NEW 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.

In all eukaryotic cells, genomic DNA in chromatine associates with histones to form nucleosomes. Each nucleosome consists of a protein octamer made up of two copies of each histones H2A, H2B, H3 and H4. DNA winds around this protein core, with the basic amino acids of the histones interacting with the negatively charged phosphate groups of the DNA. The most common posttranslational modification of these core histones is the reversible acetylation of the ε-amino groups of conserved, highly basic N-terminal lysine residues. The steady state of histone acetylation is established by the dynamic equilibrium between competing histone acetyltransferase(s) and histone deacetylase(s) herein referred to as "HDAC". Histone acetylation and deacetylation has long been linked to transcriptional control. The recent cloning of the genes encoding different histone acetyltransferases and histone deacetylases provided a possible explanation for the relationship between histone acetylation and transcriptional control. The reversible acetylation of histones can result in chromatin remodelling and as such act as a control mechanism for gene transcription. In general, hyperacetylation of histones facilitates gene expression, whereas histone deacetylation is correlated with transcriptional repression. Histone acetyltransferases were shown to act as transcriptional coactivators, whereas histone deacetylases were found to belong to transcriptional repression pathways.
The dynamic equilibrium between histone acetylation and deacetylation is essential for normal cell growth. Inhibition of histone deacetylase results in cell cycle arrest, cellular differentiation, apoptosis and reversal of the transformed phenotype. Therefore HDAC inhibitors can have great therapeutic potential in the treatment of cell proliferative diseases or conditions (Marks et al., Nature Reviews: Cancer 1: 194-202, 2001)

The study of inhibitors of histone deacetylases (HDAC) indicates that indeed these enzymes play an important role in cell proliferation and differentiation. The inhibitor Trichostatin A (TSA) 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).

Patent application WO01/38322 published on May 31, 2001 discloses amongst others inhibitors of histone deacetylase of general formula Cy-L^1-Ar-Y^1-C(O)-NH-Z, providing compositions and methods for treating cell proliferative diseases and conditions.

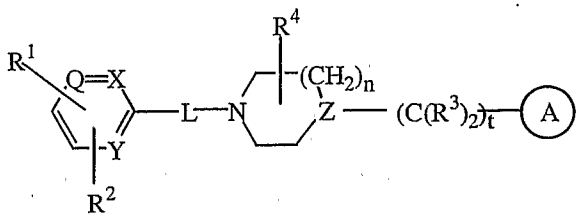
Patent application WO01/70675 published on 27 September, 2001 discloses inhibitors of histone deacetylase of formula Cy^2-Cy^1-X- Y^1-W and further provides compositions and methods for treating cell proliferative diseases and conditions.

The problem to be solved is to provide histone deacetylase inhibitors with high enzymatic activity and also show advantageous properties such as cellular activity and increased bioavailability, preferably oral bioavailability, and have little or no side effects.

The novel compounds of the present invention solve the above described problem. The compounds differ from the prior art in structure.

The compounds of the present invention show excellent *in-vitro* histone deacetylase inhibiting enzymatic activity. The present compounds have advantageous properties with regard to cellular activity and specific properties with regard to inhibition of cell cycle progression at both G1 and G2 checkpoints (p21 induction capacity). The compounds of the present invention show good metabolic stability and high bioavailability and more particular they show oral bioavailability.

This invention concerns compounds of formula (I)



(I)

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

n is 0, 1, 2 or 3 and when n is 0 then a direct bond is intended;

t is 0, 1, 2, 3 or 4 and when t is 0 then a direct bond is intended;

each Q is nitrogen or ;

each X is nitrogen or ;

each Y is nitrogen or ;

each Z is nitrogen or ;

R^1 is $-C(O)NR^7R^8$, $-NHC(O)R^9$, $-C(O)-C_{1-6}alkanediylSR^9$, $-NR^{10}C(O)N(OH)R^9$, $-NR^{10}C(O)C_{1-6}alkanediylSR^9$, $-NR^{10}C(O)C=N(OH)R^9$ or another Zn-chelating-group

wherein R^7 and R^8 are each independently selected from hydrogen, hydroxy, C_{1-6}alkyl, hydroxyC_{1-6}alkyl, aminoC_{1-6}alkyl or aminoaryl;

R^9 is independently selected from hydrogen, C_{1-6}alkyl, C_{1-6}alkylcarbonyl, arylC_{1-6}alkyl, C_{1-6}alkylpyrazinyl, pyridinone, pyrrolidinone or methylimidazolyl;

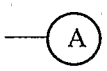
R^{10} is independently selected from hydrogen or C_{1-6}alkyl;

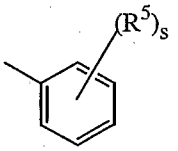
R^2 is hydrogen, halo, hydroxy, amino, nitro, C_{1-6}alkyl, C_{1-6}alkyloxy, trifluoromethyl, di(C_{1-6}alkyl)amino, hydroxyamino or naphtalenylsulfonylpyrazinyl;

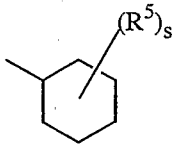
-L- is a direct bond or a bivalent radical selected from C_{1-6}alkanediyl, C_{1-6}alkyloxy, amino, carbonyl or aminocarbonyl;

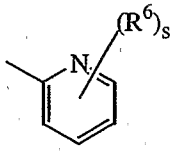
each R^3 independently represents a hydrogen atom and one hydrogen atom can be replaced by a substituent selected from aryl;

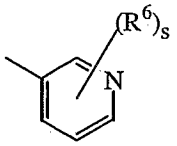
R^4 is hydrogen, hydroxy, amino, hydroxyC_{1-6}alkyl, C_{1-6}alkyl, C_{1-6}alkyloxy, arylC_{1-6}alkyl, aminocarbonyl, hydroxycarbonyl, aminoC_{1-6}alkyl, aminocarbonylC_{1-6}alkyl, hydroxycarbonylC_{1-6}alkyl, hydroxyaminocarbonyl, C_{1-6}alkyloxycarbonyl, C_{1-6}alkylaminoC_{1-6}alkyl or di(C_{1-6}alkyl)aminoC_{1-6}alkyl;

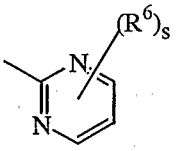

is a radical selected from

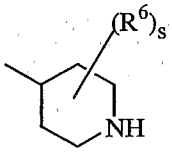

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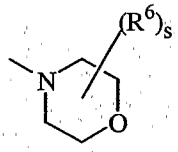

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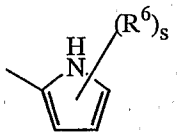

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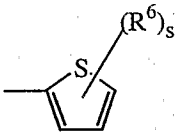

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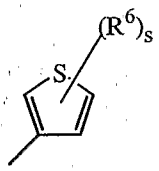

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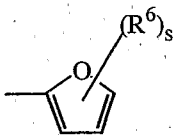

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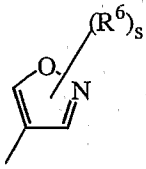

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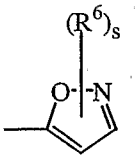

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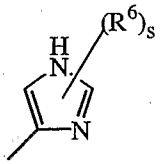

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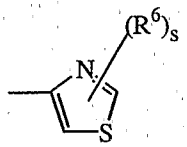

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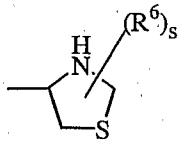

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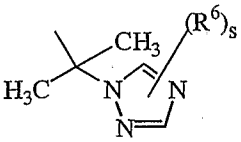

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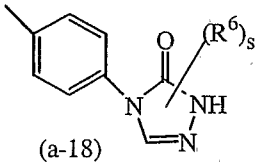

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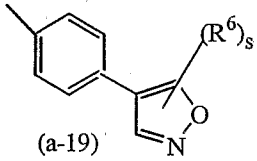

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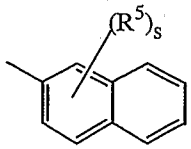

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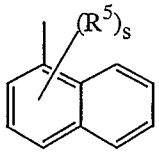

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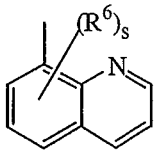

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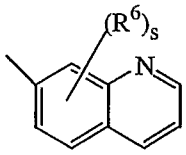

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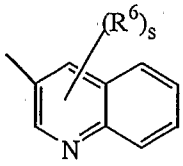
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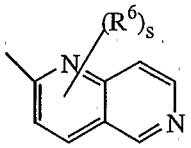
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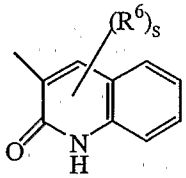
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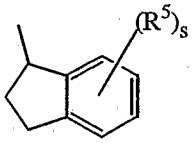
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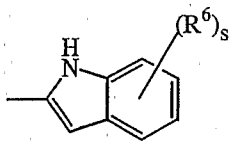
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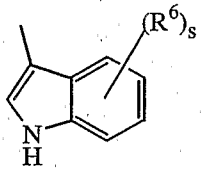
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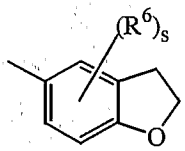
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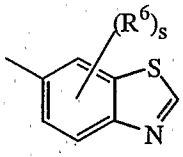
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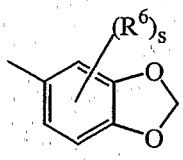
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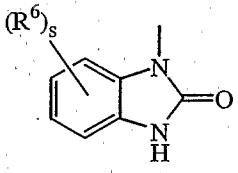
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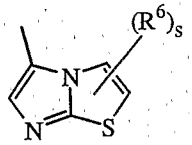
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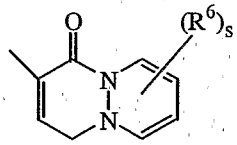
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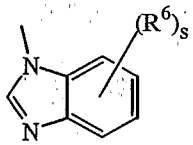
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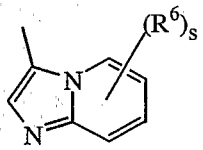
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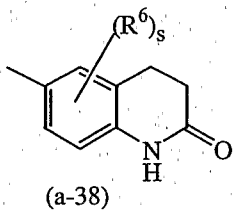
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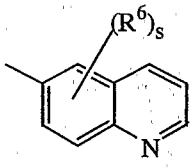
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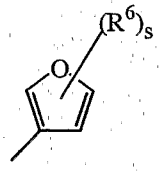
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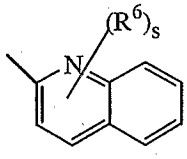
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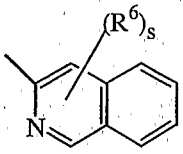
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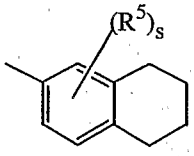
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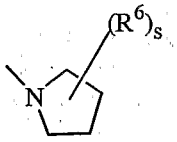
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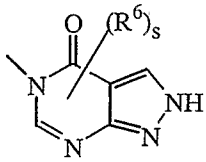
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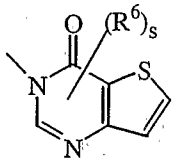
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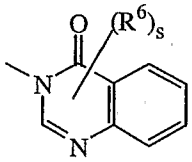
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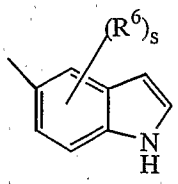
(a-45)



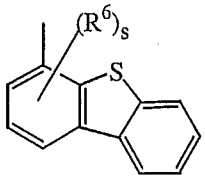
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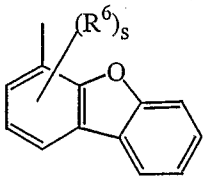


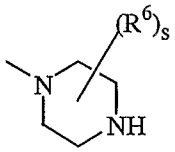
(a-47)



(a-48)


(a-49)


(a-50)


(a-51)

wherein each s is independently 0, 1, 2, 3, 4 or 5;

each R^5 and R^6 are independently selected from hydrogen; halo; hydroxy; amino; nitro; trihaloC_{1-6}alkyl; trihaloC_{1-6}alkyloxy; C_{1-6}alkyl; C_{1-6}alkyl substituted with aryl and C_{3-10}cycloalkyl; C_{1-6}alkyloxy; C_{1-6}alkyloxyC_{1-6}alkyloxy; C_{1-6}alkylcarbonyl; C_{1-6}alkyloxycarbonyl; C_{1-6}alkylsulfonyl; cyanoC_{1-6}alkyl; hydroxyC_{1-6}alkyl; hydroxyC_{1-6}alkyloxy; hydroxyC_{1-6}alkylamino; aminoC_{1-6}alkyloxy; di(C_{1-6}alkyl)aminocarbonyl; di(hydroxyC_{1-6}alkyl)amino; (aryl)(C_{1-6}alkyl)amino; di(C_{1-6}alkyl)aminoC_{1-6}alkyloxy; di(C_{1-6}alkyl)aminoC_{1-6}alkylamino; di(C_{1-6}alkyl)aminoC_{1-6}alkylaminoC_{1-6}alkyl; arylsulfonyl; arylsulfonylamino; aryloxy; aryloxyC_{1-6}alkyl; arylC_{2-6}alkenediyl; di(C_{1-6}alkyl)amino; di(C_{1-6}alkyl)aminoC_{1-6}alkyl; di(C_{1-6}alkyl)amino(C_{1-6}alkyl)amino; di(C_{1-6}alkyl)amino(C_{1-6}alkyl)aminoC_{1-6}alkyl; di(C_{1-6}alkyl)aminoC_{1-6}alkyl(C_{1-6}alkyl)amino; di(C_{1-6}alkyl)aminoC_{1-6}alkyl(C_{1-6}alkyl)aminoC_{1-6}alkyl; aminosulfonylamino(C_{1-6}alkyl)amino; aminosulfonylamino(C_{1-6}alkyl)aminoC_{1-6}alkyl; di(C_{1-6}alkyl)aminosulfonylamino(C_{1-6}alkyl)amino; di(C_{1-6}alkyl)aminosulfonylamino(C_{1-6}alkyl)aminoC_{1-6}alkyl; cyano; thiophenyl; thiophenyl substituted with di(C_{1-6}alkyl)aminoC_{1-6}alkyl(C_{1-6}alkyl)aminoC_{1-6}alkyl, di(C_{1-6}alkyl)aminoC_{1-6}alkyl, C_{1-6}alkylpiperazinylC_{1-6}alkyl, hydroxyC_{1-6}alkylpiperazinylC_{1-6}alkyl, hydroxyC_{1-6}alkyloxyC_{1-6}alkylpiperazinylC_{1-6}alkyl, di(C_{1-6}alkyl)aminosulfonylpiperazinylC_{1-6}alkyl, C_{1-6}alkyloxypiperidinyl, C_{1-6}alkyloxypiperidinylC_{1-6}alkyl, morpholinylC_{1-6}alkyl, hydroxyC_{1-6}alkyl(C_{1-6}alkyl)aminoC_{1-6}alkyl, or di(hydroxyC_{1-6}alkyl)aminoC_{1-6}alkyl; furanyl; furanyl substituted with hydroxyC_{1-6}alkyl; benzofuranyl; imidazolyl; oxazolyl; oxazolyl substituted with aryl and C_{1-6}alkyl; C_{1-6}alkyltriazolyl; tetrazolyl; pyrrolidinyl; pyrrolyl; piperidinylC_{1-6}alkyloxy; morpholinyl; C_{1-6}alkylmorpholinyl; morpholinylC_{1-6}alkyloxy; morpholinylC_{1-6}alkyl; morpholinylC_{1-6}alkylamino; morpholinylC_{1-6}alkylaminoC_{1-6}alkyl; piperazinyl; C_{1-6}alkylpiperazinyl;

C_{1-6}alkylpiperazinylC_{1-6}alkyloxy; piperazinylC_{1-6}alkyl; naphtalenylsulfonylpiperazinyl; naphtalenylsulfonylpiperidinyl; naphtalenylsulfonyl; C_{1-6}alkylpiperazinylC_{1-6}alkyl; C_{1-6}alkylpiperazinylC_{1-6}alkylamino; C_{1-6}alkylpiperazinylC_{1-6}alkylaminoC_{1-6}alkyl; C_{1-6}alkylpiperazinylsulfonyl; aminosulfonylpiperazinylC_{1-6}alkyloxy; aminosulfonylpiperazinyl; aminosulfonylpiperazinylC_{1-6}alkyl; di(C_{1-6}alkyl)aminosulfonylpiperazinyl; di(C_{1-6}alkyl)aminosulfonylpiperazinylC_{1-6}alkyl; hydroxyC_{1-6}alkylpiperazinyl; hydroxyC_{1-6}alkylpiperazinylC_{1-6}alkyl; C_{1-6}alkyloxypiperidinyl; C_{1-6}alkyloxypiperidinylC_{1-6}alkyl; piperidinylaminoC_{1-6}alkylamino; piperidinylaminoC_{1-6}alkylaminoC_{1-6}alkyl; (C_{1-6}alkylpiperidinyl)(hydroxyC_{1-6}alkyl)aminoC_{1-6}alkylamino; (C_{1-6}alkylpiperidinyl)(hydroxyC_{1-6}alkyl)aminoC_{1-6}alkylaminoC_{1-6}alkyl; hydroxyC_{1-6}alkyloxyC_{1-6}alkylpiperazinyl; hydroxyC_{1-6}alkyloxyC_{1-6}alkylpiperazinylC_{1-6}alkyl; (hydroxyC_{1-6}alkyl)(C_{1-6}alkyl)amino; (hydroxyC_{1-6}alkyl)(C_{1-6}alkyl)aminoC_{1-6}alkyl; hydroxyC_{1-6}alkylaminoC_{1-6}alkyl; di(hydroxyC_{1-6}alkyl)aminoC_{1-6}alkyl; pyrrolidinylC_{1-6}alkyl; pyrrolidinylC_{1-6}alkyloxy; pyrazolyl; thiopyrazolyl; pyrazolyl substituted with two substituents selected from C_{1-6}alkyl or trihaloC_{1-6}alkyl; pyridinyl; pyridinyl substituted with C_{1-6}alkyloxy, aryloxy or aryl; pyrimidinyl; tetrahydropyrimidinylpiperazinyl; tetrahydropyrimidinylpiperazinylC_{1-6}alkyl; quinolinyl; indole; phenyl; phenyl substituted with one, two or three substituents independently selected from halo, amino, nitro, C_{1-6}alkyl, C_{1-6}alkyloxy, hydroxyC_{1-4}alkyl, trifluoromethyl, trifluoromethyloxy, hydroxyC_{1-4}alkyloxy, C_{1-4}alkylsulfonyl, C_{1-4}alkyloxyC_{1-4}alkyloxy, C_{1-4}alkyloxycarbonyl, aminoC_{1-4}alkyloxy, di(C_{1-4}alkyl)aminoC_{1-4}alkyloxy, di(C_{1-4}alkyl)amino, di(C_{1-4}alkyl)aminocarbonyl, di(C_{1-4}alkyl)aminoC_{1-4}alkyl, di(C_{1-4}alkyl)aminoC_{1-4}alkylaminoC_{1-4}alkyl, di(C_{1-4}alkyl)amino(C_{1-4}alkyl)amino, di(C_{1-4}alkyl)amino(C_{1-4}alkyl)aminoC_{1-4}alkyl, di(C_{1-4}alkyl)aminoC_{1-4}alkyl(C_{1-4}alkyl)amino, di(C_{1-4}alkyl)aminoC_{1-4}alkyl(C_{1-4}alkyl)aminoC_{1-4}alkyl, aminosulfonylamino(C_{1-4}alkyl)amino, aminosulfonylamino(C_{1-4}alkyl)aminoC_{1-4}alkyl, di(C_{1-4}alkyl)aminosulfonylamino(C_{1-4}alkyl)amino, di(C_{1-4}alkyl)aminosulfonylamino(C_{1-4}alkyl)aminoC_{1-6}alkyl, cyano, piperidinylC_{1-4}alkyloxy, pyrrolidinylC_{1-4}alkyloxy, aminosulfonylpiperazinyl, aminosulfonylpiperazinylC_{1-4}alkyl, di(C_{1-4}alkyl)aminosulfonylpiperazinyl, di(C_{1-4}alkyl)aminosulfonylpiperazinylC_{1-4}alkyl, hydroxyC_{1-4}alkylpiperazinyl, hydroxyC_{1-4}alkylpiperazinylC_{1-4}alkyl, C_{1-4}alkyloxypiperidinyl,

C_{1-4}alkyloxypiperidinylC_{1-4}alkyl, hydroxyC_{1-4}alkyloxyC_{1-4}alkylpiperazinyl, hydroxyC_{1-4}alkyloxyC_{1-4}alkylpiperazinylC_{1-4}alkyl, (hydroxyC_{1-4}alkyl)(C_{1-4}alkyl)amino, (hydroxyC_{1-4}alkyl)(C_{1-4}alkyl)aminoC_{1-4}alkyl, di(hydroxyC_{1-4}alkyl)amino, di(hydroxyC_{1-4}alkyl)aminoC_{1-4}alkyl, furanyl, furanyl substituted with –CH=CH-CH=CH-, pyrrolidinylC_{1-4}alkyl, pyrrolidinylC_{1-4}alkyloxy, morpholinyl, morpholinylC_{1-4}alkyloxy, morpholinylC_{1-4}alkyl, morpholinylC_{1-4}alkylamino, morpholinylC_{1-4}alkylaminoC_{1-4}alkyl, piperazinyl, C_{1-4}alkylpiperazinyl, C_{1-4}alkylpiperazinylC_{1-4}alkyloxy, piperazinylC_{1-4}alkyl, C_{1-4}alkylpiperazinylC_{1-4}alkyl, C_{1-4}alkylpiperazinylC_{1-4}alkylamino, C_{1-4}alkylpiperazinylC_{1-4}alkylaminoC_{1-6}alkyl, tetrahydropyrimidinylpiperazinyl, tetrahydropyrimidinylpiperazinylC_{1-4}alkyl, piperidinylaminoC_{1-4}alkylamino, piperidinylaminoC_{1-4}alkylaminoC_{1-4}alkyl, (C_{1-4}alkylpiperidinyl)(hydroxyC_{1-4}alkyl)aminoC_{1-4}alkylamino, (C_{1-4}alkylpiperidinyl)(hydroxyC_{1-4}alkyl)aminoC_{1-4}alkylaminoC_{1-4}alkyl, pyridinylC_{1-4}alkyloxy, hydroxyC_{1-4}alkylamino, hydroxyC_{1-4}alkylaminoC_{1-4}alkyl, di(C_{1-4}alkyl)aminoC_{1-4}alkylamino, aminothiadiazolyl, aminosulfonylpiperazinylC_{1-4}alkyloxy, or thiophenylC_{1-4}alkylamino;

each R^5 and R^6 can be placed on the nitrogen in replacement of the hydrogen;

aryl in the above is phenyl, or phenyl substituted with one or more substituents each independently selected from halo, C_{1-6}alkyl, C_{1-6}alkyloxy, trifluoromethyl, cyano or hydroxycarbonyl.

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_{1-4}alkyl defines straight and branched chain saturated hydrocarbon radicals having from 1 to 4 carbon atoms such as, e.g. methyl, ethyl, propyl, butyl, 1-methylethyl, 2-methylpropyl and the like; C_{1-6}alkyl includes C_{1-4}alkyl and the higher homologues thereof having 5 to 6 carbon atoms such as, for example, pentyl, 2-methyl-

butyl, hexyl, 2-methylpentyl and the like; C_{1-6}alkanediyl defines bivalent straight and branched chained saturated hydrocarbon radicals having from 1 to 6 carbon atoms such as, for example, methylene, 1,2-ethanediyl, 1,3-propanediyl 1,4-butanediyl, 1,5-pentanediyl, 1,6-hexanediyl and the branched isomers thereof such as, 2-methylpentanediyl, 3-methylpentanediyl, 2,2-dimethylbutanediyl, 2,3-dimethylbutanediyl and the like; trihaloC_{1-6}alkyl defines C_{1-6}alkyl containing three identical or different halo substituents for example trifluoromethyl; C_{2-6}alkenediyl defines bivalent straight and branched chain hydrocarbon radicals containing one double bond and having from 2 to 6 carbon atoms such as, for example, ethenediyl, 2-propenediyl, 3-butenediyl, 2-pentenediyl, 3-pentenediyl, 3-methyl-2-butenediyl, and the like; aminoaryl defines aryl substituted with amino;
C_{3-10}cycloalkyl includes cyclic hydrocarbon groups having from 3 to 10 carbons, such as cyclopropyl, cyclobutyl, cyclopentyl, cyclopentenyl, cyclohexyl, cyclohexenyl, cycloheptyl, cyclooctyl and the like.

The term "another Zn-chelating group" refers to a group, which is capable of interacting with a Zn-ion, which can be present at an enzymatic binding site.

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 might 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.

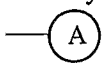
The *N*-oxide forms of the compounds of formula (I) are meant to comprise those compounds of formula (I) wherein one or several nitrogen atoms are oxidized to the so-called *N*-oxide, particularly those *N*-oxides wherein one or more of the piperidine-, piperazine or pyridazinyl-nitrogens are *N*-oxidized.

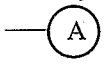
Some of the compounds of formula (I) may also exist in their tautomeric forms. Such forms although not explicitly indicated in the above formula are intended to be included within the scope of the present invention.

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 and HDAC-10. 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 one or more of the following restrictions apply:

a) n is 1 or 2;
b) t is 0, 1, 2 or 4;
c) each Q is ;
d) R^1 is –C(O)NH(OH);
e) R^2 is hydrogen or nitro;
f) -L- is a direct bond or a bivalent radical selected from C_{1-6}alkanediyl;
g) R^4 is hydrogen;
h)  is a radical selected from (a-1),(a-2), (a-3), (a-5), (a-6), (a-11), (a-18), (a-20), (a-21), (a-32), (a-33), (a-47) or (a-51);
i) each s is independently 0, 1, 2, or 4;
j) each R^5 and R^6 are independently selected from hydrogen; halo; trihaloC_{1-6}alkyl; C_{1-6}alkyl; C_{1-6}alkyl substituted with aryl and C_{3-10}cycloalkyl; C_{1-6}alkyloxy; C_{1-6}alkylcarbonyl; benzofuranyl; naphtalenylsulfonyl; pyridinyl substituted with aryloxy; phenyl; or phenyl substituted with one substituent independently selected from hydroxyC_{1-4}alkyl or morpholinylC_{1-4}alkyl.

A second group of interesting compounds consists of those compounds of formula (I) wherein one or more of the following restrictions apply:
a) n is 1;
b) t is 0, 1 or 2;
c) each Q is ;
d) each X is nitrogen;
e) each Y is nitrogen;
f) R^1 is –C(O)NH(OH);
g) R^2 is hydrogen;
h) -L- is a direct bond;
i) each R^3 independently represents a hydrogen atom;
j) R^4 is hydrogen;
k)  is a radical selected from (a-6), (a-11), (a-20), (a-47) or (a-51);
l) each s is independently 0, 1, or 4;
m) each R^5 and R^6 are independently selected from hydrogen; C_{1-6}alkyl; C_{1-6}alkyloxy; naphtalenylsulfonyl; or phenyl substituted with hydroxyC_{1-4}alkyl or morpholinylC_{1-4}alkyl.

A third group of interesting compounds consists of those compounds of formula (I) wherein one or more of the following restrictions apply:
a) R^1 is –C(O)NH(OH);

b) -L- is a direct bond.

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

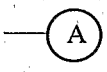
a) t is 1, 2, 3, or 4;

b) R^1 is $-C(O)NR^7R^8$, $-C(O)-C_{1-6}alkanediylSR^9$, $-NR^{10}C(O)N(OH)R^9$, $-NR^{10}C(O)C_{1-6}alkanediylSR^9$, $-NR^{10}C(O)C=N(OH)R^9$ or another Zn-chelating-group wherein R^7 and R^8 are each independently selected from hydrogen, hydroxy, $hydroxyC_{1-6}alkyl$ or $aminoC_{1-6}alkyl$;

c) R^2 is hydrogen, halo, hydroxy, amino, nitro, $C_{1-6}alkyl$, $C_{1-6}alkyloxy$, trifluoromethyl or $di(C_{1-6}alkyl)amino$;

d) -L- is a direct bond or a bivalent radical selected from $C_{1-6}alkanediyl$, $C_{1-6}alkanediyloxy$, amino or carbonyl;

e) R^4 is hydrogen, hydroxy, amino, $hydroxyC_{1-6}alkyl$, $C_{1-6}alkyl$, $C_{1-6}alkyloxy$, $arylC_{1-6}alkyl$, aminocarbonyl, $aminoC_{1-6}alkyl$, $C_{1-6}alkylaminoC_{1-6}alkyl$ or $di(C_{1-6}alkyl)aminoC_{1-6}alkyl$;

f)  is a radical selected from (a-1), (a-3), (a-4), (a-5), (a-6), (a-7), (a-8), (a-9), (a-10), (a-11), (a-12), (a-13), (a-14), (a-15), (a-16), (a-17), (a-18), (a-19), (a-20), (a-21), (a-22), (a-23), (a-24), (a-25), (a-26), (a-28), (a-29), (a-30), (a-31), (a-32), (a-33), (a-34), (a-35), (a-36), (a-37), (a-38), (a-39), (a-40), (a-41), (a-42), (a-44), (a-45), (a-46), (a-47), (a-48) and (a-51);

g) each s is independently 0, 1, 2, 3 or 4;

h) R^5 is hydrogen; halo; hydroxy; amino; nitro; $trihaloC_{1-6}alkyl$; $trihaloC_{1-6}alkyloxy$; $C_{1-6}alkyl$; $C_{1-6}alkyloxy$; $C_{1-6}alkylcarbonyl$; $C_{1-6}alkyloxycarbonyl$; $C_{1-6}alkylsulfonyl$; $hydroxyC_{1-6}alkyl$; aryloxy; $di(C_{1-6}alkyl)amino$; cyano; thiophenyl; furanyl; furanyl substituted with $hydroxyC_{1-6}alkyl$; benzofuranyl; imidazolyl; oxazolyl; oxazolyl substituted with aryl and $C_{1-6}alkyl$; $C_{1-6}alkyltriazolyl$; tetrazolyl; pyrrolidinyl; pyrrolyl; morpholinyl; $C_{1-6}alkylmorpholinyl$; piperazinyl; $C_{1-6}alkylpiperazinyl$; $hydroxyC_{1-6}alkylpiperazinyl$; $C_{1-6}alkyloxypiperidinyl$; pyrazoly; pyrazolyl substituted with one or two substituents selected from $C_{1-6}alkyl$ or $trihaloC_{1-6}alkyl$; pyridinyl; pyridinyl substituted with $C_{1-6}alkyloxy$, aryloxy or aryl; pyrimidinyl; quinolinyl; indole; phenyl; or phenyl substituted with one or two substituents independently selected from halo, $C_{1-6}alkyl$, $C_{1-6}alkyloxy$ or trifluoromethyl;

i) R^6 is hydrogen; halo; hydroxy; amino; nitro; $trihaloC_{1-6}alkyl$; $trihaloC_{1-6}alkyloxy$; $C_{1-6}alkyl$; $C_{1-6}alkyloxy$; $C_{1-6}alkylcarbonyl$; $C_{1-6}alkyloxycarbonyl$;

C_{1-6}alkylsulfonyl; hydroxyC_{1-6}alkyl; aryloxy; di(C_{1-6}alkyl)amino; cyano; pyridinyl; phenyl; or phenyl substituted with one or two substituents independently selected from halo, C_{1-6}alkyl, C_{1-6}alkyloxy or trifluoromethyl.

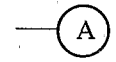
A group of preferred compounds consists of those compounds of formula (I) wherein t is 1, 2, 3, or 4;

R^1 is $-C(O)NR^7R^8$, $-C(O)-C_{1-6}alkanediylSR^9$, $-NR^{10}C(O)N(OH)R^9$, $-NR^{10}C(O)C_{1-6}alkanediylSR^9$, $-NR^{10}C(O)C{=}N(OH)R^9$ or another Zn-chelating-group wherein R^7 and R^8 are each independently selected from hydrogen, hydroxy, hydroxyC_{1-6}alkyl or aminoC_{1-6}alkyl;

R^2 is hydrogen, halo, hydroxy, amino, nitro, C_{1-6}alkyl, C_{1-6}alkyloxy, trifluoromethyl or di(C_{1-6}alkyl)amino;

-L- is a direct bond or a bivalent radical selected from C_{1-6}alkanediyl, C_{1-6}alkanediyloxy, amino or carbonyl;

R^4 is hydrogen, hydroxy, amino, hydroxyC_{1-6}alkyl, C_{1-6}alkyl, C_{1-6}alkyloxy, arylC_{1-6}alkyl, aminocarbonyl, aminoC_{1-6}alkyl, C_{1-6}alkylaminoC_{1-6}alkyl or di(C_{1-6}alkyl)aminoC_{1-6}alkyl;


is a radical selected from (a-1), (a-3), (a-4), (a-5), (a-6), (a-7), (a-8), (a-9), (a-10), (a-11), (a-12), (a-13), (a-14), (a-15), (a-16), (a-17), (a-18), (a-19), (a-20), (a-21), (a-22), (a-23), (a-24), (a-25), (a-26), (a-28), (a-29), (a-30), (a-31), (a-32), (a-33), (a-34), (a-35), (a-36), (a-37), (a-38), (a-39), (a-40), (a-41), (a-42), (a-44), (a-45), (a-46), (a-47), (a-48) and (a-51);

each s is independently 0, 1, 2, 3 or 4;

R^5 is hydrogen; halo; hydroxy; amino; nitro; trihaloC_{1-6}alkyl; trihaloC_{1-6}alkyloxy; C_{1-6}alkyl; C_{1-6}alkyloxy; C_{1-6}alkylcarbonyl; C_{1-6}alkyloxycarbonyl; C_{1-6}alkylsulfonyl; hydroxyC_{1-6}alkyl; aryloxy; di(C_{1-6}alkyl)amino; cyano; thiophenyl; furanyl; furanyl substituted with hydroxyC_{1-6}alkyl; benzofuranyl; imidazolyl; oxazolyl; oxazolyl substituted with aryl and C_{1-6}alkyl; C_{1-6}alkyltriazolyl; tetrazolyl; pyrrolidinyl; pyrrolyl; morpholinyl; C_{1-6}alkylmorpholinyl; piperazinyl; C_{1-6}alkylpiperazinyl; hydroxyC_{1-6}alkylpiperazinyl; C_{1-6}alkyloxypiperidinyl; pyrazolyl; pyrazolyl substituted with one or two substituents selected from C_{1-6}alkyl or trihaloC_{1-6}alkyl; pyridinyl; pyridinyl substituted with C_{1-6}alkyloxy, aryloxy or aryl; pyrimidinyl; quinolinyl; indole; phenyl; or phenyl substituted with one or two substituents independently selected from halo, C_{1-6}alkyl, C_{1-6}alkyloxy or trifluoromethyl;

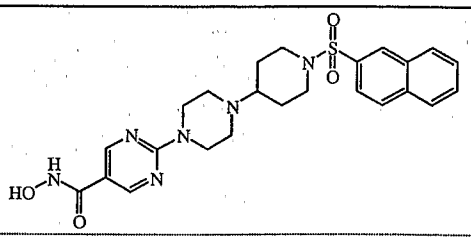
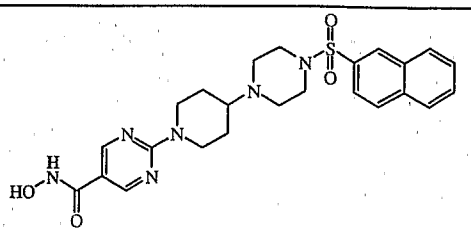
R^6 is hydrogen; halo; hydroxy; amino; nitro; trihaloC_{1-6}alkyl; trihaloC_{1-6}alkyloxy;

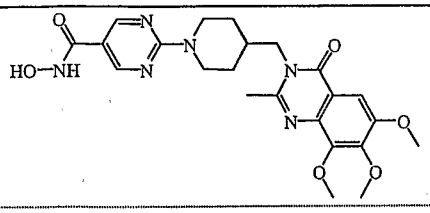
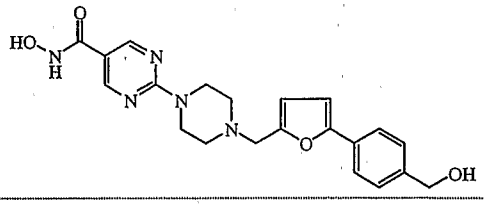
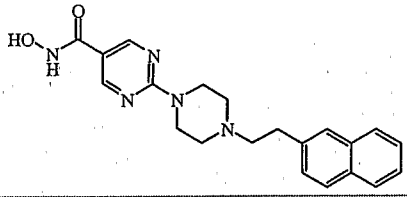
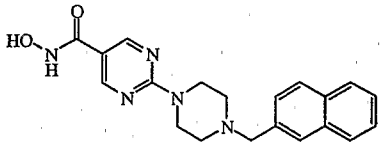
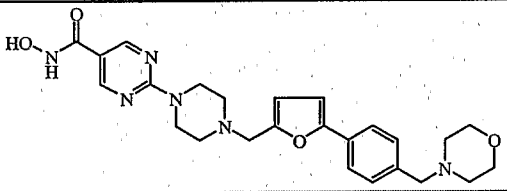
C_{1-6}alkyl; C_{1-6}alkyloxy; C_{1-6}alkylcarbonyl; C_{1-6}alkyloxycarbonyl; C_{1-6}alkylsulfonyl; hydroxyC_{1-6}alkyl; aryloxy; di(C_{1-6}alkyl)amino; cyano; pyridinyl; phenyl; or phenyl substituted with one or two substituents independently selected from halo, C_{1-6}alkyl, C_{1-6}alkyloxy or trifluoromethyl.

A further group of preferred compounds are those compounds of formula (I) wherein n is 1 or 2; t is 0, 1, 2 or 4; each Q is —C≡; R^1 is –C(O)NH(OH); R^2 is hydrogen or nitro; -L- is a direct bond or a bivalent radical selected from C_{1-6}alkanediyl; R^4 is hydrogen; —(A) is a radical selected from (a-1),(a-2), (a-3), (a-5), (a-6), (a-11), (a-18), (a-20), (a-21), (a-32), (a-33), (a-47) or (a-51); each s is independently 0, 1, 2, or 4; each R^5 and R^6 are independently selected from hydrogen; halo; trihaloC_{1-6}alkyl; C_{1-6}alkyl; C_{1-6}alkyl substituted with aryl and C_{3-10}cycloalkyl; C_{1-6}alkyloxy; C_{1-6}alkylcarbonyl; benzofuranyl; naphtalenylsulfonyl; pyridinyl substituted with aryloxy; phenyl; or phenyl substituted with one substituent independently selected from hydroxyC_{1-4}alkyl or morpholinylC_{1-4}alkyl.

A group of more preferred compounds are those compounds of formula (I) wherein n is 1; t is 0 or 1; each Q is —C≡; each X is nitrogen; each Y is nitrogen; R^1 is –C(O)NH(OH); R^2 is hydrogen; -L- is a direct bond; each R^3 independently represents a hydrogen atom; R^4 is hydrogen; —(A) is a radical selected from (a-6), (a-11), (a-20), (a-47) or (a-51); each s is independently 0, 1, or 4; and each R^5 and R^6 are independently selected from hydrogen; C_{1-6}alkyl; C_{1-6}alkyloxy; naphtalenylsulfonyl; or aryl substituted with hydroxyC_{1-4}alkyl or morpholinylC_{1-4}alkyl.

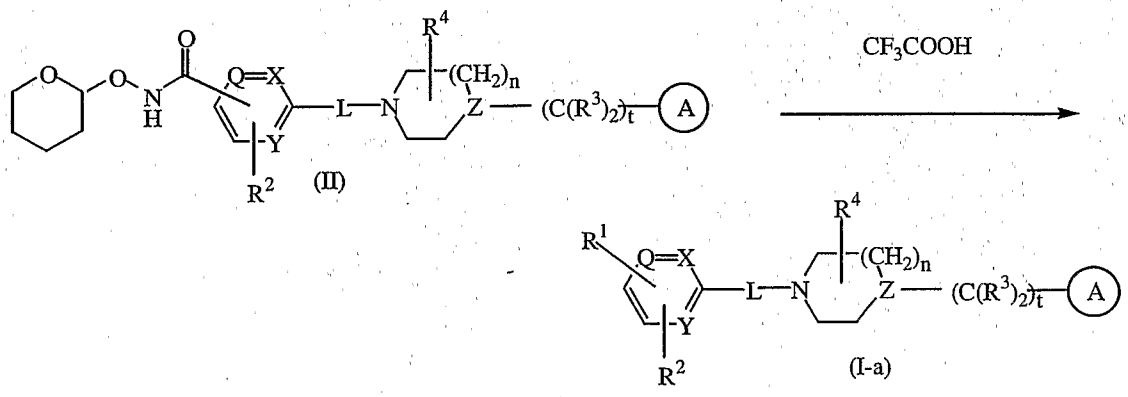
Most preferred compounds are compounds No. 3, No. 4, No. 8, No. 5, No. 7, No. 6 and No 9.

	
0.91 $C_2HF_3O_2$; Co. No. 3	0.86 $C_2HF_3O_2$;Co. No. 4

	
$C_2HF_3O_2$ (1:1);Co. No. 8	0.83 $C_2HF_3O_2$;Co. No. 5
	
0.79 $C_2HF_3O_2$;Co. No. 7	0.83 $C_2HF_3O_2$;Co. No. 6
	
0.47 H_2O .1.99 $C_2HF_3O_2$; Co. No. 9	

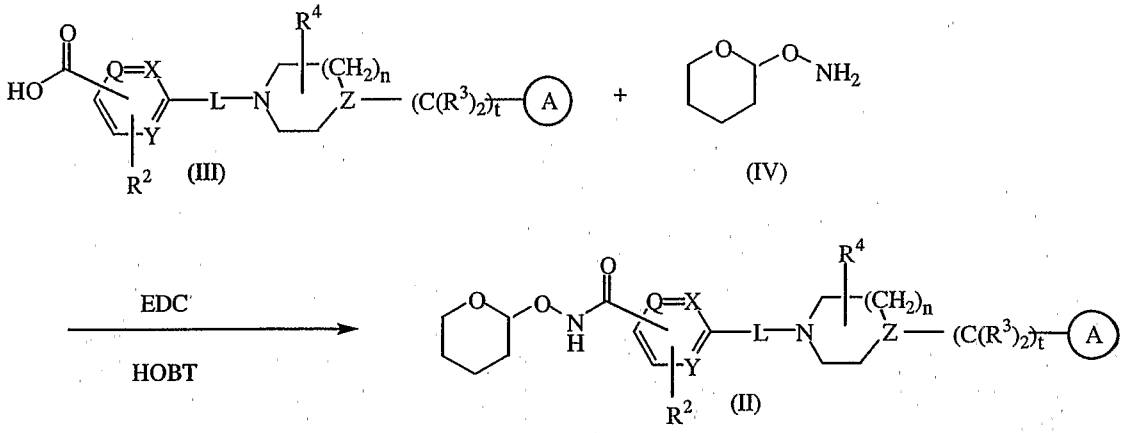
The compounds of formula (I) and their pharmaceutically acceptable salts and N-oxides and stereochemically isomeric forms thereof may be prepared in a conventional manner. A general synthesis route is encompassed as example:

a) Hydroxamic acids of formula (I) wherein R^1 is –C(O)NH(OH), said compounds being referred to as compounds of formula (I-a), may be prepared by reacting an intermediate of formula (II) with an appropriate acid, such as for example, trifluoro acetic acid. Said reaction is performed in an appropriate solvent, such as, for example, methanol.

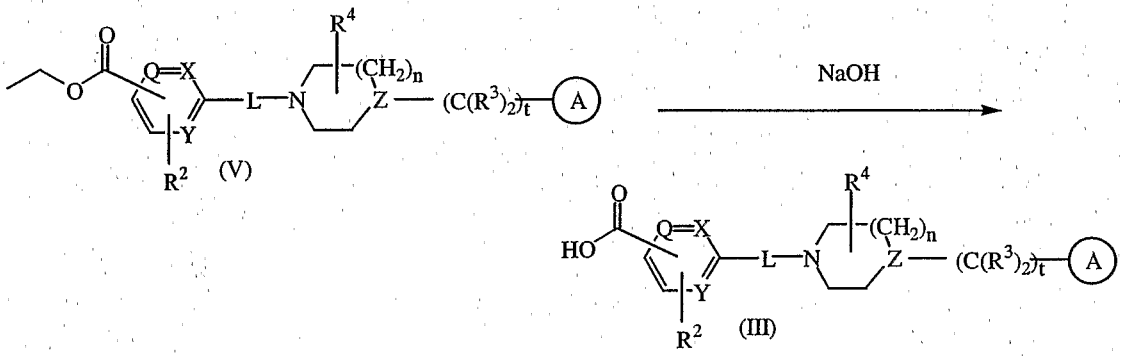


b) intermediates of formula (II) may be prepared by reacting an intermediate of formula (III) with an intermediate of formula (IV) in the presence of appropriate reagents such as *N*'-(ethylcarbonimidoyl)-*N*,*N*-dimethyl-1,3-propanediamine, monohydrochloride

(EDC) and 1-hydroxy-1*H*-benzotriazole (HOBT). The reaction may be performed in a suitable solvent such as a mixture of DCM and THF.



c) intermediates of formula (III) may be prepared by reacting an intermediate of formula (V) with an appropriate base such as NaOH in the presence of a suitable solvent such as ethanol.



The compounds of formula (I) can also conveniently be prepared using solid phase synthesis techniques. In general, solid phase synthesis involves reacting an intermediate in a synthesis with a polymer support. This polymer-supported intermediate can then be carried on through a number of synthesis steps. After each step, filtering the resin and washing it numerous times with various solvents remove impurities. At each step the resin can be split up to react with various intermediates in the next step thus allowing for the synthesis of a large number of compounds. After the last step in the procedure the resin is treated with a reagent or process to cleave the resin from the sample. More detailed explanation of the techniques used in solid phase chemistry is described in for example "The Combinatorial Index" (B.Bunin, Academic Press) and Novabiochem's 1999 Catalogue & Peptide Synthesis Handbook (Novabiochem AG, Switzerland) both incorporated herein by reference.

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

n) treating spinal muscular atrophy;

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

p) enhancing gene therapy.

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; or
- other HDAC inhibitors.

The term "platinum coordination compound" is used herein to denote any tumor 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 "topisomerase 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-tumor 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 tumors 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 bond to the ER, induces a change in the three-dimensional shape of the receptor, inhibiting 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:

- short-chain fatty acids for example butyrate, 4-phenylbutyrate or valproic acid;
- hydroxamic acids for example suberoylanilide hydroxamic acid (SAHA), biaryl hydroxamate A-161906, bicyclic aryl-*N*-hydroxycarboxamides, pyroxamide, CG-1521, PXD-101, sulfonamide hydroxamic acid, LAQ-824, trichostatin A (TSA), oxamflatin, scriptaid, m-carboxy cinnamic acid bishydroxamic acid, or trapoxin-hydroxamic acid analogue;
- cyclic tetrapeptides for example trapoxin, apidicin or depsipeptide;
- benzamides for example MS-275 or CI-994, or
- depudecin.

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 combinations 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 250mg/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-tumor 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-tumor 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 100mg 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

The following examples are provided for purposes of illustration.

Hereinafter "EDC" means *N*'-(ethylcarbonimidoyl)-*N*,*N*-dimethyl-1,3-propanediamine, monohydrochloride "DCM" means dichloromethane, "DIEA" means

diisopropylethylamine, "DIPE" means diisopropyl ether, "DMF" means dimethylformamide, "EtOAc" means ethyl acetate, "iPrOH" means isopropyl, "MeOH" means methanol, "EtOH" means ethanol, "PyBrOP" means bromo-tris-pyrrolidino-phosphonium hexafluorophosphate, "TEA"means triethylamine, "TFA" means trifluoroacetic acid and "THF" means tetrahydrofuran.

A. Preparation of the intermediates

Example A1

a) A solution of hexahydro-1*H*-1,4-diazepine (0.20 mol) and 1-fluoro-4-nitro-benzene (0.10 mol) in DCM (300 ml) was stirred at room temperature for 24 hours under nitrogen atmosphere. Yellow crystals precipitated. The precipitate was collected on filter, washed with ether and dried. The residue (21.1g, 87%) was taken up in water and treated with a 3 N NaOH solution. This mixture was extracted with DCM (3 x 500 ml), dried (Na_2SO_4), filtered, and the solvent was removed under reduced pressure and dried under vacuum at room temperature for 16 hours, yielding 18.3 g of hexahydro-1-(4-nitrophenyl)- 1*H*-1,4-diazepine (interm. 1), melting point 115-116°C.

b) Bis-(1,1-dimethylethyl)-dicarbonic acid, ester (0.090 mol) in DCM (100 ml) was added to a cooled (ice bath) solution of interm. 1 (0.090 mol) in DCM (200 ml), with stirring. Evolution of gas was observed. After the evolution of CO_2 stopped, the ice-bath was removed and stirring was continued for 3 hours at room temperature. The reaction mixture was washed with a 1 N HCl solution and water, then dried. The solvent was evaporated, yielding 20.5 g (70%) of hexahydro-4-(4-nitrophenyl)- 1*H*-1,4-diazepine-1-carboxylic acid, 1,1-dimethylethyl ester (interm.2), melting point 128-130°C.

c) Interm. 2 (0.047 mol) was added portionwise (over a 20-min period) to a refluxing mixture of hydrazine monohydrate (15 ml) in methanol (700 ml) and Raney Nickel (16.1 g). The reaction mixture was stirred and refluxed until the yellow color was discharged. The mixture was continued refluxing for an additional half an hour. The catalyst was removed by filtration. The solvent was evaporated, yielding 13.0 g of 4-(4-aminophenyl)hexahydro-1*H*-1,4-diazepine-1-carboxylic acid, 1,1-dimethylethyl ester (interm. 3).

d) A mixture of interm. 3 (0.045 mol) and (1-ethoxyethylidene)-hydrazinecarboxylic acid, ethyl ester (0.090 mol) was heated neat on an oil bath at 130°C for 1 hour, with stirring. After additional two hours, the reaction mixture was cooled and 2-propanol (about 100 ml) was added with trituration. The solid was filtered and dried to give 14 g of solid. This solid was triturated with ether, filtered off and dried, yielding 13.0 g (77%) of 1*H*-1,4-diazepine-1-carboxylic acid, 4-[4-(1,5-dihydro-3-methyl-5-oxo-4*H*-

1,2,4-triazol-4-yl)phenyl]hexahydro-1*H*-1,4-diazepine-1-carboxylic acid, 1,1-dimethylethyl ester (interm. 4) , melting point 238-240°C.

e) Reaction under Ar atmosphere. 1,1,1-Trimethyl-*N*-(trimethylsilyl)- silanamine, sodium salt (0.01 mol; 10 ml, 1 M/THF) was added (via syringe) to interm. 4 (0.01 mol) in DMF (200 ml), at room temperature. Solid sodium salt started to form. It was stirred vigorously and additional DMF (200 ml) was added. A solution of 2-bromo-butane (0.02 mol) in DMF (100 ml) was added slowly at room temperature. The reaction mixture was stirred at room temperature for 18 hours. The DMF solvent was removed (vacuum pump). Water was added to the residue and the resulting oily product was taken up with ether (500 ml). The ether solution was concentrated to give 2.70 g (oily material which solidified on standing; 62% yield) which was further purified by flash column chromatography over silica gel (eluent: 1% (10% NH_4OH/CH_3OH)/CH_2Cl_2) yielding 0.55 g of 4-[4-[1,5-dihydro-3-methyl-1-(1-methylpropyl)-5-oxo-4*H*-1,2,4-triazol-4-yl]phenyl]hexahydro-1*H*-1,4-diazepine-1-carboxylic acid, 1,1-dimethylethyl ester (interm. 5), melting point: 119-120°C.

f) Interm. 5 (0.0014 mol) was added into a cooled solution of TFA (5 ml; ice-bath) for 30 min. The solvent was removed under reduced pressure. Water was added and the mixture was saturated with potassium carbonate. This mixture was extracted with ethylacetate (2 x 50 ml), dried over sodium sulfate, filtered and the solvent was removed, yielding 0.35 g (65%) of 4-[4-(hexahydro-1*H*-1,4-diazepin-1-yl)phenyl]-2,4-dihydro-5-methyl-2-(1-methylpropyl)- 3*H*-1,2,4-triazol-3-one (interm. 6).

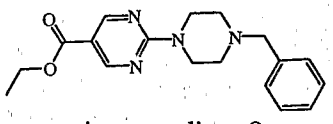
g) A mixture of interm. 6 (0.00076 mol), 4-bromo-benzoic acid, methyl ester (0.00304 mol), (1R)-[1,1'-binaphthalene]-2,2'-diylbis[diphenyl- phosphine (0.016 g), $Pd_2(dba)_3$ (0.008 g) and Cs_2CO_3 (0.40 g) in toluene (10 ml) was placed in a pressure tube (containing a small magnetic stirring bar) in a glove box under argon. The pressure tube was capped tightly and was heated in an oil bath at 120°C for 12 hours with stirring). More (1R)-[1,1'-binaphthalene]-2,2'-diylbis[diphenyl- phosphine (0.016 g), $Pd_2(dba)_3$ (0.008 g) and Cs_2CO_3 (0.40 g) were added. The mixture was heated at 120°C in the oil bath for 24 hours. The reaction mixture was filtered to remove the inorganic solid material and was washed with about 20 ml of $CHCl_3$. The filtrate was concentrated under reduced pressure to dryness. The residue was purified by flash chromatography on silica gel (eluent: $NH_4OH/CH_3OH/CH_2Cl_2$ 0.1/0.9/99). The desired fractions were combined and the solvent was evaporated and dried at room temperature under vacuum for 16 hours, yielding 0.21 g (60%) of 4-[4-[4-[1,5-dihydro-3-methyl-1-(1-methylpropyl)-5-oxo-4*H*-1,2,4-triazol-4-yl]phenyl]hexahydro-1*H*-1,4-diazepin-1-yl]-benzoic acid, methyl ester (interm. 7), melting point 152°C-153°C.

Example A2

A mixture of 4-[4-(phenylmethyl)-1-piperazinyl]- benzoic acid (0.0145 mol), O-(tetrahydro-2*H*-pyran-2-yl)- hydroxylamine (0.029 mol), *N*,*N*'-methanetetrayl-biscyclohexanamine (0.0145 mol) and 1-hydroxy-1*H*-benzotriazole (0.021 mol) in DCM p.a. (200 ml) was stirred at room temperature over the weekend. The reaction mixture was washed with water, dried ($MgSO_4$), filtered and the solvent was evaporated. The residue was crystallized from EtOAc, filtered off and dried, yielding 3.5 g and another crop: 2.0 g , total yielding 5.5 g (95%) of 4-[4-(phenylmethyl)-1-piperazinyl]-*N*-[(tetrahydro-2*H*-pyran-2-yl)oxy]- benzamide (interm. 8).

Example A3

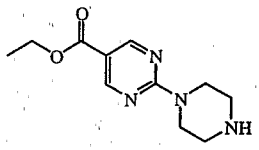
a) Preparation of



intermediate 9

A solution of 1-(phenylmethyl)- piperazine (0.068 mol) in acetonitrile p.a. (135 ml) was added gradually to a solution of potassium carbonate (0.18 mol) and 2-(methylsulfonyl)- 5-pyrimidinecarboxylic acid, ethyl ester (0.082 mol) in acetonitrile p.a. (135 ml) and the reaction mixture was stirred for 45 min at room temperature. Then, the reaction mixture was stood overnight. DCM (400 ml) was added. Water (300 ml) was added and the organic layer was separated, dried ($MgSO_4$), filtered and the solvent was evaporated. The residue (28g) was purified by column chromatography over silica gel (eluent: DCM/MeOH 95/5). The pure fractions were collected and the solvent was evaporated. The residue was crystallized from acetonitrile, filtered off and dried in vacuo, yielding 15.1 g of intermediate 9.

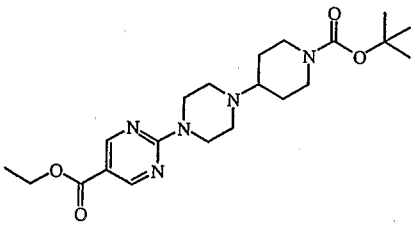
b) Preparation of



intermediate 10

A mixture of intermediate 9 (0.03 mol) in EtOH (250 ml) was hydrogenated at 50 °C with Pd/C 10% (2 g) as a catalyst. After uptake of H_2 (1 equiv), the catalyst was filtered off and the filtrate was evaporated. The residue was purified by column chromatography over silica gel (eluent: DCM/($MeOH/NH_3$) 90/10). The product fractions were collected and the solvent was evaporated, yielding 6.8 g (>96%) of intermediate 10.

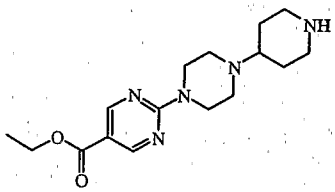
c) Preparation of



intermediate 11

Titanium tetrakis(ethoxide) (0.0029 mol) was added to a mixture of intermediate 10 (0.0022 mol) and 4-oxo-1-piperidinecarboxylic acid, 1,1-dimethylethyl ester (0.0027 mol) in 1,2-dichloro- ethane (6ml). The mixture was stirred at 50°C for 18 hours, then cooled to room temperature. $NaBH(OAc)_3$ (0.0029 mol) was added portionwise. The mixture was stirred at room temperature for 2 hours. Water was added. The mixture was extracted with DCM and filtered over celite. The organic layer was separated, dried ($MgSO_4$), filtered, and the solvent was evaporated till dryness. The residue (1.1g) was crystallized from diethyl ether/DIPE. The precipitate was filtered off and dried, yielding 0.49g (51%) of intermediate 11.

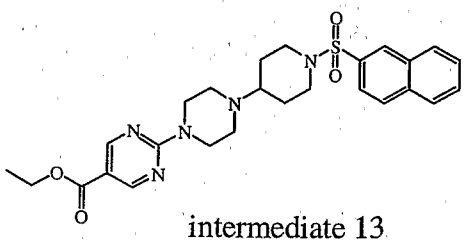
d) Preparation of



.HCl
intermediate 12

A mixture of intermediate 11 (0.0011 mol) in HCl/iPrOH (5ml) was stirred at 50°C for 1 hour, then cooled to room temperature. The precipitate was filtered, washed with EtOH, then with diethyl ether and dried, yielding 0.38g (94%) of intermediate 12 (HCl salt).

e) Preparation of

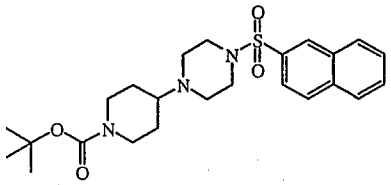


intermediate 13

A solution of 2-naphthalenesulfonyl chloride (0.0011 mol) in DCM (1ml) was added at 5°C to a mixture of intermediate 12 (0.001 mol) and TEA (0.0032 mol) in DCM (4ml) under N_2 flow. The mixture was stirred at room temperature overnight. Potassium carbonate 10% was added. The mixture was extracted with DCM. The organic layer was separated, dried ($MgSO_4$), filtered, and the solvent was evaporated till dryness. The residue (0.53g) was taken up in diethyl ether/DIPE. The precipitate was filtered off and dried yielding 0.42g (77%) of intermediate 13.

Example A4

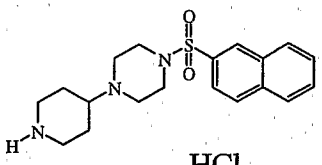
a) Preparation of



intermediate 14

Tetrakis (2-propanolato) titanium (0.0023 mol) was added to a mixture of 1-(2-naphthalenylsulfonyl)- piperazine (0.0018 mol) and 4-oxo-1-piperidinecarboxylic acid, 1,1-dimethylethyl ester (0.0021 mol) in 1,2-dichloro- ethane (6ml) under N_2 flow. The mixture was stirred at 50°C for 18 hours, then cooled to room temperature. $NaBH(OAc)_3$ (0.0023 mol) was added portionwise. The mixture was stirred at room temperature for 4 hours. Water was added. The mixture was filtered over celite. The mixture was extracted with DCM. The filtrate was decanted. The organic layer was separated, dried ($MgSO_4$), filtered, and the solvent was evaporated till dryness. The residue (1.3g) was purified by column chromatography over silica gel (70-200μm) (eluent: DCM 100 to DCM/MeOH 99/1). The pure fractions were collected and the solvent was evaporated, yielding 0.72g (86%) of intermediate 14.

b) Preparation of



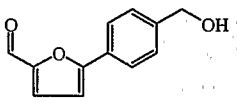
.HCl

intermediate 15

A mixture of intermediate 14 (0.0014 mol) in HCl/iPrOH 5N (10ml) was stirred at 50°C for 18 hours, then cooled to room temperature, filtered, washed with diethyl ether and dried, yielding 0.53g (95%) of intermediate 15 (HCl salt), melting point 260°C.

Example A5

Preparation of



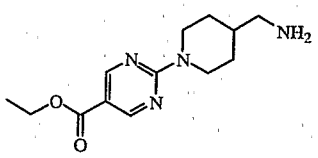
intermediate 16

A solution of 5-bromo-2-furancarboxaldehyde (0.0171 mol) in 1,2-dimethoxyethane (15ml) was added dropwise at room temperature to a solution of tetrakis(triphenylphosphine)- palladium (0.0045 mol) in 1,2-dimethoxyethane (50ml) under N_2 flow. The mixture was stirred for 20 minutes. A suspension of [4-(hydroxymethyl)phenyl]- boronic acid (0.0257 mol) in EtOH (18ml) was added. The

mixture was stirred for 20 minutes. Sodiumcarbonate (0.15 mol) was added. The mixture was stirred and refluxed for 4 hours, then brought to room temperature. The organic layer was evaproated. The residue was taken up in DCM and washed with water. The organic layer was separated, dried ($MgSO_4$), filtered, and the solvent was evaporated. The residue (4.1g) was purified by column chromatography over silica gel (15-40μm) (eluent: DCM/MeOH 99/1). The pure fractions were collected and the solvent was evaporated, yielding 2.8g (82%) of intermediate 16.

Example A6

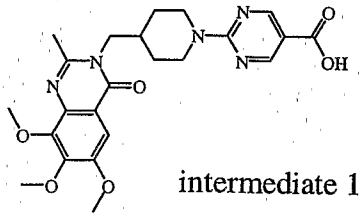
a) Preparation of



intermediate 17

A solution of 2-(methylsulfonyl)- 5-pyrimidinecarboxylic acid, ethyl ester (0.0434 mol) in acetonitrile (100ml) was added dropwise at 10°c to a solution of 4-piperidinemethanamine (0.0868 mol) and potassium carbonate (0.0434 mol) in acetonitrile (200ml) under N_2 flow. The mixture was stirred at room temperature for 2 hours, poured out into ice water and extracted with DCM. The organic layer was separated, dried ($MgSO_4$), filtered, and the solvent was evaporated. The residue (14.18g) was purified by column chromatography over silica gel (20-45μm) (eluent: DCM/MeOH/NH_4OH 90/10/1 to 80/20/2). The pure fractions were collected and the solvent was evaporated, yielding 3.7g (32%) of intermediate 17.

b) Preparation of

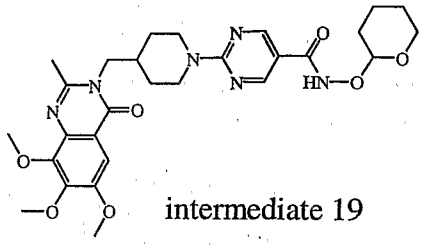


intermediate 18

To *N*-cyclohexylcarbodiimide, *N'*-methyl polystyrene (153mg) (NovaBiochem Cat. Nr. 01-64-0211) was added *N*-acetyl-3,4,5-trimethoxy- anthranilic acid(0.2 mmol), dissolved in THF (0.5ml), followed by 1-hydroxy-1*H*-benzotriazole, dissolved in THF (0.5ml). This mixture was shaken for 10 minutes at room temperature. Subsequently, intermediate 17 (0.1 mmol), dissolved in DCM (1ml) was added to the suspension after which the solvents were evaporated by means of a nitrogen flow at 50°C and subsequently, the neat reaction mixture was left overnight at 90°C under a flow of nitrogen. The product was dissolved in dichloromethane (2 mL) and filtered through a glass filter. The residue was rinsed with DCM (2 x 2ml) and the filtrate was

concentrated. The product was then treated with a mixture of THF(1ml) and aqueous sodium hydroxide (1ml, 1N) for 48 hours at room temperature. Finally, the mixture was neutralized by the addition of aqueous HCl (1ml, 1N), and dried at 70°C under a nitrogen flow, yielding intermediate 18.

c) Preparation of

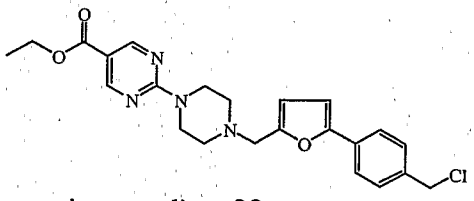


intermediate 19

To product intermediate 18 (0.1 mmol) was added a solution of 1-hydroxy-1*H*-benzotriazole (0.1 mmol), EDC (0.1 mmol) and TEA (0.12 mmol) in a mixture of DCM/THF (3/4, 7ml). The reaction mixture was shaken for 5 minutes at room temperature, after which O-(tetrahydro-2H-pyran-2-yl)- hydroxylamine (0.1 mmol) was added. The resulting solution was allowed to shake overnight at room temperature. The reaction mixture was then concentrated under a nitrogen flow at 50 °C to a volume of 5ml and then polymer supported methyl isocyanate (1.25 mmol/g, 0.25 mmol, Argonaut, Cat. Nr. 800261) and (polystyrylmethyl)trimethylammonium bicarbonate, (0.6 mmol) (NovaBiochem Cat. Nr. 01-64-0419) were added and this mixture was shaken for an additional 2 hours. The reaction mixture was filtered and the filtrate concentrated. Preparative HPLC purification of the product, yielded 29 mg of intermediate 19.

Example A7

Preparation of

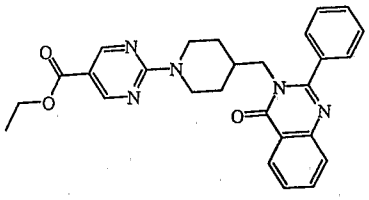


intermediate 20

Methanesulfonyl chloride (0.006 mol) was added at 0°C to a solution of intermediate 27 (0.001 mol) and TEA (0.008 mol) in DCM (10ml) under N_2 flow. The mixture was stirred at 0°C for 3 hours, poured out into ice water and extracted with DCM. The organic layer was separated, dried ($MgSO_4$), filtered, and the solvent was evaporated, yielding 0.44g of intermediate 20. This product was used directly in the next reaction step.

Example A8

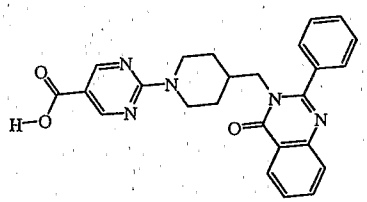
a) Preparation of



intermediate 21

A sample of intermediate (0.1 mmol) and 2-phenyl-4*H*-3,1-benzoxazin-4-one (0.1 mmol) were weighed into a vessel and toluene (2ml) was added. The vessel was sealed and the mixture heated for 10 hours at 90°C. Next, the solvent was evaporated and the residue was taken up in DCM (4ml), followed by addition of ethanedioyl dichloride (1 mmol) and DMF (1 mmol). The resulting mixture was shaken overnight at 90°C. The reaction mixture was then washed with a 10% aqueous sodium bicarbonate solution (2 X 1ml), the phases were separated and the organic layer was concentrated, yielding intermediate 21.

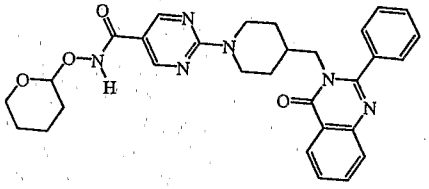
b) Preparation of



intermediate 22

Intermediate 21(crude) was treated with a mixture of THF/1N aq. NaOH/MeOH (1/1/0.2, 2.2ml) while stirred vigorously overnight at room temperature. Next, an aqueous HCl (1N, 1ml) was added to neutralize the solution. The solvents were evaporated in vacuo, yielding intermediate 22.

c) Preparation of



intermediate 23

To intermediate 22 was subsequently added 1-hydroxy-1*H*-benzotriazole (0.13 mmol), dissolved in THF (dry, 1ml), EDC (0.13 mmol), dissolved in DCM (dry, 1ml), and TEA (0.15 mmol). The mixture was stirred at room temperature for 10 minutes. O-(tetrahydro-2H-pyran-2-yl)- hydroxylamine (0.13 mmol), dissolved in THF (dry 1ml) was added and the reaction mixture was stirred at room temperature overnight. The

solvents were evaporated and the product was purified using reverse phase-HPLC, yielding intermediate 23, melting point (219°C).

B. Preparation of final compounds

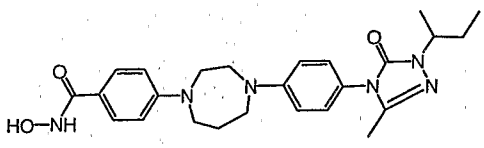
Example B1

N-Fmoc-hydroxylamine 2-chlorotrityl resin (Novabiochem, 01-64-0165) was deprotected by 50% piperidine in DMF (RT, 24 hr). The resin was washed several times with DCM and DMF and swelled in DMF. Two equivalents of acid[1], PyBrOP and 4 equivalents of DIEA were added as one portion. The mixture was shaken for 24 hr, liquid was drained and the resin was washed several times by DCM and DMF. The resin was swelled in DMF containing 2 equivalents of amine, was shaken 24 hr at RT, the liquid was drained and the resin was washed by DCM and DMF. The final product was cleaved by 5% TFA in DCM, analyzed by HPLC and MS and evaporated in the pre-weighted test-tubes.

[1]. Based on the loading of the resin.

Example B2

Preparation of



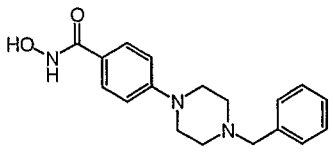
.H_2O

Compound 1

A mixture of interm. 7 (0.00075 mol), NH_2OH/H_2O 50% (5 ml), KCN (0.001 mol), MeOH (10 ml) and THF (10 ml) was stirred at room temperature for 48 hours. The solvent was evaporated. The residue was extracted with DCM and was washed with water. The solvent was removed and the oily residue (0.225 g) was purified by column chromatography over silica gel (eluent: $NH_4OH/CH_3OH/CH_2Cl_2$ 0.05/0.95/99; then with $NH_4OH/CH_3OH/CH_2Cl_2$ 0.1/0.9/99). The pure fractions were collected and the solvent was evaporated, yielding 0.114 g of compound 1 .H_2O, melting point 182-184°C and 0.040 g of 4-[4-[4-[1,5-dihydro-3-methyl-1-(1-methylpropyl)-5-oxo-4*H*-1,2,4-triazol-4-yl]phenyl]hexahydro-1*H*-1,4-diazepin-1-yl]- benzoic acid, melting point 244-245°C.

Example B3

Preparation of

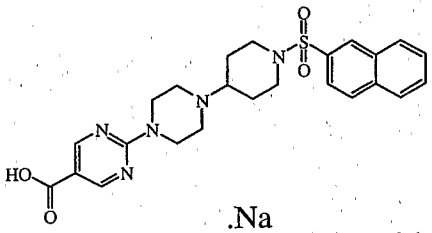


Compound 2

Interm. 8 (0.000088 mol) was stirred in 5% TFA/MeOH (5 ml) for 24 hours at 25°C. Then, the reaction mixture was poured out into water (5 ml) + 1 equiv of $NaHCO_3$. This mixture was extracted with DCM (5 ml, 2 x). The separated organic layer was dried ($MgSO_4$), filtered and the solvent evaporated (blown dry under N_2 flow at 50°C). The residue was dried (vacuum, 50°C), yielding 0.018 g (65%) of compound 2, melting point 196°C.

Example B4

a) Preparation of

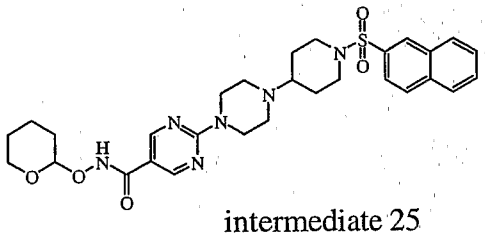


.Na

intermediate 24

A mixture of intermediate 13 (0.0008 mol) and sodium hydroxide (0.0016 mol) in EtOH (10ml) was stirred and refluxed for 2 hours, then cooled to room temperature. The precipitate was filtered, washed with EtOH, then with diethyl ether and dried, yielding 0.425g (>100%) of intermediate 24 (sodium salt).

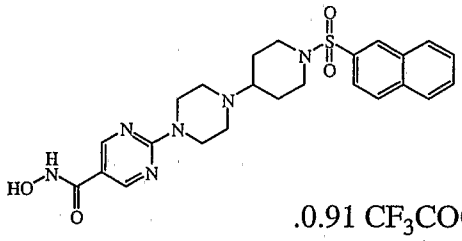
b) Preparation of



intermediate 25

EDC (0.001 mol) was added to a mixture of intermediate 24 (0.0008 mol), O-(tetrahydro-2H-pyran-2-yl)- hydroxylamine (0.001 mol) and 1-hydroxy-1*H*-benzotriazole (0.001 mol) in DCM/THF (10ml) under N_2 flow. The mixture was stirred at room temperature for 18 hours. The precipitate was filtered, washed with THF, then with diethyl ether and dried, yielding 0.4g (83%) of intermediate 25, melting point 260°C.

c) Preparation of

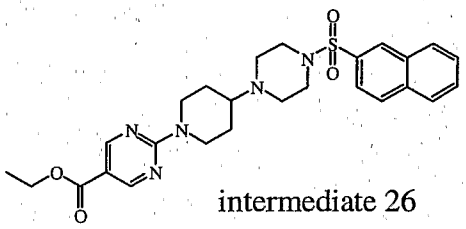


.0.91 CF_3COOH
compound 3

TFA(0.5ml) was added to a mixture of intermediate 25 (0.0006 mol) in MeOH (10ml). The mixture was stirred at room temperature for 5 days. The precipitate was filtered, washed with diethyl ether and dried.. The residue was taken up in water, stirred for 30 minutes, filtered off and dried, yielding 0.143g (38%) of compound 3 (.0.91 CF_3COOH), melting point 210°C.

Example B5

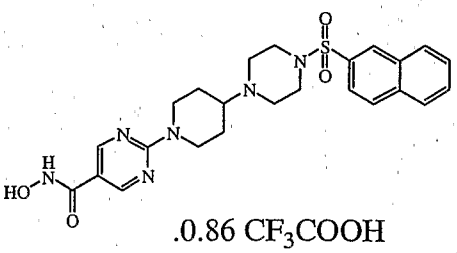
Preparation of



intermediate 26

A mixture of intermediate 15 (0.0013 mol), 2-(methylsulfonyl)- 5-pyrimidinecarboxylic acid, ethyl ester (0.0017 mol) and potassium carbonate (0.0039 mol) in acetonitrile (10ml) was stirred at 80°C for 18 hours. Water was added. The mixture was extracted with DCM. The organic layer was separated, dried ($MgSO_4$), filtered and the solvent was evaporated till dryness. The residue (0.5g) was crystallized from CH_3CN/DIPE. The precipitate was filtered off and dried, yielding 0.25g (37%) of intermediate 26.

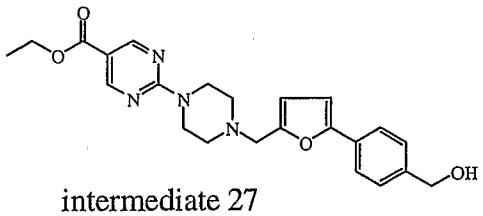
Intermediate 26 was handled analogously as described in example [B4] to give 0.126g (74%) of compound 4 (.0.86 CF_3COOH), melting point 230°C.



.0.86 CF_3COOH
compound 4

Example B6

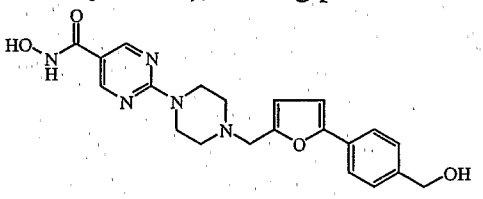
Preparation of



intermediate 27

A mixture of intermediate 10 (0.0015 mol) and intermediate 16 (0.0015 mol) in MeOH (6ml) was stirred at 60°C for 20 hours, then cooled to 0°C. Sodium hydroborate (0.0022 mol) was added. The mixture was brought to room temperature, then stirred for 4 hours, poured out into ice water and extracted with DCM. The organic layer was separated, dried ($MgSO_4$), filtered, and the solvent was evaporated. The residue (0.8g) was purified by column chromatography over silica gel (15-40μm) (eluent: DCM/MeOH/NH_4OH 97/3/0.1 to 95/5/0.1). The pure fractions were collected and the solvent was evaporated, yielding 0.28g (45%) of intermediate 27.

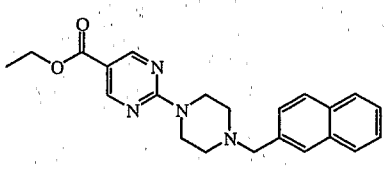
Intermediate 27 was handled analogously as described in example [B4] to give 0.143g (89%) of compound 5 (.0.83 CF_3COOH), melting point 219°C.



. 0.83 CF_3COOH
compound 5

Example B7

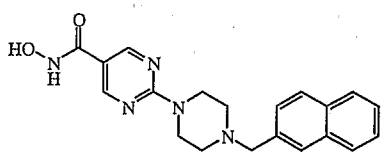
Preparation of



intermediate 28

A mixture of intermediate 10 (0.0042 mol) and 2-naphthalenecarboxaldehyde (0.005 mol) in 1,2-dichloro-ethane (10ml) was stirred at 50°C for 3 hours, then cooled to room temperature. $NaBH(OAc)_3$ (0.0055 mol) was added portionwise. The mixture was stirred at room temperature for 2 hours. Water was added. The mixture was extracted with DCM. The organic layer was separated, dried ($MgSO_4$), filtered, and the solvent was evaporated till dryness. The residue (2g) was taken up in CH_3CN/DIPE. The precipitate was filtered off and dried, yielding 1.2g (75%) of intermediate 28, melting point 147°C.

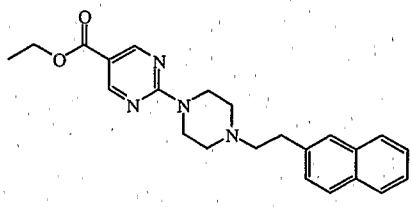
Intermediate 28 was handled analogously as described in example [B4] to give 0.724g (88%) of compound 6 (.0.83 CF_3COOH) , melting point > 260°C.



.0.83 CF_3COOH
compound 6

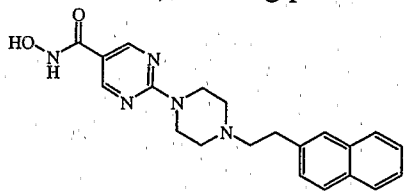
Example B8

Preparation of



intermediate 29

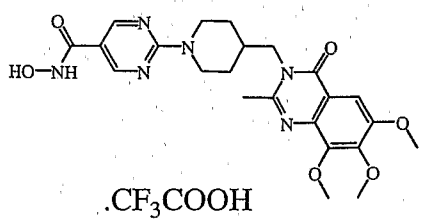
A solution of 2-naphthaleneethanol, methanesulfonate (0.0029 mol) in acetonitrile (3ml) was added at 5°C to a mixture of intermediate 10 (0.0024 mol) and potassium carbonate (0.0048 mol) in acetonitrile (6ml). The mixture was stirred at room temperature for 18 hours, then stirred and refluxed overnight and cooled to room temperature. Water was added. The mixture was filtered, washed with water, then with diethyl ether and dried, yielding 0.45g (48%) of intermediate 29, melting point 128°C. Intermediate 29 was handled analogously as described in example [B4] to give 0.254g (86%) of compound 7 (.0.79 CF_3COOH), melting point 209°C.



.0.79 CF_3COOH
compound 7

Example B9

Preparation of



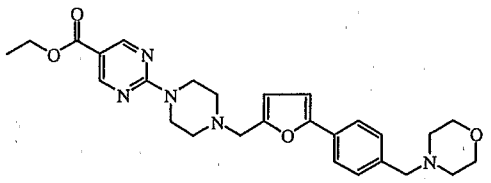
.CF_3COOH
compound 8

Intermediate 19 (0.05 mmol) was treated with TFA (2ml , in DCM/MeOH 1/1) for 10 days at room temperature. Next, the solvents were evaporated at room temperature

under a flow of nitrogen, followed by addition of 1,4-dioxane and repeating the evaporation procedure. Then the sample was dried under a nitrogen flow overnight at 40°C, yielding compound 8 (.CF_3COOH).

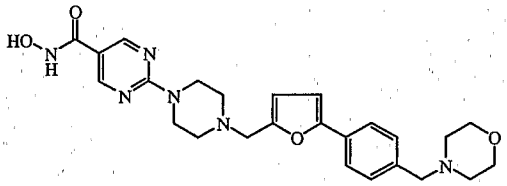
Example B10

Preparation of



intermediate 30

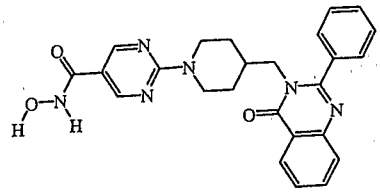
A mixture of intermediate 20 (0.001 mol), morpholine (0.0013 mol) and potassium carbonate (0.002 mol) in acetonitrile (6ml) was stirred and refluxed overnight, then cooled, poured out into ice water and extracted with EtOAc. The organic layer was separated, dried ($MgSO_4$), filtered, and the solvent was evaporated. The residue (0.79g) was purified by column chromatography over silica gel (10μm) (eluent: DCM/MeOH/NH_4OH 96/4/0.1). The pure fractions were collected and the solvent was evaporated. The residue (0.183g) was crystallized from DIPE. The precipitate was filtered off and dried, yielding 0.086g (47%) of intermediate 30, melting point 120°C. Intermediate 30 was handled analogously as described in example [B4] to give 0.432g (87%) of compound 9 (.0.47 H_2O .1.99 CF_3COOH), melting point 140°C .



compound 9
.0.47 H_2O
.1.99 CF_3COOH

Example B11

Preparation of

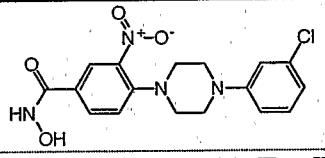
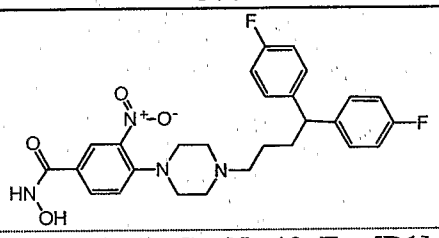
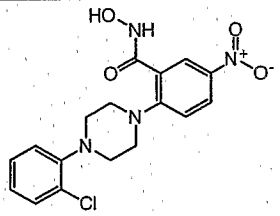
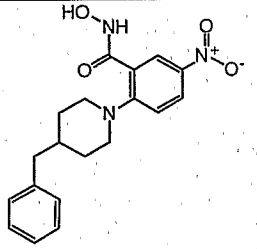
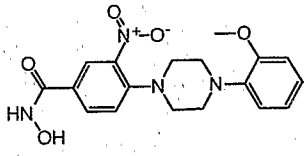
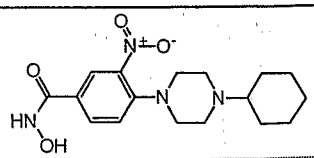
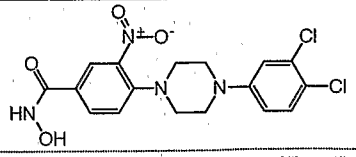


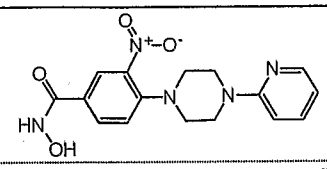
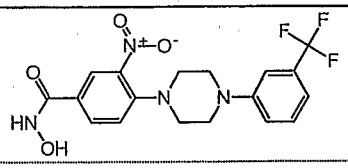
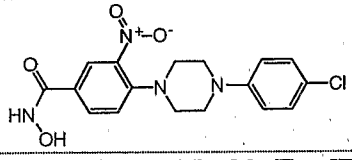
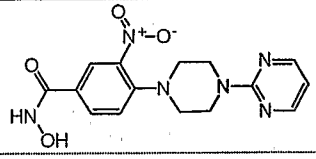
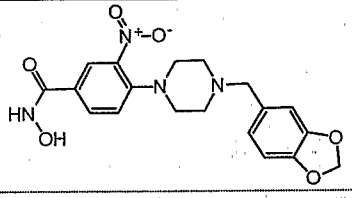
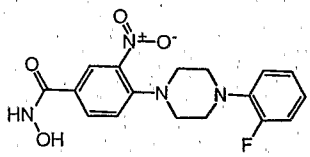
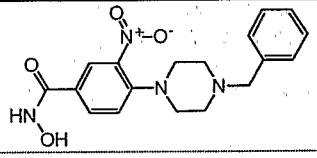
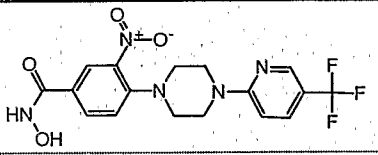
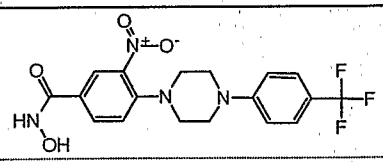
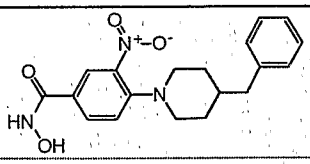
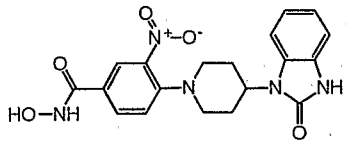
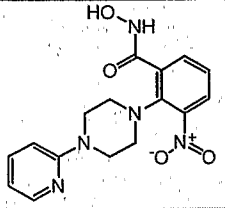
compound 10
.CF_3COOH

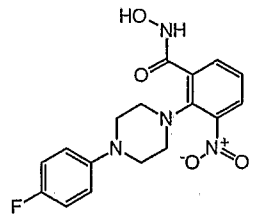
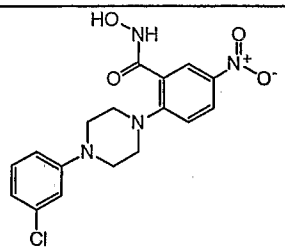
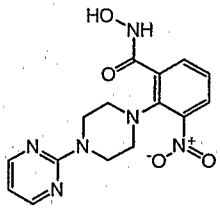
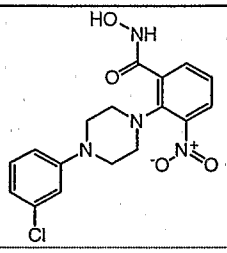
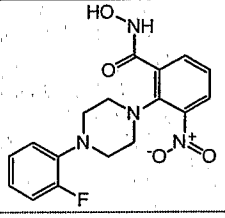
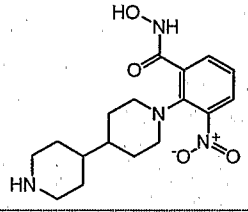
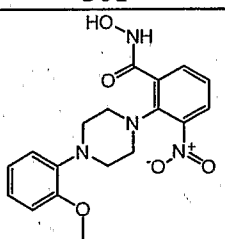
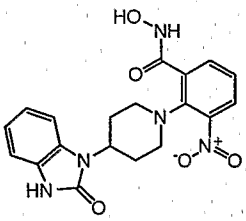
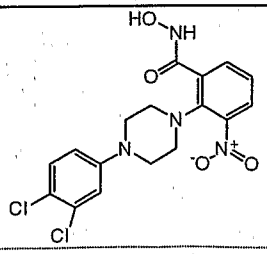
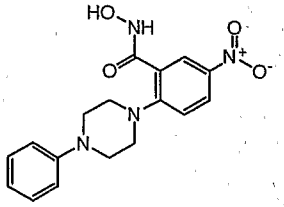
A solution of TFA (5% in DCM/MeOH 1/1, 2ml) was added to intermediate 23 and the reaction mixture was stirred for 5 days at room temperature. Next, the solvents were evaporated at room temperature under a flow of nitrogen, followed by addition of 1,4-dioxane and the concentrating procedure was repeated. Then, the sample was dried under a nitrogen flow overnight at 40°C, yielding compound 10 (.CF_3COOH).

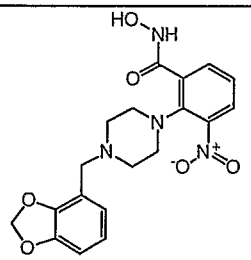
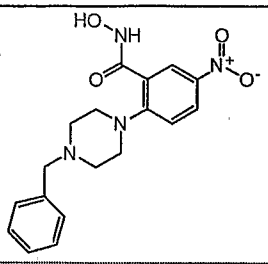
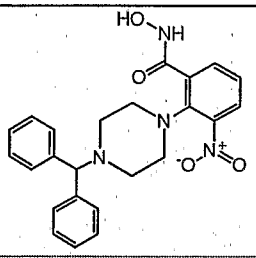
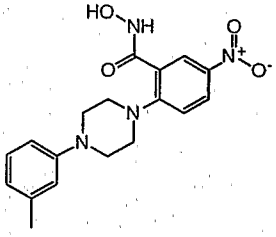
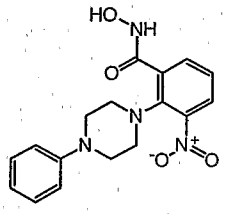
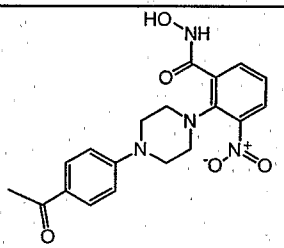
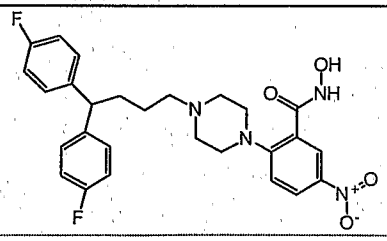
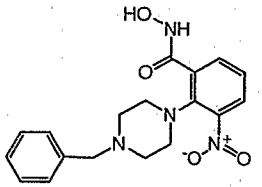
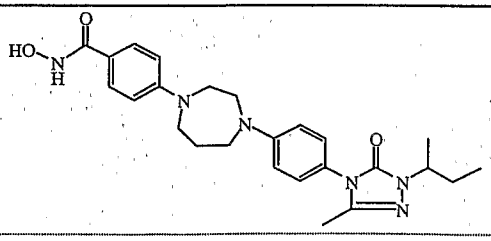
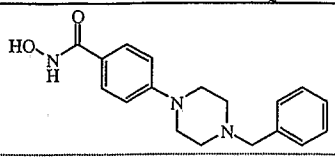
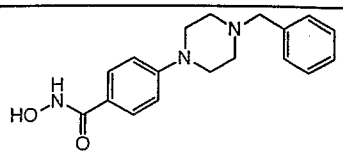
Table F-1 lists the compounds that were prepared according to one of the above Examples. The following abbreviations were used in the tables : .$C_2HF_3O_2$ stands for the trifluoroacetate salt.

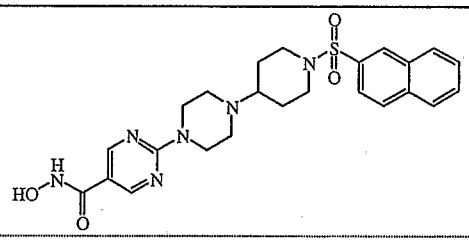
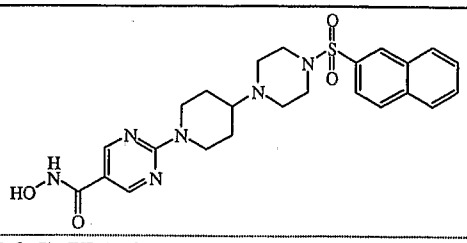
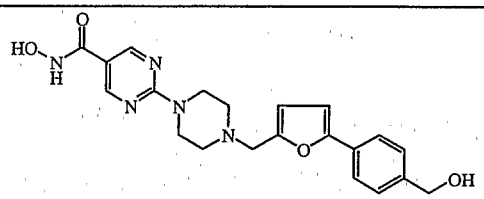
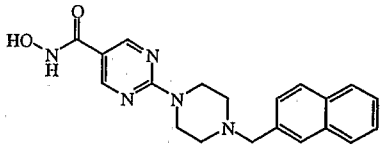
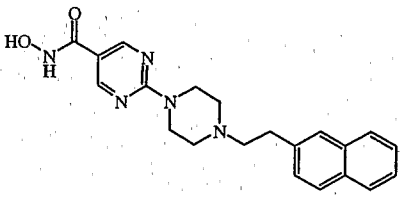
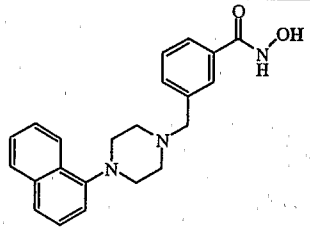
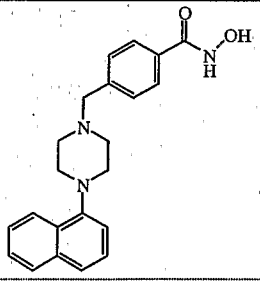
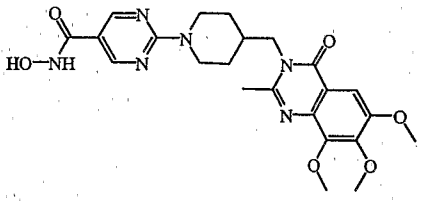
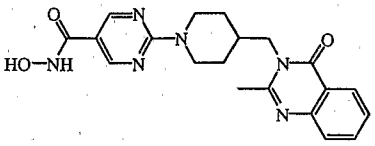
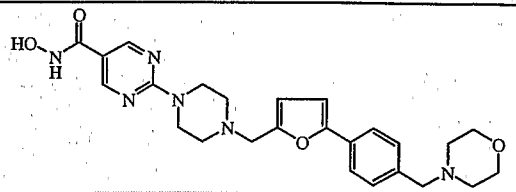
Table F-1

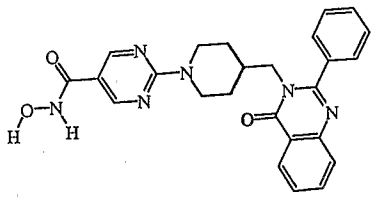
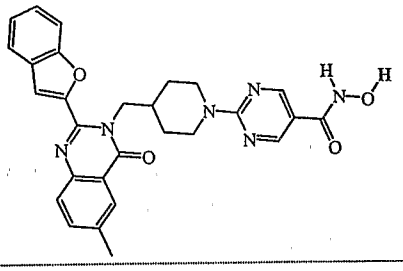
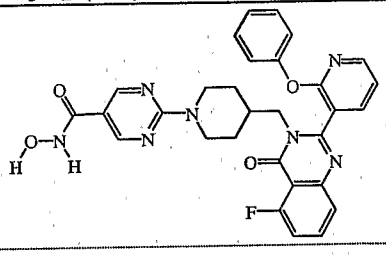
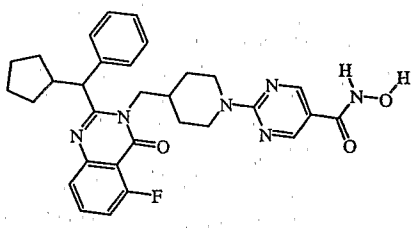
	
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.$C_2HF_3O_2$ (1:1), Co. No.14; Ex. [B1]; ms. 356	.$C_2HF_3O_2$ (1:2), Co. No.15; Ex. [B1]; ms. 373
	
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.$C_2HF_3O_2$ (1:2), Co. No.20; Ex. [B1]; ms. 378	.$C_2HF_3O_2$ (1:2), Co. No.21; Ex. [B1]; ms. 345
	
.$C_2HF_3O_2$ (1:2), Co. No. 22; Ex. [B1]; ms. 401	.$C_2HF_3O_2$ (1:2), Co. No. 23; Ex. [B1]; ms. 361
	
.$C_2HF_3O_2$ (1:2), Co. No.24; Ex. [B1]; ms. 357	.$C_2HF_3O_2$ (1:2), Co. No.25; Ex. [B1]; ms. 412
	
.$C_2HF_3O_2$ (1:2), Co. No.26; Ex. [B1]; ms. 411	.$C_2HF_3O_2$ (1:1), Co. No.27; Ex. [B1]; ms. 356
	
.$C_2HF_3O_2$ (1:1), Co. No.28; Ex. [B1]; ms. 397	.$C_2HF_3O_2$ (1:2), Co. No.29; Ex. [B1]; ms. 344

	
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.$C_2HF_3O_2$ (1:2), Co. No.32; Ex. [B1]; ms. 345	.$C_2HF_3O_2$ (1:2), Co. No.33; Ex. [B1]; ms. 377
	
.$C_2HF_3O_2$ (1:2), Co. No.34; Ex. [B1]; ms. 361	.$C_2HF_3O_2$ (1:2), Co. No.35; Ex. [B1]; ms. 349
	
.$C_2HF_3O_2$ (1:2), Co. No.36; Ex. [B1]; ms. 373	.$C_2HF_3O_2$ (1:1), Co. No.37; Ex. [B1]; ms. 398
	
.$C_2HF_3O_2$ (1:2), Co. No.38; Ex. [B1]; ms. 411	.$C_2HF_3O_2$ (1:2), Co. No.39; Ex. [B1]

	
.$C_2HF_3O_2$ (1:2), Co. No.40; Ex. [B1]; ms. 401	.$C_2HF_3O_2$ (1:2), Co. No.41; Ex. [B1]
	
.$C_2HF_3O_2$ (1:2), Co. No.42; Ex. [B1]; ms. 433	$C_2HF_3O_2$ (1:2), Co. No.43; Ex. [B1]
	
$C_2HF_3O_2$ (1:2), Co. No.44; Ex. [B1]; ms. 343	$C_2HF_3O_2$ (1:2), Co. No.45; Ex. [B1]; ms. 385
	
$C_2HF_3O_2$ (1:2), Co. No.46; Ex. [B1]	$C_2HF_3O_2$ (1:2), Co. No.47; Ex. [B1]; ms. 357
	
. H2O Co. No. 1; Ex. [B2]; mp. 182-184°C	
	
Co. No. 2; Ex. [B3]; mp. 196°C	.$C_2HF_3O_2$ (1:1) , Co. No.48; Ex. [B3]

	
0.91 $C_2HF_3O_2$; Co. No. 3; Ex. [B4]; mp. 210°C	0.86 $C_2HF_3O_2$; Co. No. 4; Ex. [B5]; mp. 230°C
	
0.83 $C_2HF_3O_2$;Co. No. 5; Ex. [B6]; mp. 219°C	0.83 $C_2HF_3O_2$;Co. No. 6; Ex. [B7]; mp. >260°C
	
0.79 $C_2HF_3O_2$; Co. No. 7; Ex. [B8]; mp. 209°C	$C_2HF_3O_2$ (1:1); Co. No.49; Ex. [B3]
	
$C_2HF_3O_2$ (1:1); Co. No.50; Ex. [B3]	Co. No. 8; Ex. [B9]
	
.$C_2HF_3O_2$ (1:1); Co. No.51; Ex. [B9]	0.47 H_2O .1.99 $C_2HF_3O_2$; Co. No. 9; Ex. [B10]

	
$C_2HF_3O_2$ (1:1); Co. No. 10; Ex. [B11]	$C_2HF_3O_2$ (1:1); Co. No.52; Ex. [B11]
	
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C. Pharmacological example:

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).

Kinetic solubility in aqueous media measures the ability of a compound to stay in aqueous solution upon dilution (see example C.3).
DMSO-stock solutions are diluted with a single aqueous buffer solvent in 3 consecutive steps. For every dilution turbidity is measured with a nephelometer.

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.

The tumour suppressor p53 transcriptionally activates a number of genes including the WAF1/CIP1 gene in response to DNA damage. The 21 kDa product of the WAF1 gene is found in a complex involving cyclins, cyclin dependent kinases (CDKs), and proliferating cell nuclear antigen (PCNA) in normal cells but not transformed cells and appears to be a universal inhibitor of CDK activity. One consequence of p21WAF1 binding to and inhibiting CDKs is to prevent CDK-dependent phosphorylation and subsequent inactivation of the Rb protein, which is essential for cell cycle progression. Induction of p21WAF1 in response to cellular contact with a HDAC inhibitor is therefore a potent and specific indicator of inhibition of cell cycle progression at both the G1 and G2 checkpoints.

The capacity of the compounds to induce p21WAF1 was measured with the p21WAF1 enzyme linked immunosorbent assay (WAF1 ELISA of Oncogene). The p21WAF1 assay is a "sandwich" enzyme immunoassay employing both mouse monoclonal and rabbit polyclonal antibodies. A rabbit polyclonal antibody, specific for the human WAF1 protein, has been immobilized onto the surface of the plastic wells provided in the kit. Any p21WAF present in the sample to be assayed will bind to the capture antibody. The biotinylated detector monoclonal antibody also recognizes human p21WAF1 protein, and will bind to any p21WAF1, which has been retained by the capture antibody. The detector antibody, in turn, is bond by horseradish peroxidase-

conjugated streptavidin. The horseradish peroxidase catalyses the conversion of the chromogenic substrate tetra-methylbenzidine from a colorless solution to a blue solution (or yellow after the addition of stopping reagent), the intensity of which is proportional to the amount of p21WAF1 protein bond to the plate. The colored reaction product is quantified using a spectrophotometer. Quantitation is achieved by the construction of a standard curve using known concentrations of p21WAF1 (provided lyophilised)(see example C.6).

Example C.1: *In Vitro* Assay for Inhibition of histone deacetylase :

HeLa nuclear extracts (supplier: Biomol) were incubated at 60 µg/ml with $2x10^{-8}$ M of radiolabeled peptide substrate. As a substrate for measuring HDAC activity a synthetic peptide, i.e. the amino acids 14-21 of histone H4, was used. The substrate is biotinylated at the NH_2-terminal part with a 6-aminohexanoic acid spacer, and is protected at the COOH-terminal part by an amide group and specifically [^{3}H]acetylated at lysine 16. The substrate, biotin-(6-aminohexanoic)Gly-Ala-([^{3}H]-acetyl-Lys-Arg-His-Arg-Lys-Val-NH_2), was added in a buffer containing 25 mM Hepes, 1 M sucrose, 0.1 mg/ml BSA and 0.01% Triton X-100 at pH 7.4. After 30 min the deacetylation reaction was terminated by the addition of HCl and acetic acid. (final concentration 0.035 mM and 3.8 mM respectively). After stopping the reaction, the free ^{3}H-acetate was extracted with ethylacetate. After mixing and centrifugation, the radioactivity in an aliquot of the upper (organic) phase was counted in a β-counter.
For each experiment, controls (containing HeLa nuclear extract and DMSO without compound), a blank incubation (containing DMSO but no HeLa nuclear extract or compound) and samples (containing compound dissolved in DMSO 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^{-12}M. 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 radioactivity 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). All tested compounds showed enzymatic activity at a test concentration of 10^{-5}M and 28 compounds had a $pIC_{50} \geq 5$ (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). Most of the tested compounds showed cellular activity at a test concentration of 10^{-6} M and 9 compounds had a $pIC_{50} \geq 5$ (see table F-2)

Example C.3: Kinetic solubility in aqueous media

In the first dilution step, 10 µl of a concentrated stock-solution of the active compound, solubilized in DMSO (5mM), was added to 100 µl phosphate citrate buffer pH 7.4 and mixed. In the second dilution step, an aliquot (20 µl) of the first dilution step was further dispensed in 100 µl phosphate citrate buffer pH 7.4 and mixed. Finally, in the third dilution step, a sample (20 µl) of the second dilution step was further diluted in 100 µl phosphate citrate buffer pH 7.4 and mixed. All dilutions were performed in 96-well plates. Immediately after the last dilution step the turbidity of the three consecutive dilution steps were measured with a nephelometer. Dilution was done in triplicate for each compound to exclude occasional errors. Based on the turbidity measurements a ranking is performed into 3 classes. Compounds with high solubility obtained a score of 3 and for this compounds the first dilution is clear. Compounds with medium solubility obtained a score of 2. For these compounds the first dilution is unclear and the second dilution is clear. Compounds with low solubility obtained a score of 1 and for these compounds both the first and the second dilution are unclear. The solubility of 6 compounds was measured. From these compounds 3 showed a score of 3, 2 obtained a score of 2 and 1 demonstrated a score of 1 (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 $< 0.5 \times 10^{-6}$ cm/s; score 1), a medium permeability (1×10^{-6} cm/s > mean effect $\geq 0.5 \times 10^{-6}$ cm/s; score 2) or a high permeability ($\geq 0.5 \times 10^{-6}$ cm/s; score 3). Two compounds were tested and showed at least a score of 2 at one of the pH's measured.

Example C.5: Metabolic stability

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 % - $\left(\left(\frac{\text{Total Ion Current (TIC) of E(act) at t=15}}{\text{TIC of E(act) at t=0}}\right) \times 100\right)$. Compounds that had a percentage metabolism less than 20 % were defined as highly metabolic stable. Compound that had a metabolism between 20 and 70 % were defined as intermediately stable and compounds that showed a percentage metabolism higher than 70 were defined as low metabolic stable. 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 percentage metabolism less than 20 % and two had a metabolism between 20 and 70 %.

Example C.6: p21 induction capacity

The following protocol has been applied to determine the p21 protein expression level in human A2780 ovarian carcinoma cells. The A2780 cells (20000 cells /180 µl) were seeded in 96 microwell plates in RPMI 1640 medium supplemented with 2 mM L-glutamine, 50 µg/ml gentamicin and 10 % fetal calf serum. 24 hours before the lysis of the cells, compounds were added at final concentrations of 10^{-5}, 10^{-6}, 10^{-7} and 10^{-8} M. All compounds tested were dissolved in DMSO and further dilutions were made in culture medium. 24 hours after the addition of the compound, the supernatants were removed from the cells. Cells were washed with 200 µl ice-cold PBS. The wells were aspirated and 30 µl of lysisbuffer (50 mM Tris.HCl (pH 7.6), 150 mM NaCl, 1 % Nonidet p40 and 10 % glycerol) was added. The plates were incubated overnight at –70 °C.
The appropriate number of microtiter wells were removed from the foil pouch and placed into an empty well holder. A working solution (1x) of the Wash Buffer (20x plate wash concentrate: 100 ml 20-fold concentrated solution of PBS and surfactant. Contains 2 % chloroacetamide) was prepared. The lyophilised p21WAF standard was reconstituted with distilled H_2O and further diluted with sample diluent (provided in the kit)
The samples were prepared by diluting them 1:4 in sample diluent. The samples (100 µl) and the p21WAF1 standards (100 µl) were pipetted into the appropriate wells and incubated at room temperature for 2 hours. The wells were washed 3 times with 1x wash buffer and then 100 µl of detector antibody reagent (a solution of biotinylated monoclonal p21WAF1 antibody) was pipetted into each well. The wells were incubated at room temperature for 1 hour and then washed three times with 1x wash buffer. The 400x conjugate (peroxidase streptavidine conjugate: 400-fold concentrated solution) was diluted and 100 µl of the 1x solution was added to the wells. The wells were incubated at room temperature for 30 min and then washed 3 times with 1x wash buffer and 1 time with distilled H_2O. Substrate solution (chromogenic substrate)(100 µl) was added to the wells and the wells were incubated for 30 minutes in the dark at room temperature. Stop solution was added to each well in the same order as the previously added substrate solution. The absorbance in each well was measured using a spectrophotometric plate reader at dual wavelengths of 450/595 nm.

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 p21WAF1 induction (in absorbance units) was expressed as the percentage of the value for p21WAF1 present in the control. Percentage induction higher than 130 % was defined as significant induction. Two compounds were tested in this assay, they both showed significant induction.

Table F-2: Table F-2 lists the results of the compounds that were tested according to example C.1, C.2, and C.3.

Co. No.	Enzyme activity pIC50	Cellular activity pIC50	Solubility Score
1	5.121	4.825	3
2	6.377	5.04	3
3	7.28	6.016	1
4	7.306	6.182	3
5	8.035	6.814	3
6	8.148	7.227	3
7	7.952	6.353	3
8	7.365	6.429	
9	7.889	7.009	3
10	7.427	6.182	
11	<5		
12	>5		
13	<5		
14	<5		
15	>5		
16	5.698		
17	>5		
18	>5		
19	>5		
20	<5		
21	5		
22	>5		
23	>5		
24	<5		
25	5		
26	>5		
27	<5		
28	>5		

Co. No.	Enzyme activity pIC50	Cellular activity pIC50	Solubility Score
48	6.343	5.367	3
49	<5		2
50	5.206		2
51	7.561	5.929	
52	7.235	5.93	
53	7.295		
54	6.176		

D. Composition example: Film-coated tablets

Preparation of tablet core

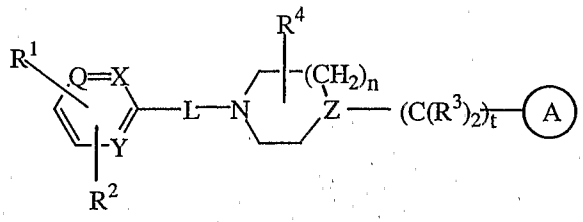
A mixture of 100 g of a compound of formula (I), 570 g lactose and 200 g starch is mixed well and thereafter humidified with a solution of 5 g sodium dodecyl sulphate and 10 g polyvinyl-pyrrolidone in about 200 ml of water. The wet powder mixture is sieved, dried and sieved again. Then there is added 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

n is 0, 1, 2 or 3 and when n is 0 then a direct bond is intended;

t is 0, 1, 2, 3 or 4 and when t is 0 then a direct bond is intended;

each Q is nitrogen or ;

each X is nitrogen or ;

each Y is nitrogen or ;

each Z is nitrogen or ;

R^1 is $-C(O)NR^7R^8$, $-NHC(O)R^9$, $-C(O)-C_{1-6}$alkanediylSR^9, $-NR^{10}C(O)N(OH)R^9$, $-NR^{10}C(O)C_{1-6}$alkanediylSR^9, $-NR^{10}C(O)C{=}N(OH)R^9$ or another Zn-chelating-group

wherein R^7 and R^8 are each independently selected from hydrogen, hydroxy, C_{1-6}alkyl, hydroxyC_{1-6}alkyl, aminoC_{1-6}alkyl or aminoaryl;

R^9 is independently selected from hydrogen, C_{1-6}alkyl, C_{1-6}alkylcarbonyl, arylC_{1-6}alkyl, C_{1-6}alkylpyrazinyl, pyridinone, pyrrolidinone or methylimidazolyl;

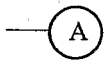
R^{10} is independently selected from hydrogen or C_{1-6}alkyl;

R^2 is hydrogen, halo, hydroxy, amino, nitro, C_{1-6}alkyl, C_{1-6}alkyloxy, trifluoromethyl, di(C_{1-6}alkyl)amino, hydroxyamino or naphtalenylsulfonylpyrazinyl;

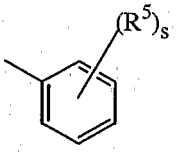
-L- is a direct bond or a bivalent radical selected from C_{1-6}alkanediyl, C_{1-6}alkanediyloxy, amino, carbonyl or aminocarbonyl;

each R^3 independently represents a hydrogen atom and one hydrogen atom can be replaced by a substituent selected from aryl;

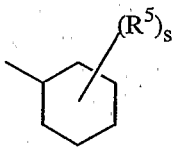
R^4 is hydrogen, hydroxy, amino, hydroxyC_{1-6}alkyl, C_{1-6}alkyl, C_{1-6}alkyloxy, arylC_{1-6}alkyl, aminocarbonyl, hydroxycarbonyl, aminoC_{1-6}alkyl, aminocarbonylC_{1-6}alkyl, hydroxycarbonylC_{1-6}alkyl, hydroxyaminocarbonyl, C_{1-6}alkyloxycarbonyl, C_{1-6}alkylaminoC_{1-6}alkyl or di(C_{1-6}alkyl)aminoC_{1-6}alkyl;



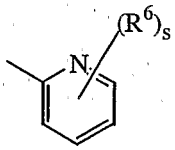
is a radical selected from



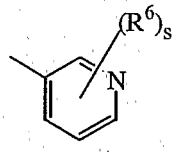
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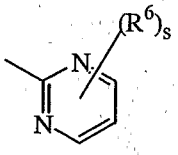
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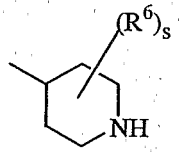
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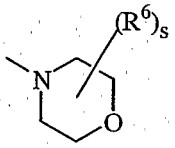
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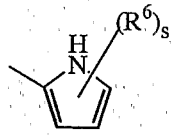
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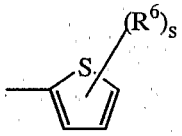
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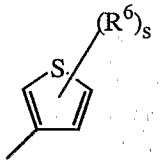
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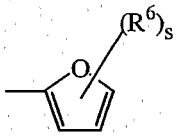
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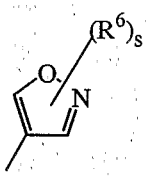
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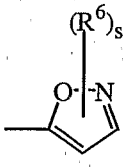
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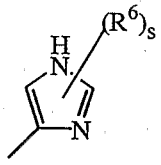
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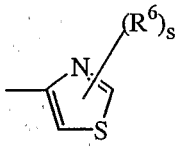
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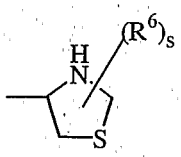
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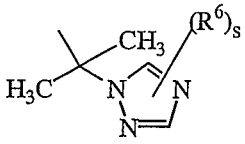
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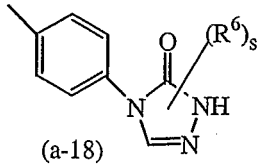
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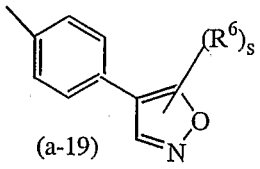
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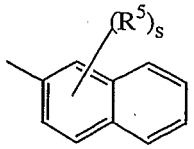
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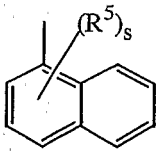
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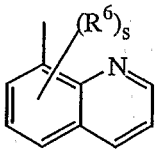
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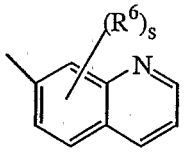
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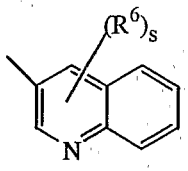
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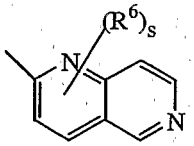
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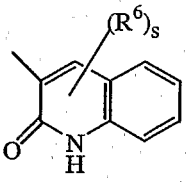
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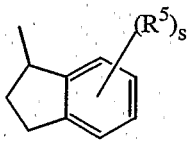
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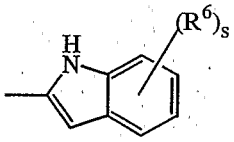
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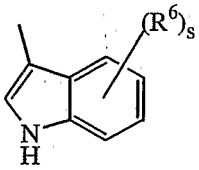
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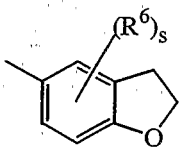
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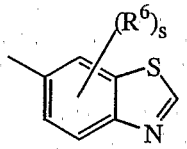
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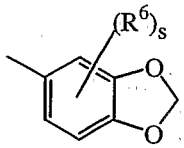
(a-29)



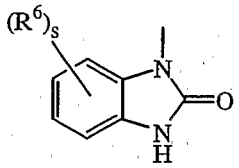
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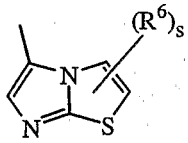
(a-31)



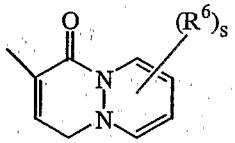
(a-32)



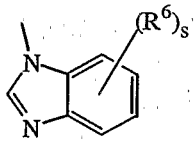
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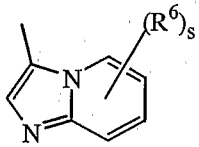
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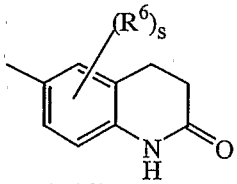
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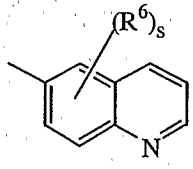
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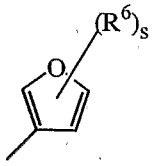
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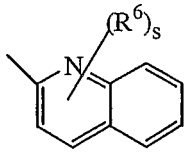
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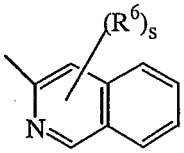
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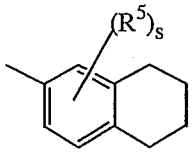
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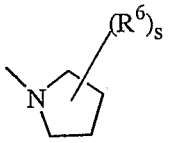
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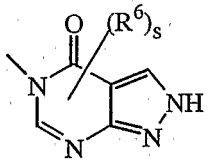
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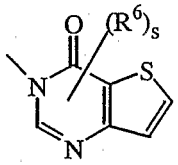
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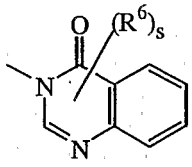
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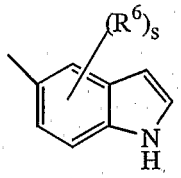
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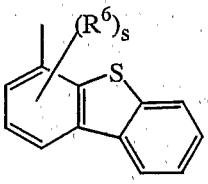
(a-46)



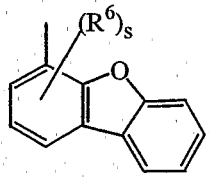
(a-47)



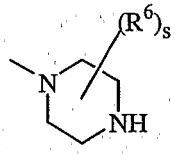
(a-48)



(a-49)



(a-50)



(a-51)

wherein each s is independently 0, 1, 2, 3, 4 or 5;

each R^5 and R^6 are independently selected from hydrogen; halo; hydroxy; amino; nitro; trihaloC_{1-6}alkyl; trihaloC_{1-6}alkyloxy; C_{1-6}alkyl; C_{1-6}alkyl substituted with aryl and C_{3-10}cycloalkyl; C_{1-6}alkyloxy; C_{1-6}alkyloxyC_{1-6}alkyloxy; C_{1-6}alkylcarbonyl; C_{1-6}alkyloxycarbonyl; C_{1-6}alkylsulfonyl; cyanoC_{1-6}alkyl; hydroxyC_{1-6}alkyl; hydroxyC_{1-6}alkyloxy; hydroxyC_{1-6}alkylamino; aminoC_{1-6}alkyloxy; di(C_{1-6}alkyl)aminocarbonyl; di(hydroxyC_{1-6}alkyl)amino; (aryl)(C_{1-6}alkyl)amino; di(C_{1-6}alkyl)aminoC_{1-6}alkyloxy; di(C_{1-6}alkyl)aminoC_{1-6}alkylamino; di(C_{1-6}alkyl)aminoC_{1-6}alkylaminoC_{1-6}alkyl; arylsulfonyl; arylsulfonylamino; aryloxy; aryloxyC_{1-6}alkyl; arylC_{2-6}alkenediyl; di(C_{1-6}alkyl)amino; di(C_{1-6}alkyl)aminoC_{1-6}alkyl; di(C_{1-6}alkyl)amino(C_{1-6}alkyl)amino; di(C_{1-6}alkyl)amino(C_{1-6}alkyl)aminoC_{1-6}alkyl; di(C_{1-6}alkyl)aminoC_{1-6}alkyl(C_{1-6}alkyl)amino; di(C_{1-6}alkyl)aminoC_{1-6}alkyl(C_{1-6}alkyl)aminoC_{1-6}alkyl; aminosulfonylamino(C_{1-6}alkyl)amino; aminosulfonylamino(C_{1-6}alkyl)aminoC_{1-6}alkyl; di(C_{1-6}alkyl)aminosulfonylamino(C_{1-6}alkyl)amino; di(C_{1-6}alkyl)aminosulfonylamino(C_{1-6}alkyl)aminoC_{1-6}alkyl; cyano; thiophenyl; thiophenyl substituted with di(C_{1-6}alkyl)aminoC_{1-6}alkyl(C_{1-6}alkyl)aminoC_{1-6}alkyl, di(C_{1-6}alkyl)aminoC_{1-6}alkyl, C_{1-6}alkylpiperazinylC_{1-6}alkyl, hydroxyC_{1-6}alkylpiperazinylC_{1-6}alkyl,

hydroxyC_{1-6}alkyloxyC_{1-6}alkylpiperazinylC_{1-6}alkyl, di(C_{1-6}alkyl)aminosulfonylpiperazinylC_{1-6}alkyl, C_{1-6}alkyloxypiperidinyl, C_{1-6}alkyloxypiperidinylC_{1-6}alkyl, morpholinylC_{1-6}alkyl, hydroxyC_{1-6}alkyl(C_{1-6}alkyl)aminoC_{1-6}alkyl, or di(hydroxyC_{1-6}alkyl)aminoC_{1-6}alkyl; furanyl; furanyl substituted with hydroxyC_{1-6}alkyl; benzofuranyl; imidazolyl; oxazolyl; oxazolyl substituted with aryl and C_{1-6}alkyl; C_{1-6}alkyltriazolyl; tetrazolyl; pyrrolidinyl; pyrrolyl; piperidinylC_{1-6}alkyloxy; morpholinyl; C_{1-6}alkylmorpholinyl; morpholinylC_{1-6}alkyloxy; morpholinylC_{1-6}alkyl; morpholinylC_{1-6}alkylamino; morpholinylC_{1-6}alkylaminoC_{1-6}alkyl; piperazinyl; C_{1-6}alkylpiperazinyl; C_{1-6}alkylpiperazinylC_{1-6}alkyloxy; piperazinylC_{1-6}alkyl; naphtalenylsulfonylpiperazinyl; naphtalenylsulfonylpiperidinyl; naphtalenylsulfonyl; C_{1-6}alkylpiperazinylC_{1-6}alkyl; C_{1-6}alkylpiperazinylC_{1-6}alkylamino; C_{1-6}alkylpiperazinylC_{1-6}alkylaminoC_{1-6}alkyl; C_{1-6}alkylpiperazinylsulfonyl; aminosulfonylpiperazinylC_{1-6}alkyloxy; aminosulfonylpiperazinyl; aminosulfonylpiperazinylC_{1-6}alkyl; di(C_{1-6}alkyl)aminosulfonylpiperazinyl; di(C_{1-6}alkyl)aminosulfonylpiperazinylC_{1-6}alkyl; hydroxyC_{1-6}alkylpiperazinyl; hydroxyC_{1-6}alkylpiperazinylC_{1-6}alkyl; C_{1-6}alkyloxypiperidinyl; C_{1-6}alkyloxypiperidinylC_{1-6}alkyl; piperidinylaminoC_{1-6}alkylamino; piperidinylaminoC_{1-6}alkylaminoC_{1-6}alkyl; (C_{1-6}alkylpiperidinyl)(hydroxyC_{1-6}alkyl)aminoC_{1-6}alkylamino; (C_{1-6}alkylpiperidinyl)(hydroxyC_{1-6}alkyl)aminoC_{1-6}alkylaminoC_{1-6}alkyl; hydroxyC_{1-6}alkyloxyC_{1-6}alkylpiperazinyl; hydroxyC_{1-6}alkyloxyC_{1-6}alkylpiperazinylC_{1-6}alkyl; (hydroxyC_{1-6}alkyl)(C_{1-6}alkyl)amino; (hydroxyC_{1-6}alkyl)(C_{1-6}alkyl)aminoC_{1-6}alkyl; hydroxyC_{1-6}alkylaminoC_{1-6}alkyl; di(hydroxyC_{1-6}alkyl)aminoC_{1-6}alkyl; pyrrolidinylC_{1-6}alkyl; pyrrolidinylC_{1-6}alkyloxy; pyrazolyl; thiopyrazolyl; pyrazolyl substituted with two substituents selected from C_{1-6}alkyl or trihaloC_{1-6}alkyl; pyridinyl; pyridinyl substituted with C_{1-6}alkyloxy, aryloxy or aryl; pyrimidinyl; tetrahydropyrimidinylpiperazinyl; tetrahydropyrimidinylpiperazinylC_{1-6}alkyl; quinolinyl; indole; phenyl; phenyl substituted with one, two or three substituents independently selected from halo, amino, nitro, C_{1-6}alkyl, C_{1-6}alkyloxy, hydroxyC_{1-4}alkyl, trifluoromethyl, trifluoromethyloxy, hydroxyC_{1-4}alkyloxy, C_{1-4}alkylsulfonyl, C_{1-4}alkyloxyC_{1-4}alkyloxy, C_{1-4}alkyloxycarbonyl, aminoC_{1-4}alkyloxy, di(C_{1-4}alkyl)aminoC_{1-4}alkyloxy, di(C_{1-4}alkyl)amino, di(C_{1-4}alkyl)aminocarbonyl, di(C_{1-4}alkyl)aminoC_{1-4}alkyl, di(C_{1-4}alkyl)aminoC_{1-4}alkylaminoC_{1-4}alkyl, di(C_{1-4}alkyl)amino(C_{1-4}alkyl)amino, di(C_{1-4}alkyl)amino(C_{1-4}alkyl)aminoC_{1-4}alkyl,

di(C_{1-4}alkyl)aminoC_{1-4}alkyl(C_{1-4}alkyl)amino,
di(C_{1-4}alkyl)aminoC_{1-4}alkyl(C_{1-4}alkyl)aminoC_{1-4}alkyl,
aminosulfonylamino(C_{1-4}alkyl)amino,
aminosulfonylamino(C_{1-4}alkyl)aminoC_{1-4}alkyl,
di(C_{1-4}alkyl)aminosulfonylamino(C_{1-4}alkyl)amino,
di(C_{1-4}alkyl)aminosulfonylamino(C_{1-4}alkyl)aminoC_{1-6}alkyl, cyano,
piperidinylC_{1-4}alkyloxy, pyrrolidinylC_{1-4}alkyloxy, aminosulfonylpiperazinyl,
aminosulfonylpiperazinylC_{1-4}alkyl, di(C_{1-4}alkyl)aminosulfonylpiperazinyl,
di(C_{1-4}alkyl)aminosulfonylpiperazinylC_{1-4}alkyl, hydroxyC_{1-4}alkylpiperazinyl,
hydroxyC_{1-4}alkylpiperazinylC_{1-4}alkyl, C_{1-4}alkyloxypiperidinyl,
C_{1-4}alkyloxypiperidinylC_{1-4}alkyl, hydroxyC_{1-4}alkyloxyC_{1-4}alkylpiperazinyl,
hydroxyC_{1-4}alkyloxyC_{1-4}alkylpiperazinylC_{1-4}alkyl,
(hydroxyC_{1-4}alkyl)(C_{1-4}alkyl)amino, (hydroxyC_{1-4}alkyl)(C_{1-4}alkyl)aminoC_{1-4}alkyl,
di(hydroxyC_{1-4}alkyl)amino, di(hydroxyC_{1-4}alkyl)aminoC_{1-4}alkyl, furanyl, furanyl
substituted with –CH=CH-CH=CH-, pyrrolidinylC_{1-4}alkyl, pyrrolidinylC_{1-4}alkyloxy,
morpholinyl, morpholinylC_{1-4}alkyloxy, morpholinylC_{1-4}alkyl,
morpholinylC_{1-4}alkylamino, morpholinylC_{1-4}alkylaminoC_{1-4}alkyl, piperazinyl,
C_{1-4}alkylpiperazinyl, C_{1-4}alkylpiperazinylC_{1-4}alkyloxy, piperazinylC_{1-4}alkyl,
C_{1-4}alkylpiperazinylC_{1-4}alkyl, C_{1-4}alkylpiperazinylC_{1-4}alkylamino,
C_{1-4}alkylpiperazinylC_{1-4}alkylaminoC_{1-6}alkyl, tetrahydropyrimidinylpiperazinyl,
tetrahydropyrimidinylpiperazinylC_{1-4}alkyl, piperidinylaminoC_{1-4}alkylamino,
piperidinylaminoC_{1-4}alkylaminoC_{1-4}alkyl,
(C_{1-4}alkylpiperidinyl)(hydroxyC_{1-4}alkyl)aminoC_{1-4}alkylamino,
(C_{1-4}alkylpiperidinyl)(hydroxyC_{1-4}alkyl)aminoC_{1-4}alkylaminoC_{1-4}alkyl,
pyridinylC_{1-4}alkyloxy,
hydroxyC_{1-4}alkylamino, hydroxyC_{1-4}alkylaminoC_{1-4}alkyl,
di(C_{1-4}alkyl)aminoC_{1-4}alkylamino, aminothiadiazolyl,
aminosulfonylpiperazinylC_{1-4}alkyloxy, or thiophenylC_{1-4}alkylamino;

each R^5 and R^6 can be placed on the nitrogen in replacement of the hydrogen;

aryl in the above is phenyl, or phenyl substituted with one or more substituents each independently selected from halo, C_{1-6}alkyl, C_{1-6}alkyloxy, trifluoromethyl, cyano or hydroxycarbonyl.

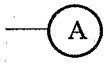
2. A compound as claimed in claim 1 wherein n is 1 or 2; t is 0, 1, 2 or 4; each Q is —C≤; R^1 is –C(O)NH(OH); R^2 is hydrogen or nitro; -L- is a direct bond or a bivalent radical selected from C_{1-6}alkanediyl; R^4 is hydrogen; —(A) is a radical

selected from (a-1),(a-2), (a-3), (a-5), (a-6), (a-11), (a-18), (a-20), (a-21), (a-32), (a-33), (a-47) or (a-51); each s is independently 0, 1, 2, or 4; each R^5 and R^6 are independently selected from hydrogen; halo; trihaloC_{1-6}alkyl; C_{1-6}alkyl; C_{1-6}alkyl substituted with aryl and C_{3-10}cycloalkyl; C_{1-6}alkyloxy; C_{1-6}alkylcarbonyl; benzofuranyl; naphtalenylsulfonyl; pyridinyl substituted with aryloxy; phenyl; or phenyl substituted with one substituent independently selected from hydroxyC_{1-4}alkyl or morpholinylC_{1-4}alkyl.

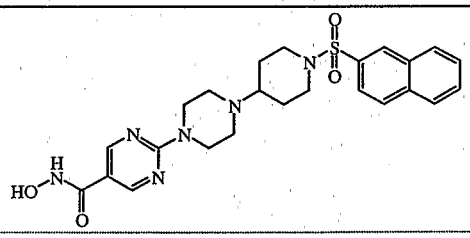
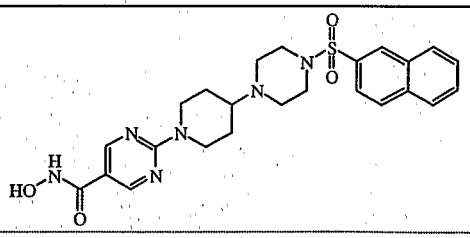
3. A compound as claimed in claim 1 wherein t is 1, 2, 3, or 4;
R^1 is $-C(O)NR^7R^8$, $-C(O)-C_{1-6}$alkanediylSR^9, $-NR^{10}C(O)N(OH)R^9$, $-NR^{10}C(O)C_{1-6}$alkanediylSR^9, $-NR^{10}C(O)C{=}N(OH)R^9$ or another Zn-chelating-group wherein R^7 and R^8 are each independently selected from hydrogen, hydroxy, hydroxyC_{1-6}alkyl or aminoC_{1-6}alkyl;
R^2 is hydrogen, halo, hydroxy, amino, nitro, C_{1-6}alkyl, C_{1-6}alkyloxy, trifluoromethyl or di(C_{1-6}alkyl)amino;
-L- is a direct bond or a bivalent radical selected from C_{1-6}alkanediyl, C_{1-6}alkanediyloxy, amino or carbonyl;
R^4 is hydrogen, hydroxy, amino, hydroxyC_{1-6}alkyl, C_{1-6}alkyl, C_{1-6}alkyloxy, arylC_{1-6}alkyl, aminocarbonyl, aminoC_{1-6}alkyl, C_{1-6}alkylaminoC_{1-6}alkyl or di(C_{1-6}alkyl)aminoC_{1-6}alkyl;
—(A) is a radical selected from (a-1), (a-3), (a-4), (a-5), (a-6), (a-7), (a-8), (a-9), (a-10), (a-11), (a-12), (a-13), (a-14), (a-15), (a-16), (a-17), (a-18), (a-19), (a-20), (a-21), (a-22), (a-23), (a-24), (a-25), (a-26), (a-28), (a-29), (a-30), (a-31), (a-32), (a-33), (a-34), (a-35), (a-36), (a-37), (a-38), (a-39), (a-40), (a-41), (a-42), (a-44), (a-45), (a-46), (a-47), (a-48) and (a-51);
each s is independently 0, 1, 2, 3 or 4;
R^5 is hydrogen; halo; hydroxy; amino; nitro; trihaloC_{1-6}alkyl; trihaloC_{1-6}alkyloxy; C_{1-6}alkyl; C_{1-6}alkyloxy; C_{1-6}alkylcarbonyl; C_{1-6}alkyloxycarbonyl; C_{1-6}alkylsulfonyl; hydroxyC_{1-6}alkyl; aryloxy; di(C_{1-6}alkyl)amino; cyano; thiophenyl; furanyl; furanyl substituted with hydroxyC_{1-6}alkyl; benzofuranyl; imidazolyl; oxazolyl; oxazolyl substituted with aryl and C_{1-6}alkyl; C_{1-6}alkyltriazolyl; tetrazolyl; pyrrolidinyl; pyrrolyl; morpholinyl; C_{1-6}alkylmorpholinyl; piperazinyl; C_{1-6}alkylpiperazinyl; hydroxyC_{1-6}alkylpiperazinyl; C_{1-6}alkyloxypiperidinyl; pyrazoly; pyrazolyl substituted with one or two substituents selected from C_{1-6}alkyl or trihaloC_{1-6}alkyl; pyridinyl; pyridinyl substituted with C_{1-6}alkyloxy, aryloxy or aryl; pyrimidinyl; quinolinyl; indole;

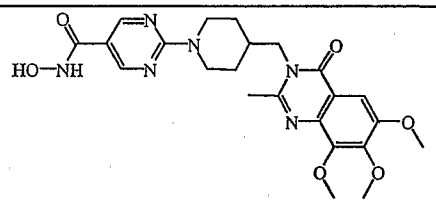
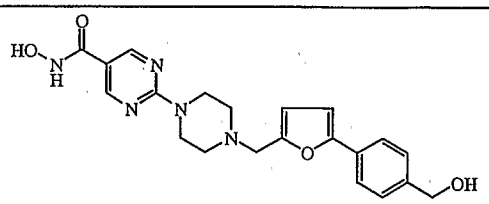
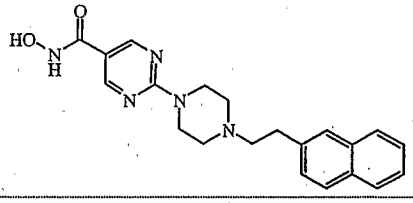
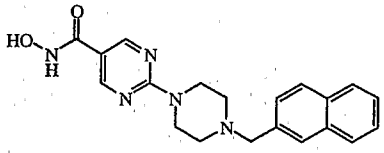
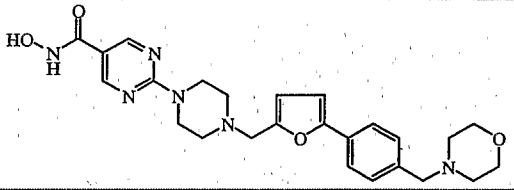
phenyl; or phenyl substituted with one or two substituents independently selected from halo, C_{1-6}alkyl, C_{1-6}alkyloxy or trifluoromethyl;

R^6 is hydrogen; halo; hydroxy; amino; nitro; trihaloC_{1-6}alkyl; trihaloC_{1-6}alkyloxy; C_{1-6}alkyl; C_{1-6}alkyloxy; C_{1-6}alkylcarbonyl; C_{1-6}alkyloxycarbonyl; C_{1-6}alkylsulfonyl; hydroxyC_{1-6}alkyl; aryloxy; di(C_{1-6}alkyl)amino; cyano; pyridinyl; phenyl; or phenyl substituted with one or two substituents independently selected from halo, C_{1-6}alkyl, C_{1-6}alkyloxy or trifluoromethyl.

4. A compound as claimed in claim 1 and 2 wherein n is 1; t is 0 or 1; each Q is ; each X is nitrogen; each Y is nitrogen; R^1 is –C(O)NH(OH); R^2 is hydrogen; -L- is a direct bond; each R^3 independently represents a hydrogen atom; R^4 is hydrogen;  is a radical selected from (a-6), (a-11), (a-20), (a-47) or (a-51); each s is independently 0, 1, or 4; and each R^5 and R^6 are independently selected from hydrogen; C_{1-6}alkyl; C_{1-6}alkyloxy; naphtalenylsulfonyl; or phenyl substituted with hydroxyC_{1-4}alkyl or morpholinylC_{1-4}alkyl.

5. A compound according to claim 1, 2 and 4 selected from compounds No. 3, No. 4, No. 8, No. 5, No. 7, No. 6 and No. 9.

	
0.91 $C_2HF_3O_2$; Co. No. 3	0.86 $C_2HF_3O_2$; Co. No. 4

	
$C_2HF_3O_2$ (1:1); Co. No.8	0.83 $C_2HF_3O_2$; Co. No.5
	
0.79 $C_2HF_3O_2$; Co. No.7	0.83 $C_2HF_3O_2$; Co. No.6
	
0.47 H_2O .1.99 $C_2HF_3O_2$; Co. No.9	

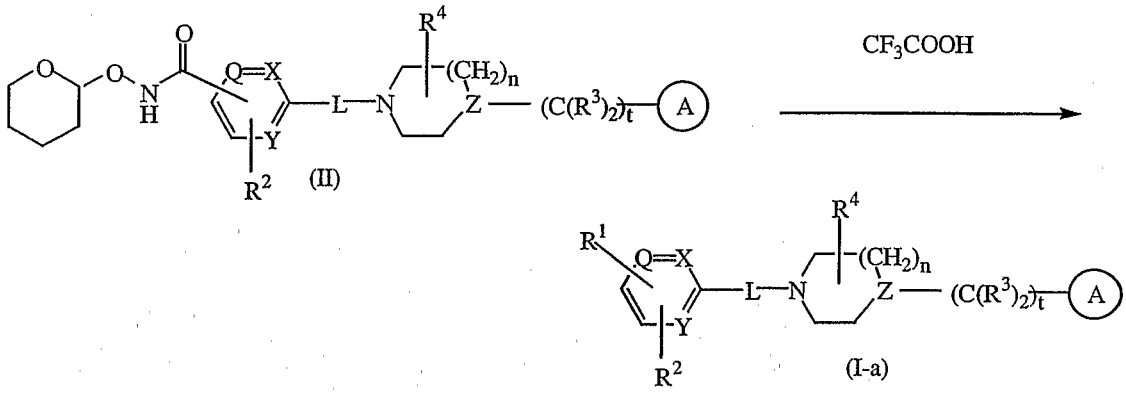
6. A pharmaceutical composition comprising pharmaceutically acceptable carriers and as an active ingredient a therapeutically effective amount of a compound as claimed in claim 1 to 5.

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

8. A compound as claimed in any of claims 1 to 5 for use as a medicine.

9. Use of a compound as claimed in any of claims 1 to 5 for the manufacture of a medicament for the treatment of proliferative diseases.

10. A process for preparing a compound as claimed in claim 1, characterized by reacting an intermediate of formula (II) with an appropriate acid, such as for example, trifluoro acetic acid, yielding a hydroxamic acid of formula (I-a), wherein R^1 is –C(O)NH(OH)



11. A method of detecting or identifying a HDAC in a biological sample comprising detecting or measuring the formation of a complex between a labelled compound as defined in claim (I) and a HDAC.

12. A combination of an anti-cancer agents and a HDAC inhibitor as claimed in any of claims 1 to 5.