The trials and tribulations of generating a fully synthetic peroxide based antimalarial drug candidate

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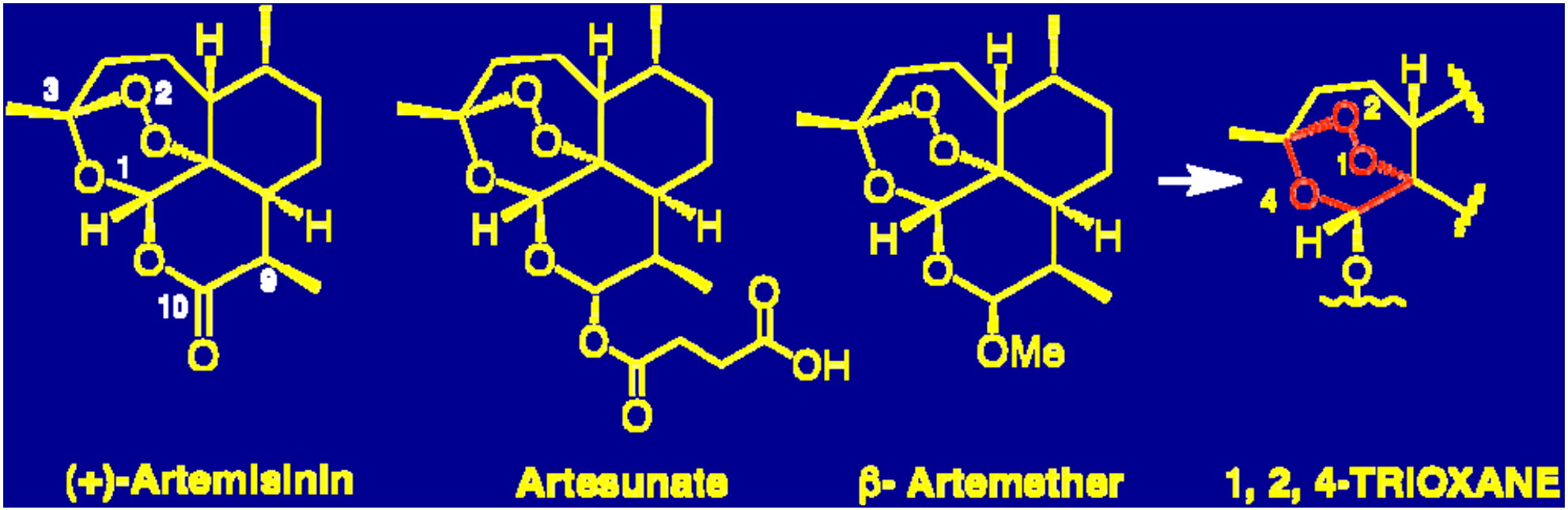
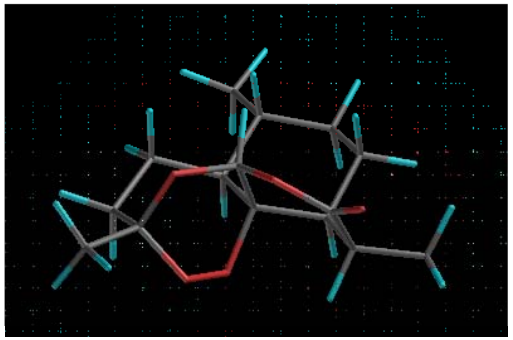
JITMM 2013

Endoperoxide Antimalarials



ARTEMISININ is a sesquiterpene lactone peroxide and is also known as (qinghaosu), (1). Derivatives such as artemether and artesunate are now routinely used clinically for the treatment of multidrug resistant *Plasmodium falciparum* malaria.

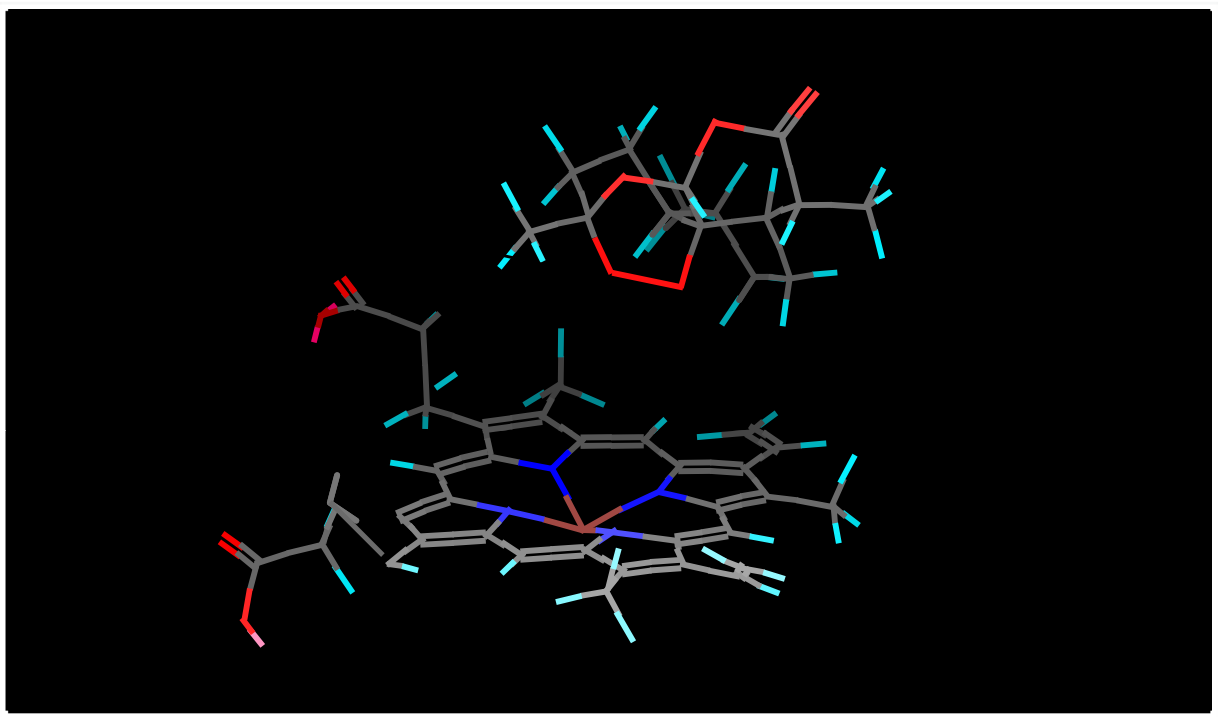


Artemisia annua

LSTM

Posner, G.H;. O'Neill,P.M. *Accounts of Chemical Res*. **2004**, *37*, 397-404

Endoperoxide Antimalarials - Proposal for the Mechanism of Action



Homolytic Cleavage (Reduction) of the Peroxide Bond takes place following docking of artemisinin onto Heme

HEME Fe^{2+}

ARTEMISININ → RADICAL SPECIES → ALKYLATION OF VITAL PARASITE PROTEINS/ HEME

Food Vacuole

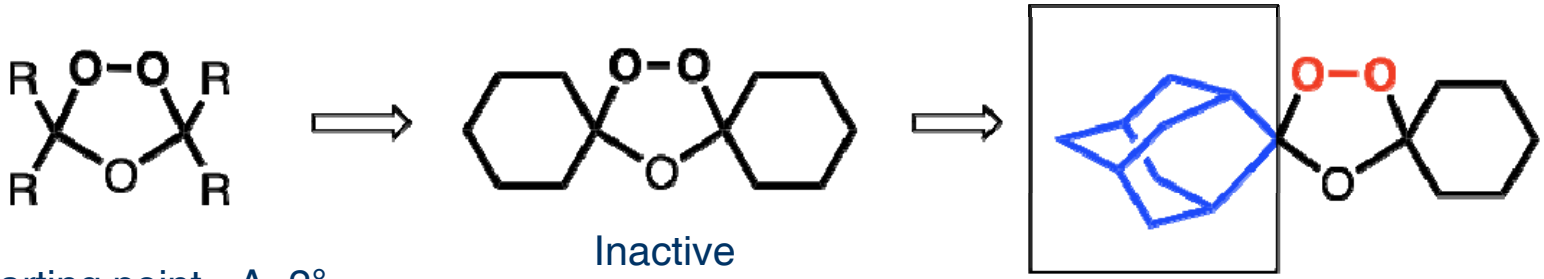
$R^{\cdot}$

LSTM

O'Neill, P.M.; Posner, G.H., A Medicinal Chemistry Perspective on Artemisinin Related Endoperoxides, *J. Med. Chem.*, **2004**, *47*, 2945-2964

1,2,4-TRIOXALANES v's 1,2,4,5-TETRAOXANES - 2 CHEMICALLY DISTINCT GROUPS

1,2,4-TRIOXALANES:



The starting point - A 2° Ozonide (1,2,4-Trioxolane)

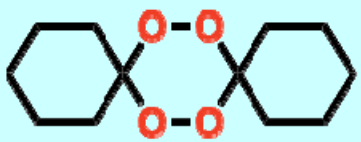
Inactive Unstable and reactive

Highly potent antimalarial

1,2,4,5-TETRAOXANES:

Two endoperoxide "war-heads"

Simple Spiro 1,2,4,5 -Tetraoxanes are chemically quite stable - even without fusion to an adamantane ring sytem



The template is achiral in contrast to the ozonides

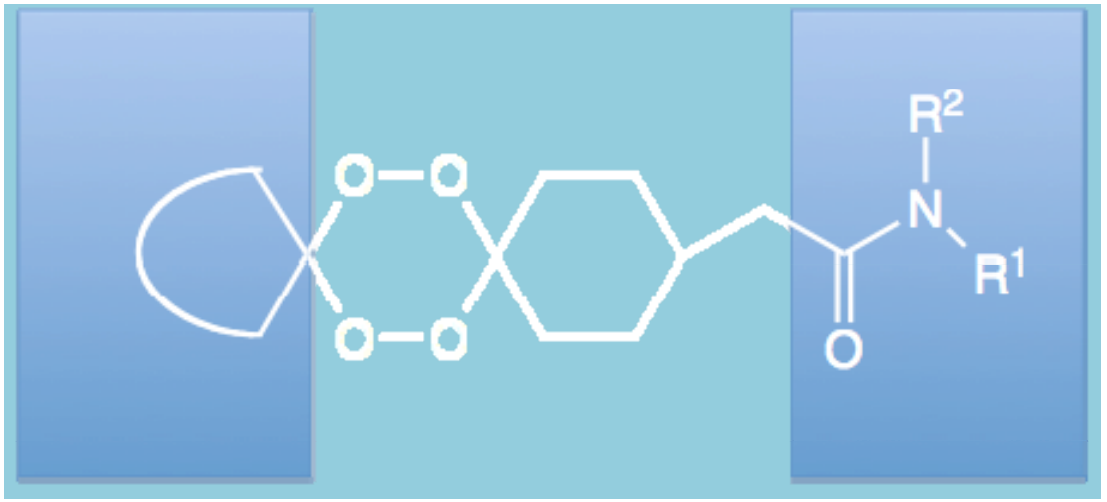
Many methods available for synthesis

This parent compound has an IC50 in the nanomolar range (25 nM) versus 3D7

LSTM

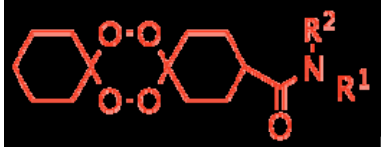
MEDICINAL CHEMISTRY OPTIMISATION

Potency;
In vitro
& In vivo
Stability



PK
Solubility

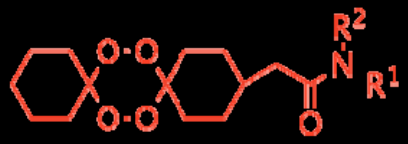
NO ORAL ACTIVITY
IN MOUSE MODEL



5 compounds

R = morpholinyl
IC50 = 23.60 nM

MARGINAL ACTIVITY

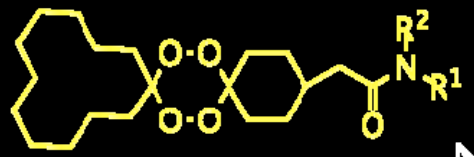


15 compounds

R = morpholinyl

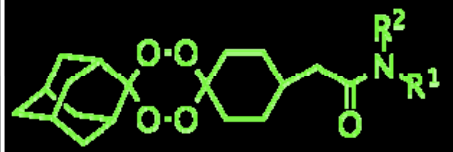
IC50 =19.85nM
Inhibition at 30mg/kg = 33 %

MODERATE IN VIVO
ACTIVITY/ HIGH CLOGP



13 compounds

NR^1R^2 = cyclopropylaminyl
IC50 = 16.7 nM
ED50 = 17.2 mg/kg
ED90 = >50mg/kg
Artesunate = ED50/ED90 :
3.23/>10 mg/kg



90 compounds- HIT SERIES 1

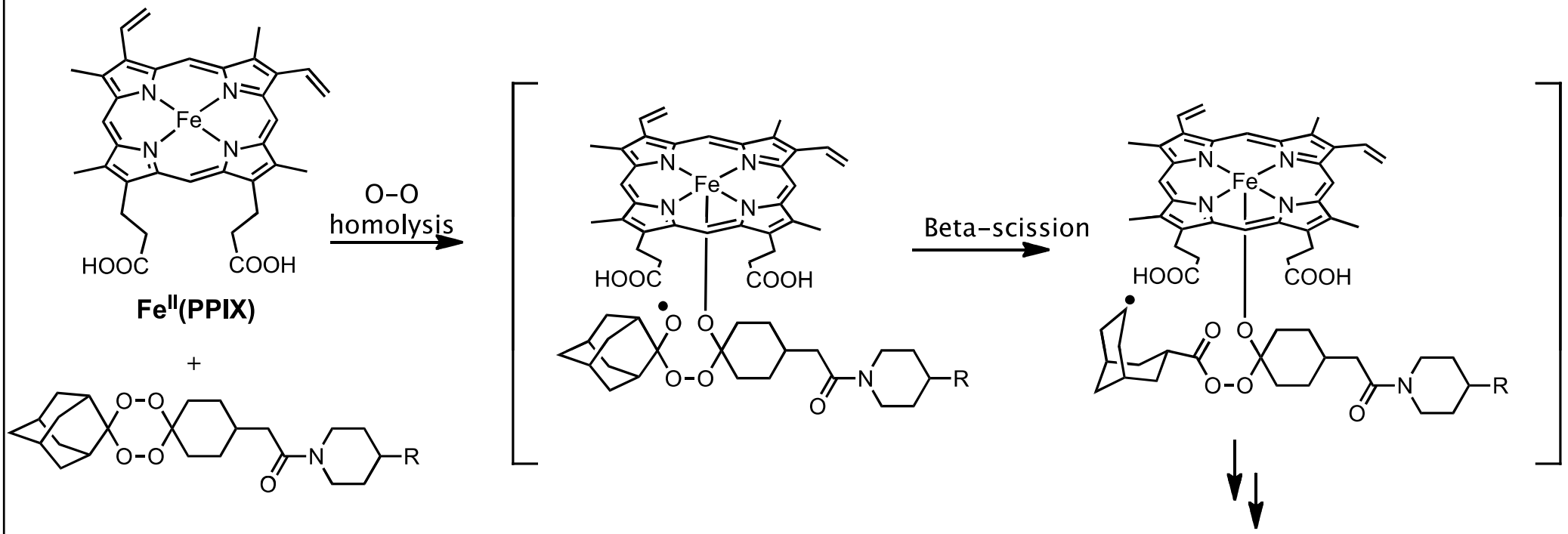
NR^1R^2 = 1-Methyl-4-(4-piperidino) piperazinyl

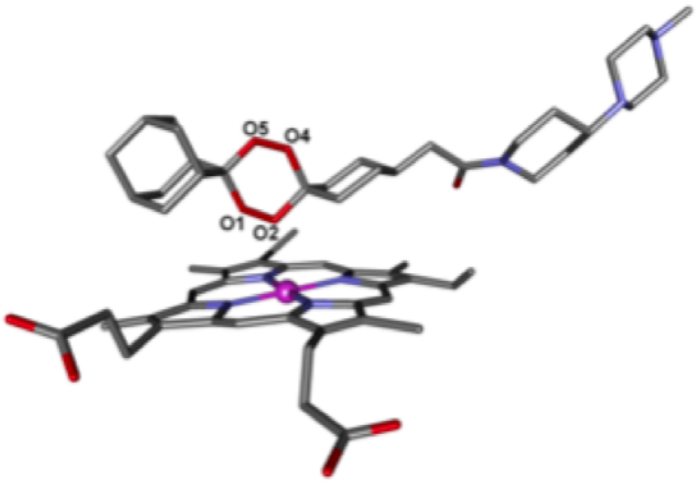
IC50 = 0.6 nM
ED50 = 1mg/kg
ED90 = 4.2 mg/kg
Artesunate = ED50/ED90 :
3.33/12.52 mg/kg

INCREASING IN VITRO AND IN VIVO ACTIVITY, AND ENHANCED PK/EXSPOSURE PROFILES

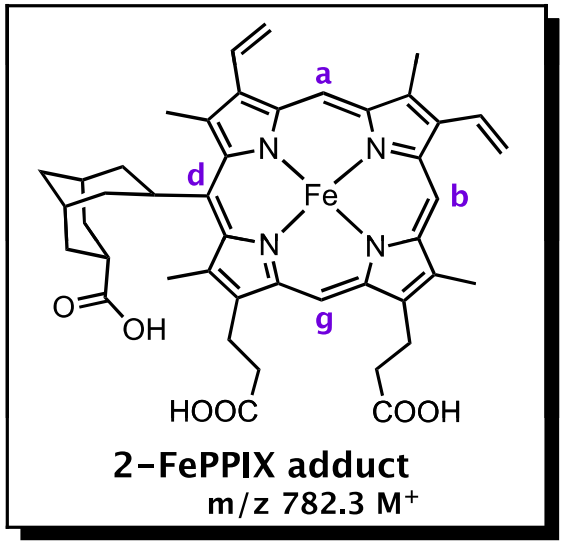
TPP EXCEEDED

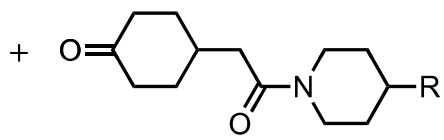
POSSIBLE MECHANISM OF ACTION - HEME ALKYLATION





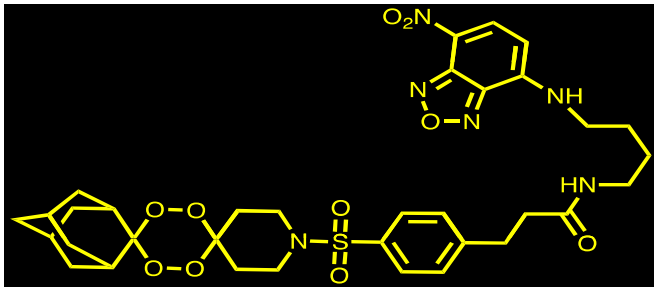
F. Bousejra ElGarah; J. Med. Chem., 2011, 54, 6443

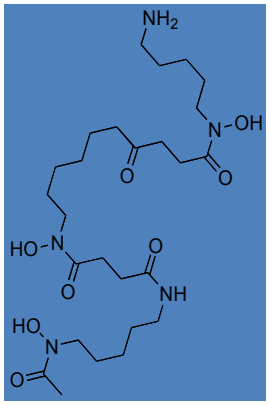




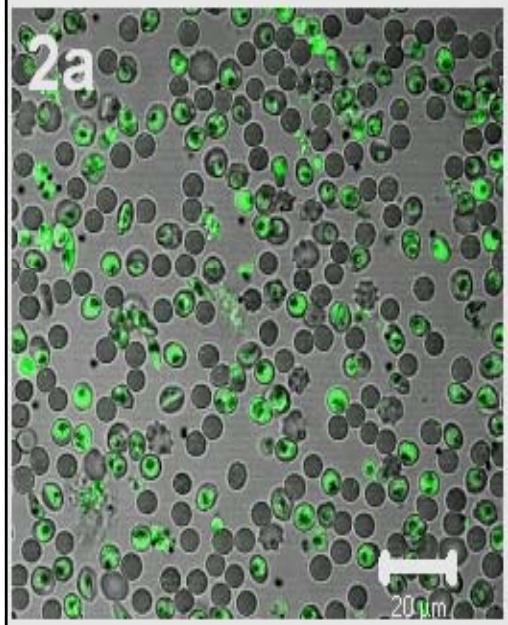
Confocal Imaging Studies - Effects of Iron Chelators

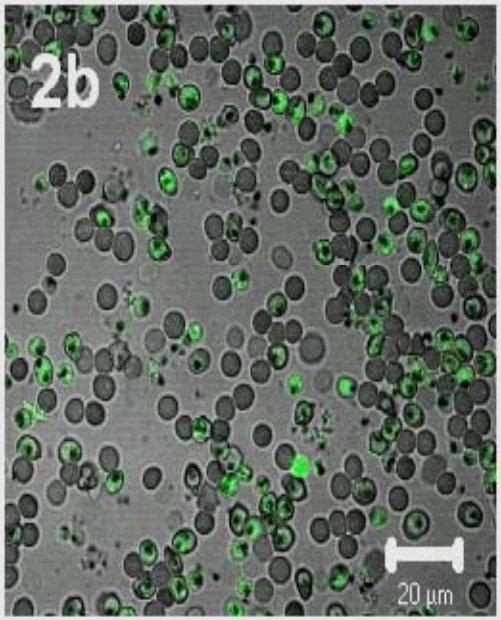
O'Neill, P.M. et al. *Angew. Chem. Int. Ed* .**2007**, *46*, 6278

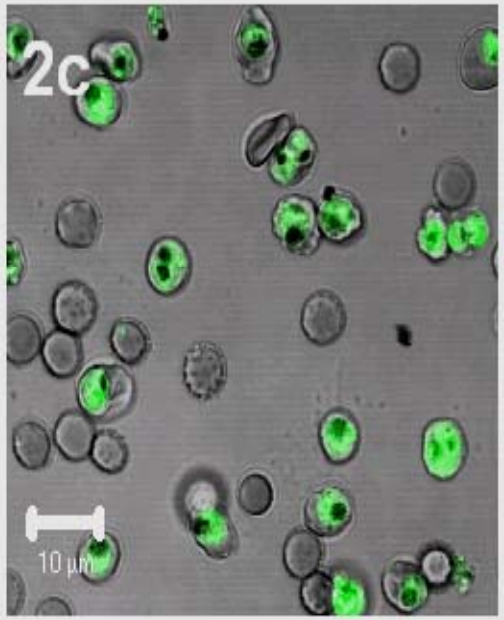


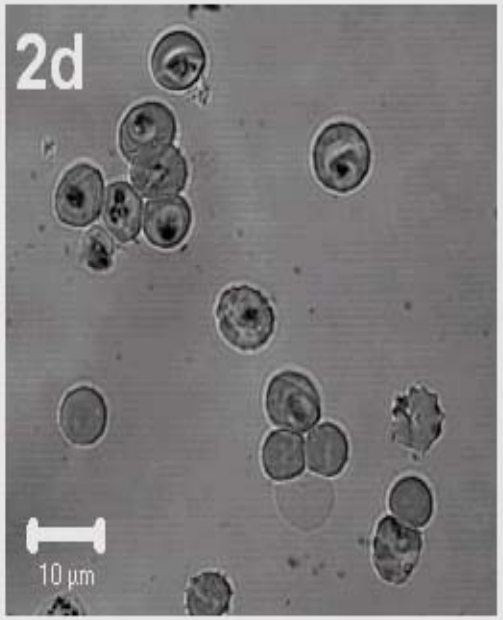


Desferrioxamine (DFO)









Drug is added to chamber containing Infected RBCs

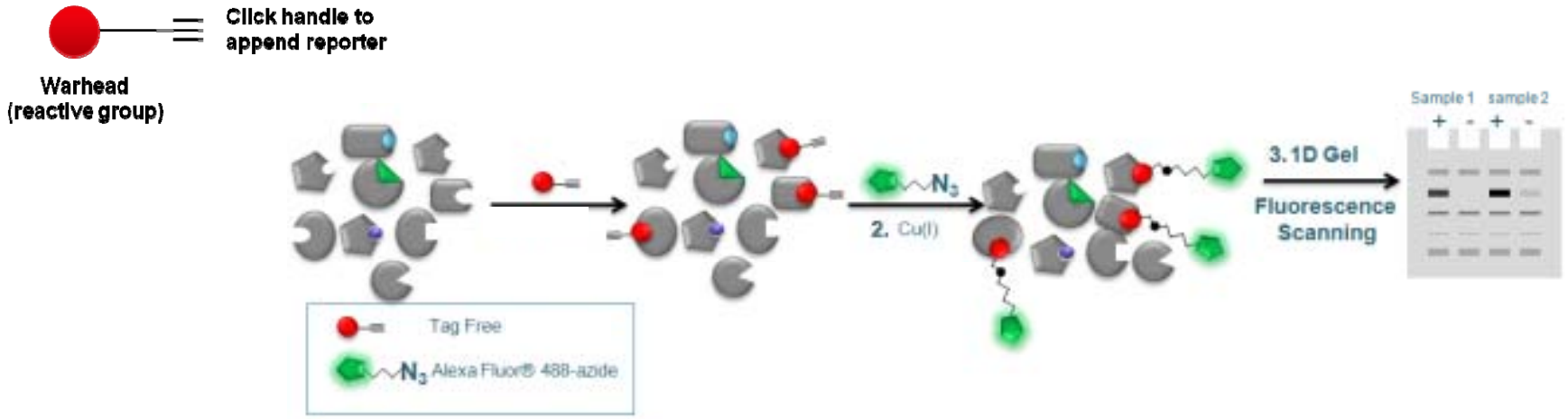
Cells are washed with buffer; note how drug is retained.

Cells pre-treated with DFO prior to addition of drug

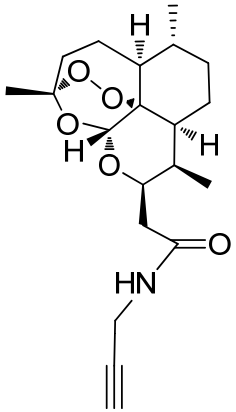
Fluorescent drug can be completely washed out

Activity Based Protein Probes for MOA

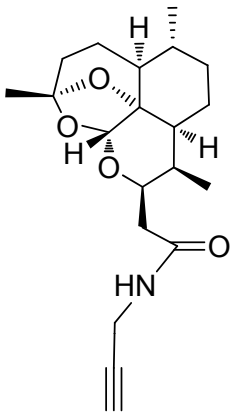
1. Clickable activity based protein probe/ copper catalysed click reaction



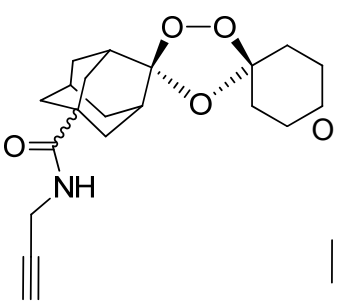
Inhibitor
+



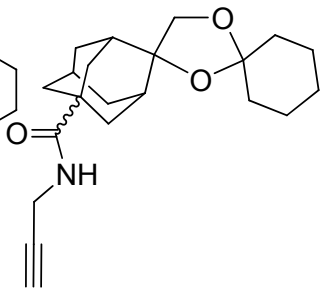
Control
-



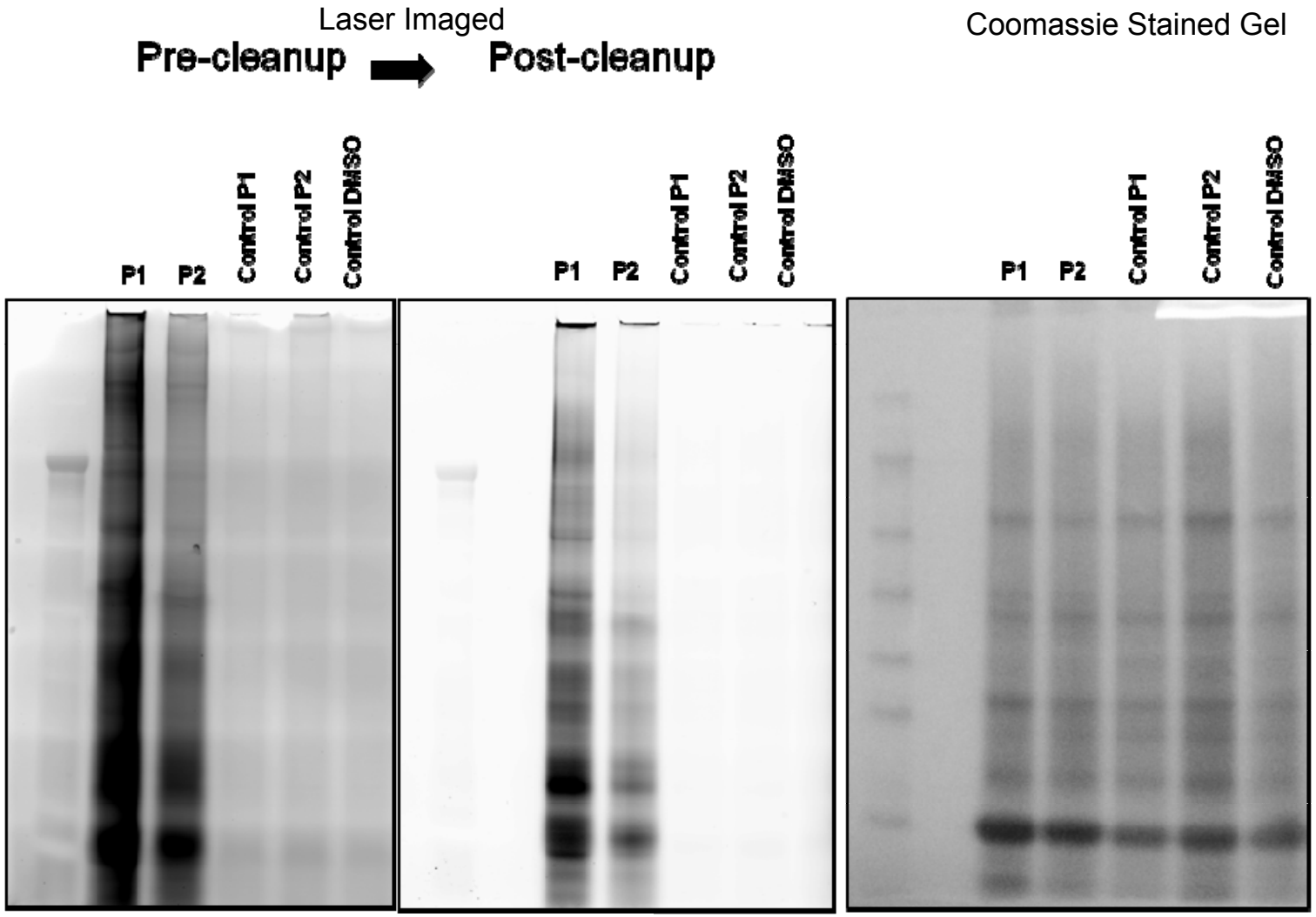
Inhibitor
+



Control
-



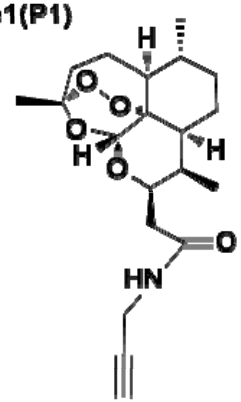
Labelling profile of P1, P2 and control probes



Proteins captured and identified by treatment of 3D7 with Probe 1 (Human and PF-3D7 Proteins)

Score	MW [kDa]	Score	Description
600	246.3		Spectrin beta chain, erythrocyte OS=Homo sapiens GN=SPTB PE=1 SV=5 - [SPTB1_HUMAN]
550	206.1		Ankyrin-1 OS=Homo sapiens GN=ANK1 PE=1 SV=3 - [ANK1_HUMAN]
500	187-196.2		Merozoite surface protein 1 OS=Plasmodium falciparum (isolate Camp / Malaysia) GN=MSP-1 PE=3 SV=2 - [MSP1_PLAFC]
450			Merozoite surface protein 1 OS=Plasmodium falciparum (isolate ro-33 / Ghana) GN=MSP-1 PE=2 SV=2 - [MSP1_PLAF3]
400			Merozoite surface protein 1 OS=Plasmodium falciparum (isolate Wellcome) GN=MSP-1 PE=1 SV=2 - [MSP1_PLAFW]
350	142-162		Multidrug resistance protein OS=Plasmodium falciparum (isolate FC27 / Papua New Guinea) GN=MDR1 PE=3 SV=1 - [MDR_PLAFF]
300			Putative cell division cycle ATPase OS=Plasmodium falciparum (isolate 3D7) GN=PF07_0047 PE=3 SV=2 - [CDAT_PLAF7]
250	101.7		Band 3 anion transport protein OS=Homo sapiens GN=SLC4A1 PE=1 SV=3 - [B3AT_HUMAN]
200	72-77		Erythrocyte membrane protein band 4.2 OS=Homo sapiens GN=EPB42 PE=1 SV=3 - [EPB42_HUMAN]
150			Heat shock 70 kDa protein OS=Plasmodium falciparum PE=2 SV=2 - [HSP70_PLAFA]
100			78 kDa glucose-regulated protein homolog OS=Plasmodium falciparum (isolate NF54) PE=3 SV=1 - [GRP78_PLAFO]
50	65-69		V-type proton ATPase catalytic subunit A OS=Plasmodium falciparum (isolate 3D7) GN=vapA PE=3 SV=1 - [VATA_PLAF7]
0			Keratin, type II cytoskeletal 2 epidermal OS=Homo sapiens GN=KRT2 PE=1 SV=2 - [K22E_HUMAN]
	52-60		T-complex protein 1 subunit eta OS=Plasmodium falciparum (isolate 3D7) GN=PFC0350c PE=3 SV=1 - [TCPH_PLAF7]
			Hexokinase OS=Plasmodium falciparum GN=HK PE=3 SV=1 - [HXK_PLAFA]
			Adenosylhomocysteinase OS=Plasmodium falciparum (isolate 3D7) GN=PFE1050w PE=1 SV=2 - [SAHH_PLAF7]
			Selenium-binding protein 1 OS=Homo sapiens GN=SELENBP1 PE=1 SV=2 - [SBP1_HUMAN]
	48-50		Elongation factor 1-alpha OS=Plasmodium falciparum (isolate K1 / Thailand) GN=MEF-1 PE=3 SV=1 - [EF1A_PLAFK] ★
			Enolase OS=Plasmodium falciparum (isolate 3D7) GN=ENO PE=3 SV=1 - [ENO_PLAF7] ★
	45-47		Alpha-1-antitrypsin OS=Homo sapiens GN=SERPINA1 PE=1 SV=3 - [A1AT_HUMAN]
			Ornithine aminotransferase OS=Plasmodium falciparum (isolate 3D7) GN=OAT PE=3 SV=1 - [OAT_PLAF7] ★
			Phosphoglycerate kinase OS=Plasmodium falciparum (isolate 3D7) GN=PGK PE=1 SV=1 - [PGK_PLAF7]
	40-43		Solute carrier family 2, facilitated glucose transporter member 1 (Fragment) OS=Ovis aries GN=SLC2A1 PE=2 SV=1 - [GTR1_SHEEP]
			Fructose-bisphosphate aldolase OS=Plasmodium falciparum (isolate 3D7) GN=PF14_0425 PE=3 SV=1 - [ALF_PLAF7]
	34 -36KDa		Glyceraldehyde-3-phosphate dehydrogenase OS=Homo sapiens GN=GAPDH PE=1 SV=3 - [G3P_HUMAN]
			Glyceraldehyde-3-phosphate dehydrogenase OS=Omphalotus olearius GN=GPD PE=3 SV=1 - [G3P_OMPOL]
			Glyceraldehyde-3-phosphate dehydrogenase OS=Candida albicans GN=TDH1 PE=1 SV=1 - [G3P_CANAL]
			60S acidic ribosomal protein P0 OS=Plasmodium falciparum (isolate 7G8) GN=RPLP0 PE=2 SV=2 - [RLA0_PLAF8]
			L-lactate dehydrogenase OS=Plasmodium falciparum (isolate CDC / Honduras) PE=1 SV=1 - [LDH_PLAFD] ★
	19-30 Kda		40S ribosomal protein S3a OS=Plasmodium falciparum (isolate 3D7) GN=MAL3P7.35 PE=3 SV=1 - [RS3A_PLAF7]
			40S ribosomal protein SA OS=Plasmodium berghei (strain Anka) GN=PB000415.00.0 PE=3 SV=1 - [RSSA_PLABA]
			Hypoxanthine-guanine-xanthine phosphoribosyltransferase OS=Plasmodium falciparum (isolate FCR-3 / Gambia) GN=LACZ PE=1 SV=1 - [HGXR_PLAFG]
			GTP-binding nuclear protein Ran OS=Plasmodium falciparum PE=2 SV=1 - [RAN_PLAFA]
			Glutathione S-transferase P OS=Homo sapiens GN=GSTP1 PE=1 SV=2 - [GSTP1_HUMAN]
			Peroxiredoxin-2 OS=Pongo abelii GN=PRDX2 PE=2 SV=3 - [PRDX2_PONAB]
	15-16KDa		Hemoglobin subunit beta OS=Callicebus torquatus GN=HBB PE=2 SV=3 - [HBB_CALTO]
			Hemoglobin subunit beta OS=Saimiri sciureus GN=HBB PE=1 SV=2 - [HBB_SAISC]
			Hemoglobin subunit delta OS=Gorilla gorilla gorilla GN=HBD PE=1 SV=2 - [HBD_GORGO]
			Hemoglobin subunit beta OS=Homo sapiens GN=HBB PE=1 SV=2 - [HBB_HUMAN] ★
			Hemoglobin subunit beta-S/F OS=Spermophilus townsendii PE=1 SV=1 - [HBB_SPETO]
			Hemoglobin subunit delta OS=Ateles fusciceps GN=HBD PE=1 SV=1 - [HBD_ATEFU]
			Hemoglobin subunit beta OS=Tadarida brasiliensis GN=HBB PE=1 SV=1 - [HBB_TADBR]
			Hemoglobin subunit alpha OS=Homo sapiens GN=HBA1 PE=1 SV=2 - [HBA_HUMAN]
			Hemoglobin subunit alpha OS=Mesocricetus auratus GN=HBA PE=1 SV=1 - [HBA_MESAU]
			Hemoglobin subunit alpha-2 OS=Bos mutus grunniens PE=1 SV=1 - [HBA2_BOSMU]
	11		**Thioredoxin OS=Plasmodium falciparum (isolate 3D7) GN=PF14_0545 PE=1 SV=1 - [THIO_PLAF7]** ★

Probe1(P1)



Significant score threshold 49

ORIGINAL ARTICLE

Artemisinin Resistance in *Plasmodium falciparum* Malaria

Arjen M. Dondorp, M.D., François Nosten, M.D., Poravuth Yi, M.D., Debashish Das, M.D., Aung Phae Phyo, M.D., Joel Tarning, Ph.D., Khin Maung Lwin, M.D., Frank Ariey, M.D., Ph.D., Warunee Hanpithakpong, Ph.D., Sue J. Lee, Ph.D., Pascal Ringwald, M.D., Kamolrat Silamut, Ph.D., Mallika Imwong, Ph.D., Kesinee Chotivanich, Ph.D., Pharath Lim, M.D., Trent Herdman, Ph.D., Sen Sam An, Shunmay Yeung, Ph.D., Pratap Singhasivanon, M.D., Nicholas P.J. Day, D.M., Niklas Lindegardh, Ph.D., Duong Socheat, M.D., and Nicholas J. White, F.R.S.

ABSTRACT

BACKGROUND

Artemisinin-based combination therapies are the recommended first-line treatments of falciparum malaria in all countries with endemic disease. There are recent concerns that the efficacy of such therapies has declined on the Thai–Cambodian border, historically a site of emerging antimalarial-drug resistance.

METHODS

In open-label, randomized trials, we compared the efficacies of two treatments for uncomplicated falciparum malaria in Pailin, western Cambodia, and Wang Pha, northwestern Thailand: oral artesunate given at a dose of 2 mg per kilogram of body weight per day, for 7 days, and artesunate given at a dose of 4 mg per kilogram per day, for 3 days, followed by mefloquine at two doses totaling 25 mg per kilogram. We assessed the results in relation to in vitro drug susceptibility, artesunate pharmacokinetics, and molecular markers of resistance.

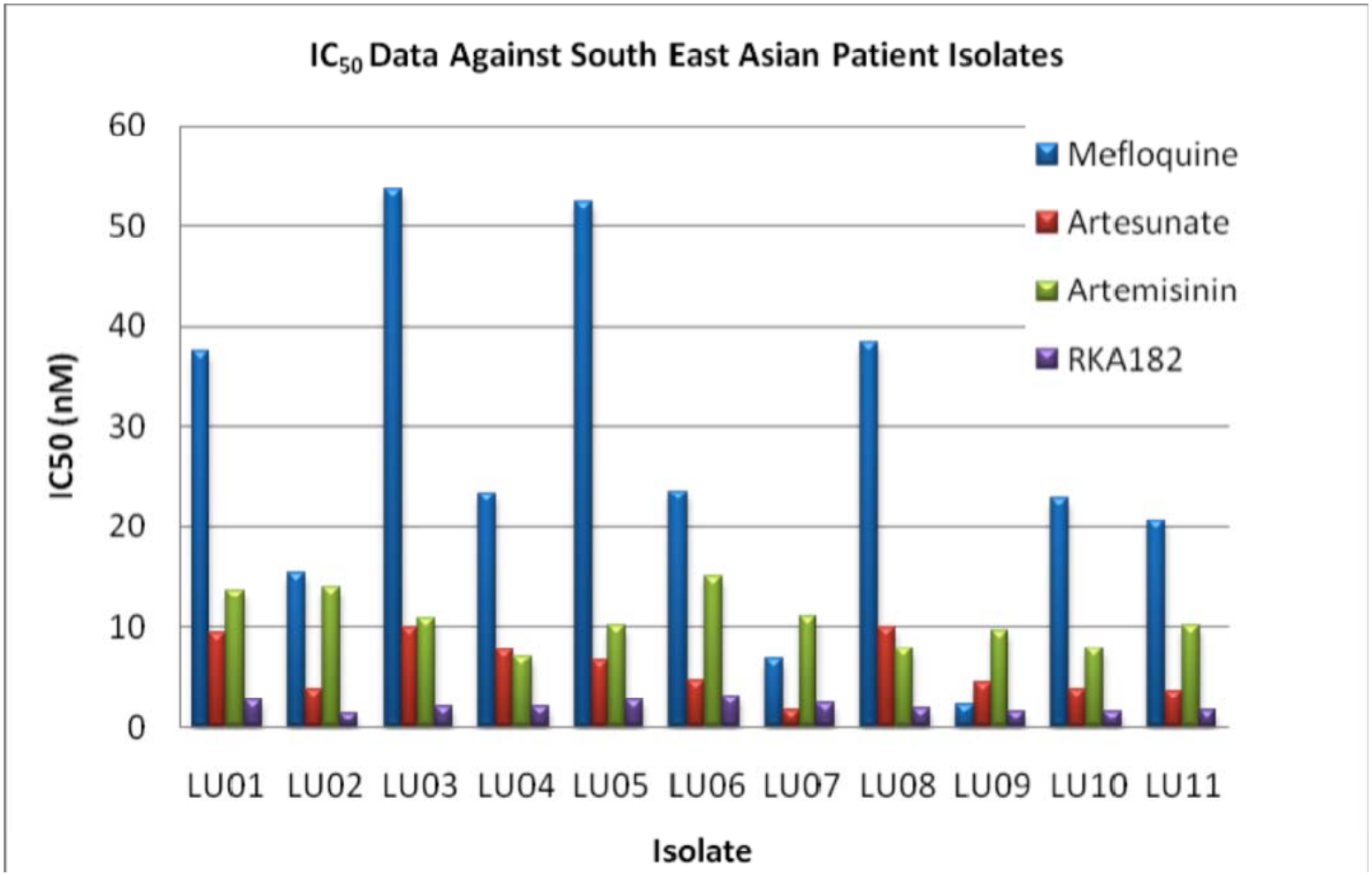
RESULTS

We studied 40 patients in each of the two locations. The overall median parasite clearance times were 84 hours (interquartile range, 60 to 90) in Pailin and 48 hours (interquartile range, 36 to 60) in Wang Pha ($P<0.001$). Parasite clearance as judged by means of polymerase-chain-reaction assay occurred in 6 of 20 patients (30%) receiving artesunate monotherapy and 1 of 20 (5%) receiving artesunate–mefloquine therapy in Pailin, as compared with 2 of 20 (10%) and 1 of 20 (5%), respectively, in Wang Pha ($P=0.31$). These markedly different parasitologic responses were not explained by differences in age, artesunate or dihydroartemisinin pharmacokinetics, results of

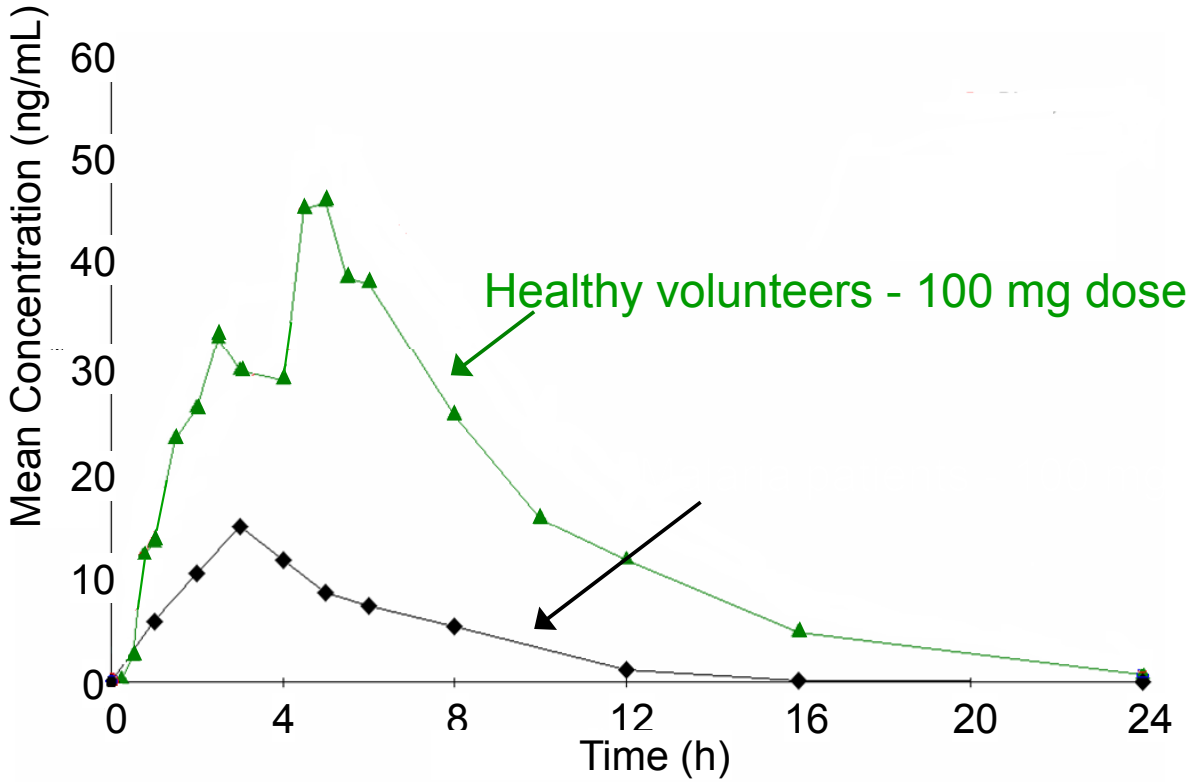
From the Mahidol-Oxford Research Unit, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand (A.M.D., F.N., P.Y., D.D., A.P.P., J.T., K.M.L., W.H., S.J.L., P.R., M.I., K.C., T.H., S.Y., P.L., S.S.A., K.S., P.S., N.P.J.D., N.L., N.J.W.); Shoklo Malaria Research Unit, Mae Sot (F.N., M.A.P., K.M.L.), and Family Health International, Bangkok (F.A., P.L.) — all in Thailand; Centre for Tropical Medicine, Churchill Hospital, Oxford, United Kingdom (A.M.D., F.N., J.T., S.J.L., N.P.J.D., N.J.W.); National Center for Parasitology, Entomology and Malaria Control, Phnom Penh, Cambodia (P.Y., P.L., D.S.); Institut Pasteur du Cambodge (P.A., P.L.); and the Global Malaria Program, World Health Organization, Geneva (P.R.). Address reprint requests to Dr. Dondorp at the Faculty of Tropical Medicine, Mahidol University, 420/6 Rajvithi Rd., Bangkok 10400, Thailand, or at arjen@tropmed.mahidol.ac.th.

N Engl J Med 2009;361:455-67.

In Vitro Activity of RKA182 versus South East Asian Isolates –Thai/Cambodia

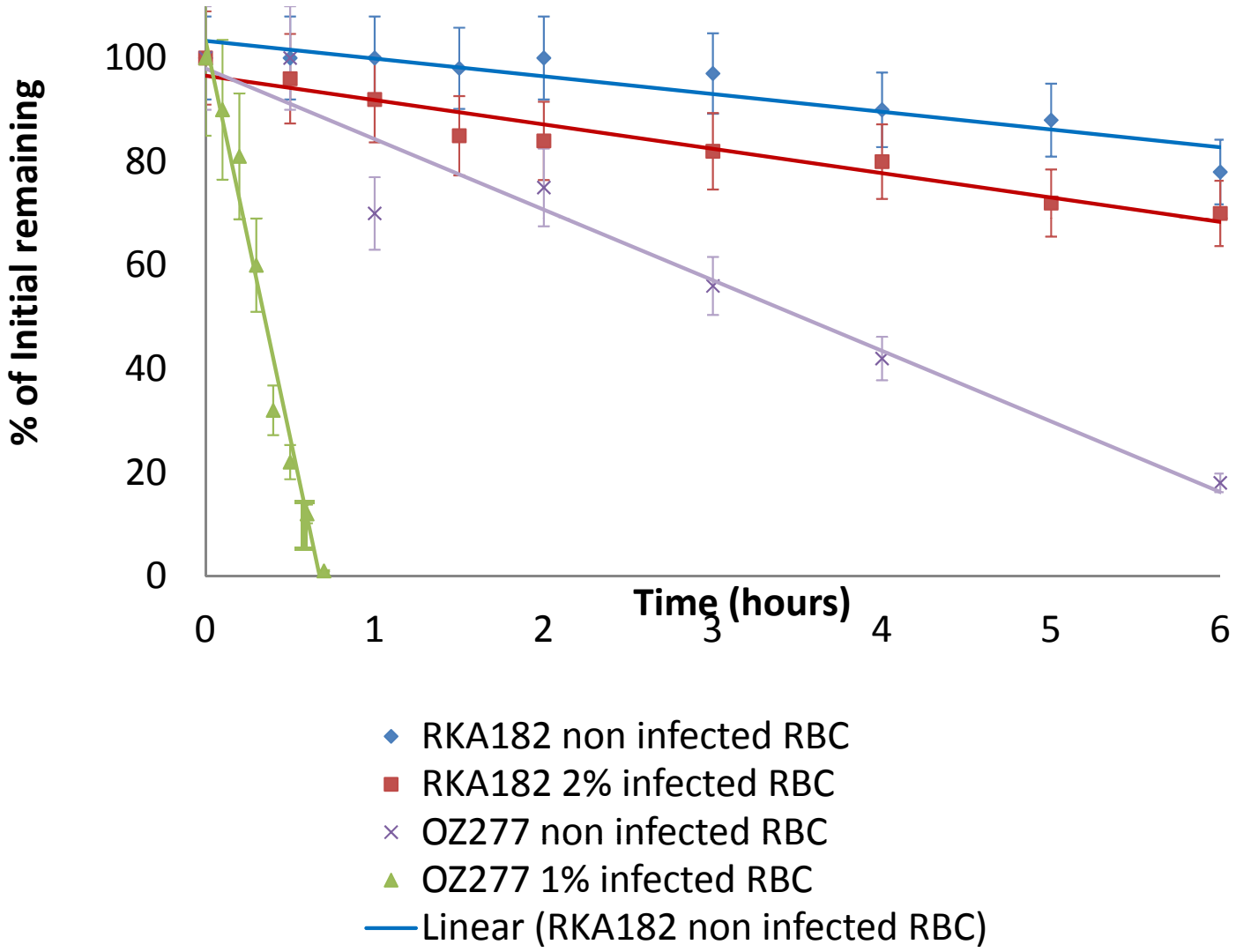


The impact of infection on PK for OZ277

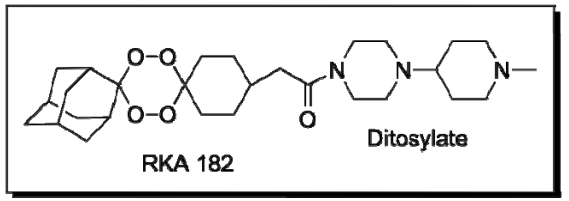


- **Significant reduction in drug plasma concentrations in malaria patients…**
- **Reduced exposure meant that it was unlikely to meet 3-day treatment regimen**
- **Phase II: Approx 70% efficacy (28 APR) with 7 days treatment**

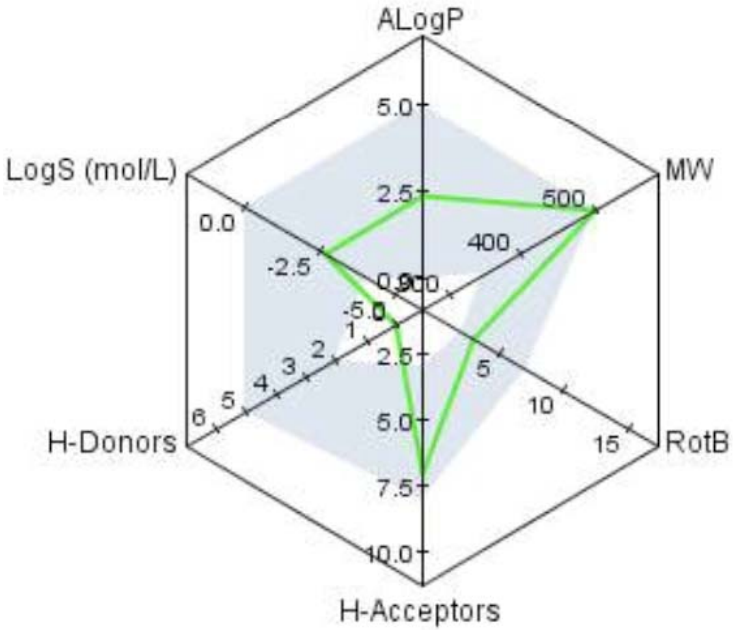
Pharmacokinetics: *RBC stability; Infected & Non-Infected*



Target product profile For Synthetic Peroxide SUMMARY FOR RKA 182

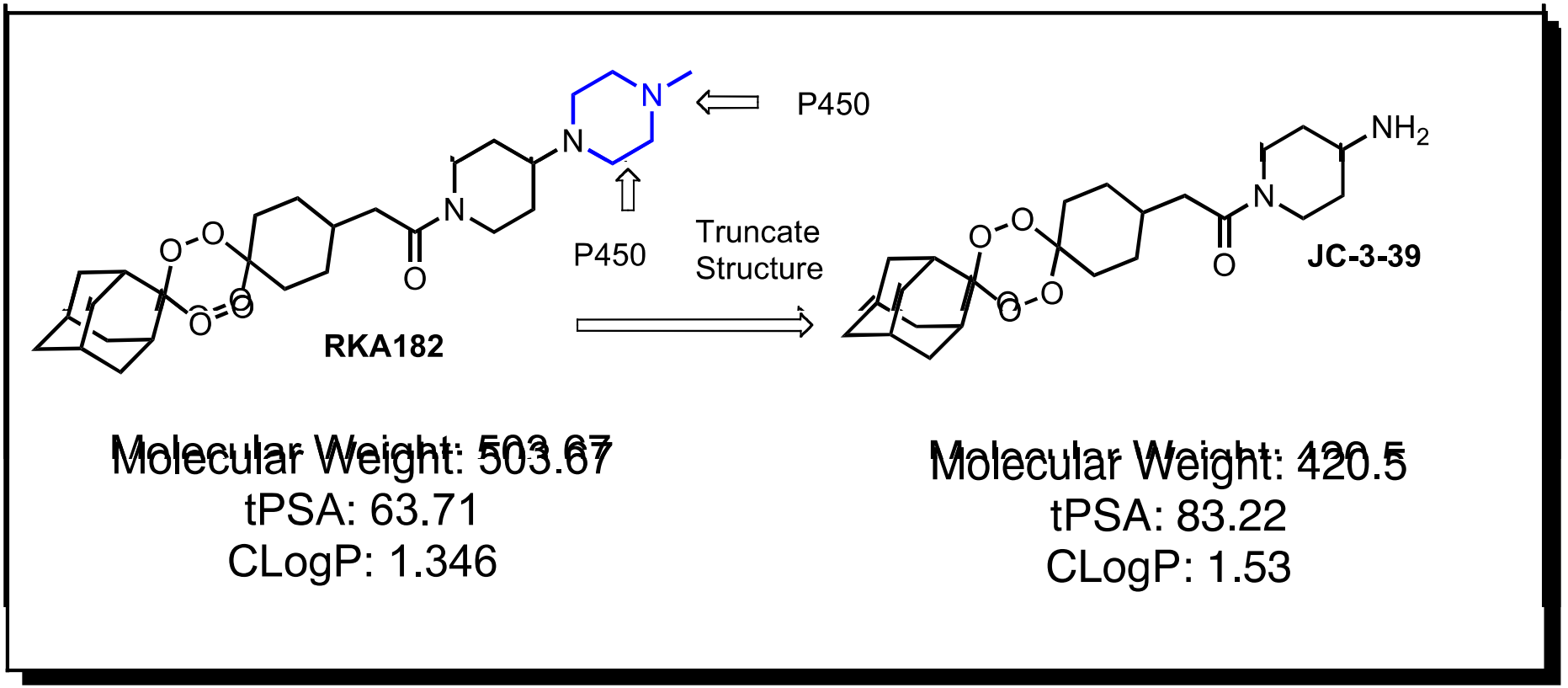


- Simple synthesis / achiral – 4 steps scalable
- Water solubility 4.5 < ClogP < 3. Clog P 2.5
- Activity IC50 < 10 nM *in vitro* 2-5nM
- *In vivo* activity ED90 < 10mg/kg 4mg/kg
- Acceptable toxicity
  - Ames –Ve
  - HERG –Ve (>10μM)
  - MTD 400mg/kg rat
  - MTD 80mg/kg dog
- ADME profiles (F= >25%)
  - F = 38% (possibly 70%)



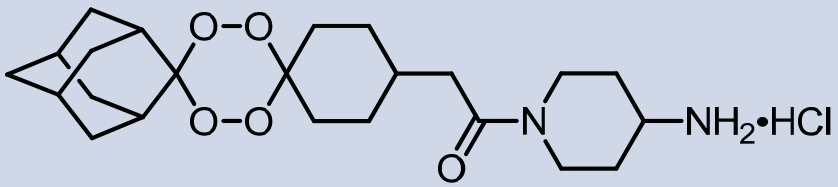
FULL PRECLINICAL COMPLETED MEETS ORIGINAL TPP

MMV's TPP – Aims for Molecules with PK Properties Capable of Delivering Single Dose Cure (TPP1)



PK of RKA182 – whilst improved over semi-synthetics half-life is too short; MMV require longer half-life endoperoxide analogues for combination chemotherapy.

JC3-39 – An improved Variant of RKA 182

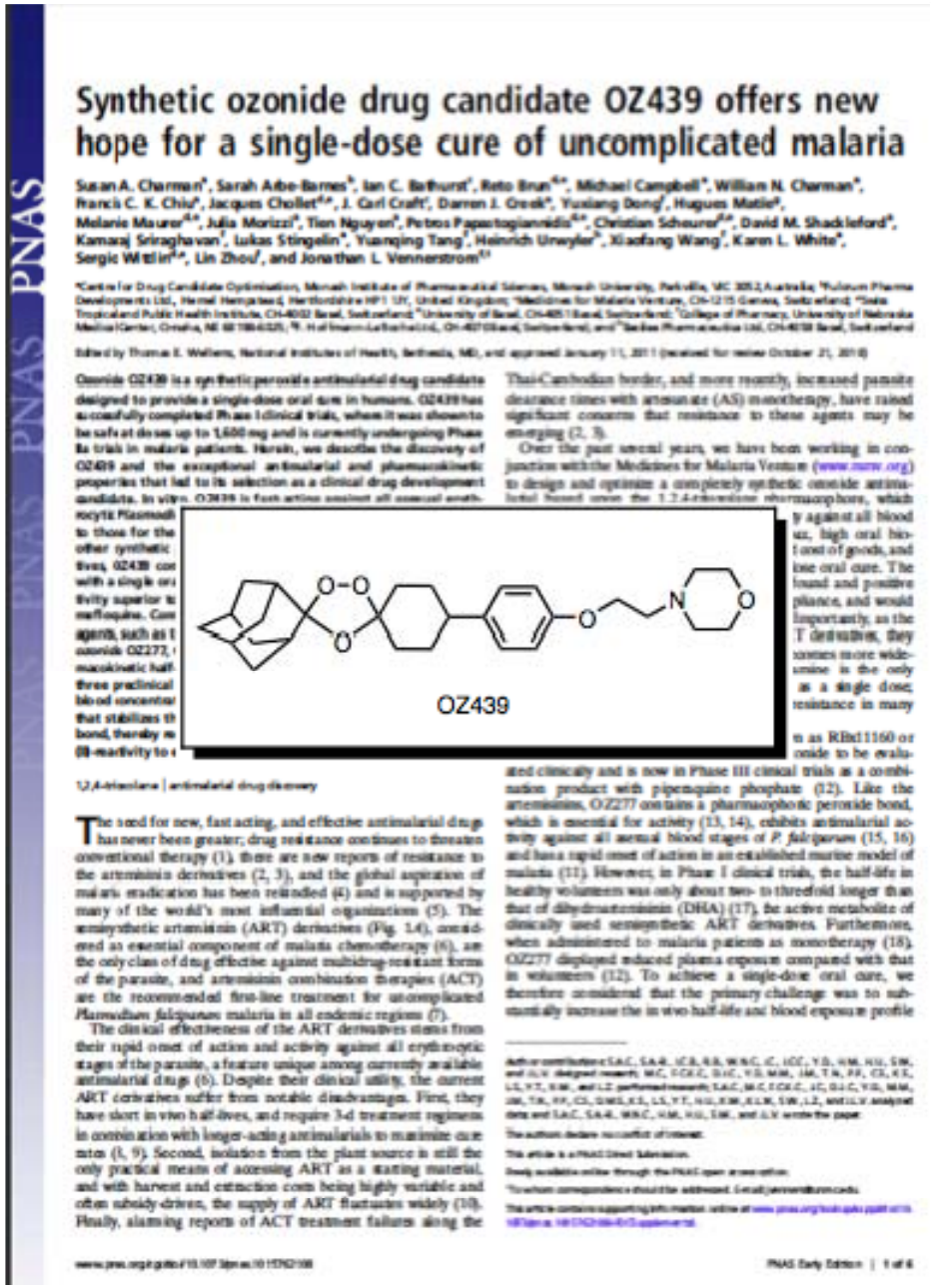
JC3-39

IC_{50}(3D7) = 1.4 nM
ED_{50} = 0.5 mg kg^{-1}
ED_{90} = 3.5 mg kg^{-1}
Mouse survival 3 × 10 mg kg^{-1} p.o. dosing =17 days (Free base) (Artes = 8 days)
PK in Rat (2 mg kg^{-1} *i.v.*/20 mg kg^{-1} *p.o.*) **T½ (*p.o.*) = 7 h, T½ (*i.v.*) =2h** **F = 76 %**
Solubility >50 mg ml^{-1} in H_2O (22 °C)

Truncation of Side-chain

Key Features

- Maintained Antimalarial Activity
- Increased metabolic stability/simpl profile
- Enhanced Drug Exposure/ F(%) (7
- Less toxic than RKA 182 in repeat dose toxicity studies in preclinical animal studies
- PK profile and Single dose

PNAS **February 7, 2011**



Synthetic ozonide drug candidate OZ439 offers new hope for a single-dose cure of uncomplicated malaria

Susan A. Charman[a], Sarah Arbe-Barnes[b], Ian C. Bathurst[c], Reto Brun[d,e], Michael Campbell[a], William N. Charman[a], Francis C. K. Chiu[a], Jacques Chollet[d,e], J. Carl Craft[a], Darren J. Creek[a], Yuxiang Dong[f], Hugues Matile[g], Melanie Maurer[d,e], Julia Morizzi[g], Tien Nguyen[g], Petros Papastogiannidis[d,e], Christian Scheurer[d,e], David M. Shackleford[a], Kamaraj Sriraghavan[f], Lukas Stingelin[d], Yuanqing Tang[f], Heinrich Urwyler[b], Xiaofang Wang[f], Karen L. White[a], Sergio Wittlin[d,e], Lin Zhou[f], and Jonathan L. Vennerstrom[a,1]

Edited by Thomas E. Wellems, National Institutes of Health, Bethesda, MD, and approved January 11, 2011 (received for review October 21, 2010)

Ozonide OZ439 is a synthetic peroxide antimalarial drug candidate designed to provide a single-dose oral cure in humans. OZ439 has successfully completed Phase I clinical trials, where it was shown to be safe at doses up to 1,600 mg and is currently undergoing Phase IIa trials in malaria patients. Herein, we describe the discovery of OZ439 and the exceptional antimalarial and pharmacokinetic properties that led to its selection as a clinical drug development candidate. In vitro, OZ439 is fast acting against all asexual erythrocyte Plasmodi… to those for the other synthetic… tives, OZ439 co… with a single or… tivity superior to… mefloquine. Cure… agents, such as t… ozonide OZ277, … macokinetic half… three preclinical… blood concentra… that stabilizes th… bond, thereby re… (i)-activity to …

OZ439

1,2,4-trioxolane | antimalarial drug discovery

The need for new, fast acting, and effective antimalarial drugs has never been greater; drug resistance continues to threaten conventional therapy (1), there are new reports of resistance to the artemisinin derivatives (2, 3), and the global aspiration of malaria eradication has been rekindled (4) and is supported by many of the world's most influential organizations (5). The semisynthetic artemisinin (ART) derivatives (Fig. 1A), considered as essential component of malaria chemotherapy (6), are the only class of drug effective against multidrug-resistant forms of the parasite, and artemisinin combination therapies (ACT) are the recommended first-line treatment for uncomplicated *Plasmodium falciparum* malaria in all endemic regions (7).

The clinical effectiveness of the ART derivatives stems from their rapid onset of action and activity against all erythrocytic stages of the parasite, a feature unique among currently available antimalarial drugs (6). Despite their clinical utility, the current ART derivatives suffer from notable disadvantages. First, they have short in vivo half-lives, and require 3-d treatment regimens in combination with longer-acting antimalarials to maximize cure rates (1, 9). Second, isolation from the plant source is still the only practical means of accessing ART as a starting material, and with harvest and extraction costs being highly variable and often subsidy-driven, the supply of ART fluctuates widely (10). Finally, alarming reports of ACT treatment failures along the Thai-Cambodian border, and more recently, increased parasite clearance times with artesunate (AS) monotherapy, have raised significant concerns that resistance to these agents may be emerging (2, 3).

Over the past several years, we have been working in conjunction with the Medicines for Malaria Venture (www.mmv.org) to design and optimize a completely synthetic ozonide antimalarial based upon the 1,2,4-trioxolane pharmacophore, which … y against all blood … ss, high oral bio… t cost of goods, and … ose oral cure. The … found and positive … pliance, and would … importantly, as the … T derivatives, they … comes more wide… umine is the only … as a single dose; … resistance in many …

… n as RBx11160 or … oxide to be evalu… ated clinically and is now in Phase III clinical trials as a combination product with piperaquine phosphate (12). Like the artemisinins, OZ277 contains a pharmacophoric peroxide bond, which is essential for activity (13, 14), exhibits antimalarial activity against all asexual blood stages of *P. falciparum* (15, 16) and has a rapid onset of action in an established murine model of malaria (11). However, in Phase I clinical trials, the half-life in healthy volunteers was only about two- to threefold longer than that of dihydroartemisinin (DHA) (17), the active metabolite of clinically used semisynthetic ART derivatives. Furthermore, when administered to malaria patients as monotherapy (18), OZ277 displayed reduced plasma exposure compared with that in volunteers (12). To achieve a single-dose oral cure, we therefore considered that the primary challenge was to substantially increase the in vivo half-life and blood exposure profile

Author contributions: S.A.C., S.A.-B., I.C.B., R.B., W.N.C., J.C., J.C.C., Y.D., H.M., H.U., S.W., and J.L.V. designed research; M.C., D.C.C., D.J.C., Y.D., M.M., J.M., T.N., P.P., C.S., K.S., L.S., Y.T., X.W., and L.Z. performed research; S.A.C., M.C., F.C.K.C., J.C., D.J.C., Y.D., M.M., J.M., T.N., P.P., C.S., D.M.S., K.S., L.S., Y.T., H.U., X.W., K.L.W., S.W., L.Z., and J.L.V. analyzed data; and S.A.C., S.A.-B., W.N.C., H.M., H.U., S.W., and J.L.V. wrote the paper.

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OZ439 Raises the Bar

Cures malaria with a single oral dose in *P. berghei* model (30 mg/kg single dose)

Is more effective (against *P. berghei*) than mefloquine as prophylactic agent (and faster acting)

Exhibits 5-fold lower clearance, and 9-fold longer half life than OZ277 (in mice, rats, dogs)

Has high and reproducible oral bioavailability

Has minimal toxicity in rats, negative AMES and MNT, low hERG potential, and low toxicity in rat whole embryo cultures

Strategies Adopted for Third Generation Series

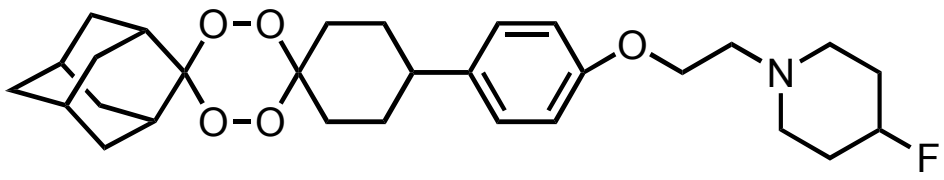
Goals:

- Investigate blood-mediated degradation (and subsequent in vivo clearance)
- Extend half-life to enhance exposure/Increase Metabolic stability

Strategy:

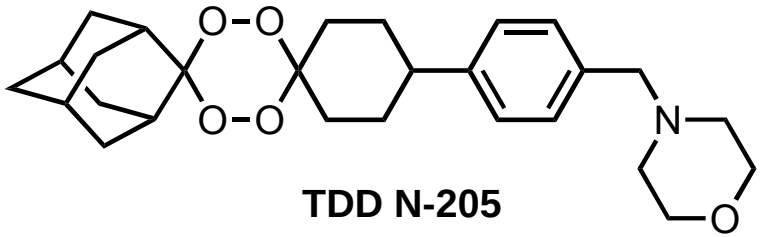
- Modify structure of JC-3-39 to include OZ439 Tetraoxane Hybrids.
- Modifications intended to alter logD, pKa and Fe(II) stability

Late Leads Identified



TDD E-209

In vitro IC50 3D7; 5.1 nM
F (rat) = 60-100 %, iv $T_{1/2}$ = 21h
Single dose ED_{90} Pfscid = 11 mg/kg
P. berghei MSD = 25 days (2/3 cures)*

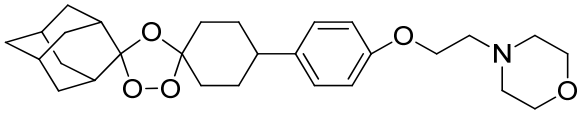
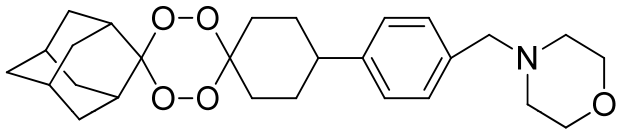
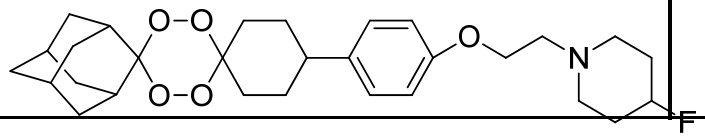


TDD N-205

In vitro IC50 3D7; 1.3 nM
F (rat) = 60-100 %, iv $T_1/_2$ = 5.7h *
Single Dose ED_{90} Pfscid = 8.6 mg/kg
P. berghei MSD = 25 days (2/3 cures)

* Shanghai Chem Partners data; may Underestimate T1/2 based on Timepoints used.

Key Results - In vitro activity

Code	Structure	IC50(nM) vrs 3D7
OZ439		8.0 ± 0.3
TDD-N205		1.3 ± 0.1
TDD-E209		5.1 ± 0.81

In vitro IC50 values for TDD-E209 against several sensitive and resistant strains of the malarial parasite.

Parasite strain	IC50 (nM)	ART	Chloroquine
***NF54**	5.2 ± 1.0	4.7 ± 1.0	5.9 ± 1.0
#K1	4.2 ± 0.8	2.8 ± 0.6	173.0 ± 29.5
***HB3**	4.2 ± 0.8	2.8 ± 0.6	9.0 ± 1.5
#7G8	2.9 ± 0.6	1.8 ± 0.4	55.0 ± 9.4
#TM90C2B	7.3 ± 1.4	3.8 ± 0.8	121.0 ± 20.6
***D6**	8.5 ±1.6	5.8 ± 1.2	8.6 ± 1.5
#V1/S	4.2 ± 0.8	2.7 ± 0.6	193.5 ± 33.0
#Dd2	7.7 ± 1.5	5.4 ± 1.1	150.5 ± 25.7
#FCB	14.0 ± 2.7	5.8 ± 1.2	66.0 ± 11.3

LSTM

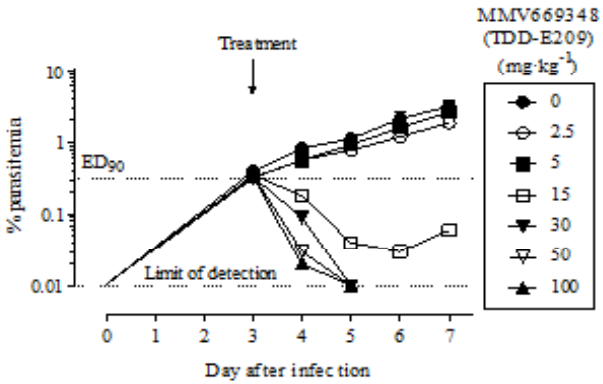
In vivo activity and Average mouse survival

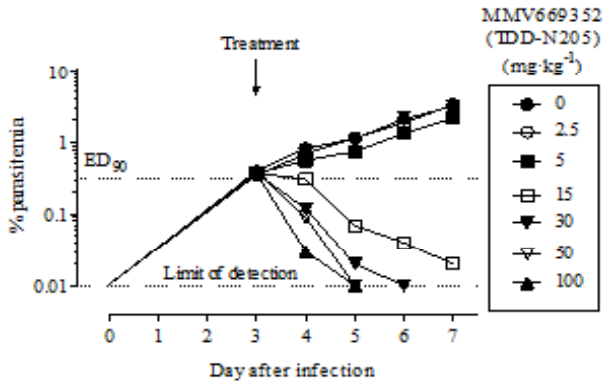
Code	% Activity	Average mouse survival Following 30mg/kg dose
OZ439	99.4	30 (30, 30, 30)
#TDD-N205	99.42	26.3 (16, 30, 30)
#TDD-E209	99.65	25.0 (15, 30, 30)
[1]TDD-N205 Mesylate	99.30	25.0 (13, 30, 30)
[2]TDD-E209 Mesylate	99.42	13.7 (13,14, 14)
Control	-	4.0 (4, 4, 4)

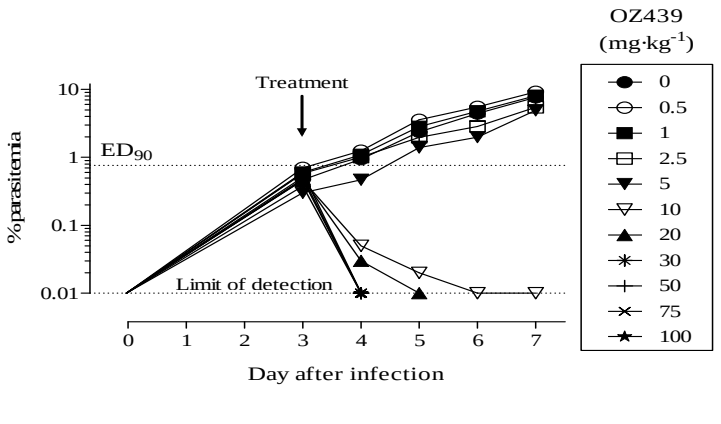
- Mean survival times were vehicle dependent.
- The citric acid formulation of E209 gave 2/3 cures whilst the mesylate salt gave no cures.
- Both the citric acid formulation the mesylate salts of N205 gave 2/3 cures.
- OZ439 consistently gave 3/3 cures.

LSTM

Therapeutic Efficacy of TDD-E209, TDD-N205







Method of estimation	Goodness of fit	Parameter	Mean	Interval of confidence of the mean at 95% (IC_{95})	Unit of parameter
TDD-E209					
log fit	0.99	ED_{90}	11.6	10.5-13	$mg \cdot kg^{-1}$
log fit	0.99	AUC_{ED90}	1.2	1-1.4	$\mu g \cdot h \cdot ml^{-1}$
log fit	0.99	AUC_{PCC}	3	2.3-4.4	$\mu g \cdot h \cdot ml^{-1}$
TDD-N205					
log fit	0.99	ED_{90}	8.6	7.7-9.6	$mg \cdot kg^{-1}$
log fit	-	AUC_{ED90}	< 0.75	-	$\mu g \cdot h \cdot ml^{-1}$
***OZ439**					
log fit	0.99	ED_{90}	6.0	5.65-6.9	$mg \cdot kg^{-1}$
log fit	0.99	AUC_{ED90}	0.68	0.6-0.9	$\mu g \cdot h \cdot ml^{-1}$

- **No cures** were observed at the dose levels administered for N205, E209 and OZ439.
- All compounds were fast acting.

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Current status

TDD-E209 and TDD-N205 have been identified as potential late leads

- in vitro potency
- DMPK single dose cure
- Iron stability
- X resistance studies (ongoing)
- Superior to OZ469

- non-GLP pre-clinical studies completed Q1 2014
- CANDIDATE DECLARATION Q1 2014

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Summary

Third Generation Tetraoxanes Show promise for full development

Several Analogues discovered with superior properties to OZ277, RKA 182 and all semi-synthetics artemisinins.
OZ439 still remains the gold standard in mouse PD model
Single dose PoC?

Clearly extending $T_{1/2}$ for this class of drug has implications in terms of potential toxicity (neurotoxicity, embryotoxicity) and this aspect will be addressed Should TPP1 features be achieved.

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