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54. TITULO METODOS Y USOS TERAPEUTICOS DE SAPOGENINAS Y SUS DERIVADOS

51. CLASIFICACION INTERNACIONAL _____

71. SOLICITANTE PHYTOPHARM PLC

DOMICILIO Godmanchester, Cambridgeshire, Reino Unido

74. APODERADO EMILIO FERRERO

C.C. 438,019 de Usaquén.

22. BOGOTA, D.C., _____

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PETITORIO

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1 SOLICITUD DE:			
☒ Patente de Invención.		☐ Patente de Modelo de Utilidad.	
☐ Capítulo I.		☒ Capítulo II.	
2 SOLICITANTE (71)	Nombre: PHYTOPHARM PLC sociedad constituida y existente bajo las leyes del Reino Unido. Dirección: Corpus Christi House, 9 West Street, Godmanchester, Cambridgeshire PE29 2HY, Reino Unido Domicilio: Godmanchester, Cambridgeshire, Reino Unido		IDENTIFICACIÓN C.C. ☐ NIT ☐ C.E. ☐ Otro ☐ Cual _____ Número _____
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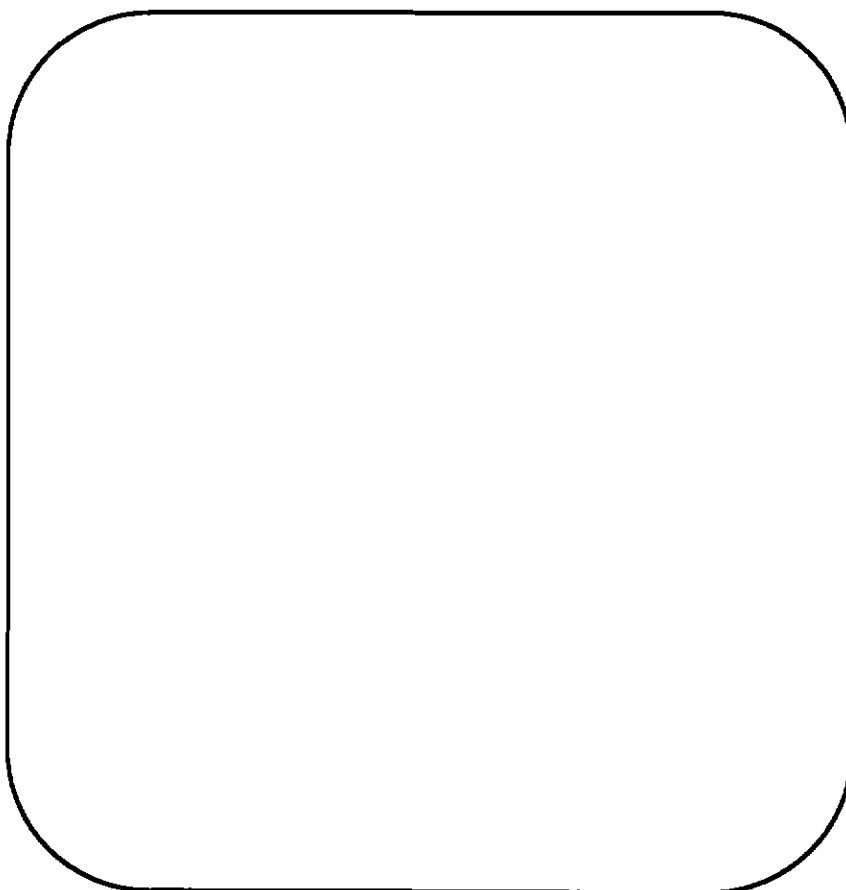
Comprobante de pago No.: 04-79.372Fecha: 11 de Octubre de 2004

10

Anexos:

- ☒ Comprobante de pago de la tasa de presentación de la solicitud por \$ 422.000.00.
- ☐ Comprobante de pago por reivindicación de prioridad.
- ☒ Documento que acredita la existencia y representación legal cuando el solicitante sea persona jurídica.
- ☐ Poderes, si fuere el caso.
- ☐ Documento de cesión del inventor al solicitante o a su causante.
- ☐ Declaraciones.
- ☒ Copia de la solicitud internacional. (Resumen, descripción, reivindicaciones, dibujos)
- ☒ Traducción de la solicitud internacional al castellano. (Resumen, descripción, reivindicaciones, dibujos)
- ☒ Copia del examen preliminar efectuado por la IPEA.
- ☒ Traducción del examen preliminar efectuado por la IPEA.
- ☐ De ser el caso, copia del contrato de acceso.
- ☐ De ser el caso, documento que acredite la licencia o autorización de uso de conocimientos tradicionales de las comunidades indígenas.
- ☐ De ser el caso, certificado de depósito del material biológico.
- ☐ Arte final 12x12
- ☐ De ser el caso, información sobre otras solicitudes de patente o títulos obtenidos en el extranjero por el mismo titular o su causante, relacionadas parcial o totalmente con la invención de esta solicitud.
- ☐ Otros

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FIGURA CARACTERÍSTICA

12

Solicito la concesión de la patente,

NOMBRE:

EMILIO FERRERO

FIRMA:

C.C. 438.019 de Usaquén T.P. 31.929

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- (72) Inventors; and
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(54) Title: THERAPUTIC METHODS AND USES OF SAPOGENINS AND THEIR DERIVATIVES

(57) Abstract: The invention discloses therapeutic methods and uses of certain steroidal saponins, related compounds and derivatives thereof, in the treatment of non-cognitive neurodegeneration, non-cognitive neuromuscular degeneration, motor-sensory neurodegeneration or receptor dysfunction or loss in the absence of cognitive, neural and neuromuscular impairment.

THERAPEUTIC METHODS AND USES OF SAPOGENINS AND THEIR DERIVATIVES

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Field of the Invention

The present invention relates to therapeutic methods and uses of sapogenins, related compounds and their derivatives.

10

The uses of the sapogenins, related compounds and their derivatives are in the treatment of non-cognitive neurodegeneration, non-cognitive neuromuscular degeneration, motor-sensory neurodegeneration, or receptor dysfunction or loss. In a further aspect, the invention relates to compositions for use in such treatments.

15

Background of the Invention

Cognitive dysfunction is a characteristic of dementia conditions and syndromes, such as Alzheimer's disease (AD), senile dementia of the Alzheimer's type (SDAT), Lewy body dementia and vascular dementia. A lesser degree of cognitive dysfunction is also a characteristic of certain non-dementia conditions and syndromes, such as mild cognitive impairment (MCI), age-associated memory impairment (AAMI), autism and neuroimpairment.

25 Non-cognitive neurodegeneration (i.e. neurodegeneration in the absence of cognitive dysfunction), non-cognitive neuromuscular degeneration (i.e. neuromuscular degeneration in the absence of cognitive dysfunction) and motor-sensory neurodegeneration are characteristic of conditions and syndromes such as Parkinson's disease, muscular dystrophy including facioscapulohumeral muscular dystrophy (FSH), Duchenne muscular dystrophy, 30 Becker muscular dystrophy and Bruce's muscular dystrophy, Fuchs' dystrophy, myotonic dystrophy, corneal dystrophy, reflex sympathetic dystrophy syndrome (RSDSA), neurovascular dystrophy, myasthenia gravis, Lambert Eaton disease, Huntington's disease, amyotrophic lateral sclerosis (ALS) and multiple sclerosis.

35 Receptor dysfunction or loss - particularly dysfunction or loss of nicotinic and/or muscarinic acetylcholine receptors and/or dopamine receptors and/or adrenoceptors - is a

characteristic of some or all of the above conditions and syndromes. Receptor dysfunction or loss in the absence of cognitive, neural and neuromuscular impairment is also a characteristic of conditions and syndromes such as postural hypotension, chronic fatigue syndrome, asthma, susceptibility to heart failure and macular degeneration.

5

The above conditions and syndromes are grave and growing problems in all societies where, because of an increase in life expectancy and control of adventitious disease, the demographic profile is increasingly extending towards a more aged population. Agents which can treat, or help in the management or prevention of such disorders, are urgently required.

10

DE-A-4303214, the disclosure of which is incorporated herein by reference, suggests the use of saponins and sapogenins in the treatment of viral diseases, but with no data that would allow one skilled in the art to select a particular group of compounds for any particular viral disease.

15

WO-A-99/16786, the disclosure of which is incorporated herein by reference, suggests the use of certain saponins and sapogenins in the treatment of dementia.

20 WO-A-99/48482, WO-A-99/48507, WO-A-01/23406, WO-A-01/23407, WO-A-01/23408, the disclosures of which are incorporated herein by reference, relate to the use of certain saponins, sapogenins and derivatives thereof in the treatment of cognitive dysfunction and allied conditions.

25 Chinese Patent Application No. CN-A-1096031, the disclosure of which is incorporated herein by reference, suggests a bioactivity of the spirostane sapogenin, sarsasapogenin, in the two-way regulation of β -adrenergic and M-cholinergic receptors. No specific pharmaceutical activity is suggested. However, in "Synthesis and Applications of Isotopically Labelled Compounds", 1998, pages 315-320, Yi *et al* describe the use of sarsasapogenin in the treatment of senile dementia.

30

There exist a number of so-called "spectrum" disorders, in which a wide range of combinations of symptoms, in a wide range of relative severities, present themselves. The severity of each symptom and the particular combination of symptoms will vary from individual to individual and according to the stage of progression of the disorder. In the cases of Parkinson's disease, myasthenia gravis, Lambert Eaton disease, postural

35

hypotension and chronic fatigue syndrome, for example, cognitive dysfunction is not a primary symptom, although it may be present as one of a number of possible secondary symptoms. Moreover, these conditions are not viral diseases or dementias. Many of these disorders are so-called "spectrum" disorders. Therefore, in many instances, a treatment for cognitive dysfunction (e.g. dementia) is not necessary.

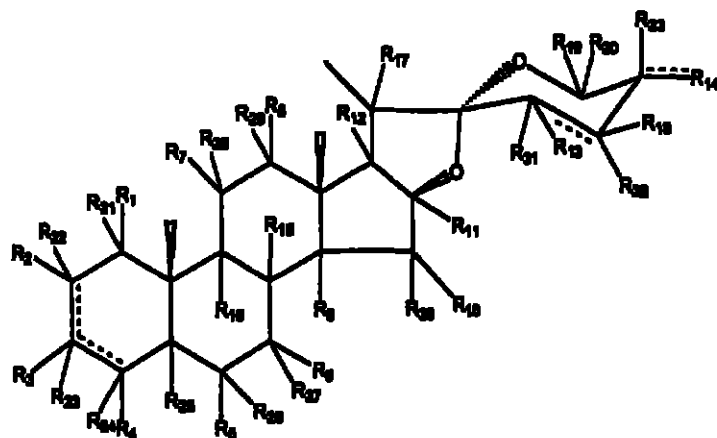
The present invention is based upon our finding that certain saponins and their derivatives, including saponins, have a surprising disease-modifying activity against non-cognitive neurodegeneration, non-cognitive neuromuscular degeneration, motor-sensory neurodegeneration as well as against receptor dysfunction or loss in the absence of cognitive, neural and neuromuscular impairment, and thus actively prevent and reverse the conditions. This finding enables improved treatment of certain non-viral, spectrum and non-spectrum, disorders in which cognitive dysfunction is not a primary symptom.

Brief Description of the Invention

According to one aspect of the present invention, there is provided the use of the active agents (as herein defined) in the treatment or prevention of, or in the preparation of compositions (e.g. pharmaceutical compositions, foodstuffs, food supplements and beverages) for the treatment or prevention of, (i) non-cognitive neurodegeneration, (ii) non-cognitive neuromuscular degeneration, (iii) motor-sensory neurodegeneration (iv) receptor dysfunction or loss in the absence of cognitive, neural and neuromuscular impairment, in human and non-human animals suffering therefrom or susceptible thereto.

The expression "active agents" refers to compounds of the following general formulae I, II and III, saponin derivatives as defined below by reference to derivatives bearing at least one defined X radical substituent, sugar-substituted derivatives of such compounds as defined below, all their stereoisomers and racemic mixtures, all their pharmaceutically acceptable pro-drugs and salts, and all mixtures and combinations thereof:

Formula I:



(I)

wherein in the general formula (I):

- 5 - $R_1, R_2, R_3, R_4, R_5, R_6, R_7, R_8, R_{10}, R_{11}, R_{12}, R_{13}, R_{14}, R_{15}, R_{16}, R_{17}, R_{18}, R_{19}, R_{20}, R_{21}, R_{22}, R_{23}, R_{24}, R_{25}, R_{26}, R_{27}, R_{28}, R_{29}, R_{30}, R_{31}, R_{32}$, are, independently of each other, either H, OH, =O, halo atom, (Me-S-), (Me-SO-), (Me-SO₂-), N₃-, NH₂-, MeSO₂NH-, alkyl or absent or OR where R = alkyl or acyl group;

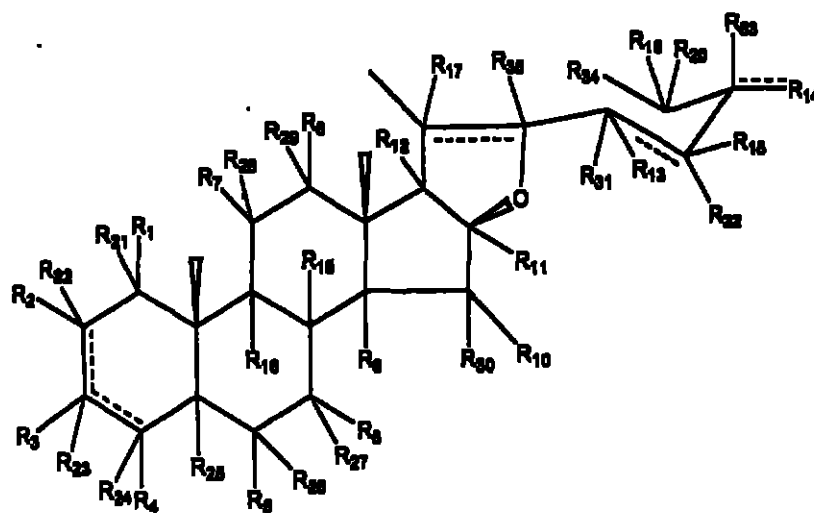
- $R_9, R_{12}, R_{13}, R_{14}, R_{15}, R_{16}, R_{17}, R_{21}, R_{22}$ can be either a H, OH, halo atom, (Me-S-), (Me-SO-), (Me-SO₂-), N₃-, NH₂-, MeSO₂NH-, alkyl or absent or OR where R = alkyl or acyl group;

--- represents an optional double bond,

wherein in addition to the above

- either R_{33} or R_{34} = alkyl group;

15 Formula II:



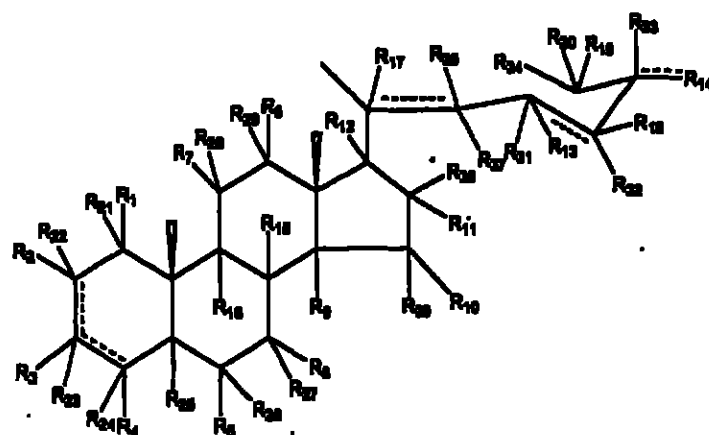
(II)

wherein in the general formula (II):

- $R_1, R_2, R_3, R_4, R_5, R_6, R_7, R_8, R_{10}, R_{13}, R_{15}, R_{19}, R_{20}, R_{21}, R_{22}, R_{23}, R_{24}, R_{25}, R_{27}, R_{28}, R_{29}, R_{30}, R_{31}, R_{32}, R_{34}$ are, independently of each other, either H, OH, =O, halo atom, (Me-S-), (Me-SO-), (Me-SO₂-), N₃-, NH₂-, MeSO₂NH-, alkyl, OR where R = alkyl or acyl group, or absent;
- $R_9, R_{11}, R_{12}, R_{14}, R_{16}, R_{17}, R_{21}, R_{23}, R_{25}$ can be either a H, OH, halo atom, (Me-S-), (Me-SO-), (Me-SO₂-), N₃-, NH₂-, MeSO₂NH-, alkyl, OR where R = alkyl or acyl group, or absent;
- 10 --- represents an optional double bond,
- wherein in addition to the above
- either R_{13} or R_{14} = alkyl group;

Formula III:

15



(III)

wherein in the general formula (III):

- 20 - $R_1, R_2, R_3, R_4, R_5, R_6, R_7, R_8, R_{10}, R_{13}, R_{14}, R_{15}, R_{16}, R_{17}, R_{18}, R_{20}, R_{21}, R_{22}, R_{24}, R_{25}, R_{27}, R_{28}, R_{29}, R_{30}, R_{31}, R_{32}, R_{33}, R_{34}, R_{35}, R_{36}, R_{37}$ are, independently of each other, either H, OH, =O, halo atom, (Me-S-), (Me-SO-), (Me-SO₂-), N₃-, NH₂-, MeSO₂NH-, alkyl, OR where R = alkyl or acyl group, or absent;
- $R_9, R_{11}, R_{12}, R_{15}, R_{16}, R_{17}, R_{23}$ can be either H, OH, halo atom, (Me-S-), (Me-SO-), (Me-SO₂-), N₃-, NH₂-, MeSO₂NH-, alkyl, OR where R = alkyl or acyl group, or absent;
- 25 --- represents an optional double bond,
- wherein in addition to the above
- either R_{13} or R_{14} = alkyl group, and

the stereochemistry of R_{35} is in the β orientation;

sapogenin derivatives, particularly but not exclusively steroidal spirostane sapogenin derivatives, bearing at least one X radical substituent,

5 wherein X is chosen from the group consisting of :

- halo atom,
- (Me-S-), (Me-SO-), (Me-SO₂-),
- N₃-, NH₂-, MeSO₂NH-, and
- alkyl; and

10

derivative forms of any of the above compounds, in which the carbon atom at the 3-position (i.e. the carbon to which R_3 is attached, or the corresponding position in the sapogenin derivatives defined above without a formula) or, in the case of Formulae II and III, the 3-position carbon atom, the 26-position (i.e. the carbon to which R_{34} is attached) or each of
15 the carbon atoms at the 3- and 26-positions, carries an O-sugar moiety wherein the sugar group is a mono-, di- or tri-saccharide.

Sugar-carrying derivative forms of sapogenin active agents are generally referred to in the art as saponins. The expression "carbohydrate" used herein includes particularly such sugar
20 groups.

The active agents used in the present invention are preferably the non-estrogenic steroidal sapogenins, saponins, and derivatives thereof within the terms of the above definition, including all physiologically acceptable pro-drugs and salts thereof.

25

The active agents may be naturally occurring or non-naturally occurring. Non-naturally occurring active agents may suitably be prepared by modification of side groups and/or side atoms of naturally occurring compounds, as described below and as known in the art.

30

The invention also provides corresponding methods for the treatment of human and non-human animals, and compositions containing the active agents for use in the said treatment methods.

The active agents of the invention may, if desired, be co-administered with one or more
35 additional active agent, for example one or more agent selected from, but not limited to, cholinesterase inhibitors, dopamine agonists (e.g. L-dopa), COMT inhibitors, MAO-B

inhibitors, anti-cholinergics, acetylcholine agonists, serotonin agonists, AMPA receptor agonists, GABA receptor agonists, NMDA receptor agonists, β -adrenoceptor agonists, digoxin, dobutamine, anti-inflammatories, neurotrophic factors, statins, adenosine A2a receptor antagonists, aldose reductase inhibitors, immunomodulators, cannabinoid agonists, interferon β or tricyclic anti-depressants.

The active agents may be applied therapeutically or prophylactically to human and non-human animals suffering from, or susceptible to, conditions and diseases that are characterised by non-cognitive neurodegeneration, non-cognitive neuromuscular degeneration, motor-sensory neurodegeneration, or receptor dysfunction or loss. Examples of such conditions and diseases are provided below.

The present invention therefore also provides the use of the active agents (as herein defined) in the treatment or prevention of, or in the preparation of compositions (e.g. pharmaceutical compositions, foodstuffs, food supplements and beverages) for the treatment or prevention of, one or more of said conditions and diseases in human and non-human animals suffering therefrom or susceptible thereto.

Detailed Description of the Invention

Examples of Active Agents

The following classes of active agents are particularly mentioned:

1. Compounds of the above general formula I, wherein:
 - $R_1, R_2, R_3, R_4, R_5, R_6, R_7, R_8, R_{10}, R_{11}, R_{12}, R_{13}, R_{20}, R_{21}, R_{22}, R_{23}, R_{24}, R_{25}, R_{26}, R_{27}, R_{28}, R_{29}, R_{30}, R_{31}, R_{32}$ are, independently of each other, either H, OH, =O, halo atom, (Me-S-), (Me-SO-), (Me-SO₂-), N₃-, NH₂-, MeSO₂NH-, alkyl or absent or OR where R = alkyl or aryl group;
 - $R_9, R_{11}, R_{12}, R_{14}, R_{15}, R_{16}, R_{17}, R_{25}, R_{33}$ can be either a H, OH, halo atom, (Me-S-), (Me-SO-), (Me-SO₂-), N₃-, NH₂-, MeSO₂NH-, alkyl or absent or OR where R = alkyl or aryl group;
 - represents an optional double bond,
 - wherein in addition to the above
 - either R_{13} or R_{14} = alkyl group,
- and the stereochemistry of R_{25} is in the β orientation;

2. Compounds of the above general formula I, wherein:

- $R_1, R_2, R_3, R_4, R_5, R_6, R_7, R_8, R_{10}, R_{11}, R_{12}, R_{13}, R_{14}, R_{15}, R_{16}, R_{17}, R_{18}, R_{19}, R_{20}, R_{21}, R_{22}, R_{23}, R_{24}, R_{25}, R_{26}, R_{27}, R_{28}, R_{29}, R_{30}, R_{31}, R_{32}$ are, independently of each other, either H, OH, =O, halo atom, (Me-S-), (Me-SO-), (Me-SO₂), N₃-, NH₂-, MeSO₂NH-, alkyl or absent or OR where R = alkyl or acyl group;

- $R_9, R_{12}, R_{15}, R_{16}, R_{17} = H$,

- $R_{11}, R_{14}, R_{25}, R_{30}$ can be either a H, OH, halo atom, (Me-S-), (Me-SO-), (Me-SO₂-), N₃-, NH₂-, MeSO₂NH-, alkyl, or absent or OR where R = alkyl or acyl group;

_____ represents an optional double bond

10 wherein in addition to the above

- either R_{33} or $R_{34} = \text{alkyl group}$,

and the stereochemistry of R_{25} is in the β orientation;

3. Compounds of the above general formula I, wherein:

15 - $R_1 = R_2 = R_3 = R_4 = R_5 = R_6 = R_7 = R_8 = R_{10} = R_{11} = R_{12} = R_{13} = R_{15} = R_{16} = R_{17} = R_{18} = R_{19} = R_{20} = R_{21} = R_{22} = R_{23} = R_{24} = R_{25} = R_{26} = R_{27} = R_{28} = R_{29} = R_{30} = R_{31} = R_{32} = H$,

- either R_{33} or $R_{34} = \text{CH}_3$,

_____ represents a single bond,

- the methyl group at C25 may be either in the R or S configuration

20 - the stereochemistry of R_{25} is in the β orientation and

wherein in addition to the above

at least one of R_3 or R_{20} is a X radical, the possible remaining substituent being H, OH, =O, and OR where R = alkyl or acyl group or absent,

and X is chosen from the group consisting of:

25 - halo atom,

- (Me-S-), (Me-SO-), (Me-SO₂-), and

- N₃-, NH₂-, MeSO₂NH-

- alkyl;

30 4. Compounds of the above general formula I, wherein:

- $R_1 = R_2 = R_3 = R_4 = R_5 = R_6 = R_7 = R_8 = R_{10} = R_{11} = R_{12} = R_{13} = R_{15} = R_{16} = R_{17} = R_{18} = R_{19} = R_{20} = R_{21} = R_{22} = R_{23} = R_{24} = R_{25} = R_{26} = R_{27} = R_{28} = R_{29} = R_{30} = R_{31} = R_{32} = H$,

- $R_{14} = R_{33} = \text{CH}_3$,

_____ represents a single bond,

35 - the stereochemistry of R_{25} is in the β orientation and

wherein in addition to the above

at least one of R_3 or R_{23} is a X radical, the possible remaining substituent being H, OH, =O, and OR where R = alkyl or acyl group or absent, and X is chosen from the group consisting of:

- halo atom,

5 - (Me-S-), (Me-SO-), (Me-SO₂-), and

- N₃-, NH₂-, MeSO₂NH-

- alkyl;

5. Compounds of the above general formula II, wherein

10 - $R_1, R_2, R_3, R_4, R_5, R_6, R_7, R_8, R_{10}, R_{12}, R_{13}, R_{15}, R_{20}, R_{21}, R_{22}, R_{23}, R_{24}, R_{25}, R_{27}, R_{28}, R_{29}, R_{30}, R_{31}, R_{32}, R_{34}$ are, independently of each other, either H, OH, =O, halo atom, (Me-S-), (Me-SO-), (Me-SO₂-), N₃-, NH₂-, MeSO₂NH-, alkyl, OR where R = alkyl or acyl group, or absent;

- $R_9, R_{11}, R_{14}, R_{16}, R_{17}, R_{26}, R_{33}, R_{35}$ can be either a H, OH, halo atom, (Me-S-),

15 (Me-SO-), (Me-SO₂-), N₃-, NH₂-, MeSO₂NH-, alkyl, OR where R = alkyl or acyl group, or absent;

... represents an optional double bond,

wherein in addition to the above

- either R_{23} or R_{34} = alkyl group,

20 and the stereochemistry of R_{25} is in the β orientation;

6. Compounds of the above general formula II and carbohydrate derivatives thereof, wherein:

25 - $R_1, R_2, R_3, R_4, R_5, R_6, R_7, R_8, R_{10}, R_{12}, R_{13}, R_{15}, R_{20}, R_{21}, R_{22}, R_{23}, R_{24}, R_{25}, R_{27}, R_{28}, R_{29}, R_{30}, R_{31}, R_{32}$ are, independently of each other, either H, OH, =O, halo atom, (Me-S-), (Me-SO-), (Me-SO₂-), N₃-, NH₂-, MeSO₂NH-, alkyl, OR where R = alkyl or acyl group, or absent;

- $R_9, R_{12}, R_{15}, R_{16}, R_{17}$ = H,

- R_{34} = either H, OH, =O, and OR where R = alkyl, acyl or carbohydrate and

30 - $R_{11}, R_{14}, R_{26}, R_{33}, R_{35}$ can be either H, OH, halo atom, (Me-S-), (Me-SO-), (Me-SO₂-), N₃-, NH₂-, MeSO₂NH-, alkyl, OR where R = alkyl or acyl group, or absent;

... represents an optional double bond,

wherein in addition to the above

- either R_{23} or R_{34} = alkyl group,

35 and the stereochemistry of R_{25} is in the β orientation;

7. Compounds of the above general formula II and carbohydrate derivatives thereof, wherein:

$-R_1 = R_2 = R_3 = R_4 = R_5 = R_6 = R_7 = R_8 = R_{10} = R_{11} = R_9 = R_{12} = R_{13} = R_{15} = R_{16} = R_{17} = R_{18} = R_{19} = R_{20} =$
 $R_{21} = R_{22} = R_{23} = R_{24} = R_{25} = R_{26} = R_{27} = R_{28} = R_{29} = R_{30} = R_{31} = R_{32} = R_{33} = H,$

5 $-R_{14} = CH_3,$

$-R_{34} = -OH$ or $-OR$ where $R =$ alkyl, acyl or carbohydrate and

$R_{35} = H$ or is absent

$\sim$ represents an optional double bond, and

- the methyl group at C25 may be either in the R or S configuration and

10 and the stereochemistry of R_{35} is in the β orientation

wherein in addition to the above

at least one of R_3 or R_{23} is a X radical, the possible remaining substituent being H, OH, $=O$,

and OR where $R =$ alkyl or acyl group or absent,

and X is chosen from the group consisting of:

15 - halo atom,

- (Me-S-), (Me-SO-), (Me-SO₂-), and

- N₃-, NH₂-, MeSO₂NH-

- alkyl;

20 8. Compounds of the above general formula II and carbohydrate derivatives thereof, wherein:

$-R_1 = R_2 = R_3 = R_4 = R_5 = R_6 = R_7 = R_8 = R_{10} = R_{11} = R_9 = R_{12} = R_{13} = R_{14} = R_{16} = R_{17} = R_{18} = R_{19} = R_{20} =$
 $R_{21} = R_{22} = R_{23} = R_{24} = R_{25} = R_{26} = R_{27} = R_{28} = R_{29} = R_{30} = R_{31} = R_{32} = H,$

$-R_{14} = R_{33} = CH_3,$

25 $-R_{34} = -OH$ or $-OR$ where $R =$ alkyl, acyl or carbohydrate and

$R_{35} = H$ or is absent

$\sim$ represents an optional double bond, and

the stereochemistry of R_{35} is in the β orientation, and

wherein in addition to the above

30 at least one of R_3 or R_{23} is a X radical, the possible remaining substituent being H, OH, $=O$,

and OR where $R =$ alkyl or acyl group or absent,

and X is chosen from the group consisting of:

- halo atom,

- (Me-S-), (Me-SO-), (Me-SO₂-), and

35 - N₃-, NH₂-, MeSO₂NH-

- alkyl;

9. Compounds of the above general formula III, wherein:

- $R_1, R_2, R_3, R_4, R_5, R_6, R_7, R_8, R_{10}, R_{13}, R_{14}, R_{18}, R_{19}, R_{20}, R_{21}, R_{22}, R_{23}, R_{24}, R_{26}, R_{27}, R_{28}, R_{29}, R_{30}, R_{31}, R_{32}, R_{33}, R_{34}, R_{35}, R_{36}, R_{37}$ are, independently of each other, either H, OH, =O, halo atom, (Me-S-), (Me-SO-), (Me-SO₂-), N₃-, NH₂-, MeSO₂NH-, alkyl, OR where R = alkyl or acyl group, or absent;
- $R_9, R_{11}, R_{12}, R_{15}, R_{16}, R_{17}, R_{25}$ can be either H, OH, halo atom, (Me-S-), (Me-SO-), (Me-SO₂-), N₃-, NH₂-, MeSO₂NH-, alkyl, OR where R = alkyl or acyl group, or absent;
- ... represents an optional double bond,
- 10 wherein in addition to the above
- either R_{33} or R_{14} = alkyl group, and
- the stereochemistry of R_{25} is in the β orientation;

10. Compounds of the above general formula III, wherein:

- 15 - $R_1, R_2, R_3, R_4, R_5, R_6, R_7, R_8, R_{10}, R_{13}, R_{14}, R_{18}, R_{19}, R_{20}, R_{21}, R_{22}, R_{23}, R_{24}, R_{26}, R_{27}, R_{28}, R_{29}, R_{30}, R_{31}, R_{32}, R_{33}, R_{34}, R_{35}, R_{36}, R_{37}$ are, independently of each other, either H, OH, =O, halo atom, (Me-S-), (Me-SO-), (Me-SO₂-), N₃-, NH₂-, MeSO₂NH-, alkyl, OR where R = alkyl or acyl group, or absent;
- $R_9, R_{12}, R_{15}, R_{16}, R_{17}$ = H,
- 20 - R_{34} = H, OH, =O, halo atom, (Me-S-), (Me-SO-), (Me-SO₂-), N₃-, NH₂-, MeSO₂NH-, alkyl, OR where R = alkyl, acyl or carbohydrate, or absent;
- R_{11}, R_{25} can be either H, OH, halo atom, (Me-S-), (Me-SO-), (Me-SO₂-), N₃-, NH₂-, MeSO₂NH-, alkyl, OR where R = alkyl or acyl group, or absent;
- ... represents an optional double bond,
- 25 wherein in addition to the above
- either R_{33} or R_{14} = alkyl group, and
- the stereochemistry of R_{25} is in the β orientation;

11. Compounds of the above general formula III and carbohydrate derivatives thereof,

30 wherein:

- $R_1 = R_2 = R_3 = R_4 = R_5 = R_6 = R_7 = R_8 = R_{10} = R_{11} = R_{12} = R_{13} = R_{15} = R_{16} = R_{17} = R_{18} = R_{19} = R_{20} = R_{21} = R_{22} = R_{23} = R_{24} = R_{26} = R_{27} = R_{28} = R_{29} = R_{30} = R_{31} = R_{32} = R_{33} = R_{34} = R_{35} = R_{36} = R_{37} = R_{38} = R_{39} = R_{40} = R_{41} = R_{42} = R_{43} = R_{44} = R_{45} = R_{46} = R_{47} = R_{48} = R_{49} = R_{50} = R_{51} = R_{52} = R_{53} = R_{54} = R_{55} = R_{56} = R_{57} = R_{58} = R_{59} = R_{60} = R_{61} = R_{62} = R_{63} = R_{64} = R_{65} = R_{66} = R_{67} = R_{68} = R_{69} = R_{70} = R_{71} = R_{72} = R_{73} = R_{74} = R_{75} = R_{76} = R_{77} = R_{78} = R_{79} = R_{80} = R_{81} = R_{82} = R_{83} = R_{84} = R_{85} = R_{86} = R_{87} = R_{88} = R_{89} = R_{90} = R_{91} = R_{92} = R_{93} = R_{94} = R_{95} = R_{96} = R_{97} = R_{98} = R_{99} = R_{100} = H,$
- $R_{14} = CH_3,$
- $R_{34} = -OH$ or $-OR$ where R = alkyl, acyl or carbohydrate and
- 35 $R_{25} = H$ or is absent
- $R_{37} = H, -OH$ or $=O$

$R_{36} = H$ or $-OH$

... represents a single bond, and

- the methyl group at C25 may be either in the R or S configuration and the stereochemistry of R_{35} is in the β orientation,

5 wherein in addition to the above

at least one of R_3 or R_{23} is a X radical, the possible remaining substituent being H, OH, =O, and OR where R = alkyl or acyl group or absent, and X is chosen from the group consisting of:

- halo atom,

10 - (Me-S-), (Me-SO-), (Me-SO₂-), and

- N₃-, NH₂-, MeSO₂NH-

- alkyl;

12. Compounds of the above general formula III and carbohydrate derivatives thereof,

15 wherein:

- $R_1 = R_2 = R_3 = R_4 = R_5 = R_6 = R_7 = R_8 = R_{10} = R_{11} = R_9 = R_{12} = R_{13} = R_{15} = R_{16} = R_{17} = R_{18} = R_{19} = R_{20} =$

$R_{21} = R_{22} = R_{23} = R_{24} = R_{25} = R_{26} = R_{27} = R_{28} = R_{29} = R_{30} = R_{31} = R_{32} = R_{33} = R_{34} = H,$

- $R_{14} = R_{35} = CH_3,$

- $R_{34} = -OH$ or $-OR$ where R = alkyl, acyl or carbohydrate and

20 $R_{31} = H$ or is absent

$R_{37} = H, -OH$ or $=O$

$R_{38} = H$ or $-OH$

... represents a single bond, and

- the methyl group at C25 may be either in the R or S configuration and

25 the stereochemistry of R_{35} is in the β orientation

wherein in addition to the above

at least one of R_3 or R_{23} is a X radical, the possible remaining substituent being H, OH, =O, and OR where R = alkyl or acyl group or absent,

and X is chosen from the group consisting of:

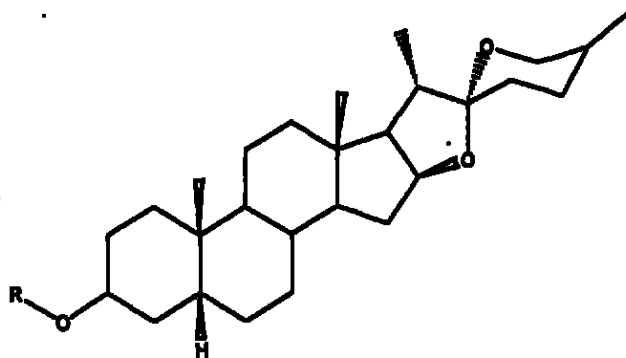
30 - halo atom,

- (Me-S-), (Me-SO-), (Me-SO₂-), and

- N₃-, NH₂-, MeSO₂NH-

- alkyl;

13. Substituted sapogenins, particularly but not exclusively steroidal spirostane sapogenins, wherein at least one OH-group of the sapogenin is substituted with X, chosen from the group consisting of:
- halo atom,
 - 5 - (Me-S-), (Me-SO-), (Me-SO₂-),
 - N₃-, NH₂-, MeSO₂NH-, and
 - alkyl;
14. Sapogenins defined above, particularly but not exclusively steroidal spirostane sapogenins, wherein in the definition of X the halo atom is a fluoro atom;
15. Substituted sapogenins selected from:
- (3 β -fluoro-5 β , 20 α ,22 α ,25R-spirostane), (3,3-difluoro-5 β , 20 α ,22 α ,25R-spirostane), (3 α -methylsulphonylamino-5 β ,20 α ,22 α ,25R-spirostane), (3 α -azido-5 β , 20 α ,22 α ,25R-spiro-
- 15 stane), (3 α -amino-5 β ,20 α ,22 α ,25R-spirostane), and their stereoisomers and racemic mixtures, their pharmaceutically acceptable pro-drugs and salts;
16. Substituted sapogenins wherein the parent sapogenin which is then substituted with at least one X radical as defined above is selected from sarsapogenin, episarsapogenin,
- 20 smilagenin, episamilagenin, and anurogenin-D;
17. Compounds of the general formula Ia:



(Ia)

25

wherein the group R is selected from hydrogen; alkylcarbonyl; alkoxy carbonyl; alkyl-carbamoyl; or arylcarbonyl; or sulpho (HO,S); phosphono ((HO)₂P(O)-); or a mono-, di- or

tri-saccharide; wherein any alkyl group is optionally substituted with aryl, amino, mono- or di-alkyl-amino, a carboxylic acid residue (-COOH), or any combination thereof;

18. Derivative forms of the above compounds as defined as items 1 to 17, in which the 3-position carbon atom or, in the case of Formulae II and III, the 3-position carbon atom, the 26-position carbon atom or each of the carbon atoms at the 3- and 26-positions, carries an O-sugar moiety wherein the sugar group is a mono-, di- or tri-saccharide, for example a mono aldose or ketose having 5 or 6 carbon atoms, preferably in the cyclised furanose or pyranose form, either as the α or β anomer and having D or L optical isomerism, or any di- and tri-oligosaccharide combination thereof; acylated forms of sugar residues are also to be included within the term "sugar"; examples of suitable sugars include glucose, mannose, fructose, galactose, maltose, cellobiose, sucrose, rhamnose, xylose, arabinose, fucose, quinovose, apiose, lactose, galactose-glucose, glucose-arabinose, fucose-glucose, rhamnose-glucose, glucose-glucose-glucose, glucose-rhamnose, mannose-glucose, glucose-(rhamnose)-glucose, glucose-(rhamnose)-rhamnose, glucose-(glucose)-glucose, galactose-(rhamnose)-galactose and acylated (e.g. acetylated) derivatives thereof.

In the above compound definitions:

- 20 Optional amino, mono-alkyl-amino and di-alkyl-amino substituents of alkyl groups, where present, are preferably a mono-substituent at the α position of the alkyl group.

Optional -COOH substituents of alkyl groups, where present, may be at the terminal or any other position of the alkyl group.

- 25 "Alkyl" means an aliphatic hydrocarbon group which may be straight or branched having about 1 to about 20 carbon atoms in the chain. Preferred alkyl groups have 1 to about 12 carbon atoms in the chain. Branched means that one or more lower alkyl groups such as methyl, ethyl or propyl are attached to a linear alkyl chain. "Lower alkyl" means about 1 to about 4 carbon atoms in the chain which may be straight or branched. Exemplary alkyl groups include methyl, ethyl, *n*-propyl, *i*-propyl, *n*-butyl, *i*-butyl, *t*-butyl, *n*-pentyl, 3-pentyl.

"Aryl" means any group comprising an aromatic ring or system of fused rings, and preferably contains up to 12 carbon atoms. An exemplary aryl group is the phenyl group.

- 35 An aryl group may optionally be mono- or poly-substituted, for example by substituents

independently selected from halo (e.g. chloro or bromo), alkyl, cycloalkyl, hydroxy, alkoxy, amino, nitro, acylamino, carboxy and alkoxycarbonyl.

"Carboxylic acid residue" means the group -COOH.

5

"Acyl" means an H-CO- or Alkyl-CO- group wherein the alkyl group is as defined below. Preferred acyls contain a lower alkyl. Exemplary acyl groups include formyl, acetyl, propanoyl, 2-methylpropanoyl, butanoyl and palmitoyl;

- 10 "Optionally substituted" means that the said group may be substituted with one or more substituents, which may be the same or different, preferably one or more substituents which individually have a size which is small (e.g. less than about 20% of the largest molecular dimension) in relation to the parent group being substituted; suitable substituents include halo (e.g. chloro or bromo), alkyl, cycloalkyl, hydroxy, alkoxy, amino, acylamino, aryl, 15 aroylamino, carboxy, alkoxycarbonyl, aralkoxycarbonyl, heteroaralkoxycarbonyl, and optionally substituted carbamoyl, preferably subject to the size limitation set out above ;

- "Pharmaceutically acceptable" means it is, within the scope of sound medical and veterinary judgment, suitable for use in contact with the cells of humans and lower animals 20 without undue toxicity, irritation, allergic response and the like, and are commensurate with a reasonable benefit/risk ratio. "Pharmaceutically acceptable prodrugs" means those prodrugs of the compounds which are, within the scope of sound medical and veterinary judgment, suitable for use in contact with the tissues of humans and lower animals without undue toxicity, irritation, allergic response, and the like, commensurate with a reasonable 25 benefit/risk ratio, and effective for their intended use, as well as the zwitterionic forms, where possible, of the compounds. The term "prodrug" means compounds that are rapidly transformed *in vivo* to yield the parent compound of the above formula, for example by hydrolysis in blood. Functional groups which may be rapidly transformed, by metabolic cleavage, *in vivo* form a class of groups reactive with the carboxyl group. Because of the 30 ease with which the metabolically cleavable groups of the compounds are cleaved *in vivo*, the compounds bearing such groups act as pro-drugs. A thorough discussion of prodrugs is provided in the following: Design of Prodrugs, H. Bundgaard, ed., Elsevier, 1985; Methods in Enzymology, K. Widder et al, Ed., Academic Press, 42, p.309-396, 1985; A Textbook of Drug Design and Development, Krogsgaard-Larsen and H. Bundgaard, ed., Chapter 5; 35 Design and Applications of Prodrugs p.113-191, 1991; Advanced Drug Delivery Reviews, H. Bundgaard, 8, p.1-38, 1992; Journal of Pharmaceutical Sciences, 77, p. 285, 1988; Chem.

Pharm. Bull., N. Nakaya et al, 32, p. 692, 1984; Pro-drugs as Novel Delivery Systems, T. Higuchi and V. Stella, Vol. 14 of the A.C.S. Symposium Series, and Bioreversible Carriers in Drug Design, Edward B. Roche, ed., American Pharmaceutical Association and Pergamon Press, 1987, which are incorporated herein by reference;

5

"Pharmaceutically acceptable salts" means the relatively non-toxic, inorganic and organic acid addition salts, and base addition salts, of compounds of the present invention. These salts can be prepared *in situ* during the final isolation and purification of the compounds. In particular, acid addition salts can be prepared by separately reacting the purified compound in its free base form with a suitable organic or inorganic acid and isolating the salt thus formed. See, for example S. M. Berge, et al., Pharmaceutical Salts, J. Pharm. Sci., 66: p.1-19 (1977) which is incorporated herein by reference. Base addition salts can also be prepared by separately reacting the purified compound in its acid form with a suitable organic or inorganic base and isolating the salt thus formed. Base addition salts include pharmaceutically acceptable metal and amine salts. Examples of suitable acid addition salts are those formed with acids selected from hydrochloric, sulphuric, phosphoric and nitric acids. Examples of suitable base addition salts are those formed with bases selected from sodium hydroxide, potassium hydroxide and ammonium hydroxide.

10

15

20 A particularly preferred class of active agents are the compounds of the general formula Ia.

In some of the compounds of formula Ia, the C₁₅ methyl group is in the S configuration; these compounds of the invention are *sansaspogenin* and *episansaspogenin* or derivatives thereof. In other compounds of formula Ia, the C₁₅ methyl group is in the R configuration; these compounds of the invention are *amilagenin* and *epiamilagenin* or derivatives thereof.

25

In the above formula Ia, -OR may, for example, be selected from the following (unless excluded by proviso): hydroxy, ethylate (ethoxycarbonyloxy), acetate, succinate, cinnamate, ferulate, propionate, butyrate, valerate, isovalerate, caproate, isocaproate, diethylacetate, octanoate, decanoate, laurate, myristate, palmitate, stearate, benzoate, phenylacetate, phenylpropionate, cinnamate, p-nitrobenzoyloxy, 3,5-dinitrobenzoyloxy, p-chlorobenzoyloxy, 2,4-dichlorobenzoyloxy, p-bromobenzoyloxy, m-bromobenzoyloxy, p-methoxybenzoyloxy, phthalyl, glycinate, alaninate, valinate, phenylalaninate, isoleucinate, methioninate, argininate, asparaginate, aspartate, cysteinate, glutamate, histidinate, lysinate, proline, serinate, threoninate, tryptophanate, tyrosinate, fumarate or maleate.

30

35

Of the compounds of general formula Ia and their pharmaceutically acceptable salts, particularly preferred are the following compounds:

sarsasapogenin

sarsasapogenin cathylate

5 sarsasapogenin acetate

sarsasapogenin succinate and pharmaceutically acceptable salts thereof

sarsasapogenin glycinate and pharmaceutically acceptable salts thereof

sarsasapogenin alaninate and pharmaceutically acceptable salts thereof

sarsasapogenin valinate and pharmaceutically acceptable salts thereof

10 sarsasapogenin phenylalaninate and pharmaceutically acceptable salts thereof

sarsasapogenin isoleucinate and pharmaceutically acceptable salts thereof

sarsasapogenin methioninate and pharmaceutically acceptable salts thereof

episarsasapogenin

episarsasapogenin cathylate

15 episarsasapogenin acetate

episarsasapogenin succinate and pharmaceutically acceptable salts thereof

episarsasapogenin glycinate and pharmaceutically acceptable salts thereof

episarsasapogenin alaninate and pharmaceutically acceptable salts thereof

episarsasapogenin valinate and pharmaceutically acceptable salts thereof

20 episarsasapogenin phenylalaninate and pharmaceutically acceptable salts thereof

episarsasapogenin isoleucinate and pharmaceutically acceptable salts thereof

episarsasapogenin methioninate and pharmaceutically acceptable salts thereof

smilagenin

smilagenin cathylate

25 smilagenin acetate

smilagenin succinate and pharmaceutically acceptable salts thereof

smilagenin glycinate and pharmaceutically acceptable salts thereof

smilagenin alaninate and pharmaceutically acceptable salts thereof

smilagenin valinate and pharmaceutically acceptable salts thereof

30 smilagenin phenylalaninate and pharmaceutically acceptable salts thereof

smilagenin isoleucinate and pharmaceutically acceptable salts thereof

smilagenin methioninate and pharmaceutically acceptable salts thereof

epismilagenin

epismilagenin cathylate

35 epismilagenin acetate

epismilagenin succinate and pharmaceutically acceptable salts thereof

epimilagenin glycinate and pharmaceutically acceptable salts thereof
epimilagenin alaninate and pharmaceutically acceptable salts thereof
epimilagenin valinate and pharmaceutically acceptable salts thereof
epimilagenin phenylalaninate and pharmaceutically acceptable salts thereof
5 epimilagenin isoleucinate and pharmaceutically acceptable salts thereof
epimilagenin methioninate and pharmaceutically acceptable salts thereof.

Of the saponin (R = sugar) compounds of general formula Ia, particularly preferred are the following compounds: sarsasapogenin, episarsasapogenin, smilagenin and epimilagenin in
10 which, in each case, the 3-position carbon atom carries an O-sugar moiety wherein the sugar group is selected from glucose, mannose, fructose, galactose, maltose, cellobiose, sucrose, rhamnose, xylose, arabinose, fucose, quinovose, apiose, lactose, galactose-glucose, glucose-arabinose, fucose-glucose, rhamnose-glucose, glucose-glucose-glucose, glucose-rhamnose, mannose-glucose, glucose-(rhamnose)-glucose, glucose-(rhamnose)-rhamnose,
15 glucose-(glucose)-glucose, galactose-(rhamnose)-galactose and acylated (e.g. acetylated) derivatives thereof.

Further examples of suitable active agents include 16,22-epoxycoprostan-3 β -ol, smilagenone, coprosterol and pharmaceutically acceptable pro-drugs and salts thereof.

20

Compositions and Uses

The present invention thus enables and provides a method for treating or preventing non-cognitive neurodegeneration, non-cognitive neuromuscular degeneration, motor-sensory
25 neurodegeneration or receptor dysfunction or loss in the absence of cognitive, neural or neuromuscular impairment (particularly but not exclusively in relation to the specific disease states mentioned above) in a human or non-human animal in need thereof, which comprises administering to the said human or non-human animal an effective dosage of an active agent (as defined herein) or a pharmaceutically acceptable salt thereof.

30

The active agent may be administered in the form of a composition comprising the active agent and any suitable additional component. The composition may, for example, be a pharmaceutical composition (medicament), a foodstuff, food supplement or beverage. Such a composition may contain a mixture of the specified compounds, and/or of their
35 pharmaceutically acceptable salts.

According to a further aspect of the present invention, there is provided a composition having activity against, and for use in treating, non-cognitive neurodegeneration, non-cognitive neuromuscular degeneration, motor-sensory neurodegeneration or receptor dysfunction or loss in the absence of cognitive, neural or neuromuscular impairment in a human or non-human animal, which comprises an effective amount of a compound of the active agent.

The term "pharmaceutical composition" in the context of this invention means a composition comprising an active agent and comprising additionally pharmaceutically acceptable carriers, diluents, adjuvants, excipients, or vehicles, such as preserving agents, fillers, disintegrating agents, wetting agents, emulsifying agents, suspending agents, sweetening agents, flavoring agents, perfuming agents, antibacterial agents, antifungal agents, lubricating agents and dispensing agents, depending on the nature of the mode of administration and dosage forms.

The terms "foodstuff", "food supplement" and "beverages" used herein have the normal meanings for those terms, and are not restricted to pharmaceutical preparations.

The dosage of the active agent will vary widely, depending on the severity of the symptoms to be treated or prevented. The selection of appropriate dosages is within the ability of one of ordinary skill in this art, without undue burden. The dosage of the active agent may, for example, be greater than about 0.1 mg/kg body weight, for example greater than about 0.3 mg/kg body weight, preferably administered once per day. More typically, the dosage will be between about 1 and about 25 mg/kg, e.g. between about 1 and about 10 mg/kg, preferably administered once per day. For human use, the dosage may conveniently be between about 70 and about 700 mg per day.

"Pharmaceutically acceptable dosage forms" means dosage forms of the compounds or compositions of the invention, and includes, for example, tablets, dragees, powders, elixirs, syrups, liquid preparations, including suspensions, sprays, inhalants, tablets, lozenges, emulsions, solutions, granules, capsules and suppositories, as well as liquid preparations for injections, including liposome preparations. Techniques and formulations generally may be found in Remington, Pharmaceutical Sciences, Mack Publishing Co., Easton, PA, latest edition.

In general, reference herein to the presence of one of a specified group of compounds includes within its scope the presence of a mixture of two or more of such compounds.

This invention provides for the treatment of (i) non-cognitive neurodegeneration, (ii) non-cognitive neuromuscular degeneration, (iii) motor-sensory neurodegeneration, or (iv) receptor dysfunction or loss in the absence of cognitive, neural and neuromuscular impairment, in a human or non-human animal subject suffering from, or susceptible to, any of: Parkinson's disease, postencephalitic Parkinsonism, depression, schizophrenia, muscular dystrophy including facioscapulohumeral muscular dystrophy (FSH), Duchenne muscular dystrophy, Becker muscular dystrophy and Bruce's muscular dystrophy, Fuchs' dystrophy, myotonic dystrophy, corneal dystrophy, reflex sympathetic dystrophy syndrome (RSDSA), neurovascular dystrophy, myasthenia gravis, Lambert Eaton disease, Huntington's disease, motor neurone diseases including amyotrophic lateral sclerosis (ALS), multiple sclerosis, postural hypotension, traumatic neurodegeneration e.g. following stroke or following an accident (for example, traumatic head injury or spinal cord injury), Batten's disease, Cockayne syndrome, Down syndrome, corticobasal ganglionic degeneration, multiple system atrophy, cerebral atrophy, olivopontocerebellar atrophy, dentatorubral atrophy, pallidolubal atrophy, spinobulbar atrophy, optic neuritis, sclerosing pan-encephalitis (SSPE), attention deficit disorder, post-viral encephalitis, post-polio myelitis syndrome, Fahr's syndrome, Joubert syndrome, Guillain-Barre syndrome, lissencephaly, Moyamoya disease, neuronal migration disorders, autistic syndrome, polyglutamine disease, Niemann-Pick disease, progressive multifocal leukoencephalopathy, pseudotumor cerebri, Refsum disease, Zellweger syndrome, supranuclear palsy, Friedreich's ataxia, spinocerebellar ataxia type 2, Rhet syndrome, Shy-Drager syndrome, tuberous sclerosis, Pick's disease, chronic fatigue syndrome, neuropathies including hereditary neuropathy, diabetic neuropathy and mitotic neuropathy, prion-based neurodegeneration, including Creutzfeldt-Jakob disease (CJD), variant CJD, new variant CJD, bovine spongiform encephalopathy (BSE), GSS, FFI, kuru and Alper's syndrome, Joseph's disease, acute disseminated encephalomyelitis, arachnoiditis, vascular lesions of the central nervous system, loss of extremity neuronal function, Charcot-Marie-Tooth disease, susceptibility to heart failure, asthma, and macular degeneration.

The invention therefore includes methods of treating or preventing the above diseases and conditions in a human or non-human animal suffering therefrom or susceptible thereto, which comprises administering to the said human or non-human animal an effective amount

of an active agent as defined herein, as well as uses of the active agents in the preparation of compositions for said treatment or prevention.

In the cases of Parkinson's disease, postencephalitic Parkinsonism, postural hypotension, autistic syndromes, chronic fatigue syndrome, myasthenia gravis, and Lambert Eaton disease, as well as any other conditions within the range of disease states to which the present invention relates which are disclosed in, or obvious from, the treatments made available or disclosed in the prior art acknowledged above, the present invention may be subject to the proviso that either symptoms of cognitive dysfunction are absent, or any symptoms of cognitive dysfunction presented by a subject to be treated are secondary or ancillary to the symptoms of non-cognitive neurodegeneration, non-cognitive neuromuscular degeneration, motor-sensory neurodegeneration or receptor dysfunction or loss in the absence of cognitive, neural and neuromuscular impairment.

15 *Preparation of Compounds for Use in the Invention*

Smilagenin, epismilagenin and sarsasapogenin are commercially available materials. Suppliers include, for example, Sigma Aldrich, Research Plus Inc. and Steraloids Inc. Preparative methods for these materials are also to be found in the literature (e.g. a preparation of episarsasapogenin is given in JACS p.5225 (1959)). Episarsasapogenin can be prepared by reduction of sarsasapogenone using a metal hydride reducing agent. Sarsasapogenone can be prepared using the method of Lajis et al, Steroids, 1993, 58, 387-389.

25 Also, as starting materials, unsubstituted saponins and sapogenins may occur naturally in a range of plant species, notably plants of the genus Smilax, Asparagus, Anemarrhena, Yucca or Agave. Where smilagenin or sarsasapogenin is used in accordance with this invention, it may be in the form of a plant extract, or dry powdered plant material, derived from a plant of the genus Smilax, Asparagus, Anemarrhena, Yucca or Agave.

30 Methods for preparing the active agents are well known to one of ordinary skill in this art. Examples are shown, for example, in WO-A-02/079221 (Examples 5 to 16 therein, which describe the preparation of sarsasapogenin cathylate, episarsasapogenin cathylate, episarsasapogenin succinate, epismilagenin cathylate, episarsasapogenin glyoinate hydrochloride, sarsasapogenin glycinate hydrochloride, epismilagenin glycinate hydrochloride, epismilagenin L-alaninate hydrochloride, epismilagenin L-valinate

hydrochloride, epismilagenin L-isoleucinate hydrochloride, epismilagenin L-phenylalaninate hydrochloride and epismilagenin L-methioninate hydrochloride). The compounds of formula Ia, other than those with R = H, can be prepared using conventional techniques from compounds in which R = H.

5

The preferred reaction is a nucleophilic substitution reaction, in which a compound having OH at the 3-position is reacted with a compound of formula



10

in which R is selected from alkylcarbonyl; alkoxy carbonyl; alkylcarbamoyl; or arylcarbonyl; or wherein any alkyl group is optionally substituted with aryl, amino, mono-alkyl-amino, di-alkyl-amino, a carboxylic acid residue (-COOH), or any combination thereof; and L is a leaving group, under conditions suitable for nucleophilic substitution.

15

The compound L-R may, for example, be a carboxylic acid or, if appropriate, an anhydride, or an acyl halide (e.g. an acyl chloride). For example, where R is a cathylate (ethoxycarbonyl) moiety, the compound L-R may suitably be ethyl chloroformate.

20 The reaction is suitably performed in a base such as pyridine, optionally in the presence of an acid such as hydrochloric acid.

The reaction details for nucleophilic substitution reactions are well known. See, for example, RC Larock, in Comprehensive Organic Transformations, VCH publishers, 1989.

25

Dihydrosarsasapogenin may be made using the method described in Marker and Rohrmann (1939), Sterols LIII; The structure of the side chain of sarsasapogenin, J. Am. Chem. Soc. 61, pp 846-851. 16,22-Epoxycoprostan-3 β -ol may be made using the method described in Scheer et al, (1955), The C-25 isomerism of smilagenin and sarsasapogenin: J. Am. Chem. Soc. 77, pp 641-646.

30

In the reactions described herein it may be necessary to protect reactive functional groups, for example hydroxy, carboxy or amino groups, where these are desired in the final product, to avoid their unwanted participation in the reactions. Conventional protecting groups may be used in accordance with standard practice. For example, see TW Green and PGM Wuts, in "Protective Groups in Organic Chemistry", John Wiley & Sons, 1991; JFW

35

McOmie in "Protective Groups in Organic Chemistry", Plenum Press, 1973. For protecting amino substituents in compounds of formula L-R wherein R is amino-substituted, it is preferred to use an alkoxycarbonyl protecting group, whereby the amino function is present as an alkoxycarbonylamino group (preferably t-butoxycarbonylamino) during the synthetic steps, until deprotection in acid conditions in a dry solvent.

The compound thus prepared may be recovered from the reaction mixture by conventional means. For example, the compound may be recovered by distilling off the solvent from the reaction mixture or, if necessary after distilling off the solvent from the reaction mixture, pouring the residue into water, followed by extraction with a water-miscible solvent and distilling off the solvent from the extract. Additionally, the product can, if desired, be further purified by various well known techniques, such as recrystallisation, reprecipitation, or the various chromatography techniques, notably column chromatography or preparative thin layer chromatography.

15

Discussion of the Basis for the Activity

The therapeutic uses underlying the present invention arise from a number of novel observations which are documented in detail in the Examples below. To understand the rationale of the invention, it is useful to summarise the observations and to explain how they predict the therapeutic activities claimed in this invention across the range of active agents defined above.

Smilagenin, epismilagenin, sarsapogenin and episarsapogenin restore the loss of muscarinic acetylcholine receptors and adrenoceptors in cells expressing such receptors *in vitro*. These results demonstrate that these compounds restore towards normal cellular receptor loss (Example 1).

Sarsapogenin, episarsapogenin cathylate, episarsapogenin, epismilagenin and smilagenin prevent chemically induced neurodegeneration in rat cortical neurones *in vitro*. These results demonstrate that these compounds are neuroprotective and prevent neurodegeneration and neuroimpairment *in vitro* (Example 2).

Sarsapogenin, smilagenin, 16,22-epoxycoprostan-3 α -ol, smilagenone, smilagenin glycinate hydrochloride and coprosterol reverse the chemically induced neurodegeneration

in rat cortical neurones *in vitro*. These results demonstrate that the compounds reverse sensory neurodegeneration and neuroimpairment *in vitro* (Example 3).

Smilagenin reverses the chemical induced apoptosis in neurones, demonstrating that this
5 compound is anti-apoptotic and neuroprotective *in vitro* (Example 4).

Smilagenin and sarsasapogenin increase the neurite outgrowth (neurite number and neurite branching) in rat cortical neurones *in vitro*, demonstrating their neurotrophic effects *in vitro* (Example 5).

10

Smilagenin and sarsasapogenin prevent and reverse neurotoxin-induced neurodegeneration (neurotoxin 1-methyl-4-phenylpyridinium (MPP⁺) in mesencephalic dopaminergic neurones *in vitro*. These results demonstrate that these compounds prevent and reverse neurodegeneration and neuroimpairment *in vitro* (Examples 6 and 7).

15

Sarsasapogenin and smilagenin reverse the chemically induced neurodegeneration in rat spinal motor neurones *in vitro*. These results demonstrate that the compounds reverse neurodegeneration and neuroimpairment of motor neurones *in vitro* (Example 8).

20

Sarsasapogenin, episarsasapogenin cathylate and smilagenin reduce the number of wrong responses in a cognitive ability test *in vivo* in aged rats, which correlates with an increase in muscarinic acetylcholine receptor density in the brains of aged rats following treatment with the compounds tested. These results demonstrate that the compounds reverse neuroimpairment *in vivo* (Example 9).

25

Smilagenin and sarsasapogenin reverse the decline of muscarinic acetylcholine and dopamine receptors and the decline in brain derived neurotrophic factor (BDNF) in aged animals. These results demonstrate that the compounds reverse motor-sensory neurodegeneration and neuroimpairment and are neurotrophic *in vivo* (Example 9).

30

Episarsasapogenin cathylate, sarsasapogenin cathylate, episarsasapogenin and epismilagenin, reduce the number of wrong responses in a cognitive ability test *in vivo* in young rats exposed to neurotoxic agents (ibotenic acid and amyloid β), and increase in muscarinic acetylcholine receptor density in the brains. These results demonstrate that the
35 compounds reverse neuroimpairment *in vivo* (Example 10).

Smilagenin and zararsapogenin improve survival and motor-sensory neurodegeneration and neuroimpairment in a mouse model of amyotrophic lateral sclerosis (ALS) and Charcot-Marie-Tooth disease (Example 11).

- 5 In summary, the compounds have been found to slow or reverse certain aspects of neuronal degeneration. These include the reversal of adverse changes in the cell body, atrophy of neuronal extensions (neurites), reduction in release of neurotrophic factors such as neurotrophins (e.g. BDNF, NGF, NT-3, NT4/5), TGF- β super-family neurotrophic factors (e.g. GDNF) and neurokines (e.g. CNTF, LIF), and neuronal toxicity or death (apoptosis).
- 10 The compounds are strongly neuroprotective, stimulative of neurite outgrowth, and preventive of neurotoxicity. The compounds have also been found to slow or reverse decreases in cholinergic and dopaminergic function, for example, decreases in muscarinic acetylcholine and dopamine receptor density. Furthermore, we have found that the neuroprotective and the reversal of receptor loss effects are actively regulated effects, in
- 15 which past deterioration is reversed towards the normal or young state with protection against continued deterioration. Still further, we have found that the reversal of apoptotic effect of the compounds appears to be regulated in the non-neoplastic domain of cell life, and does not appear likely to trigger neoplasia.
- 20 The above data, taken together, indicate activity against the disease states already listed in this application. Furthermore, the above data indicates a likely absence of severe or life-threatening side effects such as cancer. The active agents are typically non-estrogenic.

- 25 The prior art acknowledged above shows the sound basis of prediction for extension of the above observations and sound predictions of therapeutic activity to the related chemical structures and derivatives embraced by the term "active agents" in the present invention.

- 30 It is well known in the art and in pharmacology that sugar, ester and other groupings at suitable locations on steroid molecules, particularly at the 3- and/or 26-positions, can be easily cleaved off by *in vivo* hydrolysis, and the same effects would be expected to be observed at other carbon atoms of the molecules. Further, it is well known in the art and in pharmacology that salts, free acids and free bases within the terms of the expression "active agents" as used herein are readily convertible *in vivo* between each other according to the pH of the body fluid in which the active agent is present. Further, it is well known that side
- 35 group substituents, in a wide range of forms, can be present in a complex carbon skeleton without substantially adversely affecting the pharmacological activity of the structure,

particularly when the side groups are small in comparison with the overall size of the molecule.

For all these reasons, the claims of beneficial pharmacological activity made in the present application are seen to be reasonable and based on sound and credible prediction from the test data assembled and presented herein.

Without wishing to be bound by theory, it is believed that one physiological effect of the active agents is the ability to increase the synthesis, or release of, or to reduce the rate of degradation of, neurotrophic factors such as brain derived neurotrophic factor and/or nerve growth factor or their receptors. These effects on growth factors might be due to an effect of the compound on a cytosolic or nuclear receptor, or the binding of a compound to a promoter region with a consequent effect directly on the rate of production of mRNA for the growth factor, or as a consequence of increasing the production of another material factor.

In addition, the compounds appear to regulate receptors. For example, some of these compounds have been found to prevent or reverse the loss of muscarinic acetylcholine or dopamine receptors in the brain. It is believed that the compounds function by rectifying a deficiency in receptor number or function or turnover.

Brief Description of the Drawings

In order to illustrate the invention further by way of non-limiting example, reference will now be made to the accompanying drawings and to the Examples which follow.

In the drawings:

Figure 1 shows the effect of epismilagenin acetate on m3 and $\beta 2$ adrenoceptor density at day 5 in a CHO- $\beta 2$ /m3 co-transfected cell line;

Figure 2 shows the effects of sarsasapogenin, episarsasapogenin cathylate and smilagenin on glutamate induced neurodegeneration in rat primary cortical neurons;

Figure 3 shows the effects of sarsasapogenin, episarsasapogenin cathylate and smilagenin on the learning ability and memory of aged rats;

Figure 4 shows the effects of sarsasapogenin, episarsasapogenin cathylate and smilagenin on muscarinic receptor number;

Figure 5 shows the survival profile of SOD-1 mice following oral administration of smilagenin; and

Figure 6 shows the survival profile of pmu mice following oral administration of sarsasapogenin.

Detailed Description of the Drawings and Examples

EXAMPLE 1

Restoration of receptor loss *in vitro*

The effects of epismilagenin cathylate, sarsasapogenin cathylate, episarsasapogenin cathylate, episarsasapogenin succinate, epismilagenin acetate and sarsasapogenin on the expression of muscarinic acetylcholine receptor (m) in CHO cells or $\beta 2$ and m3 receptors in CHO cells transfected with either a vector for the m receptor or co-transfected with the vector for $\beta 2$ and m3 receptors were investigated.

The results are illustrated in Table 1 below and in Figure 1 of the drawings. Over the culturing period of CHO cells transfected with a vector for the m receptor, treatment with epismilagenin cathylate, sarsasapogenin cathylate, episarsasapogenin cathylate, episarsasapogenin succinate and sarsasapogenin each prevents the decrease in m receptor number. Over the culturing period of the CHO cells co-transfected with the vector for $\beta 2$ and m3 receptors, the density of the m3 receptor did not alter; whereas the density of the $\beta 2$ adrenoceptors decreased. Incubation with epismilagenin acetate (Figure 1) did not significantly alter the density of m3 receptors; but significantly prevented the decrease in $\beta 2$ adrenoceptors.

Table 1 - Effect of epismilagenin cathylate, sarsasapogenin cathylate, episarsasapogenin cathylate, episarsasapogenin succinate and sarsasapogenin on restoration of m acetylcholine receptor density.

Compound	Concentration [microM]	Activity
epismilagenin cathylate	10	++
sarsasapogenin cathylate	10	++
episarsasapogenin cathylate	10	++
episarsasapogenin succinate	10	++
Sarsasapogenin	10	++

Thus, the experiments indicate that each of epismilagenin cathylate, sarsasapogenin cathylate, episarsasapogenin cathylate, episarsasapogenin succinate, epismilagenin acetate and sarsasapogenin were able to prevent decline in receptor number with time and also tend to restore receptor number to normal levels when given to cells in which the receptor level is depressed.

EXAMPLE 2

Neuroprotective effect of sarsasapogenin, episarsasapogenin cathylate, episarsasapogenin, epismilagenin and smilagenin in neurones

The objective of this study was to examine the effects of sarsasapogenin, episarsasapogenin cathylate, episarsasapogenin, epismilagenin and smilagenin on the survival of rat primary cortical neurones exposed to glutamate, which is known to induce neurodegeneration.

Rat cortical neurones were cultured for 10 days; at day 10 the medium was changed to a serum-free defined medium. On day 12, 24 hours before glutamate exposure, cultures were washed and medium was replaced with fresh medium containing positive control (β -oestradiol), test compounds (sarsasapogenin, episarsasapogenin : cathylate, episarsasapogenin, epismilagenin and smilagenin) or vehicle control (DMSO, 0.25%) or diosgenin as negative control.

On day 13, cultures were exposed to glutamate. After the incubation period, the cultures were washed with and placed in fresh medium, supplemented with relevant compounds or vehicle to evaluate their protective effects, 24 h after glutamate exposure.

Neuronal cell survival was evaluated by measuring lactate dehydrogenase (LDH) activity released in the media 24 h after test compound treatment or glutamate + test compound exposure, using the CytoTox 96 non-radioactive kit and quantitated by measuring wavelength absorbance at 450 nm.

5

Following exposure of rat primary cortical cultures with glutamate, there was a significant degeneration of cortical neurones, 24 h post-treatment, demonstrated by an increase in lactate dehydrogenase release into the culture medium.

10 In primary cortical cultures pre-treated with the compounds for 24 h, there was also a significant reduction in the glutamate-induced neurodegeneration (Figure 2; Table 2).

Table 2 - Effect of sarsasapogenin, episarsasapogenin cathylate, episarsasapogenin, epismilagenin and smilagenin on prevention of glutamate-induced neurodegeneration

15

Conditions	Mean $\pm$ s.e.m (%)
Control	100
+ glutamate	66 $\pm$ 3
+ glutamate + sarsasapogenin (30 nM)	79 $\pm$ 3
Control	100
+ glutamate	65 $\pm$ 3
+ glutamate + episarsasapogenin cathylate (30 nM)	74 $\pm$ 3
Control	100
+ glutamate	68 $\pm$ 4
+ glutamate + episarsasapogenin (30 nM)	88 $\pm$ 3
Control	100
+ glutamate	71 $\pm$ 2
+ glutamate + epismilagenin (30 nM)	79 $\pm$ 2
Control	100
+ glutamate	68 $\pm$ 4
+ glutamate + smilagenin (30 nM)	91 $\pm$ 4
Control	100
+ glutamate	68 $\pm$ 4
+ glutamate + diosgenin (30 nM) negative control	72 $\pm$ 4

Sarsasapogenin, episarsasapogenin cathylate, episarsasapogenin, epismilagenin and smilagenin all displayed significant neuroprotective effects against glutamate-induced neurodegeneration in rat primary cortical neurones *in vitro*.

EXAMPLE 3

Reversal of neurodegeneration by sarsasapogenin, smilagenin, 16,22-epoxycoprostan-3 β -ol, smilagenone, smilagenin glycinate hydrochloride and coprosterol in neurones

As above mentioned, exposure of rat primary cortical cultures to glutamate (100 μ M; 10 min) caused an increase in lactate dehydrogenase (LDH) activity measured after 24 h, indicating a significant neurodegeneration. Treatment with 17 β -oestradiol after glutamate exposure produced a significant decrease in the LDH activity compared to neurones exposed to glutamate, suggesting a significant neuroprotective effect. Similarly, treatment with sarsasapogenin, smilagenin, 16,22-epoxycoprostan-3 β -ol, smilagenone, smilagenin glycinate hydrochloride and coprosterol produced a significant decrease in the LDH activity compared to neurones exposed to glutamate, suggesting a significant neuroprotective effect (Table 3).

Table 3 - Effect of different compounds on cortical neurones previously exposed to glutamate

Condition	Mean $\pm$ s.e.m (%)
Control	100 $\pm$ 4
Glutamate [100 μ M]	66 $\pm$ 2
Glutamate + 17 β -oestradiol [3 nM]	69 $\pm$ 2
Glutamate + 17 β -oestradiol [30 nM]	75 $\pm$ 5
Control	100 $\pm$ 1
Glutamate [100 μ M]	67 $\pm$ 3
Glutamate + Sarsasapogenin [3 nM]	101 $\pm$ 3
Glutamate + Sarsasapogenin [30 nM]	112 $\pm$ 1
Glutamate + Smilagenin [3 nM]	109 $\pm$ 6
Glutamate + Smilagenin [30 nM]	104 $\pm$ 1
Control	100 $\pm$ 8
Glutamate [100 μ M]	40 $\pm$ 1
Glutamate + Diosgenin [30 nM, negative control]	49 $\pm$ 6
Control	100 $\pm$ 5
Glutamate [100 μ M]	64 $\pm$ 4
Glutamate + 16,22-epoxycoprostan-3 β -ol [3 nM]	114 $\pm$ 7
Glutamate + 16,22-epoxycoprostan-3 β -ol [30 nM]	134 $\pm$ 5
Glutamate + Smilagenone [3 nM]	119 $\pm$ 7

Glutamate + Smilagenone [30 nM]	119 ± 4
Control	100 ± 4
Glutamate [100 µM]	58 ± 3
Glutamate + Smilagenin glycinate hydrochloride [3 nM]	117 ± 4
Glutamate + Smilagenin glycinate hydrochloride [30 nM]	141 ± 6
Glutamate + Coprosterol [3 nM]	126 ± 5
Glutamate + Coprosterol [30 nM]	116 ± 4

In conclusion, in rat primary cortical neurones sarsapogenin, smilagenin, 16,22-epoxycoprostan-3β-ol, smilagenone, smilagenin glycinate hydrochloride and coprosterol reversed the neurodegeneration induced by glutamate, suggesting a therapeutic potential in neurodegenerative disorders.

EXAMPLE 4

10 Anti-apoptotic effect of smilagenin in neurones

The objective of this study was to examine the anti-apoptotic effect of smilagenin on the caspase-3 activity, a marker of apoptosis, in rat primary cortical cultures exposed to glutamate

15

Primary cultures of cortical neurones

Rat cortical neurones were cultured for 6 days. At day 6, glutamate (100 µM, 10 min) was added. Then the cultures were washed and medium was replaced with fresh medium containing smilagenin or vehicle control (DMSO, 0.25%) for 6 h. After 6 h treatment, apoptosis was evaluated by measuring caspase 3 activity. Caspase 3 activity was detected by the cleavage of p-nitroaniline from a colorimetric caspase-3 substrate, acetyl-Asp-Glu-Val-Asp p-nitroanilide. p-Nitroalanine has a high absorbance at 405 nm. Relative caspase-3 activity was measured as optical density. In addition the relative activity of caspase-3 was standardised to protein concentration of the sample, which was also measured as an optical density (Du et al, J Neurochem., 69, 1382-1388, 1997; Sawada et al, FASEB J., 14, 1202-1214, 2000).

Smilagenin reverses the increase in glutamate induced caspase 3 activity in rat primary cortical neurones, demonstrating the anti-apoptotic effect of smilagenin (Table 4)

Table 4 - Effect of smilagenin on glutamate induced caspase 3 activity in cortical neurones

Conditions	Caspase activity (% of control)
Control	100
+ Glutamate	131
+ Glutamate + smilagenin (300 nM)	105

EXAMPLE 5

- 10 Neurodegenerative disorders are characterised by a progressive loss of neurones and degradation of neuronal processes (neurites). Agents that induce neurite outgrowth may promote the formation of new connections between neurones and ameliorate the symptoms of neurodegenerative conditions (Katzman et al, FASEB J., 5, 278-286, 1991).
- 15 Exposure to 17 β -oestradiol (0.3, 3, 30 pM) significantly increased the length of existing neurites in rat primary cortical neurones (Table 5). Exposure to 17 β -oestradiol (3, 30 pM) significantly increased the percentage of neurones displaying neurites in rat primary cortical neurones (Table 6). Exposure to smilagenin and sarsasapogenin (0.3, 3, 30 pM) significantly increased the length of existing neurites and the percentage of neurones
- 20 displaying neurites in rat primary cortical neurones (Tables 5 and 6).

In conclusion, smilagenin and sarsasapogenin have neurotrophic effects *in vitro*.

Table 5 - Effect of 17 β -oestradiol, smilagenin and sarsasapogenin on neurite length measured using optical micrometry

Control	100 $\pm$ 4
17 β -oestradiol (0.3 pM)	154 $\pm$ 5
17 β -oestradiol (3 pM)	163 $\pm$ 4
17 β -oestradiol (30 pM)	183 $\pm$ 5
Smilagenin (0.3 pM)	159 $\pm$ 5
Smilagenin (3 pM)	190 $\pm$ 8
Smilagenin (30 pM)	204 $\pm$ 6

Sarsasapogenin (0.3 pM)	177 ± 5
Sarsasapogenin (3 pM)	197 ± 5
Sarsasapogenin (30 pM)	211 ± 6

Table 6 - Effect of 17 β -oestradiol, smilagenin and sarsasapogenin on the number of neurones displaying neurites

5

Control	47 ± 2
17 β -oestradiol (0.3 pM)	48 ± 2
17 β -oestradiol (3 pM)	60 ± 2
17 β -oestradiol (30 pM)	59 ± 2
Smilagenin (0.3 pM)	63 ± 2
Smilagenin (3 pM)	63 ± 2
Smilagenin (30 pM)	66 ± 2
Sarsasapogenin (0.3 pM)	55 ± 2
Sarsasapogenin (3 pM)	61 ± 1
Sarsasapogenin (30 pM)	62 ± 2

EXAMPLE 6

10 Smilagenin and sarsasapogenin prevent the neurodegeneration caused by exposure to the neurotoxin, 1-methyl-4-phenylpyridinium (MPP⁺) in rat mesencephalic dopaminergic neurones; a model of Parkinson's disease *in vitro*.

15 Damage caused by the neurotoxin, MPP⁺, a metabolite of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) mimics the degeneration of nigrostriatal dopaminergic neurones observed in neurodegenerative disorders such as Parkinson's disease (Mytilineou et al, Science, 225, 529-531, 1984). The most prominent biochemical changes induced by this toxin include decreased levels of dopamine and its metabolites in the substantia nigra pars compacta and in the caudate nucleus (Burns et al, Proc Natl Acad Sci U.S.A., 80, 4546-4550, 1983) and a reduction in dopamine uptake in nigrostriatal synaptosomal preparations (Heikkila et al, J Neurochem., 44, 310-313, 1985).

20 Pre-treatment of dopaminergic neurones with smilagenin and sarsasapogenin significantly reduced the neuronal death following exposure of the dopaminergic specific neurotoxin MPP⁺ (2 μ M) when compared with MPP⁺ alone. Glial cell line-derived neurotrophic factor

(GDNF) and brain-derived neurotrophic factor (BDNF), molecules that are involved in neuronal growth, were used as positive controls. Pre-treatment with smilagenin and sarsasapogenin produced a significant increase in the neuronal survival compared to neurones exposed to MPP⁺ alone, suggesting a significant neuroprotective effect (Table 7).

5

Table 7 - Effect of pre-treatment with BDNF and GDNF, smilagenin and sarsasapogenin on dopaminergic neurones after MPP⁺ (2 μ M) exposure

Control	100 $\pm$ 3
+ MPP ⁺ (2 μ M)	55 $\pm$ 3
+ MPP ⁺ (2 μ M) + BDNF (1.85 nM) & GDNF (0.17 nM)	122 $\pm$ 7
+ MPP ⁺ (2 μ M) + smilagenin (30 nM)	98 $\pm$ 4
+ MPP ⁺ (2 μ M) + sarsasapogenin (30 nM)	89 $\pm$ 3

10

In this model of Parkinson's disease *in vitro*, pre-treatment with smilagenin and sarsasapogenin significantly prevented the neuronal degeneration following exposure to the dopaminergic specific neurotoxin, MPP⁺, demonstrating a neuroprotective effect.

15 EXAMPLE 7

Smilagenin and sarsasapogenin also reverse the neurodegeneration caused by exposure to the neurotoxin 1-methyl-4-phenylpyridinium (MPP⁺) in rat mesencephalic dopaminergic neurones; a model of Parkinson's disease *in vitro*.

20

Treatment of dopaminergic neurones with smilagenin and sarsasapogenin significantly reduced the neuronal death following exposure to the dopaminergic specific neurotoxin MPP⁺ (2 μ M) when compared with MPP⁺ alone. Glial cell line-derived neurotrophic factor (GDNF) and brain-derived neurotrophic factor (BDNF), molecules that are involved in neuronal growth, and 17 β -oestradiol were used as positive controls. Treatment with smilagenin and sarsasapogenin produced a significant increase in the neuronal survival compared to neurones exposed to MPP⁺ alone (Table 8).

25

Table 8: Effect of treatment with BDNF and GDNF, smilagenin, sarsasapogenin and 17 β -oestradiol on dopaminergic neurones after MPP⁺ (2 μ M) exposure

Control	100 $\pm$ 6
+ MPP ⁺ (2 μ M)	76 $\pm$ 4
+ MPP ⁺ (2 μ M) + BDNF (1.85 nM) & GDNF (0.17 nM)	98 $\pm$ 5
+ MPP ⁺ (2 μ M) + smilagenin (0.03 nM)	111 $\pm$ 6
+ MPP ⁺ (2 μ M) + sarsasapogenin (0.03 nM)	112 $\pm$ 6
+ MPP ⁺ (2 μ M) + 17 β -oestradiol (0.03 nM)	106 $\pm$ 5

5 Exposure to MPP⁺ caused not only a significant decrease in dopaminergic number but also in the percentage of neurites. This study shows that smilagenin and sarsasapogenin significantly increased the number of neurites of the neurones *in vitro* Table 9. These results demonstrate that the compounds reverse motor neurodegeneration.

10 **Table 9 - Effect of smilagenin and sarsasapogenin on percentage of neurites in dopaminergic neurones after MPP⁺ (2 μ M) exposure**

Control	41 $\pm$ 4
+ MPP ⁺ (2 μ M)	27 $\pm$ 5
+ MPP ⁺ (2 μ M) + smilagenin (0.03 nM)	41 $\pm$ 4
+ MPP ⁺ (2 μ M) + sarsasapogenin (0.03 nM)	43 $\pm$ 4

15 **EXAMPLE 8**

Neuroprotective effect of sarsasapogenin and smilagenin in spinal motor neurones

20 The objective of this study was to examine the effects of sarsasapogenin and smilagenin on the survival of rat primary spinal motor neurones exposed to glutamate, which is known to induce neurodegeneration in this model of motor neurodegeneration. 17 β -oestradiol and BDNF were used as positive controls.

25 **Primary cultures of spinal motor neurones**

Rat motor neurones were prepared according to the method described by (Martinou et al, Neuron, 8, 737-744, 1992). On day 10, medium was removed and the cultures were

exposed to glutamate (4 microM) for 10 min at 37°C in defined medium. After the glutamate exposure, cultures were washed with Dulbecco modified Eagle medium at 37°C then placed in fresh culture medium containing test compounds. After 48 h, the extent of spinal motor neurone degeneration was determined by measuring the amount of lactate dehydrogenase (LDH) released into the culture medium as above.

Results

Following exposure of glutamate, there was a significant degeneration of rat primary spinal motor neurones, 48 h post-treatment, demonstrated by an increase in lactate dehydrogenase release into the culture medium.

In primary rat primary spinal motor neurones treated with sarsasapogenin or smilagenin for 48 h, there was a significant reduction in the glutamate-induced neurodegeneration (Table 10).

Table 10 - Effect of sarsasapogenin and smilagenin on glutamate-induced neurodegeneration in spinal motor neurones

Control + DMSO [0.25 %]	100 ± 1
Glutamate [4 microM] + DMSO [0.25 %]	94 ± 1
Glutamate + BDNF [3 nM]	148 ± 8
Glutamate + 17β-oestradiol [0.03 nM]	102 ± 2
Glutamate + 17β-oestradiol [3 nM]	110 ± 1
Glutamate + 17β-oestradiol [300 nM]	116 ± 6
Glutamate + sarsasapogenin [0.03 nM]	123 ± 2
Glutamate + sarsasapogenin [3 nM]	137 ± 1
Glutamate + sarsasapogenin [300 nM]	136 ± 6
Glutamate + smilagenin [0.03 nM]	128 ± 4
Glutamate + smilagenin [3 nM]	154 ± 1
Glutamate + smilagenin [300 nM]	144 ± 4

Sarsasapogenin and smilagenin reversed the glutamate-induced neurodegeneration in rat spinal motor neurones in this *in vitro* model of motor neurodegeneration

EXAMPLE 9

In the second half of life (in humans from the age of 40 onwards) the density of neurones in the brain decreases (Selkoe, D J, Sci. Am. 267, 134-142, 1992). The alterations in cortical function may be due to a reduction in the number of neurones, their interconnections, a decrease in neurotrophins such as brain derived neurotrophic factor (BDNF; Bothwell, M, Functional interactions of neurotrophins and neurotrophin receptors, Annu. Rev. Neurosci., 18, 223-253, 1995), a decrease in acetylcholine receptor density (muscarinic and nicotinic) and/or a decrease in their coupling function in cortical areas (Rinne et al, Brain Res., 336, 19-25, 1985; Selkoe, D J, Sci. Am. 267, 134-142, 1992). Furthermore during ageing, muscarinic acetylcholine receptor binding is significantly reduced in the hippocampus (Narang, N, Mech. Ageing Dev., 78, 221-239, 1995) and striatum of older rats (Biegon et al, Neurobiol. Aging., 10, 305-310, 1989) and humans (Rinne et al, Brain Res., 336, 19-25, 1985). In addition, in Alzheimer's disease, the decline in cholinergic activity is associated with amyloid β plaque deposition (von der Kammer et al, Biochem. Soc. Symp. 131-140, 2001). Other neurodegenerative disorders, such as Parkinson's disease, show a characteristic decline in dopaminergic activity (Drukarch et al, Expert. Opin. Investig. Drugs, 10, 1855-1868, 2001).

Oral administration of sarsasapogenin, episarsasapogenin cathylate or amilagenin to aged rats (Sprague-Dawley rats 20 month old), for a two or three month period reverses the impairment in learning and memory ability, the decline in muscarinic acetylcholine and dopamine receptors and the decline in the neurotrophin BDNF, alterations that are characteristic of the ageing process.

Aged Sprague-Dawley rats aged were divided into different groups, one control and groups treated for 2-3 months with either sarsasapogenin, episarsasapogenin cathylate or amilagenin ($18 \text{ mg kg}^{-1} \text{ day}^{-1}$, $n = 10$). A control group ($n=14$) of untreated young rats was also included in the study. The daily dose of drug was mixed in a minimum amount of food and was administered every morning separately to each rat.

A Y-maze apparatus was used for the learning and memory test. On the floor of each arm of the Y-maze is an array of copper rods to which electric current is applied whenever needed, with adjustable voltage. Each arm is 45 cm long and has a 15 W lamp at the end, which is turned on when needed. After 3 months drug administration, each rat was trained for 7 consecutive days, as follows. For each training session, the rat was put into one arm

of the Y-maze, after two minutes rest, an electrical current was applied to the copper rods and the lamp of the clockwise arm was illuminated to indicate the non-stimulation area. If the rat went into that arm, one correct response was recorded, otherwise, one wrong response was recorded. This stimulation-response test was repeated 20 times each day, with a pause of 5 sec between each two consecutive tests. The number of correct responses following the twenty tests on the seventh day was used to express learning ability, (the higher the number the better the learning ability). The rats were then left resting for 30 days and the procedure was repeated once more. The number of correct responses of the 20 tests after the 30 day rest period was used to represent the memory ability

10

Muscarinic acetylcholine receptor density in the brain was measured. Tissue was prepared as follows: brains were removed quickly after decapitation, frozen in dry ice, and transferred to a freezer. The brains were homogenised and the pellet was finally suspended in buffer.

15

The dual-site competitive ligand binding assay was used to measure muscarinic acetylcholine receptor density.

The results are shown in Figures 3 and 4 of the drawings. The Y-maze experiments revealed that both the learning ability and memory are impaired in aged rats. Sarasapogenin, episarasapogenin cathylate and smilagenin restored the learning and memory ability following administration in aged rats. Muscarinic acetylcholine receptor density was markedly reduced in aged rats. Sarasapogenin, episarasapogenin cathylate and smilagenin significantly restored the muscarinic acetylcholine receptor density.

25

Young rats showed a significantly higher dopamine (D) 1 and 2 receptor density (157.5 ± 33.2 ; 200.6 ± 50.9 fmol/mg protein, respectively) compared to aged rats (129.2 ± 36.8 ; 153.8 ± 40.5 fmol/mg protein, D₁ and D₂, respectively). By contrast, smilagenin and sarasapogenin treatment in aged rats for 3 months restored the D₁ and D₂ receptor density (smilagenin 177 ± 10.9 ; 217 ± 45.7 fmol/mg protein; sarasapogenin 172.0 ± 44.0 ; 206.4 ± 60.5 respectively).

30

Young rats showed significantly higher BDNF levels (1.647 ± 0.277 ng/g tissue) compared to aged rats (1.205 ± 0.219 ng/g tissue). By contrast, smilagenin and sarasapogenin treatment in aged rats for 3 months, partially restored BDNF levels (1.342 ± 0.07 ; 1.410 ± 0.232 ng/g tissue, respectively).

35

Thus, the compounds reverse the neuroimpairment, the decline in BDNF levels, and the decline in the muscarinic acetylcholine and dopamine receptor density that occur in aged rats.

5

EXAMPLE 10

10

Alzheimer's disease model as a model of neurodegeneration

An *in vivo* model of Alzheimer's disease was used to model neurodegeneration. In this model the neurotoxic agents (amyloid β and ibotenic acid) are injected into the brain of the rat. This leads to neuronal loss, receptor loss and cognitive impairment. Previous studies showed that local injection of amyloid β in the nucleus basalis of the rat brain caused cholinergic hypofunction and behavioural impairment up to two months post surgery (Giovannelli et al., 1995: Neuroscience, 56, 781-792.). In addition the co-injection of amyloid β with a small amount of ibotenic acid into the rat hippocampus synergistically produces neuronal loss with infiltration of glial cells not only adjacent but also far from the injected site (Morimoto et al., 1998: Neuroscience, 84, 479-487).

Our studies used the method of Morimoto (Morimoto et al., 1998: Neuroscience, 84, 479-487) with some modifications (unilateral instead of bilateral injection). Three months old, Sprague Dawley rats, were randomly divided into different groups. Injection of amyloid β , and ibotenic acid (both from Sigma) was accomplished by means of a stereotaxic instrument (Stoelting Co.) and the coordinates were AP= -0.5mm (right to medial line), L= -2.8mm (backward from bregma), H= -7.0mm (ventral to dura). The dose for each rat was amyloid β (4 μ g) and ibotenic acid (1 μ g) in 1 μ l of saline. The injection was completed in 20 min, and the needle was withdrawn 10 min later. Then the skin was sutured.

The 8 groups were:

- Operated control injected with normal saline (control)
- Model (control injected with amyloid β + ibotenic acid)
- Model + Epistasapogenin cathylate (18 mg/kg/day)*
- Model + Sarsapogenin cathylate (18 mg/kg/day)*
- Model + Epistasapogenin ethylsuccinate (18 mg/kg/day) (comparison)

35

Model + Episaraspogenin (18 mg/kg/day)*

Model + Epismilagenin (18 mg/kg/day)*

Model + Diosgenin (i.e. negative control, 18 mg/kg/day)

5 * Compounds in accordance with the present invention

10 Episaraspogenin cathylate, sarasaspogenin cathylate, episaraspogenin ethylsuccinate (comparison compound), episaraspogenin, epismilagenin and diosgenin (all at a dosage of 18 mg/kg/day) were administered to animals as stable suspensions in CMC-Na (0.5%) once daily through a gastric tube. The control and the model group were given the same volume of CMC-Na (0.5%) once daily. The drugs and vehicles were given for a period of two months, starting 20 days before operation.

15 Muscarinic acetylcholine receptor density was assessed. The brain samples were homogenised, centrifuged, and the pellet of centrifugation at 27000×g was re-homogenised and used for measurement. The concentration of ³H-QNB was chosen at the saturation range. After incubation and separation, the bound portion was measured by liquid scintillation counter.

20 Step-Through Test: learning and memory. The effect of test compounds on learning and memory was assessed using the step-through test. A 60 × 15 × 15 cm box, divided into 2 equally sized rooms, one dark room with copper rod base, which was electrically charged (40 V ac) when in use, while the other was a light room but not electrically charged. Between the two rooms there is an opening (hole) for the rat to go through. The experiment is carried out for each rat on two consecutive days. The first day is for training, when the rat is adapted in the box for the first 3 min, then put in the light room, with its back toward the hole, and the copper rods of the dark room are charged for 5 min. The second day is for testing, when the number of crosses in 5 min are recorded. Improvements in memory are signalled by a reduction in the number of crosses.

30 The muscarinic acetylcholine receptor density in the neurodegeneration model brains was significantly lower than control. Episaraspogenin cathylate, sarasaspogenin cathylate, episaraspogenin and epismilagenin produced a significant elevation in brain muscarinic acetylcholine receptor density, whereas diosgenin and episaraspogenin ethylsuccinate did not significantly change the muscarinic acetylcholine receptor density. Thus the experiments indicate that the compounds of this invention act to normalise receptor number,

i.e. they tend to restore receptor number to normal levels when given to animals in which the receptor level is depressed.

The number of wrong responses (error number) in 5 min was significantly higher in the neurodegeneration model group than the control group, indicating an impairment of memory (see Table 11). Epismilagenin, episarsapogenin cathylate, episarsapogenin and sarsapogenin cathylate each significantly decreased the number of wrong responses, whereas diosgenin and episarsapogenin ethylsuccinate were both ineffective in decreasing the number of wrong responses.

Table 11

Group	M receptor density	Learning and memory Step through test
	(fmol/mg/protein)	Error No
Control (n=10)	859±101	0.60±0.70
Model (n=10)	713±48	4.00±2.40
+ Episarsapogenin cathylate (n=10)	877±89 *	1.36±0.92*
+ Sarsapogenin cathylate (n=11)	916±158 *	1.36±1.03 *
+ Episarsapogenin ethylsuccinate (n=11)	774±79	3.73±1.35
+ Episarsapogenin (n=10)	869±104*	1.50±1.18*
+ Epismilagenin (n=11)	877±90*	1.73±0.91*
+ Diosgenin (negative control n=8)	770±68	3.75±1.49

Statistical analysis using unpaired Student t test. * denotes p<0.05

EXAMPLE 11

Amyotrophic lateral sclerosis (ALS) is a progressive fatal neurodegenerative disorder that causes motor neurone degeneration, skeletal atrophy, paralysis and death. The cause of this disease is heterogeneous: mutations in the Cu/Zn superoxide dismutase (SOD-1) gene is responsible for some forms of human ALS. Animal models of this disease include the SOD-1 transgenic mice over-expressing SOD-1 gene and the progressive motor neuropathy (pnm, a model of Charcot-Marie-Tooth) mice. Smilagenin and sarsapogenin increase the lifespan and improve the behavioural deficits of the superoxide dismutase (SOD) mouse

(Figure 5) and psm mouse (Figure 6), two models of amyotrophic lateral sclerosis (ALS) and Charcot-Marie-Tooth disease.

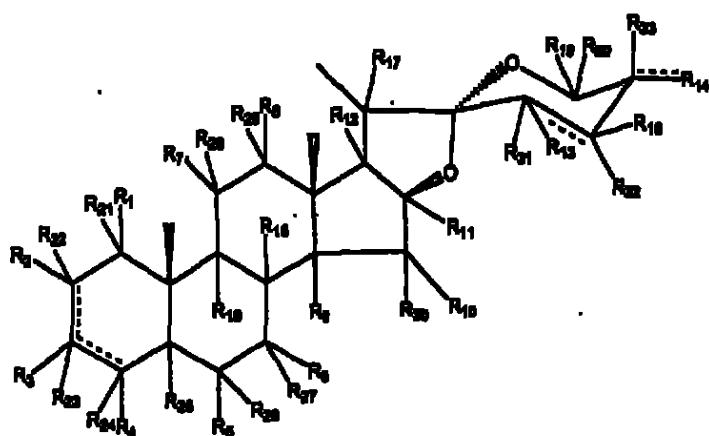
The foregoing broadly describes the present invention without limitation. Variations and
5 modifications as will be readily apparent to those of ordinary skill in this art are intended to be within the scope of this application and any subsequent patent(s).

CLAIMS

1. Use of one or more active agent selected from:

5

A. compounds of Formula I:



10

(I)

wherein in the general formula (I):

- R₁, R₂, R₃, R₄, R₅, R₆, R₇, R₈, R₉, R₁₀, R₁₁, R₁₂, R₁₃, R₁₄, R₁₅, R₁₆, R₁₇, R₁₈, R₁₉, R₂₀, R₂₁, R₂₂, R₂₃, R₂₄, R₂₅, R₂₆, R₂₇, R₂₈, R₂₉, R₃₀, R₃₁, R₃₂ are, independently of each other, either H, OH, =O, halo atom, (Me-S-), (Me-

15 SO-), (Me-SO₂-), N₃-, NH₂-, MeSO₂NH-, alkyl or absent or OR where R = alkyl or acyl group;

- R₉, R₁₁, R₁₂, R₁₄, R₁₅, R₁₆, R₁₇, R₂₅, R₂₉ can be either a H, OH, halo atom, (Me-S-), (Me-SO-), (Me-SO₂-), N₃-, NH₂-, MeSO₂NH-, alkyl or absent or OR where R = alkyl or acyl group;

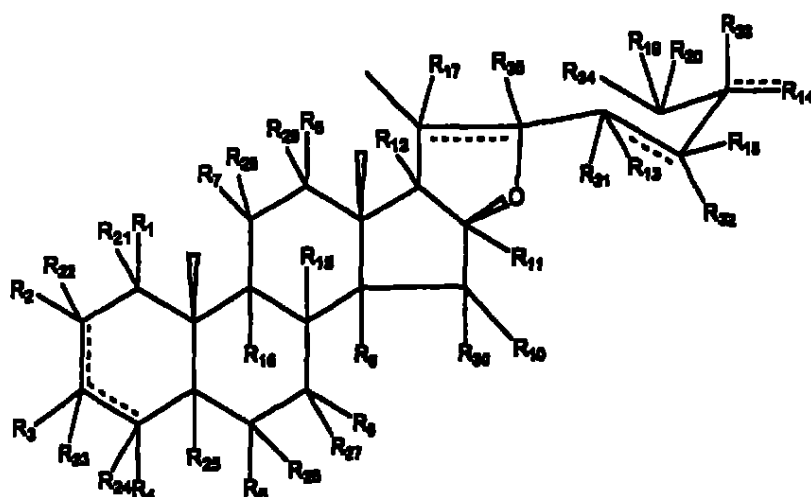
20 --- represents an optional double bond,

wherein in addition to the above

- either R₃₃ or R₁₄ = alkyl group;

B. compounds of Formula II:

25



(II)

wherein in the general formula (II):

- R₁, R₂, R₃, R₄, R₅, R₆, R₇, R₈, R₉, R₁₀, R₁₁, R₁₂, R₁₃, R₁₄, R₁₅, R₁₆, R₁₇, R₁₈, R₁₉, R₂₀, R₂₁, R₂₂, R₂₃, R₂₄, R₂₅, R₂₆, R₂₇, R₂₈, R₂₉, R₃₀, R₃₁, R₃₂, R₃₃, R₃₄ are, independently of each other, either H, OH, =O, halo atom, (Me-S-), (Me-SO-), (Me-SO₂-), N₃-, NH₂-, MeSO₂NH-, alkyl, OR where R = alkyl or acyl group, or absent;

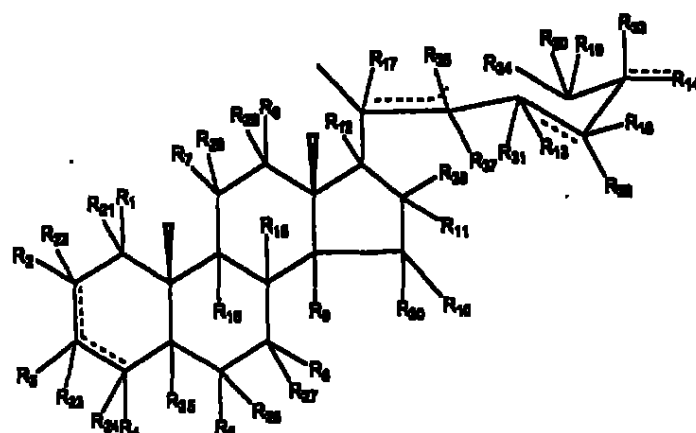
- R₉, R₁₁, R₁₂, R₁₄, R₁₅, R₁₆, R₁₇, R₂₀, R₂₁, R₂₃ can be either a H, OH, halo atom, (Me-S-), (Me-SO-), (Me-SO₂-), N₃-, NH₂-, MeSO₂NH-, alkyl, OR where R = alkyl or acyl group, or absent;

..... represents an optional double bond.

wherein in addition to the above

- either R₃₃ or R₁₄ = alkyl group;

15 C. compounds of Formula III:



(III)

wherein in the general formula (III):

- $R_1, R_2, R_3, R_4, R_5, R_6, R_7, R_8, R_{10}, R_{11}, R_{14}, R_{16}, R_{18}, R_{20}, R_{21}, R_{22}, R_{23}, R_{24}, R_{26}, R_{27}, R_{28}, R_{29}, R_{30}, R_{31}, R_{32}, R_{33}, R_{34}, R_{35}, R_{36}, R_{37}$ are, independently of each other, either H, OH, =O, halo atom, (Me-S-), (Me-SO-), (Me-SO₂-), N₃-, NH₂-, MeSO₂NH-, alkyl, OR where R = alkyl or acyl group, or absent;
- $R_9, R_{11}, R_{12}, R_{13}, R_{15}, R_{17}, R_{25}$ can be either H, OH, halo atom, (Me-S-), (Me-SO-), (Me-SO₂), N₃-, NH₂-, MeSO₂NH-, alkyl, OR where R = alkyl or acyl group, or absent;
- represents an optional double bond,

10 wherein in addition to the above

- either R_{33} or R_{34} = alkyl group, and
- the stereochemistry of R_{43} is in the β orientation;

D. sapogenin derivatives bearing at least one X radical substituent,

15 wherein X is chosen from the group consisting of:

- halo atom,
- (Me-S-), (Me-SO-), (Me-SO₂-),
- N₃-, NH₂-, MeSO₂NH-, and
- alkyl; and

20

E. derivative forms of any of the above compounds, in which the carbon atom at the 3-position or, in the case of Formulae II and III, the 3-position carbon atom, the 26-position or each of the carbon atoms at the 3- and 26-positions, carries an O-sugar moiety wherein the sugar group is a mono-, di- or tri-saccharide;

25

all their stereoisomers and racemic mixtures, all their pharmaceutically acceptable pro-drugs and salts, and all mixtures and combinations thereof

30 in the treatment or prevention of, or in the preparation of compositions for the treatment or prevention of, (i) non-cognitive neurodegeneration, (ii) non-cognitive neuromuscular degeneration, (iii) motor-sensory neurodegeneration, or (iv) receptor dysfunction or loss in the absence of cognitive, neural and neuromuscular impairment, in human and non-human animals suffering therefrom or susceptible thereto.

35 2. A use according to claim 1, wherein the active agent, or at least one of the active agents, is selected from:

a. Compounds of the above general formula I, wherein:

- $R_1, R_2, R_3, R_4, R_5, R_6, R_7, R_8, R_{10}, R_{11}, R_{12}, R_{13}, R_{22}, R_{21}, R_{22}, R_{23}, R_{24}, R_{25}, R_{27}, R_{28}, R_{29}, R_{30}, R_{31}, R_{32}$ are, independently of each other, either H, OH, =O, halo atom, (Me-S-), (Me-SO-), (Me-SO₂-), N₃-, NH₂-, MeSO₂NH-, alkyl or absent or OR where R = alkyl or acyl group;

- $R_9, R_{11}, R_{12}, R_{14}, R_{15}, R_{16}, R_{17}, R_{23}, R_{33}$ can be either a H, OH, halo atom, (Me-S-), (Me-SO-), (Me-SO₂-), N₃-, NH₂-, MeSO₂NH-, alkyl or absent or OR where R = alkyl or acyl group;
 represents an optional double bond,

10 wherein in addition to the above

- either R_{33} or R_{14} = alkyl group,

and the stereochemistry of R_{25} is in the β orientation;

b. Compounds of the above general formula I, wherein:

15 - $R_1, R_2, R_3, R_4, R_5, R_6, R_7, R_8, R_{10}, R_{11}, R_{12}, R_{22}, R_{21}, R_{22}, R_{23}, R_{24}, R_{25}, R_{27}, R_{28}, R_{29}, R_{30}, R_{31}, R_{32}$ are, independently of each other, either H, OH, =O, halo atom, (Me-S-), (Me-SO-), (Me-SO₂-), N₃-, NH₂-, MeSO₂NH-, alkyl or absent or OR where R = alkyl or acyl group;

- $R_9, R_{12}, R_{15}, R_{16}, R_{17}$ = H,

20 - $R_{11}, R_{14}, R_{23}, R_{33}$ can be either a H, OH, halo atom, (Me-S-), (Me-SO-), (Me-SO₂-), N₃-, NH₂-, MeSO₂NH-, alkyl or absent or OR where R = alkyl or acyl group;

represents an optional double bond

wherein in addition to the above

- either R_{33} or R_{14} = alkyl group,

25 and the stereochemistry of R_{25} is in the β orientation;

c. Compounds of the above general formula I, wherein:

- $R_1 = R_2 = R_3 = R_4 = R_5 = R_6 = R_7 = R_8 = R_{10} = R_{11} = R_{12} = R_{13} = R_{15} = R_{16} = R_{17} = R_{18} = R_{19} = R_{20} = R_{21} = R_{22} = R_{23} = R_{24} = R_{25} = R_{26} = R_{27} = R_{28} = R_{29} = R_{30} = R_{31} = R_{32} = H$,

30 - either R_{33} or R_{14} = CH₃

represents a single bond,

- the methyl group at C25 may be either in the R or S configuration

- the stereochemistry of R_{25} is in the β orientation and

wherein in addition to the above

35 at least one of R_3 or R_{23} is a X radical, the possible remaining substituent being H, OH, =O, and OR where R = alkyl or acyl group or absent,

and X is chosen from the group consisting of:

- halo atom,
- 5 - (Me-S-), (Me-SO-), (Me-SO₂-), and
- N₃-, NH₂-, MeSO₂NH-

5 - alkyl;

d. Compounds of the above general formula I, wherein:

-R₁=R₂=R₃=R₄=R₅=R₆=R₇=R₈=R₉=R₁₀=R₁₁=R₁₂=R₁₃=R₁₄=R₁₅=R₁₆=R₁₇=R₁₈=R₁₉=R₂₀=
R₂₁=R₂₂=R₂₃=R₂₄=R₂₅=R₂₆=R₂₇=R₂₈=R₂₉=R₃₀=R₃₁=R₃₂=H,

10 -R₃₁=R₃₂=CH₃,

... represents a single bond,

- the stereochemistry of R₄₅ is in the β orientation and

wherein in addition to the above

at least one of R₃ or R₄₅ is a X radical, the possible remaining substituent being H, OH, =O,

15 and OR where R = alkyl or acyl group or absent,

and X is chosen from the group consisting of:

- halo atom,
- (Me-S-), (Me-SO-), (Me-SO₂-), and
- N₃-, NH₂-, MeSO₂NH-

20 - alkyl;

e. Compounds of the above general formula II, wherein

- R₁, R₂, R₃, R₄, R₅, R₆, R₇, R₈, R₉, R₁₀, R₁₁, R₁₂, R₁₃, R₁₄, R₁₅, R₁₆, R₁₇, R₁₈, R₁₉, R₂₀, R₂₁, R₂₂, R₂₃, R₂₄, R₂₅, R₂₆, R₂₇, R₂₈, R₂₉, R₃₀, R₃₁, R₃₂, R₃₃ are, independently of each other, either H, OH, =O, halo atom, (Me-S-),
25 (Me-SO-), (Me-SO₂-), N₃-, NH₂-, MeSO₂NH-, alkyl, OR where R = alkyl or acyl group, or absent;

- R₉, R₁₁, R₁₂, R₁₄, R₁₅, R₁₆, R₁₇, R₂₃, R₃₁, R₃₃ can be either a H, OH, halo atom, (Me-S-), (Me-SO-), (Me-SO₂-), N₃-, NH₂-, MeSO₂NH-, alkyl, OR where R = alkyl or acyl group, or absent;

30 ... represents an optional double bond,

wherein in addition to the above

- either R₃₃ or R₁₄ = alkyl group,

and the stereochemistry of R₄₅ is in the β orientation;

35 f. Compounds of the above general formula II or carbohydrate derivatives thereof, wherein:

- $R_1, R_2, R_3, R_4, R_5, R_6, R_7, R_8, R_{10}, R_{11}, R_{14}, R_{15}, R_{22}, R_{21}, R_{22}, R_{23}, R_{24}, R_{25}, R_{27}, R_{28}, R_{29}, R_{30}, R_{31}, R_{32}$ are, independently of each other, either H, OH, =O, halo atom, (Me-S-), (Me-SO-), (Me-SO₂-), N₃-, NH₂-, MeSO₂NH-, alkyl, OR where R = alkyl or acyl group, or absent;
- 5 - $R_9, R_{12}, R_{13}, R_{16}, R_{17} = H$,
 - R_{34} = either H, OH, =O, and OR where R = alkyl, acyl or carbohydrate and
 - $R_{11}, R_{14}, R_{25}, R_{33}, R_{35}$ can be either H, OH, halo atom, (Me-S-), (Me-SO-), (Me-SO₂-), N₃-, NH₂-, MeSO₂NH-, alkyl, OR where R = alkyl or acyl group, or absent;
 $\Delta_{11,12}$ represents an optional double bond,
- 10 wherein in addition to the above
 - either R_{33} or R_{34} = alkyl group,
 and the stereochemistry of R_{25} is in the β orientation;
- g. Compounds of the above general formula II or carbohydrate derivatives thereof,
- 15 wherein:
 $-R_1 = R_2 = R_3 = R_4 = R_5 = R_6 = R_7 = R_8 = R_{10} = R_{11} = R_9 = R_{12} = R_{13} = R_{14} = R_{15} = R_{16} = R_{17} = R_{18} = R_{19} = R_{20} =$
 $R_{21} = R_{22} = R_{23} = R_{24} = R_{25} = R_{26} = R_{27} = R_{28} = R_{29} = R_{30} = R_{31} = R_{32} = H$,
 $-R_{14} = CH_3$,
 $-R_{34} = -OH$ or $-OR$ where R = alkyl, acyl or carbohydrate and
 $R_{33} = H$ or is absent
 $\Delta_{11,12}$ represents an optional double bond, and
 - the methyl group at C25 may be either in the R or S configuration and
 and the stereochemistry of R_{25} is in the β orientation
 wherein in addition to the above
- 25 at least one of R_1 or R_{25} is a X radical, the possible remaining substituent being H, OH, =O,
 and OR where R = alkyl or acyl group or absent,
 and X is chosen from the group consisting of :
 - halo atom,
 - (Me-S-), (Me-SO-), (Me-SO₂-), and
- 30 - N₃-, NH₂-, MeSO₂NH-
 - alkyl;

- h. Compounds of the above general formula II or carbohydrate derivatives thereof,
 wherein:
- 35 $-R_1 = R_2 = R_3 = R_4 = R_5 = R_6 = R_7 = R_8 = R_{10} = R_{11} = R_9 = R_{12} = R_{13} = R_{14} = R_{15} = R_{16} = R_{17} = R_{18} = R_{19} = R_{20} =$
 $R_{21} = R_{22} = R_{23} = R_{24} = R_{25} = R_{26} = R_{27} = R_{28} = R_{29} = R_{30} = R_{31} = R_{32} = H$,

- R₁₄ = R₃₃ = CH₃,
 - R₃₄ = -OH or -OR where R = alkyl, acyl or carbohydrate and
 R₃₅ = H or is absent
 ... represents an optional double bond, and
- 5 the stereochemistry of R₂₅ is in the β orientation and
 wherein in addition to the above
 at least one of R₃ or R₂₁ is a X radical, the possible remaining substituent being H, OH, =O,
 and OR where R = alkyl or acyl group or absent,
 and X is chosen from the group consisting of :
- 10 - halo atom,
 - (Me-S-), (Me-SO-), (Me-SO₂-), and
 - N₃-, NH₂-, MeSO₂NH-
 - alkyl;
- 15 i. Compounds of the above general formula III, wherein:
 - R₁, R₂, R₃, R₄, R₅, R₆, R₇, R₈, R₁₀, R₁₂, R₁₄, R₁₆, R₁₈, R₂₀, R₂₁, R₂₂, R₂₃, R₂₄, R₂₆, R₂₇, R₂₈,
 R₂₉, R₃₀, R₃₁, R₃₂, R₃₃, R₃₄, R₃₅, R₃₆, R₃₇ are, independently of each other, either H, OH, =O,
 halo atom, (Me-S-), (Me-SO-), (Me-SO₂-), N₃-, NH₂-, MeSO₂NH-, alkyl, OR where R =
 alkyl or acyl group, or absent;
- 20 -R₉, R₁₁, R₁₃, R₁₅, R₁₇, R₂₅ can be either H, OH, halo atom, (Me-S-), (Me-SO-), (Me-
 SO₂), N₃-, NH₂-, MeSO₂NH-, alkyl, OR where R = alkyl or acyl group, or absent;
 ... represents an optional double bond,
 wherein in addition to the above
 - either R₃₃ or R₁₄ = alkyl group, and
- 25 the stereochemistry of R₂₅ is in the β orientation;
- j. Compounds of the above general formula III or carbohydrate derivatives thereof,
 wherein:
- 30 - R₁, R₂, R₃, R₄, R₅, R₆, R₇, R₈, R₁₀, R₁₂, R₁₄, R₁₆, R₁₈, R₂₀, R₂₁, R₂₂, R₂₃, R₂₄, R₂₆, R₂₇, R₂₈,
 R₂₉, R₃₀, R₃₁, R₃₂, R₃₃, R₃₄, R₃₅, R₃₆, R₃₇ are, independently of each other, either H, OH, =O, halo
 atom, (Me-S-), (Me-SO-), (Me-SO₂-), N₃-, NH₂-, MeSO₂NH-, alkyl, OR where R = alkyl or
 acyl group, or absent;
 - R₉, R₁₂, R₁₅, R₁₆, R₁₇ = H,
 - R₃₄ = H, OH, =O, halo atom, (Me-S-), (Me-SO-), (Me-SO₂-), N₃-, NH₂-, MeSO₂NH-,
 35 alkyl, OR where R = alkyl, acyl or carbohydrate, or absent;

- R_{11} , R_{25} , can be either H, OH, halo atom, (Me-S-), (Me-SO-), (Me-SO₂-), N₃-, NH₂-, MeSO₂NH-, alkyl, OR where R = alkyl or acyl group, or absent;

..... represents an optional double bond,

wherein in addition to the above

- 5 - either R_{21} or R_{14} = alkyl group, and
the stereochemistry of R_{25} is in the β orientation;

k. Compounds of the above general formula III, wherein:

- $R_1=R_2=R_3=R_4=R_5=R_6=R_7=R_8=R_{10}=R_{11}=R_9=R_{12}=R_{13}=R_{15}=R_{16}=R_{17}=R_{18}=R_{19}=R_{20}=$

- 10 $R_{21}=R_{22}=R_{23}=R_{24}=R_{25}=R_{26}=R_{27}=R_{28}=R_{29}=R_{30}=R_{31}=R_{32}=R_{33}=H,$

- $R_{14}=CH_3,$

- $R_{34}=-OH$ or $-OR$ where R = alkyl, acyl or carbohydrate and

$R_{25}=H$ or is absent

$R_{37}=H, -OH$ or $=O$

- 15 $R_{38}=H$ or $-OH$

..... represents a single bond, and

- the methyl group at C25 may be either in the R or S configuration and

the stereochemistry of R_{25} is in the β orientation

wherein in addition to the above

- 20 at least one of R_3 or R_{21} is a X radical, the possible remaining substituent being H, OH, $=O$,

and OR where R = alkyl or acyl group or absent,

and X is chosen from the group consisting of :

- halo atom,

- (Me-S-), (Me-SO-), (Me-SO₂-), and

- 25 - N₃-, NH₂-, MeSO₂NH-

- alkyl;

l. Compounds of the above general formula III or carbohydrate derivatives thereof, wherein:

- 30 - $R_1=R_2=R_3=R_4=R_5=R_6=R_7=R_8=R_{10}=R_{11}=R_9=R_{12}=R_{13}=R_{15}=R_{16}=R_{17}=R_{18}=R_{19}=R_{20}=$

$R_{21}=R_{22}=R_{23}=R_{24}=R_{25}=R_{26}=R_{27}=R_{28}=R_{29}=R_{30}=R_{31}=R_{32}=R_{33}=H,$

- $R_{14}=R_{33}=CH_3,$

- $R_{34}=-OH$ or $-OR$ where R = alkyl, acyl or carbohydrate and

$R_{25}=H$ or is absent

- 35 $R_{37}=H, -OH$ or $=O$

$R_{38}=H$ or $-OH$

... represents a single bond, and

- the methyl group at C25 may be either in the R or S configuration and the stereochemistry of R₂₄ is in the β orientation wherein in addition to the above

- 5 at least one of R₃ or R₄₁ is a X radical, the possible remaining substituent being H, OH, =O, and OR where R = alkyl or acyl group or absent, and X is chosen from the group consisting of :
- halo atom,
 - (Me-S-), (Me-SO-), (Me-SO₂-), and
 - 10 - N₃-, NH₂-, MeSO₂NH-
 - alkyl;

m. Substituted sapogenins wherein at least one OH-group of the sapogenin is substituted with X, chosen from the group consisting of :

- 15 - halo atom,
- (Me-S-), (Me-SO-), (Me-SO₂-),
 - N₃-, NH₂-, MeSO₂NH-, and
 - alkyl;

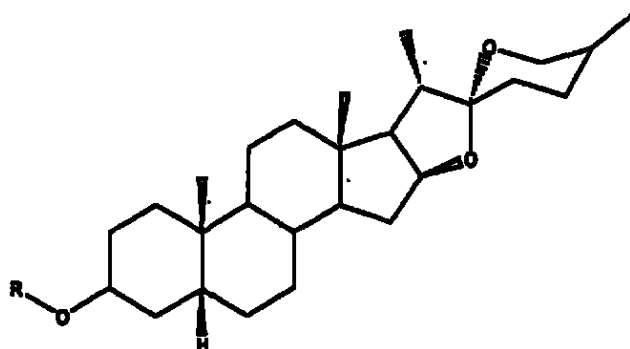
- 20 n. Sapogenins defined above wherein in the definition of X the halo atom is a fluoro atom;

o. Substituted sapogenins selected from :

- 25 (3β-fluoro-5β, 20α,22α,25R-spirostane), (3,3-difluoro-5β, 20α,22α,25R-spirostane), (3α-methylsulphonylamino-5β,20α,22α,25R-spirostane), (3α-azido-5β, 20α,22α,25R-spirostane), (3α-amino-5β,20α,22α,25R-spirostane), and their stereoisomers and racemic mixtures, their pharmaceutically acceptable pro-drugs and salts;

- p. Substituted sapogenins wherein the parent sapogenin which is then substituted with at least one X radical as defined above is selected from sarsapogenin, episarsapogenin, smilagenin, epismilagenin, and anzuogenin-D;
- 30

q. Compounds of the general formula Ia:



(Ia)

5 wherein the group R is selected from hydrogen; alkylcarbonyl; alkoxy carbonyl; alkyl-
 carbamoyl; or arylcarbonyl; or sulfo (HO_2S); phosphono ($(\text{HO})_2\text{P}(\text{O})-$); or a mono-, di- or
 tri-saccharide; wherein any alkyl group is optionally substituted with aryl, amino, mono- or
 di-alkyl-amino, a carboxylic acid residue ($-\text{COOH}$), or any combination thereof; and

10 r. Derivative forms of the above compounds as defined as items a to q, in which the
 3-position carbon atom or, in the case of Formulas II and III, the 3-position carbon atom,
 the 26-position carbon atom or each of the carbon atoms at the 3- and 26-positions, carries
 an O-sugar moiety wherein the sugar group is a mono-, di- or tri-saccharide, and acylated
 15 derivatives thereof.

3. A use according to claim 1 or claim 2, wherein the active agent, or at least one of
 the active agents, is selected from compounds of the general formula Ia.

20 4. A use according to any one of the preceding claims, wherein the active agent, or at
 least one of the active agents, is selected from:

- sarsasapogenin
- sarsasapogenin ceftrylate
- sarsasapogenin acetate
- 25 sarsasapogenin succinate and pharmaceutically acceptable salts thereof
- sarsasapogenin glycinate and pharmaceutically acceptable salts thereof
- sarsasapogenin alaninate and pharmaceutically acceptable salts thereof
- sarsasapogenin valinate and pharmaceutically acceptable salts thereof

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- sarsasapogenin phenylalaninate and pharmaceutically acceptable salts thereof
sarsasapogenin isoleucinate and pharmaceutically acceptable salts thereof
sarsasapogenin methioninate and pharmaceutically acceptable salts thereof
episarsasapogenin
- 5 episarsasapogenin cathylate
episarsasapogenin acetate
episarsasapogenin succinate and pharmaceutically acceptable salts thereof
episarsasapogenin glycinate and pharmaceutically acceptable salts thereof
episarsasapogenin alaninate and pharmaceutically acceptable salts thereof
- 10 episarsasapogenin valinate and pharmaceutically acceptable salts thereof
episarsasapogenin phenylalaninate and pharmaceutically acceptable salts thereof
episarsasapogenin isoleucinate and pharmaceutically acceptable salts thereof
episarsasapogenin methioninate and pharmaceutically acceptable salts thereof
smilagenin
- 15 smilagenin cathylate
smilagenin acetate
smilagenin succinate and pharmaceutically acceptable salts thereof
smilagenin glycinate and pharmaceutically acceptable salts thereof
smilagenin alaninate and pharmaceutically acceptable salts thereof
- 20 smilagenin valinate and pharmaceutically acceptable salts thereof
smilagenin phenylalaninate and pharmaceutically acceptable salts thereof
smilagenin isoleucinate and pharmaceutically acceptable salts thereof
smilagenin methioninate and pharmaceutically acceptable salts thereof
epismilagenin
- 25 epismilagenin cathylate
epismilagenin acetate
epismilagenin succinate and pharmaceutically acceptable salts thereof
epismilagenin glycinate and pharmaceutically acceptable salts thereof
epismilagenin alaninate and pharmaceutically acceptable salts thereof
- 30 epismilagenin valinate and pharmaceutically acceptable salts thereof
epismilagenin phenylalaninate and pharmaceutically acceptable salts thereof
epismilagenin isoleucinate and pharmaceutically acceptable salts thereof
epismilagenin methioninate and pharmaceutically acceptable salts thereof.
- 35 saponin derivatives of sarsasapogenin, episarsasapogenin, smilagenin and epismilagenin in which, in each case, the 3-position carbon atom carries an O-sugar moiety wherein the

sugar group is selected from glucose, mannose, fructose, galactose, maltose, cellobiose, sucrose, rhamnose, xylose, arabinose, fucose, quinovose, apiose, lactose, galactose-glucose, glucose-arabinose, fucose-glucose, rhamnose-glucose, glucose-glucose-glucose, glucose-rhamnose, mannose-glucose, glucose-(rhamnose)-glucose, glucose-(rhamnose)-rhamnose, glucose-(glucose)-glucose, galactose-(rhamnose)-galactose and acylated derivatives thereof;

16,22-epoxycoprostan-3 β -ol, smilagenone, coprostanol, and pharmaceutically acceptable pro-drugs and salts thereof.

10

5. A use according to any one of the preceding claims, wherein the active agent is present in a composition selected from pharmaceutical compositions, foodstuffs, food supplements and beverages.

15

6. A use according to any one of the preceding claims, wherein the active agent is present with one or more additional active agent.

20

7. A use according to claim 6, wherein the one or more additional active agent is selected from, but not limited, to cholinesterase inhibitors, dopamine agonists, COMT inhibitors, MAO-B inhibitors, anti-cholinergics, acetylcholine agonists, serotonin agonists, AMPA receptor agonists, GABA receptor agonists, NMDA receptor agonists, β -adrenoreceptor agonists, digoxin, dobutamine, anti-inflammatories, neurotrophic factors, statins, adenosine A2a receptor antagonists, aldose reductase inhibitors, immunomodulators, cannabinoid agonists, interferon β or tricyclic anti-depressants.

25

8. A use according to any one of the preceding claims, wherein the human or non-human animal is suffering from, or is susceptible to, any of: Parkinson's disease, postencephalitic Parkinsonism, depression, schizophrenia, muscular dystrophy including facioscapulohumeral muscular dystrophy (FSH), Duchenne muscular dystrophy, Becker muscular dystrophy and Bruce's muscular dystrophy, Fuchs' dystrophy, myotonic dystrophy, corneal dystrophy, reflex sympathetic dystrophy syndrome (RSDSA), neurovascular dystrophy, myasthenia gravis, Lambert Eaton disease, Huntington's disease, motor neurone diseases including amyotrophic lateral sclerosis (ALS), multiple sclerosis, postural hypotension, traumatic neurodegeneration e.g. following stroke or following an accident (for example, traumatic head injury or spinal cord injury), Batten's disease, Cockayne syndrome, Down syndrome, corticobasal ganglionic degeneration, multiple

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system atrophy, cerebral atrophy, olivopontocerebellar atrophy, dentatorubral atrophy, pallidolusian atrophy, spinobulbar atrophy, optic neuritis, sclerosing pan-encephalitis (SSPE), attention deficit disorder, post-viral encephalitis, post-poliomyelitis syndrome, Fahr's syndrome, Joubert syndrome, Guillain-Barre syndrome, lissencephaly, Moyamoya
5 disease, neuronal migration disorders, autistic syndrome, polyglutamine disease, Niemann-Pick disease, progressive multifocal leukoencephalopathy, pseudotumor cerebri, Refsum disease, Zellweger syndrome, supranuclear palsy, Friedreich's ataxia, spinocerebellar ataxia type 2, Rhett syndrome, Shy-Drager syndrome, tuberous sclerosis, Pick's disease, chronic fatigue syndrome, neuropathies including hereditary neuropathy, diabetic neuropathy and
10 mitotic neuropathy, prion-based neurodegeneration, including Creutzfeldt-Jakob disease (CJD), variant CJD, new variant CJD, bovine spongiform encephalopathy (BSE), GSS, FFI, kuru and Alper's syndrome, Joseph's disease, acute disseminated encephalomyelitis, arachnoiditis, vascular lesions of the central nervous system, loss of extremity neuronal function, Charcot-Marie-Tooth disease, susceptibility to heart failure, asthma, and macular
15 degeneration.

Figure 1

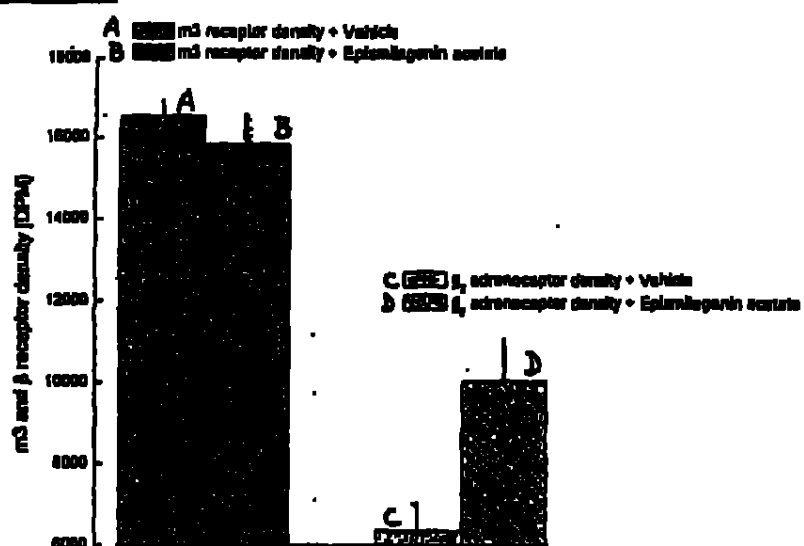


Figure 2

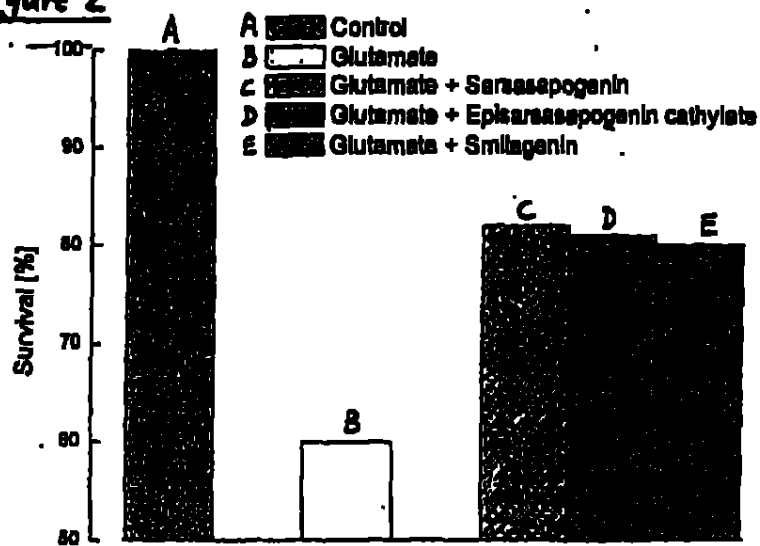


Figure 3

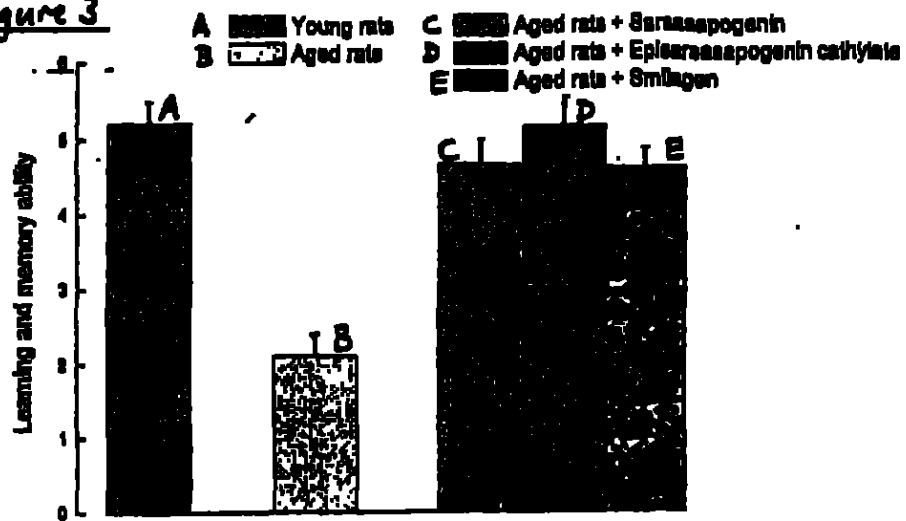


Figure 4

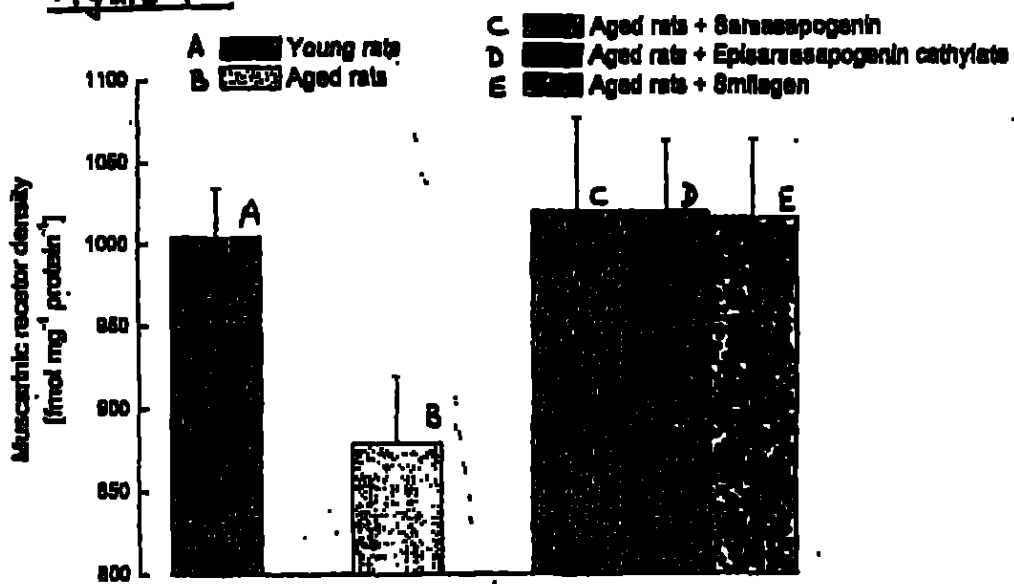


Figure 5

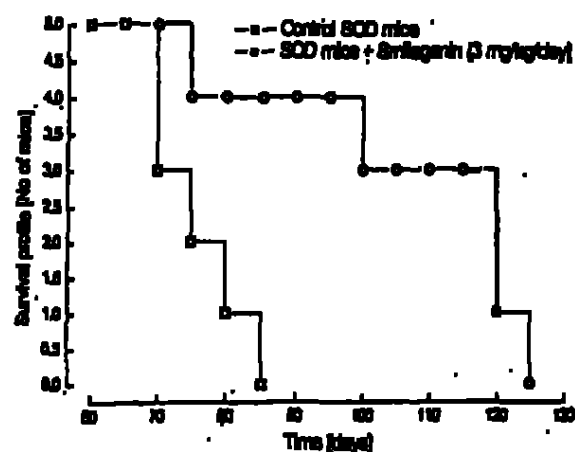
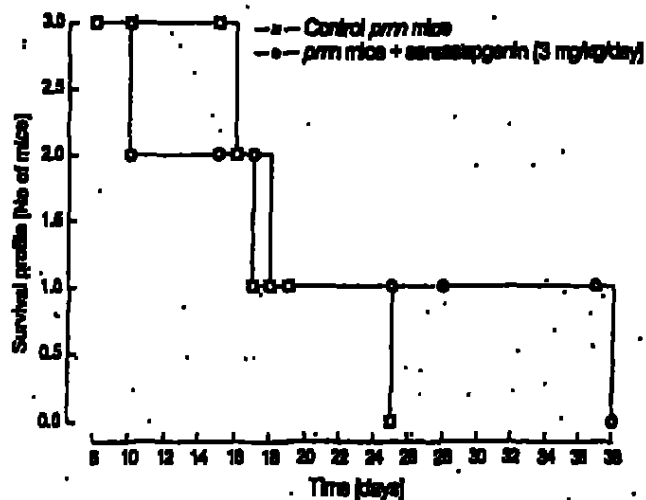


Figure 6



PCT

REQUEST

The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty.

For receiving Office use only	
International Application No.	
International Filing Date	
Name of receiving Office and "PCT International Application"	
Applicant's or agent's file reference (if desired) (12 characters maximum) HL81711/001/DLB	

Box No. I TITLE OF INVENTION Therapeutic Methods and Uses of Sapogenins and Their Derivatives	
Box No. II APPLICANT ☐ This person is also inventor	
Name and address: (Family name followed by given names; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence (if no State of residence is indicated below.)	Telephone No.
PHYTOPHARM PLC Corpus Christi House 9, West Street, Godmanchester, Cambridgeshire PE29 2HY United Kingdom (GB)	Facsimile No.
	Teleprinter No.
	Applicant's registration No. with the Office
State (that is, country) of nationality: GB	State (that is, country) of residence: GB
This person is applicant for the purposes of: ☐ all designated States ☒ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box	
Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)	
Name and address: (Family name followed by given names; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence (if no State of residence is indicated below.)	This person is:
REES, Daryl c/o Phytopharm plc, Corpus Christi House 9, West Street, Godmanchester, Cambridgeshire PE29 2HY United Kingdom (GB)	☐ applicant only
	☒ applicant and inventor
	☐ inventor only (if this check-box is marked, do not fill in below.)
Applicant's registration No. with the Office	
State (that is, country) of nationality: GB	State (that is, country) of residence: GB
This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box	
☒ Further applicants and/or (further) inventors are indicated on a continuation sheet.	
Box No. IV AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE	
The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as: ☒ agent ☐ common representative	
Name and address: (Family name followed by given names; for a legal entity, full official designation. The address must include postal code and name of country.)	Telephone No.
BROWN, David Leslie HASELTINE LAKE Imperial House, 15-19 Kingsway London, WC2B 6UD United Kingdom (GB)	+44 20 7420 0500
	Facsimile No.
	+44 20 7420 0505
Teleprinter No.	
Agent's registration No. with the Office	
☐ Address for correspondence: Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.	

Continuation of Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

If none of the following sub-boxes is used, this sheet should not be included in the request.

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this box is the applicant's State (that is, country) of residence (if no State of residence is indicated below.)

GUNNING, Phil
c/o Phytopharm plc, Corpus Christi House
9, West Street, Godmanchester,
Cambridgeshire PE29 2HY
United Kingdom (GB)

This person is:

- ☐ applicant only
☒ applicant and inventor
☐ inventor only (If this check-box is marked, do not fill in below.)

Applicant's registration No. with the Office

State (that is, country) of nationality:

GB

State (that is, country) of residence:

GB

This person is applicant for the purposes of:

- ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this box is the applicant's State (that is, country) of residence (if no State of residence is indicated below.)

ORSI, Antonia
c/o Phytopharm plc, Corpus Christi House
9, West Street, Godmanchester,
Cambridgeshire PE29 2HY
United Kingdom (GB)

This person is:

- ☐ applicant only
☒ applicant and inventor
☐ inventor only (If this check-box is marked, do not fill in below.)

Applicant's registration No. with the Office

State (that is, country) of nationality:

GB

State (that is, country) of residence:

GB

This person is applicant for the purposes of:

- ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this box is the applicant's State (that is, country) of residence (if no State of residence is indicated below.)

XU, Zongqin
Flat 403, Block 8, 1
Jian-De Road, Shanghai
200025 China (CN)

This person is:

- ☐ applicant only
☒ applicant and inventor
☐ inventor only (If this check-box is marked, do not fill in below.)

Applicant's registration No. with the Office

State (that is, country) of nationality:

CN

State (that is, country) of residence:

CN

This person is applicant for the purposes of:

- ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this box is the applicant's State (that is, country) of residence (if no State of residence is indicated below.)

HU, Yaer
Flat 1209, Block 1, Lane 357;
Ma-Dang Road, Shanghai
200025 China (CN)

This person is:

- ☐ applicant only
☒ applicant and inventor
☐ inventor only (If this check-box is marked, do not fill in below.)

Applicant's registration No. with the Office

State (that is, country) of nationality:

CN

State (that is, country) of residence:

CN

This person is applicant for the purposes of:

- ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

☐ Further applicants and/or (further) inventors are indicated on another continuation sheet.

Box No. V DESIGNATION OF STATES

Mark the applicable check-boxes below; at least one must be marked.

The following designations are hereby made under Rule 4.9(a):

Regional Patent

- ☐ AP ARIPO Patent: GH Ghana, GM Gambia, KE Kenya, LS Lesotho, MW Malawi, MZ Mozambique, SD Sudan, SL Sierra Leone, SZ Swaziland, TZ United Republic of Tanzania, UG Uganda, ZM Zambia, ZW Zimbabwe, and any other State which is a Contracting State of the Harare Protocol and of the PCT (if other kind of protection or treatment desired, specify on dotted line)
- ☐ EA Eurasian Patent: AM Armenia, AZ Azerbaijan, BY Belarus, KG Kyrgyzstan, KZ Kazakhstan, MD Republic of Moldova, RU Russian Federation, TJ Tajikistan, TM Turkmenistan, and any other State which is a Contracting State of the Eurasian Patent Convention and of the PCT
- ☐ EP European Patent: AT Austria, BE Belgium, BG Bulgaria, CH & LI Switzerland and Liechtenstein, CY Cyprus, CZ Czech Republic, DE Germany, DK Denmark, EE Estonia, ES Spain, FI Finland, FR France, GB United Kingdom, GR Greece, IE Ireland, IT Italy, LU Luxembourg, MC Monaco, NL Netherlands, PT Portugal, SE Sweden, SK Slovakia, TR Turkey, and any other State which is a Contracting State of the European Patent Convention and of the PCT
- ☐ OA OAPI Patent: BF Burkina Faso, BJ Benin, CF Central African Republic, CG Congo, CI Côte d'Ivoire, CM Cameroon, GA Gabon, GN Guinea, GQ Equatorial Guinea, GW Guinea-Bissau, ML Mali, MR Mauritania, NE Niger, SN Senegal, TD Chad, TG Togo, and any other State which is a member State of OAPI and a Contracting State of the PCT (if other kind of protection or treatment desired, specify on dotted line)

National Patent (if other kind of protection or treatment desired, specify on dotted line):

- | | | |
|--|---|---|
| ☐ AE United Arab Emirates | ☐ GM Gambia | ☐ NZ New Zealand |
| ☐ AG Antigua and Barbuda | ☐ HR Croatia | ☐ OM Oman |
| ☐ AL Albania | ☐ HU Hungary | ☐ PH Philippines |
| ☐ AM Armenia | ☐ ID Indonesia | ☐ PL Poland |
| ☐ AT Austria | ☐ IL Israel | ☐ PT Portugal |
| ☐ AU Australia | ☐ IN India | ☐ RO Romania |
| ☐ AZ Azerbaijan | ☐ IS Iceland | ☐ RU Russian Federation |
| ☐ BA Bosnia and Herzegovina | ☐ JP Japan | |
| ☐ BB Barbados | ☐ KE Kenya | ☐ SD Sudan |
| ☐ BG Bulgaria | ☐ KG Kyrgyzstan | ☐ SE Sweden |
| ☐ BR Brazil | ☐ KP Democratic People's Republic of Korea | ☐ SG Singapore |
| ☐ BY Belarus | ☐ KR Republic of Korea | ☐ SI Slovenia |
| ☐ BZ Belize | ☐ KZ Kazakhstan | ☐ SK Slovakia |
| ☐ CA Canada | ☐ LC Saint Lucia | ☐ SL Sierra Leone |
| ☐ CH & LI Switzerland and Liechtenstein | ☐ LK Sri Lanka | ☐ TJ Tajikistan |
| ☐ CN China | ☐ LR Liberia | ☐ TM Turkmenistan |
| ☐ CO Colombia | ☐ LS Lesotho | ☐ TN Tunisia |
| ☐ CR Costa Rica | ☐ LT Lithuania | ☐ TR Turkey |
| ☐ CU Cuba | ☐ LU Luxembourg | ☐ TT Trinidad and Tobago |
| ☐ CZ Czech Republic | ☐ LV Latvia | |
| ☐ DE Germany | ☐ MA Morocco | ☐ TZ United Republic of Tanzania |
| ☐ DK Denmark | ☐ MD Republic of Moldova | ☐ UA Ukraine |
| ☐ DM Dominica | | ☐ UG Uganda |
| ☐ DZ Algeria | ☐ MG Madagascar | ☐ US United States of America |
| ☐ EC Ecuador | ☐ MK The former Yugoslav Republic of Macedonia | |
| ☐ EE Estonia | ☐ MN Mongolia | ☐ UZ Uzbekistan |
| ☐ ES Spain | ☐ MW Malawi | ☐ VN Viet Nam |
| ☐ FI Finland | ☐ MX Mexico | ☐ YU Yugoslavia |
| ☐ GB United Kingdom | ☐ MZ Mozambique | ☐ ZA South Africa |
| ☐ GD Grenada | ☐ NO Norway | ☐ ZM Zambia |
| ☐ GE Georgia | | ☐ ZW Zimbabwe |
| ☐ GH Ghana | | |

Check-boxes below reserved for designating States which have become party to the PCT after issuance of this sheet:

- ☐ and all other states which have become ☐
- ☐ bound by the PCT, since July 2002..... ☐

Precautionary Designation Statement: In addition to the designations made above, the applicant also makes under Rule 4.9(b) all other designations which would be permitted under the PCT except any designation(s) indicated in the Supplemental Box as being excluded from the scope of this statement. The applicant declares that these additional designations are subject to confirmation and that any designation which is not confirmed before the expiration of 15 months from the priority date is to be regarded as withdrawn by the applicant at the expiration of that time limit. (Confirmation (including fees) must reach the receiving Office within the 15-month time limit.)

Box No. VI PRIORITY CLAIM				
The priority of the following earlier application(s) is hereby claimed:				
Filing date of earlier application (day/month/year)	Number of earlier application	Where earlier application is:		
		national application: country or Member of WTO	regional application: regional Office	international application: receiving Office
item (1) 27.03.2002	P020101170	AR		
item (2) 28.03.2002	60/368178	USA		
item (3) 28.03.2002	PCT/GB02/01578	PCT (designating <i>inter alia</i> GB)		GB
item (4)				
item (5)				

☐ Further priority claims are indicated in the Supplemental Box.

The receiving Office is requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) (only if the earlier application was filed with the Office which for the purposes of this international application is the receiving Office) identified above as:

☐ all items ☐ item (1) ☐ item (2) ☐ item (3) ☐ item (4) ☐ item (5) ☐ other, see Supplemental Box

* Where the earlier application is an ARIPO application, indicate at least one country party to the Paris Convention for the Protection of Industrial Property or one Member of the World Trade Organization for which that earlier application was filed (Rule 4.10(b)(10)):

Box No. VII INTERNATIONAL SEARCHING AUTHORITY		
Choice of International Searching Authority (ISA) (if two or more International Searching Authorities are competent to carry out the international search, indicate the Authority chosen; the two-letter code may be used):		
ISA / EPO		
Request to use results of earlier search; reference to that search (if an earlier search has been carried out by or requested from the International Searching Authority):		
Date (day/month/year)	Number	Country (or regional Office)

Box No. VIII DECLARATIONS		
The following declarations are contained in Boxes Nos. VIII (i) to (v) (mark the applicable check-boxes below and indicate in the right column the number of each type of declaration):		Number of declarations
☐ Box No. VIII (i)	Declaration as to the identity of the inventor	:
☐ Box No. VIII (ii)	Declaration as to the applicant's entitlement, as at the international filing date, to apply for and be granted a patent	:
☐ Box No. VIII (iii)	Declaration as to the applicant's entitlement, as at the international filing date, to claim the priority of the earlier application	:
☐ Box No. VIII (iv)	Declaration of inventorship (only for the purposes of the designation of the United States of America)	:
☐ Box No. VIII (v)	Declaration as to non-prejudicial disclosures or exceptions to lack of novelty	:

Box No. IX CHECK LIST; LANGUAGE OF FILING		
This international application contains: (a) the following number of sheets in paper form: request (including declaration sheets) : 5 description (excluding sequence listing part) : 42 claims : 13 abstract : 1 drawings : 3 Sub-total number of sheets : 64 sequence listing part of description (actual number of sheets if filed in paper form, whether or not also filed in computer readable form; see (b) below) : _____ Total number of sheets : 64 (b) sequence listing part of description filed in computer readable form (i) ☐ only (under Section 801(a)(i)) (ii) ☐ in addition to being filed in paper form (under Section 801(a)(ii)) Type and number of carriers (diskette, CD-ROM, CD-R or other) on which the sequence listing part is contained (additional copies to be indicated under item 9(ii), in right column): _____		This international application is accompanied by the following item(s) (mark the applicable check-boxes below and indicate in right column the number of each item): 1. ☐ fee calculation sheet : _____ 2. ☐ original separate power of attorney : _____ 3. ☐ original general power of attorney : _____ 4. ☐ copy of general power of attorney; reference number, if any: _____ 5. ☐ statement explaining lack of signature : _____ 6. ☐ priority document(s) identified in Box No. VI as item(s): _____ 7. ☐ translation of international application into (language): _____ 8. ☐ separate indications concerning deposited microorganism or other biological material : _____ 9. ☐ sequence listing in computer readable form (Indicate also type and number of carriers (diskette, CD-ROM, CD-R or other)) (i) ☐ copy submitted for the purposes of international search under Rule 13ter only (and not as part of the international application) : _____ (ii) ☐ (only where check-box (b)(i) or (b)(ii) is marked in left column) additional copies including, where applicable, the copy for the purposes of international search under Rule 13ter : _____ (iii) ☐ together with relevant statement as to the identity of the copy or copies with the sequence listing part mentioned in left column : _____ 10. ☐ other (specify): _____
Figure of the drawings which should accompany the abstract: _____		Language of filing of the international application: ENGLISH

Box No. X SIGNATURE OF APPLICANT, AGENT OR COMMON REPRESENTATIVE
Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the request).  NASH, David Allen Authorized Representative, for Heseltine Lake.

For receiving Office use only	
1. Date of actual receipt of the purported international application: _____	2. Drawings: ☐ received: ☐ not received:
3. Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application: _____	
4. Date of timely receipt of the required corrections under PCT Article 11(2): _____	
5. International Searching Authority (if two or more are competent): ISA /	6. ☐ Transmittal of search copy delayed until search fee is paid
For International Bureau use only	
Date of receipt of the record copy by the International Bureau: _____	

CORRECTED VERSION

(19) World Intellectual Property
Organization
International Bureau



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(10) International Publication Number
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- (51) International Patent Classification: C07J
- (74) Agent: BROWN, David, Leslie; Haseltine Lake, Imperial House, 15-19 Kingway, London WC2B 6UD (GB).
- (21) International Application Number: PCT/GB2003/001380
- (31) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GH, GM, GR, HU, ID, IL, IN, IS, JP, KR, KG, KP, KZ, LC, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW.
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- (34) Designated States (regional): ARIPO patent (GH, GM, KR, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).
- (71) Applicant (for all designated States except US): PHYTOPHARM PLC (GB/GB); Corpus Christi House, 9 West Street, Godmanchester, Cambridgeshire PE29 2HY (GB).
- (72) Inventors; and
- (73) Inventors/Applicants (for US only): REES, Daryl (GB/GB); Phytopharm plc, Corpus Christi House, 9, West Street, Godmanchester, Cambridgeshire PE29 2HY (GB). GUNNING, Phil (GB/GB); Phytopharm plc, Corpus Christi House, 9, West Street, Godmanchester, Cambridgeshire PE29 2HY (GB). ORRIS, Antonio (GB/GB); Phytopharm plc, Corpus Christi House, 9, West Street, Godmanchester, Cambridgeshire PE29 2HY (GB). XIA, Zongqin (CN/CN); Flat 403, Block 8, 1, Jian-De Road, Shanghai 200025 (CN). HU, Yues (CN/CN); Flat 1209, Block 1, Lane 357, Ma-Dang Road, Shanghai 200025 (CN).
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- For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 2003/082893 A2

(54) Title: THERAPUTIC METHODS AND USES OF SAPOGENINS AND THEIR DERIVATIVES

(57) Abstract: The invention discloses therapeutic methods and uses of certain steroidal sapogenins, related compounds and derivatives thereof, in the treatment of non-cognitive neurodegeneration, non-cognitive neuromuscular degeneration, motor-sensory neurodegeneration or receptor dysfunction or loss in the absence of cognitive, neural and neuromuscular impairment.

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1. 下列各句，没有语病的一句是（ ）

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: THERAPUTIC METHODS AND USES OF SAPOGENINS AND THEIR DERIVATIVES

2003/082893 A3

INTERNATIONAL SEARCH REPORT

PCT/GB 03/01380

A. CLASSIFICATION OF SUBJECT MATTER					
IPC 7	A61K31/585	A61P9/02	A61P9/04	A61P11/06	A61P21/00
	A61P21/04	A61P25/08	A61P25/16	A61P25/28	A61P27/00
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 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)					
CHEM ABS Data, SCISEARCH, BIOSIS, MEDLINE, EMBASE, WPI Data, EPO-Internal, PAJ					
C. DOCUMENTS CONSIDERED TO BE RELEVANT					
Category *	Citation of document, with indication, where appropriate, of the relevant passages				Relevant to claim No.
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-/--					
☒ Further documents are listed in the continuation of box C. ☒ Patent family members are listed in annex.					
* Special categories of cited documents : 'A' document defining the general state of the art which is not considered to be of particular relevance 'E' earlier document but published on or after the international filing date 'L' document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another claim 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. 'Z' 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 February 2004			16.02.2004		
Name and mailing address of the ISA European Patent Office, P.O. Box 5818 Patentlaan 2 NL - 2200 HV Rijswijk			Authorized officer		

INTERNATIONAL SEARCH REPORT

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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X	WO 01 23406 A (HU YAER ;XIA ZONGQIN (CN); GUNNING PHIL (GB); HANSON JIM (GB); PHY) 5 April 2001 (2001-04-05) cited in the application page 1, line 4 - line 33 page 6, line 17 -page 9, line 17 page 13, line 4 - line 23 page 13, line 28 - line 30 page 15 -page 19; table 1 claims 1-15	1-8
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INTERNATIONAL SEARCH REPORT

PCT/GB 03/01380

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	DATABASE WPI Section Ch, Week 199343 Derwent Publications Ltd., London, GB; Class B04, AN 1993-339641 XP002157713 & JP 05 246866 A (KATO K), 24 September 1993 (1993-09-24) abstract	1-8
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INTERNATIONAL SEARCH REPORT

PCT/GB 03/01380

C (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	KYOUNG SOOK KIM, ET AL.: "Effects of cholestanol feeding on corneal dystrophy in mice." BIOCHIMICA ET BIOPHYSICA ACTA, vol. 1085, 1991, pages 343-349, XP008027217 the whole document	1-8
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PCT/G8 03/01380

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

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Although claims 1-8 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☒ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

see additional sheet

1. ☒ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☒ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Present claims 1-8 relate to a large number of undefined compounds in terms of "derivative" (claims 1-8) and "prodrug" (1-3 and 5-8). In fact, the claims contain so many options that a lack of clarity (and/or conciseness) within the meaning of Article 6 PCT arises to such an extent as to render a meaningful search of the claims impossible. Furthermore, present claim 1-7 relate to the treatment of a disease which actually is not well defined.

The use of the definition "non-cognitive neurodegeneration", "non-cognitive neuromuscular degeneration" or "motor-sensory neurodegeneration" or "receptor dysfunction or loss in the absence of cognitive, neural and neuromuscular impairment" in the present context is considered to lead to a lack of clarity within the meaning of Article 6 PCT. It is not fully possible to determine the diseases for which protection might legitimately be sought. The lack of clarity is such as to render a meaningful complete search over the whole scope of the claims impossible.

Consequently, the search has been carried out for those parts of the application which do appear to be clear and concise, namely the use of the compounds according to formula (I), (II), (IV) and sapogenins and saponins in relation to the treatment of diseases as specifically mentioned in claim 8.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

FURTHER INFORMATION CONTINUED FROM PCT/GB 03 01380

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-2 (partially), 3, 4-8 (partially)

The use of compounds according to formula (I) and (Ia) for the manufacture of a medicament for the treatment of non-cognitive neurodegeneration, non-cognitive neuromuscular degeneration, motor-sensory neurodegeneration or receptor dysfunction or loss in the absence of cognitive, neural and neuromuscular impairment. The use of said compounds as active ingredients present in foodstuffs, food supplement and beverages.

2. Claims: 1-2 (partially) and 5-8 (partially)

The use of compounds according to formula (II) for the manufacture of a medicament for the treatment of non-cognitive neurodegeneration, non-cognitive neuromuscular degeneration, motor-sensory neurodegeneration or receptor dysfunction or loss in the absence of cognitive, neural and neuromuscular impairment. The use of said compounds as active ingredients present in foodstuffs, food supplement and beverages.

3. Claims: 1-2 (partially) and 5-8 (partially)

The use of compounds according to formula (III) for the manufacture of a medicament for the treatment of non-cognitive neurodegeneration, non-cognitive neuromuscular degeneration, motor-sensory neurodegeneration or receptor dysfunction or loss in the absence of cognitive, neural and neuromuscular impairment. The use of said compounds as active ingredients present in foodstuffs, food supplement and beverages.

4. Claims: 1-2 (partially) and 4-8 (partially).

The use of sapogenin derivatives bearing at least one X radical substituent wherein X is chosen from an halo atom, (Me-S-), (Me-SO-), (Me-SO₂-), N₃-, Nh₂-, Me-SO₂-NH-, and alkyl (i.e. compounds according to D. of claim 1) for the manufacture of a medicament for the treatment of non-cognitive neurodegeneration, non-cognitive neuromuscular degeneration, motor-sensory neurodegeneration or receptor dysfunction or loss in the absence of cognitive, neural and neuromuscular impairment, as far as not comprised by subjects 1 to 3. The use of said compounds as active ingredients present in foodstuffs, food supplement and beverages.

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International Application No. PCT/AB 03 01380

FURTHER INFORMATION CONTINUED FROM PCT/SA/ 210

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INTERNATIONAL SEARCH REPORT

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INTERNATIONAL SEARCH REPORT

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PATENT COOPERATION TREATY

From the INTERNATIONAL SEARCHING AUTHORITY

PCT

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NOTIFICATION OF TRANSMITTAL OF
 THE INTERNATIONAL SEARCH REPORT
 OR THE DECLARATION

(PCT Rule 44.1)

Applicant's or agent's file reference HL81711/001/DLB	Date of mailing (day/month/year) 16/02/2004
International application No. PCT/GB 03/01380	International filing date (day/month/year) 27/03/2003
Applicant PHYTOPHARM PLC	

1. ☒ The applicant is hereby notified that the International Search Report has been established and is transmitted herewith.

Filing of amendments and statement under Article 18:

The applicant is entitled, if he so wishes, to amend the claims of the International Application (see Rule 46):

When? The time limit for filing such amendments is normally 2 months from the date of transmittal of the International Search Report; however, for more details, see the notes on the accompanying sheet.

Where? Directly to the International Bureau of WIPO
 34, chemin des Colombettes
 1211 Geneva 20, Switzerland
 Facsimile No.: (41-22) 740.14.35

For more detailed instructions, see the notes on the accompanying sheet.

2. ☐ The applicant is hereby notified that no International Search Report will be established and that the declaration under Article 17(2)(a) to that effect is transmitted herewith.

3. ☐ With regard to the protest against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:

☐ the protest together with the decision thereon has been transmitted to the International Bureau together with the applicant's request to forward the texts of both the protest and the decision thereon to the designated Offices.

☐ no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. Further action(s): The applicant is reminded of the following:

Shortly after 18 months from the priority date, the International application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the International application, or of the priority claim, must reach the International Bureau as provided in Rules 90bis.1 and 90bis.3, respectively, before the completion of the technical preparations for International publication.

Within 18 months from the priority date, a demand for International preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase until 30 months from the priority date (in some Offices even later).

Within 20 months from the priority date, the applicant must perform the prescribed acts for entry into the national phase before all designated Offices which have not been elected in the demand or in a later election within 18 months from the priority date or could not be elected because they are not bound by Chapter II.

Name and mailing address of the International Searching Authority

 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) 340-3018

Authorized officer

Barbara Klaver

COMM-IT

18 FEB 2004

NOTES TO FORM PCT/ISA/220

These Notes are intended to give the basic instructions concerning the filing of amendments under article 19. The Notes are based on the requirements of the Patent Cooperation Treaty, the Regulations and the Administrative Instructions under that Treaty. In case of discrepancy between these Notes and those requirements, the latter are applicable. For more detailed information, see also the PCT Applicant's Guide, a publication of WIPO.

In these Notes, "Article", "Rule", and "Section" refer to the provisions of the PCT, the PCT Regulations and the PCT Administrative Instructions, respectively.

INSTRUCTIONS CONCERNING AMENDMENTS UNDER ARTICLE 19

The applicant has, after having received the international search report, one opportunity to amend the claims of the international application. It should however be emphasized that, since all parts of the international application (claims, description and drawings) may be amended during the international preliminary examination procedure, there is usually no need to file amendments of the claims under Article 19 except where, e.g. the applicant wants the letter to be published for the purposes of provisional protection or has another reason for amending the claims before international publication. Furthermore, it should be emphasized that provisional protection is available in some States only.

What parts of the international application may be amended?

Under Article 19, only the claims may be amended.

During the international phase, the claims may also be amended (or further amended) under Article 34 before the International Preliminary Examining Authority. The description and drawings may only be amended under Article 34 before the International Examining Authority.

Upon entry into the national phase, all parts of the international application may be amended under Article 28 or, where applicable, Article 41.

When?

Within 2 months from the date of transmittal of the international search report or 18 months from the priority date, whichever time limit expires later. It should be noted, however, that the amendments will be considered as having been received on time if they are received by the International Bureau after the expiration of the applicable time limit but before the completion of the technical preparations for international publication (Rule 46.1).

Where not to file the amendments?

The amendments may only be filed with the International Bureau and not with the receiving Office or the International Searching Authority (Rule 46.2).

Where a demand for international preliminary examination has been/is filed, see below.

How?

Either by cancelling one or more entire claims, by adding one or more new claims or by amending the text of one or more of the claims as filed.

A replacement sheet must be submitted for each sheet of the claims which, on account of an amendment or amendments, differs from the sheet originally filed.

All the claims appearing on a replacement sheet must be numbered in Arabic numerals. Where a claim is cancelled, no renumbering of the other claims is required. In all cases where claims are renumbered, they must be renumbered consecutively (Administrative Instructions, Section 205(b)).

The amendments must be made in the language in which the international application is to be published.

What documents must/may accompany the amendments?

Letter (Section 205(b)):

The amendments must be submitted with a letter.

The letter will not be published with the international application and the amended claims. It should not be confused with the "Statement under Article 19(1)" (see below, under "Statement under Article 19(1)").

The letter must be in English or French, at the choice of the applicant. However, if the language of the international application is English, the letter must be in English; if the language of the international application is French, the letter must be in French.

NOTES TO FORM PCT/ISA/220 (continued)

The letter must indicate the differences between the claims as filed and the claims as amended. It must, in particular, indicate, in connection with each claim appearing in the international application (it being understood that identical indications concerning several claims may be grouped), whether

- (i) the claim is unchanged;
- (ii) the claim is cancelled;
- (iii) the claim is new;
- (iv) the claim replaces one or more claims as filed;
- (v) the claim is the result of the division of a claim as filed.

The following examples illustrate the manner in which amendments must be explained in the accompanying letter:

1. [Where originally there were 48 claims and after amendment of some claims there are 51]:
"Claims 1 to 28, 31, 32, 34, 35, 37 to 48 replaced by amended claims bearing the same numbers; claims 30, 33 and 36 unchanged; new claims 49 to 51 added."
2. [Where originally there were 15 claims and after amendment of all claims there are 11]:
"Claims 1 to 15 replaced by amended claims 1 to 11."
3. [Where originally there were 14 claims and the amendments consist in cancelling some claims and in adding new claims]:
"Claims 1 to 6 and 14 unchanged; claims 7 to 13 cancelled; new claims 15, 16 and 17 added." or
"Claims 7 to 13 cancelled; new claims 15, 16 and 17 added; all other claims unchanged."
4. [Where various kinds of amendments are made]:
"Claims 1-10 unchanged; claims 11 to 13, 16 and 19 cancelled; claims 14, 15 and 18 replaced by amended claim 14; claim 17 subdivided into amended claims 15, 16 and 17; new claims 20 and 21 added."

"Statement under article 19(1)" (Rule 48.4)

The amendments may be accompanied by a statement explaining the amendments and indicating any impact that such amendments might have on the description and the drawings (which cannot be amended under Article 19(1)).

The statement will be published with the international application and the amended claims.

It must be in the language in which the international application is to be published.

It must be brief, not exceeding 500 words if in English or if translated into English.

It should not be confused with and does not replace the letter indicating the differences between the claims as filed and as amended. It must be filed on a separate sheet and must be identified as such by a heading, preferably by using the words "Statement under Article 19(1)".

It may not contain any disparaging comments on the international search report or the relevance of citations contained in that report. Reference to citations, relevant to a given claim, contained in the international search report may be made only in connection with an amendment of that claim.

Consequence if a demand for international preliminary examination has already been filed

If, at the time of filing any amendments and any accompanying statement, under Article 19, a demand for international preliminary examination has already been submitted, the applicant must preferably, at the time of filing the amendments (and any statement) with the International Bureau, also file with the International Preliminary Examining Authority a copy of such amendments (and of any statement) and, where required, a translation of such amendments for the procedure before that Authority (see Rules 55.3(a) and 82.2, first sentence). For further information, see the Notes to the demand form (PCT/IPEA/401).

Consequence with regard to translation of the international application for entry into the national phase

The applicant's attention is drawn to the fact that, upon entry into the national phase, a translation of the claims as amended under Article 19 may have to be furnished to the designated/elected Offices, instead of, or in addition to, the translation of the claims as filed.

For further details on the requirements of each designated/elected Office, see Volume II of the PCT Applicant's Guide.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference HL81711/001/DLB	FOR FURTHER ACTION see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 6 below.	
International application No. PCT/GB 03/01380	International filing date (day/month/year) 27/03/2003	(Earliest) Priority Date (day/month/year) 27/03/2002
Applicant PHYTOPHARM PLC		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 12 sheets.

☒ It is also accompanied by a copy of each prior art document cited in this report.

1. Basis of the report

a. With regard to the language, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

☐ the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

b. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of the sequence listing:

☐ contained in the international application in written form.

☐ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

☐ the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. ☒ Certain claims were found unsearchable (See Box I).

3. ☒ Unity of invention is lacking (see Box II).

4. With regard to the title,

☒ the text is approved as submitted by the applicant.

☐ the text has been established by this Authority to read as follows:

5. With regard to the abstract,

☒ the text is approved as submitted by the applicant.

☐ the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the drawings to be published with the abstract is Figure No.

☐ as suggested by the applicant.

☐ because the applicant failed to suggest a figure.

☐ because this figure better characterizes the invention.

☒ None of the figures.

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB 03/01380

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

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Although claims 1-8 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☒ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 8.4(a).

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

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

see additional sheet

1. ☒ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☒ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box 1.2

Present claims 1-8 relate to a large number of undefined compounds in terms of "derivative" (claims 1-8) and "prodrug" (1-3 and 5-8). In fact, the claims contain so many options that a lack of clarity (and/or conciseness) within the meaning of Article 6 PCT arises to such an extent as to render a meaningful search of the claims impossible. Furthermore, present claim 1-7 relate to the treatment of a disease which actually is not well defined.

The use of the definition "non-cognitive neurodegeneration", "non-cognitive neuromuscular degeneration" or "motor-sensory neurodegeneration" or "receptor dysfunction or loss in the absence of cognitive, neural and neuromuscular impairment" in the present context is considered to lead to a lack of clarity within the meaning of Article 6 PCT. It is not fully possible to determine the diseases for which protection might legitimately be sought. The lack of clarity is such as to render a meaningful complete search over the whole scope of the claims impossible.

Consequently, the search has been carried out for those parts of the application which do appear to be clear and concise, namely the use of the compounds according to formula (I), (II), (IV) and saponins and saponins in relation to the treatment of diseases as specifically mentioned in claim 8.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

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FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-2 (partially), 3, 4-8 (partially)

The use of compounds according to formula (I) and (Ia) for the manufacture of a medicament for the treatment of non-cognitive neurodegeneration, non-cognitive neuromuscular degeneration, motor-sensory neurodegeneration or receptor dysfunction or loss in the absence of cognitive, neural and neuromuscular impairment. The use of said compounds as active ingredients present in foodstuffs, food supplement and beverages.

2. Claims: 1-2 (partially) and 5-8 (partially)

The use of compounds according to formula (II) for the manufacture of a medicament for the treatment of non-cognitive neurodegeneration, non-cognitive neuromuscular degeneration, motor-sensory neurodegeneration or receptor dysfunction or loss in the absence of cognitive, neural and neuromuscular impairment. The use of said compounds as active ingredients present in foodstuffs, food supplement and beverages.

3. Claims: 1-2 (partially) and 5-8 (partially)

The use of compounds according to formula (III) for the manufacture of a medicament for the treatment of non-cognitive neurodegeneration, non-cognitive neuromuscular degeneration, motor-sensory neurodegeneration or receptor dysfunction or loss in the absence of cognitive, neural and neuromuscular impairment. The use of said compounds as active ingredients present in foodstuffs, food supplement and beverages.

4. Claims: 1-2 (partially) and 4-8 (partially).

The use of sapogenin derivatives bearing at least one X radical substituent wherein X is chosen from an halo atom, (Me-S-), (Me-SO-), (Me-SO₂-), N₃-, Nh₂-, Me-SO₂-NH-, and alkyl (i.e. compounds according to D. of claim 1) for the manufacture of a medicament for the treatment of non-cognitive neurodegeneration, non-cognitive neuromuscular degeneration, motor-sensory neurodegeneration or receptor dysfunction or loss in the absence of cognitive, neural and neuromuscular impairment, as far as not comprised by subjects 1 to 3. The use of said compounds as active ingredients present in foodstuffs, food supplement and beverages.

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FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 03/01380

A. CLASSIFICATION OF SUBJECT MATTER IPC 7 A61K31/585 A61P9/02 A61P9/04 A61P11/06 A61P21/00 A61P21/04 A61P25/08 A61P25/16 A61P25/28 A61P27/00							
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 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) CHEM ABS Data, SCISEARCH, BIOSIS, MEDLINE, EMBASE, WPI Data, EPO-Internal, PAJ							
C. DOCUMENTS CONSIDERED TO BE RELEVANT							
Category *	Citation of document, with indication, where appropriate, of the relevant passages				Relevant to claim No.		
X	EP 1 024 146 A (INST OF RADIATION MEDICINE ACA) 2 August 2000 (2000-08-02) page 19, line 11 - line 12 page 33, line 46 -page 34, line 27 claims 1,3-20				1-7		
X	--- WO 01 49703 A (HU YAER ;XIA ZONGQIN (CN); GUNNING PHIL (GB); HANSON JIM (GB); PHY) 12 July 2001 (2001-07-12) page 1, line 1 - line 22 page 7, line 11 -page 15, line 4 page 15, line 6 -page 16, line 24 page 22, line 32 - line 34 page 23, line 3 - line 35 claims 1-20,22,26,28,29 --- -/--				1-8		
☒ Further documents are listed in the continuation of box C. ☒ Patent family members are listed in annex.							
* Special categories of cited documents: <table border="0"> <tr> <td> "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 </td> <td> "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 "Z" document member of the same patent family </td> </tr> </table>						"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 "Z" document member of the same patent family
"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 "Z" document member of the same patent family						
Date of the actual completion of the international search 9 February 2004			Date of mailing of the international search report 16. 02. 2004				
Name and mailing address of the ISA European Patent Office, P.B. 6818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3018			Authorized officer van der Kooij, M				

INTERNATIONAL SEARCH REPORT

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International Application No
PCT/GB 03/01380

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 99 48507 A (HU YAER ;XIA ZONGQIN (CN); BROSTOFF JONATHAN (GB); RUBIN IAN (GB);) 30 September 1999 (1999-09-30) cited in the application page 1, line 5 - line 24 page 7, line 6 -page 8, line 4 page 9, line 5 - line 13 page 10, line 4 - line 11 page 15, line 9 -page 16, line 3 page 23, line 23 -page 24, line 8 claims 1-32	1-8
X	WO 01 23406 A (HU YAER ;XIA ZONGQIN (CN); GUNNING PHIL (GB); HANSON JIM (GB); PHY) 5 April 2001 (2001-04-05) cited in the application page 1, line 4 - line 33 page 6, line 17 -page 9, line 17 page 13, line 4 - line 23 page 13, line 28 - line 30 page 15 -page 19; table 1 claims 1-15	1-8
X	WO 01 23408 A (HU YAER ;XIA ZONGQIN (CN); GUNNING PHIL (GB); HANSON JIM (GB); PHY) 5 April 2001 (2001-04-05) cited in the application page 1, line 4 - line 22 page 6, line 16 -page 9, line 17 page 12, line 16 - line 24 page 13, line 16 -page 14, line 27 claims 1-13	1-8
X	DE 43 03 214 A (MARKS WOLFGANG) 11 August 1994 (1994-08-11) cited in the application page 6, line 15 - line 26 page 7, line 10 - line 15 claims 1-3,7,14	1-8
X	SUN, HUI ET AL: "Retinal stimulates ATP hydrolysis by purified and reconstituted ABCR, the photoreceptor-specific ATP-binding cassette transporter responsible for Stargardt disease" JOURNAL OF BIOLOGICAL CHEMISTRY (1999), 274(12), 8269-8281 , XP001165795 figures 2,3,9,10 page 8280, column 2, paragraph 3	1-3,5,8
X	US 4 680 289 A (APPLEZWEIG NORMAN) 14 July 1987 (1987-07-14) tables I,II claims 16,18,19	5

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INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 03/01380

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	DATABASE WPI Section Ch, Week 199343 Derwent Publications Ltd., London, GB; Class B04, AN 1993-339641 XP002157713 & JP 05 246866 A (KATO K), 24 September 1993 (1993-09-24) abstract ---	1-8
X	WO 01 23407 A (HU YAER ;XIA ZONGQIN (CN); GUNNING PHIL (GB); HANSON JIM (GB); PHY) 5 April 2001 (2001-04-05) page 6, line 20 -page 8, line 32 claims 1-5,8,14 ---	1,2,5-8
X	PATENT ABSTRACTS OF JAPAN vol. 2002, no. 05, 3 May 2002 (2002-05-03) & JP 2002 030096 A (KYORIN PHARMACEUT CO LTD), 29 January 2002 (2002-01-29) abstract ---	1,2,5-8
A	PATENT ABSTRACTS OF JAPAN vol. 1997, no. 05, 30 May 1997 (1997-05-30) & JP 09 002957 A (MITSUBISHI CHEM CORP), 7 January 1997 (1997-01-07) abstract ---	1-8
A	PATENT ABSTRACTS OF JAPAN vol. 1997, no. 05, 30 May 1997 (1997-05-30) & JP 09 002956 A (MITSUBISHI CHEM CORP), 7 January 1997 (1997-01-07) abstract ---	1-8
A	WO 98 02450 A (INFLAZYME PHARMACEUTICALS LTD) 22 January 1998 (1998-01-22) example 25 page 178, line 9 - line 10 claims 27,29 ---	1-8
A	EP 0 659 762 A (MITSUBISHI CHEM CORP) 28 June 1995 (1995-06-28) page 108, line 23 - line 36 claims 1,3,27 ---	1-8
A	INOUE K ET AL: "Cholestanol induces apoptosis of cerebellar neuronal cells." BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS. UNITED STATES 5 MAR 1999, vol. 256, no. 1, 5 March 1999 (1999-03-05), pages 198-203, XP001188107 ISSN: 0006-291X the whole document ---	1-8
5	--- -/--	

INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 03/01380

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	KYOUNG SOOK KIM, ET AL.: "Effects of cholestanol feeding on corneal dystrophy in mice." BIOCHIMICA ET BIOPHYSICA ACTA, vol. 1085, 1991, pages 343-349, XP008027217 the whole document ---	1-8
X	EP 0 162 330 A (ROECAR HOLDINGS NV) 27 November 1985 (1985-11-27) page 5, paragraph 2 - paragraph 3 claim 3 ---	1,2,4-8
E	WO 03 077869 A (SAMARITAN PHARMACEUTICALS INC ;UNIV GEORGETOWN (US)) 25 September 2003 (2003-09-25) figure 1 page 5, line 14 - line 21 claims 1-8,10-14 -----	1,2,4-8

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/GB 03/01380

Patent document cited in search report		Publication date		Patent family member(s)		Publication date
EP 1024146	A	02-08-2000	CN	1212966 A		07-04-1999
			AU	9336098 A		23-04-1999
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			HR	20000638 A1		30-04-2001
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			ID	26781 A		08-02-2001
			JP	2003524581 T		19-08-2003
			JP	2002507572 T		12-03-2002
			NO	20004803 A		27-11-2000
			NO	20004804 A		09-11-2000
			PL	343423 A1		13-08-2001
			PL	343508 A1		27-08-2001
			TR	200002773 T2		21-11-2001
			TR	200002774 T2		21-06-2001
			US	2002193317 A1		19-12-2002
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			US	6258386 B1		10-07-2001
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			CN	1377368 T		30-10-2002
			EP	1224205 A1		24-07-2002

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/GB 03/01380

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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		RU 2196143 C2	10-01-2003
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INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/GB 03/01380

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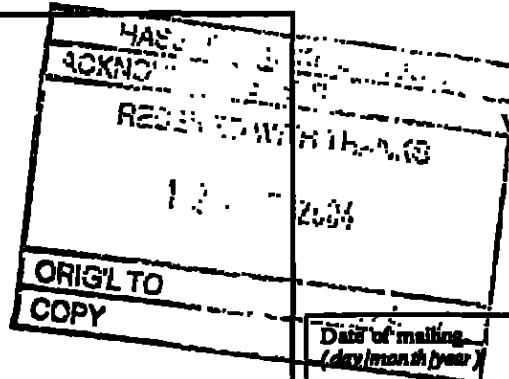
PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

PCT

To:

Brown, David L.
HASELTINE LAKE
Imperial House
15-19 Kingsway
London WC2B 6UD
GRANDE BRETAGNE



WRITTEN OPINION

(PCT Rule 66)

Applicant's or agent's file reference P81711PC00/DLB		Date of mailing (day/month/year) 12/03/2004
Applicant PHYTOPHARM PLC et al.		REPLY DUE within 1 / 00 months/days from the above date of mailing
International application No. PCT/GB03/01380	International filing date (day/month/year) 27/03/2003	Priority date (day/month/year) 27/03/2002
International Patent Classification (IPC) or both national classification and IPC A61K31/585		

1. This written opinion is the first drawn up by this International Preliminary Examining Authority.
2. This opinion contains indications relating to the following items:
 - I ☒ Basis of the opinion
 - II ☐ Priority
 - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
 - IV ☒ Lack of unity of invention
 - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
 - VI ☐ Certain documents cited
 - VII ☐ Certain defects in the international application
 - VIII ☐ Certain observations on the international application
3. The applicant is hereby invited to reply to this opinion.

When? See the time limit indicated above. The applicant may, before the expiration of that time limit, request this Authority to grant an extension, see Rule 66.2(d).

How? By submitting a written reply, accompanied, where appropriate, by amendments, according to Rule 66.3. For the form and the language of the amendments, see Rules 66.8 and 66.9.

Also For an additional opportunity to submit amendments, see Rule 66.4.
 For the examiner's obligation to consider amendments and/or arguments, see Rule 66.4bis.
 For an informal communication with the examiner, see Rule 66.6.

If no reply is filed, the international preliminary examination report will be established on the basis of this opinion.
4. The final date by which the international preliminary examination report must be established according to Rule 69.2 is: 27/07/2004

Name and mailing address of the IPEA/ European Patent Office, P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Netherlands Tel.: (+31-70) 340-2040 Fax: (+31-70) 340-3016	Authorized officer Examiner Formalities officer (incl. extension of time limits) Tel. (+49-89) 2399 2828
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Form PCT/IPEA/408 (cover sheet) (march 2002)



WRITTEN OPINION

International application No.

PCT/GB03/01380

I. Basis of the opinion

The basis of this written opinion is the application as originally filed.

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The question of whether the claimed invention appears to be novel, to involve an inventive step, or to be industrially applicable has not been and will not be the subject of the international preliminary examination in respect of the claims which have not been searched (Article 17(2)(a) or (3) and Rule 66.1(a) PCT; see also international search report).

IV. Lack of unity of invention

The objection as to lack of unity raised in the international search report is maintained. The reasons for the objection are the same as those indicated in the international search report.

V. Reasoned statement under Rule 66.2(a)(II) with regard to novelty, inventive step or industrial applicability

1. To the extent that the international preliminary examination has been carried out (see item III above), the following is pointed out:
2. In light of the documents cited in the international search report, it is considered that the invention as defined in at least some of the claims, which have been the subject of an international search report, does not appear to meet the criteria mentioned in Article 33(1) PCT, i.e. does not appear to be novel and/or to involve an inventive step (see international search report, in particular the documents cited X and/or Y and corresponding claim references).
3. If amendments are filed, the applicant should comply with the requirements of Rule 66.8 PCT and indicate the basis of the amendments in the documents of the application as originally filed (Article 34 (2) (b) PCT) otherwise these amendments may not be taken into consideration for the establishment of the international preliminary examination report. The attention of the applicant is drawn to the fact that if the application contains an unnecessary plurality of independent claims, no examination of any of the claims will be carried out.

NB: Should the applicant decide to request detailed substantive examination, then an international preliminary examination report will normally be established directly. Exceptionally the examiner may draw up a second written opinion, should this be explicitly requested.

PATENT COOPERATION TREATY

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From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

PCT

To:

Brown, David L.
HASELTINE LAKE
Imperial House
15-19 Kingsway
London WC2B 6UD
GRANDE BRETAGNE

10 JUN 2004

NOTIFICATION OF TRANSMITTAL OF
INTERNATIONAL PRELIMINARY
EXAMINATION REPORT

(PCT Rule 71.1)

Date of mailing
(day/month/year)

09/06/2004

Applicant's or agent's file reference

P81711PC00/DLB

IMPORTANT NOTIFICATION

International application No.

PCT/GB03/01380

International filing date (day/month/year)

27/03/2003

Priority date (day/month/year)

27/03/2002

Applicant

PHYTOPHARM PLC et al

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the International preliminary examination report and its annexes, if any, established on the International application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. REMINDER

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

The applicant's attention is drawn to Article 33(5), which provides that the criteria of novelty, inventive step and industrial applicability described in Article 33(2) to (4) merely serve the purposes of international preliminary examination and that "any Contracting State may apply additional or different criteria for the purposes of deciding whether, in that State, the claimed inventions is patentable or not" (see also Article 27(5)). Such additional criteria may relate, for example, to exemptions from patentability, requirements for enabling disclosure, clarity and support for the claims.

Name and mailing address of the IPEA/

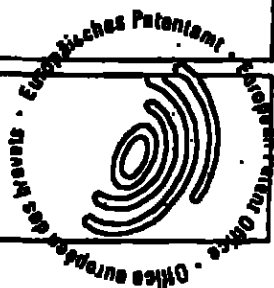


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Authorized officer

JANZING M A K

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PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

(Rationalised Report according to the Notice of the President of the EPO published in the OJ11/2001)

Applicant's or agent's file reference P81711PC00/DLB	FOR FURTHER ACTION See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPBA/416)	
International application No. PCT/GB03/01380	International filing date (day/month/year) 27/03/2003	Priority date (day/month/year) 27/03/2002
International Patent Classification (IPC) or national classification and IPC A61K31/585		
Applicant PHYTOPHARM PLC et al		

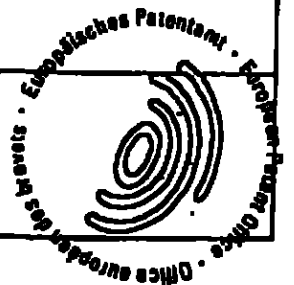
1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 2 sheets, including this cover sheet.
☐ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consists of a total of _____ sheets.

3. This report contains indications relating to the following items:
 - I ☒ Basis of the report
 - II ☐ Priority
 - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
 - IV ☒ Lack of unity of invention
 - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
 - VI ☐ Certain documents cited
 - VII ☐ Certain defects in the international application
 - VIII ☐ Certain observations on the international application

Date of submission of the demand 22/10/2003	Date of completion of this report 04/06/2004
Name and mailing address of the IPEA/  European Patent Office, P.B. 3318 Patentlaan 2 NL-2280 HV Rijswijk - Netherlands Tel.: (+31-70) 340-2040 Fax: (+31-70) 340-3016	Authorized officer HOEKSTRA S S Tel. (+49-49) 2399 2828

Form PCT/IPBA/409 (cover sheet) P20476 (October 2002)



**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No.

PCT/GB03/01380

I. Basis of the report

The basis of this international preliminary examination is the application as originally filed.

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The question of whether the claimed invention appears to be novel, to involve an inventive step, or to be industrially applicable has not been the subject of the international preliminary examination in respect of the claims which have not been searched (Article 17(2)(a) or (3) and Rule 66.1(e) PCT; see also international search report).

IV. Lack of unity of invention

The objection as to lack of unity raised in the international search report is maintained. The reasons for the objection are the same as those indicated in the international search report.

V. Reasoned statement under Rule 66.2(a)(II) with regard to novelty, inventive step or industrial applicability

To the extent that the international preliminary examination has been carried out (see item III above), the following is pointed out:

In light of the documents cited in the international search report, it is considered that the invention as defined in at least some of the claims, which have been the subject of an international search report, does not appear to meet the criteria mentioned in Article 33(1) PCT, i.e. does not appear to be novel and/or to involve an inventive step (see international search report, in particular the documents cited X and/or Y and corresponding claim references).

Traducción

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TRATADO DE COOPERACIÓN DE PATENTES
PCT
INFORME DE EXAMEN PRELIMINAR INTERNACIONAL
(Artículo 36 y Regla 70 del PCT)

Referencia del expediente del agente o solicitante P61711PC00/DLB	PARA ACTUACIONES ADICIONALES	Ver notificación de Transmisión del Reporte de Examen Preliminar (Formato PCT/IPEA416)
Solicitud Internacional No. PCT/GB03/01380	Fecha de presentación internacional (día/mes/año) 27/03/2003	Fecha de prioridad (día/mes/año) 27/03/2002
Clasificación Internacional de Patentes (IPC) o clasificación nacional e IPC A61K 31/585		
Solicitante PHYTOPHARM PLC et al		

1. Este informe de examen preliminar internacional ha sido preparado por esta Administración encargada del Examen Preliminar Internacional y se envía al solicitante en concordancia con el artículo 36. 2. Este INFORME consta de una total de 2 hojas, incluyendo la portada ☐ Este informe también se acompaña de ANEXOS, esto es documentos de la descripción, reivindicaciones y/o dibujos que han sido corregidos y que son la base de este Informe y/o documentos que contienen rectificaciones realizadas ante esta Administración (Ver Regla 70.16 y Sección 607 de las Instrucciones Administrativas según el PCT) Estos anexos constan de un total de ____ hojas.
3. Este informe contiene Indicaciones relativas a los siguientes aspectos: I ☒ Fundamentos del Informe II ☐ Prioridad III ☒ No se establece concepto relativo a la novedad, nivel inventivo y aplicación Industrial IV ☒ Carencia de unidad de la invención V ☒ Declaración razonada según el artículo 35(2), en relación con la novedad, nivel inventivo o aplicación industrial; citaciones y explicaciones que soportan dicha declaración VI ☐ Ciertos documentos citados VII ☐ Ciertos defectos de la solicitud internacional VIII ☐ Ciertas observaciones respecto a la solicitud internacional

Fecha de presentación de la demanda 22/10/2003	Fecha de terminación de este reporte 04/06/2004
Nombre y dirección de correo de la autoridad encargada del examen preliminar  Oficina Europea de Patentes D-80299 Munich Tel. +49 89 23699 - 0 Tlx 523658 epmu d Fax: +49 89 23699 - 4485	Funcionario Autorizado HOEKSTRA S S Teléfono No. (+49-89) 2369 2828

Formato PCT/IPEA/409 (portada) (julio 1998)

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I. Bases del Informe

La base del presente examen preliminar internacional es la solicitud como se presentó originalmente.

III. No-establecimiento de opinión con respecto a la novedad, nivel inventivo y aplicabilidad industrial.

La pregunta de si la invención reivindicada parece tener novedad, nivel inventivo o que sea aplicable industrialmente no ha sido sujeto de examen preliminar internacional con respecto a las reivindicaciones que no han sido sometidas a búsqueda (Artículo 17(2)(a) o (3) y Regla 66.1(e) del PCT; ver también el Informe de búsqueda internacional).

IV. Falta de Unidad de Invención

Se mantiene la objeción con respecto a la unidad de invención objetada en el Informe de búsqueda internacional. Las razones de la objeción son las mismas a las indicadas en el Informe de búsqueda internacional.

V. Declaración motivada según el Artículo 35(2) en cuanto a la novedad, el nivel inventivo y la aplicación industrial; citas y explicaciones que apoyan esta declaración.

Hasta el punto en que el examen preliminar ha sido llevado a cabo (ver ítem III arriba), puede señalarse lo siguiente:

A la luz de los documentos citados en el Informe de búsqueda internacional se considera que la invención como se define en por lo menos algunas de las reivindicaciones, que han sido sujeto del Informe de búsqueda internacional, parece no cumplir con los criterios mencionados en el Artículo 33(1) del PCT, es decir, no parece ser novedoso y/o alcanzar el nivel inventivo (ver Informe de búsqueda internacional, en particular los documentos citados con Xy/o Y y las correspondientes reivindicaciones referenciadas).

MÉTODOS Y USOS TERAPÉUTICOS DE SAPOGENINAS Y SUS

DERIVADOS

Campo de la Invención

La presente invención se refiere a métodos y usos terapéuticos de sapogeninas, compuestos relacionados y derivados.

Los usos de sapogeninas, compuestos relacionados y sus derivados se encuentran en el campo del tratamiento de la neurodegeneración no cognitiva, degeneración neuromuscular no cognitiva, neurodegeneración motora-sensorial o disfunción o pérdida de los receptores. En otro aspecto, la invención se refiere a composiciones para el uso en dichos tratamientos.

Antecedentes de la Invención

La disfunción cognitiva es una característica de condiciones y síntomas de demencia, tales como la enfermedad de Alzheimer, demencia senil de tipo Alzheimer, demencia corporal Lewy y demencia vascular. Un grado menor de disfunción cognitiva también es una característica de ciertas condiciones y síndromes distintos de la demencia, tales como deficiencia cognitiva leve, deficiencia de la memoria asociada con la edad, autismo y neurodeficiencia.

La neurodegeneración no cognitiva (es decir, neurodegeneración en ausencia de disfunción cognitiva), degeneración neuromuscular no cognitiva (es decir, degeneración neuromuscular en ausencia de disfunción cognitiva) y neurodegeneración motora-sensorial son

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características de condiciones y síndromes tales como la enfermedad de Parkinson, distrofia muscular, incluyendo distrofia muscular facioescapulohumeral, distrofia muscular de Duchenne, distrofia muscular de Becker y distrofia muscular de Bruce, distrofia de Fuchs, distrofia miotónica, distrofia corneal, síndrome de distrofia simpática refleja, distrofia neurovascular, miastenia grave, enfermedad de Lambert Eaton, enfermedad de Huntington, esclerosis lateral amiotrófica y esclerosis múltiple.

La disfunción o pérdida de los receptores, particularmente disfunción o pérdida de receptores nicotínicos y/o muscarínicos de acetilcolina y/o receptores de dopamina y/o adrenoreceptores, es una característica de algunas o todas las condiciones y síndromes antes mencionados. La disfunción o pérdida de los receptores en ausencia de deficiencia cognitiva, neural o neuromuscular también es una característica de las condiciones y síndromes tales como hipotensión postural, síndrome de fatiga crónica, asma, susceptibilidad a insuficiencia cardíaca y degeneración macular.

Las condiciones y síndromes antes mencionados son problemas graves y crecientes en todas las sociedades en donde, debido a un incremento en la expectativa de vida y el control de enfermedades extrañas, el perfil demográfico se expande de una manera creciente hacia una población de mayor edad. Los agentes que pueden tratar o ayudar al manejo o

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prevención de dichos trastornos, son urgentemente necesarios.

DE-A-4303214, cuya divulgación se incorpora al presente por referencia, sugiere el uso de saponinas y sapogeninas en el tratamiento de enfermedades virales, pero sin datos que permitirían a un experto en la técnica seleccionar un grupo particular de compuestos para cualquier enfermedad viral particular.

WO-A-99/16786, cuya divulgación se incorpora al presente por referencia, sugiere el uso de ciertas saponinas y sapogeninas en el tratamiento de la demencia.

WO-A-99/48482, WO-A-99/48507, WO-A-01/23406, WO-A-01/23407, WO-A-01/23408, cuyas divulgaciones se incorporan al el presente por referencia, se refieren al uso de ciertas saponinas, sapogeninas y sus derivados en el tratamiento de la disfunción cognitiva y condiciones asociadas.

La Solicitud de la Patente China No.CN-A-1096031, cuya divulgación se incorpora al presente por referencia, sugiere una bioactividad de sapogenina de espiroestano, sarsasapogenina, en la regulación de doble vía de receptores β -adrenérgicos y M-colinérgicos. No se sugiere ninguna actividad farmacéutica específica. Sin embargo, en "Síntesis y Aplicaciones de Compuestos Etiquetados Isotópicamente", 1998, páginas 315-320, Yi et al describe el uso de sarsasapogenina en el tratamiento de la demencia senil.

Existe un número de trastornos llamados "espectros", en los que se presenta un amplio rango de combinaciones de

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síntomas, en un amplio rango de gravedades relativas. La gravedad de cada síntoma y la combinación particular de síntomas y la combinación particular de síntomas variará según cada paciente y de acuerdo con la etapa del avance del trastorno. En los casos de la enfermedad de Parkinson, miastenia grave, enfermedad de Lambert Eaton, hipotensión postural, síndrome de fatiga crónica, por ejemplo, la disfunción cognitiva no es un síntoma primario, a pesar de que puede estar presente como uno de los posibles síntomas secundarios. Más aún, estas condiciones no son enfermedades virales o demencias. A muchos de estos trastornos se les conoce como "espectros". Por lo tanto, en muchos casos, un tratamiento para la disfunción cognitiva (por ejemplo, demencia) no es necesario.

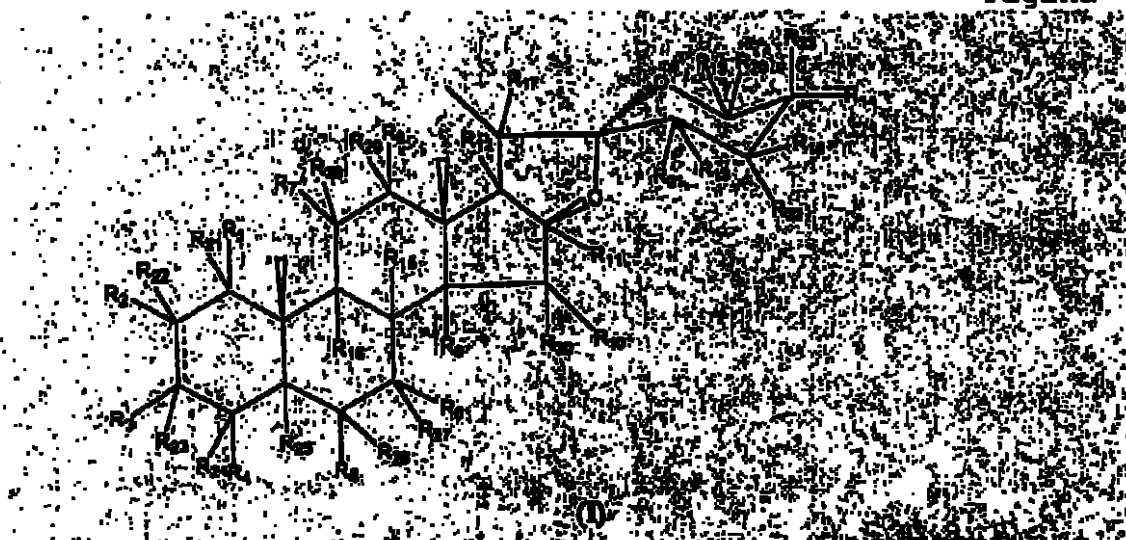
La presente invención se basa en nuestro descubrimiento de que ciertas sapogeninas y sus derivados, incluyendo saponinas, tienen una sorprendente actividad que modifica la enfermedad contra la neurodegeneración no cognitiva, degeneración neuromuscular no cognitiva, neurodegeneración motora-sensorial, así como contra la disfunción o pérdida de receptores en ausencia de deficiencia cognitiva, neural y neuromuscular y, por tanto, previenen y revierten activamente las condiciones. Este descubrimiento permite el tratamiento mejorado de ciertos trastornos no virales, espectros y no espectros cuya disfunción cognitiva no es un síntoma primario.

Breve Descripción de la Invención

De acuerdo con un aspecto de la presente invención, se contempla el uso de agentes activos (como se define en el presente) en el tratamiento o prevención, o en la preparación de composiciones (por ejemplo, composiciones farmacéuticas, productos alimenticios, suplementos alimenticios y bebidas) para el tratamiento o prevención de, (i) neurodegeneración no cognitiva, (ii) degeneración neuromuscular no cognitiva, (iii) neurodegeneración motora-sensorial, (iv) disfunción o pérdida del receptor en ausencia de deficiencia cognitiva, neural y neuromuscular en humanos y animales que sufren de las mismas o son susceptibles a ellas.

La expresión "agentes activos" se refiere a los compuestos de las siguientes fórmulas generales I, II y III, derivados de sapogenina como se define a continuación por referencia a derivados que presentan al menos un sustituto del radical X, derivados sustituidos con azúcar de dichos compuestos como se define más adelante, todos sus esteroisómeros y mezclas racémicas, todos sus pro-fármacos y sales farmacéuticamente aceptables, y todas las mezclas y combinaciones de los mismos:

Fórmula I:



caracterizados porque en la fórmula general (I):

$-R_1, R_2, R_3, R_4, R_5, R_6, R_7, R_8, R_{10}, R_{13}, R_{18}, R_{19}, R_{20}, R_{21}, R_{22}, R_{23}, R_{24}, R_{26}, R_{27}, R_{28}, R_{29}, R_{30}, R_{31}, R_{32}$, son independientemente H, OH, =O, átomo halo, (Me-S-), (Me-SO-), (Me-SO₂-), N₃-NH₂-MeSO₂NH-, alquilo o ausente u OR, en donde R = grupo alquilo o acilo;

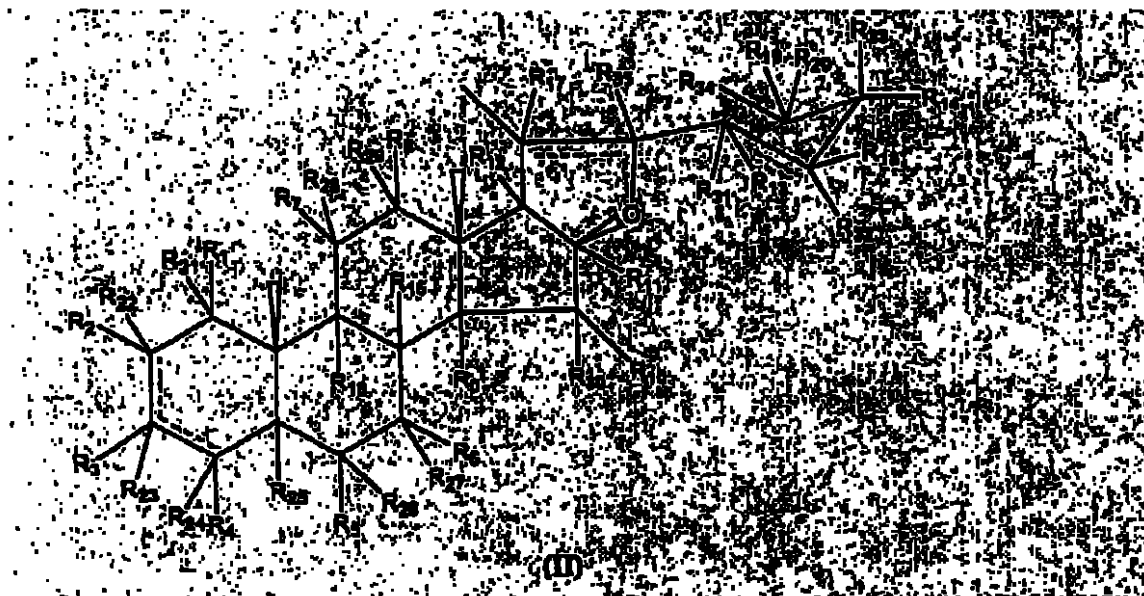
$-R_9, R_{11}, R_{12}, R_{14}, R_{15}, R_{16}, R_{17}, R_{25}, R_{33}$, pueden ser un H, OH, átomo halo, (Me-S-), (Me-SO-), (Me-SO₂-), N₃-NH₂-MeSO₂NH-, alquilo o ausente u OR, en donde R = grupo alquilo o acilo;

..... representa un enlace doble opcional;

en donde además de lo anterior

R_{33} o R_{14} = grupo alquilo;

Fórmula II:



caracterizados porque en la fórmula general (II):

$-R_1, R_2, R_3, R_4, R_5, R_6, R_7, R_8, R_{10}, R_{13}, R_{18}, R_{19}, R_{20}, R_{21}, R_{22}, R_{23}, R_{24}, R_{26}, R_{27}, R_{28}, R_{29}, R_{30}, R_{31}, R_{32}, R_{34}$, son independientemente H, OH, =O, átomo halo, (Me-S-), (Me-SO-), (Me-SO₂-), N₃-NH₂-MeSO₂NH-, alquilo, o ausente u OR, en donde R = grupo alquilo o acilo, o ausente ;

$-R_9, R_{11}, R_{12}, R_{14}, R_{15}, R_{16}, R_{17}, R_{25}, R_{33}, R_{35}$ pueden ser H, OH,

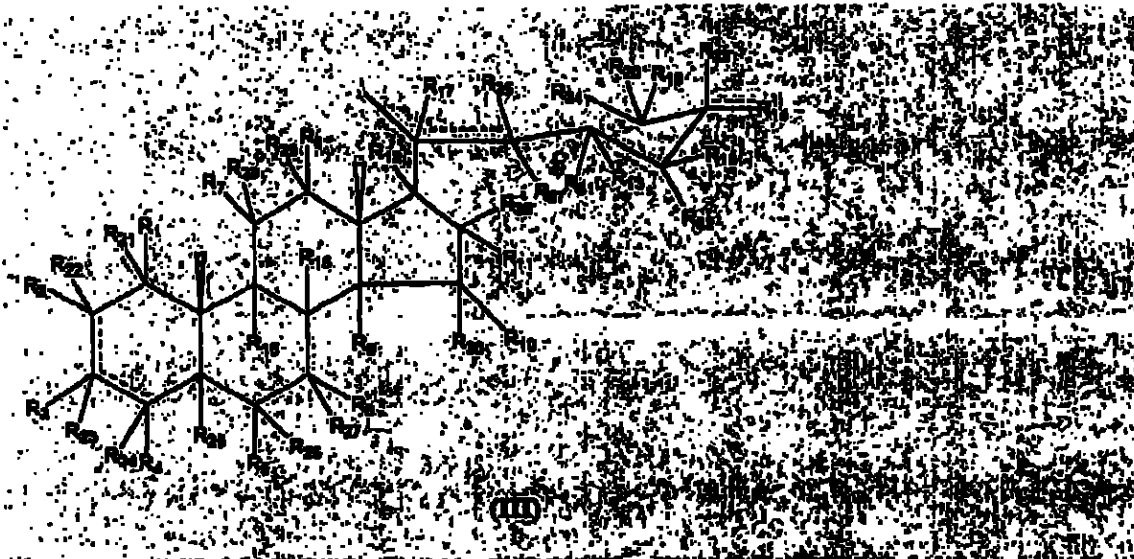
átomo halo , (Me-S-), (Me-SO-), (Me-SO₂-), N₃- NH₂- MeSO₂NH-,
alquilo o ausente u OR, en donde R = grupo alquilo o acilo o
ausente;

..... representa un enlace doble opcional;

en donde además de lo anterior

R₃₃ o R₁₄ = grupo alquilo;

Fórmula III:



caracterizado porque en la fórmula general (III):

-R₁, R₂, R₃, R₄, R₅, R₆, R₇, R₈, R₁₀, R₁₃, R₁₄, R₁₈, R₁₉, R₂₀, R₂₁, R₂₂,
R₂₃, R₂₄, R₂₆, R₂₇, R₂₈, R₂₉, R₃₀, R₃₁, R₃₂, R₃₃, R₃₄, R₃₅, R₃₆, R₃₇, son

independientemente H, OH, =O, átomo halo , (Me-S-), (Me-SO-), (Me-SO₂-), N₃- NH₂- MeSO₂NH-, alquilo o ausente , O R donde R = grupo alquilo o acilo, o ausente ;

-R₉, R₁₁, R₁₂, R₁₅, R₁₆, R₁₇, R₂₃, pueden ser H, OH, átomo halo , (Me-S-), (Me-SO-), (Me-SO₂-), N₃- NH₂- MeSO₂NH-, alquilo o ausente O R = grupo alquilo o acilo, o ausente ;

..... representa un enlace doble opcional;

en donde además de lo anterior

R₃₃ o R₁₄ = grupo alquilo; y

la estereoquímica de R₂₅ está en la orientación β,

los derivados de sapogenina, en particular pero no exclusivamente derivados de sapogenina de espiroestano esteroideas que presentan al menos un sustituto de radical X,

en donde X se escoge del grupo que consiste en:

-átomo halo ,

-(Me-S-), (Me-SO-), (Me-SO₂-),

- N₃-, NH₂-, MeSO₂NH-, y

-alquilo, y

formas derivadas de alguno de los compuestos antes mencionados, en los que el átomo de carbono en la posición 3 (es decir, el carbono al cual se enlaza R₃, o la posición correspondiente en los derivados de sapogenina definidos anteriormente sin una fórmula) o, en el caso de las Fórmulas II y III, el átomo de carbono en la posición 3, la posición 26 (es decir, el carbono al cual se enlaza R₃₄) o cada uno de los átomos de carbono en las posiciones 3 y 26, transporta una

porción de azúcar-O en donde el grupo azúcar es un mono, di o trisacárido.

A las formas derivadas que transportan azúcar de agentes activos de sapogenina generalmente se les conoce en la técnica como saponinas. La expresión "carbohidrato" usada en el presente incluye particularmente dichos grupos de azúcar.

Los agentes activos usados en la presente invención son preferiblemente sapogeninas esteroideas no estrogénicas, saponinas, y derivados de los mismos dentro de los términos de la definición antes mencionada, incluyendo todos los productos intermedios y sales de los mismos fisiológicamente aceptables.

Los agentes activos pueden ser naturales o no naturales. Los agentes activos no naturales pueden prepararse adecuadamente mediante la modificación de grupos secundarios y/o átomos secundarios de compuestos naturales, como se describe a continuación y como se conoce en la técnica.

La invención también proporciona métodos para el tratamiento de humanos y animales, y composiciones que contiene agentes activos para el uso en dichos métodos de tratamiento.

Si se deseara, los agentes activos de la invención pueden coadministrarse con uno o más agentes activos adicionales, por ejemplo, uno o más agentes seleccionados, a título enunciativo, de inhibidores de colinesterasa, agonistas de dopamina (por ejemplo, L-dopa), inhibidores COMT, inhibidores MAO-B, anti-colinérgicos, agonistas de acetilcolina, agonistas

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de serotonina, agonistas de receptores AMPA, agonistas de receptores GABA, agonistas de receptores NMDA, agonistas de β -adrenoceptores, digoxina, dobutamina, antiinflamatorios, factores neurotróficos, estatinas, antagonistas de receptores de adenosina A2a, inhibidores de aldosa reductasa, inmunomoduladores, agonistas de cannabinoides, interferón β o antidepresivos tricíclicos.

Los agentes activos se pueden aplicar terapéutica o profilácticamente a humanos y animales que sufren o son susceptibles a condiciones y enfermedades que se caracterizan por la neurodegeneración no cognitiva, degeneración neuromuscular no cognitiva, neurodegeneración motora-sensorial, o disfunción o pérdida de receptores. Ejemplos de dichas condiciones y enfermedades se proporcionan más adelante.

La presente invención, por tanto, también proporciona el uso de agentes activos (como se define en el presente) en el tratamiento o prevención de, o en la preparación de composiciones (por ejemplo composiciones farmacéuticas, alimentos, suplementos alimenticios y bebidas) para el tratamiento o prevención de una o más de dichas condiciones y enfermedades en humanos y animales que sufren de las mismas o son susceptibles a ellas.

Descripción Detallada de la Invención

Ejemplos de Agentes Activos

Las siguientes clases de agentes activos se mencionan en forma

particular:

1. Compuestos de la fórmula general I, caracterizados porque:

-R₁, R₂, R₃, R₄, R₅, R₆, R₇, R₈, R₁₀, R₁₃, R₁₈, R₁₉, R₂₀, R₂₁, R₂₂, R₂₃, R₂₄, R₂₆, R₂₇, R₂₈, R₂₉, R₃₀, R₃₁, R₃₂, son independientemente H, OH, =O, átomo halo , (Me-S-), (Me-SO-), (Me-SO₂-), N₃- NH₂- MeSO₂NH- , alquilo o ausente OR donde R = grupo alquilo o acilo;

-R₉, R₁₁, R₁₂, R₁₄, R₁₅, R₁₆, R₁₇, R₂₅, R₃₃, pueden ser H, OH, átomo halo , (Me-S-), (Me-SO-), (Me-SO₂-), N₃- NH₂- MeSO₂NH- alquilo o ausente u OR, en donde R = grupo alquilo o acilo;

..... representa un enlace doble opcional;

en donde además de lo anterior

R₃₃ o R₁₄ = grupo alquilo;

y la estereoquímica de R₂₅ está en la orientación β;

1. Compuestos de la fórmula general I, caracterizados porque:

-R₁, R₂, R₃, R₄, R₅, R₆, R₇, R₈, R₁₀, R₁₃, R₁₈, R₁₉, R₂₀, R₂₁, R₂₂, R₂₃, R₂₄, R₂₆, R₂₇, R₂₈, R₂₉, R₃₀, R₃₁, R₃₂, son independientemente H, OH, =O, átomo halo , (Me-S-), (Me-SO-), (Me-SO₂-), N₃- NH₂- MeSO₂NH- , alquilo o ausente u OR, en donde R = grupo alquilo o acilo;

-R₉, R₁₂, R₁₅, R₁₆, R₁₇ = H,

- R₁₁, R₁₄, R₂₅, R₃₃, pueden ser un H, OH, átomo halo , (Me-S-), (Me-SO-), (Me-SO₂-), N₃- NH₂- MeSO₂NH-, alquilo o ausente u OR, en donde R = grupo alquilo o acilo;

..... representa un enlace doble opcional;

en donde además de lo anterior

R₃₃ o R₁₄ = grupo alquilo;

y la estereoquímica de R₂₅ está en la orientación β;

1. Compuestos de la fórmula general I, caracterizados porque:

-R₁ = R₂ =R₄ =R₅ =R₆ =R₇ =R₈ =R₁₀ =R₁₁ =R₉ =R₁₂ =R₁₃ =R₁₅ =R₁₆ =R₁₇ =R₁₈
 =R₁₉ =R₂₀ =R₂₁ =R₂₂ =R₂₃ =R₂₄ =R₂₅ =R₂₆ =R₂₇ =R₂₈ =R₂₉ =R₃₀ =R₃₁ =R₃₂ =R₃₃ =
 H,

- R₃₃ o R₁₄ = CH₃

..... representa un enlace simple,

- el grupo de metilo a C25 puede estar en la configuración R o
 S,

- la estereoquímica de R₂₅ está en la orientación β; y

en donde además de lo anterior

al menos R₃ o R₂₃ es un radical X, el posible sustituyente
 restante es H, OH, =O, y OR, en donde R = grupo alquilo o
 acilo o ausente ,

y X se escoge del grupo que consiste en:

- átomo halo ,

(Me-S-), (Me-SO-), (Me-SO₂-), y

N₃- NH₂- MeSO₂NH-,

alquilo;

1. Compuestos de la fórmula general I, caracterizado porque:

-R₁ = R₂ =R₄ =R₅ =R₆ =R₇ =R₈ =R₁₀ =R₁₁ =R₉ =R₁₂ =R₁₃ =R₁₅ =R₁₆ =R₁₇ =R₁₈
 =R₁₉ =R₂₀ =R₂₁ =R₂₂ =R₂₃ =R₂₄ =R₂₅ =R₂₆ =R₂₇ =R₂₈ =R₂₉ =R₃₀ =R₃₁ =R₃₂ =R₃₃ =
 H,

- R₁₄ = R₃₃ = CH₃

..... representa un enlace simple,

- la estereoquímica de R₂₅ está en la orientación β; y

en donde además de lo anterior

al menos R_3 o R_{23} es un radical X, el posible sustituyente restante es H, OH, =O, y OR, en donde R = grupo alquilo o acilo o ausente,

y X se escoge del grupo que consiste en:

- átomo halo ,

(Me-S-), (Me-SO-), (Me-SO₂-), y

N₃- NH₂- MeSO₂NH-,

alquilo;

1. Compuestos de la fórmula general II, caracterizados porque:

- $R_1, R_2, R_3, R_4, R_5, R_6, R_7, R_8, R_{10}, R_{13}, R_{18}, R_{19}, R_{20}, R_{21}, R_{22}, R_{23}, R_{24}, R_{26}, R_{27}, R_{28}, R_{29}, R_{30}, R_{31}, R_{32}, R_{34}$ son independientemente H, OH, =O, átomo halo , (Me-S-), (Me-SO-), (Me-SO₂-), N₃- NH₂- MeSO₂NH-, alquilo, OR, en donde R = grupo alquilo o acilo, o ausente ;

- $R_9, R_{11}, R_{12}, R_{14}, R_{15}, R_{16}, R_{17}, R_{25}, R_{33}, R_{35}$ pueden ser un H, OH, átomo halo , (Me-S-), (Me-SO-), (Me-SO₂-), N₃- NH₂- MeSO₂NH-, alquilo, OR, en donde R = grupo alquilo o acilo, o ausente

..... representa un enlace doble opcional;

en donde además de lo anterior

R_{33} o R_{14} = grupo alquilo;

y la estereoquímica de R_{25} está en la orientación β ;

1. Compuestos de la fórmula general II y los derivados de carbohidrato de los mismos, caracterizado porque:

- $R_1, R_2, R_3, R_4, R_5, R_6, R_7, R_8, R_{10}, R_{13}, R_{18}, R_{19}, R_{20}, R_{21}, R_{22}, R_{23},$

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$R_{24}, R_{26}, R_{27}, R_{28}, R_{29}, R_{30}, R_{31}, R_{32}$, son independientemente H, OH, =O, átomo halo, (Me-S-), (Me-SO-), (Me-SO₂-), N₃-NH₂-MeSO₂NH-, alquilo, OR, en donde R = grupo alquilo o acilo, o ausente;

$-R_9, R_{12}, R_{15}, R_{16}, R_{17} = H$,

- $R_{34} = H, OH, =O$, y OR, en donde R = alquilo, acilo o carbohidrato, y,

- $R_{11}, R_{14}, R_{25}, R_{33}, R_{35}$, pueden ser un H, OH, átomo halo, (Me-S-), (Me-SO-), (Me-SO₂-), N₃-NH₂-MeSO₂NH-, alquilo, OR, en donde R = grupo alquilo o acilo, o ausente;

..... representa un enlace doble opcional;

en donde además de lo anterior

R_{33} o $R_{14} =$ grupo alquilo;

y la estereoquímica de R_{25} está en la orientación β ;

1. Compuestos de la fórmula general II y los derivados de carbohidrato de los mismos, caracterizado porque:

$-R_1 = R_2 = R_4 = R_5 = R_6 = R_7 = R_8 = R_{10} = R_{11} = R_9 = R_{12} = R_{13} = R_{15} = R_{16} = R_{17} = R_{18} = R_{19} = R_{20} = R_{21} = R_{22} = R_{23} = R_{24} = R_{25} = R_{26} = R_{27} = R_{28} = R_{29} = R_{30} = R_{31} = R_{32} = R_{33} = H$,

- $R_{14} = CH_3$

$R_{34} = -OH$ o OR donde R = alquilo, acilo o carbohidrato, y

$R_{35} = H$ o ausencia

..... representa un enlace doble opcional, y

- el grupo metilo en C25 puede estar en la configuración R o S, y

la estereoquímica de R_{25} está en la orientación β ;

en donde además de lo anterior

al menos R_3 o R_{23} es un radical X, el posible sustituyente restante es H, OH, =O, y OR, en donde R = grupo alquilo o acilo o ausente ,

y X se escoge del grupo que consiste en:

- átomo halo ,

(Me-S-), (Me-SO-), (Me-SO₂-), y

- N₃- NH₂- MeSO₂NH-,

- alquilo;

1. Compuestos de la fórmula general II y los derivados de carbohidrato de los mismos, caracterizado porque:

- $R_1 = R_2 = R_4 = R_5 = R_6 = R_7 = R_8 = R_{10} = R_{11} = R_9 = R_{12} = R_{13} = R_{15} = R_{16} = R_{17} = R_{18} = R_{19} = R_{20} = R_{21} = R_{22} = R_{23} = R_{24} = R_{25} = R_{26} = R_{27} = R_{28} = R_{29} = R_{30} = R_{31} = R_{32} = R_{33} =$
H,

- $R_{14} = R_{33} = \text{CH}_3$

- $R_{34} = \text{-OH U -OR}$, en donde R = alquilo, acilo o carbohidrato,
y

- $R_{35} = \text{H o ausente}$

..... representa un enlace doble opcional, y

- la esteoquímica de R_{24} está en la orientación β ;

en donde además de lo anterior

al menos R_3 o R_{23} es un radical X, el posible sustituyente restante es H, OH, =O, y OR, en donde R = grupo alquilo o acilo o ausente ,

y X se escoge del grupo que consiste en:

- átomo halo ,

(Me-S-), (Me-SO-), (Me-SO₂-), y

N₃- NH₂- MeSO₂NH-,

alquilo;

1. Compuestos de la fórmula general III, caracterizado porque:

-R₁, R₂, R₃, R₄, R₅, R₆, R₇, R₈, R₁₀, R₁₃, R₁₄, R₁₈, R₁₉, R₂₀, R₂₁, R₂₂, R₂₃, R₂₄, R₂₆, R₂₇, R₂₈, R₂₉, R₃₀, R₃₁, R₃₂, R₃₃, R₃₄, R₃₅, R₃₆, R₃₇ son independientemente H, OH, =O, átomo halo, (Me-S-), (Me-SO-), (Me-SO₂-), N₃- NH₂- MeSO₂NH-, alquilo, OR, en donde R = grupo alquilo o acilo, o ausencia ;

-R₉, R₁₁, R₁₂, R₁₅, R₁₆, R₁₇, R₂₅, pueden ser un H, OH, átomo halo, (Me-S-), (Me-SO-), (Me-SO₂-), N₃- NH₂- MeSO₂NH-, alquilo, OR en donde R = grupo alquilo o acilo, o ausente

..... representa un enlace doble opcional;

en donde además de lo anterior

R₃₃ o R₁₄ = grupo alquilo; y

y la estereoquímica de R₂₅ está en la orientación β;

1. Compuestos de la fórmula general III, caracterizado porque:

-R₁, R₂, R₃, R₄, R₅, R₆, R₇, R₈, R₁₀, R₁₃, R₁₄, R₁₈, R₁₉, R₂₀, R₂₁, R₂₂, R₂₃, R₂₄, R₂₆, R₂₇, R₂₈, R₂₉, R₃₀, R₃₁, R₃₂, R₃₃, R₃₅, R₃₆, R₃₇ son independientemente H, OH, =O, átomo halo, (Me-S-), (Me-SO-), (Me-SO₂-), N₃- NH₂- MeSO₂NH-, alquilo, OR, en donde R = grupo alquilo o acilo, o ausente ;

-R₉, R₁₂, R₁₅, R₁₆, R₁₇, = H,

-R₃₄ = H, OH, =O, átomo halo, (Me-S-), (Me-SO-), (Me-SO₂-), N₃-

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 $\text{NH}_2\text{-MeSO}_2\text{NH-}$, alquilo, OR, en donde R = grupo alquilo, acilo o carbohidrato, o ausente ;

- R_{11} , R_{25} , pueden ser un H, OH, átomo halo , (Me-S-), (Me-SO-), (Me-SO₂-), $\text{N}_3\text{-NH}_2\text{-MeSO}_2\text{NH-}$, alquilo, OR, en donde R = grupo alquilo o acilo, o ausente

..... representa un enlace doble opcional;

en donde además de lo anterior

R_{33} o R_{14} = grupo alquilo; y

y la estereoquímica de R_{25} está en la orientación β ;

1. Los compuestos de la fórmula general III y derivados de carbohidrato de los mismos, caracterizado porque:

$\text{-R}_1 = \text{R}_2 = \text{R}_4 = \text{R}_5 = \text{R}_6 = \text{R}_7 = \text{R}_8 = \text{R}_{10} = \text{R}_{11} = \text{R}_9 = \text{R}_{12} = \text{R}_{13} = \text{R}_{15} = \text{R}_{16} = \text{R}_{17} = \text{R}_{18} = \text{R}_{19} = \text{R}_{20} = \text{R}_{21} = \text{R}_{22} = \text{R}_{23} = \text{R}_{24} = \text{R}_{25} = \text{R}_{26} = \text{R}_{27} = \text{R}_{28} = \text{R}_{29} = \text{R}_{30} = \text{R}_{31} = \text{R}_{32} = \text{R}_{33} = \text{H},$

- $\text{R}_{14} = \text{CH}_3$

- $\text{R}_{34} = \text{-OH}$ o -OR , en donde R = alquilo, acilo o carbohidrato, y

$\text{R}_{35} = \text{H}$ o está ausente

$\text{R}_{37} = \text{H}$, -OH o $=\text{O}$

$\text{R}_{36} = \text{H}$ o -OH

..... representa un enlace simple, y

el grupo metilo en C25 puede estar en la configuración R o S,

la estereoquímica de R_{25} está en la orientación β ; y

en donde además de lo anterior

al menos R_3 o R_{23} es un radical X, el posible sustituyente

restante es H, OH, =O, y OR, en donde R = grupo alquilo o acilo o ausente ,

y X se escoge del grupo que consiste en:

- átomo halo,

(Me-S-), (Me-SO-), (Me-SO₂-), y

N₃- NH₂- MeSO₂NH-,

alquilo;

1. Compuestos de la fórmula general III y derivados de carbohidrato de los mismos, caracterizado porque:

-R₁ = R₂ =R₄ =R₅ =R₆ =R₇ =R₈ =R₁₀ =R₁₁ =R₉ =R₁₂ =R₁₃ =R₁₅ =R₁₆ =R₁₇ =R₁₈
 =R₁₉ =R₂₀ =R₂₁ =R₂₂ =R₂₃ =R₂₄ =R₂₅ =R₂₆ =R₂₇ =R₂₈ =R₂₉ =R₃₀ =R₃₁ =R₃₂ =R₁₉ =
 R₂₀ =H,

- R₁₄ =R₃₃ = CH₃

R₃₄ = -OH o -OR, en donde R = alquilo, acilo o carbohidrato, y

R₃₅ = H o está ausente

R₃₇ = H, -OH o =O

R₃₆ = H o -OH

..... representa un enlace simple, y

el grupo metilo en C25 puede estar en la configuración R o S,

la estereoquímica de R₂₅ está en la orientación β; y

en donde además de lo anterior

al menos R₃ o R₂₃ es un radical X, el posible sustituyente restante es H, OH, =O, y OR, en donde R = grupo alquilo o acilo o ausente ,

y X se escoge del grupo que consiste en:

- átomo halo ,

- (Me-S-), (Me-SO-), (Me-SO₂-), y

N₃- NH₂- MeSO₂NH-,

alquilo;

13. Sapogeninas sustituidas, en particular pero no exclusivamente sapogeninas de espiroestano esteroideas, caracterizadas porque al menos un grupo OH de la sapogenina se sustituye con X, seleccionado del grupo que consiste en:

- halo átomo,

-(Me-S-), (Me-SO-), (Me-SO₂-),

- N₃- NH₂- MeSO₂NH-, y

alquilo;

13. Las sapogeninas definidas líneas arriba en particular pero no exclusivamente sapogeninas de espiroestano esteroideas, caracterizadas porque en la definición de X el átomo halo es un átomo de fluoro;

Las sapogeninas sustituidas seleccionadas de:

(3β-fluoro-5β, 20α,22α,25R-espiroestano), (3,3-difluor -

5β,20α,22α,25R-espiroestano), (3α-metilsulfonilamino-5

β,20α,22α,25R-espiroestano), (3α-azido-5β,20α,22α,25R-

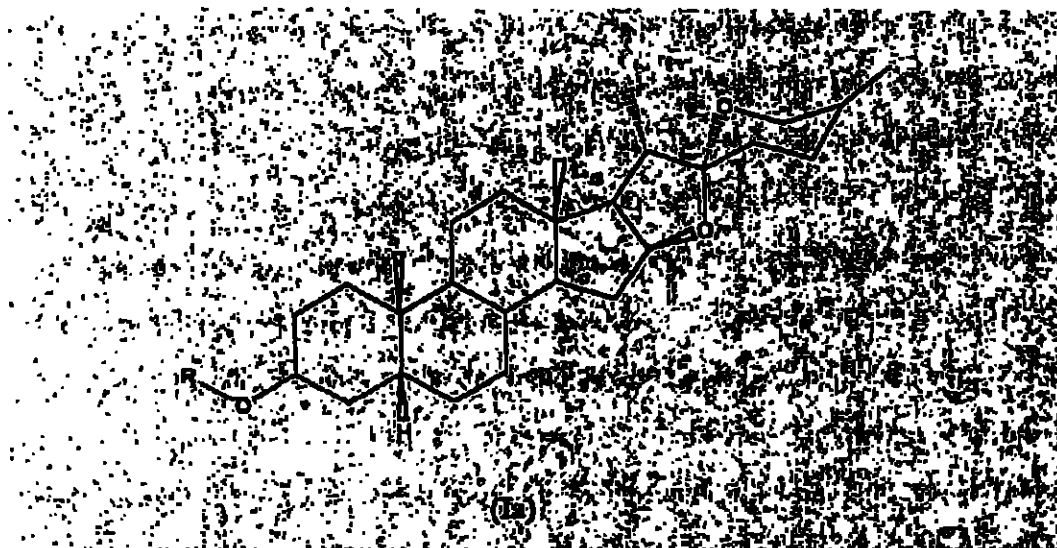
espiroestano), (3α-amino-5β,20α,22α,25R-espiroestano), y

sus estereoisómeros y mezclas racémicas, sus productos intermedios y sales farmacéuticamente aceptables;

16. Las sapogeninas sustituidas caracterizadas porque la

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sapogenina precursora, que luego se sustituye con al
menos un radical X como se define anteriormente, se
selecciona de sarsasapogenina, episarsasapogenina,
esmilagenina, epismilagenina, y anzurogenina-D;

13. Compuestos de la fórmula general Ia:



caracterizado porque el grupo R se selecciona de hidrógeno; alquilcarbonilo; alkoxycarbonilo; alquilcarbamoilo; o arilcarbonilo; o sulfo (HO_2S); fosfono($(\text{HO})_2\text{P}(\text{O})-$); o un mono-, di- o trisacárido; en donde cualquier grupo de alquilo es opcionalmente sustituido con arilo, amino, mono- o di-alquil-amino, un residuo de ácido carboxílico ($-\text{COOH}$), o cualquier

combinación de los mismos;

13. Las formas derivadas de los compuestos antes mencionados como se define en los puntos 1 a 17 en donde el átomo de carbono en la posición 3 o, en caso de las Fórmulas II y III, el átomo de carbono en la posición 3, el átomo de carbono en la posición 26 cada uno de los átomos de carbono en las posiciones 3 y 26, transporta una porción de O-azúcar en donde el grupo de azúcar es un mono-, di- o trisacárido, por ejemplo, una mono aldosa o cetosa que tiene 5 o 6 átomos de carbono, preferiblemente en la forma de furanosa o piranosa ciclizada, ya sea el anómero α o β y que tenga isomerismo óptico D o L, o cualquier combinación de di- y trioligosacáridos de los mismos; formas aciladas de residuos de azúcar también se incluyen dentro del término "azúcar"; ejemplos de azúcares adecuados incluyen glucosa, manosa, fructosa, galactosa, maltosa, celobiosa, sacarosa, ramnosa, xilosa, arabinosa, fucosa, quinovosa, apiosa, lactosa, galactosa-glucosa, glucosa-arabinosa, fucosa-glucosa, ramnosa-glucosa, glucosa-glucosa-glucosa, glucosa-ramnosa, manosa-glucosa, glucosa-(ramnosa)-glucosa, glucosa-(ramnosa)-ramnosa, glucosa-(glucosa)-glucosa, galactosa-(ramnosa)-galactosa y derivados acilados de

éstos (por ejemplo, acetilados).

En las definiciones del compuesto antes mencionado:

Los sustituyentes opcionales de amino, mono-alquil-amino y di-alquil-amino de los grupos alquilo, en donde se encuentran presentes, son preferiblemente un sustituyente mono en la posición α del grupo alquilo.

Los sustituyentes opcionales -COOH de los grupos alquilo, en donde se encuentran presentes, pueden estar en el terminal o en cualquier otra posición del grupo alquilo.

Por "alquilo" se entenderá un grupo de carbohidratos alifáticos que pueden ser rectos o ramificados que tienen entre de 1 átomos de carbono a 20 átomos de carbono en la cadena. Los grupos de alquilo recomendados tienen 1 carbono a 12 átomos de carbono en la cadena. Ramificado significa que uno o más grupos de alquilo inferiores tales como metilo, etilo o propilo se enlazan a una cadena de alquilo lineal. Por "alquilo inferior" se entenderá entre 1 carbono a 4 átomos de carbono en la cadena que pueden ser rectos o ramificados. Los grupos de alquilo a manera de ejemplo incluyen metilo, etilo, n-propilo, i-propilo, n-butilo, t-butilo, s-butilo, n-pentilo, 3-pentilo.

P r "arilo" . se entenderá cualquier grupo que comprende un anillo aromático o sistema de anillos condensados, y preferiblemente contiene hasta 12 átomos de carbono. Un grupo arilo es el grupo fenilo. Un grupo arilo opcionalmente puede ser mono o poli sustituido, p r ejemplo por sustituyentes independiente seleccionados de halo (por ejemplo, cloro o bromo), alquilo, cicloalquilo, hidroxilo, alcoxilo, amino, nitro, acilamino, carboxi y alcoxycarbonilo.

Por "residuo ácido carboxílico" se entenderá el grupo -COOH.

Por "acilo" se entenderá un grupo H-CO o Alquilo-CO caracterizado porque el grupo alquilo es como se define a continuación. Los acilos recomendados contienen un alquilo inferior. Los ejemplos grupo acilo incluyen formilo, acetilo, propanoilo, 2-metilpropanoil , butanoilo y palmitoilo;

Por "opcionalmente sustituido" se entenderá que dicho grupo se puede sustituir con uno o más sustituyentes, que pueden ser los mismos o diferentes, de preferencia uno o más sustituyentes que individualmente tienen un tamaño que es pequeño (por ejemplo, menos de alrededor de 20% de la dimensión molecular más grande) en relación con el grupo precursor que se sustituye; los sustituyentes

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adecuados incluyen halo (p r ejemplo, cloro o bromo),
alquilo, cicloalquilo, hidroxí, alcoxi, amino, acilamino,
arilo, aroylamino, carboxi, alkoxycarbonilo,
aralkoxycarbonilo, heteroaralkoxycarbonilo, y
opcionalmente carbamoilo sustituido, de preferencia con
sujeción a la limitación del tamaño estipulado
anteriormente;

Por "farmacéuticamente aceptable" se entenderá,
dentro del marco de la opinión sólida veterinaria y
médica, que es adecuado para uso en contacto con las
células de humanos y animales inferiores sin indebida
toxicidad, irritación, reacción alérgica y similares, y
están de acuerdo con una relación riesgo/benefici
razonable. Por "profármacos farmacéuticamente
aceptables" se entenderá aquellos profármacos de l s
compuestos que, dentro del marco de la opinión sólida
veterinaria y médica, son adecuados para uso en contacto
con los tejidos humanos y animales inferiores sin
indebida toxicidad, irritación, reacción alérgica y
similares, y están de acuerdo con una relación
riesgo/beneficio razonable, y son efectivos para el uso
que se pretende darles, así como las formas
zwiteriónicas, en la medida de lo posible, de l s
compuestos. Por "profármaco" se entenderán los

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compuestos que rápidamente se transforman *in vivo* para
obtener el compuesto padre de la fórmula antes
mencionada, por ejemplo mediante hidrólisis en la sangre.
Los grupos funcionales que se pueden transformar
rápidamente, mediante división metabólica, forman *in vivo*
una clase de grupos reactivos con el grupo carboxilo.
Debido a la facilidad con la que los grupos de los
compuestos metabólicamente divisibles se dividen *in vivo*,
los compuestos que presentan dichos grupos actúan como
profármacos. Una discusión detallada sobre los
profármacos se proporciona en las siguientes
publicaciones: Design of Prodrugs, H. Bundgaard, ed.,
Elsevier, 1985; Methods in Enzimology, K. Widder y otros,
Ed., Academic Press, 42, p. 309-396, 1985; A Textbook f
Drug Design and Development, Krogsgaard-Larsen y H.
Bundgaard, ed. Capítulo 5; Design and Applications of
Prodrugs p.113-191, 1991; Advanced Drug Delivery Reviews,
H. Bundgard, 8, p. 1-38, 1992; Journal of Pharmaceutical
Sciences, 77, p. 285, 1988; Chem. Pharm. Bull., N. Nakeya
y otros, 32, p. 692, 1984; Pro-drugs as Novel Delivery
Systems, T. Higuchi y V. Stella, Vol. 14 de A.C.S.
Symposium Series, y Bioreversible Carriers in Drug
Design, Edward B. Roche, ed., American Pharmaceutical
Association and Pergamon Press, 1987, los cuales se

incorporan al presente por referencia;

Por "sales farmacéuticamente aceptables" se entenderá las sales de adición ácidos relativamente atóxicas, inorgánicas y orgánicas, y las sales de adición básicas, de los compuestos de la presente invención. En particular, las sales de adición ácidas se pueden preparar sometiendo a reacción separadamente el compuesto purificado en su forma base libre con un ácido orgánico inorgánico adecuado y aislando la sal formada de este modo. Vea, por ejemplo, S.M. Berge y otros, Pharmaceutical Salts, J. Pharm. Sci., 66: p.1-19 (1977) que se incorpora al presente por referencia. Las sales de adición básicas también se pueden preparar sometiendo a reacción separadamente el compuesto purificado en su forma ácida con una base orgánica o inorgánica adecuada y aislando la sal formada de este modo. Las sales de adición básicas incluyen sales de metal y amina farmacéuticamente aceptables. Ejemplos de sales de adición ácidas adecuadas son aquellas formadas con ácidos seleccionados de ácido clorhídrico, sulfúrico, fosfórico y nítrico. Ejemplos de sales de adición básicas adecuadas son aquellas formadas con bases seleccionadas de hidróxido de sodio, hidróxido de potasio e hidróxido de amonio.

Una clase particularmente recomendada de agentes activos son los compuestos de la fórmula general Ia.

En algunos de los compuestos de la fórmula Ia, el grupo C₂₅ metil está en la configuración S; estos compuestos de la invención son sarsasapogenina y episarsasapogenina o sus derivados. En otros compuestos de la fórmula Ia, el grupo C₂₅ metil está en la configuración R; estos compuestos de la invención son esmilagenina y epismilagenina o sus derivados.

En la fórmula anterior Ia, -OR pueden seleccionarse, por ejemplo, de los siguientes compuestos (a menos que se excluya por condición): hidroxí, catilato (etoxicarboniloxi), acetato, succinato, cinamato, ferulato, propionato, butirato, valerato, isovalerato, caproato, isocaproato, dietilacetato, octanoato, decanoato, laurato, miristato, palmitato, estearato, benzoato, fenilacetato, fenilpropionato, cinamato, p-nitrobenzoiloxi, 3,5-dinitrobenzoiloxi, p-clorobenzoiloxi, 2,4-diclorobenzoiloxi, p-bromobenzoiloxi, m-bromobenzoiloxi, p-clorobenzoiloxi, 2,4-diclorobenzoiloxi, p-bromobenzoiloxi, m-bromobenzoiloxi, p-metoxibenzoiloxi, ftalil, glicinato, alaninato, valinato, fenilalaninato, isoleucinato, metioninato, argininato, asparaginato, aspartato,

cisteinato, glutamato, histidinato, lisinato, prolinato, serinato, treoninato, triptofanato, tirosinato, fumarato o maleato.

De los compuestos de la fórmula general Ia y sus sales farmacéuticamente aceptables, se recomienda en particular los siguientes compuestos:

sarsasapogenina

catilato de sarsasapogenina

acetato de sarsasapogenina

succinato de sarsasapogenina y sales farmacéuticamente aceptables del mismo

glicinato de sarsasapogenina y sales farmacéuticamente aceptables del mismo

alaninato de sarsasapogenina y sales farmacéuticamente aceptables del mismo

valinato de sarsasapogenina y sales farmacéuticamente aceptables del mismo

fenilalaninato de sarsasapogenina y sales farmacéuticamente aceptables del mismo

isoleucinato de sarsasapogenina y sales farmacéuticamente aceptables del mismo

metioninato de sarsasapogenina y sales farmacéuticamente aceptables del mismo

episarsasapogenina

catilato de episarsasapogenina

acetato de episarsasapogenina

succinato de episarsasapogenina y sales farmacéuticamente
aceptables del mismo

glicinato de episarsasapogenina y sales farmacéuticamente
aceptables del mismo

alaninato de episarsasapogenina y sales farmacéuticamente
aceptables del mismo

valinato de episarsasapogenina y sales farmacéuticamente
aceptables del mismo

fenilalaninato de episarsasapogenina y sales
farmacéuticamente aceptables de la misma

isoleucinato de episarsasapogenina y sales
farmacéuticamente aceptables del mismo

metioninato de episarsasapogenina y sales
farmacéuticamente aceptables del mismo

esmilagenina

catilato de esmilagenina

acetato de esmilagenina

succinato de esmilagenina y sales farmacéuticamente
aceptables del mismo

glicinato de esmilagenina y sales farmacéuticamente
aceptables de la misma

alaninato de esmilagenina y sales farmacéuticamente

aceptables del mism

valinato de esmilagenina y sales farmacéuticamente

aceptables del mismo

fenilalaninato de esmilagenina y sales farmacéuticamente

aceptables del mismo

isoleucinato de esmilagenina y sales farmacéuticamente

aceptables del mismo

metioninato de esmilagenina y sales farmacéuticamente

aceptables del mismo

epismilagenina

catilato de epismilagenina

acetato de epismilagenina

succinato de epismilagenina y sales farmacéuticamente

aceptables del mismo

glicinato de epismilagenina y sales farmacéuticamente

aceptables del mismo

alaninato de epismilagenina y sales farmacéuticamente

aceptables del mismo

valinato de epismilagenina y sales farmacéuticamente

aceptables del mismo

fenilalaninato de epismilagenina y sales

farmacéuticamente aceptables del mismo

isoleucinato de epismilagenina y sales farmacéuticamente

aceptables del mismo

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metioninato de epismilagenina y sales farmacéuticamente
aceptables del mismo

De los compuestos de saponina (R=azúcar) de la fórmula general Ia, se recomienda en particular los siguientes compuestos: sarsasapogenina, episarsasapogenina, esmilagenina y epismilagenina en los que, en cada caso, el átomo de carbono en la posición 3 transporta una porción de azúcar-O caracterizada porque el grupo de azúcar se selecciona de glucosa, manosa, fructosa, galactosa, maltosa, celobiosa, sacarosa, ramnosa, xilosa, arabinosa, fucosa, quinovosa, apiosa, lactosa, galactosa-glucosa, glucosa-arabinosa, fucosa-glucosa, ramnosa-glucosa, glucosa-glucosa-glucosa, glucosa-ramnosa, manosa-glucosa, glucosa-(ramnosa)-glucosa, glucosa-(ramnosa)-ramnosa, glucosa-(glucosa)-glucosa, galactosa-(ramnosa)-galactosa y derivados acilados de éstos (por ejemplo, acetilados).

Otros ejemplos de agentes activos adecuados incluyen 16,22-epoxicoprostan-3 β -ol, esmilagenona, coprosterol y profármacos farmacéuticamente aceptables y sales de los mismos.

Composiciones y Usos

La presente invención permite y proporciona un método para tratar la neurodegeneración no cognitiva,

degeneración neuromuscular no cognitiva, neurodegeneración motora-sensorial o disfunción o pérdida de receptores en ausencia de deficiencia cognitiva, neural o neuromuscular (en particular pero no exclusivamente en relación con los estados de la enfermedad específica antes mencionada) en humanos o animales que lo necesiten, que comprende administrar a dichos humanos o animales una dosificación efectiva de un agente activo (como se define en el presente) o una sal farmacéuticamente aceptable del mismo.

El agente activo se puede administrar en la forma de una composición que comprende el agente activo y cualquier componente adicional adecuado. La composición puede por ejemplo, ser una composición farmacéutica (medicamento), un alimento, suplemento alimenticio o bebida. Dicha composición puede contener una mezcla de los compuestos específicos, y/o sus sales farmacéuticamente aceptables.

De acuerdo con otro aspecto de la presente invención, se proporciona una composición que tiene actividad para combatir y para usarse en el tratamiento de neurodegeneración no cognitiva, degeneración neuromuscular no cognitiva, neurodegeneración motora-sensorial o disfunción o pérdida de receptores en ausencia de deficiencia cognitiva, neural o

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neuromuscular en un humano o animal no humano, la cual comprende una cantidad efectiva de n compuesto del agente activo.

La expresión "composición farmacéutica", en el contexto de la presente invención, significa una composición que comprende un agente activo y que comprende además portadores, diluyentes, adyuvantes, excipientes o vehículos farmacéuticamente aceptables, tales agentes preservantes, productos de carga, agentes desintegrantes, humidificantes, emulsificantes, agentes de suspensión, edulcorantes, saborizantes, aromatizantes, antibacterianos, antifúngicos, lubricantes y agentes de dispersión, dependiendo de la naturaleza del modo de administración y las formas de dosificación.

Las expresiones "producto alimenticio", "suplemento alimenticio" y "bebida" que se usan en el presente tienen el significado normal para dichas expresiones, y no se limitan a preparaciones farmacéuticas.

La dosificación del agente activo variará notablemente, dependiendo de la severidad de los síntomas que se van a tratar o prevenir. La selección de dosificaciones adecuadas se encuentra dentro de la capacidad de un experto en la técnica, sin un inconveniente indebido. La dosificación del agente

activo, por ejemplo, puede ser mayor que alrededor de 0,1 mg/kg de peso corporal, por ejemplo mayor que alrededor de 0,3 mg/kg de peso corporal, administrándose de preferencia una vez al día. Más típicamente, la dosificación será entre 1 mg/kg y 25 a mg/kg, por ejemplo, entre 1 mg/kg a 10 mg/kg, administrándose de preferencia una vez al día. Para el uso humano, la dosificación puede ser de preferencia entre 70 mg a 700 mg al día.

La expresión "formas de dosificación farmacéuticamente aceptable" significa formas de dosificación de los compuesto o composiciones de la invención, e incluye, por ejemplo, grageas, polvos, elixires, jarabes, preparaciones líquidas, incluyendo suspensiones, aerosoles, inhalantes, tabletas, pastillas, emulsiones, soluciones, gránulos, cápsulas y supositorios, así como preparaciones líquidas para inyecciones, incluyendo, preparaciones de liposomas. Por lo general, las técnicas y formulaciones pueden encontrarse en Remington, Pharmaceutical Sciences, Mack Publishing Co., Easton, PA, última edición..

En general, la referencia que se hace en el presente documento a la presencia de un compuesto de un grupo de compuestos especificados incluye dentro de su alcance la

presencia de dos o más de dichos compuestos.

La presente invención contempla el tratamiento de (i) neurodegeneración no cognitiva, (ii) degeneración neuromuscular no cognitiva, (iii) neurodegeneración motora-sensorial, o (iv) disfunción o pérdida de receptores en ausencia de deficiencia cognitiva, neural y neuromuscular, en un humano o animal no humano que sufre o es susceptible de sufrir cualquiera de las siguientes enfermedades: enfermedad de Parkinson, parkinsonismo postencefálico, depresión, esquizofrenia, distrofia muscular, incluyendo distrofia muscular facioescapulohumeral, distrofia muscular de Duchene, distrofia muscular de Becker y distrofia muscular de Bruce, distrofia de Fuchs, distrofia miotónica, distrofia corneal, síndrome de distrofia simpática refleja, distrofia neurovascular, miastenia grave, enfermedad de Lambert Eaton, enfermedad de Huntington, enfermedades de las neuronas motoras, incluyendo esclerosis lateral amiotrófica, esclerosis múltiple, hipotensión postural, neurodegeneración traumática, por ejemplo, luego de apoplejía o luego de un accidente (por ejemplo, lesión traumática de la cabeza o lesión de la médula espinal), enfermedad de Batten, síndrome de Cockayne, Síndrome de Down, degeneración ganglionar corticobasal, atrofia de

múltiples sistemas, atrofia cerebral, atrofia olivopontocerebelar, atrofia dentatorubral, atrofia palidolusiana, atrofia espinobulbar, neuritis óptica, panencefalitis esclerosante, trastorno de déficit de atención, encefalitis postviral, síndrome postpoliomielitis, síndrome de Fahr, síndrome de Joubert, síndrome de Guillain-Barre, lisencefalía, enfermedad de Moyamoya, desorden de migración neuronal, síndrome autístico, enfermedad de poliglutamina, enfermedad de Niemann-Pick, leucoencefalopatía multifocal progresiva, pseudomotor cerebral, enfermedad de Refsum-Thiébaud, síndrome de Zellweger, parálisis supranuclear, ataxia de Friedrich, ataxia espinocerebelar de tipo 2, síndrome de Rhett, síndrome de Shy-Drager, esclerosis tuberosa, enfermedad de dic, síndrome de fatiga crónica, neuropatías, incluyendo neuropatías hereditarias, neuropatía diabética y neuropatía mitótica, neurogeneración basada en priones, incluyendo enfermedad de Creutzfeldt-Jakob, variante de la enfermedad de Creutzfeldt-Jakob, nueva variante de la enfermedad de Creutzfeldt-Jakob, encefalopatía espongiforme bovina (EEB), GSS, FFI, kuru y síndrome de Alper, enfermedad de Joseph, encefalomiелitis diseminada aguda, aracnoiditis, lesiones vasculares del sistema nervioso central, pérdida

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de la función neuronal de las extremidades, enfermedad de
Charcot-Marie-Tooth, susceptibilidad a insuficiencia
cardíaca, asma y degeneración macular.

Por lo tanto, la invención incluye métodos para tratar o prevenir las enfermedades y las condiciones antes mencionadas en un humano o animal que sufre de éstas o es susceptible a las mismas, los cuales comprenden administrar a dicho humano o animal una cantidad efectiva de un agente efectivo como se define en el presente, así como los usos de los agentes activos en la preparación de composiciones para dicho tratamiento o prevención.

En los casos de la enfermedad de Parkinson, parkinsonismo postencefálico, hipotensión postural, síndrome autista, síndrome de fatiga crónica, mistenia grave y la enfermedad de Lambert Eaton, así como cualquier otra condición dentro del rango de enfermedades a las cuales se refiere la presente invención que se divulgan, o son obvias, los tratamientos puestos a disposición o divulgados en la técnica anterior reconocida líneas arriba, la presente invención puede supeditarse con la condición de que cualquiera de los síntomas de disfunción cognitiva estén ausentes, o cualquiera de los síntomas de disfunción cognitiva

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presentados por un paciente que va a recibir tratamiento
son secundarios o auxiliares a los síntomas de
neurodegeneración no cognitiva, degeneración
neuromuscular no cognitiva, neurodegeneración motora-
sensorial o disfunción o pérdida de receptores n
ausencia de deficiencia cognitiva, neural y
neuromuscular.

Preparación de Compuestos para Usarse en la Invención

La esmilagenina, epismilagenina y sarsasapogenina s n
materiales que están disponibles en el mercado. L s
proveedores incluyen, por ejemplo, Sigma Aldrich,
Research Plus Inc. y Steraloids Inc. Los métodos de
preparación para estos materiales también se encuentran
en la literatura (por ejemplo, una preparación de
episarsapogenina se incluye en JACS p. 5225 (1959)). La
episarsasapogenina puede prepararse por reducción de
sarsasapogenona usando un agente reductor de hidruro de
metal. La sarsasapogenona puede prepararse usando el
método de Lajis y otros, Steroids, 1993, 58, 387-389.

Además como materiales de inicio las saponinas y
sapogeninas pueden encontrarse de manera natural en un
rango de especies de plantas, en particular plantas del
género Smilax, Asparagus, Anemarrhena, Yucca o Agave.
Aunque la esmilagenina o sarsasapogenina se usa de

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acuerdo con esta invención, puede encontrarse en forma de un extracto vegetal, o material de la planta en polvo deshidratada, derivada de una planta del género Smilax, Asparagus, Anemarrhena, Yucca o Agave.

Los métodos para preparar los agentes activos son bien conocidos para los expertos en la técnica. Se encuentran ejemplos, por ejemplo, en WO-A-02/079221 (Ejemplos 5 a 16 de dicho documento describen la preparación de catilato de sarsasapogenina, catilato de episarsasapogenina, succinato de episarsasapogenina, catilato de epismilagenina, clorhidrato de glicinato de episarsasapogenina, clorhidrato de glicinato de sarsasapogenina, clorhidrato de glicinato de epismilagenina, clorhidrato de L-alaninato de epismilagenina, clorhidrato de L-valinato de epismilagenina, clorhidrato de L-isoleucinato de epismilagenina, clorhidrato de L-fenilalaninato de epismilagenina y clorhidrato de L-metioninato de epismilagenina). Los compuestos de la fórmula Ia, distintos de aquellos cuya fórmula es $R=H$, pueden prepararse usando técnicas convencionales en donde $R=H$.

La reacción recomendada es una reacción de sustitución nucleofílica, en la cual un compuesto que tiene OH en la posición 3 se hace reaccionar con un

compuesto de fórmula

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$$L-R,$$

en donde R se selecciona de alquilcarbonilo, alcoxycarbonilo; alquilcarbomoilo, o arylcarbonilo, o en donde cualquier grupo alquilo es opcionalmente sustituido con arilo, amino, mono-alquil-amino, di-alquil-amino, un residuo de ácido carboxílico ($-COOH$), o cualquier combinación de éstos; y L es un grupo saliente, bajo condiciones adecuadas para la sustitución nucleofílica.

Por ejemplo, el compuesto L-R puede ser ácido carboxílico, o si fuera adecuado, un anhídrido, o un haluro de acilo (por ejemplo, cloruro de acilo). Por ejemplo, cuando R es una porción catilato (etoxycarbonilo), el compuesto L-R puede ser cloroformato de etilo.

La reacción se realiza de preferencia en una base como piridina, opcionalmente en presencia de un ácido como ácido clorhídrico.

Los detalles de la reacción para las reacciones de sustitución nucleofílica son bien conocidas. Véase, por ejemplo, RC Larock, en Comprehensive Organic Transformations, editores VCH, 1989.

La dihidrosarsasapogenina se puede preparar usando el método descrito en Marker y Rohrmann (1939), Sterols

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LIH; The structure of the side Chain of sarsasappogenin (Esteroles LIH, Estructura de la Cadena secundaria de sarsasapogenina), J. Am. Chem. Soc. 61, pp. 846-851. Se puede preparar 16,22-Epoxicoprostan-3 β -ol usando el método descrito en Scheer y otros (1955), The C-25 isomerism of smilgenin and sarsasapogenin (Isomerismo C-25 de esmilageina y sarsasapogenina): J. Am. Chem. Soc. 77, pp. 641-646.

En las reacciones descritas en el presente quizá sea necesario proteger grupos funcionales reactivos, por ejemplo, grupos hidroxilo, carboxilo o amino, en donde éstos son deseados en el producto final, para evitar su participación no deseada en las reacciones. Pueden usarse grupos protectores convencionales de acuerdo con la práctica estándar. Para conocer ejemplos, véase TW Green y PGM Wuts, en "Protective Groups in Organic Chemistry (Grupos Protectores en Química Orgánica)", John Wiley & Sons, 1991; JFW McOmie en "Protective Groups in Organic Chemistry (Grupos Protectores en la Química Orgánica)", Plenum Press, 1973. Para proteger sustituyentes amino en los compuestos de la fórmula L-R en donde R es amino-sustituido, se prefiere usar un grupo protector de alcóxicarbonilo, en donde la función amino está presente como un grupo alcóxicarbonilamino (de

preferencia t-butoxicarbonilamino) durante los pasos de síntesis, hasta la desprotección en condiciones ácidas en un solvente seco.

El compuesto preparado de este modo puede recuperarse de la mezcla de reacción por mecanismos convencionales. Por ejemplo, el compuesto puede recuperarse retirando por destilación el solvente de la mezcla de reacción, o si fuera necesario, retirando por destilación el solvente de la mezcla de reacción, vertiendo el residuo en agua, con una posterior extracción con un solvente miscible en agua y retirando por destilación el solvente del extracto. Adicionalmente, si se desea, el producto puede purificarse luego mediante varias técnicas conocidas, como recristalización, reprecipitación, o las distintas técnicas de cromatografía, en particular cromatografía en columna o cromatografía en capa fina preparatoria.

Discusión de la Base para la Actividad

Los usos terapéuticos que subyacen a la presente invención surgen de un número de observaciones nuevas que se documentan en detalle en los ejemplos que se incluyen más adelante. Para entender el fundamento de la invención, resulta útil resumir las observaciones y explicar cómo éstas predicen las actividades terapéuticas reivindicadas en la presente invención a través del rango

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de agentes activos que se han definido líneas arriba.

La esmilagenina, epismilagenina, sarsasapogenina y episarsasapogenina restauran la pérdida de receptores de acetilcolina muscarínicos y adrenoceptores en células que expresan dichos receptores *in vitro*. Estos resultados demuestran que estos compuestos restauran la pérdida de receptores celulares normal (Ejemplo 1).

La sarsasapogenina, catilato de episarsasapogenina, episarsasapogenina, epismilagenina y esmilagenina previenen la neurodegeneración inducida químicamente en neuronas corticales de ratas *in vitro*. Estos resultados demuestran que estos compuestos son neuroprotectores y previenen la neurodegeneración y la neurodeficiencia *in vitro* (Ejemplo 2).

La sarsasapogenina, esmilagenina, 16,22-epoxicoprostan-3 β -ol, esmilagenona, clorhidrato de glicinato de esmilagenina y el coprosterol revierten la neurodegeneración químicamente inducida en neuronas corticales de ratas *in vitro*. Estos resultados demuestran que los compuestos revierten la neurodegeneración sensorial y la neurodeficiencia *in vitro* (Ejemplo 3).

La esmilagenina revierte la apoptosis inducida

químicamente en las neuronas, demostrando que este compuesto es antiapoptósico y neuroprotector *in vitro* (Ejemplo 4).

La esmilagenina y sarsasapogenina aumentan el crecimiento de las neuritas (número y ramificación de las neuritas) en neuronas corticales de ratas *in vitro*, demostrando sus efectos neurotróficos *in vitro* (Ejemplo 5).

La esmilagenina y sarsasapogenina previenen y revierten la neurodegeneración inducida por neurotoxinas (neurotoxina 1-metil-4-fenilpiridinio (MMP⁺) en neuronas dopaminérgicas mesencefálicas *in vitro*. Estos resultados demuestran que estos compuestos previenen y revierten la neurodegeneración y neurodeficiencia *in vitro* (Ejemplos 6 y 7).

La sarsasapogenina y esmilagenina revierten la neurodegeneración inducida químicamente en neuronas motoras de la espina dorsal de ratas *in vitro*. Estos resultados demuestran que los compuestos revierten la neurodegeneración y neurodeficiencia de neuronas motoras *in vitro* (Ejemplo 8).

La sarsasapogenina, episarsasapogenina y esmilagenina reducen el número de respuestas equivocadas en una prueba

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de habilidad cognitiva en ratas de edad avanzada, lo cual se relaciona con un aumento en la densidad de los receptores muscarínicos de acetilcolina en los cerebros de las ratas de edad avanzada luego del tratamiento con los compuestos evaluados. Estos resultados demuestran que los compuestos revierten la neurodeficiencia *in vivo* (Ejemplo 9).

La esmilagenina y sarsasapogenina revierten la disminución de receptores muscarínicos de acetilcolina y de dopamina y la disminución del factor neurotrófico derivado del cerebro (BDNF) en animales de edad avanzada. Estos resultados demuestran que los compuestos revierten la neurodegeneración motora-sensorial y neurodeficiencia *in vivo* (Ejemplo 9).

El catilato de episarsasapogenina, catilato de sarsasapogenina, episarsasapogenina y epismilagenina reducen el número de respuestas equivocadas en una prueba de habilidad cognitiva *in vivo* en ratas jóvenes expuestas a agentes neurotóxicos (ácido iboténico y amiloide - β), y aumentan la densidad de los receptores muscarínicos de acetilcolina en los cerebros. Estos resultados demuestran que los compuestos revierten la neurodeficiencia *in vivo* (Ejemplo 10).

La esmilagenina y sarsasapogenina mejoran la

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supervivencia y la neurodegeneración motora-sensorial y neurodeficiencia en un modelo de ratón de esclerosis lateral amiotrófica y enfermedad de Charcot-Marie-Tooth (Ejemplo 11).

En resumen, se ha descubierto que los compuestos retardan o revierten ciertos aspectos de la degeneración neuronal. Esto incluye la reversión de cambios adversos en el cuerpo de las células, atrofia de extensiones neuronales (neuritas), reducción en la liberación de factores neurotróficos tales como neurotrofinas (por ejemplo, BDNF, NGF, NT-3, NT4/5), factores neurotróficos de la super-familia de TGF- β (por ejemplo, GDNF) y neuroquinas (por ejemplo, CNTF, LIF) y toxicidad o muerte neuronal (apoptosis). Los compuestos son potentemente neuroprotectores, estimulantes del crecimiento de las neuritas y agentes que previenen la neurotoxicidad. También se ha descubierto que los compuestos retrasan o revierten los aumentos en la función colinérgica y dopaminérgica, por ejemplo, aumentos en la densidad de los receptores muscarínicos de acetilcolina y dopamina. Además, hemos encontrado que los efectos neuroprotectores y la reversión de la pérdida de receptores son efectos regulados de manera activa, en los cuales el deterioro anterior se revierte al estado normal o joven con

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protección contra el deterioro continuo. ^{Página} Además, hemos descubierto que la reversión del efecto apoptótico de los compuestos parece ser regulado en el dominio no neoplásico de la vida celular y parece que no desencadena una neoplasia.

Los datos anteriores, tomados juntos, indican la actividad contra los estados de enfermedad que ya se han mencionado en esta solicitud. Asimismo, los datos anteriores indican una probable ausencia de efectos colaterales severos o que amenazan la vida, como el cáncer. Los agentes activos por lo general son no estrogénicos.

La técnica anterior reconoce la base sólida de la predicción de la extensión de las observaciones antes mencionadas y sólidas predicciones de la actividad terapéutica para las estructuras químicas asociadas y los derivados comprendidos por la expresión "agentes activos" en la presente invención.

Es bien sabido en la técnica y en la farmacología que el azúcar, éster y otros grupos en ubicaciones adecuadas en moléculas esteroideas, en particular en las posiciones 3- y/o 26, pueden escindirse por hidrólisis *in vivo*, y podría esperarse observar los mismos efectos en otros átomos de carbono de las moléculas. Además, es bien

sabido en la técnica y en la farmacología que las sales, ácidos libres y bases libres dentro de los términos de la expresión "agentes activos" como se usan en el presente se pueden convertir fácilmente entre sí *in vivo* de acuerdo con el pH del fluido corporal en el cual el agente activo está presente. Además, es bien conocido que los substituyentes de grupos secundarios, en un amplio rango de formas, pueden estar presentes en un esqueleto de carbono sin afectar sustancialmente de manera adversa la actividad farmacológica de la estructura, en particular cuando los grupos secundarios son pequeños en comparación con el tamaño general de la molécula.

Por todas estas razones, las reivindicaciones de la actividad farmacológica beneficiosa realizadas en la presente invención se consideran razonables y se basan en predicciones sólidas y creíbles de los datos de la prueba reunidos y presentados en el presente documento.

Sin pretender confínos a la teoría, se considera que un efecto fisiológico de los agentes activos es la capacidad de aumentar la síntesis, o la liberación, o reducir la velocidad de degradación de factores neurotróficos como factor neurotrófico derivado del cerebro y/o factor de crecimiento de los nervios o sus

receptores. Estos efectos en los factores de crecimiento pueden deberse a un efecto del compuesto en un receptor sistólico o nuclear, o al enlace del compuesto a una región promotora con un consecuente efecto directamente en la tasa de productividad de ARNm para el factor de crecimiento, o como una consecuencia de aumentar la producción de otro factor importante.

Además, parece que los compuestos regulan los receptores. Por ejemplo, se ha descubierto que algunos de esos compuestos previenen o revierten la pérdida de receptores de acetilcolina muscarínica o de dopamina en el cerebro. Se considera que los compuestos funcionan rectificando una deficiencia en el número o la función o el recambio de los receptores.

Breve Descripción de los Dibujos

A fin de ilustrar más la invención a manera de ejemplo no limitativo, ahora se hará referencia a los dibujos adjuntos y a los Ejemplos que siguen.

En los dibujos:

La Figura 1 muestra el efecto del acetato de epismilagenina en la densidad del adrenoceptor α_3 y α_2 el día 5 en una línea celular cotransfectada de CHO- α_3/α_2 .

La Figura 2 muestra los efectos de sarsasapogenina, catilato de episarsasapogenina y esmilagenina en la

neur degeneración . inducida por glutamato en neuronas
corticales primarias de ratas. Página

La Figura 3 muestra los efectos de sarsasapogenina, catilato de episarsasapogenina y esmilagenina en la capacidad para aprender y en la memoria de las ratas de edad avanzada.

La Figura 4 muestra los efectos de la sarsasapogenina, catilato de episarsasapogenina y esmilagenina en el número de receptores muscarínicos.

La Figura 5 muestra el perfil de supervivencia de ratones SOD-1 luego de la administración oral de la esmilagenina.

La Figura 6 muestra el perfil de supervivencia de ratones luego de la administración oral de la sarsapogenina.

Descripción Detallada de los Dibujos y Ejemplos

EJEMPLO 1

Restauración de la pérdida de receptores *in vitro*

Se investigaron los efectos del catilato de epismilagenina, catilato de sarsasapogenina, catilato de episarsasapogenina, succinato de episarsasapogenina, acetato de epismilagenina y sarsasapogenina en el receptor muscarínico de acetilcolina (m) en las células de ovario de cuyo chino o receptores $\beta 2$ y m3 en células de ovario de cuyo chino trasfectadas con un vector para el receptor m o cotransfectado con el vector para l s

receptores β_2 y α_3 .

Los resultados se ilustran en la Tabla 1 que se incluye a continuación y en la Figura 1 de los dibujos. Durante el periodo de cultivo de células de ovario de cuyo chino, las células transfectadas con un vector para el receptor α_3 , el tratamiento con catilato de epismilagenina, catilato de sarsasapogenina, catilato de episarsasapogenina, succinato de episarsasapogenina y sarsasapogenina, independientemente, previene el aumento del número de receptores α_3 . Durante el periodo de cultivo de células de ovario de cuyo chino cotransfectadas con el vector para receptores β_2 y α_3 , la densidad del receptor α_3 no se alteró, mientras que la densidad de los adrenoreceptores β_2 disminuyó. La incubación acetato de epismilagenina (Figura 1) no alteró de manera significativa la densidad de los receptores α_3 ; pero evitó de manera significativa la disminución en los adrenoreceptores β_2 .

Tabla 1 - Efecto de catilato de epismilagenina, catilato de sarsasapogenina, catilato de episarsasapogenina, succinato de episarsasapogenina y sarsasapogenina en la restauración de la densidad de los receptores de acetilcolina α_3 .

Compuesto	Concentración [microm]	Actividad

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Catilato de epismilagenina	10	++
Catilato de sarsasapogenina	10	++
Catilato de episarsasapogenina	10	++
Succinato de episarsasapogenina	10	++
Sarsapogenina	10	++

Así, los experimentos indican que el catilato de epismilagenina, catilato de sarsasapogenina, catilato de episarsasapogenina, succinato de episarsasapogenina, acetato de epismilagenina y sarsasapogenina, independientemente, pudieron prevenir la disminución del número de receptores con el tiempo y también tienden a restaurar el número de receptores a niveles normales cuando se administra a células en las que el nivel de los receptores ha disminuido.

EJEMPLO 2

Efecto neuroprotector de la sarsapogenina, catilato de episarsasapogenina, episarsasapogenina, epismilagenina y esmilagenina en las neuronas

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El objetivo del presente estudio fue examinar los efectos de la sarsasapogenina, catilato de episarsasapogenina, episarsasapogenina, epismilagenina y esmilagenina en la supervivencia de las neuronas corticales primarias de ratas expuestas a glutamato, del cual se sabe que induce neurodegeneración.

Las neuronas corticales de rata se cultivaron durante 10 días, al décimo día el medio se cambió a un medio definido libre de suero. El día 12, 24 horas antes de la exposición al glutamato, los cultivos se lavaron y el medio se reemplazó con un medio fresco que contenía control positivo (β -estradiol), compuestos de prueba (sarsapogenina, catilato de episarsasapogenina, episarsasapogenina, epismilagenina y esmilagenina) o control de vehículo (DMSO, 0,25%) o diosgenina como control negativo.

El día 13, los cultivos se expusieron a glutamato. Después del período de incubación, los cultivos se lavaron con un medio fresco suplementado con compuestos un vehículo relevantes, y se colocaron en dicho medio, para evaluar sus efectos protectores, 24 horas después de la exposición al glutamato.

La supervivencia celular neuronal se evaluó midiendo la actividad de lactato-deshidrogenasa (LDH) liberada en

el medio 24 horas después de la exposición al tratamiento de prueba o glutamato + compuesto de prueba, usando el kit no radioactivo CytoTox 96 y se cuantificó midiendo la absorbancia de la longitud de onda a 450 nm.

Luego de la exposición de los cultivos corticales primarios de rata al glutamato, hubo una degeneración significativa de las neuronas corticales, lo cual se demostró 24 horas después del tratamiento con un aumento en la liberación de lacticodeshidrogenasa en el medio de cultivo.

Los cultivos corticales primarios tratados previamente con los compuestos durante 24 horas, también hubo una reducción significativa en la neurodegeneración inducida por glutamato (Figura 2; Tabla 2).

Tabla 2 - Efecto de sarsasapogenina, catilato de episarsasapogenina, episarsasapogenina, episamilagenina y esmilagenina en la prevención de la neurodegeneración inducida por glutamato

Condiciones	Media $\pm$ s.e.m. (%)
Control	100
+ glutamato	66 $\pm$ 3
+ glutamato + sarsasapogenina (30 nM)	79 $\pm$ 3

Control	100
+ glutamato	65 ± 3
+ glutamato + catilato de episarsasapogenina (30 nM)	74 ± 3
Control	100
+ glutamato	68 ± 4
+ glutamato + episarsasapogenina (30 nM)	88 ± 3
Control	100
+ glutamato	71 ± 2
+ glutamato + epismilagenina (30 nM)	79 ± 2
Control	100
+ glutamato	68 ± 4
+ glutamato + esmilagenina (30 nM)	91 ± 4
Control	100
+ glutamato	68 ± 4
+ glutamato + diosgenina (30 nM)	72 ± 4
control negativo	

La sarsasapogenina, catilato de episarsasapogenina, episarsasapogenina, epismilagenina y esmilagenina

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mostraron efectos neuroprotectores significativos contra la neurodegeneración inducida por glutamato en neuronas corticales primarias de rata *in vitro*.

EJEMPLO 3

Reversión de la neurodegeneración por sarsasapogenina, esmilagenina, 16,22-epoxicoprostan-3 β -ol, esmilagenona, clorhidrato de glicinato de esmilagenina y coprosterol en neuronas

Como se ha mencionado líneas arriba, la exposición de cultivos corticales de rata a glutamato (100 μ M; 10 min.) causó un aumento en la actividad de lacticodeshidrogenasa (LDH) medida después de 24 horas, indicando una neurodegeneración significativa. El tratamiento con 17 β -estradiol después de la exposición a glutamato produjo un aumento de la actividad de LDH en comparación con las neuronas expuestas a glutamato, sugiriendo un efecto neuroprotector significativo. De modo similar, el tratamiento con sarsasapogenina, esmilagenina, 16,22-epoxicoprostan-3 β -ol, esmilagenona, esmilagenina, clorhidrato de glicinato de esmilagenina y coprosterol produjo una disminución significativa en la actividad de LDH en comparación con las neuronas expuestas a glutamato, sugiriendo un efecto neuroprotector

significativo (Tabla 3).

Tabla 3 - Efecto de distintos compuestos en neuronas corticales expuestas previamente a glutamato

Condición	Media $\pm$ s.e.m. (%)
Control	100 $\pm$ 4
Glutamato [100 μ M]	66 $\pm$ 2
Glutamato + 17 β -estradiol [3 nM]	69 $\pm$ 2
Glutamato + 17 β -estradiol [30 nM]	75 $\pm$ 5
Control	100 $\pm$ 1
Glutamato [100 μ M]	67 $\pm$ 3
Glutamato + sarsasapogenina [3 nM]	101 $\pm$ 3
Glutamato + sarsasapogenina [30 nM]	112 $\pm$ 1
Glutamato + esmilagenina [3 nM]	109 $\pm$ 6
Glutamato + esmilagenina [30 nM]	104 $\pm$ 1
Control	100 $\pm$ 8
Glutamato [100 μ M]	40 $\pm$ 1
Glutamato + diosgenina [30 nM, control negativo]	49 $\pm$ 6
Control	100 $\pm$ 5
Glutamato [100 μ M]	64 $\pm$ 4

Glutamato + 16,22-epoxicoprostan-3 β -ol [3nM]	114 $\pm$ 7
Glutamato + 16,22-epoxicoprostan-3 β -ol [30 nM]	134 $\pm$ 5
Glutamato + esmilagenona [3 nM]	119 $\pm$ 7
Glutamato + esmilagenona [30 nM]	119 $\pm$ 4
Control	100 $\pm$ 4
Glutamato [100 μ M]	58 $\pm$ 3
Glutamato + clorhidrato de glicinato de esmilagenina [3 nM]	117 $\pm$ 4
Glutamato + clorhidrato de glicinato de esmilagenina [30 nM]	141 $\pm$ 6
Glutamato + coprosterol [3 nM]	126 $\pm$ 5
Glutamato + coprosterol [30 nM]	116 $\pm$ 4

En conclusión, en neuronas corticales primarias de ratas, la sarsasapogenina, esmilagenina, 16,22-epoxicoprostan-3 β -ol, esmilagenona, clorhidrato de glicinato de esmilagenina y coprosterol revirtió la neurodegeneración inducida por glutamato, sugiriendo un potencial terapéutico en los trastornos neurodegenerativos.

EJEMPLO 4**Efecto antiapoptótico de la esmilagenina en las neuronas**

El objetivo de este estudio era analizar el efecto antiapoptótico de la esmilagenina en la actividad de la caspasa-3, un marcador de apoptosis, en cultivos corticales primarios de rata expuestos a glutamato.

Cultivos primarios de neuronas corticales

Se cultivaron neuronas corticales de rata durante 6 días. El día 6, se agregó glutamato (100 microM, 10 min.). luego, los cultivos se lavaron, y el medio se reemplazó con un medio fresco que contenía esmilagenina o control de vehículo (DMSO, 0,25%) durante 6 horas. Después de 6 horas de tratamiento, se evaluó la apoptosis midiendo la actividad de la caspasa 3. La actividad de la caspasa 3 se detectó por la escisión de p-nitroanilina de un sustrato de caspasa-3 colorimétrico, acetil-Asp-Glu-Val-Asp p-nitroaniluro. La p-nitro-alanina tiene una alta absorbancia a 405 nM. La actividad de la caspasa-3 se midió como densidad óptica. Además, la actividad relativa de la caspasa-3 se estandarizó a la concentración de proteína de la muestra, la cual se midió también como una densidad óptica (Du y otros, J. Neurochem. 69, 1382-1388,1997; (Sawada y otros, Faseb J., 14, 1202-1214,2000).

La esmilagenina revierte el aumento de la actividad de la caspasa-3 en las neuronas corticales primarias de rata, demostrando el efecto antiapoptósico de la esmilagenina (Tabla 4).

Tabla 4- Efecto de la esmilagenina en la actividad de la caspasa-3 inducida por glutamato en neuronas corticales

C ndiciones	Actividad de la caspasa (% de control)
Control	100
+ Glutamato	131
+ Glutamato + esmilagenina (300 nM)	105

EJEMPLO 5

Los trastornos neurodegenerativos se caracterizan por una pérdida progresiva de neuronas y la degradación de procesos neuronales (neuritas). Los agentes que inducen el crecimiento de las neuritas pueden promover la formación de nuevas conexiones entre las neuronas y mejorar los síntomas de condiciones neurodegenerativas (Katzman y otros, Faseb J., 5-278-286,1991).

La exposición a 17 β -estradiol (0,3; 3; 30 pM) aumentó significativamente la longitud de las neuritas existentes en las neuronas corticales primarias de ratas (Tabla 5).

La exposición a 17 β -estradiol (3, 30 pM) aumentó significativamente el porcentaje de neuronas que muestran neuritas en las neuronas corticales primarias de rata (Tabla 6). La exposición a esmilagenina y sarsasapogenina (0,3; 3; 30 pM) aumentó significativamente la longitud de las neuritas existentes y el porcentaje de neuronas que muestran neuritas en neuronas corticales primarias de ratas (Tablas 5 y 6).

En conclusión, la esmilagenina y sarsasapogenina tienen efectos neurotróficos *in vitro*.

Tabla 5- Efecto de 17 β -estradiol, esmilagenina y sarsasapogenina en longitud de neurita medida usando micrometría óptica

Condiciones	Media $\pm$ s.e.m. (%)
Control	100 $\pm$ 4
17 β -estradiol (0,3 pM)	154 $\pm$ 5
17 β -estradiol (3 pM)	163 $\pm$ 4
17 β -estradiol (30 pM)	183 $\pm$ 5
Esmilagenina (0,3 pM)	159 $\pm$ 5
Esmilagenina (3 pM)	190 $\pm$ 8
Esmilagenina (30 pM)	204 $\pm$ 6
Sarsasapogenina (0,3 pM)	177 $\pm$ 5
Sarsasapogenina (3 pM)	197 $\pm$ 5
Sarsasapogenina (30 pM)	211 $\pm$ 6

Tabla 6 - Efecto de 17 β -estradiol, esmilagenina y sarsasapogenina en el número de neuronas que muestran neuritas

Condiciones	Media $\pm$ s.e.m. (%)
Control	47 $\pm$ 2
17 β -estradiol (0,3 pM)	48 $\pm$ 2
17 β -estradiol (3 pM)	60 $\pm$ 2
17 β -estradiol (30 pM)	59 $\pm$ 2
Esmilagenina (0,3 pM)	63 $\pm$ 2
Esmilagenina (3 pM)	63 $\pm$ 3
Esmilagenina (30 pM)	66 $\pm$ 2
Sarsasapogenina (0,3 pM)	55 $\pm$ 2
Sarsasapogenina (3 pM)	61 $\pm$ 1
Sarsasapogenina (30 pM)	62 $\pm$ 2

EJEMPLO 6

La esmilagenina y sarsasapogenina previenen la neurodegeneración causada por la exposición a la neurotoxina, 1-metil-4-fenilpiridinio (MPP⁺) en neuronas dopaminérgicas mesencefálicas; un modelo de la enfermedad de Parkinson *in vitro*.

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El daño causado por la neurotoxina, MPP^+ , un metabolito de 1-metil-4-fenil-1,2,3,6-tetrahidropiridina (MPTP) se asemeja a la degeneración de las neuronas dopaminérgicas nigrostriatales observada en los trastornos neurodegenerativos como la enfermedad de Parkinson (Mytinlineou y otros, Science, 225, 529-531, 1984). Los cambios bioquímicos más importantes inducidos por esta toxina incluyen aumento de los niveles de dopamina y sus metabolitos en la *substantia nigra pars compacta* y en el núcleo caudado (Burns y otros, Proc Natl Acad Sci USA, 80, 4546-4550, 1983) y una reducción en la absorción de dopamina en preparaciones sinaptosomales nigrostriatales (Heikkila y otros, J. Neurochem, 44, 310-313, 1985).

El tratamiento previo de las neuronas dopaminérgicas con esmilagenina y sarsasapogenina redujo de manera significativa la neurotoxina específica MPP^+ (2 μM) en comparación con el MPP^+ solo. El factor neurotrófico derivado de línea celular glial (GDNF) y el factor neurotrófico derivado del cerebro (BDNF), y las moléculas que estaban involucradas en el crecimiento neuronal se usaron como controles positivos. El tratamiento previo con esmilagenina y sarsasapogenina produjo un aumento

significativo en la supervivencia neuronal en comparación con las neuronas expuestas a MPP⁺ solo, sugiriendo un efecto neuroprotector significativo (Tabla 7).

Tabla 7 - Efecto del tratamiento previo con BDNF y GDNF, esmilagenina y sarsasapogenina en neuronas dopaminérgicas después de exposición a MPP⁺ (2 µM)

Condición	Media ± s.e.m. (%)
Control	100 ± 3
+ MPP ⁺ (2 µM)	55 ± 3
MPP ⁺ (2 µM) + BDNF (1,85 nM) & GDNF (0,17 nM)	122 ± 7
MPP ⁺ (2 µM) + esmilagenina (30 nM)	98 ± 4
MPP ⁺ (2 µM) + sarsasapogenina (30 nM)	89 ± 3

En este modelo de la enfermedad de Parkinson in vitro, el tratamiento previo con esmilagenina y sarsasapogenina previno significativamente la degeneración neuronal luego de la exposición a la neurotoxina específica MPP⁺, demostrando un efecto neuroprotector.

EJEMPLO 7

La esmilagenina y sarsasapogenina también revierten la neurodegeneración causada por la exposición a la neurotoxina 1-metil-4-fenilpiridinio (MPP⁺) en neuronas dopaminérgicas mesencefálicas; un modelo de la enfermedad de Parkinson *in vitro*.

El tratamiento de neuronas dopaminérgicas con esmilagenina y sarsasapogenina redujo significativamente la muerte neuronal luego de la exposición a la neurotoxina dopaminérgica específica MPP⁺ (2 µM), en comparación con MPP⁺ solo. El factor neurotrófico derivado de línea celular glial (GDNF) y el factor neurotrófico derivado del cerebro (BDNF), las moléculas que estaban involucradas en el crecimiento neuronal y 17β-estradiol se usaron como controles positivos. El tratamiento previo con esmilagenina y sarsasapogenina produjo un aumento significativo en la supervivencia neuronal en comparación con las neuronas expuestas a MPP⁺ solo (Tabla 8).

Tabla 8 - Efecto del tratamiento previo con BDNF y GDNF, esmilagenina, sarsasapogenina y 17β-estradiol en neuronas dopaminérgicas después de exposición a MPP⁺ (2 µM)

Condición	Media ± s.e.m. (%)
Control	100 ± 6

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+ MPP ⁺ (2 μ M)	76 $\pm$ 4
+ MPP ⁺ (2 μ M) + BDNF (1,85 nM) & GDNF (0,17 nM)	98 $\pm$ 5
+ MPP ⁺ (2 μ M) + esmilagenina (0,03 nM)	111 $\pm$ 6
+ MPP ⁺ (2 μ M) + sarsasapogenina (0,03 nM)	112 $\pm$ 6
+ MPP ⁺ (2 μ M) + 17 β -estradiol (0,03 nM)	106 $\pm$ 5

La exposición a MPP⁺ causó no sólo una disminución significativa en el número dopaminérgico sino también en el porcentaje de neuritas. Este estudio muestra que la esmilagenina y sarsasapogenina aumentaron de manera significativa el número de las neuronas *in vitro* en la Tabla 9. Estos resultados demuestran que los compuestos revierten la neurodegeneración motora.

Tabla 9 - Efecto de la esmilagenina y sarsasapogenina en el porcentaje de neuritas en neuronas dopaminérgicas después de la exposición a MPP⁺ (2 μ M)

Condición	Media $\pm$ s.e.m. (%)
Control	41 $\pm$ 4
+ MPP ⁺ (2 μ M)	27 $\pm$ 5

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MPP ⁺ (2 μ M) + esmilagenina (0,03 nM)	41 $\pm$ 4
MPP ⁺ (2 μ M) + sarsasapogenina (0,03 nM)	43 $\pm$ 4

EJEMPLO 8

Efecto neuroprotector de la sarsasapogenina y esmilagenina en las neuronas motoras de la espinal dorsal

El objetivo de este estudio fue analizar los efectos de la sarsasapogenina en la supervivencia de las neuronas motoras de la espina dorsal primarias de ratas expuestas a glutamato, del cual se conoce que induce la neurodegeneración en este modelo de neurodegeneración motora. Se usó 17 β -estradiol y BDNF como controles positivos.

Cultivos primarios de neuronas motoras de la espina dorsal

Se prepararon neuronas motoras de rata de acuerdo con el método descrito por (Martinou y otros, Neuron, 8, 737-744, 1992). El día 10, el medio se retiró, y los cultivos se expusieron a glutamato (4 μ M) durante 10 min a 37°C en un medio definido. Después de la exposición al glutamato, los cultivos se lavaron con medio Eagle modificado por Dulbecco a 37°, y luego se colocaron en un medio fresco que contenía compuestos de prueba. Después de 48 horas, el grado de degeneración de las neuronas

motoras se determinó midiendo la cantidad de lactato-deshidrogenosa (LDH) liberada en el medio de cultivo como se ha señalado líneas arriba.

Resultados

Luego de la exposición a glutamato, hubo una degeneración de las neuronas motoras primarias de la espina dorsal de ratas, 48 horas después del tratamient , demostrada por un aumento en la liberación de lacticodeshidrogenasa en el medio de cultivo.

En las neuronas motoras primarias de espina dorsal de ratas tratadas con sarsasapogenina o esmilagenina durante 48 horas, hubo una reducción significativa en la neurodegeneración inducida por glutamato. (Tabla 10)

Tabla 10 - Efecto de la sarsasapogenina y esmilagenina en la neurodegeneración inducida por glutamano en las neuronas motoras de la espinal dorsal.

Condición	Media ± s.e.m. (%)
Control + DMSO [0,25%]	100 ± 1
Glutamato [4 microM] + DMSO [0,25%]	94 ± 1
Glutamato + BDNF [3 nM]	148 ± 8
Glutamato + 17β-estradiol [0,03 nM]	102 ± 2
Glutamato + 17β-estradiol [3 nM]	110 ± 1

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Glutamato + 17 β -estradiol [300 nM]	116 $\pm$ 6
Glutamato + sarsasapogenina [0,03 nM]	123 $\pm$ 2
Glutamato + sarsasapogenina [3 nM]	137 $\pm$ 1
Glutamato + sarsasapogenina [300 nM]	136 $\pm$ 6
Glutamato + esmilagenina [0,03 nM]	128 $\pm$ 4
Glutamato + esmilagenina [3 nM]	154 $\pm$ 1
Glutamato + esmilagenina [300 nM]	144 $\pm$ 4

La sarsapogenina y esmilagenina revirtieron la neurodegeneración inducida en neuronas motoras de la espina dorsal de ratas en este modelo *in vitro* de neurodegeneración motora.

EJEMPLO 9

En la segunda mitad de la vida (en humanos a partir de los 40 años de edad) la densidad de las neuronas en el cerebro disminuye (Selkoe, D J, Sci. Am. 267, 134-142, 1992). Las alteraciones en la función cortical pueden producirse debido a la reducción en el número de neuronas, sus interconexiones, una disminución en neurofinas como el factor neurotrófico derivado del cerebro (BDNF; Bothwell, M, Interacciones funcionales de neurotrofinas y receptores de neurotrofina, Ann. Rev. Neuroscie. 18, 223-253, 1995), una disminución en la

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densidad del receptor de acetilcolina (muscarínico y nicotínico) y/o una disminución en su función de acoplamiento en las áreas corticales (Rinne y otros, Brain Res., 336, 19-25, 1985; Selkoe, D J, Sci.Am 267, 134-142, 1992). Además, durante el envejecimiento, el enlace del receptor muscarínico de acetilcolina se reduce significativamente en el hipocampo (Narang, N, Mech. Ageing Dev., 78, 221-239, 1995) y estriado de ratas mayores (Biegón y otros, Neurobiol. Aging, 10, 305-310, 1989) y humanos (Rinne y otros, Brain Res., 336, 19-25, 1985). Además, en la enfermedad de Alzheimer, la disminución en la actividad colinérgica se asocia con la deposición de placa β amiloide (von der Kammer y otros, Biochem. Soc. Symp. 131-140, 2001). Otros trastornos neurodegenerativos, como la enfermedad de Parkinson, muestran una disminución característica en la actividad dopaminérgica (Drukarch y otros, Expert. Opin. Investig. Drugs, 10, 1855-1868, 2001).

La administración oral de sarsasapogenina, catilato de episarsasapogenina o esmilagenina a ratas de edad avanzada (ratas Sprague-Dawley de 20 meses de edad), por un período de dos o tres meses revierte la deficiencia de capacidad de aprendizaje y memoria, la disminución en receptores muscarínicos de acetilcolina y dopamina y la disminución en la BDNF de neurotrofina, alteraciones que son características del proceso de envejecimiento.

Las ratas Sprague-Dawley adultas se dividieron en grupos diferentes, un grupo de control y grupos tratados durante 2-3

meses con sarsasapogenina, catilato de episarsasapogenina o esmilagenina ($18 \text{ mg kg}^{-1} \text{ día}^{-1}$, $n=10$). Un grupo de control ($n=14$) de ratas jóvenes no tratadas también se incluyó en el estudio. La dosis diaria del fármaco se mezcló en una cantidad mínima de alimento y se administró cada mañana de manera separada a cada rata.

Se usó un aparato con laberinto en Y para la prueba de aprendizaje y memoria. En el piso de cada brazo del laberinto en Y se encuentra un conjunto de varillas de cobre a las cuales se les aplica corriente eléctrica cada vez que sea necesario, con voltaje regulable. Cada brazo es de 45 cm de largo y tiene una lámpara de 15 W en el extremo, la cual se enciende cada vez que sea necesario. Después de 3 meses de administración del fármaco, cada rata fue entrenada durante 7 días consecutivos, de la siguiente manera. Para cada sesión de entrenamiento, la rata se colocó en un brazo del laberinto en Y, después de dos minutos de descanso, se aplicó corriente eléctrica a las varillas de cobre y la lámpara del brazo en sentido del reloj se iluminaba para indicar el área de no estímulo. Si la rata se dirigía a dicho brazo, se registraba una respuesta correcta, de lo contrario, se registraba una respuesta incorrecta. Esta prueba de estímulo-respuesta se repitió 20 veces cada día, con una pausa de 5 segundos entre dos pruebas consecutivas. El número de respuestas correctas siguientes a las veinte pruebas en el séptimo día se usó para expresar la capacidad de aprendizaje, (a mayor número, mejor

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capacidad de aprendizaje). Luego se dejó reposar a las ratas por 30 días y el procedimiento se repitió una vez más. El número de respuestas correctas de las 20 pruebas después del período de descanso de 30 días se usó para representar la capacidad de memoria.

Se midió la densidad del receptor muscarínico de acetilcolina en el cerebro. El tejido se preparó de la siguiente manera: los cerebros se retiraron rápidamente después de la decapitación, se congelaron en hielo seco, y se transfirieron a un congelador. Los cerebros se homogenizaron y la pella se suspendió finalmente en amortiguador.

El ensayo de enlace de ligando competitivo de sitio doble se usó para medir la densidad del receptor muscarínico de acetilcolina.

Los resultados se muestran en las Figuras 3 y 4 de los dibujos. Los experimentos del laberinto en Y revelaron que tanto la capacidad de aprendizaje como de memoria se deterioran en las ratas de edad avanzada. Sarsasapogenina, catilato de episarsasapogenina y esmilagenina restauraron la capacidad de aprendizaje y memoria después de la administración en ratas de edad avanzada. La densidad del receptor muscarínico de acetilcolina se redujo marcadamente en las ratas de edad avanzada. Sarsasapogenina, catilato de episarsasapogenina o esmilagenina aumentaron significativamente la densidad del receptor muscarínico de acetilcolina.

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Las ratas jóvenes mostraron una densidad del receptor 1 y 2 de dopamina (D) significativamente mayor ($157,5 \pm 33,2$; $200,6 \pm 50,9$ fmol/mmg proteína, respectivamente) en comparación con las ratas de edad avanzada ($129,2 \pm 36,8$; $153,8 \pm 40,5$ fmol/mg proteína, D₁ y D₂, respectivamente). En contraste, el tratamiento con esmilagenina y sarsasapogenina en ratas de edad avanzada por 3 meses restauró la densidad del receptor de D₁ y D₂, (esmilagenina $177 \pm 10,9$; $217 \pm 45,7$ fmol/mg proteína; sarsasapogenina $172,0 \pm 44,0$; $206,4 \pm 60,5$, respectivamente).

Las ratas jóvenes mostraron niveles de BDNF significativamente mayores ($1,647 \pm 0,277$ ng/g tejido) en comparación con las ratas de edad avanzada ($1,205 \pm 0,219$ ng/g tejido). En contraste, el tratamiento con esmilagenina y sarsasapogenina en ratas de edad avanzada por 3 meses restauró parcialmente los niveles de BDNF ($1,342 \pm 0,07$; $1,410 \pm 0,232$ ng/g tejido, respectivamente).

De esta manera, los compuestos revierten la neurodeficiencia, la disminución en los niveles de BDNF, y la disminución en la densidad del receptor muscarínico de acetilcolina y dopamina que ocurre en ratas de edad avanzada.

Ejemplo 10

Modelo de enfermedad de Alzheimer como modelo de neurodegeneración

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Un modelo *in vivo* de la enfermedad de Alzheimer se usó para modelar la neurodegeneración. En este modelo, los agentes neurotóxicos (ácido amiloide β e iboténico) se inyectaron en el cerebro de la rata. Esto conlleva a la pérdida neuronal, pérdida del receptor y deficiencia cognitiva. Estudios previos mostraron que la inyección local de amiloide β en el núcleo vasalis del cerebro de la rata causó hipofunción colinérgica y deficiencia conductual hasta dos meses después de la cirugía (Giovannelli y otros, 1995; Neuroscience, 66, 781-792). Además, la co-inyección de amiloide β con una pequeña cantidad de ácido iboténico en el hipocampo de la rata produce sinérgicamente pérdida neuronal con infiltración de células de la glía no sólo adyacentes sino también distantes del sitio inyectado (Morimoto y otros, 1998; Neuroscience, 84, 479-487).

Nuestros estudios usaron el método de Morimoto (Morimoto y otros, 1998; Neuroscience, 84, 479-487) con ciertas modificaciones (inyección unilateral en vez de inyección bilateral). Las ratas Sprague-Dawley de tres meses de edad se dividieron al azar en grupos diferentes. La inyección de amiloide β_{1-40} y ácido iboténico (ambas de Sigma) se realizó mediante un instrumento estererotáxico (Stoelting Co.) y las coordenadas fueron AP=-0,5mm (a la línea derecha de la línea media), L=-2,8mm (atrás de bregma), H=-7,0mm (ventral hacia dura). La dosis para cada rata fue amiloide β_{1-40} (4 μ g) y ácido iboténico (1 μ g) de solución salina. La inyección se

completó en 20 minutos y la aguja se retiró 10 minutos después. Posteriormente se suturó la piel.

Los 8 grupos fueron:

Control operado inyectado con solución salina normal (control)

Modelo (control inyectado con amiloide β y ácido iboténico)

Modelo + catilato de Episarsasapogenina (18 mg/kg/día)*

Modelo + catilato de Sarsasapogenina (18 mg/kg/día)*

Modelo + etilsuccinato de Episarsasapogenina (18 mg/kg/día) (comparación)

Modelo + Episarsasapogenina (18 mg/kg/día)*

Modelo + Epismilagenina (18 mg/kg/día)*

Modelo + Diosgenina (es decir, control negativo, 18 mg/kg/día)

*Compuestos de acuerdo con la presente invención

Catilato de episarsasapogenina, catilato de sarsasapogenina, etilsuccinato de episarsasapogenina (compuesto de comparación), episarsasapogenina, epismilagenina y diosgenina (todos en una dosis de 18 mg/kg/día) fueron administrados a animales en suspensiones estables en CMC-Na (0,5%) una vez al día a través de un tubo gástrico. Al grupo de control y al grupo modelo se le administró el mismo volumen de CMC-NA (0,5%) una vez al día. Los fármacos y los vehículos se proporcionaron durante un período de dos meses, empezando 20 días antes de la operación.

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Se evaluó la densidad del receptor muscarínico de acetilcolina. Las muestras de cerebro se homogenizaron, centrifugaron, y la pella de centrifugación a 27000xg se rehomogenizó y usó como medida. La concentración de ^3H -QNB se seleccionó en el rango de saturación. Después de la incubación y separación. La porción de combinación se midió mediante contador de centelleo líquido.

Prueba Paso Por Paso: aprendizaje y memoria. El efecto de los compuestos de prueba sobre el aprendizaje y memoria se evaluó usando la prueba paso por paso. Una caja de 60 cm x 15 cm x 15 cm, dividida en 2 cuartos del mismo tamaño, un cuarto oscuro con base de varilla de cobre, la cual estaba cargada eléctricamente (40 V ca) al usarse, mientras que el otro era un cuarto iluminado pero no cargado eléctricamente. Entre los dos cuartos hay una abertura (orificio) para que la rata pase. El experimento se realiza para cada rata en dos días consecutivos. El primer día es para entrenamiento; cuando la rata queda atrapada en la caja durante los primeros 3 minutos, luego se le coloca en el cuarto iluminado, de espaldas contra el orificio, y las varillas de cobre del cuarto oscuro se cargaron por 5 minutos. El segundo día es para la prueba, cuando se registra el número de cruces en 5 minutos. Las mejoras en memoria se señalan mediante una reducción en el número de cruces.

La densidad del receptor muscarínico de acetilcolina en cerebros modelo de neurodegeneración fue significativamente

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inferior que en el grupo de control. El catilato de episarsasapogenina, catilato de sarsasapogenina, episarsasapogenina y epismilagenina produjeron una elevación significativa en la densidad del receptor muscarínico de acetilcolina, mientras que la diosgenina y etilsuccinato de episarsasapogenina no cambiaron significativamente la densidad del receptor muscarínico de acetilcolina. Por consiguiente, los experimentos indicaron que los compuestos de esta invención actúan para normalizar el número del receptor, es decir, tienden a restaurar el número del receptor a niveles normales cuando son administrados a animales en los que el nivel del receptor está deprimido.

El número de respuestas incorrectas (número de errores) en 5 minutos fue significativamente mayor en el grupo modelo de neurodegeneración que en el grupo de control, indicando una deficiencia de memoria (véase Tabla 11). Epismilagenina, catilato de episarsasapogenina, episarsasapogenina, y catilato de sarsasapogenina redujeron significativamente el número de respuestas incorrectas, mientras que la diosgenina y el etilsuccinato de episarsasapogenina fueron inefectivos en la disminución del número de respuestas incorrectas.

Tabla 11

Grupo	Densidad del receptor M	Prueba paso por paso de aprendizaje y memoria
	(fmol/mg/proteína)	No. Errores
Control (n=10)	859±101	0,60±0,70
Modelo (n=10)	713±48*	4,00±2,40
+Catilato de episarsasapogenina (n=10)	877±89*	1,36±0,92*
+Catilato de sarsasapogenina (n=11)	916±158*	1,36±1,03*
+Etilsuccinato de episarsasapogenina (n=11)	774±79	3,73±1,35
+Episarsasapogenina (n=10)	869±104*	1,50±1,18*
+Epismilagenina (n=11)	877±90*	1,73±0,91*
+Diosgenina (control negativo n=8)	770±68	3,75±1,49

Análisis estadístico usando prueba de alumno no apareado.

*denota $p < 0,05$

EJEMPLO 11

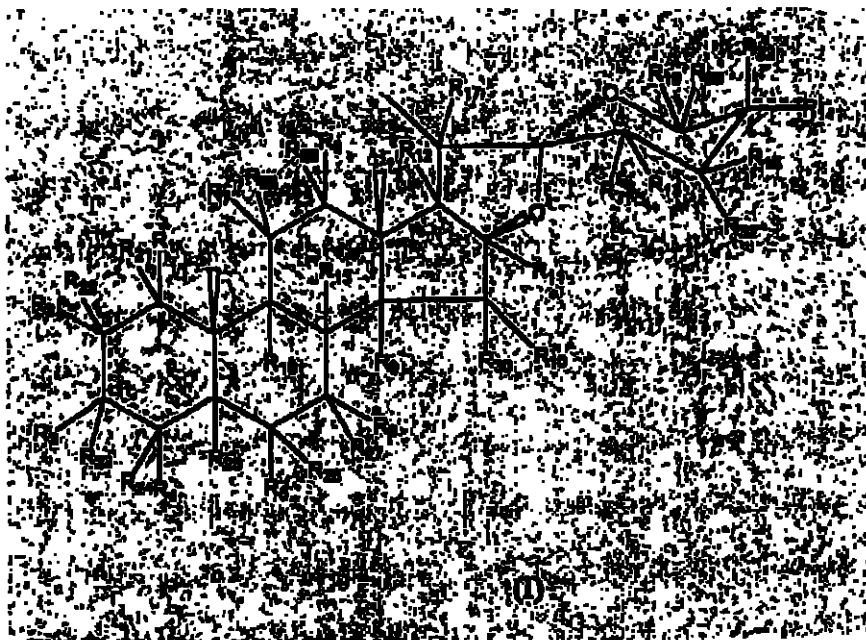
La esclerosis lateral amiotrófica (ALS) es un trastorno neurodegenerativo fatal progresivo que causa degeneración motora de las neuronas, atrofia esquelética, parálisis y muerte. La causa de esta enfermedad es heterogénea: mutaciones en el gen de dismutasa de superóxido (SOD-1) de Cu/Zn es responsable de algunas formas de ALS humana. Los modelos animales de esta enfermedad incluyen el gen trasgénico de SOD-

1 de ratones que sobreexpresan SOD-1 y los ratones con neuropatía motora progresiva (pmm, un modelo de Charcot-Marie-Tooth). La esmilagenina y sarsapogenina incrementa el ciclo de vida y mejora los déficits conductuales del ratón con dismutasa de superóxido (SOD) (Figura 5) y pmm ratón (fig. 6), dos modelos de esclerosis lateral amiotrófica (ALS) y enfermedad de Charcot-Marie-Tooth.

Lo anterior describe ampliamente la presente invención sin carácter limitativo. Las variaciones y modificaciones que serán aparentes para los expertos en la técnica pretenden estar dentro del alcance de esta solicitud y de cualquier patente(s) subsiguiente.

REIVINDICACIONES

1. Uso de uno o más agentes activos seleccionados de:
- A. compuestos de la Fórmula I:



caracterizado porque en la fórmula general (I):

$$-R_1, R_2, R_3, R_4, R_5, R_6, R_7, R_8, R_{10}, R_{13}, R_{18}, R_{19}, R_{20}, R_{21}, R_{22}, R_{23},$$

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R₂₄, R₂₆, R₂₇, R₂₈, R₂₉, R₃₀, R₃₁, R₃₂, son independientemente H, OH, =O, átomo halo, (Me-S-), (Me-SO-), (Me-SO₂-), N₃-, NH₂-, MeSO₂NH-, alquilo o ausente u OR donde R = grupo alquilo o acilo;

-R₉, R₁₁, R₁₂, R₁₄, R₁₅, R₁₆, R₁₇, R₂₅, R₃₃, pueden ser un H, OH, átomo halo, (Me-S-), (Me-SO-), (Me-SO₂-), N₃-, NH₂-, MeSO₂NH-, alquilo o ausente u OR donde R = grupo alquilo o acilo;

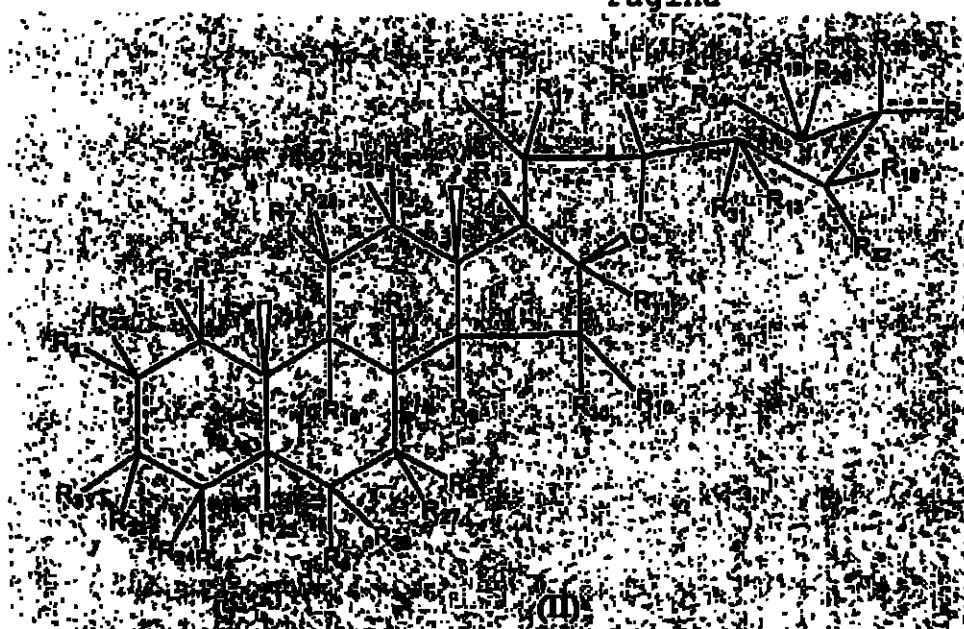
..... representa un enlace doble opcional;

caracterizado porque además de lo anterior

R₃₃ o R₁₄ = grupo alquilo;

A. Compuestos de la Fórmula II:

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caracterizado porque en la fórmula general (II):

$-R_1, R_2, R_3, R_4, R_5, R_6, R_7, R_8, R_{10}, R_{13}, R_{18}, R_{19}, R_{20}, R_{21}, R_{22}, R_{23}, R_{24}, R_{26}, R_{27}, R_{28}, R_{29}, R_{30}, R_{31}, R_{32}, R_{34}$, son independientemente H, OH, $=O$, átomo halo, (Me-S-), (Me-SO-), (Me-SO₂-), N₃-, NH₂-, MeSO₂NH-, alquilo, OR donde

R = grupo alquilo o acilo o ausente;

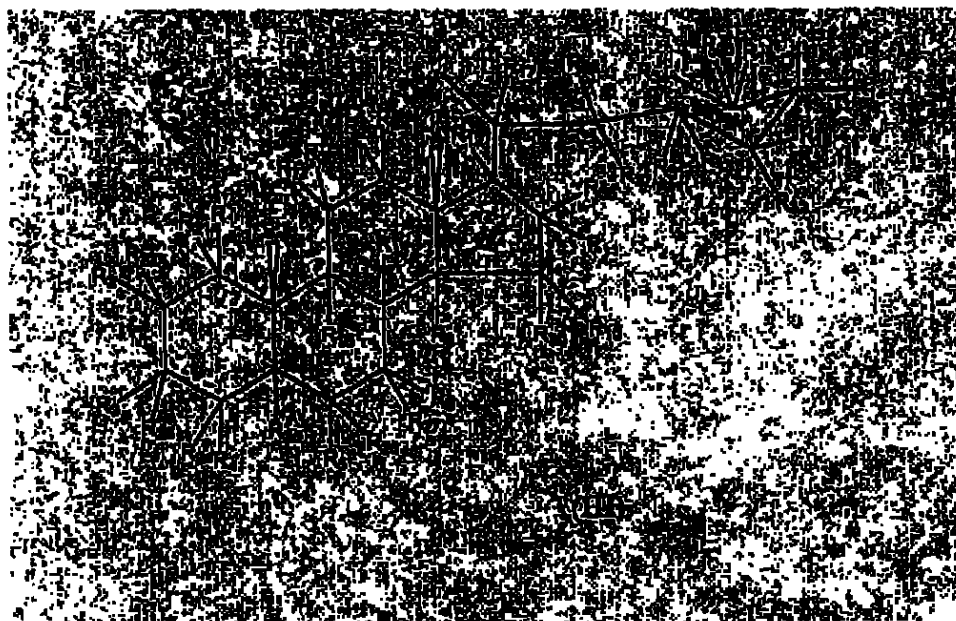
$-R_9, R_{11}, R_{12}, R_{14}, R_{15}, R_{16}, R_{17}, R_{25}, R_{33}, R_{35}$, pueden ser un H, OH, átomo halo, (Me-S-), (Me-SO-), (Me-SO₂-), N₃-, NH₂-,

MeSO₂NH-, alquilo, OR donde R = grupo alquilo o acilo, o ausente;

..... representa un enlace doble opcional;
caracterizado porque además de lo anterior

R₃₃ o R₁₄ = grupo alquilo;

A. Compuestos de la Fórmula III:



caracterizados porque en la fórmula general (III):

$-R_1, R_2, R_3, R_4, R_5, R_6, R_7, R_8, R_{10}, R_{13}, R_{14}, R_{18}, R_{19}, R_{20}, R_{21}, R_{22}, R_{23}, R_{24}, R_{26}, R_{27}, R_{28}, R_{29}, R_{30}, R_{31}, R_{32}, R_{33}, R_{34}, R_{35}, R_{36}, R_{37}$ son independientemente H, OH, =O, átomo halo, (Me-S-), (Me-SO-), (Me-SO₂-), N₃- NH₂- MeSO₂NH-, alquilo, OR donde R = grupo alquilo o acilo o ausente;

$-R_9, R_{11}, R_{12}, R_{15}, R_{16}, R_{17}, R_{25}$ pueden ser un H, OH, átomo halo, (Me-S-), (Me-SO-), (Me-SO₂-), N₃-, NH₂-, MeSO₂NH-, alquilo, OR donde R = grupo alquilo o acilo, o ausente;

..... representa un enlace doble opcional;

caracterizado porque además de lo anterior

R_{33} o R_{14} = grupo alquilo, y

La estereoquímica de R_{25} se encuentra en la orientación β ;

A. Derivados de sapogenina que soportan al menos un sustituyente de radical X,

Caracterizado porque X se selecciona del grupo que consiste de:

- átomo halo
- (Me-S-), (Me-SO-), (Me-SO₂-),
- N₃-, NH₂-, MeSO₂NH-
- alquilo; y

A. Formas derivadas de cualquiera de los compuestos

anteriores, en las que el átomo de carbono en la posición 3 o, en el caso de las Fórmulas II y III, el átomo de carbono en la posición 3, la posición 26 o cada uno de los átomos de carbono en las posiciones 3 y 26, porta una porción de azúcar O- caracterizada porque el grupo es un mono, di- o tri-sacarido;

todos sus estereoisómeros y mezclas racémicas, todos sus profármacos y sales farmacéuticamente aceptables, y todas las mezclas y combinaciones de los mismos.

En el tratamiento o prevención, o en la preparación de composiciones para el tratamiento o prevención de, (i) neurodegeneración no-cognitiva, (ii) degeneración neuromuscular no-cognitiva, (iii) neurodegeneración motora-sensorial, o (iv) disfunción o pérdida del receptor en ausencia de deficiencia cognitiva, neural y neuromuscular, en humanos y animales que sufren de los mismos o son susceptibles a éstos.

2. Un uso de acuerdo con la reivindicación 1, caracterizado porque el agente activo, o al menos uno de los agentes activos, se selecciona de:

a. Compuestos de la fórmula general I, caracterizados porque:

-R₁, R₂, R₃, R₄, R₅, R₆, R₇, R₈, R₁₀, R₁₃, R₁₈, R₁₉, R₂₀, R₂₁, R₂₂, R₂₃, R₂₄, R₂₆, R₂₇, R₂₈, R₂₉, R₃₀, R₃₁, R₃₂, son independientemente H, OH,

=O, átomo halo, (Me-S-), (Me-SO-), (Me-SO₂-), N₃-, NH₂- MeSO₂NH-,
, alquilo o ausente u OR donde R = grupo alquilo o acilo;

-R₉, R₁₁, R₁₂, R₁₄, R₁₅, R₁₆, R₁₇, R₂₅, R₃₃, pueden ser un H, OH,
átomo halo, (Me-S-), (Me-SO-), (Me-SO₂-), N₃-, NH₂-, MeSO₂NH-,
alquilo o ausente, u OR donde R = grupo alquilo o acilo, o
ausente;

..... representa un enlace doble opcional;

caracterizado porque además de lo anterior

-R₃₃ o R₁₄ = grupo alquilo, y

la estereoquímica de R₂₅ se encuentra en la orientación
β;

b. Los compuestos de la fórmula I general anterior,
caracterizados porque:

-R₁, R₂, R₃, R₄, R₅, R₆, R₇, R₈, R₁₀, R₁₃, R₁₈, R₁₉, R₂₀, R₂₁, R₂₂, R₂₃,
R₂₄, R₂₆, R₂₇, R₂₈, R₂₉, R₃₀, R₃₁, R₃₂, son independientemente H, OH,
=O, átomo halo, (Me-S-), (Me-SO-), (Me-SO₂-), N₃-, NH₂- MeSO₂NH-
, alquilo o ausente o OR donde R = grupo alquilo o acilo;

-R₉, R₁₂, R₁₅, R₁₆, R₁₇ = H,

-R₁₁, R₁₄, R₂₅, R₃₃ pueden ser un H, OH, átomo halo, (Me-S-),
(Me-SO-), (Me-SO₂-), N₃-, NH₂-, MeSO₂NH-, alquilo o ausente u OR
donde R = grupo alquilo o acilo;

..... representa un enlace doble opcional;

caracterizado porque además de lo anterior

-R₃₃ o R₁₄ = grupo alquilo, y

la estereoquímica de R₂₅ se encuentra en la orientación

β ;

C. Los compuestos de la fórmula general I, caracterizados porque:

$-R_1 = R_2 = R_4 = R_5 = R_6 = R_7 = R_8 = R_{10} = R_{11} = R_9 = R_{12} = R_{13} = R_{15} = R_{16} = R_{17} = R_{18}$
 $= R_{19} = R_{20} = R_{21} = R_{22} = R_{23} = R_{24} = R_{25} = R_{26} = R_{27} = R_{28} = R_{29} = R_{30} = R_{31} = R_{32} = R_{33} =$
 H,

- R_{33} o $R_{14} = CH_3$

..... representa un enlace simple,

- el grupo metilo a C25 puede estar en la configuración R o S,

- la esteoquímica de R_{23} está en la orientación β ; y

caracterizado porque además de lo anterior

al menos R_3 o R_{23} es un radical X, el posible sustituyente restante es H, OH, =O, y OR donde R = grupo alquilo o acilo o ausente,

y X se selecciona del grupo que consiste de:

- átomo halo,

- (Me-S-), (Me-SO-), (Me-SO₂-), y

- N₃- NH₂- MeSO₂NH-,

- alquilo;

d. Los compuestos de la fórmula I general anterior, caracterizados porque:

$-R_1 = R_2 = R_4 = R_5 = R_6 = R_7 = R_8 = R_{10} = R_{11} = R_9 = R_{12} = R_{13} = R_{15} = R_{16} = R_{17} = R_{18}$
 $= R_{19} = R_{20} = R_{21} = R_{22} = R_{23} = R_{24} = R_{25} = R_{26} = R_{27} = R_{28} = R_{29} = R_{30} = R_{31} = R_{32} = R_{33} =$
 H,

- R_{14} o $R_{33} = CH_3$

..... representa un enlace simple,

- la estereoquímica de R_{25} está en la orientación β ; y

caracterizado porque además de lo anterior

-al menos R_3 o R_{23} es un radical X, el posible sustituyente restante es H, OH, =O, y OR donde R = grupo alquilo o acilo o ausente,

y X se selecciona del grupo que consiste de:

- átomo halo,
- (Me-S-), (Me-SO-), (Me-SO₂-), y
- N₃- NH₂- MeSO₂NH-,
- alquilo;

e. Los compuestos de la fórmula II general anterior, caracterizados porque:

- R_1 , R_2 , R_3 , R_4 , R_5 , R_6 , R_7 , R_8 , R_{10} , R_{13} , R_{18} , R_{19} , R_{20} , R_{21} , R_{22} , R_{23} , R_{24} , R_{26} , R_{27} , R_{28} , R_{29} , R_{30} , R_{31} , R_{32} , R_{34} son independientemente H, OH, =O, átomo halo, (Me-S-), (Me-SO-), (Me-SO₂-), N₃-, NH₂-, MeSO₂NH-, alquilo, o OR donde R = grupo alquilo o acilo o ausente;

- R_9 , R_{11} , R_{12} , R_{14} , R_{15} , R_{16} , R_{17} , R_{25} , R_{33} , R_{35} pueden ser un H, OH, átomo halo, (Me-S-), (Me-SO-), (Me-SO₂-), N₃-, NH₂-, MeSO₂NH-, alquilo, OR donde R = grupo alquilo o acilo, o ausente;

..... representa un enlace doble opcional;

caracterizado porque además de lo anterior

- R_{33} o R_{14} = grupo alquilo, y

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la estereoquímica de R_{25} se encuentra en la orientación β ;

f. Los compuestos de la fórmula II general anterior o derivados de carbohidratos de los mismos, caracterizados porque:

$-R_1, R_2, R_3, R_4, R_5, R_6, R_7, R_8, R_{10}, R_{13}, R_{18}, R_{19}, R_{20}, R_{21}, R_{22}, R_{23}, R_{24}, R_{26}, R_{27}, R_{28}, R_{29}, R_{30}, R_{31}, R_{32}$, son independientemente H, OH, =O, átomo halo, (Me-S-), (Me-SO-), (Me-SO₂-), N₃-, NH₂-, MeSO₂NH-, alquilo u OR donde R = grupo alquilo o acilo o ausente;

$-R_9, R_{12}, R_{15}, R_{16}, R_{17} = H$,

$-R_{34} = H, OH, =O$ y OR donde R = alquilo, acilo o carbohidrato y

$-R_{11}, R_{14}, R_{25}, R_{33}, R_{35}$ pueden ser un H, OH, átomo halo, (Me-S-), (Me-SO-), (Me-SO₂-), N₃-, NH₂-, MeSO₂NH-, alquilo, OR donde R = grupo alquilo o acilo o ausente;

..... representa un enlace doble opcional;

caracterizado porque además de lo anterior

$-R_{33}$ o $R_{14} =$ grupo alquilo, y

la estereoquímica de R_{25} se encuentra en la orientación β ;

g. Los compuestos de la fórmula II general anterior o derivados de carbohidratos de los mismos, caracterizados porque:

$-R_1 = R_2 = R_4 = R_5 = R_6 = R_7 = R_8 = R_{10} = R_{11} = R_9 = R_{12} = R_{13} = R_{15} = R_{16} = R_{17} = R_{18}$

$= R_{19} = R_{20} = R_{21} = R_{22} = R_{23} = R_{24} = R_{25} = R_{26} = R_{27} = R_{28} = R_{29} = R_{30} = R_{31} = R_{32} = R_{33} =$

H,

$-R_{14} = CH_3$

$-R_{34} = -OH$ o $-OR$ donde R = alquilo, acilo o carbohidrato y

$-R_{35} = H$ o está ausente

..... representa un enlace doble opcional,

- el grupo metilo en C25 puede estar en la configuración R o S,

- la estereoquímica de R_{25} está en la orientación β ; y

caracterizado porque además de lo anterior

al menos R_3 o R_{23} es un radical X, el posible sustituyente restante es H, OH, =O, y O donde R = grupo alquilo o acilo o ausente,

y X se selecciona del grupo que consiste de:

- átomo halo,
- (Me-S-), (Me-SO-), (Me-SO₂-), y
- N₃- NH₂- MeSO₂NH-,
- alquilo;

g. Los compuestos de la fórmula II general anterior o derivados de carbohidratos de los mismos, caracterizados porque:

$-R_1 = R_2 = R_4 = R_5 = R_6 = R_7 = R_8 = R_{10} = R_{11} = R_9 = R_{12} = R_{13} = R_{15} = R_{16} = R_{17} = R_{18}$

$= R_{19} = R_{20} = R_{21} = R_{22} = R_{23} = R_{24} = R_{25} = R_{26} = R_{27} = R_{28} = R_{29} = R_{30} = R_{31} = R_{32} = H,$

$-R_{14} = R_{33} = CH_3$

-R₃₄ = -OH u -OR donde R = alquilo, acilo o carbohidrato y

-R₃₅ = H o está ausente

..... representa un enlace doble opcional,

- la stereoquímica de R₂₅ está en la orientación β; y

caracterizado porque además de lo anterior

al menos R₃ o R₂₃ es un radical X, el posible sustituyente restante es H, OH, =O, u OR donde R = grupo alquilo o acilo o ausente,

y X se selecciona del grupo que consiste de:

- átomo halo,
- (Me-S-), (Me-SO-), (Me-SO₂-), y
- N₃- NH₂- MeSO₂NH-,
- alquilo;

i. Los compuestos de la fórmula III general anterior, caracterizados porque:

-R₁, R₂, R₃, R₄, R₅, R₆, R₇, R₈, R₁₀, R₁₃, R₁₈, R₁₉, R₂₀, R₂₁, R₂₂, R₂₃, R₂₄, R₂₆, R₂₇, R₂₈, R₂₉, R₃₀, R₃₁, R₃₂, R₃₃, R₃₄, R₃₅, R₃₆, R₃₇ son independientemente H, OH, =O, átomo halo, (Me-S-), (Me-SO-), (Me-SO₂-), N₃-, NH₂-, MeSO₂NH-, alquilo u OR donde R = grupo alquilo o acilo o ausente;

-R₉, R₁₁, R₁₂, R₁₅, R₁₆, R₁₇, R₂₅, puede ser H, OH, átomo halo, (Me-S-), (Me-SO-), (Me-SO₂-), N₃-, NH₂-, MeSO₂NH-, alquilo, u OR donde R = grupo alquilo o acilo o ausente;

..... representa un enlace doble opcional;

caracterizado porque además de lo anterior

-R₃₃ o R₁₄ = grupo alquilo, y

la estereoquímica de R₂₅ se encuentra en la orientación β ;

j. Los compuestos de la fórmula II general anterior o derivados de carbohidratos de los mismos, caracterizados porque:

-R₁, R₂, R₃, R₄, R₅, R₆, R₇, R₈, R₁₀, R₁₃, R₁₈, R₁₉, R₂₀, R₂₁, R₂₂, R₂₃, R₂₄, R₂₆, R₂₇, R₂₈, R₂₉, R₃₀, R₃₁, R₃₂, R₃₃, R₃₅, R₃₆, R₃₇ son independientemente H, OH, =O, átomo halo, (Me-S-), (Me-SO-), (Me-SO₂-), N₃-, NH₂-, MeSO₂NH-, alquilo u OR donde R = grupo alquilo o acilo o ausente;

-R₉, R₁₂, R₁₅, R₁₆, R₁₇, - H,

-R₃₄ = H, OH, átomo halo, (Me-S-), (Me-SO-), (Me-SO₂-), N₃-, NH₂-, MeSO₂NH-, alquilo, OR donde R = grupo alquilo, acilo o carbohidrato o ausente;

- R₁₁, R₂₅, pueden ser H, OH, átomo halo, (Me-S-), (Me-SO-), (Me-SO₂-), N₃-, NH₂-, MeSO₂NH-, alquilo, OR donde R = grupo alquilo o acilo o ausente;

..... representa un enlace doble opcional;

caracterizado porque además de lo anterior

-R₃₃ o R₁₄ = grupo alquilo, y

la estereoquímica de R₂₅ se encuentra en la orientación β ;

k. Los compuestos de la fórmula III general caracterizados

porque:

$-R_1 = R_2 = R_4 = R_5 = R_6 = R_7 = R_8 = R_{10} = R_{11} = R_9 = R_{12} = R_{13} = R_{15} = R_{16} = R_{17} = R_{18}$
 $= R_{19} = R_{20} = R_{21} = R_{22} = R_{23} = R_{24} = R_{25} = R_{26} = R_{27} = R_{28} = R_{29} = R_{30} = R_{31} = R_{32} = R_{33}$
 $= H,$

$-R_{14} = CH_3$

$-R_{34} = -OH$ o $-OR$ donde $R =$ alquilo, acilo o carbohidrato y

$-R_{35} = H$ o está ausente

$-R_{37} = H, -OH$ o $=O$

$-R_{36} = H$ o $-OH$

..... representa un enlace simple,

-el grupo metilo en C25 puede estar en la configuración R o S
y

la estereoquímica de R_{25} está en la orientación β ;

caracterizado porque además de lo anterior

al menos R_3 o R_{23} es un radical X, el posible sustituyente
restante es H, OH, $=O$, o OR donde $R =$ grupo alquilo o acilo o
ausente,

y X se selecciona del grupo que consiste de:

- átomo halo,
- (Me-S-), (Me-SO-), (Me-SO₂-), y
- N₃- NH₂- MeSO₂NH-,
- alquilo;

1. Los compuestos de la fórmula III general anterior o
derivados de carbohidratos de los mismos, caracterizados

porque:

-R₁ = R₂ = R₄ = R₅ = R₆ = R₇ = R₈ = R₁₀ = R₁₁ = R₉ = R₁₂ = R₁₃ = R₁₅ = R₁₆ = R₁₇ = R₁₈
 = R₁₉ = R₂₀ = R₂₁ = R₂₂ = R₂₃ = R₂₄ = R₂₅ = R₂₆ = R₂₇ = R₂₈ = R₂₉ = R₃₀ = R₃₁ = R₃₂ = R₁₉
 = R₂₀ = H

-R₃₄ = -OH o -OR donde R = alquilo, acilo o carbohidrato y

-R₃₅ = H o está ausente

-R₃₇ = H, -OH o =O

-R₃₆ = H o -OH

..... representa un enlace simple,

-el grupo metilo en C25 puede estar en la configuración R o S
 y

la estereoquímica de R₂₅ está en la orientación β;

caracterizado porque además de lo anterior

al menos R₃ o R₂₃ es un radical X, el posible sustituyente
 restante es H, OH, =O, o OR donde R = grupo alquilo o acilo o
 ausente,

y X se selecciona del grupo que consiste de:

- átomo halo,
- (Me-S-), (Me-SO-), (Me-SO₂-), y
- N₃- NH₂- MeSO₂NH-,
- alquilo;

m. Sapogeninas sustituidas caracterizadas porque al menos un
 grupo OH- de la sapogenina se sustituye con X, seleccionado
 del grupo que consiste de:

- (Me-S-), (Me-SO-), (Me-SO₂-), y

- N₃- NH₂- MeSO₂NH-,

- alquilo;

n. Las sapogeninas definidas anteriormente caracterizadas porque en la definición de X el átomo halo es un átomo de fluoro.

o. Las sapogeninas sustituidas seleccionadas de:

(3 β -fluoro-5 β ,20 α ,22 α ,25R-espirostano), (3,3-difluoro-

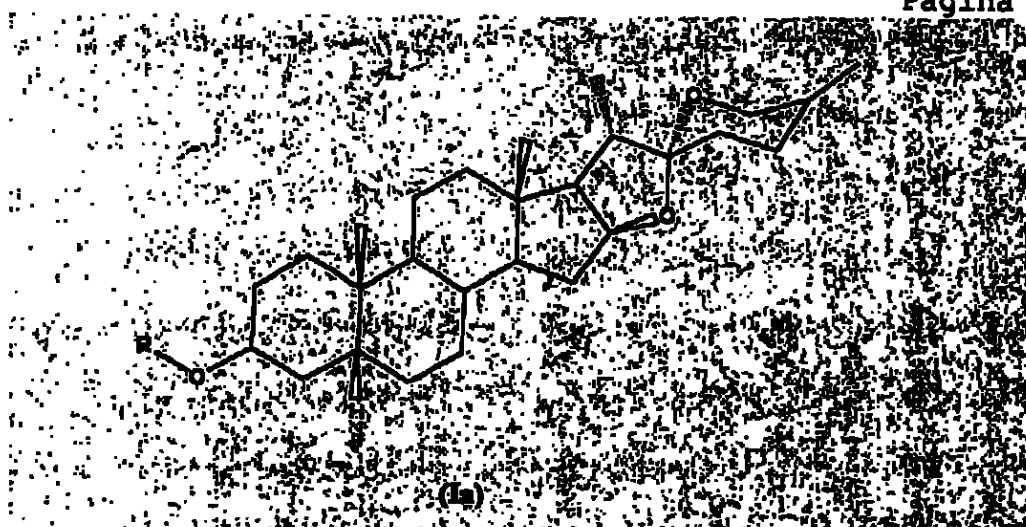
5 β ,20 α ,22 α ,25R-espirostano), (3 α -metilsulfonilamino-

5 β ,20 α ,22 α ,25R-espirostano), (3 α -azido-5 β ,20 α ,22 α ,25R-

espirostano), (3 α -amino-5 β ,20 α ,22 α ,25R-espirostano), y sus estereoisómeros y mezclas racémicas, sus pro-fármacos y sales farmacéuticamente aceptables;

o. Las sapogeninas sustituidas caracterizadas porque la sapogenina precursora, la cual es sustituida luego al menos con un radical X como se define anteriormente, se selecciona de sarsasapogenina, episarsasapogenina, esmilagenina, epismilagenina, y anzurogenina-D;

o. Los compuestos de la fórmula general Ia:



caracterizados porque el grupo R se selecciona de hidrógeno; alquilcarbonilo, alcoxicarbonilo; alquilcarbamoilo; o arilcarbonilo; o sulfo (HO_3S); fosfono ($(\text{HO})_2\text{P}(\text{O})-$); o un mono-, di- o tri-sacarido; caracterizado porque cualquier grupo alquilo es opcionalmente sustituido con arilo, amino, mono- o di-alquil-amino, un residuo de ácido carboxílico ($-\text{COOH}$), o cualquier combinación de los mismos; y

o. Las formas de derivados de los compuestos anteriores como se definen en los puntos a-q, en las que el átomo de carbono en la posición 3 o, en el caso de las Fórmulas II y III, el átomo

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de carbono en la posición 3, el átomo de carbono en la posición 26 o cada uno de los átomos de carbono en las posiciones 3 y 26, porta una porción de O-azúcar caracterizada porque el grupo de azúcar es un mono-, di- o tri-sacarido, y derivados acilados de los mismos.

3. Un uso de acuerdo con la reivindicación 1 o la reivindicación 2, caracterizado porque el agente activo, o al menos uno de los agentes activos, se selecciona de los compuestos de la fórmula general Ia.

4. Un uso de acuerdo con cualquiera de las reivindicaciones precedentes, caracterizado porque el agente activo, o al menos uno de los agentes activos, se selecciona de:

sarsasapogenina

catilato de sarsasapogenina

acetato de sarsasapogenina

succinato de sarsasapogenina y sales de los mismos farmacéuticamente aceptables

glicinatos de sarsasapogenina y sales de los mismos farmacéuticamente aceptables

alaninato de sarsasapogenina y sales de los mismos farmacéuticamente aceptables

valinato de sarsasapogenina y sales de los mismos farmacéuticamente aceptables

fenilalaninato de sarsasapogenina y sales de los mismos farmacéuticamente aceptables

isoleucinato de sarsasapogenina y sales de los mismos

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farmacéuticamente aceptables

metioninato de sarsasapogenina y sales de los mismos
farmacéuticamente aceptables

episarsasapogenina

catilato de episarsasapogenina

acetato de episarsasapogenina

succinato de episarsasapogenina y sales de los mismos
farmacéuticamente aceptables

glicinato de episarsasapogenina y sales de los mismos
farmacéuticamente aceptables

alaninato de episarsasapogenina y sales de los mismos
farmacéuticamente aceptables

valinato de episarsasapogenina y sales de los mismos
farmacéuticamente aceptables

fenilalaninato de episarsasapogenina y sales de los mismos
farmacéuticamente aceptables

isoleucinato de episarsasapogenina y sales de los mismos
farmacéuticamente aceptables

metioninato de episarsasapogenina y sales de los mismos
farmacéuticamente aceptables

esmilagenina

catilato de esmilagenina

acetato de esmilagenina

succinato de esmilagenina y sales de los mismos
farmacéuticamente aceptables

glicinato de esmilagenina y sales de los mismos

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farmacéuticamente aceptables

alaninato de esmilagenina y sales de los mismos

farmacéuticamente aceptables

valinato de esmilagenina y sales de los mismos

farmacéuticamente aceptables

fenilalaninato de esmilagenina y sales de los mismos

farmacéuticamente aceptables

isoleucinato de esmilagenina y sales de los mismos

farmacéuticamente aceptables

metioninato de esmilagenina y sales de los mismos

farmacéuticamente aceptables

epismilagenina

catilato de epismilagenina

acetato de epismilagenina

succinato de epismilagenina y sales de los mismos

farmacéuticamente aceptables

glicinato de epismilagenina y sales de los mismos

farmacéuticamente aceptables

alaninato de epismilagenina y sales de los mismos

farmacéuticamente aceptables

valinato de epismilagenina y sales de los mismos

farmacéuticamente aceptables

fenilalaninato de epismilagenina y sales de los mismos

farmacéuticamente aceptables

isoleucinato de epismilagenina y sales de los mismos

farmacéuticamente aceptables

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metioninato de epismilagenina y sales de los mismos Página
farmacéuticamente aceptables.

Derivados de saponina de sarsasapogenina, episarsasapogenina, esmilagenina y epismilagenina en los cuales, en cada caso, el átomo de carbono en la posición 3- porta una porción de O-azúcar caracterizada porque el grupo de azúcar se selecciona de glucosa, manosa, fructosa, galactosa, maltosa, celobiosa, sacarosa, ramnosa, xilosa, arabinosa, fucosa, quínovosa, apiosa, lactosa, galactosa-glucosa, glucosa-arabinosa, fucosa-glucosa, ramnosa-glucosa, glucosa-glucosa-glucosa, glucosa-ramnosa, manosa-glucosa, glucosa-(ramnosa)-glucosa, glucosa-(ramnosa)-ramnosa, glucosa-(glucosa)-glucosa, galactosa-(ramnosa)-galactosa y derivados acilados de los mismos;

16,22-epoxicoprostan-3 β -ol, esmilagenona, coprosterol, y pro-fármacos y sales de los mismos farmacéuticamente aceptables.

4. Un uso de acuerdo con cualquiera de las reivindicaciones precedentes, caracterizado porque el agente activo está presente en una composición seleccionada de las composiciones farmacéuticas, productos alimenticios, suplementos alimenticios y bebidas.

4. Un uso de acuerdo con cualquiera de las reivindicaciones precedentes, caracterizado porque el agente activo está presente con un agente activo adicional o más.

4. Un uso de acuerdo con la reivindicación 6, caracterizado

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porque un agente activo adicional o más se selecciona, sin carácter limitativo, de inhibidores de colinesterasa, agonistas de dopamina, inhibidores de COMT, inhibidores de MAO-B, anticolinérgicos, agonistas de acetilcolina, agonistas de serotonina, agonistas del receptor AMPA, agonistas del receptor GABA, agonistas del receptor NMDA, agonistas del adrenoreceptor β -, digoxina, dobutamina, anti-inflamatorios, factores neurotróficos, estatinas, antagonistas del receptor A2a de adenosina, inhibidores de reductasa de aldosa, inmunomoduladores, agonistas de canabinoide, interferon β o anti-depresivos tricíclicos.

8. Un uso de acuerdo con cualquiera de las reivindicaciones precedentes, caracterizado porque el humano o animal está sufriendo de, o es susceptible a, cualquiera de las siguientes: enfermedad de Parkinson, Parkinsonismo post-encefalítico, depresión, esquizofrenia, distrofia muscular incluyendo distrofia muscular facioescapulohumeral, distrofia muscular de Duchenne, distrofia muscular de Becker y distrofia muscular de Bruce, distrofia de Fuch, distrofia miotónica, distrofia corneal, síndrome de distrofia simpatética refleja, distrofia neurovascular, miastenia grave, enfermedad de Lambert Eaton, enfermedad de Huntington, enfermedades motoras neuronales incluyendo esclerosis lateral amiotrófica, esclerosis múltiple, hipotensión postural, neurodegeneración traumática, por ejemplo, después de una apoplejía o después de

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un accidente (por ejemplo, lesión traumática de la cabeza o lesión de la espina dorsal), enfermedad de Batten, síndrome de Cockayne, síndrome de Down, degeneración gangliónica corticobasal, atrofia del sistema múltiple, atrofia cerebral, atrofia olivopontocerebelosa, atrofia dentatorubral, atrofia palidoluisiana, atrofia espinobulbar, neuritis óptica, pan-encefalitis esclerosante, trastorno por déficit de la atención, encefalitis post-viral, síndrome post-poliomelitis, síndrome de Fahr, síndrome de Joubert, síndrome de Guillain-Barre, lisencefalia, enfermedad de Moyamoya, trastornos de migración neuronal, síndrome autístico, enfermedad de poliglutamina, enfermedad de Niemann-Pick, leucoencefalopatía multifocal progresiva, pseudotumor cerebral, enfermedad de Refsum, síndrome de Zellweger, parálisis supranuclear, ataxia de Friedreich, ataxia espinocerebelosa tipo 2, síndrome de Rhett, síndrome de Shy-Drager, esclerosis tuberosa, enfermedad de Pick, síndrome de fatiga crónica, neuropatías incluyendo neuropatía hereditaria, neuropatía diabética y neuropatía mitótica, neurodegeneración con base de prión, incluyendo enfermedad de Creutzfeldt-Jakob (CJD), CJD variante, CJD nueva variante, encefalopatía espongiiforme bovina GSS, FFI, kuru y síndrome de Alper, enfermedad de Joseph, encefalomiелitis diseminada aguda, aracnoiditis, lesiones vasculares del sistema nervioso central, pérdida de función neuronal de las extremidades, enfermedad de Charcot-Marie-Tooth, susceptibilidad a insuficiencia cardíaca, asma y

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degeneración macular.



RESUMENMÉTODOS Y USOS TERAPÉUTICOS DE SAPOGENINAS Y SUS DERIVADOS

Esta invención divulga métodos y usos terapéuticos de ciertas sapogeninas esteroidales, compuestos relacionados y derivados de los mismos, en el tratamiento de la neurogeneración no cognitiva, degeneración neuromuscular no cognitiva, neurodegeneración motora-sensorial o disfunción o pérdida del receptor en ausencia de la deficiencia cognitiva, neural y neuromuscular.

MP-MG-LU/BM1401A3/97,7pp

FIGURA 1

Densidad del receptor de m_3 y β (DPM)

Densidad del receptor de m_3 + Vehículo

Densidad del receptor de m_3 + Acetato de epismilagenina

Densidad del adrenoceptor B_2 + Vehículo

Densidad del adrenoceptor B_2 + Acetato de Epismilagenina

FIGURA 2

Sobrevivencia (%)

Control

Glutamato

Glutamato + Sarsasapogenina

Glutamato + Catilato de Episarsasapogenina

Glutamato + Esmilagenina

FIGURA 3

Capacidad de aprendizaje y memoria

A Ratas jóvenes

B Ratas de edad avanzada

C Ratas de edad avanzada + Sarsasapogenina

D Ratas de edad avanzada + Episarsasapogenina

E Ratas de edad avanzada + Esmilagenina

FIGURA 4

Densidad del receptor muscarínico

(fmol mg⁻¹ proteína⁻¹)

- A Ratas jóvenes
- B Ratas adultos
- C Ratas de edad avanzada + Sarsasapogenina
- D Ratas de edad avanzada + Episarsasapogenina
- E Ratas de edad avanzada + Esmilagenina

FIGURA 5

Perfil de sobrevivencia (No. de ratones)

Ratones SOD de Control

Ratones SOD + Esmilagenina (3mg/kg/día)

Tiempo (días)

211

NIT : 800176089-2

RECIBO OFICIAL DE CAJA : 04 - 79,372
FECHA : OCTUBRE 11 DE 2004

SUPERINDUSTRIA Y COMERCIO
Radicacion : 84105085 00000000
Fecha (AMB) : 2004-10-21 16:39:53
Tramite : 011 PCT-PATENT 379 FASE NACI 411 PRESENTAC
Dependencia: 2808 DIVISION DE NUEVAS CREACIONES
Folios: 211

***** CONSIGNACION *****

DEPOSITANTE	TIPO PAGO	BANCO	CUENTA	No. PAGO	FECHA PAGO	Vr. PAGO
CAVELIER ABOGADOS	CONSIGNACION	BANCO POPULAR	050-00110-6	32758688	11/10/2004	55,880,000.00

***** CONCEPTO *****

CANT. RENTISTICO	CONCEPTO	TOTAL CONCEPTO
1 50005-01-01 SOLICITUDES	1 TRAMITES DE SOL. DE PATENTE DE IN	422,000.00
	TOTAL :	422,000.00

SON: CUATROCIENTOS VEINTIDOS MIL PESOS

RESPONSABLE : 

RECIBO DE CAJA APLICADO AL EXPEDIENTE No. _____

Qdo 14475-841-001-9

212

SUPERINDUSTRIA Y COMERCIO
Radicación : 04105005 00000000 Folios: 211
Fecha (AMD): 2004-10-21 16:59:53
Trámite : B11 PCT-PATENT 379 FASE INCI 411 PRESENTAC
Normativa: 2020 ESTATUTO DE UNIÓN REGISTRADA

REQUISITOS NECESARIOS PARA PRESENTACION Y AGILIZACION DEL TRAMITE

	USUARIO	RADICADOR
PATENTE DE INVENCION ✓	()	(✓)
- Indicación de que se solicita la concesión de una patente.	()	(✓)
- Datos de identificación del solicitante o de la persona que se presenta la solicitud.	()	(✓)
- Descripción de la invención.	()	(✓)
- Dibujos de ser estos pertinentes.	()	(✓)
- Comprobante de Pago de las tasas establecidas.	()	(✓)

COMPLETA ()	INCOMPLETA ()
--------------	----------------

DISEÑO INDUSTRIAL ()

- Indicación de que se solicita el registro de Diseño Industrial	()	()
- Datos de identificación del solicitante o de la persona que presenta la solicitud.	()	()
- Representación gráfica o fotográfica del Diseño Industrial o muestra del Material que incorpora el diseño.	()	()
- Comprobante de pago de las tasas establecidas	()	()

COMPLETA ()	INCOMPLETA ()
--------------	----------------

ESQUEMA DE TRAZADO ()

- Indicación de que solicita el registro de un Esquema de trazado.	()	()
- Datos de identificación del solicitante o de la persona que presenta La solicitud.	()	()
- Representación gráfica del esquema de trazado	()	()
- Comprobante de pago de las tasas establecidas.	()	()

COMPLETA ()	INCOMPLETA ()
--------------	----------------

Firma Responsable: Claudia M Neva.

Fecha:

2020

Bogotá D C

Doctor(a)
EMILIO FERRERO
Apoderado
PHYTOPHARM PLC.

Oficio N° **10481**

ASUNTO:

Radicación N°	04-105805
Solicitud internacional	PCT/GB03/01380
Fecha inicio fase nacional	2004-10-27
Trámite	011
Evento	379
Actuación	430
Folios	001

Como resultado del examen de forma, realizado con fundamento en el artículo 38 de la Decisión 486 de la Comisión de la Comunidad Andina, artículo 27 del Tratado de Cooperación en Materia de Patentes (PCT), regla 51 bis del Reglamento y Circular Única de la Superintendencia de Industria y Comercio, se le comunica que:

- La solicitud no contiene copia del documento en que consta la cesión del derecho de patente del inventor al solicitante o a su causante (Art. 26-k Decisión 486).
- La solicitud no contiene el poder debidamente otorgado, con el lleno de requisitos exigidos por los artículos 63 y subsiguientes del Código de Procedimiento Civil. Adicionalmente deberá presentar el documento que acredita la existencia y representación legal de la sociedad solicitante, cuando éste fuere una persona jurídica

En cumplimiento de lo dispuesto por el artículo 39 de la Decisión 486, se le requiere para que dentro del término de dos meses siguientes a la fecha de notificación del presente oficio, complete los requisitos anteriormente enunciados. En caso de no dar cumplimiento al presente requerimiento, la solicitud se considerará abandonada y perderá su prelación.

Notifíquese el presente oficio conforme a lo dispuesto en el numeral 5.2, literal e), del capítulo quinto del título primero de la Circular Única N° 10 de 2001.

ALIX CARMENZA CÉSPEDES DE VERGEL
Jefe de la División de Nuevas Creaciones.

El anterior requerimiento fue notificado por fijación en lista de fecha,

202063

172 NOV. 2004

~~12 NOV 2004~~