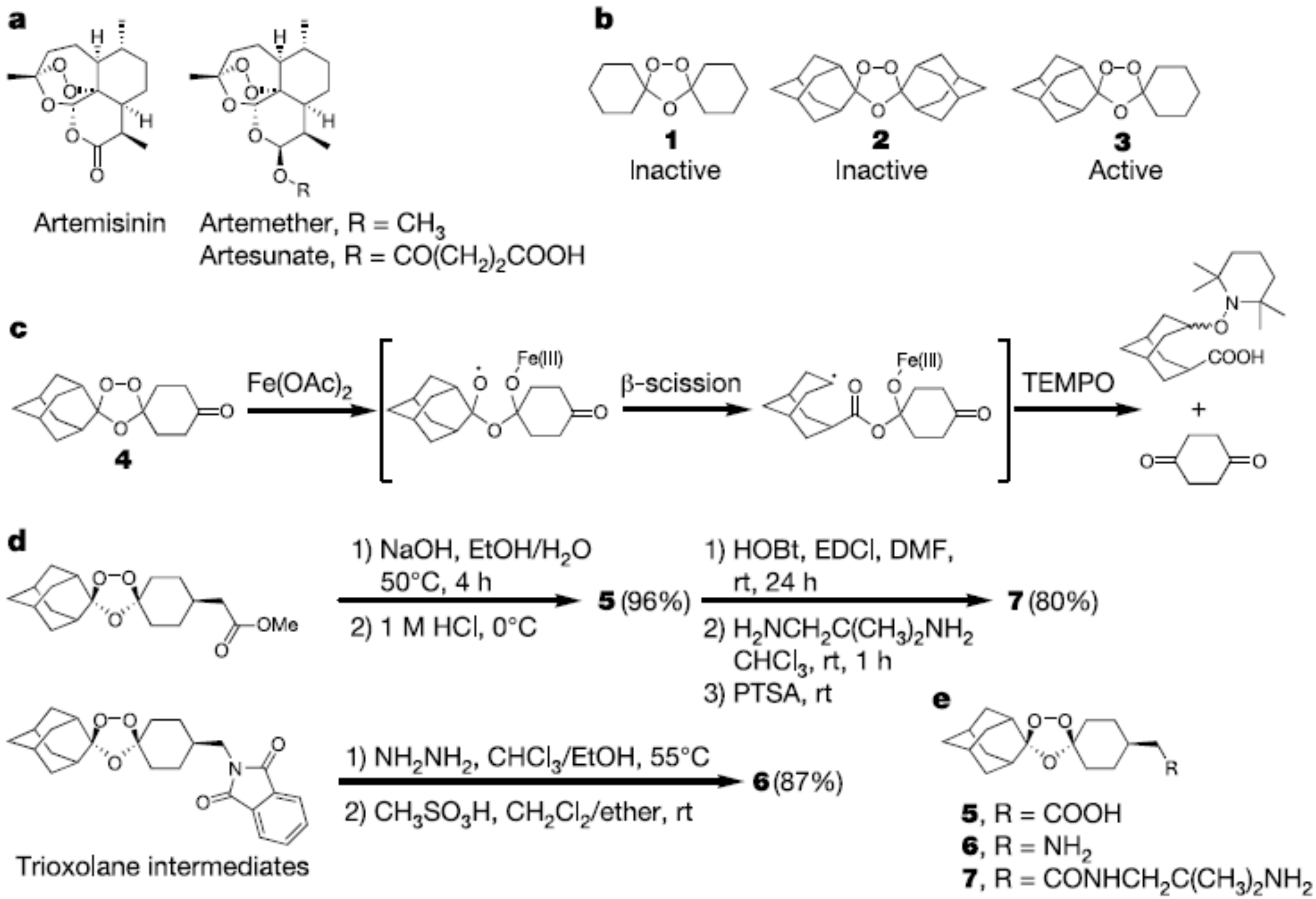
Enantioselective Synthesis and in Vivo Evaluation of Regioisomeric Analogues of the Antimalarial Arterolane

Brian R. Blank, Jiri Gut, Philip J. Rosenthal and Adam R. Renslo*

J. Med. Chem. 2017, 60, 6400–6407

Shikha Singh Chauhan
Current Literature 09/02/17
Wipf group

Identification of an antimalarial synthetic trioxolane drug development candidate



Vennerstrom et al. *Nature*, 2004, *430*, 900-904

PK/PD properties

Table 1 **Half-life and bioavailability values after a single oral dose to healthy rats**

Compound	Dose (mg kg^{-1})	Half life (h)	Bioavailability (%)
Trioxolane **5**	50.0	2.0 ± 0.3 (*n* = 3)	74.1 ± 19.1 (*n* = 3)
Trioxolane **6**	18.8	1.8, 1.6‡	27.5, 17.3‡
Trioxolane **7**	17.4	1.4 ± 0.2 (*n* = 3)	35.0 ± 6.8 (*n* = 3)
Artemether	50.0	ND	1.4 ± 0.6 (*n* = 3)
Artesunate	10.0	0.47, 0.48*‡	23.3, 32.3†‡

Values are mean ± s.d.
*Half life for dihydroartemisinin after dosing with artesunate.
†Oral bioavailability based on artesunate concentrations.
‡Only two measurements available.

Vennerstrom et al. *Nature* , 2004, *430*, 900-904

In Vitro and In vivo activities

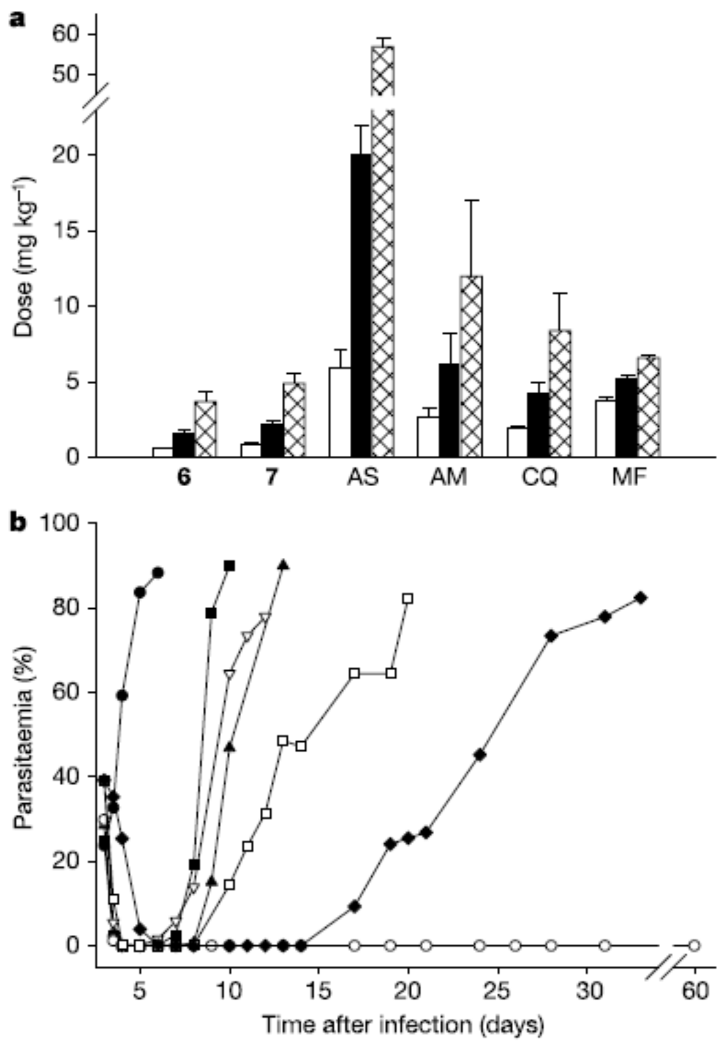
Table 2 ***In vitro* activity against *P. falciparum* and *in vivo* activity in *P. berghei*-infected mice**

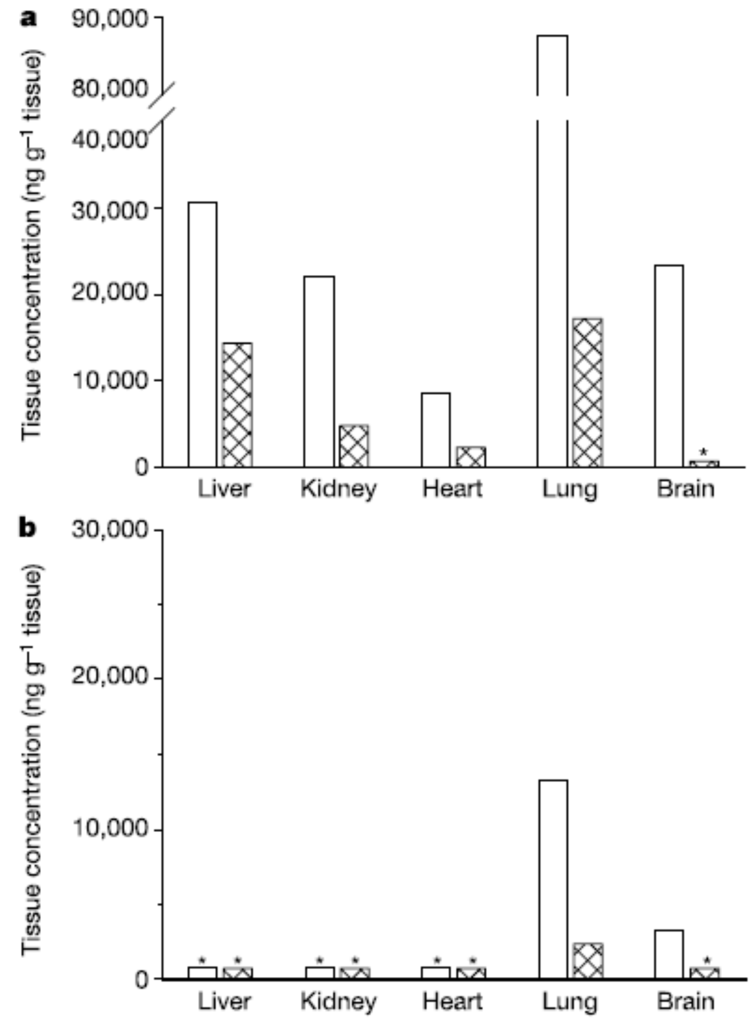
Compound	IC_{50} (ng ml^{-1}) *		1 × 3 mg kg^{-1} (oral)		3 × 10 mg kg^{-1} (oral)		
	Strain K1†	Strain NF54‡	Activity (%)	Survival (days)	Activity (%)	Survival (days)	Cure (%)§
Control	–	–	0	5.2	0	5.2	0
Trioxolane **5**	34 ± 6	45 ± 6	50	9.0	NT	–	–
Trioxolane **6**	0.39 ± 0.06	0.42 ± 0.06	99	10.0	>99.99	30.0	100
Trioxolane **7**	1.0 ± 0.1	0.91 ± 0.12	98	9.0	>99.99	26.2	67
Artesunate	1.3 ± 0.2	1.6 ± 0.1	33	6.6	97	11.0	0
Artemether	0.74 ± 0.11	1.2 ± 0.1	56	8.0	>99.99	22.3	0
Chloroquine	62 ± 4	5.1 ± 0.8	85	7.9	99.99	18.2	0
Mefloquine	3.0 ± 0.1	5.8 ± 0.2	18	7.0	99.92	24.3	0

NT, not tested.
*Mean ± s.e.m. ($n \geq 10$).
†Chloroquine-resistant (Thailand).
‡Chloroquine-sensitive (airport, unknown origin).
§No detectable parasites at 30 days after infection.

Vennerstrom et al. *Nature* , 2004, *430*, 900-904

Comparison of activities and toxicities of 6 & 7





Vennerstrom et al. *Nature* , 2004, *430*, 900-904

Toure *et al. Malar J (2015) 14:469*
DOI 10.1186/s12936-015-0982-y

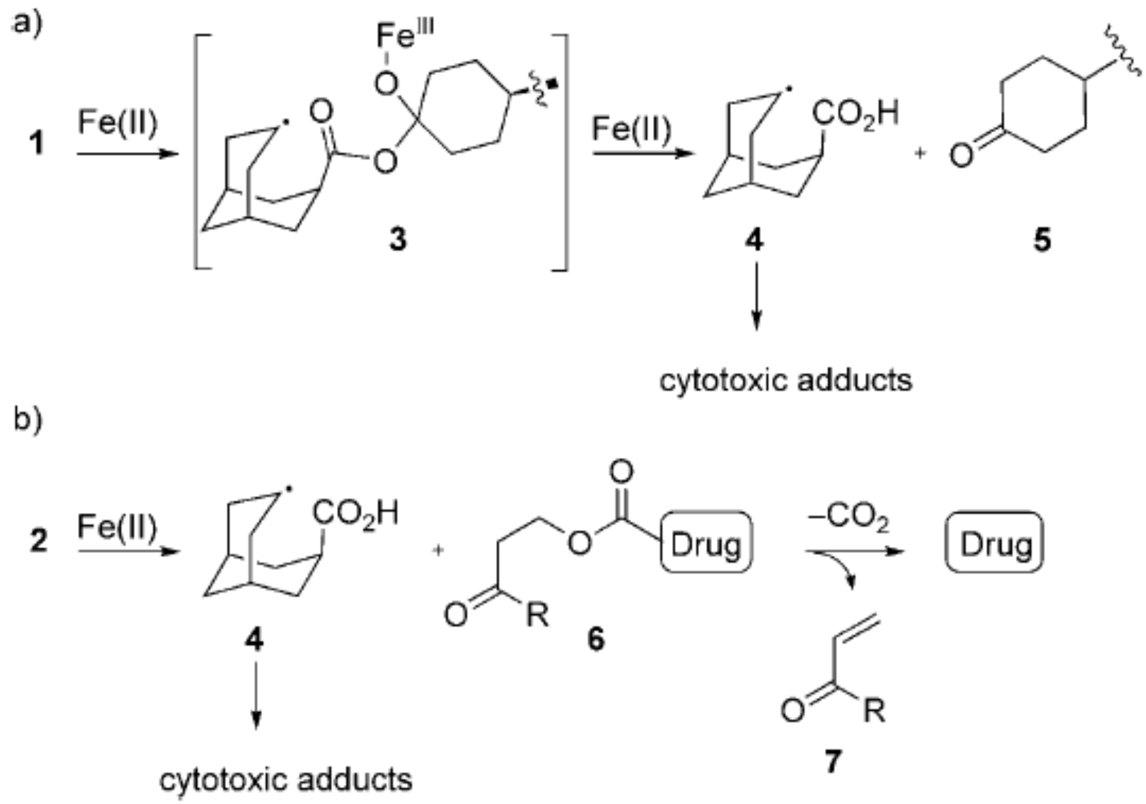
MALARIA JOURNAL

RESEARCH Open Access

Efficacy and safety of fixed dose combination of arterolane maleate and piperaquine phosphate dispersible tablets in paediatric patients with acute uncomplicated *Plasmodium falciparum* malaria: a phase II, multicentric, open-label study

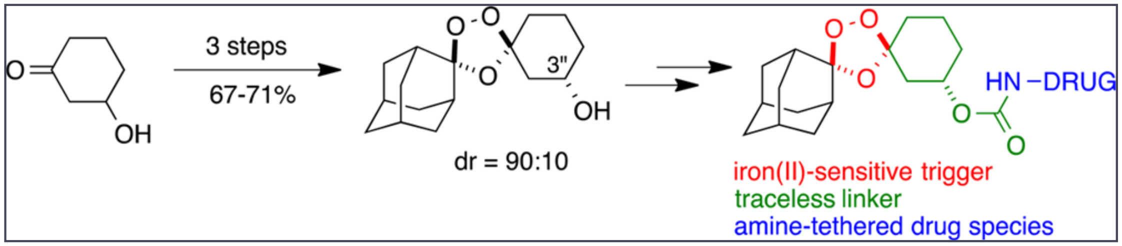
Offianan Andre Toure[1], Stephen Rulisa[2], Anupkumar R. Anvikar[3], Ballamudi S. Rao[4], Pitabas Mishra[5], Rajinder K. Jalali[6], Sudershan Arora[7], Arjun Roy[8], Nilanjan Saha[9], Sunil S. Iyer[10], Pradeep Sharma[10] and Neena Valecha[3*]

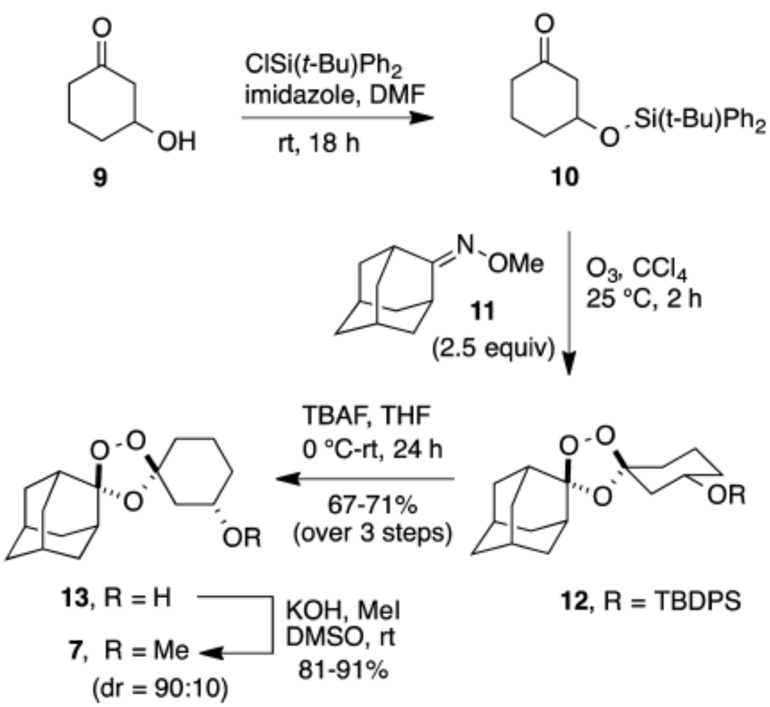
A Fragmenting Hybrid Approach for Targeted Delivery of Multiple Therapeutic Agents to the Malaria Parasite

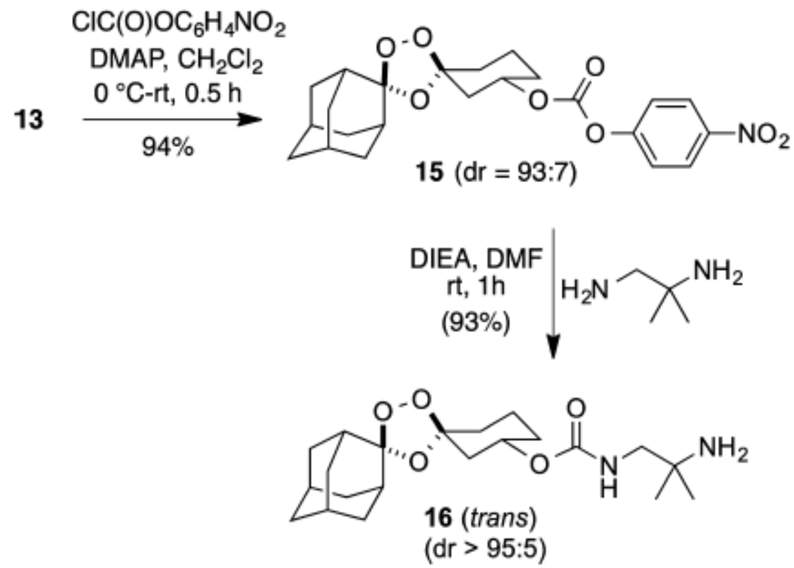


Mahajan et al. *ChemMedChem,* 2011, *6*, 415 – 419
Deu et al. *PNAS,* 2013, *110*, 18244-18249
Fontaine et al. *ChemMedChem,* 2015, *10*, 47 – 51

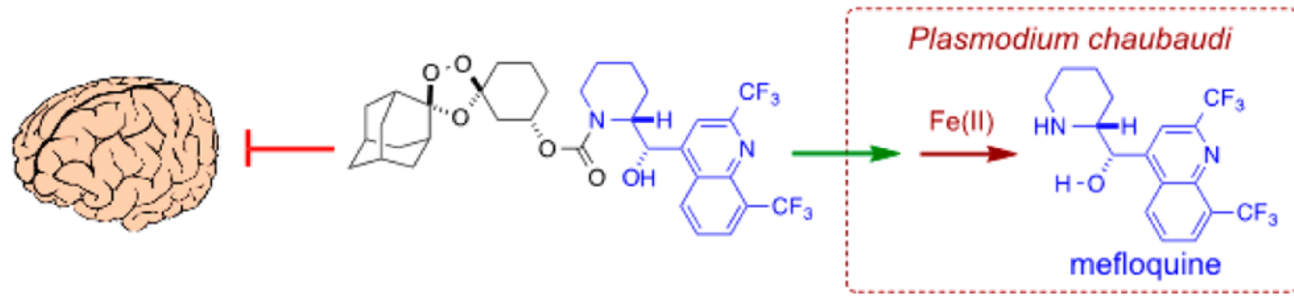
Efficient and Stereocontrolled Synthesis of 1,2,4-Trioxolanes Useful for Ferrous Iron-Dependent Drug Delivery

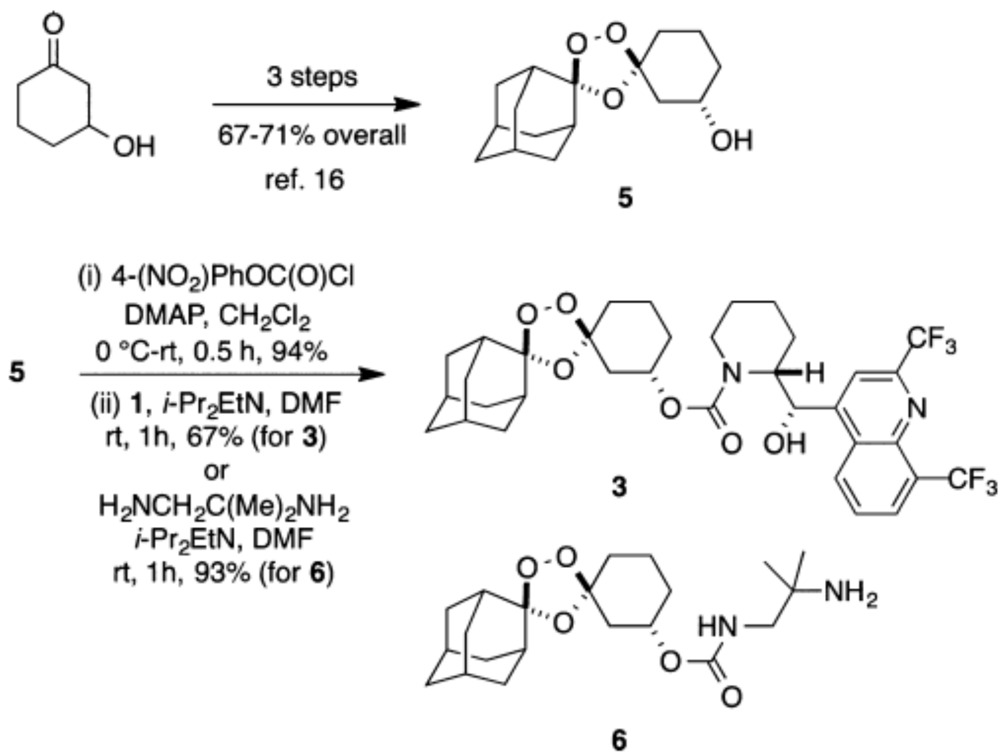


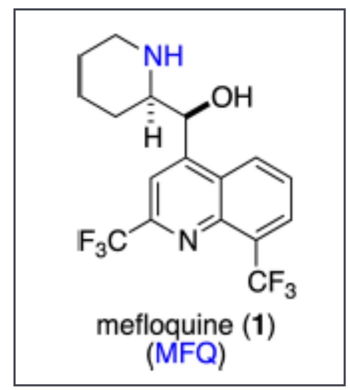




Trioxolane-Mediated Delivery of Mefloquine Limits Brain Exposure in a Mouse Model of Malaria







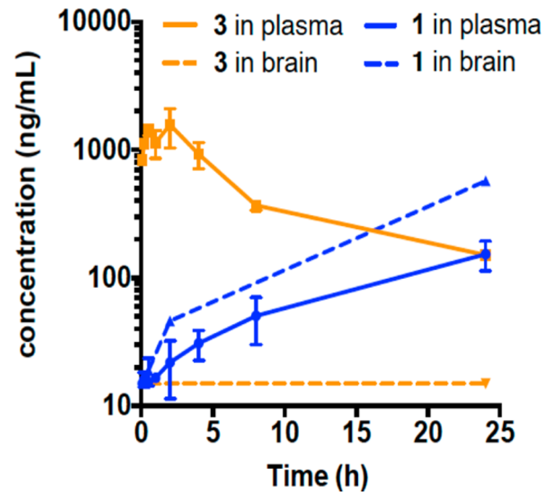
Lauterwasser et al. *ACS Med. Chem. Lett.* 2015, *6*, 1145−1149

Biological activity

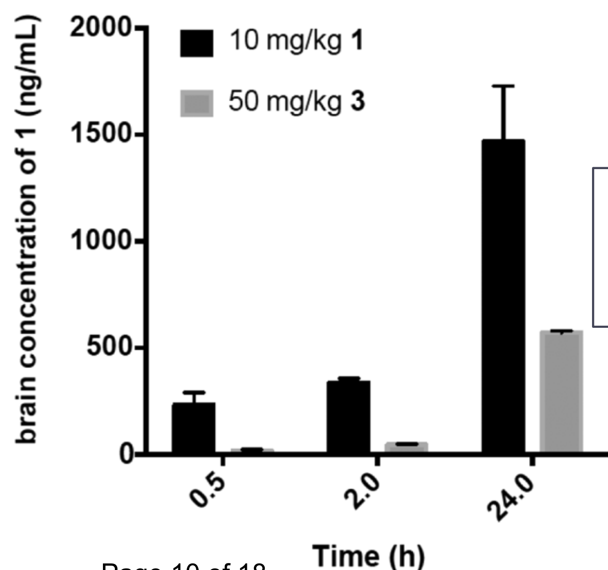
Parasitemia of P. chabaudi Infected Mice Treated with a Single Intraperitoneal Dose of 1 or 3

cmpd	dose (mg/kg)	mean parasitemia ± SEM (%) day 4[a]	day 7	day 13
vehicle		4.6 ± 0.5	34 ± 2.3	b
1	10	4.8 ± 0.5	0.02 ± 0.01	0
3	8	4.8 ± 0.4	33 ± 1.5	b
3	16.5	4.5 ± 1.1	0.7 ± 0.2	0.65 ± 0.65
3	50	5.4 ± 0.6	0	0

[a]Day postinoculation; animals dosed on day 4. [b]Animals sacrificed on day 8 due to poor health, as dictated by study protocol.



Time course of plasma and brain concentrations of 1 (blue) and 3 (orange) in P. chabaudi infected mice treated i.p. with 50 mg/kg 3.



Brain concentrations of mefloquine (1) in P. chabaudi infected mice at three time points following i.p. administration of 10 mg/kg 1 (black bars) or 50 mg/kg 3 (gray bars).

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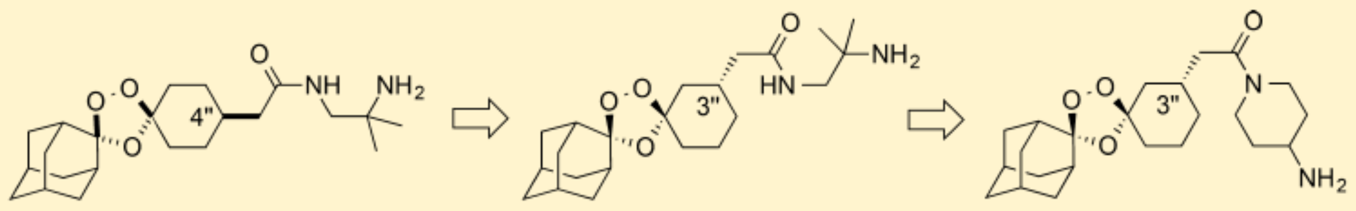
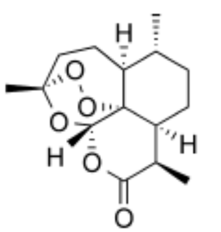
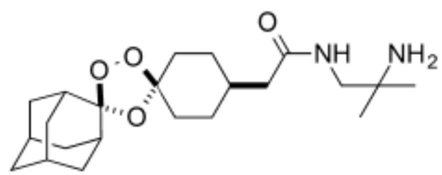


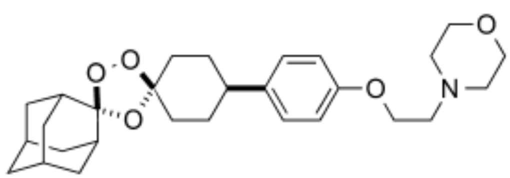
arterolane (**2a**)	(*R*,*R*)-**12a**	(*R*,*R*)-**12i**
W2 *P. falc.* EC_{50} = 2.5 ± 1.5 nM	W2 *P. falc.* EC_{50} = 3.0 ± 0.7 nM	W2 *P. falc.* EC_{50} = 3.9 ± 0.7 nM
P. berghei PD_{100} = 10 mg/kg/day	*P. berghei* PD_{100} = 10 mg/kg/day	*P. berghei* PD_{100} = 4 mg/kg/day



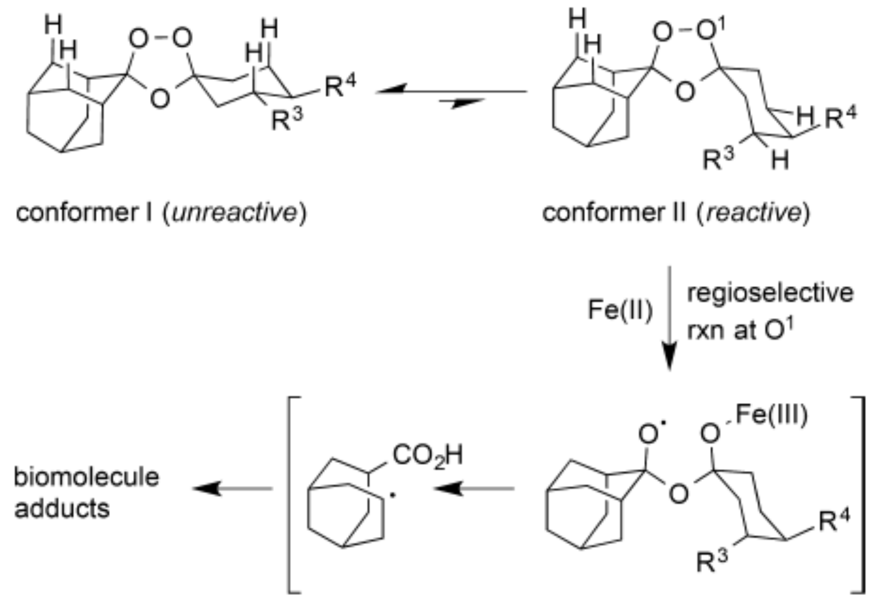
artemisinin/qinghaosu (**1**)



arterolane/OZ277 (**2a**)



artefenomel/OZ439 (**3**)



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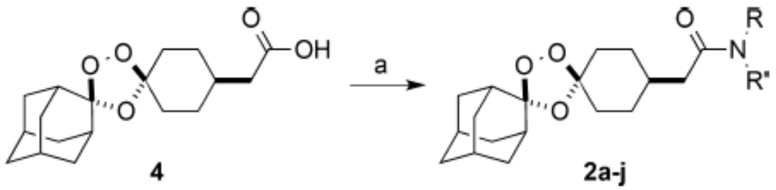
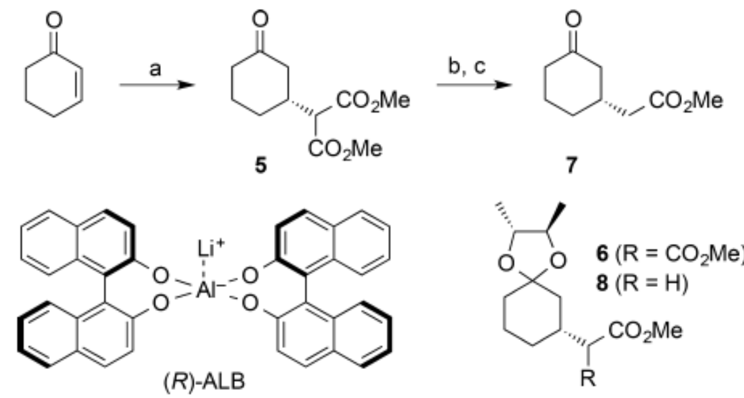
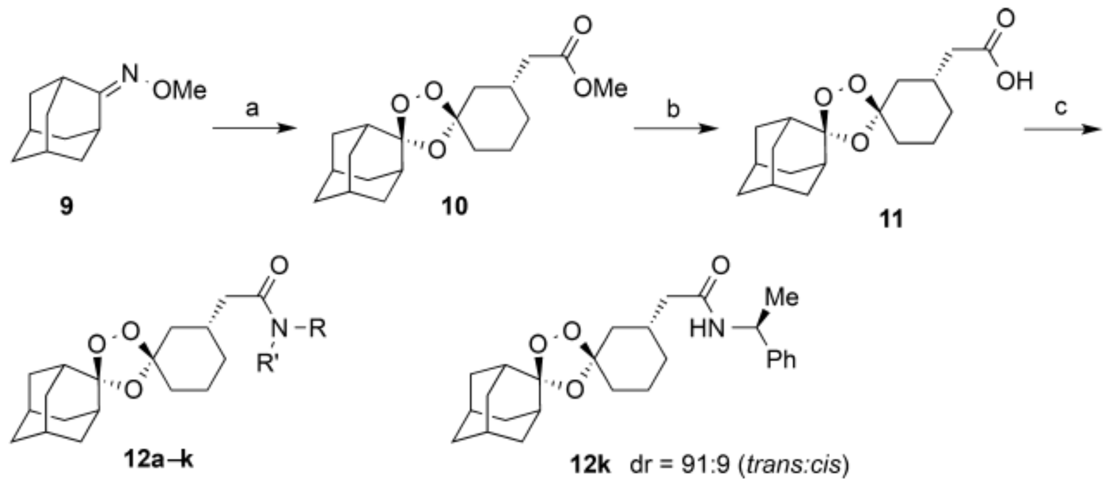
Synthesis



[a]Reagents and conditions: (a) ethyl chloroformate, Et_3N, CH_2Cl_2, −10 °C, 75 min; R(R′)NH, CH_2Cl_2, −10 °C to rt, 2–24 h, 71–95%.



[a]Reagents and conditions: (a) dimethyl malonate, (*R*)-ALB (1 mol %), *t*-BuOK (0.9 equiv relative to ALB), 4 Å MS, THF, rt, 68 h, 87%; (b) NaOH, H_2O/THF (11:1), 0 °C, 2 h; (c) DMSO, 160 °C, 4 h, 85% over two steps.



[a]Reagents and conditions: (a) 0.5 equiv of 7, O_3, CCl_4, 0 °C, 3 h, 95%; (b) NaOH, EtOH/H_2O, 50 °C, 4 h, 95%; (c) ethyl chloroformate, Et_3N, CH_2Cl_2, −10 °C, 75 min; R(R′)NH, CH_2Cl_2, −10 °C to rt, 2–24 h, 68–95%.

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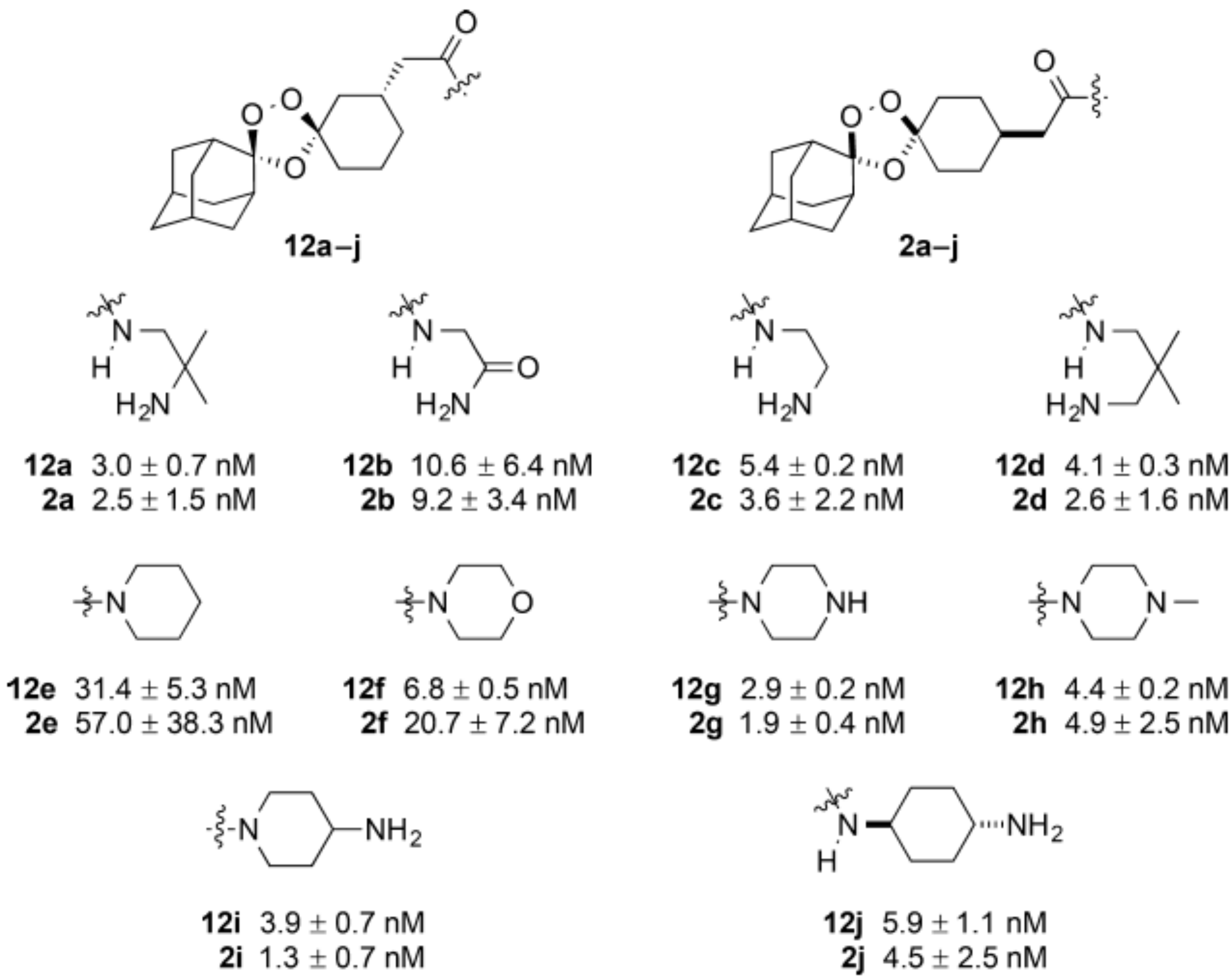
In Vitro Activity of Trioxolanes 2a-j & 12a-j against W2 *P. falciparum* Parasites (EC50 ± SEM)



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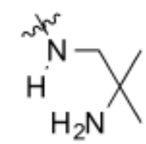
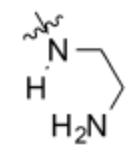
In Vitro ADME Data for Selected Trioxolane Analogues and Controls

cmpd	$T_{1/2}$ (min)[a]	CL_{int}[b]	$T_{1/2}$ (min) no NADPH	solubility (μM)[c]
2a	128	5.4	stable	433
12a	169	4.1	stable	429
2b	124	5.6	stable	
12b	37.3	18.6	stable	
2c	64.2	10.8	70	
12c	84.5	8.2	136	
2d	161	4.3	stable	
12d	64.2	10.8	stable	
2g	48.1	14.4	88.9	
12g	25.5	27.2	stable	
2i	277	2.5	stable	
12i	84.5	8.2	stable	
midazolam	1.65	420		
diclofenac	55.5	12.5		
amiodarone				<3
testosterone				315



12a 3.0 ± 0.7 nM
2a 2.5 ± 1.5 nM



12c 5.4 ± 0.2 nM
2c 3.6 ± 2.2 nM

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In Vivo Efficacy of Trioxolanes 12a and 2a in *P. berghei*-Infected Mice

Treated for 4 Days

treatment	salt form	dose (mg kg^{-1} day^{-1})	mice cured[b] (%)
2a	tosylate	13.6	100
12a	tosylate	13.6	100
12a	free base	9.5	100
chloroquine		30	80
vehicle treated			0
untreated			0

Treated with 4 Daily doses

treatment	dose[a] (mg kg^{-1} day^{-1})	mice cured[b] (%)
12a	1	0
	4	0
	6	60
	10	100
2a	1	0
	4	80
	6	100
	10	100
vehicle		0
untreated		0

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In Vivo Efficacy of Matched Analogue Pairs in *P. berghei*-Infected Mice

compd	dose (mg kg^{-1} day^{-1})	mice cured[b] (%)
12a	4	60
	6	100
2a	4	20
	6	80
12b	4	0
	6	0
2b	4	0
	6	0
12c	4	100
	6	100
2c	4	0
	6	20
12d	4	0
	6	0
2d	4	40
	6	100
12e	4	0
	6	0
2e	4	0
	6	0

compd	dose (mg kg^{-1} day^{-1})	mice cured[b] (%)
12f	4	0
	6	0
2f	4	0
	6	0
12g	4	0
	6	20
2g	4	100
	6	100
12h	4	0
	6	0
2h	4	0
	6	0
12i	4	100
	6	80
2i	4	100
	6	100
12j	4	60
	6	100
2j	4	80
	6	80
chloroquine	30	40
vehicle		0

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Comparison of In Vivo Efficacy of 12c & 12i in *P. berghei*-Infected Mice

Various Dosing Levels

compd	dose (mg kg^{-1} day^{-1})	mice cured[b] (%)
12c	0.5	0
	1	0
	2	0
	4	100
12i	0.5	0
	1	0
	2	0
	4	100

Less frequent dosing

compd	dose (mg/kg)	number of doses	mice cured[b] (%)
12c	4	4	100
	4	3	20
	4	2	0
	4	1	0
12i	4	4	100
	4	3	60
	4	2	0
	4	1	0

Single or Repeated Dose

compd	dose (mg/kg)	number of doses	mice cured[b] (%)
3 (artefenomel)	40	1	100
	80	1	100
12c	40	2	100
	40	1	0
	80	1	20
12i	40	2	100
	40	1	20
	80	1	60

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Conclusions

- Unusual Fe(II)-dependent pharmacology of antimalarial 1,2,4-trioxolanes necessitates an equally unusual approach to their optimization
- New endoperoxides needs to be identified that more rapidly kill *P. falciparum* K13 mutant ring forms while retaining stability toward endogenous Fe(II) sources in the host.
- The trans-3″ side chain modulates peroxide reactivity in a pharmacologically relevant regime
- Two novel analogues (12c & 12i) were identified that exhibited in vivo properties superior to 2a in the *P. berghei* model and one (12i) that afforded single-dose cures at higher doses
- The ultimate potential of the new 3″-substituted chemotype will only be revealed with the examination of a more diverse set of side chains, and in particular, those designed to exploit steric and conformational effects unique to this substitution pattern.

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