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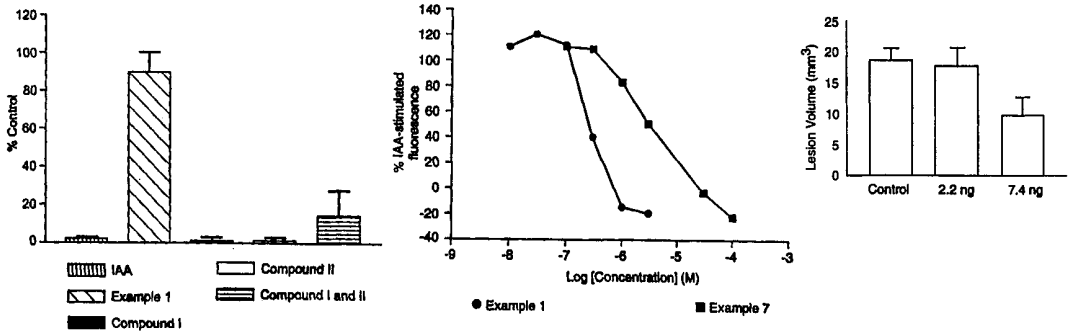

(57) Abstract

A compound of formula (1): wherein A is (AI) or (AII) wherein R^1, R^2 and R^3 are independently selected from H and alkyl; wherein X is O, S, NR^4 or a direct bond, wherein R^4 is H or alkyl; wherein Z is a saturated hydrocarbyl chain comprising from 1 to 10 carbon atoms; wherein q is 1, 2 or 3, wherein if q is 2 or 3, then each A can be the same or different; wherein the or each R^5 is independently selected from H or alkyl; wherein the or each R^6 is independently selected from alkyl; wherein n is 1 to 5; wherein p is 0 to 4; and wherein the sum of n and p is less than 6, or a pharmaceutically acceptable salt thereof, and the therapeutic use thereof, in particular for the treatment of a condition resulting in oxidative stress.

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ORTHO-HYDROXYPYRIDINONE DERIVATIVES AS IRON CHELATING AND ANTIOXIDANT AGENTS

The present invention relates to compounds containing both ortho-hydroxypyridinone and oxygenated aryl functionalities, which possess the dual ability to chelate iron and scavenge reactive oxygen species (ROS). In particular, the invention relates to specific compounds containing a 3-hydroxy-4(1*H*)-pyridinone or a 3-hydroxy-2(1*H*)-pyridinone iron chelating moiety as well as a phenolic antioxidant moiety. The present invention also relates to the synthesis of such compounds, to pharmaceutical preparations comprising such compounds and to the use of such compounds in the treatment and prophylaxis of conditions resulting in oxidative stress, particularly oxidative damage to the central nervous system.

One particularly relevant example of a condition involving oxidative damage to the central nervous system, which can be treated with compounds of the present invention, is stroke. Stroke is the third leading cause of death in major industrialised countries and the commonest cause of permanent disability (Hunter *et al.*, Trends in Pharmacological Sciences, 1995, 16, 123-128). Each year, in the US and Europe, approximately 1 million people suffer acute stroke (Dorman *et al.*, CNS Drugs, 1996, 5, 457-474). Between 25% and 35% of these patients die within the first three weeks, and of the survivors 25% to 50% will be totally dependant on family or institutional care for the rest of their lives. The incidence of stroke increases with age, roughly doubling with each passing decade, with 30% of persons aged over 65 years being affected (Babikian *et al.*, Cerebrovascular disease in the elderly. In Clinical Neurology of Aging, Eds Albert M.L. and Knoefel J.E., OUP, New York, 1994). These statistics translate into an annual incidence of 0.1 to 0.2% in the US and Europe, with the world-wide market for stroke estimated to be worth $3 billion in 1995 and projected to rise to $10 billion in 2005.

Stroke is defined as an interruption of the blood flow to the brain or leakage of blood out of the brain, resulting in oxygen deprivation (ischaemia) and subsequent neuronal cell death. Strokes can be divided into two classes, ischaemic and haemorragic. The former accounts for approximately 83% of all strokes and is caused by thrombosis (65%) and/or detachment of a previously formed clot (embolus, 18%). Haemorrhagic strokes, which account for the remaining 17% of all strokes, can be subdivided into subarachnoid

haemorrhage (7%) and cerebral haemorrhage (10%).

Markers of oxidative damage have been detected in the brains of ischaemic animals, and a variety of antioxidant molecules have been demonstrated to be neuroprotective in ischaemic stroke models. This provides conclusive evidence that cerebral ischaemia leads to the production of reactive free radicals. Defined as a chemical species containing one or more unpaired electrons, and capable of independent existence, free radicals are highly destructive towards cellular membrane lipids, DNA and proteins. This "oxidative-modification" of cellular components ultimately leads to a loss of cell function. One example of this oxidative process is lipid peroxidation (LP), a process which increases membrane fluidity leading to failure of normal membrane potential, disturbance of calcium homeostasis and transmembrane signalling, with the ultimate result of neuronal cell death.

A combination of the brains high-energy demand and subsequent high rate of oxygen consumption, with its limited endogenous antioxidant defences (superoxide dismutase (SOD), glutathione peroxidase (GSPx), and catalase) makes the brain very susceptible to free radical attack. Additionally, neuronal cell membranes, being rich in polyunsaturated fatty acids, are especially vulnerable to oxidative modification. The brains vulnerability to free radical attack is further exacerbated by relatively high levels of iron in the brain. Iron is the fundamental catalyst in the production of the hydroxyl radical ($^{\cdot}OH$) (Fenton and Haber-Weiss Reactions), reportedly the most destructive of all free radicals (Palmer, C., Metals and Oxidative Damage in Neurological Disorders, Ed. Connor, Plenum Press, New York, 1997, pp 205-236).

Exposure to free radicals is a natural consequence of aerobic respiration, to which the human body possesses a variety of natural antioxidant mechanisms. However, during pathological conditions such as stroke homeostatic mechanisms break down, and the balance between the generation of free radicals and natural antioxidant defences is shifted, resulting in a state of oxidative stress (Beal M.F., Ann. Neurol., 1995, 31, 119-130; Gerlach *et al.*, J. Neurochem., 1994, 63, 793-807).

In animal models of stroke, supplementation of antioxidant defences with exogenous

antioxidant molecules has resulted in neuroprotection, as assessed both histologically and behaviourally (Hall E.H., Metals and Oxidative Damage in Neurological Disorders, *supra*, pp 325-339). Furthermore, transgenic animals overexpressing SOD have been demonstrated to be more resistant to cerebral ischaemia than their wild type littermates (Chan *et al.*, Ann. N.Y. Acad. Sci., 1994, 738, 93-103).

The iron chelator deferiprone (1,2-dimethyl-3-hydroxy-4(1*H*)-pyridinone) has also been shown to possess antioxidant properties. For example the use of deferiprone to inhibit free radical formation has been disclosed by Kontochiorghes *et al.* (Free Rad. Res. Comms., 1986, 2, 115-124) and by Mostert *et al.* (Free Rad. Res. Comms., 1987, 3, 379-388). The use of deferiprone in conjunction with an antioxidant is also disclosed in WO 94/03169 for use in the treatment of sickle cell disease, and by Antonius *et al.* (Circulation, 1989, 80, 158-164) for use in the prevention of postischemic cardiac injury. Deferiprone has recently been in clinical trials as a potential treatment for iron overload in thalassemia major, and has also been disclosed for the treatment of parasitic infections, anemia and Alzheimer's disease.

There are many cellular systems known to be inappropriately activated or regulated as a result of oxygen starvation to the brain (e.g. glutamate receptors, voltage dependent ion channels). A major consequence of this is a loss of calcium homeostasis and inappropriate enzyme activation via several routes. Generation of free radicals is a common biochemical end point to many of the processes that are inappropriately regulated following cerebral ischaemia (Dorman *et al.*, *supra*, Hall E.H. *supra*; Patt *et al.*, J. Pediatric Surg., 1990, 25, 224-227). Hence intervention "down stream" with an antioxidant molecule at a point where many of these processes converge, is considered to be strategically sound owing to a universal applicability to many intracellular processes.

Based on the above rationale, a low molecular weight molecule designed to simultaneously trap radicals and chelate iron is a novel, scientifically relevant approach towards the treatment of conditions involving oxidative stress, in particular cerebral ischaemia/stroke.

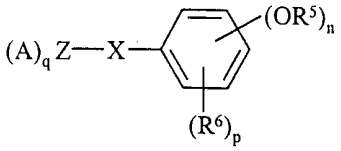
There have been three related reports describing molecular entities with dual iron chelating and anti-oxidant capabilities. The first report, Sato *et al* (Bioconjugate Chem., 1995, 6, 249-54), describes Cu,Zn-superoxide dismutase and desferrioxamine conjugated via polyoxyethylene. This high molecular weight conjugate was not used to show protection against oxidative stress *in vitro*, nor was it investigated for its effectiveness in *in vivo* models of oxidative stress. The second report, Rojanasakul *et al* (Biochim. Biophys. Acta, 1996, 1315(1), 21-8), describes a transferrin-catalase conjugate which gave increased protection to cells from oxidative stress as a result of its increased uptake in to cells *via* the transferrin receptor. The paper suggests the potential therapeutic use of the "...conjugate for the treatment of pathological processes in the lung". This high molecular weight conjugate was not investigated for its effectiveness in *in vivo* models of oxidative stress. The third report, Tilbrook *et al* (WO 9825905), claims compounds containing iron chelator units linked to a group containing reducing –SH groups for the treatment of Alzheimer's disease and related neurodegenerative diseases. The compounds were not investigated for their effectiveness in *in vivo* models of oxidative stress.

Currently there are only two recognised forms of treatment available for stroke victims. The first, Altepase® (recombinant tissue plasminogen activator, rTPA) is a clot busting drug only suitable for cerebral thrombosis. Therapeutic thrombolysis can, however, be complicated by a) systemic haemorrhage, b) intracerebral haemorrhage, c) distal embolism of a partially digested clot leading to secondary infarction and d) cerebral oedema secondary to reperfusion injury. It is, therefore, necessary to exclude the possibility of haemorrhagic stroke by computerised tomographic (CT) scanning of patients before administering Alteplase. The second recognised treatment, carotid endarterectomy is a surgical procedure for unblocking the carotid artery. However, both treatments have the potential to exacerbate and complicate the original injury, and neither treatment is neuroprotective nor universal for all types of stroke. A third treatment, the antioxidant idebenone is licensed for the treatment of stroke in Japan. There is therefore a large unmet medical need for an effective neuroprotective compound for the treatment of stroke.

It is an object of this invention to provide compounds which, unlike the current therapies

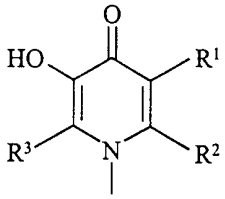
used for the treatment of stroke, protect against damage due to reperfusion injury, and are neuroprotective. Such compounds are potentially useful for all types of stroke. It is a further object of this invention to provide compounds which may be used in the treatment of oxidative stress generally and particularly oxidative damage to the central nervous system.

According to the present invention there is provided a compound of the formula (1):



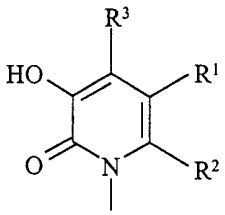
(1)

wherein A is



(AI)

or



(AII)

wherein R^1, R^2 and R^3 are independently selected from H and alkyl;

wherein X is O, S, NR^4 or a direct bond, wherein R^4 is H or alkyl;

wherein Z is a saturated hydrocarbyl chain comprising from 1 to 10 carbon atoms;

wherein q is 1, 2 or 3, wherein if q is 2 or 3, then each A can be the same or different;

wherein the or each R^5 is independently selected from H or alkyl;

wherein the or each R^6 is independently selected from alkyl;

wherein n is 1 to 5;

wherein p is 0 to 4; and

wherein the sum of n and p is less than 6,

or a pharmaceutically acceptable salt thereof.

It has been found that the compounds of the present invention which have a combined antioxidant and iron-chelating activity can be used for treating oxidative stress, particularly oxidative damage to the central nervous system.

It has further been found that the compounds of the present invention are surprisingly more effective in vitro, especially at low concentrations, than the simultaneous use of the separate hydroxypyridone iron-chelating compound and the phenolic antioxidant compound.

As used herein, the term "alkyl" means a branched or unbranched, cyclic or acyclic, saturated or unsaturated (e.g. alkenyl or alkynyl) hydrocarbyl radical. Where cyclic, the alkyl group is preferably C_3 to C_{12}, more preferably C_5 to C_{10}, more preferably C_5 to C_7. Where acyclic, the alkyl group is preferably C_1 to C_{10}, more preferably C_1 to C_6, more preferably methyl, ethyl, propyl, isopropyl, butyl, t-butyl, pentyl and iso-pentyl.

The alkyl groups may be substituted or unsubstituted. Where substituted, there will generally be 1 to 3 substituents present. Substituents may include carbon containing groups such as:

alkyl,

aryl,

arylalkyl (e.g. substituted and unsubstituted phenyl, substituted and unsubstituted benzyl);

halogen atoms and halogen containing groups such as

haloalkyl (e.g. trifluoromethyl);

oxygen containing groups such as

alcohols (e.g. hydroxy, hydroxyalkyl, aryl(hydroxy)alkyl),

ethers (e.g. alkoxy, alkoxyalkyl, poly(alkoxyalkyl), aryloxyalkyl),

ketones (e.g. alkylcarbonyl, alkylcarbonylalkyl,

arylcarbonyl, arylalkylcarbonyl, arylcarbonylalkyl)

acids (e.g. carboxy, carboxyalkyl),

acid derivatives such as esters

(e.g. alkoxycarbonyl, alkoxycarbonylalkyl, alkycarbonyloxy, alkycarbonyloxyalkyl)

and amides

(e.g. aminocarbonyl, mono- or dialkylaminocarbonyl, aminocarbonylalkyl, mono- or dialkylaminocarbonylalkyl, arylaminocarbonyl);

and nitrogen containing groups such as

amines (e.g. amino, mono- or dialkylamino, aminoalkyl, mono- or dialkylaminoalkyl).

As used herein, the term "alkoxy" means alkyl-O-. Alkoxy substituent groups or alkoxy-containing substituent groups may be substituted by one or more alkyl groups.

As used herein, the term "halogen" means a fluorine, chlorine, bromine or iodine radical, preferably a fluorine or chlorine radical.

In a preferred embodiment, the alkyl groups are either unsubstituted or substituted by substitution of a hydrogen atom with a group selected from OR^7, $OCOR^7$, $COOR^7$, NHR^7, $NHCOR^7$ and $CONHR^7$ wherein R^7 is H or alkyl.

As used herein, the term "pharmaceutically acceptable salt means any pharmaceutically acceptable salt of the compound of formula (1). Salts may be prepared from pharmaceutically acceptable non-toxic acids and bases including inorganic and organic acids and bases. Such acids include acetic, benzenesulfonic, benzoic, camphorsulfonic, citric, ethenesulfonic, dichloroacetic, fumaric, gluconic, glutamic, hippuric, hydrobromic, hydrochloric, isethionic, lactic, maleic, malic, mandelic, methanesulfonic, mucic, nitric, pamoic, pantothenic, phosphoric, succinic, sulfuric, tartaric, oxalic, p-toluenesulfonic and the like. Particularly preferred are hydrochloric, hydrobromic, phosphoric, sulfuric and methanesulfonic acids, and most particularly preferred is the methanesulfonate salt. Acceptable base salts include alkali metal (e.g. sodium, potassium), alkaline earth metal

(e.g. calcium, magnesium) and aluminium salts.

According to the present invention, the or each A may independently be attached to the chain Z at any carbon atom of the chain.

According to the present invention, when A is AI, preferably R^2 and R^3 are independently selected from H, unsubstituted alkyl, CH_2OR^7, CH_2OCOR^7, $COOR^7$, CH_2NHR^7, CH_2NHCOR^7 and $CONHR^7$ wherein R^7 is H or alkyl. Preferably, R^1 is selected from H and unsubstituted alkyl. More preferably, R^1, R^2 and R^3 are independently selected from H and unsubstituted alkyl. More preferably, R^1 and R^2 are H and R^3 is unsubstituted alkyl. It is further preferred that R^3 is methyl.

According to the present invention, when A is AII, preferably R^1, R^2 and R^3 are independently selected from H, unsubstituted alkyl, CH_2OR^7, CH_2OCOR^7, $COOR^7$, CH_2NHR^7, CH_2NHCOR^7 and $CONHR^7$ wherein R^7 is H or alkyl. Preferably, R^1, R^2 and R^3 are independently selected from H and unsubstituted alkyl. More preferably, R^1, R^2 and R^3 are H.

Preferably, the present invention provides compounds wherein A is AI.

Preferably, the present invention provides compounds wherein q=1.

Preferably, the present invention provides compounds wherein Z is $(CH_2)_m$ wherein m is 1 to 10.

In a preferred embodiment, the present invention provides compounds wherein Z is a hydrocarbyl chain having from 2 to 10 carbon atoms, preferably from 2 to 6 carbon atoms, more preferably 2, 3 or 4 carbon atoms.

In an alternative preferred embodiment, the present invention provides compounds wherein Z is a hydrocarbyl chain having from 1 to 6 carbon atoms, preferably from 2 to 6 carbon atoms, more preferably 2, 3 or 4 carbon atoms.

According to the present invention, the saturated hydrocarbyl chain Z may be branched or unbranched, optionally substituted by one or more alkyl groups, and may be cyclic.

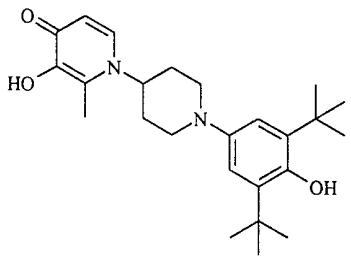
As used herein to describe the hydrocarbyl chain Z, the term "cyclic" means either that Z may comprise a cyclic hydrocarbyl group of from 3 to 10 carbon atoms, preferably 5, 6 or 7 carbon atoms; or that a cyclic group is present as a result of cyclisation of R^5 or R^6 onto Z; or that, where X is NR^4 and R^4 is alkyl, a cyclic group is present as a result of cyclisation of R^4 onto Z. It is preferred that a cyclic group formed as a result of cyclisation of R^4, R^5 or R^6 onto Z is a 5, 6 or 7-membered ring.

Preferably Z is an unbranched hydrocarbyl chain.

Preferably the present invention provides compounds wherein X is O, S or a direct bond, more preferably X is O or a direct bond.

According to the present invention, where X is NR^4, R^4 is preferably alkyl.

In an embodiment of the present invention, when X is NR^4 and R^4 is alkyl, R^4 may be cyclized onto the chain defined as Z. An example of a compound of formula (1) where R^4 is cyclized onto the chain defined as Z is:

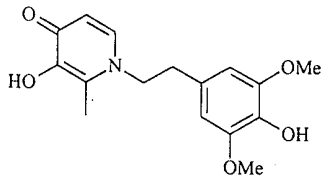


According to the present invention, R^5 is preferably selected from H and C_{1-10} alkyl. Where R^5 is C_{1-10} alkyl, R^5 is preferably methyl. Most preferably, R^5 is H. It is preferred that at least one R^5 is H.

Preferably, the present invention provides compounds wherein n is 1 to 3, more preferably n is 1 or 2.

According to the present invention, when R^5 is H, preferably OR^5 is positioned in the ortho or para position in the ring with respect to X. More preferably OR^5 is positioned in the para position with respect to X.

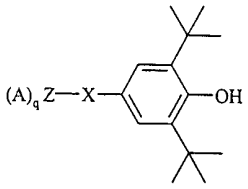
According to the present invention, when n is greater than 1, it is preferred that the OR^5 groups are positioned ortho to each other to give, for example, a compound of formula:



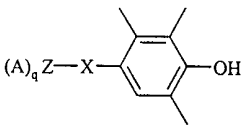
According to the present invention, the or each R^6 is preferably independently selected from C_{1-10} alkyl, preferably C_{1-4} alkyl, most preferably methyl, isopropyl or t-butyl.

Preferably the present invention provides compounds wherein p is 2, 3 or 4.

According to the present invention, when p is 1 or 2, alkyl groups represented by R^6 is/are preferably positioned ortho to OR^5, preferably in the meta-position of the ring with respect to X to give, for example, a compound of formula:-

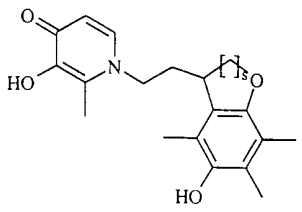


According to the present invention, when p is 3, alkyl groups represented by R^6 are preferably positioned to give, for example where three R^6 are methyl, a compound of formula:-



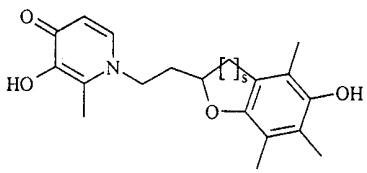
In an embodiment of the present invention, R^5 or R^6 can be cyclized on to the chain defined as Z to form a ring. An example of a compound of formula (1) where R^5 is cyclized on to

the chain defined as Z and where X is a direct bond is:



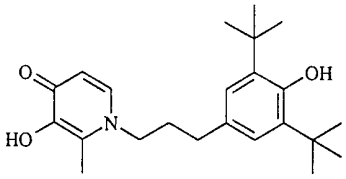
wherein s is 1 or 2.

An example of a compound of formula (I) where R^6 is cyclized onto the chain defined as Z and where X = O is:



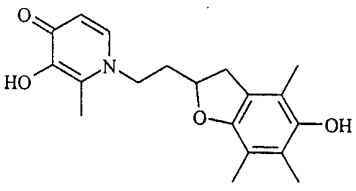
wherein s is 1 or 2.

In a preferred embodiment, in the compound of formula (1), A is AI, R^1 and R^2 are H, R^3 is methyl, m is 2 or 3, X is a direct bond, R^5 is H, R^6 is t-butyl, n is 1 and p is 2. In a more preferred embodiment, the compound of formula (1) is 1-(3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)propyl)-3-hydroxy-2-methyl-4(1*H*)-pyridinone (2a):



(2a)

In a further preferred embodiment, in the compound of formula (1), A is AI, R^1 and R^2 are H, R^3 is methyl, m is 3, X is O, R^5 is H, R^6 is methyl, n is 2 and p is 4. In a more preferred embodiment, the compound of formula (1) is 1-(2-(2,3-dihydro-5-hydroxy-4,6,7-trimethylbenzofuran-2-yl)ethyl)-3-hydroxy-2-methyl-4(1*H*)-pyridinone (2b):

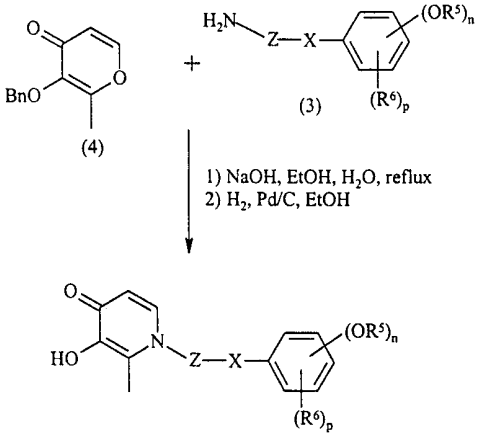


(2b)

According to a further aspect of the present invention there is provided a method of preparing the compounds of the present invention. The compounds of the present invention may be prepared using standard synthetic chemistry.

A general method for the synthesis of compounds where q=1 and A is a 3-hydroxy-4(1*H*)-pyridinone moiety comprises condensation of the primary amine of the respective antioxidant unit (3) with 3-benzyloxy-2-methyl-4-pyrone (4), followed by removal of the benzyl protecting group, as illustrated in Reaction Scheme 1. (For the condensation of (4) with simple primary amines, see Dobbin *et al.*, J. Med. Chem., 1993, 36, 2448-2458).

Reaction Scheme 1

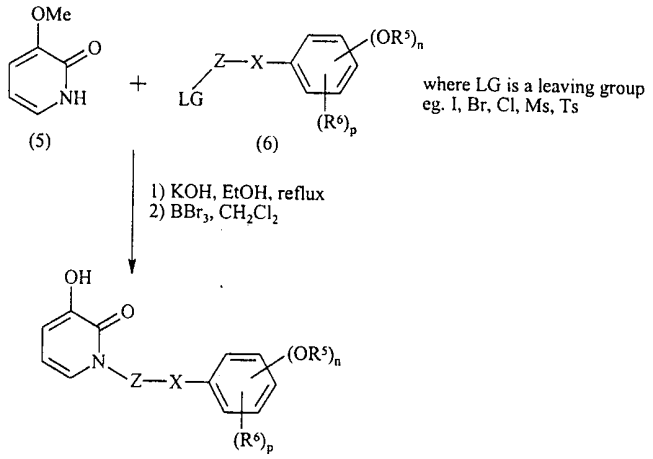


The primary amines, of the respective antioxidants, (3) can be prepared using standard synthetic chemistry. The -OH of the antioxidant unit can be optionally protected during synthetic manipulation (for example, as a benzyl ether). Deprotection to reveal the –OH of the antioxidant unit can be carried out simultaneously with removal of the benzyl protecting group of the hydroxy pyridone unit, in the last step of the sequence.

A general method for the synthesis of compounds where q=1 and A is a 3-hydroxy-2(1*H*)-pyridinone moiety comprises N-alkylation of 3-methoxy-2(1*H*)-pyridinone (5) with the halide of the respective antioxidant unit (6) (or an alternative electrophilic alkylating derivative of the antioxidant unit, for example, mesylate or tosylate), followed by removal of the methyl protecting group as illustrated in Reaction Scheme 2. (For the alkylation of

(5) with simple alkyl iodides, see Ellis *et al.*, J. Med. Chem., 1996, 39, 3659-3670).

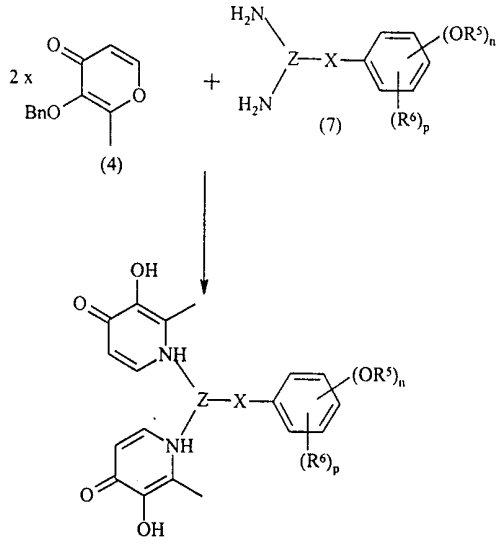
Reaction Scheme 2



The primary halides, (mesylates and tosylates) of the antioxidants, (6) can be prepared using standard synthetic chemistry. The -OH of the antioxidant unit can be optionally protected during synthetic manipulation (for example, as a benzyl ether). Deprotection to reveal the –OH of the antioxidant unit can be carried out simultaneously with removal of the methyl protecting group of the hydroxy pyridone unit, in the last step of the sequence.

Compounds of the present invention which contain more than one hydroxy-pyridone Fe-chelating unit (where q = 2 or 3) may be prepared via condensation of the bis-primary amine of the respective antioxidant unit (7) with two equivalents of 3-benzyloxy-2-methyl-4-pyrone (4) (in the presence of base, for example NaOH in a MeOH:water solution), followed by removal of the benzyl protecting groups (with for example H_2 and Pd/C), as illustrated in Reaction Scheme 3. (For the reaction of (4) with 1, 6-diaminohexane see Orvig *et al.*, Can. J. Chem., 1988, 66, 123-131).

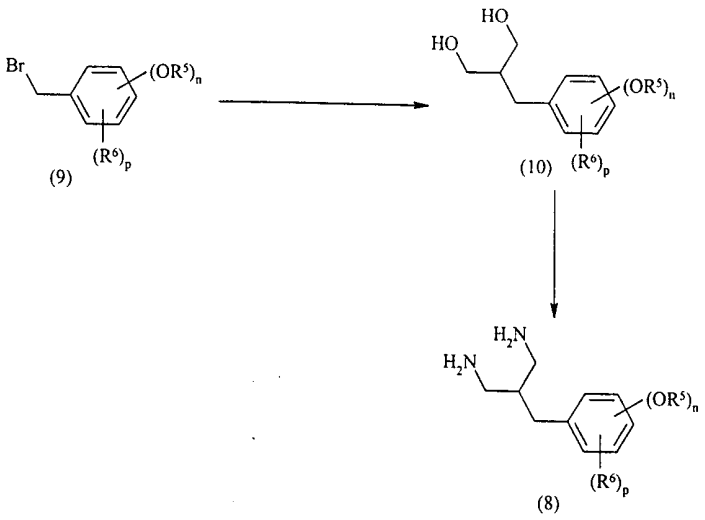
Reaction Scheme 3



The bis-primary amine (7), may be prepared using standard synthetic chemistry.

Reaction Scheme 4 illustrates a method of preparation of bis-primary amine (8). Such a compound is an intermediate in the synthesis of a compound of the type exemplified as Example 11 herein. Thus, the bis-primary amine (8) may be prepared from bromide (9) via reaction with dimethylmalonate (in the presence of a suitable base, for example NaH) to produce the dimethylester, followed by reduction (using for example $BH_3.Me_2S$) to produce the diol (10). Diol (10) may then be converted to the dimesylate (using methanesulphonyl chloride and a suitable base, for example triethylamine), reacted with sodium azide, and reduced (using for example H_2 and Pd/C) to produce the bis-primary amine (8) (Palmer *et al.* J. Med Chem., 1990, 33, 3008-3014).

Reaction Scheme 4



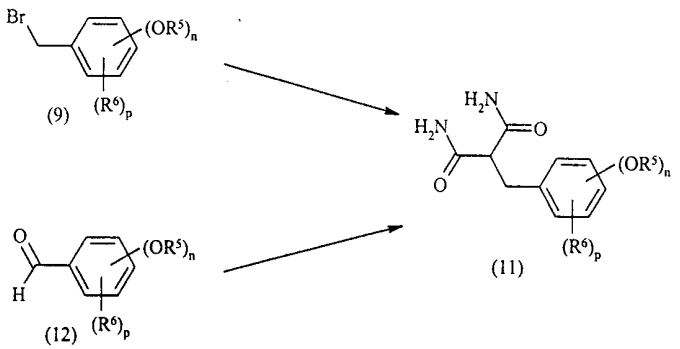
Alternatively the bis-primary amine (8), may be prepared from the bis-diamide (11) via reduction (with for example lithium aluminium hydride, or borane), as illustrated in Reaction Scheme 5.

Reaction Scheme 5

The bis-amide (11) may be prepared using standard synthetic chemistry procedures. For example, the bis-diamide (11) may be prepared from the benzyl bromide (9); via reaction with malonamide in the presence of a suitable base (for example NaOH in liquid ammonia) (Asami *et al* Sci. Rep. Res. Inst., 1957, 335-337); or alternatively via reaction with diethyl malonate in the presence of a suitable base (for example NaH in DMF) to produce the diester, followed by di-amidation (using for example ammonia). Alternatively, bis-diamide (11) may be prepared from benzaldehyde (12), via reaction with diethylmalonate in the presence of a suitable base (for example piperidine in EtOH) to produce the diester olefin, followed by reduction of the olefin (using for example H_2 and Pd/C), and finally di-amidation (using for example ammonia in EtOH) (Sekiya *et al.* Chem. Pharm. Bull., 1964, 12, 674-677); or via reaction with malononitrile in the presence of a

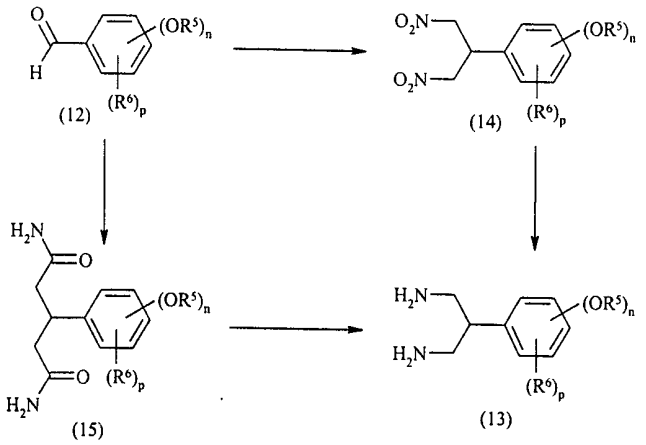
suitable base (for example piperidine in EtOH) to produce the dinitrile olefin (Gazit *et al*, J. Med. Chem., 1989, 32, 2344; Katsumi *et al*, Chem. Pharm. Bull., 1986, 34, 1619-1627), followed by reduction of the olefin (with for example, formic acid, Nanjo, Chem. Pharm. Bull., 1977, 25, 2396; or triethyltin hydride, Sommer *et al*, Justus Liebigs Ann. Chem., 1968, 11-23) and finally hydrolysis (using for example aqueous sulphuric acid). Reaction Scheme 6 illustrates these methods of preparation of the bis-diamide (11).

Reaction Scheme 6



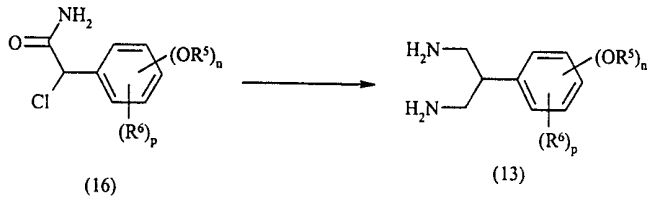
Reaction Scheme 7 illustrates a method of preparation of a bis-primary amine (13). Such a compound is an intermediate in the synthesis of a compound of the type exemplified as Example 12 herein. Thus, the bis-primary amine (13) may be prepared from aldehyde (12) via reaction with nitromethane in the presence of a suitable base (using for example catalytic butyl amine) to produce the dinitro compound (14) (Cassels *et al.* Rev. Latinoam. Quim. 1988, 19, 25-8), followed by reduction (with for example with lithium aluminium hydride, or H_2 and Pd/C, or H_2 and Raney-nickel). Alternatively, the bis-primary amine (13), may be prepared from aldehyde (12) via reaction with cyanoacetic acid in the presence of a suitable base (using for example pyridine and sodium acetate in toluene) to produce the dinitrile (Erion *et al.* J. Med. Chem., 1993, 36, 3771-3783), followed by hydrolysis (using for example aqueous sulphuric acid) to produce the bis-amide (15), and finally Hofmann rearrangement (using for example NaOH and bromine) to produce the bis-primary amine (13) (Weinhardt *et al.* J. Med Chem., 1985, 28, 694-698).

Reaction Scheme 7



As a further alternative method, bis-primary amine (13) may be prepared from chloroamide (16) via reaction with sodium cyanide (in a suitable solvent such as DMF), followed by reduction (using for example lithium aluminium hydride) (Jahn *et al*, Can. J. Chem., 1988, 66, 123-131), as illustrated in Reaction Scheme 8.

Reaction Scheme 8



The compounds of the present invention may contain one or more asymmetric carbon atoms, so that the compounds exist in different stereoisomeric forms. The compounds can be, for example, racemates or optically active forms. The optically active forms can be obtained by resolution of the racemates or by asymmetric syntheses.

The compounds of the present invention may also be prepared in a prodrug form wherein some or all the free –OH groups of the preferred compounds are derivatised (for example, via an ester, amide or phosphate bond) with a suitable group (the group may contain, for example, an alkyl, aryl, phosphate, sugar, amine, glycol, sulfonate or acid function) which is suitably labile so as it will be removed / cleaved (eg. by hydrolysis) to

reveal the preferred compound sometime after administration or when exposed to the desired biological environment.

To further increase their efficacy, compounds of the present invention may also contain additional non-covalently linked components such as dextrans or cyclodextrins, which aid stability and dispersion, and decrease metabolism of the active ingredient.

According to a further aspect of the present invention there is provided a compound of the present invention for use in therapy.

According to a further aspect of the present invention there is provided use of a compound of the present invention in the manufacture of a medicament for the treatment of a condition resulting in oxidative stress, particularly oxidative damage of the central nervous system.

The term "treatment" as used herein includes prophylaxis.

Diseases, disorders and medical treatments/procedures resulting in oxidative stress that can be treated with the compounds of the present invention include: aging; acute intermittent porphyria; adriamycin-induced cardiomyopathy; AIDS dementia and HIV-1 induced neurotoxicity; Alzheimer's disease; atherosclerosis; cateract; cerebral ischaemia; cerebral palsy; cerebral tumour; chemotherapy-induced organ damage; cisplatin-induced nephrotoxicity; coronary artery bypass surgery; diabetic neuropathy; Down's syndrome; drowning; epilepsy and post-traumatic epilepsy; Friedrich's ataxia; frontotemporal dementia; glaucoma; glomerulopathy; haemochromatosis; haemodialysis; haemolysis; haemolytic uraemic syndrome (Weil's disease); haemorrhagic stroke; heart attack and reperfusion injury; Huntington's disease; Lewy body disease; intermittent claudication; ischaemic stroke; inflammatory bowel disease; macular degeneration; malaria; methanol-induced toxicity; meningitis (aseptic and tuberculous); motor neuron disease; multiple sclerosis; multiple system atrophy; myocardial ischaemia; neoplasia; Parkinson's disease; peri-natal asphyxia; Pick's disease; progressive supra-nuclear palsy; radiotherapy-induced organ damage; restenosis after angioplasty; retinopathy; senile dementia; schizophrenia; sepsis; septic shock; spongiform encephalopathies;

subharrachnoid haemorrage/cerebral vasospasm; subdural haematoma; surgical trauma, including neurosurgery; thalassemia; transient ischaemic attack (TIA); traumatic brain injury (TBI); traumatic spinal injury; transplantation; vascular dementia; viral meningitis; and viral encephalitis.

Additionally, compounds of the present invention may also be used to potentiate the effects of other treatments, for example to potentiate the neuroprotective effects of brain derived nerve growth factor.

The invention is particularly directed to conditions which induce oxidative damage of the central nervous system, including acute and chronic neurological disorders such as traumatic brain injury, spinal cord injury, cerebral ischaemia, stroke (ischaemic and haemorragic), subharrachnoid haemorrage/cerebral vasospasm, cerebral tumour, Alzheimer's disease, Huntington's disease, Parkinson's disease, Friedrich's ataxia, motor neuron disease and multiple sclerosis.

The invention further provides a method of treating a condition resulting in oxidative stress, particularly oxidative damage of the central nervous system, comprising administering to a patient in need of such treatment an effective dose of a compound of the present invention.

The invention further provides a pharmaceutical composition comprising a compound of the present invention in combination with a pharmaceutically acceptable carrier or excipient and a method of making such a composition comprising combining a compound of the present invention with a pharmaceutically acceptable carrier or excipient.

Compounds of the present invention may be administered in a form suitable for oral use, for example a tablet, capsule, granule powder, aqueous or oily solution, suspension or emulsion; for topical use including transmucosal and transdermal use, for example a cream, ointment, gel, aqueous or oil solution or suspension, salve, patch or plaster; for nasal use, for a example a snuff, nasal spray or nasal drops; for vaginal or rectal use, for example a suppository; for administration by inhalation, for example a finely divided powder or a liquid aerosol; for sub-lingual or buccal use, for example a tablet or capsule; or for

parenteral use (including intravenous, subcutaneous, intramuscular, intravascular, intrathecal or infusion), for example a sterile aqueous or oil solution or suspension. In general the above compositions may be prepared in a conventional manner using conventional excipients, using standard techniques well known to those skilled in the art of pharmacy. Preferably, the compound is administered orally for chronic disorders such as Alzheimer's and Parkinson's disease, and intravenously for acute disorders such as stroke and TBI.

For oral administration, the compounds of the invention will generally be provided in the form of tablets or capsules or as an aqueous solution or suspension.

Tablets for oral use may include the active ingredient mixed with pharmaceutically acceptable excipients such as inert diluents, disintegrating agents, binding agents, lubricating agents, sweetening agents, flavouring agents, colouring agents and preservatives. Suitable inert diluents include sodium and calcium carbonate, sodium and calcium phosphate, cyclodextrin, and lactose, while corn starch and alginic acid are suitable disintegrating agents. Binding agents may include starch and gelatin, while the lubricating agent, if present, will generally be magnesium stearate, stearic acid or talc. If desired, the tablets may be coated with a material such as glyceryl monostearate or glyceryl distearate, to delay absorption in the gastrointestinal tract.

Capsules for oral use include hard gelatin capsules in which the active ingredient is mixed with a solid diluent, and soft gelatin capsules wherein the active ingredient is mixed with water or an oil such as peanut oil, liquid paraffin or olive oil.

For intramuscular, intraperitoneal, subcutaneous and intravenous use, the compounds of the invention will generally be provided in sterile aqueous solutions or suspensions, buffered to an appropriate pH and isotonicity. Suitable aqueous vehicles include Ringer's solution and isotonic sodium chloride. Aqueous suspensions according to the invention may include suspending agents such as cellulose derivatives, lipids, sodium alginate, polyvinyl-pyrrolidone, cyclodextrins, gum tragacanth, polyethylene glycol, propylene glycol, N,N-dimethylacetamide, cremophors, polysorbates, liposomes and wetting agents such as lecithin. It has been found that 1-(3-(3,5-di-tert-butyl-4-hydroxyphenyl)propyl)-3-hydroxy-

2-methyl-4(1*H*)-pyridinone (2a) is particularly soluble in hydroxypropyl-β-cyclodextrin. Suitable preservatives for aqueous suspensions include ethyl and n-propyl *p*-hydroxybenzoate.

It will be appreciated that the dosage levels used may vary over quite a wide range depending upon the compound used, the severity of the symptoms exhibited by the patient and the patient's body weight.

The invention will now be described in detail with reference to the following examples and figures. It will be appreciated that the invention is described by way of example only and modification of detail may be made without departing from the scope of the invention.

Figure 1 shows the protective effect of a compound of the present invention on cerebellar granular cells exposed to IAA-induced oxidative damage.

Figure 2 shows the effect of compounds of the present invention on intracellular oxidation of dichlorodihydrofluorescin (DCFH) to dichlorofluorecin (DCF). IAA-stimulated fluorescence values are given as a function of concentration of the test compound.

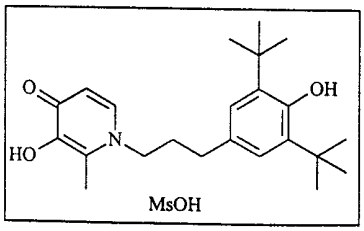
Figure 3 and Figure 4 show the *in vivo* activity of compounds of the present invention in the malonic acid lesion model of oxidative stress.

EXPERIMENTAL

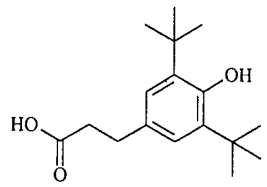
Synthetic Examples

Example 1

1-(3-(3,5-Di-*tert*-butyl-4-hydroxyphenyl)propyl)-3-hydroxy-2-methyl-4(1*H*)-pyridinone mesylate

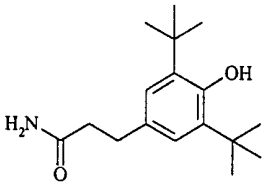


3-(3,5-Di-tert-butyl-4-hydroxyphenyl)propanoic acid



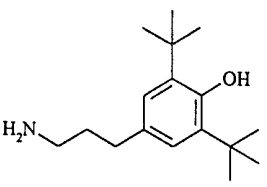
3,5-Di-*tert*-butyl-4-hydroxycinnamic acid (10 g, 36.2 mmol) in EtOH (250 ml) was hydrogenated over 10 % Pd/C (1.0 g) at 50 psi for 3 h. The solution was filtered and concentrated *in vacuo* to give the *product* (9.89 g, 98 %) essentially pure as an oil: IR υ_{max} (Nujol)/cm^{-1} 3628, 2698, 2615, 1706, 1234, 1217, 1143, and 876; NMR δ_H (400 MHz, $CDCl_3$) 1.43 (18H, s), 2.66 (2H, t, *J* 7.7Hz), 2.89 (2H, t, *J* 7.7Hz), 5.09 (1H, s), and 7.01 (2H, s).

3-(3,5-Di-tert-butyl-4-hydroxyphenyl)propanamide



Thionyl chloride (34 mL, 466 mmol) and DMF (ca. 1 mL) were added dropwise to 3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)propanoic acid (29.5 g, 106 mmol) in CH_2Cl_2 (175 mL) and toluene (350 mL) at room temperature, stirred for 2 h, concentrated *in vacuo*, taken up in THF (200 mL), treated with NH_4OH (350 mL) and stirred at 0°C for 15 min. The volatiles were removed *in vacuo*, the resulting aqueous residue was extracted with CH_2Cl_2, dried (Na_2SO_4) and concentrated *in vacuo* to give the *product* (28.6 g, 97 %), essentially pure as an oil: IR υ_{max} (Nujol)/cm^{-1} 3620, 3397, 3201, 1653, 1235, 1164, 1120 and 656; NMR δ_H (400 MHz, $CDCl_3$) 1.43 (18H, s), 2.51 (2H, t, *J* 7.7Hz), 2.89 (2H, t, *J* 7.7Hz), 5.09 (1H, s), 5.43 (1H, br s), 5.67 (1H, br s), and 7.01 (2H, s).

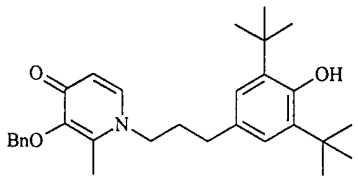
3-(3,5-Di-tert-butyl-4-hydroxyphenyl)propylamine



1.0-M $LiAlH_4$ in Et_2O (135 mL, 135 mmol) was added to 3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)propanamide in Et_2O (500 mL) at 0 °C over 5 min. After 2.5 h at room temperature, 1.0-M $LiAlH_4$ in Et_2O (40 mL, 40 mmol) was added, and the reaction refluxed for 5.5 h, then allowed to cool. Water (6.6 mL), 1.0-M NaOH (20 mL), water (6.6 mL), and Na_2SO_4 were added sequentially, and the mixture stirred at room temperature. The precipitate was removed by filtration, the filtrate concentrated *in vacuo* and purified by chromatography [SiO_2; CH_2Cl_2-MeOH (95:5)] to give contaminated fractions (8.5 g) plus pure *product* (9.1 g, 45 %) as a white solid: mp 110-113 °C; IR υ_{max} (Nujol)/cm^{-1} 3641, 3356, 3293, 1591, 1233, 1121, 1056 and 890; NMR δ_H (400 MHz, $CDCl_3$) 1.43 (18H, s), 1.81 (2H, pent, *J* 7.5 Hz), 2.57 (2H, t, *J* 7.5 Hz), 2.80 (2H, t, *J* 7.5 Hz), 3.0 (2H, br s), 5.0 (1H, br s), and 6.97 (2H, s); Anal. Calcd. for $C_{17}H_{29}NO{\cdot}0.5H_2O$: C, 74.95; H, 11.10; N, 5.14. Found: C, 74.85; H, 10.70; N, 4.63.

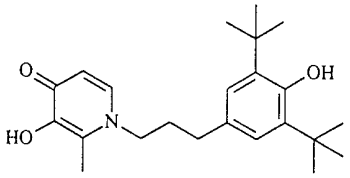
Preparation of the above compound is also described in US-3748347 the disclosure of which is incorporated herein by reference.

3-Benzyloxy-1-(3-(3,5-di-tert-butyl-4-hydroxyphenyl)propyl)-2-methyl-4(1H)-pyridinone



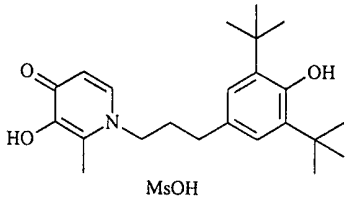
3-(3,5-Di-*tert*-butyl-4-hydroxyphenyl)propylamine (4.13 g, 15.7 mmol), 3-benzyloxy-2-methyl-4-pyrone [Harris, R. L. N., Aust. J. Chem., (1976), 29, 1329-1334] (3.39 g, 15.7 mmol) and 5.0-M NaOH (6.3 mL, 31.4 mmol) in EtOH (50 mL) and water (30 mL) were refluxed for 4.5 h. The reaction was cooled, adjusted to pH 3-4 with 1.0-M HCl, concentrated *in vacuo*, extracted with $CHCl_3$, dried ($MgSO_4$), concentrated *in vacuo* and purified by chromatography [SiO_2; CH_2Cl_2-MeOH (95:5)] to give contaminated fractions (1.1 g) plus pure *product* (2.9 g, 40 %) as light brown crystals: mp 50-55 °C; IR υ_{max} (Nujol)/cm^{-1} 3637, 3600-3100, 1626, 1565, 1250, 1218, 737 and 702; NMR δ_H (400 MHz, $CDCl_3$) 1.43 (18H, s), 1.94 (2H, pent, *J* 7.5 Hz), 2.01 (3H, s), 2.54 (2H, t, *J* 7.5 Hz), 3.76 (2H, t, *J* 7.5 Hz), 5.14 (1H, s), 5.22 (2H, s), 6.49 (1H, d, *J* 7.5 Hz), 6.92 (2H, s), 7.21 (2H, d, *J* 7.5 Hz) and 7.3-7.4 (5H, m); Anal. Calcd. for $C_{30}H_{39}NO_3{\cdot}0.5H_2O$: C, 76.56; H, 8.57; N, 2.98. Found: C, 76.31; H, 8.60; N, 2.39.

1-(3-(3,5-Di-tert-butyl-4-hydroxyphenyl)propyl)-3-hydroxy-2-methyl-4(1H)-pyridinone



3-Benzyloxy-1-(3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)propyl)-2-methyl-4(1*H*)-pyridinone (5.48 g, 11.9 mmol) and 10% Pd/C (2.5 g) in EtOH (280 mL) was stirred under a H_2 atmosphere for 22 h. The mixture was filtered through Celite®, concentrated *in vacuo*, and purified by chromatography [Sephadex® LH-20; CH_2Cl_2-MeOH (90:10)] to give the *product* (3.72 g, 84 %) as a buff solid: mp 231 °C; IR υ_{max} (Nujol)/cm^{-1} 3646, 3137, 1626, 1570, 1509, 1353, 1228, 1030 and 836; NMR δ_H (400 MHz, $CDCl_3$) 1.43 (18H, s), 2.04 (2H, pent, *J* 7.5Hz), 2.30 (3H, s), 2.60 (2H, t, *J* 7.5 Hz), 3.86 (2H, t, *J* 7.5 Hz), 5.04 (2H, br s), 6.39 (1H, d, *J* 7.0 Hz), 6.94 (2H, s), and 7.20 (1H, d, *J* 7.0 Hz); Anal. Calcd. for $C_{23}H_{33}NO_3$: C, 74.36; H, 8.95; N, 3.79. Found: C, 74.25; H, 9.01; N, 3.72.

1-(3-(3,5-Di-tert-butyl-4-hydroxyphenyl)propyl)-3-hydroxy-2-methyl-4(1H)-pyridinone mesylate

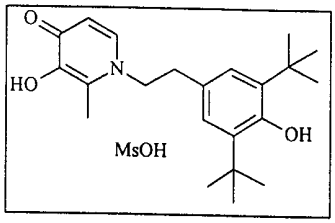


MsOH

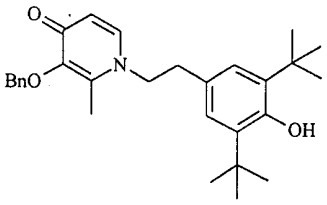
Methanesulphonic acid (175 μL, 2.7 mmol) was added dropwise to 1-(3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)propyl)-3-hydroxy-2-methyl-4(1*H*)-pyridinone (1.0 g, 2.7 mmol) in Et_2O (50 mL) and CH_2Cl_2 (50 mL). The mixture was stirred for 1.5 h, concentrated *in vacuo*, suspended in $CHCl_3$ and the solid collected by filtration to give the *title compound* (1.1 g, 87 %) as a white solid: mp 191 °C; IR υ_{max} (Nujol)/cm^{-1} 3573, 2551, 1628, 1341, 1210, 1146, 1111, 1040, 1023 and 772; NMR δ_H (400 MHz, DMSO-d_6) 1.36 (18H, s), 2.04 (2H, pent, *J* 7.5Hz), 2.32 (3H, s), 2.46 (3H, s), 2.56 (2H, t, *J* 7.5 Hz), 4.32 (2H, t, *J* 7.5 Hz), 6.75 (1H, br s), 6.91 (2H, s), 7.08 (1H, d, *J* 7.0 Hz), and 8.22 (1H, d, *J* 7.0 Hz); NMR δ_C (100 MHz, DMSO-d_6) 158.69, 152.39, 143.26, 141.65, 139.58, 138.47, 131.55, 124.51, 110.98, 56.05, 34.62, 32.08, 31.50, 30.56, 12.69; Anal. Calcd. for $C_{24}H_{37}NO_6S$: C, 61.65; H, 7.97; N, 2.99; S, 6.86. Found: C, 61.04; H, 7.95; N, 2.90; S, 6.89.

Example 2

1-(2-(3,5-Di-tert-butyl-4-hydroxyphenyl)ethyl)-3-hydroxy-2-methyl-4(1H)-pyridinone mesylate

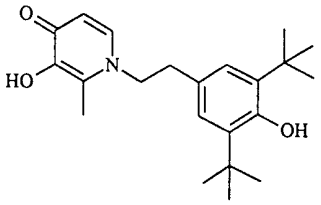


3-Benzyloxy-1-(2-(3,5-di-tert-butyl-4-hydroxyphenyl)ethyl)-2-methyl-4(1H)-pyridinone



This was prepared from 2-(3,5-di-tert-butyl-4-hydroxphenyl)ethylamine [Dreckmann-Behrendt, Bruno *et al.* EP-0404039-A1] according to the method described in Example 1 to give the *product* (1.61 g, 24 %), as a pale-brown solid: mp 145-146 °C; IR υ_{max} (Nujol)/cm^{-1} 2917, 1736, 1627, 1559, 1464, 1260, 1223, 751, 706 and 470; NMR δ_H (400 MHz, DMSO-d_6) 1.33 (18H, s), 1.99 (3H, s), 2.79 (2H, t, *J* 7.0 Hz), 4.00-4.06 (2H, m), 4.96 (2H, s), 6.08 (1H, d, *J* 7.3 Hz), 6.76-6.79 (3H, m) and 7.30-7.44 (6H, m).

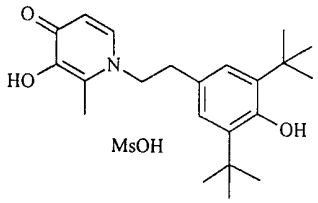
1-(2-(3,5-Di-tert-butyl-4-hydroxyphenyl)ethyl)-3-hydroxy-2-methyl-4(1H)-pyridinone



This was prepared from 3-benzyloxy-1-(2-(3,5-di-tert-butyl-4-hydroxyphenyl)ethyl)-2-methyl-4(1H)-pyridinone according to the method described in Example 1 to give the *product* (1.28 g, 100 %) as a pale-brown solid: IR υ_{max} (Nujol)/cm^{-1} 3244, 2926, 1263, 1570, 1510, 1232, and 825; NMR δ_H (400 MHz, DMSO-d_6) 1.31 (18 H, s), 2.03 (3H, s), 2.83 (2H, t, *J* 6.2 Hz), 4.07 (2H, t, *J* 6.6 Hz), 6.08 (1H, d, *J* 7.3 Hz), 6.76 (2H, s) and 7.44 (1H, d, *J* 7.0 Hz).

1-(2-(3,5-Di-tert-butyl-4-hydroxyphenyl)ethyl)-3-hydroxy-2-methyl-4(1H)-pyridinone

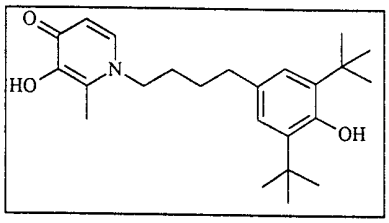
mesylate



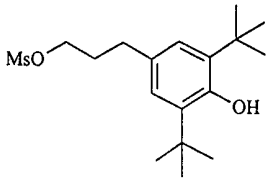
To a stirred solution of 1-[2-(3,5-di-tert-butyl-4-hydroxyphenyl)ethyl]-3-benzyloxy-1,4-dihydro-2-methylpyridin-4-one (0.80 g, 2.24 mmol) in CH_2Cl_2-Et_2O (2:1) (30 mL) was added methanesulphonic acid (0.15 mL, 2.24 mmol) and mixture allowed to stir for 2 h. The solid which formed was filtered off and dried to give the *title compound* (0.94 g, 92 %) as a white solid: mp 254-255 °C; IR υ_{max} (Nujol)/cm^{-1} 3644, 3077, 2925, 1634, 1518, 1242, 1147, 1047, 773 and 552; NMR δ_H (400 MHz, DMSO-d_6) 1.30 (18H, s), 2.24 (3H, s), 2.33 (3H, s), 2.96 (2H, t, *J* 6.2 Hz), 4.51 (2H, t, *J* 6.2 Hz), 6.69 (2H, s), 6.82 (1H, br s),7.06 (1H, d, *J* 6.6 Hz), and 8.03 (1H, d, *J* 6.6 Hz).

Example 3

1-(4-(3,5-Di-tert-butyl-4-hydroxyphenyl)butyl)-3-hydroxy-2-methyl-4(1H)-pyridinone



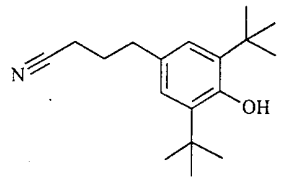
3-(3,5-Di-tert-butyl-4-hydroxphenyl)propyl methanesulphonate



A solution of 3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)propanol [A. W. White and R. S. Beavers; US 4910286] (9.8 g, 37 mmol) in CH_2Cl_2 (200 mL) at room temperature was treated sequentially with triethylamine (10.3 mL, 74 mmol) and a solution of methanesulphonyl chloride (2.9 mL, 37 mmol) in CH_2Cl_2 (50 mL) dropwise. The resulting mixture was stirred for 0.5 h, then washed with 1.0-M HCl, followed by brine. The organic layer was then dried ($MgSO_4$) and concentrated *in vacuo* to give the crude product.

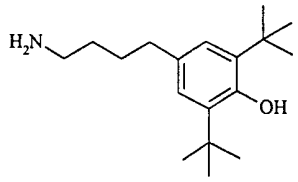
Trituration with Et_2O gave the *product* (7.5 g, 59 %), essentially pure as a pale yellow solid: mp 113-113.5 °C; IR υ_{max} (Nujol)/cm^{-1} 3592, 2925, 2855, 1459, 1437, 1349, 1171, 1140, 972, 942, 847 and 533; NMR δ_H (400 MHz, $CDCl_3$) 1.43 (18H, s), 2.01-2.08 (2H, m), 2.65 (2H, t, *J* 7.5 Hz), 3.00 (3H, s), 4.25 (2H, t, *J* 6.5 Hz), 5.09 (1H, s), and 7.02 (2H, s).

3-(3,5-Di-tert-butyl-4-hydroxphenyl)propyl cyanide



A stirred solution of 4-(3,5-di-tert-butyl-4-hydroxphenyl)propyl methanesulphonate (3.40 g, 8.71 mmol) in DMF containing NaCN (0.85 g, 17.4 mmol) was heated at 140 °C for 4 h. The mixture was poured into H_2O and extracted with CH_2Cl_2 (3 x 30 mL). The combined extracts were dried ($MgSO_4$), concentrated *in vacuo* and the residue purified by column chromatography [SiO_2; hexane-EtOAc (7:1)] to give the *product* (1.50 g, 63 %) as a thick viscous oil, which solidified on standing: mp 71-72 °C; IR υ_{max} (Nujol)/cm^{-1} 3586, 2925, 2248, 1455, 1434, 1237, 1119 and 883; NMR δ_H (400 MHz, $CDCl_3$) 1.43 (18H, s), 1.95 (2H, m), 2.34 (2H, t, J 7.1 Hz), 2.68 (2H, t, J 7.8 Hz), 5.10 (1H, s) and 6.96 (2H, s).

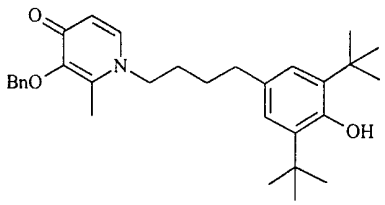
4-(3,5-Di-tert-butyl-4-hydroxphenyl)butylamine



To a stirred solution of 3-(3,5-di-tert-butyl-4-hydroxphenyl)propyl cyanide (1.50 g, 5.49 mmol) in dry THF, under argon was added 1.0-M $BH_3.SMe_2$ solution (11 mL, 10.98 mmol) and the mixture heated under reflux for 2 h. The mixture was cooled, conc. HCl was added cautiously and the mixture was heated under reflux for a further 30 min. The solution was diluted with H_2O, concentrated *in vacuo* and the residue made alkaline with NaOH pellets. The solution was extracted with CH_2Cl_2 (3x30 mL), the extracts dried ($MgSO_4$) and evaporated *in vacuo* to give the *product* (1.68 g, 100 %) as a thick viscous

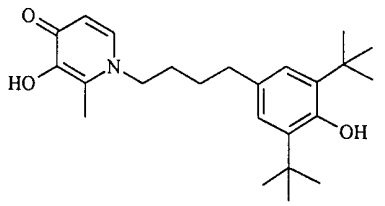
oil. IR υ_{max} (thin film)/cm^{-1} 3644, 2955, 1434, 1233, 888, and 770; NMR δ_H (400 MHz, $CDCl_3$) 1.43(9H, s), 1.48-1.65 (3H, m), 1.65-1.80 (1H, m), 1.85-1.95 (1H, m), 2.54 (1H, t, *J* 6.9 Hz), 2.78 (1H, m), 3.68-3.71 (1H, m), 5.04 (1H, br s) and 6.97 (2H, s).

3-Benzyloxy-1-(4-(3,5-di-tert-butyl-4-hydroxyphenyl)butyl)-2-methyl-4(1H)-pyridinone



This was prepared from 4-(3,5-di-tert-butyl-4-hydroxphenyl)butylamine according to the method described in Example 1 to give the *product* (2.46 g, 94 %) as a brown viscous oil. This material was used directly in the next reaction without further purification.

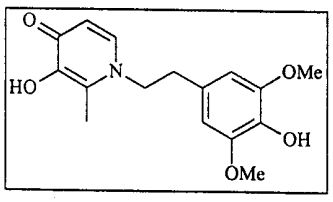
1-(4-(3,5-Di-tert-butyl-4-hydroxyphenyl)butyl)-3-hydroxy-2-methyl-4(1H)-pyridinone



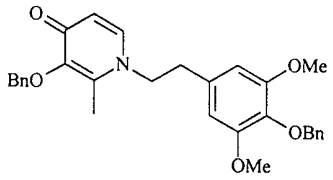
This was prepared from 3-benzyloxy-1-(4-(3,5-di-tert-butyl-4-hydroxyphenyl)butyl)-2-methyl-4(1H)-pyridinone according to the method described in Example 1 to give the title compound (0.36 g, 18 %) as a pale-brown solid; mp darkens 185 °, melts 192-193 °C; IR ν_{max} (Nujol)/cm^{-1} 3802, 2925, 1626, 1561, 1509, 1220, 1031 and 826; NMR δ_H (400 MHz, DMSO-d_6) 1.34 (9H, s), 1.50-1.53 (2H, m), 1.62-1.65 (2H, m), 2.23 (3H, s), 2.45-2.49 (2H, m), 3.93 (3H, t, J 6.9 Hz), 6.08 (1H, d, J 7.2 Hz), 6.87 (3H, s) and 7.54 (1H, d, J 7.5 Hz); Anal.Calcd. for $C_{24}H_{35}NO_3.0.5\ H_2O$: C, 73.06; H, 9.20; N, 3.55. Found: C, 72.84; H, 9.06; N, 3.36.

Example 4

1-[2-(3,5-Dimethoxy-4-hydroxyphenyl)ethyl]-3-hydroxy-2-methyl-4(1H)-pyridinone

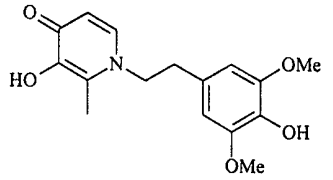


3-Benzyloxy-1-[2-(4-benzyloxy-3,5-dimethoxyphenyl)ethyl]-2-methyl-4(1H)-pyridinone



This was prepared from 2-(4-benzyloxy-3,5-dimethoxyphenyl)ethylamine [Borchardt, R.T. *et al.*,J. Med. Chem 1975, 18 (2), 152-8] according to the method described in Example 1 to give the *product* (1.5 g, 34 %) as a viscous yellow gum: NMR δ_H (400 MHz, $CDCl_3$) 2.01 (3H, s), 2.82 (2H, t, *J* 6.6 Hz), 3.73 (6H, s), 3.97 (2H, t, *J* 6.4 Hz), 4.98 (2H, s), 5.20 (2H, s), 6.13 (2H, s), 6.40 (1H, d, *J* 7.6 Hz), 6.93 (1H, d, *J* 7.6 Hz) and 7.26-7.45 (10H, m).

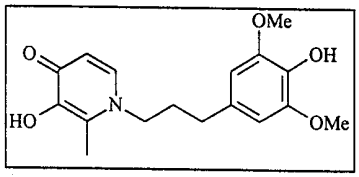
1-[2-(3,5-Dimethoxy-4-hydroxyphenyl)ethyl]-3-hydroxy-2-methyl-4(1H)-pyridinone



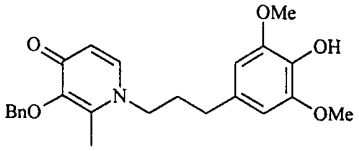
This was prepared from 3-benzyloxy-1-[2-(4-benzyloxy-3,5-dimethoxyphenyl)ethyl]-2-methyl-4(1H)-pyridinone according to the method described in Example 1 to give the title compound (0.48g, 55 %) as an off-white solid: mp 214-216 °C; IR υ_{max} (Nujol)/cm^{-1} 3613, 3256, 2923, 1629, 1506, 1462, 1238, 1114 and 824; NMR δ_H (400 MHz, $CDCl_3$) 2.20 (3H, s), 2.48 (1H, d, J 1.90 Hz), 2.81 (2H, t, J 7.6 Hz), 3.15 (1H, s), 3.66 (6H, s), 4.08 (2H, t, J 7.2 Hz), 6.04 (1H, d, J 7.0 Hz), 6.38 (2H, s) and 7.40 (1H, d, J 7.3 Hz); Anal.Calcd. for $C_{16}H_{19}NO_5$: C, 62.94; H, 6.27; N, 4.59. Found: C, 62.53; H, 6.28; N, 4.52.

Example 5

1-[3-(3,5-Dimethoxy-4-hydroxyphenyl)propyl]-3-hydroxy-2-methyl-4(1H)-pyridinone

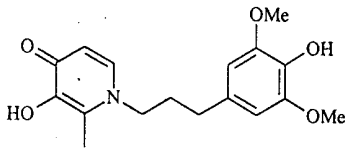


3-Benzyloxy-1-[3-(3,5-dimethoxy-4-hydroxyphenyl)propyl]-2-methyl-4(1H)-pyridinone



This was prepared from 3-(3,5-dimethoxy-4-hydroxyphenyl)propylamine [Nichols, D.E. *et al.*, J. Med. Chem. (1977), 20(2), 299-301] according to the method described in Example 1 to give the *product* (1.4 g, 58 %); IR υ_{max} (CH_2Cl_2)/cm^{-1} 3062, 2938, 1626, 1560, 1518, 1459, 1248, 1117 and 734; NMR δ_H (400 MHz, $CDCl_3$) 1.91-1.97 (2H, m), 2.01 (3H, s), 2.53 (2H, t, *J* 7.6 Hz), 3.76 (2H, t, *J*, 17.4 Hz), 3.85 (6H, s), 5.20 (3H, s), 6.32 (3H, s), 6.46 (1H, d, *J* 7.6 Hz), 7.18 (1H, d, *J* 7.5 Hz), and 7.22-7.38 (5H, m).

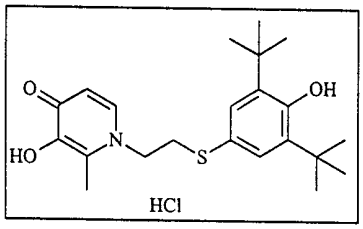
1-[3-(3,5-Dimethoxy-4-hydroxyphenyl)propyl]-3-hydroxy-2-methyl-4(1H)-pyridinone



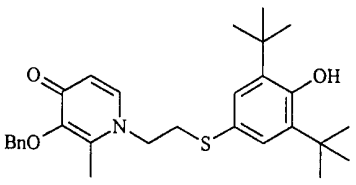
This was prepared from 3-benzyloxy-1-[3-(3,5-dimethoxy-4-hydroxyphenyl)propyl]-2-methyl-4(1H)-pyridinone according to the method described in Example 1 to give the *title compound* (0.59 g, 57 %) as a pale-orange foam: mp softens over 174-175 °C; IR υ_{max} (Nujol)/cm^{-1} 2924, 1625, 1506, 1460, 1243 113 and 827; NMR δ_H (400 MHz, $CDCl_3$) 0.89-0.94 (2H, m), 1.21 (3H, s), 1.46-1.49 (3H, s), 2.70 (6H, s), 2.88 (2H, t, *J* 7.7 Hz), 5.08 (1H, d, *J* 7.5 Hz), 5.44 (2H, s), and 6.52 (1H, d, *J* 7.6 Hz); Anal.Calcd. for $C_{17}H_{21}NO_5$.0.25 H_2O: C, 63.05; H, 6.69; N, 4.32. Found: C, 62.88; H, 6.60; N, 4.25.

Example 6

1-(2-(3,5-Di-tert-butyl-4-hydroxyphenylthio)ethyl)-3-hydroxy-2-methyl-4(1H)-pyridinone hydrochloride



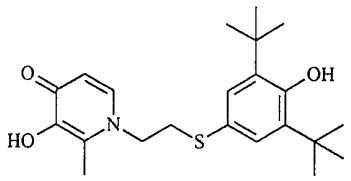
3-Benzyloxy-1-(2-(3,5-di-tert-butyl-4-hydroxyphenylthio)ethyl)-2-methyl-4(1H)-pyridinone



This was prepared according to the method described in Example 1 using 2-(3,5-di-*tert*-

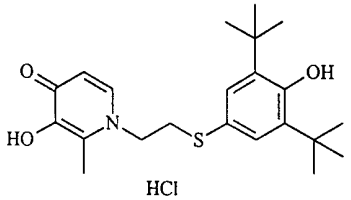
butyl-4-hydroxyphenylthio)ethylamine [*a.* Medvedev, A. I. *et al.*, Synthesis and properties of some new derivatives of 3,5-di-tert-butyl-4-hydroxythiophenol. Tezisy Dokl. Nauchn. Sess. Khim. Tekhnol. Org. Soedin. Sery Sernistykh Neftei, 13th (1974), 123-4. Editor: Gal'pern, G. D. Publisher: "Zinatne", Riga, USSR. *b.* Medvedev, A. I. *et al.*, Standard methods of producing sulfur-containing stabilizers. Izv. Vyssh. Uchebn. Zaved., Khim. Khim. Tekhnol. (1977), 20(4), 568-74], to give the *product* (0.2 g, 59 %) as a brown glass: IR υ_{max} (CH_2Cl_2)/cm^{-1} 3627, 2959, 1626, 1568, 1557, 1425 and 1250; NMR δ_H (400 MHz, $CDCl_3$) 1.43 (18H, s), 1.90 (3H, s), 2.95 (2H, t, *J* 7.5 Hz), 3.90 (2H, t, *J* 7.5 Hz), 5.19 (2H, s), 5.35 (1H, s), 6.51-6.56 (1H, m), 7.18-7.20 (1H, m), 7.24 (2H, s), 7.28-7.32 (3H, m), and 7.35-7.38 (2H, m).

1-(2-(3,5-Di-tert-butyl-4-hydroxyphenylthio)ethyl)-3-hydroxy-2-methyl-4(1H)-pyridinone



3-Benzyloxy-1-(2-(3,5-di-*tert*-butyl-4-hydroxyphenylthio)ethyl)-2-methyl-4(1*H*)-pyridinone (0.2 g, 0.4 mmol) in CH_2Cl_2 (10 mL) was treated dropwise with $BCl_3.SMe_2$ (0.4 mL, 0.8 mmol) at room temperature. The reaction was stirred for 1 h, then 6.0-M HCl (10 mL) was added and the mixture was extracted with CH_2Cl_2. The solvent was removed under reduced pressure to provide the *product* (0.16 g, 95 %) as a off white solid: IR υ_{max} (Nujol)/cm^{-1} 3630, 2925, 1627, 1507, 1464, 1425 and 1235; NMR δ_H (400 MHz, DMSO-d_6) 1.35 (18H, s), 2.34 (3H, s), 3.35 (2H, obscured t, *J* 6.5 Hz), 4.47 (2H, t, *J* 6.5 Hz), 7.05 (2H, s), 7.10 (1H, d, *J* 7.0 Hz), and 8.14 (1H, d, *J* 7.0 Hz).

1-(2-(3,5-Di-tert-butyl-4-hydroxyphenylthio)ethyl)-3-hydroxy-2-methyl-4(1H)-pyridinone hydrochloride

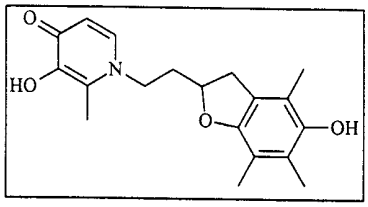


1.0-M HCl in Et_2O (0.62 mL, 0.62 mmol) was added dropwise to 1-(2-(3,5-di-*tert*-butyl-4-hydroxyphenylthio)ethyl)-3-hydroxy-2-methyl-4(1*H*)-pyridinone (0.12 g, 0.3 mmol) in

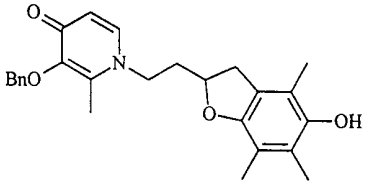
CH_2Cl_2 (2.5 mL) and Et_2O (2.5 mL). The mixture was stirred for 0.5 h, concentrated *in vacuo*, and purified by chromatography [SiO_2; CH_2Cl_2-MeOH gradient (100:0 to 90:10)] to afford the *title compound* (0.13 g, 95 %) as a white solid: mp 155-158 °C; IR υ_{max} (Nujol)/cm^{-1} 3629, 2925, 1646, 1560, 1524, 1489, 1464, 1062, 887 and 720; NMR δ_H (400 MHz, DMSO-d_6) 1.36 (18H, s), 2.35 (3H, s), 3.40 (2H, t, *J* 6.5 Hz), 4.51 (2H, t, *J* 6.5 Hz), 7.03 (2H, s), 7.17 (1H, d, *J* 7.0 Hz), and 8.37 (1H, d, *J* 7.0 Hz).; Anal. Calcd. for $C_{22}H_{32}ClNO_3S$: C, 62.02; H, 7.33; N, 3.28; Found: C, 62.05; H, 7.07; N, 3.16.

Example 7

1-(2-(2,3-Dihydro-5-hydroxy-4,6,7-trimethylbenzofuran-2-yl)ethyl)-3-hydroxy-2-methyl-4(1H)-pyridinone

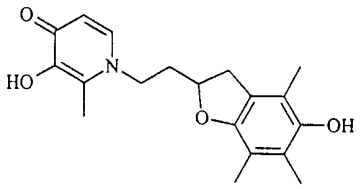


3-Benzyloxy-1-(2-(2,3-dihydro-5-hydroxy-4,6,7-trimethylbenzofuran-2-yl)ethyl)-2-methyl-4(1H)-pyridinone



This was prepared from 2-(2,3-dihydro-5-hydroxy-4,6,7-trimethylbenzofuran-2-yl)ethylamine [Ceccarelli, S. *et al.*, J. Heterocycl. Chem. (1993), 30(3), 679-90] according to the method described in Example 1 to give the *product* (1.17 g, 47 %) as a yellow solid: mp 155-156 °C; IR υ_{max} (Nujol)/cm^{-1} 3460, 2924, 1457, 1377, 1236, 1078 and 749; NMR δ_H (400 MHz, $CDCl_3$) 1.91 (3H, s), 2.00 (5H, m), 2.32 (3H, s), 2.48 (3H, s), 2.71 (1H, dd, *J* 15.4, 7.4 Hz), 4.25 (2H, t, *J* 7.6 Hz), 4.61-4.64 (1H, m), 5.02 (2H, s), 6.72 (1H, d, *J*, 6.9 Hz), 7.29-7.41 (5H, m) and 8.02 (1H, d, *J* 7.3 Hz); Anal.Calcd. for $C_{26}H_{29}NO_4.1.5\ H_2O$: C, 69.93; H, 7.22; N, 3.14. Found: C, 69.69; H, 6.88; N, 3.13.

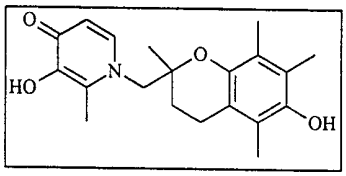
1-(2-(2,3-Dihydro-5-hydroxy-4,6,7-trimethylbenzofuran-2-yl)ethyl)-3-hydroxy-2-methyl-4(1H)-pyridinone



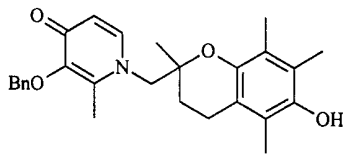
This was prepared from 3-benzyloxy-1-(2-(2,3-dihydro-5-hydroxy-4,6,7-trimethylbenzofuran-2-yl)ethyl)-2-methyl-4(1*H*)-pyridinone according to the method described in Example 1 to give the *title compound* (0.48 g, 76 %) as a pale brown solid: mp darkens 126 °C, melts 135-136 °C; IR υ_{max} (Nujol)/cm^{-1} 3217, 2924, 1624, 1561, 1508, 1462, 1236, 1079 and 829; NMR δ_H (400 MHz, DMSO-d_6); 1.97 (3H, s), 1.97-2.01 (2H, m); 2.00 (3H, s), 2.01 (3H, s), 2.30 (3H, s), 2.72 (1H, dd, *J* 15.2, 7.1 Hz), 3.15 (1H, dd, *J* 15.0 Hz), 4.07 (2H, m), 4.59-4.66 (2H, m), 6.10 (1H, d, *J* 7.8 Hz), and 7.58 (1H, d, *J* 7.4 Hz); Anal.Calcd. for $C_{19}H_{23}NO_4.1.5\ H_2O$: C, 64.03; H, 7.35; N, 3.93. Found: C, 64.59; H, 7.12; N, 4.04.

Example 8

1-(1-(3,4-Dihydro-6-hydroxy-2,5,7,8-tetramethyl-2H-benzopyran-2-yl)methyl)-3-hydroxy-2-methyl-4(1*H*)-pyridinone



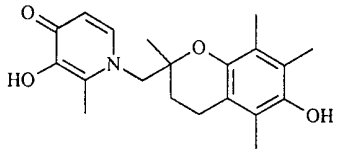
3-Benzyloxy-1-(1-(3,4-dihydro-6-hydroxy-2,5,7,8-tetramethyl-2H-benzopyran-2-yl)methyl)-2-methyl-4(1H)-pyridinone



This was prepared from (3,4-dihydro-6-hydroxy-2,5,7,8-tetramethyl-2*H*-benzopyran-2-yl)methylamine (Shiono, M. *et al.*, EP-0183869-A1) according to the method described in Example 1 to give the *product* (0.57 g, 26 %) as a yellow foam: IR υ_{max} (Nujol)/cm^{-1} 2925, 1626, 1461, 1378 and 1247; NMR δ_H (400 MHz, DMSO-d_6) 1.00 (3H, s), 1.46-1.64 (1H, m), 1.70-1.85 (1H, m), 1.93 (3H, s), 2.00 (3H, s), 2.04 (3H, s), 2.20 (3H, s),

2.49 (2H, m), 4.10 (2H, q, *J* 15.3 Hz), 5.01 (2H, q, *J* 11.0 Hz, 6.12 (1H, d, *J*, 7.5 Hz) and 7.26-7.50 (6H, m).

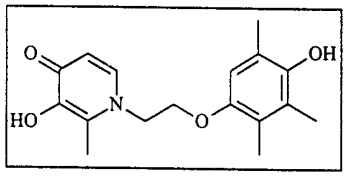
1-(1-(3,4-Dihydro-6-hydroxy-2,5,7,8-tetramethyl-2H-benzopyran-2-yl)methyl)-3-hydroxy-2-methyl-4(1H)-pyridinone



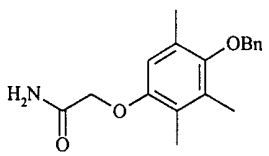
This was prepared from 3-benzyloxy-1-(1-(3,4-dihydro-6-hydroxy-2,5,7,8-tetramethyl-2*H*-benzopyran-2-yl)methyl)-2-methyl-4(1*H*)-pyridinone according to the method described in Example 1 to give the *title compound* (0.32 g, 72 %) as a pale-brown solid: mp 123-124 °C (dec); IR υ_{max} (Nujol)/cm^{-1} 3205, 2925, 1626, 1563, 1508, 1461, 1378, 1236, 1040 and 830; NMR δ_H (400 MHz, DMSO-d_6) 1.07 (3H, s), 1.58-1.61 (1H, m), 1.82-1.88 (1H, m), 1.96 (3H, s), 2.00 (3H, s), 2.04 (3H, s), 2.34 (3H, s), 2.56-2.57 (2H, m), 4.16 (2H, q, *J* 17.6 Hz), 6.10 (1H, d, *J* 7.4 Hz) and 7.46 (1H, d, *J* 7.4 Hz); Anal.Calcd. for $C_{20}H_{25}NO_4$.1.25 H_2O: C, 65.64; H, 7.57 N, 3.83. Found: C, 65.78; H, 7.42; N, 3.69.

Example 9

3-Hydroxy-1-(2-(4-hydroxy-2,3,5-trimethylphenoxy)ethyl)-2-methyl-4(1*H*)-pyridinone



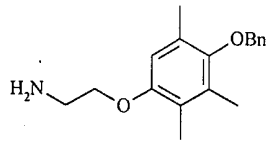
2-(4-Benzyloxy-2,3,5-trimethylphenoxy)acetamide



A solution of 4-benzyloxy-2,3,5-trimethylphenol (2.0 g, 8.3 mmol) and chloroacetamide (1.2 g, 12.5 mmol) in dry DMF (50 mL), containing K_2CO_3 (4.60 g, 33.3 mmol) and NaI (0.63 g, 4.2 mmol) was heated at 80 °C for 8 h, then at 100 °C for 12 h. The solution

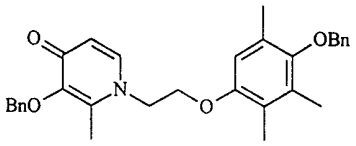
was cooled, poured into water (200 mL) and extracted with Et_2O (3x30 mL). The extracts were dried ($MgSO_4$), evaporated *in vacuo* and the residue re-crystallised from hexane/EtOAc to give the *product* (1.1 g, 43 %) as an off-white solid: IR υ_{max} (Nujol)/cm^{-1} 3373, 3192, 2925, 1662, 1219, 1128, 1090 and 751; NMR δ_H (400 MHz, $CDCl_3$) 2.18 (3H, 2.25 (3H, s), 2.29 (3H, s), 4.47 (2H, q_{AB}, *J* 20 Hz), 4.73 (2H, q_{AB}, *J* 20 Hz), 5.89 (1H, br s), 6.53 (1H, s) and 7.33-7.50 (5H, m).

2-(4-Benzyloxy-2,3,5-trimethylphenoxy)ethylamine



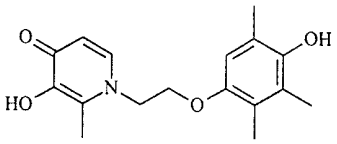
To a solution of 2-(4-benzyloxy-2,3,5-trimethylphenoxy)acetamide (0.75 g, 2.5 mmol) in THF (15 mL) was added $BH_3.SMe_2$ (0.72 mL, 7.6 mmol), and the mixture heated under reflux for 4 h. 6-M HCl (3 mL) was added cautiously, followed after 10 min by 6-M NaOH (4 mL). The solution was poured into H_2O, extracted with Et_2O (3x15 mL), dried ($MgSO_4$) and concentrated *in vacuo* to give the *product* (0.11 g, 15 %) as a pale-brown solid: IR υ_{max} (Nujol)/cm^{-1} 3360, 3029, 2924, 1583, 1464, 1372, 1224, 1122, 1089, 998 and 694; NMR δ_H (400 MHz, $CDCl_3$) 2.15 (3H, s), 2.24 (3H, s), 2.29 (3H, s), 3.01 (1H, br s), 3.95 (2H, m), 4.73 (2H, s), 5.30 (2H, q_{AB}, *J* 20 Hz), 6.56 (1H, s) and 7.33-7.50 (5H, m).

3-Benzyloxy-1-(2-(4-benzyloxy-2,3,5-trimethylphenoxy)ethyl)-2-methyl-4(1H)-pyridinone



This was prepared from *2-(4-benzyloxy-2,3,5-trimethylphenoxy)ethylamine* according to the method described in Example 1 to give the *product* (0.91 g, 30 %) as a pale-yellow syrup: NMR δ_H (400 MHz, $CDCl_3$) 2.01 (3H,s), 2.20 (3H, s), 2.21 (3H, s), 2.27 (3H, s), 4.07-4.09 (2H, m), 4.22-4.25 (2H, m), 4.71 (2H, s), 5.21 (2H, s), 6.44 (1H, s), 6.58 (1H, m) and 7.26-7.48 (11H, m).

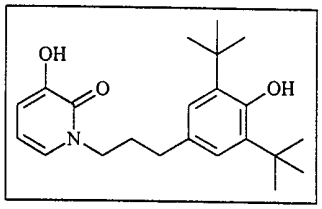
3-Hydroxy-1-(2-(4-hydroxy-2,3,5-trimethylphenoxy)ethyl)-2-methyl-4(1H)-pyridinone



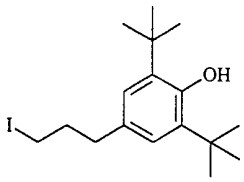
This was prepared from 3-benzyloxy-1-(2-(4-benzyloxy-2,3,5-trimethylphenoxy)ethyl)-2-methyl-4(1*H*)-pyridinone according to the method described in Example 1 to give the *title compound* (0.12 g, 21 %) as a pink solid: IR υ_{max}(Nujol)/cm^{-1} 2992, 1622, 1554, 1463, 1378, 1240, 1132 and 844; NMR δ_H (400 MHz, DMSO-d_6) 1.89 (3H, s), 2.02 (3H, s), 2.08 (3H, s), 2.32 (3H, s), 4.05-4.07 (2H, m), 4.30-4.32 (2H, m), 6.10 (1H, d, *J* 7.1 Hz), 6.49 (1H, s) and 7.60 (1H, d, *J* 7.4 Hz); m/z (ES+) 304 (100 %).

Example 10

1-(3-(3,5-Di-*tert*-butyl-4-hydroxyphenyl)propyl)-3-hydroxy-2(1*H*)-pyridinone

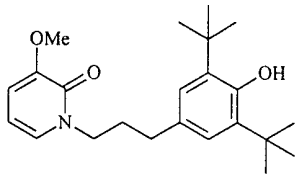


4-(3-Iodopropyl)-2,6-di-tert-butylphenol



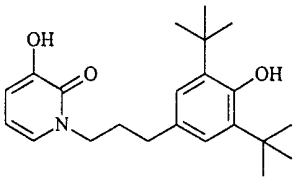
A solution of 3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)propyl methanesulphonate (7.3 g, 21 mmol) in acetone (214 mL) at room temperature was treated with sodium iodide (6.4 g, 43 mmol) and K_2CO_3 (0.3 g, 2.1 mmol). The reaction mixture was refluxed for 6.5 h, then filtered to remove the precipitate. The filtrate was reduced *in vacuo*, taken up in EtOAc and washed with water, dried ($MgSO_4$), reduced *in vacuo* and then purified by chromatography [SiO_2; Heptane-EtOAc gradient (100:0 to 95:5)] to give the *product* (7.3 g, 91 %) as a yellow solid: mp 76 °C; IR υ_{max} (Nujol)/cm^{-1} 3644, 3619, 2924, 2856, 1457, 1434, 1377, 1380, 1229, 1213, 1168, 877 and 787; NMR δ_H (400 MHz, $CDCl_3$) 1.43 (18H, s), 2.04-2.13 (2H, m), 2.63 (2H, t, *J* 7.5 Hz), 3.19 (2H, t, *J* 7.0 Hz), 5.07 (1H, s), and 6.98 (2H, s).

1-(3-(3,5-Di-tert-butyl-4-hydroxyphenyl)propyl)-3-methoxy-2(1H)-pyridinone



To a solution of 3-methoxy-2(1*H*)-pyridinone (0.7 g, 5.3 mmol) and powdered KOH (0.45 g, 8.0 mmol) in dry EtOH (34 mL) was added 4-(3-iodopropyl)-2,6-di-*tert*-butylphenol (4.0 g, 10.7 mmol) at room temperature. The reaction was stirred at room temperature for ¼ h then heated at reflux for 5 h. After cooling the reaction was concentrated *in vacuo*. The resulting residue was taken up in CH_2Cl_2, washed with water, dried (Na_2SO_4), reduced *in vacuo* and then purified by chromatography [SiO_2; CH_2Cl_2-MeOH gradient (100:0 to 96:4)] to give the *product* (1.6 g, 82 %) as a pale brown solid: mp 89-92 °C; IR υ_{max} (Nujol)/cm^{-1} 3175, 2922, 2856, 1649, 1592, 1562, 1461, 1436, 1262, 1252 and 1202; NMR δ_H (400 MHz, $CDCl_3$) 1.42 (18H, s), 2.03-2.11 (2H, m), 2.60 (2H, t, *J* 8.0 Hz), 3.81 (3H, s), 4.02 (2H, t, *J* 7.5 Hz), 5.06 (1H, br s), 6.09 (1H, t, *J* 7.0 Hz), 6.59 (1H, dd, *J* 7.0, 1.5 Hz), 6.86 (1H, dd, *J* 7.0, 1.5 Hz), and 6.97 (2H, s).

1-(3-(3,5-Di-tert-butyl-4-hydroxyphenyl)propyl)-3-hydroxy-2(1H)-pyridinone

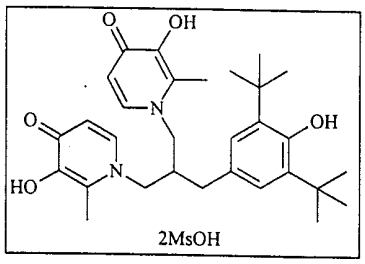


A solution of 1-(3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)propyl)-3-methoxy-2(1*H*)-pyridinone (1.0 g, 2.8 mmol) in dry CH_2Cl_2 (18 mL) at –70 °C was treated dropwise with 1.0-M BBr_3 (5.5 mL, 5.5 mmol). The reaction was allowed to warm to room temperature and stirred for 21.5 h. This was then re-cooled to –70 °C and MeOH (36 mL) was added dropwise followed by water (2 x 18 mL). The mixture was concentrated *in vacuo*, the residue adjusted to pH 7 with 2.0-M NaOH, and the resulting aqueous residue extracted with CH_2Cl_2 (3 x 18 mL). The combined organic extracts were dried (Na_2SO_4), reduced *in vacuo* and purified by chromatography [SiO_2; CH_2Cl_2-MeOH (98:2)] to give the *title compound* (0.6 g, 58 %) as a off white solid: mp 149 °C; IR υ_{max} (Nujol)/cm^{-1} 3642, 3627, 3263, 2922, 2855, 1652, 1593, 1563, 1465, 1437, 1409, 1377, 1269 and 1247; NMR δ_H (400 MHz, $CDCl_3$) 1.43 (18H, s), 2.05-2.13 (2H, m), 2.61 (2H, t, *J* 8.0 Hz), 4.03 (2H, t, *J*

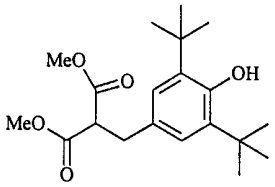
7.5 Hz), 5.07 (1H, br s), 6.15 (1H, t, *J* 7.0 Hz), 6.78-6.85 (2H, m), and 6.97 (2H, s); Anal. Calcd. for $C_{22}H_{31}NO_3 \cdot 0.2H_2O$: C, 73.54; H, 8.76; N, 3.88. Found: C, 73.08; H, 8.68; N, 3.91.

Example 11

1-(3-(3,5-Di-*tert*-butyl-4-hydroxyphenyl)-2-(1,4-dihydro-3-hydroxy-2-methyl-4-oxo-1-pyridinylmethyl)propyl)-3-hydroxy-2-methyl-4(1*H*)-pyridinone dimesylate

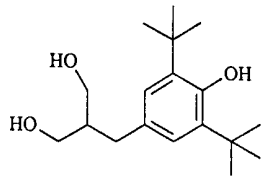


Dimethyl 2-(3,5-di-tert-butyl-4-hydroxybenzyl)malonate



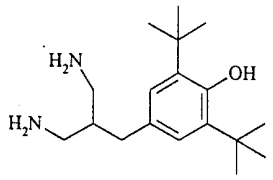
A suspension of hexane-washed NaH (2.91g of 60 % dispersion in mineral oil, 73 mmol) in a mixture of THF (80 mL) and DMF (30 mL) is cooled to 0 °C under Ar and treated dropwise with dimethyl malonate (7.9 mL, 69 mmol). After a further 10 min at 20 °C, a solution of 3,5-di-*tert*-butyl-4-hydroxybenzyl bromide (69 mmol) in a mixture of THF (20 mL) and DMF (20 mL) is added, and the mixture is heated under gentle reflux for 1 h. The reaction is then poured into brine, extracted with EtOAc and concentrated *in vacuo*. Kugelrohr distillation at 130 °C (20mm Hg) is used to remove unreacted dimethyl malonate. The residue is purified by chromatography [SiO_2; EtOAc-Hexane] to give the product.

2-(3,5-Di-tert-butyl-4-hydroxybenzyl)propane-1,3-diol



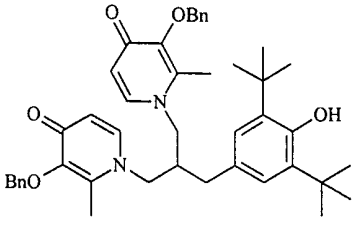
BH_3-Me_2S (130 mmol) is added to a mixture of dimethyl 2-(3,5-di-*tert*-butyl-4-hydroxybenzyl)malonate (40 mmol) and THF (100 mL) under Ar. The resulting solution is heated under reflux for 40 h. MeOH is added slowly to the cooled solution to destroy excess reagent. The mixture is diluted with brine, extracted with EtOAc and concentrated *in vacuo*. The residue is purified by chromatography [SiO_2; EtOAc-Hexane] to give the *product*.

2-(3,5-Di-tert-butyl-4-hydroxybenzyl)propane-1,3-diamine dihydrochloride



A solution of 2-(3,5-di-*tert*-butyl-4-hydroxybenzyl)propane-1,3-diol (37.8 mmol) in CH_2Cl_2 (150 mL) and Et_3N (15.8 mL, 113 mmol) is treated dropwise at 0 °C with methanesulphonyl chloride (6.5 mL, 83.3 mmol). After 15 min the solution is washed with water, $NaHCO_3$ and brine, and concentrated *in vacuo*. The crude mesylate is then immediately dissolved in DMF (30 mL), NaN_3 (15 g, 230 mmol) is added, and the suspension is stirred at 120 °C for 1 h. The cooled mixture is diluted with brine, extracted with EtOAc and concentrated *in vacuo*. The residue is purified by chromatography [SiO_2; EtOAc-Hexane] to give the diazide. This is immediately dissolved in EtOH (100 mL) and hydrogenated over 10% Pd/C (500 mg) at 60 psi for 20 h. The catalyst is removed by filtration and washed well with EtOH. The filtrate is immediately saturated with HCl gas and concentrated *in vacuo*. The residue is crystallised from MeOH:Et_2O to give the *product*.

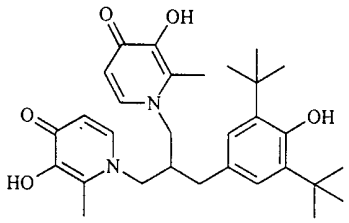
1-(3-(3,5-Di-tert-butyl-4-hydroxyphenyl)-2-(3-benzyloxy-1,4-dihydro-2-methyl-4-oxo-1-pyridinylmethyl)propyl)-3-benzyloxy-2-methyl-4(1H)-pyridinone



2-(3,5-Di-*tert*-butyl-4-hydroxybenzyl)propane-1,3-diamine dihydrochloride (69.0 mmol), 3-benzyloxy-2-methyl-4-pyrone [Harris, R. L. N., Aust. J. Chem., (1976), 29, 1329-1334]

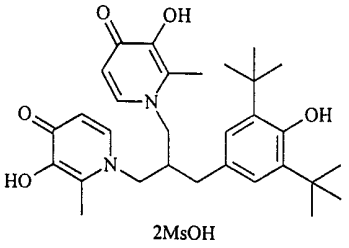
(3.0 g, 14.0 mmol) and 5.0-M NaOH (160 mmol) in EtOH (50 mL) and water (30 mL) is refluxed for 24 h. The reaction is cooled, adjusted to pH 3-4 with 1.0-M HCl, concentrated *in vacuo*, extracted with $CHCl_3$, dried ($MgSO_4$), concentrated *in vacuo* and purified by chromatography [SiO_2; CH_2Cl_2-MeOH (95:5)] to give the *product* in low yield.

1-(3-(3,5-Di-tert-butyl-4-hydroxyphenyl)-2-(1,4-dihydro-3-hydroxy-2-methyl-4-oxo-1-pyridinylmethyl)propyl)-3-hydroxy-2-methyl-4(1H)-pyridinone



This is prepared from 1-(3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)-2-(3-benzyloxy-1,4-dihydro-2-methyl-4-oxo-1-pyridinylmethyl)propyl)-3-benzyloxy-2-methyl-4(1*H*)-pyridinone according to the method described in Example 1 to give the product.

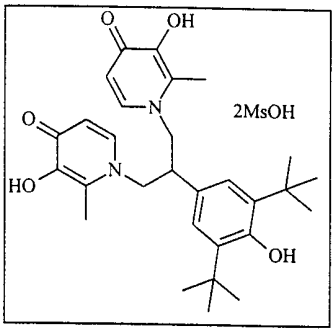
1-(3-(3,5-Di-tert-butyl-4-hydroxyphenyl)-2-(1,4-dihydro-3-hydroxy-2-methyl-4-oxo-1-pyridinylmethyl)propyl)-3-hydroxy-2-methyl-4(1H)-pyridinone dimesylate



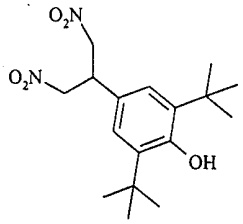
Methanesulphonic acid (175 µL, 2.7 mmol) is added dropwise to 1-(3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)-2-(1,4-dihydro-3-hydroxy-2-methyl-4-oxo-1-pyridinylmethyl)propyl)-3-hydroxy-2-methyl-4(1*H*)-pyridinone (1.3 mmol) in Et_2O (50 mL) and CH_2Cl_2 (50 mL). The mixture is stirred for 1.5 h, concentrated *in vacuo*, suspended in $CHCl_3$ and the solid collected by filtration to give the title compound.

Example 12

1-(2-(3,5-Di-*tert*-butyl-4-hydroxyphenyl)-2-(1,4-dihydro-3-hydroxy-2-methyl-4-oxo-1-pyridinylmethyl)ethyl)-3-hydroxy-2-methyl-4(1*H*)-pyridinone dimesylate

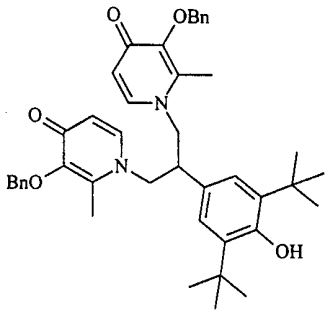


2,6-Di-tert-butyl-4-(1-(nitromethyl)-2-nitroethyl)phenol



A solution of 3,5-di-*tert*-butyl-4-hydroxybenzaldehyde (69 mmol) in nitromethane (40 mL) and butylamine (cat.) is heated at reflux for 24 h. The reaction is then poured into brine, extracted with EtOAc and concentrated *in vacuo*. Kugelrohr distillation at 130 °C (20mm Hg) is used to remove unreacted nitromethane. The residue is purified by chromatography [SiO_2; EtOAc-Hexane] to give the product.

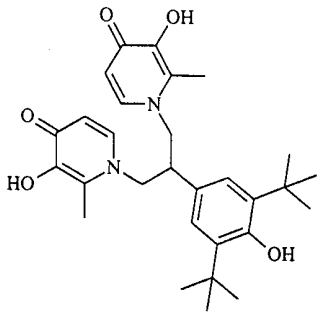
1-(2-(3,5-Di-tert-butyl-4-hydroxyphenyl)-2-(3-benzyloxy-1,4-dihydro-2-methyl-4-oxo-1-pyridinylmethyl)ethyl)-3-benzyloxy-2-methyl-4(1H)-pyridinone



A solution of 2,6-di-tert-butyl-4-(1-(nitromethyl)-2-nitroethyl)phenol in EtOH is hydrogenated over 10% Pd/C (500 mg) for 20 h. The catalyst is removed by filtration and washed well with EtOH. The filtrate is immediately saturated with HCl gas and concentrated *in vacuo*. The residue is crystallised from MeOH:Et_2O to give the diamine dihydrochloride intermediate. The diamine dihydrochloride is immediately mixed with 3-

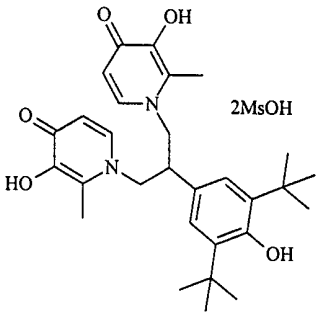
benzyloxy-2-methyl-4-pyrone [Harris, R. L. N., Aust. J. Chem., (1976), 29, 1329-1334] in 5.0-M NaOH (160 mmol) and EtOH:water (50 mL : 30 mL). The solution is refluxed for 24 h. The reaction is cooled, adjusted to pH 3-4 with 1.0-M HCl, concentrated *in vacuo*, extracted with $CHCl_3$, dried ($MgSO_4$), concentrated *in vacuo* and purified by chromatography [SiO_2; CH_2Cl_2-MeOH (95:5)] to give the *product* in low yield.

1-(2-(3,5-Di-tert-butyl-4-hydroxyphenyl)-2-(1,4-dihydro-3-hydroxy-2-methyl-4-oxo-1-pyridinylmethyl)ethyl)-3-hydroxy-2-methyl-4(1H)-pyridinone



This is prepared from 1-(2-(3,5-di-tert-butyl-4-hydroxyphenyl)-2-(3-benzyloxy-1,4-dihydro-2-methyl-4-oxo-1-pyridinylmethyl)ethyl)-3-benzyloxy-2-methyl-4(1*H*)-pyridinone according to the method described in Example 1 to give the *product*.

1-(2-(3,5-Di-tert-butyl-4-hydroxyphenyl)-2-(1,4-dihydro-3-hydroxy-2-methyl-4-oxo-1-pyridinylmethyl)ethyl)-3-hydroxy-2-methyl-4(1H)-pyridinone dimesylate



Methanesulphonic acid (2 eq) is added dropwise to 1-(2-(3,5-di-*tert*-butyl-4-hydroxyphenyl)-2-(1,4-dihydro-3-hydroxy-2-methyl-4-oxo-1-pyridinylmethyl)ethyl)-3-hydroxy-2-methyl-4(1*H*)-pyridinone in Et_2O and CH_2Cl_2. The mixture is stirred for 1.5 h, concentrated *in vacuo*, suspended in $CHCl_3$ and the solid collected by filtration to give the title compound.

Biological Testing Procedures and Data

The ability of the compounds of the present invention to protect against oxidative damage has been shown in both *in vitro* and *in vivo* models. These models are briefly explained below.

Lipid Peroxidation Assay

Lipid peroxidation in rat brain homogenates is a general procedure used to measure the antioxidant capacity of molecules in a biological environment (Das N.P. and Ratty A.K., Biochem. Med. Metab. Biol. 1987, 37, 256-264). Compounds of the present invention have been shown to be potent inhibitors of lipid peroxidation.

Iodoacetate induced Cell Toxicity in culture

Iodoacetate, via its alkylation of glyceraldehyde-3-phosphate dehydrogenase (GAPDH), is a potent inhibitor of glycolysis and hence energy production in cells. This form of chemical hypoxia has been demonstrated to result in a state of oxidative stress, from which the cells can be rescued by antioxidant molecules (Uto A. *et al.*, J. Neurochem. 1995, 64, 2185-2192; Malcolm C.S. *et al.*, Soc. Neurosci. Abstr. 1998, in press). The compounds of the present invention have been shown to protect cerebellar granule cells from this chemical induced oxidative stress. Furthermore, synergistic behaviour has been demonstrated for the compound of Example 1 over the combination of a single action Fe-chelator and single action antioxidant.

Intracerebral Malonic acid administration.

Malonic acid is an inhibitor of succinate dehydrogenase. It depletes intracellular ATP production, and intrastriatal injection has previously been demonstrated to cause a NMDA receptor mediated lesion (Greene *et al.*, J. Neurochem. 1993, 61, 1151-5). Compounds of the present invention have shown neuroprotective ability in this animal model of oxidative stress.

The ability of the compound of Example 1 to rapidly penetrate the brain has also been proven in pharmacokinetic studies.

1. *In Vitro* Assays

1a. Lipid Peroxidation

Procedure

Rat cortex was homogenised in 20 volumes of ice cold 0.32-M sucrose and centrifuged at 1,000 g for 10 min. The pellet was discarded whilst the resulting supernatant was centrifuged at 15,000 g for 20 min at 4°C to yield p2 pellets. The pellet was resuspended in ice-cold 50.0-mM phosphate buffer and centrifuged at 30,000 g for 20 min at 4°C. The pellet was resuspended in 30 vols of 50.0-mM phosphate buffer and used immediately in the assay.

Assays contained 500-μM L-ascorbic acid to induce lipid peroxidation, plus various concentrations of test compound, with the tissue preparation in a total volume of 500 μL. This was incubated at 37°C for 30 min. Lipid peroxidation was measured by adding to the reaction mixture, 300 μL 40% (w/v) trichloroacetic acid, 150 μL 5.0-M HCl and 300 μL 2% (w/v) 2-thiobarbituric acid (TBA). Samples were then heated at 90°C in a water bath for 15 min, and centrifuged at 15,000 g for 10 min. The pink colour of the supernatant was assessed spectrophotometrically at a wavelength of 532 nm. The amount of malondialdehyde (MDA) in the samples was calculated using a standard curve prepared with malondialdehyde tetrabutylammonium salt (Das, N.P. and Ratty, A.K. Biochem. Med. Metab. Biol. 1987, 37, 256-264).

Data

Results are expressed as IC_{50} values (μM) and presented in Table 1 below. The IC_{50} values show the concentration of test compound required to inhibit the lipid peroxidation by 50%.

Table 1.

Lipid Peroxidation	
Compound	**IC_{50} (μM)**
Example 1	0.4
Example 2	0.3
Example 3	2.9
Example 4	3.3
Example 5	2.0
Example 6	0.4
Example 7	0.4
Example 8	0.3
Example 9	0.3
Example 10	0.3

The results demonstrate that the compounds of the present invention inhibit the peroxidation of lipid membranes induced by ascorbic acid.

1b Cell Death

Procedure

After 6-8 days in culture, cerebellar granule cell (CGC) cultures in 96-well plates (250,000 cells per well) were prepared for hypoglycaemia. Iodoacetate (IAA) was made up in a balanced salt solution (BSS) (NaCl, 154.0-mM; KCl, 5.6-mM; $MgCl_2$, 1.0-mM; $CaCl_2$, 2.5-mM; N-[2-hydroxyethyl]piperazine-N'-[2-ethanesulfonic acid] (HEPES), 10.0-mM; D-glucose, 5.6-mM; pH 7.4) and any neuroprotective agents were made up in pre-warmed tissue culture media and allowed to equilibrate in a controlled environment (5% CO_2, 95% air). The assay was initiated by aspiration of the maintenance media, which was replaced by either BSS (control) or 30-μM IAA in BSS, both solutions containing 10-μM of the NMDA receptor antagonist MK-801. The exposure to IAA was for 30 min only at 37 °C, after which time the BSS was aspirated and replaced with fresh, pre-equilibriated maintenance media containing various concentrations of test compound. All conditions were performed at least in duplicate in each 96 well plate. The final volume for each well was always 200-μL. Following a 24 hour incubation, visual inspection of the cells was followed by quantification of neuronal cell death by

measuring 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT)-reductase activity as described previously (Malcolm *et al.*, J. Neurochem. 1996, 66, 2350-2360).

Data

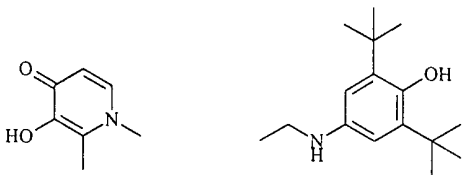
Results are expressed as EC_{50} values (μM) and presented in Table 2 below.

Table 2.

Cell Toxicity	
Compound	**EC_{50} (μM)**
Example 1	0.3
Example 2	0.3
Example 3	0.6
Example 4	33.3
Example 5	26.8
Example 6	0.4
Example 7	2.4
Example 8	3.0
Example 9	3.0
Example 10	1.2

The results demonstrate the protective effect of the compounds of the invention on neuronal cells exposed to oxidative damage induced by IAA.

Figure 1 shows the protective effect from 30-μM IAA toxicity by 1-μM concentrations of test compound. CGC are exposed to 30-μM IAA for 30 mins in a physiological salt solution. This is replaced with maintenance media containing 1-μM test compound and the cells are then tested for viability 24 hrs later. The compounds tested were Example 1, compound I (below), compound II (below) and a mixture of compounds I and II



I II

The results, illustrated in Figure 1, show that the compounds of the present invention are considerably more effective in protecting cells from oxidative damage than the separate 3-hydroxy-pyridinone iron-chelator compound and the phenolic antioxidant, either alone or in combination.

1c. Measurement of oxidative stress

Procedure

Intracellular oxidative stress was measured using the oxidant-sensitive fluorescent dye 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA) (Molecular Probes). The non-fluorescent DCFH-DA readily crosses cell membranes whereupon it is trapped within the cytoplasm by deacetylation to the non-membrane permeable form dichlorodihydrofluorescein (DCFH). Upon oxidation, DCFH yields the highly fluorescent product dichlorofluorescein (DCF). Briefly, growth medium was aspirated from cultures and replaced with 200µl BSS (plus 10.0-µM MK-801) or BSS (plus 10.0-µM MK-801) containing 30.0-µM IAA alone or in combination with various concentrations of antioxidants. After incubation at 37°C for 30 minutes, cultures were aspirated once more and 10.0-µM DCFH-DA (200µL) added to all wells. After a further 20 minute incubation at 37°C, fluorescence was measured in a Cytofluor II fluorescent plate reader (λ_{ex}=485nm; λ_{em}=530nm). Background fluorescence values from wells treated with 2% Triton X-100 were subtracted from both basal and stimulated fluorescence values and the effects of test compounds expressed as a percent inhibition of the IAA-stimulated fluorescence increase over basal. Figure 2 shows IAA-stimulated fluorescence values as a function of concentration for the compounds of Examples 1 and 7

Data

Results are expressed as EC_{50} values (μM) and presented in Table 3 below. The EC_{50} value gives the effective concentration of test compound required to block the oxidation of DCFH to DCF by 50%. The EC_{50} value is derived from Figure 2 by extrapolating from the point at which the fluoresence value is reduced to 50% of its original maximum value.

Table 3.

Oxidative Stress	
Compound	**EC_{50} (μM)**
Example 1	0.28
Example 7	3.7

The results demonstrate that the compounds of the invention inhibit oxidation of DCFH to DCF in a similar dose dependant manner to that of their inhibition of IAA-induced cell death. This confirms that the neuronal toxicity induced by IAA is a result of oxidative stress, and that the compounds of the present invention protect neuronal cells from oxidative stress.

2. *In Vivo* Assay

Malonic Acid Lesion Model

Procedure

Malonic acid is a competitive inhibitor of succinate dehydrogenase, a key enzyme in both the tricarboxylic acid cycle and oxidative phosphorylation. Injection of malonic acid into the striatum causes ATP depletion, resulting in an excitotoxic lesion (Greene *et al.*, J. Neurochem., 1993, 61, 1151-1154). 2 μL of a 0.5-M malonic acid solution is injected into the right striatum of rats, with or without test compounds. 24 hours after surgery the animals are sacrificed and the size of the lesion measured using TTC histochemistry.

Data

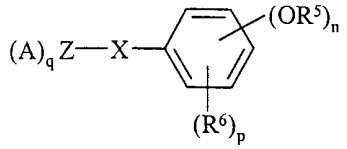
The observed activity of the compounds of Example 1 and Example 7 are displayed in Figures 3 and 4, respectively. (2.2 ng of the compound of Example 1 is the equivalent of

2 μL of a 3.0-μM solution. 7.4 ng of the compound of either Example 1 or Example 7 is the equivalent of 2 μL of a 10.0-μM solution. 74 ng of the compound of Example 7 is the equivalent of 2 μL of a 100.0-μM solution.)

The results demonstrate that the compounds of the present invention have neuroprotective ability in an animal model of oxidative stress.

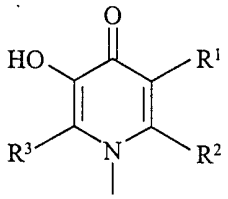
CLAIMS

1. A compound of the formula (1):



(1)

wherein A is



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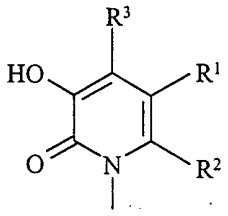
or



(AII)

wherein R^1, R^2 and R^3 are independently selected from H and alkyl;

wherein X is O, S, NR^4 or a direct bond, wherein R^4 is H or alkyl;

wherein Z is a saturated hydrocarbyl chain comprising from 1 to 10 carbon atoms;

wherein q is 1, 2 or 3, wherein if q is 2 or 3, then each A can be the same or different;

wherein the or each R^5 is independently selected from H or alkyl;

wherein the or each R^6 is independently selected from alkyl;

wherein n is 1 to 5;

wherein p is 0 to 4; and

wherein the sum of n and p is less than 6,

or a pharmaceutically acceptable salt thereof.

2. A compound according to claim 1 wherein R^1, R^2 and R^3 are independently selected from H and unsubstituted alkyl.

3. A compound according to claim 1 wherein A is AI and R^2 and R^3 are independently selected from H, unsubstituted alkyl, CH_2OR^7, CH_2OCOR^7, $COOR^7$, CH_2NHR^7, CH_2NHCOR^7 and $CONHR^7$ wherein R^7 is selected from H and alkyl.

4. A compound according to claim 3 wherein R^1 is H or unsubstituted alkyl.

5. A compound according to claim 1 wherein A is AI and R^1 and R^2 are H and R^3 is unsubstituted alkyl.

6. A compound according to claim 1, 2, 3, 4 or 5 wherein R^3 is methyl.

7. A compound according to claim 1 wherein A is AII and R^1, R^2 and R^3 are independently selected from H, unsubstituted alkyl, CH_2OR^7, CH_2OCOR^7, $COOR^7$, CH_2NHR^7, CH_2NHCOR^7 and $CONHR^7$ wherein R^7 is selected from H and alkyl.

8. A compound according to claim 1 wherein A is AII, and R^1, R^2 and R^3 are H.

9. A compound according to any one of claims 1 to 8 wherein q = 1.

10. A compound according to any one of claims 1 to 9 wherein Z is $(CH_2)_m$, wherein m is 1 to 10.

11. A compound according to any preceding claim wherein Z is a hydrocarbyl chain having 2 to 10 carbon atoms.

12. A compound according to any one of claims 1 to 10 wherein Z is a hydrocarbyl chain having 1, 2, 3, 4, 5 or 6 carbon atoms.

13. A compound according to any one of claims 1 to 12 wherein X is O, S or a direct

bond.

14. A compound according to any one of claims 1 to 12 wherein X is NR^4 and R^4 is alkyl.

15. A compound according to claim 14 wherein R^4 is cyclised on to the chain defined as Z.

16. A compound according to any one of the previous claims wherein R^5 is selected from H and C_{1-10} alkyl.

17. A compound according to claim 16 wherein R^5 is H or methyl.

18. A compound according to any preceding claim wherein at least one R^5 is H.

19. A compound according to any one of the preceding claims wherein R^6 is C_{1-10} alkyl.

20. A compound according to any one of the preceding claims wherein R^6 is methyl or t-butyl.

21. A compound according to any one of the preceding claims wherein n is 1 to 3.

22. A compound according to any one of the preceding claims wherein p is 2, 3 or 4.

23. A compound according to any of claims 1 to 14 wherein R^5 is cyclised onto the chain defined as Z.

24. A compound according to any of claims 1 to 14 wherein R^6 is cyclised onto the chain defined as Z.

25. A compound according to claim 10 wherein A is AI, R^1 and R^2 are H, R^3 is methyl, m is 2 or 3, X is a direct bond, R^5 is H, R^6 is t-butyl, n is 1 and p is 2.

26. A compound according to claim 1 which is 1-(3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)propyl)-3-hydroxy-2-methyl-4(1*H*)-pyridinone.

27. A compound according to claim 10 wherein A is AI, R^1 and R^2 are H, R^3 is methyl, m is 3, X is O, R^5 is H, R^6 is methyl, n is 2 and p is 4.

28. A compound according to claim 1 which is 1-(2-(2,3-dihydro-5-hydroxy-4,6,7-trimethylbenzofuran-2-yl)ethyl)-3-hydroxy-2-methyl-4(1*H*)-pyridinone.

29. A compound according to any one of claims 1 to 28 for use in therapy.

30. Use of a compound according to any one claims 1 to 28 in the manufacture of a medicament for the treatment of a condition resulting in oxidative stress.

31. A method of treating a condition resulting in oxidative stress comprising administering to a patient in need of such treatment an effective dose of a compound according to any one of claims 1 to 28.

32. A use or method according to claim 30 or 31 wherein said oxidative stress is oxidative damage of the central nervous system.

33. A use or method according to claim 30 or 31 wherein said condition is an acute or chronic neurological disorder.

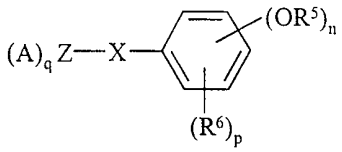
34. A use or method according to claim 33, wherein said neurological disorder is traumatic brain injury, spinal cord injury, cerebral ischaemia, stroke (ischaemic or haemorragic), subharrachnoid haemorrage/cerebral vasospasm, cerebral tumour, Alzheimer's disease, Huntington's disease, Parkinson's disease, Friedrich's ataxia, motor neuron disease or multiple sclerosis.

35. A pharmaceutical composition comprising a compound according to any one of claims 1 to 28 in combination with a pharmaceutically acceptable carrier or excipient.

AMENDED CLAIMS

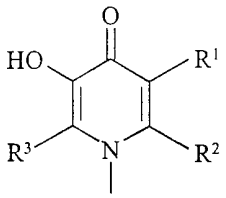
[received by the International Bureau on 21 April 1999 (21.04.99);
original claims 1-35 replaced by amended claims 1-34;
original claim 18 cancelled (4 pages)]

1. A compound of the formula (1):



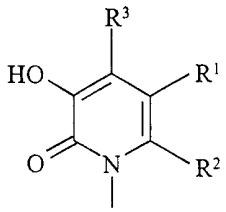
(1)

wherein A is



(AI)

or



(AII)

wherein R^1, R^2 and R^3 are independently selected from H and alkyl;

wherein X is O, S, NR^4 or a direct bond, wherein R^4 is H or alkyl;

wherein Z is a saturated hydrocarbyl chain comprising from 1 to 10 carbon atoms;

wherein q is 1, 2 or 3, wherein if q is 2 or 3, then each A can be the same or different;

wherein the or each R^5 is independently selected from H or alkyl provided that at least one R^5 is H;

wherein the or each R^6 is independently selected from alkyl;

wherein n is 1 to 5;

wherein p is 0 to 4; and

wherein the sum of n and p is less than 6,

or a pharmaceutically acceptable salt thereof.

2. A compound according to claim 1 wherein R^1, R^2 and R^3 are independently selected from H and unsubstituted alkyl.

3. A compound according to claim 1 wherein A is AI and R^2 and R^3 are independently selected from H, unsubstituted alkyl, CH_2OR^7, CH_2OCOR^7, $COOR^7$, CH_2NHR^7, CH_2NHCOR^7 and $CONHR^7$ wherein R^7 is selected from H and alkyl.

4. A compound according to claim 3 wherein R^1 is H or unsubstituted alkyl.

5. A compound according to claim 1 wherein A is AI and R^1 and R^2 are H and R^3 is unsubstituted alkyl.

6. A compound according to claim 1, 2, 3, 4 or 5 wherein R^3 is methyl.

7. A compound according to claim 1 wherein A is AII and R^1, R^2 and R^3 are independently selected from H, unsubstituted alkyl, CH_2OR^7, CH_2OCOR^7, $COOR^7$, CH_2NHR^7, CH_2NHCOR^7 and $CONHR^7$ wherein R^7 is selected from H and alkyl.

8. A compound according to claim 1 wherein A is AII, and R^1, R^2 and R^3 are H.

9. A compound according to any one of claims 1 to 8 wherein $q = 1$.

10. A compound according to any one of claims 1 to 9 wherein Z is $(CH_2)_m$, wherein m is 1 to 10.

11. A compound according to any preceding claim wherein Z is a hydrocarbyl chain having 2 to 10 carbon atoms.

12. A compound according to any one of claims 1 to 10 wherein Z is a hydrocarbyl chain having 1, 2, 3, 4, 5 or 6 carbon atoms.

13. A compound according to any one of claims 1 to 12 wherein X is O, S or a direct bond.

14. A compound according to any one of claims 1 to 12 wherein X is NR^4 and R^4 is alkyl.

15. A compound according to claim 14 wherein R^4 is cyclised on to the chain defined as Z.

16. A compound according to any one of the previous claims wherein R^5 is selected from H and C_{1-10} alkyl.

17. A compound according to claim 16 wherein R^5 is H or methyl.

18. A compound according to any one of the preceding claims wherein R^6 is C_{1-10} alkyl.

19. A compound according to any one of the preceding claims wherein R^6 is methyl or t-butyl.

20. A compound according to any one of the preceding claims wherein n is 1 to 3.

21. A compound according to any one of the preceding claims wherein p is 2, 3 or 4.

22. A compound according to any of claims 1 to 14 wherein R^5 is cyclised onto the chain defined as Z.

23. A compound according to any of claims 1 to 14 wherein R^6 is cyclised onto the chain defined as Z.

24. A compound according to claim 10 wherein A is Al, R^1 and R^2 are H, R^3 is methyl, m is 2 or 3, X is a direct bond, R^5 is H, R^6 is t-butyl, n is 1 and p is 2.

25. A compound according to claim 1 which is 1-(3-(3,5-di-*tert*-butyl-4-

hydroxyphenyl)propyl)-3-hydroxy-2-methyl-4(1*H*)-pyridinone.

26. A compound according to claim 10 wherein A is AI, R^1 and R^2 are H, R^3 is methyl, m is 3, X is O, R^5 is H, R^6 is methyl, n is 2 and p is 4.

27. A compound according to claim 1 which is 1-(2-(2,3-dihydro-5-hydroxy-4,6,7-trimethylbenzofuran-2-yl)ethyl)-3-hydroxy-2-methyl-4(1*H*)-pyridinone.

28. A compound according to any one of claims 1 to 27 for use in therapy.

29. Use of a compound according to any one claims 1 to 27 in the manufacture of a medicament for the treatment of a condition resulting in oxidative stress.

30. A method of treating a condition resulting in oxidative stress comprising dministering to a patient in need of such treatment an effective dose of a compound according to any one of claims 1 to 27.

31. A use or method according to claim 29 or 30 wherein said oxidative stress is oxidative damage of the central nervous system.

32. A use or method according to claim 29 or 30 wherein said condition is an acute or chronic neurological disorder.

33. A use or method according to claim 32, wherein said neurological disorder is traumatic brain injury, spinal cord injury, cerebral ischaemia, stroke (ischaemic or haemorragic), subharrachnoid haemorrage/cerebral vasospasm, cerebral tumour, Alzheimer's disease, Huntington's disease, Parkinson's disease, Friedrich's ataxia, motor neuron disease or multiple sclerosis.

34. A pharmaceutical composition comprising a compound according to any one of claims 1 to 27 in combination with a pharmaceutically acceptable carrier or excipient.

Statement Under Article 19

The claims of this Application have been amended by the incorporation into original claim 1 of the features of original claim 18. Amended claim 1 now requires that, in the compounds of the present invention, at least one R^5 group is hydrogen, i.e. that the phenyl ring of the compounds of formula (1) of the present invention must be substituted by at least one hydroxy group.

None of the documents cited in the International Search Report disclose compounds containing a hydroxy-substituted phenyl ring. Accordingly, the claims as amended are novel over the prior art.

The category X citations relate to two articles, M. M. Jones *et al.* (Arz. Forsch. Drug Res. 46(12), 1996, 1158-1162) and P. K. Singh *et al.* (Arz. Forsch. Drug. Res. 47(3), 1997, 311-315). Both documents disclose compounds having trypanocidal activity as a result of their ability to inactivate crucial intracellular enzymes of *Trypanosoma Cruzi* such as superoxide dismutase (SOD). SOD is an anti-oxidant enzyme that removes the oxidising species superoxide. It follows that these compounds would therefore be pro-oxidant and that administration of such compounds would be expected to lead to a condition of oxidative stress.

In view of this prior art it is therefore unexpected that, rather than being pro-oxidant, the compounds of the present Application show anti-oxidant properties and have been found to reduce oxidative stress upon administration *in vivo* and *in vitro*. It is believed that the presence of the hydroxy group on the phenyl ring is important for the anti-oxidant activity of the compounds of the present Application.

Accordingly, the claims are novel and inventive over this prior art.

FIG. 1

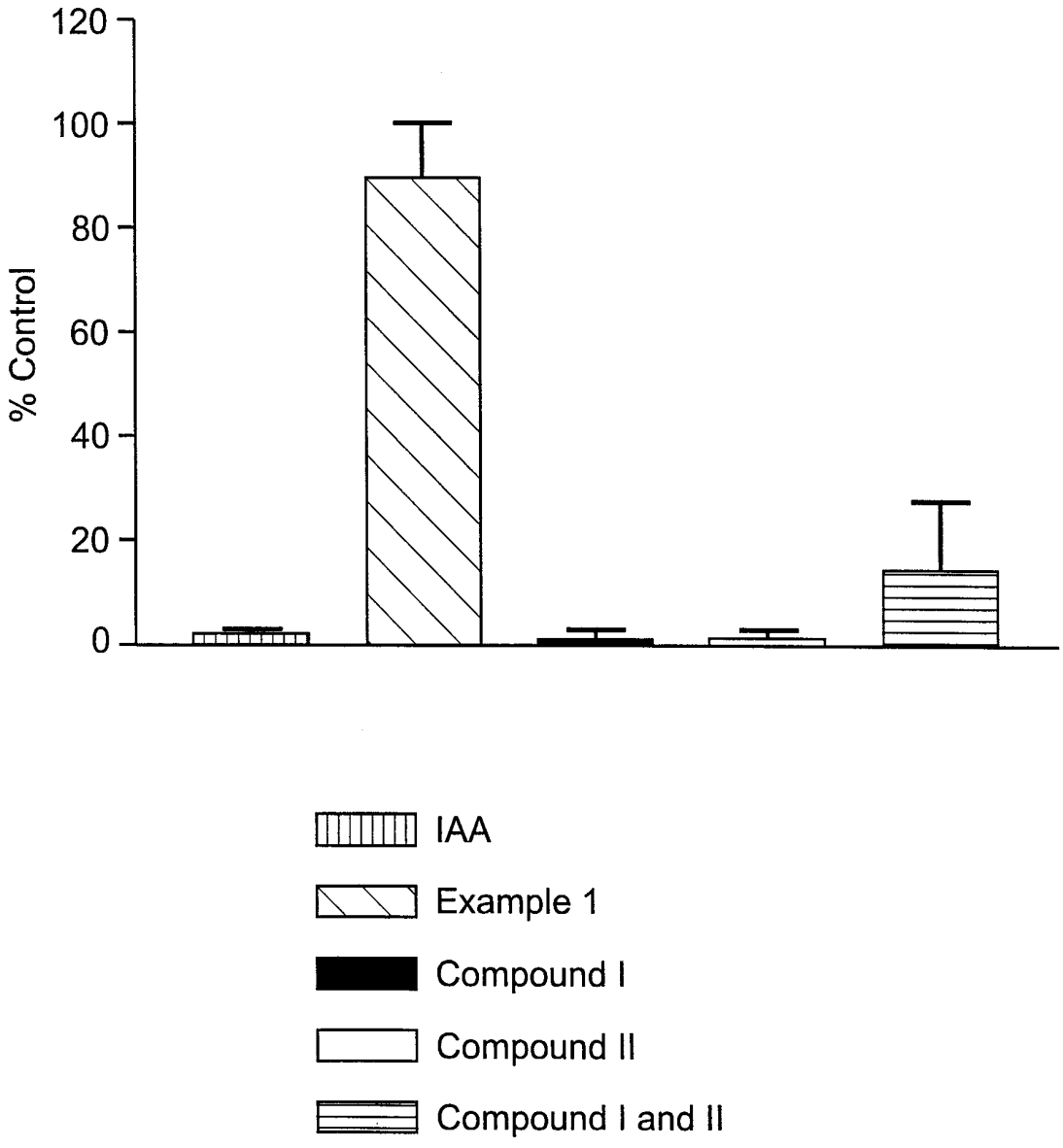

120
100
80
60
40
20
0
% Control
IAA
Example 1
Compound I
Compound II
Compound I and II

FIG. 2

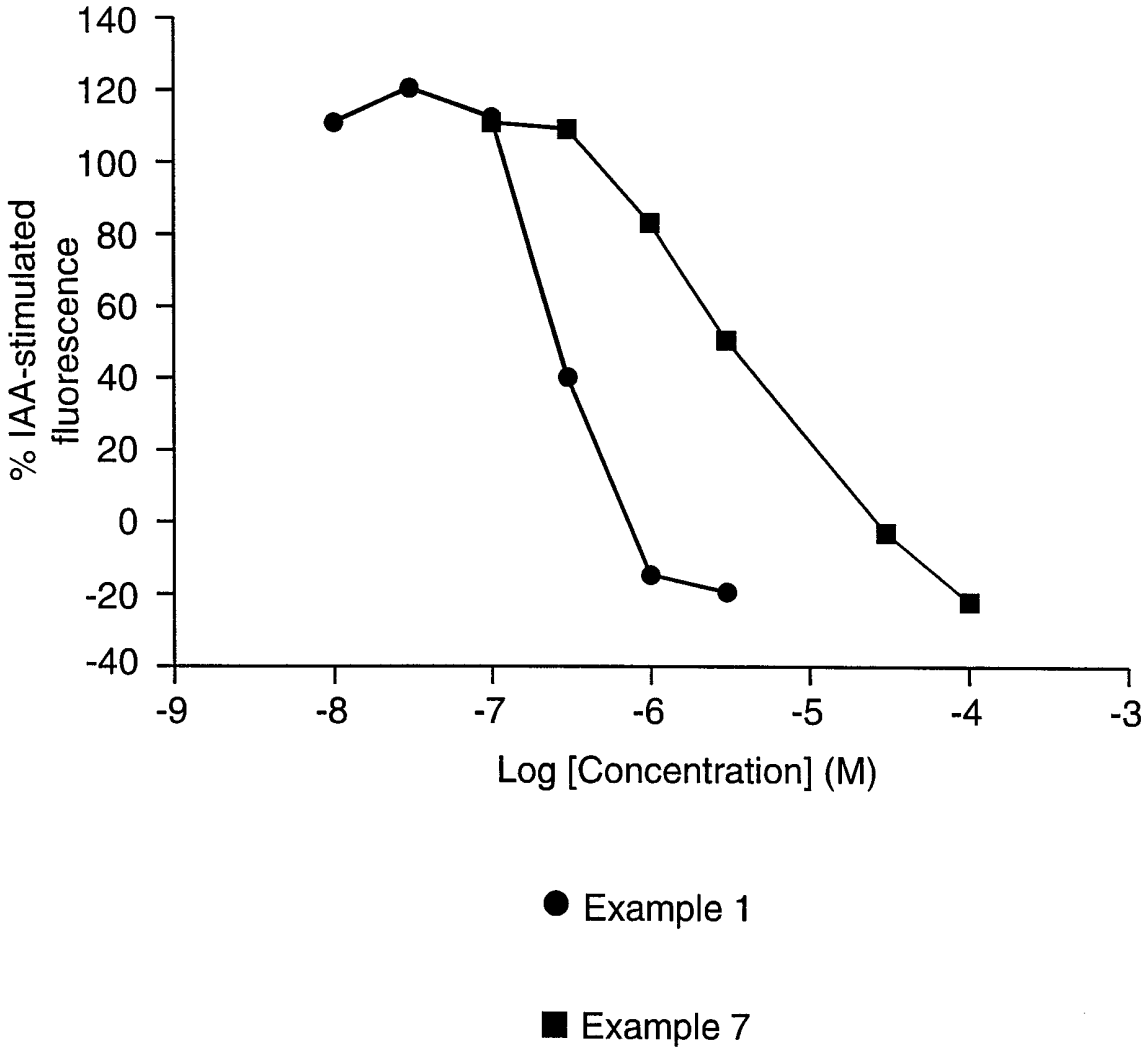

140
120
100
80
60
40
20
0
-20
-40
% IAA-stimulated fluorescence
-9
-8
-7
-6
-5
-4
-3
Log [Concentration] (M)
Example 1
Example 7

FIG. 3

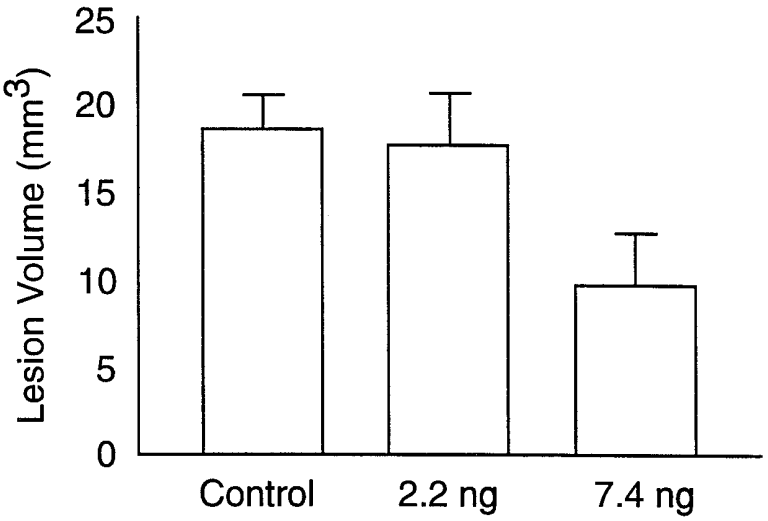
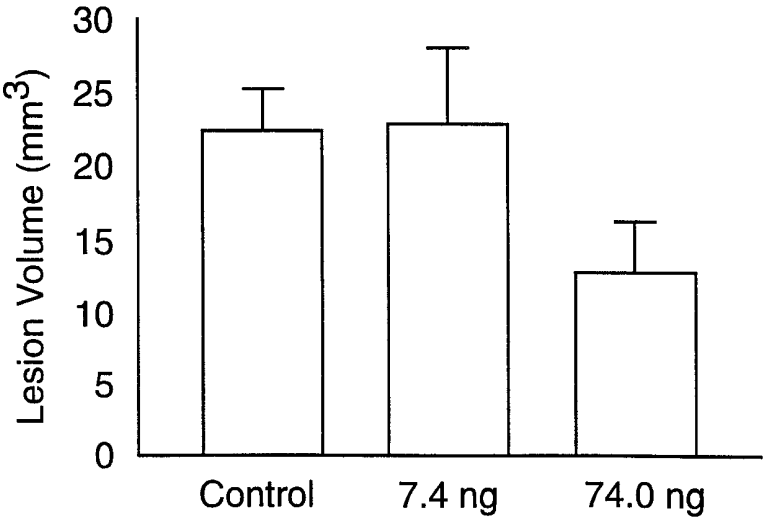

Lesion Volume (mm^3)
25
20
15
10
5
0
Control
2.2 ng
7.4 ng

FIG. 4


Lesion Volume (mm^3)
30
25
20
15
10
5
0
Control
7.4 ng
74.0 ng

INTERNATIONAL SEARCH REPORT

Interi nal Application No
PCT/GB 98/03244

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07D213/69 C07D405/06 C07D401/04 A61K31/44
//(C07D405/06,307:00,211:00),(C07D405/06,311:00,211:00)

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 6 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)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CHEMICAL ABSTRACTS, vol. 126, no. 7, 17 February 1997 Columbus, Ohio, US; abstract no. 84159, XP002093472	1,35
Y	see the whole document & M.M. JONES ET AL.: ARZNEIMITTEL FORSCHUNG DRUG RESEARCH., vol. 46, no. 12, 1996, pages 1158-1162, AULENDORF DE & FILE REGISTRY (DATABASE STN CAS):	1-35
X	see RN 185743-68-4, 185743-69-5	1,35

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

[X] 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	Date of mailing of the international search report
15 February 1999	02/03/1999

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, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Frelon, D

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INTERNATIONAL SEARCH REPORT

Interi nal Application No
PCT/GB 98/03244

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	CHEMICAL ABSTRACTS, vol. 126, no. 22, 2 June 1997 Columbus, Ohio, US; abstract no. 287657, XP002093473	1,35
Y	see the whole document & P.K. SINGH ET AL.: ARZNEIMITTEL FORSCHUNG DRUG RESEARCH., vol. 47, no. 3, 1997, pages 311-315, AULENDORF DE & FILE REGISTRY (DATABASE STN CAS):	1-35
X	see RN 189131-44-0	1,35

Y	EP 0 120 670 A (NATIONAL RESEARCH DEVELOPMENT CORP.) 3 October 1984 see abstract; claim 1	1-35

Y	EP 0 768 302 A (NOVARTIS A.G.) 16 April 1997 see abstract; claim 1	1-35

Y	US 5 624 901 A (RAYMOND ET AL.) 29 April 1997 see abstract; claim 1	1-35

A	P.S. DOBBIN ET AL.: JOURNAL OF MEDICINAL CHEMISTRY, vol. 36, 1993, pages 2448-2458, XP002064973 WASHINGTON US see the whole document	1-35

A	M. STREATER ET AL.: JOURNAL OF MEDICINAL CHEMISTRY, vol. 33, 1990, pages 1749-1755, XP002064972 WASHINGTON US see the whole document	1-35

A	CHEMICAL ABSTRACTS, vol. 121, no. 21, 21 November 1994 Columbus, Ohio, US; abstract no. 248239, XP002093474 see abstract & J.J. MOLENDA ET AL: CHEM. RES. TOXICOL., vol. 7, no. 6, 1994, pages 815-822,	1-35

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INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/GB 98/03244

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	CHEMICAL ABSTRACTS, vol. 121, no. 9, 29 August 1994 Columbus, Ohio, US; abstract no. 99755, XP002093475 see abstract & JOHN JOSEF MOLENDA ET AL.: J. INORG. BIOCHEM., vol. 55, no. 2, 1994, pages 131-146, ---	1-35
A,P	WO 98 25905 A (CENES LTD) 18 June 1998 cited in the application see abstract ---	1-35
A	WO 93 10822 A (THE DU PONT MERCK PHARMACEUTICAL COMPANY) 10 June 1993 see abstract; example 4 -----	1-35

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FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

The drafting of the claims is sometimes combersome or unclear, like, for instance, where impossible references are made in claims 3, 15, 23 or 24 to claim 1 due to different definitions of same groups or like in claims 32 to 34 referring to claim 31 which a non permissible claim wording (see remark). The search report is therefore based on the subject-matter as illustrated by the examples and onto the general idea of the alleged invention.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inter ional Application No
PCT/GB 98/03244

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 120670 A	03-10-1984	DE 3475706 A	26-01-1989
		DK 165984 A,B,	25-09-1984
		GB 2136806 A,B	26-09-1984
		HK 5190 A	02-02-1990
		JP 1897304 C	23-01-1995
		JP 6025120 B	06-04-1994
		JP 59181258 A	15-10-1984
		US 4650793 A	17-03-1987
EP 768302 A	16-04-1997	AU 6584596 A	10-04-1997
		BR 9603929 A	09-06-1998
		CA 2186716 A	30-03-1997
		CN 1151399 A	11-06-1997
		CZ 9602848 A	16-04-1997
		HU 9602681 A	29-09-1997
		JP 9124603 A	13-05-1997
		NO 964095 A	01-04-1997
		NZ 299452 A	26-06-1998
		US 5688815 A	18-11-1997
US 5624901 A	29-04-1997	NONE	
WO 9825905 A	18-06-1998	AU 7847198 A	03-07-1998
WO 9310822 A	10-06-1993	US 5336482 A	09-08-1994
		AU 3230993 A	28-06-1993
		EP 0614378 A	14-09-1994
		HU 69717 A	28-09-1995
		JP 7502501 T	16-03-1995