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SEOR
SUPERINTENDENTE DE INDUSTRIA Y COMERCIO

L.

E. S. D.

REF: Expediente 06-078-330

TITULO SOLICITADO: PIRAZOLOPIRIMIDINAS NOVEDOSAS COMO
INHIBIDORES DE QUINASA CICLINA-
DEPENDIENTES

PUBLICACION; Gaceta 583 N° de publicación 708

PRIORIDAD: US 101776.988 de 11/0212004

SOLICITANTE: SCHERING CORPORATION Y PHARMACOPEIA
DRUG DISCOVERY, INC.

APODERADO: J. IAN RAISBECK

TRAMITE: SUSTENTACION DE OPOSICION

JOSÉ LUIS REYES VILLAMIZAR, mayor de edad, identificado como aparece al pie de ml firma, abogado en ejercicio, titular de la tarieta profesional nümero 44655 del Consejo Superior de la Judicatura, obrando en calidad de apoderado de la sociedad LAFRANCOL S.A., dentro del trÆmitede la referenda, en ejercicio de 10 dispuesto por el artIculo 42 inciso 21 de la Decision 486 de 2000, por media del presente escrito, procedo a sustentar y complementar la oposiciOn presentada en los siguientes tØrminos:

I. COMPLEMENTO DE INFORMACION

Comedidamente me perrnito presentar los argumentos que demuestran que la solicitud de la referencia incumple los requisitos de patentabilidad establecidos por

Carrera 17 No. 88-23 Of. 205 Tels.: +57 1 655 7795, +57 1621263 1, Fax: +57 1 621 2542 inforeyes-abogados.com - www.reyes-abogados.com - Bogota, Colombia.

los artIculos 14, 16, 18, 20 d) y 21 de la Decision 486 de 2000, y que por ello no merece recibir el beneficio patentario, veamos:

> En primer lugar, la lectura simple del capItulo reivindicatorio de la solicitud colombiana en trÆmite, especIficamente en lasreivindicaciones 32 a 40, revela que se pretende patentar un mØtodo terapŒuticoUtil en el tratamiento de diversos tipos de cancer, que comprende la inhibiciOn de quinasas ciclina-dependientes a partir de la utilización de compuestos de la fOrmula:

In

HNR

solos o en combinaciOn con otros agentes
antineoplÆsicosy/o radioterapia; con Ia cual dicha solicitud en trÆmite incurre directamente en uria improcedencia de patentabilidad, ya que como explIcitamente se menciona en la DecisiOn 486 de 2000, especificamente en el articulo 20 literal d) del titulo II, capitulo I de dicha decision, "No serÆn patentables (..) d) los mØtodos terapØuticos o quirÆrgicospara el tratamiento humano a animal, asi como los mØtodos de diagnostico aplicados a los seres humanos o a animales"

’ La patente americana US 6,099,593 publicada el 31 de diciembre de 1997 y titulada "COMPOSITIONS FOR DYEING KERATIN FIBERS CONTAINING PYRAZOLO (1,5-A) PYRIMIDINE DERIVATIVES AND DYEING PROCESSES" y equivalente a su vez de la solicitud internacional WO 97/49378 titulada "PYRAZOLO-(1,5-a)-PYRIMIDINE DERIVATIVES, THEIR PREPARATION AND HAIR DYES CONTAINING THESE" revela nuevas composiciones para la tinciOn oxidativa de fibras de queratina, que comprende al menos un derivado especifico de pirazolo [1,5-al pirimidina, un proceso de tinciOn usando esa composiciOn, nuevos derivados de pirazolo [1.5-al pirimiding y procesos para su preparaciOn.

El ejemplo 4 de cualquiera de los dos documentos mericionados, se refiere a la obtenciôn de un compuesto coma es el 3-amino-7--hydroxyethyIamino-5-methylpyrazolo [1,5-a] pyrimidine Dihydrochloride de fOrmula:

1 - 3f

HO
NH
WN

H3CN
NH2

Este compuesto tal como se ha venido mencionando, es un derivado de pirazolo [1,5-a] pirimidina el cuat a su vez es muy similar a los compuestos revelados en la solicitud colombiana en trÆmite de formula:

3 Fe
N

R4rNN
HNR

ya que en ambos casos los compuestos poseen el
mismo nücleo principal y son denvados de pirazolo [1,5-a] pirirnidina (ver recuadro verde); esto nos Hevarla a afirmar que dichos compuestos carecen de nivel inventivo puesto que tienen el mismo nCicleo principal y sobre ellos pueden sugenrse y reempiazarse por un gran nümero de sustituyentes.

Ahora bien, podriamos estar hablando de compuestos idØnticos Si se tiene en cuenta no solo el nucleo principal sino tambiØn algunos de los sustituyentes mencionados en La sohcitud colombiana en trÆmite, veamos:
US 6,099593 SOLICITUD EN TRAMITE Co 06-078-330
Ejempto4: Rvl,3,5-7,1O,19:

Fe

H3C"eN R
NH

- H-

R= aiquilo sustituido con por ejemplo OR5

R2=R9

R3= Aiquilo
R4=H
R5=H
R9= NR5R10

R10=H

Como se observa, los sustituyentes mencionados en las reivindicaciones 1, 2, 3, 5, \N3
6, 7, 10 y 19 de la solicitud colombiana en trÆmitey su reemplazo sobre el nücleo
principal de pirazolo [1,5-a] pirimidina permiten obtener compuestos idØnticos a los revelados en las anterioridades mencionadas (ejemplo 4), afectando en consecuencia la novedad de la solicitud en trÆmite(Rvs 1,2,3,5-7, 10 y 19) y de las composiciones que los contienen (Rv 41).

Otro punto a resaltar es que los compuestos revelados en la US 6,099,593 son utilizados en la tinción oxidativa de fibras queratinicas, especificamente en la tinciôn de las fibras del cabello humano:

The invention relates to novel compositions for Lwoxidation dyeing of keratinL.s, comprising at least

thedye’Ing process usmig this composition, to novel pyrazo, M11
[1,5-a]pyrimidine derivatives and to a process for the

It is known to dye keratin fibres, fltIh 1r’!I1rTsvh:Iu1
hair, with dye compositions containing oxidation dy
precursors, in particular ortho- or para-phenylenediamin
ortho- orpar&-aminophenols andi
h as diarninopyrazole derivatives,iare generaW referredN’ oxidation
pounds which, when combined with oxidizing products, give rise to coloured and dyeing compounds by a process oxidative IJPPL.!’lP.

Si tenemos en cuenta Jo anterior, podriamos afirmar que la solicitud colombiana en tramite incurrirla nuevamente en una improcedencia de patentabilidad al pretender un uso difererite o un segundo uso, en este caso terapØutico - tratamiento de cancer a dichos compuestos; como es de amplio conocimiento, en la Decision 486 de 2000, especIficamente en el articulo 21 del titulo II, capitulo I de dicha decision se menciona que "Los productos o procedimientos ya patentados, comprendidos en el estado de la tØcnica, de conformidad con el articulo 16 de Ia presente decisiOn, no serÆn objetode nueva patente, por el simple hecho de atnbuirse un uso distinto al originalmente comprendido porla patente inicial".

> Por su parte la patente americana US 2003/0191086 publicada el 09 de octubre de 2003 y titulada "SUBSTITUTED NITROGEN HETEROCYCLIC

DERIVATIVES AND PHARMACEUTICAL USE THEREOF" revela distintos

derivados heterocIclicos sustituidos con NitrOgeno que incluyen

pirrolopirimidinas, pirazolopirimidinas, purinas e imidazopiridinas y sus sales,

Utiles como medicamentos, particularmente como antimicôticos, antivirales,

anticanceriqenos, antidegenerativos, inmunosupresores y antimicrobianos.

Adicionalmente describe que ciertas purinas tri o tetrasustituidas y

pirazolopirimidinas proporcionan la inhibiciôn de la protelna cinasa

dependientes de ciclina ("cdk"), virus e inmunoestimulación.

MÆs adelante senala que este tipo de compuestos pueden incrementar el

potencial terapØutico de agentes antitumorales actualmente empleados:

[0185] In addition, studies carried out on the derivatives of
the invention have demonstrated strong effect on apoptosis
of many cancer cell lines. It has been seen that apoptosis can
be induced at stage GI or 02 and following damage of the
DNA, some cells stop at stage Gi and p53-dependent
apoptotic pathway is then induced. In other situations, it seems that cells stop at G2ftt4 stage in response to damage caused to the DNA, and activation of an independent p53 apoptolic path is observed. This path has proved to be particularly significant in the therapy of tumours in which a less of active p53 is observed. The interest is therefore assessed by application of the derivatives of the invention for stimulating on of p53-independent apoptosis in the cells, which have stopped at stage 02 through damage to the DNA

(...)

[0199] The therapeutical composition comprise about 1% to about 95% of the active ingredient, single-dose forms of administration preferably comprising about 20% to about 90% of the active ingredient and administration forms which are not single-dose preferably comprising about 5% to about 20% of the active ingredient. Unit dose forms are, for

Como se observa, esta anterioridad demuestra claramente que la actividad inhibitoria de los diversos derivados de pirazolopirimidina sabre las kinasas dependientes de ciclina es una actividad que se reconoce con anterioridad como Util en el tratamiento de cÆnceres, asi mismo, dichos derivados pueden potenciar la actividad anticancerIgena de otros principios activos; tambiØn se describen las distintas formulaciones farmacØuticas en las que pueden ser incluidos dichos derivados como principios activos. Si bien es cierto que los compuestos descritos en Østa anterioridad no son idØnticos a los descritos en la solicitud colombiana en trÆmite, no Ia es menos que la reconocida actividad farmacológica de estos compuestos estrechamente relacionados de manera estructural (derivados todos de pirazolopirimidina) afecta el nivel inventivo de lo contenido en el capItulo reivindicatorio de la solicitud colombiana, especificamente en lo que se refiere al usa de los mismos en el tratamiento de diversos cÆnceresa partir de la reconocida inhibición de kinasas dependientes de ciclinas.

> En el mismo sentido y en adición a Ia anteriormente expuesto, tenemos que si se conoce en el estado del arte Ia actividad inhibitoria de distintos compuestos (entre ellos las pirazolopirimidina) sabre las cinasas dependientes de ciclinas y si ademÆs se conoceque dicho mecanismo de acción es ütil en el tratamiento de diversos tipos de canceres, la solicitud colombiana en trÆmitenuevamente carecerla de nivel inventivo al buscar utilizar dichos derivados de pirazolopirimidina (con dicho mecanismo de acciôn previamente reconocidolactüan como inhibidores de la cinasa dependiente de ciclina) en el tratamiento de cancer. La publicaciones cientIficas que se seæalan mÆs adelante si bien es cierto no se refieren o no especifican compuestos de tipa pirazolopirimidina, permiten demostrar la relación existente entre las cinasas dependientes de ciclinas, la inhibición de las mismas y el cancer, informaciôn con la que cualquier persona medianamente versada en la materia podrIa deducir su aplicabilidad en dicha terapia anticancerIgena y su extrapolaciôn por su mecanismo de acción a compuestos coma los que pretende proteger la solicitud colombiana en trÆmite, veamos:

47

\y Antiestrogen Inhibition of Cell Cycle Progression in Breast Cancer Cells
Is Associated with Inhibition of Cyclin-Dependent Kinase Activity and

Decreased Retinoblastoma Protein Phosphorylation1.

Abstract: To define the mechanisms by which antiestrogens inhibit breast cancer cell proliferation, the effects of the antiestrogen IC! 182788 on G, cyclins and their cyclin-dependent kinase (CDK) partners were investigated in MCF-7 cells. Inhibition of entry
into S phase became evident g h after treatment, with the proportion of cells in S phase reaching a minimum by 24 h. IC! 182788 increased the proportion of the hypophosphorylated, growth inhibitory form of the retinoblastoma protein (pRB). This change began at 4-8 h, preceding effects on S phase. This suggests that there are early effects on the activities of CDKs that target pRB that are not merely a consequence of changes in cell cycle progression. The kinase activity of Cdk2 decreased to low levels at 18-24 h when changes in S phase and pRB phosphoiylation were well advanced. An earlier effect was seen on kinase activity associated with immunoprecipitated cyclin Dl, which was reduced approximately 48% by 12 h, with further decreases at 18-24 h. Cdk2 and Cdk4 protein levels remained constant over 24 h. Cyclin Dl messenger RNA and protein were down-regulated by IC! 182780 from 2 h, with levels halved at 8 h. IC! 182788 also increased the expression of the CDK inhibitors p27 and p21 at later times. These observations are compatible with the hypothesis that antiestrogens block entry of cells into S phase and inhibit cell proliferation as the consequence of an early decline in pRB phosphorylation contributed to by reduced cyclin Dl/Cdk4 activity. At later times, increased CDK inhibitor abundance may act to repress Cdk2 and Cdk4 activities, causing additional reductions in pRB phosphoay!ation, thus maintaining the antiestrogen blockade of cell cycle progression.

Cancer Dormancy: Role of Cyclin-dependent Kinase Inhibitors in

Induction of Cell Cycle Arrest Mediated via Membrane 1gM2.

Abstract: Anti-idiotype (anti-Id) antibody can induce tumor dormancy in a murine B lymphoma, BCL,, by its ability to induce cell cycle arrest and apoptosis (negative signaling). In human B lymphoma, there is accumulating evidence that the antitumor effect of anti-Id or several other B cell-reactive antibodies relates to their ability to act as agonists rather than conventional effector antibodies. In this study, we sought to elucidate the role of cyclins, cyclin-dependent kinases (CDKs), and their inhibitors in anti-/g/0-induced cell cycle arrest to better understand the mechanisms underlying cancer dormancy. To accomplish this, we have performed in vitro studies with a human lymphoma cell line (Daudi) because its response to anti-Id (or anti-lgM) is similar to that of a BCL, cell line, more reagents are available, and the results would be particularly pertinent to therapy of human B cell lymphomas. Our results show that cross-linking of membrane 1gM on Daudi cells induces an arrest late in G, and prevents pRb from becoming phosphoiylated. The G, arrest is correlated with an induction of the CDK inhibitor p21 and reduced CDK2 activity, although the level of CDK2 protein was not changed. Coprecipitation of CDK2 with p21 in anti-lgM-treated cells and the unchanged level of cyclin E suggest that p21 is responsible for the reduction of CDK2 activity and therefore blockade of the cell cycle. The induction of p21 was not accompanied by changes in p53 levels. As a result of the G, block, cyclin A levels sharply declined by 24 h after anti-lgM treatment. There was no evidence for

’Cohn K. W. Watts, Andrea Brady, Boris Sarcevic, Anna defazio, Elizabeth A. Musgrove, and Robert L.

Sutherland. Molecular Endocrinology. Vol 9 N° 12 (1995). 2 Radu Marches, Richard H. Scheuermarin, and Jonathan W. Uhr. [CANCER RESEARCH 58. 691-697.
February 15. 1998]

/1*
involvement of CDK4 or CDK6 in the blockade. These results provide evidence that membrane 1gM cross-linking on Daudi cells induces expression of p21 and a subsequent inhibition of the cyclin E-CDK2 kinase complex resulting in a block to pRb phosphorylation and cell cycle arrest late in 01.

o Promoting Apoptosis: A Novel Activity Associated with the Cyclin-

dependent Kinase Inhibitor p273.

Abstract: p27KIpI, a cyclin-dependent kinase inhibitor, is recognized as a negative regulator of the cell cycle. In this paper, we report that overexpression of p27 triggers apoptosis in several different human cancer cell lines. Using a recombinant adenoviral vector that expresses p27 (Adp27), we found that overexpression of p27 in MDA-MB-231 breast cancer cells induces apoptosis that was seen by a number of different techniques, including flow cytometry and in situ terminal deoxynucleotidyl transferase-mediated nick end labeling, flow cytometric assay for sub-0, population, and 4ç6-diamindino-2-phenylindole staining. Cleavage of poly-(ADP-ribose) polymerase and degradation of cydin BI, events that are known to be associated with apoptosis, were also observed following overexpression of p27. This is the first report indicating a role for p27 induction of apoptosis.

o Cyclin D-Dependent Kinases, INK4 Inhibitors And Cancer4.

Abstract: The Cyclin D-Cdk4, 6/lNK4/Rb/E2F pathway plays a key role in controlling cell growth by integrating multiple mitogenic and antimitogenic stimuli. The

components of this pathway are gene families with a high level of structural and functional redundancy and are expressed in an overlapping fashion in most tissues and cell types. Using classical transgenic technology as well as gene-targeting in ES cells, a series of mouse models have been developed to study the in vivo function of individual components of this pathway in both normal homeostasis and tumor development. These models have proven to be useful to define specific as well as redundant roles among members of these cell cycle regulatory gene families. This

pathway is deregulated in the vast majority of human tumors by genetic and epigenetic alterations that target at least some of its key members such as Cyclin Dl, Cdk4, INK4a and lNK0, pRb etc. As a consequence, some of these molecules are currently being considered as targets for cancer therapy, and several novel molecules, such as
Cdk inhibitors, are under development as potential anti-cancer drugs.

o Cyclin-Dependent Kinase Inhibitors Block Proliferation Of Human Gastric

Cancer Cells5.

Abstract: Olomoucine and roscovitine are novel compounds that are designed to

inhibit cyclin-dependent kinases (e.g., Cdk2 and cdc2). Cdks regulate progression through key checkpoints of the cell cycle. The purpose of this study was to determine

(1) whether olomoucine and roscovitine inhibit Cdk2 and cdc2 kinase activities of the human gastric cancer cell line SIIA and (2) whether olomoucine and roscovitine block cell proliferation and cell cycle progression. METHODS: SIIA cells were treated with olomoucine or roscovitine and examined for Cdk2 and cdc2 activities by using histone

3 Y Katayose, Min Kim, Amol N. S. Rakkar, Zhuangwu Li, Kenneth H. Cowan, and Prem Scili. [CANCER RESEARCH 57, 5441-5445, December 15, 19971
4Ortega S. Maluinbres M, Barbacid M. Biochim Biophys Acta. 2002 Mar 14;1602(1):73-87.

iseki H, Ko TC, Xue XY, Seapan A, Heilmich MR, Townsend CM Jr. Surgery. 1997 Aug; 122(2):187-94; discussion 194-5.

HI as the substrate. Cell numbers were counted with a Coulter counter. Cell cycle distribution was analyzed by DNA flow cytometry. RESULTS: Olomoucine and roscovitine completely blocked Cdk2 and cdc2 activities in SIIA cells. Both compounds were also able to inhibit proliferation of SIIA cells, as well as three other human gastric cancer cell lines (AGS, MKN45-630, and SNU-I). Cell cycle analysis showed that treatment with olomoucine or roscovitine for 24 hours led to a decrease in the S phase population and an increase in the G2IM population. CONCLUSIONS: We have shown that Cdk inhibitors, olomoucine and roscovitine, are a new class of antineoplastic molecules with potential therapeutic benefits for gastric cancers.

Cabe anotar que esta es una pequena muestra de la gran extension de artIculos cientIficos que se pueden encontrar y que ponen en evidencia Ia utilidad de los compuestos que inhiben las cinasas dependientes de ciclinas en el tratamiento de

cancer6.

> Finalmente, tambiØn se puede desvirtuar el nivel inventivo de la asociación de los compuestos derivados de pirazolopirimidina de la solicitud colombiana en trÆmite con diversospnncipios activos para el tratamiento de cancer, si ademÆs de tener en cuenta la informaciOn anteriormente expuesta, se admite que para cualquier persona medianamente versada en la materia la asociaciôn de compuestos con mecanismos de acciOn eficientes sobre una misma disfunciOn
o patologia pueden potenciar sus respectivas efectividades farmacologicas; asI mismo y a manera de complemento es importante tener en cuenta que existen varias publicaciones de carÆctercientIfico que describen con anterioridad los efectos de la asociaciOn entre inhibidores de las cinasas dependientes de ciclina y otros agentes anticancerIgenos mencionados por la solicitud colombiana en trÆmite, veamos:

o Phase I Study of the Cyclin-Dependent Kinase Inhibitor Flavopiridol in Combination With Paclitaxel in Patients With Advanced Solid Tumors7.

Abstract: Purpose: Preclinical studies indicate that the cvclindependent kinase inhibitor flavopiridol potentiates the induction of apoptosis by Daclitaxel. provided paclitaxel is followed by flavopiridol. We therefore designed a phase I clinical trial of sequential paclitaxel and flavopiridol. Patients and Methods: Paclitaxel was administered at a fixed dose, as either a 24- or 3-hour infusion on day I, followed by a 24-hour infusion of flavopiridol on day 2. Doses of flavopiridol were escalated in

successive cohorts according to a modified Fibonacci design. Flavopiridol pharmacokinetics were obtained on all patients. Results: Dose-limiting neutropenia developed with 24-hour paclitaxel doses of 135 and 100 mg/m2 and flavopiridol doses

By Gary K. Schwartz, Eileen O’Reilly, David Ilson, Leonard Saltz, Sunil Sharma, William Tong, Peter Maslak,, Maxine Stoltz, Larry Eden, Pam Perkins, Sandra Endres, John Barazzoul, David Spriggs, and David Kelsen. J Clin Oncol 20:2157-2170. '2002 by American Society ofClinical Oncology.

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of 10 and 20 mglm2, respectively. With 3-hour paclitaxel at 100 mg/m2, flavopiridol could be escalated to 70 mg/m2 without dose-limiting toxicity. With 3-hour paclitaxel next escalated to 135 mg/m2, dose-limiting neutropenia and pulmonary toxicity occurred when flavopiridol was escalated to 94 mg1m2. This did not correlate with any change in flavopiridol orpaclitaxel pharmacokinetics. At a 3-hour paclitaxel dose of 175 mg/m2, dose-limiting pulmonary toxicity occurred in only one patient at flavopiridol doses under 94 mg/m2. Clinical activity was observed in patients with esophagus, lung, and prostate cancer, including patients who had progressed on paclitaxel. Conclusion: The recommended phase II doses will be a 3-hour infusion ofpaclitaxel at 175 mg/m2 on day I followed by a 24-hour infusion of flavopiridol at 70 mg/m2 on day
2. Flavopiridol dose escalations to 80 mg/m2 are possible. At these doses, toxicities are manageable and clinical activity is promising.

o The Use Of Cyclin-Dependent Kinase Inhibitors Alone Or In Combination

With Established Cytotoxic Drugs In Cancer Chemotherapy8.

Abstract: Cyclin-dependent kinase (CDK) inhibitors are small molecule inhibitors of the kinases required for the orderly progression of cells, both normal and neoplastic, through the cell cycle. Because cell cycle dysregulation is such a common occurrence in neoplasia, the search for agents that might block cell cycle traverse has been the focus of intense interest. These efforts have led to the identification of a broad array of compounds that interfere directly with the function of CDKs. Two of these agents (flavopiridol and UCN-
01) have now entered the clinical arena, and others are scheduled to do so in the near future. In preclinical studies, CDK inhibitors have shown the ability not only to block neoplastic cell proliferation, but also to induce, through a variety of mechanisms, programmed cell death. The latter capacity may stem from the

diverse effects that CDK inhibitors exert on multiple kinases and apoptotic regulatory molecules. In addition, there is abundant predilnical evidence that
CDK inhibitors can potentiate, generally in a dose- and sequence-dependent manner, the anti-tumor effects of many established cytotoxic agents. In clinical studies in humans, flavopiridol and UCN-01 have been shown to be tolerable,

although clear evidence of single agent activity or enhancement of the efficacy of established agents has not yet emerged. This may reflect a failure to

optimize drug schedulesipharmacokinetics, or to identify the critical molecular targets of these agents. Finally, in recent years, a rationale has emerged for

combining CDK inhibitors with other molecularly targeted agents (i.e. differentiation-inducers and signal transduction modulators). Current research
has basically two goals: (a). to identify CDK inhibitor concentrations and schedules that inhibit the growth of and induce apoptosis in specific tumor cell types; and (b). to establish a rational basis for combining CDK inhibitors with more conventional cytotoxic agents to enhance antitumor efficacy. This review gives a brief summary of such efforts, with an emphasis on agents and combinations that are in or near clinical development.

o La publicacion internacional WO 02/076484 publicada el 03 de octubre de 2003

y titulada "COMBINATION OF A TAXANE AND A CYCLIN-DEPENDENT

8Grant S. Roberts JD. Drug Resist Updat. 2003 Feb;6(1): 15-26.

KINASE" revela la combinaciôn de diversos Taxanos con flavopiridol, un reconocido inhibidor de las cinasas dependientes de ciclinas9:

Among substances which may be used In association or in combination
with TaxoP TaxotereP or their analogues, there may be mentioned enzymes such as L-asparaginase and cyclin-dependent kinases, such as flavoplildol, quercltin and genistein. Various agents such as biological response modifiers or growth factor inhibitors, such as interferons or Interteukkis, may also be used. Since the activity of the products depends on the doses used, It Is possible to use higher doses and to increase the activity while decreasing the toxicity phenomena or delaying their onset by combining growth factors of the haematopoietic type such as G-CSF or GM-CSF or certain interleukins with TaxoP, Taxotere their analogues or their combinations with other therapeutically active substances.

More especially, the invention relates to combinations of Taxo, TaxotereP and their analogues with the cyclin-dependent kinase, flavoplridol. Cyclin-dependent kinases (CDKs) are Important regulators that control
the timing and coordination of the cell cycle. CDKs form reversible complexes with their obligate cyclin partners to control transition through key Junctures in the cell cycle. For example, the activated CDK4-cyclin Dl complex controls progression through the (31 phase while the CDKI.cyclln BI complex controls entry Into the mitotic phase of the cell cycle. Endogenous cyclin dependent kinase inhibitory proteins (CDKIs) are known which bind eitherthe CDKorcydin component and Inhibit the kinase actMty. In many tumors such as melanomas, pancreatic and esophageal cancers, these natural CDKIs are either absent or mutated. Thus, selective CDX Inthibitors may prove to be effective chemotherapeutic agents.

Flavoplridol (cs-517-dlhydroxy-2-(2-chlorophenyl)-8-[4-(3.hydroxy-1 - methyl).piperldlnyl]-1-benzopyran4-one) Is a synthetic fiavone that has been shown to have antitumor activity against various tumor cells lines such as human lung carcinoma, breast carcinoma, and also inhibits tumor growth in xenograft models. It has been shown to Induce arrest in both the GI and G2 phases ofthe cell cycle. Flavopiridol is a potent and selective inhibitor of the CDKs, and its antitumor activity is related to Its CDX Inhibitory activity. Studios have shown that its tumor cell growth Inhibitory activity occurs in acell cycle specific manner. See BioorQ. & Med. Chem. Letters 10:1037-1041(2000).

Flavopiridol: the first cyclin-dependent kinase inhibitor in human clinical trials. Senderowicz AM. Invest

En conclusion, la solicitud colombiana en trÆmite carece de los requerimientos necesarios para otorgarsele el privilegio de patente de invenciôn, fundamentalmente porque incurre en dos improcedencias directas de patentabilidad tales coma son la reivindicar un mØtodo de tratamiento y dare un uso nuevo a sustancias que se reconocian para otras aplicaciones y porque ademÆs dicho mØtodo detratamiento incluye sustancias descritas en el estado de la tOcnica y recurre a informaciOn reconocida y que cualquier persona medianamente versada en la materia puede aplicar para Ilegar a emplear los compuestos activos mencionados y sus asociaciones farmacØuticas en el tratamiento del cancer.

Par todo lo anterior, ruego se analicen los argumentos expuestos can el rigor y detenimiento habituales y que en consecuencia se decrete, ademÆs de la negaciOn de la solicitud en comento, el archivo del expediente que la contiene.

II. ANEXOS

)’ La patente americana US 6,099,593 publicada el 31 de diciembre de 1997 y titulada "COMPOSITIONS FOR DYEING KERATIN FIBERS CONTAINING PYRAZOLO (1,5-A) PYRIMIDINE DERIVATIVES AND DYEING PROCESSES" columnas I y 12 a 14.
’ La patente americana US 2003/0191086 publicada el 09 de octubre de 2003 y titulada ’SUBSTITUTED NITROGEN HETEROCYCLIC DERIVATIVES AND PHARMACEUTICAL USE THEREOF" paginas 11, 12 13.
> Abstracts de los artIculos cientificos Antiestrogen Inhibition of Cell Cycle Progression in Breast Cancer Cells Is Associated with Inhibition of Cyclin-Dependent Kinase Activity and Decreased Retinoblastoma Protein Phosphonylation; Cancer Dormancy: Role of Cyclin-dependent Kinase Inhibitors in Induction of Cell Cycle Arrest Mediated via Membrane 1gM; Promoting Apoptosis: A Novel Activity Associated with the Cyclin-dependent Kinase Inhibitor p27; Cyclin D-Dependent Kinases, 1NK4 Inhibitors And Cancer; Cyclin-Dependent Kinase Inhibitors Block Proliferation Of Human Gastric Cancer Cells y Flavopiridol: the first cyclin-dependent kinase inhibitor in human clinical trials.

New Drugs. 1999;17(3):313-20.

13

> Copias de las Paginas de resultados de la büsqueda en el metabuscador de httpIIwww.sinab.unaI.educo de articulos relacionados a inhibidores de kinasas dependientes de ciclinas.
> La publicación internacional WO 02/076484 publicada el 03 de octubre de 2003 y titulada "COMBINATION OF A TAXANE AND A CYCLIN-DEPENDENT KINASE" pagina 2.

Seæor Superinte

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C.C. 79.152.473 de Usaquen
T.P. 44655 del C. S de la J.

United States Patent [19]
Terranova et at.

[54] COMPOSITIONS FOR DYEING KERATIN
FIBERS CONTAINING PYRAZOLO(1,5-A) PYRIMIDINE DERIVATIVES AND DYEING PROCESSES

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Coupvray, all of France

[73] Assignee: L’Oreal, France

*] Notice: This patent issued on a continued pros-
ecution application filed under 37 CFR
1.53(d), and is subject to the twenty year
patent term provisions of 35 U.S.C.
154(aXZ).

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[86] PCT No.: PT/FR97/01057

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8/573

[58] Field of Search................................8/406, 407, 408,
8/409, 423, 567, 573; 544/281; 5481360.5

ThfflUllhIliHIOUHllft

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[11] Patent Number: 690999593

[45] Date of Patent: *Aug. 8, 20OO

[56] References Cited

U.S. PATENT DOCUMENTS

2,566,658 911951 Fry..5 /281
3,626,064 12/1971 Hiichings ci at........................14258
5,234,818 8/1993 Zimmermann et at................ 5441281
5,356,897 10/1994 Oka et al...........................,..., 544/281
5,380,340 1/1995 Neunhoeffer ci at.......................8/409
5,457,200 10/1995 Zimmermann et at.................544/281

FOREIGN PAIENT DOCUMENTS

0 433 854 6/1991 European Pat. OIL.
4029324 3/1992 Germany.
OTHER PUBLICATIONS

Chemical Abstracts, vol. 67, No. 3, Jul. 17, 1967, Abstract No. 11498c.

William E. Kirkpatrick ci al., "3-Halo--5,7-dimethylpyra-zolo[1,2-a]pyrimidines, a Nonbenzodiazepinoid Class of Antianxiety Agents Devoid of Potentiation of Central Ner-vous System Depressant Effects of Ethanol or Barbiturates",

Journal of Medicinal Chemistry, vol. 20, No. 3, 1977, pp. 386-393.
Primary ExaminerCaroline D. Lion
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Garrett & Dunner

1571 ABSTRACT

Novel compositions for the oxidation dyeing of keratin fibres, comprising at least one specific pyrazolo[1,5-a] pyrimidine derivative, a dyeing process using these compositions, novel pyrazolo[1,5-a]pyrimidine derivatives and a process for their preparation.

26 Claims, No Drawings

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COMPOSITIONS FOR DYEING KERATIN A first subject of the invention is thus a composition for
FIBERS CONTAINING PYRAZOLO(1,5-A) the oxidation dyeing of keratin fibres, and in particular
PYRIMIDINE DERIVATIVES AND DYEING human keratin fibres such as the hair, characterized in that it
PROCESSES comprises, in a medium which is suitable for dyeing, at least

one pyrazolo[1,5-a]pyrimidine derivative of formula (1) The invention relates to novel compositions for the below as oxidation base and/or one of the addition salts oxidation dyeing of keratin lihies, comprising at least one thereof with an acid or with a base and/or one of the pyrazolo[1,5-a}pyrimidine derivative as oxidation to

the dyeing process using this composition, to novel pyrazolo tautomeric forms thereof, when a tautomeric equilibrium
[1,5-a]pyrimidine derivatives and to a process for their 10 exists:
preparation.
It is known to dye keratin fibres, and in particular human (I) hair, with dye compositions containing oxidation dye
precursors, in particular ortho- or para-phenylenediamines, (X)

ortho- or para-aminophcnols and heterocyclic compounds 15 (OH) 4 nic/ INR3R4]q such as diaminopyrazole derivatives, which are generally N

referred to as oxidation bases. Oxidation dye precursors, or
oxidation bases, are colourless or weakly-coloured com-

pounds which, when combined with oxidizing products, can in which: give rise to coloured and dyeing compounds by a process of 20

oxidative condensation.

It is also known that the shades obtained with R1, R2, R3 and R41 which may be identical or different,
oxidation bases can be varied by combining them with denote a hydrogen atom, a C1-C4 alkyl radical, an aryl
couplers or colour modifiers, the latter being chosen in radical, a C1-C4 hydmxyalkyl radical, a C2-C4 poly-
particular from aromatic meta-diamines, meta- 25 hydroxyalkyl radical, a (C1-C4)alkoxy(C1-C4)alkyl
aminophenols, meta-diphenols and certain heterocyclic radical, a C1-C4 aminoalkyl radical (it being possible
compounds. For the amine to be protected with an acetyl, an arnido

The variety of compounds used as regards the oxidation or a sulphonl), a (C1-C4)alkylamino(C--C4)alkyl bases and the couplers allows a wide range of colours to be radical, a di[(C1-C4)alkyl]amino(C1--C4)alkyl radical

obtained. 30

The so-called "permanent" coloration obtained by means (it being possible for the dialkyls to form a 5- or a
of these oxidation dyes must moreover satisfy a certain 6-membered aliphatic or heterocyclic ring), or a
number ofrequirements. Thus, it must have no toxicological hydroxy(C1-C4)alkylamino(C1-C4)alkyl or
drawbacks, it must be able to give shades of the desired di[hydroxy(C1-C4)alkyl]amino(C1--C4)alkyl radical;
intensity and it must be able to withstand external agents 35 the radicals X, which may be identical or different, denote
(light, had weather, washing, permanent waving, a hydrogen atom, a C1-C4 alkyl radical, an aryl radical,

perspiration, rubbing). a C1-C4 hydroxyalkyl radical, a C2-C4 polyhydroxy- The dyes must also be able to cover grey hair and, lastly, alkyl radical, a C1-C4 aminoalkyl radical, a (C1-C4) they must be as unselective as possible, that to say that allcylamino(C1-C4)alkyl radical, a di[(C1-C4)alkyl] they must make it possible to obtain the smallest possible

colour differences along the entire length ofthe same keratin amino(C1-C4)alkyl radical (it being possible for the
fibre, which may, in fact, be differently sensitized (i.e. dialkyls to form a or 6-membered aliphatic or
damaged) between its tip and its root. They must also have heterocyclic ring), a hydroxy(C1-C4)alkylamino
good chemical stability in the formulations. They must have (C1-C4)alkyl radical, a di[hydroxy(C,-C4)alkyl]amino
a good toxicological profile. (C1-.C4)alkyl radical, an amino radical, a (C1-C4)
It has already been proposed, in particular in patent alkylamino radical or a dl[(C1-C4)alkyl]amino radical,

application 4,029,324, to use certain pyrazolo[1,5-a] a halogen atom, a carboxylic acid group or a sulphonic pyrimidine derivatives which can be substituted with acid group; alkyl radicals in position 5 and/or as couplers for the equal to 2 or oxidation dyeing of keratin fibres.

It has also been proposed in patent application equal to or

4,133,957 to use certain pyrazolo[1,5-a]pyrimidine deriva- q equal to or lives belonging In the telrahydrnpyraxolo{l,5-ajpyrimidine n equal to or family as oxidation dye precursors for the oxidation dyeing

of keratin fibres. with the proviso that:
The Applicant has discovered, entirely unexpectedly the sum p+q other than
and surprisingly, a novel family pyrazolo[1,5-a] when p+q equal to then n equal and the

pyrimidine derivatives of formula defined below, which groups NR1R2 and NR,R4 positions are partly novel per se, these derivatives possibly being (56)’ (6,7); (3,5) or (3,7); suitable for use as oxidation dye precursors, but in addition

making it possible to obtain dye compositions which lead to when equal to then n equal to and the
intense colorations and which have good resistance to exter-
nal agents (light, bad weather, washing, permanent waving, positions (5,6); (6,7); (3,5) or (3,7).
perspiration and rubbing). Lastly, these compounds prove to When the compounds formula are that they
he readily synthesixable and are chemically stable. They contain an group on one of the positions 5 7 to
have a good toxicological profile, a nitrogen atom, a tautomeric equilibrium exists
These discoveries form the basis the present invention, represented, for example, by the following scheme:

X

6,099,593

11

stirrer and equipped with a condenser and a thermometer. The medium was refluxed for 5 h and the precipitate was
then filtered off while hot. 1.2 g of a yellow solid were obtained. Alter chromatography on silica gel (Merck: 230-400 mesh; EcOAc/MeOH=9/1), 0.4 g of 3-nitropyrazolo[1,5-a]pyrimidin-7-ol was collected in the form of a yellow powder. (Yield-18%).

12

NMR (DMSO-d6): 2.42 (s, 311); 6.03 (s, 1H); 8.61 (d,
1l-1) 12.69 (s, 1H);
ELEMENTAL ANALYSIS: C7H6N403 MW-194.15

N 0

L.alculalea I7) ’&t’J ..JL

NMR (DMSO-d6: 6.19 (d; 1H; 7.98 (d; 111; 8.75 (s; Found (%) 43.12 3.11 28.77 24.65
11-1); 13.10 (OH). 10

2nd step: 3-AMINOPYRAZOLO[1,5-a]PYRIMIDIN-7-OL
HYDROCHLORIDE

0.35 g of 3-nitrnpyrazolo[1,5-a]pyrimidin-7-ol, 20 cc of
acetic acid, 1.6 g of cyclohexene and 85 mg of 10%
palladium-on-charcoal were introduced into a 50 cc 3-necked round-bottomed flask fitted with a magnetic stirrer and equipped with a condenser and a thermometer. The medium was refluxed for 1h30’ and the catalyst was then filtered off through Celite. Alter evaporation of the acetic acid, the solid obtained was taken up in 2 cc of refluxing concentrated hydrochloric acid for 2 h 39. After evaporation of the solvent, an off-white solid was collected.

NMR (DO): 5.93 (d; 11-1); 7.87 (d; 111); 8.04 (s; 111).

EXAMPLE 3

3-AMINO-5-METHYLPYRAZOLO[1,5-a]
PYRIMIDIN-7-OL HYDROCHLORIDE

1st step: 3-NITRO-5-METHYLPYRAZOLO[1,5-a] PYRIMIDIN-7-OL

011

50 g of 4-nitro-2H-pyrazol-3-ylamine hydrochloride (prepared according to H. Dorn and H. Dilcher, LiebigsAnn. Chcm., 707, 141,1967) and 60 gofethyl acetoacetate in 160 cc of acetic acid were introduced into a 500 cc three-necked round-bottomed flask equipped with a magnetic stirrer, a thermometer and a condenser. The reaction medium was refluxed for 12 h. The precipitate formed was filtered off at about 90° C. It was rinsed with dilsopropyl ether and dried under vacuum over phosphorus pentoxide. 50 g of 3-nitro-5-methylpyrazolo[1,5-ajpyrimidin-7-ol were obtained in the form of yellow crystals. (Yield-84.5%; melting point’s290° C. with decomposition).

2nd step: 3-AMINO-5-METHYLPYRAZOLO[1,5-a] PYRIIvIIDIN-7-OL HYDROCHLORIDE

OH 15

I-lU

20

150 cc of acetic acid and 150 cc of water were introduced

into a 1 litre autoclave, followed by 10 g of 3-nitro-5-25 methylpyrazolo[1,5-a]pyrimidin-7-ol and 1 g of 5% palladium-on-charcoal containing 50% water (Engelhard). 5 bar of hydrogen were introduced into the reactor, preheated to 300 C. After reacting for 1 h, the catalyst was filtered off through Ceite. The filtrate was acidified with 100 cc of 7M 30 hydrochloric acid solution. ’l’he hydrochloride precipitates out with stirring. It was filtered off and washed with diiso-propyl ether. 4.2 g of 3-amino-5-mcthylpyrazolo{1,5-a] pyrimidin-7-ol hydrochloride were obtained in the form of white crystals. (Yield-41%).
35 NMR (DMSO-d6): 2.37 (s, 311); 5.71 (s, 111); 8.00 (s, 111); 10.32 (broad s, 31-1); 13.09 (broad s, 11-I);
ELEMENTALANALYSIS: C7115N40HC1 MW200.63

40
C H N 0 Cl

45

Calculated (%) 41.91 4.52 27.93 7.97 17.67
Found (%) 41.43 4.57 27.69 8.90 17.66

EXAMPLE 4

3-AMINO-7--HYDROXYETHYLAMINO-5-

METHYLPYRAZOLO[1,5-a]PYRThIIDINE 50 DIIIYDROCIILORIDE
1st step: 7-CHLORO-5-METHYL-3-NITROPYRAZOLO [1,5-a]PYRIMIDINE

Cl
55
’ C"

_?_N_; 60 NO2

230 cc of phosphorus oxychloride, 15.4 g of N,N-dimethylani]ine and 23.3 g of 3-amino-5-methylpyrazolo{1, ns 5-a}pyrimidin-7-ol were introduced into a 500 cc three-necked flask equipped with a magnetic stirrer, a thermometer and a condenser. The reaction medium was

6,099,593

13

refluxed for 2 h 30’. After evaporation of the phosphorus oxychioride under reduced pressure, a very viscous green oil
was obtained, to which were added about 400 g of ice. A brown solid precipitated out. After stirring for 30 minutes,
the precipitate was filtered off and rinsed with petroleum

ether and then with diisopropyl ether. After drying under vacuum over phosphorus pentoxide, 21.4 g of 7-chloro-5-

methyl-3-nitropyrazolo[1,5-a]pyrimidine were obtained in the form of a brown solid. (Yield-83.9%).
NMR (DMSO-d6): 2.70 (%,3H), 7.82 (s, 1H); 9.10 (a, 1H). 2nd step: 7-1-HYDROXYET11YLAMINO-5-MET11YL-3-NITROPYRAZOLO[1,5-a]PYRIMIDJNE

14

chioric acid solution. The hydrochloride precipitated out with stirring. It was filtered off and washed with diisopropyl ether. 10 g of 3-amino-7-3-hydroxyethylamino-5-

methylpyrazolo[1,5-a]pyrimidine dihydrochioride were 5 obtained in the form of slightly grey crystals. (Yield-60%)
NMR (D20): 2.73 (s, 311); 3.91 (m, 211); 3.98 (m, 211); 6.66 (s, IH); 8.39 (s, 111);

10 ELEMENTAL ANALYSIS: C9H13N502 HCI MW=280.16

ll

NH 15

113)
20
NO2

15 g of 7-chloro-5-mcthyl-3-nitropyrazolo[1,5-a]

pyrimidine in 100 cc of ethanol were introduced into a 250 cc three-necked round-bottomed flask equipped with a mag 25
nctic stirrer, a thermometer and a condenser. 5 g of ethano-lamine were added dropwise and the medium was refluxed for 30 minutes. After cooling to room temperature, the

yellow precipitate was filtered off. This precipitate was rinsed with diisoprnpyl ether. After drying under vacuum 30
over phosphorus pentoxide, 14.2 g of 7-1-hydroxyethylamino-5-methyl-3-nitropyrazolo[1,5-a] pyrimidine were obtained in the form of yellow crystals. (Yield-86%, melting point=231° C.).
NMR (DMSO-d6): 232 (s, 3H); 3.52 (m, 211); 3.66 (m, 35 211); 4.96 (I, 111); 6.64 (s, 111); 8.48 (t, 111); 8.89 (s, 111); ELEMENTAL ANALYSIS: C9H11N503 MW237.22

Calculated (%) 45.57 4.67 29.52 20.23
Found (%) 45.09 4.59 29.40 20.63

3rd step: 3-AMlNO-7-0-HYDROXYETHYLAMINO-5-METHYLPYRAZLO[1,5-a]PYRIMIDINE DIHYDRO-CHLORIDE

HaC’C

Ha

NH2

C H N 0 Cl

Calculated (%) 3839 5.4 25.05 5.71 25.31
Found (7e) 38.52 5.32 2439 6.42 25.15

EXAMPLE 5

2-METHYLPYRAZOLO[1,5-a]PYRIM!DINE-3.7-DIAMINE DIHYDROCHLORIDE

1st step: 2-METHYLPYRAZOLO[1,5-a]PYRIMIDIN-7 YLAMINE HYDROCHLORIDE

NH2

HCI

so 150 cc of 35% hydrochloric acid were introduced into a 500 cc three-necked flask equipped with a magnetic stirrer,
a thermometer and a condenser, and 473 g of 3-amino-5-methylpyrazole dissolved in 100 cc of water were added dropwise. The temperature rose to 600 C. 47.5 g of 45 3-ethoxyacryloni[rile were then added and the reaction was refluxed for 1 h. The reaction medium was cooled and concentrated under reduced pressure. 50 cc of acetone were added and the precipitate obtained was filtered off. It was rinsed with diisopropyl ether. After drying under vacuum o over phosphorus pentoxide, 78.7 g of 2-methylpyrazolo[1, 5-a]pyrimidin-7-ylamine hydrochloride were obtained in the form of white crystals. (Yield-83%).

NMR (DMSO-d6): 2.43 (s, 311); 6.42 (s, 1H); 6.46 (d,
55 111) 8.26 (d, 111); 9.55 (broad s, 111); 10.32 (broad s, 1H);

14 g of 7-0-hydroxyethylainino-5-methy1-3-nitropyrazolo [1,5-a]pyrimidine in 150 cc of acetic acid and 150 cc of su water were introduced into a 500 cc autoclave, followed by I g of 5% palladium-on-charcoal containing 5001c water (Engelhard). The reaction medium was preheated to 300 C. and an 8-bar pressure of hydrogen was introduced. I’he reaction started immediately and the temperature reached 55 60° C. At the end of the reaction, the catalyst was filtered off through Celite. The filtrate was acidified with 7M hydro-

ELEMENTAL ANALYSIS: C7118N4HCI0.5 11,0 MW493.63

C H N 0 Cl

Calculated (Ti) 43.42 5.20 28.93 4.13 18.30
Found (%) 43.68 5.20 28.83 4.66 18.58

ILIIfflHIHuIODOfflHHHUIflHhlIll1OflUjj43 US 20030191086A1
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Hanus et al. (43) Pub. Date: Oct. 9, 2003

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USE THEREOF Jan. 26, 1999 (CZ)... PV 273-99

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(75) inventors: Jan Hanes, Praha (CZ); Vladimir (51) mt. C1.7..................A61K 31/7076; A61K 31/675; Krystof, Ostrava (CZ); Marlin 07H 19/16; C07D 471/02;

Hajduch, Olomouc (CZ); Jaroslav C07D 473/18; CO7D 473/16;
Vesely, Bohunovice (CA); Mlroslav A6IK 31/522; A6IK 31/52
Strnad, Olomouc (CZ) (52) U.S. Cl......................... 514/46; 514/263.23; 514/81;
514/263.2; 514/263.38; 536/27.3;
544/269; 544/276; 544/277;
Correspondence Address: 514/303; 546/118
NOTARO & MICHALOS P.C.

suite 110 (57) ABSTRACT

100 Dutch 11111 Road Various substituted nitrogen heterocyclic derivatives and
Orangeburg, NY 10962-2100 (US) their pharmaceutically acceptable salt derivatives are pro-
vided for use as medicaments, and particularly, as antimi-

(73) Assignee: USTAV EXPERIMENTALNI ROT- totic, anti-viral, anti-cancer, anti-degenerative, inimunosup-
ANIKY AV CR pressive, and anti-microbial drugs or. vaccines. These
heterocyclic derivatives can be used as an active agent in a

(21) AppI. No.: 101358,674 pharmaceutical, as well as a diagnostic utility. To this end,

several families of heterocyclic derivatives are provided

(22) Filed: Feb. 5, 2003 including pyrrolopyrimidmes, pyraaolopyrimidines,
purines, and imidazopyridines. In particular, certain tn-
Related U.S. Application Data substituted and tetra-substituted purines and pyrazolopyni-
midines and their deaza analogues are provided for inhibit-
(62) Division of application No. 09/889,176, filed on Jul. jag cycin-dependent kinase ("cdk") proteins, viruses, and
12, 2001, now Pat. No. 6,552,192. immIlflflstimUlaLU)U.

US 2003/0191086 Al

11

antineurodegcncrativc, immunosuppressive and antimicro-bial activity, characterised in that it comprises, in addition to auxiliary pharmaceutical matters, at least one substituted nitrogen heterocyclic derivative of formula I.

[0174] The invention also relates to a combined pharma-ceutical composition with cytostatic effect, characterised in that it comprises, in addition to auxiliary pharmaceutical matters, a combination of at least one substituted nitrogen heterocyclic derivative of formula I and a cytostatic agent selected from a group consisting of mitoxantron, cis- and carbo-platin, methotrexate, taxol and doxorubicin.

[0175] The invention also relates to a use of substituted nitrogen heterocyclic derivatives of formula I for preparing medicaments destined for treating tumours, cancers, psoria-sis, rheumatoid arthritis, lupus, type I diabetes, multiple sclerosis, restenosis, polycydic kidney disease, host graft disease and gout, parasitoses, such as those caused by fungi or protists, or Alzheimer’s disease, or for preparing antineurodegenerative drugs and suppress immunostimula-tion agents.

[0176] The novel compounds ofthisinvention have a wide variety of diagnostic, therapeutic and industrial utilities.

[0177] The compounds of this invention are suitable as intermediates for use in the preparation of affinity absorption matrices. For example, the phosphonate groups in matrix hound form are useful in the chromatographic separation of positively charged molecules. Other immobilised examples of the compounds herein are useful in purifying proteins, e.g., cell cycle enzymes (cdk’s), enzymes involved in rec-ognition of the compound of this invention, e.g. transport proteins. Suitable methods of incorporation of the com-pounds ofthis invention into polymeric resins will be readily apparent to the skilled artisan, for instance the compounds are incorporated by cross-linking hydroxyl groups of the

phosphonate or hydroxymethyl substituents using cross- linking agents heretofore known. Linking through a group
other than the heterocyclic base will produce a resin useful in hydrophobic affinity chromatography. Other suitable link-ing methods are described in Cihiar (supra).

[0178] The compounds of the formula I and their phar-maceutically acceptable salts inhibit selectively the enzyme p34°’Jcyclin 13 kinase and related cdk.s (cdk2, cdk-5, cdk7, erki, erk2).

[0179] In another embodiment, this invention is a method for inhibiting cdks and cell proliferation in mammals com-prising administering a therapeutically effective amount of the composition of claim 1 to the mammal. The cdk inhib-iting molecules are useful lbr treating cell proliferation disorders such as rheumatoid arthritis, lupus, type! diabetes, multiple sclerosis, cancer, restenosis, Alzheimer’s disease, growth of parasites (animal, protists), host graft disease, and gout.

[0180] In another embodiment, this invention is a compo-sition useful for treating fungal infections(fungi) in humans, animal, and in plants.

[0181] 2,6,8,9-tetrasubstituted and 6,8,9- or 2,8,9-trisub-stituted adenine derivatives exhibit extremely high potency against DNA viruses on the part of the defined compounds. Such compounds otherwise have been considered to have little or no activity against DNAviruses. Moreover, surpris-

Oct. 9, 2003

ingly the chirally enriched or pure (S)-enantiomcr is antivi-rally active. Heretofore, only the (R)-enantiomer was nota-bly antivirally active, and then only against reiroviruses.

[0182] In addition to other cdc2-related kinases, this kinase controls certain steps of cell division cycles, in particular the transition from GI phase into the S phase and in particular the transition from the 02 phase into the M phase. Out the basis of this findings, it can be expected that the compounds of the formula I, II and their pharmaceuti-cally acceptable salts can be used as antimitotic compounds or for treatment of bypoproliferative diseases, such as can-cer, restenosis and Alzheimer’s disease. Thus in very low concentration (micromolar and lower), they are capable of inhibiting cell cycle transitions 021M, M-phase/metaphase) carried out on the different animal bodies and embryos. Furthermore, the compounds are useful in treating autoimune diseases, e.g. rheumatoidal arthritis, lupus, type I diabetes, multiple sclerosis, etc., in treating of cancer, car-diovascular disease such as restenosis, host vs graft disease, gout, polycystic kidney disease and other proliferative dis-eases whose pathogenesis involves abnormal cell prolifera-tion.

[0183] This invention also concerns novel compounds that have been discovered to be potent and specific inhibitors of kB-a kinase which prevents signal induced NF-KB activa-tion and cytokinc synthesis in vitro and in vivo. Such inhibitors are expected to inhibit synthesis of cytokines and adhesion proteins whose synthesis is transcriptionally regu-lated by NP-KB. Pm-inflammatory cytokines such as IL-I, IL-6, 1’NF and adhesion proteins (e.g. [CAM, VCAM and selections) belong to this class of molecules and have implicated in the pathogenesis of inflammatory diseases. Thus a potent inhibitor of 1KB-a kinase is useful in the clinical management of diseases where the NP-KB activation is required for disease induction.

[0184] It also relates to novel compounds activating p53, the mammal cell’s own natural brake gene for stopping uncontrolled cell proliferation (cancer), thus being able to switch off the cancer. p53 as well as retinoblasioma (Rh) are two well-characterised tumour suppressors whose inactiva-tion may led to uncontrolled cell proliferation and malig-nancy. Phosphorylation of these two proteins, which are involved in the cell cycle regulatory mechanisms, is known to modulate their function. Thus a potent cdk inhibitor represent a good toll for treatment of cancers due to induc-tion of wild type p53 protein in cancers expressing mutant p53.

[0185] In addition, studies carried out on the derivatives of the invention have demonstrated strong effect on apoptosis ofmany cancer cell lines. It has been seen that apoptosis can be induced at stage Gi or G2 and following damage of the DNA, some cells stop at stage 01 and p53-dependent apoptotic pathway is then induced. In other situations, it seems that cells stop at G2IM stage in response to damage caused to the DNA, and activation of an independent p53 apoptotic path is observed. This path has proved to be particularly significant in the therapy of tumours in which a less of active p53 is observed. The interest is therefore assessed by application of the derivatives of the invention for stimulating on of p53-independent apoptosis in the cells, which have stopped at stage 02 through damage to the DNA using agents such as mitoxantrone or cis-platinum. The cdk

US 2003/0191086 Al

12

inhibitors of this invention can thus increase the therapeutic potential of the anti-tumour agents currently used.

[0186] The compounds of this invention will generally be terminally incorporated into the oligonucleolide. If they do not contain phosphonyl group attached to the hydroxyl group, they optionally are incorporated internally into the sequence of the oligonucleotide. Terminally incorporated diphosphonyl compounds of this invention which contain no free hydroxyl capable of participating in chain elongation also are useful in DNA sequencing in essentially the same manner as deoxyNTPs have been used in the past (see example 8 of U.S. Pat. No. 5,276,143). The nucleotide analogues of the invention (when diphosphorylated) are useful as chain terminators for dideoxynucleotide-type DNA sequencing protocols, provided that the nucleotide analogue lacks a free hydroxyl group suitable for polymerase medi-ated chain elongation. These compounds will not have R’=hydroxymethyl and do not possess a cyclic structure incorporating the phosphorus atom (although compounds having such excluded structures can be intermediates). The nucleotide analogue is included in a kit with other reagents (such as Kienow polymcrasc or T4 polymcrasn, dNTPs, eta) needed for DNA sequencing (Otvos, et al. ’Nucl. Acids. Res." 15:1763-1777 (1987).

[0187] If the oligonucleotide-incorporated compound of this invention is bindingcompctcnt for its complementary sequence, i.e., if it is capable of base pairing, then this nucleotide monomer will participate in bybridisation. It is not necessary, however, that the incorporated nucleotide analogue of this invention base pair or otherwise participate in hybridisation. If it is located at the terminus of the oligonucleotide it will be useful as an immunological rec-ognition site, or haptenic recognition site, to facilitate detec-tion of the oigonucleotide by an antibody capable of bind-ing the compound of this invention.

[0188] The compounds of this invention also are useful as linkers or spacers in preparation affinity absorption matrices (as opposed to functioning as affinity moieties per se as noted above), immohilised enzymes for process control, or immunoassay reagents. The compounds herein contain a multiplicity of functional groups that are suitable assites for cross-linking desired substances. For example, it is conven-tional to link affinity reagents such as hormones, peptides, antibodies, drugs, and the like to insoluble substrates. These insolubilised reagents are employed in known fashion to absorb binding partners for the affinity reagents from manu-factured preparations, diagnostic samples and other impure mixture. Similarly, immobilised enzymes are used to per-form catalytic conversions with facile recovery of enzyme. Bifunctional compounds are commonly used to link analytes to detectable groups in preparing diagnostic reagents.

[0189] Many functional groups present in the compounds of this invention are suitable for use in cross-linking. For example, the phosphonic acid is used to form esters with alcohols or amides with amines. The R groups substituted with OH, azido (which is reduced to amino if desired before cross-linking) or vinyl are exemplary suitable sites. Simi-larly, the amino, halo, acyl and other reactive sites found on group B are suitable. Suitable protection of reactive groups will he used where necessary while assembling the cross-linked reagent. In general, the compounds here are used by linking them through phosphonic acid or amino group to the

Oct. 9, 2003

hydroxyl or amino groups of the linking partner in the same fashion as shown herein, and covalently bonded to the other binding partner through an R group. For example a first binding partner such as a steroid hormone is esterified and then this conjugate is cross-linked through hydroxymethyl R to cyanogen bromide activated Sepharose, whereby immo-bilised steroid is obtained. Other chemistries for conjugation are well known. See for example Maggio, "Enzymelmmu-noassay" (CRC, 1988, pp 71-135) and references cited
therein.

[0190] The oligonucleotides of this invention are labeled with any conventional detectable label, e.g. a fluorescent moiety such a fluorescein, radioisotopes such as 14C or stable free radicals, avidin, biotin and the like all of which previously have been used as labels for immunoassays or diagnostic probes. The label will be present on the oligo-nucleotide or on the residue of an analogue ofthisinvention. Suitable labelling methods are well known and are readily used with reactive groups such as hydroxyl, allyl and the like. A simple method is to label the compound of this invention with H3 by proton exchange. The compounds also are biotinylated using conventional methods. Sec for instance U.S. Pat. No. 5,276,143 for analogous structures. However, the compounds of this invention also are useful directly in diagnostic probe assays without an exogenous detectable label. In one embodiment of this alternative, antibodies are raised against the compounds of this inven-tion. Such antibodies (which in turn are labelled or used in a double antibody configuration) bind to the analogue ofthis invention and thereby are useful in detecting its presence as label for a protein or oligonucleotide.

[0191] The compounds of the invention are useful for treatment of microbial infections, for treatment of tumours or for other indications described below. Microbial infec-tions treatable by the compounds of this invention include viruses, parasites, yeast and fungi, but it is believed that the compounds are most effective against viruses, which con-stitutes the preferred utility. Exemplary viral infections include infections caused by DNA or RNAviruses including herpes viruses (CMV, HSV 1, HSV 2, EBV, varicclla zoster virus (VZV), bovid herpesvirus type 1, equid herpesvirus type 1, HVV-6, papillomaviruses(HPV types 1-55 including carcinogenic HPV), flaviviruacs (including yellow fever virus, African swine fever virus and Japanese encephalitis virus), togaviruses (including Venezuelan equine encepha-lomyelitis virus), influenza viruses (typesA-C), retroviruses (HIV-1, HIV-2, HTIV-1, HTLV-ll, STY, FeLV FlY, MoMSV), adenoviruses (types 1-8), poxviruses (vaccinia virus), enteroviruses (poliovirus types 1-3, Coxsackie, hepa-titisA vinis, and EChO virus), gastroenteritis viruses (Nor-walk viruses, rotaviruses), hantaviruses (Hantaan virus), polyomavinis, papovaviruses, rhinoviruses, parainfluenza virus types 1-4, rabies virus, respiratory synctial virus (RSV), hepatitis viruses A, B, C and E, and the like.

[0192] The antiviral activity of individual compounds is determined by routine assay of antiviral (or other antimi-crobial) activity using enzyme inhibition assays, tissue cul-ture assays, animal model assays and the like as will be understood by those skilled in the art.

[0193] Prntoxoan parasite infections are treated using the compounds of the invention. The term protozoa include those members of the subphyla Sarcomastigophora and

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13

Sporozoa of the phylum Protozoa. More particularly, the term protozoa as used herein include genera of parasitic protozoa, which are important to man, because they either cause disease in man or in his domestic animals. These genera for the most part are classified in the superclass Mastigophora of the subphylum Sarcomastigophora and the class Telesporea of the subphylum Sporozoa in the classi-fication according to Baker (1969). Illustrative genera of these parasitic protozoa include Histomonas, Pneumocystis, Trypanosorna, Giardia, Trichomonas, Eimeria, Isopua, Leishmania, tintamoeba, ’loxoplasma and Plasmodium. Parasitic protozoans include Plasmodium fakiparurn, Plas-modium berghei, Plasmodiunz malariae, Pla.smodiwn viva Leishmania braziliensis, Leishmania donovani, Trypano-soma cruz4 Trypanosoma brucei, Trypanosorna rlwd-esiense, Pnewnocysiis carinii, Entarnoeba histolytica, Tn-chomonas vaginaiis and the like (de Vries, E., et al, "Mol. Biochem. Parasitol" 47:43-50 (1991) and trypanosomes (Kaminsky et al. "J.Parasitot" 80(6): 1026-30 (1994). The compounds in which R is CHOH and B is 3-deazaadenine are particularly interesting in the treatment of malarial parasites.

[0194] Compounds of the invention are used to treat yeast or fungal infections caused by Candida glabrata, Candida ropicalis, Candida albicans, and other Candida species, Cryptococcus species including C,yptococcus neoformans, Blastomyces species including Blastomyces dermatidis, Torulopsis species including Torulopsis glabrata, Coccidio-ides species including Coccidiaides immitis, Asporgillus species and the like.

[0195] The compounds of the invention can also be (1) applied to tissue culture systems to eliminate or reduce viral spread or growth during the production of biopharmaceuti-cal or other products (such as proteins or vaccines), (2) used to eliminate or reduce viral spread or growth in clinical sample (such as blood), and (3) used to stop growth oftissue culture cells while leaving the cells to carry on with protein production.

[0196] The compounds herein have been found to sup-press immunostimulation. Accordingly, they can suppress metabolic activities ofT-lymphocytes stimulated by diverse agents, e.g. concavalin A, they principally will find appli-cation in the treatment of autuimmune diseases, e.g. arthritis, or in suppression of transplant rejection. Their therapeuti-cally active concentrations are in the range of 1 mg/kg to 50 mg/’kg of body weight.

[0197] Therapeutic administration

[0198] Suitable mutes for administration include oral, rectal, vassal, topical (including ocular, buccal and sublin-gual), vaginal and parental (including subcutaneous, intra-muscular, intravitreous, intravenous, intradermal, intrathecal and epidural). The preferred route of administration will depend upon the condition of the patient, the toxicity of the compound and the site of infection, among other consider-ations known to the clinician.

[0199] The therapeutical composition comprise about 1% to about 95% of the active ingredient, single-dose forms of administration preferably comprising about 20% to about 900A of the active ingredient and administration farina which are not single-dose preferably comprising about 5% to about 20% of the active ingredient. Unit dose forms are, for

Oct. 9, 2003

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example, coated tablets, tablets, ampoules, vials, supposito-ries or capsules. Other forms of administration are, for example, ointments, creams, pastes, foams, tinctures, lip-sticks, drops, sprays, dispersions-and the like. Examples are capsules containing from about 0.05 g to about 1.0 g of the active ingredient

[0200] The pharmaceutical compositions of the present invention are prepared in a manner known per se, for example by means of convential mixing, granulating, coat-ing, dissolving or lyophilising processes.

[0201] Preferably,solutions ofthe active ingredient, and in addition also suspensions or dispersions, especially isotonic aqueous solutions, dispersions or suspensions, are used, it being possible for these to be prepared before use, for example in the case of lyophiliscd compositions which comprise the active substance by itself or together with a carrier, for example mamiitol. The pharmaceutical compo-sitions can be sterilised and/or comprise excipients, for example preservatives, stabilisers, wetting agents and/or emulsifiers, solubilizing agents, salts for regulating the osmotic pressure and/or buffers, and they are prepared in a manner known per se, for example by means of convential dissolving or lyophilising processes. The solutions or sus-pensions mentioned can comprise viscosity-increasing sub-stances, such assodium carboxymethylcellulose, carboxym-ethylcellulose, dextran, polyvinylpyrrolidone or gelatin.

[0202] Suspensions in oil comprise, as the oily compo-nent, the vegetable, synthetic or semisynthetic oils custom-ary for injection purposes. Oils which may be mentioned are, in particular, liquid fatty acid esters which contain, as the acid component, a long-chain fatty acid having 8-22, in particular 12-22, carbon atoms, for example lauric acid, tridecylic acid, myristic acid, pentadecylic acid, palmitic acid, margarie acid, stearic acid, arachidinic acid, behenic acid or corresponding unsaturated acids, for example oleic acid, elaidic acid, curie acid, brasidic acid or linoleic acid, if appropriate with the addition of antioxidants, for example vitamin F., (j3-carotene or 3,5-di-tert-butyl4 hydroxytolu-ene. The alcohol component of these fatty acid esters has not more than 6 carbon atoms and is mono- or polyhydric, for example mono-, di- or trihydric alcohol, for example metha-nol, ethanol, propanol, butanol, or penlanol, or isomers thereof, but in particular glycol and glycerol. Fatty acid esters are therefore, for example: ethyl oleate, isopropyl myristate, isopmpyl palmitate, "I.ahrafil M 2375" (polyoxy-ethylene glycerol trioleate from Gattefosee, Paris), "Labrafil M 1944 CS" (unsaturated polyglycolated glycerides pre-pared by an alcoholysis of apricot kernel oil and made up of glycerides and polyethylene glycol esters; from Gattefosec, Paris), "Labrasol" (saturated pulyglywlated glycerides pre-pared by an alcoholysis of TCM and made up of glycerides and polyethylene glycol esters; from Gattefosce, Paris) and/or "Miglyol 812" (triglyceride ofsaturated fatty acids of chain length C, to C12 from Hills AG, Germany), and in particular vegetable oils, such as cottonseed oil, almond oil, olive oil, castor oil, sesame oil, soybean oil and, in particu-lar, groundnut oil.
[0203] The preparation of the injection compositions is carried out in the customary manner understerile conditions, as arc bottling, for example in ampoules or vials, and closing
of the containers.

[0204] For example, pharmaceutical compositions for oral use can be obtained by combining the active ingredient with

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Antiestrogen Inhibition of Cell Cycle 1/41

Progression in Breast Cancer Cells /
Is Associated with Inhibition of (Cyclin-Dependent Kinase Activity \
and Decreased Retinoblastoma

Protein Phosphorylation

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To define the mechanisms by which antiestrogens inhibit breast cancer cell proliferation, the effects
of the antiestrogen ICI 182780 on G1 cydlins and
their cyclin-dependent kinase (CDK) partners were Investigated In MCF-7 cells. Inhibition of entry Into
$ phase became evident 9 h after treatment, with the proportion of cells in S phase reaching a min-imum by 24 h. ICI 182780 increased the proportion of the hypophosphorylated, growth Inhibitory form of the retinoblastoma protein (pRB). This change began at 4-6 h, preceding effects on S phase. This suggests that there are early effects on the activ-ities of CDKs that target pRB that are not merely a consequence of changes in cell cycle progression. The kinase activity of Cdk2 decreased to low levels at 18-24 h when changes in S phase and pRB phosphorylation were well advanced. An earlier ef-fect was seen on kinase activity associated with immunopreclpitated cyclin DI, which was reduced approximately 40% by 12 h, with further decreases at 18-24 h. Cdk2 and Cdk4 protein levels remained constant over 24 h. Cyclin Dl messenger RNA and protein were down-regulated by ICI 182780 from 2 h, with levels halved at 8 h. ICI 182780 also in-creased the expression of the CDK inhibitors p27 and pWAFlICIP1 at later times.
These observations are compatible with the hy-pothesis that antlestrogens blockentry of cells into S phase and inhibit cell proliferation as the conse-quence of an early decline In pRB phosphorylation contributed to by reduced cyclin D1/Cdk4 activity. At later times, increased CDK inhibitor abundance may act to repress Cdk2 and Cdk4 activities, caus-ing additional reductions in pRB phosphorylatlon, thus maintaining the antiestrogen blockade of cell

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cycle progression. (Molecular Endocrinology 9: 1804-1813,1995)

INTRODUCTION

Although antiestrogen therapy is widely used for the treatment of hormone-dependent breast cancer, the mechanisms by which antiestrogens inhibit breast cancer cell proliferation have not yet been defined. These compounds are predominantly cytostatic rather than cytotoxic in vitro and arrest cells in the G1 phase of the cell cycle with a resulting decrease in the relative proportion of cells synthesizing DNA (S phase) (1-5). Only those cells in early to mid-G1 phase are suscep-tible to growth arrest; synchronized cells treated with cytostatic concentrations of tamoxifen in later phases of the cell cycle complete the cycle, divide, and arrest in the subsequent G1 phase (6). The effects of antles-trogens at submicromolar concentrations can gener-ally be reversed by the simultaneous or subsequent addition of estrogen (7, 8). Antiestrogen action is, therefore, believed to be mediated primarily through competitive binding to the estrogen receptor (ER), with direct effects on the transactivation of estrogen-re-sponsive genes, which, in turn, can subsequently alter the expression and activity of numerous additional gene products. The role of the subset of such genes specifically Involved in the control of progression through G1 to S phase by the steroidal antiestrogen ICI 184780 Is the subject of this study.
Cell cycle control is achieved through the action of a family of cyclin-dependent protein kinases (CDKs) that initiate phosphorylation events to allow progres-sion through checkpoints in the cell cycle (9-13). Al-though antiestrogen action could be mediated by ef-fects on CDK activity, as yet no published studies have examined this possibility. CDK activity is controlled at

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Cancer Dormancy: Role of Cyclin-dependent Kinase Inhibitors in Induction of Cell Cycle Arrest Mediated via Membrane 1gM1’2

Radu Marches, Richard H. Scheuermann, and Jonathan W. UIir3

Carte., lnunwtoblology Center and DeparOnents of Microbiology (L M.. I. W. U.! and Pathology IF H. It University of Texas Sosthweste,n Medical Center at Dallas. Dallas.
Texas 75235.8576

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And-Idlotype (anti-Id) antibody can Induce tumor dormancy In a mu-
ring B lymphoma, DCLI, by Its ability to Induce call cycle arrest and
apoptoals (negative signaling). In human B lymphoma, there Is accumu-
lating evidence that the antitumor effect of anti-Id or several other B cell-reactive antibodies relates to their ability to act as agoniets rather than conventional effector antibodies.In thisstudy, we sneght to elucidate the rule of cycihis, cyclin-dependent kinases (CUKs), and their Inhibitors In and-%M-Induced cell cycle arrest to better understand the mechanisms underlying cancer dormancy. To accomplish this, we have performed in vtau studies with a human lymphoma cell line (Daudi) became Its re-

spouse to anti-Id (or antI-IgM) is similar to that of a BCL5 cell line, more reagents are available, and the results would be particularly pertinent to
therapy of human B all lymphomas. Our results show that cram-linking ofmembrane 1gM on Daudi cellsInduces an arrest late InC1 and prevents pRb from becoming phsepboryisted. The G arrest Is correlated with an Induction of the CDK InhIbitor p21 and reduced CDK2 activity, although the level ofCDK2 protein was not changed. Coprecipltailon o CDK2 with P21 In and-%N-treaied cells and the wiah.ngset level of cydlln E
that p21 Is responsible for the rviL.rflmi of CDK2 activity and theediwe blockade ofthe cell cycle. The induction of p21 was not accompanied by changes In p53 levels. As a result of the G1 block, cyclin A levels sharply declined by 24 h alter anhI-IgM tr$n’t- There was no evidence for Involvement of CDK4 or CDK6 In the blockade.
These results provide evidence that membrane 1gM cross-linking Daudl cells Induces expression of p21 and a subsequent Inhibition of theon cyclin E-CDK2 kinase complex resulting Ins block to pith phosphoryla-tkrn and cell cycle arrest tate In C,.

INTRODUCTION

Tumor-reactive Abs4 have been used in clinical trials for more than a decade, and the most promising results have been obtained in NHL (1, 2). Thus, administration of anti-Id can induce long-lasting remis-sions (dormancy) in a proportion of these patients. However, patients with the follicular form of NHL invariably relapse and eventually die of their disease. It appears that efficacy of these Ab reagents is dictated by their ability to act as signal transduction agonis*s rather than through immunological effector mechanisms because the respon-sive patients are ones whose tumor cells are signaled by the anti-Id in vitro, as evidenced by phosphorylation of intracellular substrate pro-teins (3). In a murine B cell lymphoma model, anti-Id can induce long-lasting tumor dormancy (4-6). When the dormant tumor cells are examined by flow cytometly, there is marked cell cycle arrest in

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The costs of psiblicathm of (his article were dcfiayed in past by the payment of page charges. This article most thuefore be hereby mwked advenicentesu in accordance with 18 U.S.C. Secslon 1734 solely to Indicate this fact.
’This work was supported by NIH Giant 5-ROl-CA28149 and ACS Giant RPG-95-079.03-CCG

’This Is paper no.6 in a series.
’To whom reipseals for repaints should be addressed. at Cancer Immunahialogy Center. University of Tc,&s Southwestern Medical Center at Dallas. 60110 Hntry Hines Boulevard. Dallas, TX 75235-8376

4 7f abbreviations used or Ab. antibody, MAb. monoclonal Ab; CDL cydlin-dependent kinase 16. ldhaype mIgM, meniheane 1gM; HIlL nan-Hodgklna lymplxana pRb, rthiobtastomaciiz p21, p21’; p27. p27’; GST. gIutathlo.teansfeiase TOF. transforming growth fsetar STAT, signal transducers and activators of transcrip. tiore BtdUrd. bnnnodeoxyutidine.

this population (5). In vitro studies of murine lymphoma (4-10) and human lymphoma cell lines (11. 12) indicate that the anti-Id or anti-lgM antibodies cause cell cycle arrest and apoptosis. Maximal negative crosslinked’ signaling is achieved in vitro when mIgM is hyper-(Ii). There is a correlation between the magnitude of the in
vitro effects and the dormancy-inducing effects of anti-IgM on murine lymphoma cell lines (11). This indicates that these signaling pathways can have profound effects on tumor growth in vivo and that signal transduction mechanisms are strong enough to overcome the genetic lesions leading to the malignant phenotype.
Eukaryolic cell cycle progression is controlled by cyclins and CDKs counterbalanced by CDK inhibitors (13). During G. these two activities respond to regulatory signals and determine whether a cell will complete the division cycle. The CDKs that control progres-sion include the catalytic subunit CDK2, associated with the activat-ing subunit cyclin F., and CDK4 or CDK6, associated with any one of the three cyclin D ’asoforms. A prominent substrate for G CDKs is pRb (14). pRb phosphorylation in late G releases members of the E2F family of transcriptional regulators (IS), which can transactivate genes, the products of which are important for entry into S phase (16, 17). CDK4 or CDK6 is thought to catalyze the first wave of pRb phosphorylalion (18), whereas CDK2, which is active toward the end of G1, may help keep pRb and other substrates in the phosphorylated state (19).
The mammalian CDK inhibitors described to date fall into two

families. One family includes pl6°4’ (20), p15INK4B (21), p18, and related proteins of 15-20 kDa (22). The INK4s are specific inhibitors ofCDK4 and CDK6 and appear to compete with cyclin D for binding to these kinases (20). The other CDK inhibitor family includes p21’’" (23-25), p27’ (26), and p57’5 (27), proteins that are structurally unrelated to the INK4s. In vitro, p21 and p27 have broad specificity inhibiting the kinase activity of preactivated cyclin E-CDK2, cyclin D-CDK4, the S phase CDK. cyclin A-CDK2, and, to a lesser extent, the mitotic cyclin B-cdc2 complex (24-26). When overexpressed in transfected cells, p21 and p27 cause G1 arrest suggesting that despite their broad specificity in vitro, these CDK inhibitors act mainly on 01 CDKs. In many cases, e.g., radiation-induced DNA damage, the G arrest is mediated by the tumor sup-pressor gene p53 that elevates p21 levels transcriptionally, leading to CDK inhibition (23, 28). However, p21 can be expressed at high levels in p53-negative cells, indicating that the gene can also be regulated by other transcription factors (29-31). Although p53-de-pendent apoptosis has been established in many systems (32), a role for p21 in programmed cell death is not clear. It has been suggested that cell cycle arrest via expression of p21 is an early event in the sequence by which activation of p53 leads to cell death (33), but mice with a p21 null mutation have unimpaired p53-dependent apoptosis (34). There Is increasing evidence suggesting that p21 is responsible for the induction of G arrest and, notably, maintenance of cell

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5T11C term ’%ypet-cross-linkin(Indicates dial optimal induction of cell cycle wrest andior upuptosis is not achieved by satwating migM on lymphoma cells with ann-lgM MAli but Is accomplished by Inducing a greater degree of cross4lnklng using polyelonal and-ISM or anti-Id, a mixtuic of several van-crass-reactive MAli specific to distinct epilnpcs on the ja chain or a second Ab against monoclonal anti-si aided to lymphoma
cells.

Phase I Study of the CyclinDepeudent Kinase Inhibitor Flavopiridol in Combination With Paclitaxel in Patients With Advanced Solid Tumors

By Gary K. Schwartz, Eileen O’Reilly, David Ilson, Leonard Sotfz, Sunil Sharma, William Tong, Peter Maslok,
Maxine Stoltz, Lorry Eden, Pam Perkins, Sandra Endres, John Barazzoul, David Spriggs, and David Kelsen

Pu,ose: Prdinicel studies indicate that the
dependent kinese inhibitor flavopiridol potentiates the
induction of apoptosis by poditexel, provided pacli-
taxel followed by flevopiridel. We therefore de-
signed o phase B clinical trial of sequential paclitexel
and flovopiridal.
gents andMethods: Padlitwcel was administered
at a fixed dose, as either a 24- or 3-hour Infusion on day 1, followed by a 24-hour infusion of flavopiridol on day 2. Doses of flovephidel were escalated in successive cohorts according to a modified Fibonacci design. Flavopiridel phermecokinetics were obtained on all patients.
Results: Dose-limiting rteutrepenio developed with 24-hour paditexel doses of 135 and 100 mg/rn2 and flavopiridol doses of 10 and 20 mg/ms, respectively. With 3-hour peditaxel at 100 mg/ms, flavopiridol could be escalated to 70 Mg/M2 without dose-limiting

FLAVOPIRIDOL (L86-8275) is a synthetic flavone. It is I identical to a compound obtained by derivation from Dysoxyhim hinecff/Łrurn, a plant indigenous to India) Even though its exact mechanism of action remains unknown, it is the first anticancer agent to enter clinical trials as a potent inhibitor of the cyclin-dependent kinases (CDt(s). Inhibi-tion of CDK2 and CDK4 has been reported with concen-trations that inhibit enzymatic activity by 50% (IC51) in the 100-nmoJIL range.2 It also directly inhibits CDK1 enzy-i-antic activity with an inhibitory constant (K1) for adenosine triphosphate (ATP) of 41 nmol/L and inhibits CDKI phos-pborylaiion.34 Although it exhibits a relative selectivity For the CDKs, it is still not specific for this class of enzymes. It has been reported to inhibit protein kinese C at an IC0 of 6 smolrL, cyclic adenosine monophosphate--dependent pro-tein kinase at an IC. of 145;Lmol/L, and epidermal growth factor-receptor kinase at art IC50 of 25 mo1JL.3 It has been reported to suppress the transcription of cell cycle--specific genes, including cyclin D1,5 block human immunodefi-ciency virus Tat transactivation and viral replication through inhibition ofpositwe transcription elongation factor b,6 and inhibit glycogen phosphorylase.7
The activation ofthe CDKs is required for transit ofthe cell between the different phases of the cell cycle, whether it is 61 to S or G2 to M. Thus, flavopiridol, as a direct inhibitor of the CDKs, has been found at nanomolar concentrations to induce a block in cell cycle progression at

toxicity. With 3-hour paclitaxel next escalated to 135 mg/me, dose-limiting neutropenia and pulmonary tax-icily occurred when flavopiridol was escalated to 94 mg/rn2. This did not correlate with any change in flo-vopiridol or paditexel phormacokinetics. At a 3-hour paditezel dose of 175 mg/rn2, dose-limiting pulmo-nary toxicity occurred in only one patient atflavepiridol doses under 94 mg/m2. Clinical activity was observed in patients with esophagus, lung, and prostate cancer, including patients who had progressed on peditaxet. Conclusion: The recommended phase II doses will be a 3-hour infusion of puclitaxel at 175 mg/rn2 on day 1 followed by a 24-hour infusion of flavopiridol at 70 mg/m" on day 2. Flavopilidol dose escalations to 80 mg/vi" are possible. At these doses, toxldtles are man-ageable and clinical activity is promising.
J Cliii Oncal 20:2157-2170. s. 2002 by American Society of Clinical Oncology.

the G., -S and G2-M interfaces. Exposure of MDA-MB-468 human breast cancer cells to flavopiridol for 72 hours induces cell cycle arrest." In other cell lines it has been shown to induce apoptosis.9° -
Phase I trials with single-agent:flavopiridol have been completed at both the University of Wisconsin and the National Cancer Institute) Because the best activity in tumor xenografIs was demonstrated with repeated drug treatment, a 72-hour continuous intravenous schedule was selected for the phase I clinical trial. The 3 -day flavopiridol treatment was repeated every 2 weeks. The dose-limiting toxicity was secretory diarrhea at a flavopiridol dose of 62.5 rng/rn2/d. The diarrhea could be controlled with loperamide or cholestyramine and further dose escalations were possi-ble with antidiarrheat prophylaxis. in the presence of

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Submitted February 23 2001: accepted Jamwsy 28, 2002. Supponed by grant nos. R01 C467819 and Util 69856 04fr&u the

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Advances in Brief \S’

Promoting Apoptosis: A Novel Activity Associated with the Cyclin-dependent
Kinsice Inhibitor p27

Yu Katayose, Mlii Kim, Amol N. S. Rnkknr, Zhuangwu LI, Kenneth H. Cowan, and Prem Seth’

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p27°’, a cyciln.depeudent kinase lnhibitnr, Is ru5uLacd is a ueg.thr
regulator ofthe cell cycle. In this paper, we report that uvax’ezpresslon of
p27KIPI friers apoptasis In several different human cancer cell lines.
Using a recombinant adenoviral vector that expresses p27’(Adp27), we
found that overesprunlon of p27TM51 In MDA-MB-231 breast omwer cells
Induces apopteals that was seen by a number of different techniques, 6d..dlng flow cytosnetry and in situ terminal deoxynucleotidyl train-frrase.medlated nick end labeling, flow cytomdrk assay for sub-C, pop-ulation, and 4’,6-d1amlndlno-2-pbenyllndcie ituInhiig Cleavage at poly-(ADP-rlbase) polyrnerase and degradation of cydlin 81, events that are known to be associated with apoptosis, were also observed following overexpresslon of p27aiPt. This Is the first teport hwilcathtg a role for p27P’ In Induction of apoptosla.

Introduction

Growth arrest and programmed cell death (or apoptosis) are two critical cellular responses to a variety of genotoxic (DNA-damaging) stimuli. An arrest in the progression of the cell cycle provides the DNA repair machinery an opportunity to repair the damaged DNA. whereas the process of apoptosis eliminates cells with extensive, and presumably irreparable. DNA damage. Therefore, it is not surprising that one or both of these processes are frequently dysfimctional in
cancerous cells.

The p53 protein is a pivotal component of pathways leading to growth arrest as well as apoptosis, suggesting that the two processes may act in concert (I). Many studies have established that DNA damage leads to up-regulation and activation of p53, which can result either in arrest at the 01-S checkpoint, by transcriptional activation of a gene encoding the CD1W irjbftt p2lWtPt, or the induction of apoptosis. Therefore, it is reasonable to investigate whether other proteins involved in the regulation of the cell cycle, besides p53, may play a role in inducing apoptosis. One such protein is p271, aCDKI that is integrally involved in ensuring an orderly progression through the cell cycle. p27’°". a member of a family of proteins that includes

121WafIICIpt and p572, is a universal CDKI that negatively regu-lates cyclin D-, E-, A-, and B-dependent kinases (2, 3). Levels of p27’°’ are high in the quiescent cells but decrease in the cycling cells, when it gets sequestered into excess cyclin D-CDK complexes. This removal of p27’’-mediated repression of cyclin E-CDK2 and cyclin A4CDK2 allows progression through the 0,-S restriction point. The importance of p27’ in regulating the cell cycle, and thereby

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’To whom requests for relates should be addressed, at Medical Breast Cancer Section, Medicine Branch, National Cancer institute, NIH. Building 10. Room 12N226, Bethesda, MD 20892. Phooe: (301) 496-9517: Fix. (301)402.0172; E-mail: pseth@box.
p.flhlLgoV.

21’lw abbreviations used CDKI, cyclin-dependent kinase inhlbltee pin, plaque- forming unit(s); TUNEL terminal deoxynucleoddyl nansfetase-mediated nick cud label-

ing; FACS. thao.ctceo.-activated cell eating; DAN, 4’,ediam[ndboo-2-plwnylhrdole; PARP. poly(ADP-ribose) polyinerase.

cell size and number, is underscored by the fact that mice nullizygous for the gene encoding p27’°" grow faster, exhibit frank organo-megaly, and have a higherincidence ofpituitary tumors in comparison to littermate controls (4-6).
We have previously reported that overexpiession of p27‘P1 in cancer cells results in 0,-S arrest (7). Furthermore, overexpression of p27’ appeared to be more cytotoxic to cancer cells relative to the effects of overexpiession of another universal CDXI, p2lvI (7). In the studies reported here, we explored the mechanism of p27 cytotoxicity and its possible role in inducing apoptosis.

Materials and Methods

Cell Culture. Breast cancer cell Lines MDA-MB-231 (ATCC HTB26), BT-549 (ATCC HTBI22), MCFa7 (Mriamycln-resistant MCF-7 cells), and H-355 (a lung cancer cell line, kindly provided by I. Minus, University of Texas, Dallas. ’1() were cultured in Improved Minimal Essential Medium (Life Technologies, Inc., Gaithersburg, MD) containing 10% fetal bovine serum as described previously (7). A bile duct cancer cell line (TFK-1. kindly provided by Dr. Kudo. Cancer Cell Repository. Toboku University. Sendai. Japan), was cultured in RPM! 1640 (Life Technologies. Inc.. Gaithersburg. MD) containing 10% fetal bovine serum as described prevIously (8). Coustructlon ofAdenoviral Vectors and Infection Procedure. Adp27.a replication-deficient recombinant adenovirus that expresses p27", was con-stricted in oter laboratory as described previously (7). AdNull is a control recombinant adenovirus that does not carry any tsansgcne but has a similar adenoviral backbone as Adp27.
Infection with Adp27 and the control virus (AdNull) was accomplished by incubating cells with various doses of the virus, expressed In pfulcell, in serum-free medium for 2 It. followed by the addition of medium containing 10% serum and further incubation for the required time.
Assays for Apoptosis. For detection of apoptosis by the TUNEL method, cells were Infected with the adenoviral vectors, collected by tzpsln-EDTA. and washed two limes with M. TUNEL was performed using MEBSTAIN Apoptonis kit (Medical and Biological laboratories Co., Ltd., Nagoya, Japan) according to manufacturer’s directions. FACS analysis was performed on FACSCalibur instrument (Beaten Dickinson). Cytospins mounted on glass slides were viewed under the fluorescence microscope.
Analyses ofthe sub-G, (sub-diploid) population was done by measuring the fluorescence activity of propidlum iodide-stained DNA (9) of permeabilized and fixed cells on the FACSCalibur instrument (Becton Dickinson):

Number of cells below 0, % sub-G, population = X 100

Total number analyzed (10,000)

For detection of apoptosis using DAN staining, cultured and infected cells were washed with PBS and harvested using 0.02% trypsin in PBS. 50,000 cells were resuspended in 50 gel of PBS. 10 M1 ofbovine serum albumin (BSA) were added to the sample, and this suspension was pipetted into the bottom of a cytofunnel mounted with a microscope slide and spun at 500 rpm for 5mm. Slides were then dried for 30 mlii at room temperature and washed with PBS. 200 gel ofDAFt (2.5gegIml in 0.05 hI phosphate buffer. pH72) was applied for 30 mlii at room temperature. After washing with PBS. samples were stored in the dark at 4° C and viewed under the fluorescence microscope.
Western Blot Analysis. Cells (2 X were Infected by adenoviral vectors and scraped, and the lysates were subjected to Western Blot analysis as

Cyclin D-dependent kinases, INK4 inhibitors and ca...[Biochim Biophys Acta. 2002] -... PÆgina

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Cyclin 0-dependent kinases, INK4 Inhibitors and cancer.. CarofP1ii2.f
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Molecular Oncology Program, Centro National de Investigaciones Oncológlcas, Metchor Pi.;up.nt n’arl kare:ric:n. Fernandez Almagro 3, 28029 Madrid, Spain. t? C’S IeatL
The Cyclin D-Cdk4,6/INK4/Rb/E2F pathway plays a key role in controlling cell sncu;znc p nerfis mmembats
growth by Integrating multiple mitogenic and antimitogenic stimuli. The of th* ll’iM heniiy of epa;ttkn
components of this pathway are gene families with a high level of structural and ttt..0i3]

functional redundancy and are expressed In an overlapping fashion in most ,;, actt, ax ct tissues and cell types. Using classical transgenlc technology as well as gene- v P;3i./AihziOip7tia.;3i 1

targeting in ES cells, a series of mouse models have been developed to study the ajsij in Iman
In vivo function of individual components of this pathway in both normal

homeostasis and tumor development These models have proven to be useful to - define specific as well as redundant roles among members of these cell cycleregu% (XX4 i’" ’’r i ’S regulatory gene families. This pathway Is deregulated In the vast majority of cm:r-43

human tumors by genetic and epigenetic alterations that target at least some of
Its key members such as Cyclin Dl, Cdk4, INK4a and XNK4b, pRb etc. As a
consequence, some of these molecules are currently being considered as targets
for cancer therapy, and several novel molecules, such as cdk inhibitors, are
under development as potential anti-cancer drugs.
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Cyclin-dependent kinase Inhibitors block proliferation of human gastric cancer
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Department of Surgery, University of Texas Medical Branch, Galveston 77555-0542, USA. and t?.jenn:bto; f ffie, cy;.<.efliersi k:es
dc2,c2:ds.

BACKGROUND: Olomoucine and roscovitine are novel compounds that are
designed to inhibit cyclin-dependent kinases (e.g., Cdk2 and cdc2). Cdks regulate
progression through key checkpoints of the cell cycle. The purpose of this study
was to determine (1) whether olomoucine and roscovitine inhibit Cdk2 and cdc2
kinase activities of the human gastric cancer cell line SIIA and (2) whether olomoucine and roscovitine block cell proliferation and cell cycle progression. METHODS: SIIA cells were treated with olomoucine or roscovitine and examined for Cdk2 and cdc2 activities by using histone Hi as the substrate. Cell numbers were counted with a Coulter counter. Cell cycle distribution was analyzed by DNA flow cytometry. RESULTS: Olomoucine and roscovitine completely blocked Cdk2 and cdc2 activities in SIIA cells. Both compounds were also able to inhibit proliferation of SIIA cells, as well as three other human gastric cancer cell lines (AGS, MKN45-630, and SNU-1). Cell cycle analysis showed that treatment with olomoucine or roscovitine for 24 hours led to a decrease in the S phase population and an increase in the G2/M population. CONCLUSIONS: We have shown that Cdk inhibitors, olomoucine and roscovitine, are a new class of antineoplastic molecules with potential therapeutic benefits for gastric cancers.

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Flavopiridol: the first cyclin-dependent kinase in...[Invest New Drugs. 1999] PubMed.

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Flavopiridol: the first cyclin-dependent kinase inhibitor In human clinical trials.

Senderowics AN.

DTP Clinical Trials Unit, Developmental Therapeutics Program, Division of Cancer Treatment
and Diagnosis. National Cancer Institute, Bethesda, MD 20892, USA. sendero@heiix.nih.gov

The discovery and cloning of the cyclin-dependent kinases (cdks), main
regulators of cell cycle progression, allowed several investigators to design novel modulators of cdk activity. Flavopiridol (HMR 1275, L86-8275), a flavonoid derived from an indigenous plant from India, demonstrated potent and specific in vitro inhibition of all cdks tested (cdks 1, 2, 4 and 7) with clear block In cell cycle progression at the GuS and G2/M boundaries. Moreover, preclinical studies demonstrated the capacity of flavopiridol to induce programmed cell death, promote differentiation, inhibit anglogenic processes and modulate transcriptional events. The relationship between the latter effects and cdk inhibition Is still unclear. Initial testing in early clinical human trials with infusionai flavopiridol showed activity In some patients with non-Hodgkin’s lymphoma, renal, prostate, colon and gastric carcinomas. Main side effects were secretory diarrhea and a pro-inflammatory syndrome associated with hypotension. Biologically active plasma concentrations of flavopiridol (approximately 300-500 nM) are easily achievable in patients receiving infusional flavopiridol. Phase 2 trials with Infusional flavopiridol in several tumor types, other schedules and combination with standard chemotherapies are being assessed. In conclusion, flavopiridol is the first cdk inhibitor to be tested in clinical trials. Although important questions remain to be answered, this positive experience will stimulate the development of novel cdk modulators for cancer therapy.

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The use of cyclin.dependent kinase inhibitors alone or In combination with
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Grant S. Roberts 313.

Department of Medicine, Medical College of Virginia, Virginia Commonwealth University,
McV Station Box 230, Richmond, VA 23298, USA. stgrant@hsc.vcu.edu

Cyclin-dependent kinase (CDK) inhibitors are small molecule inhibitors of the
kinases required for the orderly progression of cells, both normal and neoplastic, through the cell cycle. Because cell cycle dysregulation is such a common occurrence in neoplasia, the search for agents that might block cell cycle traverse has been the focus of intense interest. These efforts have led to the identification of a broad array of compounds that interfere directly with the function of CDKs. Two of these agents (flavopiridol and UCN-01) have now entered the clinical arena, and others are scheduled to do so in the near future. In preclinical studies, CDK inhibitors have shown the ability not only to block neoplastic cell proliferation, but also to induce, through a variety of mechanisms, programmed cell death. The latter capacity may stem from the diverse effects that CDK inhibitors exert on multiple kinases and apoptotic regulatory molecules. In addition, there is abundant preclinical evidence that CDK inhibitors can potentiate, generally in a dose- and sequence-dependent manner, the anti-tumor effects of many established cytotoxic agents. In clinical studies In humans, flavopiridol and UCN-01 have been shown to be tolerable, although clear evidence of single agent activity or enhancement of the efficacy of established agents has not yet emerged. This may reflect a failure to optimize drug schedules/pharmacokinetics, or to identify the critical molecular targets of these agents. Finally, in recent years, a rationale has emerged for combining CDK inhibitors with other molecularly targeted agents (i.e. differentiation-Inducers and signal transduction modulators). Current research has basically two goals: (a). to identify CDK inhibitor concentrations and schedules that inhibit the growth of and induce apoptosis in specific tumor cell types; and (b). to establish a rational basis for combining CDK inhibitors with more conventional cytotoxic agents to enhance antitumor efficacy. This review gives a brief summary of such efforts, with an emphasis on agents and combinations that are in or near clinical development.

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] 1 Registro Completo
Tituic: Amplification and overexpression of cyclin Dl in breast cancer detected by immunohistochemical
Autores. C Gillett
Fuente: Cancer Research, 1994
En: Google Scholar

2 Registro Complete

Thu/c: Role of the cyclin-dependent kinase inhibitors in the development of cancer.
Autores: T l-ilrama; HP Koeftier
Fuente: Blood, 1995
En: Google Scholar

ff] 3 Registro Completo
lieu/c:.. cycle progression in breast cancer cells In associated with inhibition of cyclin-dependent kinase

Autores: CK Watts
Molecular Endocrinology, 1995
En: Google Scholar

Registro Completo

Tftu!:... of the cyclin-dependent kinase inhibitors p16INK4, p15INK413 and p21WAF1/CIP1 in human
breast cancer

Autores: EA Musgrove; B Lilischkis; AL Cornish; CS Lee
Fuente: mt j Cancer, 1995
En: Google Scholar

tJ 5

Thu/c: Inhibitors of cyclin-dependent kinase and cancer
Autores: JR Biggs; AS Kraft
Fuente: Journal of Molecular Medicine, 1995
En: Google Scholar

lltu/o: Deregulation of cyclin E in breast cancer.
Autores: K Keyomarsi; D Conte Jr; W Toyofuku; MP Fox
Fuente: Oncogene, 1995
En: Google Scholar

i;:i 7

Registro Complete

Registro Complete

Registro Complete

Tst.j!o activity of cyclin-dependent kinases and retinoblastoma protein phosphorylation In breast cancer

Autores: IS Foster

Fuente: Molecular Endocrinology, 1996
En: Google Scholar

1 S Registro Complete

Tituic:... the cyclin-dependent kinase inhibitor genes p15INK4b/MTS2, p16INK4/MTS1 p18 and p19 in

http://www.sinab.uiial.Ødu.co:2080itnuse/seMetlMuSePeer 04/06/2008

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human cancer

/-wto es A Gemma, S Takenoshita, K Hagiwara, A Okamoto, EA
Fuente: mt 3 Cancer, 1996
En: Google Scholar

Registro Completo

711tu10 UCN-01, 7-Hydroxyl-stauràsporine, Inhibits Kinase Activity of Cyclin-Dependent Kinases and
Reduces
Autores: K Kawakami; H Futami; 3 Takahara; K Yamaguchi
Fuente; Biochemical and Biophysical Research Communications, 1996
En: Google Scholar

!] 10 Registro Completo
Tkulo: 17beta-Estradiol induces cyclin Dl gene transcription, p36Dlp34cdk4 complex activation and
plO5Rb...
Autores: L Aitucci; R Addeo; L Cicatiello; S Dauvois; MG
Fuente: Oncogene, 1996
En: Google Scholar

ii Rgistro Completo L

ti.;a Iron Deprivation Inhibits Cyclin-Dependent Kinase Activity and Decreases Cyclin D/CDK4 Protein
.4utores: KS Kup; SL Green; PR Vülllet
Fuente: Experimental Cell Research, 1996
En: Google Scholar

12 Registro Completo

Titulo: Prognostic role of the cyclin-dependent Idnase Inhibitor p27 in non-small cell lung cancer
4utores: V Esposito
Fuente: Cancer Research, 1997
En: Google Scholar

13 Registro Complete

lituIo: Loss of the cyclin-dependent kinase inhibitor p27(Kipl) protein in human prostate cancer
correlates
Autores: V Guo; 43N Sklar; A Borkowski; N Kyprlanou
Fuente: CliA Cancer Res, 1997
En: Google Scholar

(j 14 Registro Completo _111 _____
Thu/c 27 Kip 1 and cyclin E, alone and in combination, correlate with survival in young breast cancer

Autores PL Porter, KE Malone, P3 Heagerty, GM Alexander, LA
Fuente: Nature Medicine, 1997
En: Google Scholar

15 Registro Complete Snuff

11W/b: Regulation of Breast Cancer Cell Growth by Progesterone: Role of the Cyclin-Dependent Kinase Autores: SD Groshong; GI Owen; S Grimison; IE Schauer; MC
Fuente: Molecular Endocrinology, 1997
En: Google Scholar

jj 16 Registro Completo

http://www.sinab.unal.edu.co:2080/muse/servlet/MusePeet 04/06/2008

SearchSolver PÆgina3 de 7

TItQ!o: IFNa induces the expression of the cyclindependent kinase Inhibitor p 21 in human prostate cancer

4utores: AC Hobeika; PS Subramaniarn; HM Johnson
Fuerste: Oncogene, 1997
En, Google Scholar

!1 17 Registro Completo

Titulo:.. cancer and two polymorphisms occurring together in the p 21 Waf 1/Cip 1 cyclln-dependent
kinase...
AutOreS: EA Father; P43 Becich; A Deka; JC Law
Fuente: Cancer, 1997
En: Google Scholar

jJ 18 Registro Completo _______

71w/n: Cyclin-dependent kinase inhibitors block proliferation of human gastric cancer cells.
Autores: H Iseki; TC Ko; XV Xue; A Seapan; MR Hellrnich; CM
Fuente: Surgery, 1997
En: Google Scholar

19 Registro Completo t" I

7Lo level expression of pZlklpl and cyclin Dl in some human breast cancer celis Inverse correlation

(,. AutOres: S Fredersdorf; 3 Burns; AM Mime; G Packham
Fuente: Proceedings of the National Academy of Sciences, 1997
En: Google Scholar

LII 20 Registro Completo

Tituin:... Progression in T-47D Breast Cancer Cells Is Accompanied by Induction of the Cyclin-Dependent
Kinase

Auton: EA Musgrove; CSL Lee; AL Cornish; A Swarbrick; RL
Fuente: Molecular Endocrinology, 1997
En: Google Scholar

21 Registro Completo Li"H? ED

Tku!o... Homozygous Deletion of cyclin-Dependent Kinase Inhibitor 2 (MTS1, p15) in Superficial Bladder
Cancer...
4ut:es
Fuente:

P4 Balazs, P Carroll; R Kerschrnann, G Sauter, FM
GENES, CHROMOSOMES CANCER, 1997
En: Google Scholar

IIIi 22 Registro Complete

TItu/o;... Expression of Cyclin-dependent Klnase-6 and Induces a Gi Cell Cycle Arrest of Human Breast
Cancer

Autores: CM Cover; 5) Hsieh; SH Tran; G Hallden; GS Kim; LF
Fuente: Journal of Biological Chemistry, 1998
En: Google Scholar

23 Registro Completo

T,tuJ D3 In Human Prostate Cancer Cell Line LNCaP Involves Reduction of Cyclin-Dependent Kinase 2 Autores: 511 Zhuang; KL Burnstein
Fuente: Endocrinology, 1998
En: Google Scholar

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edu.co:2080/muselservlet/MusePeer 04/06/2008

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is1

24 Registro Completo

TIW1c: Role of p27Kipi and Cyclin-Dependent Kinase 2 in the Proliferation of Non-Small Cell Lung Cancer \\\
Autores: H Kawaria; 3 Tarnaru; TTanaka; A Hirai; V Saito
Fuente: American Journal of Pathology, 1998
En: Google Scholar

Li25 Registro Completo

Titulo: Cyclin-dependent kinase inhibitors and human cancer.
Autores: A Kamb

Fuente: Curr Top Microbiol Immunol, 1998
En: Google Scholar

26 Registro Completo

71W/c; Cancer dormancy: role of cyclin-dependent kinase inhibitors in induction of cell cycle arrest Autores; R. Marches
Fuerite; Cancer Research, 1998
En: Google Scholar

27 Registro Completo

77W/c: Cyclins, cyclin-dependent kinases, cyclin-dependent kinase Inhibitors and their role In head and
neck cancer
Autores’ JEANNON, J. -PS; WILSON, 3; A

Fuente; Clinical Otolaryngology & Allied Sciences. 23(5):420-424, October 1998. Complete ReferenceTable of Contents En; OVID - ournals@Ovid

28 Registro Completo

iituic: THE PROGNOSTIC SIGNIFICANCE OF ALTERED CYCLIN-DEPENDENT KINASE INHIBITORS IN HUMAN CANCER

Autores: 3 Tsihlias; MD; FRCSC; L Kapusta; MD; FRCPC
FL’efltC: Annual Reviews in Medicine, 1999
En: Google Scholar

Fl 29 Registro Completo

Ttuio:... Loss of Expression of the Cyclin-dependent Kinase Inhibitor p27 In Epithelial Ovarian Cancer 1 Autores: V Masciullo; A Sgambato; C PacUlo; B Pucci
Fuente: Cancer Research, 1999
En: Google Scholar

30 Registro Completo

Titulo; Androgen and Epidermal Growth Factor Down-Regulate Cyclin-dependent Kinase Inhibitor p27Kipl
and
Autores; D Ye; 3 Mendelsohn; Z Fan
Fjente: Clinical Cancer Research, 1999
En: Google Scholar

LJ31 Registro Completo

Tltu/c... Cyclin-dependent Kinase Inhibitor p16 Is Associated with Tumor Recurrence in Human Prostate
Cancer

Autores; CT Lee; P Capotheci; I Osman; M Fazzari; 3 Ferrara
Fuente: Clinical Cancer Research, 1999
En; Google Scholar

http://www.sinab.unal.edu.co:2080/muse/servlet/MusePeer 04/06/2008

SearchSolver PÆgina5 de 7

L1 32 Registro Completo 4 3

TItulo:... subcellular localization of the cyclin-dependent kinase Inhibitor 27 Kip 1 In human colon cancer
Autores: A Sgambato; C Ratto; B Faraglia; M Merico
Fuente: Molecular Carcinogeriesls, 1999
En: Google Scholar

E] 33 Registro Completo
Titulo:... cyclin-dependent kinase inhibitor p27 as a prognostic factor In advanced non-small cell lung
cancer

.4utores: S Ishihara; K Minato; H Hoshino; R Saito; F Hara
Lung Cancer, 1999
En: Google Scholar

34 Registro Completo

Titulo: Prognostic Impact of Cyclin-Dependent Kinase Inhibitor p27 kipi in Node-Positive Breast Cancer
Atjtors: A TSUCHIYA; GUOJUN ZHANG; M KANNO
Journal of Surgical Oncology, 1999
En: Google Scholar

35 Registro Completo

Titulo.- A cyclin-dependent kinase Inhibitor Inducing cancer cell differentiation: Biochemical identification

Autores: GR Rosania; I Merlie Jr; N Gray; VT Chang; PG
Fuente: Proceedings of the National Academy of Sciences of the..., 1999
En: Google Scholar

j 36 Registro Completo

Titulo: Synthetic cyclin dependent kinase inhibitors. New generation of potent anti-cancer drugs
Autores: M Hajduch; L Havlieek; I Vesely; R Novotny
Fuente: Adv Exp Med Biol, 1999
En: Google Scholar

Li 37 Registro Completo

Titulo: Expression of the cyclin-dependent kinase inhibitor p27/KIP1 in laryngeal cancer
,4utores: JEANNON, 1.-P.; SOAMEI, J. V.; WILSON, J. A.; LUNEC, 0. 3. +; KELLY, C; ++
Fuente., Clinical Otolaryngology & Allied Sciences. 24(1):83, February 1999. Complete ReferenceTable of Contents
En: OVID - iournals@Ovid

38 Registro Completo

L.) Titulo: Flavopiridol, A Novel cyclin-Dependent Kinase Inhibitor, in Metastatic Renal Cancer: A University of

Chicago Phase II Consortium Study
Autores: Stadler, Walter N.; vogelzang, Nicholas I.; Amato, Robert; Sosman, Jeffery; Taber, David; Liebowitz, David;
yokes, Everett E
Journal of Clinical Oncology. 18(2):371-375, January 2000. AbstractComplete ReferenceTable of Contents
En: OVID - Journais@Ovid

Li 39 Registro Completo

Titulo:... for Inactivation of the KIP Family cyclin-dependent Kinase Inhibitor Genes In Gastric Cancer
Cells
.4utores: IV Shin; F-IS Kim; 3 Park; lB Park; IV Lee
Fuente: Cancer Research, 2000

http://www.sinab.unal.edu.co:2080/muse/servlet/MusePeer 04/06/2008

SearchSolver

En; Google Scholar

Li 40

PÆgina6 de 7

IIs
Registro Completo

Iltulo: Flavopiridol, A Novel Cyclin-Dependent Kinase Inhibitor, in Metastatic Renal Cancer: A University of

Autores: WM Stadler; NJ Vogelzang; R Amato; J Sosman
Fuente: Journal of Clinical Oncology, 2000
En; Google Scholar

141 Registro Completo

Titulo:... between Polymorphism In p2lWafl/Clpl Cyclin-dependent Kinase Inhibitor Gene and Human
Oral Cancer

Aurores R Ralhan; S Agarwal; M Mathur; B Wasylyk
Fuente: Clinical Cancer Research, 2000
En: Google Scholar

Li 42 Registro Completo

71tuio: Expression of cyclin-dependent kinase inhibitor p21.(WAF1) and p53 tumour suppressor gene in
laryngeal cancer
Autores: JEANNON, 3.-P.; SOAMES, J. +; LUNEC, 3. ++; AWWAD, S.; ASHTON, V.; WILSON, 3; A
Fuente: Clinical Otolaryngology & Allied Sciences. 25(1):23-27, February 2000. AbstractComplete ReferenceTable of
Contents
En: OVID - ournals@Ovid

Li43 Registro Completo

lltuio: Expression of cyclin-dependent kinase Inhibitor p2i(WAFI) and p53 tumour suppressor gene in
laryngeal cancer
Autores: JEANNON, J. -P; SOAMES, 3; LUNEC, 3; AWWAAD, S; PSHTON, V; WILSON, 3; A
Fuente: Clinical Otolaryngology & Allied Sciences. 25(5):431, October 2000. Complete ReferenceTable of Contents
En: OVID - lournals@Ovid

LII 44 Registro Completo

Titulo: A Phase II Trial of the Cyclin-dependent Kinase Inhibitor Flavopiridol In Patients with Previously
Autores: GI Shapiro; JG Supko; A Patterson; C Lynch
Fuente: Clinical Cancer Research, 2001
En: Google Scholar

1145 Registro Completo

rltulo: Cyclin E is the Only Cyclin-dependent Kinase 2-associated Cyclin that Predicts Metastasis and
Autores: C MuDer-Tidow; R Metzger; K Kugler; S Dederichs
Riente: Cancer Research, 2001
En: Google Scholar

Li 46

Tituic: Cyclin-Dependent Kinase Inhibitors forTreating Cancer
Autores: PL Toogood
Fuente: Medicinal Research Reviews, 2001
En: Google Scholar

H 47

Registro Completo

Registro Completo

Thuio:... in the domain of cancer research: Reviewl] Negative regulators of cyclin-dependent kinases and
their

http://www.sinab.unal.edu.co:2080/muse/servlet/MusePeer 04/06/2008

SearchSolver PÆgina

Autores: MH Lee; HY Yang
Fjeciee: Cellular and Molecular Life Sciences (CMLS), 2001
En: Google Scholar

48 Registro Completo

cyclin-dependent kinase inhibitor p 27 Kip 1 by PKB/Akt-mediated phosphorylation In breast
cancer

Autores: G Viglietto; ML Motti; P Brunl; RM Melillo; AD
Fuente; Nature Medicine, 2002
En; Google Scholar

49 Registro Completo

Tltu/o:... in Human Prostate Cancer: Association with Loss of the Cyclin-dependent Kinase Inhibitor p27 and

Autores: G Yang; G Ayala; AD Mar20; W Tian; A Frolov; TM
Fuente: Clinical Cancer Research, 2002
En: Google Scholar

ji 50 Registro Completo

fltuio: and cyclin dependent kinase pathways: defining a distinction between RB and p16 loss In lung
cancer

Autores: F3 Kaye
Fuente: Oncogene, 2002
En: Google Scholar

http://www.sinab.unal.edu.co:2080/muse/servlet/MusePeer 04/06/2008

SearchSolver Pªgina 1 de 4

Lista de resultados combinados I PubMed (103) I EBM Reviews, Cochrane Database (0) I Evidence Based MedicKe
Review EBMR (0) I Nedline OVID (140) I OVID)ournals@Ovid (7) I Google Scholar (422) I FindArticles (Free Afticles
Only) (0) I MEDLINE with Full Text (101)

51 Registro Completo

71W/c:... cell-cycle arrest in the epithelial cancer cell line via up-regulation of cyclin dependent kinase Autores: TToyoshima; R Kamijo; KTakzawa; KSurnitani
Fuente: Br) cancer, 2002
En: Google Scholar

52 Registro Completo

Titui:... breast cancer cells: association with selective cooperative inhibition of cyclin Di-dependent kinase
Autores: K Wu; C Wang; MDAmico; Ri Lee; C Albanese; RG
Fuente: Mol Cancer Ther, 2002
En: Google Scholar

53 Registro Completo

Ti/u/c: Cyclin D-dependent kinases, INK4 Inhibitors and cancer
Autores: S Ortega; H Malumbres; H Barbacid
Fuente: BBA-Reviews on Cancer, 2002
En: Google Scholar

54 Registro Completo

TItulo:.. Between Skp2 Ubiquitin Ligase and the Cyclin Dependent Kinase Inhibitor p27Klpl in Prostate Cancer
Autores; 0 BEN-IZHAK; S LAHAV-BARATZ; S MERETYK; S BEN-
Fuente; The Journal of urology, 2003
En: Google Scholar

Registro Completo

Tituic:... and Stabilization of Cyclin-Dependent Kinase Inhibitor p27KIP1 in Vitamin D Analog-Treated Cancer

Autores: R Lin; TT Wang; WH Miller;)H White
Fuente: Endocrinology, 2003
En: Google Scholar

56 Registro Completo

iltuio: Inverse Relationship Between Skp2 Ubiquitin Ligase and the Cyclin Dependent Kinase Inhibitor
p27Kipl in Prostate Cancer

Li Autores: BEN-IZHAK, OFER; LAHAV-BARATZ, SHIRLY; MERETYK, SHIMON; BEN-ELIEZER, SHOSHANA; SABO, EDMOND;
DIRNFELD, MARTHA; COHEN, SHAJ; CIECHANOVER, AARON +
Fuente: Journal of Urology. 170(1):241-245, July 2003. Abstractcomplete ReferenceTable of Contents
En: OVID - Joursials@Ovid

Li 57 Registro Completo

Titulo: Molecular markers in dysplasia of the larynx: expression of cyclin-dependent kinase inhibitors p21
and p27, and p53 tumour suppressor gene in predicting cancer risk
Autores: JEANNON, 3.-P.; SOAMES, 3. V. +; WILSON, 3; A
Fuente: Clinical Otolaryngology & Allied Sciences. 28(6):552, December 2003. Complete ReferenceTable of Contents
En: OVID - Journals@Ovid

58 Registro Completo

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For two-letter codes and other abbreviations, refer to the "Guid-
ance Notes on Codes andAbbreviations" appearing at the begin-
ning ofeach regular issue ofthe PCT Gazette.

((54) Title: COMBINATION OF A TAXANE AND A CYCLIN-DEPENDENT KINASE

0 (57) Abstract A pharmaceutical composition comprised ofTaxolfi, Taxoterefi. or derivatives and a cyclin-dependent kinase is de-scribed, as well as a form of administration wherein the taxane is given intermittently and the cydlin-dependent kinase is given repeatedly within the same cycle.

WO 02/076484 PCTIEP02/04083

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considerably Improved when they are administered in combination with at least one substance which is therapeutically useful in anticancertreatments and has a mechanism identical to or different from these taxanes.

Among substances which may be used in association or In combination
5 with TaxoP, TaxotereP or their analogues, there may be mentioned enzymes
such as L-asparaginase and cyclin-dependent kinases, such as flavopiridol, quercitin and genistein. Various agents such as biological response modifiers or growth factor Inhibitors, such as interferons or interleukins, may also be used. Since the activity of the products depends on the doses used, it is
io possible to use higher doses and to increase the activity while decreasing the
toxicity phenomena or delaying their onset by combining growth factors of the haematopoietic type such as G-CSF or GM-CSF or certain interleukins with Taxolfi, Taxoterefi, their analogues or their combinations with other therapeutically active substances.
15 More especially, the Invention relates to combinations of TaxoP, Taxoterefi and their analogues with the cyclin-dependent kinase, fiavopiridol. Cyclin-dependent kinases (CDKs) are important regulators that control
the timing and coordination of the cell cycle. CDKs form reversible complexes with their obligate cyclin partners to control transition through key junctures In
20 the cell cycle. For example, the activated CDK4.cyclin Dl complex controls
progression through the GI phase while the CDKI-cyclin BI complex controls entry into the mitotic phase of the cell cycle. Endogenous cyclin dependent kinase inhibitory proteins (CDKIs) are known which bind eitherthe CDKor cyclin component and inhibit the kinase activity. In many tumors such as melanomas,
25 pancreatic and esophageal cancers, these natural CDKIs are either absent or
mutated. Thus, selective CDK inihibitors may prove to be effective chemotherapeutic agents.
Flavopiridol (cis-5,7-dihydro)y-2-(2-chioropheflyi)-8-E4-(3-hYdrOXY-1 - methyl)-piperidinylj-1-benzopyran-4-one) is a synthetic fiavone that has been
30 shown to have antitumor activity against various tumor cells lines such as
human lung carcinoma, breast carcinoma, and also inhibits tumor growth in xenograft models. It has been shown to induce arrest in both the GI and G2 phases of the cell cycle. Flavopiridol is a potent and selective Inhibitor of the CDKs, and its antitumor activity is related to its CDK inhibitory activity. Studies
35 have shown that its tumor cell growth inhibitory activity occurs in a cell cycle
specific manner. See Bioorq.& Med. Chem. Letters 10:1037-1041(2000).

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2020
Bogota, D.C.,

Doctor:
Carlos Reinaldo Olarte Garcia
Apoderado

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Oficio No:! 1 32

Con fundamento en lo previsto por el articulo 43 de la Decision 486 de la Cornisión de
la Comunidad Andina, se le comunica que la oposiciOn presentada por el doctor JosØ
Luis Reyes Villamizar, en su calidad de apoderado de Laboratorlo Franco Colombiano - Lafrancol S.A., reUne los requisitos formales exigidos por la ley at momento de su presentación.

En cumplimiento de lo dispuesto en la citada norma, se le concede el tØrmino de sesenta (60) dias hÆbiles siguientesa la fecha de notificaciôn del presente oficio, para que haga valer sus argumentaciones y presente pruebas.

Nottliquese el presente oficio conforme a to dispuesto en el numeral 5.2, literal e), del capItulo quinto del titulo primero de la Circular Unica N° 10 de 2001.

ALIX CARMENZA CESPEDES DE VERGEL
Jefe de la U$ivisión de Nuevas Creaciones.

5 SET 2006

El anterior oficio fue notificado por fijaciOn en lista de fecha,______________

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