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Preliminary communication

PRELIMINARY COMMUNICATION

7,8,15,16-tetraoxa-dispiro[5.2.5.2]hexadecane-3-carboxylic acid derivatives and their antimalarial activity*

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Abstract: Several C2 symmetrical mixed tetraoxanes were prepared starting from a gemdihydroperoxide and a ketone. The obtained tetraoxanes showed pronounced antimalarial activity against *P. falciparum* chloroquine resistant W2 and chloroquine susceptible D6 strains, with *N*-(2-dimethylamino)ethyl-7,8,15,16-tetraoxa-dispiro[5.2.5.2]hexadecane-3-carboxamide being as active as artemisinin.

Keywords: mixed tetraoxane, malaria, *Plasmodium falciparum*, gem-dihydroperoxide.

INTRODUCTION

Malaria is a serious infectious disease affecting 300–500 million people per year.[1] The disease is caused by multiplication of the protozoan parasite *Plasmodium falciparum* in erythrocytes. Increased resistance to standard, and affordable, antimalarial drugs, such as chloroquine (CQ), further complicates the treatment of infected individuals. The emergence of peroxide antimalarias of the 1,2,4-trioxacyclohexane class (trioxanes), such as artemisinin and its derivatives, opened new possibilities for treating the parasitemia. Another peroxide class of compounds, 1,2,4,5-tetraoxacyclohexanes (tetraoxanes),[2] has also proved to be effective antimalarials, although their pharmacological properties have been less explored than those of the trioxanes.

In addition to steroidal tetraoxanes, a significant number of dicyclohexylidene tetraoxanes have been synthesised and their antimalarial activity evalueated *in vitro* and *in vivo*.[3] However, the structure of the previously evaluated dicyclohexyli-

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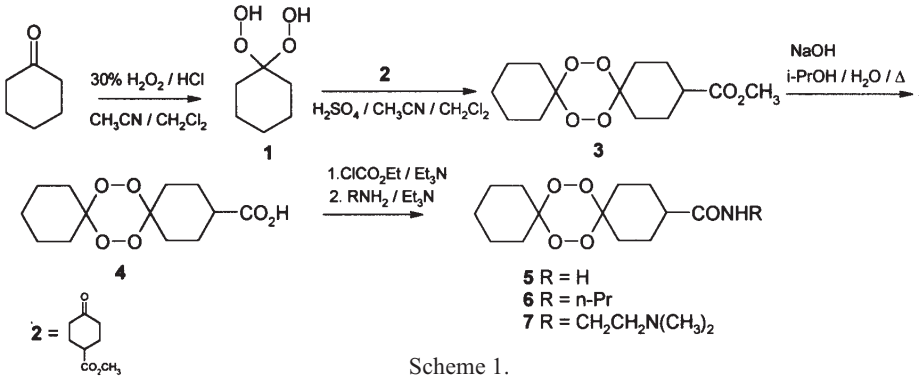
dene tetraoxanes was limited by the mode of their synthesis: only bis compounds could be obtained directly from the corresponding ketones.[4] Recent syntheses of mixed tetraoxanes[5,6] opened new possibilities for the controlled preparation of this class of promising antimalarials.

In this paper, the synthesis and initial results of biological evaluation of mixed tetraoxanes, derivatives of 7,8,15,16-tetraoxa-dispiro[5.2.5.2]hexadecane-3-carboxylic acid are reported. The present class of compounds was designed with the aim of obtaining the simplest amphiphilic structures of C2 symmetry in an effort to minimise the influence of steric effects of the tetraoxane antimalarials on their activity, and to investigate the direct influence of various functionalities.

CHEMISTRY

Gem-dihydroperoxide **1** was obtained in 50 % yield from cyclohexanone using 30 % hydrogen peroxide and HCl as a catalyst. Compound **1** was identified by comparison of its IR, ^{1}H-NMR and ^{13}C-NMR data[7] to those of previously synthe-



Scheme 1.

sised gem-dihydroperoxides.[5a] The obtained gem-dihydroperoxide was coupled to ketone **2** according to a recently developed procedure[5a] to yield the parent mixed tetraoxane, methyl 7,8,15,16-tetraoxa-dispiro[5.2.5.2]hexadecane-3-carboxylate (**3**) in 28 % yield. The identity of **3** was established from its spectral data.[8] Using the ester→acid→amide sequence (Scheme 1) desired amides were obtained in 68–80 % overall yield.

ANTIMALARIAL ACTIVITY

The tetraoxanes were screened against *Plasmodium falciparum* CQ resistant W2 and CQ susceptible D6 strains following the protocol given in Ref. 2c. All the synthesised tetraoxanes exhibited pronounced antimalarial activity. In accordance with previous findings,[2c] the acid **4** was less active than the methyl ester **3**, and significantly less active than the corresponding amides **5**–**7** against both clones. According to a current hypothesis suggesting that peroxides exert their antimalarial activity in the food-vacuole (FV) of *P. falciparum* at pH ≈ 5.5, amide **7**, possessing

TABLE I. *In vitro* antimalarial activity of the synthesised tetraoxanes

Compound	W2 (IC_{50})/(ng/mL)	D6 (IC_{50})/(ng/mL)
3	11.57	8.36
4	112.51	116.89
5	5.47	6.5
6	6.89	6.04
7	3.33	3.85
CQ	111.75	4.39
Artemisinin	2.2[a]	4.7[a]

[a]Data taken from Ref. 2a

an *N*,*N*-dimethylamino group, was designed in the expectation that protonation of the basic nitrogen would mediate the efflux through the FV membranes, hence increasing its concentration at the site of action. The *in vitro* antimalarial activity of tetraoxane **7** (Table I) confirms our expectations, and the results of further *in vitro* and *in vivo* screening will be reported in due time elsewhere.

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ИЗВОД

АНТИМАЛАРИЈСКА АКТИВНОСТ ДЕРИВАТА 7,8,15,16-ТЕТРАОКСА-ДИСПИРО[5.2.5.2]ХЕКСАДЕКАН-3-КАРБОКСИЛНЕ КИСЕЛИНЕ

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У овом раду приказана је синтеза серије C2 мешовитих тетраоксана полазећи од гем-дихидропероксида циклохексанона. Добијеним дериватима испитана је *in vitro* активност према W2 и D6 сојевима *P. falciparum*. Утврђено је да дериват *N*-(2-диметиламино)етил-7,8,15,16-тетраокса-диспиро[5.2.5.2]хексадекан-3-карбоксамид показује активност врло блиску активности познатог антималарика артемизинина.

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7. IR: Characteristic intramolecular OH bonding at 3419 cm^{-1} (film) and 3424 cm^{-1} (CCl_4); ^{1}H-NMR ($CDCl_3$): 9.60 ppm, (2H, HOO–C(1), exhangeable with D_2O); ^{13}C-NMR ($CDCl_3$): 110.94 ppm (C(1))
8. Spectral data for **3**: colourles foam, softenes at 75–80 ºC. IR (KBr): 3444*w*, 3007*w*, 2938*s*, 2865*m*, 1736*s*, 1452*s*, 1329*m*, 1270*m*, 1201*s*, 1074*s*, 1010*w*, 931*m*, 838*m* cm^{-1}. ^{1}H-NMR (200 MHz, $CDCl_3$): 3.67 (*s*, CH_3O_2C–C(3)), 2.87 (*bs*, H–C(3)), 2.5–2.1 (*m*, 3H), 2.0–1.4 (*m*, 15H). ^{13}C-NMR (50 MHz, $CDCl_3$): 174.82, 108.15, 107.04, 51.47, 41.24, 31.50, 30.06, 29.37, 27.90, 25.15, 24.40, 23.67, 21.80. ESI-MS (*m*/*z* (%)): 339.36 (100), 327.35 ($[M+Na+H_2O]^+$, 50), 313.40 ($[M+CH+H]^+$, 35) 304.37 ($[M+H_2O]^+$, 35), 288.34 (55), 285.37 ($[M]^+$, 95), 257.25 (45), 244.31 (85), 142.26 (65), 209.23 (10), 187.19 (10), 155.20 (45), 141.20 (25), 118.24 (15), 100.32 (10), 68.41 (30).