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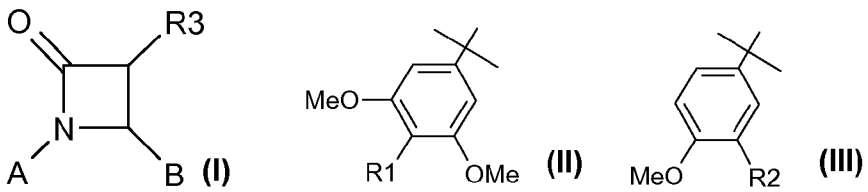
(54) Title: 1,4-DIARYL-2-AZETIDINONES WITH ANTI-TUMORAL ACTIVITY




(57) Abstract: Disclosed are compounds of formula (I): wherein one of A and B is the group:(II) wherein $R_1$ is selected from H and $OCH_3$, and the other is the group:(III) wherein $R_2$ is selected from H, OH, $NO_2$, $NH_2$; $R_3$ is selected from OH and $NH_2$;15 the salts, enantiomers and diastereoisomers thereof; with the exclusion of the following compounds: 3,4-cis-3-hydroxy-4-(3-nitro-4-methoxyphenyl)-1-(3,4,5- trimethoxyphenyl)azetidin-2-one; 3,4-cis-4-(3-amino-4-methoxyphenyl)-3-hydroxy-1-(3,4,5-20 trimethoxyphenyl)-azetidin-2-one; for use as antitumor agents.

# 1,4-DIARYL-2-AZETIDINONES WITH ANTI-TUMORAL ACTIVITY


## Summary of the invention

Disclosed are combretastatin A4 analogues having a 1,4-diaryl-2-azetidinone structure substituted at the 3-position by a hydroxyl or amino group. The compounds are useful as antitumor agents.


## Field of invention

The present invention relates to the use of 1,4-diaryl-2-azetidinones substituted at the 3-position by a hydroxyl or amino group in the treatment of tumors. A further object of the invention is novel enantiomeric forms of said 1,4-diaryl-2-azetidinones and pharmaceutical compositions containing them.

## State of the art

Combretastatins are a series of natural compounds extracted in the late 1980s by Pettit (Pettit et al., 1989) from an African plant called *Combretum caffrum*. These compounds have a stilbene structure, in the *cis* geometry with a double bond, and have, as a constant, the presence of three methoxy groups on an aromatic ring (ring A) and one methoxy group on the second ring (ring B), as shown in Figure 1.

Combretastatins have mainly antitumor activities and act on tubulin, where they bind to the site of the colchicine ligand, preventing its polymerization. Their action is antivascular. The active compounds rupture the vascular endothelium, leading to bleeding in the area of the tumour. Numerous derivatives have been prepared to date in which the substituents present in rings A and B are modified. See, for example, G. Tron et al., Journal of Medicinal Chemistry, 2006, 49(11), 3033-3044.

Among the compounds studied, those of greatest interest in view of their biological activity are combretastatin A4 and its amine derivative AC7739, both of which are at an advanced stage of clinical trials in oncology

patients in the form of water-soluble prodrugs, known as A4P (combretastatin A4 phosphate; Fosbretabulin) and AVE8062 (Ombrabulin) respectively.

The numerous structural modifications examined, in addition to those of the substituents on rings A and B, included the preparation of analogues with reduced conformational mobility, to prevent easy isomerization of the double bond from *cis* to *trans* with consequent loss of biological activity. In particular, the *cis* double bond was replaced by various rigid heterocyclic structures which allowed the *cis* ratio between the two aromatic rings A and B to be maintained.

In particular, rigid analogues of combretastatin A4 wherein the *cis* double bond was substituted by a 2-azetidinone ring are described by Carr et al, European Journal of Medicinal Chemistry, 2010, 45(12), 5752-5766. The compounds have a 1,4-diaryl-2-azetidinone structure wherein position 3 of the azetidinone ring is not substituted, or is substituted with one or two methyl groups.

O Boyle et al, Journal of Medicinal Chemistry 2010, 53(24), 8569-8584, describe 1,4-diaryl-2-azetidinones substituted at the 3-position of the azetidinone ring with an aryl group, and more recently with a heteroaryl, naphthyl, vinyl or functionalized alkyl group, Biorg. Med. Chem. 2011, 19, 2306-2325, WO 2011073211 A1.

1,4-diaryl-2-azetidinones substituted at the 3-position by a hydroxy, methoxy or acetoxy group are described by Sun et al, Bioorganic & Medicinal Chemistry Letters, 2004,14(9), 2041-2046. The products are obtained solely as *cis* cycloadducts, in which the reaction products are present as an equimolar mixture of enantiomers (R,S) and (S,R).

Inhibiting effects on tubulin polymerization and antiproliferative activity against tumour lines are reported for all the azetidinone derivatives described above.

**List of figures**

FIGURE 1 shows the structures of combretastatin A4, AC7739 and their prodrugs combretastatin A4 phosphate (Fosbretabulin) A4-P and AVE8062 (Ombrabulin).

FIGURE 2 shows the survival rate of HuTu-80 duodenal adenocarcinoma cells and Fhs74 normal small intestine cells after 72 h incubation with a 10 μM concentration of compounds representative of the invention or combretastatin A4.

FIGURE 3 shows the survival rate of human cell lines belonging to different tumour histotypes in the presence of 30 nM concentrations of compounds representative of the invention or combretastatin A4.

FIGURE 4 shows the DNA content evaluated by flow cytofluorometry (FACS) of HuTu-80 duodenal adenocarcinoma cells treated for 48 or 72 hours with 30 nM concentrations of compounds representative of the invention and combretastatin A4.

FIGURE 5 shows the sub-G1 DNA content, evaluated by flow cytofluorometry (FACS), of HuTu-80 duodenal adenocarcinoma cells treated for 48 or 72 hours with 30 nM concentrations of compounds representative of the invention or combretastatin A4.

FIGURE 6 shows the proteolytic fragments of caspase 3, evaluated by Western blot analysis with specific antibodies (anti-caspase 3 purchased from Cell Signaling Technologies) on cell extracts obtained from HuTu-80 duodenal adenocarcinoma cells treated for 24 hours with 30 nM concentrations of compounds representative of the invention or combretastatin A4.

FIGURE 7 shows the proteolytic fragments of PARP (poly-ADP-ribose polymerase) evaluated by Western blot analysis with specific antibodies (anti-PARP purchased from Cell Signaling Technologies) on cell extracts

obtained from HuTu-80 duodenal adenocarcinoma cells treated for 24 hours with 30 nM concentrations of compounds representative of the invention or combretastatin A4.

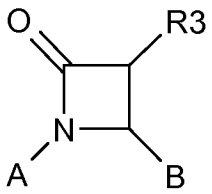
FIGURE 8 shows the phosphorylation of AMPK on Thr172, evaluated by Western blot analysis with specific antibodies (anti-phospho-AMPKα, Thr172, purchased from Cell Signaling Technologies) on cell extracts obtained from HuTu-80 duodenal adenocarcinoma cells treated for 24 hours with 30 nM concentrations of compounds representative of the invention or combretastatin A4.

FIGURE 9 shows the phosphorylation of oncosuppressor p53 on Ser15, evaluated by Western blot analysis with specific antibodies (anti-phospho-p53, Ser15, purchased from Cell Signaling Technologies) on cell extracts obtained from HuTu-80 duodenal adenocarcinoma cells treated for 24 hours with 30 nM concentrations of compounds representative of the invention or combretastatin A4.

**Description of the invention**

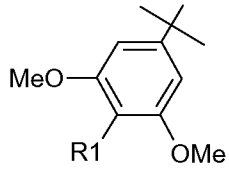
It has now been found that combretastatin A4 analogues having a 1,4-diaryl-2-azetidinone structure substituted at the 3-position by a hydroxy or amino group possess more advantageous properties than combretastatins and the azetidinone derivatives thereof previously described, which make them particularly useful as antitumor agents.

One object of this invention is compounds of formula (I)

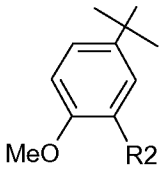



**(I)**

wherein one of A and B is the group:




wherein $R_1$ is selected from H and $OCH_3$, and the other is the group:




wherein $R_2$ is selected from H, OH, $NO_2$, $NH_2$;

$R_3$ is selected from OH and $NH_2$;

the salts, enantiomers and diastereoisomers thereof;

with the exclusion of the following compounds:

- 3,4-*cis*-3-hydroxy-4-(3-nitro-4-methoxyphenyl)-1-(3,4,5-trimethoxy-phenyl)-azetidin-2-one (compound N);
- 3,4-*cis*-4-(3-amino-4-methoxyphenyl)-3-hydroxy-1-(3,4,5-trimethoxy-phenyl)-azetidin-2-one (compound O);

for use as antitumor agents.

Examples of the compounds of the invention are:

- (±) 3,4-*cis*-1-(3,5-dimethoxyphenyl)-3-hydroxy-4-(4-methoxyphenyl)-azetidin-2-one (**rac-7**-*cis;* compound **A**);
- (±) 3,4-*trans*-1-(3,5-dimethoxyphenyl)-3-hydroxy-4-(4-methoxy-phenyl)-azetidin-2-one (**rac-7**-*trans;* compound **B**);
- (±) 3,4-*cis*-3-amino-1-(3,5-dimethoxyphenyl)-4-(4-methoxyphenyl)-azetidin-2-one (**rac-15**-*cis;* compound **U**);
- (±) 3,4-*trans*-3-amino-1-(3,5-dimethoxyphenyl)-4-(4-methoxyphenyl)-azetidin-2-one (**rac-15**-*trans;* compound **V**);
- (±) 3,4-*trans*-3-hydroxy-4-(3-hydroxy-4-methoxyphenyl)-1-(3,4,5-trimethoxy-phenyl)azetidin-2-one (**rac-8**-*trans;* compound **E**);
- (±) 3,4-*cis*-3-hydroxy-4-(3-hydroxy-4-methoxyphenyl)-1-(3,4,5-

trimethoxyphenyl)azetidin-2-one (**rac-8**-*cis*; compound **F**);

- (±) 3,4-*trans*-3-amino-4-(3-hydroxy-4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (**rac-16**-*trans*; compound **M**);
- (±) 3,4-*cis*-3-amino-4-(3-hydroxy-4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (**rac-16**-*cis;* compound **K**);
- (±) 3,4-*trans*-3-hydroxy-4-(3-nitro-4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (**rac-26**-*trans;* compound **Z**);
- 3,4-t*rans*-4-(3-amino-4-methoxyphenyl)-3-hydroxy-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (**rac-27**-*trans;* compound **Y**);
- (±) 3,4-*trans*-4-(3,5-dimethoxyphenyl)-3-hydroxy-1-(4-methoxy-phenyl)-azetidin-2-one (**rac-11**-*trans;* compound **C**);
- (±) 3,4-*cis*-4-(3,5-dimethoxyphenyl)-3-hydroxy-1-(4-methoxyphenyl)azetidin-2-one (**rac-11**-*cis;* compound **D**);
- (±) 3,4-*cis*-3-hydroxy-1-(3-hydroxy-4-methoxyphenyl)-4-(3,4,5-trimethoxyphenyl)azetidin-2-one (**rac-12**-*cis;* compound **G**);
- (±) 3,4-*trans*-3-hydroxy-1-(3-hydroxy-4-methoxyphenyl)-4-(3,4,5-trimethoxyphenyl)azetidin-2-one (**rac-12**-*trans;* compound **T***)*;
- (±) 3,4-*trans*-3-amino-1-(3-hydroxy-4-methoxyphenyl)-4-(3,4,5-trimethoxyphenyl)azetidin-2-one (**rac-20**-*trans;* compound **I**);
- (±) 3,4-*cis*-3-amino-1-(3-hydroxy-4-methoxyphenyl)-4-(3,4,5-trimethoxyphenyl)azetidin-2-one (**rac-20**-*cis;* compound **R**).

The preferred compounds of the invention are:

- (±) 3,4-*trans*-1-(3,5-dimethoxyphenyl)-3-hydroxy-4-(4-methoxyphenyl)azetidin-2-one (**rac-7**-*trans;* compound **B**);
- (±) 3,4-*trans*-3-amino-1-(3,5-dimethoxyphenyl)-4-(4-methoxyphenyl)-azetidin-2-one (**rac-15**-*trans;* compound **V**);
- (±) 3,4-*trans*-3-hydroxy-4-(3-hydroxy-4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (**rac-8**-*trans;* compound **E**);

- (±) 3,4-*trans*-3-amino-4-(3-hydroxy-4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (**rac-16**-*trans;* compound **M**);
- (±) 3,4-*trans*-3-hydroxy-4-(3-nitro-4-methoxyphenyl)-1-(3,4,5-trimethoxy-phenyl)azetidin-2-one (**rac-26**-*trans;* compound **Z**);
- (±) 3,4-t*rans*-4-(3-amino-4-methoxyphenyl)-3-hydroxy-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (**rac-27**-*trans;* compound **Y**);
- (±) 3,4-*trans*-4-(3,5-dimethoxyphenyl)-3-hydroxy-1-(4-methoxyphenyl)azetidin-2-one (**rac-11**-t*rans;* compound **C**);
- (±) 3,4-*trans*-3-hydroxy-1-(3-hydroxy-4-methoxyphenyl)-4-(3,4,5-trimethoxyphenyl)azetidin-2-one (**rac-12**-*trans;* compound **T**);
- (±) 3,4-*trans*-3-amino-1-(3-hydroxy-4-methoxyphenyl)-4-(3,4,5-trimethoxyphenyl)azetidin-2-one (**rac-20**-*trans;* compound **I**).

Particularly preferred compounds of the invention are:

- (±) 3,4-*trans*-1-(3,5-dimethoxyphenyl)-3-hydroxy-4-(4-methoxyphenyl)azetidin-2-one (**rac-7**-*trans;* compound **B**);
- (±) 3,4-*trans*-3-hydroxy-4-(3-hydroxy-4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (**rac-8**-*trans;* compound **E**).

The compounds of formula (I) can form pharmaceutically acceptable basic or acid addition salts with inorganic or organic bases, or with inorganic or organic acids, respectively. Said salts are also included in the scope of the present invention. Examples of inorganic or organic bases useful for this purpose are sodium, potassium and ammonium hydroxides; aliphatic amines such as triethylamine; amino alcohols such as ethanolamine: amino acids, such as glycine; and amino glycosides such as glucosamine. Examples of inorganic or organic acids are hydrochloric, sulphuric or phosphoric acid; citric acid, maleic acid, fumaric acid, tartaric acid, succinic acid and methanesulphonic acid.

The compounds of the invention can be prepared according to known

methods, such as the Staudinger reaction, which involves cycloaddition of a suitable imine of formula (II):

A-N = CH-B

**(II)**

wherein A and B are as defined for the compounds of formula (I), with a ketene derivative generated *in situ* from acetoxy acetyl chloride or N-phthaloylglycinyl chloride, to give a compound of formula (I) wherein $R_3$ is an acetoxy group or an N-phthalimido group respectively, which then undergoes hydrazinolysis to give compounds of formula (I) wherein $R_3$ is hydroxy or amino.

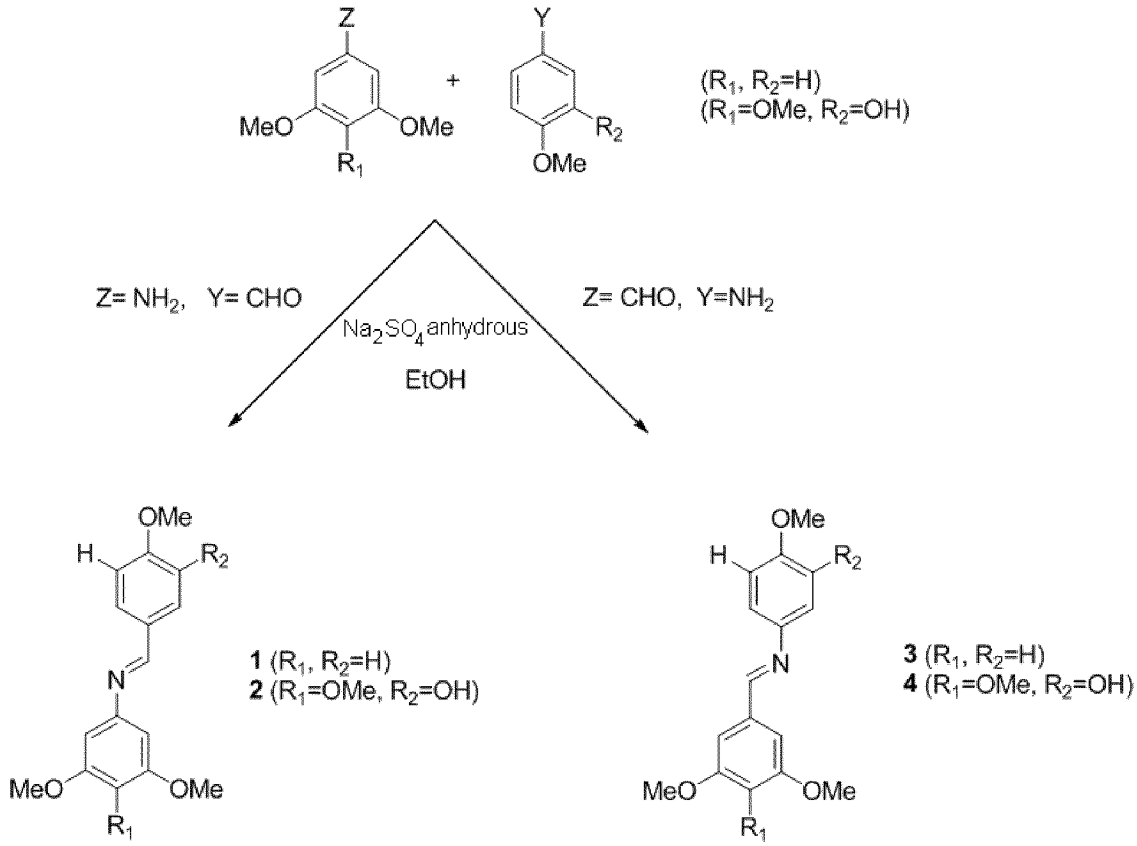
Synthesis schemes 1-5 exemplify the preparation of specific compounds of the invention.

Scheme **1** depicts the synthesis of imines **1-4** belonging to general formula (II).

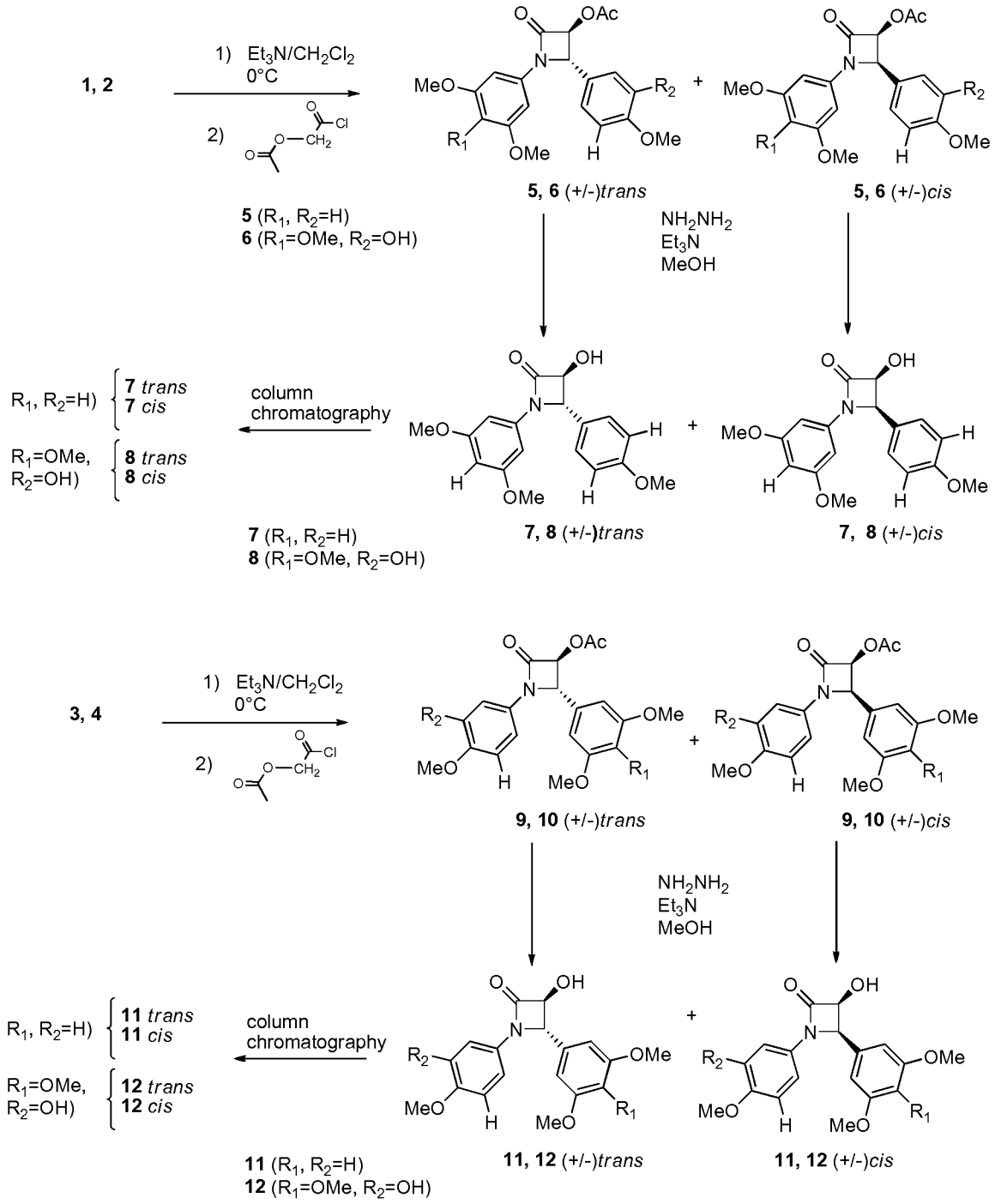
Scheme **2** depicts the synthesis of the *cis* and *trans* isomers (racemates) of compounds **7, 8, 11** and **12** according to the invention (compounds **A**, **B**, **E**, **F**, **C**, **D**, **G** and **T**).

Scheme **3** depicts the synthesis of the *cis* and *trans* isomers of compounds **15, 16** and **20** according to the invention (compounds **V**, **U**, **M**, **K**, **I** and **R**).

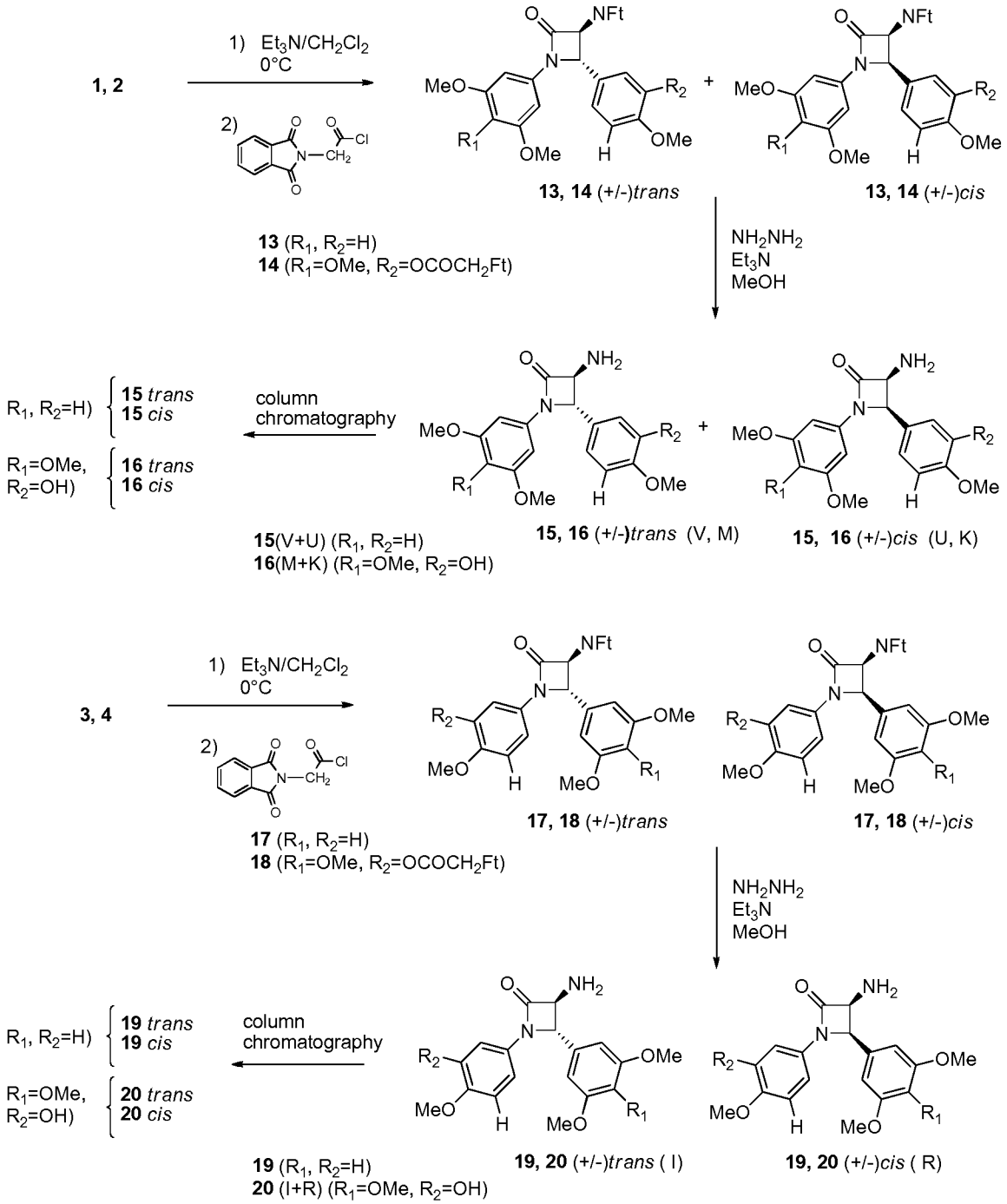
Scheme **4** depicts the synthesis of the *trans* isomers of nitro-derivative **26** and amino-derivative **27** according to the invention (compounds **Z** and **Y**).


Z
Y
MeO
OMe
R1
R2
OMe
(R1, R2=H)
(R1=OMe, R2=OH)
Z= NH2, Y= CHO
Na2SO4 anhydrous
EtOH
Z= CHO, Y=NH2
OMe
H
R2
N
MeO
OMe
R1
1 (R1, R2=H)
2 (R1=OMe, R2=OH)
3 (R1, R2=H)
4 (R1=OMe, R2=OH)

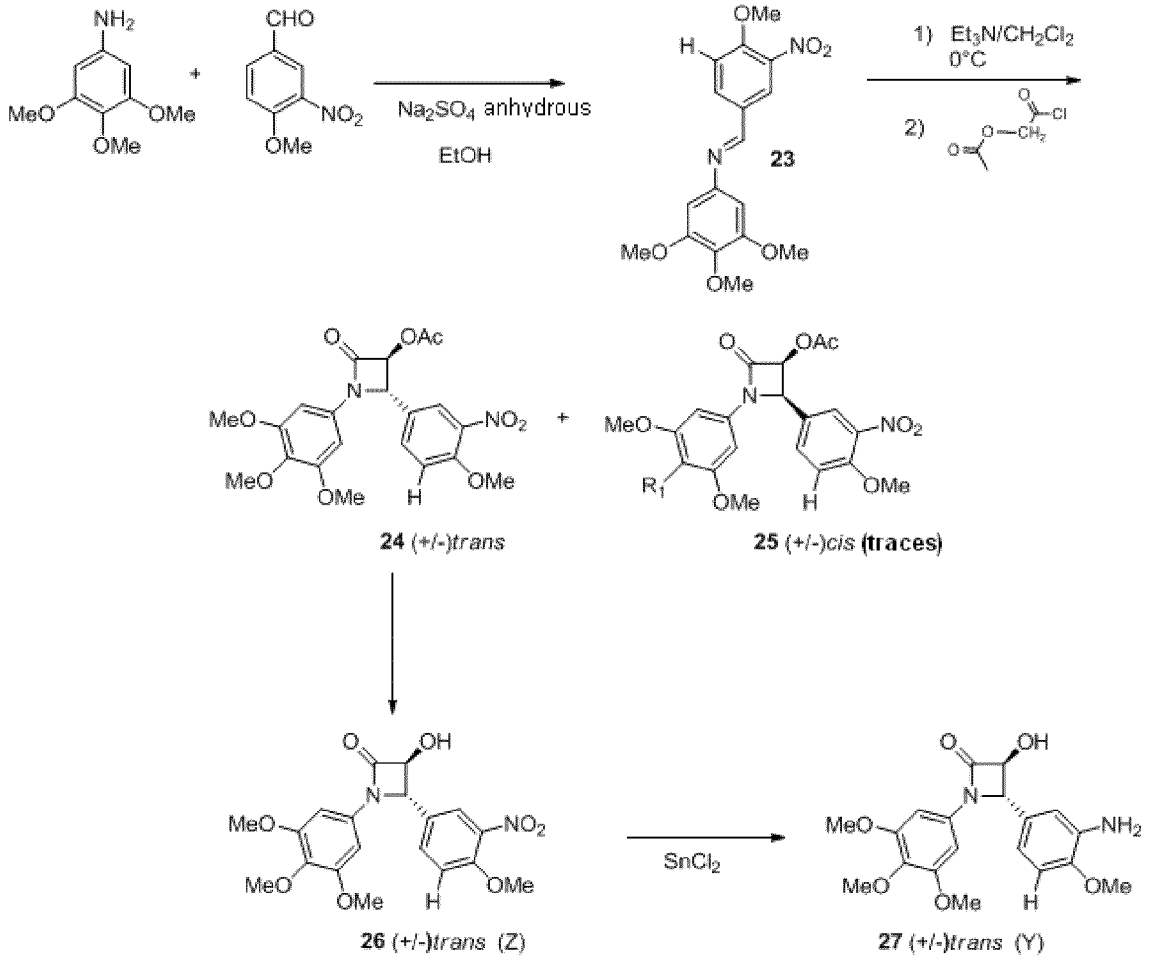
**Scheme 1**


1, 2
1) Et3N/CH2Cl2
0°C
2)
5 (R1, R2=H)
6 (R1=OMe, R2=OH)
5, 6 (+/-)trans
5, 6 (+/-)cis
NH2NH2
Et3N
MeOH
R1, R2=H)
7 trans
7 cis
R1=OMe,
R2=OH)
8 trans
8 cis
column
chromatography
7 (R1, R2=H)
8 (R1=OMe, R2=OH)
7, 8 (+/-)trans
7, 8 (+/-)cis
3, 4
1) Et3N/CH2Cl2
0°C
2)
9, 10 (+/-)trans
9, 10 (+/-)cis
NH2NH2
Et3N
MeOH
R1, R2=H)
11 trans
11 cis
R1=OMe,
R2=OH)
12 trans
12 cis
column
chromatography
11 (R1, R2=H)
12 (R1=OMe, R2=OH)
11, 12 (+/-)trans
11, 12 (+/-)cis

**Scheme 2**


1, 2
1) Et3N/CH2Cl2 0°C
2)
13, 14 (+/-)trans
13, 14 (+/-)cis
13 (R1, R2=H)
14 (R1=OMe, R2=OCOCH2Ft)
NH2NH2 Et3N MeOH
R1, R2=H)
15 trans
15 cis
R1=OMe, R2=OH)
16 trans
16 cis
column chromatography
15, 16 (+/-)trans (V, M)
15, 16 (+/-)cis (U, K)
15(V+U) (R1, R2=H)
16(M+K) (R1=OMe, R2=OH)
3, 4
17, 18 (+/-)trans
17, 18 (+/-)cis
17 (R1, R2=H)
18 (R1=OMe, R2=OCOCH2Ft)
19 trans
19 cis
20 trans
20 cis
19, 20 (+/-)trans ( I)
19, 20 (+/-)cis ( R)
19 (R1, R2=H)
20 (I+R) (R1=OMe, R2=OH)

**Scheme 3**

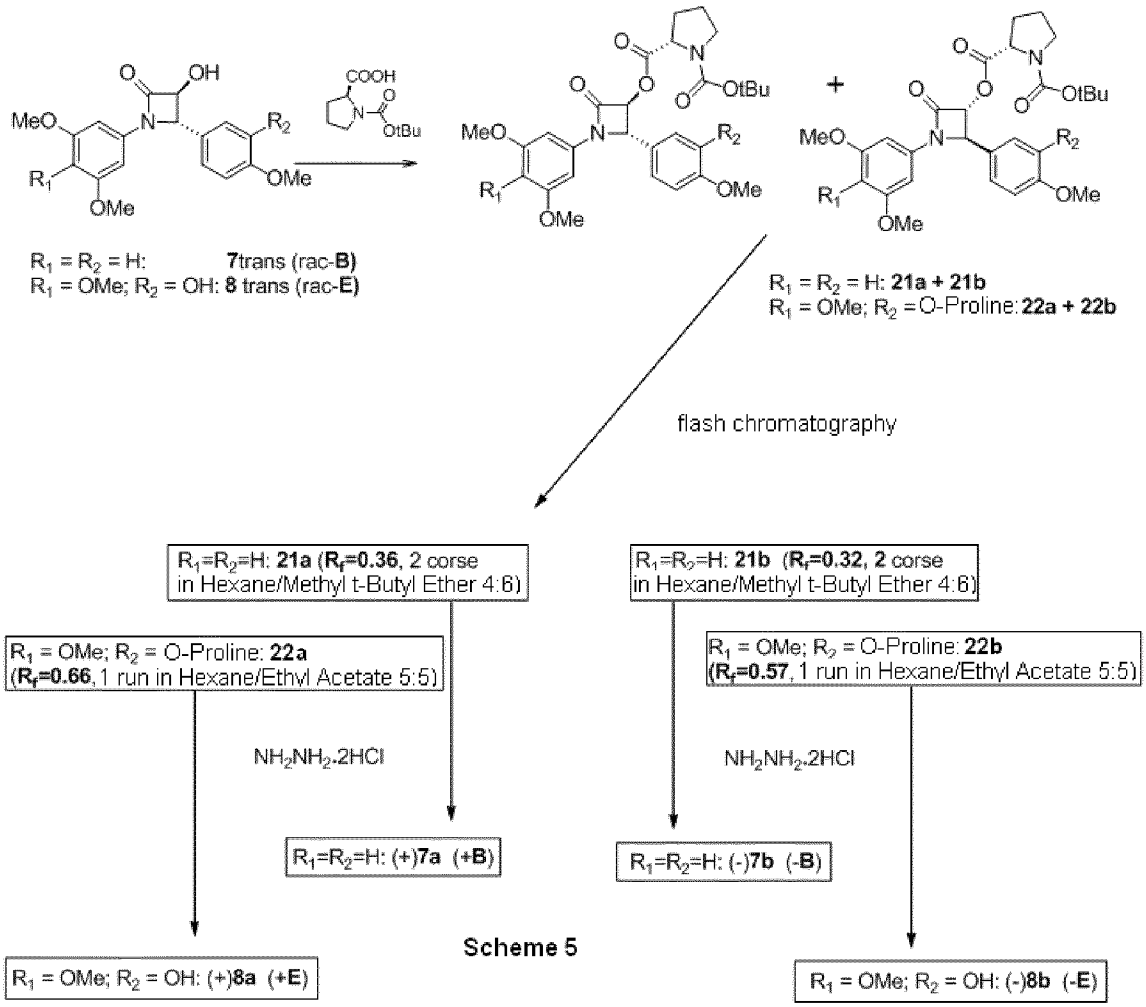



**Scheme 4**

According to the experimental conditions used, and detailed in the examples of the present invention, the compounds of the invention can be obtained in the form of *cis* or *trans* isomers with reference to the substituents present in the 3 and 4 positions of the azetidinone ring. Using the known conditions of the Staudinger reaction, the *cis* and *trans* isomers of the compounds of the invention are obtained as racemic mixtures, which can be resolved into the single enantiomers by conventional methods, such as chiral chromatography or derivatization with chiral reagents to give diastereoisomeric mixtures which can be separated into the single diastereoisomers by crystallisation or chromatography techniques and then converted to the single enantiomers.

One example of this last method is illustrated in scheme 5, which shows

the resolution of compounds **7** and **8** (**B** and **E**) and the production of enantiomers **7a**, **7b**, **8a** and **8b** (also indicated by the letters **B(+)**, **B(-)**, **E(+)** and **E(-)**). With the appropriate variations, well-known to the skilled person, this scheme can also be used to prepare the single enantiomers of the racemic mixtures of the other compounds of general formula (I).




**Scheme 5**

Compounds **7a**, **7b**, **8a**, and **8b** are novel. A further object of the invention is therefore a compound selected from:

- **(+)**-3,4-*trans*-1-(3,5-dimethoxyphenyl)-3-hydroxy-4-(4-methoxyphenyl)azetidin-2-one ((+)*trans* **7a;** compound **B(+)**);
- **(-)**-3,4-*trans*-1-(3,5-dimethoxyphenyl)-3-hydroxy-4-(4-methoxyphenyl)azetidin-2-one ((-) *trans* **7b;** compound **B(-)**);
- (+)-3,4-*trans*-3-hydroxy-4-(3-hydroxy-4-methoxyphenyl)-1-(3,4,5-

trimethoxyphenyl)azetidin-2-one ((+) *trans* **8a;** compound **E(+)**);

- (-)-3,4-*trans*-3-hydroxy-4-(3-hydroxy-4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one ((-) *trans* **8b;** compound **E(-)**).

Particularly preferred are the compounds

- **(+)**-3,4-*trans*-1-(3,5-dimethoxyphenyl)-3-hydroxy-4-(4-methoxyphenyl)azetidin-2-one ((+)*trans* **7a;** compound **B(+)**);
- (+)-3,4-*trans*-3-hydroxy-4-(3-hydroxy-4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one ((+) *trans* **8a;** compound **E(+)**).

The compounds of the invention have been pharmacologically tested on human tumour lines. After 72 hours' incubation of the cells with the compound to be tested, the cytotoxicity was determined with the MTT test, which quantifies cell viability by assaying mitochondrial dehydrogenase (L. Fang et. al., J Cancer Res Clin Oncol., 2008, 134(12): 1337-45). The data obtained demonstrate that the compounds according to the present invention possess marked activity against cell lines belonging to different human tumour histotypes, such as duodenal adenocarcinoma, colon adenocarcinoma, rectal adenocarcinoma, cervical cancer, breast cancer and neuroblastoma, whereas it possesses no cytotoxic effects against normal cell lines. In particular, the compounds of the invention are markedly more potent than the azetidinone derivatives 3,4-*cis*-3-hydroxy-4-(3-nitro-4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (compound N) and 3,4-*cis*-4-(3-amino-4-methoxyphenyl)-3-hydroxy-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (compound O) described by Sun et al, Bioorganic & Medicinal Chemistry Letters, 2004,14(9), 2041-2046.

The compounds of the invention arrest cell proliferation and markedly induce cell death due to apoptosis, mediated by activation of AMPK protein kinase and oncosuppressor p53. This effect is specific to tumour cell lines.

The compounds of the invention, when administered to mammals with

tumors, are therefore useful to control tumour growth and the formation of metastases, in particular in the treatment of tumors whose growth is supported by abnormal neo-vascularization processes.

Examples of tumors which can be advantageously treated with the compounds of the invention are duodenal adenocarcinoma, colorectal adenocarcinoma, cervical cancer, breast cancer and neuroblastoma. The compounds could also be used for the treatment of lung cancer, large-cell lung cancer, hepatocarcinoma, pancreatic adenocarcinoma, renal carcinoma, prostate cancer, testicular cancer, ovarian cancer, bladder cancer and melanoma.

The compounds according to the present invention can be administered at doses ranging from 0.01 mg to 1 g per kg of body weight a day. A preferred method of administration employs a dose of approx. 1 mg to approx. 50 mg per kg of body weight a day, using unit doses which administer approx. 70 mg to approx. 3.5 g of active substance in 24 hours to a patient weighing approx. 70 Kg. Such a method of administration can be adjusted to obtain a better therapeutic effect. For example, the doses can be adjusted on the basis of the patient's therapeutic situation. The compounds of the invention can be administered orally, intravenously, intramuscularly or subcutaneously.

When administered according to well-known therapeutic procedures, in combination with other agents used to induce the regression of tumors, the compounds of the invention synergically increase the antitumor effects of said compounds. Examples of compounds which can be used in combination with the compounds of the invention are cisplatin, carboplatin, doxorubicin, topotecan, taxol, taxotere, vincristine, 5-fluorouracil and dacarbazine, or in combination with radiotherapy.

A further aspect of the present invention relates to a compound selected from:

- **(+)**-3,4-*trans*-1-(3,5-dimethoxyphenyl)-3-hydroxy-4-(4-methoxyphenyl)azetidin-2-one ((+)*trans* **7a;** compound **B(+)**);
- **(-)**-3,4-*trans*-1-(3,5-dimethoxyphenyl)-3-hydroxy-4-(4-methoxyphenyl)azetidin-2-one ((-) *trans* **7b**; compound **B(-)**);
- (+)-3,4-*trans*-3-hydroxy-4-(3-hydroxy-4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one ((+) *trans* **8a;** compound **E(+)**);
- (-)-3,4-*trans*-3-hydroxy-4-(3-hydroxy-4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one ((-) *trans* **8b;** compound **E(-)**);

as a medicament, in particular as an antitumor agent.

Particularly preferred are the compounds:

- **(+)**-3,4-*trans*-1-(3,5-dimethoxyphenyl)-3-hydroxy-4-(4-methoxyphenyl)azetidin-2-one ((+)*trans* **7a;** compound **B(+)**);
- (+)-3,4-*trans*-3-hydroxy-4-(3-hydroxy-4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one ((+) *trans* **8a;** compound **E(+)**);

Another aspect of the invention relates to a pharmaceutical composition containing a therapeutically effective quantity of a compound selected from:

- **(+)**-3,4-*trans*-1-(3,5-dimethoxyphenyl)-3-hydroxy-4-(4-methoxyphenyl)azetidin-2-one ((+)*trans* **7a;**compound **B(+)**);
- **(-)**-3,4-*trans*-1-(3.5-dimethoxyphenyl)-3-hydroxy-4-(4-methoxyphenyl)azetidin-2-one ((-) *trans* **7b;** compound **B(-)**);
- (+)-3,4-*trans*-3-hydroxy-4-(3-hydroxy-4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one ((+) *trans* **8a;** compound **E(+)**);
- (-)-3,4-*trans*-3-hydroxy-4-(3-hydroxy-4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one ((-) *trans* **8b;** compound **E(-)**);

mixed with a compatible excipient for pharmaceutical use.

For therapeutic use, the compounds of the invention can be suitably formulated with physiologically acceptable excipients or carriers. The suitable pharmaceutical forms can vary according to the specific compound and the

administration route. The dose of active ingredient will be determined on each occasion, on the basis of the severity of the disease to be treated and the patient's general condition. Suitable pharmaceutical compositions can be prepared in accordance with the indications reported in Remington's Pharmaceutical Sciences, 18th ed., Mack Publishing Co.

The pharmaceutical compositions according to the present invention contain therapeutically effective quantities of at least one compound according to the invention, mixed with excipients compatible with pharmaceutical use.

The oral compositions generally contain an inert diluent or an edible carrier, and can be enclosed in gelatin capsules or compressed into tablets. Other possible forms for oral administration are capsules, pills, elixirs, suspensions and syrups.

Said tablets, pills, capsules and similar compositions can contain the following ingredients in addition to the compound of formula I: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; a carrier such as starch or lactose, a disintegrating agent such as alginic acid, primogel, corn starch or the like; a lubricant such as magnesium stearate; a fluidifier such as colloidal silicon dioxide; a sweetener such as saccharose or saccharine or a flavouring such as mint flavouring, methyl salicylate or orange flavouring. When the selected composition is in capsule form, it can also contain a liquid carrier such as a fatty oil. Other compositions can contain various materials, e.g. coating agents (for tablets and pills) such as sugar or shellac. The material used to prepare the compositions should be pharmaceutically pure, and non-toxic at the doses used.

To prepare pharmaceutical compositions for parenteral administration, the active ingredient can be included in solutions or suspensions, which can also contain the following constituents: a sterile diluent such as water for injectables, saline solution, oil, polyethylene glycol, glycerin, propylene

glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol; antioxidants such as ascorbic acid or sodium bisulphite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates, and agents to adjust the tonicity of the solution, for example sodium chloride or dextrose. The parenteral preparations can be enclosed in vials, disposable syringes, and glass or plastic bottles.

The invention will now be further illustrated by the following examples.

**Example 1 - Preparation of the compounds of formula (I)**

- ***Synthesis of imine 1 (Scheme 1)***

3,5-Dimethoxy aniline (3.0 g, 19.6 mmol) and 4-methoxy benzaldehyde (2.67 g, 19.6 mmol) are dissolved in 6 mL of EtOH under stirring at room temperature, and anhydrous $Na_2SO_4$ (4 g) is added. After 48 hours the mixture is filtered and the solvent is evaporated at a low pressure. **1** is obtained as a brown oil (5.0 g, 18.4 mmol, yield = 94%).

$^1$H-NMR ($CDCl_3$, 200 MHz): $\delta$ = 8.41 (1H, s), 7.86 (2H, d, J = 8.77), 7.00 (2H, d, J = 8.77), 6.45-6.35 (3H, m), 3.88 (3H, s), 3.83 (6H, s). EI-MS (m/z): 271

- ***Synthesis of imine 2 (Scheme 1)***

3,4,5-Trimethoxy aniline (1.9 g, 10.1 mmol) and 3-hydroxy-4-methoxy benzaldehyde (1.5 g, 10.1 mmol) are dissolved in 6 mL of EtOH under stirring at room temperature, and anhydrous $Na_2SO_4$ (4 g) is added. After 48 hours a yellow precipitate is obtained together with the drying agent. The solid is filtered and washed with 10 mL of cold EtOH. The precipitate is solubilized in methylene chloride and filtered to separate it from the drying agent. The solvent is evaporated at low pressure, and **3** is obtained as an amorphous yellow solid (3.1 g, 9.9 mmol, yield = 98%).

$^1$H-NMR ($CDCl_3$): $\delta$ 8.41 (1H, s), 7.63 (1H, s), 7.46 (1H, d, J = 7.2 Hz), 6.96 (1H, d, J = 7.2 Hz), 6.53 (2H, s), 5.77 (1H, s), 3.99 (3H, s), 3.92

(6H, s), 3.88 (3H, s). EI-MS (m/z): 318 ($M^+$).

- ***Synthesis of azetidin-2-ones (trans+cis) of formula (I) (Procedure A)***

**3,4-*trans*-3-hydroxy-4-(3-hydroxy-4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one, (8-*trans*) (E), and 3,4-*cis*-3-hydroxy-4-(3-hydroxy-4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (8-*cis*) (compound F)**

Imine **2** (1.8 g, 5.8 mmol) and TEA (4.7 g, 6.4 mL, 46.2 mmol) are dissolved in 140 mL of anhydrous methylene chloride in nitrogen atmosphere at 0°C. 2-acetoxy acetyl chloride (3.9 g, 28.9 mmol) diluted in 30 mL of anhydrous methylene chloride is then dripped. The mixture is left to stand at room temperature and left under stirring for 24 hours. The volume of solvent is reduced at low pressure and the solvent is purified by flash chromatography using the mixture hexane: ethyl acetate = 1:1 as eluent. A mixture of rac-**6**-*cis* and rac-**6**-*trans* is obtained as an amorphous white solid (1.14 g, 3.34 mmol, yield = 60%).

**rac-6-*cis*.** $^1$H-NMR ($CDCl_3$, 200 MHz), δ: 7,22 (1H, dd, J = 8.41, 2.04), 7.05 (1H, d, J = 2.04), 6.96 (1H, d, J = 8.41), 6.57 (2H, s), 5.90 (1H, d, J = 4.82), 5.28 (1H, d, J = 4.82), 3.82 (3H, s), 3.78 (3H, s), 3.73 (6H, s), 2.19 (3H, s), 1.79 (3H, s). $^{13}$C-NMR ($CDCl_3$, 50.3 MHz), δ: 170.37, 169.57, 166.00, 161.81, 153.81, 151.71, 139.27, 132.98, 127.17, 124.86, 122.54, 113.28, 95.45, 76.48, 61.20, 61.11, 60.48, 56.32, 20.62, 20.06. ESI-MS, (m/z): 417

**rac-6-*trans*.** $^1$H-NMR ($CDCl_3$, 200 MHz), δ: 7.23 (1H, dd, J = 8.49, 2.19), 7.00 (1H, d, 8.49), 6.53 (2H, s), 5.38 (1H, d, J = 1.74), 4.68 (2H,s), 4.58 (1H, d, J = 1.74), 3.85 (3H, s), 3.79 (3H, s), 3.74 (6H,s), 2.19 (6H, s).

ESI-MS, (m/z): 417

**6** (*cis* + *trans*) (1.14 g, 3.34 mmol) is dissolved in 50 mL of methanol in nitrogen atmosphere under stirring at 0°C, and hydrazine dichloride (2.4 g, 23 mmol) is added. TEA (6.6 g, 9.1 mL, 65 mmol) is dripped and left to stand

at room T, then heated under reflux for 4 hours. The solvent is evaporated and the residue is treated with 30 mL of an 0.1 N HCl solution and extracted with ethyl acetate (3 x 30 mL). The organic phases are combined and dried ($Na_2SO_4$). The solvent is evaporated at low pressure, and the mixture is separated by gradient flash chromatography (eluent hexane:tert-butyl-methyl ether = 6:4 up to 2:8). The isomers **8** (*trans*)(rac-**E**) (0.125 g, 0.33 mmol, yield = 9.9%) and **8** (*cis*)(rac-**F**) (0.350 g, 0.93 mmol, yield = 28%) are isolated as amorphous white solids.

**rac-8**-*trans*. $^1$H-NMR ($CDCl_3$, 200 MHz) δ: 6.8-7.0 (2H), 6.49 (1H, s), 5.91 (1H, s, disappears on deuteration), 4.83 (1H, s, disappears on deuteration), 4.74 (1H, d, J = 1.56), 4.71 (1H, d, J = 1.56), 3.86 (3H,s), 3.74 (3H,s), 3.67 (6H,s). $^{13}$C-NMR ($CDCl_3$, 50.3 MHz), δ:167.68, 154.05, 146.94, 135.39, 133.80, 129.81, 118.80, 112.91, 111.68, 96.14, 84.14, 66.35, 61.57, 55.67. ESI-MS (m/z): 398 (M+ $Na^+$).

**rac-8**-*cis*. $^1$H-NMR ($CDCl_3$+$D_2O$, 200 MHz): δ 7.10 (1H, d, J = 8.8 Hz), 7.00−

6.95 (2H, m), 6.81 (2H, s), 5.35 (1H, d, J = 5.0 Hz), 5.28 (1H, d, J =5.0 Hz), 4.00 (3H, s), 3.86 (6H, s), 3.84 (3H, s).. $^{13}$C-NMR ($CDCl_3$, 50.3 MHz), δ:167.25, 153.28, 145.02, 143.25, 133.51, 126.47, 119.17, 114.49, 111.14, 95.13, 76.50, 62.69, 59.65, 54.93. ESI-MS (m/z): 398 (M+ $Na^+$).

**3,4-*trans*-1-(3,5-dimethoxyphenyl)-3-hydroxy-4-(4-methoxyphenyl)azetidin-2-one (7-*trans*) (compound B) and 3,4-*cis*-1-(3,5-dimethoxyphenyl)-3-hydroxy-4-(4-methoxyphenyl)azetidin-2-one (7-*cis*) (compound A)**

Imine **1** (2.97 g, 10.9 mmol) and TEA (17.6 g, 24.3 mL, 174 mmol) are dissolved in 90 mL of anhydrous methylene chloride in nitrogen atmosphere, at 0°C under stirring. 2-acetoxy acetyl chloride (7.44 g, 54.5 mmol), diluted in 30 mL of anhydrous methylene chloride, is then dripped. The mixture is left to

stand at room temperature and left under stirring for 24 hours. The reaction mixture is concentrated and purified by flash chromatography using the mixture hexane: ethyl acetate = 1:1 as eluent. A mixture of the due diastereoisomers rac-**5**-*cis* and rac-**5**-*trans* (1.67 g, 4.5 mmol, yield = 41%) is obtained as an amorphous white solid.

**rac-5-** *trans*. $^{1}$H-NMR ($CDCl_3$, 200 MHz) δ: 7,23 (2H,d, J = 7.69), 6.85 (2H, d, J = 7.69), 6.42 (2H, d, J = 2.29), 6.13 (1H, t, J = 2.29), 5.30 (1H, d, J = 2), 4.82 (1H, d, J = 2), 3.73 (3H, s), 3.63 (6H, s), 2.12 (3H, s). $^{13}$C-NMR ($CDCl_3$, 50.3 MHz) δ: 169.81, 162.20, 161.30, 160.30, 138.66, 127.80, 127.22, 114.75, 97.10, 96.43, 82.72, 63.74, 55.49, 55.45, 20.59 EI-EI-MS (m/z): 371

**rac-5-***cis*. $^{1}$H-NMR($CDCl_3$, 200 MHz) δ: 7.23 (2H, d, J = 7.70), 6.87 (2H, d, J = 7.70), 6.53 (2H, d, J = 2.18), 6.22 (1H, t, J = 2.18), 5.89 (1H, d, J = 4.91), 5.29 (1H, d, J = 4.91), 3.81 (3H, s), 3.73 (6H, s), 1.74 (3H, s). $^{13}$C-NMR ($CDCl_3$, 50.3 MHz) δ: 169.81, 162.43, 161.36, 160.15, 138.82, 129.32, 124.05,114.14, 97.12, 96.31, 77.82, 61. 53, 55.52, 55.49, 20.02 EI-MS (m/z): 371.

**5** (*cis* + *trans*) (1.67 g, 4.5 mmol) is dissolved in 65 mL of methanol in nitrogen atmosphere under stirring at 0°C, and hydrazine dichloride (3.3 g, 31.5 mmol) is added. TEA (9.11 g, 12.5 mL, 90 mmol) is dripped and left to stand at room T, then heated under reflux for 4 hours. The solvent is evaporated and the residue is treated with a saturated solution of $KHSO_4$ and extracted with ethyl acetate (3 x 30 mL). The organic phases are combined and dried ($Na_2SO_4$). The solvent is evaporated at low pressure and the two diastereoisomers are separated by gradient flash column chromatography in (eluent hexane:tert-butyl-methyl ether = 7:3 up to 3:7). The isomers rac-**7** (*trans)* (0.050 g, 0.15 mmol, yield = 3.3%) and rac-**7** (*cis*) (0.246 g, 0.75 mmol, yield = 17%) are isolated as amorphous white solids.

**rac-7-***trans*. $^{1}$H-NMR ($CDCl_3$, 200 MHz) δ: 7.27 (2H, d, J = 8.6), 6.94

(2H, d, J = 8.6),6.54 (2H, d, J = 2.2), 6.20 (1H, t, J = 2.2), 5.22 (1H, d, J = 5.2), 5.16 (2H), 3.81 (3H,s), 3.72 (6H, s). $^{13}$C-NMR ($CDCl_3$, 50.3 MHz) δ: 166.67, 161.33, 138.80, 128.90, 124.77, 114.80, 96.93, 96.41, 77.10, 65.69, 55.57.

EI-MS (m/z): 329 ($M^+$), 272.

**rac-7-*cis***. $^1$H-NMR ($CDCl_3$, 200 MHz) δ: 7.23 (2H, d, J = 8.7), 6.88 (2H, d, J = 8.7), 6.42 (2H, d, J = 2.2), 6.15 (1H, t, J = 2.2), 4.9-4.6 (3H), 3.80 (3H,s), 3.67 (6H, s). $^{13}$C-NMR ($CDCl_3$, 50.3 MHz) δ: 167.77, 161.16, 160.09, 138,67, 128.13, 127.60, 114.75, 97.07, 96.52, 83.77, 65.82, 55.49. EI-MS (m/z) 329 ($M^+$), 272.

- ***Synthesis of the azetidin-2-ones (trans) of formula (I) (Procedure B)***

**3,4-*trans*-3-hydroxy-4-(3-hydroxy-4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (8 –*trans*) (compound E)**

Compound **2** (1.0 g, 3.15 mmol) and 2-acetoxy acetyl chloride (0.86 g 0.68 mL, 6.3 mmol) are dissolved in 20 mL of anhydrous toluene in nitrogen atmosphere, at 0°C under stirring. The mixture is left to stand at room T and heated to 100°C. Anhydrous TEA (0.70 g, 0.96 mL, 6.94 mmol) is dripped. After 5 hours the reaction mixture is concentrated and purified by flash chromatography using the mixture hexane:ethyl acetate = 1:1 as eluent. The sole diastereoisomer rac-**6** (*trans)* (0.578 g, 1.11 mmol, yield = 35%) is obtained as an amorphous white solid.

Rac-**6** *trans* (4.3 g, 8.3 mmol) is dissolved in 100 mL of methanol in nitrogen atmosphere under stirring at 0°C, and hydrazine dichloride (7.0 g, 66.4 mmol) is added. TEA (13.4 g, 18.5 mL, 133 mmol) is dripped and left to stand at room T, then heated under reflux for 4 hours. The solvent is evaporated at low pressure and 30 mL of the residue is treated with an 0.1 N HCl solution and extracted with ethyl acetate (3 x 50 mL). The organic phases are combined and dried ($Na_2SO_4$) and the solvent is then evaporated at low

pressure. The crude product is purified by flash chromatography (eluent hexane:ethyl acetate = 1:1). Rac-**8**-(*trans*) (compound **E**) (1.0 gm, 2.7 mmol, 32%) is isolated as an amorphous white solid.

**3,4-*trans*-1-(3,5-dimethoxyphenyl)-3-hydroxy-4-(4-methoxyphenyl)-azetidin-2-one (7-*trans*) (compound B)**

Amine **1** (5.0 g, 18.4 mmol) and 2-acetoxy acetyl chloride (2.5 g, 1.97 mL, 18.4 mmol) are dissolved in 50 mL of anhydrous toluene in nitrogen atmosphere at 0°C under stirring. The mixture is left to stand at room T and heated to 100°C. Anhydrous TEA (2.0 g, 2.75 mL, 20.2 mmol) is dripped. After 5 hours the reaction mixture is concentrated and purified by flash chromatography using the mixture hexane:ethyl acetate = 1:1 as eluent. The sole diastereoisomer **5**-*trans* (3.4 g, 9.2 mmol, yield = 50%) is obtained as an amorphous white solid.

**5**-*trans* (2.5 g, 6.8 mmol) is dissolved in 30 mL of methanol in nitrogen atmosphere under stirring at 0°C, and hydrazine dichloride (2.85 g, 27.2 mmol) is added. TEA (5.5 g, 7.6 mL, 54 mmol) is dripped and left to stand at room T, then heated under reflux for 4 hours. The solvent is evaporated and the residue is treated with a saturated solution of $KHSO_4$ and extracted with ethyl acetate (3 x 50 mL). The organic phases are combined and dried ($Na_2SO_4$); the solvent is then evaporated at low pressure and the product is purified by flash chromatography (eluent hexane:ethyl acetate = 1:1). Rac-**7**- (*trans*)(compound **B**) (2.2 g, 6.8 mmol, quantitative yield) is isolated as an amorphous white solid.

- ***Resolution of rac-7-(trans) (Scheme 5)***

Rac-**7**-(*trans*) (2.2 g, 6.8 mmol) and (L)-Boc-proline (3.0 g, 14 mmol) are dissolved in 30 mL of anhydrous acetonitrile in nitrogen atmosphere under stirring. HBTU (5.6 g, 15 mmol) is added and DIPEA (72.3 mL, 420 mmol) is dripped. After 24 hours the solvent is evaporated and the residue is diluted

with water. The resulting solution is extracted with methylene chloride (3 x 50 mL). The combined organic phases are washed with a saturated solution of $KHSO_4$, then with a saturated solution of $NaHCO_3$, and finally with brine. After drying ($Na_2SO_4$) and evaporation of the solvent at low pressure the product is purified by gradient flash chromatography (eluent hexane:ethyl acetate = 8:2 up to 6:4). A mixture of the two diastereoisomers of **21a** + **21b** is isolated as an amorphous white solid (3.1 g, 5.9 mmol, yield = 84%). The two diastereoisomers are separated by gradient flash chromatography (eluent hexane:tert-butyl-methyl ether = 8:2 up to 2:8) as amorphous white solids.

**21a**. (diastereoisomer with Rf = 0.36; 2 runs in hexane/methyl t-butyl ether 4:6; $So_2$): $[\alpha]_D$ = + 1.25 ((c = 30 mg/10 ml). $^1$H-NMR (DMSO-$d_6$, 80°C, 500 MHz), δ: 7.38 (2H, d, J = 8.5), 6.97 (2H, d, J = 8.5), 6.43 (2H, d, J = 2.2), 6.27 (1H, t, J = 2.2), 5.48 (1H, bs), 5.12 (1H, d, J = 1.85), 4.32 (1H, dd, J = 8.6, 4.3), 3.79 (3H, s), 3.67 (6H, s), 3.41-3.37 (2H, m), 2.35-2.26 (1H, m), 2.06-1.98 (1H, m), 1.90-1.88 (2H, m), 1.41 (9H, s).

$^{13}$C-NMR ($CDCl_3$, 25°C, 125 MHz) (two conformers): δ 171.68 and 171.48, 161.03 and 160.76, 160.45 and 160.38, 159.51 and 159.27, 153.81 and 152.86, 137.80 and 137.76, 127.2 and 126.9, 126.96 and 126.83, 126.41 and 126.08, 113.96 and 113.73, 96.39 and 96.28, 95.68 and 95.58, 82.23 and 82.11, 79.50 and 79.38, 62.90, 57.87, 54.66, 45.94 and 45.68, 30.30 and 29.17, 27.74, 23.81 and 23.0.

ESI-MS (m/z) : 549

**21b (**diastereoisomer with Rf = 0.32; 2 runs in hexane/methyl t-butyl ether 4:6; $SiO_2$), $[\alpha]_D$ = - 5.94 (c = 30 mg/10 ml). $^1$H-NMR ((DMSO-$d_6$, 80°C, 500 MHz) δ: 7.38 (2H, d, J = 8.41), 6.98 (2H, d, J = 8.41), 6.43 (2H, d, J = 2.2), 6.27 (1H, t, J = 2.2), 5.48 (1H, bs), 5.12 (1H, d, J = 1.75),4.35 (1H, dd, J = 8.5, 4.3), 3.79 (3H, s), 3.67 (6H, s), 3.43-3.36 (1H, m), 2.35-2.26 (1H, m),

2.06-1.99 (1H, m), 1.83-1.86 (2H, m), 1.39 (9H, s). $^{13}$C-NMR ($CDCl_3$, 25°C, 125 MHz) (two conformers): δ 171.35 and 171.19, 161.10 and 160.80, 160.42 and 160.42, 159.51 and 159.29, 153.80 and 152.86, 137.86 and 137.73, 127.19 and 126.89, 126.35 and 126.13, 113.97 and 113.82, 96.34 and 95.52, 82.04 and 82.04, 79.63 and 79.36, 62.71 and 62.66, 58.39 and 58.07, 54.66, 45.92 and 45.65, 30.36 and 29.32, 27.74, 23.92 and 22.99.

(-)-**21b** (0.33 g, 0.63 mmol) is dissolved in 30 mL of methanol in nitrogen atmosphere under stirring at 0°C, and hydrazine dichloride (0.53 g, 5 mmol) is added. TEA (1.0 g, 1.4 mL, 10 mmol) is dripped and left to stand at room T, then heated under reflux for 4 hours. The solvent is evaporated at low pressure and the residue is treated with a saturated solution of $KH_2SO_4$ and extracted with ethyl acetate (3 x 30 mL). The organic phases are combined and washed with a saturated solution of $NaHCO_3$, and then with brine. After drying the solvent is evaporated at low pressure and the product is purified by flash chromatography (eluent hexane:ethyl acetate = 1:1). (-) **7b** (compound **B(-)**) (111m g, 0.34 mmol, yield = 54%) is isolated as an amorphous white solid. $[\alpha]_D$ = - 29.64 ($CHCl_3$, c = 111 mg/10 mL).

The same protocol applied to **21a** supplied enantiomer **7a** (+)(compound **B(+)**): $[\alpha]_D$ = + 29.10 ($CHCl_3$, c = 101 mg/5 mL). (81 mg, yield = 52%)

Enantiomers (+)**7a** (compound **B(+)**) and (-)**7b** (compound **B(-)**) were tested by chiral column HPLC by comparison with their racemate, and showed a purity of 97%.

- ***Resolution of rac*-8 (Scheme 5)**

Rac-**8**-*trans* (2.66 mmol, 1.00 g), (L)-Boc-proline (7.98 mmol, 1.7 g), DIPEA (29.9 mmol, 4.6 g) and HBTU (7.98 mmol, 3.0 g) are dissolved in 75 mL of anhydrous acetonitrile in nitrogen atmosphere under stirring. After 24 hours the solvent is evaporated and the residue is diluted with water. The

resulting mixture is extracted with methylene chloride (3 x 50 mL). The combined organic phases are washed with a saturated solution of $KHSO_4$, then with a saturated solution of $NaHCO_3$, and finally with brine. After drying ($Na_2SO_4$) and evaporation of the solvent at low pressure the product is purified by gradient flash chromatography (eluent hexane:ethyl acetate = 8:2 up to 6:4). A mixture of the two diastereoisomers of **22a** + **22b** is isolated as an amorphous white solid (1.47 g, 1.9 mmol, yield = 71.4%). The two diastereoisomers are separated by gradient flash chromatography (eluent hexane:ethyl acetate = 8:2 up to 1:1) as amorphous white solids.

**22a** (diastereoisomer with Rf = 0.66; hexane/ethyl acetate 5:5, $SiO_2$): $[\alpha]_D$ = -5.83 (MeOH, c = 120 mg/10 mL). $^1$H-NMR (DMSO-$d_6$, 80°C, 500 MHz), δ: 7.38 (1H, dd, J = 8.41, 1.5), 7.19 (1H, d, J = 8.41), 7.19 (1H, m), 6.57 (2H, s), 5.52 (1H, bs), 5.18 (1H, d, J = 1.5), 4.45 (1H, dd, J = 8.76, 3.83), 4.33 (1H, dd, J = 8.61, 4.22), 3.80 (3H, s), 3.68 (6H, s), 3.64 (3H, s), 3.44-3.37 (4H, m), 2.36-2.29 (2H, m), 2.17-2.10 (1H, m), 2.00-1.87 (5H, m), 1.41 (18H, bs). $^{13}$C-NMR ($CDCl_3$, 25°C, 125 MHz) (two conformers): δ 172.32 and 172.29, 171.08 and 170.84, 161.53 and 161.45, 154.08 and 153.97, 153.62, 153.30 and 153.17, 151.65 and 151.57, 139.63 and 139.50, 134.91 and 134.87, 132.71, 128.27 and 128.17, 126.30 and 126.22, 121.78, 113.92 and 113.85, 95.94, 82.36 and 82.31, 79.72 and 79.68, 62.21 and 62.07, 60.55, 58.84 and 58.76, 56.51 and 56.21, 46.85 and 46.67, 38.71, 30.91 and 30.87, 29.94, and 29.82, 28.53 and 28.38, 24.54 and 24.34, 23.69 and 23.55. EI-MS (m/z) : 769.

**22b** (diastereoisomer with Rf = 0.57; hexane/ethyl acetate 5:5) in $SiO_2$: $[\alpha]_D$ = -56.12 (MeOH, c = 131 mg/10 mL). $^1$H-NMR (DMSO-$d_6$, 80°C, 500 MHz), δ: 7.38 (1H, d, J = 7.87), 7.20 7.16 (1H, m), (1H, d, J = 7.87), 6.56 (2H, s), 5.52 (1H, bs), 5.18 (1H, bs), 5.52 (1H, bs), 5.18 (1H, d, J = 1.5), 4.45 (1H, dd, J = 8.98, 3.91), 4.34 (1H, dd, J = 8.98, 4.27), 3.80 (3H, s), 3.66 (6H, s), 3.63 (3H, s), 3.44-3.37 (4H, m), 2.36-2.31 (2H, m), 2.17-2.15 (1H, m),

2.04-2.00 (1H, m), 1.93-1.88 (4H, m), 1.37 (18H). $^{13}$C-NMR ($CDCl_3$, 25°C, 125 MHz) (two conformers), δ: 172.38 and 171.99, 171.10 and 170.88, 161.46 and 161.37, 154.03 and 153.97, 153.71, 153.31 and 153.21, 151.65 and 151.59, 139.65 and 139.49, 134.89, 132.74 and 132.66, 128.28 and 128.17, 126.46 and 126.14, 122.42 and 122.26, 121.73 and 121.62, 113.79, 96.03 and 95.83, 82.29 and 82.05, 79.66 and 79.37, 62.38 and 61.93, 60.54, 58.87 and 58.63, 56.46 and 56.23, 46.84 and 46.44, 38.79, 30.92 and 30.74, 29.97 and 29.77, 28.49 and 28.17, 24.48 and 24.37, 23.64 and 23.52 EI-MS (m/z): 769.

**22a** (0.227 g, 0.30 mmol) is dissolved in 30 mL of methanol in nitrogen atmosphere under stirring at 0°C, and hydrazine dichloride (0.478 g, 2.36 mmol) is added. TEA (0.478 g, 0.66 mL, 4.72 mmol) is dripped and left to stand at room T, then heated under reflux for 4 hours. The solvent is evaporated at low pressure and the residue is treated with a saturated solution of $KHSO_4$ and extracted with ethyl acetate (3 x 20 mL). The organic phases are combined and washed with a saturated solution of $NaHCO_3$, and then with brine. After drying the solvent is evaporated at low pressure and the product is purified by flash chromatography (eluent hexane:ethyl acetate = 1:1). (+)**8a** (compound **E(+)**) (94 mg, yield = 83.5%) is isolated as an amorphous white solid. $[\alpha]_D$ = +16.20 ($CHCl_3$, c = 75 mg/5 mL).

The same protocol applied to **22b** supplied enantiomer (-)**8b** (compound **E(-)**): $[\alpha]_D$ = -15.43 ($CHCl_3$, c = 97 mg/10 mL). Yield: (97 mg, yield 58%).

**Example 2 - Evaluation of cytotoxic effects of the compounds of the invention**

The compounds representative of the invention **rac-7-*cis*** (compound **A**), **rac-7-*trans*** (compound **B**), **rac-8-*trans*** (compound **E**), **rac-8-*cis*** (compound **F**), **rac-12-*cis*** (compound **G**) and **rac-12-*trans*** (compound **T**), the reference compound combretastatin A4 and the azetidinone derivatives previously described 3,4-*cis*-3-hydroxy-4-(3-nitro-4-methoxyphenyl)-1-(3,4,5-

trimethoxyphenyl)azetidin-2-one (compound **N**) and 3,4-*cis*-4-(3-amino-4-methoxyphenyl)-3-hydroxy-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (compound **O**), dissolved in DMSO, were tested for their ability to inhibit the growth of human cell cultures by a spectrophotometry assay, which allows cell viability to be quantified by assaying the activity of mitochondrial dehydrogenase (MTT test, Fang *et al.*, 2008, J. Cancer Res. Clin. Oncol.134(12):1337-45). The following cell lines were used: duodenal adenocarcinoma cells, HuTu-80 and healthy small intestine cells, Fhs74. The compounds were initially supplied at the concentration of 10 μM for 72 h and DMSO (0.1% w/v) was used as negative control.

As shown in Figure 2, at the concentration of 10 μM the compounds of the invention demonstrated a high capacity to inhibit the cell viability of HuTu-80 cells (approx. 98%), equal to that of the reference compound combretastatin A4. Conversely, none of said compounds affected the viability of Fhs74 cells, suggesting specific inhibition of the tumour line by said compounds.

When the MTT assay was repeated, varying the concentration of the compounds in order to construct dose-response curves, the IC50 values (concentration required to inhibit 50% of cell viability) shown in Table 1 were obtained. The compounds present IC50 values ranging from a concentration of 9 nM to a concentration of 2.5 μM (for compound **G**). Compounds **B** and **E** proved to be the most efficient compounds in inhibiting cell viability, with an IC50 of approx. 9 nM for compound **B** and 13 nM for compound **E**. It is interesting to note that compounds **N** and **O**, previously described by Sun et al, Bioorganic & Medicinal Chemistry Letters, 2004,14(9), 2041-2046, present an activity approx. 50 and 10 times lower than compounds B and E to which the present invention relates.

**Table 1**

| Compound | IC50 nM (Hutu80 cells, 72 h) |
|---|---|
| A | 124.60 ± 27.01 |
| B | 9.05 ± 1.57 |
| E | 13.36 ± 1.47 |
| F | 28.79 ± 3.36 |
| G | 2549 ± 383.25 |
| T | 405.50 ± 48.65 |
| N | 528.55 ± 27.93 |
| O | 101.17 ± 6.75 |

The results shown in Table 1 were obtained on the racemic mixtures of the products.

Table 2 shows the results obtained in the same experimental model with the single enantiomers of the two most active compounds, **B** and **E**. For both compounds enantiomer **(+)** proved to be the most active, with an IC50 value of 8.02 nM for **B(+)** and 3.05 nM for **E(+)**.

**Table 2**

| Compound | IC50 nM (Hutu80 cells, 72 h) |
|---|---|
| B | 9.95 ± 0.10 |
| B(+) | 8.02 ± 0.71 |
| B(-) | 83.55 ± 14.33 |
| E | 13.36 ± 1.47 |
| E(+) | 3.05 ± 1.42 |
| E(-) | 589.9 ± 98.71 |

The effect of compounds **B** and **E** compared with combretastatin A4 was also evaluated on the following human tumour cell lines:

- SW48: colon adenocarcinoma cells
- HeLa: cervical tumour cells
- MCF-7: breast cancer cells
- SKNBE: neuroblastoma cells

Said lines were treated with compounds **B** and **E** at the concentration of 30 nM. DMSO (0.1% w/v) was used as negative control. The results are shown in Figure 3. As already shown in Figure 2, the growth of the normal Fhs74 cells was unaffected. Conversely, the cell viability of all the tumour lines was drastically reduced by compounds **B** and **E,** to an extent varying according to the line and the compound.

**Example 3 - Cell death induced by the compounds of the invention**

On the basis of microscopic observation of the cell lines treated with the compounds of the invention described in Example 2, evident cell death was observed in the tumour lines treated with the compounds. This cell death effect was analysed in detail using Hutu-80 cells as model system.

The Hutu-80 cells were treated with a 30 nM concentration of compounds **B**, **E** and combretastatin A4 for 48 h and 72 h, and their DNA content was analysed by flow cytofluorometry (FACS).

FACS analysis (Figure 4) clearly indicated that the treated cells presented an increase in the fraction of cells with a 4C DNA (post-replicative) content and a marked reduction in cells with a 2C DNA content (phase G1 of cell cycle), in agreement with the findings reported in the literature for combretastatin A4 (Shen et al., 2010, Br. J. Pharmacol. 160(8):2008-27). The most significant finding was the appearance of a large fraction of cells with a Sub-G1 DNA content (approx. 25% after 48 h and 35% after 72 h), due to the DNA fragmentation typical of dead cells (Figure 5).

To distinguish whether this cell death effect was due to necrosis or apoptosis, molecular analysis was conducted on the effector caspase 3, which is known to be involved in activating the apoptosis process. Caspase 3 is responsible for proteolytic cleavage of many key proteins, including the nuclear enzyme poly(ADP)ribose polymerase (PARP), which is involved in DNA damage repair mechanisms in response to environmental stress. PARP is

required to maintain cell viability, and its proteolytic cleavage by caspase 3 is an important indicator of cells which have activated an apoptotic process (Nicholson *et al.*, 1995, Nature 376(6535):37-43). The activation of caspase 3 also requires proteolytic processing of its inactive form (35 kDa) into its two active fragments p17 and p12 (Nicholson *et al.*, 1995, Nature 376(6535): 37-43; Fernandes-Alnemri *et al.*, 1994, J Biol Chem. 269(49):30761-4).

The HuTu-80 tumour cells were treated for 24 h with compounds **B** and **E** and combretastatin A4 (30 nM), and Western blot analysis was then conducted to establish whether proteolytic fragments of caspase 3 and PARP, indicators of apoptotic cells, were present. The Western blot analysis clearly demonstrated that all three compounds induced cleavage of caspase 3 (Figure 6) and PARP (Figure 7). This finding, in association with the preceding one, which shows the appearance of cells with a sub-G1 DNA content, indicates that compounds **B** and **E**, like combretastatin A4, induce apoptosis in HuTu-80 duodenal tumour cells.

**Example 4 - Inducement of activation of the AMPK protein kinase and accumulation and phosphorylation of oncosuppressor p53**

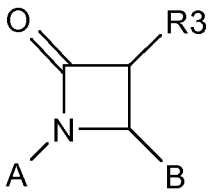
p53 performs a key role in tumour suppression, mainly inducing the arrest of cell proliferation, apoptosis and senescence (Wang *et al.*, 2010, Transl. Oncol. 3(1):1-12.). It is also known that in response to different types of stress, p53 accumulates and/or is extensively phosphorylated on various residues, including Ser15 (Wang *et al.*, 2010, Transl. Oncol. 3(1):1-12). AMPK (AMP-activated protein kinase) -dependent activation of p53 performs an essential role in inducing the process of apoptosis; this event in turn is closely connected with the activating phosphorylation of AMPK kinase on the Thr172 residue (Wang *et al.*, 2009, Acta Physiol. 196(1):55-63).

Compounds **B** and **E** according to the invention induce the activation of AMPK protein kinase, as reported for combretastatin A4 (Zhang *et al.*, 2008),

and the accumulation and phosphorylation of oncosuppressor p53. The Western blot analysis conducted on protein extracts of HuTu-80 tumour cells treated for 24 hours with 30 nM concentrations of the three compounds demonstrated clear phosphorylation of AMPK on Thr172 (Figure 8). It was also observed that p53 accumulates and is phosphorylated on Ser15 in cells treated with compounds **B** and **E** and combretastatin A4 (Figure 9).

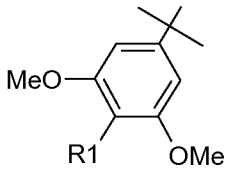
## CLAIMS

1. Compounds of formula I:




(I)

wherein one of A and B is the group:




wherein $R_1$ is selected from H and $OCH_3$, and the other is the group:


wherein $R_2$ is selected from H, OH, $NO_2$, $NH_2$;

$R_3$ is selected from OH and $NH_2$;

the salts, enantiomers and diastereoisomers thereof;

with the exclusion of the following compounds:

- 3,4-*cis*-3-hydroxy-4-(3-nitro-4-methoxyphenyl)-1-(3,4,5-trimethoxy-phenyl)azetidin-2-one;
- 3,4-*cis*-4-(3-amino-4-methoxyphenyl)-3-hydroxy-1-(3,4,5-trimethoxy-phenyl)azetidin-2-one;

for use as antitumor agents.

2. A compound as claimed in claim 1 selected from:

- (±) 3,4-*cis*-1-(3,5-dimethoxyphenyl)-3-hydroxy-4-(4-methoxyphenyl)-

azetidin-2-one;

- (±) 3,4-*trans*-1-(3,5-dimethoxyphenyl)-3-hydroxy-4-(4-methoxyphenyl)azetidin-2-one;
- (±) 3,4-*cis*-3-amino-1-(3,5-dimethoxyphenyl)-4-(4-methoxyphenyl)-azetidin-2-one;
- (±) 3,4-*trans*-3-amino-1-(3,5-dimethoxyphenyl)-4-(4-methoxyphenyl)-azetidin-2-one;
- (±) 3,4-*trans*-3-hydroxy-4-(3-hydroxy-4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one;
- (±) 3,4-*cis*-3-hydroxy-4-(3-hydroxy-4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one;
- (±) 3,4-*trans*-3-amino-4-(3-hydroxy-4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one;
- (±) 3,4-*cis*-3-amino-4-(3-hydroxy-4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one;
- (±) 3,4-*trans*-3-hydroxy-4-(3-nitro-4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one;
- (±) 3,4-*trans*-4-(3-amino-4-methoxyphenyl)-3-hydroxy-1-(3,4,5-trimethoxyphenyl)azetidin-2-one;
- (±) 3,4-*trans*-4-(3,5-dimethoxyphenyl)-3-hydroxy-1-(4-methoxyphenyl)azetidin-2-one;
- (±) 3,4-*cis*-4-(3,5-dimethoxyphenyl)-3-hydroxy-1-(4-methoxyphenyl)-azetidin-2-one;
- (±) 3,4-*cis*-3-hydroxy-1-(3-hydroxy-4-methoxyphenyl)-4-(3,4,5-trimethoxyphenyl)azetidin-2-one;
- (±) 3,4-*trans*-3-hydroxy-1-(3-hydroxy-4-methoxyphenyl)-4-(3,4,5-trimethoxyphenyl)azetidin-2-one;
- (±) 3,4-*trans*-3-amino-1-(3-hydroxy-4-methoxyphenyl)-4-(3,4,5-

trimethoxyphenyl)azetidin-2-one;

- (±) 3,4-*cis*-3-amino-1-(3-hydroxy-4-methoxyphenyl)-4-(3,4,5-trimethoxyphenyl)azetidin-2-one.

3. A compound as claimed in claims 1 and 2, selected from:

- (±) 3,4-*trans*-1-(3,5-dimethoxyphenyl)-3-hydroxy-4-(4-methoxyphenyl)azetidin-2-one;
- (±) 3,4-*trans*-3-amino-1-(3,5-dimethoxyphenyl)-4-(4-methoxyphenyl)-azetidin-2-one;
- (±) 3,4-*trans*-3-hydroxy-4-(3-hydroxy-4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one;
- (±) 3,4-*trans*-3-amino-4-(3-hydroxy-4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one;
- (±) 3,4-*trans*-3-hydroxy-4-(3-nitro-4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one;
- (±) 3,4-*trans*-4-(3-amino-4-methoxyphenyl)-3-hydroxy-1-(3,4,5-trimethoxyphenyl)azetidin-2-one;
- (±) 3,4-*trans*-4-(3,5-dimethoxyphenyl)-3-hydroxy-1-(4-methoxyphenyl)azetidin-2-one;
- (±) 3,4-*trans*-3-hydroxy-1-(3-hydroxy-4-methoxyphenyl)-4-(3,4,5-trimethoxyphenyl)azetidin-2-one;
- (±) 3,4-*trans*-3-amino-1-(3-hydroxy-4-methoxyphenyl)-4-(3,4,5-trimethoxyphenyl)azetidin-2-one.

4. A compound as claimed in claims 1, 2 or 3, selected from:

- (±) 3,4-*trans*-1-(3,5-dimethoxyphenyl)-3-hydroxy-4-(4-methoxyphenyl)azetidin-2-one;
- (±) 3,4-*trans*-3-hydroxy-4-(3-hydroxy-4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one.

5. A compound selected from:

- (+)-3,4-*trans*-1-(3,5-dimethoxyphenyl)-3-hydroxy-4-(4-methoxyphenyl)azetidin-2-one;
- (-)-3,4-*trans*-1-(3,5-dimethoxyphenyl)-3-hydroxy-4-(4-methoxyphenyl)azetidin-2-one;
- (+)-3,4-*trans*-3-hydroxy-4-(3-hydroxy-4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one;
- (-)-3,4-*trans*-3-hydroxy-4-(3-hydroxy-4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one.

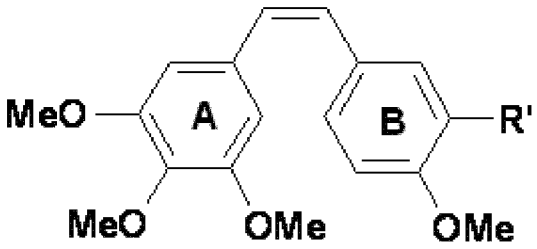
6. A compound as claimed in claim 5 selected from:

- (+)-3,4-*trans*-1-(3,5-dimethoxyphenyl)-3-hydroxy-4-(4-methoxyphenyl)azetidin-2-one;
- (+)-3,4-*trans*-3-hydroxy-4-(3-hydroxy-4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one.

7. A compound as claimed in claim 5 or 6 as a medicament.

8. A compound as claimed in claim 5 or 6 as an antitumor agent.

9. Pharmaceutical composition containing a compound as claimed in claim 5 or 6 and at least one excipient suitable for pharmaceutical use.




| | |
|---|---|
| **A4** | R' = OH (Combretastatin A4) |
| **A4P** | R' = $OPO_3Na_2$ (Combretastatin A4 phosphate; Fosbretabulin) |
| **AC7739** | R' = $NH_2$ |
| **AVE8062** | R' = $NHCOCH(NH_2)CH_2OH$ (Ombrabulin) |

**Figure 1**

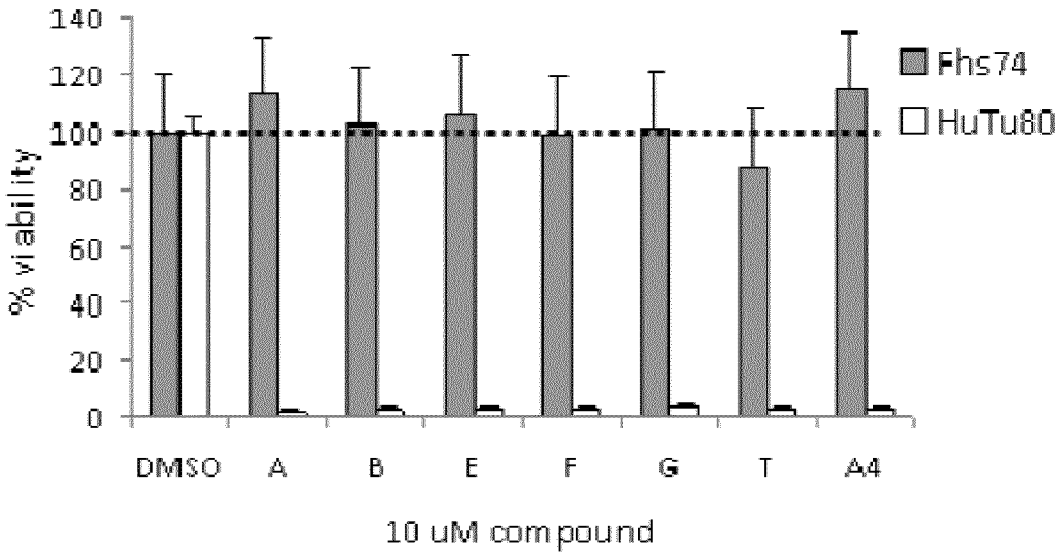



**Figure 2**

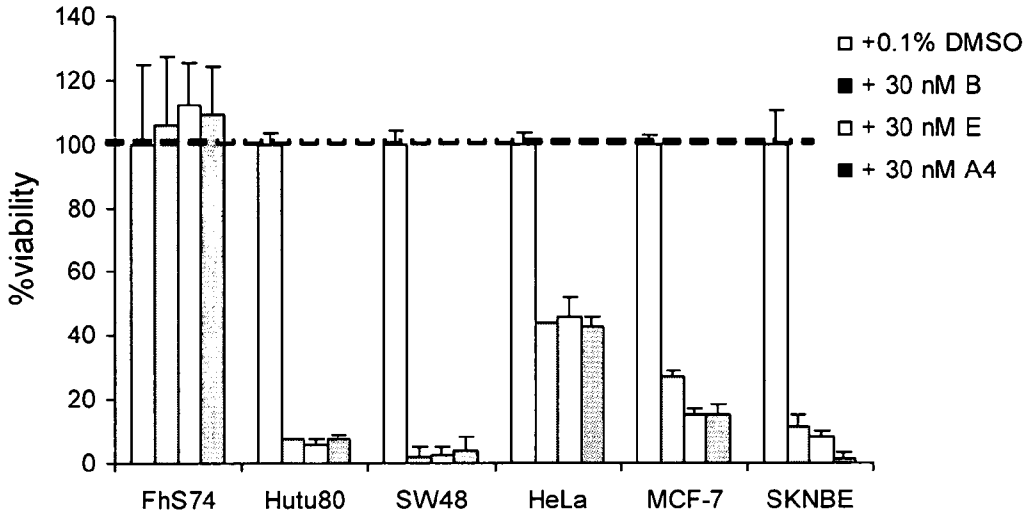



**Figure 3**

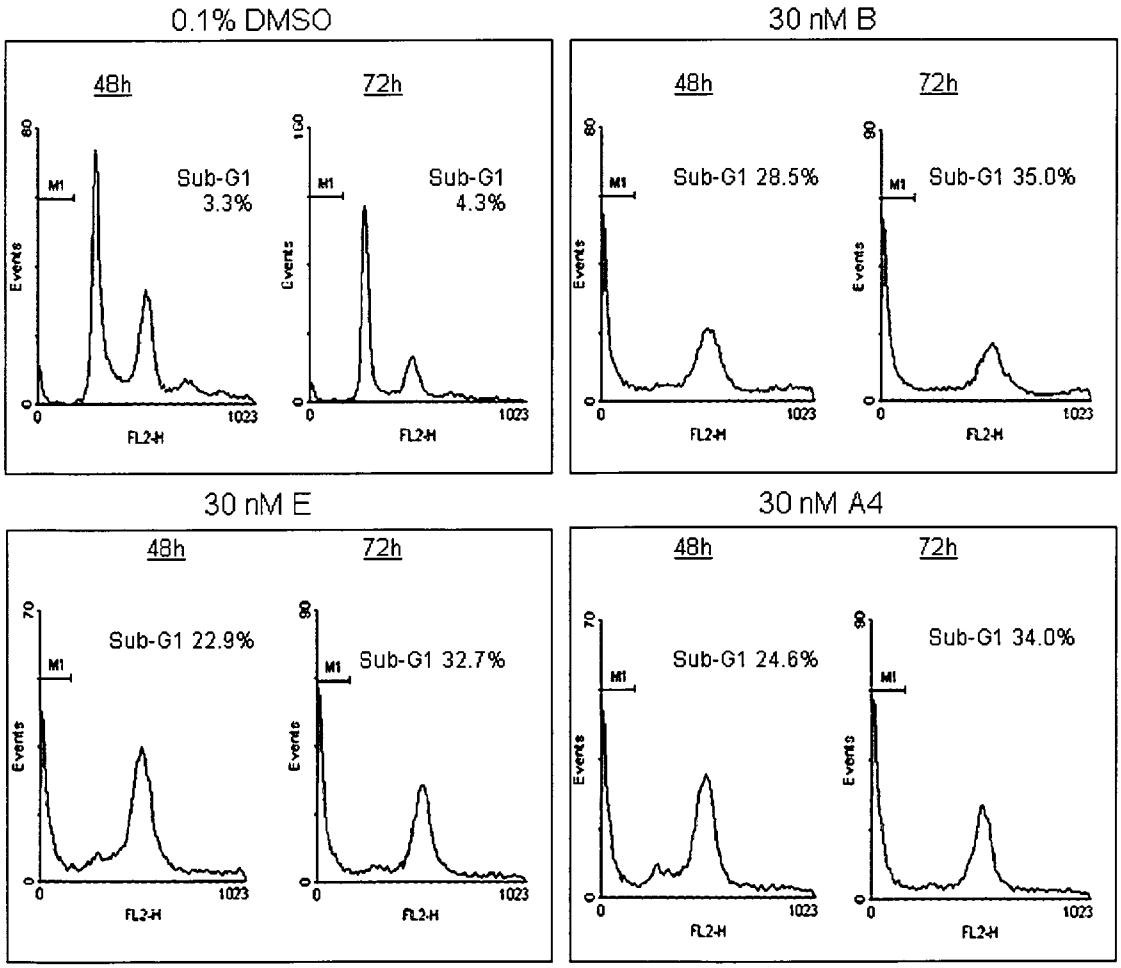



**Figure 4**

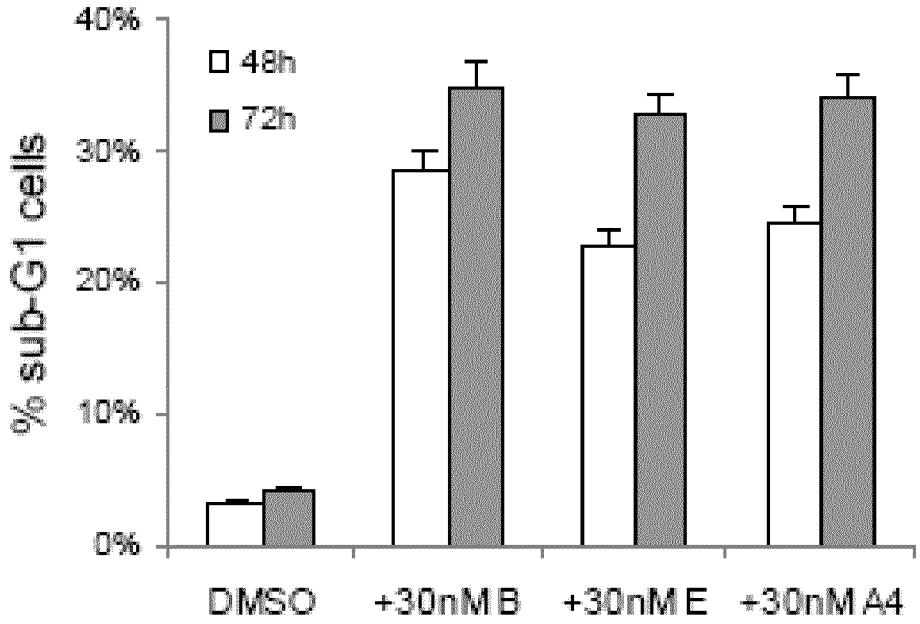



Figure 5

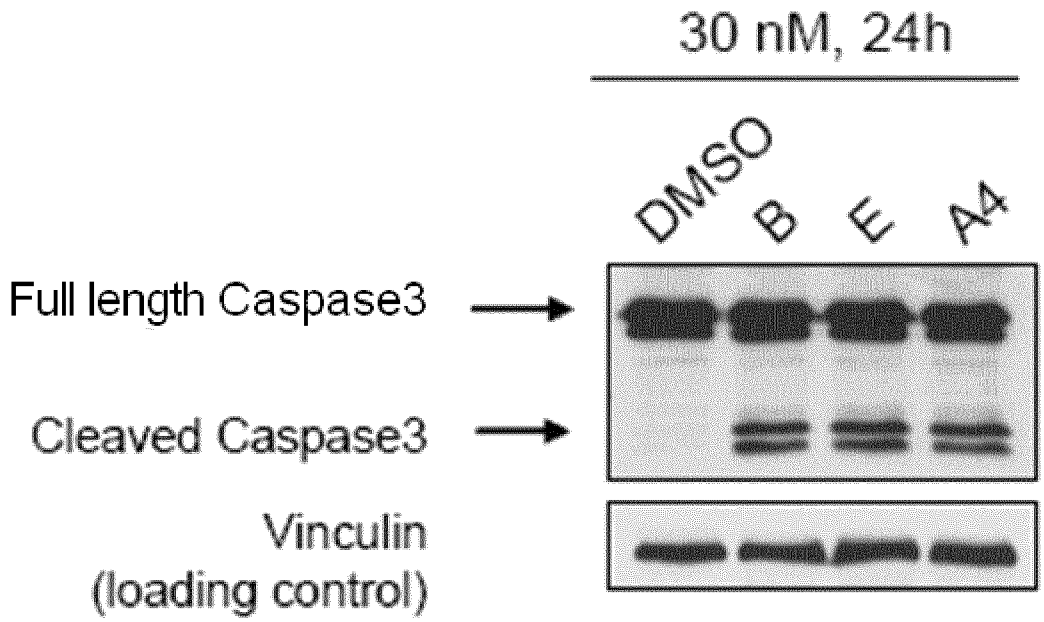



Figure 6

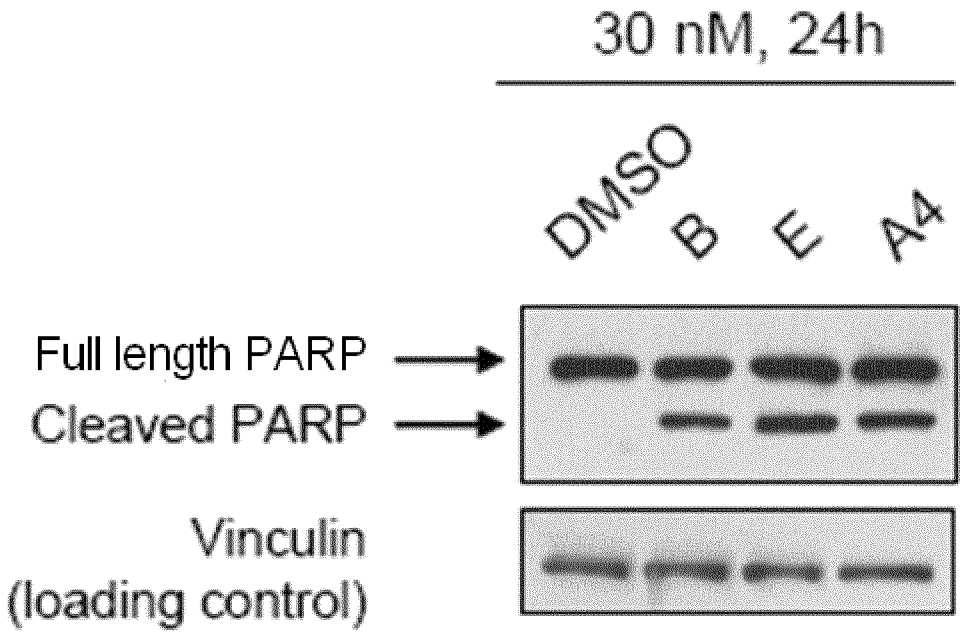



**Figure 7**

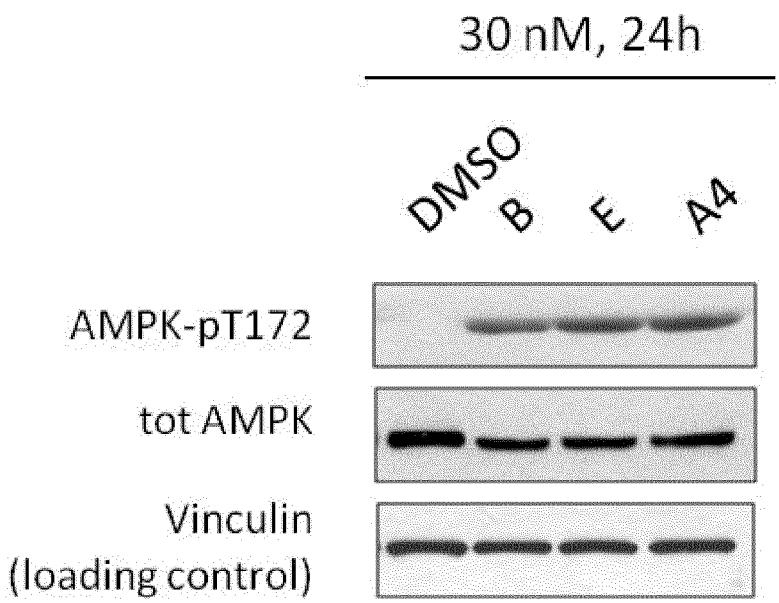



**Figure 8**

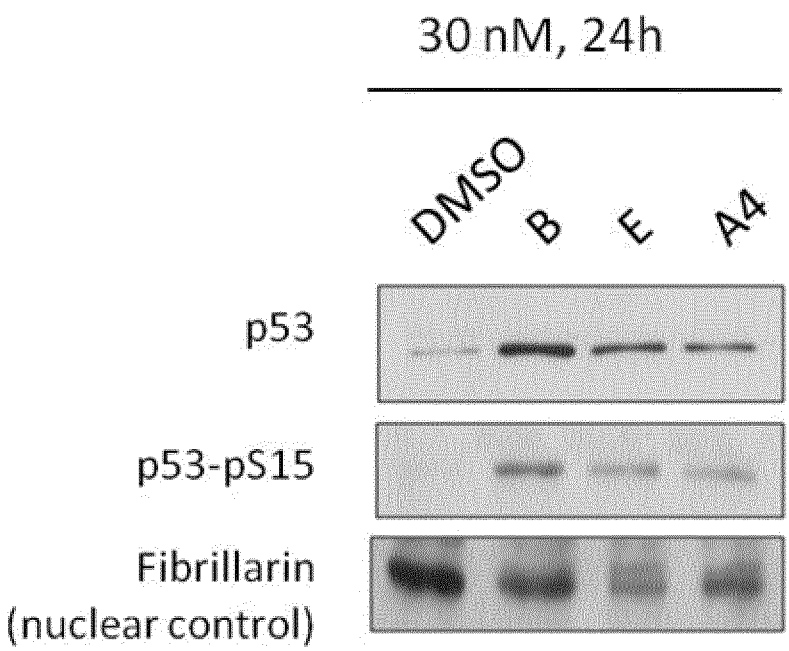

30 nM, 24h
DMSO
B
E
A4
p53
p53-pS15
Fibrillarin
(nuclear control)

**Figure 9**

# INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2012/064825

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07D205/08 C07D205/085 A61K31/397 A61P35/00
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07D A61K A61P

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 practicable, search terms used)
EPO-Internal, EMBASE, WPI Data, BEILSTEIN Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | SUN LICHUN ET AL: "Examination of the 1,4-disubstituted azetidinone ring system as a template for combretastatin A-4 conformationally restricted analogue design", BIOORGANIC & MEDICINAL CHEMISTRY LETTERS, PERGAMON, ELSEVIER SCIENCE, GB, vol. 14, no. 9, 3 May 2004 (2004-05-03), pages 2041-2046, XP002580234, ISSN: 0960-894X, DOI: 10.1016/J.BMCL.2004.02.050 [retrieved on 2004-03-12] cited in the application figure 5; compounds 2, 4, 6, 9 ----- -/-- | 1 |

[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

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"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

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"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 |
|---|---|
| 4 September 2012 | 11/09/2012 |

| 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,<br>Fax: (+31-70) 340-3016 | Moriggi, J |

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