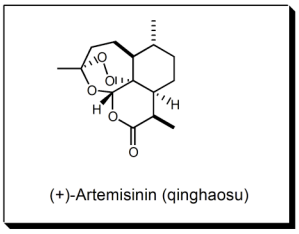
## *Potent Antimalarial Agent Artemisinin - Synthesis and Mode of Action*

*Ioana Drutu*
*MacMillan Group Meeting*
*September 24, 2003*



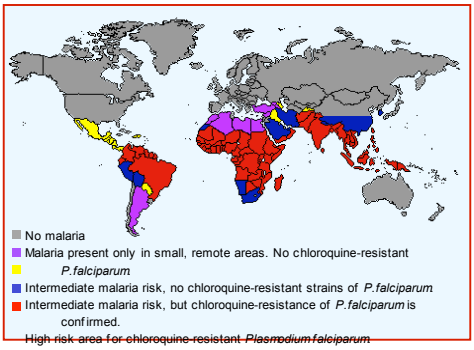
(+)-Artemisinin (qinghaosu)

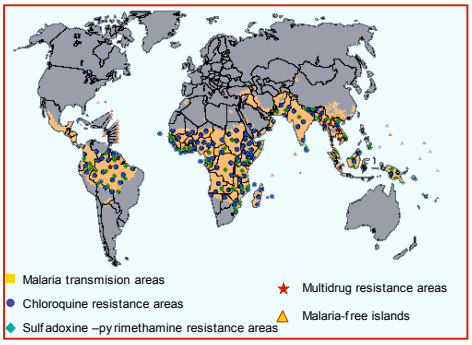
- What is malaria and why is it such a huge global health problem?
- Artemisinin and related antimalarial agents - natural occurence, biological activity and mechanism of action
- Total syntheses of artemisinin and related compounds

## *Epidemiology of Malaria*

*drug-resistant malaria is a rapidly spreading global health threat*

- Malaria is a public health problem today in more than 90 countries, inhabited by 2,400 million people – 40% of the world's population
- An estimated 300 to 500 million cases each year cause 1.5 to 2.7 million deaths, more than 90% of which occur in children under age 5 in Africa. **Malaria kills one child every 30 seconds**
- Progresses made in the last 50 years in restricting the geographical areas affected by malaria are being eroded recently, due to changes in land use, global climate changes, armed conflicts/movement of refugees, easy international travel and **development of multi-drug resistant strains of parasite**.
- The vast majority of areas of endemic malaria show resistance to chloroquine (the oldest, cheapest treatment). Resistance to sulfadoxine-pyrimidine is emerging in most affected areas. Resistence to mefloquine has been observed in South-east Asia (areas of multi-drug resistance)




No clinically relevant resistance has been observed with artemisinin and related derivatives

## *What is Malaria?*

- Malaria is a parasitic disease caused by a protozoon of the genus *Plasmodium.* The four subspecies active in humans are *P. falciparum*, *P. vivax*, *P. malariae* and *P. ovale*, with *P. falciparus* being responsible for most malaria-related deaths.

- Symptoms include cyclical bouts of fever, with muscle stiffness, shaking and sweating. The most severe manifestations are cerebral malaria, anemia and kidney, liver and lung disfunctions

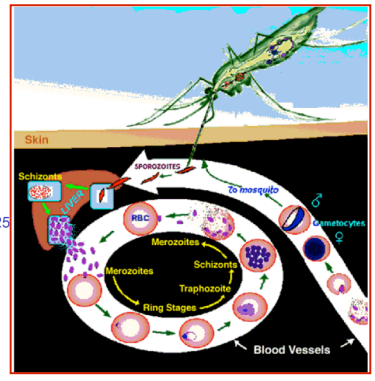
- Malaria is transmited by the bites of the female mosquitoes of the genus *Anopheles*, especially *Anopheles gambiae*

## *The Life Cycle of Plasmodium Falciparum*

- Asexual phase - in human
- Sexual phase - in mosquito

**4**. Oocyst divides asexually into sporozoites which are ready to be injected into human.

**3**. Zygote transforms into ookinete which penetrates the gut wall and becomes an oocyst




**2**. Male and female gametes fuse to form a zygote

**1**. Gametocytes enter mosquito and continue development

**1**. Mosquito injects sporozoites into the human

**2**. Sporozoites enter liver cells (30 min) and reproduce asexually - hepatocites eventually burst, releasing parasite into blood stream (the pre-patent period, 8-25 days)

**6**. Some merozoites undergo transformation into gametocytes - male and female.

**3**. The parasites attach to the red blood cell membrane and then enter the cell through a process of invagination

**4**. Asexual division starts and the parasites develop through stages of rings, trophozoites, schizonts containing thousands of merozoites

**5**. Blood cells burst, releasing the merozoites, and the cycle repeats every 48hrs. This phase stimulates production of TNF-a, resulting in the characteristic clinical manifestations of the disease

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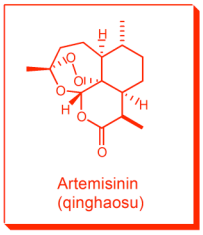
## *Artemisinin-a Rediscovered Ancient Remedy*



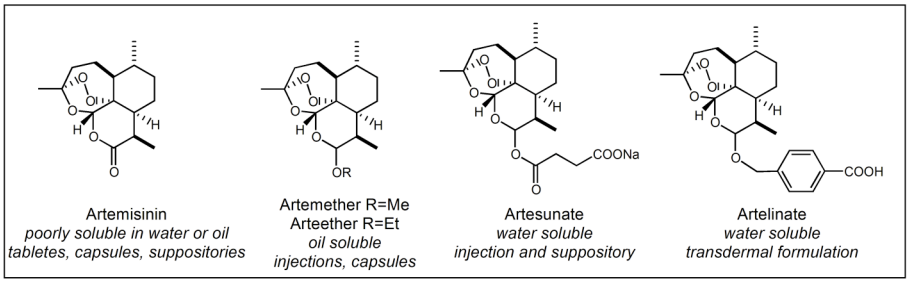

Artemisinin
(qinghaosu)

- Artemisinin is a natural sesquiterpene isolated from the leafy portions of *Artemisia annua* (qinghao) (~2%)
- First mentioned by Chinese herbalists in 168 B.C as a treatment for hemorrhoids, then in 340 A.D. as a fever reducing agent.
- Isolated and characterized by Chinese scientists in 1972, reisolated in the US in 1984
- Artemisinin is one of the very few naturally occuring endoperoxides. It is structurally related to the cadinane or amorphane class of sesquiterpene characterized by their *cis*-decalin skeleton.

China Cooperative Research Group on Qinghaosu as and its Derivatives as Antimalarials *J. Trad. Chin. Med.* **1982**, *2*, 3
Klayman *J. Nat. Prod.* **1984**, *47*, 715

## *Artemisinin and Derivatives as Active Antimalarials*

- Artemisinin and related compounds are the fastest acting antimalarials on the market




Artemisinin
*poorly soluble in water or oil*
*tabletes, capsules, suppositories*

Artemether R=Me
Arteether R=Et
*oil soluble*
*injections, capsules*

Artesunate
*water soluble*
*injection and suppository*

Artelinate
*water soluble*
*transdermal formulation*

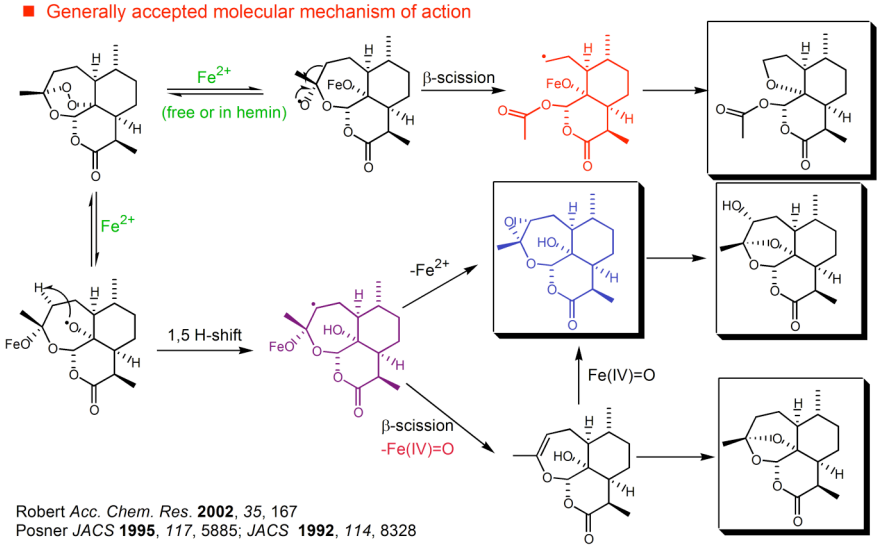
- Artemisinin and derivatives are active at nanomolar concentrations *in vitro*, even against multi-drug resistant strains of *P. falciparum*
- Parasiticidal activity is manifested against the erythrocytic phase of plasmodium. The compounds are effective at the ring, trophozoite and gametocyte stage of the blood phase, and inactive on liver-stage parasite
- The onset of action is very rapid, with clearance of parasites from the blood within 48 hours in most cases. However, because of the very short half-life of the drug (1-3hrs) a high rate of recrudescence was observed if treatment was not continued for at least five-seven days, or combined with a longer-acting antimalarial
- No neurotoxicity or other serious side efects have been observed in humans at therapeutic doses


Agtmael *Trends in Pharmacol. Science* ***1999***, *20*, 199
Robert *Pure Appl. Chem.* **2001**, *73*, 1173
Meshnick *Microbiol. Rev.* **1996**, *60*, 301


## *Artemisinin - an Elusive Mode of Action*

*reactive radical or electrophilic species are involved in the killing mechanism*

- Erythrocytes infected with P. falciparum take up and concentrate $^{14}$C-artemisinin at 100-fold higher concentrations than uninfected cells, resulting in highly selective cytotoxicity
- Peroxide bridge is essential for biological activity, and activation by Fe(II) is necessary
- Generally accepted molecular mechanism of action



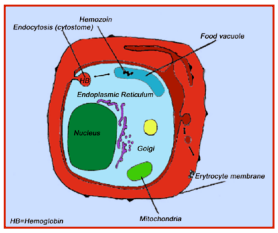


Robert *Acc. Chem. Res.* **2002**, *35*, 167
Posner *JACS* **1995**, *117*, 5885; *JACS* **1992**, *114*, 8328

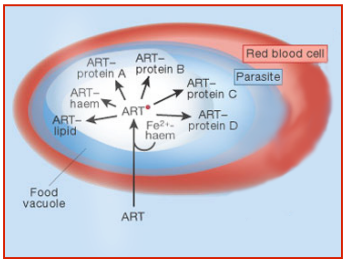
## *Artemisinin - Searching for a Cellular Target*

*where does the cytotoxic process take place?*

- P. falciparum obtains the aminoacids needed for growth by ingesting and degrading up to 80% of the host's hemoglobin in a compartment called a food vacuole. $Fe^{2+}$-heme is released and oxidized to $Fe^{3+}$-hematin (toxic for the parasite). Detoxification occurs by agregation of hematin into a crystalline pigment called hemozoin.




- Because artemisinin is activated by $Fe^{2+}$ it was believed that the specific antimalarial effect was due to the drug's entry in the food vacuole followed by interaction with $Fe^{2+}$-heme. This would set of a "cluster bomb" of free radicals, inhibiting several key parasite components and leading to parasite death.




Meshnick *Microbiol. Rev* **1996**, *60*, 301
Olliaro *Parasitol. Today* **1999**, *11*, 294
Ridley, *Nature*, **2003**, *424*, 887

## *Artemisinin - Searching for a Cellular Target*

*wealth of information, scarcity of understanding*

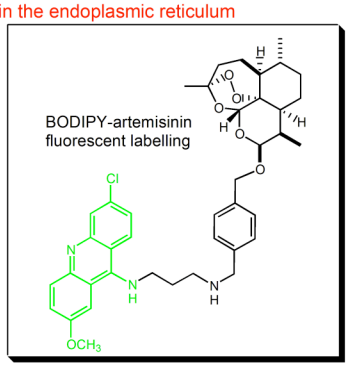
- Free radicals seem to be part of the cytotoxic pathway
  - *in vitro* antimalarial activity is enhanced by high oxygen tension and addition of other free-radical generating compounds, such as doxorubicin, miconazole, castecin
  - antioxidants such as $\alpha$-tocopherol, catalase, dithiothreitol, ascorbate, reduced glutathione inhibit activity
  - unlike other free-radical-generating drugs, artemisinin does not cause "oxidant damage" through generation of oxygen free radicals such as superoxide. Very high concentrations are necessary to induce lipid peroxidation and membrane protein thiol oxidation and there is no selectivity between parasite and uninfected erythrocytes
- Contradicting data about the importance of the artemisinin-$Fe^{2+}$-heme interaction for molecular activation
  -chloroquine (known antimalarial that binds tightly to heme) is antagonistic to artemisinin in vitro **BUT** iron chelators also antagonize the antiparasitic effect of artemisinin, suggesting involvment of free $Fe^{2+}$
  - *in vivo* activation could occur by a different mechanism involving malarial hemoproteins outside of the food vacuole
- Heme seems to be not only an activator but also a target for artemisinin
  - a covalent artemisinin-hemine adduct has been isolated and potential mechanisms cytotoxic pathways were proposed
  - the artemisinin-hemine adduct could be intrinsically toxic to the parasite (similar to chloroquine-hemine) **BUT** addition of the preformed adduct to cell cultures yields no antimalarial activity
  - the iron released upon artemisinin-hemine agregation could be toxic for *plasmodium* **BUT** no detectable increase in free iron has been observed in artemisinin treated parasites
  -the artemisinin-hemine adduct might inhibit detoxification by blocking hemozoin formation (similar to the chloroquine mechanism of action) or by hemozoin degradation **BUT** no decrease in hemozoin content has been observed in artemisinin treated parasites
  -formation of the artemisinin-hemine adduct might be completely unrelated to the mechanism of action
- Artemisinin is capable of alkylating specific proteins at therapeutic doses
  - treatment of malaria-infected erythrocytes with radioactive artemisinin specifically labels 6 proteins with masses of 25, 32, 42, 50, 65 and >200 kDa
  - no labelling is observed in intact erythrocytes
  - the 25 kDa protein was identified as the malarial translationallhy controlled tumor protein (TCTP), but since the physiological role of this protein is unknown it is not clear this binding is relevant for the antimalarial activity of artemisinin

Meshnick *Microbiol. Rev* **1996**, *60*, 301
Meshnick *Int. J. Parasitol.* **2002**, *32*, 1655

## *Recent Insights into the Mechanism of Action*

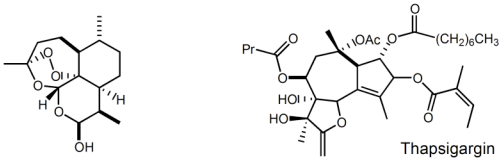
*from radical "cluster bomb" to selective enzyme inhibitor*

- Artemisinine DOES NOT in fact concentrate in the food vacuole, but in the membranous structures and in the endoplasmic reticulum




*could artemisine target be an endoplasmic reticulum enzyme, for instance an ATPase?*

- Structural similarities to known mammalian Sarco/endoplasmic reticulum calcium-dependent ATPase (SERCA) inhibitor thapsigargin strenghten the hypothesis



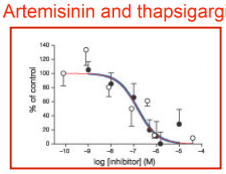
Thapsigargin

Krishna *Nature*, **2003**, *424*, 957

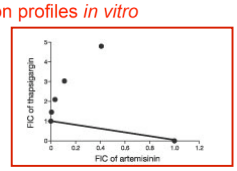
## *Recent Insights into the Mechanism of Action*

*artemisin is a selective pfATP6 inhibitor*

- The genome for *Plasmodium falciparium* encodes for only one SERCA-type $Ca^{2+}$ ATPase - PfATP6, which proved to be very similar to mammalian SERCA
- Artemisinin and thapsigargin have identical PfATP6 inhibition profiles *in vitro*




*Ki inhibition constants*
*artemisinin● and thapsigargin o*




*Isobologram for thapsigargin and artemisinin showing antagonist action (points above the isobole line)*

- Artemisinin and thapsigargin are competitive inhibitors of PfATP6 *in vivo*

*cells incubated with BODIPY-thapsigargin*

*cells preincubated with thapsigargin, then BODIPY-thapsigargin*

*cells preincubated with artemisinin then BODIPY-thapsigargin*

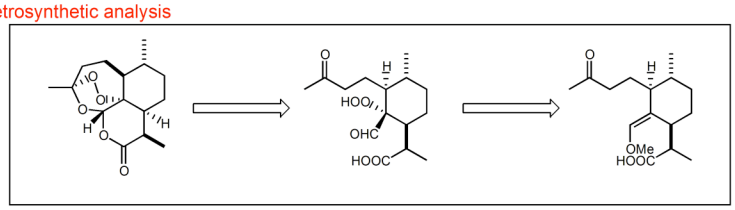
*cells preincubated with artemisinin/desferrioxamine then BODIPY-thapsigargin*

At cellular level, exposure to artemisins causes rapid swelling of endoplasmic reticulum in parasites, providing a morphological correlate for the proposed site of action

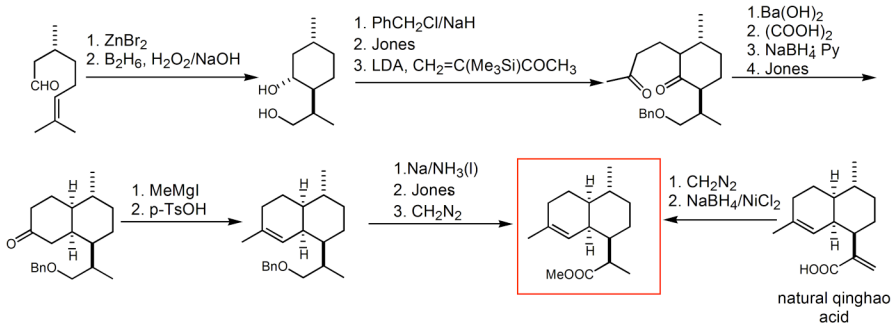
Krishna *Nature*, **2003**, *424*, 957

## *The First Total Synthesis of Artemisinin - Zhou et al.*

- Retrosynthetic analysis



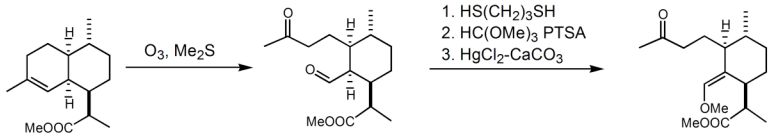
- Synthesis of an advanced decaline intermediate



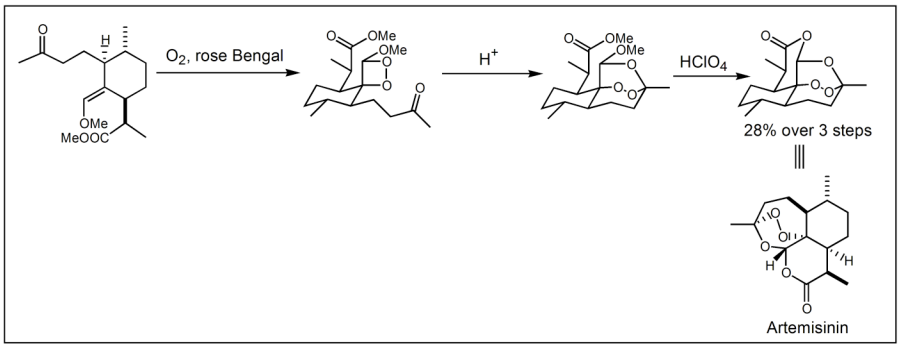

Zhou *Acc. Chem. Res* ***1994***, *27*, 211

## *The First Total Synthesis of Artemisinin - Zhou et al.*

- Synthesis of the key ketone methyl enol ether



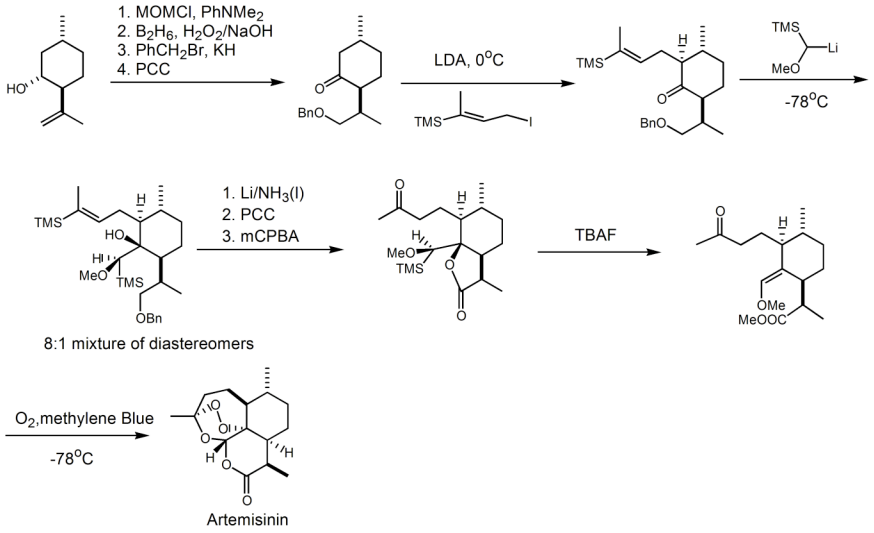

- Completion of the synthesis through a stereoselective singlet oxygen cycloaddition




Zhou *Acc. Chem. Res* ***1994***, *27*, 211

## *The Schmid and Hofheinz synthesis of Artemisinin*

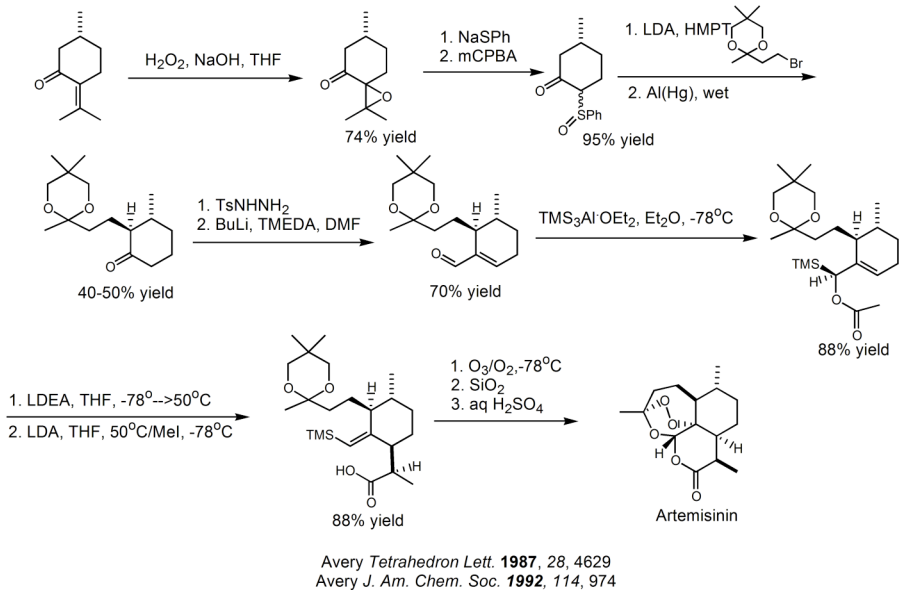
- The same intermediate enol-ether is used, accessed from (-)-isopulegol




Hofheinz *J.Am.Chem.Soc* ***1983***, *105*, 624

## *The Avery Synthesis of Artemisinin*

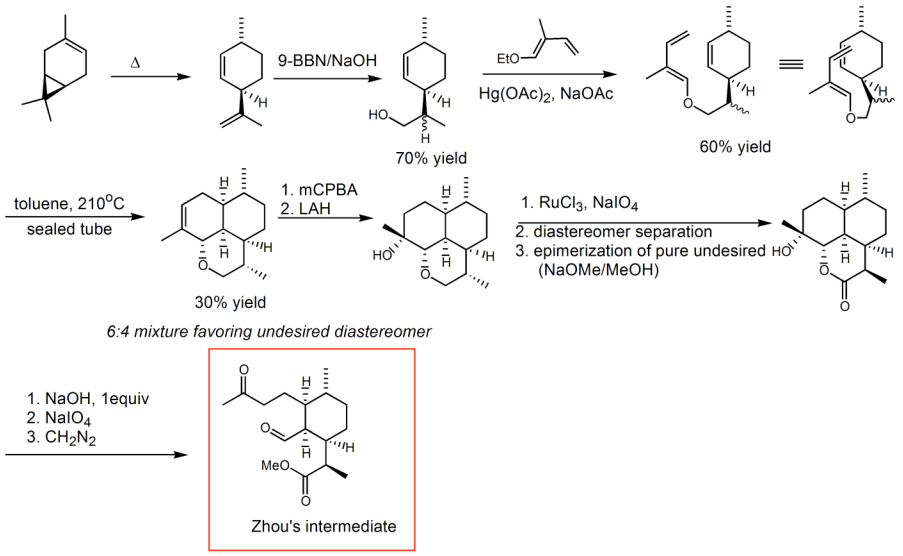
- Endo-peroxide unit is installed via the abnormal "ozonolysis" of a vinyl silane




Avery *Tetrahedron Lett.* **1987**, *28*, 4629
Avery *J. Am. Chem. Soc.* ***1992***, *114*, 974

## *Ravindranathan's Formal Synthesis of Artemisinin*

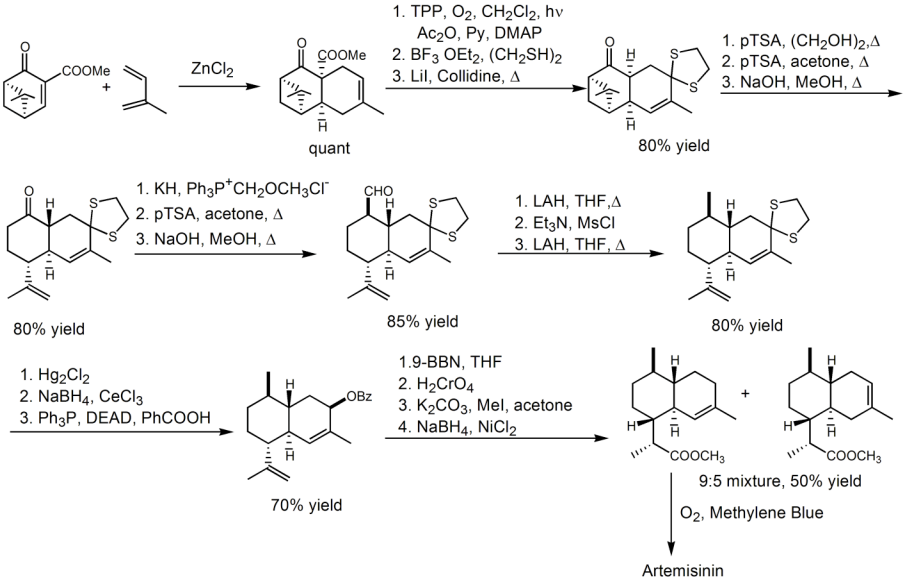
- Intramolecular Diels-Alder reaction furnishes the cis-decalin skeleton




Ravindranathan *Tetrahedron Lett.* **1990**, *31*, 755

## *Liu, Yeh and Chew - Total Synthesis of Artemisinin*

- Intermolecular Diels-Alder affords a trans decaline




Liu *Tetrahedron Lett.* **1993**, *34*, 4435
Liu *Heterocycles* **1996**, *42*, 493

*Conclusions and Future Directions*

- Artemisinin and its more bioavailable congeners are currently the state of the art treatment of uncomplicated multidrug resistent malaria. To delay the onset of parasite resistance, caution should be exerted in using artemisinins as a first line of intervention in areas where more traditional drugs are still active

- The molecular mechanism of action against *P. falciparum* has been investigated and the role of active radical or electrophilic species generated through oxidation by iron (II) is well established. More work needs to be done to elucidate the exact source of iron used for activation (free iron, iron-heme, iron bound to cytoplasmic metalloproteines)

- A potential target for the parasitocide activity has been identified to be the malarial PfATP6 enzyme. More proof is required to establish if PfATP6 inhibition is actually part of the killing mechanism. The sequencing of parasite genome should provide more insights into other potential artemisinin targets, and shed light on the mechanism of resistance to antimalarials

- A number of syntheses have been reported to date, involving mostly ene reactions or singlet oxygen additions to an olefin, followed by a rearrangement cascade to furnish the 1, 2, 4 trioxane ring