COMBINATION OF A BIS-THIAZOLIUM SALT OR A PRECURSOR THEREOF AND ARTEMISININ OR A DERIVATIVE THEREOF FOR TREATING ACUTE MALARIA

The present invention relates to a novel combination of antimalarial active ingredients, namely a bisthiazolium salt or a precursor thereof and artemisinin or derivatives thereof, and also to a pharmaceutical composition comprising such a combination, of use in the treatment and/or prevention of malaria.

Malaria is one of the primary infectious causes of mortality in the world and annually affects more than 500 million individuals, among whom 1.5 to 3 million die each year. This scourge affects mainly sub-Saharan Africa, south-east Asia and Latin America.

Four types of parasites of the *Plasmodium* genus (*P. falciparum, P. malariae, P. vivax* and *P. ovale*), transported by Anopheles mosquitoes, propagate malaria. *Plasmodium falciparum*, which is widespread in Africa, is the most virulent parasite and is responsible for most of the lethal forms of the disease.

The strong upsurge in the disease observed over the last few years is due to several factors, among which is the resistance of many strains of *Plasmodium falciparum* to the medicaments currently used, such as chloroquine, mefloquine, amodiaquine or else the antifolics and antifolinics, such as pyrimethamine and sulfadoxine.

Many individuals suffering from malaria exhibit an acute infection, with few revealing physical signs apart from a slight anemia and an increase in volume of the spleen (splenomegaly). The symptoms depend on the variety of malaria. Nevertheless, the general clinical characteristics are: feeling unwell, hyperthermia (fever), headaches, digestive problems such as nausea, vomiting and/or abdominal pain, diarrhea, asthenia, muscle pain, arthralgia (joint pain) and jaundice, in particular.

The treatment is generally carried out by administration of chloroquine (or of basic quinine hydrochloride in the event of resistance to chloroquine) until an improvement and disappearance of the parasites in the blood are obtained (usually between 3 and 5 days). The patient then generally takes a single medicament constituted of 1.5 grams of sulfadoxine and 75 mg of pyrimethamine.

Other treatments based on antifolics and on antifolinics, or alternatively based on malarone, are also used.

Severe malaria caused by *Plasmodium falciparum* is a cerebral malaria which combines a substantially elevated temperature (40°C) and a coma which has a poor prognosis despite the treatment and for which mortality sometimes rises to 20% in adults and 15% in children. The appearance of severe malaria is either gradual or abrupt. It begins after instantaneous and transient convulsions of one or more muscles, followed by decontractions. They are localized or generalized throughout the body. This variety of

malaria is accompanied by nystagmus (incessant jumping of the eyes from side to side), sometimes by a stiff neck and by a disturbance of the reflexes. Severe malaria can also be accompanied by retinal bleeding, hypoglycemia, pulmonary edema, kidney involvement, anemia and/or hematemesis (reflux of blood originating from the damaged organs and which is swallowed and then reappears in the form of hemorrhaging accompanying vomiting).

In the case of *Plasmodium falciparum* causing complicated malaria or cerebral malaria and also the other severe manifestations, it is a medical emergency when 1% of the red corpuscles (red blood cells) or more are parasitized in an individual who has not yet been immunized against this disease.

In the case of severe malaria, the priority of the treatment is to massively and rapidly (within 24 to 48 hours following commencement of treatment) reduce the parasitemia in order to rule out the lethal prognosis. Once the parasitemia is under control and the vital prognosis is confirmed, a more conventional antimalarial treatment, such as chloroquine or basic quinine hydrochloride, for example, can be administered.

The treatment for severe malaria is difficult to administer orally or rectally, since the patients are often suffering from vomiting accompanied by considerable diarrhea.

Artemisinin, which was isolated in 1972 from the plant *Artemisia annua* (qinghaosu), and has been used for centuries in China, has a powerful antimalarial activity. Derivatives with improved pharmacological properties, such as artemether, arteether and artesunate, are also commercially available.

Artemisinin and derivatives thereof, in particular artesunate, are today part of the active ingredients that are most effective against *Plasmodium falciparum*. However, it is difficult for these compounds to produce complete recovery, and many upsurges are noted. The use of artemisin or derivatives thereof in monotherapy could therefore be a causal factor in the selection of resistant parasitic strains.

The scientific community now recommends the use of combinations of active ingredients, and in particular of combinations of artemisin or derivatives thereof with other antimalarial active ingredients. These polytherapies, known as ACTs (artemisinin-based combination therapies), have been recommended since 2002 by the World Health Organization (WHO). They offer multiple advantages: improved therapeutic efficacy on resistant strains, mutual protection of the two active ingredients against the appearance of resistance, reduction in disease transmission and in resistance propagation.

Several combinations of this type are known.

The combination between artemether and lumefantrine, sold under the name Coartem®, has, for example, been proposed, as has the combination of artesunate and amodiaquine (Arsucam®).

The combination of ferroquine with an artemisinin derivative is described in

document WO 2006/111647.

However, the beneficial effect of ACT polytherapies is neither obvious nor foreseeable. For example, the publication by C. Snyder et al., Experimental Parasitology, 115 (2007), 296-300, discloses that the combination of piperaquine with the compound OZ277 appears to be more promising than the combination of piperaquine with an artemisinin derivative such as artemether.

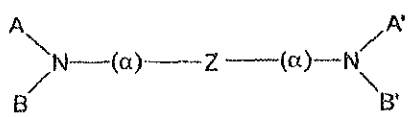
Similarly, the combination of chloroquine and artesunate does not achieve satisfactory efficacy levels (Am. J. Trop. Med. Hyg., 2003, *69(1)*, 19-25 and Transactions of the Royal Society of Tropical Medicine and Hygiene, 2003, 97, 429-433) and can result in the selection of resistant strains, in particular resistant to chloroquine.

Thus, even though the ACT strategy is recommended by the WHO, the search for new combinations of antimalarial active ingredients is, all the same, not easy and should be continued.

The present invention relates to a combination of a bisthiazolium salt, which is a compound of formula (VI) or a precursor thereof as defined below, and artemisin or a derivative thereof. Among the artemisinin derivatives of use in the present invention, mention may, for example, be made of artesunate, artemether, arteether, hydroartemisinin or dihydroartemisinin.

Such a combination shows an advantageous and unforeseeable synergy and, consequently, is of use in the prevention and/or treatment of malaria, in particular of severe malaria.

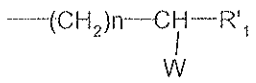
The compounds of formula (VI), and also precursors thereof for the purpose of the present invention, are described in the patent published under number EP 1 196 371. The precursors of the bisthiazolium salts of formula (VI) for the purpose of the present invention correspond to formulae (I), (II), (III), (IV) and (V) of EP 1 196 371. This patent in fact describes precursors of drugs with an antimalarial effect, characterized in that they are products capable of generating bisquaternary ammonium salts and that they correspond to general formula (I):



(I)

in which

- A and A' are identical to or different from one another and represent:
  . either, respectively, a group A_1 and A'_1 of formula:



where n is an integer from 2 to 4; R'_1 represents a hydrogen atom, a C_1 to C_5 alkyl radical, optionally substituted with an aryl radical (in particular a phenyl radical), a hydroxyl, an alkoxy, in which the alkyl radical contains from 1 to 5 carbon atoms, or aryloxy (in particular phenoxy);

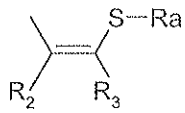
and W represents a halogen atom chosen from chlorine, bromine or iodine, or a nucleofugal group, such as the tosyl radical CH_3-C_6H_4-SO_3, the mesityl radical CH_3-SO_3, the CF_3-SO_3 radical or the NO_2-C_6H_4-SO_3 radical,

. or a group A_2 which represents a formyl radical -CHO or an acetyl radical -CO-CH_3,

- B and B' are identical to or different from one another and represent:

. either, respectively, the groups B_1 and B'_1, if A and A' represent, respectively, A_1 and A'_1, B_1 and B'_1 representing a group R_1 which has the same definition as R'_1 above, but cannot be a hydrogen atom,

. or, respectively, the groups B_2 and B'_2, if A and A' represent A_2, B_2 or B'_2 being the group R_1 as defined above, or a group of formula:



in which -Ra represents an RS- or RCO- group, where R is a linear, branched or cyclic, C_1 to C_6, in particular C_1 to C_5, alkyl radical, as appropriate substituted with one or more hydroxyl, alkoxy or aryloxy groups, or an amino group and/or a -COOH or COOM group, where M is a C_1 to C_3 alkyl; a phenyl or benzyl radical, in which the phenyl radical is, as appropriate, substituted with at least one C_1 to C_5 alkyl or alkoxy radical, said radicals being optionally substituted with an amino group, or with a nitrogenous or oxygenated heterocycle, a -COOH group or a -COOM group; or a group -CH_2-heterocycle, in which the heterocycle has 5 or 6 elements and is nitrogenous and/or oxygenated;

R_2 represents a hydrogen atom, a C_1 to C_5 alkyl radical or a -CH_2-COO(C_1 to C_5)alkyl group;

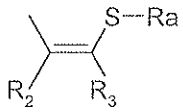
and R_3 represents a hydrogen atom; a C_1 to C_5 alkyl or alkenyl radical, as appropriate substituted with -OH, a phosphate group, an alkoxy radical, in which the alkyl radical is

C_1 to C_3, or aryloxy; or an alkyl (or aryl)carbonyloxy group;

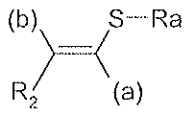
or else R_2 and R_3 together form a ring containing 5 or 6 carbon atoms; R and R_3 may be linked so as to form a ring containing 5- to 7 atoms (carbon, oxygen, sulfur);

- α represents:

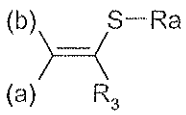
. either a single bond, when A and A' represent A1 and A'1: or when A and A' represent A_2, i.e. a -CHO or $-COCH_3$ group, and B_2 and B'_2 represent:



. or, when A and A' represent A_2 and B_2 and B'_2 represent R_1, a group of formula:



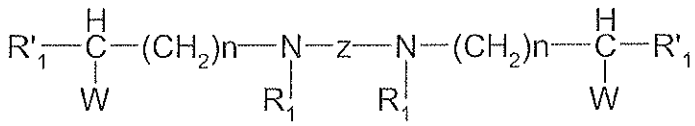
or a group of formula:



in which (a) represents a bond towards Z and (b) a bond towards the nitrogen atom,

- Z represents a C_6 to C_{21}, in particular C_{12} or C_{13} to C_{21}, alkyl radical, as appropriate with insertion of one or more multiple bonds, and/or one or more heteroatoms O and/or S, and/or one or more aromatic rings, and the pharmaceutically acceptable salts of these compounds, with the proviso that R'_1 does not represent H or a C_1 or C_2 alkyl radical, when n = 3 or 4, R_1 represents a C_1 to C_4 alkyl radical and Z represents a C_6 to C_{10} alkyl radical.

EP 1 196 371 also describes a subgroup of precursor compounds as described above, corresponding to general formula (II):



(II)

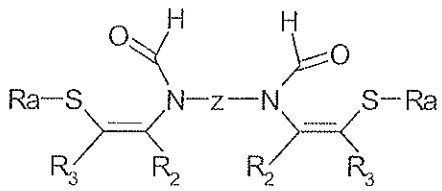
in which R_1, R'_1, W, n and Z are as defined above.

EP 1 196 371 also describes another subgroup of precursor compounds, constituted of the precursors as described above, for which Z represents a C_{13} to C_{21} alkyl radical.

EP 1 196 371 also describes a subgroup of precursor compounds constituted of the precursors as described in the previous subgroup, for which Z represents a $-(CH_2)_{16}-$ group.

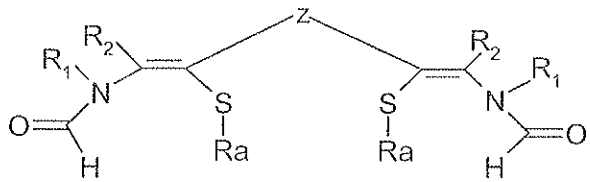
EP 1 196 371 describes, in particular, the precursors chosen from N,N'-dimethyl-N,N'-(5-chloropentyl)-1,16-hexadecanediamine dihydrochloride or N,N'-dimethyl-N,N'-(4-chloropentyl)-1,16-hexadecanediamine dihydrochloride.

EP 1 196 371 describes, in addition, a group of precursors, corresponding to general formula (III):



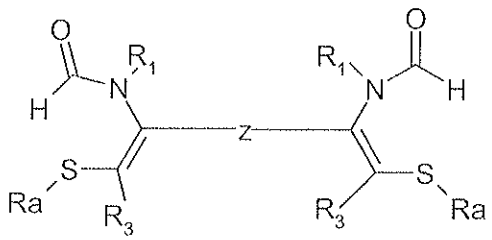
(III)

of to general formula (IV):



(IV)

or to general formula (V):



(V)

in which Ra, R_1, R_2, R_3 and Z are as defined above.

EP 1 196 371 describes, in particular, precursor compounds of formulae (III), (IV) and (V) as described above, chosen from:

- N,N'-diformyl-N,N'-di[1-methyl-2-S-thiobenzoyl-4-methoxybut-1-enyl]-1,12-diaminododecane (TE4c),
- N,N'-diformyl-N,N'-di[1-methyl-2-S-(p-diethylaminomethylphenylcarboxy)thio-4-methoxybut-1-enyl]-1,12-diaminododecane (TE4f),
- N,N'-diformyl-N,N'-di[1-methyl-2-S-(p-morpholinomethylphenylcarboxy)-thio-4-methoxybut-1-enyl]-1,12-diaminododecane (TE4g),
- N,N'-diformyl-N,N'-di[1-methyl-2-S-thiobenzoyl-4-methoxybut-1-enyl]-1,16-diaminohexadecane (TE8),
- N, N'-diformyl-N, N'-di[1(2-oxo-4,5-dihydro-1,3-oxathian-4-ylidene)ethyl]1,12-diaminododecane (TE3);

or else from:

- N,N'-diformyl-N,N'-di[1-methyl-2-tetrahydrofurfurylmethyldithio-4-hydroxybut-1-enyl]-1,12-diaminododecane (TS3a),
- N,N'-diformyl-N,N'-di[1-methyl-2-propyldithio-4-hydroxybut-1-enyl]-1,12-diaminododecane (TS3b),
- N,N'-diformyl-N,N'-di[1-methyl-2-benzyldithio-4-hydroxybut-1-enyl]-1,12-diaminododecane (TS3c),
- N,N'-diformyl-N,N'-di[1-methyl-2-(2-hydroxyethyl)dithio-4-hydroxybut-1-enyl]-1,12-diaminododecane (TS3d),
- N,N'-diformyl-N,N'-di[1-methyl-2-propyldithio-4-methoxybut-1-enyl]-1,12-diaminododecane (TS3d), and
- N,N'-diformyl-N,N'-di[1-methyl-2-propyldithioethenyl]-1,12-diaminododecane (TS6b),

or else:

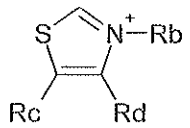
- 2,17-(N,N'-diformyl-N,N'-dimethyl)diamino-3,16-S-thio-p-methoxybenzoyl-6,13-dioxaoctadeca-2,16-diene (TE9),
- 2,17-(N,N'-diformyl-N,N'-dibenzyl)diamino-3,16-S-thio-p-methoxybenzoyl-6,13-dioxaoctadeca-2,16-diene (TE10),
- ethyl 3,18-(N,N'-diformyl-N,N'-dimethyldiamino-4,17-S-thiobenzoyleicosa-3,17-dienedioate (TE12),
- ethyl 3,18-(N,N'-diformyl-N,N'-dibenzyl)diamino-4,17-S-thiobenzoyleicosa-3,17-dienedioate (TE13),

or finally:

- 2,15-(N,N'-diformyl-N,N'-dimethyl)diamino-1,16-S-thiobenzoylhexadeca-1,15-diene (TE15),

- 2,15-(N,N'-diformyl-N,N'-dibenzyl)diamino-1,16-S-thiobenzoylhexadeca-1,15-diene (TE16).

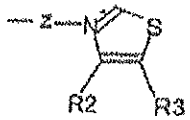
EP 1 196 371 describes, furthermore, cyclized derivatives generated from precursors of thiazolium salts, having good antimalarial activity and corresponding to general formula (VI):



(VI)

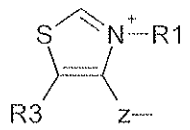
in which

. Rb represents R_1 or T, T representing the group of formula:

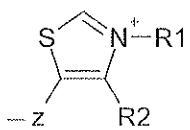


with the proviso that Z does not represent a C_6 to C_8 alkyl radical, when Rc, Rd, R_2 and R_3 represent a methyl radical, or when Rc and Rd, on the one hand, and R_2 and R_3, on the other hand, together form aromatic rings containing 6 carbon atoms,

. Rd represents R_2 or P, P representing the group of formula:



. Rc represents R_3 or U, U representing the group of formula:



R_1, R_2, R_3 and Z being as defined above,

it being understood that Rb = T, if Rc = R_3 and Rd = R_2; Rd = P, if Rc = R_3 and Rb = R_1; and Rc = U, if Rb = R_1 and Rd = R_2.

According to the present invention, the compounds of formula (VI) may be in the form of a free base, of a salt, of a hydrate or of a solvate.

In particular, EP 1 196 371 mentions, among the cyclized derivatives described above, the derivatives chosen from:

- 1,12-dodecamethylenebis[4-methyl-5-(2-hydroxyethyl)thiazolium] dibromide (T3);
- 1,12-dodecamethylenebis[4-methyl-5-(2-methoxyethyl)thiazolium] diiodide (T4);
- 1,12-dodecamethylenebis(4-methylthiazolium) diiodide (T6);
- 1,16-hexadecamethylenebis[4-methyl-5-(2-methoxyethyl)thiazolium] diiodide (T8);
- 3,10-dioxadodecamethylenebis[5-(1,4-dimethyl)thiazolium] diiodide (T9);
- 3,10-dioxadodecamethylenebis[5-(1-benzyl-4-methyl)thiazolium] dibromide (T10);
- dodecamethylenebis[5-(1-methyl-4-ethoxycarbonylethyl)thiazolium] diiodide (T12);
- dodecamethylenebis[5-(1-methyl-4-ethoxycarbonylethyl)thiazolium] dibromide (T13);
- dodecamethylenebis[4-(1-methyl)thiazolium] diiodide (T15);
- dodecamethylenebis[4-(1-benzyl)thiazolium] dibromide (T16).

Finally, EP 1 196 371 describes the process for obtaining the bisthiazolium salts and bisthiazolium precursors mentioned above, in particular the synthesis of the compounds of formula (VI) such as T3, T4, T6, T8, T9, T10, T12, T13, T15 and T16. In particular, the synthesis of T3 is described on page 18 of EP 1 196 371. Similarly, the antimalaria activity of the T3 compound is described in Table 13, page 28 of this same document.

Thus, the invention relates to a combination comprising, as active ingredients, a bisthiazolium salt, which is a compound of formula (VI) or a precursor thereof, as described above, and artemisinin or a derivative thereof.

According to a second subject, the invention relates to a combination of a bisthiazolium salt, which is a compound of formula (VI) or a precursor thereof, as described above, and artesunate.

According to a third subject, the invention relates to a combination of a bisthiazolium salt, which is a compound of formula (VI), chosen from:

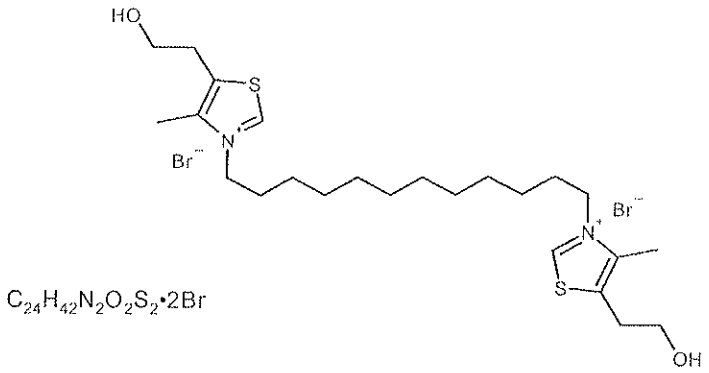
- 1,12-dodecamethylenebis[4-methyl-5-(2-hydroxyethyl)thiazolium] dibromide (T3);
- 1,12-dodecamethylenebis[4-methyl-5-(2-methoxyethyl)thiazolium] diiodide (T4);
- 1,12-dodecamethylenebis(4-methylthiazolium) diiodide (T6);
- 1,16-hexadecamethylenebis[4-methyl-5-(2-methoxyethyl)thiazolium] diiodide (T8);
- 3,10-dioxadodecamethylenebis[5-(1,4-dimethyl)thiazolium] diiodide (T9);
- 3,10-dioxadodecamethylenebis[5-(1-benzyl-4-methyl)thiazolium] dibromide (T10);
- dodecamethylenebis[5-(1-methyl-4-ethoxycarbonylethyl)thiazolium] diiodide (T12);
- dodecamethylenebis[5-(1-methyl-4-ethoxycarbonylethyl)thiazolium] dibromide (T13);
- dodecamethylenebis[4-(1-methyl)thiazolium] diiodide (T15);
- dodecamethylenebis[4-(1-benzyl)thiazolium] dibromide (T16);

and artesunate.

According to a fourth subject, the invention relates to a combination of a bisthiazolium salt of formula (VI), which is 1,12-dodecamethylenebis[4-methyl-5-(2-hydroxyethyl)-thiazolium dibromide (T3), and artesunate.

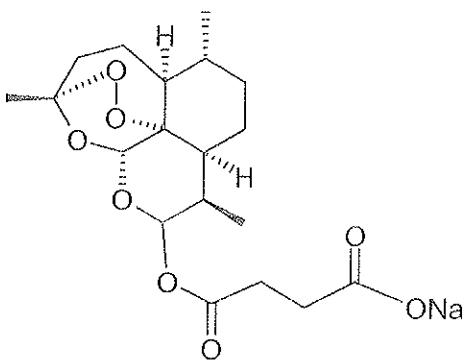
In these subjects, each of the active ingredients is used at a concentration that reflects their respective potency in curing the infection. In each of the combinations, the respective amounts of the compounds depend on these active doses.

The 1,12-dodecamethylenebis[4-methyl-5-(2-hydroxyethyl)thiazolium] dibromide (T3) present in the combinations according to the invention has the structure which follows:



$C_{24}H_{42}N_2O_2S_2 \cdot 2Br$

The artesunate present in the combinations according to the invention has the formula:



A subject of the invention is also a pharmaceutical composition comprising, as active ingredient, a combination of a bisthiazolium salt of formula (VI) or a precursor thereof, in particular T3, and artemisinin or a derivative thereof, in particular artesunate, as defined above.

Such a pharmaceutical composition contains therapeutically effective doses of a bisthiazolium salt, which is a compound of formula (VI) or a precursor thereof, and of artemisinin or of derivatives thereof, and also at least one pharmaceutically acceptable excipient. Said excipients are chosen, according to the pharmaceutical form and the method of administration desired, from the usual excipients which are known to those skilled in the art. The ranges of proportions of each of the compounds are defined according to the doses thereof which exert an antimalarial activity in monotherapy and reflect their respective potency in curing the infection.

The appropriate unit administration forms comprise oral forms such as tablets, soft or hard gel capsules, powders, granules and oral solutions or suspensions, sublingual, buccal, intratracheal, intraocular or intranasal administration forms, forms for administration by inhalation, topical, transdermal, subcutaneous, intramuscular or intravenous (bolus or perfusion) administration forms, rectal administration forms, and implants. For topical application, the compounds according to the invention may be used in creams, gels, ointments or lotions.

Administration routes envisaged are oral, rectal and parenteral routes, in particular the muscular, intrarectal and intravenous route, and especially via a drip.

For parenteral administration, use is made of aqueous suspensions, isotonic saline solutions or sterile and injectable solutions which contain pharmacologically compatible dispersing agents and/or wetting agents, for example propylene glycol or butylene glycol. The suspensions may also contain, if necessary, antioxidant adjuvants, or the active ingredients according to the invention may be diluted in appropriate solutions, such as glucose or NaCl, in order to be subsequently administered via a drip.

With parenteral administration, and in particular via a drip, the preferred daily doses of each of the two active ingredients of the combination according to the invention are as indicated above:

- bisthiazolium salt of formula (VI), in particular T3: between 0.01 and 3 mg/kg;
- artemisinin or a derivative thereof, in particular artesunate: between 1 and 5 mg/kg.

There may be specific cases where higher or lower doses are suitable; such dosages do not depart from the context of the invention. According to the usual practice, the dosage suitable for each patient is determined by the physician according to the method of administration and the weight and response of said patient.

The combination according to the invention is intended to be administered for 1 to

4 consecutive days, as one or more daily intakes of each of the two active ingredients, preferably 1 to 3 intakes (oral or rectal) or bolus(es) per day. This treatment time preferably limited to 4 days or less is particularly advantageous for massively and rapidly reducing the parasitemia and allowing the patient, having warded off his or her lethal prognosis, to be subsequently treated against the malaria per se. This subsequent treatment can be carried out by monotherapy with artemisinin derivatives, by any other known treatment for malaria, but also by administration of the combination according to the invention.

An example of dosage and of method of administration may be 2.4 mg/kg/day of artesunate and 1 mg/kg/day of T3 for the first 24 hours of treatment of the patient (D1) exhibiting severe malaria, and then 1.2 mg/kg/day of artesunate and 0.8 mg/kg/day of T3 for the next 48 hours (D2 and D3), the artesunate and the T3 being administered via a drip, in a single drip bag at D1 and in separate drip bags at D2 and D3.

The administration of each of the two active ingredients may be carried out simultaneously, or else separately or spread out over time (sequential administration).

When the administration is carried out simultaneously, the two active ingredients may be combined within a single pharmaceutical form (fixed combination), such as a tablet or a gel capsule suitable for oral administration, or in the same drip bag, or a formulation suitable for intrarectal administration.

The two active ingredients of the combination according to the invention may also, whether the administration thereof is simultaneous or nonsimultaneous, be present in distinct pharmaceutical forms. To this effect, the combinations according to the invention may be in the form of a kit comprising, on the one hand, the compound of formula (VI) or a precursor thereof, in particular T3 or a salt, hydrate or solvent, and, on the other hand, artemisinin or a derivative thereof, in particular artesunate, said compound of formula (VI) such as T3 and the artesunate being in distinct compartments and being intended to be administered simultaneously, separately or in a manner spread out over time (sequential administration).

By way of example, a unit administration form of T3 in tablet form may comprise the following components:

T3	6 mg
Mannitol	224 mg
Sodium croscaramellose	6 mg
Maize starch	15 mg
Hydroxypropylmethylcellulose	2 mg
Magnesium stearate	3 mg

Equally, by way of example, a unit administration form of artesunate in tablet form may comprise 50 or 100 mg of artesunate and customary excipients, for example lactose, croscarmellose, anhydrous colloidal silica, microcrystalline cellulose and magnesium stearate.

Equally, by way of example, a unit administration form comprising a combination of artesunate and T3 may comprise 1.5 mg of T3 and 50 mg of artesunate, and also customary excipients such as those mentioned above, the daily dose for an adult being achieved in 4 intakes of one tablet or in one intake of 4 tablets.

The same dosages of T3, of artesunate, or of T3 and artesunate combination are used for the unit forms for rectal administration, with the excipients known to those skilled in the art.

For a method of administration by parenteral injection, a unit administration form of T3 may comprise 1 mg of T3 in a vial, for example, and also customary excipients well known to those skilled in the art, such as glycerol and a phosphate buffer, the vial being designed for 10 kg of body weight.

Equally, by way of example, a unit administration form of artesunate and of T3 in a vial may comprise 24 mg of artesunate and 1 mg of T3, and also customary excipients such as those mentioned above, the vial being administered at a rate of one vial per 10 kg of body weight.

A subject of the present invention is also a method of treating and/or preventing severe malaria, which comprises the administration, to a patient, of an effective therapeutic dose of at least one thiazolium salt, which is a compound of formula (VI) or a precursor thereof, in particular T3, and of an effective therapeutic dose of artemisinin or a derivative thereof, in particular artesunate, said doses being administered simultaneously or else sequentially to said patient, as is previously described.

The present invention also relates to a method as defined above, for treating and/or preventing malaria, in particular severe malaria.

A subject of the invention is also the use of a combination comprising, as active ingredients, a bisthiazolium salt, which is a compound of formula (VI) or a precursor thereof, as described above, and artemisinin or a derivative thereof, in particular artesunate, for the preparation of a medicament for use in the treatment or prevention of malaria, in particular of severe malaria.

Finally, a subject of the invention is

– a kit for the treatment or prevention of severe malaria, comprising, on the one hand, at least one bisthiazolium salt, which is a compound of formula (VI) or a precursor thereof, and, on the other hand, at least artemisinin or a derivative thereof, in particular artesunate, said compound of formula (VI)/precursor and artemisinin/derivative being in distinct drip bags or compartments and being intended to be administered simultaneously or sequentially, and

– a kit as defined above, characterized in that it comprises, on the one hand, at least 1,12-dodecamethylenebis[4-methyl-5-(2-hydroxyethyl)thiazolium] dibromide, and, on the other hand, at least artesunate.

The combination according to the invention was the subject of pharmacological and biochemical tests *in vivo* in mice infected with a plasmodium of *Plasmodium falciparum* type (strain *Plasmodium vinckei petteri*), making it possible to demonstrate its efficacy and the synergistic effect that it provides, for the treatment of malaria.

In vivo measurement of the antimalarial activity, in mice infected with _Plasmodium vinckei petteri_, of the combination of 1,12-dodecamethylenebis[4-methyl-5-(2-hydroxyethyl)thiazolium] dibromide (T3) with artesunate

Definitions of the terms used in this test:

- ED_{50} = dose in mg/kg/day which brings about a 50% decrease in parasitaemia, observed in the infected mice,
- ED_{90} = dose in mg/kg/day which brings about a 90% decrease in parasitaemia, observed in the infected mice,
- ip = intraperitoneal administration,
- iv = intravenous administration,
- ir = intrarectal administration.

1. Description of the _in vivo_ test used

- Infection of mice

On day 0 (D0), female "Swiss" mice (OF1, 22-26 g) (Charles River Laboratories France, 59 rue de la Paix, 76410 Saint-Aubin-les-Elbeuf) are inoculated, by iv injection in the caudal vein, with 10^8 parasitized erythrocytes suspended in 200 µl of NaCl (0.9%). The parasites used are of *Plasmodium vinckei petteri* type *(strain 279 BY, supplied by Dr. I Landau, Paris, France).*

The mice are acclimatized beforehand for two weeks. They are given food and

drink *ad libitum.*

The injection of 10^8 parasitized erythrocytes results, at day 1 (D1), in a parasitemia level of between 5% and 10% (7.2% ± 0.2%). The *Plasmodium vinckei petteri* strain is maintained by weekly infection in mice with 10^7 to 10^8 parasitized erythrocytes suspended in a saline phosphate buffer (0.9%) (infection by intraperitoneal administration).

- Preparation of the solutions of T3 and of artesunate

The products are dissolved in a 0.3M phosphate buffer (pH = 8.1). The final concentration of the T3 solution is between 40 and 200 mg/l depending on the dose. The final concentration of the artesunate solution is between 100 and 500 mg/l depending on the dose.

The products are administered ip. The administration volume is of the order of 100 µl, but depends on the weight of the mouse.

- Treatments

The mice are treated once a day for 4 days, on days D1, D2, D3 and D4 (Peters, W., J. H. Portus, and B. L. Robinson. 1975. The chemotherapy of rodent malaria. XXII. The value of drug-resistant strains of P. berghei in screening for blood schizonticidal activity. Ann. Trop. Med. Parasitol. 69:155–171; and: Ancelin ML, Calas M., Bonhoure A., Herbute S. and Vial H., In vivo antimalarial activity of mono and bis quaternary ammonium salts interfering with Plasmodium phospholipid metabolism. Antimicrob. Agents Chemother., 2003, 47, 2598-2605). Four mice are used per dose.

The animal is given, by ip administration, according to the case, T3, artesunate or the mixture of the two active ingredients solubilized.

Measurement of the parasitemia

At the 5th day, a few drops of blood are taken from the tail of the mouse in order to determine the parasitemia by FACS (fluorescence-activated cell sorter) and to perform a blood smear. The parasitemia is first determined by FACS on 20 000 cells. The red blood cells taken for the FACS are fixed with glutaraldehyde and then labeled with a fluorochrome (YOYO1®) which labels DNA and therefore only the parasitized cells (Barkan, D., Ginsburg, H., and Golenser, J. 2000. Optimisation of flow cytometric measurement of parasitaemia in plasmodium-infected mice. Int. J. Parasitol. 30: 649-653). The parasitemias below 15% are subsequently recounted on smears. The smears are fixed with methanol and then stained with Giemsa stain. The number of parasitized blood cells is counted under a microscope. The parasitemia is expressed as percentage of infected erythrocytes present in the specimen, on a sample of 2000 cells. The ED_{50} and ED_{90} values are determined at D5. The parasitemia is determined from the 1st to the

10th day of treatment, and then at the 15th, 22nd and 47th days.

The mice for which the smear at D5 reveals no trace of parasites will be checked again for at least one month after the end of the treatment, in order to detect any possible upsurge of parasites. The curative dose is the dose of product which ensures the survival of the entire treated batch of animals after one month.

Determination of ED_{50} values

0% inhibition corresponds to the mean of the parasitemias observed in the nontreated, infected mice. 100% inhibition corresponds to a very weak or null parasitemia, below 0.01%. The ED_{50} values are determined by linear interpolation of the dose-response curve represented in logarithm of concentrations.

2. Measurement of the antimalarial activity of T3 and of artesunate on a *Plasmodium vinckei petteri* parasite strain *in vivo*

In order to demonstrate a synergistic effect between the two compounds, it is important to know their activity when they are administered alone, in order to combine them at doses close to their ED_{50}. The case of parenteral administration thereof corresponds to the clinical situation of severe malaria. In this case, it is important to rapidly reduce the parasitemia over the first days of treatment. At the doses tested, artesunate administered alone does not reduce the parasitemia on the first day, and likewise on the following days (Figure 1). T3 effectively reduces the parasitemia after 4 days of treatment at a dose of 1 mg/kg/d, but does not prevent an increase in parasitemia after the first day of treatment (Figure 2).

The ED_{50} values obtained are given in Table 1 which follows:

Table I

	ED_{50} (mg/kg/day)
T3	0.55
artesunate	>2.5

The lower the ED_{50}, the better the activity. Thus, T3 alone shows a better activity on the parasitemia (0.55 mg/kg/day) than artesunate alone (>2.5 mg/kg/day). Furthermore, T3 alone, at a dose of 0.5 mg/kg/day, allows complete recovery after one month (results not shown), whereas artesunate alone, at a dose of 2.5 mg/kg/day, does not.

3. Measurement of the antimalarial activity of the T3/artesunate combination on a *Plasmodium vinckei petteri* parasite strain *in vivo*

For this combination study, the dose of 2.5 mg/kg/d of artesunate (slightly below but close to the ED_{50}) was chosen, the dose of 0.5 mg/kg/d being completely inactive on the parasitemia at 4 days. T3 was evaluated in combination at a lower dose, close to and above the ED_{50}, i.e. 0.2, 0.5 and 1 mg/kg/d.

Surprisingly, as indicated in Figures 3, 4 and 5, the combination comprising T3 (at all the abovementioned doses) with artesunate present at a dose of 2.5 mg/kg/day makes it possible to significantly reduce the level of parasitemia, by comparison with the separate administration of T3 alone or of artesunate alone. In particular, it is important to note that, 24 h after the first administration (day 2) and whatever the dose of T3 administered, a decrease in the parasitemia of the mice is observed, whereas, at this time point, the products administered individually are inactive. Table II (and Figures 3, 4 and 5) give the average of the parasitemias recorded at this day 2 (4 animals per dose). It should be noted that there is a statistically synergistic effect 24 hours after the first administration (day 2) for T3 at doses of 0.2, 0.5 and 1 mg/kg in combination with artesunate at a dose of 2.5 mg/kg. This very significant synergistic effect after the first day of treatment is particularly important in the case of the treatment of severe malaria, where the clinician is precisely seeking a massive and rapid reduction in the patient's parasitemia in order to ward off the lethal prognosis.

Table II

Artesunate (mg/kg/d)	T3 (mg/kg/d)	parasitaemia D1	parasitaemia D2	parasitaemia D5
0	0	7.20%	26.90%	death
2.5	0	7.00%	32.90%	58.20%
2.5	0.2	8.10%	5.70%	54.90%
2.5	0.5	8.30%	3.40%	15.10%
2.5	1	6.10%	5.10%	10.70%
0	0.2	6.60%	35.00%	55.20%
0	0.5	7.80%	34.00%	56.80%
0	1	7.20%	27.90%	19.40%

Starting from the T3 dose of 0.5 mg/kg/day combined with 2.5 mg/kg/day artesunate, the parasitemia is maintained at its initial value before treatment, throughout the duration of the treatment, whereas the individual administrations of products do not

enable this (Table II, Figures 3, 4 and 5).

The T3 compound is responsible for the predominant effect on complete recovery of the mice after one month. The coadministration of artesunate at 2.5 mg/kg/day does not improve the curative dose obtained with T3 alone.

Table III summarizes the results discussed above.

Table III

		T3 (mg/kg/d)			
		0	0.2	0.5	1
Artesunate (mg/kg/d)	0		Dose-dependent reduction in parasite growth from D3 to D5	Dose-dependent reduction in parasite growth from D3 to D5, curative dose	
	2.5	Dose-dependent reduction in parasite growth from D3 to D5	Reduction in parasitemia compared to D0, at D1	Reduction in parasitemia compared to D0, throughout the duration of the treatment	

Thus, when the 2 active ingredients are administered in combination, the parasitemia decreases significantly during the first 4 days of treatment. The effect is very much greater at doses comparable to, or even lower than, those determined for the administration of each of the two active ingredients taken individually.

In particular when T3 is administered in combination with artesunate, the artesunate being present at a dose of at least 2.5 mg/kg/day, a beneficial effect is observed on the decrease in parasitemia. It is therefore understood that the combination of the two active ingredients is particularly effective in a window defined by the minimum dose of one of the compounds and the ratio of one of the compounds relative to the other, within this window.

The results obtained *in vivo* in mice infected with *P. vinckei petteri* prove that the combination according to the invention, of artemisinin or a derivative thereof (in particular, artesunate), and of a bisthiazolium salt, which is a compound of formula (VI), in particular T3, is advantageous for the treatment of malaria, and in particular of severe malaria.

CLAIMS

1. Combination comprising, as active ingredients,

- a bisthiazolium salt, which is a compound of formula (VI):



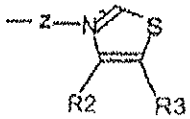
(VI)

in which

. Rb represents:

- a group R_1 which itself represents a hydrogen atom, a C_1 to C_5 alkyl radical, optionally substituted with an aryl radical (in particular a phenyl radical), a hydroxyl, an alkoxy, in which the alkyl radical contains from 1 to 5 carbon atoms, or aryloxy (in particular phenoxy),

- or else a group T, T representing the group of formula:



in which:

- R_2 represents a hydrogen atom, a C_1 to C_5 alkyl group or a -CH_2-COO-(C_1 to C_5)alkyl group;

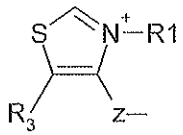
- R_3 represents a hydrogen atom, a C_1 to C_5 alkyl or alkenyl radical, as appropriate substituted with -OH, a phosphate group, an alkoxy radical, in which the alkyl radical is C_1 to C_3, or aryloxy; or an alkyl (or aryl)carbonyloxy group;

or else R_2 and R_3 together form a ring containing 5 or 6 carbon atoms; R_2 and R_3 may be linked so as to form a ring or a heterocycle containing 5 to 7 members, optionally comprising one or more heteroatoms O and/or S;

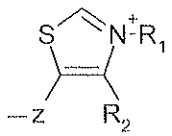
- Z represents a C_6 to C_{21}, in particular C_{12} or C_{13} to C_{21}, alkyl radical, as appropriate with insertion of one or more multiple bonds, and/or one or more heteroatoms O and/or S, and/or of one or more aromatic rings, and the pharmaceutically acceptable salts of these compounds,

with the proviso that Z does not represent a C_6 to C_8 alkyl radical, when Rc, Rd, R_2 and R_3 represent a methyl radical, or when Rc and Rd, on the one hand, and R_2 and R_3, on the other hand, together form aromatic rings containing 6 carbon atoms;

. Rd represents R_2 as defined above or P, P representing the group of formula:



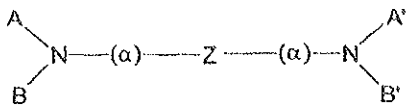
. Rc represents R_3 as defined above or U, U representing the group of formula:



it being understood that Rb = T, if Rc = R_3 and Rd = R_2; Rd = P, if Rc = R_3 and Rb = R_1; and Rc = U, if Rb = R_1, and Rd = R_2, in the form of a free base, of a salt, of a hydrate or of a solvate,

▪ or a precursor thereof chosen from the compounds of formulae (I) to (V),

▪▪ the compound (I) corresponding to the formula:

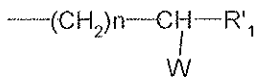


(I)

in which

- A and A' are identical to or different from one another and represent:

. either, respectively, a group A_1 and A'_1 of formula:



where n is an integer from 2 to 4; R'_1 represents a hydrogen atom, a C_1 to C_5 alkyl radical, optionally substituted with an aryl radical (in particular a phenyl radical), a hydroxyl, an alkoxy, in which the alkyl radical contains from 1 to 5 carbon atoms, or

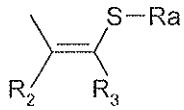
aryloxy (in particular phenoxy);
and W represents a halogen atom chosen from chlorine, bromine or iodine, or a nucleofugal group, such as the tosyl radical CH_3-C_6H_4-SO_3, the mesityl radical CH_3-SO_3, the CF_3-SO_3 radical or the NO_2-C_6H_4-SO_3 radical,

. or a group A_2 which represents a formyl radical -CHO or an acetyl radical -CO-CH_3,

- B and B' are identical to or different from one another and represent:

. either, respectively, the groups B_1 and B'_1, if A and A' represent, respectively, A_1 and A'_1, B_1 and B'_1 representing a group R_1 which has the same definition as R'_1 above, but cannot be a hydrogen atom,

. or, respectively, the groups B_2 and B'_2, if A and A' represent A_2, B_2 or B'_2 being the group R_1 as defined above, or a group of formula:



in which -Ra represents an RS- or RCO- group, where R is a linear, branched or cyclic, C_1 to C_6, in particular C_1 to C_5, alkyl radical, as appropriate substituted with one or more hydroxyl, alkoxy or aryloxy groups, or an amino group and/or a -COOH or COOM group, where M is a C_1 to C_3 alkyl; a phenyl or benzyl radical, in which the phenyl radical is, as appropriate, substituted with at least one C_1 to C_5 alkyl or alkoxy radical, said radicals being optionally substituted with an amino group, or with a nitrogenous or oxygenated heterocycle, a -COOH group or a -COOM group; or a group -CH_2-heterocycle, in which the heterocycle has 5 or 6 elements and is nitrogenous and/or oxygenated;

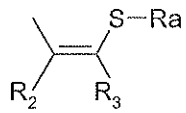
R_2 represents a hydrogen atom, a C_1 to C_5 alkyl radical or a -CH_2-COO(C_1 to C_5)alkyl group;

and R_3 represents a hydrogen atom; a C_1 to C_5 alkyl or alkenyl radical, as appropriate substituted with -OH, a phosphate group, an alkoxy radical, in which the alkyl radical is C_1 to C_3, or aryloxy; or an alkyl (or aryl)carbonyloxy group;

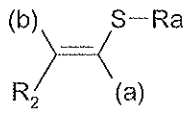
or else R_2 and R_3 together form a ring containing 5 or 6 carbon atoms; R and R_3 may be linked so as to form a ring containing 5- to 7 atoms (carbon, oxygen, sulphur);

- α represents:

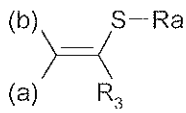
. either a single bond, when A and A' represent A1 and A'1: or when A and A' represent A_2, i.e. a -CHO or -$COCH_3$ group, and B_2 and B'_2 represent:



. or, when A and A' represent A_2 and B_2 and B'_2 represent R_1, a group of formula:



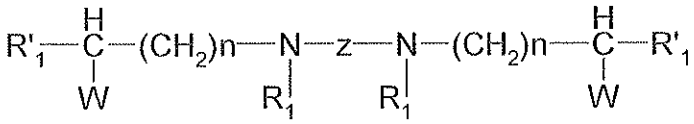
or a group of formula:



in which (a) represents a bond towards Z and (b) a bond towards the nitrogen atom,

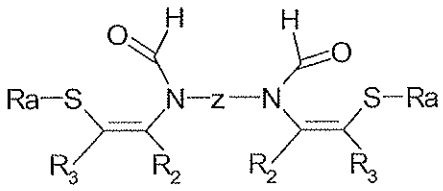
- Z represents a C_6 to C_{21}, in particular C_{12} or C_{13} to C_{21}, alkyl radical, as appropriate with insertion of one or more multiple bonds, and/or one or more heteroatoms O and/or S, and/or one or more aromatic rings, and the pharmaceutically acceptable salts of these compounds, with the proviso that R'_1 does not represent H or a C_1 or C_2 alkyl radical, when n = 3 or 4, R_1 represents a C_1 to C_4 alkyl radical and Z represents a C_6 to C_{10} alkyl radical;

▪▪ the compound (II) corresponding to the formula:



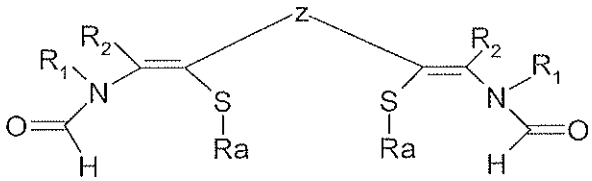
(II);

▪▪ the compound (III) corresponding to the formula:



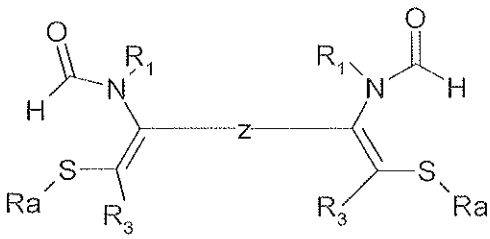
(III);

▪▪ the compound (IV) corresponding to the formula:



(IV);

▪▪ the compound (V) corresponding to the formula:



(V)

in which Ra, R_1, R'_1, R_2, R_3, W, n and Z are as defined above,

and

▪ artemisinin or a derivative thereof, chosen from artesunate, artemether, arteether, hydroartemisinin or dihydroartemisinin,
for the treatment and/or prevention of severe malaria.

2. Combination according to Claim 1, characterized in that the bisthiazolium salt, which is a compound of formula (VI), is chosen from:
- 1,12-dodecamethylenebis[4-methyl-5-(2-hydroxyethyl)thiazolium] dibromide;
- 1,12-dodecamethylenebis[4-methyl-5-(2-methoxyethyl)thiazolium] diiodide;
- 1,12-dodecamethylenebis(4-methylthiazolium) diiodide;
- 1,16-hexadecamethylenebis[4-methyl-5-(2-methoxyethyl)thiazolium] diiodide;
- 3,10-dioxadodecamethylenebis[5-(1,4-dimethyl)thiazolium] diiodide;
- 3,10-dioxadodecamethylenebis[5-(1-benzyl-4-methyl)thiazolium] dibromide;
- dodecamethylenebis[5-(1-methyl-4-ethoxycarbonylethyl)thiazolium] diiodide;
- dodecamethylenebis[5-(1-methyl-4-ethoxycarbonylethyl)thiazolium] dibromide;
- dodecamethylenebis[4-(1-methyl)thiazolium] diiodide;
- dodecamethylenebis[4-(1-benzyl)thiazolium] dibromide.

3. Combination according to either one of Claims 1 and 2, characterized in that each of the active ingredients is intended to be administered simultaneously or sequentially.

4. Pharmaceutical composition comprising therapeutically effective doses of artemisinin or of a derivative thereof, and of at least one bisthiazolium salt, which is a compound of formula (VI), or a precursor thereof, as defined in Claim 1, and also at least one pharmaceutically acceptable excipient, for the treatment and/or prevention of severe malaria.

5. Pharmaceutical composition according to Claim 4, characterized in that the bisthiazolium salt, which is a compound of formula (VI), is 1,12-dodecamethylenebis[4-methyl-5-(2-hydroxyethyl)thiazolium] dibromide.

6. Pharmaceutical composition according to Claim 4 or Claim 5, characterized in that the artemisinin derivative is artesunate.

7. Pharmaceutical composition according to any one of Claims 4 to 6, characterized in that it is suitable for oral, rectal or injectable administration, and preferably suitable for intravenous injection, particularly in the form of a drip.

8. Use of a combination according to any one of Claims 1 to 3, for the preparation of a medicament for use in the treatment or prevention of severe

malaria.

9. Kit for the treatment or prevention of severe malaria, comprising, on the one hand, at least one bisthiazolium salt, which is a compound of formula (VI) or a precursor thereof, and, on the other hand, at least artemisinin or a derivative thereof, as defined in Claim 1, said compound of formula (VI) or a precursor thereof and the artemisinin or a derivative thereof being in distinct compartments and being intended to be administered simultaneously or sequentially.

10. Kit according to Claim 10, characterized in that it comprises, on the one hand, at least 1,12-dodecamethylenebis[4-methyl-5-(2-hydroxyethyl)thiazolium] dibromide, and, on the other hand, at least artesunate.