Title: TREATMENT OF RETINAL DEGENERATION

Abstract: The use of a compound of general formula (I): or a pharmaceutically acceptable salt thereof, in the manufacture of a medicament for the treatment or prevention of a disease or condition characterised by apoptosis or degeneration of mammalian cells, wherein: R1 is a alkoxy, alky, ether or ester group; R2 is H or has the formula wherein Y is linear or branched, saturated or unsaturated, aliphatic group with from 2 to 23 carbon atoms, or a cyclic group, and which can contain substituents selected from the group consisting of hydroxy, alkoxy, amino, carboxyl, cyano, nitro, alkyloxynitro or halogen atoms, is O or S, and R3 is any substituent.
before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(b))

Published:

— with international search report (Art. 21(3))
TREATMENT OF RETINAL DEGENERATION

TECHNICAL FIELD

The invention relates to a method of treating or preventing a disease or condition characterised by apoptosis or degeneration of mammalian cells, especially retinal photoreceptive cells.

BACKGROUND TO THE INVENTION

The loss of retinal cells in Age-related Macular Degeneration (AMD) and Glaucoma are the two leading causes of blindness in the developed world. Retinitis Pigmentosa (RP) is a rarer related condition that also leads to loss of sight. RP is a group of hereditary disorders of the retina caused by mutations in numerous genes involved in photoreceptor structure or function. The disease is characterized by early loss of photoreceptors leading to blindness. Glaucoma is caused by a number of different pathological mechanisms that in most cases result in elevated intraocular pressure (IOP) within the eye. Like RP, it is a multiple gene-related disease and genetic factors play a complex role in glaucoma predisposition. Over time, the increase in IOP causes damage to the optic nerve and gradual and continuous loss of retinal ganglion cells. Cell loss in AMD (Age-related Macular Degeneration) also occurs as a result of apoptosis of retinal pigment epithelial (RPE) cells followed by apoptosis of photoreceptors. A central feature of each of the above diseases is that retinal cell loss occurs by a cell death process known as apoptosis.

There are no drugs on the market for the treatment of RP. There are a small number in early stage development, but most of them rely on repeated intravitreal injections directly into the eye for their effect or a gene therapy approach. For RP, there is a market realisation that preventing apoptosis may be a treatment option. In the case of glaucoma there are several treatment options currently available for this very large market segment. Most of these rely on the delivery of the drug in the form of eye drops. The goal of such treatments is to reduce the intraocular pressure that is a causative factor that leads to the
loss of retinal ganglion cells. Like RP there are no current treatments focused on the prevention of retinal ganglion cell apoptosis. Finally, for AMD of which there are two main forms there are a number of treatment options ranging from laser therapy to the use of inhibitors that prevent blood vessel proliferation which is a characteristic of the condition and leads to loss of photoreceptor cells. Again each of these treatment options is invasive and requires repeated hospital visits.

It is an object of the invention to overcome at least one of the above problems.

BRIEF SUMMARY OF THE INVENTION

Accordingly, the invention relates to a method of treating or preventing a disease or condition characterised by apoptosis or degeneration of mammalian cells, especially retinal photoreceptive cells. The method of the invention comprises a step of treating an individual with a therapeutically effective amount of a compound of general formula (I)

\[
\begin{align*}
R_1 & \quad R_2 \quad O \\
R_2 & \quad O \quad R_3
\end{align*}
\]

or a pharmaceutically acceptable salt thereof, wherein:

R1 is a alkoxy, alkyl, ether or ester group;
R2 is H or has the formula \( Y - X \) wherein Y is linear or branched, saturated or unsaturated, aliphatic group with from 2 to 23 carbon atoms, or a cyclic group, and which can contain substituents selected from the group consisting of hydroxyl, alkoxy, amino, carboxyl, cyano, nitro, alkylsulphonyl or halogen atoms, \( X = O \) or \( S \); and \( R3 \) is any substituent (hereafter “Active”).

In a preferred embodiment of the invention, \( R1 \) is a methoxy group, and \( R2 \) is typically H.

In one embodiment, the Active is a compound of general formula (II),

\[ \begin{align*}
&\text{or a pharmaceutically acceptable salt thereof, in which: } Y \text{ is linear or branched, saturated or unsaturated, aliphatic group with from 2 to 23 carbon atoms, or a cyclic group, and which can contain substituents selected from the group consisting of hydroxyl, alkoxy, amino, carboxyl, cyano, nitro, alkylsulphonyl or halogen atoms; } X = O \text{ or } S; \text{ and } R3 \text{ is any substituent.} \\
&\text{Suitably, } \begin{align*}
Y - X
\end{align*}\text{ is selected from the group consisting of tert-butanoyl, hexanoyl, 2-ethylhexanoyl, octanoyl, decanoyl, lauroyl, myristoyl, palmitoyl, stearoyl, oleoyl, or lineoayl.}
\end{align*} \]
Typically, Y is an alicyclic group, or an aromatic cyclic group, or a heterocyclic group.

In one embodiment, \( Y \) is selected from the group consisting of \(-\text{CO-(CH}_2\text{)}_6\text{phenyl}, -\text{CO-(CH}_2\text{)}_6\text{(1-napthyl)}, -\text{CO-(CH}_2\text{)}_6\text{(2-napthyl)}, -\text{CO-(CH}_2\text{)}_6.\)
\(6\text{CH(phenyl)}_2\), -\text{CO-(2-fluorophenyl)}, -\text{CO-cyclohexyl, \( \alpha \)-lipooyl, L-prolyl, D-prolyl, biotinyl-CO-(4-imidazolyl), -CO-(2-pyridyl), -CO-(2-thienyl), -CO-(2-furyl), -CO-(3-furyl).}\)

In a preferred embodiment of the invention, \( X \) is O.

In one embodiment, \( R_1 \) is a methoxy group and \( OR_2 \) is a hydroxyl group (BP – Figure 1A).

In another embodiment, \( R_1 \) is a methoxy group and \( OR_2 \) is an acetate ester (Derivative BP-1 – Figure 1B).

In another embodiment, \( R_1 \) is a methoxy group and \( OR_2 \) is a pivalate ester (Derivative BP-2 – Figure 1C).

In one embodiment, \( R_1 \) is a methoxy group and \( OR_2 \) is a laureate ester (Derivative BP-4 – Figure 1E).

In another embodiment, \( R_1 \) is a methoxy group and \( OR_2 \) is a 2-methylhexanate ester (Derivative BP-3 – Figure 1D).

In one embodiment, \( R_1 \) is a methoxy group and \( OR_2 \) is a phenyl ester (Derivative BP-5 – Figure 1F).
In another embodiment, \( R_1 \) is a methoxy group and \( OR_2 \) is a \( \alpha \)-fluorophenyl ester (Derivative BP-6 – Figure 1G).

In one preferred embodiment, the Active is 3,4-dihydro-6-hydroxy-7-methoxy-2,2-dimethyl-1(2H)-benzopyran (BP).

In another embodiment, the Active is a compound of general formula (III),

![Chemical structure](image)

or a pharmaceutically acceptable salt thereof, in which \( X \) is \( O \) or \( S \), and \( R_3 \) is any substituent.

In another embodiment, the Active is a compound of general formula (IV),

![Chemical structure](image)

or a pharmaceutically acceptable salt thereof, in which \( R_3 \) is any substituent.

Typically \( R_3 \) is selected from the group consisting of: \( H \); halogen; lower alkyl; lower alkoxy; hydroxyl; amine; thiol; \( \text{NHR}_4 \); or a substituted or unsubstituted aromatic ring structure in which the substituents (if included) are selected from the groups consisting of \( H \), halogen, lower alkyl, lower alkoxy, hydroxyl, amine, and thiol, and wherein \( R_4 \) is any substituent. Suitably, \( R_4 \) is selected from the group consisting of: halogen; lower alkyl;
lower alkoxy; hydroxyl; amine; thiol; NHR4; or a substituted or unsubstituted aromatic ring structure in which the substituents (if included) are selected from the groups consisting of H, halogen, lower alkyl, lower alkoxy, hydroxyl, amine, and thiol.

In a preferred embodiment, R3 and R4 are, independently, C4 to C8 straight alkyl chains, preferably a C5 to C7 straight alkyl chain, and ideally a C6 straight alkyl chain.

In one embodiment, the Active is selected from the group consisting of:

The Active is administered in a therapeutically effective amount to treat or prevent the disease or condition. When the invention relates to therapy, as opposed to prophylaxis, the individual is generally one in need of such treatment such as a patient having a retinal degenerative condition. Suitably, the disease or condition is an retinal degenerative disease, such as, for example, Retinitis Pigmentosa (RP), Glaucoma, or Age-related Macular Degeneration (AMD). In an alternative embodiment, the disease or condition is a mammalian degenerative disease, such as a neurodegenerative disease.

The invention also relates to the use of the Active as a medicament. Suitably, the medicament is for treating a retinal degenerative disease, especially Retinitis Pigmentosa (RP), Glaucoma, or Age-related Macular Degeneration (AMD).

The invention also relates to the use of the Active in the manufacture of a medicament for the treatment or prevention of a disease or condition characterised by apoptosis or degeneration of mammalian cells. In particular, the invention relates to the use of the
Active in the manufacture of a medicament for the treatment or prevention of an retinal degenerative condition such as Retinitis Pigmentosa (RP), Glaucoma, or Age-related Macular Degeneration (AMD).

The invention also relates to the Active compounds of Formula (I), (II) or (III), or pharmaceutically acceptable salts thereof. The invention also relates to the Active compound of Figure 1D, or pharmaceutically acceptable salts thereof.

The invention also relates to a pharmaceutical formulation comprising an Active compound of the invention, in combination with a suitable pharmaceutical excipient. The invention also relates to the use of an Active compound of the invention as a medicament.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1: Shows the chemical structure of BP and BP derivatives, including BP (Fig. 1A), BP-1 (Fig. 1B), BP-2 (Fig. 1C), BP-3 (Fig. 1D), BP-4 (Fig. 1E), BP-5 (Fig. 1F), BP-6 (Fig. 1G), BP-X (Fig. 1H), and BP-Y (Fig. 1I).

Figure 2: Is a histogram of results showing the effectiveness of a 25μM concentration of each of the BP derivatives BP-1 to BP6 against increasing concentrations of SNP in photoreceptor cells. Error bars are +/- standard deviation-SD.

Figure 3: Is a histogram showing the scavenging ability of each of the BP derivatives BP-1 to BP6 at 25μM in photoreceptor cells in the presence of increasing concentrations of SNP. Error bars are +/- SD.

Figure 4: Is a histogram of % protection afforded by a 25μM concentration of each BP derivative BP1 to BP6 in photoreceptor cells as a function of the lipophilicity of each derivative. (P is a logarithmic value that indicates the lipophilicity of a compound).
While BP4 appears to have the greater protective capacity this derivative is very lipophilic rendering it problematic for administration. It is also a highly unstable compound.

Lipophilicity of the compounds is as follows: BP1<BP2<BP3=BP5=BP6<BP4
With lower lipophilicity, BP3 is far more stable than BP4, and hence the chosen derivative.

**Figures 5 and 6:** These figures show that both BP and BP3 protect photoreceptor cells from SNP induced apoptosis. Both BP and BP3 are effective at 25μM but BP3 is equally as effective at a concentration 10 times lower than BP.

**Figure 7:** In this figure the study was extended from *ex vivo* cultures to the light damage model *in vivo*. In this acute model of retinal disease albino balb/c mice are exposed to excessive white light causing the photoreceptor cells to die by apoptosis. This figure shows that mice exposed to bright white light undergo extensive apoptosis at 24, 48 and 72 hours post light damage. 1 hour pre-treatment with 200mg/kg BP3 protects from the retinal damage observed. The protection extends to 48 hours with only a single injection of BP3. Administration of a second dose, or a ‘top up’ of 200mg/kg BP3 at 24 hours post light damage affords significant protection at 72 hours.

**Figure 8** is a histogram of the results from Figure 7. TUNEL positive cells in three independent retinas were counted. Counts were performed on the central 40x field of the outer nuclear layer (ONL) and graphed with error bars (+/- standard deviation-SD). The graph demonstrates approx 40% protection from light induced cell death in the central retina at 24, 48 and 72 hours.

**Figure 9A** shows the results obtained in a chronic model of retinal degeneration called the rd10 model. In this model photoreceptor cells degenerate more slowly than the light model, over weeks rather than hours to days. Cell death is evident from postnatal day 18 (P18) and by P25 a significant loss of photoreceptor cell layers is observed. Figure 9 A shows protection from photoreceptor cell death in the ONL when mice are injected daily
with 200mg/kg BP3. In this model, the central and peripheral retina degenerate at different rates. The loss of photoreceptors is greater in the central retina than the periphery between P18 and P25.

**Figure 9B,** a histogram of the data shown in Figure 9A, show that both the central and peripheral (divided into inferior and superior regions as illustrated by the schematic) areas of the retina are protected. Furthermore Figure 9B shows that BP3 injection on alternate days affords the same level of protection as a daily injection regimen in all retinal areas.

**Figure 10:** The ONL comprises two types of cell; rod photoreceptors and cone photoreceptors. Figure 10 identifies cell types which are protected by BP3 treatment when administered on alternate days from P18 to P25. Rhodopsin is a protein specifically found in the outer segments (OS) of rod photoreceptors. Immunofluorescent staining using an antibody specific to rhodopsin indicates that more rhodopsin positive cells remain in the ONL of BP3 treated mice compared to vehicle. Peanut agglutinin (PNA) is a lectin which binds to carbohydrates found in the outer membrane of cone photoreceptors but which are absent from rods. Staining of retinal sections with PNA indicates that more cone cells are found in the ONL following BP3 treatment. Therefore the cells of the ONL involved in both colour and black/white vision are protected in the rd10 model by BP3.

**Figure 11** shows the results obtained in an acute model of Glaucoma: NMDA-induced excitotoxicity. In this model intravitreal injection of 40mM NMDA results in the progressive loss of ganglion cells from the ganglion cell layer (GCL), accompanied by a thinning of the inner plexiform layer (IPL) over time. Figure 11 shows the loss of cells in the GCL and the reduced IPL at 48 and 72 hours post insult. Administration of 200mg/kg BP3 1 hour prior to NMDA injection significantly attenuates both ganglion cell loss and IPL thinning.
Figure 12 shows the onset of apoptotic cell death in the GCL at 4 hours post NMDA injection. Ganglion cell death is significantly greater by 24 hours post damage at which point, cells of the inner nuclear layer (INL) also undergo cell death. By 48 and 72 hours post excitotoxicity the eventual loss of cells of the ONL is evident. Intraperitoneal (I.P.) injection of 200mg/kg BP3 1hour prior to NMDA injection significantly protects retinal cells in all layers at all time points examined, indicating universal protection by BP3 in the retina.

Figure 13 is a histogram of the data from Figure 12. TUNEL positive cells were counted across all the retinal layers from the inferior through the central to the periphery at the indicated timepoints. The graph indicates significant protection from NMDA-induced cell death at 4, 24, 48 and 72 hours.

DETAILED DESCRIPTION OF THE INVENTION

The therapeutic method, and therapeutic products, of the invention are directed against diseases or conditions characterised by apoptosis or degeneration of mammalian cells. In one embodiment of the invention, the disease or condition characterised by apoptosis or degeneration of mammalian cells is an ocular disease or condition, especially a retinal degenerative condition or disease. The invention is particularly applicable for the treatment/prevention of retinal dystrophies. In one embodiment of the invention, the disease or condition characterised by apoptosis or degeneration of mammalian cells, is a neurodegenerative disease. Typically, the neurodegenerative disease is selected from the group comprising: motor neurone disease (ALS) or variants thereof including primary lateral sclerosis and spinal muscular atrophy; prion disease; Huntington’s disease; Parkinson’s disease; Parkinson’s plus; Tauopathies; Chromosome 17 dementias; Alzheimer’s disease; Multiple sclerosis (MS); hereditary neuropathies; and diseases involving cerebellar degeneration.
In a preferred embodiment of the invention, the retinal degenerative condition is selected from the group comprising: RP; Glaucoma; retinopathies; and AMD.

“Lower alkyl” means an alkyl group, as defined below, but having from one to ten carbons, more preferable from one to six carbon atoms (eg. “C – C – alkyl”) in its backbone structure. “Alkyl” refers to a group containing from 1 to 8 carbon atoms and may be straight chained or branched. An alkyl group is an optionally substituted straight, branched or cyclic saturated hydrocarbon group. When substituted, alkyl groups may be substituted with up to four substituent groups, at any available point of attachment. When the alkyl group is said to be substituted with an alkyl group, this is used interchangeably with “branched alkyl group”. Exemplary unsubstituted such groups include methyl, ethyl, propyl, isopropyl, a-butyl, isobutyl, pentyl, hexyl, isohexyl, 4, 4-dimethylpentyl, octyl, 2,2,4-trimethylpentyl, nonyl, decyl, undecyl, dodecyl, and the like. Exemplary substituents may include but are not limited to one or more of the following groups: halo (such as F, Cl, Br, I), Haloalkyl (such as CC13 or CF13), alkoxy, alkylthio, hydroxyl, carboxy (-COOH), alkoxy carbonyl (-C(O)R), alkylcarbonyloxy (-OCOR), amino (-NH2), carbamoyl (-NHCOR-or-OCONHR), urea (-NHCONHR-) or thiol (-SH). Alkyl groups as defined may also comprise one or more carbon double bonds or one or more carbon to carbon triple bonds.

“Lower alkoxy” refers to O-alkyl groups, wherein alkyl is as defined hereinabove. The alkoxy group is bonded to the core compound through the oxygen bridge. The alkoxy group may be straight-chained or branched; although the straight-chain is preferred. Examples include methoxy, ethoxyloxy, propanoxy, butyloxy, t-butyloxy, i-propoxy, and the like. Preferred alkoxy groups contain 1-4 carbon atoms, especially preferred alkoxy groups contain 1-3 carbon atoms. The most preferred alkoxy group is methoxy.

“Halogen” means the non-metal elements of Group 17 of the periodic table, namely bromine, chlorine, fluorine, iodine and astatine.
“Salt” is a pharmaceutically acceptable salt and can include acid addition salts such as the hydrochlorides, hydrobromides, phosphates, sulphates, hydrogen sulphates, alkylsulphates, aroylsulphonates, acetates, benzoates, citrates, maleates, fumarates, succinates, lactates, and tartrates; alkali metal cations such as Na, K, Li; alkali earth metal salts such as Mg or Ca; or organic amine salts. Exemplary organic amine salts are tromethamine (TRIS) salts and amino acid salts (e.g. histidine salts) of the compounds of the invention.

In this specification the term “therapeutically effective amount” should be taken to mean an amount which results in a clinically significant reduction of degeneration or apoptosis in cells having a phenotype characteristic of a degenerative condition (i.e. retinal photoreceptor cells from a patient with a retinal dystrophy, for example AMD or RP). Suitably, the Active is administered at a dose of between 1 microgram and 10 milligrams per ml, preferably between 10 micrograms and 5 milligrams per ml, more preferably between 100 micrograms and 2 milligrams per ml. Typically, it is given as a bolus dose. However, when continuous infusion is used, such as by intrathecal pump, the Active may be administered at a dosage rate of between 5 and 20 μg/kg/minute, preferably between 7 and 15 μg/kg/minute. In the context of the therapeutic aspects of the present invention, the term “individual in need thereof” shall be taken to mean an individual who is afflicted with a disease or condition which involves apoptosis or degeneration of mammalian cells, especially apoptosis or degeneration of the photoreceptive cell. Retinal degenerative conditions or diseases such as RP, Glaucoma, Retinopathies, and AMD, and variants thereof as described herein, are examples of such diseases or conditions.

In one embodiment of the invention, an individual in treated with the Active by direct delivery of the Active by a means selected from the group: intravenous delivery; intraperitoneal delivery; oral delivery; intramuscular delivery; intrathecal delivery; and inhaled delivery. Methods for achieving these means of delivery will be well known to those skilled in the art of drug delivery. Specific examples are provided below:

- Intramuscular- delivery directly into muscle(s) by syringe or mini osmotic pump (Azzouz et al., Nat Med. 2005;11(4):429-33).
- Subcutaneous- for systemic administration. Directly administered below the skin by syringe (Reinholz et al., Exp Neurol. 1999;159(1):204-16).
- Intraventricular- direct administration to the ventricles in the brain, by injection or using small catheter attached to an osmotic pump.(Sathasivam et al., 2005 Neuropath App Neurobiol; 31(5): 467)
- Implant- Active can be prepared in an implant (eg small silicon implant) that will release the active. Implant can be placed at muscles or directly onto the spinal cord (Kieran and Greensmith, 2004 Neurosci 125(2):427-39).

In a particularly preferred embodiment of the invention, in which the indication is a retinal dystrophy, the active may be administered by direct intraocular or intravitreal injection, by topical application by means of eye drops, or by oral gavage.

In one embodiment of the therapy of the invention, the Active is linked to a coupling partner, e.g. an effector molecule, a label, a drug, a toxin and/or a carrier or transport molecule. Techniques for coupling the Active of the invention to both peptidyl and non-peptidyl coupling partners are well known in the art.

The invention provides methods of treatment and prevention of diseases or conditions characterized by apoptosis or degeneration of mammalian cells, especially photoreceptive cells, by administration to a subject in need of such treatment of a therapeutically or prophylactically effective amount of the Active. The subject is preferably an animal, including, but not limited to, animals such as monkeys, cows, pigs, horses, chickens, cats, dogs, etc., and is preferably a mammal, and most preferably human.

Apart from the specific delivery systems embodied below, various delivery systems are known and can be used to administer the Active of the invention, e.g., encapsulation in
liposomes, microparticles, microcapsules. Methods of introduction include but are not limited to intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, and oral routes. The Active may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (e.g., oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents. Administration can be systemic or local. In addition, it may be desirable to introduce the Active of the invention into the central nervous system by any suitable route, including intraventricular and intrathecal injection; intraventricular injection may be facilitated by an intraventricular catheter, for example, attached to a reservoir, such as an Ommaya reservoir. Pulmonary administration can also be employed, e.g., by use of an inhaler or nebulizer, and formulation with an aerosolizing agent.

In a specific embodiment, it may be desirable to administer the Active of the invention locally to the area in need of treatment; this may be achieved, for example, by means of eye drops, intraocular injection, or an implant, said implant being of a porous, non-porous, or gelatinous material, including membranes, such as sialastic membranes, or fibers.

In another embodiment, the Active can be delivered in a vesicle, in particular a liposome (see Langer, Science 249:1527-1533 (1990); Treat et al., in Liposomes in the Therapy of Infectious Disease and Cancer, Lopez-Berestein and Fidler (eds.), Liss, New York, pp. 353-365 (1989); Lopez-Berestein, ibid., pp. 317-327; see generally ibid.)

In yet another embodiment, the Active can be delivered in a controlled release system. In one embodiment, a pump may be used (see Langer, supra; Sefton, CRC Crit. Ref. Biomed., Eng. 14:201 (1987); Buchwald et al., Surgery 88:75 (1980); Saudek et al., N. Engl. J. Med. 321:574 (1989)). In another embodiment, polymeric materials can be used (see Medical Applications of Controlled Release, Langer and Wise (eds.), CRC Pres., Boca Raton, Fla. (1974); Controlled Drug Bioavailability, Drug Product Design and Performance, Smolen and Ball (eds.), Wiley, New York (1984); Ranger and Peppas, J.
Macromol. Sci. Rev. Macromol. Chem. 23:61 (1983); see also Levy et al., Science 228:190 (1985); During et al., Ann. Neurol. 25:351 (1989); Howard et al., J. Neurosurg. 71:105 (1989)). In yet another embodiment, a controlled release system can be placed in proximity of the therapeutic target, thus requiring only a fraction of the systemic dose (see, e.g., Goodson, in Medical Applications of Controlled Release, supra, vol. 2, pp. 115-138 (1984)). Other controlled release systems are discussed in the review by Langer (Science 249:1527-1533 (1990)).

The present invention also provides pharmaceutical compositions comprising the Active. Such compositions comprise a therapeutically effective amount of the Active, and a pharmaceutically acceptable carrier. In a specific embodiment, the term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans.

The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the Active is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is a preferred carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene glycol, water, ethanol and the like.

The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. These compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations and the like.
The composition can be formulated as a suppository, with traditional binders and carriers such as triglycerides. Oral formulation can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc. Examples of suitable pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by E. W. Martin. Such compositions will contain a therapeutically effective amount of the therapeutic, preferably in purified form, together with a suitable amount of carrier so as to provide the form for proper administration to the patient. The formulation should suit the mode of administration.

In a preferred embodiment, the composition is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous administration to human beings. Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anesthetic such as lignocaine to, ease pain at the, site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

The Active of the invention can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with free amino groups such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with free carboxyl groups such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, etc.
The amount of the Active of the invention which will be effective in the treatment of a particular disorder or condition will depend on the nature of the disorder or condition, and can be determined by standard clinical techniques. In addition, in vivo and/or in vitro assays may optionally be employed to help predict optimal dosage ranges. The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of the disease or disorder, and should be decided according to the judgment of the practitioner and each patient's circumstances.

The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

It has been demonstrated that there is a linear relationship between blood brain barrier (BBB) permeability and lipid solubility providing the MW of the molecule is under a 400-600 Da threshold. However, the presence of a hydroxyl group in the chemical structure of BP can significantly reduce its permeation through biological barriers. In the present Application, two approaches have been applied to increase the lipid solubility of the lead compound BP, namely (a) block the hydroxyl (R') group by transforming it into an ester group or (b) substitute the methoxy residue (R) in BP for an alkoxy group containing a higher number of methylene groups. Six ester derivatives (BP1-6) of BP were synthesised using a parallel synthetic approach (BP is the common starting material of all the reactions). Optimizing the size of the side chain is an important consideration. If it's too large the compound can be sequestered by fatty tissue and may not reach its target. If it is too small the compound loses the ability to cross membranes and may be quickly excreted. The compounds synthesized using the first strategy included the acetate (BP-1), the pivalate (BP-2) and the laureate (BP-4) esters of the lead compound BP, two aromatic derivatives: the phenyl (BP-5) and o-fluophenyl (BP-6) esters and one α-substituted compound: the 2-methylhexanate ester (BP-3) of the lead compound BP.
Results shown below from retinal cells and retinal explants indicate that the BP-3 performs very effectively, inhibiting apoptosis both in retinal cells and explants. This suggests that BP-3 has greater lipophilicity than the lead compound BP, improving its bioavailability and allowing more of the compound to access the cell compartment.

**Experimental**

**Synthesis of 3,4-dihydro-6-hydroxy-7-methoxy-2,2-dimethyl-1(2H)-benzopyran (BP) derivatives**

A solution of BP (1 equivalent) in anhydrous dichloromethane was added dropwise to a mixture of DCC \((N,N\)-dicyclohexylcarbodiimide; 1 equivalent), DMAP (dimethylaminopyridine; 0.1 equivalents) and the corresponding acid in each case (i.e. palmitic acid for BP3 synthesis; 1 equivalent of acid) in anhydrous dichloromethane. The mixture was stirred for 3 h at room temperature. Formation of the corresponding BP derivative was monitored by thin layer chromatography. Next, the mixture was filtered to remove the appearance of the urea precipitate. The dichloromethane was evaporated under reduced pressure and the crude product was redissolved in hexane. The solution was kept overnight at 4°C and filtered again the following day. Finally, the crude product was purified by column chromatography (silica gel, hexane: ethyl acetate 20:1) to obtain the pure BP derivative. The chemical structure of BP derivatives is shown in Figure 1.

**Ex-vivo Methods**

**Retinal explant culture:** Eyes from postnatal day 10, C57BL/6 mice were removed and cleaned with 70% ethanol. The anterior segment, vitreous body, and sclera were removed and the retina mounted on Millicell nitrocellulose inserts (Millipore, Billerica, MA) photoreceptor-side down. Explants were cultured without retinal pigment epithelium (RPE) in 1.2 ml of R16 specialised media (from Dr. P. A. Ekstrom, Wallenberg Retina Centre, Lund University, Lund, Sweden) without additional serum. Treated explants were cultured in medium containing 300µM of the nitric oxide donor SNP (sodium nitroprusside) for 24 h. Pre-treatment with the Active was for 1 hour. Figure 5 shows that
photoreceptors are protected from SNP induced apoptosis by increasing concentrations of
norgestrel.

**Apoptosis detection by Terminal deoxynucleotidyl transferase-mediated biotinylated UTP nick end labeling (TUNEL):** Retinal explants were fixed in 10% neutral buffered formalin overnight at 4°C, followed by cryoprotection in 25% sucrose overnight at 4°C. Frozen sections (7μm) were incubated with terminal deoxynucleotidyl transferase (MSC, Dublin, Republic of Ireland) and fluorescein-12-dUTP (Roche, Lewes, UK) according to manufacturers' instructions at 37°C for 1 hr. Sections were mounted and viewed under a fluorescence microscope (Leica DM LB2; Leica, Nussloch, Germany) using an FITC filter. Figure 6 shows that BP and a BP derivative, BP-3, protect photoreceptive cells from light damage in an ex-vivo retinal explant model, with BP-3 providing better protection.

**Immunocytochemistry:** Eyes were fixed in 10% neutral buffered formalin overnight at 4°C, followed by cryoprotection in 25% sucrose overnight at 4°C. Frozen sections (7μm) were blocked with 0.1% bovine serum albumin (BSA) in 0.1% tween/PBS for 1 hour at room temperature. Sections were incubated with anti-rhodopsin antibody (LAB VISION Corporation, Fremont, CA., USA) overnight at 4°C. Sections were washed and incubated with FITC conjugated secondary mouse antibody (Dako, Glostrup, Denmark) for 1 hour at room temperature. Following further washes, sections were mounted and viewed under a fluorescence microscope (Leica DM LB2; Leica, Nussloch, Germany) using a FITC filter.

**Peanut agglutinin (PNA) staining:** Eyes were fixed in 10% neutral buffered formalin overnight at 4°C, followed by cryoprotection in 25% sucrose overnight at 4°C. Frozen sections (7μm) were blocked with 0.1% bovine serum albumin (BSA) in 0.1% tween/PBS for 30 minutes at room temperature. Sections were incubated with rhodamine conjugated PNA (Invitrogen, Dun Laoghaire, Ireland) for 20 minutes at room temperature as per manufacturers’ instructions. Sections were mounted and viewed under a fluorescence microscope (Leica DM LB2; Leica, Nussloch, Germany) using a TRITC filter.
**Hematoxylin staining:** Eyes were fixed in 10% neutral buffered formalin overnight at 4°C, followed by cryoprotection in 25% sucrose overnight at 4°C. Frozen sections (7μm) were stained in Hematoxylin (Sigma, Dublin, Ireland) for 10 seconds followed by a 15 minute water wash and 2-3 dips in acid alcohol. Following further washing, sections were placed in a 2% sodium bicarbonate (Sigma, Dublin, Ireland) solution for 30 seconds then dehydrated through an alcohol gradient. Sections were cleared in Histoclear (Sigma, Dublin, Ireland) for 5 minutes then mounted in DPX (BDH, VWR International Ltd., Poole, England) and viewed under a light microscope (Leica DM LB2; Leica, Nussloch, Germany).

**In-vivo Methods**

**Light damage model:** Balb/c mice were dark adapted for 18 h prior to exposure to constant light. Mice were injected intraperitoneally with the Active 1 hour prior to light damage. Immediately prior to light exposure their pupils were dilated with 0.5% cyclopentolate under red light. Retinal light damage was induced by exposure to 2 h of cool white fluorescent light at an illumination of 5000 lux. Following exposure to constant light, animals were placed in the dark for 24 h then killed immediately by cervical dislocation. TUNEL staining was performed as described above. Figures 4 and 5 show that 2 hrs light damage induces apoptosis after 24 hours in the ONL. Photoreceptors are protected by IP injection of 200mg/kg of a BP derivative, BP-3.

**Rd10 model**

The rd10 mouse strain exhibits autosomal recessive retinal degeneration and has a point mutation in exon 13 of the Pde6b gene. It is a better model of the slow progression of typical human autosomal recessive RP than the acute light model as photoreceptor cells are lost over a period of weeks rather than days. Loss of photoreceptors in the rd10 mouse begins at approximately 2 weeks of age, with the peak of photoreceptor death occurring at postnatal day (P) 25.

**Intravitreal Injections:** Adult balb/c mice were anaesthetised using an intraperitoneal injection of ketamine hydrochloride 35-50mg/kg (Pharmacia, Corby, Northamptonshire,
UK) and xylazine hydrochloride 5-10mg/kg (Chanelle Pharmaceuticals, Loughrea, Co. Galway, Ireland), and animals were placed in the pronate position. Injections were performed using a 5µL syringe (Hamilton, Reno, NV, USA) on which was mounted a 30-gauge cannula, and visualised using a binocular operating microscope. Using a 30.5-gauge needle (Becton-Dickinson, Drogheda, Ireland), an initial puncture was fashioned through the conjunctiva and sclera immediately posterior to the superonasal limbus. The cannula mounted on the 5µL syringe was then introduced to the vitreous cavity through this opening, and directed backwards towards the optic nerve until the tip was easily visualised within the vitreous cavity behind the lens. NMDA (Sigma, Dublin Ireland) was diluted to 40mM in PBS and 2µL of solution (vehicle or NMDA) was slowly injected. The cannula was left in place for one minute then slowly withdrawn.

The invention is not limited to the embodiment hereinbefore described which may be varied in construction and detail without departing from the spirit of the invention.
CLAIMS

1. Use of a compound of general formula (I)

R₁

R₂O

R₃

or a pharmaceutically acceptable salt thereof, in the manufacture of a medicament for the treatment or prevention of a disease or condition characterised by apoptosis or degeneration of mammalian cells, wherein:

R₁ is a alkoxy, alkyl, ether or ester group;

R₂ is H or has the formula

wherein Y is linear or branched, saturated or unsaturated, aliphatic group with from 2 to 23 carbon atoms, or a cyclic group, and which can contain substituents selected from the group consisting of hydroxyl, alkoxy, amino, carboxyl, cyano, nitro, alkylsulphonyl or halogen atoms,

X is O or S; and R₃ is any substituent.

2. Use as claimed in Claim 1 in which the disease or condition characterized by apoptosis or degeneration of mammalian cells is a retinal dystrophy.

3. Use as claimed in Claim 2 in which the retinal dystrophy is selected from the group consisting of: Retinitis Pigmentosa (RP); Glaucoma; or Age-related Macular Degeneration (AMD).
4. Use as claimed in Claim 1 in which the disease or condition characterized by apoptosis or degeneration of mammalian cells is a neurodegenerative disease.

5. Use as claimed in any preceding Claim, wherein R1 is a methoxy group, and R2 is H.

6. Use as claimed in any of Claim 1 to 4 in which the compound of general formula (I) has a general formula (II), or a pharmaceutically acceptable salt thereof,

![Chemical Structure]

7. Use as claimed in any preceding Claim in which is selected from the group consisting of: tert-butanoyl; hexanoyl; 2-ethylhexanoyl; octanoyl; decanoyl; lauroyl; myristoyl; palmitoyl; stearoyl; oleoyl; or lineoyl.

8. Use as claimed in any preceding Claim in which Y is an alicyclic group, or an aromatic cyclic group, or a heterocyclic group.

![Chemical Structure]

9. Use as claimed in any preceding Claim in which, is selected from the group consisting of: -CO-(CH₂)₀₋₆phenyl; -CO-(CH₂)₀₋₆(1-naphthyl); -CO-(CH₂)₀₋₆(2-naphthyl); -CO-(CH₂)₀₋₆CH(phenyl)₂; -CO-(2-fluorophenyl); -CO-cyclohexyl; α-lipoyl; L-prolyl; D-prolyl; biotinyl-CO-(4-imidazolyl); -CO-(2-pyridyl); -CO-(2-thienyl); -CO-(2-furyl); and -CO-(3-furyl).
10. Use as claimed in any preceding Claim in which X is O.

11. Use as claimed in any preceding Claim in which R₁ is a methoxy group and OR₂ is a hydroxyl group.

12. Use as claimed in any of Claims 1 to 10 in which R₁ is a methoxy group and OR₂ is an acetate ester.

13. Use as claimed in any of Claims 1 to 10 in which R₁ is a methoxy group and OR₂ is a pivalate ester.

14. Use as claimed in any of Claims 1 to 10 in which R₁ is a methoxy group and OR₂ is a laureate ester.

15. Use as claimed in any of Claims 1 to 10 in which R₁ is a methoxy group and OR₂ is a 2-methylhexanate ester.

16. Use as claimed in any of Claims 1 to 10 in which R₁ is a methoxy group and OR₂ is a phenyl ester.

17. Use as claimed in any of Claims 1 to 10 in which R₁ is a methoxy group and OR₂ is a o-fluorophenyl ester.

18. Use as claimed in any of Claims 1 to 10 in which the compound of general formula (I) is 3,4-dihydro-6-hydroxy-7-methoxy-2,2-dimethyl-1(2H)-benzopyran.

19. Use as claimed in any of Claims 1 to 4 in which the compound of general formula (I) is a compound of general formula (III),
or a pharmaceutically acceptable salt thereof, in which X is O or S, and R3 is any substituent.

20. Use as claimed in any of Claims 1 to 4 in which the compound of general formula (I) is a compound of general formula (IV),

or a pharmaceutically acceptable salt thereof, in which R3 is any substituent.

21. Use as claimed in Claim 19 or 20 in which R3 is selected from the group consisting of: H; halogen; lower alkyl; lower alkoxy; hydroxyl; amine; thiol; NHR4; or a substituted or unsubstituted aromatic ring structure in which the substituents (if included) are selected from the groups consisting of H, halogen, lower alkyl, lower alkoxy, hydroxyl, amine, and thiol, and wherein R4 is any substituent.

22. Use as claimed in Claim 21 in which R4 is selected from the group consisting of: halogen; lower alkyl; lower alkoxy; hydroxyl; amine; thiol; or a substituted or unsubstituted aromatic ring structure in which the substituents (if included) are selected from the groups consisting of H, halogen, lower alkyl, lower alkoxy, hydroxyl, amine, and thiol.
23. Use as claimed in Claims 19 or 20 in which R3 and R4 are, independently, C4 to C8 straight alkyl chains.

24. Use as claimed in Claim 23 in which R3 and R4 are, independently, C5 to C7 straight alkyl chain and ideally a C6 straight alkyl chain.

25. Use as claimed in Claim 24 in which R3 and R4 are, independently, C6 straight alkyl chain.

26. Use as claimed in Claim 20 in which the compound of general formula (IV) is selected from the group consisting of:

\[
\begin{align*}
&\text{HO} & \text{CH}_2(\text{CH}_2)_4\text{CH}_3 \\
&\text{HO} & \text{NH}(\text{CH}_2)_5\text{CH}_3
\end{align*}
\]

27. Use as claimed in any preceding Claim in which the compound of general formula (I) is administered to the eye.

28. A pharmaceutical composition formulated as a solution suitable for local delivery to the eye, the composition comprising a compound of general formula (I)
or a pharmaceutically acceptable salt thereof, in the manufacture of a medicament for the treatment or prevention of a disease or condition characterised by apoptosis or degeneration of mammalian cells, wherein:

R1 is a alkoxy, alkyl, ether or ester group;

\[ \text{Y} \quad \text{X} \]

R2 is H or has the formula wherein Y is linear or branched, saturated or unsaturated, aliphatic group with from 2 to 23 carbon atoms, or a cyclic group, and which can contain substituents selected from the group consisting of hydroxyl, alkoxy, amino, carboxyl, cyano, nitro, alkylsulphonyl or halogen atoms, X is O or S; and R3 is any substituent.

29. A pharmaceutical formulation as claimed in Claim 28 in a form selected from the group consisting of: eye-drops; solution suitable for intraocular injection; and solution suitable for intraocular injection.
Figure 1

A

B

C

D

E

F
Figure 4

Protection V's Lipophilicity

Protection (%) vs log P

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Protection (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BP1</td>
<td></td>
</tr>
<tr>
<td>BP2</td>
<td></td>
</tr>
<tr>
<td>BP3</td>
<td></td>
</tr>
<tr>
<td>BP4</td>
<td></td>
</tr>
<tr>
<td>BP5</td>
<td></td>
</tr>
<tr>
<td>BP6</td>
<td></td>
</tr>
</tbody>
</table>

4/12
Figure 5

Untd

ONL
INL
GCL

0.3mM SNP

ONL
INL
GCL

0.3mM SNP + 25μM BP

ONL
INL
GCL

0.3mM SNP + 2.5μM BP3

Figure 6

P10 retinal explants pretreated for 1hr with BP or BP3 then for 24h with SNP

![Graph showing the number of TUNEL+ cells per 40X field with different concentrations of SNP and BP3.](image)

- Untd
- SNP
- SNP+BP
- SNP+BP3

No. of TUNEL+ photoreceptor per 40X field

<table>
<thead>
<tr>
<th>Conc (μM)</th>
<th>Untd</th>
<th>300</th>
<th>25</th>
<th>5</th>
<th>2.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
Figure 8

No. TUNEL +ve cells

<table>
<thead>
<tr>
<th>Time</th>
<th>Light only</th>
<th>BP3 + Light</th>
</tr>
</thead>
<tbody>
<tr>
<td>24h</td>
<td></td>
<td></td>
</tr>
<tr>
<td>48h</td>
<td></td>
<td></td>
</tr>
<tr>
<td>72h Top up</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 10

Rhodopsin Staining

C57 wildtype  
Vehicle  
250mg/kg BP3

OS  
ONL  
INL  

OS  
ONL  
INL  
GCL  
rd10

ONL  

OS  
ONL  
INL  
GCL  
rd10

ONL

PNA Staining

C57 wildtype  
Vehicle  
250mg/kg BP3

OS  
ONL  
INL  
GCL  
rd10

ONL  

OS  
ONL  
INL  
GCL  
rd10

ONL

9/12
Figure 13

- 40mM NMDA
- 48h
- 24h
- 48h
- 72h

Total TUNEL+ve cells
A. CLASSIFICATION OF SUBJECT MATTER

INV. A61K31/353 A61P27/02 A61P25/00

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)
A61K A61P

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
</table>

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

X See patent family annex.

* Special categories of cited documents:
  *"A" document defining the general state of the art which is not considered to be of particular relevance
  *"E" earlier document but published on or after the international filing date
  *"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
  *"O" document referring to an oral disclosure, use, exhibition or other means
  *"P" document published prior to the international filing date but later than the priority date claimed

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

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

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

"S" document member of the same patent family

Date of the actual completion of the international search 14 December 2009

Date of mailing of the international search report 30/12/2009

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

Authorized officer
Nyeki, Agnes

Form PCT/ISA/210 (second sheet) (April 2005)
<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>SANVICENS NURIA ET AL: &quot;Oxidative stress-induced apoptosis in retinal photoreceptor cells is mediated by calpains and caspases and blocked by the oxygen radical scavenger CR-6&quot; JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 279, no. 38, 17 September 2004 (2004-09-17), pages 39268-39278, XP002560058 ISSN: 0021-9258 abstract page 39274, left-hand column, paragraph 1 - right-hand column, paragraph 1 page 39275; figure 5</td>
<td>1-3,5-29</td>
</tr>
<tr>
<td>X</td>
<td>SANVICENS NURIA ET AL: &quot;The radical scavenger CR-6 protects SH-SY5Y neuroblastoma cells from oxidative stress-induced apoptosis: effect on survival pathways&quot; JOURNAL OF NEUROCHEMISTRY, vol. 98, no. 3, August 2006 (2006-08), pages 735-747, XP002560059 ISSN: 0022-3042 abstract page 741, left-hand column, paragraph 2 - page 742, left-hand column, paragraph 1 figure 3</td>
<td>1,4-26</td>
</tr>
<tr>
<td>A</td>
<td>MACKLEY A M ET AL: &quot;Redox survival signalling in retina-derived 661W cells&quot; CELL DEATH AND DIFFERENTIATION, vol. 15, no. 8, April 2008 (2008-04), pages 1291-1303, XP002560060 ISSN: 1350-9047 the whole document figures 1e,1f</td>
<td>1-29</td>
</tr>
<tr>
<td>Patent document cited in search report</td>
<td>Publication date</td>
<td>Patent family member(s)</td>
</tr>
<tr>
<td>---------------------------------------</td>
<td>-----------------</td>
<td>-------------------------</td>
</tr>
<tr>
<td>JP 2004323486 A</td>
<td>18-11-2004</td>
<td>NONE</td>
</tr>
</tbody>
</table>

Form: PCT/ISA210 (patent family annex) (April 2005)