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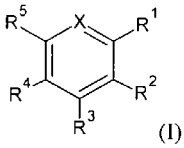
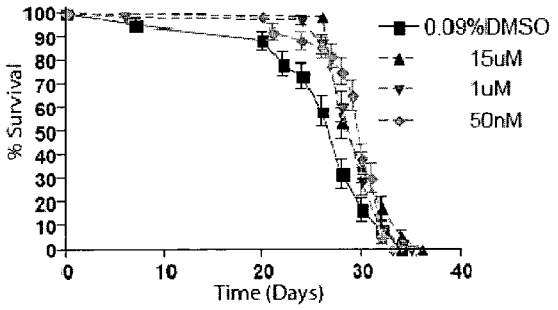
(54) Title: COMPOUNDS FOR THE TREATMENT OF MITOCHONDRIAL DISEASES



(I)

(57) Abstract: The present invention relates to the use of compounds of general formula (I): (I) for the preparation of medicaments that act against mitochondrial pathologies involving a deficiency in ATP production via the oxidative phosphorylation pathway, such as mitochondrial diseases.



Compound of Formula 13

FIGURE 8

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COMPOUNDS FOR THE TREATMENT OF MITOCHONDRIAL DISEASES

The present invention relates to the isolation and development of drugs to treat mitochondrial pathologies involving a deficiency in ATP production via the oxidative phosphorylation pathway, such as NARP syndrome.

NARP (Neuropathy, Ataxia and Retinitis Pigmentosa) is a maternally transmitted hereditary syndrome characterized by retarded development, and accompanied by retinitis pigmentosa (RP), dementia, ataxia, proximal neurological muscle weakness and sensory neuropathies (Schon *et al.*, J. Bioenerg. Biomembr., 1994, 26, 291-299; Graeber, M.B. and Müller, U., J. Neurol. Sci., 1998, 153, 251-263, for review). This disease is in general a pathology which occurs in children, but it has also been reported in rarer cases in adults. The clinical manifestations are varied and can take more or less severe forms. Thus, the ophthalmic manifestations can range from a simple "salt and pepper" changing of the retina to severe RP, accompanied by maculopathy. Similarly, there is a broad spectrum of neurological manifestations, which ranges from simple migraines to severe dementia and to "Leigh's disease" (subacute necrotising encephalomyelopathy; Ortiz *et al.*, Arch., Ophtalmol., 1993, 111, 1525-1530). Many retinitis pigmentosa-related syndromes exist, such as Usher's syndrome in which both the sight and the hearing are affected, or else macular dystrophy, also called inverse RP.

In 1990, Holt *et al.* (Am. J. Hum. Genet., 46, 428-433) described for the first time the presence of the T8993G mutation in the mitochondrial DNA of patients showing NARP syndrome/Leigh's disease. It was subsequently postulated by Tatuch and Robinson (Biochem. Biophys. Res. Commun., 1993, 192, 124-128) that this mutation -occurring in the ATP6 subunit- resulted in a reduction in ATP synthesis by impairing the mitochondrial ATP synthase complex. This mutation is thought to be responsible for an ATP synthase assembly/stability defect (Nijtmans *et al.*, J. Biol. Chem., 2001, 276, 6755-6762). Other *ATP6* gene mutations have also been detected, in association with NARP syndrome/Leigh's disease; T8993C, T9176G, T9176C, T8851C, T9185C and T9191C (Schon *et al.*, Cell & Dev. Biol., 2001, 12, 441-448 and Kucharczyk *et al.*, Biochimica et Biophysica Acta, 2009, 1793, 186-199). In addition, a T9101C mutation has been involved in the LHON (*Leber's Hereditary Optic Neuropathy)* syndrome, another mitochondrial syndrome (Kucharczyk *et al.*, Biochimica et Biophysica Acta, 2009, 1793, 186-199). Simple point mutations are therefore responsible for these syndromes, which have many more or less serious

forms. The great diversity of the pathological manifestations is attributed to the heteroplasmic nature of this mutation in patients, i.e. the coexistence of mutated and wild-type mitochondrial DNA molecules in the cells or tissues. The mutated mitochondrial DNA load is closely correlated with the seriousness of the symptoms observed (Uziel *et al.*, J. Neurol. Neurosurg. Psychiatry, 1997, 63, 16-22; Carelli *et al.*, Arch. Neurol., 2002, 59, 264-270).

The ATP synthase complex, which is the target of the T8993G mutation (and of the other mutations mentioned above), is located in the inner mitochondrial membrane (Figures 1 and 2A). It catalyzes the last steps of oxidative phosphorylation, a process which allows cells to extract the chemical energy of metabolites and to store this energy in ATP molecules. In order to synthesize ATP, the ATP synthase complex uses the electrochemical proton gradient on either side of the inner membrane, generated by other complexes located in this membrane, the respiratory complexes (Figure 1). The latter transfer to oxygen the reducing equivalents of the substrates that are oxidized in the mitochondrion. These transfers are coupled to proton transports (hydrogen ions, H^+) across the inner membrane, from the inside (the mitochondrial matrix) into the space between the outer and inner membranes (intermembrane space) of the organelle. The result is a proton concentration that is higher at the outer periphery of the inner membrane than at its inner periphery. The membrane domain F_o (Figure 1) of ATP synthase enables a channeled return of the protons into the mitochondrial matrix. This transport is coupled to ATP synthesis in the catalytic domain F_1 of ATP synthase located outside the membrane, in the mitochondrial matrix. ATP synthase operates like a rotary turbine: the passage of protons in F_o is coupled to the rotation of a subcomplex (the rotor) of the enzyme. This rotation results in conformational changes in F_1 which promote the synthesis of ATP from ADP and inorganic phosphate (Boyer P.D., Annu, Rev., Biochem., 1997, 66, 717-747). The neosynthesized ATP molecules can, via a specific transporter located in the inner membrane (ADP/ATP translocase), leave the mitochondrial compartment so as to supply the entire cell with energy. ATP synthase comprises about twenty different protein subunits for a mass of approximately 600 KDa. In humans, two ATP synthase subunits (Atp6p and Atp8p, Figure 2A) are encoded by the mitochondrial genome, all the other subunits being encoded by nuclear genes. The subunits of nuclear origin are synthesized in the cytosol and then imported into the mitochondrion, whereas the Atp6p and Atp8p subunits encoded by the

mitochondrial genome are actually synthesized inside the mitochondrion (Figure 2A).

The T8993G mutation associated with NARP syndrome is located within the mitochondrial *ATP6* gene (Figure 2B). The latter encodes ATP synthase subunit 6 (Atp6p) which is essential for proton transport across F_o. The T8993G mutation results in the replacement, with arginine, of a leucine residue conserved in all the known sequences of Atp6p, from bacteria to humans. This leucine residue is in an Atp6p region presumed to be transmembrane and essential for ATP synthase proton translocation activity. Studies carried out in the *Escherischia coli* bacterium or with NARP cybrids (human cells in which the mitochondria are enriched, up to 100%, in T8993G alleles) indicate that the T8993G mutation clearly affects the functioning of the ATP synthase proton channel and that this defect is the primary cause of the disease (Schon *et al.*, Cell & Dev. Biol., 2001, 12, 441-448; Nijtmans *et al.*, J. Biol. Chem., 2001, 276, 6755-6762).

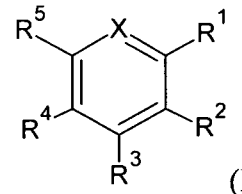
There is currently no effective medicament for the treatment of mitochondrial disorders induced by F_1F_0-ATP synthase dysfunctions.

A cellular model for the NARP syndrome has recently been developed consisting in yeast strains carrying within their mitochondrial genome, the equivalent of mitochondrial *ATP6* gene mutations responsible for NARP syndrome in humans (see the International PCT Application WO 2007/125225).

These yeast mutants make it possible to identify molecules capable of correcting the effects of the mutation by restoring either ATP synthase function, or sufficient production of ATP in the mitochondria, via a pathway other than that of oxidative phosphorylation.

The invention concerns compounds which have been selected for their ability to restore respiratory growth of the yeast *ATP6* mutant (Figures 3A and 3B).

An object of the present invention concerns the use of compounds having the general formula (I):



(I)

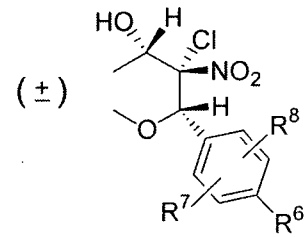
wherein

- **X** is a carbon atom or a nitrogen atom, both atoms being optionally

substituted by an oxygen atom;

- R^1 is a hydrogen atom, a halogen atom or a sulphur atom;
- R^2 is a hydrogen atom or

R^1 and R^2 taken together with the carbon atoms to which they are attached may form the following cycle



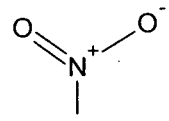
with the proviso that when R^1 and R^2 form the above defined cycle, **X** is a carbon atom;

- R^3 is a hydrogen atom; a halogen atom; an alkyl radical containing 1 to 6 carbon atoms, preferably 1 to 3 carbon atoms, optionally interrupted by one oxygen atom or one ester function or an alkenylene radical containing 2 to 6 carbon atoms, preferably 2 to 3 carbon atoms,

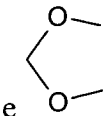
with the proviso that when R^1 and R^2 do not correspond to the above defined cycle, R^3 is a hydrogen atom;

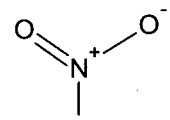
- R^4 is:
  - a hydrogen atom,
  - an alkyl radical containing 1 to 6 carbon atoms, preferably 1 to 3 carbon atoms, optionally interrupted by one oxygen atom,
  - a linear chain containing between 5 and 15 carbon atoms and between 1 and 10 nitrogen atoms,

  said chain optionally contains 1 to 5 oxygen atoms and is optionally interrupted by a benzyl group optionally substituted by one halogen atom; carbon and/or nitrogen atoms of said chain being optionally substituted by 1 or 2 alkyl radicals containing 1 to 3 carbon atoms, preferably a methyl radical, and carbon atoms of said chain being optionally substituted with =NH function;

- R^5 is a hydrogen atom, a halogen atom, or a group  ;

R^4 and R^5 taken together with the carbon atoms to which they are attached may form

the following cycle  ;

- R^6, R^7 and R^8, which may be identical or different, are:
  - a hydrogen atom;
  - a halogen atom;
  - an alkyl radical containing 1 to 6 carbon atoms, preferably 1 to 3 carbon atoms, optionally interrupted by one oxygen atom or one ester function and optionally containing a benzyl group;
  - - a group $-NH_2$;
  - a group 

R^6 and R^8 taken together with the carbon atoms to which they are attached may form a benzyl group;

with the proviso that

when R^1 and R^2 correspond to the above defined cycle, $R^4 = R^5 = R^7 = R^8 = H$ and $R^6 = Cl$, R^3 is not $-O-CH_3$ or

when R^1 and R^2 correspond to the above defined cycle, $R^3 = R^5 = R^4 = R^8 = H$, and R^7 is in ortho-position of R^6, R^6 and R^7 are not simultaneously $-O-CH_3$,

or a pharmaceutical acceptable salt

for the preparation of a drug for the prevention and/or the treatment of disorders or diseases related to an insufficiency or a lack of ATP synthesis by F_1F_0-ATP synthase or related to an excessive accumulation of reactive oxygen species (ROS) chosen amongst mitochondrial diseases, normal or physiological ageing and neurodegenerative diseases.

By "halogen atom" is intended to mean fluorine, chlorine, bromine or iodine.

By "alkyl radical" containing 1 to 6 carbon atoms, is intended to mean a saturated, linear or branched, chain of 1 to 6 carbon atoms, such as methyl, ethyl, propyl, isopropyl, butyl, pentyl, hexyl...

By "alkenylene radical containing 2 to 6 carbon atoms", is intended to mean an unsaturated, linear or branched, chain of 2 to 6 carbon atoms containing one carbon-to-carbon double bond.

Examples of alkyl radicals containing 1 to 6 carbon atoms interrupted by one oxygen atom are of the general formula $-(CH_2)_n-O-(CH_2)_{n'}-CH_3$, n and n' being two integers comprised between 0 and 6 such as $n + n' \leq 6$; an example of such radical is $-O-CH_3$.

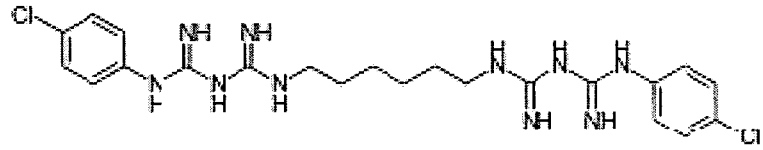
Example of alkyl radicals containing 1 to 6 carbon atoms, preferably 1 to 3 carbon atoms, optionally interrupted by one oxygen atom or one ester function and optionally containing a benzyl group are: $-O-CH_3$, $-O-CH_2-C_6H_6$, $-CO-O-CH_3$, and $-CO-O-CH_2-C_6H_6$...

A non-limitating example of linear chain containing between 5 and 15 carbon atoms and between 1 and 10 nitrogen atoms; said chain being optionally interrupted by a benzyl group optionally substituted by one halogen atom; carbon atoms of said chain being optionally substituted with =NH function is the following: -NH-C(NH)-NH-C(NH)-NH-$(CH_2)_6$-NH-C(NH)-NH-C(NH)-NH-C_6H_4-Cl.

A non-limitating example of linear chain containing between 5 and 15 carbon atoms and between 1 and 10 nitrogen atoms, said chain optionally containing 1 to 5 oxygen atoms and being optionally interrupted by a benzyl group; carbon and/or nitrogen atoms of said chain being optionally substituted by 1 or 2 alkyl radicals containing 1 to 3 carbon atoms, preferably a methyl radical; is the following: -$CH_2-NH_2-(CH_2)_2-O-(CH_2)_2-O-C_6H_4-C(CH_3)_2-CH_2-C(CH_3)_2-CH_3$.

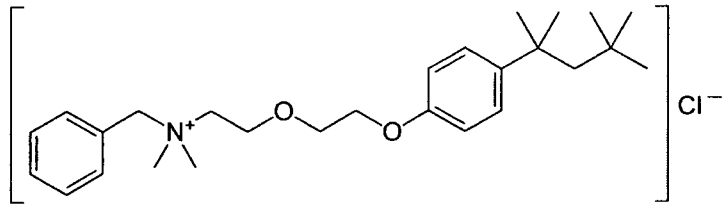
In a first mode of carrying out the invention, compounds of formula (I) are such as R^1 en R^2 do not correspond to the above-defined cycle; then preferred compounds are selected in the following compounds :

Compound of Formula A. Chlorhexidine



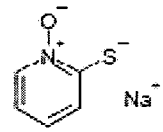
wherein X = C, R^1 = -Cl, $R^2 = R^3 = R^5$ = -H and R^4 = -NH-C(NH)-NH-C(NH)-NH-$(CH_2)_6$-NH-C(NH)-NH-C(NH)-NH-C_6H_6-Cl

Compound of Formula B. Benzethonium chloride



wherein X = C, $R^1 = R^2 = R^3 = R^5 = -H$ and $R^4 = -CH_2-NH_2-(CH_2)_2-O-(CH_2)_2-O-C_6H_6-C(CH_3)_2-CH_2-C(CH_3)_2-CH_3$

Compound of Formula D. Sodium pyrithione



wherein $X = N^+-O^-$, $R^1 = S$ and $R^2 = R^3 = R^4 = R^5 = -H$

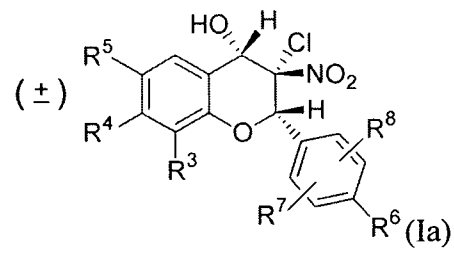
Chlorhexidine is known to have antiseptic properties, it shows also bactericidal properties, it kills both gram-positive and gram-negative bacteria.

Benzethonium chloride is a synthetic quaternary ammonium salt with surfactant, antiseptic and anti infective properties.

Sodium pyrithione (CAS Registry number: 15922-78-8 ; 3811-73-2) is a large spectrum antimicrobial agent; it inhibits the growth of fungi, yeast, mold and bacteria.

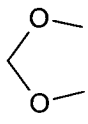
Those three compounds are commercially available; their syntheses are reported in the literature: chlorexidine may be prepared according to US Patent 2,684,924; benzethonium chloride may be prepared according to US Patents 2,115,250, 2,170,111 and 2,229,024; clotrimazole may be prepared according to South African Patents 68 05,392 and 69 00,039 (Bayer) and sodium pyrithione may be prepared according to Shaw *et al.* J. Amer. Chem Soc. 72, 4362 (1950) or to US Patent 2,745,826.

In another mode of carrying out the invention, compounds of formula (I) are such as R^1 and R^2 form the above-defined cycle; the preferred compounds are those defined by the general formula (Ia):



wherein R^3, R^5, R^6, R^7 and R^8 are defined as above;

- R^4 is:
  - a hydrogen atom;
  - an alkyl radical containing 1 to 6 carbon atoms, preferably 1 to 3 carbon atoms, optionally interrupted by one oxygen atom; or

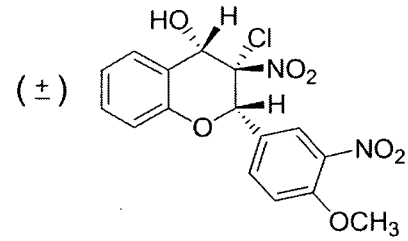
R^4 and R^5 taken together with the carbon atoms to which they are attached may form the cycle  ,

or a pharmaceutical acceptable salt of compounds of general formula (Ia).

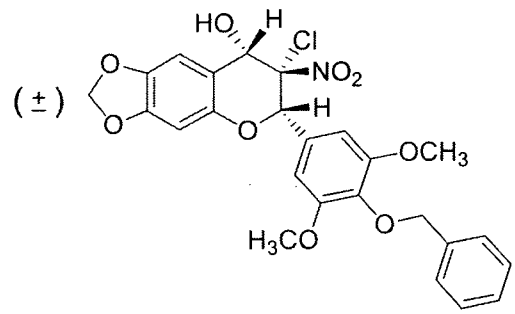
According to a particular object, the invention relates to compounds of general formula (Ia) as drug, particularly, as active agents.

The following compounds of formula (Ia) are preferred:

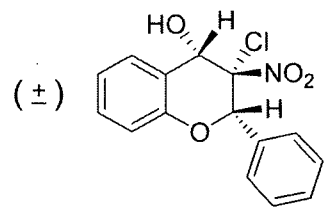
Compound of Formula 1. (ICC005-L-001-A11)



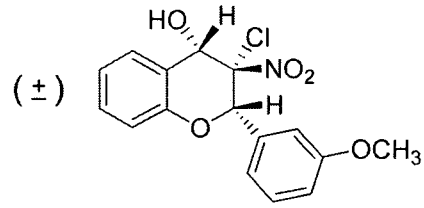
Compound of Formula 2. (ICC005-M204-C05)



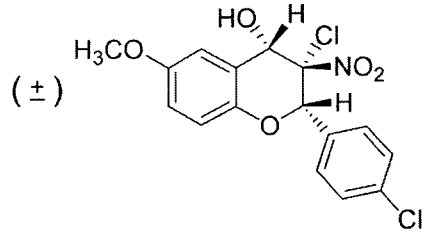
Compound of Formula 3. (ICC005-L006-D11)



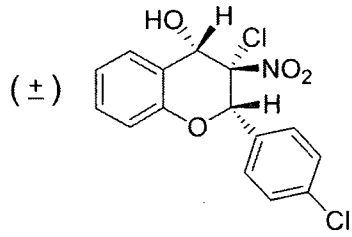
Compound of Formula 4. (ICC005-L028-D02)



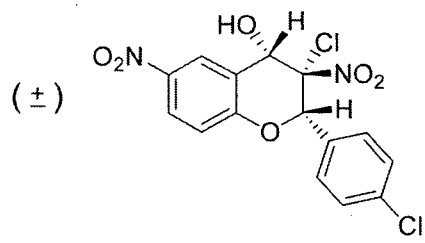
Compound of Formula 5. (ICC005-L025-A02)



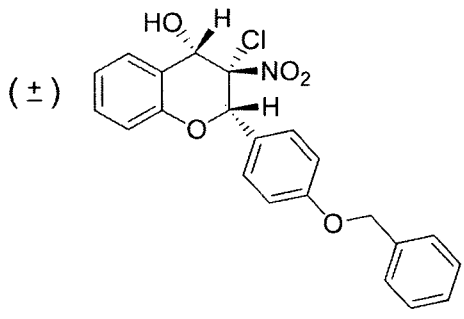
Compound of Formula 6. (ICC005-L023-B02)



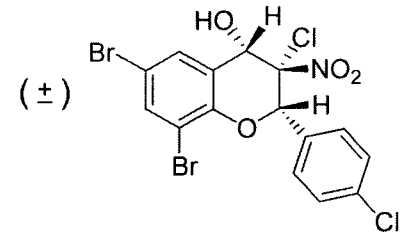
Compound of Formula 7. (ICC005-L040-D06)



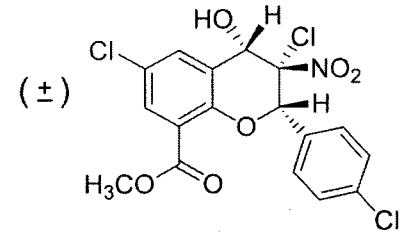
Compound of Formula 8. (ICC005-L134-H07)



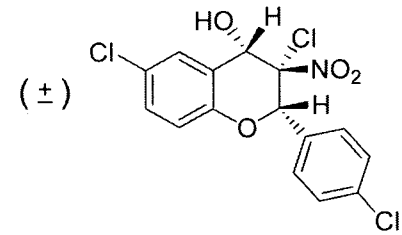
Compound of Formula 9. (ICC005-L022-B05)



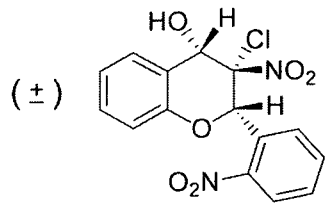
Compound of Formula 10. (ICC005-L040-E11)



Compound of Formula 11. (ICC005-L037-H11)



Compound of Formula 12. (ICC005-L037-G07)



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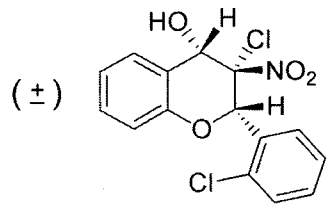

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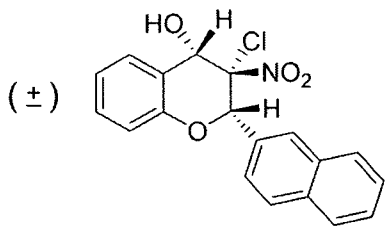

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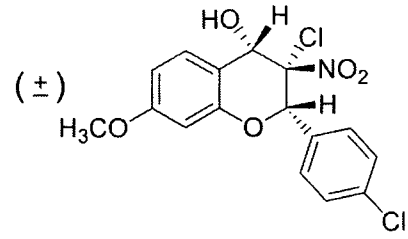

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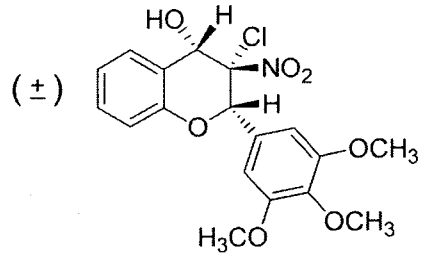
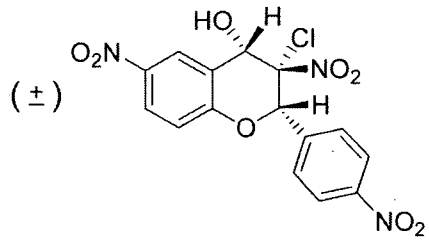


Compound of Formula 17. (ICC005-L033-E02)



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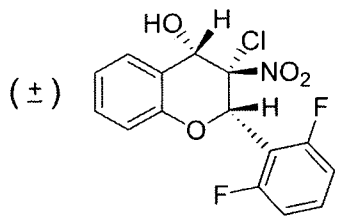

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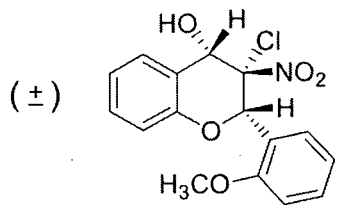
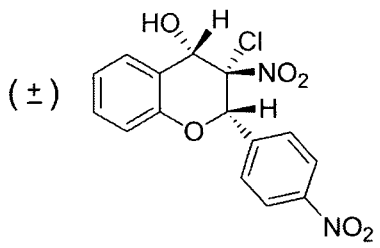


Compound of Formula 20. (ICC005-L018-E05)



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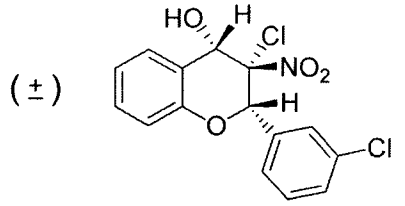

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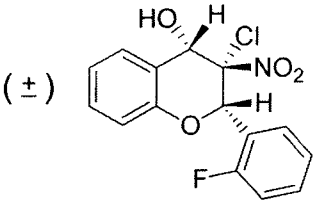

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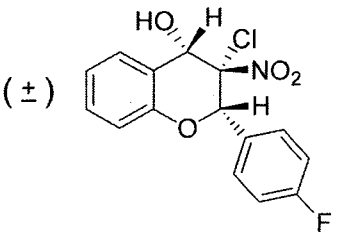

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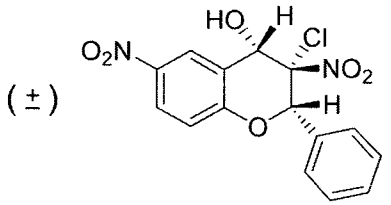

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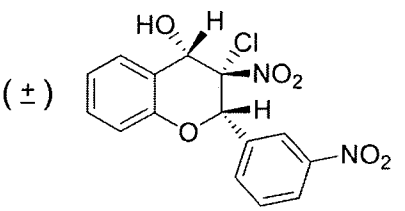
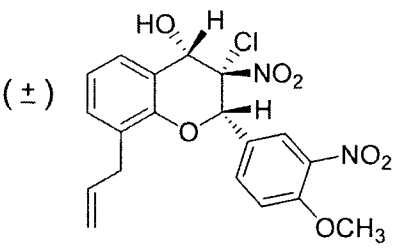


Compound of Formula 26. (ICC005-L001-B11)



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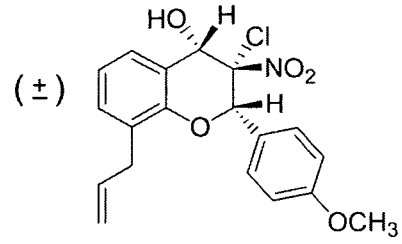
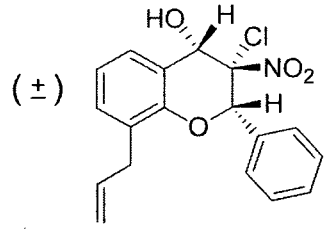
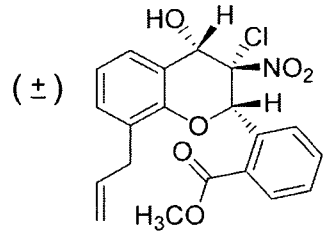


Compound of Formula 28. (ICC005-M204-C06)



Compound of Formula 29. (ICC005-L145-E05)



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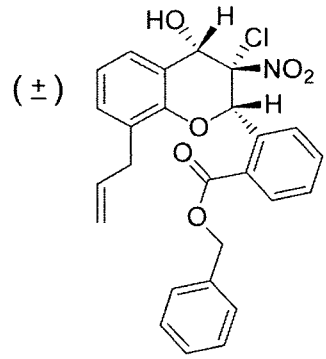

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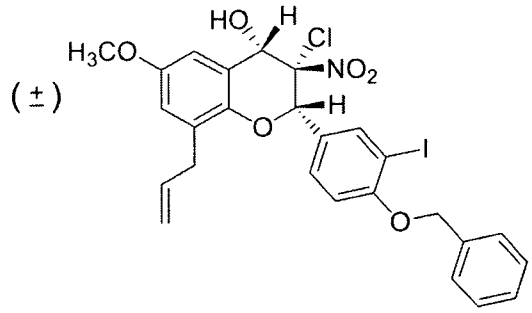
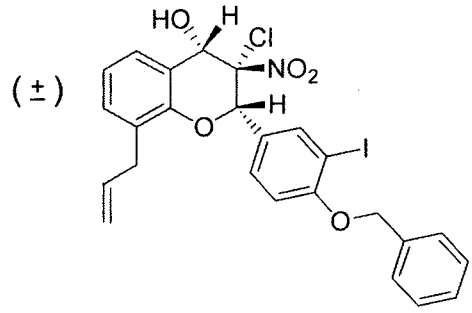
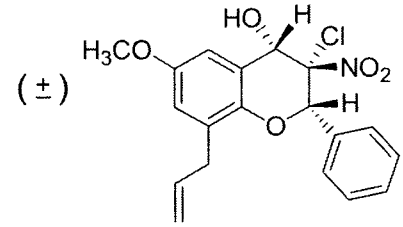


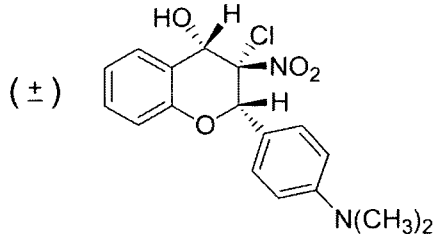
Compound of Formula 32. (ICC005-L145-H06)



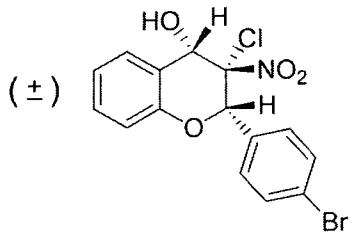
Compound of Formula 33. (ICC005-L046-F07)



Compound of Formula 34. (ICC005-L044-H06)



Compound of Formula 35. (ICC005-L046-C11)



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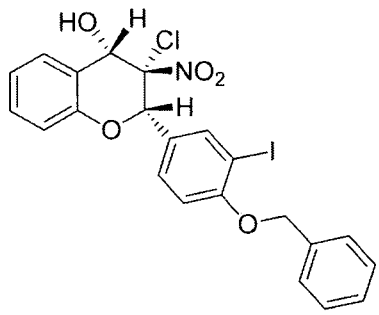
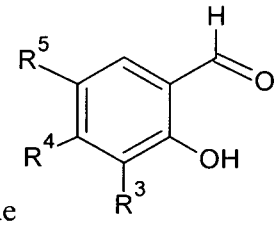
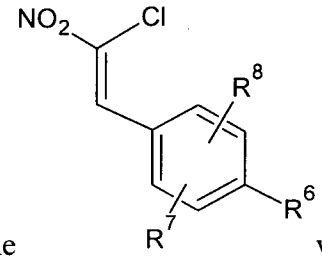


The synthesis of compounds of general formula (Ia) is described below.

Compounds of general formula (Ia) may be prepared by the

condensation of a salicylic aldehyde  and a (*Z*) β-chloro-β-nitrostyrene  with triethylamine in THF in strictly anhydrous conditions.

More specifically,

- Compounds of Formula 3, 4, 5, 6, 7, 9, 11, 12, 13, 14, 15, 16, 19, 20, 21 and 25 may be prepared according to the article of D. DAUZONNE *et al.* (*Synthesis*, 66-70 (1990));

- Compounds of Formula 1, 17, 18, 22, 23, 24 and 28 may be prepared according to the article of D. DAUZONNE *et al.* (*Eur. J. Med. Chem.*, *32*, 71-82 (1997));

- Compounds of Formula 2 may be prepared according to the article of A. GONZALEZ DE PEREDO *et al.* (*Chem. Pharm. Bull. 46*, 79-83 (1998));

- Compounds of Formula 26, 27, 29 and 30 may be prepared according to the article of B. BAUVOIS *et al.* (*J. Med. Chem. 46*, 3900-3913 (2003)).

Another object of the present invention relates to compounds of Formula 8, 10, 31, 32, 33, 34, 35 and 36.

The hitherto unknown compounds 8, 10, 31, 32, 33, 34, 35 and 36 may be prepared, starting from the appropriate salicylaldehydes and (*Z*) b-chloro-b-nitrostyrenes, according to the methodology reported in the article of D. DAUZONNE et P. DEMERSEMAN (*Synthesis*, 66-70 (1990)).

According to the present invention, preferred compounds of general formula (Ia) are compounds 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 18, 20, 21, 22, 23, 24, 25, 27, 32 and 36.

More preferred compounds of general formula (Ia) are compounds 5, 6, 7, 8, 10, 11, 13, 15, 18, 21, 22, 23, 32 and 36.

The biological activities of the compounds of general formula (I)

have been evaluated.

The ability of compounds of general formula (I) to restore respiratory growth of the yeast mutant has been evaluated with a yeast strain bearing a mutation on *ATP6* (Figure 3 and Example 1) and also with a yeast *fmc1Δ* mutant which affect the assembly of the ATPase complex.

These properties make said compounds as well as their salts suitable for use as drugs in the treatment of disorders or diseases linked with alteration of the F_1F_0-ATP synthase pathway; such diseases or disorders are those resulting from an insufficiency or a lack of ATP synthesis by F_1F_0-ATP synthase; and those related to an excessive accumulation of reactive oxygen species (ROS) which results from insufficiency or lack of ATP synthesis.

The use of the compounds of general formula (I) leads to an increase in ATP production and/or a decrease in ROS accumulation in cells.

As a consequence, compounds of general formula (I) are useful for the preparation of a drug for preventing and/or treating mitochondrial diseases, in particular for the treatment of mammals, such as human.

As already explained, mitochondrial diseases often result from a deficiency in ATP production –via the oxidative phosphorylation- which makes high energy-demanding tissues or organs such as heart, brain, and muscles, the main targets for these disorders.

Other mitochondrial alterations also contribute to pathologies amongst which an increased production of reactive oxygen species (ROS), responsible for important cellular oxidative damages, a low rate of NADH reoxidation and defective calcium storage within the organelle; examples of these pathologies are the syndromes NARP, LHON, MILS (*Maternally Inherited Leigh Syndrome*), MERRF (*Myoclonic Epilepsy with Ragged-Red Fibers*) and HSP (*Hereditary Spastic Paraplegia*).

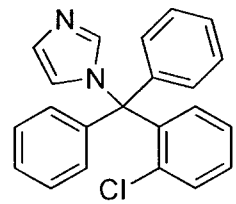
Symptoms of mitochondrial diseases usually include slow growth, loss of muscle coordination, muscle weakness, visual defect, hearing defects, learning disabilities, mental retardation, heart disease, liver disease, kidney disease, gastrointestinal disorders, respiratory disorders, neurological problems, and dementia.

Compounds of general formula (I) appear to be also useful for the preparation of a drug for preventing and/or treating an acceleration of normal or physiological ageing. Physiological ageing is characterized by numerous phenomena;

it may be characterized by the decrease of cells renewal; by the decrease of cells life and/or by the decrease of the number of cells in an organ, leading to atrophy and sometimes to dysfunction of said organ. Other evidences of ageing are modification of the appearance such as loss and/or graying of the hair, modification of appearance of the skin...

As mitochondrial disorders are observed during the progress of neurodegenerative diseases, the present invention also relates to the use of compounds of general formula (I) for preventing and/or treating neurodegenerative diseases such as Alzheimer's disease, myasteny disease, Parkinson's disease...

In another embodiment, the present invention relates to the use of compound of Formula C (Clotrimazole)



for the preparation of a drug for preventing and/or treating the symptoms of the mitochondrial diseases chosen in the group consisting of slow growth, muscle weakness, visual defect, hearing defect, heart disease, liver disease, kidney disease, gastrointestinal disorders and respiratory disorders, in particular for the treatment of mammals, such as human.

Clotrimazole is an antifungal agent.

This compound is commercially available; its synthese is reported in the literature: clotrimazole may be prepared according to South African Patents 68 05,392 and 69 00,039 (Bayer).

In addition to the above provisions, the invention also comprises other provisions which will become clear from the description which follows, which refers to examples illustrating the biological activity of compounds of general formula (I), and also to the attached drawings in which:

Figure 1 is a schematic representation of the mitochondrial energy transduction apparatus and of the genes controlling the formation thereof.

Figures 2A and 2B illustrate the structure of the ATP synthase and the genes encoding this protein complex: the ATP synthase is composed of subunits which are encoded by the nuclear DNA and by the mitochondrial DNA (Atp6p, Atp8p) and assembled in the mitochondrial matrix. Figure 2B focuses on the

mitochondrial DNA and the mutations which are associated with human mitochondrial disease (from mitomap.org).

Figures 3A and 3B are pictures of Petri dishes showing the ability of compounds of general formula (I) to restore respiratory growth of the ATP synthase yeast mutant on nonfermentable medium. The three upper panels of figure 3A show that the ATP synthase mutants strains (three NARP strains and the *fmc1Δ*) grow very slowly on a respiratory medium (nonfermentable carbon source) due to a defect of the ATP synthase (Example 1).

The three lower panels of Figure 3A illustrate the improvement of the respiratory growth when compounds (Chlorhexidine, Benzethonium Chloride or Clotrimazole instead of DMSO, the negative control) are added into the agar medium at different concentrations.

On Figure 3B, the mutant cells are plated out in a layer at the surface of an agar medium containing nonfermentable carbon source and compounds are added on filters and disposed on Petri dish. The negative control, DMSO and the positive control, oleate, are loadded on the upper left filter and on the lower right filter respectively.

Figures 4 to 8 are graphs showing the effect of compounds of general formula (I) on life expectancy of *Drosophila* having mitochondrial encephalomyopathy (Example 2).

Figure 9 is a graph showing the effect of compound of Formula A (chlorhexidine, CH) on growth of a NARP mammalian model (Example 3).

Example 1: Demonstration of the capacity of compounds of general formula (I) to restore respiratory growth of mutant yeasts on nonfermentable medium

The *ATP6* yeast mutants grow very slowly from a nonfermentable carbon source due to a dysfunction of the ATP synthase. These yeast mutants are therefore used to identify molecules capable of correcting the effects of the mutation by restoring either ATP synthase function, or ATP production by the mitochondria allowing the yeasts to grow on nonfermentable medium.

Mutant strains used in this experiment are:

- MC6 (*fmc1Δ*) (Lefebvre-Legendre L. *et al.*, J. Biol. Chem., 2001, Mar2, 276 (9) : 6789-96)

Genotype: MC6: *Mat α, ade2, leu2, ura3, trp1, his3, fmc1::HIS3*

- MR14 (NARP T8993G) which may be obtained according to International PCT Application WO 2007/125225.

Genotype: MR14: *Mat a, ade2, leu2, ura3, trp1, his3, arg8::HIS3[rho$^+$ FY1679; atp6 T8993G]*

The principle of the activity test is the following:

Step 1: the mutant yeast is cultured in medium containing glucose (YPAD medium).

Step 2: the mutant cells are plated out in a layer at the surface of an agar medium containing a nonfermentable carbon source such as glycerol (YPG medium).

Step 3: filters which each contain a defined amount of one of the test molecules are placed on the Petri dish, the molecules diffuse in the medium and establish a concentration gradient around the filters.

Step 4: the dishes are incubated at 35 or 36°C.

Under these conditions, a growth halo is seen to appear around the filters containing a substance capable of counteracting the effects of the mutation.

Media

YPG medium contains 1% Yeast Extract; 2% Bactopeptone; 2% Bactoagar only for solid medium; 2% glycerol (expressed in weight/volume); Adenine 60mg/lL and spenicilline 30 000UI/L.

Liquid YPAD medium contains 1% Yeast Extract; 2% Bactopeptone; 2% glucose (expressed in weight/volume) and Adenine 60mg/L.

The day before

For each of the MC6 and MR14 strains, one colony from a YPAD agar plate has been inoculated in 4 ml of liquid YPAD medium.

The day of assay

In the morning, the optical density (OD) of the culture is measured; the culture is diluted in liquid YPAD medium (50 or 100 µl in 4ml of medium if the broth is saturated).

After 4-5 h, OD of the culture is measured; the culture is diluted in YPG medium until the OD is 0.2.

240 µl of the culture is introduced in squared dishes (12cm /12cm) and plated with sterile glass beads.

Sterile filters are introduced into the dishes.

Then filters are impregnated with either:

- 1 µl/filter of positive control (oleate 100 mM);

- 1 µl/filter of negative control (DMSO, compounds vehicle, pure from Sigma);

- 2.5 µl/filter of compounds having general formula (Ia) 10 mg/ml in DMSO;

- 2.5 µl/filter of compounds A, B, C and D 5 mg/ml in DMSO.

The dishes are incubated at 35°C for MR14 yeast strain or 36°C for MC6 yeast strain.

Results

Results for chlorhexidine, benzethonium chloride or clotrimazole are illustrated in **Figures 3A**.

Activity of the compounds is detected when mutant yeasts grow around the filters forming a growth halo; the size and the density of said halo allow defining a qualitative value for activity. All the selected drugs are active on both mutant strains (MR14 and *fmc1Δ*).

Compounds	activity
5	++
6	++
7	++
8	+++
9	+
10	++
11	++
12	+
13	++
14	+
15	++
18	++
20	+
21	++
22	++
23	++

24	+
25	+
27	+
32	++
36	++
A- Chlorhexidine	+++
B- Benzethonium	++
C- Clotrimazole	+++
D- Sodium Pyrithione	++

Example 2 – Demonstration of the capacity of compounds of general formula (I) to improve lifespan of *Drosophila* suffering from mitochondrial encephalomyopathy

Compounds of general formula (I) have then been tested on a *Drosophila* model described by Celotto *et al.* (Mitochondrial Encephalomyopathy in *Drosophila*, The Journal of Neuroscience, January 18, 2006 – 26(3):810-820). These Drosophila mutants are known to show a shorter lifespan.

Methods for Drosophila Drug Screen

mtATP6[1];sesB[1]/sesB[1] flies were outcrossed once to produce females with the genotype mtATP6[1];sesB[1]/+ for study.

Each drug was dissolved in 0.09% DMSO to the following final concentrations: 15 μM, 1 μM, 50 nM and 2.5 nM.

Approximately 20 flies were tested per vial and 3 independent vials were tested for each concentration of each drug.

Newly eclosed females were counted and placed into a vial with approximately 10 milliliters standard cornmeal molasses media. Test compounds were applied (25 microliters at the specified concentrations) to a semi-circle of filter paper covering about 1/2 of the surface of the media.

Longevity experiments were performed using 12:12 light dark regime at 25 C.

Flies were counted every other day at which time food, filter paper and drug were replaced until all flies had expired. Survival curves were generated and analyzed using Prism 4.0b and log rank tests were performed to determine significance from the vehicle only controls.

Results

Graphs of Figures 4 to 8 show a significant increase in Drosophila's life after treatment with Compound of Formula A – Chlorhexidine (**Figure 4**), Compound of Formula B - Benzethonium chloride (**Figure 5**), Compound of Formula C – clotrimazole (**Figure 6**), Compound of Formula D - Pyrithione sodium salt (**Figure 7**) and Compound of Formula 13 (**Figure 8**).

Example 3 – Demonstration of the capacity of compound of Formula A (chlorhexidine) to improve the growth of a NARP mammalian model in medium deprivated of glucose

After the isolation of active drugs on the NARP yeast models, compounds' activity has been validated on mammalian models for the considered diseases.

Due to the limited number of cell divisions and the instability of heteroplasmy of mtDNA of the fibroblasts from the NARP patient, it has been decided to use the transmitochondrial cybrids, a cell line which is obtained by fusion of NARP patient's platelets containing heteroplasmic level of the mtDNA mutation with human osteosarcoma cells devoided of mtDNA. As fibroblasts, cybrids are using mainly a glycolytic metabolism. Therefore, in glucose medium, glycolysis provides the majority of the cellular ATP and both WT and NARP cybrids (JCP213 and JCP239 lines) present the same growth curves. On the contrary, in a medium supplemented with pyruvate and uridine but without glucose, the cells are forced to use a more oxidative metabolism (dependent on mitochondrial ATP production) (Weber, BioChem 2002).

The ability of JICP239 to grow in medium deprivated of glucose has been tested and used as a readout of the ability of the drugs selected in Example 1 to suppress NARP phenotype in human.

- Cell lines and culture conditions

The cybrid lines JCP213 and JCP239 were generated by fusion of the human osteosarcoma cell line 143BK-ρ^0 with platelets from wild-type patients or patients with T8993G mutation (Manfredi, G. *et al.* (1999). J Biol Chem *274*, 9386-9391).

JCP213 contain 100% of WT mtDNA and JCP239 contain 84 ± 4% of mtDNA with T8993G transversion and were cultivated in Dulbecco's modified Eagle's medium (DMEM), high glucose (4.5 $g.l^{-1}$) supplemented with 5% fetal bovine

serum (FBS Gold, PAA), 1 mM sodium pyruvate, 4 mM glutamine, 200 µM uridine and 20 $U.ml^{-1}$ penicillin/streptomycin at 37 °C in the atmosphere of 5% CO_2.

For growth rate measurements, 10^4 cells were plated in a 24 well plates containing DMEM with glucose, as described above except the antibiotics. After 24 h, the growth medium is removed, the cells are washed with PBS and DMEM without glucose containing the drug or DMSO is added. For each condition of treatment, four wells were used.

Chlorhexidine (CH) solution in DMSO was diluted 1,000 times in the medium and used at final concentrations from 12.5 to 80 nM for CH.

DMSO and dihydrolipoic acid (DHLA) at 200 µM are respectively used as negative and positive controls.

After three days of incubation with the drugs, cell proliferation was estimated by Neutral Red staining (Aure, K. *et al.* (2007) Neuromuscul Disord *17*, 368-37). Briefly, cells were incubated during 4 h at 37°C in presence of 33 $\mu g.ml^{-1}$ Neutral Red in DMEM without glucose, washed twice in PBS, and air-dried during 15 min. Neutral Red was then solubilized in 1 ml of 50% ethanol 1% acetic acid and quantified by its absorbance (540 nm).

Experiments were done at least three times per condition.

- Measurement of the chlorhexidine activity

The NARP cybrids show a much slower growth than the WT cybrids. In order to evaluate the efficiency of the drugs isolated as active in the yeast-based screening, the above-described conditions of culture were used to test the effect of the drugs on the cell viability/proliferation of the NARP cybrids.

Hence, 24 hours after the seeding of the cybrids in 24 wells-plates in glucose medium, the medium was withdrawn, cells were washed and medium deprived of glucose (with pyruvate and uridine) and containing the tested compound was added. DMSO served as a negative control. After three days in presence of the drug, cell proliferation was estimated by Neutral Red staining (Aure *et al.* 2007). Each condition was performed using four wells and each experiment was done at least in triplicate. DMSO served as a negative control.

As for the yeast screening, the first step to validate this cybrid-based assay was to find a positive control.

The DHLA previously shown to partially correct the NARP fibroblasts deficiencies (Mattiazzi, M. *et al.* (2004) Hum Mol Genet *13*, 869-879) and

also uses in therapy to treat patients affected by mitochondrial neuropathies (DiMauro, S. *et al.* (2006). Muscle Nerve *34*, 265-283) was tested as a positive control on the NARP cybrids growth.

After three days in a medium deprivated of glucose, the number of the NARP cybrids, JCP239, was increased by 2.2 fold in presence of 200 µM of DHLA. As for the yeast screening, the DHLA was used as a positive control.

Next CH has been tested; this compound has been shown active on the NARP yeast model using DHLA as a positive control: the day following the seeding, the glucose was removed from the medium and CH was added at concentrations ranging from 5 nM to 1 µM.

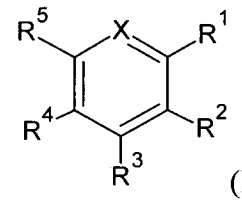
- Results (**Figure 9**)

For concentrations ranging between 100 nM to 1 µM, CH was toxic and induced the cells death. In contrast, at concentrations comprised between 12.5 nM and 50 nM, NARP cybrids growth was clearly improved. In presence of 12.5, 25 and 50 nM of CH, the NARP cybrids growth was increased by 1.4, 1.5 and 1.2 respectively (see **Figure 9**).

Therefore, the treatment with DHLA or CH improved NARP cybrids growth in condition where oxidative metabolism was necessary.

CLAIMS

1. Use of the compounds having the general formula (I):

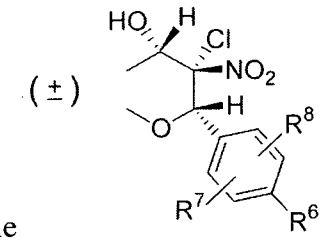


(I)

wherein

- **X** is a carbon atom or a nitrogen atom, both atoms being optionally substituted by an oxygen atom;
- $\mathbf{R^1}$ is a hydrogen atom, a halogen atom or a sulphur atom;
- $\mathbf{R^2}$ is a hydrogen atom or

$\mathbf{R^1}$ and $\mathbf{R^2}$ taken together with the carbon atoms to which they are attached may form



the following cycle

with the proviso that when $\mathbf{R^1}$ and $\mathbf{R^2}$ form the above defined cycle, **X** is a carbon atom;

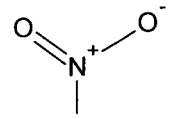
- $\mathbf{R^3}$ is a hydrogen atom; a halogen atom; an alkyl radical containing 1 to 6 carbon atoms, preferably 1 to 3 carbon atoms, optionally interrupted by one oxygen atom or one ester function or an alkenylene radical containing 2 to 6 carbon atoms, preferably 2 to 3 carbon atoms,

with the proviso that when $\mathbf{R^1}$ and $\mathbf{R^2}$ do not correspond to the above defined cycle, $\mathbf{R^3}$ is a hydrogen atom;

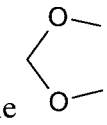
- $\mathbf{R^4}$ is:
  - a hydrogen atom,
  - an alkyl radical containing 1 to 6 carbon atoms, preferably 1 to 3 carbon atoms, optionally interrupted by one oxygen atom,
  - a linear chain containing between 5 and 15 carbon atoms and between 1 and 10 nitrogen atoms,

said chain optionally contains 1 to 5 oxygen atoms and is optionally interrupted by a benzyl group optionally substituted by one halogen atom; carbon and/or nitrogen atoms of said chain being optionally substituted by 1 or

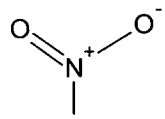
2 alkyl radicals containing 1 to 3 carbon atoms, preferably a methyl radical, and carbon atoms of said chain being optionally substituted with =NH function;



- R^5 is a hydrogen atom, a halogen atom, or a group ;

R^4 and R^5 taken together with the carbon atoms to which they are attached may form the following cycle  ;

- R^6, R^7 and R^8, which may be identical or different, are:
  - a hydrogen atom;
  - a halogen atom;
  - an alkyl radical containing 1 to 6 carbon atoms, preferably 1 to 3 carbon atoms, optionally interrupted by one oxygen atom or one ester function and optionally containing a benzyl group;
  - - a group $-NH_2$;



  - a group

R^6 and R^8 taken together with the carbon atoms to which they are attached may form a benzyl group;

with the proviso that

when R^1 and R^2 correspond to the above defined cycle, $R^4 = R^5 = R^7 = R^8 = H$ and $R^6 = Cl$, R^3 is not $-O-CH_3$ or

when R^1 and R^2 correspond to the above defined cycle, $R^3 = R^5 = R^4 = R^8 = H$, and R^7 is in ortho-position of R^6, R^6 and R^7 are not simultaneously $-O-CH_3$,

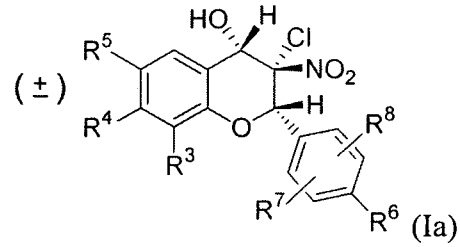
or a pharmaceutical acceptable salt

for the preparation of a drug for the prevention and/or the treatment of disorders or diseases related to an insufficiency or a lack of ATP synthesis or related to an excessive accumulation of reactive oxygen species (ROS).

2. Use according to claim 1, wherein said compounds are chosen amongst: compounds A, B and D.

3. Use according to claim 1, characterized in that said compounds

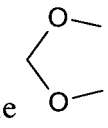
are defined by the general formula (Ia):



(Ia)

wherein R^3, R^5, R^6, R^7 and R^8 are defined as above;

- R^4 is:
  - a hydrogen atom;
  - an alkyl radical containing 1 to 6 carbon atoms, preferably 1 to 3 carbon atoms, optionally interrupted by one oxygen atom; or

R^4 and R^5 taken together with the carbon atoms to which they are attached may form the cycle  ,

or pharmaceutical acceptable salt of compounds of general formula (Ia).

4. Use according to claim 3, wherein said compounds are chosen amongst: 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 18, 20, 21, 22, 23, 24, 25, 27, 32 and 36.

5. Use according to anyone of claims 1 to 4, wherein said disorders or diseases are mitochondrial diseases.

6. Use according to claim 4, wherein said mitochondrial diseases are the syndromes NARP (*Neuropathy, Ataxia and Retinitis Pigmentosa*), LHON (*Leber's Hereditary Optic Neuropathy)*, MILS (*Maternally Inherited Leigh Syndrome*), MERRF (*Myoclonic Epilepsy with Ragged-Red Fibers*) and HSP (*Hereditary Spastic Paraplegia*).

7. Use according to anyone of claims 1 to 4, wherein said compounds are used for the preparation of drug for preventing and/or treating an acceleration of physiological ageing.

8. Use according to anyone of claims 1 to 4, wherein said compounds are used for the preparation of drug for preventing and/or treating neurodegenerative diseases such as Alzheimer's disease, myasteny disease, Parkinson's disease.

9. Compounds of general formula (Ia) as defined above as drug.

10. Compounds of Formula 8, 10, 31, 32, 33, 34, 35 and 36.

11. Use of compound of Formula C (Clotrimazole) for the preparation of a drug for preventing and/or treating the symptoms of the mitochondrial diseases chosen in the group consisting of slow growth, muscle weakness, visual defect, hearing defect, heart disease, liver disease, kidney disease, gastrointestinal disorders and respiratory disorders.

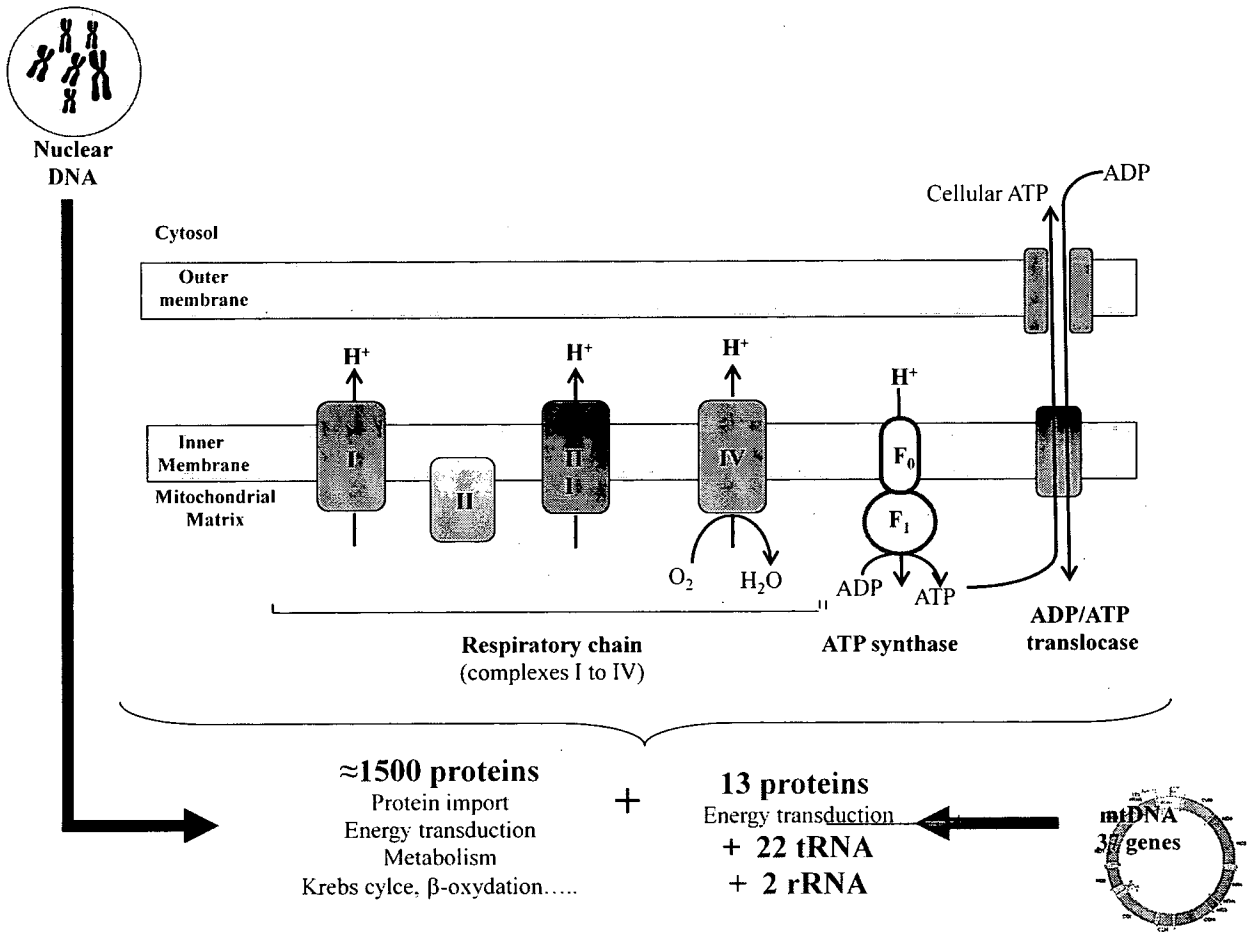

Nuclear DNA
Cytosol
Outer membrane
Inner Membrane
Mitochondrial Matrix
Cellular ATP
ADP
H+
I
II
III
IV
F0
F1
O2
H2O
ADP
ATP
Respiratory chain
(complexes I to IV)
ATP synthase
ADP/ATP translocase
≈1500 proteins
Protein import
Energy transduction
Metabolism
Krebs cylce, β-oxydation.....
+
13 proteins
Energy transduction
+ 22 tRNA
+ 2 rRNA
mtDNA
37 genes

FIGURE 1

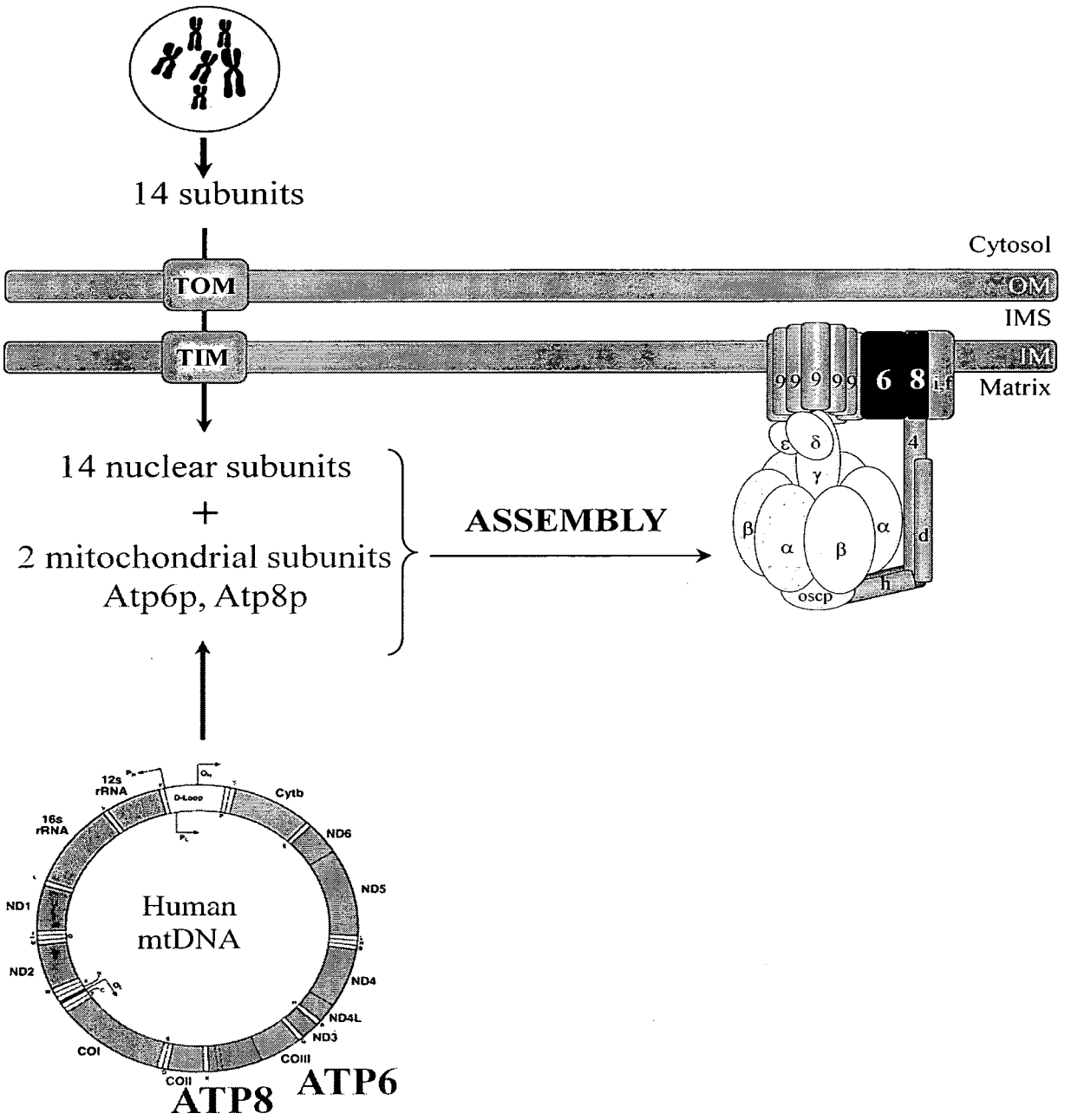

14 subunits
Cytosol
TOM
OM
IMS
TIM
IM
Matrix
14 nuclear subunits
+
2 mitochondrial subunits
Atp6p, Atp8p
ASSEMBLY
Human
mtDNA
12s rRNA
16s rRNA
D-Loop
Cytb
ND6
ND5
ND4
ND4L
ND3
COIII
COII
COI
ND2
ND1
ATP8
ATP6
oscp

FIGURE 2A

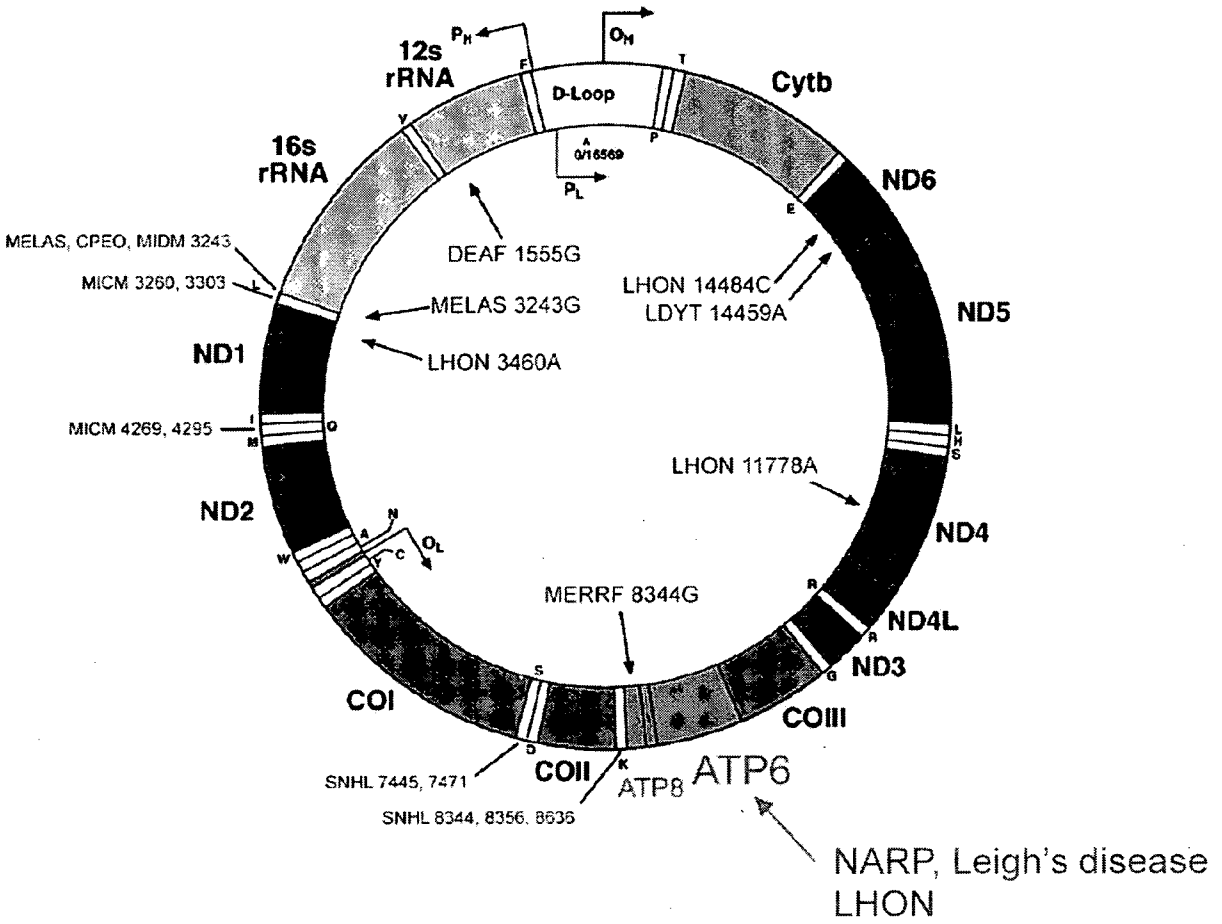

12s rRNA
D-Loop
Cytb
16s rRNA
ND6
MELAS, CPEO, MIDM 3243
MICM 3260, 3303
DEAF 1555G
MELAS 3243G
LHON 3460A
LHON 14484C
LDYT 14459A
ND5
ND1
MICM 4269, 4295
LHON 11778A
ND2
ND4
MERRF 8344G
ND4L
ND3
COI
COIII
COII
ATP8
ATP6
SNHL 7445, 7471
SNHL 8344, 8356, 8636
NARP, Leigh's disease
LHON

FIGURE 2B

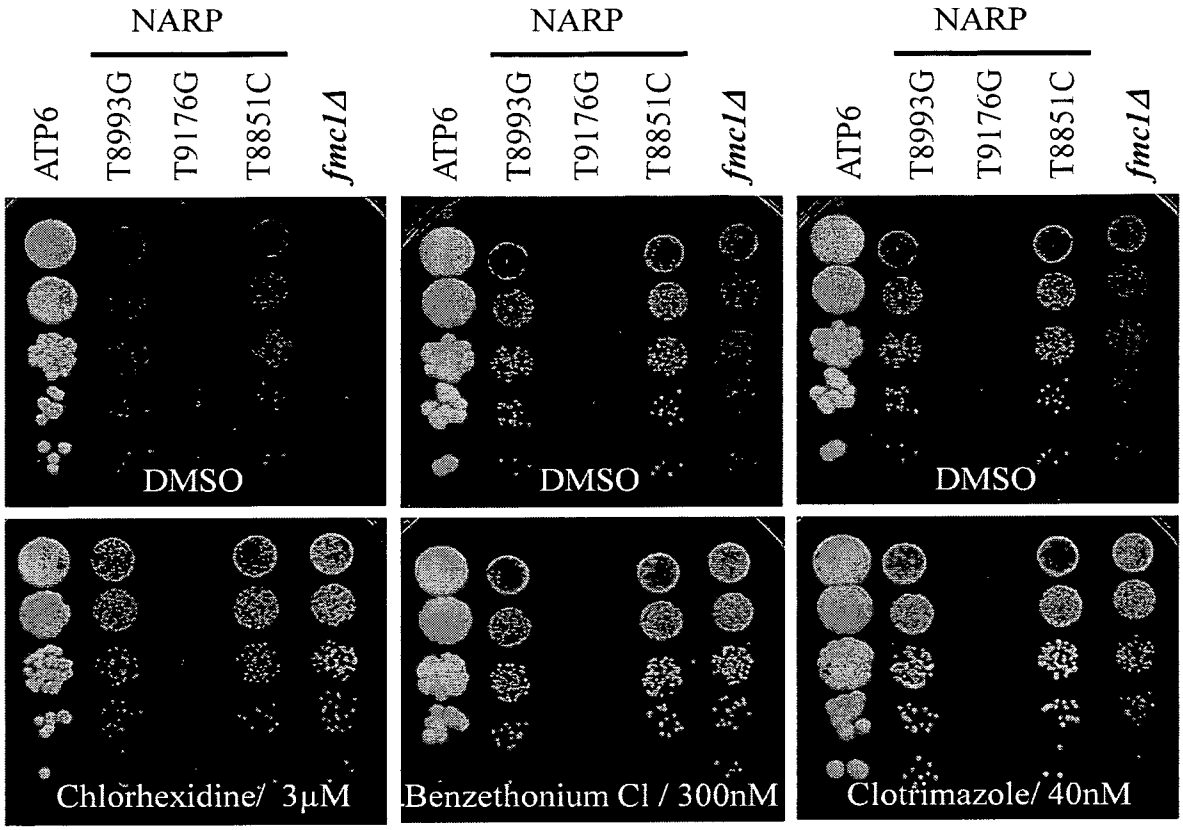


FIGURE 3A

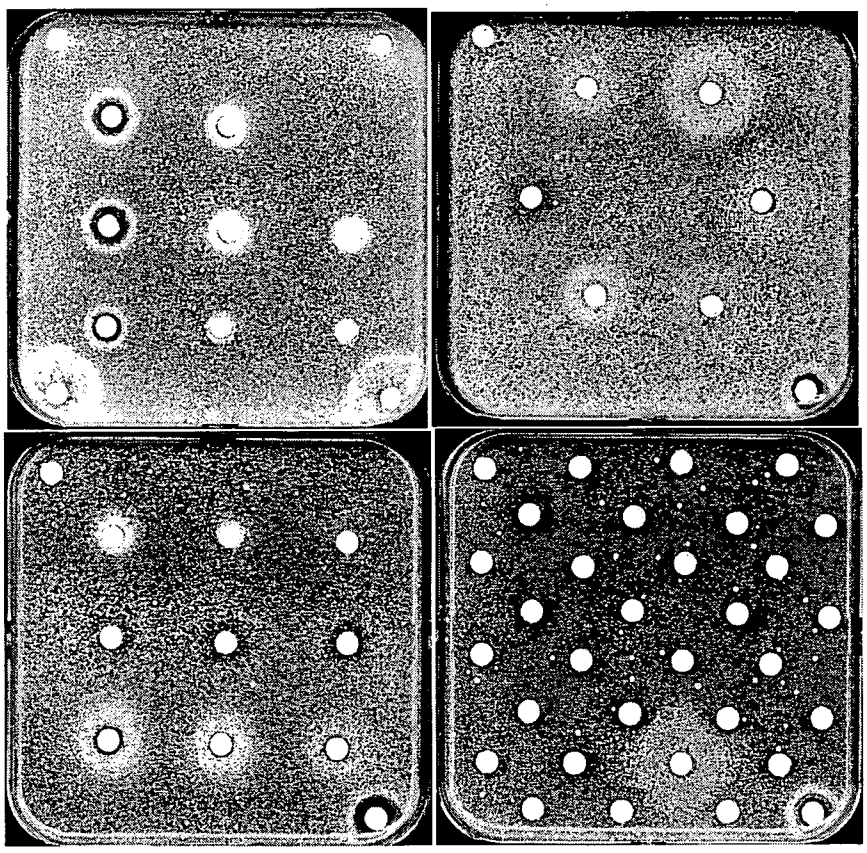
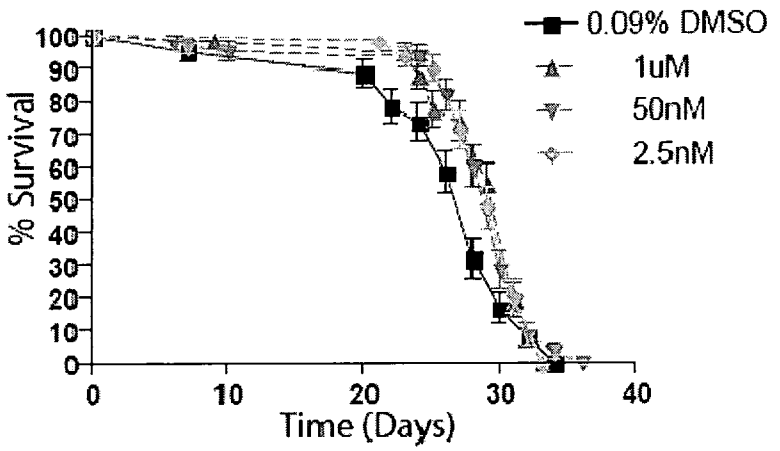
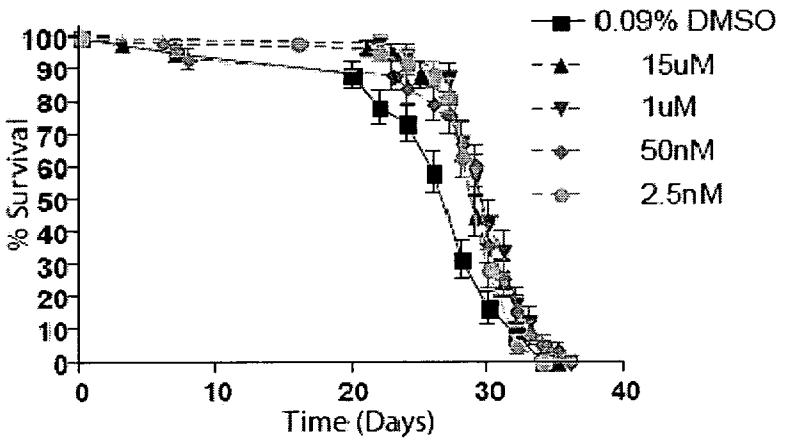


FIGURE 3B



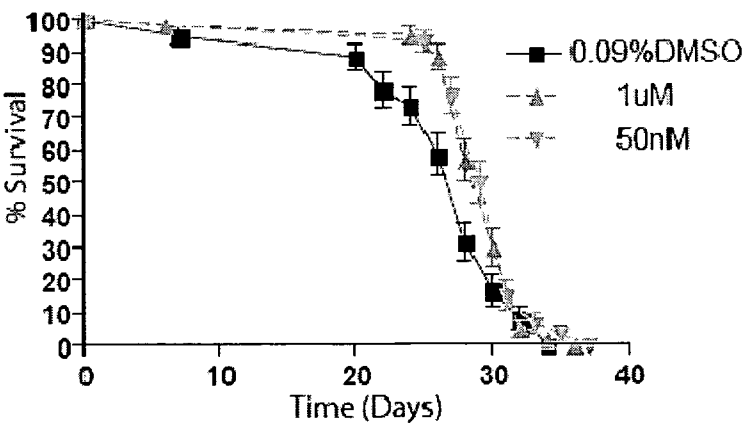
Compound of Formula A - Chlorhexidine

FIGURE 4



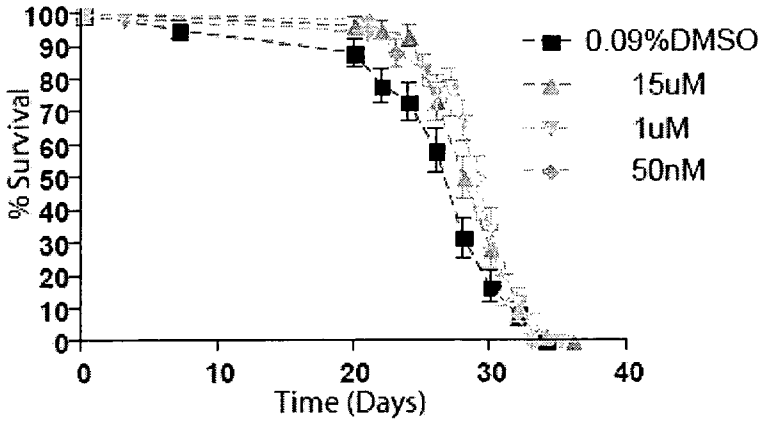
Compound of Formula B - Benzethonium chloride

FIGURE 5



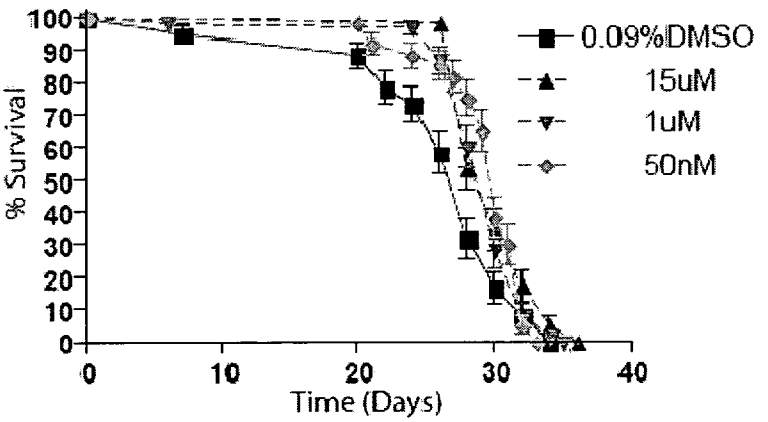
Compound of Formula C – clotrimazole

FIGURE 6



Compound of Formula D - Pyrithione sodium salt

FIGURE 7



Compound of Formula 13

FIGURE 8

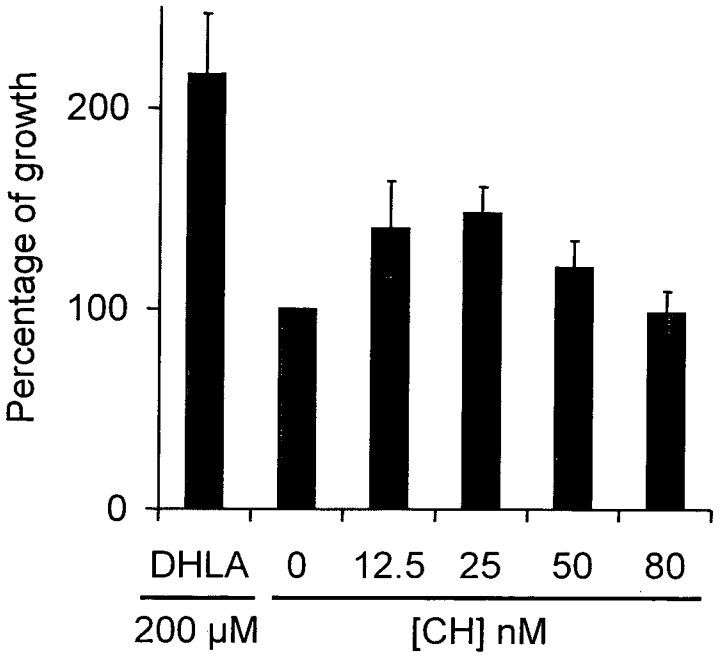


FIGURE 9

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2010/001006

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K31/14 A61K31/155 A61K31/352 A61K31/4164 A61K31/4425
C07D311/00 A61P25/00 A61P25/16 A61P25/28
ADD.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

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

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 01/29034 A1 (ASTRAZENECA AB [SE]; SCHMIESING RICHARD [US]) 26 April 2001 (2001-04-26) claims 1,9-19	2-11
X	ROTA M T ET AL: "Reduction of oral acetaldehyde levels using a controlled-release chlorhexidine chip as a prevention strategy against upper digestive tract cancer." MEDICAL HYPOTHESES JUN 2003 LNKD-PUBMED:12699713, vol. 60, no. 6, June 2003 (2003-06), pages 856-858, XP009136018 ISSN: 0306-9877 * abstract page 858, paragraph conclusion	2,5,7

-/--

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

[X] See patent family annex.

* Special categories of cited documents :

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
12 July 2010	19/07/2010

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

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	YIP KENNETH W ET AL: "Benzethonium chloride: a novel anticancer agent identified by using a cell-based small-molecule screen." CLINICAL CANCER RESEARCH : AN OFFICIAL JOURNAL OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH 15 SEP 2006 LNKD-PUBMED:17000693, vol. 12, no. 18, 15 September 2006 (2006-09-15), pages 5557-5569, XP009136017 ISSN: 1078-0432 * abstract	2,5,7
X	US 2 745 826 A (SERGE SEMENOFF ET AL) 15 May 1956 (1956-05-15) cited in the application column 2, paragraph 2-mercaptopyridine 1-oxide; claims 1-6	7
X	DAUZONNE D ET AL: "Synthesis and in vitro cytotoxicity of a series of 3-aminoflavones" EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY, vol. 32, no. 1, 1 January 1997 (1997-01-01), pages 71-82, XP004034181 ISSN: 0223-5234 cited in the application * abstract; compounds 7a-7r, 7s table I; compound 7j	3,5,7,9
X	DAUZONNE D ET AL: "A convenient synthesis of 3-chloro-3,4-dihydro-4-hydroxy-3-nitro-2-phenyl-2H-1-benzopyrans" SYNTHESIS, vol. 1990, no. 1, 1990, pages 66-70, XP009121832 ISSN: 0039-7881 cited in the application compounds 5AA, 5BA, 5CA, 5DA, 5EA, 5FA, 5GA, 5HA, 5IA, 5KA, compounds 5MA, 5DC, 5DD, 5DF, 5DG, 5DH	7
X	DAUZONNE, DANIEL ET AL: "Synthesis of 2-aryl-3-nitro-4H-1-benzopyran-4-ones" SYNTHESIS, vol. 1992, no. 7, July 1992 (1992-07), pages 677-680, XP009121862 ISSN: 0039-7881 compounds 1A-1I, 1K, 1L, 1N, 1P, 1Q, 1R	7

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INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2010/001006

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	GONZALEZ DE PEREDO A ET AL: "Synthesis and biological evaluation of flavanones and flavones related to podophyllotoxin." CHEMICAL & PHARMACEUTICAL BULLETIN, vol. 46, no. 1, January 1998 (1998-01), pages 79-83, XP009121834 ISSN: 0009-2363 cited in the application compound 9B	7
X	BAUVOIS B ET AL: "Synthesis and biological evaluation of novel flavone-8-acetic acid derivatives as reversible inhibitors of aminopeptidase N/CD13" JOURNAL OF MEDICINAL CHEMISTRY, vol. 46, no. 18, 28 August 2003 (2003-08-28), pages 3900-3913, XP001181149 ISSN: 0022-2623 cited in the application compounds 17A, 17G, 17H, 17K, 17L	7
X	DAUZONNE D ET AL: "Synthesis of the 3-aminoflavone-8-acetic acid" TETRAHEDRON LETTERS, vol. 36, no. 11, 13 March 1995 (1995-03-13), pages 1845-1848, XP004028502 ISSN: 0040-4039 compound 6	7
X	WO 2007/002497 A (ENVIVO PHARMACEUTICALS INC [US]; SHAPIRO GIDEON [US]; CUMMINGS CHRISTO) 4 January 2007 (2007-01-04) * abstract; claims 1-8; figure 1; example 6 page 1, paragraph 2	11
A	WO 95/19170 A (RHONE POULENC RORER SA [FR]; DELUMEAU JEAN CHRISTOPHE [FR]; MARTINET M) 20 July 1995 (1995-07-20) * abstract; claims 1-4	2-11
A	EP 1 550 442 A (KOGA YASUTOSHI [JP]; AJINOMOTO PHARMA CO LTD [JP] KOGA YASUTOSHI [JP];) 6 July 2005 (2005-07-06) * abstract; claims 1-6	2-11
A	WO 2007/095630 A (UNIV CALIFORNIA [US]; LIPSHUTZ BRUCE H [US]) 23 August 2007 (2007-08-23) * abstract; claims 17-24	2-11

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box II.2

Claims Nos.: 1

The present independent claim 1 relates to an extremely large number of possible compounds. In fact, claim 1 cibtains so many options, variable, possible permutations and different possibilities of attachment of the different option that lack of clarity within the meaning of Article 6 PCT areises to such an extent as to render a meaningful search of claim 1 impossible. In addition, support and disclosure in the sense of Article 5 and 6 PCT is to be found for only a very small proportion of the compounds, namely the support is to be found for chlorhexidine (compound A), benzethonium chloride (compound B), sodium pyrithione (compound D) and compounds of formula (Ia), see Example 1 and from page 8, lines 11 to page 14, line 10. The non-compliance with the substantive provisions is to such an extent, that a meaningful search of the whole claimed subject-matter of the claim could not be carried out (PCT Guidelines, 9.19 and 9.24). The extent of the search was consequently limited. The search was restricted to those claimed compounds which appear to be supported, namely the search was restricted to the compounds A, B, D of claim 2 and compounds of formula (Ia) of claim 3. Broad claim 1 was not searched.

The applicant's attention is drawn to the fact that claims relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure. If the application proceeds into the regional phase before the EPO, the applicant is reminded that a search may be carried out during examination before the EPO (see EPO Guideline C-VI, 8.2), should the problems which led to the Article 17(2) declaration be overcome.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IB2010/001006

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

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

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

2. ☒ Claims Nos.: 1
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:

see FURTHER INFORMATION sheet PCT/ISA/210

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

Box 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:

see additional sheet

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

2. ☒ As all searchable claims could be searched without effort justifying an additional 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 claims Nos.:

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

Remark on Protest

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

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 2, 5, 7, 8(all partially)

   Compound A (chlrohexidine) for use in the treatment of disorders or diseases related to an insufficiency or a lack of ATP synthesis or related to an excessive accumulation of ROS

   ---

2. claims: 2, 5, 7, 8(all partially)

   Compound B (benzethonium chloride) for use in the treatment of disorders or diseases related to an insufficiency or a lack of ATP synthesis or related to an excessive accumulation of ROS

   ---

3. claims: 2, 5, 7, 8(all partially)

   Compound D (sodium pyrithione) for use in the treatment of disorders or diseases related to an insufficiency or a lack of ATP synthesis or related to an excessive accumulation of ROS

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4. claims: 3, 4, 6, 9, 10(completely); 5, 7, 8(partially)

   Compound of formula (Ia) and its medical application for use in the treatment of disorders or diseases related to an insufficiency or a lack of ATP synthesis or related to an excessive accumulation of ROS

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5. claim: 11

   Compound D (clotrimazole) for use in the treatment of symptoms of the mitochondrial diseases chosen in the group consisting of slow growth, muscle weakness, visual defect, hearing defect, heart disease, liver disease, kidney disease, gastrointestinal disorders and respiratory disorders

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Information on patent family members

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