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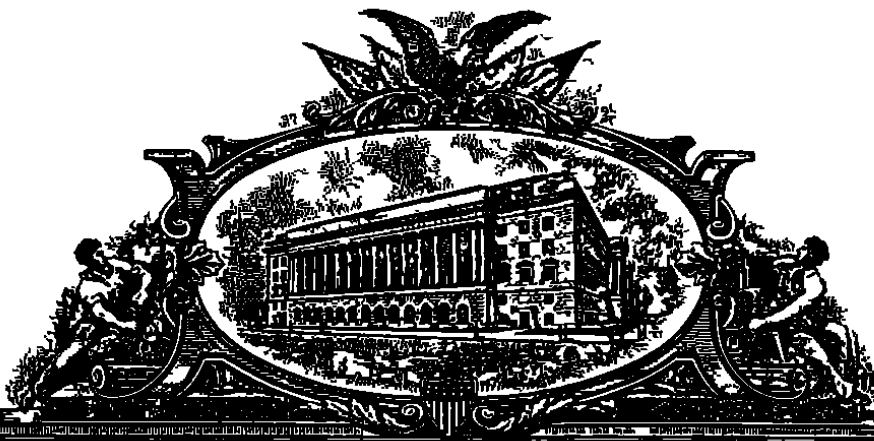
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SMITHKLINE BEECHAM
CORPORATION

En relación con el Expediente de la referencia y con el fin de dar cumplimiento a la preceptuado por el artículo 4o literal d) numeral 3 de la Convención de París adjunto a este escrito sirvanse encontrar la copia certificada de la solicitud de patente de la cual se reivindica prioridad la cual se encuentra debidamente traducida en sus apartes pertinentes

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APPLICATION NUMBER 60/152,780

FILING DATE September 07, 1999



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PROVISIONAL APPLICATION COVER SHEET

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

Docket No			P51017P
INVENTOR(s) / APPLICANT(s)			
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TITLE OF THE INVENTION (280 characters max)

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ENCLOSED APPLICATION PARTS (check all that apply)

☒ Specification	Number of Pages	54	☐ Small Entity Statement
☐ Drawings	Number of Sheets		☐ Other (specify)

METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT

☒ The Commissioner is hereby authorized to charge filing fees and credit Deposit Account No. 19-2570	PROVISIONAL FILING FEE AMOUNT (\$)	\$150 00
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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

Mary E. McCarthy

Date

Sept 7, 1999

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32,197

O Additional inventors are being named on separately numbered sheets attached hereto *

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TITLE

Vitronectin Receptor Antagonists

FIELD OF THE INVENTION

5 This invention relates to pharmaceutically active compounds which inhibit the vitronectin receptor and are useful for the treatment of inflammation cancer and cardiovascular disorders such as atherosclerosis and restenosis and diseases wherein bone resorption is a factor such as osteoporosis

10 BACKGROUND OF THE INVENTION

Integrins are a superfamily of cell adhesion receptors which are transmembrane glycoproteins expressed on a variety of cells. These cell surface adhesion receptors include gpIIb /IIIa (the fibrinogen receptor) and $\alpha_v\beta_3$ (the vitronectin receptor). The fibrinogen receptor gpIIb /IIIa is expressed on the platelet surface, and mediates platelet aggregation and the formation of a hemostatic clot at the site of a bleeding wound. Philips et al., *Blood*. 1988, 71 831. The vitronectin receptor $\alpha_v\beta_3$ is expressed on a number of cells including endothelial smooth muscle, osteoclast, and tumor cells, and thus it has a variety of functions. The $\alpha_v\beta_3$ receptor expressed on the membrane of osteoclast cells mediates the adhesion of osteoclasts to the bone matrix a key step in the bone resorption process. Ross et al., *J Biol Chem*. 1987 262 7703. A disease characterized by excessive bone resorption is osteoporosis. The $\alpha_v\beta_3$ receptor expressed on human aortic smooth muscle cells mediates their migration into neointima, a process which can lead to restenosis after percutaneous coronary angioplasty. Brown, et al. *Cardiovascular Res*. 1994, 28, 1815. Additionally Brooks, et al. *Cell* 1994 79 1157 has shown that an $\alpha_v\beta_3$ antagonist is able to promote tumor regression by inducing apoptosis of angiogenic blood vessels. Thus, agents that block the vitronectin receptor would be useful in treating diseases, such as osteoporosis restenosis and cancer.

The vitronectin receptor is now known to refer to three different integrins designated $\alpha_v\beta_1$ $\alpha_v\beta_3$ and $\alpha_v\beta_5$. Horton, et al. *Int. J Exp Pathol*. 1990 71 741. $\alpha_v\beta_1$ binds fibronectin and vitronectin. $\alpha_v\beta_3$ binds a large variety of ligands, including fibrin fibrinogen laminin, thrombospondin, vitronectin, von Willebrand's factor osteopontin and bone sialoprotein I. $\alpha_v\beta_5$ binds vitronectin. The vitronectin receptor $\alpha_v\beta_5$ has been shown to be involved in cell adhesion of a variety of cell types, including microvascular endothelial cells, (Davis et al. *J Cell Biol*. 1993 51 206) and its role in angiogenesis has been confirmed. Brooks, et al. *Science* 1994, 264 569. This integrin is expressed on blood vessels in human wound granulation tissue, but not in normal skin.

The vitronectin receptor is known to bind to bone matrix proteins which contain the tri peptide Arg-Gly Asp (or RGD) motif. Thus, Horton, et al *Exp Cell Res* 1991 195 368 disclose that RGD-containing peptides and an anti vitronectin receptor antibody (23C6) inhibit dentine resorption and cell spreading by osteoclasts. In addition, Sato et al *J Cell Biol* 1990 111 1713 discloses that echistatin, a snake venom peptide which contains the RGD sequence, is a potent inhibitor of bone resorption in tissue culture and inhibits attachment of osteoclasts to bone.

It has now been discovered that certain compounds are potent inhibitors of the $\alpha_v\beta_3$ and $\alpha_v\beta_5$ receptors. In particular, it has been discovered that such compounds are more potent inhibitors of the vitronectin receptor than the fibrinogen receptor.

SUMMARY OF THE INVENTION

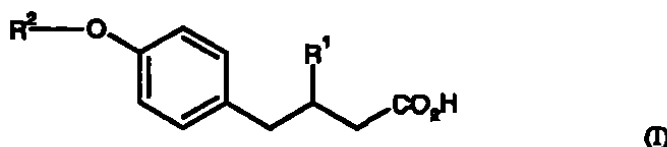
This invention comprises compounds of the formula (I) as described hereinafter which have pharmacological activity for the inhibition of the vitronectin receptor and are useful in the treatment of inflammation, cancer and cardiovascular disorders, such as atherosclerosis and restenosis, and diseases wherein bone resorption is a factor, such as osteoporosis.

This invention is also a pharmaceutical composition comprising a compound according to formula (I) and a pharmaceutically carrier.

This invention is also a method of treating diseases which are mediated by the vitronectin receptor. In a particular aspect, the compounds of this invention are useful for treating atherosclerosis, restenosis, inflammation, cancer and diseases wherein bone resorption is a factor, such as osteoporosis.

DETAILED DESCRIPTION

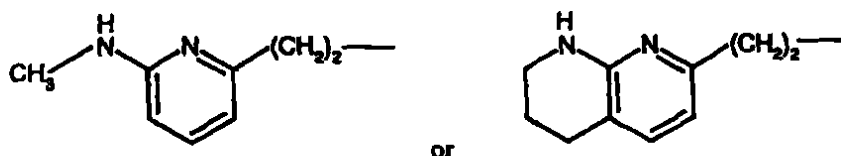
This invention comprises novel compounds which are more potent inhibitors of the vitronectin receptor than the fibrinogen receptor. This invention comprises compounds of formula (I).



wherein

R^1 is Het or Ar

R^2 is



or a pharmaceutically acceptable salt thereof

Also included in this invention are pharmaceutically acceptable addition salts and complexes of the compounds of this invention. In cases wherein the compounds of this invention may have one or more chiral centers unless specified, this invention includes each unique nonracemic compound which may be synthesized and resolved by conventional techniques. According to the present invention the (S) configuration of the formula (I) compounds is preferred.

In cases in which compounds have unsaturated carbon carbon double bonds both the cis (Z) and trans (E) isomers are within the scope of this invention. In cases wherein compounds may exist in tautomeric forms, such as keto-enol tautomers such as

and and each tautomeric form is contemplated as being included within this invention whether existing in equilibrium or locked in one form by appropriate substitution with R

The compounds of formula (I) inhibit the binding of vitronectin and other RGD-containing peptides to the vitronectin receptor. Inhibition of the vitronectin receptor on osteoclasts inhibits osteoclastic bone resorption and is useful in the treatment of diseases wherein bone resorption is associated with pathology such as osteoporosis and osteoarthritis

In another aspect, this invention is a method for stimulating bone formation which comprises administering a compound which causes an increase in osteocalcin release. Increased bone production is a clear benefit in disease states wherein there is a deficiency of mineralized bone mass or remodeling of bone is desired, such as fracture healing and the prevention of bone fractures. Diseases and metabolic disorders which result in loss of bone structure would also benefit from such treatment. For instance, hyperparathyroidism, Paget's disease, hypercalcemia of malignancy, osteolytic lesions produced by bone metastasis, bone loss due to immobilization or sex hormone deficiency, Behçet's disease, osteomalacia, hyperostosis and osteopetrosis, could benefit from administering a compound of this invention.

Additionally, since the compounds of the instant invention inhibit vitronectin receptors on a number of different types of cells, said compounds would be useful in the treatment of inflammatory disorders, such as rheumatoid arthritis and psoriasis and cardiovascular diseases, such as atherosclerosis and restenosis. The compounds of Formula

(I) of the present invention may be useful for the treatment or prevention of other diseases including but not limited to thromboembolic disorders, asthma, allergies, adult respiratory distress syndrome, graft versus host disease, organ transplant rejection septic shock, eczema contact dermatitis inflammatory bowel disease and other autoimmune diseases

5 The compounds of the present invention may also be useful for wound healing

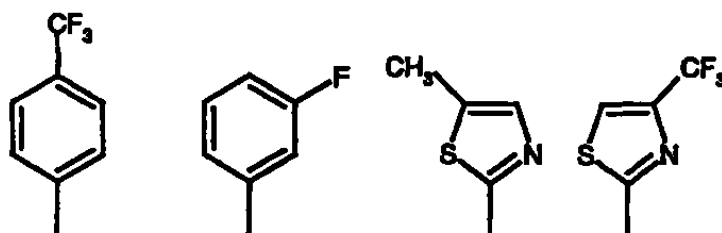
The compounds of the present invention are also useful for the treatment, including prevention of angiogenic disorders The term angiogenic disorders as used herein includes conditions involving abnormal neovascularization Where the growth of new blood vessels is the cause of or contributes to the pathology associated with a disease, inhibition of angiogenesis will reduce the deleterious effects of the disease An example of such a disease target is diabetic retinopathy Where the growth of new blood vessels is required to support growth of a deleterious tissue, inhibition of angiogenesis will reduce the blood supply to the tissue and thereby contribute to reduction in tissue mass based on blood supply requirements Examples include growth of tumors where neovascularization is a continual requirement in order that the tumor grow and the establishment of solid tumor metastases Thus, the compounds of the present invention inhibit tumor tissue angiogenesis thereby preventing tumor metastasis and tumor growth

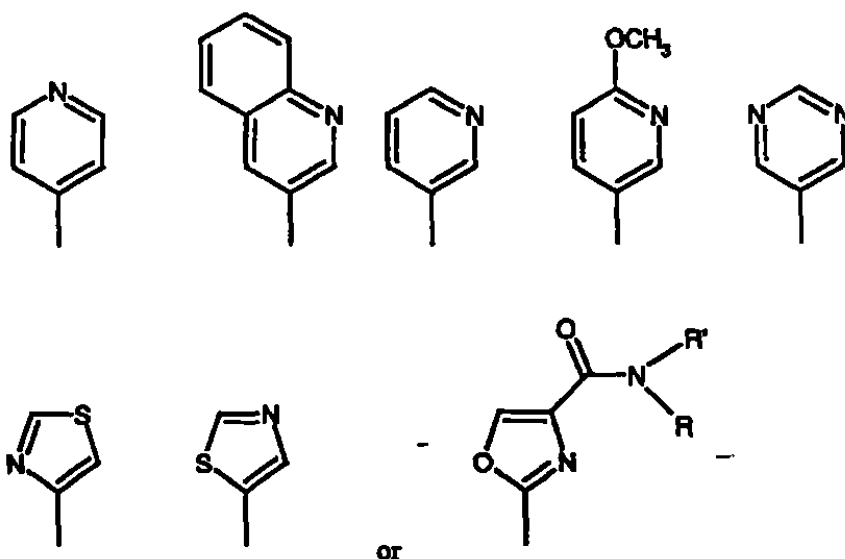
Thus according to the methods of the present invention, the inhibition of angiogenesis using the compounds of the present invention can ameliorate the symptoms of the disease, and, in some cases, can cure the disease.

Another therapeutic target for the compounds of the instant invention are eye diseases characterized by neovascularization Such eye diseases include corneal neovascular disorders such as corneal transplantation herpetic keratitis lentic keratitis, pterygium and neovascular pannus associated with contact lens use Additional eye diseases also include age-related macular degeneration, presumed ocular histoplasmosis, retinopathy of prematurity and neovascular glaucoma.

This invention further provides a method of inhibiting tumor growth which comprises administering stepwise or in physical combination a compound of formula (I) and an antineoplastic agent, such as topotecan and cisplatin.

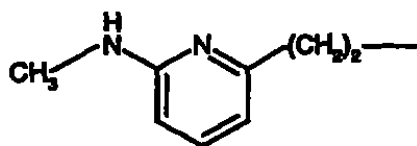
30 With respect to formula (I) suitably R^1 is





in which R is C₁₋₄ alkyl and R' is phenyl, benzyl or -CH₂CF₃ or R and R' are joined to form a morpholinyl ring

Suitably R² is



Representative of the novel compounds of this invention are the following

(±)-4-[4-[2-[6-(methylamino)pyridin-2-yl] 1-ethoxy]phenyl] 3-[4-(trifluoromethyl)phenyl]butanoic acid

(±)-4-[4-[2-[6-(methylamino)pyridin-2-yl] 1-ethoxy]phenyl] 3-[4-[(N-methyl-N-phenylamino)carbonyl] 1,3-oxazol-2-yl]butanoic acid,

(±)-4-[4-[2-[6-(methylamino)pyridin-2-yl] 1-ethoxy]phenyl] 3-[4-[(morpholin-4-yl)carbonyl] 1,3-oxazol-2-yl]butanoic acid,

(±)-4-[4-[2-[6-(methylamino)pyridin-2-yl] 1-ethoxy]phenyl] 3-[4-[(N-methyl-N-(2,2,2-trifluoroethyl)amino)carbonyl] 1,3-oxazol-2-yl]butanoic acid,

(±)-4-[4-[2-[6-(methylamino)pyridin-2-yl] 1-ethoxy]phenyl] 3-[4-(trifluoromethyl)thiazol-2-yl]butanoic acid, and

(±)-4-[4-[2-[6-(methylamino)pyridin-2-yl] 1-ethoxy]phenyl] 3-(3-methylthiazol-2-yl)butanoic acid

or a pharmaceutically acceptable salt thereof

In cases wherein the compounds of this invention may have one or more chiral centers, unless specified, this invention includes each unique nonracemic compound which may be synthesized and resolved by conventional techniques.

In cases in which compounds have unsaturated carbon-carbon double bonds both the cis (Z) and trans (E) isomers are within the scope of this invention. The meaning of any substituent at any one occurrence is independent of its meaning, or any other substituent's meaning at any other occurrence.

Also included in this invention are prodrugs of the compounds of this invention. Prodrugs are considered to be any covalently bonded carriers which release the active parent drug according to formula (I) *in vivo*. Thus, in another aspect of this invention are novel prodrugs which are also intermediates in the preparation of formula (Ia) compounds of formula (II).

Abbreviations and symbols commonly used in the peptide and chemical arts are used herein to describe the compounds of this invention. In general, the amino acid abbreviations follow the IUPAC IUB Joint Commission on Biochemical Nomenclature as described in *Eur J Biochem.* 158 9 (1984).

C₁₋₄alkyl as applied herein means an optionally substituted alkyl group of 1 to 4 carbon atoms, and includes methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl and t-butyl. Any C₁₋₄alkyl may be optionally substituted with the group R^x which may be on any carbon atom that results in a stable structure and is available by conventional synthetic techniques. Suitable groups for R^x are C₁₋₄alkyl, OR^{*}, SR^{*}, C₁₋₄alkylsulfonyl, C₁₋₄alkylsulfoxyl, -CN, N(R^{*})₂, CH₂N(R^{*})₂, NO₂, -CF₃, -CO₂R^{*}, -CON(R^{*})₂, -COR^{*}, SO₂N(R^{*})₂, NR^{*}C(O)R^{*}, F, Cl, Br, I, or CF₃S(O)_r wherein r is 0, 1 or 2, and R^{*} is H, C₁₋₄alkyl, phenyl or benzyl.

Halogen or halo means F, Cl, Br, and I.

Ar or aryl, as applied herein, means phenyl or naphthyl or phenyl or naphthyl substituted by one to three substituents, such as those defined above for alkyl, especially C₁₋₄alkyl, C₁₋₄alkoxy, C₁₋₄alkylthio, CF₃, NH₂, OH, F, Cl, Br or I.

Het, or heterocycle, indicates an optionally substituted five or six membered monocyclic ring, or a nine or ten-membered bicyclic ring containing one to three heteroatoms chosen from the group of nitrogen, oxygen and sulfur which are stable and available by conventional chemical synthesis. Illustrative heterocycles are benzofuran, benzimidazole, benzopyran, benzothiophene, benzothiazole, furan, imidazole, indoline, morpholine, piperidine, piperazine, pyrrole, pyrrolidine, tetrahydropyridine, pyridine, thiazole, oxazole, thiophene, quinoline, isoquinoline, and tetra- and perhydro-quinoline and isoquinoline. Any accessible combination of up to three substituents on the Het ring, such as those defined above for alkyl, that are available by chemical synthesis and are stable are within the scope of this invention.

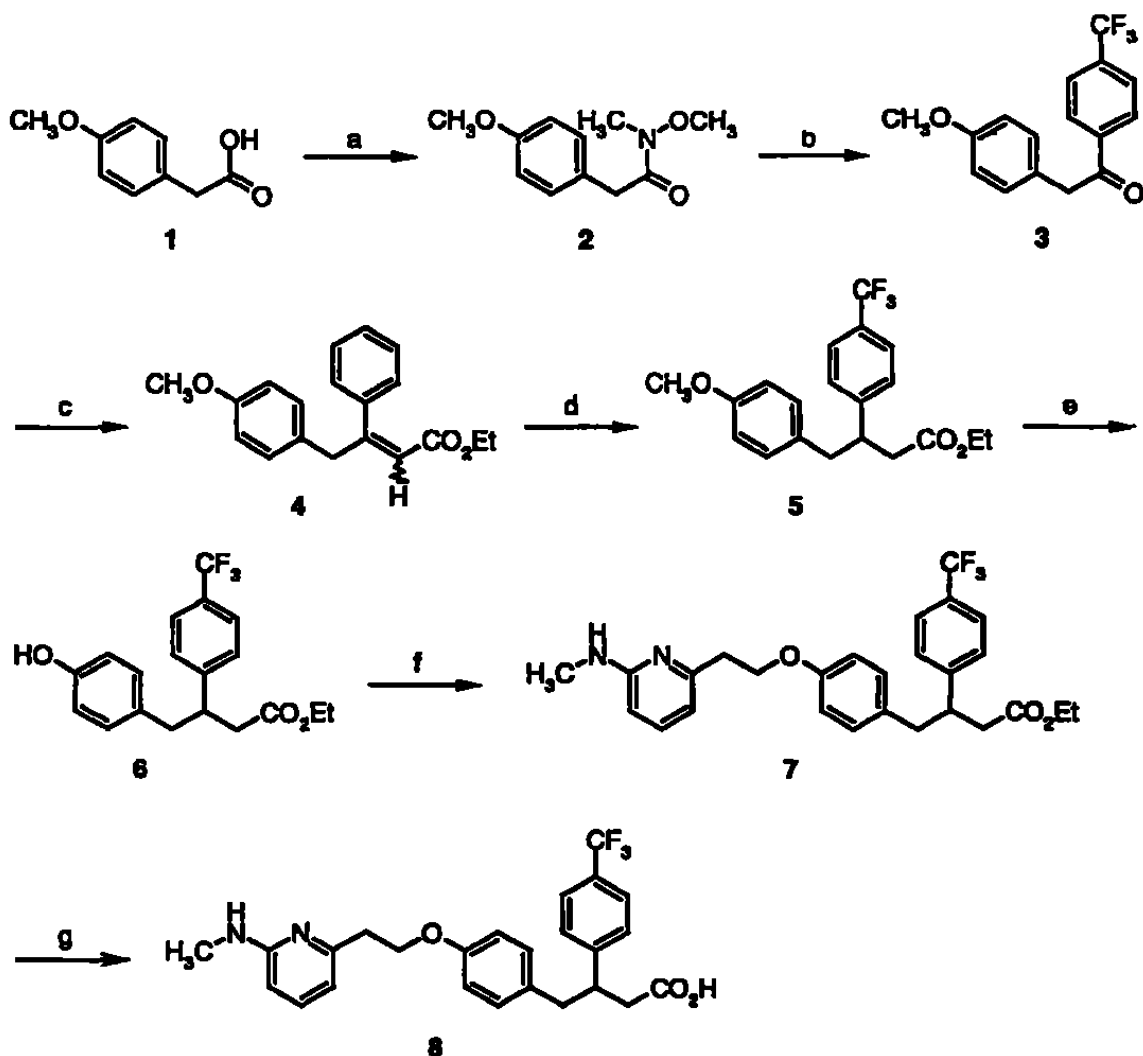
Certain radical groups are abbreviated herein. t Bu refers to the tertiary butyl radical, Boc refers to the t-butyloxycarbonyl radical. Fmoc refers to the

fluorenylmethoxycarbonyl radical, Ph refers to the phenyl radical Cbz refers to the benzyloxycarbonyl radical, Bn refers to the benzyl radical, Me refers to methyl Et refers to ethyl Ac refers to acetyl Alk refers to C₁₋₄alkyl Nph refers to 1 or 2-naphthyl and cHex refers to cyclohexyl Tet refers to 5 tetrazolyl

- 5 Certain reagents are abbreviated herein DCC refers to dicyclohexylcarbodiimide, DMAP refers to dimethylaminopyridine, DIEA refers to diisopropylethyl amine, EDC refers to 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride. HOBt refers to 1 hydroxybenzotriazole, THF refers to tetrahydrofuran DIEA refers to diisopropylethylamine, DEAD refers to diethyl azodicarboxylate, PPh₃ refers to triphenylphosphine, DIAD refers to diisopropyl azodicarboxylate DME refers to dimethoxyethane, DMF refers to dimethylformamide NBS refers to N bromosuccinimide, Pd/C refers to a palladium on carbon catalyst, PPA refers to polyphosphonic acid, DPPA refers to diphenylphosphoryl azide, BOP refers to benzotriazol 1 yloxy tris(dimethyl amino)phosphonium hexafluorophosphate, HF refers to hydrofluoric acid, TEA refers to triethylamine, TFA refers to trifluoroacetic acid, PCC refers to pyridinium chlorochromate
- 10
- 15

Compounds of this invention are prepared by the general methods described in Schemes I-IV

Scheme I

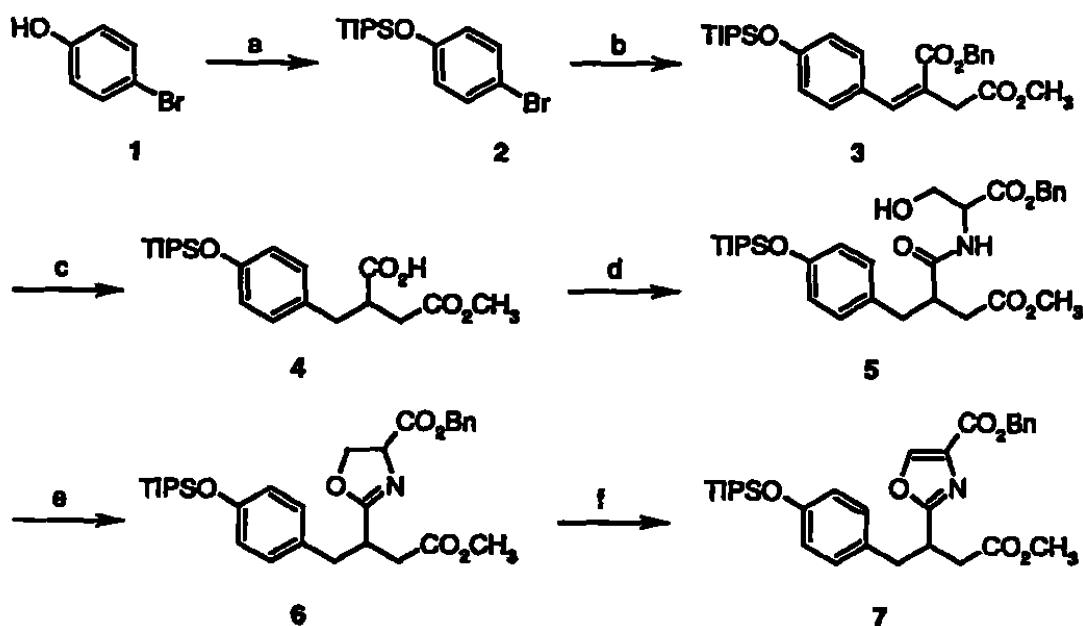


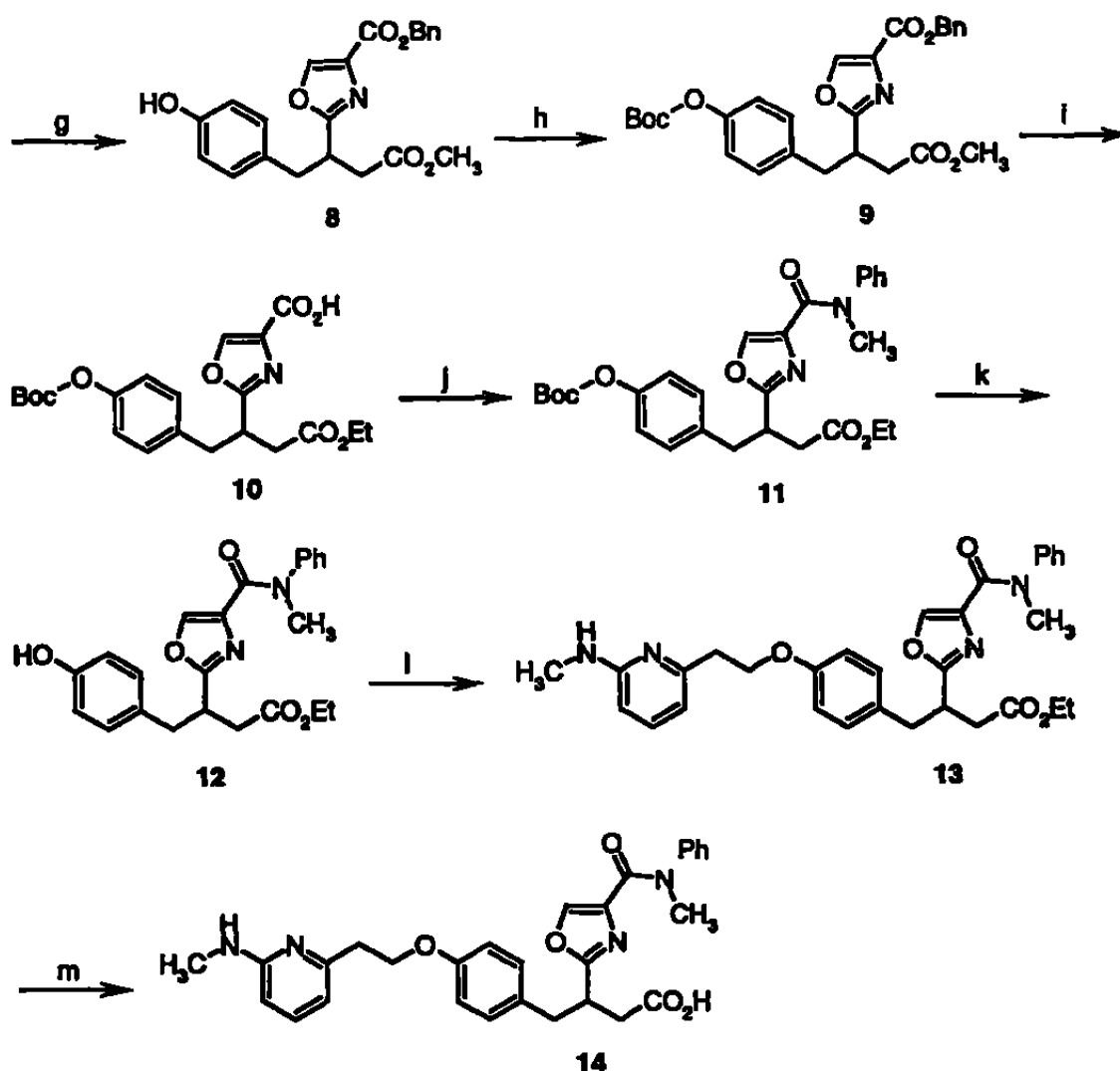
- 5 (a) $\text{CH}_3\text{NH}(\text{OCH}_3)$ HCl, EDC, HOBT, H_2O , Et_3N , DMF (b) 4-bromobenzotrifluoride, sec-BuLi , THF (c) $(\text{EtO})_2\text{P}(\text{O})\text{CH}_2\text{CO}_2\text{Et}$, NaH, toluene; (d) H_2 , 10% Pd/C, EtOH, (e) BBr_3 , CH_2Cl_2 (f) 6-(methylamino)-2-pyridylethanol, DIAD, $(\text{Ph})_3\text{P}$, THF (g) 1.0 N NaOH, MeOH, then acidification.

- 10 An appropriate alkoxyphenylacetic acid, for instance 4-methoxyphenylacetic acid (I 1) is converted to the deoxybenzoin derivative I-3 by reaction of the corresponding N-methoxy N-methylamide (I 2) with a metalated aromatic derivative, according to the general method of Weinreb (*Tetrahedron Lett.* 1981 22, 3815). Compound I-3 is converted to the α,β-unsaturated ester I-4 through the well-known Wittig reaction.
- 15 Optimally the reaction is conducted using triethyl phosphonoacetate in the presence of a suitable base, generally sodium hydride or lithium bis(trimethylsilyl)amide, in an aprotic

solvent, such as toluene THF or mixtures thereof Reduction of the olefin group of I-4 is optimally accomplished by hydrogenation in the presence of a palladium catalyst, for instance palladium on activated charcoal in a suitable solvent, such as EtOAc, MeOH, EtOH, i PrOH, or mixtures thereof The methyl ether of I 5 can be cleaved with boron tribromide (BBr₃) in an inert solvent, preferably CH₂Cl₂, or with ethanethiol (EtSH) and aluminum trichloride (AlCl₃) in an inert solvent, such as CH₂Cl₂ Other useful methods for removal of methyl ether protecting groups are described in Greene "Protective Groups in Organic Synthesis" (published by Wiley Interscience) The resulting phenol I 6 is reacted with 6-(methylamino) 2-pyridylethanol in a Mitsunobu type coupling reaction (*Organic Reactions* 1992, 42, 335-656; *Synthesis* 1981, 1, 28) to afford I 7 The reaction is mediated by the complex formed between an azodicarboxylate diester such as diethyl azodicarboxylate or diisopropyl azodicarboxylate, and triphenylphosphine and is conducted in an aprotic solvent, for instance THF CH₂Cl₂, or DMF The ethyl ester of I 7 is hydrolyzed using aqueous base, for example, LiOH in aqueous THF or NaOH in aqueous methanol or ethanol and the intermediate carboxylate salt is acidified with a suitable acid, for instance TFA or HCl to afford the carboxylic acid I-8 Alternatively the intermediate carboxylate salt can be isolated, if desired, or a carboxylate salt of the free carboxylic acid can be prepared by methods well known to those of skill in the art.

Scheme II





- 5 (a) Triisopropylsilyl chloride, imidazole, DMF (b) methyl 3-(benzyloxycarbonyl)-3-butenate, $\text{Pd}(\text{OAc})_2$, $\text{P}(\text{tol})_3$, $(i\text{-Pr})_2\text{NEt}$, propionitrile (c) H_2 10% Pd/C , EtOAc (d) serine benzyl ester hydrochloride, EDC, HOBT H_2O Et_3N DMF (e) Burgess reagent, THF (f) Cl_3CBr DBU CH_2Cl_2 (g) TBAF THF (h) $(\text{Boc})_2\text{O}$ pyridine, THF (i) H_2 , 10% Pd/C , EtOH , (j) N-methylaniline, EDC, BOPFPH, $(i\text{-Pr})_2\text{NEt}$, pyridine, DMF (k) 4 N HCl/dioxane (l) 6-(methylamino)-2-pyridylethanol, DIAD $(\text{Ph})_3\text{P}$ THF (m) LiOH, THF H_2O then acidification

A halophenol derivative, for instance 4-bromophenol (II 1), is converted to a suitably protected derivative, for instance 4-bromo-1-(triisopropylsilyloxy)benzene (II 2)

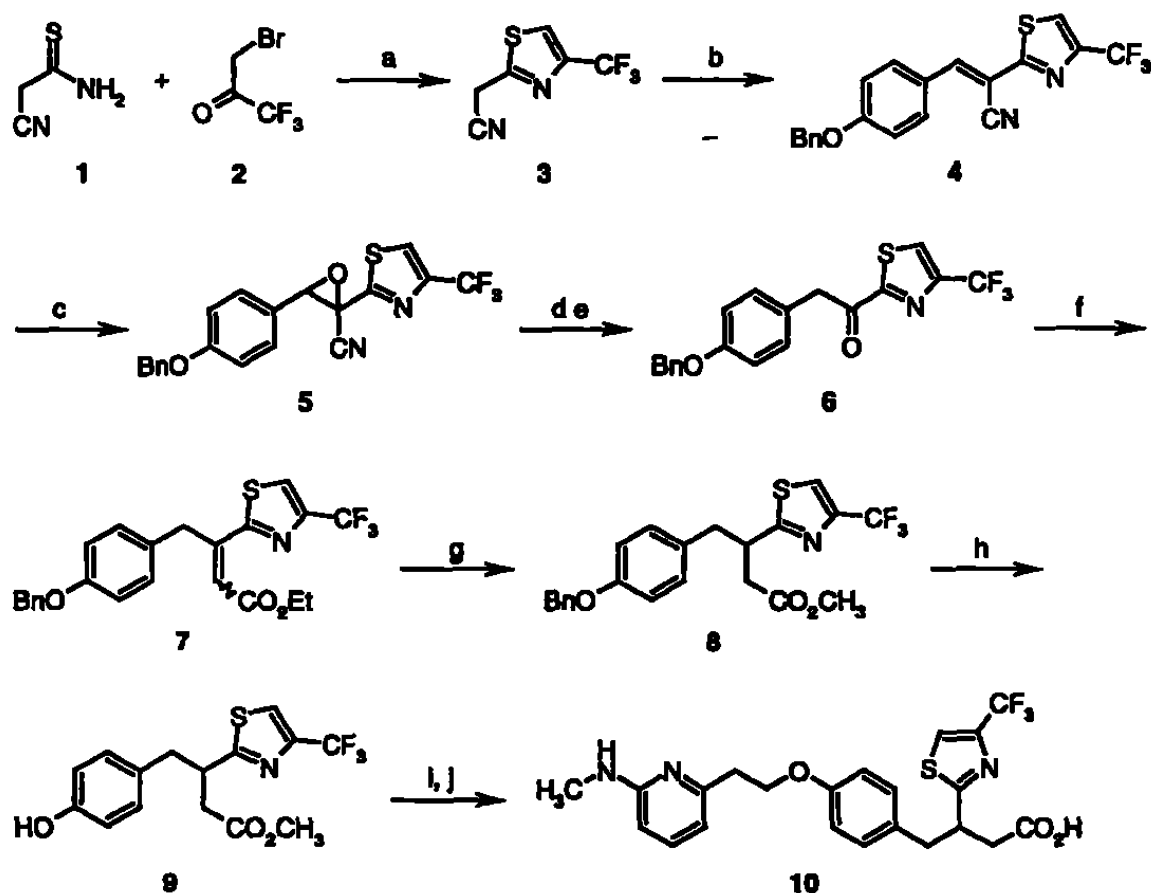
- 15 The protecting group for the phenol must be compatible with subsequent chemistry and also must be able to be removed selectively when desired. Methods for the protection of phenols are described in standard reference volumes, such as Greene, "Protective Groups in Organic Synthesis" (published by Wiley-Interscience). Compound II 2 reacts with methyl

3-(benzyloxycarbonyl)-3-butenolates in a Heck type reaction (see Heck, *Org. Reactions* 1982, 27 345) to afford II-3. The reaction is mediated by a palladium(0) species and generally is conducted in an inert solvent, such as CH₃CN, propionitrile or toluene in the presence of an appropriate acid scavenger such as triethylamine (Et₃N) or diisopropylethylamine ((i-Pr)₂NEt). Typical sources of the palladium(0) species include palladium(II) acetate (Pd(OAc)₂) and palladium(II) chloride (PdCl₂) and oftentimes phosphine ligands for instance triphenylphosphine (PPh₃) or tri-ortho-tolylphosphine (P(tol)₃) are included. The α,β-unsaturated ester II-3 is reduced to the saturated compound II-4 by reaction with hydrogen gas in the presence of a suitable catalyst, preferably palladium metal on activated carbon (Pd/C) in an inert solvent, generally MeOH, EtOH, EtOAc or mixtures thereof. The carboxylic acid of II-4 is converted to an activated form using for example, EDC and HOBT, SOCl₂ or 1,1'-carbonyldiimidazole (CDI) and the activated form is subsequently reacted with an appropriate amine, for instance serine benzyl ester in a suitable solvent, such as DMF to afford II-5. Depending on whether acid neutralization is required, an added base, such as triethylamine (Et₃N) diisopropylethylamine ((i-Pr)₂NEt) or pyridine, may be used. Many additional methods for converting a carboxylic acid to an amide are known and can be found in standard reference books, such as "Compendium of Organic Synthetic Methods Vol. I-VI (published by Wiley Interscience), or Bodansky "The Practice of Peptide Synthesis (published by Springer Verlag). II-5 is then converted to the oxazole derivative II-7. Several methods are known for the conversion of amidoalcohols to oxazoles (Meyers *Tetrahedron* 1994 50 2297-2360; Wipf *J. Org. Chem.* 1993 58 3604-3606). For example, the amidoalcohol II-5 can be converted first to the oxazoline II-6. This transformation is generally accomplished under dehydrating conditions such as reaction with Burgess reagent in THF. Oxazoline II-6 is then oxidized to oxazole II-7 using, for instance, bromotrichloromethane and DBU in CH₂Cl₂ (Williams *Tetrahedron Letters* 1997 38 331-334) or CuBr₂ and DBU in an appropriate solvent, such as EtOAc/CHCl₃ or CH₂Cl₂ (Barnish, *J. Org. Chem.* 1993, 58, 4494-4496). Removal of the silyl protecting group under standard fluoride conditions (see Greene above) affords phenol II-8, which is converted to the tert-butyloxy carbonate derivative II-9 using di-tert-butyl dicarbonate in the presence of a base, generally pyridine, in a polar neutral solvent, such as THF. This new protecting group is selected as described above. Compound II-9 is converted to the carboxylic acid derivative II-10 by hydrogenation as described above, and II-10 is converted to amide II-11 according to the general procedure of Carpino and Ayman (*J. Am. Chem. Soc.* 1995 117 5401-5402). Under these conditions carboxylic acid II-10 is reacted with a suitable amine, for instance N-methylamine, in the presence of bis(tetramethylene)fluoroformamidinium hexafluorophosphate (BPFPH) and an appropriate

base, generally Et_3N ($i\text{-Pr}$) $_2\text{NEt}$, pyridine, or mixtures thereof in a polar neutral solvent, such as DMF. The tert butyloxy carbamate protecting group is removed under standard acidic conditions (see Greene above) and the resulting phenol II 12 is converted to II 14 by the methods described in Scheme I

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Scheme III



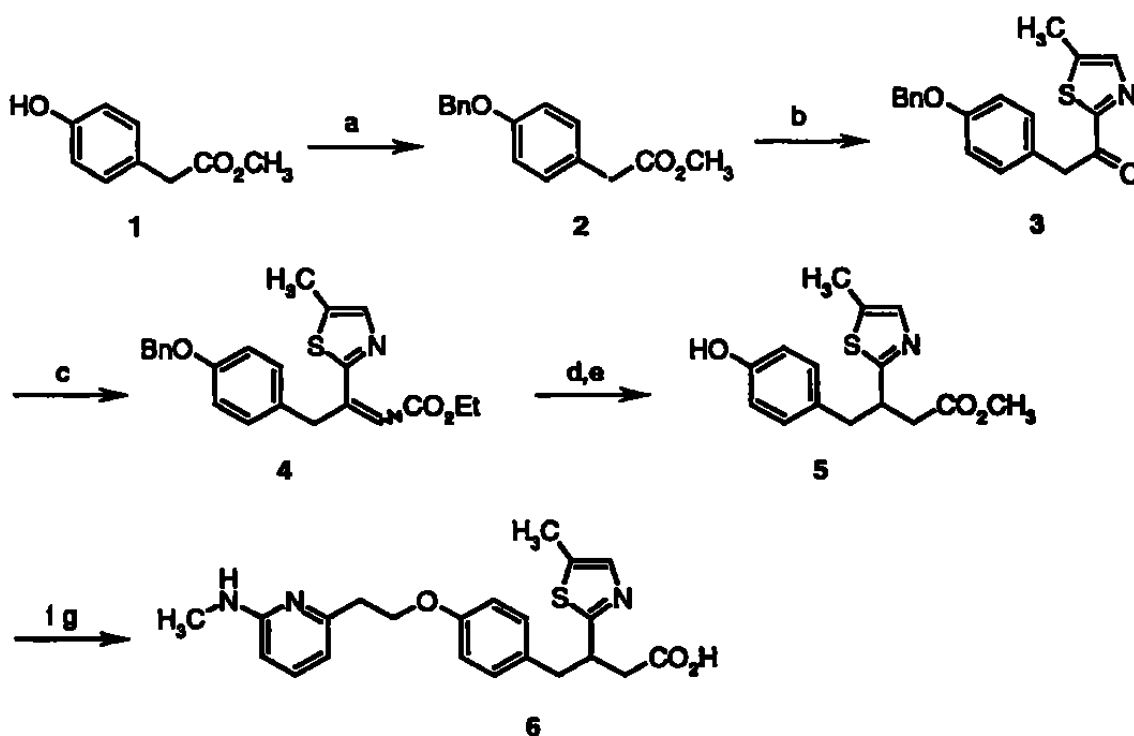
- 10 (a) EtOH , reflux, (b) 4-(benzyloxy)benzaldehyde, NaOEt , EtOH , (c) Al_2O_3 , NaOCl , CH_3CN (d) Et_3SiH , $\text{BF}_3 \cdot \text{OEt}_2$, CH_2Cl_2 , (e) $n\text{-Bu}_4\text{NF}$, THF (f) $(\text{EtO})_2\text{P}(=\text{O})\text{CH}_2\text{CO}_2\text{Et}$, NaH , THF reflux, (g) Mg , MeOH , (h) $\text{BF}_3 \cdot \text{OEt}_2$, EtSH , (i) 6-(methylamino)-2-pyridylethanol, DIAD , $(\text{Ph})_3\text{P}$, THF (j) LiOH , THF , H_2O then acidification

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Thiazole derivative III-3 is prepared by condensation of 2-cyanoacetamide (III 1) and 3-bromo-1,1,1-trifluoroacetone (III 2) in refluxing ethanol, by appropriate modification of the procedure of Schaefer and Gewald (*J Prakt. Chem.* 1974, 316 684-692) for the preparation of 4-phenyl 2-(cyanomethyl)thiazole. Aldol condensation of III-3

with an appropriately substituted benzaldehyde derivative, for example 4-(benzyloxy)benzaldehyde, gives III-4. The reaction is catalyzed by a suitable base, preferably sodium ethoxide, and is conducted in a polar solvent generally ethanol. Epoxidation of III-4 to afford III-5 is accomplished with sodium hypochlorite in the presence of neutral alumina according to the general method of Foucaud (*Synthesis* 1987 9 854-856). Other well known epoxidation conditions, such as mCPBA, basic hydrogen peroxide, or basic tert butyl hydroperoxide might also be used, as long as the conditions are compatible with the functionality in the substrate. On exposure to triethylsilane in the presence of a suitable Lewis acid such as $\text{BF}_3 \cdot \text{OEt}_2$ or a suitable protic acid for instance TFA or HCl, the epoxide ring of III-5 is reductively opened in a highly regioselective manner to afford ketone III-6 together with the corresponding trimethylsilyl cyanohydrin of the III-6. This cyanohydrin is conveniently converted to ketone III-6 on exposure to tetrabutylammonium fluoride in an appropriate solvent, generally THF. This ketone reacts in a Wittig type reaction with triethyl phosphonoacetate in the presence of a suitable base for instance $\text{LiN}(\text{TMS})_2$ or NaH, in a polar aprotic solvent, preferably THF to afford the α,β -unsaturated ester III-7 as a mixture of olefinic isomers. On reaction with magnesium metal in MeOH, the olefin of III-7 is selectively reduced to afford the saturated derivative III-8. The ethyl ester is converted to a methyl ester under these conditions. Removal of the benzyl group is accomplished with ethanethiol and $\text{BF}_3 \cdot \text{OEt}_2$, to afford the phenol III-9 which is converted to III-10 according to the methods described in Scheme I.

Scheme IV



- 5 (a) BnCl , K_2CO_3 acetone (b) 5-methylthiazole, $n\text{-BuLi}$ THF (c) $(\text{EtO})_2\text{P}(=\text{O})\text{CH}_2\text{CO}_2\text{Et}$, NaH , THF (d) Mg , MeOH , (e) $\text{BF}_3 \cdot \text{OEt}_2$, EtSH (f) 6-(methylamino)-2-pyridylethanol, DIAD, $(\text{Ph})_3\text{P}$ THF (g) LiOH , THF, H_2O then acidification

10 The phenol group of commercially available methyl 4-hydroxyphenylacetate (V 1) is protected with a suitable protecting group for instance a methyl ether, a benzyl ether or a trisopropylsilyl ether. Protection of phenols is well known to those of skill in the art, and representative protecting groups are described in standard reference volumes such as Greene "Protective Groups in Organic Synthesis" (published by Wiley Interscience). The resulting compound (IV 2) reacts with suitable Grignard or organolithium reagents to afford ketones. For example, 2-lithio-5-methylthiazole, prepared from 5-methylthiazole and n-butyllithium, reacts with IV 2 in an ethereal solvent, such as THF or DMB, to afford the ketone derivative IV-3. This ketone is then converted to IV-6 according to the methods described in Scheme III.

20 Amide coupling reagents as used herein denote reagents which may be used to form peptide bonds. Typical coupling methods employ carbodiimides, activated anhydrides and esters and acyl halides. Reagents such as EDC, DCC, DPPA, BOP reagent, HOBt, N-hydroxysuccinimide and oxalyl chloride are typical.

Coupling methods to form peptide bonds are generally well known to the art. The methods of peptide synthesis generally set forth by Bodansky *et al* THE PRACTICE OF PEPTIDE SYNTHESIS Springer Verlag Berlin 1984 Ali *et al* in *J Med. Chem.* 29 984 (1986) and *J Med. Chem.* 30 2291 (1987) are generally illustrative of the technique and are incorporated herein by reference

Typically the amine or aniline is coupled via its free amino group to an appropriate carboxylic acid substrate using a suitable carbodiimide coupling agent, such as N,N'-dicyclohexyl carbodiimide (DCC) optionally in the presence of catalysts such as 1-hydroxybenzotriazole (HOBt) and dimethylamino pyridine (DMAP) Other methods such as the formation of activated esters anhydrides or acid halides, of the free carboxyl of a suitably protected acid substrate, and subsequent reaction with the free amine of a suitably protected amine, optionally in the presence of a base, are also suitable For example a protected Boc-amino acid or Cbz-amino benzoic acid is treated in an anhydrous solvent, such as methylene chloride or tetrahydrofuran (THF), in the presence of a base, such as N-methyl morpholine, DMAP or a trialkylamine, with isobutyl chloroformate to form the activated anhydride which is subsequently reacted with the free amine of a second protected amino acid or aniline.

Useful intermediates for preparing formula (I) compounds in which R² is a benzimidazole are disclosed in Nestor *et al J Med. Chem.* 1984 27 320 Representative methods for preparing benzimidazole compounds useful as intermediates in the present invention are also common to the art and may be found, for instance, in EP A 0 381 033

Acid addition salts of the compounds are prepared in a standard manner in a suitable solvent from the parent compound and an excess of an acid, such as hydrochloric, hydrobromic hydrofluoric, sulfuric phosphoric acetic, trifluoroacetic maleic succinic or methanesulfonic Certain of the compounds form inner salts or zwitterions which may be acceptable. Cationic salts are prepared by treating the parent compound with an excess of an alkaline reagent, such as a hydroxide, carbonate or alkoxide, containing the appropriate cation or with an appropriate organic amine. Cations such as Li⁺ Na⁺ K⁺ Ca⁺⁺ Mg⁺⁺ and NH₄⁺ are specific examples of cations present in pharmaceutically acceptable salts

This invention also provides a pharmaceutical composition which comprises a compound according to formula (I) and a pharmaceutically acceptable carrier Accordingly the compounds of formula (I) may be used in the manufacture of a medicament. Pharmaceutical compositions of the compounds of formula (I) prepared as heretofore described may be formulated as solutions or lyophilized powders for parenteral administration. Powders may be reconstituted by addition of a suitable diluent or other pharmaceutically acceptable carrier prior to use. The liquid formulation may be a buffered, isotonic, aqueous solution Examples of suitable diluents are normal isotonic

19
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saline solution standard 5% dextrose in water or buffered sodium or ammonium acetate solution Such formulation is especially suitable for parenteral administration, but may also be used for oral administration or contained in a metered dose inhaler or nebulizer for insufflation It may be desirable to add excipients such as polyvinylpyrrolidone, gelatin, hydroxy cellulose, acacia, polyethylene glycol mannitol, sodium chloride or sodium citrate

Alternately these compounds may be encapsulated, tableted or prepared in a emulsion or syrup for oral administration. Pharmaceutically acceptable solid or liquid carriers may be added to enhance or stabilize the composition, or to facilitate preparation of the composition. Solid carriers include starch, lactose, calcium sulfate dihydrate terra alba, magnesium stearate or stearic acid, talc, pectin acacia, agar or gelatin Liquid carriers include syrup peanut oil olive oil saline and water The carrier may also include a sustained release material such as glyceryl monostearate or glyceryl distearate, alone or with a wax The amount of solid carrier varies but, preferably will be between about 20 mg to about 1 g per dosage unit The pharmaceutical preparations are made following the conventional techniques of pharmacy involving milling mixing, granulating, and compressing, when necessary for tablet forms or milling mixing and filling for hard gelatin capsule forms When a liquid carrier is used, the preparation will be in the form of a syrup, elixir emulsion or an aqueous or non-aqueous suspension Such a liquid formulation may be administered directly p o or filled into a soft gelatin capsule

For rectal administration, the compounds of this invention may also be combined with excipients such as cocoa butter glycerin, gelatin or polyethylene glycols and molded into a suppository

The compounds described herein are antagonists of the vitronectin receptor and are useful for treating diseases wherein the underlying pathology is attributable to ligand or cell which interacts with the vitronectin receptor For instance, these compounds are useful for the treatment of diseases wherein loss of the bone matrix creates pathology Thus, the instant compounds are useful for the treatment of osteoporosis, hyperparathyroidism, Paget's disease, hypercalcemia of malignancy osteolytic lesions produced by bone metastasis, bone loss due to immobilization or sex hormone deficiency The compounds of this invention are also believed to have utility as antitumor anti-angiogenic, antiinflammatory and anti metastatic agents, and be useful in the treatment of atherosclerosis and restenosis

The compound is administered either orally or parenterally to the patient, in a manner such that the concentration of drug is sufficient to inhibit bone resorption, or other such indication The pharmaceutical composition containing the compound is administered at an oral dose of between about 0.1 to about 50 mg/kg in a manner consistent with the

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condition of the patient. Preferably the oral dose would be about 0.5 to about 20 mg/kg. For acute therapy, parenteral administration is preferred. An intravenous infusion of the peptide in 5% dextrose in water or normal saline, or a similar formulation with suitable excipients, is most effective, although an intramuscular bolus injection is also useful.

Typically the parenteral dose will be about 0.01 to about 100 mg/kg, preferably between 0.1 and 20 mg/kg. The compounds are administered one to four times daily at a level to achieve a total daily dose of about 0.4 to about 400 mg/kg/day. The precise level and method by which the compounds are administered is readily determined by one routinely skilled in the art by comparing the blood level of the agent to the concentration required to have a therapeutic effect.

This invention further provides a method for treating osteoporosis or inhibiting bone loss which comprises administering stepwise or in physical combination a compound of formula (I) and other inhibitors of bone resorption such as bisphosphonates (i.e., allendronate), hormone replacement therapy, anti-estrogens, or calcitonin. In addition, this invention provides a method of treatment using a compound of this invention and an anabolic agent, such as the bone morphogenic protein, iproflavone, useful in the prevention of bone loss and/or to increase bone mass.

Additionally, this invention provides a method of inhibiting tumor growth which comprises administering stepwise or in physical combination a compound of formula (I) and an antineoplastic agent. Compounds of the camptothecin analog class, such as topotecan, irinotecan and 9-aminocamptothecin, and platinum coordination complexes, such as cisplatin, ormaplatin and tetraplatin, are well known groups of antineoplastic agents. Compounds of the camptothecin analog class are described in U.S. Patent Nos. 5,004,758; 4,604,463; 4,473,692; 4,545,880; 4,342,776; 4,513,138; 4,399,276; EP Patent Application Publication Nos. 0,418,099 and 0,088,642; Wani, et al., *J. Med. Chem.* 1986, 29:2358; Wani, et al., *J. Med. Chem.* 1980, 23:554; Wani, et al., *J. Med. Chem.* 1987, 30:1774; and Nitta, et al., *Proc. 14th International Congr. Chemotherapy 1985 Anticancer Section 1*, 28, the entire disclosure of each which is hereby incorporated by reference. The platinum coordination complex, cisplatin, is available under the name Platinol® from Bristol Myers-Squibb Corporation. Useful formulations for cisplatin are described in U.S. Patent Nos. 5,562,925 and 4,310,515, the entire disclosure of each which is hereby incorporated by reference.

In the method of inhibiting tumor growth which comprises administering stepwise or in physical combination a compound of formula (I) and an antineoplastic agent, the platinum coordination compound, for example cisplatin, can be administered using slow intravenous infusion. The preferred carrier is a dextrose/saline solution containing mannitol. The dose schedule of the platinum coordination compound may be on the basis

of from about 1 to about 500 mg per square meter (mg/m^2) of body surface area per course of treatment. Infusions of the platinum coordination compound may be given one to two times weekly and the weekly treatments may be repeated several times. Using a compound of the camptothecin analog class in a parenteral administration, the course of therapy generally employed is from about 0.1 to about 300.0 mg/m^2 of body surface area per day for about five consecutive days. Most preferably, the course of therapy employed for topotecan is from about 1.0 to about 2.0 mg/m^2 of body surface area per day for about five consecutive days. Preferably, the course of therapy is repeated at least once at about a seven day to about a twenty-eight day interval.

The pharmaceutical composition may be formulated with both the compound of formula (I) and the antineoplastic agent in the same container, but formulation in different containers is preferred. When both agents are provided in solution form, they can be contained in an infusion/injection system for simultaneous administration or in a tandem arrangement.

For convenient administration of the compound of formula (I) and the antineoplastic agent at the same or different times, a kit is prepared comprising, in a single container, such as a box, carton or other container, individual bottles, bags, vials or other containers each having an effective amount of the compound of formula (I) for parenteral administration, as described above, and an effective amount of the antineoplastic agent for parenteral administration as described above. Such kit can comprise, for example, both pharmaceutical agents in separate containers or the same container, optionally as lyophilized plugs, and containers of solutions for reconstitution. A variation of this is to include the solution for reconstitution and the lyophilized plug in two chambers of a single container, which can be caused to admix prior to use. With such an arrangement, the antineoplastic agent and the compound of this invention may be packaged separately, as in two containers, or lyophilized together as a powder and provided in a single container.

When both agents are provided in solution form, they can be contained in an infusion/injection system for simultaneous administration or in a tandem arrangement. For example, the compound of formula (I) may be in an i.v. injectable form, or infusion bag, linked in series, via tubing, to the antineoplastic agent in a second infusion bag. Using such a system, a patient can receive an initial bolus-type injection or infusion of the compound of formula (I) followed by an infusion of the antineoplastic agent.

The compounds may be tested in one of several biological assays to determine the concentration of compound which is required to have a given pharmacological effect.

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H2**Inhibition of vitronectin binding**

Solid Phase [³H]-SK&F 107260 Binding to $\alpha_v\beta_3$ Human placenta or human platelet $\alpha_v\beta_3$ (0.1-0.3 mg/mL) in buffer T (containing 2 mM CaCl₂ and 1% octylglucoside) was diluted with buffer T containing 1 mM CaCl₂, 1 mM MnCl₂, 1 mM MgCl₂ (buffer A) and 0.05% NaN₃ and then immediately added to 96-well ELISA plates (Corning, New York, NY) at 0.1 mL per well. 0.1-0.2 μ g of $\alpha_v\beta_3$ was added per well. The plates were incubated overnight at 4 °C. At the time of the experiment, the wells were washed once with buffer A and were incubated with 0.1 mL of 3.5% bovine serum albumin in the same buffer for 1 hr at room temperature. Following incubation the wells were aspirated completely and washed twice with 0.2 mL buffer A.

Compounds were dissolved in 100% DMSO to give a 2 mM stock solution, which was diluted with binding buffer (15 mM Tris-HCl (pH 7.4), 100 mM NaCl, 1 mM CaCl₂, 1 mM MnCl₂, 1 mM MgCl₂) to a final compound concentration of 100 μ M. This solution is then diluted to the required final compound concentration. Various concentrations of unlabeled antagonists (0.001-100 μ M) were added to the wells in triplicates, followed by the addition of 50 nM of [³H]-SK&F 107260 (65-86 Ci/mmol).

The plates were incubated for 1 hr at room temperature. Following incubation the wells were aspirated completely and washed once with 0.2 mL of ice cold buffer A in a well to well fashion. The receptors were solubilized with 0.1 mL of 1% SDS and the bound [³H]-SK&F-107260 was determined by liquid scintillation counting with the addition of 3 mL Ready Safe in a Beckman LS Liquid Scintillation Counter with 40% efficiency. Nonspecific binding of [³H]-SK&F-107260 was determined in the presence of 2 μ M SK&F 107260 and was consistently less than 1% of total radioligand input. The IC₅₀ (concentration of the antagonist to inhibit 50% binding of [³H]-SK&F-107260) was determined by a nonlinear least squares curve-fitting routine, which was modified from the LUNDON 2 program. The K_d (dissociation constant of the antagonist) was calculated according to the equation $K_d = IC_{50}/(1 + L/K_d)$ where L and K_d were the concentration and the dissociation constant of [³H]-SK&F-107260, respectively.

Compounds of the present invention inhibit vitronectin binding to SK&F 107260 in the concentration range of about 0.060 to about 0.003 micromolar.

Compounds of this invention are also tested for *in vitro* and *in vivo* bone resorption in assays standard in the art for evaluating inhibition of bone formation, such as the pit formation assay disclosed in EP 528 587 which may also be performed using human osteoclasts in place of rat osteoclasts, and the ovariectomized rat model, described by Wronski *et al. Cells and Materials* 1991, Sup 1 69-74.

Vascular smooth muscle cell migration assay

Rat or human aortic smooth muscle cells were used. The cell migration was monitored in a Transwell cell culture chamber by using a polycarbonate membrane with pores of 8 μ m (Costar). The lower surface of the filter was coated with vitronectin. Cells were suspended in DMEM supplemented with 0.2% bovine serum albumin at a concentration of 2.5×10^6 cells/mL, and were pretreated with test compound at various concentrations for 20 min at 20°C. The solvent alone was used as control. 0.2 mL of the cell suspension was placed in the upper compartment of the chamber. The lower compartment contained 0.6 mL of DMEM supplemented with 0.2% bovine serum albumin. Incubation was carried out at 37°C in an atmosphere of 95% air/5% CO₂ for 24 hr. After incubation, the non-migrated cells on the upper surface of the filter were removed by gentle scraping. The filter was then fixed in methanol and stained with 10% Giemsa stain. Migration was measured either by a) counting the number of cells that had migrated to the lower surface of the filter or by b) extracting the stained cells with 10% acetic acid followed by determining the absorbance at 600 nm.

Thyroparathyroidectomized rat model

Each experimental group consists of 5-6 adult male Sprague-Dawley rats (250-400g body weight). The rats are thyroparathyroidectomized (by the vendor Taconic Farms) 7 days prior to use. All rats receive a replacement dose of thyroxine every 3 days. On receipt of the rats, circulating ionized calcium levels are measured in whole blood immediately after it has been withdrawn by tail venipuncture into heparinized tubes. Rats are included if the ionized Ca level (measured with a Ciba-Corning model 634 calcium pH analyzer) is <1.2 mM/L. Each rat is fitted with an indwelling venous and arterial catheter for the delivery of test material and for blood sampling respectively. The rats are then put on a diet of calcium-free chow and deionized water. Baseline Ca levels are measured and each rat is administered either control vehicle or human parathyroid hormone 1-34 peptide (hPTH1-34, dose 1.25 μ g/kg/h in saline/0.1% bovine serum albumin, Bachem, CA) or a mixture of hPTH1-34 and test material, by continuous intravenous infusion via the venous catheter using an external syringe pump. The calcemic response of each rat is measured at two-hourly intervals during the infusion period of 6-8 hours.

Human osteoclast resorption and adhesion assays

Pit resorption and adhesion assays have been developed and standardized using normal human osteoclasts derived from osteoclastoma tissue. Assay 1 was developed for the measurement of osteoclast pit volumes by laser confocal microscopy. Assay 2 was developed as a higher throughput screen in which collagen fragments (released during resorption) are measured by competitive ELISA.

Assay 1 (using laser confocal microscopy)

- Aliquots of human osteoclastoma-derived cell suspensions are removed from liquid nitrogen storage, warmed rapidly at 37°C and washed x1 in RPMI 1640 medium by centrifugation (1000rpm, 5 mins at 4°C)
- The medium is aspirated and replaced with murine anti HLA DR antibody then diluted 1:3 in RPMI 1640 medium. The suspension is incubated for 30 mins on ice and mixed frequently
- The cells are washed x2 with cold RPMI 1640 followed by centrifugation (1000 rpm, 5 mins at 4°C) and the cells are then transferred to a sterile 15 ml centrifuge tube. The number of mononuclear cells are enumerated in an improved Neubauer counting chamber
- Sufficient magnetic beads (5 / mononuclear cell) coated with goat anti mouse IgG (Dyna, Great Neck, NY) are removed from their stock bottle and placed into 5 ml of fresh medium (this washes away the toxic azide preservative) The medium is removed by immobilizing the beads on a magnet and is replaced with fresh medium.
- The beads are mixed with the cells and the suspension is incubated for 30 mins on ice. The suspension is mixed frequently
- The bead-coated cells are immobilized on a magnet and the remaining cells (osteoclast rich fraction) are decanted into a sterile 50 ml centrifuge tube.
- Fresh medium is added to the bead-coated cells to dislodge any trapped osteoclasts. This wash process is repeated x10. The bead-coated cells are discarded
- The viable osteoclasts are enumerated in a counting chamber using fluorescein diacetate to label live cells. A large-bore disposable plastic pasteur pipet is used to add the sample to the chamber
- The osteoclasts are pelleted by centrifugation and the density adjusted to the appropriate number in EMEM medium (the number of osteoclasts is variable from tumor to tumor), supplemented with 10% fetal calf serum and 1.7g/liter of sodium bicarbonate.
- 3ml aliquots of the cell suspension (per compound treatment) are decanted into 15ml centrifuge tubes. The cells are pelleted by centrifugation.
- To each tube, 3ml of the appropriate compound treatment are added (diluted to 50 µM in the EMEM medium). Also included are appropriate vehicle controls: a positive control (anti vitronectin receptor murine monoclonal antibody [87MEM1] diluted to 100 µg/ml) and an isotype control (IgG₁ diluted to 100 µg/ml). The samples are incubated at 37°C for 30 mins

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- 0.5 ml aliquots of the cells are seeded onto sterile dentine slices in a 48 well plate and incubated at 37°C for 2 hours. Each treatment is screened in quadruplicate.
- The slices are washed in six changes of warm PBS (10 ml / well in a 6-well plate) and then placed into fresh medium containing the compound treatment or control samples. The samples are incubated at 37°C for 48 hours.

Tartrate resistant acid phosphatase (TRAP) procedure (selective stain for cells of the osteoclast lineage)

- The bone slices containing the attached osteoclasts are washed in phosphate buffered saline and fixed in 2% glutaraldehyde (in 0.2M sodium cacodylate) for 5 mins.
- They are then washed in water and are incubated for 4 minutes in TRAP buffer at 37°C (0.5 mg/ml naphthol AS-BI phosphate dissolved in N,N-dimethylformamide and mixed with 0.25 M citrate buffer (pH 4.5) containing 10 mM sodium tartrate).
- Following a wash in cold water the slices are immersed in cold acetate buffer (0.1 M pH 6.2) containing 1 mg/ml fast red garnet and incubated at 4°C for 4 minutes.
- Excess buffer is aspirated, and the slices are air dried following a wash in water.
- The TRAP positive osteoclasts (brick red/ purple precipitate) are enumerated by bright field microscopy and are then removed from the surface of the dentine by sonication.
- Pit volumes are determined using the Nikon/LaserTec ILM21W confocal microscope.

Assay 2 (using an ELISA readout)

- The human osteoclasts are enriched and prepared for compound screening as described in the initial 9 steps of Assay 1. For clarity these steps are repeated herein below.
- Aliquots of human osteoclastoma-derived cell suspensions are removed from liquid nitrogen storage, warmed rapidly at 37°C and washed x1 in RPMI 1640 medium by centrifugation (1000rpm, 5 mins at 4°C).
 - The medium is aspirated and replaced with murine anti HLA-DR antibody then diluted 1:3 in RPMI 1640 medium. The suspension is incubated for 30 mins on ice and mixed frequently.
 - The cells are washed x2 with cold RPMI 1640 followed by centrifugation (1000 rpm, 5 mins at 4°C) and the cells are then transferred to a sterile 15 ml centrifuge tube. The number of mononuclear cells are enumerated in an improved Neubauer counting chamber.
 - Sufficient magnetic beads (5 / mononuclear cell), coated with goat anti mouse IgG (Dyna, Great Neck, NY) are removed from their stock bottle and placed into 5 ml of

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fresh medium (this washes away the toxic azide preservative) The medium is removed by immobilizing the beads on a magnet and is replaced with fresh medium

- The beads are mixed with the cells and the suspension is incubated for 30 mins on ice The suspension is mixed frequently

5 • The bead-coated cells are immobilized on a magnet and the remaining cells (osteoclast rich fraction) are decanted into a sterile 50 ml centrifuge tube

- Fresh medium is added to the bead-coated cells to dislodge any trapped osteoclasts This wash process is repeated x10 The bead-coated cells are discarded

10 • The viable osteoclasts are enumerated in a counting chamber using fluorescein diacetate to label live cells A large-bore disposable plastic pasteur pipet is used to add the sample to the chamber

- The osteoclasts are pelleted by centrifugation and the density adjusted to the appropriate number in EMEM medium (the number of osteoclasts is variable from tumor to tumor) supplemented with 10% fetal calf serum and 1.7g/liter of sodium bicarbonate

15 In contrast to the method described above in Assay 1 the compounds are screened at 4 doses to obtain an IC_{50} as outlined below

- The osteoclast preparations are preincubated for 30 minutes at 37°C with test compound (4 doses) or controls

20 • They are then seeded onto bovine cortical bone slices in wells of a 48-well tissue culture plate and are incubated for a further 2 hours at 37°C

- The bone slices are washed in six changes of warm phosphate buffered saline (PBS) to remove non-adherent cells, and are then returned to wells of a 48 well plate containing fresh compound or controls

25 • The tissue culture plate is then incubated for 48 hours at 37°C.

- The supernatants from each well are aspirated into individual tubes and are screened in a competitive ELISA that detects the C-telopeptide of type I collagen which is released during the resorption process This is a commercially available ELISA (Osteometer Denmark) that contains a rabbit antibody that specifically reacts with an 8 amino acid sequence (Glu Lys-Ala-His- Asp-Gly-Gly Arg) that is present in the carboxy-terminal telopeptide of the $\alpha 1$ -chain of type I collagen The results are expressed as % inhibition of resorption compared to a vehicle control

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Human osteoclast adhesion assay

35 The human osteoclasts are enriched and prepared for compound screening as described above in the initial 9 steps of Assay 1 For clarity these steps are repeated hereinbelow

- Aliquots of human osteoclastoma-derived cell suspensions are removed from liquid nitrogen storage, warmed rapidly at 37°C and washed x1 in RPMI 1640 medium by centrifugation (1000rpm, 5 mins at 4°C)
- The medium is aspirated and replaced with murine anti HLA DR antibody then diluted 1:3 in RPMI 1640 medium. The suspension is incubated for 30 mins on ice and mixed frequently
- The cells are washed x2 with cold RPMI 1640 followed by centrifugation (1000 rpm, 5 mins at 4°C) and the cells are then transferred to a sterile 15 ml centrifuge tube. The number of mononuclear cells are enumerated in an improved Neubauer counting chamber
- Sufficient magnetic beads (5 / mononuclear cell) coated with goat anti mouse IgG (Dyna, Great Neck, NY) are removed from their stock bottle and placed into 5 ml of fresh medium (this washes away the toxic azide preservative). The medium is removed by immobilizing the beads on a magnet and is replaced with fresh medium.
- The beads are mixed with the cells and the suspension is incubated for 30 mins on ice. The suspension is mixed frequently
- The bead-coated cells are immobilized on a magnet and the remaining cells (osteoclast rich fraction) are decanted into a sterile 50 ml centrifuge tube.
- Fresh medium is added to the bead-coated cells to dislodge any trapped osteoclasts. This wash process is repeated x10. The bead-coated cells are discarded
- The viable osteoclasts are enumerated in a counting chamber using fluorescein diacetate to label live cells. A large-bore disposable plastic pasteur pipet is used to add the sample to the chamber
- The osteoclasts are pelleted by centrifugation and the density adjusted to the appropriate number in EMEM medium (the number of osteoclasts is variable from tumor to tumor), supplemented with 10% fetal calf serum and 1.7g/liter of sodium bicarbonate
- Osteoclastoma-derived osteoclasts are precubated with compound (4 doses) or controls at 37°C for 30 minutes.
- The cells are then seeded onto osteopontin-coated slides (human or rat osteopontin 2.5ug/ml) and incubated for 2 hours at 37°C.
- Non adherent cells are removed by washing the slides vigorously in phosphate buffered saline and the cells remaining on the slides are fixed in acetone.
- The osteoclasts are stained for tartrate-resistant acid phosphatase (TRAP) a selective marker for cells of this phenotype (see steps 15-17), and are enumerated by light microscopy. The results are expressed as % inhibition of adhesion compared to a vehicle control

Cell Adhesion Assay**Cells and Cell Culture**

Human embryonic kidney cells (HEK293 cells) were obtained from ATCC (Catalog No CRL 1573). Cells were grown in Earle's minimal essential medium (EMEM) medium containing Earle's salts, 10% fetal bovine serum, 1% glutamine and 1% Penicillin Streptomycin.

Constructs and Transfections

A 3.2 kb EcoRI KpnI fragment of the α_v subunit and a 2.4 kb XbaI XhoI fragment of the β_3 subunit were inserted into the EcoRI EcoRV cloning sites of the pCDN vector (Aryar et al 1994) which contains a CMV promoter and a G418 selectable marker by blunt end ligation. For stable expression, 80×10^6 HEK 293 cells were electrotransformed with α_v - β_3 constructs (20 μ g DNA of each subunit) using a Gene Pulser (Hensley et al 1994) and plated in 100 mm plates (5×10^5 cells/plate). After 48 hr the growth medium was supplemented with 450 μ g/mL Geneticin (G418 Sulfate, GIBCO-BRL, Bethesda, MD). The cells were maintained in selection medium until the colonies were large enough to be assayed.

Immunocytochemical analysis of transfected cells

To determine whether the HEK 293 transfectants expressed the vitronectin receptor, the cells were immobilized on glass microscope slides by centrifugation, fixed in acetone for 2 min at room temperature and air dried. Specific reactivity with 23C6, a monoclonal antibody specific for the $\alpha_v\beta_3$ complex was demonstrated using a standard indirect immunofluorescence method.

Cell Adhesion Studies

Corning 96-well ELISA plates were precoated overnight at 4°C with 0.1 mL of human vitronectin (0.2 μ g/mL in RPMI medium). At the time of the experiment, the plates were washed once with RPMI medium and blocked with 3.5% BSA in RPMI medium for 1 hr at room temperature. Transfected 293 cells were resuspended in RPMI medium, supplemented with 20 mM HEPES, pH 7.4 and 0.1% BSA at a density of 0.5×10^6 cells/mL. 0.1 mL of cell suspension was added to each well and incubated for 1 hr at 37°C, in the presence or absence of various $\alpha_v\beta_3$ antagonists. Following incubation, 0.025 mL of a 10% formaldehyde solution, pH 7.4 was added and the cells were fixed at room temperature for 10 min. The plates were washed 3 times with 0.2 mL of RPMI medium and the adherent cells were stained with 0.1 mL of 0.5% toluidine blue for 20 min at room

temperature. Excess stain was removed by extensive washing with deionized water. The toluidine blue incorporated into cells was eluted by the addition of 0.1 mL of 50% ethanol containing 50 mM HCl. Cell adhesion was quantitated at an optical density of 600 nm on a microtiter plate reader (Titertek Multiskan MC, Sterling, VA).

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Solid Phase $\alpha_v\beta_3$ Binding Assay

The vitronectin receptor $\alpha_v\beta_3$ was purified from human placenta. Receptor preparation was diluted with 50 mM Tris-HCl, pH 7.5, 100 mM NaCl, 1 mM CaCl_2 , 1 mM MnCl_2 , 1 mM MgCl_2 (buffer A) and was immediately added to 96-well ELISA plates at 0.1 mL per well. 0.1–0.2 μg of $\alpha_v\beta_3$ was added per well. The plates were incubated overnight at 4°C. At the time of the experiment, the wells were washed once with buffer A and were incubated with 0.1 mL of 3.5% bovine serum albumin in the same buffer for 1 hr at room temperature. Following incubation the wells were aspirated completely and washed twice with 0.2 mL buffer A.

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In a [^3H]-SK&F-107260 competition assay, various concentrations of unlabeled antagonists (0.001–100 μM) were added to the wells, followed by the addition of 5.0 nM of [^3H]-SK&F-107260. The plates were incubated for 1 hr at room temperature. Following incubation the wells were aspirated completely and washed once with 0.2 mL of ice cold buffer A in a well-to-well fashion. The receptors were solubilized with 0.1 mL of 1% SDS and the bound [^3H]-SK&F-107260 was determined by liquid scintillation counting with the addition of 3 mL Ready Safe in a Beckman LS 6800 Liquid Scintillation Counter with 40% efficiency. Nonspecific binding of [^3H]-SK&F-107260 was determined in the presence of 2 μM SK&F-107260 and was consistently less than 1% of total radioligand input. The IC_{50} (concentration of the antagonist to inhibit 50% binding of [^3H]-SK&F-107260) was determined by a nonlinear least squares curve-fitting routine, which was modified from the LUNDON 2 program. The K_i (dissociation constant of the antagonist) was calculated according to Cheng and Prusoff equation, $K_i = \text{IC}_{50} / (1 + L/K_d)$, where L and K_d were the concentration and the dissociation constant of [^3H]-SK&F-107260, respectively.

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Inhibition of RGD-mediated GPIIb-IIIa binding

Purification of GPIIb-IIIa

Ten units of outdated, washed human platelets (obtained from Red Cross) were lysed by gentle stirring in 3% octylglucoside, 20 mM Tris-HCl, pH 7.4, 140 mM NaCl, 2 mM CaCl_2 at 4°C for 2 h. The lysate was centrifuged at 100,000g for 1 h. The supernatant obtained was applied to a 5 mL lentil lectin sepharose 4B column (E.Y. Labs) pre-equilibrated with 20 mM Tris-HCl, pH 7.4, 100 mM NaCl, 2 mM CaCl_2 , 1%

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octylglucoside (buffer A) After 2 h incubation the column was washed with 50 mL cold buffer A The lectin-retained GPIIb-IIIa was eluted with buffer A containing 10% dextrose All procedures were performed at 4°C The GPIIb IIIa obtained was >95% pure as shown by SDS polyacrylamide gel electrophoresis

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Incorporation of GPIIb IIIa in Liposomes

A mixture of phosphatidylserine (70%) and phosphatidylcholine (30%) (Avanti Polar Lipids) were dried to the walls of a glass tube under a stream of nitrogen. Purified GPIIb-IIIa was diluted to a final concentration of 0.5 mg/mL and mixed with the phospholipids in a protein phospholipid ratio of 1:3 (w/w). The mixture was resuspended and sonicated in a bath sonicator for 5 min. The mixture was then dialyzed overnight using 12,000-14,000 molecular weight cutoff dialysis tubing against a 1000-fold excess of 50 mM Tris-HCl pH 7.4, 100 mM NaCl, 2 mM CaCl₂ (with 2 changes). The GPIIb-IIIa containing liposomes were centrifuged at 12,000g for 15 min and resuspended in the dialysis buffer at a final protein concentration of approximately 1 mg/mL. The liposomes were stored at -70°C until needed.

Competitive Binding to GPIIb-IIIa

The binding to the fibrinogen receptor (GPIIb-IIIa) was assayed by an indirect competitive binding method using [³H]-SK&F-107260 as an RGD-type ligand. The binding assay was performed in a 96-well filtration plate assembly (Millipore Corporation, Bedford, MA) using 0.22 µm hydrophilic durapore membranes. The wells were precoated with 0.2 mL of 10 µg/mL polylysine (Sigma Chemical Co., St. Louis, MO) at room temperature for 1 h to block nonspecific binding. Various concentrations of unlabeled benzazepines were added to the wells in quadruplicate. [³H]-SK&F-107260 was applied to each well at a final concentration of 4.5 nM, followed by the addition of 1 µg of the purified platelet GPIIb-IIIa-containing liposomes. The mixtures were incubated for 1 h at room temperature. The GPIIb-IIIa-bound [³H]-SK&F-107260 was separated from the unbound by filtration using a Millipore filtration manifold, followed by washing with ice-cold buffer (2 times, each 0.2 mL). Bound radioactivity remaining on the filters was counted in 1.5 mL Ready Solve (Beckman Instruments, Fullerton, CA) in a Beckman Liquid Scintillation Counter (Model LS6800) with 40% efficiency. Nonspecific binding was determined in the presence of 2 µM unlabeled SK&F-107260 and was consistently less than 0.14% of the total radioactivity added to the samples. All data points are the mean of quadruplicate determinations.

Competition binding data were analyzed by a nonlinear least-squares curve fitting procedure. This method provides the IC₅₀ of the antagonists (concentration of the

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antagonist which inhibits specific binding of [³H]-SK&F-107260 by 50% at equilibrium)
The IC₅₀ is related to the equilibrium dissociation constant (K_i) of the antagonist based on
the Cheng and Prusoff equation $K_i = IC_{50}/(1+L/K_d)$ where L is the concentration of [³H]-
SK&F-107260 used in the competitive binding assay (4.5 nM) and K_d is the dissociation
5 constant of [³H]-SK&F 107260 which is 4.5 nM as determined by Scatchard analysis

Preferred compounds of this invention have an affinity for the vitronectin receptor
relative to the fibrinogen receptor of greater than 10:1. Most preferred compounds have a
ratio of activity of greater than 100:1.

10 The efficacy of the compounds of formula (I) alone or in combination with an
antineoplastic agent may be determined using several transplantable mouse tumor models.
See U.S. Patent Nos. 5,004,758 and 5,633,016 for details of these models.

The examples which follow are intended in no way to limit the scope of this
15 invention, but are provided to illustrate how to make and use the compounds of this
invention. Many other embodiments will be readily apparent to those skilled in the art.

General

20 Proton nuclear magnetic resonance (¹H NMR) spectra were recorded at either 250
300, or 400 MHz. Chemical shifts are reported in parts per million (δ) downfield from the
internal standard tetramethylsilane (TMS). Abbreviations for NMR data are as follows: s =
singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = doublet of doublets, dt =
doublet of triplets, app = apparent, br = broad. J indicates the NMR coupling constant
25 measured in Hertz. CDCl₃ is deuteriochloroform, DMSO-d₆ is
hexadeuteriodimethylsulfoxide, and CD₃OD is tetradeuteriomethanol. Infrared (IR)
spectra were recorded in transmission mode and band positions are reported in inverse
wavenumbers (cm⁻¹). Mass spectra were obtained using electrospray (ES) or FAB
ionization techniques. Elemental analyses were performed either in-house or by
30 Quantitative Technologies Inc., Whitehouse, NJ. Melting points were taken on a Thomas-
Hoover melting point apparatus and are uncorrected. All temperatures are reported in
degrees Celsius. Analtech Silica Gel GF and E. Merck Silica Gel 60 F-254 thin layer plates
were used for thin layer chromatography. Both flash and gravity chromatography were
carried out on E. Merck Kieselgel 60 (230-400 mesh) silica gel. Analytical and preparative
35 HPLC were carried out on Rainin or Beckman chromatographs. ODS refers to an
octadecylsilyl derivatized silica gel chromatographic support. 5 μ Apex-ODS indicates an
octadecylsilyl derivatized silica gel chromatographic support having a nominal particle size

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of 5 μ , made by Jones Chromatography Littleton Colorado YMC ODS-AQ® is an ODS chromatographic support and is a registered trademark of YMC Co Ltd Kyoto, Japan PRP 1® is a polymeric (styrene-divinylbenzene) chromatographic support, and is a registered trademark of Hamilton Co Reno Nevada. Celite® is a filter aid composed of acid washed diatomaceous silica, and is a registered trademark of Manville Corp. Denver Colorado

Preparation 1

10 Preparation of 6-(methylamino) 2-pyridylethanol

a) 2-(*tert* Butoxycarbonylamino)-6-picoline

A solution of 2-amino-6-picoline (21.63 g, 200 mmole) and di-*tert*-butyl dicarbonate (52.38 g, 240 mmole) in CH_2Cl_2 (200 mL) was concentrated on the rotavap at 50 °C, and the resulting residue was allowed to rotate on the rotavap at 50 °C under vacuum. After 21.5 hr the reaction was diluted with hexanes (400 mL) and filtered through silica gel (hexanes followed by 20% EtOAc/hexanes). Concentration left the title compound (41.84 g, quantitative) as a light yellow oil which gradually solidified on standing. ^1H NMR (250 MHz, CDCl_3) δ 7.71 (d, $J = 8.3$ Hz, 1 H) 7.40–7.65 (m, 2 H) 6.80 (d, $J = 7.5$ Hz, 1 H) 2.43 (s, 3 H) 1.50 (s, 9 H). MS (ES) m/e 153 ($M + H$, C_4H_9)⁺

b) 2-[(*tert* Butoxycarbonyl)methylamino]-6-picoline

NaH (60% in mineral oil, 3.60 g, 90 mmole) was added in portions over several min to a solution of 2-(*tert*-butoxycarbonylamino)-6-picoline (15.62 g, 75 mmole) and iodomethane (9.3 mL, 150 mmole) in anhydrous DMSO (75 mL) at 15 °C (cool water bath). The internal temperature rose to 35 °C. When gas evolution had subsided, the cool water bath was removed and the reaction was allowed to stir at RT. After 0.5 hr the dark yellow mixture was poured onto ice/ H_2O (300 mL) and extracted with Et_2O (3 x 300 mL). The combined organic layers were washed sequentially with H_2O (2 x 75 mL) and brine (75 mL). Drying (MgSO_4) and concentration left a yellow oil which was chromatographed on silica gel (7% EtOAc/hexanes). The title compound (13.01 g, 78%) was obtained as a faintly yellow oil. ^1H NMR (250 MHz, CDCl_3) δ 7.51 (app t, 1 H) 7.37 (d, $J = 8.2$ Hz, 1 H) 6.86 (d, $J = 7.2$ Hz, 1 H) 3.38 (s, 3 H) 2.49 (s, 3 H) 1.50 (s, 9 H). MS (ES) m/e 223 ($M + H$)⁺

c) Ethyl-6-[(*tert* butoxycarbonyl)methylamino] 2-pyridylacetate

LDA was prepared at 0 °C under argon from diisopropylamine (19.5 mL, 139.14 mmole) and 2.5 M *n*-BuLi in hexanes (46.4 mL, 115.95 mmole) in dry THF (350 mL).

This solution was cooled to -78 °C and a solution of 2-[(*tert*

- 5 butoxycarbonyl)methylamino]-6-picoline (10.31 g, 46.38 mmole) in dry THF (46 mL) was added dropwise over 10 min. Additional dry THF (2 mL) was used in transfer. The orange solution was stirred at -78 °C for 15 min, then diethyl carbonate (6.2 mL, 51.02 mmole) was added rapidly. The red solution was stirred at -78 °C for 15 min then was quenched with half saturated NH₄Cl (175 mL). The mixture was warmed to +5 °C and extracted with EtOAc (175 mL) then with CH₂Cl₂ (2 x 100 mL). The combined organics were washed with brine (100 mL), dried (MgSO₄) and concentrated. The cloudy yellow oil was chromatographed on silica gel (15% EtOAc/hexanes) to afford the title compound (10.72 g, 79%) as a light yellow oil. ¹H NMR (250 MHz, CDCl₃) δ 7.51-7.63 (m, 2 H), 6.91-7.03 (m, 1 H), 4.19 (q, *J* = 7.1 Hz, 2 H), 3.77 (s, 2 H), 3.38 (s, 3 H), 1.27 (t, *J* = 7.1 Hz, 3 H), 1.51 (s, 9 H). MS (ES) *m/e* 295 (M + H)⁺.

d) 6-[(*tert* Butoxycarbonyl)methylamino] 2-pyridylethanol

A solution of 2 N LiBH₄ in THF (7 mL, 14 mmole) was added via syringe to a stirred solution of ethyl-6-[(*tert* butoxycarbonyl)methylamino] 2-pyridylacetate (6.97 g, 23.7 mmole) in anhydrous THF (30 mL) under argon. The reaction was then slowly heated to reflux (initial exotherm). After 16 h at reflux, the reaction was cooled to 0 °C and carefully quenched with water (50 mL). The mixture was extracted with EtOAc (150 mL) and the organic layer was washed with brine, dried (Na₂SO₄) and concentrated. Purification by flash chromatography on silica gel (35% EtOAc/hexane) gave the title compound (5.26 g, 88%) as a clear oil. ¹H NMR (400 MHz, CDCl₃) δ 7.57 (m, 2 H), 6.88 (d, *J* = 7.2 Hz, 1 H), 4.01 (t, 2 H), 3.39 (s, 3 H), 3.00 (t, 2 H), 1.53 (s, 9 H). MS (ES) *m/e* 253.2 (M + H)⁺.

e) 6-(Methylamino)-2-pyridylethanol

- 30 To 6-[(*tert*-butoxycarbonyl)methylamino]-2-pyridylethanol (17.9 g, 71 mmole) was added a solution of 4N HCl in dioxane (200 mL). The reaction was stirred at room temperature for 1 h (gentle gas evolution was observed) then was concentrated to dryness. The product as the hydrochloride salt solidified under vacuum. The solid was dissolved in NaCl-saturated 1.0 N NaOH solution (75 mL) and the solution was extracted with Et₂O (2 x 200 mL). The combined organic layers were washed with brine, dried (Na₂SO₄) and concentrated to afford the title compound (9.12 g, 85%) as a waxy solid. ¹H NMR (400

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MHz, CDCl₃) δ 7.37 (t, 1 H) 6.42 (d, J = 7.3 Hz, 1 H) 6.27 (d, J = 8.3 Hz, 1 H) 4.62 (br s, 1 H) 3.96 (t, 2 H) 2.90 (d, J = 5.2 Hz, 3 H) 2.84 (t, 2 H) MS (ES) m/e 153 (M + H)⁺

Preparation 2

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Preparation of 2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)-1-ethanol

a) 2-(Pivaloylamino)pyridine

To a solution of 2-aminopyridine (94.12 g, 1 mole) and Et₃N (167.3 mL, 1.2 mole) in CH₂Cl₂ (1 L) was added pivaloyl chloride (135.5 mL, 1.1 mole) dropwise at 0 °C. The mixture was allowed to warm to RT as the bath warmed. After 18 hr the mixture was filtered. The filtrate was washed sequentially with H₂O (1.5 L) and saturated NaHCO₃ (2 x 1.5 L) then was dried (MgSO₄) and concentrated under reduced pressure to give the title compound (183 g, 103%) as an off white solid. ¹H NMR (300 MHz, CDCl₃) δ 8.28 (m, 2 H) 8.00 (br s, 1 H) 7.70 (m, 1 H) 7.03 (m, 1 H) 1.31 (s, 9 H) MS (ES) m/e 179 (M + H)⁺

Note ¹H NMR showed the presence of a small amount of *tert* butyl containing impurities, but the material is pure enough for use in the next step

b) 2-(Pivaloylamino)-3-pyridinecarboxaldehyde

To a solution of 2-(pivaloylamino)pyridine (17.8 g, 100 mmole) in dry THF (250 mL) at 20 °C was added n-BuLi (2.5 M solution in hexanes, 100 mL, 250 mmole) dropwise over 30 min. After 2 hr DMF (21 mL, 275 mmole) was added dropwise over 30 min. The mixture was allowed to warm to RT as the bath warmed. After 18 hr the mixture was quenched with saturated NH₄Cl (300 mL), and the resulting mixture was extracted with EtOAc (3 x 400 mL). The combined organic layers were dried (MgSO₄) and concentrated to give the title compound as a 2:1 mixture with 2-(pivaloylamino)pyridine (22 g). ¹H NMR (300 MHz, CDCl₃) δ 10.95 (br s, 1 H) 9.95 (s, 1 H) 8.69 (m, 1 H) 8.02 (m, 2 H) 7.20 (m, 1 H) 1.38 (s, 9 H) MS (ES) m/e 207 (M + H)⁺

Note The above procedure was repeated using 1-formylpiperidine (30.5 mL, 275 mmole) in place of DMF to give the title compound as a 4:1 mixture with 2-(pivaloylamino)pyridine (21 g)

c) 2-Amino-3-pyridinecarboxaldehyde

Crude 2-(pivaloylamino)-3-pyridinecarboxaldehyde (from step b, 43 g) was

dissolved in 3 M HCl (500 mL) and the solution was heated to reflux. After 18 hr the mixture was cooled to RT and the pH was carefully adjusted to 7 using solid K_2CO_3 . The aqueous solution was extracted with EtOAc (3 x 500 mL). The combined organic layers were dried ($MgSO_4$) and concentrated to give the title compound (24.57 g, 101%) as a

5 reddish brown solid. ^1H NMR (300 MHz, $CDCl_3$) δ 9.85 (s, 1 H), 8.24 (m, 1 H), 7.80 (m, 1 H), 6.90 (br s, 2 H), 6.74 (m, 2 H). MS (ES) m/e 123 ($M + H$) $^+$

d) 2-Methyl 1,8-naphthyridine

10 To a solution of 2-amino-3-pyridinecarboxaldehyde (from step c, 24.57 g) in $EtOH$ (750 mL) was added proline (2.3 g, 20 mmole). Then the mixture was heated to reflux. After 48 hr the mixture was cooled to RT, filtered, and concentrated. Flash column chromatography on silica gel (35% acetone/hexanes) gave the title compound (18.5 g, 64% over 3 steps) as an orangish-yellow solid. ^1H NMR (300 MHz, $CDCl_3$) δ 9.07 (m, 1H), 8.10 (m, 2 H), 7.40 (m, 2 H), 2.80 (s, 3 H). MS (ES) m/e 145 ($M + H$) $^+$

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e) 2-Methyl 5,6,7,8-tetrahydro-1,8-naphthyridine

A mixture of 2-methyl 1,8-naphthyridine (18.5 g, 128 mmole), 10% Pd/C (5 g) and absolute $EtOH$ (150 mL) was shaken under hydrogen (15 psi) on a Parr apparatus. After 24 hr the mixture was filtered through celite® and the filter pad was washed sequentially with absolute $EtOH$ and EtOAc. The filtrate was concentrated to dryness to leave the title compound (18.85 g, 99%) as an off-white solid. ^1H NMR (300 MHz, $CDCl_3$) δ 7.02 (d, $J = 7.5$ Hz, 1 H), 6.34 (d, $J = 7.5$ Hz, 1 H), 4.80 (br s, 1 H), 3.38 (m, 2 H), 2.74 (m, 2 H), 2.30 (s, 3 H), 1.88 (m, 2 H). MS (ES) m/e 149 ($M + H$) $^+$

25 f) 2-Methyl 8-(*tert*-butoxycarbonyl)-5,6,7,8-tetrahydro-1,8-naphthyridine

To a solution of 2-methyl 5,6,7,8-tetrahydro-1,8-naphthyridine (23.32 g, 157 mmole) and di-*tert*-butyl dicarbonate (44.74 g, 205 mmole) in dry THF (750 mL) at 0 °C was added LiHMDS (1.0 M solution in THF, 205 mL, 205 mmole) dropwise. 30 min after the addition was complete, the mixture was quenched with saturated NH_4Cl (500 mL) and extracted with EtOAc (3 x 500 mL). The combined organic layers were dried ($MgSO_4$) and concentrated under reduced pressure. Flash column chromatography on silica gel (40% EtOAc/hexanes) gave the title compound (32.3 g, 83%) as a light yellow oil. ^1H NMR (300 MHz, $CDCl_3$) δ 7.27 (d, $J = 7.6$ Hz, 1 H), 6.81 (d, $J = 7.6$ Hz, 1 H), 3.69–3.79 (m, 2 H), 2.65–2.75 (m, 2 H), 2.48 (s, 3 H), 1.83–1.98 (m, 2 H), 1.52 (s, 9 H). MS (ES) m/e 249 ($M + H$) $^+$

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g) Ethyl [8-(*tert* butoxycarbonyl)-5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl]acetate

To a solution of diisopropylamine (47.7 mL, 364 mmole) in dry THF (250 mL) at 0 °C was added *n*-BuLi (2.5 M in hexanes, 145.6 mL, 364 mmole) dropwise. After 15 min this solution was added dropwise to a solution of 2-methyl 8-(*tert* butoxycarbonyl)-5,6,7,8-tetrahydro-1,8-naphthyridine (32.3 g, 130 mmole) and diethyl carbonate (56.8 mL, 481 mmole) in dry THF (400 mL) at 78 °C. After 30 min the mixture was quenched with saturated NH₄Cl (500 mL), warmed to RT and extracted with EtOAc (3 x 500 mL). The combined organic extracts were dried (MgSO₄), filtered, and concentrated under reduced pressure. Drying under high vacuum overnight gave the title compound (42.52 g, 102%) as a light yellow oil. ¹H NMR (300 MHz, CDCl₃) δ 7.35 (d, *J* = 7.5 Hz, 1 H), 6.96 (d, *J* = 7.5 Hz, 1 H), 4.18 (t, 2H), 3.75 (m, 4 H), 2.72 (t, 2 H), 1.91 (m, 2 H), 1.52 (s, 9 H), 1.24 (m, 3H). MS (ES) *m/e* 321 (M + H)⁺.

Note: ¹H NMR showed a small amount of diethyl carbonate present in the product, but the material is pure enough for use in the next step.

h) 2-(5,6,7,8-Tetrahydro-1,8-naphthyridin-2-yl)-1-ethanol

To a solution of ethyl [8-(*tert* butoxycarbonyl)-5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl]acetate (42.52 g, 130 mmole) in dry THF (650 mL) at RT was added LiBH₄ (2.0 M in THF, 65 mL, 130 mmole) and the resulting mixture was heated to reflux. After 18 hr the mixture was cooled to 0 °C and carefully quenched with H₂O (300 mL). After 10 min, the mixture was extracted with EtOAc (3 x 500 mL). The combined organic extracts were dried (MgSO₄), filtered, and concentrated under reduced pressure.

The above residue was dissolved in CH₂Cl₂ (300 mL). To this solution was added 4 N HCl in dioxane (300 mL) slowly at RT. After 4 hr the mixture was concentrated under reduced pressure. The residue was taken up in a 1:1 mixture of 1.0 N NaOH and saturated NaCl (300 mL) and extracted with CH₂Cl₂ (3 x 300 mL). The combined organic extracts were dried (MgSO₄), filtered, and concentrated under reduced pressure. The residue was taken up in Et₂O (250 mL) and 96% formic acid (130 mmole) was added dropwise. The resulting solid was collected by filtration and washed with Et₂O (2 x 50 mL). The solid was dissolved in a 1:1 mixture of 1.0 N NaOH and saturated NaCl (300 mL) and the solution was extracted with CH₂Cl₂ (3 x 300 mL). The combined organic extracts were dried (MgSO₄), filtered, and concentrated under reduced pressure to give the title compound (11.1 g, 48% over 4 steps) as a yellow solid. ¹H NMR (300 MHz, CDCl₃) δ 7.05 (d, *J* = 7.6 Hz, 1 H), 6.33 (d, *J* = 7.6 Hz, 1 H), 3.90 (t, 2H), 3.39 (m, 2 H), 2.75 (t, 2 H), 2.70 (t, 2 H), 1.90 (m, 2 H). MS (ES) *m/e* 179 (M + H)⁺.

Preparation 3Preparation of ethyl (±)-4-(4-hydroxyphenyl)-3-[4-(trifluoromethyl)phenyl]butanoate

5 a) N-Methoxy-N-methyl-2-(4-methoxyphenyl)acetamide

To a solution of 4-methoxyphenylacetic acid (3.3 g, 20 mmole) in dry DMF (75 mL) was added N-methoxy-N-methylamine hydrochloride (1.95 g, 20 mmole), Et₃N (3.1 mL, 22 mmole), HOBt · H₂O (1.95 g, 22 mmole), and EDC (2.7 g, 22 mmole). The solution was stirred at RT overnight, then was concentrated in vacuum. The residue was taken up in 5% Na₂CO₃ solution (10 mL) and extracted with CH₂Cl₂ (3 x 20 mL). The combined organic layers were dried over MgSO₄, filtered and concentrated. The residue was chromatographed on silica gel (3% MeOH/CH₂Cl₂) to give the title compound (1.54 g, 37%) as white solid. MS (ES) *m/e* 210 (M + H)⁺.

15 b) 2-(4-Methoxyphenyl)-1-[4-(trifluoromethyl)phenyl]ethanone

To a solution of *sec*-BuLi (1.3 M in THF, 22.6 mL, 29.5 mmole) in dry THF (50 mL) was added 4-bromobenzotrifluoride (3.3 g, 14.7 mmole) in dry THF (20 mL) dropwise at 78 °C. After 20 min, N-methoxy-N-methyl-2-(4-methoxyphenyl)acetamide (1.5 g, 7.4 mmole) in dry THF (20 mL) was added dropwise. After 1 hr the mixture was quenched with saturated NH₄Cl (10 mL), warmed to RT, and extracted with Et₂O (3 x 20 mL). The combined organic layers were dried over MgSO₄, filtered, and concentrated. The residue was chromatographed on silica gel (15% EtOAc/hexanes) to give the title compound (2.2 g, 100%) as a slightly yellow solid. TLC (15% EtOAc/hexanes) *R_f* 0.81. ¹H NMR (300 MHz, CDCl₃) δ 8.10 (d, *J* = 8.2 Hz, 2 H), 7.72 (d, *J* = 8.2 Hz, 2 H), 7.18 (d, *J* = 8.7 Hz, 2 H), 6.88 (d, *J* = 8.7 Hz, 2 H), 4.25 (s, 2 H), 3.79 (s, 3 H).

c) Ethyl (±)-4-(4-methoxyphenyl)-3-[4-(trifluoromethyl)phenyl]crotonate

To a suspension of 60% NaH (350 mg, 8.84 mmole) in dry toluene (30 mL) was added triethyl phosphonoacetate (2.0 g, 8.84 mmole) in dry toluene (20 mL) dropwise at RT. After 15 min, a solution of 2-(4-methoxyphenyl)-1-[4-(trifluoromethyl)phenyl]ethanone (1.3 g, 4.42 mmole) in dry toluene (15 mL) was added dropwise, and the solution was heated to reflux. After 16 hr the reaction was quenched with saturated NH₄Cl (10 mL) and the mixture was extracted with EtOAc (3 x 20 mL). The combined organic layers were dried over MgSO₄, filtered, and concentrated. The title compound (2.2 g, 56% a mixture of two components) was obtained as a light yellow oil. TLC (5% EtOAc/hexanes) *R_f* 0.42, 0.44. This material was used without further purification.

d) Ethyl (±)-4-(4-methoxyphenyl)-3-[4-(trifluoromethyl)phenyl]butanoate

To a suspension of 10% Pd/C (600 mg) in absolute EtOH (50 mL) was added ethyl (±)-4-(4-methoxyphenyl)-3-[4-(trifluoromethyl)phenyl]crotonate (2.2 g, 6 mmole) and the mixture was shaken on a Parr apparatus at RT under H₂ (50 psi). After 4 hr the mixture was filtered through a pad of celite® and the filtrate was concentrated. This reaction sequence was repeated three times. The residue was chromatographed on silica gel (35% EtOAc/hexanes) to afford the title compound (900 mg, 56%) as an oil. TLC (5% EtOAc/hexanes) R_f 0.46. ¹H NMR (300 MHz, CDCl₃) δ 7.51 (d, J = 8.2 Hz, 2 H), 7.24 (d, J = 8.2 Hz, 2 H), 6.94 (d, J = 8.7 Hz, 2 H), 6.76 (d, J = 8.7 Hz, 2 H), 3.99 (q, J = 7.1 Hz, 2 H), 3.76 (s, 3 H), 3.35 (s, 3 H), 3.50 (m, 1 H), 2.75 (m, 2 H), 2.93 (m, 2 H), 2.69 (dd, J = 15.6, 6.4 Hz, 1 H), 2.60 (dd, J = 15.6, 8.9 Hz, 1 H), 1.11 (t, J = 7.1 Hz, 3 H).

e) Ethyl (±)-4-(4-hydroxyphenyl)-3-[4-(trifluoromethyl)phenyl]butanoate

To a solution of ethyl (±)-4-(4-methoxyphenyl)-3-[4-(trifluoromethyl)phenyl]butanoate (450 mg, 1.23 mmole) in CH₂Cl₂ (5 mL) was added BBr₃ (1.5 mL, 1.48 mmole) at 10 °C. After 3 hr the mixture was carefully quenched with EtOH (10 mL) and the solution was allowed to warm to RT. The mixture was concentrated and the residue was chromatographed on silica gel (20% EtOAc/hexanes) to give the title compound (270 mg, 63%) as a yellow oil. TLC (20% EtOAc/hexanes) R_f 0.22.

Preparation 425 Preparation of methyl (±)-3-[4-carboxy-1,3-oxazol-2-yl]-4-[4-[(tert-butylloxycarbonyl)oxy]phenyl]butanoate

a) 4-Bromo-1-(trisopropylsilyloxy)benzene

To a solution of 4-bromophenol (17.3 g, 100 mmole) in dry DMF (100 mL) at RT was added imidazole (13.62 g, 200 mmole) followed by trisopropylsilyl chloride (22.5 mL, 105 mmole). After 4 hr the mixture was diluted with H₂O (50 mL) and extracted with hexanes (3 x 75 mL). The combined organic layers were dried over MgSO₄, filtered, and concentrated to give the title compound (32.23 g, 100%) as a clear oil which was used without purification. ¹H NMR (300 MHz, CDCl₃) δ 7.29 (d, J = 6 Hz, 2 H), 6.71 (d, J = 6 Hz, 2 H), 1.22 (m, 3 H), 1.09 (m, 18 H).

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b) Methyl 3-(benzyloxycarbonyl)-3 butenoate

Diisopropyl azodicarboxylate (32.8 mL, 166 mmole) was added to a solution of methyl 3-carboxy 3-butenate (20 g, 139 mmole) benzyl alcohol (17.2 mg, 166 mmole) and triphenylphosphine (43.7 g, 166 mmole) in anhydrous THF (500 mL) at 0 °C. The mixture was allowed to warm as the bath warmed to RT. After 3 hr the mixture was concentrated and the residue was chromatographed on silica gel (10% EtOAc/hexanes). The title compound (29.46 g, 91%) was obtained as a colorless oil. ¹H NMR (300 MHz, CDCl₃) δ 7.35 (m, 5 H) 6.48 (s, 1 H) 5.71 (s, 1 H) 5.20 (s, 2 H) 3.63 (s, 3 H) 3.37 (s, 2 H)

c) Methyl (±)-4-(4-(transpropylsilyloxy)phenyl) 3-carboxybutanoate

A solution of 4-bromo-1-(transpropylsilyloxy)benzene (33.23 g, 100 mmole) methyl 3-(benzyloxycarbonyl)-3-butenate (28.11 g, 120 mmole) Pd(OAc)₂ (2.24 g, 10 mmole) P(o-tolyl)₃ (6.09 g, 20 mmole) and (i-Pr)₂NEt (34.8 mL, 200 mmole) in propionitrile (350 mL) was deoxygenated (3 x evacuation/N₂ purge cycles) then was heated to reflux. After 18 hr the mixture was concentrated and the residue was chromatographed on silica gel (10% EtOAc/hexanes) to give a yellow oil. The oil was taken up in 5% EtOAc/hexanes (100 mL), and the solution was allowed to stand at RT. After 72 hr the mixture was filtered and the filtrate was concentrated to give crude methyl (±)-4-(4-(transpropylsilyloxy)phenyl)-3-(benzyloxycarbonyl)-3 butenoate as a mixture of olefin isomers. This was used immediately in the next step.

The above olefin mixture was divided into two parts. Each part was reacted in the following manner then combined after filtration. To a suspension of 10% Pd/C (7.4 g) in EtOAc (100 mL) was added the above olefin mixture. The mixture was deoxygenated (3 x evacuation/N₂ purge cycles) then was charged with H₂ (50 psi). After 4 hr the H₂ was removed and the mixture was filtered through a pad of celite®. The filtrate was concentrated to afford the title compound (24.64 g, 89% from 4-bromo-1-(transpropylsilyloxy)benzene) as a clear oil. ¹H NMR (300 MHz, CDCl₃) δ 7.01 (d, J = 6 Hz, 2 H) 6.80 (d, J = 6 Hz, 2 H) 3.62 (s, 3 H) 3.05 (m, 2 H) 2.65 (m, 1 H) 2.40 (m, 2 H) 1.21 (m, 3 H) 1.09 (m, 18 H)

d) (±)-N [2-[4-(transpropylsilyloxy)benzyl] 3-(carbomethoxy)propionyl]serine benzyl ester

To a solution of methyl (±)-3-carboxy-4-[4-(transpropylsilyloxy)phenyl]butanoate (5.00 g, 12.67 mmole) in dry DMF (60 mL) at RT was added serine benzyl ester hydrochloride (3.52 g, 15.21 mmole) HOBt (2.06 g, 15.21 mmole) Et₃N (5.3 mL, 38.01 mmole) and EDC (2.92 g, 15.21 mmole). After 18 hr the mixture was concentrated. The

residue was chromatographed on silica gel (80% EtOAc/hexanes) to give the title compound (5.76 mg, 79%) as a pale yellow oil MS (ES) m/e 572 (M + H)⁺

e) Methyl (±)-3-[4-(benzyloxycarbonyl)-1,3-oxazolin-2-yl]-4-[4-(*triisopropylsilyloxy*)phenyl]butanoate

To a solution of (±) N [2-[4-(*triisopropylsilyloxy*)benzyl]-3-(carbomethoxy)propionyl]serine benzyl ester (5.76 g, 10.07 mmole) in dry THF (50 mL) was added Burgess reagent (2.88 g, 12.08 mmole) then the mixture was heated to reflux. After 2 hr the mixture was cooled to RT and concentrated. The residue was chromatographed on silica gel (35% EtOAc/hexanes) to give the title compound (4.45 g, 80%) as a clear oil MS (ES) m/e 554 (M + H)⁺

f) Methyl (±)-3-[4-(benzyloxycarbonyl)-1,3-oxazol-2-yl]-4-[4-(*triisopropylsilyloxy*)phenyl]butanoate

To a solution of methyl (±)-3-[4-(benzyloxycarbonyl)-1,3-oxazolin-2-yl]-4-[4-(*triisopropylsilyloxy*)phenyl]butanoate (4.45 g, 8.03 mmole) in CH₂Cl₂ (40 mL) at 0 °C was added DBU (1.4 mL, 9.64 mmole) followed by bromotrichloromethane (0.95 mL, 9.64 mmole). The mixture was allowed to warm to RT as the bath warmed. After 18 hr the mixture was concentrated. The residue was chromatographed on silica gel (20% EtOAc/hexanes) to give the title compound (2.23 g, 50%) as a clear oil MS (ES) m/e 552 (M + H)⁺

g) Methyl (±)-3-[4-(benzyloxycarbonyl)-1,3-oxazol-2-yl]-4-(4-hydroxyphenyl)butanoate

To a solution of methyl (±)-3-[4-(benzyloxycarbonyl)-1,3-oxazol-2-yl]-4-[4-(*triisopropylsilyloxy*)phenyl]butanoate (2.23 g, 4.04 mmole) in dry THF (20 mL) at 0 °C was added a solution of TBAF in THF (1.0 M, 6.06 mL, 6.06 mmole). After 2 hr the mixture was diluted with saturated NH₄Cl (10 mL) and extracted with CH₂Cl₂ (3 x 15 mL). The combined organic layers were dried over MgSO₄, filtered, and concentrated. The residue was chromatographed on silica gel (40% EtOAc/hexanes) to give the title compound (1.4 g, 88%) as an off white foam. MS (ES) m/e 396 (M + H)⁺

h) Methyl (±)-3-[4-(benzyloxycarbonyl)-1,3-oxazol-2-yl]-4-[4-[(*tert*-butyloxycarbonyl)oxy]phenyl]butanoate

To a solution of methyl (±)-3-[4-(benzyloxycarbonyl)-1,3-oxazol-2-yl]-4-(4-hydroxyphenyl)butanoate (700 mg, 1.77 mmole) and di-*tert*-butyl dicarbonate (463 mg, 2.12 mmole) in dry THF (10 mL) was added pyridine (0.17 mL, 2.12 mmole) at RT. After

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18 hr the mixture was concentrated. The residue was triturated with hexanes, filtered, and dried *in vacuo* to give the title compound (765 mg, 87%) as a white solid. MS (ES) *m/e* 496 (M + H)⁺

- 5 1) Methyl (±) 3-[4-carboxy-1,3-oxazol-2-yl]-4-[4-[(*tert*-butyloxycarbonyl)oxy]phenyl]butanoate

A mixture of methyl (±)-3-[4-(benzyloxycarbonyl)-1,3-oxazol-2-yl]-4-[4-[(*tert*-butyloxycarbonyl)oxy]phenyl]butanoate (765 mg, 1.54 mmole) and 10% Pd/C (164 mg) in EtOH (20 mL) was deoxygenated (3 x evacuation/N₂ purge cycles) then was charged with H₂ (50 psi). After 4 hr the H₂ was removed and the mixture was filtered through a pad of celite®. The filtrate was concentrated to afford the title compound (659 mg, 100%) as a white solid. MS (ES) *m/e* 811 (2M + H)⁺

Preparation 5

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Preparation of methyl (±)-3-[4-(trifluoromethyl)thiazol-2-yl]-4-(4-hydroxyphenyl)butanoate

- a) 2-Cyanomethyl-4-(trifluoromethyl)thiazole

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2-Cyanothioacetamide (1.00 g, 9.99 mmole) and 3-bromo-1-trifluoropropan-2-one (1.04 mL, 9.99 mmole) were combined in absolute EtOH (50 mL) and heated to reflux. After 18 hr the mixture was cooled to RT and concentrated. The residue was chromatographed on silica gel (15% EtOAc/hexanes) to give the title compound (1.24 g) as a 2:1 mixture with ethyl 2-cyanoacetate. MS (ES) *m/e* 385 (2M + H)⁺

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- b) 3-(4-Benzyloxyphenyl)-2-[(4-trifluoromethyl)thiazol-2-yl]acrylonitrile

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NaH (516 mg, 60% dispersion in mineral oil, 12.9 mmole) was reacted with absolute EtOH (10 mL) at 0 °C. After gas evolution had ceased the mixture was warmed to RT. 4-Benzyloxybenzaldehyde (2.05 g, 9.68 mmole) was added all at once as a solid. To this mixture was added dropwise a solution of 2-cyanomethyl-4-(trifluoromethyl)thiazole (1.24 g) in absolute EtOH (20 mL). The reaction was stirred for 4 hr then the solid was collected by filtration and washed with hexanes to give the title compound (1.64 g, 42% over 2 steps) as a yellow solid. ¹H NMR (300 MHz, CDCl₃) δ 8.25 (s, 1 H), 8.00 (d, J = 6 Hz, 2 H), 7.79 (s, 1 H), 7.40 (m, 5 H), 7.10 (d, J = 6 Hz, 2 H), 5.18 (s, 2 H).

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c) 3-(4-Benzoyloxyphenyl)-2-cyano-2-[(4-trifluoromethyl)thiazol 2-yl]oxirane

To a solution of 3-(4-benzoyloxyphenyl)-2-[(4-trifluoromethyl)thiazol 2-yl]acrylonitrile (500 mg, 1.29 mmole) in CH₃CN (5 mL) was added neutral alumina (1.3 g). Clorox bleach (5 mL) was added dropwise at RT. After 1 hr the mixture was filtered. The filtrate was diluted with H₂O (20 mL) and extracted with CH₂Cl₂ (3 x 20 mL). The combined organic layers were dried over MgSO₄, filtered, and concentrated to give the title compound (425 mg, 82%) as a yellow oil which was sufficiently pure for use in the next step. ¹H NMR (300 MHz, CDCl₃) δ 7.90 (s, 1 H), 7.40 (m, 7 H), 7.06 (d, J = 6 Hz, 2 H), 5.10 (s, 2 H), 4.59 (s, 1 H).

d) 2-(4-Benzoyloxyphenyl)-1-[4-(trifluoromethyl)thiazol 2-yl]ethanone

To a solution of 3-(4-benzoyloxyphenyl)-2-cyano-2-[(4-trifluoromethyl)thiazol 2-yl]oxirane (425 mg, 1.06 mmole) in CH₂Cl₂ (7 mL) was added Et₃SiH (0.85 mL, 5.3 mmole) then BF₃·OEt₂ (0.39 mL, 3.17 mmole) dropwise at 0 °C. After 2 hr the mixture was poured into H₂O (20 mL) and extracted with CH₂Cl₂ (3 x 20 mL). The combined organic layers were dried over MgSO₄, filtered, and concentrated.

To the above residue in dry THF (7 mL) was added a solution of TBAF in THF (1.0 M, 1.6 mL, 1.6 mmole) at 0 °C. After 1 hr the mixture was poured into saturated NH₄Cl (20 mL) and extracted with EtOAc (3 x 20 mL). The combined organic layers were dried over MgSO₄, filtered, and concentrated. The residue was chromatographed on silica gel (5% EtOAc/hexanes) to give the title compound (167 mg, 40%) as a white solid. ¹H NMR (300 MHz, CDCl₃) δ 8.05 (s, 1 H), 7.35 (m, 7 H), 6.92 (d, J = 6 Hz, 2 H), 5.05 (s, 2 H), 4.40 (s, 1 H).

e) Methyl (±)-3-[4-(trifluoromethyl)thiazol-2-yl]-4-(4-hydroxyphenyl)butanoate

To a suspension of NaH (35 mg, 0.88 mmole) in dry THF (2 mL) was added triethyl phosphonoacetate (0.17 mL, 0.88 mmole) dropwise at RT. After 15 min, a solution of 2-(4-benzoyloxyphenyl)-1-[4-(trifluoromethyl)thiazol-2-yl]ethanone (167 mg, 0.44 mmole) in dry THF (2 mL) was added dropwise, and the mixture was heated to reflux. After 18 hr the mixture was cooled to RT, quenched with saturated NH₄Cl (10 mL) and extracted with EtOAc (3 x 20 mL). The combined organic layers were dried over MgSO₄, filtered, and concentrated to afford crude ethyl (±)-3-[4-(trifluoromethyl)thiazol 2-yl]-4-(4-benzoyloxyphenyl)crotonate as an oil. This was used without purification.

Ethyl (±)-3-[4-(trifluoromethyl)thiazol 2-yl]-4-(4-benzoyloxyphenyl)crotonate (0.44 mmole, crude) was dissolved in MeOH (4 mL) and magnesium turnings (53 mg, 2.20 mmole) were added at RT. After 72 hr the mixture was poured into 10% HCl (75 mL) and extracted with CH₂Cl₂ (3 x 50 mL). The combined organic layers were dried over

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MgSO₄ filtered and concentrated to afford crude methyl (±) 3[4-(trifluoromethyl)thiazol 2-yl]-4-(4-benzyloxyphenyl)butanoate. This was used without purification

- To a solution of methyl (±)-3[4-(trifluoromethyl)thiazol 2-yl]-4-(4-benzyloxyphenyl)butanoate (0.44 mmole, crude) in EtSH (5 mL) at RT was added BF₃ OEt₂ (0.3 mL). After 18 hr additional BF₃ OEt₂ (0.3 mL) was added. After another 18 hr the mixture was cooled to 0 °C and carefully quenched with saturated NaHCO₃. The resulting mixture was extracted with CH₂Cl₂ (3 x 25 mL). The combined organic layers were dried over MgSO₄, filtered and concentrated. The residue was chromatographed on silica gel (30% EtOAc/hexanes) to give the title compound (123 mg, 81% over 3 steps) as a yellow oil. MS (ES) *m/e* 346 (M + H)⁺.

Preparation 6

Preparation of methyl (±)-3-(5-methylthiazol 2-yl)-4-(4-hydroxyphenyl)butanoate

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a) Methyl 4-(benzyloxy)phenylacetate

- To a suspension of K₂CO₃ (20.7 g, 150 mmole) in acetone (50 mL) was added methyl 4-hydroxyphenyl acetate (5.0 g, 30 mmole) and benzyl chloride (10.4 mL, 90 mmole) and the mixture was heated to reflux. After 24 hr the mixture was cooled to RT, filtered and concentrated. The residue was chromatographed on silica gel (10% EtOAc/hexanes) to afford the title compound (7.7 g, 100%) as a white solid. ¹H NMR (300 MHz, CDCl₃) δ 7.40 (m, 5 H), 7.21 (d, J = 6.6 Hz, 2 H), 6.95 (d, J = 6.6 Hz, 2 H), 5.05 (s, 2 H), 3.70 (s, 3 H), 3.59 (s, 2 H).

25 b) 2-(4-Benzyloxyphenyl)-1-(5-methylthiazol 2-yl)ethanone

- To a solution of 5-methylthiazole (0.21 mL, 2.34 mmole) in dry THF (10 mL) was added *n*-BuLi (0.94 mL, 2.5 M solution in hexanes, 2.34 mmole) dropwise at -78 °C. After 15 min methyl 4-benzyloxyphenyl acetate (0.5 g, 1.95 mmole) in dry THF (5 mL) was added dropwise. After 30 min the mixture was quenched with saturated NH₄Cl (10 mL), warmed to RT and extracted with EtOAc (3 x 20 mL). The combined organic layers were dried over MgSO₄, filtered, and concentrated. The residue was chromatographed on silica gel (15% EtOAc/hexanes) to give the title compound as a white solid (532 mg, 51%). MS (ES) *m/e* 324 (M + H)⁺.

35 c) Ethyl (±)-4-(4-benzyloxyphenyl)-3-(5-methylthiazol 2-yl)crotonate

To a suspension of NaH (79 mg, 1.98 mmole) in dry THF (2 mL) was added triethyl phosphonoacetate (0.39 mL, 1.98 mmole) dropwise at RT. After 15 min a solution

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of 2-(4-benzyloxyphenyl)-1-(5-methylthiazol-2-yl)ethanone (320 mg, 0.99 mmole) in dry THF (3 mL) was added dropwise, and the mixture was heated to reflux. After 18 hr the mixture was cooled to RT, quenched with saturated NH_4Cl (10 mL) and extracted with EtOAc (3 x 20 mL). The combined organic layers were dried over MgSO_4 , filtered, and concentrated. The resulting yellow oil was used in the next step without purification. MS (ES) m/e 394 ($\text{M} + \text{H}$)⁺.

d) Methyl (±)-3-(5-methylthiazol-2-yl)-4-(4-benzyloxyphenyl)butanoate

Ethyl (±)-3-(5-methylthiazol-2-yl)-4-(4-benzyloxyphenyl)crotonate (0.99 mmole, crude) was dissolved in MeOH (4 mL) and magnesium turnings (120 mg, 4.95 mmole) were added at RT. After 72 hr the mixture was poured into 10% HCl (75 mL) and extracted with CH_2Cl_2 (3 x 50 mL). The combined organic layers were dried over MgSO_4 , filtered, and concentrated to afford the crude title compound. This was used without purification. MS (ES) m/e 382 ($\text{M} + \text{H}$)⁺.

e) Methyl (±)-3-(5-methylthiazol-2-yl)-4-(4-hydroxyphenyl)butanoate

To a solution of methyl (±)-3-(5-methylthiazol-2-yl)-4-(4-benzyloxyphenyl)butanoate (0.99 mmole, crude) in EtSH (5 mL) at RT was added $\text{BF}_3 \cdot \text{OEt}_2$ (0.6 mL). After 18 hr additional $\text{BF}_3 \cdot \text{OEt}_2$ (0.6 mL) was added. After another 18 hr the mixture was cooled to 0 °C and carefully quenched with saturated NaHCO_3 . The resulting mixture was extracted with CH_2Cl_2 (3 x 25 mL). The combined organic layers were dried over MgSO_4 , filtered, and concentrated. The residue was chromatographed on silica gel (50% EtOAc/hexanes) to give the title compound (249 mg, 86% over 3 steps) as a yellow solid. MS (ES) m/e 292 ($\text{M} + \text{H}$)⁺.

Example 1

Preparation of (±)-4-[4-[2-[6-(methylamino)pyridin-2-yl]-1-ethoxy]phenyl]-3-[4-(trifluoromethyl)phenyl]butanoic acid

a) Ethyl (±)-4-[4-[2-[6-(methylamino)pyridin-2-yl]-1-ethoxy]phenyl]-3-[4-(trifluoromethyl)phenyl]butanoate

Diisopropyl azodicarboxylate (0.15 mL, 0.767 mmole) was added over 2 min to a solution of ethyl (±)-4-(4-hydroxyphenyl)-3-[4-(trifluoromethyl)phenyl]butanoate (270 mg, 0.767 mmole), 6-(methylamino)-2-pyridylethanol (130 mg, 0.92 mmole), and triphenylphosphine (200 mg, 0.767 mmole) in anhydrous THF (5 mL) at 0 °C under N_2 . The yellow solution was kept at 0 °C for 10 min, then was warmed to RT. After 24 hr the

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reaction was concentrated and the residue was chromatographed on silica gel (20 EtOAc/hexanes) The title compound (200 mg, 54%) was obtained as a colorless oil MS (ES) m/e 487 ($M + H$)⁺

- 5 b) (±)-4-[4-[2-[6-(Methylamino)pyridin-2-yl]-1-ethoxy]phenyl]-3-[4-(trifluoromethyl)phenyl]butanoic acid

1 0 N NaOH (8.2 mL, 0.823 mmole) was added dropwise to a cooled (15 °C) solution of ethyl (±)-4-[4-[2-[6-(methylamino)pyridin-2-yl]-1-ethoxy]phenyl]-3-[4-(trifluoromethyl)phenyl]butanoate (200 mg, 0.41 mmole) in MeOH (3 mL) and the mixture
10 was stirred at RT for 24 hr. The resulting solution was concentrated in vacuum and the residue was dissolved in H₂O (5 mL). The pH was adjusted to 5 with 1.0 N HCl and the precipitate was collected, washed with small amount of water and dried in vacuum at 60 °C. The title compound (120 mg, 64%) was obtained as a white, foamy solid. MS (ES) m/e 459 ($M + H$)⁺. Anal. Calcd for C₂₅H₂₅FN₂O₃ · 0.85 H₂O: C, 63.38; H, 5.68; N, 5.91. Found: C, 63.23; H, 5.41; N, 5.73.

Example 2

20 Preparation of (±)-4-[4-[2-[6-(methylamino)pyridin-2-yl]-1-ethoxy]phenyl]-3-[4-[(N-methyl-N-phenylamino)carbonyl]-1,3-oxazol-2-yl]butanoic acid

- a) Methyl (±)-3-[4-[(N-methyl-N-phenylamino)carbonyl]-1,3-oxazol-2-yl]-4-(4-hydroxyphenyl)butanoate

To a solution of methyl (±)-3-[4-carboxy-1,3-oxazol-2-yl]-4-[4-[(*tert*-butyloxycarbonyl)oxy]phenyl]butanoate (150 mg, 0.37 mmole) (*i*-Pr)₂NEt (0.1 mL, 0.56 mmole), pyridine (0.09 mL, 1.11 mmole), and *N*-methylaniline (0.06 mL, 0.56 mmole) in dry DMF (2 mL) was added BPPFH (382 mg, 1.11 mmole) at RT. After 18 hr the mixture was concentrated. The residue was taken up in 10% HCl (20 mL) and extracted with CH₂Cl₂ (3 x 20 mL). The combined organic layers were dried over MgSO₄, filtered, and
30 concentrated.

The above residue was dissolved in 4 N HCl in dioxane (10 mL) at RT. After 18 hr the mixture was concentrated. The residue was chromatographed on silica gel (75% EtOAc/hexanes) to give the title compound (146 mg) as an orange foam contaminated with bis(pentamethylene)urea. MS (ES) m/e 417 ($M + H$)⁺

b) Methyl (±)-4-[4-[2-(6-methylaminopyridin-2-yl)-1-ethoxy]phenyl]-3-[4-[(N-methyl N-phenylamino)carbonyl]-1,3-oxazol-2-yl]]butanoate

Diisopropyl azodicarboxylate (0.15 mL, 0.74 mmole) was added to a solution of methyl (±)-3-[4-[(N-methyl N-phenylamino)carbonyl]-1,3-oxazol-2-yl]-4-(4-hydroxyphenyl)butanoate (146 mg, 0.37 mmole), 6-(methylamino)-2-pyridylethanol (113 mg, 0.74 mmole) and triphenylphosphine (194 mg, 0.74 mmole) in CH₂Cl₂ (2 mL) at 0 °C. The mixture was allowed to warm to RT as the bath warmed. After 18 hr the mixture was concentrated and the residue was chromatographed on silica gel (50% THF/hexanes). Fractions containing the product were concentrated to give the title compound (322 mg) contaminated with triphenylphosphine oxide. MS (ES) *m/e* 529 (M + H)⁺

c) (±)-4-[4-[2-[6-(Methylamino)pyridin-2-yl]-1-ethoxy]phenyl]-3-[4-[(N-phenyl N-methylamino)carbonyl]-1,3-oxazol-2-yl]]butanoic acid

To a solution of methyl (±)-4-[4-[2-(6-methylaminopyridin-2-yl)-1-ethoxy]phenyl]-3-[4-[(N-methyl N-phenylamino)carbonyl]-1,3-oxazol-2-yl]]butanoate (322 mg) in 1:1 THF/H₂O (2 mL) at RT was added 1.0 N LiOH (0.35 mL, 0.35 mmole). After 18 hr the mixture was acidified to pH 6 using 10% HCl then was concentrated to dryness. The residue was purified by reverse-phase HPLC (gradient: 10-80% CH₃CN/H₂O containing 0.1% TFA). The fractions containing the product were combined and concentrated to remove CH₃CN. The resulting aqueous solution was lyophilized to give the title compound (33 mg, 29% over 3 steps) as a white solid. MS (ES) *m/e* 515 (M + H)⁺. Anal. Calcd for C₂₉H₃₀N₄O₅ · 1.85 TFA: C, 54.13; H, 4.42; N, 7.72. Found: C, 54.17; H, 4.50; N, 7.52.

Example 3

Preparation of (±)-4-[4-[2-[6-(methylamino)pyridin-2-yl]-1-ethoxy]phenyl]-3-[4-(morpholin-4-yl)carbonyl]-1,3-oxazol-2-yl]-butanoic acid

a) Methyl (±)-3-[4-(morpholin-4-yl)carbonyl]-1,3-oxazol-2-yl]-4-(4-hydroxyphenyl)butanoate

To a solution of methyl (±)-3-[4-carboxy-1,3-oxazol-2-yl]-4-[4-[(*tert*-butyloxycarbonyl)oxy]phenyl]butanoate (150 mg, 0.37 mmole), (i-Pr)₂NEt (0.1 mL, 0.56 mmole), pyridine (0.09 mL, 1.11 mmole) and morpholine (0.05 mL, 0.56 mmole) in dry DMF (2 mL) was added BPPFH (382 mg, 1.11 mmole) at RT. After 18 hr the mixture was concentrated. The residue was taken up in 10% HCl (20 mL) and extracted with CH₂Cl₂.

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(3 x 20 mL) The combined organic layers were dried over MgSO_4 filtered and concentrated

The above residue was dissolved in 4 N HCl in dioxane (10 mL) at RT After 18 hr the mixture was concentrated The residue was chromatographed on silica gel (100%

5 EtOAc) to give the title compound (122 mg, 88%) as a clear oil ^1H NMR (300 MHz, CDCl_3) δ 8.05 (s, 1 H) 7.88 (d, $J = 6.6$ Hz, 2 H) 6.70 (d, $J = 6.6$ Hz, 2 H) 5.9 (s, 1 H) 3.73 (bs, 8 H) 3.62 (s, 3 H) 2.85 (m, 5 H)

10 b) Methyl ($\pm$)-4-[4-[2-(6-methylaminopyridin-2-yl)-1-ethoxy]phenyl]-3-[4-(morpholin-4-yl)carbonyl]-1,3-oxazol-2-yl]butanoate

Diisopropyl azodicarboxylate (0.13 mL, 0.66 mmole) was added to a solution of methyl ($\pm$)-3-[4-(morpholin-4-yl)carbonyl]-1,3-oxazol-2-yl]-4-(4-hydroxyphenyl)butanoate (122 mg, 0.37 mmole) 6-(methylamino)-2-pyridylethanol (100 mg, 0.66 mmole) and triphenylphosphine (173 mg, 0.66 mmole) in CH_2Cl_2 (2 mL) at 0 °C. The mixture was
15 allowed to warm to RT as the bath warmed. After 18 hr the mixture was concentrated and the residue was chromatographed on silica gel (100% EtOAc) Fractions containing the product were concentrated to give the title compound (94 mg) contaminated with triphenylphosphine oxide MS (ES) m/e 509 ($\text{M} + \text{H}$)⁺

20 c) ($\pm$)-4-[4-[2-[6-(Methylamino)pyridin-2-yl]-1-ethoxy]phenyl]-3-[4-(morpholin-4-yl)carbonyl]-1,3-oxazol-2-yl]-butanoic acid

To a solution of methyl ($\pm$)-4-[4-[2-(6-methylaminopyridin-2-yl)-1-ethoxy]phenyl]-3-[4-(morpholin-4-yl)carbonyl]-1,3-oxazol-2-yl]butanoate (94 mg, 0.18 mmole) in 1:1 THF/ H_2O (2 mL) at RT was added 1.0 N LiOH (0.25 mL, 0.25 mmole)
25 After 18 hr the mixture was acidified to pH 6 using 10% HCl then was concentrated to dryness The residue was purified by reverse-phase HPLC (gradient, 15-35% $\text{CH}_3\text{CN}/\text{H}_2\text{O}$ containing 0.1% TFA) The fractions containing the product were combined and concentrated to remove CH_3CN The resulting aqueous solution was lyophilized to give the title compound (23 mg, 26% over 2 steps) as a white solid MS (ES) m/e 495 ($\text{M} + \text{H}$)⁺ Anal. Calcd for $\text{C}_{26}\text{H}_{30}\text{N}_4\text{O}_6$ 1.75 TFA. C, 49.76 H, 4.78 N 7.87 Found C, 49.93 H, 4.97 N 7.84
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Example 4

Preparation of (±)-4-[4-[2-[6-(methylamino)pyridin 2-yl] 1-ethoxy]phenyl] 3-[4-[[N methyl N-(2,2,2-trifluoroethyl)amino]carbonyl] 1,3-oxazol 2-yl] butanoic acid

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a) Methyl (±) 3 [4-[[N methyl N (2,2,2-trifluoroethyl)amino]carbonyl]-1 3-oxazol 2-yl] 4-(4-hydroxyphenyl)butanoate

To a solution of methyl (±)-3 [4-carboxy 1,3-oxazol 2-yl]-4-[4-[(*tert* butyloxycarbonyl)oxy]phenyl]butanoate (150 mg, 0.37 mmole) (i Pr)₂NHt (0.1 mL, 0.56 mmole) pyridine (0.09 mL, 1.11 mmole) and N methyl N-(2,2,2 trifluoroethyl)amine hydrochloride (84 mg, 0.56 mmole) in dry DMF (2 mL) was added BPPFH (382 mg, 1.11 mmole) at RT. After 18 hr the mixture was concentrated. The residue was taken up in 10% HCl (20 mL) and extracted with CH₂Cl₂ (3 x 20 mL). The combined organic layers were dried over MgSO₄, filtered, and concentrated.

The above residue was dissolved in 4 N HCl in dioxane (10 mL) at RT. After 18 hr the mixture was concentrated. The residue was chromatographed on silica gel (60% EtOAc/hexanes) to give the title compound (298 mg) as a clear oil contaminated with bis(pentamethylene)urea. The oil was used in the next step without further purification.

b) Methyl (±)-4-[4-[2-(6-methylaminopyridin 2-yl) 1-ethoxy]phenyl] 3-[4-[[N methyl N (2,2,2-trifluoroethyl)amino]carbonyl] 1 3-oxazol 2-yl]]butanoate

Diisopropyl azodicarboxylate (0.15 mL, 0.74 mmole) was added to a solution of methyl (±) 3-[4-[[N methyl N-(2,2,2-trifluoroethyl)amino]carbonyl] 1 3-oxazol 2-yl]-4-(4-hydroxyphenyl)butanoate (298 mg, 0.37 mmole), 6-(methylamino)-2-pyridylethanol (113 mg, 0.74 mmole) and triphenylphosphine (194 mg, 0.74 mmole) in CH₂Cl₂ (2 mL) at 0

C. The mixture was allowed to warm to RT as the bath warmed. After 18 hr the mixture was concentrated and the residue was chromatographed on silica gel (40% EtOAc/hexanes). Fractions containing the product were concentrated to give the title compound (115 mg) contaminated with triphenylphosphine oxide. MS (ES) *m/e* 535 (M + H)⁺

c) (±)-4-[4-[2-[6-(Methylamino)pyridin 2-yl] 1-ethoxy]phenyl]-3-[4-[[N methyl N (2,2,2-trifluoroethyl)amino]carbonyl]-1,3-oxazol 2-yl]-butanoic acid

To a solution of methyl (±)-4-[4-[2-(6-methylaminopyridin 2-yl)-1 ethoxy]phenyl] 3-[4-[[N methyl-N-(2,2,2-trifluoroethyl)amino]carbonyl]-1,3-oxazol 2-yl]]butanoate (115 mg, 0.22 mmole) in 1:1 THF/H₂O (2 mL) at RT was added 1.0 N LiOH (0.32 mL, 0.32 mmole). After 18 hr the mixture was acidified to pH 6 using 10% HCl then

was concentrated to dryness. The residue was purified by reverse-phase HPLC (gradient 10-80% CH₃CN/H₂O containing 0.1% TFA). The fractions containing the product were combined and concentrated to remove CH₃CN. The resulting aqueous solution was lyophilized to give the title compound (42 mg, 37% over 3 steps) as a white solid. MS (ES)

5 *m/e* 522 (M + H)⁺ Anal. Calcd for C₂₅H₂₇F₃N₄O₅ · 1.4 TFA C 49.09 H 4.21 N 8.24 Found C, 49.18 H, 4.18 N 8.22

Example 5

10 Preparation of (±)-4-[4-[2-[6-(methylamino)pyridin-2-yl]-1-ethoxy]phenyl]-3-[4-(trifluoromethyl)thiazol-2-yl]butanoic acid

a) Methyl (±)-4-[4-[2-[6-(methylamino)pyridin-2-yl]-1-ethoxy]phenyl]-3-[4-(trifluoromethyl)thiazol-2-yl]butanoate

15 Diisopropyl azodicarboxylate (0.14 mL, 0.71 mmole) was added to a solution of methyl (±)-3-[4-(trifluoromethyl)thiazol-2-yl]-4-hydroxyphenyl]butanoate (123 mg, 0.37 mmole), 6-(methylamino)-2-pyridylethanol (108 mg, 0.71 mmole) and triphenylphosphine (186 mg, 0.71 mmole) in CH₂Cl₂ (2 mL) at 0 °C. The mixture was allowed to warm to RT as the bath warmed. After 18 hr the mixture was concentrated and the residue was
20 chromatographed on silica gel (40% EtOAc in 1:1 toluene/hexanes). Fractions containing the product were concentrated to give the title compound (114 mg, contaminated with reduced DIAD). MS (ES) *m/e* 480 (M + H)⁺

b) (±)-4-[4-[2-[6-(Methylamino)pyridin-2-yl]-1-ethoxy]phenyl]-3-[4-(trifluoromethyl)thiazol-2-yl]butanoic acid

25 To a solution of methyl (±)-4-[4-[2-[6-(methylaminopyridin-2-yl)-1-ethoxy]phenyl]-3-[4-(trifluoromethyl)thiazol-2-yl]butanoate (114 mg, 0.24 mmole) in 1:1 THF/H₂O (2 mL) at RT was added 1.0 N LiOH (0.36 mL, 0.36 mmole). After 18 hr the mixture was acidified to pH 6 using 10% HCl. The resulting solid was collected by
30 filtration and dried *in vacuo* at 50 °C to give the title compound (43 mg, 42%) as a white solid. MS (ES) *m/e* 466 (M + H)⁺ Anal. Calcd for C₂₂H₂₂F₃N₃O₃S · 0.2H₂O C 56.33 H 4.81 N 8.96 Found C, 56.34 H, 4.69 N 8.88

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140Example 6Preparation of (±)-4-[4-[2-(6-methylaminopyridin-2-yl)-1-ethoxy]phenyl]-3-(5-methylthiazol-2-yl)butanoic acid

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a) Methyl (±)-4-[4-[2-(6-Methylaminopyridin-2-yl)-1-ethoxy]phenyl]-3-(5-methylthiazol-2-yl)butanoate

Dusopropyl azodicarboxylate (0.25 mL, 1.28 mmole) was added to a suspension of methyl (±)-4-[4-(4-hydroxyphenyl)-3-(5-methylthiazol-2-yl)butanoate (249 mg, 0.85 mmole) 10 6-(methylamino)-2-pyridylethanol (194 mg, 1.28 mmole) and triphenylphosphine (336 mg, 1.28 mmole) in TBME (5 mL) at 0 °C. The mixture was allowed to warm to RT as the bath warmed. After 72 hr the mixture was concentrated and the residue was chromatographed on silica gel (30% EtOAc/CHCl₃). Fractions containing the product were concentrated to give the title compound (385 mg, contaminated with triphenylphosphine oxide). MS (ES) 15 *m/e* 426 (M + H)⁺

b) (±)-4-[4-[2-(6-Methylaminopyridin-2-yl)-1-ethoxy]phenyl]-3-(5-methylthiazol-2-yl)butanoic acid

To a solution of methyl (±)-4-[4-[2-(6-methylaminopyridin-2-yl)-1-ethoxy]phenyl]-3-(5-methylthiazol-2-yl)butanoate (0.85 mmole) in 1:1 THF/H₂O (5 mL) 20 was added 1.0 N NaOH (1.28 mL, 1.28 mmole). After 18 hr the mixture was acidified to pH 6 using 10% HCl then concentrated to dryness. The residue was chromatographed on silica gel (EtOH) to give the title compound as a yellow solid (217 mg, 62% over 2 steps). MS (ES) *m/e* 412 (M + H)⁺. Anal. Calcd for C₂₂H₂₅N₃O₃S 1.2 H₂O: C, 61.01; H, 6.38; N 9.70. Found: C, 61.25; H, 6.06; N 9.32. 25

Examples 7-38

The following compounds are prepared using the procedures described in the previous examples 30

Example 7 (±)-4-[4-[2-(6-(Methylamino)pyridin-2-yl)-1-ethoxy]phenyl]-3-(pyridin-4-yl)butanoic acid

Example 8. (±)-4-[4-[2-(6-(Methylamino)pyridin-2-yl)-1-ethoxy]phenyl]-3-(pyridin-3-yl)butanoic acid,

35 Example 9 (±)-4-[4-[2-(6-(Methylamino)pyridin-2-yl)-1-ethoxy]phenyl]-3-(quinolin-3-yl)butanoic acid,

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Example 10 (±)-4-[4-[2-[6-(Methylamino)pyridin-2-yl]-1-ethoxy]phenyl]-3-(6-methoxypyridin-3-yl)butanoic acid,

Example 11 (±)-4-[4-[2-[6-(Methylamino)pyridin-2-yl]-1-ethoxy]phenyl]-3-(pyrimidin-5-yl)butanoic acid,

5 Example 12 (±)-4-[4-[2-[6-(Methylamino)pyridin-2-yl]-1-ethoxy]phenyl]-3-(3-fluorophenyl)butanoic acid

Example 13 (±)-4-[4-[2-[6-(Methylamino)pyridin-2-yl]-1-ethoxy]phenyl]-3-(thiazol-4-yl)butanoic acid

10 Example 14 (±)-4-[4-[2-[6-(Methylamino)pyridin-2-yl]-1-ethoxy]phenyl]-3-(thiazol-5-yl)butanoic acid —

Example 15 (S)-4-[4-[2-[6-(Methylamino)pyridin-2-yl]-1-ethoxy]phenyl]-3-(pyridin-4-yl)butanoic acid,

Example 16 (S)-4-[4-[2-[6-(Methylamino)pyridin-2-yl]-1-ethoxy]phenyl]-3-(pyridin-3-yl)butanoic acid

15 Example 17 (S)-4-[4-[2-[6-(Methylamino)pyridin-2-yl]-1-ethoxy]phenyl]-3-(quinolin-3-yl)butanoic acid

Example 18 (S)-4-[4-[2-[6-(Methylamino)pyridin-2-yl]-1-ethoxy]phenyl]-3-(6-methoxypyridin-3-yl)butanoic acid,

20 Example 19 (S)-4-[4-[2-[6-(Methylamino)pyridin-2-yl]-1-ethoxy]phenyl]-3-(pyrimidin-5-yl)butanoic acid

Example 20 (S)-4-[4-[2-[6-(Methylamino)pyridin-2-yl]-1-ethoxy]phenyl]-3-(3-fluorophenyl)butanoic acid

Example 21 (S)-4-[4-[2-[6-(Methylamino)pyridin-2-yl]-1-ethoxy]phenyl]-3-(thiazol-4-yl)butanoic acid,

25 Example 22 (S)-4-[4-[2-[6-(Methylamino)pyridin-2-yl]-1-ethoxy]phenyl]-3-(thiazol-5-yl)butanoic acid

Example 23 (±)-3-(Pyridin-4-yl)-4-[4-[2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)-1-ethoxy]phenyl]butanoic acid

30 Example 24 (±)-3-(Pyridin-3-yl)-4-[4-[2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)-1-ethoxy]phenyl]butanoic acid

Example 25 (±)-3-(Quinolin-3-yl)-4-[4-[2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)-1-ethoxy]phenyl]butanoic acid,

Example 26 (±)-3-(6-Methoxypyridin-3-yl)-4-[4-[2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)-1-ethoxy]phenyl]butanoic acid

35 Example 27 (±)-3-(Pyrimidin-5-yl)-4-[4-[2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)-1-ethoxy]phenyl]butanoic acid

138
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+42

Example 28 (±)-3-(3-Fluorophenyl)-4-[4-[2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)-1-ethoxy]phenyl]butanoic acid

Example 29 (±)-3-(Thiazol-4-yl)-4-[4-[2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)-1-ethoxy]phenyl]butanoic acid

5 Example 30 (±)-3-(Thiazol-5-yl)-4-[4-[2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)-1-ethoxy]phenyl]butanoic acid,

Example 31 (S)-3-(Pyridin-4-yl)-4-[4-[2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)-1-ethoxy]phenyl]butanoic acid

10 Example 32 (S)-3-(Pyridin-3-yl)-4-[4-[2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)-1-ethoxy]phenyl]butanoic acid

Example 33 (S)-3-(Quinolin-3-yl)-4-[4-[2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)-1-ethoxy]phenyl]butanoic acid

Example 34 (S)-3-(6-Methoxypyridin-3-yl)-4-[4-[2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)-1-ethoxy]phenyl]butanoic acid

15 Example 35 (S)-3-(Pyrimidin-5-yl)-4-[4-[2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)-1-ethoxy]phenyl]butanoic acid

Example 36 (S)-3-(3-Fluorophenyl)-4-[4-[2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)-1-ethoxy]phenyl]butanoic acid

20 Example 37 (S)-3-(Thiazol-4-yl)-4-[4-[2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)-1-ethoxy]phenyl]butanoic acid and

Example 38 (S)-3-(Thiazol-5-yl)-4-[4-[2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)-1-ethoxy]phenyl]butanoic acid

Example 39

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Parenteral Dosage Unit Composition

A preparation which contains 20 mg of the compound of Example 1 as a sterile dry powder is prepared as follows: 20 mg of the compound is dissolved in 15 mL of distilled water. The solution is filtered under sterile conditions into a 25 mL multi-dose ampoule and lyophilized. The powder is reconstituted by addition of 20 mL of 5% dextrose in water (DSW) for intravenous or intramuscular injection. The dosage is thereby determined by the injection volume. Subsequent dilution may be made by addition of a metered volume of this dosage unit to another volume of DSW for injection, or a metered dose may be added to another mechanism for dispensing the drug, as in a bottle or bag for IV drip infusion or other injection/infusion system.

Example 40Oral Dosage Unit Composition

- 5 A capsule for oral administration is prepared by mixing and milling 50 mg of the compound of Example 1 with 75 mg of lactose and 5 mg of magnesium stearate. The resulting powder is screened and filled into a hard gelatin capsule

Example 41

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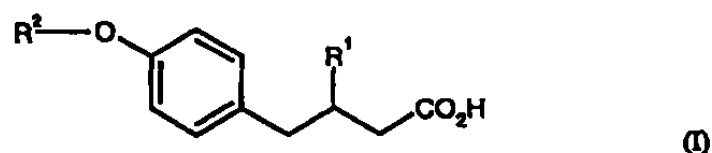
Oral Dosage Unit Composition

- 15 A tablet for oral administration is prepared by mixing and granulating 20 mg of sucrose, 150 mg of calcium sulfate dihydrate and 50 mg of the compound of Example 1 with a 10% gelatin solution. The wet granules are screened, dried, mixed with 10 mg starch, 5 mg talc and 3 mg stearic acid, and compressed into a tablet.

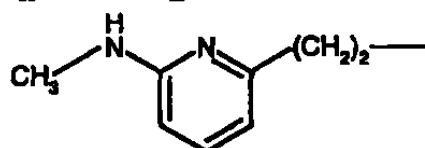
- 20 The above description fully discloses how to make and use the present invention. However, the present invention is not limited to the particular embodiments described hereinabove, but includes all modifications thereof within the scope of the following claims. The various references to journals, patents and other publications which are cited herein comprises the state of the art and are incorporated herein by reference as though fully set forth.

What is claimed is

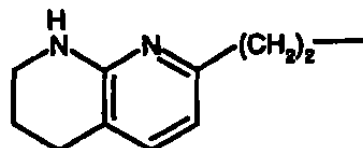
- 1 A compound according to formula (I)



wherein

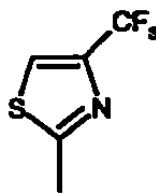
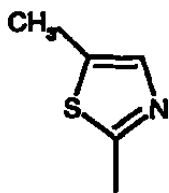
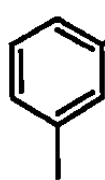
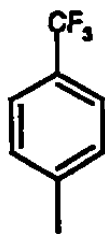
 R^1 is Het or Ar R^2 is

or

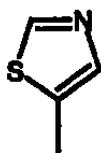
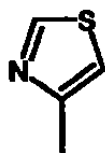
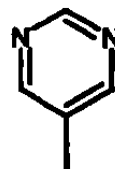
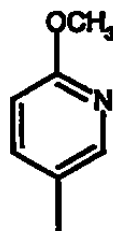
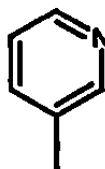
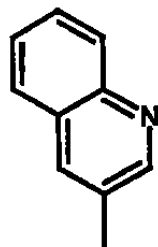
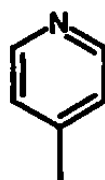


- 10 or a pharmaceutically acceptable salt thereof

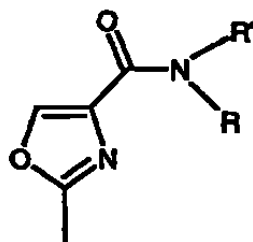
2. A compound according to claim 1 in which R^1 is



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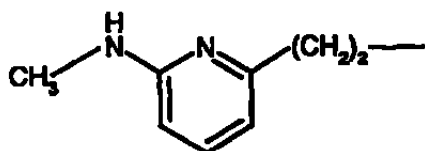
or



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in which R is C₁₋₄alkyl and R' is phenyl, benzyl or -CH₂CF₃ or R and R' are joined to form a morpholinyl ring.

3 A compound according to claim 1 in which R² is



4 A compound which is.

(±)-4-[4-[2-[6-(methylamino)pyridin-2-yl] 1-ethoxy]phenyl] 3-[4-(trifluoromethyl)phenyl]butanoic acid,

(±)-4-[4-[2-[6-(methylamino)pyridin-2-yl] 1-ethoxy]phenyl] 3-[4-[(N methyl N phenylamino)carbonyl] 1,3-oxazol-2-yl]butanoic acid,

(±)-4-[4-[2-[6-(methylamino)pyridin-2-yl] 1-ethoxy]phenyl] 3-[4-[(morpholin-4-yl)carbonyl] 1,3-oxazol-2-yl]butanoic acid

(±)-4-[4-[2-[6-(methylamino)pyridin-2-yl] 1-ethoxy]phenyl] 3-[4-[[N methyl N (2,2,2-trifluoroethyl)amino]carbonyl]-1,3-oxazol-2-yl]butanoic acid

(±)-4-[4-[2-[6-(methylamino)pyridin-2-yl] 1-ethoxy]phenyl] 3-[4-(trifluoromethyl)thiazol-2-yl]butanoic acid or

(±)-4-[4-[2-[6-(methylamino)pyridin-2-yl] 1-ethoxy]phenyl]-3-(3-methylthiazol-2-yl)butanoic acid

or a pharmaceutically acceptable salt thereof

5 A pharmaceutical composition which comprises a compound according to claim 1 and a pharmaceutically acceptable carrier

6 A pharmaceutical composition which comprises a compound according to claim 1 an antineoplastic agent and a pharmaceutically acceptable carrier

7 The pharmaceutical composition according to claim 6 wherein the antineoplastic agent is topotecan or cisplatin.

8 A pharmaceutical composition which comprises a compound according to claim 1 an inhibitor of bone resorption and a pharmaceutically acceptable carrier

142
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446

9 A method of treating a disease state in which antagonism of the $\alpha_v\beta_3$ receptor is indicated which comprises administering to a subject in need thereof a compound according to claim 1

5 10 A method of treating a disease state in which antagonism of the $\alpha_v\beta_5$ receptor is indicated which comprises administering to a subject in need thereof a compound according to claim 1

10 11 A method of treating osteoporosis which comprises administering to a subject in need thereof a compound according to claim 1

12 A method for inhibiting angiogenesis tumor growth or tumor metastasis which comprises administering to a subject in need thereof a compound according to claim 1

15 13 A method of treating atherosclerosis, restenosis or inflammation which comprises administering to a subject in need thereof a compound according to claim 1

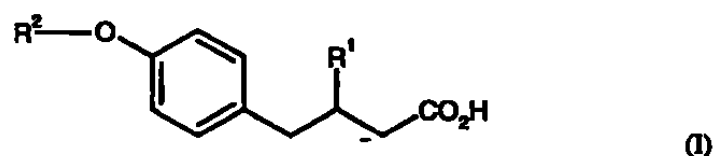
20 14 A method of inhibiting tumor growth which comprises administering stepwise or in physical combination a compound according to claim 1 and an antineoplastic agent

25 15 The method according to claim 14 wherein the antineoplastic agent is topotecan or cisplatin

16 A method of treating osteoporosis or inhibiting bone loss which comprises administering stepwise or in physical combination a compound according to claim 1 and an inhibitor of bone resorption.

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~~157~~
~~147~~ABSTRACT OF THE DISCLOSUREVitronectin Receptor Antagonists

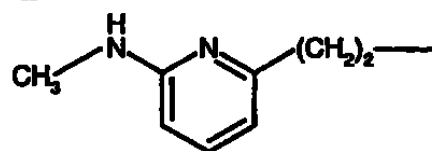
Compounds of the formula (I) are disclosed which are vitronectin receptor antagonists and are useful in the treatment of osteoporosis



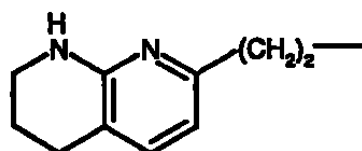
wherein

R¹ is Het or Ar

R² is



or



or a pharmaceutically acceptable salt thereof

144₅₈
448

ESTADOS UNIDOS DE AMÉRICA

A TODOS A QUIENES LLEGUE EL PRESENTE

DEPARTAMENTO DE COMERCIO DE LOS ESTADOS UNIDOS

Oficina de Marcas y Patentes

01 de septiembre del 2000

LA PRESENTE CERTIFICA QUE LA ADJUNTA ES UNA COPIA TOMADA DE LOS REGISTROS DE LA OFICINA DE MARCAS Y PATENTES DE LOS ESTADOS UNIDOS DE LOS DOCUMENTOS DE LA SOLICITUD DE PATENTE ABAJO IDENTIFICADA Y LA CUAL CUMPLE CON LOS REQUISITOS PARA QUE LE SEA CONFERIDA UNA FECHA DE RADICACIÓN SEGUN 35 USC 111

NUMERO DE SOLICITUD 60/152 780

FECHA DE RADICACIÓN 07 de septiembre de 1999

Por Autoridad del

COMISIONADO DE MARCAS Y PATENTES

(FIRMA) T WALLACE

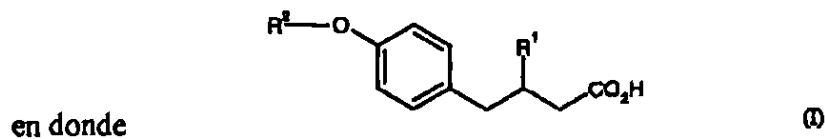
Oficial de Certificaciones

(SELLO DORADO DE OBLEA)

ANTAGONISTAS DEL RECEPTOR DE VITRONECTINA

REIVINDICACIONES

- 1 Un compuesto segun la formula (I)



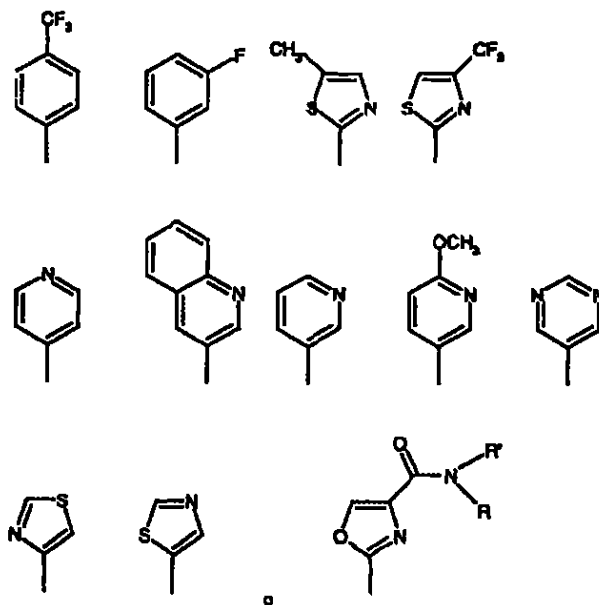
R¹ es Het o Ar

R² es



o una sal farmacéuticamente aceptable de lo mismo

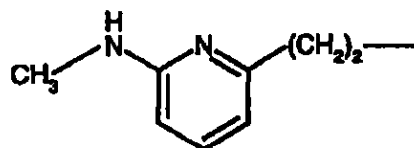
- 2 Un compuesto segun la reivindicacion 1, en el cual R¹ es



146₆₀
150

en el cual R' es alquil C₁₋₄ y R es fenil, bencil o -CH₂CF₃, o R' y R'' se unen para formar un anillo de morfolinilo

3 Un compuesto segun la reivindicacion 1 en el cual R² es



4 Un compuesto que es

(±)-4-[4-[2-[6-(metilamino)piridin-2-il]-1-etoxi]fenil]-3-[4-(trifluorometil)fenil]acido butanoico,

(±)-4-[4-[2-[6-(metilamino)piridin-2-il]-1-etoxi]fenil]-3-[4-[(N metil-N-fenilamino)carbonil]-1,3-oxazol-2-il]acido butanoico,

(±)-4-[4-[2-[6-(metilamino)piridin-2-il]-1-etoxi]fenil]-3-[4-[(morfolin-4-il)carbonil]-1,3-oxazol-2-il]acido butanoico,

(±)-4-[4-[2-[6-(metilamino)piridin-2-il]-1-etoxi]fenil]-3-[4-[(N-metil-N-(2,2,2-trifluoroetil)amino)carbonil]-1,3-oxazol-2-il]acido butanoico,

(±)-4-[4-[2-[6-(metilamino)piridin-2-il]-1-etoxi]fenil]-3-[4-(trifluorometil)tiazol-2-il]acido butanoico, o

(±)-4-[4-[2-[6-(metilamino)piridin-2-il]-1-etoxi]fenil]-3-(3-metiltiazol-2-il) acido butanoico,

o una sal farmaceuticamente aceptable de lo mismo

5 Una composicion farmaceutica que comprende un compuesto segun la reivindicacion 1 y un vehiculo farmaceuticamente aceptable

147
81
151

6 Una composicion farmaceutica que comprende un compuesto segun la reivindicacion 1, un agente antineoplastico y un vehiculo farmaceuticamente aceptable

7 La composicion farmaceutica segun la reivindicacion 6, en la cual el agente antieoplastico es topotecan o cisplatin

8 Una composicion farmaceutica que comprende un compuesto segun la reivindicacion 1, un inhibidor de resorcion osea y un vehiculo farmaceuticamente aceptable

9 Un metodo para el tratamiento de un estado de enfermedad en el cual se indica el antagonismo del receptor $\alpha_v\beta_3$, que consiste en administrar a un sujeto que lo requiera, un compuesto segun la reivindicacion 1

10 Un metodo para el tratamiento de un estado de enfermedad en el cual se indica el antagonismo del receptor $\alpha_v\beta_5$, que consiste en administrar a un sujeto que lo requiera, un compuesto segun la reivindicacion 1

11 Un metodo para el tratamiento de osteoporosis que consiste en administrar a un sujeto que lo requiera, un compuesto segun la reivindicacion 1

12 Un metodo para inhibir angiogenesis, crecimiento tumoral o metastasis tumoral que consiste en administrar a un sujeto que lo requiera, un compuesto segun la reivindicacion 1

13 Un metodo para el tratamiento de aterosclerosis, restenosis o inflamacion que consiste en administrar a un sujeto que lo requiera, un compuesto segun la reivindicacion 1

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14 Un metodo para inhibir crecimiento tumoral que consiste en administrar por paso o en combinacion fisica, un compuesto segun la reivindicacion 1 y un agente antineoplastico

15 El metodo segun la reivindicacion 14, en donde el agente antineoplastico es topotecan o cisplatino

16 Un metodo para el tratamiento de osteoporosis o para inhibir perdida osea, que consiste en administrar por pasos o en combinacion fisica, un compuesto segun la reivindicacion 1 y un inhibidor de resorcion osea

2do EXAMEN DE FORMA PATENTE DE INVENCION

Folio 184

Radicación no 00-67158

Concepto Técnico no 836

Clasificación Internacional de Patentes A61K 31/435
31/295

Practicado el examen de acuerdo a lo establecido en el Art. 38 de la Decisión 486 se determinó que esta solicitud de **Patente de Invención**

- SI [☒] NO [] Contiene un resumen de la invención (Art.26-e)
- SI [☒] NO [] Contiene el título o nombre de la invención (Art.27 d)
- SI [☒] NO [] Contiene la descripción de la invención (Art.26-b)
- SI [☒] NO [] NO ES APLICABLE [] Contiene los dibujos necesarios para comprender la invención (Art.26 d)
- SI [☒] NO [] Contiene una o más reivindicaciones (Art.26-c)
- SI [] NO [] NO ES APLICABLE [☒] Han sido desarrollados los productos y/o procedimientos a partir de recursos genéticos o de sus productos derivados de los que cualquiera de los Países Miembros es país de origen (Art. 26- h)
- SI [] NO [] NO ES APLICABLE [☒] Los productos y/o procedimientos han sido obtenidos o desarrollados a partir de los conocimientos tradicionales de las comunidades indígenas afroamericanas o locales de los países miembros de acuerdo a lo establecido en la Decisión 391 y sus modificaciones y reglamentaciones vigentes (Art.26-i)
- SI [] NO [☒] La invención se refiere a un producto relativo a un material biológico (Art.26-J)
- SI [☒] NO [] La prioridad coincide con la materia reivindicada
- SI [☒] NO [] **CUMPLE LOS REQUISITOS TÉCNICOS**

OBSERVACIONES Y OTROS SI [] NO [☒] ver hoja(s) anexa(s)

EXAMINADOR TÉCNICO
202039

Bogotá D C 15 Abril / 2002

2020
Bogotá D C

Doctor(a)
GERMÁN CASTILLO GRAU
Apoderado(a)

REFERENCIA	Radicación No	00-67158
	Trámite	002
	Evento	001
	Actuación	416
	Oficio No	811
	Folios	001

Teniendo en cuenta que la solicitud de privilegio de patente que se tramita bajo el expediente indicado en la referencia reúne los requisitos de forma establecidos en los artículos 26 y 27 de la Decisión 486 de la Comisión de la comunidad andina y en las demás disposiciones vigentes sobre la materia, se envía el extracto de la solicitud a la Oficina de comunicaciones para efecto de su publicación en la Gaceta de Propiedad Industrial conforme lo establece el artículo 40 de la misma Decisión

Cúmplase

Dado en Bogotá D C a los

06 JUN 2002

ALIX CÉSPEDES DE VERGEL

Jefe de la División de Nuevas Creaciones

Bogotá, D C

Gaceta de Propiedad Industrial
expiró el

El extracto de esta solicitud fue publicado en el numero 520 de la
de fecha El término hábil señalado por la ley
y SI___NO___ se presentaron oposiciones por parte de terceros

202039

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DIVISION DE NUEVAS CREACIONES

RADICACIÓN EXPEDIENTE No 00-67158

EL EXTRACTO DE LA PRESENTE SOLICITUD DE **PATENTE DE INVENCION** FUE PUBLICADO EN
LA GACETA DE PROPIEDAD INDUSTRIAL No 520 DE FECHA 27 DE **SEPTIEMBRE DE 2002** EL
TERMINO HABIL SEÑALADO POR LA LEY EXPIRA EL 26 DE **DICIEMBRE DE 2002**

NOTA Dentro del plazo de los seis (6) meses siguientes a partir de la fecha de publicación en la gaceta de Propiedad Industrial deberá solicitar y cancelar el examen de patentabilidad so pena de que la solicitud carga en abandono de acuerdo con lo establecido en el Artículo 44 de la Decisión 486/2000

SE PRESENTARON OPOSICIONES POR PARTE DE TERCEROS

SI _____

NO X