

Señor
SUPERINTENDENTE DE INDUSTRIA Y COMERCIO
DIVISION DE NUEVAS CREACIONES

E. _____ S. _____

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SUPERINDUSTRIA Y COMERCIO
Radicación : 00073355 (0000000)
Fecha (ACD): 2020-11-24 16:00:01
Trasite : C32 PATENTES D 1 REGISTRAD/ 323 COTIZACIONES
Dependencia: 2020 DIVISION DE NUEVAS CREACIONES
Folios: 16

Re: P1-F. HOFFMANN-LA ROCHE A.G.-Solicitud de Patente de
Invención para "NUEVOS DERIVADOS DE PIRIMIDINA-2,4,6-
TRIONA".-Clase A.61.-Expediente número 00-073.355.-

1. Me refiero a mi solicitud arriba nombrada de fecha 27 de Septiembre de 2000 .

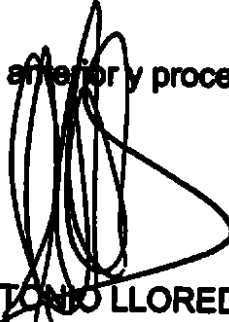
2. Anexas me permito presentar a usted:

(1) Copia auténtica de la primera solicitud de patente presentada para el invento arriba nombrado respecto de la cual reclamo prioridad, a saber la solicitud europea número 99119506.6 de 1 de Octubre de 1999, junto con su traducción oficial No. LLC-081-2000, correspondiente a las legalizaciones y reivindicaciones de dicho documento; y

(2) Copias debidamente autenticadas por el Notario Sexto del Circulo de Bogotá, de los documento que acreditan la calidad de traducción Oficial del señor Gabriel Rueda Chadid quien elaboró Traductor de la solicitud europea número 99119506.6, respecto de la cual se reivindica prioridad en el presente caso.

3. Ruego a usted tomar nota de lo anterior y proceder de conformidad.

Señor Superintendente,


JOSE ANTONIO LLOREDA

T.P. 10.784

Código 231

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**EL SUSCRITO JEFE DEL AREA DE
LEGALIZACIONES DE MINISTERIO DE
RELACIONES EXTERIORES.**

HACE CONSTAR

Que el señor Gabriel Rueda, se encuentra debidamente registrado ante este Ministerio, como traductor e intérprete oficial para los idiomas INGLES -ESPAÑOL, ESPAÑOL - INGLES.

Para efectos de ésta certificación el interesado presentó copia autenticada de la certificación del Ministerio de Justicia acerca de la vigencia de la resolución 15925 1982.

Dada en Santafé de Bogotá, D.C., a los diez y nueve días (19) del mes de diciembre de 1994.


Neilson Sánchez Torres
Jefe Área de Legalizaciones

NST/gtm.

COMO NOTARIO SEXTO ENCARGADO
DE SANTAFE DE BOGOTA
HAGO CONSTAR
QUE LA FOTOCOPIA CONCIDE CON LA FOTOCOPIA
AUTENTICA QUE HE TENIDO A LA VISTA
SANTAFE DE BOGOTA
01 SET 2000
PAULO MENDEZ GARAJAS

COMO NOTARIO SEXTO ENCARGADO DEL CIRCULO
DE SANTAFE DE BOGOTA, HAGO CONSTAR QUE
ESTA COPIA FOTOSTATICA CONCIDE CON EL ORIGINAL
QUE HE TENIDO A LA VISTA
01 JUL 1998
PAULO MENDEZ GARAJAS

21 AGO 1998



Ministerio de Justicia y del Derecho

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EL SECRETARIO GENERAL DEL MINISTERIO DE
JUSTICIA Y DEL DERECHO

CERTIFICA:

Que por Resolución 5996 del 29 de noviembre de 1982, le fue expedida licencia para ejercer funciones de Traductor e Intérprete Oficial en los idiomas INGLES-ESPAÑOL, ESPAÑOL-INGLES a GABRIEL RUEDA CHADID, identificado con la cédula de ciudadanía No. 17.124.145 expedida en Bogotá. Que dicha resolución se encuentra vigente en la fecha.

A solicitud del interesado, en Santafé de Bogotá D.C., a los 17 de los meses de noviembre de 1994.

[Firma manuscrita]
JORGE LUIS SABOGAL SABOGAL

COMO NOTARIO SEXTO ENCARGADO
DE SANTAFÉ DE BOGOTÁ
HAGO CONSTAR
QUE LA FOTOCOPIA CONCIDE CON LA FOTOCOPIA
AUTÉNTICA QUE HE TENIDO A LA VISTA
SANTAFÉ DE BOGOTÁ
PABLO MENDEZ

COMO NOTARIO SEXTO ENCARGADO
DE SANTAFÉ DE BOGOTÁ
HAGO CONSTAR
QUE LA FOTOCOPIA CONCIDE CON LA FOTOCOPIA
AUTÉNTICA QUE HE TENIDO A LA VISTA
SANTAFÉ DE BOGOTÁ
PABLO MENDEZ BARRAL
NOTARIO III

[Firma manuscrita]

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**TRIBUNAL SUPERIOR DEL DISTRITO JUDICIAL DE SANTAFE DE BOGOTA
SECRETARIA GENERAL**

LA SUSCRITA SECRETARIA DEL H. TRIBUNAL SUPERIOR DEL DISTRITO JUDICIAL
DE SANTAFE DE BOGOTA,

C E R T I F I C A :

Que el Acuerdo No. 183 de diciembre 3 de 1982 emanado de esta Corporación, por medio del cual dió posesión al señor GABRIEL RUEDA CHES, identificado con Cédula de Ciudadanía número 17.124.145, de Bogotá, como TRADUCTOR E INTERPRETE OFICIAL de los idiomas INGLES-ESPAÑOL-ESPAÑOL-INGLES, en virtud de la Resolución No. 5996, de 1994, es VIGENTE.

COMO NOTARIO SEXTO ENCARGADO
DE SANTAFE DE BOGOTA
HAGO CONSTAR
QUE LA FOTOCOPIA CONCIDE CON LA FOTOCOPIA
AUTENTICA QUE HE TENIDO A LA VISTA.
01 SET 2000
PABLO MENDEZ BARRAJAS
NOTARIO
24 AGO 1998

Se expide en Santafé de Bogotá D.C., a dieciseis (16) de noviembre
de mil novecientos noventa y cuatro (1994).


MARIA INES RODRIGUEZ TORRES
SECRETARIA

cm./

COMO NOTARIO SEXTO ENCARGADO
DE SANTAFE DE BOGOTA
HAGO CONSTAR
QUE LA FOTOCOPIA CONCIDE CON LA FOTOCOPIA
AUTENTICA QUE HE TENIDO A LA VISTA.
SANTAFE DE BOGOTA, 01 SET 1998
PABLO MENDEZ BARRAJAS
NOTARIO

COMO NOTARIO SEXTO DE
SANTAFE DE BOGOTA
HAGO CONSTAR
QUE LA FOTOCOPIA CONCIDE CON LA FOTOCOPIA
AUTENTICA QUE HE TENIDO A LA VISTA.
SANTAFE DE BOGOTA, 01 JUL 1998
JUAN MANUEL BOTERO MEDINA
NOTARIO
23 NOV 1998
JUAN MANUEL BOTERO MEDINA
NOTARIO

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F. Hoffmann-La Roche AG. C-20508

REF: LLC-081-2000. La presente es Traducción Oficial de un documento presentado originalmente en inglés. Esta traducción es elaborada por Gabriel Rueda Chadid (Traductor Oficial).

OFICINA EUROPEA DE PATENTES

CERTIFICADO

Los documentos adjuntos son fiel copia de la solicitud de patente Europea según la descripción que aparece en la siguiente página, presentada originalmente.

No. de Solicitud de la Patente 89119506.6

El Presidente de la Oficina Europea de Patentes

I.L.C. HATTEN-HECKMAN

(Firmado)

LA HAYA. 07 / 09 / 00


GABRIEL RUEDA CHADID
TRADUCTOR OFICIAL
RESOLUCION 5996 DE NOV. 29-82
MINISTERIO DE JUSTICIA

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OFICINA EUROPEA DE PATENTES

Solicitud No.: 99119506.6
Fecha de Presentación: 01 / 10 / 99

Solicitante:
F. HOFFMANN-LA ROCHE AG.
4070 Basel
SWITZERLAND

Título del Invento:

Nuevos derivados de la pirimidina-2,4,6-triona, proceso para su producción y agentes farmacéuticos que contienen dichos compuestos.

Clasificación Internacional de la Patente:

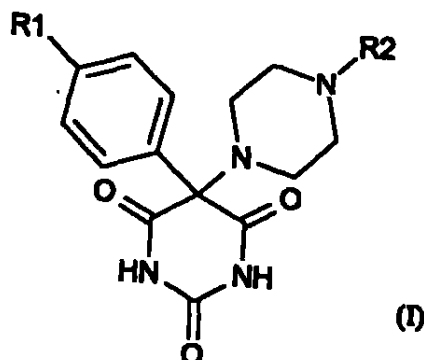
Estados Contratantes Designados en la Fecha de la Presentación:

AT/BE/CH/CY/DE/DK/ES/FI/FR/GB/GR/IE/IT/LI/LU/MC/NL/PT/SE

GABRIEL BUEDA CHADID
TRADUCTOR OFICIAL
RESOLUCION 3996 DE NOV. 20-83
MINISTERIO DE JUSTICIA

REIVINDICACIONES DE LA PATENTE

1. Composiciones de la formula



donde:

R₁ representa un fenilo, fenoxi, feniltio, fenilsulfinilo, fenilsulfonilo, fenilamino o residuo de fenilmetilo, donde el medio de fenilo puede estar sustituido por uno o más átomos de halógeno, alcoxi C₁-C₈, alquilo C₁-C₈, grupos ciano o nitro, y donde R₂ representa un grupo arilo o heteroarilo opcionalmente sustituidos, como también sus sales o precursores de droga farmacéuticamente aceptables de las composiciones de la formula I.

2. Composiciones de la formula I de acuerdo con la Reivindicación 1 donde R₁ es fenoxi.

3. Composiciones de la formula I de acuerdo con la Reivindicación 1 donde R₁ es fenoxi sustituido por cloro, bromo, metilo o tert butilo.

4. Composiciones de la formula I de acuerdo con la Reivindicación 1, donde R₂ es pirimidina o pirazina.

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5. Composiciones farmacéuticas que contienen como ingrediente activo una composición de acuerdo con las reivindicaciones 1 a 4 que se mezclan con excipientes o diluyentes farmacéuticamente aceptables.

6. El uso de compuestos de acuerdo con las Reivindicaciones 1 a 4 para la preparación de un medicamento que ejerce una actividad inhibidora de la metalo-proteinasa.

7. La utilización de composiciones de acuerdo con la Reivindicación 6 que tienen actividad antitumoral y/o antimetastásica.

La anterior es Traducción Oficial de un documento presentado originalmente en inglés. Esta traducción fue realizada por GABRIEL RUEDA CHADID (Traductor Oficial). Resolución 5996 del Minjusticia, por lo cual estampo mi Sello y Firmo.

Bogotá D.C., Septiembre 29 del 2000.


GABRIEL RUEDA CHADID
TRADUCTOR OFICIAL
RESOLUCION 5996 DE NOV. 29-02
MINISTERIO DE JUSTICIA



**Europäisches
Patentamt**

**European
Patent Office**

**Office européen
des brevets**

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Bescheinigung

Certificate

Attestation

Die angehefteten Unterla-
gen stimmen mit der
ursprünglich eingereichten
Fassung der auf dem näch-
sten Blatt bezeichneten
europäischen Patentanmel-
dung überein.

The attached documents
are exact copies of the
European patent application
described on the following
page, as originally filed.

Les documents fixés à
cette attestation sont
conformes à la version
initialement déposée de
la demande de brevet
européen spécifiée à la
page suivante.

Patentanmeldung Nr. Patent application No. Demande de brevet n°

99119506.6

Der Präsident des Europäischen Patentamts:
im Auftrag

For the President of the European Patent Office

Le Président de l'Office européen des brevets
p.o.

I.L.C. HATTEN-HECKMAN

**DEN HAAG, DEN
THE HAGUE, 07/09/00
LA HAYE, LE**



Europäisches
Patentamt

European
Patent Office

Office européen
des brevets

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**Blatt 2 der Bescheinigung
Sheet 2 of the certificate
Page 2 de l'attestation**

Anmeldung Nr.:
Application no.: 99119506.6
Demande n°:

Anmeldetag:
Date of filing: 01/10/99
Date de dépôt:

Anmelder:
Applicant(s):
Demandeur(s):
F. HOFFMANN-LA ROCHE AG
4070 Basel
SWITZERLAND

Bezeichnung der Erfindung:
Title of the invention:
Titre de l'invention:

New pyrimidine-2,4,6-trione derivatives, process for their production and pharmaceutical agents containing these compounds

In Anspruch genommene Priorität(en) / Priority(ies) claimed / Priorité(s) revendiquée(s)

Staat:
State:
Pays:

Tag:
Date:
Date:

Aktenzeichen:
File no.
Numéro de dépôt:

Internationale Patentklassifikation:
International Patent classification:
Classification internationale des brevets:

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Am Anmeldetag benannte Vertragsstaaten:
Contracting states designated at date of filing: AT/BE/CH/CY/DE/DK/ES/FI/FR/GB/GR/IE/IT/LU/MC/NL/PT/SE
Etats contractants désignés lors du dépôt:

Bemerkungen:
Remarks:
Remarques:

5324/00/EP

**New Pyrimidine-2,4,6-trione derivatives, processes for their
production and pharmaceutical agents containing these compounds**

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This invention relates to new derivatives of 5,5-disubstituted pyrimidine-2,4,6-triones. These compounds show a marked antitumor and antimetastatic activity

10 In normal tissue there is an equilibrium between synthesis and degradation. Extracellular matrix is degraded by proteinases which belong to at least three groups of matrix metalloproteinases. These are the collagenases, gelatinases and stromelysins. Normally there are specific inhibitors for these catabolic enzymes such as α_2 macroglobulins and TIMP (= tissue inhibitor of metalloproteinases (MMP)) so that
15 an excessive degradation of extracellular matrix does not occur. Adamalysins are a related group of proteinases. A prominent member of the adamalysins is TACE (TNF- α -converting enzyme).

At least 17 different and yet highly homologous MMP species have been characterized,
20 including the interstitial fibroblast collagenase (MMP-1, HFC), the neutrophil collagenase (MMP-8, HNC), two gelatinases, stromelysins (such as HSL-1) and HPUMP (for a recent review, see Birkedal-Hansen, H., Moore, W.G.I., Bodden, M.K., Windsor, L.J., Birkedal-Hansen, B., DeCarlo, A., Engler, J.A., Critical Rev. Oral Biol.Med. (1993) 4, 197-250. These proteinases share a number of structural and
25 functional features but differ somewhat in their substrate specificity. Only HNC and HFC are capable of cleaving type I, II and III native triple-helical collagens at a single bond with the production of fragments 3/4 and 1/4 of the native chain length. This lowers the collagen melting point and makes them accessible to further attack by other matrix degrading enzymes.

30 However, the uncontrolled excessive degradation of this matrix is a characteristic of many pathological states such as e.g. in the clinical picture of rheumatoid arthritis, osteoarthritis and multiple sclerosis, in the formation of tumor metastases, corneal ulceration, inflammatory diseases and invasion and in diseases of bone and teeth.

35 It can be assumed that the pathogenesis of these clinical pictures can be favourably influenced by the administration of matrix metalloproteinase inhibitors. In the meantime

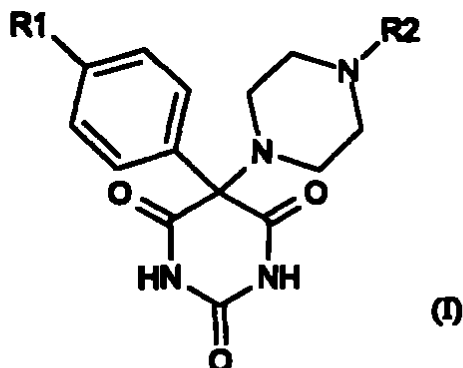
a number of compounds are known from the literature (see e.g. the review article of D.E. Levy, A.M. Ezrin *Emerging Drugs* 2, 205-230 (1997), M. Whittaker, P. Brown, *Curr. Opin. Drug Discovery Dev.* (1998), 1(2), 157-164. or are described in the patent literature, mainly with a hydroxamic acid residue, a thiol or phosphine group as a zinc binding group (see e.g. WO-A-9209563 by Glycomed, EP-A-497 192 by Hoffmann-LaRoche, WO-A-9005719 by British Biotechnology, EP-A-489 577 by Celltech, EP-A-320 118 by Beecham, US-A-459 5700 by Searle, WO 97/20824 by Agouron Pharmaceuticals, WO 96/15096 by Bayer Corporation among others).

10 Some of these compounds show a high activity as inhibitors of matrix metalloproteinases but their oral availability is very low. Also such compounds often show broad spectrum inhibition of metalloproteinases which may be associated to undesired side-effects and toxicity.

15 Pyrimidine-2,4,6-trione derivatives have been described in EP0869947 as inhibitors of matrix metalloproteinases. However, there is still a high need for new compounds having low toxicity, no side-effects and a marked inhibitory activity against metalloproteinases, especially as candidates for a chronic treatment against tumor growth and metastasis.

20 It has now been found that the claimed new pyrimidine-2,4,6-trione derivatives have improved activity as matrix metallo-proteinase inhibitors over the compounds claimed in EP0869947 and also show good oral availability.

25 The present invention therefore concerns compounds of the general formula I



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in which

R_1 represents a phenyl, phenoxy, phenylthio, phenylsulfinyl, phenylsulfonyl, phenylamino or phenylmethyl residue, wherein the phenyl moiety can be substituted by one or more halogen atoms, alkoxy, C_1-C_6 alkyl, cyano, or nitro groups, preferred are

5 substitutions in para and/or meta position by one to two substituents.

R_2 represents an optionally substituted aryl or hetaryl group.

The present invention also encompasses pharmaceutically acceptable salts or prodrugs of the compounds of formula I as well as the use of these compounds to produce pharmaceutical agents.

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The aryl group listed in case of R_2 consists of a phenyl ring. The hetaryl group is understood as a cyclic unsaturated or saturated ring system consisting of 5 to 7 ring atoms which can be selected from one or more carbon, nitrogen, oxygen or sulfur atoms. Preferred are electron deficient hetaryl residues such as the nitrogen containing

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6 membered rings like pyridines, pyrimidines, pyrazines or 1,3,5-triazines. Most preferred are the hetaryl residues pyrimidinyl or pyrazinyl.

The aryl or hetaryl rings may be substituted by one or more substituents selected from halogen, hydroxy, alkoxy, amino, dialkylamino, cyano, lower alkyl, lower alkenyl, lower alkynyl, lower acyl, lower alkylthio, lower alkylsulfonyl, lower

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alkylaminocarbonyl, aminocarbonyl, lower alkylamidossulfonyl, amidosulfonyl, nitro, lower alkoxycarbonyl, carboxy. Preferred are substitutions in para and/or meta position by one to two of the above listed substituents.

Lower alkyl in residue R_2 as such or in combinations with other residues denotes C_1-C_6 alkyl, preferred are methyl, ethyl, propyl, isopropyl or tert.-butyl.

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Lower alkenyl denotes C_2-C_6 alkenyl, preferably allyl or pentadienyl. Lower alkynyl denotes C_2-C_6 alkynyl, preferably propargyl.

Lower acyl in the residue R_2 above all denotes $-C(O)-C_1-C_6$ -alkyl or $-C(O)H$, preferred for an acetyl group.

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The alkyl residues in R_2 , can optionally be interrupted once or several times by heteroatoms (O, S, NH).

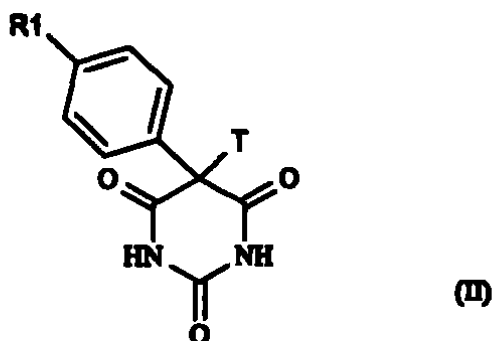
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Halogen is understood as fluorine, chlorine, bromine, iodine, preferably chlorine or bromine.

If compounds of the general formula I contain one or several asymmetric carbon atoms, the optically active compounds of the general formula I are also a subject matter of the present invention.

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Compounds of the general formula I can be synthesized by well-known processes preferably in that compounds of the general formula II



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in which R_1 has the above-mentioned meaning and T represents a leaving group such as Hal or OSO_2R_3 , Hal denoting chlorine, bromine or iodine and R_3 , denoting an aryl or a methyl residue, are reacted with a compound of the general formula III



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in which R_2 has the meaning stated above and optionally converted into pharmaceutically acceptable salts.

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Compounds of the general formula II can be synthesized by analogy to known literature procedures. Thus for example pyrimidine-2,4,6-triones brominated in the 5-position can be synthesized by reacting the appropriate bromomalonic acid dialkyl esters with urea (e.g. Acta Chim. Acad. Sci. Hung. 107 (2), 139 (1981)). The corresponding brominated or chlorinated compounds of the general formula II can be obtained by reacting

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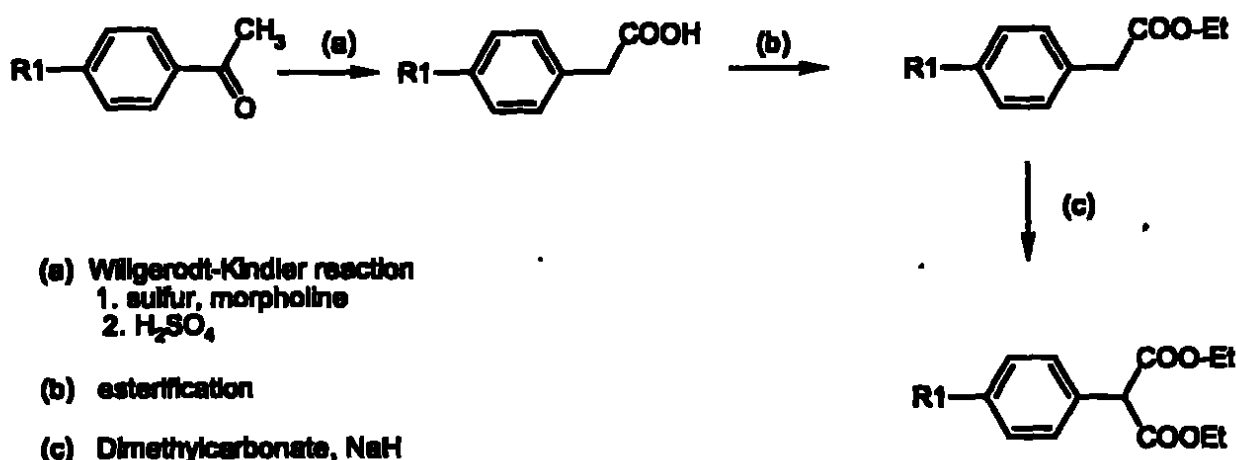
pyrimidine-2,4,6-triones substituted by R_1 -Phenyl in the 5-position with bromine (analogous to J. Prakt. Chemie 136, 329 (1933) or J. Chem. Soc. 1931, 1870) or sulfuric chloride (J. Chem. Soc. 1938, 1622) or N-bromo-succinimide or similar brominating agents. Such procedures are also described in EP0869947.

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Amines of the general formula III are commercially available or are usually known in the literature.

Pyrimidine-2,4,6-triones of formula II with T representing hydrogen can be prepared according to known methods by reacting malonic acid esters with urea (see for example J. Med. Chem. 10, 1078 (1967) or Helvetica Chim. Acta 34, 459 (1959), Pharmacie 38 (1), 65 (1983)) or EP0869947. The reactions are usually carried out in alcohols such as methanol, ethanol or butanol in the presence of an appropriate sodium alcoholate at temperatures between 40°C and 100°C

The malonic acid esters which are needed for the preparation of pyrimidine-2,4,6-triones are known from the literature or can be produced according to processes known from the literature. A convenient process for the preparation of malonic acids where R1 has the above mentioned meaning is described in the following scheme:



Examples for these reactions can be found in Houben-Weyl Vol E5/2, J. Org. Chem. 46, 2999 (1981) and Arch. Pharm. 323, 579 (1990)

Compounds of the general formula I can contain one or several chiral centres and can then be present in a racemic or in an optically active form. The racemates can be separated according to known methods into the enantiomers. Preferably diastereomeric salts which can be separated by crystallization are formed from the racemic mixtures by reaction with an optically active acid such as e.g. D- or L-tartaric acid, mandelic acid, malic acid, lactic acid or camphorsulfonic acid or with an optically active amine such as e.g. D- or L-α-phenyl-ethylamine, ephedrine, quinidine or cinchonidine.

Alkaline salts, earth alkaline salts like Ca or Mg salts, ammonium salts, acetates or hydrochlorides are mainly used as pharmaceutically acceptable salts which are produced in the usual manner e.g. by titrating the compounds with inorganic or organic bases or inorganic acids such as e.g. sodium hydroxide, potassium hydroxide, aqueous ammonia, C₁-C₄-alkyl-amines such as e.g. triethylamine or hydrochloric acid. The salts are usually purified by reprecipitation from water/acetone.

The new compounds of formula I and salts thereof according to the invention can be administered enterally or parenterally in a liquid or solid form. In this connection all the usual forms of administration come into consideration such as for example tablets, capsules, coated tablets, syrups, solutions, suspension etc. Water which contains additives such as stabilizers, solubilizers and buffers that are usual in injection solutions is preferably used as the injection medium.

Such additives are e.g. tartrate and citrate buffer, ethanol, complexing agents (such as ethylenediaminetetra-acetic acid and non-toxic salts thereof), high-molecular polymers (such as liquid polyethylene oxide) to regulate viscosity. Liquid carrier substances for injection solutions have to be sterile and are preferably dispensed into ampoules. Solid carrier substances are e.g. starch, lactose, mannitol, methylcellulose, talcum, highly dispersed silicic acids, higher molecular fatty acids (such as stearic acid), gelatins, agar-agar, calcium phosphate, magnesium stearate, animal and vegetable fats, solid high-molecular polymers (such as polyethylene glycols); suitable preparations for oral application can optionally also contain flavourings and sweeteners.

The dosage depends on various factors such as manner of administration, species, age and/or individual state of health. The doses to be administered daily are about 10-1000 mg/human, preferably 100-500 mg/human and can be taken singly or distributed over several administrations.

Prodrugs of the compounds of the invention are such which are converted in vivo to the pharmacological active compound. The most common prodrugs are carboxylic acid esters.

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Example 1

5-(4-(4-Chloro-phenoxy)-phenyl)-5-(4-pyrimidine-2-yl-piperazine)-pyrimidine-2,4,6-trione

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A) 1-(4-(4-Chloro-phenoxy)-phenyl)-ethanone

4-Fluoro-acetophenone (24.4 g) is dissolved in dimethylformamide (180ml), 4-Chlorophenol (22.8 g) and potassium carbonate (29.5 g) are added. The mixture is heated with stirring for 7 hrs. under reflux. After cooling the mixture is diluted with water and extracted with methylene chloride. The organic phase is washed with water, dried and evaporated to yield 38 g of a crystalline solid. M.p.66-68 °C.

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B) 2-(4-(4-Chloro-phenoxy)-phenyl)-morpholine-4-yl-ethanthione

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12.4 g of the product obtained by the above procedure are mixed with sulfur (4 g) and morpholine (8.8 ml). The mixture is heated to 150 °C for 2 hrs, cooled in an ice bath and treated with ethanol(20 ml) for 30 minutes. The precipitated crystals are collected and recrystallized from ethanol to yield 13 g of the title compound. M.p. 104-105 °C.

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C) (4-(4-Chloro-phenoxy)-phenyl)-acetic acid

10.4 g of the compound prepared in step B are heated together with 50% sulfuric acid (200 ml) to 130 °C for 8 hrs. After cooling to room temperature, the reaction mixture is diluted with water (300 ml) and extracted with ethyl acetate. The organic phase is washed with water and subsequently extracted with 2N sodium carbonate solution. The aqueous phase is acidified with dilute hydrochloric acid, ethyl acetate is added, the organic phase is separated, dried and evaporated to yield 5.1 g of a brownish residue. m.p.98-100 °C.

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D) (4-(4-Chloro-phenoxy)-phenyl)-acetic acid methyl ester

5.1 g of the product from step C are dissolved in methanol (50 ml). The solution is cooled to -10 °C and treated with thionyl chloride (3 ml) and subsequently heated under reflux for 1 hour. The reaction mixture is evaporated and the residue dissolved in ether. The ether phase is washed with water, dried and evaporated to yield 5.1 g of a reddish-brown oil.

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E) 2-(4-(4-Chloro-phenoxy)-phenyl)-malonic acid dimethyl ester

A suspension of sodium hydride (350 mg) in dimethyl carbonate (10 ml) is treated at room temperature with the product obtained in step D. The mixture is heated to 90 °C for 1 hour, cooled and poured into ice water and extracted with methylene chloride. The extract is dried and evaporated to yield 5.7 of the title compound as an oil.

F) 5-(4-(4-Chloro-phenoxy)-phenyl)-pyrimidine,2,4,-6-trione

Sodium (800 mg) is dissolved in ethanol (80 ml). To this solution is added urea (1.65 g) and a solution of the compound obtained above in ethanol (5.5 g). The mixture is heated for 3 hours under reflux, cooled to room temperature, treated with ice water (100 ml) and acidified with dilute hydrochloric acid. The precipitate is collected, washed with water and dried to yield 5g of the title compound. M.p. 257-258 °C.

G) 5-Bromo 5-(4-(4-Chloro-phenoxy)-phenyl)-pyrimidine,2,4,-6-trione

A suspension of the compound obtained in step F (6.3 g), N-bromo-succinimide (4.1 g) and dibenzoylperoxide (100 mg) in carbon tetrachloride (120 ml) is stirred for 3 hours at room temperature. The mixture is evaporated, the residue extracted with ethyl acetate. The organic phase is dried and evaporated to yield 7.5 g of the title compound as a thick oil.

H) 5-(4-(4-Chloro-phenoxy)-phenyl)-5-(4-pyrimidine-2-yl-piperazine)-pyrimidine-2,4,6-trione

A solution of the compound from step G (410 mg) in methanol (5 ml) is treated with N-(pyrimidin-2-yl)-piperazin (330 mg). The mixture is stirred for 24 hours. The residue obtained after evaporation of the reaction mixture is chromatographed on silica gel with methylenchloride/methanol 5% as eluent. Pooling of the relevant fractions yields 410 mg of the title compound as an amorphous solid identified by mass spectroscopy: m/e 492.

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Example 2

5-[4-(4-Chloro-phenoxy)-phenyl]-5-(2,3,5,6-tetrahydro-[1,2']bipyrazinyl-4-yl)-pyrimidine-2,4,6-trione

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The title compound was prepared by analogy to example 1 step H using 330 mg 1-(pyrazin-2-yl)-piperazine instead of the N-(pyrimidin-2-yl)-piperazine yielding 460 mg of the title compound as an amorphous product identified by mass spectrometry: m/e : 492

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Example 3

The following compounds were prepared using the procedures of example 1 replacing 4-chlorophenol by the corresponding phenols. The final products were identified by mass spectrometry

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Number	Chemical name	m/e
1	5-[4-(3,4-Dichloro-phenoxy)-phenyl]-5-(4-pyrimidin-2-yl-piperazin-1-yl)-pyrimidine-2,4,6-trione	526
2	5-[4-(3,4-Dichloro-phenoxy)-phenyl]-5-(2,3,5,6-tetrahydro-[1,2']bipyrazinyl-4-yl)-pyrimidine-2,4,6-trione	526
3	5-[4-(2,4-Dichloro-phenoxy)-phenyl]-5-(4-pyrimidin-2-yl-piperazin-1-yl)-pyrimidine-2,4,6-trione	526
4	5-[4-(2,4-Dichloro-phenoxy)-phenyl]-5-(2,3,5,6-tetrahydro-[1,2']bipyrazinyl-4-yl)-pyrimidine-2,4,6-trione	526
5	5-[4-(2-Chloro-phenoxy)-phenyl]-5-(4-pyrimidin-2-yl-piperazin-1-yl)-pyrimidine-2,4,6-trione	492
6	5-[4-(2-Chloro-phenoxy)-phenyl]-5-(2,3,5,6-tetrahydro-[1,2']bipyrazinyl-4-yl)-pyrimidine-2,4,6-trione	492
7	5-[4-(Phenoxy)-phenyl]-5-(4-pyrimidin-2-yl-piperazin-1-yl)-pyrimidine-2,4,6-trione	458
8	5-[4-(Phenoxy)-phenyl]-5-(2,3,5,6-tetrahydro-[1,2']bipyrazinyl-4-yl)-pyrimidine-2,4,6-trione	458
9	5-[4-(4-Methyl-phenoxy)-phenyl]-5-(4-pyrimidin-2-yl-piperazin-1-yl)-pyrimidine-2,4,6-trione	472

10	5-[4-(4-Methyl-phenoxy)-phenyl]-5-(2,3,5,6-tetrahydro-[1,2']bipyrazinyl-4-yl)-pyrimidine-2,4,6-trione	472
11	5-[4-(4- <i>tert</i> -Butyl-phenoxy)-phenyl]-5-(4-pyrimidin-2-yl-piperazin-1-yl)-pyrimidine-2,4,6-trione	514
12	5-[4-(4- <i>tert</i> -Butyl-phenoxy)-phenyl]-5-(2,3,5,6-tetrahydro-[1,2']bipyrazinyl-4-yl)-pyrimidine-2,4,6-trione	514
13	5-[4-(3,4-Dimethyl-phenoxy)-phenyl]-5-(4-pyrimidin-2-yl-piperazin-1-yl)-pyrimidine-2,4,6-trione	486
14	5-[4-(3,4-Dimethyl-phenoxy)-phenyl]-5-(2,3,5,6-tetrahydro-[1,2']bipyrazinyl-4-yl)-pyrimidine-2,4,6-trione	486
15	5-[4-(4-Bromo-phenoxy)-phenyl]-5-(4-pyrimidin-2-yl-piperazin-1-yl)-pyrimidine-2,4,6-trione	537
16	5-[4-(4-Bromo-phenoxy)-phenyl]-5-(2,3,5,6-tetrahydro-[1,2']bipyrazinyl-4-yl)-pyrimidine-2,4,6-trione	537

Example 4

- 5 In order to determine the inhibition of MMPs, for example HNC (MMP-8), the catalytic domain (isolation and purification see for example Schnierer, S., Kleine, T., Gote, T., Hillemann, A., Knäuper, V., Tschesche, H., Biochem. Biophys. Res. Commun. (1993) 191, 319-326) is incubated with inhibitors having various concentrations. Subsequently, the initial reaction rate in the conversion of a standard substrate is measured in a manner
- 10 analogous to Grams F. et al., FEBS 335 (1993) 76-80).

The results are evaluated by plotting the reciprocal reaction rate against the concentration of the inhibitor. The inhibition constant (K_i) is obtained as the negative section of the abscissa by the graphical method according to Dixon, M., Biochem. J. (1953) 55, 170-202.

15

The synthetic collagenase substrate is a heptapeptide which is coupled, at the C terminus, with DNP (dinitrophenol). Said DNP residue quenches by steric hindrance the fluorescence of the adjacent tryptophans of the heptapeptide. After cleavage of a tripeptide which includes the DNP group, the tryptophane fluorescence increases. The

20 proteolytic cleavage of the substrate therefore can be measured by the fluorescence value.

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a) First method

The assay was performed at 25 °C in a freshly prepared 50 mM Tris buffer (pH 8.0) treated with dithiozone to remove traces of heavy metals. 4 mM CaCl₂ was added and the buffer saturated with argon. Stock solutions of adamalysin II were prepared by centrifugation of the protein from an ammonium sulfate suspension and subsequent dissolution in the assay buffer. Stock solutions of collagenase were diluted with the assay buffer. Enzyme concentrations were determined by uv measurements ($\epsilon_{280} = 2.8 \cdot 10^4 \text{ M}^{-1} \text{ cm}^{-1}$, $\epsilon_{238} = 2.2 \cdot 10^4 \text{ M}^{-1} \cdot \text{cm}^{-1}$) and the stock solutions were stored in the cold. This solution was diluted 1:100 to obtain the final 16 nM assay concentration. The fluorogenic substrate DNP-Pro-Leu-Gly-Leu-Trp-Ala-D-Arg-NH₂ with a K_m of 52 µM was used at a concentration of 21.4 µM; for the K_i determination a 12.8 µM concentration has also been used. Substrate fluorescence was measured at an excitation and emission wavelength of $\lambda = 320$ and 420 nm, respectively, on a spectrofluorimeter (Perkin Elmer, Model 650-40) equipped with a thermostated cell holder. Substrate hydrolysis was monitored for 10 min. immediately after adding the enzyme. All reactions were performed at least in triplicate. The K_i values of the inhibitors were calculated from the intersection point of the straight lines obtained by the plots of v_0/v_i vs. [concentration of inhibitor], whereas IC₅₀ values were calculated from plots of v_i/v_0 [concentration of inhibitor] by non-linear regression with simple robust weighting.

b) Second method

Assay buffer:

50 mM Tris/HCl pH 7.6 (Tris= Tris-(hydroxymethyl)-aminomethan)
100 mM NaCl/10 mM CaCl₂/5 % MeOH (if necessary)
Enzyme: 8 nM catalytic domain (Met80-Gly242) of human neutrophil collagenase (MMP-8)

Substrate: 10 microM DNP-Pro-Leu-Gly-Leu-Trp-Ala-D-Arg-NH₂
Total assay volume: 1 ml

A solution of the enzyme and inhibitor in assay buffer (25 °C) was prepared. The reaction was started by giving the substrate into the solution. The cleavage of the fluorogenic substrate was followed by fluorescence spectroscopy with an excitation and emission wavelength of 280 and 350 nm, respectively. The IC₅₀ value was calculated as

the inhibitor concentration, which is necessary to decrease the velocity of the reaction to the half in comparison to the reaction without inhibitor.

Table 1 shows the IC₅₀ values found in comparison with the compounds from example
5 26 and preferred compound no. 118 cited in the patent application EP0869947

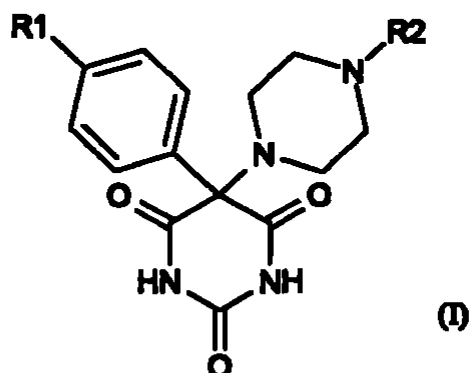
Table 1: IC₅₀ Values of MMP-Inhibitor (vs. MMP-8, catalytic domain)

Reference Compound from EP0869947	IC ₅₀ [nM]
preferred no. 118	60
example 26	15

Compounds from this invention	IC ₅₀ [nM]
Example 1	10
Example 2	4
Example 3 – no. 1	4
Example 3 – no. 2	2
Example 3 – no. 15	4

Patent claims

1. Compounds of formula



in which

10 R_1 represents a phenyl, phenoxy, phenylthio, phenylsulfinyl, phenylsulfonyl, phenylamino or phenylmethyl residue, wherein the phenyl moiety can be substituted by one or more halogen atoms, C_1-C_6 alkoxy, C_1-C_6 alkyl, cyano or nitro groups, and R_2 represents an optionally substituted aryl or heteroaryl group, as well as their pharmaceutically acceptable salts or prodrugs of the compounds of formula I.

- 15
2. Compounds of formula I according to claim 1 wherein R_1 is phenoxy,

- 20
3. Compounds of formula I according to claim 1 wherein R_1 is phenoxy substituted by chlorine, bromine, methyl or tert. butyl,

4. Compounds of formula I according to claim 1, wherein R_2 is pyrimidine or pyrazine.

- 25
5. Pharmaceutical compositions containing as active ingredient a compound according to claims 1 to 4 in admixture with pharmaceutically acceptable excipients or diluents.

6. Use of compounds according to claims 1 to 4 for the preparation of a medicament having metallo-proteinase inhibitor activity.
7. Use of compounds according to claim 6 having antitumor and/or antimetastatic activity.

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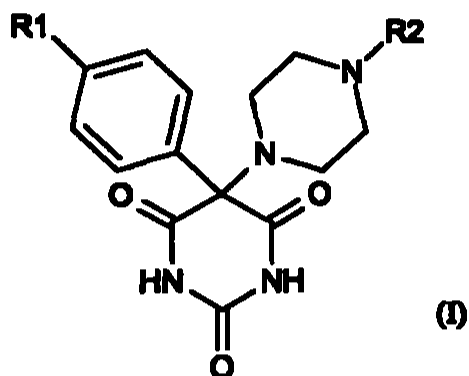
Abstract

EPO - Munich
63

01. Okt. 1999

Compounds of formula I

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in which

- 10 R_1 represents a substituted or unsubstituted phenoxy, phenylthio, phenylsulfinyl, phenylsulfonyl, phenylamino or phenylmethyl residue, and R_2 represents an optionally substituted aryl or heteroaryl residue, with metallo-proteinase inhibitor activity.