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4-Aminobicyclo[2.2.2]octanone Derivatives with Antiprotozoal Activities

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Summary. 4-Aminobicyclo[2.2.2]octanones which were prepared in one-pot-reactions from benzylidene acetone and dialkylammonium rhodanides were reduced stereoselectively to their corresponding alcohols. The activities of the bicyclic compounds against causative organisms of tropical deseases were examined. The 4-aminobicyclo[2.2.2]octan-2-ols were in general more active against *Trypanosoma b. rhodesiense* and *Plasmodium falciparum* than the corresponding keto compounds.

Keywords. Antiprotozoal activity; Bicyclo[2.2.2]octane derivatives; Cyclizations; Hydrogenations; Stereoselectivity.

Introduction

Bicyclo[2.2.2]octanones **1** serve as intermediates in the synthesis of natural products such as terpenes [1–10] and alkaloids [11]. Some of them show inhibitory activity on macrophomate synthase, an enzyme which catalyses the conversion of oxalacetate and 2-pyrone to benzoate [12]. Usually they have been prepared from electron-deficient alkenes by *Diels-Alder* reaction [13–17] with cyclohexa-1,3-dienes **2** or by tandem *Michael* additions [3–8, 18–23] with deprotonated cyclohexenone **3** (Scheme 1).

The *endo*- and *exo*-isomers of the 6-amino compounds **4** and **5** have demonstrated stereoselectivity in their inhibition of the binding of [^{3}H]-ketanserin to $5HT_2$ sites in the cerebrocorticoidal membranes of mice [24]. Compound **6** has been tested for its inhibitory potency in [^{3}H]-*WIN* 35,428 binding at the dopamine (*DA*) transporter and [^{3}H]-*DA* uptake assays showing moderate to weak activity [25].

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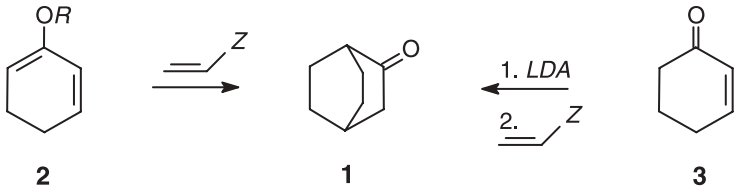
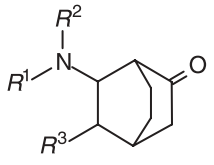
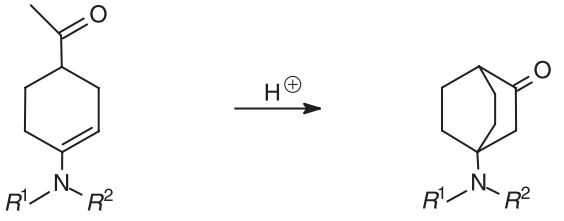


2 **1** **3**

Scheme 1



4: R^1, $R^2 = -(CH_2)_5-$, $R^3 = Ph$
5: R^1, $R^2 = -(CH_2)_4-$, R^3 = 3-indolyl
6: $R^1 = R^2 = CH_3$, $R^3 = Ph$



7: R^1, R^2 = alkyl **8:** R^1, R^2 = alkyl

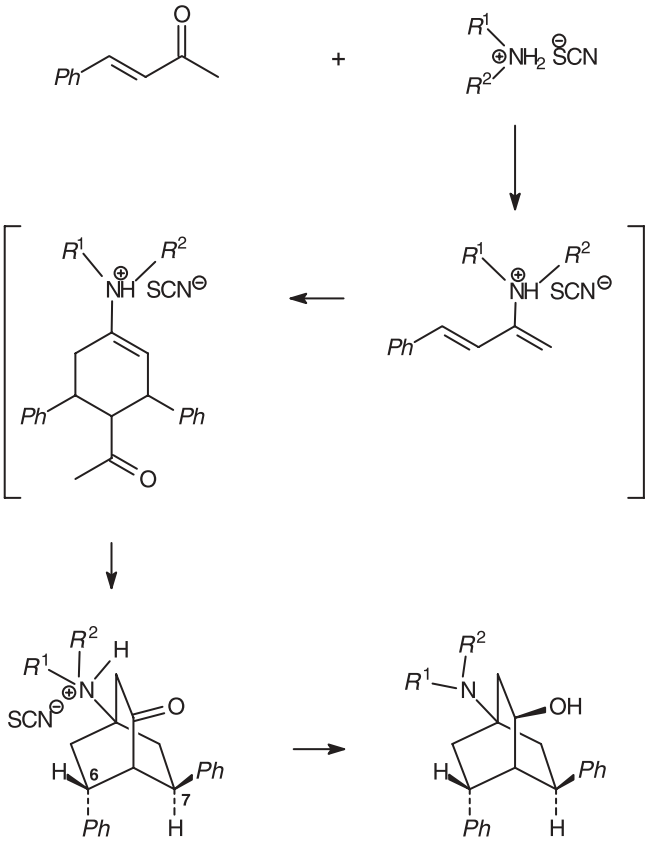
Scheme 2

The 4-amino compounds **8** are available by intramolecular cyclization of 1-(4-dialkylaminocyclohex-3-en-1-yl)ethanones **7** [26, 27], but no biological activities of those compounds have so far been published (Scheme 2).

Results and Discussion

We have reported the first one-pot synthesis of 4-dialkylamino-bicyclo[2.2.2]octan-2-ones by heating benzylidene acetone and dialkylammonium rhodanides [28]. The first step of this reaction is the formation of an enamine, which reacts with unchanged benzylidene acetone in the sense of a *Diels-Alder* addition to 4-acetylcyclohex-1-en-1-ylammoniumsalts. Those have been cyclized to bicyclo-octanones **9**–**12**. Compounds **9**–**11** have been obtained in good and compound **12** in moderate yields (Scheme 3).

Compounds **9**–**12** were reduced stereoselectively to the racemic (2*SR*,6*RS*,7*RS*)-bicyclo[2.2.2]octanols **13**–**16** using $LiAlH_4$ at ambient temperature (Scheme 3). In their NMR spectra no diastereomers were observed. The structures of **13**–**16** were established by NMR spectroscopy. In the ^{13}C NMR spectra the signal of C-2 was shifted from 213 ppm in compounds **9**–**12** to 72 ppm in compounds **13**–**16** due to reduction of the keto group. The inserted proton 2-H resonates typically at *ca*. 4.3 ppm in ^1H NMR spectra and couples with the 1-H proton



9: $R^1 = R^2 = CH_3$
10: R^1, $R^2 = -(CH_2)_2-O-(CH_2)_2-$
11: R^1, $R^2 = -(CH_2)_4-$
12: R^1, $R^2 = -(CH_2)_5-$

13: $R^1 = R^2 = CH_3$
14: R^1, $R^2 = -(CH_2)_2-O-(CH_2)_2-$
15: R^1, $R^2 = -(CH_2)_4-$
16: R^1, $R^2 = -(CH_2)_5-$

Scheme 3

($J \approx 4$ Hz). The configuration in position 2 was determined by N*O*E measurements. Irradiation of 2-H in **13** gave a 10% N*O*E to 6-H (Fig. 1).

Compounds **9–16** were investigated for their activity against causative organisms of tropical deseases including *Plasmodium falciparum, Leishmania donovani, Trypanosoma brucei rhodesiense*, and *Trypanosoma cruzi*. Furthermore, the compounds were tested for their cytotoxicity against L6 cells (Table 1).

Plasmodium falciparum K_1 is the causative organism for *Malaria tropica* and already resistant against chloroquine and pyrimethamine. The bicyclo[2.2.2]octanols **13–16** are in general more active against this protozoal organism than the ketones **9–12** with the exception of the 4-pyrrolidino compound **11**, which exhibits higher activity than the corresponding alcohol **15**. However, the 4-piperidino bicyclo[2.2.2]octanol **16** was the most active compound.

Trypanosoma rhodesiense is a causative organism of sleeping illness. Again the bicyclo[2.2.2]octanol group has higher activities but this time only the

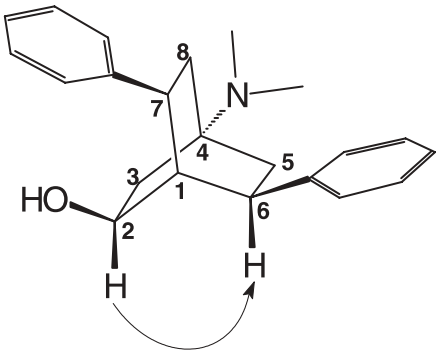


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Fig. 1. N*O*E from 2-H to 6-H

Table 1. Activities of compounds **9–16**, expressed as IC_{50} ($\mu g/cm^3$)[a]

Compound	*L. donovani*	*P. falciparum* K_1	*T. b. rhodesiense*	*T. cruci*	Cytotox. L6
9	>9	>4.0	3.78	41.9	9.3
10	>30	>5.0	48.9	49.4	n.t.
11	>30	0.48	3.25	13.4	10.7
12	n.t.	1.653	3.4	n.t.	19.6
13	22.2	>5.0	0.947	49.0	42.6
14	>30	0.881	7.56	43.5	n.t.
15	>9	0.832	1.48	31.3	9.3
16	n.t.	0.303	1.93	n.t.	n.t.
standard	5.5	0.0018	0.00155	0.23	4.3

[a] Values represent the average of four determinations (two determinations of two independent experiments), n.t.: not tested

4-morpholino compounds **10** and **14** exhibit significantly lower activities. The most active compound was the 4-dimethylaminobicyclo[2.2.2]octanol **13**.

All tested compounds have very low cytotoxicity. Despite of the similarity of the amino substituents the activity range was relatively wide. Further variations of the substitution patterns will be followed by racemic resolutions of the most active compounds.

Experimental

Yields are calculated on the basis of crystallised products. 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. ^1H and ^{13}C resonances were assigned using $^1H,^1H$ and $^1H,^{13}C$ correlation spectra. ^1H and ^{13}C resonances are numbered as given in the formulae. MS: Kratos profile spectrometer 70 eV electron impact. Microanalyses: Microanalytical Laboratory at the Institute of Physical Chemistry, Vienna; their values were in satisfactory agreement with the calculated ones. Materials: thin-layer chromatography (TLC): TLC

plates (Merck, silica gel 60 F_{254} 0.2 mm 200 × 200 mm); the compounds were detected in UV light at 254 nm.

Preparation of **9**–**12**

The preparation of the hydrorhodanides of **9**–**11** has already been reported [28].

(6RS,7RS)-(±)-6,7-Diphenyl-4-piperidinobicyclo[2.2.2]octan-2-one × HNCS (**12**, $C_{26}H_{30}N_2OS$)

Benzylidene acetone (67.4 g; 0.46 mol) and 39.0 g of piperidinium rhodanide (0.27 mol) were suspended in 250 cm^3 of *DMF* and refluxed for 4 h at 220°C using a water separator. After cooling to room temperature, the solvent was removed *in vacuo* and the residue crystallised over night. After recrystallisation from ethanol, 15.9 g (17%) of **12** were obtained as beige crystals; mp 264°C; IR (KBr): $\bar{\nu} = 2958$ (s), 2877 (m), 2601 (w), 2444 (s), 1725 (s), 1497 (s), 1452 (m), 1361 (m), 1330 (m), 755 (s), 698 (s) cm^{-1}; UV (CH_3OH): λ ($\log \varepsilon$) = 212 (4.005) nm; ^{1}H NMR (*DMSO*-d_6, 400 MHz): $\delta = 1.40$–1.54 (m, 1H, CH_2), 1.64–1.80 (m, 3H, CH_2, $(CH_2)_2$), 1.82–1.96 (m, 3H, 8-H, $(CH_2)_2$), 2.35 (t, b, $J = 9.5$ Hz, 1H, 5-H), 2.53 (s, 1H, 1-H), 2.57 (ddd, $J = 12.5$, 10.2, 2.3 Hz, 1H, 5-H), 2.80 (ddd, $J = 13.7$, 10.4, 3.3 Hz, 1H, 8-H), 2.83 (dd, $J = 17.4$, 2.2 Hz, 1H, 3-H), 3.01 (dd, $J = 17.7$, 3.2 Hz, 1H, 3-H), 2.90–3.34 (m, 2H, $N(CH_2)_2$), 3.40 (t, $J = 9.4$ Hz, 1H, 7-H), 3.54 (t, $J = 9.3$ Hz, 1H, 6-H), 3.64 (d, b, $J = 10.2$ Hz, 1H, $N(CH_2)_2$), 3.77 (d, b, $J = 10.5$ Hz, 1H, $N(CH_2)_2$), 7.08–7.49 (m, 10H, aromatic H), 9.24 (s, b, 1H, NH) ppm; ^{13}C NMR (*DMSO*-d_6, 100 MHz): $\delta = 21.63$ (CH_2), 23.62 ($(CH_2)_2$), 29.60 (C-5), 34.35 (C-8), 34.58 (C-7), 36.41 (C-6), 43.46 (C-3), 47.50, 48.01 ($N(CH_2)_2$), 53.61 (C-1), 64.40 (C-4), 126.95, 127.18, 127.76, 128.83, 128.94 (aromatic C), 140.39, 142.91 (aromatic C_q), 207.83 (C-2) ppm; MS (base, EI^+): m/z (%) = 359 (100.0, M^+), 316 (11.6), 268 (24.8), 255 (66.7), 227 (36.4), 213 (23.3), 178 (12.0), 136 (14.0), 91 (10.9).

Preparation of **13**–**16**

On an ice bath dry ether was added dropwise under stirring to $LiAlH_4$. To this suspension, an etheral solution of **9**, **10**, **11**, or **12** was added under the same conditions. The reaction mixture was stirred overnight at room temperature and the reaction was quenched carefully by addition of ice/H_2O under stirring and cooling. After that, the reaction mixture was extracted four times with CH_2Cl_2. The organic layers were washed twice with H_2O and dried (Na_2SO_4). After filtration, the solvents were removed *in vacuo* at room temperature. The residue was recrystallised from $CHCl_3$ or ethanol/H_2O.

(2SR,6RS,7RS)-(±)-4-Dimethylamino-6,7-diphenylbicyclo[2.2.2]octan-2-ol (**13**, $C_{22}H_{27}NO$)

Reaction of 0.8 g of **9** (2.5 mmol) with 0.8 g of $LiAlH_4$ (21 mmol) in 20 cm^3 of dry ether gave after crystallisation from $CHCl_3$ 0.5 g of **13** (62%); mp 111°C; IR (KBr): $\bar{\nu} = 3421$ (m), 3161 (m), 3089 (m), 3059 (m), 3027 (m), 2952 (s), 2871 (m), 2835 (m), 2791 (w), 1497 (s), 1448 (m), 1072 (m), 1035 (s), 746 (s), 697 (s) cm^{-1}; UV (CH_2Cl_2): λ ($\log \varepsilon$) = 238 (2.555), 254 (2.545) nm; ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 1.20$ (d, $J = 4.0$ Hz, 1H, OH), 1.74 (d, b, $J = 13.7$ Hz, 1H, 3-H), 1.90 (ddd, $J = 12.1$, 9.2, 2.5 Hz, 1H, 5-H), 1.99–2.05 (m, 2H, 3-H, 5-H), 2.11–2.17 (m, 2H, 8-H), 2.39 (s, 6H, $N(CH_3)_2$), 2.50 (d, $J = 4.5$ Hz, 1H, 1-H), 2.94 (t, $J = 9.2$ Hz, 1H, 6-H), 3.23 (t, $J = 9.8$ Hz, 1H, 7-H), 4.34–4.40 (m, 1H, 2-H), 7.13–7.42 (m, 10H, aromatic H) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 30.70$ (C-8), 31.76 (C-5), 34.79 (C-7), 37.36 (C-3), 38.34 ($N(CH_3)_2$), 39.43 (C-6), 43.82 (C-1), 56.55 (C-4), 71.87 (C-2), 125.73, 126.29, 127.12, 127.34, 128.25, 128.53 (aromatic C), 143.34, 145.11 (aromatic C_q) ppm; MS (EI^+): m/z (%) = 322 (13.5, $M + H^+$), 321 (58.1, M^+), 276 (33.8), 216 (81.1), 200 (52.0), 173 (57.4), 172 (52.0), 140 (52.7), 113 (75), 96 (100.0), 91 (47.3), 70 (50.7).

(2SR,6RS,7RS)-(±)-4-Morpholino-6,7-diphenylbicyclo[2.2.2]octan-2-ol
(**14**, $C_{24}H_{29}NO_2$)

Reaction of 1.63 g of **10** (4.4 mmol) with 1.7 g of $LiAlH_4$ (45 mmol) in 50 cm^3 of dry ether gave after crystallisation from ethanol/H_2O 1.06 g of **14** (66%); mp 136°C; IR (KBr): $\bar{\nu} = 3342$ (s), 2967 (s), 2942 (s), 2871 (s), 2851 (s), 1497 (m), 1451 (m), 1290 (m), 1115 (s), 960 (s), 859 (m), 746 (s), 696 (s) cm^{-1}; UV (CH_2Cl_2): λ (log ε) = 235 (2.556), 259 (2.600) nm; ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 1.32$ (s, 1H, OH), 1.71 (d, b, $J = 14.6$ Hz, 1H, 3-H), 1.89 (dt, $J = 12.0$, 2.1 Hz, 1H, 5-H), 1.96–2.02 (m, 2H, 3-H, 5-H), 2.09 (dt, $J = 11.1$, 2.7 Hz, 1H, 8-H), 2.17 (t, $J = 11.1$ Hz, 1H, 8-H), 2.49 (d, $J = 4.1$ Hz, 1H, 1-H), 2.65–2.78 (m, 4H, $(NCH_2)_2$), 2.91 (t, $J = 9.2$ Hz, 1H, 6-H), 3.21 (t, $J = 9.9$ Hz, Hz, 1H, 7-H), 3.77 (t, $J = 4.4$ Hz, 4H, $(OCH_2)_2$), 4.31–4.37 (m, 1H, 2-H), 7.12–7.42 (m, 10H, aromatic H) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 31.36$ (C-8), 31.67 (C-5), 34.71 (C-7), 37.52 (C-3), 39.32 (C-6), 43.91 (C-1), 46.20 ($N(CH_2)_2$), 56.75 (C-4), 67.60 ($O(CH_2)_2$), 71.83 (C-2), 125.80, 126.35, 127.06, 127.33, 128.31, 128.56 (aromatic C), 143.21, 145.01 (aromatic C_q) ppm; MS (EI^+): m/z (%) = 364 (20.9, $M + H^+$), 363 (91.2, M^+), 318 (41.2), 272 (29.7), 258 (94.6), 242 (58.1), 215 (81.8), 182 (83.1), 155 (70.3), 138 (100.0), 129 (32.4), 115 (43.9), 91 (78.4).

(2SR,6RS,7RS)-(±)-6,7-Diphenyl-4-pyrrolidinobicyclo[2.2.2]octan-2-ol
(**15**, $C_{24}H_{29}NO$)

Reaction of 0.4 g of **11** (1.2 mmol) with 0.4 g of $LiAlH_4$ (11 mmol) in 12 cm^3 of dry ether gave after crystallisation from ethanol/H_2O 0.27 g of **15** (65%); mp 128°C; IR (KBr): $\bar{\nu} = 3085$ (w), 3023 (w), 2965 (m), 2930 (m), 2869 (m), 2841 (m), 1035 (m), 1025 (m), 793 (m), 760 (m), 748 (m), 697 (s) cm^{-1}; UV (CH_2Cl_2): λ (log ε) = 235 (3.006) nm; ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 1.29$ (d, $J = 3.5$ Hz, 1H, OH), 1.72 (d, b, $J = 13.3$ Hz, 1H, 3-H), 1.81 (s, b, 4H, $(CH_2)_2$), 1.93–2.02 (m, 2H, 5-H), 2.07 (dd, $J = 9.0$, 3.0 Hz, 1H, 3-H), 2.12 (dt, $J = 9.9$, 3.2 Hz, 1H, 8-H), 2.22 (dt, $J = 10.1$, 1.7 Hz, 1H, 8-H), 2.45 (d, $J = 4.1$ Hz, 1H, 1-H), 2.70–2.83 (m, 4H, $(NCH_2)_2$), 2.94 (t, $J = 9.4$ Hz, 1H, 6-H), 3.22 (t, $J = 9.8$ Hz, 1H, 7-H), 4.29–4.35 (m, 1H, 2-H), 7.10–7.41 (m, 10H, aromatic H) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 23.55$ ($(CH_2)_2$), 31.75 (C-8), 32.44 (C-5), 34.66 (C-7), 38.40 (C-3), 39.60 (C-6), 44.20 (C-1), 45.46 ($N(CH_2)_2$), 54.98 (C-4), 72.04 (C-2), 125.70, 126.23, 127.11, 127.41, 128.25, 128.49 (aromatic C), 143.49, 145.24 (aromatic C_q) ppm; MS (EI^+): m/z (%) = 347 (70.9, $M + H^+$), 346 (20.9, M^+), 302 (43.9), 256 (32.4), 242 (87.8), 226 (69.6), 212 (25.0), 200 (31.1), 199 (84.5), 166 (74.3), 152 (24.3), 139 (100.0), 122 (51.4), 115 (32.4), 96 (28.4), 91 (58.1), 70 (38.5), 55 (23.0).

(2SR,6RS,7RS)-(±)-6,7-Diphenyl-4-piperidinobicyclo[2.2.2]octan-2-ol
(**16**, $C_{25}H_{31}NO$)

Reaction of 2.59 g of **12** (7.2 mmol) with 3 g of $LiAlH_4$ (79 mmol) in 100 cm^3 of dry ether gave after crystallisation from ethanol/H_2O 2.1 g of **16** (81%); mp 145°C; IR (KBr): $\nu = 3472$ (m), 3328 (m), 2980 (m), 2930 (s), 2853 (s), 1496 (m), 1446 (m), 1113 (m), 955 (m), 788 (m), 740 (s), 695 (s) cm^{-1}; UV (CH_2Cl_2): λ (log ε) = 236 (2.952) nm; ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 1.34$ (d, $J = 3.5$ Hz, 1H, OH), 1.44–1.50 (m, 2H, CH_2), 1.60–1.68 (m, 4H, $(CH_2)_2$), 1.73 (d, b, $J = 13.6$ Hz, 1H, 3-H), 1.90 (ddd, $J = 12.1$, 9.7, 2.4 Hz, 1H, 5-H), 1.95–2.04 (m, 2H, 3-H, 5-H), 2.07–2.20 (m, 2H, 8-H), 2.47 (d, $J = 4.4$ Hz, 1H, 1-H), 2.58–2.74 (m, 4H, $(NCH_2)_2$), 2.87 (t, $J = 9.4$ Hz, 1H, 6-H), 3.18 (t, $J = 9.8$ Hz, Hz, 1H, 7-H), 4.27–4.33 (m, 1H, 2-H), 7.09–7.40 (m, 10H, aromatic H) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 24.96$ (CH_2), 26.77 ($(CH_2)_2$), 31.68 (C-8), 31.96 (C-5), 34.86 (C-7), 37.72 (C-3), 39.49 (C-6), 43.80 (C-1), 46.81 ($N(CH_2)_2$), 57.03 (C-4), 72.03 (C-2), 125.66, 126.22, 127.11, 127.35, 128.22, 128.48 (aromatic C), 143.53, 145.33 (aromatic C_q) ppm; MS (EI^+): m/z (%) = 361 (100.0, M^+), 316 (30.4), 270 (34.1), 262 (26.5), 256 (70.2), 240 (36.7), 213 (49.6), 180 (52.0), 153 (34.7), 136 (57.2).

Biological Tests

Trypanosoma cruzi

Rat skeletal myoblasts (L-6 cells) were seeded in 96-well microtiter plates at 2000 cells/well/100 mm^3 in RPMI 1640 medium with 10% FBS and 2 m*M* *L*-glutamine. After 24 hours 5000 trypomastigotes of *T. cruzi* (Tulahuen strain C2C4 containing the galactosidase (Lac Z) gene) were added in 100 µl per well with 2x of a serial drug dilution. The plates were incubated at 37°C in 5% CO_2 for 4 days. After 96 hours the minimum inhibitory concentration (*MIC*) was determined microscopically. For measurement of the IC_{50} the substrate CPRG/Nonidet was added to the wells. The colour reaction which developed during the following 2–4 hours was read photometrically at 540 nm. From the sigmoidal inhibition curve IC_{50} values were calculated. Benznidazole was used as standard.

Trypanosoma b. rhodesiense and Cytotoxicity

Minimum Essential Medium (50 mm^3) supplemented according to *Baltz et al.* [29] with 2-mercaptoethanol and 15% heat-inactivated horse serum was added to each well of a 96-well microtiter plate. Serial drug dilutions were added to the wells. Then 50 mm^3 of trypanosome suspension (*T. b. rhodesiense* STIB 900) was added to each well and the plate incubated at 37°C under a 5% CO_2 atmosphere for 72 hours. Alamar Blue (10 mm^3) was then added to each well and incubation continued for a further 2–4 hours. The plate was then read with a Millipore Cytofluor 2300 using an excitation wavelength of 530 nm and emission wavelength of 590 nm [30]. Fluorescence development was expressed as percentage of the control, and IC_{50} values were determined. Melarsoprol served as standard. Cytotoxicity was assessed using the same assay and L-6 cells using mefloquine as standard.

Leishmania donovani

Mouse peritoneal macrophages were seeded in RPMI 1640 medium with 10% heat-inactivated FBS into Lab-tek 16-chamber slides. After 24 hours *Leishmania donovani* amastigotes were added at a ratio of 3:1 (amastigotes to macrophages). The medium containing free amastigotes was replaced by fresh medium 4 hours later. Next day the medium was replaced by fresh medium containing different drug concentrations. The slides were incubated at 37°C under a 5% CO_2 atmosphere for 96 hours. Then the medium was removed, the slides fixed with methanol and stained with *Giemsa* dye. The ratio of infected to non-infected macrophages was determined microscopically, expressed as percentage of the control and the IC_{50} value calculated by linear regression. Standard was pentamidine.

Plasmodium falciparum

Antiplasmodial activity was determined using the K1 strain of *P. falciparum*. A modification of the [^{3}H]-hypoxanthine incorporation assay was used [31]. Briefly, infected human red blood cells in RPMI 1640 medium with 5% Albumax were exposed to serial drug dilutions in microtiter plates for 48 hours. Viability was assessed by measuring the incorporation of [^{3}H]-hypoxanthine by liquid scintillation counting 24 hours after the addition of the radiolabel. The counts were expressed as percentage of the control cultures, sigmoidal inhibition curves were drawn and IC_{50} values calculated. Standard was artemisinine.

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References

[1] Mori K, Matsushima Y (1995) Synthesis 845
[2] Jung ME, Mc Combs CA (1978) J Am Chem Soc **100**: 5207
[3] Hagiwara H, Akama T, Okano A, Uda H (1993) J Chem Soc Perkin Trans I 2173
[4] Spitzner D, Wagner P, Simon A, Peters R (1989) Tetrahedron Lett **30**: 547
[5] Roberts R, Schlessinger RH (1981) J Am Chem Soc **103**: 724
[6] Hagiwara H, Uda H, Kodama T (1980) J Chem Soc Perkin Trans I 963
[7] Martin SF, Austin RE, Dantanarayana AP, Fishpaugh JR, Gluchonski C, Guinn DE, Hartmann M, Tanaka T, Wagner R, White JB (1995) Tetrahedron **51**: 3455
[8] Nagaoka H, Kobayashi R, Okamura T, Yamada Y (1987) Tetrahedron Lett **28**: 6641
[9] Hagiwara H, Hisashi U (1986) J Chem Soc Perkin Trans I 629
[10] Gibbons EG (1980) J Org Chem **45**: 1540
[11] Ihara M, Ishida Y, Abe M, Toyota M, Fukumoto K (1988) J Chem Soc Perkin Trans I 1155
[12] Oikawa H, Yagi K, Ohashi S, Watanabe K, Mie T, Ichihara A, Honma M, Kobayashi K (2000) Biosci Biotechnol Biochem **64**: 2368
[13] Hickmott PW, Simpson R (1992) J Chem Research (*S*) 302
[14] Brownbridge P (1983) Synthesis 85
[15] Petrzilka M, Grayson JI (1981) Synthesis 753
[16] Nazaki H, Yamaguti T, Ueda S, Kondo K (1968) Tetrahedron **24**: 1445
[17] Cimarusti CM, Wolinsky J (1968) J Am Chem Soc **90**: 113
[18] Nagaoka H, Shibuya K, Kobayashi K, Miura I, Muramatsu M, Yamada Y (1993) Tetrahedron Lett **34**: 4039
[19] Schinzer D, Kalesse M (1991) Tetrahedron Lett **36**: 4691
[20] Albrecht H, Dietz M, Schön C, Baumann V (1991) Synthesis 133
[21] Spitzner D, Engler A (1988) Org Syntheses **66**: 37
[22] Nagaoka H, Ohsawa K, Takata T, Yamada Y (1984) Tetrahedron Lett **25**: 5389
[23] White KB, Reusch W (1978) Tetrahedron **34**: 2439
[24] Schlecht MF, Tsarouhtsis D, Lipovac MN, Debler EA (1990) J Med Chem **33**: 386
[25] Javanmard S, Deutsch HM, Collard DM, Kuhar MJ, Schweri MM (1999) J Med Chem **42**: 4836
[26] Ahmed SA, Hickmott PW (1979) J Chem Soc Perkin Trans I 2180
[27] Morita KJ, Kobayashi T (1966) J Org Chem **31**: 3106
[28] Weis R, Schweiger K, Seebacher W, Belaj F (1998) Tetrahedron **54**: 14015
[29] Baltz T, Baltz D, Giroud C, Crockett J (1985) EMBO J **4**: 1273
[30] Räz B, Iten M, Grether-Bühler Y, Kaminsky R, Brun R (1997) Acta Trop **68**: 139
[31] Matile H, Pink JRL (1990) In: Lefkovits I, Pernis B (ed) Immunological Methods, Academic Press, San Diego p 221