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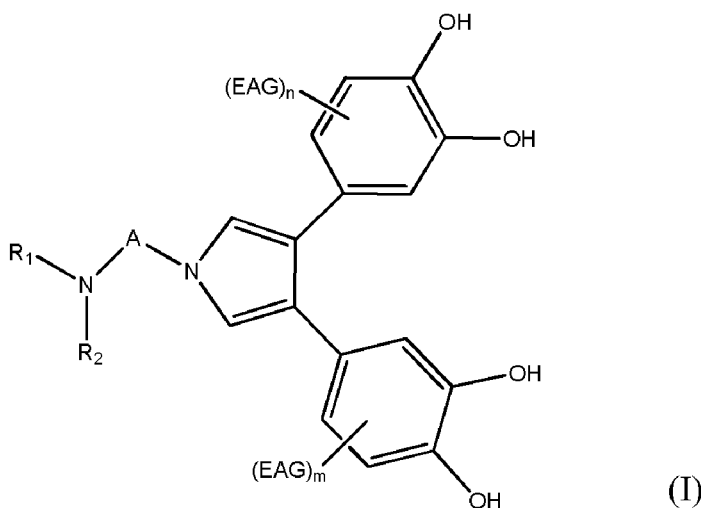
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(54) Title : N-SUBSTITUTED 3,4-BIS (CATECHOL) PYRROLE COMPOUNDS, AND THE PREPARATION AND USE THEREOF IN THE TREATMENT OF CANCER

(54) Titre : COMPOSES 3,4-BIS(CATECHOL)PYRROLE-N-SUBSTITUES, LEUR PREPARATION ET UTILISATION DANS LE TRAITEMENT DU CANCER



(57) Abstract : The present invention relates to a compound of formula (I) in which: - m is an integer from 0 to 3, preferably from 0 to 2; n is an integer from 0 to 3, preferably from 0 to 2; m + n ≥ 1; EAG is an electro-attractive group chosen independently from among a halogen atom, an NO₂, CF₃, CCl₃, CN, CO₂H, (C=O)NR₂, CH=NR, (C=S)OR, (C=O)SR, CS₂R, SO₂R, SO₂NR₂, SO₃R, P(O)(OR)₂, P(O)(R)₂, B(OR)₂ group where R is a (C₁ - C₆) alkyl radical, a phenyl group or a hydrogen atom; A is a saturated or unsaturated, linear or branched hydrocarbon chain including 1 to 10 atoms of carbon; and R₁ and R₂ each represent independently from one another a hydrogen atom, a CO-(C₁ - C₆)-alkyl, (C₁ - C₆) alkyl, phenyl or phenyl-(C₁ - C₆)-alkyl group, in which R₁ and R₂ form, together with the nitrogen atom they carry, a 5- to 15-member heterocycle, optionally substituted by a (C₁ - C₆) alkyl group; including its stereoisomers and the mixtures thereof, or a pharmaceutically acceptable salt of same.

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La présente invention concerne un composé de formule (I) dans laquelle : - m est un entier de 0 à 3, de préférence de 0 à 2; - n est un entier de 0 à 3, de préférence de 0 à 2; - m + n ≥ 1; - EAG représente un groupe électroattracteur indépendamment choisi parmi un atome d'halogène, un groupe NO₂, CF₃, CCl₃, CN, CO₂H, (C=O)NR₂, CH=NR, (C=S)OR, (C=O)SR, CS₂R, SO₂R, SO₂NR₂, SO₃R, P(O)(OR)₂, P(O)(R)₂, B(OR)₂, où R est un radical (C₁ — C₆) alkyle, un groupe phényle ou un atome d'hydrogène; - A représente une chaîne hydrocarbonée, saturée ou insaturée, linéaire ou ramifiée, comprenant de 1 à 10 atomes de carbone; et - R₁ et R₂ représentent chacun, indépendamment l'un de l'autre, un atome d'hydrogène, un groupe CO-(C₁ - C₆)-alkyle, (C₁ — C₆) alkyle, phényle ou phényle-(C₁ — C₆)-alkyle, où R₁ et R₂ forment ensemble, avec l'atome d'azote qui les porte, un hétérocycle à 5 à 15 chaînons, éventuellement substitué par un groupe (C₁ — C₆) alkyle; y compris ses stéréoisomères et leurs mélanges, ou un sel d'acide pharmaceutiquement acceptable de celui-ci.

N-SUBSTITUTED 3,4-BIS (CATHECHOL) PYRROLE COMPOUNDS,
AND THE PREPARATION AND USE THEREOF IN THE TREATMENT OF
CANCER

The present invention relates to novel N-substituted 3,4-bis (catechol) pyrrole compounds, the preparation and use thereof in the treatment of cancer.

Tumour cells originate from innate or acquired
5 chromosomal modifications resulting from translocation,
genic amplification, retroviral insertion, mutation,
genetic deletion, and epigenetic disorders. These
anomalies affect numerous genes involved in controlling
the cell cycle, apoptosis, growth and survival, and DNA
10 repair and stabilisation and detoxification genes.

The large majority of conventional anticancer
chemotherapy is essentially based on the use of drugs
wherein the mode of action involves blocking cell
multiplication, either in the S phase of the cell cycle
15 by attacking cell DNA or in the G2/M phase by attacking
the microtubules of the mitotic spindle required for
cell division. Further anticancer agents are intended
to trigger programmed cell death by apoptosis, by
inducing oxidative stress. The drawback of these

approaches is the relative non-specificity of the drugs which are unable to spare healthy cells. Multiple side-effects follow. More recently, novel therapeutic approaches have been developed no longer using a cytotoxic action, as the curing means, but rather the use of an impediment to slow down or stop tumour growth. This targeted chemotherapy targets the intimate mechanisms of cell proliferation by blocking cell multiplication, by inhibiting growth factor signal transmission pathways, or by inhibiting angiogenesis.

However, with these novel targeted anticancer treatments, new forms of chemoresistance have emerged. They differ from the known chemoresistance mechanisms essentially associated with drug expulsion membrane system expression: ABC (ATP-Binding Cassette) protein including the MDR1 (Multi Drug Resistance-1) system. In this case, cancer cells develop chemoresistance by stimulating the survival pathway, associated with pro-apoptotic pathway inhibition. This survival pathway is activated by tyrosine kinase activity receptors (EGFR, PDGFR, IGFR), themselves activated by growth factors. It consists of PI3K (Phosphatidyl inositol 3 OH kinase), PDK (phosphoinositide-dependent tyrosine kinase), Akt (serine/threonine protein kinase PKB), PTEN (phosphatase and tensin homologue deleted on chromosome 10), FOXO (forkhead box O), mTOR (mammalian target of rapamycin) proteins, associated with the Src and Ras oncogenes. As a general rule, the survival pathway has a protective effect against apoptosis and may induce autophagy.

5 The link between the survival pathway and cancerogenesis is now well established. Indeed, mutations in the genes coding for the survival pathway are present in more than 30% of cancers including 70% of breast cancers: activating mutations of the PIK3CA, PIK3CB, AKT1, AKT2 genes, loss of PTEN function, and overexpression of the src and Ras oncogenes, regulating this pathway.

10 As such, for some years, compounds inhibiting this survival pathway have been developed to effectively combat cancer cells, which, in association with other drugs, block growth factor signal transmission.

15 Recent clinical trials have revealed that inhibiting the survival pathway blocked tumorigenesis. As such, the use of survival pathway inhibitors as an anticancer treatment may be considered as a new weapon against cancer.

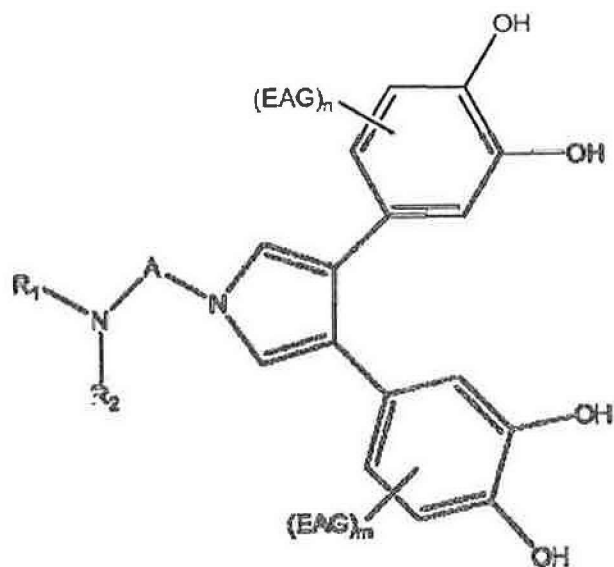
20 One aspect of anticancer drug pharmacology is thus ongoing research on novel drugs liable to display superior therapeutic efficacy, via specific molecular targets, associated with the absence of chemoresistance and the absence of toxicity on healthy cells.

25 These fundamental criteria have been adopted for the selection of a novel antitumour drug.

30 The discussion of documents, acts, materials, devices, articles and the like is included in this specification solely for the purpose of providing a context for the present invention. It is not suggested or represented that any or all of these matters formed part of the prior art base or were common general knowledge in the field relevant to the present invention as it existed before the priority date of each claim of this application.

Where the terms "comprise", "comprises", "comprised" or "comprising" are used in this specification (including the claims) they are to be interpreted as specifying the presence of the stated features, integers, steps or components, but not precluding the presence of one or more other features, integers, steps or components, or group thereof.

The present invention relates to novel N-substituted 3,4-bis (catechol) pyrrole compounds having the general formula (I) hereinafter, the preparation thereof and the antimitotic action thereof and the mode of action thereof with respect to cancer cells.

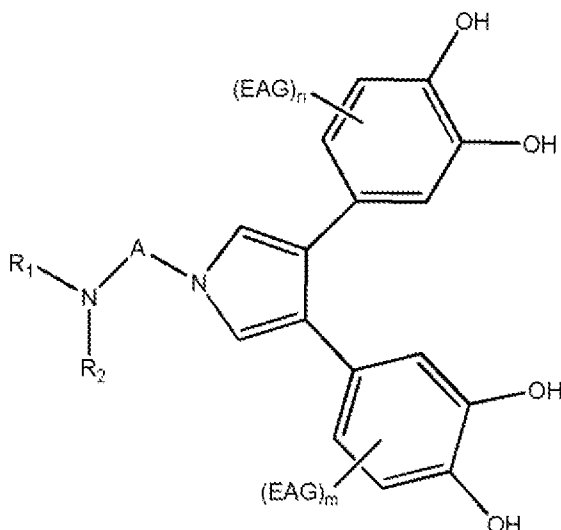


wherein:

- m is an integer from 0 to 3, preferably from 0 to 2;
- 5 - n is an integer from 0 to 3, preferably from 0 to 2;
- $m + n \geq 1$
- EAG is an electro-attractive group chosen independently from among a halogen atom, an NO_2 , CF_3 , CCl_3 , CN , CO_2H , $(\text{C}=\text{O})\text{NR}_2$, $\text{CH}=\text{NR}$, $(\text{C}=\text{S})\text{OR}$, $(\text{C}=\text{O})\text{SR}$,
 10 CS_2R , SO_2R , SO_2NR_2 , SO_3R , $\text{P}(\text{O})(\text{OR})_2$, $\text{P}(\text{O})(\text{R})_2$, $\text{B}(\text{OR})_2$ group, where R is an alkyl ($\text{C}_1\text{-C}_6$) radical, a phenyl group or a hydrogen atom;
- A is a saturated or unsaturated, linear or branched hydrocarbon chain including 1 to 10 atoms of carbon;
- 15 and
- R_1 and R_2 each represent, independently from one another, a hydrogen atom, a $\text{CO-(C}_1\text{-C}_6\text{)-alkyl}$, $(\text{C}_1\text{-C}_6)$ alkyl, phenyl or phenyl- $(\text{C}_1\text{-C}_6)$ -alkyl group, where R_1 and R_2 form, together with the nitrogen atom bearing

same, a 5- to 15-member heterocycle, optionally substituted by a (C₁-C₆) alkyl group; including the stereoisomers and the mixtures thereof, or a pharmaceutically acceptable acid salt thereof.

In one aspect, the present invention relates to a compound having the following formula (I):



wherein:

- m is an integer from 0 to 3;
- n is an integer from 0 to 3;
- $m + n \geq 1$

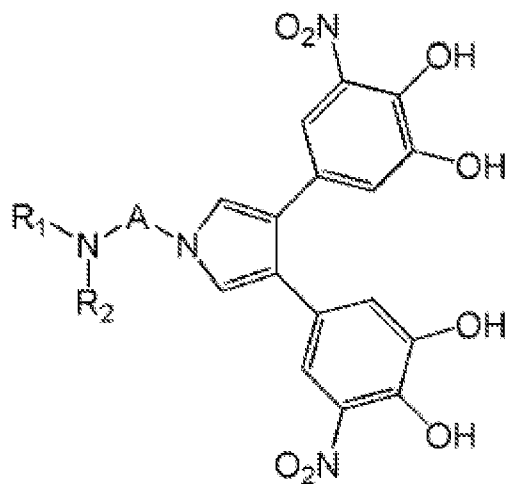
- EAG is an electro-attractive group selected from the group consisting of a halogen atom, NO₂, CF₃, CCl₃, CN, CO₂H, (C=O)NR₂, CH=NR, (C=S)OR, (C=O)SR, CS₂R, SO₂R, SO₂NR₂, SO₃R, P(O)(OR)₂, P(O)(R)₂, and B(OR)₂, where R is an alkyl (C₁-C₆) radical, a phenyl group or a hydrogen atom;

- A is a saturated or unsaturated, linear or branched hydrocarbon chain comprising 1 to 10 atoms of carbon; and

5a

5 - R₁ and R₂ each represent, independently from one another, a hydrogen atom, a CO-(C₁-C₆)-alkyl, (C₁-C₆) alkyl, phenyl or phenyl-(C₁-C₆)-alkyl group, or R₁ and R₂ form, together with the nitrogen atom bearing same, a 5- to 15-member heterocycle, optionally substituted by a (C₁-C₆) alkyl group; or the stereoisomers and the mixtures thereof, or a pharmaceutically acceptable acid salt thereof.

10 The present invention particularly relates to the compound having formula (Ia) and the use thereof as a cancer treatment drug.



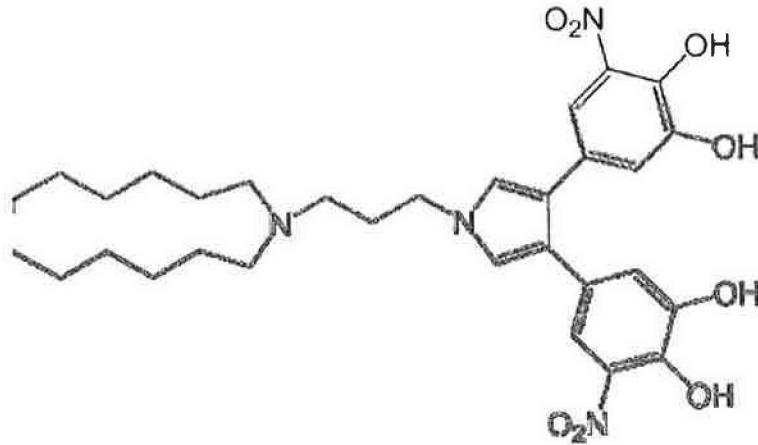
(Ia)

15 In particular, the radicals R₁ and R₂ form, together with the nitrogen atom bearing same, a heterocycle which is saturated and does not comprise any other heteroatom than the nitrogen atom bearing R₁ and R₂. More particularly, R₁ and R₂ form, together with the nitrogen atom bearing same, a 10- to 15-member, preferably 13-member, heterocycle.

20 In particular, the chain A is a saturated linear hydrocarbon chain comprising 3 to 6 carbon atoms, preferably 3 carbon atoms.

5b

The present invention particularly relates to the compound having formula (1) or a pharmaceutically acceptable acid salt thereof.



(1)

and the use thereof as a cancer treatment drug.

It should be noted that, in vitro, the compound (1), used at concentrations encompassing the
 5 IC₅₀ thereof, does not exhibit any cytotoxicity with respect to normal human cells.

In particular, the preferred pharmaceutically acceptable acid salt of the general formula (I), more particularly the compound (Ia) and preferentially the
 10 compound (1), is a hydrochloride.

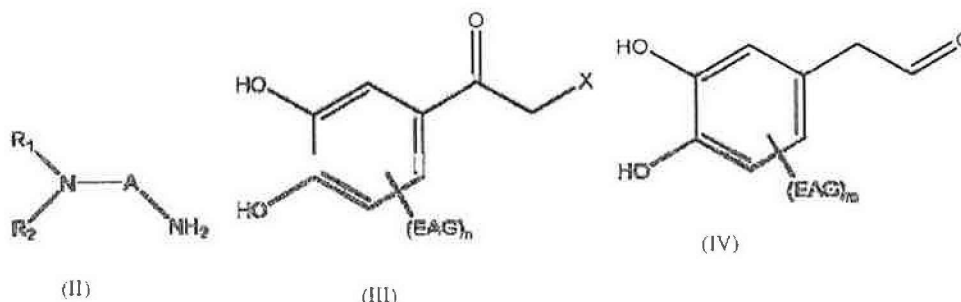
The present invention also applies to a pharmaceutical composition comprising at least one compound having general formula (I), more particularly the compound (Ia) and preferentially the compound (1),
 15 with at least one pharmaceutically acceptable excipient.

The present invention relates to the use of the compounds (I), more particularly (Ia) and (1), as a drug in the treatment of cancer, in particular lung, breast, liver, stomach, colon, rectal, oesophageal,
 20 laryngeal, nasopharyngeal, pancreatic, prostate, kidney, bladder, duodenal, endometrial, pleural, skin, testicular, ovarian, uterine, brain, bone, oral, ocular

cancer, or haematopoietic cancers such as leukaemias, myeloid leukaemias, lymphomas, bone marrow cancer or tumours of neuroectodermal origin such as glioblastomas.

The invention also relates to a process for preparing a compound having formula (I), more particularly (Ia) and (1) comprising:

a) reaction between an amine having the following formula (II), a halide having the following formula (III), and an aldehyde having the following formula (IV),



wherein n and m, EAG, R₁, R₂ and A are as defined above with respect to the general formula (I), and X represents a halogen atom, to arrive at a compound having formula (I).

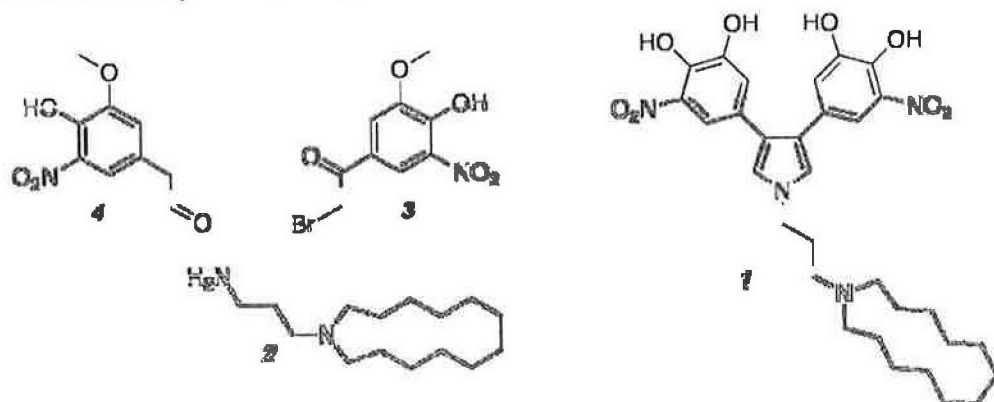
b) optionally, formation of a pharmaceutically acceptable salt from the compound having formula (I) obtained in the previous step a).

In this preparation process, the halide (III) and the amine (II) are first allowed to react with one another before adding the aldehyde (IV). Preferably, the radical X of the halide (III) is a chlorine, bromine or iodine atom.

RESULTS

Chemical synthesis

The compound 5,5'-(1-(3-(azacyclotridecan-1-yl)propyl)-1H-pyrrole-3,4-diyl)bis(3-nitrobenzene-1,2-diol) (1) was prepared by condensing the phenacyl halide 3 with the primary amine 2 and the phenylacetaldehyde 4, represented hereinafter:



N-(3-aminopropyl)azatricyclodecane (2) ((a) G. Koren-Goldshlager, Y. Kashman, M. Schleyer, J. Nat. Prod., 1998, 61, 282-284; (b) D. E. Williams, K. S. Craig, B. Patrick, L. M. McHardy, R. van Soest, M. Roberge, R. J. Andersen, J. Org. Chem., 2002, 67, 245-258) was obtained from commercial laurolactam as per diagram 1 hereinafter:

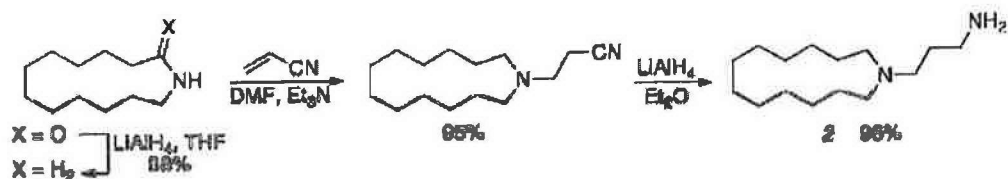
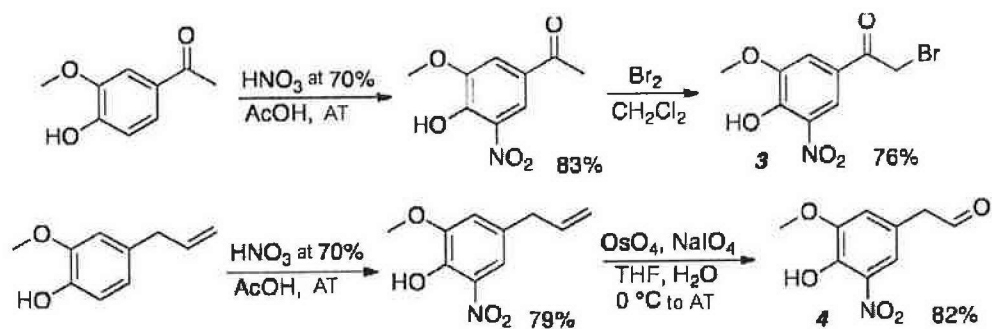


Diagram 1

Compounds 3 (J. Borgulya, H. Bruderer, K. Bernauer, G. Zürcher, M. Da Prada, Helv. Chim. Acta, 1989, 72,

952-968) and 4 were prepared as per diagram 2 hereinafter:



5

Diagram 2

A monotope formation method was developed for the synthesis of the pyrrole 5 from the amine 2, the halide 3 and the phenylacetaldehyde 4 (Diagram 3).

10

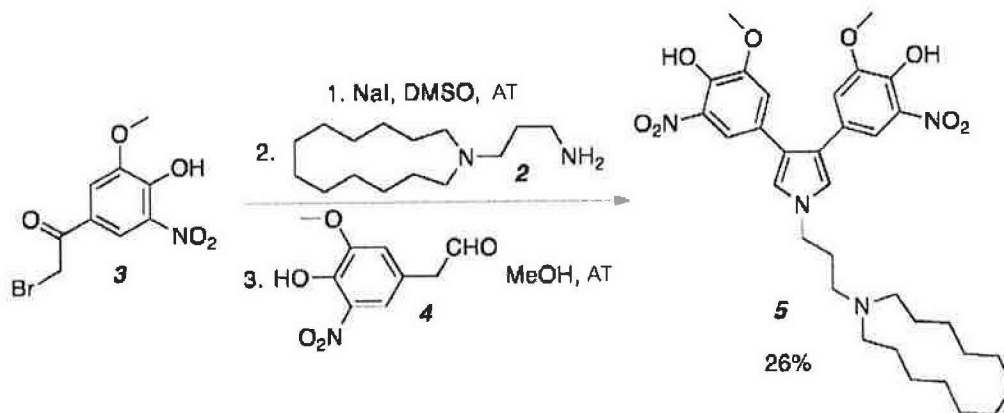


Diagram 3

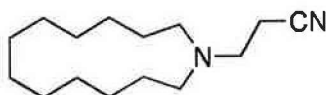
By treating compound 5 with a solution of BBr_3 in dichloromethane, the demethylated product (1) was obtained (Diagram 4).

phase is then settled and the precipitate is washed three times with a 1:1 ether/pentane mixture. The solvent is removed at reduced pressure to arrive at azacyclotridecane (3.9 g; 98%) in colourless oil form.

5 ^1H NMR (CDCl_3 ; 300 MHz) δ (ppm) 2.62 (4 H; t; $J = 5.1$ Hz); 1.57-1.25 (20 H; m). ^{13}C NMR (CDCl_3 ; 75 MHz) δ (ppm) 47.9; 27.9; 26.5; 26.0; 25.4; 24.6.

3-(Azacyclotridecan-1-yl)propanenitrile

10



To a solution of azacyclotridecane (3.8 g; 20.7 mmol) and triethylamine (50 μl ; 0.35 mmol) in DMF (15 ml) in argon, acrylonitrile (7.7 ml; 115 mmol) is

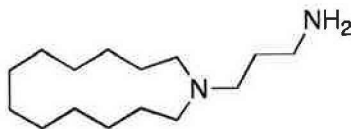
15 added and the reaction mixture is stirred for 16 hours at 70°C. The reaction mixture is extracted with ether, the organic phase is washed with brine, dried on Na_2SO_4 , and evaporated at reduced pressure to arrive at 3-

(azacyclotridecan-1-yl)propanenitrile in colourless oil form (4.64 g; 95%). ^1H NMR (CDCl_3 ; 300 MHz) δ (ppm) 2.76 (2 H; t; $J = 7.2$ Hz); 2.50-2.35 (6 H; m); 1.55-1.28 (20 H; m). ^{13}C NMR (CDCl_3 ; 75 MHz) δ (ppm) 53.7; 49.7; 26.5; 26.2; 25.23; 25.18; 15.7.

20

25 3-(Azacyclotridecan-1-yl)propan-1-amine (2) ((a) G. Koren-Goldshlager, Y. Kashman, M. Schleiter, J. Nat. Prod., 1998, 61, 282-284; (b) D. E. Williams, K. S. Craig, B. Patrick, L. M. McHardy, R. van Soest, M. Roberge, R. J. Andersen, J. Org. Chem., 2002, 67, 245-

30 258).

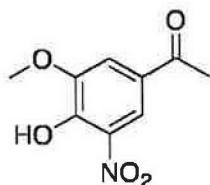


To a solution of 3-(azacyclotridecan-1-yl)propanenitrile (0.5 g; 2.1 mmol) in ether (15 ml) at 0°C in argon, LiAlH₄ (0.3 g; 7.9 mmol) is gradually added and the reaction mixture is stirred at 33°C for 15 min. A saturated Na₂SO₄ solution is then gradually added at 0°C to neutralise the excess LiAlH₄, and solid K₂CO₃ is then added to absorb the entire aqueous phase. The organic phase is settled and the precipitate is washed three times with ether. The solvent is removed at reduced pressure to arrive at 2 (0.49 g, 96%) in colourless oil form. ¹H NMR (CDCl₃; 500 MHz) δ (ppm) 2.74 (2 H; t; J = 7.0 Hz); 2.40-2.28 (6 H; m); 1.57 (2 H; quint; J = 7.0 Hz); 1.44-1.32 (22 H; m). ¹³C NMR (CDCl₃; 125 MHz) δ (ppm) 54.4; 52.8; 41.2; 31.7; 26.7; 26.5; 26.3; 25.8; 25.6.

Preparation of 2-bromo-1-(4-hydroxy-3-methoxy-5-nitrophenyl)ethanone (3)

20

1-(4-Hydroxy-3-methoxy-5-nitrophenyl)ethanone ((a) L. E. Kiss, H. S. Ferreira, L. Torrão, M. J. Bonifácio, P. N. Palma, P. Soares-da-Silva, D. A. Learnmonth, J. Med. Chem., 2010, 53, 3396-3411; (b) X. Lu, S. Wan, J. Jiang, X. Jiang, W. Yang, P. Yu, L. Xu, Z. Zhang, G. Zhang, L. Shan, Y. Wang, Eur. J. Med. Chem., 2011, 46, 2691-2698).

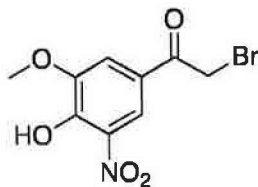


To a solution of 4'-hydroxy-3'-methoxyacetophenone (10 g; 60.2 mmol) in acetic acid (140 ml) at AT, 70% HNO₃ is added (4.2 ml; 66.3 mmol). The reaction mixture is stirred for 30 min and the yellow crystals are filtered, washed with ether and dried to arrive at 1-(4-Hydroxy-3-methoxy-5-nitrophenyl)ethanone (10.5 g; 83%), mp 158-159°C (lit. 159-161°C, ethanol, X. Lu, S. Wan, J. Jiang, X. Jiang, W. Yang, P. Yu, L. Xu, Z. Zhang, G. Zhang, L. Shan, Y. Wang, Eur. J. Med. Chem., 2011, 46, 2691-2698). ¹H NMR (CDCl₃; 300 MHz) δ (ppm) 11.09 (1 H; s), 8.30 (1 H; d; J = 1.5 Hz); 7.75 (1 H; d; J = 1.5 Hz); 4.01 (3 H; s); 2.62 (3 H; s). ¹³C NMR (CDCl₃; 75 MHz) δ (ppm) 194.9; 150.4; 150.1; 133.0; 128.3; 117.7; 115.3; 56.9; 26.0. IR (neat) ν_{max} cm⁻¹ 3228; 3091; 2988; 2925; 1676; 1612; 1535; 1413; 1378; 1350; 1329; 1218; 1136; 1047; 869; 735; 709; HRMS (DE) (m/z) [M-H]⁻ calculated for C₉H₈NO₅ 210.0402; obtained 210.0404.

20

2-Bromo-1-(4-hydroxy-3-methoxy-5-nitrophenyl)ethanone (3) (J. Borgulya, H. Bruderer, K. Bernauer, G. Zürcher, M. Da Prada, Helv. Chim. Acta, 1989, 72, 952-968).

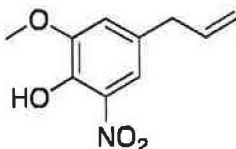
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To a solution of 1-(4-hydroxy-3-methoxy-5-nitrophenyl)ethanone (9.6 g; 45.5 mmol) in CH₂Cl₂ (200 ml) at AT, bromine (2.4 ml; 46.8 mmol) is gradually added and the reaction mixture is stirred for 10 min. During this period, the colour of the bromine disappears completely. Pentane (400 ml) is then added. After 10 min, the yellow crystals are filtered under a hood, washed with a 1:1 pentane/ether mixture, and dried to arrive at 3 (10 g; 76%), mp 143-145°C (lit. 147-149°C; J. Borgulya, H. Bruderer, K. Bernauer, G. Zürcher, M. Da Prada, *Helv. Chim. Acta*, 1989, 72, 952-968). ¹H NMR (CDCl₃; 300 MHz) δ (ppm) 11.18 (1 H; s), 8.36 (1 H; d; J = 1.8 Hz); 7.77 (1 H; d; J = 1.8 Hz); 4.44 (2 H; s); 4.03 (3 H; s). ¹³C NMR (CDCl₃; 75 MHz) δ (ppm) 188.7; 150.7; 150.6; 133.0; 125.0; 118.3; 115.9; 57.0; 29.4. IR (neat) ν_{max} cm⁻¹ 3366; 3093; 2991; 2942; 1765; 1689; 1608; 1535; 1386; 1246; 1151; 1060; 912; 887; 850; 762. HRMS (DE) (m/z) [M-H]⁻ calculated for C₉H₇⁷⁹BrNO₅ 287.9508; obtained 287.9480.

Preparation of 2-(4-hydroxy-3-methoxy-5-nitrophenyl)acetaldehyde (4)

4-Allyl-2-methoxy-6-nitrophenol (D. E. Lewin, A. Lowy, *J. Am. Chem. Soc.*, 1933, 55, 1995-2000).

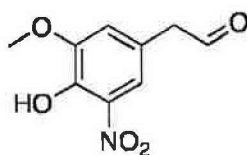


To a solution of eugenol (6.76 g; 41.2 mmol) in acetic acid (100 ml) at AT, 70% HNO₃ is added (2.8 ml; 44.2 mmol). The reaction mixture is stirred for 15 min, extracted with ether, washed vigorously with brine, and finally with a phosphate buffer (pH = 7). The ether solution is diluted with the same volume of pentane, filtered on silica gel and evaporated under reduced pressure to arrive at 4-allyl-2-methoxy-6-nitrophenol (6.36 g, 79%) in the form of dark red oil. ¹H NMR (CDCl₃; 300 MHz) δ (ppm) 10.61 (1 H; s), 7.48 (1 H; d; J = 1.5 Hz); 6.96 (1 H; d; J = 1.5 Hz); 5.91 (1 H; m); 5.20-5.08 (2 H; m); 3.92 (3 H; s); 3.35 (2 H; d; J = 6.6 Hz). ¹³C NMR (CDCl₃; 75 MHz) δ (ppm) 149.8; 144.8; 135.9; 133.6; 131.2; 118.6; 117.1; 115.0; 56.7; 39.4. IR (neat) ν_{max} cm⁻¹ 3225; 3080; 2976; 2939; 1639; 1539; 1428; 1390; 1326; 1259; 1133; 1061; 916; 763. HRMS (DE) (m/z) [M+Na]⁺ calculated for C₁₀H₁₁NNaO₄ 232.0586; obtained 232.0578.

20

2-(4-Hydroxy-3-methoxy-5-nitrophenyl)acetaldehyde

(4)

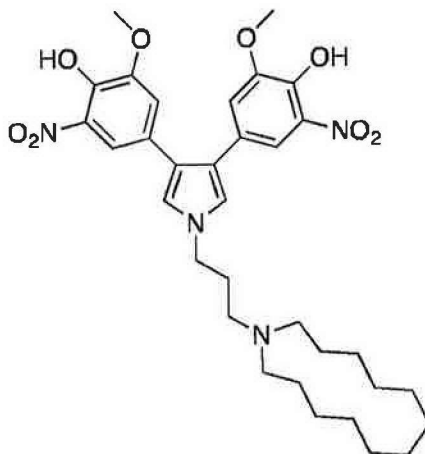


To a solution of 4-allyl-2-methoxy-6-nitrophenol (2.0 g; 9.6 mmol) in THF (23 ml) and water (8 ml) at 0°C, a 0.166 M osmium tetroxide solution

25

(23 mg/0.09 mmol) in a 7:5 THF/water mixture (0.55 ml) is added. After 5 min, NaIO₄ (4.8 g; 22 mmol) is added. The reaction mixture is stirred for 1 hour at 0°C, allowed to return gradually to AT for 1 hour, extracted
5 with a 1:1 CH₂Cl₂/ether mixture and the organic phase is washed once with brine, dried on Na₂SO₄, and evaporated at reduced pressure at 25°C. The residue is dissolved in the minimum volume of CH₂Cl₂ and a 1:1 pentane/ether mixture is added. The solvent is
10 evaporated at reduced pressure at 25°C until yellow crystals are formed; pentane is added and the solution is concentrated once again. The crystals are filtered to arrive at 4 (1.66 g, 82%), mp 130-132°C. ¹H NMR (CDCl₃; 300 MHz) δ (ppm) 10.71 (1 H; s), 9.80 (1 H; t; J = 1.8 Hz);
15 7.57 (1 H; d; J = 1.8 Hz), 6.97 (1 H; d; J = 1.8 Hz), 3.96 (3 H; s); 3.74 (2 H; d; J = 1.8 Hz). ¹³C NMR (CDCl₃; 75 MHz) δ (ppm) 197.8; 150.4; 145.8; 133.8; 123.1; 119.0; 116.6; 56.8; 49.5. IR (neat) ν_{max} cm⁻¹ 3226; 1720; 1583; 1449; 1333; 1264; 1238; 1134;
20 1064; 921; 764. HRMS (DE) (m/z) [M-H]⁻ calculated for C₉H₈NO₅ 210.0402; obtained 210.0399.

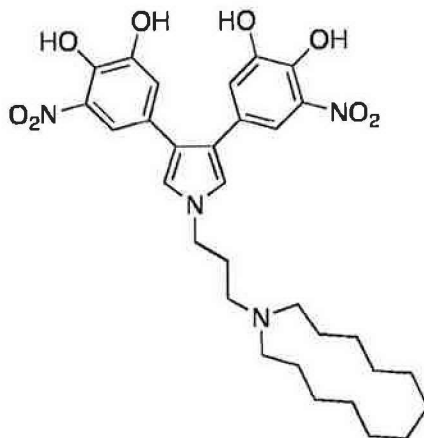
5-(1-(3-(Azacyclotridecan-1-yl)propyl)-4-(4-hydroxy-3-methoxy-5-nitrophenyl)1-H-pyrrol-3-yl)-3-nitrobenzene-1,2-diol (5)
25



Phenacyl bromide 3 (1.23 g; 4.2 mmol) and NaI (3.2 g; 21 mmol) are added to a solution of amine 2 (1 g, 4.2 mmol) in DMSO (20 ml) at AT in argon and the reaction mixture is stirred for 15 min at AT. A solution of aldehyde 4 (900 mg, 4.3 mmol) in methanol (60 ml) is then added and the reaction mixture is stirred for 16 hours at AT. It is extracted with CH_2Cl_2 and the organic phase is washed with a saturated NaHCO_3 solution, brine, dried on Na_2SO_4 , evaporated at reduced pressure and silica gel chromatography (elution gradient of CH_2Cl_2 with 80:1 $\text{CH}_2\text{Cl}_2/\text{MeOH}$) to arrive at 5 (682 mg, 26%) in the form of an amorphous red solid. ^1H NMR (CDCl_3 ; 500 MHz) δ (ppm) 7.65 (2 H; d; $J = 2.0$ Hz); 7.01 (2 H; d; $J = 2.0$ Hz); 6.87 (2 H; s); 4.05 (2 H; t; $J = 7.0$ Hz); 3.77 (6 H; s); 2.70-2.43 (6 H; m); 2.15 (2 H; quint; $J = 7.5$ Hz); 1.66-1.34 (20 H; m). ^{13}C NMR (CDCl_3 ; 75 MHz) δ (ppm) 149.7; 144.8; 133.9; 126.7; 121.0; 120.7; 118.7; 114.5; 56.8; 53.0; 51.7; 47.7; 27.9; 25.7; 25.6; 25.5; 25.2; 23.9. IR (neat) ν_{max} cm^{-1} 3255; 2930; 2859; 1552; 1522; 1464; 1337; 1240; 1156;

919; 732. HRMS (DE) (m/z) $[M+H]^+$ calculated for $C_{33}H_{45}N_4O_8$ 625.3237; obtained 625.3228.

5,5'-(1-(3-(Azacyclotridecan-1-yl)propyl)-1H-pyrrole-3,4-diyl)bis(3-nitrobenzene-1,2-diol) (1)



To compound 5 (50 mg; 0.080 mmol) in argon at AT, BBr_3 (1M solution in CH_2Cl_2 , 2 ml; 2 mmol). The heterogeneous mixture is stirred for 30 min at AT. A
 10 1:1 CH_2Cl_2 /pentane mixture (30 ml) is added and, after 5 min, the precipitate is separated, washed with the same solvent mixture and dissolved in the minimum volume of 10:1 CH_2Cl_2 /ethanol. The solution is
 15 CH_2Cl_2 /ethanol and evaporated at reduced pressure to arrive at 1 in the form of red-orange amorphous solid (44 mg; 92%). 1H NMR (CD_3OD ; 300 MHz) δ (ppm) 7.44 (2 H; d; $J = 1.8$ Hz); 7.00 (2 H; s); 6.97 (2 H; d; $J = 1.8$ Hz); 4.12 (2 H; t; $J = 6.3$ Hz); 3.30-3.12 (6 H; m);
 20 2.32 (2 H; quint; $J = 6.0$ Hz); 1.85-1.70 (4 H; m); 1.60-1.39 (16 H; m). ^{13}C NMR (CD_3OD ; 75 MHz) δ (ppm) 147.5; 141.9; 134.7; 127.0; 121.4; 121.3; 120.5; 113.5; 52.4; 52.0; 46.1; 25.9; 25.3; 24.6; 24.2; 23.9; 21.2.

IR (neat) ν_{\max} cm^{-1} 3379; 2932; 1621; 1555; 1471; 1300; 1236; 803; 762; 667. HRMS (DE) (m/z) $[\text{M}+\text{H}]^+$ calculated for $\text{C}_{31}\text{H}_{41}\text{N}_4\text{O}_8$ 597.2924; obtained 597.2938.

5 Hydrochloride 1•HCl

To a solution of 1 in a minimum volume of dichloromethane, a solution of 2 M HCl in methanol (5 equiv) diluted in ether is added. The precipitate formed is then filtered and dried. MP 179-184°C
10 (settled). ^1H NMR (DMSO- d_6 ; 300 MHz) δ (ppm) 7.19 (2 H; d; $J = 3.0$ Hz); 7.06 (2 H; s); 6.90 (2 H; d; $J = 3.0$ Hz); 4.00 (2 H; t; $J = 6.0$ Hz); 3.15-2.97 (6 H; m); 2.24 (2 H; quint; $J = 6.0$ Hz); 1.75-1.58 (4 H; m); 1.44-1.28 (16 H; m). ^{13}C NMR (DMSO- d_6 ; 75 MHz) δ (ppm) 148.0;
15 140.4; 137.7; 126.7; 121.3; 120.5; 120.1; 113.5; 51.9; 51.2; 46.7; 25.9; 25.7; 24.9; 24.7; 24.4; 20.9. IR (neat) ν_{\max} cm^{-1} 3111; 2930; 2861; 1552; 1463; 1293; 1233; 1061; 866; 801; 762. HRMS (DE) (m/z) $[\text{M}-\text{HCl}-\text{H}]^-$ calculated for $\text{C}_{31}\text{H}_{39}\text{N}_4\text{O}_8$ 595.2768; obtained 595.2773.

20

In vitro biological study

The biological activity of compound (1) was studied in vitro on 3 different cancer cell lines:

- HCT-116 (colorectal carcinoma)
- 25 - U87 (glioblastoma)
- K562 (myeloid leukaemia)

and on 3 normal non-cancerous cell types:

- HUVEC (human umbilical vascular endothelial cells)
- 30 - NHDF (human dermal fibroblasts)
- HFDPC (human hair follicle dermal papilla cells).

The cells selected for this study were incubated at 37° in the presence of compound (1) added in the culture medium at different concentrations and at different times.

5 The set of experiments conducted made it possible to determine the degree of toxicity of the compound under test, study the effect thereof on the progression of the cell cycle and the capability thereof of inducing an autophagic response and cell death by
10 apoptosis. We studied the mode of action thereof in order to determine the molecular target thereof.

1. Study of the effect of compound (1) on cell growth

The effect of compound (1) on HCT-116, U87 and
15 K562 cell growth was evaluated using a cell growth test (CellTiter-Blue™ Cell Viability Assay, Promega). The growth curves and the IC50 values (concentration of compound inducing a 50% reduction of cell growth) determined after 72 hours of cell treatment with
20 compound (1) are presented in Figure 1. They are 30 nM for HCT-116 cells, 45 nM for U87 cells and 70 nM for K562.

The effect of compound (1) on cell growth was described in more detail on the HCT-116 cell line. This
25 study was conducted as a function of the concentration and exposure time of the cells to the molecule under test (24 hrs, 48 hrs and 72 hrs) by counting the number of live cells after excluding dead cells by means of trypan blue staining.

Figure 2 represents the effect of compound (I) on the number and viability of HCT-116 cells as a function of the concentration thereof and the treatment time.

The results presented in Figure 2a demonstrate that the number of cells decreases as a function of the concentration thereof and the treatment time with compound (1). Compound (1) at the 25 nM concentration already exhibits an activity from 24 hours of treatment by reducing the number of cells by 40%, reaching up to 80% inhibition from 48 hours for concentrations greater than the IC₅₀. This inhibitory effect is however associated with a lack of modification of cell viability (Figure 2b). This indicates that the action of compound (1) is cytostatic and not cytotoxic.

In order to determine the specificity of action of compound (1) on cancer cells, we evaluated the effect thereof with respect to normal human HUVEC, NHDF and HFDPC cells and the HCT-116, U87 and K652 cancer cell annotations. The results presented in Figure 3 demonstrated that the IC₅₀ determined after 72 hours of treatment with compound (1) is 2 μ M for HUVEC cells, 5 μ M for NHDF cells and 10 μ M for HFDPC.

The IC₅₀ values are thus up to 300 times higher for normal cells compared to those obtained for cancer cells. This clearly indicates that malignant cells are significantly more sensitive to the action of compound (1) compared to healthy cells.

2. Study of the effect of compound (1) on the cell cycle

The flow cytometry analysis (FC500, Beckman Coulter) of HCT-116 cells treated with compound (1)

demonstrated that this compound blocks cell division in the G0/G1 phase. This effect is significant after 24 hours of exposure of the cells to compound (1) used at the 30 and 50 nM concentrations (Figure 4).

5 3. Study of the effect of compound (1) on the expression of the proteins p21 and p27 involved in stopping the cell cycle

On the basis of the above results demonstrating a stoppage of the cycle in G0/G1, associated with a reduction in the number of cells in the absence of toxicity, we evaluated the expression of the proteins p21 and p27 associated with stopping the cell cycle in G0/G1. The gene of the protein p27 is described as being regulated by FOXO3, whereas that of the protein p21 is controlled by the p53 transcription factor, stimulated inter alia by oxidative stress. The HCT-116 cells were treated with compound (1) for 24 hours. The cell extracts were then analysed by Western Blot. The results presented in Figure 5 demonstrate an increase in the expression of protein p27 following the treatment with compound (1) at the 25 and 50 nM concentrations. Compound (1) has no significant effect on the expression of the protein p21.

20 4. Study of the effect of compound (1) on apoptosis induction

In order to maintain that compound (1) does not induce cell death by apoptosis, the HCT-116 cells treated for 24 hours were analysed to evaluate the activity of caspases 3 and 7 and the modification of mitochondrial transmembrane potential.

The activity of caspases 3 and 7, marker enzymes of apoptosis, was measured using the Apo-ONE (Promega) test after treating HCT-116 cells with compound (1) at concentrations varying from 5 nM to 75 nM. The results presented in Figure 6a demonstrate the lack of variations in the activity of these enzymes following the treatment with the molecule under test.

The study of the mitochondrial transmembrane potential, $\Delta\psi_m$, was conducted by flow cytometry, using the JC-1 probe (MitoProbe™ JC-1 assay, Molecular Probes). JC-1 is a lipophilic anionic compound which selectively penetrates the mitochondria, the colour whereof changes from green to red when the transmembrane potential increases. Unlike healthy cells, cells in apoptosis exhibit a low $\Delta\psi_m$ which is associated with a strong green colour of the JC-1 probe.

The results presented in Figure 6b demonstrate that treating HCT-116 cells with compound (1) at concentrations varying from 25 nM to 100 nM does not modify $\Delta\psi_m$ significantly. All these results indicate that compound (1) does not induce the caspase-dependent apoptotic pathway (type I programmed cell death).

5. Study of autophagy

In the aim of determining whether the stoppage of the cell cycle induced by compound (1) induces an autophagic reaction, the HCT-116 cells were treated with compound (1) at different doses for 24 hours. The cell extracts then underwent Western Blot analysis in order to view the expression of the protein LC3-II. Indeed, the protein LC3 exists in the cell either in cytosolic form (LC3-I) or associated with phagosomes

(LC3-II). The latter is the marker of the autophagic reaction.

The results presented in Figure 7 clearly show an increase in the expression of LC3-II in the presence of compound (1) used at concentrations varying from 5 nM to 100 nm.

6. Study of the mode of action of compound (1)

In view of the stoppage of the cell cycle in the G0/G1 phase associated with an autophagic response in the absence of apoptosis following treatment with compound (1), we studied the action of this compound on the survival pathway involving PI3 kinase - Akt - Foxo. This signalling pathway is very frequently found to be disrupted (by stimulation) in a large number of cancer cells and contributes to the survival thereof.

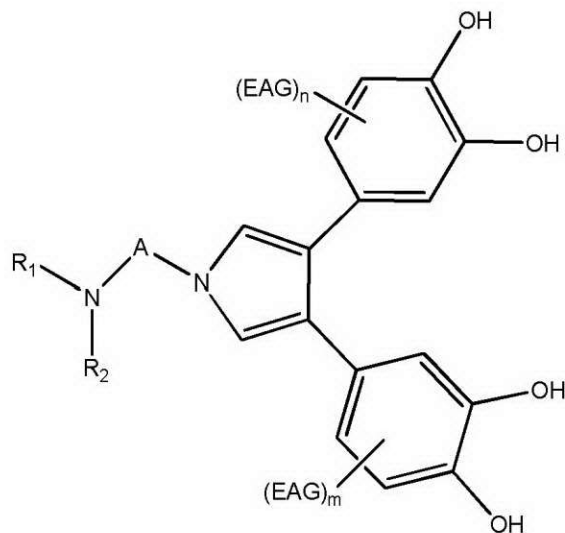
The results presented in Figure 8 demonstrate that compound (1) blocks this survival pathway. Indeed, HCT-116 cells were exposed for 24 hours to the action of compound (1) at concentrations varying from 5 nM to 100 nm. The Western Blot analysis of the cell extracts revealed that compound (1), from the 25 nm concentration, totally suppresses Akt protein serine 473 phosphorylation (Figure 8a). It stimulates the expression of the protein FOXO3 (Figure 8b), the synthesis whereof is reduced by the Akt-phosphorylated protein. It stimulates the expression of the protein peroxiredoxin 1, the gene whereof is described as being under the control of the FOXO3 factor (Figure 8c).

The protein peroxiredoxin is an enzyme having a peroxidase activity, controlling the intracellular hydrogen peroxide (H_2O_2) concentration. Therefore, we

evaluated the effect of compound (1) on the intracellular H_2O_2 concentration, using DCF-DA (2',7'-dichlorofluorescein diacetate, Sigma) as a marker of intracellular H_2O_2 . Hydrogen peroxide is an intracellular second messenger. It regulates, according to the concentration thereof, numerous functions such as proliferation or apoptosis. A reduction of the concentration thereof is associated with a decrease in proliferation. The flow cytometry analysis results of HCT-116 cells treated with compound (1) at the 50 nM concentration demonstrate a decrease by approximately 50% of the intracellular H_2O_2 concentration after 18 hours of treatment (Figure 9a). The effect observed is dose dependent (Figure 9b).

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS

1. A compound having the following formula (I):



(I)

5 wherein:

- m is an integer from 0 to 3;
- n is an integer from 0 to 3;
- $m + n \geq 1$;

10 - EAG is an electro-attractive group selected from the group consisting of a halogen atom, NO₂, CF₃, CCl₃, CN, CO₂H, (C=O)NR₂, CH=NR, (C=S)OR, (C=O)SR, CS₂R, SO₂R, SO₂NR₂, SO₃R, P(O)(OR)₂, P(O)(R)₂, and B(OR)₂, where R is an alkyl (C₁-C₆) radical, a phenyl group or a hydrogen atom;

15 - A is a saturated or unsaturated, linear or branched hydrocarbon chain comprising 1 to 10 atoms of carbon; and

20 - R₁ and R₂ each represent, independently from one another, a hydrogen atom, a CO-(C₁-C₆)-alkyl, (C₁-C₆) alkyl, phenyl or phenyl-(C₁-C₆)-alkyl group, or R₁ and R₂ form, together with the nitrogen atom bearing same,

a 5- to 15-member heterocycle, optionally substituted by a (C₁-C₆) alkyl group;

or the stereoisomers and the mixtures thereof, or a pharmaceutically acceptable acid salt thereof.

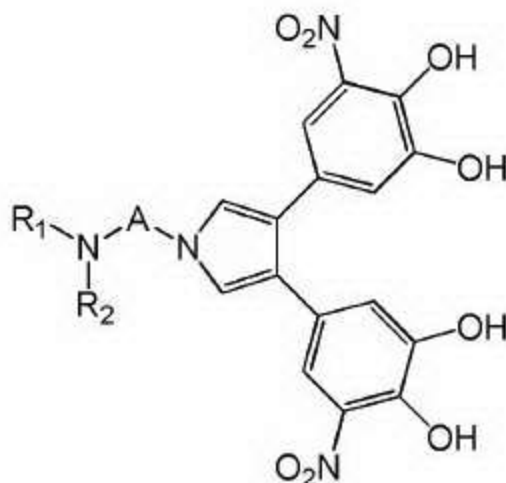
2. The compound according to claim 1, wherein:

m is an integer from 0 to 2;

n is an integer from 0 to 2; and

$m + n \geq 1$.

3. The compound according to claim 1 or claim 2, having the following formula (Ia):



(Ia)

wherein the radicals A, R₁ and R₂ have the meanings given in claim 1, or the stereoisomers and the mixtures thereof, or a pharmaceutically acceptable acid salt thereof.

4. The compound according to any one of claims 1 to 3, wherein the radicals R₁ and R₂ form, together with the nitrogen atom bearing same, a heterocycle which is saturated and does not comprise any other heteroatom than the nitrogen atom bearing R₁ and R₂.

5. The compound according to any one of claims 1 to 3, wherein the radicals R_1 and R_2 form, together with the nitrogen atom bearing same, a 10- to 15-member heterocycle.

5

6. The compound according to claim 5, wherein the radicals R_1 and R_2 form, together with the nitrogen atom bearing same, a 13-member heterocycle.

10

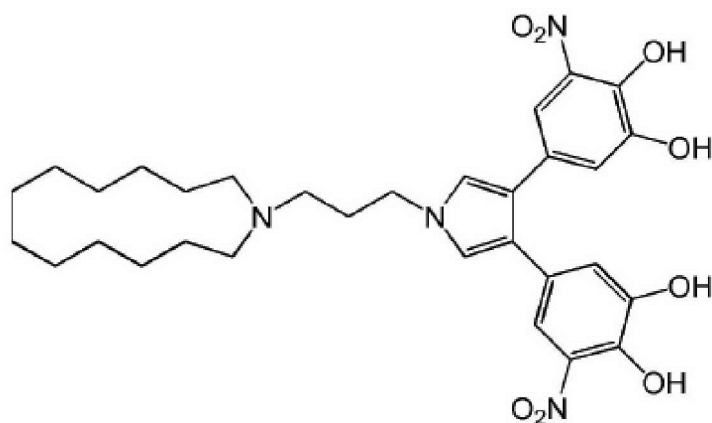
7. The compound according to any one of claims 1 to 6, wherein A is a saturated linear hydrocarbon chain comprising 3 to 6 carbon atoms.

15

8. The compound according to claim 7, wherein A is a saturated linear hydrocarbon chain comprising 3 carbon atoms.

20

9. The compound according to any one of claims 1 to 8, corresponding to the compound having the following formula (1):



(1)

or a pharmaceutically acceptable acid salt thereof.

10. The compound according to any one of claims 1 to 9, wherein the pharmaceutically acceptable acid salt is a hydrochloride.

5 11. A pharmaceutical composition comprising at least one compound according to any one of claims 1 to 10, and at least one pharmaceutically acceptable excipient.

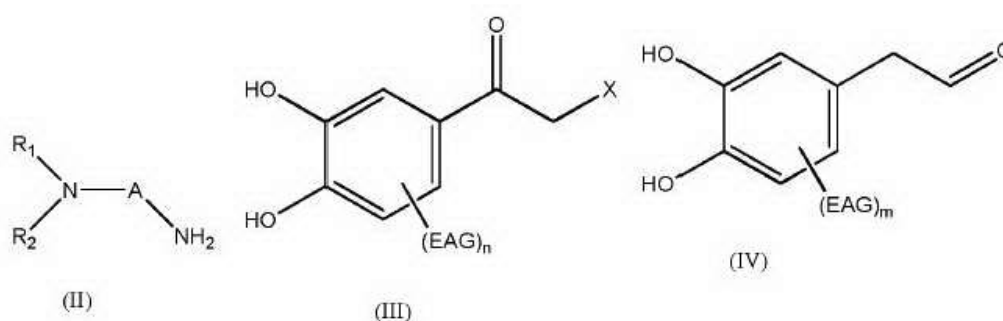
10 12. A method for treating cancer, said method comprising administering to a patient suffering from cancer an effective amount of a compound according to any one of claims 1 to 10, wherein said cancer is lung, breast, colon, rectal, kidney, skin, or bone cancer, or haematopoietic cancers, or tumours of neuroectodermal origin.

15 13. The method according to claim 12, wherein said haematopoietic cancer is leukaemia, myeloid leukaemia, lymphoma or bone marrow cancer.

20 14. The method according to claim 12, wherein said tumour of neuroectodermal origin is glioblastoma.

25 15. A process for preparing a compound having formula (I) according to claim 1, comprising:

a) reaction between an amine having the following formula (II), a halide having the following formula (III), and an aldehyde having the following formula (IV):



wherein n and m, EAG, R₁, R₂ and A are as defined in claim 1 and X represents a halogen atom, to arrive at a compound having formula (I) according to claim 1.

16. The process according to claim 15, further comprising:

b) formation of a pharmaceutically acceptable salt from the compound having formula (I) obtained in the previous step (a).

17. The process for preparing a compound having formula (I) according to claim 15 or claim 16, wherein the halide (III) and the amine (II) are first allowed to react with one another before adding the aldehyde (IV).

18. The process for preparing a compound having formula (I) according to any one of claims 15 to 17, wherein X is a chlorine, bromine or iodine atom.

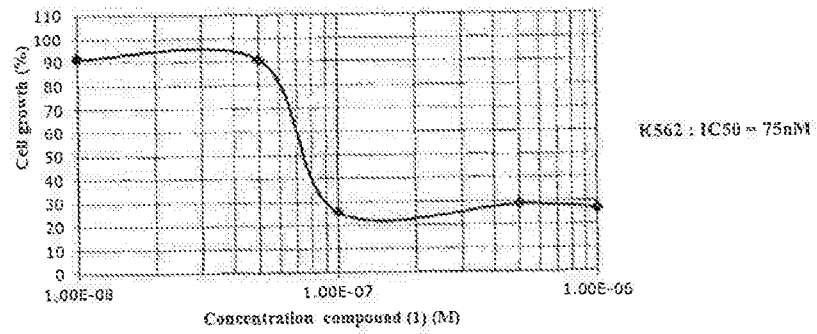
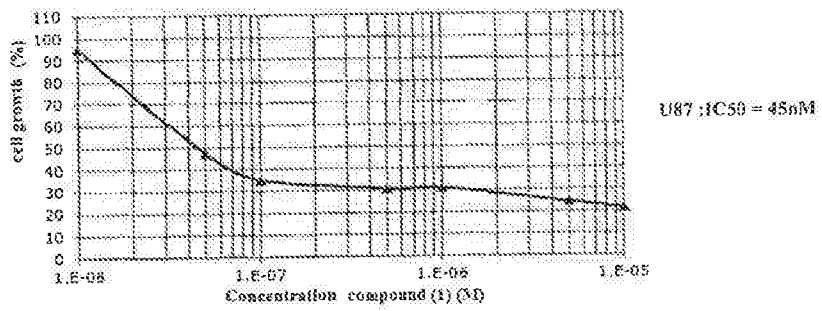
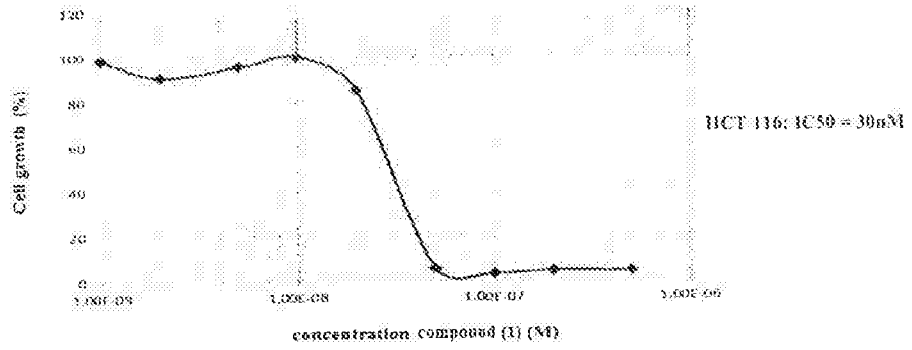


FIGURE 1

	24hrs	48hrs	72hrs
10nM	-12%	-18%	-20%
25nM	-40%	-52%	-50%
50nM	-44%	-53%	-91%
100nM	-44%	-53%	-92%
200nM	-45%	-56%	-94%

FIGURE 2a

	24hrs	48hrs	72hrs
	% viability	% viability	% viability
C	98	99	99
10nM	98	99	99
25nM	98	98	99
50nM	97	96	95
100nM	97	97	95
200nM	98	94	95

FIGURE 2b

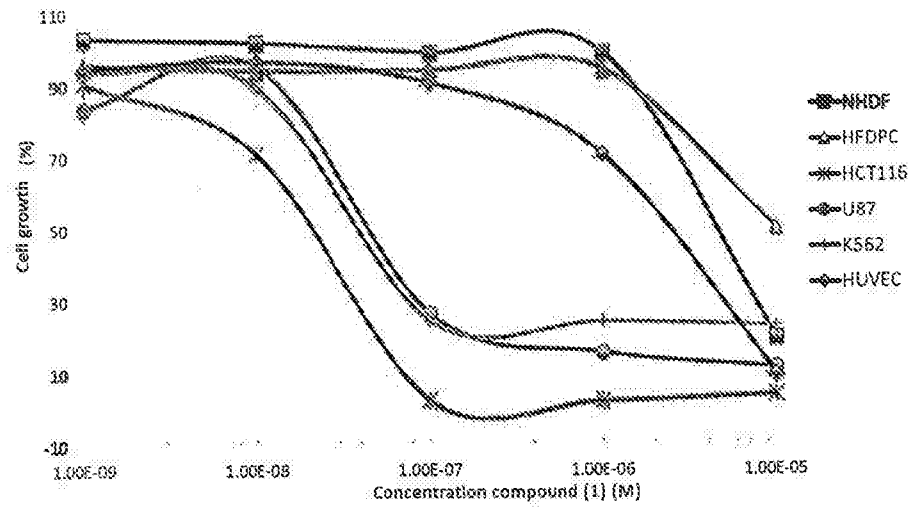


FIGURE 3

	G0/G1 %	S %	G2/M %
NT1	27	47	26
NT2	26	43	31
NT: non-treated			
10 nM	28	47	25
30 nM	42	40	18
50nM	64	21	15

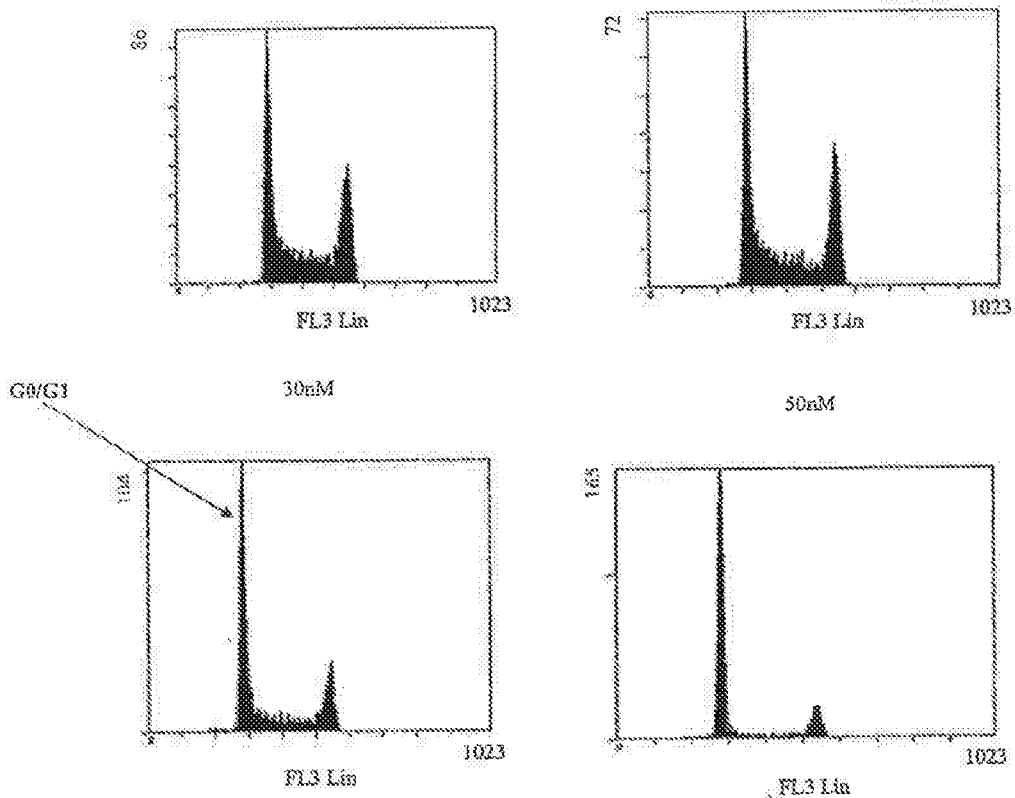


FIGURE 4

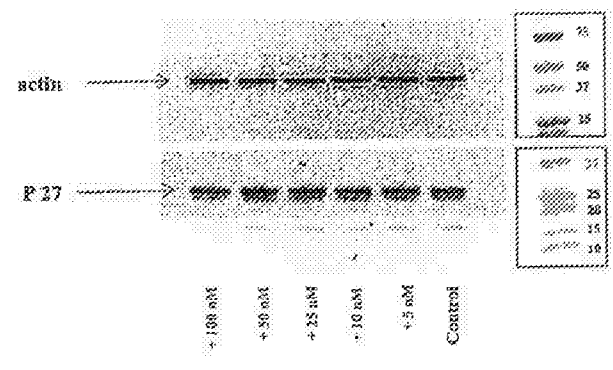
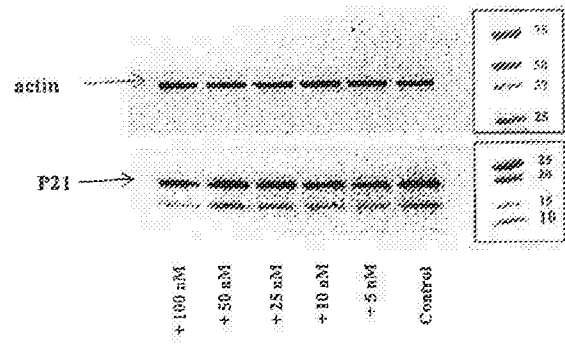


FIGURE 5

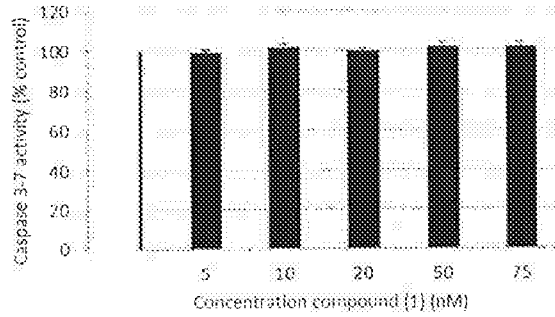


FIGURE 6a

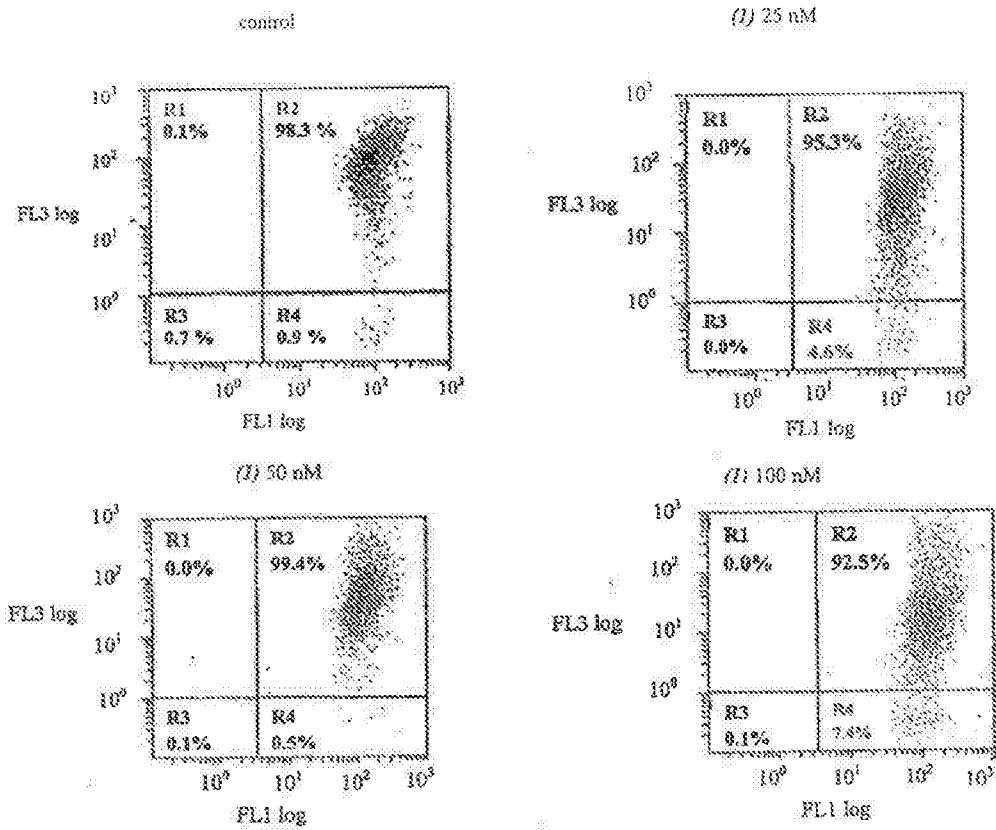


FIGURE 6b

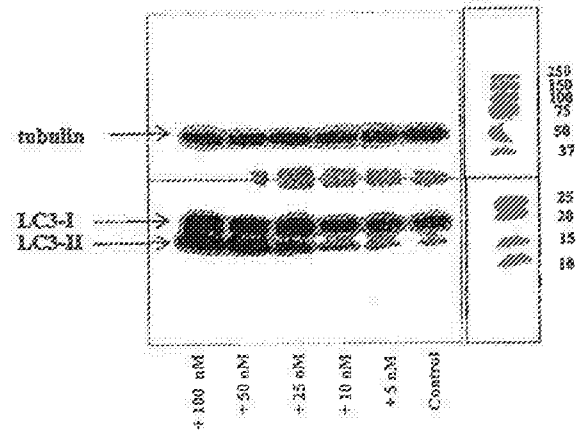


FIGURE 7

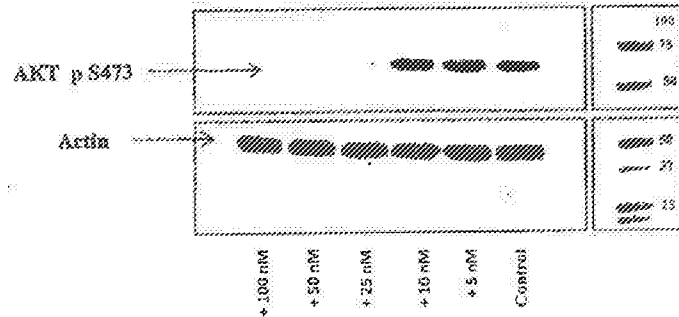


FIGURE 8a

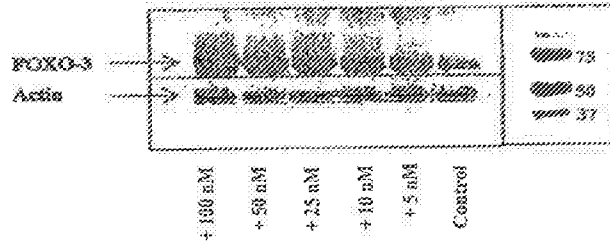


FIGURE 8b

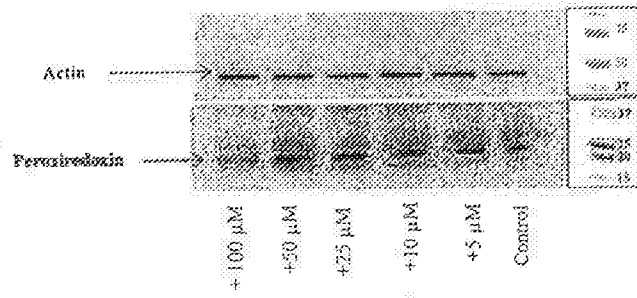


FIGURE 8c

Overlap diagram 1

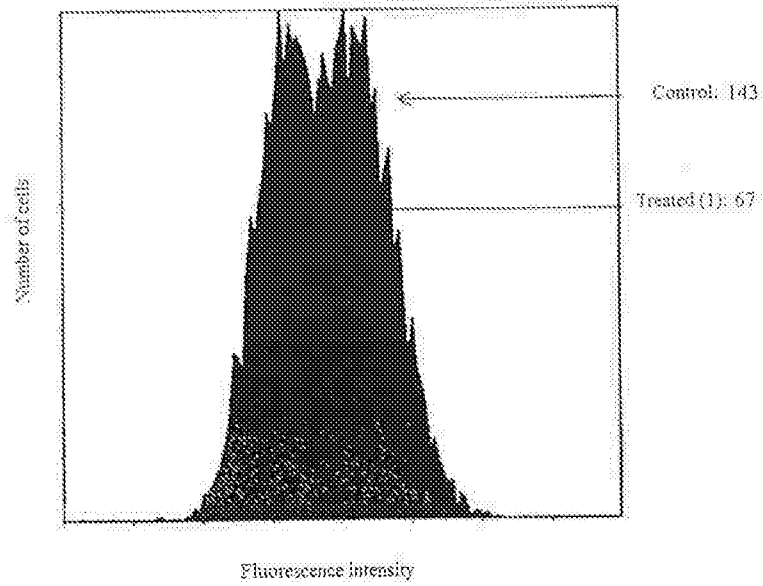


FIGURE 9a

DCF (H₂O₂), HCT, 18h

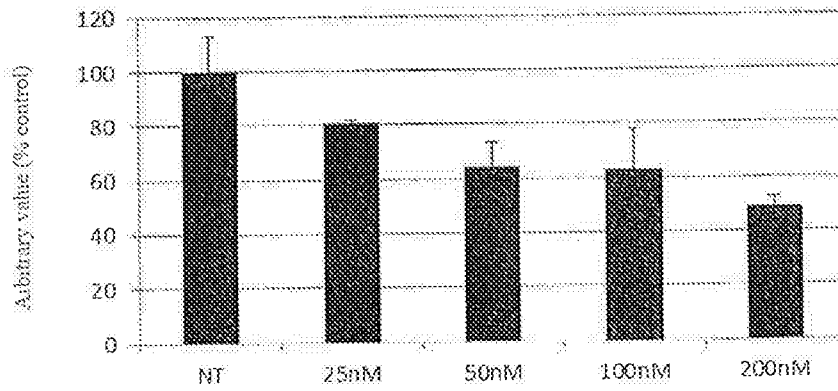


FIGURE 9b