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(54) Title: A COMBINATION KIT USED IN THE TREATMENT OF MALARIA

(57) Abstract: A combination kit for the treatment of malaria caused by *Plasmodium vivax* (*P. vivax*) having individual doses of an anti-malarial agent, 3-[1-[4-[(6-methoxy-8-quinolinyl)amino]penty]amino]ethylidene]-dihydro-2(3H)-furanone (I) in the form of capsules; individual doses of the anti-malarial agent, chloroquine in the form of tablets; and instruction material for the administration of the two anti-malarial drugs. The combination kit is particularly suited for a 6 days treatment regimen where the treatment is rendered by five tablets containing 500 mg of chloroquine phosphate (equivalent to 300 mg base), three to be taken on day one and one each on days two and three; and five capsules containing 25 mg of 3-[1-[4-[(6-methoxy-8-quinolinyl)amino]penty]amino]ethylidene]-dihydro-2(3H)-furanone (I), one each to be taken on days two to six.



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A Combination Kit Used In The Treatment Of Malaria

FIELD OF INVENTION:

The present invention relates to a combination kit for use in the treatment of malaria and a method for producing such a kit. Particularly, 5 the invention relates to a combination kit comprising anti-malarial agents, 3-[1-[[4-[(6-methoxy-8-quinoliny]amino]pentyl]amino]-ethylidene]-dihydro-2(3H)furanone and chloroquine. More particularly, the present invention relates to the use of the combination kit containing an anti-malarial agent, 3-[1-[[4-[(6-methoxy-8-quinoliny]amino]pentyl]- 10 amino]ethylidene]-dihydro-2(3H)-furanone and chloroquine against relapsing malaria caused by *Plasmodium vivax* for better patient compliance. The present invention further relates to a method for the treatment of malaria caused by *P. vivax*.

BACKGROUND OF THE INVENTION:

15 Malaria, caused by a parasitic protozoan called *Plasmodium*, is one of the most serious and complex tropical parasitic diseases. Generally human malaria is caused by four species of malarial parasites which are *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale* and *Plasmodium malariae*. Of these *P. falciparum* and *P. vivax* are most 20 widespread and cause most of the mortality and morbidity associated with these types of infections.

It is known that the malarial parasites undergo complex life cycle in humans, which is initiated through the bite of an infected female Anopheles mosquito. When the mosquito bites a host, some of the 25 sporozoites are injected into the bloodstream of the host and through the circulation they reach the liver where they multiply and liberate merozoites into the bloodstream which then invade the erythrocytes. In case of infections caused by *P. vivax*, most of the time the parasites

remain dormant in the liver which stage is termed hypnozoites.

Hypnozoites are reactivated and reinitiate blood stage parasitemias causing relapse. It has often been observed that people infected with *P. vivax* do not experience any symptoms for a very long period after their
5 initial illness but become symptomatic after certain period (Korean J. Intern Med, 1999 Jul; 14(2): 86-9).

A number of drugs ranging from those of natural origin to synthetic ones have been developed for the treatment of malaria. Quinine and artemisinin are the commonly known drugs of natural origin, which are
10 mostly used for the treatment of malaria. A number of synthetic anti-malarial drugs such as chloroquine, mefloquine, primaquine, halofantrine, amodiaquine, proguanil, maloprim are known in the literature. Of all the synthetic anti-malarial agents chloroquine has been the most widely prescribed drug for the treatment of malaria of all the
15 types, for more than last 60 years.

Chloroquine has been the effective treatment so far for the *P. vivax* malarial infections, however, some strains of *P. vivax* have shown resistance to this well known drug (*Ann. Trop. Med. Parasitol.*, 1999 Apr; 93(3): 225-230). In recent years drug resistant malaria has become one of
20 the most serious problems in malaria control. Drug resistance necessitates the use of drugs which are more expensive and may have dangerous side effects. To overcome the problems associated with drug resistance, treatments comprising combinations of anti-malarial agents are on the rise. A number of anti-malarial combinations are already
25 known in the malarial chemotherapy. For example, a combination of amodiaquine and tetracycline, a combination of sulfadoxine and pyrimethamine known as fansidar, are known therapies for the treatment of *P. falciparum*. Also fansimef, a combination of mefloquine with

sulfadoxine and pyrimethamine is used against multidrug resistant strains of *P. falciparum*.

United States Patent No. 5 998 449 describes a method for the treatment of malaria wherein combination of atovaquone and proguanil is used for the treatment of malaria. In US Patent No. 5 834 505, combination of
5 fenozan with another anti-malarial agent selected from artemisinin, sodium artesunate, chloroquine, mefloquine is described for the prophylactic and curative treatment of malaria.

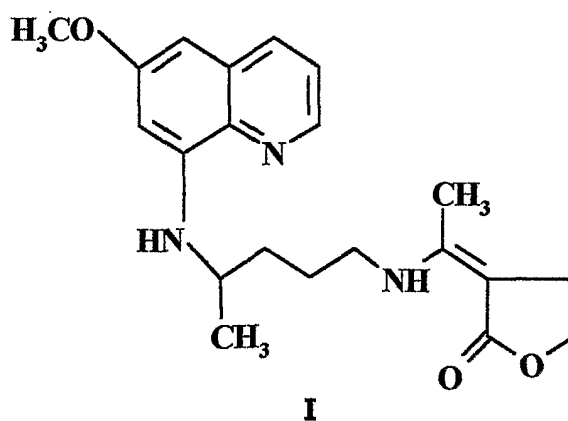
All the aforementioned anti-malarial combinations reported heretofore are
10 generally used for the treatment of *P. falciparum*. None of the standard anti-malarial combination treatment regimens have been found to be favourable for the treatment of *P. vivax* malaria which is the most relapsing type of malaria. For a very long time chloroquine was used for the treatment of infections caused by *P. vivax*, however, chloroquine
15 eradicates only the asexual erythrocytic stages of *P. vivax* and does not eliminate the hypnozoites. Until recently primaquine has been the drug of choice for the treatment of malarial relapse. Generally the standard therapy for the *P. vivax* malarial infection comprises of a sequential chloroquine-primaquine combination treatment regimen wherein
20 primaquine is administered for 14 days following the 3 days course of chloroquine. WHO (World Health Organisation) also recommends a 14 days primaquine treatment for *P. vivax* malarial infection. A shorter duration of chloroquine-primaquine treatment regimen was also tried out wherein primaquine was administered only for 5 days following the
25 chloroquine course. However, the outcome of the treatment was not encouraging, since the percentage relapse was more than the standard 14 days primaquine treatment regimen (Trans. R. Soc. Trop. Med. Hyg., 93(6), 641-643). Also primaquine is known to cause hemolytic anemia in persons deficient in the enzyme glucose-6-phosphate dehydrogenase

(G6PD) (Pharmacol Rev. 21: 73-103 (1969); Rev. Cubana Med trop, 1997; 49 (2):136-8). Moreover, methemoglobin toxicity is another predictable dose-related adverse effect associated with primaquine. Needless to say that in the case of sequential combination therapy the patient may not
5 complete the course once the symptoms of malaria are diminished, hence this may increase the chances of relapse. Thus, the chloroquine-primaquine treatment regimen is not safe with respect to toxicity of primaquine and has a further limitation from the standpoint of patient compliance due to longer duration of treatment.

10 Another anti-relapse agent namely tafenoquine is disclosed in United States Patent 4 617 394. Though more effective than primaquine, the drug was found to cause methemoglobin toxicity almost three times more than that of primaquine (Fundam. Appl. Toxicol. 1988, 10(2), 270-275), hence has drawbacks in terms of safety.

15 The compound, 3-[1-[[4-[(6-methoxy-8-quinolinyl)amino]pentyl]amino]ethylidene]-dihydro-2(3H)furanone] is a derivative of primaquine. It was described in Indian Patent Specification No. 158111 as 6-methoxy-8-(4-N-(3'-aceto-4',5'-dihydro-2-furanyl)amino)-1-methylbutylamino)quinoline , the structure of which was revised to that represented by the following
20 formula I. As per the revised structure, the compound is named 3-[1-[[4-[[6-methoxy-8-quinolinyl)amino]pentyl]amino]ethylidene]-dihydro-2(3H)-furanone (hereinafter referred to as compound I). The revised structure is described in WHO Drug Information Vol. 13, No. 4, pg. 268 (1999).

25



The compound of formula (I) has been found to be safer and less toxic than the parent compound primaquine (Am. J. Trop. Med. Hyg, 1989 Dec.; 41(6): 635-637). Its anti-relapse activity has been found to be comparable to primaquine.

5 Over the years primaquine was the only drug used for the radical cure of malaria caused by *P. vivax*. Primaquine is associated with a number of severe adverse effects, therefore there is a need to develop agents which are more effective and/or less toxic than primaquine. The compound I has been found to exhibit anti-relapse activity comparable to Primaquine
10 (Am. J. Trop. Med. Hyg., 41(6): 633-637 (1989)). However, this compound has been shown to cause less methemoglobin formation (Am. J. Trop Hyg., 41(6): 638-642 (1989)) and also has less effect on anti-oxidant defence enzymes than primaquine (Biochem Pharmacol. 46(10):1859-1860 (1993)). Thus, this primaquine derivative (I) is found to be less toxic
15 as compared to the parent drug, primaquine.

Therefore, there is a longfelt need for a more practical, effective, patient compliant and safe remedy for the radical cure of *P. vivax* malarial infection.

The inventors have found that the longfelt need may be fulfilled by
20 providing a treatment regimen consisting of regulated use of chloroquine and 3-[1-[[4-[(6-methoxy-8-quinolinyl)amino]pentyl]amino]ethylidene]-dihydro-2(3H)furanone of formula I over a period of between 5 to 8 days.

It has also been found that the treatment regimen may be executed most effectively and in a user friendly manner by providing a combination kit
25 which comprises two anti-malarial agents, namely chloroquine and 3-[1-[[4-[(6-methoxy-8-quinolinyl)amino]pentyl]amino]ethylidene]-dihydro-2(3H)furanone and an instruction material containing instructions for the administration of two anti-malarial agents during the period of treatment.

Thus the present invention relates to a combination kit for the treatment of *P. vivax* malaria for a period of between 5 to 8 days which comprises

- a) a predetermined dose of a first anti-malarial agent namely chloroquine;
- 5 b) a predetermined dose of second anti-malarial agent namely 3-[1-[[4-[(6-methoxy-8-quinoliny)amino]pentyl]amino]-ethylidene]-dihydro-2(3H)furanone;
- c) an instruction manual containing instructions for administering the two anti-malarial agents during the treatment period.

10 The present invention also relates to a method for producing a combination kit for the treatment of *P. vivax* malaria for a period of between 5 to 8 days which comprises:

- a) providing a predetermined dose of a first anti-malarial agent namely chloroquine;
- 15 b) providing a predetermined dose of second anti-malarial agent namely 3-[1-[[4-[(6-methoxy-8-quinoliny)amino]pentyl]-amino]ethylidene]-dihydro-2(3H)furanone;
- c) providing an instruction manual containing instructions for administering the two anti-malarial agents during the treatment
20 period.

The present invention further relates to a method of treatment of malaria caused by *P. vivax* comprising administering a first anti-malarial agent, chloroquine and a second anti-malarial agent, 3-[1-[[4-[(6-methoxy-8-quinoliny)amino]pentyl]amino]ethylidene]-dihydro-2(3H)furanone in
25 predetermined doses and in a predetermined sequence for a period of between 5 to 8 days.

DETAILED DESCRIPTION OF THE INVENTION

According to a preferred aspect of the invention there is provided a combination kit for the treatment of malaria *caused by P. vivax* for a period of between 5 to 8 days comprising:

- 5 1. individual doses of the anti-malarial agent, chloroquine in the form of tablets and;
2. individual doses of the anti-malarial agent, 3-[1-[[4-[(6-methoxy-8-quinolinyl)amino]pentyl]amino]ethylidene]-dihydro-2(3H)-furanone (I) in the form of capsules
- 10 3. instruction material for the administration of the two anti-malarial drugs.

In accordance with a typical embodiment of the invention, there is provided a combination kit for 6 days treatment of malaria caused *by P. vivax* comprising:

- 15 a) five tablets containing 500 mg of chloroquine phosphate (equivalent to 300 mg base).
- b) five capsules containing 25 mg of 3-[1-[[4-[(6-methoxy-8-quinolinyl)amino]pentyl]amino]ethylidene]-dihydro-2(3H)-furanone;
- 20 c) instruction material having directions to administer treatment in the following manner:
 - (i) to administer three tablets containing 500 mg of chloroquine phosphate (equivalent to 300 mg base) on day one;
 - 25 (ii) to administer one capsule containing 25 mg of compound I concurrently with a tablet containing 500 mg of

chloroquine phosphate (equivalent to 300 mg base)
from day two to three; and

(iii) to administer one capsule containing 25 mg of compound
I from day four to day six.

5 In accordance with a further typical embodiment of the invention, there is
provided a method for the treatment of malaria caused *by P. vivax* for a
period of six days comprising:

1. administering three tablets containing 500 mg of chloroquine
phosphate (equivalent to 300 mg base) on day one;
- 10 2. administering one capsule containing 25 mg of compound I
concurrently with a tablet containing 500 mg of chloroquine
phosphate (equivalent to 300 mg base) from day two to three; and
3. administering one capsule containing 25 mg of compound I from day
four to day six.

15 **THE KIT**

The kit used in the present invention may be one or more strips in which
the anti-malarial agents are packed individually or in combination. The
kit may further comprise an enclosure in the form of a small carton or
otherwise. The instruction is in the form of printed instructions provided
20 inside the carton. The instructions may also be printed on the carton
and/or on the strip or strips themselves. The instructions may be in
English and/or in any national or regional language. An illustration of an
instruction material for a 6 days treatment regimen is shown in Table 1
below, although other forms of instruction materials are not excluded
25 from the scope of the invention. The combination kit is prepared by
packing the strip/strips in a cardboard packaging on which the
instructions for the administration of the anti-malarial agents are
contained.

TABLE 1

INSTRUCTION MANUAL FOR 6 DAYS TREATMENT OF MALARIA
CAUSED BY *Plasmodium vivax*

	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6
CHLOROQUINE TABLET	Start with 2 tablets	1 tablet	1 tablet	X	X	X
	1 tablet after 6 hours	+	+			
CAPSULE OF COMPOUND OF FORMULA (I)	X	1 capsule	1 capsule	1 capsule	1 capsule	1 capsule

5

The radical curative anti-malarial activity and toxicity of the compound I was evaluated and compared with that caused by primaquine. The curative activity of compound I was evaluated against *P. cynomolgi* (a *Plasmodium* species which closely resembles the human malarial parasite *P. vivax*), in rhesus monkeys for 7 days and the compound was found to be 100% curative in the dose range of 1.25 mg/kg to 4 mg/kg Also there was no relapse observed up to 100 days after stopping the treatment.

The efficacy of the combination kit of the present invention, containing compound I (25 mg) was clinically evaluated and compared with primaquine (15 mg) used in combination with chloroquine in a group of

15

patients over a one year surveillance study. The overall study confirmed that the combination kit of the present invention can be used effectively in *P. vivax* malarial infection and that the compound I is as effective as primaquine with respect to radical curative activity.

- 5 Although equipotent to primaquine, compound I has been found to be safer than primaquine with respect to the toxicity related to methemoglobin formation. In a comparative toxicity study of compound I with primaquine in healthy human volunteers, it was found that the methemoglobin level increased from 2.29 % to 3.02 % in case of
10 compound I (25 mg daily for 7 days) as against the increase in methemoglobin level from 3.97 % to 16.23% in case of primaquine (at 15 mg daily for 7 days). Thus, the methemoglobin toxicity study proves that the compound I is distinctly safer than primaquine.

- The invention thus includes the use of this safer anti-relapse agent,
15 compound I with chloroquine in the form of a combination kit for the radical cure of *P. vivax* malaria. According to a typical aspect described above, the total course of treatment wherein compound I is administered concurrently with chloroquine lasts for 6 days only whereas in the chloroquine-primaquine treatment regimen, the total course of the
20 effective treatment is 17 days.

Thus, the novel combination therapy of the present invention comprising the use of compound I in combination with chloroquine has distinct advantages in terms of safety and improved patient compliance due to shorter duration of treatment.

- 25 Hence, the use of the combination kit of the present invention containing chloroquine and the compound I becomes a very useful treatment from the standpoint of low toxicity. In addition to this, concurrent administration of the drugs and shorter duration of the treatment may also improve patient compliance.

The chloroquine tablets and capsules of compound I may be obtained commercially or prepared by conventional methods. For instance, the capsules containing compound I may be prepared by first mixing appropriate quantities of compound I along with the excipients lactose, colloidal silicon dioxide and magnesium stearate in an octagonal blender to obtain a powdered mixture and further filling hard gelatine capsule shells with the resulting mixture. The capsules are blister packed using approved PVC film and aluminium foil.

The dosage of the drugs depends on the need of an individual and the dosages described herein are adult doses. However, this invention is not limited to the dosage of the combination regimen described herein and may be varied according to medical advice. Accordingly the specific dosage described in the typical embodiment is only illustrative and non-limiting combination kits for other dosage forms are also included in the scope of this invention.

The term relapse is used herein to indicate that the symptoms of malaria recur.

The invention will now be described in further details with respect to the following non-limiting examples:

20 Example 1:

Combination kit consisting of chloroquine tablets and capsules of 3-[1-[[4-[(6-methoxy-8-quinolinyl)amino]pentyl]amino]ethylidene]-dihydro-2-(3H)furanone (I)

A. Each tablet contains 500 mg of chloroquine phosphate equivalent to 300 mg chloroquine base. The tablet containing chloroquine may be prepared by conventional techniques.

B. Each capsule contains 25 mg of 3-[1-[[4-[(6-methoxy-8-quinolinyl)amino]pentyl]amino]ethylidene]-dihydro-2(3H)furanone (I).

The capsule containing compound I may be prepared according to the following formulation by the procedure as described:

5	Compound I	25 mg
	Lactose	250 mg
	Colloidal silicon dioxide	2 mg
	Magnesium stearate	10 mg

Procedure:

10 Compound I (25 mg), lactose (250 mg), colloidal silicon dioxide (2 mg) are separately sifted through an S. S. screen no. 40 fitted on a vibratory sifter and transferred to a octagonal blender and the contents are mixed for 40-45 minutes. Then magnesium stearate (10 mg) is sifted through a S. S. screen no. 40 fitted on a vibratory sifter
15 and transferred to the octagonal blender. The contents are further mixed for 10-15 minutes. The resulting powdered mixture is then filled in size '2' double locking gelatine capsule shells which are further polished using a capsule polishing machine.

20 C. The patients may be given the following treatment over a period of six days.

- Day 1 : Three tablets of chloroquine phosphate 500 mg (equivalent to 300 mg base).
- Day 2 : One tablet of chloroquine phosphate 500 mg (equivalent to 300 mg base) and one capsule containing 25 mg of compound I.
- 25 Day 3 : One tablet of chloroquine phosphate 500 mg (equivalent to 300 mg base) and one capsule containing 25 mg of compound I.

Day 4 : One capsule containing 25 mg of compound I.

Day 5 : One capsule containing 25 mg of compound I.

Day 6 : One capsule containing 25 mg of compound I.

Example 2 :

- 5 Combination regimen of chloroquine and 3-[1-[[4-[(6-methoxy-8-quinolinyl)amino]pentyl]amino]ethylidene]-dihydro-2(3H)furanone(I) tested against *P. cynomolgi* in rhesus monkeys.

The therapeutic effect of the combination regimen consisting of chloroquine and 3-[1-[[4-[(6-methoxy-8-quinolinyl)amino]pentyl]amino]ethylidene]-4,5-dihydro-2(3H)furanone (I) was tested against *P. cynomolgi* in rhesus monkeys. 3-[1-[[4-[(6-methoxy-8-quinolinyl)-amino]pentyl]amino]ethylidene]-dihydro-2(3H)furanone(I) was administered in the dose level of 4.00 mg/kg (to 3 monkeys), 3.00 mg/kg (to 3 monkeys), 2.50 mg/kg (to 4 monkeys), 2.00 mg/kg (to 3 monkeys) and 1.25 mg/kg (to 9 monkeys) for 7 days. Chloroquine was administered as a companion drug at 5mg /kg (base) dose. Minimum curative dose of compound I was found 1.25 mg/kg in 9 monkeys. Doses higher than 1.25mg/kg i.e. 2.0 mg/kg (base), 3.00 mg/kg (base), 2.50 mg/kg (base), and 4.00 mg/kg (base) were also found to be curative in 3 monkeys, 3 monkeys, 4 monkeys and 3 monkeys respectively. After stopping the treatment all the monkeys were observed over a period of 100 days and the blood smears remained negative in all the monkeys.

Example 3:

Toxicity study data:

- 25 3-[1-[[4-[(6-methoxy-8-quinolinyl)amino]pentyl]amino]ethylidene]-dihydro-2(3H)furanone (I) was evaluated vis-à-vis primaquine for toxicity related to methemoglobin formation in human.

The methemoglobin toxicity of compound I was evaluated vis-à-vis that of primaquine in normal human volunteers. It was found that when the human subjects were administered with 25 mg daily dose of compound I for 7 days the methemoglobin level rose from 2.29 % to 3.02 % and in
5 case of 15 mg daily dose of primaquine for 7 days the methemoglobin level rose from 3.97 % to 16.23 %.

It is thus evident from above data that the compound I is safer than primaquine.

Claim

1. A combination kit for the treatment of malaria caused by *Plasmodium vivax* (*P. vivax*) which comprises:
 - a) a predetermined dose of a first anti-malarial agent namely chloroquine;
 - b) a predetermined dose of second anti-malarial agent namely 3-[1-[[4-[(6-methoxy-8-quinolinyl)amino]pentyl]amino]-ethylidene]-dihydro-2(3H)furanone];
 - c) an instruction manual containing instructions for administering the two anti-malarial agents during the treatment period.
2. A combination kit as claimed in claim 1, wherein there is provided;
 - 1) individual doses of an anti-malarial agent, 3-[1-[[4-[(6-methoxy-8-quinolinyl)amino]pentyl]amino]ethylidene]-dihydro-2(3H)-furanone (I) in the form of capsules and;
 - 2) individual doses of the anti-malarial agent, chloroquine in the form of tablets.
 - 3) instruction material for the administration of the two anti-malarial drugs.
3. A combination kit according to claim 1 or 2, wherein the anti-malarial agents are provided for 6 days treatment in the following manner:
 - a) five tablets containing 500 mg of chloroquine phosphate equivalent to 300 mg base);
 - b) five capsules containing 25 mg of 3-[1-[[4-[(6-methoxy-8-quinolinyl)amino]pentyl]amino]ethylidene]-dihydro-2(3H)-furanone (I);

- c) instruction material having directions to administer treatment in the following manner:
- (i) to administer three tablets containing 500 mg of chloroquine phosphate (equivalent to 300 mg base) on day one;
 - 5 (ii) to administer one capsule containing 25 mg of compound I concurrently with a tablet containing 500 mg of chloroquine phosphate (equivalent to 300 mg base) from day two to three; and
 - (iii) to administer one capsule containing 25 mg of
10 compound 1 from day four to day six.
4. A combination kit as claimed in anyone of the preceding claims, which is in the form of one or more strips in which the anti-malarial agents are packed.
5. A combination kit as claimed in anyone of the preceding claims, which
15 is in the form of a small carton or otherwise, in which the instruction is in the form of a printed instructions provided inside the carton.
6. A combination kit as claimed in any of claims 1 to 5, wherein the instructions are in the form of prints on the carton and/or on the strip or strips.
- 20 7. A method for producing the combination kit according to claim 1, comprising :
- a) providing a predetermined dose of a first anti-malarial agent namely chloroquine;
 - b) providing a predetermined dose of a second anti-malarial agent namely 3-[1-[[4-[(6-methoxy-8-quinoliny]amino]-
25 pentyl]amino]ethylidene]-dihydro-2(3H)furanone (I); and

- c) providing an instruction manual containing instructions for administering the two anti-malarial agents during the treatment period.
8. A method for producing a combination kit as claimed in claim 7, wherein there is provided
- 1) individual doses of the anti-malarial agent chloroquine are provided in the form of tablets;
 - 2) individual doses of an anti-malarial agent namely 3-[1-[[4-[(6-methoxy-8-quinoliny]amino]pentyl]amino]ethylidene]-dihydro-2(3H)furanone(I) are provided in the form of capsules;
 - 3) instruction material is provided for the administration of two anti-malarial drugs.
9. A method of treatment of malaria caused by *P. vivax* comprising administration of a first anti-malarial agent, chloroquine and a second anti-malarial agent 3-[1-[[4-[(6-methoxy-8-quinoliny]amino]pentyl]- amino]ethylidene]-dihydro-2(3H)furanone (I) in predetermined doses and a predetermined sequence over a period of between 5 to 8 days.
10. A method for the treatment of *P. vivax* malaria as claimed in claim 7, for a treatment period of 6 days comprising:
- 1) administering three tablets containing 500 mg of chloroquine phosphate (equivalent to 300 mg base) on day one;
 - 2) administering one capsule containing 25 mg of compound I concurrently with a tablet containing 500 mg of chloroquine phosphate (equivalent to 300 mg base) from day two to three; and

- 3) administering one capsule containing 25 mg of compound I from day four to day.

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