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On peroxide antimalarials

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Abstract: Several dicyclohexylidene tetraoxanes were prepared in order to gain a further insight into structure–activity relationship of this kind of antimalarials. The tetraoxanes **2**–**5**, obtained as a *cis*/*trans* mixture, showed pronounced antimalarial activity against *Plasmodium falciparum* chloroquine susceptible D6, chloroquine resistant W2 and multidrug-resistant TM91C235 (Thailand) strains. They have better than or similar activity to the corresponding desmethyl dicyclohexylidene derivatives. Two chimeric endoperoxides with superior antimalarial activity to the natural product ascaridole were also synthesized.

Keywords: mixed tetraoxanes, endoperoxides, malaria, *P. falciparum*.

INTRODUCTION

Malaria affects more than 500 million people per annum, causing more than one million deaths, mostly in Africa.[1] Infants, young children and pregnant women are particularly at risk; in fact, it has been estimated that a child dies of malaria every 30 seconds. Furthermore, the disease has as an immeasurable negative impact, both personally and socioeconomically, on families and communities in endemic areas. Although malaria is treatable, increased resistance of the protozoan parasite *Plasmodium falciparum* to standard and affordable anti-malarial drugs, such as chloroquine (CQ), complicates the treatment of infected individuals. Peroxide antimalarials of the 1,2,4-trioxacyclohexane class (artemisinin and its derivatives)[2] and drugs of the trioxolane class[3] offer some new possibilities for treating malaria.

Compounds of another peroxide class, the 1,2,4,5-tetraoxacyclohexanes (tetraoxanes), although less investigated, also have been shown to have potent anti-

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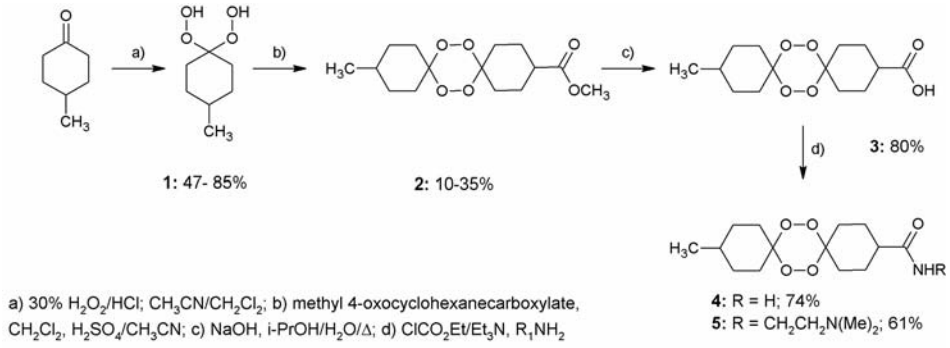
malarial activity.[4] Subsequently, the syntheses of mixed tetraoxanes[5,6] enabled the controlled preparation of a new generation of this promising class of antimalarials.* In addition to the steroidal tetraoxanes, a significant number of dicyclohexylidene tetraoxanes have been synthesized and their anti-malarial activity evaluated both *in vitro* and *in vivo*.[8,9]

Monoterpene ascaridole (Scheme 1), a natural bicyclic [2.2.2] endoperoxide with moderate antimalarial properties, was used as a model for the synthesis of a series of diaryl substituted ascaridole-type endoperoxides, which showed higher activity as compared to ascaridole or dihydroascaridole.[10] More recently, the syntheses of less volatile ascaridole and dihydroascaridole derivatives have been accomplished starting from perillyl and nopol derivatives.[11]



Scheme 1.

Here are reported the synthesis and results of anti-malarial screening of two types of anti-malarial peroxides: mixed tetraoxanes, the derivatives of 12-methyl--7,8,15,16-tetraoxadispiro[5.2.5.2]hexadecane-3-carboxylic acid, and endoperoxides, monoterpene ascaridole derivatives, bound to another anti-malarial pharmacophore, 4-amino-7-chloroquinoline. In addition, the results of *in vivo* screening of some mixed steroidal tetraoxanes are also discussed.

RESULTS AND DISCUSSION

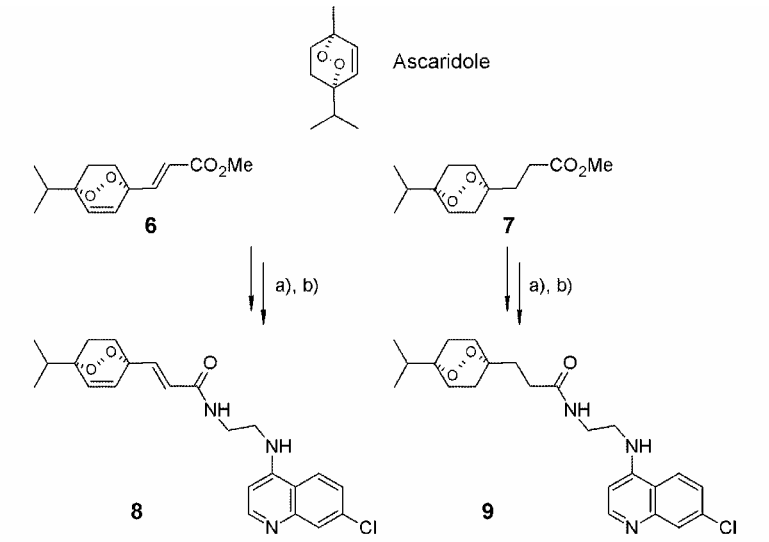
Chemistry

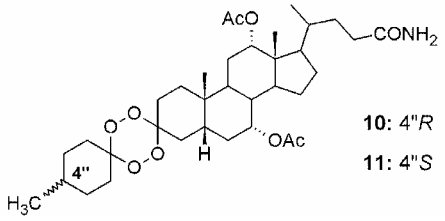
The class of dicyclohexylidene tetraoxanes (**2**–**5**) was designed with varying substituents at the C(4") position in order to obtain further insight into the structure–activity relationship related to spiro-substituents in 1,2,4,5-tetraoxacyclohexane at C(3) and C(6). Gem-dihydroperoxide **1** was prepared from 4-methylcyclohexanone using 30 % hydrogen peroxide and HCl as a catalyst.[5a] Compound **1** was isolated in 47–85 % yield and was pure enough (Scheme 2) to be directly used in the next step.** This gem-dihydroperoxide was coupled to 4-oxocyclohe-

* The structure of the previously evaluated dicyclohexylidene tetraoxanes was limited by a synthetic constraint: only bis compounds could be obtained directly from the corresponding ketones.[7]

** For alternative excellent syntheses of gem-dihydroperoxides, see reference 12.

xanecarboxylate according to a previously developed procedure[5a] to yield the parent mixed tetraoxane, achiral methyl 12-methyl-7,8,15,16-tetraoxadispiro[5.2.5.2]hexadecane-3-carboxylate (**2**; 10–35 %). Further transformations using the ester → → acid → amide sequence (Scheme 2) afforded the desired amides **4** and **5** in 59 % and 49 % yield, respectively.





Scheme 2.

Ascaridole derivatives **6** and **7** synthesized earlier[11] were hydrolysed with LiOH into the corresponding acids and further coupled to *N*-(7-chloroquinolin-4--yl)ethane-1,2-diamine to afford the final aminoquinoline products **8** and **9**, respectively (Scheme 1).

Antimalarial activity

The synthesized peroxides were screened *in vitro* against three *Plasmodium falciparum* strains: D6 (chloroquine-susceptible), W2 (chloroquine-resistant, sus-

ceptible to mefloquine), and TM91C235 (Thailand), a multidrug-resistant strain, following the protocol given in the literature.[5a] All the synthesized tetraoxanes exhibited interesting antimalarial activity. In accordance with previous findings,[5a] the acid **3** was less active (in this particular case – practically inactive) than the methyl ester **2** and the corresponding amides **4** and **5** against all parasite strains. One of the current hypotheses regarding the mechanism of antimalarial action of peroxides is that they act in the food vacuole (FV) of *P. falciparum*, which has a pH around 5.5. As such, amide **5** was designed to have an *N,N*-dimethylamino group, expecting that protonation of the basic nitrogen would enhance the flux of the compound through the FV membrane, thereby increasing its concentration at the desired site of action. However, the *in vitro* antimalarial activity data for tetraoxane **5** (Table I) does not support this hypothesis, since amide **4** (which possesses only an acidic hydrogen at the nitrogen) and ester **2** exhibit very similar activity (both IC_{50} and IC_{90}) against all the tested parasites. In addition, the activities of the dicyclohexylidene tetraoxanes presented in this paper are better or equivalent to similar desmethyl compounds.[6d,9]

TABLE I. *In vitro* antimalarial activities of tetraoxanes **2**–**9** against *P. falciparum* D6,[a] W2,[b] and TM91C235[c] strains

Compd.	IC_{50} / nM			IC_{90} / nM		
	D6	W2	TM91C235	D6	W2	TM91C235
2	12.9	6.1	16.0	32.0	20.5	57.5
3	206.6	131.4	300.4	506.9	487.1	1110.1
4	13.6	8.4	18.8	22.1	22.5	41.1
5	14.9	6.1	22.6	23.9	15.8	45.5
6	109.0	42.7	98.2	196.5	68.5	338.9
7	41.4	20.2	47.4	62.5	ND	112.7
8	101.1	58.6	99.7	156.4	140.8	221.4
9	38.3	30.4	60.6	111.6	89.7	200.7
Chloroquine	8.6	354.3	113.7	10.7	653.0	166.0
Mefloquine	17.1	4.7	49.5	36.1	15.5	121.0
Artemisinin[d]	9.0	6.7	13.0	12.8	11.5	17.4

[a]*P. falciparum* African D6 clone; [b]*P. falciparum* Indochina W2 clone; [c]*P. falciparum* multidrug resistant TM91C23 strain (Thailand); [d]average of greater than eight replicates

The antimalarial activity of compounds **6**–**9** appears to be strongly dependent on the degree of saturation: the saturated bicyclic endoperoxides (**7**,**9**) are more potent antimalarials relative to their unsaturated counterparts (Table I), and both unsaturated (**6**,**8**) and saturated (**7**,**9**) endoperoxides are 5–12 times more active than ascaridole and dihydroascaridole themselves. These results indicate that peroxide–aminoquinoline chimeras, in addition to the trioxaquines,[13] might represent a promising addition to the existing antimalarial arsenal.

Finally, the results of an *in vivo* study on the epimeric mixed steroidal tetraoxanes **10** and **11** (Table II) are presented.[5a] The *in vitro* activity of the com-

pounds differs 20- to 30-fold, with the (4"*R*)-epimer **10** being more active; this structure-activity relationship qualitatively holds for other epimeric pairs at the same carbon.[5] Docking calculations of **10** and **11** with heme[14] are consistent with the observation that the proximity of the heme iron to the oxygen atom of the tetraoxane moiety favours potent *in vitro* activity of both compounds. The more potent analogues have much lower energy minimized docked structures. In addition, preliminary metabolic stability assays and metabolite identification were performed using human and mouse liver microsomes to aid the estimation of the first-pass metabolism of the drug candidates in relevant species (Table II).[5c] It is important to note that no scission of peroxide bond was observed (the tetraoxane moiety is stable in this assay); only monohydroxylations occurred. In this assay,[5c] stable compounds were defined as having half-lives > 60 min. Both compounds have similar metabolic half-lives ($t_{1/2}$(mouse) ≅ 30 min). Hence, it would be logical that the observed difference in *in vitro* activity would also be seen in the *in vivo* efficacy test in mice. However, the collective *in vivo* results presented in Table II are not consistent with results from the *in vitro* screening and with the docking calculations: epimers **10** and **11** exert similar *in vivo* activity in mice, regardless of whether they were administered orally or subcutaneously (Table II). Furthermore, at 600 mg/kg total dose p.o. and 480 mg/kg total dose s.c. of tetraoxane **10**, 4/5 and 5/5 mice cured were observed and the respective survival times were 28 and 31 days. At a total dose 150 mg/kg p.o. and 120 mg/kg s.c., very similar survival times were observed.

Analogous results were also seen for epimer **11**. In addition, at comparable total doses of 150 mg/kg p.o. and 120 mg/kg s.c., compounds **10** and **11** both cured more animals orally than subcutaneously. This apparent higher bioavailability by the oral route may have resulted from a lack of absorption due to s.c. depot formation: a subcutaneous sterile pocket of oil and unabsorbed test compound was commonly found in each animal. Alternatively, one of the hydroxylated metabolites formed after oral administration may be more active than the administered parent drug. For both compounds, **10** and **11**, no toxic effects were observed at any tested concentration or applied protocol.

To conclude, several dicyclohexylidene tetraoxanes were prepared and tested in order to gain additional insight into the structure-activity relationship of this structural class of antimalarials. The tetraoxanes **2**–**5** obtained as *cis*/*trans* mixtures exerted better, or similar activity, than the corresponding desmethyl dicyclohexylidene derivatives.[6d,9] The initial work on ascaridole-related compounds[11] enabled the synthesis of two chimeric endoperoxides with superior antimalarial activity to the natural product, ascaridole. The present results indicate that further efforts should be put into research of peroxide–aminoquinoline chimeras as potential antimalarial drug leads. Finally, the results of *in vivo* tests of two epimeric tetraoxanes with a steroidal carrier indicate that subcutaneous testing using an oil vehicle for these compounds may be unsuitable.

TABLE II. *In vivo* activity of tetraoxanes **10** and **11** against *Plasmodium berghei*[a]

Compd.	mg/kg per day	mg/kg total	Admin.	Mice dead/day died	Mice alive day 31/total	Survival time days[b]	Metabolic stability $t_{1/2}$ /min	Metabolite identification	*In vitro* (IC_{50} / nM)[c]	
									D6	W2
10	600	1800	p.o.[d]	1/26	4/5	30	human, 15	hydroxylation (3)	1.17	0.58
	200	600	p.o.[d]	1/16	4/5	28				
	50	150	p.o.[d]	1/12 1/16 1/19	2/5	21.8	mouse, 29	hydroxylation (3)		
	160	480	s.c.		5/5	31				
	40	120	s.c.	1/15, 1/16, 1/18, 1/23	1/5	20.6				
	20	60	s.c.	2/12, 1/17, 2/20	0/5	16.2				
11	600	1800	p.o.[d]		5/5	31	human, 32	hydroxylation (2)	20.03	14.10
	200	600	p.o.[d]	1/15 1/16 1/22 1/26	1/5	22				
	50	150	p.o.[d]	2/13 1/20	2/5	21.6	mouse, 30	hydroxylation (2)		
	160	480	s.c.	1/18	4/5	28.4				
	40	120	s.c.	1/12, 1/16, 1/17, 1/20, 1/24	0/5	17.8				
	20	60	s.c.	1/8, 1/11, 1/16, 2/17	0/5	13.8				

[a]Groups of five *P. berghei* (KBG 173 strain) infected CD-1 mice were treated on days 3, 4, and 5 post infection with tetraoxanes suspended in sesame oil. Mice alive on day 31 with no parasites in a blood film are considered cured; [b]Including cured mice; [c]Taken from ref. 5a; [d]Taken from ref. 5c.

EXPERIMENTAL

General remarks

For details, please see references 5a, 5b, 5c.

ESI MS spectra of the synthesized compounds were recorded on an Agilent Technologies 6210 Time-of-Flight LC/MS instrument in the positive ion mode using CH_3CN/H_2O = 1/1 with 0.2 % HCOOH as the carrying solvent solution. The samples were dissolved in pure acetonitrile (HPLC grade). The selected values were as follows: capillary voltage 4 kV; gas temperature 350 °C; drying gas 12 l min^{-1}; nebuliser pressure 45 psig; fragmentator voltage 70 V.

*1,1-Dihydroperoxy-4-methylcyclohexane (**1**)*

4-Methylcyclohexanone (1.1 ml, 8.9 mmol) was dissolved at r.t. in a CH_2Cl_2/CH_3CN mixture (20 ml, 1:3 v/v), followed by addition of 30 % H_2O_2 (10.4 ml, 0.1 mol) and a few drops of conc. HCl. The reaction mixture was stirred for 2 h at r.t. and then quenched with saturated $NaHCO_3$ and CH_2Cl_2. The organic layer was separated, the water layer was additionally extracted with EtOAc (3×50 ml), and the combined organic layers were dried over anhydrous $MgSO_4$ and evaporated to dryness. The crude product (680 mg, 47 %) was used in the following step without further purification. IR (film, cm^{-1}): 3420*s*, 2935*s*, 2865*s*, 1712*m*, 1637*w*, 1554*m*, 1378*m*, 1357*m*, 1265*m*, 1200*w*, 1158*m*, 1104*m*, 1050*m*, 1017*m*, 980*m*, 910*m*, 861*m* cm^{-1}. IR (CCl_4, cm^{-1}): 3431*m*, 2957*s*, 2930*s*, 2865*m*, 1712*w*, 1551*s*, 1454*m*, 1384*m*, 1357*m*, 1255*s*, 1222*s*, 1162*w*, 1103*m*, 1071*m*, 1012*s*, 980s. ^{1}H-NMR (200 MHz, $CDCl_3$, δ, ppm): 9.40−9.00 (*m*, 2×HOO−C(1)), 2.30−2.00 (*m*), 1.70−1.30 (*m*), 1.30−1.10 (*m*), 0.93 (*d*, H_3C−C(4), J = 6.2 Hz). ^{13}C-NMR (50 MHz, $CDCl_3$, δ, ppm): 110.73, 40.83, 31.59, 30.58, 29.03, 21.38.

*Methyl 12-methyl-7,8,15,16-tetraoxadispiro[5.2.5.2]hexadecane-3-carboxylate (**2**)*

To a cooled solution (ice bath) of dihydroperoxide **1** (1.2 g, 7.4 mmol) in CH_2Cl_2 (20 ml), methyl 4-oxocyclohexanecarboxylate was added and after stirring for 30 min at the same temperature, 1.66 ml of an ice-bath cooled H_2SO_4/CH_3CN mixture (1:10, v/v) was added dropwise. After an additional 50 min stirring, the reaction mixture was worked-up in the usual manner and purified by SiO_2 column chromatography (Lobar B, LichroPrep Si 60, eluent: heptane/EtOAc = 95/5) affording 222 mg (10 %) **2**.* **2**: Colourless foam, softens at 57−59 °C. IR (KBr, cm^{-1}): 3449*w*, 2958*s*, 2938*m*, 2860*m*, 1736*s*, 1442*m*, 1368*m*, 1329*m*, 1265*m*, 1201*s*, 1182*m*, 1133*m*, 1074*s*, 976*m*, 932*m*, 897*m*, 828*w*. ^{1}H-NMR (200 MHz, $CDCl_3$, δ, ppm): 3.68 (*s*, CH_3CO_2−C(1)), 2.98 (*bs*, 2 H), 2.51−2.32 (*m*, 1H), 2.02−1.14 (*m*, 15 H), 0.93 (*d*, CH_3−C(12), J = 6.6 Hz). ^{13}C-NMR (50 MHz, $CDCl_3$, δ, ppm): 175.01, 108.33, 107.22, 51.64, 41.51, 41.30, 31.61, 31.55, 31.26, 30.21, 28.86, 28.09, 24.53, 23.76, 21.30. (+)ESI–MS (*m*/*z* (%)): 304.4 (100), 284.4 (58), 244.3 (25), 164.2 (21), 159.2 (51). Anal. calcd. for $C_{15}H_{24}O_6 \cdot 1/4\ H_2O$: C 59.10, H 8.10; Found: C 59.52, H 8.72.

*12-Methyl-7,8,15,16-tetraoxadispiro[5.2.5.2]hexadecane-3-carboxylic acid (**3**)*

Methyl ester **2** (400 mg, 1.3 mmol) was hydrolyzed at 80 °C with NaOH (72.8 mg, 1.82 mmol) in an *i*-PrOH/H_2O mixture (12 ml, 3:1 v/v). After 15 min, the reaction mixture was cooled and diluted with 20 ml H_2O and 50 ml CH_2Cl_2. The water layer was acidified to pH 2 with dilute HCl, and layers were separated. The water layer was further extracted with CH_2Cl_2 (3×20 ml); the combined organic layers were washed with water and brine, dried over anh. $MgSO_4$, and evaporated to dryness. Triturating with Et_2O afforded 305 mg (80 %) of product. m.p. 134−137 °C. IR (KBr, cm^{-1}): 3449*m*, 2948*s*, 2870*m*, 1707*s*, 1447*m*, 1324*w*, 1270*m*, 1226*m*, 1069*m*, 976*m*, 936*m*. ^{1}H-NMR (200 MHz, $CDCl_3$, δ, ppm): 2.99 (*bs*, 2H), 2.60−2.31 (*m*, 1H), 2.03−1.12 (*m*, 15 H), 0.93 (*d*, CH_3−C(12), J = 6.2 Hz). ^{13}C-NMR (50 MHz, $CDCl_3$, δ, ppm): 180.73, 108.44, 107.19, 41.30, 41.08, 31.65, 31.61, 30.24, 24.29, 21.38. (–)ESI–MS (*m*/*z* (%)): 285.0 ($[M-H]^-$, 89), 104.9 (100). Anal. calcd. for $C_{14}H_{22}O_6 \cdot 1/4\ H_2O$: C 57.52, H 7.82; Found: C 57.99, H 8.39.

* The yields varied within 10–35 %.

General procedure for the preparation of amides

A solution of acid **3** (103 mg, 0.36 mmol) in dry CH_2Cl_2 (20 ml), with added Et_3N (51 µl, 0.36 mmol) and $ClCO_2Et$ (35 µl, 0.36 mmol) was stirred for 90 min at 0 °C. A given amount of amine was added and after 30 min stirring, the reaction mixture was warmed to r.t. After 90 min it was diluted with H_2O, the layers were separated and the organic layer was washed with brine, dried over anh. $MgSO_4$ and evaporated to dryness.

*12-Methyl-7,8,15,16-tetraoxadispiro[5.2.5.2]hexadecane-3-carboxamide (**4**)*

Acid **3** was transformed into amide **4** (75 mg, 74 %), which upon triturating with Et_2O afforded a sample having m.p. 161−165 °C. IR (KBr, cm^{-1}): 3402*s*, 3210*m*, 2949*m*, 2860*w*, 1653*s*, 1441*m*, 1372*w*, 1234*m*, 1077*m*, 978*w*, 940*w*, 904*w*. ^{1}H-NMR (200 MHz, CD_3OD, δ, ppm): 3.40−3.10 (*bs*, 2H), 2.60−2.40 (*m*, 1 H), 2.10−1.20 (*m*, 15 H), 1.12 (*d*, CH_3−C(12), J = 6.3 Hz). ^{13}C-NMR (50 MHz, CD_3OD, δ, ppm): 180.79, 109.27, 108.36, 44.28, 32.92, 32.30, 31.61, 29.99, 29.43, 26.59, 25.69, 21.83, 9.29, 7.58. (+)ESI–MS (*m*/*z* (%)): 327.3 (18), 286.2 ([M+H]$^+$, 15), 152.2 (28), 150.2 (90), 142.2 (25), 102.3 (32), 100.3 (55), 83.3 (100). Anal. calcd. for $C_{14}H_{23}NO_5 \cdot 2/3\ H_2O$: C 56.55, H 8.25, N 4.71; Found: C 56.24, H 8.59, N 5.03.

N-*(2-dimethylamino-ethyl)-12-methyl-7,8,15,16-tetraoxadispiro[5.2.5.2]hexadecane-3-carboxamide (**5**)*

Acid **3** (103 mg, 0.36 mmol) was transformed into amide **5** (79 mg, 61 %) using 10 equivalents of $(CH_3)_2NCH_2CH_2NH_2$ in dry CH_2Cl_2 (25 ml). Upon triturating with Et_2O, a colourless foam was obtained, softness at 140−145 °C. IR (KBr, cm^{-1}): 3443*m*, 3317*s*, 2946*s*, 2873*m*, 2824*m*, 2775*m*, 1649*s*, 1556*s*, 1449*m*, 1371*w*, 1264*w*, 1220*m*, 1069*m*, 976*w*, 927*m*. ^{1}H-NMR (200 MHz, $CDCl_3$, δ, ppm): 6.20 (*bs*, HN−CO), 3.40−3.30 (*m*, $(CH_3)_2NCH_2C\mathbf{H}_2NH$−CO)), 3.10 (*bs*, H–C(1)), 2.45−2.35 (*m*, $(CH_3)_2NC\mathbf{H}_2CH_2NH$−CO)), 2.35−2.10 (*m*, $(C\mathbf{H}_3)_2NCH_2CH_2NH$−CO)), 1.90−1.10 (*m*, 15H), 0.93 (*d*, CH_3−C(12), J = 6.2 Hz). ^{13}C-NMR (50 MHz, $CDCl_3$, δ, ppm): 174.50, 108.39, 107.31, 57.65, 45.01, 43.99, 43.77, 36.45, 31.65, 31.57, 28.42, 24.76, 21.32. (+)ESI–MS (*m*/*z* (%)): 357.3 ([M+H]$^+$, 100). Anal. calc. for $C_{18}H_{32}N_2O_5$ 1/2 H_2O: C 59.16, H 9.10, N 7.67; Found: C 59.39, H 9.61, N 7.75.

*(E)-N-{2-[(7-chloro-4-quinolinyl)amino]ethyl}-3-(4-isopropyl-2,3-dioxabicyclo[2.2.2]oct-5-en-1--yl)-2-propenamide (**8**)*

Employing the above procedure, compound **6** was hydrolysed and the intermediate acid[11] was transformed into amide **8** (31 mg, 91 %), which was then triturated with Et_2O. Oil. **8**: ^{1}H-NMR (200 MHz, $CDCl_3$, δ, ppm): 8.42 (*m*, H−C(2')), 7.90 (*m*, H−C(5')), 7.83 (*m*, H−C(8')), 7.36 (*m*, H−C(6')), 7.03 (*d*, 1H, J = 15.6 Hz), 6.95 (*bs*, NH−Ar), 6.62 (*bs*, NH−CO), 6.58 (*d*, 1H, J = 8.7 Hz), 6.49 (*d*, 1H, J = 8.7 Hz), 6.26 (*m*, H−C(3')), 6.14 (*d*, 1H, J = 15.6 Hz), 3.80−3.70 (*m*, $CONHCH_2C\mathbf{H}_2NHAr$), 3.48−3.36 (*m*, $CONHC\mathbf{H}_2CH_2NHAr$), 2.20−1.95 (*m*, 4H), 1.50−1.70 (*m*, 1H), 1.02 (*m*, 6H). ^{13}C-NMR (50 MHz, $CDCl_3$, δ, ppm): 167.53, 150.90, 150.54, 147.94, 143.44, 141.80, 135.30, 133.77, 127.36, 125.59, 123.83, 122.30, 117.00, 98.08, 80.79, 75.60, 45.43, 38.93, 32.06, 28.91, 24.54, 17.53, 17.08. (+)ESI–HRMS (*m*/*z* (%)): 428.1735 ([M+H]$^+$, 100); calculated 428.1736.

*N-{2-[(7-chloro-4-quinolinyl)amino]ethyl}-3-(4-isopropyl-2,3-dioxabicyclo[2.2.2]oct-1-yl)propanamide (**9**)*

Employing the above procedure, compound **7** was hydrolysed and the intermediate acid[11] was transformed into amide **9** (32 mg, 85 %), which was then triturated with Et_2O. Oil. **9**: ^{1}H-NMR (200 MHz, $CDCl_3$, δ, ppm): 8.45 (*m*, H−C(2')), 7.88 (*m*, H−C(5')), 7.78 (*m*, H−C(8')), 7.33 (*m*, H−C(6')), 6.65 (*bs*, NH−Ar), 6.45 (*bs*, NH−CO), 6.24 (m, H−C(3')), 3.69−3.65 (*m*, $CONHCH_2C\mathbf{H}_2NHAr$), 3.39−3.37 (*m*, $CONHC\mathbf{H}_2CH_2NHAr$), 2.31−2.28 (*t*, 2H, J = 9.0 Hz), 1.94−1.88 (*m*, 4H), 1.84−1.78 (*m*, 2H), 1.67−1.57 (*m*, 5H), 0.86 (*d*, 6H, J = 7.0 Hz). ^{13}C-NMR (50 MHz, $CDCl_3$, δ, ppm): 175.56, 151.80, 150.15, 148.93, 134.91, 128.24, 125.43, 122.07, 117.26, 98.17, 79.70, 76.08, 45.61, 38.79, 34.15, 33.23, 30.25, 28.87, 25.68, 16.85. (+)ESI-HRMS (*m*/*z* (%)): 432.2050 ([M+H]$^+$, 100); calculated 432.2048.

In vitro *antimalarial activity*

The *in vitro* antimalarial drug susceptibility screen was a modification of the procedures first published by Desjardins *et al*.,[15] with modifications developed by Milhous *et al*.,[16] with the details given in ref. 4c.

In vivo *antimalarial activity*

The *P. berghei* mouse efficacy tests were conducted using a modified version of the Thompson test. On day 0, each mouse was inoculated intraperitoneally with 0.1 ml, 1.0×10^6 *P. berghei* P-line infected red blood cells from donor mice. The test drugs at 10, 20, and 80 mg/kg were suspended or dissolved in sesame oil and administered s.c. beginning on day 3 post-infection. Drug administrations were performed twice per day at 12 hours intervals for 3 days. The dose levels are given in Table II. Cure was defined as survival until day 31 post-treatment. Untreated control mice typically died on day 7−9 post-infection.

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ИЗВОД

О ПЕРОКСИДНИМ АНТИМАЛАРИЦИМА

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У овом раду приказана је синтеза неколико дицикилохексилиденских тетраоксана у циљу сагледавања односа структура–активност ове врсте антималарика. Једињења **2**–**5** добијена као (cis,trans)-смесе показала су изражену антималаријску активност према D6, W2 и TM91C235 (Thailand) сојевима *P. falciparum*. Она имају бољу или сличну активност од одговарајућих десметил циклохексилиденских деривата. Синтетисана су и два ендопероксида химерне структуре знатно израженије активности од природног производа аскаридола.

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