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# Antimalarial qinghaosu/artemisinin: The therapy worthy of a Nobel Prize

Jerapan Krungkrai[1*], Sudaratana Rochanakij Krungkrai[2]

[1]*Department of Biochemistry, Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand*

[2]*Unit of Biochemistry, Department of Medical Science, Faculty of Science, Rangsit University, Patumthani 12000, Thailand*

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ABSTRACT

Malaria is a major cause of human morbidity and mortality in the tropical endemic countries worldwide. This is largely due to the emergence and spread of resistance to most antimalarial drugs currently available. Based on the World Health Organization recommendation, artemisinin-based combination therapies are now used as first-line treatment for *Plasmodium falciparum* malaria. Artemisinin or qinghaosu (Chinese name) and its derivatives are highly potent, rapidly acting antimalarial drugs. Artemisinin was discovered in 1971 by a Chinese medical scientist Youyou Tu, who was awarded the Nobel Prize in 2015 on her discovering the antimalarial properties of qinghaosu from the traditional Chinese qinghao plant. Nevertheless, artemisinin resistance in falciparum malaria patients has first emerged on the Thai-Cambodian border in 2009, which is now prevalent across mainland Southeast Asia from Vietnam to Myanmar. Here, we reviewed malaria disease severity, history of artemisinin discovery, chemical structure, mechanism of drug action, artemisinin-based combination therapies, emergence and spread of drug resistance, including the recent findings on mechanism of resistance in the falciparum malaria parasite. This poses a serious threat to global malaria control and prompts renewed efforts for the urgent development of new antimalarial drugs.

## 1. Malaria burden and cause of deaths

Malaria is one of the oldest and important parasitic diseases in humans, with almost half the world's population at risk of infection, responsible for 515 million cases in 96 subtropical and tropical endemic countries. The countries of sub-Saharan Africa account for the majority of all malaria cases, with the remainder mostly concentrated in Brazil, Turkey, India, Afghanistan, Sri Lanka, Indonesia, Vietnam, Myanmar, Cambodia, Thailand, and China. The death toll is reported at 1.3 million people each year, mostly young children in sub-Saharan Africa (90%), Southeast Asia (7%) and the Eastern Mediterranean Region (2%) [1,2]. Transmitted through the bite of an infected female *Anopheles* spp. mosquito, malaria parasite is a single cell eukaryotic organism that belongs to the genus *Plasmodium*. In humans, *Plasmodium falciparum* (*P. falciparum*), the most dangerous one of the five human malaria parasites that include *Plasmodium vivax*, *Plasmodium ovale*, *Plasmodium malariae*, and *Plasmodium knowlesi* (a simian parasite that occasionally infects man), is responsible for the majority of deaths.

The morbidity and mortality in young children under 5-year-old can be due to (1) infection in pregnant women, resulting to low birth weight and death in the first month of life; (2) overwhelming acute infections, resulting to coma (*i.e.*, cerebral malaria), respiratory distress, and hypoglycemia; (3) chronic and repeated infections, leading to severe anemia [3] (Figure 1).

For many decades, efforts to eradicate malaria have been met with the emergence of resistance to most antimalarial drugs such as chloroquine, mefloquine, amodiaquine, sulfadoxine–pyrimethamine combination (fansidar), insecticidal resistance and other ecological concerns [4,5]. It is also well known that malaria chemotherapy has relied on a limited number of drugs, consequently resulting to the acquisition and spread of drug resistance leading to increasing morbidity and mortality in malaria endemic countries in recent years [6]. In view of this status, the World Health Organization (WHO) has recommended the use of artemisinin-based combination therapy (ACT) as the first-line drug for the treatment of patients with

*Corresponding author: Prof. Dr. Jerapan Krungkrai, Department of Biochemistry, Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand.
Tel: +662 2564482
Fax: +662 2524986
E-mails: jerapan.k@chula.ac.th, jerapan.k@gmail.com

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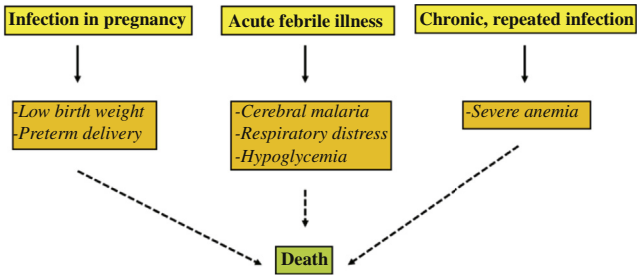
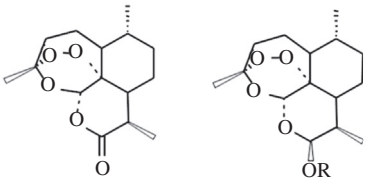



**Figure 1.** Malaria morbidity and mortality in young children under 5-year-old.
Most of the deaths occurred in the sub-Saharan Africa (90%), followed by the Southeast Asia (7%) and the Eastern Mediterranean Region (2%).



| | | |
|---|---|---|
| Artemisinin (Qinghaosu) | Dihydroartemisinin | R = H |
| | Arteether | R = Et |
| | Artemether | R = Me |
| | Artesunic acid | R = $(OC)CH_2CH_2$ COOH |
| | Artelinic acid | R = $CH_2Ph(COOH)$-P |

**Figure 2.** Chemical structures of qinghaosu/artemisinin and its derivatives.

uncomplicated falciparum malaria since 2001 [7]. The implementation of ACT, and the use of insecticidal-treated mosquito bed nets and indoor residual spraying resulted in substantial recent declines in morbidity and mortality due to malaria [8].

## 2. Qinghaosu/artemisinin discovery and the Nobel Prize award

In 1967, a drug discovery project was set up in China, named Project 523, conducted by Youyou Tu at the Chinese Academy of Medical Sciences in Beijing. Her team had screened over 2 000 traditional Chinese recipes and made 380 herbal extracts which were tested on malaria-infected mice [9]. A herbal extract used for over 1 600 years in traditional Chinese therapy for "intermittent fever" the hallmark symptom of malaria, was found effective [10]. The extract from qinghao or huanghuahao (*Artemisia annua* L.), named qinghaosu, was isolated by low temperature ethyl ether extraction and chemically characterized in 1971. The active antimalarial moieties and the physicochemical properties were determined *in vitro* and *in vivo* in both animal models and in human. The drug was distributed to the rest of the world in 1979 [10]. Tu was awarded the Nobel Prize in Medicine on October 5, 2015, for her discovery of qinghaosu/artemisinin and the more potent derivative dihydroartemisinin, effective antimalarial drugs which saved millions of lives [11].

## 3. Qinghaosu/artemisinin structure and mechanism of action

The chemical structure of qinghaosu/artemisinin is a 15-carbon sesquiterpene lactone bearing an endoperoxide group, which is essential for antimalarial activity [12]. Dihydroartemisinin is an active metabolite. To increase solubility of qinghaosu/artemisinin, arteether and artemether were synthesized as lipid soluble, and artesunate and artelinic acid as water soluble derivatives. The chemical structure qinghaosu/artemisinin and its derivatives are illustrated in Figure 2.

Artemisinin derivatives are the most rapid acting and efficacious antimalarial drugs currently available. Antimalarial activity, defined as $IC_{50}$, against *P. falciparum in vitro* culture is ~0.6–1.1 nmol/L for asynchronous or mixed intra-erythrocytic stages. The ring-stage parasite is more susceptible at $IC_{50}$ of ~0.3 nmol/L, compared to trophozoite and schizont stages ($IC_{50}$ ~5.0 nmol/L) [13]. The drugs are also effective for young *P. falciparum* sexual gametocytes, the parasite stage which transmit the infection to others, as well as *P. vivax* asexual blood stages, but have no effect on the liver stages or the pre-erythrocytic parasite development.

After oral or parenteral administration of artemisinin in both healthy humans and in patients infected with *P. falciparum*, the half-life of the drug is very short (within ~1 h). When measured in the blood, the drug and its major metabolite, dihydroartemisinin, peaks ~1–2 h after administration with concentrations of ~10–30 μmol/L (~2.4–4.0 mg/kg body weight). The drug is then eliminated to inactive metabolites by human cytochrome P2B6 glucuronidation [12].

At present, the mechanism of the antimalarial action of artemisinin remains a topic of considerable debate. Artemisinin might have multiple sites of action for its rapid killing effect [12–14]. One of the well recognized mechanisms is summarized. Free or heme-bound iron (Fe) catalyzes the conversion of drug to free radicals, *i.e.*, the reduction of the endoperoxide bridge by an electron from $Fe^{2+}$ to a free radical and the ferrous iron to $Fe^{3+}$. The free radicals alkylate and oxidize proteins as well as lipids, resulting in the rapid killing of the parasite (Figure 3) [15]. The mode of action is consistent to the first evidence reported that artemisinin's antimalarial activity is potentiated by oxidizing agents and attenuated by reducing agents [13]. However, this phenomenon should occur in the food vacuole of the parasite, especially during hemoglobin digestion to release amino acids for survival. Other targets for artemisinin action include: (1)

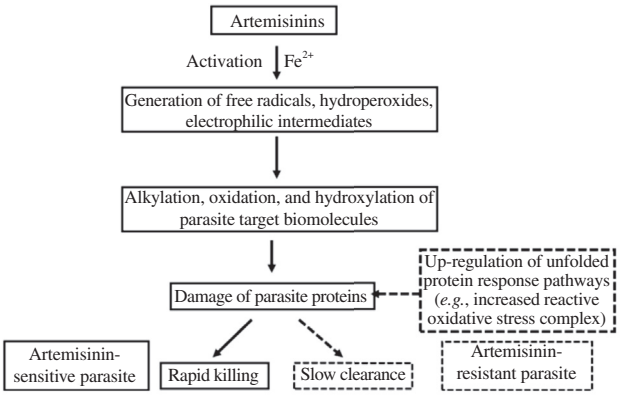



**Figure 3.** Mechanism of action and resistance to qinghaosu/artemisinin.
The activation by $Fe^{2+}$ releases free radicals, hydroperoxides, and other intermediates, and the addition of these products to target biomolecules, (*e.g.*, proteins, forming damaged proteins), leads to rapid parasite death (shown in solid line symbols). The resistant parasites have up-regulated unfolded protein response pathways including reactive oxidative stress complex, decreasing protein damage caused by artemisinin action and slowing parasite growth rate (shown in broken line symbols).

activation of mitochondrial electron transport system resulting in reactive oxygen species production [16,17]; (2) inhibition of mitochondrial oxygen utilization through cytochrome c oxidase complex [18]; (3) inhibition of sarcoplasmic reticulum calcium adenosine triphosphatase [19]. To date, research studies underscore the incompletely understood mode of action for artemisinin.

## 4. Qinghaosu/artemisinin treatment as ACT

In monotherapy, artemisinin acts rapidly against the parasites and has faster clearance of the parasites from the blood than any other antimalarial drugs, resulting to faster relief of clinical symptoms [12,20]. However, patients must take the drug for at least 7 days to maximize cure rates due to its very short half-life, otherwise some parasites could escape from the action of the drug during treatment. An approach to circumvent this problem is to use combination therapy comprising of artemisinin derivatives plus another antimalarial drug with longer half-life and a different mode of action. This approach is known as ACT [7]. The partner of antimalarial drugs for ACTs were shown in Figure 4. ACTs can be taken for shorter durations (less than 3 days) than artemisinin monotherapy, and importantly, can increase patient compliance thus reducing the risk of resistant parasites arising during therapy. ACTs such as artesunate-mefloquine, artemether-lumefantrine, artesunate-amodiaquine and artesunate-sulfadoxine/pyrimethamine are currently used in many disease endemic countries (Table 1) [5,12,20–22]. The first ACT, artesunate-mefloquine, was deployed on the north-west border of Thailand in 1994, an area of the mefloquine-resistant parasites, and has retained efficacy over 14 years [12]. ACTs are now recommended by the WHO as the first-line treatment for all falciparum malaria parasites in malaria endemic countries of the world [23].

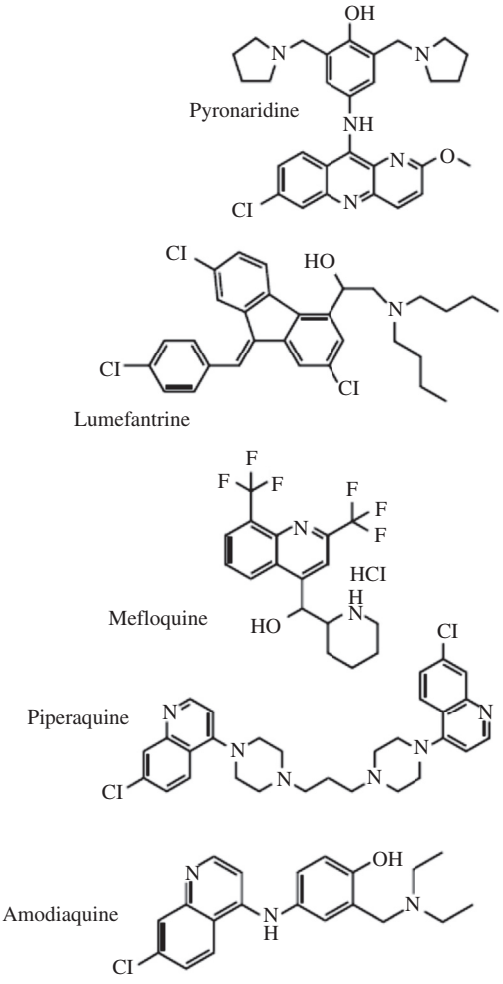



**Figure 4.** Chemical structures of partner drugs for qinghaosu/artemisinin-based combination.

## 5. Resistance of qinghaosu/artemisinin in human falciparum malaria patients and spread of resistance across mainland Southeast Asia

In 2009, there was a first report on artemisinin resistance or decreased efficacy by slowing parasite clearance in artesunate-mefloquine treatment in the falciparum malaria patients on the Thai–Cambodian border, where artemisinin remains, until that time, highly effective since its use in 1994 [24]. The WHO containment program of artemisinin resistant malaria in a limited area have also not been successful [25,26]. In 2014, Ashley *et al.* reported the spread of artemisinin resistance to *P. falciparum*, which became prevalent across mainland Southeast Asia from Vietnam to Myanmar [27].

## 6. Mechanism of qinghaosu/artemisinin resistance and molecular marker

The phenotype of artemisinin-resistant parasites in human patients is not linked to known candidate genes for drug resistance and genetic polymorphism in *pfatp6, pfcrt, pfmdr1, ubp-1*, and the 6-kb mitochondrial genome [28]. However, using a large multicenter genome-wide association study with 1 063 human malaria patient isolates in 2015, it was demonstrated that the gene polymorphisms of ferredoxin, *arps10*, *pfcrt*, and *pfmdr2*, are strongly associated with artemisinin resistance across 15 locations in Southeast Asia [29]. Moreover, resistance was linked to a point mutation in the propeller domain of the *P. falciparum* kelch protein encoded by the gene *kelch* on chromosome 13, otherwise known as K13-propeller [29,30]. As mutations on this region confer artemisinin resistance, the molecular marker for K13-propeller becomes an important tool, substantiated from recent findings on the spread of artemisinin resistance across mainland Southeast Asia [27,29,30].

By using population transcriptomics of 1 043 *P. falciparum* isolates from patients with acute malaria, Mok *et al.* have found that artemisinin resistance is associated with increased expression of unfolded protein response pathways involving at least 13 proteins in two chaperonin complexes, *e.g.*, reactive oxidative stress complex [31]. The up-regulated unfolded protein response pathways mitigate protein damage caused by $Fe^{2+}$-activated artemisinin in the artemisinin-resistant parasites. The resistant parasites increase their capacity to quickly repair or degrade proteins that are damaged by the action of artemisinin in human patients (Figure 3).

## 7. Conclusions and future perspectives

Up to now, the efficacy of artemisinin has declined in human falciparum patients across mainland Southeast Asia. The WHO has launched a program for prevention or containment of the

**Table 1**
Pipeline of qinghaosu/artemisinin-based combination therapies.

| Active ingredients (qinghaosu-partner drug) | Partnership | Product name |
|---|---|---|
| Artesunate-mefloquine | Far-Manguinhos, DNDi | – |
| Artesunate-amodiaquine | Sanofi-aventis, DNDi | Coarsucam |
| Artesunate-pyronaridine | Shin Poong, MMV | Pyramax |
| Artesunate-ferroquine | Sanofi-aventis | Ferroquine |
| Artemether-lumefantrine | Novartis, MMV | Coartem |
| Dihydroartemisinin-piperaquine | ST, MMV; CQ, Holley | Eurartesim, Artekin, Duocotexin |
| Artesunate-S/P | – | – |

Artesunate S/P: Artesunate-sulfadoxine/pyrimethamine; DNDi: Drugs for neglected disease initiative; MMV: Medicine for malaria ventur; ST: Sigma-Tau; CQ: Chongqing.

artemisinin-resistant parasite. The Tracking Resistance to Artemisinin Collaboration was created in 2011 to provide evidence and tools to halt or slow the spread of resistance [27]. The stage is also set to further support the development of new antimalarial drugs to fight against this disease with high morbidity and mortality rates in many endemic countries. Hopefully, there are at least 17 novel antimalarial compounds that are in preclinical and clinical phase I–III development [32,33]. Genomics, transcriptomics and proteomics offer great benefit for understanding not only artemisinin resistance and also new drug discovery [29–34]. A combination of new drugs and malaria vaccines with efficient vector control measures will lead to effective global malaria control programs for our malaria-free world [35,36].

## Conflict of interest statement

We declare that we have no conflict of interest.

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## References

[1] Guerin PJ, Olliaro P, Nosten F, Druilhe P, Laxminarayan R, Blinka F, et al. Malaria: current status of control, diagnosis, treatment, and a proposed agenda for research and development. *Lancet Infect Dis* 2002; **2**: 564-73.
[2] Hay SI, Okiro EA, Gething PW, Patil AP, Tatem AJ, Guerra CA, et al. Estimating the global clinical burden of *Plasmodium falciparum* malaria in 2007. *PLoS Med* 2010; **7**(6): e1000290.
[3] Murphy SC, Breman JG. Gaps in the childhood malaria burden in Africa: cerebral malaria, neurological sequelae, anemia, respiratory distress, hypoglycemia, and complications of pregnancy. *Am J Trop Med Hyg* 2001; **64**: 57-67.
[4] Fidock DA, Eastman RT, Ward SA, Meshnick SR. Recent highlights in antimalarial drug resistance and chemotherapy research. *Trends Parasitol* 2008; **24**: 537-44.
[5] Olliaro P, Wells TN. The global portfolio of new antimalarial medicines under development. *Clin Pharmacol Ther* 2009; **85**: 584-95.
[6] World Health Organization. World malaria report 2008. Geneva: World Health Organization; 2008. [Online] Available from: http://apps.who.int/iris/bitstream/10665/43939/1/9789241563697_eng.pdf [Accessed on 1st December, 2015]
[7] World Health Organization. Antimalarial drug combination therapy (archived): report of WHO technical consultation. Geneva: World Health Organization; 2001. [Online] Available from: http://apps.who.int/iris/bitstream/10665/66952/1/WHO_CDS_RBM_2001.35.pdf [Accessed on 1st December, 2015]
[8] World Health Organization. *Fact sheet: world malaria report 2015*. Geneva: World Health Organization; 2015.
[9] Miller LH, Su X. Artemisinin: discovery from the Chinese herbal garden. *Cell* 2011; **146**: 855-8.
[10] Antimalaria studies on qinghaosu. *Chin Med J (Engl)* 1979; **92**: 811-6.
[11] Nobel Media AB. Youyou Tu – facts. Stockholm: Nobel Media AB; 2015. [Online] Available from: http://www.nobelprize.org/nobel_prizes/medicine/laureates/2015/tu-facts.html [Accessed on 13th November, 2015]
[12] White NJ. Qinghaosu (artemisinin): the price of success. *Science* 2008; **320**: 330-4.
[13] Krungkrai SR, Yuthavong Y. The antimalarial action on *Plasmodium falciparum* of qinghaosu and artesunate in combination with agents which modulate oxidant stress. *Trans R Soc Trop Med Hyg* 1987; **81**: 710-4.
[14] Krungkrai J, Kanchanaphum P, Pongsabut S, Krungkrai SR. Putative metabolic roles of the mitochondria in asexual blood stages and gametocytes of *Plasmodium falciparum*. *Asian Pac J Trop Med* 2008; **1**: 31-49.
[15] Meshnick SR, Taylor TE, Kamchonwongpaisan S. Artemisinin and the antimalarial endoperoxides: from herbal remedy to targeted chemotherapy. *Microbiol Rev* 1996; **60**: 301-15.
[16] Li W, Mo W, Shen D, Sun L, Wang J, Lu S, et al. Yeast model uncovers dual roles of mitochondria in action of artemisinin. *PLoS Genet* 2005; **1**(3): e36.
[17] Wang J, Huang L, Li J, Fan Q, Long Y, Li Y, et al. Artemisinin directly targets malarial mitochondria through its specific mitochondrial activation. *PLoS One* 2010; **5**(3): e9582.
[18] Krungkrai J, Burat D, Kudan S, Krungkrai S, Prapunwattana P. Mitochondrial oxygen consumption in asexual and sexual blood stages of the human malarial parasite *Plasmodium falciparum*. *Southeast Asian J Trop Med Public Health* 1999; **30**: 636-42.
[19] Eckstein-Ludwig U, Webb RJ, Van Goethem ID, East JM, Lee AG, Kimura M, et al. Artemisinins target SERCA of *Plasmodium falciparum*. *Nature* 2003; **424**: 957-61.
[20] White NJ. Antimalarial drug resistance. *J Clin Invest* 2004; **113**: 1084-92.
[21] Kremsner PG, Krishna S. Antimalarial combinations. *Lancet* 2004; **364**: 285-94.
[22] Sinclair D, Zani B, Donegan S, Olliaro P, Garner P. Artemisinin-based combination therapy for treating uncomplicated malaria. *Cochrane Database Syst Rev* 2009; http://dx.doi.org/10.1002/14651858.CD007483.pub2.
[23] World Health Organization. *Guidelines for the treatment of malaria*. 2nd ed. Geneva: World Health Organization; 2010.

[24] Dondorp AM, Nosten F, Yi P, Das D, Phyo AP, Tarning J, et al. Artemisinin resistance in *Plasmodium falciparum* malaria. *N Engl J Med* 2009; **361**: 455-67.
[25] World Health Organization. Global malaria control and elimination: report of a meeting on containment of artemisinin tolerance. Geneva: World Health Organization; 2008. [Online] Available from: http://apps.who.int/iris/bitstream/10665/43917/1/9789241596817_eng.pdf [Accessed on 2nd December, 2015]
[26] Dondorp AM, Yeung S, White L, Nguon C, Day NP, Socheat D, et al. Artemisinin resistance: current status and scenarios for containment. *Nat Rev Microbiol* 2010; **8**: 272-80.
[27] Ashley EA, Dhorda M, Fairhurst RM, Amaratunga C, Lim P, Suon S, et al. Spread of artemisinin resistance in *Plasmodium falciparum* malaria. *N Engl J Med* 2014; **371**: 411-23.
[28] Imwong M, Dondorp AM, Nosten F, Yi P, Mungthin M, Hanchana S, et al. Exploring the contribution of candidate genes to artemisinin resistance in *Plasmodium falciparum*. *Antimicrob Agents Chemother* 2010; **54**: 2886-92.
[29] Miotto O, Amato R, Ashley EA, MacInnis B, Almagro-Garcia J, Amaratunga C, et al. Genetic architecture of artemisinin-resistant *Plasmodium falciparum*. *Nat Genet* 2015; **47**: 226-34.
[30] Ariey F, Witkowski B, Amaratunga C, Beghain J, Langlois AC, Khim N, et al. A molecular marker of artemisinin-resistant *Plasmodium falciparum* malaria. *Nature* 2014; **505**: 50-5.
[31] Mok S, Ashley EA, Ferreira PE, Zhu L, Lin Z, Yeo T, et al. Drug resistance. Population transcriptomics of human malaria parasites reveals the mechanism of artemisinin resistance. *Science* 2015; **347**: 431-5.
[32] Flannery EL, Chatterjee AK, Winzeler EA. Antimalarial drug discovery-approaches and progress towards new medicines. *Nat Rev Microbiol* 2013; **11**: 849-62.
[33] White NJ, Pukrittayakamee S, Phyo AP, Rueangweerayut R, Nosten F, Jittamala P, et al. Spiroindolone KAE609 for falciparum and vivax malaria. *N Engl J Med* 2014; **371**: 403-10.
[34] Winzeler EA, Manary MJ. Drug resistance genomics of the antimalarial drug artemisinin. *Genome Biol* 2014; **15**: 544.
[35] Roll Back Malaria. The global malaria action plan for a malaria-free world. Geneva: Roll Back Malaria; 2008. [Online] Available from: http://www.unhcr.org/4afac5629.pdf [Accessed on 5th January, 2016]
[36] World Health Organization. Global technical strategy for malaria 2016–2030. Geneva: World Health Organization; 2015. [Online] Available from: http://www.who.int/malaria/areas/global_technical_strategy/en/ [Accessed on 5th January, 2016]