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Bicyclo[2.2.2]octyl esters of dialkylamino acids as antiprotozoals

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Abstract—A couple of bicyclo[2.2.2]octyl esters of 2-dialkylaminoacetic acids were prepared. Their antiplasmodial and antitrypanosomal activities against *Trypanosoma brucei rhodesiense* (STIB 900) and the K_1 strain of *Plasmodium falciparum* (resistant to chloroquine and pyrimethamine) were determined using microplate assays. Structure–activity relationships were discussed. The antiprotozoal activities were remarkably increased by insertion of a second basic centre. The selectivity indices of the most active compounds are superior in the bicyclo-octane series.

1. Introduction

Malaria, one of the most important infectious diseases, kills over 1 million people annually throughout the world.[1,2] Furthermore approximately 500 million people get contagioned with this disease every year.[3] The causative protozoon is the *Plasmodium* parasite. The *Plasmodium falciparum* subspecies is the most hazardous organism causing malaria tropica. Today the most effective drug against malaria is artemisinin which is seen as the last defence against the disease, because resistance to the other available malaria drugs is on the rise everywhere.[1] But even against artemisinin derivatives resistance has been shown repeatedly in vitro.[4–6] Besides, those drugs are yet too expensive for widespread use.[7] So there is still need for a cheap drug which is active against chloroquine-resistant strains to replace chloroquine as first choice weapon against plasmodium parasites.

East African and West African are two variants of Human African Trypanosomiasis (HAT) or sleeping sickness. The disease is elicited by protozoan parasites of the genus *Trypanosoma* and kills ca. 50.000 people yearly.[2] The annual incidence of the disease is approximately 300.000 cases and about 60 million people worldwide are at risk of developing the sickness.[8] Only a single new drug, eflornithine, has entered medicinal therapy against HAT in the past 25 years.[9] Moreover all four drugs in use (suramin, pentamidine, melarsoprol and eflornithine) can cause severe side effects and are not effective against all stages and against all strains and species of trypanosomes.[10,11] Especially the therapy of the late stage of East African Trypanosomiasis is problematic, because melarsoprol, which is the only effective drug, causes an encephalopathy in 10% of the patients, killing half of them.[12]

Since the pharmaceutical industry does not bother that much for the relatively unprofitable development of antitrypanosomal drugs, there is an urgent need for new drugs with less undesired side effects.

The 4-dialkylaminobicyclo[2.2.2]octan-2-ol **1c** (Scheme 1) has shown antiplasmodial activity against the K_1 strain of *P. falciparum* (resistant to chloroquine and pyrimethamine), whereas **1a** was weakly active against *Trypanosoma brucei rhodesiense* (causative organism of the more hazardous East African Trypanosomiasis).[13] Since then a number of ester derivatives have been prepared, which exhibit higher antiprotozoal activities.[14–16]

In the acetate series the antiprotozoal activities have been improved by introduction of more lipophilic

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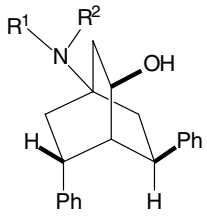
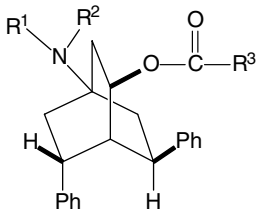
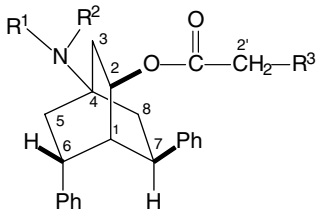


1a-1c



2a-2c, 3a-3c, 4a-4c, 5a-5c



6a-6c, 7a-7c, 8a-8c, 9a-9c

a: $R^1 = R^2 = CH_3$
b: $R^1 + R^2 = -(CH_2)_4-$
c: $R^1 + R^2 = -(CH_2)_5-$

2a-2c: R^3 = tert. butyl	**6a-6c**: R^3 = chlorine
3a-3c: R^3 = methyl	**7a-7c**: R^3 = N,N-diethylamine
4a-4c: R^3 = (3,4-methylendioxy)phenyl	**8a-8c**: R^3 = piperidine
5a-5c: R^3 = (2-phenyl)vinyl	**9a-9c**: R^3 = pyrrolidine

Scheme 1. Structures of bicyclo[2.2.2]octane derivatives.

substituents. Some of the pivalates **2** show quite good antitrypanosomal (IC_{50} = 0.62–2.69 μM) and antiplasmodial (IC_{50} = 0.42–7.93 μM) activities surpassing those of the corresponding acetates **3** by far (Table 1). The most active of the benzoates are esters of piperonylic acid **4**, with **4b** exhibiting the highest antitrypanosomal activity (IC_{50} = 0.82 μM) and **4a** as the most active antiplasmodial agent (IC_{50} = 0.34 μM).

In the present paper, the synthesis of some new ester analogues of 4-dialkylaminobicyclo[2.2.2]octan-2-ols **1** will be described. As an extension of the aryl ester series compounds **1** were esterified to the corresponding cinnamoates **5**. The structure of the alkyl esters was modified by insertion of basic substituents in the alkyl chain of the acid moiety.

All new compounds were characterized and tested for their activity against *T. brucei rhodesiense* and the K_1 strain of *P. falciparum* using in vitro assays. The results were compared to those of drugs in use and some formerly prepared 4-dialkylaminobicyclo[2.2.2]octyl esters.

2. Results

2.1. Chemistry

The dialkylaminobicyclo[2.2.2]octan-2-ols **1** were prepared in good yields by stereoselective reduction of the corresponding ketones, which are available in a one-pot reaction from benzylidene acetone and dialkylammonium thiocyanates.[17] Compounds **1** were treated with cinnamoyl chloride in the presence of 4-DMAP giving cinnamoates **5**. Likewise the 2-chloro acetates **6** were obtained. The amino acid compounds **7**–**9** were prepared by treatment of **6** with excess secondary amine.

2.2. Antiplasmodial and antitrypanosomal activity

The IC_{50} values for the antitrypanosomal and antiplasmodial activities and the cytotoxicity of compounds **1**–**5** and **7**–**9** are shown in Table 1.

3. Discussion

The antitrypanosomal activity of the newly prepared cinnamoates **5** (IC_{50} = 0.83–1.41 μM) is similar to that of the most active of the benzoate series, the piperonylates **4** (IC_{50} = 0.82–2.51 μM), but they are more toxic. With the exception of **9a** (IC_{50} = 1.99 μM) the amino acid esters **7**–**9** (IC_{50} = 0.21–0.87 μM) are more active than the corresponding pivalates **2** (IC_{50} = 0.62–2.69 μM) and, in addition, their selectivity index (SI) is distinctly better (**2**: SI < 9, **7**–**9**: SI = 14.70–186.7). Taking the activity of the corresponding acetates **3** (IC_{50} = 4.75–9.89 μM) as a reference, the influence of the newly inserted amino substituent on the antitrypanosomal activity is outstanding. The 2′-pyrrolidino compounds **9a**, **9b** (IC_{50} = 0.21–0.23 μM) are the so far most active antitrypanosomal bicyclo-octane derivatives and exhibit a quite good selectivity index (SI > 155). Nevertheless, esters **7**–**9** are still less active than the drugs in use.

With the exception of **5c** the cinnamoates **5** (IC_{50} = 0.45–0.69 μM) show lower activity against *P. falciparum* than the corresponding piperonylates **4** (IC_{50} = 0.34–0.55 μM). The new amino acid esters **7b**, **7c** and **8c** (IC_{50} = 0.18–0.25 μM) exhibit higher activity than the pivalates **2** (IC_{50} = 0.42–7.93 μM). The remarkable influence of the amino substituent in α-position of the acid moiety of **7** can be seen by comparison with the activities of the corresponding acetates **3** (IC_{50} = >10 μM). The antiplasmodial activity of the most active compounds **7b** and **8c** against the chloroquine resistant K_1 strain is comparable to that of

Table 1. In vitro activities of **1–9**, expressed as IC_{50} (μM)[a]

Compound	*Trypanosoma brucei rhodesiense*	SI = IC_{50} (Cytotox.)/IC_{50} (*T. brucei rhodesiense*)	*Plasmodium falciparum* K_1	SI = IC_{50} (Cytotox.)/IC_{50} (P.falc.)	Cytotoxicity IC_{50}, μM
1a	2.95	44.92	>15.55	<8.53	132.5
1b	4.26	6.28	2.39	11.20	26.76
1c	5.34	6.99	0.84	44.45	37.34
2a	0.62	8.16	0.42	12.05	5.06
2b	1.38	5.52	0.99	7.70	7.62
2c	2.69	4.46	7.93	1.51	12.00
3a	4.75	—	>12.50	—	n.t.
3b	4.93	—	>11.74	—	n.t.
3c	9.89	—	>10.99	—	n.t.
4a	0.98	5.43	0.34	15.65	5.32
4b	0.82	6.89	0.39	14.49	5.65
4c	2.51	5.07	0.55	23.15	12.73
5a	0.83	4.60	0.69	5.54	3.82
5b	1.04	3.37	0.63	5.56	3.50
5c	1.41	7.43	0.45	23.27	10.47
7a	0.61	49.41	2.72	11.08	30.14
7b	0.72	68.06	0.20	245.0	49.00
7c	0.56	42.71	0.25	95.68	23.92
8a	1.99	14.70	0.52	56.27	29.26
8b	0.37	38.43	0.37	38.43	14.22
8c	0.87	69.41	0.18	335.5	60.39
9a	0.21	155.5	0.71	46.00	32.66
9b	0.23	186.7	0.70	61.34	42.94
9c	0.32	51.19	0.47	34.85	16.38
mel	0.0039	1995			7.78
art			0.0064	70391	450.5
chl			0.12[b]	1571	188.5
mef					11.37

n.t., not tested; art, artemisinin; chl, chloroquine; mel, melarsoprol; mef, mefloquine.
[a] Values represent the average of four determinations (two determinations of two independent experiments).
[b] Against sensitive *P. falciparum* strains.

chloroquine (IC_{50} = 0.12 μM) against sensitive strains. In addition the selectivity indices of compounds **7b** and **8c** (SI = 245–335.5) are the highest of the so far prepared bicyclo-octane derivatives.

4. Conclusion

This paper reports the synthesis and the antitrypanosomal and antiplasmodial activity of new bicyclo[2.2.2]octyl esters of dialkylamino acids. Particularly worth mentioning is the fact that the insertion of an amino group in the acid component of the acetates remarkably improved both antiprotozoal activities and the selectivity indices. Two of the new compounds showed antiplasmodial activity comparable to chloroquine, whereas two other were the so far most active antitrypanosomal bicyclo-octyl derivatives. Furthermore, their selectivity indices were the most promising within the aminobicyclo-octane series. Although their properties are not yet a challenge for the drugs in use, they will serve as leads for further structural modifications.

5. Experimental

5.1. Instrumentation and chemicals

Melting points were measured on a digital melting point apparatus Electrothermal IA 9200 and are uncorrected. IR spectra were recorded on a Perkin-Elmer infrared spectrometer system 2000 FT on KBr discs. UV–vis spectra were recorded on a Perkin-Elmer Lambda 17 UV–vis-spectrometer. NMR spectra were recorded in 5 mm tubes at 25 °C on a Varian Unity Inova 400 (400 MHz) using TMS as an internal reference. ^1H and ^{13}C resonances were assigned using ^1H, ^1H and ^1H, ^{13}C correlation spectra (gCOSY, gHSQC, gHMBC, optimized on 8 Hz) and are numbered as given in the formulas. Analyses were carried out at the Microanalytical Laboratory at the Institute of Physical Chemistry in Vienna on a Carlo Erba EA 1108 CHNS-O apparatus. HRMS: Kratos profile spectrometer. Materials: column chromatography (CC): aluminium oxide for chromatography (pH: 9.5, Fluka) or silica gel 60 (Merck) (70–230 mesh), pore-diameter 60 Å; thin-layer chromatography (TLC): TLC plates (Merck, silica gel 60 F_{254} 0.2 mm, 200 mm × 200 mm); the substances were detected in UV light at 254 nm.

5.2. Syntheses

5.2.1. General procedure for the synthesis of (2*SR*,6*RS*,7*RS*)-(±)-4-dialkylamino-6,7-diphenylbicyclo[2.2.2]octan-2-ols (1a–1c). The bicyclic ketones were dissolved in dry MeOH and $NaBH_4$ was added under stirring and cooling. Then the mixture was stirred overnight. After it was carefully quenched with icewater, the mixture was shaken four times with $CHCl_3$. The combined organic layers were shaken three times with water, dried over sodium sulfate and

filtered. The solvent was removed in vacuo and the residue was purified by crystallization.

5.2.1.1. (2*SR*,6*RS*,7*RS*)-(±)-4-Dimethylamino-6,7-diphenylbicyclo[2.2.2]octan-2-ol (1a). The corresponding ketone (10 g, 31 mmol) and $NaBH_4$ (2.35 g, 62 mmol) in dry MeOH (80 mL) gave an oily residue. Recrystallization from EtOH/H_2O yielded **1a**[13] (9.5 g, 30 mmol, 94%) as a white powder.

5.2.1.2. (2*SR*,6*RS*,7*RS*)-(±)-4-Pyrrolidino-6,7-diphenylbicyclo[2.2.2]octan-2-ol (1b). The corresponding ketone (5 g, 14 mmol) and $NaBH_4$ (1.06 g, 28 mmol) in dry MeOH (40 mL) gave an oily residue. Recrystallization from EtOH/H_2O yielded **1b**[13] (4.52 g, 13 mmol, 93%) as a white powder.

5.2.1.3. (2*SR*,6*RS*,7*RS*)-(±)-4-Piperidino-6,7-diphenylbicyclo[2.2.2]octan-2-ol (1c). The corresponding ketone (9 g, 25 mmol) and $NaBH_4$ (1.89 g, 50 mmol) in dry MeOH (80 mL) gave an oily residue. Recrystallization from EtOH/H_2O yielded **1c**[13] (8.37 g, 23 mmol, 93%) as a white powder.

5.2.2. (2*SR*,6*RS*,7*RS*)-(±)-4-Dialkylamino-6,7-diphenylbicyclo[2.2.2]octan-2-yl pivalates (2a–2c), (2*SR*,6*RS*,7*RS*)-(±)-4-dialkylamino-6,7-diphenylbicyclo[2.2.2]octan-2-yl acetates (3a–3c) and (2*SR*,6*RS*,7*RS*)-(±)-4-dialkylamino-6,7-diphenylbicyclo[2.2.2]octan-2-yl piperonylates (4a–4c). The synthesis of **2a**–**2c**, **3a**–**3c** and **4a**–**4c** has already been reported.[14,16]

5.2.3. General procedure for the synthesis of (2*SR*,6*RS*,7*RS*)-(±)-4-dialkylamino-6,7-diphenylbicyclo[2.2.2]-octan-2-yl cinnamoates (5a–5c). The bicyclo-octanols **1** and 4-DMAP were dissolved in CH_2Cl_2 and cooled with an icebath. Under stirring the cinnamoyl chloride in 2 mL CH_2Cl_2 was added. After 1 h the icebath was removed and the solution was stirred overnight at room temperature in an argon atmosphere. Then it was shaken five times with water, another five times with 2 N NaOH, washed four times with water, dried over sodium sulfate and filtered. The solvent was removed in vacuo and the residue was purified by crystallization.

5.2.3.1. (2*SR*,6*RS*,7*RS*)-(±)-4-Dimethylamino-6,7-diphenylbicyclo[2.2.2]octan-2-yl cinnamoate (5a). Compound **1a** (0.321 g, 1 mmol), (*E*)-cinnamoyl chloride (0.333 g, 2 mmol) and 4-DMAP (0.244 g, 2 mmol) in dry CH_2Cl_2 (10 mL) yielded **5a** (0.408 g, 0.9 mmol) as an oily residue, which was recrystallized from MeOH. Mp 126 °C; yield: 90%. IR (KBr) 2940, 1698, 1601, 1497, 1447, 1299, 1279, 1203, 1169, 1045, 1020, 985, 771, 743, 698 cm^{-1}. UV (CH_2Cl_2, nm, (log ε)): 279 (4.331), 228 (3.587). ^1H NMR ($CDCl_3$, 400 MHz) δ (ppm) 1.78 (dd, *J* = 14.0, 1.6 Hz, 1H, 3-H), 1.94 (ddd, *J* = 12.2, 9.3, 2.5 Hz, 1H, 5-H), 2.07–2.14 (m, 2H, 5-H, 8-H), 2.17 (ddd, *J* = 13.9, 9.0, 3.2 Hz, 1H, 3-H), 2.27 (ddd, *J* = 12.7, 9.9, 3.2 Hz, 1H, 8-H), 2.41 (s, 6H, $N(CH_3)_2$), 2.85 (d, *J* = 4.2 Hz, 1H, 1-H), 3.07 (t, *J* = 9.4 Hz, 1H, 6-H), 3.25 (t, *J* = 9.8 Hz, 1H, 7-H), 5.39 (dd, *J* = 8.8, 4.6 Hz, 1H, 2-H), 5.80 (d, *J* = 15.9 Hz, 1H, CH–CO), 6.94 (d, *J* = 15.9 Hz, 1H, CH–Ph), 7.00–7.43 (m, 15H, Ar-H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm) 31.28 (C-5), 31.90 (C-8), 34.07 (C-7), 35.14 (C-3), 38.43 ($N(CH_3)_2$), 38.80 (C-6), 39.50 (C-1), 56.30 (C-4), 73.05 (C-2), 117.97 (*C*H–CO), 125.28, 126.41, 126.58, 127.39, 127.90, 127.94, 128.55, 128.64, 129.92, 134.47, 142.87 (aromatic C), 143.81 (CH–Ph), 144.68 (aromatic C), 166.28 (COO). Anal. Calcd for $C_{31}H_{33}NO_2$: C, 82.45; H, 7.37; N, 3.10. Found: C, 82.56; H, 7.20; N, 3.03.

5.2.3.2. (2*SR*,6*RS*,7*RS*)-(±)-6,7-Diphenyl-4-pyrrolidinobicyclo[2.2.2]octan-2-yl cinnamoate (5b). Compound **1b** (0.348 g, 1 mmol), (*E*)-cinnamoyl chloride (0.333 g, 2 mmol) and 4-DMAP (0.244 g, 2 mmol) in dry CH_2Cl_2 (10 mL) yielded **5b** (0.474 g, 1 mmol) as an oily residue, which was recrystallized from EtOH. Mp 96 °C; yield: 99%. IR (KBr) 2967, 2934, 1697, 1601, 1496, 1449, 1347, 1298, 1280, 1205, 1164, 1016, 984, 773, 750, 705, 696 cm^{-1}. UV (CH_2Cl_2, nm, (log ε)): 278 (4.334), 230 (3.561). ^1H NMR ($CDCl_3$, 400 MHz) δ (ppm) 1.80 (dd, *J* = 14.4, 2.3 Hz, 1H, 3-H), 1.81–1.86 (m, 4H, $(CH_2)_2$), 2.00 (ddd, *J* = 12.0, 9.0, 2.3 Hz, 1H, 5-H), 2.08–2.15 (m, 1H, 5-H), 2.16–2.33 (m, 3H, 3-H, 8-H), 2.74–2.84 (m, 4H, $N(CH_2)_2$), 2.83 (d, *J* = 4.7 Hz, 1H, 1-H), 3.10 (t, *J* = 9.4 Hz, 1H, 6-H), 3.27 (t, *J* = 9.7 Hz, 1H, 7-H), 5.38 (dd, *J* = 8.8, 4.7 Hz, 1H, 2-H), 5.79 (d, *J* = 16.0 Hz, 1H, CH–CO), 6.93 (d, *J* = 16.0 Hz, 1H, CH–Ph), 6.99 (t, *J* = 7.0 Hz, 1H, p-Ar-H), 7.16–7.44 (m, 14H, Ar-H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm) 23.55 ($(CH_2)_2$), 31.63 (C-5), 33.02 (C-8), 33.96 (C-7), 36.18 (C-3), 38.90 (C-6), 39.83 (C-1), 45.54 ($N(CH_2)_2$), 54.87 (C-4), 73.10 (C-2), 117.99 (*C*H–CO), 125.21, 126.35, 126.61, 127.44, 127.88, 127.89, 128.51, 128.62, 129.87, 134.46, 142.96 (aromatic C), 143.73 (CH–Ph), 144.78 (aromatic C), 166.27 (COO). Anal. Calcd for $C_{33}H_{35}NO_2$: C, 82.98; H, 7.39; N, 2.93. Found: C, 82.68; H, 7.60; N, 2.84.

5.2.3.3. (2*SR*,6*RS*,7*RS*)-(±)-6,7-Diphenyl-4-piperidinobicyclo[2.2.2]octan-2-yl cinnamoate (5c). Compound **1c** (0.362 g, 1 mmol), (*E*)-cinnamoyl chloride (0.333 g, 2 mmol) and 4-DMAP (0.244 g, 2 mmol) in dry CH_2Cl_2 (10 mL) yielded **5c** (0.464 g, 0.9 mmol) as an oily residue, which was recrystallized from EtOH. Mp 108–109 °C; yield: 94%. IR (KBr) 2938, 1696, 1601, 1496, 1449, 1281, 1205, 1168, 1012, 982, 745, 699 cm^{-1}. UV (CH_2Cl_2, nm, (log ε)): 277 (4.331), 230 (3.679). ^1H NMR ($CDCl_3$, 400 MHz) δ (ppm) 1.45–1.53 (m, 2H, CH_2), 1.61–1.69 (m, 4H, $2CH_2$), 1.79 (dd, *J* = 14.2, 1.6 Hz, 1H, 3-H), 1.93 (ddd, *J* = 12.3, 9.5, 2.3 Hz, 1H, 5-H), 2.08–2.15 (m, 2H, 5-H, 8-H), 2.31 (ddd, *J* = 12.3, 9.8, 2.8 Hz, 1H, 8-H), 2.59–2.75 (m, 4H, $N(CH_2)_2$), 2.85 (d, *J* = 4.4 Hz, 1H, 1-H), 3.04 (t, *J* = 9.5 Hz, 1H, 6-H), 3.23 (t, *J* = 9.9 Hz, 1H, 7-H), 5.38 (dd, *J* = 8.8, 4.6 Hz, 1H, 2-H), 5.79 (d, *J* = 16.0 Hz, 1H, CH–CO), 6.92 (d, *J* = 16.0 Hz, 1H, CH–Ph), 6.97–7.43 (m, 15H, Ar-H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm) 24.96 (CH_2), 26.81 ($2CH_2$), 31.33 (C-5), 32.81 (C-8), 34.10 (C-7), 35.61 (C-3), 38.78 (C-6), 39.48 (C-1), 46.87 ($N(CH_2)_2$), 56.78 (C-4), 73.14 (C-2), 118.01 (*C*H–CO), 125.21, 126.33, 126.56, 127.39, 127.87, 127.90, 128.50, 128.62, 129.87, 134.48, 143.00 (aromatic C), 143.71 (CH–Ph), 144.86 (aromatic

C), 166.26 (COO). Anal. Calcd for $C_{34}H_{37}NO_2$: C, 83.06; H, 7.59; N, 2.85. Found: C, 83.10; H, 7.46; N, 2.82.

5.2.4. General procedure for the synthesis of (2*SR*,6*RS*,7*RS*)-(±)-4-dialkylamino-6,7-diphenylbicyclo[2.2.2]octan-2-yl 2′-chloroacetates (6a–6c). The bicyclo-octanols **1** and 4-DMAP were dissolved in dry CH_2Cl_2 (35 mL) and cooled with an icebath. Under stirring the chloroacyl chloride in dry CH_2Cl_2 (2 mL) was added. After 1 h the icebath was removed and the solution was stirred overnight at room temperature in an argon atmosphere. Then it was carefully shaken five times with water, dried over sodium sulfate and filtered. The solvent was removed in vacuo. The esters **6a**–**6c** were converted into **7a**–**7c**, **8a**–**8c**, **9a**–**9c** without further purification.

5.2.4.1. (2*SR*,6*RS*,7*RS*)-(±)-6,7-Diphenyl-4-dimethylaminobicyclo[2.2.2]octan-2-yl 2′-chloroacetate (6a). Compound **1a** (1.93 g, 6 mmol), chloroacetyl chloride (1.36 g, 12 mmol) and 4-DMAP (1.47 g, 12 mmol) yielded **6a** (1.64 g, 3.5 mmol, 69%) as an oily residue.

5.2.4.2. (2*SR*,6*RS*,7*RS*)-(±)-6,7-Diphenyl-4-pyrrolidinobicyclo[2.2.2]octan-2-yl 2′-chloroacetate (6b). Compound **1b** (2.09 g, 6 mmol), chloroacetyl chloride (1.36 g, 12 mmol) and 4-DMAP (1.47 g, 12 mmol) yielded **6b** (1.48 g, 3.5 mmol, 58%) as an oily residue.

5.2.4.3. (2*SR*,6*RS*,7*RS*)-(±)-6,7-Diphenyl-4-piperidinobicyclo[2.2.2]octan-2-yl 2′-chloroacetate (6c). Compound **1c** (2.17 g, 6 mmol), chloroacetyl chloride (1.36 g, 12 mmol) and 4-DMAP (1.47 g, 12 mmol) yielded **6c** (1.93 g, 4.4 mmol, 73%) as an oily residue.

5.2.5. General procedure for the synthesis of (2*SR*,6*RS*,7*RS*)-(±)-4-dialkylamino-6,7-diphenylbicyclo[2.2.2]octan-2-yl 2′-dialkylaminoacetates (7–9). Compounds **6a**–**6c** were dissolved in excess secondary amine and a catalytical amount of KI in H_2O was added. Then the solution was cooled to 4 °C for 24 h. After that the amine was removed in vacuo and the residue was shaken five times with CH_2Cl_2/H_2O, dried over sodium sulfate and filtered. Then the solvent was removed in vacuo and the residue was purified by crystallization or by means of CC. Alternatively hydrochlorides were afforded by treatment of the acetone solution of the residue with equivalent amounts of a 1 M solution of hydrogen chloride in diethyl ether. The precipitate was sucked off and washed with a mixture of ethyl acetate and EtOH.

5.2.5.1. (2*SR*,6*RS*,7*RS*)-(±)-6,7-Diphenyl-4-dimethylaminobicyclo[2.2.2]octan-2-yl 2′-diethylaminoacetate (7a). Compound **6a** (0.359 g, 0.90 mmol) gave with diethylamine (2 mL) **7a** (0.267 g, 0.61 mmol) as an oily residue which was purified by means of CC over silica gel using CH_2Cl_2/EtOH (9:1) as eluent; yield: 68%. IR (KBr) 2968, 1721, 1601, 1497, 1380, 1203, 1173, 701 cm^{-1}. UV (MeOH, nm, ($\log\varepsilon$)): 259 (3.281), 209 (4.289). ^1H NMR ($CDCl_3$, 400 MHz) δ (ppm) 0.89 (t, J = 7.1 Hz, 6H, $2CH_3$), 1.82 (br d, J = 14.0 Hz, 1H, 3-H), 2.03 (br dd, J = 12.5, 9.2 Hz, 1H, 5-H), 2.15 (d, J = 17.2 Hz, CH–CO), 2.13–2.29 (m, 4H, 3-H, 5-H, 8-H), 2.40 (q, J = 7.1 Hz, 2H, $N(CH_2–CH_3)$), 2.41 (q, J = 7.1 Hz, 2H, $N(CH_2–CH_3)$), 2.50 (s, 6H, $N(CH_3)_2$), 2.72 (d, J = 17.2 Hz, CH–CO), 2.82 (d, J = 4.8 Hz, 1H, 1-H), 3.05 (t, J = 9.3 Hz, 1H, 6-H), 3.22 (t, J = 9.7 Hz, 1H, 7-H), 5.33 (dd, J = 8.9, 4.8 Hz, 1H, 2-H), 7.12–7.40 (m, 10H, Ar-H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm) 12.27 ($2CH_3$), 30.94 (C-8), 31.12 (C-5), 33.96 (C-7), 34.60 (C-3), 38.27 ($N(CH_3)_2$), 38.59 (C-6), 39.96 (C-1), 47.33 ($N(CH_2)_2$), 53.23 (CH_2–CO), 58.26 (C-4), 72.22 (C-2), 125.60, 126.47, 126.64, 127.33, 128.18, 128.66, 142.11, 143.95 (aromatic C), 170.80 (COO). Anal. Calcd for $C_{28}H_{38}N_2O_2$: C, 77.38; H, 8.81; N, 6.45. Found: C, 77.63; H, 8.89; N, 6.12.

5.2.5.2. (2*SR*,6*RS*,7*RS*)-(±)-6,7-Diphenyl-4-pyrrolidinobicyclo[2.2.2]octan-2-yl 2′-diethylaminoacetate (7b). Compound **6b** (0.319 g. 0.75 mmol) gave with diethylamine (2 mL) **7b** (0.234 g, 0.50 mmol) as an oily residue. For analytical and test purposes it was recrystallized from acetone. Mp 109-110 °C; yield: 66%. IR (KBr) 2964, 1742, 1600, 1498, 1193, 1170, 699 cm^{-1}. UV (MeOH, nm, ($\log\varepsilon$)): 259 (2.906), 209 (4.226). ^1H NMR ($CDCl_3$, 400 MHz) δ (ppm) 0.88 (t, J = 7.1 Hz, 6H, $2CH_3$), 1.74 (br d, J = 13.4 Hz, 1H, 3-H), 1.79–1.86 (m, 4H, $(CH_2)_2$), 1.94–2.01 (m, 1H, 5-H), 2.03–2.10 (m, 1H, 5-H), 2.15 (d, J = 17.4 Hz, 1H, CH–CO), 2.13–2.20 (m, 3H, 3-H, 8-H), 2.40 (q, J = 7.1 Hz, 4H, $N(CH_2–CH_3)_2$), 2.70 (d, J = 17.4 Hz, 1H, CH–CO), 2.74 (d, J = 4.4 Hz, 1H, 1-H), 2.74–2.82 (m, 4H, $N(CH_2)_2$), 3.04 (t, J = 9.4 Hz, 1H, 6-H), 3.19 (t, J = 9.9 Hz, 1H, 7-H), 5.31 (dd, J = 8.7, 4.4 Hz, 1H, 2-H), 7.09–7.42 (m, 10H, Ar-H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm) 12.33 ($2CH_3$), 23.57 ($CH_2)_2$, 31.75 (C-5), 32.17 (C-8), 33.98 (C-7), 35.81 (C-3), 38.90 (C-6), 40.48 (C-1), 45.54 ($N(CH_2)_2$), 47.31 ($N(CH_2–CH_3)_2$), 53.27 (CH_2–CO), 54.79 (C-4), 72.72 (C-2), 125.32, 126.37, 126.63, 127.48, 128.02, 128.52, 142.91, 144.75 (aromatic C), 170.98 (COO). HRMS (MALDI): calcd for $C_{30}H_{41}N_2O_2$ [MH^+]: 461.3168; found: 461.3194.

5.2.5.3. (2*SR*,6*RS*,7*RS*)-(±)-6,7-Diphenyl-4-piperidinobicyclo[2.2.2]octan-2-yl 2′-diethylaminoacetate (7c). Compound **6c** (0.183 g, 0.42 mmol) gave with diethylamine (2 mL) **7c** (0.157 g, 0.33 mmol) as an oily residue. For analytical and test purposes it was recrystallized from acetone. Mp 130 °C; yield: 78%. IR (KBr) 2969, 1742, 1600, 1498, 1447, 1356, 1193, 697 cm^{-1}. UV (CH_2Cl_2, nm, ($\log\varepsilon$)): 259 (2.795), 231 (3.292). ^1H NMR ($CDCl_3$, 400 MHz) δ (ppm) 0.88 (t, J = 7.2 Hz, 6H, $2CH_3$), 1.45–1.53 (m, 2H, CH_2), 1.61–1.69 (m, 4H, $2CH_2$), 1.75 (br d, J = 14.0 Hz, 1H, 3-H), 1.94 (br dd, 12.6, 9.3 Hz, 1H, 5-H), 2.04–2.12 (m, 4H, 2-H, 3-H, 5-H, 8-H), 2.13 (d, J = 17.4 Hz, 1H, CH–CO), 2.20 (br dd, J = 12.0, 9.5 Hz, 1H, 8-H), 2.39 (q, J = 7.2 Hz, 4H, $N(CH_2–CH_3)_2$), 2.58–2.74 (m, 4H, $N(CH_2)_2$), 2.69 (d, J = 17.1 Hz, 1H, CH–CO), 2.76 (d, J = 4.1 Hz, 1H, 1-H), 2.98 (t, J = 9.6 Hz, 1H, 6-H), 3.15 (t, J = 9.7 Hz, 1H, 7-H), 5.30 (dd, J = 8.8, 4.8 Hz, 1H, 2-H), 7.08–7.41 (m, 10H, Ar-H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm) 12.32 ($2CH_3$), 24.96 (CH_2), 26.80 ($2CH_2$), 31.46 (C-5), 31.96 (C-8), 34.07 (C-7), 35.07 (C-3), 38.75 (C-6), 40.14 (C-1), 46.88 ($N(CH_2)_2$), 47.30 ($N(CH_2–$

$CH_3)_2$), 53.24 (CH_2–CO), 56.77 (C-4), 72.72 (C-2), 125.31, 126.35, 126.55, 127.42, 128.02, 128.50, 142.90, 144.79 (aromatic C), 170.96 (COO). HRMS (MALDI): calcd for $C_{31}H_{43}N_2O_2$ [MH^+]: 475.3325; found: 475.3411.

5.2.5.4. (2*SR*,6*RS*,7*RS*)-(±)-6,7-Diphenyl-4-dimethylaminobicyclo[2.2.2]octan-2-yl 2′-piperidinoacetate (8a). **6a** (0.324 g, 0.81 mmol) gave with piperidine (2 mL) **8a** (0.285 g, 0.64 mmol) as an oily residue which was purified by means of CC over aluminium oxide using CH_2Cl_2/EtOH (14:1) as eluent; yield: 79%. IR (KBr) 2937, 1744, 1602, 1166, 1130, 698 cm^{-1}. UV (MeOH, nm, (log ε)): 259 (3.324), 209 (4.336). ^1H NMR ($CDCl_3$, 400 MHz) δ (ppm) 1.32–1.39 (m, 2H, CH_2), 1.47–1.54 (m, 4H, $2CH_2$), 1.73 (dd, J = 14.0, 1.8 Hz, 1H, 3-H), 1.90 (ddd, J = 12.5, 9.3, 1.6 Hz, 1H, 5-H), 1.98 (d, J = 17.2 Hz, 1H, CH–CO), 2.02–2.10 (m, 3H, 3-H, 5-H, 8-H), 2.12–2.22 (m, 1H, 8-H), 2.19–2.24 (m, 4H, $N(CH_2)_2$), 2.38 (s, 6H, $N(CH_3)_2$), 2.59 (d, J = 17.2 Hz, 1H, CH–CO), 2.78 (d, J = 4.4 Hz, 1H, 1-H), 3.01 (t, J = 9.3 Hz, 1H, 6-H), 3.16 (t, J = 9.8 Hz, 1H, 7-H), 5.30 (dd, J = 9.0, 4.7 Hz, 1H, 2-H), 7.08–7.41 (m, 10H, Ar-H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm) 23.79 (CH_2), 25.70 ($2CH_2$), 31.08 (C-5), 31.16 (C-8), 33.94 (C-7), 34.67 (C-3), 38.37 ($N(CH_3)_2$), 38.67 (C-6), 39.98 (C-1), 53.74 ($N(CH_2)_2$), 56.13 (C-4), 58.82 (CH_2–CO), 72.74 (C-2), 125.28, 126.36, 126.51, 127.35, 128.05, 128.49, 142.73, 144.58 (aromatic C), 170.14 (COO). HRMS (MALDI): calcd for $C_{29}H_{39}N_2O_2$ [MH^+]: 447.3012; found: 447.3041.

5.2.5.5. (2*SR*,6*RS*,7*RS*)-(±)-6,7-Diphenyl-4-pyrrolidinobicyclo[2.2.2]octan-2-yl 2′-piperidinoacetate (8b). Compound **6b** (0.349 g, 0.82 mmol) gave with piperidine (2 mL) **8b** (0.320 g, 0.68 mmol) as an oily residue which was converted to its hydrochloride. Mp (HCl: ethyl acetate/EtOH) 182 °C; yield: 83%. IR (KBr) 2954, 1750, 1601, 1448, 1208, 1192, 702 cm^{-1}. UV (MeOH, nm, (log ε)): 259 (2.835), 237 (2.646). ^1H NMR ($CDCl_3$, 400 MHz) δ (ppm) 1.33–1.38 (m, 2H, CH_2), 1.47–1.54 (m, 4H, $2CH_2$), 1.74 (br d, J = 13.5 Hz, 1H, 3-H), 1.81–1.85 (m, 4H, $2CH_2$), 1.98 (d, J = 17.0 Hz, 1H, CH–CO), 1.95–2.01 (m, 1H, 5-H), 2.04–2.23 (m, 8H, 3-H, 5-H, 8-H, $N(CH_2)_2$), 2.58 (d, J = 17.0 Hz, 1H, CH–CO), 2.75 (d, J = 4.4 Hz, 1H, 1-H), 2.74–2.83 (m, 4H, $N(CH_2)_2$), 3.04 (t, J = 9.4 Hz, 1H, 6-H), 3.19 (t, J = 9.7 Hz, 1H, 7-H), 5.30 (dd, J = 8.6, 4.4 Hz, 1H, 2-H), 7.07–7.42 (m, 10H, Ar-H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm) 23.58 ($(CH_2)_2$), 23.86 (CH_2), 25.78 ($2CH_2$), 31.70 (C-5), 32.17 (C-8), 33.96 (C-7), 35.72 (C-3), 38.86 (C-6), 40.42 (C-1), 45.56 ($N(CH_2)_2$), 53.77 ($N(CH_2)_2$), 54.87 (C-4), 58.87 (CH_2–CO), 72.80 (C-2), 125.29, 126.38, 126.63, 127.46, 128.07, 128.52, 142.87, 144.71 (aromatic C), 170.19 (COO). HRMS (MALDI): calcd for $C_{31}H_{41}N_2O_2$ [MH^+]: 473.3168; found: 473.3182.

5.2.5.6. (2*SR*,6*RS*,7*RS*)-(±)-6,7-Diphenyl-4-piperidinobicyclo[2.2.2]octan-2-yl 2′-piperidinoacetate (8c). Compound **6c** (0.497 g, 1.12 mmol) gave with piperidine (2 mL) **8c** (0.328 g, 0.67 mmol) as an oily residue. For analytical and test purposes it was recrystallized from EtOH. Mp 146 °C; yield: 60%. IR (KBr) 2975, 1746, 1600, 1445, 1286, 1192, 1170, 697 cm^{-1}. UV (MeOH, nm, (log ε)): 259 (2.880), 210 (4.201). ^1H NMR ($CDCl_3$, 400 MHz) δ (ppm) 1.31–1.38 (m, 2H, CH_2), 1.44–1.53 (m, 6H, $3CH_2$), 1.61–1.68 (m, 4H, $(CH_2)_2$), 1.74 (dd, J = 14.0, 1.8 Hz, 1H, 3-H), 1.91 (br t, J = 10.6 Hz, 1H, 5-H), 1.96 (d, J = 6.8 Hz, 1H, CH–CO), 2.03–2.13 (m, 3H, 3-H, 5-H, 8-H), 2.18–2.26 (m, 5H, 8-H, $N(CH_2)_2$), 2.57 (d, J = 6.8 Hz, 1H, CH–CO), 2.58–2.74 (m, 4H, $N(CH_2)_2$), 2.77 (d, J = 4.3 Hz, 1H, 1-H), 2.98 (br t, J = 9.5 Hz, 1H, 6-H), 3.14 (br t, J = 9.8 Hz, 1H, 7-H), 5.30 (dd, J = 9.1, 4.4 Hz, 1H, 2-H), 7.07-7.41 (m, 10H, Ar-H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm) 23.87 (CH_2), 24.98 (CH_2), 25.79 ($2CH_2$), 26.82 ($2CH_2$), 31.43 (C-5), 32.01 (C-8), 34.06 (C-7), 35.02 (C-3), 38.73 (C-6), 40.11 (C-1), 46.89 ($N(CH_2)_2$), 53.76 ($N(CH_2)_2$), 56.75 (C-4), 58.85 (CH_2–CO), 72.84 (C-2), 125.26, 126.34, 126.55, 127.40, 128.05, 128.49, 142.89, 144.78 (aromatic C), 170.15 (COO). Anal. Calcd for $C_{32}H_{42}N_2O_2$: C, 78.97; H, 8.70; N, 5.76. Found: C, 78.68; H, 9.00; N, 5.60.

5.2.5.7. (2*SR*,6*RS*,7*RS*)-(±)-6,7-Diphenyl-4-dimethylaminobicyclo[2.2.2]octan-2-yl 2′-pyrrolidinoacetate (9a). Compound **6a** (0.307 g, 0.77 mmol) gave with pyrrolidine (2 mL) **9a** (0.304 g, 0.70 mmol) as an oily residue which was purified by means of CC over aluminium oxide using CH_2Cl_2/EtOH (14:1) as eluent; yield: 91%. IR (KBr) 2953, 1747, 1602, 1447, 1184, 698 cm^{-1}. UV (MeOH, nm, (log ε)): 259 (2.976), 210 (4.197). ^1H NMR ($CDCl_3$, 400 MHz) δ (ppm) 1.69–1.76 (m, 5H, 3-H, $(CH_2)_2$), 1.90 (dd, J = 12.0, 9.2 Hz, 1H, 5-H), 2.02–2.10 (m, 4H, 3-H, 5-H, 8-H, CH–CO), 2.18 (ddd, J = 9.9, 9.1, 2.6 Hz, 1H, 8-H), 2.35–2.42 (m, 4H, $N(CH_2)_2$), 2.39 (s, 6H, $N(CH_3)_2$), 2.74 (d, J = 17.2 Hz, 1H, CH–CO), 2.81 (d, J = 4.0 Hz, 1H, 1-H), 3.01 (t, J = 9.2 Hz, 1H, 6-H), 3.17 (t, J = 9.9 Hz, 1H, 7-H), 5.31 (dd, J = 8.8, 4.0 Hz, 1H, 2-H), 7.08–7.41 (m, 10H, Ar-H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm) 23.68 ($(CH_2)_2$), 31.16 (C-5), 31.26 (C-8), 33.89 (C-7), 34.62 (C-3), 38.39 ($N(CH_3)_2$), 38.63 (C-6), 39.77 (C-1), 53.42 ($N(CH_2)_2$), 55.34 (CH_2–CO), 56.17 (C-4), 72.81 (C-2), 125.27, 126.39, 126.47, 127.36, 128.09, 128.52, 142.76, 144.63 (aromatic C), 170.42 (COO). Anal. Calcd for $C_{28}H_{36}N_2O_2$: C, 77.74; H, 8.39; N, 6.48. Found: C, 77.51; H, 8.49; N, 6.20.

5.2.5.8. (2*SR*,6*RS*,7*RS*)-(±)-6,7-Diphenyl-4-pyrrolidinobicyclo[2.2.2]octan-2-yl 2′-pyrrolidinoacetate (9b). Compound **6b** (0.397 g, 0.94 mmol) gave with pyrrolidine (2 mL) **9b** (0.309 g, 0.67 mmol) as an oily residue which was converted to its hydrochloride. Mp (HCl: ethyl acetate/EtOH) 190 °C; yield: 72%. IR (KBr) 2961, 1750, 1599, 1452, 1378, 1223, 1137, 702 cm^{-1}. UV (CH_2Cl_2, nm, (log ε)): 259 (2.856), 237 (2.684). ^1H NMR ($CDCl_3$, 400 MHz) δ (ppm) 1.68–1.74 (m, 4H, $(CH_2)_2$), 1.75 (br dd, J = 14.0, 2.0 Hz, 1H, 3-H), 1.82–1.86 (m, 4H, $(CH_2)_2$), 1.98 (br t, J = 12.4 Hz, 1H, 5-H), 2.08 (d, J = 17.2 Hz, 1H, CH—CO), 2.04–2.22 (m, 4H, 3-H, 5-H, 8-H), 2.34–2.40 (m, 4H, $N(CH_2)_2$), 2.73 (d, J = 17.0 Hz, 1H, CH–CO), 2.74-2.83 (m, 4H, $N(CH_2)_2$),

2.78 (d, $J = 4.5$ Hz, 1H, 1-H), 3.04 (t, $J = 9.5$ Hz, 1H, 6-H), 3.19 (t, $J = 9.9$ Hz, 1H, 7-H), 5.32 (dd, $J = 9.0$, 4.5 Hz, 1H, 2-H), 7.08–7.42 (m, 10H, Ar-H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm) 23.58 ($(CH_2)_2$), 23.74 ($(CH_2)_2$), 31.79 (C-5), 32.21 (C-8), 33.89 (C-7), 35.64 (C-3), 38.78 (C-6), 40.20 (C-1), 45.59 ($N(CH_2)_2$), 53.44 ($N(CH_2)_2$), 54.96 (C-4), 55.35 (CH_2-CO), 72.83 (C-2), 125.27, 126.39, 126.56, 127.45, 128.09, 128.52, 142.84, 144.72 (aromatic C), 170.44 (COO). HRMS (MALDI): calcd for $C_{30}H_{39}N_2O_2$ [MH^+]: 459.3012; found: 459.3061.

5.2.5.9. (2*SR*,6*RS*,7*RS*)-(±)-6,7-Diphenyl-4-piperidinobicyclo[2.2.2]octan-2-yl 2′-pyrrolidinoacetate (9c). Compound **6c** (0.460 g, 1.05 mmol) gave with pyrrolidine (2 mL) **9c** (0.385 g, 0.81 mmol) as an oily residue. For analytical and test purposes it was recrystallized from EtOH. Mp 127 °C; yield: 77%. IR (KBr) 2935, 1744, 1600, 1497, 1281, 1185, 698 cm^{-1}. UV (CH_2Cl_2, nm, ($\log \varepsilon$)): 259 (2.774), 231 (3.349). ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm) 1.45–1.53 (m, 2H, CH_2), 1.60–1.78 (m, 9H, $2CH_2$, $(CH_2)_2$, 3-H), 1.91 (br t, 10.2 Hz, 1H, 5-H), 2.03-2.13 (m, 3H, 3-H, 5-H, 8-H), 2.06 (d, $J = 17.2$ Hz, 1H, CH—CO), 2.21 (br t, $J = 9.5$ Hz, 1H, 8-H), 2.33-2.40 (m, 4H, $N(CH_2)_2$), 2.59–2.74 (m, 4H, $N(CH_2)_2$), 2.71 (d, $J = 17.2$ Hz, 1H, CH–CO), 2.81 (d, $J = 4.6$ Hz, 1H, 1-H), 2.98 (br t, $J = 9.5$ Hz, 1H, 6-H), 3.15 (br t, $J = 9.9$ Hz, 1H, 7-H), 5.31 (dd, $J = 8,9$, 4.6 Hz, 1H, 2-H), 7.08–7.41 (m, 10H, Ar-H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm) 23.74 ($(CH_2)_2$), 24.98 (CH_2), 26.83 ($2CH_2$), 31.53 (C-5), 32.09 (C-8), 34.00 (C-7), 34.96 (C-3), 38.67 (C-6), 39.89 (C-1), 46.89 ($N(CH_2)_2$), 53.44 ($N(CH_2)_2$), 55.33 (CH_2–CO), 56.74 (C-4), 72.89 (C-2), 125.21, 126.32, 126.48, 127.38, 128.06, 128.48, 142.89, 144.81 (aromatic C), 170.41 (COO). Anal. Calcd for $C_{31}H_{40}N_2O_2$: C, 78.77; H, 8.53; N, 5.93. Found: C, 78.49; H, 8.76; N, 5.80.

5.3. Biological tests

5.3.1. In vitro microplate assay against *P. falciparum* K_1. Antiplasmodial activity was examined using the K_1 strain of *P. falciparum* (resistant to chloroquine and pyrimethamine). Viability is determined by the incorporation of [^{3}H]-hypoxanthine into living protozoal cells by a modification of a reported assay.[18] Briefly, infected human red blood cells in RPMI 1640 medium with 5% Albumax were exposed to serial drug dilutions ranging from 5 to 0.078 μg/mL in microtitre plates. After 48 h of incubation at 37 °C in a reduced oxygen atmosphere, 0.5 μCi ^{3}H-hypoxanthine was added to each well. Cultures were incubated for a further 24 h before they were harvested onto glass-fibre filters and washed with distilled water. The radioactivity was counted using a BetaplateTM liquid scintillation counter (Wallac, Zurich, Switzerland). The results were recorded as counts per minute (CPM) per well at each drug concentration and expressed as percentage of the untreated controls. From the sigmoidal inhibition curves IC_{50} values were calculated. Assays were run in duplicate and repeated once. Standard was artemisinin.

5.3.2. In vitro microplate assay against *T. brucei rhodesiense*, cytotoxicity. Minimum Essential Medium (50 μl) supplemented according to a known procedure with 2-mercaptoethanol and 15% heat-inactivated horse serum was added to each well of a 96-well microtitre plate.[19] Serial drug dilutions were prepared covering a range from 90 to 0.123 μg/mL. Then 10^4 bloodstream forms of *T. brucei rhodesiense* STIB 900 in 50 μl were added to each well and the plate incubated at 37 °C under a 5% CO_2 atmosphere for 72 h. Ten microliters of Alamar blue (containing 0.0125 g resazurin dissolved in 1000 mL distilled water) was then added to each well and incubation continued for a further 2–4 h. The Alamar blue dye is an indicator of cellular growth and/or viability. The blue, non-fluorescent, oxidized form becomes pink and fluorescent upon reduction by living cells. The plate was then read in a Spectramax Gemini XS microplate fluorometer (Molecular Devices Cooperation, Sunnyvale, CA, USA) using an excitation wavelength of 536 nm and emission wavelength of 588 nm.[20] Fluorescence development was measured and expressed as percentage of the control. Data were transferred into the graphic programme Softmax Pro (Molecular Devices) which calculated IC_{50} values. Melarsoprol served as standard. Cytotoxicity was assessed using the same assay and rat skeletal myoblasts (L-6 cells) with mefloquine as standard.

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