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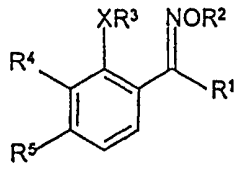

(I)

(57) Abstract

The present invention relates to ion channel modulating agents. More paricularly, the present invention relates to a particular class of oxime derivatives represented by general formula (I) that has proven useful as modulators of SK_{Ca}, IK_{Ca} and BK_{Ca} channels. In further aspects, the present invention relates to the use of these SK/IK/BK channel modulating agents for the manufacture of medicaments, and pharmaceutical compositions comprising the SK/IK/BK channel modulating agents. The SK/IK/BK channel modulating agents of the invention are useful for the treatment or alleviation of diseases and conditions associated with the SK/IK/BK channels.

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ION CHANNEL MODULATING AGENTS

TECHNICAL FIELD

The present invention relates to ion channel modulating agents. More particularly, the present invention relates to a particular class of chemical compounds that has proven useful as modulators of SK_{Ca}, IK_{Ca} and BK_{Ca} channels. In further aspects, the present invention relates to the use of these SK/IK/BK channel modulating agents for the manufacture of medicaments, and pharmaceutical compositions comprising the SK/IK/BK channel modulating agents.

The SK/IK/BK channel modulating agents of the invention are useful for the treatment or alleviation of diseases and conditions associated with the SK/IK/BK channels.

BACKGROUND ART

Ion channels are transmembrane proteins, which catalyse the transport of inorganic ions across cell membranes. The ion channels participate in processes as diverse as the generation and timing of action potentials, synaptic transmissions, secretion of hormones, contraction of muscles, etc.

Many drugs exert their effects via modulation of ion channels. Examples are anti-epileptic compounds like Phenytoin and Lamotrigine, which block voltage dependent Na^+-channels in the brain, anti-hypertensive drugs like Nifedipine and Diltiazem, which block voltage dependent Ca^{2+}-channels in smooth muscle cells, and stimulators of insulin release like Glibenclamide and Tolbutamide, which block an ATP-regulated K^+-channel in the pancreas.

All mammalian cells express potassium (K^+) channels in their cell membranes, and the channels play a dominant role in the regulation of the membrane potential. In nerve and muscle cells they regulate the frequency and form of the action potential, the release of neurotransmitters, and the degree of broncho- and vasodilation.

From a molecular point of view, the K^+ channels represent the largest and most diverse group of ion channels. For an overview they can be divided into five

large subfamilies: Voltage-activated K^+ channels (K_v), long QT related K^+ channels (KvLQT), inward rectifiers (K_{IR}), two-pore K^+ channels (K_{TP}), and calcium-activated K^+ channels (K_{ca}).

The latter group, the Ca^{2+}-activated K^+ channels, consists of three well-defined subtypes: SK channels, IK channels and BK channels. SK, IK and BK refer to the single-channel conductance (**S**mall, **I**ntermediate and **B**ig conductance **K** channel). The SK, IK, and BK channels exhibit differences in e.g. voltage- and calcium-sensitivity, pharmacology, distribution and function.

Ca^{2+}-activated SK channels are present in many central neurons and ganglia, where their primary function is to hyperpolarize nerve cells following one or several action potentials to prevent long trains of epileptogenic activity to occur. The SK channels are also present in several peripheral cells including skeletal muscle, gland cells, liver cells, and T-lymphocytes.

The significance of SK channels in normal skeletal muscle is not clear, but their number is significantly increased in denervated muscle, and the large number of SK channels in the muscle of patients with myotonic muscle dystrophia suggest a role in the pathogenesis of the disease.

A number of blockers of SK channels exist, e.g. apamin, atracurium, pancuronium, and tubocurarine, and they are all positively charged molecules which act as pore blockers.

The Ca^{2+}-activated IK channel shares a number of characteristics with the Ca^{2+}-activated SK channel, since it is highly K-selective, is activated by sub-micromolar concentrations of Ca^{2+}, and has an inwardly rectifying conductance. However, there are also striking differences. The unit conductance of the IK channel is 4-5 fold higher than that of the SK channel, and the distribution of the IK channel is restricted to the blood and vasculature. Thus, the IK channel is not expressed in the nervous system and in muscle, but in endothelial cells, cells of epithelial origin and in red blood cells.

In the red blood cells, where the IK channel has been denominated the Gardos channel, a rise in the concentration of intracellular Ca^{2+} opens the channel and causes potassium loss and cell dehydration, a condition which is exacerbated in sickle cell anemia. Promising therapeutic approaches for sickle cell anemia involve specific block of the IK channel.

IK channels have also been implicated in the microvasculature of the kidney, where they may be responsible for the vasodilatory effects of bradykinin. The decrease in blood pressure during septic shock is caused by an increased NO production by the endothelial cells, and the IK channels in these cells are responsible for maintaining the Ca^{2+} influx activating the Ca^{2+}-sensitive NO-synthase.

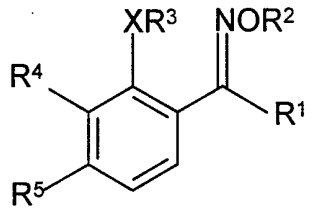
In brain capillary endothelial cells, IK channels, activated by endothelin that is produced by neurons and glia, shunt excess K^+ into the blood. Neurotrophilic granulocytes, i.e. mobile phagocytic cells that defend the body against microbial invaders, undergo large depolarisation subsequent to agonistic stimulation, and IK channels have been implicated in depolarising the stimulated granulocyte.

The Ca^{2+}-activated BK channels present in many cells including most central and peripheral nerve cells, striated muscle cells, cardiac cells, smooth muscle cells of the airways, the vasculature, the gastrointestinal tract and bladder, in endo- and exocrine glands including pancreatic b-cells and in kidney tubules.

SUMMARY OF THE INVENTION

According to the present invention it has now been found that a particular group of oxime derivatives possess valuable activity as modulators of SK_{Ca}, IK_{Ca} and/or BK_{Ca} channels.

In its first aspect the invention relates to novel oxime derivatives represented by the general formula



(I)

wherein

R^1 represents hydrogen; an alkyl group; a cycloalkyl group; hydroxy; an alkoxy group; an acyl group; a phenyl or a benzyl group, which phenyl and benzyl groups may be substituted one or more times with substituents selected from halogen, $-NO_2$, -CN, $-CF_3$, alkyl, cycloalkyl, hydroxy, and alkoxy; a group of the formula $-CH_2CN$; a group of the formula $-CH_2CO_2R'$, wherein R' represents hydrogen or an alkyl group; a group of the formula $-CH_2CONR^{IV}R^V$, wherein R^{IV} and R^V

independently represents hydrogen or an alkyl group; or a group of the formula -$CH_2C(=NOH)NH_2$;

R^2 represents hydrogen, an alkyl group, a cycloalkyl group, a phenyl or a benzyl group, which phenyl and benzyl groups may be substituted one or more times with substituents selected from halogen, -NO_2, -CN, -CF_3, alkyl, cycloalkyl, hydroxy, and alkoxy;

X represents oxygen, sulphur, or an amino group, preferably an amino group of the formula NHR^3;

R^3 represents hydrogen, an alkyl group, a cycloalkyl group, a phenyl or a benzyl group, which phenyl and benzyl groups may be substituted one or more times with substituents selected from halogen, -NO_2, -CN, -CF_3, alkyl, cycloalkyl, hydroxy, and alkoxy; and

R^4 and R^5, independently of each another represents hydrogen; halogen; -NO_2; -CN; -CF_3; an alkyl group; an alkoxy group; a phenyl or a benzyl group, which phenyl and benzyl groups may be substituted one or more times with substituents selected from halogen, -NO_2, -CN, -CF_3, alkyl, cycloalkyl, hydroxy, and alkoxy; or a group of the formula -$SO_2NR''R'''$, wherein R'' and R''' independently of each another represents hydrogen or an alkyl group;

or R^4 and R^5 together form an additional 4 to 7 membered fused ring, which fused ring may be aromatic or partially saturated, and which fused ring may optionally be substituted one or more times with substituents selected from the group consisting of halogen, -NO_2, -CN, -CF_3, and a group of the formula -$SO_2NR''R'''$, wherein R'' and R''' independently of each another represents hydrogen or an alkyl group.

In a second aspect, the invention provides a pharmaceutical composition comprising a therapeuticallly-effective amount of an oxime derivative of the invention, or a pharmaceutically-acceptable addition salt thereof, together with at least one pharmaceutically-acceptable carrier or diluent, for the treatment or alleviation of diseases, disorders or conditions responsive to modulation of SK_{Ca}, IK_{Ca} and/or BK_{Ca} channels.

In a third aspect the invention relates to the use of an oxime derivative of the invention, or a pharmaceutically-acceptable addition salt thereof, for the manufacture of a pharmaceutical composition for the treatment or alleviation of a disease or a disorder or a condition of a mammal, including a human, which disease, disorder or condition is responsive to modulation of SK_{Ca}, IK_{Ca} and/or BK_{Ca} channels.

In a final aspect, the invention provides a method for the treatment or alleviation of diseases or disorders or conditions of a living animal body, including a human, which diseases, disorders or conditions are responsive to modulation of SK_{Ca}, IK_{Ca} and/or BK_{Ca} channels, comprising the step of administering to such a living animal body, including a human, in need thereof a therapeutically effective amount of an oxime derivative of the invention.

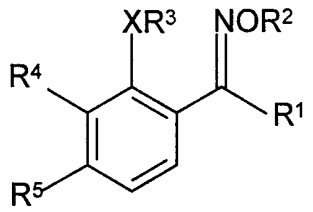
DETAILED DISCLOSURE OF THE INVENTION

According to the present invention it has now been found that a particular group of oxime derivatives possess valuable activity as modulators of Sk_{Ca}, IK_{Ca} and/or BK_{Ca} channels.

SK/IK/BK Modulating Agents

In the context of this invention, chemical compounds capable of affecting Sk_{Ca}, IK_{Ca} and/or BK_{Ca} channels are designated SK/IK/BK channel modulating agents. The SK/IK/BK channel modulating agents of the invention may affect the ion channels by opening (activating) the channels or by inhibiting (blocking) the channels.

The SK/IK/BK channel modulating agents of the invention belongs to a particular class of oxime derivatives, represented by the following general formula



(I)

wherein

R^1 represents hydrogen; an alkyl group; a cycloalkyl group; hydroxy; an alkoxy group; an acyl group; a phenyl or a benzyl group, which phenyl and benzyl groups may be substituted one or more times with substituents selected from halogen, $-NO_2$, -CN, $-CF_3$, alkyl, cycloalkyl, hydroxy, and alkoxy; a group of the formula $-CH_2CN$; a group of the formula $-CH_2CO_2R'$, wherein R' represents hydrogen or an alkyl group; a group of the formula $-CH_2CONR^{IV}R^V$, wherein R^{IV} and R^V

independently represents hydrogen or an alkyl group; or a group of the formula -$CH_2C(=NOH)NH_2$;

R^2 represents hydrogen, an alkyl group, a cycloalkyl group, a phenyl or a benzyl group, which phenyl and benzyl groups may be substituted one or more times with substituents selected from halogen, -NO_2, -CN, -CF_3, alkyl, cycloalkyl, hydroxy, and alkoxy;

X represents oxygen, sulphur, or an amino group, preferably an amino group of the formula NHR^3;

R^3 represents hydrogen, an alkyl group, a cycloalkyl group, a phenyl or a benzyl group, which phenyl and benzyl groups may be substituted one or more times with substituents selected from halogen, -NO_2, -CN, -CF_3, alkyl, cycloalkyl, hydroxy, and alkoxy; and

R^4 and R^5, independently of each another represents hydrogen; halogen; -NO_2; -CN; -CF_3; an alkyl group; an alkoxy group; a phenyl or a benzyl group, which phenyl and benzyl groups may be substituted one or more times with substituents selected from halogen, -NO_2, -CN, -CF_3, alkyl, cycloalkyl, hydroxy, and alkoxy; or a group of the formula -$SO_2NR''R'''$, wherein R'' and R''' independently of each another represents hydrogen or an alkyl group;

or R^4 and R^5 together form an additional 4 to 7 membered fused ring, which fused ring may be aromatic or partially saturated, and which fused ring may optionally be substituted one or more times with substituents selected from the group consisting of halogen, -NO_2, -CN, -CF_3, and a group of the formula -$SO_2NR''R'''$, wherein R'' and R''' independently of each another represents hydrogen or an alkyl group.

In a preferred embodiment, R^1 represents hydrogen or an alkyl group.

In another preferred embodiment, R^2 represents hydrogen, an alkyl group, or a phenyl or benzyl group, which phenyl and benzyl groups may be substituted one or more times with substituents selected from halogen, -NO_2, -CN, -CF_3, alkyl, cycloalkyl, hydroxy, and alkoxy.

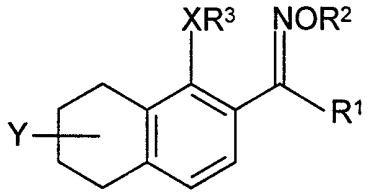
In a third preferred embodiment, X represents oxygen, or an amino group of the formula NHR^3, in which formula R^3 represents hydrogen, alkyl, benzyl, or acetyl.

In a fourth preferred embodiment, R^4 and R^5, independently of each another represents hydrogen, halogen, alkyl, or alkoxy.

In a fifth preferred embodiment, R^4 and R^5 together form an additional 6 membered fused ring, which fused ring may be aromatic or partially saturated, and

which fused ring may optionally be substituted one or more times with substituents selected from the group consisting of halogen, $-NO_2$, -CN, $-CF_3$, and a group of the formula $-SO_2NR''R'''$, wherein R'' and R''' independently of each another represents hydrogen or an alkyl group.

In yet another preferred embodiment, the oxime derivative of the invention may be represented by the general formula



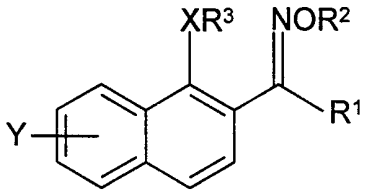
(II)

wherein

X, R1, R2 and R3 are as defined above; and

Y represents hydrogen, halogen, $-NO_2$, -CN, $-CF_3$, or a group of the formula $-SO_2NR''R'''$, wherein R'' and R''' independently of each another represents hydrogen or an alkyl group.

In still another preferred embodiment, the oxime derivative of the invention may be represented by the general formula



(III)

wherein

X, R1, R2 and R3 are as defined above; and

Y represents hydrogen, halogen, $-NO_2$, -CN, $-CF_3$, or a group of the formula $-SO_2NR''R'''$, wherein R'' and R''' independently of each another represents hydrogen or an alkyl group.

In its most preferred embodiment, the oxime derivative of the invention is

2-Aldoximo-1-naphtol;

2-Aldoximo-5,6-dimethylphenol;

2-Aldoximo-5,6-dichlorophenol;

O-Benzyl 2-formyl-5,6-dichlorophenol oxime;

O-Methyl 2-formyl-5,6-dichlorophenol oxime;

2-Aldoximo-5,6,7,8-tetrahydro-1-naphtol;

1-Hydroxy-2-acetonaphtone oxime;

1-Methoxy-2-acetonaphtone oxime;

O-Ethyl 1-methoxy-2-acetonaphtone oxime;

1-Ethoxy-2-acetonaphtone oxime;

1-Benzyloxy-2-acetonaphtone oxime;

2-Hydroxy-3,4-dimethylacetophenone oxime;

2-Methoxy-3,4-dimethylacetophenone oxime;

2-Hydroxy-3,4-dimethoxyacetophenone oxime;

2,3,4-Trimethoxyacetophenone oxime;

1-Hydroxy-5,6,7,8-tetrahydro-2-acetonaphtone oxime;

1-Methoxy-5,6,7,8-tetrahydro-2-acetonaphtone oxime;

1-(2-Propyloxy)-2-acetonaphtone oxime; or

N-(2-acetylphenyl)acetamide oxime.

Definition of Substituents

In the context of this invention halogen represents a fluorine, a chlorine, a bromine or a iodine atom.

In the context of this invention an alkyl group designates a univalent saturated, straight or branched hydrocarbon chain. The hydrocarbon chain preferably contain of from one to twelve carbon atoms (C_{1-12}-alkyl), more preferred of from one to six carbon atoms (C_{1-6}-alkyl; lower alkyl), including pentyl, isopentyl, neopentyl, tertiary pentyl, hexyl and isohexyl. In a preferred embodiment alkyl represents a C_{1-4}-alkyl group, including butyl, isobutyl, secondary butyl, and tertiary butyl. In a most preferred embodiment alkyl represents a C_{1-3}-alkyl group, which may in particular be methyl, ethyl, propyl or isopropyl.

In the context of this invention a cycloalkyl group designates a cyclic alkyl group, preferably containing of from three to seven carbon atoms (C_{3-7}-cycloalkyl), including cyclopropyl, cyclobutyl, cyclopentyl, and cyclohexyl.

In the context of this invention an alkoxy group designates an "alkyl-O-" group, wherein alkyl is as defined above.

In the context of this invention an amino group may be a primary ($-NH_2$), secondary ($-NH-R^3$), or tertiary ($-N(alkyl)R^3$) amino group.

In the context of this invention an acyl group designates a carboxy group or an alkylcarbonyl group, wherein alkyl is as defined above. Examples of preferred acyl groups of the invention are carboxy, acetyl, and propionyl.

Pharmaceutically Acceptable Salts

The SK/IK/BK channel modulating agents of the invention may be provided in any form suitable for the intended administration. Suitable forms include pharmaceutically (i.e. physiologically) acceptable salts, and pre- or prodrug forms of the chemical compound of the invention.

Examples of pharmaceutically acceptable addition salts include, without limitation, the non-toxic inorganic and organic acid addition salts such as the acetate derived from acetic acid, the aconate derived from aconitic acid, the ascorbate derived from ascorbic acid, the benzenesulfonate derived from benzensulfonic acid, the benzoate derived from benzoic acid, the cinnamate derived from cinnamic acid, the citrate derived from citric acid, the embonate derived from embonic acid, the enantate derived from enanthic acid, the formate derived from formic acid, the fumarate derived from fumaric acid, the glutamate derived from glutamic acid, the glycolate derived from glycolic acid, the hydrochloride derived from hydrochloric acid, the hydrobromide derived from hydrobromic acid, the lactate derived from lactic acid, the maleate derived from maleic acid, the malonate derived from malonic acid, the mandelate derived from mandelic acid, the methanesulfonate derived from methane sulphonic acid, the naphthalene-2-sulphonate derived from naphtalene-2-sulphonic acid, the nitrate derived from nitric acid, the perchlorate derived from perchloric acid, the phosphate derived from phosphoric acid, the phthalate derived from phthalic acid, the salicylate derived from salicylic acid, the sorbate derived from sorbic acid, the stearate derived from stearic acid, the succinate derived from succinic acid, the sulphate derived from sulphuric acid, the tartrate derived from tartaric acid, the toluene-p-sulphonate derived from p-toluene sulphonic acid, and the like. Such salts may be formed by procedures well known and described in the art.

Other acids such as oxalic acid, which may not be considered pharmaceutically acceptable, may be useful in the preparation of salts useful as intermediates in obtaining a chemical compound of the invention and its pharmaceutically acceptable acid addition salt.

Metal salts of a chemical compound of the invention includes alkali metal salts, such as the sodium salt of a chemical compound of the invention containing a carboxy group.

The chemical compound of the invention may be provided in unsolved or solvated forms together with a pharmaceutically acceptable solvents such as water, ethanol, and the like. Solvated forms may also include hydrated forms such as the monohydrate, the dihydrate, the hemihydrate, the trihydrate, the tetrahydrate, and the like. In general, solvated forms are considered equivalent to unsolved forms for the purposes of this invention.

Steric Isomers

The SK/IK/BK channel modulating agents of the present invention may exist in (+) and (-) forms as well as in racemic forms. The racemates of these isomers and the individual isomers themselves are within the scope of the present invention.

Racemic forms can be resolved into the optical antipodes by known methods and techniques. One way of separating the diastereomeric salts is by use of an optically active acid, and liberating the optically active amine compound by treatment with a base. Another method for resolving racemates into the optical antipodes is based upon chromatography on an optical active matrix. Racemic compounds of the present invention can thus be resolved into their optical antipodes, e.g., by fractional crystallisation of d- or l- (tartrates, mandelates, or camphorsulphonate) salts for example.

The chemical compounds of the present invention may also be resolved by the formation of diastereomeric amides by reaction of the chemical compounds of the present invention with an optically active activated carboxylic acid such as that derived from (+) or (-) phenylalanine, (+) or (-) phenylglycine, (+) or (-) camphanic acid or by the formation of diastereomeric carbamates by reaction of the chemical compound of the present invention with an optically active chloroformate or the like.

Additional methods for the resolving the optical isomers are known in the art. Such methods include those described by *Jaques J, Collet A, & Wilen S* in "Enantiomers, Racemates, and Resolutions", John Wiley and Sons, New York (1981).

Moreover, some of the chemical compounds of the invention being oximes, may thus exist in two forms, syn- and anti-form (Z- and E-form), depending on the arrangement of the substituents around the -C=N- double bond. A chemical

compound of the present invention may thus be the syn- or the anti-form (Z- and E-form), or it may be a mixture hereof.

Biological Activity

According to the present invention it has now been found that the isatin derivatives of the invention possess valuable activity as modulators of SK_{Ca}, IK_{Ca} and/or BK_{Ca} channels.

The SK/IK/BK channel modulating activity may be monitored using conventional electrophysiological methods such as patch-clamp techniques, or conventional spectroscopic methods such as FLIPR assay (Fluorescence Image Plate Reader; available from Molecular Devices). These methods generally comprises subjecting an SK_{Ca}, IK_{Ca} or BK_{Ca} containing cell to the action of the chemical compound of the invention, followed by monitoring the membrane potential of the SK_{Ca}, IK_{Ca} or BK_{Ca} containing cell in order to identify changes in the membrane potential caused by the action of the compound of the invention.

In Example 7 the biological activity of the compounds of the invention is demonstrated using electrophysiologic patch-clamp techniques.

Based on their biological activity the compounds of the invention are considered useful for the treatment or alleviation of diseases or conditions responsive to modulation of SK_{Ca}, IK_{Ca} and/or BK channels, including diseases or conditions like respiratory diseases such as asthma, cystic fibrosis, chronic obstructive pulmonary disease and rhinorrhea, convulsions, vascular spasms, coronary artery spasms, renal disorders, polycystic kidney disease, bladder spasms, urinary incontinence, bladder outflow obstruction, irritable bowel syndrome, gastrointestinal dysfunction, secretory diarrhoea, ischaemia, cerebral ischaemia, ischaemic hearth disease, angina pectoris, coronary hearth disease, traumatic brain injury, psychosis, anxiety, depression, dementia, memory and attention deficits, Alzheimer's disease, dysmenorrhea, narcolepsy, Reynaud's disease, intermittent claudication, Sjorgren's syndrome, migraine, arrhythmia, hypertension, absence seizures, myotonic muscle dystrophia, xerostomi, diabetes type II, hyperinsulinemia, premature labour, baldness, cancer, and immune suppression.

The compounds of the invention is considered particularly useful for reducing or inhibiting undesired immunoregulatory actions. In a preferred embodiment, therefore, the compounds of the may be used in the treatment or

alleviation of a diseases, disorders or condition related to immune dysfunction, or in order to obtain immune suppression in an individual in need herefore.

In another, and still more preferred embodiment, the invention relates to the use of a compound of the invention in a combination therapy with known immune-suppressants for the treatment or alleviation of a diseases, disorders or condition related to immune dysfunction, or for obtaining immune suppression. Preferred immune-suppressants to combine with the compounds of the invention include the calcineurin inhibitors (i.e. protein phosphatase 2B inhibitors), in particular Cyclosporin, and FK506.

Conditions which may benefit from this treatment include, but are not limited to diseases, disorders or conditions such as autoimmune diseases, e.g. Addison's disease, alopecia areata, Ankylosing spondylitis, haemolytic anemia (anemia haemolytica), pernicious anemia (anemia perniciosa), aphthae, aphthous stomatitis, arthritis, arteriosclerotic disorders, osteoarthritis, rheumatoid arthritis, aspermiogenese, asthma bronchiale, autoimmune asthma, autoimmune haemolysis, Bechet's disease, Boeck's disease, inflammatory bowel disease, Burkitt's lymphoma, Chron's disease, chorioiditis, colitis ulcerosa, Coeliac disease, cryoglobulinemia, dermatitis herpetiformis, dermatomyositis, insulin-dependent type I diabetes, juvenile diabetes, idiopathic diabetes insipidus, insulin-dependent diabetes mellisis, autoimmune demyelinating diseases, Dupuytren's contracture, encephalomyelitis, encephalomyelitis allergica, endophthalmia phacoanaphylactica, enteritis allergica, autoimmune enteropathy syndrome, erythema nodosum leprosum, idiopathic facial paralysis, chronic fatigue syndrome, febris rheumatica, glomerulo nephritis, Goodpasture's syndrome, Graves' disease, Hamman-Rich's disease, Hashimoto's disease, Hashimoto's thyroiditis, sudden hearing loss, sensoneural hearing loss, hepatitis chronica, Hodgkin's disease, haemoglobinuria paroxysmatica, hypogonadism, ileitis regionalis, iritis, leucopenia, leucemia, lupus erythematosus disseminatus, systemic lupus erythematosus, cutaneous lupus erythematosus, lymphogranuloma malignum, mononucleosis infectiosa, myasthenia gravis, traverse myelitis, primary idiopathic myxedema, nephrosis, ophthalmia symphatica, orchitis granulomatosa, pancreatitis, pemphigus, pemphigus vulgaris, polyarteritis nodosa, polyarthritis chronica primaria, polymyositis, polyradiculitis acuta, psoreasis, purpura, pyoderma gangrenosum, Quervain's thyreoiditis, Reiter's syndrome, sarcoidosis, ataxic sclerosis, progressive systemic sclerosis, scleritis, sclerodermia, multiple

sclerosis, sclerosis disseminata, acquired spenic atrophy, infertility due to antispermatozoan antobodies, thrombocytopenia, idiopathic thrombocytopenia purpura, thymoma, acute anterior uveitis, vitiligo, AIDS, HIV, SCID and Epstein Barr virus associated diseases such as Sjorgren's syndrome, virus (AIDS or EBV) associated B cell lymphoma, parasitic diseases such as Lesihmania, and immunosuppressed disease states such as viral infections following allograft transplantations, graft vs. Host syndrome, transplant rejection, or AIDS, cancers, chronic active hepatitis diabetes, toxic chock syndrome, food poisoning, and transplant rejection.

Pharmaceutical Compositions

In another aspect the invention provides novel pharmaceutical compositions comprising a therapeutically effective amount of a chemical compound having SK_{Ca}, IK_{Ca} or BK_{Ca} modulating activity.

While a chemical compound of the invention for use in therapy may be administered in the form of the raw chemical compound, it is preferred to introduce the active ingredient, optionally in the form of a physiologically acceptable salt, in a pharmaceutical composition together with one or more adjuvants, excipients, carriers, buffers, diluents, and/or other customary pharmaceutical auxiliaries.

In a preferred embodiment, the invention provides pharmaceutical compositions comprising the SK/IK/BK channel modulating agents of the invention, or a pharmaceutically acceptable salt or derivative thereof, together with one or more pharmaceutically acceptable carriers therefor, and, optionally, other therapeutic and/or prophylactic ingredients, know and used in the art. The carrier(s) must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not harmful to the recipient thereof.

Pharmaceutical compositions of the invention may be those suitable for oral, rectal, bronchial, nasal, topical (including buccal and sub-lingual), transdermal, vaginal or parenteral (including cutaneous, subcutaneous, intramuscular, and intravenous injection) administration, or those in a form suitable for administration by inhalation or insufflation.

The chemical compound of the invention, together with a conventional adjuvant, carrier, or diluent, may thus be placed into the form of pharmaceutical compositions and unit dosages thereof. Such forms include solids, and in particular

tablets, filled capsules, powder and pellet forms, and liquids, in particular aqueous or non-aqueous solutions, suspensions, emulsions, elixirs, and capsules filled with the same, all for oral use, suppositories for rectal administration, and sterile injectable solutions for parenteral use. Such pharmaceutical compositions and unit dosage forms thereof may comprise conventional ingredients in conventional proportions, with or without additional active compounds or principles, and such unit dosage forms may contain any suitable effective amount of the active ingredient commensurate with the intended daily dosage range to be employed.

The chemical compound of the present invention can be administered in a wide variety of oral and parenteral dosage forms. It will be obvious to those skilled in the art that the following dosage forms may comprise, as the active component, either a chemical compound of the invention or a pharmaceutically acceptable salt of a chemical compound of the invention.

For preparing pharmaceutical compositions from a chemical compound of the present invention, pharmaceutically acceptable carriers can be either solid or liquid. Solid form preparations include powders, tablets, pills, capsules, cachets, suppositories, and dispersible granules. A solid carrier can be one or more substances which may also act as diluents, flavouring agents, solubilizers, lubricants, suspending agents, binders, preservatives, tablet disintegrating agents, or an encapsulating material.

In powders, the carrier is a finely divided solid which is in a mixture with the finely divided active component.

In tablets, the active component is mixed with the carrier having the necessary binding capacity in suitable proportions and compacted in the shape and size desired.

The powders and tablets preferably contain from five or ten to about seventy percent of the active compound. Suitable carriers are magnesium carbonate, magnesium stearate, talc, sugar, lactose, pectin, dextrin, starch, gelatin, tragacanth, methylcellulose, sodium carboxymethylcellulose, a low melting wax, cocoa butter, and the like. The term "preparation" is intended to include the formulation of the active compound with encapsulating material as carrier providing a capsule in which the active component, with or without carriers, is surrounded by a carrier, which is thus in association with it. Similarly, cachets and lozenges are included. Tablets, powders,

capsules, pills, cachets, and lozenges can be used as solid forms suitable for oral administration.

For preparing suppositories, a low melting wax, such as a mixture of fatty acid glyceride or cocoa butter, is first melted and the active component is dispersed homogeneously therein, as by stirring. The molten homogenous mixture is then poured into convenient sized moulds, allowed to cool, and thereby to solidify.

Compositions suitable for vaginal administration may be presented as pessaries, tampons, creams, gels, pastes, foams or sprays containing in addition to the active ingredient such carriers as are known in the art to be appropriate.

Liquid preparations include solutions, suspensions, and emulsions, for example, water or water-propylene glycol solutions. For example, parenteral injection liquid preparations can be formulated as solutions in aqueous polyethylene glycol solution.

The chemical compound according to the present invention may thus be formulated for parenteral administration (e.g. by injection, for example bolus injection or continuous infusion) and may be presented in unit dose form in ampoules, pre-filled syringes, small volume infusion or in multi-dose containers with an added preservative. The compositions may take such forms as suspensions, solutions, or emulsions in oily or aqueous vehicles, and may contain formulation agents such as suspending, stabilising and/or dispersing agents. Alternatively, the active ingredient may be in powder form, obtained by aseptic isolation of sterile solid or by lyophilization from solution, for constitution with a suitable vehicle, e.g. sterile, pyrogen-free water, before use.

Aqueous solutions suitable for oral use can be prepared by dissolving the active component in water and adding suitable colorants, flavours, stabilising and thickening agents, as desired.

Aqueous suspensions suitable for oral use can be made by dispersing the finely divided active component in water with viscous material, such as natural or synthetic gums, resins, methylcellulose, sodium carboxymethylcellulose, or other well known suspending agents.

Also included are solid form preparations which are intended to be converted, shortly before use, to liquid form preparations for oral administration. Such liquid forms include solutions, suspensions, and emulsions. These preparations may contain, in addition to the active component, colorants, flavours, stabilisers, buffers,

artificial and natural sweeteners, dispersants, thickeners, solubilizing agents, and the like.

For topical administration to the epidermis the oxime derivative of the invention may be formulated as ointments, creams or lotions, or as a transdermal patch. Ointments and creams may, for example, be formulated with an aqueous or oily base with the addition of suitable thickening and/or gelling agents. Lotions may be formulated with an aqueous or oily base and will in general also contain one or more emulsifying agents, stabilising agents, dispersing agents, suspending agents, thickening agents, or colouring agents.

Compositions suitable for topical administration in the mouth include lozenges comprising the active agent in a flavoured base, usually sucrose and acacia or tragacanth; pastilles comprising the active ingredient in an inert base such as gelatin and glycerine or sucrose and acacia; and mouthwashes comprising the active ingredient in a suitable liquid carrier.

Solutions or suspensions are applied directly to the nasal cavity by conventional means, for example with a dropper, pipette or spray. The compositions may be provided in single or multi-dose form. In the latter case of a dropper or pipette, this may be achieved by the patient administering an appropriate, predetermined volume of the solution or suspension. In the case of a spray, this may be achieved for example by means of a metering atomising spray pump.

Administration to the respiratory tract may also be achieved by means of an aerosol formulation in which the active ingredient is provided in a pressurised pack with a suitable propellant such as a chlorofluorocarbon (CFC) for example dichlorodifluoromethane, trichlorofluoromethane, or dichlorotetrafluoroethane, carbon dioxide, or other suitable gas. The aerosol may conveniently also contain a surfactant such as lecithin. The dose of drug may be controlled by provision of a metered valve.

Alternatively the active ingredients may be provided in the form of a dry powder, for example a powder mix of the compound in a suitable powder base such as lactose, starch, starch derivatives such as hydroxypropylmethyl cellulose and polyvinylpyrrolidone (PVP). Conveniently the powder carrier will form a gel in the nasal cavity. The powder composition may be presented in unit dose form for example in capsules or cartridges of, e.g., gelatin, or blister packs from which the powder may be administered by means of an inhaler.

In compositions intended for administration to the respiratory tract, including intranasal compositions, the compound will generally have a small particle size for example of the order of 5 microns or less. Such a particle size may be obtained by means known in the art, for example by micronization.

When desired, compositions adapted to give sustained release of the active ingredient may be employed.

The pharmaceutical preparations are preferably in unit dosage forms. In such form, the preparation is subdivided into unit doses containing appropriate quantities of the active component. The unit dosage form can be a packaged preparation, the package containing discrete quantities of preparation, such as packaged tablets, capsules, and powders in vials or ampoules. Also, the unit dosage form can be a capsule, tablet, cachet, or lozenge itself, or it can be the appropriate number of any of these in packaged form.

Tablets or capsules for oral administration and liquids for intravenous administration and continuous infusion are preferred compositions.

Further details on techniques for formulation and administration may be found in the latest edition of Remington's Pharmaceutical Sciences (Maack Publishing Co., Easton, PA).

A therapeutically effective dose refers to that amount of active ingredient which ameliorates the symptoms or condition. Therapeutic efficacy and toxicity, e.g. ED_{50} and LD_{50}, may be determined by standard pharmacological procedures in cell cultures or experimental animals. The dose ratio between therapeutic and toxic effects is the therapeutic index and may be expressed by the ratio LD_{50}/ED_{50}. Pharmaceutical compositions which exhibit large therapeutic indexes are preferred.

The dose administered must of course be carefully adjusted to the age, weight and condition of the individual being treated, as well as the route of administration, dosage form and regimen, and the result desired, and the exact dosage should of course be determined by the practitioner.

The actual dosage depend on the nature and severity of the disease being treated, and is within the discretion of the physician, and may be varied by titration of the dosage to the particular circumstances of this invention to produce the desired therapeutic effect. However, it is presently contemplated that pharmaceutical compositions containing of from about 0.1 to about 500 mg of active ingredient per

individual dose, preferably of from about 1 to about 100 mg, most preferred of from about 1 to about 10 mg, are suitable for therapeutic treatments.

The active ingredient may be administered in one or several doses per day. A satisfactory result can, in certain instances, be obtained at a dosage as low as 0.1 µg/kg i.v. and 1 µg/kg p.o. The upper limit of the dosage range is presently considered to be about 10 mg/kg i.v. and 100 mg/kg p.o. Preferred ranges are from about 0.1 µg/kg to about 10 mg/kg/day i.v., and from about 1 µg/kg to about 100 mg/kg/day p.o.

Methods of Therapy

In another aspect the invention provides a method for the treatment or alleviation of diseases or disorders or conditions of living animal bodies, including humans, which diseases, disorders or conditions are responsive to modulation of SK_{Ca}, IK_{Ca} and/or BK_{Ca} channels, and which method comprises administering to such a living animal body, including a human, in need thereof an effective amount of an oxime derivative of the invention.

In a more preferred embodiment, the disease, disorder or condition is asthma, cystic fibrosis, chronic obstructive pulmonary disease and rhinorrhea, convulsions, vascular spasms, coronary artery spasms, renal disorders, polycystic kidney disease, bladder spasms, urinary incontinence, bladder outflow obstruction, irritable bowel syndrome, gastrointestinal dysfunction, secretory diarrhoea, ischaemia, cerebral ischaemia, ischaemic hearth disease, angina pectoris, coronary hearth disease, traumatic brain injury, psychosis, anxiety, depression, dementia, memory and attention deficits, Alzheimer's disease, dysmenorrhea, narcolepsy, Reynaud's disease, intermittent claudication, Sjorgren's syndrome, migraine, arrhythmia, hypertension, absence seizures, myotonic muscle dystrophia, xerostomi, diabetes type II, hyperinsulinemia, premature labour, baldness, cancer, and immune suppression.

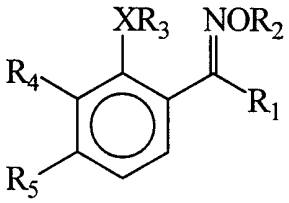
It is at present contemplated that suitable dosage ranges are 0.1 to 1000 milligrams daily, 10-500 milligrams daily, and especially 30-100 milligrams daily, dependent as usual upon the exact mode of administration, form in which administered, the indication toward which the administration is directed, the subject involved and the body weight of the subject involved, and further the preference and experience of the physician or veterinarian in charge.

EXAMPLES

The invention is further illustrated with reference to the following examples which are not intended to be in any way limiting to the scope of the invention as claimed.

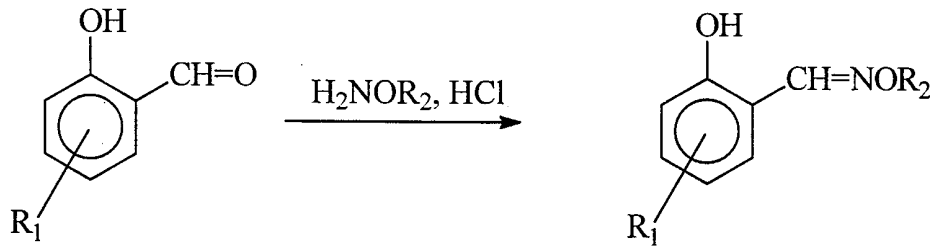
Table 1

Oximes of substituted Benzaldehydes and Acetophenones



Entry	R_1	R_2	R_3	R_4	R_5	X	Mp.	Examples
1a	H	H	H	-CH=CH-CH=CH-		O	143-4	1, 2
1b	Me	H	H	-CH=CH-CH=CH-		O	165-5.5	3, 5
1c	H	H	H	Me	Me	O	101-3	1, 2
1d	H	H	H	Cl	Cl	O	188-90	1, 2
1e	H	Bz	H	Cl	Cl	O	64-9	1, 2
1f	H	Me	H	Cl	Cl	O	82-3	1, 2
1g	Me	H	Me	-CH=CH-CH=CH-		O	118-9	3, 4, 5
1h	Me	Et	Me	-CH=CH-CH=CH-		O	oil	3, 4, 5
1i	Me	H	Ac	H	H	NH	99-100	6
1j	Me	H	Et	-CH=CH-CH=CH-		O	115-6	3, 4, 5
1k	Me	H	Bz	-CH=CH-CH=CH-		O	153-5	3, 4, 5
1l	Me	H	H	Me	Me	O	152-4	3, 5
1m	Me	H	Me	Me	Me	O	108-20	3, 4, 5
1n	Me	H	H	OMe	OMe	O	131-2	3, 5
1o	Me	H	Me	OMe	OMe	O	75-8	3, 4, 5
1p	Me	H	H	$-(CH_2)_4-$		O	147-9	3, 5
1q	Me	H	Me	$-(CH_2)_4-$		O	115-20	3, 4, 5
1r	H	H	H	$-(CH_2)_4-$		O	122-3	1, 2
1s	Me	H	i-Pr	-CH=CH-CH=CH-		O	90-90.3	3, 4, 5

Example 1



The following aldoximes were prepared by treatment of the corresponding aldehydes with the appropriate hydroxylamines in refluxing ethanol.

2-Aldoximo-1-naphtol (**1a**). From 2-formyl-1-naphtol and hydroxylamine, HCl. Mp. 143-144°C.

2-Aldoximo-5,6-dimethylphenol (**1c**). From 2-formyl-5,6-dimethylphenol and hydroxylamine, HCl. Mp. 101-103°C.

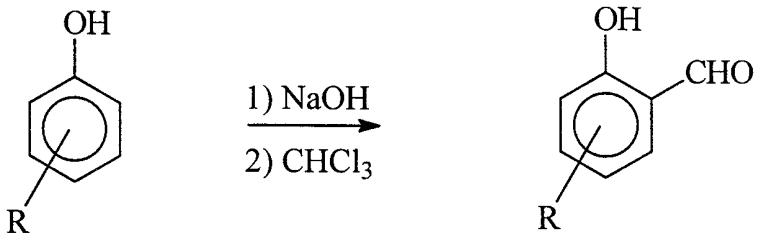
2-Aldoximo-5,6-dichlorophenol (**1d**). From 2-formyl-5,6-dichlorophenol and hydroxylamine, HCl. Mp. 188-190°C.

O-Benzyl 2-formyl-5,6-dichlorophenol oxime (**1e**). From 2-formyl-5,6-dichlorophenol and *O*-benzylhydroxylamine, HCl. Mp. 64-69°C.

O-Methyl 2-formyl-5,6-dichlorophenol oxime (**1f**). From 2-formyl-5,6-dichlorophenol and *O*-methylhydroxylamine, HCl. Mp. 82-83°C.

2-Aldoximo-5,6,7,8-tetrahydro-1-naphtol (**1r**). From 2-formyl-5,6,7,8-tetrahydro-1-naphtol and hydroxylamine, HCl. Mp. 122-123°C.

Example 2



The following 2-formylphenols were prepared in analogy with the procedure described in Organic Syntheses, Coll. Vol. III, p. 463 from the corresponding phenols. The products were purified by column-chromatography.

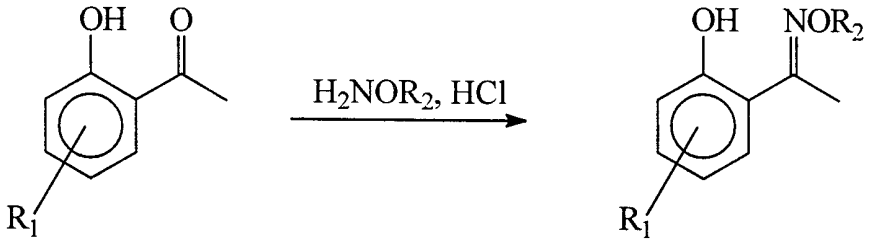
2-Formyl-1-naphtol from 1-napthol.

2-Formyl-5,6-dimethylphenol from 2,3-dimethylphenol.

2-Formyl-5,6-dichlorophenol from 2,3-dichlorophenol.

2-Formyl-5,6,7,8-tetrahydro-1-naphtol from 5,6,7,8-tetrahydro-1-naphtol.

Example 3



The following ketoximes were prepared by treatment of the corresponding ketones with the appropriate hydroxylamines in refluxing ethanol.

1-Hydroxy-2-acetonaphtone oxime (**1b**). From 1-hydroxy-2-acetonaphtone and hydroxylamine, HCl. Mp. 165-165.5°C.

1-Methoxy-2-acetonaphtone oxime (**1g**). From 1-methoxy-2-acetonaphtone and hydroxylamine, HCl. Mp. 118-119°C.

O-Ethyl 1-methoxy-2-acetonaphtone oxime (**1h**). From 1-methoxy-2-acetonaphtone and O-ethyl hydroxylamine, HCl. Mp. oil. m/z: 251 (53%), 184 (53%), 183 (100%), 158 (29%), 114 (26%).

1-Ethoxy-2-acetonaphtone oxime (**1j**). From 1-ethoxy-2-acetonaphtone and hydroxylamine, HCl. Mp. 115-116°C.

1-Benzyloxy-2-acetonaphtone oxime (**1k**). From 1-benzyloxy-2-acetonaphtone and hydroxylamine, HCl. Mp. 153-155°C.

2-Hydroxy-3,4-dimethylacetophenone oxime (**1l**). From 2-hydroxy-3,4-dimethylacetophenone and hydroxylamine, HCl. Mp. 152-154°C.

2-Methoxy-3,4-dimethylacetophenone oxime (**1m**). From 2-methoxy-3,4-dimethylacetophenone and hydroxylamine, HCl. Mp. 108-120°C.

2-Hydroxy-3,4-dimethoxyacetophenone oxime (**1n**). From 2-hydroxy-3,4-dimethoxyacetophenone and hydroxylamine, HCl. Mp. 131-132°C.

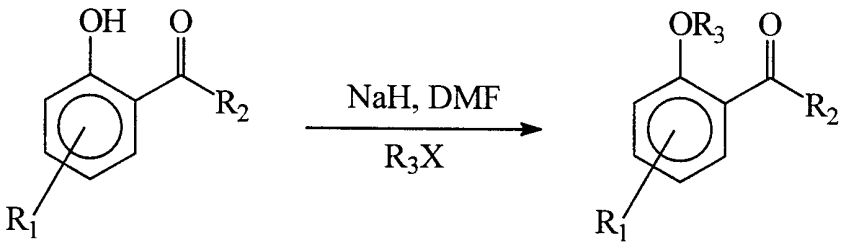
2,3,4-Trimethoxyacetophenone oxime (**1o**). From 2,3,4-trimethoxyacetophenone and hydroxylamine, HCl. Mp. 75-78°C.

1-Hydroxy-5,6,7,8-tetrahydro-2-acetonaphtone oxime (**1p**). From 1-hydroxy-5,6,7,8-tetrahydro-2-acetonaphtone and hydroxylamine, HCl. Mp. 147-149°C.

1-Methoxy-5,6,7,8-tetrahydro-2-acetonaphtone oxime (**1q**). From 1-Methoxy-5,6,7,8-tetrahydro-2-acetonaphtone and hydroxylamine, HCl. Mp. 115-120°C.

1-(2-Propyloxy)-2-acetonaphtone oxime (**1s**). From 1-(2-propyloxy)-2-acetonaphtone and hydroxylamine, HCl. m/z: 243 (36%), 201 (76%), 183 (100%), 168 (38%), 140 (41%). Mp. 90-90.3°C.

Example 4



1-Methoxy-2-acetonaphtone. To a solution of 1-hydroxy-2-acetonaphtone (4.0 g; 21.5 mmol) in anhydrous DMF (20 ml) was added sodium hydride (25.8 mmol; 1.03 g of a 60% dispersion in mineral oil). When the evolution of hydrogen had ceased iodomethane (2.4 ml; 25.8 mmol) was added and the mixture was stirred at ambient temperature overnight. The mixture was poured into four volumes of water and extracted with ethyl acetate. The organic phase was washed with aqueous sodium hydroxide (1 M) and water, successively, dried over sodium sulphate and concentrated under reduced pressure. The concentrate was purified by column-chromatography on silica gel, using a mixture of ethyl acetate and ligroin (8:2) using the eluent. Yield: 3.6 g (84%).

The following compounds were prepared analogously:

1-Ethoxy-2-acetonaphtone. From 1-hydroxy-2-acetonaphtone and bromoethane.

1-Benzyloxy-2-acetonaphtone. From 1-hydroxy-2-acetonaphtone and benzylbromide.

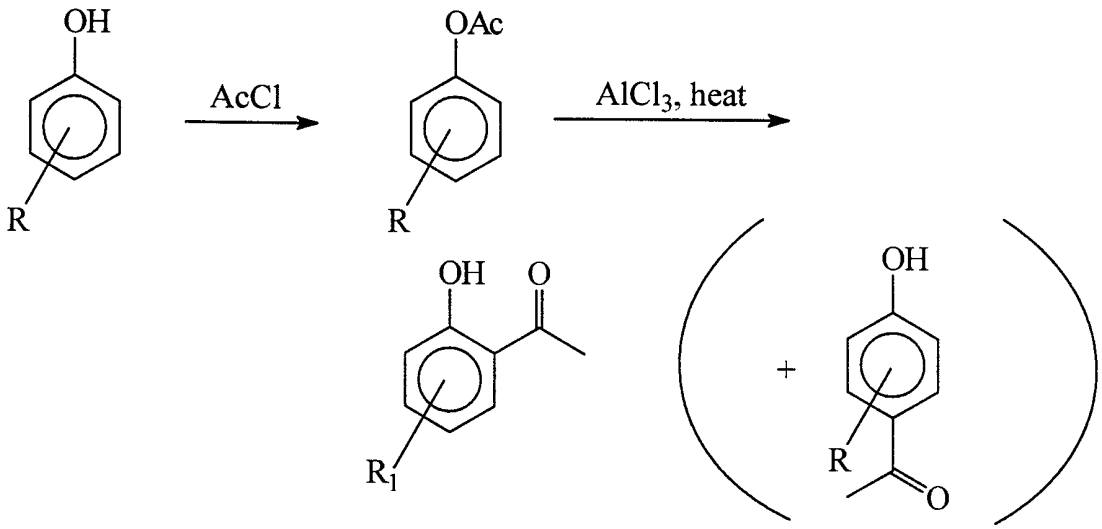
1-(2-Propyloxy)-2-acetonaphtone. From 1-hydroxy-2-acetonaphtone and 2-bromopropane.

2-Acetyl-5,6-dimethylanisole. From 2-hydroxy-3,4-dimethylacetophenone and iodomethane.

2,3,4-Trimethoxyacetophenone. From 2-hydroxy-3,4-dimethoxyacetophenone and iodomethane.

1-Methoxy-5,6,7,8-tetrahydro-2-acetonaphtone. From 1-hydroxy-5,6,7,8-tetrahydro-2-acetonaphtone.

Example 5



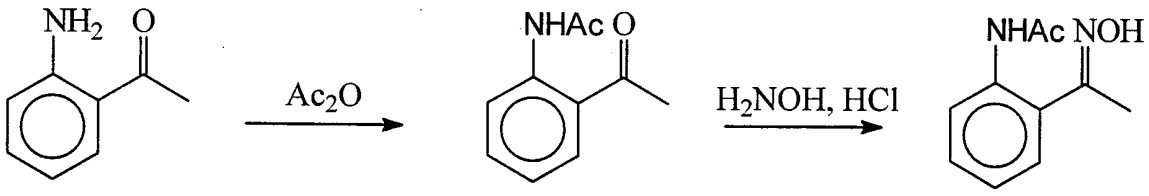
The following 2-acetylphenols were prepared according to the above Scheme in analogy with the procedure described in Organic Syntheses, Coll. Vol. III, p. 543 from the appropriate phenols.

2-Hydroxy-3,4-dimethylacetophenone. From 2,3-dimethylphenol.

1-Hydroxy-5,6,7,8-tetrahydro-2-acetonaphtone. From 5,6,7,8-tetrahydro-1-naphtol.

2-Hydroxy-3,4-dimethoxyacetophenone. From 2,3-dimethoxyphenol.

Example 6



N-(2-acetylphenyl)acetamide oxime (**1i**). A mixture of 2-acetylaniline (1 ml; 8.3 mmol) and acetic anhydride (10 ml) was stirred overnight at ambient temperature. Saturated aqueous sodium carbonate was added till basic reaction and the resulting mixture was extracted with ethyl acetate. The organic phase was dried over sodium sulphate and evaporated under reduced pressure to leave N-(2-acetylphenyl)acetamide quantitatively. This product was dissolved in ethanol (40 ml) and hydroxylamine, hydrochloride (0.94 g; 13.6 mmol) was added. The mixture was heated to reflux for 7 hours and then left at ambient temperature overnight. The solvent was removed by evaporation and the residue was partitioned between ethyl acetate and aqueous sodium carbonate. The organic phase was concentrated and subjected to chromatography on silica gel using a mixture of ethyl acetate and methanol (9:1 v/v) as the eluent. Yield: 0.26 g (15%). Mp. 99-100°C.

Example 7

Electrophysiological Experiments

In this example, the biological activity of the compounds of the invention is demonstrated using electrophysiologic patch-clamp techniques.

Intermediate-conductance Ca^{2+}-activated K^+ channels (IK channels) have been cloned from human placenta and stably expressed in HEK293 cells. The ionic current through the channels is recorded in the whole-cell mode of the patch-clamp technique.

Stable Expression of IK in HEK293 Cells

Human IK (hIK) was excised from pT3T7 (GenBank Acc. No. N56819) using EcoR I and Not I, and subcloned into the mammalian expression vector pNS1Z

(NeuroSearch), a custom designed derivative of pcDNA3Zeo (InVitrogen), to give the plasmid construct pNS1Z_hIK.

HEK293 tissue culture cells were grown in DMEM (Dulbecco's Modified Eagle Medium) supplemented with 10% FCS (foetal calf serum) at 37°C in 5% CO_2. One day prior to transfection, 10^6 cells were plated in a cell culture T25 flask. The following day, cells were transfected using lipofection (20 µL Lipofectamin™, Life Technologies, with 2.5 µg of the plasmid pNS1Z_hIK in a total volume of 540 µL).

The lipofection mixture was overlaid on the cells and incubated at 37°C for 5 hours. The cells were then rinsed with regular media and grown for 72 hours in DMEM, 10% FCS at 37°C in 5% CO_2.

72 hours post transfection, cells transfected with pNS1Z_hIK were selected in media supplemented with 0.25mg/ml Zeocin. Single clones were picked and propagated in selection media until sufficient cells for freezing were available. Hereafter the cells were cultured in regular medium without selection agent.

Expression of functional hIK channels was verified by patch-clamp measurements.

Whole Cell Recordings

Experiments are carried out on one of several patch-clamp set-ups. Cells plated on coverslips are placed in a 15 µl perfusion chamber (flowrate ~1 ml/min) mounted on a IMT-2 microscope equipped with Nomarski or Hoffmann optics. The microscopes are placed on vibration-free tables in grounded Faraday cages. All experiments are performed at room temperature (20 - 22°C). EPC-9 patch-clamp amplifiers (HEKA-electronics, Lambrect, Germany) are connected to Macintosh computers via ITC16 interfaces. Data are stored directly on the hard disk and analysed by the IGOR software (WaveMetrics, Lake Oswega, USA).

The whole-cell configuration of the patch clamp technique is applied. The tip of a borosilicate pipette (resistance 2-4 MΩ) is gently (remote control system) placed on the cell membrane. Light suction results in a giga seal (pipette resistance increases to more than 1 GΩ) and the cell membrane is then ruptured by more powerful suction. Cell capacitance is electronically compensated and the resistance between the pipette and the cell interior (the series resistance, Rs) is measured and compensated for. Usually the cell capacitance ranges from 5 to 20 pF (depending on

cell size) and the series resistance is in the range 3 to 6 MΩ. Rs- as well as capacitance compensation are updated during the experiments (before each stimulus).

All experiments with drifting Rs-values are discharged. Leak-subtractions are not performed.

Solutions

Compounds 1a-h, 1j and 2l-r (i.e. sixteen compounds) of Table 1 were subjected to this experiment.

The extracellular (bath) solution contains: 144 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM HEPES (pH = 7.4). Test compounds are dissolved in DMSO from stock solution and then diluted to a final concentration of about 10 µM in the extracellular solution. The concentration of $CaCl_2$ is 7.6 mM and that of $MgCl_2$ is 1.2 mM to give calculated free concentrations of 300 nM and 1 mM, respectively.

Quantification

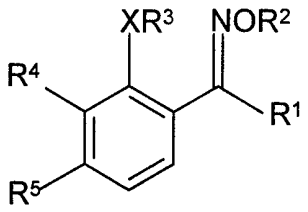
After establishment of the whole-cell configuration, voltage-ramps (usually -100 to +100 mV) are applied to the cell every 5 sec. A stable baseline current is obtained within a period of 100-300 seconds, and the compounds are then added by changing to an extracellular solution containing the compound to be tested. Very little endogen current (<200 pA at 100 mV, compared to 2-20 nA IK current) are activated under these circumstances in native HEK293 cells.

Results

All sixteen compounds tested in this experiment showed activity at a final concentration of about 10 µM, and these compounds therefore are SK/IK/BK channel modulating agents of the invention.

CLAIMS

1. An oxime derivative represented by the general formula



(I)

or a pharmaceutically acceptable salt or an oxide or a hydrate thereof, wherein,

X represents oxygen, sulphur, or an amino group, preferably an amino group of the formula NHR^3;

R^1 represents hydrogen; an alkyl group; a cycloalkyl group; hydroxy; an alkoxy group; an acyl group; a phenyl or a benzyl group, which phenyl and benzyl groups may be substituted one or more times with substituents selected from halogen, $-NO_2$, -CN, $-CF_3$, alkyl, cycloalkyl, hydroxy, and alkoxy; a group of the formula $-CH_2CN$; a group of the formula $-CH_2CO_2R'$, wherein R' represents hydrogen or an alkyl group; a group of the formula $-CH_2CONR^{IV}R^V$, wherein R^{IV} and R^V independently represents hydrogen or an alkyl group; or a group of the formula $-CH_2C(=NOH)NH_2$;

R^2 represents hydrogen, an alkyl group, a cycloalkyl group, a phenyl or a benzyl group, which phenyl and benzyl groups may be substituted one or more times with substituents selected from halogen, $-NO_2$, -CN, $-CF_3$, alkyl, cycloalkyl, hydroxy, and alkoxy;

R^3 represents hydrogen, an alkyl group, a cycloalkyl group, a phenyl or a benzyl group, which phenyl and benzyl groups may be substituted one or more times with substituents selected from halogen, $-NO_2$, -CN, $-CF_3$, alkyl, cycloalkyl, hydroxy, and alkoxy; and

R^4 and R^5, independently of each another represents hydrogen; halogen; $-NO_2$; -CN; $-CF_3$; an alkyl group; an alkoxy group; a phenyl or a benzyl group, which phenyl and benzyl groups may be substituted one or more times with substituents selected from halogen, $-NO_2$, -CN, $-CF_3$, alkyl, cycloalkyl, hydroxy, and alkoxy; or a group of the formula $-SO_2NR''R'''$, wherein R'' and R''' independently of each another represents hydrogen or an alkyl group;

or R^4 and R^5 together form an additional 4 to 7 membered fused ring, which fused ring may be aromatic or partially saturated, and which fused ring may optionally be substituted one or more times with substituents selected from the group consisting of halogen, $-NO_2$, -CN, $-CF_3$, and a group of the formula $-SO_2NR''R'''$, wherein R'' and R''' independently of each another represents hydrogen or an alkyl group.

2. The oxime derivative according to claim 1, wherein R^1 represents hydrogen or an alkyl group.

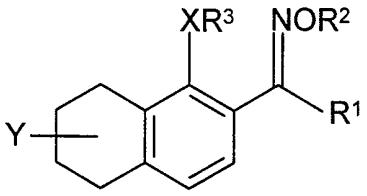
3. The oxime derivative according to either of claims 1-2, wherein R^2 represents hydrogen, an alkyl group, or a phenyl or benzyl group.

4. The oxime derivative according to any of claims 1-3, wherein X represents oxygen, or an amino group of the formula NHR^3, in which formula R^3 represents hydrogen, alkyl, benzyl, or acetyl.

5. The oxime derivative according to any of claims 1-4, wherein R^4 and R^5, independently of each another represents hydrogen, halogen, alkyl, or alkoxy.

6. The oxime derivative according to any of claims 1-4, wherein R^4 and R^5 together form an additional 6 membered fused ring, which fused ring may be aromatic or partially saturated, and which fused ring may optionally be substituted one or more times with substituents selected from the group consisting of halogen, $-NO_2$, -CN, $-CF_3$, and a group of the formula $-SO_2NR''R'''$, wherein R'' and R''' independently of each another represents hydrogen or an alkyl group.

7. The oxime derivative according to claim 6, wherein the oxime derivative is represented by the general formula



(II)

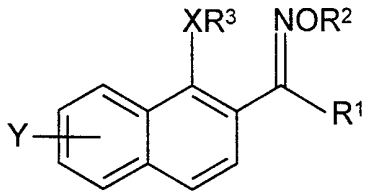
or a pharmaceutically acceptable salt or an oxide or a hydrate thereof,

wherein,

X, R1, R2 and R3 are as defined above; and

Y represents hydrogen, halogen, $-NO_2$, -CN, $-CF_3$, or a group of the formula $-SO_2NR''R'''$, wherein R'' and R''' independently of each another represents hydrogen or an alkyl group.

8. The oxime derivative according to 6, wherein the oxime derivative is represented by the general formula



(III)

or a pharmaceutically acceptable salt or an oxide or a hydrate thereof, wherein,

X, R1, R2 and R3 are as defined above; and

Y represents hydrogen, halogen, $-NO_2$, -CN, $-CF_3$, or a group of the formula $-SO_2NR''R'''$, wherein R'' and R''' independently of each another represents hydrogen or an alkyl group.

9. The oxime derivative according to claim 1, wherein the chemical compound is

2-Aldoximo-1-naphtol;

2-Aldoximo-5,6-dimethylphenol;

2-Aldoximo-5,6-dichlorophenol;

O-Benzyl 2-formyl-5,6-dichlorophenol oxime;

O-Methyl 2-formyl-5,6-dichlorophenol oxime;

2-Aldoximo-5,6,7,8-tetrahydro-1-naphtol;

1-Hydroxy-2-acetonaphtone oxime;

1-Methoxy-2-acetonaphtone oxime;

O-Ethyl 1-methoxy-2-acetonaphtone oxime;

1-Ethoxy-2-acetonaphtone oxime;

1-Benzyloxy-2-acetonaphtone oxime;

2-Hydroxy-3,4-dimethylacetophenone oxime;

2-Methoxy-3,4-dimethylacetophenone oxime;

2-Hydroxy-3,4-dimethoxyacetophenone oxime;

2,3,4-Trimethoxyacetophenone oxime;

1-Hydroxy-5,6,7,8-tetrahydro-2-acetonaphtone oxime;

1-Methoxy-5,6,7,8-tetrahydro-2-acetonaphtone oxime;

1-(2-Propyloxy)-2-acetonaphtone oxime; or

N-(2-acetylphenyl)acetamide oxime;

or a pharmaceutically acceptable salt or an oxide or a hydrate thereof.

10. A pharmaceutical composition comprising a therapeuticallly-effective amount of an oxime derivative according to any of claims 1-9, or a pharmaceutically-acceptable addition salt thereof, together with at least one pharmaceutically-acceptable carrier or diluent, for the treatment or alleviation of a disease or a disorder or a condition that is responsive to modulation of SK_{Ca}, IK_{Ca} or BK_{Ca} channels.

11. The pharmaceutical composition according to claim 10, for the treatment or alleviation of respiratory diseases such as asthma, cystic fibrosis, chronic obstructive pulmonary disease and rhinorrhea, convulsions, vascular spasms, coronary artery spasms, renal disorders, polycystic kidney disease, bladder

spasms, urinary incontinence, bladder outflow obstruction, irritable bowel syndrome, gastrointestinal dysfunction, secretory diarrhoea, ischaemia, cerebral ischaemia, ischaemic hearth disease, angina pectoris, coronary hearth disease, traumatic brain injury, psychosis, anxiety, depression, dementia, memory and attention deficits, Alzheimer's disease, dysmenorrhea, narcolepsy, Reynaud's disease, intermittent claudication, Sjorgren's syndrome, migraine, arrhythmia, hypertension, absence seizures, myotonic muscle dystrophia, xerostomi, diabetes type II, hyperinsulinemia, premature labour, baldness, cancer, and immune suppression.

12. Use of an oxime derivative according to any of claims 1-9, or a pharmaceutically-acceptable addition salt thereof, for the manufacture of a pharmaceutical composition for the treatment or alleviation of a disease or a disorder or a condition of a mammal, including a human, which disease, disorder or condition is responsive to modulation of SK_{Ca}, IK_{Ca} and/or BK_{Ca} channels.

13. The use according to claim 12, wherein the disease, disorder or condition is asthma, cystic fibrosis, chronic obstructive pulmonary disease and rhinorrhea, convulsions, vascular spasms, coronary artery spasms, renal disorders, polycystic kidney disease, bladder spasms, urinary incontinence, bladder outflow obstruction, irritable bowel syndrome, gastrointestinal dysfunction, secretory diarrhoea, ischaemia, cerebral ischaemia, ischaemic hearth disease, angina pectoris, coronary hearth disease, traumatic brain injury, psychosis, anxiety, depression, dementia, memory and attention deficits, Alzheimer's disease, dysmenorrhea, narcolepsy, Reynaud's disease, intermittent claudication, Sjorgren's syndrome, migraine, arrhythmia, hypertension, absence seizures, myotonic muscle dystrophia, xerostomi, diabetes type II, hyperinsulinemia, premature labour, baldness, cancer, and immune suppression.

14. A method of treatment or alleviation of a disease or a disorder or a condition of a living animal body, including a human, which disease, disorder or condition is responsive to modulation of SK_{Ca}, IK_{Ca} and/or BK_{Ca} channels, comprising the step of administering to such a living animal body, including a human, in need

thereof a therapeutically effective amount of an oxime derivative according to any of claims 1-9.

15. The method according to claim 14, wherein the disease, disorder or condition is asthma, cystic fibrosis, chronic obstructive pulmonary disease and rhinorrhea, convulsions, vascular spasms, coronary artery spasms, renal disorders, polycystic kidney disease, bladder spasms, urinary incontinence, bladder outflow obstruction, irritable bowel syndrome, gastrointestinal dysfunction, secretory diarrhoea, ischaemia, cerebral ischaemia, ischaemic hearth disease, angina pectoris, coronary hearth disease, traumatic brain injury, psychosis, anxiety, depression, dementia, memory and attention deficits, Alzheimer's disease, dysmenorrhea, narcolepsy, Reynaud's disease, intermittent claudication, Sjorgren's syndrome, migraine, arrhythmia, hypertension, absence seizures, myotonic muscle dystrophia, xerostomi, diabetes type II, hyperinsulinemia, premature labour, baldness, cancer, and immune suppression.

INTERNATIONAL SEARCH REPORT

Inter onal Application No
PCT/DK 99/00680

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07C251/48 C07C251/50 A61K31/15 A61P43/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07C

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

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 99 37149 A (BRIGHAM & WOMENS HOSPITAL ;KAUL MARCUS (US); LE DEAN (US); LIPTON) 29 July 1999 (1999-07-29) claims 1-10,12,17,18 ---	1-15
X	DATABASE STN INTERNATIONAL [Online] KARHU S ET AL: "Salicylaldoxime blocks K+ and Ca2+ currents in rat cardiac myocytes" retrieved from CAPLUS, accession no. 1995:608546 Database accession no. 123:47649 XP002900888 abstract & EUR. J. PHARMACOL., vol. 279, no. 1, 1995, pages 7-13, ISSN: 0014-2999 --- -/--	1-15

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

[X] Patent family members are listed in annex.

° Special categories of cited documents :

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
9 March 2000	27. 04. 2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Gerd Strandell

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Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Interr nal Application No
PCT/DK 99/00680

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE STN INTERNATIONAL [Online] PHAM-HUU-CHANH ET AL: "Pharmacology of oximes. 2-Hydroxy-3-methoxybenzaldoxime" retrieved from CAPLUS, accession no. 1970:119785 Database accession no. 72:119785 XP002900889 abstract & ARCH. INT. PHARMACODYN. THER., vol. 182, no. 2, 1969, pages 443-458, ---	1-15
X	DATABASE STN INTERNATIONAL [Online] PHAM-HUU-CHANH ET AL: "Comparative study of the action of 2-hydroxy-3-methoxybenzaldoxime and corresponding aldehyde on the smooth muscle" retrieved from CAPLUS, accession no. 1970:529247 Database accession no. 73:129247 XP002900890 abstract & THERAPIE, vol. 25, no. 4, 1970, pages 649-654, ---	1-15
X	EP 0 149 242 B (VEB FAHLBERG-LIST MAGDEBURG) 31 October 1990 (1990-10-31) the whole document ---	1-15
X	US 5 840 758 A (LEE WENDY ET AL) 24 November 1998 (1998-11-24) claims ---	1-15
X	WO 95 01157 A (PROCTER & GAMBLE) 12 January 1995 (1995-01-12) page 4, line 8 -page 7 ---	1-11
X	EP 0 012 158 A (CIBA GEIGY AG) 25 June 1980 (1980-06-25) page 20, compounds 3, 4, 8; claims 22-24 ---	1-9
X	EP 0 254 426 A (ICI PLC) 27 January 1988 (1988-01-27) page 6 - page 13, compounds 5-7, 8-11, 21-28, 52-55, 66, 67 claims 1-6 -----	1-9

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK99/00680

Box I **Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

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

1. ☒ Claims Nos.: 14, 15
because they relate to subject matter not required to be searched by this Authority, namely:

see next sheet

2. ☒ Claims Nos.: 14, 15
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

The search revealed so many documents relevant to the issue of novelty that only a selection is cited.

Box II **Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

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

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

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

Remark on Protest

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

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK99/00680

Claims 14, 15 relate to methods of treatment of the human or animal body by surgery or by therapy/ diagnostic methods practised on the human or animal body/Rule 39.1.(iv). Nevertheless, a search has been executed for these claims. The search has been based on the alleged effects of the compounds/compositions.

INTERNATIONAL SEARCH REPORT

...ormation on patent family members

Inter ...nal Application No
PCT/DK 99/00680

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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