Title: TREATMENTS FOR LEISHMANIASIS

Abstract: A method for the treatment of leishmaniasis, comprising administering a therapeutically effective amount of a compound of Formula I wherein R₁, R₂, R₃, and R₄ are H, OH, OR₅, or OR₆, and R₇ and/or R₈, and/or together forming a methylenedioxy or other bridging group of the form -O-(CH₂)ₙ-O- where n is 1, 2 or 3, where R₅ is a carbohydrate residue, phosphate residue, sulfate residue or lower alkyl, and R₆ is H or lower alkyl. Lower alkyl may be a linear, branched or cyclic group having less than about 6-10 carbons, optionally including one or more single or double bonds. The method also includes administering a pharmaceutically acceptable salt of a compound of Formula I and administration of a pharmaceutical composition containing a compound of Formula I together with one or more pharmaceutically acceptable ingredients.
TREATMENTS FOR LEISHMANIASIS

This work was supported by U.S. National Institutes of Health grant No. 1U01 TW01021-01 from the International Cooperative Biodiversity Groups program.

Background of the Invention

Field of the Invention

The present invention relates generally to the use of known chemical compounds for the treatment of leishmaniasis in humans and animals. More particularly the invention relates to the use of Xylopine and related compounds for the treatment of leishmaniasis in humans and animals.

Related Art

The leishmaniases are a globally widespread group of parasitic diseases generally caused by one species of flagellate protozoa belonging to the genus *Leishmania*. According to the World Health Organization (http://www.who.int/inf-fs/en/fact116.html, accessed May 20, 2002 and reference 1) leishmaniasis is transmitted by the bite of the infected female phlebotomine sandfly.

About 30 species of sandflies can become infected when taking a blood meal from a reservoir host, including infected humans, wild animals, for example rodents, and domestic animals, for example dogs. Most leishmaniases are zoonotic (transmitted to humans from animals), and humans become infected only when accidentally exposed to the natural transmission cycle. However, in the anthroponotic forms (those transmitted from human to human through the sandfly vector), humans are the sole reservoir host.

Leishmaniasis presents itself in humans in four different forms with a broad range of clinical manifestations. All forms can have devastating consequences. The primary forms are visceral leishmaniasis (VL or kala azar), mucocutaneous leishmaniasis (MCL or espundia), cutaneous leishmaniasis (CL), and diffuse cutaneous leishmaniasis (DCL).

Visceral leishmaniasis is the most severe form of the disease, which, if untreated, has a mortality rate of almost 100%. It is characterized by irregular bouts of fever, substantial weight loss, swelling of the spleen and liver, and anaemia.

Mucocutaneous leishmaniasis produces lesions which can lead to extensive and disfiguring destruction of mucous membranes of the nose, mouth and throat cavities.
[0008] Cutaneous leishmaniasis can produce large numbers of skin ulcers—as many as 200 in some cases—on the exposed parts of the body, such as the face, arms and legs, causing serious disability and leaving the patient permanently scarred. Diffuse cutaneous leishmaniasis never heals spontaneously and tends to relapse after treatment. The cutaneous forms of leishmaniasis are the most common and represent 50-75% of all new cases.

[0009] The leishmaniases are now endemic in 88 countries on five continents—Africa, Asia, Europe, North America and South America—with a total of 350 million people at risk. It is believed that worldwide 12 million people are affected by leishmaniasis, including those with overt disease and those with no apparent symptoms. Of the 1.5-2 million new cases of leishmaniasis estimated to occur annually, only 600,000 are officially declared.

[0010] The geographical distribution of leishmaniasis is limited by the distribution of the sandfly, its susceptibility to cold climates, its tendency to take blood from humans or animals only and its capacity to support the internal development of specific species of *Leishmania.*

[0011] The pentavalent antimonials, including sodium stibogluconate (Pentostam, Glaxo-Wellcome) and meglumine antimoniate (Glucantime, Rhone-Poumlec), are currently the drugs of choice for treating Leishmaniasis, and have been in use since the early 1950’s. Pentostam is available through the CDC Drug Service in the United States. Second-line drugs include Amphotericin B deoxycholate or the liposomal-formulation of Amphotericin B (Ambisome) and pentamidine isethionate. Ambisome recently became the first drug licensed in the United States for clinically apparent leishmaniasis. Hexadecylphosphocholine (Miltefosine) is under investigation for the oral treatment of Indian visceral leishmaniasis.

[0012] However, pentavalent antimonials must be administered over prolonged periods and are often associated with serious side effects including cardiotoxicity, pancreatitis and musculoskeletal affections when used at the therapeutic doses. Other treatments for leishmaniasis such as Amphotericin-B and pentamidine are associated with multiple adverse side effects such as bone marrow suppression, renal toxicity and glucose metabolism disturbances. The only potential oral treatment, miltefosine, is still in clinical trials and is a potential teratogen. Due to the shortcomings described above, a recent review of contemporary leishmaniasis treatments states “In short, there remains a pressing need for new anti-leishmanials.”
This invention satisfies a long felt need for a for safe oral treatments for leishmaniasis. The invention utilizes known compounds having heretofore unknown anti-leishmaniasis properties. Known therapeutic uses of the compounds used in the present invention are unrelated to leishmaniasis and, therefore, the anti-leishmaniasis uses described herein are unexpected.

**Summary of the Invention**

No plant-derived natural products have been approved in the United States or elsewhere for the treatment of leishmaniasis. Accordingly, the present invention raises the possibility that additional novel mechanisms for treating leishmaniasis may be discovered. The present invention provides a novel treatment for leishmaniasis; none of the current treatments for leishmaniasis utilize compounds having a chemical structure comparable to the compounds of the present invention, which belong to the class of substances known as aporphine alkaloids.

In summary, the present invention is a method for the treatment of leishmaniasis in an animal, which can be human or non-human, comprising administering a therapeutically effective amount of a compound of Formula I:

![Formula I](image-url)

$R_1, R_2, R_4$ and $R_5$ can be the same or different and may be, for example, H, OH, OR$_6$, where $R_6$ is a carbohydrate residue, phosphate residue, sulfate residue or lower alkyl. Alternatively, $R_1$ and $R_2$ and/or $R_4$ and $R_5$ can together form a methylenedioxy or other bridging group of the form $-O-(CH_2)n-O-$, where $n$ is 1, 2 or 3. $R_3$ can be, for example, H or lower alkyl. As used herein, lower alkyl includes linear, branched and cyclic groups having less than about 6-10 carbons, optionally including one or more single or double bonds. The use of pharmaceutically acceptable salts of a compound of Formula I is also contemplated by the invention. Exemplary compounds useful for practicing the invention include: Xylopine,
Cryptodorine, Normantene and Normuciferine, compounds 1, 2, 3 and 4, respectively, as set forth below.

The use of a carbohydrate residue, a phosphate residue or a sulfate residue as R₆, such that one or more of R₁, R₂, R₃ and R₄, comprise a carbohydrate, phosphate or sulfate, is exemplary. Useful compounds may be present as a racemic mixture, enantiomerically pure, or enantiomerically enriched. In other exemplary embodiments, R₃ is selected from H and methyl. In certain instances, when R₃ is methyl, R₁, R₂, R₄, and R₅ are not all methoxy. In yet another embodiment, R₃=H and R₁, R₂, R₄ and R₅ are the same or different and are selected from H and OCH₃, or R₁ and R₂ and/or R₄ and R₅ together form a methylenedioxy bridge.

The method is useful for treating leishmaniasis as the result of infection by one or more of the parasites: *Leishmania aethiopica*, *L. amazonensis*, *L. archibaldi*, *L. braziliensis*, *L. chagasi*, *L. donovani*, *L. garnhami*, *L. guyanensis*, *L. infantum*, *L. killickii*, *L. major*, *L. mexicana*, *L. panamensis*, *L. peruviana*, *L. pifanoi*, *L. tropica* and *L. venezuelensis*. The method has a demonstrated utility against *Leishmania mexicana* and *L. panamensis* in *in vitro* testing. The leishmaniasis may be manifested as cutaneous leishmaniasis, visceral leishmaniasis, mucocutaneous leishmaniasis and disseminated leishmaniasis.

The method also includes treatment of leishmaniasis by administering a pharmaceutical composition comprising one of the above described compounds or a pharmaceutically acceptable salt thereof, together with one or more pharmaceutically acceptable excipients.

The invention also includes a kit that includes a pharmaceutical composition and labeling instructions for the use of said pharmaceutical composition for the treatment of
leishmaniasis. The kit can also include a container for the pharmaceutical composition, wherein the instructions can be associated with the container.

[0019] Compounds useful in the present invention are broadly classified as aporphines. A class of compounds related to the aporphines, known as the oxoaporphines, have been shown to inhibit *Leishmania sp.*\(^{54,62}\). Dicentrinone (5) (CA Index number 16408-78-9) was shown by Del Rayo Camacho et al. to inhibit *Leishmania donovani* promastigotes with an \(LD_{50}\) of 30 \(\mu\)M\(^{54}\). In the same study, Liriodenine (6) (CA Index number 475-75-2) and N-Methyl-liriodendronine (7) (CA Index number 132872-61-8), were also shown to inhibit *Leishmaniasis donovani* promastigotes with \(LD_{90}\) values of 15 \(\mu\)M and 19 \(\mu\)M, respectively\(^{54}\). Waechter and co-workers showed that Liriodenine (6) had IC\(_{100}\) values of 9 \(\mu\)M against *Leishmania major* and *L. donovani*\(^ {62}\).

Dicentrinone (5)  
Liriodenine (6)  
N-Methyl-liriodendronine (7)

[0020] However, the oxidation of aporphines to oxoaporphines can actually lead to a loss in anti-*Leishmania sp.* activity. It has been found that the oxidation of Normuciferine (4) to the oxoaporphine, Lysicamine (8), resulted in a 10-fold loss in activity against *Leishmania*
mexicana. Thus, contrary to expectations, the aporphine, Normuciferine (4), shows greater anti-\textit{Leishmania mexicana} activity than the corresponding oxoaporphine, Lysicamine (8).

Dicetrinone (5) has also been shown to be an inhibitor of DNA topoisomerase I\textsuperscript{55}. Oxoaporphines such as Dicetrinone, Liriodenine and N-Methylliriodendronine have more planar structures than 1, 2, 3 or 4 which may account for the ability of Dicetrinone to inhibit topoisomerases\textsuperscript{56}, a property which may be associated with DNA intercalation; not necessarily a desirable quality for an anti-leishmaniasis treatment.

The antimalarial properties of some aporphines, for example compounds 1 and 2, of the present invention have been published. However, based on the medicines currently used to treat malaria and leishmaniasis, there is no reason to expect that treatments that are found to be effective for treatment of malaria will be effective against leishmaniasis. Typically, the medicines that are routinely prescribed for treatment of one disease are not known to be useful treatments for the other. Furthermore, the parasites that cause leishmaniasis and malaria are not closely related, belonging to two entirely different phyla (the leishmaniasis causing parasites belong to the phylum Sarcomastigophora while the malarial causing parasites are in the phylum Apicomplexa). Accordingly, drugs for treatment of malaria infection would not be expected to be useful in the treatment of leishmaniasis.

Further objectives and advantages, as well as the structure and function of preferred embodiments will become apparent from a consideration of the description, drawings, and examples.

\textit{Detailed Description}

Embodiments of the invention are discussed in detail below. In describing embodiments, specific terminology is employed for the sake of clarity. However, the invention is not intended to be limited to the specific terminology so selected. All references cited herein are incorporated by reference as if each had been individually incorporated.

A preferred embodiment of the invention is discussed in detail below. While specific exemplary embodiments are discussed, it should be understood that this is done for illustration purposes only. A person skilled in the relevant art will recognize that other components and configurations can be used without parting from the spirit and scope of the invention.

The genus \textit{Guatteria}, from the neotropical Annonaceae, has 279 species including \textit{Guatteria amplifolia} Triana & Planch and \textit{Guatteria dumetorum} R.E. Fr. (Craoatr,


The compounds 1, 2, 3 and 4, the structures of which are shown below, belong to the class of compounds known as the aporphine alkaloids which are associated with a wide range of biological activities including dopaminergic properties, antimicrobial properties, antimalarial properties, cytotoxic properties, and antiplatelet and vasorelaxing properties.
Activities of the various compounds useful for the invention further suggests that the methylenedioxy group is not necessary for, and probably does not contribute to, the anti-Leishmania sp. properties of these compounds. This finding is contrary to the art which would point to the importance of incorporating the methylenedioxy substituent.

There are also reports in the scientific literature for the anti-malarial activity of Xyloptine (1). The same publication also describes the "general cytotoxicity" against a panel of 12 mammalian cancer cell lines for Xyloptine (1). Other activities described for Xyloptine include: antiplatelet and vasorelaxing actions\(^{15,40}\) antimicrobial activities\(^{20,50,51}\) and binding to alpha adrenoceptors\(^{52,53}\). In a separate publication, the anti-malarial properties of Cryptodorine (2) have been described\(^{34}\). A recent publication describes the anti-leishmaniasis and anti-malarial properties of Guatteria amplifolia but does not describe any discrete chemical compounds that may contribute to activity\(^{53}\). The antimalarial properties of an alkaloidal preparation of Guatteria lehmannii were described by Saez et al.\(^{34}\), but the activities were not associated with any purified substance. Among the numerous alkaloids encountered in Guatteria lehmannii was Nornuciferine.

Villar et al.\(^{51}\) showed that normantenine (3) inhibits Candida albicans ATCC26555. Philipov et al.\(^{37}\) showed that normantenine has cytotoxic activity against L 929 transformed cells.

Nornuciferine (4) is considered to have potential antidepressant activity due to its ability to bind to 5-HT1A receptors\(^{58,98}\). Nornuciferine was also shown to inhibit protein tyrosine phosphatase by Miski et al.\(^{70}\) Burkman et al. showed that certain derivatives of nornuciferine when administered i.p. to mice provoked clonic convulsions\(^{98}\). Despite the wide range of biological activities attributed to the aporphines, there are relatively few examples of aporphine alkaloids that are in clinical use. A novel sublingual formulation of
the compound apomorphine (an aporphine alkaloid) is marketed by Britannia Pharmaceuticals for the treatment of Parkinson’s disease (www.britannia-pharm.co.uk).

[0033] The present invention is related to the use of known compounds for the treatment of human patients and animals suffering from the parasitic disease, leishmaniasis. Currently, sodium stibogluconate and meglumine antimoniate are the main compounds for the treatment of leishmaniasis. These compounds are useful against all of the leishmaniasis in any of its clinical presentations including cutaneous, visceral, mucocutaneous and diffuse cutaneous leishmaniasis. The seventeen known pathogenic Leishmania sp. parasites causing leishmaniasis include: Leishmania aethiopica, L. amazonensis, L. archibaldi, L. braziliensis, L. chagasi, L. donovani, L. garnhami, L. guyanensis, L. infantum, L. killicki, L. major, L. mexicana, L. panamensis, L. peruviana, L. pijanoi, L. tropica and L. venezuelensis. Consistent with the general nature of treatment regimens, the present invention is useful for each of these 17 parasites.

[0034] The chemical compounds useful for practicing the present invention include Xylopine (1, CAS Registry Number: 517-71-5), Cryptodorine (2, CAS Registry Number: 41787-55-7, also known as Nornelotisine), Normantenine (3, CAS Registry Number: 15401-66-8) and Nornuciferine (4, CAS Registry Number: 4846-19-9) as well as their analog and derivatives as described herein. The four parent compounds have been shown to possess activity against the promastigote form of Leishmania mexicana and L. panamensis in vitro, which suggests their potential utility as general treatments for leishmaniasis in general. A description of the biological assay used to measure the activity of the compounds of the invention against L. mexicana and L. panamensis and analytical data that supports the chemical structures follows.

[0035] The chemical compounds 1, 2, 3 and 4 were shown to have the following activities against the leishmaniasis-causing parasite, Leishmania mexicana (LD50 represents the concentration of the substance necessary to kill 50% of the Leishmania mexicana parasite in vitro).

[0036] 1 Xylopine \( \text{LD}_{50} = 1 \ \mu\text{g/mL (3 \mu M)} \)

[0037] 2 Cryptodorine \( \text{LD}_{50} = 1 \ \mu\text{g/mL (3 \mu M)} \)

[0038] 3 Normantenine \( \text{LD}_{50} = 8 \ \mu\text{g/mL (26 \mu M)} \)

[0039] 4 Nornuciferine \( \text{LD}_{50} = 4 \ \mu\text{g/mL (14 \mu M)} \)
To confirm the toxicity of the compounds 1-4 to all of the Leishmania sp. parasites, they were also tested in Leishmania panamensis, yielding results virtually identical to those presented above for L. mexicana. These data suggest that compounds 1-4 will be active against all Leishmania sp. parasites. Preliminary studies designed to assess the toxicity of compounds 1-4 to mammalian cells were performed with murine macrophage J774 and human fibroblast cell lines. At concentrations up to 4 μg/mL, a concentration that is four-fold greater than the LD50 of compounds 1 or 2, none of the four compounds elicited any significant cytotoxicity towards the mammalian cells. At 40 μg/mL, the highest concentration tested, the mammalian cell lines demonstrated the following percentage survival: Xylopine, 45%; Cryptodorine, 30%; Normantenine 100%; and Nornuciferne, 70%. These preliminary but highly encouraging results suggest the potential therapeutic applications of compounds 1-4. For example, there is about a 40-fold difference between the comparably lethal doses of Xylopine (1) to Leishmania mexicana (1 μg/mL) and the mammalian cell lines (40 μg/mL).

The present invention is a method to inhibit the parasites that cause leishmaniasis such as Leishmania mexicana and L. panamensis. The invention satisfies a longstanding need by providing alternatives to the standard leishmaniasis treatments discussed above, which have known undesirable side effects. The compounds useful in the invention have not previously been shown to have properties against the parasites that causes leishmaniasis.

The present invention includes both R and S configurations at the 6a position. The isolated, naturally derived compounds show the following stereochemistry at the 6a position: Xylopine (1): R, Cryptodorine (2): S, Normantenine (3): S, and Nornuciferine (4): R. The variation in absolute stereochemistry without a concomitant loss in activity indicates that no particular absolute stereochemistry is required for activity. Accordingly, the present invention includes the use of racemic mixtures, pure enantiomers and/or enantiomerically enriched compounds.

The free base form of 1, 2, 3 and 4, is soluble in chloroform, methanol, ethanol and DMSO and poorly soluble in water. The salts formed upon treatment with acid would be expected, like most alkaloids, to show greatly enhanced water-solubility.

As free bases, many alkaloids are susceptible to N-oxidation. Accordingly compounds 1, 2, 3 and 4 as free bases are expected to show the same susceptibility.
The oxidation of Noromuciferine (4) occurs at room temperature in the presence of trace amounts of silica gel to yield Lysicamine (8). The conversion of Noromuciferine (4) to Lysicamine (8) suggests that a similar transformation may be responsible for the formation of Oxoxylopine (9) (CA Index number 23740-25-2) from Xylopine (1); and for the formation of Cassameridine (10) (CA Index number 16408-76-7) from Cryptodorine (2). These transformations could likely be avoided by formation of the corresponding salts.

For reasons of stability and solubility, the substances of the invention may be administered as salts, including, but not limited to, formulations as hydrochloride salts, tartrate salts, acetate salts, etc. The compounds useful in the invention may be administered orally, parentally, intravenously or intramuscularly. Topical application of the compounds is also contemplated would be as some cutaneous forms of the disease can be treated in this fashion.

Alkylation of the nitrogen functionality in the aporphines has been documented, producing certain N-alkylated derivatives that have lower affinities for dopaminergic receptors than their non-alkylated precursors. Accordingly, variation of the N-alkyl moiety of compounds 1-4 provides a potential means of enhancing the anti-Leishmania sp. properties of 1, 2, 3 and 4 while reducing side effects.

Accordingly, N-alkyl derivatives are useful for practicing the invention. These correspond to compounds 1-4 where R₃=alkyl, for example a lower alkyl group having less than about 6 or 10 carbon atoms that may be branched linear or cyclic, for example methyl, ethyl, propyl, isopropyl, butyl, isobutyl, tert-butyl, sec-butyl, cyclopentyl, cyclohexyl, etc. N-alkyl substituents also include those having one or more double or triple bonds, for example vinyl, allyl and propynyl. Preparation of N-alkyl substituted derivatives of alkaloid compounds having a basic nitrogen are well known in the art.
Derivatives and analogues of 1, 2 or 3 based on variations at the C-9 and/or C-10 positions, as described below, may be exploited to further enhance solubility or specificity.

**Derivatives of Xylopine (1).** Derivatives of 1 in which the O-methyl attached to the C-9 position, i.e. $R_3$ is replaced by O-$R_6$, in which $R_6$ may be, but is not limited to, carbohydrates or polar substituents such as phosphate and sulfate. Aporphine glycosides have been reported in the literature. Derivatives having OR$_6$=lower alkoxy, where lower alkoxy includes a lower alkyl group as defined above are also useful. Anolobine (CA Index number 58072-87-0) is identical to Xylopine (1) except that Anolobine has a hydroxyl moiety at the 9 position ($R_2 = \text{OH}$); synthesis of Xylopine derivatives at the 9 position from Anolobine is therefore straightforward.

**Derivatives of Normantenine (3).** Derivatives of 3 in which the O-methyl attached to the C-1 or C-2 position is replaced by OR$_6$, in which $R_6$ may be, but is not limited to, carbohydrates or polar substituents such as phosphate and sulfate as well as lower alkyl (as previously defined) are also useful for practicing the present invention. Aporphine glycosides have been reported in the literature.

Pharmaceutical formulations according to the invention comprise compounds 1-4, their analogs and derivatives or a pharmaceutically acceptable salt thereof as an active ingredient together with one or more pharmaceutically acceptable carriers, excipients or diluents. Any conventional technique may be used for the preparation of pharmaceutical formulations according to the invention. The active ingredient may be contained in a formulation that provides quick release, sustained release or delayed release after administration to the patient.

Pharmaceutical compositions that are useful in the methods of the invention may be prepared, packaged, or sold in formulations suitable for oral, parenteral and topical administration. Other contemplated formulations include nanoparticles, liposomal preparations, resealed erythrocytes containing the active ingredient, and immunologically-based formulations.

The formulations of the pharmaceutical compositions described herein may be prepared by any method known or hereafter developed. In general, preparation includes bringing the active ingredient into association with a carrier or one or more other additional components, and then, if necessary or desirable, shaping or packaging the product into a
desired single- or multi-dose unit. As used herein, “additional components” include, but are
not limited to, one or more of the following: excipients; surface active agents; dispersing
agents; inert diluents; granulating and disintegrating agents; binding agents; lubricating
agents; sweetening agents; flavoring agents; coloring agents; preservatives; physiologically
degradable compositions such as gelatin; aqueous vehicles and solvents; oily vehicles and
solvents; suspending agents; dispersing or wetting agents; emulsifying agents, demulcents;
buffers; salts; thickening agents; fillers; emulsifying agents; antioxidants; antibiotics;
antifungal agents; stabilizing agents; pharmaceutically acceptable polymeric or hydrophobic
materials as well as other components.

[0055] Although the descriptions of pharmaceutical compositions provided herein are
principally directed to pharmaceutical compositions which are suitable for administration to
humans, it will be understood by the skilled artisan, based on this disclosure, that such
compositions are generally suitable for administration to any mammal. Preparation of
compositions suitable for administration to various animals is well understood, and the
ordinarily skilled veterinary pharmacologist can design and perform such modifications with
routine experimentation based on pharmaceutical compositions for administration to humans.

[0056] A pharmaceutical composition of the invention may be prepared, packaged, or
sold in bulk, as a single unit dose, or as a plurality of single unit doses. As used herein, a
“unit dose” is a discrete amount of the pharmaceutical composition comprising a
predetermined amount of the active ingredient. The amount of the active ingredient in each
unit dose is generally equal to the total amount of the active ingredient which would be
administered or a convenient fraction of a total dosage amount such as, for example, one-half
or one-third of such a dosage.

[0057] A formulation of a pharmaceutical composition of the invention suitable for
oral administration may in the form of a discrete solid dosage unit. Solid dosage units
include, for example, a tablet, a caplet, a hard or soft capsule, a cachet, a troche, or a lozenge.
Each solid dosage unit contains a predetermined amount of the active ingredient, for
example a unit dose or fraction thereof. Other formulations suitable for administration
include, but are not limited to, a powdered or granular formulation, an aqueous or oily
suspension, an aqueous or oily solution, or an emulsion. As used herein, an “oily” liquid is
one which comprises a carbon or silicon based liquid that is less polar than water.
A tablet comprising the active ingredient may be made, for example, by compressing or molding the active ingredient, optionally containing one or more additional components. Compressed tablets may be prepared by compressing, in a suitable device, the active ingredient in a free-flowing form such as a powder or granular preparation, optionally mixed with one or more of a binder, a lubricant, a glidant, an excipient, a surface active agent, and a dispersing agent. Molded tablets may be made by molding, in a suitable device, a mixture of the active ingredient, a pharmaceutically acceptable carrier, and at least sufficient liquid to moisten the mixture.

Tablets may be non-coated or they may be coated using methods known in the art or methods to be developed. Coated tablets may be formulated for delayed disintegration in the gastrointestinal tract of a subject, for example, by use of an enteric coating, thereby providing sustained release and absorption of the active ingredient. Tablets may further comprise a sweetening agent, a flavoring agent, a coloring agent, a preservative, or some combination of these in order to provide pharmaceutically elegant and palatable preparation.

Hard capsules comprising the active ingredient may be made using a physiologically degradable composition, such as gelatin. Such hard capsules comprise the active ingredient, and may further comprise additional components including, for example, an inert solid diluent. Soft gelatin capsules comprising the active ingredient may be made using a physiologically degradable composition, such as gelatin. Such soft capsules comprise the active ingredient, which may be mixed with water or an oil medium.

Liquid formulations of a pharmaceutical composition of the invention which are suitable for administration may be prepared, packaged, and sold either in liquid form or in the form of a dry product intended for reconstitution with water or another suitable vehicle prior to use.

Liquid suspensions, in which the active ingredient is dispersed in an aqueous or oily vehicle, and liquid solutions, in which the active ingredient is dissolved in an aqueous or oily vehicle, may be prepared using conventional methods or methods to be developed. Liquid suspension of the active ingredient may be in an aqueous or oily vehicle and may further include one or more additional components such as, for example, suspending agents, dispersing or wetting agents, emulsifying agents, demulcents, preservatives, buffers, salts, flavorings, coloring agents, and sweetening agents. Oily suspensions may further comprise a thickening agent. Liquid solutions of the active ingredient may be in an aqueous or oily
vehicle and may further include one or more additional components such as, for example, preservatives, buffers, salts, flavorings, coloring agents, and sweetening agents.

[0063] Powdered and granular formulations according to the invention may be prepared using known methods or methods to be developed. Such formulations may be administered directly to a subject, or used, for example, to form tablets, to fill capsules, or to prepare an aqueous or oily suspension or solution by addition of an aqueous or oily vehicle thereto. Powdered or granular formulations may further comprise one or more of a dispersing or wetting agent, a suspending agent, and a preservative. Additional excipients, such as fillers and sweetening, flavoring, or coloring agents, may also be included in these formulations.

[0064] A pharmaceutical composition of the invention may also be prepared, packaged, or sold in the form of oil-in-water emulsion or a water-in-oil emulsion. Such compositions may further comprise one or more emulsifying agents. These emulsions may also contain additional components including, for example, sweetening or flavoring agents.

[0065] Pharmaceutical compositions according to the invention can be provided as part of a kit. Such a kit can include a pharmaceutical composition according to the invention together with labeling instructions for the use of the composition for the treatment of leishmaniasis. Such instructions can include information regarding the dosage, or number of unit dosage forms, and frequency of administration. The labeling may specify the particular type of leishmaniasis for which use is indicated, for example, cutaneous leishmaniasis, visceral leishmaniasis, mucocutaneous leishmaniasis and disseminated leishmaniasis, as described above. The pharmaceutical composition and instructions may be contained in a container. Alternatively, the pharmaceutical composition may be contained in a container and the labeling instructions associated with the container, for example by adhering a label with instructions to a container. As yet another alternative, the labeling may be provided in marketing or advertising referring to the pharmaceutical composition or otherwise as contemplated in the meaning of "labeling" under the U.S. Pure Food and Drug Act.
EXAMPLES

IR spectra were measured on a Perkin-Elmer 1699 spectrophotometer. NMR spectra were recorded on 300 and 500 MHz Bruker NMR spectrometers. Low resolution EI/MS (70 eV) were collected on a Jeol SX 102A mass spectrometer. Optical rotations were determined on a Perkin-Elmer 241 polarimeter.

Leaves of Guatteria amplifolia and G. dumetorum were collected in the Barro Colorado Natural Monument in the Republic of Panama. The taxonomy was confirmed by Professor Mireya Correa of the Smithsonian Tropical Research Institute. Vouchers were deposited at the University of Panama (G. amplifolia and G. dumetorum voucher numbers PMA 50979 and PMA 50980 respectively).

EXAMPLE 1: Extraction of Guatteria amplifolia

Fresh young leaves from Guatteria amplifolia were homogenized in 30 g aliquots with 240 mL of MeOH for 30 s in a Waring blender followed by treatment with a Polytron homogenizer (Brinkmann Instruments). After filtration, the marc was washed with 150 mL of EtOAc. The crude extract (30 g) was partitioned between hexane and MeOH. The residue from the MeOH fraction (18.5 g) was partitioned between EtOAc and water. The EtOAc fraction was subjected to acid-base extraction yielding Fraction A (2.3 g). Fr. A was chromatographed on a column of basic alumina (4 x 40 cm) (Merck type T, 70-230 mesh) and eluted with CHCl₃/MeOH (700 mL), CHCl₃/MeOH (75:25, 400 mL), CHCl₃/MeOH (67:73, 750 mL), and 100% MeOH (400 mL), and the fractions were combined according to their TLC profile into Frs. 1-5. Fr. 1 yielded laudanosine 11 (370 mg; tR between 240-810 mL; Rf=0.65), detection of eluates by TLC (see below). Fr. 2 (1.03 g), between 810-1370 mL, was chromatographed by preparative reverse phase TLC (RP-18, Merck) with CHCl₃/acetone/NH₄OH (4:1:0.1), yielding compound 4 (40 mg; Rf=0.57), and Fr. 2a (730 mg; Rf=0.5). Fr. 2a was subjected to preparative TLC (Whatman, PK5F, 500 μm) with CHCl₃/hexane/NH₄OH (2:1:1), yielding Fr. 2b (57 mg; Rf=0.42), which was purified by reverse-phase HPLC (YMC ODS-A S-5 μm, 4.6 x 250 mm) with MeOH/water/Et₃N (70:30:0.1), 1.0 mL/min, yielding compound 1 (37 mg, tR:20 min). Lysicamine (8) was obtained from 153 g of Fr. A by column chromatography on silica gel 60 (37-75 microns, 1.5 x 15 cm), 100% acetone (1300 mL), acetone/MeOH (91:9, 600 mL) yielding compound 8 (90-mg; tR between 950 and 1550 mL; Rf=0.22).
EXAMPLE 2: Extraction of *Guatteria dumetorum*

The crude extract (20 g) from *G. dumetorum* was subjected to solvent partition and acid-base extraction as described for *G. amplifolia* yielding fraction B (645 mg). Fr. B was chromatographed on a column of silica gel 60 (37-75 μM, 2.5 x 28 cm) and eluted with 800 mL of CHCl₃/hexane/Н₂О (2:1:1), yielding a mixture of compounds 2 and 3 (96 mg, tR of the mixture of 2 and 3: between 190 mL-230 mL), which was then subjected to preparative TLC (Whatman, PK5F, 500 μM) and developed with CHCl₃/hexane/Н₂О (2:1:1), yielding compound 2 (50 mg; Rf=0.25) and compound 3 (15 mg; Rf=0.20), monitoring of eluates by TLC (silica gel) CHCl₃/hexane/Н₂О 2:1:1, detection Dragendorff reagent.

EXAMPLE 3: Isolation

Isolation of Xylopine (1)

In the present work, the pulverized young leaves from the plant, *Guatteria amplifolia*, were subjected to a liquid–liquid partition using hexanes, ethyl acetate, methanol and water. The ethyl acetate and methanol phases were subjected to preparative thin-layer chromatography (TLC) using normal-phase silica gel and eluted with chloroform-hexanes-ammonium hydroxide (2:1:1) followed by purification by conventional reverse-phase high-performance liquid chromatography (HPLC) using a gradient of water and increasing amounts of acetonitrile.

Xylopine has also been isolated from *Monodora junodii* by Nishiyama et al.⁹, from *Fissistigma glaucescens* by Lo et al.¹⁰, from *Talauma ovata* by Stefanello et al.¹¹, from *Annona cherimola* by Chen et al.⁸,¹² and Simeon et al.²⁰,²¹, from *Xylopia papuana* by Bermejo et al.¹³ and Johns et al.¹⁴, from *Annona reticulata* by Chang et al.¹⁵, from *Desmos longiflorus* by Hossain et al.¹⁶, from *Stephania pierrei* by Likhitwitayawuid et al.¹⁷, from *Fissistigma oldhamii* by Wu et al.¹⁸, from *Talauma gittingensis* by Nonato et al.¹⁹, from...
Guatteria sagotiana by Rasamizafy et al.\textsuperscript{22}, from Talauma obovata Korth. by Plantinet et al.\textsuperscript{23}, from Fissistigma and Goniothalamus species by Lu et al.\textsuperscript{24}, from Guatteria schomburgkiana by Cortes et al.\textsuperscript{25}, from Duguettia obovata by Roblot et al.\textsuperscript{26}, from Xylopia frutescens Aubl. by Leboeuf et al.\textsuperscript{27}, from Annona montana Macf. by Leboeuf et al.\textsuperscript{28}, from Guatteria scandens by Hocquemiller et al.\textsuperscript{29}, from Xylopia buxifolia and Xylopia danguyella by Hocquemiller et al.\textsuperscript{30}, from Annona squamosa by Bhaumik et al.\textsuperscript{31}, from Xylopia pancheri by Nieto et al.\textsuperscript{32} and from Xylopia discreta by Schmutz et al.\textsuperscript{33}.

[0076] Isolation of Cryptodorine (2) and Nornantenine (3)

In the present work, the crude methanolic extract of pulverized young leaves from the plant Guatteria dumetorum was subjected to liquid–liquid partition with hexanes, ethyl acetate, methanol and water. The ethyl acetate partition was subjected to acid-base partition and the alkaloid-bearing fraction was chromatographed on normal-phase silica gel and eluted with chloroform-hexanes-ammonium hydroxide (2:1:1). The biologically active fractions were subjected to preparative TLC using normal-phase silica gel and eluted with chloroform-hexanes-ammonium hydroxide (2:1:1) yielding Cryptodorine (2) and Nornantenine (3), respectively.

[0078] Cryptodorine has been previously isolated from Guatteria lehmannii by Saez et al.\textsuperscript{34}, from Laurus nobilis by Pech et al.\textsuperscript{35} and from Cryptocarya odorata by Bick et al.\textsuperscript{36}

[0079] Nornantenine has been isolated from Uvaria chamae P. Beauv by Philipov et al.\textsuperscript{37}, from Siparuna tonduziana by Lopez et al.\textsuperscript{38}, from Cyclea atjehensis by Tantisiewie et al.\textsuperscript{39}, from Annona cherimolia by Villar et al.\textsuperscript{40}, from Xylopia frutescens Aubl. by Leboeuf et al.\textsuperscript{27}, from Laurelia sempervirens and Laurelia philippiana by Urzua et al.\textsuperscript{41,85,105}, from Xylopia danguyella by Hocquemiller et al.\textsuperscript{30}, from Hernandia cordigera by Lavault et al.\textsuperscript{42,100} and Bruneton et al.\textsuperscript{101}, from Hernandia nympheafolia by Yakushijin et al.\textsuperscript{102}, from Nandina domestica by Kunitomo et al.\textsuperscript{103}, and from Cassytha racemosa by Johns et al.\textsuperscript{104}.

[0080] Isolation of Nornuciferine (4)

[0081] The crude extract of Guatteria amplifolia was subjected to liquid-liquid partition with hexanes, ethyl acetate, methanol and water. The crude alkaloid mixture from the ethyl acetate partition was chromatographed on a column of basic alumina and eluted with a gradient of chloroform and chloroform:methanol. Nornuciferine was purified by preparative TLC on a 10 x 20 cm sheet of reverse-phase silica gel with 4:1:0.1
chloroform:acetone:ammonium hydroxide as solvent. The compounds were eluted with 1:1 chloroform:methanol followed by washing with 100% methanol.

[0082] Norcuciferine has also been isolated from *Duguetia flagellaris* by Navarro et al.\(^ {64}\), from Dasymaschalon sootepense by Sinz et al.\(^ {65}\), from *Anomianthus dulcis* by Sinz et al.\(^ {66}\), from *Guatteria lehmannii* by Saez et al.\(^ {34}\), from *Papaver sp.* by Shafiee et al.\(^ {67,69}\), from *Annona muricata* by Hasrat et al.\(^ {68}\), from *Rollinia ulei* by Miski et al.\(^ {70}\), from *Annona squamosa* by Wu et al.\(^ {71}\), from *Neolitsea konishii* by Lee et al.\(^ {72}\), from *Trivalvaria macrophylla* by Cortes et al.\(^ {73}\), from *Zizyphus vulgaris* by Han et al.\(^ {74}\), from *Artabotrys maingayi* by Cortes et al.\(^ {75}\), from *Zizyphi fructus* by Han et al.\(^ {76}\), from *Oxandra cf. major* by Arango et al.\(^ {77}\), from *Guatteria ouregou* by Cortes et al.\(^ {79}\) and Leboeuf et al.\(^ {82}\), from *Guatteria chrysopetala* by Hocquemiller et al.\(^ {80}\), from *Isolona sp.* by Hocquemiller et al.\(^ {81}\) from *Xylopia frutescens* by Leboeuf et al.\(^ {27}\), from *Chasmanthera dependens* by Ohiri et al.\(^ {83}\), from *Hexalobus crispiflorus* by Achenbach et al.\(^ {84}\), from *Laurelia sempervirens* by Urzua Moll et al.\(^ {85}\), from *Xylopia buxifolia* and *Xylopia danguyella* by Hocquemiller et al.\(^ {30}\), from *Magnolia sp.* and *Liriodendron sp.* by Furmanowa et al.\(^ {86}\), from *Croton spasiiflorus* by Bhakuni et al.\(^ {87}\), from *Isolona campanulata* by Hocquemiller et al.\(^ {88}\), from *Zizyphus sativa* by Khokhar et al.\(^ {89}\), from *Isolona pilosa* by Hocquemiller et al.\(^ {90}\), from *Nelumbo lutea* by Zelenski et al.\(^ {91}\), from *Anona glabra* by Yang et al.\(^ {92}\), from *Colubrina faralaotra* by Guinaudeau et al.\(^ {93}\), from *Nelumbo nucifera* by Kunitomo et al.\(^ {94,96}\), from *Nelumbo nucifera* by Yang et al.\(^ {95}\), and from *Magnolia grandiflora* by Tomita et al.\(^ {97}\).


[0084] Optical rotations, Infrared (IR) and mass spectrometric data.

[0085] Xylopine (1) (CAS Registry Number: 517-71-5)

[0086] \([\alpha]_D^0: -28.18^\circ, (\text{MeOH} , c 0.001). \text{IR (cm}^{-1}) : 2922, 1610, 1506, 1466, 1306, 1236, 1124, 1049, 945, 820. \text{MS (Electrospray positive ion) m/z: 296.2 (M+H).}\)

[0087] Cryptodorine (2) (CAS Registry Number: 41787-55-7)

[0088] \([\alpha]_D^0: +19.67^\circ \ (\text{CHCl}_3 , c 0.001). \text{IR (cm}^{-1}) : 2901, 1650, 1506, 1458, 1232, 1040, 935, 866, 797. \text{MS (Electrospray positive ion) m/z: 310.2 (M+H).}\)

[0089] Normantenine (3) (CAS Registry Number: 15401-66-8)

[0090] \([\alpha]_D^0: +32.30^\circ \ (\text{EtOH} , c 0.01). \text{IR (cm}^{-1}) : 2930, 1645, 1576, 1472, 1418, 1313, 1244, 1118, 1036, 937. \text{MS (Electrospray positive ion) m/z: 326.2 (M+H).}\)

[0091] Norcuciferine (4) (CAS Registry Number: 4846-19-9)
[0092] \[\alpha\]D: -138° (CHCl₃, c 0.06), IR (film, cm⁻¹): 3388, 2921, 2848, 1593, 1495, 1453, 1424, 1363, 1257, 1032, 753, 664, 443. EIMS (low resolution, 70 eV) m/z: 281 (M⁺, 73%), 280 (100%), 266 (18%), 252 (9%), 250 (19%), 237 (8%), 221 (12%), 165 (8%), 152 (3%), 149 (5%).

5 [0093] NMR data

[0094] Carbon and proton NMR data for isolated compounds 1-4 is presented in Tables 1 and 2, respectively. Carbon and proton positions are designated as follows:

![Chemical Structure](image)

**Table 1. **$^{13}$C-NMR Data for Alkaloids 1, 2, 3 and 4.

<table>
<thead>
<tr>
<th>C</th>
<th>1 (CDCl₃)</th>
<th>2 (DMSO)</th>
<th>3 (CDCl₃)</th>
<th>4 (CDCl₃)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>142.2</td>
<td>142.0</td>
<td>143.7</td>
<td>145.9</td>
</tr>
<tr>
<td>2</td>
<td>147.3</td>
<td>146.9</td>
<td>146.8</td>
<td>153.4</td>
</tr>
<tr>
<td>3</td>
<td>106.9</td>
<td>107.7</td>
<td>108.6</td>
<td>111.4</td>
</tr>
<tr>
<td>4</td>
<td>28.6</td>
<td>29.8</td>
<td>28.5</td>
<td>25.9</td>
</tr>
<tr>
<td>5</td>
<td>43.0</td>
<td>43.7</td>
<td>43.0</td>
<td>41.5</td>
</tr>
<tr>
<td>6a</td>
<td>53.5</td>
<td>53.9</td>
<td>53.8</td>
<td>52.9</td>
</tr>
<tr>
<td>7</td>
<td>36.7</td>
<td>37.7</td>
<td>37.0</td>
<td>34.4</td>
</tr>
<tr>
<td>8</td>
<td>112.2</td>
<td>108.9</td>
<td>111.5</td>
<td>128.0</td>
</tr>
<tr>
<td>9</td>
<td>159.6</td>
<td>147.0</td>
<td>153.0</td>
<td>126.9</td>
</tr>
<tr>
<td>10</td>
<td>113.5</td>
<td>147.0</td>
<td>147.0</td>
<td>127.7</td>
</tr>
<tr>
<td>11</td>
<td>128.6</td>
<td>107.9</td>
<td>109.4</td>
<td>126.2</td>
</tr>
<tr>
<td>3a</td>
<td>126.8</td>
<td>128.1</td>
<td>127.0</td>
<td>128.2</td>
</tr>
<tr>
<td>1b</td>
<td>126.7</td>
<td>127.1</td>
<td>126.3</td>
<td>128.6</td>
</tr>
<tr>
<td>1a</td>
<td>116.3</td>
<td>117.0</td>
<td>118.1</td>
<td>122.4</td>
</tr>
<tr>
<td></td>
<td>11a</td>
<td>124.1</td>
<td>125.0</td>
<td>126.0</td>
</tr>
<tr>
<td>-------</td>
<td>------</td>
<td>-------</td>
<td>-------</td>
<td>-------</td>
</tr>
<tr>
<td>7a</td>
<td>136.9</td>
<td>130.0</td>
<td>129.0</td>
<td></td>
</tr>
<tr>
<td>1,2-OCH₂O</td>
<td>100.8</td>
<td>100.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9,10-OCH₂O</td>
<td>-</td>
<td>101.3</td>
<td>101.3</td>
<td></td>
</tr>
<tr>
<td>1-OMe</td>
<td>-</td>
<td></td>
<td>56.3</td>
<td></td>
</tr>
<tr>
<td>2-OMe</td>
<td>-</td>
<td></td>
<td>60.6</td>
<td></td>
</tr>
<tr>
<td>9-OMe</td>
<td>54.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10-OMe</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2. ¹H-NMR Data for Alkaloids 1, 2, 3 and 4.

<table>
<thead>
<tr>
<th>H</th>
<th>1 (CD₂OD)</th>
<th>2 (DMSO)</th>
<th>3 (CDCl₃)</th>
<th>4 (CDCl₃)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>6.48 (s)</td>
<td>6.59 (s)</td>
<td>6.61 (s)</td>
<td>6.60 (s)</td>
</tr>
<tr>
<td>4</td>
<td>2.80-2.91, 1H (m)</td>
<td>2.73-2.80, 1H (m)</td>
<td>2.99-3.06, 1H (m)</td>
<td>2.90, 1H (dd) J= 3.7 and 16.9 Hz 3.46-3.60, 1H (m)</td>
</tr>
<tr>
<td></td>
<td>2.61-2.72, 1H (m)</td>
<td>2.36-2.51, 1H (m)</td>
<td>2.70-2.81, 1H (m)</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>3.25-3.32, 1H (m)</td>
<td>3.10-3.11, 1H (m)</td>
<td>2.99-3.06, 1H (m)</td>
<td>3.79 (dd, 1H) J= 6.3, 13.2 HZ 3.14-3.40 (m, 1H)</td>
</tr>
<tr>
<td></td>
<td>2.80-2.91, 1H (m)</td>
<td>2.73-2.80, 1H (m)</td>
<td>3.43-3.48, 1H (m)</td>
<td></td>
</tr>
<tr>
<td>6a</td>
<td>3.74 – 3.76 (m)</td>
<td>3.66 (dd) J= 4.8 and 14.0 Hz</td>
<td>3.84-3.88 (m)</td>
<td>4.22 (dd) J= 4.7, 12.9 Hz</td>
</tr>
<tr>
<td>7</td>
<td>2.80-2.91, 1H (m)</td>
<td>2.36-2.51, 2H (m)</td>
<td>2.99-3.06, 1H (m)</td>
<td>3.14-3.40 (m)</td>
</tr>
<tr>
<td></td>
<td>2.61-2.72, 1H (m)</td>
<td>2.70-2.81, 1H (m)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>6.79 (s)</td>
<td>6.90 (s)</td>
<td>6.73 (s)</td>
<td>7.3 (m)</td>
</tr>
<tr>
<td>9</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>7.3 (m)</td>
</tr>
<tr>
<td>10</td>
<td>6.81 (d) J = 8.5 Hz</td>
<td>-</td>
<td>-</td>
<td>7.3 (m)</td>
</tr>
<tr>
<td>11</td>
<td>7.95 (d) J = 8.5Hz</td>
<td>7.48 (s)</td>
<td>7.96 (s)</td>
<td>8.4 (d) J=7.8</td>
</tr>
<tr>
<td>1,2-OCH₂O</td>
<td>6.47 (d), 5.86 (d) J = 1.1 Hz</td>
<td>5.95 (d), 5.88 (d) J = 0.92 Hz</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>9,10-OCH₂O</td>
<td>-</td>
<td>6.08 (d), 6.02 (d) J = 0.92 Hz</td>
<td>5.98 (2H) (d) J = 1.4 Hz</td>
<td>-</td>
</tr>
</tbody>
</table>
[0095] **EXAMPLE 5: Biological Testing**

[0096] While there are well established biological assays that employ the promastigote form of the *Leishmania sp.* parasite, a novel method was employed for measuring the viability of the *Leishmania sp.* promastigote in the presence of the test substance.

[0097] Approximately, 10,000 *Leishmania mexicana* parasites per well were initially tested in duplicate at concentrations of 300, 42 and 6 μg/mL of the test substance for a 72 hour period. A colorimetric assay was used to measure parasite viability based on the reduction of the tetrazolium salt, sodium-2,3-bis-(2-methoxy-4-nitro-5-sulphophenyl)-2H-tetrazolium-5-carboxanilide (XTT) by the mitochondrial dehydrogenase of the parasite. The product of XTT reduction is a water-soluble formazan which was monitored by measuring the optical density at 450 nm. The parasite survival at each concentration of the test substance was compared to control experiments which were identical in every respect except that they contain no test substance. A linear-regression operation was then performed in order to determine the value at which 50% of the parasites were killed (LD₅₀). When necessary, test substances were diluted and the biological assay repeated in order to obtain accurate LD₅₀ values. The anti-leishmaniasis drug, Amphotericin B, was used as internal control to evaluate the sensitivity and reliability of each assay. The typical response of *Leishmania mexicana* promastigote to this drug was between 80-100 ng/mL.

[0098] Xylopine (1) was tested as a purified compound in the *Leishmania sp.* promastigote assay described above. The assay was terminated 72 hours later, and it was shown that at the lowest concentration tested, 6 μg/mL, all of the parasites had been killed. An identical replicate of the sample was tested on the same date in a different 96 well plate and yielded the identical result.
[0009] In order to accurately determine the LD$_{50}$ values, Xylophone (1) was retested at 4.65, 1.32 and 0.377 μg/mL, yielding an LD$_{50}$ value of 1 μg/mL. The positive control with Amphotericin B was 88.7 ng/mL, confirming the reliability of the data.

[00100] The anti-\textit{Leishmania mexicana} activity of Cryptodorine (2) and Normantenine (3) was also tested using this method. The LD$_{50}$ value measured for Normantenine was 8 μg/mL and the LD$_{50}$ value for Cryptodorine was below the detection limit of 6 μg/mL. The Amphotericin B positive control was 85.4 ng/mL, confirming the reliability of the data.

[00101] In order to accurately determine the LD$_{50}$ value, Cryptodorine (2) was retested, using concentrations of 15, 2.14 and 0.31 μg/mL, yielding an LD$_{50}$ value of 1 μg/mL. Two samples of Normuciferine (4) were tested in duplicate and the LD$_{50}$ value measured were 4 μg/mL and 8 μg/mL.

[00102] EXAMPLE 6: Selectivity Evaluation

[00103] The activity and selectivity of compounds 1-4 was evaluated. Compounds 1 and 2 showed the greatest leishmanicidal activity (IC$_{50}$ = 3 μM against \textit{L. mexicana}) followed by 3 and 4 (24 and 14 μM, respectively). Comparable results were obtained with \textit{L. panamensis}. (See Table 1). Neither laudanosine (11) nor lysicamine (8) showed significant leishmanicidal activity at the concentration tested (data not shown). Compounds 1-4 were evaluated in macrophages and in human foreskin fibroblasts. The Selectivity Index in Table 3 indicates a 37-fold higher cytotoxicity of 1 towards \textit{L. mexicana} than macrophages, the regular host cells of \textit{Leishmainia}.

<table>
<thead>
<tr>
<th>Table 3 – Activity of Compounds 1-4 and Amphotericin B Against \textit{Leishmania} spp. and Mammalian Cell Lines</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compound</td>
</tr>
<tr>
<td>----------</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>4</td>
</tr>
<tr>
<td>Amphotericin B</td>
</tr>
</tbody>
</table>

$^a$ IC$_{50}$ (Inhibitory Concentration) is the concentration of compound that inhibited 50% growth of the parasite or cell line. Mean values of the IC$_{50}$ (μM ± standard deviation) were determined by testing the concentration of each sample in triplicate.

$^b$ Human foreskin fibroblasts.
SI (Selectivity Index) is a measure of the IC$_{50}$ values obtained in macrophages and HFF, respectively, divided by the IC$_{50}$ in L. mexicana.

Not Determined.

[00104] **EXAMPLE 7: Toxicity Testing**

[00105] Norantine (3) was tested in three groups of six BALB/c mice: Group 1 served as a control, receiving only the vehicle for Norantine (1% Tween 20 in phosphate buffered saline). Group 2 received a dose of 4 mg/kg Norantine while Group 3 received a dose of 40 mg/kg Norantine.

[00106] Groups 1 and 2 (control and 4 mg/kg, respectively) showed no behavioral differences while Group 3 (40 mg/kg) demonstrated some evidence of hyperactivity and aggression. All animals were sacrificed and their brains, liver, heart, kidneys and intestines were fixed in formalin for analyses. Norantine (3) shows no toxicity.

[00107] **EXAMPLE 8: In vivo Testing**

[00108] A rodent model with BALB/c mice was developed in order to determine their *in vivo* toxicity and efficacy. Three groups of 15 Balb/c mice were injected in the pad of their rear right foot with three different strains of Leishmania (50,000 injected parasites per mouse). In each case, the rear left foot received no injection of the leishmanial parasite.

Group 4 received *Leishmania panamensis* (a causative agent of cutaneous leishmaniasis), Group 5 received *Leishmania braziliensis* (a causative agent of cutaneous leishmaniasis) while Group 6 received *Leishmania donovani* (a causative agent of visceral leishmaniasis). In each case, there is evidence of leishmanial infection. Compounds 1-4 were administered to the three groups.

[00109] **EXAMPLE 9: Efficacy**

[00110] Evidence of leishmanial infection is analyzed for each of groups 4-6 pre- and post-administration of compounds 1-4. Compound 1 shows efficacy against leishmaniasis. Compound 2 shows efficacy against leishmaniasis. Compound 3 shows efficacy against leishmaniasis. Compound 4 shows efficacy against leishmaniasis.

[00111] The embodiments illustrated and discussed in this specification are intended only to teach those skilled in the art the best way known to the inventors to make and use the invention. Nothing in this specification should be considered as limiting the scope of the present invention. All examples presented are representative and non-limiting. The above-described embodiments of the invention may be modified or varied, without departing from the invention, as appreciated by those skilled in the art in light of the above teachings. It is
therefore to be understood that, within the scope of the claims and their equivalents, the invention may be practiced otherwise than as specifically described.
WHAT IS CLAIMED IS:

1. A method for the treatment of leishmaniasis, comprising administering to an animal in need thereof a therapeutically effective amount of a compound of Formula I

   \[
   \text{R}_4 \quad \text{N} \quad \text{R}_3 \\
   \text{R}_5 \quad \text{R}_1 \quad \text{R}_2 \\
   \text{I}
   \]

   wherein \( \text{R}_1, \text{R}_2, \text{R}_4 \) and \( \text{R}_5 \) are the same or different and are selected from \( \text{H}, \text{OH}, \text{OR}_6 \), and \( \text{R}_1 \) and \( \text{R}_2 \) and/or \( \text{R}_4 \) and \( \text{R}_5 \) together forming a methylenedioxy or other bridging group of the form \( -\text{O}-(\text{CH}_2)_n-\text{O} \) where \( n \) is 1, 2 or 3, and \( \text{R}_3 \) is selected from the group \( \text{H} \) and lower alkyl,

   wherein \( \text{R}_6 \) is a carbohydrate residue, phosphate residue, sulfate residue or lower alkyl, and

   wherein lower alkyl includes linear, branched and cyclic groups having less than about 6-10 carbons, optionally including one or more single or double bonds

or a pharmaceutically acceptable salt thereof; and thereby treating leishmaniasis.

2. The method of claim 1, wherein the animal is not a human.

3. The method of claim 1, wherein the animal is a human.

4. The method of claim 1, wherein \( \text{R}_3 \) is selected from \( \text{H} \) and methyl, with the proviso that when \( \text{R}_3 \) is methyl, \( \text{R}_1, \text{R}_2, \text{R}_4, \) and \( \text{R}_5 \) are not all methoxy.
5. The method of claim 1, wherein R_3 = H and R_1, R_2, R_4 and R_5 are the same or different and are selected from H and OCH_3, or R_1 and R_2 and/or R_4 and R_5 together form a methylenedioxy bridge.

6. The method of claim 1, wherein the compound of Formula I is selected from Xyloplane, Cryptodorine, Normantenine and Normuciferine.

7. The method of claim 1, wherein the lower alkyl group contains 6 or fewer carbons.

8. The method of claim 1, wherein R_6 is selected from a carbohydrate residue, a phosphate residue and a sulfate residue.

9. The method of claim 1, wherein the compound of Formula I is enantiomerically pure.

10. The method of claim 1, wherein the compound of Formula I is present as a racemic mixture.

11. The method of claim 1, wherein said leishmaniasis is the result of infection by one or more parasites selected from *Leishmania aethiopica, L. amazonensis, L. archibaldi, L. braziliensis, L. chagasi, L. donovani, L. garnhami, L. guyanensis, L. infantum, L. killicki, L. major, L. mexicana, L. panamensis, L. peruviana, L. pifanoi, L. tropica* and *L. venezuelensis*.

12. The method of claim 1, wherein the parasite is *Leishmania mexicana*.

13. The method of claim 1, wherein the leishmaniasis is selected from cutaneous leishmaniasis, visceral leishmaniasis, mucocutaneous leishmaniasis and disseminated leishmaniasis.

administering to an animal in need thereof a pharmaceutical composition
comprising a therapeutically effective amount of a compound of Formula I

\[
\begin{align*}
\text{wherein } R_1, R_2, R_4 \text{ and } R_5 \text{ are the same or different and are selected from }
\text{H, OH, OR}_6, \text{ and } R_1 \text{ and } R_2 \text{ and/or } R_4 \text{ and } R_5 \text{ together forming a methylenedioxy}
\text{or other bridging group of the form } -\text{O-(CH}_2\text{)}_n\text{-O- where } n \text{ is 1, 2 or 3, and } R_3 \text{ is}
\text{selected from the group } H \text{ and lower alkyl,}
\end{align*}
\[
\begin{align*}
\text{wherein } R_6 \text{ is a carbohydrate residue, phosphate residue, sulfate residue or lower alkyl, and }
\end{align*}
\[
\begin{align*}
\text{wherein lower alkyl includes linear, branched and cyclic groups}
\end{align*}
\[
\begin{align*}
\text{having less than about 6-10 carbons, optionally including one or more}
\text{single or double bonds,}
\end{align*}
\[
\begin{align*}
\text{or a pharmaceutically acceptable salt thereof together with one or more}
\text{pharmaceutically acceptable excipients; and}
\end{align*}
\[
\begin{align*}
\text{thereby treating leishmaniasis.}
\end{align*}
\]

15. The method of claim 14, wherein the animal is a human.

16. The method of claim 14, wherein R₃ is selected from H and methyl
with the proviso that, when R₃ is methyl, R₁, R₂, R₃, and R₄ are not all methoxy..

17. The method of claim 14, wherein R₃=H and R₁, R₂, R₄ and R₅ are
the same or different and are selected from H and OCH₃, or R₁ and R₂ and/or R₄
and R₅ together form a methylenedioxy bridge.
18. The method of claim 14, wherein the compound of Formula I is selected from Xyloplane, Cryptodore, Norntenine and Noruciferine.

19. A kit comprising a pharmaceutical composition that comprises a therapeutically effective amount of a compound of Formula I

\[
\begin{array}{c}
\text{R}_1 \\
\text{R}_2 \\
\text{R}_3 \\
\text{R}_4 \\
\text{R}_5 \\
\text{R}_6 \\
\end{array}
\]

wherein \( R_1, R_2, R_4 \) and \( R_5 \) are the same or different and are selected from \( H, OH, OR_6 \), and \( R_1 \) and \( R_2 \) and/or \( R_4 \) and \( R_5 \) together forming a methylenedioxy or other bridging group of the form \(-O-(CH_2)_n-O\) where \( n \) is 1, 2 or 3, and \( R_3 \) is selected from the group \( H \) and lower alkyl,

wherein \( R_6 \) is a carbohydrate residue, phosphate residue, sulfate residue or lower alkyl, and

wherein lower alkyl includes linear, branched and cyclic groups having less than about 6-10 carbons, optionally including one or more single or double bonds,

or a pharmaceutically acceptable salt thereof together with one or more pharmaceutically acceptable excipients and labeling for the use of said pharmaceutical composition for the treatment of leishmaniasis.

20. The kit of claim 19, further comprising a container for the pharmaceutical composition.

21. The kit of claim 20, wherein said labeling is associated with the container.
REFERENCES


10. Lo, W.-L., Chang, F.-R., Wu, Y.-C. Alkaloids from the leaves of


22. Rasamizafy, S., Hocquemiller, R., Cave, A., Jacquemin, H.
Alkaloids of the


-32-


64. Navarro, V.R., Sette, I.M.F., Da-Cunha, E.V.L., Silva, M.S., Barbosa-Filho, J.M., Maia, J.G.S. Alkaloids from Dugueta flagellaris Huber

-35-


74. Han, B.H., Park, M.H., Han, Y.N. Aporphine and tetrahydrobenzylisoquinoline alkaloids from the seeds of Zizyphus vulgaris var.


86. Furmanowa, M., Jozefowicz, J. Alkaloids as taxonomic markers in


98. Isoquinoline derivatives isolated from the fruit of Annona muricata


