The present invention relates to a GABA_b receptor agonist for use in the prevention and treatment of a disease associated with retinal ganglion cell degeneration. The present invention also relates to a pharmaceutical composition for the prevention and treatment of a disease associated with retinal ganglion cell degeneration which comprises a GABA_b receptor agonist and optionally one or more pharmaceutically acceptable excipients.
FIELD OF THE INVENTION:
The present invention relates to a GABA \_B receptor agonist for use in the prevention and treatment of a disease associated with retinal ganglion cell degeneration.

BACKGROUND OF THE INVENTION:
Glaucoma denotes a group of diseases of the optic nerve involving loss of retinal ganglion cells in a characteristic pattern of optic neuropathy. Raised intraocular pressure is a significant risk factor for developing glaucoma (above 22mmHg). Untreated glaucoma leads to permanent damage of the optic nerve resulting in visual field loss, which can progress to blindness. Glaucoma can be divided roughly into two main categories, open-angle glaucoma or chronic glaucoma and angle-closure or acute glaucoma. Angle-closure glaucoma appears suddenly, with often painful side effects. Accordingly, this form is usually diagnosed quickly, although damages and loss of vision can also occur very suddenly. By contrast, open-angle glaucoma can be asymptomatic for a long time since the progression is very slow. Glaucoma has been nicknamed the "sneak thief of sight" because the loss of visual field often occurs gradually and may be recognized when it is quite advanced. Once lost, this damaged visual field can never be recovered. Worldwide, glaucoma is the second cause leading to blindness since it affects one in two hundred people aged fifty and younger, and one in ten over the age of eighty.

Thus, there is a need in the art for medicaments that would allow preventing and treating glaucoma. Prevention of retinal ganglion cell degeneration may also be useful for the treatment of other forms of optic nerve atrophy like the Leber hereditary optic neuropathy or pathologies with retinal ischemia, like vascular occlusions.

The nature of the mechanistic link between high intraocular pressure and loss of retinal ganglion cells is not firmly established. Although less direct insults have occasionally been suggested, trauma at the optic nerve head, the location where the axons of the ganglion cells join together to leave the globe, has been a leading possibility. Generally speaking, this could occur by compression or by pressure on the axons at their point of exit, but the exact pathophysiological events remain unknown (Quigley, 1987; Quigley, 1999; Libby et al, 2005; Whitmore et al., 2005).
GABA (y-aminobutyric acid) is an endogenous neurotransmitter in the central and peripheral nervous systems. Receptors for GABA have traditionally been divided into GABA<sub>A</sub> and GABA<sub>B</sub> receptor subtypes. GABA<sub>B</sub> receptors (for review see Kerr and Ong, 1995) belong to the superfamily of G-protein coupled receptors. In the CNS, GABA is known to exert its actions through at least two distinct receptor types: an ionotropic GABA<sub>A</sub> receptors (which form chloric channels) and metabotropic GABA<sub>B</sub> receptors (members of the G protein-coupled receptors family). GABA<sub>B</sub> receptor agonists have been described for use in the treatment of various diseases, such as CNS disorders, but their role for the prevention of ganglion cell degeneration has not yet been investigated.

SUMMARY OF THE INVENTION:
The present invention relates to a GABA<sub>B</sub> receptor agonist for use in the prevention and treatment of a disease associated with retinal ganglion cell degeneration.

The present invention also relates to a pharmaceutical composition for the prevention and treatment of a disease associated with retinal ganglion cell degeneration which comprises a GABA<sub>B</sub> receptor agonist and optionally one or more pharmaceutically acceptable excipients.

DETAILED DESCRIPTION OF THE INVENTION:
The inventors have demonstrated that Baclofen (i.e. a GABA<sub>B</sub> receptor agonist) prevents retinal ganglion cells degeneration, and therefore may be useful for the prevention and treatment of a disease associated with retinal ganglion cell degeneration.

Thus, an object of the present invention relates to a GABA<sub>B</sub> receptor agonist for use in the prevention and treatment of a disease associated with retinal ganglion cell degeneration.

The term "GABA receptor" means a class of receptors that responds to the neurotransmitter gamma-aminobutyric acid (GABA), the major inhibitory neurotransmitter in the vertebrate central nervous system. There are two classes of GABA receptors: GABA<sub>A</sub> and GABA<sub>B</sub>. GABA<sub>A</sub> receptors are ligand-gated ion channels (also known as ionotrophic receptors), whereas GABA<sub>B</sub> receptors are G protein-coupled receptors (also known as metabotropic receptors).
As used herein, the term "GABA₄ receptor agonist" has its general meaning in the art and refers to a compound that when administered to a human or an animal activates the GABA₄ receptor. Therefore, the term refers to any GABA₄ receptor agonist that is currently known in the art or that will be identified in the future, and includes any entity that, upon administration to a patient, results in activation or up-regulation of a biological activity associated with activation of GABA₄ receptors in the patient, including any of the downstream biological effects otherwise resulting from the binding to GABA₄ receptor of its natural ligand (GABA).

The GABA₄ agonistic activity of a compound may be determined using various methods well known in the art. Binding experiments had described the affinities of different GABA₄ receptor agonists, like baclofen and analogues (Karla et al. 1999) and GABA₄ receptor antagonists, like phaclofen (Frydenvang et al., 1994). GABA₄ receptor is a heterodimeric protein composed by two sub-units GABA₄-R1 and GABA₄-R2 (Brauner-Osborne et. Al, 1999). Transduction mechanisms triggered following the GABA₄ receptor stimulation, involve the activation of G-protein, leading to an inhibition of calcium channels (Kamatchi et. al, 1990). In a general manner, current in vitro screening experiments to identify GABA₄ receptor agonists either rely on binding assays in rat brain membranes or consist in functional screening assays, such as c-AMP responses or effects on Ca²⁺ and K⁺ channels performed in cells expressing a recombinant GABA₄ receptor. In some of these functional assays the GABA₄ receptors may be co-expressed with G-proteins, e.g. Gal 6 or Gqi5 or the chimeric G-protein G aq-z5, increasing G-protein coupling (Brauner-Osborne et al., 1999). Alternatively, the US Patent Application Publication No US 2006/0216749 describes the development of a Chinese Hamster Ovary (CHO) cell line co-expressing the human GABA₄ receptor subunits GABAfi-R1a and GABA₄-R2 useful for screening GABA₄ receptor agonists.

In the context of the present invention, GABA₄ receptor agonists are preferably selective for the GABA₄ receptor as compared with the related receptors such as GABA₅. By "selective" it is meant that the affinity of the modulator for the GABA₄ receptor is at least 10-fold, preferably 25-fold, more preferably 100-fold, still preferably 500-fold higher than the affinity for the related receptors.

In one embodiment, the GABA₄ receptor agonist is a small organic molecule.
The term "small organic molecule" refers to a molecule of a comparable size to those organic molecules generally used in pharmaceuticals. The term excludes biological macromolecules (e.g., proteins, nucleic acids, etc.) The preferred small organic molecule ranges in sizes were up to about 5000 Da, more preferably up to 2000 Da, and most preferably up to about 1000 Da.


In addition, GABA_B receptor agonists are disclosed in EP 0356128; EP 0181833, EP 0399949, EP 0463969, and FR 2,722,192, each of which is hereby incorporated by reference. More particularly EP 463969 Al and FR 2722192 Al disclose 4-aminobutanoic acid derivatives having different heterocyclic substitutes at the 3-carbon of the butyl chain. EP 181833 Al discloses substituted 3-aminopropylphosphinic acids having high affinities towards GABA_B receptor sites. EP 399949 Al discloses derivatives of (3-aminopropyl)methylphosphinic acid, which are described as potent GABA_B receptor agonists. Still other (3-aminopropyl)methylphosphinic acids and (3-aminopropyl)phosphinic acids have been disclosed in WO 01/41743 Al and WO 01/42252 Al, respectively. Structure-activity relationships of several phosphinic acid analogues with respect to their affinities to the GABA_B receptor are discussed in J. Med. Chem. (1995), 38, 3297-3312. Sulphinic acid analogues and their GABA_B receptor activities are described by Carruthers et al. (1998) (for review, see Kerr and Ong, 2001).

In one embodiment, the GABA B receptor agonist according to the invention is Baclofen. Baclofen is a GABA B receptor agonist that has the chemical name 4-amino-3-(4-chlorophenyl)butanoic acid. Procedures for the preparation of baclofen are described in U.S. Pat. No. 3,471,548. The pharmacological properties are described by Hudgson and Weightman (1971) and S. Ahuja in Analytical Profiles of Drug Gabab receptor agonists vol. 14, K. Florey, Ed. (Academic Press, New York, 1985) pp 527-548. Baclofen is also referred to as Baclon, Lioresal, Kemstro and Myospan.

In a particular embodiment, baclofen may be administrated in a prodrug format. "Prodrug" refers to a derivative of a drug molecule that requires a transformation within the body to release the active drug. Prodrugs are frequently, although not necessarily, pharmacologically inactive until converted to the parent drug. For example acyloxyalkyl carbamate prodrugs of baclofen and analogs thereof have been shown to provide enhanced bioavailability of baclofen following oral administration as described in co-pending application Gallop et al., International Publication No. WO 2005/019163 entitled "Acyloxyalkyl Carbamate Prodrugs, Methods, Synthesis and Use," filed Aug. 20, 2004.

In one embodiment, the GABA B receptor agonists according to the invention are selected from the group consisting of 2,6-Di-tert-butyl-4-(3-hydroxy-2,2-dimethylpropyl)phenol (CGP7930), 3-(3,5-di-tert-butyl-4-hydroxyphenyl)-2,2-dimethylpropanal and N,N-Dicyclopentyl-2-methylsulfanyl-5-nitro-pyrimidine-4,6-diamine.

Other examples of compounds having agonistic activity to GABAB receptors include CGP54626, (R,S)-5,7-di-tert-butyl-3-hydroxy-3-trifluoromethyl-3H-benzofuran-2-one (rac-BHFF), GS39783, GS39783 and CGP13501. 2,6-Di-tert-butyl-4-(3-hydroxy-2,2-dimethylpropyl)phenol (CGP7930) and 3-(3,5-di-tert-butyl-4-hydroxyphenyl)-2,2-dimethylpropanal (disclosed in US 5,304,685) have been described to exert positive allosteric modulation of native and recombinant GABA B receptor activity (Urwyler et al; 2001).

N,N-Dicyclopentyl-2-methylsulfanyl-5-nitro-pyrimidine-4,6-diamine has been described to exert positive allosteric modulation of the GABA B receptor (The Journal of Pharmacology and Experimental Therapeutics, 307 (2003), 322-330).
Many other examples of compounds having agonistic activity to GABA_B receptors are known and include certain amino acids, aminophosphonic acids, aminophosphinic acids, aminophosphonous acids, and aminosulfmic acids such as, for example:

- (2R)-(3-amino-2-fluoropropyl)sulfinic acid;
- (3-amino-2-fluoropropyl)sulfonic acid;
- (3-amino-2-hydroxypropyl)sulfonic acid;
- (3-amino-2-hydroxypropyl)sulfinic acid;
- (3-amino-1-hydroxypropyl)methylphosphinic acid;
- (3-amino propyl)methylphosphinic acid;
- (3-amino-2-(4-fluorophenyl)propyl)phosphonous acid;
- (3-amino-2-benzylpropyl)phosphonous acid;
- (3-amino-2-cyclohexylpropyl)phosphonous acid;
- (3-amino-2-hydroxypropyl)(di fluoromethyl)phosphinic acid;
- (3-amino-2-phenylpropyl)phosphonous acid;
- (3-amino-propyl)(di fluoromethyl)phosphinic acid;
- (3-amino-1-hydroxypropyl)methylphosphinic acid;
- (3-amino-1-hydroxypropyl)methylphosphinic acid,
- (3-amino-2-(4-chlorophenyl)-2-hydroxypropyl)phosphonous acid,
- (3-amino-2-(4-chlorophenyl)propyl)phosphonous acid,
- (3-amino-2-(4-chlorophenyl)sulfinic acid,
- (3-amino-2-(4-chlorophenyl)propyl)sulfamic acid,
- (3-amino-2-benzylpropyl)phosphonous acid,
- (3-amino-2-benzylpropyl)phosphonous acid;
- (3-amino-2-cyclohexylpropyl)phosphonous acid,
- (3-amino-2-cyclohexylpropyl)phosphonic acid,
- (3-amino-2-fluoropropyl)sulfinic acid;
- (3-amino-2-hydroxypropyl)(difluoromethyl)phosphinic acid,
- (3-amino-2-hydroxypropyl)methylphosphinic acid,
- (3-amino-2-hydroxypropyl)phosphonous acid,
- (3-amino-2-methylpropyl)phosphonous acid,
- (3-amino-2-oxo-propyl)methyl phosphinic acid,
- (3-amino-2-oxopropyl)sulfamic acid.
- (3-amino-2-phenylpropyl)phosphonous acid,
(3-aminopropyl)(difluoromethyl)phosphinic acid,
(3-aminopropyl)hydroxymethylphosphinic acid,
(3-aminopropyl)methylphosphinic acid,
(3-aminopropyl)phosphonous acid,
(4-amino-1,1,1-trifluorobut-2-yl)methylphosphinic acid;
(4-aminobut-2-yl)methylphosphinic acid,
(4-aminobut-2-yl)phosphonous acid,
(5-aminopent-3-yl)methylphosphinic acid,
(E)-(3-aminopropen-1-yl)methylphosphinic acid;
(E)-(3-aminopropen-1-yl)phosphonous acid,
(E)-(3-aminopropen-1-yl)phosphonous acid;
(E)-(3-aminopropen-1-yl)methylphosphinic acid;
[3-amino-2-(4-chlorophenyl)-2-hydroxypropyl]phosphonous acid;
[3-amino-2-(4-methoxyphenyl)propyl]phosphonous acid;
[3-amino-2-(4-trifluoromethylphenyl)propyl]phosphonous acid;
[beta]-phenyl-GABA;
[gamma]-hydroxybutyrate;
1-(4-chlorophenyl)-4-(3,5-dimethoxybenzoyl)-piperazine;
1-(aminomethyl)cyclohexaneacetic acid.
2-(7-chloro-1,8-naphthyridin-2-yl)-3-[(1,4-dioxo-8-azaspiro[4,5]dec-8-
yl)carbonylmethyl]-isoindolin-1-one
2-aminoethanesulfonic acid;
3-hydroxy-baclofen;
3-(aminopropyl)methylphosphinic acid;
3-amino-2-(4-chlorophenyl)-1-nitropropane;
3-aminopropyl-(P-methyl)-phosphinic acid;
3-aminopropylphosphinic acid;
3-aminopropylsulfonic acid
4'-ethyl-2-methyl-3-pyrrolidinopropiophenone;
4-(3-hydroxy-pyridin-2-yl)butyro lactam,
4-[[a-(4-chlorophenyl)-5-fluoro-2-hydroxybenzylidene]amino]butyramide;
4-amino-[beta]-(5-chloro-thien-2-yl)-butanoic acid;
4-amino-3-(2-chlorophenyl)butanoic acid;
4-amino-3-(2-imidazolyl)butanoic acid;
4-amino-3-(4-chlorophenyl)-3-hydroxyphenylbutanoic acid;
4-amino-3-(5-chlorothien-2-yl)butanoic acid;
4-amino-3-(5-chlorothien-2-yl)butanoic acid;
4-amino-3-(5-bromothien-2-yl)butanoic acid;
4-amino-3-(5-methylthien-2-yl)butanoic acid,
4-amino-3-(thien-2-yl)butanoic acid,
4-amino-3-hydroxybutanoic acid,
4-amino-3-phenylbutanoic acid,
4-amino-3-methoxybenzofuran-2-yl)butanoic acid;
4-aminobutanoic acid
4-guanidino-3-(4-chlorophenyl)butanoic acid;

Methods for synthesizing the above compounds are disclosed supra and in GB 1017439, e.g. baclofen, U.S. Pat. No. 4,656,298, e.g. 3-aminopropylphosphonous acid (3-aminopropylphosphinic acid), EP 0356128, i.e. 3-(aminopropyl)methyl phosphinic acid, and EP0463969, e.g. 3-(2-imidazolyl)-4-aminobutanoic acid, which disclosures are incorporated herein by reference.

In a particular embodiment, the GABA_B receptor agonist according to the invention is not taurine.

The use of pharmaceutically acceptable salts of GABA_B agonists for the disclosed purposes of the invention is also included in the invention. Most known GABA_B agonists such as for example baclofen, (3-aminopropyl)methylphosphinic acid and (3-amino-2-(S)-hydroxypropyl)-methylphosphinic acid are of amphoteric nature and may be present in the form of internal salts. They also can form acid addition salts and salts with bases. Such salts are particularly pharmaceutically acceptable acid addition salts, as well as pharmaceutically...
acceptable salts formed with bases. Suitable acids for the formation of such salts include, for example, mineral acids such as hydrochloric, hydrobromic, sulfuric or phosphoric acid or organic acids such as organic sulfonic acids and organic carboxylic acids. Salts of GABA_\textsubscript{B} agonists with bases are, for example, alkali metal salts, e.g., sodium or potassium salts, or alkaline earth metal salts, e.g., calcium or magnesium salts as well as ammonium salts, such as those with ammonia or organic amines.

The use of optical isomers of GABA_\textsubscript{B} agonists for the disclosed purposes is also included in the invention. Many known GABA_\textsubscript{B} agonists such as for example baclofen and (3-amino-2-\textregistered-(S)-hydroxypropyl)methylphosphinic acid are chiral compounds due to the presence of an asymmetric carbon atom. Depending on the presence of asymmetric atoms, the GABA_\textsubscript{B} agonists may be in the form of mixtures of isomers, particularly racemates, or in the form of pure isomers, especially enantiomers.

Baclofen as currently used is a racemate. The dominant GABA_\textsubscript{B} agonist activity is associated with the (R)-isomer. There is also evidence that there is a stereoselective transport of the (R)-isomer across the blood brain barrier, and that the (R)-isomer shows a lower metabolic clearance, longer half-life, and higher systemic exposure than the S-isomer. In all embodiments, the baclofen can comprise racemic baclofen, enriched (i.e., at least 51%) (R)-baclofen, substantially pure (i.e., at least 90%) (R)-baclofen, or a pharmaceutically acceptable salt thereof.

Alternatively, the GABA_\textsubscript{B} receptor agonist may consist in an antibody (the term including "antibody fragment"). In particular, the GABA_\textsubscript{B} receptor modulator may consist in an antibody directed against the GABA_\textsubscript{B} receptor, in such a way that said antibody activates the receptor.

Antibodies can be raised according to known methods by administering the appropriate antigen or epitope to a host animal selected, e.g., from pigs, cows, horses, rabbits, goats, sheep, and mice, among others. Various adjuvants known in the art can be used to enhance antibody production. Although antibodies useful in practicing the invention can be polyclonal, monoclonal antibodies are preferred. Monoclonal antibodies can be prepared and isolated using any technique that provides for the production of antibody molecules by continuous cell lines in culture. Techniques for production and isolation include but are not limited to the hybridoma technique originally described by Kohler and Milstein (1975); the human B-cell hybridoma technique (Cote et al., 1983); and the EBV-hybridoma technique.
(Cole et al. 1985). Alternatively, techniques described for the production of single chain antibodies (see, e.g., U.S. Pat. No. 4,946,778) can be adapted to produce anti-GABA_B receptor single chain antibodies. The GABAB receptor agonist useful in practicing the present invention also include anti-GABAB receptor antibody fragments including but not limited to F(ab')2 fragments, which can be generated by pepsin digestion of an intact antibody molecule, and Fab fragments, which can be generated by reducing the disulfide bridges of the F(ab')2 fragments. Alternatively, Fab and/or scFv expression libraries can be constructed to allow rapid identification of fragments having the desired specificity to GABA_B receptor.

Humanized antibodies and antibody fragments thereof can also be prepared according to known techniques. "Humanized antibodies" are forms of non-human (e.g., rodent) chimeric antibodies that contain minimal sequence derived from non-human immunoglobulin. For the most part, humanized antibodies are human immunoglobulins (recipient antibody) in which residues from a hypervariable region (CDRs) of the recipient are replaced by residues from a hypervariable region of a non-human species (donor antibody) such as mouse, rat, rabbit or nonhuman primate having the desired specificity, affinity and capacity. In some instances, framework region (FR) residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, humanized antibodies may comprise residues that are not found in the recipient antibody or in the donor antibody. These modifications are made to further refine antibody performance. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the hypervariable loops correspond to those of a non-human immunoglobulin and all or substantially all of the FRs are those of a human immunoglobulin sequence. The humanized antibody optionally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. Methods for making humanized antibodies are described, for example, by Winter (U.S. Pat. No. 5,225,539) and Boss (Celltech, U.S. Pat. No. 4,816,397).

Then after raising antibodies as above described, the skilled man in the art can easily select those activating the GABA_B receptor.

In another embodiment the GABAB receptor modulator is an aptamer. Aptamers are a class of molecule that represents an alternative to antibodies in term of molecular recognition. Aptamers are oligonucleotide or oligopeptide sequences with the capacity to recognize virtually any class of target molecules with high affinity and specificity. Such ligands may be isolated through Systematic Evolution of Ligands by Exponential enrichment (SELEX) of a
random sequence library, as described in Tuerk C. and Gold L., 1990. The random sequence library is obtainable by combinatorial chemical synthesis of DNA. In this library, each member is a linear oligomer, eventually chemically modified, of a unique sequence. Possible modifications, uses and advantages of this class of molecules have been reviewed in Jayasena S.D., 1999. Peptide aptamers consists of a conformationally constrained antibody variable region displayed by a platform protein, such as E. coli Thioredoxin A that are selected from combinatorial libraries by two hybrid methods (Colas et al, 1996).

Then after raising aptamers directed against the GABA$_B$ receptors as above described, the skilled man in the art can easily select those activating the GABA$_B$ receptor.

In a particular embodiment, diseases associated with retinal ganglion cell degeneration include but are not limited to glaucoma and other forms of optic nerve atrophy like the Leber hereditary optic neuropathy or pathologies with retinal ischemia like vascular occlusions.

In another embodiment, diseases associated with retinal ganglion cell degeneration also include but are not limited to arteritic ischemic optic neuropathy (giant cell arteritis), nonarteritic ischemic optic neuropathy, infiltrative optic neuropathy (sarcoidosis), infectious optic neuropathy (syphilis, lyme, toxoplasmosis, herpes zoster), optic neuritis from demyelinating disease, posradiation optic neuropathy, acrodermatitis enteropathica, hereditary optic neuropathy (Leber's hereditary optic neuropathy, dominant optic neuropathy), compressive optic neuropathy (orbital pseudotumor, thyroid eye disease), autoimmune optic neuropathy (Lupus), and diabetic retinopathy.

In another embodiment, the GABA$_B$ receptor agonist according to the invention may be useful for the treatment of cholestatic liver disease, nutritional optic neuropathy, ketogenic diet, thiamine deficiency.

In another embodiment, the GABA$_B$ receptor agonist according to the invention may be useful for preventing the retinal ganglion cell degeneration induced by antimicrobial or anti-malaria drug such as chloramphenicol, chloroquine, clioquinol, dapsone, ethambutol, iodochlorhydroxyquinoline, isoniazide, linezolid, streptomycin.

In another particular embodiment, the invention relates to an antimicrobial or anti-malaria composition comprising a GABA$_B$ receptor agonist according to the invention and at least one active ingredient selected from the group consisting of chloramphenicol,
In another embodiment, the GABA$_B$ receptor agonist according to the invention may be useful for preventing the retinal ganglion cell degeneration induced by an immunomodulator or immunosuppressive drug such as cyclosporine, interferon-alpha, tacrolimus (FK506).

In another particular embodiment, the invention relates to an immunomodulator or immunosuppressive composition comprising a GABA$_B$ receptor agonist according to the invention and at least one active ingredient selected from the group consisting of cyclosporine, interferon-alpha, tacrolimus (FK506).

In another embodiment, the GABA$_B$ receptor agonist according to the invention may be useful for preventing the retinal ganglion cell degeneration induced by a chemotherapeutics drug such as carboplatin, chlorambucil, cisplatin, 5-fluorouracil, methotrexate, nitrosoureas (BCNU, CCNU, ACNU), paclitaxel, tamoxifen, 5-vincristine, cytosine arabinoside, purine analogues, procarbazine, cyclophosphamide, vinca alkaloids.

In another particular embodiment, the invention relates to a chemotherapeutic composition comprising a GABA$_B$ receptor agonist according to the invention and at least one active ingredient selected from the group consisting of carboplatin, chlorambucil, cisplatin, 5-fluorouracil, methotrexate, nitrosoureas (BCNU, CCNU, ACNU), paclitaxel, tamoxifen, 5-vincristine, cytosine arabinoside, purine analogues, procarbazine, cyclophosphamide, vinca alkaloids.

In another embodiment, the GABA$_B$ receptor agonist according to the invention may be useful for preventing the retinal ganglion cell degeneration induced by a drug such as amiodarone, amantidine amoxproen, cafegrot, chlorpropamide, cimetidine, clomiphene citrate, deferoxamine, disulfiram, emetine, infliximab, pheniprazine, quinine, PDE inhibitors (sildenafil, tadalafil, vardenafil), bendroflumethiazide, chorothiazide, chlortalidone, hydrochlorothiazide, hydroflumethiazide, indapamide, methyclothiazide, metolazone, polythiazide, trichlormethiazide, antiepileptic drugs such as vigabatrin valproate, tiagabine, gabapentin, valproate, levetiracetam, topiramate, felbamate, benzodiazepines like diazepam, clonazepam and clobazam or barbiturates like primidone and phenobarbitone.
In another particular embodiment, the invention relates to a therapeutic composition comprising a GABA_B receptor agonist according to the invention and at least one active ingredient selected from the group consisting of amiodarone, amantidine amoproxen, cafergot, chlorpropamide, cimetidine, clomiphene citrate, deferoxamine, disulfiram, emetine, infliximab, pheniprazine, quinine, PDE inhibitors (sildenafil, tadalafil, vardenafil), bendroflumethiazide, choroethiazide, chlortahdone, hydrochlorothiazide, hydroflumethiazide, indapamide, methyclothiazide, metolazone, polythiazide, trichlormethiazide, antiepileptic drugs such as vigabatrin valproate, tiagabine, gabapentin, valproate, levetiracetam, topiramate, felbamate, benzodiazepines like diazepam, clonazepam and clobazam or barbiturates like primidone and phenobarbitalone.

In another embodiment, the GABA_B receptor agonist according to the invention may be useful for preventing the toxicity induced by a molecule such as alcohol, arsacetin, caron monoxide, carbon disulfide, carbon tetrachloride, cobalt chloride, ethchoryvynol, ethylene glycol, hexachlorophene, iodoform, lead, mercury, methanol, methyl acetate, methyl bromide, octamoxin, organic solvents, perchloroethylene, pheniprazine, plasmocid, styrene, thallium, trichloroethylene, triethyl tin, tobacco, toluene.

This invention also relates to a therapeutic method for the prevention or treatment of a disease associated with retinal ganglion cell degeneration, wherein said method comprises a step of administering to a subject in need thereof with an effective amount of a GABA_B receptor agonist of the invention.

According to the invention, the term "subject" or "patient" and "subject in need thereof" or "patient in need thereof" is intended for a human or a non-human mammal.

Generally speaking, a "therapeutically effective amount", or "effective amount", or "therapeutically effective", as used herein, refers to that amount which provides a therapeutic effect for a given condition and administration regimen. This is a predetermined quantity of active material calculated to produce a desired therapeutic effect in association with the required additive and diluent; i.e., a carrier, or administration vehicle. Further, it is intended to mean an amount sufficient to reduce and most preferably prevent a clinically significant deficit in the activity, function and response of the host. Alternatively, a therapeutically effective amount is sufficient to cause an improvement in a clinically significant condition in
a host. As is appreciated by those skilled in the art, the amount of a compound may vary depending on its specific activity. Suitable dosage amounts may contain a predetermined quantity of active composition calculated to produce the desired therapeutic effect in association with the required diluents; i.e., carrier, or additive.

The present invention also pertains to pharmaceutical compositions comprising a GABA<sub>B</sub> receptor agonist for the prevention and treatment of a disease associated with retinal ganglion cell degeneration. In a pharmaceutical composition according to the invention, the amount of the GABA<sub>B</sub> receptor agonist, is adapted so that the said pharmaceutical composition is adapted so that the dosage form used allows the administration of an amount of GABA<sub>B</sub> receptor agonist ranging from 10 µg to 10 grams per day for a human adult patient having a mean weight of 80 kilos.

Indeed, in a pharmaceutical composition, the active ingredient is used in combination with one or more pharmaceutically or physiologically acceptable excipients.

Generally, a pharmaceutical composition according to the invention comprises an amount of excipient(s) that ranges from 0.1% to 99.9% by weight, and usually from 10% to 99% by weight, based on the total weight of the said pharmaceutical composition.

By "physiologically acceptable excipient or carrier" is meant solid or liquid filler, diluents or GABA<sub>B</sub> receptor agonist which may be safely used in systemic or topical administration. Depending on the particular route of administration, a variety of pharmaceutically acceptable carriers well known in the art include solid or liquid fillers, diluents, hydrotropes, surface active agents, and encapsulating GABA<sub>B</sub> receptor agonists.

Pharmaceutically acceptable carriers for systemic administration that may be incorporated in the composition of the invention include sugar, starches, cellulose, vegetable oils, buffers, polyols and alginic acid. Specific pharmaceutically acceptable carriers are described in the following documents, all incorporated herein by reference: U.S. Pat. No. 4,401,663, Buckwalter et al. issued August 30, 1983; European Patent Application No. 089710, LaHann et al. published Sept. 28, 1983; and European Patent Application No. 0068 592, Buckwalter et al. published Jan. 5, 1983. Preferred carriers for parenteral administration include propylene glycol, pyrrolidone, ethyl oleate, aqueous ethanol, and combinations thereof.

Representative carriers include acacia, agar, alginates, hydroxyalkylcellulose, hydroxypropyl methylcellulose, carboxymethylcellulose, carboxymethylcellulose sodium, carrageenan, powdered cellulose, guar gum, cholesterol, gelatin, gum agar, gum arabic, gum
karaya, gum ghatti, locust bean gum, octoxynol 9, oleyl alcohol, pectin, poly(acrylic acid) and its homologs, polyethylene glycol, polyvinyl alcohol, polyacrylamide, sodium lauryl sulfate, poly(ethylene oxide), polyvinylpyrrolidone, glycol monostearate, propylene glycol monostearate, xanthan gum, tragacanth, sorbitan esters, stearyl alcohol, starch and its modifications. Suitable ranges vary from about 0.5% to about 1%.

For formulating a pharmaceutical composition according to the invention, the one skilled in the art will advantageously refer to the last edition of the European pharmacopoeia or of the United States pharmacopoeia.

Preferably, the one skilled in the art will refer to the fifth edition “2005” of the European Pharmacopoeia, or also to the edition USP 28-NF23 of the United States Pharmacopoeia.

Pharmaceutical composition according to the invention may also contain other compounds, which may be biologically active or inactive. For example, GABA \( \text{B} \) receptor agonist according to the invention may be combined with another agent, in a treatment combination, and administered according to a treatment regimen of the present invention. Such combinations may be administered as separate compositions, combined for delivery in a complementary delivery system, or formulated in a combined composition, such as a mixture or a fusion compound.

The pharmaceutical composition of the invention may be formulated for a topical, oral, intranasal, parenteral, intraocular, intravenous, intramuscular or subcutaneous or eye drop administration and the like.

In another particular embodiment, the invention relates to a pharmaceutical composition comprising a GABA \( \text{B} \) receptor agonist according to the invention and at least one active ingredient selected from the group consisting latanoprost, timolol, travoprost, dorzolamide, brimonidine, bimatoprost, apraclonidine, dipivephrine, propine, acetazomide, brinzolamide.

In another preferred embodiment, the pharmaceutical composition comprising a GABA \( \text{B} \) receptor agonist according to the invention and at least one active ingredient selected from the group consisting latanoprost, timolol, travoprost, dorzolamide, brimonidine, bimatoprost, apraclonidine, dipivephrine, propine, acetazomide, brinzolamide, is useful for the treatment of glaucoma.
In another embodiment, the pharmaceutical composition comprising a GABA_B receptor agonist according to the invention and at least one active ingredient selected from the group consisting of latanoprost, timolol, travoprost, dorzolamide, brimonidine, bimatoprost, apraclonidine, dipivephrine, propine, acetazolamide, brinzolamide may be formulated for eye drop administration.

The invention will be further illustrated by the following figures and examples. However, these examples and figures should not be interpreted in any way as limiting the scope of the present invention.

FIGURES:

Figure 1: Protective effect of Baclofen on the survival of pure retinal ganglion cells (RGCs) in culture. Quantification of RGC survival at 6 days in vitro (6 DIV) either in control condition (negative control) or in presence of 10 µM of Baclofen; addition of the B27 supplement to the culture medium was taken as a positive control. Data were normalized with respect to the cell number in the control conditions. They are provided as mean ± SEM of 6 independent experiments.

EXAMPLE 1: PROTECTIVE EFFECT OF BACLOFEN ON THE SURVIVAL OF PURE CULTURED RETINAL GANGLION CELLS (RGCS)

Material & Methods:

Primary cultures of pure ganglion cells: Primary cultures of retinal ganglion cells (RGC) were isolated from retinas of adult Long Evans rat (8-week old) with an immunopanning technique, according the protocol previously described in young rats by Barres et al. (1988). Briefly, animals were anesthetized and killed by cerebral dislocation and their eyes removed and placed in a solution of phosphate-buffered saline (PBS) containing 1g/l of glucose (PBS-glucose; Invitrogen, Carlsbad, CA, USA). After one rinse in PBS-glucose, retinas were incubated in the same medium containing 33 U/l/ml of papain (Worthington, Lakewood, NJ, USA) and 200 U/ml of DNase (Sigma-Aldrich, St-Louis, MO, USA) for 30 min at 37°C. They were then rinsed in PBS-glucose, containing 0.15% ovomucoid (Roche Diagnosis, Basel, Switzerland) and 0.15% bovine serum albumin (BSA;
Sigma-Aldrich). Retina were dissociated in PBS-glucose containing 0.15% ovomucoid, 015% BSA, 333 Ul/ml of DNAse and a rabbit anti-rat macrophage (~5mg/ml; Accurate Chemical & Scientific Corporation, Westbury, NY, USA) in three steps, using pipettes with decreasing tip diameters. The cell suspension was centrifuged at 115g during 13 min at room temperature. The supernatant was removed and cells were suspended in PBS-glucose, containing 1% ovomucoid and 1% BSA. After a second centrifugation (115g, 13 min), cells were suspended in the PBS-glucose, containing 0.02% Bovine Serum Albumin (BSA). Cell suspension was filtrated using a Sefar Nitrex mesh (48µm, Dutsch, Brumath, France) and then incubated in a dish (diameter 150mm), previously coated with a goat anti rabbit IgG (Jackson Immunoresearch, West Grove, PA, USA), during 36 min at room temperature. After a vigorous shaking of the dish, the cell suspension was moved into a second dish (diameter 150mm), previously coated with the same antibody, and incubated during 33 min at room temperature. After another vigorous shaking, the remaining cell suspension was transferred into a dish (diameter 100mm), previously coated successively with (i) a goat anti-mouse IgM (Jackson Immunoresearch, West Grove, PA, USA) and (ii) an hybridoma extract prepared in our laboratory from a T11D7 hybridoma cell line (ATCC, Manassas, VA, USA). After 45 min incubation, the dish was rinsed ten times with PSB-glucose. Adherent cells remaining into the dish were RGC specifically selected by Thy-1 antibody contained in the hybridoma extract. Cells were incubated with Earle's Balanced Salts Solution (EBSS; Sigma-Aldrich) containing 0.125% of trypsin (Sigma-Aldrich) for 10 min at 37°C, in humidified atmosphere (5% CO₂).

Trypsin action was blocked by addition into dish of PBS-glucose, containing 30% inactive foetal bovine serum (FBS; Invitrogen). Cells were detached by ~10 successive pipette flows of PBS-glucose-30%FBS, and the resulting cell suspension was centrifuged at 115g for 15min. Pure RGC were then suspended in Neurobasal-A medium (Invitrogen) supplemented 2mM L-glutamine (Invitrogen) and cells were seeded in the 48-well plate at an initial density of 2x10⁴ cells/well, on 8mm in diameter coverslips, previously coated by successively poly-D-lysine (2 µg/cm² for 45 min; Sigma-Aldrich) and laminin (~8g/cm² overnight; Sigma-Aldrich). Cultures were kept in a humidified chamber at 37°C containing 5% CO₂, for 1 to 6 Days in vitro (DIV).

**Viability test and RGC counting:** RGC viability was assessed with the "lived-dead" test (Invitrogen), which consists in labelling viable cells with calcein AM detected as a green fluorescence, whereas dead cell were labelled with ethidium producing a red fluorescence. Briefly, coverslips were incubated in a mixture of calceinAM and ethidium homodimer-1
(performed in a PBS medium) for 1 hour in the incubator (humidified chamber, 37°C, 5%CO₂). Only lived RCG were counted from seven fields taken on each coverslip using a microscope (Leica DM 5000B, Solms, Germany) equipped for epifluorescence. Viable RCG were counted at 1 day in vitro (DIV) and 6 DIV to calculate the percentage of cell survival.

**Results:**

To investigate if Baclofen has a direct effect on RGC survival, it was applied on pure adult rat RGC in culture. After 6 days in culture, 10µM of Baclofen increased the density of viable cells by 87.5±57.2% with respect to the control condition (Figure 1). This result indicated that Baclofen can affect directly RGC survival.

**REFERENCES:**

Throughout this application, various references describe the state of the art to which this invention pertains. The disclosures of these references are hereby incorporated by reference into the present disclosure.


Quigley, 1987; Quigley, 1999; Libby et al, 2005; Whitmore et al., 2005).

CLAIMS:

1. A GABA_B receptor agonist for use in the prevention and treatment of a disease associated with retinal ganglion cell degeneration.

2. The GABA_B receptor agonist according to claim 1 which is selected from the group consisting of small organic molecules, antibodies or aptamers.

3. The GABA_B receptor agonist according to claim 2 which is selected from the group consisting of 4-amino-3-(4-chlorophenyl)butanoic acid (baclofen), (2R)-(3-amino-2-fluoropropyl)sulfinic acid, (2R)-(3-amino-2-hydroxypropyl)sulfamic acid; (2S)-(3-amino-2-fluoropropyl)sulfinic acid; (2S)-(3-amino-2-hydroxypropyl)sulfamic acid; (3-amino-1-hydroxypropyl)methylphosphinic acid; (3-amino-2-(4-fluorophenyl)propyl)phosphonous acid; (3-amino-2-benzylpropyl)phosphonous acid; (3-amino-2-cyclohexylpropyl)phosphonous acid; (3-amino-2-hydroxypropyl)(difluoromethyl)phosphinic acid; (3-amino-1-hydroxypropyl)methylphosphinic acid; (3-amino-1-hydroxypropyl)methylphosphinic acid, (3-amino-2-(4-chlorophenyl)-2-hydroxypropyl)phosphonous acid, (3-amino-2-(4-chlorophenyl)propyl)phosphonous acid; (3-amino-2-(4-chlorophenyl)propyl)sulfinic acid, (3-amino-2-(4-fluorophenyl)propyl)phosphonous acid, (3-amino-2-benzylpropyl)phosphonous acid, (3-amino-2-cyclohexylpropyl)phosphonous acid, (3-amino-2-hydroxypropyl)sulfinic acid; (3-amino-2-fluoropropyl)sulfinic acid; (3-amino-2-benzylpropyl)phosphonous acid, (3-amino-2-cyclohexylpropyl)phosphonous acid; (3-amino-2-hydroxypropyl)(difluoromethyl)phosphinic acid; (3-amino-1-hydroxypropyl)methylphosphinic acid; (3-amino-1-hydroxypropyl)methylphosphinic acid, (3-amino-2-hydroxypropyl)methylphosphinic acid, (3-amino-2-fluoropropyl)phosphonous acid, (3-amino-2-methylpropyl)phosphonous acid, (3-amino-2-oxo-propyl)methyl phosphinic acid, (3-amino-2-oxopropyl)sulfamic acid, (3-amino-2-phenylpropyl)phosphonous acid, (3-aminobutyl)phosphonous acid, (3-aminobutyrrl)(difluoromethyl)phosphinic acid; (3-aminobutyl)phosphonous acid, (3-aminobutyl)hydroxymethylphosphinic acid, (3-aminopropyl)methylphosphinic acid, (3-aminopropyl)phosphonous acid, (4-amino-1,1,1-trifluorobut-2-yl)methylphosphinic acid; (4-aminobut-2-yl)methylphosphinic acid, (4-aminobut-2-yl)methylphosphinic acid,
yl)phosphonous acid, (5-aminopent-3-yl)methylphosphinic acid, (E)-(3-aminopropen-1-yl)methylphosphinic acid; (E)-(3-aminopropen-1-yl)phosphonous acid, (E)-(3-aminopropen-1-yl)methylphosphinic acid; [3-amino-2-(4-chlorophenyl)-2-hydroxypropyl]phosphonous acid; [3-amino-2-(4-methoxyphenyl)propyl]phosphonous acid; [3-amino-2-(4-trifluoromethylphenyl)propyl]phosphonous acid; [3-amino-2-(4-chlorophenyl)-2-hydroxypropyl]phosphonous acid; [3-amino-2-(4-methoxyphenyl)propyl]phosphonous acid; [3-amino-2-(4-trifluoromethylphenyl)propyl]phosphonous acid; [3-amino-2-(4-chlorophenyl)-2-hydroxypropyl]phosphonous acid; [3-amino-2-(4-methoxyphenyl)propyl]phosphonous acid; [3-amino-2-(4-trifluoromethylphenyl)propyl]phosphonous acid; [3-amino-2-(4-chlorophenyl)-2-hydroxypropyl]phosphonous acid; [3-amino-2-(4-methoxyphenyl)propyl]phosphonous acid; [3-amino-2-(4-trifluoromethylphenyl)propyl]phosphonous acid; [beta]-phenyl-GABA; [gamma]-hydroxybutyrate; 1-(4-chlorophenyl)-4-(3,5-dimethoxybenzoyl)piperazine; 1-(aminomethyl)cyclohexanecarboxylic acid, 2-(7-chloro-1,8-naphthyridin-2-yl)-3-[(1,4-dioxo-8-azaspiro[4,5]dec-8-yl)carbonylmethyl]-isoindolin-1-one, 2-aminoethanesulfonic acid; 3-hydroxy-baclofen; 3-(ampropyl)methylphosphinic acid; 3-amino-2-(4-chlorophenyl)-1-nitropropane; 3-amino-2-(P-methyl)phosphinic acid; 3-amino-2-propylphosphonic acid; 3-amino-3-propylsulfonic acid, 4'-ethyl-2-methyl-3-pyrrolidinopropiophenone; 4-(3-hydroxy-pyridin-2-yl)butyrolactam, 4-[[alpha]-(4-chlorophenyl)-5-5-fluoro-2-hydroxybenzylidene]amino]butyramide; 4-amino-[beta]-(5-chloro-thien-2-yl)butanoic acid; 4-amino-[beta]-(5-chloro-thien-2-yl)butanoic acid; 4-amino-3-(2-chlorophenyl)butanoic acid; 4-amino-3-(2-imidazolyl)butanoic acid; 4-amino-3-(4-chlorophenyl)-3-hydroxyphenylbutanoic acid; 4-amino-3-(5-chlorothien-2-yl)butanoic acid; 4-amino-3-(5-chlorothien-2-yl)butanoic acid; 4-amino-3-(thien-2-yl)butanoic acid; 4-amino-3-hydroxybutanoic acid; 4-amino-3-(4-fluorophenyl)butanoic acid; 4-amino-3-(5-bromothien-2-yl)butanoic acid; 4-amino-3-(5-chlorothien-2-yl)butanoic acid; 4-amino-3-(thien-2-yl)butanoic acid; 4-amino-3-hydroxybutanoic acid; 4-amino-3-phenylbutanoic acid; 4-amino-5-methoxybenzofuran-2-yl)butanoic acid; 4-aminobutanoic acid (GABA), 4-guanidino-3-(4-chlorophenyl)butanoic acid; 2,6-Di-tet-butyl-4-(3-hydroxy-2,2-dimethylpropyl)phenol (CGP7930), 3-(3,5-di-tet-butyl-4-hydroxyphenyl)-2,2-dimethylpropanal and N,N-Dicyclopentyl-2-methy sulfanyl-5-nitro-pyrimidine-4,6-diamine.

4. The GABA₉ receptor agonist according to any of claims 1 to 3 wherein said disease associated with retinal ganglion cell degeneration is selected from the group consisting
of glaucoma and other forms of optic nerve atrophy like the Leber hereditary optic neuropathy or pathologies with retinal ischemia like vascular occlusions.

5. The GABA$_B$ receptor agonist according to claim 4 wherein said diseases associated with retinal ganglion cell degeneration is selected from the group consisting of arteritic ischemic optic neuropathy (giant cell arteritis), nonarteritic ischemic optic neuropathy, infiltrative optic neuropathy (sarcoidosis), infectious optic neuropathy (syphilis, lyme, toxoplasmosis, herpes zoster), optic neuritis from demyelinating disease, posradiation optic neuropathy, acrodermatitis enteropathica, hereditary optic neuropathy (Leber's hereditary optic neuropathy, dominant optic neuropathy), compressive optic neuropathy (orbital pseudotumor, thyroid eye disease), autoimmune optic neuropathy (Lupus), and diabetic retinopathy.

6. The GABA$_B$ receptor agonist according to claim 4 wherein said diseases associated with retinal ganglion cell degeneration is selected from the group consisting of cholestatic liver disease, nutritional optic neuropathy, ketogenic diet, thiamine deficiency.

7. A pharmaceutical composition for use in the prevention and treatment of a disease associated with retinal ganglion cell degeneration comprising a GABA$_B$ receptor agonist according to any of claims 1 to 6, and optionally one or more pharmaceutically acceptable excipients.

8. A pharmaceutical composition for use in the treatment or prevention of glaucoma comprising a GABA$_B$ receptor agonist according to any of claims 1 to 6 and at least one active ingredient selected from the group consisting latanoprost, timolol, travoprost, dorzolamide, brimonidine, bimatoprost, apraclonidine, dipivephrine, propine, acetazolamide, brinzolamide.

9. An antimicrobial or anti-malaria composition comprising a GABA$_B$ receptor agonist according to any of claims 1 to 6 and at least one active ingredient selected from the group consisting of chloramphenicol, chloroquine, clioquinol, dapsone, ethambutol, iodochlorhydroxyquinoline, isoniazide, linezolid, streptomycin.

10. An immunomodulator or immunosuppressive composition comprising a GABA$_B$ receptor agonist according to any of claims 1 to 6 and at least one active ingredient
selected from the group consisting of cyclosporine, interferon-alpha, tacrolimus (FK506).

11. A chemotherapeutic composition comprising a GABA$_B$ receptor agonist according to any of claims 1 to 6 and at least one active ingredient selected from the group consisting of carboplatin, chlorambucil, cisplatin, 5-fluorouracil, methotrexate, nitrosureas (BCNU, CCNU, ACNU), paclitaxel, tamoxifen, 5-vincristine, cytosine arabinoside, purine analogues, procarbazine, cyclophosphamide, vinca alkaloids.

12. A therapeutic composition comprising a GABA$_B$ receptor agonist according to any of claims 1 to 6 and at least one active ingredient selected from the group consisting of amiodarone, amantidine amoproxen, cafergot, chlorpropamide, cimetidine, clomiphene citrate, deferoxamine, disulfiram, emetine, infliximab, pheniprazine, quinine, PDE inhibitors (sildenafil, tadalafil, vardenafil), bendroflumethiazide, chorothiazide, chlortalidone, hydrochlorothiazide, hydroflumethiazide, indapamide, methyclothiazide, metolazone, polythiazide, trichlormethiazide.

13. A GABA$_B$ receptor agonist according any of claims 1 to 3 for use in the prevention of the retinal ganglion cell degeneration induced by a drug such as amiodarone, amantidine amoproxen, cafergot, chlorpropamide, cimetidine, clomiphene citrate, deferoxamine, disulfiram, emetine, infliximab, pheniprazine, quinine, PDE inhibitors (sildenafil, tadalafil, vardenafil), bendroflumethiazide, chorothiazide, chlortalidone, hydrochlorothiazide, hydroflumethiazide, indapamide, methyclothiazide, metolazone, polythiazide, trichlormethiazide, antiepileptic drugs such as vigabatrin valproate, tiagabine, gabapentin, valproate, levetiracetam, topiramate, felbamate, benzodiazepines like diazepam, clonazepam and clobazam or barbiturates like primidone and phenobarbitone.
### A. CLASSIFICATION OF SUBJECT MATTER

- **A61K31/197**  
- **A61K45/06**  
- **A61P27/02**  
- **A61P27/06**

According to International Patent Classification (IPC) and 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
- BIOSIS
- WPI Data
- EMBASE
- FSTA

### C. DOCUMENTS CONSIDERED TO BE RELEVANT

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| X        | wo 93/18762 A2 (ALLERGAN INC [US])  
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page 1, lines 5-11  
page 4, line 5 - page 5, line 3; claim 20; figure 2 | 1-4,7 |

Further documents are listed in the continuation of Box C.

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
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11 May 2011

Date of actual completion of the international search

18/05/2011

Date of mailing of the international search report

Houyvet-Landri
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