Synthesis of 2-azabicyclo[3.2.2]nonanes from bicyclo[2.2.2]octan-2-ones and their activities against *Trypanosoma brucei rhodesiense* and *Plasmodium falciparum* K_1

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ABSTRACT **Purpose:** New 2-azabicyclo[3.2.2]nonanes were prepared from antiprotozoal bicyclo[2.2.2]octan-2-ones to investigate the influence of the replacement of the rigid bicyclo-octane structure by the more flexible bicyclo-nonane system on the antiplasmodial and antitrypanosomal activity. **Methods:** The 2-azabicyclo[3.2.2]nonanes were synthesized via a one-step procedure from bicyclo[2.2.2]octan-2-ones and tested for their activities against *Trypanosoma b. rhodesiense* and *Plasmodium falciparum* K_1 (resistant to chloroquine and pyrimethamine) using in vitro microplate assays. **Results:** 2-azabicyclo[3.2.2]non-5-ylamines exhibit higher antiprotozoal activities than 4-aminobicyclo[2.2.2]octanes, 4-aminobicycl [2.2.2]octan-2-ones and 4-amino-2-azabicyclo[3.2.2]nonan-3-ones. (7, 8-Diphenyl-2-azabicyclo[3.2.2]non-5-yl)-dimethylamine shows enhanced anti-trypanosomal (IC_{50} = 0.60 μM) and remarkable antiplasmodial (IC_{50} = 0.28 μM) activity. However, the in vivo activity of this compound against *Plasmodium berghei* in mice is moderate. **Conclusions:** Due to their promising in vitro antiprotozoal activity and their low cytotoxicity, 2-azabicyclo[3.2.2]nonanes should serve as lead compounds for further modifications.

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INTRODUCTION

Trypanosoma brucei gambiense and *T. b. rhodesiense* are protozoan parasites causing Human African trypanosomiasis. About 0.5 million people are infected with this disease in central Africa and 50.000 of them die per year (1, 2). If untreated, the initial peripheral phase of the disease is always followed by a lethal CNS infection. Only four drugs, pentamidine, suramine, melarsoprol and eflornithine, are in use for treatment. Melarsoprol is active against all strains and stages of trypanosomes. Unfortunately, it causes the fatal side effect of encephalopathy in up to 5% of the patients (3). Eflornithine is ineffective against *T. b. rhodesiense* (4). Pentamidine and suramine are not able to cross the blood-brain barrier efficiently and therefore will not cure CNS infections (5). Furthermore, increasing resistance of trypanosomes against the above-mentioned drugs has been observed (6-9). Since only melarsoprol is active against CNS infection with the causative organism of East African Human trypanosomiasis, *T. b. rhodesiense*, there is an urgent need for new lipophilic antitrypanosomals.

Malaria is a parasitic disease of major global health significance that causes an estimated 2.7 million deaths each year (10). It is caused by four species and *Plasmodium falciparum* is the most virulent and potentially deadly. It is responsible for more than one million deaths in African children per year (11). Many of the antimalarial drugs are loosing their effectiveness in the face of multidrug-resistant strains of *Plasmodium* (12-14) so that traditional therapeutics such as chloroquine and pyrimethamine that were once highly effective, are almost useless in many parts of the world (15, 16). Even for the most recently introduced artemisinine derivatives loss of sensitivity has been observed (17-21). Hence that, there is great demand for new antimalarials which are active against resistant strains of *Plasmodium falciparum*.

Recently, we reported the synthesis of 4-aminobicyclo[2.2.2]octane-2-ones **1** from acyclic starting material (22) via a one-pot procedure, which could be advantageous for a later industrial synthetic process in large-scale. Those compounds and their reduction products **2** showed activity against *Plasmodium falciparum* K_1, which is resistant to chloroquine and pyrimethamine, and *Trypanosoma b. rhodesiense* (23,24). Now we present the synthesis of 2-azabicyclo[3.2.2]nonane derivatives **3** from **1** in one step and their reduction to bicyclic amines **4**. All new compounds were tested against *P. falciparum*

K_1, *T. b. rhodesiense* and for their cytotoxicity against L6 cells.

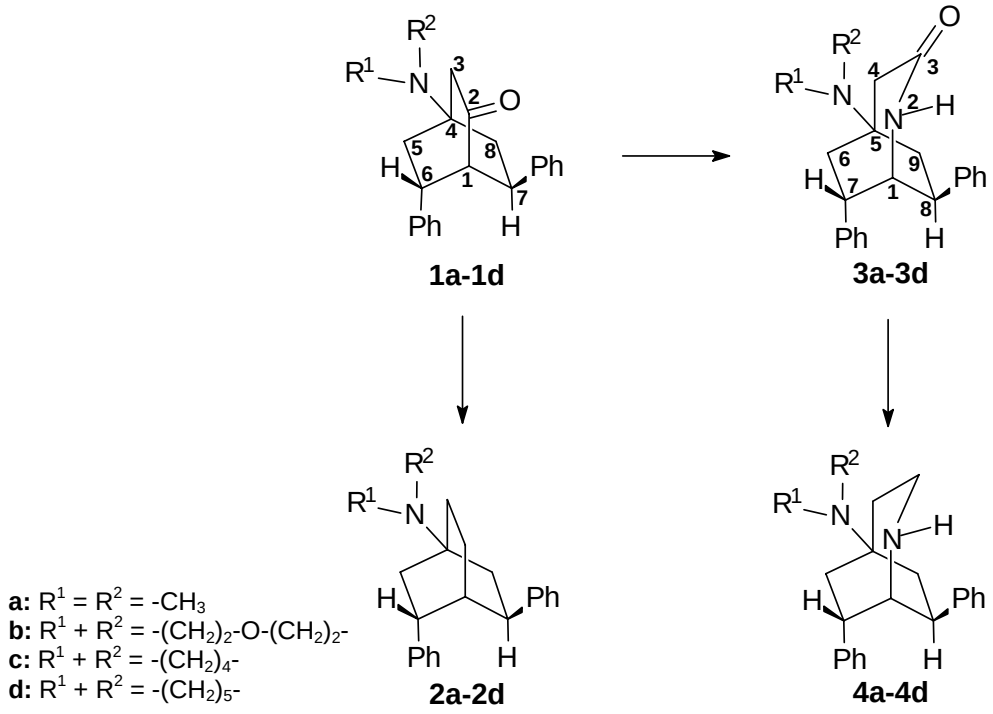
The tests were performed using in vitro microplate assays. The results are compared to the activities of the previously synthesized compounds.

MATERIAL AND METHODS

Melting points were obtained on a digital melting point apparatus Electrothermal IA 9200 and are uncorrected. IR spectra: infrared spectrometer system 2000 FT (Perkin Elmer). UV/VIS: Lambda 17 UV/VIS-spectrometer (Perkin Elmer). NMR spectra: Varian Inova 400 (300 K) 5 mm tubes, solvent resonance as internal standard. ^{1}H- and ^{13}C-resonances were assigned using ^{1}H,^{1}H- and ^{1}H,^{13}C-correlation spectra. ^{1}H- and ^{13}C-resonances are numbered as given in the formulas. MS, HR-MS: Kratos profile spectrometer 70 eV electron impact. GC-MS: HP-6890 (Hewlett-Packard) 70 eV electron impact. Microanalyses: EA 1108 CHNS-O apparatus (Carlo Erba), Microanalytical Laboratory at the Institute of Physical Chemistry, Vienna. Materials: column-chromatography (CC): 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 x 200 mm); the substances were detected in UV light at 254 nm.

Chemistry

Compounds **1** have been prepared from benzylidene acetone and dialkylammonium thiocyanates in one-pot reactions (22,23). Their reduction by a Wolff-Kishner procedure yielded the bicyclic amines **2** (24). The formation of the 2-azabicyclo[3.2.2]nonan-3-ones **3** succeeded by a Beckmann rearrangement of compounds **1** using hydroxylamine-O-sulfonic acid in glacial acetic acid. The 2-azabicyclo[3.2.2]nonanes **4** were prepared in good yields by the reduction of **3** with $LiAlH_4$ in ether. The 5-amino-2-azabicyclo[3.2.2]nonane derivatives **3** and **4** are representatives of a new class of compounds (Scheme).



Scheme: Preparation of **3**&**4**

Their structures were established by NMR spectroscopy. Compound **3a** was investigated thoroughly by 2D-NMR experiments. We observed a cross peak from N-H to 1-H in H,H-COSY measurements. The assignments were done with the aid of through-space interactions (NOE) and w-couplings.

Preparation of compounds 1a-1d and 2a-2d.
The synthesis of compounds **1** and **2** is briefly described above.

Preparation of compounds 3a-3d.
Ketones **1** were suspended in glacial acetic acid and hydroxylamine-O-sulfonic acid was added and the mixture refluxed over night at 145°C. The brown solution was poured on ice, alkalized with 2M NaOH and extracted 5 times with CH_2Cl_2. The combined organic layers were washed 3 times with water, dried over Na_2SO_4 and filtered. After evaporation of the solvent in vacuo, the residue was dissolved in the minimum amount of hot ethanol. The products **3** crystallized over night and were further purified.

(7RS,8RS)-(±)-5-Dimethylamino-7,8-diphenyl-2-azabicyclo[3.2.2]nonan-3-one **(3a)**. Compound **1a** (2.94 g, 9.2 mmol) and hydroxylamine-O-sulfonic acid (3.12 g, 27.6 mmol) in glacial acetic acid (35 ml) gave after workup a precipitate which was purified by means of CC, using CH_2Cl_2 : MeOH = 8:2 as eluent giving pure **3a** as a colourless resin. Yield: 1.2 g (39%); m.p. (ethanol): 212°C; IR (KBr, $\bar{\nu}$): 1646 (s, C=O), 754 (s, =CH, deform.), 702 (s, =CH deform.) cm^{-1}; UV (CH_2Cl_2, λ ($\log\varepsilon$)): 230 (3.121), 290 (2.012) nm; ^{1}H NMR ($CDCl_3$, δ, 400 MHz): 2.02 (ddd, *J* = 13.4, 11.3, 2.1 Hz, 1H, 6-H), 2.10 (ddd, *J* = 13.7, 10.6, 1.5 Hz, 1H, 9-H), 2.21 (ddd, *J* = 13.8, 8.7, 2.1 Hz, 1H, 9-H), 2.32 (s, 6H, $N(CH_3)_2$), 2.38 (ddd, *J* = 13.3, 8.1, 1.5 Hz, 1H, 6-H), 2.71 (dd, *J* = 17.8, 2.3 Hz, 1H, 4-H), 2.94 (d, *J* = 17.7 Hz, 1H, 4-H), 3.23 (d, *J* = 7.1 Hz, 1H, 1-H), 3.33 (br, t, *J* = 9.6 Hz, 1H, 8-H), 3.47 (dd, *J* = 11.3, 8.1 Hz, 1H, 7-H), 6.15 (d, *J* = 6.6 Hz, 1H, N-H), 7.22 - 7.41 (m, 10H, aromatic H) ppm; ^{13}C NMR ($CDCl_3$, δ, 100 MHz): 35.32 (C-9), 35.75 (C-6), 37.86 ($N(CH_3)_2$), 40.94 (C-4), 41.59 (C-8), 46.38 (C-7), 55.06 (C-5), 57.67 (C-1), 126.72, 127.05, 127.84, 128.63, 128.97, 141.96, 142.99 (aromatic C), 173.96 (C-3) ppm; MS (base, EI^+): *m/z* (%) = 334 (49.0) [M^+], 275 (100.0), 229 (32.3), 184 (42.2), 153 (24.6), 139 (14.8), 104 (12.9), 91 (23.0), 85 (60.2), 70 (25.0); Anal. Calcd for $C_{22}H_{26}N_2O$ (334.46): C 79.01, H 7.84, N 8.38; found: C 78.74, H 8.09, N 8.32; HRMS (EI+): calcd. ($C_{22}H_{26}N_2O$): 334.20451; found: 334.20446.

(7RS,8RS)-(±)-5-(1-Morpholinyl)-7,8-diphenyl-2-azabicyclo[3.2.2]nonan-3-one **(3b)**. Compound **1b** (3.6 g, 9.9 mmol) and hydroxylamine-O-sulfonic acid (3.5 g, 31 mmol) in glacial acetic acid (30 ml) gave after workup a precipitate. Yield: 2.3 g (62%). For analytical and biological test purposes the crystallizate was purified by means of CC, using CH_2Cl_2 : MeOH = 9:1 as eluent giving **3b** as a colourless resin.; m.p. (ethanol): 219°C; IR (KBr, $\bar{\nu}$): 1659 (s, C=O), 756 (m, =CH deform.), 704 (m, =CH deform) cm^{-1}; UV (CH_2Cl_2, λ ($\log\varepsilon$)): 231 (2.955) nm; ^{1}H NMR ($CDCl_3$, δ, 400 MHz): 1.98 (ddd, *J* = 13.6, 11.4, 1.7 Hz, 1H, 6-H), 2.05 (br, t, *J* = 11.9 Hz, 1H, 9-H), 2.19 (ddd, *J* = 13.4, 9.1, 1.7 Hz, 1H, 9-H), 2.32 (ddd, *J* = 12.9, 8.3, 1.5 Hz, 1H, 6-H), 2.55 - 2.71 (m, 5H, 4-H, $N(CH_2)_2$), 2.93 (d, *J* = 17.6 Hz, 1H, 4-H), 3.27 (d, *J* = 6.9 Hz, 1H, 1-H), 3.34 (br, t, *J* = 9.7 Hz, 1H, 8-H), 3.45 (dd, *J* = 11.4, 8.1 Hz, 1H, 7-H), 3.68 - 3.71 (m, 4H, $O(CH_2)_2$), 6.56 (d, *J* = 6.9 Hz, 1H, N-H), 7.22 - 7.40 (m, 10H, aromatic H) ppm; ^{13}C NMR ($CDCl_3$, δ, 100 MHz): 35.27 (C-9), 35.42 (C-6), 41.65 (C-8), 42.32 (C-4), 45.75 ($N(CH_2)_2$), 46.26 (C-7), 55.21 (C-5), 57.56 (C-1), 67.47 ($O(CH_2)_2$), 126.67, 127.09, 127.80, 128.66, 128.98, 141.83, 142.92 (aromatic C), 173.64 (C-3) ppm; MS (base, EI^+): *m/z* (%) = 376 (59.9) [M^+], 317 (100.0), 271 (27.2), 226 (23.3), 195 (21.0), 127 (24.1), 91 (27.2); Anal. Calcd for $C_{24}H_{28}N_2O_2$ (376.49): C 76.56, H 7.50, N 7.44; found: C 76.26, H 7.75, N 7.33; HRMS (EI+): calcd. ($C_{24}H_{28}N_2O_2$): 376.21508; found: 376.21536.

(7RS,8RS)-(±)-7,8-Diphenyl-5-(1-pyrrolidyl)-2-azabicyclo[3.2.2]nonan-3-one **(3c)**. Compound **1c** (3.2 g, 9.3 mmol) and hydroxylamine-O-sulfonic acid (3.2 g, 28 mmol) in glacial acetic acid (25 ml) gave after workup a precipitate. Yield: 2.1 g (63%). For analytical and biological test purposes further purification was done by means of CC, using CH_2Cl_2 : MeOH = 9:1 as eluent giving **3c** as a colourless resin. IR (KBr, $\bar{\nu}$): 1656 (s, C=O), 756 (s, =CH deform.), 700 (s, =CH, deform.) cm^{-1}; UV (CH_2Cl_2, λ ($\log\varepsilon$)): 230 (3.220) nm; ^{1}H NMR ($CDCl_3$, δ, 400 MHz): 1.78 (br, s, 4H, 2 CH_2), 2.10 (br, dd, *J* = 12.7, 11.7 Hz, 1H, 9-H), 2.16 (br, dd, *J* = 12.4, 12.1 Hz, 1H, 6-H), 2.26 - 2.35 (m, 2H, 6-H, 9-H), 2.68 - 2.78 (m, 4H, $N(CH_2)_2$), 2.81 (d, *J* = 18.0 Hz, 1H, 4-H), 2.95 (d, *J* = 17.7 Hz, 1H, 4-H), 3.24 (d, *J* = 7.1 Hz, 1H, 1-H), 3.34 (br, t, *J* = 9.6 Hz, 1H, 8-H), 3.48 (dd, *J* = 11.4, 8.4 Hz, 1H, 7-H), 6.53 (d, *J* = 7.1 Hz, 1H, N-H), 7.19 - 7.40 (m, 10H, aromatic H) ppm; ^{13}C NMR ($CDCl_3$, δ, 100 MHz): 23.67 ($2CH_2$), 35.09 (C-9), 37.04 (C-6), 41.48 (C-8), 42.58 (C-4), 45.19 ($N(CH_2)_2$), 46.42 (C-7), 54.16 (C-5), 57.81 (C-1), 126.73, 127.01, 127.83, 128.61, 128.95, 142.00, 143.06 (aromatic C), 174.14 (C-3) ppm; MS (base, EI^+): *m/z* (%) = 360 (31.1) [M^+], 301 (100.0), 255 (23.3), 199 (21.0),

179 (16.3), 111 (35.0), 91 (19.5); Anal. Calcd for $C_{24}H_{28}N_2O$ (360.49): C 79.96, H 7.83, N 7.77; found: C 79.72, H 8.10, N 7.66; HRMS (EI+): calcd. ($C_{24}H_{28}N_2O$): 360.22016; found: 360.31889.

(7RS,8RS)-(±)-7,8-Diphenyl-5-(1-piperidyl)-2-azabicyclo[3.2.2]nonan-3-one **(3d)**. Compound **1d** (3.9 g, 10.7 mmol) and hydroxylamine-O-sulfonic acid (3.7 g, 32.7 mmol) in glacial acetic acid (30 ml) gave after workup a precipitate. Yield: 2.18 g (53%). For analytical and biological test purposes further purification was done by means of CC, using CH_2Cl_2 : MeOH = 9:1 as eluent giving pure **3d** as a colourless resin. m.p. (ethanol): 204°C; IR (KBr, $\tilde{\nu}$): 1656 (s, C=O), 754 (m, =CH deform.), 699 (s, =CH deform.) cm^{-1}; UV (CH_2Cl_2, λ (logε)): 230 (3.309) nm; ^{1}H NMR ($CDCl_3$, δ, 400 MHz): 1.42 - 1.50 (m, 2H, CH_2), 1.59 (br, s, 4H, $2CH_2$), 2.00 (br, dd, *J* = 12.3, 12.1 Hz, 1H, 6-H), 2.10 (br, dd, *J* = 13.1, 10.9 Hz, 1H, 9-H), 2.21 (br, dd, *J* = 13.1, 8.6 Hz, 1H, 9-H), 2.40 (br, dd, *J* = 13.1, 8.0 Hz, 1H, 6-H), 2.50 - 2.68 (m, 4H, $N(CH_2)_2$), 2.68 (d, *J* = 18.0 Hz, 1H, 4-H), 2.94 (d, *J* = 17.5 Hz, 1H, 4-H), 3.24 (d, *J* = 6.9 Hz, 1H, 1-H), 3.32 (br, t, *J* = 9.5 Hz, 1H, 8-H), 3.43 (dd, *J* = 11.5, 8.0 Hz, 1H, 7-H), 6.46 (d, *J* = 6.7 Hz, 1H, N-H), 7.19 - 7.38 (m, 10H, aromatic H) ppm; ^{13}C NMR ($CDCl_3$, δ, 100 MHz): 24.92 (CH_2), 26.72 ($2CH_2$), 35.53 (C-6), 36.14 (C-9), 41.74 (C-8), 41.92 (C-4), 46.36 ($N(CH_2)_2$), 46.41 (C-7), 55.55 (C-5), 57.71 (C-1), 126.75, 127.01, 127.83, 128.62, 128.93, 142.08, 143.10 (aromatic C), 174.15 (C-3) ppm; MS (base, EI^+): *m/z* (%) = 374 (43.6) [M^+], 315 (100.0), 283 (18.7), 269 (27.2), 224 (22.6), 193 (19.5), 125 (21.8), 91 (17.9); Anal. Calcd for $C_{25}H_{30}N_2O$ (374.52): C 80.17, H 8.07, N 7.48; found: C 79.95, H 8.14, N 7.42; HRMS (EI+): calcd. ($C_{25}H_{30}N_2O$): 374.23581; found: 374.23665.

Preparation of compounds 4a-4d.

5-Dialkylamino-7,8-diphenyl-2-azabicyclo[3.2.2]nonan-3-ones **3** were suspended in dry ether. After cooling on an ice bath, $LiAlH_4$ was added in portions. The reaction mixture was refluxed at 55°C over night. After cooling to room temperature, the reaction was cooled with an ice bath and quenched carefully with ice water and 2M NaOH. The mixture was extracted 5 times with ether, the combined organic layers were washed 3 times with water, dried over sodium sulfate, filtered and the solvent evaporated. The remaining brownish oil was purified by distillation giving pure **4a-4d** as oils. The dihydrochlorides were prepared by treatment of a solution of **4** in dichloromethane with etheral HCl (2M) and subsequent evaporation of the solvents in vacuo. The residues crystallized from ethanol/ethyl acetate.

(7RS,8RS)-(±)-(7,8-Diphenyl-2-azabicyclo[3.2.2]non-5-yl)-dimethylamine **(4a)**. Compound **3a** (1.3 g, 3.9 mmol) in dry ether (50 ml) gave with $LiAlH_4$ (560 mg, 14.8 mmol) after the above described workup 800 mg (64%) of **4a** as a colourless oil. m.p. (HCl): 306°C; IR (HCl, KBr, $\tilde{\nu}$): 2785, 2609, 2480 (s, N^+HR_3), 700 (s, =CH deform.) cm^{-1}; UV (base, CH_2Cl_2, λ (logε)): 208 (4.261) nm; ^{1}H NMR (base, $CDCl_3$, δ, 400 MHz): 1.88 - 1.94 (m, 3H, 4-H, 6-H), 2.12 (dd, *J* = 13.2, 10.8 Hz, 1H, 9-H), 2.25 (ddd, *J* = 13.4, 8.9, 2.3 Hz, 1H, 9-H), 2.31 (s, 6H, $N(CH_3)_2$), 2.33 (ddd, *J* = 12.8, 9.1, 2.1 Hz, 1H, 6-H), 3.10 - 3.15 (m, 3H, 1-H, 3-H), 3.29 (ddd, *J* = 11.0, 8.5, 2.5 Hz, 1H, 8-H), 3.44 (t, *J* = 9.4 Hz, 1H, 7-H), 7.18 - 7.40 (m, 10H, aromatic H) ppm; ^{13}C NMR (base, $CDCl_3$, δ, 100 MHz): 31.70 (C-4), 35.66 (C-9), 35.59 (C-6), 37.95 ($N(CH_3)_2$), 39.62 (C-8), 41.86 (C-3), 47.11 (C-7), 57.99 (C-5), 61.54 (C-1), 126.10, 126.24, 127.15, 127.78, 128.44, 128.55, 144.20, 145.51 (aromatic C) ppm; MS (base, EI^+): *m/z* (%) = 320 (93.8) [M^+], 275 (37.5), 188 (24.2), 176 (100.0), 145 (48.4), 104 (19.9), 91 (28.1), 85 (92.6), 70 (25.0), 44 (25.8); Anal. Calcd for $C_{22}H_{30}N_2Cl_2$ (393.39): C 67.17, H 7.69, N 7.12, Cl 18.02; found: C 66.91, H 7.91, N 6.94, Cl 18.01; HRMS (EI+): calcd. ($C_{22}H_{28}N_2$): 320.22525; found: 320.22451.

(7RS,8RS)-(±)-1-(7,8-Diphenyl-2-azabicyclo[3.2.2]non-5-yl)-morpholine **(4b)**. Compound **3b** (1.42 g, 3.8 mmol) in dry ether (48 ml) gave with $LiAlH_4$ (540 mg, 14.2 mmol) after the above described workup 818 mg (60%) of **4b**. m.p. (HCl): 310°C (decomp.); IR (HCl, KBr, $\tilde{\nu}$): 2870, 2663, 2424 (s, N^+HR_3), 703 (s, =CH deform) cm^{-1}; UV (HCl, CH_3OH, λ (logε)): 209 (4.213) nm; ^{1}H NMR (base, $CDCl_3$, δ, 400 MHz): 1.87 (dd, *J* = 13.0, 10.1 Hz, 2H, 6-H, NH), 1.93 - 1.96 (m, 2H, 4-H), 2.11 (dd, *J* = 13.3, 10.8 Hz, 1H, 9-H), 2.22 (ddd, *J* = 13.3, 11.2, 2.3 Hz, 1H, 9-H), 2.29 (ddd, *J* = 12.8, 9.0, 2.3 Hz, 1H, 6-H), 2.59 - 2.71 (m, 4H, $N(CH_2)_2$), 3.09 - 3.17 (m, 3H, 1-H, 3-H), 3.29 (ddd, *J* = 11.1, 8.5, 2.6 Hz, 1H, 8-H), 3.42 (br, t, *J* = 9.5 Hz, 1H, 7-H), 3.69 - 3.74 (m, 4H, $O(CH_2)_2$), 7.18 - 7.39 (m, 10H, aromatic H) ppm; ^{13}C NMR (base, $CDCl_3$, δ, 100 MHz): 32.98 (C-4), 35.62 (C-9), 36.24 (C-6), 39.69 (C-8), 41.83 (C-3), 45.66 ($N(CH_2)_2$), 47.09 (C-7), 58.18 (C-5), 61.35 (C-1),

67.63 ($O(CH_2)_2$), 126.13, 126.26, 127.06, 127.72, 128.45, 128.55, 144.13, 145.45 (aromatic C) ppm; MS (base, EI^+): *m/z* (%) = 362 (100.0) [M^+], 275 (31.1), 230 (22.6), 218 (91.8), 171 (27.2), 145 (68.5), 127 (35.8), 104 (24.9), 91 (24.1); Anal. Calcd for $C_{24}H_{30}N_2O$ (362.51): C 79.52, H 8.34, N 7.73; found: C 79.25, H 8.61, N 7.69; HRMS (EI+): calcd. ($C_{24}H_{30}N_2O$): 362.23581; found: 362.23411.

(7RS,8RS)-(±)-1-(7,8-Diphenyl-2-azabicyclo[3.2.2]non-5-yl)-pyrrolidine **(4c)**. Compound **3c** (1.16 g, 3.2 mmol) in dry ether (47 ml) gave with $LiAlH_4$ (521 mg, 13.7 mmol) after the above described workup 850 mg (76%) of **4c**. m.p. (HCl): 306°C; IR (HCl, KBr, $\bar{\nu}$): 2876, 2677, 2435 (s, N^+HR_3), 700 (s, =CH deform.) cm^{-1}; UV (HCl, CH_3OH, λ ($\log\varepsilon$)): 207 (4.182) nm; ^1H NMR (base, $CDCl_3$, δ, 400 MHz): 1.75 - 1.79 (m, 4H, $2CH_2$), 1.91 - 2.06 (m, 4H, 4-H, 6-H, NH), 2.18 - 2.35 (m, 3H, 6-H, 9-H), 2.69 - 2.77 (m, 4H, $N(CH_2)_2$), 3.10 - 3.20 (m, 3H, 1-H, 3-H), 3.32 (ddd, *J* = 11.1, 8.5, 2.6 Hz, 1H, 8-H), 3.46 (br, t, *J* = 9.5 Hz, 1H, 7-H), 7.17 - 7.41 (m, 10H, aromatic H) ppm; ^{13}C NMR (base, $CDCl_3$, δ, 100 MHz): 23.61 ($2CH_2$), 33.55 (C-4), 36.08 (C-9), 37.43 (C-6), 39.53 (C-8), 41.94 (C-3), 45.17 ($N(CH_2)_2$), 47.13 (C-7), 57.05 (C-5), 61.76 (C-1), 126.06, 126.21, 127.21, 127.80, 128.44, 128.55, 144.29, 145.70 (aromatic C) ppm; MS (base, EI^+): *m/z* (%) = 346 (100.0) [M^+], 301 (49.8), 255 (35.0), 241 (24.9), 214 (26.5), 202 (59.1), 145 (23.3), 130 (20.2), 111 (78.6), 91 (27.2); Anal. Calcd for $C_{24}H_{30}N_2$ (346.51): C 83.19, H 8.73, N 8.08; found: C 82.93, H 8.52, N 8.04; HRMS (EI+): calcd. ($C_{24}H_{30}N_2$): 346.24090; found: 346.24023.

(7RS,8RS)-(±)-1-7,8-Diphenyl-2-azabicyclo[3.2.2]non-5-yl)-piperidine **(4d)**. Compound **3d** (1.3 g, 3.5 mmol) in dry ether (45 ml) gave with $LiAlH_4$ (500 mg, 13.2 mmol) after the above described workup 710 mg (57%) of **4d**. m.p. (HCl): 260°C; IR (HCl, KBr, $\bar{\nu}$): 2868, 2661, 2533 (s, N^+HR_3), 702 (s, =CH deform.) cm^{-1}; UV (HCl, CH_3OH, λ ($\log\varepsilon$)): 209 (4.137) nm; ^1H NMR (base, $CDCl_3$, δ, 400 MHz): 1.42 - 1.48 (m, 2H, CH_2), 1.55 - 1.64 (m, 4H, $2CH_2$), 1.88 (dd, *J* = 12.6, 10.7 Hz, 2H, 6-H, NH), 1.94 - 1.98 (m, 2H, 4-H), 2.13 (dd, *J* = 13.1, 10.7 Hz, 1H, 9-H), 2.25 (ddd, *J* = 13.1, 9.2, 2.2 Hz, 1H, 9-H), 2.33 (ddd, *J* = 12.9, 8.7, 2.2 Hz, 1H, 6-H), 2.52 - 2.68 (m, 4H, $N(CH_2)_2$), 3.07 - 3.20 (m, 3H, 1-H, 3-H), 3.29 (ddd, *J* = 11.0, 8.7, 2.4 Hz, 1H, 8-H), 3.40 (br, t, *J* = 9.5 Hz, 1H, 7-H), 7.18 - 7.40 (m, 10H, aromatic H) ppm; ^{13}C NMR (base, $CDCl_3$, δ, 100 MHz): 25.06 (CH_2), 26.79 ($2CH_2$), 32.94 (C-4), 35.53 (C-9), 36.59 (C-6), 40.09 (C-8), 42.16 (C-3), 46.26 ($N(CH_2)_2$), 47.62 (C-7), 58.59 (C-5), 61.42 (C-1), 126.07, 126.18, 127.08, 127.73, 128.44, 128.51, 144.36, 145.68 (aromatic C) ppm; MS (base, EI^+): *m/z* (%) = 360 (100.0) [M^+], 315 (34.2), 269 (31.1), 255 (16.3), 228 (21.8), 216 (44.4), 171 (17.1), 145 (19.5), 125 (48.2), 104 (20.2), 91 (18.7); Anal. Calcd for $C_{25}H_{32}N_2 \times 0.125H_2O$ (362.80): C 82.77, H 8.96, N 7.72; found: C 82.85, H 9.11, N 7.54; HRMS (EI+): calcd. ($C_{25}H_{32}N_2$): 360.25655; found: 360.25496.

ANTIPROTOZOAL TESTS

In vitro microplate assay against Plasmodium falciparum K_1

Antiplasmodial activity was examined using the K_1 strain of *P. falciparum* (resistant to chloroquine and pyrimethamine). Viability was determined by the incorporation of [^3H]-hypoxanthine into living protozoal cells by a modification of a reported assay (25). 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 microtiter plates. After 48 hours of incubation at 37°C in a reduced oxygen atmosphere, 0.5 µCi ^3H-hypoxanthine were added to each well. Cultures were incubated for a further 24 h before they were harvested onto glass-fiber 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. The standard was artemisinine.

In vitro microplate assay against Trypanosoma b. rhodesiense, Cytotoxicity

Minimum Essential Medium (50 µl) supplemented according to a known procedure (26) with 2-mercaptoethanol and 15% heat-inactivated horse serum was added to each well of a 96-well microtiter plate. Serial drug dilutions were prepared covering a range from 90 to 0.123 µg/ml. Then 10^4 bloodstream forms of *Trypanosoma b. 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 hours. Alamar Blue (10 µl containing 12.5 mg

resazurin dissolved in 1000 ml distilled water) was then added to each well and incubation continued for a further 2-4 hours. 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 (27). 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.

In vivo antimalarial activity of compound 4a

Male mice (Fü albino; specific pathogen free) weighing 20± 2g were infected intravenously with $2x10^7$ *P.berghei* ANKA strain-infected erythrocytes from donor mice on day 0 of the experiment. Heparinized blood was taken from donor mice with approximately 30% parasitemia and was diluted in physiological saline to 10^8 parasitized erythrocytes/ml. An aliquot (0.2 ml) of this suspension was injected intravenously into experimental groups of three mice and a control group of five mice.

In untreated control mice, parasitemias rise regularly to 30% to 40% by day +3 after infection and to 70 to 80% by day +4. The mice die between days +5 and +7 after infection.

On day +3 (48 hours after treatment) blood smears of all animals were prepared and stained with Giemsa. Parasitemia was determined microscopically by counting 1000 red blood cells. For low parasitemias (<1%) 2000 rbc's have to be counted. The difference between the mean value for the control group (taken as 100%) and that for each experimental group was calculated and expressed as percent reduction (=activity).

The mean survival day (MSD, in days) was recorded as well as observations concerning any side effects of the drug.

Compound **4a** was prepared at appropriate concentrations, as solutions containing 3% ethanol and 7% Tween 80. They were administered intraperitoneal (i.p.) and intravenous (i.v.) in a total volume of 0.01 ml per g of body weight on day +1 (24 hours after infection) of the experiment.

In vivo studies were carried out by a protocol approved by an animal ethics committee.

RESULTS

The new compounds **3a**-**3d** and **4a**-**4d** were screened using the assays described above. Antiprotozoal activities and cytotoxicity of these new compounds and as well as those of compounds **1** and **2** are presented in table 1.

Compound **4a** with the highest antiplasmodial activity was screened in an in vivo assay against *Plasmodium berghei*. The results are presented in table 2.

Table 1. In vitro activities of 1-4, expressed as IC_{50} (μM)[a]

Comp.	*Trypanosoma brucei rhodesiense*	*Plasmodium falciparum K1*	Cytotoxicity L6
1a	9.99	>10.57	24.57
1b	116.3	>11.89	n.t.
1c	8.03	1.19	26.45
1d	8.12	3.95	46.82
2a	1.64	2.50	23.40
2b	14.85	5.77	48.18
2c	1.47	3.64	16.03
2d	1.49	1.55	11.52
3a	37.97	1.40	>269.1
3b	138.4	>13.28	>239.1
3c	37.94	8.76	>249.7
3d	36.60	13.00	233.6
4a	0.60	0.28	108.8
4b	9.44	6.84	>206.7
4c	1.16	0.56	120.4
4d	6.57	0.64	89.74
mel	0.0039		7.78
sur	0.0075		4724.5
art		0.0064	450.5
chl		0.12[b]	188.5
mef			11.37

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

Table 2. In vivo activity of 4a against *P. berghei*

Comp.	dose in mg/kg	application	MSD (days)	Activity in %
4a	4×50	i.p.	6.3	35.1
4a	4×10	i.v.	6.3	18.4
ctrl	-----		5.9	---
chl	4×10	i.p.	20	99.6

chl = chloroquine, ctrl = control

DISCUSSION

The ring enlargement from bicyclo-octanones **1** to bicyclo-nonanones **3** leads to a remarkable decrease of cytotoxicity against L6 cells. In general the antiprotozoal activity is lost, however, the 5-dimethylamino-2-azabicyclo[3.2.2]nonan-3-one **3a** exhibits distinctly higher antiplasmodial activity (IC_{50} = 1.40 μM) than its bicyclo-octan-2-one analogue **1a** (IC_{50} > 10.57 μM). The positive contribution of the dimethylamino group to the antiplasmodial activity has also been observed for compounds **4**. Due to the good antiplasmodial activity and the very low cytotoxicity (IC_{50} > 269.1 μM) of compound **3a** it is the most promising antiplasmodial agent of the so far prepared aminobicyclo-alkanones. The low cytotoxicity of compounds **3** results obviously from the replacement of the keto by an amido group. Maybe, the change from the bicyclo-octane to the far more flexible bicyclo-nonane skeleton is the reason for an additional sterical contribution. The bicyclo[2.2.2]octan-4-amines **2** which were prepared from **1** using a Wolff-Kishner procedure, possess higher antitrypanosomal activity than the ketones **1**. Their antiplasmodial properties and their cytotoxicity are comparable to those of compounds **1**.

In contrast, compounds **4a**,**c** show distinctly higher antiplasmodial (IC_{50} = 0.28 - 0.56 μM) and antitrypanosomal activities (IC_{50} = 0.60 - 1.16 μM). (7,8-Diphenyl-2-azabicyclo[3.2.2]non-5-yl)-dimethylamine **4a** is the most active compound of both series against *Plasmodium falciparum* K_1 and *Trypanosoma b. rhodesiense*. The antitrypanosomal acitivity of **4a** (IC_{50} = 0.60 μM) is distinctly higher than that of all other compounds of the bicyclo-octane and the bicyclo-nonane series, but it is far less active than melarsoprol (IC_{50} = 0.0039 μM) and suramine (IC_{50} = 0.0075 μM). With reference to the commonly used antimalarial chloroquine (IC_{50} = 0.12 μM against sensitive strains) **4a** possesses a comparable antiplasmodial activity (IC_{50} = 0.28 μM) and at the same time low cytotoxicity (IC_{50} = 108.8 μM). However, the in vivo activity of **4a** against *Plasmodium berghei* is moderate (i.p.: 35.1% inhibition; i.v.: 18.4% inhibition) in the case of both applications. This could be caused by fast degradation in the mouse liver or insufficient bioavailability due to their relatively high lipophilicity or binding to other sites. Therefore, further structural modifications are in progress.

CONCLUSIONS

2-Azabicyclo[3.2.2]nonanes exhibit promising in vitro antiprotozoal activity and low cytotoxicity. Therefore, they will serve as lead compounds for further modifications. More hydrophilic compounds will be prepared by alkylation of the nitrogen in ring position 2 with substituents bearing polar groups. In order to investigate the influence of its basicity, acylation to amides is considered. In both cases, residues with differing length, polarity and acidity will be inserted.

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