Abstract: Triterpenoid iridals and derivatives thereof have anti-trypanosomal activity.
ANTITRYPANOSOMAL COMPOUNDS AND METHODS

This application claims the benefit of U.S. Provisional Application Serial Nos. 62/185,948, filed June 29, 2015, and 62/327,632, filed April 26, 2016, each of which is incorporated herein by reference in its entirety.

BACKGROUND

Neglected tropical diseases constitute a diverse group of diseases that impact primarily poorest populations, affecting more than 1.4 billion people worldwide (World Health Organization, Neglected Tropical Diseases, available from http://www.who.int/neglected_diseases/diseases/en/). Several of these diseases, exemplified by Chagas disease (American trypanosomiasis), human African trypanosomiasis (HAT or sleeping sickness), and the Leishmaniases (a set of trypanosomal diseases) are caused by parasitic protozoa known as trypanosomatids, which are transmitted to human and animal hosts by hematophagous insect vectors. HAT is mainly caused by Trypanosoma brucei and is transmitted by Tsetse flies; Chagas disease is caused by Trypanosoma cruzi and is transmitted by triatomine bugs; and leishmaniases are caused by various species of Leishmania and are transmitted by sandflies.

An estimated 30,000 people are infected and up to 70 million are at risk of developing HAT, which causes severe and progressively fatal central nervous system impairment. The disease is endemic to the African continent where several subspecies of the parasite T. brucei are spread by the Tsetse fly. Although HAT is primarily caused by two subspecies of T. brucei, rhodesiense and gambiense, there have been reports of human infections caused by T. evansi, T. lewisi, T. brucei brucei, and T. congolense. (WHO fact sheet 2014). Species and subspecies such as T. brucei, T. congolense, T. equiperdum, T. simiae, T. suis and T. vivax are known to cause disease (e.g., nagana) in wild animals and domestic animals, such as cattle.

The choice of treatment during the early stage of HAT depends on the subspecies of T. brucei responsible for the infection (U. S. Centers for Disease Control and Prevention, African Trypanosomiasis - Resources for Health Professionals, available from http://www. cdc.
Pentamidine, which was discovered in 1941, is given by intravenous infusion and is typically used to treat first stage infections caused by *T. b. gambiense*. The drug can have significant side effects including leukopenia, thrombopenia, hypotension, arrhythmias, gastrointestinal distress hepatoaegaly, hepatitis, hypoglycemia, neurological issues including seizures, and nephrotoxicity. Suramin is used to treat first stage HAT when caused by *T. b. rhodesiense*. Suramin was developed in 1916 by Bayer and is also sold under the tradename GERMANIN. In addition to nausea and vomiting, more than 50% of those treated with suramin will experience adrenal cortical damage which is usually temporary.

Treatments for the second stage of HAT are more challenging to develop due to the need to cross the blood-brain barrier to be effective. Melarsoprol (currently produced by Sanofi-Aventis) was discovered to be effective against late stage HAT in 1949. It is used as a treatment for both forms of HAT and is the only treatment available for late stage infections caused by *T. b. rhodesiense*. Melarsoprol is an arsenic derivative and has significant side effects that are similar to arsenic poisoning. Due to the dangers of treatment, it is only administered by injection with close physician supervision. Eflornithine, marketed by Sanofi-Aventis as Ornidyl in the United States, is used to treat second stage disease caused by *T. b. gambiense*. Although considered somewhat safer than melarsoprol, the side effects of eflornithine can include seizures, fever, neutropenia, hypertension and diarrhea. The treatment regimen, which includes multiple intravenous injections over 14 days, is strict and very difficult to administer, especially in a rural setting. Strains that are resistant to eflornithine have been reported since the 1980s, which has prompted the more recent use of eflornithine in combination with nifurtimox.

Nifurtimox (marketed under the tradename LAMPIT by Bayer) was originally developed for treatment of Chagas Disease, but has been approved by the WHO for treatment of HAT.

Side effects of nifurtimox include gastrointestinal, cardiac and neurological issues and it should not be used by patients with neurologic or psychiatric disorders. Nifurtimox and eflornithine combination therapy (NECT) to treat late stage HAT caused by *T. b. gambiense* was introduced in 2009 World Health Organization, Trypanosomiasis, human African (sleeping sickness): Media Centre Fact Sheet, available from: http://www.who.int/mediacentre/factsheets/fs259/en/). The WHO now provides NECT at no cost to endemic countries.
Chagas disease affects approximately 6 million people per year and kills approximately 12,000 people per year. Flu-like symptoms may follow the initial exposure to the infectious agent, *T. cruzi*. Most infected persons remain asymptomatic for the remainder of their lives; however, approximately 30% of infected persons will progress to the chronic form of Chagas disease after 10-30 years, when cardiac and gastrointestinal damage usually results in death. *T. cruzi* is transmitted through the feces of triatomines, a blood-sucking Reduvid insect vector. Triatomine bugs are also known as reduviid bugs, "kissing" bugs, assassin bugs, cone-nosed bugs, and blood suckers. Reduvid is endemic in South America and has also been found in the southern United States where Chagas disease has recently been classified as an emerging infectious disease threat. In addition to the human toll it causes, Chagas disease also affects wild animals, companion animals and domesticated animals.

Benznidazole, originally marketed under the tradenames ROCHAGAN and RADANIL by Hoffman-LaRoche, who later donated rights to the Brazilian government, is used to treat the initial (acute) stage of Chagas disease (U. S. Centers for Disease Control and Prevention, Parasites: American Trypanosomiasis, available from: http://www.cdc.gov/parasites/chagas/epi.html). Although the drug may provide some symptomatic relief or slow progression in the chronic stage, there is no known cure for Chagas disease in the chronic stage. Side effects of benznidazole treatment include rash, gastrointestinal distress, and peripheral neuropathy. Although nifurtimox is still used as a mainline treatment for Chagas disease, benznidazole is the preferred treatment due to the serious side effects and contraindications of nifurtimox.

The leishmaniases encompass three presentations of disease caused by various subspecies of *Leishmania*, which is spread by the bite of the sandfly. More than 12 million people in almost 100 countries worldwide are infected with one of the forms. Cutaneous leishmaniasis (CL) is the most common presentation with an estimated 1.3 million new cases annually. The infection causes ulcerative skin lesions, permanent disfigurement and serious disability. Mucocutaneous leishmaniasis (ML, also called espundia) causes even more severe disability and disfigurement due to the destruction of the naso-oropharyngeal mucosa. The most severe form of the disease, visceral leishmaniasis (VL, also known as kala-azar), is caused by various species of Leishmania such as *L. donovani* and *L. major*. In this presentation of the disease, those infected exhibit high fever and weight loss, swelling of the spleen and liver, and anemia. The disease is fatal without
treatment. Leishmaniasis, like other trypanosomal infections, is also known to affect wild animals, companion animals and domesticated animals.

Current therapies for leishmaniasis depend on the form of the disease (U. S. Centers for Disease Control and Prevention. Leishmaniasis - Resources for Health Professionals, 2014, available from: http://www. cdc). Pentavalent antimonials administered by daily intravenous or intramuscular administration for 10 - 28 days have been used to treat all three presentations. Sodium stibogluconate (manufactured by GlaxoSmithKline as PENTOSTAM®), is the only formulation available in the United States, where it has an IND approval from the FDA and is only available through the CDC Drug Service. Some of the more serious side effects of treatment include phlebitotoxicity, pancreatitis, cardiac conduction abnormalities, and anaphylaxis.

Infusion of liposomal amphotericin B (marketed as AmBisome®) has been approved to treat visceral leishmaniasis (VL) since 1997. Severe histamine-related reactions have been noted within hours of treatment. Side effects also include kidney and liver damage, electrolyte imbalance, leukopenia, thrombopenia, and cardiac arrhythmias and heart failure. The conventional amphotericin B deoxycholate (non-liposomal) is also considered to be very effective for treating VL but it is much more toxic than the liposomal formulation.

Other treatments for leishmaniasis include the aminoglycoside paromomycin sulfate and pentamidine to treat cutaneous leishmaniasis (CL) and miltefosine, which was approved by the FDA in 2014 as an oral treatment for all three forms of leishmaniasis.

Trypanosomal infection also affects domesticated and companion animals, as well as animals in the wild. Animal African trypanosomiasis (AAT), also known as nagana, dourine and surra, is caused by trypanosome species and subspecies other than those affecting human beings. Trypanosoma brucei, Trypanosoma congoense, Trypanosoma equiperdum, Trypanosoma simiae, Trypanosoma suis and Trypanosoma vivax are some of the species and subspecies causing diseases in wild and domestic animals. T brucei, for example, affects cattle and is a major impediment to proper nutrition and economic development in affected areas of Africa. See, e.g., Seek et al., Parasite, 17(3):257-65, describing parasitological and serological prevalence data of AAT in Senegal. Leishmaniasis also affects cows, horses, dogs, and other companion and domesticated animals. Chagas, caused by T cruzi, is known to affect dogs and other animals; "rapid tests" are available for canine Chagas, but there are no effective treatments
available once diagnosed. All these diseases have a serious economic impact on the
development of agriculture in the affected areas. Those affecting cattle are particularly
devastating since they are a major cause for reduced meat and milk production as well as animal
power for agricultural production (WHO fact sheet available at
http://www.who.int/trypanosomiasis_african/parasite/en/).

Additionally, animals (as well as humans) serve as disease reservoirs. Typical reservoirs
for *T. cruzi*, for example, include wild animals such as armadillos, raccoons, opossums, and
rodents, as well as domesticated and companion animals such as dogs, cattle and guinea-pigs
(Reza, Chagas Disease, available at http://www.austincc.edu/microbio/2704t/tc). Humans are
the main reservoir for *Trypanosoma brucei gambiense*, but this protozoan can also be found in
animals. Wild game animals are the main reservoir of *T. b. rhodesiense* (Centers for Disease
humans, have been found as natural reservoir hosts of Leishmania parasites (World Health

At present, few drugs are available to treat or prevent human and animal diseases caused
by trypanosomatids, and most of them are toxic, may be ineffective, and are difficult to
administer in a rural setting.

**SUMMARY OF THE INVENTION**

The present invention provides compounds and methods for treating or preventing
infection and disease, more particularly, for treating or preventing trypanosomatid infections in a
vertebrate subject.

In one aspect, the invention provides a method for treating or preventing a
trypanosomatid infection in a subject. In exemplary embodiments, the method involves
administering to the subject a triterpenoid iridal compound having Formula I, Formula II,
Formula III, Formula IV, and/or Formula V, as shown below.
Formula 1:

wherein $R_i$ is H, alkyl, or -C(0)$R_7$; $R_2$, $R_4$, $R_5$ and $R_6$ are each independently H, OH, or OAc; $R_3$ is CH$_3$ or CH$_2$OH; and $R_7$ is H, a substituted or unsubstituted alkyl, a substituted or unsubstituted alkenyl, or a substituted or unsubstituted aryl. In one embodiment of Formula I, $R_i$ is H; in another embodiment, $R_i$ is -C(0)$R_7$ and $R_7$ is from a plant fatty acid.

Formula II:

wherein $R_i$ is H, alkyl, or -C(0)$R_7$; $R_2$, $R_4$, $R_5$ and $R_6$ are each independently H, OH, or OAc; $R_3$ is CH$_3$ or CH$_2$OH; $R_7$ is H, a substituted or unsubstituted alkyl, a substituted or unsubstituted alkenyl, or a substituted or unsubstituted aryl; $R_8$ is H, alkyl, or -C(0)$R_9$; and $R_9$ is
H, a substituted or unsubstituted alkyl, a substituted or unsubstituted alkenyl, or a substituted or unsubstituted aryl. In one embodiment of Formula II, R_i is H; in another embodiment, R_i is C(0)R_7 and R_7 is from a plant fatty acid. In one embodiment, R_8 is H.

Formulas III, IV and V:

Exemplary compounds having Formula I, Formula II, Formula III, Formula IV or Formula V that are suitable for use in the method of the invention include iridals such as iridogermanals, iso-iridogermanals, belachinals, anhydrobelachinals, and spiroiridals, as well as derivatives thereof. An iridal useful in the method of the invention may include an aldehyde at carbon position 1. The presence of an aldehyde at carbon position 1 can, however, cause the iridal to exhibit instability; thus in a preferred embodiment, the iridal used in the method of the invention does not include an aldehyde at carbon position 1. Alternatively or additionally, the iridal used in the method of the invention optionally includes a fatty acid ester.

In another aspect, the invention provides a novel bioactive triterpenoid iridal compound. In exemplary embodiments, the compound has the structure of Formula I, Formula II, Formula III, Formula IV or Formula V. The compound may be naturally occurring, or not naturally occurring. If naturally occurring, the compound is isolated from its natural environment and optionally purified. Exemplary compounds having Formula I, Formula II, Formula III, Formula IV or Formula V include iridals such as iridogermanals, iso-iridogermanals, belachinals, anhydrobelachinals, and spiroiridals, as well as derivatives thereof. The iridal of the invention may include an aldehyde at carbon position 1. The presence of an aldehyde at carbon position 1 can, however, cause the iridal to exhibit instability; thus in a preferred embodiment, the iridal of
the invention does not include an aldehyde at carbon position 1. Alternatively or additionally, the iridal of the invention optionally includes a fatty acid ester.

A pharmaceutical composition that includes the compound according to one or more of Formulas I, II, III, IV and/or V is also provided. The pharmaceutical composition optionally includes a pharmaceutical carrier and/or at least one additional active agent. The additional active agent can be a therapeutic or prophylactic agent. The pharmaceutical composition of the invention is optionally formulated for use in the prevention or treatment of a trypanosomatid infection.

In another aspect, the method involves administering to a subject, for purposes of prevention or treatment of a trypanosomatid infection, a composition having an effective amount of a triterpenoid iridal compound. In exemplary embodiments, the compound has the structure of Formula I, Formula II, Formula III, Formula IV and/or Formula V. In one embodiment, the method involves administering to the subject a composition having an effective amount of compound 2, compound 3, compound 4, compound 5, compound 6, compound 7, compound 8 and/or compound 9. In another embodiment, the method involves administering to the subject a composition having an effective amount of an extract from a plant that is a member of the *Iris* genus. The extract can include a root extract or a rhizome extract.

The subject to whom the active agent is administered can be a human or an animal, such as a companion animal, a domesticated animal, or a wild animal. Exemplary animals include a dog and a cow (cattle).

The trypanosomatid infection can include human African trypanosomiasis (HAT), animal African trypanosomiasis (AAT), American trypanosomiasis (Chagas Disease) or a leishmaniasis. Exemplary trypanosomatid infections include a *Trypanosoma brucei* infection, a *Trypanosoma cruzi* infection, and a *Leishmania amazonensis* infection.

The composition optionally includes a pharmaceutically acceptable carrier.

The composition optionally includes, in addition the iridal as a first active agent, a second active agent. The second active agent can be a prophylactic or therapeutic agent. The second active agent can include at least one additional naturally occurring and/or at least one non-naturally occurring active agent.

In another aspect, the invention provides a plant extract that includes an iridal, such as an iridogermanal, iso-iridogermanal, or a derivative thereof, for use as a prophylactic or therapeutic
agent for treatment or prevention of a trypanosomatid infection. The plant extract can be prepared from a member of the *Iris* genus.

In another aspect, the invention provides a compound of Formula I, Formula II, Formula III, Formula IV or Formula V for use as a prophylactic or therapeutic agent for treatment or prevention of a trypanosomatid infection. Also provided is a use of a compound of Formula I, Formula II, Formula III, Formula IV or Formula V for preparation of a medicament for the treatment or prevention of trypanosomatid infection, and the use of a plant extract comprising an iridal such as an iridogermanal, iso-iridogermanal, or a derivative thereof for preparation of a medicament for the treatment or prevention of a trypanosomatid infection. The plant extract can be prepared from a member of the *Iris* genus.

BRIEF DESCRIPTION OF THE FIGURES

FIG. 1A shows belamcandal (R = acetyl) and 28-deacetylbelamcandal (R = H).

FIG. 1B shows compound 2, an iso-iridogermanal myristoyl ester (R = myristoyl).

FIG. 1C shows iridal NSC 631941 (Shao et al., J. Med. Chem., 2001, 44(23):3872-80)

FIG. 1D shows compound 1, a seco-iridoid (Cardona Zuleta et al., Phytochem. 2003, 64(2):549-53).


DESCRIPTION OF ILLUSTRATIVE EMBODIMENTS

The present disclosure provides compounds, compositions and methods relating to triterpenoid iridals, such as iridogermanals, iso-iridogermanals, belachinals, anhydrobelachinals, spiroiridals, and derivatives thereof, and their use as a prophylactic or therapeutic agent, for example, to prevent or treat parasitic protozoan infections. Iridals such as iridogermanal, iso-iridogermanal, and derivatives thereof can be isolated or extracted from naturally occurring sources or they can be chemically or enzymatically synthesized. An iridal of the invention may be naturally occurring, or not naturally occurring. If naturally occurring, the iridal is isolated from its natural environment and optionally purified. A preferred iridal, such as an iridogermanal, iso-iridogermanal, is one that is chemically or enzymatically synthesized and, additionally, is not naturally occurring. A preferred iridal may be a structural derivative of an
iridal that is naturally occurring, which structural derivative is not naturally occurring. Iridals such as iridogermanal, iso-iridogermanal, and derivatives can be administered alone or in combination with other therapeutics via a variety of routes of administration. Although the invention is described primarily with respect to iso-iridogermanal and derivatives thereof, in the interest of brevity and conciseness, it is to be understood that in and for each embodiment, other iridals including iridogermanal and derivatives thereof can likewise be utilized.

Triterpenoid iridals are a major lipid component of Iris rhizomes, and include monocyclic seco-A-ring structures such as iso-iridogermanal, and spiro-bicyclic structures such as belamcandal. The present invention relates to monocyclic triterpenoid iridals that typically contain 30 carbon atoms and possess a squalenoid side chain. They also possess a characteristic aldehyde functionality, wherein carbonyl group of the aldehyde is conjugated to an alkene to yield an α,β-unsaturated carbonyl. The squalenoid side chain can be hydroxylated, for example at carbon position 21 (as in iridogermanal) or carbon position 16 (as in iso-iridogermanal).

Compounds of the present invention, structurally based on monocyclic triterpenoid iridals and their derivatives, were found to have therapeutic effect. More particularly, these compounds were found to have anti-trypanosomal activity.

A representative compound of the invention is shown as Formula I:

![Formula I](image)

wherein Ri is H, alkyl, or -C(0)R7; R2, R4, R5, and R6 are each independently H, OH, or OAc; R3 is CH3 or CH2OH; and R7 is H, a substituted or unsubstituted alkyl, a substituted or unsubstituted alkenyl, or a substituted or unsubstituted aryl. Preferably R7 is H, substituted or unsubstituted (C5-C20)alkyl, or substituted or unsubstituted (C5-C20)alkenyl. In one embodiment, the compound of Formula I is a free alcohol at carbon position 3, wherein Ri is H.
In another embodiment, the compound of Formula I is esterified at carbon position 3. An exemplary esterified derivative is a compound of Formula I wherein R₇ is from a fatty acid, such as a plant fatty acid, including a saturated fatty acid such as capric (IOC), lauric (12C), myristic (14C), palmitic (16C) or stearic (18C) acid, or an unsaturated fatty acid such as linolenic or linoleic acid. The squalenoid side chain, which in Formula I is triply unsaturated, may in other embodiments of the compound be hydrogenated so as to yield a saturated side chain.

Another representative compound of the invention is shown as Formula II:

![Formula II graphic](image)

wherein R₁ is H, alkyl, or -C(0)R₇; R₂, R₄, R₅ and R₆ are each independently H, OH, or OAc; R₃ is CH₃ or CH₂OH; R₇ is H, a substituted or unsubstituted alkyl, a substituted or unsubstituted alkenyl, or a substituted or unsubstituted aryl; R₈ is H, alkyl, or -C(0)R₉; and R₉ is H, a substituted or unsubstituted alkyl, a substituted or unsubstituted alkenyl, or a substituted or unsubstituted aryl. Preferably R₇ is H, substituted or unsubstituted (C5-C20)alkyl, or substituted or unsubstituted (C5-C20)alkenyl. In one embodiment, the compound of Formula II is a free alcohol at carbon position 3, wherein R₁ is H. In another embodiment, the compound of Formula II is esterified at carbon position 3. An exemplary esterified derivative is a compound of Formula II wherein R₇ is from a fatty acid, such as a plant fatty acid, including a saturated fatty acid such as capric (IOC), lauric (12C), myristic (14C), palmitic (16C) or stearic (18C) acid, or an unsaturated fatty acid such as linolenic or linoleic acid.

Exemplary compounds of Formula I, which have an aldehyde at carbon position 1, include iridogermanal (R₂ = R₆ = OH; R₁ = R₄ = R₅ = H; R₃ = CH₃) and iso-iridogermanal (R₂ =
R₄ = OH; R₁ = R₅ = R₆ = H; R₃ = CH₃). The reduced forms of these compounds (dihydroiridogermanal and dihydroisoiridogermanal) are exemplified by corresponding Formula II having a free alcohol (CH₂OH) at carbon position 1 instead of the aldehyde (COH) as in Formula I.

In either of Formula I or Formula II, any or all of the alcohol groups at any of positions R₂, R₄, R₅ and R₆ can be derivatized. For example, one or more alcohol can be acetylated or alkylated. An example of an acetylated compound of Formula I or II is 16-O-acetyl iso-iridogermanal.

Other representative compounds of the invention are shown as Formula III, IV, and IV:

An iridal derivative, such as a derivative of iridogermanal, iso-iridogermanal, belachinal, anydrobelachinal, or spiroiridal, includes but is not limited to an iridogermanal, iso-iridogermanal, belachinal, anydrobelachinal, or spiroiridal 1that has been modified by adding one or more functional groups to the parent compound, or by altering or removing one or more functional groups of the parent compound. For example, in one derivative, the aldehyde functionality is reduced, for example to an alcohol. Additionally or alternatively, the double bonds of the terpenoid chain can be reduced, for example by hydrogenation. A derivative of an iridal of the invention may include one or more alcohol functional groups. In some embodiments, one or more of the alcohol groups is esterified or otherwise derivatized. In some embodiments, the iridal derivative, such as an iridogermanal or iso-iridogermanal derivative, includes one or more fatty acids or fatty acid esters. Illustrative esterifications can include a myristoyl ester, a palmitoyl and/or a lauril ester. A derivative of an iridal of the invention, such as an iridogermanal or iso-iridogermanal derivative, also includes a conjugate. Iridals of the invention, such as iridogermanal and iso-iridogermanal, or derivatives iridals of the invention,
can be chemically or enzymatically conjugated to any desired molecule, such as a protein, carbohydrate, nucleic acid, small molecule carrier, and the like. A derivative of an iridal of the invention, such as iridogermanal or iso-iridogermanal, can be synthetic, or it can be a naturally occurring variant of the iridal.

A representative iso-iridogermanal derivative is compound 2, the myristoyl ester as shown in FIG 1B and below:

![iso-iridogermanal (R = myristoyl)](attachment:iso-iridogermanal.png)

Shown below is an exemplary process for making an iso-iridogermanal derivative in which the α,β-unsaturated aldehyde of an iso-iridogermanal for example compound 2, is reduced to an alcohol by reaction with a hydride reagent such as, for example, NaBH₄. The process below shows the myristoyl ester but any esterified or non-esterified iridogermanal or iso-iridogermanal can be used to yield the corresponding iridogermanal or iso-iridogermanal alcohol.

For reduction of non-esterified iridogermanal or iso-iridogermanal compounds, another exemplary suitable hydride reagent is LiAlH₄.

![iso-iridogermanal (R = myristoyl)](attachment:iso-iridogermanal.png)

It has been surprisingly discovered that iso-iridogermanal compounds and other iridals (e.g., Example 1, compounds 2, 3, 4, 5, 6, 7, 8 and 9, and their derivatives), have anti-trypansosomal activity. Iridogermanal compounds and their derivatives may also exhibit anti-
trypanosomal activity, in view of their structural similarity. Other iridal compounds and their derivatives are also expected to show anti-trypanosomal activity. Reduced iridal derivatives, such as reduced iridogermanal and iso-iridogermanal derivatives, wherein the aldehyde at carbon position 1 (as in Formula I) has been reduced to a hydroxyl (as in Formula II, \( R_8 = \text{H} \)) are especially preferred, because the reduced form of iso-iridogermanal was found to be more stable while still preserving anti-trypanosomal activity.

**Isolation or synthesis of iso-iridogermanal**

Iridogermanal, iso-iridogermanal and their naturally occurring derivatives, as well as other iridals, can be extracted and/or isolated from members of the *Iris* genus which belongs to the family *Iridaceae*. Exemplary plants include *Iris domestica* (formerly as *Belamcanda chinensis*), *I japonica*, *I. germanica*, including *I. germanica* Linn, *I. tectorum*, *I. versicolor*, including *I. versicolor* Linn, *I. missouriensis*, *I. pseudocorus*, *I. pallida* and *I. sibirica* Linn. In 2005, based on molecular DNA sequence evidence, *Belamcanda chinensis*, the sole species in the genus *Belamcanda*, was transferred to the genus *Iris* and renamed *Iris domestica*.

Both fatty acid esters and free alcohol forms of the iridogermanal and iso-iridogermanal compounds can be obtained from plant sources (Marner et al., Curr. Org. Chem. 1997, 1(2): 153-186; Marner et al. Z. Naturforsch, 1992,47c, 21; Marner et al., Phytochemistry, 1993, 33:573). Typically the esterified compounds are esterified at carbon position 3 with a plant fatty acid.

Any convenient plant part can serve as a source of iridals such as iridogermanal, iso-iridogermanal or their naturally occurring derivatives, including, without limitation, the seeds, leaves, stems, roots, rhizomes, flowers, or a whole plant. Preferably, the compounds are obtained from the rhizomes and roots. For example, iridogermanal or iso-iridogermanal compounds can be obtained from a whole plant extract or a rhizome extract. The plant part is optionally dried. An exemplary extraction utilizes a solvent, such as petroleum ether, methanol, ethyl acetate or chloroform. Chemical synthesis of iridogermanal, iso-iridogermanal and their derivatives should also be possible, for example using synthetic methods described in Arsenyadis et al., Org. Lett. 2007, 9:4745.
Pharmaceutical compositions

The present disclosure also provides a pharmaceutical composition that includes, as an active agent, and at least one iridal such as an iridogermanal, iso-iridogermanal, belachinal, anhydrobelachinal, spiroiridal, or derivative thereof, and a pharmaceutically acceptable carrier. It should be understood that although pharmaceutical compositions and methods of treatment are described with respect to iso-iridogermanal compounds, other iridals, including iridogermanal compounds, can be utilized in the compositions and methods mutatis mutandis. The active agent is formulated in a pharmaceutical composition and then, in accordance with the method of the invention, administered to a vertebrate, particularly mammal, such as a human patient, companion animal, or domesticated animal, in a variety of forms adapted to the chosen route of administration. The formulations include those suitable for oral, rectal, vaginal, topical, nasal, ophthalmic or parenteral (including subcutaneous, intramuscular, intraperitoneal, and intravenous) administration.

The pharmaceutically acceptable carrier can include, for example, an excipient, a diluent, a solvent, an accessory ingredient, a stabilizer, a protein carrier, or a biological compound. Non-limiting examples of a protein carrier includes keyhole limpet hemocyanin (KLH), bovine serum albumin (BSA), ovalbumin, or the like. Non-limiting examples of a biological compound which can serve as a carrier include a glycosaminoglycan, a proteoglycan, and albumin. The carrier can be a synthetic compound, such as dimethyl sulfoxide or a synthetic polymer, such as a polyalkyleneglycol. Ovalbumin, human serum albumin, other proteins, polyethylene glycol, or the like can be employed as the carrier. In some embodiments, the pharmaceutically acceptable carrier includes at least one compound that is not naturally occurring or a product of nature.

In some embodiments, the active agent, for example iso-iridogermanal, or derivative thereof (i.e., a first active agent), is formulated in combination with one or more additional active agents (i.e., one or more second active agents) such an antiprotozoan and/or antiparasitic compound, or more generally any anti-infective agent. For example, an iso-iridogermanal, or derivative thereof, can be formulated in combination with one or more antiprotozoan or antiparasitic agents currently used to treat trypanosomatid infections.

Chagas Disease (American trypanosomiasis) is a trypanosomatid infection that is typically caused by T. cruzi. T. cruzi is transmitted via triatomines, a blood-sucking Reduvid insect vector. Exemplary therapeutic agents that have been used to treat Chagas Disease include,
without limitation, nifurtimox, benznidazole, or a combination of both drugs. An iso-iridogermanal, or derivative thereof, can be formulated in combination with either or both of nifurtimox or benznidazole.

Human African trypanosomiasis (HAT or sleeping sickness) can be caused by any of a number of trypanosomatids including, but not limited to, *T. brucei rhodesiense*, *T. brucei gambiense*, *T. evansi*, *T. lewisi*, *T. brucei brucei*, and *T. congolense*. The analogous disease in animals, animal African trypanosomiasis (AAT) can be caused by any number of trypanosomatids including, but not limited to, *T. brucei*, *T. congolense*, *T. equiperdum*, *T. simiae*, *T. suis* and *T. vivax*. Tsetse flies are the main vector for transmission of disease. Exemplary therapeutic agents that have been used to treat HAT include, without limitation, pentamidine, suramin, melarsoprol, eflornithine, and nifurtimox, including combinations thereof, such as nifurtimox and eflornithine combination therapy (NECT). An iso-iridogermanal, or derivative thereof, can be formulated in combination with one or more of pentamidine, suramin, melarsoprol, eflornithine, and/or nifurtimox.

Leishmaniasis represent a set of trypanosomal diseases caused by various species of *Leishmania*, for example *L. donovani*, *L. major*, and *L. amazonensis*, and are transmitted by sandflies. Exemplary therapeutic agents that have been used to treat leishmaniasis include, without limitation, pentavalent antimonials, sodium stibogluconate, liposomal amphotericin B, conventional amphotericin B deoxycholate (non-liposomal), aminoglycoside paromomycin sulfate and pentamidine, and miltefosine, including combinations thereof. An iso-iridogermanal, or derivative thereof, can be formulated in combination with one or more of a pentavalent antimonial, sodium stibogluconate, liposomal amphotericin B, conventional amphotericin B deoxycholate (non-liposomal), aminoglycoside paromomycin sulfate and pentamidine, and/or miltefosine.

The invention is not intended to be limited by the therapeutic agent that can be included as an additional active agent.

The action of the additional active agent in the combination therapy can be cumulative to the iso-iridogermanal or derivative thereof or it can be complementary, for example to manage side effects or other aspects of the patient's medical condition. The additional active agent can, for example, improve the uptake or stability of the iso-iridogermanal or derivative thereof. Other examples of an additional active agent include, without limitation, an immunomodulator, an anti-
inflammatory agent, an antibiotic, and a pain medication. The additional active agent may act synergistically with the iso-iridogermanal or derivative thereof. In one embodiment, the combination therapy includes at least one compound that is not naturally occurring or a product of nature.

The pharmaceutical composition can contain purified iridal, such as iso-iridogermanal or derivative thereof, or it can contain a partially purified plant extract that contains iso-iridogermanal or derivative thereof.

The formulations can be conveniently presented in unit dosage form and can be prepared by any of the methods well-known in the art of pharmacy. All methods include the step of bringing the active agent into association with a pharmaceutical carrier. In general, the formulations are prepared by uniformly and intimately bringing the active compound into association with a liquid carrier, a finely divided solid carrier, or both, and then, if necessary, shaping the product into the desired formulations.

Formulations of the present invention suitable for oral administration can be presented as discrete units such as tablets, troches, capsules, lozenges, wafers, or cachets, each containing a predetermined amount of the active agent as a powder or granules, as liposomes, or as a solution or suspension in an aqueous liquor or non-aqueous liquid such as a syrup, an elixir, an emulsion, or a draught. The tablets, troches, pills, capsules, and the like can also contain one or more of the following: a binder such as gum tragacanth, acacia, corn starch or gelatin; an excipient such as dicalcium phosphate; a disintegrating agent such as corn starch, potato starch, alginic acid, and the like; a lubricant such as magnesium stearate; a sweetening agent such as sucrose, fructose, lactose, or aspartame; and a natural or artificial flavoring agent. When the unit dosage form is a capsule, it can further contain a liquid carrier, such as a vegetable oil or a polyethylene glycol. Various other materials can be present as coatings or to otherwise modify the physical form of the solid unit dosage form. For instance, tablets, pills, or capsules can be coated with gelatin, wax, shellac, sugar, and the like. A syrup or elixir can contain one or more of a sweetening agent, a preservative such as methyl- or propylparaben, an agent to retard crystallization of the sugar, an agent to increase the solubility of any other ingredient, such as a polyhydric alcohol, for example glycerol or sorbitol, a dye, and flavoring agent. The material used in preparing any unit dosage form is substantially nontoxic in the amounts employed. The active agent can be
incorporated into preparations and devices in formulations that may or may not be designed for sustained release.

Formulations suitable for parenteral administration conveniently include a sterile aqueous preparation of the active agent, or dispersions of sterile powders of the active agent, which are preferably isotonic with the blood of the recipient. Parenteral administration of iridal of the invention, such as iso-iridogermanal or derivative thereof (e.g., through an I.V. drip), is one form of administration. Isotonic agents that can be included in the liquid preparation include sugars, buffers, and sodium chloride. Solutions of the active agent can be prepared in water, optionally mixed with a nontoxic surfactant and/or emulsifier. Dispersions of the active agent can be prepared in water, ethanol, a polyol (such as glycerol, propylene glycol, liquid polyethylene glycols, and the like), vegetable oils, glycerol esters, and mixtures thereof. The ultimate dosage form is sterile, fluid, and stable under the conditions of manufacture and storage. The necessary fluidity can be achieved, for example, by using liposomes or micelles, by employing the appropriate particle size in the case of dispersions, or by using surfactants.

Sterilization of a liquid preparation can be achieved by any convenient method that preserves the bioactivity of the active agent, preferably by filter sterilization. Preferred methods for preparing powders include vacuum drying and freeze drying of the sterile injectable solutions. Subsequent microbial contamination can be prevented using various antimicrobial agents, for example, antibacterial, antiviral and antifungal agents including parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. Absorption of the active agents over a prolonged period can be achieved by including agents for delaying, for example, aluminum monostearate and gelatin.

Nasal spray formulations include purified aqueous solutions of the active agent with preservative agents and isotonic agents. Such formulations are preferably adjusted to a pH and isotonic state compatible with the nasal mucous membranes. Formulations for rectal or vaginal administration can be presented as a suppository with a suitable carrier such as cocoa butter, or hydrogenated fats or hydrogenated fatty carboxylic acids. Ophthalmic formulations are prepared by a similar method to the nasal spray, except that the pH and isotonic factors are preferably adjusted to match that of the eye. Topical formulations include the active agent dissolved or suspended in one or more media such as mineral oil, petroleum, polyhydroxy alcohols, or other bases used for topical pharmaceutical formulations. Topical formulations can be provided in the
form of a bandage, wherein the formulation is incorporated into a gauze or other structure and brought into contact with the skin.

**Administration of the active agent**

The iridal active agent, such as iso-iridogermanal or derivatives thereof, can be administered to a subject alone or in a pharmaceutical composition that includes the active agent and a pharmaceutically acceptable carrier. The active agent is administered to a vertebrate, more preferably a mammal, such as a human patient, a companion animal, or a domesticated animal, in an amount effective to produce the desired effect. Iso-iridogermanal can be administered in a variety of routes, including orally, parenterally, intraperitoneally, intravenously, intraarterially, transdermally, sublingually, intramuscularly, rectally, transbuccally, intranasally, liposomally, via inhalation, vaginally, intraocularly, via local delivery by catheter or stent, subcutaneously, intraadiposally, intraarticularly, intrathecally, or in a slow release dosage form.

The formulations can be administered as a single dose or in multiple doses. Useful dosages of the active agents can be determined by comparing their *in vitro* activity and the *in vivo* activity in animal models. Methods for extrapolation of effective dosages in mice, and other animals, to humans are known in the art.

Dosage levels of the active agent, including but not limited to iso-iridogermanal or derivatives thereof, in the pharmaceutical compositions of this invention can be varied so as to obtain an amount of the active agent which is effective to achieve the desired therapeutic response for a particular subject, composition, and mode of administration, without being toxic to the subject. The selected dosage level will depend upon a variety of factors including the activity of the particular compound of the present invention employed, or the ester, salt or amide thereof, the route of administration, the time of administration, the rate of excretion of the particular compound being employed, the duration of the treatment, other drugs, compounds and/or materials used in combination with the iso-iridogermanal or derivatives thereof, the age, sex, weight, condition, general health and prior medical history of the subject being treated, and like factors well known in the medical arts.

Dosages and dosing regimens that are suitable for other prophylactic and therapeutic anti-protozoan agents are likewise suitable for therapeutic or prophylactic administration of iridals such as iso-iridogermanal or derivative thereof. For example, dosages or dosing regimens in use
for other plant-derived compounds, such as the anti-malarial botanical artemisinin, may serve as guideposts for developing suitable animal and human dosages and dosing regimens. Examples
of other antiparasitic therapies which can form the basis for determining dosages and dosing
regimens for iridals such as iso-iridogermanal and derivatives thereof can be found in
purified iso-iridogermanal or a derivative thereof can be administered orally in an amount of
between 5 mg and 100 mg at least once per day, as a medication, nutritional supplement, or food
additive. As another example, iso-iridogermanal or a derivative thereof can be administered in
dosages ranging from 0.01 mg/kg to 20 mg/kg body weight, or higher; or in a form sufficient to
provide a daily dosage of 0.01 mg/kg body weight to about 20 mg per/kg body weight of the
subject to which it is to be administered. As a further example, iso-iridogermanal or a derivative
thereof can be administered intravenously or intramuscularly in an amount between 5 mg and
100 mg at least once per day.

The iridal compound, such as iso-iridogermanal and derivatives thereof, can be
administered in purified, partially purified, or unpurified form. It can be administered as an
extract obtained from a plant source, such as a rhizome.

A physician or veterinarian having ordinary skill in the art can readily determine and
prescribe the effective amount of the pharmaceutical composition required. For example, the
physician could start doses of the iso-iridogermanal or derivative thereof of the invention
employed in the pharmaceutical composition at levels lower than that required in order to
achieve the desired therapeutic effect and gradually increase the dosage until the desired effect is
achieved.

Methods of treatment

Iridals such as iridogermanal and iso-iridogermanal compounds, as well as others
described herein, can be used to treat or prevent parasitic infections, particularly protozoan
infections caused by trypanosomes. The terms "trypanosome," "trypanosomatid" and
"trypanosomal" are used to indicate or describe organisms, or human or animal diseases caused
by protozoa in the family Trypanosomatidae which includes the genera Trypanosoma and
Leishmania. Exemplary trypanosomatid infections include, but are not limited to, human
African trypanosomiasis (HAT), animal African trypanosomiasis (AAT), American
trypanosomiasis (Chagas Disease), and leishmaniasis. The invention provides a therapeutic method of treating a subject suffering from infection with a parasitic protozoan by administering an iridal, such as iso-iridogermanal, or a derivative thereof, to the subject. Therapeutic treatment is initiated after diagnosis or the development of symptoms of infection with a parasitic protozoan.

An iridal such as iso-iridogermanal, or a derivative thereof, can also be administered prophylactically, to prevent or delay the development of infection with a parasitic protozoan. Treatment that is prophylactic, for instance, can be initiated before a subject manifests symptoms of infection with a parasitic protozoan. An example of a subject that is at particular risk of developing infection with a parasitic protozoan is a person traveling to an area in which infection with a parasitic protozoan is prevalent. Treatment can be performed before, during, or after the diagnosis or development of symptoms of infection. Treatment initiated after the development of symptoms may result in decreasing the severity of the symptoms of one of the conditions, or completely removing the symptoms. The iridal can be introduced into the mammal at any stage of trypanosomal infection including, for example, during acute, early congenital, and/or reactivated trypanosomal infection.

Administration of the iridal, such as iso-iridogermanal, or derivative thereof, can occur before, during, and/or after other treatments. Such combination therapy can involve the administration of the iridal, or derivative thereof, before, during and/or after the use of other anti-trypanosomal agents. The administration of the iridal, or derivative thereof, can be separated in time from the administration of other anti-trypanosomal agents by hours, days, or even weeks. Exemplary medications that can be administered in combination with an iridal include, without limitation, one or more of nifurtimox, benznidazole, pentamidine, suramin, melarsoprol, eflornithine, a pentavalent antimonial, sodium stibogluconate, liposomal amphotericin B, conventional amphotericin B deoxycholate (non-liposomal), aminoglycoside paromomycin sulfate and pentamidine, and/or miltefosine. The different therapeutic agents in combination therapy can be administered together or separately, using the same or different modes of administration, in accordance with a dosing schedule as determined by a physician, veterinarian, or other skilled artworker.

The compounds of the invention find utility in the treatment, control or prevention of trypanosomal infection and disease not only in humans but also in animals. Compounds of the
invention can be administered to companion animals, domesticated animals such as farm animals, or animals in the wild. Companion animals include, but are not limited to, dogs, cats, hamsters, gerbils and guinea pigs. Domesticated animals include, but are not limited to, cattle, horses, pigs, goats and llamas. In one embodiment, the compound of the invention is administered to an animal, such as a companion animal or domesticated animal, that has been diagnosed with, or is exhibiting symptoms of, or is at risk of developing, a trypanosomal infection. In another embodiment, the compound of the invention is administered in an animal or animal population that serves, may serve, or is suspected of serving as a trypanosomatid reservoir, regardless of the presence of symptoms. Administration can be, for example, part of a small or large scale public health infection control program. The compound of the invention can, for example, be added to animal feed as a prophylactic measure for reducing, controlling or eliminating trypanosomal infection in a wild or domestic animal population. The compound can, for example, be administered as part of routine or specialized veterinary treatment of a companion or domesticated animal or animal population. It should be understood that administration of the compound of the invention can be effective to reduce or eliminate trypanosomal infection or the symptoms associated therewith; to halt or slow the progression of infection or symptoms within a subject; and/or to control, limit or prevent the spread of infection within a population, or movement of infection to another population.

**Nutritional supplement and food additive**

An iridal compound, such as an iso-iridogermanal, or a derivative thereof, can be packaged as a nutritional, health or dietary supplement (e. g., in pill or capsule form). Additionally, an iridal such as iso-iridogermanal, or derivative thereof, can be added to a food product to yield what is commonly referred to as a "nutraceutical" food or "functional" food. Foods to which the iridal can be added include, without limitation, animal feed, cereals, yoghurts, cottage cheeses and other milk products, oils including hydrogenated or partially hydrogenated oils, soups and beverages. Esterified derivatives are preferably incorporated into oily or fatty food products, to facilitate solubilization.

**Bait composition**

An iridal compound, such as an iso-iridogermanal or derivative thereof, can be
formulated as a bait composition. In some embodiments, the bait composition is formulated for consumption by a disease vector, for example, for consumption by an insect vector such as, without limitation, a triatomine bug, a tsetse fly, and/or a sandfly. The bait composition includes an iridal compound and, optionally, at least one of an attractant, a food substance, and/or a preservative. Exemplary attractants and/or food substances include an odor, a pheromone, a plant extract, a hematogenous substance or mimic thereof, a sweetener such as honey, molasses, sucrose, fructose, glucose, maltose, aspartame, sucralose, and the like. Preferably, the bait composition attracts a target species of insect, and does not attract a non-target species of insect or other animal. The invention includes feeding a bait composition to the insect or depositing a bait composition in locations where the insect is likely to come in contact with the bait composition and smell, touch, taste, or eat the bait composition.

Also provided are kits that include a bait composition as described herein or components for making a bait composition, and, optionally, instructions for use. The components of the kits may be provided in any suitable form (e.g., liquid form or lyophilized form). Kits further may include buffers or solvents for resuspending or dissolving one or more of the bait components.

The words "preferred" and "preferably" refer to embodiments of the invention that may afford certain benefits, under certain circumstances. However, other embodiments may also be preferred, under the same or other circumstances. Furthermore, the recitation of one or more preferred embodiments does not imply that other embodiments are not useful, and is not intended to exclude other embodiments from the scope of the invention.

The terms "comprises" and variations thereof do not have a limiting meaning where these terms appear in the description and claims.

Unless otherwise specified, "a," "an," "the," and "at least one" are used interchangeably and mean one or more than one.

Also herein, the recitations of numerical ranges by endpoints include all numbers subsumed within that range (e.g., 1 to 5 includes 1, 1.5, 2, 2.75, 3, 3.80, 4, 5, etc.).

For any method disclosed herein that includes discrete steps, the steps may be conducted in any feasible order. And, as appropriate, any combination of two or more steps may be conducted simultaneously.
EXAMPLES

The present invention is illustrated by the following examples. It is to be understood that the particular examples, materials, amounts, and procedures are to be interpreted broadly in accordance with the scope and spirit of the invention as set forth herein.

Example 1. Anti-trypanosomal activity of an iridal from *Iris domestica*

The need to develop better therapies for human African trypanosomiasis (HAT or Sleeping Sickness) and Chagas disease has turned attention to screening of traditional plant-based medicines. The efforts have resulted in a few promising options for the treatment of HAT, however no drugs have been approved (Freiburghaus et al. 1996, 1998; Hoet et al. 2004). Screening of hundreds of extracts from plants used in Traditional Chinese Medicine (TCM) led to the identification of a petroleum ether extract of *Iris domestica* (formerly known as *Belamcanda chinensis*) as having activity against *T. brucei brucei*.

*Iris domestica* (formerly known as *Belamcanda chinensis*) belongs to the family *Iridaceae*, which comprises approximately 60 genera and 800 species worldwide and has been used as Chinese traditional medicine for treating throat ailments such as asthma and tonsillitis and has also seen use in Thai traditional medicine for menstrual disorders (Liu et al. 2012; Monthakantirat 2005). FIGs. 1A-1E show structures of iridoid compounds isolated from *Iris domestica*. Previous phytochemical studies on this plant led to the isolation of iridal-type triterpenoids, including belamcandal (FIG. 1A), and isoflavonoids such as iridin from the rhizomes, as well as phenols, benzoquinones and benzo furans from the seed (Abe et al. 1991; Ha et al. 2009; Takahashi et al. 2000; Marner et al. 1990). Flavonoids isolated from *Belamcanda chinensis* (now known as *Iris domesticus*) showed strong anti-inflammatory effects and isoflavones were reported to have selective estrogen receptor modulating (SERM) properties (Ito et al. 2001; Lee et al. 2011). The triterpenoid iridals are the major lipid component of Iris rhizomes. Structures include monocyclic seco-A-ring structures such as iso-iridogermanal, and spiro-bicyclic structures such as belamcandal. A common feature is the α,β-unsaturated aldehyde, which presumably contributes to the reported instability of many of the structures.
The only prior report of activity of against trypanosomes or other protozoa is from seco-iridoids such as compound 1 (FIG. ID), which was reported to be active against *T. cruzi* (Cardona Zuleta et al. 2003). Prior reports of biological activity of spiro-bicyclic iridals include "throat stimulation" and tumor promotion by belamcandal. The 28-deacetyl analog has been shown to be an activating ligand for protein kinase C (PKC), and induces production of TNF-a (Liu et al. 2012; Monthakantirat et al. 2005). The side chain isomer, NSC 631941 (FIG. 1C), acts as a tumor inhibitor. Also, the iridal-type triterpenoid shown in FIG. IE was recently reported to have neuroprotective activity (Zhang et al. 2014).

Bioassay-guided fractionation of the ethanol extract of *Iris domestica* led to the isolation of compound iso-iridogermanal as its myristate ester. It exhibited 98% inhibition of *T. brucei* at 6.25mg/ml. As previously reported, the compound is unstable (Marner et al. 1990; Abe et al. 1991). It was postulated that the α,β-unsaturated carbonyl might introduce instability and reactivity into the compound, since the electrophilic α,β-unsaturated carbonyl could be subject to attack by nucleophiles at the β carbon in a vinylogous reaction. Therefore, in an attempt to improve stability, the compound's aldehyde was reduced to the corresponding novel alcohol 3, which retained high activity (Scheme 1).
Scheme 1. Reduction of iso-iridogermanal and synthesis of iridal derivatives

Materials and methods

Instrumentation and chromatography material

NMR data were obtained using a 500 MHz FT-NMR model ECA-500 JEOL (Peabody, MA) purchased with funding provided by the National Science Foundation through the NSF-MRI program (#0321211).

High resolution ESI-MS was performed at Notre Dame University, Notre Dame, Indiana.

Thin layer chromatography (TLC) was performed on glass plates coated with silica gel and UV active backing purchased from Fisher Scientific, Pittsburgh, PA. The TLC plates were analyzed with a short wavelength (254 nm) UV light and subsequently stained with phosphomolybdic acid (reagent grade, Aldrich, Milwaukee, WI) prepared as a 10% solution in ethanol. Gravity column chromatography was performed with silica gel, 63-200 micron 70-230 mesh ASTM (reagent grade, Fisher Scientific, Pittsburgh, PA). Flash column chromatography was performed with silica gel, 60 A 230-400 mesh ASTM, reagent grade, Fisher Scientific, (Pittsburgh, PA). Methylene chloride, methanol, acetone, ethyl acetate, ethanol, and hexanes
were purchased from Fisher Scientific (Pittsburgh, PA). FIPLC was performed using a Breeze Waters System with a normal phase Waters Spherisorb column (10 x 250 mm).

Plant material

The petroleum ether extract of the whole plant of *B. chinensis* exhibited cytotoxic activity against *T. brucei brucei* with 100% inhibition value at 50µg/ml. The whole plant of *B. chinensis* (now known as *I. domesticus*) was collected at the Cultivation Center at Guangxi Botanical Garden of Medicinal Research, Nanning, Guangxi Autonomous Region, P. R. China, in Jun 2009, and identified by Dr. Xueyan Huang (Guangxi Botanical Garden of Medicinal Research, Nanning, P. R. China). A voucher specimen (No. 200906010) has been deposited in the natural product library at Guangxi Botanical Garden of Medicinal Research.

Extraction and isolation

Dried and powdered plant material was extracted with petroleum ether, and the solvent was evaporated. The extract was found to exhibit significant inhibitory activity against *T. brucei brucei* with 100% inhibition value at 50 µg/ml.

The petroleum ether extract (1g) was purified by gravity column chromatography on silica gel. The gravity column was eluted successively with hexane, mixtures of hexanes and EtOAc, pure EtOAc, respectively, to yield seven fractions (F1 to F7). F4 (392mg) was eluted at 45% EtOAc/hexanes. Flash column chromatography of F4 eluting successively with hexanes, mixtures of hexanes and EtOAc, and pure EtOAc afforded three fractions (F4A, F4B and F4C). F4C (70mg) eluted with 10% EA/Hex was identified as iso-iridogermanal (2) by NMR and MS. Attempts at further purification by HPLC led to decomposition. This compound has previously been reported as being unstable. *^1^H-NMR* and *^{13}C-NMR* (*C_6D_6*) data were identical to that previously reported in the literature, and confirmed by HRMS. (Abe, et. al. 1991).

Chemical Derivatization

To a solution of iso-iridogermanal (75.0 mg, 0.10 mmol) in 6 mL methanol was added sodium borohydride (43.0 mg, 1.13 mmol). The resulting solution was stirred at room temperature for 4 hours. The solution was poured into 1M HC1, and extracted twice with ethyl acetate. The organic layer was washed with aqueous sodium bicarbonate and brine and then dried over magnesium sulfate, filtered and evaporated. The crude product was purified by flash
column chromatography on silica gel, eluting with hexanes, 1:20, 1:10, and 1:5 ethyl acetate-hexane and ethyl acetate to afford 20.4 mg of alcohol 3 (27%). Rf 0.75 [ethyl acetate-hexane 1:1]. [α]D = +6.6° (CHCl3, c = 0.01).

To a solution of iso-iridogerananal (2) (75.0 mg, 0.10 mmol) in 6 mL methanol was added sodium borohydride (43.0 mg, 1.13 mmol). The resulting solution was stirred at room temperature for 4 h. The solution was poured into 1 M HCl, and extracted twice with ethyl acetate. The organic layer was washed with aqueous sodium bicarbonate and brine and then dried over magnesium sulfate, filtered and the solvent was evaporated. The crude product was purified by flash column chromatography on silica gel, eluting with hexanes, 1:20, 1:10, and 1:5 ethyl acetate-hexane and ethyl acetate to afford 20.4 mg of alcohol, iso-iridogerananal (3) (27.2%). Rf 0.75 [ethyl acetate-hexane 1:1]. [α]D = +6.6° (CHCl3, c = 0.01). MS C44 H78 O5 Na, calculated, 709.5747, found, 709.5741.

Compound (3) (50.0 mg, 0.07 mmol) was dissolved in 5 mL NaOMe/methanol. The resulting solution was stirred at room temperature for 24 h. The solution was poured into 1 M HCl, and extracted twice with ethyl acetate. The organic layer was washed with aqueous sodium bicarbonate and brine and then dried over magnesium sulfate, filtered and evaporated. The crude product was purified by flash column chromatography on silica gel, eluting with 20%, 30%, 40%, 60%, and 80% ethyl acetate-hexane and ethyl acetate to afford 25 mg of compound (4) (75%). Rf 0.18 [ethyl acetate-hexane 1:1]. [α]D = +40.64° (CHCl3, c = 0.5). MS C30 H52 O4 Na, calculated, 499.3763, found, 499.3758.

Compound (3) (50.0 mg, 0.07 mmol) was dissolved in 5 mL EtOAC and then added 50.0 mg of 10% palladium on carbon. The mixture was placed on a Parr shaker, purged twice with 10 psi of hydrogen gas, once with 12 psi of hydrogen gas, and then allowed to equilibrate to 8 psi. The mixture was shaken overnight, filtered with celite 545 filter aid, and washed with ethyl acetate. The filtrate was gravity filtered and evaporated. The crude product was purified by flash column chromatography on silica gel, eluting with hexane only, 5%, 10%, 15%, ethyl acetate-hexane to afford 12.7 mg of compound (5) (10.4%). Rf 81.5% [ethyl acetate-hexane 1:1]. [α]D = +29.29° (CHCl3, c = 0.5).

Flash column chromatography of F6 (117 mg), eluting successively with hexanes, mixtures of hexanes and EtOAc, and pure EtOAc afforded two fractions (F6A and F6B). F6A (53 mg) eluted with 15% EA/Hex as a white powder, was identified as anhydrobelachinal (6) by
NMR and MS (C_{30} H_{40} O_{4}, calculated, 469.3240, found, 469.3328). Rf 0.72 [ethyl acetate-hexane 1:1]. [a]_D = + 70.6 (CHC1_3, c =0.5).

245 mg of compound (6) was dissolved in 30 mL methanol then added sodium borohydride (186.2 mg, 4.9 mmol). The resulting solution was stirred at room temperature for 4h. The solution was poured into IM HCl, and extracted twice with ethyl acetate. The organic layer was washed with aqueous sodium bicarbonate and brine and then dried over magnesium sulfate, filtered and evaporated. The crude product was purified with preparative thin layer chromatography, eluting with 1:1 ethyl acetate-hexane to afford 14.9 mg of alcohol. Reduced form of anhydrobelachinal (7) was identified by NMR and MS. MS C_{50} H_{60} O_{4} Na, calculated, 493.3294, found, 493.3266. Rf 0.52 [ethyl acetate-hexane 1:1]. [a]_D = +30.77 (CHC1_3, c =0.01).

To a solution of F3 (375.0mg, 0.55mmol) in 30 mL methanol was added sodium borohydride (240.0 mg, 6.25 mmol). The resulting solution was stirred at room temperature for 4h. The solution was poured into IM HCl, and extracted twice with ethyl acetate. The organic layer was washed with aqueous sodium bicarbonate and brine and then dried over magnesium sulfate, filtered and the solvent was evaporated. The crude product was purified by flash column chromatography on silica gel, eluting with hexanes, 5%, 10% ethyl acetate-hexane and ethyl acetate only afford 43.2 mg of product, which was formed a white precipitate and a greenish solvent with CDC1_3. The greenish solvent was again purified by flash column chromatography on silica gel, eluting with hexanes, 5% and 10% ethyl acetate-hexane to afford three fractions. 35.5 mg of alcohol (9.4%) eluted with 10% EA/Hex as a green oily liquid, was identified as reduced spiroiridal (8) by NMR and MS for C_{44} H_{74} O_{5} Na, found, 705.5428 calculated 705.5434. Rf 0.7 [ethyl acetate-hexane 2:3]. [a]_D = +54.16 (CHC1_3, c =0.5).

To a solution of F5, (300.Omg) in 25 mL methanol, sodium borohydride (190.0 mg, 5.0mmol) was added. The resulting solution was stirred at room temperature for overnight. The solution was poured into IM HCl, and extracted twice with ethyl acetate. The organic layer was washed with aqueous sodium bicarbonate and brine and then dried over magnesium sulfate, filtered and the solvent was evaporated. The crude product was purified by flash column chromatography on silica gel, eluting with 10%, 20%, and 30%, 40% ethyl acetate-hexane and ethyl acetate to afford 43 mg of alcohol (9). Rf 0.92[ethyl acetate-hexane 1:1]. [csl]_D = +59.2° (CHC1_3, c = 0.5). MS C_{50} H_{87} O_{6} calculated, 783.6503, found, 783.5258.
Bioassays

L6 rat skeletal myoblasts (ATCC CRL-1458, ATCC, Rockville, MD) were maintained in 75 cm² tissue culture flasks (Corning, Corning, NY) by subculturing every 2-3 days. Cells were grown in DMEM (HyClone, Logan, UT) supplemented with 10% heat inactivated fetal calf serum (FCS, Atlanta Biological, Atlanta, GA) and 50 IU/mL penicillin and 50 µg/mL streptomycin solution (P/S, HyClone, Logan, UT) with incubation at 37°C and 5% CO₂. Log phase myoblasts were trypsinized and adjusted in fresh media to deliver 5x10³ cells per well in 90 µL into black wall, clear bottom microtiter plates (Corning, Corning, NY). Extract samples were dissolved in DMSO and diluted in fresh media such that the addition of 10 µL would produce final well concentrations ranging from 6.25 µg/mL - 100 µg/mL. Maximum well concentrations of DMSO did not exceed 1%. Control wells were treated with podophyllotoxin (Sigma-Aldrich, St. Louis, MO), DMSO, or media only. Each condition was plated in triplicate. Treated samples were incubated at 37°C and 5% CO₂ for 71.5 hours, at which time 11 µL of the resazurin-based indicator, PrestoBlue (Invitrogen, Frederick, MD), was added to each well. Plates were incubated for an additional 30 minutes under the same conditions. At 72 hours, fluorescent intensity was measured on a SpectraMax M5 plate reader (Molecular Devices, Sunnyvale, CA) using excitation and emission wavelengths of 560 and 590 nm, respectively. Toxicity was calculated based on the difference between fluorescent intensity levels of treated and untreated wells.

Trypanosoma brucei brucei 427 cells (Cross 1973) were maintained in 75 cm² tissue culture flasks (Corning, Corning, NY) by subculturing every 2-3 days. Cells were grown in HMI-9 medium supplemented with 10% FCS and P/S with incubation at 37°C and 5% CO₂. Log phase trypanosomes were adjusted in fresh media to deliver 1x10⁴ cells per well in 90 µL into clear 96 well microtiter plates (Corning, Corning, NY). Extract samples were dissolved in DMSO and diluted in fresh media such that the addition of 10 µL would produce final well concentrations ranging from 3.125 µg/mL - 50 µg/mL. Maximum well concentrations of DMSO did not exceed 0.5%. Control wells were treated with pentamidine (Sigma-Aldrich, St. Louis, MO), DMSO, or media only. Each condition was plated in triplicate. Treated samples were incubated at 37°C and 5% CO₂ for 48 hours, at which time 11 µL of Presto Blue was added to each well. Plates were incubated for an additional 24 hours under the same conditions. At 72 hours, 60 µL of a 4% paraformaldehyde solution (Santa Cruz Biotechnology, Dallas, TX) was
added to each well and fluorescent intensity was measured and inhibition calculated as previously described.

*Trypanosoma cruzi* (Tulahuen) cells expressing β-galactosidase (Buckner, 1996) were maintained by infecting L6 cells with freshly burst trypomastigotes in 75 cm² tissue culture flasks (Corning, Corning, NY). Infected cultures were maintained in DMEM without phenol red supplemented with 10% FCS and 1% penicillin-streptomycin-L-glutamine solution (PSG, Gibco, Carlsbad, CA) with incubation at 37°C and 5% CO₂. Freshly burst trypomastigotes were collected 5-7 days following infection. Separation of trypomastigote forms from amastigotes and host cells was accomplished by centrifugation at 2,700 rpm for 7 minutes, followed by incubation for 3 hours to allow trypomastigotes to swim out of the pellet. Separated trypomastigotes were collected then used to infect L6 stock cultures and for assays. Trypomastigotes were adjusted to deliver 4x10⁴ cells/well in 90 µL in clear 96 well plates. Extract samples were dissolved in DMSO and diluted in fresh media such that the addition of 10 µL would produce final well concentrations ranging from 0.39 - 50 µg/mL. Maximum well concentrations of DMSO did not exceed 0.5%. Control wells were treated with Amphotericin B (Sigma-Aldrich, St. Louis, MO), DMSO, or media only. Treated samples were incubated at 37°C and 5% CO₂ for 24 hours, at which time 45 µL of the luciferin-lucerase reagent, CellTiter Glo (Promega, Madison, WI), was added. Plates were shaken for 10 minutes, and incubated for an additional 15 minutes before transferring 100 µL from each well to an opaque microtiter plate (Corning, Corning, NY). Luminescence was measured using the aforementioned plate reader and inhibition calculated as described.

*Leishmania amazonensis* promastigotes expressing β-lactamase (Buckner, 2005) were maintained in capped 15 mL centrifuge tubes (Thermo Scientific, Waltham, MA) by subculturing every 5-7 days. Cells were grown in RPMI-1640 without phenol red supplemented with 10%, FCS and 1% PSG with incubation at 27°C. Stationary phase cultures were adjusted in fresh media to deliver 2x10⁴ cells per well in 90 µL into clear 96 well microtiter plates. Extract samples were dissolved in DMSO and diluted in fresh media such that the addition of 10 µL would produce final well concentrations ranging from 0.39 - 50 µg/mL. Maximum well concentrations of DMSO did not exceed 0.5%. Control wells were treated with Amphotericin B (Sigma-Aldrich, St. Louis, MO), DMSO, or media only. Treated samples were incubated at 37°C and 5% CO₂ for 24 hours, at which time 45 µL of the CellTiter Glo reagent was added.
Plates were shaken for 10 minutes, and incubated for an additional 15 minutes before transferring 100 µL from each well to an opaque microtiter plate. Luminescence was measured using the aforementioned plate reader and inhibition calculated as described.

5 Results and discussion

Through broad screening of extracts from plants used in TCM, the petroleum ether extract of dried plant material from Iris domestica was identified as having activity against T. brucei brucei. Bio-assay guided fractionation led to the isolation of the known iridal isoiridogermanal (myristate ester) as the active component. The pure compound inhibited growth by 98% at 6 µg/ml concentration. It was also found to be non-toxic to rat muscle L6 cells, with high selectivity. Similar activity was seen for the reduced analogs, indicating that the aldehyde is not necessary for activity. Results are summarized in Table 1.

Table 1. Anti-trypanosomal activity, toxicity, and selectivity of selected compounds.

<table>
<thead>
<tr>
<th>Comp.</th>
<th>IC₅₀(µg/mL)</th>
<th>Selectivity Multiple*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T. brucei (BSF)</td>
<td>T. cruzi (TM)</td>
</tr>
<tr>
<td>2</td>
<td>3.6</td>
<td>NT</td>
</tr>
<tr>
<td>3</td>
<td>5.4</td>
<td>&gt;50</td>
</tr>
<tr>
<td>4</td>
<td>6.21</td>
<td>22.39</td>
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<tr>
<td>5</td>
<td>&gt;50</td>
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</tr>
<tr>
<td>6</td>
<td>5.42</td>
<td>&gt;50</td>
</tr>
<tr>
<td>7</td>
<td>2.13</td>
<td>17.09</td>
</tr>
<tr>
<td>8</td>
<td>2.40</td>
<td>14.68</td>
</tr>
<tr>
<td>9</td>
<td>2.00</td>
<td>25.30</td>
</tr>
</tbody>
</table>
BSF = bloodstream form trypomastigotes, TM = T. cruzi trypomastigotes, PM = L. amazonensis promastigotes. NT = not tested, ND = not determined. Mean of 2 or more independent trials. Standard error of the mean <3, except as noted.

SEM = 3.19

SEM = 5.8

Selectivity estimated using L6 IC$_{50}$ = 100 µg/mL.

This is the first report of the possible use of *Iris domestica* extracts to treat trypanosomal diseases. Although iridals have previously been reported as having several types of bioactivity, there are no prior reports of trypanosomal activity of iso-iridogermanal. Iso-iridogermanal has previously been isolated from *I. domestica* as well as other Iris species (Jaenicke and Marner, 1986). Reported bioactivity includes induction of HL-60 cell adhesion and cytotoxicity to tumor cells (Wong et al. 1986). Treatments for trypanosomal diseases are extremely limited, and the investigation of plant extracts has led to some examples of active compounds, however none have been developed as yet. The addition of iridals and their derivatives as potential treatments may ultimately lead to novel drugs to treat these neglected tropical diseases.

References


The complete disclosures of all patents, patent applications including provisional patent applications, publications including patent publications and nonpatent publications, and electronically available material (e.g., GenBank amino acid and nucleotide sequence submissions) cited herein are incorporated by reference. The foregoing detailed description and examples have been provided for clarity of understanding only. No unnecessary limitations are to be understood therefrom. The invention is not limited to the exact details shown and described; many variations will be apparent to one skilled in the art and are intended to be included within the invention defined by the claims.
WHAT IS CLAIMED IS:

1. A compound having Formula II:

   \[
   \text{(II)}
   \]

   wherein \( \text{R}_{i} \) is H, alkyl, or \(-\text{C}(0)\text{R}_{7}\); \( \text{R}_{2}, \text{R}_{4}, \text{R}_{5} \) and \( \text{R}_{6} \) are each independently H, OH, or OAc; \( \text{R}_{3} \) is CH₃ or CH₂OH; \( \text{R}_{7} \) is H, a substituted or unsubstituted alkyl, a substituted or unsubstituted alkenyl, or a substituted or unsubstituted aryl; \( \text{R}_{8} \) is H, alkyl, or \(-\text{C}(0)\text{R}_{9}\); and \( \text{R}_{9} \) is H, a substituted or unsubstituted alkyl, a substituted or unsubstituted alkenyl, or a substituted or unsubstituted aryl.

2. The compound of claim 1, wherein \( \text{R}_{i} \) is H.

3. The compound of claim 1 wherein \( \text{R}_{i} \) is \(-\text{C}(0)\text{R}_{7}\) and \( \text{R}_{7} \) is from a plant fatty acid.

4. The compound of any of claims 1-3, wherein \( \text{R}_{8} \) is H.

5. A compound having Formulas III, IV or V:
6. A method for treating or preventing a trypanosomatid infection in a subject, the method comprising:

administering to the subject a composition comprising an effective amount of an active agent comprising a compound of any of claims 1-5.

7. A method for treating or preventing a trypanosomatid infection in a subject, the method comprising:

administering to the subject a composition comprising an effective amount of an active agent comprising a compound having Formula 1:

wherein Ri is H, alkyl, or -C(0)R; R2, R4, R5 and R6 are each independently H, OH, or OAc; R3 is CH3 or CH2OH; and R7 is H, a substituted or unsubstituted alkyl, a substituted or unsubstituted alkenyl, or a substituted or unsubstituted aryl.
8. The method of claim 7, wherein \( R_i \) is H.

9. The method of claim 7, wherein \( R_i \) is \(-C(0)R_7\) and \( R_7\) is from a plant fatty acid.

10. A method for treating or preventing a trypanosomatid infection in a subject, the method comprising:
    administering to the subject a composition comprising an effective amount of an active agent comprising at least one iridal selected from the group consisting of an iridogermanal, an iso-iridogermanal, a belachinal, an anhydrobelachinal, a spiroiridal, and derivative thereof.

11. The method of claim 10, wherein the iridal comprises an aldehyde.

12. The method of claim 11 or 12, wherein the iridal comprises a fatty acid ester.

13. A method for treating or preventing a trypanosomatid infection in a subject, the method comprising:
    administering to the subject a composition comprising an effective amount of an active agent comprising at least one of compound 2, 3, 4, 5, 6, 7, 8, and 9.

14. A method for treating or preventing a trypanosomatid infection in a subject, the method comprising:
    administering to the subject a composition comprising an effective amount of an extract from a plant that is a member of the Iris genus.

15. The method of claim 14, wherein the extract comprises a root extract or a rhizome extract.

16. The method of any of claims 6-15, wherein the subject is a human.

17. The method of any of claims 6-15, wherein the subject is an animal.
18. The method of claim 17, wherein the animal is a companion animal, a domesticated animal, or a wild animal.

19. The method of claim 17, wherein the animal is a dog or a cow.

20. The method of claim 16, wherein the trypanosomatid infection comprises human African trypanosomiasis (HAT).

21. The method of any of claims 17-19, wherein the trypanosomatid infection comprises animal African trypanosomiasis (AAT).

22. The method of any of claims 6-19, wherein the trypanosomatid infection comprises American trypanosomiasis (Chagas Disease) or a leishmaniasis.

23. The method of any of claims 6-19, wherein the trypanosomatid infection comprises at least one of a Trypanosoma brucei infection or a Trypanosoma cruzi infection.

24. The method of any of claims 6-20 wherein the trypanosomatid infection is selected from the group consisting of T. cruzi infection, T. brucei infection, T. brucei rhodesiense infection, T. brucei gambiense infection, T. evansi infection, T. lewisi infection, T. brucei brucei infection, T. congoense infection, T. equiperdum infection, T. simiae infection, T. suis infection, T. vivax infection, Leishmania infection, L. donovani infection, L. major infection, and L. amazonensis infection.

25. The method of any of claims 6-24, wherein the composition further comprises a pharmaceutically acceptable carrier.

26. The method of any of claims 6-25, wherein the composition further comprises at least one second active agent.
27. The method of claim 26 wherein the second active agent is selected from the group consisting of nifurtimox, benznidazole, pentamidine, suramin, melarsoprol, eflornithine, a pentavalent antimonial, sodium stibogluconate, liposomal amphotericin B, conventional amphotericin B deoxycholate (non-liposomal), aminoglycoside paromomycin sulfate and pentamidine, and miltefosine.

28. The method of claim 26 or 27 wherein the second active agent comprises a non-naturally occurring compound.

29. A pharmaceutical composition comprising an active agent comprising a compound of any of claims 1-5.

30. The pharmaceutical composition of claim 29, further comprising a pharmaceutically acceptable carrier.

31. The pharmaceutical composition of claim 29 or 30 further comprising at least one second active agent.

32. The pharmaceutical composition of claim 31 wherein the second active agent is selected from the group consisting of nifurtimox, benznidazole, pentamidine, suramin, melarsoprol, eflornithine, a pentavalent antimonial, sodium stibogluconate, liposomal amphotericin B, conventional amphotericin B deoxycholate (non-liposomal), aminoglycoside paromomycin sulfate and pentamidine, and miltefosine.

33. The pharmaceutical composition of claim 31 or 32 wherein the second active agent comprises a non-naturally occurring compound.

34. The pharmaceutical composition of any of claims 29-33, further comprising at least one solubilizing agent selected from the group consisting of a surfactant, an emulsifier, a polyalkylene glycol, a liposome, and a micelle.
35. A plant extract comprising an iridal for use as a prophylactic or therapeutic agent for treatment or prevention of a trypanosomatid infection.

36. The plant extract of claim 35 prepared from a member of the Iris genus.

37. A compound as in any of Formula I, Formula II, Formula III, Formula IV or Formula V:

(Ⅰ)

wherein \( R_i \) is H, alkyl, or \(-\text{C}(\text{O})R\) \(_7\); \( R_2, R_4, R_5\) and \( R_6\) are each independently H, OH, or OAc; \( R_3\) is CH\(_3\) or CH\(_2\)OH; and \( R_7\) is H, a substituted or unsubstituted alkyl, a substituted or unsubstituted alkenyl, or a substituted or unsubstituted aryl;

(Ⅱ)
wherein \( R_1 \) is H, alkyl, or -C(0)R; \( R_2, R_5 \) and \( R_6 \) are each independently H, OH, or OAc; \( R_3 \) is CH\(_3\) or CH\(_2\)OH; \( R_7 \) is H, a substituted or unsubstituted alkyl, a substituted or unsubstituted alkenyl, or a substituted or unsubstituted aryl; \( R_8 \) is H, alkyl, or -C(0)R; and \( R_9 \) is H, a substituted or unsubstituted alkyl, a substituted or unsubstituted alkenyl, or a substituted or unsubstituted aryl;

for use as a prophylactic or therapeutic agent for treatment or prevention of a trypanosomatid infection.

38. Use of a compound as in any of Formula I, Formula II, Formula III, Formula IV or Formula V:
wherein $R_i$ is H, alkyl, or -C(0)R$_7$; R$_2$, R$_4$, R$_5$, and R$_6$ are each independently H, OH, or OAc; R$_3$ is CH$_3$ or CH$_2$OH; and R$_7$ is H, a substituted or unsubstituted alkyl, a substituted or unsubstituted alkenyl, or a substituted or unsubstituted aryl;

wherein $R_i$ is H, alkyl, or -C(0)R$_7$; R$_2$, R$_4$, R$_5$, and R$_6$ are each independently H, OH, or OAc; R$_3$ is CH$_3$ or CH$_2$OH; R$_7$ is H, a substituted or unsubstituted alkyl, a substituted or unsubstituted alkenyl, or a substituted or unsubstituted aryl; R$_8$ is H, alkyl, or -C(0)R$_9$; and R$_9$ is H, a substituted or unsubstituted alkyl, a substituted or unsubstituted alkenyl, or a substituted or unsubstituted aryl;
for preparation of a medicament for the treatment or prevention of trypanosomatid infection.

39. Use of a plant extract comprising an iridal for preparation of a medicament for the treatment or prevention of a trypanosomatid infection.

40. The use of claim 39, wherein the extract is prepared from a member of the *Iris* genus.

41. A bait composition comprising compound as in any of Formula I, Formula II, Formula III, Formula IV or Formula V:

\[
\text{(I)}
\]

wherein \( R_1 \) is H, alkyl, or \(-\text{C}(\text{O})R_7\); \( R_2, R_4, R_5 \) and \( R_6 \) are each independently H, OH, or OAc; \( R_3 \) is CH\(_3\) or CH\(_2\)OH; and \( R_7 \) is H, a substituted or unsubstituted alkyl, a substituted or unsubstituted alkenyl, or a substituted or unsubstituted aryl;
wherein \( R_i \) is H, alkyl, or -C(0)R; \( R_j \); \( R_k \); \( R\ell \); \( R_4 \); \( R_5 \); and \( R_6 \) are each independently H, OH, or OAc; \( R_3 \) is CH₃ or CH₂OH; \( R_7 \) is H, a substituted or unsubstituted alkyl, a substituted or unsubstituted alkenyl, or a substituted or unsubstituted aryl; \( R_8 \) is H, alkyl, or -C(0)R; \( R_9 \) is H, a substituted or unsubstituted alkyl, a substituted or unsubstituted alkenyl, or a substituted or unsubstituted aryl;

42. The bait composition of claim 41, further comprising at least one component selected from the group consisting of an attractant, a food substance, and a preservative.

43. A compound, composition, or method including one or more of the features described herein.
INTERNATIONAL SEARCH REPORT

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:

2. ❌ Claims Nos.: 43 because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

   The claims do not comply with Rule 6.2(a) because they rely on references to the description and/or drawings. (omnibus claim)

3. ❌ Claims Nos.: 616-34 because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

- see supplemental page-

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 additional fees, this Authority did not invite payment of additional fees.

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. 1-4, 41-42 (in part)

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.
INTERNATIONAL SEARCH REPORT

International application No. PCT/US 16/40112

A. CLASSIFICATION OF SUBJECT MATTER

IPPC(8) - A01N 43/04 (2016.01)
CPC - A61K 31/7004, A61K 31/70, A61K 45/06

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)

IPPC(8) - A01N 43/04 (2016.01)
CPC - A61K 31/7004, A61K 31/70, A61K 45/06

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

USPC - 514/23

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

Patbase, Google Patent, Google Web

Search terms used - Trypanosomiasis trypetereinoid extract leishmaniasis Iridogermanal irid plant root iris

Pubchem substructure search

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>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>WO 97/36604 A1 (UNIVERSITE DE MONTPELLIER &amp; SCIENCES ET TECHNIQUES DU LANGUEDOC) 09 October 1997 (09.10.1997); Figure A</td>
<td>1-2, 4/(1-2)</td>
</tr>
<tr>
<td>Y</td>
<td>&quot;Pubchem CID 44550965&quot; Date Created: 26 January 2010 (26.01.2010) Date Accessed: 17 October 2016 (17.10.2016); Figure A</td>
<td>3, 4/3, 41-42</td>
</tr>
<tr>
<td>Y</td>
<td>US 6,543,181 B1 (Baker et al.) 08 April 2003 (08.04.2003); col 3, in 13-19, col 6, in 56-65</td>
<td>41-42</td>
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Further documents are listed in the continuation of Box C.

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

Date of the actual completion of the international search: 17 October 2016

Date of mailing of the international search report: 07 NOV 2016

Name and mailing address of the ISA/US

Madison PCT, Attn: ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450
Facsimile No. 571-273-8300

Authorized officer: Lee W. Young

PCT Helpdesk: 571-272-4300
PCT OSP: 571-272-7774
This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1.

Group I: Claims 1-4, 41-42 (in part) directed to compounds having general formula II.

Group II: Claim 5, 41-42 (in part) directed to compounds having general formula III, IV, or V.

Group III: Claims 7-15 and 35-40, directed to a method for treating or preventing a trypanosomatid infection in a subject administering to the subject a composition comprising an extract from a plant that is a member of the Iris genus or comprising an iridal and derivatives thereof (including compounds of the general formula I, II, III, IV, or V).

The group of inventions listed above do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Special Technical Features:

Group I includes the technical feature of a compound of formula II, not required by Group II.

Group II includes the technical feature of a compound of formula III, IV, or V, not required by Group I.

Group III includes the technical feature of a method for treating or preventing a trypanosomatid infection in a subject, not required by Groups I and II.

Common technical features:

Groups I, II, and III share the technical feature of a compound which is a triterpenoid iridal derivative wherein an alpha, beta unsaturated aldehyde is reduced to an alcohol, ether or ester (including compounds of formula II, III, IV, or V).

Groups II-III share the technical feature of a compound of formula I.

These shared technical features, however, do not provide a contribution over the prior art, as being obvious over WO 1997/036604 A1 to UNIVERSITE DE MONTPELLIER II SCIENCES ET TECHNIQUES DU LANGUEDOC (hereinafter Montpellier). Montpellier discloses a compound similar to the compound of formula II wherein R1 is H; R2 is OH, R4 is H, R5 is OH and R6 is H; R3 is CH3 (figure A, compound III), but does not disclose a compound wherein C1 is bound to two hydrogens and wherein R8 is H. It would have been obvious to one with skill in the art to prepare the disclosed compound and reduce the aldehyde moiety, giving a compound wherein C1 is bound to two hydrogens and wherein R8 is H, in order to prepare a more stable compound less prone to oxidation/reduction reactions for use in pharmaceutical compositions. Montpellier further discloses a compound of formula I, wherein R1 is H; R2 is OH, R4 is H, R5 is OH and R6 is H; R3 is CH3 (figure A, compound III) and compounds similar to the compounds of formula IV (figure C, compound XIX) and V (Figure D, compound X).

As said compounds were known in the art at the time of the invention, these cannot be considered special technical features that would otherwise unify the inventions of Groups I, II, and III.

The inventions of Group I, II and III, thus lack unity under PCT Rule 13.

Note:
claims 6, 16-34 are determined unsearchable because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a). Claim 43 is unsearchable because it's an omnibus claim.