Synthetic Peroxides: A Viable Alternative to Artemisinins for the Treatment of Uncomplicated Malaria?

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Why do we need a new class of peroxides?

- The clinical utility of peroxide antimalarials is well established; recent alarming reports of ACT treatment failures
- Fully synthetic peroxides would provide a substantial benefit in relation to cost and availability relative to artemisinins
- Design of new peroxides, with improved properties, could:

  (i) provide more convenient dosing regimens

  (ii) help to limit the 'fallout' if artemisinin resistance develops

  (iii) possibly, remove the current contraindication in pregnancy

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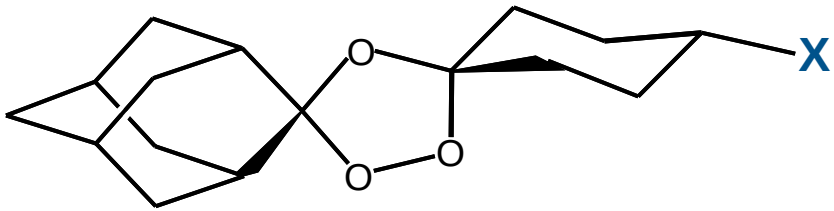
Synthetic Peroxides



1,2,4-trioxolanes
(secondary ozonides)

- Potent inhibitors of *P. falciparum in vitro* (IC_{50} < 2 nM) and *P. berghei in vivo* (single dose ED_{90} < 5 mg/kg)
- Effective against all blood stages of the parasite with rapid onset of action
- Peroxide bond is required for biological activity
- Fe(II) reactivity is a necessary, but insufficient, requirement for antimalarial activity
- 100-fold less potent than artemisinin in inhibiting *Pf*ATP6

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Objectives of the Synthetic Peroxide Project

- First Generation OZ project aimed to:
  - identify a new class of fully synthetic peroxides
  - more potent than the available semi-synthetic artemisinins
  - low cost when used in combination
  - 3-day treatment regimen when used in combination

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  - low cost when used in combination
  - 3-day treatment regimen when used in combination
- Next Generation OZ project aimed to extend these goals:
  - provide a single-dose oral cure (in combination) for patients with uncomplicated *P. falciparum* malaria
  - potential for prophylactic treatment and intermittent preventative treatment in pregnant women and infants (IPTp and IPTi)

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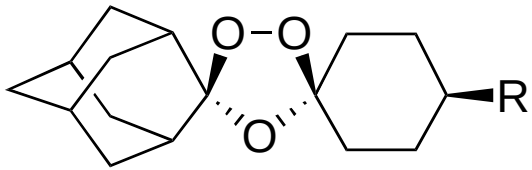
To Achieve Next Generation Goals....

- First generation OZ:
  - significantly improved biopharmaceutical properties compared to artemisinins, but overall exposure still limited
- Single dose oral cure and prophylaxis would require:
  - dramatic increase in the exposure profile following oral administration…but without sacrificing potency
  - understand the mechanisms of *in vivo* clearance
  - structural design modifications to reduce clearance and increase the half life

Suspected Clearance Mechanisms



First Generation OZ

Blood-mediated clearance
- *in vitro* studies in blood
- inherent instability of peroxide bond required for activity

CYP450-mediated clearance
- *in vitro* studies in hepatic microsomes
- *in vivo* PK studies in rats

Relative contribution of these processes was unknown

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Clearance Mechanisms of First Generation OZ

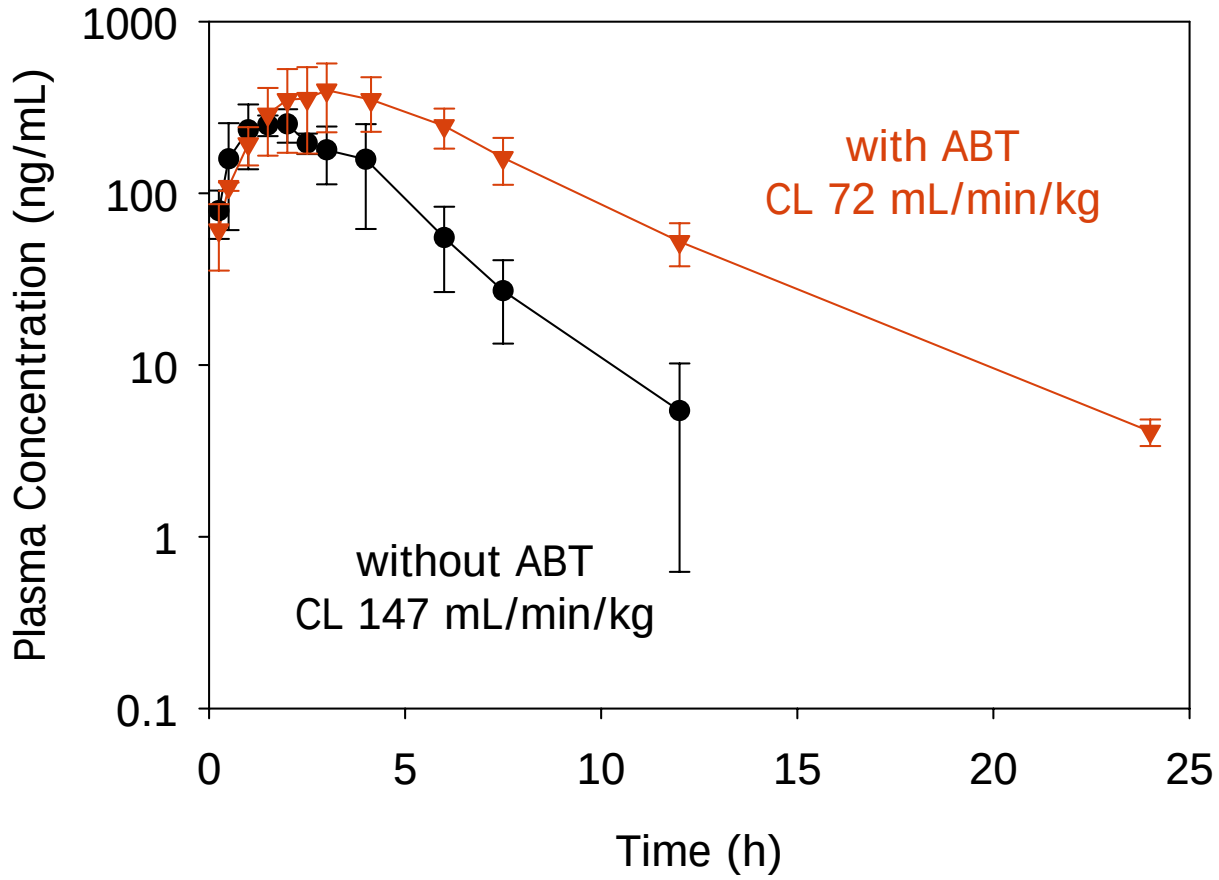
determine contribution of CYP450 metabolism → administer an irreversible CYP inhibitor (ABT)

1-Aminobenzotriazole

- irreversible inhibitor of Cytochrome P450 enzymes
- safe in animals at doses up to 100 mg/kg
- for compounds highly metabolised by CYP enzymes, there is a substantial reduction (80-90%) in *in vivo* clearance following pre-dosing with ABT

Clearance Mechanisms of First Generation OZ

Oral administration of first generation OZ (30 mg/kg PO) ± pre-dose with ABT (100 mg/kg PO) in rats



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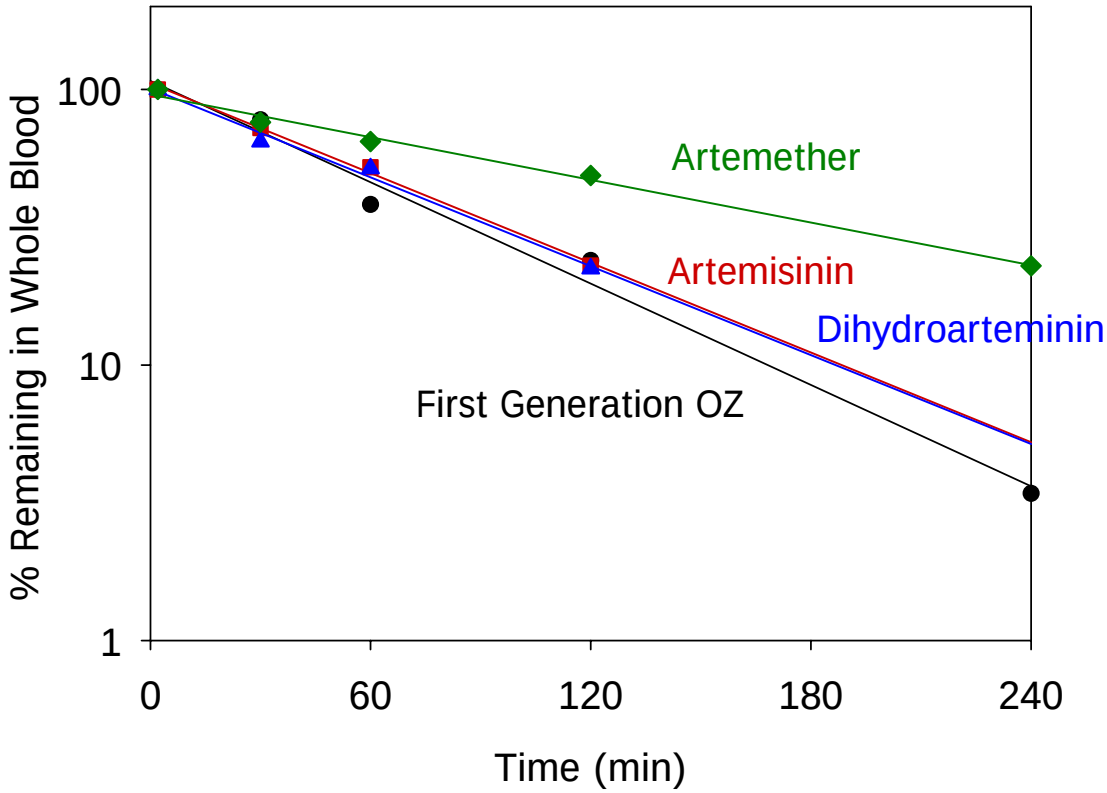
First Generation OZ Stability in Rat Blood

Relatively rapid degradation of first generation OZ and Artemisinins in rat whole blood *in vitro* (37°C)



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Structural Modifications to Reduce Clearance

CYP450 metabolism

– blocking known site of metabolism adversely affected activity

Blood instability

– cleavage of peroxide, and subsequent formation of free radicals required for activity

– could we reduce blood instability without sacrificing potency?

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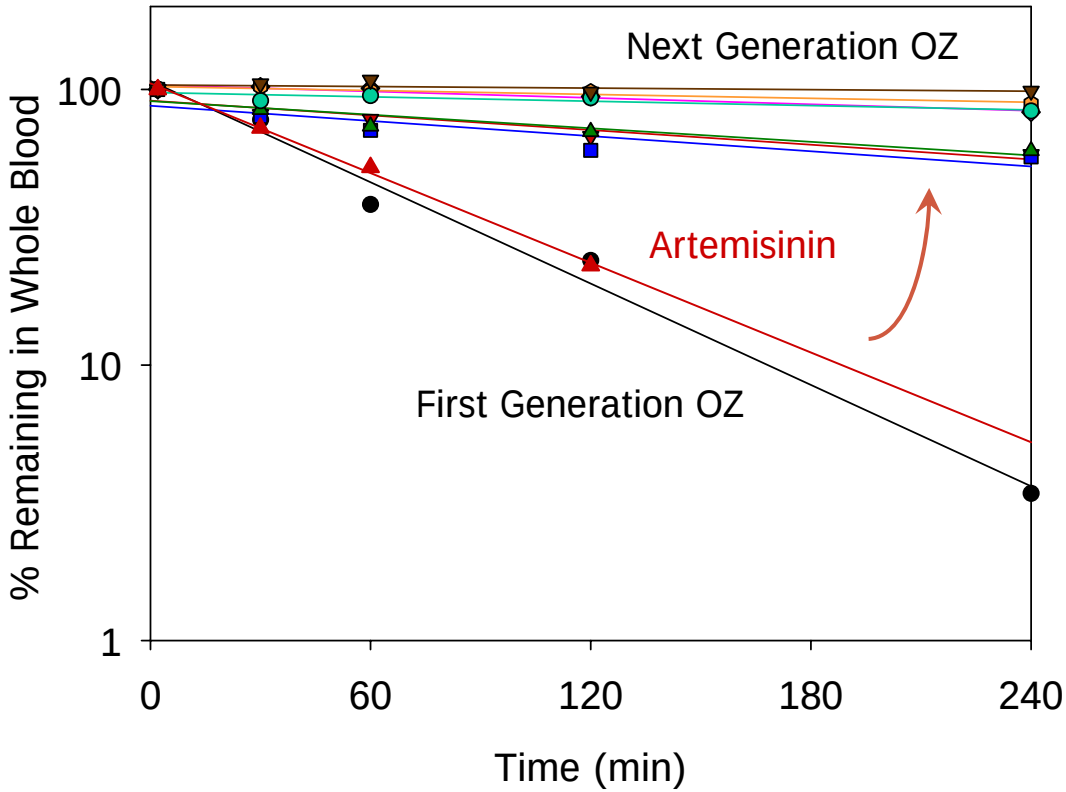
Next Generation OZ Stability in Rat Blood

Structural changes led to a significant increase in rat blood stability *in vitro* (37°C)



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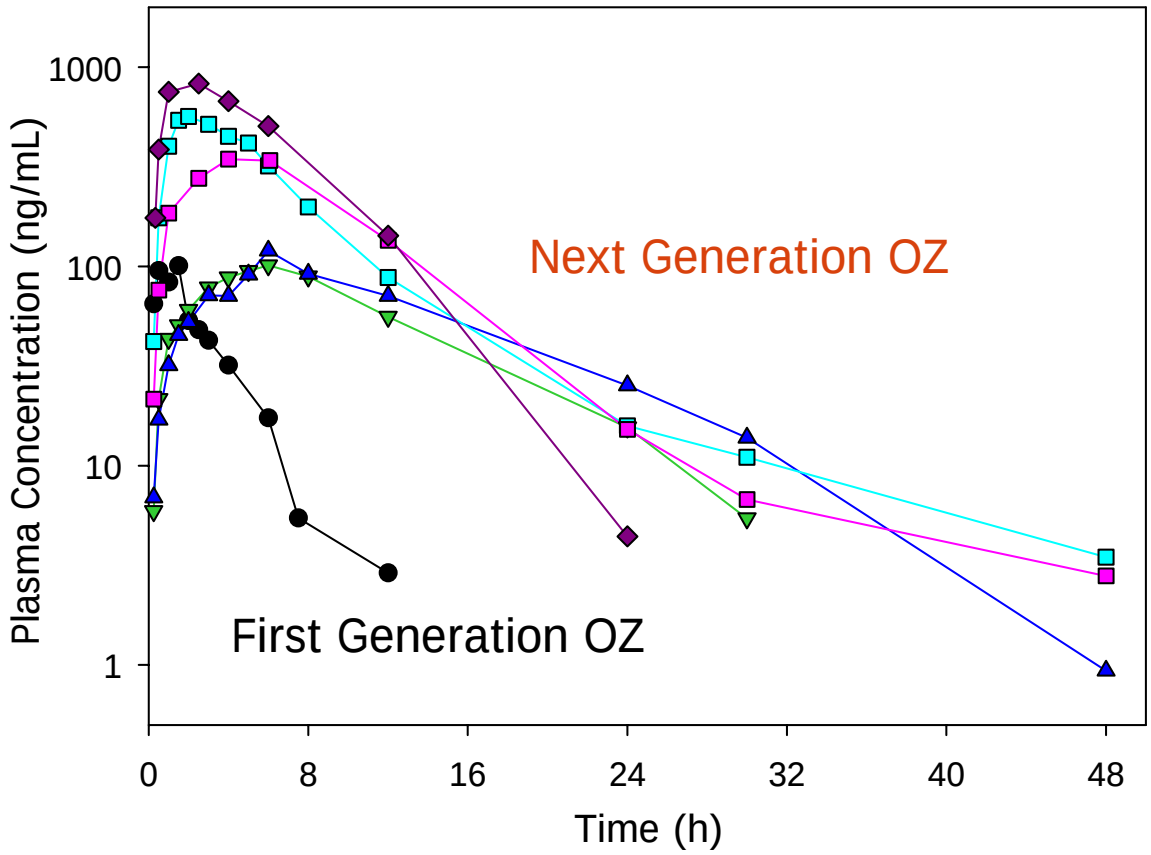
In Vivo Plasma Profiles in Rats after Oral Dosing

Substantial decrease in clearance and increase in exposure profile in rats (each dosed at 10 mg/kg PO)



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But what about antimalarial activity?

Single 30 mg/kg oral dose to mice (*P. berghei*)

Compound (mg/kg)	Survival (d), Cure (%)
Artesunate	9 d, 0%
Artemether	9 d, 0%
Chloroquine	10 d, 0%
Mefloquine	22 d, 0%
First Generation OZ	11 d, 0%
No treatment	6 d, 0%

Limited survival; no cures with artemisinin derivatives, conventional drugs or First Generation OZ

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Compound (mg/kg)	Survival (d), Cure (%)
OZ401	25 d, 64%
OZ439	**>30 d, 100%**
OZ460	25 d, 60%
OZ461	**>30 d, 100%**
OZ464	**>30 d, 100%**
OZ466	**>30 d, 100%**
OZ482	**>30 d, 100%**
OZ485	25 d, 40%
OZ493	**>30 d, 100%**

Survival and 100% cure for Next Generation OZ

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...And prophylaxis?

Single 30 mg/kg oral dose 24 h prior to *P. berghei* infection (mice)

Compound (mg/kg)	Survival (d), Prophylaxis (%)
Artesunate	7 d, 0%
Artemether	not tested
Chloroquine	7 d, 0%
Mefloquine	27 d, 60%
First Generation OZ	7 d, 0%
No treatment	6 d, 0%

Only mefloquine has prophylactic properties

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Three next generation OZ
are 100% prophylactic and
all are equivalent to mefloquine

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Characteristics of Next Generation OZ

- Specific structural design concepts of the next generation OZ have resulted in:
  - improved stability in blood...without compromising biological activity
  - reduced *in vivo* clearance, prolongation of half-life and increased exposure in rats (as well as mice and dogs)...and better oral bioavailability
  - enhanced biological activity in a well-established mouse model of malaria
  - excellent prophylactic activity in mice – exceeds that of the benchmark chemoprophylactic mefloquine

Steps Forward...

- Chemistry: straightforward synthesis, low COG — confirmed
  characterisation of different salt forms — on-going
  metabolite synthesis — planned
  expertise in process chemistry/scale up — planned
- Efficacy: *Aotus P. falciparum* monkey model — on-going
  synergism/antagonism studies — on-going
  cross resistance studies — on-going
- ADME: PK properties in mice, rats, dogs — completed
  in vitro metabolism, CYP inhibition — near completion
  PK studies with potential partner drugs — planned
- Toxicology: exploratory toxicity studies in rats — completed
  safety pharmacology studies — on-going
  exploratory embryotoxicity studies — on-going
- Selection of development candidate planned for 1H 2008

The Synthetic Peroxide Discovery Team