Antiprotozoal Activity and Cytotoxicity of Novel 1,7-Dioxadispiro[5.1.5.2]pentadeca-9,12-dien-11-one Derivatives from *Amomum aculeatum*

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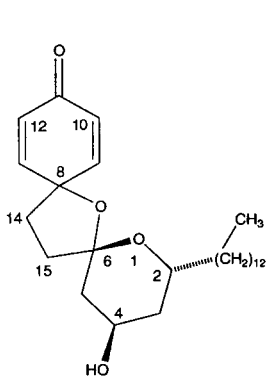
Cytotoxicity against the KB cancer cell line as a lead bioactivity-guided fractionation of the petroleum ether extract of rhizomes of *Amomum aculeatum* ROXB. led to the isolation of three novel dioxadispiro[5.1.5.2]-pentadeca-9,12-dien-11-one derivatives. The structures of aculeatin A (**1**), aculeatin B (**2**), and aculeatin C (**3**) were established as *rel*-(2*R*,4*R*,6*S*)- and *rel*-(2*R*,4*R*,6*R*)-4-hydroxy-2-tridecyl-1,7-dioxadispiro[5.1.5.2]pentadeca-9,12-dien-11-one (**1** and **2**, resp.) and *rel*-(2*R*,4*R*,6*R*)-2-[4-(3-dodecyl-2-heptyl-3-hydroxy-6-oxocylohexa-1,4-dienyl)-2-oxobutyl]-4-hydroxy-1,7-dioxadispiro[5.1.5.2]pentadeca-9,12-dien-11-one (**3**) by extensive spectroscopic analyses, particularly ^{13}C-NMR, inverse-gated ^{13}C, HMQC, HMBC, NOESY, and INADEQUATE NMR experiments as well as mass spectrometry. The aculeatins represent a novel type of natural products. All compounds showed high cytotoxicity against the KB cell line: **1**, $IC_{50} = 1.7$ μM; **2**, $IC_{50} = 2.0$ μM; **3**, $IC_{50} = 1.6$ μM. Additional testing against two *Plasmodium falciparum* strains as well as against trypomastigote forms of *Trypanosoma brucei rhodesiense* and *Trypanosoma cruzi* showed strong activities, particularly against *P. falciparum* strain K1 (**1**, $IC_{50} = 0.18$ μM; **2**, $IC_{50} = 0.43$ μM; **3**, $IC_{50} = 0.37$ μM).

1. Introduction. – Hitherto only a few species of the genus *Amomum* (Zingiberaceae) have been phytochemically and biologically investigated. Eicosenones and methylated flavonols have been isolated from the fruits of *Amomum koenigii* J. F. GMELIN [1]. A diterpene peroxide with antiplasmodial activity has been reported from *Amomum krervanh* PIERRE *ex* GAGNEP. [2], and the phytochemical investigation of the essential oil of *Amomum subulatum* ROXB. revealed monoterpenic hydrocarbons, oxygenated monoterpenes, and sesquiterpenes [3]. In our research on new cytotoxic and antiprotozoal compounds from plants, we investigated the petroleum ether extract of the rhizomes of *Amomum aculeatum* ROXB. and report here the chemical and biological characterization of the active compounds.

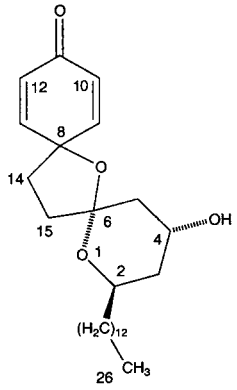
2. Results and Discussion. – The petroleum ether extract of *A. aculeatum* rhizomes showed strong antiplasmodial activity and cytotoxicity against the KB cell line. Vacuum liquid chromatography on silica gel provided three main active compounds, which were further separated by open column chromatography on silica gel and *Sephadex LH-20*.

Compound **1** was isolated as an optically active oil. Its DEI-MS (positive mode) displayed a $[M - H_2O]^+$ pseudomolecular ion at *m/z* 400, whereas the FAB-MS revealed a $[M + Na]^+$ at *m/z* 441. ^{13}C-NMR Experiments including DEPT 135 and inverse-gated ^{13}C sorted the C-atoms into three C, six CH, 16 CH_2, and one Me. ^{1}H,^{1}H-COSY, ^{1}H,^{13}C-HSQC, and ^{1}H,^{13}C-HMBC Experiments allowed the complete assignment of all proton

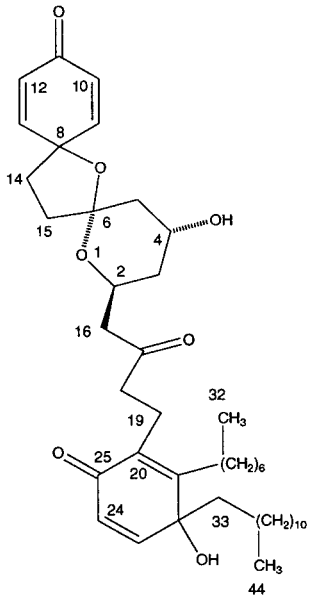
and C-atoms of *rel*-(2*R*,4*R*,6*S*)-4-hydroxy-2-tridecyl-1,7-dioxadispiro[5.1.5.2]pentadeca-9,12-dien-11-one (**1**) for which we propose the name aculeatin A (**1**) (see *Tables 1* and *2*). The configuration of **1** was established by a NOESY experiment. The structure of **1** was finally confirmed by an INADEQUATE experiment, which revealed the $^{13}C,^{13}C$ couplings.



1 Aculeatin A



2 Aculeatin B



3 Aculeatin C

Resonances of the quarternary C(11) of **1** at δ 185.3 and the four olefinic atoms C(13), C(9), C(12), and C(10) at δ 150.9, 148.7, 127.2, and 127.0, respectively, pointed to the presence of a dienone structure [4]. Eight CH_2 signals between δ 29.3 and 29.6 revealed the presence of an alkyl chain. In the ^{1}H-NMR spectrum, four protons at δ 6.85 and 6.76 (each *dd*, $J = 10.0$ and 2.9 Hz, each 1 H) and δ 6.14 and 6.11 (each *dd*, $J = 10.0$ and 2.0 Hz, each 1 H) confirmed the dienone moiety.

Key correlations included long-range correlations of both spiro centers of **1**. Spiro atom C(8) at δ 79.7 coupled with the olefinic protons of the dienone system and with CH_2(14) at δ 2.37 and 2.00, whereas spiro atom C(6) at δ 109.0 coupled with CH_2(15) at δ 2.37 and 2.22 and CH_2(5) at δ 2.00 and 1.93, as well as with H–C(2) at δ 4.10. The O-bearing atom C(2) at δ 65.3 showed correlations to the four methylene protons CH_2(3) (δ 1.79 and 1.42) and CH_2(16) (both δ 1.50). The protons CH_2(3) were correlated with C(4) at δ 64.8, and CH_2(16) coupled with the alkyl-chain C(17) at δ 25.6. The number of C-atoms in the side chain was determined by an ^{13}C inverse-gated experiment.

Changing the solvent from $CDCl_3$ to C_6D_6 led to a complete separation of the ^{1}H-NMR signals of H–C(2) and H–C(4), which partly overlapped in $CDCl_3$. That NOE effects were lacking between both protons as well as NOEs between H–C(4), CH_2(15), and the alkyl-chain CH_2(16), indicated that H–C(4) and the alkyl chain were on the same side of the ring.

The DEI-MS of compound **2** revealed a pseudomolecular ion at *m/z* 419 ($[M + H]^+$) and, therefore, an identical molecular mass for aculeatin A (**1**) and B (**2**). The ^{1}H- and ^{13}C-NMR spectra resembled those of compound **1**, except for remarkable shift differences of H–C(2) (+0.24 ppm), H–C(4) (–0.23 ppm) and H–C(15a)

Table 1. *^{1}H-NMR Data* (500 MHz) *of Compounds* **1**–**3** *in $CDCl_3$*. Chemical shifts in ppm, *J* in Hz. Arbitrary numbering.

	1	**2**	**3**
H–C(2)	4.10 (*m*)	3.86 (*m*)	4.03 (*m*)
CH_2(3)	1.79 (*ddd*, *J* = 2.1, 5.1, 13.7); 1.42 (*m*[a]))	1.62 (*m*); 1.55 (*m*)	1.48 (*m*[a]))
H–C(4)	4.12 (*m*)	4.35 (*m*)	4.26 (*m*)
CH_2(5)	2.00 (*m*[a])); 1.93 (*m*)	2.08 (*m*); 1.88 (*m*)	2.09 (*m*[a])); 1.82 (*dd*, *J* = 2.1, 14.2)
H–C(9)	6.76 (*dd*, *J* = 2.9, 10)	6.78 (*dd*, *J* = 2.9, 10)	6.75 (*dd*, *J* = 2.9, 9.9)
H–C(10)	6.11 (*dd*, *J* = 2, 10)	6.11 (*dd*, *J* = 2, 10)	6.11 (*dd*, *J* = 2, 9.9)
H–C(12)	6.14 (*dd*, *J* = 2, 10)	6.13 (*dd*, *J* = 2, 10)	6.14 (*dd*, *J* = 2, 10)
H–C(13)	6.85 (*dd*, *J* = 2.9, 10)	6.99 (*dd*, *J* = 2.9, 10)	7.10 (*dd*, *J* = 2.9, 10)
CH_2(14)	2.37 (*m*[a])); 2.00 (*m*[a]))	2.30 (*m*); 2.04 (*m*)	2.27 (*m*); 1.98 (*m*)
CH_2(15)	2.37 (*m*[a])); 2.22 (*m*)	2.69 (*ddd*, *J* = 1.2, 6.2, 12.1); 1.92 (*m*)	2.45 (*m*); 2.06 (*m*[a]))
CH_2(16)	1.50 (*m*[a]))	1.59 (*m*); 1.42 (*m*[a]))	2.55 (*m*[a]))
CH_2(17)	1.49 (*m*[a])); 1.35 (*m*[a]))	1.48 (*m*[a])); 1.35 (*m*[a]))	
CH_2(18)	1.35–1.25 (*m*[a]))	1.35–1.25 (*m*[a]))	2.08 (*m*[a]))
CH_2(19)	1.35–1.25 (*m*[a]))	1.35–1.25 (*m*[a]))	2.50 (*m*[a]))
CH_2(20) to CH_2(22)	1.35–1.25 (*m*[a]))	1.35–1.25 (*m*[a]))	
CH_2(23) or H–C(23)	1.35–1.25 (*m*[a]))	1.35–1.25 (*m*[a]))	6.81 (*d*, *J* = 10)
CH_2(24) or H–C(24)	1.35–1.25 (*m*[a]))	1.35–1.25 (*m*[a]))	6.20 (*d*, *J* = 10)
CH_2(25)	1.35–1.25 (*m*[a]))	1.35–1.25 (*m*[a]))	
CH_2(26)	1.35–1.25 (*m*[a]))	1.35–1.25 (*m*[a]))	2.06 (*m*[a]))
CH_2(27)	1.35–1.25 (*m*[a]))	1.35–1.25 (*m*[a]))	1.38 (*m*[a])); 1.30 (*m*[a]))
Me(28) or CH_2(28)	0.88 (*t*, *J* = 6.9)	0.87 (*t*, *J* = 6.9)	1.35–1.25 (*m*[a]))
CH_2(29), CH_2(30)			1.35–1.25 (*m*[a]))
CH_2(31)			1.35–1.25 (*m*[a]))
Me(32)			0.88 (*t*, *J* = 6.9)
CH_2(33)			1.48 (*m*[a])); 1.38 (*m*[a]))
CH_2(34)			1.30 (*m*[a]))
CH_2(35) to CH_2(43)			1.35–1.25 (*m*[a]))
Me(44)			0.88 (*t*, *J* = 6.9)

[a]) Coupling constants not assignable due to overlap.

(−0.32 ppm) as well as a downfield shift of C(2) (−4.6 ppm). ^{1}H,^{1}H-COSY, HSQC, and HMBC experiments established identical correlations as for aculeatin A (**1**) confirming that **1** and **2** are stereoisomers. NOESY and ROESY Experiments revealed, in contrast to compound **1**, a strong NOE between H–C(2) and one H–C(15), whereas no NOEs could be observed between H–C(4) and protons of the alkyl side chain (no NOE between H–C(2) and H–C(4)). A pure solution of aculeatin B (**2**) in $CDCl_3$ containing traces of H^+ led to a mixture of the two epimers aculeatin A (**1**) and B (**2**) within a day. This can be explained by an acid-catalyzed temporary opening of the ketal at C(6) and corroborated the NMR-based structure elucidation [5].

The DEI-MS of aculeatin C (**3**) displayed a pseudomolecular ion at *m/z* 662 ($[M - H_2O]^+$), and the DCI-MS revealed a pseudomolecular peak at *m/z* 679 ($[M - H]^+$). The structure and configuration of **3** was established by ^{13}C- and ^{1}H-NMR (see *Tables 1* and *2*) correlation, NOESY, and ROESY data and confirmed by an INADEQUATE experiment.

Table 2. *^{13}C-NMR* (125 MHz) *Data of Compounds* **1**–**3** *in $CDCl_3$*. Chemical shifts in ppm. Arbitrary numbering.

	1	**2**	**3**
C(2)	65.3 (*d*)	69.4 (*d*)	67.8 (*d*)
C(3)	37.9 (*t*)	37.8 (*t*)	38.4 (*t*)
C(4)	64.8 (*d*)	65.0 (*d*)	70.1 (*d*)
C(5)	39.0 (*t*)	40.5 (*t*)	44.6 (*t*)
C(6)	109.0 (*s*)	108.5 (*s*)	108.4 (*s*)
C(8)	79.7 (*s*)	77.5 (*s*)	78.1 (*s*)
C(9)	148.7 (*d*)	149.3 (*d*)	149.4 (*d*)
C(10)	127.0 (*d*)	127.0 (*d*)	126.8 (*d*)
C(11)	185.3 (*s*)	185.7 (*s*)	185.7 (*s*)
C(12)	127.2 (*d*)	127.0 (*d*)	127.1 (*d*)
C(13)	150.9 (*d*)	152.4 (*d*)	152.4 (*d*)
C(14)	34.1 (*t*)	35.2 (*t*)	38.6 (*t*)
C(15)	39.0 (*t*)	35.3 (*t*)	35.0 (*t*)
C(16)	35.8 (*t*)	35.7 (*t*)	49.4 (*t*)
C(17)	25.6 (*t*)	25.9 (*t*)	210.6 (*s*)
C(18)	29.6 (*t*)	29.6 (*t*)	32.8 (*t*)
C(19)	29.6 (*t*)	29.6 (*t*)	37.8 (*t*)
C(20)	29.6 (*t*)	29.6 (*t*)	126.8 (*s*)
C(21)	29.6 (*t*)	29.6 (*t*)	150.4 (*s*)
C(22)	29.6 (*t*)	29.6 (*t*)	69.2 (*s*)
C(23)	29.6 (*t*)	29.6 (*t*)	150.4 (*d*)
C(24)	29.6 (*t*)	29.6 (*t*)	128.5 (*d*)
C(25)	29.3 (*t*)	29.3 (*t*)	185.2 (*s*)
C(26)	31.9 (*t*)	31.9 (*t*)	35.0 (*t*)
C(27)	22.6 (*t*)	22.7 (*t*)	25.4 (*t*)
C(28)	14.1 (*q*)	14.1 (*q*)	29.6 (*t*)
C(29)			29.6 (*t*)
C(30)			31.9 (*t*)
C(31)			22.7 (*t*)
C(32)			14.1 (*q*)
C(33)			36.6 (*t*)
C(34)			25.1 (*t*)
C(35) to C(40)			29.6 (*t*)
C(41)			29.3 (*t*)
C(42)			31.9 (*t*)
C(43)			22.7 (*t*)
C(44)			14.1 (*q*)

Besides the typical signals of the dioxydispiro[5.1.5.2]pentadeca-9,12-dien-11-one skeleton, the ^{13}C-NMR spectrum of **3** showed the additional carbonyl resonance of C(25) at δ 185.2. Furthermore, the two quaternary atoms C(20) and C(21) at δ 126.8 and 150.4, respectively, the two olefinic C(24) and C(23) at δ 128.5 and 150.4, respectively, as well as the *s* of C(22) at δ 69.2 pointed to a second substituted cyclohexadienone moiety. The signal of C(17) at δ 210.6 indicated the presence of a further keto group, and the split signal of the terminal Me group in the ^{13}C inverse-gated experiment suggested the presence of two alkyl chains in **3**. The ^{1}H-NMR spectrum showed close similarities to that of **2**. As expected, the signal of the terminal Me at δ 0.88 integrated to six protons, and the two additional protons H–C(23) and H–C(24) resonated at δ 6.81 and 6.20 (each *d*, J = 10.0 Hz, each 1 H). ^{1}H,^{1}H-COSY, HSQC, and HMBC experiments with **3** allowed again the linkage of the substructures and the complete assignment of all ^{1}H- and ^{13}C-NMR signals. Strong HMBC correlations were observed between CH_2(16) at δ 2.55 and C(17) as well as between CH_2(16) and C(2). Further key correlations were CH_2(19)/C(20), CH_2(26)/C(21), and CH_2(33)/C(22). The NOESY data were identical to those of **2**.

The pure compounds **1**–**3** were evaluated for cytotoxicity against the KB cell line (see *Table 3*). In KB cells, aculeatin A (**1**) and aculeatin C (**3**) showed identical activity ($IC_{50} = 1.7$ and 1.6 μM, resp.), closely followed by aculeatin B (**2**; $IC_{50} = 2.0$ μM). *In vitro* screening for antiprotozoal activity against *P. falciparum*, *T. brucei rhodesiense*, and *T. cruzi* revealed a strong antiplasmodial activity of the aculeatins against the two different *P. falciparum* strains K1 and NF-54. Against *P. falciparum* K1 strain, aculeatin A (**1**) showed the highest activity ($IC_{50} = 0.18$ μM), followed by aculeatin C (**3**; $IC_{50} =$ 0.37 μM) and B (**2**, $IC_{50} = 0.43$ μM). Activity of aculeatins against trypomastigote forms of *T. brucei rhodensiense* and *T. cruzi* was somewhat lower, but in the same range (see *Table 3*). Aculeatin A (**1**) was again more active than aculeatin B (**2**) and C (**3**).

Table 3. *Antiprotozoal Activity and Cytotoxicity of Compounds* **1**–**3**. Values given as IC_{50} in μM, cytotoxicity $n = 4$, other $n = 3$.

	Antiprotozoal activity				Cytotoxicity
	P. falciparum K1	*P. falciparum* NF 54	*T. cruzi*	*T. b. rhodesiense*	KB cells
Aculeatin A (**1**)	0.18	0.20	0.3	0.9	1.7
Aculeatin B (**2**)	0.43	0.62	1	1.1	2.0
Aculeatin C (**3**)	0.37	0.51	2.2	3	1.6
Chloroquine	0.12	0.01			
Melarsoprol				0.01	
Benznidazol			2.3		
Podophyllotoxin					0.01

The aculeatins represent a novel type of compounds with a 1,7-dioxadispiro-[5.1.5.2]pentadecan skeleton. Except for the unsubstituted 1,7-dioxaspiro[5.1.5.2]dec-14-ene and a *tert*-butyl derivative sythesized by *Whitby* and *Kocienski* [6][7], there is no report in the literature on the synthesis or isolation of compounds with the same skeleton until now. The high cytotoxicity against the KB cancer cell line and activity against three protozoan parasites indicates a general toxicity of the aculeatins. Our ongoing research focuses now on additional testing against other cancer cell lines, the mechanism of cytotoxicity, and, due to the different activity of the stereoisomers **1** and **2** against the protozoan parasites, on the preparation of semisynthetic compounds with the aim to find analogues with a more specific activity against the parasites.

Experimental Part

General. See also [8][9]. UV Spectra: MeOH solns; λ_{max} (log ε) in nm. NMR Spectra: *Bruker-DRX-500* spectrometer, at 500.13 MHz for ^1H and 125.77 for ^{13}C, at 295 K; $CDCl_3$ solns. (NOESY: additionally in C_6D_6); δ in ppm referenced against residual $CHCl_3$ (δ(H) 7.27) and $CDCl_3$ (δ(C) 77.0); for INADEQUATE measurements acetylacetonatochromium(III) was added. DEI-MS (70 eV) and DCI-MS (NH_3 as reactant gas): *Hitachi-Perkin-Elmer-RMUGM* mass spectrometer; *m/z* (rel. %). FAB-MS: *ZAB-2-SEQ* spectrometer at 8.3 keV; 3-nitrobenzyl alcohol as matrix.

Collection and Extraction. Rhizomes and roots of *Amomum aculeatum* ROXB. were collected in the Central Province of Papua New Guinea in September 1996. A voucher specimen is deposited in the *Rijksherbarium* (Leiden, The Netherlands) with the identification number ETH 96/1. Air-dried plant material (659 g) was extracted successively with petroleum ether, CH_2Cl_2, MeOH, MeOH/H_2O 7:3, and H_2O. The extracts were stored under N_2 at $-20°$.

Isolation. The petroleum ether extract (11.1 g) was subjected to vacuum LC (silica gel, 35–70 μm, step gradient hexane, hexane/CH_2Cl_2, CH_2Cl_2, CH_2Cl_2/AcOEt, AcOEt, AcOEt/MeOH, and MeOH). *Aculeatin A* (**1**) and *aculeatin B* (**2**) were eluted with CH_2Cl_2/AcOEt 6:4. The following fraction (CH_2Cl_2/AcOEt 4:6) yielded a mixture of **2** and *aculeatin C* (**3**). The mixture **1**/**2** (2.9 g) was subjected to open-column chromatography (silica gel (60–200 μm), CH_2Cl_2/MeOH 9.75:0.25). Fractions containing **1** and **2** were purified on *Sephadex LH-20* (cyclohexane/CH_2Cl_2/MeOH 7:4:1): pure **1** (200 mg) and **2** (300 mg). The mixture **2**/**3** (0.77 g) was subjected to CC (silica gel, $CHCl_3$/toluene/MeOH 7:4:1): 140 mg of pure **3**.

Aculeatin A (= rel-(*2*R,*4*R,*6*S)*-4-Hydroxy-2-tridecyl-1,7-dioxadispiro[5.1.5.2]pentadeca-9,12-dien-11-one*; **1**). Pale yellow oil. $[\alpha]_D^{23} = -5.3$ ($c = 0.9$, $CHCl_3$). UV (MeOH): 230 (4.17). DEI-MS (pos.): 400 (< 1, $C_{26}H_{40}O_3^+$, $[M - H_2O]^+$), 285 (1), 235 (2), 208 (5), 165 (42), 107 (100). FAB-MS (pos.): 441 ($[M + Na]^+$).

Aculeatin B (= rel-(*2*R,*4*R,*6*R)*-4-Hydroxy-2-tridecyl-1,7-dioxadispiro[5.1.5.2]pentadeca-9,12-dien-11-one*; **2**). Yellow oil. $[\alpha]_D^{23} = +50.0$ ($c = 0.2$, $CHCl_3$). UV (MeOH): 230 (4.14). DEI-MS (pos.): 419 (2, $C_{26}H_{43}O_4^+$, $[M + H]^+$), 400 (2), 208 (17), 165 (100), 107 (78).

Aculeatin C (= rel-(*2*R,*4*R,*6*R)*-2-[4-(3-Dodecyl-2-heptyl-3-hydroxy-6-oxocyclohexa-1,4-dienyl)-2-oxobutyl]-4-hydroxy-1,7-dioxadispiro[5.1.5.2]pentadeca-9,12-dien-11-one*; **3**). Pale yellow oil. $[\alpha]_D^{23} = +1.4$ ($c = 1.3$, $CHCl_3$). UV (MeOH): 227 (4.18). DEI-MS (pos.): 662 (< 1, $C_{42}H_{61}O_6^+$, $[M - H_2O]^+$), 386 (2), 368 (5), 293 (5), 280 (4), 222 (9), 194 (13), 168 (7), 110 (67), 84 (100). DCI-MS (pos.): 679 (1, $[M - H]^+$), 293 (100).

In-vitro Assays of WHO Screening. Plasmodium falciparum. Antiplasmodial activity was determined with the NF 54 strain of *P. falciparum* (sensitive to all known drugs) and K1 strain (resistant to chloroquine and pyrimethamine). A modification of the [^{3}H]hypoxanthine-incorporation assay was used [10]. Briefly, infected human red blood cells were exposed to serial drug dilutions in microtiter plates for 48 h at 37° in a gas mixture with reduced O_2 and elevated CO_2 content. [^{3}H]hypoxanthine was added to each well, and after further incubation for 24 h, the wells were harvested on glass fiber filters and counted in a liquid scintillation counter. From the sigmoidal inhibition curve, the IC_{50} value was calculated.

Trypanosoma cruzi. Rat skeletal myoblasts (L-6 cells) were seeded in 96-well microtiter plates at 2000 cells/well/100 μl in *RPMI-1640* medium with 10% FBS and 2 mM L-glutamine. After 24 h, 5000 trypomastigotes of *T. cruzi* (Tulahuen strain C2C4 containing the galactosidase (Lac Z) gene) were added in 100 μl per well with 2 × of a serial drug dilution. The plates were incubated at 37° under a 5% CO_2 atmosphere for 4 days. For measurement of the IC_{50}, the substrate 'CPRG/Nonidet' was added to the wells. The color reaction which developed during the following 2–4 h was read photometrically at 540 nm. From the sigmoidal inhibition curve, IC_{50} values were calculated.

Trypanosoma brucei rhodesiense. 'Minimum essential medium' (50 μl) supplemented with 2-mercaptoethanol and 15% heat-inactivated horse serum was added to each well of a 96-well microtiter plate [11]. Serial drug dilutions were added to the wells. Then 50 μl of trypanosome suspension (*T. b. rhodesiense* STIB 900) was added to each well and the plate incubated at 37° under a 5% CO_2 atmosphere for 72 h. 'Alamar Blue' (10 μl) was then added to each well and incubation continued for a further 2–4 h. The plate was then read with a *Millipore Cytofluor 2300* using an excitation wavelength of 530 nm and emission wavelength of 590 nm [12]. Fluorescence development was expressed as percentage of the control, and IC_{50} values were determined.

KB Cell Assay. The screen for activity against the KB cell line (ATC CCL 17) was performed as described previously [9].

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