The present invention discloses 4-nerolidylcatechol and its derivatives isolated from South American/Amazon plants (Pothomorphe species) and their potential use as therapeutic agent for treatment of malarial symptoms, including malarial patients resistant to traditional drugs. The present invention also discloses a method for producing 4-nerolidylcatechol and their derivatives.
Description

DERIVATIVES OF 4-NEROLIDYLCATECHOL, PHARMACEUTICAL COMPOSITIONS COMPRISING THEM AND PROCESS FOR PRODUCING THE SAME

Field of the Invention

The present invention discloses 4-nerolidylcatechol derivatives and their use in pharmaceutical compositions, as composition for the treatment of malaria in general. The present invention also discloses a method for producing 4-nerolidylcatechol derivatives.

Background of the invention

Malaria

Malaria is the main cause of economic loss and high morbidity in the world today. It is an infectious disease caused by protozoan parasites and continues to be endemic at tropical regions, such as Amazon.

In the Brazilian Amazon, 1.6 million positive plates in a total of 8 million diagnostic tests for malaria were registered. The lack of an effective vaccine and the increasing expansion of strains of Plasmodium falciparum presenting resistance towards commonly used, low cost antimalarials make control of this disease difficult. As a result, the WHO has been promoting research on natural product based drugs for treatment of disease and many plant species have been evaluated for antimalarial activity [Weninger, 2004].

The triage of useful and effective plants is at the heart of traditional medicinal knowledge and is an extremely important source of therapeutic compounds in use today. Important semi-synthetic, low cost, highly effective antimalarial drugs such as the quinolines (chloroquine, mefloquine and primaquine) and artemisinin derivatives (sodium artesunate, arteether, artemether) owe their initial discovery to the isolation and structural identification of antimalarial natural products (quinine and artemisinin) from...
traditionally used antimalarial plant species (*Cinchona* spp. and *Artemisia annua*) [Rosenthal (2003)].

Recent studies on traditionally used antimalarial remedies have revealed plants which produce indole and isoquinoline alkaloids, sesqui-, di- and triterpenes, flavonoids and other substances presenting proven in vitro activity against *P. falciparum* [Frederich (1999)].

There is a high risk of contracting highly chloroquine-resistant falciparum malaria in the Amazon, especially at the "legal Amazon" (Amazon, Mato Grosso and Maranhao), but it also exists in adjacent parts of Colombia, Peru, Bolivia and Venezuela. For those patients using chloroquine, around 20% of treatment failure rates have been reported in some areas of Amazon. Furthermore, sulfadoxine pyramethamine (a second-line antimalarial drug) resistance has been reported in Colombia, Peru and Venezuela.

Studies on the macromolecular profiles of these parasites in association with analysis of genetic resistance markers should contribute to elucidate the possible mechanisms of resistance of the parasites to the natural products tested as well as aid in the discovery of new targets and/or new mechanisms of action for antimalarial chemotherapy [Mu (2007)].

**Medicinal Plants**

Species of the *Pothomorphe* genus (*P. umbellata* and *P. petalta*) are commonly found in Brazil. *Pothomorphe peltata* (L) Miquel is a small tree, around 1 to 2 m height and can be found at Central and South America. [Missouri Botanical Garden (2002)]. It is known by many popular names, as caapeba, pariparoba, caapeba-do-Norte, malvarisco, aguaxima, among others.

This species is commonly used in traditional medicine to treat patients with malaria [Bastos (1998)], liver diseases and ulcers [Desmarchelier (1997)], headaches, hepatite and conjuntivite [Egg (1999)], erysipelas, leishmaniose [Mors (2000)], burns [Di Stasi (2002)]. It is also used as antiinflammatory, diuretic, abortive, stimulating [Jardim (2002)], feed [Egg (1999)], sudoriferous and tonic [Rodrigues (1989)]
The extract of *P. umbellata* roots and 4-nerolidylcatechol compound, a catechol prenylated found in both species, can be used as cosmetics and pharmaceutical formulations. Many models were tested to evaluate the antioxidant and photo protection activity *in vitro* and *in vivo* of this compound.

The hydroalcoholic extract of roots and the compound 4-nerolidylcatechol were tested in gel formulations to establish the chemical stability and the factor of solar protection using irradiation with UVB lamps. (SILVA et al., 2005). ROPKE (2002, 2003) studied the cutaneous permeation and the effects of the topical application of formulations containing 4-nerolidylcatechol (gel, cream-gel and creams). This study involved *in vivo* analyses about the chronic and acute problems within the UV radiation. It showed that the gel was the most effective vehicle to be used on the skin. In 2002, a Brazilian patent protected the right to use the *P. umbellata* extract as a topical cosmetic and pharmaceutical formulation to prevent/oppose the effects of photo oxidatives on the skin, skin aging and/or skin cancer (INPI, 2004).

In order to allow the use of 4-nerolidylcatechol in pharmaceutical formulations, some studies were done about stability, solubility and bioavailability of complexes of 4-nerolidylcatechol (which has low water solubility) containing hydroxypropyl-β-cyclodextrines (HP-β-CD). The increase of hydroxypropyl-β-cyclodextrines concentration lead to an increase of A-nerolidylcatechol water solubility (VALERIANO et al., 2005).

In vivo studies showed that the hydroalcoholic extract of roots having 25,4% of 4-nerolidylcatechol can be used as a protector agent (topical use) against the damage caused by the UV irradiation (ROPKE et al., 2003, 2005).

Besides, the ethanolic extract of the *P. umbellata* roots showed a greater inhibitory effect of metalloproteases (MMPs - enzymes which regulates the exposition to radiation UV) *in vivo* than the 4-nerolidylcatechol compound (ROPKE et al., 2006).

Many studies disclose the properties of *P. petalta* and *P. umbellata* as antioxidants, as well as the alkylcatechol 4-nerolidylcatechol. *In vitro* studies
showed that *P. umbellata* leaf methanol extract had antioxidant activity (AGBOR et al., 2005).

Another example of antioxidant activity given by leaf methanol extracts could be seen analyzing hydroperoxide luminescence of liver homogenates (DESMARCHELIER et al., 1997a).

The capability to capture the peroxyl radical was determined through the total reactive antioxidant potential (TRAP) and total antioxidant reactivity (TAR) using 2,2-azo-bis(2-amidinopropano) (ABAP) to modulate the luminol luminescence. This capability was as high for the leaf methanol extracts as for the 4-nerolidylcatechol compound. The latter also exhibited hydroxyl inhibition mediated by DNA damage induced by Fe (II) salts. (DESMARCHELIER et al., 1997b; BARROS et al., 1996).

PASQUALOTO et al. (2001) found evidence that the antioxidant activity of 4-nerolidylcatechol is associated with the oxidation of its alkyl side chain. This was observed in alkenyl resorcinol compounds, where the electrostatic and lipophilic potentials were measured at the aromatic rings and lateral chains. 4-nerolidylcatechol presented *in vitro* antioxidant activity 20 times greater than α-tocoferol, using tests based on co-oxidation of substrate (β-carotene, linoleic acid) and simultaneous lipoperoxidation can be seen in rat brain homogenates.

The water-alcohol extract of roots showed a greater antioxidant effect compared to the leaves and stamen extracts (DE FREITAS, 1999).

**Objects of the Invention**

It is an objective of the 4-nerolidylcathecol derivative invention according to general formulas (I) and/or (II):

(I)
wherein:

R\(_1\) and R\(_2\) are, independently, chosen from the group comprising OH, OC(=O)C\(_6\)H\(_5\), acetate, OCH\(_2\)(C\(_6\)H\(_5\)), OCH\(_3\), and mixtures thereof; and

It is a further object of the invention a pharmaceutical compositions comprising:

a) 4-nerolidylcatechol derivatives according to general formulas (I) and/or (II):

wherein:

R\(_1\) and R\(_2\) are, independently, chosen from the group comprising OH, OC(=O)C\(_6\)H\(_5\), acetate, OCH\(_2\)(C\(_6\)H\(_5\)), OCH\(_3\), and mixtures thereof; and
Its salts, solvates, hydrates and/or isomers; and

b) a pharmaceutically acceptable vehicle

In a preferred embodiment, said composition has antimalarial activity.

It is a further object of the present invention a method for producing A-nerolidylcatecol derivatives comprising the steps of:

a) isolating 4-nerolidylcatecol from a plant material;

b) performing at least one of the following reactions:

Methylation;

Benzylation;

Benzoylation;

Epoxidation;

Acetylation;

**Detailed Description of the Invention**

The following examples are illustrative of the present invention and are by no means intended to limit the scope of the present invention.

As used here, the expression "pharmaceutical compositions" should be understood as any and all compositions with an active principle, prophylactic or healing to maintain or restoring the homeostase, formulated for topical, oral, parenteral, intranasal, intravenous, intramuscular, subcutaneous or intraocular administration and the like. The meaning of the expression "active principle" comprises all or any compounds of general formula (I) and (II) and their salts, solvates, hydrates and/or isomers.

**4-nerolidylcatecol Derivatives**

The 4-nerolidylcatecol derivatives disclosed herein are compounds having structures according to general formulas (I) and/or (II) below:
wherein:
R\textsubscript{1} and R\textsubscript{2} are, independently, chosen from the group comprising H, OH, CrC\textsubscript{8} alkyl, Ci-C\textsubscript{5} acolxy, OC(=O)C\textsubscript{6}H\textsubscript{5}, formate, acetate, propionate, butanoate, pentanoate, OCH\textsubscript{2}(C\textsubscript{6}H\textsubscript{5}) and mixtures thereof.

In a preferred embodiment, the following structures are synthesized:

Compound 1 - General formula (I) wherein R\textsubscript{1} is OCOC\textsubscript{6}H\textsubscript{5} and R\textsubscript{2} is OCOC\textsubscript{6}H\textsubscript{5}.

Compound 2 - General formula (I) wherein R\textsubscript{1} is OCH\textsubscript{2}(C\textsubscript{6}H\textsubscript{5}) and R\textsubscript{2} is OCH\textsubscript{2}(C\textsubscript{6}H\textsubscript{5}).

Compound 3 - General formula (I) wherein R\textsubscript{1} is, OCH\textsubscript{2}(C\textsubscript{6}H\textsubscript{5}) and R\textsubscript{2} is OH.

Compound 4 - General formula (I) wherein R\textsubscript{1} is and R\textsubscript{2} is OCH\textsubscript{2}(C\textsubscript{6}H\textsubscript{5}).

Compound 5 - General formula (I) wherein R\textsubscript{1} is, acetate and R\textsubscript{2} is acetate.

Compound 6 - General formula (II) wherein R\textsubscript{1} is acetate and R\textsubscript{2} is acetate.

Compound 7 - General formula (I) wherein R\textsubscript{1} is OCH\textsubscript{3} and R\textsubscript{2} is OH.

Compound 8 - General formula (I) wherein R\textsubscript{1} is OH and R\textsubscript{2} is OCH\textsubscript{3}.
4-nerolidylcathecol
The starting compound 4-nerolidylcathecol can be obtained from plant extracts of Pothomorphe (P. umbellata and P. peltata). The extract of Pothomorphe can be obtained through several methods disclosed in the art. The starting plant material can be any part of the plant (leaves, roots, stems and inflorescence). The most common methods are:

a) Cold Maceration - known amounts of plant material are put in contact with a solvent, which is changed every 48 hours, filtered and concentrated in rotary evaporator at low temperatures. Solvents such as chloroform, methanol and chloroform:methanol 1:1 were evaluated.

b) Soxhlet - known amounts of plant material are put into filter-paper cartidges and the chosen solvent is added (about 400 ml). Several solvents were tested, such as chloroform, ethyl acetate, ethanol, hexane, methanol, and mixtures chloroform:ethanol 1:1 and chloroform:methanol 1:1, as well as nitrogen atmosphere, varying the extraction time.

c) Ultrasound - known amounts of plant material (ca. 5g) were weighed and put into a flask with 150 ml of a chosen solvent such as chloroform, ethyl acetate, acetone, hexane, methanol, and mixtures chloroform:ethanol 1:1, for 15 minutes under ultrasound, for a total of 3 extractions. The extract is filtered, concentrated in rotary-evaporator and lyophilized.

The most preferred method is the Ultrasound method, due to its speed and efficiency. After the extracts were produced, the 4-nerolidylcathecol was isolated from them.

Synthesis of the semi-synthetic derivatives
All the synthesized derivatives were characterized by spectroscopic methods such as (NMR iH, 13C, Cosy, HMBC e HSQC). The semi-synthetic derivatives were prepared from the natural product 4-nerolidylcathecol, through several reactions such as:

1) Methylation reactions - using methyl iodide in potassium carbonate and dichloromethane and/or diazomethane.
2) Benzylation reactions - using benzyl bromide or chloride, dimethylformamide in double-boiling for 20-30 min. The reaction remained under stirring in room temperature for 18 h.

3) Benzylation reactions - using pyridine or triethylamine in benzoyl chloride, under N₂ atmosphere and stirring for 1-24 hours.

4) Epoxidation reactions - using meta-chloroperbenzoic acid in dichloromethane at 0°C, under N₂ atmosphere and stirring for 4.5-72 hours.

5) Acetylation reactions - using acetic anhydride and pyridine, under N₂ atmosphere and stirring for 4.5-72 hours.

The yields for each reaction vary from 10 to 80%, and the derivatives were isolated by chromatography.

Example 1 - Isolation of 4-nerolidylcathecol

19.5 g of Chloroform:ethanol (1:1) extract from roots of P. peltata (prepared by ultrasound method) were passed through a silica-gel chromatographic column ((0 = 5.0 cm; h = 38 cm), using chloroform:ethanol (9:1) as mobile phase and methanol. 14 fractions were obtained and were compared with a standard sample with 4-nerolidylcathecol.

The fractions containing 4-nerolidylcathecol were then purified with preparative TLC using as elution system a mixture of chloroform:ethanol (85:15). The final yield of 4-nerolidylcathecol was 8.6 g (44%) and a total yield of 5.7% based on the dry and ground root (w/w).

Example 2 - Semi-Synthesis of 4-nerolidylcathecol derivatives

2.1 Benzylation reaction

4-nerolidylcathecol was solubilized in pyridine under nitrogen atmosphere and stirring, followed by drop addition of benzoyl chloride. The solution was heated for 20 min at 110°C under stirring. The reaction remained at room temperature under agitation for 139 h. The post-reaction protocol began with the addition of 5 ml of cold water to the medium, extracting with chloroform (2 x 5 mL) and 3 x 5 mL of water, in an alternate fashion.
The chloroform phase was washed with HCl 0.1 N (2 x 5 mL), diluted NaHCO₃ (2 x 5 mL), water (5 mL) and saturated NaCl (2 x 5 mL). The solution of CHCl₃ was dried with anhydrous sodium sulphate, filtered and the solvent removed by a rotary evaporator.

The product was purified by column chromatography, yielding a mixture of monobenzoylated derivatives and separated by preparative thin layer chromatography.

2.2 Benzylation reaction

4-nerolidycathecol was solubilized in DMF under Nitrogen atmosphere and stirring, followed by addition of potassium carbonate and benzyl chloride. The reaction remained at room temperature under agitation for 158 h. 5 mL of water was added, extracted with 20 mL of chloroform. The organic phase was washed with water (5 mL), saturated sodium chloride solution (6.5 mL) and dried with MgSO₄, filtered and concentrated via rotary evaporator.

The product was purified by column chromatography, yielding a mixture of monobenzylated derivatives and separated by preparative thin layer chromatography.

2.3 Acetylation Reaction

4-nerolidycathecol was added to acetic anhydride and pyridine, under nitrogen atmosphere and stirring for 24 hours. The post-reaction protocol began with the addition of 3 mL of water to the reaction, extraction with chloroform (5 mL).

The chloroform phase was washed with HCl 0.1 N (2 x 3 mL), saturated solution of NaHCO₃ (2 x 3 mL), water (3 mL) and saturated NaCl (2 x 3 mL). The solution of CHCl₃ was dried with anhydrous sodium sulphate, filtered and the solvent removed by a rotary evaporator.

The product was purified by column chromatography, yielding a diacetylated derivative.

2.4 Epoxidation Reaction of the Diacetylated Reaction
The diacetylated 4-nerolidylcathecol derivative from the previous item was dissolved in dichloromethane with 1.2 %M of meta-chloroperbenzoic acid (m-CPBA) in dichloromethane. The flask remained, in the first two hours, in a NaCl ice bath at -5°C and then at room temperature, under nitrogen atmosphere and stirring for 11 days.

After this period, the reaction was treated with aqueous Na₂S₂O₃ (to neutralizing the oxidative power), followed by aqueous NaHCO₃ (for neutralization of acids). The solution was filtered and extracted with chloroform. The organic phases were washed with water and dried with anhydrous NaSO₄. The product was purified by flash chromatography.

2.5 Methylation Reaction

4-nerolidylcathecol (410 mg) was treated with diazomethane (10 ml) at room temperature. The solvent was removed by rotary evaporator and the product was purified by column chromatography, obtaining monomethylated derivatives and separated by preparative thin layer chromatography.

Example 3 - Biological Assays

\textit{In vitro test to evaluate the growth inhibition of P.falciparum}

Initially, the parasites were sincronized to trophozoite stage with the ring shape, using the sorbitol treatment (LAMBROS, VANDERBERG, 1979). Stock solutions of each extract, pure compound or derivative were done in DMSO or ethanol with an initial concentration of 50 mg/mL for the extracts and 10 mg/mL for pure substances. A volume of 20 µL of each stock solution was transferred to microtubes containing growth medium RPMI complete.

Aliquots of 150 µL were added to microplates containing 96 wells each. To those wells were added 100 µL of parasited blood (final volume of 250 µL), 1% of parasitemy and initial concentration of 500 µg/mL for the extracts and 50 µg/mL for the pure substances. Control was prepared exchanging the stock solution to DMSO or ethanol (negative control) or antimalarial chloroquine, quinine or arthemisinine (positive control).
The plates were left to rest without direct light at 37° C, 5% of CO₂ atmosphere, 5% of O₂ and 90% of N₂ and the antiplasmodial activity was evaluated after 48 h for extracts and 24 h for pure substances or semi-synthetic derivatives. The parasitemy of each well was determined by the colored blood smear exam using the Panotic method in an optic microscope, with lens of 100 times.

The parasitemy was expressed in percentage of viable erythrocytes shapes observed during the counting of 3000 red blood cell and the inhibition was expressed in percentage. All the clinical studies were made in triplicate.

The concentration of the samples required to reduce in 50% the growth of the parasite (CL₅₀) were calculated by linear regression using dose-response curves.

Results

The benzoylated derivatives, monobenzoylated, benzylated, monobenzylated, epoxide and monomethylated presented growth inhibition potential of stocks K₁ of *P. falciparum in vitro*, comparable to the natural substance and standard substance.
**Claims**

DERIVATIVES OF \^\text{NEROLIDYLCATECHOL}, PHARMACEUTICAL COMPOSITIONS COMPRISING THEM AND PROCESS FOR PRODUCING THE SAME

1. Derivative of 4-nerolidylcatehol characterized by the fact that it has a structure according to general formulas (I) and/or (II):

![Structure of derivative](image)

wherein:

R1 and R2 are, independently, chosen from the group comprising OH, OC(=O)C6H5, acetate, OCH2(C6H5), OCH3 and mixtures thereof; and its salts, solvates, hydrates and/or isomers thereof.

2. Derivative, according to claim 1, characterized by the fact that it has general formula (I) wherein R1 is OCOC6H5 and R2 is OCOC6H5.

3. Derivative, according to claim 1, characterized by the fact that it has general formula (I) wherein R1 is OCH2(C6H5) and R2 is OCH2(C6H5).

4. Derivative, according to claim 1, characterized by the fact that it has general formula (I) wherein R1 is OCH2(C6H5) and R2 is OH.
5. Derivative, according to claim 1, characterized by the fact that it has general formula (I) wherein R1 is OH and R2 is OCH\(_2\)(C\(_6\)H\(_5\)).

6. Derivative, according to claim 1, characterized by the fact that it has general formula (I) wherein R1 is acetate and R2 is acetate.

7. Derivative, according to claim 1, characterized by the fact that it has general formula (II) wherein R1 is acetate and R2 is acetate.

8. Derivative, according to claim 1, characterized by the fact that it has general formula (I) wherein R1 is OCH\(_3\) and R2 is OH.

9. Derivative, according to claim 1, characterized by the fact that it has general formula (I) wherein R1 is OH and R2 is OCH\(_3\).

10. Pharmaceutical composition characterized by the fact that it comprises:

    a) 4-nerolidylcatechol derivative according to general formulas (I) and/or (M):

    ![Diagram of Compound (I)]

    ![Diagram of Compound (II)]

    wherein:

    R1 and R2 are, independently, chosen from the group comprising OH, O=C(O)C\(_6\)H\(_5\), acetate, OCH\(_2\)(C\(_6\)H\(_5\)), OCH\(_3\), and mixtures thereof; and

    Its salts, solvates, hydrates and/or isomers; and
b) a pharmaceutically acceptable vehicle

11. Pharmaceutical composition, according to claim 10, characterized by the fact that it comprises the general formula (I) wherein R1 is OCOC₆H₅ and R2 is OCOC₆H₅.

12. Pharmaceutical composition, according to claim 10, characterized by the fact that it comprises the general formula (I) wherein R1 is OCH₂(C₆H₅) and R2 is OCH₂(C₆H₅).

13. Pharmaceutical composition, according to claim 10, characterized by the fact that it comprises the general formula (I) wherein R1 is OCH₂(C₆H₅) and R2 is OH.

14. Pharmaceutical composition, according to claim 10, characterized by the fact that it comprises the general formula (I) wherein R1 is OH and R2 is OCH₂(C₆H₅).

15. Pharmaceutical composition, according to claim 10, characterized by the fact that it comprises the general formula (I) wherein R1 is acetate and R2 is acetate.

16. Pharmaceutical composition, according to claim 10, characterized by the fact that it comprises the general formula (II) wherein R1 is acetate and R2 is acetate.

17. Pharmaceutical composition, according to claim 10, characterized by the fact that it comprises the general formula (I) wherein R1 is OCH₃ and R2 is OH.

18. Pharmaceutical composition, according to claim 10, characterized by the fact that it comprises the general formula (I) wherein R1 is OH and R2 is OCH₃.

19. Process for producing 4-nerolidylcathecol derivatives characterized by the fact that it comprises the steps of:
   a) isolating 4-nerolidylcathecol from a plant material; and
   b) performing at least one of the following reactions:
      Methylation;
      Benzylation;
Benzoylation;
Epoxidation;
Acetylation;

20. Process of production, according to claim 19, characterized by the fact that the starting compound 4-nerolidylcatechol of a) can be obtained from plant extracts of plants selected from the genus *Pothomorphe*.

21. Process of production, according to claim 20, characterized by the fact that the plant is *P. umbellata* and/or *P. peltata*.

22. Process of production, according to claim 20, characterized by the fact that the extract of *Pothomorphe* can be obtained from leaves, roots, stems and/or inflorescence.

23. Process of production, according to claim 20, characterized by the fact that the methods used to obtain the plant extracts are chosen from the group comprising cold maceration, soxhlet and ultrasound.

24. Process of production, according to claim 22, characterized by the fact that the method is ultrasound.

25. Process of production, according to claim 19, characterized by the fact that the derivatives were isolated by chromatography.
1. Derivative of 4-nerolidycatechol characterized by the fact that is has a structure according to general formulas (I) and/or (II):

wherein:

R1 and R2 are, independently, chosen from the group comprising OH, OC(=O)C₆H₅, acetate, OCH₂(C₆H₅), OCH₃ and mixtures thereof; and

its salts, solvates, hydrates and/or isomers thereof;

with the proviso that:

when the derivative possesses formula (I) and either R1 or R2 is OH, then R2 or R1 is chosen from the group comprising OC(=O)C₆H₅, OC(=O)CH₃, OCH₂(C₆H₅), OCH₃ and mixtures thereof; and

when the derivative possesses formula (I) and R1 or R2 is OCH₃, R2 or R1 is chosen from the group comprising OH, OC(=O)C₆H₅, OC(=O)CH₃, OCH₂(C₆H₅), and mixtures thereof.
10. Pharmaceutical composition characterized by the fact that it comprises:

a) 4-nerolidylcathecol derivative according to general formulas (I) and/or (I.I):

\[ \text{Chemical structure of 4-nerolidylcathecol derivative} \]

(wherein:

R₁ and R₂ are, independently, chosen from the group comprising OH, OC(\(^=\)O)C₆H₅, acetate, OCH₂(C₆H₅), OCH₃, and mixtures thereof; and

its salts, solvates, hydrates and/or isomers;

with the proviso that:

when the derivative possesses formula (I) and R₁ or R₂ is OH, R₂ or R₁ is chosen from the group comprising OC(\(^=\)O)C₆H₅, OC(\(^=\)O)CH₃, OCH₂(C₆H₅), OCH₃ and mixtures thereof; and

when the derivative possesses formula (I) and R₁ or R₂ is OCH₃, R₂ or R₁ is chosen from the group comprising OH, OC(\(^=\)O)C₆H₅, acetate, OCH₂(C₆H₅), and mixtures thereof; and

b) a pharmaceutically acceptable vehicle.
PCT

Statement under Article 19(1)

Rio de Janeiro, Mai 06, 2008.

To
International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Ref: PCT/BR2007/000373
Our reference: PCTNerolidil

Dear Mr Gallay,

In response to the Communication in cases for which no other form is applicable (PCT/IB/345 from GALLAY LAURENCE) dated April 10, 2008, we would like to submit this letter with its enclosed pages as our "Statement under Article 19(1)". Additionally we tried to adapt this petition to the Rule 46.4, as recommended.

Further to the Written Opinion of the International Searching Authority (PCT/ISA/237 from MULLER-HIEL R.) dated March 03, 2008, which one we thank you, please accept this petition containing claims amended in response to the Written Opinion. These new amended sheets (that are being sent in duplicate) including the new claims 1, 10 as being the one to be protected. In the new set of claims: new independent claims 1, 10 were amended so as to better define the Compound I and Pharmaceutical composition, respectively.

Please, acknowledge receipt of this letter and its enclosed pages and process this submission as an "Statement under Article 19(1)".

Yours faithfully,

Bernardo Atem Francischetti
Patent Attorney
### A. CLASSIFICATION OF SUBJECT MATTER

IPC 5: C07C 39/08 (2006.01); C07C 43/215 (2006.01); C07C 41/01 (2006.01); C07C 69/017 (2006.01); C07C 67/00 (2006.01); C07D 303/12 (2006.01); C07D 301/03 (2006.01); A61K31/05 (2006.01); A61K 3/336 (2006.01)

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

### B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 8: C07C, C07D, A61K

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

Electronic database consulted during the international search (name of database and, where practicable, search terms used)

STN databases, WPI, EPODOC

### C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category*</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No</th>
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<td>X</td>
<td>BRPI0504720 A (UNIV SAO PAULO USP) 15 May 2007 (15.05.2007) Abstract</td>
<td>1,10</td>
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<tr>
<td>X</td>
<td>WO2004/026323 A1 (FUNDACAO AMPARO A PESQUISA DO ESTADO) 1 April 2004 (01.04.2004) Claim 1,4,5</td>
<td>1,10</td>
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[X] Further documents are listed in the continuation of Box C

[X] See patent family annex

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

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

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

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

"&" document member of the same patent family

### Date of the actual completion of the international search

7 February 2008 (07.02.2008)

### Date of mailing of the international search report

3 March 2008 (03.03.2008)

Name and mailing address of the ISA/ A T

Austrian Patent Office

Dresdner Straße 87, A-1200 Vienna

Facsimile No +43 / 1 / 534 24 / 535

Authorized officer

MULLER-HIEL R.

Telephone No +43 / 1 / 534 24 / 434

Form PCT/ISA/210 (second sheet) (January 2004)
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