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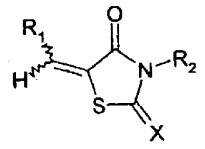
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(54) **Title:** USE OF THIAZOLIDINONE DERIVATIVES AS ANTIANGIOGENIC AGENTS




(I)

(57) **Abstract:** The invention relates to the use of compounds of general formula (I), in which $R_1$, $R_2$ and X are as defined in the description for the preparation of pharmaceutical compositions for the treatment of pathologies in which inhibition of the interaction between HIF-1α and p300 is beneficial, in particular as antiangiogenic medicaments for the therapy of solid tumors.

WO 2006/066846 A1

# USE OF THIAZOLIDINONE DERIVATIVES AS ANTIANGIOGENIC AGENTS

## Field of the invention

The invention relates to thiazolidinone compounds capable of inhibiting the interaction between HIF-1α transcription factor and its coactivator p300 and of preventing the production of Vascular Endothelial cell Growth Factor.

## Background of the invention

Vascular Endothelial Cell Growth Factor plays a key role in the processes of physiological and physiopathological angiogenesis. A number of mechanisms are involved in the regulation of the VEGF gene, among which a fundamental role is played by the tissue oxygen tension, as proved by the reversible increase in VEGF mRNA levels under *in vivo* and *in vitro* hypoxia conditions. Increased expression of VEGF mRNA is mainly mediated by the transcription factor HIF-1 (hypoxia-inducible factor-1), which binds to a recognition site in the promoter region of the VEGF gene.

A great number of experimental data show that HIF-1 is a global regulator of oxygen homeostasis and that an impaired activity of HIF-1 promotes survival, proliferation, invasion and metastatization of tumoral cells (1). It has been therefore suggested that therapeutic strategies focusing on the inhibition of HIF-1 activity could increase the survival of cancer patients (2).

HIF-1 is a heterodimer consisting of HIF-1α and HIF-1β sub-units, which dimerize and bind to DNA through the bHLH-PAS domain (3). The expression of the HIF-1α sub-unit is strictly regulated by the tissue oxygen tension (4) through processes of ubiquitination and proteasome degradation, mediated by the binding of VHL protein to HIF-1α. Such interaction only takes place when HIF-1α has been hydroxylated at the 402 and 564 proline residues. Oxygen is the limiting substrate for prolyl-hydroxylase which

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modifies HIF-1α (5). The expression of HIF-1α exponentially increases as $O_2$ concentration decreases and determines the HIF-1 global activity levels.

The function of HIF-1α transactivation domain is also subject to negative regulation, controlled by oxygen partial pressure. The N-terminal transactivation domain is negatively regulated through the recruitment of hystone deacylase by VHL and by HIF-1 inhibiting factor (FIH-1), which binds to both VHL and HIF-1α (6).

HIF-1 activation takes place through p300/CBP coactivators which physically interact with the activation of the HIF-1 domain to promote transcription of genes like VEGF (7). Both p300 and CBP are co-activators also for other transcription factors, such as Stat-3, NF-κB, p53.

The interaction of p300/CBP with HIF-1 is essential to transcription, and recent publications have proved the importance of the HIF-1/p300 interaction for tumor growth (8). HIF-1α C-terminal trans-activation domain (C-TAD) binds to a p300 and CBP domain known as CH1. The binding of CBP and p300 to HIF-1α is negatively regulated through oxygen-dependent hydroxylation of asparagine 803 in the C-terminal activation domain by FIH-1. Thus, hypoxia causes both stabilization to proteasome degradation and transcriptional activity of HIF-1.

Structural details of the interaction between HIF-1α TAD-C and the CH1 domain of p300 or CBP have been elucidated (9, 10). Details of the interaction between p300/CBP and the CITED2 protein (also known as $p35^{srj}$), which is considered a negative regulator of Hif-1α activity (11), have also been published.

HIF-1 activation induces the transcription of a number of genes involved in the production of angiogenic factors, glucose carriers, glycolytic enzymes, survival, migration and invasion factors, which are particularly important for tumor progression.

Aberrant expression of Hif-1α was observed in more than 70% of human tumors and their metastases and was connected with an increase in vascularization and tumor progression (12-14). In clinical practice, aberrant expression of Hif-1α was associated to therapy failure and mortality increase in a number of tumoral pathologies, such as non-small cells lung carcinoma (15), oropharyngeal squamous cell cancer (16), early-stage cervical cancer (17), head-and-neck cancer (18), mutated p53 ovary cancer (19), oligodendrioglioma (20) and BCL-2 positive esophageal cancer (21).

Various approaches for inhibiting HIF-1 activity have been described in the literature. Some of them suggested the use of antisense oligonucleotides for Hif-1α or negative dominant forms of Hif-1α.

Among the pharmacological approaches, Hif-1α activity inhibitors acting through indirect mechanisms have been described, such as PI3K-mTOR inhibitors (22-23) and MEKK (24) inhibitors which act on the transduction of signals controlling Hif-1α activity; inhibitors of HSP90 chaperone protein (25); thioredoxin reductase inhibitors, which act modifying the cell redox state (26); molecules which destabilize microtubules, such as 2-methoxyestradiol (27) and epothilones (28). Recently, both constitutive and hypoxia-induced inhibition of Hif-1α levels by PX-478 (Melphalan N-oxide) in human tumors transplanted in nude mice was reported. The compound shows marked antitumoral effects. However, the mechanism of action of this compound has yet to be completely clarified (29).

Finally, chaetomine, a dithiodioxopiperazine metabolite of Chaetomium sp fungi, has recently been reported to interfere with the binding of Hif-1α to p300. The compound acts altering the CH1 domain structure of p300, thus preventing its interaction with Hif-1α. Chaetomine administration to tumor-bearing mice inhibits hypoxia-induced transcription in the tumor and tumor growth (30). It would therefore be advantageous to provide further

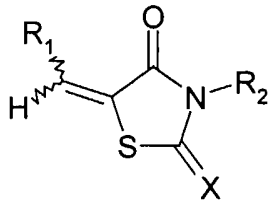
compounds capable of inhibiting the binding between HIF-1α to p300.

US 2004/002526 A1 discloses thiazolidinone compounds for use as inhibitors of phospholipase D and WO 98/53790 discloses thiazolidinones useful as antitumor agents.

**Disclosure of the invention**

It has now been found that some thiazolidinones inhibit the interaction between Hif-1α and p300 and prevent VEGF production in tumour cells under hypoxia conditions. The compounds are therefore useful for the control of angiogenesis and tumor growth.

Accordingly, the present invention relates to the use of compounds of general formula (**I**)



(I)

in which

$R_1$ is aryl or heteroaryl;

$R_2$ is alkyl, alkenyl, cycloalkyl, heterocycloalkyl, aryl or heteroaryl;

X is oxygen or sulphur

for the preparation of pharmaceutical compositions for the treatment of pathologies in which inhibition of interaction between HIF-1α and p300 is beneficial.

For the purposes of the present application:

"alkyl" is a straight or branched hydrocarbon comprising 1 to 10 carbon atoms, preferably 1 to 8 carbon atoms, optionally substituted with one or more groups independently selected from cycloalkyl, heterocycloalkyl, aryl, heteroaryl, haloalkyl, halogen, hydroxy, alkoxy, mercapto, cyano, sulfonamido, aminosulfonyl, acyl, acyloxy and substituted amino;

"aryl" is an unsaturated cyclic aromatic hydrocarbon comprising 6 to 14 carbon atoms, optionally substituted with one or more substituents independently selected from alkyl, aryl, haloalkyl, halogen, hydroxy, alkoxy, mercapto, cyano, sulfonamido, aminosulfonyl, acyl, acyloxy, nitro and substituted amino.

"heteroaryl" is a 5- to 10-membered monocyclic or condensed aromatic ring containing one or more atoms preferably selected from nitrogen, oxygen and sulphur, optionally substituted with one or more groups independently selected from alkyl, aryl, haloalkyl, halogen, hydroxy, alkoxy, mercapto, cyano, sulfonamido, aminosulfonyl, acyl, acyloxy, nitro and substituted amino;

"cycloalkyl" means cyclic alkyl groups which contain from 3 to 8 carbon atoms, optionally substituted with one or more substituents selected from alkyl, aryl, haloalkyl, halogen, hydroxy, alkoxy, mercapto, cyano, sulfonamido, aminosulfonyl, acyl, acyloxy, nitro and substituted amino;

"heterocycloalkyl" is a saturated or partially unsaturated monocyclic or condensed ring, containing one or more atoms preferably selected from nitrogen, oxygen and sulphur, and optionally substituted with one or more groups independently selected from alkyl, aryl, haloalkyl, halogen, hydroxy, alkoxy, mercapto, cyano, sulfonamido, aminosulfonyl, acyl, acyloxy, nitro and substituted amino;

"alkenyl" is a straight, cyclic or branched monovalent hydrocarbon radical, comprising at least one carbon-carbon double bond, optionally substituted with one or more groups independently selected from alkyl, acyl, cycloalkyl, heterocycloalkyl, aryl, haloalkyl, alkoxy and substituted amino;

"alkoxy" is a straight or branched $C_1$-$C_4$ alkoxy group;

"substituted amino" is a -NRR group, in which each R group is independently selected from from hydrogen, acyl, alkyl, cycloalkyl, aryl, or

the R groups can form, together with the nitrogen atom, a 5- or 6-membered heterocyclic ring;

"halogen" or the prefix "halo" indicate fluorine, chlorine, bromine and iodine;

"acyl" means a -C(O)-R" group, in which R" is preferably selected from hydrogen, hydroxy, alkyl, haloalkyl, cycloalkyl, aryl optionally substituted with one or more alkyl groups, haloalkyl, alkoxy, halogen and substituted amino groups, heteroaryl optionally substituted with one or more alkyl groups, haloalkyl, alkoxy, halo and substituted amino groups and rings optionally substituted with one or more alkyl, haloalkyl, alkoxy, halo and substituted amino groups. Preferred compounds according to the invention are those in which:

$R_1$ is substituted or unsubstituted furan;

$R_2$ is substituted or unsubstituted phenyl or benzyl, or

(2-thienyl)-methyl, morpholinyl-propyl, phenylethyl and (3,4-dimethoxyphenyl)ethyl and

X is sulphur.

"Substituted furan" preferably means a furan group substituted with a phenyl group, optionally substituted with one or more groups selected from those indicated above, preferably carboxy, methoxycarbonyl, cyano, sulfonamido and hydroxyethoxy. "Substituted phenyl or benzyl" preferably means phenyl or benzyl substituted with one or more groups selected from methyl, trifluoromethyl, methoxy, methylenedioxy.

Most preferred are the following compounds:

2-[5-(3-benzyl-4-oxo-2-thioxo-thiazolidine-5-ylidenemethyl)-furan-2-yl]benzoic acid methyl ester (**1**);

4-{5-[3-(3,4,5-trimethoxybenzyl)-4-oxo-2-thioxo-thiazolidine-5-ylidenemethyl]-furan-2-yl}benzoic acid (**2**);

4-{5-[3-(3,4,5-trimethoxybenzyl)-4-oxo-2-thioxo-thiazolidine-5-ylidenemethyl]-furan-2-yl}benzoic acid methyl ester (**3**);

4-{5-[3-(2-trifluoromethylbenzyl)-4-oxo-2-thioxo-thiazolidine-5-ylidenemethyl]-furan-2-yl}benzoic acid methyl ester (**4**);

4-[5-(3-benzyl-4-oxo-2-thioxo-thiazolidine-5-ylidenemethyl)furan-2-yl-] benzonitrile (**5**);

4-{5-[3-(4-methylbenzyl)-4-oxo-2-thioxo-thiazolidine-5-ylidenemethyl]furan-2-yl-}benzonitrile (**6**);

4-[5-(3-benzyl-4-oxo-2-thioxo-thiazolidine-5-ylidenemethyl)-furan-2-yl]benzenesulfonamide (**7**);

4-{5-[3-(4-methylbenzyl)-4-oxo-2-thioxo-thiazolidine-5-ylidenemethyl]-furan-2-yl}benzenesulfonamide (**8**);

4-{5-[3-(4-methylbenzyl)-4-oxo-2-thioxo-thiazolidine-5-ylidenemethyl]-furan-2-yl}benzoic acid methyl ester (**9**);

4-{5-[3-(4-methylbenzyl)-4-oxo-2-thioxo-thiazolidine-5-ylidenemethyl]-furan-2-yl}benzoic acid (**10**);

4-{5-[3-(3,4-methylenedioxybenzyl)-4-oxo-2-thioxo-thiazolidine-5-ylidenemethyl]-furan-2-yl}benzenesulfonamide (**11**);

4-{5-[3-(3,4-methylenedioxybenzyl)-4-oxo-2-thioxo-thiazolidine-5-ylidenemethyl]-furan-2-yl}benzoic acid methyl ester (**12**);

4-{5-[3-(2-thienyl)methyl-4-oxo-2-thioxo-thiazolidine-5-ylidenemethyl]-furan-2-yl}benzoic acid methyl ester (**13**);

4-{5-[3-(2-thienyl)methyl-4-oxo-2-thioxo-thiazolidine-5-ylidenemethyl]-furan-2-yl}benzonitrile (**14**);

4-{5-[3-(2-thienyl)methyl-4-oxo-2-thioxo-thiazolidine-5-ylidenemethyl]-furan-2-yl}benzenesulfonamide (**15**);

4-{5-[3-(morpholine-4yl-propyl)-4-oxo-2-thioxo-thiazolidine-5-ylidenemethyl]-furan-2-yl}benzoic acid methyl ester (**16**);

4-{5-[3-(3,4-dimethoxyphenyl)-4-oxo-2-thioxo-thiazolidine-5-ylidenemethyl]-furan-2-yl}benzoic acid (**17**);

4-{5-[3-[2-(3,4-dimethoxyphenyl)ethyl]-4-oxo-2-thioxo-thiazolidine-5-ylidenemethyl]-furan-2-yl}benzonitrile (**18**);

4-{5-[3-(2-phenyl)ethyl-4-oxo-2-thioxo-thiazolidine-5-ylidenemethyl]-furan-2-yl}benzonitrile (**19**);

4-{5-[3-[2-(phenyl)ethyl]-4-oxo-2-thioxo-thiazolidine-5-ylidenemethyl]-furan-2-yl}benzoic acid methyl ester (**20**);

4-{5-[3-[2-(3,4-dimethoxyphenyl)ethyl]-4-oxo-2-thioxo-thiazolidine-5-ylidenemethyl]-furan-2-yl}benzoic acid methyl ester (**21**);

2-{5-[4-[3-(2-phenyl)ethyl-4-oxo-2-thioxo-thiazolidine-5-ylidenemethyl]-furan-2-yl]phenoxy}ethanol (**22**);

3-benzyl-5-[[5-(2-methoxyphenyl)furan-2-ylidenemethyl]-4-oxo-2-thioxo-thiazolidine (**23**);

2-[5-(3-benzyl-4-oxo-2-thioxo-thiazolidine-5-ylidenemethyl)-furan-2-yl]benzoic acid (**24**).

Among these compounds, the most preferred is 2-{5-[3-(4-methylbenzyl)-4-oxo-2-thioxo-thiazolidine-5-ylidenemethyl]-furan-2-yl}benzoic acid methyl ester.

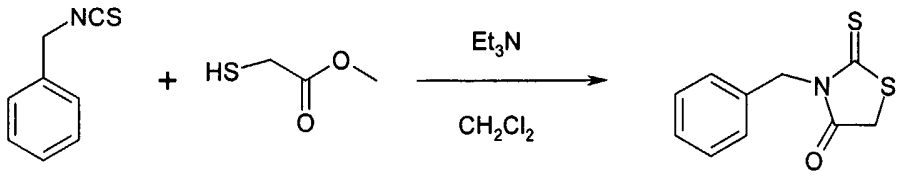
The above-mentioned compounds, except for compounds **1** and **16**, are a further object of the invention.

The compounds of the invention can be synthesised according to known methods, for example those described in US 2004/0002526 A1, which comprise the condensation between a 2-thioxo-thiazolidine-4-one, suitably substituted at the 3- position and a suitably substituted furanaldehyde, or according to the methods described in *Tetrahedron Lett.*, **44**, 4257 (2003), *Bioorg. Med. Chem. Lett.*, **12**, 2681 (2002), *J. Med. Chem.*, **41**, 2390 (1998).

The following schemes illustrate in particular the synthesis of 4-{5-[3-

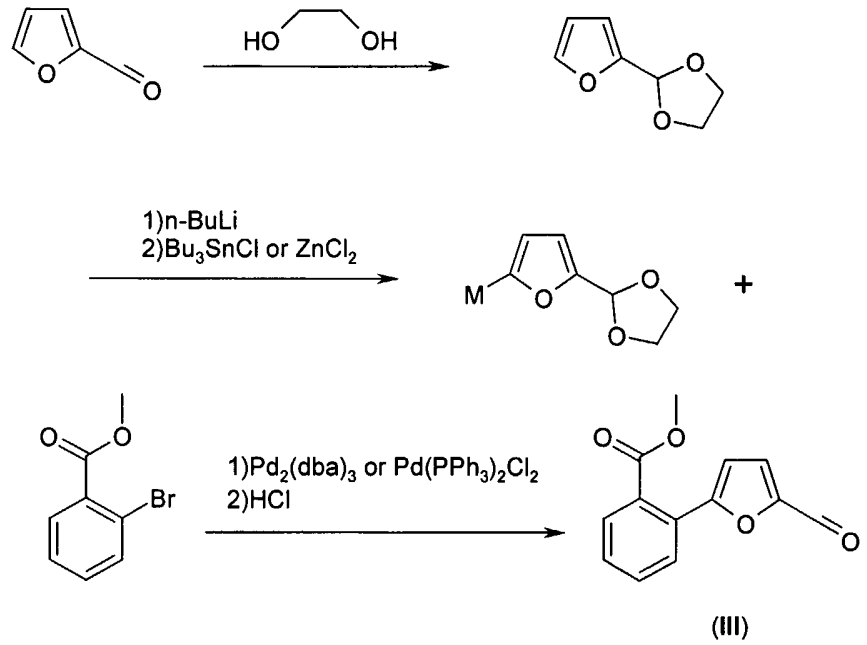
(4-methylbenzyl)-4-oxo-2-thioxo-thiazolidine-5-ylidenemethyl]-furan-2-yl}benzoic acid methyl ester.

**Scheme 1**




(II)

**Scheme 2**

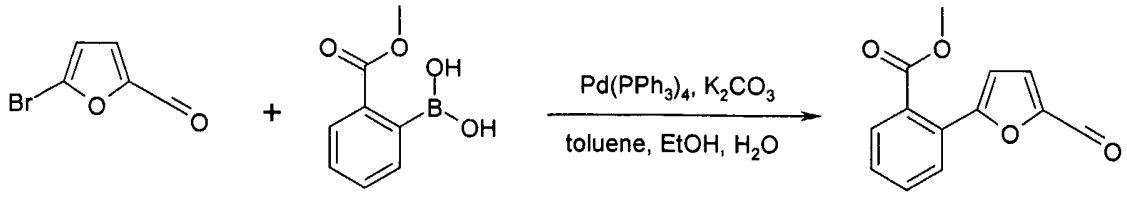



(III)

Wherein M is Li, Zn or $Bu_3Sn$ and DBA is dibenzylidenacetone

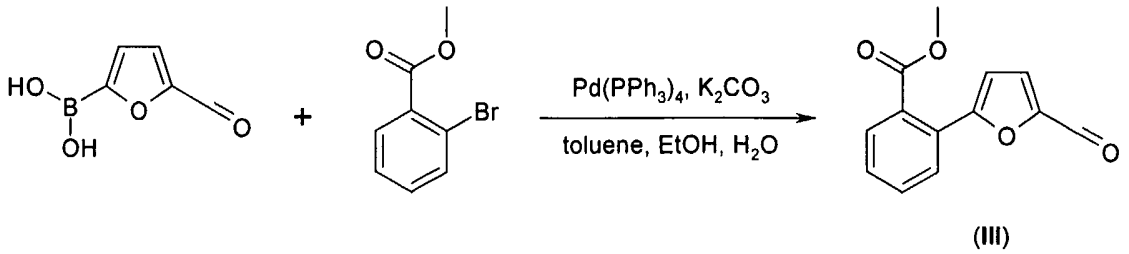
Alternatively, compound (**III**) can be synthesized according to the following schemes:

**Scheme 3**



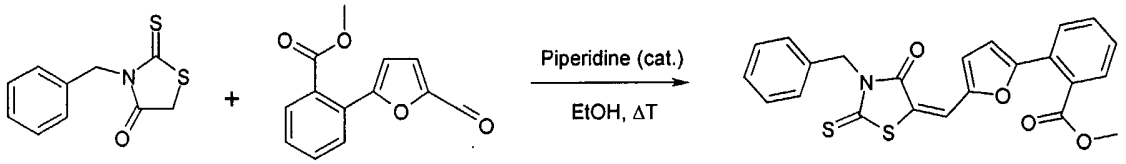

(III)

**Scheme 4**




(III)

**Scheme 5**




(II) (III)

All the reagents reported in Schemes 1-5 can be prepared with known methods and are commercially available.

The compounds of the invention inhibit the interaction between HIF-1α and p300 with $IC_{50}$ ranging from 0.5 to 25 μM and the production of VEGF in tumour cells under hypoxia conditions with $IC_{50}$ ranging from 0.10 to 100 μM.

The compounds can therefore be used for the preparation of pharmaceutical compositions for the treatment of pathologies in which inhibition of the interaction between HIF-1α and p300 is beneficial. The compositions can be solid, semi-solid or liquid, preferably in the form of solutions, suspensions, powders, granules, tablets, capsules, syrups, suppositories, aerosol or controlled-release systems. The compositions can be administered through different routes, in particular through the oral, transdermal, subcutaneous, intravenous, intramuscular, rectal and intranasal route. The amount of active ingredient per dosage unit depends on the form and on the administration route, the compound, the disease to treat, but in general ranges from 0.1 to 1000 mg, preferably from 1 to 600 mg. The principles and methods for the preparation of pharmaceutical compositions are known to those

skilled in the art and are described, for example, in Remington's Pharmaceutical Science, Mack Publishing Company, Easton (PA).

The compounds of the invention, suitably formulated, can be used for the treatment of a number of pathologies in which angiogenesis is involved as pathogenesis factor, for example different forms of solid tumors, diabetic retinopathy, rheumatoid arthritis, psoriasis, emangioma, sclerodermia, neovascular glaucoma. Solid tumors particularly sensitive to the compounds of formula (**I**) comprise lung carcinoma, mammary carcinoma, prostate carcinoma, neuroblastoma, glioblastoma multiforme, melanoma, central nervous system cancer, squamous cell oropharyngeal cancer, cervical cancer, ovary, esophageal, kidney, colon, head-and-neck cancer and oligodendrioglioma.

Therefore, the invention relates also to a method for inhibiting VEGF production in a cell, which method comprises contacting said cell with an effective amount of a compound of formula (**I**).

The invention will be illustrated in greater detail in the following experimental section.

**EXPERIMENTAL SECTION**

**Synthesis of the compounds**

**General procedure A**

**Example - 4-(5-Formyl-furan-2-yl)-benzoic acid methyl ester**

A solution of 5-bromofuraldehyde (2.43 g, 13.9 mmoles), 4-(methoxycarbonyl)phenylboronic acid (2.50 g, 13.9 mmoles), tris(dibenzylideneacetone)palladium (0) (192 mg, 0.2 mmoles) and potassium fluoride (2.42 g, 41.7 mmoles) in 1,4-dioxane (100 ml) was added with a solution of tri-*tert*-butylphosphine in hexane (10% by weight, 101 mg, 0.5 mmoles). After heating at 65-70°C for 4 hours, the mixture was cooled to room temperature and treated with methylene chloride (150 ml). After stirring for 10 minutes, the mixture was filtered through Celite and the filtrate was

concentrated under reduced pressure. The residue was purified by flash silica gel chromatography eluting with an ethyl acetate-hexane mixture (1:1) to give 4-(5-formyl-furan-2-yl)-benzoic acid methyl ester (2.6 g, 81% yield).

$^1$H NMR (300 MHz, $CDCl_3$): δ 9.70 (s, 1H), 8.10 (d, 2H), 7.90 (d, 2H), 7.35 (d, 1H), 6.95 (d, 1H), 3.98 (s, 3H).

**General procedure B**

**Example - 3-(3-Morpholino-4-yl-propyl)-2-thioxo-thiazolidine-4-one**

A suspension of bis(carboxymethyl)trithiocarbonate (498 mg, 2.2 mmoles), potassium carbonate (138 mg, 1.0 mmoles) and 4-(3-aminopropyl)morpholine (288 mg, 2.0 mmoles) in water (20 ml) was refluxed for 12 hours. After addition of water (10 ml), the mixture was cooled to room temperature and extracted with 10% methanol-methylene chloride (3x50 ml). The combined extracts were dried over anhydrous magnesium sulphate and concentrated under reduced pressure. The residue was purified by flash silica gel chromatography eluting with 10% methanol-methylene chloride to give 3-(3-morpholino-4-yl-propyl)-2-thioxo-thiazolidine-4-one (252 mg, 48% yield). $^1$H NMR (300 MHz, $CDCl_3$): δ 4.10 (dd, 2H), 3.95 (s, 2H), 3.55-3.70 (m, 4H), 2.30-2.50 (m, 6H), 1.70-1.90 (m, 2H).

**General procedure C**

**Example - 4{5-[3-(3-Morpholino-4-yl-propyl)-4-oxo-2-thioxo-thiazolidine-5-ylidenemethyl]-furan-2-yl}-benzoic acid methyl ester**

A solution of 4-(5-formyl-furan-2-yl)-benzoic acid methyl ester (59 mg, 0.27 mmoles) and 3-(3-morpholino-4-yl-propyl)-2-thioxo-thiazolidine-4-one (72 mg, 0.27 mmoles) in ethanol (10 ml) was added with piperidine (1 drop). After heating under reflux for 6 hours, the mixture was concentrated under reduced pressure. The residue was purified by flash silica gel chromatography eluting with 10% methanol-methylene chloride to give the title compound (32 mg, 25%yield).

$^1$H NMR (300 MHz, DMSO-$d_6$): δ 8.15 (d, 2H), 7.75 (d, 2H), 7.50 (s, 1H), 6.95-7.05 (m, 2H), 4.20 (t, 2H), 3.95 (s, 3H), 3.90 (t, 2H), 3.58-3.75 (m, 2H), 2.30-2.60 (m, 6H), 1.80-2.00 (m, 2H).

**4-(5-Formyl-furan-2-yl)benzenesulfonyl amide**

A mixture 4-(tributylstannyl)benzenesulfonyl amide (2.77 g, 6.21 mmoles) and bis(triphenylphosphine) palladium (II) chloride (436 mg, 0.62 mmoles) in ethanol (65 ml) was refluxed for 18 hours under argon atmosphere. After cooling to room temperature, ethyl ether (200 ml) was added and the mixture was vacuum filtered through Celite. The filtrate was concentrated under reduced pressure and the residue was purified by flash silica gel chromatography eluting with 20% ethyl acetate-hexane to give the title compound (450 mg, 29% yield).

$^1$H NMR (300 MHz, DMSO-$d_6$): δ 9.70 (s, 1H), 7.90-8.10 (m, 4H), 7.40 (d, 1H), 7.00 (d, 1H), 4.90 (br s, 2H).

**3-Benzyl-2-thioxo-thiazolidine-4-one**

Following general procedure B, bis(carboxymethyl)-trithiocarbonate (2.50 g, 11.0 mmoles), was reacted with benzylamine (1.07 g, 10.0 mmoles). The title compound was obtained after flash silica gel chromatography eluting with 70% methylene chloride-hexane, (960 mg, 43% yield).

**4-[5-(3-Benzyl-4-oxo-2-thioxo-thiazolidine-5-ylidenemethyl)-furan-2-yl]-benzene sulfonamide**

A solution of 4-(5-formyl-furan-2-yl)benzenesulfonyl amide (50 mg, 0.2 mmoles) and 3-benzyl-2-thioxo-thiazolidine-4-one (44.6 mg. 0.2 mmoles) in ethanol (5 ml) was added with piperidine (1 drop) and the mixture was refluxed for 30 minutes. After cooling to 0-5°C, the solid was filtered, washed with cold ethanol (2x5 ml) and dried under vacuum to give the title compound (73 mg, 80% yield).

$^1$H NMR (300 MHz, DMSO-$d_6$): d 7.95-8.25 (m, 4H), 7.75 (s, 1H),

7.20-7.55 (m, 10H), 5.25 (s, 2H).

**4-(5-Formyl-furan-2-yl)-benzonitrile**

Following general procedure A, 5-bromo furaldehyde (3.50 g, 0.2 moles) was reacted with 4-cyanoboronic acid (2.94 g, 0.2 moles). After usual work-up of the mixture and chromatographic purification on silica gel column eluting with 50% ethyl acetate-hexane the title compound was obtained (2.60 g, 66% yield).

$^1$H NMR (300 MHz, $CDCl_3$): δ 9.70 (s, 1H), 8.00 (d, 2H), 7.80 (d, 2H), 7.40 (d, 1H), 7.00 (d, 1H).

**2-Thioxo-3-(3,4-dimethoxy-phenylethyl)-thiazolidine-4-one**

Following general procedure B, bis(carboxymethyl) thiocarbonate (905 mg, 4.0 mmoles), was reacted with 3,4-dimethoxy-benzylamine (363 mg, 2.0 mmoles) and, after flash silica gel chromatography eluting with 70% methylene chloride-hexane, the title compound was obtained (420 mg, 71% yield).

$^1$H NMR (300 MHz, $CDCl_3$): δ 6.70-6.90 (m, 3H), 4.20 (t, 2H), 4.00 (s, 2H), 3.90 (s, 3H), 3.85 (s, 3H), 2.95 (t, 2H).

**4-(5-{3-[2-(3,4-Dimethoxy-phenyl)-ethyl]-oxo-2-thioxo-thiazolidine-5-ylidenemethyl}-furan-2-yl)-benzonitrile**

Following general procedure C, 4-(5-formyl-furan-2-yl)-benzonitrile (39.4 mg, 0.2 mmoles) was reacted with 2-thioxo-3-(3,4-dimethoxy-benzyl)-thiazolidine-4-one (59.4 mg, 0.2 mmoles) to give the title compound (58 mg, 61% yield).

$^1$H NMR (300 MHz, DMSO-$d_6$): δ 7.95-8.25 (m, 4H), 7.75 (s, 1H), 7.60 (d, 1H), 7.40 (d, 1H), 6.7-6.90 (m, 3H), 4.25 (t, 2H), 3.75 (s, 3H), 3.70 (s, 3H), 2.95 (s, 2H).

**3-(2-Phenylethyl)-2-thioxo-thiazolidine-4-one**

Following general procedure B, bis(carboxymethyl)trithiocarbonate

(905 mg, 4.0 mmoles), was reacted with 2-phenylethylamine (242.4 mg, 2.0 mmoles). The title compound was obtained after flash silica gel chromatography eluting with 70% methylene chloride-hexane, (246 mg, 52% yield).

$^1$HMR (300 MHz, $CDCl_3$): δ 7.15-7.40 (m, 5H), 4.20 (t, 2H), 3.95 (s, 2H), 3.00 (t, 2H).

**4-[5-(4-Oxo-3-(2-phenylethyl)-2-thioxo-thiazolidine-5-ylidenemethyl)-furan-2-yl]-benzonitrile**

Following general procedure C, 4-(5-formyl-furan-2-yl)-benzonitrile (39.4 mg, 0.2 mmoles) was reacted with 3-(2-phenylethyl)-2-thioxo-thiazolidine-4-one (47.4 mg, 0.2 mmoles) to give the title compound (64 mg, 76% yield).

$^1$H NMR (300 MHz, DMSO-$d_6$): δ 7.95-8.25 (m, 4H), 7.75 (s, 1H), 7.55 (d, 1H), 7.40 (d, 1H), 7.10-7.35 (m, 5H), 4.24 (t, 2H), 3.00 (t, 2H).

**3-Thiophenemethyl-2-thioxo-thiazolidine-4-one**

Following general procedure B, bis(carboxymethyl)trithiocarbonate (905 mg, 4.0 mmoles), was reacted with 2-(aminomethyl)thiophene (226.4 mg, 2.0 mmoles) to give the title compound (320 mg, 70% yield). $^1$HNMR ($CDCl_3$): δ 7.15-7.35 (m, 2H), 6.85-7.05 (m, 1H), 5.35 (s, 2H), 4.00 (s, 2H).

**4-[5-(4-Oxo-3-thiophene-2-yl-methyl-2-thioxo-thiazolidine-5-ylidenemethyl)-furan-2-yl]-benzoic acid methyl ester**

Following general procedure C, 4-(5-formyl-furan-2-yl)-benzoic acid methyl ester (46.0 mg, 0.2 mmoles) was reacted with 3-thiophenemethyl-2-thioxo-thiazolidine-4-one (45.8 mg, 0.2 mmoles) to give the title compound (38.7 mg, 44% yield).

$^1$H NMR (300 MHz, $CDCl_3$): δ 8.15 (d, 2H), 7.85 (d, 2H), 7.50 (s, 1H), 7.20-7.30 (m, 2H), 6.90-7.05 (m, 3H), 5.50 (s, 2H), 3.98 (s, 3H).

**4-[5-(4-Oxo-3-(2-phenylethyl)-2-thioxo-thiazolidine-5-ylidenemethyl)-furan-2-yl]-benzoic acid methyl ester**

Following general procedure C, 4-(5-formyl-furan-2-yl)-benzoic acid methyl ester (46.0 mg, 0.2 mmoles) was reacted with 3-(2-phenylethyl)-2-thioxo-thiazolidine-4-one (47.4 mg, 0.2 mmoles) to give the title compound (39.2 mg, 44% yield).

$^1$H NMR (300 MHz, $CDCl_3$): δ 8.00 (d, 2H), 7.65 (d, 2H), 6.70-7.15 (m, 8H), 4.15 (t, 2H), 3.88 (s, 3H), 2.85 (t, 2H).

**4-(5-{3-[2-(3,4-Dimethoxyphenyl)-ethyl]-4-oxo-2-thioxo-thiazolidine-5-ylidenemethyl}-furan-2-yl)-benzoic acid methyl ester**

Following general procedure C, 4-(5-formyl-furan-2-yl)-benzoic acid methyl ester (46.0 mg, 0.2 mmoles) was reacted with 2-thioxo-3-[2-(3,4-dimethoxy-phenylethyl)]-thiazolidine-4-one (59.4 mg, 0.2 mmoles (59.4 mg, 0.20 mmoles) to give the title compound (46.7 mg, 46% yield)

$^1$H NMR (300 MHz, $CDCl_3$): δ 8.20 (d, 2H), 7.85 (d, 2H), 7.50 (s, 1H), 6.85-7.05 (m, 5H), 4.45 (t, 2H), 4.00 (s, 3H), 3.88 (s, 3H), 3.85(s, 3H), 2.95 (t, 2H).

**3-(4-Methyl-benzyl)-2-thioxo-thiazolidine-4-one**

Following general procedure B, bis(carboxymethyl) trithiocarbonate (905 mg, 4.0 mmoles), was reacted with 4-methyl-benzyl amine (242 mg, 2.0 mmoles) to give the title compound (250 mg, 53% yield).

$^1$HNMR ($CDCl_3$): δ 7.40 (d, 2H), 7.15 (d, 2H), 5.20 (s, 2H), 4.00 (s, 2H), 2.35 (s, 3H).

**4-{5-[3-(4-Methyl-benzyl)-4-oxo-2-thioxo-thiazolidine-5-ylidenemethyl]-furan-2-yl}-benzoic acid methyl ester**

Following general procedure C, 4-(5-formyl-furan-2-yl)-benzoic acid methyl ester (46.0 mg, 0.2 mmoles) was reacted with 3-(4-methyl-benzyl)-2-thioxo-thiazolidine-4-one (47.4 mg, 0.2 mmoles) to give the title compound

(46.7 mg, 46% yield).

$^1$H NMR (300 MHz, $CDCl_3$): δ 8.20 (d, 2H), 7.85 (d, 2H), 7.50 (s, 1H), 7.40 (d, 1H), 7.15 (d, 1H), 6.90-7.05 (m, 4H), 5.30 (s, 2H), 3.95 (s, 2H), 2.30 (s, 3H).

**Primary biochemical assay for the inhibition of Biot-HIF-1α$^{786-826}$/GST-p300$^{323/423}$**

The compounds were evaluated for their ability to inhibit the interaction between Hif-1α and p300 using a fluorescence assay (DELFIA™). The procedure described by Freedman SJ at al., Nature Structural Biology 2003, 10 (7), 504-512 was suitably modified.

The human biotinylated Hif-1α fragment corresponding to C-terminal 786-826 amino acids (Biotinylated Hif-1α$^{786-826}$) was obtained from AnaSpec Inc (San Josè, California, USA) and used without further purifications.

A construct expressing the GST-p300$^{323-423}$ fragment was transformed in the BL21 strain (DE3) of E. coli. Said construct was obtained by cloning in the expression vector pGEX- 4T-1 (Amersham No. 27-45-80-01) the DNA sequence which encodes for the p300 region ranging from amino acids 323 to 423; the DNA sequence was obtained by PCR (Polymerase Chain Reaction). Protein expression was induced with 1 mM isopropyl-1-thio-ß-D-galactopyranoside (IPTG). The bacteria were lysed by sonication in the presence of a suitable buffer (50 mM Tris.HCl pH 8.00, 100 mM NaCl, 0.1 mM $ZnSO_4$, 1 mM DTT, 0.1 mg/ml lysozyme and a tablet of Complete EDTA-free Protease Inhibitor Cocktail Tablets (Roche, catalogue number 1 873 580)) and the GST fusion protein present in the soluble fraction was purified on a Glutathione-Sepharose 4B resin (Amersham Biosciences; no. 27-4574-01). The protein final concentration was determined according to Bradford with the Biorad assay (Bradford M., Anal. Biochem.,72, 248, (1976)) and sample purity was evaluated by SDS-PAGE. The samples were

stored at -80°C in 50% glycerol.

The assay was carried out using NUNC Maxisorp 96-well plates as follows.

C96 NUNC Maxisorp plates (from Nunc, product No. 446612) were incubated overnight with streptavidin (Sigma; product No. S 4762) at a final concentration of 1 µg/ml in PBS buffer (Phosphate Buffered Saline 10 mM sodium phosphate, 150 mM sodium chloride pH 7.4). Each well was subsequently washed with 3x300 µl of TBST buffer (50 mM Tris-HCl pH 8.0, 150 mM NaCl, 0.05% (v/v) Tween 20). Each well was then added with 100 µl of a 10 nM solution of biotinylated Hif-1$\alpha^{786-826}$ in TBSB (50 mM Tris-HCl pH 8.0, 150 mM NaCl, 5% (w/v) BSA (Sigma, product No. A 2153)) and incubated for 1h at 25°C. In the last row of each plate, TBSB buffer only was added. Each well was subsequently washed with 3 x 300 µl of TBST buffer. The thus prepared plate represented the assay plate.

Separately, a plate (daughter plate) containing in each well 10 µl of each test compound dissolved in DMSO to a concentration of 10 µM was prepared. This plate was added with 100 µl of a 111 pM solution of GST-p300$^{323-423}$ diluted in the incubation buffer (TBSB added with 0.1% (v/v) Tween 20, 0.5 mM DTT, 10 µM $ZnCl_2$) and the whole was mixed. 100 µl of the mixture contained in the daughter plate was immediately transferred to the assay plate.

Each daughter plate was prepared with 80 different compounds at a 10 µM concentration, safe for the last two well rows, wherein each well was added with 10 µl of DMSO. These two rows represented the positive (row 11, + Hif-1) and negative (row 12, - Hif-1) controls.

After incubation for 1 h at 25°C, each well was washed with 3 x 300 µl of TBST buffer (50 mM Tris-HCl pH 8.0, 150 mM NaCl, 0.05% (v/v) Tween 20). Each well was then added with 60.8 ng of an europium-labelled anti-GST

antibody (DELFIA Eu-N1 labeled; Perkin Elmer; product no. A D 0251) dissolved in 100 µl of TBST buffer containing 10 µM $ZnCl_2$. After incubation for 1 h at room temperature, each well was washed with 3 x 300 µl of TBST buffer, then 100 µl of signal amplification solution (Enhancement Solution, Perkin Elmer product No. 1244-105) was added.

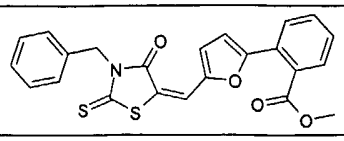
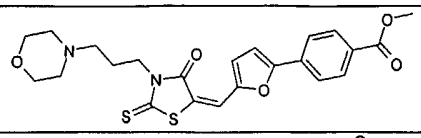
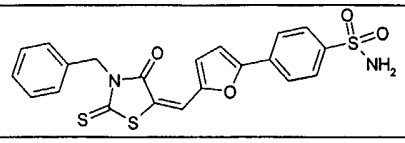
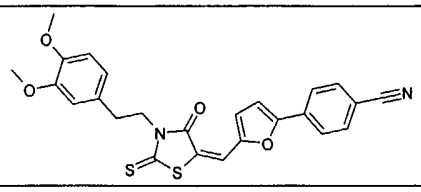
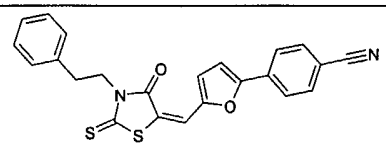
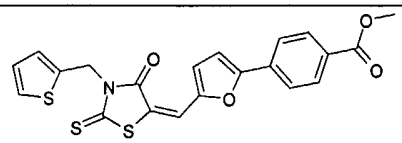
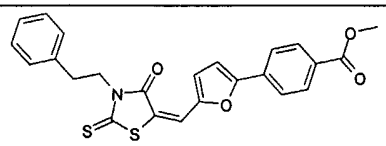
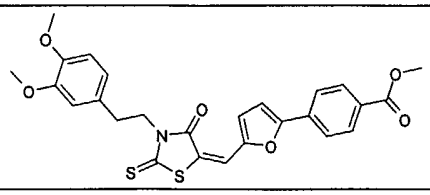
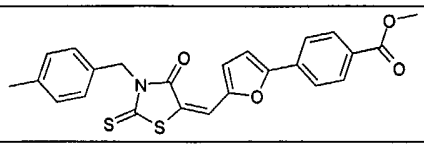
The plates were then read using a FUSION alpha-FP-HT reader (Perkin Elmer) in fluorescence mode for time resolution.

The activity of the compounds was calculated as follows. The fluorescence mean value of negative controls in row 12 of the plate test was subtracted from the fluorescence value of all the other wells. The resulting fluorescence value for each well was then divided by the fluorescence mean value of the positive controls in row 11 (which represent the maximum signal value, 100%) and expressed as percentage value. The inhibition value was expressed as the difference to 100 of the signal percentage calculated for each well.

Using daughter plates in which the compounds were present at 10 different concentrations ranging from 90 µM to 0.178 µM in each row, a dose-response curve could be calculated from which the $IC_{50}$ value (concentration of the compound necessary to inhibit the signal by 50%) was obtained. Rows 11 and 12 containing the vehicle only were the controls.

The $IC_{50}$ values obtained for some compounds of the present invention are reported in Table 1. The data are the mean of two independent experiments.

**Table 1**

| Structure | Compound | HIF/p300 ($IC_{50}$, μM) |
|---|---|---|
|  | 1 | 8.6 |
|  | 16 | 11.08 |
|  | 7 | 4.9 |
|  | 18 | 29.9 |
|  | 19 | 1.4 |
|  | 13 | 14.7 |
|  | 20 | 4.2 |
|  | 21 | 7.4 |
|  | 9 | 5.9 |

**Inhibition of VEGF production**

The compounds having inhibitory activity in the Hif-1α/p300 assay described above were evaluated using a cellular test on genetically modified human hepatocarcinoma Hep3B cells (Hep3B-VEGF*Luciferase*) in order to stably express a vector in which the Open Reading Frame of *firefly Luciferase*

is under the control of the rat VEGF gene promoter.

Hif-1 induction by deferoxamine (which causes hypoxia) induces luciferase transcription through activation of the VEGF promoter, which in turn increases luciferase activity, which can be measured with a commercially available kit. The compounds interfering with the Hif-1α/p300 complex inhibit Hif-dependent luciferase activation, resulting in a reduction of luciferase activity. Therefore, this assay allows to evaluate the activity of the compounds towards the VEGF promoter, which is essential to VEGF production and subsequent tumor angiogenesis.

The Hep-3B-VEGF *Luciferase* cell line was obtained according to the following procedure.

Human hepatocarcinoma cells Hep-3B (ATCC reference No. HB-8064) were seeded onto 6-well plates at a concentration of 2.5 x $10^5$ cells/well in 2 ml DMEM/10% FCS and the following day were transfected using Fugene 6 (Roche Biochemicals®). In each well, the transfection mixtures contained 6 µl of the transfection reagent Fugene 6, 1 µg of the pxp2-VEGF-luciferase reporter plasmid (VEGF rat promoter, NCBI GenBank No. of accession U22373, Levy et al., J. Biol. Chem. 270 (22), 13333-13340, 1995), and 10 ng of pcDNA3.1(+)plasmid (INVITROGEN) which makes the cells resistant to neomycin. Transfection was carried out according to the manufacturer's instructions.

A suitable cell population (phenotypically resistant to neomycin) was selected by means of a cloning approach based on the "dilution limit" procedure (Sambrook J., Fritsch E.F. and Maniatis T. (1989) Molecular Cloning, A Laboratory Manual; Cold Spring Harbor Laboratories). The subsequent assays for Luciferase expression/activity (Luciferase assay) and for the quantification of secreted VEGF in the supernatant (ELISA secreted VEGF test) were carried out with the stably transfected selected cells.

The following experimental protocol was used:

Day 1. Hep-3B –VEGF Luciferase cells were seeded onto "blank" 96-wells plates (Greiner) at a density of 1 x $10^4$ cells/well/125 µl of medium, then left to adhere overnight in thermostat (37°C/5% $CO_2$).

Day 2. 75 µl of "3.2x working solutions" of compound (previously prepared in culture medium so that the DMSO concentration is 1.6% v/v) were added to the cells (partial volume/well = 200 µl, partial concentration of compound = 1.2 x, partial concentration of DMSO = 0.6%). After 1 hour incubation in thermostat, hypoxia was induced chemically by addition of 40 µl/well of a 6x (600 µM) stock solution of deferoxamine (final volume/well = 240 µl, final concentration of compound = 1x, final concentration of DMSO = 0.5%, final concentration of deferoxamine = 1x ≈ 100 µM). The plates were then thermostated for further 18-20 hours.

Day 3. The Luciferase assay and the secreted VEGF ELISA test were carried out as follows.

**Secreted VEGF ELISA test**

Quantification of secreted VEGF was performed using the "DuoSet Elisa Development System human VEGF" kit (R&D Systems). 100 µl/well of the supernatant from the "blank" 96-well plates with the cells of the Hep3B/VEGF Luciferase clone were transferred into transparent 96-well plates (Maxisorp) and assayed according to the indications of the kit manufacturer.

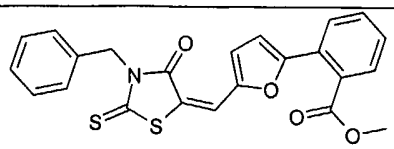
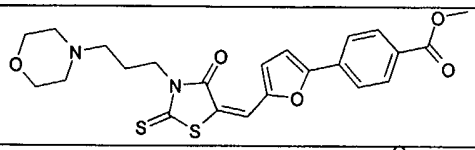
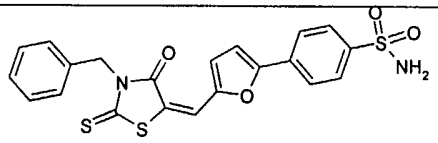
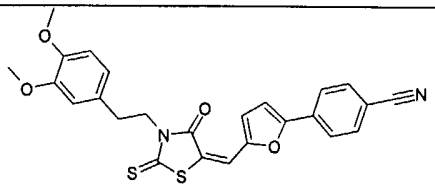
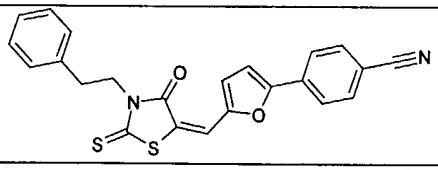
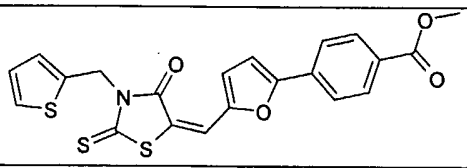
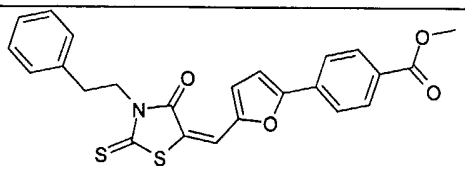
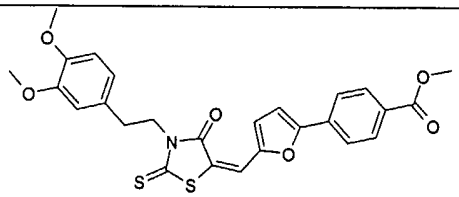
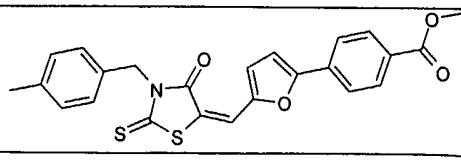
**Luciferase assay**

Quantification of the expression of the Luciferase reporter gene was performed by means of the Bright Glo Reagent (Promega). After removing the supernatant and washing once with PBS, 40 µl/well of Bright Glo Reagent were added to blank 96-well plates with Hep3B/VEGF-Luciferase cells. The expression levels of the reporter gene were determined by reading the plates with a luminometer.

$IC_{50}$ values (concentration of the compound that causes 50% inhibition

of the luciferase signal or 50% reduction of secreted VEGF) for some compounds of the invention are reported in table 2.

**Table 2**

| Compound | Structure | $IC_{50}$ (μM) | |
|---|---|---|---|
| | | Luciferase assay | ELISA test (secreted VEGF) |
| 1 |  | 6.66 | 0.37 |
| 16 |  | 2.07 | 0.70 |
| 7 |  | 3.16 | 0.87 |
| 18 |  | 3.17 | 0.49 |
| 19 |  | 7.29 | 0.33 |
| 13 |  | 9.64 | 0.58 |
| 20 |  | 10.47 | 0.46 |
| 21 |  | 4.86 | 0.24 |
| 9 |  | > 5.0 | 0.18 |

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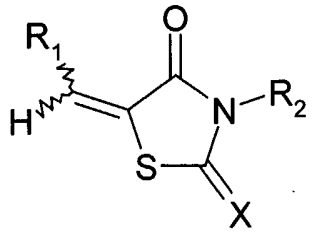
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## CLAIMS

1. Use of compounds of general formula (I)



(I)

in which

$R_1$ is aryl or heteroaryl;

$R_2$ is alkyl, alkenyl, cycloalkyl, heterocycloalkyl, aryl or heteroaryl;

X is oxygen or sulphur

and in which

"alkyl" is a straight or branched hydrocarbon containing 1 to 10 carbon atoms, preferably 1 to 8 carbon atoms, optionally substituted with one or more groups independently selected from cycloalkyl, heterocycloalkyl, aryl, heteroaryl, haloalkyl, halogen, hydroxy, alkoxy, mercapto, cyano, sulfonamido, aminosulfonyl, acyl, acyloxy and substituted amino;

"aryl" is an unsaturated cyclic aromatic hydrocarbon from 6 to 14 carbon atoms, optionally substituted with one or more substituents independently selected from alkyl, aryl, haloalkyl, halogen, hydroxy, alkoxy, mercapto, cyano, sulfonamido, aminosulfonyl, acyl, acyloxy, nitro, and substituted amino;

"heteroaryl" is a monocyclic or fused 5- to 10-membered aromatic ring containing one or more atoms preferably selected from nitrogen, oxygen and sulphur, optionally substituted with one or more groups independently selected from alkyl, aryl, haloalkyl, halogen, hydroxy,

alkoxy, mercapto, cyano, sulfonamido, aminosulfonyl, acyl, acyloxy, nitro and substituted amino;

"cycloalkyl" is a cyclic alkyl group containing 3 to 8 carbon atoms, optionally substituted with one or more substituents selected from alkyl, aryl, haloalkyl, halogen, hydroxy, alkoxy, mercapto, cyano, sulfonamido, aminosulfonyl, acyl, acyloxy, nitro and substituted amino;

"heterocycloalkyl" is a saturated or partially unsaturated monocyclic or condensed ring, containing one or more atoms preferably selected from nitrogen, oxygen and sulphur, and optionally substituted with one or more groups independently selected from alkyl, aryl, haloalkyl, halogen, hydroxy, alkoxy, mercapto, cyano, sulfonamido, aminosulfonyl, acyl, acyloxy, nitro and substituted amino;

"alkenyl" is a straight, cyclic or branched monovalent hydrocarbon radical, comprising at least one carbon-carbon double bond, optionally substituted with one or more groups independently selected from alkyl, acyl, cycloalkyl, heterocycloalkyl, aryl, haloalkyl, alkoxy and substituted amino;

"alkoxy" is a straight or branched $C_1$-$C_4$ alkoxy group;

" substituted amino " is a -NRR group, in which each R group is independently selected from hydrogen, acyl, alkyl, cycloalkyl, aryl, or the R groups can form, together with the nitrogen atom, a 5- or 6-membered heterocyclic ring;

"halogen" or the "halo" prefix means fluorine, chlorine, bromine and iodine;

"acyl" is a -C(O)-R" group, in which R" is preferably selected from hydrogen, hydroxy, alkyl, haloalkyl, cycloalkyl, aryl optionally substituted with one or more alkyl groups, haloalkyl, alkoxy, halogen and substituted amino groups, heteroaryl optionally substituted with one

or more alkyl groups, haloalkyl, alkoxy, halo and substituted amino groups and rings optionally substituted with one or more alkyl, haloalkyl, alkoxy, halo and substituted amino groups

for the preparation of pharmaceutical compositions for the treatment of pathologies in which inhibition of the interaction between HIF-1α and p300 is beneficial.

2. Use as claimed in claim 1 wherein in the compound of formula (**I**)

$R_1$ is furan, optionally substituted with a phenyl or substituted phenyl group with one or more groups selected from carboxy, methoxycarbonyl, cyano, sulfonamido and hydroxyethoxy;

$R_2$ is phenyl or benzyl, optionally substituted with one or more groups selected from methyl, trifluoromethyl, methoxy, methylenedioxy, or (2-thienyl)-methyl, morpholinyl-propyl, phenylethyl and (3,4-dimethoxyphenyl)ethyl

and X is sulphur.

3. Use as claimed in claim 2 in which the compound of formula (**I**) is selected from

2-[5-(3-benzyl-4-oxo-2-thioxo-thiazolidine-5-ylidenemethyl)-furan-2-yl]benzoic acid methyl ester (**1**);

4-{5-[3-(3,4,5-trimethoxybenzyl)-4-oxo-2-thioxo-thiazolidine-5-ylidenemethyl]-furan-2-yl}benzoic acid (**2**);

4-{5-[3-(3,4,5-trimethoxybenzyl)-4-oxo-2-thioxo-thiazolidine-5-ylidenemethyl]-furan-2-yl}benzoic acid methyl ester (**3**);

4-{5-[3-(2-trifluoromethylbenzyl)-4-oxo-2-thioxo-thiazolidine-5-ylidenemethyl]-furan-2-yl}benzoic acid methyl ester (**4**);

4-[5-(3-benzyl-4-oxo-2-thioxo-thiazolidine-5-ylidenemethyl)furan-2-yl-]benzonitrile (**5**);

4-{5-[3-(4-methylbenzyl)-4-oxo-2-thioxo-thiazolidine-5-

ylidenemethyl]furan-2-yl-}benzonitrile (**6**);

4-[5-(3-benzyl-4-oxo-2-thioxo-thiazolidine-5-ylidenemethyl)-furan-2-yl]benzenesulfonamide (**7**);

4-{5-[3-(4-methylbenzyl)-4-oxo-2-thioxo-thiazolidine-5-ylidenemethyl]-furan-2-yl}benzenesulfonamide (**8**);

4-{5-[3-(4-methylbenzyl)-4-oxo-2-thioxo-thiazolidine-5-ylidenemethyl]-furan-2-yl}benzoic acid methyl ester (**9**);

4-{5-[3-(4-methylbenzyl)-4-oxo-2-thioxo-thiazolidine-5-ylidenemethyl]-furan-2-yl}benzoic acid (**10**);

4-{5-[3-(3,4-methylenedioxybenzyl)-4-oxo-2-thioxo-thiazolidine-5-ylidenemethyl]-furan-2-yl}benzenesulfonamide (**11**);

4-{5-[3-(3,4-methylenedioxybenzyl)-4-oxo-2-thioxo-thiazolidine-5-ylidenemethyl]-furan-2-yl}benzoic acid methyl ester (**12**);

4-{5-[3-(2-thienyl)methyl-4-oxo-2-thioxo-thiazolidine-5-ylidenemethyl]-furan-2-yl}benzoic acid methyl ester (**13**);

4-{5-[3-(2-thienyl)methyl-4-oxo-2-thioxo-thiazolidine-5-ylidenemethyl]-furan-2-yl}benzonitrile (**14**);

4-{5-[3-(2-thienyl)methyl-4-oxo-2-thioxo-thiazolidine-5-ylidenemethyl]-furan-2-yl}benzenesulfonamide (**15**);

4-{5-[3-(morpholine-4yl-propyl)-4-oxo-2-thioxo-thiazolidine-5-ylidenemethyl]-furan-2-yl}benzoic acid methyl ester (**16**);

4-{5-[3-(3,4-dimethoxyphenyl)-4-oxo-2-thioxo-thiazolidine-5-ylidenemethyl]-furan-2-yl}benzoic acid (**17**);

4-{5-[3-[2-(3,4-dimethoxyphenyl)ethyl]-4-oxo-2-thioxo-thiazolidine-5-ylidenemethyl]-furan-2-yl}benzonitrile (**18**);

4-{5-[3-(2-phenyl)ethyl-4-oxo-2-thioxo-thiazolidine-5-ylidenemethyl]-furan-2-yl}benzonitrile (**19**);

4-{5-[3-[2-(phenyl)ethyl]-4-oxo-2-thioxo-thiazolidine-5-

ylidenemethyl]-furan-2-yl}benzoic acid (**20**);

4-{5-[3-[2-(3,4-dimethoxyphenyl)ethyl]-4-oxo-2-thioxo-thiazolidine-5-ylidenemethyl]-furan-2-yl}benzoic acid methyl ester (**21**);

2-{5-[4-[3-(2-phenyl)ethyl-4-oxo-2-thioxo-thiazolidine-5-ylidenemethyl]-furan-2-yl]phenoxy}ethanol (**22**);

3-benzyl-5-[[5-(2-methoxyphenyl)furan-2-ylidenemethyl]-4-oxo-2-thioxo-thiazolidine (**23**);

2-[5-(3-benzyl-4-oxo-2-thioxo-thiazolidine-5-ylidenemethyl)-furan-2-yl]benzoic acid (**24**).

4. Use according to any one of claims 1 to 3 for the preparation of antiangiogenic medicaments.

5. Use according to any one of claims 1 to 4 for the preparation of medicaments for the therapy of solid tumors.

6. Use as claimed in claim 5 in which the tumors are selected from lung carcinoma, mammary carcinoma, prostate carcinoma, neuroblastoma, glioblastoma multiforme, melanoma, central nervous system cancer, squamous cell oropharyngeal cancer, cervical cancer, ovary, esophageal, kidney, colon, head-and-neck cancer and oligodendrioglioma.

7. A method for inhibiting VEGF production in a cell which comprises contacting said cell with an effective amount of compound of formula (**I**).

8. A compound selected from:

4-{5-[3-(3,4,5-trimethoxybenzyl)-4-oxo-2-thioxo-thiazolidine-5-ylidenemethyl]-furan-2-yl}benzoic acid (**2**);

4-{5-[3-(3,4,5-trimethoxybenzyl)-4-oxo-2-thioxo-thiazolidine-5-ylidenemethyl]-furan-2-yl}benzoic acid methyl ester (**3**);

4-{5-[3-(2-trifluoromethylbenzyl)-4-oxo-2-thioxo-thiazolidine-5-ylidenemethyl]-furan-2-yl}benzoic acid methyl ester (**4**);

4-[5-(3-benzyl-4-oxo-2-thioxo-thiazolidine-5-ylidenemethyl)furan-2-yl-]

benzonitrile (**5**);

4-{5-[3-(4-methylbenzyl)-4-oxo-2-thioxo-thiazolidine-5-ylidenemethyl]furan-2-yl-} benzonitrile (**6**);

4-[5-(3-benzyl-4-oxo-2-thioxo-thiazolidine-5-ylidenemethyl)-furan-2-yl]benzenesulfonamide (**7**);

4-{5-[3-(4-methylbenzyl)-4-oxo-2-thioxo-thiazolidine-5-ylidenemethyl]-furan-2-yl}benzenesulfonamide (**8**);

4-{5-[3-(4-methylbenzyl)-4-oxo-2-thioxo-thiazolidine-5-ylidenemethyl]-furan-2-yl}benzoic acid methyl ester (**9**);

4-{5-[3-(4-methylbenzyl)-4-oxo-2-thioxo-thiazolidine-5-ylidenemethyl]-furan-2-yl}benzoic acid (**10**);

4-{5-[3-(3,4-methylenedioxybenzyl)-4-oxo-2-thioxo-thiazolidine-5-ylidenemethyl]-furan-2-yl}benzenesulfonamide (**11**);

4-{5-[3-(3,4-methylenedioxybenzyl)-4-oxo-2-thioxo-thiazolidine-5-ylidenemethyl]-furan-2-yl}benzoic acid methyl ester (**12**);

4-{5-[3-(2-thienyl)methyl-4-oxo-2-thioxo-thiazolidine-5-ylidenemethyl]-furan-2-yl}benzoic acid methyl ester (**13**);

4-{5-[3-(2-thienyl)methyl-4-oxo-2-thioxo-thiazolidine-5-ylidenemethyl]-furan-2-yl}benzonitrile (**14**);

4-{5-[3-(2-thienyl)methyl-4-oxo-2-thioxo-thiazolidine-5-ylidenemethyl]-furan-2-yl}benzenesulfonamide (**15**);

4-{5-[3-(3,4-dimethoxyphenyl)-4-oxo-2-thioxo-thiazolidine-5-ylidenemethyl]-furan-2-yl}benzoic acid (**17**);

4-{5-[3-[2-(3,4-dimethoxyphenyl)ethyl]-4-oxo-2-thioxo-thiazolidine-5-ylidenemethyl]-furan-2-yl}benzonitrile (**18**);

4-{5-[3-(2-phenyl)ethyl-4-oxo-2-thioxo-thiazolidine-5-ylidenemethyl]-furan-2-yl}benzonitrile (**19**);

4-{5-[3-[2-(phenyl)ethyl]-4-oxo-2-thioxo-thiazolidine-5-

ylidenemethyl]-furan-2-yl}benzoic acid (**20**);

4-{5-[3-[2-(3,4-dimethoxyphenyl)ethyl]-4-oxo-2-thioxo-thiazolidine-5-ylidenemethyl]-furan-2-yl}benzoic acid methyl ester (**21**);

2-{5-[4-[3-(2-phenyl)ethyl-4-oxo-2-thioxo-thiazolidine-5-ylidenemethyl]-furan-2-yl]phenoxy}ethanol (**22**);

3-benzyl-5-[[5-(2-methoxyphenyl)furan-2-ylidenemethyl]-4-oxo-2-thioxo-thiazolidine (**23**);

2-[5-(3-benzyl-4-oxo-2-thioxo-thiazolidine-5-ylidenemethyl)-furan-2-yl]benzoic acid (**24**).

# INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2005/013650

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K31/427 A61K31/5377 C07D417/06 C07D417/14 A61P35/00
A61P19/02 A61P17/06 A61P17/00 A61P27/02 A61P27/06

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K C07D

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | US 2004/002526 A1 (KLEIN J. PETER ET AL)<br>1 January 2004 (2004-01-01)<br>cited in the application<br>page 1, paragraph 3<br>page 2, paragraph 14-18<br>page 3, paragraph 36<br>compounds 26-28,34,35,37,38<br>page 17, paragraphs 49,50<br>claims 1,2,5-9,16-18,21-25,32,33,36-40<br>-----<br>-/-- | 1-8 |

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

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 25 April 2006 | 18/05/2006 |

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

# INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2005/013650

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | WO 2004/028535 A (PINTEX PHARMACEUTICALS, INC) 8 April 2004 (2004-04-08)<br>page 3, lines 12-14<br>page 10, line 15 - page 12, line 8<br>page 12, lines 29-35<br>page 41, column 2, paragraph 12<br>page 70, column 3, paragraph 5<br>page 88, lines 4-7<br>page 93, lines 1-20<br>page 93, line 35 - page 94, line 20<br>claims 1,6-14,17-22,24-26 | 1-8 |
| X | WO 98/53790 A (TEXAS BIOTECHNOLOGY CORPORATION; SCOTT, IAN, L; BIEDIGER, RONALD, J; M) 3 December 1998 (1998-12-03)<br>cited in the application<br>page 1, lines 6-9<br>page 10, line 16 - page 11, line 31<br>compound 1<br>page 16, lines 15-20<br>claims 3,5 | 1-5,7,8 |
| X | WO 2004/043955 A (RIGEL PHARMACEUTICALS, INC; SINGH, RAJINDER; RAMESH, USHA, V; GOFF, DA) 27 May 2004 (2004-05-27)<br>page 3, paragraph 13<br>page 5, paragraphs 19,20<br>page 6, paragraphs 25,26<br>compounds 155,178,181,184<br>page 52, paragraph 64-67 - page 53<br>claims 15-17 | 1-5,7,8 |
| X | DATABASE WPI 2 November 1999 (1999-11-02), Derwent Publications Ltd., London, GB;<br>Class 000,page 7, AN 2000-075331<br>XP002378133<br>KOBAYASHI K., NISHIYAMA T., NAKADE S.:<br>"Thiazolidine derivative, and medicine containing the same as active ingredient"<br>& JP 11 302280 A (ONO PHARMACEUTICAL CO)<br>2 November 1999 (1999-11-02)<br>abstract | 1-5,7,8 |
| X | -& Computer-generated translation of JP11302280<br>XP002378169<br>abstract<br>page 4, paragraph 7<br>page 3; compound 6<br>page 15, paragraph 56<br>table 6<br>table 12; compound 4<br>page 29, paragraph 95 | 1-5,7,8 |

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# INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2005/013650

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | WO 03/105840 A (THE PENNSYLVANIA STATE RESEARCH FOUNDATION; SMITH, CHARLES, D; FRENCH,) 24 December 2003 (2003-12-24)<br>page 1, lines 12-19<br>page 4, line 19 - page 5, line 22<br>page 15, line 3 - page 16, line 19<br>page 29, lines 16,17<br>table 1; compound 1<br>page 32, lines 2-9<br>page 33, lines 13-21<br>page 38, lines 18-29<br>page 45, line 15 - page 47, line 16<br>claims 1,12,26,27,49-51 | 1,4-7 |
| X | WO 2004/024058 A (CONSELHO NACIONAL DE DESENVOLVIMENTO CIENTIFICO E; DA ROCHA PITTA, IVA) 25 March 2004 (2004-03-25)<br>page 1, lines 2-7<br>page 1, line 28 - page 2, line 4<br>page 2, line 27 - page 3, line 7<br>claims 1-3,9<br>figures 1,3 | 1,4,5,7 |
| X | FR 2 169 334 A (BOEHRINGER SOHN,DT) 7 September 1973 (1973-09-07)<br>page 1, lines 1-16<br>page 2, lines 9-37<br>claims 1-3,9 | 1,4,5,7 |
| A | NICOLAUS B J R: "Symbiotic Approach to Drug Design"<br>DECISION MAKING IN DRUG RESEARCH, 1983, pages 173-186, XP002197412<br>the whole document | |
| P,X | US 2004/266846 A1 (YUAN JUNYING ET AL) 30 December 2004 (2004-12-30)<br>figures 1,2,4,11<br>page 1, paragraphs 6,8,10-13<br>page 2, paragraph 22-30<br>page 3, paragraph 43-49<br>page 4, paragraph 58 | 1,4-7 |

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# INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2005/013650

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| P,X | WO 2005/016227 A (INSIGHT BIOPHARMACEUTICALS LTD; VAN-GELDER, JOEL, M; MIRON, DAPHNA)<br>24 February 2005 (2005-02-24)<br>page 6, lines 1-12<br>page 9, line 17 - page 12, line 23<br>page 55, line 31 - page 56, line 27<br>page 62, lines 15-19<br>page 62, line 27 - page 64, line 9<br>page 70, line 22 - page 72, line 2<br>table 7<br>compounds 47,48<br>tables 8,9<br>page 103, line 3 - page 104, line 12<br>page 109, line 16 - page 111, line 12<br>claims<br>1,3,9,24,27-29,116,118,124,139,142,143,173<br>,331 | 1,2,4,5,<br>7 |
| P,X | WO 2005/076695 A (PAINCEPTOR PHARMA CORPORATION; QUEEN'S UNIVERSITY AT KINGSTON; ROSS, G)<br>25 August 2005 (2005-08-25)<br>page 8, line 21 - page 9, line 8<br>page 10, lines 26-31<br>page 11, lines 29-34<br>page 12, lines 15-19<br>page 12, line 26 - page 13, line 6<br>page 24, lines 3-10<br>page 43, lines 19-24<br>table 1; compounds<br>10,12,14,15,24,29,30,34,35,37,45,52,60,61<br>table 2; compounds<br>67,69,73,74,76,79,80,83,84,B<br>table 3; compounds<br>98,113,115-118,120,121,125,143<br>page 75, lines 1-4<br>page 75, line 16 - page 76, line 5<br>page 89, lines 26-33<br>page 96, line 9 - page 97, line 5<br>claims 10,34,36,37,51,52,56,57,97 | 1,2,4-7 |
| P,X | WO 2005/007141 A (PROTEOLOGICS, INC; ALROY, IRIS; TUVIA, SHMUEL; REISS, YUVAL; LEVI-HACH) 27 January 2005 (2005-01-27) | 1,4-7 |
| P,A | page 21, lines 1-7<br>page 65, lines 11-15<br>page 65, line 21 - page 66, line 5<br>table 7; compounds 27,109,110,115<br>table 9<br>claims 40,44 | 2 |

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# INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2005/013650

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| P,X | DATABASE WPI 24 March 2005 (2005-03-24), Derwent Publications Ltd., London, GB; Class 052,page 5, AN 2005-242351 XP002378134 ITAI A, KUBO A, MUTO S, SOTOME T, YAMAGUCHI Y: "plasminogen activator inhibitor-1 inhibitor" & WO 2005/026127 A (INST MEDICINAL MOLECULAR DESIGN INC) 24 March 2005 (2005-03-24) abstract | 1,2,4-7 |
| P,X | -& WO 2005/026127 A (INSTITUTE OF MEDICINAL MOLECULAR DESIGN. INC; MUTO, SUSUMU; KUBO, ASAK) 24 March 2005 (2005-03-24) pages 116-143 page 123; compound 64 pages 144-177 | 1,2,4-7 |

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# INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2005/013650

## Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claim 7 is directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

**Remark on Protest**

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2005/013650

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