, heteroarilo, $-C(O)R^{17}$ y $-C(O)R^{10}$;

R^6 y R^7 se pueden combinar para formar un grupo seleccionado del grupo formado por $-(CH_2)_4-$, $-(CH_2)_5-$ y $-(CH_2)_6-$;

con la condición de que al menos uno de R^5, R^6 o R^7 debe ser $-C(O)R^{10}$;

R^8 y R^9 se seleccionan independientemente del grupo formado por hidrógeno, alquilo y arilo;

R^{10} se selecciona del grupo formado por hidroxi, alcoxi, ariloxi, $-N(R^{11})(CH_2)_nR^{12}$, $-NR^{13}R^{14}$;

R^{11} se selecciona del grupo formado por hidrógeno y alquilo;

R^{12} se selecciona del grupo formado por $-NR^{13}R^{14}$, hidroxi, $-C(O)R^{15}$, arilo y heteroarilo;

R^{13} y R^{14} se seleccionan independientemente del grupo formado por hidrógeno, alquilo, cicloalquilo, arilo y heteroarilo;

R^{13} y R^{14} se pueden combinar para formar un grupo seleccionado del grupo formado por $-(CH_2)_4-$, $-(CH_2)_5-$, $-(CH_2)_2O(CH_2)_2-$, y $-(CH_2)_2N(CH_3)(CH_2)_2-$;

R^{15} se selecciona del grupo formado por hidrógeno, hidroxi, alcoxi y ariloxi;

R^{16} se selecciona del grupo formado por hidroxi, $-C(O)R^{15}$, $-NR^{13}R^{14}$ y $-C(O)NR^{13}R^{14}$;

R^{17} se selecciona del grupo formado por alquilo, cicloalquilo, arilo y heteroarilo; y

n y r son independientemente 1, 2, 3, o 4.

o una sal aceptable para uso farmacéutico de dicho compuesto.

2. El compuesto o sal de la reivindicación 1 en el cual :

R^1 y R^4 son hidrógeno;

R^2 se selecciona del grupo formado por hidrógeno, halo, alcoxi inferior, $-C(O)R^{15}$ y $-S(O)_2NR^{13}R^{14}$;

R^3 se selecciona del grupo formado por hidrógeno, alcoxi inferior, $-C(O)R^{15}$, -

$S(O)_2NR^{13}R^{14}$, arilo y heteroarilo;

R^5 es $-C(O)R^{10}$;

R^6 se selecciona del grupo formado por hidrógeno y alquilo inferior; y

R^7 se selecciona del grupo formado por hidrógeno, alquilo inferior y $-C(O)R^{17}$.

3. El compuesto o sal de la reivindicación 2 en el cual :

R^{10} se selecciona del grupo formado por hidroxi, alcoxi inferior y $-NR^{11}(CH_2)_nR^{12}$, donde

n es 2 o 3;

R^{11} se selecciona del grupo formado por hidrógeno y alquilo inferior; y,

R^{12} se selecciona del grupo formado por arilo y $-NR^{13}R^{14}$.

4. El compuesto o sal de la reivindicación 3, en el cual R^{13} y R^{14} se seleccionan independientemente del grupo formado por hidrógeno, alquilo inferior, y, en combinación,$-(CH_2)_4-$, $-(CH_2)_5-$, $-CH_2)_2O(CH_2)_2-$ o $-(CH_2)_2N(CH_3)(CH_2)_2-$.

5. El compuesto o sal de la reivindicación 1 en el cual :

R^1 se selecciona del grupo formado por hidrógeno, alquilo inferior, $-(CH_2)_nR^{16}$ y $-C(O)NR^8R^8$;

R^2 se selecciona del grupo formado por hidrógeno, halógeno, arilo y $-S(O)_2NR^{13}R^{14}$;

R^3 se selecciona del grupo formado por hidrógeno, alquilo inferior, alcoxi inferior, arilo, heteroarilo y $-C(O)R^{15}$;

R^4 es hidrógeno;

R^5 se selecciona del grupo formado por hidrógeno y alquilo inferior;

R^6 es $-C(O)R^{10}$;

R^7 se selecciona del grupo formado por hidrógeno, alquilo inferior y arilo;

R^{16} se selecciona del grupo formado por hidroxi y $-C(O)R^{15}$; y,

r es 2 o 3.

6. El compuesto o sal de la reivindicación 5, en el cual R^3 es arilo opcionalmente sustituido con uno o más grupos seleccionados del grupo formado por alquilo inferior, alcoxi inferior y halo.

7. El compuesto o sal de la reivindicación 5, en el cual R^{10} se selecciona del grupo formado por hidroxi, alcoxi inferior, $-NR^{13}R^{14}$ y $-NR^{11}(CH_2)_nR^{12}$, donde

n es 1, 2 o 3,

R^{11} es hidrógeno, y

R^{12} se selecciona del grupo formado por hidroxi, alcoxi inferior, $-C(O)R^{15}$, heteroarilo y $-NR^{13}R^{14}$.

8. El compuesto o sal de la reivindicación 7 en el cual R^{13} y R^{14} se seleccionan independientemente del grupo formado por hidrógeno, alquilo inferior, heteroarilo y, en combinación, $-(CH_2)_4-$, $-(CH_2)_5-$, $-(CH_2)_2O(CH_2)_2-$, o $-(CH_2)_2N(CH_3)(CH_2)_2-$.

9. El compuesto o sal de la reivindicación 5, en el cual R^1 es $-C(O)NR^8R^9$, donde;

R^8 es hidrógeno; y

R^9 es arilo opcionalmente sustituido con uno o más grupos seleccionados del grupo formado por halo, hidroxi y alcoxi inferior.

10. El compuesto de la reivindicación 1, en el cual:

R^1 se selecciona del grupo formado por hidrógeno y alquilo inferior;

R^2 se selecciona del grupo formado por hidrógeno, halo, alcoxi inferior, arilo, $-C(O)R^{15}$ y $-S(O)_2NR^{13}R^{14}$;

R^3 se selecciona del grupo formado por hidrógeno, halo, arilo, heteroarilo y –

$C(O)R^{15}$;

R^4 es hidrógeno;

R^5 es $-C(O)R^{10}$; y,

R^6 y R^7 se combinan para formar un grupo $-(CH_2)_4-$.

11. El compuesto de la reivindicación 10, en el cual:

R^{10} se selecciona del grupo formado por hidroxi, alcoxi,$-NR^{13}R^{14}$ y $-NH(CH_2)_nNR^{13}R^{14}$; y

donde n es 2 o 3.

12. El compuesto de la reivindicación 11, en el cual R^{13} y R^{14} se seleccionan independientemente del grupo formado por hidrógeno, alquilo inferior, y, en combinación, $-(CH_2)_4-$, $-(CH_2)_5-$, $-(CH_2)_2O(CH_2)_2-$ o $-(CH_2)_2N(CH_3)(CH_2)_2-$.

13. El compuesto de la reivindicación 1, en el cual:

R^1 y R^4 se seleccionan del grupo formado por hidrógeno, alquilo inferior, alcoxi inferior y halógeno;

R^2 se selecciona del grupo formado por hidrógeno, alquilo inferior, alcoxi inferior, halo y $-S(O)_2NR^{13}R^{14}$;

R^3 se selecciona del grupo formado por hidrógeno, alquilo inferior, alcoxi inferior, halo, arilo y $-C(O)R^{15}$;

R^5 y R^6 se seleccionan del grupo formado por hidrógeno y alquilo inferior; y

R^7 es $-C(O)R^{10}$

14. El compuesto de la reivindicación 13, en el cual:

R^{10} se selecciona del grupo formado por hidroxi, alcoxi, $-NR^{13}R^{14}$ y $-NR^{11}(CH_2)_nR^{12}$;

donde

n es 2 o 3;

R^{11} se selecciona del grupo formado por hidrógeno y alquilo inferior; y

R^{12} es $-NR^{13}R^{14}$;donde

R^{13} y R^{14} se seleccionan independientemente del grupo formado por hidrógeno, alquilo inferior, y, en combinación,$-(CH_2)_4-$, $-(CH_2)_5-$, $-CH_2)_2O(CH_2)_2-$ o $-(CH_2)_2N(CH_3)(CH_2)_2-$.

15. Una composición farmacéutica, que comprende un compuesto o sal de la reivindicación 1; y

un vehículo o excipiente aceptable para uso farmacéutico.

16. Un método para la modulación de la actividad catalítica de una proteinquinasa que comprende poner en contacto dicha proteinquinasa con un compuesto o sal de la reivindicación 1.

17. El método de la reivindicación 16 en el cual dicha proteinqüinasa se selecciona del grupo formado por un receptor de tirosinquinasa, un no receptor de tirosinquinasa y una serin-treoninquinasa.

18. Un método para tratar o prevenir una afección relacionada con la proteinquinasa en un organismo que comprende administrar una cantidad eficaz en términos terapéuticos de un compuesto o sal de la reivindicación 1 a dicho organismo.

19. El método de la reivindicación 18 en el cual dicha afección relacionada con la proteinquinasa se selecciona del grupo formado por una afección relacionada con un receptor de tirosinquinasa , una afección relacionada con un no receptor de tirosinquinasa y una afección relacionada con una serin-treoninquinasa.

20. El método de la reivindicación 18 en el cual dicha afección relacionada con una proteinquinasa se selecciona del grupo formado por una afección relacionada con los EGFR, una afección relacionada con los PDGFR, una afección relacionada con los IGFR y una afección relacionada con los FLK.

21. El método de la reivindicación 18 en el cual dicha afección relacionada con la proteinquinasa es un cáncer seleccionado del grupo formado por carcinoma de células escamosas, astrocitoma, sarcoma de Kaposi, glioblastoma, cáncer de pulmón, cáncer de vejiga, cáncer de cabeza y cuello, melanoma, cáncer de ovarios, cáncer de próstata, cáncer de mama, cáncer de células pequeñas de pulmón, glioma, cáncer colorrectal, cáncer genitourinario y cáncer gastrointestinal.

22. El método de la reivindicación 18 en el cual dicha afección relacionada con la proteinquinasa se selecciona del grupo formado por diabetes, un trastorno autoinmune , un trastorno hiperproliferativo, restenosis, fibrosis, psoriasis, enfermedad de von Heppel-Lindau, osteoartritis, artritis reumatoide, angiogenesis, un trastorno inflamatorio, una enfermedad inmunológica y una enfermedad cardiovascular.

23. El método de la reivindicación 18 en el cual dicho organismo es un ser humano.

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APPLICATION NUMBER: ***60/243,532***
FILING DATE: ***October 27, 2000***

By Authority of the
COMMISSIONER OF PATENTS AND TRADEMARKS

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Atty. Dkt. No. 038602/0997

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Peng Cho TANG *et al.*

Appl. No.: To be assigned

Filing Date: October 27, 2000

Examiner: To be assigned

Art Unit: To be assigned

PYRROLE SUBSTITUTED 2-INDOLINONE PROTEIN KINASE INHIBITORS

PROVISIONAL PATENT APPLICATION TRANSMITTAL

Commissioner for Patents
Box PROVISIONAL PATENT APPLICATION
Washington, D.C. 20231

Sir:

Transmitted herewith for filing under 37 C.F.R. § 1.53(c) is the provisional patent application of:

Peng Cho TANG
Todd MILLER
Xiaoyuan LI
Connie SUN
Chung Chen WEI
Sheri SHIRAZIAN

Enclosed are:

[X] Specification, Claim(s), and Abstract (186 pages).

[ ] Informal drawings (sheets, Figures).

[ ] Assignment of the invention to

[ ] Assignment Recordation Cover Sheet.

[ ] Check in the amount of $40.00 for Assignment recordation.

[ ] Small Entity statement.

Atty. Dkt. No. 038602/0897

The filing fee is calculated below:

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Basic Fee		$150.00		$150.00
[ ]	Small Entity Fees Apply (subtract ½ of above):		=	$ 00.00
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[X] The Commissioner is hereby authorized to charge any additional fees which may be required regarding this application under 37 C.F.R. §§ 1.16-1.17, or credit any overpayment, to Deposit Account No. 19-0741. Should no proper payment be enclosed herewith, as by a check being in the wrong amount, unsigned, post-dated, otherwise improper or informal or even entirely missing, the Commissioner is authorized to charge the unpaid amount to Deposit Account No. 19-0741.

Please direct all correspondence to the undersigned attorney or agent at the address indicated below.

Respectfully submitted,

Date October 27, 2000

By _____

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PYRROLE SUBSTITUTED 2-INDOLINONE PROTEIN KINASE INHIBITORS

U.S. Application Serial No. 60/182,710, filed February 15, 2000, is hereby incorporated by reference in its entirety.

INTRODUCTION

The present invention relates generally to organic chemistry, biochemistry, pharmacology and medicine. More particularly, it relates to pyrrole substituted 2-indolinone compounds, and their physiologically acceptable salts and prodrugs, which modulate the activity of protein kinases ("PKs") and are expected to exhibit a salutary effect against disorders related to abnormal PK activity.

BACKGROUND OF THE INVENTION

The following is offered as background information only and is not admitted to be prior art to the present invention.

PKs are enzymes that catalyze the phosphorylation of hydroxy groups on tyrosine, serine and threonine residues of proteins. The consequences of this seemingly simple activity are staggering; cell growth, differentiation and proliferation, i.e., virtually all aspects of cell life in one way or another depend on PK activity. Furthermore, abnormal PK activity has been related to a host of disorders, ranging from relatively non-life threatening diseases such as psoriasis to extremely virulent diseases such as glioblastoma (brain cancer).

The PKs can be conveniently broken down into two classes, the protein tyrosine kinases (PTKs) and the serine-threonine kinases (STKs).

One of the prime aspects of PTK activity is their involvement with growth factor receptors. Growth factor receptors are cell-surface proteins. When bound by a growth factor ligand, growth factor receptors are converted to an active form which interacts with proteins on the inner surface of a cell membrane. This leads to phosphorylation on tyrosine residues of the

receptor and other proteins and to the formation inside the cell of complexes with a variety of cytoplasmic signaling molecules that, in turn, effect numerous cellular responses such as cell division (proliferation), cell differentiation, cell growth, expression of metabolic effects to the extracellular microenvironment, etc. For a more complete discussion, see Schlessinger and Ullrich, Neuron, 9:303-391 (1992) which is incorporated by reference, including any drawings, as if fully set forth herein.

Growth factor receptors with PTK activity are known as receptor tyrosine kinases ("RTKs"). They comprise a large family of transmembrane receptors with diverse biological activity. At present, at least nineteen (19) distinct subfamilies of RTKs have been identified. An example of these is the subfamily designated the "HER" RTKs, which include EGFR (epithelial growth factor receptor), HER2, HER3 and HER4. These RTKs consist of an extracellular glycosylated ligand binding domain, a transmembrane domain and an intracellular cytoplasmic catalytic domain that can phosphorylate tyrosine residues on proteins.

Another RTK subfamily consists of insulin receptor (IR), insulin-like growth factor I receptor (IGF-1R) and insulin receptor related receptor (IRR). IR and IGF-1R interact with insulin, IGF-I and IGF-II to form a heterotetramer of two entirely extracellular glycosylated α subunits and two β subunits which cross the cell membrane and which contain the tyrosine kinase domain.

A third RTK subfamily is referred to as the platelet derived growth factor receptor ("PDGFR") group, which includes PDGFRα, PDGFRβ, CSFIR, c-kit and c-fms. These receptors consist of glycosylated extracellular domains composed of variable numbers of immunoglobin-like loops and an intracellular domain wherein the tyrosine kinase domain is interrupted by unrelated amino acid sequences.

Another group which, because of its similarity to the PDGFR subfamily, is sometimes subsumed into the later group is the fetus liver kinase ("flk") receptor subfamily. This group is believed to be made up of kinase insert domain-receptor fetal liver kinase-1 (KDR/FLK-1, VEGF-R2), flk-1R, flk-4 and fms-like tyrosine kinase 1 (flt-1).

A further member of the tyrosine kinase growth factor receptor family is the fibroblast growth factor ("FGF") receptor subgroup. This group consists of four receptors, FGFR1-4, and seven ligands, FGF1-7. While not yet well defined, it appears that the receptors consist of a glycosylated extracellular domain containing a variable number of immunoglobin-like loops and an intracellular domain in which the tyrosine kinase sequence is interrupted by regions of unrelated amino acid sequences.

Still another member of the tyrosine kinase growth factor receptor family is the vascular endothelial growth factor (VEGF") receptor subgroup. VEGF is a dimeric glycoprotein similar to PDGF but has different biological functions and target cell specificity in vivo. In particular, VEGF is presently thought to play an essential role is vasculogenesis and angiogenesis.

A more complete listing of the known RTK subfamilies is described in Plowman et al., DN&P, 7(6):334-339 (1994) which is incorporated by reference, including any drawings, as if fully set forth herein.

In addition to the RTKs, there also exists a family of entirely intracellular PTKs called "non-receptor tyrosine kinases" or "cellular tyrosine kinases." This latter designation, abbreviated "CTK," will be used herein. CTKs do not contain extracellular and transmembrane domains. At present, over 24 CTKs in 11 subfamilies (Src, Frk, Btk, Csk, Abl, Zap70, Fes, Fps, Fak, Jak and Ack) have been identified. The Src subfamily appear so far to be the largest group of CTKs and includes Src, Yes, Fyn, Lyn, Lck, Blk, Hck, Fgr and Yrk. For a

more detailed discussion of CTKs, see Bolen, Oncogene, 8:2025-2031 (1993), which is incorporated by reference, including any drawings, as if fully set forth herein.

The serine/threonine kinases, STKs, like the CTKs, are predominantly intracellular although there are a few receptor kinases of the STK type. STKs are the most common of the cytosolic kinases; i.e., kinases that perform their function in that part of the cytoplasm other than the cytoplasmic organelles and cytoskelton. The cytosol is the region within the cell where much of the cell's intermediary metabolic and biosynthetic activity occurs; e.g., it is in the cytosol that proteins are synthesized on ribosomes.

RTKs, CTKs and STKs have all been implicated in a host of pathogenic conditions including, significantly, cancer. Other pathogenic conditions which have been associated with PTKs include, without limitation, psoriasis, hepatic cirrhosis, diabetes, angiogenesis, restenosis, ocular diseases, rheumatoid arthritis and other inflammatory disorders, immunological disorders such as autoimmune disease, cardiovascular disease such as atherosclerosis and a variety of renal disorders.

With regard to cancer, two of the major hypotheses advanced to explain the excessive cellular proliferation that drives tumor development relate to functions known to be PK regulated. That is, it has been suggested that malignant cell growth results from a breakdown in the mechanisms that control cell division and/or differentiation. It has been shown that the protein products of a number of proto-oncogenes are involved in the signal transduction pathways that regulate cell growth and differentiation. These protein products of proto-oncogenes include the extracellular growth factors, transmembrane growth factor PTK receptors (RTKs), cytoplasmic PTKs (CTKs) and cytosolic STKs, discussed above.

In view of the apparent link between PK-related cellular

activities and wide variety of human disorders, it is no surprise that a great deal of effort is being expended in an attempt to identify ways to modulate PK activity. Some of this effort has involved biomimetic approaches using large molecules patterned on those involved in the actual cellular processes (e.g., mutant ligands (U.S. App. No. 4,966,849); soluble receptors and antibodies (App. No. WO 94/10202, Kendall and Thomas, Proc. Nat'l Acad. Sci., 90:10705-09 (1994), Kim, et al., Nature, 362:841-844 (1993)); RNA ligands (Jelinek, et al., Biochemistry, 33:10450-56); Takano, et al., Mol. Bio. Cell 4:358A (1993); Kinsella, et al., Exp. Cell Res. 199:56-62 (1992); Wright, et al., J. Cellular Phys., 152:448-57) and tyrosine kinase inhibitors (WO 94/03427; WO 92/21660; WO 91/15495; WO 94/14808; U.S. Pat. No. 5,330,992; Mariani, et al., Proc. Am. Assoc. Cancer Res., 35:2268 (1994)).

In addition to the above, attempts have been made to identify small molecules which act as PK inhibitors. For example, bis- monocylic, bicyclic and heterocyclic aryl compounds (PCT WO 92/20642), vinyleneazaindole derivatives (PCT WO 94/14808) and 1-cyclopropyl-4-pyridylquinolones (U.S. Pat. No. 5,330,992) have been described as tyrosine kinase inhibitors. Styryl compounds (U.S. Pat. No. 5,217,999), styryl-substituted pyridyl compounds (U.S. Pat. No. 5,302,606), quinazoline derivatives (EP App. No.0 566 266 A1), selenaindoles and selenides (PCT WO 94/03427), tricyclic polyhydroxylic compounds (PCT WO 92/21660) and benzylphosphonic acid compounds (PCT WO 91/15495) have all been described as PTK inhibitors useful in the treatment of cancer.

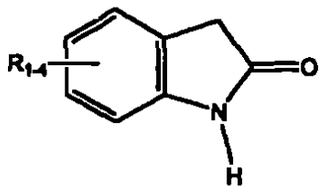
SUMMARY OF THE INVENTION

Our own efforts to identify small organic molecules which modulate PK activity and which, therefore, are expected to be useful in the treatment and prevention of disorders involving abnormal PK activity, has led us to the discovery of a family of pyrrole substituted 2-indolinone compounds, and their prodrugs

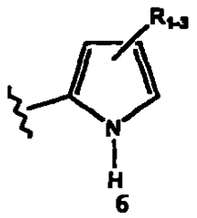
and physiologically acceptable salts, which exhibit PK modulating ability and are thereby expected to have a salutary effect against disorders related to abnormal PK activity.

Thus, the present invention relates generally to pyrrole substituted 2-indolinones and their prodrugs and physiologically acceptable salts, which modulate the activity of receptor tyrosine kinases (RTKs), non-receptor protein tyrosine kinases (CTKs) and serine/threonine protein kinases (STKs). In addition, the present invention relates to the preparation and use of pharmaceutical compositions of the disclosed compounds and their physiologically acceptable salts and prodrugs in the treatment or prevention of PK driven disorders such as, by way of example and not limitation, cancer, diabetes, hepatic cirrhosis, cardiovasacular disease such as atherosclerosis, angiogenesis, immunological disease such as autoimmune disease and renal disease.

The terms "2-indolinone," indolin-2-one and "2-oxindole" are used interchangeably herein to refer to a molecule having the chemical structure:



A "pyrrole" refers to a molecule having the chemical structure:



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"Pyrrole substituted 2-indolinone" and "3-pyrrolidenyl-2-indolinone" are used interchangeably herein to refer to a chemical compound having the general structure shown in Formula 1.

A "pharmaceutical composition" refers to a mixture of one or more of the compounds described herein, or physiologically acceptable salts or prodrugs thereof, with other chemical components, such as physiologically acceptable carriers and excipients. The purpose of a pharmaceutical composition is to facilitate administration of a compound to an organism.

A "prodrug" refers to an agent which is converted into the parent drug in vivo. Prodrugs are often useful because, in some situations, they may be easier to administer than the parent drug. They may, for instance, be bioavailable by oral administration whereas the parent drug is not. The prodrug may also have improved solubility in pharmaceutical compositions over the parent drug. An example, without limitation, of a prodrug would be a compound of the present invention which is administered as an ester (the "prodrug") to facilitate transmittal across a cell membrane where water solubility is detrimental to mobility but then is metabolically hydrolyzed to the carboxylic acid, the active entity, once inside the cell where water solubility is beneficial.

A further example of a prodrug might be a short polypeptide, for example, without limitation, a 2 - 10 amino acid polypeptide, bonded through a terminal amino group to a carboxy group of a compound of this invention wherein the polypeptide is hydrolyzed or metabolized in vivo to release the active molecule.

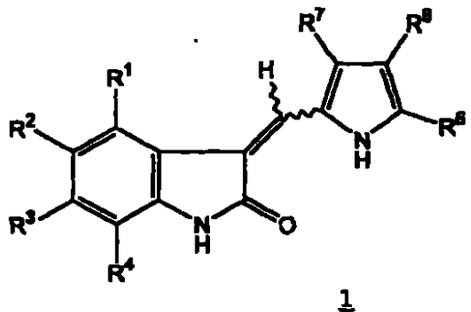
As used herein, a "physiologically acceptable carrier" refers to a carrier or diluent that does not cause significant irritation to an organism and does not abrogate the biological activity and properties of the administered compound.

An "excipient" refers to an inert substance added to a pharmaceutical composition to further facilitate administration of a compound. Examples, without limitation, of excipients include calcium carbonate, calcium phosphate, various sugars and types of starch, cellulose derivatives, gelatin, vegetable oils and polyethylene glycols.

1. **CHEMISTRY**

A. General Structural Features.

In one aspect, the present invention relates to pyrrole substituted 2-indolinones having the following chemical structure:



1

wherein:

R^1 is selected from the group consisting of hydrogen, halo, alkyl, cyclkoalkyl, aryl, heteroaryl, heteroalicyclic, hydroxy, alkoxy, $-C(O)R^{15}$, $-NR^{13}R^{14}$, $-(CH_2)_rR^{16}$ and $-C(O)NR^8R^9$;

R^2 is selected from the group consisting of hydrogen, halo, alkyl, trihalomethyl, hydroxy, alkoxy, $-NR^{13}R^{14}$, $-NR^{13}C(O)R^{14}$, $-C(O)R^{15}$, aryl, heteroaryl and $-S(O)_2NR^{13}R^{14}$;

R^3 is selected from the group consisting of hydrogen, halogen, alkyl, trihalomethyl, hydroxy, alkoxy, $-C(O)R^{15}$, $-NR^{13}R^{14}$, aryl, heteroaryl, $-NR^{13}S(O)_2R^{14}$, $-S(O)_2NR^{13}R^{14}$, $-NR^{13}C(O)R^{14}$, and $-NR^{13}C(O)OR^{14}$;

R^4 is selected from the group consisting of hydrogen, halogen, alkyl, hydroxy, alkoxy and $-NR^{13}R^{14}$;

is selected from the group consisting of hydrogen, alkyl and $-C(O)R^{10}$;

R^6 is selected from the group consisting of hydrogen, alkyl and $-C(O)R^{10}$;

R^7 is selected from the group consisting of hydrogen, alkyl, aryl, heteroaryl, $-C(O)R^{17}$ and $-C(O)R^{10}$;

R^6 and R^7 may combine to form a group selected from the group consisting of $-(CH_2)_4-$, $-(CH_2)_5-$ and $-(CH_2)_6-$;

with the proviso that at least one of R^5, R^6 or R^7 must be $-C(O)R^{10}$;

R^8 and R^9 are independently selected from the group consisting of hydrogen, alkyl and aryl;

R^{10} is selected from the group consisting of hydroxy, alkoxy, aryloxy, $-N(R^{11})(CH_2)_nR^{12}$ and $-NR^{13}R^{14}$;

R^{11} is selected from the group consisting of hydrogen and alkyl;

R^{12} is selected from the group consisting of $-NR^{13}R^{14}$, hydroxy, $-C(O)R^{15}$, aryl and heteroaryl;

R^{13} and R^{14} are independently selected from the group consisting of hydrogen, alkyl, cycloalkyl, aryl and heteroaryl;

R^{13} and R^{14} may combine to form a group selected from the group consisting of $-(CH_2)_4-$, $-(CH_2)_5-$, $-(CH_2)_2O(CH_2)_2-$, and $-(CH_2)_2N(CH_3)(CH_2)_2-$;

R^{15} is selected from the group consisting of hydrogen, hydroxy, alkoxy and aryloxy;

R^{16} is selected from the group consisting of hydroxy, $-C(O)R^{15}$, $-NR^{13}R^{14}$ and $C(O)NR^{13}R^{14}$;

R^{17} is selected from the group consisting of alkyl, cycloalkyl, aryl and heteroaryl; and,

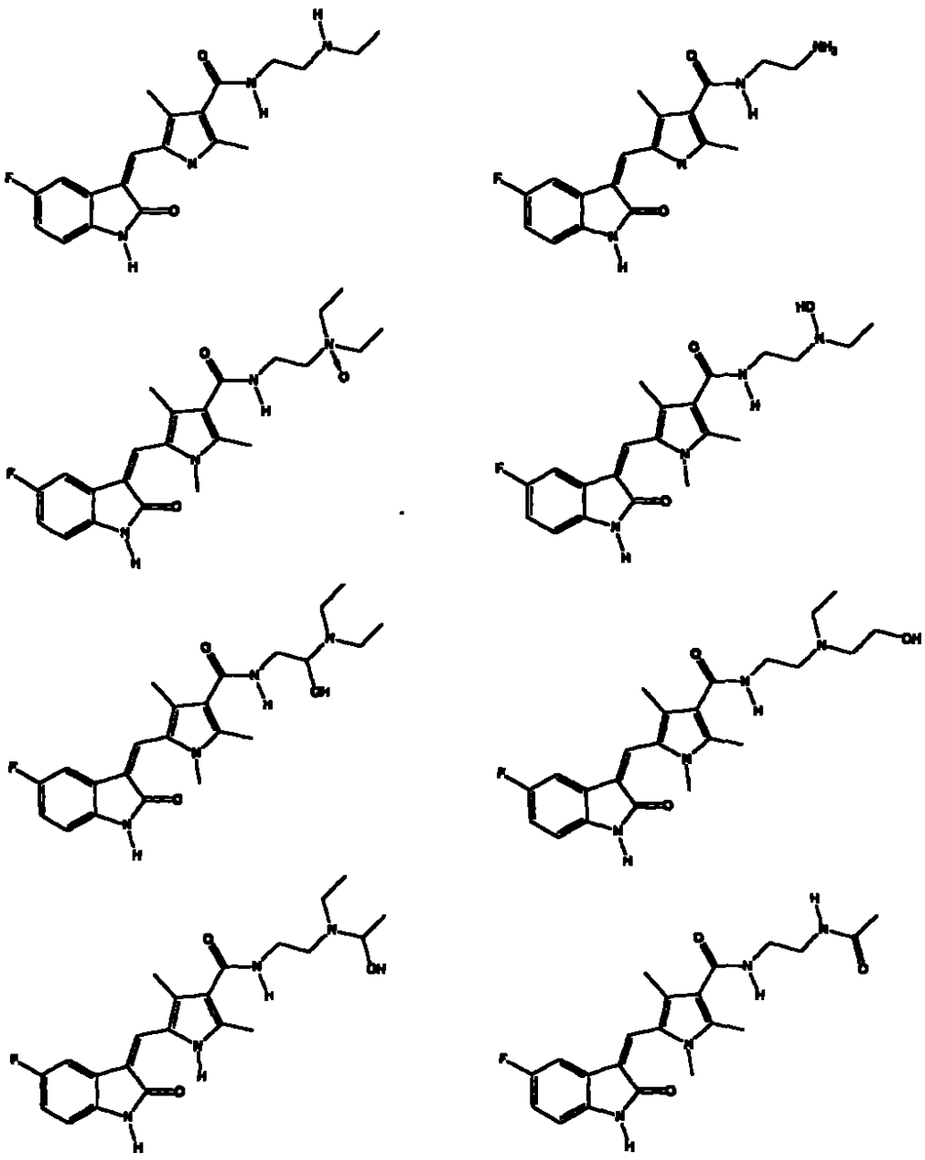
n and r are independently 1, 2, 3, or 4.

Metabolites of compounds of the preferred embodiments of the present invention are within the scope of this invention. For example, *in vivo* and *in vitro* metabolism studies indicted that

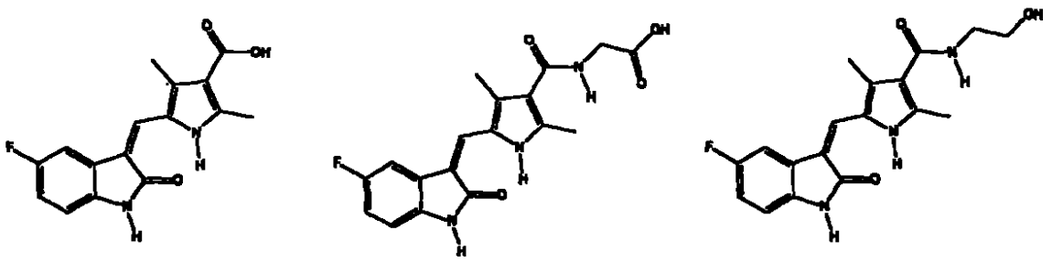
eight metabolites are formed from the metabolism of 5-(5-fluoro-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (2-diethylamino-ethyl)amide (the compound of Example 80). CYP3A4 was shown to be the major metabolic enzyme *in vitro* in human hepatic microsomes. The primary metabolite produced by this enzyme was formed by mouse, rat, dog and monkey microsomes as well as human microsomes. This metabolite was also identified in the plasma of rats, dogs and monkeys following oral administration of 5-(5-fluoro-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (2-diethylamino-ethyl)amide. The primary metabolite has similar potency to 5-(5-Fluoro-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (2-diethylamino-ethyl)amide in biochemical tyrosine kinase and cellular proliferation assays. The possible metabolites of 5-(5-fluoro-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (2-diethylamino-ethyl)amide include:

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In addition, the following metabolites may also occur and may have similar biological activity relative to 5-(5-fluoro-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (2-diethylamino-ethyl)amide:



An additional aspect of this invention is a pharmaceutically acceptable salt of a compound hereof.

As used herein, the term "pharmaceutically acceptable salt" refers to those salts which retain the biological effectiveness and properties of the compound. Pharmaceutically acceptable salts are often useful because they may have improved stability and/or solubility in pharmaceutical compositions over the free base form of the compound. A pharmaceutically acceptable salt is obtained by reaction of the free base with inorganic acids such as hydrochloric acid, hydrobromic acid, nitric acid, phosphoric acid, sulfuric acid, and perhcloric acid and the like, or with organic acids such as acetic acid, oxalic acid, malic acid, maleic acid, methanesulfonic acid, ethanesulfonic acid, *p*-toluenesulfonic acid, salicylic acid, tartaric acid, citric acid, succinic acid or malonic acid and the like.

A particularly preferred pharmaceutically acceptable salt is the L-malate salt of the free base form of the compound. The L-malate salt of 5-(5-fluoro-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid(2-diethylaminoethyl)amide is specifically contemplated by this invention.

As used herein, the term "alkyl" refers to a saturated aliphatic hydrocarbon including straight chain and branched chain groups. Preferably, the alkyl group has 1 to 20 carbon atoms . (whenever a numerical range; e.g. "1-20", is stated herein, it means that the group, in this case the alkyl group, may contain 1 carbon atom, 2 carbon atoms, 3 carbon atoms, etc. up to and

including 20 carbon atoms). More preferably, it is a medium size alkyl having 1 to 10 carbon atoms. Most preferably, it is a lower alkyl having 1 to 4 carbon atoms. The alkyl group may be substituted or unsubstituted. When substituted, the substituent group(s) is preferably one or more independently selected from the group consisting of halo, hydroxy, unsubstituted lower alkoxy, aryl optionally substituted with one or more halo, hydroxy, unsubstituted lower alkyl or unsubstituted lower alkoxy groups, aryloxy optionally substituted with one or more halo, hydroxy, unsubstituted lower alkyl or unsubstituted lower alkoxy groups, 6-member heteroaryl having from 1 to 3 nitrogen atoms in the ring, the carbons in the ring being optionally substituted with one or more halo, hydroxy, unsubstituted lower alkyl or unsubstituted lower alkoxy groups, 5-member heteroaryl having from 1 to 3 heteroatoms selected from the group consisting of nitrogen, oxygen and sulfur, the carbon atoms of the group being optionally substituted with one or more halo, hydroxy, unsubstituted lower alkyl or unsubstituted lower alkoxy groups, 5- or 6-member heteroalicyclic group having from 1 to 3 heteroatoms selected from the group consisting of nitrogen, oxygen and sulfur, the carbon and nitrogen (if present) atoms in the group being optionally substituted with one or more halo, hydroxy, unsubstituted lower alkyl or unsubstituted lower alkoxy groups, mercapto, (unsubstituted lower alkyl)thio, arylthio optionally substituted with one or more halo, hydroxy, unsubstituted lower alkyl or unsubstituted lower alkoxy groups, cyano, acyl, thioacyl, O-carbamyl, N-carbamyl, O-thiocarbamyl, N-thiocarbamyl, C-amido, N-amido, nitro, N-sulfonamido, S-sulfonamido, $R^{18}S(O)$-, $R^{18}S(O)_2$-, $-C(O)OR^{18}$, $R^{18}C(O)O$-, and $-NR^{18}R^{19}$, wherein R^{18} and R^{19} are independently selected from the group consisting of hydrogen, unsubstituted lower alkyl, trihalomethyl, unsubstituted (C_3-C_6)cycloalkyl, unsubstituted lower alkenyl, unsubstituted lower alkynyl and aryl optionally optionally substituted with one or more halo, hydroxy, unsubstituted lower alkyl or unsubstituted lower alkoxy groups.

A "cycloalkyl" group refers to a 3 to 8 member all-carbon

monocyclic ring, an all-carbon 5-member/6-member or 6-member/6-member fused bicyclic ring or a multicyclic fused ring (a "fused" ring system means that each ring in the system shares an adjacent pair of carbon atoms with each other ring in the system) group wherein one or more of the rings may contain one or more double bonds but none of the rings has a completely conjugated pi-electron system. Examples, without limitation, of cycloalkyl groups are cyclopropane, cyclobutane, cyclopentane, cyclopentene, cyclohexane, cyclohexadiene, adamantane, cycloheptane and, cycloheptatriene. A cycloalkyl group may be substituted or unsubstituted. When substituted, the substituent group(s) is preferably one or more independently selected from the group consisting of unsubstituted lower alkyl, trihaloalkyl, halo, hydroxy, unsubstituted lower alkoxy, aryl optionally substituted with one or more halo, hydroxy, unsubstituted lower alkyl or unsubstituted lower alkoxy groups, aryloxy optionally substituted with one or more halo, hydroxy, unsubstituted lower alkyl or unsubstituted lower alkoxy groups, 6-member heteroaryl having from 1 to 3 nitrogen atoms in the ring, the carbons in the ring being optionally substituted with one or more halo, hydroxy, unsubstituted lower alkyl or unsubstituted lower alkoxy groups, 5-member heteroaryl having from 1 to 3 heteroatoms selected from the group consisting of nitrogen, oxygen and sulfur, the carbon atoms of the group being optionally substituted with one or more halo, hydroxy, unsubstituted lower alkyl or unsubstituted lower alkoxy groups, 5- or 6-member heteroalicyclic group having from 1 to 3 heteroatoms selected from the group consisting of nitrogen, oxygen and sulfur, the carbon and nitogen (if present)atoms in the group being optionally substituted with one or more halo, hydroxy, unsubstituted lower alkyl or unsubstituted lower alkoxy groups, mercapto,(unsubstituted lower alkyl)thio, arylthio optionally substituted with one or more halo, hydroxy, unsubstituted lower alkyl or unsubstituted lower alkoxy groups,

cyano, acyl, thioacyl, O-carbamyl, N-carbamyl, O-thiocarbamyl, N-thiocarbamyl, C-amido, N-amido, nitro, N-sulfonamido, S-sulfonamido, $R^{18}S(O)$-, $R^{18}S(O)_2$-, $-C(O)OR^{18}$, $R^{18}C(O)O$-, and $-NR^{18}R^{19}$ are as defined above.

An "alkenyl" group refers to an alkyl group, as defined herein, consisting of at least two carbon atoms and at least one carbon-carbon double bond.

An "alkynyl" group refers to an alkyl group, as defined herein, consisting of at least two carbon atoms and at least one carbon-carbon triple bond.

An "aryl" group refers to an all-carbon monocyclic or fused-ring polycyclic (i.e., rings which share adjacent pairs of carbon atoms) groups having a completely conjugated pi-electron system. Examples, without limitation, of aryl groups are phenyl, naphthalenyl and anthracenyl. The aryl group may be substituted or unsubstituted. When substituted, the substituted group(s) is preferably one or more independently selected from the group consisting of unsubstituted lower alkyl, trihaloalkyl, halo, hydroxy, unsubstituted lower alkoxy, mercapto, (unsubstituted lower alkyl)thio, cyano, acyl, thioacyl, O-carbamyl, N-carbamyl, O-thiocarbamyl, N-thiocarbamyl, C-amido, N-amido, nitro, N-sulfonamido, S-sulfonamido, $R^{18}S(O)$-, $R^{18}S(O)_2$-, $-C(O)OR^{18}$, $R^{18}C(O)O$-, and $-NR^{18}R^{19}$, with R^{18} and R^{19} as defined above.

As used herein, a "heteroaryl" group refers to a monocyclic or fused ring (i.e., rings which share an adjacent pair of atoms) group having in the ring(s) one or more atoms selected from the group consisting of nitrogen, oxygen and sulfur and, in addition, having a completely conjugated pi-electron system. Examples, without limitation, of heteroaryl groups are pyrrole, furan, thiophene, imidazole, oxazole, thiazole, pyrazole, pyridine, pyrimidine, quinoline, isoquinoline, purine and carbazole. The heteroaryl group may be substituted or unsubstituted. When substituted, the substituted group(s) is preferably one or more independently selected from the group consisting of unsubstituted lower alkyl, trihaloalkyl, halo, hydroxy, unsubstituted lower

alkoxy, mercapto, (unsubstituted lower alkyl)thio, cyano, acyl, thioacyl, O-carbamyl, N-carbamyl, O-thiocarbamyl, N-thiocarbamyl, C-amido, N-amido, nitro, N-sulfonamido, S-sulfonamido, $R^{18}S(O)$-, $R^{18}O)_2$-, -C(O)OR^{18}, $R^{18}C(O)O$-, and -$NR^{18}R^{19}$, with R^{18} and R^{19} as defined above.

A "heteroalicyclic" group refers to a monocyclic or fused ring group having in the ring(s) one or more atoms selected from the group consisting of nitrogen, oxygen and sulfur. The rings may also have one or more double bonds. However, the rings do not have a completely conjugated pi-electron system. The heteroalicyclic ring may be substituted or unsubstituted. When substituted, the substituted group(s) is preferably one or more independently selected from the group consisting of unsubstituted lower alkyl, trihaloalkyl, halo, hydroxy, unsubstituted lower alkoxy, mercapto, (unsubstituted lower alkyl)thio, cyano, acyl, thioacyl, O-carbamyl, N-carbamyl, O-thiocarbamyl, N-thiocarbamyl, C-amido, N-amido, nitro, N-sulfonamido, S-sulfonamido, $R^{18}S(O)$-, $R^{18}S(O)_2$-, -C(O)OR^{18}, $R^{18}C(O)O$-, and -$NR^{18}R^{19}$, with R^{18} and R^{19} as defined above.

A "hydroxy" group refers to an -OH group.

An "alkoxy" group refers to both an -O-(unsubstituted alkyl) and an -O-(unsubstituted cycloalkyl) group.

An "aryloxy" group refers to both an -O-aryl and an -O-heteroaryl group, as defined herein.

A "mercapto" group refers to an -SH group.

A "alkylthio" group refers to both an S-(unsubstituted alkyl) and an -S-(unsubstituted cycloalkyl) group.

A "arylthio" group refers to both an -S-aryl and an -S-heteroaryl group, as defined herein.

An "acyl" group refers to a -C(O)-R" group, where R" is selected from the group consisting of hydrogen, unsubstituted lower alkyl, trihalomethyl, unsubstituted cycloalkyl, aryl optionally substituted with one or more unsubstituted lower alkyl, trihalomethyl, unsubstituted lower alkoxy, halo and -$NR^{18}R^{19}$ groups, heteroaryl (bonded through a ring carbon)

optionally substituted with one or more unsubstituted lower alkyl, trihaloalkyl, unsubstituted lower alkoxy, halo and $-NR^{18}R^{19}$ groups and heteroalicyclic (bonded through a ring carbon) optionally substituted with one or more unsubstituted lower alkyl, trihaloalkyl, unsubstituted lower alkoxy, halo and $-NR^{18}R^{19}$ groups.

An "aldehyde" group refers to an acyl group in which R" is hydrogen.

A "thioacyl" group refers to a $-C(S)-R"$ group, with R" as defined herein.

An "ester" group refers to a $-C(O)O-R"$ group with R" as defined herein except that R" cannot be hydrogen.

An "acetyl" group refers to a $-C(O)CH_3$ group.

A "halo" group refers to fluorine, chlorine, bromine or iodine.

A "trihalomethyl" group refers to a $-CX_3$ group wherein X is a halo group as defined herein.

A "trihalomethanesulfonyl" group refers to a $X_3CS(=O)_2-$ groups with X as defined above.

A "cyano" group refers to a $-C\equiv N$ group.

A "methylenedioxy" group refers to a $-OCH_2O-$ group where the two oxygen atoms are bonded to adjacent carbon atoms.

An "ethylenedioxy" group refers to a $-OCH_2CH_2O-$ where the two oxygen atoms are bonded to adjacent carbon atoms.

An "S-sulfonamido" group refers to a $-S(O)_2NR^{18}R^{19}$ group, with R^{18} and R^{19} as defined herein.

An "N-sulfonamido" group refers to a $-NR^{18}S(O)_2R^{19}$ group, with R^{18} and R^{19} as defined herein.

An "O-carbamyl" group refers to a $-OC(O)NR^{18}R^{19}$ group with R^{18} and R^{19} as defined herein.

An "N-carbamyl" group refers to an $R^{18}OC(O)NR^{19}-$ group, with R^{18} and R^{19} as defined herein.

An "O-thiocarbamyl" group refers to a $-OC(S)NR^{18}R^{19}$ group

with R^{18} and R^{19} as defined herein.

An "N-thiocarbamyl" group refers to a $R^{18}OC(S)NR^{19}$- group, with R^{19} and R^{19} as defined herein.

An "amino" group refers to an $-NR^{18}R^{19}$ group, wherein R^{18} and R^{19} are both hydrogen.

A "C-amido" group refers to a $-C(O)NR^{18}R^{19}$ group with R^{18} and R^{19} as defined herein.

An "N-amido" group refers to a $R^{18}C(O)NR^{19}$- group, with R^{18} and R^{19} as defined herein.

A "nitro" group refers to a $-NO_2$ group.

B. Preferred Structural Features.

A presently preferred embodiment of this invention is a compound in which:

R^1 and R^4 are hydrogen;

R^2 is selected from the group consisting of hydrogen, halo, lower alkoxy, $-C(O)R^{15}$ and $-S(O)_2NR^{13}R^{14}$;

R^3 is selected from the group consisting of hydrogen, lower alkoxy, $-C(O)R^{15}$, $-S(O)_2NR^{13}R^{14}$, aryl and heteroaryl;

R^5 is $-C(O)R^{10}$;

R^6 is selected from the group consisting of hydrogen and lower alkyl; and,

R^7 is selected from the group consisting of hydrogen, lower alkyl and $-C(O)R^{17}$.

It is another presently preferred embodiment of this invention that, in a compound having a structure as described in the previous paragraph:

R^{10} is selected from the group consisting of hydroxy, lower alkoxy and $-NR^{11}(CH_2)_nR^{12}$, wherein

n is 2 or 3;

R^{11} is selected from the group consisting of hydrogen and lower alkyl; and,

R^{12} is selected from the group consisting of aryl and $-NR^{13}R^{14}$.

It is a further presently preferred embodiment of this

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invention that, in a compound having a structure as described in the previous two paragraphs, R^{13} and R^{14} are independently selected from the group consisting of hydrogen, lower alkyl, and, combined, $-(CH_2)_4-$, $-(CH_2)_5-$, $-(CH_2)_2O(CH_2)_2-$ or $-(CH_2)_2N(CH_3)(CH_2)_2-$.

A presently preferred embodiment of this invention is a compound in which:

R^1 is selected from the group consisting of hydrogen, lower alkyl, $(CH_2)_rR^{16}$ and $-C(O)NR^8R^9$;

R^2 is selected from the group consisting of hydrogen, halogen, aryl and $-S(O)_2NR^{13}R^{14}$;

R^3 is selected from the group consisting of hydrogen, lower alkyl, lower alkoxy, aryl, heteroaryl and $-C(O)R^{15}$;

R^4 is hydrogen;

R^5 is selected from the group consisting of hydrogen and lower alkyl;

R^6 is $-C(O)R^{10}$;

R^7 is selected from the group consisting of hydorgen, lower alkyl and aryl;

R^{16} is selected from the group consisting of hydroxy and $-C(O)R^{15}$; and,

r is 2 or 3.

A presently preferred embodiment of this invention is a compound having as structure described in the paragraph just above in which R^3 is aryl optionally substituted with one or more groups selected from the group consisting of lower alkyl, lower alkoxy and halo.

Likewise, it is a presently preferred embodiment of this invention that, in a compound in which:

R^1 is selected from the group consisting of hydrogen, lower alkyl, $(CH_2)_rR^{16}$ and $-C(O)NR^8R^9$;

R^2 is selected from the group consisting of hydrogen, halogen, aryl and $-S(O)_2NR^{13}R^{14}$;

R^3 is selected from the group consisting of hydrogen, lower

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alkyl, lower alkoxy, aryl, heteroaryl and $-C(O)R^{15}$;
R^4 is hydrogen;
R^5 is selected from the group consisting of hydrogen and lower alkyl;
R^6 is $-C(O)R^{10}$;
R^7 is selected from the group consisting of hydorgen, lower alkyl and aryl;
R^{16} is selected from the group consisting of hydroxy and $-C(O)R^{15}$; and,
r is 2 or 3,
R^{10} is selected from the group consisting of hydroxy, lower alkoxy, $-NR^{13}R^{14}$ and $-NR^{11}(CH_2)_nR^{12}$, wherein n is 1, 2 or 3, R^{11} is hydrogen and R^{12} is selected from the group consisting of hydroxy, lower alkoxy, $-C(O)R^{15}$, heteroaryl and $-NR^{13}R^{14}$.

A further presently preferred embodiment of this invention is a compound having a structure as described in the paragraph immediately above in which R^{13} and R^{14} are independently selected from the group consisting of hydrogen, lower alkyl, heteroaryl and, combined, $-(CH_2)_4-$, $-(CH_2)_5-$, $-(CH_2)_2O(CH_2)_2-$, or $-(CH_2)_2N(CH_3)(CH_2)_2-$.

Another presently preferred embodiment of this invention is a compound in which:
R^1 is $-C(O)NR^8R^9$, wherein R^8 is hydrogen and R^9 is aryl optionally substituted with one or more groups selected from the group consisting of halo, hydroxy and lower alkoxy;
R^2 is selected from the group consisting of hydrogen, halogen, aryl and $-S(O)_2NR^{13}R^{14}$;
R^3 is selected from the group consisting of hydrogen, lower alkyl, lower alkoxy, aryl, heteroaryl and $-C(O)R^{15}$;
R^4 is hydrogen;
R^5 is selected from the group consisting of hydrogen and lower alkyl;
R^6 is $-C(O)R^{10}$;

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R^7 is selected from the group consisting of hydorgen, lower alkyl and aryl;
R^{16} is selected from the group consisting of hydroxy and $-C(O)R^{15}$; and,
r is 2 or 3,

A still further presently preferred embodiment of this invention is a compound in which:
R^1 is selected from the group consisting of hydrogen and lower alkyl;
R^2 is selected from the group consisting of hydrogen, halo, lower alkoxy, aryl, $-C(O)R^{15}$ and $-S(O)_2NR^{13}R^{14}$;
R^3 is selected from the group consisting of hydrogen, halo, aryl, heteroaryl and $-C(O)R^{15}$;
R^4 is hydrogen;
R^5 is $-C(O)R^{10}$; and,
R^6 and R^7 combine to form a $-(CH_2)_4-$ group.

In a compound having a structure as described in the paragraph immediately above, it is a presently preferred embodiment that R^{10} is selected from the group consisting of hydroxy, alkoxy,
$-NR^{13}R^{14}$ and $-NH(CH_2)_nNR^{13}R^{14}$ wherein n is 2 or 3.

It is a presently preferred embodiment of this invention that, in a compound having a structure as described in the two paragraphs immediately above, R^{13} and R^{14} are independently selected from the group consisting of hydrogen, lower alkyl, and, combined, $-(CH_2)_4-$, $-(CH_2)_5-$, $-(CH_2)_2O(CH_2)_2-$ or $-(CH_2)_2N(CH_3)(CH_2)_2-$.

A compound in which:
R^1 and R^4 are selected from the group consisting of hydrogen, lower alkyl, lower alkoxy and halogen;
R^2 is selected from the group consisting of hydrogen, lower alkyl, lower alkoxy, halo, and $-S(O)_2NR^{13}R^{14}$;
R^3 is selected from the group consisting of hydrogen, lower

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alkyl, lower alkoxy, halo, aryl and $-C(O)R^{15}$;
R^5 and R^6 are selected from the group consisting of hydrogen and lower alkyl; and,
R^7 is $-C(O)R^{10}$ is also a presently preferred embodiment of this invention.

Finally, with regard to chemical structure, in a compound having a structure as described in the paragraph immediately above, R^{10} is selected from the group consisting of hydroxy, alkoxy, $-NR^{13}R^{14}$ and $-NR^{11}(CH_2)_nR^{12}$, wherein n is 2 or 3, R^{11} is selected from the group consisting of hydrogen and lower alkyl; and, R^{12} is $-NR^{13}R^{14}$, wherein R^{13} and R^{14} are independently selected from the group consisting of hydrogen, lower alkyl, and, combined, $-(CH_2)_4-$, $-(CH_2)_5-$, $-(CH_2)_2O(CH_2)_2-$ or $-(CH_2)_2N(CH_3)(CH_2)_2-$.

The chemical formulae referred to herein may exhibit the phenomena of tautomerism and structural isomerism. For example, the compounds described herein may adopt an E or a Z configuration about the double bond connecting the 2-indolinone moiety to the pyrrole moiety or they may be a mixture of E and Z. This invention encompasses any tautomeric or structural isomeric form and mixtures thereof which possess the ability to modulate RTK, CTK and/or STK activity and is not limited to any one tautomeric or structural isomeric form.

2. **BIOCHEMISTRY/PHARMACOTHERAPY**

Another aspect of this invention relates to a method for the modulation of the catalytic activity of a PK by contacting a PK with a compound of this invention or a physiologically acceptable salt or prodrug thereof.

As used herein, "PK" refers to receptor protein tyrosine kinase (RTKs), non-receptor or "cellular" tyrosine kinase (CTKs) and serine-threonine kinases (STKs).

The term "method" refers to manners, means, techniques and procedures for accomplishing a given task including, but not

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limited to, those manners, means, techniques and procedures either known to, or readily developed from known manners, means, techniques and procedures by, practitioners of the chemical, pharmaceutical, biological, biochemical and medical arts.

As used herein, the term "modulation" or "modulating" refers to the alteration of the catalytic activity of RTKs, CTKs and STKs. In particular, modulating refers to the activation of the catalytic activity of RTKs, CTKs and STKs, preferably the activation or inhibition of the catalytic activity of RTKs, CTKs and STKs, depending on the concentration of the compound or salt to which the RTK, CTK or STK is exposed or, more preferably, the inhibition of the catalytic activity of RTKs, CTKs and STKs.

The term "catalytic activity" as used herein refers to the rate of phosphorylation of tyrosine under the influence, direct or indirect, of RTKs and/or CTKs or the phosphorylation of serine and threonine under the influence, direct or indirect, of STKs.

The term "contacting" as used herein refers to bringing a compound of this invention and a target PK together in such a manner that the compound can affect the catalytic activity of the PK, either directly, i.e., by interacting with the kinase itself, or indirectly, i.e., by interacting with another molecule on which the catalytic activity of the kinase is dependent. Such "contacting" can be accomplished "in vitro," i.e., in a test tube, a petri dish or the like. In a test tube, contacting may involve only a compound and a PK of interest or it may involve whole cells. Cells may also be maintained or grown in cell culture dishes and contacted with a compound in that environment. In this context, the ability of a particular compound to affect a PK related disorder, i.e., the IC_{50} of the compound, defined below, can be determined before use of the compounds in vivo with more complex living organisms is attempted. For cells outside the organism, multiple methods exist, and are well-known to those skilled in the art, to get the PKs in contact with the compounds including, but

not limited to, direct cell microinjection and numerous transmembrane carrier techniques.

A further aspect of this invention is that the modulation of the catalytic activity of PKs using a compound of this invention may be carried out in vitro or in vivo.

"In vitro" refers to procedures performed in an artificial environment such as, e.g., without limitation, in a test tube or culture medium.

As used herein, "in vivo" refers to procedures performed within a living organism such as, without limitation, a mouse, rat or rabbit.

A still further aspect of this invention is that the protein kinase whose catalytic activity is being modulated by a compound of this invention is selected from the group consisting of receptor protein tyrosine kinases, cellular (or non-receptor) tyrosine kinases and serine-threonine kinases.

It is an aspect of this invention that the receptor protein kinase whose catalytic activity is modulated by a compound of this invention is selected from the group consisting of EGF, HER2, HER3, HER4, IR, IGF-1R, IRR, PDGFRα, PDGFRβ, CSFIR, C-Kit, C-fms, Flk-1R, Flk4, KDR/Flk-1, Flt-1, FGFR-1R, FGFR-2R, FGFR-3R and FGFR-4R.

In addition, it is an aspect of this invention that the cellular tyrosine kinase whose catalytic activity is modulated by a compound of this invention is selected from the group consisting of Src, Frk, Btk, Csk, Abl, ZAP70, Fes/Fps, Fak, Jak, Ack, Yes, Fyn, Lyn, Lck, Blk, Hck, Fgr and Yrk.

Another aspect of this invention is that the serine-threonine protein kinase whose catalytic activity is modulated by a compound of this invention is selected from the group consisting of CDK2 and Raf.

A pharmaceutical composition of a compound of this invention with a pharmaceutically acceptable carrier or excipient is yet

another aspect of this invention. Such pharmaceutical composition may contain both carriers and excipients as well as other components generally known to those skilled in the formulation of pharmaceutical compositions.

A method for treating or preventing a protein kinase related disorder in an organism comprising administering a therapeutically effective amount of a compound, salt or prodrug of this invention to an organism in need of such treatment is another aspect of this invention.

As used herein, "PK related disorder," "PK driven disorder," and "abnormal PK activity" all refer to a condition characterized by inappropriate, i.e., under or, more commonly, over, PK catalytic activity, where the particular PK can be an RTK, a CTK or an STK. Inappropriate catalytic activity can arise as the result of either: (1) PK expression in cells which normally do not express PKs, (2) increased PK expression leading to unwanted cell proliferation, differentiation and/or growth, or, (3) decreased PK expression leading to unwanted reductions in cell proliferation, differentiation and/or growth. Over-activity of a PK refers to either amplification of the gene encoding a particular PK or production of a level of PK activity which can correlate with a cell proliferation, differentiation and/or growth disorder (that is, as the level of the PK increases, the severity of one or more of the symptoms of the cellular disorder increases). Under-activity is, of course, the converse, wherein the severity of one or more symptoms of a cellular disorder increase as the level of the PK activity decreases.

As used herein, the terms "prevent", "preventing" and "prevention" refer to a method for barring an organism from acquiring a PK related disorder in the first place.

As used herein, the terms "treat", "treating" and "treatment" refer to a method of alleviating or abrogating a PK mediated cellular disorder and/or its attendant symptoms. With

regard particularly to cancer, these terms simply mean that the life expectancy of an individual affected with a cancer will be increased or that one or more of the symptoms of the disease will be reduced.

The term "organism" refers to any living entity comprised of at least one cell. A living organism can be as simple as, for example, a single eukariotic cell or as complex as a mammal, including a human being.

The term "therapeutically effective amount" as used herein refers to that amount of the compound being administered which will relieve to some extent one or more of the symptoms of the disorder being treated. In reference to the treatment of cancer, a therapeutically effective amount refers to that amount which has the effect of (1) reducing the size of the tumor, (2) inhibiting (that is, slowing to some extent, preferably stopping) tumor metastasis, (3) inhibiting to some extent (that is, slowing to some extent, preferably stopping) tumor growth, and/or, (4) relieving to some extent (or, preferably, eliminating) one or more symptoms associated with the cancer.

It is an aspect of this invention that the above-referenced protein kinase related disorder is selected from the group consisting of a receptor protein tyrosine kinase related disorder, a cellular tyrosine kinase disorder and a serine-threonine kinase related disorder.

In yet another aspect of this invention, the above referenced protein kinase related disorder is selected from the group consisting of an EGFR related disorder, a PDGFR related disorder, an IGFR related disorder and a flk related disorder.

The above referenced protein kinase related disorder is a cancer selected from the group consisting of squamous cell carcinoma, sarcomas such as Kaposi's sarcoma, astrocytoma, glioblastoma, lung cancer, bladder cancer, colorectal cancer, gastrointestinal cancer, head and neck cancer, melanoma, ovarian

cancer, prostate cancer, breast cancer, small-cell lung cancer and glioma in a further aspect of this invention.

The above referenced protein kinase related disorder is selected from the group consisting of diabetes, an autoimmune disorder, a hyperproliferation disorder, von Hippel-Lindau disease, restenosis, fibrosis, psoriasis, osteoarthritis, rheumatoid arthritis, an inflammatory disorder and angiogenesis in yet another aspect of this invention.

Additional disorders which may be treated or prevented using the compounds of this invention are immunological disorders such as autoimmune disease, AIDS and cardiovasular disorders such as atherosclerosis.

It is as aspect of this invention that a chemical compound that modulates the catalytic activity of a protein kinase may be identified by contacting cells expressing said protein kinase with a compound, salt or prodrug of the present invention and then monitoring said cells for an effect.

By "monitoring" is meant observing or detecting the effect of contacting a compound with a cell expressing a particular PK. The observed or detected effect can be a change in cell phenotype, in the catalytic activity of a PK or a change in the interaction of a PK with a natural binding partner. Techniques for observing or detecting such effects are well-known in the art.

The above-referenced effect is selected from a change or an absence of change in a cell phenotype, a change or absence of change in the catalytic activity of said protein kinase or a change or absence of change in the interaction of said protein kinase with a natural binding partner in a final aspect of this invention.

"Cell phenotype" refers to the outward appearance of a cell or tissue or the biological function of the cell or tissue. Examples, without limitation, of a cell phenotype are cell size,

cell growth, cell proliferation, cell differentiation, cell survival, apoptosis, and nutrient uptake and use. Such phenotypic characteristics are measurable by techniques well-known in the art.

A "natural binding partner" refers to a polypeptide that binds to a particular PK in a cell. Natural binding partners can play a role in propagating a signal in a PK-mediated signal transduction process. A change in the interaction of the natural binding partner with the PK can manifest itself as an increased or decreased concentration of the PK/natural binding partner complex and, as a result, in an observable change in the ability of the PK to mediate signal transduction.

It is also an aspect of this invention that a compound described herein, or its salt or prodrug, might be combined with other chemotherapeutic agents for the treatment of the diseases and disorders discussed above. For instance, a compound, salt or prodrug of this invention might be combined with alkylating agents such as fluorouracil (5-FU) alone or in further combination with leukovorin; or other alkylating agents such as, without limitation, other pyrimidine analogs such as UFT, capecitabine, gemcitabine and cytarabine, the alkyl sulfonates, e.g., busulfan (used in the treatment of chronic granulocytic leukemia), improsulfan and piposulfan; aziridines, e.g., benzodepa, carboquone, meturedepa and uredepa; ethyleneimines and methylmelamines, e.g., altretamine, triethylenemelamine, triethylenephosphoramide, triethylenethiophosphoramide and trimethylolmelamine; and the nitrogen mustards, e.g., chlorambucil (used in the treatment of chronic lymphocytic leukemia, primary macroglobulinemia and non-Hodgkin's lymphoma), cyclophosphamide (used in the treatment of Hodgkin's disease, multiple myeloma, neuroblastoma, breast cancer, ovarian cancer, lung cancer, Wilm's tumor and rhabdomyosarcoma), estramustine, ifosfamide, novembrichin, prednimustine and uracil mustard (used

in the treatment of primary thrombocytosis, non-Hodgkin's lymphoma, Hodgkin's disease and ovarian cancer); and triazines, e.g., dacarbazine (used in the treatment of soft tissue sarcoma).

Likewise a compound, salt or prodrug of this invention might be expected to have a beneficial effect in combination with other antimetabolite chemotherapeutic agents such as, without limitation, folic acid analogs, e.g. methotrexate (used in the treatment of acute lymphocytic leukemia, choriocarcinoma, mycosis fungiodes breast cancer, head and neck cancer and osteogenic sarcoma) and pteropterin; and the purine analogs such as mercaptopurine and thioguanine which find use in the treatment of acute granulocytic, acute lymphocytic and chronic granulocytic leukemias.

A compound, salt or prodrug of this invention might also be expected to prove efficacious in combination with natural product based chemotherapeutic agents such as, without limitation, the vinca alkaloids, e.g., vinblastin (used in the treatment of breast and testicular cancer), vincristine and vindesine; the epipodophylotoxins, e.g., etoposide and teniposide, both of which are useful in the treatment of testicular cancer and Kaposi's sarcoma; the antibiotic chemotherapeutic agents, e.g., daunorubicin, doxorubicin, epirubicin, mitomycin (used to treat stomach, cervix, colon, breast, bladder and pancreatic cancer), dactinomycin, temozolomide, plicamycin, bleomycin (used in the treatment of skin, esophagus and genitourinary tract cancer); and the enzymatic chemotherapeutic agents such as L-asparaginase.

In addition to the above, a compound, salt or prodrug of this invention might be expected to have a beneficial effect used in combination with the platinum coordination complexes (cisplatin, etc.); substituted ureas such as hydroxyurea; methylhydrazine derivatives, e.g., procarbazine; adrenocortical suppressants, e.g., mitotane, aminoglutethimide; and hormone and hormone antagonists such as the adrenocorticosteriods (e.g.,

prednisone), progestins (e.g., hydroxyprogesterone caproate); estrogens (e.g., diethylstilbesterol); antiestrogens such as tamoxifen; androgens, e.g., testosterone propionate; and aromatase inhibitors such as anastrozole.

Finally, the combination of a compound of this invention might be expected to be particularly effective in combination with mitoxantrone or paclitaxel for the treatment of solid tumor cancers or leukemias such as, without limitation, acute myelogenous (non-lymphocytic) leukemia.

DETAILED DESCRIPTION OF THE INVENTION

1. BRIEF DESCRIPTION OF THE TABLES

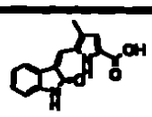
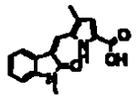
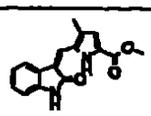
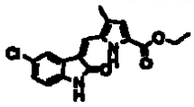
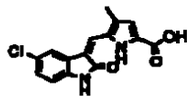
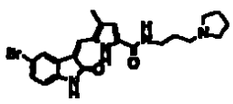
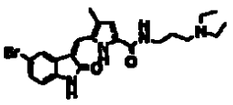
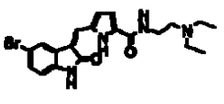
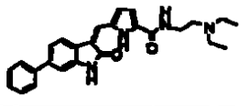
TABLE 1 shows the chemical structures of some exemplary compounds of this invention. The compound numbers correspond to the Example numbers in the Examples section. That is, the synthesis of Compound 1 in Table 1 is described in Example 1. The compounds presented in Table 1 are exemplary only and are not to be construed as limiting the scope of this invention in any manner.

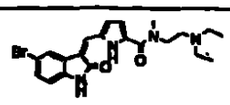
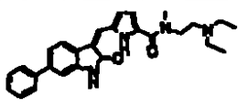
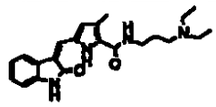
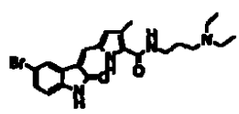
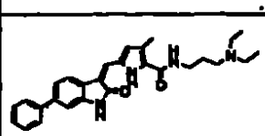
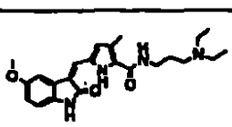
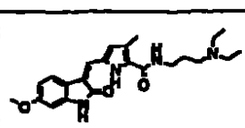
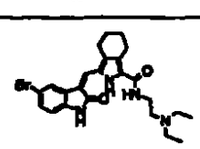
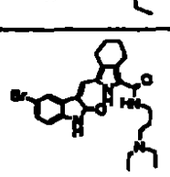
TABLE 2 shows the results of biological testing of some exemplary compounds of this invention. The results are reported in terms of IC_{50}, the micromolar (μM) concentration of the compound being tested which causes a 50% change in the activity of the target PKT compared to the activity of the PTK in a control to which no test compound has been added. Specifically, the results shown indicate the concentration of a test compound needed to cause a 50% reduction of the activity of the target PTK. Bioassays which have been or mau be used to evaluate compounds are described in detail below.

481 483

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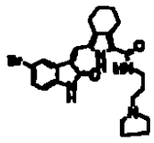
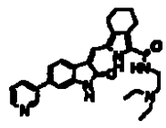
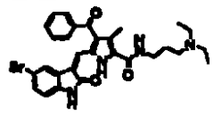
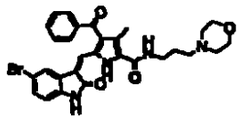
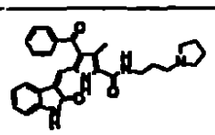
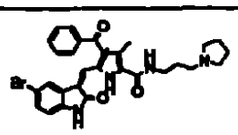
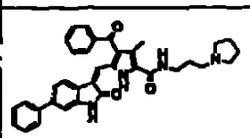
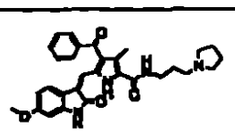
TABLE 1

Example	Structure	Name
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2		4-Methyl-5-(1-methyl-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrole-2-carboxylic acid
3		4-Methyl-5-(2-oxo-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrole-2-carboxylic acid methyl ester
4		5-(5-Chloro-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-4-methyl-1H-pyrrole-2-carboxylic acid ethyl ester
5		5-(5-Chloro-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-4-methyl-1H-pyrrole-2-carboxylic acid
6		5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-4-methyl-1H-pyrrole-2-carboxylic acid (3-pyrrolidin-1-ylpropyl)amide
7		5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-4-methyl-1H-pyrrole-2-carboxylic acid (3-diethylaminopropyl)amide
8		5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrole-2-carboxylic acid (2-diethylaminoethyl)amide
9		5-(2-Oxo-6-phenyl-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrole-2-carboxylic acid (2-diethylaminoethyl)amide

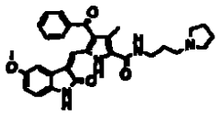
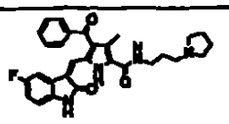
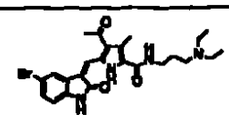
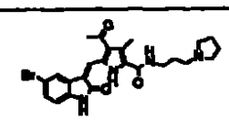
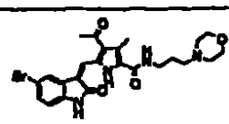
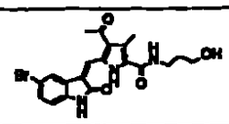
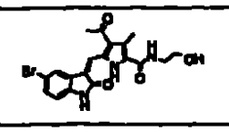
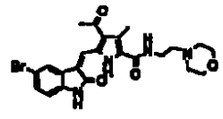
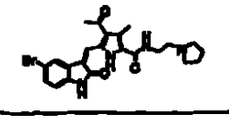
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11		5-(2-Oxo-6-phenyl-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrole-2-carboxylic acid (2-diethylaminoethyl)methylamide
12		3-Methyl-5-(2-oxo-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrole-2-carboxylic acid (3-diethylaminopropyl)amide
13		5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-3-methyl-1H-pyrrole-2-carboxylic acid (3-diethylaminopropyl)amide
14		3-Methyl-5-(2-oxo-6-phenyl-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrole-2-carboxylic acid (3-diethylaminopropyl)amide
15		5-(5-Methoxy-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-3-methyl-1H-pyrrole-2-carboxylic acid (3-diethylaminopropyl)amide
16		5-(6-Methoxy-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-3-methyl-1H-pyrrole-2-carboxylic acid (3-diethylaminopropyl)amide
17		3-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-4,5,6,7-tetrahydro-2H-isoindole-1-carboxylic acid (2-diethylaminoethyl)amide
18		3-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-4,5,6,7-tetrahydro-2H-isoindole-1-carboxylic acid (3-diethylaminopropyl)amide

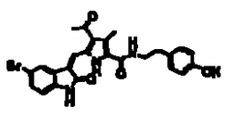
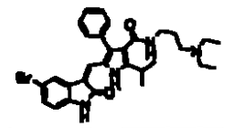
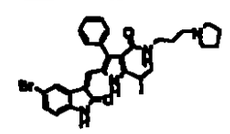
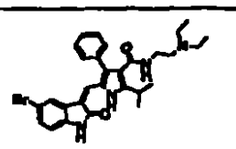
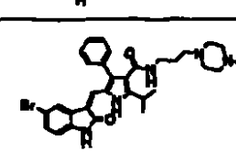
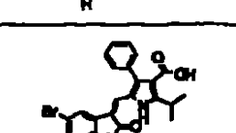
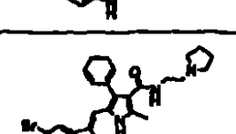
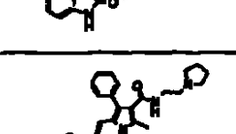
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20		3-(2-Oxo-6-pyridin-3-yl-1,2-dihydroindol-3-ylidenemethyl)-4,5,6,7-tetrahydro-2H-isoindole-1-carboxylic acid (2-diethylaminoethyl)amide
21		4-Benzoyl-5-(5-bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-3-methyl-1H-pyrrole-2-carboxylic acid (3-diethylaminopropyl)amide
22		4-Benzoyl-5-(5-bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-3-methyl-1H-pyrrole-2-carboxylic acid (3-morpholin-4-ylpropyl)amide
23		4-Benzoyl-3-methyl-5-(2-oxo-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrole-2-carboxylic acid (3-pyrrolidin-1-ylpropyl)amide
24		4-Benzoyl-5-(5-bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-3-methyl-1H-pyrrole-2-carboxylic acid (3-pyrrolidin-1-ylpropyl)amide
25		4-Benzoyl-3-methyl-5-(2-oxo-6-phenyl-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrole-2-carboxylic acid (3-pyrrolidin-1-ylpropyl)amide
26		4-Benzoyl-5-(6-methoxy-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-3-methyl-1H-pyrrole-2-carboxylic acid (3-pyrrolidin-1-ylpropyl)amide

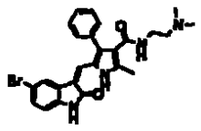
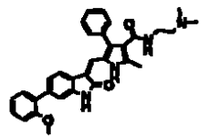
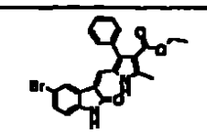
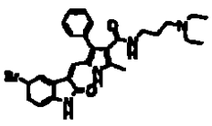
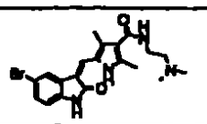
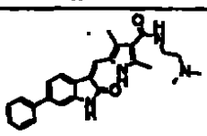
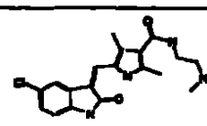
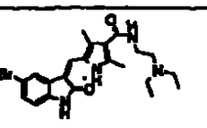
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27		4-Benzoyl-5-(5-methoxy-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-3-methyl-1H-pyrrole-2-carboxylic acid (3-pyrrolidin-1-ylpropyl)amide
28		4-Benzoyl-5-(5-fluoro-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-3-methyl-1H-pyrrole-2-carboxylic acid (3-pyrrolidin-1-ylpropyl)amide
29		4-Acetyl-5-(5-bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-3-methyl-1H-pyrrole-2-carboxylic acid (3-diethylaminopropyl)amide
30		4-Acetyl-5-(5-bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-3-methyl-1H-pyrrole-2-carboxylic acid (3-pyrrolidin-1-ylpropyl)amide
31		4-Acetyl-5-(5-bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-3-methyl-1H-pyrrole-2-carboxylic acid (3-morpholin-4-ylpropyl)amide
32		4-Acetyl-5-(5-bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-3-methyl-1H-pyrrole-2-carboxylic acid (3-hydroxy-propyl)amide
33		4-Acetyl-5-(5-bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-3-methyl-1H-pyrrole-2-carboxylic acid (2-hydroxy-ethyl)amide
34		4-Acetyl-5-(5-bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-3-methyl-1H-pyrrole-2-carboxylic acid (2-morpholin-4-yl-ethyl)amide
35		4-Acetyl-5-(5-bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-3-methyl-1H-pyrrole-2-carboxylic acid (2-pyrrolidin-1-yl-ethyl)amide

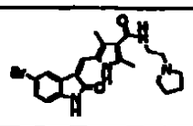
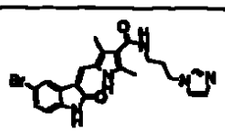
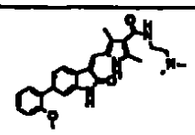
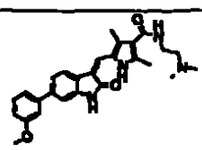
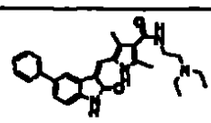
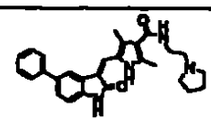
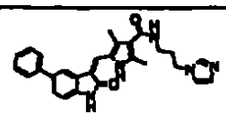
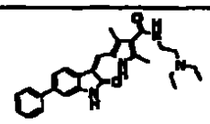
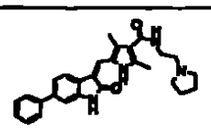
36		4-Acetyl-5-(5-bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-3-methyl-1H-pyrrole-2-carboxylic acid (2-(4-hydroxy-phenyl)-ethyl)amide
37		5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2-isopropyl-4-phenyl-1H-pyrrole-3-carboxylic acid (3-diethylaminopropyl)amide
38		5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2-isopropyl-4-phenyl-1H-pyrrole-3-carboxylic acid (3-pyrrolidin-1-ylpropyl)amide
39		5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2-isopropyl-4-phenyl-1H-pyrrole-3-carboxylic acid (2-diethylaminoethyl)amide
40		5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2-isopropyl-4-phenyl-1H-pyrrole-3-carboxylic acid [3-(4-methyl-piperazin-1-yl)-propyl]amide
41		5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2-isopropyl-4-phenyl-1H-pyrrole-3-carboxylic acid
42		5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2-methyl-4-phenyl-1H-pyrrole-3-carboxylic acid (2-pyrrolidin-1-yl-ethyl)amide
43		5-[5-(2-Methoxy-phenyl)-2-oxo-1,2-dihydroindol-3-ylidenemethyl]-2-methyl-4-phenyl-1H-pyrrole-3-carboxylic acid (2-pyrrolidin-1-yl-ethyl)amide

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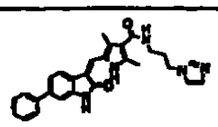
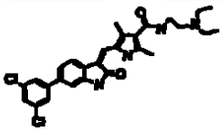
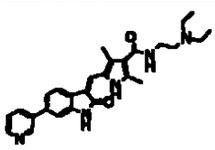
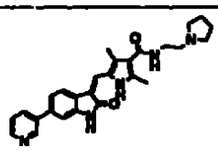
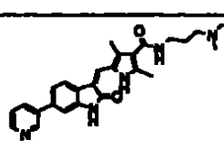
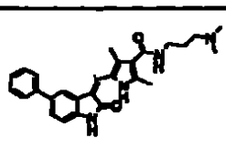
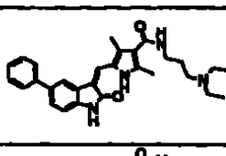
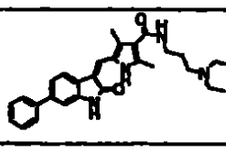
44		5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2-methyl-4-phenyl-1H-pyrrole-3-carboxylic acid (2-dimethylamino-ethyl)amide
45		5-[5-(2-Methoxy-phenyl)-2-oxo-1,2-dihydroindol-3-ylidenemethyl]-2-methyl-4-phenyl-1H-pyrrole-3-carboxylic acid (2-dimethylamino-ethyl)amide
46		5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2-methyl-4-phenyl-1H-pyrrole-3-carboxylic acid ethyl ester
47		5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2-methyl-4-phenyl-1H-pyrrole-3-carboxylic acid (3-diethylaminopropyl)amide
48		5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (2-dimethylamino-ethyl)amide
49		2,4-Dimethyl-5-(2-oxo-6-phenyl-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrole-3-carboxylic acid (2-dimethylamino-ethyl)amide
50		5-(5-Chloro-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (2-dimethylamino-ethyl)amide
51		5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (2-diethylaminoethyl)amide

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52		5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (2-pyrrolidin-1-yl-ethyl)amide
53		5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (3-imidazol-1-ylpropyl)amide
54		5-[5-(2-Methoxy-phenyl)-2-oxo-1,2-dihydroindol-3-ylidenemethyl]-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (2-dimethylamino-ethyl)amide
55		5-[5-(3-Methoxy-phenyl)-2-oxo-1,2-dihydroindol-3-ylidenemethyl]-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (2-dimethylamino-ethyl)amide
58		2,4-Dimethyl-5-(2-oxo-5-phenyl-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrole-3-carboxylic acid (2-diethylaminoethyl)amide
57		2,4-Dimethyl-5-(2-oxo-5-phenyl-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrole-3-carboxylic acid (2-pyrrolidin-1-yl-ethyl)amide
56		2,4-Dimethyl-5-(2-oxo-5-phenyl-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrole-3-carboxylic acid (3-imidazol-1-ylpropyl)amide
59		2,4-Dimethyl-5-(2-oxo-6-phenyl-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrole-3-carboxylic acid (2-diethylaminoethyl)amide
60		2,4-Dimethyl-5-(2-oxo-6-phenyl-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrole-3-carboxylic acid (2-pyrrolidin-1-yl-ethyl)amide

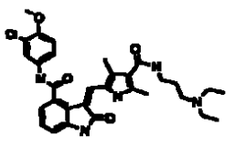
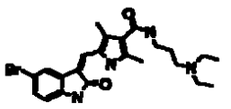
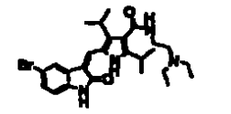
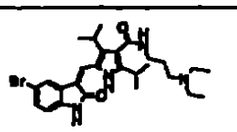
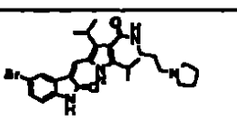
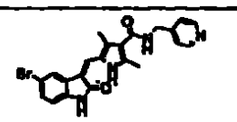
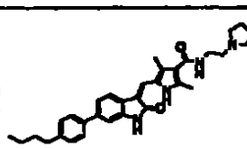
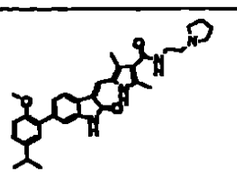
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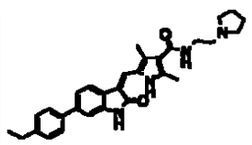
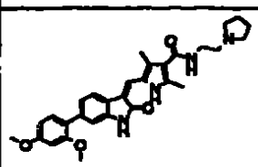
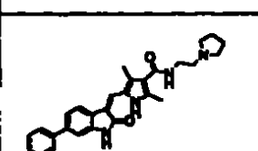
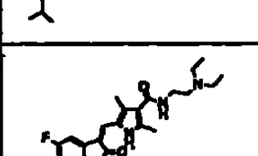
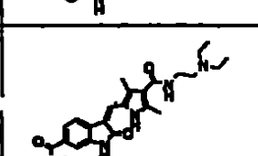
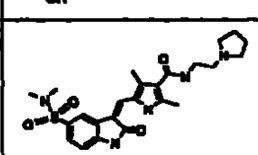
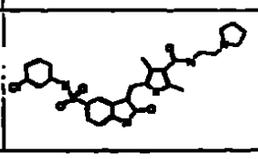
61		2,4-Dimethyl-5-(2-oxo-5-phenyl-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrole-3-carboxylic acid (3-imidazol-1-ylpropyl)amide
62		5-[5-(3,5-Dichloro-phenyl)-2-oxo-1,2-dihydroindol-3-ylidenemethyl]-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (2-diethylaminoethyl)amide
63		2,4-Dimethyl-5-(2-oxo-5-pyridin-3-yl-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrole-3-carboxylic acid (2-diethylaminoethyl)amide
64		2,4-Dimethyl-5-(2-oxo-5-pyridin-3-yl-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrole-3-carboxylic acid (2-pyrrolidin-1-yl-ethyl)amide
65		2,4-Dimethyl-5-(2-oxo-5-pyridin-3-yl-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrole-3-carboxylic acid (3-dimethylamino-propyl)amide
66		2,4-Dimethyl-5-(2-oxo-5-phenyl-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrole-3-carboxylic acid (3-dimethylamino-propyl)amide
67		2,4-Dimethyl-5-(2-oxo-5-phenyl-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrole-3-carboxylic acid (3-diethylaminopropyl)amide
68		2,4-Dimethyl-5-(2-oxo-5-phenyl-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrole-3-carboxylic acid (3-diethylaminopropyl)amide

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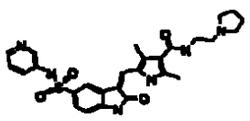
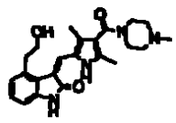
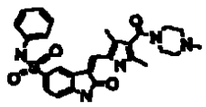
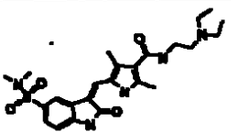
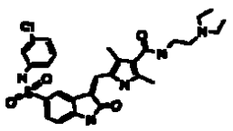
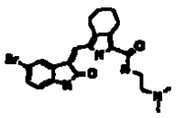
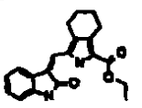
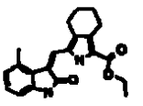
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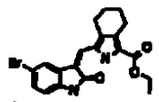
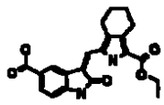
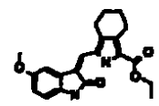
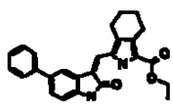
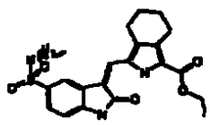
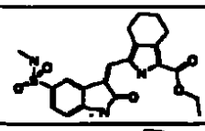
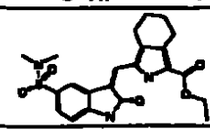
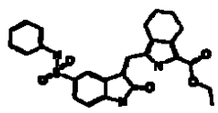
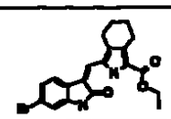
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70		5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (3-diethylaminopropyl)amide
71		5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2,4-diisopropyl-1H-pyrrole-3-carboxylic acid (2-diethylaminoethyl)amide
72		5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2,4-diisopropyl-1H-pyrrole-3-carboxylic acid (3-diethylaminopropyl)amide
73		5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2,4-diisopropyl-1H-pyrrole-3-carboxylic acid (3-pyrrolidin-1-ylpropyl)amide
74		5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (pyridin-4-ylmethyl)amide
75		5-[5-(4-Butyl-phenyl)-2-oxo-1,2-dihydroindol-3-ylidenemethyl]-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (2-pyrrolidin-1-yl-ethyl)amide
78		5-[5-(5-Isopropyl-2-methoxy-phenyl)-2-oxo-1,2-dihydroindol-3-ylidenemethyl]-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (2-pyrrolidin-1-yl-ethyl)amide

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77		5-[5-(4-Ethyl-phenyl)-2-oxo-1,2-dihydroindol-3-ylidenemethyl]-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (2-pyrrolidin-1-yl-ethyl)amide
78		5-[5-(2,4-Dimethoxy-phenyl)-2-oxo-1,2-dihydroindol-3-ylidenemethyl]-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (2-pyrrolidin-1-yl-ethyl)amide
79		5-[5-(3-Isopropyl-phenyl)-2-oxo-1,2-dihydroindol-3-ylidenemethyl]-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (2-pyrrolidin-1-yl-ethyl)amide
80		5-(5-Fluoro-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (2-diethylaminoethyl)amide
81		3-[4-(2-diethylaminoethylcarbamoyl)-3,5-dimethyl-1H-pyrrol-2-ylmethylene]-2-oxo-2,3-dihydro-1H-indole-6-carboxylic acid
82		5-(5-Dimethylsulfamoyl-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (2-pyrrolidin-1-yl-ethyl)amide
83		5-[5-(3-Chloro-phenylsulfamoyl)-2-oxo-1,2-dihydroindol-3-ylidenemethyl]-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (2-pyrrolidin-1-yl-ethyl)amide

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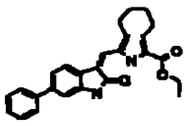
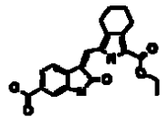
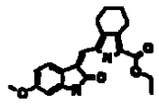
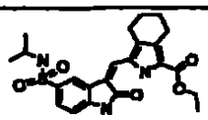
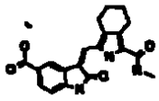
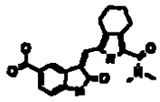
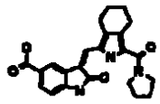
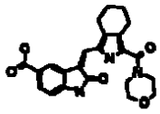
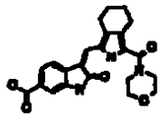
84		2,4-Dimethyl-5-[2-oxo-5-(pyridin-3-ylsulfamoyl)-1,2-dihydroindol-3-ylidenemethyl]-1H-pyrrole-3-carboxylic acid (2-pyrrolidin-1-yl-ethyl)amide
85		3-[3,5-Dimethyl-4-(4-methyl-piperazine-1-carbonyl)-1H-pyrrol-2-ylmethylene]-4-(2-hydroxy-ethyl)-1,3-dihydroindol-2-one
86		3-[3,5-Dimethyl-4-(4-methyl-piperazine-1-carbonyl)-1H-pyrrol-2-ylmethylene]-2-oxo-2,3-dihydro-1H-indole-5-sulfonic acid phenylamide
87		5-(5-Dimethylsulfamoyl-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (2-diethylaminoethyl)amide
88		5-[5-(3-Chloro-phenylsulfamoyl)-2-oxo-1,2-dihydroindol-3-ylidenemethyl]-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (2-diethylaminoethyl)amide
89		3-(5-Bromo-2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-4,5,6,7-tetrahydro-2H-isoindole-1-carboxylic acid (2-dimethylamino-ethyl)-amide
90		3-(2-Oxo-1,2-dihydro-indol-3-ylidenemethyl)-4,5,6,7-tetrahydro-2H-isoindole-1-carboxylic acid ethyl ester
91		3-(4-Methyl-2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-4,5,6,7-tetrahydro-2H-isoindole-1-carboxylic acid ethyl ester

92		3-(5-Bromo-2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-4,5,6,7-tetrahydro-2*H*-isoindole-1-carboxylic acid ethyl ester
93		3-(3-Ethoxycarbonyl-4,5,6,7-tetrahydro-2*H*-isoindol-1-ylmethylene)-2-oxo-2,3-dihydro-1*H*-indole-5-carboxylic acid
94		3-(5-Methoxy-2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-4,5,6,7-tetrahydro-2*H*-isoindole-1-carboxylic acid ethyl ester
95		3-(2-Oxo-5-phenyl-1,2-dihydro-indol-3-ylidenemethyl)-4,5,6,7-tetrahydro-2*H*-isoindole-1-carboxylic acid ethyl ester
96		3-(2-Oxo-5-sulfamoyl-1,2-dihydro-indol-3-ylidenemethyl)-4,5,6,7-tetrahydro-2*H*-isoindole-1-carboxylic acid ethyl ester
97		3-(5-Methylsulfamoyl-2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-4,5,6,7-tetrahydro-2*H*-isoindole-1-carboxylic acid ethyl ester
98		3-(5-Dimethylsulfamoyl-2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-4,5,6,7-tetrahydro-2*H*-isoindole-1-carboxylic acid ethyl ester
99		3-(2-Oxo-5-phenylsulfamoyl-1,2-dihydro-indol-3-ylidenemethyl)-4,5,6,7-tetrahydro-2*H*-isoindole-1-carboxylic acid ethyl ester
100		3-(6-Bromo-2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-4,5,6,7-tetrahydro-2*H*-isoindole-1-carboxylic acid ethyl ester

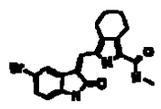
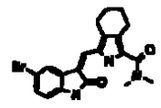
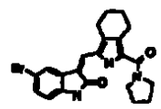
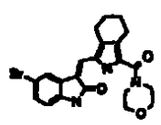
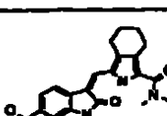
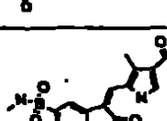
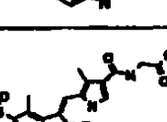
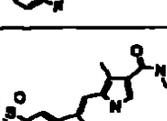
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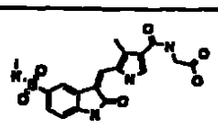
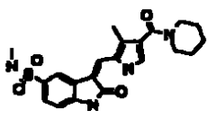
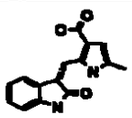
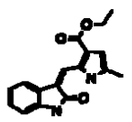
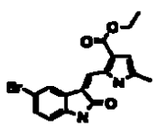
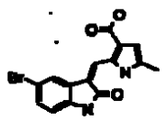
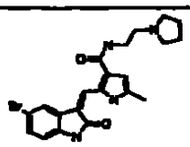
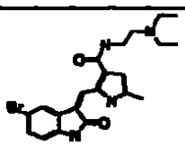
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101		3-(2-Oxo-6-phenyl-1,2-dihydro-indol-3-ylidenemethyl)-4,5,6,7-tetrahydro-2*H*-isoindole-1-carboxylic acid ethyl ester
102		3-(3-Ethoxycarbonyl-4,5,6,7-tetrahydro-2*H*-isoindol-1-ylmethylene)-2-oxo-2,3-dihydro-1*H*-indole-5-carboxylic acid
103		3-(5-Methoxy-2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-4,5,6,7-tetrahydro-2*H*-isoindole-1-carboxylic acid ethyl ester
104		3-(5-Isopropylsulfamoyl-2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-4,5,6,7-tetrahydro-2*H*-isoindole-1-carboxylic acid ethyl ester
105		3-(3-Methylcarbamoyl-4,5,6,7-tetrahydro-2*H*-isoindol-1-ylmethylene)-2-oxo-2,3-dihydro-1*H*-indole-5-carboxylic acid
106		3-(3-Dimethylcarbamoyl-4,5,6,7-tetrahydro-2*H*-isoindol-1-ylmethylene)-2-oxo-2,3-dihydro-1*H*-indole-5-carboxylic acid
107		2-Oxo-3-[3-(pyrrolidine-1-carbonyl)-4,5,6,7-tetrahydro-2*H*-isoindol-1-ylmethylene]-2,3-dihydro-1*H*-indole-5-carboxylic acid
108		3-[3-(Morpholine-4-carbonyl)-4,5,6,7-tetrahydro-2*H*-isoindol-1-ylmethylene]-2-oxo-2,3-dihydro-1*H*-indole-5-carboxylic acid
109		3-[3-(Morpholine-4-carbonyl)-4,5,6,7-tetrahydro-2*H*-isoindol-1-ylmethylene]-2-oxo-2,3-dihydro-1*H*-indole-5-carboxylic acid

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110		3-(5-Bromo-2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-4,5,6,7-tetrahydro-2H-isoindole-1-carboxylic acid methylamide
111		3-(5-Bromo-2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-4,5,6,7-tetrahydro-2*H*-isoindole-1-carboxylic acid dimethylamide
112		5-Bromo-3-[3-(pyrrolidine-1-carbonyl)-4,5,6,7-tetrahydro-2*H*-isoindol-1-ylmethylene]-1,3-dihydro-indol-2-one
113		5-Bromo-3-[3-(morpholine-4-carbonyl)-4,5,6,7-tetrahydro-2*H*-isoindol-1-ylmethylene]-1,3-dihydro-indol-2-one
114		3-[3-Dimethylcarbamoyl-4,5,6,7-tetrahydro-2*H*-isoindol-1-ylmethylene]-2-oxo-2,3-dihydro-1*H*-indole-6-carboxylic acid
115		4-Methyl-5-(5-methylsulfamoyl-2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-1*H*-pyrrole-3-carboxylic acid
116		[(4-Methyl-5-(4-methyl-5-methylsulfamoyl-2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-1*H*-pyrrole-3-carbonyl]-amino}-acetic acid ethyl ester
117		[(4-Methyl-5-(5-methylsulfamoyl-2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-1*H*-pyrrole-3-carbonyl]-amino}-acetic acid ethyl ester

118		([4-Methyl-5-(5-methylsulfamoyl-2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-1*H*-pyrrole-3-carbonyl]-amino)-acetic acid
119		3-[3-Methyl-4-(piperidine-1-carbonyl)-1*H*-pyrrol-2-ylmethylene]-2-oxo-2,3-dihydro-1*H*-indole-5-sulfonic acid methylamide
120		5-Methyl-2-(2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-1*H*-pyrrole-3-carboxylic acid
121		5-Methyl-2-(2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-1*H*-pyrrole-3-carboxylic acid ethyl ester
122		2-(5-Bromo-2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-5-methyl-1*H*-pyrrole-3-carboxylic acid ethyl ester
123		2-(5-Bromo-2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-5-methyl-1*H*-pyrrole-3-carboxylic acid
124		2-(5-Bromo-2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-5-methyl-1H-pyrrole-3-carboxylic acid (3-pyrrolidin-1-yl-ethyl)-amide
125		2-(5-Bromo-2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-5-methyl-1H-pyrrole-3-carboxylic acid (2-diethylamino-ethyl)-amide

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TABLE 2

Example	bio flkGST IC50 (nM)	bio FGFR1 IC50 (nM)	bio PDGF IC50 (nM)	bio EGF IC50 (nM)	cell EGF IC50 (nM)	Her2 Kinase IC50 (nM)	cdk2cpa C50 (nM)	bio pyk2 IC50 (nM)
1	57.68	15.16	>100	>100	>100			>100
2	>100		>100	>100	>100			
3	9.85	9.62	>100	>100	>100			>100
4	3.57	>20	>100	>100	>100	>100		
5	8.3	16.06	>100	>100	>100	>100		
6	4.04		>100	3.26	7.82	2.43		
7	7.74		>100	5.07	9.8	4.24		
8	12.1		>100	51.34	20.08	5.5		
9	0.96		>100	>100	>100	16.38		
10	5.72		>100	94.04	15.86	9.06		
11	9.77		>100	>100	>100	>100		
12	>20		21.46	>100		27.73		
13	>20		81.92	8.17		2.66		
14	13.01		42.41	>100		56.02		
15	>20		>100	>100		38.61		
16	>20		98.06	>100		23.32		
17	8.25	2.47	94.35	0.83	11.47	15.94	>10	
18	2.67			2.57	9.23	4.99		
19	7.5			6.86	34.18	3.37		
20	11.53			>100	41.16	9		
21	7.18		>100	40.34		27.69		
22	>20		>100	>100		87.67		
23	>20		>100	36.64		4.05		
24			>100	16.84		5.31		
25	12.55		>100	23.48		7.9		
26	16.03		66.87	34.67		10.04		
27			>100	26.5		3.91		
28	4.5		71.27	53.66		2.67		
29	10.12		>100	26.72		3.98		
30	9.4		>100	18.69		4.1		
31	>50		>100	9.83		47.19		
32	45.74		5.94	>100		>100		
34	>50		>100	>100		>100		

35	>20		>100	80.4		54.14		
36	>20		>100	>100		>100		
37	0.22		3.06	10.78	9.84	1.4		
38	4.17		3.06	6.04	8.97	2.16		
39	3.38		4.69	3.67	14.54	3.53		
40	4.5		7.9	6.52		6.27		
42	0.1		0.12	11.95	74.55	20.43		
43	1.12		8.38	>100	37.33	53.37		
44	<0.05		0.02	20.73	67.46	6.99		
45	1.71		>100	>100	29.95	>100		
46	30.62		6.18	>100	>100	>100		
47	0.08	1.56	0.06	11.42	41.54	8.4	>20	1.05
48	0.006	0.3	<0.78	17.88	21.58	7.93		0.09
49			<0.78	>100	43.86	>100		
50			<0.78	>100	20.34	>100		
51	0.006	1.66	0.01	18.1	21.61	23.24	16.69	0.35
52	0.08	1.26	<0.78	12.53	>100	>100	10.66	0.45
53			<0.78	>100	>100	>100		
54	1.98		<0.78	23.88	9.76	7.02		
55	0.27		0.53	6.03	35.99	77.82		
56	2.32		3.19	>100	10.03	7.11		
57	0.06		7.98	>100	9.97	6.94		
58			21.14	>100	>100	>100		
59			<0.78	>100	>100	>100		
60			<0.78	>100	>100	>100		
61			<0.78	>100	>100	>100		
62	8.00		8.32	>100	>100	>100		
63	0.21		<0.78	8.58	>100	>100		
64	0.55		<0.78	30.49	>100	>100		
65	0.37		<0.05	>100	74.36	15.97		
66	<0.05			>100	11.84	2.76		
67	0.39		24.77	31.38	19.79	2.56		
68	1.16		0.03	>100	23.52	34.13		
69	0.3		56.55	>100	97.54	>100		
70	0.09	1.50	0.0030	10.57	6.42	7.99	12.62	0.63
71	15.21		22.5	>100		9.91		
72	6.06		10.54	>100	39.94	9.65		

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73	5.95		14.12	>100	39.5	8.59		
74	1.2		0.09	46.75		>100		
75	2.7		61.55	>100		>100		
76	3.33		19.18	5.11		3.01		
77	0.49		25.01	>100		>100		
78	1.94		70.62	9.33		4.25		
79	1.49		>100	27.39		>100		
80	0.13	4.29	0.001	>100		50.19	17.19	0.29
81	0.21		0.18	>100		>100		
82	2.03	7.69	6.88	>100		>100		0.31
83	0.34	0.41	9.46	2.18		86.9		0.008
84	1.38		12.51	67.2		5.86		0.006
85	0.2	0.8	2.59	>100		3.76		
86	1.45	1.3	19.6	41.8		>100		3.58
87	3.27	7.56	6.46	>100		9.1		0.17
88	0.35	1.18	8.06	2.36		>100		0.09
89	7.84		47.58	8.53	9.67	15.97		
115	7.3		7.48	>100		>100	0.006	
116	>20		>100	>100		>100	<0.0005	
117	0.91		12.9	>100		>100	0.006	
118	1.93		1.2	>100		>100	0.002	
119	1.38		61.63	>100		>100	<0.0005	

2. INDICATIONS/TARGET DISEASES

The PKs whose catalytic activity is modulated by the compounds of this invention include protein tyrosine kinases of which there are two types, receptor tyrosine kinases (RTKs) and cellular tyrosine kinases (CTKs), and serine-threonine kinases (STKs). RTK mediated signal transduction is initiated by extracellular interaction with a specific growth factor (ligand), followed by receptor dimerization, transient stimulation of the intrinsic protein tyrosine kinase activity and phosphorylation. Binding sites are thereby created for intracellular signal transduction molecules and lead to the formation of complexes with a spectrum of cytoplasmic signaling molecules that facilitate the appropriate cellular response (e.g., cell

division, metabolic effects on the extracellular microenvironment, etc.). See, Schlessinger and Ullrich, 1992, Neuron 9:303-391.

It has been shown that tyrosine phosphorylation sites on growth factor receptors function as high-affinity binding sites for SH2 (src homology) domains of signaling molecules. Fantl et al., 1992, Cell 69:413-423, Songyang *et al.*, 1994, Mol. Cell. Biol. 14:2777-2785), Songyang et al., 1993, Cell 72:767-778, and Koch *et al.*, 1991, Science 252:668-678. Several intracellular substrate proteins that associate with RTKs have been identified. They may be divided into two principal groups: (1) substrates that have a catalytic domain, and (2) substrates which lack such domain but which serve as adapters and associate with catalytically active molecules. Songyang et al., 1993, Cell 72:767-778. The specificity of the interactions between receptors and SH2 domains of their substrates is determined by the amino acid residues immediately surrounding the phosphorylated tyrosine residue. Differences in the binding affinities between SH2 domains and the amino acid sequences surrounding the phosphotyrosine residues on particular receptors are consistent with the observed differences in their substrate phosphorylation profiles. Songyang et al., 1993, Cell 72:767-778. These observations suggest that the function of each RTK is determined not only by its pattern of expression and ligand availability but also by the array of downstream signal transduction pathways that are activated by a particular receptor. Thus, phosphorylation provides an important regulatory step which determines the selectivity of signaling pathways recruited by specific growth factor receptors, as well as differentiation factor receptors.

STKs, being primarily cytosolic, affect the internal biochemistry of the cell, often as a down-line response to a PTK event. STKs have been implicated in the signaling process which

initiates DNA synthesis and subsequent mitosis leading to cell proliferation.

Thus, PK signal transduction results in, among other responses, cell proliferation, differentiation, growth and metabolism. Abnormal cell proliferation may result in a wide array of disorders and diseases, including the development of neoplasia such as carcinoma, sarcoma, glioblastoma and hemangioma, disorders such as leukemia, psoriasis, arteriosclerosis, arthritis and diabetic retinopathy and other disorders related to uncontrolled angiogenesis and/or vasculogenesis.

A precise understanding of the mechanism by which the compounds of this invention inhibit PKs is not required in order to practice the present invention. However, while not hereby being bound to any particular mechanism or theory, it is believed that the compounds interact with the amino acids in the catalytic region of PKs. PKs typically possess a bi-lobate structure wherein ATP appears to bind in the cleft between the two lobes in a region where the amino acids are conserved among PKs. Inhibitors of PKs are believed to bind by non-covalent interactions such as hydrogen bonding, van der Waals forces and ionic interactions in the same general region where the aforesaid ATP binds to the PKs. More specifically, it is thought that the 2-indolinone component of the compounds of this invention binds in the general space normally occupied by the adenine ring of ATP. Specificity of a particular molecule for a particular PK may then arise as the result of additional interactions between the various substituents on the 2-indolinone core and the amino acid domains specific to particular PKs. Thus, different indolinone substituents may contribute to preferential binding to particular PKs. The ability to select compounds active at different ATP (or other nucleotide) binding sites makes the compounds of this invention useful for targeting any protein with

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such a site. The compounds disclosed herein may thus have utility as in vitro assays for such proteins as well as exhibiting in vivo therapeutic effects through interaction with such proteins.

In another aspect, the protein kinase, the catalytic activity of which is modulated by contact with a compound of this invention, is a protein tyrosine kinase, more particularly, a receptor protein tyrosine kinase. Among the receptor protein tyrosine kinases whose catalytic activity can be modulated with a compound of this invention, or salt thereof, are, without limitation, EGF, HER2, HER3, HER4, IR, IGF-1R, IRR, PDGFRα, PDGFRβ, CSFIR, C-Kit, C-fms, Flk-1R, Flk4, KDR/Flk-1, Flt-1, FGFR-1R, FGFR-2R, FGFR-3R and FGFR-4R.

The protein tyrosine kinase whose catalytic activity is modulated by contact with a compound of this invention, or a salt or a prodrug thereof, can also be a non-receptor or cellular protein tyrosine kinase (CTK). Thus, the catalytic activity of CTKs such as, without limitation, Src, Frk, Btk, Csk, Abl, ZAP70, Fes, Fps, Fak, Jak, Ack, Yes, Fyn, Lyn, Lck, Blk, Hck, Fgr and Yrk, may be modulated by contact with a compound or salt of this invention.

Still another group of PKs which may have their catalytic activity modulated by contact with a compound of this invention are the serine-threonine protein kinases such as, without limitation, CDK2 and Raf.

In another aspect, this invention relates to a method for treating or preventing a PK related disorder by administering a therapeutically effective amount of a compound of this invention, or a salt or a prodrug thereof, to an organism.

It is also an aspect of this invention that a pharmaceutical composition containing a compound of this invention or a salt or prodrug thereof is administered to an organism for the purpose of preventing or treating a PK related disorder.

This invention is therefore directed to compounds that modulate PK signal transduction by affecting the enzymatic activity of RTKs, CTKs and/or STKs, thereby interfering with the signals transduced by such proteins. More particularly, the present invention is directed to compounds which modulate RTK, CTK and/or STK mediated signal transduction pathways as a therapeutic approach to the treatment of many kinds of solid tumors, including but not limited to carcinomas, sarcomas including Kaposi's sarcoma, erythroblastoma, glioblastoma, meningioma, astrocytoma, melanoma and myoblastoma. Treatment or prevention of non-solid tumor cancers such as leukemia are also contemplated by this invention. Indications may include, but are not limited to brain cancers; bladder cancers, ovarian cancers, gastric cancers, pancreas cancers, colon cancers, blood cancers, lung cancers and bone cancers.

Further examples, without limitation, of the types of disorders related to inappropriate PK activity that the compounds described herein may be useful in preventing, treating and studying, are cell proliferative disorders, fibrotic disorders and metabolic disorders.

Cell proliferative disorders, which may be prevented, treated or further studied by the present invention include cancer, blood vessel proliferative disorders and mesangial cell proliferative disorders.

Blood vessel proliferative disorders refer to disorders related to abnormal vasculogenesis (blood vessel formation) and angiogenesis (spreading of blood vessels). While vasculogenesis and angiogenesis play important roles in a variety of normal physiological processes such as embryonic development, corpus luteum formation, wound healing and organ regeneration, they also play a pivotal role in cancer development where they result in the formation of new capillaries needed to keep a tumor alive. Other examples of blood vessel proliferation disorders include

arthritis, where new capillary blood vessels invade the joint and destroy cartilage, and ocular diseases, like diabetic retinopathy, where new capillaries in the retina invade the vitreous, bleed and cause blindness.

Two structurally related RTKs have been identified to bind VEGF with high affinity: the fms-like tyrosine 1 (fit-1) receptor (Shibuya et al., 1990, Oncogene,5:519-524; De Vries et al., 1992, Science, 255:989-991) and the KDR/FLK-1 receptor, also known as VEGF-R2. Vascular endothelial growth factor (VEGF) has been reported to be an endothelial cell specific mitogen with in vitro endothelial cell growth promoting activity. Ferrara & Henzel, 1989, Biochein. Biophys. Res. Comm., 161:851-858; Vaisman et al., 1990, J. Biol. Chem., 265:19461-19566. Information set forth in U.S. application Ser. Nos. 08/193,829, 08/038,596 and 07/975,750, strongly suggest that VEGF is not only responsible for endothelial cell proliferation, but also is the prime regulator of normal and pathological angiogenesis. See generally, Klagsburn & Soker, 1993, Current Biology, 3(10)699-702; Houck, et al., 1992, J. Biol. Chem., 267:26031-26037.

Normal vasculogenesis and angiogenesis play important roles in a variety of physiological processes such as embryonic development, wound healing, organ regeneration and female reproductive processes such as follicle development in the corpus luteum during ovulation and placental growth after pregnancy. Folkman & Shing, 1992, J. Biological Chem., 267(16):10931-34. Uncontrolled vasculogenesis and/or angiogenesis has been associated with diseases such as diabetes as well as with malignant solid tumors that rely on vascularization for growth. Klagsburn & Soker, 1993, Current Biology, 3(10):699-702; Folkham, 1991, J. Natl. Cancer Inst., 82:4-6; Weidner, et al., 1991, New Engl. J. Med., 324:1-5.

The surmised role of VEGF in endothelial cell proliferation and migration during angiogenesis and vasculogenesis indicates an

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important role for the KDR/FLK-1 receptor in these processes. Diseases such as diabetes mellitus (Folkman, 198, in XIth Congress of Thrombosis and Haemostasis (Verstraeta, et al., eds.), pp. 583-596, Leuven University Press, Leuven) and arthritis, as well as malignant tumor growth may result from uncontrolled angiogenesis. See e.g., Folkman, 1971, N. Engl. J. Med., 285:1182-1186. The receptors to which VEGF specifically binds are an important and powerful therapeutic target for the regulation and modulation of vasculogenesis and/or angiogenesis and a variety of severe diseases which involve abnormal cellular growth caused by such processes. Plowman, et al., 1994, DN&P, 7(6):334-339. More particularly, the KDR/FLK-1 receptor's highly specific role in neovascularization make it a choice target for therapeutic approaches to the treatment of cancer and other diseases which involve the uncontrolled formation of blood vessels.

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Thus, one aspect of the present invention relates to compounds capable of regulating and/or modulating tyrosine kinase signal transduction including KDR/FLK-1 receptor signal transduction in order to inhibit or promote angiogenesis and/or vasculogenesis, that is, compounds that inhibit, prevent, or interfere with the signal transduced by KDR/FLK-1 when activated by ligands such as VEGF. Although it is believed that the compounds of the present invention act on a receptor or other component along the tyrosine kinase signal transduction pathway, they may also act directly on the tumor cells that result from uncontrolled angiogenesis.

Although the nomenclature of the human and murine counterparts of the generic "flk-I" receptor differ, they are, in many respects, interchangeable. The murine receptor, Flk-1, and its human counterpart, KDR, share a sequence homology of 93.4% within the intracellular domain. Likewise, murine FLK-I binds human VEGF with the same affinity as mouse VEGF, and accordingly,

is activated by the ligand derived from either species. Millauer et al., 1993, Cell, 72:835-846; Quinn et al., 1993, Proc. Natl. Acad. Sci. USA, 90:7533-7537. FLK-1 also associates with and subsequently tyrosine phosphorylates human RTK substrates (e.g., PLC-γ or p85) when co-expressed in 293 cells (human embryonal kidney fibroblasts).

Models which rely upon the FLK-1 receptor therefore are directly applicable to understanding the KDR receptor. For example, use of the murine FLK-1 receptor in methods which identify compounds that regulate the murine signal transduction pathway are directly applicable to the identification of compounds which may be used to regulate the human signal transduction pathway, that is, which regulate activity related to the KDR receptor. Thus, chemical compounds identified as inhibitors of KDR/FLK-1 in vitro, can be confirmed in suitable in vivo models. Both in vivo mouse and rat animal models have been demonstrated to be of excellent value for the examination of the clinical potential of agents acting on the KDR/FLK-1 induced signal transduction pathway.

Thus, in one aspect, this invention is directed to compounds that regulate, modulate and/or inhibit vasculogenesis and/or angiogenesis by affecting the enzymatic activity of the KDR/FLK-1 receptor and interfering with the signal transduced by KDR/FLK-1. In another aspect, the present invention is directed to compounds which regulate, modulate and/or inhibit the KDR/FLK-1 mediated signal transduction pathway as a therapeutic approach to the treatment of many kinds of solid tumors including, but not limited to, glioblastoma, melanoma and Kaposi's sarcoma, and ovarian, lung, mammary, prostate, pancreatic, colon and epidermoid carcinoma. In addition, data suggest the administration of compounds which inhibit the KDR/Flk-1 mediated signal transduction pathway may also be used in the treatment of hemangioma, restenois and diabetic retinopathy.

A further aspect of this invention relates to the inhibition of vasculogenesis and angiogenesis by other receptor-mediated pathways, including the pathway comprising the flt-1 receptor.

Receptor tyrosine kinase mediated signal transduction is initiated by extracellular interaction with a specific growth factor (ligand), followed by receptor dimerization, transient stimulation of the intrinsic protein tyrosine kinase activity and autophosphorylation. Binding sites are thereby created for intracellular signal transduction molecules which leads to the formation of complexes with a spectrum of cytoplasmic signalling molecules that facilitate the appropriate cellular response, e.g., cell division and metabolic effects to the extracellular microenvironment. See, Schlessinger and Ullrich, 1992, Neuron, 9:1-20.

The close homology of the intracellular regions of KDR/FLK-1 with that of the PDGF-β receptor (50.3% homology) and/or the related flt-1 receptor indicates the induction of overlapping signal transduction pathways. For example, for the PDGF-β receptor, members of the src family (Twamley et al., 1993, Proc. Natl. Acad. Sci. USA, 90:7696-7700), phosphatidylinositol-3'-kinase (Hu et al., 1992, Mol. Cell. Biol., 12:981-990), phospholipase cγ (Kashishian & Cooper, 1993, Mol. Cell. Biol., 4:49-51), ras-GTPase-activating protein, (Kashishian et al., 1992, EMBO J., 11:1373-1382), PTP-ID/syp (Kazlauskas et al., 1993, Proc. Natl. Acad. Sci. USA, 10 90:6939-6943), Grb2 (Arvidsson et al., 1994, Mol. Cell. Biol., 14:6715-6726), and the adapter molecules Shc and Nck (Nishimura et al., 1993, Mol. Cell. Biol., 13:6889-6896), have been shown to bind to regions involving different autophosphorylation sites. See generally, Claesson-Welsh, 1994, Prog. Growth Factor Res., 5:37-54. Thus, it is likely that signal transduction pathways activated by KDR/FLK-1 include the ras pathway (Rozakis et al., 1992, Nature, 360:689-692), the PI-3'-kinase, the src-mediated and the plcγ-mediated

pathways. Each of these pathways may play a critical role in the angiogenic and/or vasculogenic effect of KDR/FLK-1 in endothelial cells. Consequently, a still further aspect of this invention relates to the use of the organic compounds described herein to modulate angiogenesis and vasculogenesis as such processes are controlled by these pathways.

Conversely, disorders related to the shrinkage, contraction or closing of blood vessels, such as restenosis, are also implicated and may be treated or prevented by the methods of this invention.

Fibrotic disorders refer to the abnormal formation of extracellular matrices. Examples of fibrotic disorders include hepatic cirrhosis and mesangial cell proliferative disorders. Hepatic cirrhosis is characterized by the increase in extracellular matrix constituents resulting in the formation of a hepatic scar. An increased extracellular matrix resulting in a hepatic scar can also be caused by a viral infection such as hepatitis. Lipocytes appear to play a major role in hepatic cirrhosis. Other fibrotic disorders implicated include atherosclerosis.

Mesangial cell proliferative disorders refer to disorders brought about by abnormal proliferation of mesangial cells. Mesangial proliferative disorders include various human renal diseases such as glomerulonephritis, diabetic nephropathy and malignant nephrosclerosis as well as such disorders as thrombotic microangiopathy syndromes, transplant rejection, and glomerulopathies. The RTK PDGFR has been implicated in the maintenance of mesangial cell proliferation. Floege et al., 1993, Kidney International 43:47S-54S.

Many cancers are cell proliferative disorders and, as noted previously, PKs have been associated with cell proliferative disorders. Thus, it is not surprising that PKs such as, for example, members of the RTK family have been associated with the

development of cancer. Some of these receptors, like EGFR (Tuzi et al., 1991, Br. J. Cancer 63:227-233, Torp et al., 1992, APMIS 100:713-719) HER2/neu (Slamon et al., 1989, Science 244:707-712) and PDGF-R (Kumabe et al., 1992, Oncogene, 7:627-633) are over-expressed in many tumors and/or persistently activated by autocrine loops. In fact, in the most common and severe cancers these receptor over-expressions (Akbasak and Suner-Akbasak et al., 1992, J. Neurol. Sci., 111:119-133, Dickson et al., 1992, Cancer Treatment Res. 61:249-273, Korc et al., 1992, J. Clin. Invest. 90:1352-1360) and autocrine loops (Lee and Donoghue, 1992, J. Cell. Biol., 118:1057-1070, Korc et al., supra, Akbasak and Suner-Akbasak et al., supra) have been demonstrated. For example, EGFR has been associated with squamous cell carcinoma, astrocytoma, glioblastoma, head and neck cancer, lung cancer and bladder cancer. HER2 has been associated with breast, ovarian, gastric, lung, pancreas and bladder cancer. PDGFR has been associated with glioblastoma and melanoma as well as lung, ovarian and prostate cancer. The RTK c-met has also been associated with malignant tumor formation. For example, c-met has been associated with, among other cancers, colorectal, thyroid, pancreatic, gastric and hepatocellular carcinomas and lymphomas. Additionally c-met has been linked to leukemia. Over-expression of the c-met gene has also been detected in patients with Hodgkins disease and Burkitts disease.

IGF-IR, in addition to being implicated in nutritional support and in type-II diabetes, has also been associated with several types of cancers. For example, IGF-I has been implicated as an autocrine growth stimulator for several tumor types, e.g. human breast cancer carcinoma cells (Arteaga et al., 1989, J. Clin. Invest. 84:1418-1423) and small lung tumor cells (Macauley et al., 1990, Cancer Res., 50:2511-2517). In addition, IGF-I, while integrally involved in the normal growth and differentiation of the nervous system, also appears to be an autocrine stimulator of human

gliomas. Sandberg-Nordqvist et al., 1993, Cancer Res. 53:2475-2478. The importance of IGF-IR and its ligands in cell proliferation is further supported by the fact that many cell types in culture (fibroblasts, epithelial cells, smooth muscle cells, T-lymphocytes, myeloid cells, chondrocytes and osteoblasts (the stem cells of the bone marrow)) are stimulated to grow by IGF-I. Goldring and Goldring, 1991, Eukaryotic Gene Expression, 1:301-326. Baserga and Coppola suggest that IGF-IR plays a central role in the mechanism of transformation and, as such, could be a preferred target for therapeutic interventions for a broad spectrum of human malignancies. Baserga, 1995, Cancer Res., 55:249-252, Baserga, 1994, Cell 79:927-930, Coppola et al., 1994, Mol. Cell. Biol., 14:4588-4595.

STKs have been implicated in many types of cancer including, notably, breast cancer (Cance, et al., Int. J. Cancer, 54:571-77 (1993)).

The association between abnormal PK activity and disease is not restricted to cancer. For example, RTKs have been associated with diseases such as psoriasis, diabetes mellitus, endometriosis, angiogenesis, atheromatous plaque development, Alzheimer's disease, restenosis, von Hippel-Lindau disease, epidermal hyperproliferation, neurodegenerative diseases, age-related macular degeneration and hemangiomas. For example, EGFR has been indicated in corneal and dermal wound healing. Defects in Insulin-R and IGF-1R are indicated in type-II diabetes mellitus. A more complete correlation between specific RTKs and their therapeutic indications is set forth in Plowman et al., 1994, DN&P 7:334-339.

As noted previously, not only RTKs but CTKs including, but not limited to, src, abl, fps, yes, fyn, lyn, lck, blk, hck, fgr and yrk (reviewed by Bolen et al., 1992, FASEB J., 6:3403-3409) are involved in the proliferative and metabolic signal transduction pathway and thus could be expected, and have been shown, to be

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involved in many PTK-mediated disorders to which the present invention is directed. For example, mutated src (v-src) has been shown to be an oncoprotein ($pp60^{v\text{-}src}$) in chicken. Moreover, its cellular homolog, the proto-oncogene $pp60^{c\text{-}src}$ transmits oncogenic signals of many receptors. Over-expression of EGFR or HER2/neu in tumors leads to the constitutive activation of $pp60^{c\text{-}src}$, which is characteristic of malignant cells but absent in normal cells. On the other hand, mice deficient in the expression of c-src exhibit an osteopetrotic phenotype, indicating a key participation of c-src in osteoclast function and a possible involvement in related disorders.

Similarly, Zap70 has been implicated in T-cell signaling which may relate to autoimmune disorders.

STKs have been associated with inflamation, autoimmune disease, immunoresponses, and hyperproliferation disorders such as restenosis, fibrosis, psoriasis, osteoarthritis and rheumatoid arthritis.

PKs have also been implicated in embryo implantation. Thus, the compounds of this invention may provide an effective method of preventing such embryo implantation and thereby be useful as birth control agents.

Finally, both RTKs and CTKs are currently suspected as being involved in hyperimmune disorders.

A method for identifying a chemical compound that modulates the catalytic activity of one or more of the above discussed protein kinases is another aspect of this invention. The method involves, for example, contacting cells expressing the desired protein kinase with a compound of this invention (or its salt or prodrug) and monitoring the cells for any effect that the compound has on them. The effect may be any observable, either to the naked eye or through the use of instrumentation, change or absence of change in a cell phenotype. The change or absence of change in the cell phenotype monitored may be, for example, without limitation, a change or absence of change in the

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catalytic activity of the protein kinase in the cells or a change or absence of change in the interaction of the protein kinase with a natural binding partner.

Examples of the effect of a number of exemplary compounds of this invention on several PTKs are shown in Table 2. The compounds and data presented are not to be construed as limiting the scope of this invention in any manner whatsoever.

3. PHARMACEUTICAL COMPOSITIONS AND USE

A compound of the present invention, a prodrug thereof or a physiologically acceptable salt of either the compound or its prodrug, can be administered as such to a human patient or can be administered in pharmaceutical compositions in which the foregoing materials are mixed with suitable carriers or excipient(s). Techniques for formulation and administration of drugs may be found in "Remington's Pharmacological Sciences," Mack Publishing Co., Easton, PA, latest edition.

Routes of Administration.

As used herein, "administer" or "administration" refers to the delivery of a compound, salt or prodrug of the present invention or of a pharmaceutical composition containing a compound, salt or prodrug of this invention to an organism for the purpose of prevention or treatment of a PK-related disorder.

Suitable routes of administration may include, without limitation, oral, rectal, transmucosal or intestinal administration or intramuscular, subcutaneous, intramedullary, intrathecal, direct intraventricular, intravenous, intravitreal, intraperitoneal, intranasal, or intraocular injections. The preferred routes of administration are oral and parenteral.

Alternatively, one may administer the compound in a local rather than systemic manner, for example, via injection of the compound directly into a solid tumor, often in a depot or sustained release formulation.

Furthermore, one may administer the drug in a targeted drug delivery system, for example, in a liposome coated with tumor-specific antibody. The liposomes will be targeted to and taken up selectively by the tumor.

Composition/Formulation.

Pharmaceutical compositions of the present invention may be manufactured by processes well known in the art, e.g., by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping or lyophilizing processes.

Pharmaceutical compositions for use in accordance with the present invention may be formulated in conventional manner using one or more physiologically acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. Proper formulation is dependent upon the route of administration chosen.

For injection, the compounds of the invention may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks' solution, Ringer's solution, or physiological saline buffer. For transmucosal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

For oral administration, the compounds can be formulated by combining the active compounds with pharmaceutically acceptable carriers well known in the art. Such carriers enable the compounds of the invention to be formulated as tablets, pills, lozenges, dragees, capsules, liquids, gels, syrups, slurries, suspensions and the like, for oral ingestion by a patient. Pharmaceutical preparations for oral use can be made using a solid excipient, optionally grinding the resulting mixture, and processing the mixture of granules, after adding other suitable auxiliaries if desired, to obtain tablets or dragee cores.

Useful excipients are, in particular, fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol, cellulose preparations such as, for example, maize starch, wheat starch, rice starch and potato starch and other materials such as gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carboxymethylcellulose, and/or polyvinyl-pyrrolidone (PVP). If desired, disintegrating agents may be added, such as cross-linked polyvinyl pyrrolidone, agar, or alginic acid. A salt such as sodium alginate may also be used.

Dragee cores are provided with suitable coatings. For this purpose, concentrated sugar solutions may be used which may optionally contain gum arabic, talc, polyvinyl pyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for identification or to characterize different combinations of active compound doses.

Pharmaceutical compositions which can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a plasticizer, such as glycerol or sorbitol. The push-fit capsules can contain the active ingredients in admixture with a filler such as lactose, a binder such as starch, and/or a lubricant such as talc or magnesium stearate and, optionally, stabilizers. In soft capsules, the active compounds may be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols. Stabilizers may be added in these formulations, also.

Pharmaceutical compositions which may also be used include hard gelatin capsules. As a non-limiting example, the active compound capsule oral drug product formulation may be as 50 and 200 mg dose strengths (formulation codes J-011248-AA-00 and J-011248-AA-01, respectively). The two dose strengths are made

from the same granules by filling into different size hard gelatin capsules, size 3 for the 50 mg capsule and size 0 for the 200 mg capsule. The composition of the formulation may be, for example, as indicated in Table 3.

TABLE 3

Ingredient Name/Grade	Concentration in Granulation (% w/w)	Amount in 50 mg Capsule (mg)	Amount in 200 mg Capsule (mg)
Formulation Code	J-011248-AA	J-011248-AA-00	J-011248-AA-01
Active Compound NF	65.0	50.0	200.0
Mannitol NF	23.5	18.1	72.4
Croscarmellose sodium NF	6.0	4.6	18.4
Povidone K 30 NF	5.0	3.8	15.2
Magnesium stearate NF	0.5	0.38	1.52
Capsule, Swedish yellow NF		Size 3	Size 0

The capsules may be packaged into brown glass or plastic bottles to protect the active compound from light. The containers containing the active compound capsule formulation must be stored at controlled room temperature (15-30°C).

For administration by inhalation, the compounds for use according to the present invention are conveniently delivered in the form of an aerosol spray using a pressurized pack or a nebulizer and a suitable propellant, e.g., without limitation, dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane or carbon dioxide. In the case of a pressurized aerosol, the dosage unit may be controlled by providing a valve to deliver a metered amount. Capsules and cartridges of, for

example, gelatin for use in an inhaler or insufflator may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

The compounds may also be formulated for parenteral administration, e.g., by bolus injection or continuous infusion. Formulations for injection may be presented in unit dosage form, e.g., in ampoules or in multi-dose containers, with an added preservative. The compositions may take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulating materials such as suspending, stabilizing and/or dispersing agents.

Pharmaceutical compositions for parenteral administration include aqueous solutions of a water soluble form, such as, without limitation, a salt, of the active compound. Additionally, suspensions of the active compounds may be prepared in a lipophilic vehicle. Suitable lipophilic vehicles include fatty oils such as sesame oil, synthetic fatty acid esters such as ethyl oleate and triglycerides, or materials such as liposomes. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Optionally, the suspension may also contain suitable stabilizers and/or agents that increase the solubility of the compounds to allow for the preparation of highly concentrated solutions.

Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, e.g., sterile, pyrogen-free water, before use.

The compounds may also be formulated in rectal compositions such as suppositories or retention enemas, using, e.g., conventional suppository bases such as cocoa butter or other glycerides.

In addition to the fomulations described previously, the compounds may also be formulated as depot preparations. Such

long acting formulations may be administered by implantation (for example, subcutaneously or intramuscularly) or by intramuscular injection. A compound of this invention may be formulated for this route of administration with suitable polymeric or hydrophobic materials (for instance, in an emulsion with a pharamcologically acceptable oil), with ion exchange resins, or as a sparingly soluble derivative such as, without limitation, a sparingly soluble salt.

A non-limiting example of a pharmaceutical carrier for the hydrophobic compounds of the invention is a cosolvent system comprising benzyl alcohol, a nonpolar surfactant, a water-miscible organic polymer and an aqueous phase such as the VPD co-solvent system. VPD is a solution of 3% w/v benzyl alcohol, 8% w/v of the nonpolar surfactant Polysorbate 80, and 65% w/v polyethylene glycol 300, made up to volume in absolute ethanol. The VPD co-solvent system (VPD:D5W) consists of VPD diluted 1:1 with a 5% dextrose in water solution. This co-solvent system dissolves hydrophobic compounds well, and itself produces low toxicity upon systemic administration. Naturally, the proportions of such a co-solvent system may be varied considerably without destroying its solubility and toxicity characteristics. Furthermore, the identity of the co-solvent components may be varied: for example, other low-toxicity nonpolar surfactants may be used instead of Polysorbate 80, the fraction size of polyethylene glycol may be varied, other biocompatible polymers may replace polyethylene glycol, *e.g.*, polyvinyl pyrrolidone, and other sugars or polysaccharides may substitute for dextrose.

Alternatively, other delivery systems for hydrophobic pharmaceutical compounds may be employed. Liposomes and emulsions are well known examples of delivery vehicles or carriers for hydrophobic drugs. In addtion, certain organic

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solvents such as dimethylsulfoxide also may be employed, although often at the cost of greater toxicity.

Additionally, the compounds may be delivered using a sustained-release system, such as semipermeable matrices of solid hydrophobic polymers containing the therapeutic agent. Various sustained-release materials have been established and are well known by those skilled in the art. Sustained-release capsules may, depending on their chemical nature, release the compounds for a few weeks up to over 100 days. Depending on the chemical nature and the biological stability of the therapeutic reagent, additional strategies for protein stabilization may be employed.

The pharmaceutical compositions herein also may comprise suitable solid or gel phase carriers or excipients. Examples of such carriers or excipients include, but are not limited to, calcium carbonate, calcium phosphate, various sugars, starches, cellulose derivatives, gelatin, and polymers such as polyethylene glycols.

Many of the PK modulating compounds of the invention may be provided as physiologically acceptable salts wherein the claimed compound may form the negatively or the positively charged species. Examples of salts in which the compound forms the positively charged moiety include, without limitation, quaternary ammonium (defined elsewhere herein), salts such as the hydrochloride, sulfate, carbonate, lactate, tartrate, maleate, succinate wherein the nitrogen atom of the quaternary ammonium group is a nitrogen of the selected compound of this invention which has reacted with the appropriate acid. Salts in which a compound of this invention forms the negatively charged species include, without limitation, the sodium, potassium, calcium and magnesium salts formed by the reaction of a carboxylic acid group in the compound with an appropriate base (e.g. sodium hydroxide (NaOH), potassium hydroxide (KOH), Calcium hydroxide ($Ca(OH)_2$), etc.).

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Dosage.

Pharmaceutical compositions suitable for use in the present invention include compositions wherein the active ingredients are contained in an amount sufficient to achieve the intended purpose, e.g., the modulation of PK activity or the treatment or prevention of a PK-related disorder.

More specifically, a therapeutically effective amount means an amount of compound effective to prevent, alleviate or ameliorate symptoms of disease or prolong the survival of the subject being treated.

Determination of a therapeutically effective amount is well within the capability of those skilled in the art, especially in light of the detailed disclosure provided herein.

For any compound used in the methods of the invention, the therapeutically effective amount or dose can be estimated initially from cell culture assays. Then, the dosage can be formulated for use in animal models so as tc achieve a circulating concentration range that includes the IC_{50} as determined in cell culture (i.e., the concentration of the test compound which achieves a half-maximal inhibition of the PK activity). Such information can then be used to more accurately determine useful doses in humans.

Toxicity and therapeutic efficacy of the compounds described herein can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., by determining the IC_{50} and the LD_{50} (both of which are discussed elsewhere herein) for a subject compound. The data obtained from these cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage may vary depending upon the dosage form employed and the route of administration utilized. The exact formulation, route of administration and dosage can be chosen by the individual physician in view of the patient's condition. (See e.g., Fingl, et al., 1975, in "The

Pharmacological Basis of Therapeutics", Ch. 1 p.1).

Dosage amount and interval may be adjusted individually to provide plasma levels of the active species which are sufficient to maintain the kinase modulating effects. These plasma levels are referred to as minimal effective concentrations (MECs). The MEC will vary for each compound but can be estimated from in vitro data, e.g., the concentration necessary to achieve 50-90% inhibition of a kinase may be ascertained using the assays described herein. Dosages necessary to achieve the MEC will depend on individual characteristics and route of administration. HPLC assays or bioassays can be used to determine plasma concentrations.

Dosage intervals can also be determined using MEC value. Compounds should be administered using a regimen that maintains plasma levels above the MEC for 10-90% of the time, preferably between 30-90% and most preferably between 50-90%.

In cases of local administration or selective uptake, the effective local concentration of the drug may not be related to plasma concentration and other procedures known in the art may be employed to determine the correct dosage amount and interval.

The amount of a composition administered will, of course, be dependent on the subject being treated, the severity of the affliction, the manner of administration, the judgment of the prescribing physician, etc.

Packaging.

The compositions may, if desired, be presented in a pack or dispenser device, such as an FDA approved kit, which may contain one or more unit dosage forms containing the active ingredient. The pack may for example comprise metal or plastic foil, such as a blister pack. The pack or dispenser device may be accompanied by instructions for administration. The pack or dispenser may also be accompanied by a notice associated with the container in a form prescribed by a governmental agency regulating the

manufacture, use or sale of pharmaceuticals, which notice is reflective of approval by the agency of the form of the compositions or of human or veterinary administration. Such notice, for example, may be of the labeling approved by the U.S. Food and Drug Administration for prescription drugs or of an approved product insert. Compositions comprising a compound of the invention formulated in a compatible pharmaceutical carrier may also be prepared, placed in an appropriate container, and labeled for treatment of an indicated condition. Suitable conditions indicated on the label may include treatment of a tumor, inhibition of angiogenesis, treatment of fibrosis, diabetes, and the like.

4. SYNTHESIS

The compounds of this invention, as well as the precursor 2-oxindoles and aldehydes, may be readily synthesized using techniques well known in the chemical arts. It will be appreciated by those skilled in the art that other synthetic pathways for forming the compounds of the invention are available and that the following is offered by way of example and not limitation.

A. General synthetic procedure.

The following general methodology may be employed to prepare the compounds of this invention:

The appropriately substituted 2-oxindole (1 equiv.), the appropriately substituted aldehyde (1.2 equiv.) and a base (0.1 equiv.) are mixed in a solvent (1-2 ml/mmol 2-oxindole) and the mixture is then heated for from about 2 to about 12 hours. After cooling, the precipitate that forms is filtered, washed with cold ethanol or ether and vacuum dried to give the solid product. If no precipitate forms, the reaction mixture is concentrated and the residue is triturated with dichloromethane/ether, the resulting solid is collected by filtration and then dried. The product may optionally be further purified by chromatography.

The base may be an organic or an inorganic base. If an organic base is used, preferably it is a nitrogen base. Examples of organic nitrogen bases include, but are not limited to, diisopropylamine, trimethylamine, triethylamine, aniline, pyridine, 1,8-diazabicyclo[5.4.1]undec-7-ene, pyrrolidine and piperidine.

Examples of inorganic bases are, without limitation, ammonia, alkali metal or alkaline earth hydroxides, phosphates, carbonates, bicarbonates, bisulfates and amides. The alkali metals include, lithium, sodium and potassium while the alkaline earths include calcium, magnesium and barium.

In a presently preferred embodiment of this invention, when the solvent is a protic solvent, such as water or alcohol, the base is an alkali metal or an alkaline earth inorganic base, preferably, a alkali metal or an alkaline earth hydroxide.

It will be clear to those skilled in the art, based both on known general principles of organic synthesis and on the disclosures herein which base would be most appropriate for the reaction contemplated.

The solvent in which the reaction is carried out may be a protic or an aprotic solvent, preferably it is a protic solvent. A "protic solvent" is a solvent which has hydrogen atom(s) covalently bonded to oxygen or nitrogen atoms which renders the hydrogen atoms appreciably acidic and thus capable of being "shared" with a solute through hydrogen bonding. Examples of protic solvents include, without limitation, water and alcohols.

An "aprotic solvent" may be polar or non-polar but, in either case, does not contain acidic hydrogens and therefore is not capable of hydrogen bonding with solutes. Examples, without limitation, of non-polar aprotic solvents, are pentane, hexane, benzene, toluene, methylene chloride and carbon tetrachloride. Examples of polar aprotic solvents are chloroform, tetrahydrofuran, dimethylsulfoxide and dimethylformamide.

In a presently preferred embodiment of this invention, the solvent is a protic solvent, preferably water or an alcohol such as ethanol.

The reaction is carried out at temperatures greater than room temperature. The temperature is generally from about 30° C to about 150° C, preferably about 80°C to about 100° C, most preferable about 75° C to about 85° C, which is about the boiling point of ethanol. By "about" is meant that the temperature range is preferably within 10 degrees Celcius of the indicated temperature, more preferably within 5 degrees Celcius of the indicated temperature and, most preferably, within 2 degrees Celcius of the indicated temperature. Thus, for example, by "about 75° C" is meant 75° C ± 10° C, preferably 75° C ± 5° C and most preferably, 75° C ± 2° C.

B. Synthetic methods used in the examples which follow:

Method A: Formylation of pyrroles

$POCl_3$ (1.1 equiv.) is added dropwise to dimethylformamide (3 equiv.)at -10° C followed by addition of the appropriate pyrrole dissolved in dimethylformamide. After stirring for two hours, the reaction mixture is diluted with H_2O and basified to pH 11 with 10 N KOH. The precipitate which forms is collected by filtration, washed with H_2O and dried in a vacuum oven to give the desired aldehyde.

Method B: Saponification of pyrrolecarboxylic acid esters

A mixture of a pyrrolecarboxylic acid ester and KOH (2 - 4 equiv.) in EtOH is refluxed until reaction completion is indicated by thin layer chromatography (TLC). The cooled reaction mixtrue is acidified to pH 3 with 1 N HCl. The precipitate which forms is collected by filtration, washed with H_2O and dried in a vacuum oven to give the desired pyrrolecarboxylic acid.

Method C: Amidation

To a stirred solution of a pyrrolecarboxylic acid dissolved

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in dimethylformamide(0.3M) is added 1-ethyl-3-(3-dimethylamino-propyl)carbodiimide (1.2 equiv.), 1-hydroxybenzotriazole (1.2 equiv.), and triethylamine (2 equiv.). The appropriate amine is added (1 equiv.) and the reaction stirred until completion is indicated by TLC. Ethyl acetate is then added to the reaction mixture and the solution washed with saturated $NaHCO_3$ and brine (with extra salt), dried over anhydrous $MgSO_4$ and concentrated to afford the desired amide.

Method D: Condensation of aldehydes and oxindoles containing carboxylic acid substituents

A mixture of the oxindole (1 equivalent), 1 equivalent of the aldehyde and 1 - 3 equivalents of piperidine (or pyrrolidine) in ethanol (0.4 M) is stirred at 90-100° C until reaction completion is indicated by TLC. The mixture is then concentrated and the residue acidified with 2N HCl. The precipitate that forms is washed with H_2O and EtOH and then dried in a vacuum oven to give the product.

Method E: Condensation of aldehydes and oxindoles not containing carboxylic acid substituents

A mixture of the oxindole (1 equivalent), 1 equivalent of the aldehyde and 1 - 3 equivalents of piperidine (or pyrrolidine) in ethanol (0.4 M) is stirred at 90-100° C until reaction completion is indicated by TLC. The mixture is cooled to room temperature and the solid which forms is collected by vacuum filtration, washed with ethanol and dried to give the product. If a precipitate does not form upon cooling of the reaction mixture, the mixture is concentrated and purified by column chromatography.

C. Examples of oxindole syntheses

The following examples of the synthesis of representative oxindoles is not to be construed as limiting the scope of this invention in any manner whatsoever. Alternate routes to the oxindoles shown as well as other oxindoles to be used to make the

compounds of this invention will become apparent to those skilled in the art based on the following disclosures. Such syntheses and oxindoles are within the scope and spirit of this invention.

5-Amino-2-oxindole

5-Nitro-2-oxindole (6.3 g) was hydrogenated in methanol over 10% palladium on carbon to give 3.0 g (60% yield) of the title compound as a white solid.

5-Bromo-2-oxindole

2-Oxindole (1.3 g) in 20 mL acetonitrile was cooled to -10 °C and 2.0 g N-bromosuccinimide was slowly added with stirring. The reaction was stirred for 1 hour at -10 °C and 2 hours at 0 °C. The precipitate was collected, washed with water and dried to give 1.9 g (90 % yield) of the title compound.

4-Methyl-2-oxindole

Diethyl oxalate (30 mL) in 20 mL of dry ether was added with stirring to 19 g of potassium ethoxide suspended in 50 mL of dry ether. The mixture was cooled in an ice bath and 20 mL of 3-nitro-o-xylene in 20 mL of dry ether was slowly added. The thick dark red mixture was heated to reflux for 0.5 hr, concentrated to a dark red solid, and treated with 10% sodium hydroxide until almost all of the solid dissolved. The dark red mixture was treated with 30% hydrogen peroxide until the red color changed to yellow. The mixture was treated alternately with 10% sodium hydroxide and 30% hydrogen peroxide until the dark red color was no longer present. The solid was filtered off and the filtrate acidified with 6N hydrochloric acid. The resulting precipitate was collected by vacuum filtration, washed with water, and dried under vacuum to give 9.8 g (45% yield) of 2-methyl-6-nitrophenylacetic acid as an off-white solid. The solid was hydrogenated in methanol over 10 % palladium on carbon to give 9.04 g of the title compound as a white solid.

7-Bromo-5-chloro-2-oxindole

5-Chloro-2-oxindole (16.8 g) and 19.6 g of N-

bromosuccinimide were suspended in 140 mL of acetonitrile and refluxed for 3 hours. Thin layer chromatography (silica, ethyl acetate) at 2 hours of reflux showed 5-chloro-2-oxindole or N-bromosuccinimide (Rf 0.8), product (Rf 0.85) and a second product (Rf 0.9) whose proportions did not change after another hour of reflux. The mixture was cooled to 10 °C, the precipitate was collected by vacuum filtration, washed with 25 mL of ethanol and sucked dry for 20 minutes in the funnel to give 14.1 g of wet product (56 % yield). The solid was suspended in 200 mL of denatured ethanol and slurry-washed by stirring and refluxing for 10 minutes. The mixture was cooled in an ice bath to 10 °C. The solid product was collected by vacuum filtration, washed with 25 mL of ethanol and dried under vacuum at 40 °C to give 12.7 g (51% yield) of 7-bromo-5-chloro-2-oxindole.

5-Fluoro-2-oxindole

5-Fluoroisatin (8.2 g) was dissolved in 50 mL of hydrazine hydrate and refluxed for 1.0 hr. The reaction mixtures were then poured in ice water. The precipitate was then filtered, washed with water and dried in a vacuum oven to afford the title compound.

5-Nitro-2-oxindole

2-Oxindole (6.5 g) was dissolved in 25 mL concentrated sulfuric acid and the mixture maintained at -10 to -15 °C while 2.1 mL of fuming nitric acid was added dropwise. After the addition of the nitric acid the reaction mixture was stirred at 0 °C for 0.5 hr and poured into ice-water. The precipitate was collected by filtration, washed with water and crystallized from 50% acetic acid. The crystalline product was then filtered, washed with water and dried under vacuum to give 6.3 g (70%) of 5-nitro-2-oxindole.

5-Aminosulfonyl-2-oxindole

To a 100 mL flask charged with 27 mL of chlorosulfonic acid was added slowly 13.3 g of 2-oxindole. The reaction temperature

was maintained below 30 °C during the addition. After the addition, the reaction mixture was stirred at room temperature for 1.5 hr, heated to 68 °C for 1 hr, cooled, and poured into water. The precipitate was washed with water and dried in a vacuum oven to give 11.0 g of 5-chlorosulfonyl-2-oxindole (50% yield) which was used without further purification.

5-Chlorosulfonyl-2-oxindole (2.1 g) was added to 10 mL of ammonium hydroxide in 10 mL of ethanol and stirred at room temperature overnight. The mixture was concentrated and the solid collected by vacuum filtration to give 0.4 g (20% yield) of the title compound as an off-white solid.

5-Isopropylaminosulfonyl-2-oxindole

To a 100 mL flask charged with 27 mL chlorosulfonic acid was slowly added 13.3 g 2-oxindole. The reaction temperature was maintained below 30° C during the addition. The reaction mixture was stirred at room temperature for 1.5 hour, heated to 68° C for 1 hour, cooled, and poured into water. The precipitate which formed was filtered, washed with water and dried in a vacuum oven to give 11.0 g (50%) of 5-chlorosulfonyl-2-oxindole which was used without further purification.

A suspension of 3 g 5-chlorosulfonyl-2-oxindole, 1.15 g isopropylamine and 1.2 mL of pyridine in 50 mL of dichloromethane was stirred at room temperature for 4 hours during which time a white solid formed. The solid was collected by vacuum filtration, slurry-washed with hot ethanol, cooled, collected by vacuum filtration and dried under vacuum at 40° C overnight to give 1.5 g (45%) of 5-isopropylaminosulfonyl-2-oxindole.

^{1}HNMR (360 MHz, DMSO-d6) δ 10.69 (s, br, 1H, NH), 7.63 (dd, J = 2 and 8 Hz, 1H), 7.59 (d, J = 2 Hz, 1H), 7.32 (d, J = 7 Hz, 1H, N*H*-SO_2-), 6.93 (d, J = 8 Hz, 1H), 3.57 (s, 2H), 3.14-3.23 (m, 1H, C*H*-$(CH_3)_2$), 0.94 (d, J = 7 Hz, 6H, 2xCH_3).

5-Phenylaminosulfonyl-2-oxindole

A suspension of 5-chlorosulfonyl-2-oxindole (1.62 g, 7 mmol), aniline (0.782 mL, 8.4 mmol) and pyridine (1 mL) in dichloromethane (20 ml) was stirred at room temperature for 4 hours. The reaction mixture was diluted with ethyl acetate (300 mL) and acidified with 1N hydrochloric acid (16 mL). The organic layer was washed with sodium bicarbonate and brine, dried and concentrated. The residue was washed with ethanol (3 mL) and then chromatographed on silica gel eluting with methanol/ dichloromethane 1:9 to give of 5-phenylaminosulfonyl-2-oxindole.

^{1}HNMR (360 MHz, DMSO-d6) δ 10.71 (s, br, 1H, NH), 10.10 (s, br, 1H, NH), 7.57-7.61 (m, 2H), 7.17-7.22 (m, 2H), 7.06-7.09 (m, 2H), 6.97-7.0 (m, 1H), 6.88 (d, J = 8.4 Hz, 1H), 3.52 (s, 2H).

2-Oxo-2,3-dihydro-1H-indole-5-sulfonic acid pyridin-3-ylamide

A solution of 5-chlorosufonyl-2-oxindole (3 g) and 3-aminopyridine (1.46g) in pyridine (15 mL) was stirred at room temperature overnight at which time a brown solid was present. The solid was filtered, washed with ethanol and dried under vacuum to yield 1.4 g (38%) of 2-oxo-2,3-dihydro-1H-indole-5-sulfonic acid pyridin-3-ylamide.

^{1}HNMR (360 MHz, DMSO-d6) δ 10.74 (s, 1H, NH), 10.39 (s, 1H, SO_2NH), 8.27-8.28 (d, 1H), 8.21-8.23 (m, 1H), 7.59-7.62 (m, 2H), 7.44-7.68 (m, 1H), 7.24-7.28 (m, 1H), 6.69-6.71 (d, 1H), 3.54 (s, 2H).

MS *m/z* (APCI+) 290.2.

5-Phenyloxindole

5-Bromo-2-oxindole (5 g, 23.5 mmol) was dissolved in 110 mL toluene and 110 mL ethanol with stirring and a little heat. Tetrakis(triphenylphosphine)palladium(0) (1.9 g, 1.6 mmol) was added followed by 40 mL (80 mmol) 2M aqueous sodium carbonate. To this mixture was added benzene boronic acid (3.7 g, 30.6 mmol) and the mixture was heated in a 100° C oil bath for 12 hours. The reaction was cooled, diluted with ethyl acetate (500 mL), washed

with saturated sodium bicarbonate (200 mL), water (200 mL), 1N HCl (200 mL) and brine (200 mL). The organic layer was dried over magnesium sulfate and concentrated to afford a brown solid. Trituration with dichloromethane afforded 3.8 g (77%) of 5-phenyl-2-oxindole as a tan solid.

^{1}H NMR (360 MHz, DMSO-d6) δ 10.4 (br s, 1H, NH), 7.57 (dd, *J* = 1.8 and 7.2 Hz, 1H), 7.5 to 7.35 (m, 5H), 7.29 (m, 1H), 6.89 (d, *J* = 8.2 Hz, 1H), 3.51 (s, 2H, CH_2CO).

MS *m/z* 209 [M^+].

In similar fashion, the following oxindoles can be prepared:

6-(3,5-Dichlorophenyl)-1,3-dihydroindol-2-one

^{1}H NMR (360 MHz, DMSO-d6) δ 10.46 (br, 1H, NH), 7.64 (d, *J* = 1.8 Hz, 2H), 7.57 (m, 1H), 7.27 (m, 2H), 7.05 (d, J = 1.1 Hz, 1H), 3.5 (s, 2H).

MS-EI *m/z* 277/279 [M]$^+$.

6-(4-Butylphenyl)-1,3-dihydroindol-2-one

^{1}H NMR (360 MHz, DMSO-d6) δ 10.39 (s, 1H, NH), 7.49 (d, *J* = 8.0 Hz, 2H), 7.25 (d, *J* = 8 Hz, 3H), 7.17 (dd, *J* = 1.5 and 7.8 Hz, 1H), 6.99 (d, *J* = 1.5 Hz, 1H), 3.48 (s, 2H, CH_2CO), 2.60 (t, *J* = 7.5 Hz, 2Hz, CH_2CH_3), 1.57 (m, 2H, CH_2), 1.32 (m, 2H, CH_2), 0.9 (t, *J* = 7.5 Hz, 3H, CH_3).

6-(5-Isopropyl-2-methoxyphenyl)-1,3-dihydroindol-2-one

^{1}H NMR (360 MHz, DMSO-d6) δ 10.29 (br s, 1H, NH), 7.16-7.21 (m, 2H), 7.08 (d, *J* = 2.4 Hz, 1H), 6.97-7.01 (m, 2H), 6.89 (d, *J* = 0.8 Hz, 1H), 3.71 (s, 3H, OCH_3), 3.47 (s, 2H, CH_2CO), 2.86 (m, 1H, $CH(CH_3)_2$), 1.19 (d, *J* = 6.8 Hz, 6H, $CH(CH_3)_2$).

MS-EI *m/z* 281 [M^+].

6-(4-Ethylphenyl)-1,3-dihydroindol-2-one

^{1}H NMR (360 MHz, DMSO-d6) δ 10.39 (br s, 1H, NH), 7.50 (d, *J* = 8.2 Hz, 2H), 7.28 (d, *J* = 8.2 Hz, 2H), 7.25 (d, *J* = 7.5 Hz, 1H), 7.17 (dd, *J* = 1.6 & 7.5 Hz, 1H), 6.99 (d, *J* = 1.6 Hz, 1H), 3.48 (s, 2H, CH_2CO), 2.63 (q, *J* = 7.6 Hz, 2H, CH_2CH_3), 1.20 (t, *J*

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= 7.6 Hz, 3H, CH_2CH_3).

MS-EI *m/z* 237 $[M]^+$.

6-(3-Isopropylphenyl)-1,3-dihydroindol-2-one

^1H NMR (360 MHz, DMSO-d6) δ 10.37 (br s, 1H, NH), 7.43 (m, 1H), 7.35-7.39 (m, 1H), 7.17-7.27 (m, 3H), 7.01 (d, *J* = 1.8 Hz, 1H), 3.49 (s, 2H, CH_2CO), 2.95 (m, 1H, $CH(CH_3)_2$), 1.24 (d, *J* = 6.8 Hz, 6H, $CH(CH_3)_2$).

MS-EI *m/z* 251 $[M]^+$.

6-(2,4-Dimethoxyphenyl)-1,3-dihydroindol-2-one

^1H NMR (360 MHz, DMSO-d6) δ 10.28 (br s, 1H, NH), 7.17 (m, 2H), 6.93 (dd, *J* = 1.6 & 7.6 Hz, 1H), 6.86 (d, *J* = 1.6 Hz, 1H), 6.63 (d, *J* = 2.4 Hz, 1H), 6.58 (dd, *J* = 2.4 & 8.5 Hz, 1H), 3.79 (s, 3H, OCH_3), 3.74 (s, 3H, OCH_3), 3.45 (s, 2H, CH_2CO).

MS-EI *m/z* 269 $[M]^+$.

6-Pyridin-3-yl-1,3-dihydroindol-2-one

^1H NMR (360 MHz, DMSO-d6) δ 10.51 (s, 1H, NH), 8.81 (d, *J* = 2.5 Hz, 1H), 8.55 (dd, *J* = 1.8 and 5.7 Hz, 1H), 8 (m, 1H), 7.45 (dd, *J* = 5.7 and 9.3 Hz, 1H), 7.3 (m, 2H), 7.05 (s, 1H), 3.51 (s, 2H, CH_2CO).

MS *m/z* 210 $[M]^+$.

2-Oxo-2,3-dihydro-1H-indole-4-carboxylic acid (3-chloro-4-ethoxyphenyl)-amide

To a solution of 4-carboxy-2-oxindole (200 mg, 1.13 mmol) and 3-chloro-4-methoxyphenylamine (178 mg, 1.13 mmol) in dimethylformamide (15 mL) at room temperature was added benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (BOP reagent, 997 mg, 2.26 mmol) followed by 4-dimethylaminopyridine (206 mg, 1.69 mmol). The mixture was stirred at room temperature for 72 hours. The reaction was then diluted with ethyl acetate (300 mL), washed with saturated sodium bicarbonate (100 mL), water, 2N hydrochloric acid (100 mL), water (3x200 mL) and brine. It was then dried over magnesium sulfate

and concentrated. The residue was triturated with ethyl acetate to give 2-oxo-2,3-dihydro-1H-indole-4-carboxylic acid (3-chloro-4-methoxyphenyl)-amide as a pink solid.

^{1}HNMR (360 MHz, DMSO-d6) δ 10.50 (s, br, 1H, NH), 10.12 (s, br, 1H, NH), 7.9 (s, *J* = 2.5 Hz, 1H), 7.62 (dd, *J* = 2.5 & 9 Hz, 1H), 7.38 (d, *J* = 7.6 Hz, 1H), 7.32 (t, *J* = 7.6 Hz, 1H), 7.13 (d, *J* = 9 Hz, 1H), 6.98 (d, *J* = 7.6 Hz, 1H), 3.83 (s, 3H, OCH_3), 3.69 (s, 2H, CH_2).

MS-EI *m/z* 316 $[M]^+$.

4-Carboxy-2-oxindole

A solution of trimethylsilyldiazomethane in hexane (2 M) was added dropwise to a solution of 2.01 g 2-chloro-3-carboxy-nitrobenzene in 20 mL methanol at room temperature until no further gas evolution occurred. Acetic acid was then added to quench excess trimethylsilyldiazomethane. The reaction mixture was evaporated under vacuum and the residue was dried in an oven overnight. The 2-chloro-3-methoxycarbonylnitrobenzene obtained was pure enough for the following reaction.

Dimethyl malonate (6.0 mL) was added to an ice-cold suspension of 2.1 g sodium hydride in 15 mL DMSO. The reaction mixture was stirred at 100° C for 1 hour and then cooled to room temperature. 2-Chloro-3-methoxycarbonylnitrobenzene (2.15 g) was added in one portion and the mixture was heated to 100° C for 1.5 hours. The reaction mixture was then cooled to room temperature, poured into ice water, acidified to pH 5 and extracted with ethyl acetate. The organic layer was washed with brine, dried over anhydrous sodium sulfate and concentrated to give 3.0 g of the dimethyl 2-methoxycarbonyl-6-nitrophenyl- malonate.

Dimethyl 2-methoxycarbonyl-6-nitrophenylmalonate (3.0 g) was refluxed in 50 mL of 6 N hydrochloric acid overnight. The mixture was concentrated to dryness, 20 mL ethanol and 1.1 g of tin(II) chloride were added and the mixture was refluxed for 2 hours. The mixture was filtered through Celite, concentrated and

chromatographed on silica gel using ethyl acetate:hexane:acetic acid as eluent to give 0.65 g (37%) of 4-carboxy-2-oxindole as a white solid.

^{1}HNMR (360 MHz, DMSO-d6) δ 12.96 (s, br, 1H, COOH), 10.74 (s, br, 1H, NH), 7.53 (d, *J* = 8Hz, 1H), 7.39 (t, *J* = 8Hz, 1H), 7.12 (d, *J* = 8Hz, 1H), 3.67 (s, 2H).

D. Examples - Synthesis of pyrrole substituted 2-indolinones.

The following syntheses of representative compounds of this invention are shown by way of example only and are not to be construed as limiting the scope of this invention as to synthetic approach or as to the compounds which comprises this invention.

Example 1

4-Methyl-5-(2-oxo-1,2-dihydroindol-3-ylidenemethyl)-1*H*-pyrrole-2-carboxylic acid

4-Methyl-2-pyrrolecarboxylic acid ethyl ester (commercially available) was formylated using method **A** to give (73%) of 5-formyl-4-methyl-2-pyrrolecarboxylic acid ethyl ester. It was then hydrolysed using method **B** to give 5-formyl-4-methyl-1*H*-pyrrole-2-carboxylic acid (58%).

Oxindole (133 mg, 1 mmol) was condensed with 5-formyl-4-methyl-1*H*-pyrrole-2-carboxylic acid (153 mg) using method **D** to give 268 mg (100%) of the title compound as an orange-red solid.

^{1}HNMR (360 MHz, DMSO-d6) δ 13.84 (s, br, 1H, N*H*), 12.84 (s, br, 1H, COO*H*), 10.98 (s, br, 1H, NH), 7.82 (d, *J* = 7.5 Hz, 1H), 7.67 (s, 1H, H-vinyl), 7.18 (t, *J* = 7.5 Hz, 1H), 7.01 (t, *J* = 7.5 Hz, 1H), 6.88 (d, *J* = 7.5 Hz, 1H), 6.71 (d, *J* = 2.2 Hz, 1H), 2.32 (s, 3H, C*H*$_3$).

MS (negative mode) 266.8 $[M-1]^+$.

Example 2

4-Methyl-5-(1-methyl-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-1*H*-pyrrole-2-carboxylic acid

1-Methyl-1,3-dihydroindol-2-one (147 mg, 1 mmol) was condensed with 5-formyl-4-methyl-1*H*-pyrrole-2-carboxylic acid (153 mg) using

method D to give 250 mg (86%) of the title compound.

^{1}HNMR (360 MHz, DMSO-d6) δ 13.82 (s, br, 1H, N*H*), 12.88 (s, br, 1H, 7.83 (d, *J* = 7.5 Hz, 1H), 7.65 (s, 1H, H-vinyl), 7.26 (t, *J* = 7.5 Hz, 1H), 7.02-7.09 (m, 2H), 6.70 (d, *J* = 2.2 Hz, 1H), 2.32 (s, 3H, C*H*$_3$).

MS *m/z* 283.0 [M+1]$^+$.

Example 3

4-Methyl-5-(2-oxo-1,2-dihydroindol-3-ylidenemethyl)-1*H*-pyrrole-2-carboxylic acid methyl ester

Oxindole (105 mg, 0.79 mmol) was condensed with 5-formyl-4-methyl-1*H*-pyrrole-2-carboxylic acid methyl ester (110 mg, 0.67 mmol) using method E to give 153.2 mg (81%) of the title compound.

^{1}HNMR (360 MHz, DMSO-d6) δ 13.98 (s, br, 1H, N*H*), 10.97 (s, br, 1H, N*H*), 7.82 (d, *J* = 7.6 Hz, 1H), 7.67 (s, 1H, H-vinyl), 7.2 (dt, *J*= 1.2 & 7.7 Hz, 1H), 7.01 (dt, *J* = 1.2, 7.7 Hz, 1H), 6.90 (d, *J* = 7.6 Hz, 1H), 6.77 (d, *J* = 2 Hz, 1H).

MS (ES) *m/z* 283 [M$^+$+1].

Example 4

5-(5-Chloro-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-4-methyl-1*H*-pyrrole-2-carboxylic acid ethyl ester

5-Chloro-1,3-dihydroindol-2-one (2.22 g, 13.2 mmol) was condensed with 5-formyl-4-methyl-1*H*-pyrrole-2-carboxylic acid ethyl ester (2.43 g) using method E to give 4.1 g (94%) of the title compound as an orange solid.

^{1}HNMR (360 MHz, DMSO-d6) δ 13.95 (s, br, 1H, N*H*), 7.98 (d, *J* = 2.2 Hz, 1H, H-4), 7.78 (s, 1H, H-vinyl), 7.18 (dd, *J*= 2.2 & 8.3 Hz, 1H, H-6), 6.87 (d, *J* = 8.3 Hz, 1H, H-7), 7.34 (d, *J* = 1.8 Hz, 1H, H-3'), 4.27 (q, *J* = 7.2 Hz, 2H, OC*H*$_2$CH$_3$), 2.33 (s, 3H, C*H*$_3$), 1.29 (t, *J* = 7.2 Hz, 3H, OCH$_2$C*H*$_3$).

MS-EI *m/z* 330 [M$^+$].

Example 5

5-(5-Chloro-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-4-methyl-1*H*-pyrrole-2-carboxylic acid

A mixture of 5-(5-chloro-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-4-methyl-1*H*-pyrrole-2-carboxylic acid ethyl ester (1.3 g, 4 mmol) and potassium hydroxide in methanol (25 mL) and ethanol (25 mL) was heated to reflux for overnight. Insoluble materials were removed by filtration and the mixture was neutralized with 6N hydrochloric acid to give 0.876 g (70%) of the title compound.

^{1}HNMR (360 MHz, DMSO-d6) δ 13.80 (s, br, 1H, N*H*), 12.90 (s, br, 1H, COO*H*), 11.06 (s, br, 1H, N*H*), 8.02 (d, *J* = 1.8 Hz, 1H, H-4), 7.81 (s, 1H, H-vinyl), 7.20 (dd, *J*= 1.8 & 8.3 Hz, 1H, H-6), 6.89 (d, *J* = 8.3 Hz, 1H, H-7), 6.72 (d, *J* = 1.8 Hz, 1H, H-3'), 2.35 (s, 3H, C*H*$_3$).

MS-EI *m/z* 302 [M$^+$].

Example 6

5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-4-methyl-1*H*-pyrrole-2-carboxylic acid (3-pyrrolidin-1-yl-propyl)amide

5-Bromo-1,3-dihydroindol-2-one (0.16 g, 0.76 mmol) was condensed with 5-formyl-4-methyl-1*H*-pyrrole-2-carboxylic acid (3-pyrrolidin-1-ylpropyl)amide (0.2 g, prepared by method C) to give 60 mg (17%) of the title compound as an orange solid.

^{1}HNMR (300 MHz, DMSO-d6) δ 13.61 (s, br, 1H, N*H*), 11.02 (s, br, 1H, N*H*), 8.42 (t, *J* = 5.8 Hz, 1H, CONHCH$_2$), 8.12 (d, *J* = 1.8 Hz, 1H, H-4), 7.78 (s, 1H, H-vinyl), 7.30 (dd, *J* = 1.8 & 8.4 Hz, 1H, H-6), 6.82 (d, *J* = 8.4 Hz, 1H, H-7), 6.77 (d, *J* = 2.4 Hz, 1H, H-3'), 3.22-3.31 (m, 2H, CH$_2$), 2.38-2.43 (m, 6H, 3xC*H*$_2$), 2.35 (s, 3H, C*H*$_3$), 1.62-1.71 (m, 6H, 3xC*H*$_2$).

MS-EI *m/z* 456 and 458 [M$^+$-1 and M$^+$+2].

536
534

Example 7

5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-4-methyl-1*H*-pyrrole-2-carboxylic acid (3-diethylamino-propyl)amide

5-Bromo-1,3-dihydroindol-2-one (0.16 g, 0.75 mmol) was condensed with 5-formyl-4-methyl-1*H*-pyrrole-2-carboxylic acid (3-diethylaminopropyl)amide (0.2 g, prepared by method C) to give 30 mg (8%) of the title compound as an orange solid.

^{1}HNMR (300 MHz, DMSO-d6) δ 13.61 (s, br, 1H, N*H*), 11.02 (s, br, 1H, N*H*), 8.40 (m, 1H, CON*H*CH_2), 8.12 (d, J = 1.5 Hz, 1H, H-4), 7.78 (s, 1H, H-vinyl), 7.30 (dd, J = 1.5 & 8.2 Hz, 1H, H-6), 6.82 (d, J = 8.2 Hz, 1H, H-7), 6.78 (d, J = 2.4 Hz, 1H, H-3'), 3.23 (m, 2H, C*H*$_2$), 2.38-2.45 (m, 6H, CH_2 & N(CH_2CH_3)$_2$), 2.35 (s, 3H, CH_3), 1.61 (m, 2H, CH_2), 0.93 (t, J = 7.1 Hz, 6H, N(CH_2CH_3)$_2$).

MS-EI *m/z* 458 and 460 [M^+-1 and M^++2].

Example 8

5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-1*H*-pyrrole-2-carboxylic acid (2-diethylaminoethyl)amide

5-Bromo-1,3-dihydroindol-2-one (212 mg, 1 mmol) was condensed with 5-formyl-1*H*-pyrrole-2-carboxylic acid (2-diethylaminoethyl)amide (prepared from ethyl pyrrole-2-carboxylate by method A, B and then C) to give 162 mg (38%) of the title compound.

^{1}H NMR (300 MHz, DMSO-d6) δ 13.53 (s, br, 1H, N*H*), 11.06 (s, br, 1H, N*H*), 8.37 (t, 1H, CON*H*CH_2), 7.89 (m, 2H), 7.32 (dd, J = 2.0 Hz, 1H), 6.96 (s, 1H), 6.80-6.84 (m, 2H), 3.3 (m, 2H, CH_2), 2.45-2.55 (m, 6H, N(CH_2CH_3)$_2$ & C*H*$_2$), 0.95 (t, J = 7.2 Hz, 6H, N(CH_2CH_3)$_2$).

MS-EI *m/z* 430 and 432 [M^+-1 and M^++ 1].

537 535 272

Example 9

5-(2-Oxo-6-phenyl-1,2-dihydroindol-3-ylidenemethyl)-1*H*-pyrrole-2-carboxylic acid (2-diethylaminoethyl)amide

6-Phenyl-1,3-dihydroindol-2-one (209 mg, 1mmol) was condensed with 5-formyl-1*H*-pyrrole-2-carboxylic acid (2-diethylaminoethyl)amide to give 182 mg (42%) of the title compound.

^{1}H NMR (300 MHz, DMSO-d6) δ 13.56 (s, br, 1H, N*H*), 11.06 (s, br, 1H, N*H*), 8.36 (t, 1H, CON*H*CH_2), 7.77 (s, 1H, H-vinyl), 7.73 (d, J = 7.8 Hz, 1H), 7.64 (d, J = 7.2 Hz, 2H), 7.46 (m, 2H), 7.32 (m, 2H), 7.11 (s, 1H), 6.96 (m, 1H), 6.80 (m, 1H), 3.31-3.32 (m, 2H, CH_2), 2.46-2.53 (m, 6H, $N(CH_2CH_3)_2$ & CH_2), 0.96 (t, J = 6.9 Hz, 6H, $N(CH_2CH_3)_2$).

MS-EI *m/z* 428 [M^+].

Example 10

5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-1*H*-pyrrole-2-carboxylic acid (2-diethylaminoethyl)-methyl-amide

5-Bromo-1,3-dihydroindol-2-one (212 mg, 1 mmol) was condensed with 5-formyl-1*H*-pyrrole-2-carboxylic acid (2-diethylaminoethyl)methylamide to give 246 mg (55%) of the title compound.

^{1}H NMR (360 MHz, DMSO-d6) δ 13.54 (s, br, 1H, N*H*), 11.06 (s, br, 1H, N*H*), 7.90 (m, 2H), 7.33 (dd, J = 1.8 & 8.4 Hz, 1H), 6.82-6.85 (m, 3H), 3.55 (s, br, 2H, CH_2), 3.25 (s, br, 3H, NCH_3), 2.57 (t, J = 6.5 Hz, 2H, CH_2), 2.45 (m, 4H, $N(CH_2CH_3)_2$), 0.91 (m, 6H, $N(CH_2CH_3)_2$).

MS-EI m/z 444 and 446 [M^+-1 and M^+ +1].

Example 11

5-(2-Oxo-6-phenyl-1,2-dihydroindol-3-ylidenemethyl)-1*H*-pyrrole-2-carboxylic acid (2-diethylaminoethyl)methylamide

6-Phenyl-1,3-dihydroindol-2-one (209 mg, 1 mmol) was condensed with 5-formyl-1*H*-pyrrole-2-carboxylic acid (2-diethylaminoethyl)methylamide to give 277 mg (63%) of the title

compound.

^{1}H NMR (360 MHz, DMSO-d6) δ 13.58 (s, br, 1H, NH), 11.04 (s, br, 1H, NH), 7.78 (s, 1H, H-vinyl), 7.73 (d, *J* = 7.8 Hz, 1H), 7.64 (d, *J* = 7.5 Hz, 2H), 7.46 (m, 2H), 7.33-7.36 (m, 2H), 7.11 (s, 1H), 6.84 (m, 1H), 6.78 (m, 1H), 3.55 (s, br, 2H, CH_2), 3.25 (s, br, 3H, NCH_3), 2.58 (t, 2H, CH_2), 2.44 (m, 4H, $N(CH_2CH_3)_2$), 0.92 (m, 6H, $N(CH_2CH_3)_2$).

Example 12

3-Methyl-5-(2-oxo-1,2-dihydroindol-3-ylidenemethyl)-1*H*-pyrrole-2-carboxylic acid (3-diethylaminopropyl)amide

Oxindole (66.5 mg, 0.5 mmol) was condensed with 5-formyl-3-methyl-1H-pyrrole-2-carboxylic acid (3-diethylaminopropyl)amide (prepared from 3-formyl-3-methyl-1H-pyrrole-2-carboxylic acid ethyl ester by method B then C) to give 39 mg (21%) of the title compound.

^{1}H NMR (300 MHz, DMSO-d6) δ 13.34 (s, br, 1H, N*H*), 10.88 (s, br, 1H, N*H*), 7.62-7.67 (m, 3H), 7.17 (m, 1H), 6.99 (m, 1H), 6.87 (d, *J* = 7.6 Hz, 1H), 6.63 (d, *J* = 1 Hz, 1H), 3.26-3.32 (m, 2H, CH_2), 2.41-2.48 (m, 6H, CH_2 & $N(CH_2CH_3)_2$), 2.29 (s, 3H, CH_3), 1.63 (m, 2H, CH_2), 0.93 (t, *J* = 7.2 Hz, 6H, $N(CH_2CH_3)_2$).

MS-EI *m/z* 380 [M^+].

Example 13

5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-3-methyl-1*H*-pyrrole-2-carboxylic acid (3-diethylamino-propyl)amide

5-Bromo-1,3-dihydroindol-2-one (106 mg, 0.5 mmol) was condensed with 5-formyl-3-methyl-1H-pyrrole-2-carboxylic acid (3-diethylaminopropyl)amide to give 35 mg (15%) of the title compound.

^{1}H NMR (300 MHz, DMSO-d6) δ 13.35 (s, br, 1H, N*H*), 11.00 (s, br, 1H, N*H*); 7.89 (d, *J* = 1.9 Hz, 1H, H-4), 7.80 (s, 1H, H-vinyl), 7.74 (t, *J* = 5.3 Hz, 1H, $CONHCH_2$), 7.31 (dd, *J* = 1.9 & 8.4 Hz, 1H, H-6), 6.83 (d, *J* = 8.4 Hz, 1H, H-7), 6.63 (s,1H, H-

3'), 3.26 (m, 2H, CH_2), 2.41-2.48 (m, 6H, CH_2 & $N(CH_2CH_3)_2$), 2.29 (s, 3H, CH_3), 1.63 (m, 2H, CH_2), 0.93 (t, J = 7.1 Hz, 6H, $N(CH_2CH_3)_2$).

MS-EI m/z 458 and 460 [M^+-1 and M^++1].

Example 14

3-Methyl-5-(2-oxo-6-phenyl-1,2-dihydroindol-3-ylidenemethyl)-1*H*-pyrrole-2-carboxylic acid (3-diethylaminopropyl)amide

6-Phenyl-1,3-dihydroindol-2-one (105 mg, 0.5 mmol) was condensed with 5-formyl-3-methyl-1H-pyrrole-2-carboxylic acid (3-diethylaminopropyl)amide to give 67.8 (30%) of the title compound.

^1H NMR (300 MHz, DMSO-d6) δ 13.37 (s, br, 1H, N*H*), 11.02 (s, br, 1H, N*H*), 7.23-7.73 (m, 11H), 3.29 (m, 2H, CH_2), 2.41-2.48 (m, 6H, CH_2 & $N(CH_2CH_3)_2$), 2.29 (s, 3H, CH_3), 1.64 (m, 2H, CH_2), 0.94 (t, J = 7.0 Hz, 6H, $N(CH_2CH_3)_2$).

MS-EI m/z 456 [M^+].

Example 15

5-(5-Methoxy-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-3-methyl-1*H*-pyrrole-2-carboxylic acid (3-diethylamino-propyl)amide

5-Methoxy-1,3-dihydroindol-2-one (82.5 mg, 0.5 mmol) was condensed with 5-formyl-3-methyl-1H-pyrrole-2-carboxylic acid (3-diethylaminopropyl)amide to give 80 mg (39%) of the title compound.

^1H NMR (300 MHz, DMSO-d6) δ 13.45 (s, br, 1H, N*H*), 10.70 (s, br, 1H, N*H*), 7.68-7.70 (m, 2H), 7.32 (d, J = 1.8 Hz, 1H), 6.72-6.79 (m, 2H), 6.60 (s, 1H), 3.73 (s, 3H, OCH_3), 3.28 (m, 2H, CH_2), 2.41-2.48 (m, 6H, CH_2 & $N(CH_2CH_3)_2$), 2.29 (s, 3H, CH_3), 1.63 (m, 2H, CH_2), 0.93 (t, J = 7.0 Hz, 6H, $N(CH_2CH_3)_2$).

MS m/z 410 [M^+].

540
538

Example 16

5-(6-Methoxy-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-3-methyl-1*H*-pyrrole-2-carboxylic acid (3-diethylamino-propyl)amide

6-Methoxy-1,3-dihydroindol-2-one (82.5 mg, 0.5 mmol) was condensed with 5-formyl-3-methyl-1H-pyrrole-2-carboxylic acid (3-diethylaminopropyl)amide to give 63 mg (31%) of the title compound.

^{1}H NMR (300 MHz, DMSO-d6) δ 13.22 (s, br, 1H, N*H*), 10.86 (s, br, 1H, N*H*), 7.39-7.63 and 6.37-6.55 (m, 6H), 3.73 (s, 3H, OC*H*$_3$), 3.3 (m, 2H, C*H*$_2$), 2.45 (m, 6H, C*H*$_2$ & N(C*H*$_2$CH$_3$)$_2$), 2.28 (s, 3H, C*H*$_3$), 1.63 (m, 2H, C*H*$_2$), 0.93 (m, 6H, N(CH$_2$C*H*$_3$)$_2$).

MS *m/z* 410 [M$^+$].

Example 17

3-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-4,5,6,7-tetrahydro-2*H*-isoindole-1-carboxylic acid (2-diethylamino-ethyl)amide

4,5,6,7-Tetrahydro-2*H*-isoindole-1-carboxylic acid ethyl ester (May, Donald A.; Lash, Timothy D.; J. Org. Chem., 1992, 57:18, 4820-4828) was formylated using method A then B to give 3-formyl-4,5,6,7-tetrahydro-2*H*-isoindole-1-carboxylic acid.

5-Bromo-1,3-dihydroindol-2-one (1.43 g, 6.8 mmol) was condensed with 3-formyl-4,5,6,7-tetrahydro-2*H*-isoindole-1-carboxylic acid (2-diethylaminoethyl)amide (1.97 g) to give 2.2 g (67%) of the title compound as a yellow-orange solid.

^{1}H NMR (360 MHz, DMSO-d6) δ 13.47 (s, 1H, N*H*), 11.0 (s, 1H, N*H*), 8.0 (d, 1H, N*H*), 7.70 (s, 1H, C*H*), 7.28 (dd, *J* = 2.1 and 8.2 Hz, 1H, Ar*H*), 7.16 (m, 1H, Ar*H*), 6.8 (d, *J* = 8.3Hz, 1H, Ar*H*), 3.3 (s, 2H, CON*H*), 2.5 (m, 6H, 3xNC*H*$_2$), 2.78 (br m, 2H, pyrrole C*H*$_2$), 2.72 (br m, 2H, pyrroleC*H*$_2$), 1.7 (br m, 4H, N(C*H*$_2$CH$_3$)$_2$), 1.74 (br s, 4H, CH$_2$C*H*$_2$C*H*$_2$CH$_2$), 0.96 (t, *J* = 7.4 Hz, 6H, N(CH$_2$C*H*$_3$)$_2$).

MS-EI *m/z* 484 and 486 [M$^+$-1 and M$^+$+1].

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Example 18

3-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-4,5,6,7-tetrahydro-2*H*-isoindole-1-carboxylic acid (3-diethylaminopropyl)amide

5-Bromo-1,3-dihydroindol-2-one (20 mg, 0.1 mmol) was condensed with 3-formyl-4,5,6,7-tetrahydro-2*H*-isoindole-1-carboxylic acid (3-diethylaminopropyl)amide (30 mg) to give 33 mg (46%) of the title compound as an orange solid.

^{1}H NMR (360 MHz, DMSO-d6) δ 10.9 (s, 1H, N*H*), 8.0 (m, 1H, N*H*), 7.68 (m, 1H, Ar*H*), 7.4 (m, 1H, Ar*H*), 7.29 (d, *J* = 1.9 and 8.5Hz, 1H, Ar*H*), 6.8 (d, *J* = 8 Hz, 1H, Ar*H*), 2.7 (br m, 4H, 2xNCH_2), 2.4 (m, 8H, 4xNCH_2), 1.7 (br m, 4H, N(CH_2CH_3)$_2$), 1.6 (br m, 2H, $CH_2CH_2CH_2$), 0.93 (t, *J* = 7.4 Hz, 6H, N(CH_2CH_3)$_2$).

MS-EI *m/z* 499 and 501 [M^+ and M^++2].

Example 19

3-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-4,5,6,7-tetrahydro-2*H*-isoindole-1-carboxylic acid (3-pyrrolidin-1-ylpropyl)amide

5-Bromo-1,3-dihydroindol-2-one (80 mg, 0.4 mmol) was condensed with 3-formyl-4,5,6,7-tetrahydro-2*H*-isoindole-1-carboxylic acid (3-pyrrolidin-1-ylpropyl)amide (120 mg) to give 43 mg (22%) of the title compound as a tan-orange solid.

^{1}H NMR (360 MHz, DMSO-d6) δ 13.4 (s, 1H, N*H*), 10.9 (s, 1H, N*H*), 8.0 (m, 1H, N*H*), 7.69 (m, 1H, Ar*H*), 7.49 (m, 1H, Ar*H*), 7.28 (d, *J* = 1.7 and 7.8 Hz, 1H, Ar*H*), 6.8 (d, *J* = 8 Hz, 1H, Ar*H*), 3.3 (br m, 2H, 2xNCH_2), 2.8 (m, 4H, 2xpyrroleCH_2), 2.5 (br m, 4H, N(CH_2CH_3)$_2$), 1.6 (br m, 8H, 2xpyrroleCH$_2$CH_2, $CH_2CH_2CH_2$ and CONHCH_2).

MS-EI *m/z* 497 and 499 [M^+ and M^++2].

Example 20

3-(2-Oxo-6-pyridin-3-yl-1,2-dihydroindol-3-ylidenemethyl)-4,5,6,7-tetrahydro-2*H*-isoindole-1-carboxylic acid (2-diethylaminoethyl)amide

6-Pyridin-3-yl-1,3-dihydroindol-2-one (60 mg, 0.4 mmol) was condensed with 3-formyl-4,5,6,7-tetrahydro-2*H*-isoindole-1-carboxylic acid (2-diethylaminoethyl)amide (80 mg) to give 50 mg (38%) of the title compound as a reddish solid.

^{1}H NMR (360 MHz, DMSO-d6) δ 13.4 (s, 1H, NH), 11 (s, 1H, NH), 8.9 (d, 1H, NH), 8.7 (dd, 1H, ArH), 8.1 (dd, 1H, ArH), 7.9 (d, 1H, ArH), 7.6 (s, 1H, CH), 7.5 (dd, 1H, ArH), 7.3 (dd, 1H, ArH), 7.1 (m, 2H, ArH), 3.35 (m, 2H, $CONHCH_2$), 2.8 (m, 4H, 2xpyrroleCH_2), 2.5 (br m, 6H, $N(CH_2CH_3)_2$ and NCH_2), 1.75 (br s, 4H, 2xpyrroleCH_2CH_2), 0.9 (t, 6H, $N(CH_2CH_3)_2$).

MS-EI *m/z* 484 [M^+].

Example 21

4-Benzoyl-5-(5-bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-3-methyl-1*H*-pyrrole-2-carboxylic acid (3-diethylaminopropyl)amide

To a mixture of benzoyl chloride (1 equiv.) and aluminum chloride (1 equiv.) in dichloroethane at 0° C was added ethyl 3,5-dimethyl-2-pyrrolecarboxylate (1 equiv.). The mixture was stirred at 80° C for 4 hr. The mixture was then extracted with ethyl acetate (EtOAc) and H_2O. The combined organic extracts were washed with saturated sodium bicarbonate and brine, dried and concentrated to give (51%) of 4-benzoyl-3,5-dimethyl-1*H*-pyrrole-2-carboxylic acid.

A mixture of 4-benzoyl-3,5-dimethyl-1*H*-pyrrole-2-carboxylic acid ethyl ester (4.13 g, 15.2 mmol) and ceric ammonium nitrate (33 g, 4equiv.) in 50 mL of tetrahydrofuran (THF):acetic acid (HOAc):H_2O 1:1:1 was refluxed overnight. The reaction mixture was then cooled, extracted with EtOAc and then basified to pH 9 with sodium carbonate. The organic layer was then washed with brine, dried ($MgSO_4$) and concentrated followed by column chromatography

to give 3.25 g (75%) of 4-benzoyl-5-formyl-3-methyl-1*H*-pyrrole-2-carboxylic acid ethyl ester as a yellow solid.

5-Bromo-1,3-dihydro-indol-2-one was condensed with 4-benzoyl-5-formyl-3-methyl-1*H*-pyrrole-2-carboxylic acid using method D to give 4-benzoyl-5-(5-bromo-2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-3-methyl-1*H*-pyrrole-2-carboxylic acid.

The above carboxylic acid was then coupled with N,N-diethyl-1,3-propanediamine using method C to give the title compound.

^{1}H NMR (360 MHz, DMSO-d6) δ 7.96 (m, 1H, CONHCH$_2$), 7.76 (d, J ≅ 7.0 Hz, 2H), 7.68 (t, 1H), 7.56 (m, 2H), 7.40 (s, 2H) 7.33 (dd, J ≅ 1.6 & 8.3 Hz, 1H, H-6), 6.84 (d, J = 8.3 Hz, 1H, H-7), 3.33 (m, 2H, CH$_2$), 2.42-2.46 (m, 6H, 3xCH$_2$), 2.10 (s, 3H, CH$_3$), 1.65 (m, 2H, CH$_2$), 0.94 (t, J = 7.0 Hz, 6H, N(CH$_2$CH$_3$)$_2$).

MS Electron Impact m/z 564 [M$^+$+1].

Example 22

4-Benzoyl-5-(5-bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-3-methyl-1*H*-pyrrole-2-carboxylic acid (3-morpholin-4-ylpropyl)amide

^{1}H NMR (360 MHz, DMSO-d6) δ 14.10 (s, 1H, NH), 11.14 (br s, 1H, NH), 7.92 (m, 1H, CONHCH$_2$), 7.75 (m, 2H), 7.69 (t, 1H), 7.56 (m, 2H), 7.42 (m, 2H), 7.33 (dd, J = 1.9 & 8.3 Hz, 1H, H-6), 6.85 (d, J = 8.3 Hz, 1H, H-7), 3.56 (m, 4H, 2xCH$_2$), 3.33 (m, 2H, CH$_2$), 2.35 (m, 6H, 3xCH$_2$), 2.10 (s, 3H, CH$_3$), 1.70 (m, 2H, CH$_2$).

Example 23

4-Benzoyl-3-methyl-5-(2-oxo-1,2-dihydroindol-3-ylidenemethyl)-1*H*-pyrrole-2-carboxylic acid (3-pyrrolidin-1-ylpropyl)amide

^{1}H NMR (300 MHz, DMSO-d6) δ 14.18 (s, 1H, NH), 11.14 (br s, 1H, NH), 8.01 (m, 1H, CONHCH$_2$), 7.74 (m, 1H), 7.67 (m, 1H), 7.55 (m, 1H), 7.32 (s, 1H, H-vinyl), 7.17 (m, 1H), 6.92 (m, 1H), 3.36 (m, 2H, CH$_2$), 2.44 (m, 6 H, 3xCH$_2$), 2.11 (s, 3H, CH$_3$), 1.65-1.75 (m, 6H, 3xCH$_2$).

MS Electron Impact m/z 482 [M$^+$].

Example 24

4-Benzoyl-5-(5-bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-3-methyl-1*H*-pyrrole-2-carboxylic acid (3-pyrrolidin-1-ylpropyl)amide

^{1}H NMR (360 MHz, DMSO-d6) δ 14.01 (s, 1H, N*H*), 11.18 (br s, 1H, N*H*), 7.98 (m, 1H, CONHCH$_2$), 7.75 (m, 2H), 7.68 (m, 1H), 7.55 (m, 2H), 7.40 (m, 2H), 7.33 (dd, *J* = 2.0 & 8.2 Hz, 1H, H-6), 6.84 (d, *J* = 8.2 Hz, 1H, H-7), 3.34 (m, 2H, C*H*$_2$), 2.42-2.47 (m, 6 H, 3xC*H*$_2$), 2.09 (s, 3H, C*H*$_3$), 1.70 (m, 2H, C*H*$_2$), 1.64 (m, 4H, 2xC*H*$_2$).

Example 25

4-Benzoyl-3-methyl-5-(2-oxo-6-phenyl-1,2-dihydroindol-3-ylidenemethyl)-1*H*-pyrrole-2-carboxylic acid (3-pyrrolidin-1-ylpropyl)amide

^{1}H NMR (300 MHz, DMSO-d6) δ 14.15 (s, 1H, N*H*), 11.16 (br s, 1H, N*H*), 7.98 (m, 1H, CONHCH$_2$), 7.77 (d, *J* = 7.7 Hz, 2H), 7.69 (m, 1H), 7.53-7.63 (m, 4H), 7.44 (m, 2H), 7.33-7.37 (m, 2H), 7.24 (s, 2H), 7.12 (s, 1H), 3.36 (m, 2H, C*H*$_2$), 2.43-2.48 (m, 6 H, 3xC*H*$_2$), 2.12 (s, 3H, C*H*$_3$), 1.74 (m, 2H, C*H*$_2$), 1.69 (m, 4H, 2xC*H*$_2$).

MS Electron Impact *m/z* 558 [M$^+$].

Example 26

4-Benzoyl-5-(6-methoxy-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-3-methyl-1*H*-pyrrole-2-carboxylic acid (3-pyrrolidin-1-ylpropyl)amide

^{1}H NMR (300 MHz, DMSO-d6) δ 13.99 (s, 1H, N*H*), 11.05 (br s, 1H, N*H*), 7.93 (m, 1H, CONHCH$_2$), 7.72 (m, 2H), 7.65 (m, 1H), 7.54 (m, 2H), 7.15 (s, 1H, H-vinyl), 7.04 (d, *J* = 8.4 Hz, 1H, H-4), 6.51 (dd, *J* = 2.3 & 8.4 Hz, 1H, H-5) , 6.44 (d, *J* = 2.3 Hz, 1H, H-7), 3.74 (s, 3H, OC*H*$_3$), 3.35 (m, 2H, C*H*$_2$), 2.42-2.46 (m, 6 H, 3xC*H*$_2$), 2.10 (s, 3H, C*H*$_3$), 1.72 (m, 2H, C*H*$_2$), 1.65 (m, 4H, 2xC*H*$_2$).

MS Electron Impact *m/z* 512 [M$^+$].

Example 27

4-Benzoyl-5-(5-methoxy-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-3-methyl-1*H*-pyrrole-2-carboxylic acid (3-pyrrolidin-1-ylpropyl)amide

^{1}H NMR (360 MHz, DMSO-d6) δ 14.24 (s, 1H, N*H*), 10.90 (br s, 1H, N*H*), 7.97 (m, 1H, CON*H*CH$_2$), 7.75 (d, *J* = 7.2 Hz, 2H), 7.69 (m, 1H), 7.56 (m, 2H), 7.24 (s, 1H, H-vinyl), 6.79 (m, 2H), 6.66 (m, 1H), 3.67 (s, 3H, OC*H*$_3$), 3.34 (m, 2H, C*H*$_2$), 2.43-2.48 (m, 6 H, 3xC*H*$_2$), 2.14 (s, 3H, C*H*$_3$), 1.71 (m, 2H, C*H*$_2$), 1.66 (m, 4H, 2xC*H*$_2$).

MS Electron Impact *m/z* 512 [M$^+$].

Example 28

4-Benzoyl-5-(5-fluoro-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-3-methyl-1*H*-pyrrole-2-carboxylic acid (3-pyrrolidin-1-ylpropyl)amide

^{1}H NMR (300 MHz, DMSO-d6) δ 14.20 (s, 1H, N*H*), 11.14 (br s, 1H, N*H*), 8.03 (m, 1H, CON*H*CH$_2$), 7.75 (m, 2H), 7.68 (m, 1H), 7.55 (m, 2H), 7.38 (s, 1H, H-vinyl), 7.08 (m, 1H), 7.01 (m, 1H), 6.87 (m, 1H), 3.34 (m, 2H, C*H*$_2$), 2.42-2.48 (m, 6 H, 3xC*H*$_2$), 2.09 (s, 3H, C*H*$_3$), 1.70 (m, 2H, C*H*$_2$), 1.65 (m, 4H, 2xC*H*$_2$).

MS Electron Impact *m/z* 500 [M$^+$].

Example 29

4-Acetyl-5-(5-bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-3-methyl-1*H*-pyrrole-2-carboxylic acid (3-diethylaminopropyl)amide

5-Bromo-1,3-dihydro-indol-2-one was condensed with 4-acetyl-5-formyl-3-methyl-1*H*-pyrrole-2-carboxylic acid (3-diethylaminopropyl)amide (prepared from 4-acetyl-5-formyl-3-methyl-1*H*-pyrrole-2-carboxylic acid ethyl ester by method B then C) to give the title compound.

^{1}H NMR (300 MHz, DMSO-d6) δ 14.19 (s, 1H, N*H*), 11.19 (br s, 1H, N*H*), 8.15 (m, 1H, CON*H*CH$_2$), 8.11 (s, 1H, H-vinyl), 7.72 (d, *J* = 1.8 Hz, 1H, H-4), 7.38 (dd, *J* = 1.8 & 8.2 Hz, 1H, H-6), 6.87

(d, J = 8.2 Hz, 1H, H-7), 3.27 (m, 2H, CH_2), 2.57 (s, 3H, CH_3CO), 2.46 (m, 9 H, CH_3 & 3xCH_2), 1.64 (m, 2H, CH_2), 0.93 (t, J = 7.1 Hz, 6H, $N(CH_2CH_3)_2$).

Example 30

4-Acetyl-5-(5-bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-3-methyl-1*H*-pyrrole-2-carboxylic acid (3-pyrrolidin-1-ylpropyl)amide

^{1}H NMR (300 MHz, DMSO-d6) δ 8.14 (m, 1H, CONHCH$_2$), 8.10 (s, 1H, H-vinyl), 7.70 (d, 1H, H-4), 7.36 (dd, J = 1.6 & 8.1 Hz, 1H, H-6), 6.85 (d, J = 8.1 Hz, 1H, H-7), 3.32 (m, 2H, CH_2), 2.57 (s, 3H, CH_3CO), 2.44 (s, 3H, CH_3), 2.35-2.48 (m, 6H, 3xCH_3), 1.65-1.71 (m, 6H, 3xCH_2).

MS m/z 499 & 501 [M^+] & [M^++2].

Example 31

4-Acetyl-5-(5-bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-3-methyl-1*H*-pyrrole-2-carboxylic acid (3-morpholin-4-ylpropyl)amide

^{1}H NMR (300 MHz, DMSO-d6) δ 14.20 (s, 1H, NH), 11.26 (br s, 1H, NH), 8.09 (m, 2H, H-vinyl & CONHCH$_2$), 7.73 (d, J = 1.5 Hz, 1H, H-4), 7.38 (dd, J = 1.5 & 8.3 Hz, 1H, H-6), 6.87 (d, J = 8.3 Hz, 1H, H-7), 3.55 (m, 4H, 2xCH_2), 3.26 (m, 2H, CH_2), 2.57 (s, 3H, CH_3CO), 2.44 (s, 3H, CH_3), 2.35 (m, 6H, 3xCH_3), 1.68 (m, 2H, CH_2).

MS-EI m/z 514 & 516 [M^+-1] & [M^-+1].

Example 32

4-Acetyl-5-(5-bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-3-methyl-1*H*-pyrrole-2-carboxylic acid (3-hydroxypropyl)amide

^{1}H NMR (360 MHz, DMSO-d6) δ 14.17 (s, 1H, NH), 11.25 (br s, 1H, NH), 8.10 (s, 1H, H-vinyl), 8.03 (m, 1H, CONHCH$_2$), 7.71 (br s, 1H, H-4), 7.37 (br d, J = 8.4 Hz, 1H, H-6), 6.87 (d, J = 8.4 Hz, 1H, H-7), 4.51 (br s, 1H, OH), 3.51 (br s, 2H, CH_2), 3.36 (m, 2H, CH_2), 2.57 (s, 3H, CH_3CO), 2.43 (s, 3H, CH_3), 1.70 (m, 2H, CH_2).

MS-EI *m/z* 445 & 447 [M^+-1] & [M^++1].

Example 34

4-Acetyl-5-(5-bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-3-methyl-1*H*-pyrrole-2-carboxylic acid (2-morpholin-4-ylethyl)amide

^{1}H NMR (360 MHz, DMSO-d6) δ 14.19 (s, 1H, N*H*), 11.14 (br s, 1H, N*H*), 8.10 (s, 1H, H-vinyl), 7.84 (m, 1H, CON*H*CH_2), 7.71 (d, *J* = 1.8 Hz, 1H, H-4), 7.38 (dd, *J* = 1.8 & 8.2 Hz, 1H, H-6), 6.87 (d, *J* = 8.2 Hz, 1H, H-7), 3.58 (m, 4H, 2xCH_2), 3.40 (m, 2H, CH_2), 2.57 (s, 3H, CH_3CO), 2.49 (m, 4H, 2xCH_2), 2.45 (m, CH_3 & CH_2).

MS-EI *m/z* 500 & 502 [M^+-1] & [M^++1].

Example 35

4-Acetyl-5-(5-bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-3-methyl-1*H*-pyrrole-2-carboxylic acid (2-pyrrolidin-1-ylethyl)amide

^{1}H NMR (360 MHz, DMSO-d6) δ 14.17 (s, 1H, N*H*), 11.23 (s, 1H, N*H*), 8.11 (s, 1H, H-vinyl), 7.91 (m, 1H, CON*H*CH_2), 7.73 (d, *J* = 1.9 Hz, 1H, H-4), 7.39 (dd, *J* = 1.9 & 8.3 Hz, 1H, H-6), 6.88 (d, *J* = 8.3 Hz, 1H, H-7), 3.40 (m, 2H, CH_2), 2.62 (m, 2H, CH_2), 2.57 (s, 3H, CH_3CO), 2.49 (m, 4H, 2xCH_2), 2.44 (s, 3H, CH_3), 1.69 (m, 4H, 2xCH_2).

Example 36

4-Acetyl-5-(5-bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-3-methyl-1*H*-pyrrole-2-carboxylic acid [2-(4-hydroxyphenyl)ethyl]amide

^{1}H NMR (300 MHz, DMSO-d6) δ 14.21 (s, 1H, N*H*), 11.18 (s, 1H,O*H*), 9.09 (s, 1H, N*H*), 8.06-8.10 (m, 2H), 7.73 (s, 1H), 7.38 (d, *J* = 7.8 Hz, 1H), 7.04 (d, *J* = 7.1 Hz, 2H), 6.88 (d, *J* = 7.8 Hz, 1H), 6.67 (d, *J* = 7.1 Hz, 2H), 3.42 (m, 2H, CH_2), 2.72 (m, 2H, CH_2), 2.56 (s, 3H, CH_3CO), 2.37 (s, 3H, CH_3).

MS-EI *m/z* 507 & 509 [M^+-1] & [M^++1].

Example 37

5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2-isopropyl-4-phenyl-1*H*-pyrrole-3-carboxylic acid (3-diethylaminopropyl)amide

A mixture of 2-aminoacetophenone hydrochloride (1 equiv.), ethyl isobutyrylacetate (1.2 equiv.) and sodium acetate (2.4 equiv.) in H_2O was stirred at 100° C for 18 hours and then cooled to room temperature. The aqueous layer was decanted off and the oil was dissolved in ethyl acetate. It was then washed with water and brine and then dried to give (93%) of 2-isopropyl-4-phenyl-1*H*-pyrrole-3-carboxylic acid ethyl ester as a red brown oil.

^{1}HNMR (300 MHz, DMSO-d6) δ 11.21 (s, br, 1H, N*H*), 7.14-7.27 (m, 5H), 6.70 (d, *J* = 2.7 Hz, 1H), 4.02 (q, *J* = 7.1 Hz, 2H, OC*H*$_2$CH$_3$), 3.65 (m, 1H, C*H*(CH$_3$)$_2$), 1.22 (d, *J* = 7.5 Hz, 6H, CH(C*H*$_3$)$_2$), 1.04 (t, *J* = 7.1 Hz, 3H, OCH$_2$C*H*$_3$).

MS-EI *m/z* 257 [M$^+$].

The above pyrrole was formylated using method **A** to give (41%) 5-formyl-2-isopropyl-4-phenyl-1*H*-pyrrole-3-carboxylic acid ethyl ester as a reddish solid.

^{1}HNMR (300 MHz, DMSO-d6) δ 12.35 (s, br, 1H, N*H*), 9.14 (s, 1H, CHO), 7.36 (s, 5H), 3.96 (q, *J* = 7.1 Hz, 2H, OC*H*$_2$CH$_3$), 3.74 (m, 1H, C*H*(CH$_3$)$_2$), 1.29 (d, *J* = 6.9 Hz, 6H, CH(C*H*$_3$)$_2$), 0.90 (t, *J* = 7.1 Hz, 3H, OCH$_2$C*H*$_3$).

MS-EI *m/z* 285 [M$^+$].

The pyrrolecarboxylic acid ester was hydrolysed using method **B** to give (57%) of 5-formyl-2-isopropyl-4-phenyl-1*H*-pyrrole-3-carboxylic acid as a beige solid.

^{1}HNMR (300 MHz, DMSO-d6) δ 12.28 (s, br, 1H, COO*H*), 12.02 (s, br, 1H, N*H*), 9.10 (s, 1H, CHO), 7.35 (s, 5H), 3.81 (m, 1H, C*H*(CH$_3$)$_2$), 1.28 (d, *J* = 6.9 Hz, 6H, CH(C*H*$_3$)$_2$).

MS-EI *m/z* 257 [M$^+$].

5-Bromo-1,3-dihydroindol-2-one (120 mg, 0.31 mmol) was condensed with 5-formyl-2-isopropyl-4-phenyl-1*H*-pyrrole-3-

carboxylic acid (3-diethylaminopropyl)amide (prepared by method C) to give 120 mg (71%) of the title compound.

^{1}HNMR (300 MHz, DMSO-d6) δ 14.23 (s, br, 1H, N*H*), 11.08 (s, br, 1H, N*H*), 7.38-7.55 (m, 7H, Ar-H & CON*H*CH_2), 7.30 (s, 1H, H-vinyl), 7.26 (dd, *J* = 1.8 & 7.8 Hz, 1H), 6.85 (d, *J* = 8.7 Hz, 1H), 3.36 (m, 1H, C*H*$(CH_3)_2$), 3.07 (m, 2H, CH_2), 2.34 (q, *J* = 7.1 Hz, 4H, N$(CH_2CH_3)_2$), 2.22 (t, *J* = 6.9 Hz, 2H, CH_2), 1.40 (m, 2H, CH_2), 1.31 (d, *J* = 6.9 Hz, 6H, CH$(CH_3)_2$), 0.86 (t, *J* = 7.1 Hz, 6H, N$(CH_2CH_3)_2$).

MS *m/z* 565.1 [M^++1].

Example 38

5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2-isopropyl-4-phenyl-1*H*-pyrrole-3-carboxylic acid (3-pyrrolidin-1-ylpropyl)amide

5-(5-bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2-isopropyl-4-phenyl-1*H*-pyrrole-3-carboxylic acid (127 mg, 0.28 mmol) was condensed with 3-pyrrolidin-1-yl-propylamine (43 mg, 0.336 mmol) to give 140 mg (66%) of the title compound.

^{1}HNMR (300 MHz, DMSO-d6) δ 14.40 (s, br, 1H, N*H*), 7.38-7.47 (m, 7H), 7.23-7.27 (m, 2H), 6.84 (d, *J* = 8.1 Hz, 1H), 3.36 (m, 1H, C*H*$(CH_3)_2$), 3.08 (m, 2H, CH_2), 2.30 (m, 4H, 2xCH_2), 2.20 (t, *J* = 7.0 Hz, 2H, CH_2), 1.62 (m, 4H, 2xCH_2), 1.42 (t, *J* = 7.0 Hz, 2H, CH_2), 1.31 (d, *J* = 7.2 Hz, 6H, CH$(CH_3)_2$).

MS-EI *m/z* 560 and 562 [M^+ -1 and M^++1].

Example 39

5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2-isopropyl-4-phenyl-1*H*-pyrrole-3-carboxylic acid (2-diethylaminoethyl)amide

5-Bromo-1,3-dihydroindol-2-one (57 ,g. 0.27 mmol) was condensed with 5-formyl-2-isopropyl-4-phenyl-1*H*-pyrrole-3-carboxylic acid (2-diethylaminoethyl)amide (120 mg) to give 78 mg (53%) of the title compound as a yellow solid.

^{1}HNMR (300 MHz, DMSO-d6) δ 14.23 (s, br, 1H, N*H*), 11.09 (s,

550 548

br, 1H, NH), 7.38-7.51 (m, 6H), 7.25-7.28 (m, 2H), 7.19 (t, 1H, CONHCH$_2$), 6.85 (d, J = 7.8 Hz,1H), 3.43 (m, 1H, CH(CH$_3$)$_2$), 3.11 (m, 2H, CH$_2$), 2.28-2.39 (m, 6H, N(CH$_2$CH$_3$)$_2$ & CH$_2$, 1.31 (d, J = 6.9 Hz, CH(CH$_3$)$_2$), 0.85 (t, J = 7.0 Hz, 6H, N(CH$_2$CH$_3$)$_2$.

MS-EI m/z 548 and 550 [M$^+$ -1 and M$^+$+1].

Example 40

5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2-isopropyl-4-phenyl-1H-pyrrole-3-carboxylic acid [3-(4-methylpiperazin-1-yl)propyl]amide

5-Bromo-1,3-dihydroindol-2-one (53 mg, 0.25 mmol) was condensed with 5-formyl-2-isopropyl-4-phenyl-1H-pyrrole-3-carboxylic acid [3-(4-methylpiperazin-1-yl)propyl]amide (300 mg) to give 65 mg of the title compound.

^{1}HNMR (300 MHz, DMSO-d6) δ 14.22 (s, br, 1H, NH), 11.08 (s, br, 1H, NH), 7.23-7.50 (m, 9H), 6.85 (d, J = 8.7 Hz, 1H), 3.37 (m, 1H, CH(CH$_3$)$_2$), 3.05 (m, 2H, CH$_2$), 2.24 (m, 8H, 4xCH$_2$), 2.11 (m, 5H, CH$_2$ & CH$_3$),1.42 (m, 2H, CH$_2$), 1.31 (d, J = 7.2 Hz, 6H, CH(CH$_3$)$_2$).

MS-EI m/z 589 and 591 [M$^+$ -1 and M$^+$+1].

Example 41

5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2-isopropyl-4-phenyl-1H-pyrrole-3-carboxylic acid

5-Bromo-1,3-dihydroindol-2-one (170 mg, 0.8 mmol) was condensed with 5-formyl-2-isopropyl-4-phenyl-1H-pyrrole-3-carboxylic acid (205 mg) using method D to give 210 mg (58%) of the title compound as a yellow solid.

^{1}HNMR (300 MHz, DMSO-d6) δ 14.31 (s, br, 1H, NH), 11.16 (s, br, 1H, NH), 7.26-7.44 (m, 7H), 7.11 (s, 1H, H-vinyl), 6.85 (d, J = 7.8 Hz, 1H), 3.78 (m, 1H, CH(CH$_3$)$_2$), 1.34 (d, J = 6.9 Hz, 6H, CH(CH$_3$)$_2$).

MS-EI m/z 452 [M$^+$+1].

Example 42

5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2-methyl-4-phenyl-1*H*-pyrrole-3-carboxylic acid (2-pyrrolidin-1-ylethyl)amide

5-Bromo-1,3-dihydroindol-2-one (44 mg, 0.21 mmol) was condensed with 5-formyl-2-methyl-4-phenyl-1*H*-pyrrole-3-carboxylic acid (2-pyrrolidin-1-ylethyl)amide (70 mg, prepared in the same manner as the isopropyl analog, above) to give 0.03 g (27%) of the title compound as a yellow solid.

^{1}HNMR (300 MHz, DMSO-d6) δ 13.87 (s, br, 1H, N*H*), 11.11 (s, br, 1H, N*H*), 7.36-7.51 (m, 6H), 7.26 (dd, *J* = 1.8 & 8.1 Hz, 1H), 7.2 (s, 1H, H-vinyl), 7.09 (m, 1H, CON*H*CH$_2$), 6.83 (d, *J* = 8.1 Hz, 1H), 3.17 (m, 2H, NC*H*$_2$), 2.48 (m, C*H*$_3$), 2.29-2.35 (m, 6H, 3xNC*H*$_2$), 1.59 (m, 4H, 2xC*H*$_2$).

MS-EI *m/z* 518 and 520 [M$^+$ -1 and M$^+$+1].

Example 43

5-[6-(2-Methoxyphenyl)-2-oxo-1,2-dihydroindol-3-ylidenemethyl]-2-methyl-4-phenyl-1*H*-pyrrole-3-carboxylic acid (2-pyrrolidin-1-ylethyl)amide

6-(2-Methoxyphenyl)-1,3-dihydroindol-2-one (50 mg, 0.21 mmol) was condensed with 5-formyl-2-methyl-4-phenyl-1*H*-pyrrole-3-carboxylic acid (2-pyrrolidin-1-ylethyl)amide (70 mg) to give 0.04 g (35%) of the title compound as a yellow-orange solid.

^{1}HNMR (300 MHz, DMSO-d6) δ 13.82 (s, br, 1H, N*H*), 11.02 (s, br, 1H, N*H*), 7.48 (m, 2H), 7.43 (m, 1H), 7.38 (m, 2H), 7.32 (m, 1H), 7.24 (m, 2H), 7.16 (s, 1H, H-vinyl), 7.08 (m, 2H), 7.03 (m, 1H), 7.0 (m, 2H), 3.74 (s, 3H, OC*H*$_3$), 3.19 (m, 2H, NC*H*$_2$), 2.49 (m, C*H*$_3$), 2.32-2.38 (m, 6H, 3xNC*H*$_2$), 1.59 (m, 4H, 2xC*H*$_2$).

MS-EI *m/z* 546 [M$^+$].

Example 44

5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2-methyl-4-phenyl-1*H*-pyrrole-3-carboxylic acid (2-dimethylaminoethyl)amide

5-Bromo-1,3-dihydroindol-2-one (46 mg, 0.22 mmol) was condensed with 5-formyl-2-methyl-4-phenyl-1*H*-pyrrole-3-carboxylic

acid (2-dimethylaminoethyl)amide (65 mg) to give 60 mg (55%) of the title compound as a yellow solid.

^{1}HNMR (360 MHz, DMSO-d6) δ 13.86 (s, br, 1H, N*H*), 11.09 (s, br, 1H, N*H*), 7.47-7.49 (m, 2H), 7.38-7.41 (m, 4H), 7.26 (dd, *J* = 2.2 & 8.3 Hz, 1H), 7.21 (s, 1H, H-vinyl), 7.04 (m, 1H, CONHCH$_2$), 6.77 (d, *J* = 8.3 Hz, 1H), 3.15 (m, 2H, NC*H*$_2$), 2.48 (m, C*H*$_3$), 2.16 (t, *J* = 6.8 Hz, 2H, 3xNC*H*$_2$), 2.02 (s, 6H, 2xNC*H*$_3$).

MS *m/z* 493 and 494.8 [M$^+$ and M$^+$+2].

Example 45

5-[6-(2-Methoxyphenyl)-2-oxo-1,2-dihydroindol-3-ylidenemethyl]-2-methyl-4-phenyl-1*H*-pyrrole-3-carboxylic acid (2-dimethylaminoethyl)amide

6-(2-Methoxyphenyl)-1,3-dihydroindol-2-one (53 mg, 0.22 mmol) was condensed with 5-formyl-2-methyl-4-phenyl-1*H*-pyrrole-3-carboxylic acid (2-dimethylaminoethyl)amide (65 mg) to give 0.05 g (44%) of the title compound as an orange gum.

^{1}HNMR (300 MHz, DMSO-d6) δ 13.82 (s, br, 1H, N*H*), 11.02 (s, br, 1H, N*H*), 7.37-7.52 (m, 5H), 7.32 (m, 1H), 7.22-7.27 (m, 2H), 7.16 (s, 1H), 7.08 (m, 2H), 7.03 (m, 1H), 7.0 (m, 2H), 3.74 (s, 3H, OC*H*$_3$), 3.15 (m, 2H, NC*H*$_2$), 2.49 (m, C*H*$_3$), 2.16 (t, *J* = 6.5 Hz, 2H, NC*H*$_2$), 2.02 (s, 6H, 2xNC*H*$_3$).

MS *m/z* 521 [M$^+$+1].

Example 46

5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2-methyl-4-phenyl-1*H*-pyrrole-3-carboxylic acid ethyl ester

5-Bromo-1,3-dihydroindol-2-one (60 mg, 0.29 mmol) was condensed with 5-formyl-2-methyl-4-phenyl-1*H*-pyrrole-3-carboxylic acid ethyl ester (75 mg) to give 78 mg (60%) of the title compound as an orange solid.

^{1}HNMR (360 MHz, DMSO-d6) δ 14.01 (s, br, 1H, N*H*), 11.13 (s, br, 1H, N*H*), 7.42-7.46 (m, 3H), 7.27-7.34 (m, 4H), 7.12 (s, 1H), 6.84 (dd, *J* = 2.2 & 8.3 Hz, 1H), 3.99-4.03 (m, 2H, OC*H*$_2$CH$_3$), 2.61

(s, 3H, CH_3), 0.98-1.03 (m, 3H, OCH_2CH_3).

MS-EI *m/z* 450 and 452 [M^+ -1 and M^++1].

Example 47

5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2-methyl-4-phenyl-1*H*-pyrrole-3-carboxylic acid (3-diethylaminopropyl)amide

5-bromo-1,3-dihydroindol-2-one (0.47 g, 2.2 mmol) was condensed with 5-formyl-2-methyl-4-phenyl-1*H*-pyrrole-3-carboxylic acid (3-diethylaminopropyl)amide (0.75 g) to give 0.11 g (42%) of the title compound as an orange solid.

^{1}HNMR (300 MHz, DMSO-d6) δ 13.86 (s, br, 1H, NH), 7.42-7.46 (m, 3H), 7.37-7.50 (m, 7H), 7.24-7.28 (m, 2H), 6.83 (d, *J* = 8.1 Hz, 1H), 3.09 (m, 2H, NCH_2), 2.45 (s, 3H, CH_3), 2.38 (q, *J* = 7.1 Hz, 4H, $2xNCH_2CH_3$), 2.26 (t, *J* = 6.9 Hz, 2H, NCH_2), 1.42 (m, 2H, NCH_2), 0.87 (t, *J* = 7.1 Hz, 6H, $2xNCH_2CH_3$).

MS-EI *m/z* 535.0 and 537 [M^+ and M^++2].

Example 48

5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-dimethylamino-ethyl)amide

A mixture of *tert*-butyl 3-oxobutyrate and sodium nitrite (1 equiv.) in acetic acid was stirred at room temperature to give *tert*-butyl-2-hydroximino-3-oxobutyrate.

Ethyl-3-oxobutyrate (1 equiv.), zinc dust (3.8 equiv.) and the crude *tert*-butyl-2-hydroximino-3-oxobutyrate in acetic acid was stirred at 60° C for 1 hr. The reaction mixture was poured into H_2O and the filtrate was collected to give (65%) 2-*tert*-butyloxycarbonyl-3,5-dimethyl-4-ethoxycarbonylpyrrole.

A mixture of 2-*tert*-butyloxycarbonyl-3,5-dimethyl-4-ethoxycarbonylpyrrole and triethyl orthoformate (1.5 equiv.) in trifluoroacetic acid was stirred at 15°C for 1 hour. The reaction was concentrated and the residue was purified to give (64%) 2,4-dimethyl-3-ethoxycarbonyl-5-formylpyrrole as yellow needles.

2,4-Dimethyl-3-ethoxycarbonyl-5-formylpyrrole was

hydrolyzed using method B to give (90%) 5-formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid.

^{1}H NMR (360 MHz, DMSO-d6) δ 12 (br s, 2H, N*H* and CO_2H), 9.58 (s, 1H, CHO), 2.44 (s, 3H, CH_3), 2.40 (s, 3H, CH_3).

MS *m/z* 267 [M^+].

5-Bromo-1,3-dihydroindol-2-one (0.17 g, 0.8 mmol) was condensed with 5-formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-dimethylaminoethyl)amide (0.2 g, prepared by method C) using method B to give 0.3 g (83%) of the title compound as a yellow solid.

^{1}HNMR (360 MHz, DMSO-d6) δ 13.60 (s, br, 1H, N*H*), 10.94 (s, br, 1H, N*H*), 8.07 (d, *J* = 1.8 Hz, 1H, H-4), 7.75 (s, 1H, H-vinyl), 7.44 (t, *J* = 5.2 Hz, 1H, CON*H*CH_2), 7.24 (dd, *J* = 1.8 & 8.4 Hz, 1H, H-6), 6.82 (d, *J* = 8.4 Hz, 1H, H-7), 3.26-3.33 (m, 2H, NCH_2), 2.42 (s, 3H, CH_3), 2.41 (s, 3H, CH_3), 2.38 (t, *J* = 6.7 Hz, 2H, NCH_2), 2.18 (s, 6H, N(CH_3)$_2$).

MS-EI *m/z* 430 and 432 [M^+-1 and M^++1].

Example 49

2,4-Dimethyl-5-(2-oxo-6-phenyl-1,2-dihydroindol-3-ylidenemethyl)-1*H*-pyrrole-3-carboxylic acid (2-dimethylaminoethyl)amide

6-Phenyl-1,3-dihydroindol-2-one (0.17 g, 0.8 mmol) was condensed with 5-formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-dimethylaminoethyl)amide (0.2 g) to give 0.13 g (36%) of the title compound as a yellow-orange solid.

^{1}HNMR (360 MHz, DMSO-d6) δ 13.59 (s, br, 1H, N*H*), 10.93 (, br, 1H, N*H*), 7.85 (d, *J* = 7.92 Hz, 1H, H-4), 7.63-7.65 (m, 3H), 7.40-7.47 (m, 3H,), 7.32-7.36 (m, 1H, Ar-*H*), 7.30 (dd, *J* = 1.6 & 7.9 Hz, 1H, H-5), 7.11 (d, *J* = 1.6 Hz, 1H, H-7), 3.28-3.34 (m, 2H, NCH_2), 2.43 (s, 3H, CH_3), 2.41 (s, 3H, CH_3), 2.38 (t, *J* = 6.8 Hz, 2H, NCH_2), 2.18 (s, 6H, N(CH_3)$_2$).

MS-EI *m/z* 428 [M^+].

Example 50

5-(5-Chloro-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-dimethylamino-ethyl)amide

5-Chloro-1,3-dihydroindol-2-one (0.1 g, 0.6 mmol) was condensed with 5-formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-dimethylaminoethyl)amide (0.15 g) to give 0.22 g (90%) of the title compound as a yellow solid.

^{1}HNMR (300 MHz, DMSO-d6) δ 13.61 (s, br, 1H, N*H*), 10.98 (, br, 1H, N*H*), 7.96 (d, *J* = 2.0 Hz, 1H, H-4), 7.75 (s, 1H, H-vinyl), 7.50 (t, *J* = 5.5 Hz, 1H, CON*H*CH$_2$), 7.12 (dd, *J* = 2.0 & 8.3 Hz, 1H, H-6), 6.86 (d, *J* = 8.3 Hz, 1H, H-7), 3.26-3.31 (m, 2H, NC*H*$_2$), 2.42 (s, 3H, C*H*$_3$), 2.40 (s, 3H, C*H*$_3$), 2.36 (t, *J* = 6.6 Hz, 2H, NC*H*$_2$), 2.17 (s, 6H, N(C*H*$_3$)$_2$).

MS-EI *m/z* 386 [M$^+$].

Example 51

5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-diethylamino-ethyl)amide

5-Bromo-1,3-dihydroindol-2-one (0.17 g, 0.8 mmol) was condensed with 5-formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-diethylaminoethyl)amide (0.2 g) to give 0.09 g (26%) of the title compound as a yellow solid.

^{1}HNMR (360 MHz, DMSO-d6) δ 13.61 (s, br, 1H, N*H*), 10.98 (, br, 1H, N*H*), 8.09 (d, *J* = 1.7 Hz, 1H, H-4), 7.76 (s, 1H, H-vinyl), 7.42 (t, *J* = 5.5 Hz, 1H, CON*H*CH$_2$), 7.24 (dd, *J* = 1.7 & 8.0 Hz, 1H, H-6), 6.82 (d, *J* = 8.0 Hz, 1H, H-7), 3.23-3.32 (m, 2H, NC*H*$_2$), 2.46-2.55 (m, 6H, 3xNC*H*$_2$), 2.43 (s, 3H, C*H*$_3$), 2.42 (s, 3H, C*H*$_3$), 0.96 (t, *J* = 7.2 Hz, 6H, 2xNCH$_2$C*H*$_3$).

MS-EI *m/z* 458 and 460 [M$^+$-1 and M$^+$+1].

Example 52

5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (2-pyrrolidin-1-yl-ethyl)amide

5-Bromo-1,3-dihydroindol-2-one (0.09 g, 0.4 mmol) was condensed with 5-formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid

(2-pyrrolidin-1-ylethyl)amide (0.1 g) to give 0.14 g (81%) of the title compound as a yellow-orange solid.

^{1}HNMR (300 MHz, DMSO-d6) δ 13.61 (s, br, 1H, N*H*), 10.98 (, br, 1H, N*H*), 8.09 (d, *J* = 1.9 Hz, 1H, H-4), 7.76 (s, 1H, H-vinyl), 7.53 (t, *J* = 5.5 Hz, 1H, CON*H*CH$_2$), 7.24 (dd, *J* = 1.9 & 8.5 Hz, 1H, H-6), 6.81 (d, *J* = 8.5 Hz, 1H, H-7), 3.29-3.35 (m, 2H, NC*H*$_2$), 2.54 (t, *J* = 6.9 Hz, 2H, NC*H*$_2$), 2.47 (m, under DMSO), 2.42 (s, 3H, C*H*$_3$), 2.40 (s, 3H, C*H*$_3$), 1.66-1.69 (m, 4H, 2xC*H*$_2$).

MS-EI *m/z* 456 and 458 [M$^+$-1 and M$^+$+1].

Example 53

5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (3-imidazol-1-yl-propyl)amide

5-Bromo-1,3-dihydroindol-2-one (0.09 g, 0.4 mmol) was condensed with 5-formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (3-imidazol-1-ylpropyl)amide (0.1 g) to give 0.1 g (59%) of the title compound as an orange solid.

^{1}HNMR (300 MHz, DMSO-d6) δ 13.63 (s, br, 1H, N*H*), 10.99 (, br, 1H, N*H*), 8.09 (d, *J* = 2.2 Hz, 1H, H-4), 7.77 (s, 1H, H-vinyl), 7.71 (t, *J* = 5.7 Hz, 1H, CON*H*CH$_2$), 7.65 (s, 1H, Ar-H), 7.25 (dd, *J* = 2.2 & 8.4 Hz, 1H, H-6), 7.20 (s, 1H, Ar-H), 6.89 (s, 1H, Ar-H), 6.81 (d, *J* = 8.4 Hz, 1H, H-7), 4.02 (t, *J* = 6.7 Hz, 2H, NC*H*$_2$), 3.18 (q, *J* = 6.7 Hz, 2H, NC*H*$_2$), 2.43 (s, 3H, C*H*$_3$), 2.41 (s, 3H, C*H*$_3$), 1.93 (m, 2H, C*H*$_2$).

MS-EI *m/z* 467 and 469 [M$^+$-1 and M$^+$+1].

Example 54

5-[6-(2-Methoxyphenyl)-2-oxo-1,2-dihydroindol-3-ylidenemethyl]-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-dimethylaminoethyl)amide

6-(2-Methoxyphenyl)-1,3-dihydroindol-2-one (30 mg, 0.13 mmol) was condensed with 5-formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-dimethylaminoethyl)amide (30 mg) to give 0.06 g (100%) of the title compound as a yellow-orange gum.

^{1}HNMR (300 MHz, DMSO-d6) δ 13.60 (s, br, 1H, N*H*), 10.89 (s, br, 1H, N*H*), 7.79 (d, *J* = 8.4 Hz, 1H), 7.63 (s, 1H, H-vinyl), 7.46 (t, *J* = 5.5 Hz, 1H, CON*H*CH$_2$), 7.28-7.35 (m, 2H), 6.99-7.11 (m, 4H), 3.76 (s, 3H, OC*H*$_3$), 3.27-3.31 (m, 2H, NC*H*$_2$), 2.43 (s, 3H, C*H*$_3$), 2.39 (s, 3H, C*H*$_3$), 2.37 (m, 2H, NC*H*$_2$), 2.18 (s, 6H, N(C*H*$_3$)$_2$).

MS-EI *m/z* 458 [M$^+$].

Example 55

5-[6-(3-Methoxyphenyl)-2-oxo-1,2-dihydroindol-3-ylidenemethyl]-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-dimethylaminoethyl)amide

6-(3-Methoxyphenyl)-1,3-dihydroindol-2-one (30 mg, 0.13 mmol) was condensed with 5-formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-dimethylaminoethyl)amide (30 mg) to give 8 mg (14%) of the title compound as a yellow-orange solid.

^{1}HNMR (360 MHz, DMSO-d6) δ 13.59 (s, br, 1H, N*H*), 10.92 (s, br, 1H, N*H*), 7.84 (d, *J* = 7.6 Hz, 1H), 7.65 (s, 1H, H-vinyl), 7.42 (m, 1H, CON*H*CH$_2$), 7.36 (d, *J* = 7.8 Hz, 1H), 7.29 (dd, *J* = 1.6 & 7.6 Hz, 1H), 7.20 (d, *J* = 7.8 Hz, 1H), 7.14 (d, *J* = 2.8 Hz, 1H), 7.11 (d, *J* = 1.6 Hz, 1H), 6.91 (dd, *J* = 2.8 & 7.8 Hz, 1H), 3.82 (s, 3H, OC*H*$_3$), 3.21-3.33 (m, 2H, NC*H*$_2$), 2.43 (s, 3H, C*H*$_3$), 2.40 (s, 3H, C*H*$_3$), 2.36-2.40 (m, 2H, NC*H*$_2$), 2.18 (s, 6H, N(C*H*$_3$)$_2$).

MS-EI *m/z* 458 [M$^+$].

Example 56

2,4-Dimethyl-5-(2-oxo-5-phenyl-1,2-dihydroindol-3-ylidenemethyl)-1*H*-pyrrole-3-carboxylic acid (2-diethylaminoethyl)amide

5-Phenyl-1,3-dihydroindol-2-one (80 mg, 0.4 mmol) was condensed with 5-formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-diethylaminoethyl)amide (0.1 g) using method B to give 79 mg (46%) of the title compound.

^{1}HNMR (300 MHz, DMSO-d6) δ 13.66 (s, br, 1H, N*H*), 10.95 (, br, 1H, N*H*), 8.15 (d, *J* = 1.2 Hz, 1H), 7.81 (s, 1H, H-vinyl),

7.71 (d, J = 7.5 Hz, 1H), 7.40-7.47 (m, 4H), 7.31 (m, 1H), 6.95 (d, J = 8.1 Hz, 1H), 3.2-3.31 (m, 2H, NCH_2), 2.46-2.55 (m, 6H, 3xNCH_2), 2.44 (s, 6H, 2xCH_3), 0.96 (t, J = 7.4 Hz, 6H, 2xNCH$_2$CH_3).

MS-EI m/z 456 [M$^+$].

Example 57

2,4-Dimethyl-5-(2-oxo-5-phenyl-1,2-dihydroindol-3-ylidenemethyl)-1*H*-pyrrole-3-carboxylic acid (2-pyrrolidin-1-ylethyl)amide

5-Phenyl-1,3-dihydroindol-2-one (0.04 g, 0.2 mmol) was condensed with 5-formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-pyrrolidin-1-ylethyl)amide (0.04 g) to give the title compound as a yellow-orange solid.

^{1}HNMR (300 MHz, DMSO-d6) δ 13.65 (s, br, 1H, N*H*), 10.96 (, br, 1H, N*H*), 8.15 (d, J = 1.0 Hz, 1H), 7.80 (s, 1H, H-vinyl), 7.71 (d, J = 7.2 Hz, 2H), 7.49 (t, J = 6.3 Hz, 1H, CON*H*CH$_2$), 7.41-7.46 (m, 3H), 7.31 (m, 1H), 6.95 (d, J = 7.8 Hz, 1H), 4.08 (m, 4H, 2x NCH_2), 3.32 (m, 2H, NCH_2), 2.55 (t, J = 7.1 Hz, 2H, NCH_2), 2.47 (m, under DMSO), 2.43 (s, 6H, 2xCH_3), 1.66 (m, 4H, 2xCH_2).

MS-EI m/z 454 [M$^+$].

Example 58

2,4-Dimethyl-5-(2-oxo-5-phenyl-1,2-dihydroindol-3-ylidenemethyl)-1*H*-pyrrole-3-carboxylic acid (3-imidazol-1-ylpropyl)amide

5-Phenyl-1,3-dihydroindol-2-one (8 mg, 0.04 mmol) was condensed with 5-formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (3-imidazol-1-ylpropyl)amide (10 mg) to give 10 mg (59%) of the title compound as an orange solid.

^{1}HNMR (300 MHz, DMSO-d6) δ 13.67 (s, br, 1H, N*H*), 10.96 (, br, 1H, N*H*), 8.16 (d, J = 1.2 Hz, 1H), 7.81 (s, 1H, H-vinyl), 7.65-7.72 (m, 4H), 7.44 (m, 3H), 7.31 (m, 1H, CON*H*CH$_2$), 7.21 (s, 1H, Ar-H), 4.02 (t, J = 6.5 Hz, 2H, NCH_2), 3.19 (q, J = 6.5 Hz, 2H, CONHCH_2), 2.44 (s, 6H, 2xCH_3), 1.93 (m, 2H, CH$_2$CH_2 CH$_2$).

MS-EI m/z 465 [M$^+$].

Example 59

2,4-Dimethyl-5-(2-oxo-6-phenyl-1,2-dihydroindol-3-ylidenemethyl)-1*H*-pyrrole-3-carboxylic acid (2-diethylaminoethyl)amide

6-Phenyl-1,3-dihydroindol-2-one (0.08 g, 0.4 mmol) was condensed with 5-formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-diethylaminoethyl)amide (0.1 g) to give 65 mg (38%) of the title compound as a yellow solid.

^{1}HNMR (300 MHz, DMSO-d6) δ 13.61 (s, br, 1H, N*H*), 10.99 (, br, 1H, N*H*), 7.86 (d, *J* = 7.8 Hz, 1H), 7.62-7.66 (m, 3H), 7.40-7.47 (m, 3H), 7.28-7.36 (m, 2H), 7.10 (d, *J* = 1.2 Hz, 1H), 3.26 (m, 2H, NCH_2), 2.46-2.55 (m, 6H, 3xNCH_2), 2.44 (s, 3H, CH_3), 2.41 (s, 3H, CH_3), 0.97 (t, *J* = 7.2 Hz, 6H, 2xNCH$_2$CH_3).

MS-EI *m/z* 456 [M$^+$].

Example 60

2,4-Dimethyl-5-(2-oxo-6-phenyl-1,2-dihydroindol-3-ylidenemethyl)-1*H*-pyrrole-3-carboxylic acid (2-pyrrolidin-1-ylethyl)amide

6-Phenyl-1,3-dihydroindol-2-one (30 mg, 0.15 mmol) was condensed with 5-formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-pyrrolidin-1-ylethyl)amide (40 mg) to give 5.9 mg (8.5%) of the title compound as a yellow-orange solid.

^{1}HNMR (300 MHz, DMSO-d6) δ 13.60 (s, br, 1H, N*H*), 10.99 (, br, 1H, N*H*), 7.86 (d, *J* = 7.8 Hz, 1H), 7.63-7.66 (m, 3H), 7.51 (m, 1H, CON*H*CH$_2$), 7.45 (m, 2H), 7.28-7.36 (m, 2H), 7.10 (d, *J* = 1.5 Hz, 1H), 3.31 (m, 6H, 3xNCH_2), 2.55 (t, *J* = 6.6 Hz, 2H, NCH_2), 2.43 (s, 3H, CH_3), 2.40 (s, 3H, CH_3), 1.67 (m, 4H, 2xCH_2).

MS-EI *m/z* 454 [M$^+$].

Example 61

2,4-Dimethyl-5-(2-oxo-6-phenyl-1,2-dihydroindol-3-ylidenemethyl)-1*H*-pyrrole-3-carboxylic acid (3-imidazol-1-ylpropyl)amide

6-Phenyl-1,3-dihydroindol-2-one (8 mg, 0.04 mmol) was condensed with 5-formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (3-imidazol-1-ylpropyl)amide (10 mg) to give 7.3 mg (43%) of the title compound as an orange solid.

^{1}HNMR (300 MHz, DMSO-d6) δ 13.62 (s, br, 1H, N*H*), 10.99 (, br, 1H, N*H*), 7.86 (d, *J* = 8.2 Hz, 1H), 7.62-7.70 (m, 5H), 7.45 (m, 2H), 7.35 (m, 1H), 7.30 (dd, *J* = 1.4 & 8.2 Hz, 1H), 7.21 (s, 1H), 7.10 (d, *J* = 1.4 Hz, 1H), 6.89 (s, 1H), 4.02 (t, *J* = 6.9 Hz, 2H, C*H*$_2$), 3.19 (m, 2H, NCH$_2$ C*H*$_2$), 2.43 (s, 3H, C*H*$_3$), 2.41 (s, 3H, C*H*$_3$), 1.93 (t, *J* = 6.9 Hz, 2H, NC*H*$_2$).

MS-EI *m/z* 465 [M$^+$].

Example 62

5-[6-(3,5-Dichlorophenyl)-2-oxo-1,2-dihydroindol-3-ylidenemethyl]-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-diethylaminoethyl)amide

6-(3,5-Dichlorophenyl)-1,3-dihydroindol-2-one (64 mg, 0.23 mmol) was condensed with 5-formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-diethylaminoethyl)amide (60 mg) to give 53 mg (44%) of the title compound as a light brown solid.

^{1}HNMR (360 MHz, DMSO-d6) δ 13.62 (s, br, 1H, N*H*), 10.99 (s, 1H, N*H*), 7.89 (d, *J* = 7.9 Hz, 1H, H-4), 7.69-7.71 (m, 3H), 7.55 (m, 1H, CON*H*CH$_2$), 7.37 (m, 2H), 7.14 (d, *J* = 1.4 Hz, 1H, H-7), 3.27 (m, 2H, NC*H*$_2$), 2.48-2.58 (m, 6H, 3xNC*H*$_2$), 2.45 (s, 3H, C*H*$_3$), 2.42 (s, 3H, C*H*$_3$), 0.97 (t, *J* = 6.8 Hz, 6H, 3xNCH$_2$C*H*$_3$).

MS *m/z* 526.9 [M$^+$+1].

Example 63

2,4-Dimethyl-5-(2-oxo-6-pyridin-3-yl-1,2-dihydroindol-3-ylidenemethyl)-1*H*-pyrrole-3-carboxylic acid (2-diethylaminoethyl)amide

6-Pyridin-3-yl-1,3-dihydroindol-2-one (40 mg, 0.19 mmol) was condensed with 5-formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-diethylaminoethyl)amide (50 mg) give 29 mg (33%) of the title compound as a light orange solid.

^{1}HNMR (300 MHz, DMSO-d6) δ 13.62 (s, br, 1H, N*H*), 11.05 (s, br, 1H, N*H*), 8.86 (s, br, 1H), 8.53 (d, *J* = 5.8 Hz, 1H), 8.04 (m, 1H), 7.91 (d, *J* = 8.1 Hz, 1H), 7.70 (s, 1H, H-vinyl), 7.40-7.48

(m, 2H), 7.35 (d, J = 7.5 Hz, 1H), 7.14 (s, 1H), 3.26 (m, 2H, NCH_2), 2.48-2.55 (m, 3xNCH_2), 2.42 (s, 3H, CH_3), 2.38 (s, 3H, CH_3), 0.96 (t, J = 6.9 Hz, 6H, 2xNCH_2CH_3).

MS-EI m/z 457 [M^+].

Example 64

2,4-Dimethyl-5-(2-oxo-6-pyridin-3-yl-1,2-dihydroindol-3-ylidenemethyl)-1*H*-pyrrole-3-carboxylic acid (2-pyrrolidin-1-ylethyl)amide

6-Pyridin-3-yl-1,3-dihydroindol-2-one (60 mg, 0.28 mmol) was condensed with 5-formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-pyrrolidin-1-ylethyl)amide (75 mg) to give 90 mg (71%) of the title compound as a light orange solid.

^{1}HNMR (300 MHz, DMSO-d6) δ 13.61 (s, br, 1H, N*H*), 11.05 (s, br, 1H, N*H*), 8.86 (d, J = 1.5 Hz, 1H), 8.54 (dd, J = 1.5 & 4.8 Hz, 1H), 8.05 (m, 1H), 7.91 (d, J = 7.8 Hz, 1H), 7.70 (s, 1H, H-vinyl), 7.44-7.53 (m, 2H), 7.36 (dd, J = 1.5 & 8.1 Hz, 1H), 7.15 (d, J = 1.2 Hz, 1H), 3.33 (m, 2H, NCH_2), 2.47-2.57 (m, 6H, 3xNCH_2), 2.43 (s, 3H, CH_3), 2.41 (s, 3H, CH_3), 1.67 (m, 4H, 2xCH_2).

MS-EI m/z 455 [M^+].

Example 65

2,4-Dimethyl-5-(2-oxo-6-pyridin-3-yl-1,2-dihydroindol-3-ylidenemethyl)-1*H*-pyrrole-3-carboxylic acid (3-dimethylaminopropyl)amide

6-Pyridin-3-yl-1,3-dihydroindol-2-one (42 mg, 0.2 mmol) was condensed with 5-formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (3-dimethylaminopropyl)amide (50 mg) to give 67 mg (75%) of the title compound as yellow-brown solid.

^{1}HNMR (360 MHz, DMSO-d6) δ 13.61 (s, br, 1H, N*H*), 11.00 (s, br, 1H, N*H*), 8.86 (s, br, 1H), 8.54 (s, br, 1H), 8.04 (m, 1H), 7.90 (d, J = 8.0 Hz, 1H), 7.69 (s, 1H, H-vinyl), 7.63 (m, 1H), 7.45-7.48 (m, 1H), 7.35 (dd, J = 1.7 & 8.0 Hz, 1H), 7.15 (d, J = 1.7 Hz, 1H), 3.21-3.27 (m, 2H, NCH_2), 2.43 (s, 3H, CH_3), 2.41 (s,

3H, CH_3), 2.28 (m, 2H, NCH_2), 2.14 (s, 6H, 2xNCH_3), 1.64 (m, 2H, CH_2).

MS-EI *m/z* 443 [M^+].

Example 66

2,4-Dimethyl-5-(2-oxo-5-phenyl-1,2-dihydroindol-3-ylidenemethyl)-1*H*-pyrrole-3-carboxylic acid (3-dimethylaminopropyl)amide

5-Phenyl-1,3-dihydroindol-2-one (67 mg, 0.32 mmol) was condensed with 5-formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (3-dimethylaminopropyl)amide (81 mg) to give 40 mg (28%) of the title compound as an orange solid.

^{1}HNMR (360 MHz, DMSO-d6) δ 13.66 (s, br, 1H, NH), 10.92 (s, br, 1H, NH), 8.14 (s, 1H), 7.79 (s, 1H), 7.71 (m, 2H), 7.62 (m, 1H), 7.44 (m, 3H), 7.32 (m, 1H), 6.95 (m, 1H), 3.33 (m, 2H, NCH_2), 2.43 (s, 6H, 2xCH_3), 2.27 (m, 2H, NCH_2), 2.13 (s, 6H, 2xNCH_3), 1.63 (m, 2H, CH_2).

MS-EI *m/z* 442 [M^+].

Example 67

2,4-Dimethyl-5-(2-oxo-5-phenyl-1,2-dihydroindol-3-ylidenemethyl)-1*H*-pyrrole-3-carboxylic acid (3-diethylaminopropyl)amide

5-Phenyl-1,3-dihydroindol-2-one (1.5 g, 7.16 mmol) was condensed with 5-formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (3-diethylaminopropyl)amide (2 g) to give 1.3 g (40%) of the title compound as a yellow-orange solid.

^{1}HNMR (360 MHz, DMSO-d6) δ 13.64 (s, 1H, NH), 10.91 (s, 1H, NH), 8.14 (d, *J* = 1.4 Hz, 1H, Ar*H*), 7.8 (s, 1H, Ar*H*), 7.7 (dd, *J* = 1.2 and 8.5 Hz, 2H, Ar*H*), 7.6 (t, *J* = 5.3 Hz, 1H, CON*H*CH_2), 7.4 (m, 3H, Ar*H*), 7.3 (t, *J* = 7.4 Hz, 1H, Ar*H*), 6.9 (d, *J* = 8.0 Hz, 1H, Ar*H*), 3.2 (m, 2H, CONHC$\underline{H_2}$), 2.5 (m, 12H, 3x$NC\underline{H_2}$ and 2x$C\underline{H_3}$), 1.61 (m, 2H, $CH_2C\underline{H_2}CH_2$), 0.93 (t, *J* = 6.7 Hz, 6H, $NCH_2C\underline{H_3}$).

MS-EI *m/z* 470 [M^+].

Example 68

2,4-Dimethyl-5-(2-oxo-6-phenyl-1,2-dihydroindol-3-ylidenemethyl)-1*H*-pyrrole-3-carboxylic acid (3-diethylaminopropyl)amide

6-Phenyl-1,3-dihydroindol-2-one (1.5 g, 7.16 mmol) was condensed with 5-formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (3-diethylaminopropyl)amide (2 g) to give 1.9 g (57%) of the title compound as an orange solid.

^{1}HNMR (360 MHz, DMSO-d6) δ 13.58 (s, 1H, NH), 10.94 (s, 1H, NH), 7.8 (d, *J* = 7.9 Hz, 1H, Ar*H*), 7.6 (m, 4H, Ar*H*), 7.4 (t, *J* = 7.5 Hz, 2H, Ar*H*), 7.3 (m, 2H), 7.1 (d, *J* = 1.4 Hz, 1H, Ar*H*), 3.2 (m, 2H, CONHC*H*$_2$), 2.5 (m, 12H, 3xNC*H*$_2$ and 2xC*H*$_3$), 1.61 (m, 2H, CH$_2$C*H*$_2$CH$_2$), 0.93 (t, *J* = 6.7 Hz, 6H, NCH$_2$C*H*$_3$).

MS-EI *m/z* 470 [M$^+$].

Example 69

3-[4-(3-Diethylaminopropylcarbamoyl)-3,5-dimethyl-1*H*-pyrrol-2-ylmethylene]-2-oxo-2,3-dihydro-1*H*-indole-4-carboxylic acid (3-chloro-4-methoxyphenyl)amide

2-Oxo-2,3-dihydro-1*H*-indole-4-carboxylic acid (3-chloro-4-methoxyphenyl)amide (1 g, 3.16 mmol) was condensed with 5-formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (3-diethylaminopropyl)amide (1 g, 3.58 mmol) to give 1.7 g (85%) of the title compound as a yellow-orange solid.

MS-EI *m/z* 578.2 [M$^+$].

Example 70

5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (3-diethylamino-propyl)amide

5-Bromo-1,3-dihydroindol-2-one (0.5 g, 2.36 mmol) was condensed with 5-formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (3-diethylaminopropyl)amide (0.51 g) to give 0.84 g of the title compound as a red-orange solid.

^{1}HNMR (360 MHz, DMSO-d6) δ 13.61 (s, 1H, NH), 10.99 (s, 1H, NH), 8.09 (d, *J* = 1.8 Hz, 1H, Ar*H*), 7.7 (m, 4H), 7.2 (dd, *J* = 1.8 and 8.3 Hz, 2H, Ar*H*), 6.8 (d, *J* = 7.8 Hz, 1H, Ar*H*), 3.3 (br s,

4H, 2xNCH_2), 3.2 (m, 2H, CONHCH_2), 2.6 (br s, 2H, NCH_2 and 2xCH_3), 2.4 (s, 6H, 2xCH_3), 1.66 (m, 2H, $CH_2CH_2CH_2$), 0.98 (t, J = 7.1 Hz, 6H, NCH_2CH_3).

MS-EI m/z 472 and 474 [M^+ -1 and M^++1].

Example 71

5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2,4-diisopropyl-1*H*-pyrrole-3-carboxylic acid (2-diethylaminoethyl)amide

5-Bromo-1,3-dihydroindol-2-one (100 mg, 0.47 mmol) was condensed with 5-formyl-2,4-diisopropyl-1*H*-pyrrole-3-carboxylic acid (2-diethylaminoethyl)amide (150 mg) to give 0.15 g (62%) of the title compound as a yellow-orange solid.

^{1}HNMR (300 MHz, DMSO-d6) δ 13.97 (s, 1H, NH), 10.95 (s, 1H, NH), 8.09 (d, J = 1.3 Hz, 1H, Ar*H*), 7.84 (m, 1H), 7.79 (s, 1H), 7.23 (dd, J = 1.3 and 8.1 Hz, 1H, Ar*H*), 6.8 (d, J = 8.1 Hz, 1H, Ar*H*), 3.5 (m, 1H, C*H*), 3.3 (m, 3H, C*H* and NHCH_2), 2.5 (br m, 6H, 3xNCH_2), 1.28 (d, J = 6.9 Hz, 6H, 2xCH_3), 1.23 (d, J = 6.6 Hz, 6H, 2xCH_3), 0.96 (m, 6H, 2xCH_2CH_3).

MS-EI m/z 514 and 516 [M^+-1 and M^++1].

Example 72

5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2,4-diisopropyl-1*H*-pyrrole-3-carboxylic acid (3-diethylaminopropyl)amide

5-Bromo-1,3-dihydroindol-2-one (90 mg, 0.42 mmol) was condensed with 5-formyl-2,4-diisopropyl-1*H*-pyrrole-3-carboxylic acid (3-diethylaminopropyl)amide (140 mg) to give 54 mg (25%) of the title compound as red-brown solid.

^{1}HNMR (300 MHz, DMSO-d6) δ 13.98 (s, 1H, NH), 10.96 (s, 1H, NH), 8.09 (d, J = 1.7 Hz, 2H), 7.78 (s, 1H, H-vinyl), 7.23 (dd, J = 1.7 and 8.1 Hz, 1H, Ar*H*), 6.82 (d, J = 8.1 Hz, 1H, Ar*H*), 3.5 (m, 1H, C*H*), 3.25 (m, 2H, NHCH_2), 3.15 (m, 1H, C*H*), 2.7 (br s, 6H, 3xNCH_2), 1.7 (br m, 2H, $CH_2CH_2CH_2$), 1.28 (d, J = 6.9 Hz, 6H, 2xCH_3), 1.24 (d, J = 5.9 Hz, 6H, 2xCH_3), 1.06 (m, 6H, 2xCH_2CH_3).

MS-EI *m/z* 528 and 530 [M^+-1 and M^++1].

Example 73

5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2,4-diisopropyl-1*H*-pyrrole-3-carboxylic acid (3-pyrrolidin-1-ylpropyl)amide

5-Bromo-1,3-dihydroindol-2-one (130 mg, 0.6 mmol) was condensed with 5-formyl-2,4-diisopropyl-1*H*-pyrrole-3-carboxylic acid (3-pyrrolidin-1-ylpropyl)amide (150 mg, 0.45 mmol) to give 36 mg (15%) of the title compound as a tan-orange solid.

^{1}HNMR (300 MHz, DMSO-d6) δ 13.98 (s, 1H, NH), 10.97 (s, 1H, NH), 8.10 (d, *J* = 1.6 Hz, 2H), 7.78 (s, 1H, H-vinyl), 7.23 (dd, *J* = 1.6 and 7.6 Hz, 1H, Ar*H*), 6.82 (d, *J* = 7.6 Hz, 1H, Ar*H*), 3.5 (m, 1H, C*H*), 3.25 (m, 2H, NHCH_2), 3.15 (m, 1H, C*H*), 2.7 (br s, 6H, 3xNCH_2), 1.7 (br m, 6H, 3xNCH$_2$CH_2), 1.28 (d, *J* = 5.6 Hz, 6H, 2xCH_3), 1.24 (d, *J* = 5.7 Hz, 6H, 2xCH_3).

MS-EI *m/z* 526 and 528 [M^+-1 and M^++1].

Example 74

5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (pyridin-4-ylmethyl)-amide

5-Bromo-1,3-dihydroindol-2-one (170 mg, 0.8 mmol) was condensed with 5-formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (pyridin-4-ylmethyl)amide (200 mg) to give 14 mg (4%) of the title compound as a yellow solid.

^{1}HNMR (300 MHz, DMSO-d6) δ 13.67 (s, 1H, NH), 11.01 (s, br, 1H, NH), 8.51 (dd, *J* = 1.6 & 4.3 Hz, 2H), 8.23 (t, *J* = 6.0 Hz, 1H, CONHCH$_2$), 8.11 (d, *J* = 1.9 Hz, 1H), 7.78 (s, 1H, H-vinyl), 7.31 (d, *J* = 6.0 Hz, 2H), 7.25 (dd, *J* = 1.9 & 8.1 Hz, 1H), 6.82 (d, *J* = 8.1 Hz, 1H), 4.45 (d, *J* = 6.0 Hz, 2H, NCH_2), 2.46 (s, 6H, 2xCH_3).

MS-EI *m/z* 450 and 452 [M^+-1 and M^++1].

564
564

Example 75

5-[6-(4-Butylphenyl)-2-oxo-1,2-dihydroindol-3-ylidenemethyl]-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-pyrrolidin-1-ylethyl)amide

5-[6-(4-Butylphenyl)]-1,3-dihydroindol-2-one (50 mg, 0.19 mmol) was condensed with 5-formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-pyrrolidin-1-ylethyl)amide (50 mg) to give 74 mg (76%) of the title compound as an orange solid.

^{1}HNMR (360 MHz, DMSO-d6) δ 13.58 (s, 1H, NH), 10.93 (s, br, 1H, NH), 7.82 (d, *J* = 7.9 Hz, 1H), 7.63 (s, 1H, H-vinyl), 7.54 (d, *J* = 7.9 Hz, 2H), 7.46 (m, 1H, CONH), 7.26 (m, 3H), 7.09 (s, 1H), 3.30 (m, 2H, CH_2), 2.52-2.63 (m, 4H, 2xCH_2), 2.49 (m, 4H, 2xCH_2), 2.43 (s, 3H, CH_3), 2.40 (s, 3H, CH_3), 1.68 (m, 4H, 2xCH_2), 1.58 (m, 2H, CH_2), 1.34 (m, 2H, CH_2), 0.91 (t, *J*= 7.2 Hz, 3H, CH_2CH_3).

MS-EI *m/z* 510 [M^+].

Example 76

5-[6-(5-Isopropyl-2-methoxyphenyl)-2-oxo-1,2-dihydroindol-3-ylidenemethyl]-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-pyrrolidin-1-ylethyl)amide

6-(5-Isopropyl-2-methoxyphenyl)-1,3-dihydroindol-2-one (50 mg, 0.17 mmol) was condensed with 5-formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-pyrrolidin-1-yl-ethyl)amide (45 mg) to give 67 mg (75%) of the title compound as an orange solid.

^{1}HNMR (360 MHz, DMSO-d6) δ 13.60 (s, 1H, NH), 10.82 (s, br, 1H, NH), 7.77 (d, *J* = 7.9 Hz, 1H), 7.61 (s, 1H, H-vinyl), 7.45 (m, 1H, CONH), 7.0-7.19 (m, 5H), 3.73 (s, 3H, OCH_3), 3.32 (m, 2H, CH_2), 2.87 (m, 1H, $CH(CH_3)_2$), 2.56 (m, 2H, CH_2), 2.48 (m, 4H, 2xCH_2), 2.43 (s, 3H, CH_3), 2.40 (s, 3H, CH_3), 1.68 (m, 4H, 2xCH_2), 1.21 (d, *J* = 6.8 Hz, 6H, $CH(CH_3)_2$).

MS *m/z* 527.2 [M^++1].

Example 77

5-[6-(4-Ethylphenyl)-2-oxo-1,2-dihydroindol-3-ylidenemethyl]-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-pyrrolidin-1-ylethyl)amide

6-(4-Ethylphenyl)-1,3-dihydroindol-2-one (45 mg, 0.19 mmol) was condensed 5-formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-pyrrolidin-1-ylethyl)amide (50 mg) to give 60 mg (65%) of the title compound as a yellow-orange solid.

^{1}HNMR (300 MHz, DMSO-d6) δ 13.59 (s, 1H, NH), 10.96 (s, br, 1H, NH), 7.83 (d, *J* = 8.4 Hz, 1H), 7.64 (s, 1H, H-vinyl), 7.51-7.56 (m, 3H), 7.25-7.30 (m, 3H), 7.08 (d, *J* = 1 Hz, 1H), 3.31 (m, 2H, CH_2), 2.63 (m, 2H, CH_2CH_3), 2.55 (m, 2H, CH_2), 2.49 (m, 4H, 2xCH_2), 2.42 (s, 3H, CH_3), 2.40 (s, 3H, CH_3), 1.67 (m, 4H, 2xCH_2), 1.20 (t, *J*= 7.5 Hz, 3H, CH_2CH_3).

MS-EI *m/z* 482 [M^+].

Example 78

5-[6-(2,4-Dimethoxyphenyl)-2-oxo-1,2-dihydroindol-3-ylidenemethyl]-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-pyrrolidin-1-ylethyl)amide

6-(2,4-Dimethoxyphenyl)-1,3-dihydroindol-2-one (51 mg, 0.19 mmol) was condensed with 5-formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-pyrrolidin-1-ylethyl)amide (50 mg) to give 30 mg (31%) of the title compound as an orange solid.

^{1}HNMR (300 MHz, DMSO-d6) δ 13.59 (s, 1H, NH), 10.86 (s, br, 1H, NH), 7.75 (d, *J* = 7.8 Hz, 1H), 7.60 (s, 1H, H-vinyl), 749 (m, 1H, CONH), 7.22 (d, *J* = 8.4 Hz, 1H), 7.03 (m, 1H), 6.97 (s, 1H), 6.58-6.65 (m, 2H), 3.79 (s, 3H, OCH_3), 3.76 (s, 3H, OCH_3), 3.33 (m, 2H, CH_2), 2.55 (m, 2H, CH_2), 2.50 (m, 4H, 2xCH_2), 2.42 (s, 3H, CH_3), 2.39 (s, 3H, CH_3), 1.67 (m, 4H, 2xCH_2).

MS-EI *m/z* 514 [M^+].

Example 79

5-[6-(3-Isopropylphenyl)-2-oxo-1,2-dihydroindol-3-ylidenemethyl]-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-pyrrolidin-1-ylethyl)amide

6-(3-Isopropylphenyl)-1,3-dihydroindol-2-one (48 mg, 0.19 mmol) was condensed with 5-formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-pyrrolidin-1-ylethyl)amide (50 mg) to give 59 mg (63%) of the title compound as an orange solid.

^{1}HNMR (300 MHz, DMSO-d6) δ 13.63 (s, 1H, N*H*), 10.97 (s, br, 1H, N*H*), 7.87 (d, *J* = 7.8 Hz, 1H), 7.68 (s, 1H, H-vinyl), 7.24-7.55 (m, 6H), 7.13 (s, 1H), 3.34 (m, 2H, C*H*$_2$), 3.30 (m, 1H, C*H*(CH$_3$)$_2$), 2.60 (m, 2H, C*H*$_2$), 2.50 (m, 4H, 2xCH$_2$), 2.45 (s, 3H, C*H*$_3$), 2.43 (s, 3H, C*H*$_3$), 1.70 (m, 4H, 2xC*H*$_2$), 1.27 (d, *J* = 6.9 Hz, 6H, CH(C*H*$_3$)$_2$).

MS-EI *m/z* 496 [M$^+$].

Example 80

5-(5-Fluoro-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-diethylamino-ethyl)amide

5-Fluoro-1,3-dihydroindol-2-one (0.54 g, 3.8 mmol) was condensed with 5-formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-diethylaminoethyl)amide to give 0.83 g (55%) of the title compound as a yellow green solid.

^{1}HNMR (360 MHz, DMSO-d6) δ 13.66 (s, 1H, N*H*), 10.83 (s, br, 1H, N*H*), 7.73 (dd, *J* = 2.5 & 9.4 Hz, 1H), 7.69 (s, 1H, H-vinyl), 7.37 (t, 1H, CON*H*CH$_2$CH$_2$), 6.91 (m, 1H), 6.81-6.85 (m, 1H), 3.27 (m, 2H, C*H*$_2$), 2.51 (m, 6H, 3xCH$_2$), 2.43 (s, 3H, C*H*$_3$), 2.41 (s, 3H, C*H*$_3$), 0.96 (t, *J* = 6.9 Hz, 6H, N(CH$_2$C*H*$_3$)$_2$).

MS-EI *m/z* 398 [M$^+$].

Example 81

3-[4-(2-Diethylaminoethylcarbamoyl)-3,5-dimethyl-1*H*-pyrrol-2-ylmethylene]-2-oxo-2,3-dihydro-1*H*-indole-6-carboxylic acid

2-Oxo-2,3-dihydro-1*H*-indole-6-carboxylic acid (80 mg, 0.45 mmol) was condensed with 5-formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-diethylaminoethyl)amide to give 210 mg (92%) of the title compound as a yellow orange solid.

^{1}HNMR (360 MHz, DMSO-d6) δ 13.6 (s, 1H, N*H*), 7.76 (d, *J* = 8.0 Hz, 1H), 7.66 (s, 1H, H-vinyl), 7.57 (dd, *J* = 1.5 & 8.0 Hz, 1H), 7.40-7.42 (m, 2H), 3.28 (m, 2H, CH_2), 2.88 (m, H-piperidine), 2.54 (m, 6H, 3xCH_2), 2.44 (s, 3H, CH_3), 2.40 (s, 3H, CH_3), 1.56 (m, H-piperidine), 0.97 (t, *J* = 6.98 Hz, 6H, N(CH_2CH_3)$_2$).

MS *m/z* 424 [M$^+$].

Example 82

5-(5-Dimethylsulfamoyl-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-pyrrolidin-1-ylethyl)amide

2-Oxo-2,3-dihydro-1*H*-indole-5-sulfonic acid dimethylamide (90 mg, 0.38 mmol) was condensed with 5-formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-pyrrolidin-1-ylethyl)amide (100 mg) to give 100 mg (54%) of the title compound as a yellow solid.

^{1}HNMR (360 MHz, DMSO-d6) δ 13.65 (s, 1H, N*H*), 11.30 (s, br, 1H, N*H*), 8.25 (d, 1H), 7.92 (s, 1H, H-vinyl), 7.48-7.53 (m, 2H), 7.07 (d, *J* = 8.2 Hz, 1H), 3.33 (m, 2H, CH_2), 2.61 (s, 6H, N(CH_3)$_2$), 2.56 (t, 2H, CH_2), 2.49 (m, 4H, 2xCH_2), 2.45 (s, 3H, CH_3), 2.44 (s, 3H, CH_3), 1.67 (m, 4H, 2xCH_2).

MS-EI *m/z* 485 [M$^+$].

Example 83

5-[5-(3-Chlorophenylsulfamoyl)-2-oxo-1,2-dihydroindol-3-ylidenemethyl]-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-pyrrolidin-1-ylethyl)amide

2-Oxo-2,3-dihydro-1*H*-indole-5-sulfonic acid (3-chlorophenyl)amide (120 mg, 0.38 mmol) was condensed with 5-formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-pyrrolidin-1-ylethyl)amide (100 mg) to give 150 mg (69%) of the title compound as a yellow orange solid.

^{1}HNMR (360 MHz, DMSO-d6) δ 13.55 (s, 1H, N*H*), 11.26 (br s, 1H, N*H*), 10.30 (br s,1H, N*H*), 8.26 (d, 1H), 7.79 (s, 1H, H-vinyl), 7.51-7.57 (m, 2H), 7.22 (t, *J* = 8.1 Hz, 1H), 7.15 (m, 1H), 7.07 (m, 1H), 7.0 (m, 2H), 3.44 (m, 2H, C*H*$_2$), 2.57 (t, *J* = 7.0 Hz, 2H, C*H*$_2$), 2.49 (m, 4H, 2xC*H*$_2$), 2.44 (s, 3H, C*H*$_3$), 2.43 (s, 3H, C*H*$_3$), 1.68 (m, 4H, 2xC*H*$_2$).

MS *m/z* 568 [M$^+$].

Example 84

2,4-Dimethyl-5-[2-oxo-5-(pyridin-3-ylsulfamoyl)-1,2-dihydroindol-3-ylidenemethyl]-1*H*-pyrrole-3-carboxylic acid (2-pyrrolidin-1-ylethyl)amide

2-Oxo-2,3-dihydro-1*H*-indole-5-sulfonic acid pyridin-3-ylamide (110 mg, 0.38 mmol) was condensed with 5-formyl-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (2-pyrrolidin-1-ylethyl)amide (100 mg) to give 150 mg (74%) of the title compound as an orange solid.

^{1}HNMR (360 MHz, DMSO-d6) δ 13.58 (s, 1H, N*H*), 8.21 (d, *J* = 2.0 Hz, 2H), 8.04 (m, 1H), 7.76 (s, 1H, H-vinyl), 7.49-7.54 (m, 2H), 7.41 (m, 1H), 7.14 (m, 1H), 6.94 (d, *J* = 8.5 Hz, 1H), 3.33 (m, 2H, C*H*$_2$), 2.56 (t, *J* = 7.06 Hz, 2H, C*H*$_2$), 2.49 (m, 4H, 2xC*H*$_2$), 2.43 (s, 6H, 2xC*H*$_3$), 1.68 (m, 4H, 2xC*H*$_2$).

MS *m/z* 535 [M$^+$].

Example 85

3-[3,5-Dimethyl-4-(4-methylpiperazine-1-carbonyl)-1*H*-pyrrol-2-ylmethylene]-4-(2-hydroxyethyl)-1,3-dihydroindol-2-one

4-(2-Hydroxyethyl)-1,3-dihydroindol-2-one (71 mg, 0.4 mmol) was condensed with 3,5-dimethyl-4-(4-methyl-piperazine-1-carbonyl)-1*H*-pyrrole-2-carbaldehyde to give 90 mg (55%) of the title compound as an orange solid.

^{1}HNMR (300 MHz, DMSO-d6) δ 14.25(s, 1H, N*H*), 10.88 (s, 1H, N*H*), 7.57 (s, 1H, H-vinyl), 7.03 (m, 1H), 6.75-6.82 (m, 2H), 4.86 (m, 1H, O*H*), 3.70 (m, 2H, CH_2), 3.04 (m, 2H, CH_2), 2.48 (m, 4H, 2xCH_2), 2.28 (br s, 7H), 2.19 (s, 3H, CH_3), 2.18 (s, 3H, CH_3).

MS *m/z* (+ve) 4.09.3 [M$^+$].

Example 86

3-[3,5-Dimethyl-4-(4-methylpiperazine-1-carbonyl)-1*H*-pyrrol-2-ylmethylene]-2-oxo-2,3-dihydro-1*H*-indole-5-sulfonic acid phenylamide

2-Oxo-2,3-dihydro-1*H*-indole-5-sulfonic acid phenylamide (110 mg, 0.4 mmol) was condensed with 3,5-dimethyl-4-(4-methylpiperazine-1-carbonyl)-1*H*-pyrrole-2-carbaldehyde (100 mg) to give 50 mg (24%) of the title compound as a yellow solid.

^{1}HNMR (300 MHz, DMSO-d6) δ 13.52(s, 1H, N*H*), 11.26 (s, 1H, N*H*), 10.08 (s, 1H, N*H*), 8.21 (d, *J* = 1.6 Hz, 1H), 7.75 (s, 1H, H-vinyl), 7.50 (dd, *J* = 1.6 & 8.3 Hz, 1H), 7.19 (m, 2H), 7.10 (m, 2H), 6.97 (m, 2H), 2.49 (m, 4H, 2xCH_2), 2.28 (m, 10H, 2xCH_3 & 2xCH_2), 2.18 (s, 3H, CH_3).

MS-EI *m/z* 519 [M$^+$].

Example 87

5-(5-Dimethylsulfamoyl-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-diethylaminoethyl)amide

2-Oxo-2,3-dihydro-1*H*-indole-5-sulfonic acid dimethylamide (90 mg, 0.38 mmol) was condensed with 5-formyl-2,4-dimethyl-1*H*-

pyrrole-3-carboxylic acid (2-diethylaminoethyl)amide (100 mg) to give 80 mg (43%) of the title compound as a yellow solid.

^{1}HNMR (300 MHz, DMSO-d6) δ 11.30 (s, 1H, N*H*), 8.27 (d, *J* = 1.7 Hz, 1H), 7.94 (s, 1H, H-vinyl), 7.49 (dd, *J* = 1.7 & 8.0 Hz, 1H), 7.44 (m, 1H, CON*H*CH_2CH_2), 7.07 (d, *J* = 8.0 Hz, 1H), 3.26 (m, 2H, CH_2), 2.60 (s, 6H, $N(CH_3)_2$), 2.53 (m, 2H, CH_2), 2.45-2.50 (m, 10H, 2xCH_3 & $N(CH_2CH_3)_2$), 0.96 (t, *J* = 7.2 Hz, 6H, $N(CH_2CH_3)_2$).

MS-EI *m/z* 487 [M$^+$].

Example 88

5-[5-(3-Chlorophenylsulfamoyl)-2-oxo-1,2-dihydroindol-3-ylidenemethyl]-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-diethylaminoethyl)amide

2-Oxo-2,3-dihydro-1*H*-indole-5-sulfonic acid (3-chlorophenyl)amide (120 mg, 3.8 mmol) was condensed with 5-formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-diethylaminoethyl)amide (100 mg) to give 80 mg (37%) of the title compound as a yellow solid.

^{1}HNMR (360 MHz, DMSO-d6) δ 13.55 (s, 1H, N*H*), 11.24 (s, 1H, N*H*), 10.29 (s, 1H, N*H*), 8.25 (d, *J* = 1.87 Hz, 1H), 7.79 (s, 1H, H-vinyl), 7.52 (dd, *J* = 1.87 & 8.3 Hz, 1H), 7.42 (m, 1H, CON*H*CH_2CH_2), 7.22 (t, *J* = 8.02 Hz, 1H), 7.15 (t, *J* = 2 Hz, 1H), 7.08 (m, 1H), 7.0 (m, 2H), 3.27 (m, 2H, CH_2), 2.48-2.57 (m, 6H, 3xCH_2), 2.45 (s, 3H, CH_3), 2.44 (s, 3H, CH_3), 0.97 (t, *J* = 7.0 Hz, 6H, $N(CH_2CH_3)_2$).

MS *m/z* 570.1 [M$^+$].

Example 95

3-(2-Oxo-5-phenyl-1,2-dihydroindol-3-ylidenemethyl)-4,5,6,7-tetrahydro-2*H*-isoindole-1-carboxylic acid ethyl ester

^{1}HNMR (360 MHz, DMSO-d6) δ 13.74 (s, 1H, NH), 11.00 (s, 1H, NH), 8.13 (d, *J* = 1.7 Hz, 1H), 7.74 (s, 1H, H-vinyl), 7.70 (d, *J* = 7.7 Hz, 2H), 7.49 (dd, *J* = 1.7 & 8.0 Hz, 1H), 7.44 (t, *J* = 7.7 Hz, 2H), 7.32 (m, 1H), 6.96 (d, *J* = 8.0 Hz, 1H), 4.26 (q, *J* = 7.0 Hz, 2H, OCH_2CH_3), 2.79 (m, 2H, CH_2), 2.72 (m, 2H, CH_2), 1.73 (m,

4H, 2xCH_2), 1.30 (t, J = 7.0 Hz, 3H, OCH$_2$CH_3).

MS-EI m/z 412 [M$^+$].

Example 99

3-(2-Oxo-5-phenylsulfamoyl-1,2-dihydroindol-3-ylidenemethyl)-4,5,6,7-tetrahydro-2*H*-isoindole-1-carboxylic acid ethyl ester

^{1}HNMR (360 MHz, DMSO-d6) δ 13.64 (s, 1H, NH), 11.33 (s, 1H, NH), 10.07 (s, 1H, NH), 8.24 (d, J = 1.8 Hz, 1H), 7.74 (s, 1H, H-vinyl), 7.57 (dd, J = 1.8 & 8.0 Hz, 1H), 7.21 (t, J = 7.6 Hz, 2H), 7.11 (d, J = 7.6 Hz, 2H), 6.99 (d, J = 8.0 Hz, 1H), 6.98 (d, J = 7.6 Hz, 1H), 4.27 (q, J = 7.0 Hz, 2H, OCH_2CH$_3$), 2.80 (m, 2H, CH_2), 2.73 (m, 2H, CH_2), 1.73 (m, 4H, 2xCH_2), 1.30 (t, J = 7.0 Hz, 3H, OCH$_2$CH_3).

MS-EI m/z 491 [M$^+$].

Example 109

3-[3-(Morpholine-4-carbonyl)-4,5,6,7-tetrahydro-2*H*-isoindol-1-ylmethylene]-2-oxo-2,3-dihydro-1*H*-indole-6-carboxylic acid

^{1}HNMR (360 MHz, DMSO-d6) δ 13.60 (s, 1H, NH), 12.75 (br s, 1H, COOH), 11.08 (s, 1H, NH), 7.85 (d, J = 7.8 Hz, 1H), 7.71 (s, 1H, H-vinyl), 7.62 (dd, J = 1.4 & 7.8 Hz, 1H), 7.41 (d, J = 1.4 Hz, 1H), 3.65 (m, 4H, 2xCH_2), 3.55 (m, 4H, 2xCH_2), 2.81 (m, 2H, CH_2), 2.54 (m, 2H, CH_2).1.73 (m, 4H, 2xCH_2).

MS-EI m/z 421 [M$^+$].

Example 112

5-Bromo-3-[3-(pyrrolidine-1-carbonyl)-4,5,6,7-tetrahydro-2H-isoindol-1-ylmethylene]-1,3-dihydro-indol-2-one

^{1}HNMR (360 MHz, DMSO-d6) δ 13.56 (s, 1H, NH), 11.00 (s, 1H, NH), 8.05 (d, J = 1.8 Hz, 1H), 7.74 (s, 1H, H-vinyl), 7.28 (dd, J = 1.3 & 8.3 Hz, 1H), 6.83 (d, J = 8.3 Hz, 1H), 3.57 (m, 4H, 2xCH_2), 2.79 (m, 2H, CH_2), 2.65 (m, 2H, CH_2), 1.88 (m, 4H, 2xCH_2), 1.71 (m, 4H, 2xCH_2).

MS-EI m/z 439 & 441 [M$^+$-1] & [M$^+$+1].

Example 114

3-(3-Dimethylcarbamoyl-4,5,6,7-tetrahydro-2*H*-isoindol-1-ylmethylene)-2-oxo-2,3-dihydro-1*H*-indole-6-carboxylic acid

^{1}HNMR (360 MHz, DMSO-d6) δ 13.60 (s, 1H, NH), 12.72 (br s, 1H, COOH), 11.05 (s, 1H, NH), 7.85 (d, *J* = 7.9 Hz, 1H), 7.72 (s, 1H, H-vinyl), 7.62 (dd, *J* = 1.3 & 7.9 Hz, 1H), 7.42 (d, *J* = 1.3 Hz, 1H), 3.03 (s, 6H, N(CH_3)$_2$), 2.81 (m, 2H, CH_2), 2.55 (m, 2H, CH_2), 1.73 (m, 4H, 2xCH_2).

MS-EI *m/z* 379 [M$^+$].

Exapmle 115

4-Methyl-5-(5-methylsulfamoyl-2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-1H-pyrrole-3-carboxylic acid

^{1}HNMR (300 MHz, DMSO-d6) □ 13.56 (br s, 1H, NH), 8.24 (d, *J* = 1.5 Hz, 1H), 7.86 (s, 1H, H-vinyl), 7.74 (d, *J* = 2.96 Hz, 1H), 7.56 (dd, *J* = 1.5 & 8.1 Hz, 1H), 7.20 (br m, 1H, N*H*CH$_3$), 7.03 (d, *J* = 8.1 Hz, 1H), 2.57 (s, 3H, CH_3), 2.41 (s, 3H, CH_3).

MS-EI *m/z* 361 [M$^+$].

Example 116

[[4-Methyl-5-(4-methyl-5-methylsulfamoyl-2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-1H-pyrrole-3-carbonyl]-amino}-acetic acid ethyl ester

4-Methyl-1*H*-pyrrole-3-carboxylic acid ethyl ester (lit. ref. D.O. Cheng, T. L. Bowman and E. LeGoff; J. Heterocyclic Chem.; 1976; 13; 1145-1147) was formylated using method A, hydrolysed using method B followed by amidation (method C) to give [(5-formyl-4-methyl-1*H*-pyrrole-3-carbonyl)-amino]-acetic acid ethyl ester.

4-Methyl-5-methylaminosulfonyl-2-oxindole (50 mg, 0.21 mmol) was condensed with [(5-formyl-4-methyl-1*H*-pyrrole-3-carbonyl)-amino]-acetic acid ethyl ester (100 mg, 0.42 mmol) and piperidine (0.1 mL) in ethanol (2 mL) to give 50 mg (52%) of the title compound.

^{1}HNMR (360 MHz, DMSO-d6) δ 13.59 (s, 1H, NH), 11.29 (v.br. s, 1H, N*H*-CO), 8.33 (t, *J* = 5.8 Hz, 1H, CON*H*CH$_2$), 7.83 (d, *J* =

3.11 Hz, 1H), 7.80 (s, 1H, H-vinyl), 7.71 (d, J = 8.5 Hz, 1H), 7.34 (br m, 1H, NHCH$_3$), 6.89 (d, J = 8.5 Hz, 1H), 4.11 (q, J = 7.1 Hz, 2H, OCH$_2$CH$_3$), 3.92 (d, J = 5.8 Hz, 2H, GlyCH$_2$), 2.86 (s, 3H, CH$_3$), 2.48 (s, 3H, CH$_3$), 2.42 (d, J = 4.71 Hz, 3H, HNCH$_3$), 1.20 (t, J = 7.1 Hz, 3H, OCH$_2$CH$_3$).

MS-EI m/z 460 [M$^+$].

Example 117

[[4-Methyl-5-(5-methylsulfamoyl-2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-1H-pyrrole-3-carbonyl]-amino}-acetic acid ethyl ester

A mixture of 5-methylaminosulfonyl-2-oxindole (0.06 g, 0.22 mmol), [(5-formyl-4-methyl-1H-pyrrole-3-carbonyl)-amino]-acetic acid ethyl ester (0.075 g, 0.27 mmol) and piperidine (2 drops) in ethanol (5 mL) was heated in a sealed tube at 90° C for 12 hrs. After cooling, the precipitate was collected by vacuum filtration, washed with ethanol, triturated with dichloromethane/ether and dried to give 0.035 g (36%) of the title compound as a yellowish brown solid.

^{1}H NMR (360 MHz, DMSO-d6) δ 13.6 (s, 1H, NH), 11 (v.br. s, 1H, NH-CO), 8.30 (t, J = 5.7 Hz, 1H, CONHCH$_2$), 8.25 (d, J = 1.2 Hz, 1H), 7.88 (s, 1H, H-vinyl), 7.84 (d, J = 3.3 Hz, 1H), 7.57 (dd, J = 1.9 & 8.5 Hz, 1H), 7.14 (br m, 1H, NHCH$_3$), 7.04 (d, J = 8.5 Hz, 1H), 4.11 (q, J = 6.7 Hz, 2H, OCH$_2$CH$_3$), 3.92 (d, J = 5.7 Hz, 2H, GlyCH$_2$), 2.55 (s, 3H, CH$_3$), 2.41 (m, 3H, NCH$_3$), 1.20 (t, J = 6.7 Hz, 3H, OCH$_2$CH$_3$).

MS m/z 446 [M$^+$].

Example 118

{[4-Methyl-5-(5-methylsulfamoyl-2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-1H-pyrrole-3-carbonyl]-amino}-acetic acid

A mixture of [(5-formyl-4-methyl-1H-pyrrole-3-carbonyl)-amino]-acetic acid ethyl ester (0.142 g, 0.59 mmol) and 1N NaOH (1.2 mL) in methanol (10 mL) was stirred at room temperature for 1 hr. The reaction was concentrated and the residue was condensed

with 5-methylaminosulfonyl-2-oxindole (0.13 g, 0.48 mmol) and piperidine (0.12 mL) in ethanol (12 mL) to give 0.11 g (52%) of the title compound.

^{1}HNMR (300 MHz, DMSO-d6) δ 13.98 (br s, 1H, NH), 8.17 (s, 1H), 7.80 (s, 1H), 7.75 (d, *J* = 3.1Hz, 1H), 7.51 (dd, *J* = 2 & 8.2 Hz, 1H), 7.21 (m on br s, 2H), 6.97 (d, *J* = 8.1 Hz, 1H), 3.41 (d, *J* = 4.2 Hz, 2H, CH_2NH), 2.54 (s, 3H, pyrrole-CH_3), 2.39 (s, 3H, Ar$C\underline{H}_3$).

MS *m/z* 417 $[M-1]^+$.

Example 120

5-Methyl-2-(2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-1H-pyrrole-3-carboxylic acid

^{1}HNMR (300 MHz, DMSO-d6) δ 13.77 (br s, 1H, NH), 12.49 (s, 1H, COOH), 11.07 (s, 1H, NH), 8.39 (s, 1H, H-vinyl), 7.43 (d, *J* = 7.47 Hz, 1H), 7.20 (t, *J* = 7.47 Hz, 1H), 7.03 (t, *J* = 7.47 Hz, 1H), 6.91 (d, *J* = 7.47 Hz, 1H), 6.49 (d, *J* = 1.53 Hz, 1H), 2.34 (s, 3H, CH_3).

MS *m/z* 269 $[M+H]^+$.

Example 121

5-Methyl-2-(2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-1H-pyrrole-3-carboxylic acid ethyl ester

^{1}HNMR (300 MHz, DMSO-d6) δ 13.79 (s, 1H, NH), 11.08 (s, 1H, NH), 8.31 (s, 1H, H-vinyl), 7.45 (d, *J* = 7.52 Hz, 1H), 7.20 (t, *J* = 7.52 Hz, 1H), 7.03 (t, *J* = 7.52 Hz, 1H), 6.91 (d, *J* = 7.52 Hz, 1H), 6.50 (d, *J* = 2.1 Hz, 1H), 4.26 (q, *J* = 7.2 Hz, 2H, OCH_2CH_3), 2.33 (s, 3H, CH_3), 1.32 (t, *J* = 7.2 Hz, 3H, OCH_2CH_3).

MS *m/z* 297.1 $[M+H]^+$.

Example 122

2-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-5-methyl-1*H*-pyrrole-3-carboxylic acid ethyl ester

^{1}HNMR (360 MHz, DMSO-d6) δ 13.72(s, 1H, NH), 11.16 (s, 1H, NH), 8.29 (s, 1H, H-vinyl), 7.53 (d, *J* = 2.0 Hz, 1H), 7.35 (dd, *J* = 2.0 & 8.05 Hz, 1H), 6.87 (t, *J* = 8.05 Hz, 1H), 6.53 (d, *J* = 2.4

Hz, 1H), 4.28 (q, J = 7.03 Hz, 2H, OCH_2CH_3), 2.35 (s, 3H, CH_3), 1.33 (t, J = 7.03 Hz, 3H, OCH_2CH_3).

MS m/z 375 & 377 $[M+H]^+$.

Example 123

2-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-5-methyl-1*H*-pyrrole-3-carboxylic acid

^{1}HNMR (300 MHz, DMSO-d6) δ 13.72(s, 1H, NH), 12.57 (s, 1H, COOH), 11.19 (s, 1H, NH), 8.36 (s, 1H, H-vinyl), 7.51 (d, J = 1.4 Hz, 1H), 7.34 (dd, J = 1.4 & 8.17 Hz, 1H), 6.87 (t, J = 8.17 Hz, 1H), 6.52 (d, J = 2.5 Hz, 1H), 2.35 (s, 3H, CH_3).

MS m/z 347 & 349 $[M+H]^+$.

Example 124

2-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-5-methyl-1*H*-pyrrole-3-carboxylic acid (2-pyrrolidin-1-ylethyl)-amide

To a solution of 2-formyl-5-methyl-1*H*-pyrrole-3-carboxylic acid (250 mg, 1.63 mmol) in dimethylformamide (3 mL) was added 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (376 mg, 1.2 equiv.), 1-hydroxybenzotriazole (265 mg, 1.2 equiv.), triethylamine (0.45 mL, 2 equiv.) and 1-(2-aminoethyl)pyrrolidine (0.23 mL. 1.1 equiv.). After stirring at room temperature overnight, the reaction was diluted with saturated sodium bicarbonate and brine (with extra salt) and extracted with 10% methanol in dichloromethane. The combined organic layers were washed with brine, dried over anhydrous sodium sulfate and concentrated to give 130 mg of 2-formyl-5-methyl-1*H*-pyrrole-3-carboxylic acid (2-pyrrolidin-1-yl-ethyl)-amide.

A mixture of 5-bromo-2-oxindole (106 mg, 0.5 mmol), 2-formyl-5-methyl-1*H*-pyrrole-3-carboxylic acid (2-pyrrolidin-1-yl-ethyl)-amide (125 mg, 1 equiv.) and piperidine (0.2 mL) in ethanol (2 mL) was heated in a sealed tube at 80°C for 1 hr and then cooled. The precipitate which formed was collected by vacuum filtration, washed with ethanol and ethyl acetate and dried to give the title compound as an orange solid.

^{1}HNMR (300 MHz, DMSO-d6) δ 13.62 (s, 1H, N*H*), 11.06 (br s, 1H, N*H*), 8.56 (s, 1H, H-vinyl), 8.15 (m, 1H, CONHCH$_2$), 7.48 (d, *J* = 1.8 Hz, 1H), 7.31 (dd, *J* = 1.8 & 7.9 Hz, 1H), 6.86 (d, *J* = 7.9 Hz, 1H), 6.60 (d, *J* = 2.3 Hz, 1H), 3.35 (m, 2H, HNC*H*$_2$CH$_2$), 2.56 (t, *J* = 6.91 Hz, 2H, HNCH$_2$C*H*$_2$), 2.35 (s, 3H, C*H*$_3$), 1.67 (m, 4H, 2xC*H*$_2$).

MS m/z 443/ 445 [M$^+$ and M$^-$+2].

Example 125

2-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-5-methyl-1*H*-pyrrole-3-carboxylic acid (2-diethylaminoethyl)-amide

To a solution of 2-formyl-5-methyl-1*H*-pyrrole-3-carboxylic acid (320 mg, 2.1 mmol) in dimethylformamide (3 mL) was added 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (483 mg, 1.2 equiv.), 1-hydroxybenzotriazole (340 mg, 1.2 equiv.), triethylamine (0.59 mL, 2 equiv.) and N,N-diethylethylenediamine (0.32 mL, 1.1 equiv.). After stirring at room temperature overnight, the reaction was diluted with saturated sodium bicarbonate and brine (with extra salt) and extracted with 10% methanol in dichloromethane. The combined organic layers were washed with brine, dried over anhydrous sodium sulfate and concentrated to give 2-formyl-5-methyl-1*H*-pyrrole-3-carboxylic acid (2-diethylaminoethyl)-amide.

A mixture of 5-bromo-2-oxindole (106 mg, 0.5 mmol), 2-formyl-5-methyl-1*H*-pyrrole-3-carboxylic acid (2-diethylamino-ethyl)-amide (126 mg, 1 equiv.) and piperidine (0.2 mL) in ethanol (2 mL) was heated in a sealed tube at 80°C for 1 hr and then cooled. The precipitate was collected by vacuum filtration, washed with ethanol and ethyl acetate and dried to give the title compound as an orange solid.

^{1}HNMR (360 MHz, DMSO-d6) δ 13.62 (s, 1H, N*H*), 11.11 (br s, 1H, N*H*), 8.54 (s, 1H, H-vinyl), 8.1 (m, 1H, CONHCH$_2$), 7.49 (d, *J* = 2.2 Hz, 1H), 7.31 (dd, *J* = 2.2 & 8.3 Hz, 1H), 6.86 (d, *J* = 8.3 Hz, 1H), 6.58 (d, *J* = 2.24 Hz, 1H), 3.31 (m, 2H, HNC*H*$_2$CH$_2$), 2.59

(m, 6H, 3xCH_2), 2.36 (s, 3H, CH_3), 0.99 (t, J = 6.8 Hz, 6H, $N(CH_2CH_3)_2$).

MS m/z 445/ 447 [M^+ and M^++2].

5. BIOLOGICAL EVALUATION

It will be appreciated that, in any given series of compounds, a spectrum of biological activity will be afforded. In its presently preferred embodiments, this invention relates to novel pyrrole substituted 2-indolinones demonstrating the ability to modulate RTK, CTK, and STK activity. The following assays are employed to find those compounds demonstrating the optimal degree of the desired activity.

A. Assay Procedures.

The following assays may be used to determine the level of activity and effect of the different compounds of the present invention on one or more of the PKs. Similar assays can be designed along the same lines for any PK using techniques well known in the art.

Several of the assays described herein are performed in an ELISA (Enzyme-Linked Immunosorbent Sandwich Assay) format (Voller, et al., 1980, "Enzyme-Linked Immunosorbent Assay," Manual of Clinical Immunology, 2d ed., Rose and Friedman, Am. Soc. Of Microbiology, Washington, D.C., pp. 359-371). The general procedure is as follows: a compound is introduced to cells expressing the test kinase, either naturally or recombinantly, for a selected period of time after which, if the test kinase is a receptor, a ligand known to activate the receptor is added. The cells are lysed and the lysate is transferred to the wells of an ELISA plate previously coated with a specific antibody recognizing the substrate of the enzymatic phosphorylation reaction. Non-substrate components of the cell lysate are washed away and the amount of phosphorylation on the substrate is detected with an antibody specifically recognizing phosphotyrosine compared with control

cells that were not contacted with a test compound.

The presently preferred protocols for conducting the ELISA experiments for specific PKs is provided below. However, adaptation of these protocols for determining the activity of compounds against other RTKs, as well as for CTKs and STKs, is well within the scope of knowledge of those skilled in the art.

Other assays described herein measure the amount of DNA made in response to activation of a test kinase, which is a general measure of a proliferative response. The general procedure for this assay is as follows: a compound is introduced to cells expressing the test kinase, either naturally or recombinantly, for a selected period of time after which, if the test kinase is a receptor, a ligand known to activate the receptor is added. After incubation at least overnight, a DNA labeling reagent such as 5-bromodeoxyuridine (BrdU) or H^3-thymidine is added. The amount of labeled DNA is detected with either an anti-BrdU antibody or by measuring radioactivity and is compared to control cells not contacted with a test compound.

GST-FLK-1 BIOASSAY

This assay analyzes the tyrosine kinase activity of GST-Flk1 on poly(glu,tyr) peptides.

Materials and Reagents:

1. Corning 96-well ELISA plates (Corning Catalog No. 5805-96).
2. poly(glu,tyr) 4:1, lyophilizate (Sigma Catalog # P0275).
3. Preparation of poly(glu,tyr)(pEY) coated assay plates: Coat 2 ug/well of poly(glu,tyr)(pEY) in 100 ul PBS, hold at room temperature for 2 hours or at 4°C overnight. Cover plates well to prevent evaporation.
4. PBS Buffer: for 1 L, mix 0.2 g KH_2PO_4, 1.15 g Na_2HPO_4, 0.2 g KCl and 8 g NaCl in approx. 900ml dH_2O. When all reagents have dissolved, adjust the pH to 7.2 with HCl.

Bring total volume to 1 L with dH_2O.

5. PBST Buffer: to 1 L of PBS Buffer, add 1.0 ml Tween-20.
6. TBB - Blocking Buffer: for 1 L, mix 1.21 g TRIS, 8.77 g NaCl, 1 ml TWEEN-20 in approximately 900 ml dH_2O. Adjust pH to 7.2 with HCl. Add 10 g BSA, stir to dissolve. Bring total volume to 1 L with dH_2O. Filter to remove particulate matter.
7. 1% BSA in PBS: To make a 1x working solution, add 10 g BSA to approx. 990 ml PBS buffer, stir to dissolve. Adjust total volume to 1 L with PBS buffer, filter to remove particulate matter.
8. 50 mM Hepes pH 7.5.
9. GST-Flklcd purified from sf9 recombinant baculovirus transformation (SUGEN, Inc.).
10. 4% DMSO in dH_2O.
11. 10 mM ATP in dH_2O.
12. 40 mM $MnCl_2$
13. Kinase Dilution Buffer (KDB): mix 10 ml Hepes (pH 7.5), 1 ml 5M NaCl, 40 µL 100 mM sodium orthovanadate and 0.4 ml of 5% BSA in dH_2O with 88.56 ml dH_2O.
14. NUNC 96-well V bottom polypropylene plates, Applied Scientific Catalog # AS-72092
15. EDTA: mix 14.12 g ethylenediaminetetraacetic acid (EDTA) to approx. 70 ml dH_2O. Add 10 N NaOH until EDTA dissolves. Adjust pH to 8.0. Adjust total volume to 100 ml with dH_2O.
16. 1^0 Antibody Dilution Buffer: mix 10 ml of 5% BSA in PBS buffer with 89.5 ml TBST.
17. Anti-phosphotyrosine monoclonal antibody conjugated to horseradish peroxidase (PY99 HRP, Santa Cruz Biotech).
18. 2,2'-Azinobis(3-ethylbenzthiazoline-6-sulfonic acid (ABTS, Moss, Cat. No. ABST).
19. 10% SDS.

Procedure:

1. Coat Corning 96-well ELISA plates with 2 µg of polyEY peptide in sterile PBS as described in step 3 of Materials and Reagents.
2. Remove unbound liquid from wells by inverting plate. Wash once with TBST. Pat the plate on a paper towel to remove excess liquid.
3. Add 100 µl of 1% BSA in PBS to each well. Incubate, with shaking, for 1 hr. at room temperature.
4. Repeat step 2.
5. Soak wells with 50 mM HEPES (pH7.5) (150 µl/well).
6. Dilute test compound with dH_2O/4% DMSO to 4 times the desired final assay concentration in 96-well polypropylene plates.
7. Add 25 µl diluted test compound to ELISA plate. In control wells, place 25 µl of dH_2O/4% DMSO.
8. Add 25 µl of 40 mM $MnCl_2$ with 4x ATP (2 µM) to each well.
9. Add 25 µl 0.5M EDTA to negative control wells.
10. Dilute GST-Flk1 to 0.005 µg(5 ng)/well with KDB.
11. Add 50 µl of diluted enzyme to each well.
12. Incubate, with shaking, for 15 minutes at room temperature.
13. Stop reaction by adding 50 µl of 250 mM EDTA (pH 8.0).
14. Wash 3X with TBST and pat plate on paper towel to remove excess liquid.
15. Add 100 µl per well anti-phosphotyrosine HRP conjugate, 1:5,000 dilution in antibody dilution buffer. Incubate, with shaking, for 90 min. at room temperature.
16. Wash as in step 14.
17. Add 100 µl of room temperature ABTS solution to each

well.

18. Incubate, with shaking, for 10 to 15 minutes. Remove any bubbles.
19. Stop reaction by adding 20 µl of 10% SDS to each well.
20. Read results on Dynatech MR7000 ELISA reader with test filter at 410 nM and reference filter at 630 nM.

PYK2 BIOASSAY

This assay is used to measure the <u>in vitro</u> kinase activity of HA epitope-tagged full length pyk2 (FL.pyk2-HA) in an ELISA assay.

<u>Materials and reagents</u>:

1. Corning 96-well Elisa plates.
2. 12CA5 monoclonal anti-HA antibody (SUGEN, Inc.)
3. PBS (Dulbecco's Phosphate-Buffered Saline (Gibco Catalog # 450-1300EB)
4. TBST Buffer: for 1 L, mix 8.766 g NaCl, 6.057 g TRIS and 1 ml of 0.1% Triton X-100 in approx. 900 ml dH_2O. Adjust pH to 7.2, bring volume to 1 L.
5. Blocking Buffer: for 1 L, mix 100 g 10% BSA, 12.1 g 100 mM TRIS, 58.44 g 1M NaCl and 10 mL of 1% TWEEN-20.
6. FL.pyk2-HA from sf9 cell lysates (SUGEN, Inc.).
7. 4% DMSO in MilliQue H_2O.
8. 10 mM ATP in dH_2O.
9. 1M $MnCl_2$.
10. 1M $MgCl_2$.
11. 1M Dithiothreitol (DTT).
12. 10X Kinase buffer phosphorylation: mix 5.0 ml 1M Hepes (pH 7.5), 0.2 ml 1M $MnCl_2$, 1.0 ml 1 M $MgCl_2$, 1.0 ml 10% Triton X-100 in 2.8 ml dH_2O. Just prior to use, add 0.1 ml 1M DTT.
13. NUNC 96-well V bottom polypropylene plates.
14. 500 mM EDTA in dH_2O.
15. Antibody dilution buffer: for 100 mL, 1 mL 5% BSA/PBS

and 1 mL 10% Tween-20 in 88 mL TBS.

16. HRP-conjugated anti-Ptyr PY99), Santa Cruz Biotech Cat. No. SC-7020.
17. ABTS, Moss, Cat. No. ABST-2000.
18. 10% SDS.

Procedure:

1. Coat Corning 96 well ELISA plates with 0.5 µg per well 12CA5 anti-HA antibody in 100 µl PBS. Store overnight at 4°C.
2. Remove unbound HA antibody from wells by inverting plate. Wash plate with dH_2O. Pat the plate on a paper towel to remove excess liquid.
3. Add 150 µl Blocking Buffer to each well. Incubate, with shaking, for 30 min at room temperature.
4. Wash plate 4x with TBS-T.
5. Dilute lysate in PBS (1.5 µg lysate/100 µl PBS).
6. Add 100 µl of diluted lysate to each well. Shake at room temperature for 1 hr.
7. Wash as in step 4.
8. Add 50 µl of 2X kinase Buffer to ELISA plate containing captured pyk2-HA.
9. Add 25 µL of 400 µM test compound in 4% DMSO to each well. For control wells use 4% DMSO alone.
10. Add 25 µL of 0.5 M EDTA to negative control wells.
11. Add 25 µl of 20 µM ATP to all wells. Incubate, with shaking, for 10 minutes.
12. Stop reaction by adding 25 µl 500 mM EDTA (pH 8.0) to all wells.
13. Wash as in step 4.
14. Add 100 µL HRP conjugated anti-Ptyr diluted 1:6000 in Antibody Dilution Buffer to each well. Incubate, with shaking, for 1 hr. at room temperature.

15. Wash plate 3X with TBST and 1X with PBS.
16. Add 100 µL of ABST solution to each well.
17. If necessary, stop the development reaction by adding 20 µL 10% SDS to each well.
18. Read plate on ELISA reader with test filter at 410 nM and reference filter at 630 nM.

FGFR1 BIOASSAY

This assay is used to measure the <u>in vitro</u> kinase activity of FGF1-R in an ELISA assay.

<u>Materials and Reagents</u>:

1. Costar 96-well Elisa plates (Corning Catalog # 3369).
2. Poly(Glu-Tyr) (Sigma Catalog # P0275).
3. PBS (Gibco Catalog # 450-1300EB)
4. 50 mM Hepes Buffer Solution.
5. Blocking Buffer (5% BSA/PBS).
6. Purified GST-FGFR1 (SUGEN, Inc.)
7. Kinase Dilution Buffer.
   Mix 500 µl 1M Hepes (GIBCO), 20 µl 5% BSA/PBS, 10 µl 100mM sodium orthovanadate and 50 µl 5M NaCl.
8. 10mM ATP
9. ATP/$MnCl_2$ phosphorylation mix: mix 20 µL ATP, 400 µL 1M $MnCl_2$ and 9.56 ml dH_2O.
10. NUNC 96-well V bottom polypropylene plates (Applied Scientific Catalog # AS-72092).
11. 0.5M EDTA.
12. 0.05% TBST
    Add 500 µL TWEEN to 1 liter TBS.
13. Rabbit polyclonal anti-phosphotyrosine serum (SUGEN, Inc.).
14. Goat anti-rabbit IgG peroxidase conjugate (Biosource, Catalog # ALI0404).
15. ABTS Solution.
16. ABTS/H_2O_2 solution.

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Procedure:

1. Coat Costar 96 well ELISA plates with 1 µg per well Poly(Glu,Tyr) in 100µl PBS. Store overnight at 4° C.
2. Wash coated plates once with PBS.
3. Add 150 µL of 5%BSA/PBS Blocking Buffer to each well. Incubate, with shaking, for 1 hr.room temperature.
4. Wash plate 2x with PBS, then once with 50mM Hepes. Pat plates on a paper towel to remove excess liquid and bubbles.
5. Add 25µL of 0.4 mM test compound in 4% DMSO or 4% DMSO alone (controls) to plate.
6. Dilute purified GST-FGFR1 in Kinase Dilution Buffer (5 ng kinase/50ul KDB/well).
7. Add 50µL of diluted kinase to each well.
8. Start kinase reaction by adding 25µl/well of freshly prepared ATP/Mn++ (0.4 ml 1M $MnCl_2$, 40 µL 10 mM ATP, 9.56 ml dH_2O), freshly prepared).
9. This is a fast kinase reaction and must be stopped with 25µL of 0.5M EDTA in a manner similar to the addition of ATP.
10. Wash plate 4x with fresh TBST.
11. Make up Antibody Dilution Buffer: Per 50 ml: Mix 5 ml of 5% BSA, 250 µl of 5% milk and 50 µl of 100mM sodium vanadate, bring to final volume with 0.05% TBST.
12. Add 100 µl per well of anti-phosphotyrosine (1:10000 dilution in ADB). Incubate, with shaking for 1 hr. at room temperature.
13. Wash as in step 10.
14. Add 100 µl per well of Biosource Goat anti-rabbit IgG peroxidase conjugate (1:6000 dilution in ADB). Incubate, with shaking for 1 hr. at room temperature.

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15. Wash as in step 10 and then with PBS to remove bubbles and excess TWEEN.
16. Add 100 µl of ABTS/H_2O_2 solution to each well.
17. Incubate, with shaking, for 10 to 20 minutes. Remove any bubbles.
18. Read assay on Dynatech MR7000 elisa reader: test filter at 410 nM, reference filtrate 630 nM.

EGFR BIOASSAY

This assay is used to the <u>in vitro</u> kinase activity of FGF1-R in an ELISA assay.

<u>Materials and Reagents</u>:

1. Corning 96-well Elisa plates.
2. SUMO1 monoclonal anti-EGFR antibody (SUGEN, Inc.).
3. PBS
4. TBST Buffer
5. Blocking Buffer: for 100 ml, mix 5.0 g Carnation Instant Non-fat Milk® with 100 ml of PBS.
6. A431 cell lysate (SUGEN, Inc.).
7. TBS Buffer:
8. TBS + 10% DMSO: for 1L, mix 1.514 g TRIS, 2.192 g NaCl and 25 ml DMSO; bring to 1 liter total volume with dH_2O.
9. ATP (Adenosine-5'-triphosphate, from Equine muscle, Sigma Cat. No. A-5394), 1.0 mM solution in dH_2O. This reagent should be made up immediately prior to use and kept on ice.
10. 1.0 mM $MnCl_2$.
11. ATP/$MnCl_2$ phosphorylation mix: to make 10 ml, mix 300 µl of 1 mM ATP, 500 µl $MnCl_2$ and 9.2 ml dH_2O. Prepare just prior to use, keep on ice.
12. NUNC 96-well V bottom polypropylene plates.
13. EDTA.

14. Rabbit polyclonal anti-phosphotyrosine serum (SUGEN, Inc.).
15. Goat anti-rabbit IgG peroxidase conjugate (Biosource Cat. No. ALI0404).
16. ABTS.
17. 30% Hydrogen peroxide.
18. ABTS/H_2O_2.
19. 0.2 M HCl.

Procedure:

1. Coat Corning 96 well ELISA plates with 0.5 µg SUMO1 in 100 µl PBS per well, store overnight at 4° C.
2. Remove unbound SUMO1 from wells by inverting plate to remove liquid. Wash 1x with dH_2O. Pat the plate on a paper towel to remove excess liquid.
3. Add 150 µl of Blocking Buffer to each well. Incubate, with shaking, for 30 min. at room temperature.
4. Wash plate 3x with deionized water, then once with TBST. Pat plate on a paper towel to remove excess liquid and bubbles.
5. Dilute lysate in PBS (7 µg lysate/100 µl PBS).
6. Add 100 µl of diluted lysate to each well. Shake at room temperature for 1 hr.
7. Wash plates as in 4, above.
8. Add 120 µl TBS to ELISA plate containing captured EGFR.
9. Dilute test compound 1:10 in TBS, place in well
10. Add 13.5 µl diluted test compound to ELISA plate. To control wells, add 13.5 µl TBS in 10% DMSO.
11. Incubate, with shaking, for 30 minutes at room temperature.
12. Add 15 µl phosphorylation mix to all wells except negative control well. Final well volume should be approximately 150 µl with 3 µM ATP/5 mM $MnCl_2$ final

concentration in each well. Incubate with shaking for 5 minutes.

13. Stop reaction by adding 16.5 µl of EDTA solution while shaking. Shake for additional 1 min.
14. Wash 4x with deionized water, 2x with TBST.
15. Add 100 µl anti-phosphotyrosine (1:3000 dilution in TBST) per well. Incubate, with shaking, for 30-45 min. at room temperature.
16. Wash as in 4, above.
17. Add 100 µl Biosource Goat anti-rabbit IgG peroxidase conjugate (1:2000 dilution in TBST) to each well. Incubate with shaking for 30 min. at room temperature.
18. Wash as in 4, above.
19. Add 100 µl of ABTS/H_2O_2 solution to each well.
20. Incubate 5 to 10 minutes with shaking. Remove any bubbles.
21. If necessary, stop reaction by adding 100 µl 0.2 M HCl per well.
22. Read assay on Dynatech MR7000 ELISA reader: test filter at 410 nM, reference filter at 630 nM.

PDGFR BIOASSAY

This assay is used to the <u>in vitro</u> kinase activity of FGF1-R in an ELISA assay.

<u>Materials and Reagents</u>:

1. Corning 96-well Elisa plates
2. 28D4C10 monoclonal anti-PDGFR antibody (SUGEN, Inc.).
3. PBS.
4. TBST Buffer.
5. Blocking Buffer (same as for EGFR bioassay).
6. PDGFR-β expressing NIH 3T3 cell lysate (SUGEN, Inc.).
7. TBS Buffer.
8. TBS + 10% DMSO.
9. ATP.

10. $MnCl_2$.

11. Kinase buffer phosphorylation mix: for 10 ml, mix 250 µl 1M TRIS, 200 µl 5M NaCl, 100 µl 1M $MnCl_2$ and 50µl 100 mM Triton X-100 in enough dH_2O to make 10 ml.

12. NUNC 96-well V bottom polypropylene plates.

13. EDTA.

14. Rabbit polyclonal anti-phosphotyrosine serum (SUGEN,Inc.).

15. Goat anti-rabbit IgG peroxidase conjugate (Biosource Cat. No. ALI0404).

16. ABTS.

17. Hydrogen peroxide, 30% solution.

18. ABTS/H_2O_2.

19. 0.2 M HCl.

Procedure:

1. Coat Corning 96 well ELISA plates with 0.5 µg 28D4C10 in 100 µl PBS per well, store overnight at 4° C.

2. Remove unbound 28D4C10 from wells by inverting plate to remove liquid. Wash 1x with dH_2O. Pat the plate on a paper towel to remove excess liquid.

3. Add 150 µl of Blocking Buffer to each well. Incubate for 30 min. at room temperature with shaking.

4. Wash plate 3x with deionized water, then once with TBST. Pat plate on a paper towel to remove excess liquid and bubbles.

5. Dilute lysate in HNTG (10 µg lysate/100 µl HNTG).

6. Add 100 µl of diluted lysate to each well. Shake at room temperature for 60 min.

7. Wash plates as described in Step 4.

8. Add 80 µl working kinase buffer mix to ELISA plate containing captured PDGFR.

9. Dilute test compound 1:10 in TBS in 96-well polypropylene plates.

10. Add 10 µl diluted test compound to ELISA plate. To control wells, add 10 µl TBS + 10% DMSO. Incubate with shaking for 30 minutes at room temperature.
11. Add 10 µl ATP directly to all wells except negative control well (final well volume should be approximately 100 µl with 20 µM ATP in each well.) Incubate 30 minutes with shaking.
12. Stop reaction by adding 10 µl of EDTA solution to each well.
13. Wash 4x with deionized water, twice with TBST.
14. Add 100 µl anti-phosphotyrosine (1:3000 dilution in TBST) per well. Incubate with shaking for 30-45 min. at room temperature.
15. Wash as in Step 4.
16. Add 100 µl Biosource Goat anti-rabbit IgG peroxidase conjugate (1:2000 dilution in TBST) to each well. Incubate with shaking for 30 min. at room temperature.
17. Wash as in Step 4.
18. Add 100 µl of ABTS/H_2O_2 solution to each well.
19. Incubate 10 to 30 minutes with shaking. Remove any bubbles.
20. If necessary stop reaction with the addition of 100 µl 0.2 M HCl per well.
21. Read assay on Dynatech MR7000 ELISA reader with test filter at 410 nM and reference filter at 630 nM.

CELLULAR HER-2 KINASE ASSAY

This assay is used to measure HER-2 kinase activity in whole cells in an ELISA format.

Materials and Reagents:

1. DMEM (GIBCO Catalog #11965-092).
2. Fetal Bovine Serum (FBS, GIBCO Catalog #16000-044), heat inactivated in a water bath for 30 min. at 56° C

3. Trypsin (GIBCO Catalog #25200-056).
4. L-Glutamine (GIBCO Catalog #25030-081)
5. HEPES (GIBCO Catalog #15630-080).
6. Growth Media
   Mix 500 ml DMEM, 55 ml heat inactivated FBS, 10 ml HEPES and 5.5 ml L-Glutamine.
7. Starve Media
   Mix 500 ml DMEM, 2.5 ml heat inactivated FBS, 10 ml HEPES and 5.5 ml L-Glutamine.
8. PBS.
9. Flat Bottom 96-well Tissue Culture Micro Titer Plates (Corning Catalog # 25860).
10. 15 cm Tissue Culture Dishes (Corning Catalog #08757148).
11. Corning 96-well ELISA Plates.
12. NUNC 96-well V bottom polypropylene plates.
13. Costar Transfer Cartridges for the Transtar 96 (Costar Catalog #7610).
14. SUMO 1: monoclonal anti-EGFR antibody (SUGEN, Inc.).
15. TBST Buffer.
16. Blocking Buffer : 5% Carnation Instant Milk® in PBS.
17. EGF Ligand: EGF-201, Shinko American, Japan. Suspend powder in 100 uL of 10mM HCl. Add 100uL 10mM NaOH. Add 800 uL PBS and transfer to an Eppendorf tube, store at -20°C until ready to use.
18. HNTG Lysis Buffer
    For Stock 5X HNTG, mix 23.83 g Hepes, 43.83 g NaCl, 500 ml glycerol and 100 ml Triton X-100 and enough dH_2O to make 1 L of total solution.
    For 1X HNTG*, mix 2 ml HNTG, 100 µL 0.1M Na_3VO_4, 250 µL 0.2M $Na_4P_2O_7$ and 100 µL EDTA.
19. EDTA.

20. Na_3VO_4. To make stock solution, mix 1.84 g Na_3VO_4 with 90 ml dH_2O. Adjust pH to 10. Boil in microwave for one minute (solution becomes clear). Cool to room temperature. Adjust pH to 10. Repeat heating/cooling cycle until pH remains at 10.

21. 200 mM $Na_4P_2O_7$.

22. Rabbit polyclonal antiserum specific for phosphotyrosine (anti-Ptyr antibody, SUGEN, Inc.).

23. Affinity purified antiserum, goat anti-rabbit IgG antibody, peroxidase conjugate (Biosource Cat # ALI0404).

24. ABTS Solution.

25. 30 % Hydrogen peroxide solution.

26. ABTS/H_2O_2.

27. 0.2 M HCl.

Procedure:

1. Coat Corning 96 well ELISA plates with SUMO1 at 1.0 ug per well in PBS, 100 ul final volume/well. Store overnight at 4°C.

2. On day of use, remove coating buffer and wash plate 3 times with dH_2O and once with TBST buffer. All washes in this assay should be done in this manner, unless otherwise specified.

3. Add 100 ul of Blocking Buffer to each well. Incubate plate, with shaking, for 30 min. at room temperature. Just prior to use, wash plate.

4. Use EGFr/HER-2 chimera/3T3-C7 cell line for this assay.

5. Choose dishes having 80-90 % confluence. Collect cells by trypsinization and centrifuge at 1000 rpm at room temperature for 5 min.

6. Resuspend cells in starve medium and count with trypan blue. Viability above 90% is required. Seed cells in starve medium at a density of 2,500 cells per well, 90

ul per well, in a 96 well microtiter plate. Incubate seeded cells overnight at 37° under 5% CO_2.

7. Start the assay two days after seeding.
8. Test compounds are dissolved in 4% DMSO. Samples are then further diluted directly on plates with starve-DMEM. Typically, this dilution will be 1:10 or greater. All wells are then transferred to the cell plate at a further 1:10 dilution (10µl sample and media into 90 µl of starve media. The final DMSO concentration should be 1% or lower. A standard serial dilution may also be used.
9. Incubate under 5% CO_2 at 37°C for 2 hours.
10. Prepare EGF ligand by diluting stock EGF (16.5 uM) in warm DMEM to 150 nM.
11. Prepare fresh HNTG* sufficient for 100 ul per well; place on ice.
12. After 2 hour incubation with test compound, add prepared EGF ligand to cells, 50 ul per well, for a final concentration of 50 nM. Positive control wells receive the same amount of EGF. Negative controls do not receive EGF. Incubate at 37° C for 10 min.
13. Remove test compound, EGF, and DMEM. Wash cells once with PBS.
14. Transfer HNTG* to cells, 100 ul per well. Place on ice for 5 minutes. Meanwhile, remove blocking buffer from ELISA plate and wash.
15. Scrape cells from plate with a micropipettor and homogenize cell material by repeatedly aspirating and dispensing the HNTG* lysis buffer. Transfer lysate to a coated, blocked, washed ELISA plate. Or, use a Costar transfer cartridge to transfer lysate to the plate.
16. Incubate, with shaking, at room temperature for 1 hr.
17. Remove lysate, wash. Transfer freshly diluted anti-Ptyr

antibody (1:3000 in TBST) to ELISA plate, 100 ul per well.

18. Incubate, with shaking, at room temperature, for 30 min.

19. Remove anti-Ptyr antibody, wash. Transfer freshly diluted BIOSOURCE antibody to ELISA plate(1:8000 in TBST, 100 ul per well).

20. Incubate, with shaking, at room temperature for 30 min.

21. Remove BIOSOURCE antibody, wash. Transfer freshly prepared ABTS/H_2O_2 solution to ELISA plate, 100 ul per well.

22. Incubate, with shaking, for 5-10 minutes. Remove any bubbles.

23. Stop reaction with the addition of 100ul of 0.2M HCl per well.

24. Read assay on Dynatech MR7000 ELISA reader with test filter set at 410 nM and reference filter at 630 nM.

CDK2/CYCLIN A ASSAY

This assay is used to measure the <u>in vitro</u> serine/threonine kinase activity of human cdk2/cyclin A in a Scintillation Proximity Assay (SPA).

<u>Materials and Reagents</u>.

1. Wallac 96-well polyethylene terephthalate (flexi) plates (Wallac Catalog # 1450-401).

2. Amersham Redivue [γ^{33}P] ATP (Amersham catalog #AH 9968).

3. Amersham streptavidin coated polyvinyltoluene SPA beads (Amersham catalog #RPNQ0007). The beads should be reconstituted in PBS without magnesium or calcium, at 20 mg/ml.

4. Activated cdk2/cyclin A enzyme complex purified from Sf9 cells (SUGEN, Inc.).

5. Biotinylated peptide substrate (Debtide). Peptide

biotin-X-PKTPKKAKKL is dissolved in dH_2O at a concentration of 5 mg/ml.

6. Peptide/ATP Mixture: for 10 ml, mix 9.979 ml dH_2O, 0.00125 ml "cold" ATP, 0.010 ml Debtide and 0.010 ml $\gamma^{33}P$ ATP. The ultimate concentration per well will be 0.5 µM "cold" ATP, 0.1 µg Debtide and 0.2 µCi $\gamma^{33}P$ ATP.
7. Kinase buffer: for 10 ml, mix 8.85 ml dH_2O, 0.625 ml TRIS(pH 7.4), 0.25 ml 1M $MgCl_2$, 0.25 ml 10% NP40 and 0.025 ml 1M DTT, added fresh just prior to use.
8. 10 mM ATP in dH_2O.
9. 1M Tris, pH adjusted to 7.4 with HCl.
10. 1M $MgCl_2$.
11. 1M DTT.
12. PBS (Gibco Catalog # 14190-144).
13. 0.5M EDTA.
14. Stop solution: For 10 ml, mix 9.25 ml PBS, 0.005 ml 100 mM ATP, 0.1 ml 0.5 M EDTA, 0.1 ml 10% Triton X-100 and 1.25 ml of 20 mg/ml SPA beads.

Procedure:

1. Prepare solutions of test compounds at 5x the desired final concentration in 5% DMSO. Add 10 ul to each well. For negative controls, use 10 ul 5% DMSO alone in wells.
2. Dilute 5 µL of cdk2/cyclin A solution with 2.1 ml 2x kinase buffer.
3. Add 20 ul enzyme to each well.
4. Add 10 µL of 0.5 M EDTA to the negative control wells.
5. To start kinase reaction, add 20 µL of peptide/ATP mixture to each well. Incubate for 1 hr. without shaking.
6. Add 200 µl stop solution to each well.
7. Hold at least 10 min.
8. Spin plate at approx. 2300 rpm for 3-5 min.

9. Count plate using Trilux or similar reader.

MET TRANSPHOSPHORYLATION ASSAY

This assay is used to measure phosphotyrosine levels on a poly(glutamic acid:tyrosine (4:1)) substrate as a means for identifying agonists/antagonists of met transphosphorylation of the substrate.

Materials and Reagents:

1. Corning 96-well Elisa plates, Corning Catalog # 25805-96.
2. Poly(glu, tyr) 4:1, Sigma, Cat. No; P 0275.
3. PBS, Gibco Catalog # 450-1300EB
4. 50 mM HEPES
5. Blocking Buffer: Dissolve 25 g Bovine Serum Albumin, Sigma Cat. No A-7888, in 500 ml PBS, filter through a 4 µm filter.
6. Purified GST fusion protein containing the Met kinase domain, Sugen, Inc.
7. TBST Buffer.
8. 10% aqueous (MilliQue H_2O) DMSO.
9. 10 mM aqueous (dH_2O) Adenosine-5'-triphosphate, Sigma Cat. No. A-5394.
10. 2X Kinase Dilution Buffer: for 100 ml, mix 10 mL 1M HEPES at pH 7.5 with 0.4 mL 5% BSA/PBS, 0.2 mL 0.1 M sodium orthovanadate and 1 mL 5M sodium chloride in 88.4 mL dH_2O.
11. 4X ATP Reaction Mixture: for 10 mL, mix 0.4 mL 1 M manganese chloride and 0.02 mL 0.1 M ATP in 9.56 mL dH_2O.
12. 4X Negative Controls Mixture: for 10 mL, mix 0.4 mL 1 M manganese chloride in 9.6 mL dH_2O.
13. NUNC 96-well V bottom polypropylene plates, Applied Scientific Catalog # S-72092
14. 500 mM EDTA.

15. Antibody Dilution Buffer: for 100 mL, mix 10 mL 5% BSA/PBS, 0.5 mL 5% Carnation Instant Milk® in PBS and 0.1 mL 0.1 M sodium orthovanadate in 88.4 mL TBST.

16. Rabbit polyclonal antophosphotyrosine antibody, Sugen, Inc.

17. Goat anti-rabbit horseradish peroxidase conjugated antibody, Biosource, Inc.

18. ABTS Solution: for 1 L, mix 19.21 g citric acid, 35.49 g Na_2HPO_4 and 500 mg ABTS with sufficient dH_2O to make 1 L.

19. ABTS/H_2O_2: mix 15 mL ABST solution with 2µL H_2O_2 five minutes before use.

20. 0.2 M HCl

Procedure:

1. Coat ELISA plates with 2 µg Poly(Glu-Tyr) in 100 µL PBS, store overnight at 4 ° C.
2. Block plate with 150 µL of 5% BSA / PBS for 60 min.
3. Wash plate twice with PBS, once with 50 mM Hepes buffer pH 7.4.
4. Add 50 µl of the diluted kinase to all wells. (Purified kinase is diluted with Kinase Dilution Buffer. Final concentration should be 10 ng/well.)
5. Add 25 µL of the test compound (in 4% DMSO) or DMSO alone (4% in dH_2O) for controls to plate.
6. Incubate the kinase/compound mixture for 15 minutes.
7. Add 25 µL of 40 mM $MnCl_2$ to the negative control wells.
8. Add 25 µL ATP/ $MnCl_2$ mixture to the all other wells (except the negative controls). Incubate for 5 min.
9. Add 25 µL 500 mM EDTA to stop reaction.
10. Wash plate 3x with TBST.
11. Add 100 µL rabbit polyclonal anti-Ptyr diluted 1:10,000 in Antibody Dilution Buffer to each well. Incubate, with shaking, at room temperature for one hour.

12. Wash plate 3x with TBST.
13. Dilute Biosource HRP conjugated anti-rabbit antibody 1: 6,000 in Antibody Dilution buffer. Add 100 µL per well and incubate at room temperature, with shaking, for one hour.
14. Wash plate 1X with PBS.
15. Add 100 µl of ABTS/H_2O_2 solution to each well.
16. If necessary, stop the development reaction with the addition of 100 µl of 0.2M HCl per well.
17. Read plate on Dynatech MR7000 elisa reader with the test filter at 410 nM and the reference filter at 630 nM.

IGF-1 TRANSPHOSPHORYLATION ASSAY

This assay is used to measure the phosphotyrosine level in poly(glutamic acid:tyrosine)(4:1) for the identification of agonists/antagonists of gst-IGF-1 transphosphorylation of a substrate.

Materials and Reagents:

1. Corning 96-well Elisa plates.
2. Poly (Glu-tyr) (4:1), Sigma Cat. No. P 0275.
3. PBS, Gibco Catalog # 450-1300EB.
4. 50 mM HEPES
5. TBB Blocking Buffer: for 1 L, mix 100 g BSA, 12.1 gTRIS (pH 7.5), 58.44 g sodium chloride and 10 mL 1%TWEEN-20.
6. Purified GST fusion protein containing the IGF-1 kinase domain (Sugen, Inc.)
7. TBST Buffer: for 1 L, mix 6.057 g Tris, 8.766 g sodium chloride and 0.5 ml TWEEN-20 with enough dH_2O to make 1 liter.
8. 4% DMSO in Milli-Q H_2O.
9. 10 mM ATP in dH_2O.
10. 2X Kinase Dilution Buffer: for 100 mL, mix 10 mL 1 M HEPES (pH 7.5), 0.4 mL 5% BSA in dH_2O, 0.2 mL 0.1 M

sodium orthovanadate and 1 mL 5 M sodium chloride with enough dH_2O to make 100 mL.

11. 4X ATP Reaction Mixture: for 10 mL, mix 0.4 mL 1 M $MnCl_2$ and 0.008 mL 0.01 M ATP and 9.56 mL dH_2O.
12. 4X Negative Controls Mixture: mix 0.4 mL 1 M manganese chloride in 9.60 mL dH_2O.
13. NUNC 96-well V bottom polypropylene plates.
14. 500 mM EDTA in dH_2O.
15. Antibody Dilution Buffer: for 100 mL, mix 10 mL 5% BSA in PBS, 0.5 mL 5% Carnation Instant Non-fat Milk® in PBS and 0.1 mL 0.1 M sodium orthovanadate in 88.4 mL TBST.
16. Rabbit Polyclonal antiphosphotyrosine antibody, Sugen, Inc.
17. Goat anti-rabbit HRP conjugated antibody, Biosource.
18. ABTS Solution.
20. ABTS/H_2O_2: mix 15 mL ABTS with 2 µL H_2O_2 5 minutes before using.
21. 0.2 M HCl in dH_2O.

Procedure:

1. Coat ELISA plate with 2.0 µg / well Poly(Glu, Tyr) 4:1 (Sigma P0275) in 100 µl PBS. Store plate overnight at 4° C.
2. ash plate once with PBS.
3. Add 100 µl of TBB Blocking Buffer to each well. Incubate plate for 1 hour with shaking at room temperature.
4. Wash plate once with PBS, then twice with 50 mM Hepes buffer pH 7.5.
5. Add 25 µL of test compound in 4% DMSO (obtained by diluting a stock solution of 10 mM test compound in 100% DMSO with dH_2O) to plate.

6. Add 10.0 ng of gst-IGF-1 kinase in 50 µl Kinase Dilution Buffer) to all wells.
7. Start kinase reaction by adding 25µl 4X ATP Reaction Mixture to all test wells and positive control wells. Add 25µl 4X Negative Controls Mixture to all negative control wells. Incubates for 10 minutes with shaking at room temperature.
8. Add 25µl 0.5M EDTA (pH 8.0) to all wells.
9. Wash plate 4x with TBST Buffer.
10. Add rabbit polyclonal anti-phosphotyrosine antisera at a dilution of 1:10,000 in 100µl Antibody Dilution Buffer to all wells. Incubate, with shaking, at room temperature for 1 hour.
11. Wash plate as in step 9.
12. Add 100 µL Biosource anti-rabbit HRP at a dilution of 1:10,000 in Antibody dilution buffer to all wells. Incubate, with shaking, at room temperature for 1 hour.
13. Wash plate as in step 9, follow with one wash with PBS to reduce bubbles and excess Tween-20.
14. Develop by adding 100µl/well ABTS/H_2O_2 to each well.
15. After about 5 minutes, read on ELISA reader with test filter at 410 nm and referenced filter at 630 nm.

BRDU INCORPORATION ASSAYS

The following assays use cells engineered to express a selected receptor and then evaluate the effect of a compound of interest on the activity of ligand-induced DNA synthesis by determining BrdU incorporation into the DNA.

The following materials, reagents and procedure are general to each of the following BrdU incorporation assays. Variances in specific assays are noted.

Materials and Reagents:

1. The appropriate ligand.

2. The appropriate engineered cells.

3. BrdU Labeling Reagent: 10 mM, in PBS (pH 7.4)(Boehringer Mannheim, Germany).

4. FixDenat: fixation solution (ready to use)(Boehringer Mannheim, Germany).

5. Anti-BrdU-POD: mouse monoclonal antibody conjugated with peroxidase (Boehringer Mannheim, Germany).

6. TMB Substrate Solution: tetramethylbenzidine (TMB, Boehringer Mannheim, Germany).

7. PBS Washing Solution : 1X PBS, pH 7.4.

8. Albumin, Bovine (BSA), fraction V powder (Sigma Chemical Co., USA).

General Procedure:

1. Cells are seeded at 8000 cells/well in 10% CS, 2 mM Gln in DMEM, in a 96 well plate. Cells are incubated overnight at 37°C in 5% CO_2.

2. After 24 hours, the cells are washed with PBS, and then are serum-starved in serum free medium (0% CS DMEM with 0.1% BSA) for 24 hours.

3. On day 3, the appropriate ligand and the test compound are added to the cells simultaneously. The negative control wells receive serum free DMEM with 0.1% BSA only; the positive control cells receive the ligand but no test compound. Test compounds are prepared in serum free DMEM with ligand in a 96 well plate, and serially diluted for 7 test concentrations.

4. After 18 hours of ligand activation, diluted BrdU labeling reagent (1:100 in DMEM, 0.1% BSA) is added and the cells are incubated with BrdU (final concentration=10 µM) for 1.5 hours.

5. After incubation with labeling reagent, the medium is removed by decanting and tapping the inverted plate on a paper towel. FixDenat solution is added (50 µl/well)

and the plates are incubated at room temperature for 45 minutes on a plate shaker.

6. The FixDenat solution is thoroughly removed by decanting and tapping the inverted plate on a paper towel. Milk is added (5% dehydrated milk in PBS, 200 µl/well) as a blocking solution and the plate is incubated for 30 minutes at room temperature on a plate shaker.
7. The blocking solution is removed by decanting and the wells are washed once with PBS. Anti-BrdU-POD solution (1:200 dilution in PBS, 1% BSA) is added (50 µl/well) and the plate is incubated for 90 minutes at room temperature on a plate shaker.
8. The antibody conjugate is thoroughly removed by decanting and rinsing the wells 5 times with PBS, and the plate is dried by inverting and tapping on a paper towel.
9. TMB substrate solution is added (100 µl/well) and incubated for 20 minutes at room temperature on a plate shaker until color development is sufficient for photometric detection.
10. The absorbance of the samples are measured at 410 nm (in "dual wavelength" mode with a filter reading at 490 nm, as a reference wavelength) on a Dynatech ELISA plate reader.

EGF-Induced BrdU Incorporation Assay

Materials and Reagents:

1. Mouse EGF, 201 (Toyobo Co., Ltd., Japan).
2. 3T3/EGFRc7.

EGF-Induced Her-2-driven BrdU Incorporation Assay

Materials and Reagents:

1. Mouse EGF, 201 (Toyobo Co., Ltd., Japan).
2. 3T3/EGFr/Her2/EGFr (EGFr with a Her-2 kinase domain).

EGF-Induced Her-4-driven BrdU Incorporation Assay

Materials and Reagents:

1. Mouse EGF, 201 (Toyobo Co., Ltd., Japan).
2. 3T3/EGFr/Her4/EGFr (EGFr with a Her-4 kinase domain).

PDGF-Induced BrdU Incorporation Assay

Materials and Reagents:

1. Human PDGF B/B (Boehringer Mannheim, Germany).
2. 3T3/EGFRc7.

FGF-Induced BrdU Incorporation Assay

Materials and Reagents:

1. Human FGF2/bFGF (Gibco BRL, USA).
2. 3T3c7/EGFr

IGF1-Induced BrdU Incorporation Assay

Materials and Reagents:

1. Human, recombinant (G511, Promega Corp., USA)
2. 3T3/IGF1r.

Insulin-Induced BrdU Incorporation Assay

Materials and Reagents:

1. Insulin, crystalline, bovine, Zinc (13007, Gibco BRL, USA).
2. 3T3/H25.

HGF-Induced BrdU Incorporation Assay

Materials and Reagents:

1. Recombinant human HGF (Cat. No. 249-HG, R&D Systems, Inc. USA).
2. BxPC-3 cells (ATCC CRL-1687).

Procedure:

1. Cells are seeded at 9000 cells/well in RPMI 10% FBS in a 96 well plate. Cells are incubated overnight at 37°C in 5% CO_2.
2. After 24 hours, the cells are washed with PBS, and then are serum starved in 100 µl serum-free medium (RPMI with 0.1% BSA) for 24 hours.

3. On day 3, 25 µl containing ligand (prepared at 1 µg/ml in RPMI with 0.1% BSA; final HGF conc. is 200 ng/ml) and test compounds are added to the cells. The negative control wells receive 25 µl serum-free RPMI with 0.1% BSA only; the positive control cells receive the ligand (HGF) but no test compound. Test compounds are prepared at 5 times their final concentration in serum-free RPMI with ligand in a 96 well plate, and serially diluted to give 7 test concentrations. Typically, the highest final concentration of test compound is 100 µM, and 1:3 dilutions are used (i.e. final test compound concentration range is 0.137-100 µM).

4. After 18 hours of ligand activation, 12.5 µl of diluted BrdU labeling reagent (1:100 in RPMI, 0.1% BSA) is added to each well and the cells are incubated with BrdU (final concentration is 10 µM) for 1 hour.

5. Same as General Procedure.

6. Same as General Procedure.

7. The blocking solution is removed by decanting and the wells are washed once with PBS. Anti-BrdU-POD solution (1:100 dilution in PBS, 1% BSA) is added (100 µl/well) and the plate is incubated for 90 minutes at room temperature on a plate shaker.

8. Same as General Procedure.

9. Same as General Procedure.

10. Same as General Procedure.

HUV-EC-C Assay

This assay is used to measure a compound's activity against PDGF-R, FGF-R, VEGF, aFGF or Flk-1/KDR, all of which are naturally expressed by HUV-EC cells.

DAY 0

1. Wash and trypsinize HUV-EC-C cells (human umbilical vein endothelial cells, (American Type Culture Collection, catalogue no. 1730 CRL). Wash with Dulbecco's phosphate-buffered saline (D-PBS, obtained from Gibco BRL, catalogue no. 14190-029) 2 times at about 1 ml/10 cm^2 of tissue culture flask. Trypsinize with 0.05% trypsin-EDTA in non-enzymatic cell dissociation solution (Sigma Chemical Company, catalogue no. C-1544). The 0.05% trypsin is made by diluting 0.25% trypsin/1 mM EDTA (Gibco, catalogue no. 25200-049) in the cell dissociation solution. Trypsinize with about 1 ml/25-30 cm^2 of tissue culture flask for about 5 minutes at 37°C. After cells have detached from the flask, add an equal volume of assay medium and transfer to a 50 ml sterile centrifuge tube (Fisher Scientific, catalogue no. 05-539-6).

2. Wash the cells with about 35 ml assay medium in the 50 ml sterile centrifuge tube by adding the assay medium, centrifuge for 10 minutes at approximately 200x g, aspirate the supernatant, and resuspend with 35 ml D-PBS. Repeat the wash two more times with D-PBS, resuspend the cells in about 1 ml assay medium/15 cm^2 of tissue culture flask. Assay medium consists of F12K medium (Gibco BRL, catalogue no. 21127-014) and 0.5% heat-inactivated fetal bovine serum. Count the cells with a Coulter Counter® (Coulter Electronics, Inc.) and add assay medium to the cells to obtain a concentration of 0.8-1.0 x 10^5 cells/ml.

3. Add cells to 96-well flat-bottom plates at 100 µl/well or 0.8-1.0 x 10^4 cells/well, incubate ~24h at 37°C, 5% CO_2.

DAY 1

1. Make up two-fold test compound titrations in separate 96-well plates, generally 50 µM on down to 0 µM. Use the same assay medium as mentioned in day 0, step 2 above. Titrations are made by adding 90 µl/well of test compound at 200 µM (4X the final well concentration) to the top well of a particular plate

column. Since the stock test compound is usually 20 mM in DMSO, the 200 µM drug concentration contains 2% DMSO.

A diluent made up to 2% DMSO in assay medium (F12K + 0.5% fetal bovine serum) is used as diluent for the test compound titrations in order to dilute the test compound but keep the DMSO concentration constant. Add this diluent to the remaining wells in the column at 60 µl/well. Take 60 µl from the 120 µl of 200 µM test compound dilution in the top well of the column and mix with the 60 µl in the second well of the column. Take 60 µl from this well and mix with the 60 µl in the third well of the column, and so on until two-fold titrations are completed. When the next-to-the-last well is mixed, take 60 µl of the 120 µl in this well and discard it. Leave the last well with 60 µl of DMSO/media diluent as a non-test compound-containing control. Make 9 columns of titrated test compound, enough for triplicate wells each for: (1) VEGF (obtained from Pepro Tech Inc., catalogue no. 100-200, (2) endothelial cell growth factor (ECGF) (also known as acidic fibroblast growth factor, or aFGF) (obtained from Boehringer Mannheim Biochemica, catalogue no. 1439 600), or, (3) human PDGF B/B (1276-956, Boehringer Mannheim, Germany) and assay media control. ECGF comes as a preparation with sodium heparin.

2. Transfer 50 µl/well of the test compound dilutions to the 96-well assay plates containing the $0.8\text{-}1.0\times10^4$ cells/100 µl/well of the HUV-EC-C cells from day 0 and incubate ~2 h at 37° C, 5% CO_2.

3. In triplicate, add 50 µl/well of 80 µg/ml VEGF, 20 ng/ml ECGF, or media control to each test compound condition. As with the test compounds, the growth factor concentrations are 4X the desired final concentration. Use the assay media from day 0 step 2 to make the concentrations of growth factors. Incubate approximately 24 hours at 37°C, 5% CO_2. Each well will have 50

µl test compound dilution, 50 µl growth factor or media, and 100 µl cells, which calculates to 200 µl/well total. Thus the 4X concentrations of test compound and growth factors become 1X once everything has been added to the wells.

DAY 2

1. Add ^{3}H-thymidine (Amersham, catalogue no. TRK-686) at 1 µCi/well (10 µl/well of 100 µCi/ml solution made up in RPMI media + 10% heat-inactivated fetal bovine serum) and incubate ~24 h at 37°C, 5% CO_2. RPMI is obtained from Gibco BRL, catalogue no. 11875-051.

DAY 3

1. Freeze plates overnight at -20°C.

DAY 4

Thaw plates and harvest with a 96-well plate harvester (Tomtec Harvester 96®) onto filter mats (Wallac, catalogue no. 1205-401), read counts on a Wallac Betaplate™ liquid scintillation counter.

IN VIVO ANIMAL MODELS

XENOGRAFT ANIMAL MODELS

The ability of human tumors to grow as xenografts in athymic mice (e.g., Balb/c, nu/nu) provides a useful in vivo model for studying the biological response to therapies for human tumors. Since the first successful xenotransplantation of human tumors into athymic mice, (Rygaard and Povlsen, 1969, Acta Pathol. Microbial. Scand. 77:758-760), many different human tumor cell lines (e.g., mammary, lung, genitourinary, gastro-intestinal, head and neck, glioblastoma, bone, and malignant melanomas) have been transplanted and successfully grown in nude mice. The following assays may be used to determine the level of activity, specificity and effect of the different compounds of the present invention. Three general types of assays are useful for evaluating compounds: cellular/catalytic, cellular/biological and in vivo. The object of the cellular/catalytic assays is to determine the effect of a

determine the effect of a compound on the ability of a TK to phosphorylate tyrosines on a known substrate in a cell. The object of the cellular/biological assays is to determine the effect of a compound on the biological response stimulated by a TK in a cell. The object of the in vivo assays is to determine the effect of a compound in an animal model of a particular disorder such as cancer.

Suitable cell lines for subcutaneous xenograft experiments include C6 cells (glioma, ATCC # CCL 107), A375 cells (melanoma, ATCC # CRL 1619), A431 cells (epidermoid carcinoma, ATCC # CRL 1555), Calu 6 cells (lung, ATCC # HTB 56), PC3 cells (prostate, ATCC # CRL 1435), SKOV3TP5 cells and NIH 3T3 fibroblasts genetically engineered to overexpress EGFR, PDGFR, IGF-1R or any other test kinase. The following protocol can be used to perform xenograft experiments:

Female athymic mice (BALB/c, nu/nu) are obtained from Simonsen Laboratories (Gilroy, CA). All animals are maintained under clean-room conditions in Micro-isolator cages with Alpha-dri bedding. They receive sterile rodent chow and water ad libitum.

Cell lines are grown in appropriate medium (for example, MEM, DMEM, Ham's F10, or Ham's F12 plus 5% - 10% fetal bovine serum (FBS) and 2 mM glutamine (GLN)). All cell culture media, glutamine, and fetal bovine serum are purchased from Gibco Life Technologies (Grand Island, NY) unless otherwise specified. All cells are grown in a humid atmosphere of 90-95% air and 5-10% CO_2 at 37°C. All cell lines are routinely subcultured twice a week and are negative for mycoplasma as determined by the Mycotect method (Gibco).

Cells are harvested at or near confluency with 0.05% Trypsin-EDTA and pelleted at 450 x g for 10 min. Pellets are resuspended in sterile PBS or media (without FBS) to a particular concentration and the cells are implanted into the hindflank of

the mice (8 - 10 mice per group, 2 - 10 x 10^6 cells/animal). Tumor growth is measured over 3 to 6 weeks using venier calipers. Tumor volumes are calculated as a product of length x width x height unless otherwise indicated. P values are calculated using the Students t-test. Test compounds in 50 - 100 µL excipient (DMSO, or VPD:D5W) can be delivered by IP injection at different concentrations generally starting at day one after implantation.

TUMOR INVASION MODEL

The following tumor invasion model has been developed and may be used for the evaluation of therapeutic value and efficacy of the compounds identified to selectively inhibit KDR/FLK-1 receptor.

Procedure

8 week old nude mice (female) (Simonsen Inc.) are used as experimental animals. Implantation of tumor cells can be performed in a laminar flow hood. For anesthesia, Xylazine/Ketamine Cocktail (100 mg/kg ketamine and 5 mg/kg Xylazine) are administered intraperitoneally. A midline incision is done to expose the abdominal cavity (approximately 1.5 cm in length) to inject 10^7 tumor cells in a volume of 100 µl medium. The cells are injected either into the duodenal lobe of the pancreas or under the serosa of the colon. The peritoneum and muscles are closed with a 6-0 silk continuous suture and the skin is closed by using wound clips. Animals are observed daily.

Analysis

After 2-6 weeks, depending on gross observations of the animals, the mice are sacrificed, and the local tumor metastases to various organs (lung, liver, brain, stomach, spleen, heart, muscle) are excised and analyzed (measurement of tumor size, grade of invasion, immunochemistry, *in situ* hybridization determination, etc.).

C-KIT ASSAY

This assay is used to detect the level of c-kit tyrosine phosphorylation.

MO7E (human acute myeloid leukemia) cells were serum starved overnight in 0.1% serum. Cells were pre-treated with the compound (concurrent with serum starvation), prior to ligand stimulation. Cells were stimulated with 250 ng/ml rh-SCF for 15 minutes. Following stimulation, cells were lysed and immunoprecipitated with an anti-c-kit antibody. Phosphotyrosine and protein levels were determined by Western blotting.

MTT PROLIFERATION ASSAY

MO7E cells were serum starved and pre-treated with compound as described for the phosphorylation experiments. Cells were plated @ $4X10^5$ cells/well in a 96 well dish, in 100 µl RPMI + 10% serum. rh-SCF (100 ng/mL) was added and the plate was incubated for 48 hours. After 48 hours, 10 µl of 5 mg/ml MTT [3-(4, 5-dimethythiazol-2-yl)-2, 5-diphenyl tetrazolium bromide) was added and allowed to incubate for 4 hours. Acid isopropanol (100 µl of 0.04N HCl in isopropanol) was added and the optical density was measured at a wavelength of 550 nm.

APOPTOSIS ASSAY

MO7E cells were incubated +/- SCF and +/- compound in 10% FBS with rh-GM-CSF(10ng/mL) and rh-IL-3 (10ng/mL). Samples were assayed at 24 and 48 hours. To measure activated caspase-3, samples were washed with PBS and permeabilized with ice-cold 70% ethanol. The cells were then stained with PE-conjugated polyclonal rabbit anti-active caspase-3 and analyzed by FACS. To measure cleaved PARP, samples were lysed and analyzed by western blotting with an anti-PARP antibody.

Additional assays

Additional assays which may be used to evaluate the compounds of this invention include, without limitation, a bio-flk-1 assay, an EGF receptor-HER2 chimeric receptor assay in

whole cells, a bio-src assay, a bio-lck assay and an assay measuring the phosphorylation function of raf. The protocols for each of these assays may be found in U. S. Application Ser. No. 09/099,842, which is incorporated by reference, including any drawings, herein.

Measurement of Cell Toxicity

Therapeutic compounds should be more potent in inhibiting receptor tyrosine kinase activity than in exerting a cytotoxic effect. A measure of the effectiveness and cell toxicity of a compound can be obtained by determining the therapeutic index, i.e., IC_{50}/LD_{50}. IC_{50}, the dose required to achieve 50% inhibition, can be measured using standard techniques such as those described herein. LD_{50}, the dosage which results in 50% toxicity, can also be measured by standard techniques as well (Mossman, 1983, J. Immunol. Methods, 65:55-63), by measuring the amount of LDH released (Korzeniewski and Callewaert, 1983, J. Immunol. Methods, 64:313, Decker and Lohmann-Matthes, 1988, J. Immunol. Methods, 115:61), or by measuring the lethal dose in animal models. Compounds with a large therapeutic index are preferred. The therapeutic index should be greater than 2, preferably at least 10, more preferably at least 50.

B. Example of Cellular Assay Results Using 5-(5-Fluoro-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-diethylamino-ethyl)amide (Compound 80).

To confirm the potency of 5-(5-Fluoro-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-diethylamino-ethyl)amide (hereinafter "Compound 80"; refer to Table 1, in which this compound is the compound of Example 80) detected in biochemical assays(*vide infra*), the ability of said compound to inhibit ligand-dependent RTK phosphorylation was evaluated in cell-based assays using NIH-3T3 mouse cells engineered to overexpress Flk-1 or human PDGFRβ. Compound 80 inhibited VEGF-dependent Flk-1 tyrosine

phosphorylation with an IC_{50} value of approximately 0.03 μM. This value is similar to the 0.009 μM K_i value determined for inhibition of Flk-1 by compound 80 determined in biochemical assays. This indicates that compound 80 readily penetrates into cells. Consistent with the biochemical data (*vide infra*) indicating that compound 80 had comparable activity against Flk-1 and PDGFRβ, it was also found that it inhibited PDGF-dependent receptor phosphorylation in cells with an IC_{50} value of approximately 0.03 μM. The ability of compound 80 to inhibit c-kit, a closely related RTK that binds stem cell factor (SCF), was determined using MO7E cells that express this receptor. In these cells, compound 80 inhibited SCF-dependent c-kit phosphorylation with an IC_{50} value of 0.01-0.1 μM. This compound also inhibited SCF-stimulated c-kit phosphorylation in acute myeloid leukemia (AML) blasts isolated from the peripheral blood of patients.

In addition to testing the ability of compound 80 to inhibit ligand-dependent receptor phosphorylation in cells, its effect on ligand-dependent proliferative response of cells was also examined <u>in vitro</u> (*see* Table 4). In these studies, cells quiesced by overnight serum starvation were induced to undergo DNA synthesis upon addition of the appropriate mitogenic ligand. As shown in Table 4, compound 80 inhibited the PDGF-induced proliferation of NIH-3T3 cells overexpressing PDGFRβ or PDGFRα with IC_{50} values 0.031 and 0.069 μM, respectively, and the SCF-induced proliferation of MO7E cells with an IC_{50} value of 0.007 μM. No attempt was made to determine the effect compound 80 on a ligand-dependent proliferative response in NIH-3T3 cells expressing Flk-1 since these cells did not proliferate in response to VEGF stimulation.

TABLE 4

Receptor	Biochemical K_i[1] (μM)	Cellular IC_{50} Receptor Phosphorylation (μM)	Cellular IC_{50} Ligand-dependent Proliferation (μM)
Flk-1/KDR	0.009	0.03[2]	0.004[3]
PDGFRα	0.008	0.03[4]	0.031[4]
PDGFRβ	ND	ND	0.069[5]
FGFR	0.83	ND	0.7[3]
c-kit	ND	0.01-0.1[6]	0.007[6]

ND = Not Determined
[1] Determined using recombinant enzyme
[2] Determined using serum-starved NIH-3T3 cells expressing Flk-1
[3] Determined using serum-starved HUVECs
[4] Determined using serum-starved NIH-3T3 cells expressing PDGFRβ
[5] Determined using serum-starved NIH-3T3 cells expressing PDGFRβ
[6] Determined using serum-starved MO7E cells

As shown in Table 4, there is a general agreement between the biochemical and cellular activities of compound 80 supporting the conclusion that this compound crosses cellular membranes. Further, it can be concluded that the cellular responses are a result of the activity of compound 80 against the indicated target. In contrast, when tested in the presence of complete growth medium in vitro, substantially higher concentrations of compound 80 (>10 μM) were required to inhibit the growth of a variety of human tumor cells (see Table 5). This indicates that the compound did not directly inhibit the growth of these cells at concentrations required to inhibit ligand-dependent receptor phosphorylation and cell proliferation.

TABLE 5

Cell Line	Origin	IC_{50} (μM)	LD_{50} (μM)
HT29	Colon carcinoma	10	22
A549	Lung carcinoma	9.5	22
NCI-H460	NSC lung carcinoma	8.9	20
SF767T	Glioma	7.9	14
A431	Epidermoid carcinoma	6.0	18

Briefly, the results shown in Table 5 were obtained by incubating cells for 48 hr in complete growth medium in the presence of serial dilutions compound 80. At the end of the growth period, the relative number of cells was determined. IC_{50} values were calculated as the concentration of compound that inhibited the growth of cells by 50% relative to untreated cells. LD_{50} values were calculated as the concentration of compound that caused a 50% reduction in the number of cells relative to those at the start of the experiment.

A more relevant cell-based assay in which to evaluate the anti-angiogenic potential of compound 80 is the <u>in vitro</u> mitogenesis assay using human umbilical vein endothelial cells (HUVECs) as a model system for the endothelial cell proliferation critical to the angiogenic process. In this assay, a mitogenic response, measured as an increase in DNA synthesis, is induced in serum-starved HUVECs upon addition of VEGF or FGF. In these cells, compound 80 inhibited the VEGF- and FGF-induced mitogenic response in a dose-dependent manner with IC_{50} values of 0.004 μM and 0.7 μM, respectively, when compound was present throughout the 48-hr assay.

Briefly, the aforementioned results were obtained using Serum-starved HUVECs that were incubated with mitogenic concentrations of VEGF (100 ng/ml) or FGF (30 ng/ml) in the presence of serial dilutions of compound 80 for 24 hrs. The

mitogenic response during the following 24 hrs. in the presence of ligand and inhibitor was quantitated by measurement of DNA synthesis based on incorporation of bromodeoxyuridine into cellular DNA.

In separate experiments, compound 80 inhibited the VEGF-dependent phosphorylation of ERK 1/2 (p42/44MAP kinase), an early downstream target of Flk-1/KDR, in a dose-dependent manner. The inhibitory activity of compound 80 was also shown to be long-lasting in this system; inhibiting VEGF-dependent phosphorylation of ERK 1/2 for as long as 48 hours after removal of compound 80 from the medium following a short (2 hr) exposure to micromolar concentrations of the compound.

VEGF has been recognized to be an important survival factor for endothelial cells. Since compound 80 inhibits the VEGF-dependent mitogenic response of HUVECs, the effect of the compound on HUVEC survival was investigated. In these experiments, cleavage of the caspase 3 substrate poly-ADP-ribosyl polymerase (PARP) was used as a readout for apoptosis. HUVECs cultured in serum-free conditions for 24 hours exhibited substantial levels of PARP cleavage, as detected by the accumulation of the 23 kDa PARP cleavage fragment. This was largely prevented by the addition of VEGF to the cell medium, indicating that VEGF acts as a survival factor in this assay. Compound 80 has been shown to inhibit KDR signaling. Accordingly, compound 80 inhibited VEGF-mediated HUVEC survival in a dose-dependent manner. Thus, these data indicate that compound 80 induced apoptosis in endothelial cells in culture in the presence of VEGF.

C. <u>In Vivo</u> Efficacy Studies

i. Efficacy Against Established Tumor Xenografts

The <u>in vivo</u> efficacy of compound 80 was studied in subcutaneous (SC) xenograft models using human tumor cells implanted into the hindflank region of athymic mice. Following

implantation, tumors were allowed to become established to a size of 100-550 mm^3 prior to starting oral treatment with the compound.

Daily oral administration of compound 80 caused a dose-dependent inhibition of A431 tumor growth when treatment was initiated after tumors had grown to a size of 400 mm^3. Statistically significant ($P < 0.05$) inhibition of tumor growth was seen at doses of 40 mg/kg/day (74% inhibition) and 80 mg/kg/day (84% inhibition) (*see* Table 6). In preliminary experiments, a higher (160 mg/kg/day) dose of the compound was not more efficacious against established A431 tumors than the 80 mg/kg/day dose. In addition, mice treated at the 160 mg/kg/day dose of the compound lost body weight, indicating that the higher dose was not as well tolerated. Similar results were obtained in an experiment in which A431 tumors were only allowed to reach 100 mm^3 in size (*see* Table 6). In this second experiment, complete regression of the tumors occurred in six of the eight animals treated at the 80 mg/kg/day for 21 days. In these six animals, the tumors did not regrow during a 110-day observation period following the end of treatment. In the two animals in which the tumors regrew to a large size (2000-3000 mm^3), the tumors regressed in response to a second round of treatment with compound 80. Importantly, in all efficacy experiments, compound 80 at 80 mg/kg/day has been well tolerated, even when dosed continuously for more than 100 days.

616 5A 25 615

TABLE 6

Initial Tumor Volume (mm^3)	Compound[1] (mg/kg/day)	% Inhibition (day)	P-Value
400	80	84 (36)	0.001
	40	74 (36)	0.003
	20	51 (36)	0.130
100	80	93 (40)	0.002
	40	75 (40)	0.015
	10	61 (40)	0.059

[1] Compound 80.

5024201 .05E+209

Briefly, the results shown in Table 6 were obtained using A431 cells (0.5 x 106 cells/mouse) which were implanted SC into the hindflank region of athymic mice. Daily oral administration of compound 80 in a Cremophore-based vehicle or vehicle control began when tumors reached the indicated average volume. Tumors were measured using vernier calipers and tumor volume was calculated as the product of length x width x height. P-values were calculated by comparing the size of the tumors for animals that were treated with compound 80 (n=8) to those of animals that were treated with a vehicle (n=16) on the last day of the experiment, using the two-tailed Student's t-test.

The efficacy compound 80 against established human tumors of different origins was determined using Colo205 (colon carcinoma), SF763T (glioma), and NCI-H460 (non-small cell lung carcinoma) xenografts (*see* Table 7). These experiments were conducted using compound 80 administered orally at 80 mg/kg/day; a dose that was effective and well tolerated.

TABLE 7

	Tumor Type	Initial Tumor Volume (mm^3)	% Inhibition (day)	P-Value
A431[1]	Epidermoid	100	93 (40)	0.002
A431[1]	Epidermoid	400	84 (36)	0.001
Colo205	Colon	370	77 (54)	0.028
NCI-H460	Lung	300	61 (54)	0.003
SF763T	Glioma	550	53 (30)	0.001

[1] Data are from experiment reported in Table 5.

In the abovementioned experiments, compound 80 was administered once daily at 80 mg/kg in a Cremophor-based vehicle once tumors reached the indicated size. Percent inhibition compared to the vehicle-treated control group was calculated at termination of the experiments. P-values were calculated by comparing tumor sizes of the animals that had been treated with the compound to tumor sizes of those animals that had been treated with the vehicle, using the two-tailed Student's t-test.

Although compound 80 inhibited the growth of all the tumor types shown in Table 7, there was a difference in the response of the different xenograft models. . Specifically, the growth of NCI-H460 and SF763T tumors was arrested or greatly slowed whereas the Colo205 tumors, like A431 tumors, regressed when treated with compound 80.

In order to determine the molecular basis for the difference in response between xenograft models, the SF763T tumors were studied. Therefore, SF763T tumors, which were less responsive to treatment with compound 80, have been evaluated at the molecular level using immunohistological techniques to determine the effect of treatment with the compound. These studies were initially conducted in this tumor type because SF763T tumors are highly vascularized with microvessels that strongly express the endothelial cell marker CD31 and are hence well suited for studies of tumor microvessel density (MVD). Immunohistological

evaluation of SF763T tumors indicated that tumors from treated animals had reduced MVD relative to vehicle-treated controls, consistent with an anti-angiogenic mechanism of action for compound 80; MVD was 24.2 ± 4.1 in animals treated with compound 80, compared to 39.3 ± 5.7 for those that were treated with just the vehicle. As anticipated from the associated tumor growth arrest, a pronounced inhibition of tumor cell proliferation was evident in tumors that were treated with compound 80. These tumors had half the mitotic index of those in vehicle-treated tumors (data not shown). The effect compound 80 on MVD and tumor cell proliferation indicates that the compound has profound anti-angiogenic and anti-tumor effects, even under conditions in which tumors do not regress.

The ability of compound 80 to inhibit PDGFR phosphorylation and subsequent signaling in vivo was also evaluated in the SF763T tumors, which express high levels of PDGFRβ. Treatment of the SF763T tumors with compound 80 strongly inhibited PDGFRβ tyrosine phosphorylation in established SF763T tumors. Compound 80 also reduced the levels of phosphorylated (activated) phospholipase C gamma (PLC-γ), an immediate downstream indicator of PDGFR activation. These data demonstrate that oral administration of compound 80 causes a direct effect on target (PDGFR) activity in tumors in vivo.

Based on the demonstration that the ability of compound 80 to inhibit VEGF-dependent signaling in HUVECs in vitro was long-lasting (*vide supra*), the efficacy of the compound was evaluated when the compound was administered infrequently in the Colo205 tumor model. As shown in Table 8, 80 mg/kg (91% inhibition) and 40 mg/kg (84% inhibition) were efficacious when administered daily, but not when administered twice weekly. In contrast, a higher dose of compound 80 (160 mg/kg) did inhibit (52% inhibition) the growth of established Colo205 tumors when administered twice weekly, suggesting that this compound can

demonstrate efficacy when administered infrequently at a higher dose. It should be noted that dosing regimens may be determined by those with ordinary skill in the art without undue experimentation.

TABLE 8

Dose (mg/kg)	Frequency	% Inhibition	P-Value
160	Twice weekly	52	0.085
	Once weekly	17	NS
80	Daily	91	0.039
	Twice weekly	19	NS
	Once weekly	0	NS
40	Daily	84	0.028
	Twice weekly	36	NS
NS: not significant (P >0.05)			

Briefly, the results shown in Table 8 were obtained using Colo205 cells (0.5 x 10^6 cells/mouse) that had been implanted SC into the hindflank region of athymic mice. Oral administration of compound 80 according to the indicated schedule began when tumors reached 400 mm^3. Tumors were measured using vernier calipers and tumor volume was calculated as the product of length x width x height. P-values were calculated by comparing the size of the tumors for animals that were treated with compound 80 to those of animals that were treated with a vehicle on the last day of the experiment, using the two-tailed Student's t-test.

ii. Efficacy of Compound 80 in a Model of Disseminated Disease

In addition to supporting the sustained growth of solid primary tumors, angiogenesis is also an essential component supporting the development of disseminated disease due to metastasis from the primary tumor. The effect of compound 80 on the development of disseminated disease was examined in the B16-F1 mouse melanoma lung colonization model. In this model, B16-F1 cells inoculated intravenously via the tail vein of athymic mice colonize the lungs and form tumors. As shown in Table 9, oral

administration of compound 80 at 80 mg/kg/day effectively reduced the burden of B16-F1 cells in the lung as evaluated by measurements of total lung weight. These data suggest that compound 80 can inhibit disseminated disease in vivo.

TABLE 9

	Lung Weight (g)	% Inhibition	P-Value
Vehicle	0.83 ± 0.07	-	-
Compound[1]	0.41 ± 0.04	50	<0.001

[1] Compound 80

Briefly, the results shown in Table 9 were obtained using athymic mice that had been inoculated with B16-F1 tumor cells ($5x10^5$ cells/mouse) via the tail vein. Mice were treated daily with orally administered compound 80 at 80 mg/kg/day (n=10) or vehicle (n=18) for 24 days after tumor cell inoculation. At the end of the treatment period, the mice were sacrificed and their lungs removed and weighed. Percent inhibition was calculated by comparing the lung weight of those animals that had been treated with compound 80, with the lung weight of the animals that had only been treated with vehicle. P-values were determined using the two-tailed Student's t-test.

D. **Examples of Biological Activity.**

Examples of the <u>in vitro</u> potency of compounds of this invention are shown in Table 2.

CONCLUSION

In studies to investigate the pharmacokinetic characteristics of the compounds of the preferred embodiments of the present invention it has been demonstrated that oral administration of a single dose of said compounds resulted in high oral bioavailability in mice. The good oral bioavailability and linear pharmacokinetics indicate that the compounds of the

preferred embodiments of the present have favorable pharmacokinetic characteristics.

In addition, the compounds of the preferred embodiments of the present invention are potent inhibitor of the tyrosine kinase activity of the split-kinase domain RTKs Flk-1/KDR and PDGFR, which are involved in angiogenesis, and the RTK c-kit, a receptor for stem cell factor (SCF), that is involved in certain hematologic cancers. At higher concentrations, the compounds of the preferred embodiments of the present invention also inhibit the tyrosine kinase activity of FGFR-1, a third RTK involved in angiogenesis. Consistent with their biochemical activity, the compounds of the preferred embodiments of the present invention inhibit the ligand-dependent tyrosine phosphorylation of target RTKs and the in vitro mitogenic response of human umbilical vein endothelial cells (HUVECs) stimulated with VEGF or FGF, of PDGFR-expressing NIH-3T3 cells stimulated with PDGF, and of MO7E acute myeloid leukemia cells stimulated with SCF . In contrast, the compounds of the preferred embodiments of the present invention do not directly inhibit the proliferation of tumor cells in complete growth medium except at concentrations 2 to 3 orders of magnitude higher than those required to inhibit the ligand-dependent mitogenic responses. In mouse xenograft studies, the compounds of the prefered embodiments of the present invention inhibited the growth of established human tumors of various origins in a dose-dependent manner and at concentrations that were well tolerated even upon extended (>100 days) dosing. At 80 mg/kg/day, the compounds of the preferred embodiments of the present invention induced regression of large established A431 and Colo205 tumors, and caused substantial growth inhibition or stasis of SF763T and NCI-H460 tumors. In mice bearing SF763T tumors, the compounds of the preferred embodiments of the present invention caused reductions in microvessel density, phosphorylation of PDGFR in the tumors, and mitotic index in the

tumor cells. At this dose, the compounds of the preferred embodiments of the present invention also inhibited lung colonization by B16-F1 tumor cells in a model of tumor metastasis. Regimen studies demonstrated that the compounds of the preferred embodiments of the present invention are most efficacious when administered daily. Direct evidence of the anti-angiogenic activity of the compounds of the preferred embodiments of the present invention was detected in SF763T tumors in which microvessel density was reduced. Direct evidence that the compounds of the preferred embodiments of the present invention inhibit PDGFR phosphorylation and signaling *in vivo* was also obtained in SF763T tumors.

Taken together, these data support the notion that orally administered compounds of the preferred embodiments of the present invention are anti-angiogenic agents for the treatment of cancers, including solid tumors and hematological malignancies in which angiogenesis and/or signaling through c-kit are important in the disease pathology.

It will be appreciated that the compounds, methods and pharmaceutical compositions of the present invention are effective in modulating PK activity and therefore are expected to be effective as therapeutic agents against RTK, CTK-, and STK-related disorders.

One skilled in the art would also readily appreciate that the present invention is well adapted to carry out the objects and obtain the ends and advantages mentioned, as well as those inherent herein. The molecular complexes and the methods, procedures, treatments, molecules, specific compounds described herein are presently representative of preferred embodiments, are exemplary, and are not intended as limitations on the scope of the invention. Changes therein and other uses will occur to those skilled in the art which are encompassed within the spirit of the invention are defined by the scope of the claims.

It will be readily apparent to one skilled in the art that varying substitutions and modifications may be made to the invention disclosed herein without departing from the scope and spirit of the invention.

All patents and publications mentioned in the specification are indicative of the levels of those skilled in the art to which the invention pertains. All patents and publications are herein incorporated by reference to the same extent as if each individual publication was specifically and individually indicated to be incorporated by reference.

The invention illustratively described herein suitably may be practiced in the absence of any element or elements, limitation or limitations which is not specifically disclosed herein. Thus, for example, in each instance herein any of the terms "comprising", "consisting essentially of" and "consisting of" may be replaced with either of the other two terms. The terms and expressions which have been employed are used as terms of description and not of limitation, and there is no intention that in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the invention claimed. Thus, it should be understood that although the present invention has been specifically disclosed by preferred embodiments and optional features, modification and variation of the concepts herein disclosed may be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this invention as defined by the appended claims.

In addition, where features or aspects of the invention are described in terms of Markush groups, those skilled in the art will recognize that the invention is also thereby described in terms of any individual member or subgroup of members of the

Markush group. For example, if X is described as selected from the group consisting of bromine, chlorine, and iodine, claims for X being bromine and claims for X being bromine and chlorine are fully described.

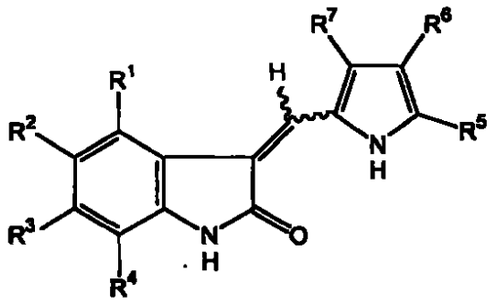
Other embodiments are within the following claims.

60720T "28562209

CLAIMS

WHAT IS CLAIMED:

1. A compound having the chemical structure:



wherein,

R^1 is selected from the group consisting of hydrogen, halo, alkyl, cyclkoalkyl, aryl, heteroaryl, heteroalicyclic, hydroxy, alkoxy, $-C(O)R^{15}$, $-NR^{13}R^{14}$, $-(CH_2)_zR^{16}$ and $-C(O)NR^8R^9$;

R^2 is selected from the group consisting of hydrogen, halo, alkyl, trihalomethyl, hydroxy, alkoxy, $-NR^{13}R^{14}$, N-amido, $C(O)R^{15}$, aryl, heteroaryl and $-S(O)_2NR^{13}R^{14}$;

R^3 is selected from the group consisting of hydrogen, halo, alkyl, trihalomethyl, hydroxy, alkoxy, $-C(O)R^{15}$, $-NR^{13}R^{14}$, aryl, heteroaryl, N-sulfonamido, S-sulfonamido, N-amido and N-carbamyl;

R^4 is selected from the group consisting of hydrogen, halogen, alkyl, hydroxy, alkoxy and $-NR^{13}R^{14}$;

R^5 is selected from the group consisting of hydrogen, alkyl and $-C(O)R^{10}$;

R^6 is selected from the group consisting of hydrogen, alkyl and $-C(O)R^{10}$;

R^7 is selected from the group consisting of hydrogen, alkyl, aryl, heteroaryl, $-C(O)R^{17}$ and $-C(O)R^{10}$;

R^6 and R^7 may combine to form a group selected from the group consisting of $-(CH_2)_4-$, $-(CH_2)_5-$ and $-(CH_2)_6-$;

with the proviso that at least one of R^5, R^6 or R^7 must be $-C(O)R^{10}$;

R^8 and R^9 are independently selected from the group consisting of hydrogen, alkyl and aryl;

R^{10} is selected from the group consisting of hydroxy, alkoxy, aryloxy, $-N(R^{11})(CH_2)_nR^{12}$ and $-NR^{13}R^{14}$;

R^{11} is selected from the group consisting of hydrogen and alkyl;

R^{12} is selected from the group consisting of $-NR^{13}R^{14}$, hydroxy, $-C(O)R^{15}$, aryl and heteroaryl;

R^{13} and R^{14} are independently selected from the group consisting of hydrogen, alkyl, cycloalkyl, aryl and heteroaryl;

R^{13} and R^{14} may combine to form a group selected from the group consisting of $-(CH_2)_4-$, $-(CH_2)_5-$, $-(CH_2)_2O(CH_2)_2-$, and $-(CH_2)_2N(CH_3)(CH_2)_2-$;

R^{15} is selected from the group consisting of hydrogen, hydroxy,

alkoxy and aryloxy;

R^{16} is selected from the group consisting of hydroxy, $-C(O)R^{15}$, $-NR^{13}R^{14}$ and $C(O)NR^{13}R^{14}$;

R^{17} is selected from the group consisting of alkyl, cycloalkyl, aryl and heteroaryl; and,

n and r are independently 1, 2, 3, or 4; or,

a pharmaceutically acceptable salt of said compound.

2. The compound or salt of claim 1, wherein

R^1 and R^4 are hydrogen;

R^2 is selected from the group consisting of hydrogen, halo, lower alkoxy, $-C(O)R^{15}$ and $-S(O)_2NR^{13}R^{14}$;

R^3 is selected from the group consisting of hydrogen, lower alkoxy, $-C(O)R^{15}$, $-S(O)_2NR^{13}R^{14}$, aryl and heteroaryl;

R^5 is $-C(O)R^{10}$;

R^6 is selected from the group consisting of hydrogen and lower alkyl; and,

R^7 is selected from the group consisting of hydrogen, lower alkyl and $-C(O)R^{17}$.

3. The compound or salt of claim 2, wherein:

R^{10} is selected from the group consisting of hydroxy, lower alkoxy and $-NR^{11}(CH_2)_nR^{12}$, wherein:

n is 2 or 3;

R^{11} is selected from the group consisting of hydrogen and lower alkyl; and,

R^{12} is selected from the group consisting of aryl and $-NR^{13}R^{14}$.

4. The compound of claim 3, wherein R^{13} and R^{14} are independently selected from the group consisting of hydrogen, lower alkyl, and, combined, $-(CH_2)_4-$, $-(CH_2)_5-$, $-(CH_2)_2O(CH_2)_2-$ or $-(CH_2)_2N(CH_3)(CH_2)_2-$.

5. The compound or salt of claim 1, wherein,

R^1 is selected from the group consisting of hydrogen, lower alkyl, $(CH_2)_cR^{16}$ and $-C(O)NR^8R^9$;

R^2 is selected from the group consisting of hydrogen, halogen, aryl and $-S(O)_2NR^{13}R^{14}$;

R^3 is selected from the group consisting of hydrogen, lower alkyl, lower alkoxy, aryl, heteroaryl and $-C(O)R^{15}$;

R^4 is hydrogen;

R^5 is selected from the group consisting of hydrogen and lower alkyl;

R^6 is $-C(O)R^{10}$;

R^7 is selected from the group consisting of hydorgen, lower alkyl and aryl;

R^{16} is selected from the group consisting of hydroxy and $-C(O)R^{15}$; and,

r is 2 or 3.

6. The compound or salt of claim 5 wherein R^3 is aryl optionally substituted with one or more groups selected from the group consisting of lower alkyl, lower alkoxy and halo.

7. The compound or salt of claim 5 wherein R^{10} is selected from the group consisting of hydroxy, lower alkoxy, $-NR^{13}R^{14}$ and $-NR^{11}(CH_2)_nR^{12}$, wherein

n is 1, 2 or 3;

R^{11} is hydrogen; and,

R^{12} is selected from the group consisting of hydroxy, lower alkoxy, $-C(O)R^{15}$, heteroaryl and $-NR^{13}R^{14}$.

8. The compound of claim 7 wherein R^{13} and R^{14} are independently selected from the group consisting of hydrogen, lower alkyl, heteroaryl and, combined, $-(CH_2)_4-$, $-(CH_2)_5-$, $-(CH_2)_2O(CH_2)_2-$, or $-(CH_2)_2N(CH_3)(CH_2)_2-$.

9. The compound or salt of claim 5, wherein R^1 is $-C(O)NR^8R^9$, wherein:

R^8 is hydrogen; and,

R^9 is aryl optionally substituted with one or more groups

selected from the group consisting of halo, hydroxy and lower alkoxy.

10. The compound of claim 1, wherein:

R^1 is selected from the group consisting of hydrogen and lower alkyl;

R^2 is selected from the group consisting of hydrogen, halo, lower alkoxy, aryl, $-C(O)R^{15}$ and $-S(O)_2NR^{13}R^{14}$;

R^3 is selected from the group consisting of hydrogen, halo, aryl, heteroaryl and $-C(O)R^{15}$;

R^4 is hydrogen;

R^5 is $-C(O)R^{10}$; and,

R^6 and R^7 combine to form a $-(CH_2)_4-$ group.

11. The compound of claim 10, wherein:
R^{10} is selected from the group consisting of hydroxy, alkoxy, $-NR^{13}R^{14}$ and $-NH(CH_2)_nNR^{13}R^{14}$; and

n is 2 or 3.

12. The compound of claim 11 wherein R^{13} and R^{14} are independently selected from the group consisting of hydrogen, lower alkyl, and, combined, $-(CH_2)_4-$, $-(CH_2)_5-$, $-(CH_2)_2O(CH_2)_2-$ or $-(CH_2)_2N(CH_3)(CH_2)_2-$.

13. The compound of claim 1, wherein:

R^1 and R^4 are selected from the group consisting of hydrogen, lower alkyl, lower alkoxy and halogen;

R^2 is selected from the group consisting of hydrogen, lower alkyl, lower alkoxy, halo, and $-S(O)_2NR^{13}R^{14}$;

R^3 is selected from the group consisting of hydrogen, lower alkyl, lower alkoxy, halo, aryl and $-C(O)R^{15}$;

R^5 and R^6 are selected from the group consisting of hydrogen and lower alkyl; and,

R^7 is $-C(O)R^{10}$.

14. The compound of claim 13, wherein:

R^{10} is selected from the group consisting of hydroxy, alkoxy, $-NR^{13}R^{14}$ and $-NR^{11}(CH_2)_nR^{12}$, wherein

n is 2 or 3;

R^{11} is selected from the group consisting of hydrogen and lower alkyl; and,

R^{12} is $-NR^{13}R^{14}$, wherein

R^{13} and R^{14} are independently selected from the group consisting of hydrogen, lower alkyl, and, combined, $-(CH_2)_4-$, $-(CH_2)_5-$, $-(CH_2)_2O(CH_2)_2-$ or $-(CH_2)_2N(CH_3)(CH_2)_2-$

15. A pharmaceutical composition, comprising

a compound or salt of claim 1; and,

a pharmaceutically acceptable carrier or excipient.

16. A method for the modulation of the catalytic activity of a protein kinase comprising contacting said protein kinase with a compound or salt of claim 1.

17. The method of claim 16 wherein said protein kinase is selected from the group consisting of a receptor tyrosine kinase, a non-receptor tyrosine kinase and a serine-threonine kinase.

18. A method for treating or preventing a protein kinase related disorder in an organism comprising administering a therapeutically effective amount of a compound or salt of claim 1 to said organism.

19. The method of claim 18 wherein said protein kinase related disorder is selected from the group consisting of a receptor tyrosine kinase related disorder, a non-receptor tyrosine kinase related disorder and a serine-threonine kinase related disorder.

20. The method of claim 18 wherein said protein kinase related disorder is selected from the group consisting of an EGFR related disorder, a PDGFR related disorder, an IGFR related disorder and a flk related disorder.

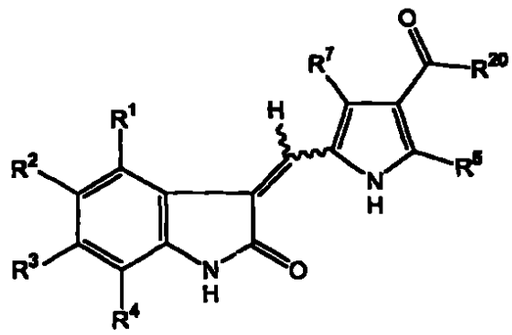
21. The method of claim 18 wherein said protein kinase related disorder is a cancer selected from the group consisting of squamous cell carcinoma, astrocytoma, Kaposi's sarcoma, glioblastoma, lung cancer, bladder cancer, head and neck

cancer, melanoma, ovarian cancer, prostate cancer, breast cancer, small-cell lung cancer, glioma, colorectal cancer, genitourinary cancer and gastrointestinal cancer.

22. The method of claim 18 wherein said protein kinase related disorder is selected from the group consisting of diabetes, an autoimmune disorder, a hyperproliferation disorder, restenosis, fibrosis, psoriasis, von Heppel-Lindau disease, osteoarthritis, rheumatoid arthritis, angiogenesis, an inflammatory disorder, an immunological disorder and a cardiovascular disorder.

23. The method of claim 18 wherein said organism is a human.

24. A compound having the chemical structure:



wherein,

R^1 is selected from the group consisting of hydrogen, halo, alkyl, cyclkoalkyl, aryl, heteroaryl, heteroalicyclic, hydroxy, alkoxy, $-C(O)R^{15}$, $-NR^{13}R^{14}$, $-(CH_2)_rR^{16}$ and $-C(O)NR^8R^9$;

R^2 is selected from the group consisting of hydrogen, halo, alkyl, trihalomethyl, hydroxy, alkoxy, $-NR^{13}R^{14}$, N-amido, $C(O)R^{15}$, aryl, heteroaryl and $-S(O)_2NR^{13}R^{14}$;

R^3 is selected from the group consisting of hydrogen, halo, alkyl, trihalomethyl, hydroxy, alkoxy, $-C(O)R^{15}$, $-NR^{13}R^{14}$, aryl, heteroaryl, N-sulfonamido, S-sulfonamido, N-amido and N-carbamyl;

R^4 is selected from the group consisting of hydrogen, halogen, alkyl, hydroxy, alkoxy and $-NR^{13}R^{14}$;

R^5 is selected from the group consisting of hydrogen, alkyl and $-C(O)R^{10}$;

R^7 is selected from the group consisting of hydrogen, alkyl, aryl, heteroaryl, $-C(O)R^{17}$ and $-C(O)R^{10}$;

R^8 and R^9 are independently selected from the group consisting of hydrogen, alkyl and aryl;

R^{10} is selected from the group consisting of hydroxy, alkoxy, aryloxy, $-N(R^{11})(CH_2)_nR^{12}$ and $-NR^{13}R^{14}$;

R^{11} is selected from the group consisting of hydrogen and alkyl;

R^{12} is selected from the group consisting of $-NR^{13}R^{14}$, hydroxy, $-C(O)R^{15}$, aryl and heteroaryl;

R^{13} and R^{14} are independently selected from the group consisting of hydrogen, alkyl, cycloalkyl, aryl and heteroaryl;

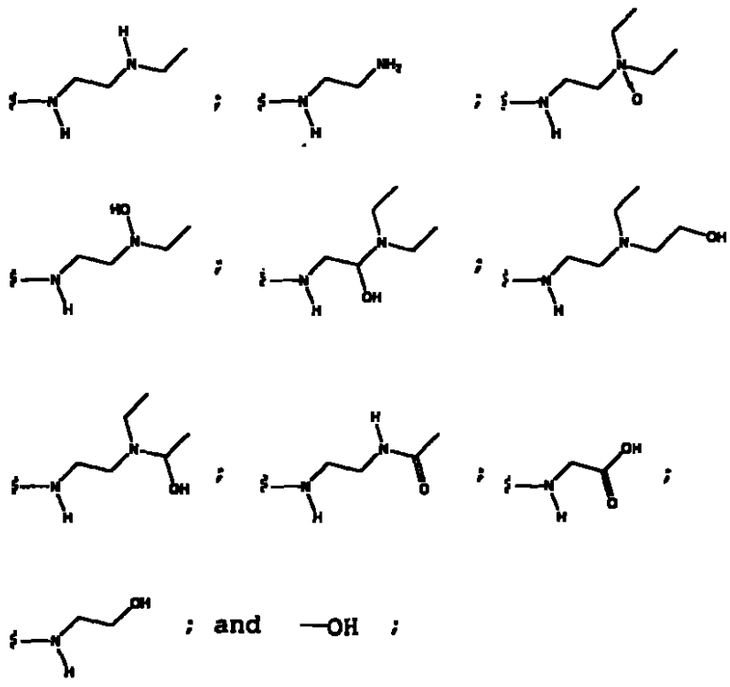
R^{13} and R^{14} may combine to form a group selected from the group consisting of $-(CH_2)_4-$, $-(CH_2)_5-$, $-(CH_2)_2O(CH_2)_2-$, and $-(CH_2)_2N(CH_3)(CH_2)_2-$;

R^{15} is selected from the group consisting of hydrogen, hydroxy, alkoxy and aryloxy;

R^{16} is selected from the group consisting of hydroxy, $-C(O)R^{15}$, $-NR^{13}R^{14}$ and $C(O)NR^{13}R^{14}$;

R^{17} is selected from the group consisting of alkyl, cycloalkyl, aryl and heteroaryl;

R^{20} is selected from the group consisting of



; and —OH ;

and,

n and r are independently 1, 2, 3, or 4; or,

a pharmaceutically acceptable salt of said compound.

ABSTRACT

The present invention relates to pyrrole substituted 2-indolinone compounds and their physiologically acceptable salts which modulate the activity of protein kinases and therefore are expected to be useful in the prevention and treatment of protein kinase related cellular disorders such as cancer.

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TRADUCCIÓN

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A quien corresponda:

DEPARTAMENTO DE COMERCIO - OFICINA DE PATENTES Y MARCAS

30 de marzo 2001

CERTIFICO QUE ADJUNTA A LA PRESENTE SE ENCUENTRA UNA COPIA FIEL DE LOS REGISTROS EN LA OFICINA DE PATENTES Y MARCAS DE LOS ESTADOS UNIDOS DE LOS DOCUMENTOS DE LA SOLICITUD DE PATENTE ABAJO IDENTIFICADA QUE REUNIAN LOS REQUISITOS PARA LA ASIGNACIÓN DE UNA FECHA DE PRESENTACIÓN EN VIRTUD DE 35 USC 111.

SOLICITUD NUMERO: 60/243.532

FECHA DE DEPÓSITO: 27 de octubre del 2000

.En nombre del COMISARIO DE PATENTES Y MARCAS.

(Firmado) E. Bornett Certificaciones.

(La presente certificación se encuentra unida en forma inviolable a sus adjuntos y lleva una oblea dorada que dice): OFICINA DE PATENTES Y MARCAS - ESTADOS UNIDOS DE NORTEAMÉRICA.

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Solicitante: Peng Cho Tang et al.

Fecha de depósito: 27 de octubre del 2000

2- INDOLINONAS SUSTITUIDAS CON PIRROLES INHIBIDORAS DE PROTEINQUINASAS

Carátula para Transmisión de Solicitud de Patente Provicinal

Destinatario: Asistente del Comisario de Patentes

Solicitudes de Patente

Washington DC 20231

Transmitimos con la presente una solicitud de Patente Provisional para su tramitación bajo el art. 1.53 (c) del CRF 37 de:

Peng Cho Tang, Todd Miller, Xiaoyuan Li, Connie Sun, Chung Chen Wei y Sheri Shirazian.

Adjuntos:

Memoria descriptiva, reivindicaciones y resumen (186 páginas)

Cálculo de aranceles:

	tasa	
Arancel básico	$150,00	$150,00
Total		$150,00

Se adjunta un cheque por $150,00 para cubrir el arancel.

El Comisario es autorizado por la presente a subsanar cualquier diferencia en el monto de los siguientes aranceles concernientes a esta solicitud bajo el art. 1.16 o 1.17 del CRF 37 a través de la cuenta número 10-0047.

Atentamente, John P. Isacson

Abogado del solicitante

Nro. de registro: 33.715

Fecha: 27 de octubre del 2000

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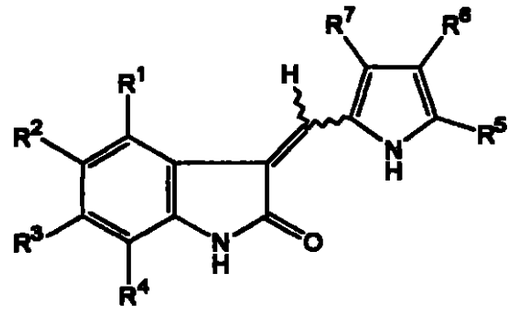
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Fecha

REIVINDICACIONES

1. Un compuesto que tiene la estructura química:



donde:

R^1 se selecciona del grupo formado por hidrógeno, halo, alquilo, cicloalquilo, arilo, heteroarilo, heteroalicíclico, hidroxi, alcoxi, $-(CO)R^{15}$, $-NR^{13}R^{14}$, $-(CH_2)_rR^{16}$ y $-C(O)NR^8R^9$;

R^2 se selecciona del grupo formado por hidrógeno, halo, alquilo, trihalometilo, hidroxi, alcoxi, $-NR^{13}R^{14}$, $-NR^{13}C(O)R^{14}$, $-C(O)R^{15}$, arilo, heteroarilo, y $-S(O)_2NR^{13}R^{14}$;

R^3 se selecciona del grupo formado por hidrógeno, halógeno, alquilo, trihalometilo, hidroxi, alcoxi, $-(CO)R^{15}$, $-NR^{13}R^{14}$, arilo, heteroarilo, $-NR^{13}S(O)_2R^{14}$, $-S(O)_2NR^{13}R^{14}$, $-NR^{13}C(O)R^{14}$,and $-NR^{13}C(O)OR^{14}$;

R^4 se selecciona del grupo formado por hidrógeno, halógeno, alquilo, hidroxi, alcoxi y $-NR^{13}R^{14}$;

R^5 se selecciona del grupo formado por hidrógeno, alquilo y $-C(O)R^{10}$;

R^6 se selecciona del grupo formado por hidrógeno, alquilo y $-C(O)R^{10}$;

R^7 se selecciona del grupo formado por hidrógeno, alquilo, arilo, heteroarilo, $-C(O)R^{17}$ y $-C(O)R^{10}$;

R^6 y R^7 se pueden combinar para formar un grupo seleccionado del

grupo formado por $-(CH_2)_4-$, $-(CH_2)_5-$ y $-(CH_2)_6-$;

con la condición de que al menos uno de R^5, R^6 o R^7 debe ser $-C(O)R^{10}$;

R^8 y R^9 se seleccionan independientemente del grupo formado por hidrógeno, alquilo y arilo;

R^{10} se selecciona del grupo formado por hidroxi, alcoxi, ariloxi, $-N(R^{11})(CH_2)_nR^{12}$ y $-NR^{13}R^{14}$;

R^{11} se selecciona del grupo formado por hidrógeno y alquilo;

R^{12} se selecciona del grupo formado por $-NR^{13}R^{14}$, hidroxi, $-C(O)R^{15}$, arilo y heteroarilo;

R^{13} y R^{14} se seleccionan independientemente del grupo formado por hidrógeno, alquilo, cicloalquilo, arilo y heteroarilo;

R^{13} y R^{14} se pueden combinar para formar un grupo seleccionado del grupo formado por $-(CH_2)_4-$, $-(CH_2)_5-$, $-(CH_2)_2O(CH_2)_2-$, y $-(CH_2)_2N(CH_3)(CH_2)_2-$;

R^{15} se selecciona del grupo formado por hidrógeno, hidroxi, alcoxi y ariloxi;

R^{16} se selecciona del grupo formado por hidroxi, $-C(O)R^{15}$, $-NR^{13}R^{14}$ y $-C(O)NR^{13}R^{14}$;

R^{17} se selecciona del grupo formado por alquilo, cicloalquilo, arilo y heteroarilo; y

n y r son independientemente 1, 2, 3, o 4.

o una sal aceptable para uso farmacéutico de dicho compuesto.

2. El compuesto o sal de la reivindicación 1 en el cual :

R^1 y R^4 son hidrógeno;

R^2 se selecciona del grupo formado por hidrógeno, halo, alcoxi inferior, -$C(O)R^{16}$ y $-S(O)_2NR^{13}R^{14}$;

R^3 se selecciona del grupo formado por hidrógeno, alcoxi inferior, -$C(O)R^{15}$, $-S(O)_2NR^{13}R^{14}$, arilo y heteroarilo;

R^5 es $-C(O)R^{10}$;

R^6 se selecciona del grupo formado por hidrógeno y alquilo inferior; y

R^7 se selecciona del grupo formado por hidrógeno, alquilo inferior y $-C(O)R^{17}$.

3. El compuesto o sal de la reivindicación 2 en el cual :

R^{10} se selecciona del grupo formado por hidroxi, alcoxi inferior y -$NR^{11}(CH_2)_nR^{12}$, donde

n es 2 o 3;

R^{11} se selecciona del grupo formado por hidrógeno y alquilo inferior; y,

R^{12} se selecciona del grupo formado por arilo y $-NR^{13}R^{14}$.

4. El compuesto o sal de la reivindicación 3, R^{13} y R^{14} se seleccionan independientemente del grupo formado por hidrógeno, alquilo inferior, y, en combinación,$-(CH_2)_4-$, $-(CH_2)_5-$, $-CH_2)_2O(CH_2)_2-$ o $-(CH_2)_2N(CH_3)(CH_2)_2-$.

5. El compuesto o sal de la reivindicación 1 en el cual :

R^1 se selecciona del grupo formado por hidrógeno, alquilo inferior, -$(CH_2)_nR^{16}$ y $-C(O)NR^8R^9$;

R^2 se selecciona del grupo formado por hidrógeno, halógeno, arilo y $-S(O)_2NR^{13}R^{14}$;

R^3 se selecciona del grupo formado por hidrógeno, alquilo inferior, alcoxi inferior, arilo, heteroarilo y $-C(O)R^{15}$;

R^4 es hidrógeno;

R^5 se selecciona del grupo formado por hidrógeno y alquilo inferior;

R^6 es $-C(O)R^{10}$;

R^7 se selecciona del grupo formado por hidrógeno, alquilo inferior y arilo;

R^{16} se selecciona del grupo formado por hidroxi y $-C(O)R^{15}$; y,

r es 2 o 3.

6. El compuesto o sal de la reivindicación 5, en el cual R^3 es arilo opcionalmente sustituido con uno o más grupos seleccionados del grupo formado por alquilo inferior, alcoxi inferior y halo.

7. El compuesto o sal de la reivindicación 5, en el cual R^{10} se selecciona del grupo formado por hidroxi, alcoxi inferior, $-NR^{13}R^{14}$ y $-NR^{11}(CH_2)_nR^{12}$, donde n es 1, 2 o 3, R^{11} es hidrógeno y R^{12} se selecciona del grupo formado por hidroxi, alcoxi inferior, $-C(O)R^{15}$, heteroarilo y $-NR^{13}R^{14}$.

8. El compuesto o sal de la reivindicación 7 en el cual R^{13} y R^{14} se seleccionan independientemente del grupo formado por hidrógeno, alquilo inferior, heteroarilo y, en combinación, $-(CH_2)_4-$, $-(CH_2)_5-$, $-(CH_2)_2O(CH_2)_2-$, o $-(CH_2)_2N(CH_3)(CH_2)_2-$.

9. El compuesto o sal de la reivindicación 5, en el cual R^1 es $-C(O)NR^8R^9$, donde;

R^8 es hidrógeno; y

R^9 es arilo opcionalmente sustituido con uno o más grupos seleccionados del grupo formado por halo, hidroxi y alcoxi inferior.

10. El compuesto de la reivindicación 1, en el cual:

R^1 se selecciona del grupo formado por hidrógeno y alquilo inferior;

R^2 se selecciona del grupo formado por hidrógeno, halo, alcoxi inferior, arilo, $-C(O)R^{15}$ y $-S(O)_2NR^{13}R^{14}$;

R^3 se selecciona del grupo formado por hidrógeno, halo, arilo, heteroarilo y $-C(O)R^{15}$;

R^4 es hidrógeno;

R^5 es $-C(O)R^{10}$; y,

R^6 y R^7 se combinan para formar un grupo $-(CH_2)_4-$.

11. El compuesto de la reivindicación 10, en el cual:

R^{10} se selecciona del grupo formado por hidroxi, alcoxi,$-NR^{13}R^{14}$ y $-NH(CH_2)_nNR^{13}R^{14}$ donde n es 2 o 3.

12. El compuesto de la reivindicación 11, en el cual R^{13} y R^{14} se seleccionan independientemente del grupo formado por hidrógeno, alquilo inferior, y, en combinación, $-(CH_2)_4-$, $-(CH_2)_5-$, $-(CH_2)_2O(CH_2)_2-$ o $-(CH_2)_2N(CH_3)(CH_2)_2-$.

13. El compuesto de la reivindicación 1, en el cual:

R^1 y R^4 se seleccionan del grupo formado por hidrógeno, alquilo inferior, alcoxi inferior y halógeno;

R^2 se selecciona del grupo formado por hidrógeno, alquilo inferior, alcoxi inferior, halo y $-S(O)_2NR^{13}R^{14}$;

R^3 se selecciona del grupo formado por hidrógeno, alquilo inferior, alcoxi inferior, halo, arilo y $-C(O)R^{15}$;

R^5 y R^6 se seleccionan del grupo formado por hidrógeno y alquilo inferior; y

R^7 es $-C(O)R^{10}$

14. El compuesto de la reivindicación 13, en el cual:

R^1 y R^4 se seleccionan del grupo formado por hidrógeno, alquilo inferior, alcoxi inferior y halógeno;

R^2 se selecciona del grupo formado por hidrógeno, alquilo inferior, alcoxi inferior, halo y $-S(O)_2NR^{13}R^{14}$;

R^3 se selecciona del grupo formado por hidrógeno, alquilo inferior, alcoxi inferior, halo, arilo y $-C(O)R^{15}$;

R^5 y R^6 se seleccionan del grupo formado por hidrógeno y alquilo inferior; y

R^7 es $-C(O)R^{10}$.

15. Una composición farmacéutica, que comprende un compuesto o sal de la reivindicación 1; y

un vehículo o excipiente aceptable para uso farmacéutico.

16. Un método para la modulación de la actividad catalítica de una proteinquinasa que comprende poner en contacto dicha proteinquinasa con un compuesto o sal de la reivindicación 1.

17. El método de la reivindicación 16 en el cual dicha proteinquinasa se selecciona del grupo formado por un receptor de tirosinquinasa, un no receptor de tirosinquinasa y una serin-treoninquinasa.

18. Un método para tratar o prevener una afección relacionada con la proteinquinasa en un organismo que comprende administrar una cantidad eficaz en términos terapéuticos de un compuesto o sal de la reivindicación 1 a dicho organismo.

19. El método de la reivindicación 18 en el cual dicha afección relacionada con la proteinquinasa se selecciona del grupo formado por una afección relacionada con un receptor de tirosinquinasa , una afección relacionada con un no receptor de tirosinquinasa y una afección relacionada con una serin-treoninquinasa.

20. El método de la reivindicación 18 en el cual dicha afección relacionada con una proteinquinasa se selecciona del grupo formado por una afección

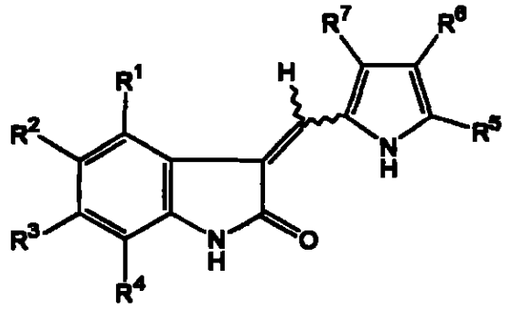
relacionada con los EGFR, una afección relacionada con los PDGFR, una afección relacionada con los IGFR y una afección relacionada con los FLK.

21. El método de la reivindicación 18 en el cual dicha afección relacionada con la proteinquinasa es un cáncer seleccionado del grupo formado por carcinoma de células escamosas, astrocitoma, sarcoma de Kaposi, glioblastoma, cáncer de pulmón, cáncer de vejiga, cáncer de cabeza y cuello, melanoma, cáncer de ovarios, cáncer de próstata, cáncer de mama, cáncer de células pequeñas de pulmón, glioma, cáncer colorrectal, cáncer genitourinario y cáncer gastrointestinal.

22. El método de la reivindicación 18 en el cual dicha afección relacionada con la proteinquinasa se selecciona del grupo formado por diabetes, un trastorno autoinmune , un trastorno hiperproliferativo, restenosis, fibrosis, psoriasis, enfermedad de von Heppel-Lindau, osteoartritis, artritis reumatoide, angiogenesis, un trastorno inflamatorio, una enfermedad inmunológica y una enfermedad cardiovascular.

23. El método de la reivindicación 18 en el cual dicho organismo es un ser humano.

24. Un compuesto que tiene la estructura química:



donde:

R^1 se selecciona del grupo formado por hidrógeno, halo, alquilo, cicloalquilo, arilo, heteroarilo, heteroalicíclico, hidroxi, alcoxi, $-(CO)R^{15}$, $-NR^{13}R^{14}$, $-(CH_2)_rR^{16}$ y $-C(O)NR^8R^9$;

R^2 se selecciona del grupo formado por hidrógeno, halo, alquilo, trihalometilo, hidroxi, alcoxi, $-NR^{13}R^{14}$, $-NR^{13}C(O)R^{14}$, $-C(O)R^{15}$, arilo, heteroarilo, y $-S(O)_2NR^{13}R^{14}$;

R^3 se selecciona del grupo formado por hidrógeno, halógeno, alquilo, trihalometilo, hidroxi, alcoxi, $-(CO)R^{15}$, $-NR^{13}R^{14}$, arilo, heteroarilo, $-NR^{13}S(O)_2R^{14}$, $-S(O)_2NR^{13}R^{14}$, $-NR^{13}C(O)R^{14}$, and $-NR^{13}C(O)OR^{14}$;

R^4 se selecciona del grupo formado por hidrógeno, halógeno, alquilo, hidroxi, alcoxi y $-NR^{13}R^{14}$;

R^5 se selecciona del grupo formado por hidrógeno, alquilo y $-C(O)R^{10}$;

R^6 se selecciona del grupo formado por hidrógeno, alquilo y $-C(O)R^{10}$;

R^7 se selecciona del grupo formado por hidrógeno, alquilo, arilo, heteroarilo, $-C(O)R^{17}$ y $-C(O)R^{10}$;

R^6 y R^7 se pueden combinar para formar un grupo seleccionado del grupo formado por $-(CH_2)_4-$, $-(CH_2)_5-$ y $-(CH_2)_6-$;

con la condición de que al menos uno de R^5, R^6 o R^7 debe ser $-C(O)R^{10}$;

R^8 y R^9 se seleccionan independientemente del grupo formado por hidrógeno, alquilo y arilo;

R^{10} se selecciona del grupo formado por hidroxi, alcoxi, ariloxi, $-N(R^{11})(CH_2)_nR^{12}$ y $-NR^{13}R^{14}$;

R^{11} se selecciona del grupo formado por hidrógeno y alquilo;

R^{12} se selecciona del grupo formado por $-NR^{13}R^{14}$, hidroxi, $-C(O)R^{15}$,

arilo y heteroarilo;

R^{13} y R^{14} se seleccionan independientemente del grupo formado por hidrógeno, alquilo, cicloalquilo, arilo y heteroarilo;

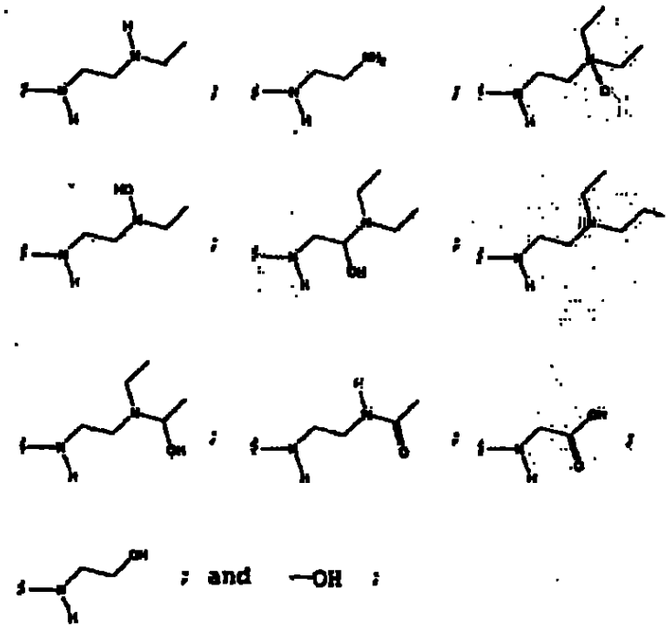
R^{13} y R^{14} se pueden combinar para formar un grupo seleccionado del grupo formado por $-(CH_2)_4-$, $-(CH_2)_5-$, $-(CH_2)_2O(CH_2)_2-$, y $-(CH_2)_2N(CH_3)(CH_2)_2-$;

R^{15} se selecciona del grupo formado por hidrógeno, hidroxi, alcoxi y ariloxi;

R^{16} se selecciona del grupo formado por hidroxi, $-C(O)R^{15}$, $-NR^{13}R^{14}$ y $-C(O)NR^{13}R^{14}$;

R^{17} se selecciona del grupo formado por alquilo, cicloalquilo, arilo y heteroarilo;

R^{20} se selecciona del grupo formadopor:



n y r son independientemente 1, 2, 3, o 4.

o una sal aceptable para uso farmacéutico de dicho compuesto.

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THE UNITED STATES OF AMERICA

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FILING DATE: ***February 15, 2000***

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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

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☐ Additional inventors are being named on the ____ separately numbered sheets attached hereto

TITLE OF THE INVENTION (280 characters max)

PYRROLE SUBSTITUTED 2-INDOLINONE PROTEIN KINASE INHIBITORS

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ENCLOSED APPLICATION PARTS (check all that apply)

☒ Specification Number of Pages 176 ☐ Small Entity Statement

☐ Drawing(s) Number of Sheets ☐ Other (specify)

METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT (check one)

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The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

☒ No.

☐ Yes, the name of the U.S. Government agency and the Government contract number are: _____

Respectfully submitted,

SIGNATURE _____ Date 2/15/2000

TYPED or PRINTED NAME Bernard F. Rose REGISTRATION NO. (If appropriate) 42,112

TELEPHONE (408) 993-1555 Docket Number: 02421772

USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

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DESCRIPTION

PYRROLE SUBSTITUTED 2-INDOLINONE PROTEIN KINASE INHIBITORS

INTRODUCTION

The present invention relates generally to organic chemistry, biochemistry, pharmacology and medicine. More particularly, it relates to pyrrole substituted 2-indolinone compounds, and their physiologically acceptable salts and prodrugs, which modulate the activity of protein kinases ("PKs") and are expected to exhibit a salutary effect against disorders related to abnormal PK activity.

BACKGROUND OF THE INVENTION

The following is offered as background information only and is not admitted to be prior art to the present invention.

PKs are enzymes that catalyze the phosphorylation of hydroxy groups on tyrosine, serine and threonine residues of proteins. The consequences of this seemingly simple activity are staggering; cell growth, differentiation and proliferation, i.e., virtually all aspects of cell life in one way or another depend on PK activity. Furthermore, abnormal PK activity has been related to a host of disorders, ranging from relatively non-life threatening diseases such as psoriasis to extremely virulent diseases such as glioblastoma (brain cancer).

The PKs can be conveniently broken down into two classes, the protein tyrosine kinases (PTKs) and the serine-threonine kinases (STKs).

One of the prime aspects of PTK activity is their involvement with growth factor receptors. Growth factor receptors are cell-surface proteins. When bound by a growth factor ligand,

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growth factor receptors are converted to an active form which interacts with proteins on the inner surface of a cell membrane. This leads to phosphorylation on tyrosine residues of the receptor and other proteins and to the formation inside the cell of complexes with a variety of cytoplasmic signaling molecules that, in turn, effect numerous cellular responses such as cell division (proliferation), cell differentiation, cell growth, expression of metabolic effects to the extracellular microenvironment, etc. For a more complete discussion, see Schlessinger and Ullrich, Neuron, 9:303-391 (1992) which is incorporated by reference, including any drawings, as if fully set forth herein.

Growth factor receptors with PTK activity are known as receptor tyrosine kinases ("RTKs"). They comprise a large family of transmembrane receptors with diverse biological activity. At present, at least nineteen (19) distinct subfamilies of RTKs have been identified. An example of these is the subfamily designated the "HER" RTKs, which include EGFR (epithelial growth factor receptor), HER2, HER3 and HER4. These RTKs consist of an extracellular glycosylated ligand binding domain, a transmembrane domain and an intracellular cytoplasmic catalytic domain that can phosphorylate tyrosine residues on proteins.

Another RTK subfamily consists of insulin receptor (IR), insulin-like growth factor I receptor (IGF-1R) and insulin receptor related receptor (IRR). IR and IGF-1R interact with insulin, IGF-I and IGF-II to form a heterotetramer of two entirely extracellular glycosylated α subunits and two β subunits which cross the cell membrane and which contain the tyrosine kinase domain.

A third RTK subfamily is referred to as the platelet derived growth factor receptor ("PDGFR") group, which includes PDGFRα,

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PDGFRβ, CSFIR, c-kit and c-fms. These receptors consist of glycosylated extracellular domains composed of variable numbers of immunoglobin-like loops and an intracellular domain wherein the tyrosine kinase domain is interrupted by unrelated amino acid sequences.

Another group which, because of its similarity to the PDGFR subfamily, is sometimes subsumed into the later group is the fetus liver kinase ("flk") receptor subfamily. This group is believed to be made up of kinase insert domain-receptor fetal liver kinase-1 (KDR/FLK-1, VEGF-R2), flk-1R, flk-4 and fms-like tyrosine kinase 1 (flt-1).

A further member of the tyrosine kinase growth factor receptor family is the fibroblast growth factor ("FGF") receptor subgroup. This group consists of four receptors, FGFR1-4, and seven ligands, FGF1-7. While not yet well defined, it appears that the receptors consist of a glycosylated extracellular domain containing a variable number of immunoglobin-like loops and an intracellular domain in which the tyrosine kinase sequence is interrupted by regions of unrelated amino acid sequences.

Still another member of the tyrosine kinase growth factor receptor family is the vascular endothelial growth factor (VEGF") receptor subgroup. VEGF is a dimeric glycoprotein similar to PDGF but has different biological functions and target cell specificity in vivo. In particular, VEGF is presently thought to play an essential role is vasculogenesis and angiogenesis.

A more complete listing of the known RTK subfamilies is described in Plowman et al., DN&P, 7(6):334-339 (1994) which is incorporated by reference, including any drawings, as if fully set forth herein.

In addition to the RTKs, there also exists a family of entirely intracellular PTKs called "non-receptor tyrosine

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kinases" or "cellular tyrosine kinases." This latter designation, abbreviated "CTK," will be used herein. CTKs do not contain extracellular and transmembrane domains. At present, over 24 CTKs in 11 subfamilies (Src, Frk, Btk, Csk, Abl, Zap70, Fes, Fps, Fak, Jak and Ack) have been identified. The Src subfamily appear so far to be the largest group of CTKs and includes Src, Yes, Fyn, Lyn, Lck, Blk, Hck, Fgr and Yrk. For a more detailed discussion of CTKs, see Bolen, Oncogene, 8:2025-2031 (1993), which is incorporated by reference, including any drawings, as if fully set forth herein.

The serine/threonine kinases, STKs, like the CTKs, are predominantly intracellular although there are a few receptor kinases of the STK type. STKs are the most common of the cytosolic kinases; i.e., kinases that perform their function in that part of the cytoplasm other than the cytoplasmic organelles and cytoskelton. The cytosol is the region within the cell where much of the cell's intermediary metabolic and biosynthetic activity occurs; e.g., it is in the cytosol that proteins are synthesized on ribosomes.

RTKs, CTKs and STKs have all been implicated in a host of pathogenic conditions including, significantly, cancer. Other pathogenic conditions which have been associated with PTKs include, without limitation, psoriasis, hepatic cirrhosis, diabetes, angiogenesis, restenosis, ocular diseases, rheumatoid arthritis and other inflammatory disorders, immunological disorders such as autoimmune disease, cardiovascular disease such as atherosclerosis and a variety of renal disorders.

With regard to cancer, two of the major hypotheses advanced to explain the excessive cellular proliferation that drives tumor development relate to functions known to be PK regulated. That is, it has been suggested that malignant cell growth results from

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a breakdown in the mechanisms that control cell division and/or differentiation. It has been shown that the protein products of a number of proto-oncogenes are involved in the signal transduction pathways that regulate cell growth and differentiation. These protein products of proto-oncogenes include the extracellular growth factors, transmembrane growth factor PTK receptors (RTKs), cytoplasmic PTKs (CTKs) and cytosolic STKs, discussed above.

In view of the apparent link between PK-related cellular activities and wide variety of human disorders, it is no surprise that a great deal of effort is being expended in an attempt to identify ways to modulate PK activity. Some of this effort has involved biomimetic approaches using large molecules patterned on those involved in the actual cellular processes (e.g., mutant ligands (U.S. App. No. 4,966,849); soluble receptors and antibodies (App. No. WO 94/10202, Kendall and Thomas, Proc. Nat'l Acad. Sci., 90:10705-09 (1994), Kim, et al., Nature, 362:841-844 (1993)); RNA ligands (Jelinek, et al., Biochemistry, 33:10450-56); Takano, et al., Mol. Bio. Cell 4:358A (1993); Kinsella, et al., Exp. Cell Res. 199:56-62 (1992); Wright, et al., J. Cellular Phys., 152:448-57) and tyrosine kinase inhibitors (WO 94/03427; WO 92/21660; WO 91/15495; WO 94/14808; U.S. Pat. No. 5,330,992; Mariani, et al., Proc. Am. Assoc. Cancer Res., 35:2268 (1994)).

In addition to the above, attempts have been made to identify small molecules which act as PK inhibitors. For example, bis- monocylic, bicyclic and heterocyclic aryl compounds (PCT WO 92/20642), vinyleneazaindole derivatives (PCT WO 94/14808) and 1-cyclopropyl-4-pyridylquinolones (U.S. Pat. No. 5,330,992) have been described as tyrosine kinase inhibitors. Styryl compounds (U.S. Pat. No. 5,217,999), styryl-substituted pyridyl compounds (U.S. Pat. No. 5,302,606), quinazoline

derivatives (EP App. No.0 566 266 A1), selenaindoles and selenides (PCT WO 94/03427), tricyclic polyhydroxylic compounds (PCT WO 92/21660) and benzylphosphonic acid compounds (PCT WO 91/15495) have all been described as PTK inhibitors useful in the treatment of cancer.

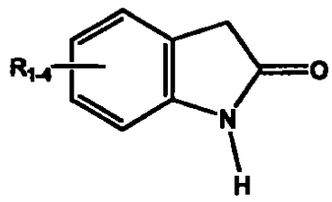
SUMMARY OF THE INVENTION

Our own efforts to identify small organic molecules which modulate PK activity and which, therefore, are expected to be useful in the treatment and prevention of disorders involving abnormal PK activity, has led us to the discovery of a family of pyrrole substituted 2-indolinone compounds, and their prodrugs and physiologically acceptable salts, which exhibit PK modulating ability and are thereby expected to have a salutary effect against disorders related to abnormal PK activity.

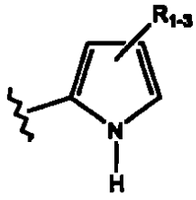
Thus, the present invention relates generally to pyrrole substituted 2-indolinones and their prodrugs and physiologically acceptable salts, which modulate the activity of receptor tyrosine kinases (RTKs), non-receptor protein tyrosine kinases (CTKs) and serine/threonine protein kinases (STKs). In addition, the present invention relates to the preparation and use of pharmaceutical compositions of the disclosed compounds and their physiologically acceptable salts and prodrugs in the treatment or prevention of PK driven disorders such as, by way of example and not limitation, cancer, diabetes, hepatic cirrhosis, cardiovasacular disease such as atherosclerosis, angiogenesis, immunological disease such as autoimmune disease and renal disease.

The terms "2-indolinone," indolin-2-one and "2-oxindole" are used interchangeably herein to refer to a molecule having the chemical structure:

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A "pyrrole" refers to a molecule having the chemical structure:



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"Pyrrole substituted 2-indolinone" and "3-pyrrolidenyl-2-indolinone" are used interchangeably herein to refer to a chemical compound having the general structure shown in Formula 1.

A "pharmaceutical composition" refers to a mixture of one or more of the compounds described herein, or physiologically acceptable salts or prodrugs thereof, with other chemical components, such as physiologically acceptable carriers and excipients. The purpose of a pharmaceutical composition is to facilitate administration of a compound to an organism.

A "prodrug" refers to an agent which is converted into the parent drug in vivo. Prodrugs are often useful because, in some situations, they may be easier to administer than the parent drug. They may, for instance, be bioavailable by oral administration whereas the parent drug is not. The prodrug may also have improved solubility in pharmaceutical compositions over the parent drug. An example, without limitation, of a prodrug

would be a compound of the present invention which is administered as an ester (the "prodrug") to facilitate transmittal across a cell membrane where water solubility is detrimental to mobility but then is metabolically hydrolyzed to the carboxylic acid, the active entity, once inside the cell where water solubility is beneficial.

A further example of a prodrug might be a short polypeptide, for example, without limitation, a 2 - 10 amino acid polypeptide, bonded through a terminal amino group to a carboxy group of a compound of this invention wherein the polypeptide is hydrolyzed or metabolized in vivo to release the active molecule.

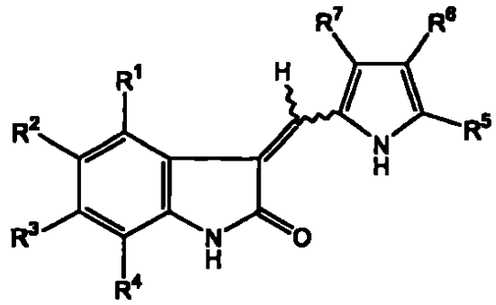
As used herein, a "physiologically acceptable carrier" refers to a carrier or diluent that does not cause significant irritation to an organism and does not abrogate the biological activity and properties of the administered compound.

An "excipient" refers to an inert substance added to a pharmaceutical composition to further facilitate administration of a compound. Examples, without limitation, of excipients include calcium carbonate, calcium phosphate, various sugars and types of starch, cellulose derivatives, gelatin, vegetable oils and polyethylene glycols.

1. CHEMISTRY

A. General Structural Features.

In one aspect, the present invention relates to pyrrole substituted 2-indolinones having the following chemical structure:



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wherein:

R^1 is selected from the group consisting of hydrogen, halo, alkyl, cyclkoalkyl, aryl, heteroaryl, heteroalicyclic, hydroxy, alkoxy, $-C(O)R^{15}$, $-NR^{13}R^{14}$, $-(CH_2)_1R^{16}$ and $-C(O)NR^8R^9$;

R^3 is selected from the group consisting of hydrogen, halo, alkyl, trihalomethyl, hydroxy, alkoxy, $-NR^{13}R^{14}$, $-NR^{13}C(O)R^{14}$, $-C(O)R^{15}$, aryl, heteroaryl and $-S(O)_1NR^{13}R^{14}$;

R^3 is selected from the group consisting of hydrogen, halogen, alkyl, trihalomethyl, hydroxy, alkoxy, $-C(O)R^{15}$, $-NR^{13}R^{14}$, aryl, heteroaryl, $-NR^{13}S(O)_2R^{14}$, $-S(O)_2NR^{13}R^{14}$, $-NR^{13}C(O)R^{14}$, and $-NR^{13}C(O)OR^{14}$;

R^4 is selected from the group consisting of hydrogen, halogen, alkyl, hydroxy, alkoxy and $-NR^{13}R^{14}$;

is selected from the group consisting of hydrogen, alkyl and $-C(O)R^{10}$;

R^6 is selected from the group consisting of hydrogen, alkyl and $-C(O)R^{10}$;

R^7 is selected from the group consisting of hydrogen, alkyl, aryl, heteroaryl, $-C(O)R^{17}$ and $-C(O)R^{10}$;

R^6 and R^7 may combine to form a group selected from the group consisting of $-(CH_2)_4-$, $-(CH_2)_5-$ and $-(CH_2)_6-$;

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with the proviso that at least one of R^5, R^6 or R^7 must be $-C(O)R^{10}$;

R^8 and R^9 are independently selected from the group consisting of hydrogen, alkyl and aryl;

R^{10} is selected from the group consisting of hydroxy, alkoxy, aryloxy, $-N(R^{11})(CH_2)_nR^{12}$ and $-NR^{13}R^{14}$;

R^{11} is selected from the group consisting of hydrogen and alkyl;

R^{12} is selected from the group consisting of $-NR^{13}R^{14}$, hydroxy, $-C(O)R^{15}$, aryl and heteroaryl;

R^{13} and R^{14} are independently selected from the group consisting of hydrogen, alkyl, cycloalkyl, aryl and heteroaryl;

R^{13} and R^{14} may combine to form a group selected from the group consisting of $-(CH_2)_4-$, $-(CH_2)_5-$, $-(CH_2)_2O(CH_2)_2-$, and $-(CH_2)_2N(CH_3)(CH_2)_2-$;

R^{15} is selected from the group consisting of hydrogen, hydroxy, alkoxy and aryloxy;

R^{16} is selected from the group consisting of hydroxy, $-C(O)R^{15}$, $-NR^{13}R^{14}$ and $C(O)NR^{13}R^{14}$;

R^{17} is selected from the group consisting of alkyl, cycloalkyl, aryl and heteroaryl; and,

n and r are independently 1, 2, 3, or 4.

An additional aspect of this invention is a pharmaceutically acceptable salt of a compound hereof.

As used herein, the term "alkyl" refers to a saturated aliphatic hydrocarbon including straight chain and branched chain groups. Preferably, the alkyl group has 1 to 20 carbon atoms (whenever a numerical range; e.g. "1-20", is stated herein, it means that the group, in this case the alkyl group, may contain 1 carbon atom, 2 carbon atoms, 3 carbon atoms, etc. up to and including 20 carbon atoms). More preferably, it is a medium size alkyl having 1 to 10 carbon atoms. Most preferably, it is a

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lower alkyl having 1 to 4 carbon atoms. The alkyl group may be substituted or unsubstituted. When substituted, the substituent group(s) is preferably one or more independently selected from the group consisting of halo, hydroxy, unsubstituted lower alkoxy, aryl optionally substituted with one or more halo, hydroxy, unsubstituted lower alkyl or unsubstituted lower alkoxy groups, aryloxy optionally substituted with one or more halo, hydroxy, unsubstituted lower alkyl or unsubstituted lower alkoxy groups, 6-member heteroaryl having from 1 to 3 nitrogen atoms in the ring, the carbons in the ring being optionally substituted with one or more halo, hydroxy, unsubstituted lower alkyl or unsubstituted lower alkoxy groups, 5-member heteroaryl having from 1 to 3 heteroatoms selected from the group consisting of nitrogen, oxygen and sulfur, the carbon atoms of the group being optionally substituted with one or more halo, hydroxy, unsubstituted lower alkyl or unsubstituted lower alkoxy groups, 5- or 6-member heteroalicyclic group having from 1 to 3 heteroatoms selected from the group consisting of nitrogen, oxygen and sulfur, the carbon and nitrogen (if present) atoms in the group being optionally substituted with one or more halo, hydroxy, unsubstituted lower alkyl or unsubstituted lower alkoxy groups, mercapto, (unsubstituted lower alkyl)thio, arylthio optionally substituted with one or more halo, hydroxy, unsubstituted lower alkyl or unsubstituted lower alkoxy groups, cyano, acyl, thioacyl, O-carbamyl, N-carbamyl, O-thiocarbamyl, N-thiocarbamyl, C-amido, N-amido, nitro, N-sulfonamido, S-sulfonamido, $R^{18}S(O)$-, $R^{18}S(O)_2$-, $-C(O)OR^{18}$, $R^{18}C(O)O$-, and $-NR^{18}R^{19}$, wherein R^{18} and R^{19} are independently selected from the group consisting of hydrogen, unsubstituted lower alkyl, trihalomethyl, unsubstituted (C_3-C_6)cycloalkyl, unsubstituted lower alkenyl, unsubstituted lower alkynyl and aryl optionally optionally

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substituted with one or more halo, hydroxy, unsubstituted lower alkyl or unsubstituted lower alkoxy groups.

A "cycloalkyl" group refers to a 3 to 8 member all-carbon monocyclic ring, an all-carbon 5-member/6-member or 6-member/6-member fused bicyclic ring or a multicyclic fused ring (a "fused" ring system means that each ring in the system shares an adjacent pair of carbon atoms with each other ring in the system) group wherein one or more of the rings may contain one or more double bonds but none of the rings has a completely conjugated pi-electron system. Examples, without limitation, of cycloalkyl groups are cyclopropane, cyclobutane, cyclopentane, cyclopentene, cyclohexane, cyclohexadiene, adamantane, cycloheptane and, cycloheptatriene. A cycloalkyl group may be substituted or unsubstituted. When substituted, the substituent group(s) is preferably one or more independently selected from the group consisting of unsubstituted lower alkyl, trihaloalkyl, halo, hydroxy, unsubstituted lower alkoxy, aryl optionally substituted with one or more halo, hydroxy, unsubstituted lower alkyl or unsubstituted lower alkoxy groups, aryloxy optionally substituted with one or more halo, hydroxy, unsubstituted lower alkyl or unsubstituted lower alkoxy groups, 6-member heteroaryl having from 1 to 3 nitrogen atoms in the ring, the carbons in the ring being optionally substituted with one or more halo, hydroxy, unsubstituted lower alkyl or unsubstituted lower alkoxy groups, 5-member heteroaryl having from 1 to 3 heteroatoms selected from the group consisting of nitrogen, oxygen and sulfur, the carbon atoms of the group being optionally substituted with one or more halo, hydroxy, unsubstituted lower alkyl or unsubstituted lower alkoxy groups, 5- or 6-member heteroalicyclic group having from 1 to 3 heteroatoms selected from the group consisting of nitrogen, oxygen and sulfur, the carbon and nitogen (if present)atoms in

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the group being optionally substituted with one or more halo, hydroxy, unsubstituted lower alkyl or unsubstituted lower alkoxy groups, mercapto, (unsubstituted lower alkyl)thio, arylthio optionally substituted with one or more halo, hydroxy, unsubstituted lower alkyl or unsubstituted lower alkoxy groups, cyano, acyl, thioacyl, O-carbamyl, N-carbamyl, O-thiocarbamyl, N-thiocarbamyl, C-amido, N-amido, nitro, N-sulfonamido, S-sulfonamido, $R^{18}S(O)$-, $R^{18}S(O)_2$-, $-C(O)OR^{18}$, $R^{18}C(O)O$-, and $-NR^{18}R^{19}$ are as defined above.

An "alkenyl" group refers to an alkyl group, as defined herein, consisting of at least two carbon atoms and at least one carbon-carbon double bond.

An "alkynyl" group refers to an alkyl group, as defined herein, consisting of at least two carbon atoms and at least one carbon-carbon triple bond.

An "aryl" group refers to an all-carbon monocyclic or fused-ring polycyclic (i.e., rings which share adjacent pairs of carbon atoms) groups having a completely conjugated pi-electron system. Examples, without limitation, of aryl groups are phenyl, naphthalenyl and anthracenyl. The aryl group may be substituted or unsubstituted. When substituted, the substituted group(s) is preferably one or more independently selected from the group consisting of unsubstituted lower alkyl, trihaloalkyl, halo, hydroxy, unsubstituted lower alkoxy, mercapto, (unsubstituted lower alkyl)thio, cyano, acyl, thioacyl, O-carbamyl, N-carbamyl, O-thiocarbamyl, N-thiocarbamyl, C-amido, N-amido, nitro, N-sulfonamido, S-sulfonamido, $R^{18}S(O)$-, $R^{18}S(O)_2$-, $-C(O)OR^{18}$, $R^{18}C(O)O$-, and $-NR^{18}R^{19}$, with R^{18} and R^{19} as defined above.

As used herein, a "heteroaryl" group refers to a monocyclic or fused ring (i.e., rings which share an adjacent pair of atoms) group having in the ring(s) one or more atoms selected from the

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group consisting of nitrogen, oxygen and sulfur and, in addition, having a completely conjugated pi-electron system. Examples, without limitation, of heteroaryl groups are pyrrole, furan, thiophene, imidazole, oxazole, thiazole, pyrazole, pyridine, pyrimidine, quinoline, isoquinoline, purine and carbazole. The heteroaryl group may be substituted or unsubstituted. When substituted, the substituted group(s) is preferably one or more independently selected from the group consisting of unsubstituted lower alkyl, trihaloalkyl, halo, hydroxy, unsubstituted lower alkoxy, mercapto, (unsubstituted lower alkyl)thio, cyano, acyl, thioacyl, O-carbamyl, N-carbamyl, O-thiocarbamyl, N-thiocarbamyl, C-amido, N-amido, nitro, N-sulfonamido, S-sulfonamido, $R^{18}S(O)$-, $R^{18}O)_2$-, $-C(O)OR^{18}$, $R^{18}C(O)O$-, and $-NR^{18}R^{19}$, with R^{18} and R^{19} as defined above.

A "heteroalicyclic" group refers to a monocyclic or fused ring group having in the ring(s) one or more atoms selected from the group consisting of nitrogen, oxygen and sulfur. The rings may also have one or more double bonds. However, the rings do not have a completely conjugated pi-electron system. The heteroalicyclic ring may be substituted or unsubstituted. When substituted, the substituted group(s) is preferably one or more independently selected from the group consisting of unsubstituted lower alkyl, trihaloalkyl, halo, hydroxy, unsubstituted lower alkoxy, mercapto, (unsubstituted lower alkyl)thio, cyano, acyl, thioacyl, O-carbamyl, N-carbamyl, O-thiocarbamyl, N-thiocarbamyl, C-amido, N-amido, nitro, N-sulfonamido, S-sulfonamido, $R^{18}S(O)$-, $R^{18}S(O)_2$-, $-C(O)OR^{18}$, $R^{18}C(O)O$-, and $-NR^{18}R^{19}$, with R^{18} and R^{19} as defined above.

A "hydroxy" group refers to an -OH group.

An "alkoxy" group refers to both an -O-(unsubstituted alkyl) and an -O-(unsubstituted cycloalkyl) group.

An "aryloxy" group refers to both an -O-aryl and an -O-heteroaryl group, as defined herein.

A "mercapto" group refers to an -SH group.

A "alkylthio" group refers to both an S-(unsubstituted alkyl) and an -S-(unsubstituted cycloalkyl) group.

A "arylthio" group refers to both an -S-aryl and an -S-heteroaryl group, as defined herein.

An "acyl" group refers to a -C(O)-R" group, where R" is selected from the group consisting of hydrogen, unsubstituted lower alkyl, trihalomethyl, unsubstituted cycloalkyl, aryl optionally substituted with one or more unsubstituted lower alkyl, trihalomethyl, unsubstituted lower alkoxy, halo and $-NR^{18}R^{19}$ groups, heteroaryl (bonded through a ring carbon) optionally substituted with one or more unsubstituted lower alkyl, trihaloalkyl, unsubstituted lower alkoxy, halo and $-NR^{18}R^{19}$ groups and heteroalicyclic (bonded through a ring carbon) optionally substituted with one or more unsubstituted lower alkyl, trihaloalkyl, unsubstituted lower alkoxy, halo and $-NR^{18}R^{19}$ groups.

An "aldehyde" group refers to an acyl group in which R" is hydrogen.

A "thioacyl" group refers to a -C(S)-R" group, with R" as defined herein.

An "ester" group refers to a -C(O)O-R" group with R" as defined herein except that R" cannot be hydrogen.

An "acetyl" group refers to a $-C(O)CH_3$ group.

A "halo" group refers to fluorine, chlorine, bromine or iodine.

A "trihalomethyl" group refers to a $-CX_3$ group wherein X is a halo group as defined herein.

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A "trihalomethanesulfonyl" group refers to a $X_3CS(=O)_2$- groups with X as defined above.

A "cyano" group refers to a -C≡N group.

A "methylenedioxy" group refers to a $-OCH_2O-$ group where the two oxygen atoms are bonded to adjacent carbon atoms.

An "ethylenedioxy" group refers to a $-OCH_2CH_2O-$ where the two oxygen atoms are bonded to adjacent carbon atoms.

An "S-sulfonamido" group refers to a $-S(O)_2NR^{18}R^{19}$ group, with R^{18} and R^{19} as defined herein.

An "N-sulfonamido" group refers to a $-NR^{18}S(O)_2R^{19}$ group, with R^{18} and R^{19} as defined herein.

An "O-carbamyl" group refers to a $-OC(O)NR^{18}R^{19}$ group with R^{18} and R^{19} as defined herein.

An "N-carbamyl" group refers to an $R^{18}OC(O)NR^{19}$- group, with R^{18} and R^{19} as defined herein.

An "O-thiocarbamyl" group refers to a $-OC(S)NR^{18}R^{19}$ group with R^{18} and R^{19} as defined herein.

An "N-thiocarbamyl" group refers to a $R^{18}OC(S)NR^{19}$- group, with R^{18} and R^{19} as defined herein.

An "amino" group refers to an $-NR^{18}R^{19}$ group, wherein R^{18} and R^{19} are both hydrogen.

A "C-amido" group refers to a $-C(O)NR^{18}R^{19}$ group with R^{18} and R^{19} as defined herein.

An "N-amido" group refers to a $R^{18}C(O)NR^{19}$- group, with R^{18} and R^{19} as defined herein.

A "nitro" group refers to a $-NO_2$ group.

B. Preferred Structural Features.

A presently preferred embodiment of this invention is a compound in which:

R^1 and R^4 are hydrogen;

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R^2 is selected from the group consisting of hydrogen, halo, lower alkoxy, $-C(O)R^{15}$ and $-S(O)_2NR^{13}R^{14}$;

R^3 is selected from the group consisting of hydrogen, lower alkoxy, $-C(O)R^{15}$, $-S(O)_2NR^{13}R^{14}$, aryl and heteroaryl;

R^5 is $-C(O)R^{10}$;

R^6 is selected from the group consisting of hydrogen and lower alkyl; and,

R^7 is selected from the group consisting of hydrogen, lower alkyl and $-C(O)R^{17}$.

It is another presently preferred embodiment of this invention that, in a compound having a structure as described in the previous paragraph:

R^{10} is selected from the group consisting of hydroxy, lower alkoxy and $-NR^{11}(CH_2)_nR^{12}$, wherein

n is 2 or 3;

R^{11} is selected from the group consisting of hydrogen and lower alkyl; and,

R^{12} is selected from the group consisting of aryl and $-NR^{13}R^{14}$.

It is a further presently preferred embodiment of this invention that, in a compound having a structure as described in the previous two paragraphs, R^{13} and R^{14} are independently selected from the group consisting of hydrogen, lower alkyl, and, combined, $-(CH_2)_4-$, $-(CH_2)_5-$, $-(CH_2)_2O(CH_2)_2-$ or $-(CH_2)_2N(CH_3)(CH_2)_2-$.

A presently preferred embodiment of this invention is a compound in which:

R^1 is selected from the group consisting of hydrogen, lower alkyl, $(CH_2)_rR^{16}$ and $-C(O)NR^8R^9$;

R^2 is selected from the group consisting of hydrogen, halogen, aryl and $-S(O)_2NR^{13}R^{14}$;

R^3 is selected from the group consisting of hydrogen, lower alkyl, lower alkoxy, aryl, heteroaryl and $-C(O)R^{15}$;

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R^4 is hydrogen;

R^5 is selected from the group consisting of hydrogen and lower alkyl;

R^6 is $-C(O)R^{10}$;

R^7 is selected from the group consisting of hydorgen, lower alkyl and aryl;

R^{16} is selected from the group consisting of hydroxy and $-C(O)R^{15}$; and,

r is 2 or 3.

A presently preferred embodiment of this invention is a compound having as structure described in the paragraph just above in which R^3 is aryl optionally substituted with one or more groups selected from the group consisting of lower alkyl, lower alkoxy and halo.

Likewise, it is a presently preferred embodiment of this invention that, in a compound in which:

R^1 is selected from the group consisting of hydrogen, lower alkyl, $(CH_2)_rR^{16}$ and $-C(O)NR^9R^9$;

R^2 is selected from the group consisting of hydrogen, halogen, aryl and $-S(O)_2NR^{13}R^{14}$;

R^3 is selected from the group consisting of hydrogen, lower alkyl, lower alkoxy, aryl, heteroaryl and $-C(O)R^{15}$;

R^4 is hydrogen;

R^5 is selected from the group consisting of hydrogen and lower alkyl;

R^6 is $-C(O)R^{10}$;

R^7 is selected from the group consisting of hydorgen, lower alkyl and aryl;

R^{16} is selected from the group consisting of hydroxy and $-C(O)R^{15}$; and,

r is 2 or 3,

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R^{16} is selected from the group consisting of hydroxy, lower alkoxy, $-NR^{11}R^{14}$ and $-NR^{11}(CH_2)_nR^{12}$, wherein n is 1, 2 or 3, R^{11} is hydrogen and R^{12} is selected from the group consisting of hydroxy, lower alkoxy, $-C(O)R^{15}$, heteroaryl and $-NR^{13}R^{14}$.

A further presently preferred embodiment of this invention is a compound having a structure as described in the paragraph immediately above in which R^{13} and R^{14} are independently selected from the group consisting of hydrogen, lower alkyl, heteroaryl and, combined, $-(CH_2)_4-$, $-(CH_2)_5-$, $-(CH_2)_2O(CH_2)_2-$, or $-(CH_2)_2N(CH_3)(CH_2)_2-$.

Another presently preferred embodiment of this invention is a compound in which:

R^1 is $-C(O)NR^8R^9$, wherein R^8 is hydrogen and R^9 is aryl optionally substituted with one or more groups selected from the group consisting of halo, hydroxy and lower alkoxy;

R^2 is selected from the group consisting of hydrogen, halogen, aryl and $-S(O)_2NR^{13}R^{14}$;

R^3 is selected from the group consisting of hydrogen, lower alkyl, lower alkoxy, aryl, heteroaryl and $-C(O)R^{15}$;

R^4 is hydrogen;

R^5 is selected from the group consisting of hydrogen and lower alkyl;

R^6 is $-C(O)R^{16}$;

R^7 is selected from the group consisting of hydorgen, lower alkyl and aryl;

R^{16} is selected from the group consisting of hydroxy and $-C(O)R^{15}$; and,

r is 2 or 3,

A still further presently preferred embodiment of this invention is a compound in which:

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R^1 is selected from the group consisting of hydrogen and lower alkyl;

R^2 is selected from the group consisting of hydrogen, halo, lower alkoxy, aryl, $-C(O)R^{15}$ and $-S(O)_2NR^{13}R^{14}$;

R^3 is selected from the group consisting of hydrogen, halo, aryl, heteroaryl and $-C(O)R^{15}$;

R^4 is hydrogen;

R^5 is $-C(O)R^{10}$; and,

R^6 and R^7 combine to form a $-(CH_2)_4-$ group.

In a compound having a structure as described in the paragraph immediately above, it is a presently preferred embodiment that R^{10} is selected from the group consisting of hydroxy, alkoxy, $-NR^{13}R^{14}$ and $-NH(CH_2)_nNR^{13}R^{14}$ wherein n is 2 or 3.

It is a presently preferred embodiment of this invention that, in a compound having a structure as described in the two paragraphs immediately above, R^{13} and R^{14} are independently selected from the group consisting of hydrogen, lower alkyl, and, combined, $-(CH_2)_4-$, $-(CH_2)_5-$, $-(CH_2)_2O(CH_2)_2-$ or $-(CH_2)_2N(CH_3)(CH_2)_2-$.

A compound in which:

R^1 and R^4 are selected from the group consisting of hydrogen, lower alkyl, lower alkoxy and halogen;

R^2 is selected from the group consisting of hydrogen, lower alkyl, lower alkoxy, halo, and $-S(O)_2NR^{13}R^{14}$;

R^3 is selected from the group consisting of hydrogen, lower alkyl, lower alkoxy, halo, aryl and $-C(O)R^{15}$;

R^5 and R^6 are selected from the group consisting of hydrogen and lower alkyl; and,

R^7 is $-C(O)R^{10}$ is also a presently preferred embodiment of this invention.

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Finally, with regard to chemical structure, in a compound having a structure as described in the paragraph immediately above, R^{10} is selected from the group consisting of hydroxy, alkoxy, $-NR^{13}R^{14}$ and $-NR^{11}(CH_2)_nR^{12}$, wherein n is 2 or 3, R^{11} is selected from the group consisting of hydrogen and lower alkyl; and, R^{12} is $-NR^{13}R^{14}$, wherein R^{13} and R^{14} are independently selected from the group consisting of hydrogen, lower alkyl, and, combined, $-(CH_2)_4-$, $-(CH_2)_5-$, $-(CH_2)_2O(CH_2)_2-$ or $-(CH_2)_2N(CH_3)(CH_2)_2-$.

The chemical formulae referred to herein may exhibit the phenomena of tautomerism and structural isomerism. For example, the compounds described herein may adopt an E or a Z configuration about the double bond connecting the 2-indolinone moiety to the pyrrole moiety or they may be a mixture of E and Z. This invention encompasses any tautomeric or structural isomeric form and mixtures thereof which possess the ability to modulate RTK, CTK and/or STK activity and is not limited to any one tautomeric or structural isomeric form.

2. BIOCHEMISTRY/PHARMACOTHERAPY

Another aspect of this invention relates to a method for the modulation of the catalytic activity of a PK by contacting a PK with a compound of this invention or a physiologically acceptable salt or prodrug thereof.

As used herein, "PK" refers to receptor protein tyrosine kinase (RTKs), non-receptor or "cellular" tyrosine kinase (CTKs) and serine-threonine kinases (STKs).

The term "method" refers to manners, means, techniques and procedures for accomplishing a given task including, but not limited to, those manners, means, techniques and procedures either known to, or readily developed from known manners, means,

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techniques and procedures by, practitioners of the chemical, pharmaceutical, biological, biochemical and medical arts.

As used herein, the term "modulation" or "modulating" refers to the alteration of the catalytic activity of RTKs, CTKs and STKs. In particular, modulating refers to the activation of the catalytic activity of RTKs, CTKs and STKs, preferably the activation or inhibition of the catalytic activity of RTKs, CTKs and STKs, depending on the concentration of the compound or salt to which the RTK, CTK or STK is exposed or, more preferably, the inhibition of the catalytic activity of RTKs, CTKs and STKs.

The term "catalytic activity" as used herein refers to the rate of phosphorylation of tyrosine under the influence, direct or indirect, of RTKs and/or CTKs or the phosphorylation of serine and threonine under the influence, direct or indirect, of STKs.

The term "contacting" as used herein refers to bringing a compound of this invention and a target PK together in such a manner that the compound can affect the catalytic activity of the PK, either directly, i.e., by interacting with the kinase itself, or indirectly, i.e., by interacting with another molecule on which the catalytic activity of the kinase is dependent. Such "contacting" can be accomplished "in vitro," i.e., in a test tube, a petri dish or the like. In a test tube, contacting may involve only a compound and a PK of interest or it may involve whole cells. Cells may also be maintained or grown in cell culture dishes and contacted with a compound in that environment. In this context, the ability of a particular compound to affect a PK related disorder, i.e., the IC_{50} of the compound, defined below, can be determined before use of the compounds in vivo with more complex living organisms is attempted. For cells outside the organism, multiple methods exist, and are well-known to those skilled in the art, to get the PKs in contact with the compounds including, but

not limited to, direct cell microinjection and numerous transmembrane carrier techniques.

A further aspect of this invention is that the modulation of the catalytic activity of PKs using a compound of this invention may be carried out in vitro or in vivo.

"In vitro" refers to procedures performed in an artificial environment such as, e.g., without limitation, in a test tube or culture medium.

As used herein, "in vivo" refers to procedures performed within a living organism such as, without limitation, a mouse, rat or rabbit.

A still further aspect of this invention is that the protein kinase whose catalytic activity is being modulated by a compound of this invention is selected from the group consisting of receptor protein tyrosine kinases, cellular (or non-receptor) tyrosine kinases and serine-threonine kinases.

It is an aspect of this invention that the receptor protein kinase whose catalytic activity is modulated by a compound of this invention is selected from the group consisting of EGF, HER2, HER3, HER4, IR, IGF-1R, IRR, PDGFRα, PDGFRβ, CSFIR, C-Kit, C-fms, Flk-1R, Flk4, KDR/Flk-1, Flt-1, FGFR-1R, FGFR-2R, FGFR-3R and FGFR-4R.

In addition, it is an aspect of this invention that the cellular tyrosine kinase whose catalytic activity is modulated by a compound of this invention is selected from the group consisting of Src, Frk, Btk, Csk, Abl, ZAP70, Fes/Fps, Fak, Jak, Ack, Yes, Fyn, Lyn, Lck, Blk, Hck, Fgr and Yrk.

Another aspect of this invention is that the serine-threonine protein kinase whose catalytic activity is modulated by a compound of this invention is selected from the group consisting of CDK2 and Raf.

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A pharmaceutical composition of a compound of this invention with a pharmaceutically acceptable carrier or excipient is yet another aspect of this invention. Such pharmaceutical composition may contain both carriers and excipients as well as other components generally known to those skilled in the formulation of pharmaceutical compositions.

A method for treating or preventing a protein kinase related disorder in an organism comprising administering a therapeutically effective amount of a compound, salt or prodrug of this invention to an organism in need of such treatment is another aspect of this invention.

As used herein, "PK related disorder," "PK driven disorder," and "abnormal PK activity" all refer to a condition characterized by inappropriate, i.e., under or, more commonly, over, PK catalytic activity, where the particular PK can be an RTK, a CTK or an STK. Inappropriate catalytic activity can arise as the result of either: (1) PK expression in cells which normally do not express PKs, (2) increased PK expression leading to unwanted cell proliferation, differentiation and/or growth, or, (3) decreased PK expression leading to unwanted reductions in cell proliferation, differentiation and/or growth. Over-activity of a PK refers to either amplification of the gene encoding a particular PK or production of a level of PK activity which can correlate with a cell proliferation, differentiation and/or growth disorder (that is, as the level of the PK increases, the severity of one or more of the symptoms of the cellular disorder increases). Under-activity is, of course, the converse, wherein the severity of one or more symptoms of a cellular disorder increase as the level of the PK activity decreases.

As used herein, the terms "prevent", "preventing" and "prevention" refer to a method for barring an organism from acquiring a PK related disorder in the first place.

As used herein, the terms "treat", "treating" and "treatment" refer to a method of alleviating or abrogating a PK mediated cellular disorder and/or its attendant symptoms. With regard particularly to cancer, these terms simply mean that the life expectancy of an individual affected with a cancer will be increased or that one or more of the symptoms of the disease will be reduced.

The term "organism" refers to any living entity comprised of at least one cell. A living organism can be as simple as, for example, a single eukariotic cell or as complex as a mammal, including a human being.

The term "therapeutically effective amount" as used herein refers to that amount of the compound being administered which will relieve to some extent one or more of the symptoms of the disorder being treated. In reference to the treatment of cancer, a therapeutically effective amount refers to that amount which has the effect of (1) reducing the size of the tumor, (2) inhibiting (that is, slowing to some extent, preferably stopping) tumor metastasis, (3) inhibiting to some extent (that is, slowing to some extent, preferably stopping) tumor growth, and/or, (4) relieving to some extent (or, preferably, eliminating) one or more symptoms associated with the cancer.

It is an aspect of this invention that the above-referenced protein kinase related disorder is selected from the group consisting of a receptor protein tyrosine kinase related disorder, a cellular tyrosine kinase disorder and a serine-threonine kinase related disorder.

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In yet another aspect of this invention, the above referenced protein kinase related disorder is selected from the group consisting of an EGFR related disorder, a PDGFR related disorder, an IGFR related disorder and a flk related disorder.

The above referenced protein kinase related disorder is a cancer selected from the group consisting of squamous cell carcinoma, sarcomas such as Kaposi's sarcoma, astrocytoma, glioblastoma, lung cancer, bladder cancer, colorectal cancer, gastrointestinal cancer, head and neck cancer, melanoma, ovarian cancer, prostate cancer, breast cancer, small-cell lung cancer and glioma in a further aspect of this invention.

The above referenced protein kinase related disorder is selected from the group consisting of diabetes, an autoimmune disorder, a hyperproliferation disorder, von Hippel-Lindau disease, restenosis, fibrosis, psoriasis, osteoarthritis, rheumatoid arthritis, an inflammatory disorder and angiogenesis in yet another aspect of this invention.

Additional disorders which may be treated or prevented using the compounds of this invention are immunological disorders such as autoimmune disease, AIDS and cardiovasular disorders such as atherosclerosis.

It is as aspect of this invention that a chemical compound that modulates the catalytic activity of a protein kinase may be identified by contacting cells expressing said protein kinase with a compound, salt or prodrug of the present invention and then monitoring said cells for an effect.

By "monitoring" is meant observing or detecting the effect of contacting a compound with a cell expressing a particular PK. The observed or detected effect can be a change in cell phenotype, in the catalytic activity of a PK or a change in the interaction of a PK with a natural binding partner. Techniques

for observing or detecting such effects are well-known in the art.

The above-referenced effect is selected from a change or an absence of change in a cell phenotype, a change or absence of change in the catalytic activity of said protein kinase or a change or absence of change in the interaction of said protein kinase with a natural binding partner in a final aspect of this invention.

"Cell phenotype" refers to the outward appearance of a cell or tissue or the biological function of the cell or tissue. Examples, without limitation, of a cell phenotype are cell size, cell growth, cell proliferation, cell differentiation, cell survival, apoptosis, and nutrient uptake and use. Such phenotypic characteristics are measurable by techniques well-known in the art.

A "natural binding partner" refers to a polypeptide that binds to a particular PK in a cell. Natural binding partners can play a role in propagating a signal in a PK-mediated signal transduction process. A change in the interaction of the natural binding partner with the PK can manifest itself as an increased or decreased concentration of the PK/natural binding partner complex and, as a result, in an observable change in the ability of the PK to mediate signal transduction.

It is also an aspect of this invention that a compound described herein, or its salt or prodrug, might be combined with other chemotherapeutic agents for the treatment of the diseases and disorders discussed above. For instance, a compound, salt or prodrug of this invention might be combined with alkylating agents such as fluorouracil (5-FU) alone or in further combination with leukovorin; or other alkylating agents such as, without limitation, other pyrimidine analogs such as UFT,

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capecitabine, gemcitabine and cytarabine, the alkyl sulfonates, e.g., busulfan (used in the treatment of chronic granulocytic leukemia), improsulfan and piposulfan; aziridines, e.g., benzodepa, carboquone, meturedepa and uredepa; ethyleneimines and methylmelamines, e.g., altretamine, triethylenemelamine, triethylenephosphoramide, triethylenethiophosphoramide and trimethylolmelamine; and the nitrogen mustards, e.g., chlorambucil (used in the treatment of chronic lymphocytic leukemia, primary macroglobulinemia and non-Hodgkin's lymphoma), cyclophosphamide (used in the treatment of Hodgkin's disease, multiple myeloma, neuroblastoma, breast cancer, ovarian cancer, lung cancer, Wilm's tumor and rhabdomyosarcoma), estramustine, ifosfamide, novembrichin, prednimustine and uracil mustard (used in the treatment of primary thrombocytosis, non-Hodgkin's lymphoma, Hodgkin's disease and ovarian cancer); and triazines, e.g., dacarbazine (used in the treatment of soft tissue sarcoma).

Likewise a compound, salt or prodrug of this invention might be expected to have a beneficial effect in combination with other antimetabolite chemotherapeutic agents such as, without limitation, folic acid analogs, e.g. methotrexate (used in the treatment of acute lymphocytic leukemia, choriocarcinoma, mycosis fungiodes breast cancer, head and neck cancer and osteogenic sarcoma) and pteropterin; and the purine analogs such as mercaptopurine and thioguanine which find use in the treatment of acute granulocytic, acute lymphocytic and chronic granulocytic leukemias.

A compound, salt or prodrug of this invention might also be expected to prove efficacious in combination with natural product based chemotherapeutic agents such as, without limitation, the vinca alkaloids, e.g., vinblastin (used in the treatment of breast and testicular cancer), vincristine and vindesine; the

epipodophylotoxins, e.g., etoposide and teniposide, both of which are useful in the treatment of testicular cancer and Kaposi's sarcoma; the antibiotic chemotherapeutic agents, e.g., daunorubicin, doxorubicin, epirubicin, mitomycin (used to treat stomach, cervix, colon, breast, bladder and pancreatic cancer), dactinomycin, temozolomide, plicamycin, bleomycin (used in the treatment of skin, esophagus and genitourinary tract cancer); and the enzymatic chemotherapeutic agents such as L-asparaginase.

In addition to the above, a compound, salt or prodrug of this invention might be expected to have a beneficial effect used in combination with the platinum coordination complexes (cisplatin, etc.); substituted ureas such as hydroxyurea; methylhydrazine derivatives, e.g., procarbazine; adrenocortical suppressants, e.g., mitotane, aminoglutethimide; and hormone and hormone antagonists such as the adrenocorticosteriods (e.g., prednisone), progestins (e.g., hydroxyprogesterone caproate); estrogens (e.g., diethylstilbesterol); antiestrogens such as tamoxifen; androgens, e.g., testosterone propionate; and aromatase inhibitors such as anastrozole.

Finally, the combination of a compound of this invention might be expected to be particularly effective in combination with mitoxantrone or paclitaxel for the treatment of solid tumor cancers or leukemias such as, without limitation, acute myelogenous (non-lymphocytic) leukemia.

DETAILED DESCRIPTION OF THE INVENTION

1. BRIEF DESCRIPTION OF THE TABLES

TABLE 1 shows the chemical structures of some exemplary compounds of this invention. The compound numbers correspond to the Example numbers in the Examples section. That is, the synthesis of Compound 1 in Table 1 is described in Example 1. The

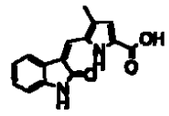
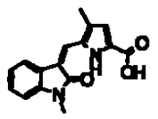
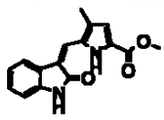
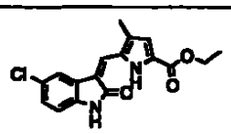
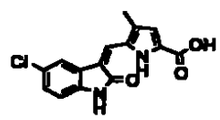
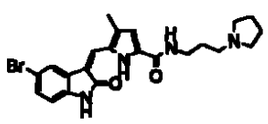
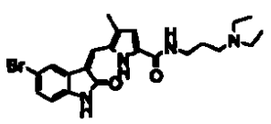
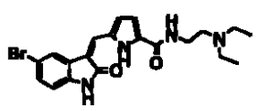
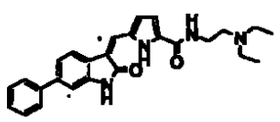
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compounds presented in Table 1 are exemplary only and are not to be construed as limiting the scope of this invention in any manner.

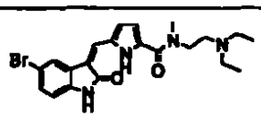
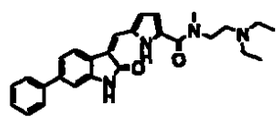
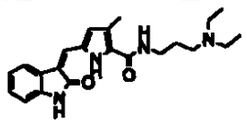
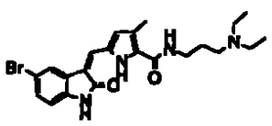
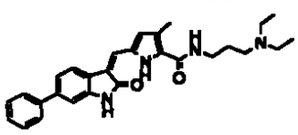
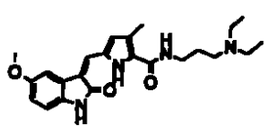
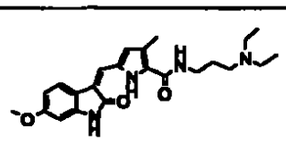
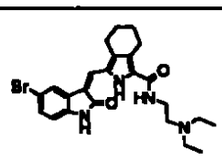
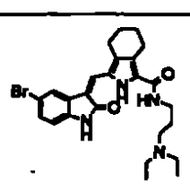
TABLE 2 shows the results of biological testing of some exemplary compounds of this invention. The results are reported in terms of IC_{50}, the micromolar (μM) concentration of the compound being tested which causes a 50% change in the activity of the target PKT compared to the activity of the PTK in a control to which no test compound has been added. Specifically, the results shown indicate the concentration of a test compound needed to cause a 50% reduction of the activity of the target PTK. Bioassays which have been or mau be used to evaluate compounds are described in detail below.

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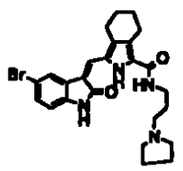
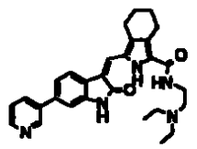
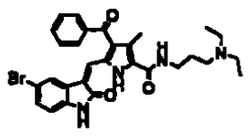
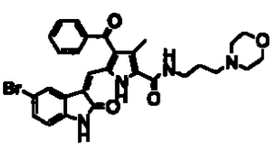
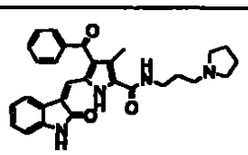
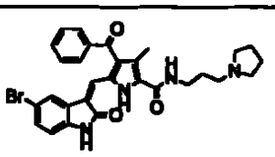
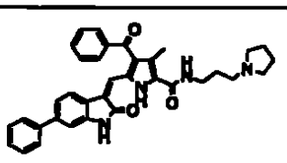
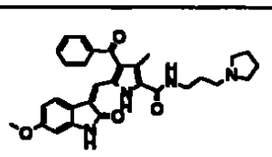
TABLE 1

Example	Structure	Name
1		4-Methyl-5-(2-oxo-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrole-2-carboxylic acid
2		4-Methyl-5-(1-methyl-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrole-2-carboxylic acid
3		4-Methyl-5-(2-oxo-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrole-2-carboxylic acid methyl ester
4		5-(5-Chloro-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-4-methyl-1H-pyrrole-2-carboxylic acid ethyl ester
5		5-(5-Chloro-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-4-methyl-1H-pyrrole-2-carboxylic acid
6		5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-4-methyl-1H-pyrrole-2-carboxylic acid (3-pyrrolidin-1-ylpropyl)amide
7		5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-4-methyl-1H-pyrrole-2-carboxylic acid (3-diethylaminopropyl)amide
8		5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrole-2-carboxylic acid (2-diethylaminoethyl)amide
9		5-(2-Oxo-6-phenyl-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrole-2-carboxylic acid (2-diethylaminoethyl)amide

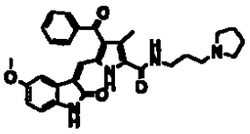
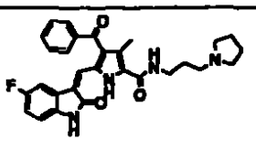
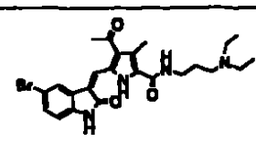
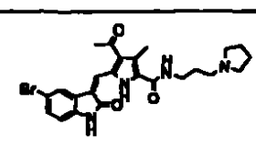
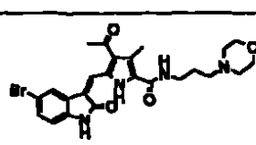
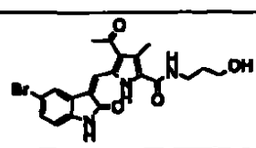
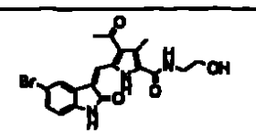
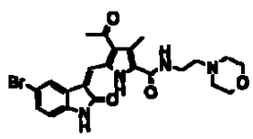
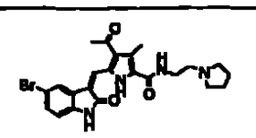
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10		5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrole-2-carboxylic acid (2-diethylaminoethyl)methylamide
11		5-(2-Oxo-6-phenyl-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrole-2-carboxylic acid (2-diethylaminoethyl)methylamide
12		3-Methyl-5-(2-oxo-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrole-2-carboxylic acid (3-diethylaminopropyl)amide
13		5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-3-methyl-1H-pyrrole-2 carboxylic acid (3-diethylaminopropyl)amide
14		3-Methyl-5-(2-oxo-6-phenyl-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrole-2 carboxylic acid (3-diethylaminopropyl)amide
15		5-(5-Methoxy-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-3-methyl-1H-pyrrole 2-carboxylic acid (3-diethylaminopropyl)amide
16		5-(6-Methoxy-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-3-methyl-1H-pyrrole 2-carboxylic acid (3-diethylaminopropyl)amide
17		3-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-4,5,6,7-tetrahydro-2H-isoindole-1-carboxylic acid (2-diethylaminoethyl)amide
18		3-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-4,5,6,7-tetrahydro-2H-isoindole-1-carboxylic acid (3-diethylaminopropyl)amide

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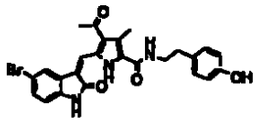
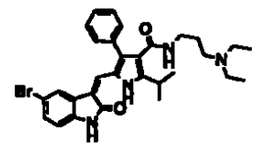
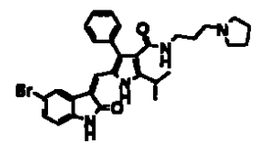
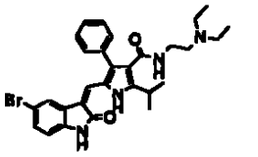
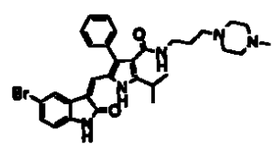
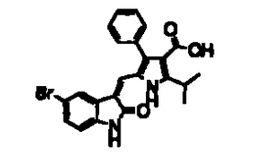
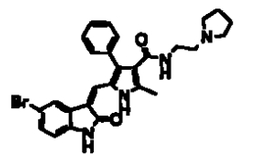
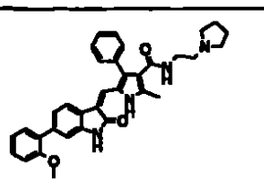
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20		3-(2-Oxo-6-pyridin-3-yl-1,2-dihydroindol-3-ylidenemethyl)-4,5,6,7-tetrahydro-2H-isoindole-1-carboxylic acid (2-diethylaminoethyl)amide
21		4-Benzoyl-5-(5-bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-3-methyl-1H-pyrrole-2-carboxylic acid (3-diethylaminopropyl)amide
22		4-Benzoyl-5-(5-bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-3-methyl-1H-pyrrole-2-carboxylic acid (3-morpholin-4-ylpropyl)amide
23		4-Benzoyl-3-methyl-5-(2-oxo-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrole-2-carboxylic acid (3-pyrrolidin-1-ylpropyl)amide
24		4-Benzoyl-5-(5-bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-3-methyl-1H-pyrrole-2-carboxylic acid (3-pyrrolidin-1-ylpropyl)amide
25		4-Benzoyl-3-methyl-5-(2-oxo-6-phenyl-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrole-2-carboxylic acid (3-pyrrolidin-1-ylpropyl)amide
26		4-Benzoyl-5-(6-methoxy-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-3-methyl-1H-pyrrole-2-carboxylic acid (3-pyrrolidin-1-ylpropyl)amide

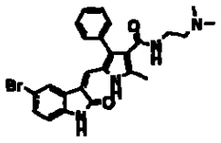
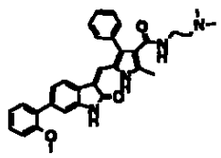
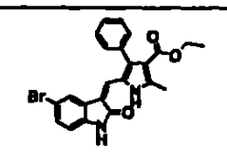
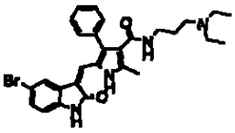
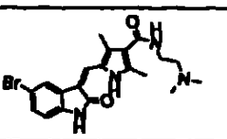
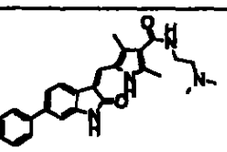
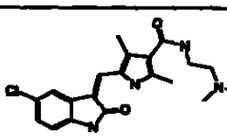
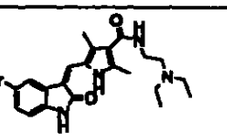
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27		4-Benzoyl-5-(5-methoxy-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-3-methyl-1H-pyrrole-2-carboxylic acid (3-pyrrolidin-1-ylpropyl)amide
28		4-Benzoyl-5-(5-fluoro-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-3-methyl-1H-pyrrole-2-carboxylic acid (3-pyrrolidin-1-ylpropyl)amide
29		4-Acetyl-5-(5-bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-3-methyl-1H-pyrrole-2-carboxylic acid (3-diethylaminopropyl)amide
30		4-Acetyl-5-(5-bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-3-methyl-1H-pyrrole-2-carboxylic acid (3-pyrrolidin-1-ylpropyl)amide
31		4-Acetyl-5-(5-bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-3-methyl-1H-pyrrole-2-carboxylic acid (3-morpholin-4-ylpropyl)amide
32		4-Acetyl-5-(5-bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-3-methyl-1H-pyrrole-2-carboxylic acid (3-hydroxy-propyl)amide
33		4-Acetyl-5-(5-bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-3-methyl-1H-pyrrole-2-carboxylic acid (2-hydroxy-ethyl)amide
34		4-Acetyl-5-(5-bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-3-methyl-1H-pyrrole-2-carboxylic acid (2-morpholin-4-yl-ethyl)amide
35		4-Acetyl-5-(5-bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-3-methyl-1H-pyrrole-2-carboxylic acid (2-pyrrolidin-1-yl-ethyl)amide

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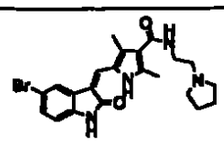
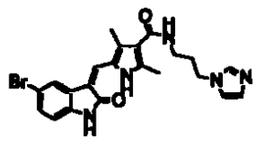
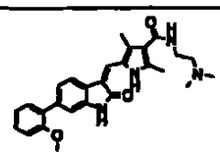
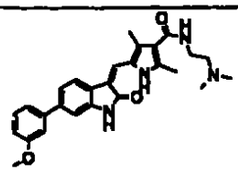
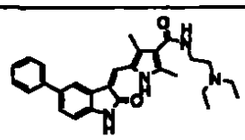
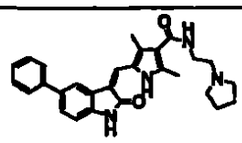
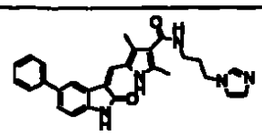
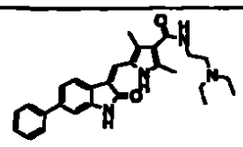
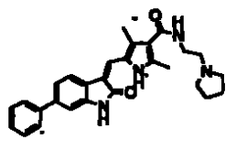
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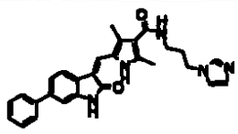
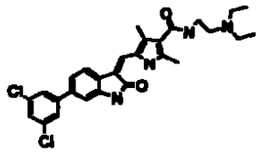
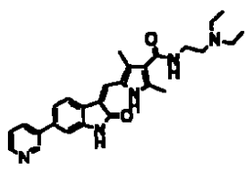
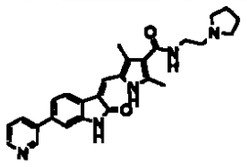
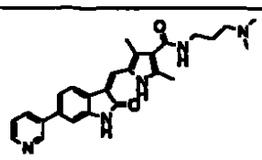
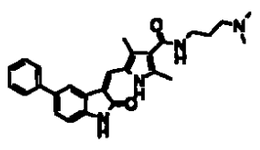
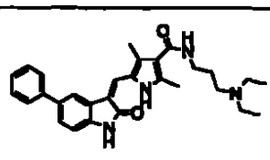
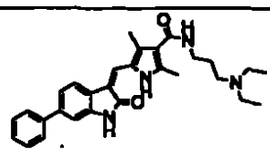
36		4-Acetyl-5-(5-bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-3-methyl-1H-pyrrole-2-carboxylic acid [2-(4-hydroxy-phenyl)-ethyl]amide
37		5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2-isopropyl-4-phenyl-1H-pyrrole-3-carboxylic acid (3-diethylaminopropyl)amide
38		5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2-isopropyl-4-phenyl-1H-pyrrole-3-carboxylic acid (3-pyrrolidin-1-ylpropyl)amide
39		5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2-isopropyl-4-phenyl-1H-pyrrole-3-carboxylic acid (2-diethylaminoethyl)amide
40		5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2-isopropyl-4-phenyl-1H-pyrrole-3-carboxylic acid [3-(4-methyl-piperazin-1-yl)-propyl]amide
41		5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2-isopropyl-4-phenyl-1H-pyrrole-3-carboxylic acid
42		5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2-methyl-4-phenyl-1H-pyrrole-3-carboxylic acid (2-pyrrolidin-1-yl-ethyl)amide
43		5-[5-(2-Methoxy-phenyl)-2-oxo-1,2-dihydroindol-3-ylidenemethyl]-2-methyl-4-phenyl-1H-pyrrole-3-carboxylic acid (2-pyrrolidin-1-yl-ethyl)amide

44		5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2-methyl-4-phenyl-1H-pyrrole-3-carboxylic acid (2-dimethylamino-ethyl)amide
45		5-[6-(2-Methoxy-phenyl)-2-oxo-1,2-dihydroindol-3-ylidenemethyl]-2-methyl-4-phenyl-1H-pyrrole-3-carboxylic acid (2-dimethylamino-ethyl)amide
46		5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2-methyl-4-phenyl-1H-pyrrole-3-carboxylic acid ethyl ester
47		5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2-methyl-4-phenyl-1H-pyrrole-3-carboxylic acid (3-diethylaminopropyl)amide
48		5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (2-dimethylamino-ethyl)amide
49		2,4-Dimethyl-5-(2-oxo-6-phenyl-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrole-3-carboxylic acid (2-dimethylamino-ethyl)amide
50		5-(5-Chloro-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (2-dimethylamino-ethyl)amide
51		5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (2-diethylaminoethyl)amide

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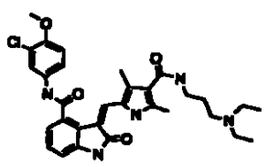
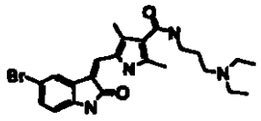
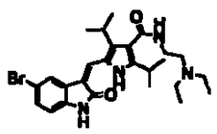
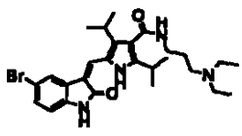
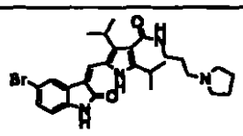
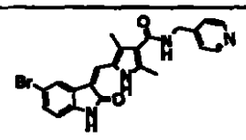
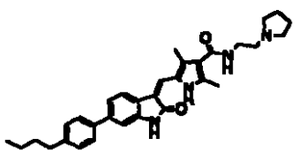
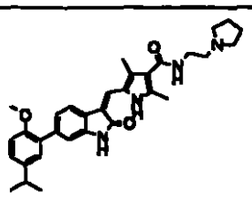
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52		5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (2-pyrrolidin-1-yl-ethyl)amide
53		5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (3-imidazol-1-yl-propyl)amide
54		5-[6-(2-Methoxy-phenyl)-2-oxo-1,2-dihydroindol-3-ylidenemethyl]-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (2-dimethylamino-ethyl)amide
55		5-[6-(3-Methoxy-phenyl)-2-oxo-1,2-dihydroindol-3-ylidenemethyl]-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (2-dimethylamino-ethyl)amide
56		2,4-Dimethyl-5-(2-oxo-5-phenyl-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrole-3-carboxylic acid (2-diethylaminoethyl)amide
57		2,4-Dimethyl-5-(2-oxo-5-phenyl-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrole-3-carboxylic acid (2-pyrrolidin-1-yl-ethyl)amide
58		2,4-Dimethyl-5-(2-oxo-5-phenyl-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrole-3-carboxylic acid (3-imidazol-1-yl-propyl)amide
59		2,4-Dimethyl-5-(2-oxo-6-phenyl-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrole-3-carboxylic acid (2-diethylaminoethyl)amide
60		2,4-Dimethyl-5-(2-oxo-6-phenyl-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrole-3-carboxylic acid (2-pyrrolidin-1-yl-ethyl)amide

61		2,4-Dimethyl-5-(2-oxo-6-phenyl-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrole-3-carboxylic acid (3-imidazol-1-ylpropyl)amide
62		5-[6-(3,5-Dichloro-phenyl)-2-oxo-1,2-dihydroindol-3-ylidenemethyl]-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (2-diethylaminoethyl)amide
63		2,4-Dimethyl-5-(2-oxo-6-pyridin-3-yl-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrole-3-carboxylic acid (2-diethylaminoethyl)amide
64		2,4-Dimethyl-5-(2-oxo-6-pyridin-3-yl-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrole-3-carboxylic acid (2-pyrrolidin-1-yl-ethyl)amide
65		2,4-Dimethyl-5-(2-oxo-6-pyridin-3-yl-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrole-3-carboxylic acid (3-dimethylamino-propyl)amide
66		2,4-Dimethyl-5-(2-oxo-5-phenyl-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrole-3-carboxylic acid (3-dimethylamino-propyl)amide
67		2,4-Dimethyl-5-(2-oxo-5-phenyl-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrole-3-carboxylic acid (3-diethylaminopropyl)amide
68		2,4-Dimethyl-5-(2-oxo-6-phenyl-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrole-3-carboxylic acid (3-diethylaminopropyl)amide

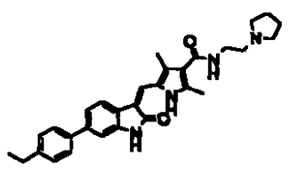
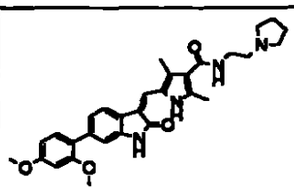
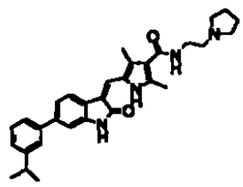
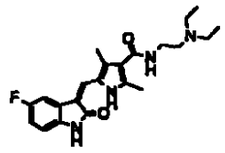
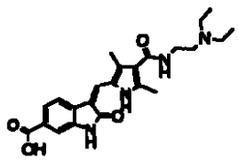
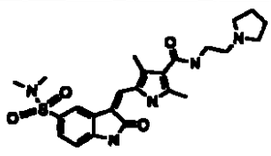
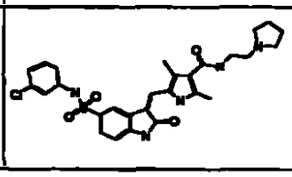
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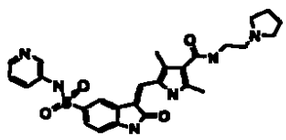
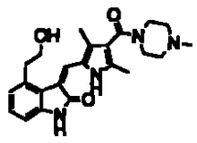
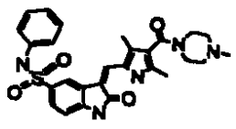
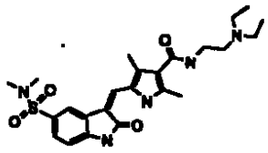
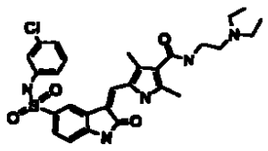
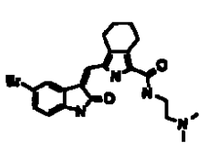
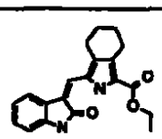
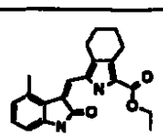
69		3-[4-(3-Diethylamino-propylcarbamoyl)-3,5-dimethyl-1H-pyrrol-2-ylmethylene]-2-oxo-2,3-dihydro-1H-indole-4-carboxylic acid (3-chloro-4-methoxy-phenyl)amide
70		5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (3-diethylaminopropyl)amide
71		5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2,4-diisopropyl-1H-pyrrole-3-carboxylic acid (2-diethylaminoethyl)amide
72		5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2,4-diisopropyl-1H-pyrrole-3-carboxylic acid (3-diethylaminopropyl)amide
73		5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2,4-diisopropyl-1H-pyrrole-3-carboxylic acid (3-pyrrolidin-1-ylpropyl)amide
74		5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (pyridin-4-ylmethyl)amide
75		5-[6-(4-Butyl-phenyl)-2-oxo-1,2-dihydroindol-3-ylidenemethyl]-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (2-pyrrolidin-1-yl-ethyl)amide
76		5-[6-(5-Isopropyl-2-methoxy-phenyl)-2-oxo-1,2-dihydroindol-3-ylidenemethyl]-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (2-pyrrolidin-1-yl-ethyl)amide

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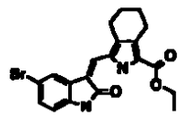
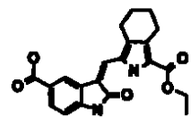
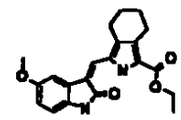
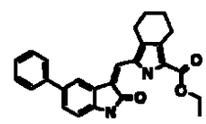
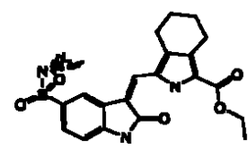
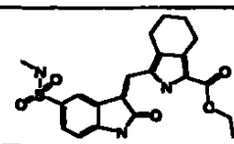
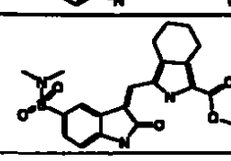
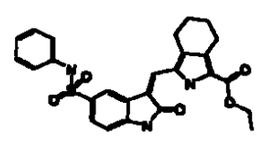
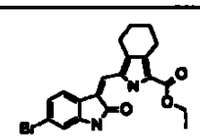
77		5-[6-(4-Ethyl-phenyl)-2-oxo-1,2-dihydroindol-3-ylidenemethyl]-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (2-pyrrolidin-1-yl-ethyl)amide
78		5-[6-(2,4-Dimethoxy-phenyl)-2-oxo-1,2-dihydroindol-3-ylidenemethyl]-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (2-pyrrolidin-1-yl-ethyl)amide
79		5-[6-(3-Isopropyl-phenyl)-2-oxo-1,2-dihydroindol-3-ylidenemethyl]-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (2-pyrrolidin-1-yl-ethyl)amide
80		5-(5-Fluoro-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (2-diethylaminoethyl)amide
81		3-[4-(2-diethylaminoethylcarbamoyl)-3,5-dimethyl-1H-pyrrol-2-ylmethylene]-2-oxo-2,3-dihydro-1H-indole-6-carboxylic acid
82		5-(5-Dimethylsulfamoyl-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (2-pyrrolidin-1-yl-ethyl)amide
83		5-[5-(3-Chloro-phenylsulfamoyl)-2-oxo-1,2-dihydroindol-3-ylidenemethyl]-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (2-pyrrolidin-1-yl-ethyl)amide

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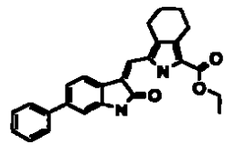
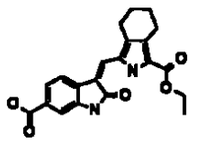
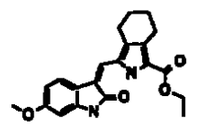
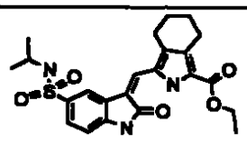
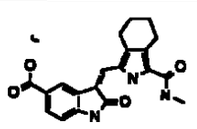
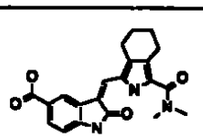
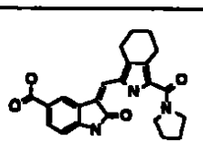
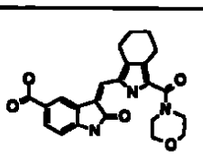
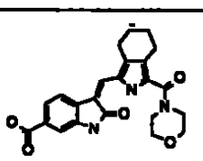
84		2,4-Dimethyl-5-[2-oxo-5-(pyridin-3-ylsulfamoyl)-1,2-dihydroindol-3-ylidenemethyl]-1H-pyrrole-3-carboxylic acid (2-pyrrolidin-1-yl-ethyl)amide
85		3-[3,5-Dimethyl-4-(4-methyl-piperazine-1-carbonyl)-1H-pyrrol-2-ylmethylene]-4-(2-hydroxy-ethyl)-1,3-dihydroindol-2-one
86		3-[3,5-Dimethyl-4-(4-methyl-piperazine-1-carbonyl)-1*H*-pyrrol-2-ylmethylene]-2-oxo-2,3-dihydro-1*H*-indole-5-sulfonic acid phenylamide
87		5-(5-Dimethylsulfamoyl-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (2-diethylaminoethyl)amide
88		5-[5-(3-Chloro-phenylsulfamoyl)-2-oxo-1,2-dihydroindol-3-ylidenemethyl]-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (2-diethylaminoethyl)amide
89		3-(5-Bromo-2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-4,5,6,7-tetrahydro-2*H*-isoindole-1-carboxylic acid (2-dimethylamino-ethyl)-amide
90		3-(2-Oxo-1,2-dihydro-indol-3-ylidenemethyl)-4,5,6,7-tetrahydro-2*H*-isoindole-1-carboxylic acid ethyl ester
91		3-(4-Methyl-2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-4,5,6,7-tetrahydro-2*H*-isoindole-1-carboxylic acid ethyl ester

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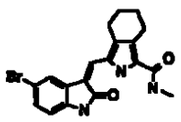
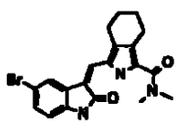
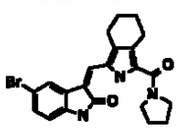
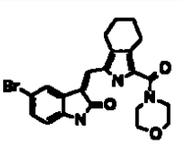
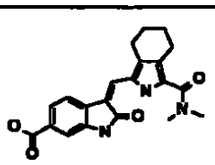
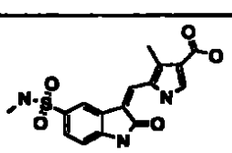
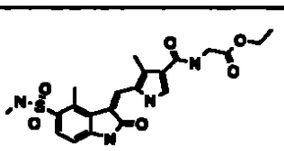
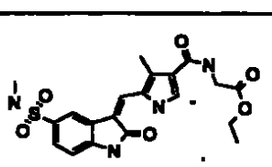
693

92		3-(5-Bromo-2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-4,5,6,7-tetrahydro-2*H*-isoindole-1-carboxylic acid ethyl ester
93		3-(3-Ethoxycarbonyl-4,5,6,7-tetrahydro-2*H*-isoindol-1-ylmethylene)-2-oxo-2,3-dihydro-1*H*-indole-5-carboxylic acid
94		3-(5-Methoxy-2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-4,5,6,7-tetrahydro-2*H*-isoindole-1-carboxylic acid ethyl ester
95		3-(2-Oxo-5-phenyl-1,2-dihydro-indol-3-ylidenemethyl)-4,5,6,7-tetrahydro-2*H*-isoindole-1-carboxylic acid ethyl ester
96		3-(2-Oxo-5-sulfamoyl-1,2-dihydro-indol-3-ylidenemethyl)-4,5,6,7-tetrahydro-2*H*-isoindole-1-carboxylic acid ethyl ester
97		3-(5-Methylsulfamoyl-2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-4,5,6,7-tetrahydro-2*H*-isoindole-1-carboxylic acid ethyl ester
98		3-(5-Dimethylsulfamoyl-2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-4,5,6,7-tetrahydro-2*H*-isoindole-1-carboxylic acid ethyl ester
99		3-(2-Oxo-5-phenylsulfamoyl-1,2-dihydro-indol-3-ylidenemethyl)-4,5,6,7-tetrahydro-2*H*-isoindole-1-carboxylic acid ethyl ester
100		3-(6-Bromo-2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-4,5,6,7-tetrahydro-2*H*-isoindole-1-carboxylic acid ethyl ester

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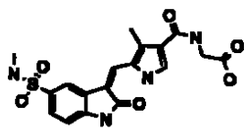
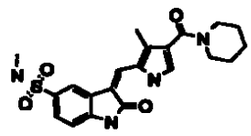
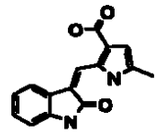
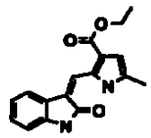
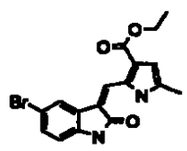
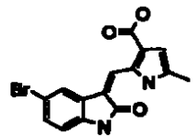
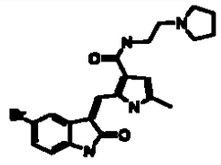
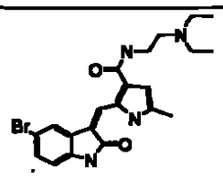
101		3-(2-Oxo-5-phenyl-1,2-dihydro-indol-3-ylidenemethyl)-4,5,6,7-tetrahydro-2*H*-isoindole-1-carboxylic acid ethyl ester
102		3-(3-Ethoxycarbonyl-4,5,6,7-tetrahydro-2*H*-isoindol-1-ylmethylene)-2-oxo-2,3-dihydro-1*H*-indole-6-carboxylic acid
103		3-(5-Methoxy-2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-4,5,6,7-tetrahydro-2*H*-isoindole-1-carboxylic acid ethyl ester
104		3-(5-Isopropylsulfamoyl-2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-4,5,6,7-tetrahydro-2*H*-isoindole-1-carboxylic acid ethyl ester
105		3-(3-Methylcarbamoyl-4,5,6,7-tetrahydro-2*H*-isoindol-1-ylmethylene)-2-oxo-2,3-dihydro-1*H*-indole-5-carboxylic acid
106		3-(3-Dimethylcarbamoyl-4,5,6,7-tetrahydro-2*H*-isoindol-1-ylmethylene)-2-oxo-2,3-dihydro-1*H*-indole-5-carboxylic acid
107		2-Oxo-3-[3-(pyrrolidine-1-carbonyl)-4,5,6,7-tetrahydro-2*H*-isoindol-1-ylmethylene]-2,3-dihydro-1*H*-indole-5-carboxylic acid
108		3-[3-(Morpholine-4-carbonyl)-4,5,6,7-tetrahydro-2*H*-isoindol-1-ylmethylene]-2-oxo-2,3-dihydro-1*H*-indole-5-carboxylic acid
109		3-[3-(Morpholine-4-carbonyl)-4,5,6,7-tetrahydro-2*H*-isoindol-1-ylmethylene]-2-oxo-2,3-dihydro-1*H*-indole-6-carboxylic acid

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110		3-(5-Bromo-2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-4,5,6,7-tetrahydro-2*H*-isoindole-1-carboxylic acid methylamide
111		3-(5-Bromo-2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-4,5,6,7-tetrahydro-2*H*-isoindole-1-carboxylic acid dimethylamide
112		5-Bromo-3-[3-(pyrrolidine-1-carbonyl)-4,5,6,7-tetrahydro-2*H*-isoindol-1-ylmethylene]-1,3-dihydro-indol-2-one
113		5-Bromo-3-[3-(morpholine-4-carbonyl)-4,5,6,7-tetrahydro-2*H*-isoindol-1-ylmethylene]-1,3-dihydro-indol-2-one
114		3-(3-Dimethylcarbamoyl-4,5,6,7-tetrahydro-2*H*-isoindol-1-ylmethylene)-2-oxo-2,3-dihydro-1*H*-indole-6-carboxylic acid
115		4-Methyl-5-(5-methylsulfamoyl-2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-1*H*-pyrrole-3-carboxylic acid
116		{[4-Methyl-5-(4-methyl-5-methylsulfamoyl-2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-1*H*-pyrrole-3-carbonyl]-amino}-acetic acid ethyl ester
117		{[4-Methyl-5-(5-methylsulfamoyl-2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-1*H*-pyrrole-3-carbonyl]-amino}-acetic acid ethyl ester

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118		([4-Methyl-5-(5-methylsulfamoyl-2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-1*H*-pyrrole-3-carbonyl]-amino)-acetic acid
119		3-[3-Methyl-4-(piperidine-1-carbonyl)-1*H*-pyrrol-2-ylmethylene]-2-oxo-2,3-dihydro-1*H*-indole-5-sulfonic acid methylamide
120		5-Methyl-2-(2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-1*H*-pyrrole-3-carboxylic acid
121		5-Methyl-2-(2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-1*H*-pyrrole-3-carboxylic acid ethyl ester
122		2-(5-Bromo-2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-5-methyl-1*H*-pyrrole-3-carboxylic acid ethyl ester
123		2-(5-Bromo-2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-5-methyl-1*H*-pyrrole-3-carboxylic acid
124		2-(5-Bromo-2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-5-methyl-1H-pyrrole-3-carboxylic acid (2-pyrrolidin-1-yl-ethyl)-amide
125		2-(5-Bromo-2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-5-methyl-1H-pyrrole-3-carboxylic acid (2-diethylamino-ethyl)-amide

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TABLE 2

Example	bio flkGST IC50 (nM)	bio FGFR1 IC50 (nM)	bio PDGF IC50 (nM)	bio EGF IC50 (nM)	cell EGF IC50 (nM)	Her2 Kinase IC50 (nM)	cdk2spa CS0 (nM)	bio pyk2 IC50 (nM)
1	57.68	15.16	>100	>100	>100			>100
2	>100		>100	>100	>100			
3	9.85	9.62	>100	>100	>100			>100
4	3.57	>20	>100	>100	>100	>100		
5	8.3	16.06	>100	>100	>100	>100		
6	4.04		>100	3.26	7.82	2.43		
7	7.74		>100	5.07	9.8	4.24		
8	12.1		>100	51.34	20.08	5.5		
9	0.96		>100	>100	>100	16.38		
10	5.72		>100	94.04	15.86	8.06		
11	9.77		>100	>100	>100	>100		
12	>20		21.46	>100		27.73		
13	>20		81.92	8.17		2.66		
14	13.01		42.41	>100		66.02		
15	>20		>100	>100		98.61		
16	>20		98.06	>100		23.32		
17	8.25	2.47	94.35	0.83	11.47	15.94	>10	
18	2.67			2.57	9.23	4.99		
19	7.5			6.86	34.18	8.37		
20	11.53			>100	41.16	8		
21	7.18		>100	40.34		27.69		
22	>20		>100	>100		87.67		
23	>20		>100	36.64		4.05		
24			>100	16.84		5.31		
25	12.55		>100	23.48		7.9		
26	16.03		66.87	34.67		10.04		
27			>100	26.5		3.91		
28	4.5		71.27	53.66		2.67		
29	10.12		>100	26.72		3.98		
30	9.4		>100	18.69		4.1		
31	>50		>100	9.83		47.19		
32	45.74		5.94	>100		>100		

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34	>50		>100	>100		>100		
35	>20		>100	80.4		54.14		
36	>20		>100	>100		>100		
37	0.22		3.06	10.78	9.84	1.4		
38	4.17		3.06	6.04	8.97	2.16		
39	3.38		4.69	3.67	14.54	3.53		
40	4.5		7.9	6.52		6.27		
42	0.1		0.12	11.95	74.55	20.43		
43	1.12		8.38	>100	37.33	53.37		
44	<0.05		0.02	20.73	67.46	6.99		
45	1.71		>100	>100	29.95	>100		
46	30.62		6.18	>100	>100	>100		
47	0.08	1.56	0.06	11.42	41.54	8.4	>20	1.05
48	0.006	0.3	<0.78	17.88	21.58	7.93		0.09
49			<0.78	>100	43.86	>100		
50			<0.78	>100	20.34	>100		
51	0.006	1.66	0.01	18.1	21.61	23.24	16.69	0.35
52	0.08	1.26	<0.78	12.53	>100	>100	10.66	0.45
53			<0.78	>100	>100	>100		
54	1.98		<0.78	23.88	9.76	7.02		
55	0.27		0.53	6.03	35.99	77.82		
56	2.32		3.19	>100	10.03	7.11		
57	0.06		7.98	>100	9.97	6.94		
58			21.14	>100	>100	>100		
59			<0.78	>100	>100	>100		
60			<0.78	>100	>100	>100		
61			<0.78	>100	>100	>100		
62	8.00		8.32	>100	>100	>100		
63	0.21		<0.78	8.59	>100	>100		
64	0.55		<0.78	30.49	>100	>100		
65	0.37		<0.05	>100	74.36	15.97		
66	<0.05			>100	11.84	2.76		
67	0.39		24.77	31.38	19.79	2.56		
68	1.16		0.03	>100	23.52	34.13		
69	0.3		56.55	>100	97.54	>100.		
70	0.09	1.50	0.0030	10.57	6.42	7.99	12.62	0.63

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71	15.21		22.5	>100		9.91		
72	6.06		10.54	>100	39.94	9.65		
73	5.95		14.12	>100	39.5	8.59		
74	1.2		0.09	46.75		>100		
75	2.7		61.55	>100		>100		
76	3.33		19.18	5.11		3.01		
77	0.49		25.01	>100		>100		
78	1.94		70.62	9.33		4.25		
79	1.49		>100	27.39		>100		
80	0.13	4.29	0.001	>100		50.19	17.19	0.28
81	0.21		0.18	>100		>100		
82	2.03	7.69	6.88	>100		>100		0.31
83	0.34	0.41	9.46	2.18		86.9		0.008
84	1.38		12.51	67.2		5.86		0.006
85	0.2	0.8	2.59	>100		3.76		
86	1.45	1.3	19.6	41.8		>100		3.58
87	3.27	7.56	6.46	>100		9.1		0.17
88	0.35	1.18	8.06	2.36		>100		0.09
89	7.84		47.58	8.53	9.67	15.97		
115	7.3		7.48	>100		>100	0.006	
116	>20		>100	>100		>100	<0.0005	
117	0.91		12.9	>100		>100	0.006	
118	1.93		1.2	>100		>100	0.002	
119	1.38		61.63	>100		>100	<0.0005	

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2. INDICATIONS/TARGET DISEASES

The PKs whose catalytic activity is modulated by the compounds of this invention include protein tyrosine kinases of which there are two types, receptor tyrosine kinases (RTKs) and cellular tyrosine kinases (CTKs), and serine-threonine kinases (STKs). RTK mediated signal transduction is initiated by extracellular interaction with a specific growth factor (ligand), followed by receptor dimerization, transient stimulation of the intrinsic protein tyrosine kinase activity and phosphorylation. Binding sites are thereby created for intracellular signal

transduction molecules and lead to the formation of complexes with a spectrum of cytoplasmic signaling molecules that facilitate the appropriate cellular response (e.g., cell division, metabolic effects on the extracellular microenvironment, etc.). See, Schlessinger and Ullrich, 1992, Neuron 9:303-391.

It has been shown that tyrosine phosphorylation sites on growth factor receptors function as high-affinity binding sites for SH2 (src homology) domains of signaling molecules. Fantl et al., 1992, Cell 69:413-423, Songyang et al., 1994, Mol. Cell. Biol. 14:2777-2785), Songyang et al., 1993, Cell 72:767-778, and Koch *et al.*, 1991, Science 252:668-678. Several intracellular substrate proteins that associate with RTKs have been identified. They may be divided into two principal groups: (1) substrates that have a catalytic domain, and (2) substrates which lack such domain but which serve as adapters and associate with catalytically active molecules. Songyang et al., 1993, Cell 72:767-778. The specificity of the interactions between receptors and SH2 domains of their substrates is determined by the amino acid residues immediately surrounding the phosphorylated tyrosine residue. Differences in the binding affinities between SH2 domains and the amino acid sequences surrounding the phosphotyrosine residues on particular receptors are consistent with the observed differences in their substrate phosphorylation profiles. Songyang et al., 1993, Cell 72:767-778. These observations suggest that the function of each RTK is determined not only by its pattern of expression and ligand availability but also by the array of downstream signal transduction pathways that are activated by a particular receptor. Thus, phosphorylation provides an important regulatory step which determines the selectivity of signaling pathways

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recruited by specific growth factor receptors, as well as differentiation factor receptors.

STKs, being primarily cytosolic, affect the internal biochemistry of the cell, often as a down-line response to a PTK event. STKs have been implicated in the signaling process which initiates DNA synthesis and subsequent mitosis leading to cell proliferation.

Thus, PK signal transduction results in, among other responses, cell proliferation, differentiation, growth and metabolism. Abnormal cell proliferation may result in a wide array of disorders and diseases, including the development of neoplasia such as carcinoma, sarcoma, glioblastoma and hemangioma, disorders such as leukemia, psoriasis, arteriosclerosis, arthritis and diabetic retinopathy and other disorders related to uncontrolled angiogenesis and/or vasculogenesis.

A precise understanding of the mechanism by which the compounds of this invention inhibit PKs is not required in order to practice the present invention. However, while not hereby being bound to any particular mechanism or theory, it is believed that the compounds interact with the amino acids in the catalytic region of PKs. PKs typically possess a bi-lobate structure wherein ATP appears to bind in the cleft between the two lobes in a region where the amino acids are conserved among PKs. Inhibitors of PKs are believed to bind by non-covalent interactions such as hydrogen bonding, van der Waals forces and ionic interactions in the same general region where the aforesaid ATP binds to the PKs. More specifically, it is thought that the 2-indolinone component of the compounds of this invention binds in the general space normally occupied by the adenine ring of ATP. Specificity of a particular molecule for a particular PK

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may then arise as the result of additional interactions between the various substituents on the 2-indolinone core and the amino acid domains specific to particular PKs. Thus, different indolinone substituents may contribute to preferential binding to particular PKs. The ability to select compounds active at different ATP (or other nucleotide) binding sites makes the compounds of this invention useful for targeting any protein with such a site. The compounds disclosed herein may thus have utility as in vitro assays for such proteins as well as exhibiting in vivo therapeutic effects through interaction with such proteins.

In another aspect, the protein kinase, the catalytic activity of which is modulated by contact with a compound of this invention, is a protein tyrosine kinase, more particularly, a receptor protein tyrosine kinase. Among the receptor protein tyrosine kinases whose catalytic activity can be modulated with a compound of this invention, or salt thereof, are, without limitation, EGF, HER2, HER3, HER4, IR, IGF-1R, IRR, PDGFRα, PDGFRβ, CSFIR, C-Kit, C-fms, Flk-1R, Flk4, KDR/Flk-1, Flt-1, FGFR-1R, FGFR-2R, FGFR-3R and FGFR-4R.

The protein tyrosine kinase whose catalytic activity is modulated by contact with a compound of this invention, or a salt or a prodrug thereof, can also be a non-receptor or cellular protein tyrosine kinase (CTK). Thus, the catalytic activity of CTKs such as, without limitation, Src, Frk, Btk, Csk, Abl, ZAP70, Fes, Fps, Fak, Jak, Ack, Yes, Fyn, Lyn, Lck, Blk, Hck, Fgr and Yrk, may be modulated by contact with a compound or salt of this invention.

Still another group of PKs which may have their catalytic activity modulated by contact with a compound of this invention are the serine-threonine protein kinases such as, without limitation, CDK2 and Raf.

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In another aspect, this invention relates to a method for treating or preventing a PK related disorder by administering a therapeutically effective amount of a compound of this invention, or a salt or a prodrug thereof, to an organism.

It is also an aspect of this invention that a pharmaceutical composition containing a compound of this invention or a salt or prodrug thereof is administered to an organism for the purpose of preventing or treating a PK related disorder.

This invention is therefore directed to compounds that modulate PK signal transduction by affecting the enzymatic activity of RTKs, CTKs and/or STKs, thereby interfering with the signals transduced by such proteins. More particularly, the present invention is directed to compounds which modulate RTK, CTK and/or STK mediated signal transduction pathways as a therapeutic approach to the treatment of many kinds of solid tumors, including but not limited to carcinomas, sarcomas including Kaposi's sarcoma, erythroblastoma, glioblastoma, meningioma, astrocytoma, melanoma and myoblastoma. Treatment or prevention of non-solid tumor cancers such as leukemia are also contemplated by this invention. Indications may include, but are not limited to brain cancers, bladder cancers, ovarian cancers, gastric cancers, pancreas cancers, colon cancers, blood cancers, lung cancers and bone cancers.

Further examples, without limitation, of the types of disorders related to inappropriate PK activity that the compounds described herein may be useful in preventing, treating and studying, are cell proliferative disorders, fibrotic disorders and metabolic disorders.

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Cell proliferative disorders, which may be prevented, treated or further studied by the present invention include cancer, blood vessel proliferative disorders and mesangial cell proliferative disorders.

Blood vessel proliferative disorders refer to disorders related to abnormal vasculogenesis (blood vessel formation) and angiogenesis (spreading of blood vessels). While vasculogenesis and angiogenesis play important roles in a variety of normal physiological processes such as embryonic development, corpus luteum formation, wound healing and organ regeneration, they also play a pivotal role in cancer development where they result in the formation of new capillaries needed to keep a tumor alive. Other examples of blood vessel proliferation disorders include arthritis, where new capillary blood vessels invade the joint and destroy cartilage, and ocular diseases, like diabetic retinopathy, where new capillaries in the retina invade the vitreous, bleed and cause blindness.

Two structurally related RTKs have been identified to bind VEGF with high affinity: the fms-like tyrosine 1 (flt-1) receptor (Shibuya et al., 1990, Oncogene,5:519-524; De Vries et al., 1992, Science, 255:989-991) and the KDR/FLK-1 receptor, also known as VEGF-R2. Vascular endothelial growth factor (VEGF) has been reported to be an endothelial cell specific mitogen with in vitro endothelial cell growth promoting activity. Ferrara & Henzel, 1989, Biochein. Biophys. Res. Comm., 161:851-858; Vaisman et al., 1990, J. Biol. Chem., 265:19461-19566. Information set forth in U.S. application Ser. Nos. 08/193,829, 08/038,596 and 07/975,750, strongly suggest that VEGF is not only responsible for endothelial cell proliferation, but also is the prime regulator of normal and pathological angiogenesis. See generally, Klagsburn

& Soker, 1993, Current Biology, 3(10)699-702; Houck, et al., 1992, J. Biol. Chem., 267:26031-26037.

Normal vasculogenesis and angiogenesis play important roles in a variety of physiological processes such as embryonic development, wound healing, organ regeneration and female reproductive processes such as follicle development in the corpus luteum during ovulation and placental growth after pregnancy. Folkman & Shing, 1992, J. Biological Chem., 267(16):10931-34. Uncontrolled vasculogenesis and/or angiogenesis has been associated with diseases such as diabetes as well as with malignant solid tumors that rely on vascularization for growth. Klagsburn & Soker, 1993, Current Biology, 3(10):699-702; Folkham, 1991, J. Natl. Cancer Inst., 82:4-6; Weidner, et al., 1991, New Engl. J. Med., 324:1-5.

The surmised role of VEGF in endothelial cell proliferation and migration during angiogenesis and vasculogenesis indicates an important role for the KDR/FLK-1 receptor in these processes. Diseases such as diabetes mellitus (Folkman, 198, in XIth Congress of Thrombosis and Haemostasis (Verstraeta, et al., eds.), pp. 583-596, Leuven University Press, Leuven) and arthritis, as well as malignant tumor growth may result from uncontrolled angiogenesis. See e.g., Folkman, 1971, N. Engl. J. Med., 285:1182-1186. The receptors to which VEGF specifically binds are an important and powerful therapeutic target for the regulation and modulation of vasculogenesis and/or angiogenesis and a variety of severe diseases which involve abnormal cellular growth caused by such processes. Plowman, et al., 1994, DN&P, 7(6):334-339. More particularly, the KDR/FLK-1 receptor's highly specific role in neovascularization make it a choice target for therapeutic approaches to the treatment of cancer and other

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diseases which involve the uncontrolled formation of blood vessels.

Thus, one aspect of the present invention relates to compounds capable of regulating and/or modulating tyrosine kinase signal transduction including KDR/FLK-1 receptor signal transduction in order to inhibit or promote angiogenesis and/or vasculogenesis, that is, compounds that inhibit, prevent, or interfere with the signal transduced by KDR/FLK-1 when activated by ligands such as VEGF. Although it is believed that the compounds of the present invention act on a receptor or other component along the tyrosine kinase signal transduction pathway, they may also act directly on the tumor cells that result from uncontrolled angiogenesis.

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Although the nomenclature of the human and murine counterparts of the generic "flk-I" receptor differ, they are, in many respects, interchangeable. The murine receptor, Flk-1, and its human counterpart, KDR, share a sequence homology of 93.4% within the intracellular domain. Likewise, murine FLK-I binds human VEGF with the same affinity as mouse VEGF, and accordingly, is activated by the ligand derived from either species. Millauer et al., 1993, Cell, 72:835-846; Quinn et al., 1993, Proc. Natl. Acad. Sci. USA, 90:7533-7537. FLK-1 also associates with and subsequently tyrosine phosphorylates human RTK substrates (e.g., PLC-γ or p85) when co-expressed in 293 cells (human embryonal kidney fibroblasts).

Models which rely upon the FLK-1 receptor therefore are directly applicable to understanding the KDR receptor. For example, use of the murine FLK-1 receptor in methods which identify compounds that regulate the murine signal transduction pathway are directly applicable to the identification of compounds which may be used to regulate the human signal

transduction pathway, that is, which regulate activity related to the KDR receptor. Thus, chemical compounds identified as inhibitors of KDR/FLK-1 *in vitro*, can be confirmed in suitable *in vivo* models. Both *in vivo* mouse and rat animal models have been demonstrated to be of excellent value for the examination of the clinical potential of agents acting on the KDR/FLK-1 induced signal transduction pathway.

Thus, in one aspect, this invention is directed to compounds that regulate, modulate and/or inhibit vasculogenesis and/or angiogenesis by affecting the enzymatic activity of the KDR/FLK-1 receptor and interfering with the signal transduced by KDR/FLK-1. In another aspect, the present invention is directed to compounds which regulate, modulate and/or inhibit the KDR/FLK-1 mediated signal transduction pathway as a therapeutic approach to the treatment of many kinds of solid tumors including, but not limited to, glioblastoma, melanoma and Kaposi's sarcoma, and ovarian, lung, mammary, prostate, pancreatic, colon and epidermoid carcinoma. In addition, data suggest the administration of compounds which inhibit the KDR/Flk-1 mediated signal transduction pathway may also be used in the treatment of hemangioma, restenois and diabetic retinopathy.

A further aspect of this invention relates to the inhibition of vasculogenesis and angiogenesis by other receptor-mediated pathways, including the pathway comprising the flt-1 receptor.

Receptor tyrosine kinase mediated signal transduction is initiated by extracellular interaction with a specific growth factor (ligand), followed by receptor dimerization, transient stimulation of the intrinsic protein tyrosine kinase activity and autophosphorylation. Binding sites are thereby created for intracellular signal transduction molecules which leads to the formation of complexes with a spectrum of cytoplasmic signalling

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molecules that facilitate the appropriate cellular response, e.g., cell division and metabolic effects to the extracellular microenvironment. See, Schlessinger and Ullrich, 1992, Neuron, 9:1-20.

The close homology of the intracellular regions of KDR/FLK-1 with that of the PDGF-β receptor (50.3% homology) and/or the related flt-1 receptor indicates the induction of overlapping signal transduction pathways. For example, for the PDGF-β receptor, members of the src family (Twamley et al., 1993, Proc. Natl. Acad. Sci. USA, 90:7696-7700), phosphatidylinositol-3'-kinase (Hu et al., 1992, Mol. Cell. Biol., 12:981-990), phospholipase cγ (Kashishian & Cooper, 1993, Mol. Cell. Biol., 4:49-51), ras-GTPase-activating protein, (Kashishian et al., 1992, EMBO J., 11:1373-1382), PTP-ID/syp (Kazlauskas et al., 1993, Proc. Natl. Acad. Sci. USA, 10 90:6939-6943), Grb2 (Arvidsson et al., 1994, Mol. Cell. Biol., 14:6715-6726), and the adapter molecules Shc and Nck (Nishimura et al., 1993, Mol. Cell. Biol., 13:6889-6896), have been shown to bind to regions involving different autophosphorylation sites. See generally, Claesson-Welsh, 1994, Prog. Growth Factor Res., 5:37-54. Thus, it is likely that signal transduction pathways activated by KDR/FLK-1 include the ras pathway (Rozakis et al., 1992, Nature, 360:689-692), the PI-3'-kinase, the src-mediated and the plcγ-mediated pathways. Each of these pathways may play a critical role in the angiogenic and/or vasculogenic effect of KDR/FLK-1 in endothelial cells. Consequently, a still further aspect of this invention relates to the use of the organic compounds described herein to modulate angiogenesis and vasculogenesis as such processes are controlled by these pathways.

Conversely, disorders related to the shrinkage, contraction or closing of blood vessels, such as restenosis, are also

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implicated and may be treated or prevented by the methods of this invention.

Fibrotic disorders refer to the abnormal formation of extracellular matrices. Examples of fibrotic disorders include hepatic cirrhosis and mesangial cell proliferative disorders. Hepatic cirrhosis is characterized by the increase in extracellular matrix constituents resulting in the formation of a hepatic scar. An increased extracellular matrix resulting in a hepatic scar can also be caused by a viral infection such as hepatitis. Lipocytes appear to play a major role in hepatic cirrhosis. Other fibrotic disorders implicated include atherosclerosis.

Mesangial cell proliferative disorders refer to disorders brought about by abnormal proliferation of mesangial cells. Mesangial proliferative disorders include various human renal diseases such as glomerulonephritis, diabetic nephropathy and malignant nephrosclerosis as well as such disorders as thrombotic microangiopathy syndromes, transplant rejection, and glomerulopathies. The RTK PDGFR has been implicated in the maintenance of mesangial cell proliferation. Floege et al., 1993, Kidney International 43:478-548.

Many cancers are cell proliferative disorders and, as noted previously, PKs have been associated with cell proliferative disorders. Thus, it is not surprising that PKs such as, for example, members of the RTK family have been associated with the development of cancer. Some of these receptors, like EGFR (Tuzi et al., 1991, Br. J. Cancer 63:227-233, Torp et al., 1992, APMIS 100:713-719) HER2/neu (Slamon et al., 1989, Science 244:707-712) and PDGF-R (Kumabe et al., 1992, Oncogene, 7:627-633) are over-expressed in many tumors and/or persistently activated by autocrine loops. In fact, in the most common and severe cancers

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these receptor over-expressions (Akbasak and Suner-Akbasak et al., 1992, J. Neurol. Sci., 111:119-133, Dickson et al., 1992, Cancer Treatment Res. 61:249-273, Korc et al., 1992, J. Clin. Invest. 90:1352-1360) and autocrine loops (Lee and Donoghue, 1992, J. Cell. Biol., 118:1057-1070, Korc et al., supra, Akbasak and Suner-Akbasak et al., supra) have been demonstrated. For example, EGFR has been associated with squamous cell carcinoma, astrocytoma, glioblastoma, head and neck cancer, lung cancer and bladder cancer. HER2 has been associated with breast, ovarian, gastric, lung, pancreas and bladder cancer. PDGFR has been associated with glioblastoma and melanoma as well as lung, ovarian and prostate cancer. The RTK c-met has also been associated with malignant tumor formation. For example, c-met has been associated with, among other cancers, colorectal, thyroid, pancreatic, gastric and hepatocellular carcinomas and lymphomas. Additionally c-met has been linked to leukemia. Over-expression of the c-met gene has also been detected in patients with Hodgkins disease and Burkitts disease.

IGF-IR, in addition to being implicated in nutritional support and in type-II diabetes, has also been associated with several types of cancers. For example, IGF-I has been implicated as an autocrine growth stimulator for several tumor types, e.g. human breast cancer carcinoma cells (Arteaga et al., 1989, J. Clin. Invest. 84:1418-1423) and small lung tumor cells (Macauley et al., 1990, Cancer Res., 50:2511-2517). In addition, IGF-I, while integrally involved in the normal growth and differentiation of the nervous system, also appears to be an autocrine stimulator of human gliomas. Sandberg-Nordqvist et al., 1993, Cancer Res. 53:2475-2478. The importance of IGF-IR and its ligands in cell proliferation is further supported by the fact that many cell types in culture (fibroblasts, epithelial cells, smooth muscle cells, T-

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lymphocytes, myeloid cells, chondrocytes and osteoblasts (the stem cells of the bone marrow)) are stimulated to grow by IGF-I. Goldring and Goldring, 1991, Eukaryotic Gene Expression,1:301-326. Baserga and Coppola suggest that IGF-IR plays a central role in the mechanism of transformation and, as such, could be a preferred target for therapeutic interventions for a broad spectrum of human malignancies. Baserga, 1995, Cancer Res., 55:249-252, Baserga, 1994, Cell 79:927-930, Coppola et al., 1994, Mol. Cell. Biol., 14:4588-4595.

STKs have been implicated in many types of cancer including, notably, breast cancer (Cance, et al., Int. J. Cancer, 54:571-77 (1993)).

The association between abnormal PK activity and disease is not restricted to cancer. For example, RTKs have been associated with diseases such as psoriasis, diabetes mellitus, endometriosis, angiogenesis, atheromatous plaque development, Alzheimer's disease, restenosis, von Hippel-Lindau disease, epidermal hyperproliferation, neurodegenerative diseases, age-related macular degeneration and hemangiomas. For example, EGFR has been indicated in corneal and dermal wound healing. Defects in Insulin-R and IGF-1R are indicated in type-II diabetes mellitus. A more complete correlation between specific RTKs and their therapeutic indications is set forth in Plowman et al., 1994, DN&P 7:334-339.

As noted previously, not only RTKs but CTKs including, but not limited to, src, abl, fps, yes, fyn, lyn, lck, blk, hck, fgr and yrk (reviewed by Bolen et al., 1992, FASEB J., 6:3403-3409) are involved in the proliferative and metabolic signal transduction pathway and thus could be expected, and have been shown, to be involved in many PTK-mediated disorders to which the present invention is directed. For example, mutated src (v-src) has been

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shown to be an oncoprotein ($pp60^{v\text{-}src}$) in chicken. Moreover, its cellular homolog, the proto-oncogene $pp60^{c\text{-}src}$ transmits oncogenic signals of many receptors. Over-expression of EGFR or HER2/neu in tumors leads to the constitutive activation of $pp60^{c\text{-}src}$, which is characteristic of malignant cells but absent in normal cells. On the other hand, mice deficient in the expression of c-src exhibit an osteopetrotic phenotype, indicating a key participation of c-src in osteoclast function and a possible involvement in related disorders.

Similarly, Zap70 has been implicated in T-cell signaling which may relate to autoimmune disorders.

STKs have been associated with inflamation, autoimmune disease, immunoresponses, and hyperproliferation disorders such as restenosis, fibrosis, psoriasis, osteoarthritis and rheumatoid arthritis.

PKs have also been implicated in embryo implantation. Thus, the compounds of this invention may provide an effective method of preventing such embryo implantation and thereby be useful as birth control agents.

Finally, both RTKs and CTKs are currently suspected as being involved in hyperimmune disorders.

A method for identifying a chemical compound that modulates the catalytic activity of one or more of the above discussed protein kinases is another aspect of this invention. The method involves, for example, contacting cells expressing the desired protein kinase with a compound of this invention (or its salt or prodrug) and monitoring the cells for any effect that the compound has on them. The effect may be any observable, either to the naked eye or through the use of instrumentation, change or absence of change in a cell phenotype. The change or absence of change in the cell phenotype monitored may be, for example, without limitation, a change or absence of change in the

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catalytic activity of the protein kinase in the cells or a change or absence of change in the interaction of the protein kinase with a natural binding partner.

Examples of the effect of a number of exemplary compounds of this invention on several PTKs are shown in Table 2. The compounds and data presented are not to be construed as limiting the scope of this invention in any manner whatsoever.

3. **PHARMACEUTICAL COMPOSITIONS AND USE**

A compound of the present invention, a prodrug thereof or a physiologically acceptable salt of either the compound or its prodrug, can be administered as such to a human patient or can be administered in pharmaceutical compositions in which the foregoing materials are mixed with suitable carriers or excipient(s). Techniques for formulation and administration of drugs may be found in "Remington's Pharmacological Sciences," Mack Publishing Co., Easton, PA, latest edition.

Routes of Administration.

As used herein, "administer" or "administration" refers to the delivery of a compound, salt or prodrug of the present invention or of a pharmaceutical composition containing a compound, salt or prodrug of this invention to an organism for the purpose of prevention or treatment of a PK-related disorder.

Suitable routes of administration may include, without limitation, oral, rectal, transmucosal or intestinal administration or intramuscular, subcutaneous, intramedullary, intrathecal, direct intraventricular, intravenous, intravitreal, intraperitoneal, intranasal, or intraocular injections. The preferred routes of administration are oral and parenteral.

Alternatively, one may administer the compound in a local rather than systemic manner, for example, via injection of the

compound directly into a solid tumor, often in a depot or sustained release formulation.

Furthermore, one may administer the drug in a targeted drug delivery system, for example, in a liposome coated with tumor-specific antibody. The liposomes will be targeted to and taken up selectively by the tumor.

Composition/Formulation.

Pharmaceutical compositions of the present invention may be manufactured by processes well known in the art, e.g., by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping or lyophilizing processes.

Pharmaceutical compositions for use in accordance with the present invention may be formulated in conventional manner using one or more physiologically acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. Proper formulation is dependent upon the route of administration chosen.

For injection, the compounds of the invention may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks' solution, Ringer's solution, or physiological saline buffer. For transmucosal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

For oral administration, the compounds can be formulated by combining the active compounds with pharmaceutically acceptable carriers well known in the art. Such carriers enable the compounds of the invention to be formulated as tablets, pills, lozenges, dragees, capsules, liquids, gels, syrups, slurries, suspensions and the like, for oral ingestion by a patient.

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Pharmaceutical preparations for oral use can be made using a solid excipient, optionally grinding the resulting mixture, and processing the mixture of granules, after adding other suitable auxiliaries if desired, to obtain tablets or dragee cores. Useful excipients are, in particular, fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol, cellulose preparations such as, for example, maize starch, wheat starch, rice starch and potato starch and other materials such as gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carboxymethylcellulose, and/or polyvinyl-pyrrolidone (PVP). If desired, disintegrating agents may be added, such as cross-linked polyvinyl pyrrolidone, agar, or alginic acid. A salt such as sodium alginate may also be used.

Dragee cores are provided with suitable coatings. For this purpose, concentrated sugar solutions may be used which may optionally contain gum arabic, talc, polyvinyl pyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for identification or to characterize different combinations of active compound doses.

Pharmaceutical compositions which can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a plasticizer, such as glycerol or sorbitol. The push-fit capsules can contain the active ingredients in admixture with a filler such as lactose, a binder such as starch, and/or a lubricant such as talc or magnesium stearate and, optionally, stabilizers. In soft capsules, the active compounds may be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid

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polyethylene glycols. Stabilizers may be added in these formulations, also.

For administration by inhalation, the compounds for use according to the present invention are conveniently delivered in the form of an aerosol spray using a pressurized pack or a nebulizer and a suitable propellant, e.g., without limitation, dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane or carbon dioxide. In the case of a pressurized aerosol, the dosage unit may be controlled by providing a valve to deliver a metered amount. Capsules and cartridges of, for example, gelatin for use in an inhaler or insufflator may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

The compounds may also be formulated for parenteral administration, e.g., by bolus injection or continuous infusion. Formulations for injection may be presented in unit dosage form, e.g., in ampoules or in multi-dose containers, with an added preservative. The compositions may take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulating materials such as suspending, stabilizing and/or dispersing agents.

Pharmaceutical compositions for parenteral administration include aqueous solutions of a water soluble form, such as, without limitation, a salt, of the active compound. Additionally, suspensions of the active compounds may be prepared in a lipophilic vehicle. Suitable lipophilic vehicles include fatty oils such as sesame oil, synthetic fatty acid esters such as ethyl oleate and triglycerides, or materials such as liposomes. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Optionally, the

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suspension may also contain suitable stabilizers and/or agents that increase the solubility of the compounds to allow for the preparation of highly concentrated solutions.

Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, e.g., sterile, pyrogen-free water, before use.

The compounds may also be formulated in rectal compositions such as suppositories or retention enemas, using, e.g., conventional suppository bases such as cocoa butter or other glycerides.

In addition to the fomulations described previously, the compounds may also be formulated as depot preparations. Such long acting formulations may be administered by implantation (for example, subcutaneously or intramuscularly) or by intramuscular injection. A compound of this invention may be formulated for this route of administration with suitable polymeric or hydrophobic materials (for instance, in an emulsion with a pharamcologically acceptable oil), with ion exchange resins, or as a sparingly soluble derivative such as, without limitation, a sparingly soluble salt.

A non-limiting example of a pharmaceutical carrier for the hydrophobic compounds of the invention is a cosolvent system comprising benzyl alcohol, a nonpolar surfactant, a water-miscible organic polymer and an aqueous phase such as the VPD co-solvent system. VPD is a solution of 3% w/v benzyl alcohol, 8% w/v of the nonpolar surfactant Polysorbate 80, and 65% w/v polyethylene glycol 300, made up to volume in absolute ethanol. The VPD co-solvent system (VPD:D5W) consists of VPD diluted 1:1 with a 5% dextrose in water solution. This co-solvent system dissolves hydrophobic compounds well, and itself produces low toxicity upon systemic administration. Naturally, the

proportions of such a co-solvent system may be varied considerably without destroying its solubility and toxicity characteristics. Furthermore, the identity of the co-solvent components may be varied: for example, other low-toxicity nonpolar surfactants may be used instead of Polysorbate 80, the fraction size of polyethylene glycol may be varied, other biocompatible polymers may replace polyethylene glycol, e.g., polyvinyl pyrrolidone, and other sugars or polysaccharides may substitute for dextrose.

Alternatively, other delivery systems for hydrophobic pharmaceutical compounds may be employed. Liposomes and emulsions are well known examples of delivery vehicles or carriers for hydrophobic drugs. In addtion, certain organic solvents such as dimethylsulfoxide also may be employed, although often at the cost of greater toxicity.

Additionally, the compounds may be delivered using a sustained-release system, such as semipermeable matrices of solid hydrophobic polymers containing the therapeutic agent. Various sustained-release materials have been established and are well known by those skilled in the art. Sustained-release capsules may, depending on their chemical nature, release the compounds for a few weeks up to over 100 days. Depending on the chemical nature and the biological stability of the therapeutic reagent, additional strategies for protein stabilization may be employed.

The pharmaceutical compositions herein also may comprise suitable solid or gel phase carriers or excipients. Examples of such carriers or excipients include, but are not limited to, calcium carbonate, calcium phosphate, various sugars, starches, cellulose derivatives, gelatin, and polymers such as polyethylene glycols.

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Many of the PK modulating compounds of the invention may be provided as physiologically acceptable salts wherein the claimed compound may form the negatively or the positively charged species. Examples of salts in which the compound forms the positively charged moiety include, without limitation, quaternary ammonium (defined elsewhere herein), salts such as the hydrochloride, sulfate, carbonate, lactate, tartrate, maleate, succinate wherein the nitrogen atom of the quaternary ammonium group is a nitrogen of the selected compound of this invention which has reacted with the appropriate acid. Salts in which a compound of this invention forms the negatively charged species include, without limitation, the sodium, potassium, calcium and magnesium salts formed by the reaction of a carboxylic acid group in the compound with an appropriate base (e.g. sodium hydroxide (NaOH), potassium hydroxide (KOH), Calcium hydroxide ($Ca(OH)_2$), etc.).

Dosage.

Pharmaceutical compositions suitable for use in the present invention include compositions wherein the active ingredients are contained in an amount sufficient to achieve the intended purpose, e.g., the modulation of PK activity or the treatment or prevention of a PK-related disorder.

More specifically, a therapeutically effective amount means an amount of compound effective to prevent, alleviate or ameliorate symptoms of disease or prolong the survival of the subject being treated.

Determination of a therapeutically effective amount is well within the capability of those skilled in the art, especially in light of the detailed disclosure provided herein.

For any compound used in the methods of the invention, the therapeutically effective amount or dose can be estimated

initially from cell culture assays. Then, the dosage can be formulated for use in animal models so as to achieve a circulating concentration range that includes the IC_{50} as determined in cell culture (i.e., the concentration of the test compound which achieves a half-maximal inhibition of the PK activity). Such information can then be used to more accurately determine useful doses in humans.

Toxicity and therapeutic efficacy of the compounds described herein can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., by determining the IC_{50} and the LD_{50} (both of which are discussed elsewhere herein) for a subject compound. The data obtained from these cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage may vary depending upon the dosage form employed and the route of administration utilized. The exact formulation, route of administration and dosage can be chosen by the individual physician in view of the patient's condition. (See e.g., Fingl, et al., 1975, in "The Pharmacological Basis of Therapeutics", Ch. 1 p.1).

Dosage amount and interval may be adjusted individually to provide plasma levels of the active species which are sufficient to maintain the kinase modulating effects. These plasma levels are referred to as minimal effective concentrations (MECs). The MEC will vary for each compound but can be estimated from in vitro data, e.g., the concentration necessary to achieve 50-90% inhibition of a kinase may be ascertained using the assays described herein. Dosages necessary to achieve the MEC will depend on individual characteristics and route of administration. HPLC assays or bioassays can be used to determine plasma concentrations.

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Dosage intervals can also be determined using MEC value. Compounds should be administered using a regimen that maintains plasma levels above the MEC for 10-90% of the time, preferably between 30-90% and most preferably between 50-90%.

In cases of local administration or selective uptake, the effective local concentration of the drug may not be related to plasma concentration and other procedures known in the art may be employed to determine the correct dosage amount and interval.

The amount of a composition administered will, of course, be dependent on the subject being treated, the severity of the affliction, the manner of administration, the judgment of the prescribing physician, etc.

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Packaging.

The compositions may, if desired, be presented in a pack or dispenser device, such as an FDA approved kit, which may contain one or more unit dosage forms containing the active ingredient. The pack may for example comprise metal or plastic foil, such as a blister pack. The pack or dispenser device may be accompanied by instructions for administration. The pack or dispenser may also be accompanied by a notice associated with the container in a form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals, which notice is reflective of approval by the agency of the form of the compositions or of human or veterinary administration. Such notice, for example, may be of the labeling approved by the U.S. Food and Drug Administration for prescription drugs or of an approved product insert. Compositions comprising a compound of the invention formulated in a compatible pharmaceutical carrier may also be prepared, placed in an appropriate container, and labeled for treatment of an indicated condition. Suitable conditions indicated on the label may include treatment of a

tumor, inhibition of angiogenesis, treatment of fibrosis, diabetes, and the like.

4. SYNTHESIS

The compounds of this invention, as well as the precursor 2-oxindoles and aldehydes, may be readily synthesized using techniques well known in the chemical arts. It will be appreciated by those skilled in the art that other synthetic pathways for forming the compounds of the invention are available and that the following is offered by way of example and not limitation.

A. General synthetic procedure.

The following general methodology may be employed to prepare the compounds of this invention:

The appropriately substituted 2-oxindole (1 equiv.), the appropriately substituted aldehyde (1.2 equiv.) and a base (0.1 equiv.) are mixed in a solvent (1-2 ml/mmol 2-oxindole) and the mixture is then heated for from about 2 to about 12 hours. After cooling, the precipitate that forms is filtered, washed with cold ethanol or ether and vacuum dried to give the solid product. If no precipitate forms, the reaction mixture is concentrated and the residue is triturated with dichloromethane/ether, the resulting solid is collected by filtration and then dried. The product may optionally be further purified by chromatography.

The base may be an organic or an inorganic base. If an organic base is used, preferably it is a nitrogen base. Examples of organic nitrogen bases include, but are not limited to, diisopropylamine, trimethylamine, triethylamine, aniline, pyridine, 1,8-diazabicyclo[5.4.1]undec-7-ene, pyrrolidine and piperidine.

Examples of inorganic bases are, without limitation, ammonia, alkali metal or alkaline earth hydroxides, phosphates, carbonates,

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bicarbonates, bisulfates and amides. The alkali metals include, lithium, sodium and potassium while the alkaline earths include calcium, magnesium and barium.

In a presently preferred embodiment of this invention, when the solvent is a protic solvent, such as water or alcohol, the base is an alkali metal or an alkaline earth inorganic base, preferably, a alkali metal or an alkaline earth hydroxide.

It will be clear to those skilled in the art, based both on known general principles of organic synthesis and on the disclosures herein which base would be most appropriate for the reaction contemplated.

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The solvent in which the reaction is carried out may be a protic or an aprotic solvent, preferably it is a protic solvent. A "protic solvent" is a solvent which has hydrogen atom(s) covalently bonded to oxygen or nitrogen atoms which renders the hydrogen atoms appreciably acidic and thus capable of being "shared" with a solute through hydrogen bonding. Examples of protic solvents include, without limitation, water and alcohols.

An "aprotic solvent" may be polar or non-polar but, in either case, does not contain acidic hydrogens and therefore is not capable of hydrogen bonding with solutes. Examples, without limitation, of non-polar aprotic solvents, are pentane, hexane, benzene, toluene, methylene chloride and carbon tetrachloride. Examples of polar aprotic solvents are chloroform, tetrahydrofuran, dimethylsulfoxide and dimethylformamide.

In a presently preferred embodiment of this invention, the solvent is a protic solvent, preferably water or an alcohol such as ethanol.

The reaction is carried out at temperatures greater than room temperature. The temperature is generally from about 30° C to about 150° C, preferably about 80°C to about 100° C, most preferable

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about 75° C to about 85° C, which is about the boiling point of ethanol. By "about" is meant that the temperature range is preferably within 10 degrees Celcius of the indicated temperature, more preferably within 5 degrees Celcius of the indicated temperature and, most preferably, within 2 degrees Celcius of the indicated temperature. Thus, for example, by "about 75° C" is meant 75° C ± 10° C, preferably 75° C ± 5° C and most preferably, 75° C ± 2° C.

B. Synthetic methods used in the examples which follow:

Method A: Formylation of pyrroles

$POCl_3$ (1.1 equiv.) is added dropwise to dimethylformamide (3 equiv.)at -10° C followed by addition of the appropriate pyrrole dissolved in dimethylformamide. After stirring for two hours, the reaction mixture is diluted with H_2O and basified to pH 11 with 10 N KOH. The precipitate which forms is collected by filtration, washed with H_2O and dried in a vacuum oven to give the desired aldehyde.

Method B: Saponification of pyrrolecarboxylic acid esters

A mixture of a pyrrolecarboxylic acid ester and KOH (2 - 4 equiv.) in EtOH is refluxed until reaction completion is indicated by thin layer chromatography (TLC). The cooled reaction mixtrue is acidified to pH 3 with 1 N HCl. The precipitate which forms is collected by filtration, washed with H_2O and dried in a vacuum oven to give the desired pyrrolecarboxylic acid.

Method C: Amidation

To a stirred solution of a pyrrolecarboxylic acid dissolved in dimethylformamide(0.3M) is added 1-ethyl-3-(3-dimethylamino-propyl)carbodiimide (1.2 equiv.), 1-hydroxybenzotriazole (1.2 equiv.), and triethylamine (2 equiv.). The appropriate amine is added (1 equiv.) and the reaction stirred until completion is indicated by TLC. Ethyl acetate is then added to the reaction

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mixture and the solution washed with saturated $NaHCO_3$ and brine (with extra salt), dried over anhydrous $MgSO_4$ and concentrated to afford the desired amide.

Method D: Condensation of aldehydes and oxindoles containing carboxylic acid substituents

A mixture of the oxindole (1 equivalent), 1 equivalent of the aldehyde and 1 - 3 equivalents of piperidine (or pyrrolidine) in ethanol (0.4 M) is stirred at 90-100° C until reaction completion is indicated by TLC. The mixture is then concentrated and the residue acidified with 2N HCl. The precipitate that forms is washed with H_2O and EtOH and then dried in a vacuum oven to give the product.

Method E: Condensation of aldehydes and oxindoles not containing carboxylic acid substituents

A mixture of the oxindole (1 equivalent), 1 equivalent of the aldehyde and 1 - 3 equivalents of piperidine (or pyrrolidine) in ethanol (0.4 M) is stirred at 90-100° C until reaction completion is indicated by TLC. The mixture is cooled to room temperature and the solid which forms is collected by vacuum filtration, washed with ethanol and dried to give the product. If a precipitate does not form upon cooling of the reaction mixture, the mixture is concentrated and purified by column chromatography.

C. Examples of oxindole syntheses

The following examples of the synthesis of representative oxindoles is not to be construed as limiting the scope of this invention in any manner whatsoever. Alternate routes to the oxindoles shown as well as other oxindoles to be used to make the compounds of this invention will become apparent to those skilled in the art based on the following disclosures. Such syntheses and oxindoles are within the scope and spirit of this invention.

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5-Amino-2-oxindole

5-Nitro-2-oxindole (6.3 g) was hydrogenated in methanol over 10% palladium on carbon to give 3.0 g (60% yield) of the title compound as a white solid.

5-Bromo-2-oxindole

2-Oxindole (1.3 g) in 20 mL acetonitrile was cooled to -10 °C and 2.0 g N-bromosuccinimide was slowly added with stirring. The reaction was stirred for 1 hour at -10 °C and 2 hours at 0 °C. The precipitate was collected, washed with water and dried to give 1.9 g (90 % yield) of the title compound.

4-Methyl-2-oxindole

Diethyl oxalate (30 mL) in 20 mL of dry ether was added with stirring to 19 g of potassium ethoxide suspended in 50 mL of dry ether. The mixture was cooled in an ice bath and 20 mL of 3-nitro-o-xylene in 20 mL of dry ether was slowly added. The thick dark red mixture was heated to reflux for 0.5 hr, concentrated to a dark red solid, and treated with 10% sodium hydroxide until almost all of the solid dissolved. The dark red mixture was treated with 30% hydrogen peroxide until the red color changed to yellow. The mixture was treated alternately with 10% sodium hydroxide and 30% hydrogen peroxide until the dark red color was no longer present. The solid was filtered off and the filtrate acidified with 6N hydrochloric acid. The resulting precipitate was collected by vacuum filtration, washed with water, and dried under vacuum to give 9.8 g (45% yield) of 2-methyl-6-nitrophenylacetic acid as an off-white solid. The solid was hydrogenated in methanol over 10 % palladium on carbon to give 9.04 g of the title compound as a white solid.

7-Bromo-5-chloro-2-oxindole

5-Chloro-2-oxindole (16.8 g) and 19.6 g of N-bromosuccinimide were suspended in 140 mL of acetonitrile and

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refluxed for 3 hours. Thin layer chromatography (silica, ethyl acetate) at 2 hours of reflux showed 5-chloro-2-oxindole or N-bromosuccinimide (Rf 0.8), product (Rf 0.85) and a second product (Rf 0.9) whose proportions did not change after another hour of reflux. The mixture was cooled to 10 °C, the precipitate was collected by vacuum filtration, washed with 25 mL of ethanol and sucked dry for 20 minutes in the funnel to give 14.1 g of wet product (56 % yield). The solid was suspended in 200 mL of denatured ethanol and slurry-washed by stirring and refluxing for 10 minutes. The mixture was cooled in an ice bath to 10 °C. The solid product was collected by vacuum filtration, washed with 25 mL of ethanol and dried under vacuum at 40 °C to give 12.7 g (51% yield) of 7-bromo-5-chloro-2-oxindole.

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5-Fluoro-2-oxindole

5-Fluoroisatin (8.2 g) was dissolved in 50 mL of hydrazine hydrate and refluxed for 1.0 hr. The reaction mixtures were then poured in ice water. The precipitate was then filtered, washed with water and dried in a vacuum oven to afford the title compound.

5-Nitro-2-oxindole

2-Oxindole (6.5 g) was dissolved in 25 mL concentrated sulfuric acid and the mixture maintained at -10 to -15 °C while 2.1 mL of fuming nitric acid was added dropwise. After the addition of the nitric acid the reaction mixture was stirred at 0 °C for 0.5 hr and poured into ice-water. The precipitate was collected by filtration, washed with water and crystallized from 50% acetic acid. The crystalline product was then filtered, washed with water and dried under vacuum to give 6.3 g (70%) of 5-nitro-2-oxindole.

5-Aminosulfonyl-2-oxindole

To a 100 mL flask charged with 27 mL of chlorosulfonic acid was added slowly 13.3 g of 2-oxindole. The reaction temperature was maintained below 30 °C during the addition. After the addition, the reaction mixture was stirred at room temperature for 1.5 hr, heated to 68 °C for 1 hr, cooled, and poured into water. The precipitate was washed with water and dried in a vacuum oven to give 11.0 g of 5-chlorosulfonyl-2-oxindole (50% yield) which was used without further purification.

5-Chlorosulfonyl-2-oxindole (2.1 g) was added to 10 mL of ammonium hydroxide in 10 mL of ethanol and stirred at room temperature overnight. The mixture was concentrated and the solid collected by vacuum filtration to give 0.4 g (20% yield) of the title compound as an off-white solid.

5-Isopropylaminosulfonyl-2-oxindole

To a 100 mL flask charged with 27 mL chlorosulfonic acid was slowly added 13.3 g 2-oxindole. The reaction temperature was maintained below 30° C during the addition. The reaction mixture was stirred at room temperature for 1.5 hour, heated to 68° C for 1 hour, cooled, and poured into water. The precipitate which formed was filtered, washed with water and dried in a vacuum oven to give 11.0 g (50%) of 5-chlorosulfonyl-2-oxindole which was used without further purification.

A suspension of 3 g 5-chlorosulfonyl-2-oxindole, 1.15 g isopropylamine and 1.2 mL of pyridine in 50 mL of dichloromethane was stirred at room temperature for 4 hours during which time a white solid formed. The solid was collected by vacuum filtration, slurry-washed with hot ethanol, cooled, collected by vacuum filtration and dried under vacuum at 40° C overnight to give 1.5 g (45%) of 5-isopropylaminosulfonyl-2-oxindole.

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^{1}HNMR (360 MHz, DMSO-d6) δ 10.69 (s, br, 1H, NH), 7.63 (dd, *J* = 2 and 8 Hz, 1H), 7.59 (d, *J* = 2 Hz, 1H), 7.32 (d, *J* = 7 Hz, 1H, *NH*-SO_2-), 6.93 (d, *J* = 8 Hz, 1H), 3.57 (s, 2H), 3.14-3.23 (m, 1H, *CH*-$(CH_3)_2$), 0.94 (d, *J* = 7 Hz, 6H, 2xCH_3).

5-Phenylaminosulfonyl-2-oxindole

A suspension of 5-chlorosulfonyl-2-oxindole (1.62 g, 7 mmol), aniline (0.782 mL, 8.4 mmol) and pyridine (1 mL) in dichloromethane (20 ml) was stirred at room temperature for 4 hours. The reaction mixture was diluted with ethyl acetate (300 mL) and acidified with 1N hydrochloric acid (16 mL). The organic layer was washed with sodium bicarbonate and brine, dried and concentrated. The residue was washed with ethanol (3 mL) and then chromatographed on silica gel eluting with methanol/ dichloromethane 1:9 to give of 5-phenylaminosulfonyl-2-oxindole.

^{1}HNMR (360 MHz, DMSO-d6) δ 10.71 (s, br, 1H, NH), 10.10 (s, br, 1H, NH), 7.57-7.61 (m, 2H), 7.17-7.22 (m, 2H), 7.06-7.09 (m, 2H), 6.97-7.0 (m, 1H), 6.88 (d, *J* = 8.4 Hz, 1H), 3.52 (s, 2H).

2-Oxo-2,3-dihydro-1H-indole-5-sulfonic acid pyridin-3-ylamide

A solution of 5-chlorosufonyl-2-oxindole (3 g) and 3-aminopyridine (1.46g) in pyridine (15 mL) was stirred at room temperature overnight at which time a brown solid was present. The solid was filtered, washed with ethanol and dried under vacuum to yield 1.4 g (38%) of 2-oxo-2,3-dihydro-1H-indole-5-sulfonic acid pyridin-3-ylamide.

^{1}HNMR (360 MHz, DMSO-d6) δ 10.74 (s, 1H, NH), 10.39 (s, 1H, SO_2NH), 8.27-8.28 (d, 1H), 8.21-8.23 (m, 1H), 7.59-7.62 (m, 2H), 7.44-7.68 (m, 1H), 7.24-7.28 (m, 1H), 6.69-6.71 (d, 1H), 3.54 (s, 2H).

MS *m/z* (APCI+) 290.2.

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5-Phenyloxindole

5-Bromo-2-oxindole (5 g, 23.5 mmol) was dissolved in 110 mL toluene and 110 mL ethanol with stirring and a little heat. Tetrakis(triphenylphosphine)palladium(0) (1.9 g, 1.6 mmol) was added followed by 40 mL (80 mmol) 2M aqueous sodium carbonate. To this mixture was added benzene boronic acid (3.7 g, 30.6 mmol) and the mixture was heated in a 100° C oil bath for 12 hours. The reaction was cooled, diluted with ethyl acetate (500 mL), washed with saturated sodium bicarbonate (200 mL), water (200 mL), 1N HCl (200 mL) and brine (200 mL). The organic layer was dried over magnesium sulfate and concentrated to afford a brown solid. Trituration with dichloromethane afforded 3.8 g (77%) of 5-phenyl-2-oxindole as a tan solid.

^{1}H NMR (360 MHz, DMSO-d6) δ 10.4 (br s, 1H, NH), 7.57 (dd, *J* = 1.8 and 7.2 Hz, 1H), 7.5 to 7.35 (m, 5H), 7.29 (m, 1H), 6.89 (d, *J* = 8.2 Hz, 1H), 3.51 (s, 2H, CH_2CO).

MS *m/z* 209 [M$^+$].

In similar fashion, the following oxindoles can be prepared:

6-(3,5-Dichlorophenyl)-1,3-dihydroindol-2-one

^{1}H NMR (360 MHz, DMSO-d6) δ 10.46 (br, 1H, NH), 7.64 (d, *J* = 1.8 Hz, 2H), 7.57 (m, 1H), 7.27 (m, 2H), 7.05 (d, J = 1.1 Hz, 1H), 3.5 (s, 2H).

MS-EI *m/z* 277/279 [M]$^+$.

6-(4-Butylphenyl)-1,3-dihydroindol-2-one

^{1}H NMR (360 MHz, DMSO-d6) δ 10.39 (s, 1H, NH), 7.49 (d, *J* = 8.0 Hz, 2H), 7.25 (d, *J* = 8 Hz, 3H), 7.17 (dd, *J* = 1.5 and 7.8 Hz, 1H), 6.99 (d, *J* = 1.5 Hz, 1H), 3.48 (s, 2H, CH_2CO), 2.60 (t, *J* = 7.5 Hz, 2Hz, CH_2CH_3), 1.57 (m, 2H, CH_2), 1.32 (m, 2H, CH_2), 0.9 (t, *J* = 7.5 Hz, 3H, CH_3).

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6-(5-Isopropyl-2-methoxyphenyl)-1,3-dihydroindol-2-one

^{1}H NMR (360 MHz, DMSO-d6) δ 10.29 (br s, 1H, NH), 7.16-7.21 (m, 2H), 7.08 (d, *J* = 2.4 Hz, 1H), 6.97-7.01 (m, 2H), 6.89 (d, *J* = 0.8 Hz, 1H), 3.71 (s, 3H, OCH_3), 3.47 (s, 2H, CH_2CO), 2.86 (m, 1H, $CH(CH_3)_2$), 1.19 (d, *J* = 6.8 Hz, 6H, $CH(CH_3)_2$).

MS-EI *m/z* 281 [M]$^+$.

6-(4-Ethylphenyl)-1,3-dihydroindol-2-one

^{1}H NMR (360 MHz, DMSO-d6) δ 10.39 (br s, 1H, NH), 7.50 (d, *J* = 8.2 Hz, 2H), 7.28 (d, *J* = 8.2 Hz, 2H), 7.25 (d, *J* = 7.5 Hz, 1H), 7.17 (dd, *J* = 1.6 & 7.5 Hz, 1H), 6.99 (d, *J* = 1.6 Hz, 1H), 3.48 (s, 2H, CH_2CO), 2.63 (q, *J* = 7.6 Hz, 2H, CH_2CH_3), 1.20 (t, *J* = 7.6 Hz, 3H, CH_2CH_3).

MS-EI *m/z* 237 [M]$^+$.

6-(3-Isopropylphenyl)-1,3-dihydroindol-2-one

^{1}H NMR (360 MHz, DMSO-d6) δ 10.37 (br s, 1H, NH), 7.43 (m, 1H), 7.35-7.39 (m, 1H), 7.17-7.27 (m, 3H), 7.01 (d, *J* = 1.8 Hz, 1H), 3.49 (s, 2H, CH_2CO), 2.95 (m, 1H, $CH(CH_3)_2$), 1.24 (d, *J* = 6.8 Hz, 6H, $CH(CH_3)_2$).

MS-EI *m/z* 251 [M]$^+$.

6-(2,4-Dimethoxyphenyl)-1,3-dihydroindol-2-one

^{1}H NMR (360 MHz, DMSO-d6) δ 10.28 (br s, 1H, NH), 7.17 (m, 2H), 6.93 (dd, *J* = 1.6 & 7.6 Hz, 1H), 6.86 (d, *J* = 1.6 Hz, 1H), 6.63 (d, *J* = 2.4 Hz, 1H), 6.58 (dd, *J* = 2.4 & 8.5 Hz, 1H), 3.79 (s, 3H, OCH_3), 3.74 (s, 3H, OCH_3), 3.45 (s, 2H, CH_2CO).

MS-EI *m/z* 269 [M]$^+$.

6-Pyridin-3-yl-1,3-dihydroindol-2-one

^{1}H NMR (360 MHz, DMSO-d6) δ 10.51 (s, 1H, NH), 8.81 (d, *J* = 2.5 Hz, 1H), 8.55 (dd, *J* = 1.8 and 5.7 Hz, 1H), 8 (m, 1H), 7.45 (dd, *J* = 5.7 and 9.3 Hz, 1H), 7.3 (m, 2H), 7.05 (s, 1H), 3.51 (s, 2H, CH_2CO).

MS *m/z* 210 [M]$^+$.

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2-Oxo-2,3-dihydro-1H-indole-4-carboxylic acid (3-chloro-4-ethoxyphenyl)-amide

To a solution of 4-carboxy-2-oxindole (200 mg, 1.13 mmol) and 3-chloro-4-methoxyphenylamine (178 mg, 1.13 mmol) in dimethylformamide (15 mL) at room temperature was added benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (BOP reagent, 997 mg, 2.26 mmol) followed by 4-dimethylaminopyridine (206 mg, 1.69 mmol). The mixture was stirred at room temperature for 72 hours. The reaction was then diluted with ethyl acetate (300 mL), washed with saturated sodium bicarbonate (100 mL), water, 2N hydrochloric acid (100 mL), water (3x200 mL) and brine. It was then dried over magnesium sulfate and concentrated. The residue was triturated with ethyl acetate to give 2-oxo-2,3-dihydro-1H-indole-4-carboxylic acid (3-chloro-4-methoxyphenyl)-amide as a pink solid.

^{1}HNMR (360 MHz, DMSO-d6) δ 10.50 (s, br, 1H, NH), 10.12 (s, br, 1H, NH), 7.9 (s, *J* = 2.5 Hz, 1H), 7.62 (dd, *J* = 2.5 & 9 Hz, 1H), 7.38 (d, *J* = 7.6 Hz, 1H), 7.32 (t, *J* = 7.6 Hz, 1H), 7.13 (d, *J* = 9 Hz, 1H), 6.98 (d, *J* = 7.6 Hz, 1H), 3.83 (s, 3H, OCH_3), 3.69 (s, 2H, CH_2).

MS-EI *m/z* 316 $[M]^+$.

4-Carboxy-2-oxindole

A solution of trimethylsilyldiazomethane in hexane (2 M) was added dropwise to a solution of 2.01 g 2-chloro-3-carboxy-nitrobenzene in 20 mL methanol at room temperature until no further gas evolution occurred. Acetic acid was then added to quench excess trimethylsilyldiazomethane. The reaction mixture was evaporated under vacuum and the residue was dried in an oven overnight. The 2-chloro-3-methoxycarbonylnitrobenzene obtained was pure enough for the following reaction.

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Dimethyl malonate (6.0 mL) was added to an ice-cold suspension of 2.1 g sodium hydride in 15 mL DMSO. The reaction mixture was stirred at 100° C for 1 hour and then cooled to room temperature. 2-Chloro-3-methoxycarbonylnitrobenzene (2.15 g) was added in one portion and the mixture was heated to 100° C for 1.5 hours. The reaction mixture was then cooled to room temperature, poured into ice water, acidified to pH 5 and extracted with ethyl acetate. The organic layer was washed with brine, dried over anhydrous sodium sulfate and concentrated to give 3.0 g of the dimethyl 2-methoxycarbonyl-6-nitrophenyl- malonate.

Dimethyl 2-methoxycarbonyl-6-nitrophenylmalonate (3.0 g) was refluxed in 50 mL of 6 N hydrochloric acid overnight. The mixture was concentrated to dryness, 20 mL ethanol and 1.1 g of tin(II) chloride were added and the mixture was refluxed for 2 hours. The mixture was filtered through Celite, concentrated and chromatographed on silica gel using ethyl acetate:hexane:acetic acid as eluent to give 0.65 g (37%) of 4-carboxy-2-oxindole as a white solid.

^{1}HNMR (360 MHz, DMSO-d6) δ 12.96 (s, br, 1H, COOH), 10.74 (s, br, 1H, NH), 7.53 (d, *J* = 8Hz, 1H), 7.39 (t, *J* = 8Hz, 1H), 7.12 (d, *J* = 8Hz, 1H), 3.67 (s, 2H).

D. Examples - Synthesis of pyrrole substituted 2-indolinones.

The following syntheses of representative compounds of this invention are shown by way of example only and are not to be construed as limiting the scope of this invention as to synthetic approach or as to the compounds which comprises this invention.

Example 1

4-Methyl-5-(2-oxo-1,2-dihydroindol-3-ylidenemethyl)-1*H*-pyrrole-2-carboxylic acid

4-Methyl-2-pyrrolecarboxylic acid ethyl ester (commercially

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available) was formylated using method A to give (73%) of 5-formyl-4-methyl-2-pyrrolecarboxylic acid ethyl ester. It was then hydrolysed using method B to give 5-formyl-4-methyl-1*H*-pyrrole-2-carboxylic acid (58%).

Oxindole (133 mg, 1 mmol) was condensed with 5-formyl-4-methyl-1*H*-pyrrole-2-carboxylic acid (153 mg) using method D to give 268 mg (100%) of the title compound as an orange-red solid.

^{1}HNMR (360 MHz, DMSO-d6) δ 13.84 (s, br, 1H, *NH*), 12.84 (s, br, 1H, COO*H*), 10.98 (s, br, 1H, NH), 7.82 (d, *J* = 7.5 Hz, 1H), 7.67 (s, 1H, H-vinyl), 7.18 (t, *J* = 7.5 Hz, 1H), 7.01 (t, *J* = 7.5 Hz, 1H), 6.88 (d, *J* = 7.5 Hz, 1H), 6.71 (d, *J* = 2.2 Hz, 1H), 2.32 (s, 3H, CH_3).

MS (negative mode) 266.8 [M-1]$^+$.

Example 2

4-Methyl-5-(1-methyl-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-1*H*-pyrrole-2-carboxylic acid

1-Methyl-1,3-dihydroindol-2-one (147 mg, 1 mmol) was condensed with 5-formyl-4-methyl-1*H*-pyrrole-2-carboxylic acid (153 mg) using method D to give 250 mg (86%) of the title compound.

^{1}HNMR (360 MHz, DMSO-d6) δ 13.82 (s, br, 1H, *NH*), 12.88 (s, br, 1H, 7.83 (d, *J* = 7.5 Hz, 1H), 7.65 (s, 1H, H-vinyl), 7.26 (t, *J* = 7.5 Hz, 1H), 7.02-7.09 (m, 2H), 6.70 (d, *J* = 2.2 Hz, 1H), 2.32 (s, 3H, CH_3).

MS m/z 283.0 [M+1]$^+$.

Example 3

4-Methyl-5-(2-oxo-1,2-dihydroindol-3-ylidenemethyl)-1*H*-pyrrole-2-carboxylic acid methyl ester

Oxindole (105 mg, 0.79 mmol) was condensed with 5-formyl-4-methyl-1*H*-pyrrole-2-carboxylic acid methyl ester (110 mg, 0.67 mmol) using method E to give 153.2 mg (81%) of the title compound.

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^{1}HNMR (360 MHz, DMSO-d6) δ 13.98 (s, br, 1H, NH), 10.97 (s, br, 1H, NH), 7.82 (d, *J* = 7.6 Hz, 1H), 7.67 (s, 1H, H-vinyl), 7.2 (dt, *J*= 1.2 & 7.7 Hz, 1H), 7.01 (dt, *J* = 1.2, 7.7 Hz, 1H), 6.90 (d, *J* = 7.6 Hz, 1H), 6.77 (d, *J* = 2 Hz, 1H).

MS (ES) *m/z* 283 [M$^+$+1].

Example 4

5-(5-Chloro-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-4-methyl-1*H*-pyrrole-2-carboxylic acid ethyl ester

5-Chloro-1,3-dihydroindol-2-one (2.22 g, 13.2 mmol) was condensed with 5-formyl-4-methyl-1*H*-pyrrole-2-carboxylic acid ethyl ester (2.43 g) using method E to give 4.1 g (94%) of the title compound as an orange solid.

^{1}HNMR (360 MHz, DMSO-d6) δ 13.95 (s, br, 1H, NH), 7.98 (d, *J* = 2.2 Hz, 1H, H-4), 7.78 (s, 1H, H-vinyl), 7.18 (dd, *J*= 2.2 & 8.3 Hz, 1H, H-6), 6.87 (d, *J* = 8.3 Hz, 1H, H-7), 7.34 (d, *J* = 1.8 Hz, 1H, H-3'), 4.27 (q, *J* = 7.2 Hz, 2H, OCH_2CH_3), 2.33 (s, 3H, CH_3), 1.29 (t, *J* = 7.2 Hz, 3H, OCH_2CH_3).

MS-EI *m/z* 330 [M$^+$].

Example 5

5-(5-Chloro-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-4-methyl-1*H*-pyrrole-2-carboxylic acid

A mixture of 5-(5-chloro-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-4-methyl-1*H*-pyrrole-2-carboxylic acid ethyl ester (1.3 g, 4 mmol) and potassium hydroxide in methanol (25 mL) and ethanol (25 mL) was heated to reflux for overnight. Insoluble materials were removed by filtration and the mixture was neutralized with 6N hydrochloric acid to give 0.876 g (70%) of the title compound.

^{1}HNMR (360 MHz, DMSO-d6) δ 13.80 (s, br, 1H, NH), 12.90 (s, br, 1H, COOH), 11.06 (s, br, 1H, NH), 8.02 (d, *J* = 1.8 Hz, 1H, H-4), 7.81 (s, 1H, H-vinyl), 7.20 (dd, *J*= 1.8 & 8.3 Hz, 1H, H-6),

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6.89 (d, J = 8.3 Hz, 1H, H-7), 6.72 (d, J = 1.8 Hz, 1H, H-3'), 2.35 (s, 3H, CH_3).

MS-EI m/z 302 [M$^+$].

Example 6

5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-4-methyl-1*H*-pyrrole-2-carboxylic acid (3-pyrrolidin-1-yl-propyl)amide

5-Bromo-1,3-dihydroindol-2-one (0.16 g, 0.76 mmol) was condensed with 5-formyl-4-methyl-1*H*-pyrrole-2-carboxylic acid (3-pyrrolidin-1-ylpropyl)amide (0.2 g, prepared by method C) to give 60 mg (17%) of the title compound as an orange solid.

^{1}HNMR (300 MHz, DMSO-d6) δ 13.61 (s, br, 1H, N*H*), 11.02 (s, br, 1H, N*H*), 8.42 (t, J = 5.8 Hz, 1H, CON*H*CH_2), 8.12 (d, J = 1.8 Hz, 1H, H-4), 7.78 (s, 1H, H-vinyl), 7.30 (dd, J = 1.8 & 8.4 Hz, 1H, H-6), 6.82 (d, J = 8.4 Hz, 1H, H-7), 6.77 (d, J = 2.4 Hz, 1H, H-3'), 3.22-3.31 (m, 2H, CH_2), 2.38-2.43 (m, 6H, 3xCH_2), 2.35 (s, 3H, CH_3), 1.62-1.71 (m, 6H, 3xCH_2).

MS-EI m/z 456 and 458 [M$^+$-1 and M$^+$+2].

Example 7

5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-4-methyl-1*H*-pyrrole-2-carboxylic acid (3-diethylamino-propyl)amide

5-Bromo-1,3-dihydroindol-2-one (0.16 g, 0.75 mmol) was condensed with 5-formyl-4-methyl-1*H*-pyrrole-2-carboxylic acid (3-diethylaminopropyl)amide (0.2 g, prepared by method C) to give 30 mg (8%) of the title compound as an orange solid.

^{1}HNMR (300 MHz, DMSO-d6) δ 13.61 (s, br, 1H, N*H*), 11.02 (s, br, 1H, N*H*), 8.40 (m, 1H, CON*H*CH_2), 8.12 (d, J = 1.5 Hz, 1H, H-4), 7.78 (s, 1H, H-vinyl), 7.30 (dd, J = 1.5 & 8.2 Hz, 1H, H-6), 6.82 (d, J = 8.2 Hz, 1H, H-7), 6.78 (d, J = 2.4 Hz, 1H, H-3'), 3.23 (m, 2H, CH_2), 2.38-2.45 (m, 6H, CH_2 & N(CH_2CH_3)$_2$), 2.35 (s, 3H, CH_3), 1.61 (m, 2H, CH_2), 0.93 (t, J = 7.1 Hz, 6H, N(CH_2CH_3)$_2$).

MS-EI m/z 458 and 460 [M$^+$-1 and M$^+$+2].

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Example 8

5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-1*H*-pyrrole-2-carboxylic acid (2-diethylaminoethyl)amide

5-Bromo-1,3-dihydroindol-2-one (212 mg, 1 mmol) was condensed with 5-formyl-1*H*-pyrrole-2-carboxylic acid (2-diethylaminoethyl)amide (prepared from ethyl pyrrole-2-carboxylate by method A, B and then C) to give 162 mg (38%) of the title compound.

^{1}H NMR (300 MHz, DMSO-d6) δ 13.53 (s, br, 1H, NH), 11.06 (s, br, 1H, NH), 8.37 (t, 1H, CONHCH$_2$), 7.89 (m, 2H), 7.32 (dd, J = 2.0 Hz, 1H), 6.96 (s, 1H), 6.80-6.84 (m, 2H), 3.3 (m, 2H, CH$_2$), 2.45-2.55 (m, 6H, N(CH$_2$CH$_3$)$_2$ & CH$_2$), 0.95 (t, J = 7.2 Hz, 6H, N(CH$_2$CH$_3$)$_2$).

MS-EI m/z 430 and 432 [M$^+$-1 and M$^+$+ 1].

Example 9

5-(2-Oxo-6-phenyl-1,2-dihydroindol-3-ylidenemethyl)-1*H*-pyrrole-2-carboxylic acid (2-diethylaminoethyl)amide

6-Phenyl-1,3-dihydroindol-2-one (209 mg, 1mmol) was condensed with 5-formyl-1*H*-pyrrole-2-carboxylic acid (2-diethylaminoethyl)amide to give 182 mg (42%) of the title compound.

^{1}H NMR (300 MHz, DMSO-d6) δ 13.56 (s, br, 1H, NH), 11.06 (s, br, 1H, NH), 8.36 (t, 1H, CONHCH$_2$), 7.77 (s, 1H, H-vinyl), 7.73 (d, J = 7.8 Hz, 1H), 7.64 (d, J = 7.2 Hz, 2H), 7.46 (m, 2H), 7.32 (m, 2H), 7.11 (s, 1H), 6.96 (m, 1H), 6.80 (m, 1H), 3.31-3.32 (m, 2H, CH$_2$), 2.46-2.53 (m, 6H, N(CH$_2$CH$_3$)$_2$ & CH$_2$), 0.96 (t, J = 6.9 Hz, 6H, N(CH$_2$CH$_3$)$_2$).

MS-EI m/z 428 [M$^+$].

Example 10

5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-1*H*-pyrrole-2-carboxylic acid (2-diethylaminoethyl)-methyl-amide

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5-Bromo-1,3-dihydroindol-2-one (212 mg, 1 mmol) was condensed with 5-formyl-1*H*-pyrrole-2-carboxylic acid (2-diethylaminoethyl)methylamide to give 246 mg (55%) of the title compound.

^{1}H NMR (360 MHz, DMSO-d6) δ 13.54 (s, br, 1H, *NH*), 11.06 (s, br, 1H, *NH*), 7.90 (m, 2H), 7.33 (dd, *J* = 1.8 & 8.4 Hz, 1H), 6.82-6.85 (m, 3H), 3.55 (s, br, 2H, CH_2), 3.25 (s, br, 3H, NCH_3), 2.57 (t, *J* = 6.5 Hz, 2H, CH_2), 2.45 (m, 4H, $N(CH_2CH_3)_2$), 0.91 (m, 6H, $N(CH_2CH_3)_2$).

MS-EI *m/z* 444 and 446 [M$^+$-1 and M$^+$+1].

Example 11

5-(2-Oxo-6-phenyl-1,2-dihydroindol-3-ylidenemethyl)-1*H*-pyrrole-2-carboxylic acid (2-diethylaminoethyl)methylamide

6-Phenyl-1,3-dihydroindol-2-one (209 mg, 1 mmol) was condensed with 5-formyl-1*H*-pyrrole-2-carboxylic acid (2-diethylaminoethyl)methylamide to give 277 mg (63%) of the title compound.

^{1}H NMR (360 MHz, DMSO-d6) δ 13.58 (s, br, 1H, NH), 11.04 (s, br, 1H, NH), 7.78 (s, 1H, H-vinyl), 7.73 (d, *J* = 7.8 Hz, 1H), 7.64 (d, *J* = 7.5 Hz, 2H), 7.46 (m, 2H), 7.33-7.36 (m, 2H), 7.11 (s, 1H), 6.84 (m, 1H), 6.78 (m, 1H), 3.55 (s, br, 2H, CH_2), 3.25 (s, br, 3H, NCH_3), 2.58 (t, 2H, CH_2), 2.44 (m, 4H, $N(CH_2CH_3)_2$), 0.92 (m, 6H, $N(CH_2CH_3)_2$).

Example 12

3-Methyl-5-(2-oxo-1,2-dihydroindol-3-ylidenemethyl)-1*H*-pyrrole-2-carboxylic acid (3-diethylaminopropyl)amide

Oxindole (66.5 mg, 0.5 mmol) was condensed with 5-formyl-3-methyl-1H-pyrrole-2-carboxylic acid (3-diethylaminopropyl)amide (prepared from 3-formyl-3-methyl-1H-pyrrole-2-carboxylic acid ethyl ester by method B then C) to give 39 mg (21%) of the title compound.

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^{1}H NMR (300 MHz, DMSO-d6) δ 13.34 (s, br, 1H, NH), 10.88 (s, br, 1H, NH), 7.62-7.67 (m, 3H), 7.17 (m, 1H), 6.99 (m, 1H), 6.87 (d, J = 7.6 Hz, 1H), 6.63 (d, J = 1 Hz, 1H), 3.26-3.32 (m, 2H, CH_2), 2.41-2.48 (m, 6H, CH_2 & $N(CH_2CH_3)_2$), 2.29 (s, 3H, CH_3), 1.63 (m, 2H, CH_2), 0.93 (t, J = 7.2 Hz, 6H, $N(CH_2CH_3)_2$).

MS-EI m/z 380 [M$^+$].

Example 13

5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-3-methyl-1*H*-pyrrole-2-carboxylic acid (3-diethylamino-propyl)amide

5-Bromo-1,3-dihydroindol-2-one (106 mg, 0.5 mmol) was condensed with 5-formyl-3-methyl-1H-pyrrole-2-carboxylic acid (3-diethylaminopropyl)amide to give 35 mg (15%) of the title compound.

^{1}H NMR (300 MHz, DMSO-d6) δ 13.35 (s, br, 1H, NH), 11.00 (s, br, 1H, NH), 7.89 (d, J = 1.9 Hz, 1H, H-4), 7.80 (s, 1H, H-vinyl), 7.74 (t, J = 5.3 Hz, 1H, $CONHCH_2$), 7.31 (dd, J = 1.9 & 8.4 Hz, 1H, H-6), 6.83 (d, J = 8.4 Hz, 1H, H-7), 6.63 (s,1H, H-3'), 3.26 (m, 2H, CH_2), 2.41-2.48 (m, 6H, CH_2 & $N(CH_2CH_3)_2$), 2.29 (s, 3H, CH_3), 1.63 (m, 2H, CH_2), 0.93 (t, J = 7.1 Hz, 6H, $N(CH_2CH_3)_2$).

MS-EI m/z 458 and 460 [M$^+$-1 and M$^+$+1].

Example 14

3-Methyl-5-(2-oxo-6-phenyl-1,2-dihydroindol-3-ylidenemethyl)-1*H*-pyrrole-2-carboxylic acid (3-diethylaminopropyl)amide

6-Phenyl-1,3-dihydroindol-2-one (105 mg, 0.5 mmol) was condensed with 5-formyl-3-methyl-1H-pyrrole-2-carboxylic acid (3-diethylaminopropyl)amide to give 67.8 (30%) of the title compound.

^{1}H NMR (300 MHz, DMSO-d6) δ 13.37 (s, br, 1H, NH), 11.02 (s, br, 1H, NH), 7.23-7.73 (m, 11H), 3.29 (m, 2H, CH_2), 2.41-2.48 (m, 6H, CH_2 & $N(CH_2CH_3)_2$), 2.29 (s, 3H, CH_3), 1.64 (m, 2H, CH_2), 0.94

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(t, J = 7.0 Hz, 6H, $N(CH_2CH_3)_2$).

MS-EI m/z 456 [M$^+$].

Example 15

5-(5-Methoxy-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-3-methyl-1*H*-pyrrole-2-carboxylic acid (3-diethylamino-propyl)amide

5-Methoxy-1,3-dihydroindol-2-one (82.5 mg, 0.5 mmol) was condensed with 5-formyl-3-methyl-1H-pyrrole-2-carboxylic acid (3-diethylaminopropyl)amide to give 80 mg (39%) of the title compound.

^{1}H NMR (300 MHz, DMSO-d6) δ 13.45 (s, br, 1H, *NH*), 10.70 (s, br, 1H, *NH*), 7.68-7.70 (m, 2H), 7.32 (d, J = 1.8 Hz, 1H), 6.72-6.79 (m, 2H), 6.60 (s, 1H), 3.73 (s, 3H, OCH_3), 3.28 (m, 2H, CH_2), 2.41-2.48 (m, 6H, CH_2 & $N(CH_2CH_3)_2$), 2.29 (s, 3H, CH_3), 1.63 (m, 2H, CH_2), 0.93 (t, J = 7.0 Hz, 6H, $N(CH_2CH_3)_2$).

MS m/z 410 [M$^+$].

Example 16

5-(6-Methoxy-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-3-methyl-1*H*-pyrrole-2-carboxylic acid (3-diethylamino-propyl)amide

6-Methoxy-1,3-dihydroindol-2-one (82.5 mg, 0.5 mmol) was condensed with 5-formyl-3-methyl-1H-pyrrole-2-carboxylic acid (3-diethylaminopropyl)amide to give 63 mg (31%) of the title compound.

^{1}H NMR (300 MHz, DMSO-d6) δ 13.22 (s, br, 1H, *NH*), 10.86 (s, br, 1H, *NH*), 7.39-7.63 and 6.37-6.55 (m, 6H), 3.73 (s, 3H, OCH_3), 3.3 (m, 2H, CH_2), 2.45 (m, 6H, CH_2 & $N(CH_2CH_3)_2$), 2.28 (s, 3H, CH_3), 1.63 (m, 2H, CH_2), 0.93 (m, 6H, $N(CH_2CH_3)_2$).

MS m/z 410 [M$^+$].

Example 17

3-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-4,5,6,7-tetrahydro-2*H*-isoindole-1-carboxylic acid (2-diethylamino-ethyl)amide

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4,5,6,7-Tetrahydro-2*H*-isoindole-1-carboxylic acid ethyl ester (May, Donald A.; Lash, Timothy D.; J. Org. Chem., 1992, 57:18, 4820-4828) was formylated using method A then B to give 3-formyl-4,5,6,7-tetrahydro-2*H*-isoindole-1-carboxylic acid.

5-Bromo-1,3-dihydroindol-2-one (1.43 g, 6.8 mmol) was condensed with 3-formyl-4,5,6,7-tetrahydro-2*H*-isoindole-1-carboxylic acid (2-diethylaminoethyl)amide (1.97 g) to give 2.2 g (67%) of the title compound as a yellow-orange solid.

^{1}H NMR (360 MHz, DMSO-d6) δ 13.47 (s, 1H, N*H*), 11.0 (s, 1H, N*H*), 8.0 (d, 1H, N*H*), 7.70 (s, 1H, C*H*), 7.28 (dd, *J* = 2.1 and 8.2 Hz, 1H, Ar*H*), 7.16 (m, 1H, Ar*H*), 6.8 (d, *J* = 8.3Hz, 1H, Ar*H*), 3.3 (s, 2H, CON*H*), 2.5 (m, 6H, 3xNC*H*$_2$), 2.78 (br m, 2H, pyrrole C*H*$_2$), 2.72 (br m, 2H, pyrroleC*H*$_2$), 1.7 (br m, 4H, N(C*H*$_2$CH$_3$)$_2$), 1.74 (br s, 4H, CH$_2$C*H*$_2$C*H*$_2$CH$_2$), 0.96 (t, *J* = 7.4 Hz, 6H, N(CH$_2$C*H*$_3$)$_2$).

MS-EI *m/z* 484 and 486 [M$^+$-1 and M$^+$+1].

Example 18

3-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-4,5,6,7-tetrahydro-2*H*-isoindole-1-carboxylic acid (3-diethylaminopropyl)amide

5-Bromo-1,3-dihydroindol-2-one (20 mg, 0.1 mmol) was condensed with 3-formyl-4,5,6,7-tetrahydro-2*H*-isoindole-1-carboxylic acid (3-diethylaminopropyl)amide (30 mg) to give 33 mg (46%) of the title compound as an orange solid.

^{1}H NMR (360 MHz, DMSO-d6) δ 10.9 (s, 1H, N*H*), 8.0 (m, 1H, N*H*), 7.68 (m, 1H, Ar*H*), 7.4 (m, 1H, Ar*H*), 7.29 (d, *J* = 1.9 and 8.5Hz, 1H, Ar*H*), 6.8 (d, *J* = 8 Hz, 1H, Ar*H*), 2.7 (br m, 4H, 2xNC*H*$_2$), 2.4 (m, 8H, 4xNC*H*$_2$), 1.7 (br m, 4H, N(C*H*$_2$CH$_3$)$_2$), 1.6 (br m, 2H, CH$_2$C*H*$_2$CH$_2$), 0.93 (t, *J* = 7.4 Hz, 6H, N(CH$_2$C*H*$_3$)$_2$).

MS-EI *m/z* 499 and 501 [M$^+$ and M$^+$+2].

Example 19

3-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-4,5,6,7-

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tetrahydro-2*H*-isoindole-1-carboxylic acid (3-pyrrolidin-1-ylpropyl)amide

5-Bromo-1,3-dihydroindol-2-one (80 mg, 0.4 mmol) was condensed with 3-formyl-4,5,6,7-tetrahydro-2*H*-isoindole-1-carboxylic acid (3-pyrrolidin-1-ylpropyl)amide (120 mg) to give 43 mg (22%) of the title compound as a tan-orange solid.

^{1}H NMR (360 MHz, DMSO-d6) δ 13.4 (s, 1H, N*H*), 10.9 (s, 1H, N*H*), 8.0 (m, 1H, N*H*), 7.69 (m, 1H, Ar*H*), 7.49 (m, 1H, Ar*H*), 7.28 (d, *J* = 1.7 and 7.8 Hz, 1H, Ar*H*), 6.8 (d, *J* = 8 Hz, 1H, Ar*H*), 3.3 (br m, 2H, 2xNCH_2), 2.8 (m, 4H, 2xpyrroleCH_2), 2.5 (br m, 4H, N(CH_2CH_3)$_2$), 1.6 (br m, 8H, 2xpyrroleCH_2CH_2, $CH_2CH_2CH_2$ and CONHCH_2).

MS-EI *m/z* 497 and 499 [M$^+$ and M$^+$+2].

Example 20

3-(2-Oxo-6-pyridin-3-yl-1,2-dihydroindol-3-ylidenemethyl)-4,5,6,7-tetrahydro-2*H*-isoindole-1-carboxylic acid (2-diethylaminoethyl)amide

6-Pyridin-3-yl-1,3-dihydroindol-2-one (60 mg, 0.4 mmol) was condensed with 3-formyl-4,5,6,7-tetrahydro-2*H*-isoindole-1-carboxylic acid (2-diethylaminoethyl)amide (80 mg) to give 50 mg (38%) of the title compound as a reddish solid.

^{1}H NMR (360 MHz, DMSO-d6) δ 13.4 (s, 1H, NH), 11 (s, 1H, NH), 8.9 (d, 1H, N*H*), 8.7 (dd, 1H, Ar*H*), 8.1 (dd, 1H, Ar*H*), 7.9 (d, 1H, Ar*H*), 7.6 (s, 1H, C*H*), 7.5 (dd, 1H, Ar*H*), 7.3 (dd, 1H, Ar*H*), 7.1 (m, 2H, Ar*H*), 3.35 (m, 2H, CONHCH_2), 2.8 (m, 4H, 2xpyrroleCH_2), 2.5 (br m, 6H, N(CH_2CH_3)$_2$ and NCH_2), 1.75 (br s, 4H, 2xpyrroleCH_2CH_2), 0.9 (t, 6H, N(CH_2CH_3)$_2$).

MS-EI *m/z* 484 [M$^+$].

Example 21

4-Benzoyl-5-(5-bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-3-methyl-1*H*-pyrrole-2-carboxylic acid (3-diethylaminopropyl)amide

To a mixture of benzoyl chloride (1 equiv.) and aluminum

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chloride (1 equiv.) in dichloroethane at 0° C was added ethyl 3,5-dimethyl-2-pyrrolecarboxylate (1 equiv.). The mixture was stirred at 80° C for 4 hr. The mixture was then extracted with ethyl acetate (EtOAc) and H_2O. The combined organic extracts were washed with saturated sodium bicarbonate and brine, dried and concentrated to give (51%) of 4-benzoyl-3,5-dimethyl-1*H*-pyrrole-2-carboxylic acid.

A mixture of 4-benzoyl-3,5-dimethyl-1*H*-pyrrole-2-carboxylic acid ethyl ester (4.13 g, 15.2 mmol) and ceric ammonium nitrate (33 g, 4equiv.) in 50 mL of tetrahydrofuran (THF):acetic acid (HOAc):H_2O 1:1:1 was refluxed overnight. The reaction mixture was then cooled, extracted with EtOAc and then basified to pH 9 with sodium carbonate. The organic layer was then washed with brine, dried ($MgSO_4$) and concentrated followed by column chromatography to give 3.25 g (75%) of 4-benzoyl-5-formyl-3-methyl-1*H*-pyrrole-2-carboxylic acid ethyl ester as a yellow solid.

5-Bromo-1,3-dihydro-indol-2-one was condensed with 4-benzoyl-5-formyl-3-methyl-1*H*-pyrrole-2-carboxylic acid using method D to give 4-benzoyl-5-(5-bromo-2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-3-methyl-1*H*-pyrrole-2-carboxylic acid.

The above carboxylic acid was then coupled with N,N-diethyl-1,3-propanediamine using method C to give the title compound.

^{1}H NMR (360 MHz, DMSO-d6) δ 7.96 (m, 1H, CONHCH$_2$), 7.76 (d, *J* = 7.0 Hz, 2H), 7.68 (t, 1H), 7.56 (m, 2H), 7.40 (s, 2H) 7.33 (dd, *J* = 1.6 & 8.3 Hz, 1H, H-6), 6.84 (d, *J* = 8.3 Hz, 1H, H-7), 3.33 (m, 2H, CH$_2$), 2.42-2.46 (m, 6H, 3xCH$_2$), 2.10 (s, 3H, CH$_3$), 1.65 (m, 2H, CH$_2$), 0.94 (t, *J* = 7.0 Hz, 6H, N(CH$_2$CH$_3$)$_2$).

MS Electron Impact *m/z* 564 [M$^+$+1].

Example 22

4-Benzoyl-5-(5-bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-3-methyl-1*H*-pyrrole-2-carboxylic acid (3-morpholin-4-ylpropyl)amide

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^{1}H NMR (360 MHz, DMSO-d6) δ 14.10 (s, 1H, NH), 11.14 (br s, 1H, NH), 7.92 (m, 1H, CONHCH$_2$), 7.75 (m, 2H), 7.69 (t, 1H), 7.56 (m, 2H), 7.42 (m, 2H), 7.33 (dd, *J* = 1.9 & 8.3 Hz, 1H, H-6), 6.85 (d, *J* = 8.3 Hz, 1H, H-7), 3.56 (m, 4H, 2xCH$_2$), 3.33 (m, 2H, CH$_2$), 2.35 (m, 6H, 3xCH$_2$), 2.10 (s, 3H, CH$_3$), 1.70 (m, 2H, CH$_2$).

Example 23

4-Benzoyl-3-methyl-5-(2-oxo-1,2-dihydroindol-3-ylidenemethyl)-1*H*-pyrrole-2-carboxylic acid (3-pyrrolidin-1-ylpropyl)amide

^{1}H NMR (300 MHz, DMSO-d6) δ 14.18 (s, 1H, NH), 11.14 (br s, 1H, NH), 8.01 (m, 1H, CONHCH$_2$), 7.74 (m, 1H), 7.67 (m, 1H), 7.55 (m, 1H), 7.32 (s, 1H, H-vinyl), 7.17 (m, 1H), 6.92 (m, 1H), 3.36 (m, 2H, CH$_2$), 2.44 (m, 6 H, 3xCH$_2$), 2.11 (s, 3H, CH$_3$), 1.65-1.75 (m, 6H, 3xCH$_2$).

MS Electron Impact *m/z* 482 [M$^+$].

Example 24

4-Benzoyl-5-(5-bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-3-methyl-1*H*-pyrrole-2-carboxylic acid (3-pyrrolidin-1-ylpropyl)amide

^{1}H NMR (360 MHz, DMSO-d6) δ 14.01 (s, 1H, NH), 11.18 (br s, 1H, NH), 7.98 (m, 1H, CONHCH$_2$), 7.75 (m, 2H), 7.68 (m, 1H), 7.55 (m, 2H), 7.40 (m, 2H), 7.33 (dd, *J* = 2.0 & 8.2 Hz, 1H, H-6), 6.84 (d, *J* = 8.2 Hz, 1H, H-7), 3.34 (m, 2H, CH$_2$), 2.42-2.47 (m, 6 H, 3xCH$_2$), 2.09 (s, 3H, CH$_3$), 1.70 (m, 2H, CH$_2$), 1.64 (m, 4H, 2xCH$_2$).

Example 25

4-Benzoyl-3-methyl-5-(2-oxo-6-phenyl-1,2-dihydroindol-3-ylidenemethyl)-1*H*-pyrrole-2-carboxylic acid (3-pyrrolidin-1-ylpropyl)amide

^{1}H NMR (300 MHz, DMSO-d6) δ 14.15 (s, 1H, NH), 11.16 (br s, 1H, NH), 7.98 (m, 1H, CONHCH$_2$), 7.77 (d, *J* = 7.7 Hz, 2H), 7.69 (m, 1H), 7.53-7.63 (m, 4H), 7.44 (m, 2H), 7.33-7.37 (m, 2H), 7.24 (s, 2H), 7.12 (s, 1H), 3.36 (m, 2H, CH$_2$), 2.43-2.48 (m, 6 H,

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3xCH_2), 2.12 (s, 3H, CH_3), 1.74 (m, 2H, CH_2), 1.69 (m, 4H, 2xCH_2).

MS Electron Impact *m/z* 558 [M$^+$].

Example 26

4-Benzoyl-5-(6-methoxy-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-3-methyl-1*H*-pyrrole-2-carboxylic acid (3-pyrrolidin-1-ylpropyl)amide

^{1}H NMR (300 MHz, DMSO-d6) δ 13.99 (s, 1H, N*H*), 11.05 (br s, 1H, N*H*), 7.93 (m, 1H, CON*H*CH$_2$), 7.72 (m, 2H), 7.65 (m, 1H), 7.54 (m, 2H), 7.15 (s, 1H, H-vinyl), 7.04 (d, *J* = 8.4 Hz, 1H, H-4), 6.51 (dd, *J* = 2.3 & 8.4 Hz, 1H, H-5) , 6.44 (d, *J* = 2.3 Hz, 1H, H-7), 3.74 (s, 3H, OCH_3), 3.35 (m, 2H, CH_2), 2.42-2.46 (m, 6 H, 3xCH_2), 2.10 (s, 3H, CH_3), 1.72 (m, 2H, CH_2), 1.65 (m, 4H, 2xCH_2).

MS Electron Impact *m/z* 512 [M$^+$].

Example 27

4-Benzoyl-5-(5-methoxy-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-3-methyl-1*H*-pyrrole-2-carboxylic acid (3-pyrrolidin-1-ylpropyl)amide

^{1}H NMR (360 MHz, DMSO-d6) δ 14.24 (s, 1H, N*H*), 10.90 (br s, 1H, N*H*), 7.97 (m, 1H, CON*H*CH$_2$), 7.75 (d, *J* = 7.2 Hz, 2H), 7.69 (m, 1H), 7.56 (m, 2H), 7.24 (s, 1H, H-vinyl), 6.79 (m, 2H), 6.66 (m, 1H), 3.67 (s, 3H, OCH_3), 3.34 (m, 2H, CH_2), 2.43-2.48 (m, 6 H, 3xCH_2), 2.14 (s, 3H, CH_3), 1.71 (m, 2H, CH_2), 1.66 (m, 4H, 2xCH_2).

MS Electron Impact *m/z* 512 [M$^+$].

Example 28

4-Benzoyl-5-(5-fluoro-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-3-methyl-1*H*-pyrrole-2-carboxylic acid (3-pyrrolidin-1-ylpropyl)amide

^{1}H NMR (300 MHz, DMSO-d6) δ 14.20 (s, 1H, N*H*), 11.14 (br s, 1H, N*H*), 8.03 (m, 1H, CON*H*CH$_2$), 7.75 (m, 2H), 7.68 (m, 1H), 7.55 (m, 2H), 7.38 (s, 1H, H-vinyl), 7.08 (m, 1H), 7.01 (m, 1H), 6.87

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(m, 1H), 3.34 (m, 2H, CH_2), 2.42-2.48 (m, 6 H, 3xCH_2), 2.09 (s, 3H, CH_3), 1.70 (m, 2H, CH_2), 1.65 (m, 4H, 2xCH_2).

MS Electron Impact *m/z* 500 [M$^+$].

Example 29

4-Acetyl-5-(5-bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-3-methyl-1*H*-pyrrole-2-carboxylic acid (3-diethylaminopropyl)amide

5-Bromo-1,3-dihydro-indol-2-one was condensed with 4-acetyl-5-formyl-3-methyl-1*H*-pyrrole-2-carboxylic acid (3-diethylaminopropyl)amide (prepared from 4-acetyl-5-formyl-3-methyl-1*H*-pyrrole-2-carboxylic acid ethyl ester by method B then C) to give the title compound.

^{1}H NMR (300 MHz, DMSO-d6) δ 14.19 (s, 1H, N*H*), 11.19 (br s, 1H, N*H*), 8.15 (m, 1H, CON*H*CH_2), 8.11 (s, 1H, H-vinyl), 7.72 (d, *J* = 1.8 Hz, 1H, H-4), 7.38 (dd, *J* = 1.8 & 8.2 Hz, 1H, H-6), 6.87 (d, *J* = 8.2 Hz, 1H, H-7), 3.27 (m, 2H, CH_2), 2.57 (s, 3H, CH_3CO), 2.46 (m, 9 H, CH_3 & 3xCH_2), 1.64 (m, 2H, CH_2), 0.93 (t, *J* = 7.1 Hz, 6H, N(CH_2CH_3)$_2$).

Example 30

4-Acetyl-5-(5-bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-3-methyl-1*H*-pyrrole-2-carboxylic acid (3-pyrrolidin-1-ylpropyl)amide

^{1}H NMR (300 MHz, DMSO-d6) δ 8.14 (m, 1H, CON*H*CH_2), 8.10 (s, 1H, H-vinyl), 7.70 (d, 1H, H-4), 7.36 (dd, *J* = 1.6 & 8.1 Hz, 1H, H-6), 6.85 (d, *J* = 8.1 Hz, 1H, H-7), 3.32 (m, 2H, CH_2), 2.57 (s, 3H, CH_3CO), 2.44 (s, 3H, CH_3), 2.35-2.48 (m, 6H, 3xCH_2), 1.65-1.71 (m, 6H, 3xCH_2).

MS *m/z* 499 & 501 [M$^+$] & [M$^+$+2].

Example 31

4-Acetyl-5-(5-bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-3-methyl-1*H*-pyrrole-2-carboxylic acid (3-morpholin-4-ylpropyl)amide

^{1}H NMR (300 MHz, DMSO-d6) δ 14.20 (s, 1H, N*H*), 11.26 (br s,

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1H, NH), 8.09 (m, 2H, H-vinyl & CONHCH$_2$), 7.73 (d, J = 1.5 Hz, 1H, H-4), 7.38 (dd, J = 1.5 & 8.3 Hz, 1H, H-6), 6.87 (d, J = 8.3 Hz, 1H, H-7), 3.55 (m, 4H, 2xCH$_2$), 3.26 (m, 2H, CH$_2$), 2.57 (s, 3H, CH$_3$CO), 2.44 (s, 3H, CH$_3$), 2.35 (m, 6H, 3xCH$_3$), 1.68 (m, 2H, CH$_2$).

MS-EI m/z 514 & 516 [M$^+$-1] & [M$^+$+1].

Example 32

4-Acetyl-5-(5-bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-3-methyl-1H-pyrrole-2-carboxylic acid (3-hydroxypropyl)amide

^{1}H NMR (360 MHz, DMSO-d6) δ 14.17 (s, 1H, NH), 11.25 (br s, 1H, NH), 8.10 (s, 1H, H-vinyl), 8.03 (m, 1H, CONHCH$_2$), 7.71 (br s, 1H, H-4), 7.37 (br d, J = 8.4 Hz, 1H, H-6), 6.87 (d, J = 8.4 Hz, 1H, H-7), 4.51 (br s, 1H, OH), 3.51 (br s, 2H, CH$_2$), 3.36 (m, 2H, CH$_2$), 2.57 (s, 3H, CH$_3$CO), 2.43 (s, 3H, CH$_3$), 1.70 (m, 2H, CH$_2$).

MS-EI m/z 445 & 447 [M$^+$-1] & [M$^+$+1].

Example 34

4-Acetyl-5-(5-bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-3-methyl-1H-pyrrole-2-carboxylic acid (2-morpholin-4-ylethyl)amide

^{1}H NMR (360 MHz, DMSO-d6) δ 14.19 (s, 1H, NH), 11.14 (br s, 1H, NH), 8.10 (s, 1H, H-vinyl), 7.84 (m, 1H, CONHCH$_2$), 7.71 (d, J = 1.8 Hz, 1H, H-4), 7.38 (dd, J = 1.8 & 8.2 Hz, 1H, H-6), 6.87 (d, J = 8.2 Hz, 1H, H-7), 3.58 (m, 4H, 2xCH$_2$), 3.40 (m, 2H, CH$_2$), 2.57 (s, 3H, CH$_3$CO), 2.49 (m, 4H, 2xCH$_2$), 2.45 (m, CH$_3$ & CH$_2$).

MS-EI m/z 500 & 502 [M$^+$-1] & [M$^+$+1].

Example 35

4-Acetyl-5-(5-bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-3-methyl-1H-pyrrole-2-carboxylic acid (2-pyrrolidin-1-ylethyl)amide

^{1}H NMR (360 MHz, DMSO-d6) δ 14.17 (s, 1H, NH), 11.23 (s, 1H, NH), 8.11 (s, 1H, H-vinyl), 7.91 (m, 1H, CONHCH$_2$), 7.73 (d, J = 1.9 Hz, 1H, H-4), 7.39 (dd, J = 1.9 & 8.3 Hz, 1H, H-6), 6.88 (d, J =

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8.3 Hz, 1H, H-7), 3.40 (m, 2H, CH_2), 2.62 (m, 2H, CH_2), 2.57 (s, 3H, CH_3CO), 2.49 (m, 4H, 2xCH_2), 2.44 (s, 3H, CH_3), 1.69 (m, 4H, 2xCH_2).

Example 36

4-Acetyl-5-(5-bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-3-methyl-1*H*-pyrrole-2-carboxylic acid [2-(4-hydroxyphenyl)ethyl]amide

^{1}H NMR (300 MHz, DMSO-d6) δ 14.21 (s, 1H, N*H*), 11.18 (s, 1H,O*H*), 9.09 (s, 1H, N*H*), 8.06-8.10 (m, 2H), 7.73 (s, 1H), 7.38 (d, *J* = 7.8 Hz, 1H), 7.04 (d, *J* = 7.1 Hz, 2H), 6.88 (d, *J* = 7.8 Hz, 1H), 6.67 (d, *J* = 7.1 Hz, 2H), 3.42 (m, 2H, CH_2), 2.72 (m, 2H, CH_2), 2.56 (s, 3H, CH_3CO), 2.37 (s, 3H, CH_3).

MS-EI *m/z* 507 & 509 [M$^+$-1] & [M$^+$+1].

Example 37

5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2-isopropyl-4-phenyl-1*H*-pyrrole-3-carboxylic acid (3-diethylaminopropyl)amide

A mixture of 2-aminoacetophenone hydrochloride (1 equiv.), ethyl isobutyrylacetate (1.2 equiv.) and sodium acetate (2.4 equiv.) in H_2O was stirred at 100° C for 18 hours and then cooled to room temperature. The aqueous layer was decanted off and the oil was dissolved in ethyl acetate. It was then washed with water and brine and then dried to give (93%) of 2-isopropyl-4-phenyl-1*H*-pyrrole-3-carboxylic acid ethyl ester as a red brown oil.

^{1}HNMR (300 MHz, DMSO-d6) δ 11.21 (s, br, 1H, N*H*), 7.14-7.27 (m, 5H), 6.70 (d, *J* = 2.7 Hz, 1H), 4.02 (q, *J* = 7.1 Hz, 2H, OCH_2CH_3), 3.65 (m, 1H, $CH(CH_3)_2$), 1.22 (d, *J* = 7.5 Hz, 6H, $CH(CH_3)_2$), 1.04 (t, *J* = 7.1 Hz, 3H, OCH_2CH_3).

MS-EI *m/z* 257 [M$^+$].

The above pyrrole was formylated using method A to give (41%) 5-formyl-2-isopropyl-4-phenyl-1*H*-pyrrole-3-carboxylic acid ethyl ester as a reddish solid.

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^{1}HNMR (300 MHz, DMSO-d6) δ 12.35 (s, br, 1H, N*H*), 9.14 (s, 1H, CHO), 7.36 (s, 5H), 3.96 (q, *J* = 7.1 Hz, 2H, OC*H*$_2$CH$_3$), 3.74 (m, 1H, C*H*(CH$_3$)$_2$), 1.29 (d, *J* = 6.9 Hz, 6H, CH(C*H*$_3$)$_2$), 0.90 (t, *J* = 7.1 Hz, 3H, OCH$_2$C*H*$_3$).

MS-EI *m/z* 285 [M$^+$].

The pyrrolecarboxylic acid ester was hydrolysed using method B to give (57%) of 5-formyl-2-isopropyl-4-phenyl-1*H*-pyrrole-3-carboxylic acid as a beige solid.

^{1}HNMR (300 MHz, DMSO-d6) δ 12.28 (s, br, 1H, COO*H*), 12.02 (s, br, 1H, N*H*), 9.10 (s, 1H, CHO), 7.35 (s, 5H), 3.81 (m, 1H, C*H*(CH$_3$)$_2$), 1.28 (d, *J* = 6.9 Hz, 6H, CH(C*H*$_3$)$_2$).

MS-EI *m/z* 257 [M$^+$].

5-Bromo-1,3-dihydroindol-2-one (120 mg, 0.31 mmol) was condensed with 5-formyl-2-isopropyl-4-phenyl-1*H*-pyrrole-3-carboxylic acid (3-diethylaminopropyl)amide (prepared by method C) to give 120 mg (71%) of the title compound.

^{1}HNMR (300 MHz, DMSO-d6) δ 14.23 (s, br, 1H, N*H*), 11.08 (s, br, 1H, N*H*), 7.38-7.55 (m, 7H, Ar-H & CONHCH$_2$), 7.30 (s, 1H, H-vinyl), 7.26 (dd, *J* = 1.8 & 7.8 Hz, 1H), 6.85 (d, *J* = 8.7 Hz, 1H), 3.36 (m, 1H, C*H*(CH$_3$)$_2$), 3.07 (m, 2H, C*H*$_2$), 2.34 (q, *J* = 7.1 Hz, 4H, N(C*H*$_2$CH$_3$)$_2$), 2.22 (t, *J* = 6.9 Hz, 2H, C*H*$_2$), 1.40 (m, 2H, C*H*$_2$), 1.31 (d, *J* = 6.9 Hz, 6H, CH(C*H*$_3$)$_2$), 0.86 (t, *J* = 7.1 Hz, 6H, N(CH$_2$C*H*$_3$)$_2$).

MS *m/z* 565.1 [M$^+$+1].

Example 38

5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2-isopropyl-4-phenyl-1*H*-pyrrole-3-carboxylic acid (3-pyrrolidin-1-ylpropyl)amide

5-(5-bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2-isopropyl-4-phenyl-1*H*-pyrrole-3-carboxylic acid (127 mg, 0.28 mmol) was condensed with 3-pyrrolidin-1-yl-propylamine (43 mg,

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0.336 mmol) to give 140 mg (66%) of the title compound.

^{1}HNMR (300 MHz, DMSO-d6) δ 14.40 (s, br, 1H, NH), 7.38-7.47 (m, 7H), 7.23-7.27 (m, 2H), 6.84 (d, J = 8.1 Hz, 1H), 3.36 (m, 1H, CH(CH$_3$)$_2$), 3.08 (m, 2H, CH$_2$), 2.30 (m, 4H, 2xCH$_2$), 2.20 (t, J = 7.0 Hz, 2H, CH$_2$), 1.62 (m, 4H, 2xCH$_2$), 1.42 (t, J = 7.0 Hz, 2H, CH$_2$), 1.31 (d, J = 7.2 Hz, 6H, CH(CH$_3$)$_2$).

MS-EI m/z 560 and 562 [M$^+$ -1 and M$^+$+1].

Example 39

5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2-isopropyl-4-phenyl-1H-pyrrole-3-carboxylic acid (2-diethylaminoethyl)amide

5-Bromo-1,3-dihydroindol-2-one (57 ,g. 0.27 mmol) was condensed with 5-formyl-2-isopropyl-4-phenyl-1H-pyrrole-3-carboxylic acid (2-diethylaminoethyl)amide (120 mg) to give 78 mg (53%) of the title compound as a yellow solid.

^{1}HNMR (300 MHz, DMSO-d6) δ 14.23 (s, br, 1H, NH), 11.09 (s, br, 1H, NH), 7.38-7.51 (m, 6H), 7.25-7.28 (m, 2H), 7.19 (t, 1H, CONHCH$_2$), 6.85 (d, J = 7.8 Hz,1H), 3.43 (m, 1H, CH(CH$_3$)$_2$), 3.11 (m, 2H, CH$_2$), 2.28-2.39 (m, 6H, N(CH$_2$CH$_3$)$_2$ & CH$_2$, 1.31 (d, J = 6.9 Hz, CH(CH$_3$)$_2$), 0.85 (t, J = 7.0 Hz, 6H, N(CH$_2$CH$_3$)$_2$.

MS-EI m/z 548 and 550 [M$^+$ -1 and M$^+$+1].

Example 40

5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2-isopropyl-4-phenyl-1H-pyrrole-3-carboxylic acid [3-(4-methylpiperazin-1-yl)propyl]amide

5-Bromo-1,3-dihydroindol-2-one (53 mg, 0.25 mmol) was condensed with 5-formyl-2-isopropyl-4-phenyl-1H-pyrrole-3-carboxylic acid [3-(4-methylpiperazin-1-yl)propyl]amide (300 mg) to give 65 mg of the title compound.

^{1}HNMR (300 MHz, DMSO-d6) δ 14.22 (s, br, 1H, NH), 11.08 (s, br, 1H, NH), 7.23-7.50 (m, 9H), 6.85 (d, J = 8.7 Hz, 1H), 3.37 (m, 1H, CH(CH$_3$)$_2$), 3.05 (m, 2H, CH$_2$), 2.24 (m, 8H, 4xCH$_2$), 2.11

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(m, 5H, CH_2 & CH_3), 1.42 (m, 2H, CH_2), 1.31 (d, J = 7.2 Hz, 6H, $CH(CH_3)_2$).

MS-EI m/z 589 and 591 [M^+ -1 and M^++1].

Example 41

5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2-isopropyl-4-phenyl-1*H*-pyrrole-3-carboxylic acid

5-Bromo-1,3-dihydroindol-2-one (170 mg, 0.8 mmol) was condensed with 5-formyl-2-isopropyl-4-phenyl-1*H*-pyrrole-3-carboxylic acid (205 mg) using method D to give 210 mg (58%) of the title compound as a yellow solid.

^{1}HNMR (300 MHz, DMSO-d6) δ 14.31 (s, br, 1H, *NH*), 11.16 (s, br, 1H, *NH*), 7.26-7.44 (m, 7H), 7.11 (s, 1H, H-vinyl), 6.85 (d, J = 7.8 Hz, 1H), 3.78 (m, 1H, $CH(CH_3)_2$), 1.34 (d, J = 6.9 Hz, 6H, $CH(CH_3)_2$).

MS-EI *m/z* 452 [M^++1].

Example 42

5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2-methyl-4-phenyl-1*H*-pyrrole-3-carboxylic acid (2-pyrrolidin-1-ylethyl)amide

5-Bromo-1,3-dihydroindol-2-one (44 mg, 0.21 mmol) was condensed with 5-formyl-2-methyl-4-phenyl-1*H*-pyrrole-3-carboxylic acid (2-pyrrolidin-1-ylethyl)amide (70 mg, prepared in the same manner as the isopropyl analog, above) to give 0.03 g (27%) of the title compound as a yellow solid.

^{1}HNMR (300 MHz, DMSO-d6) δ 13.87 (s, br, 1H, *NH*), 11.11 (s, br, 1H, *NH*), 7.36-7.51 (m, 6H), 7.26 (dd, J = 1.8 & 8.1 Hz, 1H), 7.2 (s, 1H, H-vinyl), 7.09 (m, 1H, $CONHCH_2$), 6.83 (d, J = 8.1 Hz, 1H), 3.17 (m, 2H, NCH_2), 2.48 (m, CH_3), 2.29-2.35 (m, 6H, 3xNCH_2), 1.59 (m, 4H, 2xCH_2).

MS-EI *m/z* 518 and 520 [M^+ -1 and M^++1].

Example 43

5-[6-(2-Methoxyphenyl)-2-oxo-1,2-dihydroindol-3-ylidenemethyl]-2-

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methyl-4-phenyl-1*H*-pyrrole-3-carboxylic acid (2-pyrrolidin-1-ylethyl)amide

6-(2-Methoxyphenyl)-1,3-dihydroindol-2-one (50 mg, 0.21 mmol) was condensed with 5-formyl-2-methyl-4-phenyl-1*H*-pyrrole-3-carboxylic acid (2-pyrrolidin-1-ylethyl)amide (70 mg) to give 0.04 g (35%) of the title compound as a yellow-orange solid.

^{1}HNMR (300 MHz, DMSO-d6) δ 13.82 (s, br, 1H, N*H*), 11.02 (s, br, 1H, N*H*), 7.48 (m, 2H), 7.43 (m, 1H), 7.38 (m, 2H), 7.32 (m, 1H), 7.24 (m, 2H), 7.16 (s, 1H, H-vinyl), 7.08 (m, 2H), 7.03 (m, 1H), 7.0 (m, 2H), 3.74 (s, 3H, OC*H*$_3$), 3.19 (m, 2H, NC*H*$_2$), 2.49 (m, C*H*$_2$), 2.32-2.38 (m, 6H, 3xNC*H*$_2$), 1.59 (m, 4H, 2xC*H*$_2$).

MS-EI *m/z* 546 [M$^+$].

Example 44

5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2-methyl-4-phenyl-1*H*-pyrrole-3-carboxylic acid (2-dimethylaminoethyl)amide

5-Bromo-1,3-dihydroindol-2-one (46 mg, 0.22 mmol) was condensed with 5-formyl-2-methyl-4-phenyl-1*H*-pyrrole-3-carboxylic acid (2-dimethylaminoethyl)amide (65 mg) to give 60 mg (55%) of the title compound as a yellow solid.

^{1}HNMR (360 MHz, DMSO-d6) δ 13.86 (s, br, 1H, N*H*), 11.09 (s, br, 1H, N*H*), 7.47-7.49 (m, 2H), 7.38-7.41 (m, 4H), 7.26 (dd, *J* = 2.2 & 8.3 Hz, 1H), 7.21 (s, 1H, H-vinyl), 7.04 (m, 1H, CONHCH$_2$), 6.77 (d, *J* = 8.3 Hz, 1H), 3.15 (m, 2H, NC*H*$_2$), 2.48 (m, C*H*$_3$), 2.16 (t, *J* = 6.8 Hz, 2H, 3xNC*H*$_2$), 2.02 (s, 6H, 2xNC*H*$_3$).

MS *m/z* 493 and 494.8 [M$^+$ and M$^+$+2].

Example 45

5-[6-(2-Methoxyphenyl)-2-oxo-1,2-dihydroindol-3-ylidenemethyl]-2-methyl-4-phenyl-1*H*-pyrrole-3-carboxylic acid (2-dimethylaminoethyl)amide

6-(2-Methoxyphenyl)-1,3-dihydroindol-2-one (53 mg, 0.22 mmol) was condensed with 5-formyl-2-methyl-4-phenyl-1*H*-pyrrole-3-

carboxylic acid (2-dimethylaminoethyl)amide (65 mg) to give 0.05 g (44%) of the title compound as an orange gum.

^{1}HNMR (300 MHz, DMSO-d6) δ 13.82 (s, br, 1H, N*H*), 11.02 (s, br, 1H, N*H*), 7.37-7.52 (m, 5H), 7.32 (m, 1H), 7.22-7.27 (m, 2H), 7.16 (s, 1H), 7.08 (m, 2H), 7.03 (m, 1H), 7.0 (m, 2H), 3.74 (s, 3H, OC*H*$_3$), 3.15 (m, 2H, NC*H*$_2$), 2.49 (m, C*H*$_3$), 2.16 (t, *J* = 6.5 Hz, 2H, NC*H*$_2$), 2.02 (s, 6H, 2xNC*H*$_3$).

MS *m/z* 521 [M$^+$+1].

Example 46

5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2-methyl-4-phenyl-1*H*-pyrrole-3-carboxylic acid ethyl ester

5-Bromo-1,3-dihydroindol-2-one (60 mg, 0.29 mmol) was condensed with 5-formyl-2-methyl-4-phenyl-1*H*-pyrrole-3-carboxylic acid ethyl ester (75 mg) to give 78 mg (60%) of the title compound as an orange solid.

^{1}HNMR (360 MHz, DMSO-d6) δ 14.01 (s, br, 1H, *NH*), 11.13 (s, br, 1H, N*H*), 7.42-7.46 (m, 3H), 7.27-7.34 (m, 4H), 7.12 (s, 1H), 6.84 (dd, *J* = 2.2 & 8.3 Hz, 1H), 3.99-4.03 (m, 2H, OC*H*$_2$CH$_3$), 2.61 (s, 3H, C*H*$_3$), 0.98-1.03 (m, 3H, OCH$_2$C*H*$_3$).

MS-EI *m/z* 450 and 452 [M$^+$ -1 and M$^+$+1].

Example 47

5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2-methyl-4-phenyl-1*H*-pyrrole-3-carboxylic acid (3-diethylaminopropyl)amide

5-bromo-1,3-dihydroindol-2-one (0.47 g, 2.2 mmol) was condensed with 5-formyl-2-methyl-4-phenyl-1*H*-pyrrole-3-carboxylic acid (3-diethylaminopropyl)amide (0.75 g) to give 0.11 g (42%) of the title compound as an orange solid.

^{1}HNMR (300 MHz, DMSO-d6) δ 13.86 (s, br, 1H, NH), 7.42-7.46 (m, 3H), 7.37-7.50 (m, 7H), 7.24-7.28 (m, 2H), 6.83 (d, *J* = 8.1 Hz, 1H), 3.09 (m, 2H, NC*H*$_2$), 2.45 (s, 3H, C*H*$_3$), 2.38 (q, *J* = 7.1 Hz, 4H, 2xNC*H*$_2$CH$_3$), 2.26 (t, *J* = 6.9 Hz, 2H, NC*H*$_2$), 1.42 (m, 2H,

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NCH_2), 0.87 (t, J = 7.1 Hz, 6H, 2xNCH_2CH_3).

MS-EI m/z 535.0 and 537 [M$^+$ and M$^+$+2].

Example 48

5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-dimethylamino-ethyl)amide

A mixture of tert-butyl 3-oxobutyrate and sodium nitrite (1 equiv.) in acetic acid was stirred at room temperature to give tert-butyl-2-hydroximino-3-oxobutyrate.

Ethyl-3-oxobutyrate (1 equiv.), zinc dust (3.8 equiv.) and the crude tert-butyl-2-hydroximino-3-oxobutyrate in acetic acid was stirred at 60° C for 1 hr. The reaction mixture was poured into H_2O and the filtrate was collected to give (65%) 2-tert-butyloxycarbonyl-3,5-dimethyl-4-ethoxycarbonylpyrrole.

A mixture of 2-tert-butyloxycarbonyl-3,5-dimethyl-4-ethoxycarbonylpyrrole and triethyl orthoformate (1.5 equiv.) in trifluoroacetic acid was stirred at 15°C for 1 hour. The reaction was concentrated and the residue was purified to give (64%) 2,4-dimethyl-3-ethoxycarbonyl-5-formylpyrrole as yellow needles.

2,4-Dimethyl-3-ethoxycarbonyl-5-formylpyrrole was hydrolyzed using method B to give (90%) 5-formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid.

^{1}H NMR (360 MHz, DMSO-d6) δ 12 (br s, 2H, N*H* and CO_2H), 9.58 (s, 1H, CHO), 2.44 (s, 3H, CH_3), 2.40 (s, 3H, CH_3).

MS m/z 267 [M$^+$].

5-Bromo-1,3-dihydroindol-2-one (0.17 g, 0.8 mmol) was condensed with 5-formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-dimethylaminoethyl)amide (0.2 g, prepared by method C) using method B to give 0.3 g (83%) of the title compound as a yellow solid.

^{1}HNMR (360 MHz, DMSO-d6) δ 13.60 (s, br, 1H, N*H*), 10.94 (s,

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br, 1H, NH), 8.07 (d, J = 1.8 Hz, 1H, H-4), 7.75 (s, 1H, H-vinyl), 7.44 (t, J = 5.2 Hz, 1H, CONHCH$_2$), 7.24 (dd, J = 1.8 & 8.4 Hz, 1H, H-6), 6.82 (d, J = 8.4 Hz, 1H, H-7), 3.26-3.33 (m, 2H, NCH$_2$), 2.42 (s, 3H, CH$_3$), 2.41 (s, 3H, CH$_3$), 2.38 (t, J = 6.7 Hz, 2H, NCH$_2$), 2.18 (s, 6H, N(CH$_3$)$_2$).

MS-EI m/z 430 and 432 [M$^+$-1 and M$^+$+1].

Example 49

2,4-Dimethyl-5-(2-oxo-6-phenyl-1,2-dihydroindol-3-ylidenemethyl)-1*H*-pyrrole-3-carboxylic acid (2-dimethylaminoethyl)amide

6-Phenyl-1,3-dihydroindol-2-one (0.17 g, 0.8 mmol) was condensed with 5-formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-dimethylaminoethyl)amide (0.2 g) to give 0.13 g (36%) of the title compound as a yellow-orange solid.

^{1}HNMR (360 MHz, DMSO-d6) δ 13.59 (s, br, 1H, NH), 10.93 (, br, 1H, NH), 7.85 (d, J = 7.92 Hz, 1H, H-4), 7.63-7.65 (m, 3H), 7.40-7.47 (m, 3H,), 7.32-7.36 (m, 1H, Ar-H), 7.30 (dd, J = 1.6 & 7.9 Hz, 1H, H-5), 7.11 (d, J = 1.6 Hz, 1H, H-7), 3.28-3.34 (m, 2H, NCH$_2$), 2.43 (s, 3H, CH$_3$), 2.41 (s, 3H, CH$_3$), 2.38 (t, J = 6.8 Hz, 2H, NCH$_2$), 2.18 (s, 6H, N(CH$_3$)$_2$).

MS-EI m/z 428 [M$^+$].

Example 50

5-(5-Chloro-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-dimethylamino-ethyl)amide

5-Chloro-1,3-dihydroindol-2-one (0.1 g, 0.6 mmol) was condensed with 5-formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-dimethylaminoethyl)amide (0.15 g) to give 0.22 g (90%) of the title compound as a yellow solid.

^{1}HNMR (300 MHz, DMSO-d6) δ 13.61 (s, br, 1H, NH), 10.98 (, br, 1H, NH), 7.96 (d, J = 2.0 Hz, 1H, H-4), 7.75 (s, 1H, H-vinyl), 7.50 (t, J = 5.5 Hz, 1H, CONHCH$_2$), 7.12 (dd, J = 2.0 & 8.3 Hz, 1H, H-6), 6.86 (d, J = 8.3 Hz, 1H, H-7), 3.26-3.31 (m,

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2H, NCH_2), 2.42 (s, 3H, CH_3), 2.40 (s, 3H, CH_3), 2.36 (t, J = 6.6 Hz, 2H, NCH_2), 2.17 (s, 6H, $N(CH_3)_2$).

MS-EI m/z 386 [M^+].

Example 51

5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-diethylamino-ethyl)amide

5-Bromo-1,3-dihydroindol-2-one (0.17 g, 0.8 mmol) was condensed with 5-formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-diethylaminoethyl)amide (0.2 g) to give 0.09 g (26%) of the title compound as a yellow solid.

^{1}HNMR (360 MHz, DMSO-d6) δ 13.61 (s, br, 1H, N*H*), 10.98 (, br, 1H, N*H*), 8.09 (d, J = 1.7 Hz, 1H, H-4), 7.76 (s, 1H, H-vinyl), 7.42 (t, J = 5.5 Hz, 1H, CONHCH_2), 7.24 (dd, J = 1.7 & 8.0 Hz, 1H, H-6), 6.82 (d, J = 8.0 Hz, 1H, H-7), 3.23-3.32 (m, 2H, NCH_2), 2.46-2.55 (m, 6H, 3xNCH_2), 2.43 (s, 3H, CH_3), 2.42 (s, 3H, CH_3), 0.96 (t, J = 7.2 Hz, 6H, 2xNCH_2CH_3).

MS-EI m/z 458 and 460 [M^+-1 and M^++1].

Example 52

5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (2-pyrrolidin-1-yl-ethyl)amide

5-Bromo-1,3-dihydroindol-2-one (0.09 g, 0.4 mmol) was condensed with 5-formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-pyrrolidin-1-ylethyl)amide (0.1 g) to give 0.14 g (81%) of the title compound as a yellow-orange solid.

^{1}HNMR (300 MHz, DMSO-d6) δ 13.61 (s, br, 1H, N*H*), 10.98 (, br, 1H, N*H*), 8.09 (d, J = 1.9 Hz, 1H, H-4), 7.76 (s, 1H, H-vinyl), 7.53 (t, J = 5.5 Hz, 1H, CONHCH_2), 7.24 (dd, J = 1.9 & 8.5 Hz, 1H, H-6), 6.81 (d, J = 8.5 Hz, 1H, H-7), 3.29-3.35 (m, 2H, NCH_2), 2.54 (t, J = 6.9 Hz, 2H, NCH_2), 2.47 (m, under DMSO), 2.42 (s, 3H, CH_3), 2.40 (s, 3H, CH_3), 1.66-1.69 (m, 4H, 2xCH_2).

MS-EI m/z 456 and 458 [M^+-1 and M^++1].

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Example 53

5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (3-imidazol-1-yl-propyl)amide

5-Bromo-1,3-dihydroindol-2-one (0.09 g, 0.4 mmol) was condensed with 5-formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (3-imidazol-1-ylpropyl)amide (0.1 g) to give 0.1 g (59%) of the title compound as an orange solid.

^{1}HNMR (300 MHz, DMSO-d6) δ 13.63 (s, br, 1H, *NH*), 10.99 (, br, 1H, *NH*), 8.09 (d, *J* = 2.2 Hz, 1H, H-4), 7.77 (s, 1H, H-vinyl), 7.71 (t, *J* = 5.7 Hz, 1H, CONHCH$_2$), 7.65 (s, 1H, Ar-H), 7.25 (dd, *J* = 2.2 & 8.4 Hz, 1H, H-6), 7.20 (s, 1H, Ar-H), 6.89 (s, 1H, Ar-H), 6.81 (d, *J* = 8.4 Hz, 1H, H-7), 4.02 (t, *J* = 6.7 Hz, 2H, NCH$_2$), 3.18 (q, *J* = 6.7 Hz, 2H, NCH$_2$), 2.43 (s, 3H, CH$_3$), 2.41 (s, 3H, CH$_3$), 1.93 (m, 2H, CH$_2$).

MS-EI *m/z* 467 and 469 [M$^+$-1 and M$^+$+1].

Example 54

5-[6-(2-Methoxyphenyl)-2-oxo-1,2-dihydroindol-3-ylidenemethyl]-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-dimethylaminoethyl)amide

6-(2-Methoxyphenyl)-1,3-dihydroindol-2-one (30 mg, 0.13 mmol) was condensed with 5-formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-dimethylaminoethyl)amide (30 mg) to give 0.06 g (100%) of the title compound as a yellow-orange gum.

^{1}HNMR (300 MHz, DMSO-d6) δ 13.60 (s, br, 1H, NH), 10.89 (s, br, 1H, *NH*), 7.79 (d, *J* = 8.4 Hz, 1H), 7.63 (s, 1H, H-vinyl), 7.46 (t, *J* = 5.5 Hz, 1H, CONHCH$_2$), 7.28-7.35 (m, 2H), 6.99-7.11 (m, 4H), 3.76 (s, 3H, OCH$_3$), 3.27-3.31 (m, 2H, NCH$_2$), 2.43 (s, 3H, CH$_3$), 2.39 (s, 3H, CH$_3$), 2.37 (m, 2H, NCH$_2$), 2.18 (s, 6H, N(CH$_3$)$_2$).

MS-EI *m/z* 458 [M$^+$].

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Example 55

5-[6-(3-Methoxyphenyl)-2-oxo-1,2-dihydroindol-3-ylidenemethyl]-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-dimethylaminoethyl)amide

6-(3-Methoxyphenyl)-1,3-dihydroindol-2-one (30 mg, 0.13 mmol) was condensed with 5-formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-dimethylaminoethyl)amide (30 mg) to give 8 mg (14%) of the title compound as a yellow-orange solid.

^{1}HNMR (360 MHz, DMSO-d6) δ 13.59 (s, br, 1H, N*H*), 10.92 (s, br, 1H, N*H*), 7.84 (d, *J* = 7.6 Hz, 1H), 7.65 (s, 1H, H-vinyl), 7.42 (m, 1H, CON*H*CH$_2$), 7.36 (d, *J* = 7.8 Hz, 1H), 7.29 (dd, *J* = 1.6 & 7.6 Hz, 1H), 7.20 (d, *J* = 7.8 Hz, 1H), 7.14 (d, *J* = 2.8 Hz, 1H), 7.11 (d, *J* = 1.6 Hz, 1H), 6.91 (dd, *J* = 2.8 & 7.8 Hz, 1H), 3.82 (s, 3H, OC*H*$_3$), 3.21-3.33 (m, 2H, NC*H*$_2$), 2.43 (s, 3H, C*H*$_3$), 2.40 (s, 3H, C*H*$_3$), 2.36-2.40 (m, 2H, NC*H*$_2$), 2.18 (s, 6H, N(C*H*$_3$)$_2$).

MS-EI *m/z* 458 [M$^+$].

Example 56

2,4-Dimethyl-5-(2-oxo-5-phenyl-1,2-dihydroindol-3-ylidenemethyl)-1*H*-pyrrole-3-carboxylic acid (2-diethylaminoethyl)amide

5-Phenyl-1,3-dihydroindol-2-one (80 mg, 0.4 mmol) was condensed with 5-formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-diethylaminoethyl)amide (0.1 g) using method B to give 79 mg (46%) of the title compound.

^{1}HNMR (300 MHz, DMSO-d6) δ 13.66 (s, br, 1H, N*H*), 10.95 (, br, 1H, N*H*), 8.15 (d, *J* = 1.2 Hz, 1H), 7.81 (s, 1H, H-vinyl), 7.71 (d, *J* = 7.5 Hz, 1H), 7.40-7.47 (m, 4H), 7.31 (m, 1H), 6.95 (d, *J* = 8.1 Hz, 1H), 3.2-3.31 (m, 2H, NC*H*$_2$), 2.46-2.55 (m, 6H, 3xNC*H*$_2$), 2.44 (s, 6H, 2xC*H*$_3$), 0.96 (t, *J* = 7.4 Hz, 6H, 2xNCH$_2$C*H*$_3$).

MS-EI *m/z* 456 [M$^+$].

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Example 57

2,4-Dimethyl-5-(2-oxo-5-phenyl-1,2-dihydroindol-3-ylidenemethyl)-1*H*-pyrrole-3-carboxylic acid (2-pyrrolidin-1-ylethyl)amide

5-Phenyl-1,3-dihydroindol-2-one (0.04 g, 0.2 mmol) was condensed with 5-formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-pyrrolidin-1-ylethyl)amide (0.04 g) to give the title compound as a yellow-orange solid.

^{1}HNMR (300 MHz, DMSO-d6) δ 13.65 (s, br, 1H, N*H*), 10.96 (, br, 1H, N*H*), 8.15 (d, *J* = 1.0 Hz, 1H), 7.80 (s, 1H, H-vinyl), 7.71 (d, *J* = 7.2 Hz, 2H), 7.49 (t, *J* = 6.3 Hz, 1H, CON*H*CH$_2$), 7.41-7.46 (m, 3H), 7.31 (m, 1H), 6.95 (d, *J* = 7.8 Hz, 1H), 4.08 (m, 4H, 2x NC*H*$_2$), 3.32 (m, 2H, NC*H*$_2$), 2.55 (t, *J* = 7.1 Hz, 2H, NC*H*$_2$), 2.47 (m, under DMSO), 2.43 (s, 6H, 2xC*H*$_3$), 1.66 (m, 4H, 2xC*H*$_2$).

MS-EI *m/z* 454 [M$^+$].

Example 58

2,4-Dimethyl-5-(2-oxo-5-phenyl-1,2-dihydroindol-3-ylidenemethyl)-1*H*-pyrrole-3-carboxylic acid (3-imidazol-1-ylpropyl)amide

5-Phenyl-1,3-dihydroindol-2-one (8 mg, 0.04 mmol) was condensed with 5-formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (3-imidazol-1-ylpropyl)amide (10 mg) to give 10 mg (59%) of the title compound as an orange solid.

^{1}HNMR (300 MHz, DMSO-d6) δ 13.67 (s, br, 1H, N*H*), 10.96 (, br, 1H, N*H*), 8.16 (d, *J* = 1.2 Hz, 1H), 7.81 (s, 1H, H-vinyl), 7.65-7.72 (m, 4H), 7.44 (m, 3H), 7.31 (m, 1H, CON*H*CH$_2$), 7.21 (s, 1H, Ar-H), 4.02 (t, *J* = 6.5 Hz, 2H, NC*H*$_2$), 3.19 (q, *J* = 6.5 Hz, 2H, CONHC*H*$_2$), 2.44 (s, 6H, 2xC*H*$_3$), 1.93 (m, 2H, CH$_2$C*H*$_2$ CH$_2$).

MS-EI m/z 465 [M$^+$].

Example 59

2,4-Dimethyl-5-(2-oxo-6-phenyl-1,2-dihydroindol-3-ylidenemethyl)-1*H*-pyrrole-3-carboxylic acid (2-diethylaminoethyl)amide

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6-Phenyl-1,3-dihydroindol-2-one (0.08 g, 0.4 mmol) was condensed with 5-formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-diethylaminoethyl)amide (0.1 g) to give 65 mg (38%) of the title compound as a yellow solid.

^{1}HNMR (300 MHz, DMSO-d6) δ 13.61 (s, br, 1H, N*H*), 10.99 (, br, 1H, N*H*), 7.86 (d, *J* = 7.8 Hz, 1H), 7.62-7.66 (m, 3H), 7.40-7.47 (m, 3H), 7.28-7.36 (m, 2H), 7.10 (d, *J* = 1.2 Hz, 1H), 3.26 (m, 2H, NCH_2), 2.46-2.55 (m, 6H, 3xNCH_2), 2.44 (s, 3H, CH_3), 2.41 (s, 3H, CH_3), 0.97 (t, *J* = 7.2 Hz, 6H, 2xNCH$_2$CH_3).

MS-EI *m/z* 456 [M$^+$].

Example 60

<u>2,4-Dimethyl-5-(2-oxo-6-phenyl-1,2-dihydroindol-3-ylidenemethyl)-1*H*-pyrrole-3-carboxylic acid (2-pyrrolidin-1-ylethyl)amide</u>

6-Phenyl-1,3-dihydroindol-2-one (30 mg, 0.15 mmol) was condensed with 5-formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-pyrrolidin-1-ylethyl)amide (40 mg) to give 5.9 mg (8.5%) of the title compound as a yellow-orange solid.

^{1}HNMR (300 MHz, DMSO-d6) δ 13.60 (s, br, 1H, N*H*), 10.99 (, br, 1H, N*H*), 7.86 (d, *J* = 7.8 Hz, 1H), 7.63-7.66 (m, 3H), 7.51 (m, 1H, CONHCH$_2$), 7.45 (m, 2H), 7.28-7.36 (m, 2H), 7.10 (d, *J* = 1.5 Hz, 1H), 3.31 (m, 6H, 3xNCH_2), 2.55 (t, *J* = 6.6 Hz, 2H, NCH_2), 2.43 (s, 3H, CH_3), 2.40 (s, 3H, CH_3), 1.67 (m, 4H, 2xCH_2).

MS-EI *m/z* 454 [M$^+$].

Example 61

<u>2,4-Dimethyl-5-(2-oxo-6-phenyl-1,2-dihydroindol-3-ylidenemethyl)-1*H*-pyrrole-3-carboxylic acid (3-imidazol-1-ylpropyl)amide</u>

6-Phenyl-1,3-dihydroindol-2-one (8 mg, 0.04 mmol) was condensed with 5-formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (3-imidazol-1-ylpropyl)amide (10 mg) to give 7.3 mg (43%) of the title compound as an orange solid.

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^{1}HNMR (300 MHz, DMSO-d6) δ 13.62 (s, br, 1H, NH), 10.99 (, br, 1H, NH), 7.86 (d, J = 8.2 Hz, 1H), 7.62-7.70 (m, 5H), 7.45 (m, 2H), 7.35 (m, 1H), 7.30 (dd, J = 1.4 & 8.2 Hz, 1H), 7.21 (s, 1H), 7.10 (d, J = 1.4 Hz, 1H), 6.89 (s, 1H), 4.02 (t, J = 6.9 Hz, 2H, CH_2), 3.19 (m, 2H, NCH_2 CH_2), 2.43 (s, 3H, CH_3), 2.41 (s, 3H, CH_3), 1.93 (t, J = 6.9 Hz, 2H, NCH_2).

MS-EI m/z 465 [M$^+$].

Example 62

5-[6-(3,5-Dichlorophenyl)-2-oxo-1,2-dihydroindol-3-ylidenemethyl]-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (2-diethylaminoethyl)amide

6-(3,5-Dichlorophenyl)-1,3-dihydroindol-2-one (64 mg, 0.23 mmol) was condensed with 5-formyl-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (2-diethylaminoethyl)amide (60 mg) to give 53 mg (44%) of the title compound as a light brown solid.

^{1}HNMR (360 MHz, DMSO-d6) δ 13.62 (s, br, 1H, NH), 10.99 (s, 1H, NH), 7.89 (d, J = 7.9 Hz, 1H, H-4), 7.69-7.71 (m, 3H), 7.55 (m, 1H, $CONHCH_2$), 7.37 (m, 2H), 7.14 (d, J = 1.4 Hz, 1H, H-7), 3.27 (m, 2H, NCH_2), 2.48-2.58 (m, 6H, 3xNCH_2), 2.45 (s, 3H, CH_3), 2.42 (s, 3H, CH_3), 0.97 (t, J = 6.8 Hz, 6H, 3xNCH_2CH_3).

MS m/z 526.9 [M$^+$+1].

Example 63

2,4-Dimethyl-5-(2-oxo-6-pyridin-3-yl-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrole-3-carboxylic acid (2-diethylaminoethyl)amide

6-Pyridin-3-yl-1,3-dihydroindol-2-one (40 mg, 0.19 mmol) was condensed with 5-formyl-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (2-diethylaminoethyl)amide (50 mg) give 29 mg (33%) of the title compound as a light orange solid.

^{1}HNMR (300 MHz, DMSO-d6) δ 13.62 (s, br, 1H, NH), 11.05 (s, br, 1H, NH), 8.86 (s, br, 1H), 8.53 (d, J = 5.8 Hz, 1H), 8.04 (m,

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1H), 7.91 (d, J = 8.1 Hz, 1H), 7.70 (s, 1H, H-vinyl), 7.40-7.48 (m, 2H), 7.35 (d, J = 7.5 Hz, 1H), 7.14 (s, 1H), 3.26 (m, 2H, NCH_2), 2.48-2.55 (m, 3xNCH_2), 2.42 (s, 3H, CH_3), 2.38 (s, 3H, CH_3), 0.96 (t, J = 6.9 Hz, 6H, 2xNCH_2CH_3).

MS-EI m/z 457 [M$^+$].

Example 64

2,4-Dimethyl-5-(2-oxo-6-pyridin-3-yl-1,2-dihydroindol-3-ylidenemethyl)-1*H*-pyrrole-3-carboxylic acid (2-pyrrolidin-1-ylethyl)amide

6-Pyridin-3-yl-1,3-dihydroindol-2-one (60 mg, 0.28 mmol) was condensed with 5-formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-pyrrolidin-1-ylethyl)amide (75 mg) to give 90 mg (71%) of the title compound as a light orange solid.

^{1}HNMR (300 MHz, DMSO-d6) δ 13.61 (s, br, 1H, *NH*), 11.05 (s, br, 1H, *NH*), 8.86 (d, J = 1.5 Hz, 1H), 8.54 (dd, J = 1.5 & 4.8 Hz, 1H), 8.05 (m, 1H), 7.91 (d, J = 7.8 Hz, 1H), 7.70 (s, 1H, H-vinyl), 7.44-7.53 (m, 2H), 7.36 (dd, J = 1.5 & 8.1 Hz, 1H), 7.15 (d, J = 1.2 Hz, 1H), 3.33 (m, 2H, NCH_2), 2.47-2.57 (m, 6H, 3xNCH_2), 2.43 (s, 3H, CH_3), 2.41 (s, 3H, CH_3), 1.67 (m, 4H, 2xCH_2).

MS-EI m/z 455 [M$^+$].

Example 65

2,4-Dimethyl-5-(2-oxo-6-pyridin-3-yl-1,2-dihydroindol-3-ylidenemethyl)-1*H*-pyrrole-3-carboxylic acid (3-dimethylaminopropyl)amide

6-Pyridin-3-yl-1,3-dihydroindol-2-one (42 mg, 0.2 mmol) was condensed with 5-formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (3-dimethylaminopropyl)amide (50 mg) to give 67 mg (75%) of the title compound as yellow-brown solid.

^{1}HNMR (360 MHz, DMSO-d6) δ 13.61 (s, br, 1H, *NH*), 11.00 (s, br, 1H, *NH*), 8.86 (s, br, 1H), 8.54 (s, br, 1H), 8.04 (m, 1H),

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7.90 (d, J = 8.0 Hz, 1H), 7.69 (s, 1H, H-vinyl), 7.63 (m, 1H), 7.45-7.48 (m, 1H), 7.35 (dd, J = 1.7 & 8.0 Hz, 1H), 7.15 (d, J = 1.7 Hz, 1H), 3.21-3.27 (m, 2H, NCH_2), 2.43 (s, 3H, CH_3), 2.41 (s, 3H, CH_3), 2.28 (m, 2H, NCH_2), 2.14 (s, 6H, 2xNCH_3), 1.64 (m, 2H, CH_2).

MS-EI m/z 443 [M$^+$].

Example 66

2,4-Dimethyl-5-(2-oxo-5-phenyl-1,2-dihydroindol-3-ylidenemethyl)-1*H*-pyrrole-3-carboxylic acid (3-dimethylaminopropyl)amide

5-Phenyl-1,3-dihydroindol-2-one (67 mg, 0.32 mmol) was condensed with 5-formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (3-dimethylaminopropyl)amide (81 mg) to give 40 mg (28%) of the title compound as an orange solid.

^{1}HNMR (360 MHz, DMSO-d6) δ 13.66 (s, br, 1H, NH), 10.92 (s, br, 1H, NH), 8.14 (s, 1H), 7.79 (s, 1H), 7.71 (m, 2H), 7.62 (m, 1H), 7.44 (m, 3H), 7.32 (m, 1H), 6.95 (m, 1H), 3.33 (m, 2H, NCH_2), 2.43 (s, 6H, 2xCH_3), 2.27 (m, 2H, NCH_2), 2.13 (s, 6H, 2xNCH_3), 1.63 (m, 2H, CH_2).

MS-EI m/z 442 [M$^+$].

Example 67

2,4-Dimethyl-5-(2-oxo-5-phenyl-1,2-dihydroindol-3-ylidenemethyl)-1*H*-pyrrole-3-carboxylic acid (3-diethylaminopropyl)amide

5-Phenyl-1,3-dihydroindol-2-one (1.5 g, 7.16 mmol) was condensed with 5-formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (3-diethylaminopropyl)amide (2 g) to give 1.3 g (40%) of the title compound as a yellow-orange solid.

^{1}HNMR (360 MHz, DMSO-d6) δ 13.64 (s, 1H, NH), 10.91 (s, 1H, NH), 8.14 (d, J = 1.4 Hz, 1H, Ar*H*), 7.8 (s, 1H, Ar*H*), 7.7 (dd, J = 1.2 and 8.5 Hz, 2H, Ar*H*), 7.6 (t, J = 5.3 Hz, 1H, CONHCH_2), 7.4 (m, 3H, Ar*H*), 7.3 (t, J = 7.4 Hz, 1H, Ar*H*), 6.9 (d, J = 8.0 Hz, 1H, Ar*H*), 3.2 (m, 2H, CONHCH_2), 2.5 (m, 12H, 3xNCH_2 and

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2xCH_3), 1.61 (m, 2H, $CH_2CH_2CH_2$), 0.93 (t, J = 6.7 Hz, 6H, NCH_2CH_3).

MS-EI m/z 470 [M$^+$].

Example 68

2,4-Dimethyl-5-(2-oxo-6-phenyl-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrole-3-carboxylic acid (3-diethylaminopropyl)amide

6-Phenyl-1,3-dihydroindol-2-one (1.5 g, 7.16 mmol) was condensed with 5-formyl-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (3-diethylaminopropyl)amide (2 g) to give 1.9 g (57%) of the title compound as an orange solid.

^{1}HNMR (360 MHz, DMSO-d6) δ 13.58 (s, 1H, NH), 10.94 (s, 1H, NH), 7.8 (d, J = 7.9 Hz, 1H, ArH), 7.6 (m, 4H, ArH), 7.4 (t, J = 7.5 Hz, 2H, ArH), 7.3 (m, 2H), 7.1 (d, J = 1.4 Hz, 1H, ArH), 3.2 (m, 2H, $CONHCH_2$), 2.5 (m, 12H, 3xNCH_2 and 2xCH_3), 1.61 (m, 2H, $CH_2CH_2CH_2$), 0.93 (t, J = 6.7 Hz, 6H, NCH_2CH_3).

MS-EI m/z 470 [M$^+$].

Example 69

3-[4-(3-Diethylaminopropylcarbamoyl)-3,5-dimethyl-1H-pyrrol-2-ylmethylene]-2-oxo-2,3-dihydro-1H-indole-4-carboxylic acid (3-chloro-4-methoxyphenyl)amide

2-Oxo-2,3-dihydro-1H-indole-4-carboxylic acid (3-chloro-4-methoxyphenyl)amide (1 g, 3.16 mmol) was condensed with 5-formyl-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (3-diethylaminopropyl)amide (1 g, 3.58 mmol) to give 1.7 g (85%) of the title compound as a yellow-orange solid.

MS-EI m/z 578.2 [M$^+$].

Example 70

5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (3-diethylamino-propyl)amide

5-Bromo-1,3-dihydroindol-2-one (0.5 g, 2.36 mmol) was condensed with 5-formyl-2,4-dimethyl-1H-pyrrole-3-carboxylic acid

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(3-diethylaminopropyl)amide (0.51 g) to give 0.84 g of the title compound as a red-orange solid.

^{1}HNMR (360 MHz, DMSO-d6) δ 13.61 (s, 1H, NH), 10.99 (s, 1H, NH), 8.09 (d, *J* = 1.8 Hz, 1H, Ar*H*), 7.7 (m, 4H), 7.2 (dd, *J* = 1.8 and 8.3 Hz, 2H, Ar*H*), 6.8 (d, *J* = 7.8 Hz, 1H, Ar*H*), 3.3 (br s, 4H, 2xNCH_2), 3.2 (m, 2H, $CONHCH_2$), 2.6 (br s, 2H, NCH_2 and 2xCH_3), 2.4 (s, 6H, 2xCH_3), 1.66 (m, 2H, $CH_2CH_2CH_2$), 0.98 (t, *J* = 7.1 Hz, 6H, NCH_2CH_3).

MS-EI *m/z* 472 and 474 [M$^+$ -1 and M$^+$+1].

Example 71

5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2,4-diisopropyl-1*H*-pyrrole-3-carboxylic acid (2-diethylamino-ethyl)amide

5-Bromo-1,3-dihydroindol-2-one (100 mg, 0.47 mmol) was condensed with 5-formyl-2,4-diisopropyl-1*H*-pyrrole-3-carboxylic acid (2-diethylaminoethyl)amide (150 mg) to give 0.15 g (62%) of the title compound as a yellow-orange solid.

^{1}HNMR (300 MHz, DMSO-d6) δ 13.97 (s, 1H, NH), 10.95 (s, 1H, NH), 8.09 (d, *J* = 1.3 Hz, 1H, Ar*H*), 7.84 (m, 1H), 7.79 (s, 1H), 7.23 (dd, *J* = 1.3 and 8.1 Hz, 1H, Ar*H*), 6.8 (d, *J* = 8.1 Hz, 1H, Ar*H*), 3.5 (m, 1H, C*H*), 3.3 (m, 3H, C*H* and $NHCH_2$), 2.5 (br m, 6H, 3xNCH_2), 1.28 (d, *J* = 6.9 Hz, 6H, 2xCH_3), 1.23 (d, *J* = 6.6 Hz, 6H, 2xCH_3), 0.96 (m, 6H, 2xCH_2CH_3).

MS-EI *m/z* 514 and 516 [M$^+$-1 and M$^+$+1].

Example 72

5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2,4-diisopropyl-1*H*-pyrrole-3-carboxylic acid (3-diethylamino-propyl)amide

5-Bromo-1,3-dihydroindol-2-one (90 mg, 0.42 mmol) was condensed with 5-formyl-2,4-diisopropyl-1*H*-pyrrole-3-carboxylic

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acid (3-diethylaminopropyl)amide (140 mg) to give 54 mg (25%) of the title compound as red-brown solid.

^{1}HNMR (300 MHz, DMSO-d6) δ 13.98 (s, 1H, NH), 10.96 (s, 1H, NH), 8.09 (d, *J* = 1.7 Hz, 2H), 7.78 (s, 1H, H-vinyl), 7.23 (dd, *J* = 1.7 and 8.1 Hz, 1H, Ar*H*), 6.82 (d, *J* = 8.1 Hz, 1H, Ar*H*), 3.5 (m, 1H, C*H*), 3.25 (m, 2H, NHCH_2), 3.15 (m, 1H, C*H*), 2.7 (br s, 6H, 3xNCH_2), 1.7 (br m, 2H, $CH_2CH_2CH_2$), 1.28 (d, *J* = 6.9 Hz, 6H, 2xCH_3), 1.24 (d, *J* = 5.9 Hz, 6H, 2xCH_3), 1.06 (m, 6H, 2xCH_2CH_3).

MS-EI *m/z* 528 and 530 [M$^+$-1 and M$^+$+1].

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5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2,4-diisopropyl-1*H*-pyrrole-3-carboxylic acid (3-pyrrolidin-1-ylpropyl)amide

5-Bromo-1,3-dihydroindol-2-one (130 mg, 0.6 mmol) was condensed with 5-formyl-2,4-diisopropyl-1*H*-pyrrole-3-carboxylic acid (3-pyrrolidin-1-ylpropyl)amide (150 mg, 0.45 mmol) to give 36 mg (15%) of the title compound as a tan-orange solid.

^{1}HNMR (300 MHz, DMSO-d6) δ 13.98 (s, 1H, NH), 10.97 (s, 1H, NH), 8.10 (d, *J* = 1.6 Hz, 2H), 7.78 (s, 1H, H-vinyl), 7.23 (dd, *J* = 1.6 and 7.6 Hz, 1H, Ar*H*), 6.82 (d, *J* = 7.6 Hz, 1H, Ar*H*), 3.5 (m, 1H, C*H*), 3.25 (m, 2H, NHCH_2), 3.15 (m, 1H, C*H*), 2.7 (br s, 6H, 3xNCH_2), 1.7 (br m, 6H, 3xNCH_2CH_2), 1.28 (d, *J* = 5.6 Hz, 6H, 2xCH_3), 1.24 (d, *J* = 5.7 Hz, 6H, 2xCH_3).

MS-EI *m/z* 526 and 528 [M$^+$-1 and M$^+$+1].

Example 74

5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (pyridin-4-ylmethyl)-amide

5-Bromo-1,3-dihydroindol-2-one (170 mg, 0.8 mmol) was condensed with 5-formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (pyridin-4-ylmethyl)amide (200 mg) to give 14 mg (4%) of the title compound as a yellow solid.

^{1}HNMR (300 MHz, DMSO-d6) δ 13.67 (s, 1H, NH), 11.01 (s, br, 1H, NH), 8.51 (dd, *J* = 1.6 & 4.3 Hz, 2H), 8.23 (t, *J* = 6.0 Hz, 1H, CONHCH$_2$), 8.11 (d, *J* = 1.9 Hz, 1H), 7.78 (s, 1H, H-vinyl), 7.31 (d, *J* = 6.0 Hz, 2H), 7.25 (dd, *J* = 1.9 & 8.1 Hz, 1H), 6.82 (d, *J* = 8.1 Hz, 1H), 4.45 (d, *J* = 6.0 Hz, 2H, NCH$_2$), 2.46 (s, 6H, 2xCH$_3$).

MS-EI *m/z* 450 and 452 [M$^+$-1 and M$^+$+1].

Example 75

5-[6-(4-Butylphenyl)-2-oxo-1,2-dihydroindol-3-ylidenemethyl]-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-pyrrolidin-1-ylethyl)amide

5-[6-(4-Butylphenyl)]-1,3-dihydroindol-2-one (50 mg, 0.19 mmol) was condensed with 5-formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-pyrrolidin-1-ylethyl)amide (50 mg) to give 74 mg (76%) of the title compound as an orange solid.

^{1}HNMR (360 MHz, DMSO-d6) δ 13.58 (s, 1H, NH), 10.93 (s, br, 1H, NH), 7.82 (d, *J* = 7.9 Hz, 1H), 7.63 (s, 1H, H-vinyl), 7.54 (d, *J* = 7.9 Hz, 2H), 7.46 (m, 1H, CONH), 7.26 (m, 3H), 7.09 (s, 1H), 3.30 (m, 2H, CH$_2$), 2.52-2.63 (m, 4H, 2xCH$_2$), 2.49 (m, 4H, 2xCH$_2$), 2.43 (s, 3H, CH$_3$), 2.40 (s, 3H, CH$_3$), 1.68 (m, 4H, 2xCH$_2$), 1.58 (m, 2H, CH$_2$), 1.34 (m, 2H, CH$_2$), 0.91 (t, *J*= 7.2 Hz, 3H, CH$_2$CH$_3$).

MS-EI *m/z* 510 [M$^+$].

Example 76

5-[6-(5-Isopropyl-2-methoxyphenyl)-2-oxo-1,2-dihydroindol-3-ylidenemethyl]-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-pyrrolidin-1-ylethyl)amide

6-(5-Isopropyl-2-methoxyphenyl)-1,3-dihydroindol-2-one (50 mg, 0.17 mmol) was condensed with 5-formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-pyrrolidin-1-yl-ethyl)amide (45 mg) to give 67 mg (75%) of the title compound as an orange solid.

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^{1}HNMR (360 MHz, DMSO-d6) δ 13.60 (s, 1H, NH), 10.82 (s, br, 1H, NH), 7.77 (d, *J* = 7.9 Hz, 1H), 7.61 (s, 1H, H-vinyl), 7.45 (m, 1H, CONH), 7.0-7.19 (m, 5H), 3.73 (s, 3H, OCH_3), 3.32 (m, 2H, CH_2), 2.87 (m, 1H, $CH(CH_3)_2$), 2.56 (m, 2H, CH_2), 2.48 (m, 4H, 2xCH_2), 2.43 (s, 3H, CH_3), 2.40 (s, 3H, CH_3), 1.68 (m, 4H, 2xCH_2), 1.21 (d, *J* = 6.8 Hz, 6H, $CH(CH_3)_2$).

MS *m/z* 527.2 [M$^+$+1].

Example 77

5-[6-(4-Ethylphenyl)-2-oxo-1,2-dihydroindol-3-ylidenemethyl]-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-pyrrolidin-1-ylethyl)amide

6-(4-Ethylphenyl)-1,3-dihydroindol-2-one (45 mg, 0.19 mmol) was condensed 5-formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-pyrrolidin-1-ylethyl)amide (50 mg) to give 60 mg (65%) of the title compound as a yellow-orange solid.

^{1}HNMR (300 MHz, DMSO-d6) δ 13.59 (s, 1H, NH), 10.96 (s, br, 1H, NH), 7.83 (d, *J* = 8.4 Hz, 1H), 7.64 (s, 1H, H-vinyl), 7.51-7.56 (m, 3H), 7.25-7.30 (m, 3H), 7.08 (d, *J* = 1 Hz, 1H), 3.31 (m, 2H, CH_2), 2.63 (m, 2H, CH_2CH_3), 2.55 (m, 2H, CH_2), 2.49 (m, 4H, 2xCH_2), 2.42 (s, 3H, CH_3), 2.40 (s, 3H, CH_3), 1.67 (m, 4H, 2xCH_2), 1.20 (t, *J*= 7.5 Hz, 3H, CH_2CH_3).

MS-EI *m/z* 482 [M$^+$].

Example 78

5-[6-(2,4-Dimethoxyphenyl)-2-oxo-1,2-dihydroindol-3-ylidenemethyl]-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-pyrrolidin-1-ylethyl)amide

6-(2,4-Dimethoxyphenyl)-1,3-dihydroindol-2-one (51 mg, 0.19 mmol) was condensed with 5-formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-pyrrolidin-1-ylethyl)amide (50 mg) to give 30 mg (31%) of the title compound as an orange solid.

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^{1}HNMR (300 MHz, DMSO-d6) δ 13.59 (s, 1H, NH), 10.86 (s, br, 1H, NH), 7.75 (d, *J* = 7.8 Hz, 1H), 7.60 (s, 1H, H-vinyl), 749 (m, 1H, CONH), 7.22 (d, *J* = 8.4 Hz, 1H), 7.03 (m, 1H), 6.97 (s, 1H), 6.58-6.65 (m, 2H), 3.79 (s, 3H, OCH_3), 3.76 (s, 3H, OCH_3), 3.33 (m, 2H, CH_2), 2.55 (m, 2H, CH_2), 2.50 (m, 4H, 2xCH_2), 2.42 (s, 3H, CH_3), 2.39 (s, 3H, CH_3), 1.67 (m, 4H, 2xCH_2).

MS-EI *m/z* 514 [M$^+$].

Example 79

5-[6-(3-Isopropylphenyl)-2-oxo-1,2-dihydroindol-3-ylidenemethyl]-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-pyrrolidin-1-ylethyl)amide

6-(3-Isopropylphenyl)-1,3-dihydroindol-2-one (48 mg, 0.19 mmol) was condensed with 5-formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-pyrrolidin-1-ylethyl)amide (50 mg) to give 59 mg (63%) of the title compound as an orange solid.

^{1}HNMR (300 MHz, DMSO-d6) δ 13.63 (s, 1H, NH), 10.97 (s, br, 1H, NH), 7.87 (d, *J* = 7.8 Hz, 1H), 7.68 (s, 1H, H-vinyl), 7.24-7.55 (m, 6H), 7.13 (s, 1H), 3.34 (m, 2H, CH_2), 3.30 (m, 1H, $CH(CH_3)_2$), 2.60 (m, 2H, CH_2), 2.50 (m, 4H, 2xCH_2), 2.45 (s, 3H, CH_3), 2.43 (s, 3H, CH_3), 1.70 (m, 4H, 2xCH_2), 1.27 (d, *J* = 6.9 Hz, 6H, $CH(CH_2)_2$).

MS-EI *m/z* 496 [M$^+$].

Example 80

5-(5-Fluoro-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-diethylaminoethyl)amide

5-Fluoro-1,3-dihydroindol-2-one (0.54 g, 3.8 mmol) was condensed with 5-formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-diethylaminoethyl)amide to give 0.83 g (55%) of the title compound as a yellow green solid.

501 2740.021500

^{1}HNMR (360 MHz, DMSO-d6) δ 13.66 (s, 1H, NH), 10.83 (s, br, 1H, NH), 7.73 (dd, J = 2.5 & 9.4 Hz, 1H), 7.69 (s, 1H, H-vinyl), 7.37 (t, 1H, CONHCH$_2$CH$_2$), 6.91 (m, 1H), 6.81-6.85 (m, 1H), 3.27 (m, 2H, CH$_2$), 2.51 (m, 6H, 3xCH$_2$), 2.43 (s, 3H, CH$_3$), 2.41 (s, 3H, CH$_3$), 0.96 (t, J = 6.9 Hz, 6H, N(CH$_2$CH$_3$)$_2$).

MS-EI m/z 398 [M$^+$].

Example 81

3-[4-(2-Diethylaminoethylcarbamoyl)-3,5-dimethyl-1*H*-pyrrol-2-ylmethylene]-2-oxo-2,3-dihydro-1*H*-indole-6-carboxylic acid

2-Oxo-2,3-dihydro-1*H*-indole-6-carboxylic acid (80 mg, 0.45 mmol) was condensed with 5-formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-diethylaminoethyl)amide to give 210 mg (92%) of the title compound as a yellow orange solid.

^{1}HNMR (360 MHz, DMSO-d6) δ 13.6 (s, 1H, NH), 7.76 (d, J = 8.0 Hz, 1H), 7.66 (s, 1H, H-vinyl), 7.57 (dd, J = 1.5 & 8.0 Hz, 1H), 7.40-7.42 (m, 2H), 3.28 (m, 2H, CH$_2$), 2.88 (m, H-piperidine), 2.54 (m, 6H, 3xCH$_2$), 2.44 (s, 3H, CH$_3$), 2.40 (s, 3H, CH$_3$), 1.56 (m, H-piperidine), 0.97 (t, J = 6.98 Hz, 6H, N(CH$_2$CH$_3$)$_2$).

MS m/z 424 [M$^+$].

Example 82

5-(5-Dimethylsulfamoyl-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-pyrrolidin-1-ylethyl)amide

2-Oxo-2,3-dihydro-1*H*-indole-5-sulfonic acid dimethylamide (90 mg, 0.38 mmol) was condensed with 5-formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-pyrrolidin-1-ylethyl)amide (100 mg) to give 100 mg (54%) of the title compound as a yellow solid.

^{1}HNMR (360 MHz, DMSO-d6) δ 13.65 (s, 1H, NH), 11.30 (s, br, 1H, NH), 8.25 (d, 1H), 7.92 (s, 1H, H-vinyl), 7.48-7.53 (m, 2H), 7.07 (d, J = 8.2 Hz, 1H), 3.33 (m, 2H, CH$_2$), 2.61 (s, 6H,

501 270.02150

$N(CH_3)_2$), 2.56 (t, 2H, CH_2), 2.49 (m, 4H, 2xCH_2), 2.45 (s, 3H, CH_3), 2.44 (s, 3H, CH_3), 1.67 (m, 4H, 2xCH_2).

MS-EI m/z 485 [M$^+$].

Example 83

5-[5-(3-Chlorophenylsulfamoyl)-2-oxo-1,2-dihydroindol-3-ylidenemethyl]-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-pyrrolidin-1-ylethyl)amide

2-Oxo-2,3-dihydro-1*H*-indole-5-sulfonic acid (3-chlorophenyl)amide (120 mg, 0.38 mmol) was condensed with 5-formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-pyrrolidin-1-ylethyl)amide (100 mg) to give 150 mg (69%) of the title compound as a yellow orange solid.

^{1}HNMR (360 MHz, DMSO-d6) δ 13.55 (s, 1H, N*H*), 11.26 (br s, 1H, N*H*), 10.30 (br s,1H, N*H*), 8.26 (d, 1H), 7.79 (s, 1H, H-vinyl), 7.51-7.57 (m, 2H), 7.22 (t, *J* = 8.1 Hz, 1H), 7.15 (m, 1H), 7.07 (m, 1H), 7.0 (m, 2H), 3.44 (m, 2H, CH_2), 2.57 (t, *J* = 7.0 Hz, 2H, CH_2), 2.49 (m, 4H, 2xCH_2), 2.44 (s, 3H, CH_3), 2.43 (s, 3H, CH_3), 1.68 (m, 4H, 2xCH_2).

MS *m/z* 568 [M$^+$].

Example 84

2,4-Dimethyl-5-[2-oxo-5-(pyridin-3-ylsulfamoyl)-1,2-dihydroindol-3-ylidenemethyl]-1*H*-pyrrole-3-carboxylic acid (2-pyrrolidin-1-ylethyl)amide

2-Oxo-2,3-dihydro-1*H*-indole-5-sulfonic acid pyridin-3-ylamide (110 mg, 0.38 mmol) was condensed with 5-formyl-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (2-pyrrolidin-1-ylethyl)amide (100 mg) to give 150 mg (74%) of the title compound as an orange solid..

^{1}HNMR (360 MHz, DMSO-d6) δ 13.58 (s, 1H, N*H*), 8.21 (d, *J* = 2.0 Hz, 2H), 8.04 (m, 1H), 7.76 (s, 1H, H-vinyl), 7.49-7.54 (m, 2H), 7.41 (m, 1H), 7.14 (m, 1H), 6.94 (d, *J* = 8.5 Hz, 1H), 3.33

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(m, 2H, CH_2), 2.56 (t, J = 7.06 Hz, 2H, CH_2), 2.49 (m, 4H, 2xCH_2), 2.43 (s, 6H, 2xCH_3), 1.68 (m, 4H, 2xCH_2).

MS m/z 535 [M$^+$].

Example 85

3-[3,5-Dimethyl-4-(4-methylpiperazine-1-carbonyl)-1*H*-pyrrol-2-ylmethylene]-4-(2-hydroxyethyl)-1,3-dihydroindol-2-one

4-(2-Hydroxyethyl)-1,3-dihydroindol-2-one (71 mg, 0.4 mmol) was condensed with 3,5-dimethyl-4-(4-methyl-piperazine-1-carbonyl)-1*H*-pyrrole-2-carbaldehyde to give 90 mg (55%) of the title compound as an orange solid.

^{1}HNMR (300 MHz, DMSO-d6) δ 14.25(s, 1H, NH), 10.88 (s, 1H, NH), 7.57 (s, 1H, H-vinyl), 7.03 (m, 1H), 6.75-6.82 (m, 2H), 4.86 (m, 1H, OH), 3.70 (m, 2H, CH_2), 3.04 (m, 2H, CH_2), 2.48 (m, 4H, 2xCH_2), 2.28 (br s, 7H), 2.19 (s, 3H, CH_3), 2.18 (s, 3H, CH_3).

MS m/z (+ve) 409.3 [M$^+$].

Example 86

3-[3,5-Dimethyl-4-(4-methylpiperazine-1-carbonyl)-1*H*-pyrrol-2-ylmethylene]-2-oxo-2,3-dihydro-1*H*-indole-5-sulfonic acid phenylamide

2-Oxo-2,3-dihydro-1*H*-indole-5-sulfonic acid phenylamide (110 mg, 0.4 mmol) was condensed with 3,5-dimethyl-4-(4-methylpiperazine-1-carbonyl)-1*H*-pyrrole-2-carbaldehyde (100 mg) to give 50 mg (24%) of the title compound as a yellow solid.

^{1}HNMR (300 MHz, DMSO-d6) δ 13.52(s, 1H, NH), 11.26 (s, 1H, NH), 10.08 (s, 1H, NH), 8.21 (d, J = 1.6 Hz, 1H), 7.75 (s, 1H, H-vinyl), 7.50 (dd, J = 1.6 & 8.3 Hz, 1H), 7.19 (m, 2H), 7.10 (m, 2H), 6.97 (m, 2H), 2.49 (m, 4H, 2xCH_2), 2.28 (m, 10H, 2xCH_3 & 2xCH_2), 2.18 (s, 3H, CH_3).

MS-EI m/z 519 [M$^+$].

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Example 87

5-(5-Dimethylsulfamoyl-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-diethylaminoethyl)amide

2-Oxo-2,3-dihydro-1*H*-indole-5-sulfonic acid dimethylamide (90 mg, 0.38 mmol) was condensed with 5-formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-diethylaminoethyl)amide (100 mg) to give 80 mg (43%) of the title compound as a yellow solid.

^{1}HNMR (300 MHz, DMSO-d6) δ 11.30 (s, 1H, *NH*), 8.27 (d, *J* = 1.7 Hz, 1H), 7.94 (s, 1H, H-vinyl), 7.49 (dd, *J* = 1.7 & 8.0 Hz, 1H), 7.44 (m, 1H, $CONHCH_2CH_3$), 7.07 (d, *J* = 8.0 Hz, 1H), 3.26 (m, 2H, CH_2), 2.60 (s, 6H, $N(CH_3)_2$), 2.53 (m, 2H, CH_2), 2.45-2.50 (m, 10H, $2xCH_3$ & $N(CH_2CH_3)_2$), 0.96 (t, *J* = 7.2 Hz, 6H, $N(CH_2CH_3)_2$).

MS-EI *m/z* 487 [M$^+$].

Example 88

5-[5-(3-Chlorophenylsulfamoyl)-2-oxo-1,2-dihydroindol-3-ylidenemethyl]-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-diethylaminoethyl)amide

2-Oxo-2,3-dihydro-1*H*-indole-5-sulfonic acid (3-chlorophenyl)amide (120 mg, 3.8 mmol) was condensed with 5-formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-diethylaminoethyl)amide (100 mg) to give 80 mg (37%) of the title compound as a yellow solid.

^{1}HNMR (360 MHz, DMSO-d6) δ 13.55 (s, 1H, *NH*), 11.24 (s, 1H, *NH*), 10.29 (s, 1H, *NH*), 8.25 (d, *J* = 1.87 Hz, 1H), 7.79 (s, 1H, H-vinyl), 7.52 (dd, *J* = 1.87 & 8.3 Hz, 1H), 7.42 (m, 1H, $CONHCH_2CH_2$), 7.22 (t, *J* = 8.02 Hz, 1H), 7.15 (t, *J* = 2 Hz, 1H), 7.08 (m, 1H), 7.0 (m, 2H), 3.27 (m, 2H, CH_2), 2.48-2.57 (m, 6H, $3xCH_2$), 2.45 (s, 3H, CH_3), 2.44 (s, 3H, CH_3), 0.97 (t, *J* = 7.0 Hz, 6H, $N(CH_2CH_3)_2$).

MS *m/z* 570.1 [M$^+$].

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Example 95

3-(2-Oxo-5-phenyl-1,2-dihydroindol-3-ylidenemethyl)-4,5,6,7-tetrahydro-2*H*-isoindole-1-carboxylic acid ethyl ester

^{1}HNMR (360 MHz, DMSO-d6) δ 13.74 (s, 1H, NH), 11.00 (s, 1H, NH), 8.13 (d, *J* = 1.7 Hz, 1H), 7.74 (s, 1H, H-vinyl), 7.70 (d, *J* = 7.7 Hz, 2H), 7.49 (dd, *J* = 1.7 & 8.0 Hz, 1H), 7.44 (t, *J* = 7.7 Hz, 2H), 7.32 (m, 1H), 6.96 (d, *J* = 8.0 Hz, 1H), 4.26 (q, *J* = 7.0 Hz, 2H, OCH_2CH_3), 2.79 (m, 2H, CH_2), 2.72 (m, 2H, CH_2), 1.73 (m, 4H, 2xCH_2), 1.30 (t, *J* = 7.0 Hz, 3H, OCH_2CH_3).

MS-EI *m/z* 412 [M$^+$].

Example 99

3-(2-Oxo-5-phenylsulfamoyl-1,2-dihydroindol-3-ylidenemethyl)-4,5,6,7-tetrahydro-2*H*-isoindole-1-carboxylic acid ethyl ester

^{1}HNMR (360 MHz, DMSO-d6) δ 13.64 (s, 1H, NH), 11.33 (s, 1H, NH), 10.07 (s, 1H, NH), 8.24 (d, *J* = 1.8 Hz, 1H), 7.74 (s, 1H, H-vinyl), 7.57 (dd, *J* = 1.8 & 8.0 Hz, 1H), 7.21 (t, *J* = 7.6 Hz, 2H), 7.11 (d, *J* = 7.6 Hz, 2H), 6.99 (d, *J* = 8.0 Hz, 1H), 6.98 (d, *J* = 7.6 Hz, 1H), 4.27 (q, *J* = 7.0 Hz, 2H, OCH_2CH_3), 2.80 (m, 2H, CH_2), 2.73 (m, 2H, CH_2), 1.73 (m, 4H, 2xCH_2), 1.30 (t, *J* = 7.0 Hz, 3H, OCH_2CH_3).

MS-EI *m/z* 491 [M$^+$].

Example 109

3-[3-(Morpholine-4-carbonyl)-4,5,6,7-tetrahydro-2*H*-isoindol-1-ylmethylene]-2-oxo-2,3-dihydro-1*H*-indole-6-carboxylic acid

^{1}HNMR (360 MHz, DMSO-d6) δ 13.60 (s, 1H, NH), 12.75 (br s, 1H, COOH), 11.08 (s, 1H, NH), 7.85 (d, *J* = 7.8 Hz, 1H), 7.71 (s, 1H, H-vinyl), 7.62 (dd, *J* = 1.4 & 7.8 Hz, 1H), 7.41 (d, *J* = 1.4 Hz, 1H), 3.65 (m, 4H, 2xCH_2), 3.55 (m, 4H, 2xCH_2), 2.81 (m, 2H, CH_2), 2.54 (m, 2H, CH_2).1.73 (m, 4H, 2xCH_2).

MS-EI *m/z* 421 [M$^+$].

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Example 112
5-Bromo-3-[3-(pyrrolidine-1-carbonyl)-4,5,6,7-tetrahydro-2H-isoindol-1-ylmethylene]-1,3-dihydro-indol-2-one

^{1}HNMR (360 MHz, DMSO-d6) δ 13.56 (s, 1H, NH), 11.00 (s, 1H, NH), 8.05 (d, *J* = 1.8 Hz, 1H), 7.74 (s, 1H, H-vinyl), 7.28 (dd, *J* = 1.3 & 8.3 Hz, 1H), 6.83 (d, *J* = 8.3 Hz, 1H), 3.57 (m, 4H, 2xCH_2), 2.79 (m, 2H, CH_2), 2.65 (m, 2H, CH_2), 1.88 (m, 4H, 2xCH_2), 1.71 (m, 4H, 2xCH_2).

MS-EI *m/z* 439 & 441 [M$^+$-1] & [M$^+$+1].

Example 114
3-(3-Dimethylcarbamoyl-4,5,6,7-tetrahydro-2*H*-isoindol-1-ylmethylene)-2-oxo-2,3-dihydro-1*H*-indole-6-carboxylic acid

^{1}HNMR (360 MHz, DMSO-d6) δ 13.60 (s, 1H, NH), 12.72 (br s, 1H, COOH), 11.05 (s, 1H, NH), 7.85 (d, *J* = 7.9 Hz, 1H), 7.72 (s, 1H, H-vinyl), 7.62 (dd, *J* = 1.3 & 7.9 Hz, 1H), 7.42 (d, *J* = 1.3 Hz, 1H), 3.03 (s, 6H, N(CH_3)$_2$), 2.81 (m, 2H, CH_2), 2.55 (m, 2H, CH_2), 1.73 (m, 4H, 2xCH_2).

MS-EI *m/z* 379 [M$^+$].

Example 115
4-Methyl-5-(5-methylsulfamoyl-2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-1H-pyrrole-3-carboxylic acid

^{1}HNMR (300 MHz, DMSO-d6) 13.56 (br s, 1H, NH), 8.24 (d, *J* = 1.5 Hz, 1H), 7.86 (s, 1H, H-vinyl), 7.74 (d, *J* = 2.96 Hz, 1H), 7.56 (dd, *J* = 1.5 & 8.1 Hz, 1H), 7.20 (br m, 1H, NHCH$_3$), 7.03 (d, *J* = 8.1 Hz, 1H), 2.57 (s, 3H, CH_3), 2.41 (s, 3H, CH_3).

MS-EI *m/z* 361 [M$^+$].

Example 116
{[4-Methyl-5-(4-methyl-5-methylsulfamoyl-2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-1H-pyrrole-3-carbonyl]-amino}-acetic acid ethyl ester

4-Methyl-1*H*-pyrrole-3-carboxylic acid ethyl ester (lit. ref. D.O. Cheng, T. L. Bowman and E. LeGoff; J. Heterocyclic Chem.;

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1976; 13; 1145-1147) was formylated using method A, hydrolysed using method B followed by amidation (method C) to give [(5-formyl-4-methyl-1*H*-pyrrole-3-carbonyl)-amino]-acetic acid ethyl ester.

4-Methyl-5-methylaminosulfonyl-2-oxindole (50 mg, 0.21 mmol) was condensed with [(5-formyl-4-methyl-1*H*-pyrrole-3-carbonyl)-amino]-acetic acid ethyl ester (100 mg, 0.42 mmol) and piperidine (0.1 mL) in ethanol (2 mL) to give 50 mg (52%) of the title compound.

^{1}HNMR (360 MHz, DMSO-d6) δ 13.59 (s, 1H, NH), 11.29 (v.br. s, 1H, NH-CO), 8.33 (t, *J* = 5.8 Hz, 1H, CONHCH$_2$), 7.83 (d, *J* = 3.11 Hz, 1H), 7.80 (s, 1H, H-vinyl), 7.71 (d, *J* = 8.5 Hz, 1H), 7.34 (br m, 1H, NHCH$_3$), 6.89 (d, *J* = 8.5 Hz, 1H), 4.11 (q, *J* = 7.1 Hz, 2H, OCH$_2$CH$_3$), 3.92 (d, *J* = 5.8 Hz, 2H, GlyCH$_2$), 2.86 (s, 3H, CH$_3$), 2.48 (s, 3H, CH$_3$), 2.42 (d, *J* = 4.71 Hz, 3H, HNCH$_3$), 1.20 (t, *J* = 7.1 Hz, 3H, OCH$_2$CH$_3$).

MS-EI *m/z* 460 [M$^+$].

Example 117

{[4-Methyl-5-(5-methylsulfamoyl-2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-1H-pyrrole-3-carbonyl]-amino}-acetic acid ethyl ester

A mixture of 5-methylaminosulfonyl-2-oxindole (0.06 g, 0.22 mmol), [(5-formyl-4-methyl-1*H*-pyrrole-3-carbonyl)-amino]-acetic acid ethyl ester (0.075 g, 0.27 mmol) and piperidine (2 drops) in ethanol (5 mL) was heated in a sealed tube at 90° C for 12 hrs. After cooling, the precipitate was collected by vacuum filtration, washed with ethanol, triturated with dichloromethane/ether and dried to give 0.035 g (36%) of the title compound as a yellowish brown solid.

^{1}H NMR (360 MHz, DMSO-d6) δ 13.6 (s, 1H, NH), 11 (v.br. s, 1H, NH-CO), 8.30 (t, *J* = 5.7 Hz, 1H, CONHCH$_2$), 8.25 (d, *J* =

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1.2 Hz, 1H), 7.88 (s, 1H, H-vinyl), 7.84 (d, *J* = 3.3 Hz, 1H), 7.57 (dd, *J* = 1.9 & 8.5 Hz, 1H), 7.14 (br m, 1H, $NHCH_3$), 7.04 (d, *J* = 8.5 Hz, 1H), 4.11 (q, *J* = 6.7 Hz, 2H, OCH_2CH_3), 3.92 (d, *J* = 5.7 Hz, 2H, $GlyCH_2$), 2.55 (s, 3H, CH_3), 2.41 (m, 3H, NCH_3), 1.20 (t, *J* = 6.7 Hz, 3H, OCH_2CH_3).

MS *m/z* 446 [M⁺].

Example 118

{[4-Methyl-5-(5-methylsulfamoyl-2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-1H-pyrrole-3-carbonyl]-amino}-acetic acid

A mixture of [(5-formyl-4-methyl-1*H*-pyrrole-3-carbonyl)-amino]-acetic acid ethyl ester (0.142 g, 0.59 mmol) and 1N NaOH (1.2 mL) in methanol (10 mL) was stirred at room temperature for 1 hr. The reaction was concentrated and the residue was condensed with 5-methylaminosulfonyl-2-oxindole (0.13 g, 0.48 mmol) and piperidine (0.12 mL) in ethanol (12 mL) to give 0.11 g (52%) of the title compound.

^{1}HNMR (300 MHz, DMSO-d6) δ 13.98 (br s, 1H, NH), 8.17 (s, 1H), 7.80 (s, 1H), 7.75 (d, *J* = 3.1Hz, 1H), 7.51 (dd, *J* = 2 & 8.2 Hz, 1H), 7.21 (m on br s, 2H), 6.97 (d, *J* = 8.1 Hz, 1H), 3.41 (d, *J* = 4.2 Hz, 2H, CH_2NH), 2.54 (s, 3H, pyrrole-CH_3), 2.39 (s, 3H, $ArCH_3$).

MS *m/z* 417 [M-1]⁺.

Example 120

5-Methyl-2-(2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-1H-pyrrole-3-carboxylic acid

^{1}HNMR (300 MHz, DMSO-d6) δ 13.77 (br s, 1H, NH), 12.49 (s, 1H, COOH), 11.07 (s, 1H, NH), 8.39 (s, 1H, H-vinyl), 7.43 (d, *J* = 7.47 Hz, 1H), 7.20 (t, *J* = 7.47 Hz, 1H), 7.03 (t, *J* = 7.47 Hz, 1H), 6.91 (d, *J* = 7.47 Hz, 1H), 6.49 (d, *J* = 1.53 Hz, 1H), 2.34 (s, 3H, CH_3).

MS *m/z* 269 [M+H]⁺.

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Example 121

5-Methyl-2-(2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-1H-pyrrole-3-carboxylic acid ethyl ester

^{1}HNMR (300 MHz, DMSO-d6) δ 13.79 (s, 1H, NH), 11.08 (s, 1H, NH), 8.31 (s, 1H, H-vinyl), 7.45 (d, *J* = 7.52 Hz, 1H), 7.20 (t, *J* = 7.52 Hz, 1H), 7.03 (t, *J* = 7.52 Hz, 1H), 6.91 (d, *J* = 7.52 Hz, 1H), 6.50 (d, *J* = 2.1 Hz, 1H), 4.26 (q, *J* = 7.2 Hz, 2H, OCH_2CH_3), 2.33 (s, 3H, CH_3), 1.32 (t, *J* = 7.2 Hz, 3H, OCH_2CH_3).

MS *m/z* 297.1 $[M+H]^+$.

Example 122

2-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-5-methyl-1*H*-pyrrole-3-carboxylic acid ethyl ester

^{1}HNMR (360 MHz, DMSO-d6) δ 13.72(s, 1H, NH), 11.16 (s, 1H, NH), 8.29 (s, 1H, H-vinyl), 7.53 (d, *J* = 2.0 Hz, 1H), 7.35 (dd, *J* = 2.0 & 8.05 Hz, 1H), 6.87 (t, *J* = 8.05 Hz, 1H), 6.53 (d, *J* = 2.4 Hz, 1H), 4.28 (q, *J* = 7.03 Hz, 2H, OCH_2CH_3), 2.35 (s, 3H, CH_3), 1.33 (t, *J* = 7.03 Hz, 3H, OCH_2CH_3).

MS *m/z* 375 & 377 $[M+H]^+$.

Example 123

2-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-5-methyl-1*H*-pyrrole-3-carboxylic acid

^{1}HNMR (300 MHz, DMSO-d6) δ 13.72(s, 1H, NH), 12.57 (s, 1H, COOH), 11.19 (s, 1H, NH), 8.36 (s, 1H, H-vinyl), 7.51 (d, *J* = 1.4 Hz, 1H), 7.34 (dd, *J* = 1.4 & 8.17 Hz, 1H), 6.87 (t, *J* = 8.17 Hz, 1H), 6.52 (d, *J* = 2.5 Hz, 1H), 2.35 (s, 3H, CH_3).

MS *m/z* 347 & 349 $[M+H]^+$.

Example 124

2-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-5-methyl-1*H*-pyrrole-3-carboxylic acid (2-pyrrolidin-1-ylethyl)-amide

To a solution of 2-formyl-5-methyl-1*H*-pyrrole-3-carboxylic acid (250 mg, 1.63 mmol) in dimethylformamide (3 mL) was added 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (376 mg, 1.2 equiv.),

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1-hydroxybenzotriazole (265 mg, 1.2 equiv.), triethylamine (0.45 mL, 2 equiv.) and 1-(2-aminoethyl)pyrrolidine (0.23 mL. 1.1 equiv.). After stirring at room temperature overnight, the reaction was diluted with saturated sodium bicarbonate and brine (with extra salt) and extracted with 10% methanol in dichloromethane. The combined organic layers were washed with brine, dried over anhydrous sodium sulfate and concentrated to give 130 mg of 2-formyl-5-methyl-1*H*-pyrrole-3-carboxylic acid (2-pyrrolidin-1-yl-ethyl)-amide.

A mixture of 5-bromo-2-oxindole (106 mg, 0.5 mmol), 2-formyl-5-methyl-1*H*-pyrrole-3-carboxylic acid (2-pyrrolidin-1-yl-ethyl)-amide (125 mg, 1 equiv.) and piperidine (0.2 mL) in ethanol (2 mL) was heated in a sealed tube at 80°C for 1 hr and then cooled. The precipitate which formed was collected by vacuum filtration, washed with ethanol and ethyl acetate and dried to give the title compound as an orange solid.

^{1}HNMR (300 MHz, DMSO-d6) δ 13.62 (s, 1H, N*H*), 11.06 (br s, 1H, N*H*), 8.56 (s, 1H, H-vinyl), 8.15 (m, 1H, CON*H*CH_2), 7.48 (d, *J* = 1.8 Hz, 1H), 7.31 (dd, *J* = 1.8 & 7.9 Hz, 1H), 6.86 (d, *J* = 7.9 Hz, 1H), 6.60 (d, *J* = 2.3 Hz, 1H), 3.35 (m, 2H, HNC*H*$_2$$CH_2$), 2.56 (t, *J* = 6.91 Hz, 2H, HNCH$_2$C*H*$_2$), 2.35 (s, 3H, C*H*$_3$), 1.67 (m, 4H, 2xC*H*$_2$).

MS *m/z* 443/ 445 [M$^+$ and M$^+$+2].

Example 125

2-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-5-methyl-1*H*-pyrrole-3-carboxylic acid (2-diethylaminoethyl)-amide

To a solution of 2-formyl-5-methyl-1*H*-pyrrole-3-carboxylic acid (320 mg, 2.1 mmol) in dimethylformamide (3 mL) was added 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (483 mg, 1.2 equiv.), 1-hydroxybenzotriazole (340 mg, 1.2 equiv.), triethylamine (0.59 mL, 2 equiv.) and N,N-diethylethylenediamine (0.32 mL, 1.1

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equiv.). After stirring at room temperature overnight, the reaction was diluted with saturated sodium bicarbonate and brine (with extra salt) and extracted with 10% methanol in dichloromethane. The combined organic layers were washed with brine, dried over anhydrous sodium sulfate and concentrated to give 2-formyl-5-methyl-1*H*-pyrrole-3-carboxylic acid (2-diethylaminoethyl)-amide.

A mixture of 5-bromo-2-oxindole (106 mg, 0.5 mmol), 2-formyl-5-methyl-1*H*-pyrrole-3-carboxylic acid (2-diethylaminoethyl)-amide (126 mg, 1 equiv.) and piperidine (0.2 mL) in ethanol (2 mL) was heated in a sealed tube at 80°C for 1 hr and then cooled. The precipitate was collected by vacuum filtration, washed with ethanol and ethyl acetate and dried to give the title compound as an orange solid.

^{1}HNMR (360 MHz, DMSO-d6) δ 13.62 (s, 1H, N*H*), 11.11 (br s, 1H, N*H*), 8.54 (s, 1H, H-vinyl), 8.1 (m, 1H, CO*NH*CH_2), 7.49 (d, *J* = 2.2 Hz, 1H), 7.31 (dd, *J* = 2.2 & 8.3 Hz, 1H), 6.86 (d, *J* = 8.3 Hz, 1H), 6.58 (d, *J* = 2.24 Hz, 1H), 3.31 (m, 2H, HNCH_2CH_2), 2.59 (m, 6H, 3xCH_2), 2.36 (s, 3H, CH_3), 0.99 (t, *J* = 6.8 Hz, 6H, N(CH_2CH_3)$_2$).

MS *m/z* 445/ 447 [M$^+$ and M$^+$+2].

5. **BIOLOGICAL EVALUATION**

It will be appreciated that, in any given series of compounds, a spectrum of biological activity will be afforded. In its presently preferred embodiments, this invention relates to novel pyrrole substituted 2-indolinones demonstrating the ability to modulate RTK, CTK, and STK activity. The following assays are employed to find those compounds demonstrating the optimal degree of the desired activity.

A. Assay Procedures.

The following assays may be used to determine the level of activity and effect of the different compounds of the present invention on one or more of the PKs. Similar assays can be designed along the same lines for any PK using techniques well known in the art.

Several of the assays described herein are performed in an ELISA (Enzyme-Linked Immunosorbent Sandwich Assay) format (Voller, et al., 1980, "Enzyme-Linked Immunosorbent Assay," Manual of Clinical Immunology, 2d ed., Rose and Friedman, Am. Soc. Of Microbiology, Washington, D.C., pp. 359-371). The general procedure is as follows: a compound is introduced to cells expressing the test kinase, either naturally or recombinantly, for a selected period of time after which, if the test kinase is a receptor, a ligand known to activate the receptor is added. The cells are lysed and the lysate is transferred to the wells of an ELISA plate previously coated with a specific antibody recognizing the substrate of the enzymatic phosphorylation reaction. Non-substrate components of the cell lysate are washed away and the amount of phosphorylation on the substrate is detected with an antibody specifically recognizing phosphotyrosine compared with control cells that were not contacted with a test compound.

The presently preferred protocols for conducting the ELISA experiments for specific PKs is provided below. However, adaptation of these protocols for determining the activity of compounds against other RTKs, as well as for CTKs and STKs, is well within the scope of knowledge of those skilled in the art.

Other assays described herein measure the amount of DNA made in response to activation of a test kinase, which is a general measure of a proliferative response. The general

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procedure for this assay is as follows: a compound is introduced to cells expressing the test kinase, either naturally or recombinantly, for a selected period of time after which, if the test kinase is a receptor, a ligand known to activate the receptor is added. After incubation at least overnight, a DNA labeling reagent such as 5-bromodeoxyuridine (BrdU) or H^3-thymidine is added. The amount of labeled DNA is detected with either an anti-BrdU antibody or by measuring radioactivity and is compared to control cells not contacted with a test compound.

GST-FLK-1 BIOASSAY

This assay analyzes the tyrosine kinase activity of GST-Flk1 on poly(glu,tyr) peptides.

Materials and Reagents:

1. Corning 96-well ELISA plates (Corning Catalog No. 5805-96).
2. poly(glu,tyr) 4:1, lyophilizate (Sigma Catalog # P0275).
3. Preparation of poly(glu,tyr)(pEY) coated assay plates: Coat 2 ug/well of poly(glu,tyr)(pEY) in 100 ul PBS, hold at room temperature for 2 hours or at 4°C overnight. Cover plates well to prevent evaporation.
4. PBS Buffer: for 1 L, mix 0.2 g KH_2PO_4, 1.15 g Na_2HPO_4, 0.2 g KCl and 8 g NaCl in approx. 900ml dH_2O. When all reagents have dissolved, adjust the pH to 7.2 with HCl. Bring total volume to 1 L with dH_2O.
5. PBST Buffer: to 1 L of PBS Buffer, add 1.0 ml Tween-20.
6. TBB - Blocking Buffer: for 1 L, mix 1.21 g TRIS, 8.77 g NaCl, 1 ml TWEEN-20 in approximately 900 ml dH_2O. Adjust pH to 7.2 with HCl. Add 10 g BSA, stir to

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dissolve. Bring total volume to 1 L with dH_2O. Filter to remove particulate matter.

7. 1% BSA in PBS: To make a 1x working solution, add 10 g BSA to approx. 990 ml PBS buffer, stir to dissolve. Adjust total volume to 1 L with PBS buffer, filter to remove particulate matter.
8. 50 mM Hepes pH 7.5.
9. GST-Flklcd purified from sf9 recombinant baculovirus transformation (SUGEN, Inc.).
10. 4% DMSO in dH_2O.
11. 10 mM ATP in dH_2O.
12. 40 mM $MnCl_2$
13. Kinase Dilution Buffer (KDB): mix 10 ml Hepes (pH 7.5), 1 ml 5M NaCl, 40 µL 100 mM sodium orthovanadate and 0.4 ml of 5% BSA in dH_2O with 88.56 ml dH_2O.
14. NUNC 96-well V bottom polypropylene plates, Applied Scientific Catalog # AS-72092
15. EDTA: mix 14.12 g ethylenediaminetetraacetic acid (EDTA) to approx. 70 ml dH_2O. Add 10 N NaOH until EDTA dissolves. Adjust pH to 8.0. Adjust total volume to 100 ml with dH_2O.
16. 1° Antibody Dilution Buffer: mix 10 ml of 5% BSA in PBS buffer with 89.5 ml TBST.
17. Anti-phosphotyrosine monoclonal antibody conjugated to horseradish peroxidase (PY99 HRP, Santa Cruz Biotech).
18. 2,2'-Azinobis(3-ethylbenzthiazoline-6-sulfonic acid (ABTS, Moss, Cat. No. ABST).
19. 10% SDS.

Procedure:

1. Coat Corning 96-well ELISA plates with 2 µg of polyEY peptide in sterile PBS as described in step 3 of

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Materials and Reagents.

2. Remove unbound liquid from wells by inverting plate. Wash once with TBST. Pat the plate on a paper towel to remove excess liquid.
3. Add 100 µl of 1% BSA in PBS to each well. Incubate, with shaking, for 1 hr. at room temperature.
4. Repeat step 2.
5. Soak wells with 50 mM HEPES (pH7.5) (150 µl/well).
6. Dilute test compound with dH_2O/4% DMSO to 4 times the desired final assay concentration in 96-well polypropylene plates.
7. Add 25 µl diluted test compound to ELISA plate. In control wells, place 25 µl of dH_2O/4% DMSO.
8. Add 25 µl of 40 mM $MnCl_2$ with 4x ATP (2 µM) to each well.
9. Add 25 µl 0.5M EDTA to negative control wells.
10. Dilute GST-Flk1 to 0.005 µg(5 ng)/well with KDB.
11. Add 50 µl of diluted enzyme to each well.
12. Incubate, with shaking, for 15 minutes at room temperature.
13. Stop reaction by adding 50 µl of 250 mM EDTA (pH 8.0).
14. Wash 3X with TBST and pat plate on paper towel to remove excess liquid.
15. Add 100 µl per well anti-phosphotyrosine HRP conjugate, 1:5,000 dilution in antibody dilution buffer. Incubate, with shaking, for 90 min. at room temperature.
16. Wash as in step 14.
17. Add 100 µl of room temperature ABTS solution to each well.

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18. Incubate, with shaking, for 10 to 15 minutes. Remove any bubbles.
19. Stop reaction by adding 20 µl of 10% SDS to each well.
20. Read results on Dynatech MR7000 ELISA reader with test filter at 410 nM and reference filter at 630 nM.

PYK2 BIOASSAY

This assay is used to measure the <u>in vitro</u> kinase activity of HA epitope-tagged full length pyk2 (FL.pyk2-HA) in an ELISA assay.

<u>Materials and reagents</u>:

1. Corning 96-well Elisa plates.
2. 12CA5 monoclonal anti-HA antibody (SUGEN, Inc.)
3. PBS (Dulbecco's Phosphate-Buffered Saline (Gibco Catalog # 450-1300EB)
4. TBST Buffer: for 1 L, mix 8.766 g NaCl, 6.057 g TRIS and 1 ml of 0.1% Triton X-100 in approx. 900 ml dH_2O. Adjust pH to 7.2, bring volume to 1 L.
5. Blocking Buffer: for 1 L, mix 100 g 10% BSA, 12.1 g 100 mM TRIS, 58.44 g 1M NaCl and 10 mL of 1% TWEEN-20.
6. FL.pyk2-HA from sf9 cell lysates (SUGEN, Inc.).
7. 4% DMSO in MilliQue H_2O.
8. 10 mM ATP in dH_2O.
9. 1M $MnCl_2$.
10. 1M $MgCl_2$.
11. 1M Dithiothreitol (DTT).
12. 10X Kinase buffer phosphorylation: mix 5.0 ml 1M Hepes (pH 7.5), 0.2 ml 1M $MnCl_2$, 1.0 ml 1 M $MgCl_2$, 1.0 ml 10% Triton X-100 in 2.8 ml dH_2O. Just prior to use, add 0.1 ml 1M DTT.
13. NUNC 96-well V bottom polypropylene plates.
14. 500 mM EDTA in dH_2O.

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15. Antibody dilution buffer: for 100 mL, 1 mL 5% BSA/PBS and 1 mL 10% Tween-20 in 88 mL TBS.
16. HRP-conjugated anti-Ptyr PY99), Santa Cruz Biotech Cat. No. SC-7020.
17. ABTS, Moss, Cat. No. ABST-2000.
18. 10% SDS.

Procedure:

1. Coat Corning 96 well ELISA plates with 0.5 µg per well 12CA5 anti-HA antibody in 100 µl PBS. Store overnight at 4°C.
2. Remove unbound HA antibody from wells by inverting plate. Wash plate with dH_2O. Pat the plate on a paper towel to remove excess liquid.
3. Add 150 µl Blocking Buffer to each well. Incubate, with shaking, for 30 min at room temperature.
4. Wash plate 4x with TBS-T.
5. Dilute lysate in PBS (1.5 µg lysate/100 µl PBS).
6. Add 100 µl of diluted lysate to each well. Shake at room temperature for 1 hr.
7. Wash as in step 4.
8. Add 50 µl of 2X kinase Buffer to ELISA plate containing captured pyk2-HA.
9. Add 25 µL of 400 µM test compound in 4% DMSO to each well. For control wells use 4% DMSO alone.
10. Add 25 µL of 0.5 M EDTA to negative control wells.
11. Add 25 µl of 20 µM ATP to all wells. Incubate, with shaking, for 10 minutes.
12. Stop reaction by adding 25 µl 500 mM EDTA (pH 8.0) to all wells.
13. Wash as in step 4.

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14. Add 100 µL HRP conjugated anti-Ptyr diluted 1:6000 in Antibody Dilution Buffer to each well. Incubate, with shaking, for 1 hr. at room temperature.
15. Wash plate 3X with TBST and 1X with PBS.
16. Add 100 µL of ABST solution to each well.
17. If necessary, stop the development reaction by adding 20 µL 10% SDS to each well.
18. Read plate on ELISA reader with test filter at 410 nM and reference filter at 630 nM.

FGFR1 BIOASSAY

This assay is used to measure the in vitro kinase activity of FGF1-R in an ELISA assay.

Materials and Reagents:

1. Costar 96-well Elisa plates (Corning Catalog # 3369).
2. Poly(Glu-Tyr) (Sigma Catalog # P0275).
3. PBS (Gibco Catalog # 450-1300EB)
4. 50 mM Hepes Buffer Solution.
5. Blocking Buffer (5% BSA/PBS).
6. Purified GST-FGFR1 (SUGEN, Inc.)
7. Kinase Dilution Buffer.
   Mix 500 µl 1M Hepes (GIBCO), 20 µl 5% BSA/PBS, 10 µl 100mM sodium orthovanadate and 50 µl 5M NaCl.
8. 10mM ATP
9. ATP/$MnCl_2$ phosphorylation mix: mix 20 µL ATP, 400 µL 1M $MnCl_2$ and 9.56 ml dH_2O.
10. NUNC 96-well V bottom polypropylene plates (Applied Scientific Catalog # AS-72092).
11. 0.5M EDTA.
12. 0.05% TBST
    Add 500 µL TWEEN to 1 liter TBS.

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13. Rabbit polyclonal anti-phosphotyrosine serum (SUGEN, Inc.).
14. Goat anti-rabbit IgG peroxidase conjugate (Biosource, Catalog # ALI0404).
15. ABTS Solution.
16. ABTS/H_2O_2 solution.

Procedure:

1. Coat Costar 96 well ELISA plates with 1 µg per well Poly(Glu,Tyr) in 100µl PBS. Store overnight at 4° C.
2. Wash coated plates once with PBS.
3. Add 150 µL of 5%BSA/PBS Blocking Buffer to each well. Incubate, with shaking, for 1 hr.room temperature.
4. Wash plate 2x with PBS, then once with 50mM Hepes. Pat plates on a paper towel to remove excess liquid and bubbles.
5. Add 25µL of 0.4 mM test compound in 4% DMSO or 4% DMSO alone (controls) to plate.
6. Dilute purified GST-FGFR1 in Kinase Dilution Buffer (5 ng kinase/50ul KDB/well).
7. Add 50µL of diluted kinase to each well.
8. Start kinase reaction by adding 25µl/well of freshly prepared ATP/Mn++ (0.4 ml 1M $MnCl_2$, 40 µL 10 mM ATP, 9.56 ml dH_2O), freshly prepared).
9. This is a fast kinase reaction and must be stopped with 25µL of 0.5M EDTA in a manner similar to the addition of ATP.
10. Wash plate 4x with fresh TBST.
11. Make up Antibody Dilution Buffer: Per 50 ml: Mix 5 ml of 5% BSA, 250 µl of 5% milk and 50 µl of 100mM sodium vanadate, bring to final volume with with

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0.05% TBST.

12. Add 100 µl per well of anti-phosphotyrosine (1:10000 dilution in ADB). Incubate, with shaking for 1 hr. at room temperature.
13. Wash as in step 10.
14. Add 100 µl per well of Biosource Goat anti-rabbit IgG peroxidase conjugate (1:6000 dilution in ADB). Incubate, with shaking for 1 hr. at room temperature.
15. Wash as in step 10 and then with PBS to remove bubbles and excess TWEEN.
16. Add 100 µl of ABTS/H_2O_2 solution to each well.
17. Incubate, with shaking, for 10 to 20 minutes. Remove any bubbles.
18. Read assay on Dynatech MR7000 elisa reader: test filter at 410 nM, reference filterat 630 nM.

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EGFR BIOASSAY

This assay is used to the in vitro kinase activity of FGF1-R in an ELISA assay.

Materials and Reagents:

1. Corning 96-well Elisa plates.
2. SUMO1 monoclonal anti-EGFR antibody (SUGEN, Inc.).
3. PBS
4. TBST Buffer
5. Blocking Buffer: for 100 ml, mix 5.0 g Carnation Instant Non-fat Milk® with 100 ml of PBS.
6. A431 cell lysate (SUGEN, Inc.).
7. TBS Buffer:
8. TBS + 10% DMSO: for 1L, mix 1.514 g TRIS, 2.192 g NaCl and 25 ml DMSO; bring to 1 liter total volume with dH_2O.
9. ATP (Adenosine-5'-triphosphate, from Equine muscle,

Sigma Cat. No. A-5394), 1.0 mM solution in dH_2O. This reagent should be made up immediately prior to use and kept on ice.

10. 1.0 mM $MnCl_2$.
11. $ATP/MnCl_2$ phosphorylation mix: to make 10 ml, mix 300 µl of 1 mM ATP, 500 µl $MnCl_2$ and 9.2 ml dH_2O. Prepare just prior to use, keep on ice.
12. NUNC 96-well V bottom polypropylene plates.
13. EDTA.
14. Rabbit polyclonal anti-phosphotyrosine serum (SUGEN, Inc.).
15. Goat anti-rabbit IgG peroxidase conjugate (Biosource Cat. No. ALI0404).
16. ABTS.
17. 30% Hydrogen peroxide.
18. $ABTS/H_2O_2$.
19. 0.2 M HCl.

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Procedure:

1. Coat Corning 96 well ELISA plates with 0.5 µg SUMO1 in 100 µl PBS per well, store overnight at 4° C.
2. Remove unbound SUMO1 from wells by inverting plate to remove liquid. Wash 1x with dH_2O. Pat the plate on a paper towel to remove excess liquid.
3. Add 150 µl of Blocking Buffer to each well. Incubate, with shaking, for 30 min. at room temperature.
4. Wash plate 3x with deionized water, then once with TBST. Pat plate on a paper towel to remove excess liquid and bubbles.
5. Dilute lysate in PBS (7 µg lysate/100 µl PBS).
6. Add 100 µl of diluted lysate to each well. Shake at

room temperature for 1 hr.

7. Wash plates as in 4, above.
8. Add 120 µl TBS to ELISA plate containing captured EGFR.
9. Dilute test compound 1:10 in TBS, place in well
10. Add 13.5 µl diluted test compound to ELISA plate. To control wells, add 13.5 µl TBS in 10% DMSO.
11. Incubate, with shaking, for 30 minutes at room temperature.
12. Add 15 µl phosphorylation mix to all wells except negative control well. Final well volume should be approximately 150 µl with 3 µM ATP/5 mM $MnCl_2$ final concentration in each well. Incubate with shaking for 5 minutes.
13. Stop reaction by adding 16.5 µl of EDTA solution while shaking. Shake for additional 1 min.
14. Wash 4x with deionized water, 2x with TBST.
15. Add 100 µl anti-phosphotyrosine (1:3000 dilution in TBST) per well. Incubate, with shaking, for 30-45 min. at room temperature.
16. Wash as in 4, above.
17. Add 100 µl Biosource Goat anti-rabbit IgG peroxidase conjugate (1:2000 dilution in TBST) to each well. Incubate with shaking for 30 min. at room temperature.
18. Wash as in 4, above.
19. Add 100 µl of ABTS/H_2O_2 solution to each well.
20. Incubate 5 to 10 minutes with shaking. Remove any bubbles.
21. If necessary, stop reaction by adding 100 µl 0.2 M HCl per well.
22. Read assay on Dynatech MR7000 ELISA reader: test filter

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at 410 nM, reference filter at 630 nM.

PDGFR BIOASSAY

This assay is used to the in vitro kinase activity of FGF1-R in an ELISA assay.

Materials and Reagents:

1. Corning 96-well Elisa plates
2. 28D4C10 monoclonal anti-PDGFR antibody (SUGEN, Inc.).
3. PBS.
4. TBST Buffer.
5. Blocking Buffer (same as for EGFR bioassay).
6. PDGFR-β expressing NIH 3T3 cell lysate (SUGEN, Inc.).
7. TBS Buffer.
8. TBS + 10% DMSO.
9. ATP.
10. $MnCl_2$.
11. Kinase buffer phosphorylation mix: for 10 ml, mix 250 µl 1M TRIS, 200 µl 5M NaCl, 100 µl 1M $MnCl_2$ and 50µl 100 mM Triton X-100 in enough dH_2O to make 10 ml.
12. NUNC 96-well V bottom polypropylene plates.
13. EDTA.
14. Rabbit polyclonal anti-phosphotyrosine serum (SUGEN,Inc.).
15. Goat anti-rabbit IgG peroxidase conjugate (Biosource Cat. No. ALI0404).
16. ABTS.
17. Hydrogen peroxide, 30% solution.
18. ABTS/H_2O_2.
19. 0.2 M HCl.

Procedure:

1. Coat Corning 96 well ELISA plates with 0.5 µg 28D4C10 in 100 µl PBS per well, store overnight at 4° C.

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2. Remove unbound 28D4C10 from wells by inverting plate to remove liquid. Wash 1x with dH_2O. Pat the plate on a paper towel to remove excess liquid.
3. Add 150 µl of Blocking Buffer to each well. Incubate for 30 min. at room temperature with shaking.
4. Wash plate 3x with deionized water, then once with TBST. Pat plate on a paper towel to remove excess liquid and bubbles.
5. Dilute lysate in HNTG (10 µg lysate/100 µl HNTG).
6. Add 100 µl of diluted lysate to each well. Shake at room temperature for 60 min.
7. Wash plates as described in Step 4.
8. Add 80 µl working kinase buffer mix to ELISA plate containing captured PDGFR.
9. Dilute test compound 1:10 in TBS in 96-well polypropylene plates.
10. Add 10 µl diluted test compound to ELISA plate. To control wells, add 10 µl TBS + 10% DMSO. Incubate with shaking for 30 minutes at room temperature.
11. Add 10 µl ATP directly to all wells except negative control well (final well volume should be approximately 100 µl with 20 µM ATP in each well.) Incubate 30 minutes with shaking.
12. Stop reaction by adding 10 µl of EDTA solution to each well.
13. Wash 4x with deionized water, twice with TBST.
14. Add 100 µl anti-phosphotyrosine (1:3000 dilution in TBST) per well. Incubate with shaking for 30-45 min. at room temperature.
15. Wash as in Step 4.

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16. Add 100 µl Biosource Goat anti-rabbit IgG peroxidase conjugate (1:2000 dilution in TBST) to each well. Incubate with shaking for 30 min. at room temperature.
17. Wash as in Step 4.
18. Add 100 µl of ABTS/H_2O_2 solution to each well.
19. Incubate 10 to 30 minutes with shaking. Remove any bubbles.
20. If necessary stop reaction with the addition of 100 µl 0.2 M HCl per well.
21. Read assay on Dynatech MR7000 ELISA reader with test filter at 410 nM and reference filter at 630 nM.

CELLULAR HER-2 KINASE ASSAY

This assay is used to measure HER-2 kinase activity in whole cells in an ELISA format.

Materials and Reagents:

1. DMEM (GIBCO Catalog #11965-092).
2. Fetal Bovine Serum (FBS, GIBCO Catalog #16000-044), heat inactivated in a water bath for 30 min. at 56° C
3. Trypsin (GIBCO Catalog #25200-056).
4. L-Glutamine (GIBCO Catalog #25030-081)
5. HEPES (GIBCO Catalog #15630-080).
6. Growth Media
   Mix 500 ml DMEM, 55 ml heat inactivated FBS, 10 ml HEPES and 5.5 ml L-Glutamine.
7. Starve Media
   Mix 500 ml DMEM, 2.5 ml heat inactivated FBS, 10 ml HEPES and 5.5 ml L-Glutamine.
8. PBS.
9. Flat Bottom 96-well Tissue Culture Micro Titer Plates (Corning Catalog # 25860).

10. 15 cm Tissue Culture Dishes (Corning Catalog #08757148).
11. Corning 96-well ELISA Plates.
12. NUNC 96-well V bottom polypropylene plates.
13. Costar Transfer Cartridges for the Transtar 96 (Costar Catalog #7610).
14. SUMO 1: monoclonal anti-EGFR antibody (SUGEN, Inc.).
15. TBST Buffer.
16. Blocking Buffer : 5% Carnation Instant Milk® in PBS.
17. EGF Ligand: EGF-201, Shinko American, Japan. Suspend powder in 100 uL of 10mM HCl. Add 100uL 10mM NaOH. Add 800 uL PBS and transfer to an Eppendorf tube, store at -20°C until ready to use.
18. HNTG Lysis Buffer
    For Stock 5X HNTG, mix 23.83 g Hepes, 43.83 g NaCl, 500 ml glycerol and 100 ml Triton X-100 and enough dH_2O to make 1 L of total solution.
    For 1X HNTG*, mix 2 ml HNTG, 100 µL 0.1M Na_3VO_4, 250 µL 0.2M $Na_4P_2O_7$ and 100 µL EDTA.
19. EDTA.
20. Na_3VO_4. To make stock solution, mix 1.84 g Na_3VO_4 with 90 ml dH_2O. Adjust pH to 10. Boil in microwave for one minute (solution becomes clear). Cool to room temperature. Adjust pH to 10. Repeat heating/cooling cycle until pH remains at 10.
21. 200 mM $Na_4P_2O_7$.
22. Rabbit polyclonal antiserum specific for phosphotyrosine (anti-Ptyr antibody, SUGEN, Inc.).
23. Affinity purified antiserum, goat anti-rabbit IgG antibody, peroxidase conjugate (Biosource Cat # ALI0404).

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24. ABTS Solution.
25. 30 % Hydrogen peroxide solution.
26. ABTS/H_2O_2.
27. 0.2 M HCl.

Procedure:

1. Coat Corning 96 well ELISA plates with SUMO1 at 1.0 ug per well in PBS, 100 ul final volume/well. Store overnight at 4°C.
2. On day of use, remove coating buffer and wash plate 3 times with dH_2O and once with TBST buffer. All washes in this assay should be done in this manner, unless otherwise specified.
3. Add 100 ul of Blocking Buffer to each well. Incubate plate, with shaking, for 30 min. at room temperature. Just prior to use, wash plate.
4. Use EGFr/HER-2 chimera/3T3-C7 cell line for this assay.
5. Choose dishes having 80-90 % confluence. Collect cells by trypsinization and centrifuge at 1000 rpm at room temperature for 5 min.
6. Resuspend cells in starve medium and count with trypan blue. Viability above 90% is required. Seed cells in starve medium at a density of 2,500 cells per well, 90 ul per well, in a 96 well microtiter plate. Incubate seeded cells overnight at 37° under 5% CO_2.
7. Start the assay two days after seeding.
8. Test compounds are dissolved in 4% DMSO. Samples are then further diluted directly on plates with starve-DMEM. Typically, this dilution will be 1:10 or greater. All wells are then transferred to the cell plate at a further 1:10 dilution (10µl sample and media into 90 µl of starve media. The final DMSO

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concentration should be 1% or lower. A standard serial dilution may also be used.

9. Incubate under 5% CO_2 at 37°C for 2 hours.
10. Prepare EGF ligand by diluting stock EGF (16.5 uM) in warm DMEM to 150 nM.
11. Prepare fresh HNTG* sufficient for 100 ul per well; place on ice.
12. After 2 hour incubation with test compound, add prepared EGF ligand to cells, 50 ul per well, for a final concentration of 50 nM. Positive control wells receive the same amount of EGF. Negative controls do not receive EGF. Incubate at 37° C for 10 min.
13. Remove test compound, EGF, and DMEM. Wash cells once with PBS.
14. Transfer HNTG* to cells, 100 ul per well. Place on ice for 5 minutes. Meanwhile, remove blocking buffer from ELISA plate and wash.
15. Scrape cells from plate with a micropipettor and homogenize cell material by repeatedly aspirating and dispensing the HNTG* lysis buffer. Transfer lysate to a coated, blocked, washed ELISA plate. Or, use a Costar transfer cartridge to transfer lysate to the plate.
16. Incubate, with shaking, at room temperature for 1 hr.
17. Remove lysate, wash. Transfer freshly diluted anti-Ptyr antibody (1:3000 in TBST) to ELISA plate, 100 ul per well.
18. Incubate, with shaking, at room temperature, for 30 min.
19. Remove anti-Ptyr antibody, wash. Transfer freshly diluted BIOSOURCE antibody to ELISA plate(1:8000 in TBST, 100 ul per well).

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20. Incubate, with shaking, at room temperature for 30 min.
21. Remove BIOSOURCE antibody, wash. Transfer freshly prepared ABTS/H_2O_2 solution to ELISA plate, 100 ul per well.
22. Incubate, with shaking, for 5-10 minutes. Remove any bubbles.
23. Stop reaction with the addition of 100ul of 0.2M HCl per well.
24. Read assay on Dynatech MR7000 ELISA reader woth test filter set at 410 nM and reference filter at 630 nM.

CDK2/CYCLIN A ASSAY

This assay is used to measure the <u>in vitro</u> serine/threonine kinase activity of human cdk2/cyclin A in a Scintillation Proximity Assay (SPA).

<u>Materials and Reagents</u>.

1. Wallac 96-well polyethylene terephthalate (flexi) plates (Wallac Catalog # 1450-401).
2. Amersham Redivue [γ^{33}P] ATP (Amersham catalog #AH 9968).
3. Amersham streptavidin coated polyvinyltoluene SPA beads (Amersham catalog #RPNQ0007). The beads should be reconstituted in PBS without magnesium or calcium, at 20 mg/ml.
4. Activated cdk2/cyclin A enzyme complex purified from Sf9 cells (SUGEN, Inc.).
5. Biotinylated peptide substrate (Debtide). Peptide biotin-X-PKTPKKAKKL is dissolved in dH_2O at a concentration of 5 mg/ml.
6. Peptide/ATP Mixture: for 10 ml, mix 9.979 ml dH_2O, 0.00125 ml "cold" ATP, 0.010 ml Debtide and 0.010 ml γ^{33}P ATP. The ultimate concentration per well will be 0.5 µM "cold" ATP, 0.1 µg Debtide and 0.2 µCi γ^{33}P ATP.

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7. Kinase buffer: for 10 ml, mix 8.85 ml dH_2O, 0.625 ml TRIS(pH 7.4), 0.25 ml 1M $MgCl_2$, 0.25 ml 10% NP40 and 0.025 ml 1M DTT, added fresh just prior to use.
8. 10 mM ATP in dH_2O.
9. 1M Tris, pH adjusted to 7.4 with HCl.
10. 1M $MgCl_2$.
11. 1M DTT.
12. PBS (Gibco Catalog # 14190-144).
13. 0.5M EDTA.
14. Stop solution: For 10 ml, mix 9.25 ml PBS, 0.005 ml 100 mM ATP, 0.1 ml 0.5 M EDTA, 0.1 ml 10% Triton X-100 and 1.25 ml of 20 mg/ml SPA beads.

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Procedure:

1. Prepare solutions of test compounds at 5x the desired final concentration in 5% DMSO. Add 10 ul to each well. For negative controls, use 10 ul 5% DMSO alone in wells.
2. Dilute 5 µl of cdk2/cyclin A solution with 2.1 ml 2x kinase buffer.
3. Add 20 ul enzyme to each well.
4. Add 10 µL of 0.5 M EDTA to the negative control wells.
5. To start kinase reaction, add 20 µL of peptide/ATP mixture to each well. Incubate for 1 hr. without shaking.
6. Add 200 µl stop solution to each well.
7. Hold at least 10 min.
8. Spin plate at approx. 2300 rpm for 3-5 min.
9. Count plate using Trilux or similar reader.

MET TRANSPHOSPHORYLATION ASSAY

This assay is used to measure phosphotyrosine levels on a poly(glutamic acid:tyrosine (4:1)) substrate as a means for

identifying agonists/antagonists of met transphosphorylation of the substrate.

Materials and Reagents:

1. Corning 96-well Elisa plates, Corning Catalog # 25805-96.
2. Poly(glu, tyr) 4:1, Sigma, Cat. No; P 0275.
3. PBS, Gibco Catalog # 450-1300EB
4. 50 mM HEPES
5. Blocking Buffer: Dissolve 25 g Bovine Serum Albumin, Sigma Cat. No A-7888, in 500 ml PBS, filter through a 4 µm filter.
6. Purified GST fusion protein containing the Met kinase domain, Sugen, Inc.
7. TBST Buffer.
8. 10% aqueous (MilliQue H_2O) DMSO.
9. 10 mM aqueous (dH_2O) Adenosine-5'-triphosphate, Sigma Cat. No. A-5394.
10. 2X Kinase Dilution Buffer: for 100 ml, mix 10 mL 1M HEPES at pH 7.5 with 0.4 mL 5% BSA/PBS, 0.2 mL 0.1 M sodium orthovanadate and 1 mL 5M sodium chloride in 88.4 mL dH_2O.
11. 4X ATP Reaction Mixture: for 10 mL, mix 0.4 mL 1 M manganese chloride and 0.02 mL 0.1 M ATP in 9.56 mL dH_2O.
12. 4X Negative Controls Mixture: for 10 mL, mix 0.4 mL 1 M manganese chloride in 9.6 mL dH_2O.
13. NUNC 96-well V bottom polypropylene plates, Applied Scientific Catalog # S-72092
14. 500 mM EDTA.
15. Antibody Dilution Buffer: for 100 mL, mix 10 mL 5% BSA/PBS, 0.5 mL 5% Carnation Instant Milk® in PBS and

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0.1 mL 0.1 M sodium orthovanadate in 88.4 mL TBST.

16. Rabbit polyclonal antophosphotyrosine antibody, Sugen, Inc.
17. Goat anti-rabbit horsradish peroxidase conjugatedantibody, Biosource, Inc.
18. ABTS Solution: for 1 L, mix 19.21 g citric acid, 35.49 g Na_2HPO_4 and 500 mg ABTS with sufficient dH_2O to make 1 L.
19. ABTS/H_2O_2: mix 15 mL ABST solution with 2µL H_2O_2 five minutes before use.
20. 0.2 M HCl

Procedure:

1. Coat ELISA plates with 2 µg Poly(Glu-Tyr) in 100 µL PBS, store overnight at 4 ° C.
2. Block plate with 150 µL of 5% BSA / PBS for 60 min.
3. Wash plate twice with PBS, once with 50 mM Hepes buffer pH 7.4.
4. Add 50 µl of the diluted kinase to all wells. (Purified kinase is diluted with Kinase Dilution Buffer. Final concentration should be 10 ng/well.)
5. Add 25 µL of the test compound (in 4% DMSO) or DMSOalone (4% in dH_2O) for controls to plate.
6. Incubate the kinase/compound mixture for 15 minutes.
7. Add 25 µL of 40 mM $MnCl_2$ to the negative control wells.
8. Add 25 µL ATP/ $MnCl_2$ mixture to the all other wells (except the negative controls). Incubate for 5 min.
9. Add 25 µL 500 mM EDTA to stop reaction.
10. Wash plate 3x with TBST.
11. Add 100 µL rabbit polyclonal anti-Ptyr diluted 1:10,000 in Antibody Dilution Buffer to each well. Incubate, with shaking, at room temperature for one hour.

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12. Wash plate 3x with TBST.
13. Dilute Biosource HRP conjugated anti-rabbit antibody 1:6,000 in Antibody Dilution buffer. Add 100 µL per well and incubate at room temperature, with shaking, for one hour.
14. Wash plate 1X with PBS.
15. Add 100 µl of ABTS/H_2O_2 solution to each well.
16. If necessary, stop the development reaction with the addition of 100 µl of 0.2M HCl per well.
17. Read plate on Dynatech MR7000 elisa reader with the test filter at 410 nM and the reference filter at 630 nM.

IGF-1 Transphosphorylation Assay

This assay is used to measure the phosphtyrosine level in poly(glutamic acid:tyrosine)(4:1) for the identification of agonists/antagonists of gst-IGF-1 transphosphorylation of a substrate.

Materials and Reagents:

1. Corning 96-well Elisa plates.
2. Poly (Glu-tyr) (4:1), Sigma Cat. No. P 0275.
3. PBS, Gibco Catalog # 450-1300EB.
4. 50 mM HEPES
5. TBB Blocking Buffer: for 1 L, mix 100 g BSA, 12.1 gTRIS (pH 7.5), 58.44 g sodium chloride and 10 mL 1%TWEEN-20.
6. Purified GST fusion protein containing the IGF-1 kinase domain (Sugen, Inc.)
7. TBST Buffer: for 1 L, mix 6.057 g Tris, 8.766 g sodium chloride and 0.5 ml TWEEN-20 with enough dH_2O to make 1 liter.
8. 4% DMSO in Milli-Q H_2O.
9. 10 mM ATP in dH_2O.

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10. 2X Kinase Dilution Buffer: for 100 mL, mix 10 mL 1 M HEPES (pH 7.5), 0.4 mL 5% BSA in dH_2O, 0.2 mL 0.1 M sodium orthovanadate and 1 mL 5 M sodium chloride with enough dH_2O to make 100 mL.
11. 4X ATP Reaction Mixture: for 10 mL, mix 0.4 mL 1 M $MnCl_2$ and 0.008 mL 0.01 M ATP and 9.56 mL dH_2O.
12. 4X Negative Controls Mixture: mix 0.4 mL 1 M manganese chloride in 9.60 mL dH_2O.
13. NUNC 96-well V bottom polypropylene plates.
14. 500 mM EDTA in dH_2O.
15. Antibody Dilution Buffer: for 100 mL, mix 10 mL 5% BSA in PBS, 0.5 mL 5% Carnation Instant Non-fat Milk® in PBS and 0.1 mL 0.1 M sodium orthovanadate in 88.4 mL TBST.
16. Rabbit Polyclonal antiphosphotyrosine antibody, Sugen, Inc.
17. Goat anti-rabbit HRP conjugated antibody, Biosource.
18. ABTS Solution.
20. ABTS/H_2O_2: mix 15 mL ABTS with 2 µL H_2O_2 5 minutes before using.
21. 0.2 M HCl in dH_2O.

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Procedure:

1. Coat ELISA plate with 2.0 µg / well Poly(Glu, Tyr) 4:1 (Sigma P0275) in 100 µl PBS. Store plate overnight at 4° C.
2. ash plate once with PBS.
3. Add 100 µl of TBB Blocking Buffer to each well. Incubate plate for 1 hour with shaking at room temperature.
4. Wash plate once with PBS, then twice with 50 mM Hepes

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buffer pH 7.5.

5. Add 25 µL of test compound in 4% DMSO (obtained by diluting a stock solution of 10 mM test compound in 100% DMSO with dH_2O) to plate.

6. Add 10.0 ng of gst-IGF-1 kinase in 50 µl Kinase Dilution Buffer) to all wells.

7. Start kinase reaction by adding 25µl 4X ATP Reaction Mixture to all test wells and positive control wells. Add 25µl 4X Negative Controls Mixture to all negative control wells. Incubates for 10 minutes with shaking at room temperature.

8. Add 25µl 0.5M EDTA (pH 8.0) to all wells.

9. Wash plate 4x with TBST Buffer.

10. Add rabbit polyclonal anti-phosphotyrosine antisera at a dilution of 1:10,000 in 100µl Antibody Dilution Buffer to all wells. Incubate, with shaking, at room temperature for 1 hour.

11. Wash plate as in step 9.

12. Add 100 µL Biosource anti-rabbit HRP at a dilution of 1:10,000 in Antibody dilution buffer to all wells. Incubate, with shaking, at room temperature for 1 hour.

13. Wash plate as in step 9, follow with one wash with PBS to reduce bubbles and excess Tween-20.

14. Develop by adding 100µl/well ABTS/H_2O_2 to each well.

15. After about 5 minutes, read on ELISA reader with test filter at 410 nm and referenced filter at 630 nm.

BRDU INCORPORATION ASSAYS

The following assays use cells engineered to express a selected receptor and then evaluate the effect of a compound of interest on the activity of ligand-induced DNA synthesis by

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determining BrdU incorporation into the DNA.

The following materials, reagents and procedure are general to each of the following BrdU incorporation assays. Variances in specific assays are noted.

Materials and Reagents:

1. The appropriate ligand.
2. The appropriate engineered cells.
3. BrdU Labeling Reagent: 10 mM, in PBS (pH7.4)(Boehringer Mannheim, Germany).
4. FixDenat: fixation solution (ready to use)(Boehringer Mannheim, Germany).
5. Anti-BrdU-POD: mouse monoclonal antibody conjugated with peroxidase (Boehringer Mannheim, Germany).
6. TMB Substrate Solution: tetramethylbenzidine (TMB, Boehringer Mannheim, Germany).
7. PBS Washing Solution : 1X PBS, pH 7.4.
8. Albumin, Bovine (BSA), fraction V powder (Sigma Chemical Co., USA).

General Procedure:

1. Cells are seeded at 8000 cells/well in 10% CS, 2mM Gln in DMEM, in a 96 well plate. Cells are incubated overnight at 37°C in 5% CO_2.
2. After 24 hours, the cells are washed with PBS, and then are serum-starved in serum free medium (0%CS DMEM with 0.1% BSA) for 24 hours.
3. On day 3, the appropriate ligand and the test compound are added to the cells simultaneously. The negative control wells receive serum free DMEM with 0.1% BSA only; the positive control cells receive the ligand but no test compound. Test compounds are prepared in serum free DMEM with ligand in a 96 well plate, and serially

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diluted for 7 test concentrations.

4. After 18 hours of ligand activation, diluted BrdU labeling reagent (1:100 in DMEM, 0.1% BSA) is added and the cells are incubated with BrdU (final concentration=10 μM) for 1.5 hours.

5. After incubation with labeling reagent, the medium is removed by decanting and tapping the inverted plate on a paper towel. FixDenat solution is added (50 μl/well) and the plates are incubated at room temperature for 45 minutes on a plate shaker.

6. The FixDenat solution is thoroughly removed by decanting and tapping the inverted plate on a paper towel. Milk is added (5% dehydrated milk in PBS, 200 μl/well) as a blocking solution and the plate is incubated for 30 minutes at room temperature on a plate shaker.

7. The blocking solution is removed by decanting and the wells are washed once with PBS. Anti-BrdU-POD solution (1:200 dilution in PBS, 1% BSA) is added (50 μl/well) and the plate is incubated for 90 minutes at room temperature on a plate shaker.

8. The antibody conjugate is thoroughly removed by decanting and rinsing the wells 5 times with PBS, and the plate is dried by inverting and tapping on a paper towel.

9. TMB substrate solution is added (100 μl/well) and incubated for 20 minutes at room temperature on a plate shaker until color development is sufficient for photometric detection.

10. The absorbance of the samples are measured at 410 nm (in "dual wavelength" mode with a filter reading at 490

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nm, as a reference wavelength) on a Dynatech ELISA plate reader.

EGF-Induced BrdU Incorporation Assay

Materials and Reagents:

1. Mouse EGF, 201 (Toyobo Co., Ltd., Japan).
2. 3T3/EGFRc7.

EGF-Induced Her-2-driven BrdU Incorporation Assay

Materials and Reagents:

1. Mouse EGF, 201 (Toyobo Co., Ltd., Japan).
2. 3T3/EGFr/Her2/EGFr (EGFr with a Her-2 kinase domain).

EGF-Induced Her-4-driven BrdU Incorporation Assay

Materials and Reagents:

1. Mouse EGF, 201 (Toyobo Co., Ltd., Japan).
2. 3T3/EGFr/Her4/EGFr (EGFr with a Her-4 kinase domain).

PDGF-Induced BrdU Incorporation Assay

Materials and Reagents:

1. Human PDGF B/B (Boehringer Mannheim, Germany).
2. 3T3/EGFRc7.

FGF-Induced BrdU Incorporation Assay

Materials and Reagents:

1. Human FGF2/bFGF (Gibco BRL, USA).
2. 3T3c7/EGFr

IGF1-Induced BrdU Incorporation Assay

Materials and Reagents:

1. Human, recombinant (G511, Promega Corp., USA)
2. 3T3/IGF1r.

Insulin-Induced BrdU Incorporation Assay

Materials and Reagents:

1. Insulin, crystalline, bovine, Zinc (13007, Gibco BRL, USA).
2. 3T3/H25.

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HGF-Induced BrdU Incorporation Assay

Materials and Reagents:

1. Recombinant human HGF (Cat. No. 249-HG, R&D Systems, Inc. USA).
2. BxPC-3 cells (ATCC CRL-1687).

Procedure:

1. Cells are seeded at 9000 cells/well in RPMI 10% FBS in a 96 well plate. Cells are incubated overnight at 37°C in 5% CO_2.
2. After 24 hours, the cells are washed with PBS, and then are serum starved in 100 µl serum-free medium (RPMI with 0.1% BSA) for 24 hours.
3. On day 3, 25 µl containing ligand (prepared at 1 µg/ml in RPMI with 0.1% BSA; final HGF conc. is 200 ng/ml) and test compounds are added to the cells. The negative control wells receive 25 µl serum-free RPMI with 0.1% BSA only; the positive control cells receive the ligand (HGF) but no test compound. Test compounds are prepared at 5 times their final concentration in serum-free RPMI with ligand in a 96 well plate, and serially diluted to give 7 test concentrations. Typically, the highest final concentration of test compound is 100 µM, and 1:3 dilutions are used (i.e. final test compound concentration range is 0.137-100 µM).
4. After 18 hours of ligand activation, 12.5 µl of diluted BrdU labeling reagent (1:100 in RPMI, 0.1% BSA) is added to each well and the cells are incubated with BrdU (final concentration is 10 µM) for 1 hour.
5. Same as General Procedure.

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6. Same as General Procedure.

7. The blocking solution is removed by decanting and the wells are washed once with PBS. Anti-BrdU-POD solution (1:100 dilution in PBS, 1% BSA) is added (100 µl/well) and the plate is incubated for 90 minutes at room temperature on a plate shaker.

8. Same as General Procedure.

9. Same as General Procedure.

10. Same as General Procedure.

HUV-EC-C Assay

This assay is used to measure a compound's activity against PDGF-R, FGF-R, VEGF, aFGF or Flk-1/KDR, all of which are naturally expressed by HUV-EC cells.

DAY 0

1. Wash and trypsinize HUV-EC-C cells (human umbilical vein endothelial cells, (American Type Culture Collection, catalogue no. 1730 CRL). Wash with Dulbecco's phosphate-buffered saline (D-PBS, obtained from Gibco BRL, catalogue no. 14190-029) 2 times at about 1 ml/10 cm^2 of tissue culture flask. Trypsinize with 0.05% trypsin-EDTA in non-enzymatic cell dissociation solution (Sigma Chemical Company, catalogue no. C-1544). The 0.05% trypsin is made by diluting 0.25% trypsin/1 mM EDTA (Gibco, catalogue no. 25200-049) in the cell dissociation solution. Trypsinize with about 1 ml/25-30 cm^2 of tissue culture flask for about 5 minutes at 37°C. After cells have detached from the flask, add an equal volume of assay medium and transfer to a 50 ml sterile centrifuge tube (Fisher Scientific, catalogue no. 05-539-6).

2. Wash the cells with about 35 ml assay medium in the 50 ml sterile centrifuge tube by adding the assay medium, centrifuge for 10 minutes at approximately 200x g, aspirate the supernatant,

and resuspend with 35 ml D-PBS. Repeat the wash two more times with D-PBS, resuspend the cells in about 1 ml assay medium/15 cm^2 of tissue culture flask. Assay medium consists of F12K medium (Gibco BRL, catalogue no. 21127-014) and 0.5% heat-inactivated fetal bovine serum. Count the cells with a Coulter Counter® (Coulter Electronics, Inc.) and add assay medium to the cells to obtain a concentration of 0.8-1.0 x 10^5 cells/ml.

3. Add cells to 96-well flat-bottom plates at 100 µl/well or 0.8-1.0 x 10^4 cells/well, incubate ~24h at 37°C, 5% CO_2.

DAY 1

1. Make up two-fold test compound titrations in separate 96-well plates, generally 50 µM on down to 0 µM. Use the same assay medium as mentioned in day 0, step 2 above. Titrations are made by adding 90 µl/well of test compound at 200 µM (4X the final well concentration) to the top well of a particular plate column. Since the stock test compound is usually 20 mM in DMSO, the 200 µM drug concentration contains 2% DMSO.

A diluent made up to 2% DMSO in assay medium (F12K + 0.5% fetal bovine serum) is used as diluent for the test compound titrations in order to dilute the test compound but keep the DMSO concentration constant. Add this diluent to the remaining wells in the column at 60 µl/well. Take 60 µl from the 120 µl of 200 µM test compound dilution in the top well of the column and mix with the 60 µl in the second well of the column. Take 60 µl from this well and mix with the 60 µl in the third well of the column, and so on until two-fold titrations are completed. When the next-to-the-last well is mixed, take 60 µl of the 120 µl in this well and discard it. Leave the last well with 60 µl of DMSO/media diluent as a non-test compound-containing control. Make 9 columns of titrated test compound, enough for triplicate

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wells each for: (1) VEGF (obtained from Pepro Tech Inc., catalogue no. 100-200, (2) endothelial cell growth factor (ECGF) (also known as acidic fibroblast growth factor, or aFGF) (obtained from Boehringer Mannheim Biochemica, catalogue no. 1439 600), or, (3) human PDGF B/B (1276-956, Boehringer Mannheim, Germany) and assay media control. ECGF comes as a preparation with sodium heparin.

2. Transfer 50 µl/well of the test compound dilutions to the 96-well assay plates containing the 0.8-1.0x10^4 cells/100 µl/well of the HUV-EC-C cells from day 0 and incubate ~2 h at 37° C, 5% CO_2.

3. In triplicate, add 50 µl/well of 80 µg/ml VEGF, 20 ng/ml ECGF, or media control to each test compound condition. As with the test compounds, the growth factor concentrations are 4X the desired final concentration. Use the assay media from day 0 step 2 to make the concentrations of growth factors. Incubate approximately 24 hours at 37°C, 5% CO_2. Each well will have 50 µl test compound dilution, 50 µl growth factor or media, and 100 µl cells, which calculates to 200 µl/well total. Thus the 4X concentrations of test compound and growth factors become 1X once everything has been added to the wells.

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DAY 2

1. Add ^{3}H-thymidine (Amersham, catalogue no. TRK-686) at 1 µCi/well (10 µl/well of 100 µCi/ml solution made up in RPMI media + 10% heat-inactivated fetal bovine serum) and incubate ~24 h at 37°C, 5% CO_2. RPMI is obtained from Gibco BRL, catalogue no. 11875-051.

DAY 3

1. Freeze plates overnight at -20°C.

DAY 4

Thaw plates and harvest with a 96-well plate harvester (Tomtec Harvester 96®) onto filter mats (Wallac, catalogue no. 1205-401), read counts on a Wallac Betaplate™ liquid scintillation counter.

In Vivo Animal Models

XENOGRAFT ANIMAL MODELS

The ability of human tumors to grow as xenografts in athymic mice (*e.g.*, Balb/c, nu/nu) provides a useful *in vivo* model for studying the biological response to therapies for human tumors. Since the first successful xenotransplantation of human tumors into athymic mice; (Rygaard and Povlsen, 1969, *Acta Pathol. Microbial. Scand.* 77:758-760), many different human tumor cell lines (*e.g.*, mammary, lung, genitourinary, gastro-intestinal, head and neck, glioblastoma, bone, and malignant melanomas) have been transplanted and successfully grown in nude mice. The following assays may be used to determine the level of activity, specificity and effect of the different compounds of the present invention. Three general types of assays are useful for evaluating compounds: cellular/catalytic, cellular/biological and *in vivo*. The object of the cellular/catalytic assays is to determine the effect of a compound on the ability of a TK to phosphorylate tyrosines on a known substrate in a cell. The object of the cellular/biological assays is to determine the effect of a compound on the biological response stimulated by a TK in a cell. The object of the *in vivo* assays is to determine the effect of a compound in an animal model of a particular disorder such as cancer.

Suitable cell lines for subcutaneous xenograft experiments include C6 cells (glioma, ATCC # CCL 107), A375 cells (melanoma, ATCC # CRL 1619), A431 cells (epidermoid carcinoma, ATCC # CRL 1555), Calu 6 cells (lung, ATCC # HTB 56), PC3 cells (prostate,

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ATCC # CRL 1435), SKOV3TP5 cells and NIH 3T3 fibroblasts genetically engineered to overexpress EGFR, PDGFR, IGF-1R or any other test kinase. The following protocol can be used to perform xenograft experiments:

Female athymic mice (BALB/c, nu/nu) are obtained from Simonsen Laboratories (Gilroy, CA). All animals are maintained under clean-room conditions in Micro-isolator cages with Alpha-dri bedding. They receive sterile rodent chow and water ad libitum.

Cell lines are grown in appropriate medium (for example, MEM, DMEM, Ham's F10, or Ham's F12 plus 5% - 10% fetal bovine serum (FBS) and 2 mM glutamine (GLN)). All cell culture media, glutamine, and fetal bovine serum are purchased from Gibco Life Technologies (Grand Island, NY) unless otherwise specified. All cells are grown in a humid atmosphere of 90-95% air and 5-10% CO_2 at 37°C. All cell lines are routinely subcultured twice a week and are negative for mycoplasma as determined by the Mycotect method (Gibco).

Cells are harvested at or near confluency with 0.05% Trypsin-EDTA and pelleted at 450 x g for 10 min. Pellets are resuspended in sterile PBS or media (without FBS) to a particular concentration and the cells are implanted into the hindflank of the mice (8 - 10 mice per group, 2 - 10 x 10^6 cells/animal). Tumor growth is measured over 3 to 6 weeks using venier calipers. Tumor volumes are calculated as a product of length x width x height unless otherwise indicated. P values are calculated using the Students t-test. Test compounds in 50 - 100 µL excipient (DMSO, or VPD:D5W) can be delivered by IP injection at different concentrations generally starting at day one after implantation.

TUMOR INVASION MODEL

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The following tumor invasion model has been developed and may be used for the evaluation of therapeutic value and efficacy of the compounds identified to selectively inhibit KDR/FLK-1 receptor.

Procedure

8 week old nude mice (female) (Simonsen Inc.) are used as experimental animals. Implantation of tumor cells can be performed in a laminar flow hood. For anesthesia, Xylazine/Ketamine Cocktail (100 mg/kg ketamine and 5 mg/kg Xylazine) are administered intraperitoneally. A midline incision is done to expose the abdominal cavity (approximately 1.5 cm in length) to inject 10^7 tumor cells in a volume of 100 µl medium. The cells are injected either into the duodenal lobe of the pancreas or under the serosa of the colon. The peritoneum and muscles are closed with a 6-0 silk continuous suture and the skin is closed by using wound clips. Animals are observed daily.

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Analysis

After 2-6 weeks, depending on gross observations of the animals, the mice are sacrificed, and the local tumor metastases to various organs (lung, liver, brain, stomach, spleen, heart, muscle) are excised and analyzed (measurement of tumor size, grade of invasion, immunochemistry, in situ hybridization determination, etc.).

Additional assays

Additional assays which may be used to evaluate the compounds of this invention include, without limitation, a bio-flk-1 assay, an EGF receptor-HER2 chimeric receptor assay in whole cells, a bio-src assay, a bio-lck assay and an assay measuring the phosphorylation function of raf. The protocols for each of these assays may be found in U. S. Application Ser.

No. 09/099,842, which is incorporated by reference, including any drawings, herein.

Measurement of Cell Toxicity

Therapeutic compounds should be more potent in inhibiting receptor tyrosine kinase activity than in exerting a cytotoxic effect. A measure of the effectiveness and cell toxicity of a compound can be obtained by determining the therapeutic index, i.e., IC_{50}/LD_{50}. IC_{50}, the dose required to achieve 50% inhibition, can be measured using standard techniques such as those described herein. LD_{50}, the dosage which results in 50% toxicity, can also be measured by standard techniques as well (Mossman, 1983, J. Immunol. Methods, 65:55-63), by measuring the amount of LDH released (Korzeniewski and Callewaert, 1983, J. Immunol. Methods, 64:313, Decker and Lohmann-Matthes, 1988, J. Immunol. Methods, 115:61), or by measuring the lethal dose in animal models. Compounds with a large therapeutic index are preferred. The therapeutic index should be greater than 2, preferably at least 10, more preferably at least 50.

B. Examples - Biological Activity.

Examples of the in vitro potency of compounds of this invention are shown in Table 2.

CONCLUSION

It will be appreciated that the compounds, methods and pharmaceutical compositions of the present invention are effective in modulating PK activity and therefore are expected to be effective as therapeutic agents against RTK, CTK-, and STK-related disorders.

One skilled in the art would also readily appreciate that the present invention is well adapted to carry out the objects and obtain the ends and advantages mentioned, as well as those inherent herein. The molecular complexes and the methods,

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procedures, treatments, molecules, specific compounds described herein are presently representative of preferred embodiments, are exemplary, and are not intended as limitations on the scope of the invention. Changes therein and other uses will occur to those skilled in the art which are encompassed within the spirit of the invention are defined by the scope of the claims.

It will be readily apparent to one skilled in the art that varying substitutions and modifications may be made to the invention disclosed herein without departing from the scope and spirit of the invention.

All patents and publications mentioned in the specification are indicative of the levels of those skilled in the art to which the invention pertains. All patents and publications are herein incorporated by reference to the same extent as if each individual publication was specifically and individually indicated to be incorporated by reference.

The invention illustratively described herein suitably may be practiced in the absence of any element or elements, limitation or limitations which is not specifically disclosed herein. Thus, for example, in each instance herein any of the terms "comprising", "consisting essentially of" and "consisting of" may be replaced with either of the other two terms. The terms and expressions which have been employed are used as terms of description and not of limitation, and there is no intention that in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the invention claimed. Thus, it should be understood that although the present invention has been specifically disclosed by preferred embodiments and optional features, modification and variation of the concepts herein

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disclosed may be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this invention as defined by the appended claims.

In addition, where features or aspects of the invention are described in terms of Markush groups, those skilled in the art will recognize that the invention is also thereby described in terms of any individual member or subgroup of members of the Markush group. For example, if X is described as selected from the group consisting of bromine, chlorine, and iodine, claims for X being bromine and claims for X being bromine and chlorine are fully described.

Other embodiments are within the following claims.

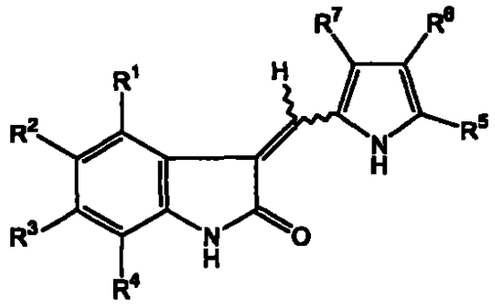
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CLAIMS

WHAT IS CLAIMED:

1. A compound having the chemical structure:



wherein,

R^1 is selected from the group consisting of hydrogen, halo, alkyl, cyclkoalkyl, aryl, heteroaryl, heteroalicyclic, hydroxy, alkoxy, $-C(O)R^{15}$, $-NR^{13}R^{14}$, $-(CH_2)_rR^{16}$ and $-C(O)NR^8R^9$;

R^2 is selected from the group consisting of hydrogen, halo, alkyl, trihalomethyl, hydroxy, alkoxy, $-NR^{13}R^{14}$, N-amido, $C(O)R^{15}$, aryl, heteroaryl and $-S(O)_2NR^{13}R^{14}$;

R^3 is selected from the group consisting of hydrogen, halo, alkyl, trihalomethyl, hydroxy, alkoxy, $-C(O)R^{15}$, $-NR^{13}R^{14}$, aryl, heteroaryl, N-sulfonamido, S-sulfonamido, N-amido and N-carbamyl;

R^4 is selected from the group consisting of hydrogen, halogen, alkyl, hydroxy, alkoxy and $-NR^{13}R^{14}$;

R^5 is selected from the group consisting of hydrogen, alkyl and $-C(O)R^{10}$;

R^6 is selected from the group consisting of hydrogen, alkyl and $-C(O)R^{10}$;

R^7 is selected from the group consisting of hydrogen, alkyl, aryl, heteroaryl, $-C(O)R^{17}$ and $-C(O)R^{10}$;

R^6 and R^7 may combine to form a group selected from the group consisting of $-(CH_2)_4-$, $-(CH_2)_5-$ and $-(CH_2)_6-$;

with the proviso that at least one of R^5, R^6 or R^7 must be $-C(O)R^{10}$;

R^8 and R^9 are independently selected from the group consisting of hydrogen, alkyl and aryl;

R^{10} is selected from the group consisting of hydroxy, alkoxy, aryloxy, $-N(R^{11})(CH_2)_nR^{12}$ and $-NR^{13}R^{14}$;

R^{11} is selected from the group consisting of hydrogen and alkyl;

R^{12} is selected from the group consisting of $-NR^{13}R^{14}$, hydroxy, $-C(O)R^{15}$, aryl and heteroaryl;

R^{13} and R^{14} are independently selected from the group consisting of hydrogen, alkyl, cycloalkyl, aryl and heteroaryl;

R^{13} and R^{14} may combine to form a group selected from the group consisting of $-(CH_2)_4-$, $-(CH_2)_5-$, $-(CH_2)_2O(CH_2)_2-$, and

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R^{13} and R^{14} may combine to form a group selected from the group consisting of $-(CH_2)_4-$, $-(CH_2)_5-$, $-(CH_2)_2O(CH_2)_2-$, and $-(CH_2)_2N(CH_3)(CH_2)_2-$;

R^{15} is selected from the group consisting of hydrogen, hydroxy, alkoxy and aryloxy;

R^{16} is selected from the group consisting of hydroxy, $-C(O)R^{15}$, $-NR^{13}R^{14}$ and $C(O)NR^{13}R^{14}$;

R^{17} is selected from the group consisting of alkyl, cycloalkyl, aryl and heteroaryl; and,

n and r are independently 1, 2, 3, or 4; or,

a pharmaceutically acceptable salt of said compound.

2. The compound or salt of claim 1, wherein

R^1 and R^4 are hydrogen;

R^2 is selected from the group consisting of hydrogen, halo, lower alkoxy, $-C(O)R^{15}$ and $-S(O)_2NR^{13}R^{14}$;

R^3 is selected from the group consisting of hydrogen, lower alkoxy, $-C(O)R^{15}$, $-S(O)_2NR^{13}R^{14}$, aryl and heteroaryl;

R^5 is $-C(O)R^{16}$;

R^6 is selected from the group consisting of hydrogen and lower alkyl; and,

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R^7 is selected from the group consisting of hydrogen, lower alkyl and $-C(O)R^{17}$.

3. The compound or salt of claim 2, wherein:

R^{10} is selected from the group consisting of hydroxy, lower alkoxy and $-NR^{11}(CH_2)_nR^{12}$, wherein:

n is 2 or 3;

R^{11} is selected from the group consisting of hydrogen and lower alkyl; and,

R^{12} is selected from the group consisting of aryl and $-NR^{13}R^{14}$.

4. The compound of claim 3, wherein R^{13} and R^{14} are independently selected from the group consisting of hydrogen, lower alkyl, and, combined, $-(CH_2)_4-$, $-(CH_2)_5-$, $-(CH_2)_2O(CH_2)_2-$ or $-(CH_2)_2N(CH_3)(CH_2)_2-$.

5. The compound or salt of claim 1, wherein,

R^1 is selected from the group consisting of hydrogen, lower alkyl, $(CH_2)_nR^{16}$ and $-C(O)NR^8R^9$;

R^2 is selected from the group consisting of hydrogen, halogen, aryl and $-S(O)_2NR^{13}R^{14}$;

R^3 is selected from the group consisting of hydrogen, lower alkyl, lower alkoxy, aryl, heteroaryl and $-C(O)R^{15}$;

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R^4 is hydrogen;

R^5 is selected from the group consisting of hydrogen and lower alkyl;

R^6 is $-C(O)R^{10}$;

R^7 is selected from the group consisting of hydorgen, lower alkyl and aryl;

R^{16} is selected from the group consisting of hydroxy and $-C(O)R^{15}$; and,

r is 2 or 3.

6. The compound or salt of claim 5 wherein R^3 is aryl optionally substituted with one or more groups selected from the group consisting of lower alkyl, lower alkoxy and halo.

7. The compound or salt of claim 5 wherein R^{10} is selected from the group consisting of hydroxy, lower alkoxy, $-NR^{13}R^{14}$ and $-NR^{11}(CH_2)_nR^{12}$, wherein

n is 1, 2 or 3;

R^{11} is hydrogen; and,

R^{12} is selected from the group consisting of hydroxy, lower alkoxy, $-C(O)R^{15}$, heteroaryl and $-NR^{13}R^{14}$.

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8. The compound of claim 7 wherein R^{13} and R^{14} are independently selected from the group consisting of hydrogen, lower alkyl, heteroaryl and, combined, $-(CH_2)_4-$, $-(CH_2)_5-$, $-(CH_2)_2O(CH_2)_2-$, or $-(CH_2)_2N(CH_3)(CH_2)_2-$.

9. The compound or salt of claim 5, wherein R^1 is $-C(O)NR^8R^9$, wherein:

R^8 is hydrogen; and,

R^9 is aryl optionally substituted with one or more groups selected from the group consisting of halo, hydroxy and lower alkoxy.

10. The compound of claim 1, wherein:

R^1 is selected from the group consisting of hydrogen and lower alkyl;

R^2 is selected from the group consisting of hydrogen, halo, lower alkoxy, aryl, $-C(O)R^{15}$ and $-S(O)_2NR^{13}R^{14}$;

R^3 is selected from the group consisting of hydrogen, halo, aryl, heteroaryl and $-C(O)R^{15}$;

R^4 is hydrogen;

R^5 is $-C(O)R^{10}$; and,

R^6 and R^7 combine to form a $-(CH_2)_4-$ group.

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11. The compound of claim 10, wherein:

R^{10} is selected from the group consisting of hydroxy, alkoxy, $-NR^{13}R^{14}$ and $-NH(CH_2)_nNR^{13}R^{14}$; and

n is 2 or 3.

12. The compound of claim 11 wherein R^{13} and R^{14} are independently selected from the group consisting of hydrogen, lower alkyl, and, combined, $-(CH_2)_4-$, $-(CH_2)_5-$, $-(CH_2)_2O(CH_2)_2-$ or $-(CH_2)_2N(CH_3)(CH_2)_2-$.

13. The compound of claim 1, wherein:

R^1 and R^4 are selected from the group consisting of hydrogen, lower alkyl, lower alkoxy and halogen;

R^2 is selected from the group consisting of hydrogen, lower alkyl, lower alkoxy, halo, and $-S(O)_2NR^{13}R^{14}$;

R^3 is selected from the group consisting of hydrogen, lower alkyl, lower alkoxy, halo, aryl and $-C(O)R^{15}$;

R^5 and R^6 are selected from the group consisting of hydrogen and lower alkyl; and,

R^7 is $-C(O)R^{10}$.

14. The compound of claim 13, wherein:

R^{10} is selected from the group consisting of hydroxy, alkoxy, $-NR^{13}R^{14}$ and $-NR^{11}(CH_2)_nR^{12}$, wherein

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n is 2 or 3;

R^{11} is selected from the group consisting of hydrogen and lower alkyl; and,

R^{12} is $-NR^{13}R^{14}$, wherein

R^{13} and R^{14} are independently selected from the group consisting of hydrogen, lower alkyl, and, combined, $-(CH_2)_4-$, $-(CH_2)_5-$, $-(CH_2)_2O(CH_2)_2-$ or $-(CH_2)_2N(CH_3)(CH_2)_2-$.

15. A pharmaceutical composition, comprising a compound or salt of claim 1; and,

a pharmaceutically acceptable carrier or excipient.

16. A method for the modulation of the catalytic activity of a protein kinase comprising contacting said protein kinase with a compound or salt of claim 1.

17. The method of claim 16 wherein said protein kinase is selected from the group consisting of a receptor tyrosine kinase, a non-receptor tyrosine kinase and a serine-threonine kinase.

18. A method for treating or preventing a protein kinase related disorder in an organism comprising administering a therapeutically effective amount of a compound or salt of claim 1 to said organism.

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19. The method of claim 18 wherein said protein kinase related disorder is selected from the group consisting of a receptor tyrosine kinase related disorder, a non-receptor tyrosine kinase related disorder and a serine-threonine kinase related disorder.

20. The method of claim 18 wherein said protein kinase related disorder is selected from the group consisting of an EGFR related disorder, a PDGFR related disorder, an IGFR related disorder and a flk related disorder.

21. The method of claim 18 wherein said protein kinase related disorder is a cancer selected from the group consisting of squamous cell carcinoma, astrocytoma, Kaposi's sarcoma, glioblastoma, lung cancer, bladder cancer, head and neck cancer, melanoma, ovarian cancer, prostate cancer, breast cancer, small-cell lung cancer, glioma, colorectal cancer, genitourinary cancer and gastrointestinal cancer.

22. The method of claim 18 wherein said protein kinase related disorder is selected from the group consisting of diabetes, an autoimmune disorder, a hyperproliferation disorder, restenosis, fibrosis, psoriasis, von Heppel-Lindau disease, osteoarthritis, rheumatoid arthritis, angiogenesis, an inflammatory disorder, an immunological disorder and a cardiovascular disorder.

23. The method of claim 18 wherein said organism is a human.

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ABSTRACT OF THE DISCLOSURE

The present invention relates to pyrrole substituted 2-indolinone compounds and their physiologically acceptable salts which modulate the activity of protein kinases and therefore are expected to be useful in the prevention and treatment of protein kinase related cellular disorders such as cancer.

TRADUCCIÓN

ESTADOS UNIDOS DE AMÉRICA

A quien corresponda:

DEPARTAMENTO DE COMERCIO - OFICINA DE PATENTES Y MARCAS

30 de marzo del 2001

CERTIFICO QUE ADJUNTA A LA PRESENTE SE ENCUENTRA UNA COPIA FIEL DE LOS REGISTROS EN LA OFICINA DE PATENTES Y MARCAS DE LOS ESTADOS UNIDOS DE LOS DOCUMENTOS DE LA SOLICITUD DE PATENTE ABAJO IDENTIFICADA QUE REUNIAN LOS REQUISITOS PARA LA ASIGNACIÓN DE UNA FECHA DE PRESENTACIÓN EN VIRTUD DE 35 USC 111.

SOLICITUD NUMERO: 60/216.422

FECHA DE DEPÓSITO: 06 de julio del 2000 del 2000

.En nombre del COMISARIO DE PATENTES Y MARCAS.

(Firmado) E. Bornett Certificaciones.

(La presente certificación se encuentra unida en forma inviolable a sus adjuntos y lleva una oblea dorada que dice): OFICINA DE PATENTES Y MARCAS - ESTADOS UNIDOS DE NORTEAMÉRICA.

Carátula para Presentación Provisional de Patente

Esta solicitud es para presentación de una SOLICITUD DE PATENTE PROVISIONAL bajo el CRF 37, Art. 1.53 (c)

INVENTOR/ES SOLICITANTE/S

Nombre, Inicial y Apellido	Residencia (Ciudad y Estado o País Extranjero)
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Chung Chen Wei	Foster City, California
Sheri Shirazian	Corte Madera, California

Título de la invención : 2- INDOLINONAS SUSTITUIDAS CON PIRROLES INHIBIDORAS DE PROTEINQUINASAS

Dirección Postal: Nro. de cliente: 22249

Adjuntos:

Memoria descriptiva: 174 páginas

Forma de pago:

Se autoriza al Comisario a cobrar los aranceles de presentación a través de la cuenta número : 12-2475. Arancel : $150,00

La invención no fue hecha por una agencia del Gobierno de los Estados Unidos ni por contrato con una agencia del Gobierno de los Estados Unidos.

Atentamente, firmado Michael A Whittaker, Nro. de registro: 46.230, para Richard J Warburg

Fecha: 06 de julio del 2000

Teléfono: (858) 720-25000

Nro. de expediente: 030597.01780,PRV2

Hay un certificado de correo expreso con el nro. EL542253548US, la fecha 06 de julio del 2000 y firmado por: Gretchen Dieckmann.

SOLICITUD PROVISIONAL

Bajo 37 CFR art. 1.53(C)

Título: 2- INDOLINONAS SUSTITUIDAS CON PIRROLES INHIBIDORAS DE PROTEINQUINASAS

Solicitantes: Peng Cho Tang, Todd Miller, Xiaoyuan Li, Connie Sun, Chung Chen Wei y Sheri Shirazian

Adjuntos: 1 página de Carátula para Presentación Provisional de Patente; 1 página de Carátula; 174 páginas de memoria descriptiva, 12 páginas de reivindicaciones; 1 página de resumen; un cheque nro. 50529 por $ 150,00: y un aviso de retorno.

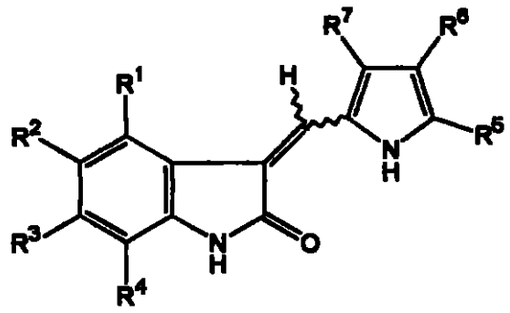
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REIVINDICACIONES

1. Un compuesto que tiene la estructura química:

das con pirrol que tienen la siguiente estructura química:



donde:

R^1 se selecciona del grupo formado por hidrógeno, halo, alquilo, cicloalquilo, arilo, heteroarilo, heteroalicíclico, hidroxi, alcoxi, -(CO)R^{15}, -$NR^{13}R^{14}$, -$(CH_2)_rR^{16}$ y -C(O)NR^8R^9;

R^2 se selecciona del grupo formado por hidrógeno, halo, alquilo, trihalometilo, hidroxi, alcoxi, -$NR^{13}R^{14}$, -NR^{13}C(O)R^{14}, -C(O)R^{15}, arilo, heteroarilo, y –$S(O)_2NR^{13}R^{14}$;

R^3 se selecciona del grupo formado por hidrógeno, halógeno, alquilo, trihalometilo, hidroxi, alcoxi, -(CO)R^{15}, -$NR^{13}R^{14}$, arilo, heteroarilo, -$NR^{13}S(O)_2R^{14}$, -$S(O)_2NR^{13}R^{14}$, -NR^{13}C(O)R^{14}, y –NR^{13}C(O)OR^{14};

R^4 se selecciona del grupo formado por hidrógeno, halógeno, alquilo, hidroxi, alcoxi y –$NR^{13}R^{14}$;

R^5 se selecciona del grupo formado por hidrógeno, alquilo y -C(O)R^{10};

R^6 se selecciona del grupo formado por hidrógeno, alquilo y -C(O)R^{10};

R^7 se selecciona del grupo formado por hidrógeno, alquilo, arilo,

heteroarilo, $-C(O)R^{17}$ y $-C(O)R^{10}$;

R^6 y R^7 se pueden combinar para formar un grupo seleccionado del grupo formado por $-(CH_2)_4-$, $-(CH_2)_5-$ y $-(CH_2)_6-$;

con la condición de que al menos uno de R^5, R^6 o R^7 debe ser $-C(O)R^{10}$;

R^8 y R^9 se seleccionan independientemente del grupo formado por hidrógeno, alquilo y arilo;

R^{10} se selecciona del grupo formado por hidroxi, alcoxi, ariloxi, $-N(R^{11})(CH_2)_nR^{12}$ y $-NR^{13}R^{14}$;

R^{11} se selecciona del grupo formado por hidrógeno y alquilo;

R^{12} se selecciona del grupo formado por $-NR^{13}R^{14}$, hidroxi, $-C(O)R^{15}$, arilo y heteroarilo;

R^{13} y R^{14} se seleccionan independientemente del grupo formado por hidrógeno, alquilo, cicloalquilo, arilo y heteroarilo;

R^{13} y R^{14} se pueden combinar para formar un grupo seleccionado del grupo formado por $-(CH_2)_4-$, $-(CH_2)_5-$, $-(CH_2)_2O(CH_2)_2-$, y $-(CH_2)_2N(CH_3)(CH_2)_2-$;

R^{15} se selecciona del grupo formado por hidrógeno, hidroxi, alcoxi y ariloxi;

R^{16} se selecciona del grupo formado por hidroxi, $-C(O)R^{15}$, $-NR^{13}R^{14}$ y $-C(O)NR^{13}R^{14}$;

R^{17} se selecciona del grupo formado por alquilo, cicloalquilo, arilo y heteroarilo; y

n y r son independientemente 1, 2, 3, o 4.

2. El compuesto o sal de la reivindicación 1 en el cual :

R^1 y R^4 son hidrógeno;

R^2 se selecciona del grupo formado por hidrógeno, halo, alcoxi inferior, $-C(O)R^{15}$ y $-S(O)_2NR^{13}R^{14}$;

R^3 se selecciona del grupo formado por hidrógeno, alcoxi inferior, $-C(O)R^{15}$, $-S(O)_2NR^{13}R^{14}$, arilo y heteroarilo;

R^5 es $-C(O)R^{10}$;

R^6 se selecciona del grupo formado por hidrógeno y alquilo inferior; y

R^7 se selecciona del grupo formado por hidrógeno, alquilo inferior y $-C(O)R^{17}$.

3. El compuesto o sal de la reivindicación 2 en el cual :

R^{10} se selecciona del grupo formado por hidroxi, alcoxi inferior y $-NR^{11}(CH_2)_nR^{12}$, donde

n es 2 o 3;

R^{11} se selecciona del grupo formado por hidrógeno y alquilo inferior; y,

R^{12} se selecciona del grupo formado por arilo y $-NR^{13}R^{14}$.

4. El compuesto o sal de la reivindicación 3, en el cual R^{13} y R^{14} se seleccionan independientemente del grupo formado por hidrógeno, alquilo inferior, y, en combinación,$-(CH_2)_4-$, $-(CH_2)_5-$, $-CH_2)_2O(CH_2)_2-$ o $-(CH_2)_2N(CH_3)(CH_2)_2-$.

5. El compuesto o sal de la reivindicación 1 en el cual :

R^1 se selecciona del grupo formado por hidrógeno, alquilo inferior, $-(CH_2)_rR^{16}$ y $-C(O)NR^8R^9$;

R^2 se selecciona del grupo formado por hidrógeno, halógeno, arilo y $-S(O)_2NR^{13}R^{14}$;

R^3 se selecciona del grupo formado por hidrógeno, alquilo inferior, alcoxi

inferior, arilo, heteroarilo y –C(O)R^{15};

R^4 es hidrógeno;

R^5 se selecciona del grupo formado por hidrógeno y alquilo inferior;

R^6 es -C(O)R^{10};

R^7 se selecciona del grupo formado por hidrógeno, alquilo inferior y arilo;

R^{16} se selecciona del grupo formado por hidroxi y –C(O)R^{15}; y,

r es 2 o 3.

6. El compuesto o sal de la reivindicación 5, en el cual R^3 es arilo opcionalmente sustituido con uno o más grupos seleccionados del grupo formado por alquilo inferior, alcoxi inferior y halo.

7. El compuesto o sal de la reivindicación 5, en el cual R^{10} se selecciona del grupo formado por hidroxi, alcoxi inferior, -$NR^{13}R^{14}$ y -$NR^{11}(CH_2)_nR^{12}$, donde

n es 1, 2 o 3,

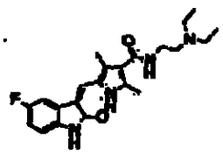
R^{11} es hidrógeno, y

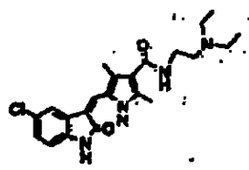
R^{12} se selecciona del grupo formado por hidroxi, alcoxi inferior, -C(O)R^{15}, heteroarilo y –$NR^{13}R^{14}$.

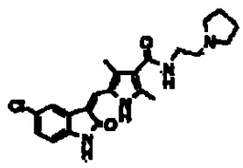
8. El compuesto o sal de la reivindicación 7 en el cual R^{13} y R^{14} se seleccionan independientemente del grupo formado por hidrógeno, alquilo inferior, heteroarilo y, en combinación, -$(CH_2)_4$-, -$(CH_2)_5$-, -$(CH_2)_2O(CH_2)_2$-, o -$(CH_2)_2N(CH_3)(CH_2)_2$-.

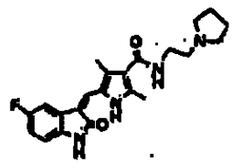
9. El compuesto o sal de la reivindicación 8, en el cual el compuesto se selecciona del grupo compuesto consistente en:

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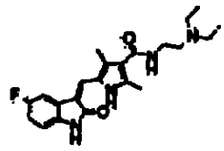








10. El compuesto de la reivindicación 9, en el cual el compuesto es::



11. Una composición farmacéutica, que comprende un compuesto o sal de la reivindicación 10; y un vehículo o excipiente aceptable para uso farmacéutico.

12. Un método para la modulación de la actividad catalítica de una proteinquinasa que comprende poner en contacto dicha proteinquinasa con un compuesto o sal de la reivindicación 10.

13. El método de la reivindicación 12 en el cual dicha proteinquinasa se selecciona del grupo formado por un receptor de tirosinquinasa, un no receptor de tirosinquinasa y una serin-treoninquinasa.

14. Un método para tratar o prevener una afección relacionada con la proteinquinasa en un organismo que comprende administrar una cantidad eficaz en términos terapéuticos de un compuesto o sal de la reivindicación 10 a

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dicho organismo.

15. El método de la reivindicación 14 en el cual dicha afección relacionada con la proteinquinasa se selecciona del grupo formado por una afección relacionada con un receptor de tirosinquinasa , una afección relacionada con un no receptor de tirosinquinasa y una afección relacionada con una serin-treoninquinasa.

16. El método de la reivindicación 14 en el cual dicha afección relacionada con una proteinquinasa se selecciona del grupo formado por una afección relacionada con los EGFR, una afección relacionada con los PDGFR, una afección relacionada con los IGFR y una afección relacionada con los FLK.

17. El método de la reivindicación 14 en el cual dicha afección relacionada con la proteinquinasa es un cáncer seleccionado del grupo formado por carcinoma de células escamosas, astrocitoma, sarcoma de Kaposi, glioblastoma, cáncer de pulmón, cáncer de vejiga, cáncer de cabeza y cuello, melanoma, cáncer de ovarios, cáncer de próstata, cáncer de mama, cáncer de células pequeñas de pulmón, glioma, cáncer colorrectal, cáncer genitourinario y cáncer gastrointestinal.

18. El método de la reivindicación 14 en el cual dicha afección relacionada con la proteinquinasa se selecciona del grupo formado por diabetes, un trastorno autoinmune , un trastorno hiperproliferativo, restenosis, fibrosis, psoriasis, enfermedad de von Heppel-Lindau, osteoartritis, artritis reumatoide, angiogenesis, un trastorno inflamatorio, una enfermedad inmunológica y una enfermedad cardiovascular.

19. El método de la reivindicación 14 en el cual dicho organismo es un ser humano.

20. El compuesto o sal de la reivindicación 5 en el cual :

R^1 es $-C(O)NR^8R^9$, donde

R^8 es hidrógeno y

R^9 es arilo opcionalmente sustituido con uno o más grupos seleccionados del grupo formado por halo, hidroxi y alcoxi inferior.

21. El compuesto de la reivindicación 1 en el cual :

R^1 se selecciona del grupo formado por hidrógeno y alquilo inferior;

R^2 se selecciona del grupo formado por hidrógeno, halo, alcoxi inferior, arilo, $-C(O)R^{15}$ y $-S(O)_2NR^{13}R^{14}$;

R^3 se selecciona del grupo formado por hidrógeno, halo, arilo, heteroarilo y $-C(O)R^{15}$;

R^4 es hidrógeno;

R^5 es $-C(O)R^{10}$; y,

R^6 y R^7 se combinan para formar un grupo $-(CH_2)_4-$.

22. El compuesto de la reivindicación 21 en el cual :

R^{10} se selecciona del grupo formado por hidroxi, alcoxi,$-NR^{13}R^{14}$ y $-NH(CH_2)_nNR^{13}R^{14}$ donde n es 2 o 3.

23. El compuesto de la reivindicación 22 en el cual :

R^{13} y R^{14} se seleccionan independientemente del grupo formado por hidrógeno, alquilo inferior, y, en combinación, $-(CH_2)_4-$, $-(CH_2)_5-$, $-(CH_2)_2O(CH_2)_2-$ o $-(CH_2)_2N(CH_3)(CH_2)_2-$.

24. El compuesto de la reivindicación 1 en el cual :

R^1 y R^4 se seleccionan del grupo formado por hidrógeno, alquilo inferior, alcoxi inferior y halógeno;

R^2 se selecciona del grupo formado por hidrógeno, alquilo inferior, alcoxi

inferior, halo y $-S(O)_2NR^{13}R^{14}$;

R^3 se selecciona del grupo formado por hidrógeno, alquilo inferior, alcoxi inferior, halo, arilo y $-C(O)R^{15}$;

R^5 y R^6 se seleccionan del grupo formado por hidrógeno y alquilo inferior; y

R^7 es $-C(O)R^{10}$ también es una realización actualmente preferida de esta invención.

25. El compuesto de la reivindicación 24 en el cual :

R^{10} se selecciona del grupo formado por hidroxi, alcoxi, $-NR^{13}R^{14}$ y $-NR^{11}(CH_2)_nR^{12}$, donde n es 2 o 3, R^{11} se selecciona del grupo formado por hidrógeno y alquilo inferior; y, R^{12} es $-NR^{13}R^{14}$,donde R^{13} y R^{14} se seleccionan independientemente del grupo formado por hidrógeno, alquilo inferior, y, en combinación,$-(CH_2)_4-$, $-(CH_2)_5-$, $-CH_2)_2O(CH_2)_2-$ o $-(CH_2)_2N(CH_3)(CH_2)_2-$.

26. Una composición farmacéutica, que comprende un compuesto o sal de la reivindicación 1; y un vehículo o excipiente aceptable para uso farmacéutico.

27. Un método para la modulación de la actividad catalítica de una proteinquinasa que comprende poner en contacto dicha proteinquinasa con un compuesto o sal de la reivindicación 1.

28. El método de la reivindicación 27 en el cual dicha proteinquinasa se selecciona del grupo formado por un receptor de tirosinquinasa, un no receptor de tirosinquinasa y una serin-treoninquinasa.

29. Un método para tratar o prevener una afección relacionada con la proteinquinasa en un organismo que comprende administrar una cantidad eficaz en términos terapéuticos de un compuesto o sal de la reivindicación 10 a dicho organismo.

30. El método de la reivindicación 29 en el cual dicha afección relacionada con la proteinquinasa se selecciona del grupo formado por una afección relacionada con un receptor de tirosinquinasa , una afección relacionada con un no receptor de tirosinquinasa y una afección relacionada con una serin-treoninquinasa.

31. El método de la reivindicación 32 en el cual dicha afección relacionada con una proteinquinasa se selecciona del grupo formado por una afección relacionada con los EGFR, una afección relacionada con los PDGFR, una afección relacionada con los IGFR y una afección relacionada con los FLK.

32. El método de la reivindicación 29 en el cual dicha afección relacionada con la proteinquinasa es un cáncer seleccionado del grupo formado por carcinoma de células escamosas, astrocitoma, sarcoma de Kaposi, glioblastoma, cáncer de pulmón, cáncer de vejiga, cáncer de cabeza y cuello, melanoma, cáncer de ovarios, cáncer de próstata, cáncer de mama, cáncer de células pequeñas de pulmón, glioma, cáncer colorrectal, cáncer genitourinario y cáncer gastrointestinal.

33. El método de la reivindicación 29 en el cual dicha afección relacionada con la proteinquinasa se selecciona del grupo formado por diabetes, un trastorno autoinmune , un trastorno hiperproliferativo, restenosis, fibrosis, psoriasis, enfermedad de von Heppel-Lindau, osteoartritis, artritis reumatoide, angiogenesis, un trastorno inflamatorio, una enfermedad inmunológica y una enfermedad cardiovascular.

34. El método de la reivindicación 29 en el cual dicho organismo es un ser humano.