Trypanocidal and Antileishmanial Dihydrochelerythrine Derivatives from *Garcinia lucida*

Jean Fotie,[†,§] D. Scott Bohle,*[,†] Martin Olivier,[‡] Maria Adelaida Gomez,[‡] and Sandra Nzimiro[§]

Department of Chemistry, McGill University, 801 Sherbrooke Street West, Montreal, Quebec H3A 2K6, Canada, Department of Microbiology and Immunology, McGill University, 3775 University Street, Montreal, Quebec H3A 2B4, Canada, and Division of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, The Ohio State University, 500 West 12th Avenue, Columbus, Ohio 43210

Received May 14, 2007

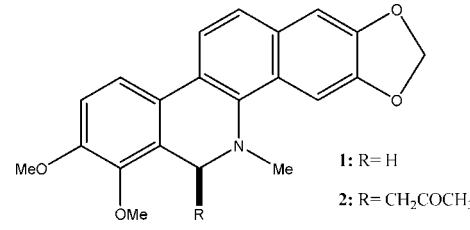
Three benzo[*c*]phenanthridine alkaloids have been isolated from the stem bark of *Garcinia lucida:* dihydrochelerythrine (**1**), 6-acetonyldihydrochelerythrine (**2**), and its new derivative, (*S*)1″-(9,10-dihydro-2′,3′-dihydroxy-7,8-dimethoxy-10-methyl-1,2-benzophenanthridin-9-yl)propan-2″-one (lucidamine A) (**3**). The new diisoprenylated derivative of lucidamine B (**4**) was obtained by semisynthesis. These dihydrochelerythrine derivatives as well as the crude extract displayed attractive antiprotozoal activity against *Trypanosoma brucei brucei* and *Leishmania donovani*, with little toxicity to Vero cells and the host cells. This is the first trypanocidal and antileishmanial bioguided study of *G. lucida*, and the activity of the crude extract as well as of the dihydrochelerythrine derivatives are reported for the first time.

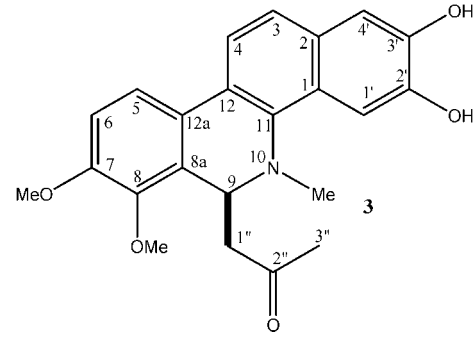
Parasitic trypanosomatids cause a number of important diseases, including human African trypanosomiasis (HAT), Chagas disease, and the leishmaniasis. Today, more than 60 million people living in 36 sub-Saharan Africa countries are at risk of contracting the sleeping sickness, caused by *Trypanosoma brucei gambiense* and *T. b. rhodesience*.[1] It is estimated that currently 300 000–500 000 people are infected, with 50 000 deaths annually.[1,2] An estimated 16 –18 million people in South America are infected with *T. cruzi*, the parasite responsible for Chagas disease.[3] For leishmaniasis, approximately 20 *Leishmania* species cause a spectrum of disease ranging from self-healing cutaneous lesions to life-threatening visceral infections, with 2 million new cases occurring annually.[4] Despite the huge impact of these diseases, the drugs used for their treatment are often toxic, marginally effective, administrated by injection, expensive, and/or compromised by the development of resistance.[5,6]

The genus *Garcinia* is exclusively tropical in distribution and contains an estimated 400 species[7,8] about 20 of which have been reported in Cameroon.[9] The bark of *Garcinia lucida* is commonly used by traditional healers in Cameroon to treat gastric infections, and as antidote against poison.[10] It is also believed to possess some aphrodisiac properties. To the best of our knowledge, only one phytochemical study of this species has been reported so far, a study that led to the isolation of three cycloartanes.[10] As part of phytochemical and pharmacological investigations of medicinal plants among the Baka pygmies of the Dja biosphere reserve of Cameroon,[11] a bioguided isolation of constituents of the stem bark of *G. lucida* (Clusiaceae) was conducted, investigations that led to the isolation of dihydrochelerythrine (**1**), 6-acetonyldihydrochelerythrine (**2**), and its new derivative, lucidamine A (**3**). The diisoprenylated derivative of lucidamine A, namely, lucidamine B (**4**), was also obtained by semisynthesis. The present report describes the isolation and structure elucidation as well as trypanocidal and antileishmanial evaluation of these constituents.

A combination of vacuum liquid chromatography (VLC), column chromatography (CC), and preparative thin layer chromatography (PTLC) on the dichloromethane/methanol (1:1) extract of the stem bark of *G. lucida* resulted in the isolation of compounds **1–3**. Stigmasterol, betulenic acid, β-*D*-glucopyranoside of β-sitosterol, sesamin, and *trans*-fagaramide were isolated as well.

The new compound (**3**), obtained as yellowish powder from a mixture of hexane:ethyl acetate (mp 212.2 °C, $\Delta H = +179.1$ J/g), showed a strong fluorescence under UV light (254 and 366 nm) and positive reactions with alkaloid-precipitating reagents such as Dragendorff's reagent; it also reacted positively to Gibbs and $FeCl_3$ reagents, indicating the presence of a phenolic group. The ESI mass spectrum in positive mode showed $[M + H]^+$ at *m/z* 394 and the HREIMS displayed $[M]^+$ at *m/z* 393.15802, corresponding to a molecular formula of $C_{23}H_{23}O_5N$, implying 12 unsaturation sites. The broad-band decoupled ^{13}C NMR spectrum showed 23 carbon signals, which were attributed by DEPT and HMQC techniques as four methyls, two of which are methoxyls, one methylene, seven methines comprising one sp^3 carbon, and eleven quaternary carbons including a carbonyl (δ 207.6), four oxygenated sp^2 carbons (δ 150.3, 149.9, 147.7, and 144.7), and six simple sp^2 carbons. The IR spectrum displayed free hydroxyl groups ($\upsilon_{max} = 3475$ and 3210 cm^{-1}), a carbonyl ($\upsilon_{max} = 1698$ cm^{-1}), and aromatic ring ($\upsilon_{max} =$ 1612, 1541 cm^{-1}) absorptions. The ^1H NMR spectrum of compound **3** analyzed by ^1H–^1H-COSY displayed, in addition to the two free hydroxyl (δ 9.63 and 9.55), two methoxyl (δ 3.88 and 3.87), and two methyl (δ 2.56 and 2.07) signals, four other sets of signals. Among these four signal sets are two aromatic AB systems corresponding to two 1,2,3,4-tetrasubstituted benzene rings, two isolated aromatic protons (singlets) corresponding to a 1,2,4,5-tetrasubstituted benzene ring, and an ABX system of three sp^3 protons suggesting a short aliphatic system. These spectrometric





* Corresponding author. E-mail: scott.bohle@mcgill.ca (D.S.B.).
† Department of Chemistry, McGill University.
§ College of Pharmacy, The Ohio State University.
‡ Department of Microbiology and Immunology, McGill University.

10.1021/np0702281 CCC: $37.00
Published on Web 09/20/2007

Scheme 1. Preparation of **4** from **3**

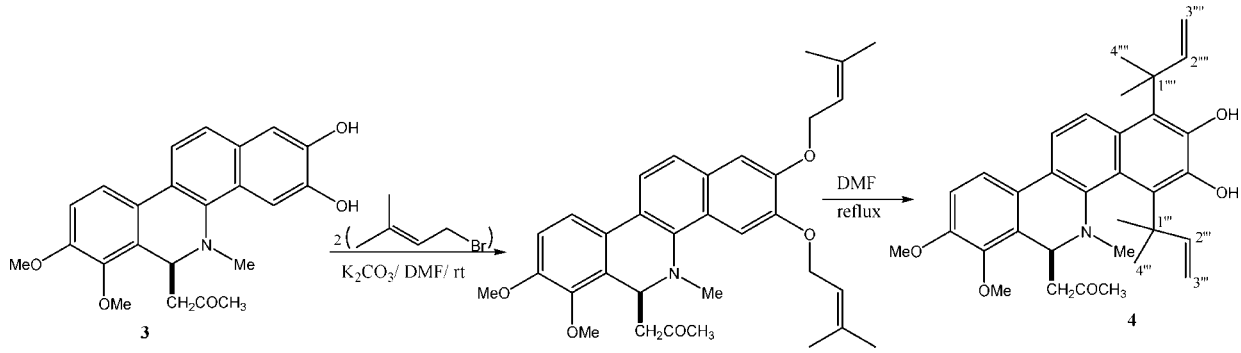


Table 1. Trypanocidal and Antileishmanial Activity of the Crude Extract and the Isolated Compounds, and Their Effect on Vero Cells[a]

compds	*T. b. brucei* $IC_{50} \pm SD$ (μM)	*L. donovani* axenic amastigotes $IC_{50} \pm SD$ (μM)	% inhibition on *L. donovani* promastigote (μg/mL; μM)[c] 100	10	1	% reduction in infection[b] 50 (μg/mL; μM)[c]	Vero cell $IC_{50} \pm SD$ (μM)
crude extract	4.9 ± 0.5 μg/mL	nd	100	25	3	67.2	>50 μg/mL
1	0.8 ± 0.2	2.0 ± 0.8	100	89	36	70.9	35.4 ± 0.3
2	3.9 ± 0.8	6.6 ± 0.6	100	87	23	57.4	15.3 ± 0.7
3	14.1 ± 2.3	10.8 ± 0.03	100	76	23	nd	11.6 ± 1.3
4	4.1 ± 0.1	6.8 ± 0.7	64	48	14	nd	17.5 ± 3.5
pentamidine	2.5 ± 0.13 nM	1.3 ± 0.92	nd	nd	nd	nd	13.7 ± 2.5
suramin	0.2 ± 0.03	nd	nd	nd	nd	nd	nd

[a] nd = not determined. [b] The % decrease of infected macrophages in treated vs nontreated wells. [c] μg/mL for the crude extract and μM for pure compounds.

and spectroscopic features are very similar to that of (*S*) 6-acetonyldihydrochelerythrine,[12–14] a compound that was also isolated during this investigation and whose crystal structure is also reported.[14] In fact, the only differences in the spectra of these two compounds are the disappearance in the ^{1}H NMR spectrum of **3** of the singlet of two protons at δ 6.04 attributed to the CH_2 of the methylenedioxy function, and the appearance in the same spectrum of the two broad singlets of two free hydroxyl groups at δ 9.55 and 9.63. These observations were confirmed in the ^{13}C NMR of **3** by the disappearance of the signal at δ 96.1 attributed to the carbon of the mentioned methylenedioxy function. Basically, compound **3** is a derivative of **2** in which the methylenedioxy function is transformed into two opened free hydroxyl groups. The stereochemistry of C-9 was also determined by close comparison of the spectroscopic data of compound **3** with those described in the literature for (*S*) 6-acetonyldihydrochelerythrinem,[12–14] in particular the specific rotation of compound **3** ($[\alpha]_D^{25}$ –133; *c* 0.2; DMSO) is comparable to that of (*S*) 6-acetonyldihydrochelerythrine ($[\alpha]_D^{25}$ –131; *c* 0.1; $CHCl_3$; lit.[13]). Compound **3** is thus characterized as (*S*)1″-(9,10-dihydro-2′,3′-dihydroxy-7,8-dimethoxy-10-methyl-1,2-benzophenanthridin-9-yl)propan-2″-one, for which we propose the trivial name of lucidamine A.

The structure of compound **3** was further chemically confirmed by an *O*-prenylation reaction followed by a Claisen rearrangement[15] that afforded compound **4** (see Scheme 1), a new semisynthetic derivative of lucidamine A, for which we propose the trivial name of lucidamine B.

Compound **2** was obtained as brown prisms (mp 197.4 °C, ΔH = +108.9 J/g; $[\alpha]_D^{25}$ –132; *c* 0.2, $CHCl_3$). Its molecular formula was deduced from its HREIMS as $C_{24}H_{23}O_5N$ (*m/z* 405.15725). All these physical features and the spectroscopic and single-crystal diffraction data of **2** were consistent with those reported for (*S*) 6-acetonyldihydrochelerythrine.[12–14] In a similar fashion, compound **1** was obtained as a yellow powder (mp 200.7 °C, ΔH = +70.77 J/g), with $C_{21}H_{19}O_4N$ (HREIMS, *m/z* 349.13245) as molecular formula. The only difference between **1** and **2** was the absence of the acetonyl side chain in **1**. The spectroscopic data of **1** matched well with those reported for dihydrochelerythrine.[16]

The crude extract of *G. lucida* displayed significant activity against *T. b. brucei* (IC_{50} 4.9 μg/mL) with no toxicity on the Vero cell. Amongst the isolated compounds, the dihydrochelerythrine derivatives (**1**–**4**) exhibited interesting activity, with IC_{50} values in the range 0.8–14.1 μM. Dihydrochelerythrine (**1**) was the most potent compound (IC_{50} 0.8 μM), with more than 44-fold selectivity for *T. b. brucei* parasites over Vero cells. Table 1 gives a comprehensive view on all the testing results.

When tested on promastigote *L. donovani*, the crude extract (100 μg/mL) and compounds **1**–**3** (100 μM) were able to clear the parasites (100% inhibition), whereas at 10 μM, these compounds were able to achieve about 89, 87, and 76% inhibition, respectively. Compound **4** displayed only 64 and 48% inhibition at 100 and 10 μM, respectively. However, when those compounds on the axenic amastigote form of the parasite are evaluated, compound **1** (IC_{50} 2.0 μM) appeared to be the most potent, followed by **2** and **4** (IC_{50} 6.6 and 6.8 μM, respectively), and finally, **3** (IC_{50} 10.8 μM). On infected macrophages, the crude extract (50 μg/mL) achieved 67.2% inhibition, and amongst the isolated compounds (at 50 μM), **1** was the most potent (70.9%) followed by **2** (57.4%). In the toxicity pretesting on noninfected macrophages, compounds **3** and **4** were toxic and thus were not evaluated on infected cells. Sesamin and *trans*-fagaramide displayed only marginal activity in all the assays, with no toxicity either to Vero cells or to healthy macrophages.

G. lucida possess a potential trypanocidal activity, and its nontoxicity to Vero cells and healthy macrophages mean this plant is an inexpensive herbal remedy to be considered for trypanosomiasis treatment. Dihydrochelerythrine and derivatives have also proven to be potential antileishmanial and trypanocidal compounds, and with the low toxicity of some derivatives, they should be considered for further structure–activity relationship investigations and possible optimization of their antiprotozoal activities.

Experimental Section

General Experimental Procedures. Melting points were determined by differential scanning calorimetric techniques on a Thermal Analysis (TA) Instruments DSC 2010 (differential scanning calorimeter). Optical rotations were recorded on a JASCO model DIP-140 digital polarimeter. UV spectra were recorded on an HP 8453

UV–visible spectrophotometer, and FTIR (KBr pellet) spectra were measured on an ABB-Bomem, MB series spectrophotometer. ESI spectra were obtained on a Finnigan LCQ^{DUO}, and EIMS and HREIMS on Kratos MS25RFA or Kratos analytical spectrometers. ^{1}H NMR (400 MHz), ^{13}C NMR (100 MHz), COSY, HMQC, HMBC, and NOESY spectra were recorded on a Varian Oxford-400 spectrometer. The following Merck chromatographic supports were used: Si gel 230–400 mesh for column and silica gel 60 F_{254} plates for analytical TLC. The Si gel GF plates (150 μm, 0.25 mm, and 2 mm thick) for preparative TLC were from Analtech, Uniplate.

Plant Material. *G. lucida* stem bark was collected in August 2005 in the Dja biosphere reserve, South Province of Cameroon, and identified by Victor NANA, botanist at the National Herbarium of Cameroon at Yaounde, where voucher specimens are deposited (N° 5768HNC and 25666HNC).

Isolation of Compounds. The pulverized, air-dried stems of *G. lucida* (1.45 kg) were extracted by maceration at room temperature in a 1:1 CH_2Cl_2:MeOH mixture for 24 h, yielding, after evaporation under reduced pressure, an oily dark-brown extract (200 g). The whole extract was fractionated by VLC on silica gel, eluted with *n*-hexanes:EtOAc mixtures of increasing polarity. A total of 100 fractions of ca. 500 mL each were collected and regrouped on the basis of TLC analysis to afford five major fractions (F_1–F_5). Fraction F_1, when eluted with a 9:1 *n*-hexanes:EtOAc mixture, afforded stigmasterol (1.8 g) and **1** (23 mg). The next fraction, F_2, was further separated by CC on silica gel, eluted with 8:2 to 7:3 *n*-hexanes:EtOAc, and yielded betulinic acid (2.8 g) and sesamin (850 mg). From fraction F_3, we obtained after further separation (7:3 *n*-hexanes:EtOAc), a solid that was recrystallized from a 1:1 *n*-hexanes:CH_2Cl_2 mixture to yield **2** as brown prisms (930 mg). From the same fraction, F_3 (6.5:3.5 *n*-hexanes:EtOAc) *trans*-fagaramide was also obtained and recrystallized from a mixture of *n*-hexanes:CH_2Cl_2 to yield colorless prisms (695 mg). Compound **3** was obtained as a yellowish powder (196 mg) from fraction F_4 when further eluted with 6:4 *n*-hexanes:EtOAC. Fraction F_5 afforded the β-*D*-glucopyranoside of β-sitosterol.

Dihydrochelerythrine (1): yellow powder (hexane:EtOAc); mp 200.7 °C; HREIMS *m/z* 349.1324 (calcd for $C_{21}H_{19}O_4N$, 349.1314). The IR, UV, ^{1}H, and ^{13}C NMR data matched those reported in the literature.[16]

6-Acetonyldihydrochelerythrine (2): brown prism (hexane:EtOAc); mp 197.4 °C; $[\alpha]^{23}_D$ –135 (*c* 0.1, $CHCl_3$)[13]; HREIMS *m/z* 405.1572 (calcd for $C_{24}H_{23}O_5N$, 405.1576). The IR, UV, ^{1}H, and ^{13}C NMR data as well as the X-ray crystal structure matched well with the literature data.[12–14]

Lucidamine A (3): yellowish powder (hexane:EtOAc); mp 212.2 °C, $[\alpha]^{25}_D$ –133 (*c* 0.2, DMSO); UV(DMSO) λ_{max} (log ε) 286 (2.22), 326 (0.71) nm; IR (KBr) υ_{max} 3475, 3210, 2939, 1698, 1612, 1541, 1502, 1028, 872, 782 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.63 (1H, brs, C-2′-OH), 9.55 (1H, brs, C-3′-OH), 7.71 (1H, d, J = 8.4 Hz, H-4), 7.49 (1H, d, J = 8.8 Hz, H-5), 7.44 (1H, d, J = 8.4 Hz, H-3), 7.36 (1H, s, H-1′), 7.14 (1H, s, H-4′), 6.91 (1H, d, J = 8.8 Hz, H-6), 4.91 (1H, dd, J = 14.0, 11.2 Hz, H-9), 3.88 (3H, s, C-7-OMe), 3.87 (3H, s, C-8-OMe), 2.56 (3H, s, N-Me), 2.34 (1H, dd, J = 11.2, 3.6 Hz, H-1″a), 2.19 (1H, dd, J = 14.0, 3.6 Hz, H-1″b), 2.07 (3H, s, Me); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 207.6 (C, C-2″), 150.3 (C, C-7), 149.9 (C, C-3′), 147.7 (C, C-2′), 144.7 (C, C-8), 138.3 (C, C-11), 130.3 (C, C-2), 128.1 (C, C-12a), 125.2 (C, C-1), 123.4 (C, C-8a), 123.2 (CH, C-3), 122.6 (C, C-12), 120.0 (CH, C-4), 119.4 (CH, C-5), 116.7 (CH, C-6), 111.0 (CH, C-4′), 103.1 (CH, C-1′), 60.8 (CH_3, C-8-OMe), 55.9 (CH_3, C-7-OMe), 55.5 (CH, C-9), 47.5 (CH_2, C-1″), 43.0 (CH_3, N-Me), 31.3 (CH_3, C-3″); ESIMS *m/z* 394 $[M + H]^+$; EIMS *m/z* 393 $[M]^+$ (15), 336 (100), 321 (32), 306 (7), 160 (6), 147 (4), 129 (15). HREIMS *m/z* 393.1580 (calcd for $C_{23}H_{23}O_5N$, 393.1576).

Semisynthesis of 4. A mixture of **3** (136 mg, 3.46 × 10^{-4} mol), 3,3-dimethylallyl bromide (105 mg, 7 × 10^{-4} mol) and potassium carbonate (104 mg, 7.54 × 10^{-4} mol) in DMF (50 mL) was stirred at room temperature for 48 h. The reaction mixture was then quenched with cold water, extracted with ethyl acetate, and purified on silica gel column eluted with a 7:3 *n*-hexane:EtOAc mixture to yield the oily di-*O*-prenylated intermediate (132 mg, 72%). This intermediate was then refluxed overnight in DMF (160 °C). The reaction mixture was then treated with water and chloroform and purified on silica gel column eluted with a 1:1 *n*-hexane:EtOAc mixture to yield **4** (105.6 mg, 80%).

Lucidamine B (4): viscous brown gum; $[\alpha]^{25}_D$ −130 (*c* 0.2; $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 206 (4.36), 289 (4.53), 335 (2.86) nm; IR (KBr) υ_{max} 3567, 3225, 3005, 1652, 1619, 1457, 1379, 1218, 1167, 1094 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.74 (1H, brs, C-3′-OH), 9.65 (1H, brs, C-2′-OH),7.54 (1H, d, J = 8.6 Hz, H-4), 7.36 (1H, d, J = 8.6 Hz, H-3), 7.12 (1H, d, J = 8.9 Hz, H-5), 6.88 (1H, d, J = 8.9 Hz, H-6), 6.30 (1H, m, H-2‴), 6.28 (1H, m, H-2‴), 4.90–4.93 (4H, m, H-3‴ and H-3‴), 4.57 (1H, dd, J = 14.5, 11.4 Hz, H-9), 3.73 (3H, s, C-7-OMe), 3.72 (3H, s, C-8-OMe), 2.46 (3H, s, N-Me), 2.35 (1H, dd, J = 14.5, 3.4 Hz, H-1″a), 2.20 (1H, dd, J = 11.4, 3.4 Hz, H-1″b), 2.09 (3H, s, H-3″), 1.65 (6H, brs, H-4‴), 1.62 (6H, brs, H-4‴); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 207.7 (C, C-2‴), 149.9 (C, C-7), 148.7 (C, C-8), 146.8 (CH, C-2‴), 146.5 (CH, C-2‴), 144.8 (C, C-3′), 144.1 (C, C-2′), 135.7 (C, C-11), 129.7 (C, C-2), 126.7 (C, C-12a), 124.4 (C, C-1), 122.6 (CH, C-4), 121.4 (C, C-12), 121.0 (CH, C-5), 119.1 (CH, C-3), 116.4 (C, C-4′), 114.6 (CH, C-6), 112.7 (C, C-1′), 110.8 ($2CH_2$, C-3‴ and C-3‴), 59.4 (CH_3, C-7-OMe), 56.7 (CH_3, C-8-OMe), 53.2 (CH, C-9), 45.9 (CH_2, C-1″), 40.8 (CH_3, N-Me), 38.3 (C, C-1‴), 37.9 (C, C-1‴), 30.9 (CH_3, C-3″), 27.8 ($2CH_3$, C-4‴), 27.5 ($2CH_3$, C-4‴); ESIMS *m/z* 530 $[M + H]^+$; EIMS *m/z* 529 $[M]^+$ (46), 472 (19), 324 (100), 206 (16), 148 (44); HREIMS *m/z* 529.6670 (calcd for $C_{33}H_{39}O_5N$, 529.6665).

Antitrypanosomal Assay. The strain of *Trypanosoma brucei brucei* used in this study was donated by Dr. Karl Werbovetz, and the trypanocidal assays were performed at The Ohio State University College of Pharmacy. Compounds were tested for their activity against the bloodstream-form *T. b. brucei* (MITat 1.2, variant 221) axenically cultured in HMI-9 medium following a protocol reported previously.[18,19] Pentamidine and suramin standards were obtained from Sigma. The results are the mean IC_{50} values obtained for three tests on different days.

Antileishmanial Assay. The susceptibility of *L. donovani* amastigote-like parasites (WHO designation MHOM/SD/62/1S-$CL2_D$) to growth inhibition was measured in a 3-day assay using tetrazolium dye-based CellTiter reagent (Promega, Madison, WI).[18,19] The RAW 264.7 murine macrophage cell line was kept in DMEM (Gibco-BRL) containing 10% heat-inactivated fetal bovine serum (FBS), 100 μg/mL streptomycin, 100 units/mL penicillin, and 2 mM L-glutamine at 37 °C and 5% CO_2. *L. donovani* promastigotes strain 2211 and a *L. donovani* strain stably transfected with the luciferase reporter gene were kept in SDM-79 (Schneider Drosophilia Medium-79 supplemented with 10% heat-inactivated FBS and 5 mg/mL hemin) at 25 °C.[20] To monitor the impact of the compounds, we transferred parasites (1 × 10^7 promastigotes) into 5 mL of SDM containing increasing concentrations of test compounds (0–100 μM). Protozoan growth was monitored for 6 days by measuring the absorbance at 600 nm using an automated microplate reader.[21] To test the effect on macrophages, we either treated or did not treat RAW cells (1 × 10^5) with the compounds for 48 h and subjected them to the cell growth and cytotoxicity indicator alamarBlue (Biosource International, Camarillo, CA). At dose-inhibiting parasite growth, compounds showing toxicity (**3** and **4**) were not used for the treatment of *Leishmania*-infected cells in vitro. Macrophages (1 × 10^6) were then plated in six-well plates for 2 h and thereafter infected with stationary phase *L. donovani* promastigotes for 6 h in a 20:1 *L. donovani*:macrophage ratio. Noninternalized parasites were removed by PBS washing and parasite survival estimated by luciferase activity 24 h later using a MiniLumat LB 9506 portable luminometer (EG&G Berthold). [20] Experiments were performed in triplicate. Data were converted to percent parasite survival over control and are values measured 24 h after incubation with the various compounds.

Cytotoxicity Assay. Cytotoxicity was evaluated against vero cells (ATCC, Rockville, MD). The cells were grown in Eagle's minimum essential medium with Earle's balanced salt solution (ATCC) supplemented with 50 units/mL penicillin, 50 μg/mL streptomycin, and 10% fetal bovine serum (FBS). Vero cells (1000 cells/well) were seeded together with serial dilutions of the test compounds in the individual wells of 96-well plates (final volume 100 μL/well). After 72 h of incubation at 37 °C in a humidified 5% CO_2 incubator, cell viability was determined using the CellTiter reagent by adding 20 μL of assay solution to each well. After 12–14 h of incubation at 37 °C to allow color development, the absorbance of each well at 490 nm was measured in a SpectraMax Pro microplate reader.

Acknowledgment. We thank Dr. K. Werbovetz, Ohio State University College of Pharmacy, for providing parasites and means for trypanocidal, antileishmanial (amastigote form), and Vero cell testing.

Support from the CIHR in the form of an operating grant to D.S.B and M.O. is gratefully acknowledged as is the CRC for a Tier I Chair to D.S.B..

References and Notes

(1) WHO. *African trypansosomiasis (sleeping sickness)*;WHO fact sheet no. 259; World Health Organization: Geneva, Switzerland, 2001 (updated Aug 2007); http://www.who.int/mediacentre/factsheets/fs259/en/.
(2) Fairlamb, A. *Trends Parasitol.* **2003**, *19*, 488–494.
(3) WHO. 2004, http://www.who.int/ctd/chagas/burdens.htm.
(4) *Leishmaniasis:background information* **2005** http://www.who.int/leishmaniasis/en/
(5) Croft, S. L; Barrett, M. P; Urbina, J. A *Trends Parasitol.* **2005**, *21*, 508–512.
(6) Ouellette, M.; Drummelsmith, J.; Papadopoulou, B. *Drug Res. Updates* **2004**, *7*, 257–266.
(7) Bamps, P. *Flore du Congo, du Rwanda et du Burundi. Guttifere Spermatophytes*; Jardin Botanique National de Belgique: Brussels, Belgium; 1970.
(8) Trease, G. E.; Evans, W. C. *Pharmacognosy*, 11th ed.; Baillere: Tindall, London, 1978; p 99.
(9) Letouzey, R. *Constat et recueil desusages aux Cameroun*; National Herbarium of Cameroon and IRA of Yaoundé: Yaoundé, Cameroon; 1985.
(10) Nyemba, A. M.; Ngando, M. T.; Connolly, J. D.; Rycroft, D. S. *Phytochemistry* **1990**, *29*, 994–998.
(11) Fotie, J.; Bohle, D. S.; Leimanis, M. L.; Georges, E.; Rukunga, G.; Nkengfack, A. E. *J. Nat. Prod.* **2006**, *69*, 62–67.
(12) Chen, J. J.; Fang, H. Y.; Duh, C. Y.; Chen, I. S. *Planta Med.* **2005**, *71*, 470–475.
(13) Chaaib, F.; Queiroz, E. F.; Ndjoko, K.; Diallo, D.; Hostettmann, K. *Planta Med.* **2003**, *69*, 316–320.
(14) Martinez-Martinez, F. J.; Padilla-Martinez, I. I.; Hernàndez-Carlos, B.; Pérez-Guitiérrez, R. M.; Garcia-Bàez, E. V. J. *Chem. Cryst.* **2002**, 3263–3268.
(15) Iikubo, K.; Ishikawa, Y.; Ando, N.; Umezawa, K.; Nishiyama, S. *Tetrahedron Lett.* **2002**, *43*, 291–293.
(16) Decaudain, N.; Kunesch, N.; Poisson, J. *Phytochemistry* **1974**, *13*, 505–507.
(17) Bohle, D. S.; Fotie, J. *Acta Crystallogr., Sect. E* **2006**, *62*, 3537–3538.
(18) Werbovetz, K.; Brendle, J.; Sackett, D. *Mol. Biochem. Parasitol.* **1999**, *98*, 53–65.
(19) Havens, C.; Bryant, N.; Asher, L.; Lamoreaux, L.; Perfetto, S.; Brendle, J.; Werbovetz, K. *Mol. Biochem. Parasitol.* **2000**, *110*, 223–236.
(20) Roy, G.; Dumas, C.; Sereno, D.; Wu, Y.; Singh, A. K.; Tremblay, M. J.; Ouellette, M.; Olivier, M.; Papadopoulou, B. *Mol. Biochem. Parasitol.* **2000**, *110*, 195–206.
(21) White, T. C.; Fase-Fowler, F.; Van Luenen, H.; Calafat, J.; Borst, P. *J. Biol. Chem.* **1988**, *263*, 16977–16983.

NP0702281