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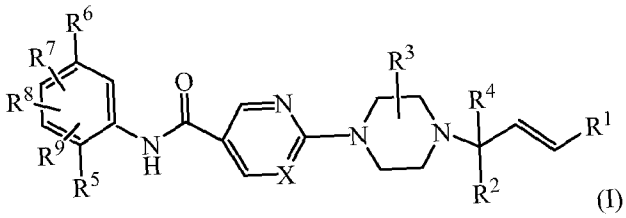
(54) **Title:** PYRIDINE AND PYRIMIDINE DERIVATIVES AS INHIBITORS OF HISTONE DEACETYLASE



(I)

(57) **Abstract:** This invention comprises the novel compounds of formula (I) wherein R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, R^9 and X have defined meanings, having histone deacetylase inhibiting enzymatic activity; their preparation, compositions containing them and their use as a medicine.

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PYRIDINE AND PYRIMIDINE DERIVATIVES AS INHIBITORS OF HISTONE DEACETYLASE

This invention concerns compounds having histone deacetylase (HDAC) inhibiting enzymatic activity. It further relates to processes for their preparation, to compositions comprising them, as well as their use, both *in vitro* and *in vivo*, to inhibit HDAC and as a medicine, for instance as a medicine to inhibit proliferative conditions, such as cancer and psoriasis.

Nuclear histones are known as integral and dynamic components of the machinery responsible for regulating gene transcription and other DNA-templated processes such as replication, repair, recombination, and chromosome segregation. They are the subject of post-translational modifications including acetylation, phosphorylation, methylation, ubiquitination, and ADP-ribosylation.

Histone deacetylase(s), herein referred to as "HDACs", are enzymes that catalyze the removal of the acetyl modification on lysine residues of proteins, including the core nucleosomal histones H2A, H2B, H3 and H4. Together with histone acetyltransferase(s), herein referred to as "HATs", HDACs regulate the level of acetylation of the histones. The balance of acetylation of nucleosomal histones plays an important role in transcription of many genes. Hypoacetylation of histones is associated with condensed chromatin structure resulting in the repression of gene transcription, whereas acetylated histones are associated with a more open chromatin structure and activation of transcription.

Eleven structurally related HDACs have been described and fall into two classes. Class I HDACs consist of HDAC 1, 2, 3, 8 and 11 whereas class II HDACs consist of HDAC 4, 5, 6, 7, 9 and 10. Members of a third class of HDACs are structurally unrelated to the class I and class II HDACs. Class I/II HDACs operate by zinc-dependent mechanisms, whereas class III HDACs are NAD-dependent.

In addition to histones, other proteins have also been the substrate for acetylation, in particular transcription factors such as p53, GATA-1 and E2F; nuclear receptors such as the glucocorticoid receptor, the thyroid receptors, the estrogen receptors; and cell-cycle regulating proteins such as pRb. Acetylation of proteins has been linked with protein stabilization, such as p53 stabilization, recruitment of cofactors and increased DNA binding. p53 is a tumour suppressor that can induce cell cycle arrest or apoptosis

in response to a variety of stress signals, such as DNA damage. The main target for p53-induced cell cycle arrest seems to be the p21 gene. Next to its activation by p53, p21 has been identified by virtue of its association with cyclin/cyclin-dependent kinase complexes resulting in cell cycle arrest at both G1 and G2 phases, its up-regulation during senescence, and its interaction with the proliferating cell nuclear antigen.

The study of inhibitors of HDACs indicates that they play an important role in cell cycle arrest, cellular differentiation, apoptosis and reversal of transformed phenotypes.

The inhibitor Trichostatin A (TSA), for example, causes cell cycle arrest at both G1 and G2 phases, reverts the transformed phenotype of different cell lines, and induces differentiation of Friend leukemia cells and others. TSA (and suberoylanilide hydroxamic acid SAHA) have been reported to inhibit cell growth, induce terminal differentiation, and prevent the formation of tumours in mice (Finnin et al., Nature, 401: 188-193, 1999).

Trichostatin A has also been reported to be useful in the treatment of fibrosis, e.g. liver fibrosis and liver chirrhosis (Geerts et al., European Patent Application EP 0 827 742, published 11 March, 1998).

The pharmacophore for HDAC inhibitors consists of a metal-binding domain, which interacts with the zinc-containing active site of HDACs, a linker domain, and a surface recognition domain or capping region, which interacts with residues on the rim of the active site.

Inhibitors of HDACs have also been reported to induce p21 gene expression. The transcriptional activation of the p21 gene by these inhibitors is promoted by chromatin remodelling, following acetylation of histones H3 and H4 in the p21 promotor region. This activation of p21 occurs in a p53-independent fashion and thus HDAC inhibitors are operative in cells with mutated p53 genes, a hallmark of numerous tumours.

In addition HDAC inhibitors can have indirect activities such as augmentation of the host immune response and inhibition of tumour angiogenesis and thus can suppress the growth of primary tumours and impede metastasis (Mai et al., Medicinal Research Reviews, 25: 261-309, 2005).

In view of the above, HDAC inhibitors can have great potential in the treatment of cell proliferative diseases or conditions, including tumours with mutated p53 genes.

Patent application EP1472216 published on August 14, 2003 discloses bicyclic hydroxamates as inhibitors of histone deacetylase.

Patent applications EP1485099, EP1485348, EP1485353, EP1485354, EP1485364, EP1485365, EP1485370, EP1485378 published on 18 September, 2003, amongst others, disclose substituted piperazinylpyrimidinylhydroxamic acids as inhibitors of histone deacetylase furthermore EP1485365 discloses R306465.

Patent application EP1492534 published on 9 October, 2003, discloses carbamic acid compounds comprising a piperazine linkage, as HDAC inhibitors.

Patent application EP1495002 published on 23 October, 2003, disclose substituted piperazinyl phenyl benzamide compounds, as histone deacetylase inhibitors.

Patent application EP1501508 published on 13 November, 2003, discloses benzamides as histone deacetylase inhibitors.

Patent application WO04/009536 published on 29 January, 2004, discloses derivatives containing an alkyl linker between the aryl group and the hydroxamate, as histone deacetylase inhibitors.

Patent application EP1525199 published on 12 February, 2004, discloses (hetero)arylalkenyl substituted bicyclic hydroxamates, as histone deacetylase inhibitors.

Patent application EP1572626 published on 24 June 2004, discloses arylene-carboxylic acid (2-amino-phenyl)-amide derivatives as pharmacological agents.

Patent application EP1581484 published on 29 July 2004, discloses derivatives of *N*-hydroxy-benzamide derivatives with anti-inflammatory and antitumour activity.

Patent application EP1585735 published on 29 July 2004, discloses substituted aryl hydroxamate derivatives as histone deacetylase inhibitors.

Patent application EP1592667 published on 19 August 2004, discloses mono-acylated O-phenylendiamines derivatives as pharmacological agents.

Patent application EP1590340 published on 19 August 2004, discloses diaminophenylene derivatives as histone deacetylase inhibitors.

Patent application EP1592665 published on 26 August 2004, discloses benzamide derivatives as histone deacetylase inhibitors.

Patent application WO04/072047 published on 26 August 2004, discloses indoles, benzimidazoles and naphhimidazoles as histone deacetylase inhibitors.

Patent application EP1608628 published on 30 September 2004, discloses hydroxamates linked to non-aromatic heterocyclic ring systems as histone deacetylase inhibitors.

Patent application EP1613622 published on 14 October 2004, discloses oxime derivatives as histone deacetylase inhibitors.

Patent application EP1611088 published on 28 October 2004, discloses hydroxamate derivatives as histone deacetylase inhibitors.

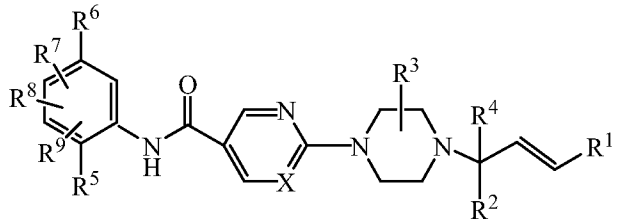
Patent application WO05/028447 published on 31 March 2005, discloses benzimidazoles as histone deacetylase inhibitors.
Patent applications WO05/030704 and WO05/030705 published on 7 April 2005, discloses benzamides as histone deacetylase inhibitors.
Patent application WO05/040101 published on 6 May 2005, disloses acylurea connected and sulfonylurea connected hydroxamates as histone deacetylase inhibitors.
Patent application WO05/040161 also published on 6 May 2005, discloses biaryl linked hydroxamates as histone deacetylase inhibitors.
Patent application WO05/075469 published on 18 August 2005, discloses thiazolyl hydroxamic acids and thiadiazolyl hydroxamic acids as histone deacetylase inhibitors.
Patent application WO05/086898 published on 22 September 2005, discloses heteropentacyclic hydroxamic acids as histone deacetylase inhibitors.
Patent application WO05/092899 published on 6 October 2005, discloses alkenylbenzamides as histone deacetylases.

The compounds of the present invention differ from the prior art in structure, in their pharmacological activity and/or pharmacological potency.

The problem to be solved is to provide histone deacetylase inhibitors with high enzymatic and cellular activity that have increased bioavailability and/or *in vivo* potency.

The novel compounds of the present invention solve the above-described problem. The compounds of the present invention show useful cellular activity. They have a high capacity to activate the p21 gene, both at the cellular and the in vivo level. They can have a desirable pharmacokinetic profile and low affinity for the P450 enzymes, which reduces the risk of adverse drug-drug interaction allowing also for a wider safety margin. Advantageous features of the present compounds can be metabolic stability, solubility and/or p21 induction capacity.

This invention concerns compounds of formula (I)



(I)

the *N*-oxide forms, the pharmaceutically acceptable addition salts and the stereochemically isomeric forms thereof, wherein

X is N or CH;

R^1 is phenyl, naphtalenyl or heterocyclyl; wherein
each of said phenyl or naphtalenyl is optionally substituted with one or two substituents each independently selected from halo, C_{1-6}alkyl, C_{1-6}alkyloxy, polyhaloC_{1-6}alkyl, aryl, hydroxy, cyano, amino, C_{1-6}alkylcarbonylamino, C_{1-6}alkylsulfonylamino, hydroxycarbonyl, C_{1-6}alkyloxycarbonyl, hydroxyC_{1-6}alkyl, C_{1-6}alkyloxymethyl, aminomethyl, C_{1-6}alkylaminomethyl, C_{1-6}alkylcarbonylaminomethyl, C_{1-6}alkylsulfonylaminomethyl, aminosulfonyl, C_{1-6}alkylaminosulfonyl or heterocyclyl;

R^2 is $-CH_2-R^{10}$, trifluoromethyl, $-C(=O)-R^{11}$, or $-CH_2-NR^{12}R^{13}$; wherein
each R^{10} is independently selected from hydrogen, hydroxy, C_{1-6}alkyloxy, C_{1-6}alkyloxyC_{1-6}alkyloxy, C_{1-6}alkylcarbonyloxy, piperazinyl, *N*-methylpiperazinyl, morpholinyl, thiomorpholinyl, imidazolyl or triazolyl;
each R^{11} is independently selected from hydroxy, C_{1-6}alkyloxy, amino or mono- or di(C_{1-6}alkyl)amino, C_{1-6}cycloalkylamino, hydroxyC_{1-6}alkylamino, piperazinyl, mono- or di(C_{1-6}alkyl)aminoC_{1-6}alkylamino *N*-methylpiperazinyl, morpholinyl or thiomorpholinyl;
each R^{12} and R^{13} are independently selected from hydrogen, C_{1-6}alkyl, C_{1-6}alkylcarbonyl, C_{1-6}alkylsulfonyl, or mono-or di(C_{1-4}alkyl)aminosulfonyl;

R^3 is hydrogen, hydroxymethyl, aminomethyl or mono- or di(C_{1-6}alkyl)aminomethyl;

R^4 is hydrogen or C_{1-6}alkyl;

R^5 is hydroxy or amino;

R^6 is hydrogen, thienyl, furanyl or phenyl and each thienyl, furanyl or phenyl is optionally substituted with one or two substituents each independently selected from halo, amino, nitro, cyano, hydroxy, phenyl, C_{1-6}alkyl, (diC_{1-6}alkyl)amino, C_{1-6}alkyloxy, phenylC_{1-6}alkyloxy, hydroxyC_{1-6}alkyl, C_{1-6}alkyloxycarbonyl, hydroxycarbonyl, C_{1-6}alkylcarbonyl, polyhaloC_{1-6}alkyloxy, polyhaloC_{1-6}alkyl, C_{1-6}alkylsulfonyl, hydroxycarbonylC_{1-6}alkyl, C_{1-6}alkylcarbonylamino,

aminosulfonyl, aminosulfonylC_{1-6}alkyl, isoxazolyl, aminocarbonyl, phenylC_{2-6}alkenyl, phenylC_{3-6}alkynyl or pyridinylC_{3-6}alkynyl;

R^7, R^8 and R^9 are each independently hydrogen, amino, nitro, furanyl, halo, C_{1-6}alkyl, C_{1-6}alkyloxy, trifluoromethyl, thienyl, phenyl, C_{1-6}alkylcarbonylamino, aminocarbonylC_{1-6}alkyl or -C≡C-CH_2-R^{14};
wherein R^{14} is hydrogen, C_{1-6}alkyl, hydroxy, amino or C_{1-6}alkyloxy;

aryl in the above is phenyl or naphtalenyl; wherein
each of said phenyl or naphtalenyl is optionally substituted with one or two substituents each independently selected from halo, C_{1-6}alkyl, C_{1-6}alkyloxy, trifluoromethyl, cyano or hydroxycarbonyl; and

heterocyclyl in the above is furanyl, thienyl, pyrrolyl, pyrrolinyl, pyrolidinyl, dioxolyl, oxazolyl, thiazolyl, imidazolyl, imidazolinyl, imidazolidinyl, pyrazolyl, pyrazolinyl, pyrazolidinyl, isoxazolyl, isothiazolyl, oxadiazolyl, triazolyl, thiadiazolyl, pyranyl, pyridinyl, piperidinyl, dioxanyl, morpholinyl, dithianyl, thiomorpholinyl, pyridazinyl, pyrimidinyl, pyrazinyl, piperazinyl, triazinyl, trithianyl, indolizinyl, indolyl, indolinyl, benzofuranyl, benzothiophenyl, indazolyl, benzimidazolyl, benzthiazolyl, purinyl, quinolizinyl, quinolinyl, cinnolinyl, phthlazinyl, quinazolinyl, quinoxalinyl or naphthyridinyl; wherein
each of said heterocycles is optionally substituted with one or two substituents each independently selected from halo, C_{1-6}alkyl, C_{1-6}alkyloxy, cyano, amino, mono-or di(C_{1-4}alkyl)amino.

The term "histone deacetylase inhibitor" or "inhibitor of histone deacetylase" is used to identify a compound, which is capable of interacting with a histone deacetylase and inhibiting its activity, more particularly its enzymatic activity. Inhibiting histone deacetylase enzymatic activity means reducing the ability of a histone deacetylase to remove an acetyl group from a histone. Preferably, such inhibition is specific, i.e. the histone deacetylase inhibitor reduces the ability of a histone deacetylase to remove an acetyl group from a histone at a concentration that is lower than the concentration of the inhibitor that is required to produce some other, unrelated biological effect.

As used in the foregoing definitions and hereinafter, halo is generic to fluoro, chloro, bromo and iodo; C_{1-2}alkyl defines straight chain saturated hydrocarbon radicals having 1 or 2 carbon atoms such as, e.g. methyl or ethyl; C_{1-6}alkyl defines C_{1-2}alkyl and straight and branched chain saturated hydrocarbon radicals having from 3 to 6 carbon

atoms such as, e.g. propyl, butyl, 1-methylethyl, 2-methylpropyl, pentyl, 2-methyl-butyl, hexyl, 2-methylpentyl and the like; polyhaloC_{1-6}alkyl defines C_{1-6}alkyl containing three identical or different halo substituents for example trifluoromethyl; C_{2-6}alkenyl defines straight and branched chain hydrocarbon radicals containing one double bond and having from 2 to 6 carbon atoms such as, for example, ethenyl, 2-propenyl, 3-butenyl, 2-pentenyl, 3-pentenyl, 3-methyl-2-butenyl, and the like; C_{3-6}alkynyl defines straight and branched chain hydrocarbon radicals containing one triple bond and having from 3 to 6 carbon atoms, such as, for example, 2-propynyl, 3-butynyl, 2-butynyl, 2-pentynyl, 3-pentynyl, 3-hexynyl, and the like; C_{3-7}cycloalkyl includes cyclic hydrocarbon groups having from 3 to 7 carbons, such as cyclopropyl, cyclobutyl, cyclopentyl, cyclopentenyl, cyclohexyl, cyclohexenyl, cycloheptyl and the like.

Pharmaceutically acceptable addition salts encompass pharmaceutically acceptable acid addition salts and pharmaceutically acceptable base addition salts. The pharmaceutically acceptable acid addition salts as mentioned hereinabove are meant to comprise the therapeutically active non-toxic acid addition salt forms, which the compounds of formula (I) are able to form. The compounds of formula (I) which have basic properties can be converted in their pharmaceutically acceptable acid addition salts by treating said base form with an appropriate acid. Appropriate acids comprise, for example, inorganic acids such as hydrohalic acids, e.g. hydrochloric or hydrobromic acid; sulfuric; nitric; phosphoric and the like acids; or organic acids such as, for example, acetic, trifluoroacetic, propanoic, hydroxyacetic, lactic, pyruvic, oxalic, malonic, succinic (*i.e.* butanedioic acid), maleic, fumaric, malic, tartaric, citric, methanesulfonic, ethanesulfonic, benzenesulfonic, *p*-toluenesulfonic, cyclamic, salicylic, *p*-amino-salicylic, pamoic and the like acids.
The compounds of formula (I) which have acidic properties may be converted in their pharmaceutically acceptable base addition salts by treating said acid form with a suitable organic or inorganic base. Appropriate base salt forms comprise, for example, the ammonium salts, the alkali and earth alkaline metal salts, e.g. the lithium, sodium, potassium, magnesium, calcium salts and the like, salts with organic bases, e.g. the benzathine, *N*-methyl-D-glucamine, hydrabamine salts, and salts with amino acids such as, for example, arginine, lysine and the like.

The term “acid or base addition salts” also comprises the hydrates and the solvent addition forms, which the compounds of formula (I) are able to form. Examples of such forms are e.g. hydrates, alcoholates and the like.

The term "stereochemically isomeric forms of compounds of formula (I)", as used herein, defines all possible compounds made up of the same atoms bonded by the same sequence of bonds but having different three-dimensional structures, which are not interchangeable, which the compounds of formula (I) may possess. Unless otherwise mentioned or indicated, the chemical designation of a compound encompasses the mixture of all possible stereochemically isomeric forms, which said compound may possess. Said mixture may contain all diastereomers and/or enantiomers of the basic molecular structure of said compound. All stereochemically isomeric forms of the compounds of formula (I) both in pure form or in admixture with each other are intended to be embraced within the scope of the present invention.

The *N*-oxide forms of the compounds of formula (I) are meant to comprise those compounds of formula (I) wherein one or several nitrogen atoms are oxidized to the so-called *N*-oxide, particularly those *N*-oxides wherein one or more of the piperidine-, piperazine or pyridazinyl-nitrogens are *N*-oxidized.

Some of the compounds of formula (I) may also exist in their tautomeric forms. Such forms although not explicitly indicated in the above formula are intended to be included within the scope of the present invention.

Whenever used hereinafter, the term "compounds of formula (I)" is meant to include also the pharmaceutically acceptable addition salts and all stereoisomeric forms.

As used herein, the terms "histone deacetylase" and "HDAC" are intended to refer to any one of a family of enzymes that remove acetyl groups from the ε-amino groups of lysine residues at the N-terminus of a histone. Unless otherwise indicated by context, the term "histone" is meant to refer to any histone protein, including H1, H2A, H2B, H3, H4, and H5, from any species. Human HDAC proteins or gene products, include, but are not limited to, HDAC-1, HDAC-2, HDAC-3, HDAC-4, HDAC-5, HDAC-6, HDAC-7, HDAC-8, HDAC-9 HDAC-10 and HDAC-11. The histone deacetylase can also be derived from a protozoal or fungal source.

A first group of interesting compounds consists of those compounds of formula (I) wherein R^6 is other than hydrogen.

A second group of interesting compounds consists of those compounds of formula (I) wherein one or more of the following restrictions apply:

a) X is N or CH;

b) R^1 is phenyl optionally substituted with one or two substituents each independently selected from halo, C_{1-6}alkyl, C_{1-6}alkyloxy, polyhaloC_{1-6}alkyl or aryl;
c) R^2 is $-CH_2-R^{10}$ or $-C(=O)-R^{11}$;
d) each R^{10} is independently selected from hydrogen, hydroxy, C_{1-6}alkyloxy, C_{1-6}alkyloxyC_{1-6}alkyloxy, C_{1-6}alkylcarbonyloxy, *N*-methylpiperazinyl, morpholinyl, or imidazolyl;
e) each R^{11} is independently selected from C_{1-6}alkylamino, C_{1-6}cycloalkylamino, hydroxyC_{1-6}alkylamino, di(C_{1-6}alkyl)aminoC_{1-6}alkylamino or morpholinyl;
f) R^3 is hydrogen;
g) R^4 is hydrogen or C_{1-6}alkyl;
h) R^5 is amino;
i) R^6 is hydrogen or thienyl; and
j) R^7, R^8 and R^9 are each hydrogen.

A third group of interesting compounds consists of those compounds of formula (I) wherein one or more of the following restrictions apply:

a) X is N or CH;
b) R^1 is phenyl;
c) R^2 is $-CH_2-R^{10}$ or $-C(=O)-R^{11}$;
d) each R^{10} is independently selected from hydrogen, hydroxy, C_{1-6}alkyloxy, C_{1-6}alkyloxyC_{1-6}alkyloxy, C_{1-6}alkylcarbonyloxy, *N*-methylpiperazinyl, morpholinyl, or imidazolyl;
e) each R^{11} is independently selected from C_{1-6}alkylamino, C_{1-6}cycloalkylamino, hydroxyC_{1-6}alkylamino, di(C_{1-6}alkyl)aminoC_{1-6}alkylamino or morpholinyl;
f) R^3 is hydrogen;
g) R^4 is hydrogen or C_{1-6}alkyl;
h) R^5 is amino;
i) R^6 is hydrogen or thienyl; and
j) R^7, R^8 and R^9 are each hydrogen.

A preferred group of interesting compounds consists of those compounds of formula (I) wherein one or more of the following restrictions apply:

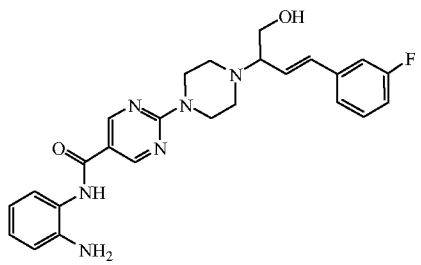
a) X is N;
b) R^1 is phenyl;
c) R^2 is $-CH_2-R^{10}$ wherein R^{10} is hydroxy or C_{1-6}alkyloxy;

d) R^3 is hydrogen;
e) R^4 is hydrogen;
f) R^5 is amino;
g) R^6 is hydrogen or thienyl; and
h) R^7, R^8 and R^9 are each hydrogen.

A further preferred group of interesting compounds consists of those compounds of formula (I) wherein one or more of the following restrictions apply:

a) X is N;
b) R^1 is phenyl substituted with a halo substituent;
c) R^2 is $-CH_2-R^{10}$ wherein R^{10} is hydroxy or C_{1-6}alkyloxy;
d) R^3 is hydrogen;
e) R^4 is hydrogen;
f) R^5 is amino;
g) R^6 is hydrogen or thienyl; and
h) R^7, R^8 and R^9 are each hydrogen.

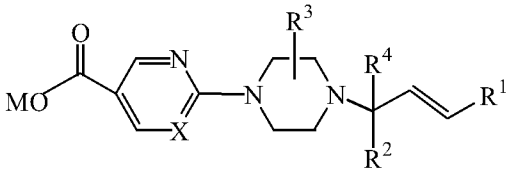
An especially preferred compound of formula (I) is Compound No. 3, namely the compound of formula



The compounds of formula (I), their pharmaceutically acceptable salts and *N*-oxides and stereochemically isomeric forms thereof may be prepared in conventional manner. The starting materials and some of the intermediates are known compounds and are commercially available or may be prepared according to conventional reaction procedures as generally known in the art or as described in patent applications EP1485099, EP1485348, EP1485353, EP1485354, EP1485364, EP1485365, EP1485370, and EP1485378.

Some preparation methods will be described hereinafter in more detail. Other methods for obtaining final compounds of formula (I) are described in the examples.

Compounds of formula (I) may be prepared by reacting an intermediate of formula (II) wherein M represents hydrogen or an alkali metal for example sodium or lithium, with a compound of formula (III), in the presence of a base such as for example triethylamine, and benzotriazol-1-yloxy-tripyrrolidino-phosphonium hexafluorophosphate (PyBOP). Said reaction is performed in an appropriate solvent, such as, for example, tetrahydrofuran or dichloromethane or a mixture thereof



(II)

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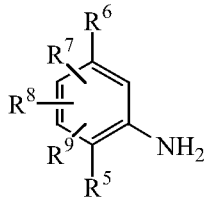

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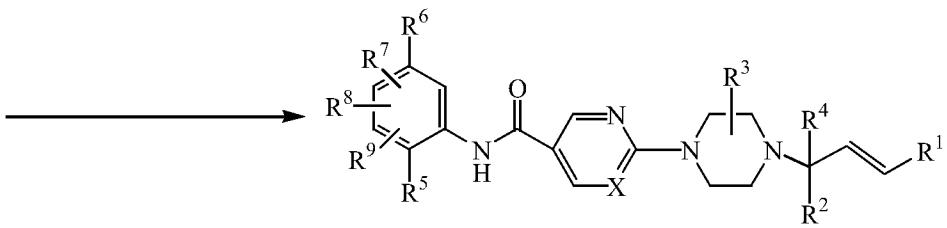


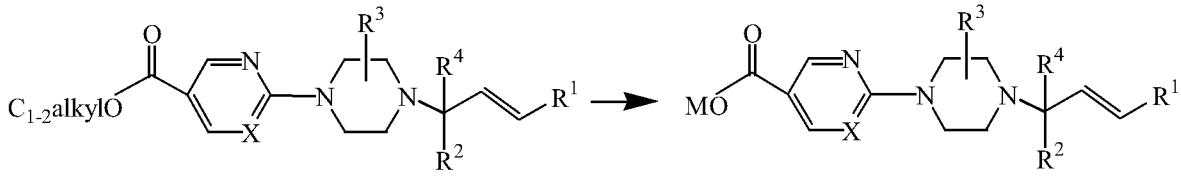
(I)

Alternatively, compounds of formula (I) can be prepared by converting a compound of formula (II) into an acid chloride for example by treatment with sulfonyl chloride ($SOCl_2$) in a suitable solvent such as dichloromethane, and then reacting the resulting acid chloride compound with a compound of formula (III) in the presence of a base such as pyridine in a suitable solvent such as dichloromethane or THF.

The compounds of formula (I) may also be converted into each other via art-known reactions or functional group transformations, depending on the sensitivity of other groups in the molecule, for example hydrolysis of carboxylic esters to the corresponding carboxylic acid or alcohol; hydrolysis of amides to the corresponding carboxylic acids or amines; hydrolysis of nitriles to the corresponding amides; amino groups on phenyl may be replaced by a hydrogen by art-known diazotation reactions and subsequent replacement of the diazo-group by hydrogen; alcohols may be converted into esters and ethers; primary amines may be converted into secondary or tertiary amines; double bonds may be hydrogenated to the corresponding single bond; an iodo radical on a phenyl group may be converted in to an ester group by carbon monoxide insertion in the presence of a suitable palladium catalyst.

Intermediates of formula (II) may be prepared by reacting an intermediate of formula (III) with an appropriate acidic solution, e.g. hydrochloric acid, or an appropriate

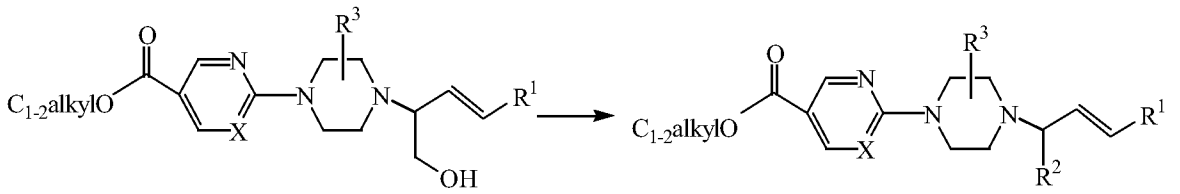
alkali metal base, e.g. sodium hydroxide, in a suitable solvent such as ethanol, generally under reflux.



(III) (II)

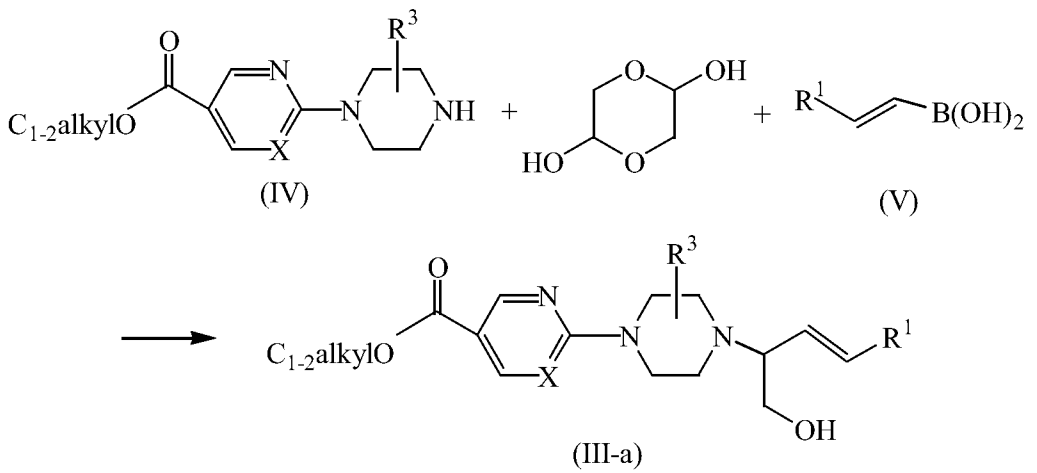
The intermediates of formula (III) can be prepared as follows:

a) The intermediates of formula (III), wherein R^2 is $-CH_2OH$, and R^4 is hydrogen, herein referred to as intermediates of formula (III-a), can be converted into intermediates of formula (III) wherein R^2 is other than $-CH_2OH$, herein referred to as intermediates of formula (III-b), via art-known reactions or functional group transformations. For example the alcohols of formula (III-a) can be converted into amines, esters and ethers. The amines can be transformed into the corresponding amides and the primary amines may be converted into secondary or tertiary amines.



(III-a) (III-b)

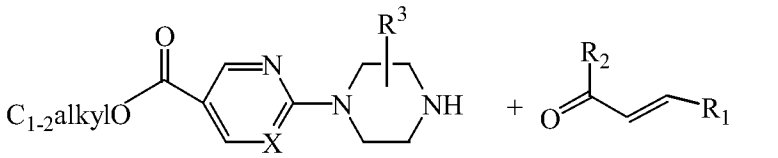
b) The intermediates of formula (III-a) can be prepared in a single step by reacting the intermediate of formula (IV), with 1,4-dioxane-2,5-diol and the appropriate boronic acid of formula (V), wherein R^1 is as defined above, in a suitable solvent, e.g. an alcohol, such as ethanol.



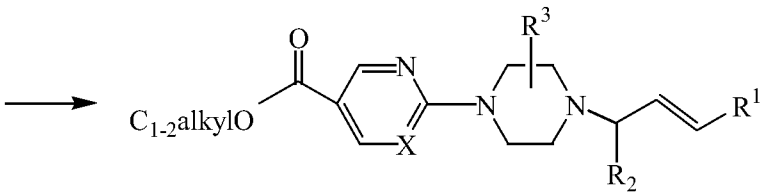
(IV) (V)

(III-a)

c) The intermediates of formula (III-b) can be prepared by reacting the intermediates of formula (IV) with the appropriate ketone of formula (VI) in the presence of an appropriate reagent, such as tetrakis(ethanolato)titanium or a sodium borohydride, in a suitable solvent e.g. 1,2-dichloroethane.



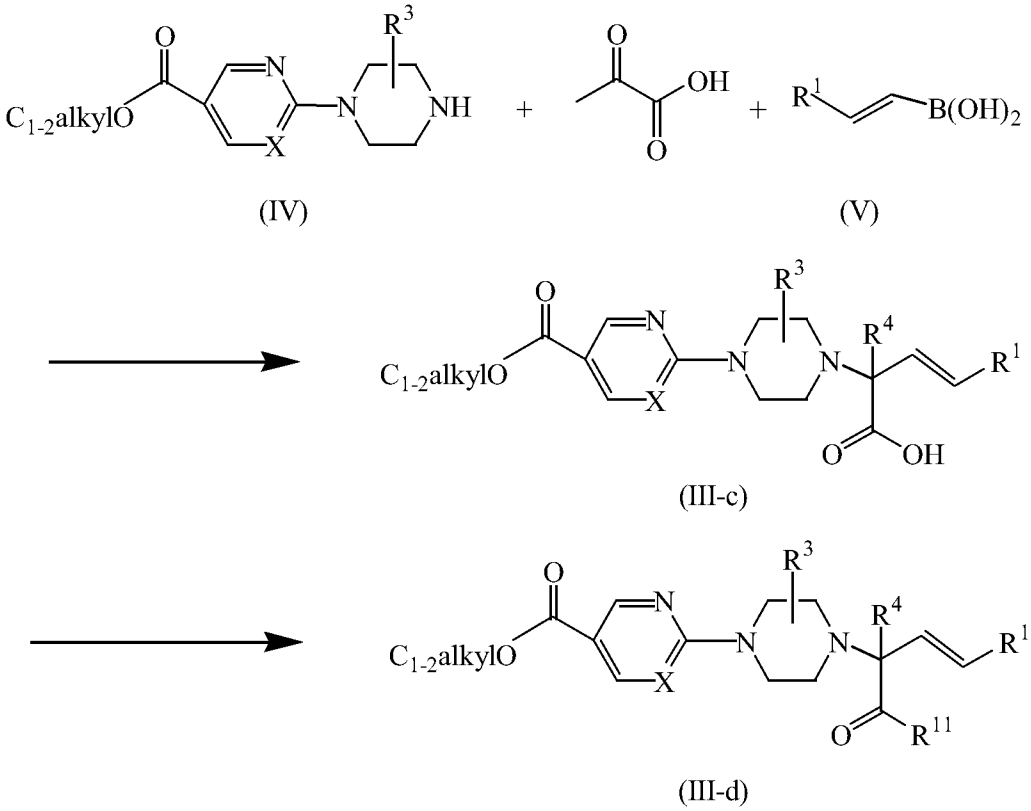
(IV) (VI)



(III-b)

d) The intermediates of formula (III), wherein R^2 is -COOH herein referred to as compounds of formula (III-c) can be prepared in a single step by reacting the intermediate of formula (IV), with 2-oxo- propanoic acid and the appropriate boronic acid of formula (V), wherein R^1 is as defined above, in a suitable solvent, e.g. 1,2-dichloromethane; and

e) The intermediates of formula (III-c), wherein R^2 is –COOH, can be converted into intermediates of formula (III) wherein R^2 is $-C(=O)-R^{11}$ in which R^{11} is other than hydroxy, herein referred to as compounds of formula (III-d), via art-known reactions or functional group transformations, for example the conversion into amines and amides.



The compounds of formula (I) and some of the intermediates may have at least one stereogenic centre in their structure. This stereogenic centre may be present in an R or an S configuration.

The compounds of formula (I) as prepared in the hereinabove described processes are generally racemic mixtures of enantiomers, which can be separated from one another following art-known resolution procedures. The racemic compounds of formula (I) may be converted into the corresponding diastereomeric salt forms by reaction with a suitable chiral acid. Said diastereomeric salt forms are subsequently separated, for example, by selective or fractional crystallization and the enantiomers are liberated there from by alkali. An alternative manner of separating the enantiomeric forms of the compounds of formula (I) involves liquid chromatography using a chiral stationary phase. Said pure stereochemically isomeric forms may also be derived from the corresponding pure stereochemically isomeric forms of the appropriate starting materials, provided that the reaction occurs stereospecifically. Preferably if a specific stereoisomer is desired, said compound would be synthesized by stereospecific methods of preparation. These methods will advantageously employ enantiomerically pure starting materials.

The compounds of formula (I), the pharmaceutically acceptable acid addition salts and stereoisomeric forms thereof have valuable pharmacological properties in that they have a histone deacetylase (HDAC) inhibitory effect.

This invention provides a method for inhibiting the abnormal growth of cells, including transformed cells, by administering an effective amount of a compound of the invention. Abnormal growth of cells refers to cell growth independent of normal regulatory mechanisms (e.g. loss of contact inhibition). This includes the inhibition of tumour growth both directly by causing growth arrest, terminal differentiation and/or apoptosis of cancer cells, and indirectly, by inhibiting neovascularization of tumours.

This invention also provides a method for inhibiting tumour growth by administering an effective amount of a compound of the present invention, to a subject, e.g. a mammal (and more particularly a human) in need of such treatment. In particular, this invention provides a method for inhibiting the growth of tumours by the administration of an effective amount of the compounds of the present invention. Examples of tumours which may be inhibited, but are not limited to, lung cancer (e.g. adenocarcinoma and including non-small cell lung cancer), pancreatic cancers (e.g. pancreatic carcinoma such as, for example exocrine pancreatic carcinoma), colon cancers (e.g. colorectal carcinomas, such as, for example, colon adenocarcinoma and colon adenoma), prostate cancer including the advanced disease, hematopoietic tumours of lymphoid lineage (e.g. acute lymphocytic leukemia, B-cell lymphoma, Burkitt's lymphoma), myeloid leukemias (for example, acute myelogenous leukemia (AML)), thyroid follicular cancer, myelodysplastic syndrome (MDS), tumours of mesenchymal origin (e.g. fibrosarcomas and rhabdomyosarcomas), melanomas, teratocarcinomas, neuroblastomas, gliomas, benign tumour of the skin (e.g. keratoacanthomas), breast carcinoma (e.g. advanced breast cancer), kidney carcinoma, ovary carcinoma, bladder carcinoma and epidermal carcinoma.

The compound according to the invention may be used for other therapeutic purposes, for example:

a) the sensitisation of tumours to radiotherapy by administering the compound according to the invention before, during or after irradiation of the tumour for treating cancer;

b) treating arthropathies and osteopathological conditions such as rheumatoid arthritis, osteoarthritis, juvenile arthritis, gout, polyarthritis, psoriatic arthritis, ankylosing spondylitis and systemic lupus erythematosus;

c) inhibiting smooth muscle cell proliferation including vascular proliferative disorders, atherosclerosis and restenosis;

d) treating inflammatory conditions and dermal conditions such as ulcerative colitis, Crohn's disease, allergic rhinitis, graft vs. host disease, conjunctivitis, asthma, ARDS, Behcets disease, transplant rejection, uticaria, allergic dermatitis, alopecia areata, scleroderma, exanthema, eczema, dermatomyositis, acne, diabetes, systemic lupus erythematosis, Kawasaki's disease, multiple sclerosis, emphysema, cystic fibrosis and chronic bronchitis;

e) treating endometriosis, uterine fibroids, dysfunctional uterine bleeding and endometrial hyperplasia;

f) treating ocular vascularisation including vasculopathy affecting retinal and choroidal vessels;

g) treating a cardiac dysfunction;

h) inhibiting immunosuppressive conditions such as the treatment of HIV infections;

i) treating renal dysfunction;

j) suppressing endocrine disorders;

k) inhibiting dysfunction of gluconeogenesis;

l) treating a neuropathology for example Parkinson's disease or a neuropathology that results in a cognitive disorder, for example, Alzheimer's disease or polyglutamine related neuronal diseases;

m) treating psychiatric disorders for example schizophrenia, bipolar disorder, depression, anxiety and psychosis;

n) inhibiting a neuromuscular pathology, for example, amylotrophic lateral sclerosis;

o) treating spinal muscular atrophy;

p) treating other pathologic conditions amenable to treatment by potentiating expression of a gene;

q) enhancing gene therapy;

r) inhibiting adipogenesis;

s) treating parasitosis such as malaria.

Hence, the present invention discloses the compounds of formula (I) for use as a medicine as well as the use of these compounds of formula (I) for the manufacture of a medicament for treating one or more of the above-mentioned conditions.

The compounds of formula (I), the pharmaceutically acceptable acid addition salts and stereoisomeric forms thereof can have valuable diagnostic properties in that they can be

used for detecting or identifying a HDAC in a biological sample comprising detecting or measuring the formation of a complex between a labelled compound and a HDAC.

The detecting or identifying methods can use compounds that are labelled with labelling agents such as radioisotopes, enzymes, fluorescent substances, luminous substances, etc. Examples of the radioisotopes include ^{125}I, ^{131}I, ^{3}H and ^{14}C. Enzymes are usually made detectable by conjugation of an appropriate substrate which, in turn catalyses a detectable reaction. Examples thereof include, for example, beta-galactosidase, beta-glucosidase, alkaline phosphatase, peroxidase and malate dehydrogenase, preferably horseradish peroxidase. The luminous substances include, for example, luminol, luminol derivatives, luciferin, aequorin and luciferase.

Biological samples can be defined as body tissue or body fluids. Examples of body fluids are cerebrospinal fluid, blood, plasma, serum, urine, sputum, saliva and the like.

In view of their useful pharmacological properties, the subject compounds may be formulated into various pharmaceutical forms for administration purposes.

To prepare the pharmaceutical compositions of this invention, an effective amount of a particular compound, in base or acid addition salt form, as the active ingredient is combined in intimate admixture with a pharmaceutically acceptable carrier, which carrier may take a wide variety of forms depending on the form of preparation desired for administration. These pharmaceutical compositions are desirably in unitary dosage form suitable, preferably, for administration orally, rectally, percutaneously, or by parenteral injection. For example, in preparing the compositions in oral dosage form, any of the usual pharmaceutical media may be employed, such as, for example, water, glycols, oils, alcohols and the like in the case of oral liquid preparations such as suspensions, syrups, elixirs and solutions; or solid carriers such as starches, sugars, kaolin, lubricants, binders, disintegrating agents and the like in the case of powders, pills, capsules and tablets.

Because of their ease in administration, tablets and capsules represent the most advantageous oral dosage unit form, in which case solid pharmaceutical carriers are obviously employed. For parenteral compositions, the carrier will usually comprise sterile water, at least in large part, though other ingredients, to aid solubility for example, may be included. Injectable solutions, for example, may be prepared in which the carrier comprises saline solution, glucose solution or a mixture of saline and glucose solution. Injectable suspensions may also be prepared in which case

appropriate liquid carriers, suspending agents and the like may be employed. In the compositions suitable for percutaneous administration, the carrier optionally comprises a penetration enhancing agent and/or a suitable wetting agent, optionally combined with suitable additives of any nature in minor proportions, which additives do not cause a significant deleterious effect to the skin. Said additives may facilitate the administration to the skin and/or may be helpful for preparing the desired compositions. These compositions may be administered in various ways, e.g., as a transdermal patch, as a spot-on or as an ointment.

It is especially advantageous to formulate the aforementioned pharmaceutical compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used in the specification and claims herein refers to physically discrete units suitable as unitary dosages, each unit containing a predetermined quantity of active ingredient, calculated to produce the desired therapeutic effect, in association with the required pharmaceutical carrier. Examples of such dosage unit forms are tablets (including scored or coated tablets), capsules, pills, powder packets, wafers, injectable solutions or suspensions, teaspoonfuls, tablespoonfuls and the like, and segregated multiples thereof.

Those skilled in the art could easily determine the effective amount from the test results presented hereinafter. In general it is contemplated that a therapeutically effective amount would be from 0.005 mg/kg to 100 mg/kg body weight, and in particular from 0.005 mg/kg to 10 mg/kg body weight. It may be appropriate to administer the required dose as two, three, four or more sub-doses at appropriate intervals throughout the day. Said sub-doses may be formulated as unit dosage forms, for example, containing 0.5 to 500 mg, and in particular 10 mg to 500 mg of active ingredient per unit dosage form.

As another aspect of the present invention a combination of a HDAC-inhibitor with another anticancer agent is envisaged, especially for use as a medicine, more specifically in the treatment of cancer or related diseases.

For the treatment of the above conditions, the compounds of the invention may be advantageously employed in combination with one or more other medicinal agents, more particularly, with other anti-cancer agents. Examples of anti-cancer agents are:

- platinum coordination compounds for example cisplatin, carboplatin or oxalyplatin;
- taxane compounds for example paclitaxel or docetaxel;

- topoisomerase I inhibitors such as camptothecin compounds for example irinotecan or topotecan;
- topoisomerase II inhibitors such as anti-tumour podophyllotoxin derivatives for example etoposide or teniposide;
- anti-tumour vinca alkaloids for example vinblastine, vincristine or vinorelbine;
- anti-tumour nucleoside derivatives for example 5-fluorouracil, gemcitabine or capecitabine;
- alkylating agents such as nitrogen mustard or nitrosourea for example cyclophosphamide, chlorambucil, carmustine or lomustine;
- anti-tumour anthracycline derivatives for example daunorubicin, doxorubicin, idarubicin or mitoxantrone;
- HER2 antibodies for example trastuzumab;
- estrogen receptor antagonists or selective estrogen receptor modulators for example tamoxifen, toremifene, droloxifene, faslodex or raloxifene;
- aromatase inhibitors such as exemestane, anastrozole, letrazole and vorozole;
- differentiating agents such as retinoids, vitamin D and retinoic acid metabolism blocking agents (RAMBA) for example accutane;
- DNA methyl transferase inhibitors for example azacytidine;
- kinase inhibitors for example flavoperidol, imatinib mesylate or gefitinib;
- farnesyltransferase inhibitors;
- other HDAC inhibitors;
- inhibitors of the ubiquitin-proteasome pathway for example Velcade; or
- Yondelis.

The term "platinum coordination compound" is used herein to denote any tumour cell growth inhibiting platinum coordination compound which provides platinum in the form of an ion.

The term "taxane compounds" indicates a class of compounds having the taxane ring system and related to or derived from extracts from certain species of yew (Taxus) trees.

The term "topoisomerase inhibitors" is used to indicate enzymes that are capable of altering DNA topology in eukaryotic cells. They are critical for important cellular functions and cell proliferation. There are two classes of topoisomerases in eukaryotic cells, namely type I and type II. Topoisomerase I is a monomeric enzyme of approximately 100,000 molecular weight. The enzyme binds to DNA and introduces a transient single-strand break, unwinds the double helix (or allows it to unwind) and

subsequently reseals the break before dissociating from the DNA strand. Topisomerase II has a similar mechanism of action which involves the induction of DNA strand breaks or the formation of free radicals.

The term "camptothecin compounds" is used to indicate compounds that are related to or derived from the parent camptothecin compound which is a water-insoluble alkaloid derived from the Chinese tree Camptothecin acuminata and the Indian tree Nothapodytes foetida.

The term "podophyllotoxin compounds" is used to indicate compounds that are related to or derived from the parent podophyllotoxin, which is extracted from the mandrake plant.

The term "anti-tumour vinca alkaloids" is used to indicate compounds that are related to or derived from extracts of the periwinkle plant (Vinca rosea).

The term "alkylating agents" encompass a diverse group of chemicals that have the common feature that they have the capacity to contribute, under physiological conditions, alkyl groups to biologically vital macromolecules such as DNA. With most of the more important agents such as the nitrogen mustards and the nitrosoureas, the active alkylating moieties are generated *in vivo* after complex degradative reactions, some of which are enzymatic. The most important pharmacological actions of the alkylating agents are those that disturb the fundamental mechanisms concerned with cell proliferation in particular DNA synthesis and cell division. The capacity of alkylating agents to interfere with DNA function and integrity in rapidly proliferating tissues provides the basis for their therapeutic applications and for many of their toxic properties.

The term "anti-tumour anthracycline derivatives" comprise antibiotics obtained from the fungus *Strep. peuticus var. caesius* and their derivatives, characterised by having a tetracycline ring structure with an unusual sugar, daunosamine, attached by a glycosidic linkage.

Amplification of the human epidermal growth factor receptor 2 protein (HER 2) in primary breast carcinomas has been shown to correlate with a poor clinical prognosis for certain patients. Trastuzumab is a highly purified recombinant DNA-derived humanized monoclonal IgG1 kappa antibody that binds with high affinity and specificity to the extracellular domain of the HER2 receptor.

Many breast cancers have estrogen receptors and growth of these tumours can be stimulated by estrogen. The terms "estrogen receptor antagonists" and "selective estrogen receptor modulators" are used to indicate competitive inhibitors of estradiol binding to the estrogen receptor (ER). Selective estrogen receptor modulators, when bound to the ER, induces a change in the three-dimensional shape of the receptor, modulating its binding to the estrogen responsive element (ERE) on DNA.

In postmenopausal women, the principal source of circulating estrogen is from conversion of adrenal and ovarian androgens (androstenedione and testosterone) to estrogens (estrone and estradiol) by the aromatase enzyme in peripheral tissues. Estrogen deprivation through aromatase inhibition or inactivation is an effective and selective treatment for some postmenopausal patients with hormone-dependent breast cancer.

The term "antiestrogen agent" is used herein to include not only estrogen receptor antagonists and selective estrogen receptor modulators but also aromatase inhibitors as discussed above.

The term "differentiating agents" encompass compounds that can, in various ways, inhibit cell proliferation and induce differentiation. Vitamin D and retinoids are known to play a major role in regulating growth and differentiation of a wide variety of normal and malignant cell types. Retinoic acid metabolism blocking agents (RAMBA's) increase the levels of endogenous retinoic acids by inhibiting the cytochrome P450-mediated catabolism of retinoic acids.

DNA methylation changes are among the most common abnormalities in human neoplasia. Hypermethylation within the promotors of selected genes is usually associated with inactivation of the involved genes. The term "DNA methyl transferase inhibitors" is used to indicate compounds that act through pharmacological inhibition of DNA methyl transferase and reactivation of tumour suppressor gene expression.

The term "kinase inhibitors" comprises potent inhibitors of kinases that are involved in cell cycle progression and programmed cell death (apoptosis).

The term "farnesyltransferase inhibitors" is used to indicate compounds that were designed to prevent farnesylation of Ras and other intracellular proteins. They have been shown to have effect on malignant cell proliferation and survival.

The term "other HDAC inhibitors" comprises but is not limited to:

- carboxylates for example butyrate, cinnamic acid, 4-phenylbutyrate or valproic acid;
- hydroxamic acids for example suberoylanilide hydroxamic acid (SAHA), piperazine containing SAHA analogues, biaryl hydroxamate A-161906 and its carbozolylether-, tetrahydropyridine- and tetralone- analogues, bicyclic aryl-*N*-hydroxycarboxamides, pyroxamide, CG-1521, PXD-101, sulfonamide hydroxamic acid, LAQ-824, LBH-589, trichostatin A (TSA), oxamflatin, scriptaid, scriptaid related tricyclic molecules, m-carboxy cinnamic acid bishydroxamic acid (CBHA), CBHA-like hydroxamic acids, trapoxin-hydroxamic acid analogue, CRA-024781, R306465 and related benzoyl- and heteroaryl-hydroxamic acids, aminosuberates and malonyldiamides;
- cyclic tetrapeptides for example trapoxin, apidicin, depsipeptide, spiruchostatin-related compounds, RedFK-228, sulfhydryl-containing cyclic tetrapeptides (SCOPs), hydroxamic acid containing cyclic tetrapeptides (CHAPs), TAN-174s and azumamides;
- benzamides for example MS-275 or CI-994, or
- depudecin.

The term "inhibitors of the ubiquitin-proteasome pathway" is used to identify compounds that inhibit the targeted destruction of cellular proteins in the proteasome, including cell cycle regulatory proteins.

For the treatment of cancer the compounds according to the present invention may be administered to a patient as described above, in conjunction with irradiation. Irradiation means ionising radiation and in particular gamma radiation, especially that emitted by linear accelerators or by radionuclides that are in common use today. The irradiation of the tumour by radionuclides can be external or internal.

The present invention also relates to a combination according to the invention of an anti-cancer agent and a HDAC inhibitor according to the invention.

The present invention also relates to a combination according to the invention for use in medical therapy for example for inhibiting the growth of tumour cells.

The present invention also relates to a combination according to the invention for inhibiting the growth of tumour cells.

The present invention also relates to a method of inhibiting the growth of tumour cells in a human subject which comprises administering to the subject an effective amount of a combination according to the invention.

This invention further provides a method for inhibiting the abnormal growth of cells, including transformed cells, by administering an effective amount of a combination according to the invention.

The other medicinal agent and HDAC inhibitor may be administered simultaneously (e.g. in separate or unitary compositions) or sequentially in either order. In the latter case, the two compounds will be administered within a period and in an amount and manner that is sufficient to ensure that an advantageous or synergistic effect is achieved. It will be appreciated that the preferred method and order of administration and the respective dosage amounts and regimes for each component of the combination will depend on the particular other medicinal agent and HDAC inhibitor being administered, their route of administration, the particular tumour being treated and the particular host being treated. The optimum method and order of administration and the dosage amounts and regime can be readily determined by those skilled in the art using conventional methods and in view of the information set out herein.

The platinum coordination compound is advantageously administered in a dosage of 1 to 500mg per square meter (mg/m^2) of body surface area, for example 50 to 400 mg/m^2, particularly for cisplatin in a dosage of about 75 mg/m^2 and for carboplatin in about 300mg/m^2 per course of treatment.

The taxane compound is advantageously administered in a dosage of 50 to 400 mg per square meter (mg/m^2) of body surface area, for example 75 to 250 mg/m^2, particularly for paclitaxel in a dosage of about 175 to 250 mg/m^2 and for docetaxel in about 75 to 150 mg/m^2 per course of treatment.

The camptothecin compound is advantageously administered in a dosage of 0.1 to 400 mg per square meter (mg/m^2) of body surface area, for example 1 to 300 mg/m^2, particularly for irinotecan in a dosage of about 100 to 350 mg/m^2 and for topotecan in about 1 to 2 mg/m^2 per course of treatment.

The anti-tumour podophyllotoxin derivative is advantageously administered in a dosage of 30 to 300 mg per square meter (mg/m^2) of body surface area, for example 50 to

250 mg/m^2, particularly for etoposide in a dosage of about 35 to 100 mg/m^2 and for teniposide in about 50 to 250 mg/m^2 per course of treatment.

The anti-tumour vinca alkaloid is advantageously administered in a dosage of 2 to 30 mg per square meter (mg/m^2) of body surface area, particularly for vinblastine in a dosage of about 3 to 12 mg/m^2, for vincristine in a dosage of about 1 to 2 mg/m^2 , and for vinorelbine in dosage of about 10 to 30 mg/m^2 per course of treatment.

The anti-tumour nucleoside derivative is advantageously administered in a dosage of 200 to 2500 mg per square meter (mg/m^2) of body surface area, for example 700 to1500 mg/m^2, particularly for 5-FU in a dosage of 200 to 500mg/m^2, for gemcitabine in a dosage of about 800 to 1200 mg/m^2 and for capecitabine in about 1000 to 2500 mg/m^2 per course of treatment.

The alkylating agents such as nitrogen mustard or nitrosourea is advantageously administered in a dosage of 100 to 500 mg per square meter (mg/m^2) of body surface area, for example 120 to 200 mg/m^2, particularly for cyclophosphamide in a dosage of about 100 to 500 mg/m^2, for chlorambucil in a dosage of about 0.1 to 0.2 mg/kg, for carmustine in a dosage of about 150 to 200 mg/m^2 , and for lomustine in a dosage of about 100 to 150 mg/m^2 per course of treatment.

The anti-tumour anthracycline derivative is advantageously administered in a dosage of 10 to 75 mg per square meter (mg/m^2) of body surface area, for example 15 to 60 mg/m^2, particularly for doxorubicin in a dosage of about 40 to 75 mg/m^2, for daunorubicin in a dosage of about 25 to 45mg/m^2, and for idarubicin in a dosage of about 10 to 15 mg/m^2 per course of treatment.

Trastuzumab is advantageously administered in a dosage of 1 to 5mg per square meter (mg/m^2) of body surface area, particularly 2 to 4mg/m^2 per course of treatment.

The antiestrogen agent is advantageously administered in a dosage of about 1 to 100 mg daily depending on the particular agent and the condition being treated. Tamoxifen is advantageously administered orally in a dosage of 5 to 50 mg, preferably 10 to 20 mg twice a day, continuing the therapy for sufficient time to achieve and maintain a therapeutic effect. Toremifene is advantageously administered orally in a dosage of about 60mg once a day, continuing the therapy for sufficient time to achieve and maintain a therapeutic effect. Anastrozole is advantageously administered orally in a dosage of about 1mg once a day. Droloxifene is advantageously administered orally

in a dosage of about 20-100mg once a day. Raloxifene is advantageously administered orally in a dosage of about 60mg once a day. Exemestane is advantageously administered orally in a dosage of about 25mg once a day.

These dosages may be administered for example once, twice or more per course of treatment, which may be repeated for example every 7,14, 21 or 28 days.

In view of their useful pharmacological properties, the components of the combinations according to the invention, i.e. the other medicinal agent and the HDAC inhibitor may be formulated into various pharmaceutical forms for administration purposes. The components may be formulated separately in individual pharmaceutical compositions or in a unitary pharmaceutical composition containing both components.

The present invention therefore also relates to a pharmaceutical composition comprising the other medicinal agent and the HDAC inhibitor together with one or more pharmaceutical carriers.

The present invention also relates to a combination according to the invention in the form of a pharmaceutical composition comprising an anti-cancer agent and a HDAC inhibitor according to the invention together with one or more pharmaceutical carriers.

The present invention further relates to the use of a combination according to the invention in the manufacture of a pharmaceutical composition for inhibiting the growth of tumour cells.

The present invention further relates to a product containing as first active ingredient a HDAC inhibitor according to the invention and as second active ingredient an anticancer agent, as a combined preparation for simultaneous, separate or sequential use in the treatment of patients suffering from cancer.

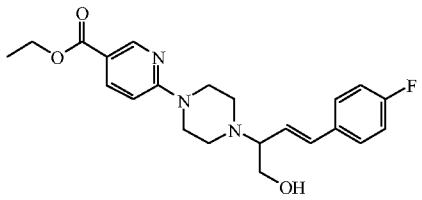
Experimental part

Hereinafter, the term 'EtOAc' means ethyl acetate, '$MgSO_4$' means magnesium sulphate, 'K_2CO_3' means potassium carbonate, 'Et_3N' means triethylamine, 'CH_2Cl_2' means dichloromethane, 'PyBOP' means benzotriazol-1-yloxy-tripyrrolidino-phosphonium hexafluorophosphate and 'THF' means tetrahydrofuran, 'DIPE' means diisopropyl ether, 'NH_4OH' means ammonium hydroxide, , 'iPrOH' means 2-propanol.

A. Preparation of the intermediates

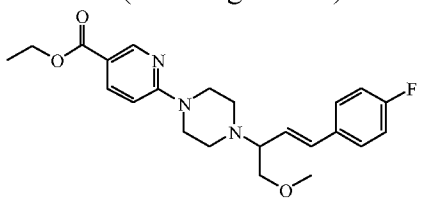
Example A1

a) Preparation of intermediate 1



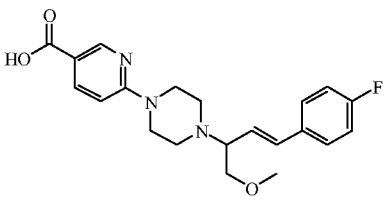
1,4-Dioxane-2,5-diol (0.0093 mol) was added to a solution of [2-(4-fluorophenyl)ethenyl]boronic acid (0.0093 mol) in ethanol (200ml). 6-(1-Piperazinyl)-3-pyridinecarboxylic acid ethyl ester (0.0085 mol) was added. The mixture was stirred at room temperature for 15 hours, then filtered. The filtrate was evaporated. The residue was taken up in EtOAc. The organic layer was washed with saturated sodium chloride, dried ($MgSO_4$), filtered and the solvent was evaporated. The obtained fraction (3.3g) was dissolved in diethyl ether. HCl 5-6N (2ml) was added dropwise at 5°C. The precipitate was filtered, washed with diethyl ether and dried This fraction (3g) was taken up in H_2O and then K_2CO_3 was added. The mixture was extracted with CH_2Cl_2. The organic layer was separated, dried ($MgSO_4$), filtered and the solvent was evaporated, yielding 2.7g (79%) of intermediate 1 (E-configuration).

b) Preparation of intermediate 2



A 60% sodium hydride solution (0.0068 mol) was added portionwise at 5°C to a solution of intermediate 1 (0.0042 mol) in THF (20ml). The mixture was stirred at 5°C for 30 minutes. Iodomethane (0.0063 mol) was added dropwise. The mixture was stirred at 5°C for 30 minutes, then stirred at room temperature for 7 hours, poured onto ice and extracted with EtOAc. The organic layer was washed with saturated sodium chloride, dried ($MgSO_4$), filtered and the solvent was evaporated. This fraction (1.2g) was purified by column chromatography over silica gel (eluent: $CH_2Cl_2/CH_3OH/NH_4OH$ 99/1/0.4 to 94/6/0.6; 5µm). The pure fractions were collected and the solvent was evaporated, yielding 0.86g (50%) (oil) of intermediate 2 (E-configuration).

c) Preparation of intermediate 3

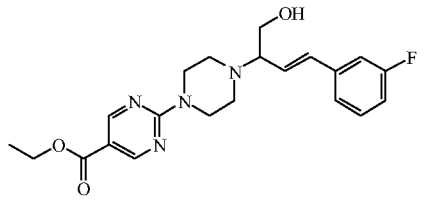


A mixture of intermediate 2 (0.002 mol) and lithium hydroxide (0.0041 mol) in THF (40ml) and H_2O (20ml) was stirred at room temperature for 48 hours. THF was

evaporated. The mixture was extracted with EtOAc. The aqueous layer was neutralized with HCl 3N and extracted with CH_2Cl_2 three times. The organic layer was separated, dried ($MgSO_4$), filtered and the solvent was evaporated, yielding 0.74g (96%) of intermediate 3 (E-configuration).

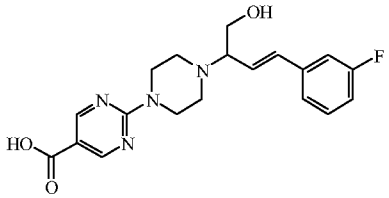
Example A2

a) Preparation of intermediate 4



A mixture of 2-(1-piperazinyl)-5-pyrimidinecarboxylic acid ethyl ester (0.0055 mol), [2-(3-fluorophenyl)ethenyl]boronic acid (0.006 mol) and 1,4-dioxane-2,5-diol (0.72g) in ethanol (70ml) was stirred at room temperature for 15 hours, then evaporated to dryness. The residue was taken up in H_2O. The mixture was extracted with CH_2Cl_2. The organic layer was separated, dried ($MgSO_4$), filtered and the solvent was evaporated. This fraction (2.6g) was purified by column chromatography over silica gel (eluent: $CH_2Cl_2/CH_3OH/NH_4OH$ 97/3/0.1; 15-40µm). The pure fractions were collected and the solvent was evaporated, yielding 1.4g (64%) (oil) of intermediate 4 (E-configuration).

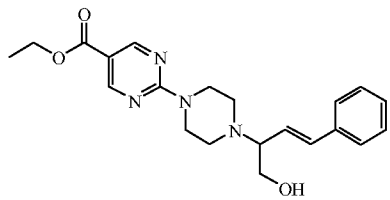
b) Preparation of intermediate 5



A mixture of intermediate 4 (0.0012 mol) and lithium hydroxide (0.0025 mol) in THF (25ml) and H_2O (12ml) was stirred at room temperature for 15 hours, then cooled to room temperature and evaporated, HCl 3N was added to a pH of 4-5. THF was evaporated. The precipitate was filtered, washed with H_2O, then with diethyl ether and dried, yielding 0.44g (95%) of intermediate 5 as the hydrochloride salt (.HCl) (E-configuration).

Example A3

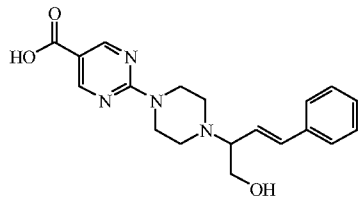
a) Preparation of intermediate 6



A solution of 1,4-dioxane-2,5-diol (0.0021 mol) in ethanol (300ml) was added to [(1E)-2-phenylethenyl] boronic acid (0.0021 mol). Then 2-(1-piperazinyl)-5-pyrimidinecarboxylic acid ethyl ester (0.0021 mol) was added. The mixture was stirred

at room temperature for 24 hours. The precipitate was filtered. The filtrate was evaporated. This fraction (9.6g) was purified by column chromatography over silica gel (eluent: $CH_2Cl_2/CH_3OH/NH_4OH$ 97/3/0.1; 15-40µm). The pure fractions were collected and the solvent was evaporated, yielding 3.2g (39%) of intermediate 6 (E-configuration).

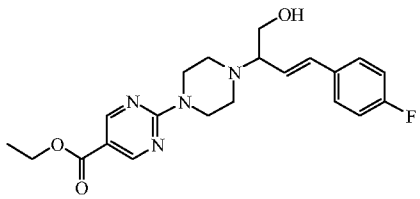
b) Preparation of intermediate 7



A mixture of intermediate 6 (0.007 mol) and lithium hydroxide (aq.) (0.0141 mol) in THF (80ml) and H_2O (40ml) was stirred at room temperature for 3 days. HCl 1N was added. THF was evaporated. The precipitate was filtered, washed with a minimum of H_2O, then with diethyl ether and dried, yielding 2.3g of intermediate 7 (E-configuration).

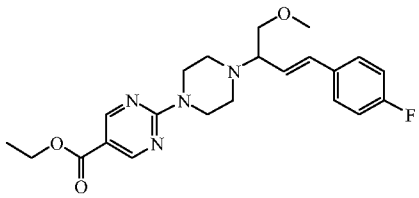
Example A4

a) Preparation of intermediate 8



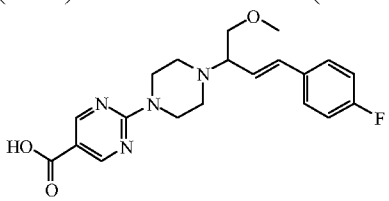
1,4-Dioxane-2,5-diol (0.0186 mol) was added to a solution of [2-(4-fluorophenyl)ethenyl]boronic acid (0.0186 mol) in ethanol (400ml). 2-(1-Piperazinyl)-5-pyrimidinecarboxylic acid ethyl ester (0.0169 mol) was added. The mixture was stirred at room temperature for 15 hours, then filtered. The filtrate was evaporated. The residue was taken up in EtOAc. The organic layer was washed with H_2O and saturated NaCl, dried ($MgSO_4$), filtered and the solvent was evaporated. This fraction (6.8g) was purified by column chromatography over silica gel (eluent: $CH_2Cl_2/CH_3OH/NH_4OH$ 98/2/0.1; 15-40µm). The pure fractions were collected and the solvent was evaporated. This fraction (4.6g) was dissolved in diethyl ether (200ml). HCl 5-6N/iPrOH (3ml) was added. The precipitate was filtered, washed with diethyl ether and dried. This fraction (4.1g) was taken up in H_2O. K_2CO_3 was added. The mixture was extracted with CH_2Cl_2. The organic layer was separated, dried ($MgSO_4$), filtered, and the solvent was evaporated, yielding 4.1g (oil) of intermediate 8 (E-configuration).

b) Preparation of intermediate 9



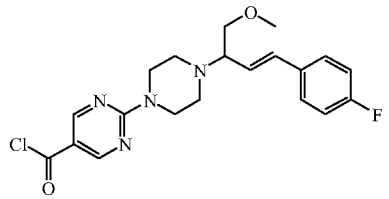
A 60% sodium hydride solution (0.008 mol) was added portionwise at 5°C to a solution of intermediate 8 (0.005 mol) in THF (35ml) under N_2 flow. The mixture was stirred at 5°C for 30 minutes. Iodomethane (0.0074 mol) was added dropwise. The mixture was stirred at 5°C for 30 minutes, then stirred at room temperature for 15 hours, poured onto ice and extracted twice with EtOAc. The organic layer was washed with saturated NaCl, dried ($MgSO_4$), filtered and the solvent was evaporated to dryness. This fraction (1.45g) was purified by column chromatography over silica gel (eluent: $CH_2Cl_2/CH_3OH/NH_4OH$ 99/1/0.1 to 96/4/0.4; 5µm). The pure fractions were collected and the solvent was evaporated, yielding 1g (48%) of intermediate 9 (E-configuration).

c) Preparation of intermediate 10



A mixture of intermediate 9 (0.0023 mol) and lithium hydroxide (0.0047 mol) in THF (50ml) and H_2O (25ml) was stirred at room temperature for 15 hours. THF was evaporated. The mixture was acidified with HCl 3N and extracted with CH_2Cl_2. The organic layer was separated, dried ($MgSO_4$), filtered, and the solvent was evaporated, yielding 0.9g (90%) of intermediate 10 as a hydrochloric acid salt (.HCl) (E-configuration).

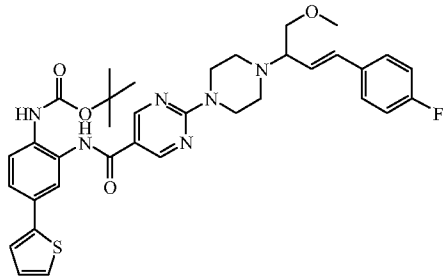
d) Preparation of intermediate 11



Thionyl chloride (0.0083 mol) was added dropwise to a solution of intermediate 10 (0.0007 mol) in 1,2-dichloroethane (12ml). The mixture was stirred at 50°C for 15 hours, then evaporated to dryness, yielding 0.16g of intermediate 11 as a hydrochloric acid salt (.HCl) (E-configuration).

Example A5

a) Preparation of intermediate 12

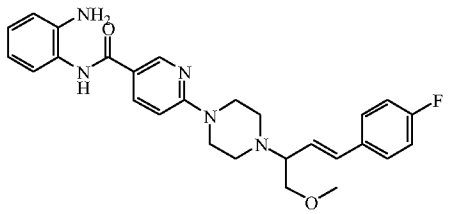


A solution of intermediate 11 (0.0003 mol) in CH_2Cl_2 (3ml) was added dropwise to a solution of [2-amino-4-(2-thienyl)phenyl]-1,1-dimethylethyl ester carbamic acid (0.0006 mol) in pyridine (7ml). The mixture was stirred at room temperature for 15 hours, then evaporated to dryness. The residue was taken up in CH_2Cl_2. The organic layer was washed with H_2O several times, dried ($MgSO_4$), filtered and the solvent was evaporated. This fraction (0.35g) was purified by column chromatography over silica gel (eluent: $CH_2Cl_2/CH_3OH/NH_4OH$ 99/1/0 to 97/3/0.1; 15-40µm). The pure fractions were collected and the solvent was evaporated, yielding 0.2g (80%) of intermediate 12 (E-configuration).

B. Preparation of the compounds

Example B1

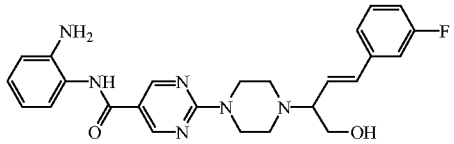
Preparation of compound 1



PyBOP (0.0016 mol) was added to a mixture of intermediate 3 (0.0011 mol), 1,2-benzenediamine monohydrochloride (0.0026 mol) and Et_3N (0.005 mol) in THF/CH_2Cl_2 (50/50) (45ml). The mixture was stirred at room temperature for 5 hours, poured out into H_2O and extracted with CH_2Cl_2. The organic layer was separated, dried ($MgSO_4$), filtered and the solvent was evaporated. The residue was purified by column chromatography over silica gel (eluent: $CH_2Cl_2/CH_3OH/NH_4OH$ 96/4/0.2; 15-40µm). The pure fractions were collected and the solvent was evaporated. This fraction (0.41g) was crystallized from DIPE/diethyl ether. The precipitate was filtered off and dried, yielding 0.28g (32%) (M.P.: 214°C) of compound 1 (E-configuration).

Example B2

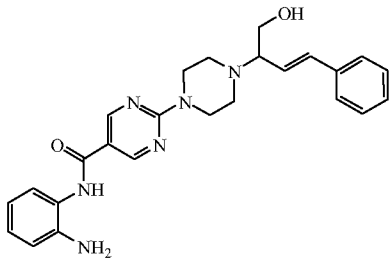
Preparation of compound 2



PyBOP (0.0009 mol) then Et_3N (0.0028 mol) were added to a solution of intermediate 5 (0.0006 mol) and 1,2-benzenediamine monohydrochloride (0.0015 mol) in THF/CH_2Cl_2 (25ml). The mixture was stirred at room temperature for 5 hours, then poured out into H_2O and extracted with CH_2Cl_2. The organic layer was separated, dried ($MgSO_4$), filtered and the solvent was evaporated. This fraction (0.75g) was purified by column chromatography over silica gel (eluent: $CH_2Cl_2/CH_3OH/NH_4OH$ 98/2/0.2 to 92/8/0.8; 5µm). The pure fractions were collected and the solvent was evaporated. This fraction (0.18g) was crystallized from DIPE. The precipitate was filtered off and dried, yielding 0.14g (45%) (M.P.: 230°C) of compound 2 (E-configuration).

Example B3

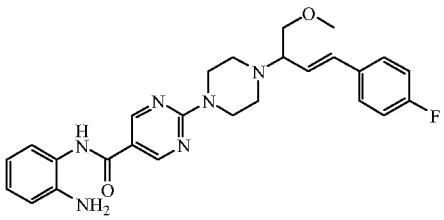
Preparation of compound 3



PyBOP (0.0015 mol) then Et_3N (0.0048 mol) then 1,2-benzenediamine monohydrochloride (0.0026 mol) were added to a solution of intermediate 7 (0.0011 mol) in THF/CH_2Cl_2 (50/50) (40ml). The mixture was stirred at room temperature for 4 hours, then poured out into H_2O and extracted with CH_2Cl_2. The organic layer was washed with saturated $NaHCO_3$, dried ($MgSO_4$), filtered and the solvent was evaporated. This fraction (0.59g) was purified by column chromatography over silica gel (eluent: $CH_2Cl_2/CH_3OH/NH_4OH$ 99/1/0.05 to 95/5/0.25; 5µm). The pure fractions were collected and the solvent was evaporated. This fraction (0.36g) was crystallized from diethyl ether. The precipitate was filtered off and dried, yielding 0.32g (51%) (M.P.: 150°C) of compound 3 (E-configuration).

Example B4

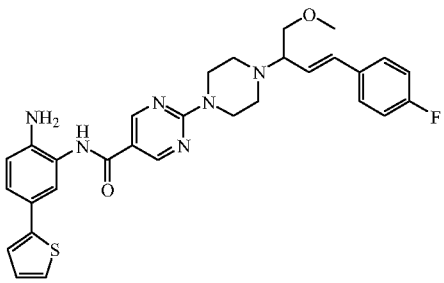
Preparation of compound 4



A solution of intermediate 11 (0.0003 mol) in CH_2Cl_2 (3ml) was added dropwise at 5°C to a solution of 1,2-benzenediamine monohydrochloride (0.0012 mol) in pyridine (7ml). The mixture was stirred at room temperature for 15 hours, then evaporated to dryness. The residue was taken up in CH_2Cl_2. The organic layer was washed several times with H_2O, dried ($MgSO_4$), filtered and the solvent was evaporated. This fraction (0.2g) was purified by column chromatography over silica gel (eluent: CH_2Cl_2/CH_3OH 97/3; 10µm). The pure fractions were collected and the solvent was evaporated. This fraction (0.09g) was crystallized from DIPE. The precipitate was filtered off and dried, yielding 0.05g of compound 4 (E-configuration).

Example B5

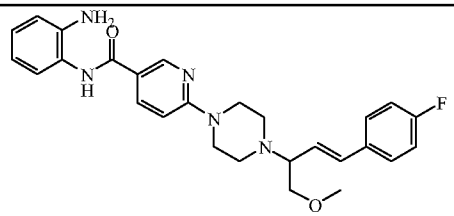
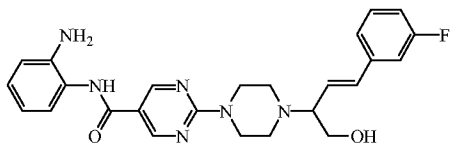
Preparation of compound 5



Trifluoroacetic acid (0.7ml) was added dropwise at 5°C to a solution of intermediate 12 (0.0003 mol) in CH_2Cl_2 (5ml). The mixture was stirred at room temperature for 4 hours. Ice and water were added. $NaHCO_3$ (solid) was added. The mixture was extracted with CH_2Cl_2 twice. The organic layer was separated, dried ($MgSO_4$), filtered and the solvent was evaporated. This fraction (0.2g) was purified by column chromatography over silica gel (eluent: $CH_2Cl_2/CH_3OH/NH_4OH$ 97/3/0.1; 10µm). The pure fractions were collected and the solvent was evaporated. This fraction (0.13g) was taken up in diethyl ether. The precipitate was filtered off and dried, yielding 0.1g (59%) (M.P.: 191°C) of compound 5 (E-configuration).

Table F-1 lists the compounds that were prepared according to one of the above Examples.

Table F-1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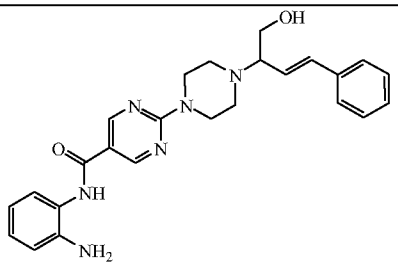	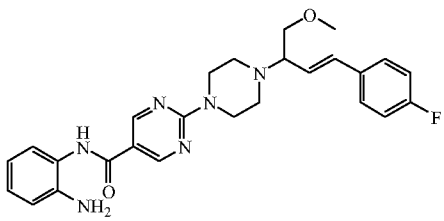
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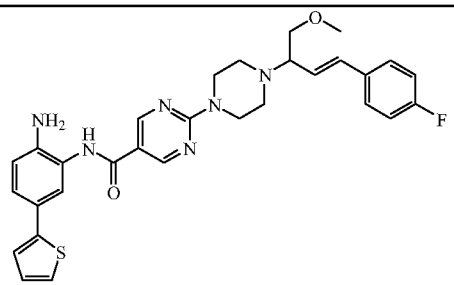
	
Co. No. 5	

C. Pharmacological Examples:

The *in vitro* assay for inhibition of histone deacetylase (see example C.1) measures the inhibition of HDAC enzymatic activity obtained with the compounds of formula (I).

Cellular activity of the compounds of formula (I) was determined on A2780 tumour cells using a colorimetric assay for cell toxicity or survival (Mosmann Tim, Journal of Immunological Methods 65: 55-63, 1983)(see example C.2).

The solubility of a compound measures the ability of a compound to stay in solution. In the first method, the ability of a compound to stay in aqueous solution upon dilution (see example C.3.a) is measured. DMSO-stock solutions are diluted with a single aqueous buffer solvent in different consecutive steps. In this method (C.3.a), mixtures

are then scanned in the BD Gentest Solubility Scanner for the occurrence of precipitation. In a second method the solubility of a compound at different pH's can be measured with the use of a chemiluminescent nitrogen detector (see example C.3.b).

A drug's permeability expresses its ability to move from one medium into or through another. Specifically its ability to move through the intestinal membrane into the blood stream and/or from the blood stream into the target. Permeability (see example C.4) can be measured through the formation of a filter-immobilized artificial membrane phospholipid bilayer. In the filter-immobilized artificial membrane assay, a "sandwich" is formed with a 96-well microtitre plate and a 96-well filter plate, such that each composite well is divided into two chambers with a donor solution at the bottom and an acceptor solution at the top, separated by a 125 µm micro-filter disc (0.45 µm pores), coated with 2%(wt/v) dodecane solution of dioleoylphosphatidyl-choline, under conditions that multi-lamellar bilayers form inside the filter channels when the system contacts an aqueous buffer solution. The permeability of compounds through this artificial membrane is measured in cm/s. The purpose is to look for the permeation of the drugs through a parallel artificial membrane at 2 different pH's: 4.0 and 7.4. Compound detection is done with UV-spectrometry at optimal wavelength between 250 and 500 nm.

Metabolism of drugs means that a lipid-soluble xenobiotic or endobiotic compound is enzymatically transformed into (a) polar, water-soluble, and excretable metabolite(s). The major organ for drug metabolism is the liver. The metabolic products are often less active than the parent drug or inactive. However, some metabolites may have enhanced activity or toxic effects. Thus drug metabolism may include both "detoxication" and "toxication" processes. One of the major enzyme systems that determine the organism's capability of dealing with drugs and chemicals is represented by the cytochrome P450 monooxygenases, which are NADPH dependent enzymes. Metabolic stability of compounds can be determined *in vitro* with the use of subcellular human tissue (see example C.5.). Here metabolic stability of the compounds is expressed as % of drug metabolised after 15 minutes incubation of these compounds with microsomes. Quantitation of the compounds was determined by LC-MS analysis.

It has been shown that a wide variety of anti-tumoral agents activate the p21 protein, including DNA damaging agents and histone deacetylase inhibitors. DNA damaging agents activate the p21 gene through the tumour suppressor p53, while histone deacetylase inhibitors transcriptionally activates the p21 gene via the transcription factor Sp1. Thus, DNA damaging agents activate the p21 promoter through the p53 responsive element while histone deacetylase inhibitors activate the p21 promoter

through sp1 sites (located at the –60 bp to +40 bp region relative to the TATA box) both leading to increased expression of the p21 protein. When the p21 promoter in a cells consists of a p21 1300 bp promoter fragment that does not comprise the p53 responsive elements it is accordingly non-responsive to DNA damaging agents.

The capacity of compounds to induce p21 can be evaluated by testing the capacity of compounds to induce p21 as the consequence of HDAC inhibition at the cellular level. The cells can be stably transfected with an expression vector containing a p21 1300bp promoter fragment that does not comprise the p53 responsive elements and wherein an increase of a reporter gene expression, compared to the control levels, identifies the compound as having p21 induction capacity. The reporter gene is a fluorescent protein and the expression of the reporter gene is measured as the amount of fluorescent light emitted (see example C.6.).

Specific HDAC inhibitors should not inhibit other enzymes like the abundant CYP P450 proteins. The CYP P450 (E.coli expressed) proteins 3A4, 2D6 en 2C9 convert their specific substrates into a fluorescent molecule. The CYP3A4 protein converts 7- benzyloxy-trifluoromethyl coumarin (BFC) into 7-hydroxy-trifluoromethyl coumarin. The CYP2D6 protein converts 3-[2-(N,N-diethyl-N-methylamino)ethyl]-7-methoxy-4-methylcoumarin (AMMC) into 3-[2-(N,N-diethylamino)ethyl]-7-hydroxy-4-methylcoumarin hydrochloride and the CYP2C9 protein converts 7-Methoxy-4-trifluoromethyl coumarin (MFC) into 7-hydroxy-trifluoromethyl coumarin. Compounds inhibiting the enzymatic reaction will result in a decrease of fluorescent signal (see example C.7).

Example C.1.: *In Vitro* Assay for Inhibition of histone deacetylase with Fluorescent-labelled substrate:

The HDAC Fluorescent Activity Assay/Drug Discovery Kit of Biomol (cat.No: AK-500-0001) was used. The HDAC Fluorescent Activity Assay is based on the Fluor de Lys (Fluorogenic Histone deAcetylase Lysyl) substrate and developer combination. The Fluor de Lys substrate, comprises an acetylated lysine side chain. Deacetylation of the substrate sensitizes the substrate so that, in the second step, treatment with the Fluor de Lys developer produces a fluorophore.

HeLa nuclear extracts (supplier: Biomol) were incubated at 60 µg/ml with 75 µM of substrate. The Fluor de Lys substrate was added in a buffer containing 25 mM Tris, 137 mM NaCl, 2.7 mM KCl and 1 mM $MgCl_2.6H_2O$ at pH 7.4. After 30 min, 1 volume of the developer was added. The fluorophore was excited with 355 nm light and the emitted light (450 nm) was be detected on a fluorometric plate reader.

For each experiment, controls (containing HeLa nuclear extract and buffer), a blank incubation (containing buffer but no HeLa nuclear extract) and samples (containing compound dissolved in DMSO and further diluted in buffer and HeLa nuclear extract) were run in parallel. In first instance, compounds were tested at a concentration of 10^{-5}M. When the compounds showed activity at 10^{-5}M, a concentration-response curve was made wherein the compounds were tested at concentrations between 10^{-5}M and 10^{-9}M. All sample were tested 4 times. In each test the blank value was substracted from both the control and the sample values. The control sample represented 100% of substrate deactylation. For each sample the fluorescence was expressed as a percentage of the mean value of the controls. When appropriate IC_{50}-values (concentration of the drug, needed to reduce the amount of metabolites to 50% of the control) were computed using probit analysis for graded data. Herein the effects of test compounds are expressed as pIC_{50} (the negative log value of the IC_{50}-value) (see Table F-2).

Example C.2: Determination of antiproliferative activity on A2780 cells

All compounds tested were dissolved in DMSO and further dilutions were made in culture medium. Final DMSO concentrations never exceeded 0.1 % (v/v) in cell proliferation assays. Controls contained A2780 cells and DMSO without compound and blanks contained DMSO but no cells. MTT was dissolved at 5 mg/ml in PBS. A glycine buffer comprised of 0.1 M glycine and 0.1 M NaCl buffered to pH 10.5 with NaOH (1 N) was prepared (all reagents were from Merck).

The human A2780 ovarian carcinoma cells (a kind gift from Dr. T.C. Hamilton [Fox Chase Cancer Centre, Pennsylvania, USA]) were cultured in RPMI 1640 medium supplemented with 2 mM L-glutamine, 50 µg/ml gentamicin and 10 % fetal calf serum. Cells were routinely kept as monolayer cultures at 37°C in a humidified 5 % CO_2 atmosphere. Cells were passaged once a week using a trypsin/EDTA solution at a split ratio of 1:40. All media and supplements were obtained from Life Technologies. Cells were free of mycoplasma contamination as determined using the Gen-Probe Mycoplasma Tissue Culture kit (supplier: BioMérieux).

Cells were seeded in NUNC™ 96-well culture plates (Supplier: Life Technologies) and allowed to adhere to the plastic overnight. Densities used for plating were 1500 cells per well in a total volume of 200 µl medium. After cell adhesion to the plates, medium was changed and drugs and/or solvents were added to a final volume of 200 µl. Following four days of incubation, medium was replaced by 200 µl fresh medium and cell density and viability was assessed using an MTT-based assay. To each well, 25 µl MTT solution was added and the cells were further incubated for 2 hours at 37°C. The medium was then carefully aspirated and the blue MTT-formazan product was solubilized by addition of 25 µl glycine buffer followed by 100 µl of DMSO. The

microtest plates were shaken for 10 min on a microplate shaker and the absorbance at 540 nm was measured using an Emax 96-well spectrophotometer (Supplier: Sopachem). Within an experiment, the results for each experimental condition are the mean of 3 replicate wells. For initial screening purposes, compounds were tested at a single fixed concentration of 10^{-6} M. For active compounds, the experiments were repeated to establish full concentration-response curves. For each experiment, controls (containing no drug) and a blank incubation (containing no cells or drugs) were run in parallel. The blank value was subtracted from all control and sample values. For each sample, the mean value for cell growth (in absorbance units) was expressed as a percentage of the mean value for cell growth of the control. When appropriate, IC_{50}-values (concentration of the drug, needed to reduce cell growth to 50% of the control) were computed using probit analysis for graded data (Finney, D.J., Probit Analyses, 2nd Ed. Chapter 10, Graded Responses, Cambridge University Press, Cambridge 1962). Herein the effects of test compounds are expressed as pIC_{50} (the negative log value of the IC_{50}-value) (see Table F-2).

Example C.3: Solubility/Stability

C.3.a. Kinetic solubility in aqueous media

DMSO-stock solutions from 5000-9.8 µM (1/2 dilutions) are made in DMSO in a 96 well stock solution plate (200 µl per well). After each dilution the samples are mixed. Aliquots of these DMSO solutions (2 µl) are then transferred into 2 other 96 well buffer plates, containing 200 µl per well aqueous buffer. Each of the buffer plates contains either aqueous buffer pH 7.4 or aqueous buffer pH 4.0. After the last dilution the buffer plates are mixed and the samples are stabilized at room temperature for ½ hour. Dilution is done in duplicate for each compound to exclude occasional errors. Mixtures are then scanned in the BD Gentest Solubility Scanner for the occurrence of precipitation. Based on the absence/presence of precipitate in the mixtures the kinetic solubility is calculated by interpolation. Ranking is performed into the 3 classes.. Compounds with high solubility obtained a score of 3 and have a solubility higher than or equal to 50 µM. Compounds with medium solubility obtained a score of 2 and have a solubility higher than 10 µM and lower than 50 µM. Compounds with low solubility obtained a score of 1 and for these compounds solubility is lower than or equal to 10 µM.

Four compounds were tested: three had a score of 1 at both pH values in the assay and one had a score of 1 at a pH value of 7.4 and a score of 2 at a pH value of 4.0.

C.3.b. Solubility at pH 2.3

The solubility of a compound, at pH 2.3, can also be measured with the use of a chemiluminescent nitrogen detector (see Table F-2).

Example C.4: Parallel artificial membrane permeability analysis

The stock samples (aliquots of 10 µl of a stock solution of 5 mM in 100 % DMSO) were diluted in a deep-well or Pre-mix plate containing 2 ml of an aqueous buffer system pH 4 or pH 7.4 (PSR4 System Solution Concentrate (pION)).

Before samples were added to the reference plate, 150 µl of buffer was added to wells and a blank UV-measurement was performed. Thereafter the buffer was discarded and the plate was used as reference plate. All measurements were done in UV-resistant plates (supplier: Costar or Greiner).

After the blank measurement of the reference plate, 150 µl of the diluted samples was added to the reference plate and 200 µl of the diluted samples was added to donorplate 1. An acceptor filter plate 1 (supplier: Millipore, type:MAIP N45) was coated with 4 µl of the artificial membrane-forming solution (1,2-Dioleoyl-sn-Glycer-3-Phosphocholine in Dodecane containing 0.1% 2,6-Di-tert-butyl-4-methylphenol and placed on top of donor plate 1 to form a "sandwich". Buffer (200 µl) was dispensed into the acceptor wells on the top. The sandwich was covered with a lid and stored for 18h at room temperature in the dark.

A blank measurement of acceptor plate 2 was performed through the addition of 150 µl of buffer to the wells, followed by an UV-measurement. After the blank measurement of acceptor plate 2 the buffer was discarded and 150 µl of acceptor solution was transferred from the acceptor filter plate 1 to the acceptor plate 2. Then the acceptor filter plate 1 was removed form the sandwich. After the blank measurement of donor plate 2 (see above), 150 µl of the donor solution was transferred from donor plate 1 to donor plate 2. The UV spectra of the donor plate 2, acceptor plate 2 and reference plate wells were scanned (with a SpectraMAX 190). All the spectra were processed to calculate permeability with the PSR4p Command Software. All compounds were measured in triplo. Carbamazepine, griseofulvin, acycloguanisine, atenolol, furosemide, and chlorothiazide were used as standards in each experiment. Compounds were ranked in 3 categories as having a low permeability (mean effect < 0.5×10^{-6} cm/s; score 1), a medium permeability (1×10^{-6} cm/s > mean effect ≥ 0.5×10^{-6} cm/s; score 2) or a high permeability (≥ 1×10^{-6} cm/s; score 3).

Example C.5: Metabolic stability

Example C.5.a.

Sub-cellular tissue preparations were made according to Gorrod *et al.* (Xenobiotica 5: 453-462, 1975) by centrifugal separation after mechanical homogenization of tissue. Liver tissue was rinsed in ice-cold 0.1 M Tris-HCl (pH 7.4) buffer to wash excess blood. Tissue was then blotted dry, weighed and chopped coarsely using surgical scissors. The tissue pieces were homogenized in 3 volumes of ice-cold 0.1 M phosphate buffer (pH 7.4) using either a Potter-S (Braun, Italy) equipped with a Teflon pestle or a Sorvall Omni-Mix homogeniser, for 7 x 10 sec. In both cases, the vessel was kept in/on ice during the homogenization process.

Tissue homogenates were centrifuged at 9000 x g for 20 minutes at 4°C using a Sorvall centrifuge or Beckman Ultracentrifuge. The resulting supernatant was stored at –80°C and is designated 'S9'.

The S9 fraction can be further centrifuged at 100.000 x g for 60 minutes (4°C) using a Beckman ultracentrifuge. The resulting supernatant was carefully aspirated, aliquoted and designated 'cytosol'. The pellet was re-suspended in 0.1 M phosphate buffer (pH 7.4) in a final volume of 1 ml per 0.5 g original tissue weight and designated 'microsomes'.

All sub-cellular fractions were aliquoted, immediately frozen in liquid nitrogen and stored at –80°C until use.

For the samples to be tested, the incubation mixture contained PBS (0.1M), compound (5 μM), microsomes (1mg/ml) and a NADPH-generating system (0.8 mM glucose-6-phosphate, 0.8 mM magnesium chloride and 0.8 Units of glucose-6-phosphate dehydrogenase). Control samples contained the same material but the microsomes were replaced by heat inactivated (10 min at 95 degrees Celsius) microsomes. Recovery of the compounds in the control samples was always 100%.

The mixtures were preincubated for 5 min at 37 degrees Celsius. The reaction was started at timepoint zero (t = 0) by addition of 0.8 mM NADP and the samples were incubated for 15 min (t = 15). The reaction was terminated by the addition of 2 volumes of DMSO. Then the samples were centrifuged for 10 min at 900 x g and the supernatants were stored at room temperature for no longer as 24 h before analysis. All incubations were performed in duplo. Analysis of the supernatants was performed with LC-MS analysis. Elution of the samples was performed on a Xterra MS C18 (50 x 4.6 mm, 5 μm, Waters, US). An Alliance 2790 (Supplier: Waters, US) HPLC system was used. Elution was with buffer A (25 mM ammoniumacetate (pH 5.2) in H_2O/acetonitrile (95/5)), solvent B being acetonitrile and solvent C methanol at a flow rate of 2.4 ml/min. The gradient employed was increasing the organic phase concentration from 0 % over 50 % B and 50 % C in 5 min up to 100 % B in 1 min in a

linear fashion and organic phase concentration was kept stationary for an additional 1.5 min. Total injection volume of the samples was 25 µl.

A Quattro (supplier: Micromass, Manchester, UK) triple quadrupole mass spectrometer fitted with and ESI source was used as detector. The source and the desolvation temperature were set at 120 and 350 °C respectively and nitrogen was used as nebuliser and drying gas. Data were acquired in positive scan mode (single ion reaction). Cone voltage was set at 10 V and the dwell time was 1 sec.

Metabolic stability was expressed as % metabolism of the compound after 15 min of incubation in the presence of active microsomes (E(act)) (% metabolism = 100 % - $\left(\frac{\text{Total Ion Current (TIC) of E(act) at t = 15}}{\text{TIC of E(act) at t = 0}}\right)$ x 100). Compounds that had a percentage metabolism less than 20 % were defined as highly metabolic stable and given a score of 3. Compound that had a metabolism between 20 and 70 % were defined as intermediately stable and given a score of 2. Compounds that showed a percentage metabolism higher than 70 were defined as low metabolic stable and given a core of 1. Three reference compounds were always included whenever a metabolic stability screening was performed. Verapamil was included as a compound with low metabolic stability (% metabolism = 73 %). Cisapride was included as a compound with medium metabolic stability (% metabolism 45 %) and propanol was included as a compound with intermediate to high metabolic stability (25 % metabolism). These reference compounds were used to validate the metabolic stability assay. Three compounds were tested, one had a score of 3 and two had a score of 2.

Example C.6: p21 induction capacity

A2780 cells (ATCC) were cultivated in RPMI 1640 medium supplemented with 10% FCS, 2 mM L-glutamine and gentamycine at 37°C in a humidified incubator with 5%CO_2. All cell culture solutions are provided by Gibco-BRL (Gaithersburg, MD). Other materials are provided by Nunc.

Genomic DNA was extracted from proliferating A2780 cells and used as template for nested PCR isolation of the p21 promoter. The first amplification was performed for 20 cycles at an annealing temperature of 55°C using the oligonucleotide pair GAGGGCGCGGTGCTTGG and TGCCGCCGCTCTCTCACC with the genomic DNA as template. The resulting 4.5 kb fragment containing the –4551 to +88 fragment relative to the TATA box was re-amplified with the oligonucleotides TCGGGTACCGAGGGCGCGGTGCTTGG and ATACTCGAGTGCCGCCGCTCTCTCACC for 20 cycles with annealing at 88°C resulting in a 4.5 kb fragment and subsequently with the oligonucleotide pair TCGGGTACCGGTAGATGGGAGCGGATAGACACATC and

ATACTCGAGTGCCGCCGCTCTCTCACC for 20 cycles with annealing at 88°C resulting in a 1.3 kb fragment containing the –1300 to +88 fragment relative to the TATA box. The restriction sites XhoI and KpnI present in the oligonucleotides (underlined sequence) were used for subcloning.

The luciferase reporter was removed from the pGL3-basic and replaced by the ZsGreen reporter (from the pZsGreen1-N1 plasmid) at KpnI and XbaI restriction sites. pGL3-basic-ZsGreen-1300 was constructed via insertion of the above mentioned 1.3 kb fragment of the human p21 promoter region into pGL3-basic-ZsGreen at the XhoI and KpnI sites. All restriction enzymes are provided by Boehringer Manheim (Germany).

A2780 cells were plated into a 6-well plate at a density of $2x10^5$ cells, incubated for 24 hours, and transfected with 2 µg of pGL3-basic-ZsGreen-1300 and 0.2 µg of pSV2neo vector by using Lipofectamine 2000 (Invitrogen, Brussels, Belgium) as described by manufacturer. The transfected cells were selected for 10 days with G418 (Gibco-BRL, Gaithersburg, MD) and single cell suspensions were grown. After three weeks, single clones were obtained.

The A2780 selected clones were expanded and seeded at 10000 cells per well into 96-well plates. 24 hours after seeding, the cells were treated for an additional 24 hours with compounds (affecting sp1 sites in the proximal p21 promoter region). Subsequently, cells were fixed with 4% PFA for 30' and counterstained with Hoechst dye. The p21 promoter activation leading to ZsGreen production and thus fluorescence, was monitored by the Ascent Fluoroskan (Thermo Labsystems, Brussels, Belgium).

For each experiment, controls (containing no drug) and a blank incubation (containing no cells or drugs) were run in parallel. The blank value was substracted from all control and sample values. For each sample, the value for p21 induction was expressed as the percentage of the value for p21 present in the control. Percentage induction higher than 130 % was defined as significant induction. Three compounds were tested and showed significant p21 induction at 10^{-6}M.

Example C.7: P450 inhibiting capacity

All compounds tested were dissolved in DMSO (5 mM) and a further dilution to 5 10^{-4} M was made in acetonitrile. Further dilutions were made in assay buffer (0.1M NaK phosphate buffer pH 7.4) and the final solvent concentration was never higher than 2 %.

The assay for the CYP3A4 protein comprises per well 15 pmol P450/mg protein (in 0.01M NaKphosphate buffer + 1.15% KCl), an NADPH generating system (3.3 mM Glucose-6-phosphate, 0.4 U/ml Glucose-6-phosphate dehydrogenase, 1.3 mM NADP and 3.3 mM $MgCl_2.6H_2O$ in assay buffer) and compound in a total assay volume of 100 µl. After a 5 min pre-incubation at 37 °C the enzymatic reaction was started with the

addition of 150 µM of the fluorescent probe substrate BFC in assay buffer. After an incubation of 30 minutes at room temperature the reaction was terminated after addition of 2 volumes of acetonitrile. Fluorescent determinations were carried out at an excitation wavelength of 405 nm and an emission wavelength of 535 nm. Ketoconazole (IC_{50}-value = 3 X 10^{-8}M) was included as reference compound in this experiment.

The assay for the CYP2D6 protein comprises per well 6 pmol P450/mg protein (in 0.01M NaKphosphate buffer + 1.15% KCl), an NADPH generating system (0.41 mM Glucose-6-phosphate, 0.4 U/ml Glucose-6-phosphate dehydrogenase, 0.0082 mM NADP and 0.41 mM $MgCl_2.6H_2O$ in assay buffer) and compound in a total assay volume of 100 µl. After a 5 min pre-incubation at 37 °C the enzymatic reaction was started with the addition of 3 µM of the fluoresent probe substrate AMMC in assay buffer. After an incubation of 45 minutes at room temperature the reaction was terminated after addition of 2 volumes of acetonitrile. Fluorescent determinations were carried out at an excitation wavelength of 405 nm and an emission wavelength of 460 nm. Quinidine (IC_{50}-value < 5 X 10^{-8} M) was included as reference compound in this experiment.

The assay for the CYP2C9 protein comprises per well 15 pmol P450/mg protein (in 0.01M NaKphosphate buffer + 1.15% KCl), an NADPH generating system (3.3 mM Glucose-6-phosphate, 0.4 U/ml Glucose-6-phosphate dehydrogenase, 1.3 mM NADP and 3.3 mM $MgCl_2.6H_2O$ in assay buffer) and compound in a total assay volume of 100 µl. After a 5 min pre-incubation at 37 °C the enzymatic reaction was started with the addition of 200 µM of the fluoresent probe substrate MFC in assay buffer. After an incubation of 30 minutes at room temperature the reaction was terminated after addition of 2 volumes of acetonitrile. Fluorescent determinations were carried out at an excitation wavelength of 405 nm and an emission wavelength of 535 nm. Sulfaphenazole (IC_{50}-value = 6.8 X 10^{-7} M) was included as reference compound in this experiment.

For initial screening purposes, compounds were tested at a single fixed concentration of 1 X 10^{-5} M. For active compounds, the experiments were repeated to establish full concentration-response curves. For each experiment, controls (containing no drug) and a blank incubation (containing no enzyme or drugs) were run in parallel. All compounds were assayed in quadruplicate. The blank value was subtracted from all control and sample values. For each sample, the mean value of P450 activity of the sample (in relative fluorescence units) was expressed as a percentage of the mean value of P450 activity of the control. Percentage inhibition was expressed as 100% minus the mean value of P450 activity of the sample. When appropriate, IC_{50}-values (concentration of the drug, needed to reduce P450 activity to 50% of the control) were calculated.

Table F-2: lists the results of the compounds that were tested according to Examples C.1, C.2, and C.3.b (a blank indicates no value is available for the relevant compound)

Table F-2

Compound No.	Enzymatic activity pIC50 C.1	Cellular activity pIC50 C.2	Solubility C.3.b. pH = 2.3 (mg/ml)
1	<6	5.6	
2	<6	6.6	
3	4.8	6.7	3.7

D. Composition example: Film-coated tablets

Preparation of tablet core

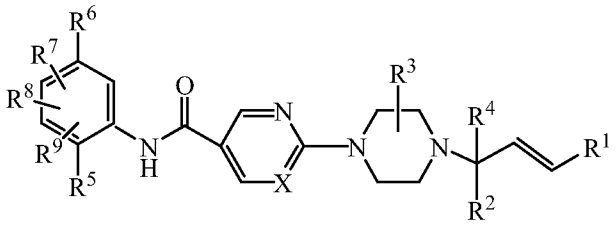
A mixture of 100 g of a compound of formula (I), 570 g lactose and 200 g starch is mixed well and thereafter humidified with a solution of 5 g sodium dodecyl sulphate and 10 g polyvinyl-pyrrolidone in about 200 ml of water. The wet powder mixture is sieved, dried and sieved again. Then there is added 100 g microcrystalline cellulose and 15 g hydrogenated vegetable oil. The whole is mixed well and compressed into tablets, giving 10.000 tablets, each comprising 10 mg of a compound of formula (I).

Coating

To a solution of 10 g methyl cellulose in 75 ml of denaturated ethanol there is added a solution of 5 g of ethyl cellulose in 150 ml of dichloromethane. Then there are added 75 ml of dichloromethane and 2.5 ml 1,2,3-propanetriol 10 g of polyethylene glycol is molten and dissolved in 75 ml of dichloromethane. The latter solution is added to the former and then there are added 2.5 g of magnesium octadecanoate, 5 g of polyvinyl-pyrrolidone and 30 ml of concentrated colour suspension and the whole is homogenated. The tablet cores are coated with the thus obtained mixture in a coating apparatus.

Claims

1. A compound of formula (I)



(I)

the *N*-oxide forms, the pharmaceutically acceptable addition salts and the stereochemically isomeric forms thereof, wherein

X is N or CH;

R^1 is phenyl, naphtalenyl or heterocyclyl; wherein
each of said phenyl or naphtalenyl is optionally substituted with one or two substituents each independently selected from halo, C_{1-6}alkyl, C_{1-6}alkyloxy, polyhaloC_{1-6}alkyl, aryl, hydroxy, cyano, amino, C_{1-6}alkylcarbonylamino, C_{1-6}alkylsulfonylamino, hydroxycarbonyl, C_{1-6}alkyloxycarbonyl, hydroxyC_{1-6}alkyl, C_{1-6}alkyloxymethyl, aminomethyl, C_{1-6}alkylaminomethyl, C_{1-6}alkylcarbonylaminomethyl, C_{1-6}alkylsulfonylaminomethyl, aminosulfonyl, C_{1-6}alkylaminosulfonyl or heterocyclyl;

R^2 is $-CH_2-R^{10}$, trifluoromethyl, $-C(=O)-R^{11}$, or $-CH_2-NR^{12}R^{13}$; wherein
each R^{10} is independently selected from hydrogen, hydroxy, C_{1-6}alkyloxy, C_{1-6}alkyloxyC_{1-6}alkyloxy, C_{1-6}alkylcarbonyloxy, piperazinyl, *N*-methylpiperazinyl, morpholinyl, thiomorpholinyl, imidazolyl or triazolyl;
each R^{11} is independently selected from hydroxy, C_{1-6}alkyloxy, amino or mono- or di(C_{1-6}alkyl)amino, C_{1-6}cycloalkylamino, hydroxyC_{1-6}alkylamino, piperazinyl, mono- or di(C_{1-6}alkyl)aminoC_{1-6}alkylamino *N*-methylpiperazinyl, morpholinyl or thiomorpholinyl;
each R^{12} and R^{13} are independently selected from hydrogen, C_{1-6}alkyl, C_{1-6}alkylcarbonyl, C_{1-6}alkylsulfonyl, or mono-or di(C_{1-4}alkyl)aminosulfonyl;

R^3 is hydrogen, hydroxymethyl, aminomethyl or mono- or di(C_{1-6}alkyl)aminomethyl;

R^4 is hydrogen or C_{1-6}alkyl;

R^5 is hydroxy or amino;

R^6 is hydrogen, thienyl, furanyl or phenyl and each thienyl, furanyl or phenyl is optionally substituted with one or two substituents each independently selected from halo, amino, nitro, cyano, hydroxy, phenyl, C_{1-6}alkyl, (diC_{1-6}alkyl)amino, C_{1-6}alkyloxy, phenylC_{1-6}alkyloxy, hydroxyC_{1-6}alkyl, C_{1-6}alkyloxycarbonyl, hydroxycarbonyl, C_{1-6}alkylcarbonyl, polyhaloC_{1-6}alkyloxy, polyhaloC_{1-6}alkyl, C_{1-6}alkylsulfonyl, hydroxycarbonylC_{1-6}alkyl, C_{1-6}alkylcarbonylamino, aminosulfonyl, aminosulfonylC_{1-6}alkyl, isoxazolyl, aminocarbonyl, phenylC_{2-6}alkenyl, phenylC_{3-6}alkynyl or pyridinylC_{3-6}alkynyl;

R^7, R^8 and R^9 are each independently hydrogen, amino, nitro, furanyl, halo, C_{1-6}alkyl, C_{1-6}alkyloxy, trifluoromethyl, thienyl, phenyl, C_{1-6}alkylcarbonylamino, aminocarbonylC_{1-6}alkyl or $-C\equiv C-CH_2-R^{14}$;
wherein R^{14} is hydrogen, C_{1-6}alkyl, hydroxy, amino or C_{1-6}alkyloxy;

aryl in the above is phenyl or naphtalenyl; wherein
each of said phenyl or naphtalenyl is optionally substituted with one or two substituents each independently selected from halo, C_{1-6}alkyl, C_{1-6}alkyloxy, trifluoromethyl, cyano or hydroxycarbonyl; and

heterocyclyl in the above is furanyl, thienyl, pyrrolyl, pyrrolinyl, pyrolidinyl, dioxolyl, oxazolyl, thiazolyl, imidazolyl, imidazolinyl, imidazolidinyl, pyrazolyl, pyrazolinyl, pyrazolidinyl, isoxazolyl, isothiazolyl, oxadiazolyl, triazolyl, thiadiazolyl, pyranyl, pyridinyl, piperidinyl, dioxanyl, morpholinyl, dithianyl, thiomorpholinyl, pyridazinyl, pyrimidinyl, pyrazinyl, piperazinyl, triazinyl, trithianyl, indolizinyl, indolyl, indolinyl, benzofuranyl, benzothiophenyl, indazolyl, benzimidazolyl, benzthiazolyl, purinyl, quinolizinyl, quinolinyl, cinnolinyl, phthlazinyl, quinazolinyl, quinoxalinyl or naphthyridinyl; wherein
each of said heterocycles is optionally substituted with one or two substituents each independently selected from halo, C_{1-6}alkyl, C_{1-6}alkyloxy, cyano, amino, mono-or di(C_{1-4}alkyl)amino.

2. A compounds according to claim 1 wherein:
   a) X is N or CH;
   b) R^1 is phenyl optionally substituted with one or two substituents each independently selected from halo, C_{1-6}alkyl, C_{1-6}alkyloxy, polyhaloC_{1-6}alkyl or aryl;

c) R^2 is $-CH_2-R^{10}$ or $-C(=O)-R^{11}$;

d) each R^{10} is independently selected from hydrogen, hydroxy, C_{1-6}alkyloxy, C_{1-6}alkyloxyC_{1-6}alkyloxy, C_{1-6}alkylcarbonyloxy, *N*-methylpiperazinyl, morpholinyl, or imidazolyl;

e) each R^{11} is independently selected from C_{1-6}alkylamino, C_{1-6}cycloalkylamino, hydroxyC_{1-6}alkylamino, di(C_{1-6}alkyl)aminoC_{1-6}alkylamino or morpholinyl;

f) R^3 is hydrogen;

g) R^4 is hydrogen or C_{1-6}alkyl;

h) R^5 is amino;

i) R^6 is hydrogen or thienyl; and

j) R^7, R^8 and R^9 are each hydrogen.

3. A compound according to claim 1 wherein:

a) X is N;

b) R^1 is phenyl;

c) R^2 is $-CH_2-R^{10}$ wherein R^{10} is hydroxy or C_{1-6}alkyloxy;

d) R^3 is hydrogen;

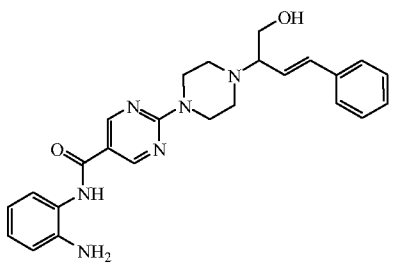
e) R^4 is hydrogen;

f) R^5 is amino;

g) R^6 is hydrogen or thienyl; and

h) R^7, R^8 and R^9 are each hydrogen.

4. A compound according to claim 1 wherein the compound is Compound No. 3 herein, namely the compound of formula



5. A pharmaceutical composition comprising pharmaceutically acceptable carriers and as an active ingredient a therapeutically effective amount of a compound as claimed in any of claims 1 to 4.

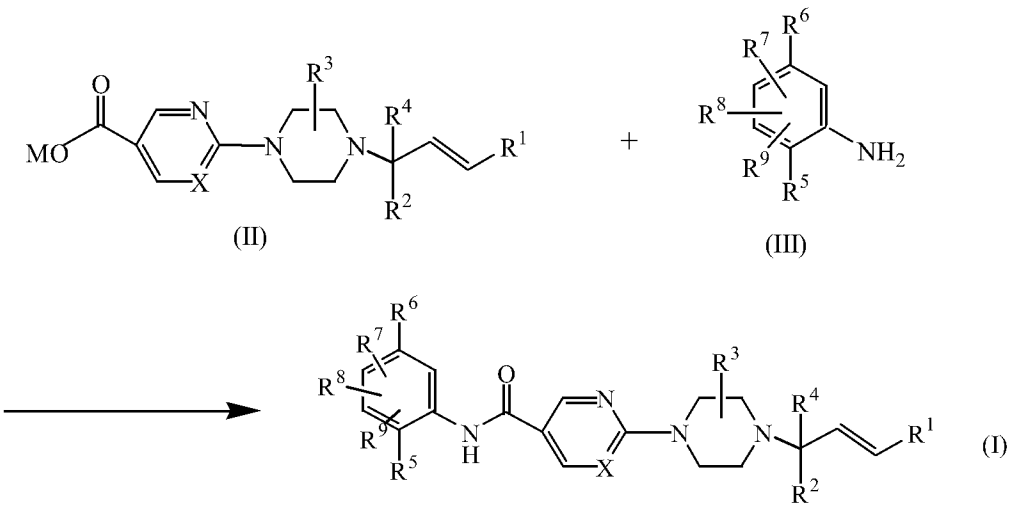
6. A process of preparing a pharmaceutical composition as claimed in claim 5 wherein the pharmaceutically acceptable carriers and a compound as claimed in any of claims 1 to 4 are intimately mixed.

7. A compound as claimed in any of claims 1 to 4 for use as a medicine.

8. Use of a compound as claimed in any of claims 1 to 4 for the manufacture of a medicament for the treatment of proliferative diseases.

9. A combination of an anti-cancer agent and a HDAC inhibitor as claimed in any of claims 1 to 4.

10. A process for preparing a compound according to claim 1 characterised by reacting an intermediate of formula (II) wherein M represents hydrogen or an alkali metal with a compound of formula (III), in the presence of a base:



(II) (III) (I)

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2007/050374

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07D239/42 C07D213/82 C07D409/10 A61K31/506 A61P35/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,A	WO 2006/010749 A (JANSSEN PHARMACEUTICA N.V; VAN BRANDT, SVEN, FRANCISCUS, ANNA; VAN EME) 2 February 2006 (2006-02-02) the whole document	1-10
Y	WO 2005/030705 A (METHYLGENE, INC; MORADEI, OSCAR; PAQUIN, ISABELLE; LEIT, SILVANA; FREC) 7 April 2005 (2005-04-07) cited in the application the whole document	1-10
Y	WO 03/092686 A (ASTRAZENECA AB; ASTRAZENECA UK LIMITED; STOKES, ELAINE, SOPHIE, ELIZAB) 13 November 2003 (2003-11-13) cited in the application the whole document	1-10

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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
23 February 2007	12/03/2007

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Von Daacke, Axel

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INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2007/050374

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 03/075929 A (JANSSEN PHARMACEUTICA N.V; VAN EMELEN, KRISTOF; VERDONCK, MARC, GUSTAA) 18 September 2003 (2003-09-18) cited in the application the whole document	1-10

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2007/050374

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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