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SPECIFICATION (CONFIDENTIAL)

1. TITLE OF INVENTION

Fluorescent-Labeled Artemisinin Derivatives as Bioimaging Probes

2. DESCRIPTION OF THE INVENTION

2.1 Field Of The Invention:

The present invention relates to the development of novel, fluorescent-tagged, artemisinin-based derivatives as bioimaging research tools for studying drug-cell interactions and tracking the dynamics of artemisinin derivatives within (or on) a live cell. With fluorescent marking, these analogues provide useful insight into the unresolved mode of action of the parent molecules.

Keywords: fluorescent-labeling, artesunate, bioimaging, live-cell imaging

2.2 Background Of The Invention:

Refer to PCT/SG2010/000240 "SYNTHESIS AND USE OF FLUOROPHORE-TAGGED ANTIMALARIALS" for the background of invention.

Malaria is the fifth most devastating infectious disease in the world, following respiratory infections, HIV/AIDS, diarrheal diseases, and tuberculosis (World Health Organisation (WHO, 2010)). According to the latest WHO estimates, there were about 219 million cases of malaria in 2010 and estimated 660,000 death cases with Africa being the most afflicted region (WHO, 2012). Malaria is caused by Apicomplexan pathogens of the genus *Plasmodium*, with *Plasmodium falciparum* being the most deadly species. Chloroquine was the key antimalarial used for hundreds of millions of treatments annually, judging by its efficacy, safety, and low cost of treatment (Greenwood *et al.*, 2005). However, since the 1960s, drug resistance worldwide has alarmed the public community to search for alternatives to chloroquine. In the early 1970s, artemisinin came to light as an effective therapeutic agent against malaria in the form of artemisinin combination therapy (ACT) (Miller *et al.*, 2013). WHO has recommended ACT to be the first-line therapy against *Plasmodium falciparum*.

Artemisinin is a naturally occurring antimalarial isolated from the Chinese remedy Qinghaosu (*Artemisia annua*), a medication for fever for over two millennia (Qinghaosu Antimalaria Coordinating Research Group, 1979). It is a sesquiterpene lactone with a 1,2,4-trioxane ring system, with no significant side effects and clinical resistance, although tolerance has been reported (Crespo-Ortiz and Wei, 2011). Despite its potency, it has low solubility in oil or water, poor bioavailability and a short half-life *in vivo* (Li *et al.*, 2007). Henceforth, artemisinin derivatives have been developed to improve the clinical efficacy and tolerability of the artemisinin family.

Artelinic acid and artesunate are semi-synthetic derivatives of artemisinin, which are prepared from dihydroartemisinin (DHA), the main active metabolite of artemisinin. Both artelinic acid and artesunate contain the endoperoxide moiety that has been shown to be responsible for killing malarial parasites. Scientific studies suggest that this endoperoxide bridge is cleaved and activated by ferrous heme or free ferrous iron (Meshnick *et al.*, 1991). Free carbon centered radicals are generated and interrupt cellular metabolism of parasites, through damage on the integrity of cellular lipids and digestive vacuole membranes; inactivation of *Plasmodium* proteins, alkylation of heme; and interference with heme sequestration into crystals (Miller *et al.*, 2013).

Interestingly, the artemisinins have also displayed a broad variety of potential applications as anti-cancer, anti-viral, anti-bacterial or anti-fungal agents. Evidence highlight the potential of artemisinin analogues to deliver a more cost-efficient therapy against highly metastatic and aggressive cancers (Xu *et al.*, 2011) as well as fighting the effect of commonly-developing drug resistance (Wang *et al.*, 2010). Besides, there is supporting data on artemisinin inducing apoptosis (Singh and Lai, 2004),

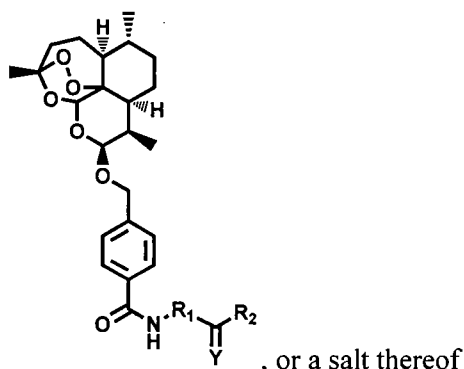
countering proliferative capacity of cancer (Woerdenbag *et al.*, 1993) and inhibiting angiogenesis (Crespo-Ortiz and Wei, 2011). Moreover, artemisinin was proved to be an effective drug against schistosomiasis, which is the second most prevalent parasitic infection after malaria (Xiao, 2005).

Despite its wide use as therapeutic drug against malaria, little has been developed on the mechanism of action of artemisinin. Although several models have been proposed by researchers, their actual targets remain ambiguous. Recent reports on the emergence of artemisinin-resistant malaria isolates have alarmed the scientific community since artemisinin serves as the front-line therapy currently. Henceforth understanding of the underlying molecular actions of artemisinin is urgently needed to enhance the development of targeted artemisinin derivatives as potential therapy. Moreover, it is essential to understand production of reactive oxygen species (ROS) upon triggering from artemisinin to design future cytotoxic agents. With that, growing research has been focusing on the mechanism of bioactivation and subsequent molecular events following artemisinin effects. Therefore, a strategy is needed to elucidate its actions.

2.3 Summary Of The Invention:

Our invention describes the synthesis of a series of new fluorescent-labeled artemisinin analogues, designed to be used for imaging of artemisinin and its metabolite (dihydroartemisinin) within a live cell. Artemisinin is widely known for its variety of biological activities, such as anti-malarial, anti-tumorigenic, anti-angiogenic and even anti-viral activities. *In vivo*, artemisinin is reduced to its active metabolite dihydroartemisinin (DHA) and medicinal chemists increase the solubility of this metabolite by synthesizing the analogues artelinic acid and artesunate.

One embodiment of the invention is a fluorescent artemisinin-analogue, represented by the following structural formula:



Wherein

R_1 is $-(CH_2)_n-NH-$, $NH-(CH_2)_n-NH-$, $-(CH_2)_n-NH-(C_2H_4X)_m-NH-$, $-(CH_2)_n-A-$ or $-(CH_2)_n-NH-(CH_2)_{x1}-B-(CH_2)_{x2}-NH-$ wherein n is an integer of from 1 to 10, m is an integer of from 1 to 100, and $x1$ and $x2$ may be the same or different and are an integer of from 0 to 10;

X is CH_2 , O or a single bond, with the proviso that when m is 2 or more, X s may be the same or different;

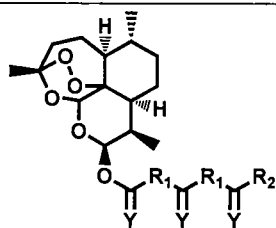
A is heterocycloalkylene of C2-C20 or heteroarylene of C2-C20, containing at least one heteroatom selected from among nitrogen, oxygen and sulfur therein;

B is cycloalkylene of C3-C20 or arylene of C6-C20;

R_2 is a fluorescent dye or a low-molecular weight organic compound capable of selectively binding to a fluorescent dye-labeled substance; and

Y is O , S or CH_2 .

Another embodiment of the invention is a fluorescent artemisinin-analogue, represented by the following structural formula:



, or a salt thereof.

Wherein

R_1 is $-(CH_2)_n-NH-$, $NH-(CH_2)_n-NH-$, $-(CH_2)_n-NH-(C_2H_4X)_m-NH-$, $-(CH_2)_n-A-$ or $-(CH_2)_n-NH-(CH_2)_{x1}-B-(CH_2)_{x2}-NH-$ wherein n is an integer of from 1 to 10, m is an integer of from 1 to 100, and x_1 and x_2 may be the same or different and are an integer of from 0 to 10;

X is CH_2 , O or a single bond, with the proviso that when m is 2 or more, X_s may be the same or different;

A is heterocycloalkylene of C2-C20 or heteroarylene of C2-C20, containing at least one heteroatom selected from among nitrogen, oxygen and sulfur therein;

B is cycloalkylene of C3-C20 or arylene of C6-C20;

R_2 is a fluorescent dye or a low-molecular weight organic compound capable of selectively binding to a fluorescent dye-labeled substance; and

Y is O , S or CH_2 .

Currently no technology has been developed to allow scientists to track the actions of artemisinin-like derivatives directly in biological systems. The fluorescent compounds described above have been validated to retain killing potencies of the parent drugs (specifically the artemisinin derivatives, artelinic acid and artesunate) against malarial parasites. The uptake of fluorescent compounds into *P. falciparum* parasites is observed to be driven by the active moiety of artemisinin or artemisinin derivatives. Furthermore, the experimental results show that these fluorescent compounds act similarly to their respective parent compounds. In addition, the fluorophores used are small and non-toxic, hence should not interfere with cellular processes, rendering the fluorescent compounds a series of mimics of artemisinin or artemisinin derivatives. Moreover, these fluorophores are safe and non-radioactive, therefore omitting the need of sophisticated lab settings and avoiding radioactive hazards.

With that, this invention is useful to reveal the unknown characteristics and biological properties of artemisinin derivatives within live cells, for instance the uptake of artemisinin derivatives into cells, mode of action and potential accumulation site of artemisinin derivatives. Furthermore, this invention can be applied on *in-vitro* study of any other related biological mechanisms such as malarial drug resistance, autophagy, tumorigenesis, and angiogenesis. For ease of application, these fluorescent compounds can be used on common laboratory settings such as confocal laser scanning microscope for imaging purpose; flow cytometer and microplate reader for quantitative measurement. This invention brings researchers an easy, direct and time-saving strategy to understand the drug actions of the artemisinin family.

2.4 Brief Description Of The Drawings

Fig. 1 shows the structure of the blue fluorescent-labeled artelinic acid (AA-Blue).

Fig. 2 shows the structure of the green fluorescent-labeled artelinic acid (AA-Green).

Fig. 3 shows the structure of the red fluorescent-labeled artelinic acid (AA-Red).

Fig. 4 shows the structure of the blue fluorescent-labeled artesunate (AS-blue).

Fig. 5 is a synthetic scheme of the blue fluorescent-labeled artelinic acid (AA-Blue).

Fig. 6 is a synthetic scheme of the green fluorescent-labeled artelinic acid (AA-Green).

Fig. 7 is a synthetic scheme of the red fluorescent-labeled artelinic acid (AA-Red).

Fig. 8 is a synthetic scheme of the blue fluorescent-labeled artesunate (AS-blue).

Fig. 9 shows the IC_{50} curve of blue fluorescent-labeled artelinic acid (AA-Blue) against *P. falciparum* 3D7 and K1, obtained from triplicates of independent experiments.

Fig. 10 shows the competition effect of parent arteminin acid and active metabolite of artemisinin, dihydroartemisinin on the uptake of blue fluorescent-labeled arteminin acid (AA-Blue) into *P. falciparum* 3D7, but not the blue fluorescent tag, coumarin linker.

Fig. 11 illustrates the localization of blue fluorescent-labeled arteminin acid (AA-Blue) in *P. falciparum* 3D7. The parasite is imaged using confocal laser scanning microscope under bright field (leftmost), dark field with excitation wavelength of 405nm (centre) and merged, after incubation with 1 μ M AA-Blue for 1 hour at 37°C.

Fig. 12 displays the image of *P. falciparum* 3D7 after incubation with 1 μ M coumarin linker used to tag on arteminin acid for 1 hour at 37°C. No fluorescence is obtained under same settings applied on Fig. 11.

Fig. 13 illustrates the localization of green fluorescent-labeled arteminin acid (AA-Green) in *P. falciparum* 3D7. The parasite is imaged using confocal laser scanning microscope under bright field (leftmost), dark field with excitation wavelength of 488nm (centre) and merged, after incubation with 1 μ M AA-Green for 1 hour at 37°C.

Fig. 14 illustrates the localization of red fluorescent-labeled arteminin acid (AA-Red) in *P. falciparum* 3D7. The parasite is imaged using confocal laser scanning microscope under bright field (leftmost), dark field with excitation wavelength of 559nm (centre) and merged, after incubation with 1 μ M AA-Red for 1 hour at 37°C.

2.5 Detailed Description Of The Invention:

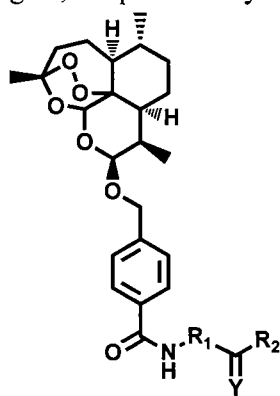
Artemisinin has displayed a wide range of use as anti-malarial, anti-cancer, anti-angiogenesis, anti-viral, anti-bacterial and anti-fungal applications. Tagging artemisinin or artemisinin derivatives with a variety of fluorophores provides a series of visualization tools to explore the underlying molecular mechanism of artemisinin across the said applications.

One embodiment of the invention is a compound for use in malaria research or any research field where artemisinin is of potential use comprising a fluorophore-tagged antimalarial, wherein the antimalarial is an artemisinin or an artemisinin derivative or a salt thereof; the fluorophore can be any small molecular fluorescence dye containing a reactive group, and the fluorophore is tagged from its reactive group to the antimalarial through a linker.

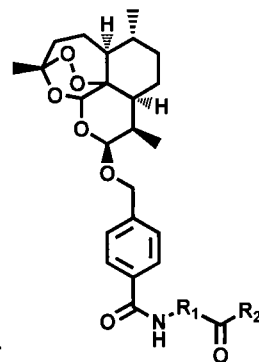
As used herein, "fluorescence dye" refers to a compound that absorbs light of a specific wavelength and reemits the light at a different (but equally specific) wavelength to produce a detectable signal. A fluorescence dye is chosen by its photostability, longevity of fluorescence and inert biological properties.

As used herein, "reactive group" is an atom or a group that allows binding of a group of molecule to another group of molecule via covalent bonding or physical forces. Reactive groups include but are not limited to, carboxylic acid, amine, alcohol, isothiocyanate, isocyanate and thiol.

In another embodiment of the invention, the fluorophore-tagged artemisinin or artemisinin derivatives analogues, is represented by the following structural formula:



, or a salt thereof



, or a salt thereof

Wherein

R_1 is $-(CH_2)_n-NH-$, $NH-(CH_2)_n-NH-$, $-(CH_2)_n-NH-(C_2H_4X)_m-NH-$, $-(CH_2)_n-A-$ or $-(CH_2)_n-NH-(CH_2)_{x1}-B-(CH_2)_{x2}-NH-$ wherein n is an integer of from 1 to 10, m is an integer of from 1 to 100, and $x1$ and $x2$ may be the same or different and are an integer of from 0 to 10;

X is CH_2 , O or a single bond, with the proviso that when m is 2 or more, Xs may be the same or different;

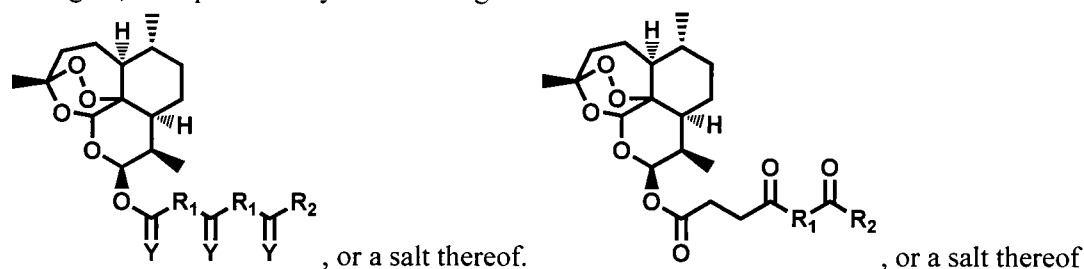
A is heterocycloalkylene of C2-C20 or heteroarylene of C2-C20, containing at least one heteroatom selected from among nitrogen, oxygen and sulfur therein;

B is cycloalkylene of C3-C20 or arylene of C6-C20;

R_2 is a fluorescent dye or a low-molecular weight organic compound capable of selectively binding to a fluorescent dye-labeled substance; and

Y is O , S or CH_2 .

In another embodiment of the invention, the fluorophore-tagged artemisinin or artemisinin derivatives analogues, are represented by the following structural formula:



Wherein

R_1 is $-(CH_2)_n-NH-$, $-NH-(CH_2)_n-NH-$, $-NH-A-NH-$, $-NH-B-NH-$, $-(CH_2)_n-NH-(C_2H_4X)_m-NH-$, $-(CH_2)_n-A-$ or $-(CH_2)_n-NH-(CH_2)_{x1}-B-(CH_2)_{x2}-NH-$ wherein n is an integer of from 1 to 10, m is an integer of from 1 to 100, and $x1$ and $x2$ may be the same or different and are an integer of from 0 to 10;

X is CH_2 , O or a single bond, with the proviso that when m is 2 or more, Xs may be the same or different;

A is heterocycloalkylene of C2-C20 or heteroarylene of C2-C20, containing at least one heteroatom selected from among nitrogen, oxygen and sulfur therein;

B is cycloalkylene of C3-C20 or arylene of C6-C20;

R_2 is a fluorescent dye or a low-molecular weight organic compound capable of selectively binding to a fluorescent dye-labeled substance; and

Y is O , S or CH_2 .

As used herein, "cycloalkylene" refers to divalent cycloalkyl groups comprising from 3 to 20 carbon atoms, and "cycloalkyl" refers to an alicyclic ring system of 3 to 20 carbon atoms which may be saturated or unsaturated. Examples of cycloalkyl groups are, but are not limited to, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl and cyclooctyl.

As used herein, "arylene" refers to divalent aryl groups comprising from 6 to 20 carbon atoms, and "aryl" refers to single-ring aromatic radicals which may include from 6 to 20 carbon atoms. Aryl groups include, but are not limited to, phenyl, biphenyl, anthracenyl, and naphthenyl.

As used herein, "heterocycloalkylene" refers to cycloalkylene comprising from 2 to 20 carbon atoms, and at least one heteroatom selected from among nitrogen, oxygen and sulfur or like.

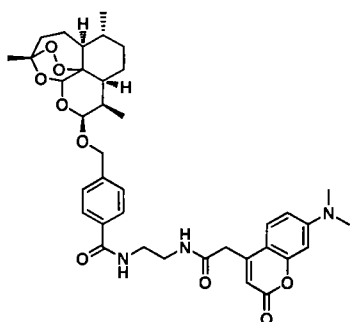
As used herein, "heteroarylene" refers to divalent heteroaryl groups having in the range of 2 up to 20 carbon atoms, and "heteroaryl" refers to a 3 to 20-membered aromatic ring containing carbon atoms and one or more heteroatoms selected from among nitrogen, oxygen and sulfur or like; or an 8- to 10-membered bicyclic or polycyclic ring system containing carbon atoms and heteroatoms, such as nitrogen, oxygen and sulfur, wherein at least one of the rings in the bicyclic system is an aromatic ring.

In another embodiment of the invention, the fluorescent dye refers to Coumarin, Cy3, Cy5, fluorescein isothiocyanate (FITC), tetramethylrhodamin (TAMRA), tetramethylrhodamin isothiocyanate (RITC), Alexa, 4,4-difluoro-4-boro-3a,4a-diaza-s-indacene (BODIPY), Texas Red,

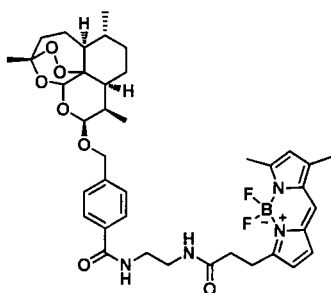
2-acetyl-6-dimethylaminonaphthalene (Acedan) and combinations thereof. These fluorescent dyes are biologically inert and thus non-toxic to living cells.

In another embodiment of the invention, the low-molecular weight organic compound capable of selectively binding to a fluorescent dye-labeled substance is biotin.

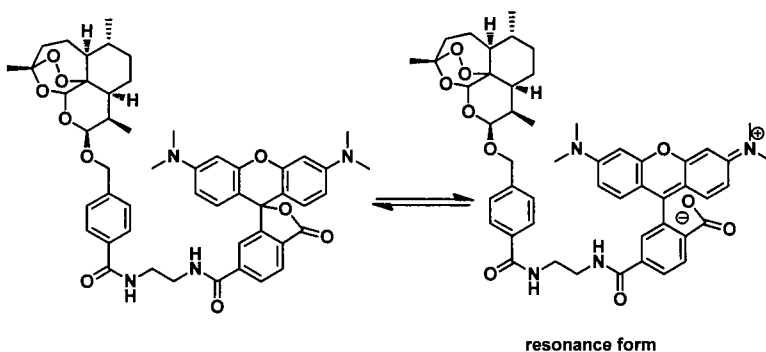
In a particular embodiment of the invention is a compound represented by the following structural formula:



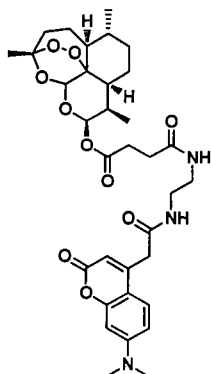
In another particular embodiment of the invention is a compound represented by the following structural formula:



In another particular embodiment of the invention is a compound represented by the following structural formula:



In another particular embodiment of the invention is a compound represented by the following structural formula:



Another embodiment of the invention is a method for determining the location of a fluorophore-tagged antimalarial within or on a cell comprising the steps of exposing the cell to the fluorophore-tagged antimalarial of any of the structural formulas (refer to Figure 1 to 4); and assaying the cell to determine the location of the fluorophore-tagged antimalarial within or on the cell; wherein the cell is visualized by confocal and/or fluorescence microscopy.

As used herein, "location" refers to any cellular components or organelles that the described fluorophore-tagged antimalarial accumulates in. A cellular component refers, but is not limited to cellular proteins and cellular membranes. An organelle refers, but is not limited to lysosomes, digestive vacuoles, nucleus, endoplasmic reticula, and mitochondria.

As used herein, "exposing the cell to the fluorophore-tagged antimalarial of any of the structural formulas" means incubating the cells with the described fluorophore-tagged antimalarial for a desired period, in the same container or solution and may come into contact; under viable environment with tolerable temperature, and suitable air composition to cultivate the cells. The solution refers to cultivating medium which provides sufficient nutrient to the cells. The fluorophore-tagged antimalarial can be added at any time, depending on the interest of study.

Another embodiment of the invention is a method for determining the binding target of a fluorophore-tagged antimalarial within or on a cell comprising the steps of exposing the cell to the fluorophore-tagged antimalarial of any of the structural formulas (refer to Figure 1 to 4); allowing the compound to bind to its target; then exposing the cell to specific fluorescent agent and assaying the cell to view the fluorescence from fluorophore-tagged antimalarial and fluorescent agent within or on the cell; wherein the cell is visualized by confocal and/or fluorescence microscopy.

As used herein, "binding target" refers to a particular cellular component to which the fluorophore-tagged antimalarial binds. A cellular component includes, but is not limited to cellular organelles, signaling and membrane molecules, structural proteins, enzymes, receptors, glycolipids, and metabolic products.

As used herein, "exposing the cell to specific fluorescent agent" means staining a particular cellular component by using a fluorescent agent that specifically target said particular cellular component. The fluorescent agent refers to a fluorescent dye, fluorescent antibodies and fluorescent ligands which possess different excitation and emission wavelengths from that of the fluorophore-tagged antimalarial. This exposure is performed after removal of fluorophore-tagged antimalarial from cultivating medium and thorough washing of cells to prevent unspecific binding of fluorophore-tagged antimalarial to fluorescent agent.

As used herein, "view the fluorescence from fluorophore-tagged antimalarial and fluorescent agent" refers to visualize the locations of fluorescent signals from fluorophore-tagged antimalarial and fluorescent agent using the above mentioned equipment via separate emission channels. Co-localization of these fluorescent signals, which fluorescent signal of fluorophore-tagged antimalarial is superimposed on the fluorescent signal of fluorescent agent, identifies the particular cellular component as the binding target of fluorophore-tagged antimalarial and vice versa.

One embodiment of the invention provides a method for measuring the uptake of a fluorophore-tagged

antimalarial into a cell comprising the steps of exposing the cell to the fluorophore-tagged antimalarial of any of the structural formulas (refer to Figure 1 to 4); and assaying the cell to quantify the uptake of the fluorophore-tagged antimalarial into the cell; wherein the cell is visualized by confocal and/or fluorescence microscopy and/or the cell is assayed by flow cytometry and/or microplate reader.

As used herein, "assaying the cell to quantify the uptake of the fluorophore-tagged antimalarial" means measuring the fluorescence intensity within the cell using above mentioned equipment after desired period of incubation and washing. Fluorescence intensity refers to the relative fluorescence readout of signal within the cell, which depicts the amount of fluorophore-tagged antimalarial in the cells.

Another embodiment of the invention provides a method for determining whether a test substance has an effect on the uptake of a fluorophore-tagged artemisinin into a cell, comprising the steps of exposing test cells to said test substance with the fluorophore-tagged antimalarial of any of the structural formulas (refer to Figure 1 to 4) under conditions whereby fluorophore-tagged antimalarial is taken into said one or more cells; and assaying the test cells compared to control cells, wherein the cell is visualized by confocal and/or fluorescence microscopy and/or the cell is assayed by flow cytometry and/or microplate reader.

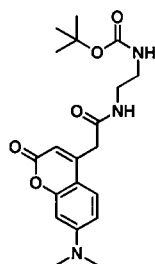
As used herein, "test substance" refers to a chemical or biological molecule that is suspected to influence the entry of the fluorophore-tagged artemisinin into a cell. One or more test substance can be applied.

As used herein, "exposing test cells to said test substance with the fluorophore-tagged antimalarial of any of the structural formulas" means incubating the cells with the test substance and described fluorophore-tagged antimalarial under said conditions, in the same container or solution and may come into contact. The test substance can be added to test cells at any time depending on the objective of investigation, for instance addition prior to, or after exposure of fluorophore-tagged antimalarial to the test cells or simultaneously.

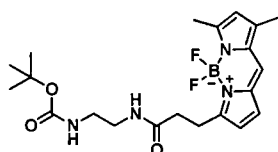
As used herein, "assaying the test cells compared to control cells" means comparing the fluorescence intensity of test cells compared to control cells, wherein a change in fluorescence depicts the effects of the test substance on uptake of the fluorophore-tagged antimalarial into the cell.

As used herein, "control cells" refers to cognate cells that are exposed to only the fluorophore-tagged antimalarial under said conditions.

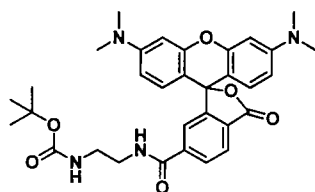
One more embodiment of the invention includes a method of preparing a fluorophore-tagged antimalarial, said method comprising the step of reacting a compound represented by the following structural formulas:



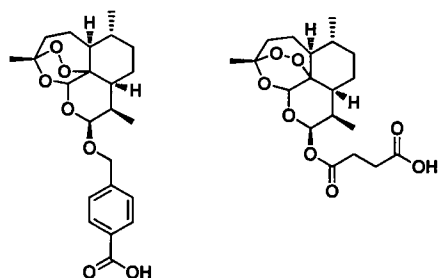
Or,



Or,



With an antimalarial represented by the following structural formulas:



Thereby forming the fluorophore-tagged antimalarials.

Artemisinin derivatives are synthesized to improve the bioavailability of artemisinin since they serve as prodrugs of dihydroartemisinin, the active metabolite of artemisinin in cells. The present invention is able to give a good indication on the mode of action of artemisinin inside a cell. These fluorophore-tagged antimalarials display comparable killing potencies with the parent drugs (artemisinin derivatives artelinic acid and artesunate) against malarial parasites *Plasmodium falciparum* using IC₅₀ reinvasion assay. For example in Fig. 9, the IC₅₀ value of blue fluorescent labeled artelinic acid (AA-Blue) is in the range of 20nM to 30nM, which are near to published IC₅₀ value of artemisinin (10nM to 15nM) (Crespo *et al.*, 2008).

Further investigations have demonstrated that only the parent drugs compete with the uptake of these fluorophore-tagged antimalarials into *P. falciparum* causing reduction in fluorescence intensity within the parasites, but not the fluorescent tag +linkers or other drugs (refer to Fig.10). These suggest the similar mode of actions of fluorophore-tagged antimalarials to their parent drugs. Similarly, confocal imaging on *P. falciparum* incubated with fluorophore-tagged antimalarials or fluorescent tag +linkers shows that only fluorophore-tagged antimalarials display bright fluorescent signals in cells (refer to Fig.11 and Fig.12). This again indicates that inert fluorophores used do not contribute to the actions of fluorophore-tagged antimalarials, wherein the active entities of parent compounds are necessary for the fluorescent compounds to enter *P. falciparum*. These results show that these fluorescent compounds act similarly to their respective parent compounds. Henceforth, present invention will benefit the research fields of malaria, cancer, bacterial and viral studies as a research use only (RUO) bioimaging tool, to image the localization, accumulation and/or dynamics of artemisinin-like derivatives within or on the cell.

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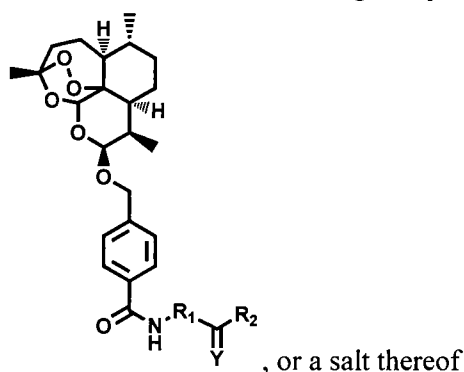
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World Health Organisation (2012). World Malaria Report 2012.

3. CLAIMS

What is claimed is:

1. A fluorescent artemisinin-analogue, represented by the following structural formula:



Wherein

R₁ is —(CH₂)_n—NH—, —A—NH—, —B—NH—, —(CH₂)_n—NH—(C₂H₄X)_n—NH—, —(CH₂)_n—A— or —(CH₂)_n—NH—(CH₂)_{x1}—B—(CH₂)_{x2}—NH— wherein n is an integer of from 1 to 10, m is an integer of from 1 to 100, and x1 and x2 may be the same or different and are an integer of from 0 to 10;

X is CH₂, O or a single bond, with the proviso that when m is 2 or more, Xs may be the same or different;

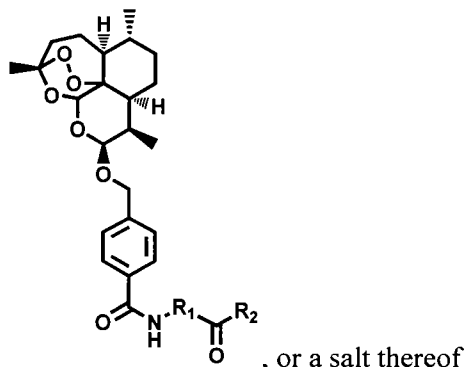
A is heterocycloalkylene of C2-C20 or heteroarylene of C2-C20, containing at least one heteroatom selected from among nitrogen, oxygen and sulfur therein;

B is cycloalkylene of C3-C20 or arylene of C6-C20;

R₂ is a fluorescent dye or a low-molecular weight organic compound capable of selectively binding to a fluorescent dye-labeled substance; and

Y is O, S or CH₂.

2. A fluorescent artemisinin-analogue, represented by the following structural formula:



Wherein

R₁ is $-(CH_2)_n-NH-$, $-A-NH-$, $-B-NH-$, $-(CH_2)_n-NH-(C_2H_4X)_m-NH-$, $-(CH_2)_n-A-$ or $-(CH_2)_n-NH-(CH_2)_{x1}-B-(CH_2)_{x2}-NH-$ wherein n is an integer of from 1 to 10, m is an integer of from 1 to 100, and x₁ and x₂ may be the same or different and are an integer of from 0 to 10;

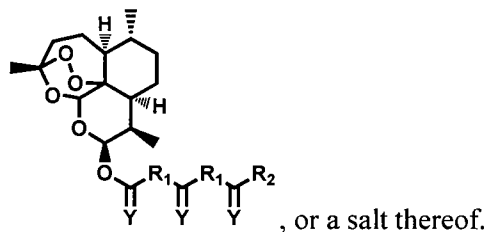
X is CH₂, O or a single bond, with the proviso that when m is 2 or more, Xs may be the same or different;

A is heterocycloalkylene of C2-C20 or heteroarylene of C2-C20, containing at least one heteroatom selected from among nitrogen, oxygen and sulfur therein;

B is cycloalkylene of C3-C20 or arylene of C6-C20;

R₂ is a fluorescent dye or a low-molecular weight organic compound capable of selectively binding to a fluorescent dye-labeled substance.

3. A fluorescent artemisinin-analogue, represented by the following structural formula:



Wherein

R₁ is $-(CH_2)_n-NH-$, $-NH-(CH_2)_n-NH-$, $-NH-A-NH-$, $-NH-B-NH-$, $-(CH_2)_n-NH-(C_2H_4X)_m-NH-$, $-(CH_2)_n-A-$ or $-(CH_2)_n-NH-(CH_2)_{x1}-B-(CH_2)_{x2}-NH-$ wherein n is an integer of from 1 to 10, m is an integer of from 1 to 100, and x₁ and x₂ may be the same or different and are an integer of from 0 to 10;

X is CH₂, O or a single bond, with the proviso that when m is 2 or more, Xs may be the same or different;

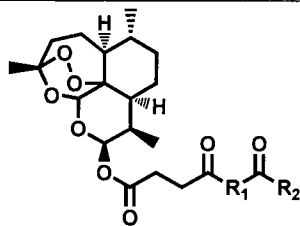
A is heterocycloalkylene of C2-C20 or heteroarylene of C2-C20, containing at least one heteroatom selected from among nitrogen, oxygen and sulfur therein;

B is cycloalkylene of C3-C20 or arylene of C6-C20;

R₂ is a fluorescent dye or a low-molecular weight organic compound capable of selectively binding to a fluorescent dye-labeled substance; and

Y is O, S or CH₂.

4. A fluorescent artemisinin-analogue, represented by the following structural formula:



, or a salt thereof.

Wherein

R_1 is $-(CH_2)_n-NH-$, $-NH-(CH_2)_n-NH-$, $-NH-A-NH-$, $-NH-B-NH-$,
 $-(CH_2)_n-NH-(C_2H_4X)_n-NH-$, $-(CH_2)_n-A-$ or
 $-(CH_2)_n-NH-(CH_2)_{x1}-B-(CH_2)_{x2}-NH-$ wherein n is an integer of from 1 to 10, m is an integer of from 1 to 100, and $x1$ and $x2$ may be the same or different and are an integer of from 0 to 10;

X is CH_2 , O or a single bond, with the proviso that when m is 2 or more, X s may be the same or different;

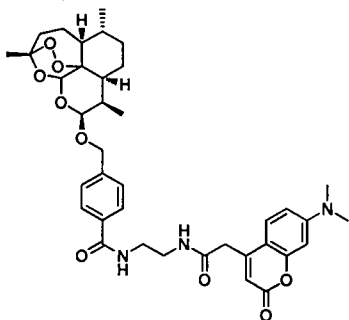
A is heterocycloalkylene of C2-C20 or heteroarylene of C2-C20, containing at least one heteroatom selected from among nitrogen, oxygen and sulfur therein;

B is cycloalkylene of C3-C20 or arylene of C6-C20;

R_2 is a fluorescent dye or a low-molecular weight organic compound capable of selectively binding to a fluorescent dye-labeled substance.

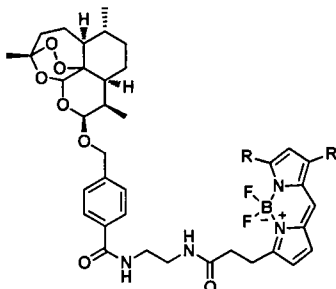
5. A fluorescent artemisinin analogue according to claim 1 and 2, wherein the fluorescent dye is selected from the group consisting of Coumarin, Cy3, Cy5, fluorescein isothiocyanate (FITC), tetramethylrhodamin (TAMRA), tetramethylrhodamin isothiocyanate (RITC), Alexa, 4,4-difluoro-4-boro-3a,4a-diaza-s-indacene (BODIPY), Texas Red, 2-acetyl-6-dimethylaminonaphthalene (Acedan) and a combination thereof, and the low-molecular weight organic compound capable of selectively binding to a fluorescent dye-labeled substance is biotin.

6. The compound according to Claim 1, wherein the compound is represented by the following structural formula:



, or a salt thereof.

7. The compound according to Claim 1, wherein the compound is represented by the following structural formula:

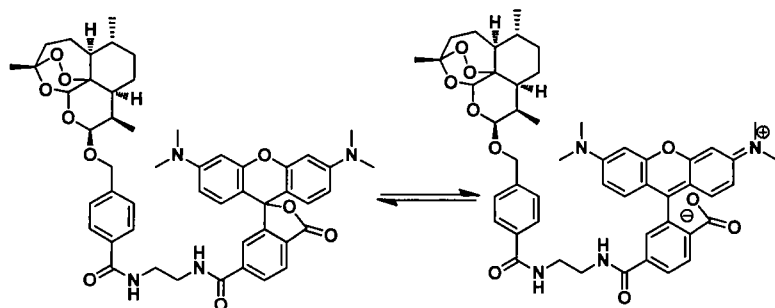


, or a salt thereof.

Wherein

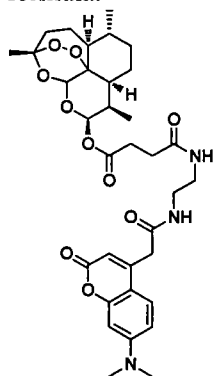
R is hydrogen, methyl, alkyl (containing 1-10 carbons), phenyl, 4-methoxyphenyl, thiophene, pyrrole.

8. The compound according to Claim 1, wherein the compound is represented by the following structural formula:



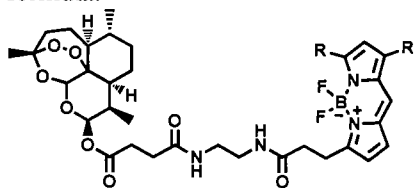
, or a salt thereof.

9. The compound according to Claim 2, wherein the compound is represented by the following structural formula:



, or a salt thereof.

10. The compound according to Claim 2, wherein the compound is represented by the following structural formula:



, or a salt thereof.

Wherein

R is hydrogen, methyl, alkyl (containing 1-10 carbons), phenyl, 4-methoxyphenyl, thiophene, pyrrole.

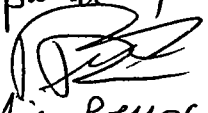
11. A method for determining the location of a fluorophore-tagged artemisinin within or on a cell, comprising the steps of:

exposing the cell to the compound according to any one of claims 1 to 10; and assaying the cell to determine the location of the compound within or on the cell.

12. A method for determining the binding target of a fluorophore-tagged artemisinin within or on a cell, comprising the steps of:

- exposing the cell to the compound according to any one of claims 1 to 10 to allow binding to its target; then
- expose the cell to said fluorescent agents that specifically binds to a cellular component; then
- assaying the cell; wherein co-localization of fluorescent signals from said fluorescent agents with that of said compound is an indication of said cellular component as the binding target of the compound within or on the cell.

13. The method according to claim 12, wherein said fluorescent agents refer to fluorescent dyes, fluorescent tagged antibodies and ligands.
14. The method according to claim 12, wherein said cellular component refers to cellular proteins, organelles, membranes and nucleic acids.
15. A method for determining whether a test substance has an effect on the uptake of a fluorophore-tagged artemisinin into a cell, comprising the steps of:
 - a) exposing said one or more test cells to said test substance with the compound according to any one of claims 1 to 10 under conditions whereby said compound is taken into said one or more cells; and
 - b) measuring the fluorescence of said one or more test cells compared to control cells, which have only been exposed to said compound, wherein a change in fluorescence within said one or more test cells compared to said control cells is an indication of effects of test substance on uptake of the said compound into the cell.
16. The method according to claim 15, wherein said one or more test cells is exposed to said test substance prior to exposing said test cells to said compound.
17. The method according to claim 15, wherein said one or more test cells is exposed to said test substance after exposing to said compound.
18. The method according to claim 15, wherein said one or more test cells is exposed simultaneously to said test substance and said compound.
19. The method according to claim 15, wherein said exposing step further includes exposing said one or more test cells with at least one second test substance in the presence of said first test substance.
20. The method according to any one of claims 11 to 19, wherein the parasite or cell is either visualized by confocal and/or fluorescence microscopy, or assayed by flow cytometry.
21. A method for synthesizing the fluorescent artemisinin analogue of claim 1 to 4, comprising:
 - a) dissolving dihydroartemisinin and hydroxymethylbenzoic acid in diethyl ether and add boron trifluoride dimethyl etherate to obtain artelinic acid
 - b) Reacting the amine reactive fluorescent dye with a Boc group-containing organic compound in an organic solvent under peptide coupling conditions followed by purification
 - c) Deprotecting the Boc protected product of step b) and reacting it with a solution of artelinic acid or artesunate under peptide coupling conditions followed by purification to obtain the compound of claim 1 to 4.

Prepared by

Bartien Reusc 06/08/13