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Synthesis of spiro-1,2-dioxolanes and their activity against *Plasmodium falciparum*

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ABSTRACT

Artemisinin-derived compounds play an integral role in current malaria chemotherapy. Given the virtual certainty of emerging resistance, we have investigated spiro-1,2-dioxolanes as an alternative scaffold. The endoperoxide functionality was generated by the $SnCl_4$-mediated annulation of a *bis*-silylperoxide and an alkene. The first set of eight analogs gave EC_{50} values of 50–150 nM against *Plasmodium falciparum* 3D7 and Dd2 strains, except for the carboxylic acid analog. A second series, synthesized by coupling a spiro-1,2-dioxolane carboxylic acid to four separate amines, afforded the most potent compound (EC_{50} ~5 nM).

Malaria is a major parasitic disease in many areas of Africa, Asia and South America. While four species of *Plasmodium* infect humans, the majority of malaria cases are caused by *Plasmodium falciparum*, and its resistance to many contemporary antimalarials has compounded the problem of disease control.[1] Several of the current combination therapies in clinical use for malaria treatment rely on endoperoxide-based compounds.[2] Artemisinin (**1a**, Fig. 1), the parent compound from which the clinically relevant endoperoxide antimalarials **1b**–**d** are derived, is a natural product isolated from the Sweet Wormwood tree (*Artemisia annua*). As *P. falciparum* has developed resistance to every other clinically deployed agent, it seemed prudent to investigate alternative artemisinin-inspired templates. Several groups have pursued the development of antimalarial endoperoxides produced by fully synthetic methods.[3] The most well known is the trioxolane OZ277 (Figs. 1 and 2). Its central trioxolane core resembles the endoperoxide of artemisinin,[4] and the amino side group was introduced to improve its pharmacokinetic properties.[3a]

In an attempt to discover low-cost, short synthetic routes towards novel endoperoxides that display antiplasmodial activity, we utilized a $SnCl_4$-mediated annulation strategy[5] to form a 1,2-dioxolane core as an alternative to the trioxolane core of OZ277-type compounds. A recent report[6] described a similar approach that afforded 1,2-dioxolanes with antiplasmodial activity. This letter summarizes the synthesis and testing of a similar series of analogs, in addition to the development of a synthetic pathway that enables the incorporation of a wide range of auxiliary groups using simple coupling methodology.

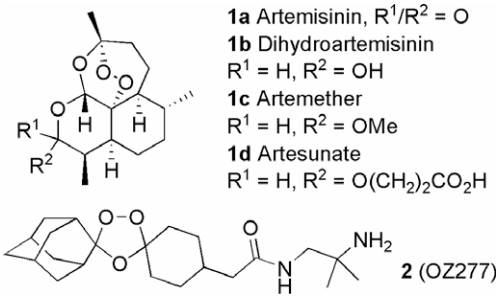

Figure 1. Artemisinin (**1a**), its semisynthetic derivatives (**1b–d**), and synthetic trioxolane **2**.

Crucial in the development of OZ277 was the observation that trioxolanes **3** and **4** were completely inactive (Fig. 2), suggesting that antimalarial activity decreases when the peroxide bond is too exposed (as in **3**) or sterically inaccessible (**4**).[3a,7] For trioxolane **5**, a compromise between stability and potency was met. Preparation of the dioxolane equivalents of **3** and **5**[6] (**6** and **7**, Fig. 2) gave

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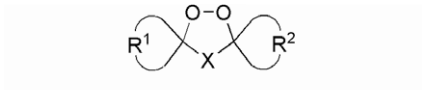
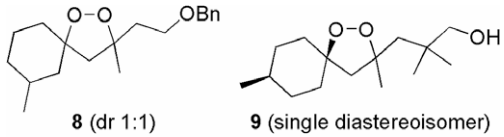


3 $R^1 = R^2$ = cyclohexyl, X = O (inactive)
4 $R^1 = R^2$ = adamantyl, X = O (inactive)
5 R^1 = cyclohexyl, R^2 = adamantyl, X = O
(EC_{50} *Pf* K1/NF54 3.67/5.30 nM)
6 $R^1 = R^2$ = cyclohexyl, X = CH_2
(EC_{50} *Pf* K1/NF54 195/523 nM)
7 R^1 = cyclohexyl, R^2 = adamantyl, X = CH_2
(EC_{50} *Pf* K1/NF54 >2 μM)

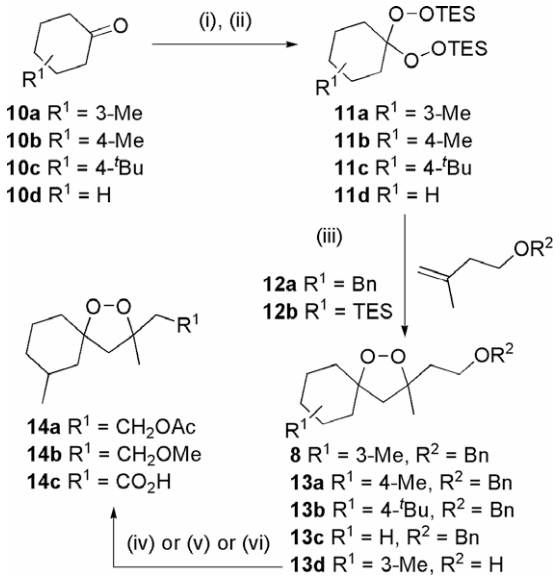
Figure 2. Development of the active trioxolane and dioxolane scaffolds.



8 (dr 1:1)
EC_{50} *Pf* 3D7 = 268 nM
EC_{50} *Pf* Dd2 = 897 nM

9 (single diastereoisomer)
EC_{50} *Pf* 3D7 = 332 nM
EC_{50} *Pf* Dd2 = 897 nM

Figure 3. Assay results for **8** and **9** against *P. falciparum* (EC_{50} values determined via a 3*H*-hypoxanthine incorporation assay[8]).



10a R^1 = 3-Me
10b R^1 = 4-Me
10c R^1 = 4-^{t}Bu
10d R^1 = H

11a R^1 = 3-Me
11b R^1 = 4-Me
11c R^1 = 4-^{t}Bu
11d R^1 = H

12a R^1 = Bn
12b R^1 = TES

14a R^1 = CH_2OAc
14b R^1 = CH_2OMe
14c R^1 = CO_2H

8 R^1 = 3-Me, R^2 = Bn
13a R^1 = 4-Me, R^2 = Bn
13b R^1 = 4-^{t}Bu, R^2 = Bn
13c R^1 = H, R^2 = Bn
13d R^1 = 3-Me, R^2 = H

Scheme 1. Reagents: (i) H_2O_2, HCl; (ii) TES–Cl, Et_3N (**11a**, 49%), (**11b**, 93%), (**11c**, 87%), (**11d**, 21%) (all yields over two steps); (iii) $SnCl_4$ (**8**, 55%), (**13a**, 59%), (**13b**, 80%), (**13c**, 46%), (**13d**, 51%); (iv) Ac_2O (**14a**, 51%); (v) MeOTf (**14b**, 43%); (vi) H_5IO_6, $RuCl_3{\cdot}H_2O$ (**14c**, 57%).

contrasting results to that of the trioxolanes, with the more active compound being the exposed dioxolane **6**.

Our efforts have focused on compounds with accessible dioxolane cores analogous to **6**. A set of compounds that possessed a variety of groups appended to the dioxolane core was tested against *P. falciparum* (see Ref. 5 for synthetic details). The two most active compounds, **8** and **9**, are displayed in Fig. 3. The main structural feature of these compounds was the presence of alkyl and cyclohexyl groups at the 3 and 5 positions of the dioxolane respectively. This core structure served as the basis for the design and synthesis of further analogs.

Synthesis of the first series of analogs is outlined in Scheme 1. Cyclohexanones **10a**–**d** were converted to the *bis*-silylperoxides **11a**–**d**, which in turn were treated with alkene **12a** in the presence of $SnCl_4$ to afford spiro-1,2-dioxolanes **8** and **13a**–**c**. *bis*-Silylperoxide **11a** was also treated with alkene **12b** to afford the hydroxyethyl spiro-1,2-dioxolane **13d**, which was then separately acetylated, methylated or oxidized to give **14a**–**c**.

All compounds were tested in a DAPI-staining based in vitro live/dead *P. falciparum* assay[9] against parasite strains 3D7 and Dd2. With the exception of the carboxylic acid **14c**, all compounds gave similar EC_{50} values (Table 1). The lower activity of **14c** most likely originates in its inability to cross cell membranes as a result of its charged carboxylate group. The greater activity seen for resynthesized **8** compared to the original material probably arises from differences in the two parasite cell viability assays. Overall, the assay results suggested that future series of analogs should focus on incorporating groups with more favorable pharmacokinetics, analogous to the development of OZ277.

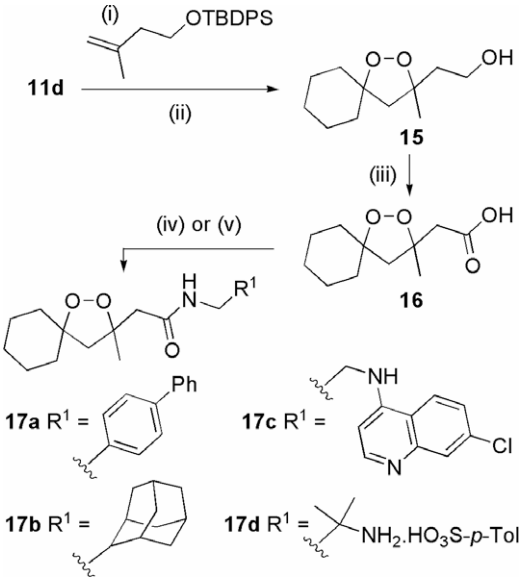
The synthesis of the second series of analogs is shown in Scheme 2. Bis-silylperoxide **11d** was treated with a TBDPS-protected hydroxyalkene, and the dioxolane was deprotected with TBAF to afford **15**. Oxidation gave carboxylic acid **16**, which was coupled to amines using PyBOP or EDCI/HOBT to afford amides **17a** and **17b**, the chloroquine (CQ) mimic **17c**, and the OZ277 side-chain mimic **17d**.

Dioxolanes **17a**, **17b**, and **17d** were equipotent to the majority of the first set of compounds (see Table 2), suggesting that the presence of an amide has no detrimental effect upon activity,

Table 1
Diastereomeric ratios and assay results for **13a**–**e** and **14a**–**c**

Compound	d.r.[a]	EC_{50} *Pf* 3D7 (nM)	EC_{50} *Pf* Dd2 (nM)
8	1:1	92	151
13[a]	8:1	545	59
13b	>99:1	86	74
13c	NA	141	146
13d	1:1	241	149
14a	1:1	109	114
14b	1:1	69	62
14c	1:1	>1000	>1000
Artemisinin	NA	4	4

[a] Diastereomeric ratios were determined by GC–MS.

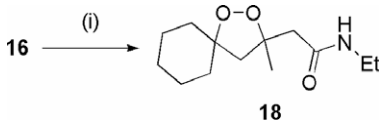


17a R^1 =
17b R^1 =
17c R^1 =
17d R^1 = $NH_2.HO_3S$-*p*-Tol

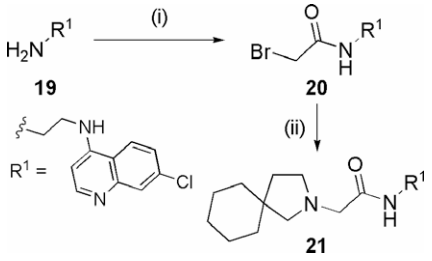
Scheme 2. Reagents: (i) $SnCl_4$; (ii) TBAF (42% over two steps); (iii) H_5IO_6, $RuCl_3{\cdot}H_2O$ (96%); (iv) Amine, PyBOP, Et_3N (**17a**, 72%), (**17b**, 65%); (v) EDCI, HOBT, then amine (**17c**, 46%), (**17d**, 58%).

Table 2
Assay results for **17a–d**

Compound	EC_{50} *Pf* 3D7 (nM)	EC_{50} *Pf* Dd2 (nM)
17a	100	77
17b	81	57
17c	5	6
17d	61	108



Scheme 3. Reagents: (i) $EtNH_2$, PyBOP, Et_3N (45%).



Scheme 4. Reagents: (i) $BrCH_2CO_2H$, EDCI (22%); (ii) Amine, K_2CO_3 (31%).

and that most auxiliary groups have little effect on potency against *P. falciparum*.

Dioxolane **17c** was the most active compound found in this study, and its potency is associated with a 7-chloroquinoline group, analogous to that possessed by CQ. Walsh et al.[10] recently demonstrated that an artemisinin–quinine adduct displayed activity greater than that of artemisinin, quinine, or a 1:1 mixture of artemisinin and quinine. The authors proposed that the adduct's increased potency may arise through its enhanced cellular uptake over that of the individual components. Hydrolysis of the 'mutual prodrug' affords the individual components and delivers them to their site of action.

Two compounds were synthesized to determine whether the high activity of **17c** resulted predominantly from the 7-chloroquinoline moiety, or whether the endoperoxide plays an additional role. Coupling of $EtNH_2$ to acid **16** afforded **18**, an analog of **17c** that lacks the 7-chloroquinoline group (Scheme 3). Additionally, a desperoxy analog of **17c** was synthesized (Scheme 4). Coupling of bromoacetic acid to **19** afforded **20**, which gave pyrrolidine **21** by the alkylation of spiroazadecane.

The assay results for dioxolane **18**, amine **19** and pyrrolidine **21** reveal the origin of the high activity of **17c** (Table 3). For comparative purposes, CQ was tested as a control, and the expected difference in potency against 3D7 (CQ susceptible) and Dd2 (CQ resistant) was observed. The individual components of **17c** (**18** and **19**) and the desperoxy analog **21** were considerably less active

Table 3
Assay results for **18**, **19**, **21**, and **18** in combination with CQ or **19**

Compound	EC_{50} *Pf* 3D7 (nM)	EC_{50} *Pf* Dd2 (nM)
18	485	99
19	57	155
21	69	78
CQ	7	66
18 + CQ	6	42
18 + **19**	50	65

Table 4
Assay results for **17c**, **18**, and **21** against three CQ-resistant *P. falciparum* strains

Compound	EC_{50} (nM)		
	7G8	FCB	106/1
17c	8	16	22
18	83	356	142
21	91	80	53
CQ	46	52	14
Artemisinin	48	8	13

than the parent compound, revealing that the high potency of **17c** results from the presence of both the endoperoxide and 7-chloroquinoline functionalities. Testing **18** in a 1:1 combination with CQ gave a similar EC_{50} value against 3D7 to that of **17c**, whereas the higher EC_{50} value against Dd2 resulted from this strain's resistance to CQ. The 1:1 combination of **18** and **19** gave similar EC_{50} values against both strains, further underlining the lower activity of **19** compared to CQ despite their analogous structures.

Dioxolanes **7**, **8**, **13a–13d**, **14a**, **14b,** and **17a–d** were subjected to an assay that tests for a compound's ability to inhibit heme crystallization in a parasite-independent assay.[11] None of these compounds displayed activity compared to the positive controls amodiaquine and CQ. Compounds **19** and **21** were also inactive in this assay, which indicates that the 7-chloroquinoline moiety possessed by these compounds and **17c** does not inhibit heme crystallization per se.

Compounds **17c**, **18**, and **21** were tested against three geographically distinct CQ-resistant *P. falciparum* strains to determine their ability to overcome various resistance mechanisms (for the Pfcrt mutations specific to each strain, see Ref. 12). Dioxolane **17c** maintained potency against all three strains, as opposed to the observed attenuation of CQ activity, particularly in strains 7G8 and FCB (Table 4). Compound **18** displayed less activity than **17c**, and it exhibited the high degree of variability in EC_{50} values against different strains that was shown against 3D7 and Dd2 (see Table 3). Finally, the absence of an endoperoxide moiety in **21** contributed to decreased activity against these strains compared to **17c**, underlining the importance of the endoperoxide for activity.

In summary, we have utilized a $SnCl_4$-mediated annulation to synthesize compounds based on a spiro-1,2-dioxolane motif. Antiplasmodial activity appeared to be relatively independent of the nature of the group appended to the 3 position, with the exception of **17c**, which possessed a 7-chloroquinoline group analogous to that of CQ. This compound displayed potent activity against a range of *P. falciparum* strains, including CQ-resistant ones, and the combination of the endoperoxide and 7-chloroquinoline moieties are required for this activity. Conversely, there is little evidence that the 7-chloroquinoline functionality present in compounds **17c**, **19**, and **21** acts in an analogous manner to CQ, as these compounds did not inhibit heme crystallization, and the potentiation of **17c** activity must arise through another mechanism.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.10.083.

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12. Strain, source, Pfcrt mutations. Dd2, Indochina/Laos, M74I, N75E, K76T, A220S, Q271E, N326S, I356T, R371I. 7G8, Brazil, C72S, M74N, K76T, A220S, N326D, I356L. FCB, SE Asia, M74I, N75E, K76T, A220S, Q271E, N326S, I356T, R371I. 106/1, Sudan, M74I, N75E, A220S, Q271E, N326S, I356T, R371I.