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ARTIMISININ A SESQUITERPENE LACTONE WITH AN ENDOPEROXIDE BRIDGE IS TOXIC TO MALARIAL PARASITES.

Abdolhossein Rustaiyan[1]*, Afsaneh Faridchehr[2] and Mehdi Bakhtiyari[3]

[1,2]Department of Chemistry, Science and Research Branch, Islamic Azad University, P.O. Box 14515-775, Tehran, Iran.

[3]Shahdara Caspian, Pharmaceutical and Cosmetic Company, Tehran, Iran.

***Corresponding Author: Abdolhossein Rustaiyan**

Department of Chemistry, Science and Research Branch, Islamic Azad University, P.O. Box 14515-775, Tehran, Iran.

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ABSTRACT

Each year there are 300-500 million cases of malaria resulting in over million deaths from the disease world-wide. Malaria is especially in acute sub-Sahara Africa, where official estimate that two children die from malaria every minute. Resistance of the malaria parasite to longstanding treatments is for new antimalarial therapies. Artemisinin and synthetic derivatives are the focus of intense efforts to develop new drugs against malaria.

KEYWORDS: *Artemisia annua*, Sesquiterpene lactone, Anti- malarial effect, New anti-malarial candidate, Biosynthesis of Artemisinin.

1. INTRODUCTION

According to the World Health Organization, Malaria is still the chief case of human death in the world, aside from natural cases.

The disease acquired its name in ancient Rome (*mala, bad, aria, air*), where it was believed to be a result of the bad air in the city. It is actually caused by a parasite of *Plasmodium* family which infects and raptures erythrocytes in the blood stream.

The organism has a complex life cycle requiring both vertebrate and invertebrate host.

Humans are infected by sporozoites of the organism which are injected into the blood stream by the bite of an infected mosquito.

Malaria is a mosquito- borne infectious diseases of humans caused by eukaryotic protists of the genus *Plasmodium*. It is widespread in tropical and subtropical regions, including much of Sub-Saharan Africa, Asia and the Americas. The disease results from the multiplication of malaria parasites within red blood cells, causing symptoms that typically include fever and headache, in severe cases progressing to coma and death.

Four species of *Plasmodium* can infect and be transmitted by humans. Severe disease is largely caused by *Plasmodium falciparum*. Malaria caused by *Plasmodium vivax*, *Plasmodium ovale* and *Plasmodium malariae* is generally a milder disease that is rarely fatal. A fifth species, *Plasmodium knowlesi*, is a zoonosis that causes malaria in macaques but can also infect humans.[1][2]

Malaria transmission can be reduced by preventing mosquito bites by distribution of mosquito nets and insect repellents, or by mosquito-control measure such as spraying insecticides inside houses and draining standing water where mosquitoes lay their eggs.

Although many are under development, the challenge of producing a widely available vaccine that provides a high level of protection for a sustained period is still to be met.[3]

A variety of antimalarial medications are available. In the last years, treatment of *P. falciparum* infections in endemic countries has been transformed by the use of combinations of drugs containing an artemisinin derivative. Severe malaria is treated with intravenous or intramuscular quinine or, increasingly, the artemisinin derivative artesunate[4] which is superior to quinine in both children and adults.[5] [6] Resistance has developed to several antimalarial drugs, most notably chloroquine.[7]

Symptoms of malaria include fever, shivering, arthralgia (joint pain), vomiting, anemia (caused by hemolysis), hemogolobinuria, retinal damage[8] and convulsions. The classic symptom of malarias cyclical occurrence of sudden coldness followed by rigor and then fever and sweating lasting four to six hours, occurring every two days in *P. vivax* and *P. ovale* infections, while every three days for *P. malariae* and *P. falciparum* can have

recurrent fever every 36-48 hours or a less pronounced and almost continuous fever. For reasons that are poorly understood, but that may be related to high intracranial pressure, children with malaria frequently exhibit abnormal posturing, a sign indicating severe brain damage.[9] Malaria has been found to cause cognitive impairments, especially in children. It causes widespread anemia during a period of rapid brain development and also direct brain damage. This neurologic damage results from cerebral malaria to which children are more vulnerable.[10] Cerebral malaria is associated with retinal whitening.[11] This may be a useful clinical sign in distinguishing malaria from other causes of fever.[12]

Severe malaria is almost exclusively caused by *P. falciparum* infection and usually arises 6-14 days after infection.[8] Consequences of severe malaria include coma and death if untreated-young children and pregnant women are especially vulnerable. Splenomegaly (enlarged spleen), severe headache, cerebral ischemia, hepatomegaly (enlarged liver), hypoglycemia and hemoglobinuria with renal failure may occur. Renal failure is a feature of black water fever, where hemoglobin from lysed red blood cells leaks into the urine. Severe malaria can progress extremely rapidly and cause death within hours or days.[13] In the most severe cases of the disease, fatality rates can exceed 20%, even with intensive care and treatment.[14] In endemic areas, treatment is often has satisfactory and the overall fatality rate for all cases of malaria can be as high as one in ten.[15] Over the longer term, developmental impairments have been documented in children who have suffered episodes of severe malaria.[16]

2. Mechanism of action

The action of artimisinin derivatives is different from that of the other antimalarial drugs, although both the artemisinin drugs and the 4-aminoquinolines interact with haem.[17] [18] Artemisinins have a very fast action and parasite clearance times are much shorter than with other malaria drugs.

Artemisinin is only active on blood-stage parasites and does not affect liver-stage parasites or stages within the mosquito. However, it does act on gametocyte development, resulting in decreased transmission in areas where artemisinin compounds are extensively used.[19]

During the blood-stage phase of parasite, more than 70% of the hemoglobin within the infected erythrocyte is digested.[20] Haem is released, which is toxic for the parasite and therefore neutralized by polymerization into haemazoin. (This polymerization is inhabited by 4-aminoquinolines such as chloroquine.[21]

It was found that haem or Fe^{2+} catalysis the opening of the peroxide bridge in artemisinin, leading to the formation of free radicals.[17] [22] Malaria parasites are known to be sensitive to free radicals.[23] A mechanistic framework for the Fe^{2+} - induced cleavage of artemisinin and its derivatives has been proposed explain the formation of metabolic products and the most important pathways (shown in fig1). This mechanism is based on careful analysis of the formed products from reaction of Fe^{2+} -salts with artemisinin compounds under different conditions.[24] [25]

The initially formed oxygen radicals rearrange to primary and secondary carbon-centered radicals intermediates in the formation of known metabolites. These intermediates are involved in the alkylation of proteins. These secondary radical at C_4 originates from a 1, 5-H shift of C_4-H to the oxygen radical. Much effort has gone into investigating the relationship of the stability of the C_4 radical and the antimalarial activity. Formation of this radical is crucial for retaining high activity in the artemisinin analogues [24]. Blocking the formation or destruction of the formed radical at C_4 reduces the activity significantly. Further support for the formation of carbon radicals has come from trapping experiments of the formed radicals using spin labels.[26]

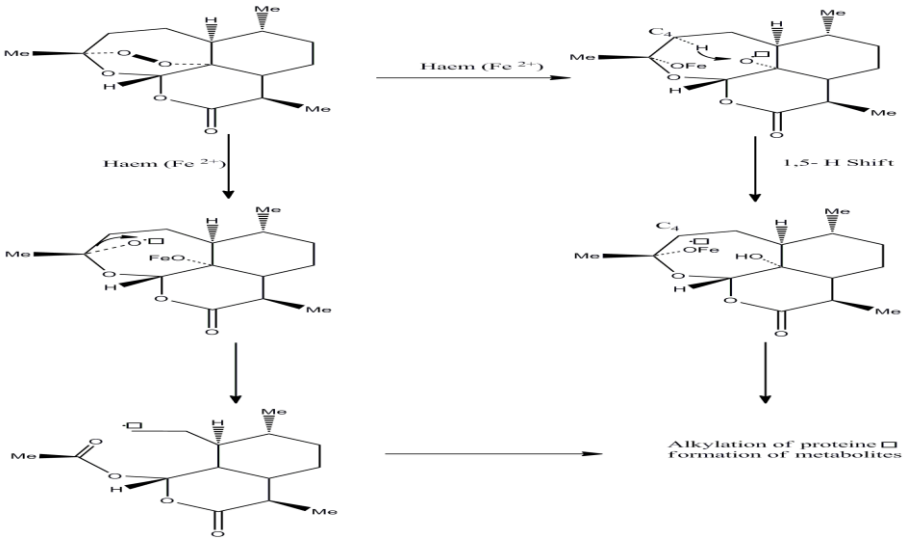

Figure1. Mechanism of artemisinin drugs action.

Upon reduction of the peroxide bridge by Fe^{2+}, two radical anions can be formed. In one of these a 1,5-H shift between the oxygen radical and hydrogen atom at C_4 occurs with formation of a carbon radical and subsequent formation of a presumed epoxide intermediate which is electrophilic and can react with proteins. Rearrangement of the other oxygen radical intermediate gives rise to primary radical involved in the alkylation of haem and parasite proteins leading to parasite death.

3. Biosynthesis of Artemisinin

The first step in artemisinin biosynthesis is the formation of amorpha-4, 11-diene from farnesyl diphosphate (show in fig.2). The reaction is catalyzed by *amorpha-4, 11-diene synthase* (EC 4.2.3.24) and an enzyme which has been isolated from the leaves of *Artemisia annua*. Like most sesquiterpene synthases it has a broad pH optimum (6.5-7.0) and its molecular mass is 56 kDa. From young leaves a cDNA clone has been isolated which contains a 1641-bp open reading frame coding for 546 amino acids. The clone was expressed in *Escherichia coli*, yielding a product with the properties of amorpha-4, 11-diene synthase. In the next step, amorpha-4, 11-diene is oxidized to artemisinic alcohol. An enzyme that catalyses this reaction has not yet been identified, but leaf microsomes from *A. annua* perform the reaction in the presence of NADPH. The following steps to produce artemisinin are not entirely clear. Theoretical pathways are presented in Fig. 2, which are supported by identification of a cDNA clone, which, when expressed in yeast, yielded a multifunctional cytochrome P450-enzyme, designated CYP71AV1, which oxidized amorpha-4, 11-diene to artemisinic alcohol and subsequently to the corresponding aldehyde and to artemisinic acid. That reduction of the C11-C13 double bond occurs after formation of artemisinic acid has not been proved. Theoretically, the oxidation of artemisinic alcohol to artemisinic acid might represent a branch in the pathway to artemisinin and, as indicated in Fig. 2, reduction of the C11-C13 double bond could occur before further oxidation of artemisinic alcohol.[27]

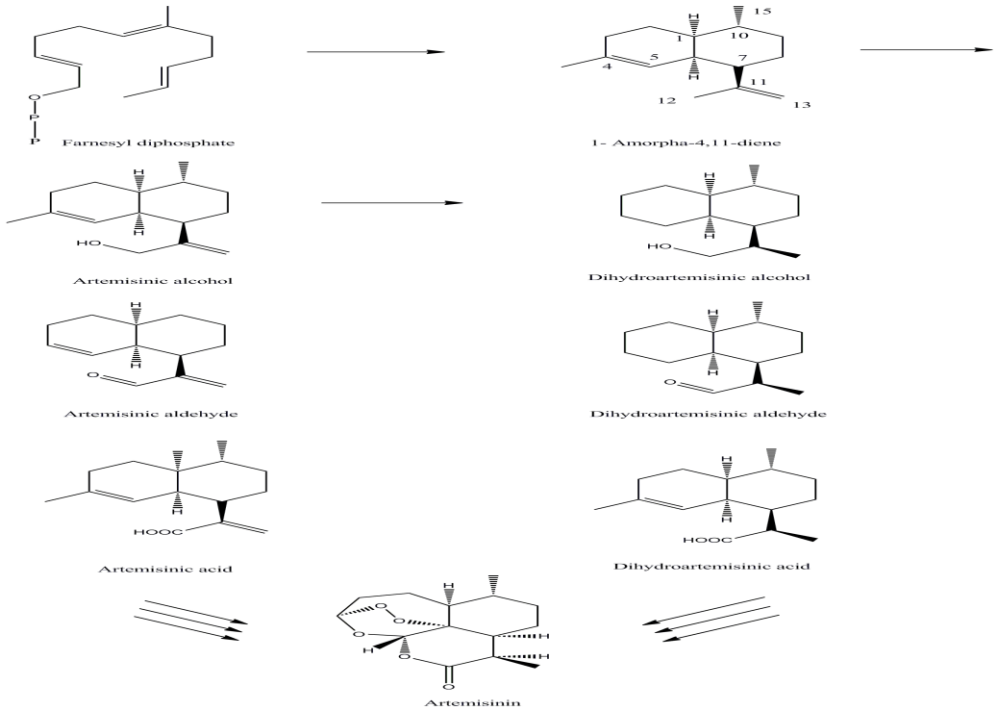

Fig 2. Biosynthesis of artemisinin

4. Artemisinin derivatives

The parasite responsible for the vast majority of fatal malaria infection, *Plasmodium falciparum*, can kill patients in a matter of hours. Malaria has traditionally been treated with quinolones such as chloroquine, quinine, mefloquine, primaquine and with antifolates such as Fansidar (sulfadoxinepyrimethamine). Unfortunately, most *P. falciparum* strains have now become resistant to chloroquine and some such as those in Southeast Asia; have also developed resistance to mefloquine and halofantriene, multidrug resistance is expected to develop in Africa soon.[28] [29]

Studies have documented over 1200 plant species from 160 families used in the treatment of malaria or fever.[30] Ethno botanical survey is an important step in identification, selection and development of the therapeutic agents from medicinal plants.

It is believed strongly that if the herbs used to treat malaria by our ancestors in Africa hundreds of years ago were not effective, malaria would have destroy Africa. More so, Missionaries that came to Africa would not have met a single on the continent of Africa.[31]

Drug discovery for the treatment of malaria has been challenging because of the emergence of parasites resistant to the conventional antimalarial drugs. Natural products have historically been important sources of antimalarial agents such as artemisinin and quinine.[32] Even though plant derived natural products are used as traditional herbal remedies, most of them have not been explored for the discovery of new targets against the malaria parasite.[33]

For this reason, more research on new antimalarial compounds from natural products is needed to develop new therapeutic agents with novel mechanisms of action against *P. falciparum.*

The endoperoxides are a promising class of antimalarial drugs which may meet the dual challenges posed by drug-resistant parasites and rapid progression of malarial illness. The first generation endoperoxides include artemisinin (qinghaosu) and several semisynthetic derivatives. (Fig 3).

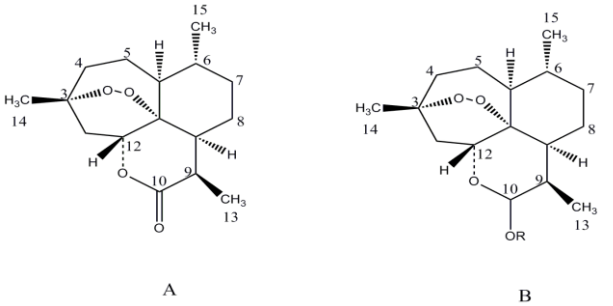

Fig 3. artemisinin (qinghaosu) and several semisynthetic derivatives

Artemisinin fig.3 First-generation artemisinin derivatives. (A) artemisinin. (B) lactol derivatives including dihydroartemisinin (R= H), artemether (R= CH_3), arteether (R= C_2H_5) and artesunate [R= OCO (CH_2) CO_2 Na].

Artemisinin, the protype, is a sesquiterpene lactone. Its structure, which includes an endoperoxide bridge (C-O-O-C), is unique among antimalarial drugs. Dihydroartemisinin is the reduced lactol derivative of artemisinin and the semisynthetic derivatives (artemether, arteether, artesunate and artelinate) are ethers or esters of lactol (Fig3).

The first generation artemisisnin drugs are being used widely in Thailand, Myanmar, Vietnam and China where multidrug resistant parasites are common.[34]

5. Artemisinin can inhibit the calmodulin-mediated activation of phosphodiesterase in comparison with Cyclosporine A

Artemisinin and Cyclosporin A were examined for their ability to inhibit the calmodulin-mediated activation of phosphodiesterase, which is based on the hydrolysis of cAMP to AMP by phosphodiesterase in the presence or absence of inhibitors, followed by quantitative analysis using spectrophotometer method. Anti-calmodulin activity of these agents was investigated by spectrofluorometry. Our results indicates that artemisinin and Cyclosporin A induced some conformational changes on calmodulin and increased the fluorescence emission, but artemisinin increased fluorescence emission of calmodulin in higher amounts compared with the Cyclosporin A. Kinetic analysis of the Artemisinin-calmodulin and Cyclosporine A-calmodulin interaction showed that these agents competitively inhibited the activation of phosphodiesterase without affecting Vmax. Artemisinin increased Km value in higher amounts compared with the Cyclosporin A. Ki values of artemisinin and Cyclosporin A were determined as 10 microM and 35 microM, respectively. The ΔG (H2O), the best parameter for the estimation of macromolecule stability, was determined for calmodulin in the absence and presence of artemisinin and Cyclosporin A. However, the degree of decrease in ΔG (H2O) value was as follows: Artemisinin>Cyclosporin A, which means artemisinin induced more instability in the calmodulin structure. In conclusion, our findings showed a good correlation between the ability of both artemisinin and Cyclosporin A to block the activation of phosphodiesterase and their ability to bind to the activator and that artemisinin is a more potent inhibitor of phosphodiesterase compared with Cyclosporin A.[35]

5.1. Tehranolide as a new Antimalarial Candidate

Since the discovery and the use of artemisinin and endoperoxide sesquiterpene lacton, particular attention has been directed to this class of compounds, we have investigate many Iranian Artemisia species.

Artemisia aucheri[36]-[38], *A. austriaca*[39], *A. biennis*[40], *A. campestris*[41], *A. deserti*[42], *A. diffusa*[43][44][45], *A. gypsacea*[46][47], *A. haussknechtii*[48], *A. kermanensis*[48], *A. kopetdaghensis*[48], *A. kulbadica*[49], *A. oliveriana*[50], *A. persica*[51], *A. santolina*[47], *A. sieberi*[52]-[54], *A. tschernieviana*[55], A. ciniformis[56], A. incana[56], *A. turanica*[57] and *A. tournefortiana*.[38]

From all these species, we discovered an unusual sesquiterpene lactone with endoperoxide group, which we have named Tehranolide.

The extract of the aerial parts of *A. diffusa* afforded several eudesmanolide and a new type of sesquiterpene lactone with unusual carbon skeleton, an eight member ring.[58]

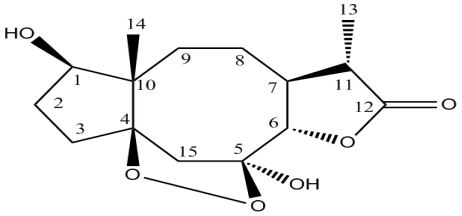

Fig4. Tehranolide

Probably Tehranolide has the same effect as the antimalarial agent Artemisinin. Recently Tehranolide (fig.4) has been confirmed and considered as a new antimalarial agent.[43] [59]

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