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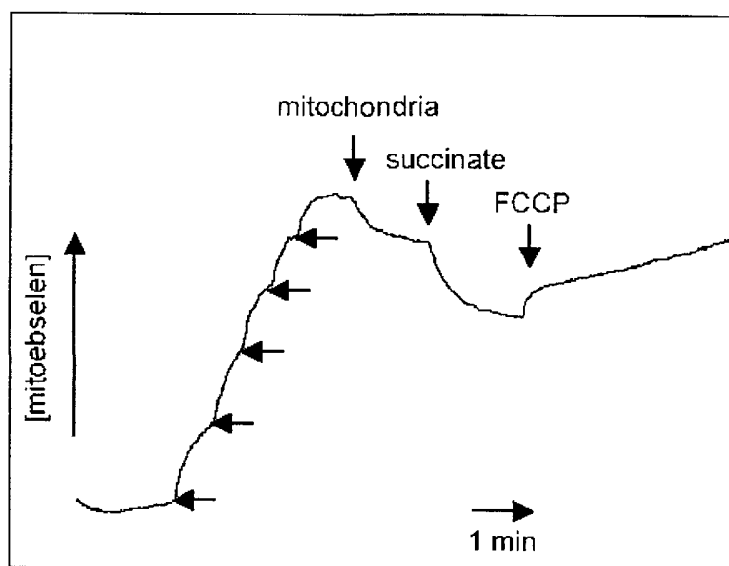
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(54) Title: MITOCHONDRIALLY TARGETED ANTIOXIDANTS



(57) Abstract: The invention provides mitochondrially targeted antioxidant compounds comprising a lipophilic cation moiety covalently coupled to a glutathione peroxidase mimetic. These compounds can be used to treat patients who would benefit from the reduction of oxidative stress.

MITOCHONDRially TARGETED ANTIOXIDANTS

The present invention relates to compounds which are mitochondrially targeted antioxidants, their use and synthesis.

Mitochondria are intracellular organelles responsible for energy metabolism. Consequently, mitochondrial defects are damaging, particularly to neural and muscle tissues which have high energy demands.

Mitochondrial dysfunction is central to a number of human degenerative diseases, and can be due to primary defects in genes encoded by mitochondrial DNA, by mutations in nuclear encoded genes, or due to secondary consequences of other defects. Oxidative damage to the mitochondrion is a major factor in the pathophysiology of these diseases, because the mitochondrial respiratory chain is the major source of reactive oxygen species (ROS) within most human cells. These diseases include Parkinson's disease, Friedreich's Ataxia, Wilson's Disease, mtDNA diseases, diabetes, motor neurone disease and the non-specific loss of vigour associated with ageing. Oxidative damage to mitochondria also contributes to the pathophysiology of inflammation and ischaemic-reperfusion injury in stroke, heart attack and during organ transplantation and surgery.

To prevent the damage caused by oxidative stress a number of antioxidant therapies have been developed. In addition, a range of therapeutically or prophylactically useful compounds designed to protect or alter mitochondrial function have been designed. The present inventors have previously disclosed (WO 99/26954) that certain classes of antioxidants can be

targeted to mitochondria by their covalent attachment to lipophilic cations by means of alkylene chain. In particular, the targeting of Vitamin E and Ubiquinol to mitochondria by linking them to the triphenyl phosphonium ion was described.

The present invention relates to the targeting of a class of antioxidant moieties to mitochondria by their attachment to lipophilic cation moieties.

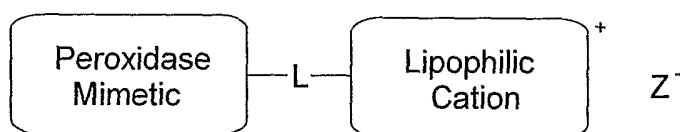
The antioxidant moieties which have been previously targeted to mitochondria are to some extent destroyed when removing reactive oxygen species, in that although the active antioxidant moiety may be regenerated to some degree by processes occurring in the mitochondria or the cell, the regeneration processes produce by-products, eventually leading to a complete loss or severe reduction of antioxidant function. To overcome this reduction in function, the antioxidant compounds must be replenished over time or be present in sufficient quantity so as to avoid a loss of efficacy of the treatment.

A first aspect of the present invention provides a compound comprising a lipophilic cation moiety covalently coupled to an antioxidant moiety which is a glutathione peroxidase mimetic.

Preferably the glutathione peroxidase mimetic is a selenoorganic compound, i.e. an organic compound comprising at least one selenium atom. Preferred classes of selenoorganic glutathione peroxidase mimetics include benzisoselenazolones, diaryl diselenides and diaryl selenides. Such compounds, are disclosed in: Sies, H., *Adv. Pharmacol.*, **38**, 229-246 (1996); Galet, V., et al., *J. Med. Chem.*, **37**, 2903-2911 (1994); Parnham, M.J., et al., *Agents and Actions*, **27**, 306-308;

Andersson, C-M., et al., *Free Radical Biol. & Med.*, **16**, 17-28 (1994); Chaudiere, J, et al., "Design of New Selenium-Containing Mimics of Glutathione Peroxidase", 165-184, in *Oxidative Processes and Antioxidants*, edited by R. Paoletti, et al., Raven Press, New York (1994), which are incorporated herein by reference.

Compounds of this aspect therefore have the following structure:



where L is a linking group and Z is an anion, and preferably a pharmaceutically acceptable anion.

A second aspect of the present invention provides a compound of the first aspect for use in methods of treatment of the human or animal body.

A third aspect of the present invention provides a pharmaceutical composition comprising a compound of the first aspect in combination with one or more pharmaceutically acceptable carriers or diluents.

A fourth aspect of the present invention provides a method of treatment of a patient who would benefit from reduced oxidative stress which comprises the step of administering to said patient a therapeutically effective amount of a compound of the first aspect.

In a further aspect, the present invention provides a method of reducing oxidative stress in a cell which comprises the

step of administering to said cell a compound of the first aspect.

Another aspect of the present invention provides the use of a compound of the first aspect for the manufacture of a medicament for use in the treatment of a condition ameliorated by reduced oxidative stress.

Conditions ameliorated by reduced oxidative stress include Parkinson's disease, Friedreich's Ataxia, Wilson's Disease, mtDNA diseases, diabetes, motor neurone disease, inflammation and ischaemic-reperfusion tissue injury in strokes, heart attacks, organ transplantation and surgery.

Preferred features of the invention will now be described by way of further definition and example, with reference to the following figures.

Figure 1 shows the variation in concentration of Compound 6 ('mitoebelsen') over time in an experiment to show the take up of compound 6 into the mitochondrial matrix;

Figure 2a shows the effect on absorbance at 340 nM caused by the oxidation of NADPH in the presence of glutathione reductase and other compounds, which were added at the time shown by the arrow: hydrogen peroxide ('basal'); hydrogen peroxide and compound 6 ('mitoebelsen'); and hydrogen peroxide and ebelsen ('ebelsen')

Figures 2b-d show how the rate of NADPH oxidation varies with increasing concentration of H₂O₂ (Figure 2b), GSH (Figure 2c), and of compound 6 ('mitoebelsen') or ebelsen;

Figure 3a shows the effect on fluorescence caused by the peroxidation of *cis*-parinaric acid (added at arrow) in mitochondria, in the presence of AAPH ('AAPH'), and how this varies in the presence of further compounds: TPMP ('TPMP'), compound **6** ('mitoebelsen') and ebelsen ('ebelsen');

Figure 3b shows the same effect except in mitochondrial membranes; and

Figure 3c shows the amount of MDA produced by mitochondria under oxidative stress, in the presence of compound **6** ('mitoebelsen'), ebelsen and TPMP.

Lipophilic cation moieties

Mitochondria have a substantial membrane potential of up to 180 mV across their inner membrane (negative inside). Because of this potential, membrane permeant, lipophilic cations accumulate several-hundred fold within the mitochondrial matrix. (Rottenberg, *Methods in Enzymology*, **55**, 547-560 (1979); Chen, *Ann. Rev. Cell Biol.*, **4**, 155-181 (1988)). Such ions are accumulated provided they are sufficiently lipophilic to screen the positive charge or delocalise it over a large surface area, also provided that there is no active efflux pathway and the cation is not metabolised or immediately toxic to a cell.

The lipophilic cation moieties of the present invention may be ammonium, phosphonium or arsonium cations, and in particular tribenzyl or triphenyl substituted cation moieties, of which the phenyl groups may be optionally substituted, for example hydroxy (OH), alkoxy (O-C₁₋₇ alkyl), nitro (NO₂), amido (CONH₂), carboxy (COOH) or C₁₋₇ alkyl, at one or more of the 3-, 4- and 5- positions. Examples include, but are not limited to, tribenzyl ammonium, tribenzyl phosphonium, tribenzyl arsonium,

and triphenyl phosphonium cations. Of these triphenyl phosphonium is preferred.

The lipophilic cation moieties may also be fluorescent or light absorbing. Examples include, but are not limited to, rhodamine 123, JC-1, N,N'-bis(2-ethyl-1,3-doxylene)kryptocyanine, pyronine Y, o-toluidine blue, chalcogenpyrilium and benzo(a)phenoxazinium (see Chen, L., *Ann. Rev. Cell Biol.*, **4**, 155-181 (1988), which is incorporated herein by reference).

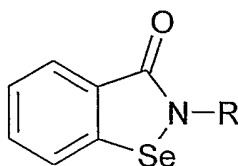
Triphenyl phosphonium is the most preferred lipophilic cation moiety for the present invention.

Glutathione Peroxidase mimetic moieties

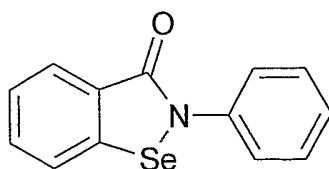
Glutathione peroxidase reduces hydrogen peroxide to water, and organic hydroperoxides to alcohol, whilst oxidising glutathione to glutathione disulphide. It is known that glutathione peroxidase has selenium as an integral part of its active site. It is therefore preferred that glutathione peroxidase mimetics are selenoorganic compounds, i.e. organic compounds comprising at least one selenium atom.

A number of classes of selenoorganic glutathione peroxidase mimetics have been disclosed in the references given above, and include benzoselenazolones, diaryl diselenides and diaryl selenides.

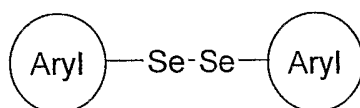
Benzisoselenazolones have the general structure:



where R can be a variety of alkyl and aryl groups. The benzene ring can bear substituents, and can also be replaced by other fused aromatic rings, for example, pyridine. The most extensively studied, and preferred, member of this class is Ebelsen (2-phenyl-benzo[d]isoseleazol-3-one):



Diaryl diselenides are of the general formula:



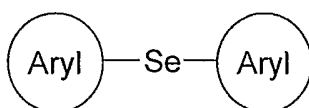
where the term "aryl", as used herein, pertains to a monovalent moiety obtained by removing a hydrogen atom from an aromatic ring atom of an aromatic compound, which moiety has from 3 to 20 ring atoms (unless otherwise specified). Preferably, each ring has from 5 to 7 ring atoms.

The ring atoms may be all carbon atoms, as in "carboaryl groups", for example those derived from benzene (i.e. phenyl) (C_6) and naphthalene (C_{10}), or the ring atoms may include one or more heteroatoms, as in "heteroaryl groups", for example those derived from pyridine (C_6), furan (C_5), thiophene (C_5) and pyrimidine (C_6).

Preferably the aryl groups are optionally substituted phenyl groups.

Examples of this class of compound include bis(2-amino)phenyl diselenide and bis(2-amino,5-acetyl)phenyl diselenide.

Diaryl selenides are of the general formula:



where the term "aryl" is as defined above. Preferably the aryl groups are optionally substituted phenyl groups.

Examples of this class of compound include di(4-aminophenyl) selenide and di(4-phenylphenyl) selenide.

Benzisoselenazolones are the preferred class of selenoorganic glutathione peroxidase mimetics, with Ebelsen being the most preferred.

Covalent Linking Groups

The covalent linking group may be any group which joins the lipophilic cation moiety to the enzyme mimetic with a covalent bond at each end, and enables the two moieties to remain bonded together while crossing the mitochondrial inner membrane into the mitochondrial matrix.

Typically the group will be an alkylene group. The term "alkylene," as used herein, pertains to a bidentate moiety obtained by removing two hydrogen atoms, either both from the same carbon atom, or one from each of two different carbon atoms, of a hydrocarbon compound having from 1 to 30 carbon atoms, which may be aliphatic or alicyclic, and which may be saturated, partially unsaturated, or fully unsaturated. Thus, the term "alkylene" includes the sub-classes alkenylene, alkynylene, cycloalkylene, etc., discussed below.

In this context, the prefixes (e.g. C₁₋₄, C₁₋₇, C₁₋₃₀, C₂₋₇, C₃₋₇, etc.) denote the number of carbon atoms, or range of number of carbon atoms. For example, the term "C₁₋₄ alkylene" as used

herein, pertains to an alkylene group having from 1 to 4 carbon atoms. Examples of groups of alkylene groups include C_{1-4} alkylene ("lower alkylene"), C_{1-7} alkylene, and C_{1-30} alkylene.

Examples of linear saturated C_{1-7} alkylene groups include, but are not limited to, $-(CH_2)_n-$ where n is an integer from 1 to 7, for example, $-CH_2-$ (methylene), $-CH_2CH_2-$ (ethylene), $-CH_2CH_2CH_2-$ (propylene), and $-CH_2CH_2CH_2CH_2-$ (butylene).

Examples of branched saturated C_{1-7} alkylene groups include, but are not limited to, $-CH(CH_3)-$, $-CH(CH_3)CH_2-$, $-CH(CH_3)CH_2CH_2-$, $-CH(CH_3)CH_2CH_2CH_2-$, $-CH_2CH(CH_3)CH_2-$, $-CH_2CH(CH_3)CH_2CH_2-$, $-CH(CH_2CH_3)-$, $-CH(CH_2CH_3)CH_2-$, and $-CH_2CH(CH_2CH_3)CH_2-$.

Examples of linear partially unsaturated C_{1-7} alkylene groups include, but are not limited to, $-CH=CH-$ (vinylene), $-CH=CH-CH_2-$, $-CH=CH-CH_2-CH_2-$, $-CH=CH-CH_2-CH_2-CH_2-$, $-CH=CH-CH=CH-$, $-CH=CH-CH=CH-CH_2-$, $-CH=CH-CH=CH-CH_2-CH_2-$, $-CH=CH-CH_2-CH=CH-$, and $-CH=CH-CH_2-CH_2-CH=CH-$.

Examples of branched partially unsaturated C_{1-7} alkylene groups include, but are not limited to, $-C(CH_3)=CH-$, $-C(CH_3)=CH-CH_2-$, and $-CH=CH-CH(CH_3)-$.

Examples of alicyclic saturated C_{1-7} alkylene groups include, but are not limited to, cyclopentylene (e.g., cyclopent-1,3-ylene), and cyclohexylene (e.g., cyclohex-1,4-ylene).

Examples of alicyclic partially unsaturated C_{1-7} alkylene groups include, but are not limited to, cyclopentenylene (e.g., 4-cyclopenten-1,3-ylene), cyclohexenylene (e.g.,

2-cyclohexen-1,4-ylene; 3-cyclohexen-1,2-ylene;
2,5-cyclohexadien-1,4-ylene).

The alkylene group may be substituted by substituent groups that increase the solubility of the molecule, increase the uptake of the molecule across the mitochondrial membrane, or decrease the rate of degradation of the molecule *in vivo*. In particular, the linking group may be substituted by: hydroxy groups, which have the formula -OH; thio groups, which have the formula -SH; amino groups, which have the formula -NH₂; carboxy groups, which have the formula -C(=O)OH; amido groups, which have the formula -C(=O)NH₂; or groups derived from sugars or sugar derivatives.

- 5 The hydroxy, thio, amino, carboxy and amido groups are preferred located at the end of branches in the linking group, so that the overall effect is that of an alcohol, thiol, amine, carboxylic acid or amide respectively attached at one end to the backbone of the linking group.

- 0 Sugars (saccharides) are carbohydrates which can be considered to be hydroxylated aldehydes and ketones. If two or more monosaccharides are linked, for example, via an acetal linkage, the compound is conventionally referred to as a
5 disaccharide (e.g. sucrose, maltose), trisaccharide, etc., and these may all be used in the invention. Polysaccharides are not intended for use in the present invention.

- 0 Monosaccharides are conventionally named according to the overall number of carbon atoms, for example, tri- (C₃), tetra- (C₄), pent- (C₅), and hex- (C₆). Monosaccharides may be in, for example, aldose, ketose, aldoketose, and dialdose form. Aldoses are conventionally named as -ose, for example, triose

(C₃), tetrose (C₄), pentose (C₅), hexose (C₆), and heptose (C₇). Ketoses are conventionally named as -ulose, for example, tetrulose (C₄), pentulose (C₅), hexulose (C₆), and heptulose (C₇). Aldoketoses are conventionally named as -osulose. Dialdoses are conventionally named as -odialdose.

Monosaccharides may have one or more chiral centres, and thus may have different stereoisomeric forms (e.g., R-, S-, D-, L-, α -, β -, (+), (-), and combinations thereof, e.g., α -D-, β -D-, α -L-, β -L-). Isomers which are superimposable mirror images are conventionally referred to as enantiomers. Isomers which differ from each other by the configuration at two or more chiral centres are conventionally referred to as diastereomers. Isomers which differ from each other by the configuration at only one chiral centre are conventionally referred to as epimers (e.g., D-ribose and D-xylose).

The configuration at each chiral centre is conventionally denoted R or S. The prefixes D- or L- are conventionally used to indicate monosaccharides with a configuration that is related to D- and L-glyceraldehyde, respectively. The prefixes (+)- and (-)- are conventionally used to indicate monosaccharides which are dextrorotatory (rotate the plane of polarised light to the right, in a clockwise direction) or levorotatory (to the left, in a counter-clockwise direction).

The prefixes erythro- and threo- denote certain tetrose (C₄) diastereomers. The prefixes arabino-, lyxo-, ribo-, and xylo- denote certain pentose (C₅) diastereomers. The prefixes allo-, altro-, gluco-, manno-, gulo-, ido-, galacto-, and talo- denote certain hexose (C₆) diastereomers.

In cyclic form (hemiacetal or hemiketal form), monosaccharides are conventionally named according to the number of ring atoms. For example, a furanose has a 5-membered ring; a pyranose has a 6-membered ring; a septanose has a 7-membered ring. The prefixes α - and β - are conventionally used to indicate the two anomers which arise from the new chiral centre which is formed upon cyclisation.

Examples of saccharides include, but are not limited to, the following, which may be in a α -D, β -D, α -L, or β -L form:

erythrose and threose;
arabinose, lyxose, ribose, and xylose;
allose, altrose, glucose, mannose, gulose, idose,
galactose, and talose;
arabinofuranose, lyxofuranose, ribofuranose, and
xylofuranose;
allofuranose, altrofuranose, glucofuranose,
mannofuranose, gulofuranose, idofuranose, galactofuranose,
talofuranose;
allopyranose, altropyranose, glucopyranose,
mannopyranose, gulopyranose, idopyranose, galactopyranose, and
talopyranose.

Many saccharides are known by their trivial names, for example, D-threose (D-threo-tetrose), D-ribose (D-ribo-pentose), D-galactose (D-galacto-hexose), D-fructose (D-arabino-2-hexulose), L-sorbose (L-xylo-2-hexulose), D-ribulose (D-erythro-2-pentulose), D-sedoheptulose (D-altro-2-heptulose).

Many saccharides derivatives are well known, for example, deoxy-saccharides (e.g., 6-deoxy-L-galactose, also known as L-fucose; 6-deoxy-L-mannose, also known as L-rhamnose;

2-deoxy-D-erythro-pentose, also known as deoxyribose or 2-deoxy-D-ribose); glycosides (e.g., methyl α -D-glucopyranoside); amino-deoxy-saccharides, also known as glucosamines (e.g., D-glucosamine, D-galactosamine); alditols (e.g., D-glutitol, also known as D-sorbitol; D-mannitol; meso-galactitol); aldonic acids, also known as glyconic acids (e.g., D-gluconic acid); uronic acids, also known as glycouronic acids (e.g., D-galactouronic acid); and aldaric acids, also known as glycaric acids (e.g., L(+)-tartaric acid).

The alkylene group may have a heteroatom, selected from O, S or NH at its end adjacent the enzyme mimetic moiety.

Preferred linking groups are C₁₋₃₀ alkylene groups, more preferably C₁₋₂₀, C₁₋₁₀ or C₁₋₄ alkylene groups, optionally terminating at the enzyme mimetic end with an O or S, for example -S-(CH₂)₃- and -O-(CH₂)₄-.

-) The linking group is preferably attached to the enzyme mimetic moiety on an aromatic ring, for example a benzene or pyridine ring.

Anions

-) Examples of suitable inorganic anions include, but are not limited to, those derived from the following inorganic acids: hydrochloric, hydrobromic, hydroiodic, sulfuric, sulfurous, nitric, nitrous, phosphoric, and phosphorous
-) Examples of suitable organic anions include, but are not limited to, those derived from the following organic acids: tetraphenylboronic, 2-acetyoxybenzoic, acetic, ascorbic, aspartic, benzoic, camphorsulfonic, cinnamic, citric, edetic,

ethanedisulfonic, ethanesulfonic, fumaric, glucheptonic, gluconic, glutamic, glycolic, hydroxymaleic, hydroxynaphthalene carboxylic, isethionic, lactic, lactobionic, lauric, maleic, malic, methanesulfonic, mucic, oleic, oxalic, palmitic, pamoic, pantothenic, phenylacetic, phenylsulfonic, propionic, pyruvic, salicylic, stearic, succinic, sulfanilic, tartaric, toluenesulfonic, and valeric.

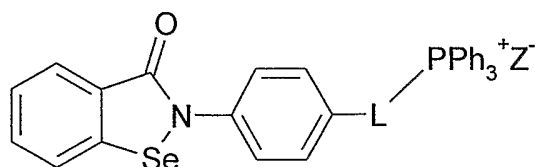
The above anions are generally pharmaceutically acceptable.

Examples of pharmaceutically acceptable salts are discussed in Berge, *et al.*, "Pharmaceutically Acceptable Salts," *J. Pharm. Sci.*, **66**, 1-19 (1977), which is incorporated herein by reference.

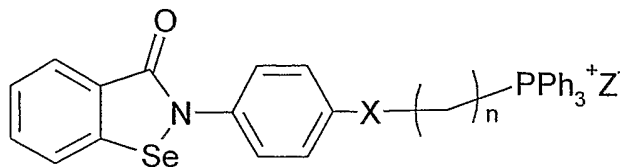
In the present invention inorganic anions are preferred, and in particular the halo anions, of which Br^- is the most preferred.

Preferred Compounds

Preferred compounds of the invention have the formula:



where Z and L are as defined above, and more preferably have the formula:



where Z is as defined above, X is O, S, or NH, and preferably O or S, and n is from 1 to 20, more preferably 3 to 6.

Treatment

The term "treatment" as used herein in the context of treating a condition, pertains generally to treatment and therapy, whether of a human or an animal (e.g., in veterinary applications), in which some desired therapeutic effect is achieved, for example, the inhibition of the progress of the condition, and includes a reduction in the rate of progress, a halt in the rate of progress, amelioration of the condition, and cure of the condition. Treatment as a prophylactic measure (i.e., prophylaxis) is also included.

The term "therapeutically-effective amount" as used herein, pertains to that amount of an active compound, or a material, composition or dosage form comprising an active compound, which is effective for producing some desired therapeutic effect, commensurate with a reasonable benefit/risk ratio.

The term "treatment" includes combination treatments and therapies, in which two or more treatments or therapies are combined, for example, sequentially or simultaneously.

The compound or pharmaceutical composition comprising the compound may be administered to a subject by any convenient route of administration, whether systemically/ peripherally or topically (i.e., at the site of desired action).

While it is possible for the active compound to be used (e.g., administered) alone, it is often preferable to present it as a formulation.

Suitable carriers, diluents, excipients, etc. can be found in standard pharmaceutical texts. See, for example, Handbook for Pharmaceutical Additives, 2nd Edition (eds. M. Ash and I.

Ash), 2001 (Synapse Information Resources, Inc., Endicott, New York, USA), Remington's Pharmaceutical Sciences, 18th edition, Mack Publishing Company, Easton, Pa., 1990; and Handbook of Pharmaceutical Excipients, 2nd edition, 1994, which are incorporated herein by reference.

The term "pharmaceutically acceptable" as used herein pertains to compounds, ingredients, materials, compositions, dosage forms, etc., which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of the subject in question (e.g. human) without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio. Each carrier, diluent, excipient, etc. must also be "acceptable" in the sense of being compatible with the other ingredients of the formulation.

The formulations may be prepared by any methods well known in the art of pharmacy. Such methods include the step of

) bringing into association the active compound with a carrier +which constitutes one or more accessory ingredients. In general, the formulations are prepared by uniformly and intimately bringing into association the active compound with carriers (e.g. liquid carriers, finely divided solid carrier, etc.), and then shaping the product, if necessary.

It will be appreciated by one of skill in the art that appropriate dosages of the compounds, and compositions comprising the compounds, can vary from patient to patient.

) Determining the optimal dosage will generally involve the balancing of the level of therapeutic benefit against any risk or deleterious side effects. The selected dosage level will depend on a variety of factors including, but not limited to,

the activity of the particular compound, the route of administration, the time of administration, the rate of excretion of the compound, the duration of the treatment, other drugs, compounds, and/or materials used in combination, the severity of the condition, and the species, sex, age, weight, condition, general health, and prior medical history of the patient. The amount of compound and route of administration will ultimately be at the discretion of the physician, veterinarian, or clinician, although generally the dosage will be selected to achieve local concentrations at the site of action which achieve the desired effect without causing substantial harmful or deleterious side-effects.

Administration can be effected in one dose, continuously or intermittently (e.g. in divided doses at appropriate intervals) throughout the course of treatment. Methods of determining the most effective means and dosage of administration are well known to those of skill in the art and will vary with the formulation used for therapy, the purpose of the therapy, the target cell(s) being treated, and the subject being treated. Single or multiple administrations can be carried out with the dose level and pattern being selected by the treating physician, veterinarian, or clinician.

In general, a suitable dose of the active compound is in the range of about 1 μ g to about 250 mg per kilogram body weight of the subject per day.

Further Uses

The compounds of the first and second aspects may also be useful for perfusing isolated organs prior to transport, and in storing frozen cells, for example, embryos.

Synthesis Routes

Methods for the chemical synthesis of compounds of the present invention are described herein. These methods may be modified and/or adapted in known ways in order to facilitate the synthesis of additional compounds within the scope of the present invention. The amounts of reactants given are for guidance. Descriptions of general laboratory methods and procedures, useful for the preparation of the compounds of the present invention, are described in Vogel's Textbook of Practical Organic Chemistry (5th edition, Ed. Furniss, B. S., Hannaford, A.J., Smith, P.W.G., Tatchell, A.R., Longmann, UK).

The synthesis of compounds of the present invention has three key steps:

- (i) formation of the antioxidant moiety;
- (ii) attachment of the linking group to the lipophilic cation moiety;
- (iii) attachment of the linking group to the antioxidant moiety.

These three steps can be carried out in any order, which will be dependent on the methods used and the nature of each of the three groups. It is possible that the formation of the antioxidant moiety can be interrupted by linking a precursor to the linking group. If necessary, protecting groups can be employed to avoid any unwanted reactions occurring during the synthesis (see *Protective Groups in Organic Synthesis*, T. Green and P. Wuts; 3rd Edition; John Wiley and Sons, 1999).

Formation of the antioxidant moiety: this will depend on the nature of the antioxidant moiety, and can usually be based on the disclosed routes for forming that moiety. It is sometimes convenient to synthesise the enzyme mimetic moiety with the

heteroatom (O, S or NH) that is on the end of the alkylene chain of the linking group adjacent the moiety, to aid the joining of the linking group to the enzyme mimetic moiety.

Linking the linking group to the lipophilic cation moiety: it is generally preferred to carry this step out by heating a halogenated precursor, preferably an iodinated or brominated precursor (RBr or RI), sometimes in an appropriate solvent with 2-3 equivalents of the lipophilic cation precursor under argon for several days. R can either be the linking group, the linking group already attached to the enzyme mimetic moiety, or the linking group attached to a precursor of the enzyme mimetic moiety. The compound is then isolated as its bromide or iodide salt. To do this the solvent is removed (if necessary), the product is then triturated repeatedly with a compound such as diethyl ether, until a solid remains. This can then be dissolved in a solvent, e.g. dichloromethane, and precipitated with diethyl ether to remove the excess unreacted cation. This can be repeated. Purification can involve recrystallisation, for example, from methylene chloride/diethyl ether or chromatography on silica gel eluting with dichloromethane/ethanol mixtures.

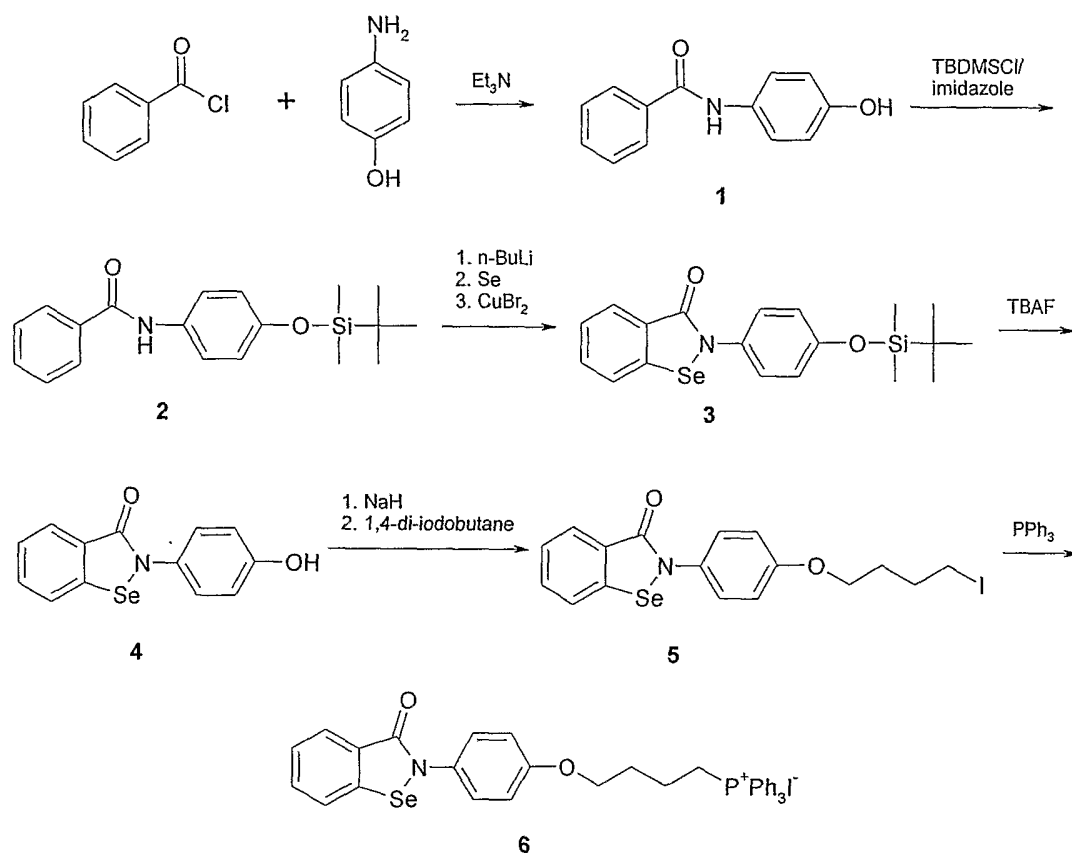
Linking the linking group to the antioxidant moiety: this will depend on the nature of the antioxidant moiety. One method of achieving this linking is to synthesise the linking group as part of the antioxidant moiety. Alternatively, if the antioxidant moiety has been synthesised with a heteroatom in place (see above), then the linking group can be joined by treating the antioxidant moiety with a strong base and reacting it with the linking group having a suitable leaving group (for example, halo).

Examples*General Experimental Details*

Preparative column chromatography was performed using silica gel (Merck) type 60, 200-400 mesh, 40-63 μm . Analytical thin layer chromatography (TLC) was performed using silica gel (Merck) 60F 254 coated on alumina roll and visualization accomplished with UV light. Nuclear magnetic resonance spectra were acquired using a Varian 300 MHz instrument.

Tetramethylsilane was used as an internal standard for ^1H and ^{13}C NMR experiments in CDCl_3 , and 85% phosphoric acid was used as an internal standard for ^{31}P NMR experiments in all solvents. Residual solvent peaks were used as internal standards in ^1H and ^{13}C NMR experiments not performed in CDCl_3 . Chemical shift (δ) data are reported in units of ppm relative to the internal standard. Peak assignment for ^{13}C NMR were made on the basis of chemical shift, relative intensity and HSQC data. Infrared absorption spectra were acquired using a Perkin Elmer Spectrum BX FTIR instrument. Samples were examined as KBr discs prepared using anhydrous KBr and units of absorption are reported in wavenumbers (cm^{-1}). Low resolution electrospray (LRES) and low resolution atmospheric pressure chemical ionisation (LRAPCI) mass spectra were acquired using a Shimadzu LCMS-QP800X liquid chromatograph mass spectrometer and data are reported as m/z values. Melting points were acquired using a Kofler Heizbank melting point bench and are reported uncorrected.

Synthesis of 2-[4-(4-triphenylphosphoniobutoxy)phenyl]-1,2-benziselenazol-3(2H)-one iodide (Compound 6)



Scheme 1

N-(4-hydroxyphenyl)-benzamide (Compound 1)

A solution of benzoyl chloride (12.9 g, 91.7 mmol) in dry THF (250 mL) was added dropwise to a solution of 4-aminophenol (10.0g, 91.7 mmol) and Et₃N (9.4 g, 92.9 mmol) in dry THF (700 mL), and the mixture stirred for 2 days under a drying tube. Solvent was removed *in vacuo* and the solid residue extracted with H₂O (3 x 250 mL) and diethyl ether (3 x 250 mL). The remaining solid material was recrystallised from aqueous ethanol, the crystals filtered, washed with diethyl ether and sucked dry, giving **1** as white crystals (14.97g, 77%). ¹H NMR (299.9 MHz, d₆-DMSO) δ 10.06 (broad s, 1H, -OH), 9.28 (s, 1H, -NH-), 7.96 (d, *J* = 7.8 Hz, 2H, Ar-H), 7.57 (m, 5, Ar-H), 6.78 (d, *J* = 7.8 Hz, Ar-H); ¹³C NMR (125.7 MHz, d₆-DMSO) δ 165.92

(carbonyl), 154.56 (aromatic), 136.09 (aromatic), 132.30 (aromatic), 131.61 (aromatic), 129.33 (protonated aromatic), 128.48 (protonated aromatic), 123.21 (protonated aromatic), 115.90 (protonated aromatic); Anal. Calcd. for $C_{13}H_{11}NO_2$: C:73.22, H: 5.20, N: 6.57, found: C: 73.33, H: 5.27, N: 6.69; m.p. 224°C.

N-[4-(*tert*-Butyldimethylsiloxy)phenyl]benzamide (Compound **2**)

A solution of **1** (14.5 g, 68.01 mmol) and imidazole (11.6g, 67.91 mmol) in dry THF (160 mL) was cooled to 0°C under a drying tube. A solution of TBDMSCl in dry THF (60 mL) was added dropwise under a drying tube to the cooled solution. The resultant suspension was stirred overnight under an argon atmosphere. The suspension was then diluted with H_2O (500 mL) and extracted with CH_2Cl_2 (2 x 500 mL). The organic extracts were combined, dried over Na_2SO_4 , and filtered. Solvent was removed from the filtrate *in vacuo*, giving a white solid. The solid was recrystallised from hexane after a hot filtration. The crystals were collected by filtration, washed with hexane and sucked dry, giving sufficiently pure **2** as white crystals (19.86 g, 89%); 1H NMR (299.9 MHz, $CDCl_3$) δ 7.86 (d, J = 8.1 Hz, 1H, Ar-**H**), 7.85 (d, J = 8.1Hz, 1H, Ar-**H**), 7.57 - 7.44 (m, 5H, Ar-**H**), 6.84 (d, J = 8.5 Hz, 2H, Ar-**H**), 0.99 (s, 9H, *t*-butyl methyl), 0.20 (s, 6H, methyl); ^{13}C NMR (125.7 MHz, $CDCl_3$)

δ 165.97 (carbonyl), 152.9 (aromatic), 135.45 (aromatic), 132.04 (protonated aromatic), 131.88 (aromatic), 129.11 (protonated aromatic), 127.31 (protonated aromatic), 122.22 (protonated aromatic), 120.80 (protonated aromatic), 26.04 (methyl), 18.56 (tertiary), -4.10 (methyl); LRAPCI MS (+formic acid) Calcd. For $C_{19}H_{24}NO_2SiSe$; 328, found: 328.

2-[4-(*tert*-Butyldimethylsiloxy)phenyl]-1,2-benzisoselenazol-3(2H)-one (Compound **3**)

To a solution of **2** (9.20g, 28.09 mmol) in dry THF (150 mL) under argon and cooled to -15°C was slowly added *n*-BuLi (1.6 M in hexane, 35 mL, 56.0 mmol) via a cannula over 45 minutes.

The resultant orange solution was stirred at -15°C for 45 minutes and selenium powder (2.22g, 28.12 mmol) was then added. The resultant suspension was stirred for 45 minutes at -15°C , giving a deep red solution. The solution was cooled to -78°C and CuBr_2 (12.55g, 56.18 mmol) was added in 3 portions over 15 minutes. The resultant suspension was stirred for 1h at -78°C then removed from the cooling bath and stirred for 22 hours. The resultant brown suspension was poured into 1%

aqueous acetic acid (600 mL) and extracted with CH_2Cl_2 (3 x 500 mL). The organic fractions were combined and filtered. The filtrate was dried over Na_2SO_4 , filtered and solvent removed from the filtrate *in vacuo*, to give a brown greasy solid. The solid was chromatographed (silica gel packed in CH_2Cl_2 , eluting with CH_2Cl_2), fractions containing product were combined and

solvent removed *in vacuo*, giving a pale tan solid. The solid was recrystallised from EtOH, the crystals filtered, washed with hexane and sucked dry, giving **3** as pale yellow crystals

(5.09g, 45 %). ^1H NMR (299.9 MHz, CDCl_3) δ 8.11 (d, $J = 7.8$ Hz, 1H Ar-**H**), 7.64-7.60 (m, 2H, Ar-**H**), 7.50-7.42 (m, 3H, Ar-**H**),

6.88 (d, $J = 9$ Hz, 2H, Ar-H), 1.00 (s, 9H, *t*-butyl methyl), 0.22 (s, 6H, methyl); ^{13}C NMR (125.7 MHz) δ 166.12 (carbonyl), 154.91 (aromatic), 138.09 (aromatic), 132.72 (protonated aromatic), 132.54 (aromatic) 129.72 (protonated aromatic), 127.78 (aromatic), 127.45 (protonated aromatic), 126.82

(protonated aromatic), 124.05 (protonated aromatic), 120.96 (protonated aromatic), 25.71 (*t*-butyl methyl), 18.27

(tertiary), -4.34 (methyl); Anal Calcd. for $\text{C}_{19}\text{H}_{23}\text{NO}_2\text{SiSe}$: C:

56.43, H: 5.73, N: 3.46, found: C: 56.72, H: 5.84, N: 3.56;
m.p. 188°C

2-(4-Hydroxyphenyl)-1,2-benzisoselenazol-3(2H)-one (Compound
4)

To a 0°C solution of **3** (2.0g, 4.95 mmol) in THF (25 mL) was added TBAF solution (1M in THF, 14 mL, 14 mmol) dropwise over 15 minutes under a nitrogen atmosphere. The mixture was stirred at 0°C for 1 hour after which time TLC analysis (silica gel, 19:1 CH₂Cl₂/diethyl ether) showed no starting material to be present. The solvent was removed *in vacuo* to give a brown oil. The oil was dissolved in CH₂Cl₂ (50 mL) and the organic phase washed with 5% HCl (150mL), and H₂O (4 x 50 mL). Upon the second water wash a yellow solid formed inside the separating funnel. The solid was collected by filtering both phases and residual solid was washed from the funnel in the final two washes. The filtered solid was washed with the final two aqueous extracts, sucked dry and recrystallised from 1:1 EtOH/THF (35 mL). The crystals were collected by filtration, washed with diethyl ether and sucked dry, giving **4** as yellow crystals (1.01g, 97%). ¹H NMR (299.9 MHz, d₆-DMSO) δ 9.65 (s, 1H, -OH), 8.10 (d, *J* = 7.8 Hz, 1H, Ar-H), 7.91 (d, *J* = 8.4 Hz, Ar-H), 7.70 (dd, 1H, Ar-H), 7.49 (dd, 1H, Ar-H), 7.39 (d, *J* = 9Hz, 2H, Ar-H), 6.86 (d, *J* = 9 Hz, 2H, Ar-H); ¹³C NMR (125.7 MHz, d₆-DMSO) δ 165.82 (carbonyl), 156.71 (aromatic), 139.94 (aromatic), 132.87 (aromatic), 131.54 (aromatic), 129.26 (aromatic), 128.75 (aromatic), 127.70 (protonated aromatic), 127.07 (aromatic), 126.71 (aromatic), 116.51 (protonated aromatic); Anal Calcd. for C₁₃H₉NO₂Se: C: 53.81, H: 3.13, N: 4.83, found: C:54.04, H: 3.03, N: 4.88; m.p. >260°C.

2-[4-(4-iodobutoxy)phenyl]-1,2-benzisoselenazol-3(2H)-one
(Compound 5)

To a suspension of NaH (60% emulsion washed with pentane and dried *in vacuo*, 60 mg, 1.50 mmol) in dry DMF (1 mL) at 0°C under argon was added a solution of **4** (300 mg, 1.03 mmol) in dry DMF (7 mL) under argon via a cannula. The resultant solution was stirred for 2 hours at room temperature then added via a cannula to a solution of 1,4-diiodobutane (3.20 g, 10.3 mmol) in dry DMF (2 mL) under argon. The resultant solution was stirred in the dark for 2 days at room temperature under argon. Water (1 mL) was carefully added and solvent removed *in vacuo* to give an oily residue. The residue was dissolved in CH₂Cl₂ (20 mL) and the organic phase washed with H₂O (20 mL), 10% Na₂S₂O₃ (20 mL), and H₂O (20 mL). The organic extract was dried over Na₂SO₄ and filtered. Solvent was removed from the filtrate *in vacuo*, giving a yellow oily residue. The residue was chromatographed (silica gel packed in CH₂Cl₂, eluting with 19:1 CH₂Cl₂/diethyl ether) and fractions containing product were combined. Solvent was removed *in vacuo*, giving sufficiently pure **5** as a white solid (254 mg, 52%). ¹H NMR (299.9 MHz, CDCl₃) δ 8.11 (d, *J* = 7.8 Hz, 2H, Ar-**H**), 7.68-7.62 (m, 2H, Ar-**H**), 7.52-7.43 (m, 3H, Ar-**H**), 6.94 (d, *J* = 9Hz, 2H, Ar-**H**), 4.02 (t, *J* = 5.9Hz, 2H, -O-CH₂-), 3.27 (t, *J* = 6.6 Hz, 2H, -CH₂-I), 1.98 (m, 4H, -CH₂-CH₂-); ¹³CNMR (125.7 MHz, CDCl₃) δ 166.20 (carbonyl), 158.0 (aromatic), 138.12 (aromatic), 132.73 (aromatic), 132.03 (aromatic), 129.73 (aromatic), 127.73 (protonated aromatic), 127.60 (aromatic), 126.85 (aromatic), 126.85 (aromatic), 124.08 (aromatic), 115.41 (protonated aromatic), 67.32 (-O-CH₂-), 30.43 (methylene), 6.64 (-CH₂-I); LRAPCI MS (+formic acid) Calcd. For C₁₇H₁₇NOSel: 473, found: 473.

2-[4-(4-triphenylphosphoniobutoxy)phenyl]-1,2-benzisoselenazol)-3(2H)-one iodide (Compound **6**)

A dry Kimax tube containing **5** (50 mg, 0.106 mmol) and triphenylphosphine (278 mg, 1.06 mmol) was purged with argon, sealed and placed in the dark. The mixture was stirred as a melt at 90°C for 2 hours. The mixture was cooled and the solid residue dissolved in CH₂Cl₂ (1 mL). Product was triturated with diethyl ether and collected by centrifugation (3500 rpm for 10 minutes). The solid residue was triturated twice more from CH₂Cl₂ with diethyl ether and collected by centrifugation. Residual solvent was removed from the precipitate *in vacuo*, giving crude **6** as a yellow solid (68 mg, 87%). ¹H NMR (299.9 MHz, CD₂Cl₂) δ 8.44 (d, *J* = 8.1 Hz, 1H, Ar-**H**), 7.90 (d, *J* = 8 Hz, 1H, Ar-**H**), 7.86-7.34 (m, 19H, Ar-**H**, -P⁺Ph₃), 6.79 (d, *J* = 9Hz, 2H, Ar-**H**), 3.97 (t, *J* = 5.7 Hz, 2H, -O-CH₂-), 3.41 (m, 2H, -CH₂-P⁺Ph₃), 2.04 (m, 2H, -O-CH₂-CH₂-), 1.87 (m, 2H, -CH₂-CH₂-P⁺Ph₃); ³¹P NMR (121.4 MHz, CD₂Cl₂) δ 24.29; LRES⁺ Calcd for [C₃₅H₃₁NO₂PSe]⁺: 608, found: 608

Accumulation of Compound 6 in mitochondria

To demonstrate that the targeting of the glutathione peroxidase mimetic to mitochondria is effective and that these compounds can cross the lipid bilayer of the mitochondrial inner membrane, compound **6** was tested in relation to isolated mitochondria.

Liver mitochondria were prepared from female Wistar rats by homogenisation followed by differential centrifugation in medium containing 250 mM sucrose, 10 mM Tris-HCL (pH 7.4) and 1mM EGTA (Chappell, J.B. and Hansford, R.G., In: *Subcellular components: preparation and fractionation* (Ed. Birnie GD), pp. 77-91. Butterworths, London, 1972, which is incorporated herein by reference.)

An ion selective electrode for compound **6** was prepared by standard procedures (Kamo, N., et al., *Journal of Membrane Biology*, **49**, 105-121 (1979) and Davey, G.P., et al., *Biochemical Journal*, **288**, 439-443 (1992), which are incorporated herein by reference), and activated by soaking in 100 μ M compound **6** for 1- 3 days.

The rat liver mitochondria (0.5mg protein/ml) were suspended in 2mL KCl medium (120 mM KCl, 10 mM HEPES, pH 7.2, 1 mM EGTA) with additions of rotenone (2 μ g/ml) in a 30°C thermostatted chamber. Compound **6** was titrated in in 1 μ M increments, as 1.5 μ l aliquots in DMSO, and the response of the electrode measured over 0-5 μ M of Compound **6**. This response was logarithmic with respect to the concentration of Compound **6**.

To energise the mitochondria, the respiratory substrate succinate (10 mM) was added. These conditions are known to generate a mitochondrial membrane potential of about 180 mV (Burns, et al., *Arch Biochem Biophys*, **332**, 60-68 (1995); Burns and Murphy, *Arch Biochem Biophys*, **339**, 33-39 (1997), which are incorporated herein by reference). As can be seen from figure 1, the concentration of compound **6** outside the mitochondria falls rapidly.

When the uncoupler FCCP (carbonyl cyanide-p-trifluoromethoxyphenylhydrazine), which prevents mitochondria establishing a membrane potential (Burns et al., 1995) (332 nM) was added to dissipate the membrane potential, the accumulation of compound **6** into mitochondria was reversed, as seen in Figure 1.

This shows that compound **6** is rapidly and selectively accumulated into mitochondria driven by the mitochondrial membrane potential. As this accumulation is rapidly (<10 s) reversed by addition of the uncoupler FCCP to dissipate the mitochondrial membrane potential after accumulation of compound **6** within the mitochondria, the mitochondrial specific accumulation of compound **6** is solely due to the mitochondrial membrane potential and is not due to specific binding or covalent interaction.

Glutathione Peroxidase Assay

The activity of the glutathione peroxidase mimetics was determined indirectly by measuring the stimulated oxidation of NADPH in the presence of glutathione reductase. The incubations were carried out at 37°C in quartz cuvettes containing 143 mM potassium phosphate buffer, 6.3 mM EDTA, pH 7.5, 250 µM NADPH, 1 mM GSH, 50 µM ebselen or compound **6** and 1 U glutathione reductase. The reaction was started with 1 mM hydrogen peroxide or 1 mM tert butyl hydroperoxide and was recorded at 340 nm with air as a reference by a Shimadzu model UV-2501PC Spectrophotometer. The reaction was further tested with increasing concentrations of ebselen and compound **6**, glutathione and oxidants.

- ;) The indirect glutathione peroxidase assay indicates that the basal oxidation of GSH by hydrogen peroxide amounts to 55 ± 2 nmol NADPH/min in control incubations without glutathione peroxidase mimetics. This basal rate was accelerated three fold by Ebselen giving a rate of 140 ± 4 nmol NADPH/min.
-) Compound **6** had about two fold increase over the basal oxidation with a rate of 115 ± 4 nmol NADPH/min (Figure 2a). The basal oxidation of GSH by t-butyl hydroperoxide was 29 ± 1 nmol NADPH/min and this was increased two fold by compound **6**

and three fold by Ebselen (data not shown). This NADPH oxidation is accelerated by increasing the concentrations of hydroperoxides (Figure 2b), GSH (Figure 2c) and compound **6** or ebselen (Figure 2d). No reaction was observed in the absence of GSH and peroxide. This confirms that compound **6** is an effective mimetic.

Antioxidant efficacy of Compound 6

To determine the antioxidant efficacy of compound **6**, mitochondria or mitochondrial membranes were incubated with *cis*-parinaric acid. This fatty acid fluoresces when incorporated into lipid bilayers. Upon oxidation of its conjugated double bond fluorophore the fluorescence is decreased and this is a measure of lipid peroxidation.

To measure *cis*-parinaric acid oxidation, rat liver mitochondria (0.5 mg protein/mL) were suspended in a 3 mL fluorometer cuvette in 100 mM KCl and 10 mM Tris-HCl, pH 7.6, 10 mM succinate, 8 µg/mL rotenone, 10 mM 2,2'-azobis(amidinopropane) dihydrochloride (AAPH) in the presence or absence of 1 µM ebselen or compound **6** ('mitoebelsen') at 37°C. Control incubations were in the presence of 1 µM TPMP. The oxidation reaction was started with the addition of 0.5 µM *cis*-parinaric acid and the fluorescence was measured ($\lambda_{\text{excite}}=324$ nm, $\lambda_{\text{emission}}=413$ nm). Control incubation of *cis*-parinaric acid into a mitochondrial suspension showed a slow decay of fluorescence over time due to self quenching. *Cis*-parinaric acid oxidation was measured using beef heart mitochondrial membranes (0.1 mg protein/mL) in 50 mM potassium phosphate buffer, pH 8.0 using the same conditions described above.

In the presence of mitochondria or mitochondrial membranes *cis*-parinaric acid fluoresces and has a slow autooxidation

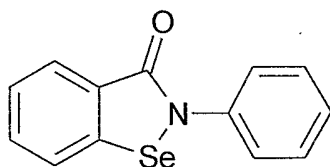
rate (Figure 3a). This basal oxidation rate is rapidly accelerated in the presence of the radical initiator of lipid peroxidation AAPH (Figure 3a). In mitochondria 1 μ M compound **6** ('mitoebbselen') prevented the oxidation of *cis*-parinaric acid by AAPH, showing its antioxidant activity, and ebselen at this concentration failed to protect *cis*-parinaric acid from oxidation (Figure 3a). Control incubation with TPMP did not slow down the oxidation of the *cis*-parinaric acid (Figure 3a). Similar experiments with mitochondrial membranes showed that 5 μ M compound **6** ('mitoebbselen') and ebselen effectively protect against oxidation of *cis*-parinaric acid (Figure 3b). The lipophilic cation TPMP has no effect on *cis*-parinaric oxidation confirming the antioxidant effect of compound **6** ('mitoebbselen') and ebselen (Figure 3b).

To quantitate the antioxidant effect of compound **6**, mitochondria were incubated with rat liver mitochondria (2 mg protein/mL) at 37°C for 15 min in 100 mM KCl and 10 mM Tris-HCl, pH 7.6, 10 mM succinate, 8 μ g/mL rotenone, 50 μ M FeCl₂ in the presence or absence of 1 μ M compound **6** or ebselen. Control incubations were in the presence of 1 μ M TPMP and FeCl₂ alone. Then 0.8 mL aliquots were mixed with, 0.1% butylated hydroxytoluene, 200 μ L of 1% thiobarbituric acid and 200 μ L 35% HClO₄, heated at 100°C for 15 min, diluted with 3 mL water, and extracted into 3 mL n-butanol. TBARS (thiobarbituric acid relative species) were determined fluorometrically (λ_{excite} =515 nm, $\lambda_{\text{emission}}$ =553 nm) in a plate reader and expressed as nanomoles of MDA by comparison with standard solutions of 1,1,3,3- tetraethoxypropane processed as above, and the accumulation of MDA was measured as a marker of lipid peroxidation.

Incubation of mitochondria with 1 μ M compound **6** ('mitoebesen') prevented the accumulation of MDA (Figure 3c). Control incubations with 1 μ M TPMP did not prevent MDA accumulation confirming that the antioxidant effect of compound **6** is due to its ebisen selenol moiety (Figure 3c).

CLAIMS

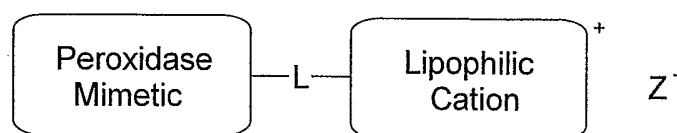
1. A compound comprising a lipophilic cation moiety covalently coupled to an antioxidant moiety which is a glutathione peroxidase mimetic.
2. A compound according to claim 1, wherein the glutathione peroxidase mimetic moiety comprises at least one selenium atom.
3. A compound according to claim 2, wherein the glutathione peroxidase mimetic moiety is selected from the group consisting of: benzisosenazolones, diaryl diselenides and diaryl selenides.
4. A compound according to claim 3, wherein the glutathione peroxidase mimetic moiety is:



5. A compound according to any one of claims 1 to 4, wherein the lipophilic cation moiety is selected from the group consisting of: ammonium cations; phosphonium cations; arsonium cations; rhodamine 123; JC-1; N,N'-bis(2-ethyl-1,3-doxylene)kryptocyanine; pyronine Y; o-toluidine blue; chalcogenpyrilium; and benzo(a)phenoxazinium.
6. A compound according to claim 5, wherein the lipophilic cation moiety is a tribenzyl or triphenyl substituted ammonium or phosphonium cation.

7. A compound according to claim 6, wherein the lipophilic cation moiety is triphenyl phosphonium.

8. A compound according to any one of claims 1 to 7 having the formula:

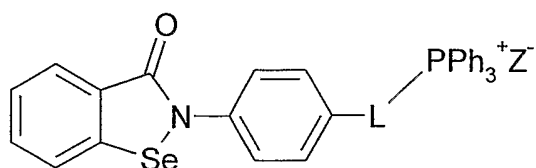


where L is a linking group and Z is an anion.

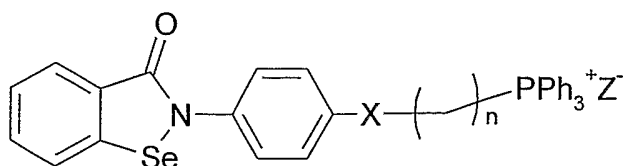
9. A compound according to claim 8, wherein the linking group is an alkylene group having 1 to 30 carbon atoms, optionally comprising O, S or NH at the glutathione peroxidase mimetic moiety end, and is optionally substituted by a group selected from: hydroxy, thio, amino, carboxy, amido and groups derived from sugars or sugar derivatives.

10. A compound according to either claim 8 or claim 9, wherein Z is a pharmaceutically acceptable anion.

11. A compound according to any one of claims 8 to 10, wherein the compound is of formula:



12. A compound according to claim 11, wherein the compound is of formula:



where X is selected from the group consisting of: O, S, and n

is from 1 to 30.

13. A compound according to any one of claims 1 to 12 for use in a method of treatment of the human or animal body.

14. A pharmaceutical composition comprising a compound according to any one of claims 1 to 12 in combination with one or more pharmaceutically acceptable carriers or diluents.

15. The use of a compound according to any one of claims 1 to 12 for the manufacture of a medicament for use in the treatment of a condition ameliorated by reduced oxidative stress.

16. The use according to claim 15, wherein the condition ameliorated by reduced oxidative stress is selected from Parkinson's disease, Friedreich's Ataxia, Wilson's Disease, mtDNA diseases, diabetes, motor neurone disease, inflammation and ischaemic-reperfusion tissue injury in strokes, heart attacks, organ transplantation and surgery.

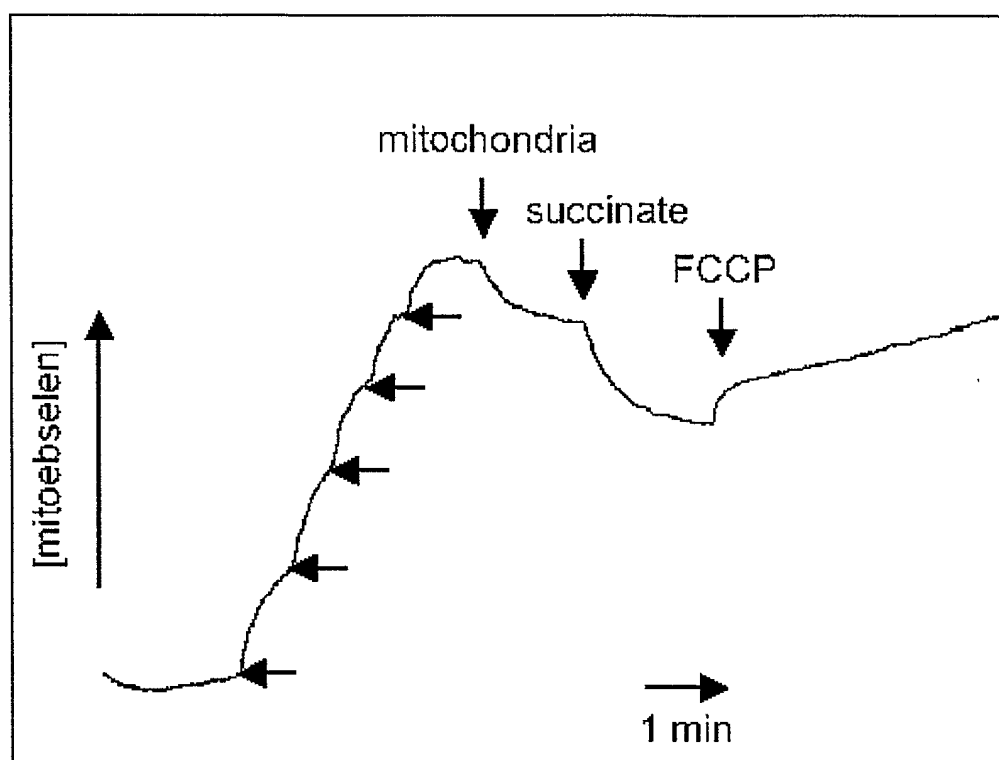


Figure 1

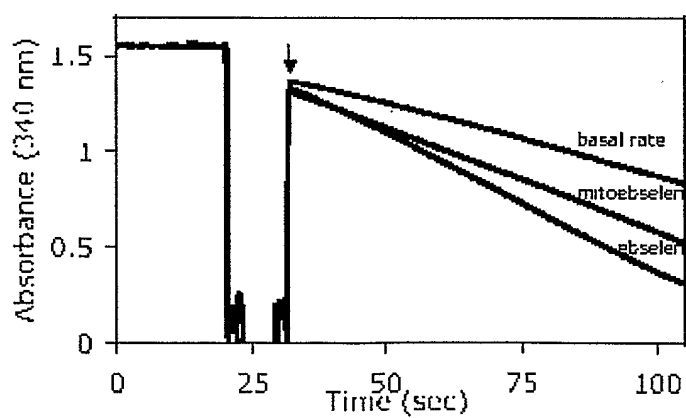


Figure 2a

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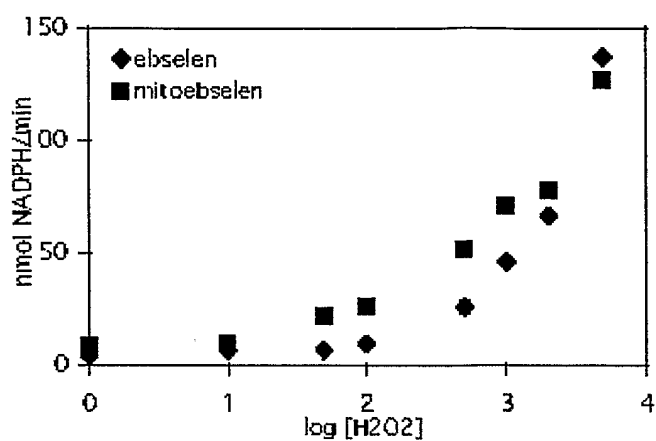


Figure 2b

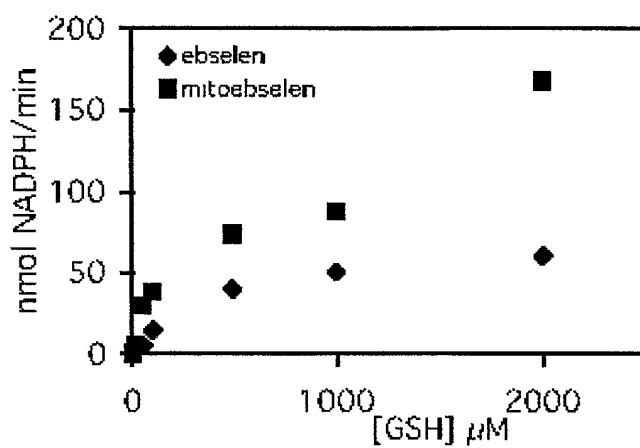


Figure 2c

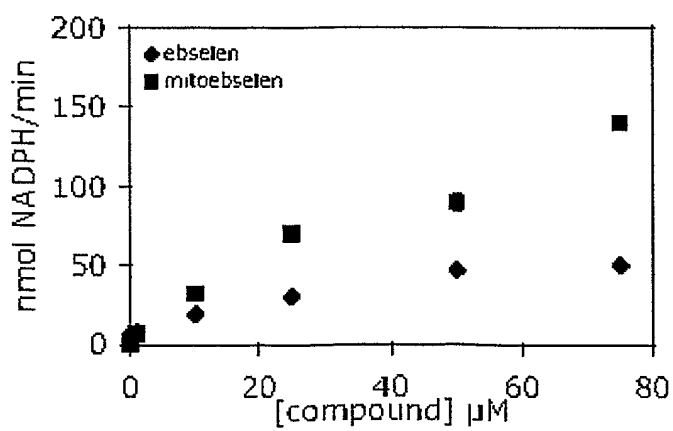


Figure 2d

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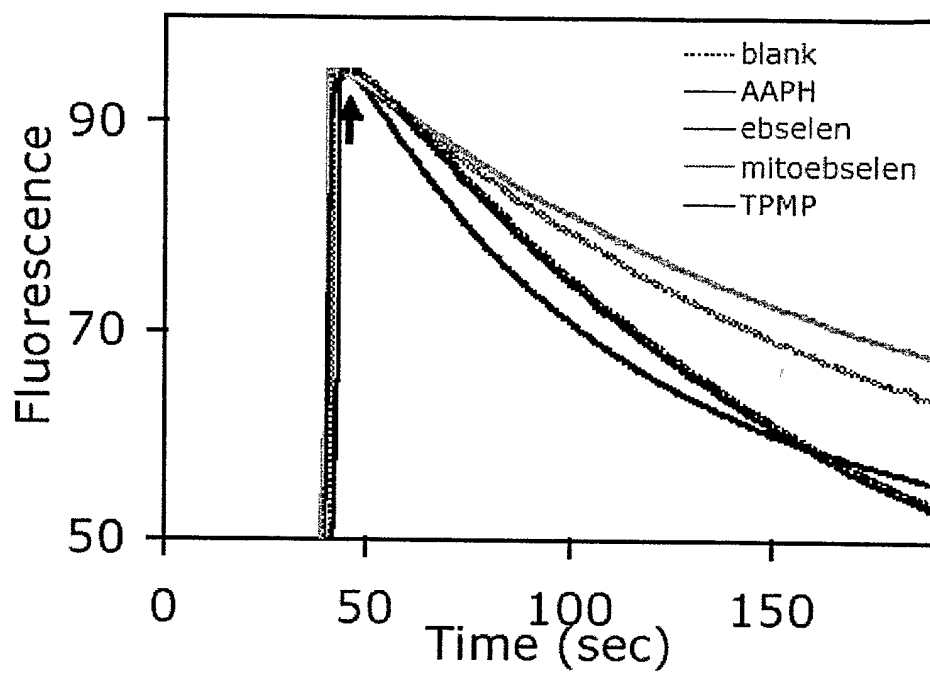


Figure 3a

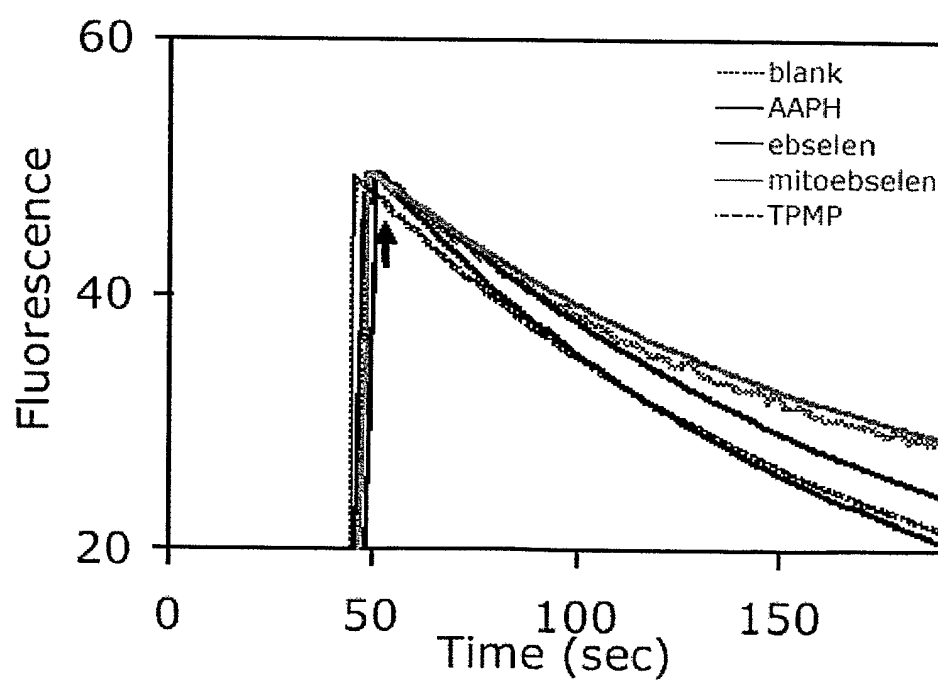


Figure 3b

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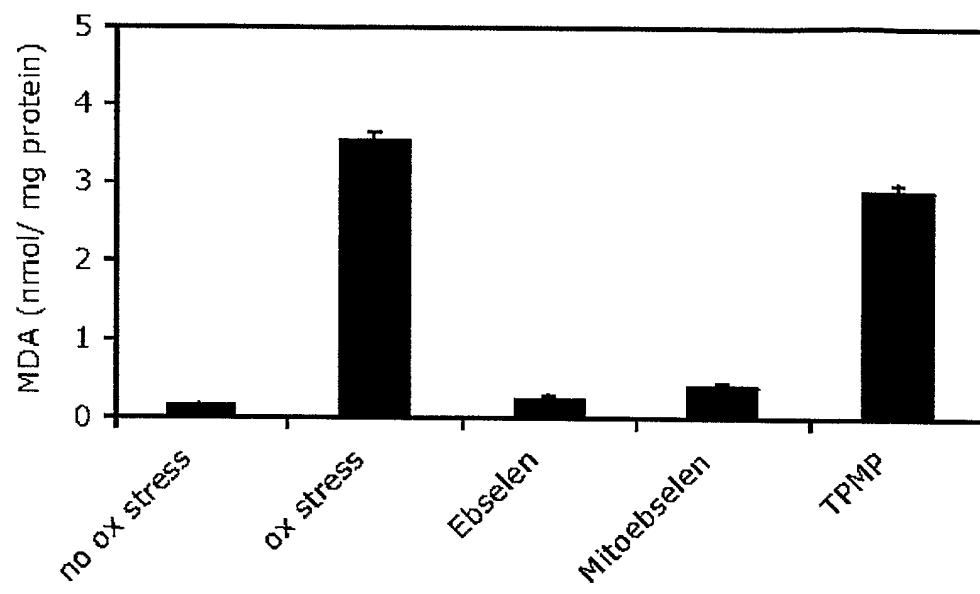


Figure 3c

INTERNATIONAL SEARCH REPORT

PCT/GB 03/03386

A. CLASSIFICATION OF SUBJECT MATTER IPC 7 C07F9/54 A61K31/66 C07D293/12		
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) IPC 7 C07D A61K		
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, CHEM ABS Data		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 99 26582 A (SMITH ROBIN A J ;UNIV OTAGO (NZ); MURPHY MICHAEL PATRICK (NZ)) 3 June 1999 (1999-06-03) page 2, line 9 -page 4, line 25 ---	1-16
Y	MUELLER A ET AL: "A NOVEL BIOLOGICALLY ACTIVE SELENO-ORGANIC COMPOUND-1. GLUTATHIONE PEROXIDASE-LIKE ACTIVITY IN VITRO AND ANTIOXIDANT CAPACITY OF PZ 51 (EBSELEN)" BIOCHEMICAL PHARMACOLOGY, PERGAMON, OXFORD, GB, vol. 33, no. 20, 15 October 1984 (1984-10-15), pages 3235-3239, XP000654591 ISSN: 0006-2952 the whole document --- -/--	1-16
☒ Further documents are listed in the continuation of box C.		
☒ Patent family members are listed in 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 10 October 2003		Date of mailing of the international search report 23/10/2003
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016		Authorized officer Usue11i, A

INTERNATIONAL SEARCH REPORT

PCT/GB 03/03386

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	NAMURA, S. ET AL: "Ebselen Reduces Cytochrome c Release From Mitochondria and Subsequent DNA Fragmentation After Transient Focal Cerebral Ischemia in Mice" STROKE, vol. 32, no. 8, 2001, pages 1906-1911, XP002257319 the whole document	1-16
P,X	WO 03 010154 A (KIM EON-KYEOM ;KOH JAE-YOUNG (KR); WIE MYUNG-BOK (KR); KIM WON-YEO) 6 February 2003 (2003-02-06) claim 1; examples 1-4	4

INTERNATIONAL SEARCH REPORT

PCT/GB 03/03386

Box I Observations where certain claims were found unsearchable (Continuation of item 1 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(part)-3(part), 5(part)-10(part), 13(part)-16(part)
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 II Observations where unity of invention is lacking (Continuation of item 2 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 an additional fee, this Authority did not invite payment of any additional fee.
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.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 1(part)-3(part),5(part)-10(part),13(part)-16(part)

Present claims relate to compounds consisting of different moieties which have been defined by reference to functional desirable characteristics.

Said moieties are:

- a lipophilic cation group
- a linking group
- an antioxidant group which is a glutathione peroxidase mimetic.

Due to the broad definitions of said moieties, the claims cover a huge number of possible products, whereas the application provides support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT for only a very limited number of such products. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible.

Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). An attempt is made to define the products by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible. Consequently, the search has been carried out for those parts of the claims which appear to be clear, supported and disclosed, namely those parts relating to the products of claims 4,11 and 12.

The applicant's attention is drawn to the fact that claims, or parts of 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.

INTERNATIONAL SEARCH REPORT

PCT/GB 03/03386

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9926582	A	03-06-1999	AU 1696499 A 15-06-1999
			AU 763179 B2 17-07-2003
			AU 1696599 A 15-06-1999
			CA 2311318 A1 03-06-1999
			CN 1282334 T 31-01-2001
			EP 1047701 A1 02-11-2000
			JP 2001524483 T 04-12-2001
			WO 9926582 A2 03-06-1999
			WO 9926954 A1 03-06-1999
			NZ 505352 A 20-12-2002
			US 2003069208 A1 10-04-2003
			US 2002052342 A1 02-05-2002
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