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Antimalarial, antimycobacterial and antiproliferative activity of phenyl substituted mixed tetraoxanes*

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Abstract: Mixed tetraoxanes of the 4"-phenyl-substituted cyclohexyl-spirotetraoxacyclohexyl-spirocholate series have been prepared and evaluated as possible antimalarials, antiproliferatives and antimycobacterials. The activity of the (4"*R* or *S*)-phenyl series against *P. falciparum* D6 and W2 strains was found to be at the level of artemisinin, with two compounds, the acid **4** and the amide **6**, exhibiting encouraging anti-TB activity as well. Very promising *in vitro* results of the said tetraoxanes were obtained against solid tumours and, in some instances, the activity against a selected number of cell lines was higher than that of the antitumor drug paclitaxel.

Keywords: mixed tetraoxane, malaria, tuberculosis, cancer, peroxide, steroid.

INTRODUCTION

Malaria, which is caused by multiplication of the protozoan parasite *Plasmodium falciparum* in erythrocytes, is a major health problem in many southern countries. The present resurgence of malaria and the lack of proper treatment effects 300–500 million people annually causing over 1.5 million deaths.[1] More than 400 million disease cases with over 1.5 million fatalities are the annual toll of *P. falciparum* infections. The development of resistance to the standard antimalarial drug chloroquine (CQ), which had been the affordable and effective antimalarial mainstay for 50 years, has severe health implications for countries in malaria endemic regions. In a recent genetic study[2] of the malaria parasite, it is found that this species is unexpectedly diverse; another study[3] points to the multiple independent origins of mutations in one parasite gene that confer resistance to a widely used drug such as CQ. The results show that, in principle, *P. falciparum* could rapidly develop resistance to multiple drugs (CQ: estimated ~6–30 years), additionally justifying further search for new drugs.

* Dedicated to Professor Miroslav Gašić on the occasion of his 70th birthday.

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The antimalarial properties of artemisinin[4] and of other peroxides such as 1,2,4,5-tetraoxacycloalkanes[5] against CQ-resistant strains opened a new approach to fighting malaria.

Our research in this area has exploited various steroid derivatives as tetraoxane pharmacophore carriers: bis-steroidal tetraoxanes,[6,7] mixed steroidal tetraoxanes,[8] and intramolecular tetraoxane[9] were explored. Our latest results indicate that cholic acid-based mixed steroidal tetraoxanes are extremely potent tetraoxane antimalarials *in vitro*, with the 4”-substituted cyclohexyl-spirotetraoxacyclohexyl-spirocholates appearing to be among the most potent antimalarials.

Tuberculosis (TB) affects 1.7 billion people per year worldwide, killing *ca.* 3 million.[10] It is estimated that about 8 million new cases emerge annually, mostly in sub-Saharan Africa, and the disease, especially targeting people with suppressed immune systems, *e.g.*, HIV positive cases, is slowly but steadily spreading in the developed countries as well. Multidrug resistant TB strains have developed,[11] and the current lack of new leads[12] additionally warrants the development of new antitubercular drugs.

The toxicity of steroidal tetraoxanes against (PBMC,[7] VERO[8]) as compared to their antimalarial activity was shown to be low. In addition, preliminary tests on the haemolytic behaviour of mixed tetraoxanes possessing a C(24) amide terminus revealed no RBC membrane lysis,[8] suggesting that antimalarial activity is the consequence of interaction specific to infected RBC, and is not the result of uncontrolled RBC membrane lysis.

In this paper, the synthesis and extensive biological evaluation (antimalarial, antitubercular and antiproliferative) of twelve new 4”-phenyl substituted mixed tetraoxanes are presented.

CHEMISTRY

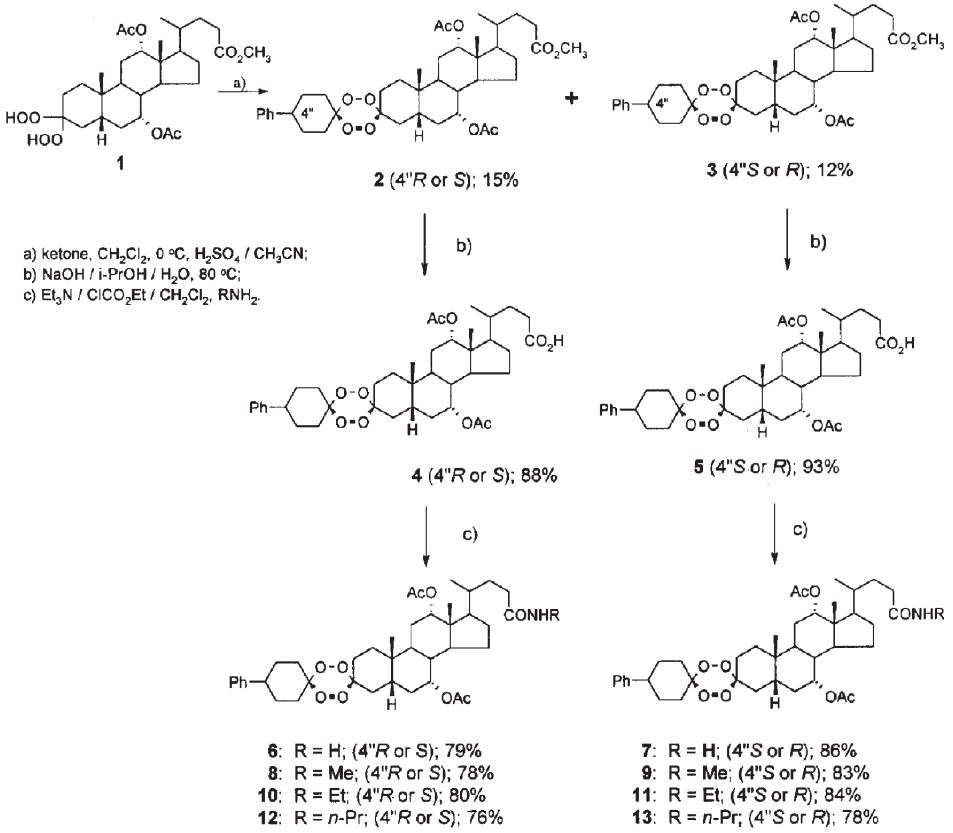
A bis-dispirotetraoxane compound[5b,7] affords little opportunity for selective functionalisation of any incorporated functionality, and such a fact was the motivation to devise a method for the synthesis of tetraoxanes possessing non-identical spiro substituents at C(3) and C(6) (“mixed tetraoxanes”).[8]

In this work, mixed tetraoxanes **2**–**13** were prepared using the same procedure as described recently in Ref. 8 (Scheme 1). *Gem*-dihydroperoxide **1** was treated with prochiral 4-phenylcyclohexanone (Scheme 1) yielding a mixture of tetraoxane diastereomers (**2** + **3**; 27 %).[13] The esters were separated and each was selectively hydrolysed into the corresponding acids **4** and **5**. Utilising a mixed anhydride procedure, the acids **4** and **5** were transformed into amides **6**, **8**, **10**, **12**, and **7**, **9**, **11**, **13**, respectively. The overall yield of amides in each series starting from *gem*-dihydroperoxide **1** was *ca.* 10 %.

BIOLOGICAL EVALUATION

Antimalarial activity

In vitro antimalarial activity was assessed against two *P. falciparum* strains: D6 Sierra Leone clone (CQ and pyrimethamine susceptible, mefloquine resistant), and W2 Indochina (CQ and pyrimethamine resistant, mefloquine susceptible). As expected from previ-



Scheme 1.

ous findings,[6–8] the two diastereomeric series exerted different activity against both strains. One series (esters, acids, amides), denoted as (4"*R* or *S*),[13] is *ca.* 2–8 times more active than the other one on both strains. While the activity of the (4"*S* or *R*) series is in the range of *ca.* 33 –91 nM, the respective diastereomers (4"*R* or *S*) are as active as artemisinin. The esters show poor activity in comparison to the corresponding amides,[7,8] and the acids are usually the least active in the series.[5b,8] However, with the (4"*R* or *S*)-phenyl series, Table I, the unique trend observed with the corresponding (4"*R*)-methyls and (4"*R* or *S*)-ethyls[8] is extended here: the (4"*R* or *S*) acid **4** is more active than the corresponding ester **2**, and is as active as the corresponding amides **6**, **8**, **10**, **12**. The established cytotoxicity against the VERO cells for compound **4** (IC_{50} = 1.53 μM) provides a good starting SI (IC_{50} VERO / IC_{50} (D6 or W2)) for further improvements of this structure.

Antitubercular activity

All the 4"-phenyl tetraoxanes **2**–**13** were screened against *Mycrobacterium tuberculosis*, strain H37Rv (Table II), within the NIAID Tuberculosis Antimicrobial Acquisition and Coordinating Facility program (TAACF).[10] Of the 12 compounds screened at level 1,

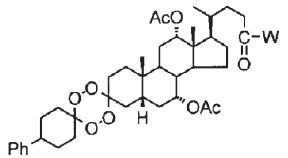


TABLE I. *In vitro* antimalarial activity of the tetraoxanes **2** – **13** against *P. falciparum* D6 and W2 Strains

Compound	(4"*R* or *S*)				Compound	(4"*S* or *R*)			
	W	D6 (nM)	W2 (nM)	RI (W2 / D6)		W	D6 (nM)	W2 (nM)	RI (W2 / D6)
2	OCH_3	17.66	16.48	0.93	**3**	OCH_3	44.67	35.17	0.79
4	OH	8.88	8.74	0.98	**5**	OH	77.83	62.93	0.80
6	NH_2	10.34	10.57	1.02	**7**	NH_2	58.18	53.82	0.92
8	NHMe	12.43	9.80	0.79	**9**	NHMe	91.02	77.07	0.85
10	NHEt	7.48	8.11	1.08	**11**	NHEt	45.30	45.00	0.99
12	$NHPr^n$	8.93	9.11	1.02	**13**	$NHPr^n$	32.90	33.26	1.05
Artemisinin [a]		8.6	7.3	0.85	Chloroquine [b]		13.76	185.38	13.47
Artemether [a]		2.92	1.00	0.34	Mefloquine [b]		28.29	5.02	0.18

[a] Taken from Ref. 5c; [b] Control drugs.

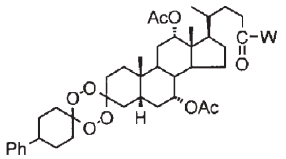


TABLE II. *In vitro* antimalarial activity of the tetraoxanes **2** – **13** against *M. tuberculosis*

Compound	W	Asaay	% Inh.	MIC (μg/mL)	Compound	W	Assay	% Inh.	MIC (μg/mL)
2	OCH_3	Alamar	37	> 6.25	**3**	OCH_3	Alamar	46	> 6.25
4	OH	Alamar	94	6.25	**5**	OH	Alamar	89	> 6.25
6	NH_2	Alamar	93	6.25	**7**	NH_2	Alamar	87	> 6.25
8	NHMe	Alamar	71	> 6.25	**9**	NHMe	Alamar	61	> 6.25
10	NHEt	Alamar	50	> 6.25	**11**	NHEt	Alamar	62	> 6.25
12	$NHPr^n$	Alamar	46	> 6.25	**13**	$NHPr^n$	Alamar	59	> 6.25
Rifampin				0.125	Isoniazid				0.05

two of them (**4** and **6**) exhibited the required > 90 % inhibition (at the single concentration of 6.25 μg/mL) for entering the next level of screening. The MICs were determined at level 2 of the screening protocol: acid **4** – 6.25 μg/mL (94 % inhibition), primary amide **6** – 6.25 μg/mL (93 % inhibition). Such high activities of these tetraoxanes indicate that tetraoxanes might represent a good new anti-TB lead.

Antiproliferative activity

Four of the compounds (**2**, **4**, **6**, and **8**) were chosen by NIH-NCI for in vitro screening.[14] All the tetraoxanes were evaluated in the 3-cell line (lung – NCI-H460, breast – MCF7, CNS – SF-268) one dose primary anticancer assay: growth percentage after 48 h, at a concentration of 100 μM of the tested compound. Two compounds were eliminated at this stage (**2** and **8**), while the acid **4** and primary amide **6** were evaluated against the full panel of 60 human tumor cell lines starting at a concentation of 10^{-4} M of the investigated compound. The assessed antiproliferative activity, expressed as GI_{50}, TGI, LC_{50} were obtained applying the 48 h continuous drug exposure protocol using the SRB (sulforhodamine B) protein assay.[14] The results, given in Table III, indicate that both compounds are strong antiproliferatives with 50 % growth inhibitory activities (GI_{50}), often at the nanomolar concentration. The highest activity was exhibited by the primary amide **6** on a melanoma cancer cell line (MALME-3M; GI_{50} = 20 nM). The compounds arrested the cancer cells growth (TGI) at concentrations within the *ca.* 0.8 – 6 μM range, with the acid **4** being a good inhibitor of the growth of the ovarian cancer cell line (IGROV1; TGI = 0.82 μM). The LC_{50} values (concentration of the compound at which 50 % of the cells are killed) for both compounds are mostly at the 10^{-6} M level indicating, together with previous results,[7] that steroidal tetraoxanes are possibly good new leads in fighting cancer. For comparison, the corresponding inhibitory activity of the antimalarial artemisinin and the antitumor drug paclitaxel are also given in Table III.

TABLE III. *In vitro* antiproliferative activity of tetraoxanes **4** and **6** (after 48 h, μM; selected data)

Cell Line		Artemisinin (NSC 369397)	Comp. **4**	Comp. **6**	Paclitaxel (NSC 125973)
IGROV1[a]	GI50	79.4	0.26	0.295	0.032
	TGI	100	0.82	1.11	79.4
	LC50	100	3.76	–	100
TK-10[b]	GI50	100	1.99	2.70	0.25
	TGI	100	5.94	4.66	50.1
	LC50	100	27.0	8.07	79.4
UO-31[b]	GI50	79.4	1.83	0.36	1.58
	TGI	100	3.51	1.35	39.8
	LC50	100	6.73	4.57	100
SR[c]	GI50	100	1.83	0.29	0.079
	TGI	100	5.92	–	63.1
	LC50	100	100	100	63.1

TABLE III. Continued

Cell Line		Artemisinin (NSC 369397)	Comp. **4**	Comp. **6**	Paclitaxel (NSC 125973)
KM-12[d]	GI50	100	1.96	1.19	0.0079
	TGI	100	5.36	2.92	79.4
	LC50	100	40.1	7.15	100
MALME-3M[e]	GI50	100	2.27	0.020	2.51
	TGI	100	9.14	3.99	50.1
	LC50	100	47.0	37.3	79.4

[a] Ovarian cancer cell line; [b] Renal cancer cell line; [c] Leukemia cell line; [d] Colon cancer cell line; [e] Melanoma cancer cell line.

DISCUSSION

In this paper, the synthesis and the activity of two diastereomeric series of 4''-phenyl mixed tetraoxanes are reported. The antimalarial activity of one series, designated as (4''*R* or *S*), is significantly higher than that of the corresponding C(4'') epimeric series, and is at the level of the proto peroxide antimalarial artemisinin (Table I). All members of the (4''*R* or *S*) series are almost equally active against both the *P. falciparum* strains tested, D6 and W2, and the determined cytotoxicity of tetraoxane **4** (IC_{50} = 1.53 μM) against VERO cells, affords solid ground for further development of these compounds as antimalarials.

In Table III, the selected antiproliferative activity data of compounds **4** and **6** are compared to these of artemisinin (NSC 369397) and the antitumor drug paclitaxel (NSC 125973). While artemisinin is ineffective as an antiproliferative, our compounds exhibit significant activity against various solid cancer types *in vitro*. The previously observed[7] pronounced activity (GI_{50}, TGI, LC_{50}) of bis-steroidal tetraoxanes against renal cancers is confirmed here, with both the acid **4** and the primary amide **6** being more active than paclitaxel on the TK-10 and UO-31 renal cancer cell lines. The acid **4** was found to be most active against the ovarian IGROV1 cell line, while the amide **6** was most active against the melanoma MALME-3M cell line.***

To conclude, mixed tetraoxanes of the 4''-phenyl series have been prepared and evaluated as possible antimalarials, cancer antiproliferatives and antimycobacterials. The activity of the (4''*R* or *S*)-phenyl series against the primary target of this investigation, *P. falciparum* D6 and W2 strains, was found to be at the level of artemisinin, with two compounds, the acid **4** and the amide **6**, exhibiting encouraging anti-TB activity as well. Very promising *in vitro* results of the above cited tetraoxanes were obtained against solid tumours and, in some instances, the activity against a selected number of cell line was higher than that of the antitumor drug paclitaxel.

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*** The tetraoxane **6** was 8 (GI_{50}), 12 (TGI) and 2 (LC_{50}) times more active than paclitaxel against the melanoma MALME-3M cell line.

velopment contract with the U.S. National Institute of Allergy and Infectious Diseases. We thank the NIH-NCI's Developmental and Therapeutics program for evaluation of our tetraoxanes. This work was partially supported by the Ministry of Science, Technologies and Development of Serbia (Grant No.1579).

EXPERIMENTAL

General

Melting points were determined on Boetius PMHK apparatus and were not corrected. Specific rotations were determined on a Perkin-Elmer 141-MC at the given temperatures. IR spectra were recorded on a Perkin-Elmer spectrophotometer FT-IR 1725X. ^{1}H- and ^{13}C-NMR spectra were recorded on a Varian Gemini-200 spectrometer (at 200 and 50 MHz, respectively) in the indicated solvent using TMS as the internal standard. Chemical shifts are expressed in ppm (δ) values and coupling constants (*J*) in Hz. Thin-layer chromatography (TLC) was performed on precoated Merck silica gel 60 F_{254} plates, using *N*,*N*-dimethyl-*p*-phenylene-diammonium dichloride peroxide reagent for the detection of peroxide moieties,[15] and Lobar LichroPrep Si 60 (40–63 μm) columns coupled to a Waters RI 401 detector were used for column chromatography. Where appropriate, the compounds are listed according to their elution order.

Methyl 3,3-dihydroperoxy-7α,12α-diacetoxy-5β-cholan-24-oate (**1**)

Gem-dihydroperoxide **1** was synthesized according to procedure described in Ref. 8.

*Methyl 7α,12α-diacetoxy-5β-cholan-24-oate-3-spiro-6'-(1',2',4',5'-tetraoxacyclohexane)-3'-spiro-1''- ((4''*R *or* S*)- and (4''*S *or* R*)-phenyl)cyclohexane (***2** *and* **3***)*

A solution of the dihydroperoxide **1** (500 mg, 0.90 mmol) in CH_2Cl_2 (14 mL) and 4-phenylcyclohexanone (314 mg, 1.80 mmol) at r.t. was cooled with stirring in an ice-bath. After 30 min, 0.6 mL of an ice-bath cooled (H_2SO_4 : CH_3CN)-mixture (1:10, v/v) was added dropwise. The reaction mixture was stirred at 0 ºC for 15 min and, after the usual work-up,[7] the crude product was purified by column chromatography (Lobar B, LichroPrep Si 60, eluent heptane / EtOAc (85:15); Lobar B, LichroPrep RP-8, eluent MeOH / H_2O (9:1)) to afford the tetraoxanes **2** and **3**.

2 (4''*R* or *S*): Yield 96 mg (15 %). Colourless foam, softens at 101–104 ºC. $[\alpha]^{20}_D$ = +34.27 (*c* =1.14, $CHCl_3$). IR (KBr): 2945 *m*, 2875 *m*, 1737 *s*, 1449 *m*, 1378 *m*, 1248 *s*, 1072 *m*, 1030 *m*, 945 *w*, 938 *w* cm^{-1}. ^{1}H-NMR (200 MHz, $CDCl_3$): 7.40–7.10 (*m*, Ph–C(4'')), 5.10 (*bs*, H–C(12)), 4.93 (*bs*, H–C(7)), 3.66 (*s*, CH_3O_2C(24)), 2.12 (*bs*, CH_3COO–), 2.10 (*bs*, CH_3COO–), 0.95 (*s*, H_3C–C(10)), 0.82 (*d*, *J* = 6.0 Hz H_3C–C(20)), 0.74 (*s*, H_3C–C(13)). ^{13}C-NMR (50 MHz, $CDCl_3$): 174.53, 170.54, 145.74, 128.43, 126.76, 126.28, 108.65, 107.83, 75.24, 70.65, 51.48, 47.29, 44.99, 43.43, 43.26, 37.63, 34.63, 34.53, 30.81, 30.68, 29.57, 28.36, 27.10, 25.64, 22.75, 22.05, 21.58, 21.32, 17.45, 12.15. Anal. Calcd. for $C_{41}H_{58}O_{10}\cdot 0.5\ H_2O$ (719.92): C 68.40, H 8.26; Found: C 68.60, H 8.30. **3** (4''*S* or *R*): Yield 77 mg (12 %). Colourless foam, softens at 186–190 ºC. $[\alpha]^{20}_D$ = +47.67 (*c* = 1.03, $CHCl_3$). IR (KBr): 2951 *m*, 2880 *m*, 1738 *s*, 1449 *m*, 1378 *m*, 1253 *s*, 1128 *w*, 1062 *w*, 1025 *w*, 970 *w*, 932 *w* cm^{-1}. ^{1}H-NMR (200 MHz, $CDCl_3$): 7.40–7.20 (*m*, Ph–C(4'')), 5.09 (*bs*, H–C(12)), 4.93 (*bs*, H–C(7)), 3.66 (*s*, CH_3O_2C(24)), 2.13 (*bs*, CH_3COO–), 2.08 (*bs*, CH_3COO–), 0.96 (*s*, H_3C–C(10)), 0.81 (*d*, *J* = 6.0 Hz H_3C–C(20)), 0.74 (*s*, H_3C–C(13)). ^{13}C-NMR (50 MHz, $CDCl_3$): 174.53, 170.60, 145.73, 128.42, 126.79, 126.28, 108.66, 107.87, 75.25, 70.64, 51.48, 47.30, 45.02, 43.56, 43.30, 37.62, 34.66, 34.53, 30.81, 30.69, 29.53, 28.42, 27.10, 25.69, 22.73, 22.06, 21.38, 17.44, 12.17. Anal. Calcd. for $C_{14}H_{58}O_{10}$ (710.91): C 69.27, H 8.22; Found: C 68.93, H 7.89.

*7α,12α-Diacetoxy-5β-cholan-24-oic acid-3-spiro-6'-(1',2',4',5'-tetraoxacyclohexane)-3'-spiro-1''-((4''*R *or* S*)-phenyl)cyclohexane* (**4**)

Methyl ester **2** (250 mg, 0.35 mmol) was hydrolysed at 90 ºC with NaOH (21.1 mg, 0.53 mmol) in *i*-PrOH / H_2O mixture (10 mL, 3:1 v/v). After 30 min, reaction mixture was cooled and diluted with 10 mL H_2O and 30 mL CH_2Cl_2. The aqueous layer was acidified to pH 2 with diluted HCl, and the layers were separated. The aqueous layer was further extracted with CH_2Cl_2 (3×20 mL). The combined organic layers were washed with water and brine, dried over anh. Na_2SO_4 and evaporated to dryness. Acid **4**: yield 215 mg (88

%), colourless foam softens at 139 – 143 ºC. $[\alpha]^{20}_D$ = +32.48 (*c* = 1.10, $CHCl_3$). IR (KBr): 3419 *w*, 2945 *m*, 2880 *w*, 1738 *s*, 1449 *w*, 1383 *m*, 1247 *s*, 1123 *w*, 1079 *w*, 1030 *w*, 970 *w*, 932 *w* cm^{-1}. ^{1}H-NMR (200 MHz, $CDCl_3$): 7.40–7.10 (*m*, Ph–C(4")), 5.10 (*bs*, H–C(12)), 4.93 (*bs*, H–C(7)), 2.13 (*bs*, CH_3COO–), 0.96 (*s*, H_3C–C(10)), 0.83 (*d*, *J* = 4.6 Hz, H_3C–C(20)), 0.74 (*s*, H_3C–C(13)). ^{13}C-NMR (50 MHz, $CDCl_3$): 170.62, 145.72, 128.42, 126.75, 126.28, 108.64, 107.84, 75.24, 70.68, 47.23, 44.98, 43.41, 37.59, 34.61, 34.46, 30.45, 29.56, 28.34, 27.05, 25.62, 24.49, 22.71, 22.04, 21.58, 21.33, 17.42, 12.16. Anal. Calcd. for $C_{40}H_{56}O_{10}\cdot H_2O$ (714.90): C 67.20, H 8.18; Found: C 67.23, H 8.31.

*7α,12α-Diacetoxy-5β-cholan-24-oic acid-3-spiro-6'-(1',2',4',5'-tetraoxacyclohexane)-3'-spiro-1"-((4"*S *or* R*)-phenyl)cyclohexane* (**5**)

Methyl ester **3** (250 mg, 0.35 mmol) was hydrolysed using the same procedure as given above for the preparation of **4**.

Acid **5**: yield 229 mg (93 %), colorless foam softens at 137–140 ºC. $[\alpha]^{20}_D$ = +43.87 (*c* = 1.06, $CHCl_3$). IR (film): 3436 *s*, 2946 *w*, 1739 *m*, 1642 *m*, 1448 *w*, 1378 *w*, 1244 *m*, 1131 *w*, 1061 *w*, 1033 *w* cm^{-1}. ^{1}H-NMR (200 MHz, $CDCl_3$): 7.40–7.10 (*m*, Ph–C(4")), 5.10 (*bs*, H–C(12)), 4.93 (*bs*, H–C(7)), 2.14 (*bs*, CH_3COO–), 2.09 (*bs*, CH_3COO–), 0.96 (*s*, H_3C–C(10)), 0.82 (*d*, *J* = 5.6 Hz, H_3C–C(20)), 0.74 (*s*, H_3C–C(13)).^{13}C-NMR (50 MHz, $CDCl_3$): 179.78, 170.68, 145.72, 128.42, 126.78, 126.28, 108.65, 107.88, 75.26, 70.68, 47.26, 45.02, 43.54, 43.28, 37.60, 34.65, 34.46, 30.45, 29.53, 28.39, 27.07, 25.69, 22.71, 22.05, 21.59, 21.40, 17.41, 12.18. Anal. Calcd. for $C_{40}H_{56}H_{10}\cdot 0.5H_2O$ (705.90): C 68.06, H 8.14; Found: C 67.97, H 7.83.

General procedure for the preparation of the amides **6**–**13**

A solution of **4** (263.4 mg, 0.38 mmol), in dry CH_2Cl_2 (20 mL), with added Et_3N (52.9 μL, 0.38 mmol) and $ClCO_2Et$ (36.31 μL, 0.5 mmol) was stirred for 60 min at 0 ºC. Given amount of amine given below was added, and after 30 min of stirring the reaction mixture was warmed to r.t. After 90 min it was diluted with H_2O, the layers were separated and the reaction mixture was worked-up in the usual manner.[7] The crude product was purified by column chromatography.

*7α,12α-Diacetoxy-5β-cholan-24-amide-3-spiro-6'-(1',2',4',5'-tetraoxacyclohexane)-3'-spiro-1"-((4"*R *or* S*)-phenyl)cyclohexane* (**6**)

Using a suspension of 10 eq. NH_4Cl and 10 eq. Et_3N in dry CH_2Cl_2 (20 mL), 209 mg (79 %) of **6** were obtained. Column chromatography: eluent EtOAc. Colourles foam softens at 142–146 ºC. $[\alpha]^{20}_D$ = +35.79 (*c* = 1.08, $CHCl_3$). IR (KBr): 3458 *m*, 2946 *s*, 2876 *m*, 1739 *s*, 1675 *m*, 1621 *w*, 1448 *m*, 1378 *m*, 1243 *s*, 1131 *w*, 1082 *m*, 1034 *m*, 969 *w*, 937 *w* cm^{-1}. ^{1}H-NMR (200 MHz, $CDCl_3$): 7.40–7.00 (*m*, Ph–C(4")), 5.80–5.40 (*m*, H_2N–C(24)), 5.10 (*bs*, H–C(12)), 4.93 (*bs*, H–C(7)), 2.12 (*bs*, CH_3COO–), 2.10 (*bs*, CH_3COO–), 0.95 (*s*, H_3C–C(10)), 0.83 (*d*, *J* = 5.6 Hz, H_3C–C(20)), 0.74 (*s*, H_3C–C(13)). ^{13}C-NMR (50 MHz, $CDCl_3$): 175.90, 170.57, 145.72, 128.43, 126.75, 126.28, 108.63, 107.83, 75.24, 70.65, 47.40, 45.01, 43.41, 43.23, 37.60, 34.61, 32.66, 31.29, 29.59, 28.34, 27.14, 25.64, 22.73, 22.04, 21.58, 21.34, 17.53, 12.18. Anal. Calcd. for $C_{40}H_{57}NO_9\cdot 0.5\ H_2O$ (704.91): C 68.16, H 8.29; Found: C 68.09, H 8.22.

N-*Methyl-7α,12α-diacetoxy-5β-cholan-24-amide-3-spiro-6'-(1',2',4',5'-tetraoxacyclohexane)-3'-spiro-1"-((4"*R *or* S*)-phenyl)cyclohexane* (**8**)

Acid **4** (263.7 mg, 0.38 mmol) was transformed into **8** (210 mg, 78 %) according to the general procedure using a suspension of 6 eq. $MeNH_3Cl$ / 6 eq. Et_3N in 20 ml dry CH_2Cl_2. Column chromatography: Lobar B, LichroPrep Si 60; eluent EtOAc. Colourless foam softens at 133–137 ºC. $[\alpha]^{20}_D$ = +28.03 (*c* = 1.09, $CHCl_3$). IR (KBr): 3353 *w*, 2945 *s*, 2880 *w*, 1738 *s*, 1656 *m*, 1553 *w*, 1455 *w*, 1378 *m*, 1253 *s*, 1128 *w*, 1079 *w*, 1030 *m*, 965 *w*, 943 *w* cm^{-1}. ^{1}H-NMR (200 MHz, $CDCl_3$): 7.40–7.10 (*m*, Ph–C(4")), 5.60–5.40 (*m*, *H*N–C(24)), 5.10 (*bs*, H–C(12)), 4.92 (*bs*, H–C(7)), 2.80 (*d*, *J* = 4.80 Hz, H_3C–NH), 2.12 (*bs*, CH_3COO–), 0.95 (*s*, H_3C–C(10)), 0.82 (*d*, *J* = 5.80 Hz, H_3C–C(20)), 0.73 (*s*, H_3C–C(13)). ^{13}C-NMR (50 MHz, $CDCl_3$): 173.93, 170.58, 145.71, 128.42, 126.74, 126.26, 108.62, 107.82, 75.25, 70.64, 47.42, 44.98, 43.40, 43.21, 37.59, 34.69, 34.60, 33.35, 31.47, 30.54, 29.55, 28.34, 27.12, 26.25, 25.62, 22.72, 22.04, 21.58, 21.33, 17.52, 12.17. Anal. Calcd. for $C_{41}H_{59}NO_9\cdot\ 0.5\ H_2O$ (718.94): C 68.50, H 8.41; Found: C 68.84, H 8.71.

N-*Ethyl-7α,12α-diacetoxy-5β-cholan-24-amide-3-spiro-6'-(1',2',4',5'-tetraoxacyclohexane)-3'-spiro-1''-((4''*R *or* S*)-phenyl)cyclohexane* (**10**)

Acid **4** (261.3 mg, 0.37 mmol) was transformed into **10** (216 mg, 80 %) according to the general procedure using a suspension of 6 eq. $EtHN_3Cl$ / 6 eq. Et_3N in 20 ml dry CH_2Cl_2. Column chromatography: Lobar B, LichroPrep Si 60; eluent EtOAc / heptane (95/5). Colourless foam, softens at 128–131 ºC. $[\alpha]^{20}_D = +34.36$ (*c* = 1.04, $CHCl_3$). IR (KBr): 3326 *w*, 2951 *m*, 2880 *m*, 1738 *s*, 1656 *m*, 1547 *w*, 1455 *m*, 1379 *m*, 1248 *s*, 1128 *w*, 1085 *w*, 1030 *m*, 965 *w*, 938 *w* cm^{-1}. ^{1}H-NMR (200 MHz, $CDCl_3$): 7.40–7.10 (*m*, Ph–C(4'')), 5.60–5.40 (*m*, *H*N–C(24)), 5.10 (*bs*, H–C(12)), 4.92 (*bs*, H–C(7)), 3.40–3.10 (*m*, CH_3CH_2–NH–), 2.12 (*bs*, CH_3COO–), 2.09 (*bs*, CH_3COO–), 1.13 (*t*, *J* = 7.2 Hz, CH_3CH_2–NH–), 0.95 (*s*, H_3C–C(10)), 0.82 (*d*, *J* = 6 Hz, H_3C–C(20)), 0.73 (*s*, H_3C–C(13)). ^{13}C-NMR (50 MHz, $CDCl_3$): 173.11, 170.58, 145.72, 128.42, 126.74, 126.28, 108.63, 107.82, 75.25, 70.63, 47.42, 44.98, 43.40, 43.22, 37.58, 34.67, 34.60, 34.25, 33.49, 31.48, 30.53, 29.43, 28.34, 27.12, 25.61, 22.71, 22.04, 21.56, 21.33, 17.54, 14.83, 12.16. Anal. Calcd. for $C_{42}H_{61}NO_9 \cdot 0.5\ H_2O$ (732.96): C 68.83, H 8.53; Found: C 68.81, H 8.57.

N-*(*n*-Propyl) -7α,12α-diacetoxy-5β-cholan-24-amide-3-spiro-6'-(1',2',4',5'-tetraoxacyclohexane)-3'-spiro-1''- ((4''* R *or* S*)-phenyl)cyclohexane* (**12**)

Acid **4** (259.2 mg, 0.37 mmol) was transformed into **12** (210 mg, 76 %) according to the general procedure using 60.24 μL, (0.74 mmol) *n*-$PrNH_2$. Column chromatography: Lobar B, LichroPrep Si 60; eluent EtOAc / heptane (95/5). Colourless foam, softens at 125–127 ºC. $[\alpha]^{20}_D = +32.25$ (*c* = 1.10, $CHCl_3$). IR (KBr): 3413 *w*, 3326 *w*, 2945 *s*, 2875 *m*, 1738 *s*, 1656 *m*, 1547 *w*, 1455 *m*, 1378 *m*, 1248 *s*, 1128 *w*, 1078 *m*, 1030 *m*, 965 *w*, 943 *w* cm^{-1}. ^{1}H-NMR (200 MHz, $CDCl_3$): 7.40–7.10 (*m*, Ph–C(4'')), 5.60–5.40 (*m*, *H*N–C(24)), 5.10 (*bs*, H–C(12)), 4.93 (*bs*, H–C(7)), 3.40–3.10 (*m*, $CH_3CH_2CH_2$–NH–), 2.12 (*bs*, CH_3COO–), 1.70–1.20 (*m*, $CH_3CH_2CH_2$–NH–), 1.00–0.80 (*m*, H_3C–C(10), $CH_3CH_2CH_2$–NH–), 0.82 (*d*, *J* = 6.0 Hz, H_3C–C(20)), 0.73 (*s*, H_3C–C(13)). ^{13}C-NMR (50 MHz, $CDCl_3$): 173.21, 170.59, 145.73, 128.43, 126.76, 126.28, 108.64, 107.83, 75.27, 70.65, 47.45, 44.99, 43.41, 43.23, 41.13, 37.59, 34.61, 33.55, 31.54, 29.55, 28.34, 27.13, 25.62, 22.83, 22.04, 21.57, 21.34, 17.54, 12.17, 11.30. Anal. Calcd. for $C_{43}H_{63}NO_9 \cdot 0.5\ H_2O$ (746.99): C 69.14, H 8.64; Found: C 68.94, H 8.85.

7α,12α-Diacetoxy-5β-cholan-24-amide-3-spiro-6'-(1',2',4',5'-tetraoxacyclohexane)-3'-spiro-1''-((4'' S *or* R*)-phenyl)cyclohexane* (**7**)

Acid **5**, (273.5 mg, 0.39 mmol) was transformed into **7** (236 mg, 86 %) using a suspension of 10 eq. NH_4Cl / 10 eq. Et_3N in 20 ml dry CH_2Cl_2. Column chromatography: Lobar B, LichroPrep Si 60; eluent EtOAc. Colourless foam, softens at 141–144 ºC. $[\alpha]^{20}_D = +41.37$ (*c* = 1.02, $CHCl_3$). IR (KBr): 3463 *w*, 3356 *w*, 2952 *m*, 2876 *m*, 1739 *s*, 1675 *m*, 1621 *w*, 1448 *m*, 1384 *m*, 1249 *s*, 1131 *w*, 1061 *w*, 1028 *m*, 969 *w*, 942 *w* cm^{-1}. ^{1}H-NMR (200 MHz, $CDCl_3$): 7.40–7.10 (*m*, Ph–C(4'')), 5.70–5.30 (*m*, H_2N–C(24)), 5.13 (*bs*, H–C(12)), 4.95 (*bs*, H–C(7)), 2.16 (*bs*, CH_3COO–), 2.12 (*bs*, CH_3COO–), 0.99 (*s*, H_3C–(10)), 0.86 (*d*, *J* = 5.8 Hz, H_3C–C(20)), 0.77 (*s*, H_3C–C(13)). ^{13}C-NMR (50 MHz, $CDCl_3$): 175.83, 170.63, 145.74, 128.43, 126.80, 126.29, 108.66, 107.89, 75.28, 70.66, 47.43, 45.06, 43.56, 43.30, 37.63, 34.67, 32.70, 31.31, 29.56, 28.42, 27.15, 25.71, 22.75, 22.06, 21.59, 21.43, 17.55, 12.21. Anal. Calcd. for $C_{40}H_{57}NO_9 \cdot 0.5\ H_2O$ (704.91): C 68.16, H 8.29; Found: C 68.27, H 8.57.

N-*Methyl-7α,12α-diacetoxy-5β-cholan-24-amide-3-spiro-6'-(1',2',4',5'-tetraoxacyclohexane)-3'-spiro-1''-((4''*S *or* R*)-phenyl)cyclohexane* (**9**)

Acid **5** (256.7 mg, 0.37 mmol) was transformed into **9** (217 mg, 83 %) according to the general procedure using a suspension of 6 eq. $MeNH_3Cl$ / 6 eq. Et_3N in 20 ml dry CH_2Cl_2. Column chromatography: Lobar B, LichroPrep Si 60; eluent EtOAc. Colourless foam, softens at 137–140 ºC. $[\alpha]^{20}_D = +47.15$ (*c* = 0.90, $CHCl_3$). IR (KBr): 3402 *w*, 3343 *w*, 2945 *s*, 2875 *m*, 1738 *s*, 1661 *m*, 1553 *w*, 1449 *m*, 1378 *m*, 1248 *s*, 1169 *w*, 1128 *w*, 1063 *m*, 1030 *m*, 970 *w*, 938 *w* cm^{-1}. ^{1}H-NMR (200 MHz, $CDCl_3$): 7.40–7.10 (*m*, Ph–C(4'')), 5.60–5.30 (*m*, *H*N–C(24)), 5.09 (*bs*, N–C(12)), 4.92 (*bs*, H–C(7)), 2.80 (*d*, *J* = 5.0 Hz, H_3C–NH), 2.13 (*bs*, CH_3COO–), 2.08 (*bs*, CH_3COO–), 0.96 (*s*, H_3C–C(10)), 0.81 (*d*, *J* = 6.0 Hz, H_3C–C(20)), 0.73 (*s*, H_3C–C(13)). ^{13}C-NMR (50 MHz, $CDCl_3$): 173.90, 170.63, 145.74, 128.43, 126.79, 126.28, 108.67, 107.88,

75.29, 70.65, 47.47, 45.04, 43.56, 43.29, 37.63, 34.67, 33.39, 31.50, 30.58, 29.60, 28.42, 27.13, 26.27, 25.70, 22.75, 22.06, 21.59, 21.41, 17.55, 12.21. Anal. Calcd. for $C_{41}H_{59}NO_9 \cdot 0.5\ H_2O$ (718.94): C 68.50, H 8.41; Found: C 68.69, H 8.30.

N-*Ethyl-7α,12α-diacetoxy-5β-cholan-24-amide-3-spiro-6'-(1',2',4',5'-tetraoxacyclohexane)-3'-spiro-1''-((4''* S *or* R*)-phenyl)cyclohexane* (**11**)

Acid **5** (256.5 mg, 0.37 mmol) was transformed into **11** (224 mg, 84 %) according to the general procedure using a suspension of 6 eq. $EtNH_3Cl$ / 6 eq. Et_3N in 20 ml dry CH_2Cl_2. Column chromatography: Lobar B, LichroPrep Si 60; eluent EtOAc / heptane (95/5). Colourless foam, softens at 129–132 ºC. $[\alpha]^{20}_D = +46.12$ ($c = 0.97$, $CHCl_3$). IR (KBr): 3440 *m*, 2951 *s*, 2880 *m*, 1738 *s*, 1655 *m*, 1547 *w*, 1449 *m*, 1378 *m*, 1248 *s*, 1128 *w*, 1063 *w*, 1030 *m*, 970 *w*, 943 *w* cm^{-1}. ^{1}H-NMR (200 MHz, $CDCl_3$): 7.40–7.10 (*m*, Ph–C(4'')), 5.60–5.40 (*m*, *H*N–C(24)), 5.10 (*bs*, H–C(12)), 4.92 (*bs*, H–C(7)), 3.40–3.10 (*m*, CH_3CH_2–NH–), 2.13 (*bs*, CH_3COO–), 2.09 (*bs*, CH_3COO–), 1.30–1.10 (*m*, CH_3CH_2–NH–), 0.96 (*s*, H_3C–C(10)), 0.82 (*d*, $J = 5.8$ Hz, H_3C–C(20)), 0.73 (*s*, H_3C–C(13)). ^{13}C-NMR (50 MHz, $CDCl_3$): 173.15, 170.60, 170.40, 145.69, 128.39, 126.76, 126.25, 108.62, 107.84, 75.26, 70.62, 47.42, 45.00, 43.51, 43.26, 37.58, 34.63, 34.24, 33.49, 31.48, 30.53, 29.48, 28.38, 27.11, 25.67, 22.70, 22.03, 21.54, 21.38, 17.53, 14.82, 12.17. Anal. Calcd. for $C_{42}H_{61}NO_9 \cdot 0.5\ H_2O$ (732.96): C 68.83, H 8.53; Found: C 68.70, H 8.89.

N-*(*n*-Propyl)-7α,12α-diacetoxy-5β-cholan-24-amide-3-spiro-6'-(1',2',4',5'-tetraoxacyclohexane)-3'-spiro-1''-((4''*S *or* R*)-phenyl)cyclohexane* (**13**)

Acid **5** (258.1 mg, 0.37 mmol) was transformed into **13** (214 mg, 78 %) according to the general procedure using 59.92 μL (0.74 mmol) *n*-$PrNH_2$. Column chromatography: Lobar B, LichroPrep Si 60; eluent EtOAc / heptane (95/5). Colourless foam, softens at 127–131 ºC. $[\alpha]^{20}_D = +45.23$ ($c = 1.07$, $CHCl_3$). IR (KBr): 3402 *w*, 3321 *w*, 2945 *s*, 2880 *m*, 1738 *s*, 1656 *m*, 1547 *w*, 1449 *m*, 1378 *m*, 1248 *s*, 1128 *w*, 1063 *w*, 1030 *m*, 970 *w*, 938 *w* cm^{-1}. ^{1}H-NMR (200 MHz, $CDCl_3$): 7.40–7.10 (*m*, Ph–C(4'')), 5.50–5.30 (*m*, *H*N–C(24)), 5.10 (*bs*, H–C(12)), 4.92 (*bs*, H–C(7)), 3.40–3.10 (*m*, $CH_3CH_2CH_2$–NH–), 2.13 (*bs*, CHCOO–), 2.08 (*bs*, CH_3COO–), 1.70–1.30 (*m*, $CH_3CH_2CH_2$–NH–), 1.00–0.80 (*m*, H_3C–C(10), $CH_3CH_2CH_2$–NH–), 0.82 (*d*, $J = 6.0$ Hz, H_3C–C(20)), 0.73 (*s*, H_3C–C(13)). ^{13}C-NMR (50 MHz, $CDCl_3$): 173.21, 170.62, 145.73, 128.42, 126.78, 126.28, 108.65, 107.87, 75.29, 70.64, 47.47, 45.03, 43.55, 43.28, 41.13, 37.61, 34.66, 33.58, 31.56, 29.53, 28.41, 27.13, 25.70, 22.85, 22.05, 21.57, 21.41, 17.55, 12.20, 11.30. Anal. Calcd. for $C_{43}H_{63}NO_9$ (737.98): C 69.99, H 8.60; Found: C 70.03, H 8.88.

ИЗВОД

АНТИМАЛАРИЈСКА, АНТИМИКОБАКТЕРИЈСКА И АНТИПРОЛИФЕРАТИВНА АКТИВНОСТ ФЕНИЛ-СУПСТИТУИСАНИХ ТЕТРАОКСАНА

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У овом раду приказана је синтеза серије мешовитих тетраоксана, 4''-фенил-супституисаних циклохексил-спиротетраоксациклохексил-спирохолата, а испитана је и њихова *in vitro* активност као могућих антималарика, анти-ТБЦ агенаса и антипролиферативних једињења. Активност (4''*R* или *S*)-фенил серије на D6 и W2 сојеве *P. falciparum* врло је слична активности познатог антималарика артемизинина. Изражену анти-ТБЦ активност исказала су једињења **4** и **6**, чија антипролиферативна *in vitro* активност према неким компактним туморима превазилази активност лека паклитаксела.

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