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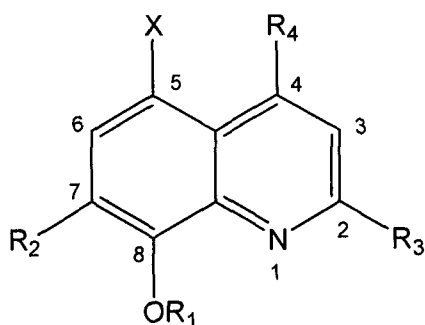
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(54) Title: QUINOLINE DERIVATIVES



(A)

(57) Abstract: The invention relates to new quinoline derivatives which are active CLK-1 inhibitors. More specifically, the CLK-1 inhibitors of the invention are compounds of formula (A). The invention also relates to pharmaceutical compositions comprising such compounds and to methods for the prophylaxis and/or treatment of disorders or their associated symptoms for which the inhibition of CLK-1 is beneficial.

QUINOLINE DERIVATIVES

FIELD OF THE INVENTION

The present invention relates to new quinoline derivatives as CLK-1 inhibitors. More specifically, the present invention relates to new quinoline derivatives or their pharmaceutically acceptable salts or pro-drugs as CLK-1 inhibitors, and to pharmaceutical compositions comprising the same and to methods for the prophylaxis and/or treatment of disorders or its associated symptoms for which the inhibition of CLK-1 is beneficial.

BACKGROUND OF THE INVENTION

Role of *clk-1* and CLK-1

The *clk-1* gene encodes a 21 kDa mitochondrial protein which is conserved amongst eukaryotes with sequence identity of 85% between mouse and human sequence and 50% with the homologous sequence of the nematode worm *Caenorhabditis elegans*. CLK-1 participates in one of the last steps of ubiquinone biosynthesis and is responsible for demethoxyubiquinone (DMQ) hydroxylation. Mutants in which CLK-1 is inactive do not synthesize detectable amounts of ubiquinone (Q), and rather accumulate DMQ (Miyadera *et al.* 2001).

Q has numerous functions in the cell. It is involved in electron transport in the inner mitochondrial membrane, the plasma membrane, and the membrane of the lysosome. It is also a cofactor for several cellular enzymes (e.g. dihydroorotate dehydrogenase, which is necessary for nucleic acid biosynthesis) and other proteins and complexes such as the mitochondrial uncoupling proteins (UCP) which regulate metabolic heat production and the mitochondrial permeability transition pore (MPTP) which regulates programmed cell death.

DMQ is a quinone that can transport electrons, although less efficiently than ubiquinone. Mouse embryos in which the *clk-1* homologue (*mclk-1*) has been

knocked out also accumulate DMQ, and are not viable (Levavasseur *et al.* 2001) but mice heterozygous for *mclk-1* display an increased lifespan phenotype. Transgenic expression of CLK-1 in the mouse knock-out rescues the lethality phenotype, and allows ubiquinone synthesis (Nakai *et al.* 2004). Interestingly, heterologous expression of *C. elegans* CLK-1 in slow-growing *coq7* (yeast *clk-1*) yeast mutants is functional, and rescues yeast slow-growth phenotype (Ewbank *et al.* 1997). Altogether, these observations indicate that CLK-1 is the only DMQ hydroxylase in the studied organisms, and that there are no functional compensatory mechanisms for CLK-1 activities.

In *C. elegans*, reduction of CLK-1 activity is also translated in an increase of lifespan. Lifespan is a cumulative phenotype that integrates variations in worm physiology, and that is sensitive to environmental cues, and to genetic mutations. As such, conditions that extend lifespan are often considered as beneficial. For example, a favourable repair vs. damage balance would manifest itself by increasing lifespan (Hekimi *et al.* 2001).

Age-related disorders

Age-related diseases refer to diseases for which the incidence and/or prevalence increase with age. These diseases include by example, cancers, cerebrovascular disease (stroke), neurodegenerative diseases, pneumonia, respiratory diseases, arthritis, heart diseases, diabetes, hearing impairment, vision impairment and kidney disease.

CLK-1 activity and age-related disorders

Inhibition of CLK-1 activity has similar lifespan-lengthening effects in mice and in nematodes. This strongly shows a causal link between CLK-1 biological activity and the physiological aging process. It is generally accepted that physiological aging is a risk factor for numerous diseases, the so-called age-dependent diseases. Many of these diseases are lethal and limit lifespan. It has been shown that when the level of mouse CLK1 (mCLK1) protein is reduced by half in *mclk1*

+/- heterozygotes (Levavasseur *et al.*, 2001), it increases the mean, median and the maximum lifespan of these animals in three different experiments. Each experiment was carried out in a different genetic background (129SV/j, C57BL/6 and 129SV/j x Balb/c) (Liu *et al.*, 2005). Animals in these backgrounds are known to die from a variety of age-dependent diseases, whose pattern is different in each background (Blackwell *et al.*, 1995; Cosgrove *et al.*, 1978; Frith *et al.*, 1983; Smith *et al.*, 1973). In all three experiments most of the wild type animals and the mutant animals die within a relatively short period of time, typical of the genotype (*mclk1* +/+ or *mclk1* +/-) (Liu *et al.*, 2005). The fact that *mclk1* reduction acts in different genetic background, and that maximum and median lifespans are increased indicates that all or most causes of lethality must have been partially suppressed by this reduction. Indeed, when a single cause of lifespan is affected by a genetic manipulation, such as cancer, lifespan lengthens only minimally or not at all (Matheu *et al.*, 2004; Miller, 2005). In contrast, manipulations, such as caloric restriction and reduction of growth hormone signaling, which are known to slow down physiological aging, reduce the incidence of age-dependent diseases (Bartke, 2005; Berrigan *et al.*, 2002; Hursting *et al.*, 2004). Moreover, animals in which the aging process has been accelerated demonstrate increase prevalence and early onset of disease (Fenton *et al.*, 2004; Takeda *et al.*, 1997). These observations, together with the evolutionary conservation of the effect of *clk-1/mclk1* on lifespan (Liu *et al.*, 2005), indicate that reducing mCLK1 activity slows down physiological aging, and therefore that inhibition of CLK-1 can delay the onset and severity of age-dependent diseases. It is well recognized that slowing down physiological aging is the best way to reduce the incidence and severity of a variety of diseases (Finkel, 2005; Miller, 2005).

Thus, inhibiting CLK-1 by using chemicals that are useful as CLK-1 inhibitors leads to the prevention or reduction of age-related diseases.

Quinoline derivatives

Compounds of the type of quinoline derivatives are disclosed in **CA 2.493.536 A1**, **US 2006/0074104 A1** and **WO 2004/087160 A1** for use in the treatment, amelioration and/or prophylaxis of neurological conditions in particular those associated with or facilitated by oxidative stress. The neurological disorders contemplated include any condition leading to cognitive impairment such as pre- or mild cognitive impairment or memory loss.

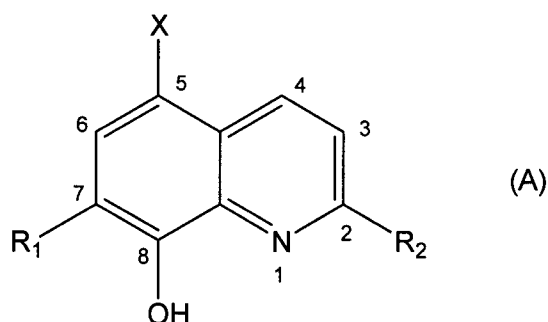
Although the above mentioned quinoline derivatives have been shown to be useful oxidative stress regulators, they are described as exerting their therapeutic effects principally through metal chelation. There is no prior art reference known to date showing that similar compounds could be used to prevent and/or treat age-related disorders for which the inhibition of CLK- is beneficial.

In this context, the present inventors have developed a new family of compounds which are active to inhibit CLK-1. Such compounds are active on age-related disorders as well as on ischemia-reperfusion injury and inflammation disorders.

SUMMARY

Therefore, an object of the present invention is to provide new quinoline derivatives of formula A, which have CLK-1 inhibitory activity.

- 5 More specifically, the present invention relates to the compounds of formula A as defined below, or their pharmaceutically acceptable salts or their pro-drugs:

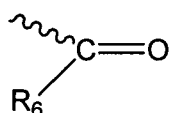


- 10 wherein X = H, methyl or halogen and the substituents R₁ and R₂ are defined as follows:

- a) when R₂ is hydrogen, then R₁ is H or halogen, or R₁ is selected from the group consisting of: amino(3,4-dimethoxyphenyl)methyl, 2-hydroxyphenyl, 3-hydroxyphenyl, 4-hydroxyphenyl, 4-dimethylaminophenyl, pyridin-4-yl, 2-methoxypyridin-3-yl and 2-acetamidophenyl;
- 15

or R₁ is -CH(R₃)NR₄R₅ with

- R₄ = H or C₁-C₄ alkyl;

- R₅ = H or 

- 20 and R₆ is selected from the group consisting of: C₁-C₆ alkyl, C₅-C₆ aryl, benzyl, dimethylaminomethyl, phenylethyl and diphenylmethyl; and

- R₃ is selected from the group consisting of:

- H, methyl, phenyl, benzyl, 2-chlorophenyl, 2-methoxyphenyl, 4-chlorophenyl, 4-methylphenyl, 4-isopropylphenyl, 3-hydroxyphenyl, 4-hydroxyphenyl, 4-methoxyphenyl, 4-cyanophenyl, 4-dimethylamino-phenyl,
- 25

4-diethylamino-phenyl, 2,4-dichlorophenyl, 3,4-dimethoxyphenyl, 4-methoxycarbonylphenyl, 3-methoxycarbonylphenyl, N-methylbenzamido, N-(3-methoxypropyl)benzamido, N,N-dimethylbenzamido, 4-(morpholin-4-ylcarbonyl)phenyl, 4-(pyridin-2-yl)phenyl, 4-(1H-pyrazol-1-yl)phenyl, pyridin-2-yl, pyridin-3-yl, pyridin-4-yl, 6-(morpholin-4-yl)pyridin-3-yl, 2-furyl, 3-furyl, 2-(morpholin-4-yl)pyridin-3-yl, 2-methoxypyridin-3-yl, 4-methoxypyridin-3-yl, 2-thienyl, 2-butyl-1H-imidazol-4-yl, quinolin-3-yl, quinolin-4-yl, 8-hydroxyquinolin-2-yl, 1H-indol-3-yl, 1H-indol-4-yl and 1H-indol-7-yl;

or R_1 is $\text{---}\overset{\text{H}}{\underset{\text{O}}{\underset{\parallel}{\text{C}}}}\text{---}\text{N---}R_7$ and R_7 is $\text{---}(\text{CH}_2)_n\text{---}R_8$ where R_8 is a $\text{C}_5\text{---}\text{C}_6$ aryl optionally substituted by one or two methoxyl groups, and $n = 0$ or 1 ; or

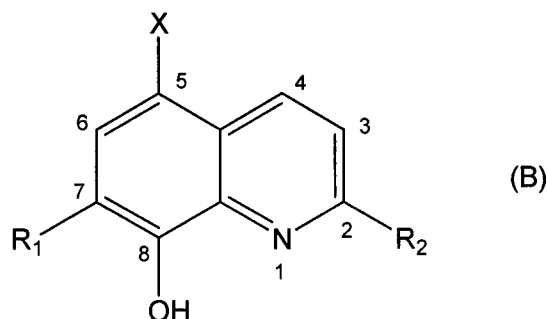
b) when X and R_1 both represent a hydrogen atom, then R_2 is selected from the group consisting of: pyridin-2-ylcarboxamido, pyridin-2-ylacetamido, 3-hydroxyphenylacetamido, 4-hydroxyphenylacetamido, ((2-methoxyphenyl)amino)methyl, ((3-methoxyphenyl)amino)methyl, ((3-methoxybenzyl)amino)methyl, 2-thienylacetamido and ((2-thienylmethyl)amino)methyl;

with the proviso that each of the compounds of formula A is not one of the compounds identified in Annex 1.

The compounds of Formula A of the invention when comprising at least one asymmetric centre are either in the form of one of their optically active isomers like enantiomers or a mixture thereof including for example the racemate.

The invention also relates to a method for inhibiting CLK-1 activity in a cell which comprises the following steps:

- a) providing a cell wherein CLK-1 activity needs to be inhibited,
- b) contacting the cell with a compound of formula (B) as defined below, or a
- 5 pharmaceutically acceptable salts or a pro-drug thereof:



wherein X, R₁ and R₂ have the same definition than previously mentioned;

- 10 the compound of formula (B), when comprising at least one asymmetric centre is in the form of one of its optically active isomer or a mixture thereof; and
- c) determining the CLK-1 activity in the cell.

The invention also relates to a method for the prophylaxis and/or treatment of a disorder or its associated symptoms for which inhibition of CLK-1 is beneficial, in

15 animal, which comprises the following steps:

- a) identifying an animal having a disorder for which inhibition of CLK-1 is beneficial; and
- b) administering to the animal a compound of formula (B), a
- pharmaceutically acceptable salts or a pro-drug thereof.

20

The invention also relates to a pharmaceutical composition comprising a compound of formula A of the invention, a pharmaceutically acceptable salt or a pro-drug thereof, and at least one pharmaceutically acceptable carrier.

- 25 The invention further relates to a pharmaceutical composition comprising a pharmaceutically effective amount of a compound of formula A or B as defined hereinabove, a pharmaceutically acceptable salt or a pro-drug thereof, and at

least one pharmaceutically acceptable carrier, to effectively reduce and/or inhibit totally or partially CLK-1 activity.

BRIEF DESCRIPTION OF THE FIGURES

5 **Figure 1:** HPLC profiles showing the induction of DMQ in quinone profiles after treatment with a CLK-1 inhibitor. Mouse macrophages (RAW264.7) were treated with a) an inactive compound or b) the active compound 135 (1 μ M) for 24hr, followed by cell lysis and hexane/ethanol extraction of quinones. Samples were run with methanol/ethanol gradients on HPLC with UV detection to determine
10 levels of ubiquinone (Q) and its precursor, demethoxyubiquinone (DMQ).

Figure 2: HPLC profiles showing relative quinone peaks in RAW264.7 cells treated with a) DMSO or b) 10 μ M of Compound 7 (Q: Ubiquinone, DMQ: Demethoxyubiquinone).
15

Figure 3: HPLC profiles showing relative quinone peaks in RAW264.7 cells treated with a) 1 μ M or b) 3 μ M of Compound 23 (Q: Ubiquinone, DMQ: Demethoxyubiquinone).

20 **Figure 4:** HPLC profiles showing relative quinone peaks in RAW264.7 cells treated with a) 5 μ M or b) 10 μ M of Compound 69 (Q: Ubiquinone, DMQ: Demethoxyubiquinone).

Figure 5: HPLC profiles showing relative quinone peaks in RAW264.7 cells
25 treated with a) 5 μ M or b) 10 μ M of Compound 47 (Q: Ubiquinone, DMQ: Demethoxyubiquinone).

Figure 6: HPLC profiles showing relative quinone peaks in RAW264.7 cells treated with a) 5 μ M or b) 10 μ M of Compound 53 (Q: Ubiquinone, DMQ: Demethoxyubiquinone).
30

Figure 7: Specificity of CLK-1 inhibitor. Effect of Compound 7 on a) mouse CLK-1 (JF496-*mclk1* testing system), and b) the bacterial DMQ hydroxylase UbiF (JF496-*ubiF* testing system). JF496 bacteria expressing a) mouse CLK-1 and b) the bacterial DMQ-hydroxylase UbiF were treated with Compound 7 for 5h. Quinones were extracted and analyzed using HPLC. The HPLC trace shows that CLK-1 activity is inhibited by 7, as ubiquinone (Q) synthesis is lowered with a concomitant increase of demethoxyubiquinone (DMQ). In contrast to that, UbiF activity is not affected, and Q is the major quinone species detected. This shows that Compound 7 has a specific CLK-1 inhibition activity.

Figure 8: Specificity of CLK-1 inhibitor. Effect of Compound 52 on a) mouse CLK-1 (JF496-*mclk1* testing system), and b) the bacterial DMQ hydroxylase UbiF (JF496-*ubiF* testing system). JF496 bacteria expressing a) mouse CLK-1 and b) the bacterial DMQ-hydroxylase UbiF were treated with Compound 52 for 5hrs. Quinones were extracted and analyzed using HPLC. The HPLC trace shows that CLK-1 activity is inhibited by 52, as ubiquinone (Q) synthesis is lowered with a concomitant increase of demethoxyubiquinone (DMQ). In contrast to that, UbiF activity is not affected, and Q is the major quinone species detected. This shows that Compound 52 has a CLK-1-specific inhibition activity.

Figure 9: ROS lowering effect of CLK-1 inhibitors (7 and 135). (A) table showing quinone profiles for CLK-1 inhibitor in mouse macrophage cells; (B) cells pre-incubated with CLK-1 inhibitors were treated with the fluorescence marker DCF, which produces green fluorescence when oxidized by cellular ROS. H₂O₂ is used to induce ROS in cells, and the reduction observed with CLK-1 inhibitors is shown as percentage relative to control; and (C) COMET assay whereby fluorescent tails of DNA leaking from cell nuclei are used to measure DNA damage induced by ROS. Again H₂O₂ was used to induce ROS, and data are expressed as percentage of cells with tails compared to control. The reduction of cells showing comets (% of total cells) in the presence of CLK-1 inhibitors is indicated.

Figure 10: Effects of Compounds 7 or 52 on serum creatinine levels in a rat model of ischemia-reperfusion.

Figure 11: Effects of Compounds 7 or 52 on Blood Urea Nitrogen (BUN) levels in
5 a rat model of ischemia-reperfusion.

Figure 12: Effects of Compounds 7 or 52 on creatinine clearance in a rat model of ischemia-reperfusion.

10 **Figure 13:** Effects of Compounds 7 or 52 on urine protein concentration (g/L) in a rat model of ischemia reperfusion.

Figure 14: Effects of Compound 7 on lipoxxygenase (LPO) levels in rats with lipopolysaccharide (LPS) induced lung injury.

15 **Figure 15:** Effects of Compound 7 on protein contents in rats with LPS induced lung injury.

Figure 16: Effects of Compound 7 on total cells and neutrophils levels in rats with
20 LPS induced lung injury.

Figure 17: Effects of Compound 7 on TNF- α levels in rats with LPS induced lung injury.

25 **Figure 18:** Effects of a treatment with Compound 138 in various dosages on soluble A β 1-40 determined by ELISA in hAPP751SL transgenic mice in the TBS fraction.

Figure 19: Effects of a treatment with Compound 138 in various dosages on
30 soluble A β 1-42 determined by ELISA in hAPP751SL transgenic mice in the TBS fraction.

Figure 20: Effects of a treatment with Compound **138** in various dosages on soluble A β 1-40 determined by ELISA in hAPP751SL transgenic mice in the Triton X-100 fraction.

- 5 **Figure 21:** Effects of a treatment with Compound **138** in various dosages on soluble A β 1-42 determined by ELISA in hAPP751SL transgenic mice in the Triton X-100 fraction.

- 10 **Figure 22:** Effects of a treatment with Compound **138** in various dosages on bound A β 1-40 determined by ELISA in hAPP751SL transgenic mice in the FA fraction.

- 15 **Figure 23:** Effects of a treatment with Compound **138** in various dosages on bound A β 1-42 determined by ELISA in hAPP751SL transgenic mice in the FA fraction.

DETAILED DESCRIPTION OF THE INVENTION

- 20 The present invention has yielded the unexpected discovery of a new class of compounds consisting of quinoline derivatives of formula A defined below which present CLK-1 inhibitory activity.

Definitions

- 25 As used herein, a **pharmaceutically acceptable salt** is meant to include any salt of the compounds of the invention that are suitable for use in contact with the tissues of human and lower animals without undue toxicity, irritation, or allergic response and are commensurate with a reasonable risk/benefit ratio. Pharmaceutically acceptable salts are well known in the art (Pharmaceutical Salts
30 Properties, Selection, and Use, Stahl, P. Heinrich / Wermuth, Camille G. (eds.), 2002. Monograph - ISBN 3-906390-26-8 - Verlag Helvetica Chimica Acta, Zürich). The salts can be prepared *in situ* during the final isolation and purification

of the compounds of the invention, or separately, by reacting a free basic function of the compounds of the present invention with a suitable acid or by reacting a free acidic function of the compounds of the invention with a suitable base such as, but not limited to, hydroxide, carbonate or bicarbonate of a pharmacologically acceptable metal cation. Pharmaceutically acceptable acid addition salts include, but are not limited to, acetate, adipate, alginate, citrate, aspartate, benzoate, benzenesulfonate, butyrate, camphorate, camphorsulfonate, digluconate, glycerophosphate, hemisulfonate, heptanoate, hexanoate, fumarate, hydrochloride, hydrobromide, hydroiodide, 2-hydroxyethanesulfonate, lactate, maleate, methanesulfonate, nicotinate, 2-naphtalenesulfonate, oxalate, pramoate, pectinate, persulfate, 3-phenylpropionate, picrate, pivalate, propionate, succinate, tartrate, thiocyanate, nitrate, sulfate, bisulfate, phosphate, acid phosphate salts, glutamate, bicarbonate, p-toluensulfonate and undecanoate. Also the basic nitrogen-containing groups can be quaternized with such agents as alkyl halides such as methyl, ethyl, propyl, and butyl chlorides, bromides and iodides; dialkyl sulfates such as dimethyl, diethyl, dibutyl and diamyl sulfates; long chain halides such as decyl, lauryl, myristyl, and stearyl chlorides, bromides and iodides; and arylalkyl halides such as benzyl and phenylethyl bromides. Pharmaceutically acceptable salts also include, but are not limited to, cations based on alkali metals or alkaline earth metals such as aluminum, calcium, lithium, magnesium, potassium, sodium salts and the like.

As used herein, a **pro-drug** is a compound that, upon *in vivo* administration, is metabolized or otherwise converted to the biologically, pharmaceutically or therapeutically active form of the parent compound, for example, by hydrolysis in blood. To produce a pro-drug, the pharmaceutically active compound is modified such that the active compound will be regenerated by metabolic processes. The pro-drug may be designed to alter the metabolic stability or the transport characteristics of a drug (including improvement of bioavailability), to mask side effects or toxicity, to improve the flavor of a drug or to alter other characteristics or properties of a drug. By virtue of knowledge of pharmacodynamic processes and drug metabolism *in vivo*, those of skill in this art, once a pharmaceutically active compound is known, could design pro-drugs of the compound.

The present invention also contemplates metabolites formed by *in vivo* transformation of compounds having formula A or B. The term metabolite refers to compounds formed by *in vivo* biotransformation of compounds having formula A or B by oxidation, reduction, hydrolysis, or conjugation.

5

As used herein, the term **alkyl** refers to the saturated aliphatic groups, including straight-chain alkyl groups, branched-chain alkyl groups, and cycloalkyl (alicyclic) groups.

10 The term **aryl** as used herein includes 5- and 6-membered single-ring aromatic groups that may include from zero to four heteroatoms (S, N, O), for example, unsubstituted or substituted benzene, pyrrole, furan, thiophene, imidazole, oxazole, thiazole, triazole, pyrazole, pyridine, pyrazine, pyridazine and pyrimidine, and the like.

15

As used herein, the term **halogen** designates F, Cl, Br or I.

As used herein, the term **optically active isomer** designates any isomeric form of a claimed compound such as but not limited to an enantiomer, a
20 diastereoisomer, a racemate or a mixture thereof.

By **pharmaceutically acceptable carrier**, it is meant a carrier medium which does not interfere with the effectiveness of the biological activity of the active ingredients of the composition, namely the compound of formula A, and which is
25 not toxic to the host or patient. Furthermore, the carrier is advantageously a compound with minimum probability of being rejected by the immune system of the subject being treated. Suitable carriers are of common knowledge to one skilled in the art and will not be further detailed.

30 As used herein, the term **treatment** refers to a process by which a disorder or its associated symptoms, for which inhibition of CLK-1 is beneficial, are alleviated or completely eliminated. For instance, one may understand that such treatment of

the disorder or its associated symptoms may be alleviated or completely eliminated by, for instance, giving a compound of the present invention in appropriate formulation at appropriate doses to patients with high risk of/early stages of disease.

5

As used herein, the term **prophylaxis** refers to a process by which a disorder, or its associated symptoms, including but not limited to an age-related disorder, is obstructed, delayed or prevented.

10 As used herein, the expression **a disorder for which inhibition of CLK-1 is beneficial** refers to a disorder or its associated symptoms for which inhibition of CLK-1 is known or anticipated to be beneficial. More particularly, this expression refers to a disorder or its associated symptoms is alleviated or eliminated upon inhibition of CLK-1. Even more particularly, this expression refers to, but is not
15 limited to, inflammation disorders, disorders caused by or exacerbated by oxidative stress and/or free radical-induced damage, including ROS mediated diseases, such as hypoxia / reoxygenation injury and ischemia / reperfusion injury. This expression also encompasses age-related disorders.

20 As used herein, the expression **an age-related disorder** refers to a disorder for which the incidence and/or prevalence increase with age, including, without being limited to, cardiovascular diseases, such as atherosclerosis, coronary artery disease and stroke; peripheral vascular disease; metabolic disorders such as Type II diabetes, dyslipidemia, and hypertriglyceridemia; cancer, such as skin
25 cancer, papillomas and age-dependent cancers; ischemia / reperfusion injury, such as renal, heart, cerebral ischemia and radiocontrast-induced nephropathy; inflammation; neurodegenerative disorders and dementia such as Alzheimer's disease, Parkinson's disease, Huntington's disease, memory disorders, and psychosis; Bladder and kidney disorders such as nephritis, nephropathy, end-stage renal disease (ESRD); Diabetes complications such as neuropathy,
30 impaired wound healing, and retinopathy; eyes disorders such as age-related macular degeneration, dry-eye disease, glaucoma, retinitis pigmentosa, and

cataracts; lung and respiratory disorders such as chronic obstructive pulmonary disease (COPD), and idiopathic pulmonary fibrosis (IPF); musculoskeletal disorders such as inflammatory arthritis, gout, and osteoporosis; and skin conditions such as skin cancer, and skin ageing like photoaging.

5

As used herein the term **inflammation** is meant to intend a localized protective response elicited by injury or destruction of tissues which serves to destroy, dilute or wall off both the injurious agent and the injured tissue, characterized in the acute form by the classical sequence of pain, heat, redness, swelling, and loss of function, and histologically involving a complex series of events, including dilatation of the arterioles, capillaries, and venules with increased permeability and blood flow, exudation of fluids including plasma proteins, and leukocyte migration into the inflammatory focus. It is also characterized by massive release of $\text{TNF-}\alpha$.

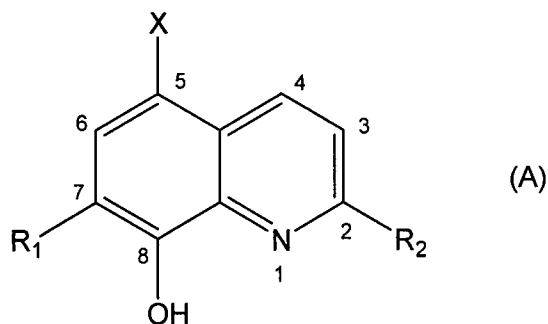
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As used herein the expression **ischemia / reperfusion injury** refers to the condition suffered by tissues and organs when deprived of blood flow, mostly due to the effects of inadequate nutrient and oxygen. Reperfusion injury refers to the tissue damage inflicted when blood flow is restored after an ischemic period of generally more than about ten minutes. Ischemia and reperfusion can cause serious or fatal damage to afflicted tissues.

20

The quinoline derivatives according to the present invention are the compounds having the following general formula (A), or their pharmaceutically acceptable salts or their pro-drugs:

25

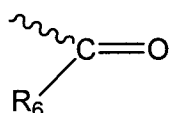


wherein X = H, methyl or halogen, and the substituents R₁ and R₂ are defined as follows:

a) when R₂ is hydrogen, then R₁ is H or halogen, or R₁ is selected from the group consisting of: amino(3,4-dimethoxyphenyl)methyl, 2-hydroxyphenyl, 3-hydroxyphenyl, 4-hydroxyphenyl, 4-dimethylaminophenyl, pyridin-4-yl, 2-methoxypyridin-3-yl and 2-acetamidophenyl;

or R₁ is -CH(R₃)NR₄R₅ with

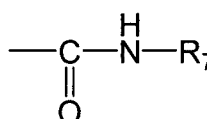
▪ R₄ = H or C₁-C₄ alkyl;

▪ R₅ = H or 

and R₆ is selected from the group consisting of: C₁-C₆ alkyl, C₅-C₆ aryl, benzyl, dimethylaminomethyl, phenylethyl and diphenylmethyl; and

▪ R₃ is selected from the group consisting of:

H, methyl, phenyl, benzyl, 2-chlorophenyl, 2-methoxyphenyl, 4-chlorophenyl, 4-methylphenyl, 4-isopropylphenyl, 3-hydroxyphenyl, 4-hydroxyphenyl, 4-methoxyphenyl, 4-cyanophenyl, 4-dimethylamino-phenyl, 4-diethylamino-phenyl, 2,4-dichlorophenyl, 3,4-dimethoxyphenyl, 4-methoxycarbonylphenyl, 3-methoxycarbonylphenyl, N-methylbenzamido, N-(3-methoxypropyl)benzamido, N,N-dimethylbenzamido, 4-(morpholin-4-ylcarbonyl)phenyl, 4-(pyridin-2-yl)phenyl, 4-(1H-pyrazol-1-yl)phenyl, pyridin-2-yl, pyridin-3-yl, pyridin-4-yl, 6-(morpholin-4-yl)pyridin-3-yl, 2-furyl, 3-furyl, 2-(morpholin-4-yl)pyridin-3-yl, 2-methoxypyridin-3-yl, 4-methoxypyridin-3-yl, 2-thienyl, 2-butyl-1H-imidazol-4-yl, quinolin-3-yl, quinolin-4-yl, 8-hydroxyquinolin-2-yl, 1H-indol-3-yl, 1H-indol-4-yl and 1H-indol-7-yl;

or R₁ is  and R₇ is -(CH₂)_n-R₈ where R₈ is a C₅-C₆ aryl

optionally substituted by one or two methoxyl groups, and n = 0 or 1; or

- b) when X and R₁ both represent a hydrogen atom, then R₂ is selected from the group consisting of: pyridin-2-ylcarboxamido, pyridin-2-ylacetamido, 3-hydroxyphenylacetamido, 4-hydroxyphenylacetamido, ((2-methoxyphenyl)amino)methyl, ((3-methoxyphenyl)amino)methyl, ((3-methoxybenzyl)amino)methyl, 2-thienylacetamido and ((2-thienylmethyl)amino)methyl;

with the proviso that each of the compounds of formula (A) is not one of the compounds identified in Annex 1.

The compounds of Formula (A) of the invention comprising at least one asymmetric centre are either in the form of one of their enantiomers or a mixture thereof.

More preferably, the compounds of formula (A) are the compounds wherein R₄ is H or methyl, R₆ is selected from methyl, ethyl, *n*-propyl, *i*-propyl, *n*-butyl, *i*-butyl, *ter*-butyl, phenyl, benzyl, dimethylaminomethyl, phenylethyl, cyclohexyl, diphenylmethyl, pyridin-2-yl and pyridin-3-yl, the other substituents having the same meaning as previously defined.

According to another preferred embodiment, the compounds of formula (A) are chosen in the group consisting of:

N-((3,4-dimethoxyphenyl)(8-hydroxyquinolin-7-yl)methyl)acetamide **2**,

N-((5-chloro-8-hydroxyquinolin-7-yl)(3,4-dimethoxyphenyl)methyl)benzamide

3,

N-((5-chloro-8-hydroxyquinolin-7-yl)(3,4-dimethoxyphenyl)methyl)-N-methylacetamide **8**,

N-((5-chloro-8-hydroxyquinolin-7-yl)(3,4-dimethoxyphenyl)methyl)-2,2-diphenylacetamide **11**,

N-((5-chloro-8-hydroxyquinolin-7-yl)(3,4-dimethoxyphenyl)methyl)-2-phenylacetamide **12**,

7-(amino(3,4-dimethoxyphenyl)methyl)-5-chloroquinolin-8-ol **13**,

- N-(1-(5-chloro-8-hydroxyquinolin-7-yl)-2-phenylethyl)acetamide **14**,
N-((2-butyl-1H-imidazol-4-yl)(5-chloro-8-hydroxyquinolin-7-yl)methyl)acetamide
15,
N-((5-chloro-8-hydroxyquinolin-7-yl)(2-methoxypyridin-3-yl)methyl)acetamide
5 **16**,
N-((5-chloro-8-hydroxyquinolin-7-yl)(quinolin-4-yl)methyl)acetamide **17**,
N-((5-chloro-8-hydroxyquinolin-7-yl)(2-morpholin-4-ylpyridin-3-yl)methyl)-
acetamide **18**,
N-((5-chloro-8-hydroxyquinolin-7-yl)(quinolin-3-yl)methyl)acetamide **19**,
10 4-((acetylamino)(5-chloro-8-hydroxyquinolin-7-yl)methyl) methylbenzoate
21,
3-((acetylamino)(5-chloro-8-hydroxyquinolin-7-yl)methyl) methylbenzoate
22,
N-((5-chloro-8-hydroxyquinolin-7-yl)(4-cyanophenyl)methyl)acetamide **23**,
15 N-((5-chloro-8-hydroxyquinolin-7-yl)(4-pyridin-2-ylphenyl)methyl)acetamide
24,
N-((5-chloro-8-hydroxyquinolin-7-yl)(4-(1H-pyrazol-1-yl)phenyl)methyl)acetamide
25,
N-((5-chloro-8-hydroxyquinolin-7-yl)(4-hydroxyphenyl)methyl)acetamide **26**,
20 N-((5-chloro-8-hydroxyquinolin-7-yl)(3-hydroxyphenyl)methyl)acetamide **27**,
N-((5-chloro-8-hydroxyquinolin-7-yl)(6-methoxypyridin-3-yl)methyl)acetamide
28,
N-((5-chloro-8-hydroxyquinolin-7-yl)(pyridin-2-yl)methyl)acetamide **29**,
N-((5-chloro-8-hydroxyquinolin-7-yl)(pyridin-4-yl)methyl)acetamide **30**,
25 N-((5-chloro-8-hydroxyquinolin-7-yl)(pyridin-4-yl)methyl)acetamidehydrochloride
31,
N-((5-chloro-8-hydroxyquinolin-7-yl)(pyridin-3-yl)methyl)acetamide **32**,
N-((5-chloro-8-hydroxyquinolin-7-yl)(pyridin-3-yl)methyl)acetamidehydrochloride
33,
30 N-((5-chloro-8 hydroxyquinolin-7-yl)(8-hydroxyquinolin-2-yl)methyl)acetamide
34,
N-((5-chloro-8-hydroxyquinolin-7-yl)(6-morpholin-4-ylpyridin-3-yl)methyl)-

- acetamide **35**,
N-((5-chloro-8-hydroxyquinolin-7-yl)(1H-indol-3-yl)methyl)acetamide **36**,
N-((5-chloro-8-hydroxyquinolin-7-yl)(1H-indol-4-yl)methyl)acetamide **37**,
N-((5-chloro-8-hydroxyquinolin-7-yl)(1H-indol-7-yl)methyl)acetamide **38**,
5 4-((acetylamino)(5-chloro-8-hydroxyquinolin-7-yl)methyl)-N,N-dimethyl-
benzamide **39**,
N-((5-chloro-8-hydroxyquinolin-7-yl)(4-(morpholin-4-ylcarbonyl)phenyl)methyl)-
acetamide **40**,
N-((5-chloro-8-hydroxyquinolin-7-yl)(3,4-dimethoxyphenyl)methyl)pyridine-2-
10 carboxamide **41**,
4-((acetylamino)(5-chloro-8-hydroxyquinolin-7-yl)methyl)-N-(3-methoxypropyl)-
benzamide **44**,
4-((acetylamino)(5-chloro-8-hydroxyquinolin-7-yl)methyl)-N-methylbenzamide
45,
15 N-((5-chloro-8-hydroxyquinolin-7-yl)(3,4-dimethoxyphenyl)methyl)-N,N-dimethyl-
glycinamide **46**,
N-(1-(5-chloro-8-hydroxyquinolin-7-yl)ethyl)acetamide **47**,
N-((8-hydroxyquinolin-7-yl)methyl)acetamide **48**,
N-(2-furylmethyl)-8-hydroxyquinolin-7-yl carboxamide **53**,
20 N-(2-thienylmethyl)-8-hydroxyquinolin-7-yl carboxamide **54**,
N-(2-methoxybenzyl)-8-hydroxyquinolin-7-yl carboxamide **55**,
5-chloro-7-(3-hydroxyphenyl)quinolin-8-ol **57**,
5-chloro-7-(4-hydroxyphenyl)quinolin-8-ol **58**,
N-(2-(5-chloro-8-hydroxyquinolin-7-yl)phenyl)acetamide **59**,
25 5-chloro-7-(2-methoxypyridin-3-yl)quinolin-8-ol **61**,
5-chloro-7-pyridin-4-ylquinolin-8-ol hydrochloride **62**,
N-(3,4-dimethoxyphenyl)-8-hydroxyquinolin-7-yl carboxamide **63**,
N-(3-methoxyphenyl)-8-hydroxyquinolin-7-yl carboxamide **64**,
N-(3-methoxybenzyl)-8-hydroxyquinolin-7-yl carboxamide **65**,
30 N-(3,4-dimethoxybenzyl)-8-hydroxyquinolin-7-yl carboxamide **66**,
N-(2-methoxyphenyl)-8-hydroxyquinolin-7-yl carboxamide **67**,
N-(pyridin-3-ylmethyl)-8-hydroxyquinolin-7-yl carboxamide **68**,

- N-(8-hydroxyquinolin-2-yl)pyridin-2-yl carboxamide 69,
N-(8-hydroxyquinolin-2-yl)-2-pyridin-2-yl acetamide 70,
2-(3-hydroxyphenyl)-N-(8-hydroxyquinolin-2-yl)acetamide 71,
2-(4-hydroxyphenyl)-N-(8-hydroxyquinolin-2-yl)acetamide 72,
5 N-(8-hydroxyquinolin-2-yl)-2-(2-thienyl)acetamide 73,
2-(((3-methoxybenzyl)amino)methyl)quinolin-8-ol 74,
2-(((3-methoxyphenyl)amino)methyl)quinolin-8-ol 75,
2-(((2-thienylmethyl)amino)methyl)quinolin-8-ol 76,
2-(((2-methoxyphenyl)amino)methyl)quinolin-8-ol 77,
10 N-[(2,4-dichlorophenyl)(8-hydroxyquinolin-7-yl)methyl]acetamide 78,
N-[(8-hydroxyquinolin-7-yl)(2-thienyl)methyl]acetamide 79,
N-[(8-hydroxyquinolin-7-yl)(4-isopropylphenyl)methyl]acetamide 80,
N-[(8-hydroxyquinolin-7-yl)(2-thienyl)methyl]butanamide 81,
N-[(8-hydroxyquinolin-7-yl)(4-isopropylphenyl)methyl]butanamide 82,
15 N-[(8-hydroxyquinolin-7-yl)(4-methylphenyl)methyl]-3-phenylpropanamide
92,
N-[(2-chlorophenyl)(8-hydroxyquinolin-7-yl)methyl]-3-phenylpropanamide
93,
N-[(2,4-dichlorophenyl)(8-hydroxyquinolin-7-yl)methyl]-3-methylbutanamide
20 94,
N-[(8-hydroxyquinolin-7-yl)(phenyl)methyl]-3-methylbutanamide 110,
N-[(8-hydroxyquinolin-7-yl)(4-methoxyphenyl)methyl]-3-phenylpropanamide
111,
N-[(3,4-dimethoxyphenyl)(8-hydroxyquinolin-7-yl)methyl]-3-methylbutanamide
25 112,
N-[(8-hydroxyquinolin-7-yl)(2-thienyl)methyl]-3-methylbutanamide 113,
N-[(4-chlorophenyl)(8-hydroxyquinolin-7-yl)methyl]cyclohexanecarboxamide
121,
N-[(2,4-dichlorophenyl)(8-hydroxyquinolin-7-yl)methyl]propanamide 122,
30 N-[(3,4-dimethoxyphenyl)(8-hydroxyquinolin-7-yl)methyl]-3-phenylpropanamide
124,
N-[(2,4-dichlorophenyl)(8-hydroxyquinolin-7-yl)methyl]pentanamide 125,

N-[(8-hydroxyquinolin-7-yl)(4-isopropylphenyl)methyl]pentanamide 126,

N-[[4-(diethylamino)phenyl](8-hydroxyquinolin-7-yl)methyl]pentanamide 127,

N-[(8-hydroxyquinolin-7-yl)(4-methoxyphenyl)methyl]cyclohexanecarboxamide
128,

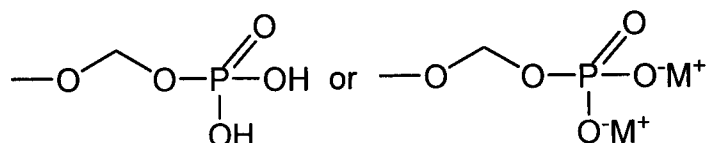
5 N-[(2,4-dichlorophenyl)(8-hydroxyquinolin-7-yl)methyl]butanamide 129,

N-[(8-hydroxyquinolin-7-yl)(4-methylphenyl)methyl]cyclohexanecarboxamide
130, and

N-[(8-hydroxyquinolin-7-yl)(2-thienyl)methyl]cyclohexanecarboxamide 131.

10

According to a preferred embodiment, the pro-drugs of the compounds of formula (A) of the invention correspond to the compounds wherein the hydroxyl group in position 8 of the quinoline moiety is replaced by one of the following groups:



15 wherein M is selected from Li, Na, Ca and K.

According to another preferred embodiment, the above defined dihydrogen phosphate pro-drugs are in the form of their hydrochloride or dihydrochloride salts.

20

The preferred pro-drugs according to the invention are the following:

((7-((acetylamino)(3,4-dimethoxyphenyl)methyl)-5-chloroquinolin-8-yl)oxy)methyl dihydrogen phosphate 5,

25 ((7-((acetylamino)(3,4-dimethoxyphenyl)methyl)-5-chloroquinolin-8-yl)oxy)methyl disodium phosphate 6,

((7-((acetylamino)(2-furyl)methyl)-5-chloroquinolin-8-yl)oxy)methyl dihydrogen phosphate 136,

((7-((acetylamino)(2-furyl)methyl)-5-chloroquinolin-8-yl)oxy)methyl disodium phosphate **137**,

((7-((acetylamino)(3,4-dimethoxyphenyl)methyl)-5-chloroquinolin-8-yl)oxy)methyl dihydrogen phosphate dihydrochloride **138**, and

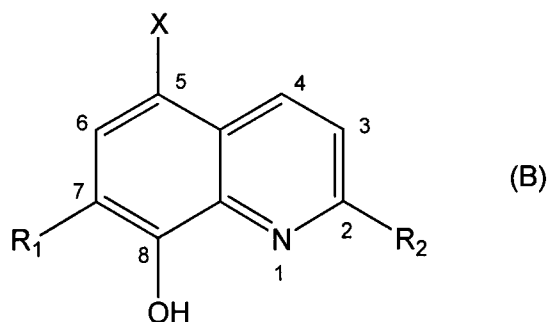
5 ((7-((acetylamino)(3,4-dimethoxyphenyl)methyl)-5-chloroquinolin-8-yl)oxy)methyl dihydrogen phosphate hydrochloride **139**.

As previously mentioned, asymmetric or chiral centers may exist in the compounds of the present invention. The present invention contemplates the
 10 various optically active isomers of the compounds of formula (A) and mixtures thereof. As an example, an individual enantiomer of a compound of the present invention is prepared synthetically from commercially available starting materials which contain asymmetric or chiral centers or by preparation of mixtures of enantiomeric compounds followed by resolution well-known to those of ordinary
 15 skill in the art. These methods of resolution are exemplified by a) attachment of a racemic mixture of enantiomers to a chiral auxiliary, separation of the resulting diastereoisomers by recrystallization or chromatography and liberation of the optically pure product from auxiliary or b) direct separation of the mixture of optical enantiomers on the chiral chromatographic columns.

20

The invention also relates to a method for inhibiting CLK-1 activity in a cell which comprises the following steps:

- a) providing a cell wherein CLK-1 activity needs to be inhibited,
- b) contacting the cell with a compound of formula (B):



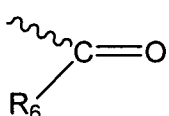
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wherein X = H, methyl or halogen and the substituents R₁ and R₂ are defined as follows:

- i) when R_2 is hydrogen, then R_1 is H or halogen, or R_1 is selected from the group consisting of: amino(3,4-dimethoxyphenyl)methyl, 2-hydroxyphenyl, 3-hydroxyphenyl, 4-hydroxyphenyl, 4-dimethylaminophenyl, pyridin-4-yl, 2-methoxypyridin-3-yl and 2-acetamidophenyl;

or R_1 is $-\text{CH}(\text{R}_3)\text{NR}_4\text{R}_5$ with

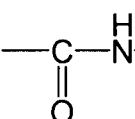
▪ $R_4 = \text{H}$ or $\text{C}_1\text{-C}_4$ alkyl;

▪ $R_5 = \text{H}$ or 

- and R_6 is selected from the group consisting of: $\text{C}_1\text{-C}_6$ alkyl, $\text{C}_5\text{-C}_6$ aryl, benzyl, dimethylaminomethyl, phenylethyl and diphenylmethyl; and

▪ R_3 is selected from the group consisting of:

- H, methyl, phenyl, benzyl, 2-chlorophenyl, 2-methoxyphenyl, 4-chlorophenyl, 4-methylphenyl, 4-isopropylphenyl, 3-hydroxyphenyl, 4-hydroxyphenyl, 4-methoxyphenyl, 4-cyanophenyl, 4-dimethylamino-phenyl, 4-diethylamino-phenyl, 2,4-dichlorophenyl, 3,4-dimethoxyphenyl, 4-methoxycarbonylphenyl, 3-methoxycarbonylphenyl, N-methylbenzamido, N-(3-methoxypropyl)benzamido, N,N-dimethylbenzamido, 4-(morpholin-4-ylcarbonyl)phenyl, 4-(pyridin-2-yl)phenyl, 4-(1H-pyrazol-1-yl)phenyl, pyridin-2-yl, pyridin-3-yl, pyridin-4-yl, 6-(morpholin-4-yl)pyridin-3-yl, 2-furyl, 3-furyl, 2-(morpholin-4-yl)pyridin-3-yl, 2-methoxypyridin-3-yl, 4-methoxypyridin-3-yl, 2-thienyl, 2-butyl-1H-imidazol-4-yl, quinolin-3-yl, quinolin-4-yl, 8-hydroxyquinolin-2-yl, 1H-indol-3-yl, 1H-indol-4-yl and 1H-indol-7-yl;

or R_1 is  and R_7 is $-(\text{CH}_2)_n\text{-R}_8$ where R_8 is a $\text{C}_5\text{-C}_6$ aryl optionally

substituted by one or two methoxyl groups, and $n = 0$ or 1 ; or

- ii) when X and R_1 both represent a hydrogen atom, then R_2 is selected from the group consisting of: pyridin-2-ylcarboxamido, pyridin-2-ylacetamido, 3-

hydroxyphenylacetamido, 4-hydroxyphenylacetamido, ((2-methoxyphenyl)amino)methyl, ((3-methoxyphenyl)amino)methyl, ((3-methoxybenzyl)amino)methyl, 2-thienylacetamido and ((2-thienylmethyl)amino)methyl;

- 5 the compound of formula (B), when comprising at least one asymmetric centre, being in the form of one of its enantiomers or a mixture thereof; and
c) determining the CLK-1 activity in the cell.

Advantageously, the compound of formula (B) used for inhibiting CLK-1 activity in
10 a cell is selected from the group of compounds identified in Table 1.

Even more advantageously, the compound of formula (B) used for inhibiting CLK-1 activity in a cell is selected from the group of:

N-[(5-chloro-8-hydroxyquinolin-7-yl)(3,4-dimethoxyphenyl)methyl]acetamide

7,

- 15 5-chloro-7-iodoquinolin-8-ol 52,
5-chloro-7-(2-hydroxyphenyl)quinolin-8-ol 56,
5-chloro-7-(4-(dimethylamino)phenyl)quinolin-8-ol 60, and
N-[(5-chloro-8-hydroxyquinolin-7-yl)(2-furyl)methyl]acetamide 135.

- 20 The invention further relates to a method for the prophylaxis and/or treatment of a disorder or its associated symptoms for which inhibition of CLK-1 is beneficial, in animals, which comprises the following steps:

- a) identifying an animal having a disorder for which inhibition of CLK-1 is beneficial; and
25 b) administering to the animal a compound of formula (B) as defined above, a pharmaceutically acceptable salt or a pro-drug thereof.

Advantageously, the compound of formula (B), its salt or its pro-drug or an optically active isomer, used for the prophylaxis and/or treatment in animal, of a
30 disorder or its associated symptoms for which inhibition of CLK-1 is beneficial, is selected from the group of compounds identified in Table 1.

Even more advantageously, the compound of formula (B), its salt or its pro-drug, used for the prophylaxis and/or treatment in animal, of a disorder or its associated symptoms for which inhibition of CLK-1 is beneficial, is selected from the group of:

- 5 ((7-((acetylamino)(3,4-dimethoxyphenyl)methyl)-5-chloroquinolin-8-yl)oxy)methyl dihydrogen phosphate 5,
((7-((acetylamino)(3,4-dimethoxyphenyl)methyl)-5-chloroquinolin-8-yl)oxy)methyl disodium phosphate 6,
N-[(5-chloro-8-hydroxyquinolin-7-yl)(3,4-dimethoxyphenyl)methyl]acetamide
- 10 7,
N-[(5-chloro-8-hydroxyquinolin-7-yl)(2-furyl)methyl]acetamide 135,
((7-((acetylamino)(2-furyl)methyl)-5-chloroquinolin-8-yl)oxy)methyldihydrogen phosphate 136,
((7-((acetylamino)(2-furyl)methyl)-5-chloroquinolin-8-yl)oxy)methyldisodium
- 15 phosphate 137,
((7-((acetylamino)(3,4-dimethoxyphenyl)methyl)-5-chloroquinolin-8-yl)oxy)methyl dihydrogen phosphate dihydrochloride 138, and
((7-((acetylamino)(3,4-dimethoxyphenyl)methyl)-5-chloroquinolin-8-yl)oxy)methyl dihydrogen phosphate hydrochloride 139.

20

According to a preferred embodiment the disorder for which inhibition of CLK-1 is beneficial is an ischemia / reperfusion injury, an inflammation or a neurodegenerative disorder or dementia.

- 25 More preferably, but not limited to, the ischemia / reperfusion injury is a renal, heart, myocardium, lung, brain, or spinal cord ischemia / reperfusion injury.

30

According to a preferred embodiment, the inflammation is a lung inflammation, a liver inflammation or an inflammation of any other organ and any clinical indications in which such inflammation is promoted.

According to another embodiment, a neurodegenerative disorder is Alzheimer's disease.

Since it has been previously shown that inhibiting CLK-1 activity in mice slows down physiological aging (Liu et al., 2005), this indicates that the inhibition of CLK-1 activity in animals can delay the onset and severity of age-dependent diseases.

In this context, the present inventors have now disclosed that the compounds of formula (B) of the present invention are also effective to treat or prevent an age-related disorder or its associated symptoms.

Thus, more preferably, the invention relates to a method for the prophylaxis and/or treatment of an age-related disorder or its associated symptoms, in animals, which comprises the following steps:

a) identifying an animal having an age-related disorder; and

b) administering to the animal a compound of formula (B), a pharmaceutically acceptable salt or a pro-drug thereof;

with the proviso that said compound of formula (B) is not one of the following compounds, a pharmaceutically acceptable salt or a pro-drug thereof:

5-chloro-7-iodoquinolin-8-ol 52,

5-chloro-7-(2-hydroxyphenyl)quinolin-8-ol 56, or

5-chloro-7-(4-(dimethylamino)phenyl)quinolin-8-ol 60.

According to a preferred embodiment the above method is used for the prophylaxis and/or treatment of an age-related disorder or its associated symptoms selected from the group consisting of: cardiovascular diseases, such as atherosclerosis, coronary artery disease and stroke; peripheral vascular disease; metabolic disorders such as Type II diabetes, dyslipidemia, and hypertriglyceridemia; cancer, such as skin cancer, papillomas and age-dependent cancers; ischemia / reperfusion injury, such as renal, heart, cerebral ischemia and radiocontrast-induced nephropathy; inflammation; neurodegenerative disorders and dementia such as Alzheimer's disease, Parkinson's disease, Huntington's disease, memory disorders, and psychosis; Bladder and kidney disorders such as nephritis, nephropathy, end-stage renal disease (ESRD);

Diabetes complications such as neuropathy, impaired wound healing, and retinopathy; eyes disorders such as age-related macular degeneration, dry-eye disease, glaucoma, retinitis pigmentosa, and cataracts; lung and respiratory disorders such as chronic obstructive pulmonary disease (COPD), and idiopathic pulmonary fibrosis (IPF); musculoskeletal disorders such as inflammatory arthritis, gout, and osteoporosis; and skin conditions, both cosmetic and dermatologic, including skin ageing like photoaging and skin cancer.

The animal to be treated with the compound of formula (B) of the invention may be a human or any animal of commercial or domestic value including but not limited to cow, horse, pig, guinea pig, hamster goat, rabbit, chicken, dog, cat and birds. Even more preferably the animal treated is a human.

The invention also relates to a pharmaceutical composition comprising a compound of formula (A), a pharmaceutically acceptable salt or a pro-drug thereof as defined herein above, or any of the pro-drugs 5, 6, 136, 137, 138 and 139 defined above, formulated together with one or more non-toxic pharmaceutically acceptable carrier.

The present inventors have also shown that the compounds of formula (A) or (B) of the invention have a CLK-1 inhibitory activity.

Accordingly, the invention also relates to a pharmaceutical composition comprising a pharmaceutically effective amount of the compound of formula (A) or (B), a pharmaceutically acceptable salt or a pro-drug thereof, to effectively reduce and/or inhibit CLK-1 activity.

The invention also relates to a pharmaceutical composition comprising a pharmaceutically acceptable amount of any one of the pro-drug 5, 6, 136, 137, 138 and 139 to effectively reduce and/or inhibit CLK-1 activity.

According to a preferred embodiment, the compounds of the present invention could be combined with another drug known to be active to treat an age-related disorder or its associated symptoms, such as for example combination with a statin for the treatment of dyslipidemia.

5

The pharmaceutical compositions according to the invention may be specially formulated for oral administration in solid or liquid form, for parenteral injection, for rectal administration or topical administration. The pharmaceutical compositions of this invention can be administered to human and other animals
10 orally, rectally, parenterally, intra cisternally, intravaginally, intraperitoneally, topically (as by powder, cream, ointment, or drops), buccally, or as an oral or nasal spray. These compositions may also contain adjuvants such as preservative, wetting agents, emulsifying agents and dispersing agents. Prevention of action of microorganisms may be ensured by the inclusion of various antibacterial and
15 antifungal agents, for example, paraben, chlorobutanol, phenol sorbic acid and the like. Prolonged absorption of the injectable pharmaceutical form may be brought about by the inclusion of agents which delay absorption such as aluminium monostearate and gelatine.

20 In some cases, in order to prolong the effect of the drug, it is desirable to slow the absorption of the drug from subcutaneous or intramuscular injection. This may be accomplished by the use of a liquid suspension of crystalline or amorphous material with poor water solubility. The rate of absorption of the drug thus depends upon its rate of dissolution which, in turn, may depend upon crystal size
25 and crystalline form. Alternatively, delayed absorption of a parenterally administered drug form is accomplished by dissolving or suspending the drug in an oil vehicle.

Solid dosage forms for oral administration include capsules, tablets, pills,
30 powders, and granules. In such solid dosage forms, the active compound is mixed with at least one inert, pharmaceutically acceptable excipient or carrier

such as sodium citrate or dicalcium phosphate and/or fillers or extenders such as starches, lactose, sucrose, glucose, mannitol.

Liquid dosage forms for oral administration include pharmaceutically acceptable emulsions, solutions, suspensions, syrups and elixirs. In addition to the active compounds, the liquid dosage forms may contain inert diluents commonly used in the art such as, for example, water or other solvents, solubilizing agents and emulsifiers such as ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, propylene glycol, glycerol, tetrahydrofurfuryl alcohol, polyethyleneglycols and fatty acid esters of sorbitan, and mixtures thereof.

The compounds of the invention may also be administered in the form of liposomes deriving from phospholipids or other lipid substances. Liposomes are formed by mono- or multi-lamellar hydrated liquid crystals that are dispersed in aqueous medium. Any non-toxic, physiologically acceptable and metabolizable liquid capable of forming liposomes can be used. The preferred lipids are the phospholipids and the phosphatidyl cholines, both natural and synthetic.

Dosage forms for topical administration of a compound of the invention include powders, sprays, ointments and inhalants. The active compound is mixed under sterile conditions with a pharmaceutically acceptable carrier and any needed preservatives, buffers, or propellants which may be required.

Actual dosage levels of active ingredients in the pharmaceutical compositions of this invention may be varied so as to obtain an amount of the active compound(s) that is effective to achieve the desired therapeutic response for a particular patient, compositions, and mode of administration. The selected dosage level will depend upon the activity of the particular compound, the route of administration, the severity of the condition being treated, and the condition and prior medical history of the patient being treated. However, it is within the skill of the art to start doses of the compound at levels lower than required for to achieve the desired

therapeutic effect and to gradually increase the dosage until the desired effect is achieved.

The present invention will be more readily understood by referring to the following
5 examples, with reference to the accompanying figures. These examples are
illustrative of the wide range of applicability of the present invention and are not
intended to limit its scope. Modifications and variations can be made therein
without departing from the spirit and scope of the invention. Although any
methods and materials similar or equivalent to those described herein can be
10 used in the practice for testing of the present invention, the preferred methods
and materials are described.

EXAMPLES

EXAMPLE 1: Specificity of CLK-1 inhibitors

Demethoxyubiquinone (DMQ) hydroxylation is carried by CLK-1 in eukaryotes and by UbiF in eubacteria. CLK-1 inhibitor molecules were tested in a modified JF496 *E. coli* strain, which carries a mutation in the *ubiF* gene, and lacks DMQ hydroxylation activity. This strain is complemented either with mouse *clk-1* gene, or with *E. coli ubiF* gene. This complementation is functional, and restores DMQ hydroxylation. As a consequence, the rescued strains are capable of synthesizing ubiquinone (Q), instead of accumulating DMQ. Test molecules are evaluated for specificity regarding Q synthesis inhibition, using the two modified strains.

Materials and methods

Bacterial strains and media

Escherichia coli strain JF496 (*ubiF*⁻) was transformed with plasmids containing the mouse *clk-1* gene, or the *E. coli ubiF* gene. For general growth, a clone of each strain is grown overnight at 37°C in 5 ml LB supplemented with ampicillin (50 µg/ml). The cultures are diluted 1/10 and OD₆₀₀ is measured. The culture is diluted to an OD₆₀₀ of 0.03 in M9-LB (0.5/0.5:v/v). This mix consists of M9 medium supplemented with (1 mM MgSO₄, 20 µM CaCl₂, 0.5 µg/mL thiamine, 0.12% casamino acids, 40 µg/mL D-L-methionine, 100 µg/mL L-asparagine, trace metals, 0.5% glucose), and of LB medium. Media contain ampicillin (50 µg/mL) for plasmid selection. For HPLC quinone analysis, the JF496-*ubiF* and JF496-*mclk-1* strains are diluted from an overnight preculture into M9-LB (0.5/0.5:v/v) to an OD₆₀₀ of 0.03. A volume of 750 µl of bacteria is transferred to 15 ml tubes. The bacterial cultures are incubated for 5 hours at 37°C with agitation.

Quinones extraction

Cells are harvested by centrifugation, washed with distilled water and centrifuged again. Pellets are frozen at -80°C overnight. Cells are then thawed and resuspended in 1 ml sodium phosphate buffer (0.1M, pH 7.0), and 50 ng Q₆ is added as a quinone extraction control. To extract quinones, 1 ml ethanol is added

and cells are vortexed 30 seconds. 1 ml hexane is subsequently added. Cells are then vortexed 2 minutes and centrifuged. The upper phase (hexane) is collected. The lower phase is extracted a second time and centrifuged. The upper phase is then collected and added to the previous extract. Upper phases are freeze-dried
5 for approximately 1 hour and the pellet is kept at -80°C. Pellets are resuspended in 300 µl of mobile phase before HPLC analysis.

HPLC method

Samples are analyzed using a Beckman System Gold HPLC with a photo diode
10 array detector and a Beckman Ultrasphere ODS (4.6mm x 25 cm) column. Data are analyzed using the 32 Karat software (Beckman). Quinones are separated using a mobile phase gradient of methanol-ethanol (beginning at 70% methanol-30% ethanol, ending 6 minutes later at 30% methanol-70% ethanol), at a flow rate of 1 ml/min for 20 minutes. Demethoxyubiquinone (DMQ) and ubiquinone (Q)
15 peaks are detected at 275 nm.

Results and short discussion on drugs specificity testing

JF496 bacteria expressing mouse CLK-1 and the bacterial DMQ-hydroxylase UbiF were treated with compound 7 (Figure 1), and with compound 52 (Figure 2).
20 The quinone distributions resulting from these treatments were analyzed using HPLC. It appears that 7 and 52 can inhibit CLK-1 DMQ hydroxylase activity, as indicated by the accumulation of the DMQ precursor. In contrast to that, UbiF activity is not challenged by any of the compounds, since DMQ is not accumulating in treated cells. This differential effect is indicative of a specificity of
25 action on CLK-1 compared to UbiF, even though these two enzymes carry an identical function which is DMQ hydroxylation. The effects seen on CLK-1 are not due to some non-specific activities of the compounds in *E. coli*, as both CLK-1 and UbiF are expressed in the same bacterial genetic background.

EXAMPLE 2: Measurement of CLK-1 inhibition

To confirm that a positive compound identified in bacterial screening was a *bona fide* inhibitor of CLK-1, we utilized an HPLC-based secondary assay to measure the quinone content of mammalian cells. CLK-1 is a demethoxyquinone (DMQ) hydroxylase that catalyzes the penultimate step in Q biosynthesis. Inhibition of CLK-1 leads to accumulation of the reaction precursor, DMQ. This assay measures the endogenous cellular levels of Q, and its precursor DMQ. In all cells tested the levels of DMQ present normally are negligible, so robust inhibition of CLK-1 leads to a profound change in the HPLC quinone profiles of treated cells.

Materials and Methods

Fresh dilutions of compounds that are identified from the bacterial screen were dissolved overnight in dimethyl sulfoxide (DMSO) at a concentration of 10 mM. Dilutions of the compound (in DMSO) were added to wells of RAW264.7 cells, an Abelson's murine leukemia virus-transformed murine macrophage cell line. The cells were plated (1×10^5 cells per well) in Dulbecco's Modified Eagle's medium (supplemented with 10% Fetal Bovine serum, 1% Penicillin/Streptomycin, 100 μ M Sodium Pyruvate) the day before compound treatment and left in a humidified CO₂ incubator (5% CO₂, 37°C) overnight. Following addition of the compound, the plates were incubated for 24 hours, in humidified CO₂ (5% CO₂, 37°C).

After 24 hours, the medium was aspirated from the wells; the cells were washed with PBS, before 500 μ l RIPA buffer was added to each well (15 minutes, room temperature) with gentle rocking. After this time, samples were removed to a 1.5 ml microcentrifuge tube. 500 μ l of HPLC grade ethanol, followed by 500 μ l of HPLC grade hexane, was added to each tube, which was then vortexed vigorously for one minute, before being centrifuged (3000 x g, 15 minutes, room temperature). After centrifugation, 2 distinct layers could be seen in each tube. The top layer was carefully removed to a fresh microcentrifuge tube, capped with a needle perforated lid, and then subjected to freeze drying.

Once the samples were dry, 320 μ l of 70% HPLC grade methanol: 30% ethanol (v/v), was added to each tube. The tubes were then vortexed vigorously for 20

seconds, centrifuged for 10 seconds (14 000 x g), before the samples were transferred to a capped HPLC vial. Each sample was then subjected to HPLC analysis. The analysis was done by passing samples through a 250 mm C18 ODS column, using an ethanol: methanol gradient and quinones were detected at 275 nm, using a photodiode array detector. Visual inspection of chromatograms was used to determine the activity of various doses of each compound of interest.

Table 1 shows a list of compounds which have been determined to be active CLK-1 inhibitors.

Results and brief discussion of the inhibition of CLK-1 in mammalian cells

Shown in Figure 3 is a typical HPLC profile obtained from quinone extraction of a sample of RAW 264.7 mouse macrophage cells. The graph in the upper panel represents the quinone extracted from untreated macrophages, wherein the major species evident is ubiquinone-9, or Q. When we treat cells with a CLK-1 inhibitor we observe the appearance of a second quinone peak, which represents the accumulation of Q's precursor, DMQ. This profile represents cells treated with compound **135**. Thus CLK-1 inhibitors are readily identified by a clear and simple visual analysis of HPLC profiles of cells treated with putative candidate inhibitors.

A variety of human and mouse cell lines were examined, with no major difference between them other than variations in levels of endogenous ubiquinone.

This means that the only conceivable fashion by which we can detect a loss of Q in cells, with a corresponding accumulation of DMQ, is by inhibition of CLK-1.

This secondary assay is thus extremely useful and very robust in its capacity to rapidly identify CLK-1 inhibitors. Figures 4-8 show the HPLC profiles of mouse macrophages treated with variable concentrations of compound **7**, **23**, **69**, **47** or **53**. Here we see the effects of increasing compound concentrations on the distribution of DMQ₉ and Q₉ in treated cells.

EXAMPLE 3: Measure the effect of CLK-1 inhibition

CLK-1 activity may have impacts on cellular ROS levels by regulating the cellular content of Q, which contributes to ROS production via the electron transport chain and ROS scavenging. Therefore, it is to be expected that inhibition of CLK-1 will lead to alterations in cellular ROS levels. Additionally, one of the macromolecules that is targeted by ROS in cells is DNA, resulting in DNA damage. One might expect that DNA damage would also be reduced as a consequence of CLK-1 inhibition. We therefore used two assays to measure these factors in living cells, as outlined below.

Materials and Methods**ROS assay**

DCF-DA (2,7-dichlorofluorescein diacetate) was used to assess cellular ROS levels. The lipophilic DCF-DA is transported across the cell membrane to the cytosol, and enzymatically converted to hydrophilic 2,7-dichlorofluorescein (DCFH) by cytosolic esterase(s). Cellular ROS oxidize DCFH to DCF, which is a fluorescent molecule. To evaluate the effect of CLK-1 inhibition on ROS production, firstly, mouse macrophage cells (RAW264.7) seeded (1×10^5 per well) in 96-well plates were treated with CLK-1 inhibitors for 24h. The cells were then incubated with 20 μ M DCF-DA in Hanks' Balanced Salt Solution (HBSS) for 30 min at 37°C. The fluorescence from cellular DCF was then monitored using a fluorescence plate reader (excitation 488 nm and emission 520 nm). The protein amount in the tested wells was determined using a commercially available BCA protein assay kit. Cellular ROS levels were subsequently expressed as mean DCF fluorescence/mg protein from 3 replicates.

COMET assay

RAW264.7 cells were seeded (2.5×10^6 cells per well) in the presence/absence of a CLK-1 inhibitory compound in 12-well plates. After 24 hours, the cells were treated with 1 mM H_2O_2 for 1 hour, to induce ROS-mediated DNA damage. The cells were then washed twice in ice-cold PBS, before being scraped into 575 μ l of

ice cold PBS. 75µl of this mixture was removed and added to 750µl ice cold PBS. A 10µl aliquot was removed and mixed with 90µl of molten low-melting point agarose. 75 µl of this cell-agarose mix was pipetted onto a glass slide, which was placed flat at 4°C, and shielded from the light for 15 minutes.

5

After this time, the slide was immersed in a pre-chilled lytic solution and left for 1 hour, at 4°C, shielded from the light. Subsequently, the slide was removed and after removal of excess lytic solution, immersed in a freshly prepared alkaline solution (pH 13). The slide was then left for 1 hour, shielded from the light, at room temperature.

10

Excess alkaline solution was removed, before the slide was immersed for 5 minutes, at room temperature in 1x Tris-Borate/EDTA buffer (TBE). Again shielded from the light, the slide was placed in a 1x TBE-filled, electrophoretic apparatus and a voltage applied for 20 minutes. The slide was removed after 20 minutes and immersed, shielded from the light, in 70% ethanol, for 5 minutes, before being allowed to air dry at 37°C until the agarose forms a thin layer on the slide.

15

Following this 37°C incubation, 50 µl of diluted SYBR green is added to each dried agarose disk for 10 min, in the dark, before the dye is washed off by immersion in distilled water. Excess water is blotted off, before the slide was allowed to air dry, once again in the dark. Once completely dry, the slide is viewed on a fluorescence microscope and examined for the presence of DNA tails (comets) trailing the nuclei of cells. Data are usually represented as percentage of cells containing comets, having counted 10 fields.

25

Results and brief discussion of the effect of CLK-1 inhibitors on cellular ROS levels

Three panels showing the effect of archetype CLK-1 inhibitors, namely compounds **7** and **135**, in quinone profiling, cellular ROS levels, and DNA damage are presented in Figure 9. These molecules were found to lower the

30

induction of ROS by H₂O₂ in macrophages, with 7 being more potent (Panel B). These molecules have a quite striking effect in DNA damage induced by H₂O₂ in macrophages (Panel C), with 7 being extremely potent. These data underline that we are reducing the cellular levels of ROS and the subsequent damage incurred.

5

EXAMPLE 4: CLK-1 inhibitors are useful to treat

kidney ischemia-reperfusion injury in the rat

The following study evaluates the utility of CLK-1 inhibitor administration for preventing and/or reducing reperfusion injuries in a model of renal ischemia.

10

Brief description of Experimental Methods

Adult (9 to 11 week-old) male Sprague-Dawley rats were anaesthetized and underwent right nephrectomy through a mid-line abdominal incision. Transient ischemia of the left renal artery was achieved by occlusion of the left renal artery and vein for 60 minutes. The treatment for all groups (Sham: no ischemia, received saline (0.9% NaCl), Naïve: ischemia plus the vehicle, Treatment: ischemia plus compound 7 or 52) was performed once daily intraperitoneally (*i.p.*) for 15 consecutive days starting three days before the surgical intervention. Treatment group received compound 7 at 2.5 mg/kg/day *i.p.*, (1 mg/ml), or compound 52 at 20 mg/kg/day (4 mg/ml). The renal functions were evaluated on Days 0, 3, 7, 11 and 14 through determination of Creatinine and Blood Urea Nitrogen (BUN) in serum and Creatinine and protein content in urine. Creatinine clearance (ml/min/100g) was calculated using the formula - $CrCl = (uCr \times uV) / (sCr \times 1440 \times \text{weight})$. Urine protein concentration in 24 hr urine samples were determined using the method of Coomassi. Histopathologic changes in renal tissue (Hematoxylin and eosin; H&E stained slides) were analyzed by light microscopy for tubular epithelial cell necrosis, tubular dilation, proteinaceous casts, and medullar congestion as suggested by Racusen (2). The statistical analysis of the collected data was performed using One-way ANOVA followed by Turkey post-hoc test when indicated. The results with $p \leq 0.05$ were considered statistically significant.

30

Brief description of ischemia-reperfusion injury Results**Renal function: Serum creatinine**

5 The initial results demonstrated that the serum creatinine level in each group was not statistically different on day 0. The serum creatinine at day 3 to day 14, in groups treated with compound 7 or compound 52, was significantly lower than in group NAÏVE. (Figure 10)

Renal function: Blood urea nitrogen (BUN)

10 Initial results demonstrated that on day 0, BUN in each group was not statistically different. On day 3, 7, 11 and 14 BUN in groups treated with compounds 7 and 52 was significantly lower than in group NAÏVE (Figure 11).

Renal function: Creatinine clearance

15 On day 0, creatinine clearance in each group was not statistically different. On days 3 and 7 creatinine clearance in group treated with compound 7 or compound 52 was significantly higher than in group NAÏVE. On days 11 and 14 creatinine clearance in group treated with compounds 7 or 52 was not statistically different compared with group NAÏVE, however was higher than in group NAÏVE
20 on day 14 (Figure 12).

Renal function: Urine protein

On day 0, 24-hr urine protein in each group was not statistically different. On day 3, 24-hr urine protein in group treated with compounds 7 or 52 was significantly
25 lower than in group NAÏVE. On day 7 and 14, 24-hr urine protein in group treated with compound 7 or 52 was not statistically different compared with group NAÏVE. On day 11, 24-hr urine protein in group treated with compounds 7 or 52 was significantly lower than in group NAÏVE (P=0.033) (Figure 13).

Brief discussion of the effect of CLK-1 inhibitors on kidney ischemia-reperfusion injury

The renal function data (serum creatinine, blood urea nitrogen (BUN), creatinine clearance and urine protein) showed that once a day intraperitoneal treatment with compound **7** (2.5 mg/kg) and compound **52** (20 mg/kg) starting three days before the ischemic event and continued for 11 consecutive days effectively prevented, in a similar extent, the renal ischemia/reperfusion injury in one kidney rat model after 60-min ischemia when compared with the Naïve control groups. In all cases, renal functions in the SHAM groups were not affected. The protective effect on several parameters of the renal function was particularly pronounced on days 3 and 7 after the ischemia/reperfusion.

EXAMPLE 5: Effects of CLK-1 inhibitors on lipopolysaccharide-induced acute lung injury

The following study evaluates the utility of CLK-1 inhibitors in an *in vivo* relevant rat model of acute lung inflammation elicited by systemic administration of lipopolysaccharide (LPS).

Brief description of Experimental Methods

Male Sprague-Dawley rats weighing 225 to 250 g (n=5 to 10 per group) were treated with lipopolysaccharide (LPS) in order to induce lung injury. Drug-treated rats received intraperitoneally (*i.p.*) Compound **7** or N-Acetyl-L-cysteine (NAC) as reference. Compound **7** was administered *i.p.* in vehicle containing DMA/PG/Tween 80 and water. Doses were given once a day, for three consecutive days, with the last dose administered 1h before LPS exposure. Control rats received drug vehicles as indicated. Freshly prepared drugs were administered in a volume of 5 or 10 ml/kg or 5 ml/kg. Twenty-four hours after *i.p.* LPS or saline administration, the animals were sacrificed by an overdose of urethane and blood alveolar fluid (BALF) was collected for total and differential cell counts, LPO and TNF- α determination and protein contents.

Brief description and discussion of LPS-induced lung injury results

The results showing the effects of Compound 7 (5 mg/kg or 10 mg/kg) given *i.p.*, once a day for three days before LPS (6 mg/kg, *i.p.*) on total cell and neutrophil counts, protein and TNF- α content, LPO levels in BALF from rats exposed to saline (control) or LPS are shown in Figures 14 to 17. N-Acetylcysteine (NAC; 0.5 g/kg, *i.p.*, once a day for three days before LPS) was given as reference compound. Briefly, Compound 7 effectively reduced the increase in BALF total cells and neutrophils to levels comparable to those observed in NAC-treated rats. This compound did not produce any effect on BALF total cell counts when given alone. Similarly, Compound 7 effectively reduced the increase in LPO in BALF to levels comparable to those observed in NAC-treated rats. Compound 7 failed to reduce the increase in BALF protein. Compound 7 (10 mg/kg) was effective to significantly reduce BALF TNF- α levels. Moreover, histological analysis (not shown) demonstrated that Compound 7 at 5 mg/kg, *i.p.* was effective to reduce the intensity of the interstitial pattern (alveolar wall is less thick in treated lungs) and the inflammatory response (lower number of inflammatory cells).

Altogether, these experiments demonstrated the utility of Compound 7 in reducing the BALF total and neutrophil counts, lipid hydroperoxide, and histological pulmonary lesions (not shown) in LPS-challenged rats. TNF- α level reduction was also observed with Compound 7 at 10 mg/kg, *i.p.* dose regimen. As neutrophil influx and LPO are well known markers of inflammation for which CLK-1 inhibitors are protective, the utility of the novel derivatives of quinoline for clinical indications in which such inflammation is promoted represents a particularly useful embodiment.

Example 6: Assessment of the effects of a treatment with Compound 138 on brain β -amyloid1-40 and β -amyloid1-42 levels of hAPP751SL transgenic mice.

Animal: Male hAPPSL transgenic (tg) mice (C57BL/6 background) aged 5 months ($\pm$ 2 weeks) at the starting date of the study. Transgenic hAPP751SL animals constitutively over-express human APP751 with the London (V717I) and the Swedish (K670M/N671L) mutations under the regulatory control of the neuronal tissue specific murine-Thy-1 promoter. Due to the London mutation, high levels of β -amyloid1-42 are expressed all over the brain but mainly in cortex and hippocampus. High levels of β -amyloid1-42 are associated with amyloid plaque formation in the CNS at a much earlier age beginning at 3 months. Enhanced brain A β 1-42 levels as seen in this mouse model are associated with earlier plaque formation. A β 1-42 accelerates amyloid deposition and promotes formation of denser deposits; while A β 1-40 might have opposite effects. Therefore, the potential to influence the generation of this fatal amyloid peptide might be advantageous, in particular with regard to a possible treatment of Alzheimer's disease patients.

Treatment: Compound **138** was formulated in carboxymethyl cellulose (CMC) and administrated *per os* (*p.o.*) to animals once daily and twice daily (at the highest dose), respectively, for 60 consecutive days. Treatment doses were 5, 20, 50 and 2 x 50 mg/kg b.w./day, respectively or with vehicle (CMC).

Tissue Preparation: Transgenic mice were transcardially perfused with physiological (0.9%) saline until the whole blood was washed out; then brains were rapidly removed and the right hemisphere was immersion fixed in fresh 4% paraformaldehyde for one hour. After that the hemispheres were transferred to a 15% sucrose solution for cryprotection. On the next day, brains were frozen on dry ice and stored at -80°C. The left brain hemisphere was immediately snap frozen on dry ice to determine β -amyloid1-40 and β -amyloid1-42 in four brain homogenate fractions.

Measurements: One brain hemisphere (including the bulbus olfactorius but without Cerebellum) of each animal was used for evaluation of brain A β 1-40 and A β 1-42 in four (4) brain fractions which are TBS, Triton X-100 and FA. A β 1-40

and A β 1-42 was using high sensitivity ELISA kits (TK40HS™; TK42HS™) manufactured by The GENETICS Company, Switzerland.

Results:

5

Levels of Soluble A β 1-40 and A β 1-42

TBS extract fractions contain the water soluble A β 1-40 and A β 1-42 fraction of the brain tissue while in the Triton x-100 fraction smaller polymers like protofibrils are solubilized. The formic acid (FA) fractions contain the insoluble polymerised A β .

10 Results are shown in Figures 18 to 23. Compound **138** significantly reduces A β 1-42 levels in the TBS (5 and 20mg/kg; Fig.19) and in the Triton x-100 fraction (5, 20 and 50mg/kg; Fig.21). In the TBS fractions (Fig. 18 and 19) but not in the Triton fractions (Fig. 20 and 21) a similar but, however, not significant effect can be seen in terms of A β 1-40 where all individual data points are very close together in
15 the two Compound **138** groups.

In the FA fraction, mean A β 1-42 values of all dosages are below that of the vehicle control whereby effects are significant in concentrations of 5 and 20mg/kg (Fig 23).

20

Thus, Compound **138** has a significant effect on APP processing and on generation of A β peptide, though the effects were more pronounced on A β 1-42. Clear A β 1-42 lowering effects can be seen across various brain fractions, which represent different polymerization states of β -amyloid (monomers, oligomers,
25 fibrils).

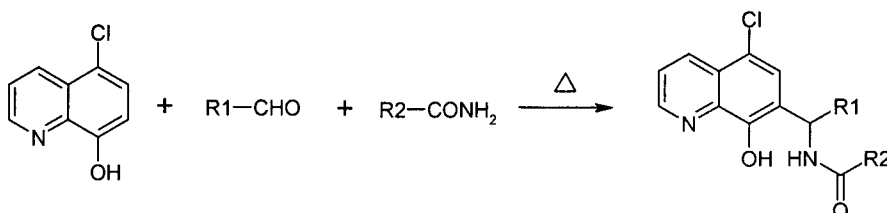
Conclusion:

In the brain, clear A β -amyloid 1-42 lowering effects of Compound **138** can be
30 seen. The most striking and unique result is the capacity of Compound **138** to penetrate across brain fractions (monomers, oligomers, fibrils) to lower A β 1-42 levels. The significance of the results is underlined by the fact that in this model,

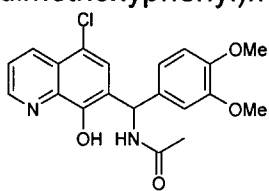
the A β 1-42 level is extremely high, even compared with other transgenic models such as Tg2576 mouse. Thus, even in this aggressive model, Compound 138 reduces A β 1-42 levels across brain fractions. Although a clearest effect was seen on A β 1-42, Compound 138 also reduced A β 1-40 to some extent in brain
5 fractions. The more pronounced effect of Compound 138 on the toxic A β 1-42 peptide leads to a reduction in the A β 1-42/A β 1-40 ratio, and this is beneficial therapeutically.

EXAMPLE 7: Synthesis of compounds from Series A

Compounds in series A can be prepared by employing the strategy depicted below. Groups other than the 5-Cl, e.g. F or Me, would be equally well tolerated in this chemistry.

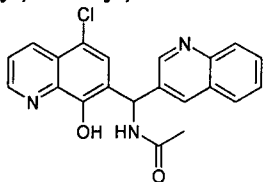
**Method A_1**

Example 7.1: N-[(5-chloro-8-hydroxyquinolin-7-yl)(3,4-dimethoxyphenyl)methyl]acetamide (Compound **7**) {Method A_1}



An intimate mixture of 5-chloro-8-hydroxyquinoline (1.5g, 8.7mmol), 3,4-dimethoxybenzaldehyde (2.85g, 17.4mmol) and acetamide (1.03g, 17.4mmol) was sealed in a microwave reactor vessel. The vessel was placed in a microwave reactor (Biotage Initiator) and heated to 180°C for 1 hour. The cooled reaction mixture was triturated with ethyl acetate and the resulting solid filtered off. This solid was further triturated with ethanol to yield the title compound as a white solid (2.2g, 65%). MS 387 (MH^+); ^1H NMR (DMSO- d_6), 400 MHz δ : 10.28 (bs, 1H), 8.93-8.97 (m, 1H), 8.74 (d, 1H), 8.47 (d, 1H), 7.69-7.75 (m, 2H), 6.91-6.94 (m, 1H), 6.87 (d, 1H), 6.74 (d, 1H), 6.62 (d, 1H), 3.70 (s, 3H), 3.71 (s, 3H), 1.94 (s, 3H).

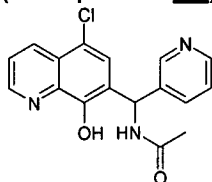
Example 7.2: N-[(5-chloro-8-hydroxyquinolin-7-yl)(quinolin-3-yl)methyl]acetamide (Compound **19**) {Method A_1}



An intimate mixture of 5-chloro-8-hydroxyquinoline (0.25g, 1.4mmol), quinoline-3-carbaldehyde (0.44g, 2.8mmol) and acetamide (0.165g, 2.8mmol) was sealed in a microwave reactor vessel. The vessel was placed in a microwave reactor (Biotage Initiator) and heated to 180°C for 0.5 hour. The cooled reaction mixture was triturated with ethyl acetate and the resulting solid filtered off. This solid was further triturated with ethanol to yield the title compound as a white solid (0.236g,

44%). MS 377 (M^+); ^1H NMR (DMSO-d_6), 400 MHz δ : 10.52 (bs, 1H), 8.96-9.01 (m, 2H), 8.85 (s, 1H), 8.51 (d, 1H), 8.11 (s, 1H), 7.95-8.01 (dd, 2H), 7.71-7.79 (m, 4H), 7.58 (t, 1H), 6.87 (d, 1H), 2.01 (s, 3H).

5 Example 7.3: N-((5-chloro-8-hydroxyquinolin-7-yl)(pyridin-3-yl)methyl)acetamide (Compound **32**) {Method A_1}

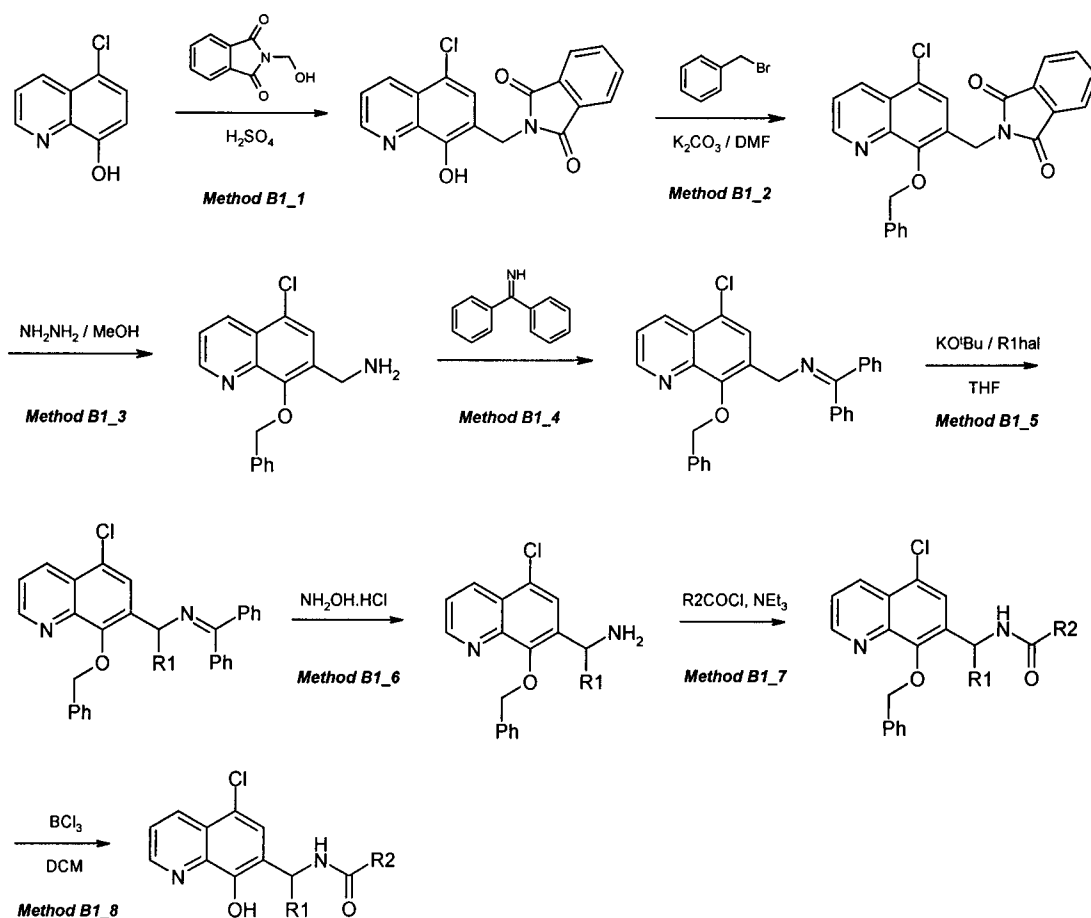


10 An intimate mixture of 5-chloro-8-hydroxyquinoline (0.25g, 1.4mmol), nicotinaldehyde (0.299g, 2.8mmol) and acetamide (0.165g, 2.8mmol) was sealed in a microwave reactor vessel. The vessel was placed in a microwave reactor (Biotage Initiator) and heated to 180°C for 0.5 hour. The cooled reaction mixture was triturated with ethyl acetate and the resulting solid filtered off. This solid was further triturated with ethanol to yield the title compound as a white solid (0.123g, 27%). MS 327 (M^+); ^1H NMR (DMSO-d_6), 400 MHz δ : 10.49 (bs, 1H), 8.97 (s, 1H), 8.90 (d, 1H), 8.47-8.51 (m, 3H), 7.72-7.77 (m, 2H), 7.64 (d, 1H), 7.33-7.36 (dd, 1H), 6.70 (d, 1H), 1.97 (s, 3H).

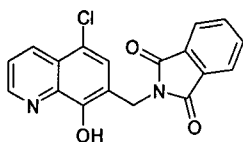
15

EXAMPLE 8: Synthesis of compounds from Series B1

Compounds in series B1 can be prepared by employing the strategy depicted below. This complements the synthesis employed for Series A compounds and is particularly appropriate for compounds where R1 is alkyl c.f. aryl. Groups other than the 5-Cl, e.g. F or Me, would be equally well tolerated in this chemistry.



Method B1_1 Synthesis of intermediate: 2-((5-chloro-8-hydroxyquinolin-7-yl)methyl)-1H-indole-1,3(2H)-dione

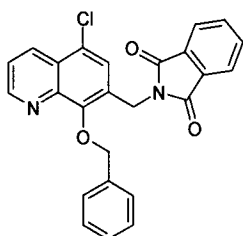


2-(Hydroxymethyl)-1H-indole-1,3(2H)-dione (14.8g, 83mmol) was added portionwise to a well stirred solution of 5-chloro-8-hydroxyquinoline (15g, 83mmol) in concentrated sulphuric acid (150ml) cooled to 0°C. After addition was complete the reaction was heated to 100 °C overnight. The mixture was allowed to cool to RT then poured onto crushed ice (1000ml) and the resulting yellow precipitate filtered, washed with water and dried *in vacuo* overnight. The solid

was triturated with ethanol and hexane to yield an off-white solid (16.7g, 59%). MS 338 (M^+); ^1H NMR (DMSO-d_6), 400 MHz δ : 10.24 (bs, 1H), 8.95 (d, 1H), 8.48 (d, 1H), 7.41-7.92 (m, 4H), 7.72 (dd, 1H), 7.57 (s, 1H), 4.96 (s, 2H).

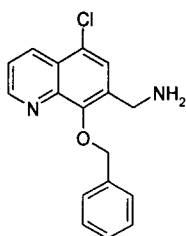
5

10 Method B1_2 Synthesis of intermediate: 2-((8-(benzyloxy)-5-chloroquinolin-7-yl)methyl)-1H-isindole-1,3(2H)-dione



15 2-((5-chloro-8-hydroxyquinolin-7-yl)methyl)-1H-isindole-1,3(2H)-dione (16.68g, 49mmol), benzyl bromide (12.63g, 73.5mmol) and potassium carbonate (13.61g, 98mmol) were dissolved/suspended in dimethylformamide (250ml) and heated to 60°C for 1.5 hours. The reaction was poured into cold water (1000ml) and the off-white precipitate filtered and washed well with water. The solid was dried *in vacuo* to yield the pure product (20.8g, 98%). MS 446 ($[\text{M}+\text{H}_2\text{O}]^+$); ^1H NMR (DMSO-d_6), 400 MHz δ : 9.09 (d, 1H), 8.55 (d, 1H), 7.83-7.91 (m, 4H), 7.75 (dd, 1H), 7.64 (s, 1H), 7.55-7.61 (m, 2H), 7.347.44 (m, 3H), 5.53 (s, 2H), 4.96 (s, 2H).

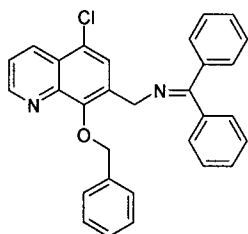
25 Method B1_3 Synthesis of intermediate: 1-(8-(benzyloxy)-5-chloroquinolin-7-yl)methanamine



30 Hydrazine monohydrate (2.28ml, 46.6mmol) was added to a suspension of 2-((8-(benzyloxy)-5-chloroquinolin-7-yl)methyl)-1H-isindole-1,3(2H)-dione (2.0g, 4.7mmol) in methanol (50ml). The mixture was refluxed for 1hour and then solvent removed *in vacuo*. The residue was triturated with hot ethanol (~10ml) and filtered hot. The ethanol was concentrated to yield a light brown solid (1.1g, 79%) used in subsequent reactions without any further purification. MS 299 (MH^+); ^1H NMR (CDCl_3), 400 MHz δ : 9.02 (d, 1H), 8.54 (d, 1H), 7.59 (s, 1H), 7.51 (dd, 1H), 7.41-7.44 (m, 2H), 7.31-7.39 (m, 3H), 5.50 (s, 2H), 3.87 (s, 2H).

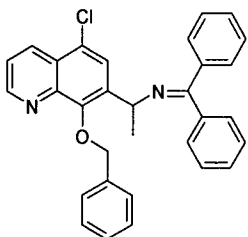
35

Method B1_4 Synthesis of intermediate: 1-(8-(benzyloxy)-5-chloroquinolin-7-yl)-N-(diphenylmethylene)methanamine

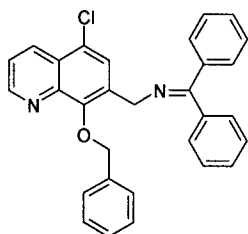


A mixture of 1-(8-(benzyloxy)-5-chloroquinolin-7-yl)methanamine (407mg, 1.36mmol) and benzophenone imine (247mg, 1.36mmol) were heated together at 60°C. After 1hour the resultant solid was triturated with methanol and filtered to yield the title compound as a pale brown solid (480mg, 76%). MS 463 (M⁺); ¹H NMR (DMSO-d₆), 400 MHz δ: 9.06 (d, 1H), 8.57 (d, 1H), 7.96 (s, 1H), 7.73 (dd, 1H), 7.54-7.62 (m, 5H), 7.39-7.48 (m, 3H), 7.20-7.31 (m, 7H), 5.29 (s, 2H), 4.61 (s, 2H).

Method B1_5 Synthesis of intermediate: 1-(8-(benzyloxy)-5-chloroquinolin-7-yl)-N-(diphenylmethylene)ethanamine

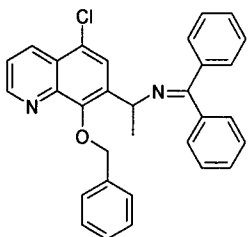


Methyl iodide (147mg, 1.03mmol) and 1-(8-(benzyloxy)-5-chloroquinolin-7-yl)-N-(diphenylmethylene)methanamine (480mg, 1.03mmol) were dissolved in anhydrous tetrahydrofuran (0.5ml) and cooled to 0°C. To this mixture was added dropwise a solution of potassium *tert*-butoxide (128mg, 1.16mmol) in tetrahydrofuran (0.13ml). The mixture was allowed to warm to RT and after 1 hour the reaction was concentrated. The residue was dissolved in ethyl acetate and washed with water and brine. The organic extract was dried over anhydrous magnesium sulphate and reduced *in vacuo* to yield the title compound as a white solid (480mg, 97%). MS 477 (M⁺); ¹H NMR (CDCl₃), 400 MHz δ: 8.89 (d, 1H), 8.47 (d, 1H), 8.15 (s, 1H), 7.60 (d, 2H), 7.42 (dd, 1H), 7.15-7.38 (m, 10H), 7.03-7.09 (m, 3H), 5.20 (d, 1H), 5.18 (q, 1H), 4.81 (d, 1H), 1.23 (d, 3H)).



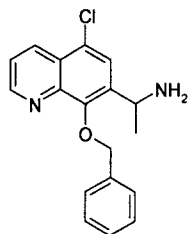
A mixture of 1-(8-(benzyloxy)-5-chloroquinolin-7-yl)methanamine (407mg, 1.36mmol) and benzophenone imine (247mg, 1.36mmol) were heated together at 60°C. After 1hour the resultant solid was triturated with methanol and filtered to yield the title compound as a pale brown solid (480mg, 76%). MS 463 (M⁺); ¹H NMR (DMSO-d₆), 400 MHz δ: 9.06 (d, 1H), 8.57 (d, 1H), 7.96 (s, 1H), 7.73 (dd, 1H), 7.54-7.62 (m, 5H), 7.39-7.48 (m, 3H), 7.20-7.31 (m, 7H), 5.29 (s, 2H), 4.61 (s, 2H).

Method B1_5 Synthesis of intermediate: 1-(8-(benzyloxy)-5-chloroquinolin-7-yl)-N-(diphenylmethylene)ethanamine



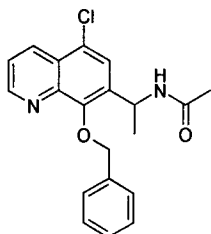
Methyl iodide (147mg, 1.03mmol) and 1-(8-(benzyloxy)-5-chloroquinolin-7-yl)-N-(diphenylmethylene)methanamine (480mg, 1.03mmol) were dissolved in anhydrous tetrahydrofuran (0.5ml) and cooled to 0°C. To this mixture was added dropwise a solution of potassium *tert*-butoxide (128mg, 1.16mmol) in tetrahydrofuran (0.13ml). The mixture was allowed to warm to RT and after 1 hour the reaction was concentrated. The residue was dissolved in ethyl acetate and washed with water and brine. The organic extract was dried over anhydrous magnesium sulphate and reduced *in vacuo* to yield the title compound as a white solid (480mg, 97%). MS 477 (M⁺); ¹H NMR (CDCl₃), 400 MHz δ: 8.89 (d, 1H), 8.47 (d, 1H), 8.15 (s, 1H), 7.60 (d, 2H), 7.42 (dd, 1H), 7.15-7.38 (m, 10H), 7.03-7.09 (m, 3H), 5.20 (d, 1H), 5.18 (q, 1H), 4.81 (d, 1H), 1.23 (d, 3H)).

Method B1_6 Synthesis of intermediate: 1-(8-(benzyloxy)-5-chloroquinolin-7-yl)ethanamine



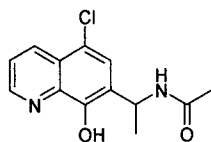
5 To a solution of 1-(8-(benzyloxy)-5-chloroquinolin-7-yl)-N-(diphenylmethylene)ethanamine (550mg, 1.15mmol) in methanol (5ml) was added hydroxylamine hydrochloride (144mg, 2.07mmol) and the mixture stirred at RT overnight. The reaction was concentrated and the residue treated with
10 hydrochloric acid (5ml, 5% aqueous solution) and extracted with ethyl acetate to remove benzophenone. The aqueous layer was basified with sodium hydroxide solution (5M) and extracted with ethyl acetate (3x5ml). The combined organic extracts were washed with brine, dried over anhydrous magnesium sulphate, filtered and concentrated to yield the title compound (230mg, 64%). MS 313 (M⁺); ¹H NMR (CDCl₃), 400 MHz δ: 9.00 (d, 1H), 8.49 (d, 1H), 7.68 (s, 1H), 7.51 (dd, 1H), 7.31-7.44 (m, 5H), 5.50 (q, 2H), 4.59 (q, 1H), 1.46 (bs, 2H), 1.26 (d, 3H)).

20 Method B1_7 Synthesis of intermediate: N-(1-(8-(benzyloxy)-5-chloroquinolin-7-yl)ethyl)acetamide



25 To an ice-cold stirred solution of 1-(8-(benzyloxy)-5-chloroquinolin-7-yl)ethanamine (220mg, 0.7mmol) and triethylamine (0.12ml, 0.84mmol) in dichloromethane (2.5ml) was added dropwise a solution of acetyl chloride (66mg, 0.84mmol) in dichloromethane (0.5ml). The reaction was stirred at RT overnight then diluted with dichloromethane (5ml). The mixture was washed with
30 sequentially with saturated sodium carbonates solution, water and brine and dried over anhydrous magnesium sulphate. Removal of solvent *in vacuo* yielded the title compound (240mg, 96%). MS 354 (M⁺); ¹H NMR (CDCl₃), 400 MHz δ: 8.90 (d, 1H), 8.44 (d, 1H), 7.54 (d, 2H), 7.40-7.45 (m, 2H), 7.26-7.37 (m, 3H), 6.22 (bd, 1H), 5.61 (d, 1H), 5.46 (d, 1H), 5.36 (q, 1H), 1.77 (s, 3H), 1.36 (d, 3H).

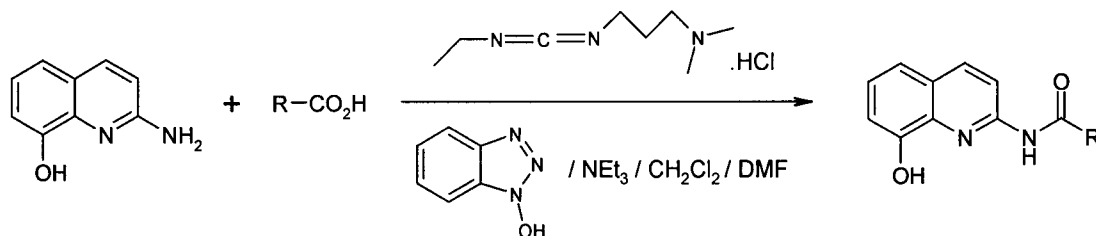
Example 8.1 Synthesis of: N-(1-(5-chloro-8-hydroxyquinolin-7-yl)ethyl)acetamide
(Compound **47**)



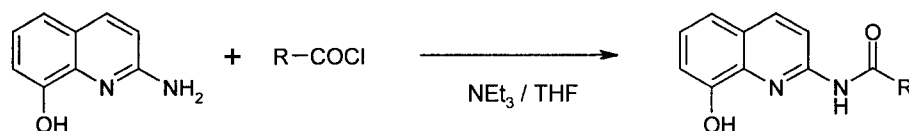
5 To an ice-cold stirred solution of N-(1-(8-(benzyloxy)-5-chloroquinolin-7-yl)ethyl)acetamide (0.7g, 1.97mmol) in dichloromethane (20ml) was added a solution of boron trichloride (1M in dichloromethane, 4ml, 4mmol) in dichloromethane (5ml) under a nitrogen atmosphere. The mixture was then
10 stirred at RT for 3 hours. The reaction was then cautiously poured into ice-cold water (100ml) and extracted with dichloromethane. The combined organic extracts were washed with brine, dried over anhydrous magnesium sulphate, filtered and concentrated. The crude material was purified by chromatography on silica (0-10%MeOH:100-90% EtOAc) to yield the title compound as a white solid
15 (0.45g, 86%). MS 265 (M⁺); ¹H NMR (DMSO-d₆), 400 MHz δ: 10.17 (bs, 1H), 8.95 (d, 1H), 8.47 (d, 1H), 8.39 (d, 1H), 7.70 (dd, 1H), 7.64 (s, 1H), 5.41 (q, 1H), 1.88 (s, 3H), 1.35 (d, 3H).

EXAMPLE 9: Synthesis of compounds from Series B2

20 Compounds in series B2 can be prepared by either of the methods depicted below:

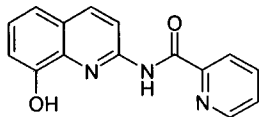


Method B2_1



Method B2_2

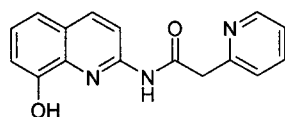
Example 9.1: N-(8-hydroxyquinolin-2-yl)pyridine-2-carboxamide (Compound **69**)
{Method B2_2}



5

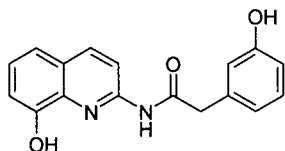
To a suspension of picolinoyl chloride hydrochloride (89mg, 0.5mmol) in tetrahydrofuran at 0°C was added triethylamine (0.42ml, 3mmol) and 2-amino-8-hydroxyquinoline (160mg, 1.0mmol). The mixture was allowed to warm to RT and left to stir overnight. Solvent was removed *in vacuo* and the crude material purified by HPLC to yield the desired product (15mg, 11%). MS 265 (M⁺); ¹H NMR (DMSO-d₆), 400 MHz δ: 10.79 (bs, 1H), 9.70 (bs, 1H), 8.81 (d, 1H), 8.53 (d, 1H), 8.43 (d, 1H), 8.28 (d, 1H), 8.16 (t, 1H), 7.75-7.80 (m, 1H), 7.36-7.41 (m, 2H), 7.08 (d, 1H).

Example 9.2: N-(8-hydroxyquinolin-2-yl)-2-pyridin-2-ylacetamide (Compound **70**)
{Method B2_1}



4-Pyridylacetic acid hydrochloride (87mg, 0.5mmol) was dissolved in dimethylformamide (4ml). To this solution was added hydroxybenzotriazole (68mg, 0.5mmol), N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (96mg, 0.5mmol) and triethylamine (0.28ml, 2.0mmol) and the mixture stirred at RT for 30 mins. 2-Amino-8-hydroxyquinoline (80mg, 0.5mmol) was added to the reaction which was stirred at RT overnight. Solvent was removed *in vacuo*, the residue dissolved in dichloromethane (2ml) and washed with water. The organic phase was concentrated *in vacuo* and the crude product purified by HPLC to yield the desired product (11mg, 8%). MS 280 (MH⁺); ¹H NMR (DMSO-d₆), 400 MHz δ: 11.00 (bs, 1H), 9.48 (bs, 1H), 8.52 (d, 2H), 8.27 (d, 1H), 8.21 (d, 1H), 7.39 (d, 2H), 7.28-7.34 (m, 2H), 7.07 (d, 1H), 3.88 (s, 2H). (10mg, 7%). MS 280 (MH⁺); ¹H NMR (DMSO-d₆), 400 MHz δ 10.92 (bs, 1H), 9.35-9.55 (bs, 1H), 8.53 (d, 1H), 8.17-8.29 (m, 2H), 7.78 (t, 1H), 7.44 (d, 1H), 7.26-7.35 (m, 3H), 7.08 (d, 1H), 4.04 (bs, 2H).

Example 9.3: 2-(3-hydroxyphenyl)-N-(8-hydroxyquinolin-2-yl)acetamide
(Compound **71**) {Method B2_1}



5

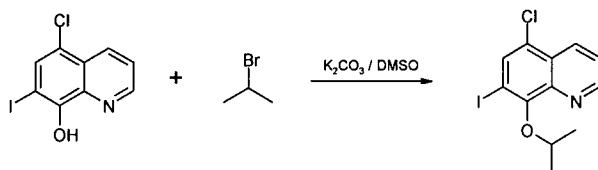
The title compound was similarly prepared according to Method B2_1 as described above in example 9.2. Yield = 12mg (9%). MS 295 (MH⁺); ¹H NMR (DMSO-d₆), 400 MHz δ 10.84 (bs, 1H), 9.43 (bs, 1H), 9.34 (bs, 1H), 8.21-8.28 (dd, 2H), 7.27-7.35 (m, 2H), 7.11 (t, 1H), 7.07 (d, 1H), 6.81 (s, 1H), 6.79 (d, 1H), 6.64 (d, 1H), 3.70 (s, 2H).

10

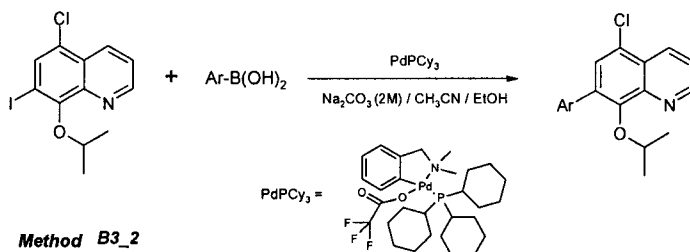
EXAMPLE 10: Synthesis of compounds from Series B3

Compounds in series B3 can be prepared by employing the scheme exemplified below:

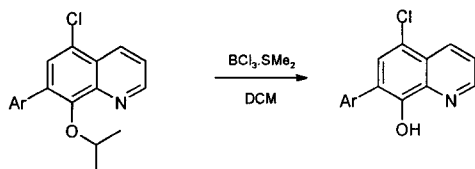
15



Method B3_1

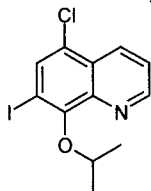


Method B3_2



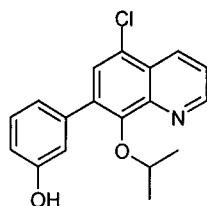
Method B3_3

Method B3_1 Synthesis of intermediate: 5-chloro-7-iodo-8-isopropoxyquinoline



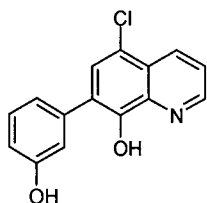
To a solution of 5-chloro-7-iodo-8-hydroxyquinoline (18g, 58.9mmol) in dimethylsulfoxide was added potassium carbonate (32.57g, 235.6mmol) and 2-bromopropane (10.87g, 88.4mmol). The mixture was stirred at RT overnight and then poured into saturated ammonium chloride solution. This mixture was extracted with dichloromethane (3 x 300ml). The combined organic extracts were washed successively with sodium hydroxide (2M), water and brine and dried over anhydrous magnesium sulphate. Removal of solvent in vacuo and chromatography on silica eluting with ethyl acetate; hexane mixtures yielded the desired compound (11.0g, 55%). MS 347 (M⁺); ¹H NMR (CDCl₃), 400 MHz δ: 8.92 (d, 1H), 8.50 (d, 1H), 7.97 (s, 1H), 7.51 (dd, 1H), 5.38 (sept, 1H), 1.42 (d, 6H).

Method B3_2 Synthesis of intermediate: 3-(5-chloro-8-isopropoxyquinolin-7-yl)phenol



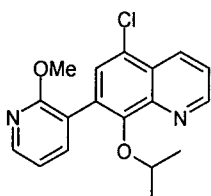
5-chloro-7-iodo-8-isopropoxyquinoline (500mg, 1.7mmol), (3-hydroxyphenyl)boronic acid (238mg, 1.7mmol) and PdPCy₃ (18mg, 0.028mmol) were dissolved/suspended in a mixture of acetonitrile/water (4ml:1ml respectively) and sodium carbonate solution (2M, 5ml) added. The mixture was then heated to 80°C under N₂ with stirring for 1 hour. TLC indicated complete consumption of starting iodo compound. The solvent was removed in vacuo, the residue dissolved/suspended in ethyl acetate and washed with water and brine. The organic phase was dried over anhydrous magnesium sulphate and concentrated in vacuo to yield essentially pure product (tlc and nmr) (350mg, 77%). MS 314 (M⁺); ¹H NMR (CDCl₃), 400 MHz δ: 9.01 (bs, 1H), 8.92 (d, 1H), 8.51 (d, 1H), 7.55 (s, 1H), 7.47 (dd, 1H), 7.35 (t, 1H), 7.32 (s, 1H), 6.97-7.02 (m, 2H), 4.36 (sept, 1H), 1.12 (d, 6H).

Example 10.1: 5-chloro-7-(3-hydroxyphenyl)quinolin-8-ol (Compound **57**)
{Method B3_3}



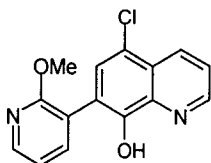
3-(5-chloro-8-isopropoxyquinolin-7-yl)phenol (475mg, 1.52mmol) was dissolved in dichloromethane (4ml) and cooled to -78°C under N₂. A solution of boron trichloride dimethylsulfide complex in dichloromethane (2M, 3.04ml, 6.08mmol) was added dropwise and the mixture stirred at -78°C for 2 hours. Methanol (20ml) was added cautiously and the reaction allowed to warm to RT. Solvent was removed in vacuo and a further aliquot of methanol (20ml) added and again concentrated in vacuo. This was repeated a further two times to yield the crude product which was purified by HPLC to give the title compound (315mg, 76%). MS 272 (MH⁺); ¹H NMR (DMSO-d₆), 400 MHz δ 9.5-10.5 (vbs, 1H), 9.02 (d, 1H), 8.56 (d, 1H), 7.78 (dd, 1H), 7.72 (s, 1H), 7.29 (t, 1H), 7.17 (bs, 1H), 7.11 (d, 1H), 6.80 (d, 1H).

Method B3_2 Synthesis of intermediate: 5-chloro-8-isopropoxy-7-(2-methoxypyridin-3-yl)quinoline



The title compound was similarly prepared according to Method B3_2 as described above. MS 329 (MH⁺); ¹H NMR (CDCl₃), 400 MHz δ 8.96 (d, 1H), 8.54 (d, 1H), 8.21 (d, 1H), 7.77 (d, 1H), 7.63 (s, 1H), 7.48-7.53 (dd, 1H), 6.99-7.02 (dd, 1H), 4.57 (sept, 1H), 3.97 (s, 3H), 1.06 (d, 6H).

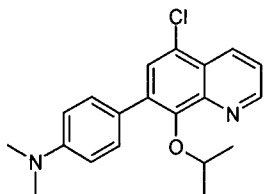
Example 10.2: 5-chloro-7-(2-methoxypyridin-3-yl)quinolin-8-ol (Compound **61**)
{Method B3_3}



The title compound was similarly prepared from 5-chloro-8-isopropoxy-7-(2-methoxypyridin-3-yl)quinoline according to Method B3_3 as described above in example 10.1. MS 288 (MH⁺); ¹H NMR (DMSO-d₆), 400 MHz δ 10.15 (bs, 1H),

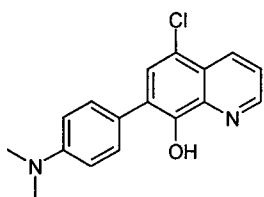
9.00 (d, 1H), 8.54 (d, 1H), 8.23 (d, 1H), 7.76-7.81 (m, 2H), 7.64 (s, 1H), 7.12 (dd, 1H), 3.86 (s, 3H).

Method B3_2 Synthesis of intermediate: 4-(5-chloro-8-isopropoxyquinolin-7-yl)-N,N-dimethylaniline



The title compound was similarly prepared according to Method B3_2 as described above. MS 341 (MH⁺); ¹H NMR (CDCl₃), 400 MHz δ 8.95 (d, 1H), 8.49 (d, 1H), 7.69 (s, 1H), 7.63 (d, 2H), 7.43-7.46 (dd, 1H), 6.80 (d, 2H), 4.51 (sept, 1H), 3.02 (s, 6H), 1.11 (d, 6H).

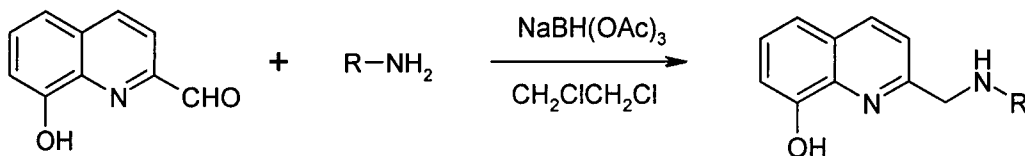
Example 10.3: 5-chloro-7-(4-(dimethylamino)phenyl)quinolin-8-ol (Compound **60**)



The title compound was similarly prepared from 4-(5-chloro-8-isopropoxyquinolin-7-yl)-N,N-dimethylaniline according to Method B3_3 as described above in example 10.1. MS 300 (MH⁺); ¹H NMR (DMSO-d₆), 400 MHz δ 9.02 (d, 1H), 8.57 (d, 1H), 7.77-7.86 (m, 4H), 7.55-7.68 (bs, 2H), 3.13 (s, 6H).

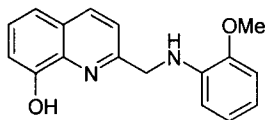
EXAMPLE 11: Synthesis of compounds from Series B4

Compounds in series B4 can be prepared by employing the scheme exemplified below:



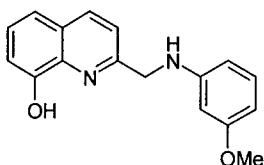
Method B4-1

Example 11.1: 2-(((2-methoxyphenyl)amino)methyl)quinolin-8-ol (Compound **77**)
{Method B4_1}



To a solution of 8-hydroxyquinoline-2-carbaldehyde (87mg, 0.5mmol) in 1,2-dichloroethane (2ml) was added 2-methoxyaniline (62mg, 0.5mmol) and the mixture stirred for 30 minutes before the addition of sodium triacetoxyborohydride (95mg, 45mmol). The reaction was then stirred at RT overnight before being evaporated to dryness *in vacuo*. The residue was taken up in dichloromethane and washed with water. The aqueous phase was back extracted with dichloromethane and the combined organic extracts dried and reduce *in vacuo* to yield the crude product which was purified by HPLC (74mg, 53%). MS 281 (MH⁺); ¹H NMR (DMSO-d₆), 400 MHz δ 9.51 (bs, 1H), 8.22 (d, 1H), 7.52 (d, 1H), 7.32-7.43 (q, 2H), 7.09 (d, 1H), 6.83 (d, 1H), 6.67 (t, 1H), 6.48-6.56 (dd, 2H), 5.96 (t, 1H), 4.59 (d, 2H), 3.84 (s, 3H).

Example 11.2: 2-(((3-methoxyphenyl)amino)methyl)quinolin-8-ol (Compound **75**)
{Method B4_1}

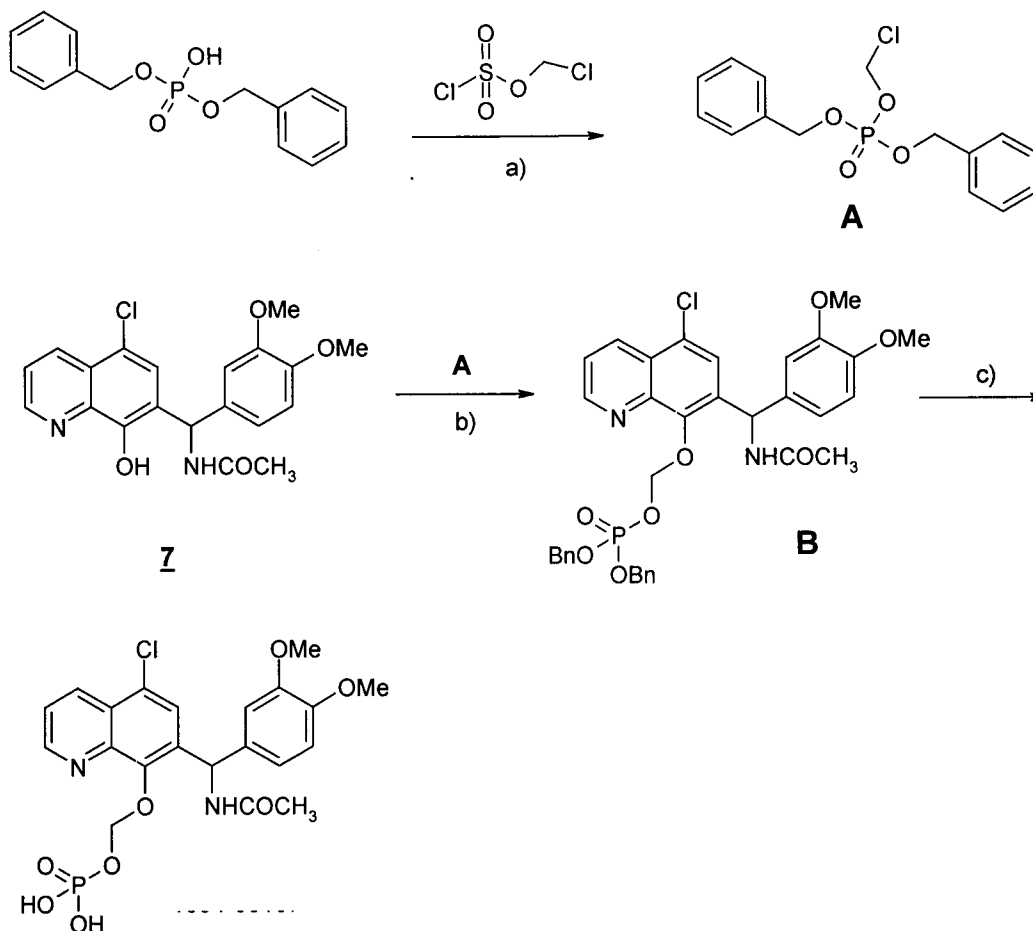


The title compound was similarly prepared according to Method B4_1 as described above in example 11.1. Yield = 71mg (51%). MS 281 (MH⁺); ¹H NMR (DMSO-d₆), 400 MHz δ 9.74 (bs, 1H), 8.27 (d, 1H), 7.53 (d, 1H), 7.36-7.45 (q, 2H), 7.11 (d, 1H), 7.08 (t, 1H), 6.70 (t, 1H), 6.36 (d, 1H), 6.32 (s, 1H), 6.16 (d, 1H), 4.54 (d, 2H), 3.69 (s, 3H).

EXAMPLE 12: Synthesis of phosphate pro-drugs

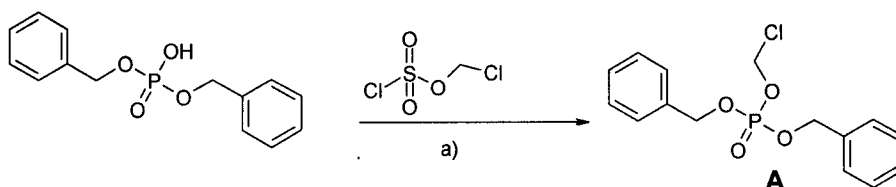
Phosphate Pro-drug (compound **5**)

The scheme and protocols discussed in this section could equally well be applied to other compounds in the series.

Synthetic scheme:

5

- a) NaHCO_3 , $n\text{-Bu}_4\text{NHSO}_4$, $\text{H}_2\text{O-CH}_2\text{Cl}_2$, $0^\circ\text{C} \sim \text{RT.}$;
 b) K_2CO_3 , DMF, 25°C ;
 c) 1,4-cyclohexadiene, 10% Pd/C, EtOH.

10 Synthesis of benzyl chloromethyl phosphate A

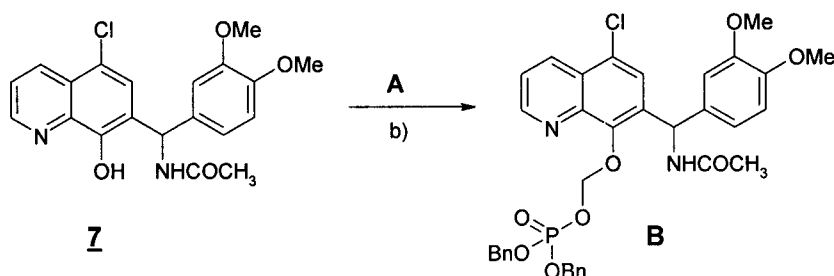
- 15 Dibenzyl phosphate (2.64 g, 9.48 mmol), sodium bicarbonate (3.18 mL, 11.97 mmol) and tetra-n-butylammonium hydrogen sulfate (3.2 g, 9.48 mmol) were dissolved in water (80 mL). Dichloromethane (DCM, 90 mL) was added and the mixture was vigorously stirred at 0°C for 10 min, followed by the addition of chloromethyl chlorosulfate (1.17 mL, 11.37 mmol) in DCM (15 mL) with
 20 continuous vigorous stirring overnight at room temperature. The organic layer

was separated, washed with brine, dried (MgSO_4) and evaporated. The residue was purified by flash silica gel column chromatography using ethyl acetate/hexane (1:1) as eluent to give 1.39 g (44.8 %) of pure material.

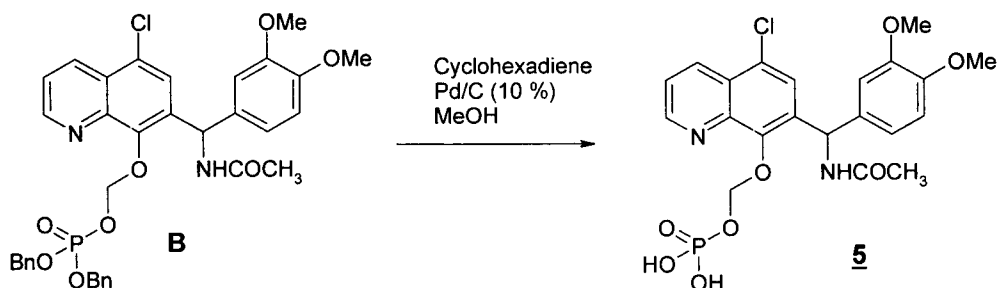
MS 327 (MH^+); ^1H NMR (CDCl_3), 400 MHz δ 5.09 (d, 2H, $J = 8$ Hz), 5.62 (d, 2H, $J = 16$ Hz), 7.41-7.28 (m, 10H).

Synthesis of the Pro-drug derivative:

Synthesis of compound 5:



- Protocol:** Compound 7 (0.5 g, 1.29 mmol), potassium carbonate solid (0.36 g, 2.58 mmol) were suspended in dry N, N-Dimethylformamide (DMF, 5 mL), and then dibenzyl chloromethyl phosphate (**A**, 0.42 g, 1.29 mmol) dissolved in DMF (2 mL) was added while stirring at 0 °C under a N_2 atmosphere. The reaction mixture was stirred for 16 hrs at room temperature under a N_2 atmosphere. The reaction mixture was poured into water (50 mL), and extracted with EtOAc (3 x 25 mL). The combined extracts were washed with brine, dried (MgSO_4) and evaporated to dryness. An LC-MS of the crude residue showed that the oily residue was 97 % pure at 254 nm. So no purification was attempted at that stage and the oily residue was used in the next step.
- MS 677 (MH^+) and isotope pattern consistent; ^1H NMR (DMSO), 400 MHz δ 1.99 (s, 3H), 3.64 (s, 3H), 3.68 (s, 3H), 4.72 (d, 1H, $J = 4$ Hz), 4.74 (d, 1H, $J = 3.5$ Hz), 4.89 (d, 2H, $J = 7.8$ Hz), 5.96-6.05 (m, 2H), 6.47 (d, 1H, $J = 8.08$ Hz), 6.58 (d, 1H, $J = 8.33$ Hz), 6.64 (dd, 1H, $J = 8.58$ & 2.02 Hz), 6.80 (d, 1H, $J = 2.02$ Hz), 7.10-7.23 (m, 10H), 7.43 (dd, 1H, $J = 8.58$ & 8.58 Hz), 7.54 (s, 1H), 7.55 (d, 1H, $J = 8.33$ Hz), 8.45 (dd, 1H, $J = 6.82$ & 1.76 Hz), 8.80 (dd, 1H, $J = 4.04$ & 1.76 Hz).

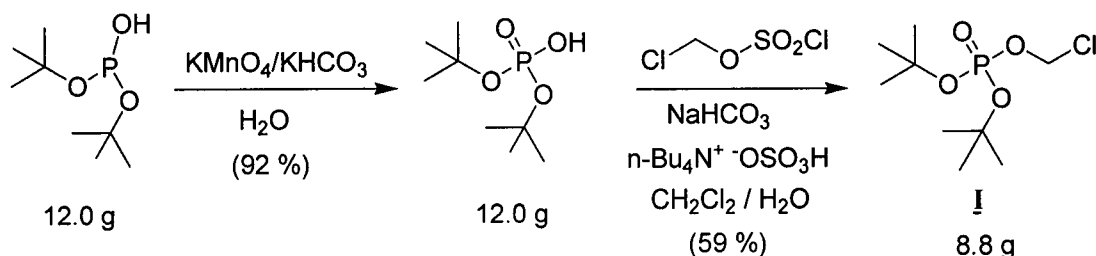
Synthesis of compound 5:

- 5 **Protocol:** To a suspension of 10 % Pd/C in EtOH was added drop-wise under a N₂ atmosphere a solution of B in EtOH (3 mL), followed by cyclohexadiene (0.25 mL; 10 eq, 2.58 mmol). The reaction mixture was stirred for 12 hrs at room temperature. The catalyst was filtered on celite and the filtrate evaporated nearly to dryness (~10 % of EtOH being still present after the evaporation). The crude residue was then triturated with dry Et₂O. The solid that precipitated was filtered and rinsed with Et₂O to give 0.065 g (60%) of pure material.
- 10 MS 497 (MH⁺) and isotope pattern consistent; ¹H NMR (DMSO), 400 MHz δ 1.52 (s, 3H), 3.3 (s, 3H), 3.49 (s, 3H), 5.63 (m, 2H), 6.40 (d, 1H, J = 9.8 Hz), 6.61 (m, 2H), 6.88 (s, 1H), 7.46 (m, 2H), 8.29 (d, 1H, J = 8.58 Hz), 8.74 (d, 1H, J = 3.2 Hz), 8.83 (d, 1H, 7.7 Hz).
- 15

Phosphate Pro-drug (compound 138)

Step 1: synthesis of P(OBut)₂(=O)(OCH₂Cl) I

- 20 I is synthesized according to the following scheme:



Step 2: synthesis of 138

- 25 I (8.8 g) is reacted with compound 7 (as synthesized in Example 7.1) in the presence of K₂CO₃ in dimethylformamide (DMF) to form ((7-((acetylamino)(3,4-dimethoxyphenyl)methyl)-5-chloroquinolin-8-yl)oxy)methyl di-ter-butyl phosphate II (9.6 g, HPLC yield 96%). II (5.0 g) is then reacted with HCl in a mixture of EtOAc/Et₂O to form 138 in a 85% yield.

Table 1

ACTIVITY TABLE OF CLK-1 INHIBITION ACTIVE COMPOUNDS		
Compound number	Compound chemical name	IC50 μM
<u>1</u>	N-[(5-chloro-8-hydroxyquinolin-7-yl)(3,4-dimethoxyphenyl)methyl]-2-methylpropanamide	5
<u>2</u>	N-((3,4-dimethoxyphenyl)(8-hydroxyquinolin-7-yl)methyl)acetamide	5
<u>3</u>	N-((5-chloro-8-hydroxyquinolin-7-yl)(3,4-dimethoxyphenyl)methyl)benzamide	10
<u>4</u>	N-[(5-chloro-8-hydroxyquinolin-7-yl)(3,4-dimethoxyphenyl)methyl]pentanamide	>10
<u>5</u>	((7-((acetylamino)(3,4-dimethoxyphenyl)methyl)-5-chloroquinolin-8-yl)oxy)methyl dihydrogen phosphate	10
<u>6</u>	((7-((acetylamino)(3,4-dimethoxyphenyl)methyl)-5-chloroquinolin-8-yl)oxy)methyl disodium phosphate	10
<u>7</u>	N-[(5-chloro-8-hydroxyquinolin-7-yl)(3,4-dimethoxyphenyl)methyl]acetamide	1.5
<u>8</u>	N-((5-chloro-8-hydroxyquinolin-7-yl)(3,4-dimethoxyphenyl)methyl)-N-methylacetamide	3
<u>9</u>	N-[(5-chloro-8-hydroxyquinolin-7-yl)(3,4-dimethoxyphenyl)methyl]butanamide	3.5
<u>10</u>	N-[(5-chloro-8-hydroxyquinolin-7-yl)(3,4-dimethoxyphenyl)methyl]propanamide	4
<u>11</u>	N-((5-chloro-8-hydroxyquinolin-7-yl)(3,4-dimethoxyphenyl)methyl)-2,2-diphenylacetamide	7.5
<u>12</u>	N-((5-chloro-8-hydroxyquinolin-7-yl)(3,4-dimethoxyphenyl)methyl)-2-phenylacetamide	10
<u>13</u>	7-(amino(3,4-dimethoxyphenyl)methyl)-5-chloroquinolin-8-ol	12.5
<u>14</u>	N-(1-(5-chloro-8-hydroxyquinolin-7-yl)-2-phenylethyl)acetamide	3.5
<u>15</u>	N-((2-butyl-1H-imidazol-4-yl)(5-chloro-8-hydroxyquinolin-7-yl)methyl)acetamide	8
<u>16</u>	N-((5-chloro-8-hydroxyquinolin-7-yl)(2-methoxypyridin-3-yl)methyl)acetamide	1.5
<u>17</u>	N-((5-chloro-8-hydroxyquinolin-7-yl)(quinolin-4-	3.5

	yl)methyl)acetamide	
<u>18</u>	N-((5-chloro-8-hydroxyquinolin-7-yl)(2-morpholin-4-ylpyridin-3-yl)methyl) acetamide	6
<u>19</u>	N-((5-chloro-8-hydroxyquinolin-7-yl)(quinolin-3-yl)methyl)acetamide	8.5
<u>20</u>	N-((5-chloro-8-hydroxyquinolin-7-yl)(3-furyl)methyl)acetamide	1.5
<u>21</u>	4-((acetylamino)(5-chloro-8-hydroxyquinolin-7-yl)methyl) methylbenzoate	1.5
<u>22</u>	3-((acetylamino)(5-chloro-8-hydroxyquinolin-7-yl)methyl) methylbenzoate	1.5
<u>23</u>	N-((5-chloro-8-hydroxyquinolin-7-yl)(4-cyanophenyl)methyl)acetamide	1.5
<u>24</u>	N-((5-chloro-8-hydroxyquinolin-7-yl)(4-pyridin-2-ylphenyl)methyl)acetamide	2
<u>25</u>	N-((5-chloro-8-hydroxyquinolin-7-yl)(4-(1H-pyrazol-1-yl)phenyl)methyl)acetamide	2
<u>26</u>	N-((5-chloro-8-hydroxyquinolin-7-yl)(4-hydroxyphenyl)methyl)acetamide	4
<u>27</u>	N-((5-chloro-8-hydroxyquinolin-7-yl)(3-hydroxyphenyl)methyl)acetamide	10
<u>28</u>	N-((5-chloro-8-hydroxyquinolin-7-yl)(6-methoxypyridin-3-yl)methyl)acetamide	3.5
<u>29</u>	N-((5-chloro-8-hydroxyquinolin-7-yl)(pyridin-2-yl)methyl)acetamide	4
<u>30</u>	N-((5-chloro-8-hydroxyquinolin-7-yl)(pyridin-4-yl)methyl)acetamide	5
<u>31</u>	N-((5-chloro-8-hydroxyquinolin-7-yl)(pyridin-4-yl)methyl)acetamidehydrochloride	10
<u>32</u>	N-((5-chloro-8-hydroxyquinolin-7-yl)(pyridin-3-yl)methyl)acetamide	5
<u>33</u>	N-((5-chloro-8-hydroxyquinolin-7-yl)(pyridin-3-yl)methyl)acetamidehydrochloride	>10
<u>34</u>	N-((5-chloro-8-hydroxyquinolin-7-yl)(8-hydroxyquinolin-2-yl)methyl)acetamide	12.5
<u>35</u>	N-((5-chloro-8-hydroxyquinolin-7-yl)(6-morpholin-4-ylpyridin-3-yl)methyl) acetamide	2.5
<u>36</u>	N-((5-chloro-8-hydroxyquinolin-7-yl)(1H-indol-3-	>10

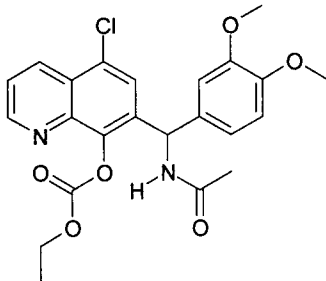
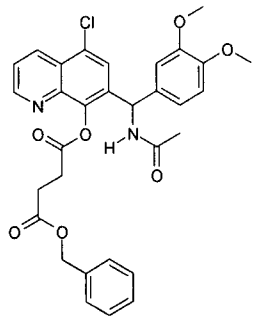
	yl)methyl)acetamide	
<u>37</u>	N-((5-chloro-8-hydroxyquinolin-7-yl)(1H-indol-4-yl)methyl)acetamide	>10
<u>38</u>	N-((5-chloro-8-hydroxyquinolin-7-yl)(1H-indol-7-yl)methyl)acetamide	>10
<u>39</u>	4-((acetylamino)(5-chloro-8-hydroxyquinolin-7-yl)methyl)-N,N-dimethyl benzamide	10-15
<u>40</u>	N-((5-chloro-8-hydroxyquinolin-7-yl)(4-(morpholin-4-ylcarbonyl)phenyl)methyl)acetamide	7.5
<u>41</u>	N-((5-chloro-8-hydroxyquinolin-7-yl)(3,4-dimethoxyphenyl)methyl)pyridine-2-carboxamide	3
<u>42</u>	N-((5-chloro-8-hydroxyquinolin-7-yl)(3,4-dimethoxyphenyl)methyl)-2-phenoxyacetamide	5-10
<u>43</u>	N-((5-chloro-8-hydroxyquinolin-7-yl)(3,4-dimethoxyphenyl)methyl)nicotinamide	>10
<u>44</u>	4-((acetylamino)(5-chloro-8-hydroxyquinolin-7-yl)methyl)-N-(3-methoxypropyl)benzamide	4
<u>45</u>	4-((acetylamino)(5-chloro-8-hydroxyquinolin-7-yl)methyl)-N-methylbenzamide	5
<u>46</u>	N-((5-chloro-8-hydroxyquinolin-7-yl)(3,4-dimethoxyphenyl)methyl)-N,N-dimethyl glycineamide	4
<u>47</u>	N-(1-(5-chloro-8-hydroxyquinolin-7-yl)ethyl)acetamide	5
<u>48</u>	N-((8-hydroxyquinolin-7-yl)methyl)acetamide	20
<u>49</u>	5,7-dichloroquinolin-8-ol	10
<u>50</u>	5,7-dibromoquinolin-8-ol	15
<u>51</u>	5,7-diiodoquinolin-8-ol	17.5
<u>52</u>	5-chloro-7-iodoquinolin-8-ol	20
<u>53</u>	N-(2-furylmethyl)-8-hydroxyquinolin-7-yl carboxamide	7.5
<u>54</u>	N-(2-thienylmethyl)-8-hydroxyquinolin-7-yl carboxamide	7.5
<u>55</u>	N-(2-methoxybenzyl)-8-hydroxyquinolin-7-yl carboxamide	12.5
<u>56</u>	5-chloro-7-(2-hydroxyphenyl)quinolin-8-ol	8
<u>57</u>	5-chloro-7-(3-hydroxyphenyl)quinolin-8-ol	12.5
<u>58</u>	5-chloro-7-(4-hydroxyphenyl)quinolin-8-ol	17.5

<u>59</u>	N-(2-(5-chloro-8-hydroxyquinolin-7-yl)phenyl)acetamide	10
<u>60</u>	5-chloro-7-(4-(dimethylamino)phenyl)quinolin-8-ol	12.5
<u>61</u>	5-chloro-7-(2-methoxypyridin-3-yl)quinolin-8-ol	10
<u>62</u>	5-chloro-7-pyridin-4-ylquinolin-8-ol hydrochloride	>10
<u>63</u>	N-(3,4-dimethoxyphenyl)-8-hydroxyquinolin-7-yl carboxamide	1.5
<u>64</u>	N-(3-methoxyphenyl)-8-hydroxyquinolin-7-yl carboxamide	1.5
<u>65</u>	N-(3-methoxybenzyl)-8-hydroxyquinolin-7-yl carboxamide	5
<u>66</u>	N-(3,4-dimethoxybenzyl)-8-hydroxyquinolin-7-yl carboxamide	2
<u>67</u>	N-(2-methoxyphenyl)-8-hydroxyquinolin-7-yl carboxamide	10
<u>68</u>	N-(pyridin-3-ylmethyl)-8-hydroxyquinolin-7-yl carboxamide	12.5
<u>69</u>	N-(8-hydroxyquinolin-2-yl)pyridin-2-yl carboxamide	7.5
<u>70</u>	N-(8-hydroxyquinolin-2-yl)-2-pyridin-2-yl acetamide	17
<u>71</u>	2-(3-hydroxyphenyl)-N-(8-hydroxyquinolin-2-yl)acetamide	17
<u>72</u>	2-(4-hydroxyphenyl)-N-(8-hydroxyquinolin-2-yl)acetamide	20
<u>73</u>	N-(8-hydroxyquinolin-2-yl)-2-(2-thienyl)acetamide	>20
<u>74</u>	2-(((3-methoxybenzyl)amino)methyl)quinolin-8-ol	15
<u>75</u>	2-(((3-methoxyphenyl)amino)methyl)quinolin-8-ol	>20
<u>76</u>	2-(((2-thienylmethyl)amino)methyl)quinolin-8-ol	>20
<u>77</u>	2-(((2-methoxyphenyl)amino)methyl)quinolin-8-ol	>20
<u>78</u>	N-[(2,4-dichlorophenyl)(8-hydroxyquinolin-7-yl)methyl]acetamide	3-4.5
<u>79</u>	N-[(8-hydroxyquinolin-7-yl)(2-thienyl)methyl]acetamide	1.5-2
<u>80</u>	N-[(8-hydroxyquinolin-7-yl)(4-isopropylphenyl)methyl]acetamide	1.5-2.5
<u>81</u>	N-[(8-hydroxyquinolin-7-yl)(2-thienyl)methyl]butanamide	2.5
<u>82</u>	N-[(8-hydroxyquinolin-7-yl)(4-	2.5

	isopropylphenyl)methyl]butanamide	
<u>83</u>	N-[(5-chloro-8-hydroxyquinolin-7-yl)(4-methylphenyl)methyl]acetamide	2.5-3
<u>84</u>	N-[(5-chloro-8-hydroxyquinolin-7-yl)(4-chlorophenyl)methyl]acetamide	2.5-3
<u>85</u>	N-[(5-chloro-8-hydroxyquinolin-7-yl)(2-chlorophenyl)methyl]acetamide	1.5-2
<u>86</u>	N-[(5-chloro-8-hydroxyquinolin-7-yl)(2,4-dichlorophenyl)methyl]acetamide	1.5-2
<u>87</u>	N-[(5-chloro-8-hydroxyquinolin-7-yl)(2-thienyl)methyl]acetamide	1.5-2
<u>88</u>	N-[(5-chloro-8-hydroxyquinolin-7-yl)(4-isopropylphenyl)methyl]acetamide	1.5-2
<u>89</u>	N-[(5-chloro-8-hydroxyquinolin-7-yl)(2-furyl)methyl]propanamide	3-4.5
<u>90</u>	N-[(5-chloro-8-hydroxyquinolin-7-yl)(2-methoxyphenyl)methyl]propanamide	2.5-3.5
<u>91</u>	N-[(5-chloro-8-hydroxyquinolin-7-yl)(4-chlorophenyl)methyl]propanamide	3-4.5
<u>92</u>	N-[(8-hydroxyquinolin-7-yl)(4-methylphenyl)methyl]-3-phenylpropanamide	2.5-3.5
<u>93</u>	N-[(2-chlorophenyl)(8-hydroxyquinolin-7-yl)methyl]-3-phenylpropanamide	5.5-7.5
<u>94</u>	N-[(2,4-dichlorophenyl)(8-hydroxyquinolin-7-yl)methyl]-3-methylbutanamide	1.5-2
<u>95</u>	N-[(5-chloro-8-hydroxyquinolin-7-yl)(phenyl)methyl]butanamide	2.5
<u>96</u>	N-[(5-chloro-8-hydroxyquinolin-7-yl)(phenyl)methyl]pentanamide	3-4.5
<u>97</u>	N-[(5-chloro-8-hydroxyquinolin-7-yl)(2-furyl)methyl]-2-methylpropanamide	5.5-7.5
<u>98</u>	N-[(5-chloro-8-hydroxyquinolin-7-yl)(2-methoxyphenyl)methyl]butanamide	2-2.5
<u>99</u>	N-[(5-chloro-8-hydroxyquinolin-7-yl)(2-methoxyphenyl)methyl]-2-methylpropanamide	5.5-7.5
<u>100</u>	N-[(5-chloro-8-hydroxyquinolin-7-yl)(4-methoxyphenyl)methyl]butanamide	3-4.5
<u>101</u>	N-[(5-chloro-8-hydroxyquinolin-7-yl)(4-methoxyphenyl)methyl]pentanamide	3-4.5

<u>102</u>	N-[(5-chloro-8-hydroxyquinolin-7-yl)(4-methylphenyl)methyl]butanamide	3-4.5
<u>103</u>	N-[(5-chloro-8-hydroxyquinolin-7-yl)(4-methylphenyl)methyl]pentanamide	3-4.5
<u>104</u>	N-[(5-chloro-8-hydroxyquinolin-7-yl)(4-methylphenyl)methyl]-2-methylpropanamide	3-4.5
<u>105</u>	N-[(5-chloro-8-hydroxyquinolin-7-yl)(4-chlorophenyl)methyl]butanamide	3-4.5
<u>106</u>	N-[(5-chloro-8-hydroxyquinolin-7-yl)(4-chlorophenyl)methyl]pentanamide	3-4.5
<u>107</u>	N-[(5-chloro-8-hydroxyquinolin-7-yl)(2-chlorophenyl)methyl]pentanamide	5
<u>108</u>	N-[(5-chloro-8-hydroxyquinolin-7-yl)(2-thienyl)methyl]-3-phenylpropanamide	6
<u>109</u>	N-[(5-chloro-8-hydroxyquinolin-7-yl)(4-isopropylphenyl)methyl]-2-methylpropanamide	5.5-7.5
<u>110</u>	N-[(8-hydroxyquinolin-7-yl)(phenyl)methyl]-3-methylbutanamide	3-4.5
<u>111</u>	N-[(8-hydroxyquinolin-7-yl)(4-methoxyphenyl)methyl]-3-phenylpropanamide	3-4.5
<u>112</u>	N-[(3,4-dimethoxyphenyl)(8-hydroxyquinolin-7-yl)methyl]-3-methylbutanamide	3-4.5
<u>113</u>	N-[(8-hydroxyquinolin-7-yl)(2-thienyl)methyl]-3-methylbutanamide	4
<u>114</u>	N-[(8-hydroxyquinolin-7-yl)(4-isopropylphenyl)methyl]-3-methylbutanamide	5.5-7.5
<u>115</u>	N-[(5-chloro-8-hydroxyquinolin-7-yl)[4-(dimethylamino)phenyl)methyl]butanamide	7.5
<u>116</u>	N-[(5-chloro-8-hydroxyquinolin-7-yl)(2-thienyl)methyl]butanamide	5.5-7.5
<u>117</u>	N-[(5-chloro-8-hydroxyquinolin-7-yl)(2-thienyl)methyl]pentanamide	5.5-7.5
<u>118</u>	N-[(5-chloro-8-hydroxyquinolin-7-yl)(2-thienyl)methyl]-2-methylpropanamide	5
<u>121</u>	N-[(4-chlorophenyl)(8-hydroxyquinolin-7-yl)methyl]cyclohexanecarboxamide	1.5-2
<u>122</u>	N-[(2,4-dichlorophenyl)(8-hydroxyquinolin-7-yl)methyl]propanamide	2.5
<u>123</u>	N-[(5-chloro-8-hydroxyquinolin-7-yl)(2,4-	5

	dichlorophenyl)methyl]propanamide	
<u>124</u>	N-[(3,4-dimethoxyphenyl)(8-hydroxyquinolin-7-yl)methyl]-3-phenylpropanamide	3-4.5
<u>125</u>	N-[(2,4-dichlorophenyl)(8-hydroxyquinolin-7-yl)methyl]pentanamide	1.5-2
<u>126</u>	N-[(8-hydroxyquinolin-7-yl)(4-isopropylphenyl)methyl]pentanamide	2.5
<u>127</u>	N-[[4-(diethylamino)phenyl](8-hydroxyquinolin-7-yl)methyl]pentanamide	2.5
<u>128</u>	N-[(8-hydroxyquinolin-7-yl)(4-methoxyphenyl)methyl]cyclohexanecarboxamide	1.5-2
<u>129</u>	N-[(2,4-dichlorophenyl)(8-hydroxyquinolin-7-yl)methyl]butanamide	10
<u>130</u>	N-[(8-hydroxyquinolin-7-yl)(4-methylphenyl)methyl]cyclohexanecarboxamide	10
<u>131</u>	N-[(8-hydroxyquinolin-7-yl)(2-thienyl)methyl]cyclohexanecarboxamide	1.5-2
<u>132</u>	N-[(5-chloro-8-hydroxyquinolin-7-yl)(phenyl)methyl]acetamide	1.5-2
<u>133</u>	N-[(5-chloro-8-hydroxyquinolin-7-yl)(2-methoxyphenyl)methyl]acetamide	1.5-2
<u>134</u>	N-[(5-chloro-8-hydroxyquinolin-7-yl)(2-methoxyphenyl)methyl]pentanamide	2-2.5
<u>135</u>	N-[(5-chloro-8-hydroxyquinolin-7-yl)(2-furyl)methyl]acetamide	10
<u>136</u>	((7-((acetylamino)(2-furyl)methyl)-5-chloroquinolin-8-yl)oxy)methyl dihydrogen phosphate	n/d
<u>137</u>	((7-((acetylamino)(2-furyl)methyl)-5-chloroquinolin-8-yl)oxy)methyl disodium phosphate	n/d
<u>138</u>	((7-((acetylamino)(3,4-dimethoxyphenyl)methyl)-5-chloroquinolin-8-yl)oxy)methyl dihydrogen phosphate dihydrochloride	n/d
<u>139</u>	((7-((acetylamino)(3,4-dimethoxyphenyl)methyl)-5-chloroquinolin-8-yl)oxy)methyl dihydrogen phosphate hydrochloride	n/d
<u>140</u>	N-[(5-chloro-8-acetoxyquinolin-7-yl)(3,4-dimethoxyphenyl)methyl]acetamide	1.5

<u>141</u>		2
<u>142</u>	((7-((acetylamino)(3,4-dimethoxyphenyl)methyl)-5-fluoroquinolin-8-yl)oxy)methyl dihydrogen phosphate	7.5
<u>143</u>	N-[(3,4-dimethoxyphenyl)(8-hydroxy-5-methylquinolin-7-yl) methyl]acetamide	6
<u>144</u>	N-[(3,4-dimethoxyphenyl)(5-fluoro-8-hydroxyquinolin-7-yl) methyl]acetamide	1.5
<u>145</u>		3.5
<u>146</u>	N-[1-(8-hydroxyquinolin-7-yl)ethyl]acetamide	10-20
<u>147</u>	N-((5-chloro-8-hydroxyquinolin-7-yl)(3,4-dimethoxyphenyl)methyl)-N,N-dimethyl glycineamide hydrochloride	4
<u>148</u>	({7-[(acetylamino)(4-cyanophenyl)methyl]-5-chloroquinolin-8-yl}oxy)methyl dihydrogen phosphate	2
<u>149</u>	[(5-chloro-7-iodoquinolin-8-yl)oxy]methyl dihydrogen phosphate	20
<u>150</u>	5-chloro-7-iodoquinolin-8-yl ethyl carbonate	20
<u>151</u>	N-[1-(5-fluoro-8-hydroxyquinolin-7-yl)ethyl]acetamide	15

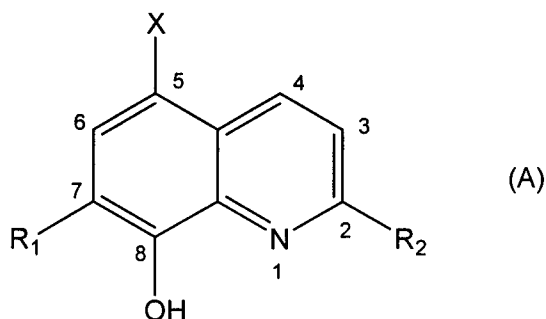
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- 25

CLAIMS

1- A compound having formula (A):



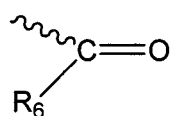
a pharmaceutically acceptable salt or a pro-drug thereof,

wherein X = H, methyl or halogen, and the substituents R₁ and R₂ are defined as follows:

a) when R₂ is an hydrogen, then R₁ is H or halogen, or R₁ is selected from the group consisting of: amino(3,4-dimethoxyphenyl)methyl, 2-hydroxyphenyl, 3-hydroxyphenyl, 4-hydroxyphenyl, 4-dimethylaminophenyl, pyridin-4-yl, 2-methoxypyridin-3-yl and 2-acetamidophenyl;

or R₁ is -CH(R₃)NR₄R₅ with

▪ R₄ = H or C₁-C₄ alkyl;

▪ R₅ = H or 

and R₆ is selected from the group consisting of: C₁-C₆ alkyl, C₅-C₆ aryl, benzyl, dimethylaminomethyl, phenylethyl and diphenylmethyl; and

▪ R₃ is selected from the group consisting of:

H, methyl, phenyl, benzyl, 2-chlorophenyl, 2-methoxyphenyl, 4-chlorophenyl, 4-methylphenyl, 4-isopropylphenyl, 3-hydroxyphenyl, 4-hydroxyphenyl, 4-methoxyphenyl, 4-cyanophenyl, 4-dimethylamino-phenyl, 4-diethylamino-phenyl, 2,4-dichlorophenyl, 3,4-dimethoxyphenyl, 4-methoxycarbonylphenyl, 3-methoxycarbonylphenyl, N-methylbenzamido, N-(3-methoxypropyl)benzamido, N,N-dimethylbenzamido, 4-(morpholin-4-

ylcarbonyl)phenyl, 4-(pyridin-2-yl)phenyl, 4-(1H-pyrazol-1-yl)phenyl, pyridin-2-yl, pyridin-3-yl, pyridin-4-yl, 6-(morpholin-4-yl)pyridin-3-yl, 2-furyl, 3-furyl, 2-(morpholin-4-yl)pyridin-3-yl, 2-methoxypyridin-3-yl, 4-methoxypyridin-3-yl, 2-thienyl, 2-butyl-1H-imidazol-4-yl, quinolin-3-yl, quinolin-4-yl, 8-hydroxyquinolin-2-yl, 1H-indol-3-yl, 1H-indol-4-yl and 1H-indol-7-yl;

or R₁ is $\text{---}\overset{\text{H}}{\underset{\text{O}}{\text{C}}}\text{---}\text{N---R}_7$ and R₇ is $\text{---}(\text{CH}_2)_n\text{---R}_8$ where R₈ is a C₅-C₆ aryl optionally

substituted by one or two methoxyl groups, and n= 0 or 1; or

b) when X and R₁ both represent a hydrogen atom, then R₂ is selected from the group consisting of: pyridin-2-ylcarboxamido, pyridin-2-ylacetamido, 3-hydroxyphenylacetamido, 4-hydroxyphenylacetamido, ((2-methoxyphenyl)amino)methyl, ((3-methoxyphenyl)amino)methyl, ((3-methoxybenzyl)amino)methyl, 2-thienylacetamido and ((2-thienylmethyl)amino)methyl;

with the proviso that said compound of formula (A) is not one of the compounds identified in Annex 1;

said compound of formula (A), when comprising at least one asymmetric centre, being in the form of one of its enantiomers, or a mixture thereof.

2- The compound of claim 1 wherein R₄ is H or methyl, and R₆ is selected from the group consisting of methyl, ethyl, *n*-propyl, *i*-propyl, *n*-butyl, *i*-butyl, *ter*-butyl, phenyl, benzyl, dimethylaminomethyl, phenylethyl, cyclohexyl, diphenylmethyl, pyridin-2-yl and pyridin-3-yl.

3- The compound of claim 2 selected from the group consisting of:

N-((3,4-dimethoxyphenyl)(8-hydroxyquinolin-7-yl)methyl)acetamide 2,

- N-((5-chloro-8-hydroxyquinolin-7-yl)(3,4-dimethoxyphenyl)methyl)benzamide
3,
- N-((5-chloro-8-hydroxyquinolin-7-yl)(3,4-dimethoxyphenyl)methyl)-N-methylacetamide 8,
- 5 N-((5-chloro-8-hydroxyquinolin-7-yl)(3,4-dimethoxyphenyl)methyl)-2,2-diphenylacetamide 11,
- N-((5-chloro-8-hydroxyquinolin-7-yl)(3,4-dimethoxyphenyl)methyl)-2-phenylacetamide 12,
- 7-(amino(3,4-dimethoxyphenyl)methyl)-5-chloroquinolin-8-ol 13,
- 10 N-(1-(5-chloro-8-hydroxyquinolin-7-yl)-2-phenylethyl)acetamide 14,
- N-((2-butyl-1H-imidazol-4-yl)(5-chloro-8-hydroxyquinolin-7-yl)methyl)acetamide
15,
- N-((5-chloro-8-hydroxyquinolin-7-yl)(2-methoxypyridin-3-yl)methyl)acetamide
16,
- 15 N-((5-chloro-8-hydroxyquinolin-7-yl)(quinolin-4-yl)methyl)acetamide 17,
- N-((5-chloro-8-hydroxyquinolin-7-yl)(2-morpholin-4-ylpyridin-3-yl)methyl)-acetamide 18,
- N-((5-chloro-8-hydroxyquinolin-7-yl)(quinolin-3-yl)methyl)acetamide 19,
- 4-((acetylamino)(5-chloro-8-hydroxyquinolin-7-yl)methyl) methylbenzoate
20 21,
- 3-((acetylamino)(5-chloro-8-hydroxyquinolin-7-yl)methyl) methylbenzoate
22,
- N-((5-chloro-8-hydroxyquinolin-7-yl)(4-cyanophenyl)methyl)acetamide 23,
- N-((5-chloro-8-hydroxyquinolin-7-yl)(4-pyridin-2-ylphenyl)methyl)acetamide
25 24,
- N-((5-chloro-8-hydroxyquinolin-7-yl)(4-(1H-pyrazol-1-yl)phenyl)methyl)acetamide
25,
- N-((5-chloro-8-hydroxyquinolin-7-yl)(4-hydroxyphenyl)methyl)acetamide 26,
- N-((5-chloro-8-hydroxyquinolin-7-yl)(3-hydroxyphenyl)methyl)acetamide 27,
- 30 N-((5-chloro-8-hydroxyquinolin-7-yl)(6-methoxypyridin-3-yl)methyl)acetamide
28,
- N-((5-chloro-8-hydroxyquinolin-7-yl)(pyridin-2-yl)methyl)acetamide 29,

- N-((5-chloro-8-hydroxyquinolin-7-yl)(pyridin-4-yl)methyl)acetamide 30,
N-((5-chloro-8-hydroxyquinolin-7-yl)(pyridin-4-yl)methyl)acetamidehydrochloride 31,
N-((5-chloro-8-hydroxyquinolin-7-yl)(pyridin-3-yl)methyl)acetamide 32,
5 N-((5-chloro-8-hydroxyquinolin-7-yl)(pyridin-3-yl)methyl)acetamidehydrochloride 33,
N-((5-chloro-8 hydroxyquinolin-7-yl)(8-hydroxyquinolin-2-yl)methyl)acetamide 34,
N-((5-chloro-8-hydroxyquinolin-7-yl)(6-morpholin-4-ylpyridin-3-yl)methyl)-
10 acetamide 35,
N-((5-chloro-8-hydroxyquinolin-7-yl)(1H-indol-3-yl)methyl)acetamide 36,
N-((5-chloro-8-hydroxyquinolin-7-yl)(1H-indol-4-yl)methyl)acetamide 37,
N-((5-chloro-8-hydroxyquinolin-7-yl)(1H-indol-7-yl)methyl)acetamide 38,
4-((acetylamino)(5-chloro-8-hydroxyquinolin-7-yl)methyl)-N,N-dimethyl-
15 benzamide 39,
N-((5-chloro-8-hydroxyquinolin-7-yl)(4-(morpholin-4-ylcarbonyl)phenyl)methyl)-
acetamide 40,
N-((5-chloro-8-hydroxyquinolin-7-yl)(3,4-dimethoxyphenyl)methyl)pyridine-2-
carboxamide 41,
20 4-((acetylamino)(5-chloro-8-hydroxyquinolin-7-yl)methyl)-N-(3-methoxypropyl)-
benzamide 44,
4-((acetylamino)(5-chloro-8-hydroxyquinolin-7-yl)methyl)-N-methylbenzamide 45,
N-((5-chloro-8-hydroxyquinolin-7-yl)(3,4-dimethoxyphenyl)methyl)-N,N-dimethyl-
25 glycineamide 46,
N-(1-(5-chloro-8-hydroxyquinolin-7-yl)ethyl)acetamide 47,
N-((8-hydroxyquinolin-7-yl)methyl)acetamide 48,
N-(2-furylmethyl)-8-hydroxyquinolin-7-yl carboxamide 53,
N-(2-thienylmethyl)-8-hydroxyquinolin-7-yl carboxamide 54,
30 N-(2-methoxybenzyl)-8-hydroxyquinolin-7-yl carboxamide 55,
5-chloro-7-(3-hydroxyphenyl)quinolin-8-ol 57,
5-chloro-7-(4-hydroxyphenyl)quinolin-8-ol 58,

- N-(2-(5-chloro-8-hydroxyquinolin-7-yl)phenyl)acetamide 59,
5-chloro-7-(2-methoxypyridin-3-yl)quinolin-8-ol 61,
5-chloro-7-pyridin-4-ylquinolin-8-ol hydrochloride 62,
N-(3,4-dimethoxyphenyl)-8-hydroxyquinolin-7-yl carboxamide 63,
5 N-(3-methoxyphenyl)-8-hydroxyquinolin-7-yl carboxamide 64,
N-(3-methoxybenzyl)-8-hydroxyquinolin-7-yl carboxamide 65,
N-(3,4-dimethoxybenzyl)-8-hydroxyquinolin-7-yl carboxamide 66,
N-(2-methoxyphenyl)-8-hydroxyquinolin-7-yl carboxamide 67,
N-(pyridin-3-ylmethyl)-8-hydroxyquinolin-7-yl carboxamide 68,
10 N-(8-hydroxyquinolin-2-yl)pyridin-2-yl carboxamide 69,
N-(8-hydroxyquinolin-2-yl)-2-pyridin-2-yl acetamide 70,
2-(3-hydroxyphenyl)-N-(8-hydroxyquinolin-2-yl)acetamide 71,
2-(4-hydroxyphenyl)-N-(8-hydroxyquinolin-2-yl)acetamide 72,
N-(8-hydroxyquinolin-2-yl)-2-(2-thienyl)acetamide 73,
15 2-(((3-methoxybenzyl)amino)methyl)quinolin-8-ol 74,
2-(((3-methoxyphenyl)amino)methyl)quinolin-8-ol 75,
2-(((2-thienylmethyl)amino)methyl)quinolin-8-ol 76,
2-(((2-methoxyphenyl)amino)methyl)quinolin-8-ol 77,
N-[(2,4-dichlorophenyl)(8-hydroxyquinolin-7-yl)methyl]acetamide 78,
20 N-[(8-hydroxyquinolin-7-yl)(2-thienyl)methyl]acetamide 79,
N-[(8-hydroxyquinolin-7-yl)(4-isopropylphenyl)methyl]acetamide 80,
N-[(8-hydroxyquinolin-7-yl)(2-thienyl)methyl]butanamide 81,
N-[(8-hydroxyquinolin-7-yl)(4-isopropylphenyl)methyl]butanamide 82,
N-[(8-hydroxyquinolin-7-yl)(4-methylphenyl)methyl]-3-phenylpropanamide
25 92,
N-[(2-chlorophenyl)(8-hydroxyquinolin-7-yl)methyl]-3-phenylpropanamide
93,
N-[(2,4-dichlorophenyl)(8-hydroxyquinolin-7-yl)methyl]-3-methylbutanamide
94,
30 N-[(8-hydroxyquinolin-7-yl)(phenyl)methyl]-3-methylbutanamide 110,
N-[(8-hydroxyquinolin-7-yl)(4-methoxyphenyl)methyl]-3-phenylpropanamide
111,

N-[(3,4-dimethoxyphenyl)(8-hydroxyquinolin-7-yl)methyl]-3-methylbutanamide
112,

N-[(8-hydroxyquinolin-7-yl)(2-thienyl)methyl]-3-methylbutanamide **113,**

N-[(4-chlorophenyl)(8-hydroxyquinolin-7-yl)methyl]cyclohexanecarboxamide

5 **121,**

N-[(2,4-dichlorophenyl)(8-hydroxyquinolin-7-yl)methyl]propanamide **122,**

N-[(3,4-dimethoxyphenyl)(8-hydroxyquinolin-7-yl)methyl]-3-phenylpropanamide

124,

N-[(2,4-dichlorophenyl)(8-hydroxyquinolin-7-yl)methyl]pentanamide **125,**

10 N-[(8-hydroxyquinolin-7-yl)(4-isopropylphenyl)methyl]pentanamide **126,**

N-[[4-(diethylamino)phenyl](8-hydroxyquinolin-7-yl)methyl]pentanamide **127,**

N-[(8-hydroxyquinolin-7-yl)(4-methoxyphenyl)methyl]cyclohexanecarboxamide

128,

N-[(2,4-dichlorophenyl)(8-hydroxyquinolin-7-yl)methyl]butanamide **129,**

15 N-[(8-hydroxyquinolin-7-yl)(4-methylphenyl)methyl]cyclohexanecarboxamide

130, and

N-[(8-hydroxyquinolin-7-yl)(2-thienyl)methyl]cyclohexanecarboxamide **131.**

4- A pro-drug selected from the group consisting of:

20 ((7-((acetylamino)(3,4-dimethoxyphenyl)methyl)-5-chloroquinolin-8-yl)oxy)methyl
dihydrogen phosphate **5,**

((7-((acetylamino)(3,4-dimethoxyphenyl)methyl)-5-chloroquinolin-8-yl)oxy)methyl
disodium phosphate **6,**

25 ((7-((acetylamino)(2-furyl)methyl)-5-chloroquinolin-8-yl)oxy)methyl dihydrogen
phosphate **136,**

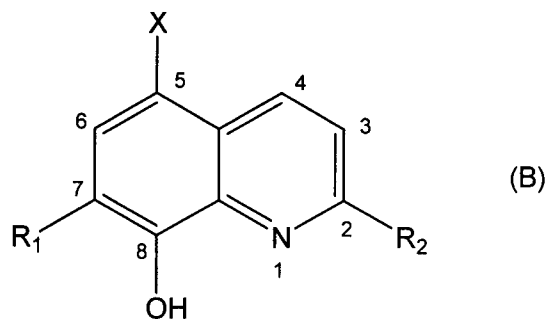
((7-((acetylamino)(2-furyl)methyl)-5-chloroquinolin-8-yl)oxy)methyl disodium
phosphate **137,**

((7-((acetylamino)(3,4-dimethoxyphenyl)methyl)-5-chloroquinolin-8-yl)oxy)methyl
dihydrogen phosphate dihydrochloride **138, and**

30 ((7-((acetylamino)(3,4-dimethoxyphenyl)methyl)-5-chloroquinolin-8-yl)oxy)methyl
dihydrogen phosphate hydrochloride **139.**

5- A method for inhibiting CLK-1 activity in a cell, said method comprising the steps of:

- a) providing a cell wherein CLK-1 activity needs to be inhibited,
- b) contacting said cell with a compound of formula (B):



wherein X = H, methyl or halogen, and the substituents R₁ and R₂ are defined as follows:

- 10 i) when R₂ is an hydrogen, then R₁ is H or halogen, or R₁ is selected from the group consisting of: amino(3,4-dimethoxyphenyl)methyl, 2-hydroxyphenyl, 3-hydroxyphenyl, 4-hydroxyphenyl, 4-dimethylaminophenyl, pyridin-4-yl, 2-methoxypyridin-3-yl and 2-acetamidophenyl;

- 15 or R₁ is -CH(R₃)NR₄R₅ with

▪ R₄ = H or C₁-C₄ alkyl;

▪ R₅ = H or

and R₆ is selected from the group consisting of: C₁-C₆ alkyl, C₅-C₆ aryl, benzyl, dimethylaminomethyl, phenylethyl and diphenylmethyl; and

- 20 ▪ R₃ is selected from the group consisting of:

H, methyl, phenyl, benzyl, 2-chlorophenyl, 2-methoxyphenyl, 4-chlorophenyl, 4-methylphenyl, 4-isopropylphenyl, 3-hydroxyphenyl, 4-hydroxyphenyl, 4-methoxyphenyl, 4-cyanophenyl, 4-dimethylamino-phenyl, 4-diethylamino-phenyl, 2,4-dichlorophenyl, 3,4-dimethoxyphenyl, 4-methoxycarbonylphenyl, 3-methoxycarbonylphenyl, N-methylbenzamido,

N-(3-methoxypropyl)benzamido, N,N-dimethylbenzamido, 4-(morpholin-4-ylcarbonyl)phenyl, 4-(pyridin-2-yl)phenyl, 4-(1H-pyrazol-1-yl)phenyl, pyridin-2-yl, pyridin-3-yl, pyridin-4-yl, 6-(morpholin-4-yl)pyridin-3-yl, 2-furyl, 3-furyl, 2-(morpholin-4-yl)pyridin-3-yl, 2-methoxypyridin-3-yl, 4-methoxypyridin-3-yl, 2-thienyl, 2-butyl-1H-imidazol-4-yl, quinolin-3-yl, quinolin-4-yl, 8-hydroxyquinolin-2-yl, 1H-indol-3-yl, 1H-indol-4-yl and 1H-indol-7-yl;

or R₁ is $\text{---}\overset{\text{H}}{\underset{\text{O}}{\underset{\parallel}{\text{C}}}}\text{---}\text{N---R}_7$ and R₇ is $\text{---}(\text{CH}_2)_n\text{---R}_8$ where R₈ is a C₅-C₆ aryl optionally

substituted by one or two methoxyl groups, and n= 0 or 1; or

ii) when X and R₁ both represent a hydrogen atom, then R₂ is selected from the group consisting of: pyridin-2-ylcarboxamido, pyridin-2-ylacetamido, 3-hydroxyphenylacetamido, 4-hydroxyphenylacetamido, ((2-methoxyphenyl)amino)methyl, ((3-methoxyphenyl)amino)methyl, ((3-methoxybenzyl)amino)methyl, 2-thienylacetamido and ((2-thienylmethyl)amino)methyl;

the compound of formula (B), when comprising at least one asymmetric centre, being in the form of one of its enantiomers or a mixture thereof; and

c) determining the CLK-1 activity of the cell.

6- The method of claim 5 wherein said compound of formula (B) is selected from the group of compounds identified in Table 1.

7- The method of claim 6 wherein said compound of formula (B) is selected from the group consisting of:

N-[(5-chloro-8-hydroxyquinolin-7-yl)(3,4-dimethoxyphenyl)methyl]acetamide

7,

5-chloro-7-iodoquinolin-8-ol 52,

5-chloro-7-(2-hydroxyphenyl)quinolin-8-ol 56,

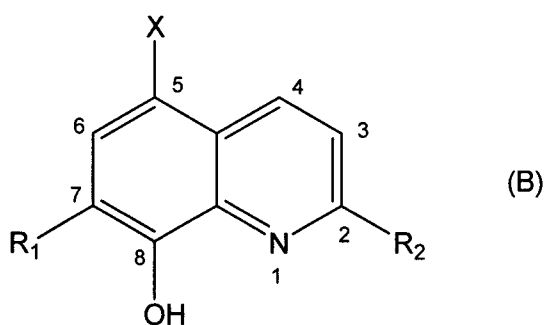
5-chloro-7-(4-(dimethylamino)phenyl)quinolin-8-ol **60**, and
 N-[(5-chloro-8-hydroxyquinolin-7-yl)(2-furyl)methyl]acetamide **135**.

8- A method for the prophylaxis and/or treatment of a disorder or its associated
 5 symptoms for which inhibition of CLK-1 is beneficial, in an animal, said method
 comprising the steps of:

a) identifying an animal having a disorder for which inhibition of CLK-1 is
 beneficial; and

b) administering to said animal a compound of formula (B)

10



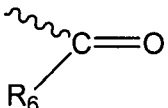
a pharmaceutically acceptable salt or a pro-drug thereof,

15 wherein X = H, methyl or halogen, and the substituents R₁ and R are defined as
 follows:

i) when R₂ is an hydrogen, then R₁ is H or halogen, or R₁ is selected from the
 group consisting of: amino(3,4-dimethoxyphenyl)methyl, 2-hydroxyphenyl, 3-
 20 hydroxyphenyl, 4-hydroxyphenyl, 4-dimethylaminophenyl, pyridin-4-yl, 2-
 methoxypyridin-3-yl and 2-acetamidophenyl;

or R₁ is -CH(R₃)NR₄R₅ with

▪ R₄ = H or C₁-C₄ alkyl;

25 ▪ R₅ = H or 

and R₆ is selected from the group consisting of: C₁-C₆ alkyl, C₅-C₆ aryl, benzyl, dimethylaminomethyl, phenylethyl and diphenylmethyl; and

▪ R₃ is selected from the group consisting of:

H, methyl, phenyl, benzyl, 2-chlorophenyl, 2-methoxyphenyl, 4-chlorophenyl, 4-methylphenyl, 4-isopropylphenyl, 3-hydroxyphenyl, 4-hydroxyphenyl, 4-methoxyphenyl, 4-cyanophenyl, 4-dimethylamino-phenyl, 4-diethylamino-phenyl, 2,4-dichlorophenyl, 3,4-dimethoxyphenyl, 4-methoxycarbonylphenyl, 3-methoxycarbonylphenyl, N-methylbenzamido, N-(3-methoxypropyl)benzamido, N,N-dimethylbenzamido, 4-(morpholin-4-ylcarbonyl)phenyl, 4-(pyridin-2-yl)phenyl, 4-(1H-pyrazol-1-yl)phenyl, pyridin-2-yl, pyridin-3-yl, pyridin-4-yl, 6-(morpholin-4-yl)pyridin-3-yl, 2-furyl, 3-furyl, 2-(morpholin-4-yl)pyridin-3-yl, 2-methoxypyridin-3-yl, 4-methoxypyridin-3-yl, 2-thienyl, 2-butyl-1H-imidazol-4-yl, quinolin-3-yl, quinolin-4-yl, 8-hydroxyquinolin-2-yl, 1H-indol-3-yl, 1H-indol-4-yl and 1H-indol-7-yl;

or R₁ is $\text{---}\overset{\text{H}}{\underset{\text{O}}{\underset{\parallel}{\text{C}}}}\text{---}\text{N---R}_7$ and R₇ is $\text{---}(\text{CH}_2)_n\text{---R}_8$ where R₈ is a C₅-C₆ aryl optionally

substituted by one or two methoxyl groups, and n= 0 or 1; or

ii) when X and R₁ both represent a hydrogen atom, then R₂ is selected from the group consisting of: pyridin-2-ylcarboxamido, pyridin-2-ylacetamido, 3-hydroxyphenylacetamido, 4-hydroxyphenylacetamido, ((2-methoxyphenyl)amino)methyl, ((3-methoxyphenyl)amino)methyl, ((3-methoxybenzyl)amino)methyl, 2-thienylacetamido and ((2-thienylmethyl)amino)methyl;

the compound of formula (B), when comprising at least one asymmetric centre, being in the form of one of its enantiomers or a mixture thereof.

9- The method of claim 8 wherein said compound of formula (B) is selected from the group of compounds identified in Table 1.

10- The method of claim 8 wherein said compound of formula (B) is selected from the group consisting of:

((7-((acetylamino)(3,4-dimethoxyphenyl)methyl)-5-chloroquinolin-8-yl)oxy)methyl dihydrogen phosphate 5,

((7-((acetylamino)(3,4-dimethoxyphenyl)methyl)-5-chloroquinolin-8-yl)oxy)methyl disodium phosphate 6,

10 N-[(5-chloro-8-hydroxyquinolin-7-yl)(3,4-dimethoxyphenyl)methyl]acetamide 7,

N-[(5-chloro-8-hydroxyquinolin-7-yl)(2-furyl)methyl]acetamide 135,

((7-((acetylamino)(2-furyl)methyl)-5-chloroquinolin-8-yl)oxy)methyl dihydrogen phosphate 136,

15 ((7-((acetylamino)(2-furyl)methyl)-5-chloroquinolin-8-yl)oxy)methyl disodium phosphate 137,

((7-((acetylamino)(3,4-dimethoxyphenyl)methyl)-5-chloroquinolin-8-yl)oxy)methyl dihydrogen phosphate dihydrochloride 138, and

20 ((7-((acetylamino)(3,4-dimethoxyphenyl)methyl)-5-chloroquinolin-8-yl)oxy)methyl dihydrogen phosphate hydrochloride 139.

11- The method of claim 8 wherein said disorder is an ischemia / reperfusion injury, or an inflammation.

12- A method for the prophylaxis and/or treatment of an age-related disorder or its associated symptoms, in an animal, comprising the following steps:

a) identifying an animal having an age-related disorder; and

b) administering to the animal a compound of formula (B) as defined in claim 8, a pharmaceutically acceptable salt or a pro-drug thereof, with the proviso that said compound of formula (B) is not one of the following compounds, a pharmaceutically acceptable salt or a pro-drug thereof:

5-chloro-7-iodoquinolin-8-ol 52,

5-chloro-7-(2-hydroxyphenyl)quinolin-8-ol 56, or

5-chloro-7-(4-(dimethylamino)phenyl)quinolin-8-ol **60.**

- 13- The method of claim 12 wherein said age-related disorder is selected from the group consisting cardiovascular diseases, peripheral vascular disease, metabolic disorders, cancers, neurodegenerative disorders, dementia, bladder and kidney disorders, diabetes complications, eyes disorders, lung and respiratory disorders, musculoskeletal disorders and skin conditions.
- 14- The method according to claim 13 wherein said neurodegenerative disorder is Alzheimer's disease.
- 15- The method of claim 11 wherein said ischemia / reperfusion injury is a renal, heart, myocardium, lung, brain, or spinal cord ischemia / reperfusion injury.
- 16- The method of claim 11 wherein said inflammation is a lung inflammation or an inflammation of any other organ.
- 17- The method of claim 8 wherein said animal is a human.
- 18- The method of claim 12 wherein said animal is a human
- 19- A pharmaceutical composition comprising a compound of formula (A), as defined in claim 1, a pharmaceutically acceptable salt or a pro-drug thereof, and at least one pharmaceutically acceptable carrier.
- 20- A pharmaceutical composition comprising a compound of formula (A), as defined in claim 2, a pharmaceutically acceptable salt or a pro-drug thereof, and at least one pharmaceutically acceptable carrier.
- 21- A pharmaceutical composition comprising a compound of formula (A), as defined in claim 3, a pharmaceutically acceptable salt or a pro-drug thereof, and at least one pharmaceutically acceptable carrier.

22- A pharmaceutical composition comprising a pro-drug of claim 4 and at least one pharmaceutically acceptable carrier.

5 23- A pharmaceutical composition comprising a pharmaceutically effective amount of the compound of formula (A), a pharmaceutically acceptable salt or a pro-drug thereof, as defined in claim 1, to effectively reduce and/or inhibit CLK-1 activity.

10 24- A pharmaceutical composition comprising a pharmaceutically effective amount of the compound of formula (B), a pharmaceutically acceptable salt or a pro-drug thereof as defined in claim 8, to effectively reduce and/or inhibit CLK-1 activity.

15 25- The pharmaceutical composition of claim 22, comprising a pharmaceutically effective amount of the pro-drug to effectively reduce and/or inhibit CLK-1 activity.

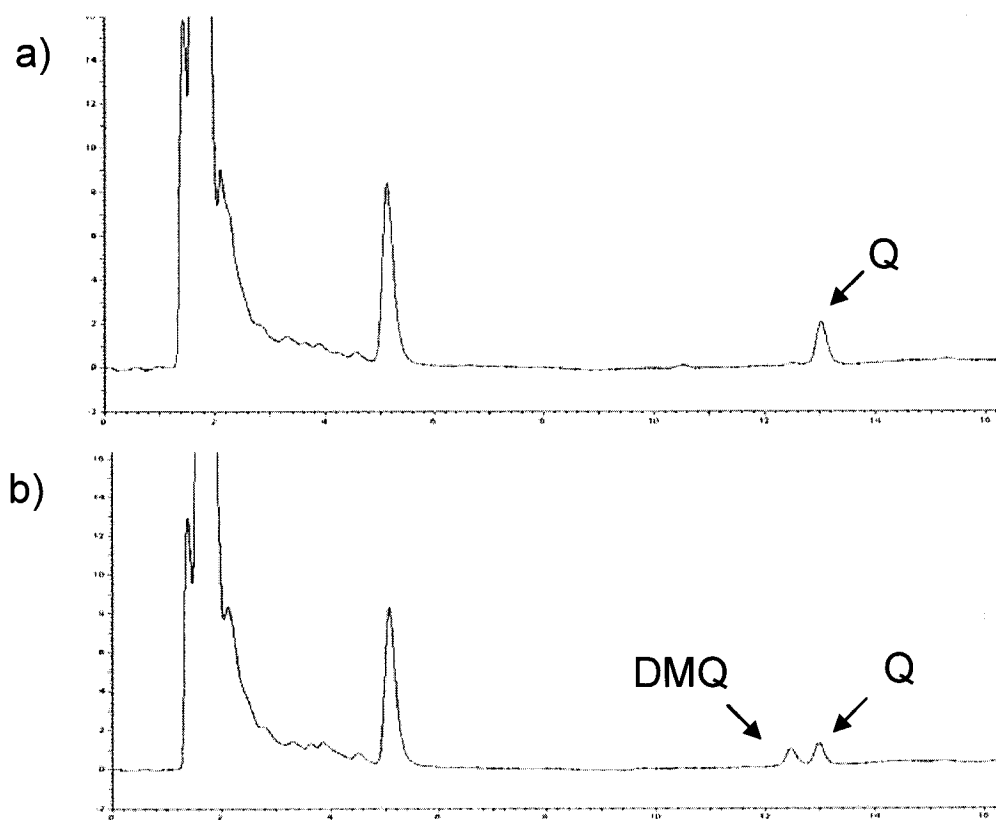
ANNEX 1

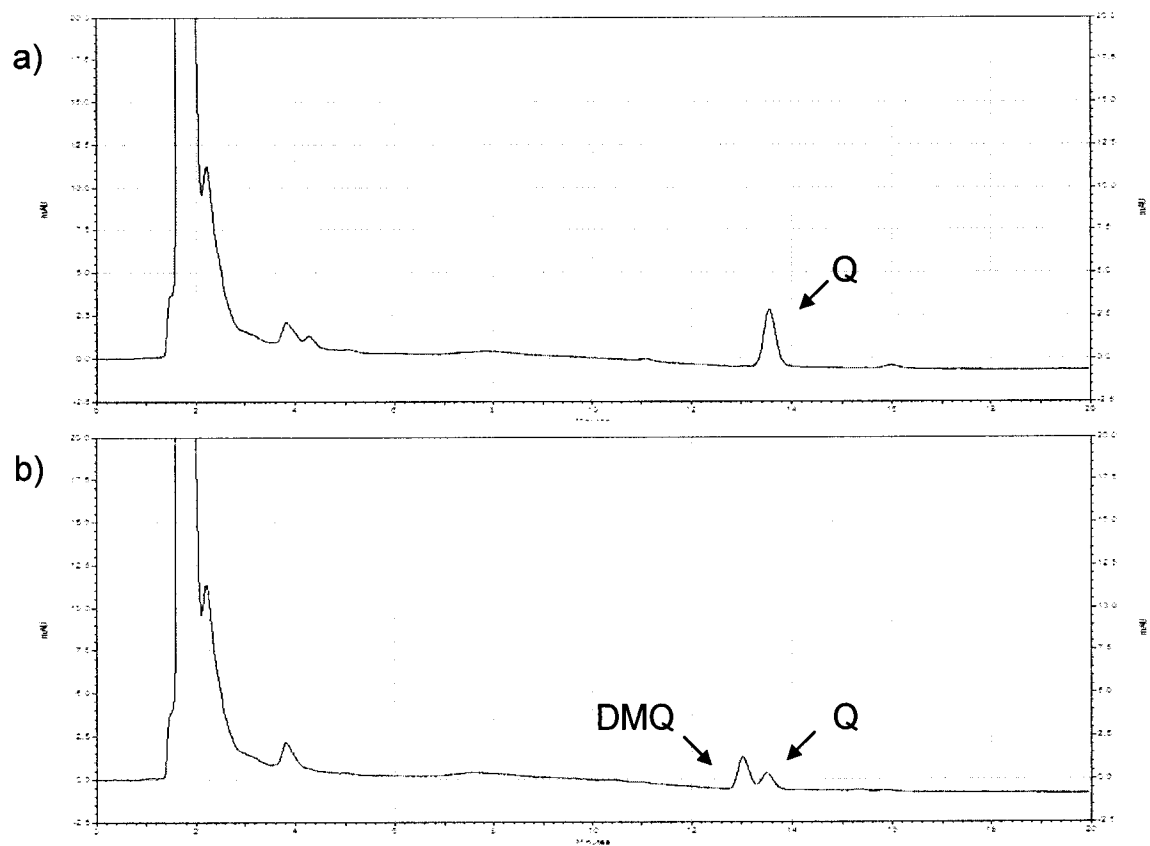
Compound number	Compound chemical name
<u>1</u>	N-[(5-chloro-8-hydroxyquinolin-7-yl)(3,4-dimethoxyphenyl)methyl]-2-methylpropanamide
<u>4</u>	N-[(5-chloro-8-hydroxyquinolin-7-yl)(3,4-dimethoxyphenyl)methyl]pentanamide
<u>7</u>	N-[(5-chloro-8-hydroxyquinolin-7-yl)(3,4-dimethoxyphenyl)methyl]acetamide
<u>9</u>	N-[(5-chloro-8-hydroxyquinolin-7-yl)(3,4-dimethoxyphenyl)methyl]butanamide
<u>10</u>	N-[(5-chloro-8-hydroxyquinolin-7-yl)(3,4-dimethoxyphenyl)methyl]propanamide
<u>20</u>	N-((5-chloro-8-hydroxyquinolin-7-yl)(3-

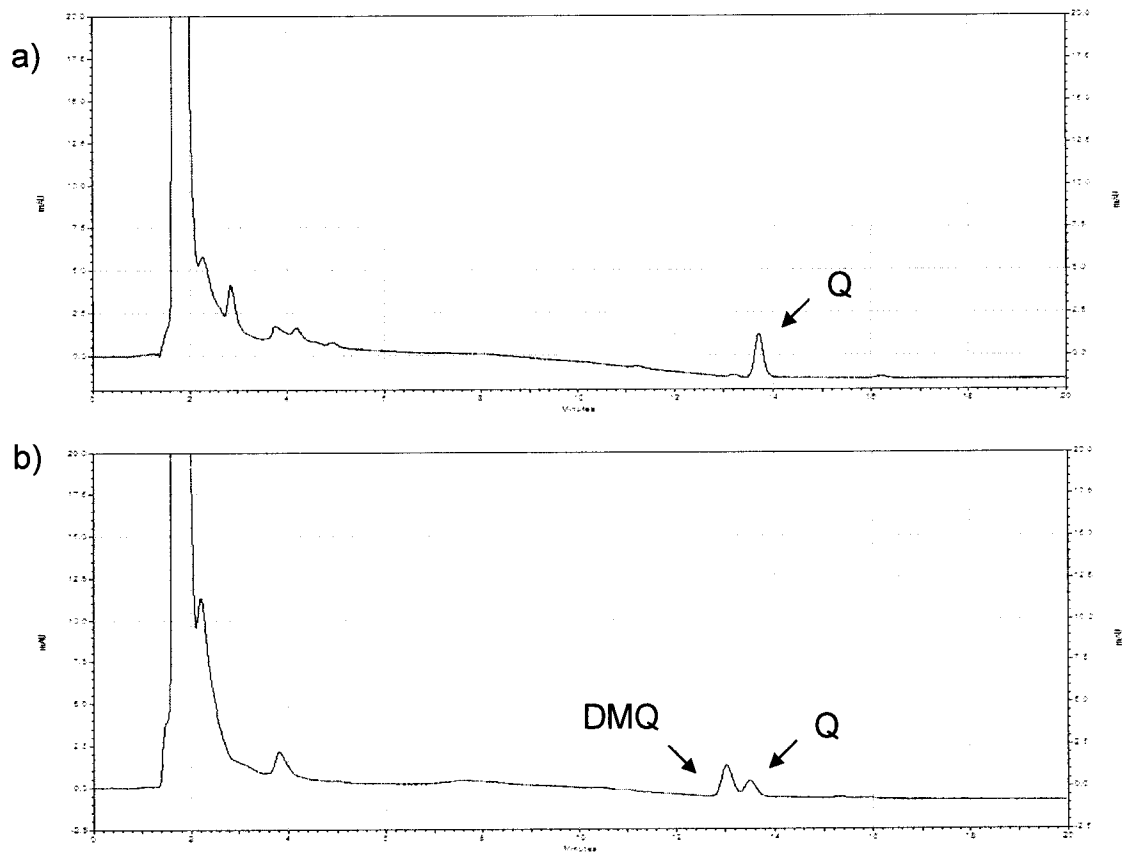
	furyl)methyl)acetamide
<u>42</u>	N-((5-chloro-8-hydroxyquinolin-7-yl)(3,4-dimethoxyphenyl)methyl)-2-phenoxyacetamide
<u>43</u>	N-((5-chloro-8-hydroxyquinolin-7-yl)(3,4-dimethoxyphenyl)methyl)nicotinamide
<u>49</u>	5,7-dichloroquinolin-8-ol
<u>50</u>	5,7-dibromoquinolin-8-ol
<u>51</u>	5,7-diiodoquinolin-8-ol
<u>52</u>	5-chloro-7-iodoquinolin-8-ol
<u>56</u>	5-chloro-7-(2-hydroxyphenyl)quinolin-8-ol
<u>60</u>	5-chloro-7-(4-(dimethylamino)phenyl)quinolin-8-ol
<u>83</u>	N-[(5-chloro-8-hydroxyquinolin-7-yl)(4-methylphenyl)methyl]acetamide
<u>84</u>	N-[(5-chloro-8-hydroxyquinolin-7-yl)(4-chlorophenyl)methyl]acetamide
<u>85</u>	N-[(5-chloro-8-hydroxyquinolin-7-yl)(2-chlorophenyl)methyl]acetamide
<u>86</u>	N-[(5-chloro-8-hydroxyquinolin-7-yl)(2,4-dichlorophenyl)methyl]acetamide
<u>87</u>	N-[(5-chloro-8-hydroxyquinolin-7-yl)(2-thienyl)methyl]acetamide
<u>88</u>	N-[(5-chloro-8-hydroxyquinolin-7-yl)(4-isopropylphenyl)methyl]acetamide
<u>89</u>	N-[(5-chloro-8-hydroxyquinolin-7-yl)(2-furyl)methyl]propanamide
<u>90</u>	N-[(5-chloro-8-hydroxyquinolin-7-yl)(2-methoxyphenyl)methyl]propanamide
<u>91</u>	N-[(5-chloro-8-hydroxyquinolin-7-yl)(4-chlorophenyl)methyl]propanamide
<u>95</u>	N-[(5-chloro-8-hydroxyquinolin-7-yl)(phenyl)methyl]butanamide
<u>96</u>	N-[(5-chloro-8-hydroxyquinolin-7-yl)(phenyl)methyl]pentanamide
<u>97</u>	N-[(5-chloro-8-hydroxyquinolin-7-yl)(2-furyl)methyl]-2-methylpropanamide
<u>98</u>	N-[(5-chloro-8-hydroxyquinolin-7-yl)(2-methoxyphenyl)methyl]butanamide

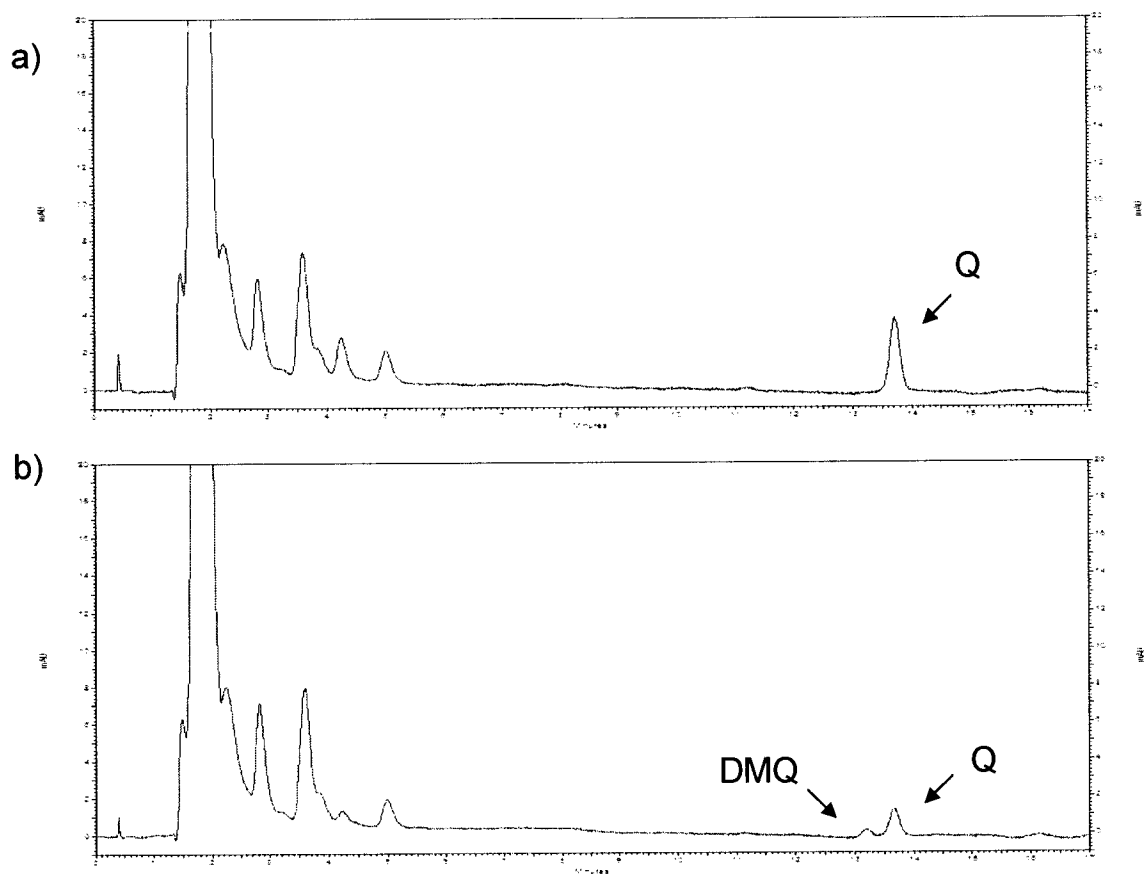
<u>99</u>	N-[(5-chloro-8-hydroxyquinolin-7-yl)(2-methoxyphenyl)methyl]-2-methylpropanamide
<u>100</u>	N-[(5-chloro-8-hydroxyquinolin-7-yl)(4-methoxyphenyl)methyl]butanamide
<u>101</u>	N-[(5-chloro-8-hydroxyquinolin-7-yl)(4-methoxyphenyl)methyl]pentanamide
<u>102</u>	N-[(5-chloro-8-hydroxyquinolin-7-yl)(4-methylphenyl)methyl]butanamide
<u>103</u>	N-[(5-chloro-8-hydroxyquinolin-7-yl)(4-methylphenyl)methyl]pentanamide
<u>104</u>	N-[(5-chloro-8-hydroxyquinolin-7-yl)(4-methylphenyl)methyl]-2-methylpropanamide
<u>105</u>	N-[(5-chloro-8-hydroxyquinolin-7-yl)(4-chlorophenyl)methyl]butanamide
<u>106</u>	N-[(5-chloro-8-hydroxyquinolin-7-yl)(4-chlorophenyl)methyl]pentanamide
<u>107</u>	N-[(5-chloro-8-hydroxyquinolin-7-yl)(2-chlorophenyl)methyl]pentanamide
<u>108</u>	N-[(5-chloro-8-hydroxyquinolin-7-yl)(2-thienyl)methyl]-3-phenylpropanamide
<u>109</u>	N-[(5-chloro-8-hydroxyquinolin-7-yl)(4-isopropylphenyl)methyl]-2-methylpropanamide
<u>114</u>	N-[(8-hydroxyquinolin-7-yl)(4-isopropylphenyl)methyl]-3-methylbutanamide
<u>115</u>	N-[(5-chloro-8-hydroxyquinolin-7-yl)[4-(dimethylamino)phenyl]methyl]butanamide
<u>116</u>	N-[(5-chloro-8-hydroxyquinolin-7-yl)(2-thienyl)methyl]butanamide
<u>117</u>	N-[(5-chloro-8-hydroxyquinolin-7-yl)(2-thienyl)methyl]pentanamide
<u>118</u>	N-[(5-chloro-8-hydroxyquinolin-7-yl)(2-thienyl)methyl]-2-methylpropanamide
<u>123</u>	N-[(5-chloro-8-hydroxyquinolin-7-yl)(2,4-dichlorophenyl)methyl]propanamide
<u>132</u>	N-[(5-chloro-8-hydroxyquinolin-7-yl)(phenyl)methyl]acetamide
<u>133</u>	N-[(5-chloro-8-hydroxyquinolin-7-yl)(2-methoxyphenyl)methyl]acetamide
<u>134</u>	N-[(5-chloro-8-hydroxyquinolin-7-yl)(2-

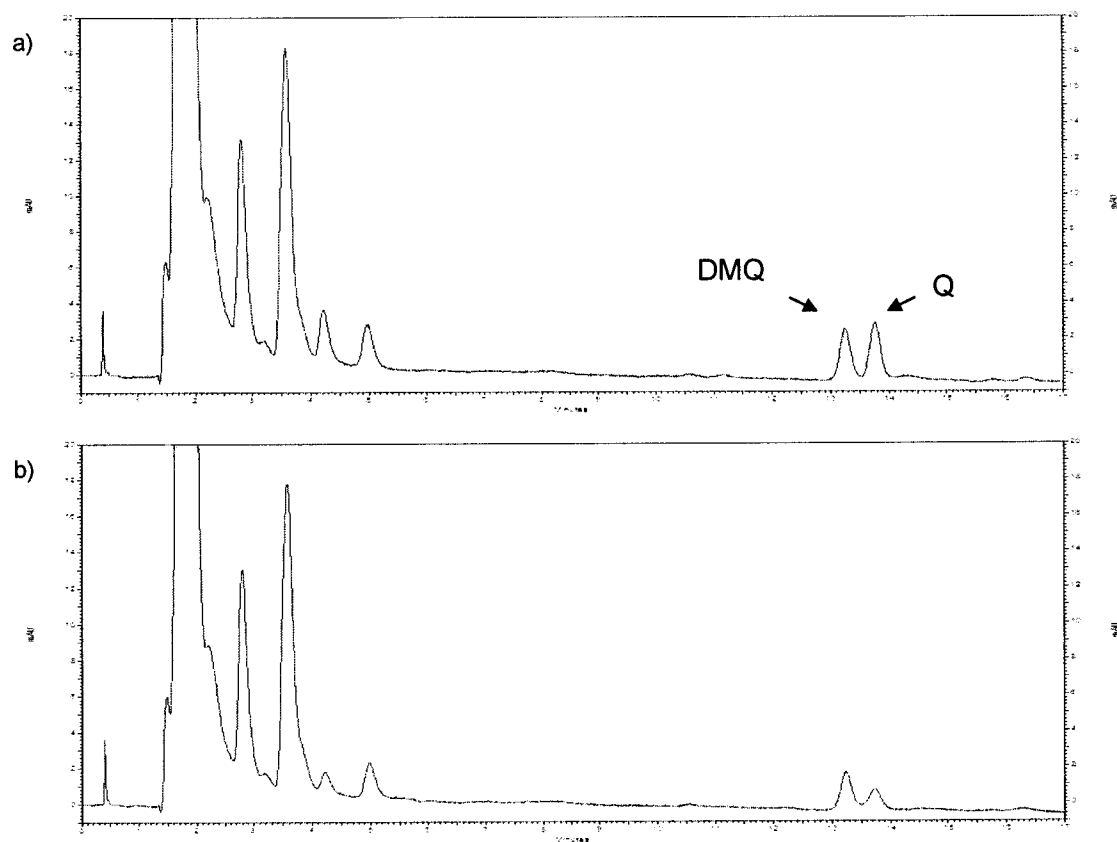
	methoxyphenyl)methyl]pentanamide
<u>135</u>	N-[(5-chloro-8-hydroxyquinolin-7-yl)(2-furyl)methyl]acetamide

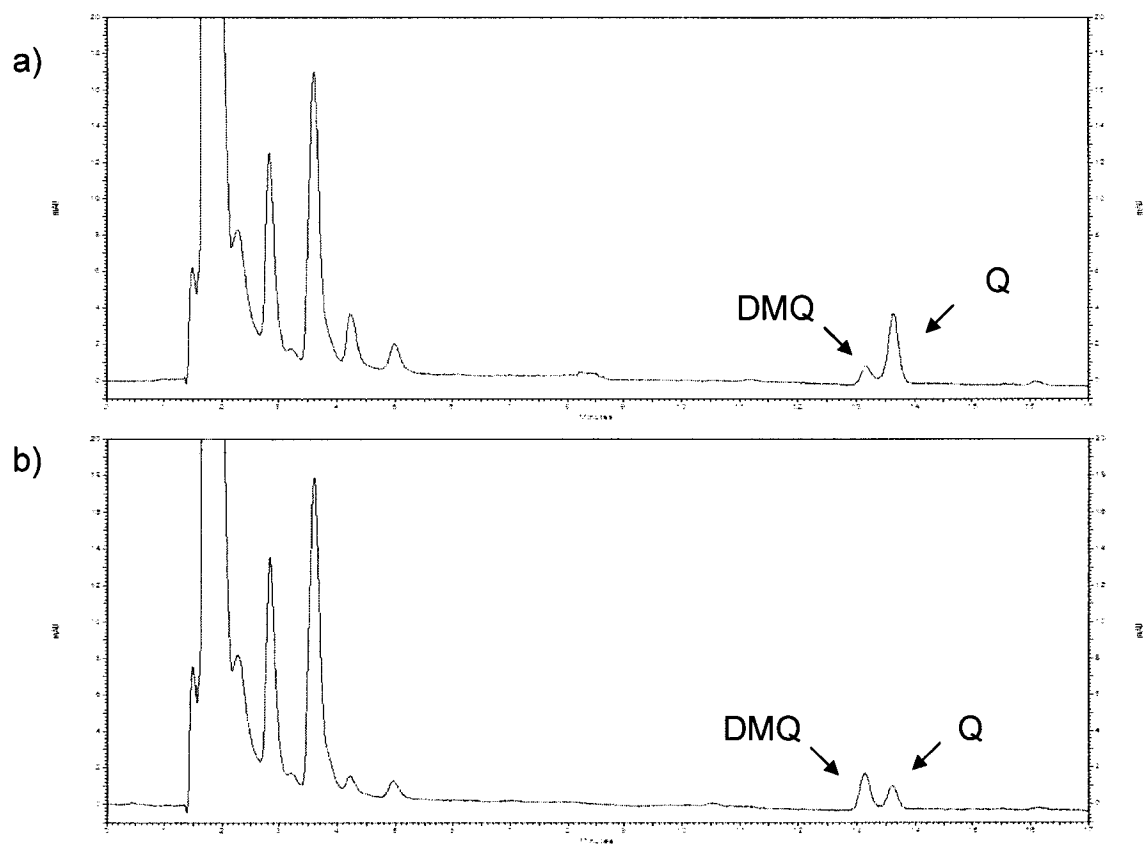
**FIG. 1**

**FIG. 2**

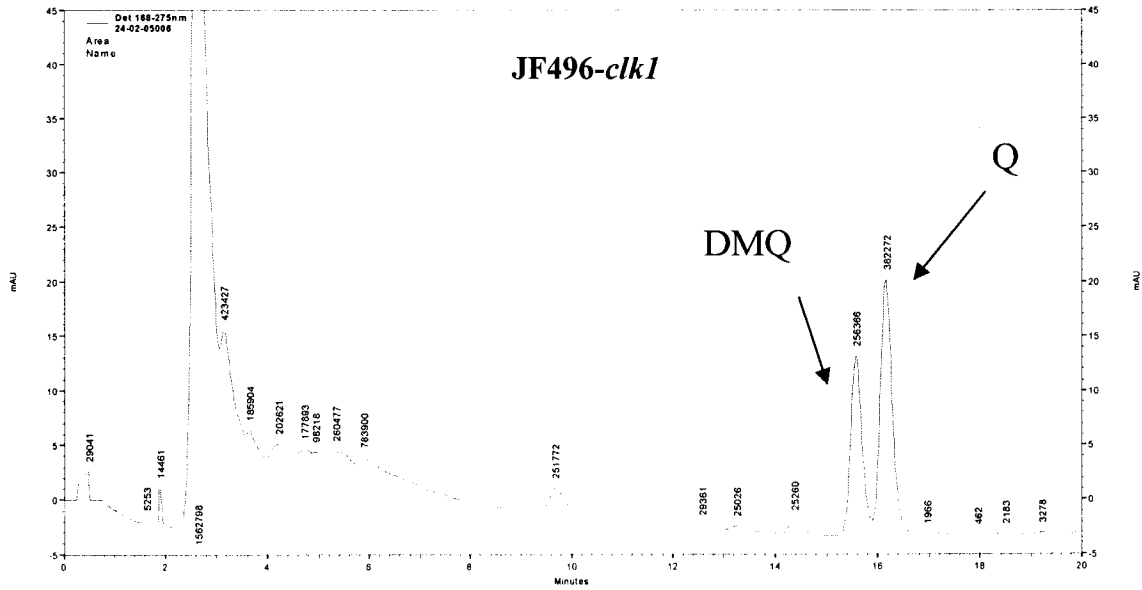
**FIG. 3**

**FIG. 4**

**FIG. 5**

**FIG. 6**

a)



b)

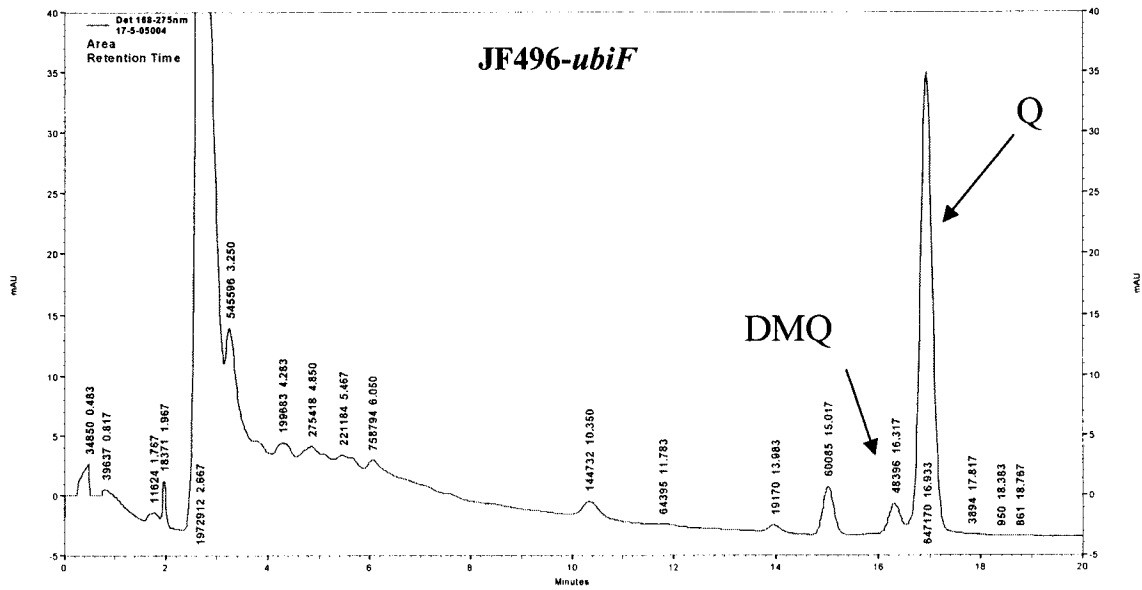
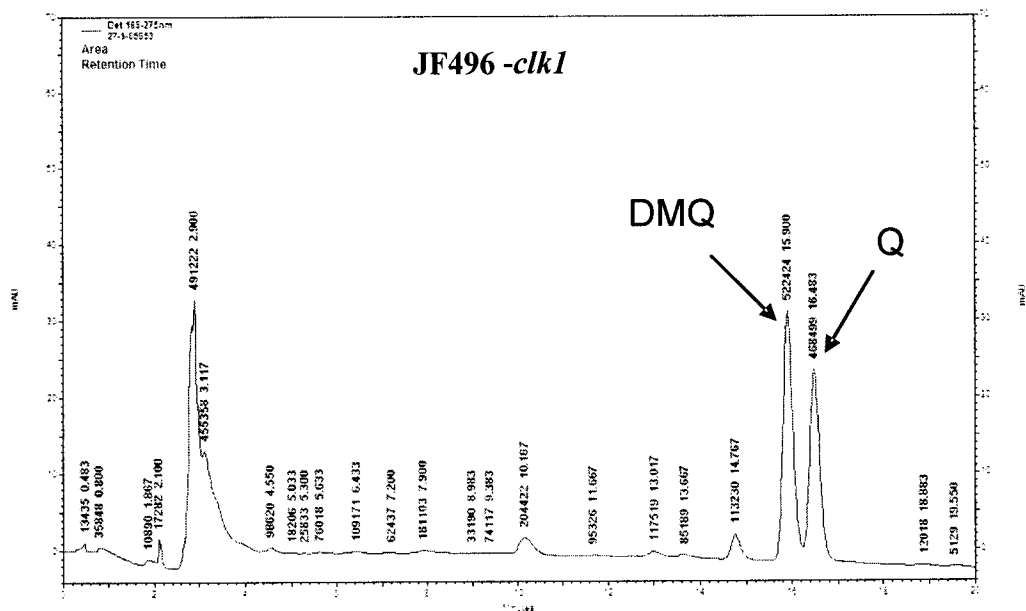


FIG. 7

a)



b)

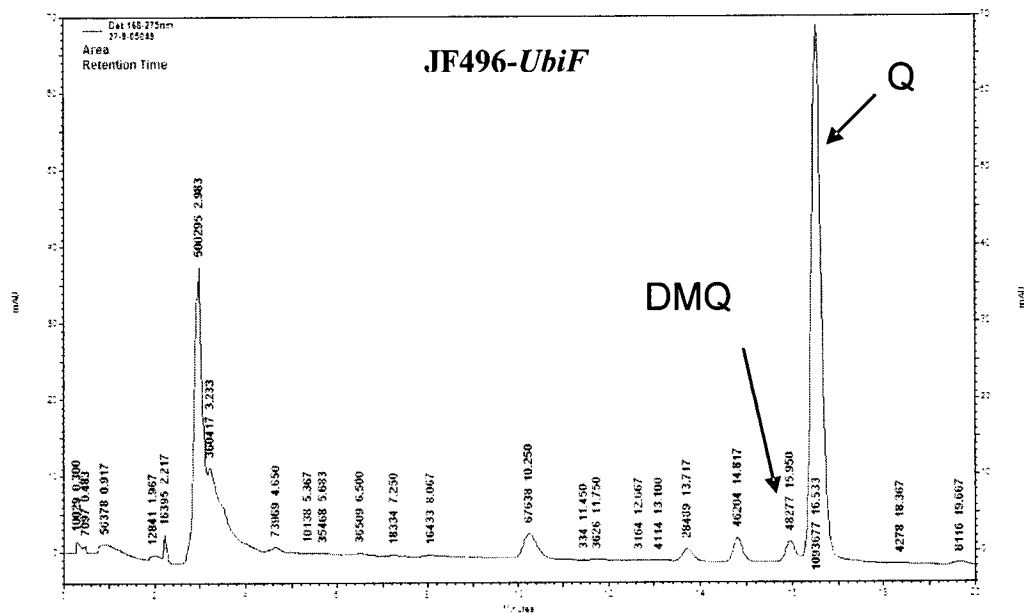


FIG. 8

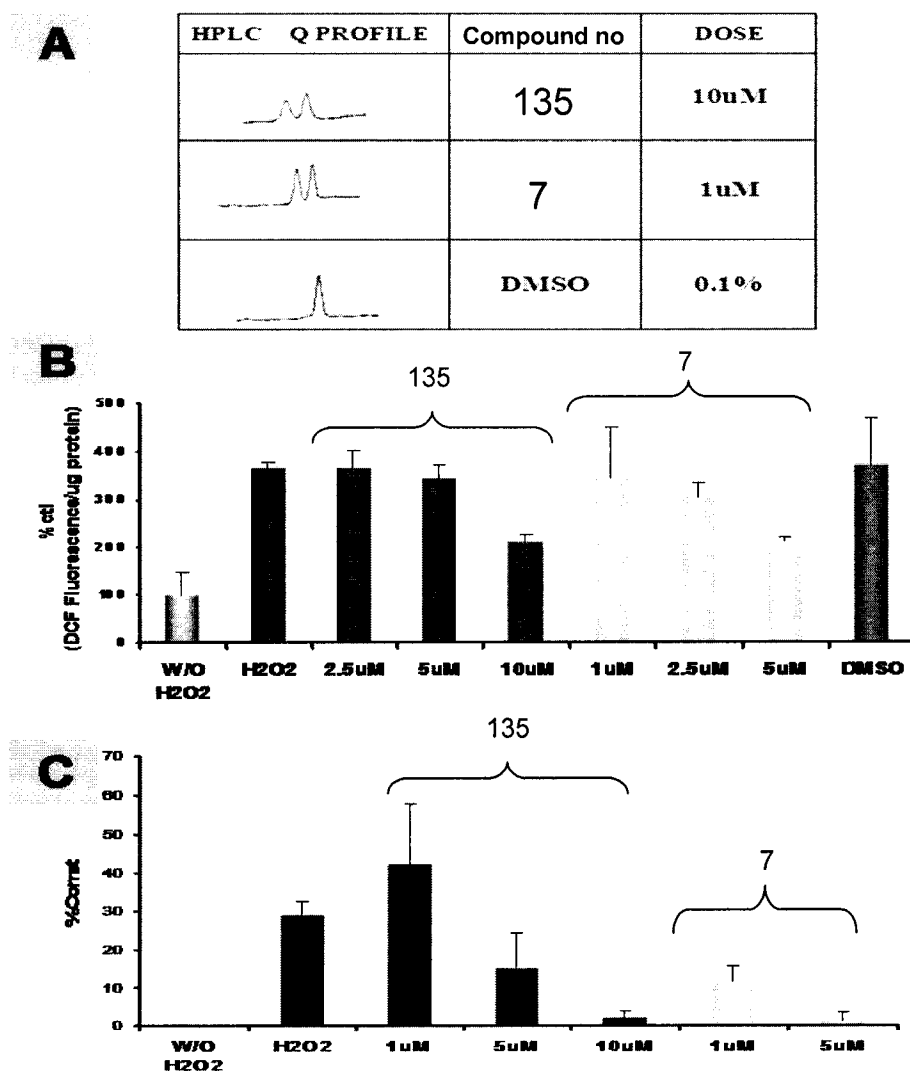
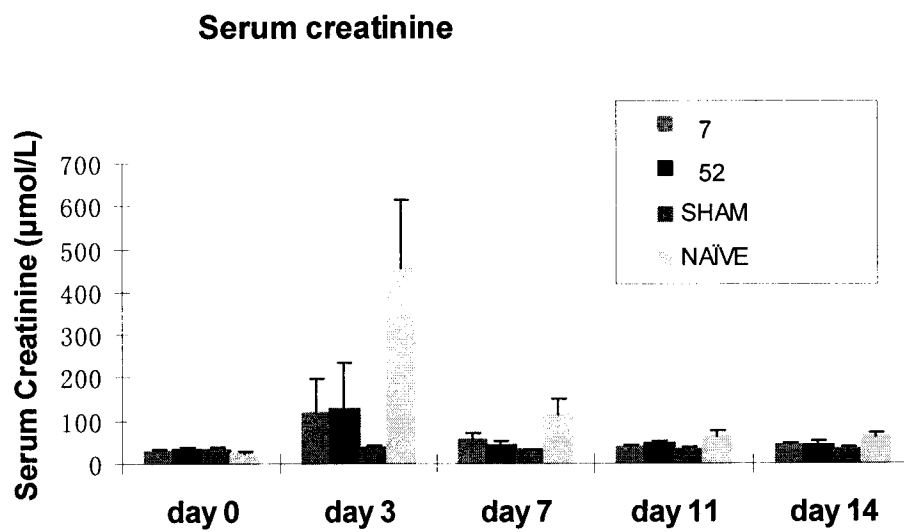
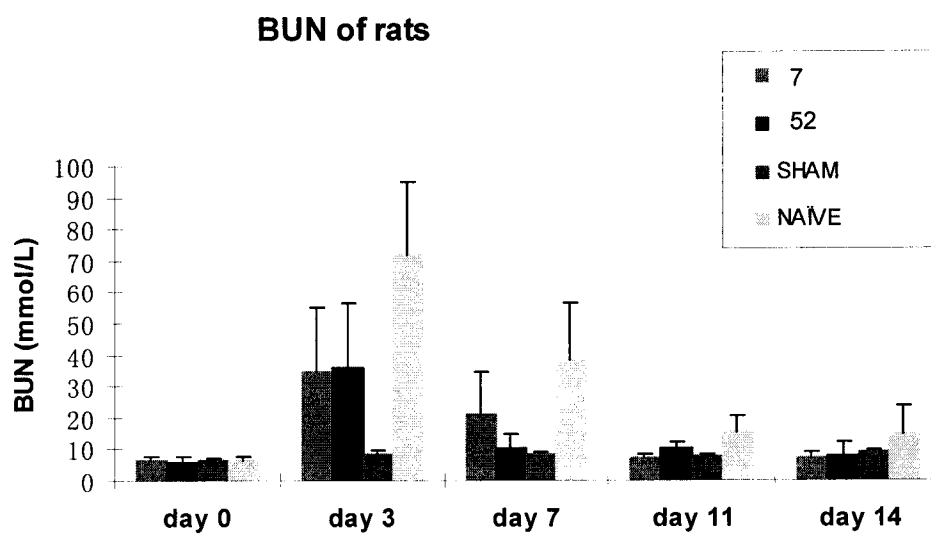
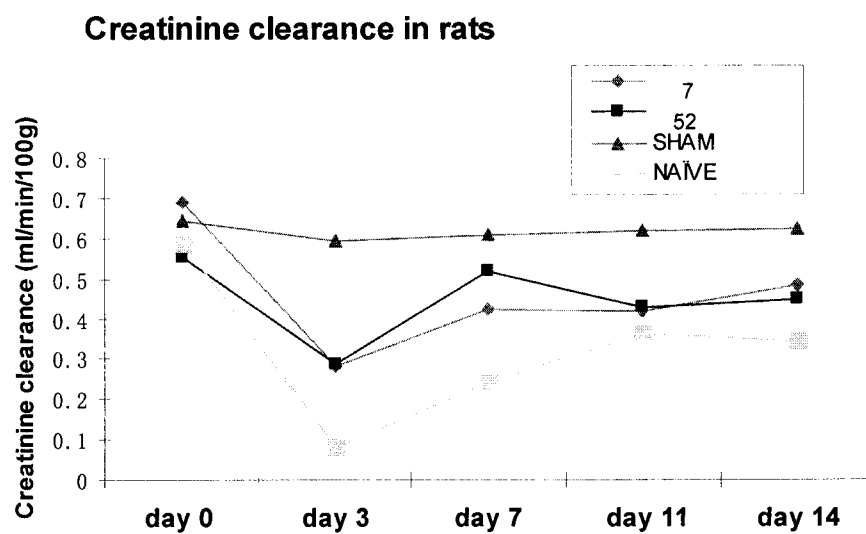
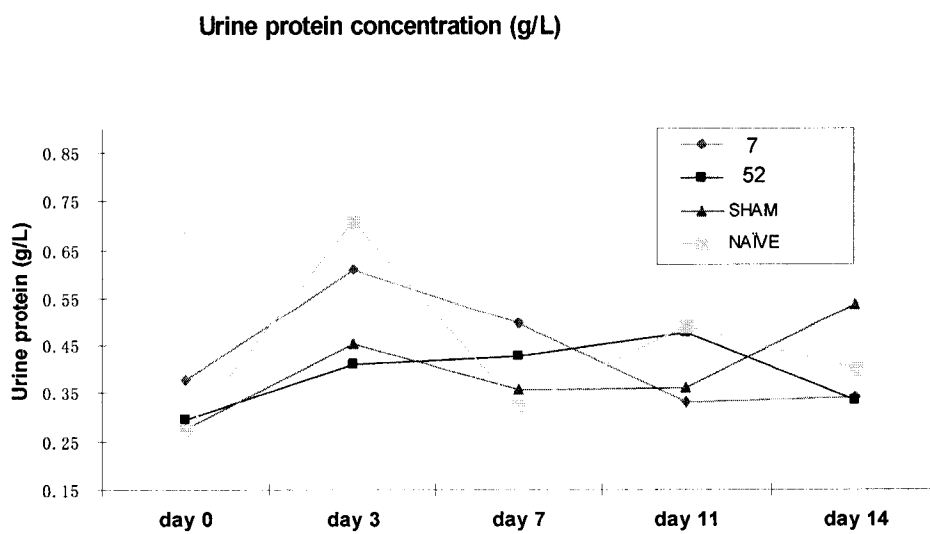


FIG. 9

**FIG. 10****FIG. 11**

**FIG. 12****FIG. 13**

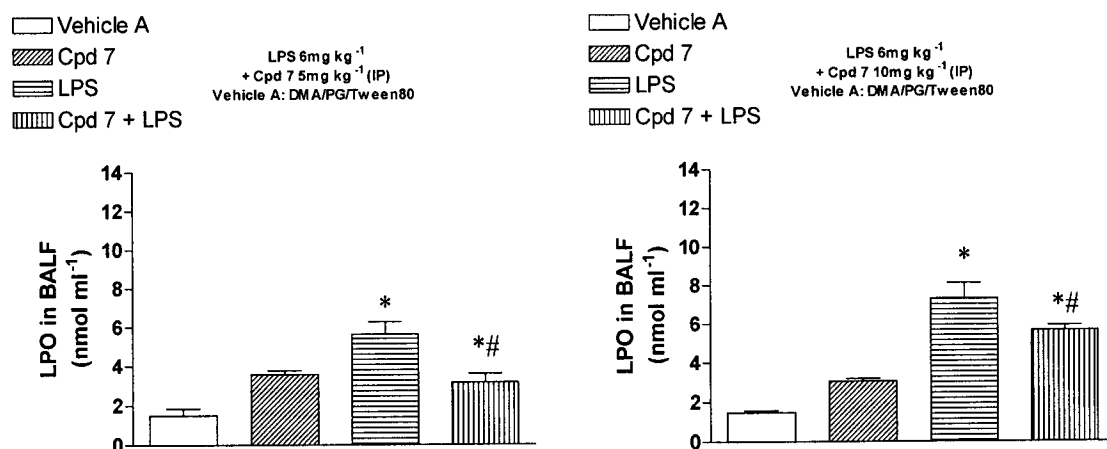


FIG. 14

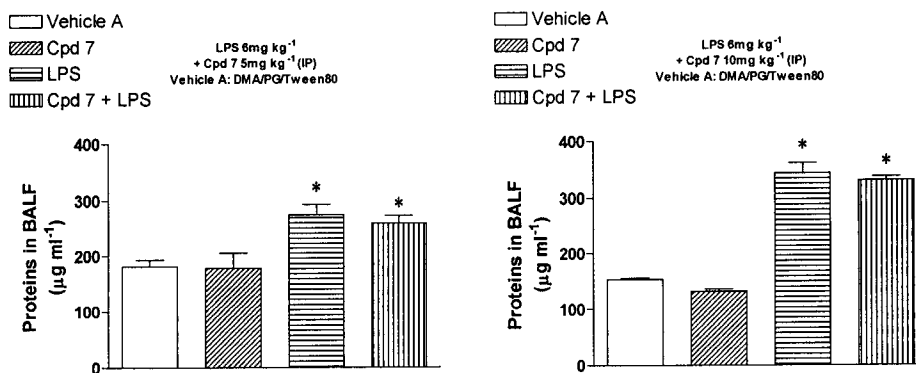


FIG. 15

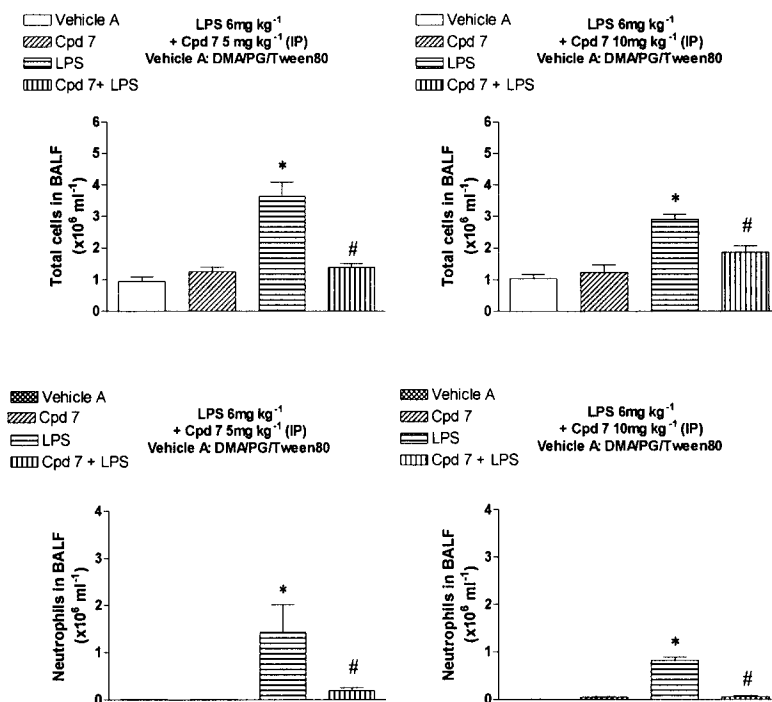


FIG. 16

TNF- α levels in BALF

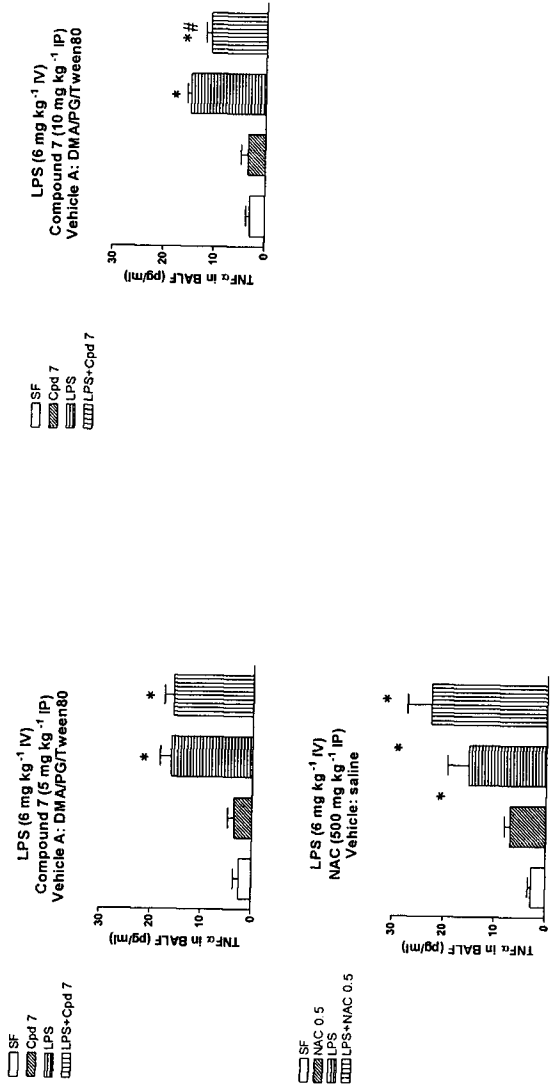
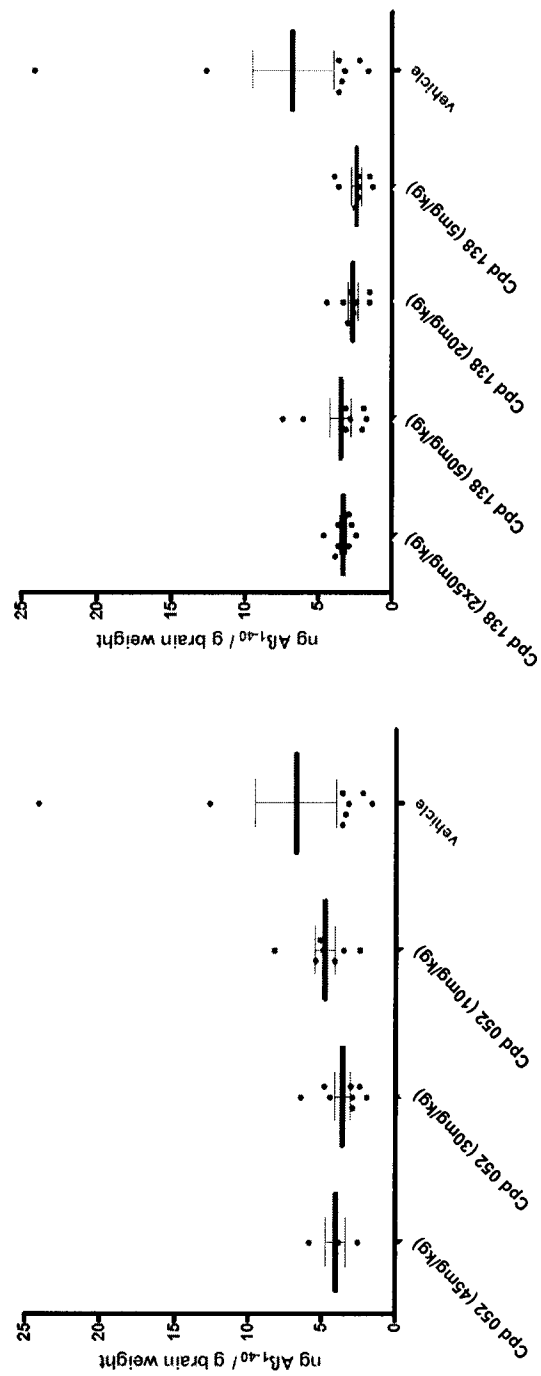


FIG 17

**FIG 18**

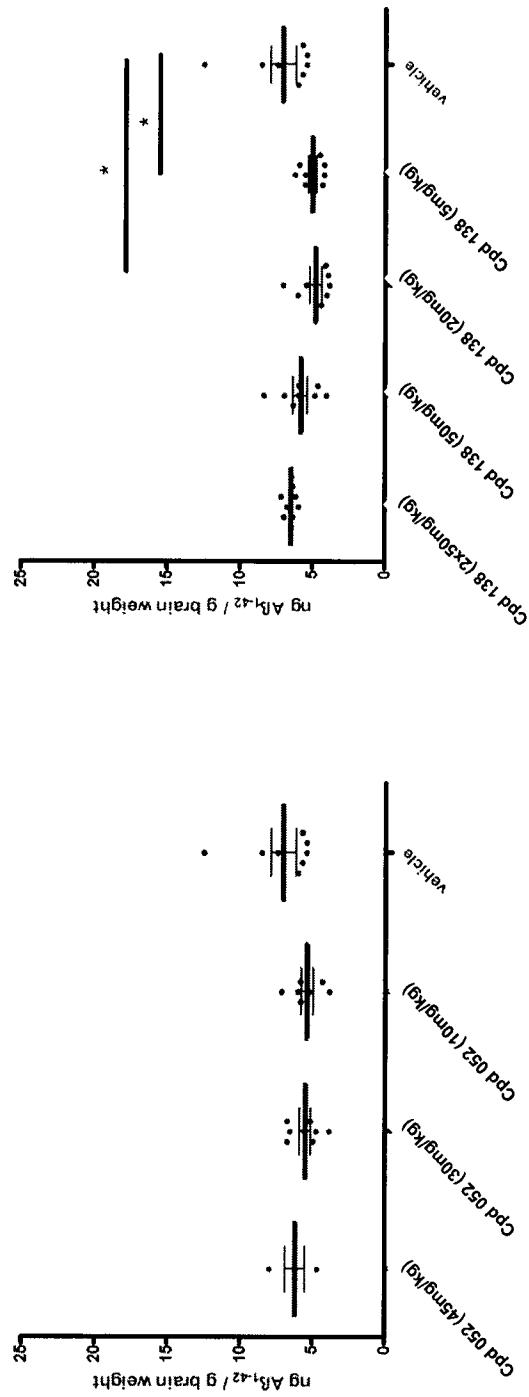


FIG 19

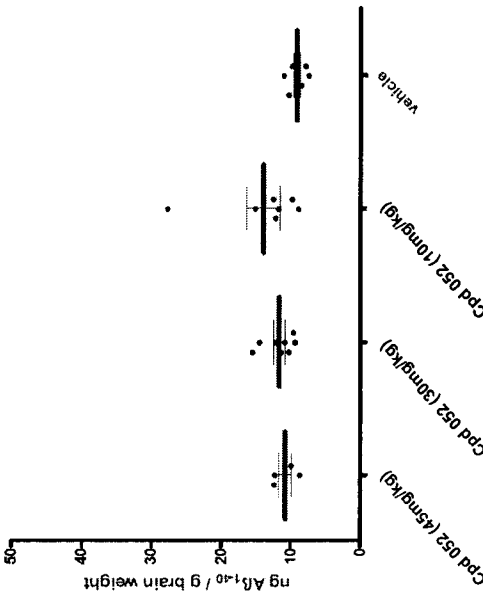
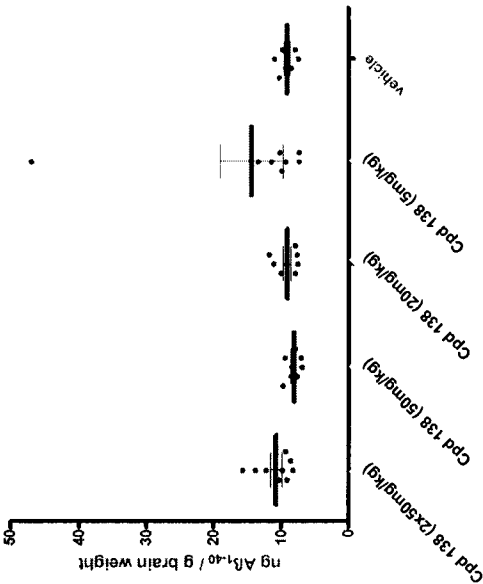


FIG 20

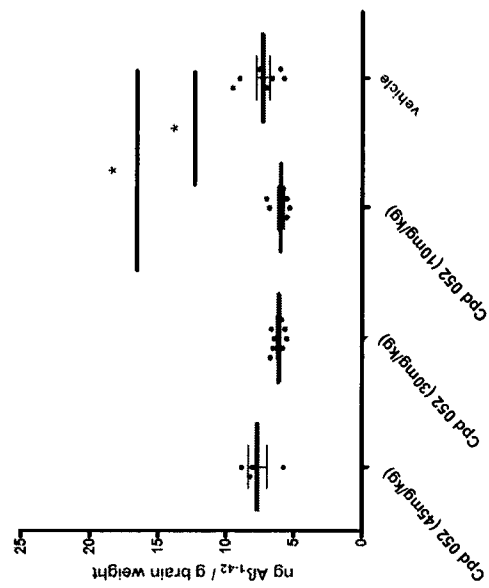
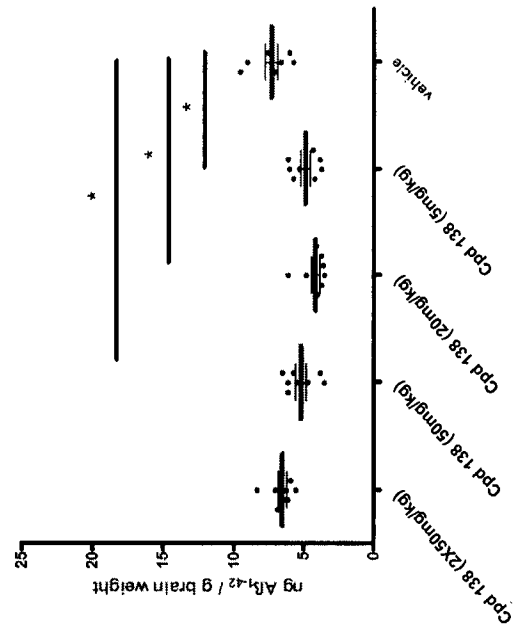


FIG 21

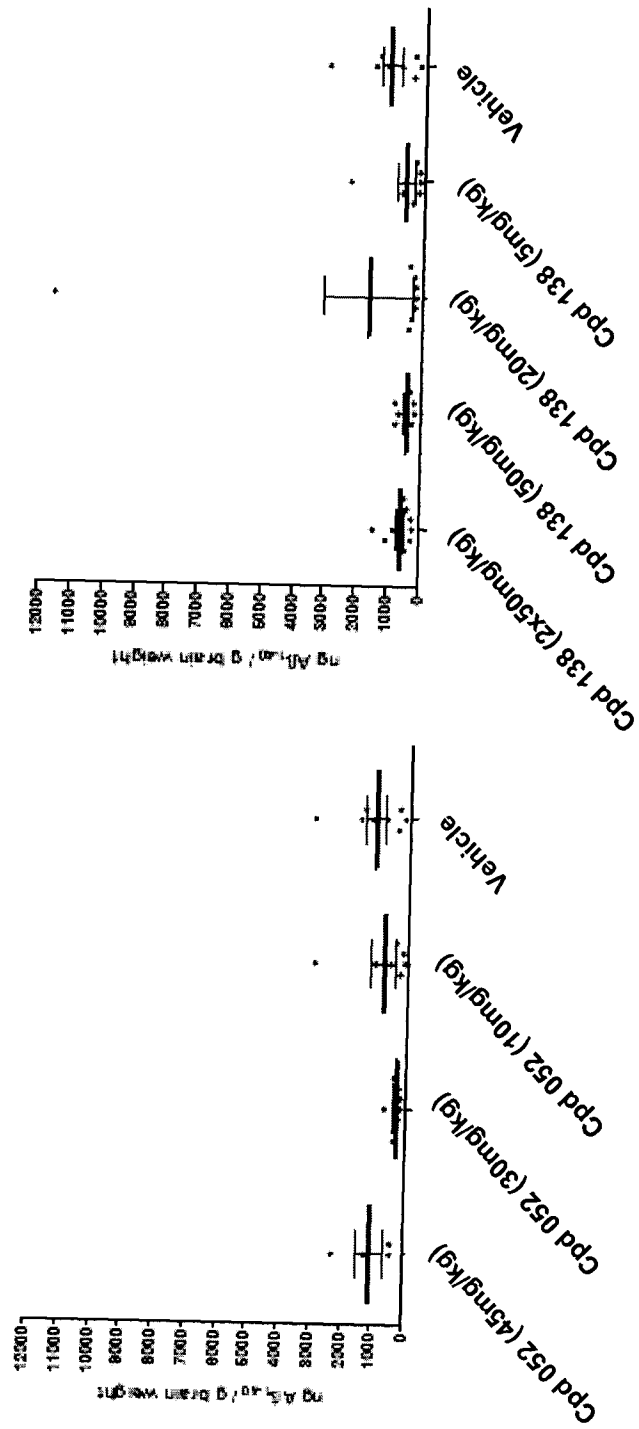


FIG 22

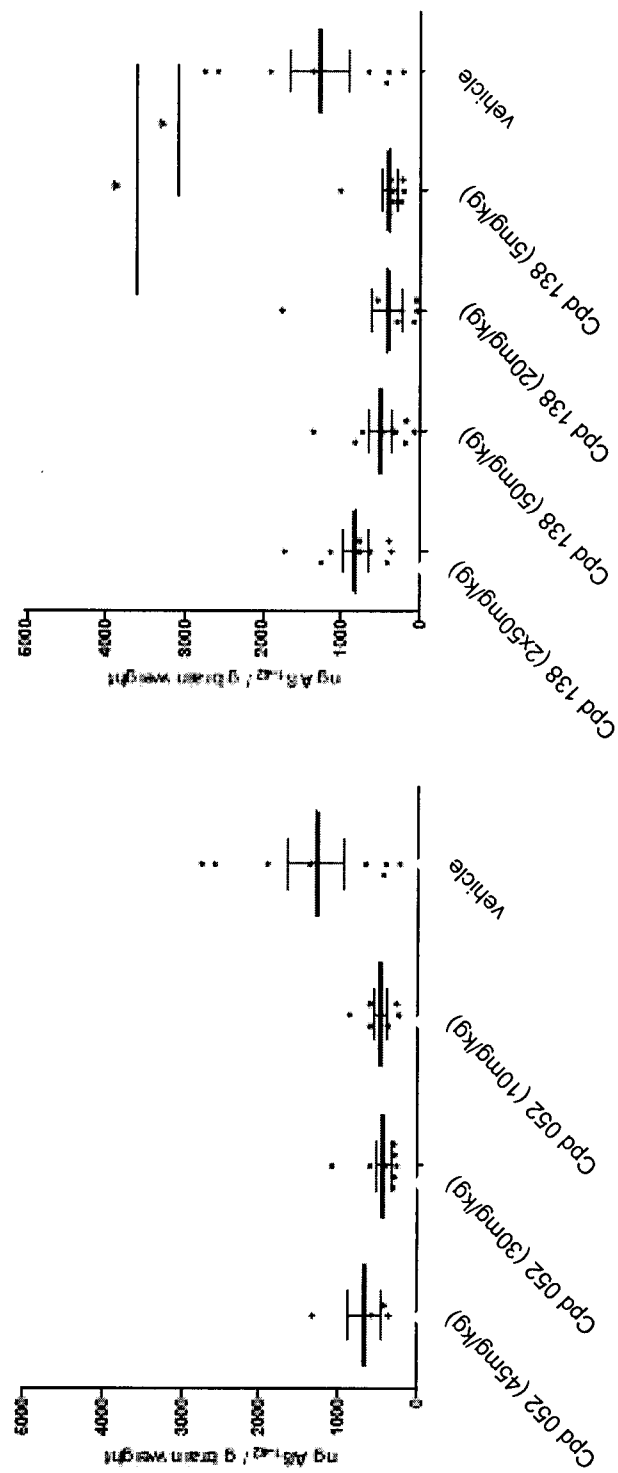


FIG 23

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2007/001324

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8): *C07D 215/24* (2006.01) , *A61K 31/47* (2006.01) , *A61K 31/4709* (2006.01) , *A61K 31/5377* (2006.01) , *C07D 215/26* (2006.01) , *C07D 215/28* (2006.01) , *C07D 215/12* (2006.01) , *C07D 215/16* (2006.01) , *C07D 401/06* (2006.01) , *C07D 413/06* (2006.01)

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC(8): *C07D 215/24* (2006.01) , *A61K 31/47* (2006.01) , *A61K 31/4709* (2006.01) , *A61K 31/5377* (2006.01) , *C07D 215/26* (2006.01) , *C07D 215/28* (2006.01) , *C07D 215/12* (2006.01) , *C07D 215/16* (2006.01) , *C07D 401/06* (2006.01) , *C07D 413/06* (2006.01)

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

Electronic database(s) consulted during the international search (name of database(s) and, where practicable, search terms used)

STN (structure), Scopus, Canadian Patent Database, keywords: hydroxyquinolin*, hydroxy*quinoline, "CLK-1 inhibitor*", "CLK*", ubiquinone, demethoxyquinone, age.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CA 2,493,536 A1 (BARNHAM ET AL.) 22 January 2004 (22-01-2004) entire document (cited in the application)	1, 2, 12-14 and 18-20
X	WO 02/30930 A2 (ANTHONY ET AL.) 18 April 2002 (18-04-2002) claims, Examples 129 and 134	1, 2, 19 and 20
X	WO 98/11073 A1 (VAILLANCOURT ET AL.) 19 March 1998 (19-03-1998) claims, compounds V4-94 page 36, Examples 152 and 231	1, 2, 19 and 20
X	WO 02/070486 A1 (FUJI ET AL.) 12 September 2002 (12-09-2002) claims, compounds 52 (page 134), I-78 (page 137), I-79 and I-80 (page 138)	1-3 and 19-21

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

[X] See patent family annex.

* Special categories of cited documents :	"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
"A" document defining the general state of the art which is not considered to be of particular relevance	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E" earlier application or patent but published on or after the international filing date	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&" document member of the same patent family
"O" document referring to an oral disclosure, use, exhibition or other means	
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

20 November 2007 (20-11-2007)

Date of mailing of the international search report

29 November 2007 (29-11-2007)

Name and mailing address of the ISA/CA
Canadian Intellectual Property Office
Place du Portage I, C114 - 1st Floor, Box PCT
50 Victoria Street
Gatineau, Quebec K1A 0C9
Facsimile No.: 001-819-953-2476

Authorized officer

Cara Weir 819- 934-2322

INTERNATIONAL SEARCH REPORTInternational application No.
PCT/CA2007/001324**Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of the first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons :

1. ☒ Claim Nos. : 5-18

because they relate to subject matter not required to be searched by this Authority, namely :

Although claims 5-18 are directed to a method of medical treatment of the human or animal body, the search has been carried out based on the alleged effects of the compounds.

2. ☐ Claim Nos. :

because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically :

3. ☐ Claim Nos. :

because they are dependant claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

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

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claim Nos. :

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim Nos. :

Remark on Protest ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2007/001324

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Chem. Ber., 1974, 107(8), MÖHRLE ET AL., "On Mannich Bases, VIII. The Structure of Products from the <i>Betti</i> Reaction with Ammonia", pages 2675-2682. N-((phenyl)-8-hydroxyquinolin-7-yl)methyl)benzamide (compound 8, page 2680) and N-((phenyl)-8-hydroxyquinolin-7-yl)methyl)acetamide (compound 8, page 2681)	1 and 2
X	J. Electrochem. Soc. India, 1978, 27(2), SHAH ET AL., "Proton ligand stability constants of some quinolinol derivatives in dioxane-water media", page 121. 7-(aminophenylmethyl)-8-quinolinol	1 and 2
P,X	Bioorg. Med. Chem. Lett., 2006, 16(21), WARSHAKOON ET AL., "Structure-based design, synthesis, and SAR evaluation of a new series of 8-hydroxyquinolines as HIF-1 α prolyl hydroxylase inhibitors", pages 5517-5522. Table 1 compounds 4, 5, 7, 16, 17 and 19 (page 5519)	1 and 2

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CA2007/001324

Patent Document Cited in Search Report	Publication Date	Patent Family Member(s)	Publication Date
CA2493536	22-01-2004	AU2002950217D D0	12-09-2002
		AU2003243836 A1	02-02-2004
		BR0312934 A	21-06-2005
		CN1681791 A	12-10-2005
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		JP2006504646T T	09-02-2006
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		CA2439666 A1	12-09-2002
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		CZ20032332 A3	12-11-2003
		EP1375486 A1	02-01-2004
		HU0400175 A2	30-07-2007
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