Antiprotozoal Compounds from *Psorothamnus polydenius*

Manar M. Salem and Karl A. Werbovetz*

Division of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, The Ohio State University, Columbus, Ohio 43210

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Bioactivity-guided fractionation of the methanolic extract of *Psorothamnus polydenius* yielded the new chalcone 2,2′,4′-trihydroxy-6′-methoxy-3′,5′-dimethylchalcone (**2**), together with six other known compounds, 2′,4′-dihydroxy-6′-methoxy-3′,5′-dimethylchalcone (**1**), dalrubone (**3**), demethoxymatteucinol (**4**), eriodictyol (**5**), and photodalrubone (**6a** and **6b**). This is the first report of chalcones in *P. polydenius.* The extracts and isolated compounds were tested in vitro for their antiprotozoal activity against *Leishmania donovani* and *Trypanosoma brucei*. Chalcones **1** and **2** and dalrubone (**3**) exhibited leishmanicidal (IC_{50} 5.0, 7.5, and 7.5 μg/mL, respectively) and trypanocidal (IC_{50} 6.3, 6.8, and 21.6 μg/mL, respectively) properties. Dalrubone (**3**) displayed 6-fold selectivity for axenic *L. donovani* parasites over Vero cells. Furthermore, treatment of *L. mexicana*-preinfected macrophages with chalcones **1** and **2** and dalrubone (**3**) (12.5, 12.5, and 25 μg/mL, respectively) reduced the number of infected macrophages by at least 96% while posing no toxicity to the host cell.

The genus *Psorothamnus*, family Fabaceae, consists of nine North American species confined to the Sonoran, Chihuahuan, and Mohave deserts and deserts of the Colorado Plateau and the Great Basin. One member, *Psorothamnus polydenius* (S. Watson) Rydb.,[1] also known as *Dalea polyadenia* or the smoke bush, is a fragrant desert shrub characterized by numerous tiny orange glands scattered over the stems that give the plant its distinguishing citrus-like odor.[2] *P. polydenius* was used by the desert Native Americans for dyeing deer skins and baskets. More importantly, they used it to treat numerous ailments ranging from colds and coughs to influenza, pneumonia, tuberculosis, smallpox, and kidney problems. *P. polydenius* has thus been described as "the medicinal cure-all of the desert tribes".[3,4] Previous investigation of *P. polydenius* resulted in the isolation of the red dye dalrubone and demethoxymatteucinol.[5] However, there is no previous report of any antiprotozoal activity associated with this plant.

In our preliminary screening of 315 American and Chinese plants for antikinetoplastid properties, the ethanolic extract of *P. polydenius* exhibited significant activity and selectivity for the parasites compared to mammalian cells. Therefore, we decided to investigate the active constituents in hope of finding new antiprotozoal compounds. Herein, we report on the isolation and characterization of compounds **1**−**6** and their activity against *Leishmania* and *Trypanosoma*.

Two chalcones, 2′,4′-dihydroxy-6′-methoxy-3′,5′-dimethylchalcone (**1**) and the new 2,2′,4′-trihydroxy-6′-methoxy-3′,5′-dimethylchalcone (**2**), two flavanones, demethoxymatteucinol (**4**)[6] and eriodictyol (**5**),[7] and two benzopyran pigments, dalrubone (**3**)[8] and photodalrubone (**6a** and **6b**),[5] were isolated from the methanolic extract of *P. polydenius.* Separations were guided by bioactivity assays using *L. donovani* axenic amastigotes. The structures of the known compounds were determined by 1D and 2D NMR techniques and confirmed by comparing the physical and spectral data with those from the literature (mp, NMR, and MS). The flavanone demethoxymatteucinol (**4**) and the red pigment dalrubone (**3**) represented the major metabolites in the extract. However, it appears that the amount of the red pigment dalrubone varies with the environmental conditions and time of collection. The plant collected in September 2002, after a relatively hot, dry summer, contained only traces of dalrubone, while the same plant collected in June 2003 in a rainy season contained copious amounts of the dye that imparted the methanolic extract with its characteristic dark reddish color. The demethoxymatteucinol content, however, was comparable in both collections.

The NMR data for compound **1** (see Table 1) confirmed a chalcone structure with a nonsubstituted A ring and a highly substituted B ring with two methyls, two hydroxyls, and a methoxy group. The methoxy group was assigned to the 6′ position on the basis of the HMBC correlation between the methoxy singlet at δ 3.66 and the C-6′ peak at δ 158.4, which was in turn correlated to the 5′-methyl protons at δ 2.16. Furthermore, the position of the methoxy group was confirmed by an NOE experiment where the OMe resonance at δ 3.66 was irradiated. The resulting NOE difference spectrum showed an enhancement of only one methyl signal (at δ 2.16) and the α-olefenic proton. Conversely, irradiation of the α-olefenic proton signal at δ 7.99 resulted in enhancement of the OMe signal at δ 3.66. This established the assignment of each atom within the molecule. The spectroscopic data for chalcone **1** are given in Table 1 for comparison with novel chalcone **2** (see following) and because of some disagreement among literature data;[9−11] this is the first report of chalcone **1** in *P. polydenius*.

Chalcone **2** was also isolated from the flavonoid fraction eluted from Sephadex LH-20 by preparative HPLC. It showed a ^{1}H NMR spectrum similar to **1** except for a broad hydroxyl peak at δ 5.35 integrating to two protons and the A ring possessing only four aromatic protons, indicating disubstitution. The ^{1}H−^{1}H COSY spectrum showed that the four aromatic protons are adjacent, indicating an *o*-substituted aryl ring. HRESIMS gave $[M + Na]^+$ at *m/z* 337.1050 consistent with the proposed molecular formula $C_{18}H_{18}O_5$. The HSQC and HMBC spectral data confirmed the proposed structure. The methyl group at δ 2.14 was assigned to the C-3′ position on the basis of its HMBC correlation to the C-2′ and C-4′ carbons, while the δ 2.15 methyl was assigned to the C-5′ position on the basis of its correlation to the C-4′ and C-6′ carbons. The methoxy

* To whom correspondence should be addressed. Tel: (614) 292-5499. Fax: (614) 292-2435. E-mail: werbovetz.1@osu.edu.

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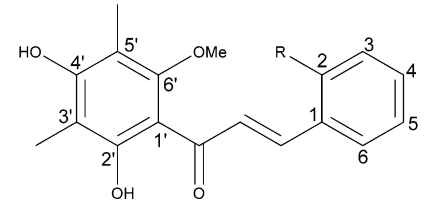
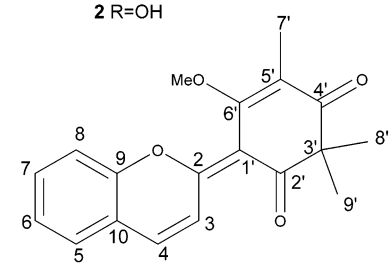
Table 1. NMR Data for Chalcones **1** and **2** in $CDCl_3$

chalcone **1**				chalcone **2**			
position	δ_C	δ_H (mult.; J_{HH})	HMBC	position	δ_C	δ_H (mult.; J_{HH})	HMBC
1	134.9			1	122.7		
2	127.9	7.65 (d; 7.2)	C-4, β	2	155.0		
3	128.4	7.41 (m)	C-1	3	116.4	6.84 (d; 8.0)	C-1, 5
4	129.7	7.41 (m)	C-2, 6	4	131.4	7.26 (ddd, 1.5, 7.3, 8.0)	C-2, 6
5	128.4	7.41 (m)	C-1	5	121.3	6.98 (dd; 7.3, 7.6)	C-1, 3
6	127.9	7.65 (d; 7.2)	C-4, β	6	129.2	7.62 (dd; 1.5, 7.6)	C-2, 4, β
α	126.2	7.99 (d; 15.7)	C-1, β, CO	α	127.6	8.05 (d; 15.8)	C-1, β, CO
β	142.4	7.84 (d; 15.7)	C-1, 2, 6, α, CO	β	137.8	8.15 (d; 15.8)	C-1, 2, 6, α, CO
1′	108.6			1′	109.2		
2′	161.6			2′	162.1		
OH-2′		13.60 (s)	C-1′, 2′, 3′	OH-2′		13.67 (s)	C-1′, 2′, 3′
3′	106.1			3′	106.5		
4′	158.7			4′	159.1		
5′	108.4			5′	108.8		
6′	158.4			6′	158.9		
CH_3-3′	7.1	2.14 (s)	C-2′, 3′, 4′	CH_3-3′	7.6	2.14 (s)	C-2′, 3′, 4′
CH_3-5′	7.7	2.16 (s)	C-4′, 5′, 6′	CH_3-5′	8.2	2.15 (s)	C-4′, 5′, 6′
OCH_3-6′	61.9	3.66 (s)	C-6′	OCH_3-6′	62.5	3.67 (s)	C-6′
CO	192.9			CO	193.7		



1 R= H
2 R=OH



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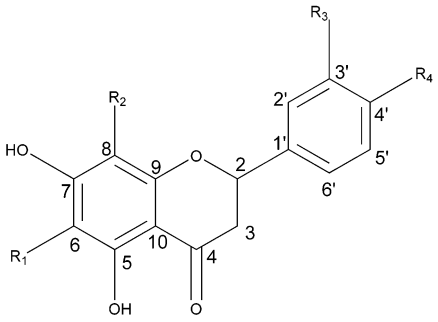
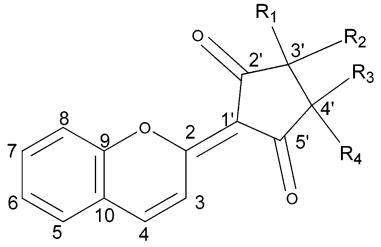


4 R_1=R_2=Me, R_3=R_4=H
5 R_1=R_2=H, R_3=R_4=OH



6a R_1 and R_2=OH and Ac, R_3=R_4=Me
6b R_1=R_2=Me, R_3 and R_4=OH and Ac

Table 2. IC_{50} Values[a] (μg/mL) of Compounds **1**−**5** against Axenic *L. donovani*, *T. brucei*, and Mammalian Cell Lines

compound	*L. donovani* axenic amastigotes	*T. b. brucei* variant 221	Vero cells	J774 A.1 macrophages
1	5.0 ± 1.3	6.3 ± 1.1	12.9 ± 0.1	7.7 ± 3.3
2	7.5 ± 0.9	6.8 ± 0.5	13.3 ± 0.5	9.3 ± 3.3
3	7.5 ± 1.0	21.6 ± 5.0	44.5 ± 2.3	30.1 ± 4.7
4	>100	28.9 ± 3.0	>50	>50
5	25.0 ± 4.4	25−50	36.5 ± 4.5	32.8 ± 12.3
pentamidine	1.4 ± 0.2	0.007 ± 0.002	>50	12.9 ± 2.1
suramin	ND[b]	0.193 ± 0.039	ND[b]	ND[b]

[a] IC_{50} values are given as the mean ± SD of three determinations. [b] ND not determined.

group was assigned to the C-6′ position on the basis of the HMBC correlation between the methoxy proton singlet at δ 3.67 and the C-6′ peak at δ 158.9 ppm, which was in turn correlated to the 5′ methyl protons at δ 2.15. This is the first report of chalcone **2**.

Compounds **6a** and **6b** eluted as one broad peak from preparative HPLC and showed a ^{13}C NMR spectrum similar to dalrubone (**3**) in the aromatic region with doubling of the peaks. The doubled peaks in the ^1H NMR spectrum consistently displayed slight differences in intensity and integration, suggesting the presence of two isomeric forms rather than a dimeric structure. HRESIMS gave $[M + Na]^+$ at *m/z* 335.0909, consistent with a molecular formula $C_{18}H_{16}O_5$. The HSQC and HMBC data confirmed the photodalrubone structure (**6a** and **6b**), which was proposed earlier by Dreyer[5] as a photodegradation product of dalrubone (**3**). **6a** and **6b** are isomeric structures that may result from rotation around the C-2−C-1′ single bond in the enol tautomer and thus were not separated.

The antiparasitic activities of compounds **1**−**5** were evaluated against *L. donovani*, the causative agent of visceral leishmaniasis, and *T. brucei brucei*, a *Trypanosoma* subspecies related to the causative agent of African sleeping sickness (see Tables 2 and 3). Compounds **1** and **2** exhibited significant activity against both parasites, with IC_{50} values in the range 5.0−7.5 μg/mL, while dalrubone (**3**) was about 3 times more active against *L. donovani* than *T. brucei brucei* (IC_{50} 7.5 and 21.6 μg/mL, respectively). Moreover, compounds **1**−**3** displayed 2.6-, 1.8- and 5.9-fold selectivity, respectively, against the *Leishmania* parasites compared to African Green Monkey kidney (Vero) cells. Dalrubone (**3**) showed low toxicity to the malignant J774A.1

Table 3. Antileishmanial Activity of Compounds **1**−**3** and **5** against Intracellular *L. mexicana*

compound	concentration (μg/mL)	% reduction in infection[a]
1	12.5	96 ± 2
	6.3	61 ± 13
	3.1	28 ± 3
2	12.5	96 ± 2
	6.3	34 ± 11
	3.1	6 ± 6
3	25	97 ± 3
	12.5	41 ± 10
	6.3	1 ± 8
5	50	76 ± 13
	25	55 ± 16
	12.5	30 ± 8
amphotericin B	0.116	95 ± 5
	0.029	85 ± 13
	0.014	59 ± 22

[a] The % decrease of infected macrophages in treated vs non-treated wells.

macrophages compared to chalcones **1** and **2** (IC_{50} 30.1, 7.7, and 9.3 μg/mL, respectively). In addition, intracellular *Leishmania mexicana* amastigotes were essentially cleared at concentrations of 12.5, 12.5, and 25 μg/mL of compounds **1**−**3**, respectively, in murine peritoneal macrophages without harming the host mammalian cells.

P. polydenius is worthy of further consideration as an inexpensive herbal remedy for leishmaniasis, as evidenced by the in vitro results. The major active metabolite, dalrubone (**3**), can be easily isolated in reasonable amounts from the aerial parts of the plant and was shown for the first time to possess significant activity and selectivity toward the *Leishmania* parasite.

Experimental Section

General Experimental Procedures. Melting points were measured on a Thomas-Hoover capillary melting point apparatus and were uncorrected. Optical rotations were determined on a Perkin-Elmer 241 polarimeter using a 100 mm glass microcell. UV−vis spectra were taken in methanol using a SPECTRAmax PLUS spectrophotometer (Molecular Devices, Sunnyvale, CA). IR spectra were obtained in KBr on a Nicolet Protégé 460 FT-IR spectrophotometer. Nuclear magnetic resonance (NMR) spectra were obtained on Bruker 250, 300, and 600 MHz spectrometers using the solvents $CDCl_3$, methanol-d_4, and acetone-d_6 (Sigma) with TMS as the internal standard. ^{1}H−^{1}H COSY, HSQC, and HMBC NMR spectra were obtained using standard Bruker pulse sequences. All accurate mass experiments were performed on a Micromass ESI-Tof II (Micromass, Wythenshawe, UK) mass spectrometer. Column chromatography was conducted using silica gel 60 (63−200 μm particle size) from EM Science or Sephadex LH-20 from Amersham Biosciences. Silica gel 60 H (15−45 μm particle size) from EM Science was used for vacuum-liquid chromatography (VLC). Precoated TLC silica gel 60 F_{254} plates from EM Science were used for thin-layer chromatography (0.25 and 2 mm layer thickness for analytical and preparative TLC, respectively). Spots were visualized using either anisaldehyde/sulfuric acid reagent or Natural Products reagent.[12] HPLC runs were carried out using a System Gold model 127 pump equipped with a model 166 UV detector (Beckman) and 4.6 × 250 mm or 10 × 250 mm C18-A Polaris columns (Varian) for analytical or semipreparative runs, respectively.

Plant Material. *Psorothamnus polydenius* (Fabaceae) was initially collected from Washoe Co., NV, Highway 50, south of Highway 395 between Fenley and Benton Springs at an elevation of approximately 5000 ft in July 1998 by Dr. Richard Spjut (Spjut 14343, WBA 3643), World Botanical Associates (Bakersfield, CA). A second collection was made near the same location in September 2002 (Spjut 14964, WBA-4401-11). A third collection of the plant was made from Inyo Co., CA, Owens Valley near Lone Pine just off Highway 193 at an elevation of ~3600 ft in June 2003 (Spjut 15358, WBA-4842-22). Voucher specimens of all collections were deposited at the U.S. National Herbarium, Smithsonian Institution, Washington, DC. Additional vouchers for WBA-4401-11 and WBA-4842-22 were deposited at the herbaria of the Botanical Research Institute of Texas (BRIT) and the World Botanical Associates (WBA, Bakersfield, CA).

Extraction and Isolation. The dried and powdered twigs with leaves and flowers (6.4 kg) were extracted with 95% EtOH. A portion (446 g) of the dried extract was suspended in water and extracted with CH_2Cl_2. The dried CH_2Cl_2 fraction (223.6 g) was suspended in 90% MeOH and extracted with hexane to give a 90% MeOH fraction (F001, 169.2 g) and a hexane fraction (F002, 59.0 g). The water layer was further extracted with EtOAc to give, after drying, an EtOAc fraction (F003, 6.8 g), a water fraction (F004, 194.3 g), and an insoluble fraction (F005, 10.7 g). The antileishmanial activities of F001, F002, F003, F004, and F005 in the axenic amastigote assay were 24, 45, >200, >200, and >200 μg/mL, respectively. F001 was further investigated on the basis of the high bioactivity. A portion of F001 (46.0 g) was subjected to silica gel (440 g) column chromatography eluted with a gradient mixture of $CHCl_3$−MeOH (1:0 → 0:1, 1 L per fraction) to give nine fractions, A−I. Demethoxymatteucinol (**4**; 970 mg) was crystallized directly from fraction A in EtOAc−hexane. Crude dalrubone (**3**) in fraction B (2.5 g, eluted with $CHCl_3$) was purified over a silica gel (71 g) column using EtOAc−hexane as the solvent system. Purified dalrubone resisted crystallization except after additional purification by preparative HPLC using the solvent system 60% MeOH in water to give dalrubone (**3**; t_R 26 min, 517 mg equivalent to 1.1% of the MeOH fraction), which was crystallized from EtOH (130 mg). The actual amount of dalrubone (**3**) in the MeOH-soluble extract is estimated at 4−5%. F002, the hexane-soluble extract, was found to contain an additional quantity of dalrubone that was responsible for the bioactivity of that fraction.

In an alternative procedure, another portion of F001 (21.0 g) was chromatographed over Sephadex LH-20 eluting with MeOH at a flow rate of 1 mL/min to give five fractions, F006−F010. F007−F010 showed activity in the bioassay (IC_{50}'s of 20.2, 11.7, 11.8, 23.0 μg/mL, respectively). VLC of F007 yielded oleanolic acid,[13] which was inactive in our assay. F008 (2.6 g) and F009 (3.1 g) contained crude dalrubone, while F010 (1.6 g) contained a mixture of flavonoids dominated by the flavanone demethoxymatteucinol (**4**). F010 (1.58 g) was subjected to VLC using a solvent gradient of EtOAc in $CHCl_3$ and then a gradient of MeOH in EtOAc (100 mL per fraction). Fractions were pooled on the basis of the TLC behavior to give 11 fractions, F011−F021. Demethoxymatteucinol (**4**, 329 mg) was separated by crystallization from F013 (454 mg) as pale yellow crystals from EtOAc−MeOH. The mother liquor left after separation of demethoxymatteucinol crystals was subjected to preparative HPLC using a solvent system composed of 70% MeOH in water with 0.05% AcOH to give chalcone **1** at t_R 33 min (22 mg) and an additional quantity of demethoxymatteucinol at t_R 17 min (**4**, 43 mg). F016 (134 mg) was applied to a preparative TLC plate and eluted with the solvent system toluene−EtOAc−AcOH (20:10:1). The band with $R_f \approx 0.63$ was separated and subjected to preparative HPLC with a gradient of 50% solvent B in A (MeOH and water, respectively, each with 0.05% AcOH) increased to 80% in 60 min to elute chalcone **2** at t_R 41 min (4.0 mg). The band with $R_f \approx 0.2$ was separated and subjected to preparative HPLC with a gradient of 40% solvent B in A increased to 45% in 20 min to elute a mixture of compounds **6a** and **6b** as one peak (t_R 14 min, 4.2 mg). F019 (206 mg, eluted with 50% EtOAc in $CHCl_3$ from VLC) was subjected to preparative HPLC using a gradient of 40% solvent B in A increased to 60% B in 40 min to give eriodictyol (**5**, 19 mg) at t_R 18 min.

2′,4′-Dihydroxy-6′-methoxy-3′,5′-dimethylchalcone (1): orange needles; mp 120−122 °C (lit.[10] 126−128 °C); UV and IR data consistent with literature values;[9−11] ^{1}H, ^{13}C, and

HMBC NMR data, see Table 1; HRESIMS *m/z* 321.1106 [M + Na]$^+$ (calcd for $C_{18}H_{18}O_4Na$, 321.1103).

2,2′,4′-Trihydroxy-6′-methoxy-3′,5′-dimethylchalcone (2): orange powder; mp 155−160 °C (dec); UV (MeOH) λ_{max} (log ϵ) 300 sh (4.16), 366 (4.35); IR ν_{max} (KBr) 3390 (br OH), 2927, 1621, 1556, 1539, 1456, 1350, 1165, 1110 cm^{-1}; ^{1}H, ^{13}C, and HMBC NMR data, see Table 1; HRESIMS *m/z* 337.1050 [M + Na]$^+$ (calcd for $C_{18}H_{18}O_5Na$, 337.1052).

Dalrubone (3): dark red plates; mp 99−100 °C (lit.[8] 98−100 °C); UV, IR, and ^{1}H and ^{13}C NMR data consistent with literature values.[8]

Demethoxymatteucinol (6,8-dimethylpinocembrin) (4): pale yellow needles; mp 201−202 °C (lit.[6] 211 °C); $[\alpha]_D$ −57.4 ° (*c* 0.5, MeOH at 24 °C); UV, IR, and ^{1}H and ^{13}C NMR data consistent with literature values;[6] HRESIMS *m/z* 307.0960 [M + Na]$^+$ (calcd for $C_{17}H_{16}O_4Na$, 307.0946).

Eriodictyol (5): white powder; mp 263−265 °C (dec, lit.[14] 268 °C); $[\alpha]_D$ −21.5° (*c* 1.0, MeOH at 25 °C); UV, IR, and ^{1}H and ^{13}C NMR data consistent with literature values;[7,13] HRESIMS *m/z* 311.0522 [M + Na]$^+$ (calcd for $C_{15}H_{12}O_6Na$, 311.0532).

Photodalrubone (6a and 6b): dark yellow residue; ^{1}H and ^{13}C NMR data consistent with literature values;[5] HRESIMS *m/z* 335.0909 [M + Na]$^+$ (calcd for $C_{18}H_{16}O_5Na$, 335.0895).

Antileishmanial Assay Using Axenic Amastigotes. The antileishmanial activity of the isolated compounds was tested in vitro against *L. donovani* amastigote-like parasites (WHO designation: MHOM/SD/62/1S-CL2$_D$) in a three-day assay using the tetrazolium dye-based CellTiter reagent (Promega) as described previously.[15,16]

Antitrypanosomal Assay. Compounds were tested for their activity against bloodstream-form *T. brucei brucei* (MITat 1.2, variant 221) axenically cultured in HMI-9 medium as described earlier[17] with minor modification. Briefly, 100 μL of late log phase parasites was incubated in 96-well plates (Costar) at an initial concentration of 10^5 cells/mL with or without test compounds at 37 °C in a humidified 5% CO_2 atmosphere for 72 h. A 25 μL portion of a 5 mg/mL solution of MTT (prepared in phosphate-buffered saline and filter sterilized) was then added to each well, and plates were reincubated at 37 °C as before for 2 h. A 100 μL portion of 10% SDS lysis buffer (prepared in 50% aqueous DMF) was added to each well, and plates were incubated as before for an additional 3−4 h. Optical densities were then measured at 570 nm using a SpectraMax Plus microplate reader. IC_{50} values, the concentration of the compound that inhibited cell growth by 50% compared to untreated control, were determined with the aid of the software program SoftMax Pro (Molecular Devices) as mentioned previously.[14]

Cytotoxicity Assay. Cytotoxicity was evaluated against two cell lines, Vero cells and J774 A.1 macrophages, obtained from the American Type Culture Collection (ATCC, Rockville, MD). Vero 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. The growth medium used for J774 A.1 cells is Dulbecco's modified Eagle's medium (DMEM, from ATCC) with the same supplements as for Vero cells. Cells (1000 Vero cells/well or 5000 J774 A.1 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 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 incubation at 37 °C to allow for color development, the absorbance of each well at 490 nm was measured in a SpectraMax Pro microplate reader as described previously.

***Leishmania*-Infected Macrophage Assay.** Mouse peritoneal macrophages, obtained from 5−6-month-old female mice (C57BL/6 strain),[18] were mixed with late log phase *L. mexicana* (WHO designation: MNYC/BCZ/62/M379) promastigotes to give a suspension containing 5×10^5 macrophages/mL and 50 $\times 10^5$ promastigotes/mL in DMEM supplemented with 4 mM L-glutamine, 50 units/mL penicillin, 50 μg/mL streptomycin, and 10% fetal bovine serum. A 200 μL aliquot of that suspension was then pipetted into the individual wells of a 16-well glass chamber slide (Lab-Tek). Infection and attachment of the macrophages were allowed to occur over a period of 24 h at 33 °C in a humidified 5% CO_2 incubator. Wells were washed with Hank's balanced salt solution (HBSS) to remove extracellular parasites, and then serial dilutions of drugs in supplemented DMEM were added to each well. After incubation for 72 h, the medium was discarded, the growth chamber was removed, and the slides were thoroughly washed with phosphate-buffered saline (PBS). The cell films were immediately fixed for 5 s in methanol and stained with 0.04% Giemsa stain (Fisher) for 35 min. After thorough washing in flowing tap water, the slides were allowed to air-dry. The percentage of infected cells was determined after examination of at least 200 cells in each sample by oil immersion microscopy.

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Supporting Information Available: The ^{1}H and ^{13}C NMR spectra for compound **2**; the physical and spectral data for compounds **3**−**6**. This material is available free of charge via the Internet at http://pubs.acs.org.

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