

Isoflavonoids and Other Compounds from *Psoralea arborescens* with Antiprotozoal Activities

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Bioactivity-guided fractionation of the root extract of *Psoralea arborescens* yielded the new isoflavone 5,7,3',4'-tetrahydroxy-2'-(3,3-dimethylallyl)isoflavone (**1a**) and the new 2-arylbenzofuran 2-(2'-hydroxy-4',5'-methylenedioxyphenyl)-6-methoxybenzofuran-3-carbaldehyde (**2**), together with seven known compounds, including three isoflavones, fremontin (**3a**), glycyrrhisoflavone (**4a**), and calycosin (**5**), two pterocarpan, maackiain (**6**) and 4-hydroxymaackiain (**7**), one triterpene, oleanolic acid (**8**), and one chalcone, isoliquiritigenin (**9**). In addition, the structure of the isoflavone fremontin was revised using spectroscopic and chemical methods and was assigned the new structure **3a**. The isoflavone **1a** and the chalcone **9** displayed leishmanicidal activity with IC₅₀ values of 13.0 and 20.7 μ M, respectively, against *Leishmania donovani* axenic amastigotes. Calycosin (**5**) exhibited selective toxicity against *Trypanosoma brucei brucei* (IC₅₀ 12.7 μ M) compared to *L. donovani* amastigotes and Vero cells (IC₅₀ 100 and 159 μ M, respectively). These results prompted us to test a small group of structurally related isoflavones for their antitrypanosomal activities. Genistein and 7,3',4'-trihydroxyisoflavone displayed promising activity (IC₅₀ values 4.2 and 7.1 μ M, respectively) and selectivity (IC₅₀ versus Vero cells: 32.9 and 135 μ M, respectively). These studies suggest that the isoflavone skeleton deserves further investigation as a template for novel antileishmanial and trypanocidal compounds.

The genus *Psoralea* (name from the Greek *psoros* and *thamnus*, meaning scurfy or scab shrub) belongs to the legume family (Fabaceae). It was shown in our laboratory to be a rich source of antiparasitic compounds.¹ The genus includes nine species found in the deserts of southwestern North America.² Previously, we isolated antileishmanial and trypanocidal compounds from *P. polydenius* (S. Watson) Rydberg that displayed selectivity for these organisms.¹ The plant *P. arborescens* (Torrey ex A. Gray) Barneby var. *minutifolia* (Parish) Barneby, Fabaceae-Papilionoideae^{2,3} [synonym *Dalea fremontii* Torrey ex A. Gray var. *minutifolia* (Parish) L. Benson]⁴ is also known as Mojave dalea⁵ or indigo bush.⁶ This plant is a small, thorny desert shrub up to 1 m in height with deep indigo blue or violet flowers and is locally common through the west-central and north Mojave Desert, California, growing chiefly on granitic and volcanic bedrock.^{2,5} This species has not been investigated before, and there are no previous reports of any antiparasitic activity associated with this plant. The closely related *P. fremontii* (*D. fremontii*), which occurs in California only on the sedimentary ranges of the far eastern Mojave Desert,² is reported to have been used by the indigenous tribes to stop internal hemorrhage and for the treatment of stomach problems.⁷

P. arborescens extracts exhibited significant activity against *Leishmania donovani* axenic amastigotes and bloodstream form *Trypanosoma brucei brucei*. Bioassay-guided fractionation of the ethanolic root extract from this plant resulted in the isolation of several bioactive compounds. In this paper, we report on the antiprotozoal activities of compounds **1a–9** and the structure elucidation of compounds **1a** and **2**. The structure of fremontin, an isoflavone previously isolated from *P. fremontii*,⁸ has also been revised.

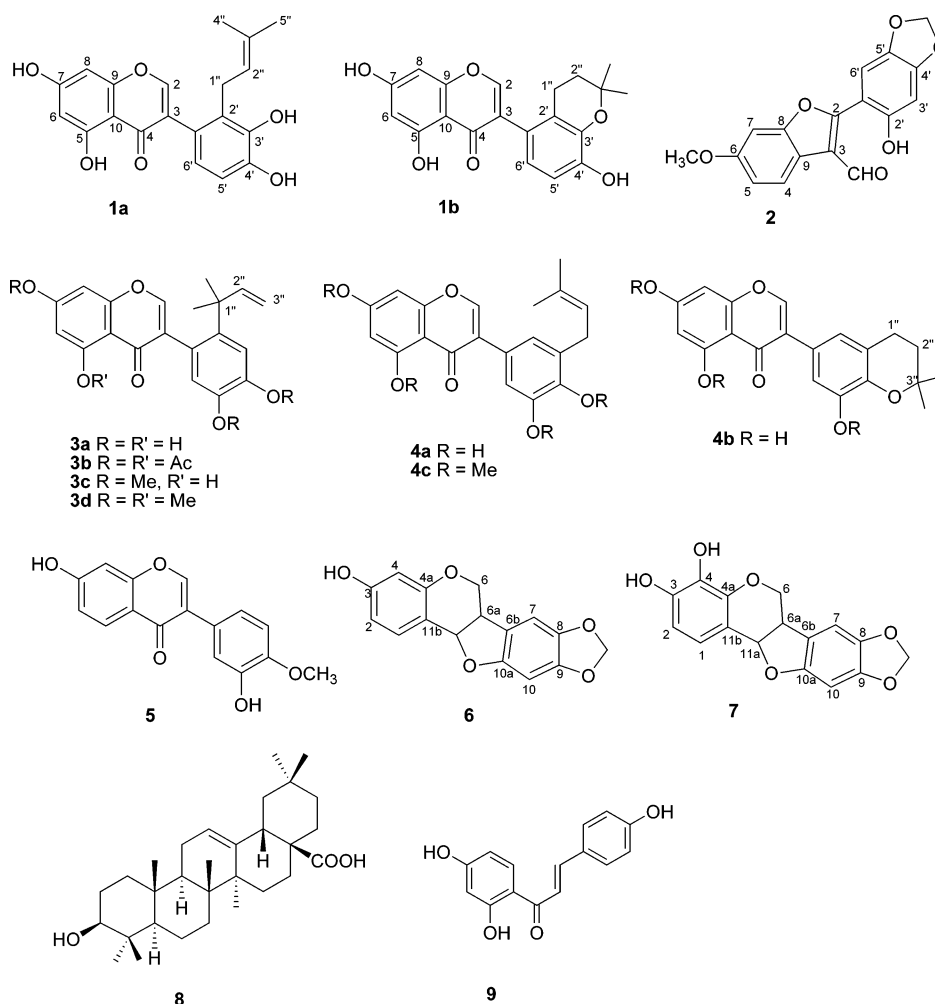
Results and Discussion

Bioactivity-guided fractionation of the ethanolic extract of *P. arborescens* yielded four isoflavones, the new 5,7,3',4'-tetrahydroxy-2'-(3,3-dimethylallyl)isoflavone (**1a**), fremontin (**3a**),⁸ glycyrrhisoflavone (**4a**),⁹ and calycosin (**5**),^{10–13} a new 2-arylbenzofuran, 2-(2'-hydroxy-4',5'-methylenedioxyphenyl)-6-methoxybenzofuran-3-carbaldehyde (**2**), the pterocarpan maackiain (**6**)^{14–17} and 4-hy-

droxymaackiain (**7**),^{17,18} the triterpene oleanolic acid (**8**),^{10,19} and the chalcone isoliquiritigenin (**9**).^{20,21} The structures of the known compounds were determined by 1D and 2D NMR techniques and confirmed by comparing the physical and spectroscopic/spectrometric data with those from the literature (NMR and MS). Compound **1a** isolated from *P. arborescens* had a ¹H NMR spectrum (see Table 1) showing a hydrogen-bonded OH group at δ 13.00, a downfield singlet at δ 7.89, two *o*-coupled aromatic protons at δ 6.56 and 6.76 (J = 8.0 Hz), two *m*-coupled aromatic protons at δ 6.29 and 6.43 (J = 1.3 Hz), and a methylene group at δ 3.30 displaying ¹H–¹H COSY correlation to a downfield methine at δ 5.08, in addition to two methyls at δ 1.43 and 1.52. The ¹³C and DEPT NMR spectra showed a carbonyl at δ 182.0, six oxygenated carbons in the aromatic region at δ 165.0, 163.8, 159.2, 155.2, 145.6, and 144.2, and three methines at δ 124.4, 123.0, and 113.1, in addition to two relatively upfield methines at δ 99.8 and 94.5, one methylene at δ 27.3, two methyls at δ 25.6 and 17.6, and five quaternary carbons at δ 130.8, 129.2, 125.3, 123.9, and 106.1. HRESIMS confirmed the deduced molecular formula (measured [M + Na]⁺ 377.1000, calcd 377.1001 for C₂₀H₁₈NaO₆). The NMR and MS data indicated an isoflavone structure with a 5,7-dihydroxylated A-ring and a 2',3',4'-trisubstituted B-ring with two hydroxyls and one isoprenyl group. This was supported by an HMBC correlation between H-2 (δ 7.89) and C-1', which is correlated to H-5' and H-1''. H-6' showed three-bond HMBC correlations to C-3 and C-2'. H-1'' and H-2'' of the isoprenyl group correlated to C-2', thus establishing the position of the isoprenyl group at C-2'. Further confirmation was provided via a selective NOE experiment in which H-2 showed NOE correlations to H-6', H-1'', and H-2''. Assignment of ring B oxygenated carbons is based on the HMBC correlation of H-1'' to C-3' and that of H-6' to C-4'. Furthermore, acid-catalyzed cyclization of the 2'-isoprenyl group gave the dihydrobenzopyran derivative **1b**, verifying the presence of an OH group at the 3' position. Further evidence against the presence of an OH group in the 2' position is the relatively downfield position of the OH-5 signal (δ 13.0); a 2'-OH group in an isoflavone structure would be expected to exert a significant shielding effect on the 5-OH, resulting in a more upfield chemical shift (δ 12.51–12.79).^{22,23} Thus compound **1a** was identified as the new 5,7,3',4'-tetrahydroxy-2'-(3,3-dimethylallyl)isoflavone. The isoflavanone arizonanol D, isolated from the root of *Sophora*

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Chart 1

**Table 1.** ^1H and ^{13}C NMR Data and HMBC Correlations for Compounds **1a** and **1b** in Acetone- d_6

position	1a			1b		
	δ_{C}	δ_{H} (mult.; J_{HH} , Hz)	HMBC	δ_{C}	δ_{H} (mult.; J_{HH} , Hz)	HMBC
2	155.2	7.89 (s)	C-3, 4, 9, 10 ^a , 1'	155.5	8.02 (s)	C-3, 4, 9, 1'
3	125.3			124.9		
4	182.0			181.5		
5	163.8			163.9		
6	99.8	6.29 (d; 1.3)	C-4 ^a , 5, 7, 8, 10	99.9	6.30 (d; 1.3)	C-4 ^a , 5, 7, 8, 10
7	165.0			165.1		
8	94.5	6.43 (d; 1.3)	C-4 ^a , 6, 7, 9, 10	94.6	6.44 (d; 1.3)	C-4 ^a , 6, 7, 9, 10
9	159.2			159.3		
10	106.1			106.0		
1'	123.9			122.9 ^b		
2'	129.2			122.7 ^b		
3'	144.2			142.4		
4'	145.6			147.5		
5'	113.1	6.76 (d; 8.0)	C-1', 2' ^a , 3'	112.7	6.70 (d; 8.0)	C-1', 3'
6'	123.0	6.56 (d; 8.0)	C-3, 2', 4', 1'' ^a	122.8	6.64 (d; 8.0)	C-3, 2', 4'
1''	27.3	3.30 (d; 6.0)	C-1', 2', 3', 2'', 3''	21.3	2.66 (br t; 6.8)	C-1', 2', 3', 2'', 3''
2''	124.4	5.08 (m)	C-2', 1'', 4'', 5''	33.3	1.78 (t; 6.8)	C-2', 1'', 3'', 4'', 5''
3''	130.8			75.2		
4''	17.6	1.43 (s)	C-2'', 3'', 5''	26.8	1.35 (s)	C-2'', 3'', 5''
5''	25.6	1.52 (s)	C-2'', 3'', 4''	26.8	1.35 (s)	C-2'', 3'', 4''
5-OH		13.00 (s)	C-5, 6, 10		12.98 (s)	C-5, 6, 10

^a Weak four-bond HMBC correlations. ^b Entries with the same superscript are interchangeable.

arizonica (Fabaceae), possesses a B-ring structure similar to that found in compound **1a**, and their ^1H and ^{13}C NMR data are in agreement.²⁴ The structure of compound **1a** is also closely related to that of the isoflavone piscidone isolated from the root bark of the Jamaican dogwood *Piscidia erythrina* (Fabaceae).²³

The new 2-arylbenzofuran **2** exhibited a ^1H NMR spectrum (see Table 2) showing a downfield singlet at δ 10.00, a 1,2,4-trisubstituted benzene ring with protons at δ 7.50 (d, J = 8.4 Hz), 6.66 (dd, J = 2.2 and 8.4 Hz), and 6.71 (J = 2.2 Hz), two isolated aromatic singlets at δ 7.52 and 7.16, a downfield methylene singlet

Table 2. ^1H and ^{13}C NMR Data and HMBC Correlations for Compound **2** in Acetone- d_6

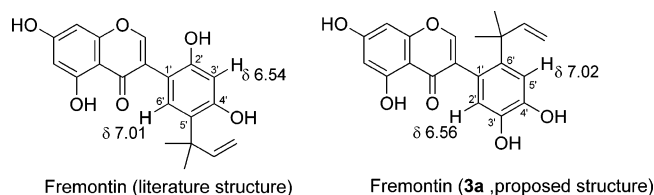
position	δ_{C}	δ_{H} (mult.; J_{HH})	HMBC
2	163.5		
3	118.3		
4	133.5	7.50 (d; 8.4)	C-6, 8
5	108.9	6.66 (dd; 2.2, 8.4)	C-6, 7, 9
6	159.9		
7	100.5	6.71 (d; 2.2)	C-5, 6, 8, 9
8	162.5		
9	109.8		
1'	119.5		
2'	150.4		
3'	94.1	7.16 (s)	C-1', 2', 5'
4'	147.8		
5'	146.7		
6'	100.9	7.52 (s)	C-2, 2', 4'
3-CHO	187.7	10.00 (s)	C-3
6-OCH ₃	56.1	3.86 (s)	C-6
-OCH ₂ O-	102.7	6.08 (s)	C-4', 5'

at δ 6.08 indicating a methylenedioxy group, and a methoxy group at δ 3.86. The ^{13}C and DEPT NMR spectra showed a carbonyl signal at δ 187.7, six oxygenated aromatic carbons at δ 163.5–146.7, five aromatic methines and three quaternary carbons at δ 133.5–94.1, one methylenedioxy group at δ 102.7, and one methoxy group at δ 56.1. The deduced molecular formula $\text{C}_{17}\text{H}_{12}\text{O}_6$ was confirmed by HRESIMS (measured $[\text{M} + \text{Na}]^+$ 335.0550, calcd 335.0532). The carbonyl signal at δ 187.7 showed HSQC correlation to the proton singlet at δ 10.00, thus indicating a carbaldehyde group that was assigned to position 3 on the basis of an HMBC correlation between the aldehydic proton and C-3. The position of the methoxy group was established by an HMBC correlation to C-6 and a NOESY correlation to H-5 and H-7. The methylenedioxy protons showed HMBC correlations to the *o*-oxygenated carbons of ring C (δ 146.7 and 147.8). The aromatic singlet at δ 7.52 was assigned to H-6' on the basis of its three-bond HMBC correlations to C-2, while that at δ 7.16 to H-3' on the basis of three-bond correlations to C-1' and 5'. Thus compound **2** was shown to be 2-(2'-hydroxy-4',5'-methylenedioxyphenyl)-6-methoxybenzofuran-3-carbaldehyde. The compound closest in structure to **2** is cicerfuran isolated from the wild chickpea *Cicer bijugum*.²⁵

Table 3. ^1H and ^{13}C NMR Assignments and HMBC Correlations for Compounds **3a** (acetone- d_6) and **3c** (CDCl_3)

position	3a			3c		
	δ_{C}	δ_{H} (mult.; J_{HH} , Hz)	HMBC	δ_{C}	δ_{H} (mult.; J_{HH} , Hz)	HMBC
2	155.0	7.79 (s)	C-3, 4, 9, 1'	153.6	7.59 (s)	C-3, 4, 9, 1'
3	126.8			126.1		
4	183.0			182.1		
5	163.7			162.6		
6	99.7	6.27 (d; 1.9)	C-5, 7, 8, 10	98.1	6.38 (d; 2.1)	C-5, 7, 8, 10
7	165.1			165.5		
8	94.4	6.39 (d; 1.9)	C-6, 7, 9, 10	92.4	6.40 (d; 2.1)	C-6, 7, 9, 10
9	159.1			158.0		
10	105.9			106.0		
1'	122.2			121.6		
2'	121.1	6.56 (s)	C-3, 3', 4', 6'	115.7	6.54 (s)	C-3, 3', 4', 6'
3'	143.5			146.9		
4'	145.7			148.7		
5'	115.4	7.02 (s)	C-1', 3', 4', 1''	111.2	7.06 (s)	C-1', 3', 4', 6', 1''
6'	140.2			140.3		
1''	42.2			42.0		
2''	150.0	6.01 (dd; 10.6, 17.6)	C-6', 1'', 4'', 5''	148.5	6.02 (dd; 10.6, 17.5)	C-1'', 4'', 5''
3''	109.4	4.77 (dd; 1.2, 17.6)	C-1'', 2''	109.8	4.81 (d; 17.5)	C-1'', 2''
		4.64 (dd; 1.2, 10.6)	C-1''		4.73 (d; 10.6)	C-1'', 2''
4''	29.4	1.29 (s)	C-6', 1'', 2'', 5''	28.9	1.37 (s)	C-6', 1'', 2'', 5''
5''	30.0	1.33 (s)	C-6', 1'', 2'', 4''	29.7	1.40 (s)	C-6', 1'', 2'', 4''
5-OH		13.01 (s)	C-5, 6, 10		12.81 (s)	C-5, 6, 10
3'-OCH ₃				55.78 ^a	3.83 (s)	C-3'
7'-OCH ₃				55.84 ^a	3.88 (s)	C-7
4'-OCH ₃				55.94 ^a	3.92 (s)	C-4'

^a Assignments are interchangeable.

**Figure 1.** Fremontin: literature and proposed structures.

The ^1H and ^{13}C NMR data (Table 3) as well as the physical data for compound **3a** closely matched those reported by Manikumar et al.⁸ for the isoflavone fremontin (see Figure 1) isolated from the related species *P. fremontii*. However, the ^{13}C NMR data showing ring B oxygenated carbons at relatively upfield positions (δ 143.1 and 147.3) seemed more consistent with the OH groups in *ortho* rather than *meta* positions to each other; oxygenated carbons with no *ortho/para* oxygenation are expected to be more downfield (δ 155–165).²⁶ Furthermore, the OH-5 signal in acetone- d_6 occurred at δ 13.00, a more downfield value than that expected for a 2'-hydroxylated isoflavone (δ 12.51–12.79),^{22,27} implying that position 2' is not hydroxylated. Tahara et al. suggested that the structure of fremontin should be reexamined on the basis of the OH-5 chemical shift²² and later proposed a possible B-ring structure with two *ortho*-OH groups at positions 3' and 4' and the isoprenyl group assigned to position 6'.²⁸ Our HMBC data (see Table 3) supported the proposed structure (Figure 1) where correlations of H-2' at δ 6.56 with C-3 and C-6', and H-5' at δ 7.02 with C-1' and -3', were observed. To shed further light on the structure of this compound, we performed selective NOE experiments to irradiate the methyl group at δ 1.33, resulting in enhancement of the H-2 and the H-5' singlets by 0.1 and 0.5%, respectively. Alternatively, irradiating the H-2 singlet enhanced the signals of the nearby isoprenyl methyl groups in the 6' positions of ring B by 0.5 and 0.6%, while irradiating H-5' strongly enhanced the isoprenyl methyls by 9.1 and 3.1%. In addition, acetylation of fremontin gave the tetraacetyl derivative **3b**, whose ^1H NMR signals for H-2' and H-5' experienced a significant downfield shift of 0.39 and 0.37 ppm, respectively, indicating that both protons are *ortho* to the acetylated OH group(s).¹¹ Since those protons are in the *para* position to each other, it follows that the only arrangement possible is that of the structure **3b**. Additional evidence came from the trimethylated

derivative **3c**, prepared by reacting fremontin with dimethyl sulfate in the presence of K_2CO_3 , followed by separating the tri- (**3c**) and tetramethylfremontin (**3d**) products. The OMe groups in **3c** at δ 3.83, 3.88, and 3.92 were assigned at the 3', 7, and 4' positions, respectively, due to HMBC correlations (see Table 3) with C-3', -7, and -4', respectively. Irradiation of H-5' at δ 7.06 resulted in enhancement of the two isoprenyl methyl signals by 3.4 and 4.1%, and only the 4'-OMe group at δ 3.92 by 6.5%, while irradiating the H-2' at δ 6.54 resulted in enhancing the 3'-OMe peak at δ 3.83 by 5.6% and the H-2 signal at δ 7.59 by 0.5%. On the other hand, irradiating the H-2 proton at δ 7.59 resulted in enhancing the H-2' signal at δ 6.54 (0.5%) and the isoprenyl group methyls at δ 1.37 and 1.40 by 0.5 and 0.7%.

Glycyrrhisoflavone (**4a**), which is closely related in structure to compound **1a**, showed 1H and ^{13}C NMR spectra consistent with those reported in the literature.⁹ However, we assigned the following signals differently due to the current availability of HMBC data. The peak at δ 6.84 is assigned to H-6' on the basis of its three-bond correlation to C-3 and C-1'', while the peak at δ 7.02 is assigned to H-2', as it shows correlation to C-3, -4', and -6'. C-5' is assigned to δ 128.8 on the basis of its correlation to H-2''. Further support for the structure was obtained in an NOE experiment where the H-2 was irradiated, resulting in enhancement of the H-6' and to a lesser extent H-2'. The cyclized (**4b**) and methylated (**4c**) derivatives were prepared for biological testing.

The NMR, UV, and IR data of maackiain (**6**) were in agreement with the literature values.^{15–17} Despite the presence of two chiral centers in the structure, only the 6a*R*,11a*R* and 6a*S*,11a*S* *cis*-configurations are sterically possible.²⁹ However, the specific rotation data did not completely match those reported for either the pure isomers or the racemate. The specific rotation is negative ($[\alpha]_D^{24} = -33.5$, $CHCl_3$), but the absolute value is much less than that reported for the optically pure (–)-isomer ($[\alpha]_D = -251.7$, $CHCl_3$).¹⁴ This suggests that the compound isolated is a mixture of (–)-6a*R*,11a*R*-maackiain and the (+)-6a*S*,11a*S*-isomer with an excess of the (–)-form. The same situation is true for the isolated 3,4-dihydroxy-8,9-methylenedioxypterocarpan (**7**), which is a 4-hydroxy analogue of maackiain (**6**). The specific rotation of compound **7** in $CHCl_3$, $[\alpha]_D^{24} = +62.2$, is less than that reported for the (+)-isomer in the same solvent ($[\alpha]_D = +154$). This, again, suggests the presence of a mixture of (–)-6a*R*,11a*R*-4-hydroxymaackiain and the (+)-6a*S*,11a*S*-isomer with an excess of the (+)-form.

The activities of the isolated compounds against *L. donovani* axenic amastigotes and *T. b. brucei*, as well as against two mammalian cell lines (Vero cells and PC-3 prostate cancer cells), are summarized in Table 4. Compounds **1a** and **9** showed significant antileishmanial and trypanocidal activities, displaying IC_{50} values below 21 μM . However, they showed low selectivity (around 3-fold) for the parasites over the mammalian Vero cells. The selectivity is worse for pterocarpan **7**, which shows higher toxicity toward Vero cells than to the parasites. On the other hand, calycosin (**5**) displayed selective toxicity (about 15-fold) toward *T. b. brucei* bloodstream forms (IC_{50} 12.7 μM) over Vero cells. Calycosin is reported to have antiparasitic,^{30,31} antileishmanial,³² and antiangiogenic effects.³³ In addition, calycosin was found to protect human umbilical vein endothelial cells from hypoxia-induced impairment.³⁴ Methylation of the isoflavones **3a** and **4a** moderately improved the trypanocidal but not the antileishmanial activity. Tetramethylglycyrrhisoflavone (**4c**) was 3.7-fold more active toward trypanosomes than the parent glycyrrhisoflavone (**4a**). Interestingly, compound **2** did not show any antikinoplast activity in our assays. Related Fabaceae-derived 2-arylbenzofurans were reported to possess antifungal²⁵ and antimalarial³⁰ activities. The trypanocidal selectivity of calycosin prompted us to assay a group of related natural isoflavones that are commercially available (see Figure 2); the results are summarized in Table 5. Genistein, a common dietary isoflavone (especially in soy-based diets), was the most potent against

Table 4. Bioactivity of Compounds **1a–9** against Axenic *L. donovani*, *T. b. brucei*, and Two Mammalian Cell Lines

compound	IC_{50} (μM) ^a			
	<i>L. donovani</i> axenic amastigotes	<i>T. b. brucei</i> variant 221	Vero cells	PC-3 cells
1a	13.0 \pm 0.8	12.1 \pm 1.9	37.5 \pm 10.2	12.7 \pm 3.1
1b	38.4 \pm 6.2	57.9 \pm 2.5	NT ^b	107 \pm 16
2	>250	>250	>250	>250
3a	123 \pm 4	75.3 \pm 5.9	134 \pm 27	78.5 \pm 27.4
3b	42.7 \pm 1.5	67.8 \pm 5.6	NT	66.2 \pm 28.5
3c	>250	32.3 \pm 2.0	NT	146 \pm 4
3d	86.0 \pm 11.0	41.7 \pm 12.2	NT	134 \pm 30
4a	46.3 \pm 2.5	33.0 \pm 2.5	52.8 \pm 11.3	62.1 \pm 7.3
4b	68.3 \pm 6.5	77.0 \pm 3.4	NT	79.3 \pm 9.6
4c	41.4 \pm 2.7	7.8 \pm 2.2	44.3 \pm 7.3	30.9 \pm 4.6
5	100 \pm 10	12.7 \pm 0.7	159 \pm 17	192 \pm 29
6	>250	130 \pm 13	>250	>250
7	37.3 \pm 1.0	3.7 \pm 0.7	3.3 \pm 1.7	28.0 \pm 10.0
8	>250	43.1 \pm 8.3	>250	211 \pm 10
9	20.7 \pm 2.3	32.8 \pm 2.0	65.6 \pm 19.5	46.4 \pm 7.8
ansamitocin P3	NT	NT	1.2 \pm 0.3 ^c	NT
pentamidine	2.3 \pm 0.4	NT	NT	NT
podophyllo-toxin	NT	NT	NT	12.5 \pm 1.1 ^c
suramin	NT	0.19 \pm 0.03	NT	NT

^a Values represent the mean \pm standard deviation of at least 3 independent experiments. ^b NT: not tested. ^c IC_{50} values in nM.

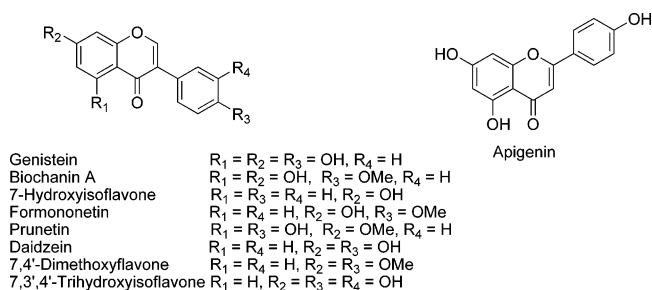


Figure 2. Structures of the additional compounds tested for antiprotozoal activity.

Table 5. Bioactivity of Selected Isoflavones against Axenic *L. donovani*, *T. b. brucei*, and Vero Cells

compound	IC_{50} (μM)		
	<i>L. donovani</i> axenic amastigotes	<i>T. b. brucei</i> variant 221	Vero cells
genistein	73.0 \pm 5.6	4.2 \pm 0.5	32.9 \pm 8.4
biochanin A	89.9 \pm 1.8	12.3 \pm 1.0	NT ^b
7-hydroxyisoflavone	113 \pm 6	94.2 \pm 4.0	NT
formononetin	NT	90.2 \pm 14.1	NT
prunetin	NT	20.2 \pm 4.3	NT
daidzein	NT	23.7 \pm 5.0	NT
7,4'-dimethoxyisoflavone	NT	>100	NT
7,3',4'-trihydroxyisoflavone	NT	7.1 \pm 1.6	135 \pm 13
apigenin	NT	11.9 \pm 0.9	NT
pentamidine	1.5 \pm 0.2	NT	NT
suramin	NT	0.183 \pm 0.025	NT

^a Values represent the mean \pm standard deviation of at least 3 independent experiments. ^b NT: not tested.

trypanosomes. This compound displayed an IC_{50} of 4.2 μM , making it 3-fold more active than calycosin (IC_{50} of 12.7 μM) on a molar basis. Past reports have shown that genistein inhibits trypanosomal protein tyrosine kinase (PTK) activity.³⁵ Later, it was shown that genistein is an efficient inhibitor of trypanosomal protein synthesis and phosphorylation (which is primarily serine and threonine phosphorylation in these organisms), indicating that the antitrypanosomal effect of this compound is not due to a specific effect on the tyrosine kinase activity.³⁶ Daidzein, a genistein analogue that is known to be inactive against PTK,³⁷ still displayed an inhibitory effect (IC_{50} of 23.7 μM) against the parasites. Genistein was also reported to show activity against both chloroquine-sensitive and -resistant *Plasmodium falciparum*.³¹ 7,3',4'-Trihydroxyisoflavone

also showed a significant antitrypanosomal effect (IC_{50} of 7.1 μ M), while being virtually nontoxic to Vero cells. Apigenin, a flavone with the same substitution pattern as that of genistein, was about 3-fold less active than genistein against *T. b. brucei*.

Our studies with compounds from *P. arborescens* and related commercially available molecules revealed several isoflavonoids with interesting antiprotozoal activities. These results indicate that the isoflavone skeleton is worthy of further investigation as a template for novel antitrypanosomal compounds.

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 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 300 and 400 MHz spectrometers using the solvents $CDCl_3$, CD_3CN , methanol- d_4 , or acetone- d_6 (Aldrich) with TMS as the internal standard. ^{13}C DEPT, 1H - 1H COSY, DPGSE-NOE (selective NOE), ^{31}P NOESY, 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 μ m particle size) from EM Science or Sephadex LH-20 from Amersham Biosciences. Silica gel 60 H (15–45 μ m particle size) from EM Science was used for vacuum-liquid chromatography (VLC). Precoated TLC silica gel 60 F₂₅₄ 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 UV light or vanillin/sulfuric acid reagent. HPLC runs were carried out using a System Gold model 127 pump equipped with a model 166 UV detector (Beckman) and 4.6 \times 250 mm or 10 \times 250 mm C18-A Polaris columns (Varian) for analytical (flow rate = 1 mL/min) or semipreparative runs (flow rate 5 mL/min), respectively. The solvents used were H_2O , MeOH, and CH_3CN , each with 0.1% HOAc, and are designated as A, B, and C, respectively. Daidzein, 7,4'-dimethoxyisoflavone, 7,3',4'-trihydroxyisoflavone, and apigenin were purchased from Alfa Aesar and genistein from Indofine Chemical Company (Somerville, NJ). Other compounds and reagents were obtained from Sigma-Aldrich.

Plant Material. *P. arborescens* var. *minutifolius* root was collected on June 11, 2003, from E. Tulare Co., California, on the eastern slopes of the southern Sierra Nevada in desert-chaparral transitional vegetation by Dr. Richard Spjut (WBA 4841-13, SPJ-15357), World Botanical Associates (Bakersfield, CA). Voucher specimens are deposited at the Botanical Research Institute of Texas (BRIT), the United States National Herbarium, the Smithsonian Institution (US), and the World Botanical Associates (WBA).

Extraction and Isolation. The dried and powdered root material (0.6 kg) was percolated with 2.9 L of 95% EtOH. The resulting gummy extract (38 g) was partitioned between H_2O and CH_2Cl_2 . The organic layer was then partitioned between 90% MeOH (F001, 12.0 g) and hexane (F002, 2.0 g). The aqueous fraction was further partitioned between EtOAc (F003, 0.5 g) and H_2O (F004, 10.4 g). The activity was concentrated in F001 (IC_{50} = 16.5 μ g/mL in the axenic amastigote assay), which was chromatographed over Sephadex LH-20 eluting with MeOH. The fractions were combined according to their TLC profile into seven pools (F006–F012). The bioactivity was found to be highest in fractions F008, F009, and F010. F008 (0.86 g) was chromatographed on a silica gel column eluting with a gradient of 20% EtOAc–hexane to 100% EtOAc. The fraction eluted with 25% EtOAc–hexane was purified using preparative HPLC with an isocratic solvent system of 90% B in A, λ_{max} 210 nm, to collect 23 mg of compound **8** (t_R 11 min). F009 (9.4 g) was subjected to silica gel chromatography using a solvent gradient from 30% EtOAc–hexane to 100% EtOAc to 25% MeOH–EtOAc to obtain nine fractions, F009-1 to F009-9. F009-4 (750 mg) was subjected to Sephadex LH-20 chromatography followed by HPLC using a gradient from 50% to 60% B in A over 30 min then isocratic 60% B in A to yield compound **2** (3.5 mg, t_R 37 min),

compound **4** (22.5 mg, t_R 53 min), and crude compound **9** (20 mg, t_R 8–12 min). The latter was purified by additional HPLC using a gradient solvent of 40% to 60% B in A in 60 min to isolate 9.1 mg of **9** (t_R = 48–54 min). F009-5 (500 mg) was chromatographed on a silica gel column using a gradient solvent of 4% EtOAc– $CHCl_3$ to 100% $CHCl_3$ to 5% MeOH– $CHCl_3$. Fractions were combined on the basis of their TLC profile to give 150 mg of crude compound **1a**, which was purified by HPLC using a solvent gradient of 50% B in A increased to 60% over 30 min then kept isocratic at 60% B, collecting fractions eluting at t_R 28–34 min to give 94 mg of pure compound **1a**. F009-8 (917 mg) was subjected to silica gel chromatography eluting with a gradient of 100% CH_2Cl_2 to 100% EtOAc. Additional silica gel column chromatography of the fraction was performed eluting with 35% EtOAc in CH_2Cl_2 (F009-8-3, 260 mg) using the isocratic solvent $CHCl_3$ –MeOH– H_2O (90:6:1, lower phase) followed by HPLC of subfraction 3 to obtain compound **5** (6 mg, isocratic 20% C in A, t_R 32 min) and subfraction 4 (50% B in A increased to 58% over 24 min, then kept isocratic, collecting fractions eluting at t_R 24–28 min) to give 42.6 mg of compound **3a**. A portion (95 mg) of F010 was purified using preparative HPLC with an isocratic solvent system of 50% solvent B in A to obtain 24 mg of compound **7** (t_R 13.5 min) and 57 mg of compound **6** (t_R 29 min).

5,7,3',4'-Tetrahydroxy-2'-(3,3-dimethylallyl)isoflavone (1a): greenish-white residue; UV (MeOH) λ_{max} (log ϵ) 210 (4.53), 260 (4.42), 285 sh (4.00) nm; IR ν_{max} (KBr) 3446, 3223 (br OH), 3081, 2976, 2927, 1704, 1654, 1619, 1570, 1492, 1444, 1360, 1304, 1200, 1051 cm^{-1} ; 1H , ^{13}C (acetone- d_6 , 300 and 75 MHz, respectively), and HMBC NMR data, see Table 1; HRESIMS m/z 377.1000 [$M + Na$] $^+$ (calcd for $C_{20}H_{18}NaO_6$, 377.1001).

Acid-Catalyzed Cyclization of Compound 1a. Compound **1a** (9.5 mg, 0.03 mmol) was stirred with 88% $HCOOH$ (1 mL) at 80 $^{\circ}C$ overnight. The product **1b** (8.0 mg, 84% yield) was purified by HPLC (50 to 100% solvent B in A in 50 min; t_R = 22 min; mp 247–249 $^{\circ}C$; 1H , ^{13}C (acetone- d_6 , 400 and 100 MHz, respectively), and HMBC NMR data, see Table 1).

2-(2'-Hydroxy-4',5'-methylenedioxyphenyl)-6-methoxybenzofuran-3-carbaldehyde (2): white residue; UV (MeOH) λ_{max} (log ϵ) 247 (4.21), 295 (3.93), 346 (4.05) nm; IR ν_{max} (KBr) 3281 (br OH), 2867, 1656, 1618, 1589, 1506, 1461, 1314, 1202, 1141, 1034, 950, 893, 802 cm^{-1} ; 1H , ^{13}C (acetone- d_6 , 400 and 100 MHz, respectively), and HMBC NMR data, see Table 2; HRESIMS m/z 335.0550 [$M + Na$] $^+$ (calcd for $C_{17}H_{12}NaO_6$, 335.0532).

Fremontin (3a): greenish-white powder; UV (MeOH) λ_{max} (log ϵ) 259 (4.37), 292 (3.99) nm; IR ν_{max} (film) 3366 (br OH), 1652, 1617, 1576, 1508, 1437, 1361, 1277, 1169 cm^{-1} ; 1H , ^{13}C (acetone- d_6 , 400 and 100 MHz, respectively), and HMBC NMR data, see Table 3; HRESIMS m/z 377.1027 [$M + Na$] $^+$ (calcd for $C_{20}H_{18}NaO_6$, 377.1001).

Acetylation of Fremontin. Fremontin (4 mg, 0.01 mmol) was acetylated with acetic anhydride (0.3 mL) in pyridine (0.3 mL) by stirring at room temperature overnight. Fremontin tetraacetate (**3b**, 3.5 mg, 59% yield) was purified by HPLC (gradient B in A, 70 to 100% in 30 min; t_R = 7.5 min; mp 195–197 $^{\circ}C$ (lit.⁸ mp 195 $^{\circ}C$); 1H NMR (acetone- d_6 , 400 MHz) δ 8.00 (1H, s, H-2), 7.39 (1H, s, H-5'), 7.35 (1H, d, J = 2.2 Hz, H-8), 6.97 (1H, d, J = 2.2 Hz, H-6), 6.95 (1H, s, H-2'), 5.99 (1H, dd, J = 10.6, 17.5 Hz, H-2''), 4.83 (1H, dd, J = 1.1, 17.5 Hz, H-3''), 4.72 (1H, dd, J = 1.1, 10.6 Hz, H-3'b), 2.34, 2.29, 2.26, and 2.25 (3H each, s, $COCH_3$), 1.38 and 1.33 (3H each, s, CH_3 -4'' and -5''); ^{13}C NMR (acetone- d_6 , 100 MHz) δ 175.9 (C, C-4), 169.3, 168.8, 168.7, and 168.5 (C each, $COCH_3$), 158.6 (C, C-9), 155.2 (C, C-7), 153.6 (CH, C-2), 151.6 (C, C-5), 148.7 (CH, C-2''), 147.3 (C, C-4'), 143.1 (C, C-3'), 141.0 (C, C-6'), 130.2 (C, C-1'), 128.9 (CH, C-2'), 128.3 (C, C-3), 123.3 (CH, C-5'), 116.1 (C, C-10), 114.8 (CH, C-6), 110.8 (CH_2 , C-3''), 109.9 (CH, C-8), 42.8 (C, C-1''), 29.9 and 29.5 (CH_3 each, CH_3 -4'' and -5''), 21.0, 21.0, 20.53, and 20.45 (CH_3 each, $COCH_3$); HMBC correlations: H-2 to C-3, -4, -9, -1', H-6 to C-5, -7, -8, -10, H-8 to C-6, -7, -9, -10, H-2' to C-3, -3', -4', -6', H-5' to C-1', -3', -6', -1'', H-2'' to C-1'', -4'', -5'', H-3'' to C-1'', -2'', CH_3 -4'' to C-6', -1'', -2'', -5'', CH_3 -5'' to C-6', -1'', -2'', -4'', $COCH_3$ to $COCH_3$.

Methylation of Fremontin. Fremontin (20 mg, 0.06 mmol) in anhydrous acetone (9 mL) was refluxed for 2 h with excess K_2CO_3 (125 mg) and Me_2SO_4 (0.1 mL, 1.06 mmol) with molecular sieves (4 Å). PTLC (2% MeOH in $CHCl_3$) of the products yielded trimethylfremontin (**3c**, 10.5 mg, 47% yield), which was recrystallized from EtOAc–hexane as grayish needles (R_f 0.47; mp 163–164 $^{\circ}C$; 1H , ^{13}C

(CDCl₃, 400 and 100 MHz, respectively), and HMBC NMR data, see Table 3) and tetramethylfremontin (**3d**, 11.0 mg, 47% yield, *R_f* 0.23, yellow residue): ¹H NMR (acetone-*d*₆, 400 MHz) δ 7.62 (1H, s, H-2), 7.05 (1H, s, H-5'), 6.59 (1H, s, H-2'), 6.56 (1H, d, *J* = 2.3 Hz, H-8), 6.48 (1H, d, *J* = 2.3 Hz, H-6), 6.05 (1H, dd, *J* = 10.6, 17.6 Hz, H-2''), 4.82 (1H, dd, *J* = 1.2, 17.6 Hz, H-3'a), 4.68 (1H, dd, *J* = 1.2, 10.6 Hz, H-3'b), 3.93 (3H, s, 7-OCH₃), 3.86 (3H, s, 5-OCH₃), 3.84 (3H, s, 4'-OCH₃), 3.75 (3H, s, 3'-OCH₃), 1.38 (3H, s, CH₃-4''), 1.33 (3H, s, CH₃-5''); ¹³C NMR (acetone-*d*₆, 100 MHz) δ 176.0 (C, C-4), 164.8 (C, C-7), 162.3 (C, C-5), 160.8 (C, C-9), 151.6 (CH, C-2), 150.2 (CH, C-2''), 149.5 (C, C-4'), 147.9 (C, C-3'), 140.8 (C, C-6'), 129.7 (C, C-3), 125.1 (C, C-1'), 118.1 (CH, C-2'), 112.7 (C, C-5'), 110.5 (C, C-10), 109.5 (CH₂, C-3''), 96.7 (CH, C-6), 93.6 (CH, C-8), 56.47, 56.25, 56.21, and 56.09 (CH₃ each, OCH₃-5, 7, 3', and 4'), 42.8 (C, C-1''), 29.7 (CH₃, CH₃-4''), 28.9 (CH₃, CH₃-5''); HMBC correlations: H-2 to C-3, -4, -9, -1', H-6 to C-5, -7, -8, -10, H-8 to C-6, -7, -9, -10, H-2' to C-3, -4', -6', H-5' to C-1', -3', -1'', H-2'' to C-1', -4'', -5'', H-3'' to C-1'', -2'', CH₃-4'' to C-6', -1'', -2'', -5'', CH₃-5'' to C-6', -1'', -2'', -4'', OCH₃-3' to C-3', OCH₃-4' to C-4', OCH₃-5 to C-5, OCH₃-7 to C-7.

Glycyrrhisoflavone (4a): brownish-white residue; UV (MeOH) λ_{max} (log ε) 261 (4.48), 292 sh (4.13) nm; IR ν_{max} (film) 3373 (br OH), 1651, 1622, 1575, 1504, 1442, 1367, 1307, 1284, 1178, 1051, 838 cm⁻¹; ¹H NMR (acetone-*d*₆, 400 MHz) δ 13.07 (1H, br s, 5-OH), 8.09 (1H, s, H-2), 7.02 (1H, d, *J* = 1.2 Hz, H-2'), 6.84 (1H, d, *J* = 1.2 Hz, H-6'), 6.40 (1H, br s, H-8), 6.28 (1H, br s, H-6), 5.37 (1H, m, H-2''), 3.37 (2H, d, *J* = 7.3 Hz, H-1''), 1.73 (3H, s, CH₃-4''), 1.70 (3H, s, CH₃-5''); ¹³C NMR (acetone-*d*₆, 100 MHz) δ 181.6 (C, C-4), 165.1 (C, C-7), 163.9 (C, C-5), 159.0 (C, C-9), 154.2 (CH, C-2), 144.9 (C, C-4'), 144.2 (C, C-3'), 132.3 (C, C-3''), 128.8 (C, C-5'), 124.3 (C, C-3), 123.7 (CH, C-2''), 122.7 (C, C-1'), 122.1 (CH, C-6'), 114.7 (CH, C-2'), 106.0 (C, C-10), 99.8 (CH, C-6), 94.4 (CH, C-8), 29.0 (CH₂, C-1''), 25.9 (CH₃, CH₃-4''), 17.8 (CH₃, CH₃-5''); HMBC correlations: H-2 to C-3, -4, -9, -1', H-6 to C-5, -7, -8, -10, H-8 to C-6, -7, -9, -10, H-2' to C-3, -3', -4', -6', H-6' to C-3, -2', -4', -1'', H-1'' to C-4', -5', -6', -2'', -3'', H-2'' to C-5', 1'', -4'', -5'', CH₃-4'' to C-2'', -3'', -5'', CH₃-5'' to C-2'', -3'', -4'', HRESIMS *m/z* 377.1012 [M + Na]⁺ (calcd for C₂₀H₁₈NaO₆, 377.1001).

Acid-Catalyzed Cyclization of Glycyrrhisoflavone. Glycyrrhisoflavone (4.0 mg, 0.01 mmol) in 1 mL of HCOOH (88%) was stirred at 90 °C for 5 h. The reaction mixture was partitioned between H₂O (2 mL) and EtOAc (3 × 2 mL). After drying the organic layer, the product **4b** (2.5 mg, 63% yield) was purified by HPLC (50 to 100% B in A in 50 min; *t_R* = 25 min): ¹H NMR (acetone-*d*₆, 400 MHz) δ 13.07 (1H, br s, 5-OH), 8.13 (1H, s, H-2), 6.93 (1H, d, *J* = 2.1 Hz, H-2'), 6.84 (1H, d, *J* = 2.1 Hz, H-6'), 6.41 (1H, d, *J* = 1.9 Hz, H-8), 6.28 (1H, d, *J* = 1.9 Hz, H-6), 2.81 (2H, t, *J* = 6.7 Hz, H-1''), 1.86 (2H, t, *J* = 6.7 Hz, H-2''), 1.35 (6H, s, CH₃-4'' and -5''); ¹³C NMR (acetone-*d*₆, 100 MHz) δ 181.5 (C, C-4), 165.8 (C, C-7), 163.6 (C, C-5), 159.1 (C, C-9), 154.2 (CH, C-2), 146.6 (C, C-3'), 142.7 (C, C-4'), 124.1^a (C, C-3), 123.2^a (C, C-1'), 122.4 (CH, C-6'), 121.9 (C, C-5'), 114.3 (CH, C-2'), 105.8 (C, C-10), 100.0 (CH, C-6), 94.6 (CH, C-8), 75.7 (C, C-3''), 33.5 (CH₂, C-2''), 27.0 and 27.0 (CH₃ each, CH₃-4'' and -5''), 22.9 (CH₂, C-1'') (assignments with the same superscript may be interchanged); HMBC correlations, H-2 to C-3, -4, -9, -1', H-6 to C-5, -7, -8, -10, H-8 to C-6, -7, -9, -10, H-2' to C-3, -3', -4', -6', H-6' to C-3, -2', -4', -1'', H-1'' to C-4', -5', -6', -2'', -3'', H-2'' to C-5', 1'', -3'', -4'', -5'', CH₃-4'' and -5'' to C-2'', -3''.

Methylation of Glycyrrhisoflavone. Glycyrrhisoflavone (**4a**, 9.0 mg, 0.03 mmol) was heated to reflux with excess K₂CO₃ (280 mg) and excess Me₂SO₄ (0.5 mL, 5.3 mmol) in 9.0 mL of anhydrous acetone under 4 Å molecular sieves for 5 h. The product **4c** (6.2 mg, 59% yield) was purified by HPLC (50 to 100% B in A in 50 min; *t_R* 34 min): ¹H NMR (CDCl₃, 400 MHz) δ 7.77 (1H, s, H-2), 7.11 (1H, d, *J* = 1.9 Hz, H-2'), 6.81 (1H, d, *J* = 1.9 Hz, H-6'), 6.45 (1H, d, *J* = 2.2 Hz, H-8), 6.38 (1H, d, *J* = 2.2 Hz, H-6), 5.28 (1H, m, H-2''), 3.94 (3H, s, 5-OCH₃), 3.89 (3H, s, 7-OCH₃), 3.88 (3H, s, 3'-OCH₃), 3.82 (3H, s, 4'-OCH₃), 3.36 (2H, d, *J* = 7.2 Hz, H-1''), 1.72 (3H, s, CH₃-4''), 1.71 (3H, s, CH₃-5''); ¹³C NMR (CDCl₃, 100 MHz) δ 175.3 (C, C-4), 163.8 (C, C-7), 161.4 (C, C-5), 159.8 (C, C-9), 152.2 (C, C-3'), 150.4 (CH, C-2), 146.8 (C, C-4'), 135.2 (C, C-5'), 132.1 (C, C-3''), 127.7 (C, C-3), 126.2 (C, C-1'), 123.0 (CH, C-2''), 122.1 (CH, C-6'), 111.8 (CH, C-2'), 109.9 (C, C-10), 96.2 (CH, C-6), 92.5 (CH, C-8), 60.5, 56.4, 55.8, and 55.7 (CH₃ each, 5-, 7-, 3', 4'-OCH₃), 28.6 (CH₂, C-1''), 25.8 (CH₃, CH₃-4''), 17.8 (CH₃, CH₃-5''); HMBC correlations, H-2 to C-3, -4, -9, -1', H-6 to C-5, -7, -8, -10, H-8 to C-6, -7, -9, -10,

H-2' to C-3, -3', -4', -6', H-6' to C-3, -2', -4', -1'', H-1'' to C-4', -5', -6', -2'', -3'', H-2'' to 1'', -4'', -5'', CH₃-4'' to C-2'', -3'', -5'', CH₃-5'' to C-2'', -3'', -4'').

Calycosin (5): white residue; UV (MeOH) λ_{max} (log ε) 248 (4.44), 260 (sh 4.41), 290 (4.21) nm; IR ν_{max} (film) 3205 (br OH), 1623, 1585, 1511, 1454, 1280, 1196, 1133, 1024, 852 cm⁻¹; ¹H, ¹³C, and HMBC NMR data consistent with literature values;¹⁰⁻¹² HRESIMS *m/z* 307.0578 [M + Na]⁺ (calcd for C₁₆H₁₂NaO₅, 307.0582).

Maackiain (6): amorphous white powder; [α]_D²⁴ -33.5 (*c* 0.13, CHCl₃); UV (MeOH) λ_{max} (log ε) 213 (4.38), 286 (3.66), 310 (3.85) nm; IR ν_{max} (KBr) 3393 (br OH), 2927, 1624, 1474, 1456, 1347, 1320, 1186, 1144, 1122 cm⁻¹; ¹H, ¹³C, and HMBC NMR data consistent with literature values;¹⁴⁻¹⁷ HRESIMS *m/z* 307.0603 [M + Na]⁺ (calcd for C₁₆H₁₂NaO₅, 307.0582).

3,4-Dihydroxy-8,9-methylenedioxypterocarpan (7): amorphous white powder; [α]_D²⁴ +62.2 (*c* 0.188, CHCl₃); UV (MeOH) λ_{max} (log ε) 213 (4.66), 310 (3.85) nm; IR ν_{max} (film) 3423 (br OH), 1625, 1476, 1459, 1338, 1206, 1144, 1052, 936 cm⁻¹; ¹H, ¹³C, and HMBC NMR data consistent with literature values;^{17,18} HRESIMS *m/z* 323.0521 [M + Na]⁺ (calcd for C₁₆H₁₂NaO₆, 323.0532).

Oleanolic acid (8): amorphous white powder; [α]_D²⁴ +72; UV (MeOH) λ_{max} < 220 nm; IR ν_{max} (KBr) 3421 (br OH), 2944, 2874, 1697 (sh), 1458, 1387, 1210, 1167, 1029 1051 cm⁻¹; ¹H, ¹³C, and HMBC NMR data consistent with literature values;^{19,39} HRESIMS *m/z* 479.3501 [M + Na]⁺ (calcd for C₃₀H₄₈NaO₃, 479.3501).

Isoliquiritigenin (9): yellow powder; UV (MeOH) λ_{max} (log ε) 242 sh (3.86), 298 sh (3.78), 371 (4.30) nm; IR ν_{max} (KBr) 3357 (br OH), 1630, 1605, 1586, 1547, 1514, 1503, 1220, 1166, 1142; ¹H, ¹³C, and HMBC NMR data consistent with literature values;^{20,21} HRESIMS *m/z* 279.0647 [M + Na]⁺ (calcd for C₁₅H₁₂NaO₄, 279.0633).

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-CL_{2D}) in a three-day assay using the tetrazolium dye-based CellTiter reagent (Promega) as described previously.⁴⁰⁻⁴²

Antitrypanosomal Assay. Compounds were tested for their activity against bloodstream-form *T. b. brucei* (MITat 1.2, variant 221) axenically cultured in HMI-9 medium as described by Bodley et al.⁴³ with minor modifications. Briefly, 100 μL of late log phase parasites were incubated in 96-well plates (Costar) at an initial concentration of 10⁵ cells/mL with or without test compounds at 37 °C in a humidified 5% CO₂ atmosphere for 72 h. Ten microliters of a 20 mg/mL solution of *p*-nitrophenyl phosphate (prepared in 1 M NaOAc, pH 5.5, 1% TritonX-100) was then added to each well, and plates were reincubated at 37 °C as before for 6–8 h. Optical densities were then measured at 405 nm using a SpectraMax Plus microplate reader (Molecular Devices). IC₅₀ 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). This program uses the dose-response equation $y = ((a - d)/(1 + (x/c)^b)) + d$, where *x* = the drug concentration, *y* = absorbance at 405 nm, *a* = upper asymptote, *b* = slope, *c* = IC₅₀, and *d* = lower asymptote. This assay has the advantage of not being subject to interference by samples with a reducing potential (such as the *o*-catechols isolated and assayed in this study), as it depends on measuring acid phosphatase activity in surviving parasites.

Cytotoxicity Assay. Cytotoxicity was evaluated against two cell lines, Vero cells and PC-3 prostate cells, obtained from the American Type Culture Collection (ATCC, Rockville, MD) as described previously.^{2,44}

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Supporting Information Available: The ¹H and ¹³C NMR spectra of compounds **1a** and **2** and the NMR data of compounds **5–9**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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