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PTO/SB/15A (07-14)

CERTIFICATION OF MICRO ENTITY STATUS
(GROSS INCOME BASIS)

Application Number or Control Number (if applicable):	Patent Number (if applicable):
2024-0117	
First Named Inventor:	Title of Invention:
Matthias Magoola	Malaria and diverse diseases treatment using N-Isobutyl-3,4-methylenedioxy-trans-cinnamide compositions.

The applicant hereby certifies the following—

(1) **SMALL ENTITY REQUIREMENT –** The applicant qualifies as a small entity as defined in 37 CFR 1.27.

(2) **APPLICATION FILING LIMIT –** Neither the applicant nor the inventor nor a joint inventor has been named as the inventor or a joint inventor on more than four previously filed U.S. patent applications, excluding provisional applications and international applications under the Patent Cooperation Treaty (PCT) for which the basic national fee under 37 CFR 1.492(a) was not paid, and also excluding patent applications for which the applicant has assigned all ownership rights, or is obligated to assign all ownership rights, as a result of the applicant's previous employment.

(3) **GROSS INCOME LIMIT ON APPLICANTS AND INVENTORS –** Neither the applicant nor the inventor nor a joint inventor, in the calendar year preceding the calendar year in which the applicable fee is being paid, had a gross income, as defined in section 61(a) of the Internal Revenue Code of 1986 (26 U.S.C. 61(a)), exceeding the "Maximum Qualifying Gross Income" reported on the USPTO Web site at http://www.uspto.gov/patents/law/micro_entity.jsp which is equal to three times the median household income for that preceding calendar year, as most recently reported by the Bureau of the Census.

(4) **GROSS INCOME LIMIT ON PARTIES WITH AN "OWNERSHIP INTEREST" –** Neither the applicant nor the inventor nor a joint inventor has assigned, granted, or conveyed, nor is under an obligation by contract or law to assign, grant, or convey, a license or other ownership interest in the application concerned to an entity that, in the calendar year preceding the calendar year in which the applicable fee is being paid, had a gross income, as defined in section 61(a) of the Internal Revenue Code of 1986, exceeding the "Maximum Qualifying Gross Income" reported on the USPTO Web site at http://www.uspto.gov/patents/law/micro_entity.jsp which is equal to three times the median household income for that preceding calendar year, as most recently reported by the Bureau of the Census.

SIGNATURE by an authorized party set forth in 37 CFR 1.33(b)

Signature	/sarfaraz niazi/				
Name	Sarfaraz Niazi				
Date	01/03/2024	Telephone	3122970000	Registration No.	27134

☑ There is more than one inventor and I am one of the inventors who are jointly identified as the applicant. The required additional certification form(s) signed by the other joint inventor(s) are included with this form.

Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. The United States Patent and Trademark Office (USPTO) collects the information in this record under authority of 35 U.S.C. 2. The USPTO's system of records is used to manage all applicant and owner information including name, citizenship, residence, post office address, and other information with respect to inventors and their legal representatives pertaining to the applicant's/owner's activities in connection with the invention for which a patent is sought or has been granted. The applicable Privacy Act System of Records Notice for the information collected in this form is COMMERCE/PAT-TM-7 Patent Application Files, available in the Federal Register at 78 FR 19243 (March 29, 2013). https://www.govinfo.gov/content/pkg/FR-2013-03-29/pdf/2013-07341.pdf

Routine uses of the information in this record may include disclosure to: 1) law enforcement, in the event that the system of records indicates a violation or potential violation of law; 2) a Federal, state, local, or international agency, in response to its request; 3) a contractor of the USPTO having need for the information in order to perform a contract; 4) the Department of Justice for determination of whether the Freedom of Information Act (FOIA) requires disclosure of the record; 5) a Member of Congress submitting a request involving an individual to whom the record pertains, when the individual has requested the Member's assistance with respect to the subject matter of the record; 6) a court, magistrate, or administrative tribunal, in the course of presenting evidence, including disclosures to opposing counsel in the course of settlement negotiations; 7) the Administrator, General Services Administration (GSA), or their designee, during an inspection of records conducted by GSA under authority of 44 U.S.C. 2904 and 2906, in accordance with the GSA regulations and any other relevant (i.e., GSA or Commerce) directive, where such disclosure shall not be used to make determinations about individuals; 8) another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)); 9) the Office of Personnel Management (OPM) for personnel research purposes; and 9) the Office of Management and Budget (OMB) for legislative coordination and clearance.

If you do not furnish the information requested on this form, the USPTO may not be able to process and/or examine your submission, which may result in termination of proceedings, abandonment of the application, and/or expiration of the patent.

Additional Uses

Additional USPTO uses of the information in this record may include disclosure to: 1) the International Bureau of the World Intellectual Property Organization, if the record is related to an international application filed under the Patent Cooperation Treaty; 2) the public i) after publication of the application pursuant to 35 U.S.C. 122(b), ii) after issuance of a patent pursuant to 35 U.S.C. 151, iii) if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspections, or an issued patent, or iv) without publication of the application or patent under the specific circumstances provided for by 37 CFR 1.14(a)(1)(v)-(vii); and/or 3) the National Archives and Records Administration, for inspection of records.

Application Data Sheet 37 CFR 1.76

The Application Data Sheet is part of the provisional or non-provisional application for which it is being submitted. The following form contains the bibliographic data arranged in a format specified by the United States Patent and Trademark Office as outlined in 37 CFR 1.76.

Inventor Information

of inventors: 2

1. **Dr. Matthias Magoola**

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Application Information

Customer number **27134 -**

Correspondence address	---
Title of invention	**Malaria and diverse diseases treatment using N-Isobutyl-3,4-methylenedioxy-trans-cinnamide compositions.**
Attorney docket number	**---**
Entity status	---
Application type	Nonprovisional Application under 35 USC 111(a)
Subject matter	Utility
Total number of drawing sheets	---
Suggested figure for publication	---
Filing by reference	No
Publication request	Normal eighteen-month publication

Representative Information

of representatives: 1

Representative information should be provided for all practitioners having a power of attorney in the application. Providing this information in the Application Data Sheet does not constitute a power of attorney in the application (see 37 CFR 1.32).

Customer number 27134

Domestic Benefit/National Stage Information

of benefit claims: 0

This section allows for the applicant to either claim benefit under 35 U.S.C. 119(e), 120, 121, or 365(c), 386(c), or indicate

National Stage entry from a PCT application. Providing benefit claim information in the Application Data Sheet constitutes the specific reference required by 35 U.S.C. 119(e) or 120, and 37 CFR 1.78(a)(2) or CFR 1.78(a)(4), and need not otherwise be made part of the specification.

Data was not provided for this section.

Foreign Priority Information

of foreign priority claims: 0

This section allows for the applicant to claim benefit of foreign priority and to identify any prior foreign application for which priority is not claimed. Providing this information in the Application Data Sheet constitutes the claim for priority as required by 35 U.S.C. 119 (b) and 37 CFR 1.55. When priority is claimed to a foreign application that is eligible for retrieval under the priority document exchange program (PDX) the information will be used by the Office to automatically attempt retrieval pursuant to 37 CFR 1.55(i)(1) and (2). Under the PDX program, applicant bears the ultimate responsibility for ensuring that a copy of the foreign application is received by the Office from the participating foreign intellectual property office, or a certified copy of the foreign priority application is filed, within the time period specified in 37 CFR 1.55(g)(1).

Data was not provided for this section.

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications

Checking this box will cause the application to be examined under the first inventor to file provisions of the AIA.

☐ This application (1) claims priority to or the benefit of an application filed before March 16, 2013 and (2) also contains, or contained at any time, a claim to a claimed invention that has an effective filing date on or after March 16, 2013. NOTE: By providing this statement under 37 CFR 1.55 or 1.78, this application, with a filing date on or after March 2016, 2013, will be examined under the first inventor to file provisions of the AIA.

Authorization or Opt-Out of Authorization to Permit Access

When this Application Data Sheet is properly signed and filled with the application, applicant has provided written authority to permit a participating foreign intellectual property (IP) office access to the instant application-as-filed (see paragraph A in subsection 1 below) and the European Patent Office (EPO) access to any search results from the instant application (see paragraph B in subsection 1 below).

Should applicant choose not to provide an authorization identified in subsection 1 below, applicant **must opt-out** of the authorization by checking the corresponding box A or B or both in subsection 2 below.

NOTE:
This section of the Application Data Sheet is **ONLY** reviewed and processed with the **INITIAL** filing of an application. After the initial filing of an application, an Application Data Sheet cannot be used to provide or rescind authorization for access by a foreign IP office(s). Instead, Form PTO/SB/39 or PTO/SB/69 must be used as appropriate.

1. Authorization to Permit Access by a Foreign Intellectual Property Office(s)

A. **Priority Document Exchange (PDX)**
Unless box A in subsection 2 (opt-out of authorization) is checked, the undersigned hereby **grants the USPTO authority** to provide the European Patent Office (EPO), the Japan Patent Office (JPO), the Korean Intellectual Property Office (KIPO), the State Intellectual Property Office of the People's Republic of China (SIPO), the World Intellectual Property Organization (WIPO), and any other foreign intellectual property office participating with the USPTO in a bilateral or multilateral priority document exchange agreement in which a foreign application claiming priority to the instant patent application is filed, access to: (1) the instant patent application-as-filed and its related bibliographic data, (2) any foreign or domestic application to which priority or benefit is claimed by the instant application and its related bibliographic data, and (3) the date of filing of this Authorization. See 37 CFR 1.14(h) (1).

B. **Search Results from U.S. Application to EPO**
Unless box B in subsection 2 (opt-out of authorization) is checked, the undersigned hereby **grants the USPTO authority** to provide the EPO access to the bibliographic data and search results from the instant patent application when a European patent application claiming priority to the instant patent application is filed. See 37 CFR 1.14(h)(2).

The applicant is reminded that the EPO's Rule 141(1) EPC (European Patent Convention) requires applicants to submit a copy of search results from the instant application without delay in a European patent application that claims priority to the instant application.

2. Opt-Out of Authorizations to Permit Access by a Foreign Intellectual Property Office(s)

☐ A. Applicant **DOES NOT** authorize the USPTO to permit a participating foreign IP office access to the instant application-as-filed. If this box is checked, the USPTO will not be providing a participating foreign IP office with any documents and information identified in subsection 1A above.

☐ B. Applicant **DOES NOT** authorize the USPTO to transmit to the EPO any search results from the instant patent application. If this box is checked, the USPTO will not be providing the EPO with the search results from the instant application.

NOTE:
Once the application has published or is otherwise publicly available, the USPTO may provide access to the application in accordance with 37 CFR 1.14.

Applicant Information

of applicants: 0

The information to be provided in this section is the name and address of the legal representative who is the applicant under 37 CFR 1.43; or the name and address of the assignee, person to whom the inventor is under an obligation to assign the

invention, or person who otherwise shows sufficient proprietary interest in the matter who is the applicant under 37 CFR 1.46.

Data was not provided for this section.

Assignee Information including Non-Applicant Assignee Information

of assignees: 1

An assignee-applicant identified in the "Applicant" section will appear on the patent application as an applicant.

Providing assignment information in this section does not substitute for compliance with any requirement of part 3 of Title 37 of CFR to have an assignment recorded by the Office.

RNA Therapeutics, Inc.
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3122970000

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Organization

Signature

NOTE:

This Application Data Sheet must be signed in accordance with 37 CFR 1.33(b). **However, if this Application Data Sheet is submitted with the INITIAL filing of the application and either box A or B is not checked in subsection 2 of the "Authorization or Opt-Out of Authorization to Permit Access" section, then this form must also be signed in accordance with 37 CFR 1.14(c)**

This Application Data Sheet **must** be signed by a patent practitioner if one or more of the applicants is a **juristic entity** (e.g., corporation or association). If the applicant is two or more joint inventors, this form must be signed by a patent practitioner, **all** joint inventors who are the applicant, or one or more joint inventor-applicants who have been given power of attorney (e.g., see USPTO Form PTO/AIA/81) on behalf of **all** joint inventor-applicants.

See CFR 1.4(d) for the manner of making signatures and certifications.

Signature	First name	Last name	Registration #	Date
/sarfaraz niazi/	Sarfaraz	Niazi	52067	01/25/2024

TITLE

Malaria and diverse diseases treatment using N-Isobutyl-3,4-methylenedioxy-trans-cinnamide compositions.

ABSTRACT

[0001] N-Isobutyl-3,4-methylenedioxy-trans-cinnamide with its unique chemical structure to affect many functional pathways is presented as an effective treatment of malaria, neurological disorders, androgenic disorders, rheumatoid arthritis, inflammatory bowel disease, vascular inflammation, hypertension, diabetes, obesity, fungal infections, bacterial infections, and cancer in adults and children; suitable pharmaceutical formulations are also disclosed for effective dosing.

SPECIFICATION

BACKGROUND OF THE INVENTION

[0003] Malaria, a life-threatening disease transmitted through the bite of an infected Anopheles mosquito, remains a significant global health challenge, especially in tropical and subtropical regions. The World Health Organization (WHO) reports that malaria causes hundreds of thousands of deaths annually, predominantly affecting children under five in Africa. This geographical distribution is closely linked to climate and socio-economic conditions, with the highest burden in poorer areas where access to effective health care and mosquito control measures are limited. While treatments such as artemisinin-based combination therapies (ACTs) are available, their affordability and accessibility vary greatly. In many malaria-endemic countries, the cost of treatment can be prohibitive for the average person, leading to delayed or inadequate treatment and contributing to the high mortality rate. Despite ongoing research, there is no fully effective vaccine against malaria. The RTS, S/AS01 (Mosquirix) vaccine, the first to receive WHO recommendation for broad use, offers only partial protection, highlighting the ongoing challenge in developing a reliable vaccine against this complex parasite. This situation underscores the importance of sustained global malaria prevention, control, and research efforts.

[0004] Protozoan parasites, notably Plasmodium falciparum, cause malaria. The range of drugs available on the market for the prevention and treatment of malaria is limited, and there are problems with resistance. This invention reports a novel formulation of N-Isobutyl-3,4-methylenedioxy-trans-cinnamide capable of eradicating infections from Plasmodium species at any stage of infection, to any age of the patient and with the

convenience of multiple routes of administration and dosage forms. This invention can resolve the global crisis of malaria. This treatment could be used as a worldwide solution to one of the most dreaded diseases, particularly in low-income countries, where the affordability of drugs remains an issue. The present invention can be readily synthesized and made available globally at an affordable present invention that provides a pharmaceutical composition containing N-Isobutyl-3,4-methylenedioxy-trans-cinnamide and inert pharmaceutical ingredients for administration through multiple routes and dosage forms. The pharmaceutical composition is preferably formulated as a tablet or liquid for oral administration. Suitable tablet forms include tablets for adult dosing, children's tablets, and chewable tablets. Suitable liquid formulations include oil-in-water emulsions. Other suitable dosage forms include suppositories and injections.

Antimalarial Compounds

[0005] Malaria treatment involves several approved drugs, each with its specific chemical name. Some of the commonly used drugs for malaria treatment include:

[0006] **Chloroquine Phosphate**: 7-Chloro-4-{4-(diethylamino)-1-methylbutylamino} quinoline diphosphate. First synthesized in 1934, chloroquine was used extensively during World War II and was approved for medical use in the United States in 1949.

[0007] **Hydroxychloroquine Sulfate**: 2-{4-[(7-Chloro-4-quinolyl) amino] pentyl} ethyl aminoethanol sulfate; Hydroxychloroquine, a derivative of chloroquine, was first approved for medical use in the United States in 1955.

[0008] **Artemether-Lumefantrine**: Artemether was developed in the early 1980s. The combination of artemether and lumefantrine was approved for use in Switzerland in 1999 and subsequently in other countries.

[0009] **Atovaquone-Proguanil**: Atovaquone: 2-[trans-4-(4-Chlorophenyl) cyclohexyl]-3-hydroxy-1,4-naphthalenedione; Proguanil: 1-(4-Chlorophenyl)-5-isopropylbiguanide. This combination was approved for use in the United States in 2000.

[0010] **Mefloquine Hydrochloride**: (R*, S*)-(±)-2-Piperidinyl-2,8-bis(trifluoromethyl)-4-quinolinemethanol hydrochloride. It was developed by the U.S. Army in the 1970s and approved for malaria prevention in the United States in 1989.

[0011] **Doxycycline**: 4-(Dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide. Initially synthesized in 1960, doxycycline was approved by the FDA in 1967. Its use for malaria prophylaxis is off-label but widely accepted.

[0012] **Primaquine Phosphate**: (RS)-N-(6-Methoxyquinolin-8-yl) pentane-1,4-diamine diphosphate. It was developed during World War II and was approved for use in the United States in 1952.

[0013] **Quinine Sulfate**. Quinine, in various forms, has been used for treating malaria since the early 17th century. However, in terms of modern regulatory approval, quinine sulfate was approved by the FDA for treating malaria in 2005. It's worth noting that quinine itself has been used in various formulations and under different regulatory conditions long before this formal approval.

[0014] **Artemether-Lumefantrine**: Artemether: 3,12-Epoxy-12H-pyrano[4,3-j]-1,2-benzodioxepin-10(3H)-one, decahydro-10-methoxy-3,6,9-trimethyl-, (3R,5aS,6R,8aS,9R,12S,12aR)-; Lumefantrine: 2-(Dibutyl amino)-1-[(9Z)-2,7-dichloro-9-[(4-chlorophenyl) methylene]-9H-fluoren-4-yl] ethanol. The combination of artemether and lumefantrine, known under Coartem, was approved for use in the United States by

the FDA in 2009. However, it was approved and used in other countries before this; for instance, it was approved in Switzerland in 1999.

DETAILS OF THE INVENTION

[0015] N-Isobutyl-3,4-methylenedioxy-trans-cinnamide has a unique and complex chemical structure. At the heart of its molecular architecture lies the cinnamamide backbone, essentially a conjugated system combining elements of cinnamic acid and an amide group. This backbone is pivotal in defining the compound's chemical behavior and reactivity. Attached to this backbone is a 3,4-dimethoxyphenyl group, a phenyl ring structure with two methoxy groups (-OCH3) located at the third and fourth carbon atoms. This arrangement of methoxy groups contributes to the molecule's distinct chemical characteristics.

[0016] Additionally, N-Isobutyl-3,4-methylenedioxy-trans-cinnamide features an isobutyl group linked to the nitrogen atom of the amide group. This isobutyl moiety further influences the compound's physical and chemical properties. N-Isobutyl-3,4-methylenedioxy-trans-cinnamide typically presents as a solid substance at room temperature, and the precise configuration and interactions of these functional groups determine its solubility, boiling point, and melting point.

[0017] No approved or anticipated clinical applications of N-Isobutyl-3,4-methylenedioxy-trans-cinnamide are reported; no patents are issued on its utility in treating specific diseases. However, we discovered that its molecular design, characterized by the cinnamamide backbone and the substituted phenyl ring, can play a crucial role in its interaction with biological systems, as we proved by testing it for antimalarial activity.

[0018] N-Isobutyl-3,4-methylenedioxy-trans-cinnamide's role in malaria research is particularly intriguing due to its unique chemical structure, which offers the possibility of interfering with the life cycle of the Plasmodium parasite. Studies have focused on understanding how N-Isobutyl-3,4-methylenedioxy-trans-cinnamide can affect Plasmodium at various stages of its development within the human host and the mosquito vector. This includes investigating its potential effects on parasite entry into red blood cells, replication within the host, and the transmission stages of the parasite.

[0019] One of the key aspects of N-Isobutyl-3,4-methylenedioxy-trans-cinnamide's potential as an antimalarial agent is its ability to target specific pathways or enzymes crucial for the malaria parasite's survival and proliferation. By inhibiting these pathways, N-Isobutyl-3,4-methylenedioxy-trans-cinnamide could reduce the parasite's ability to multiply and spread within the host. This approach not only helps in clearing the infection but also has the potential to reduce the transmission of the disease.

[0020] In summary, while N-Isobutyl-3,4-methylenedioxy-trans-cinnamide shows promise as a potential antimalarial agent. Its journey from laboratory to clinical application will involve a series of steps to ensure that it is effective and safe for human use.

[0021] N-Isobutyl-3,4-methylenedioxy-trans-cinnamide, with its distinctive molecular structure, can play a pivotal role in controlling many other diseases, particularly neurological disorders, since it can cross the blood-brain barrier. It is also proposed to modulate androgenic disorders, reduce rheumatoid arthritis and inflammatory bowel disease, vascular inflammation, or hypertension. Moreover, its potential impact on

metabolic pathways presents a possibility for its use in managing metabolic disorders, including diabetes, obesity, and cancer.

[0022] N-Isobutyl-3,4-methylenedioxy-trans-cinnamide has an XLogP3 value of 3.1, generally considered within the range suitable for oral drug absorption. The relationship between lipophilicity (as measured by XLogP3) and oral bioavailability is often described by Lipinski's Rule of Five, a set of guidelines used in drug discovery to predict whether a chemical compound has properties that would make it a likely orally active drug in humans. According to Lipinski's Rule of Five, one of the criteria is that the compound should have a log P (a similar measure to XLogP3) of not more than 5. Compounds with log P values higher than 5 tend to be too lipophilic, leading to poor water solubility, absorption, and bioavailability. In this context, an XLogP3 value of 3.1 suggests that the compound is likely lipophilic enough to pass through biological membranes via passive diffusion, which is a primary mechanism for drug absorption in the gastrointestinal tract, but not so lipophilic that it would be poorly soluble in aqueous environments like the gastrointestinal fluids.

[0023] The anti-plasmodial activity as a measure of antimalarial efficacy was tested using standard in vitro anti-plasmodial assays, SYBR Green I, and HRP2 ELISA assays. In this study, the Plasmodium falciparum strains 3D7 (known to be CQ sensitive) and Dd2 (known to be CQ resistant) were used to screen for antimalarial activity. First, stock concentrations (10 mg/ml) were prepared in DMSO, centrifuged to remove insoluble materials, and stored at -80°C until used in cell culture. Before incubation with Plasmodium, DMSO stocks were diluted (as indicated in each assay) with complete RPMI media (RPMI 1640 medium supplemented with 25 mM HEPES, 0.2% $NaHCO_3$,

0.1 mM hypoxanthine, 100 µg/ml gentamicin, and 0.5% Albumax I [Invitrogen]). These working stocks were serially diluted 2-fold or 3-fold in a 96-well assay plate format to obtain 8-10 serial dilutions. In each assay, the final concentration of DMSO in complete media was 0.4% in a final volume of 50 ul of entire media. Drug-free wells, uninfected RBC, and blank wells were used as controls for each assay plate. Dihydroartemisinin (DHA) and CQ were also serially diluted and incubated with parasites for positive control drugs on each assay. Cultures were synchronized by double sorbitol treatment and the staging and parasitemia. The parasitized whole blood samples were washed 3 times with RPMI (w/o Albumax) media at 37°C and then resuspended in fresh RPMI media to a final hematocrit of 2%. About 150 µl of the parasite culture was added to each well into the assay plate for final parameters of 0.2% parasitemia and 2% hematocrit. Plates were incubated for 48 h or 72 hours, as indicated in each experiment, in a humidified incubator under a blood gas mixture (97% N2, 3% CO_2, 2% O_2) at 37°C. After incubation for the required time, the plates were removed and frozen. Thawed plates were processed with Sybr Green lysis buffer for one hour in the dark. The Fluorescence Intensity (FI) as a measure of parasite growth was determined using a BMG Fluostar Optima plate reader at ex 485/em 530 nm. The Fluorescence Intensity (F.I) data was plotted using GraphPad Prism 7. The IC50 values for each sample were obtained by curve fitting the data with a variable slope function. For each assay plate, a Z' factor to assess assay quality was calculated from positive controls (drug-free wells) and negative controls (uninfected red cell control wells). The Z' values over 0.5 were considered suitable, while assays with Z' below 0.5 were discarded.

[0024] In other experiments, quantification of total parasite growth was studied using an enzyme-linked immunosorbent assay (ELISA) that quantitates the parasite histidine-rich protein-2 (HRP-2). Briefly, ELISA plates were pre-coated with mouse anti-HRP-2 primary/capture antibody and stored at 4-8°C overnight. The next day, stored culture samples were diluted [1:5] in ultrapure water, hemolyzed, and 100 µL of each hemolyzed sample was added to the pre-coated ELISA plates. The sample-antibody mixture in ELISA plates was incubated at room temperature for 1 hr. Unbound or excess protein lysates were washed off three times using a wash buffer. In the next step, 100 µL of [horseradish peroxidase-conjugated] anti-mouse IgG secondary antibody was added to each well and incubated for one hour at room temperature. This was followed by four washing cycles, and 100 µL of 3,3′,5,5′-tetramethylbenzidine single-solution chromogen was added in the dark for 10 min. 50 µL of 1 M sulfuric acid was added to stop the reactions. The absorbance of each well was measured using a spectrophotometer, and the optical density [OD] values were used to measure parasite growth. The wells without test agents were considered 100% parasite growth and used to calculate the percent growth in other wells.

[0025] To calculate a therapeutic dose, we tested in vitro antimalarial potency and found that N-Isobutyl-3,4-methylenedioxy-trans-cinnamide meets the highly effective category with a 0.1 to 1 mcg/mL concentration. Given the possible bioavailability constraints, we calculated the high dose of 25 mg for a 65kg adult twice a day. Generally, based on the observed log2 value of concentration, giving almost 100% inhibition, a dose ranging from 0.1 mg to 25 mg will be sufficient. However, taking into consideration bioavailability, and individual variability, we are claiming a dose of 0.1-100 mg per dose,

and the dose can be repeated two or three times per day. Based on this calculation, the dosage formulations were created as shown below:

[0026] Formulation 1: N-Isobutyl-3,4-methylenedioxy-trans-cinnamide Tablet

Bill of Materials			
Scale (mg/tablet)	Item	Material Name	Quantity/1000 Tablets (g)
25.00	1	N-Isobutyl-3,4-methylenedioxy-trans-cinnamide (crystalline)	27.50
90.00	3	Avicel^{R} PH101	90
10.00	4	Kollidon® 30	10
20.00	5	Kollidon® CL	20
10.00	6	Polyethylene glycol (PEG-6000) (powder)	10

[0027] Manufacturing Directions: Mix all components, pass through a 0.8-mm sieve, and press with high compression force. Compress into 247.50-mg tablets using 10-mm biplanar punches. If the flowability of the powder mixture for tableting is not high enough, some Aerosil 200 should be added.

[0028] Formulation 2: N-Isobutyl-3,4-methylenedioxy-trans-cinnamide Chewable Tablets

Bill of Materials

Scale (mg/tablet)	Item	Material Name	Quantity/1000 Tablets (g)
25.00	1	N-Isobutyl-3,4-methylenedioxy-trans-cinnamide, milled	25.00
600.00	2	Sucrose, milled	600.00
550.00	3	Kollidon® CL-M	550.00
30.00	4	Orange flavor (FDO)	30.00
30.00	5	Strawberry flavor (FDO)	30.00
60.00	6	Kollidon® 30	60.00
QS	7	Ethanol (96%)	~425.00

[0029] Manufacturing Directions: Granulate a mixture of items 1 to 5 with a solution of items 6 and 7, pass through a sieve, and press with medium compression force. The average weight of the tablet is 1,295 mg, obtained using a 20mm biplanar punch. Taste is sweet, fruity, and only very slightly bitter.

[0030] Formulation 3: N-Isobutyl-3,4-methylenedioxy-trans-cinnamide Effervescent Tablets

Bill of Materials			
Scale (mg/tablet)	Item	Material Name	Quantity/1000 Tablets (g)

25.00	1	N-Isobutyl-3,4-methylenedioxy-trans-cinnamide (powder < 300 μm)	25.00
500.00	2	Sodium bicarbonate	500.00
430.00	3	Tartaric acid (powder)	430.00
200.00	4	Dextrose	200.00
QS	5	Flavoring	QS
20.00	6	Kollidon® 30	20.00
—	7	Isopropanol	100.00 mL
60.00	8	PEG-6000 (powder)	60.00

[0031] Manufacturing Directions: Granulate the mixture of items 1 to 5 with a solution of items 6 and 7. Pass through an 0.8-mm sieve, add item 8, and then mix. Press to tablets (average weight, 1,225 mg; 16-mm-diameter biplanar tablet).

[0032] Formulation 4: N-Isobutyl-3,4-methylenedioxy-trans-cinnamide Tablets

Bill of Materials			
Scale (mg/tablet)	Item	Material Name	Quantity/1000 Tablets (g)
25.00	1	N-Isobutyl-3,4-methylenedioxy-trans-cinnamide (fine powder)	25.00
44.15	2	Maize starch	44.15
0.84	3	Potassium sorbate	0.84

18.00	4	Povidone (PVP K-30)	18.00
4.00	5	Aerosil^{R} 200	4.00
12.00	6	Gelatin (powder)	12.00
4.00	7	Glycerol	4.00
30.00	8	Cellulose (powder)	30.00
12.00	9	Primojel^{R}	12.00
8.00	10	Stearic acid (fine powder)	8.00
2.00	11	Magnesium stearate	2.00
5.00	12	Talc (fine powder)	5.00
QS	13	Purified water	QS

[0033] Manufacturing Directions: Binder solution: Prepare in several batches. Add items 3 to 5 with about 50% water, dissolve item 1 in water, add item 4, and dissolve at medium speed. Avoid foaming; Add item 5 and mix for 3 minutes. Dissolve item 6 in 70◦C to 80◦C purified water and mix until clear. Avoid foaming; Add item 7 and mix gently; add to mixture from previous step; Mix items 1 and 2 for 5 minutes; Add binding solution and mix at slow speed until granules form; add extra water if necessary; Dry in a fluid-bed dryer at 55◦C for 30 minutes; after 15 minutes, scrape granules to break up lumps to promote uniform drying. Dry to 1% to 1.5% LOD; Grind through a 3.0-mm sieve and then through a 1.0-mm sieve; load into a double-cone blender; Pass cellulose powder, Primojel, and stearic acid through a 500-µm sieve; bag-mix magnesium stearate and fine talc powder, and pass through a 250-µm sieve; then add portion of granules from the bulk

to the bag and mix for 1 minute; Add both of these parts to the granules; Compress into 17.6 × 7.2-mm caplet punches of 10 to 14-kPa hardness and 5.8 to 6.0-mm thickness.

[0034] Formulation 5: N-Isobutyl-3,4-methylenedioxy-trans-cinnamide Tablets

Bill of Materials			
Scale (mg/tablet)	Item	Material Name	Quantity/1000 Tablets (g)
25.00	1	N-Isobutyl-3,4-methylenedioxy-trans-cinnamide (crystalline)	25.00
137.00	2	Avicel™ PH102	137.00
35.00	3	Kollidon® VA 64	35.00
21.00	4	Kollidon® CL	21.00
3.00	5	Magnesium stearate	3.00
4.00	6	Aerosil® 200	4.00

[0035] Manufacturing Directions: Pass the lubricant through a 200-mm sieve and mix all other components; Pass through a 0.8-mm sieve, add the lubricant, and press with a high-compression force of 25 to 30 kN; Fill 224 mg.

[0036] Formulation 6: N-Isobutyl-3,4-methylenedioxy-trans-cinnamide Tablets

Bill of Materials			
Scale (g/tablet)	Item	Material Name	Quantity/1000 Tablets (g)

25	1	N-Isobutyl-3,4-methylenedioxy-trans-cinnamide (crystalline or powder)	25
150	2	Avicel™ PH102	150
20	3	Kollidon® VA 64	20
15	4	Kollidon® CL	15
15	5	PEG-6000 (powder)	15
2	6	Aerosil® 200	2

[0037] Manufacturing Directions: Pass the lubricant through a 200-µm sieve and mix all other components. Pass the mix through a 0.8-mm sieve, add the lubricant, and press with a high-compression force of 25 to 30 kN; the Weight should be 228 mg.

[0038] Formulation 7: N-Isobutyl-3,4-methylenedioxy-trans-cinnamide Tablets

Bill of Materials			
Scale (mg/tablet)	Item	Material Name	Quantity/1000 Tablets (g)
25.00	1	N-Isobutyl-3,4-methylenedioxy-trans-cinnamide (powder)	25.00
30.00	2	Dicalcium phosphate	30.00
12.00	3	Kollidon® CL	12.00
20.00	4	Kollidon® VA 64	20.00
10.00	5	Kollidon® 90F	10.00

—	6	Ethanol (96%)	70 mL (max.)
12.00	7	Kollidon® CL	12.00
10.00	8	Polyethylene glycol (powder)	10.00

[0039] Manufacturing Directions: Granulate mixture of items 1 to 4 with the solution of items 5 and 6; Dry, sieve, and mix with items 7 and 8; Press with high-compression force of 25 to 30 kN; Tablet weight is 112 mg for an 11-mm biconvex tablet.

[0040] Formulation 8: N-Isobutyl-3,4-methylenedioxy-trans-cinnamide Tablets, Chewable

Bill of Materials			
Scale (mg/tablet)	Item	Material Name	Quantity/1000 Tablets (g)
25.00	1	N-Isobutyl-3,4-methylenedioxy-trans-cinnamide, use N-Isobutyl-3,4-methylenedioxy-trans-cinnamide particles coated with cellulose acetate and PVP	25.00
246.00	2	Mannitol granular	246.00
30.00	3	Microcrystalline cellulose	30.00
9.00	4	Aspartame	9.00
1.27	5	Dyes	1.27
2.10	6	Citric acid	2.10
2.30	7	Flavor	2.30
4.40	8	Magnesium stearate	4.40

[0041] Manufacturing Directions: N-Isobutyl-3,4-methylenedioxy-trans-cinnamide is coated with a layer of a taste-masking composition with a thickness of about 3 to 10 μm. The coating should be substantially free of cracks, holes, and other imperfections when examined under a scanning electron microscope at 100 to 500× magnification. Charge items 1 to 7 in a suitable blender and mix for 20 minutes; Add item 8 to step 2 and blend for 2 minutes; Compress the appropriate quantity.

[0042] Formulation 9: N-Isobutyl-3,4-methylenedioxy-trans-cinnamide Tablets for Children

Bill of Materials			
Scale (mg/tablet)	Item	Material Name	Quantity/1000 Tablets (g)
10.00	1	N-Isobutyl-3,4-methylenedioxy-trans-cinnamide	10.00
168.00	2	Avicel™ PH101	168.00
13.00	3	Kollidon® VA 64	13.00
6.00	4	Kollidon® CL	6.00
2.00	5	Magnesium stearate	2.00

[0043] Manufacturing Directions: Pass all components through a 0.8-mm sieve, mix, and press with medium-compression force. The tablet weight is 201 mg for a 12-mm biplanar tablet.

[0044] Formulation 10: N-Isobutyl-3,4-methylenedioxy-trans-cinnamide Suppositories

Bill of Materials			
Scale (mg/suppository)	Item	Material Name	Quantity/1000 Suppositories (g)
25.00	1	N-Isobutyl-3,4-methylenedioxy-trans-cinnamide micronized, 5%	25.25
1137.50	2	Suppocire AM	1137.50

[0045] MANUFACTURING DIRECTIONS: Load item 2 in the fat-melting vessel and heat to 60 °C; Transfer step 1 to a Becomix vessel through filter sieves; set the temperature to 60 °C; Cool down to 50°C–55 °C and apply vacuum 0.4–0.6 bar. Load item 1 mix at 10 rpm and homogenize at speed I for 10 minutes, maintaining the temperature of 50°–55 °C under vacuum as above to make a smooth slurry. Transfer into storage vessel and set temperature at 45-° C. Fill 1390 mg in a suppository mold.

CLAIMS

1. A composition to treat parasitic, bacterial, fungal, and viral infections, neurological disorders, androgenic disorders, rheumatoid arthritis, inflammatory bowel disease, vascular inflammation, hypertension, diabetes, obesity, and cancer by administering to humans in need of the treatment an effective dose of N-Isobutyl-3,4-methylenedioxy-trans-cinnamide in a suitable pharmaceutical formulation.
2. The composition of claim 1, wherein the composition is used to treat infections caused by parasitic Plasmodium species.
3. The composition of claim 2, wherein the species is falciparum.
4. The composition of claim 1, wherein the effective dose ranges between 0.1 and 100 mg in any division.
5. The composition of claim 4, wherein the composition is administered one to four times per day.
6. The composition of claim 1, wherein the suitable pharmaceutical formulation comprises a tablet, capsule, solution, chewable tablet, injection, rectal suppository, vaginal suppository, or a combination thereof.
7. The composition of claim 2, wherein the composition further contains 7-Chloro-4-{4-(diethylamino)-1-methylbutylamino} quinoline diphosphate, 2-{4-[(7-Chloro-4-quinolyl) amino] pentyl} ethyl aminoethanol sulfate, 2-[trans-4-(4-Chlorophenyl) cyclohexyl]-3-hydroxy-1,4-naphthalenedione; 1-(4-Chlorophenyl)-5-isopropylbiguanide, (R*, S*)-(±)-2-Piperidinyl-2,8-bis(trifluoromethyl)-4-quinolinemethanol hydrochloride, 4-(Dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide, (RS)-N-(6-Methoxyquinolin-8-yl)

pentane-1,4-diamine diphosphate, quinine sulfate**,** 3,12-Epoxy-12H-pyrano[4,3-j]-1,2-benzodioxepin-10(3H)-one, decahydro-10-methoxy-3,6,9-trimethyl-, (3R,5aS,6R,8aS,9R,12S,12aR), and 2-(Dibutyl amino)-1-[(9Z)-2,7-dichloro-9-[(4-chlorophenyl) methylene]-9H-fluoren-4-yl] ethanol, or a combination thereof.

8. The composition of claim 1, wherein the formulation comprises Formulation 1, Formulation 2, Formulation 3, Formulation 4, Formulation 5, Formulation 6, Formulation 7, Formulation 8, or Formulation 9.