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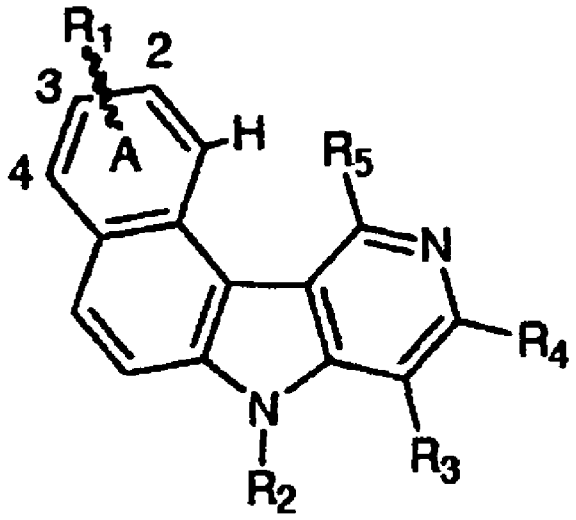
(54) Title: AMINO-SUBSTITUTED-ALKYLOXY-BENZO[E]PYRIDO[4,3-B]INDOLE DERIVATIVES AS NEW POTENT KINASE INHIBITORS



(I)

(57) Abstract: The present invention relates to a new class of benzo[e] pyrido-indole, the amino- substituted-alkyloxy-benzo [e] pyrido [4, 3 -b] indole derivatives of formula (I), having a particular kinase inhibition profile and useful as a therapeutic agent, in particular an anti - tumoralagent. wherein the cycle A, R1, R2, R3, R4 and R5 are as defined in the claims.

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Amino-substituted-alkyloxy-benzo[e]pyrido[4,3-b]indole derivatives as new potent kinase inhibitors

Field of the Invention

The present invention relates to the medical field and in particular to the oncology field.

Background of the Invention

Inhibitors of kinases represent a real hope in cancer therapies since the encouraging results obtained with imatinib in leukemia. In particular, Aurora kinases are a family of serine/threonine protein kinases that play a key role in mitosis progression. Aurora A is found to be associated first with centrosomes and finally with microtubules, whereas aurora B is a chromosomal passenger protein. Aurora A is required for centrosome duplication, entry into mitosis, formation of bipolar spindle and mitotic checkpoint. Aurora B exhibits typical passenger protein behavior during mitosis. Initially, the kinase associates with centromeres, and as mitosis proceeds, it relocates to the central spindle and the midbody. Aurora B is essential for chromosome condensation, kinetochore functions, spindle checkpoint activation and cytokinesis completion.

Aurora A and B are overexpressed in many cancers, including primary colon and breast cancer. Furthermore, the human *Aurora A* gene is localized to the 20q13 amplicon, which is associated with a poor prognosis in breast cancer. Xenografts of mouse NIH-3T3 cells overexpressing aurora A give rise to tumors in nude mice, suggesting that aurora A behaves as an oncogene. Under similar conditions, overexpression of aurora B may induce metastasis.

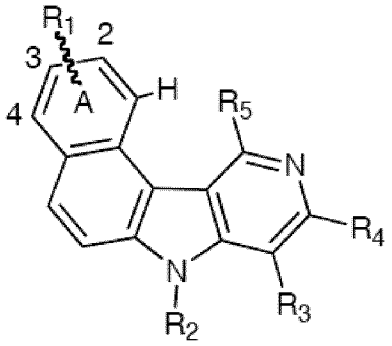
Benzo[e]pyrido-indoles have been identified as interesting mitotic kinase inhibitors (Hoang et al, 2009). These compounds were found to inhibit aurora kinases with a minimal toxicity. It was also shown that those compounds, in particular compounds C1 and C2, inhibit the growth of different cell lines derived from different carcinoma.

Despite an intensive research, two major problems in cancer therapy remain: the induction of tumor resistance to already used drugs and the balance between the toxicity of the treatment and its efficiency. Therefore, new treatments with less toxicity and resistance induction are still required.

Summary of the Invention

In the present invention, the inventors identified a new class of benzo[e]pyrido-indole, the amino-substituted-alkyloxy-benzo[e]pyrido[4,3-b]indole derivatives. This new class of compounds presents a therapeutic interest, in particular as an antiproliferative drug.

The present invention relates to a compound having the formula (I)



(I)

wherein

the benzo cycle A is mono-substituted by R1 in position 2, 3 or 4 ;

R1 is a radical –O-(C_2-C_5)alkyl-NR_aR_b, wherein (C_2-C_5)alkyl is a linear or branched alkyl, R_a and R_b, each independently, are selected from the group consisting of hydrogen, a (C_1-C_4)alkyl optionally substituted by a radical selected from the group consisting of hydroxyl, -NRR', -OPO(OR)(OR'), and -OC(=O)R, and a (C_3-C_6)cycloalkyl; or NR_aR_b may be taken together to form a heterocycle selected from the group consisting of aziridine, azetidine, pyrrolidine, pyrrole, piperidine, piperazine, morpholine, and thiomorpholine, the said heterocycle being optionally substituted by (C_1-C_4)alkyl or hydroxyl radical;

R2 is selected from the group consisting of hydrogen and a (C_1-C_3)alkyl optionally substituted by a radical OH, (C_1-C_3)alkyloxy or -NRR',

R3 and R4, each independently, are selected from the group consisting of hydrogen, (C_1-C_3)alkyl and aryl; or R3 and R4 may be taken together to form a bivalent radical of formula

- –$(CH_2)_n$- wherein n is 3, 4 or 5; or
- -CH=CH-CH=CH- , optionally substituted by a (C_1-C_3)alkyloxy;

R5 is hydrogen or halogen;

wherein R and R', identical or different, are selected from the group consisting of hydrogen and a (C_1-C_4)alkyl;

or an isomeric form thereof or a pharmaceutically acceptable salt thereof or a derivative thereof.

Preferably, the compound of formula (I) has one or several of the following features:

- R1 is a radical –O-(C_2-C_4)alkyl-NR_aR_b, wherein (C_2-C_4)alkyl is a linear or branched alkyl, R_a and R_b, each independently, are selected from the group consisting of hydrogen and a (C_1-C_3)alkyl; or NR_aR_b may be taken together to form a heterocycle selected from the group consisting of aziridine, azetidine, pyrrolidine, pyrrole, piperidine, piperazine, morpholino and thiomorpholine; and/or
- R2 is selected from the group consisting of hydrogen, methyl, ethyl, and -$(CH_2)_n$-N[(C_1-C_2)alkyl]$_2$ with n being 2 or 3; and/or
- R3 and R4, each independently, are selected from the group consisting of hydrogen, methyl, ethyl and phenyl, or may be taken together to form a bivalent radical of formula -CH=CH-CH=CH-; and/or
- R5 is hydrogen or chloride.

More preferably, the compound of formula (I) has one or several of the following features:

- R1 is a radical –O-$(CH_2)_n$-NR_aR_b with n being 2 or 3 or a radical –O-CH_2-($CHCH_3$)-CH_2-NR_aR_b, wherein R_a and R_b, each independently, are selected from the group consisting of hydrogen and a (C_1-C_2)alkyl; or NR_aR_b may be taken together to form a heterocycle selected from the group consisting of piperidine and morpholine; and/or
- R2 is hydrogen; and/or
- R3 is selected from the group consisting of hydrogen, methyl and ethyl, and R4 is hydrogen; and/or
- R5 is hydrogen or chloride.

Still more preferably, the compound of formula (I) has the following features:

- R1 is a radical –O-$(CH_2)_n$-NR_aR_b with n being 2 or 3 or a radical –O-CH_2-($CHCH_3$)-CH_2-NR_aR_b, wherein R_a and R_b, each independently, are selected from the group consisting of hydrogen and a (C_1-C_2)alkyl; or NR_aR_b may be taken together to form a heterocycle selected from the group consisting of piperidine and morpholine;
- R2 is hydrogen;
- R3 is selected from the group consisting of methyl and ethyl, and R4 is hydrogen; and
- R5 is hydrogen or chloride.

In a particular embodiment of the compound of formula (I) as defined above, R1 is –O-$(CH_2)_n$-$N(CH_3)_2$ or –O-$(CH_2)_n$-$N(CH_2CH_3)_2$ with n being 2 or 3 (preferably at position 3 of the benzo cycle A). Preferably, R1 is –O-$(CH_2)_2$-$N(CH_3)_2$ (preferably at position 3 of the benzo cycle A).

In a very particular embodiment, the compound has the formula (I) with R1 being –O-$(CH_2)_2$-$N(CH_3)_2$ (preferably at position 3 of the benzo cycle A), R2 and R4 being hydrogen, R3 being methyl and R5 being hydrogen or chloride.

In a very particular embodiment, the compound has the formula (I) has R5 being hydrogen.

In a very specific embodiment, the compound of the invention may be selected from the group consisting of

11-Chloro-3-(2-*N*,*N*-dimethylaminoethoxy)-8-methyl-7*H*-benzo(e)pyrido(4,3-b)indole (2 or CH20)

3-(2-*N*,*N*-dimethylaminoethoxy)-8-methyl-7*H*-benzo(e)pyrido(4,3-b)indole (3 or CH21)

11-Chloro-3-(3-*N*,*N*-dimethylaminopropoxy)-8-methyl-7*H*-benzo(e)pyrido(4,3-b)indole (4)

3-(3-*N*,*N*-dimethylaminopropoxy)-8-methyl-7*H*-benzo(e)pyrido(4,3-b)indole (5 or CH23)

3-(4-Aminobutyloxy)-8-methyl-7*H*-benzo(e)pyrido(4,3-b)indole (6)

11-Chloro-3-(2-*N*,*N*-diethylaminoethoxy)-8-methyl-7*H*-benzo(e)pyrido(4,3-b)indole (7)

3-(2-*N*,*N*-diethylaminoethoxy)-8-methyl-7*H*-benzo(e)pyrido(4,3-b)indole (8)

11-Chloro-3-(2-(morpholin-4-yl)ethoxy)-8-methyl-7*H*-benzo(e)pyrido(4,3-b)indole (9)

3-(2-(Morpholin-4-yl)ethoxy)-8-methyl-7*H*-benzo(e)pyrido(4,3-b)indole (10)

DL 11-Chloro-3-(3-*N*,*N*-dimethylamino-2-methylpropoxy)-8-methyl-7*H*-benzo(e)pyrido(4,3-b)indole (11)

DL 3-(3-*N*,*N*-dimethylamino-2-methylpropoxy)-8-methyl-7*H*-benzo(e)pyrido(4,3-b)indole (12)

11-Chloro-3-(3-(piperidin-1-yl)propoxy)-8-methyl-7*H*-benzo(e)pyrido(4,3-b)indole (13)

3-(3-(Piperidin-1-yl)propoxy)-8-methyl-7*H*-benzo(e)pyrido(4,3-b)indole (14)

11-Chloro-3-(2-*N*,*N*-dimethylaminoethoxy)-8-ethyl-7*H*-benzo(e)pyrido(4,3-b)indole (15), and

3-(2-*N*,*N*-dimethylaminoethoxy)-8-ethyl-7*H*-benzo(e)pyrido(4,3-b)indole (16).

Preferably, the compound of the invention is 3-(2-*N*,*N*-dimethylaminoethoxy)-8-methyl-7*H*-benzo(e)pyrido(4,3-b)indole (3 or CH21).

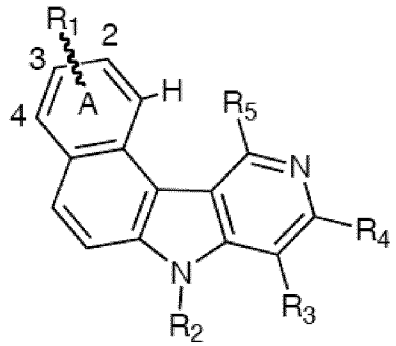
The present invention also relates to a pharmaceutical composition comprising a compound of the present invention and a pharmaceutically acceptable carrier. Optionally, the pharmaceutical composition may further comprise an additional antitumoral drug. Preferably,

the additional antitumoral drug is a DNA-damaging anti-tumoral agent. Alternatively, the pharmaceutical composition may be devoid of a DNA-damaging anti-tumoral agent.

The present invention further relates to a compound of the present invention as a drug. In particular, it relates to a compound of the present invention for use for treating cancer, inflammation, or pain, preferably for use for treating cancer. Optionally, the compound is for use for treating cancer in combination with radiotherapy, hyperthermia and/or an antitumoral chemotherapy, preferably chemotherapy with a DNA-damaging anti-tumoral agent. Alternatively, the compound is for use for treating cancer without any combination with radiotherapy, hyperthermia and/or or a chemotherapy with a DNA-damaging anti-tumoral agent. In addition, the present invention relates to a compound of the present invention for use for treating or preventing inflammation or pain.

In addition, the present invention may relate to a kit or product comprising (a) a compound of the present invention; and (b) an additional antitumoral drug, preferably a DNA-damaging anti-tumoral agent, as a combined preparation for simultaneous, separate or sequential use, in particular in the treatment of cancer.

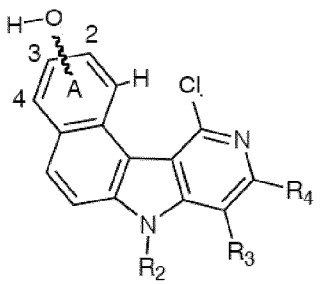
The present invention relates to a method for preparing a compound of formula (I)



(I)

wherein R1, R2, R3 and R4 are as defined in the present disclosure, and R5 is chloride;

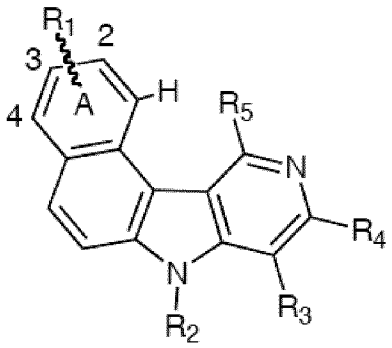
comprising reacting the reagent Y-(C_2-C_5)alkyl-NR_aR_b with the compound **1**



(1)

wherein Y is halo, or hydroxy, R_a, R_b, R2, R3 and R4 are as defined in the present disclosure, thereby obtaining the compound of formula (I).

In addition, present invention relates to a method for preparing a compound of formula (I)



(I)

wherein R1, R2, R3 and R4 are as defined in the present disclosure, and R5 is hydrogen;

comprising carrying out a palladium catalyzed hydrogenation on the compound obtained by the above-detailed method, thereby obtaining the compound of formula (I).

Brief Description of the Drawings

Figure 1: Effect of CH21 and CH23 in quiescent H358 cells.

Quiescent H358 cells were incubated with the compounds, for 96 h, in the absence of serum. Tree different concentrations of compounds were used and live cells were estimated with MTT (Promega). An increase of live cells compared to the control reveals a modification of the NAD/NADH concentrations. In the control only DMSO was added. VX means VX-680 and is described as a reference inhibitor for Aurora kinases.

Figure 2: Comparison of compound efficiencies on spheroid growth.

H358 cells were culture in 3-Dimensions. At day 1, the compound was added at the concentration of 1 μM. The spheroid growth was measured each day and expressed as a growth ratio (Vd-Vo/Vo). The evolution of the volume of the spheroids is expressed in function of the time (in days). On each panel, the spheroid growth in either the presence of C1 (A) or C2 (B) or CH20 (C) or CH23 (D) or CH21 (D) is represented by triangles and compared to the control (diamonds).

Figure 3: Full profiling of CH21 at the concentration of 1 μM

In vitro assays of inhibition of 106 kinases by 1 μM CH21 were performed in duplicate. Each vertical bar represents a kinase.

Figure 4: Determination of Aurora B kinase activity by western blotting in U2OS cells

The intensity of Histone H3 phosphorylation (Histone H3-P) reveals Aurora B activity. The decrease of signal indicates an inhibition of Aurora B by the compound. Actin is used as control of quantity. Cells were incubated overnight in the presence of either C1, or C2 or CH21 or CH23 or DMSO (C: control). All compounds were tested at 1 μM. C1 and C2 inhibit Aurora B, in cells, whereas CH21 and CH23 have no effect.

Figure 5: effect of CH21 in Nuak1 in cellulo

Nuak1 inhibition was revealed by the stabilization of Late Antigen Tumour Suppressor (LATS1). HeLA cells were treated ON by CH21 (1 μM) and whole extracts compared to control cells. Tubulin was used as loading control.

Figure 6 Analysis of cell cycle by FACS

DNA was labelled by propidium iodine and the repartition of the cells in the different phases is represented and compared to control cells (dotted lines). In A, H358 cells were treated by C1 (1 μM) and in B by CH21 (1 μM).

Figure 7: In vivo assays of CH21 efficiency

Nude mice bearing HL60 xenograft were treated by CH21 (rectangles) and compared to mice treated with the vehicle (diamonds). The evolution of the volume of the tumours established in nude mice is expressed in function of the time (in days). Mice received daily CH21 (5 mg/Kg) during the period indicated by the bar (bottom lelt).

Figure 8: HeLa cell viability upon 4 days of treatment with Benzo[e]pyridoindoles

Compounds C1, C3, C4, C7 and C21 were tested at two concentrations (500 nM and 1 μM). Cell viability was estimated by MTT and results are the average of three determinations.

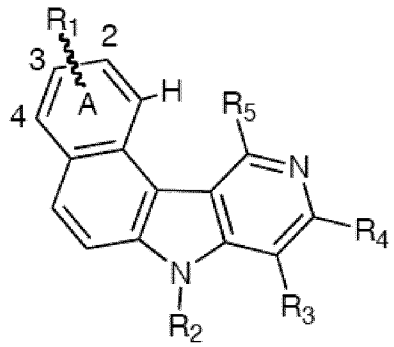
Detailed Description of the Invention

The inventors identified a new class of benzo[e]pyrido-indoles, the amino-substituted-alkyloxy-benzo[e]pyrido[4,3-b]indole derivatives. This new class of compounds presents a therapeutic interest, in particular as an antiproliferative drug with their broad anti-proliferating activities. They are structurally characterized by the presence on the benzo cycle A of a group aminoalkoxy and at position 11 of a hydrogen or halogen, in particular hydrogen or chloride. Indeed, those features provide to the compounds a kinase profile with a narrow selectivity including a MAP4K such as Gck, Nuak1 and TRkA. Indeed, the compounds of the present invention inhibit Gck, Tak1 and Nuak1, allowing anti-tumoral and anti-inflammatory effects; and they inhibit TrkA, being an oncogene and playing a role in chronic inflammatory pain. In addition, MAP4K has been identified as being important for cellular proliferation and for

signal transduction in inflammation. The compounds of the invention present the additional advantage to be soluble in water.

In particular, the compounds of the present invention have a kinase inhibition profile clearly distinct from compounds C1 and C2 (Hoang et al, 2009), leading to biological activity distinct from them. Indeed, the new class of the present invention does not present an inhibition of the full-length Aurora A and B kinases, whereas C1 compound inhibits both Aurora A and B. The compounds of the invention do not modify significantly cell cycle repartition whereas C1 induces a mitotic stop and the polyploidisation of the cells. CH21 exhibits a narrow specificity towards kinases and the inventors described an antiproliferative activity both *in cellulo* and *in vivo*.

Therefore, the present invention relates to a compound having the formula (I)



(I)

wherein

the benzo cycle A is mono-substituted by R1 in position 2, 3 or 4 ;

R1 is a radical –O-(C_2-C_5)alkyl-NR_aR_b, wherein (C_2-C_5)alkyl is a linear or branched alkyl, R_a and R_b, each independently, are selected from the group consisting of hydrogen, a (C_1-C_4)alkyl optionally substituted by a radical selected from the group consisting of hydroxyl, -NRR', OPO(OR)(OR'), and -OC(=O)R, and a (C_3-C_6)cycloalkyl; or NR_aR_b may be taken together to form a heterocycle selected from aziridine, azetidine, pyrrolidine, pyrrole, piperidine, piperazine, morpholine, thiomorpholine, the said heterocycle is optionally substituted by a (C_1-C_4)alkyl or hydroxyl radical;

R2 is selected from the group consisting of hydrogen and a (C_1-C_3)alkyl optionally substituted by a radical OH, (C_1-C_3)alkyloxy or -NRR',

R3 and R4, each independently, are selected from the group consisting of hydrogen, a (C_1-C_3)alkyl and an aryl; or R3 and R4 may be taken together to form a bivalent radical of formula

- –$(CH_2)_n$- wherein n is 3, 4 or 5; or
- -CH=CH-CH=CH- , optionally substituted by a (C_1-C_3)alkyloxy;

R5 is hydrogen or halogen;

wherein R and R', identical or different, are selected from the group consisting of hydrogen and (C_1-C_4)alkyl, preferably hydrogen and (C_1-C_2)alkyl;

or an isomeric form thereof or a pharmaceutically acceptable salt thereof or a derivative thereof.

In the context of the present invention, the term "(C_1-C_2)alkyl" more specifically means methyl or ethyl, the term "(C_1-C_3)alkyl" more specifically means methyl, ethyl, propyl, or isopropyl and "(C_1-C_4)alkyl" more specifically means methyl, ethyl, propyl, isopropyl, butyl, isobutyl, or *tert*-butyl, the term "(C_2-C_5)alkyl" more specifically means methyl, ethyl, propyl, isopropyl, butyl, isobutyl, *tert*-butyl or propyl.

"Alkoxy" groups correspond to the alkyl groups defined hereinabove bonded to the molecule by an -O- (ether) bond. (C_1-C_3)alkoxy includes methoxy, ethoxy, propyloxy, and isopropyloxy. (C_1-C_4)alkoxy includes methoxy, ethoxy, propyloxy, isopropyloxy, butyloxy, isobutyloxy, and *tert*-butyloxy.

The "aryl" or "Ar" group is mono- or bi- cyclic aromatic hydrocarbons having from 6 to 12 carbon atoms, optionally substituted. Aryl may be a phenyl, biphenyl or naphthyl. In a preferred embodiment, the aryl is a phenyl.

The term "derivative" is meant to encompass hydrate, ester, ether, conjugates, or pro-drugs thereof. For instance, the compounds with a radical -OPO(OR)(OR') as defined above is a prodrug and has an increased solubility.

(C_3-C_6)cycloalkyl includes cyclopropyl, cyclobutyl, cyclopentyl and cyclohexyl.

"Halogen" or "halo" groups are preferably selected from the group consisting of Cl (chloride), Br (bromide), I (iodide) and F (fluoride).

The pharmaceutically acceptable salts include salts of inorganic acids as well as organic acids. Representative examples of suitable inorganic acids include hydrochloric, hydrobromic, hydroiodic, phosphoric, and the like. Representative examples of suitable organic acids include formic, acetic, trichloroacetic, trifluoroacetic, propionic, benzoic, cinnamic, citric, fumaric, maleic, methanesulfonic and the like. Further examples of pharmaceutically acceptable inorganic or organic acid addition salts include the pharmaceutically acceptable salts listed in J. Pharm. Sci. 1977, 66, 2, and in Handbook of Pharmaceutical Salts: Properties, Selection, and Use edited by P. Heinrich Stahl and Camille G. Wermuth 2002. In a preferred embodiment, the salt is selected from the group consisting of maleate, chlorhydrate, bromhydrate, and methanesulfonate.

In a preferred embodiment, R1 is a radical –O-$(CH_2)_n$-NR_aR_b with n being 2 or 3 or a radical –O-CH_2-$(CHCH_3)$-CH_2-NR_aR_b, wherein R_a and R_b, each independently, are selected from the group consisting of hydrogen and a $(C_1$-$C_2)$alkyl; or NR_aR_b may be taken together to form a heterocycle selected from the group consisting of piperidine and morpholino. More preferably, R1 is a radical –O-$(CH_2)_n$-NR_aR_b with n being 2 or 3 or a radical –O-CH_2-$(CHCH_3)$-CH_2-NR_aR_b, wherein R_a and R_b, each independently, are selected from the group consisting of hydrogen and $(C_1$-$C_2)$alkyl; or NR_aR_b may be taken together to form a heterocycle selected from the group consisting of piperidine and morpholino. Preferably, R_a and R_b, each independently, are selected from the group consisting of methyl and ethyl. In a particular embodiment of the compound of formula (I) as defined above, R1 is –O-$(CH_2)_n$-$N(CH_3)_2$ or –O-$(CH_2)_n$-$N(CH_2CH_3)_2$ with n being 2 or 3. Preferably, R1 is –O-$(CH_2)_2$-$N(CH_3)_2$. More preferably, R1 is at position 3 of the benzo cycle A.

In a preferred embodiment, R2 is selected from the group consisting of hydrogen and a $(C_1$-$C_3)$alkyl, more preferably is hydrogen.

In a preferred embodiment, R3 and R4, each independently, are selected from the group consisting of hydrogen, a $(C_1$-$C_3)$alkyl and an aryl. More preferably, R3 is selected from the group consisting of a $(C_1$-$C_3)$alkyl and an aryl, and R4 is selected from the group consisting of hydrogen and a $(C_1$-$C_3)$alkyl. Still more preferably, R3 is a $(C_1$-$C_3)$alkyl, and R4 is hydrogen.

In a preferred embodiment, R5 is hydrogen or chloride. Still more preferably, R5 is hydrogen.

Preferably, the compound of formula (I) may have one or several of the following features:

- R2 is hydrogen; and/or
- R3 is selected from the group consisting of hydrogen, methyl and ethyl, and R4 is hydrogen; and/or
- R5 is hydrogen or chloride, preferably hydrogen.

Still more preferably, the compound of formula (I) may have one or several of the following features:

- R2 is hydrogen; and/or
- R3 is selected from the group consisting of methyl and ethyl; and/or
- R4 is hydrogen; and/or
- R5 is hydrogen or chloride, preferably hydrogen.

Even more preferably, the compound of formula (I) may have the following features:

- R2 is hydrogen;
- R3 is selected from the group consisting of methyl and ethyl, and R4 is hydrogen; and
- R5 is hydrogen or chloride, preferably hydrogen.

In a first very particular embodiment, the compound has the formula (I) with R1 being $-O-(CH_2)_n-N(CH_3)_2$ (preferably at position 3 of the benzo cycle A), n being 2 or 3, R2 and R4 being hydrogen, R5 being hydrogen or chloride, preferably hydrogen, and R3 being methyl or ethyl. In a first embodiment, R3 is methyl. In a second embodiment, R3 is ethyl.

In a second very particular embodiment, the compound has the formula (I) with R1 being $-O-(CH_2)_n-NH_2$ (preferably at position 3 of the benzo cycle A), n being 2, 3 or 4, R2 and R4 being hydrogen, R5 being hydrogen or chloride, preferably hydrogen, and R3 being methyl or ethyl. More specifically, n is 4. In a first embodiment, R3 is methyl. In a second embodiment, R3 is ethyl.

In a third very particular embodiment, the compound has the formula (I) with R1 being $-O-(CH_2)_n-N(CH_2CH_3)_2$ (preferably at position 3 of the benzo cycle A), n being 2 or 3, R2 and R4 being hydrogen, R5 being hydrogen or chloride, preferably hydrogen, and R3 being methyl or ethyl. More specifically, n is 2. In a first embodiment, R3 is methyl. In a second embodiment, R3 is ethyl.

In a fourth very particular embodiment, the compound has the formula (I) with R1 being $-O-(CH_2)_n$-morpholino (preferably at position 3 of the benzo cycle A), n being 2 or 3, R2 and R4 being hydrogen, R5 being hydrogen or chloride, and R3 being methyl or ethyl. More specifically, n is 2. In a first embodiment, R3 is methyl. In a second embodiment, R3 is ethyl.

In a fifth particular embodiment, the compound has the formula (I) with R1 being being $-O-CH_2-(CHCH_3)-CH_2-N(CH_3)_2$ (preferably at position 3 of the benzo cycle A), R2 and R4 being hydrogen, R5 being hydrogen or chloride, and R3 being methyl or ethyl. In a first embodiment, R3 is methyl. In a second embodiment, R3 is ethyl.

In a sixth very particular embodiment, the compound has the formula (I) with R1 being $-O-(CH_2)_n$- piperidin-1-yl (preferably at position 3 of the benzo cycle A), n being 2 or 3, R2 and R4 being hydrogen, R5 being hydrogen or chloride, and R3 being methyl or ethyl. More specifically, n is 2. In a first embodiment, R3 is methyl. In a second embodiment, R3 is ethyl.

Optionally, the compound of the invention may be selected from the group consisting of

11-Chloro-3-(2-*N*,*N*-dimethylaminoethoxy)-8-methyl-7*H*-benzo(e)pyrido(4,3-b)indole (2 or CH20)

3-(2-*N*,*N*-dimethylaminoethoxy)-8-methyl-7*H*-benzo(e)pyrido(4,3-b)indole (3 or CH21)

11-Chloro-3-(3-*N*,*N*-dimethylaminopropoxy)-8-methyl-7*H*-benzo(e)pyrido(4,3-b)indole (4)

3-(3-*N*,*N*-dimethylaminopropoxy)-8-methyl-7*H*-benzo(e)pyrido(4,3-b)indole (5 or CH23)

3-(4-Aminobutyloxy)-8-methyl-7*H*-benzo(e)pyrido(4,3-b)indole (6)

11-Chloro-3-(2-*N*,*N*-diethylaminoethoxy)-8-methyl-7*H*-benzo(e)pyrido(4,3-b)indole (7)

3-(2-*N*,*N*-diethylaminoethoxy)-8-methyl-7*H*-benzo(e)pyrido(4,3-b)indole (8)

11-Chloro-3-(2-(morpholin-4-yl)ethoxy)-8-methyl-7*H*-benzo(e)pyrido(4,3-b)indole (9)

3-(2-(Morpholin-4-yl)ethoxy)-8-methyl-7*H*-benzo(e)pyrido(4,3-b)indole (10)

DL 11-Chloro-3-(3-*N*,*N*-dimethylamino-2-methylpropoxy)-8-methyl-7*H*-benzo(e)pyrido(4,3-b)indole (11)

DL 3-(3-*N*,*N*-dimethylamino-2-methylpropoxy)-8-methyl-7*H*-benzo(e)pyrido(4,3-b)indole (12)

11-Chloro-3-(3-(piperidin-1-yl)propoxy)-8-methyl-7*H*-benzo(e)pyrido(4,3-b)indole (13)

3-(3-(Piperidin-1-yl)propoxy)-8-methyl-7*H*-benzo(e)pyrido(4,3-b)indole (14)

11-Chloro-3-(2-*N*,*N*-dimethylaminoethoxy)-8-ethyl-7*H*-benzo(e)pyrido(4,3-b)indole (15), and

3-(2-*N*,*N*-dimethylaminoethoxy)-8-ethyl-7*H*-benzo(e)pyrido(4,3-b)indole (16).

More preferably, it selected from the group consisting of

11-Chloro-3-(2-*N*,*N*-dimethylaminoethoxy)-8-methyl-7*H*-benzo(e)pyrido(4,3-b)indole (2 or CH20)

3-(2-*N*,*N*-dimethylaminoethoxy)-8-methyl-7*H*-benzo(e)pyrido(4,3-b)indole (3 or CH21)

3-(3-*N*,*N*-dimethylaminopropoxy)-8-methyl-7*H*-benzo(e)pyrido(4,3-b)indole (5 or CH23)

11-Chloro-3-(3-*N*,*N*-dimethylaminopropoxy)-8-methyl-7*H*-benzo(e)pyrido(4,3-b)indole (4)

11-Chloro-3-(2-*N*,*N*-dimethylaminoethoxy)-8-ethyl-7*H*-benzo(e)pyrido(4,3-b)indole (15), and

3-(2-*N*,*N*-dimethylaminoethoxy)-8-ethyl-7*H*-benzo(e)pyrido(4,3-b)indole (16).

Still more preferably, it selected from the group consisting of

11-Chloro-3-(2-*N*,*N*-dimethylaminoethoxy)-8-methyl-7*H*-benzo(e)pyrido(4,3-b)indole (2 or CH20)

3-(2-*N*,*N*-dimethylaminoethoxy)-8-methyl-7*H*-benzo(e)pyrido(4,3-b)indole (3 or CH21), and

3-(3-*N*,*N*-dimethylaminopropoxy)-8-methyl-7*H*-benzo(e)pyrido(4,3-b)indole (5 or CH23).

The present invention relates to

- a pharmaceutical composition comprising a) any compound having the formula (I) as disclosed above including anyone of the disclosed embodiments, and a

pharmaceutically acceptable carrier, in particular for use in the treatment of cancer, inflammation, or pain; and/or

- any compound having the formula (I) as disclosed above including anyone of the disclosed embodiments as a drug, in particular an anti-tumoral drug, an anti-inflammatory drug, or an anti-pain drug; and/or
- a pharmaceutical composition comprising a) any compound having the formula (I) as disclosed above including anyone of the disclosed embodiments, b) an additional anti-tumoral agent, preferably a DNA-damaging anti-tumoral agent, and a pharmaceutically acceptable carrier, in particular for use in the treatment of cancer; and/or
- a pharmaceutical composition comprising a) any compound having the formula (I) as disclosed above including anyone of the disclosed embodiments, and a pharmaceutically acceptable carrier but without any DNA-damaging anti-tumoral agent, in particular for use in the treatment of cancer; and/or
- a product or kit containing (a) any compound of formula (I) as disclosed above including anyone of the disclosed embodiments and (b) an additional anti-tumoral agent, preferably a DNA-damaging anti-tumoral agent, as a combined preparation for simultaneous, separate or sequential use, in particular in the treatment of cancer; and/or
- a combined preparation which comprises (a) any compound of formula (I) as disclosed above including anyone of the disclosed embodiments and (b) an additional anti-tumoral agent, preferably a DNA-damaging anti-tumoral agent, for simultaneous, separate or sequential use, in particular in the treatment of cancer; and/or
- a pharmaceutical composition comprising any compound having the formula (I) as disclosed above including anyone of the disclosed embodiments for the use in the treatment of cancer in combination with radiotherapy and/or hyperthermia; and/or
- the use of a pharmaceutical composition comprising any compound having the formula (I) as disclosed above including anyone of the disclosed embodiments for the manufacture of a medicament for the treatment of cancer, inflammation or pain, preferably cancer; and/or
- the use of a pharmaceutical composition comprising any compound having the formula (I) as disclosed above including anyone of the disclosed embodiments for the manufacture of a medicament for the treatment of cancer in combination with

radiotherapy, hyperthermia and/or or an additional anti-tumoral agent, preferably a DNA-damaging anti-tumoral agent; and/or

- the use of a pharmaceutical composition comprising any compound having the formula (I) as disclosed above including anyone of the disclosed embodiments for the manufacture of a medicament for the treatment of cancer without any combination with a DNA-damaging anti-tumoral agent or radiotherapy; and/or
- the use of a pharmaceutical composition comprising a) any compound having the formula (I) as disclosed above including anyone of the disclosed embodiments and b) an additional anti-tumoral agent, preferably a DNA-damaging anti-tumoral agent, and a pharmaceutically acceptable carrier for the manufacture of a medicament for the treatment of cancer; and/or
- a method for treating a cancer in a subject in need thereof, comprising administering an effective amount of a pharmaceutical composition comprising any compound having the formula (I) as disclosed above including anyone of the disclosed embodiments, and a pharmaceutically acceptable carrier; and/or
- a method for treating a cancer in a subject in need thereof, comprising administering an effective amount of a pharmaceutical composition comprising a) any compound having the formula (I) as disclosed above including anyone of the disclosed embodiments, b) an additional anti-tumoral agent, preferably a DNA-damaging anti-tumoral agent, and a pharmaceutically acceptable carrier; and/or
- a method for treating a cancer in a subject in need thereof, comprising administering an effective amount of a pharmaceutical composition comprising any compound having the formula (I) as disclosed above including anyone of the disclosed embodiments, and an effective amount of a pharmaceutical composition comprising an additional anti-tumoral agent, preferably a DNA-damaging anti-tumoral agent; and/or
- a method for treating a cancer in a subject in need thereof, comprising administering an effective amount of a pharmaceutical composition comprising a) any compound having the formula (I) as disclosed above including anyone of the disclosed embodiments in combination with radiotherapy and/or hyperthermia; and/or
- a method for treating a cancer in a subject in need thereof, comprising administering an effective amount of a pharmaceutical composition comprising any compound having the formula (I) as disclosed above including anyone of the

disclosed embodiments, and a pharmaceutically acceptable carrier but without any DNA-damaging anti-tumoral agent; and/or

- a method for treating an inflammation or pain in a subject in need thereof, comprising administering an effective amount of a pharmaceutical composition comprising any compound having the formula (I) as disclosed above including anyone of the disclosed embodiments, and a pharmaceutically acceptable carrier.

Whenever within this whole specification "treatment of a cancer" or the like is mentioned with reference to the pharmaceutical composition of the invention, there is meant: a) a method for treating a cancer, said method comprising administering a pharmaceutical composition of the invention to a subject in need of such treatment; b) the use of a pharmaceutical composition of the invention for the treatment of a cancer; c) the use of a pharmaceutical composition of the invention for the manufacture of a medicament for the treatment of a cancer; d) a pharmaceutical composition comprising a dose of any compound having the formula (I) as disclosed above including anyone of the disclosed embodiments and of an additional anti-tumoral agent, preferably a DNA-damaging anti-tumoral agent, that is appropriate for the treatment of a cancer; and/or e) a pharmaceutical composition of the invention for treating a cancer.

Pain includes acute pain, chronic pain, neuropathic pain, muscular pain, bone pain, postoperative pain, migraine, cancer-related pain, lumbalgia, arthrosic pain, diabetes-related pain or pain associated to AIDS. In a particularly preferred embodiment, pain is a cancer-related pain, especially a cancer with bone metastasis.

By “inflammation” or “inflammatory disorder or disease” refer to any disorder, condition, or disease characterized or caused by excessive or uncontrolled inflammation, or any aspect of inflammation such as redness, swelling, heat, pain, etc. In particular, it may refer to chronic or acute inflammation. Inflammatory diseases include, but are not limited to, irritable bowel disease, Crohn’s disease, ulcerative colitis, allergies, including allergic rhinitis/sinusitis, skin allergies such as urticaria/hives, angioedema, atopic dermatitis, food allergies, drug allergies, insect allergies, and rare allergic disorders such as mastocytosisasthma, asthma, arthritis, including osteoarthritis, rheumatoid arthritis, and spondyloarthropathies, gastrointestinal inflammation, neuroinflammatory disorders, and autoimmune disorders.

The term "pharmaceutically acceptable carrier" is meant to encompass any carrier (e.g., support, substance, solvent, etc.) which does not interfere with effectiveness of the biological activity of the active ingredient(s) and that is not toxic to the host to which it is

administered. For example, for parenteral administration, the active compounds(s) may be formulated in a unit dosage form for injection in vehicles such as saline, dextrose solution, serum albumin and Ringer's solution.

The pharmaceutical composition can be formulated as solutions in pharmaceutically compatible solvents or as emulsions, suspensions or dispersions in suitable pharmaceutical solvents or vehicule, or as pills, tablets or capsules that contain solid vehicules in a way known in the art. Formulations of the present invention suitable for oral administration may be in the form of discrete units as capsules, sachets, tablets or lozenges, each containing a predetermined amount of the active ingredient; in the form of a powder or granules; in the form of a solution or a suspension in an aqueous liquid or non-aqueous liquid; or in the form of an oil-in-water emulsion or a water-in-oil emulsion. Formulations for rectal administration may be in the form of a suppository incorporating the active ingredient and carrier such as cocoa butter, or in the form of an enema. Formulations suitable for parenteral administration conveniently comprise a sterile oily or aqueous preparation of the active ingredient which is preferably isotonic with the blood of the recipient. Every such formulation can also contain other pharmaceutically compatible and nontoxic auxiliary agents, such as, e.g. stabilizers, antioxidants, binders, dyes, emulsifiers or flavouring substances. The formulations of the present invention comprise an active ingredient in association with a pharmaceutically acceptable carrier therefore and optionally other therapeutic ingredients. The carrier must be "acceptable" in the sense of being compatible with the other ingredients of the formulations and not deleterious to the recipient thereof. The pharmaceutical compositions are advantageously applied by injection or intravenous infusion of suitable sterile solutions or as oral dosage by the digestive tract. Methods for the safe and effective administration of most of these chemotherapeutic agents are known to those skilled in the art. In addition, their administration is described in the standard literature.

Radiotherapy includes, but is not limited to, γ-rays, X-rays, and/or the directed delivery of radioisotopes to tumor cells. Other radiotherapies include microwaves and UV-irradiation. Other approaches to radiation therapy are also contemplated in the present invention. The radiotherapy may be applied before or simultaneously with the administration of the compound of the present invention. When the administration of the compound is after radiotherapy, the compound may be for instance administered 1, 2, 3, 4, 5, 6, 12, 18 or 24 h after the radiotherapy.

Hyperthermia is a medical treatment in which body tissue is exposed to high temperatures to damage and kill cancer cells or to make cancer cells more sensitive to the

effects of radiation and certain anti-cancer drugs. There are many techniques, well-known by the on skilled in the art, by which heat may be delivered. Some of the most common involve the use of focused ultrasound (FUS or HIFU), infrared sauna, microwave heating, induction heating, magnetic hyperthermia, infusion of warmed liquids, or direct application of heat such as through sitting in a hot room or wrapping a patient in hot blankets.

The anti-tumoral agent may be for instance an histone deacetylase (HDAC) inhibitor or a taxoid antitumoral agent. Such antitumoral agents are well-known by the one skilled in the art. For instance, non-exhaustively, the taxoid antitumoral agent can be selected from the group consisting of paclitaxel, docetaxel, larotaxel, XRP6258, BMS-184476, BMS-188797, BMS-275183, ortataxel, RPR 109881A, RPR 116258, NBT-287, PG-paclitaxel, ABRAXANE®, Tesetaxel, IDN 5390, Taxoprexin, DHA-paclitaxel, and MAC-321. More preferably, the molecule of the taxoid antitumoral agent is paclitaxel. Similarly, non-exhaustively, the HDAC can be selected from the group consisting of trichostatin A, vironostat, belinostat, LAQ824, panobinostat (LBH589), mocetinostat, valproic acid, romidepsin, ITF2357, benzamides entinostat (MS275), and CI994.

The DNA-damaging anti-tumoral agent may be chosen from the group consisting of inhibitors of topoisomerases I and/or II, DNA crosslinkers, DNA alkylating agents, and anti-metabolic agents. In a preferred embodiment, the DNA-damaging anti-tumoral agent is chosen from the group consisting of inhibitors of topoisomerases I and/or II, and DNA crosslinkers.

Inhibitors of topoisomerases I and/or II include, but are not limited to, etoposide, topotecan, camptothecin, irinotecan, amsacrine, intoplicin, anthracyclines such as doxorubicin, epirubicin, daunorubicin, idarubicin and mitoxantrone. Inhibitors of Topoisomerase I and II include, but are not limited to, intoplicin.

DNA crosslinkers include, but are not limited to, cisplatin, carboplatin and oxaliplatin. In a preferred embodiment, the DNA crosslinker is cisplatin.

Anti-metabolic agents block the enzymes responsible for nucleic acid synthesis or become incorporated into DNA, which produces an incorrect genetic code and leads to apoptosis. Non-exhaustive examples thereof include, without limitation, folic acid antagonists, pyrimidine analogs, purine analogs and adenosine deaminase inhibitors, and more particularly Methotrexate, Floxuridine, Cytarabine, 6-Mercaptopurine, 6- Thioguanine, Fludarabine phosphate, Pentostatine, 5-fluorouracil, gemcitabine and capecitabine.

The DNA-damaging anti-tumoral agent can be alkylating agents including, without limitation, nitrogen mustards, ethylenimine derivatives, alkyl sulfonates, nitrosoureas, metal

salts and triazenes. Non-exhaustive examples thereof include Uracil mustard, Chlormethine, Cyclophosphamide (CYTOXAN(R)), Ifosfamide, Melphalan, Chlorambucil, Pipobroman, Triethylenemelamine, Triethylenethiophosphoramine, Busulfan, Carmustine, Lomustine, cisplatin, carboplatin, oxaliplatin, thiotepa, Streptozocin, Dacarbazine, and Temozolomide.

The terms “kit”, “product” or "combined preparation", as used herein, defines especially a "kit of parts" in the sense that the combination partners (a) and (b) as defined above can be dosed independently or by use of different fixed combinations with distinguished amounts of the combination partners (a) and (b), i.e. simultaneously or at different time points. The parts of the kit of parts can then, e.g., be administered simultaneously or chronologically staggered, that is at different time points and with equal or different time intervals for any part of the kit of parts. The ratio of the total amounts of the combination partner (a) to the combination partner (b) to be administered in the combined preparation can be varied. The combination partners (a) and (b) can be administered by the same route or by different routes. In a preferred embodiment, partner (b) is administered before or simultaneously partner (a). When the administration is sequential, the first partner may be for instance administered 1, 2, 3, 4, 5, 6, 12, 18 or 24 h before the second partner.

Within the context of the invention, the term treatment denotes curative, symptomatic, and preventive treatment.

Pharmaceutical compositions, kits, products and combined preparations of the invention can be used in humans with existing cancer or tumor, including at early or late stages of progression of the cancer. The pharmaceutical compositions, kits, products and combined preparations of the invention will not necessarily cure the patient who has the cancer but will delay or slow the progression or prevent further progression of the disease, ameliorating thereby the patients’ condition. In particular, the pharmaceutical compositions, kits, products and combined preparations of the invention reduce the development of tumors, reduce tumor burden, produce tumor regression in a mammalian host and/or prevent metastasis occurrence and cancer relapse. In treating the cancer, the pharmaceutical composition of the invention is administered in a therapeutically effective amount.

By "effective amount" it is meant the quantity of the pharmaceutical composition of the invention which prevents, removes or reduces the deleterious effects of the treated disease in mammals, including humans. It is understood that the administered dose may be adapted by those skilled in the art according to the patient, the pathology, the mode of administration, etc. For instance, the compounds of the invention may be used at a dose of 0.01 to 500 mg / kg of body weight / day. In a particular embodiment, the pharmaceutical composition according to

the invention comprises 0.01 to 500 mg / kg of the compound of the invention. It is understood that the administered dose may be adapted by those skilled in the art according to the patient, the pathology, the mode of administration, etc.

The treatment may be topical, transdermal, oral, rectal, sublingual, intranasal or parenteral. The pharmaceutical composition, kit, product or combined preparation is preferably administered by injection or by intravenous infusion or suitable sterile solutions, or in the form of liquid or solid doses via the alimentary canal.

The terms "cancer", "cancerous", or "malignant" refer to or describe the physiological condition in mammals that is typically characterized by unregulated cell growth. Examples of cancer include, for example, leukemia, lymphoma, blastoma, carcinoma and sarcoma. More particular examples of such cancers include chronic myeloid leukemia, acute lymphoblastic leukemia, Philadelphia chromosome positive acute lymphoblastic leukemia (Ph+ ALL), squamous cell carcinoma, lung cancer, small-cell lung cancer, non-small cell lung cancer, glioma, gastrointestinal cancer, renal cancer, ovarian cancer, liver cancer, colorectal cancer, endometrial cancer, kidney cancer, prostate cancer, thyroid cancer, neuroblastoma, osteosarcoma, pancreatic cancer, glioblastoma multiforme, cervical cancer, stomach cancer, bladder cancer, hepatoma, breast cancer, oesophagal cancer, colon carcinoma, and head and neck cancer, gastric cancer, germ cell tumor, pediatric sarcoma, sinonasal natural killer, multiple myeloma, acute myelogenous leukemia (AML), chronic lymphocytic leukemia, mastocytosis and any symptom associated with mastocytosis.

"Leukemia" refers to progressive, malignant diseases of the blood-forming organs and is generally characterized by a distorted proliferation and development of leukocytes and their precursors in the blood and bone marrow. Leukemia is generally clinically classified on the basis of (1) the duration and character of the disease—acute or chronic; (2) the type of cell involved; myeloid (myelogenous), lymphoid (lymphogenous), or monocytic; and (3) the increase or non-increase in the number of abnormal cells in the blood—leukemic or aleukemic (subleukemic). Leukemia includes, for example, acute nonlymphocytic leukemia, chronic lymphocytic leukemia, acute granulocytic leukemia, chronic granulocytic leukemia, acute promyelocytic leukemia, adult T-cell leukemia, aleukemic leukemia, a leukocythemic leukemia, basophylic leukemia, blast cell leukemia, bovine leukemia, chronic myelocytic leukemia, leukemia cutis, embryonal leukemia, eosinophilic leukemia, Gross' leukemia, hairy-cell leukemia, hemoblastic leukemia, hemocytoblastic leukemia, histiocytic leukemia, stem cell leukemia, acute monocytic leukemia, leukopenic leukemia, lymphatic leukemia, lymphoblastic leukemia, lymphocytic leukemia, lymphogenous leukemia, lymphoid

leukemia, lymphosarcoma cell leukemia, mast cell leukemia, megakaryocyte leukemia, micromyeloblastic leukemia, monocytic leukemia, myeloblasts leukemia, myelocytic leukemia, myeloid granulocytic leukemia, myelomonocytic leukemia, Naegeli leukemia, plasma cell leukemia, plasmacytic leukemia, promyelocytic leukemia, Rieder cell leukemia, Schilling's leukemia, stem cell leukemia, subleukemic leukemia, and undifferentiated cell leukemia. In certain aspects, the present invention provides treatment for chronic myeloid leukemia, acute lymphoblastic leukemia, and/or Philadelphia chromosome positive acute lymphoblastic leukemia (Ph+ ALL).

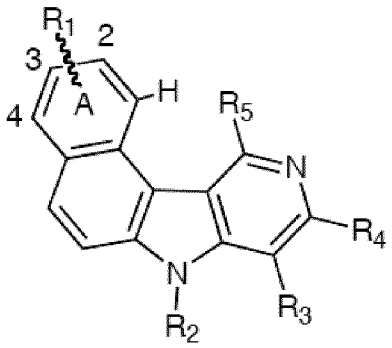
Various cancers are also encompassed by the scope of the invention, including, but not limited to, the following: carcinoma including that of the bladder (including accelerated and metastatic bladder cancer), breast, colon (including colorectal cancer), kidney, liver, lung (including small and non-small cell lung cancer and lung adenocarcinoma), ovary, prostate, testes, genitourinary tract, lymphatic system, rectum, larynx, pancreas (including exocrine pancreatic carcinoma), esophagus, stomach, gall bladder, cervix, thyroid, and skin (including squamous cell carcinoma); hematopoietic tumors of lymphoid lineage including leukemia, acute lymphocytic leukemia, acute lymphoblastic leukemia, B-cell lymphoma, T-cell lymphoma, Hodgkins lymphoma, non-Hodgkins lymphoma, hairy cell lymphoma, histiocytic lymphoma, and Burketts lymphoma; hematopoietic tumors of myeloid lineage including acute and chronic myelogenous leukemias, myelodysplastic syndrome, myeloid leukemia, and promyelocytic leukemia; tumors of the central and peripheral nervous system including astrocytoma, neuroblastoma, glioma, and schwannomas; tumors of mesenchymal origin including fibrosarcoma, rhabdomyosarcoma, and osteosarcoma; other tumors including melanoma, xenoderma pigmentosum, keratoactanthoma, seminoma, thyroid follicular cancer, and teratocarcinoma; melanoma, unresectable stage III or IV malignant melanoma, squamous cell carcinoma, small-cell lung cancer, non-small cell lung cancer, glioma, gastrointestinal cancer, renal cancer, ovarian cancer, liver cancer, colorectal cancer, endometrial cancer, kidney cancer, prostate cancer, thyroid cancer, neuroblastoma, pancreatic cancer, glioblastoma multiforme, cervical cancer, stomach cancer, bladder cancer, hepatoma, breast cancer, colon carcinoma, and head and neck cancer, retinoblastoma, gastric cancer, germ cell tumor, bone cancer, bone tumors, adult malignant fibrous histiocytoma of bone; childhood malignant fibrous histiocytoma of bone, sarcoma, pediatric sarcoma, sinonasal natural killer, neoplasms, plasma cell neoplasm; myelodysplastic syndromes; neuroblastoma; testicular germ cell tumor, intraocular melanoma, myelodysplastic syndromes; myelodysplastic/myeloproliferative diseases, synovial sarcoma. In addition, disorders include

urticaria pigmentosa, mastocytosises such as diffuse cutaneous mastocytosis, solitary mastocytoma in human, as well as dog mastocytoma and some rare subtypes like bullous, erythrodermic and teleangiectatic mastocytosis, mastocytosis with an associated hematological disorder, such as a myeloproliferative or myelodysplastic syndrome, or acute leukemia, myeloproliferative disorder associated with mastocytosis, mast cell leukemia, in addition to other cancers. Other cancers are also included within the scope of disorders including, but are not limited to, the following: carcinoma, including that of the bladder, urothelial carcinoma, breast, colon, kidney, liver, lung, ovary, pancreas, stomach, cervix, thyroid, testis, particularly testicular seminomas, and skin; including squamous cell carcinoma; gastrointestinal stromal tumors ("GIST"); hematopoietic tumors of lymphoid lineage, including leukemia, acute lymphocytic leukemia, acute lymphoblastic leukemia, B-cell lymphoma, T-cell lymphoma, Hodgkins lymphoma, non-Hodgkins lymphoma, hairy cell lymphoma and Burketts lymphoma; hematopoietic tumors of myeloid lineage, including acute and chronic myelogenous leukemias and promyelocytic leukemia; tumors of mesenchymal origin, including fibrosarcoma and rhabdomyosarcoma; other tumors, including melanoma, seminoma, teratocarcinoma, neuroblastoma and glioma; tumors of the central and peripheral nervous system, including astrocytoma, neuroblastoma, glioma, and schwannomas; tumors of mesenchymal origin, including fibrosarcoma, rhabdomyosarcoma, and osteosarcoma; and other tumors, including melanoma, xenoderma pigmentosum, keratoactanthoma, seminoma, thyroid follicular cancer, teratocarcinoma, chemotherapy refractory non-seminomatous germ-cell tumors, and Kaposi's sarcoma, and any metastasis thereof. In particular, the cancer may be selected from the group consisting of hepatoma, glioblastoma, leukemia, breast cancer, lung cancer, ovarian cancer, and osteosarcoma.

In a preferred embodiment of the present invention, the cancer is a solid tumor. The term "solid tumor" especially means breast cancer, ovarian cancer, cancer of the colon and generally the GI (gastro-intestinal) tract, cervix cancer, neuroblastoma, lung cancer, in particular small- cell lung cancer, and non-small-cell lung cancer, head and neck cancer, colorectal cancer, bladder cancer, osteosarcoma, cancer of the prostate or Kaposi's sarcoma. The present combination inhibits the growth of solid tumors, but also liquid tumors. Furthermore, a decrease of the tumor volume can be obtained. The compounds disclosed herein are also suited to prevent the metastatic spread of tumors and the growth or development of micrometastases.

Finally, the present invention relates to methods for preparing the compounds of formula (I) as disclosed above.

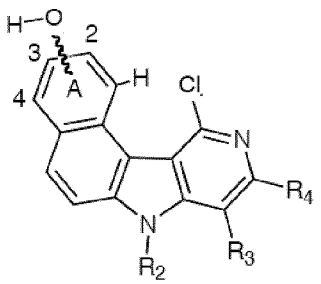
Accordingly, the present invention relates to a method for preparing a compound of formula (I)



(I)

wherein R1, R2, R3 and R4 are as defined above including anyone of the disclosed embodiments, and R5 is chloride;

comprising reacting the reagent Y-(C_2-C_5)alkyl-NR_aR_b wherein Y is halo or hydroxy with the compound **1**



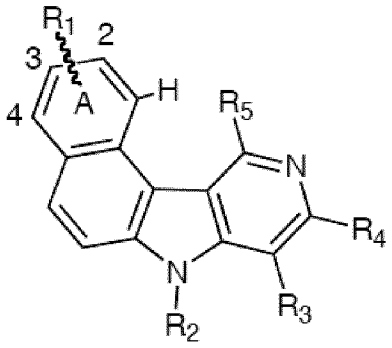
(1)

wherein R_a, R_b, R2, R3 and R4 are as defined above including anyone of the disclosed embodiments, thereby obtaining the compound of formula (I).

The reaction is carried out in the appropriate conditions. For instance, the conditions are the followings i) for the Williamson condensation using halogeno-(C_2-C_5)alkyl-NR_aR_b as reagent: the reaction was realized in phase-transfer conditions (BuOH-water) and in the presence of alkali hydroxide as a base and ii) for the Mitsunobu reaction using HO-(C_2-C_5)alkyl-NR_aR_b and dialkyl azodicarboxylate as reagents: the condensation was realized in standard conditions.

Preferably, Halogeno-(C_2-C_5)alkyl-NR_aR_b is chloro-(C_2-C_5)alkyl-NR_aR_b and dialkyl azodicarboxylate is diisopropyl azodicarboxylate.

The present invention also relates to a method for preparing a compound of formula (I)



(I)

wherein R1, R2, R3 and R4 are as defined above including anyone of the disclosed embodiments, and R5 is hydrogen;

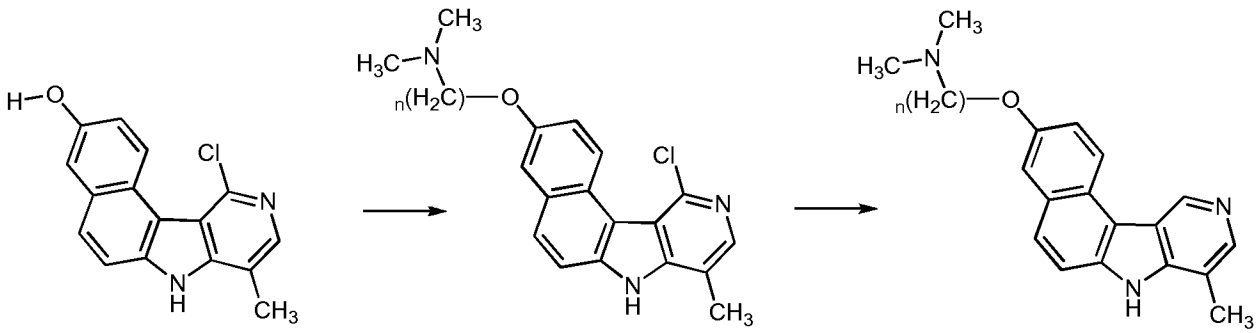
comprising carrying out an hydrogenation on the compound obtained by the above detailed method, thereby obtaining the compound of formula (I).

The reaction is carried out in the appropriate conditions. For instance, the conditions are the followings: The palladium-catalyzed (10% Pd/C) hydrogenation was realized at room temperature under atmospheric pressure of hydrogen.

Further aspects and advantages of the present invention will be disclosed in the following experimental section, which should be regarded as illustrative and not limiting the scope of the present application. A number of references are cited in the present specification; each of these cited references is incorporated herein by reference.

Examples

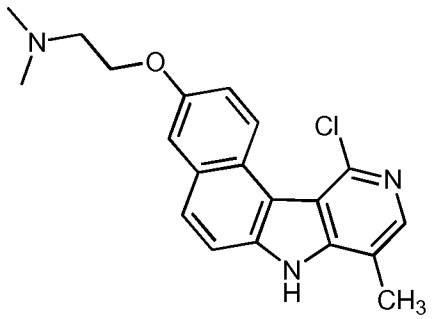
Compounds synthesis



2: n = 2; CH20 **3**: n = 2; CH21

4: n = 3 **5**: n = 3; CH23

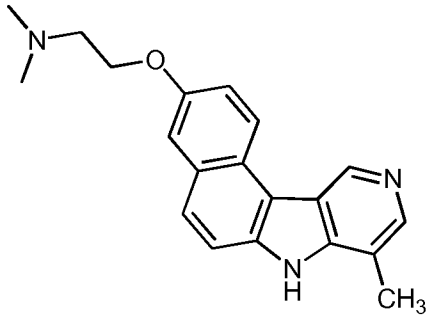
11-Chloro-3-(2-*N*,*N*-dimethylaminoethoxy)-8-methyl-7*H*-benzo(e)pyrido(4,3-b)indole (2 orCH20)



Method 1: A mixture of 11-chloro-3-hydroxy-8-methyl-7*H*-benzo(e)pyrido(4,3-b)indole (400 mg, 1.4 mmol) (prepared according to Bioconjugate Chem. (2003), **14**, 120-135), n-butanol (40 mL) and water (24 mL) was stirred during 30 min and a solution of NaOH (300 mg) in water (5 mL) was then added. After stirring during 15 min, 2-chloro-*N*,*N*-dimethylethanamine hydrochloride (240 mg, 1,7 mmol) was added and the mixture was heated under reflux for 1 h. The reaction mixture was cooled and organic layer was separated. The aqueous layer was extracted by AcOEt and the organic layers were combined, washed with brine, dried over $MgSO_4$ and evaporated under vacuum. The residue was purified by flash chromatography (neutral alumina, gradient of ethanol (0 to 2%) in dichloromethane) to give the expected compound as beige solid (220 mg, 44 %). ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 9.76 (d, 1H), 8.79 (br s, 1H), 8.10 (s, 1H), 7.85 (d, 1H), 7.62 (d, 1H), 7.39 (dd, 1H), 7.35 (d, 1H), 4.24 (t, 2H), 2.83 (t, 2H), 2.56 (s, 3H), 2.40 (s, 6H). Microanalyses, calculated for $C_{20}H_{20}ClN_3O \cdot 0.7\ H_2O$: C, 65.64; H, 5.85; N, 11.48, found: C, 65.29; H, 5.49; N, 11.31.

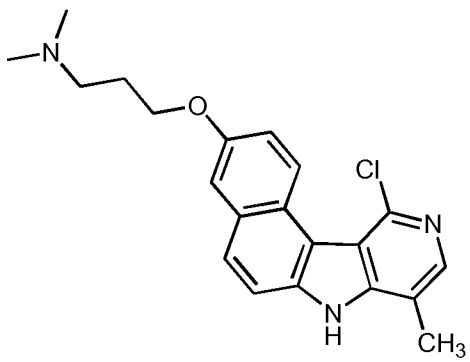
Method 2: Under N2 atmosphere, diisopropyl azodicarboxylate (280 mg, 1.4 mmol) was added to a solution of triphenylphosphine (430 mg, 1.4 mmol) in dry THF (10 mL). This mixture was added to a solution of 11-chloro-3-hydroxy-8-methyl-7*H*-benzo(e)pyrido(4,3-b)indole (200 mg, 0.7 mmol) (prepared according to Bioconjugate Chem. (2003), **14**, 120-135) and 2-(dimethylamino)ethanol (75 mg, 0 .8 mmol) in dry THF (20 mL). The final mixture was stirred for 24 h at room temperature. The solvent was evaporated. The crude residue was purified by column chromatography as described above to give the exected compound which is identical to that obtained by method 1.

3-(2-*N*,*N*-dimethylaminoethoxy)-8-methyl-7*H*-benzo(e)pyrido(4,3-b)indole (3 or CH21)



Palladium on carbon (10%, 150 mg) was added to a solution of 11-chloro-3-(2-*N*,*N*-dimethylaminoethoxy)-8-methyl-7*H*-benzo(e)pyrido(4,3-b)indole (300 mg, 0.84 mmol) in absolute ethanol (30 mL). At atmospheric pressure, hydrogen was introduced and the mixture was stirred during 18 h. The catalyst was then filtered off, washed with hot ethanol and the solvent was removed under reduced pressure. The residue was purified by flash chromatography (neutral alumina, gradient of ethanol (0 to 2%) in dichloromethane) to give the expected compound 3-(2-*N*,*N*-dimethylaminoethoxy)-8-methyl-7*H*-benzo(e)pyrido(4,3-b)indole as beige solid (190 mg, 71 %). ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 12.39 (s, 1H), 9.73 (s, 1H), 8.77 (d, 1H), 8.37 (s, 1H), 7.99 (d, 1H), 7.86 (d, 1H), 7.68 (d, 1H), 7.47 (dd, 1H), 4.48 (t, 2H), 2.79 (s, 6H), 2.65 (s, 3H), 2.53 (m, 2H). Microanalyses, calculated for $C_{20}H_{21}N_3O \cdot 0.25\ H_2O$: C, 74.18; H, 6.64; N, 12.98, found: C, 74.21; H, 6.67; N, 12.71. Formation of the maleate salt: A solution of this free base (33 mg) in hot acetone (5 mL) was poured into a solution of maleic acid (33 mg) in hot acetone (2 mL). The precipitate was collected by filtration, washed with acetone and dried in a dessicator under vacuum affording the maleat salt (43 mg), MS 320 [M+1].

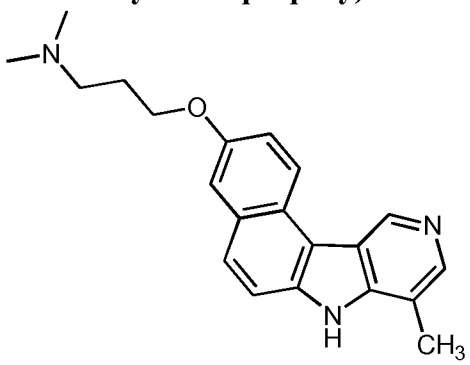
11-Chloro-3-(3-*N*,*N*-dimethylaminopropoxy)-8-methyl-7*H*-benzo(e)pyrido(4,3-b)indole (4)



A mixture of 11-chloro-3-hydroxy-8-methyl-7*H*-benzo(e)pyrido(4,3-b)indole (300 mg, 1.1 mmol) (prepared according to Bioconjugate Chem. (2003), **14**, 120-135), n-butanol

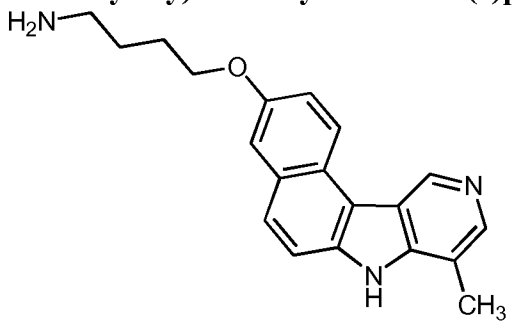
(30 mL) and water (18 mL) was stirred during 30 min and a solution of NaOH (200 mg) in water (3 mL) was then added. After stirring during 15 min, $(CH_3)_2N(CH_2)_3Cl$, HCl (200 mg, 1,3 mmol) was added and the mixture was heated under reflux for 1 h. The reaction mixture was cooled and organic layer was separated. The aqueous layer was extracted by AcOEt and the organic layers were combined, washed with brine, dried over $MgSO_4$ and evaporated under vacuum. The residue was purified by flash chromatography (neutral alumina, gradient of ethanol (0 to 2%) in dichloromethane) to give the expected compound 11-chloro-3-(3-*N*,*N*-dimethylaminopropoxy)-8-methyl-7*H*-benzo(e)pyrido(4,3-b)indole as beige solid (190 mg, 48 %). Microanalyses, calculated for $C_{21}H_{22}ClN_3O \cdot 0.5\ H_2O$: C, 66.92; H, 6.15; N, 11.15, found: C, 66.73; H, 5.92; N, 11.27.

3-(3-*N*,*N*-dimethylaminopropoxy)-8-methyl-7*H*-benzo(e)pyrido(4,3-b)indole (5 or CH23)



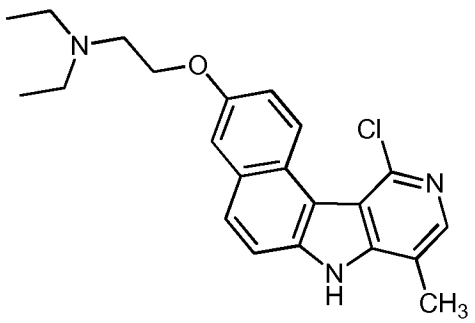
Palladium on carbon (10%, 70 mg) was added to a solution of 11-chloro-3-(3-*N*,*N*-dimethylaminopropoxy)-8-methyl-7*H*-benzo(e)pyrido(4,3-b)indole (130 mg, 0.35 mmol) in absolute ethanol (20 mL). At atmospheric pressure, hydrogen was introduced and the mixture was stirred during 18 h. The catalyst was then filtered off, washed with hot ethanol and the solvent was removed under reduced pressure. The residue was purified by flash chromatography (neutral alumina, gradient of ethanol (0 to 2%) in dichloromethane) to give the expected compound as beige solid (84 mg, 71 %). ^1H NMR (300 MHz, DMSO-d_6) δ (ppm): 12.07 (s, 1H), 9.64 (s, 1H), 8.70 (d, 1H), 8.31 (s, 1H), 7.95 (d, 1H), 7.80 (d, 1H), 7.59 (d, 1H), 7.39 (dd, 1H), 4.20 (t, 2H), 2.62 (s, 3H), 2.47 (t, 2H), 2.22 (s, 6H), 1.98 (m, 2H). Microanalyses, calculated for $C_{21}H_{23}N_3O \cdot 0.25\ H_2O$: C, 74.64; H, 7.01; N, 12.43, found: C, 74.77; H, 6.99; N, 12.45.

3-(4-Aminobutyloxy)-8-methyl-7*H*-benzo(e)pyrido(4,3-b)indole (6)



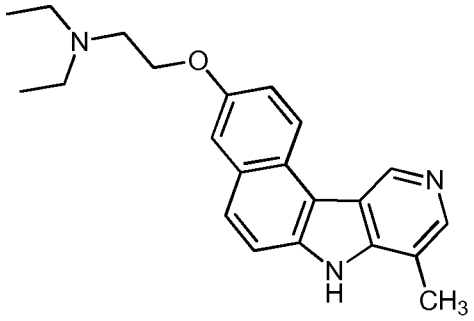
This compound was obtained in two-step transformation as described above starting from the 11-chloro-3-hydroxy-8-methyl-7*H*-benzo(e)pyrido(4,3-b)indole (prepared according to Bioconjugate Chem. (2003), **14**, 120-135), and 4-chlorobutylamine hydrochloride (prepared according to Tetrahedron Lett. (2005), **46**, 6723-6725). ^{1}H NMR (300 MHz, DMSO-d_6+ D_2O) δ (ppm): 9.63 (s, 1H), 8.69 (d, 1H), 8.30 (s, 1H), 7.96-7.77 (m, 2H), 7.58 (d, 1H), 7.39 (dd, 1H), 4.16 (t, 2H), 3.20-2.65 (m, 2H), 2.61 (s, 3H), 1.95-1.75 (m, 2H), 1.75-1.55 (m, 2H).

11-Chloro-3-(2-*N*,*N*-diethylaminoethoxy)-8-methyl-7*H*-benzo(e)pyrido(4,3-b)indole (7)



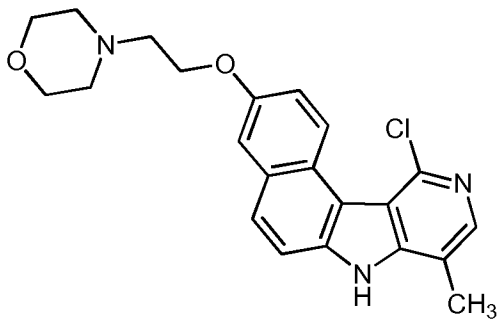
The protocol described above for the synthesis of compound 11-chloro-3-(2-*N*,*N*-dimethylaminoethoxy)-8-methyl-7*H*-benzo(e)pyrido(4,3-b)indole is applied, starting from 11-chloro-3-hydroxy-8-methyl-7*H*-benzo(e)pyrido(4,3-b)indole and using 2-chloro-*N*,*N*-diethylethanamine hydrochloride in place of 2-chloro-*N*,*N*-dimethylethanamine hydrochloride, to give the title compound.

3-(2-*N*,*N*-diethylaminoethoxy)-8-methyl-7*H*-benzo(e)pyrido(4,3-b)indole (8)



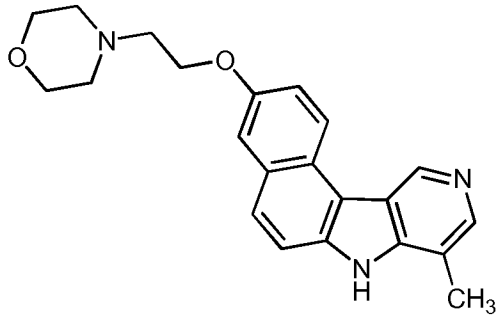
The protocol described above for the synthesis of compound 3-(2-*N*,*N*-dimethylaminoethoxy)-8-methyl-7*H*-benzo(e)pyrido(4,3-b)indole is applied, starting from 11-chloro-3-(2-*N*,*N*-diethylaminoethoxy)-8-methyl-7*H*-benzo(e)pyrido(4,3-b)indole, prepared as describe above, to give the title compound.

11-Chloro-3-(2-(morpholin-4-yl)ethoxy)-8-methyl-7*H*-benzo(e)pyrido(4,3-b)indole (9)



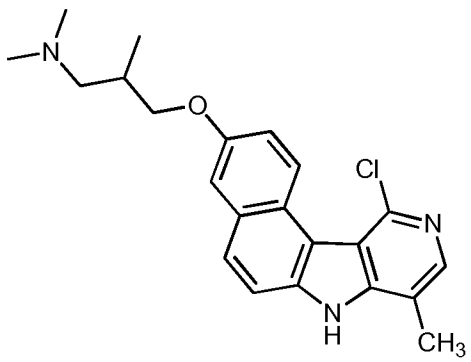
The protocol described above for the synthesis of compound 11-chloro-3-(2-*N*,*N*-dimethylaminoethoxy)-8-methyl-7*H*-benzo(e)pyrido(4,3-b)indole was applied, starting from 11-chloro-3-hydroxy-8-methyl-7*H*-benzo(e)pyrido(4,3-b)indole and using 4-(2-chloroethyl)morpholine hydrochloride in place of 2-chloro-*N*,*N*-dimethylethanamine hydrochloride, to give the title compound. ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 12.94 (br s, 1H), 9.62 (d, 1H), 8.06 (d, 1H), 7.99 (d 1H), 7.80 (d, 1H), 7.59 (d, 1H), 7.35 (dd, 1H), 4.25 (t, 2H), 3.64-3.58 (m, 4H), 2.78 (t, 2H), 2.56 (s, 3H), 2.54-2.49 (m. overlapped by DMSO signals). Microanalyses, calculated for $C_{22}H_{20}ClN_3O_2 \cdot 0.5\ H_2O$: C, 65.34; H, 5.69; N, 10.39, found: C,65.75; H,5.73; N,10.37. MS 394.1 & 396.1 [M-1].

3-(2-(Morpholin-4-yl)ethoxy)-8-methyl-7*H*-benzo(e)pyrido(4,3-b)indole (10)



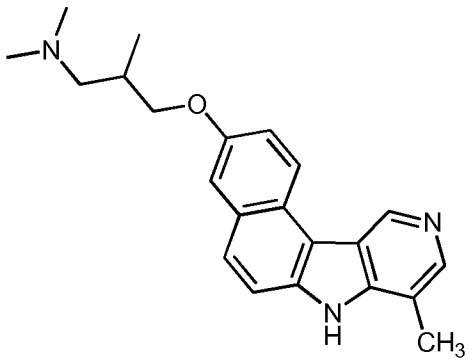
The protocol described above for the synthesis of compound 3-(2-*N*,*N*-dimethylaminoethoxy)-8-methyl-7*H*-benzo(e)pyrido(4,3-b)indole is applied, starting from 11-chloro-3-(2-(morpholin-4-yl)ethoxy)-8-methyl-7*H*-benzo(e)pyrido(4,3-b)indole prepared as describe above, to give the title compound.

DL 11-Chloro-3-(3-*N*,*N*-dimethylamino-2-methylpropoxy)-8-methyl-7*H*-benzo(e)pyrido(4,3-b)indole (11)



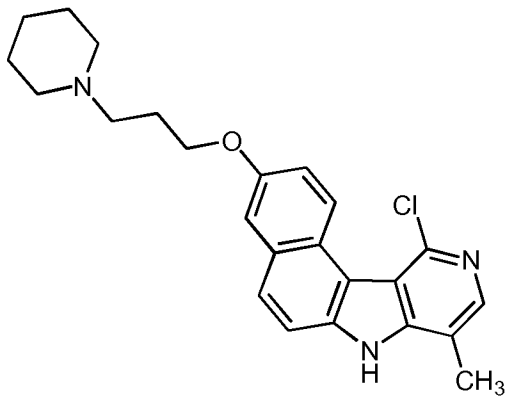
The protocol described above for the synthesis of compound 11-chloro-3-(2-*N*,*N*-dimethylaminoethoxy)-8-methyl-7*H*-benzo(e)pyrido(4,3-b)indole is applied, starting from 11-chloro-3-hydroxy-8-methyl-7*H*-benzo(e)pyrido(4,3-b)indole and using DL 3-chloro-2-methyl-*N*,*N*-dimethylpropan-1-amine hydrochloride in place of 2-chloro-*N*,*N*-dimethylethanamine hydrochloride, to give the title compound.

DL 3-(3-*N*,*N*-dimethylamino-2-methylpropoxy)-8-methyl-7*H*-benzo(e)pyrido(4,3-b)indole (12)



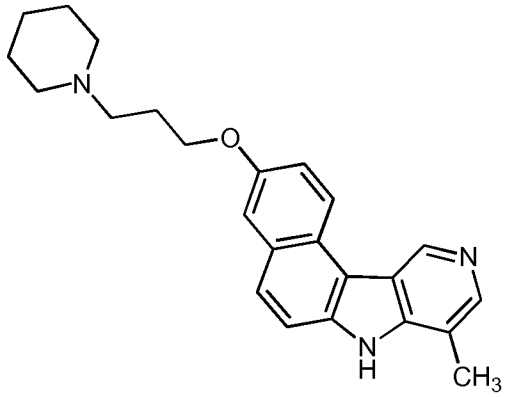
The protocol described above for the synthesis of compound 3-(2-*N*,*N*-dimethylaminoethoxy)-8-methyl-7*H*-benzo(e)pyrido(4,3-b)indole is applied, starting from DL 11-chloro-3-(3-*N*,*N*-dimethylamino-2-methylpropoxy)-8-methyl-7*H*-benzo(e)pyrido(4,3-b)indole, prepared as describe above, to give the title compound.

11-Chloro-3-(3-(piperidin-1-yl)propoxy)-8-methyl-7*H*-benzo(e)pyrido(4,3-b)indole (13)



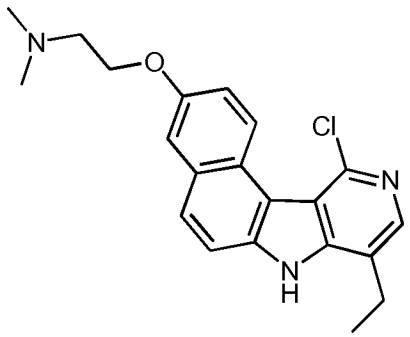
The protocol described above for the synthesis of compound 11-chloro-3-(2-*N*,*N*-dimethylaminoethoxy)-8-methyl-7*H*-benzo(e)pyrido(4,3-b)indole is applied, starting from 11-chloro-3-hydroxy-8-methyl-7*H*-benzo(e)pyrido(4,3-b)indole and using 1-(3-chloropropyl)piperidine hydrochloride in place of 2-chloro-*N*,*N*-dimethylethanamine hydrochloride, to give the title compound.

3-(3-(Piperidin-1-yl)propoxy)-8-methyl-7*H*-benzo(e)pyrido(4,3-b)indole (14)



The protocol described above for the synthesis of compound 3-(2-*N*,*N*-dimethylaminoethoxy)-8-methyl-7*H*-benzo(e)pyrido(4,3-b)indole is applied, starting from 11-chloro-3-(3-(piperidin-1-yl)propoxy)-8-methyl-7*H*-benzo(e)pyrido(4,3-b)indole, prepared as describe above, to give the title compound.

11-Chloro-3-(2-*N*,*N*-dimethylaminoethoxy)-8-ethyl-7*H*-benzo(e)pyrido(4,3-b)indole (15)

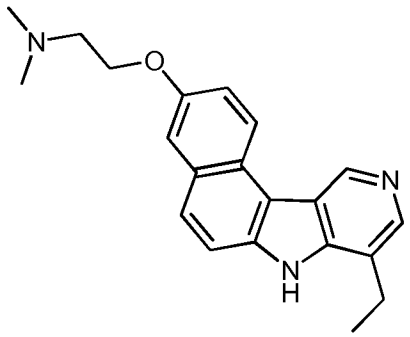


Step1: In a 50 mL sealed tube, a mixture of 11-chloro--8-ethyl-3-methoxy-7*H*-benzo(e)pyrido(4,3-b)indole (600 mg, 1.9 mmol) (prepared according to Anti-Cancer Drug Design (1992), **7**, 235-251), benzyltriethylammonium chloride (2.80 g, 12 mmol) and 37% hydrochloric acid (45 mL) was heated in an oil bath at 140°C for 24 h. The reaction mixture was then evaporated under vacuum, then water (10 mL) is added. The medium was rendered basic by addition of 28% ammonium hydroxide (2 mL), and the resulting solid was collected by filtration, then washed with water and dried using a vacuum desiccator. The intermediate 11-chloro--8-ethyl-3-hydroxy-7*H*-benzo(e)pyrido(4,3-b)indole 450 mg (78% yield) was obtained as brown powder. Microanalyses, calculated for $C_{17}H_{13}ClN_2O$ 0.25 H_2O: C, 67.78; H, 4.52; N, 9.30 ; found: C, 67.90; H, 4.62; N, 9.56. MS : 296.1/298.1 (M+H).

Step 2 : The protocol described above for the synthesis of compound 11-chloro-3-(2-*N*,*N*- dimethylaminoethoxy)-8-methyl-7*H*-benzo(e)pyrido(4,3-b)indole was applied, starting from 11-chloro-3-hydroxy-8-ethyl-7*H*-benzo(e)pyrido(4,3-b)indole and using 2-chloro-*N*,*N*-

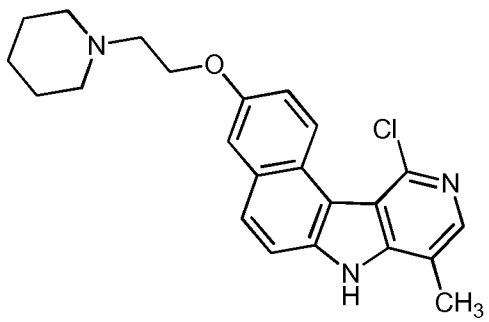
dimethylethanamine hydrochloride, to give the title compound. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 9.76 (d, 1H), 8.73 (br s, 1H), 8.13 (s, 1H), 7.86 (d, 1H), 7.62 (d, 1H), 7.43-7.34 (m, 2H), 4.25 (t, 2H), 2.97 (q, 2H), 2.84 (t, 2H), 2.40 (s, 6H), 1.46 (t, 3H). MS: 368.2 & 370.2 (M+H).

3-(2-*N*,*N*-dimethylaminoethoxy)-8-ethyl-7*H*-benzo(e)pyrido(4,3-b)indole (16)



The protocol described above for the synthesis of compound 3-(2-*N*,*N*-dimethylaminoethoxy)-8-methyl-7*H*-benzo(e)pyrido(4,3-b)indole is applied, starting from 11-chloro-3-(2-*N*,*N*-dimethylaminoethoxy)-8-ethyl-7*H*-benzo(e)pyrido(4,3-b)indole, prepared as describe above, to give the title compound.

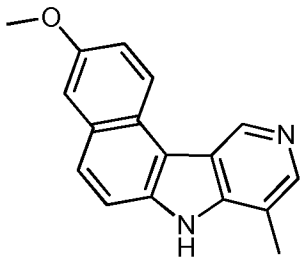
11-Chloro-3-(2-(piperidin-1-yl)ethoxy)-8-methyl-7*H*-benzo(e)pyrido(4,3-b)indole (CH 1709)



The protocole described above for the synthesis of compound 11-chloro-3-(2-*N*,*N*-dimethylaminoethyloxy)-8-methyl-7*H*-benzo(e)pyrido(4,3-b)indole was applied, starting from 11-chloro-3-hydroxy-8-methyl-7*H*-benzo(e)pyrido(4,3-b)indole and using N-(2-chloroethyl)piperidine hydrochloride in place of 2-chloro-*N*,*N*-dimethylethanamine hydrochloride, to give the title compound. ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 12.48 (br s, 1H), 9.61 (d, 1H), 8.06 (s, 1H), 7.99 (d 1H), 7.79 (d, 1H), 7.58 (d, 1H), 7.34 (dd, 1H), 4.22 (t, 2H), 2.74 (t, 2H), 2.56 (s, 3H), 2.51-2.45 (m. overlapped by DMSO signals), 1.56-1.47 (m,

4H), 1.45-1.30 (m, 2H). Microanalyses, calculated for $C_{23}H_{24}ClN_3O_2$: C, 70.13; H, 6.14; N, 10.67, found: C, 69.63; H, 6.38; N, 10.44. MS 394.1 & 396.1 [M+1].

3-Methoxy-8-methyl-7*H*-benzo(e)pyrido(4,3-b)indole (**18** or CH7 or C7)



This compound was prepared as described in J. Am. Chem. Soc. (1995), **117**, 10212-10219.

Characterization of the effects of Amino-substituted-alkyloxy-benzo[e]pyrido[4,3-b]indole family.

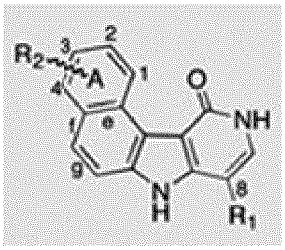
1) In vitro assays towards the kinase domain of Aurora A

The molecules were tested at two different concentrations and inhibition was expressed in percent. C1 to C7 were described in Hoang et al (Cell cycle, 2009). The three additional molecules CH20, CH21 and CH23 are thus proposed as kinase inhibitors.

Compound	R3 in formula I	R1 in formula I	R5 in formula I	Inhibition at 15 µM in %	Inhibition at 1.5 µM in %
C1	Et	3-OMe	=O	94	70
C2	Me	3-OMe	=O	90	57
C3	Me	3-H	=O	88	53
C4	H	3-OMe	=O	85	45
C7	Me	3-OMe	H	47	19
CH21	Me	3- O-$(CH_2)_2$ $N(CH_3)_2$	H	100	70
CH20	Me	3- O-$(CH_2)_2$ $N(CH_3)_2$	Cl	94	60
CH23	Me	3- O-$(CH_2)_3$ $N(CH_3)_2$	H	100	68

Table 1: Inhibition in vitro of the catalytic domain of Aurora A.

Compounds C1-C4 have been disclosed in Hoang et al, 2009 in Figure 1 as following:



with

Compound	R_1	R_2
1	Et	3-OMe
2	Me	3-OMe
3	Me	H
4	H	3-OMe

The most active molecules CH21 (3-(2-Dimethylaminoethoxy)-8-methyl-7H-benzo(e)pyrido(4,3-b)indole) and in some extent CH23 (11-Chloro-3-(3-*N*,*N*-dimethylaminopropoxy)-8-methyl-7*H*-benzo(e)pyrido(4,3-b)indole) and CH20 (11-Chloro-3-(2-*N*,*N*-dimethylaminoethoxy)-8-methyl-7*H*-benzo(e)pyrido(4,3-b)indole) were tested as anti-proliferative molecules.

2) Proliferation assays

HeLa cell growth and viability were tested in the presence of C1, C3, C4, C7 and C21 at two different concentrations 0.5 and 1 μM for 96 hours. Assays were run in triplicate. The percentage of alive cells, for both concentrations, are reported in Table 2A and represented in Figure 8. Compared to the lead C1, the substitution of the lateral in either position 3 or 8 or 11 by hydrogen (respectively compounds C3, C4 and C7) induced a loss of antiproliferative activity. In particular, C7 bearing hydrogen at position 11 has some antiproliferative activity, but was found around two fold less efficient than C1 (remaining activity of 41 % and 21 % respectively at 1 μM).

The addition of the –O-(CH2)2-N(CH3)$_2$ at position 3 restored the antiproliferative activity and even induced a high antiproliferative activity, even it bore a hydrogen at position 11 (IC50 of proliferation was found 3-fold higher than C1 or C7). Therefore, the efficiency of C21 was not expected and cannot be deducted from the analysis of the compounds described in Hoang et al, 2009.

Table 2A: Viability of HeLa cells upon compound treatments

Molecule	R1	R3	R5	Cell viability 0.5 µM (in %)	SD	Cell viability 1 µM (in %)	SD
C1	OMe	Et	O	63	6	21	2
C3	H	Me	O	96	3	90	3
C4	OMe	H	O	95	3	92	3
C7	OMe	Me	H	74	10	41	5
C21	O $CH3_2N(Me)_2$	Me	H	24	3	3	3

Among the tested molecules, the inventors found that CH21 prevents the proliferation of several cell lines in culture (see Table 2B). Assays were run on the DMSO soluble compound but its efficiency is compared to that of the water-soluble corresponding salt. Mice bearing xenografts were injected with the water-soluble counterpart.

Origin	Cell line	IC50 for CH21	IC50 for CH23
Breast	BT20	180 nM	400 nM
Breast	HCC70	900 nM	
Lung	**H1299**	**550 nM**	
Lung	H322	800 nM	
Lung	**H358**	**450 nM**	
Lung	A549	700 nM	
Leukemia	HL60	Sup 2 µM	NI
Cervix	**HeLa**	**350 nM**	
Poor differenciated Liver cells	**SkHep1-HG**	**450 nM**	
Poor differenciated Liver cells	SkHep1-MG	1 200 nM	
Poor differenciated Liver cells	**Malhavu -HG**	**510 nM**	
Poor differenciated Liver cells	**Malhavu -MG**	**520 nM**	
Poor differenciated Liver cells	Malhavu -LG	1 400 nM	
Differenciated Liver cells	HepG2	Sup 2 µM	

Osteosarcoma	U2OS	1 100 nM	
Glioblastoma	**U373**	**360 nM**	**600 nM**

Table 2B: Anti-proliferating effect of CH21 and CH23 on various cell lines. IC50: concentration of compound necessary for having only 50 % alive cells after 96 hours of treatment. HG: high glucose: 4.5 g/L, MG: medium glucose: 3; 85 g/L and LG: 3.15 g/L. NI: no inhibition.

Similar IC50 values were found for the soluble CH21 counterpart; IC50 for HeLa and H358 cells of 500 nM and 330 nM in comparison to 350 and 450 nM respectively for the DMSO soluble CH21.

Interestingly, the IC50 of viability in liver cell lines depends on the glucose concentration and on the differentiation stage of the cells, HepG2 being the most differentiated. For example, an IC50 of viability of 450 nM is noted for Sk-Hep1 in high glucose and it drops to 1 200 nM at a lower concentration of glucose. This may reflect that CH21 activity depends on cell metabolism. Taking into account that glucose uptake is increased in tumours compared to normal tissue, this may be an interesting feature for an anticancer drug. CH23, although less active than CH21, prevents proliferation of BT20 and glioblastoma U373 cells with 400 and 600 nM IC50, respectively.

3) Viability of quiescent cells in the presence of compounds

Furthermore, these compounds have no deleterious action on quiescent cells as shown in Figure 1. These data confirm that the inhibitory activity targets a cycling procedure and allow anticipating that these compounds (CH21 and CH23) may have poor toxicity on non-dividing cells.

4) Efficiency of compounds to prevent spheroid growth

The efficiency of compounds was tested on cells cultured in 3-Dimensions: (spheroids cultures). Compounds (C1, C2, CH20, CH23 and CH21) were added at day 1, at the concentration of 1 μM and then spheroid growth was measured for 13 days. Results are shown in Figure 2. In the absence of compound, spheroids expand exponentially. C1, C2 and CH20 are efficient to prevent cell growth for few days, but then, spheroids grow normally (Figure 2 A-C). In the presence of CH23, the spheroid size decreases by one third (Figure 2 D). CH21 was found to be the most efficient molecule and long-term effects are noted (Figure 2E).

5) Profiling of kinase inhibition by CH21

Taking into account the efficiency of these molecules towards the kinase domain of Aurora A, the inventors have therefore performed a kinase profiling with CH21, at the concentration of 1 μM.

The profiling was performed at the MRC (Dundee University), in duplicate, on a set of 106 kinases. The complete profiling of inhibition is represented in Figure 3 and kinases inhibited by at least 40 % are reported in Table 2.

Kinases	Accession n°	Percent of residual Activity (in %)	Deviation
GCK (Germinal central kinase)	BC047865	**32**	1
TrkA (Neurotrophic tyrosine kinase, receptor, type 1)	NM_001007792.1	**36**	2
NUAK1 (SnF1-like Kinase)	NM_014840	53	2
PHK (Phosphorylase kinase)		53	7
TAK1 (Transforming growth factor beta activated kinase)	NM_003188 (TAK1)	55	3

Table 3: Profiling results : Kinases inhibited by at least 40 % by CH21, at 1 μM are indicated. www.kinase-screen.mrc.ac.uk/kinase-panel.htm

GCK is known under the following names: Mitogen-activated protein kinase kinase kinase kinase 2, MAPK/ERK kinase kinase kinase 2, MEK kinase kinase 2, MEKKK 2, Germinal center kinase, GC kinase, Rab8-interacting protein, B lymphocyte serine/threonine-protein kinase, MAP4K2, RAB8IP, M4K2

Trk are neurotrophic tyrosine kinase receptors, Trk is the acronym for tropomyosin related kinase. The family encompasses three members A, B and C exhibiting a highly homologous ATP binding sites.

Nuak1 is an AMPK-related kinase (ARK-5). The proliferation of myc-deregulated tumours is dependent on its activity (Liu et al).

Tak1 kinase (MAP3K7) inhibition induced apoptosis in KRAS-dependent colon cancers (Singh et al).

The profiling suggested that CH21 might be a good Gck, TrkA and Nuak1 inhibitor. Taking into account that the kinase domain of Trk kinases is fully conserved among the

family, it may be anticipated that CH21 targets TrkA, B and C. Finally, the specifity of CH21 is quite narrow since only five kinases show a decreased activity of at least 40 %.

6) Determinations of IC50 of kinase inhibition in vitro

Kinase	**CH21 In nM**	**CH23 In nM**	**C1 In nM**	**C2 In nM**
Aurora A	NI	NI	**31**	40
Aurora B	NI	NI	**31**	47
Chk1	NI	NI	224	84
Chk2	649	1420	**29**	16
FLT3	**12**	**25**	**1**	**2**
GCK	**21**	ND	ND	ND
Nuak1 (ARK-5)	**284**	ND	ND	ND
Tak1	**2 000**	ND	ND	ND

Table 4: IC50, concentration that inhibits kinase activity, *in vitro*, by 50 %, are determined with recombinant kinases. IC50 were determined in duplicate .The IC50 were not determined for TrkA and phosphorylase kinase. NI: no inhibition and ND not determined.

The IC50 data presented in Table 4 confirm that the profile of inhibition of the new class of molecules differs from that of C1 and C2. Although both CH21 and CH23 inhibit efficiently, in vitro, the kinase domain of Aurora A (see Table 1), they have no activity towards the full-length kinases Aurora A and B.

These data confirms that CH21 inhibits, in vitro, GCK and Nuak1 with good efficiencies (IC50 = 21 nM and 284 nM respectively)). Note that it is also a potent Flt3 inhibitor. CH21 and CH23 are poor Chk2 inhibitors and exhibit no inhibitory activity on Chk1. The absence of targeting of Aurora B by either CH21 or CH23 is also confirmed, in cellulo, as shown in Figure 4.

The presence of a large chain in position 3 and the absence of the oxygen group, in particular oxo group, in position 11 on the benzo[e]pyridoindol scaffold confer to CH21 a highly specificity of inhibition in the kinome.

The possibility of inhibiting Nuak1, in cellulo, was evaluated through the stabilization of the Large Antigen Tumour Suppressor LATS1. As expected for Nuak1 inhibition, the

treatment of HeLa cells by CH21 (1 μM) induced a large increase, by 1.5, of LATS1 protein (Figure 5).

7) Effect of CH21 on the cell cycle

H358 cells were incubated in the presence of either C1 or CH21 (dark histograms) and their cell cycle was compared to that of control cells. Histograms representing the repartition of the cells in the different phases are in Figure 4; dotted lines for control and dark histograms for treated cells. CH21 do not modify significantly cell cycle repartition (Figure 6B) whereas C1 induces a mitotic stop and the polyploidisation of the cells (Figure 6A). CH21 slows down cell cycle progression and induces cell death. This result straightened that benzo[e]pyridoindoles CH21 and C1 functioned differently in cells. Their anti-proliferating activities are accounted by two separate signalling inhibitions.

8) Assays were also realized on mice bearing HL60 xenografts.

HL60 xenografts were established in nude mice. Mice were dispatched in each treatment so as the mean tumour volume was similar in each batch. CH21 was injected daily, for 11 days, by IP. Each mouse received either the vehicle (PBS) or 5 mg per kg of CH21 solubilized in PBS. The volume of the tumour was measured third a week and mice were weighted weekly. The inventors do not observe any loss of weight. CH21 decreases the average size of the tumour by two and long term effects are observed (Figure 7).

As a first conclusion, CH21 exhibits a narrow specificity towards kinases and the inventors described an antiproliferative activity both *in cellulo* and *in vivo*. This molecule targets, at least in vitro, the germinal center kinase that may represent a new target for cancer therapy.

9) Conclusion

CH21 (3-(2-Dimethylaminoethoxy)-8-methyl-7H-benzo(e)pyrido(4,3-b)indole) and similar compounds (CH20 and CH23) are new, water soluble, antiproliferating compounds. In addition to the *in cellulo* activities observed for the three compounds, CH21 was found efficient *in vivo*. CH21 has a narrow specificity towards kinases. It is proposed as a kinase inhibitor targeting Gck, a MAP4K (Ippeita et al., 2001), Nuak1 (Liu et al, 2012) and Trks enzymes (Thiele et al., 2009) and kinases sharing homologous ATP binding sites.

Gck is involved in signal transduction and acts very upstream in the MAP cascade. The expression of GCK is ubiquitous but is regulated by its activity. Such a feature may

explain the poor toxicity of CH21 in quiescent cells. The germinal center kinase may represent a new target for cancer therapy. Outside the cancer field, Gck was found to be a master enzyme in inflammatory process. Gck is a PAMP (Pathogen-Associated Molecular Patterns) effector coupling JNK and p38, but not ERK or NF-κB to systemic inflammation. Consequently, CH21 may have some potentialities in anti-inflammatory therapy (Jian et al., 2007; Jian et al., 2009). Trk kinases have oncogenic potency and are tyrosine kinase receptors activated by neurotrophin therefore involved in pain sensation (Harel et al., 2010; Thiele et al., 2009; Albanese et al, 2010; Jian et al., 2009). AMPK-related kinases are new actors in mitosis and Nuak1 (Ark-5) inhibition was found to be a validated strategy to eliminate tumour cells that express deregulated Myc (Humbert et al, 2010; Liu et al, 2012). To note the c-Myc protein or the c-*myc* gene is overexpressed in a wide variety of human cancers with 80% of breast cancers, 70% of colon cancer, 90% of gynecological cancers, 50% of hepatocellular carcinomas and a variety of hematological tumors possessing abnormal *myc* expression. On the basis of these frequencies, it is estimated that approximately 100,000 US cancer deaths per year are associated with changes in the c-*myc* gene or its expression.

10) Materials and Methods.

Cell culture and cell viability test. H358, H322, H1299, A549 (lung cancer cells) and HeLa (ovarian cancer) were grown in DMEM (1.5 g/L glucose) whereas HL60 (human myeloid cell line) and HCC70 (breast cancer) cells were in RPMI 1640. U2OS (a human osteosarcoma cell line) cells were cultured in McCoy's Medium 5A, BT20 (breast cancer) in MEM (Gibco-Invitrogen) and liver cells (HepG2, Malhavu and SK Hep1) in DMEM (4g/L glucose, (Gibco-Invitrogen). Media (Gibco-Invitrogen), were supplemented with 10% heat-inactivated foetal bovine serum (Gibco-Invitrogen), L-glutamine (2mM), penicillin (100 UI/ml) and streptomycin (100 µg/ml).

Cell proliferation assays were conducted in 96 well culture plates. Assays were run in triplicate. Cell viability was estimated upon 72 h of treatment with varying concentrations of compound by addition of CellTiter 96Queous one Solution Reagent (Promega) directly to culture wells under conditions defined by the manufacturer.

Cells were drawn to quiescence by serum withdrawn. After two days of serum deprivations cells were incubated with different concentrations of compounds for 96 hours, serum being still omitted. The viability is determined as described above.

Western Blot. Cells were treated by compounds, harvested and lyzed in 9M urea then supplemented with Laemmli sample buffer. Cell extracts were subjected to SDS-PAGE and

transferred to nitrocellulose filter (*GE Healthcare*). After blocking with 5% non-fat milk in PBS for at least 1 hour, the membranes were incubated with the primary antibodies. The following antibodies were used: phospho-Histone H3 Ser10 (1:2000, *Upstate Biotechnology*), anti-LATS1 (Cell Signalling), alpha-tubulin (Sigma) and anti actin (Sigma). Anti-rabbit horseradish peroxidase (1:5000, *GE Healthcare*) was used as secondary antibodies. Bands were visualized by *ECL technique (Amersham Bioscience).*

Cell cycle analysis. Cells were incubated in either the presence or the absence of compounds. For determination of cell cycle profile, cells were fixed by ice-cold 70% ethanol for 1 h and then, incubated with propidium iodide solution (50 µg/ml PI in the presence of 0.2 mg/ml RNAse) for 15 minutes at 37° C. DNA content was measured using the FACS flow cytometer equipped with CellQuest Pro software (Becton Dickinson, San Diego, CA).

Kinase profiling and in vitro IC50 determinations. The profiling was performed at the MRC (Dundee University), in duplicate, on a set of 106 kinases. The panel is representative of the major different classes of kinases. Compounds were used at the concentration of 1 µM. IC50 were determined with recombinant kinases by either the MRC or Reaction Biology Corp

The multicellular tumour spheroid (MTS) model. The inventors applied the hanging-drop method to produce H358 spheroids of similar diameters. 1400 cells were suspended on the lid of an agar coated 24-petri dishes containing culture medium. 48 h later, the spheroids were transferred to the culture medium. Spheroid volumes were measured before (Day 0) and during the drug treatment (Day N). H358 spheroids were treated by compounds. Control H358 spheroids were grown under the same conditions without drug treatment. The size of each spheroid was determined by measuring 2 diameters (d_1 and d_2) using an inverted microscope. The volume was calculated according to the formula: $V=4/3\pi r^3$ where $r=1/2\sqrt{d_1 x d_2}$. Spheroid growth was calculated by measuring the variations in volume and compared to the initial volume V_0. Data are the mean of at least three spheroids.

In vivo experiments. *In-vivo* experiments were conducted on four-week old female Swiss nude mice (*Iffa Credo, Marcy l'Etoile, France*). After one week of adaptation in the animal facility (French agreement number A38-516-01), the mice were inoculated subcutaneously with 3×10^6 HL60 cells mixed with growth factor free matrigel (1/1, BD). Tumours were established at five days post-injection. Then the mice from each cage were randomly divided into four groups (five animal by group), which allowed the equalization of the mean tumour size of each group. Tumour volumes were determined by measuring two perpendicular diameters using a clipper and then calculated as follows: $V = d_1^2 \times d_2$, where d_1 and d_2 are the smallest and the largest diameters, respectively. CH21 was injected daily, for

11 days, by IP. Each mouse received either the vehicle (PBS) or 5 mg per kg of CH21 solubilized in PBS. The volume of the tumour was measured third a week and mice were weighted weekly.

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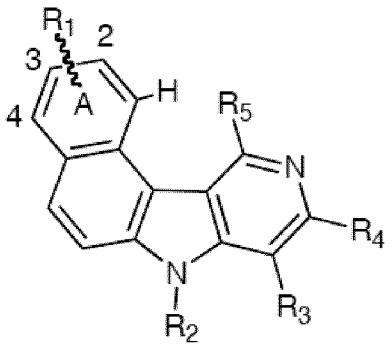
Claims

1- A compound having the formula (I)



(I)

wherein

the benzo cycle A is mono-substituted by R1 in position 2, 3 or 4 ;

R1 is a radical –O-(C_2-C_5)alkyl-NR_aR_b, wherein (C_2-C_5)alkyl is a linear or branched alkyl, R_a and R_b, each independently, are selected from the group consisting of hydrogen, (C_1-C_4)alkyl optionally substituted by a radical selected from the group consisting of hydroxyl, -NRR', OPO(OR)(OR'), and -OC(=O)R, and (C_3-C_6)cycloalkyl; or NR_aR_b may be taken together to form a heterocycle selected from the group consisting of aziridine, azetidine, pyrrolidine, pyrrole, piperidine, piperazine, morpholine, and thiomorpholine, the said heterocycle is optionally substituted by a (C_1-C_4)alkyl or hydroxyl radical;

R2 is selected from the group consisting of hydrogen and a (C_1-C_3)alkyl optionally substituted by a radical OH, (C_1-C_3)alkyloxy or -NRR',

R3 and R4, each independently, are selected from the group consisting of hydrogen, a (C_1-C_3)alkyl and an aryl; or R3 and R4 may be taken together to form a bivalent radical of formula

- –$(CH_2)_n$- wherein n is 3, 4 or 5; or
- -CH=CH-CH=CH- , optionally substituted by a (C_1-C_3)alkyloxy;

R5 is hydrogen or halogen;

wherein R and R', identical or different, are selected from the group consisting of hydrogen and a (C_1-C_4)alkyl;

or an isomeric form thereof or a pharmaceutically acceptable salt thereof.

2- The compound according to claim 1, wherein the compound of formula (I) has one or several of the following features:

- R1 is a radical –O-(C_2-C_4)alkyl-NR_aR_b, wherein (C_2-C_4)alkyl is a linear or branched alkyl, R_a and R_b, each independently, are selected from the group consisting of hydrogen and a (C_1-C_3)alkyl; or NR_aR_b may be taken together to form a heterocycle selected from the group consisting of aziridine, azetidine, pyrrolidine, pyrrole, piperidine, piperazine, morpholine, and thiomorpholine; and/or
- R2 is selected from the group consisting of hydrogen, methyl, ethyl, and -$(CH_2)_n$-N[(C_1-C_2)alkyl]$_2$ with n being 2 or 3; and/or
- R3 and R4, each independently, are selected from the group consisting of hydrogen, methyl, ethyl and phenyl, or may be taken together to form a bivalent radical of formula -CH=CH-CH=CH-; and/or
- R5 is hydrogen or chloride.

3- The compound according to claim 1 or 2, wherein the compound of formula (I) has one or several of the following features:

- R1 is a radical –O-$(CH_2)_n$-NR_aR_b with n being 2 or 3 or a radical –O-CH_2-$(CHCH_3)$-CH_2-NR_aR_b, wherein R_a and R_b, each independently, are selected from the group consisting of hydrogen and a (C_1-C_2)alkyl; or NR_aR_b may be taken together to form a heterocycle selected from piperidine and morpholine; and/or
- R2 is hydrogen; and/or
- R3 is selected from the group consisting of hydrogen, methyl and ethyl, and R4 is hydrogen; and/or
- R5 is hydrogen or chloride.

4- The compound according to anyone of claims 1-3, wherein

- R1 is a radical –O-$(CH_2)_n$-NR_aR_b with n being 2 or 3 or a radical –O-CH_2-$(CHCH_3)$-CH_2-NR_aR_b, wherein R_a and R_b, each independently, are selected from the group consisting of hydrogen and a (C_1-C_2)alkyl; or NR_aR_b may be taken together to form a heterocycle selected from piperidine and morpholine;
- R2 is hydrogen;
- R3 is selected from the group consisting of methyl and ethyl, and R4 is hydrogen; and
- R5 is hydrogen or chloride.

5- The compound according to anyone of claims 1-4, wherein R1 is $–O-(CH_2)_n-N(CH_3)_2$ or $–O-(CH_2)_n-N(CH_2CH_3)_2$ with n being 2 or 3 (preferably at position 3 of the benzo cycle A).

6- The compound according to anyone of claims 1-5, wherein R1 is $–O-(CH_2)_2-N(CH_3)_2$ (preferably at position 3 of the benzo cycle A).

7- The compound according to anyone of claims 1-5, wherein the compound has the formula (I) with R1 being $–O-(CH_2)_2-N(CH_3)_2$ or $–O-(CH_2)_2-N(CH_3)_2$ (preferably at position 3 of the benzo cycle A), R2 and R4 being hydrogen, R3 being methyl and R5 being hydrogen or chloride.

8- The compound according to anyone of claims 1-7, wherein R5 is hydrogen.

9- The compound according to anyone of claims 1-4, wherein the compound is selected from the group consisting of

11-Chloro-3-(2-*N*,*N*-dimethylaminoethoxy)-8-methyl-7*H*-benzo(e)pyrido(4,3-b)indole (2 or CH20)

3-(2-*N*,*N*-dimethylaminoethoxy)-8-methyl-7*H*-benzo(e)pyrido(4,3-b)indole (3 or CH21)

11-Chloro-3-(3-*N*,*N*-dimethylaminopropoxy)-8-methyl-7*H*-benzo(e)pyrido(4,3-b)indole (4)

3-(3-*N*,*N*-dimethylaminopropoxy)-8-methyl-7*H*-benzo(e)pyrido(4,3-b)indole (5 or CH23)

3-(4-Aminobutyloxy)-8-methyl-7*H*-benzo(e)pyrido(4,3-b)indole (6)

11-Chloro-3-(2-*N*,*N*-diethylaminoethoxy)-8-methyl-7*H*-benzo(e)pyrido(4,3-b)indole (7)

3-(2-*N*,*N*-diethylaminoethoxy)-8-methyl-7*H*-benzo(e)pyrido(4,3-b)indole (8)

11-Chloro-3-(2-(morpholin-4-yl)ethoxy)-8-methyl-7*H*-benzo(e)pyrido(4,3-b)indole (9)

3-(2-(Morpholin-4-yl)ethoxy)-8-methyl-7*H*-benzo(e)pyrido(4,3-b)indole (10)

DL 11-Chloro-3-(3-*N*,*N*-dimethylamino-2-methylpropoxy)-8-methyl-7*H*-benzo(e)pyrido(4,3-b)indole (11)

DL 3-(3-*N*,*N*-dimethylamino-2-methylpropoxy)-8-methyl-7*H*-benzo(e)pyrido(4,3-b)indole (12)

11-Chloro-3-(3-(piperidin-1-yl)propoxy)-8-methyl-7*H*-benzo(e)pyrido(4,3-b)indole (13)

3-(3-(Piperidin-1-yl)propoxy)-8-methyl-7*H*-benzo(e)pyrido(4,3-b)indole (14)

11-Chloro-3-(2-*N*,*N*-dimethylaminoethoxy)-8-ethyl-7*H*-benzo(e)pyrido(4,3-b)indole (15), and

3-(2-*N*,*N*-dimethylaminoethoxy)-8-ethyl-7*H*-benzo(e)pyrido(4,3-b)indole (16).

10- The compound according to anyone of claims 1-4, wherein the compound is 3-(2-*N*,*N*-dimethylaminoethoxy)-8-methyl-7*H*-benzo(e)pyrido(4,3-b)indole (3 or CH21).

11- A pharmaceutical composition comprising a compound according to anyone of claims 1-10, a pharmaceutically acceptable carrier, and optionally an additional antitumoral drug.

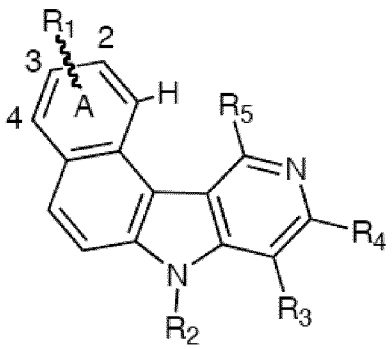
12- A compound according to anyone of claims 1-10 as a drug.

13- The compound according to claim 12 for use for treating cancer, inflammation or pain.

14- The compound according to claim 12 or 13, for use for treating cancer, optionally in combination with radiotherapy, hyperthermia and/or an antitumoral chemotherapy.

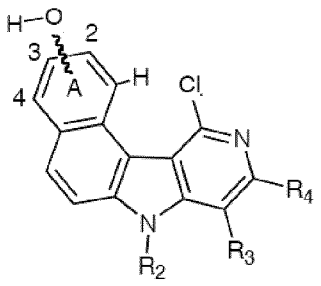
15- A kit comprising (a) a compound according to anyone of claims 1-10; and (b) an additional antitumoral drug, as a combined preparation for simultaneous, separate or sequential use, in particular in the treatment of cancer.

16- A method for preparing a compound of formula (I)



(I)

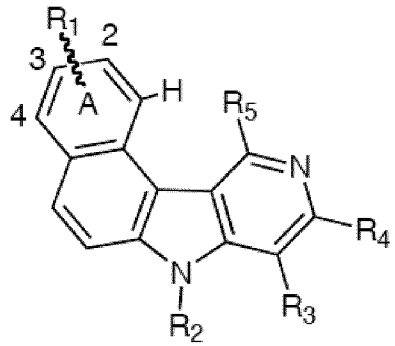
wherein R1, R2, R3 and R4 are as defined in anyone of claims 1-8, and R5 is chloride; comprising reacting the reagent Y-(C_2-C_5)alkyl-NR_aR_b with the compound **1**



(1)

wherein Y is halo, or hydroxy, R_a, R_b, R2, R3 and R4 are as defined in anyone of claims 1-8, thereby obtaining the compound of formula (I).

17- A method for preparing a compound of formula (I)



(I)

wherein R1, R2, R3 and R4 are as defined in anyone of claims 1-8, and R5 is hydrogen;

comprising carrying out a palladium catalyzed hydrogenation on the compound obtained by the method of claim 16, thereby obtaining the compound of formula (I).

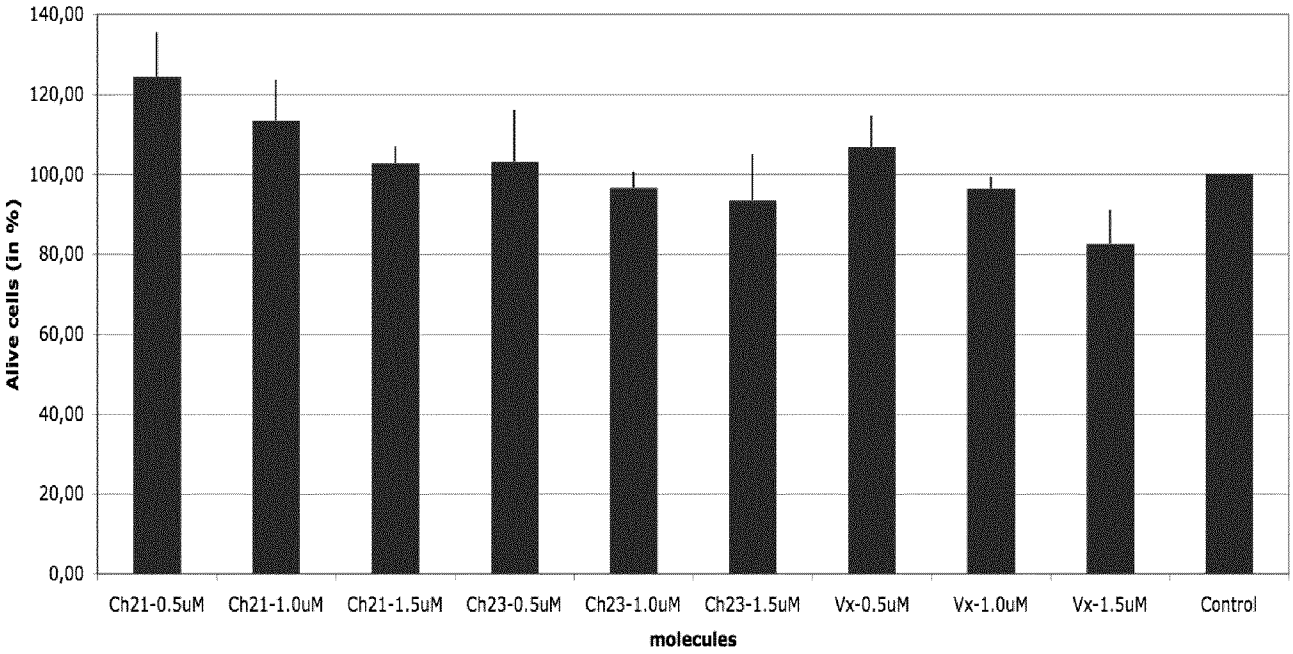

140,00
120,00
100,00
80,00
60,00
40,00
20,00
0,00
Alive cells (in %)
Ch21-0.5uM
Ch21-1.0uM
Ch21-1.5uM
Ch23-0.5uM
Ch23-1.0uM
Ch23-1.5uM
Vx-0.5uM
Vx-1.0uM
Vx-1.5uM
Control
molecules

FIGURE 1

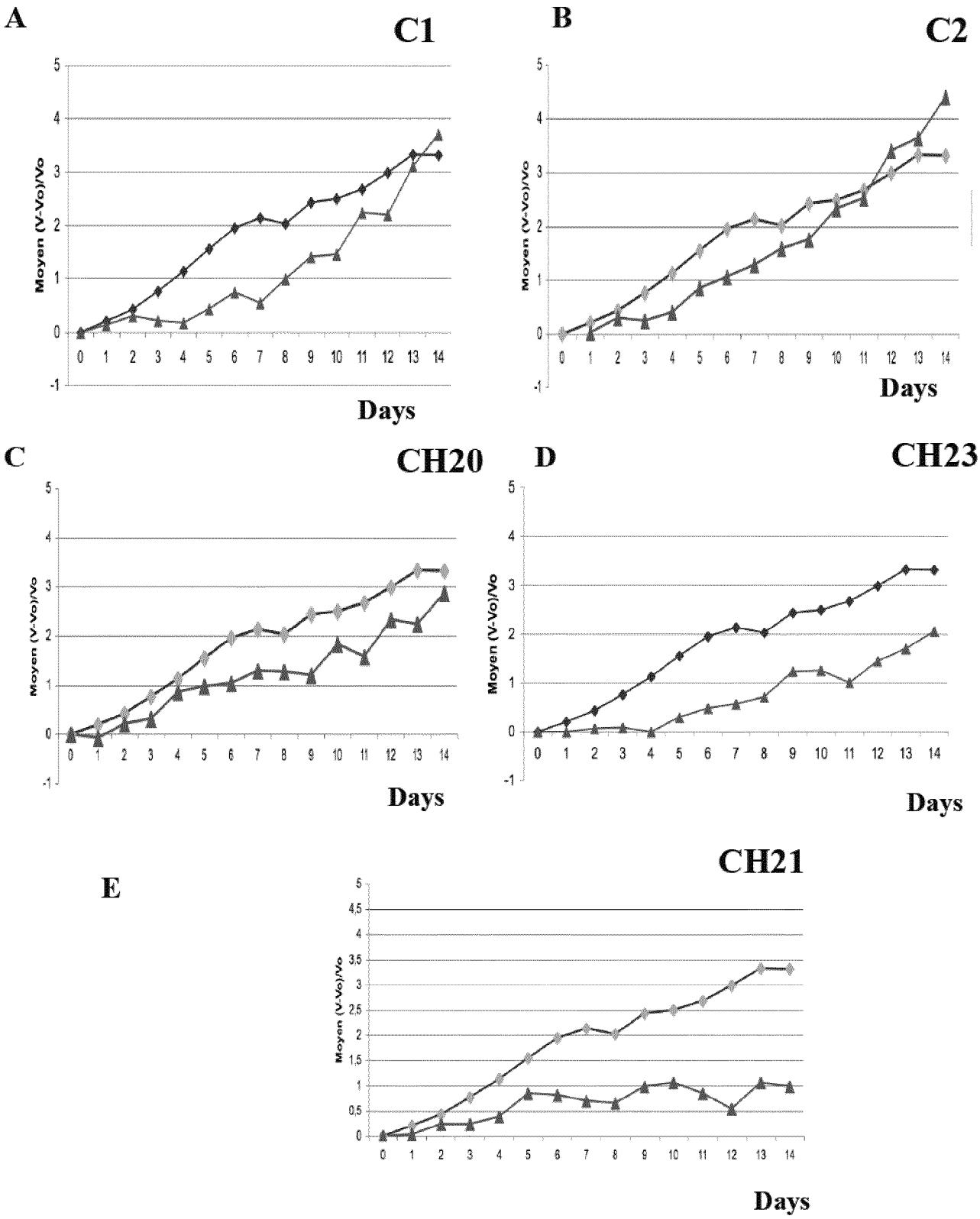

A
C1
Moyen (V-Vo)/Vo
Days
B
C2
Moyen (V-Vo)/Vo
Days
C
CH20
Moyen (V-Vo)/Vo
Days
D
CH23
Moyen (V-Vo)/Vo
Days
E
CH21
Moyen (V-Vo)/Vo
Days

FIGURE 2

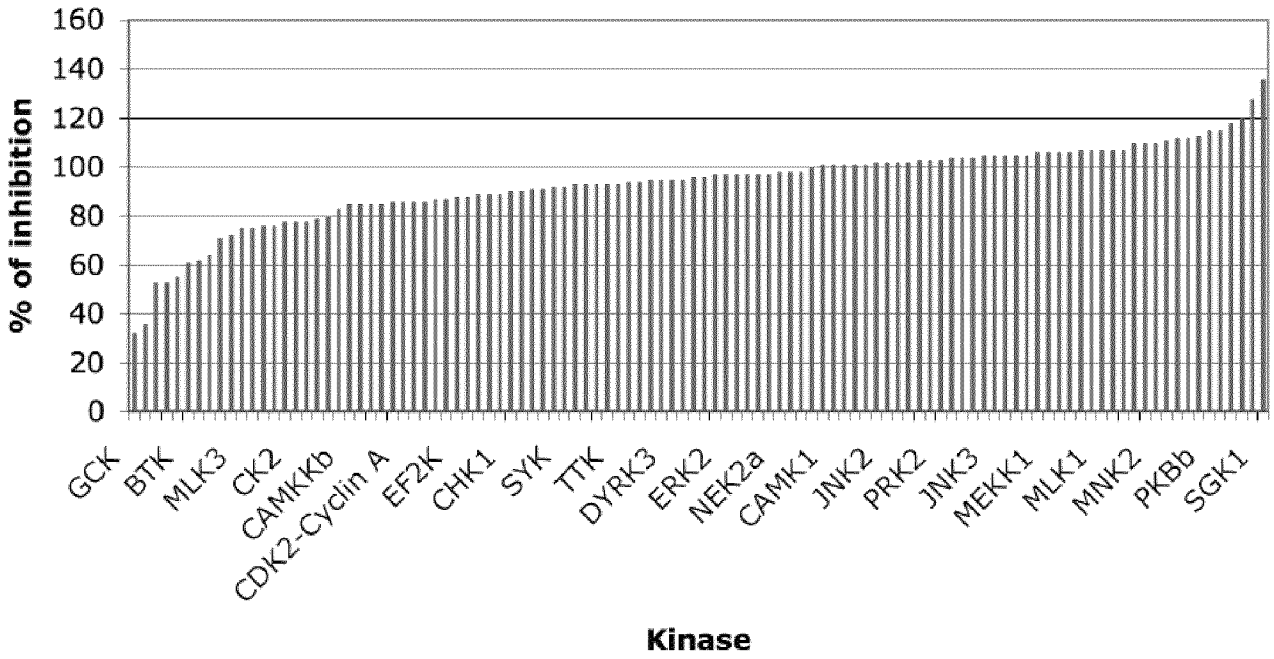


FIGURE 3

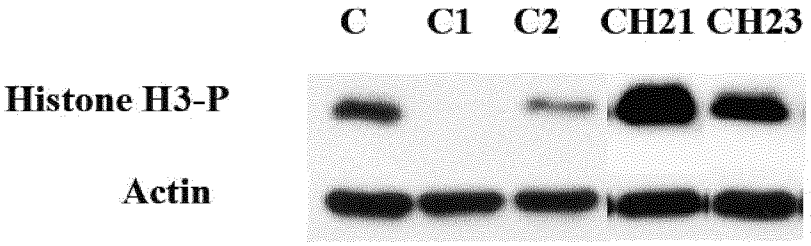


FIGURE 4

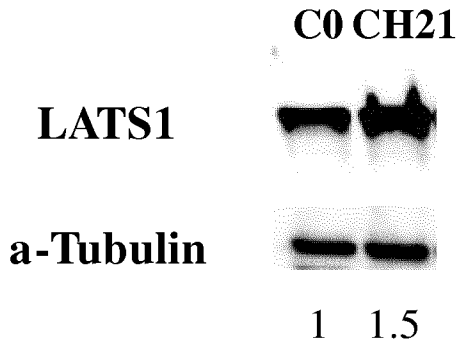


FIGURE 5

FIG 6A

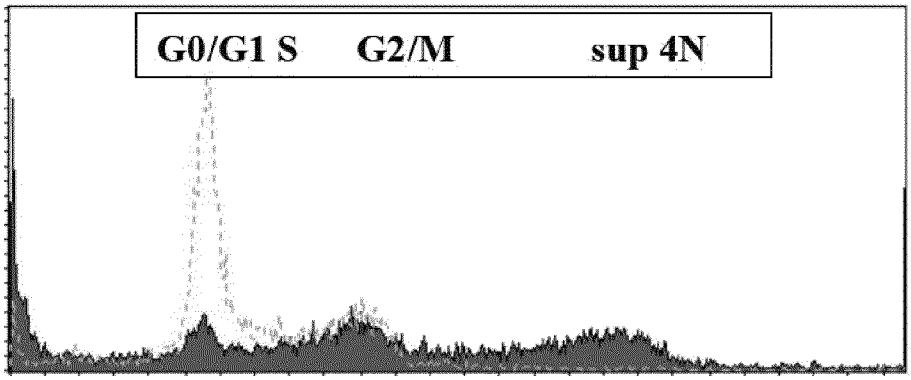


FIG 6B

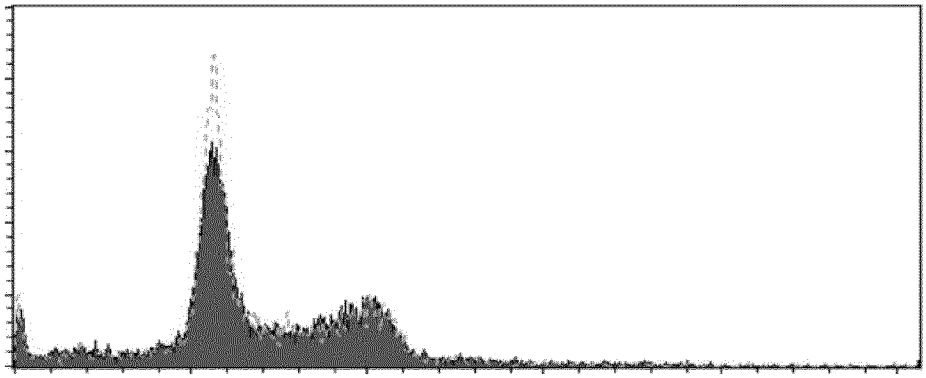


FIGURE 6

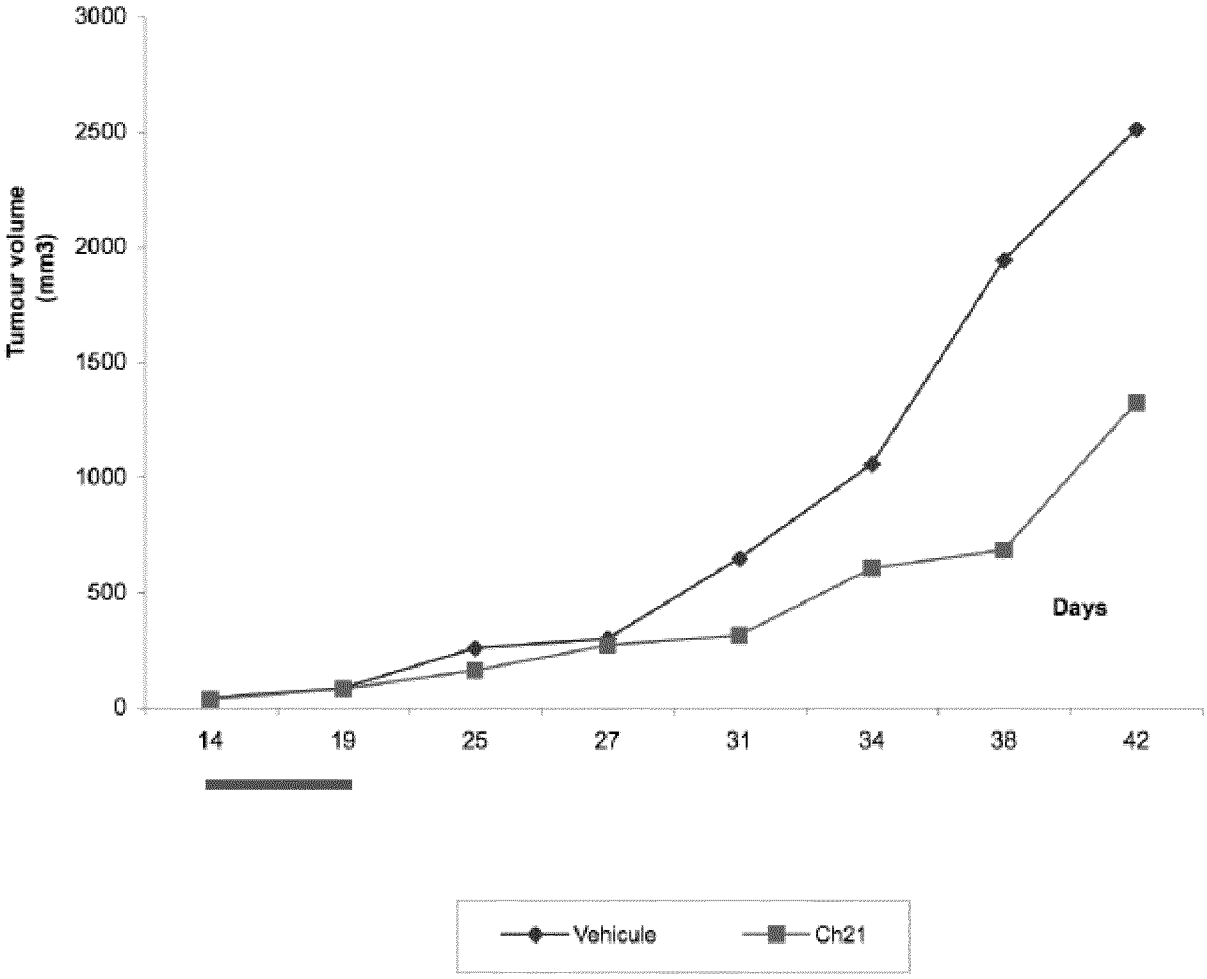


FIGURE 7

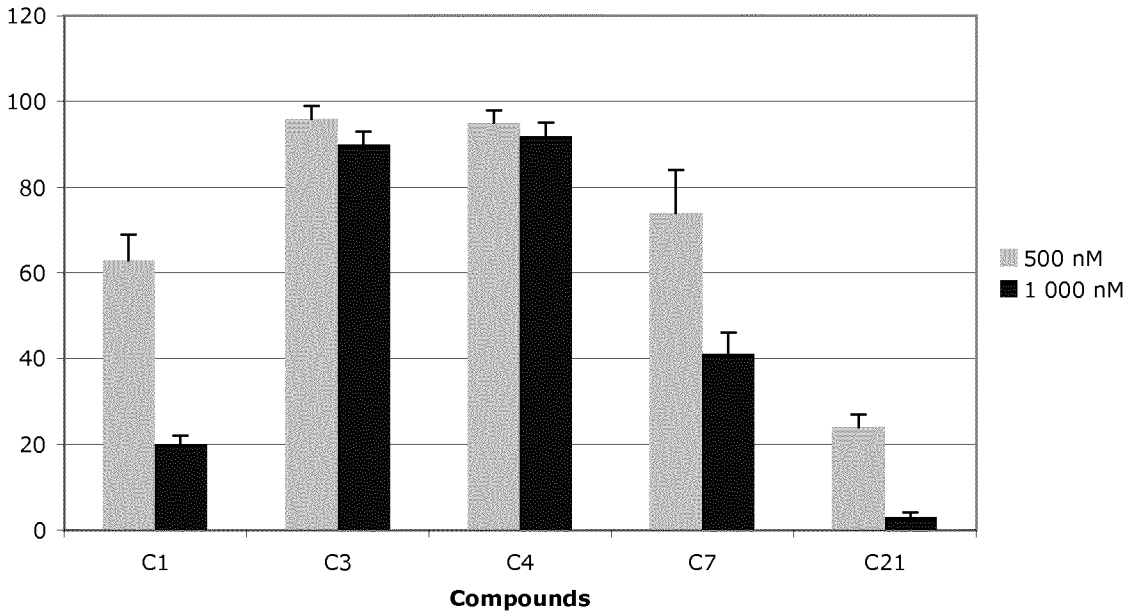


FIGURE 8

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2012/060081

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07D471/04 A61K31/437 A61P35/00 A61P29/00
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

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, BIOSIS, CHEM ABS Data, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	HOANG THI MY-NHUNG ET AL: "Benzo[e]pyridoindoles, novel inhibitors of the aurora kinases", CELL CYCLE, LANDES BIOSCIENCE, US, vol. 8, no. 5, 1 March 2009 (2009-03-01), pages 765-772, XP009120214, ISSN: 1551-4005 cited in the application Whole document, in particular Figure 1 page 766	1-17
A	EP 2 280 011 A1 (COMMISSARIAT ENERGIE ATOMIQUE [FR]; INST NAT SANTE RECH MED [FR]; INST) 2 February 2011 (2011-02-02) Claims and examples	1-17

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

[X] See patent family annex.

* Special categories of cited documents :

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

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"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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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
17 August 2012	27/08/2012

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Lécaillon, Jennifer

1

Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP 0 402 232 A1 (RHONE POULENC SANTE [FR]) 12 December 1990 (1990-12-12) Claims and examples and page 4 line 39	1-17
A	HE L ET AL: "Design of antineoplastic agents based on the '2-phenylnaphthalene-type' structural pattern-synthesis and biological activity studies of 11H-indolo[3.2-c]quinoline derivatives", EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY, EDITIONS SCIENTIFIQUE ELSEVIER, PARIS, FR, vol. 38, no. 1, 1 January 2003 (2003-01-01), pages 101-107, XP004410831, ISSN: 0223-5234, DOI: 10.1016/S0223-5234(02)01420-4 page 102; figure 2; compound 6d and 6r	1-17
A	WANG T ET AL: "Trk kinase inhibitors as new treatments for cancer and pain", EXPERT OPINION ON THERAPEUTIC PATENTS, INFORMA HEALTHCARE, GB, vol. 19, no. 3, 1 March 2009 (2009-03-01), pages 305-319, XP002557234, ISSN: 1354-3776, DOI: 10.1517/13543770902721261 the whole document	1-17

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2012/060081

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