Leishmanicidal and Antiplasmodial Activity of Constituents of *Smirnowia iranica*

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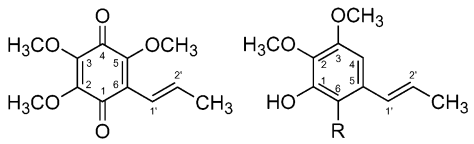
Three unusual, highly oxygenated novel phenylpropanoids (**1**−**3**) and two novel isoflavans, 8-prenylmucronulatol (**4**) and smiranicin (**6**), were isolated from *Smirnowia iranica* together with a previously described isoflavan, glyasperin H (**5**). The structures were established using homo- and heteronuclear two-dimensional NMR experiments. The isoflavans significantly inhibited the growth of extracellular stages of three *Leishmania* species in vitro, their activity against the intracellular stages being considerably lower. 8-Prenylmucronulatol (**4**) showed moderate in vitro toxicity against *Plasmodium falciparum*, without noticeable erythrocyte membrane effects at the inhibitory concentration. Because of the structural relationship of isoflavans with chalcones and aurones, some of which are potent antiprotozoal agents, the isoflavan skeleton may be a template structure in search for new compounds with leishmanicidal and antiplasmodial activity.

The genus *Smirnowia* Bunge (Leguminosae-Papilionoideae) was regarded as monotypic when described in 1876, with *Smirnowia turkestana* Bunge as the only representative.[1] In 1977, a new species endemic to Iran, *S. iranica* H. Sabeti, was described.[2] While the distinction between the two species may not be justified,[3] *S. iranica* grows in regions of central and northeast Iran (Aran, Kashan), whereas *S. turkestana* is represented in geographically wide areas of Central Asia, notably in the Quizilqum and Garagum deserts. The plant is a large shrub used for stabilization of arid, sandy soils. In the Isfahan province of Iran, roots and leaves of the plant have been used as a curative for abdominal pain and as an anthelmintic drug (unpublished information).

Previous phytochemical investigations of the genus *Smirnowia* are extremely limited, being restricted to the isolation of several bases of uncertain structure from aerial parts of *S. turkestana*.[4−6] In this work, we report on several new natural products from roots of *S. iranica*. Their growth-inhibitory activity against *Leishmania* and *Plasmodium* parasites is described.



1

2 R = H
3 R = OCH_3

Results and Discussion

Following initial fractionation of the ethyl acetate extract of *S. iranica* roots by VLC, six constituents were isolated and purified by preparative, normal-phase HPLC using heptane containing 0.5−5% of ethanol. ^{1}H NMR spectra of compounds **1**−**3** showed the presence of a characteristic spin system of an (*E*)-propenyl group. In addition to the latter, only three methoxy signals were observed in the ^{1}H NMR spectrum of **1**, the ^{13}C NMR spectrum of which disclosed the presence of two carbonyl resonances (δ 180.5 and 184.0). These data and the remaining ^{13}C chemical shifts were compatible with a 1,4-benzoquinone structure but not with an alternative 1,2-benzoquinone structure.[7−10] Compound **1** is new; in fact, only a very few 2,3,5-trialkoxy-6-alkyl-1,4-benzoquinones have been described so far.[8,9,11−15] Because of its red color, the quinone **1** is a highly characteristic constituent of the ethyl acetate extract of *S. iranica* roots.

The structures of compounds **2** and **3** were established on the basis of NOESY and HMBC experiments. Thus, **2** contained two *meta* aromatic hydrogens (J_{meta} = 1.9 Hz), both showing equally intense NOEs to the olefinic protons of the propenyl group. One of the aromatic protons showed NOE to a hydroxy group, which in turn showed NOE to one of the two methoxy groups present in the molecule. The other methoxy group showed NOE to the other aromatic hydrogen. Thus, the substituents are placed as shown in **2**, the structure being confirmed by HMBC connectivities.

^{1}H and ^{13}C NMR spectra of **3** disclosed the presence of three methoxy groups. According to the ^{13}C NMR spectrum, two of them were flanked by two *ortho* substituents (δ 61.04 and 61.21), whereas the last methoxy group had only one *ortho* substituent (δ 56.00).[16−19] A NOESY spectrum demonstrated that the single aromatic hydrogen present was *ortho* to the propenyl group and to the methoxy group at δ 56.00. The hydroxylic proton showed HMBC connectivities to three quaternary carbons: one bearing the hydroxy group, and the two flanking carbons that also exhibited HMBC cross-peaks to protons of the two methoxy groups having two *ortho* substituents. This proves the structure as **3**. The resonances of C-2 and C-6 and those of the attached methoxy groups were distinguished by NOEs from 6-OCH_3 to H-1′ and OH. Corroborative evidence was

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Table 1. 400 MHz ^{1}H NMR Spectral Data for Isoflavans **4**−**6** from *Smirnowia iranica* ($CDCl_3$)[a,b]

proton	**4**	**5**	**6**
H-2	α: 4.33 (ddd, $J_{2\alpha,2\beta} = 10.4$, $J_{2\alpha,3} = 3.6$, $J_{2\alpha,4\alpha} = 2.0$)	α: 4.35 (ddd, $J_{2\alpha,2\beta} = 10.4$, $J_{2\alpha,3} = 3.6$, $J_{2\alpha,4\alpha} = 2.0$)	α: 4.43 (ddd, $J_{2\alpha,2\beta} = 10.4$, $J_{2\alpha,3} = 3.6$, $J_{2\alpha,4\alpha} = 2.0$)
	β: 3.97 (t, $J_{2\beta,2\alpha} = J_{2\beta,3} = 10.4$)	β: 3.99 (t, $J_{2\beta,2\alpha} = J_{2\beta,3} = 10.4$)	β: 4.10 (t, $J_{2\beta,2\alpha} = J_{2\beta,3} = 10.4$)
H-3	3.53 (m)	3.55 (m)	3.64 (m)
H-4	α: 2.89 (ddd, $J_{4\alpha,4\beta} = 15.6$, $J_{4\alpha,3} = 5.8$, $J_{4\alpha,2\alpha} = 2.0$)	α: 2.87 (ddd, $J_{4\alpha,4\beta} = 15.7$, $J_{4\alpha,3} = 5.5$, $J_{4\alpha,2\alpha} = 2.0$)	α: 3.01 (ddd, $J_{4\alpha,4\beta} = 15.8$, $J_{4\alpha,3} = 5.5$, $J_{4\alpha,2\alpha} = 2.0$)
	β: 2.93 (ddd, $J_{4\beta,4\alpha} = 15.6$, $J_{4\beta,3} = 10.8$, $J_{4\beta,5} = 1.0$)	β: 2.93 (ddd, $J_{4\beta,4\alpha} = 15.7$, $J_{4\beta,3} = 11.0$, $J_{4\beta,5} = 1.0$)	β: 3.08 (ddd, $J_{4\beta,4\alpha} = 15.8$, $J_{4\beta,3} = 10.8$, $J_{4\beta,5} = 0.9$)
H-5	6.81 (d, $J_{5,6} = 8.2$)	6.83 (d, $J_{5,6} = 8.2$)	7.00 (d, $J_{5,6} = 8.4$)
H-6	6.40 (d, $J_{6,5} = 8.2$)	6.39 (dd, $J_{6,5} = 8.2$, $J_{6,1''} = 0.7$)	7.06 (dd, $J_{6,5} = 8.4$, $J_{6,1''} = 1.0$)
H-5′	6.60 (d, $J_{5',6'} = 8.6$)	6.62 (d, $J_{5',6'} = 8.6$)	6.63 (d, $J_{5',6'} = 8.6$)
H-6′	6.64 (d, $J_{6',5'} = 8.6$)	6.65 (d, $J_{6',5'} = 8.6$)	6.65 (d, $J_{6',5'} = 8.6$)
H-1″	3.40 (d, $J_{1'',2''} = 7.1$)	6.67 (dd, $J_{1'',2''} = 9.9$, $J_{1'',6} = 0.7$)	6.82 (dd, $J_{1'',2''} = 2.2$, $J_{1'',6} = 1.0$)
H-2″	5.27 (tsp, $J_{2'',1''} = 7.1$, $J_{2'',4''} \approx J_{2'',5''} \approx 1.5$)	5.58 (d, $J_{2'',1''} = 9.9$)	7.52 (d, $J_{2'',1''} = 2.2$)
3″-CH_3	*E*: 1.81 (m)	1.43 (s), 1.45 (s)	
	Z: 1.74 (m)		
2′-OCH_3	3.90 (s)	3.91 (s)	3.91 (s)
4′-OCH_3	3.88 (s)	3.90 (s)	3.89 (s)
7-OH	5.15 (br s)		
3′-OH	5.57 (s)	5.59 (s)	5.58 (s)

[a] Multiplicity of signals is given in parentheses: s, singlet; d, doublet; t, triplet; sp, septet; m, multiplet; br, broad; coupling constants (apparent splittings) are reported as numerical values in Hz. [b] Stereospecific assignments of H-2, H-4, H-4′, and H-5′ were obtained from NOESY spectra; for H-2 and H-4, α designates protons on the same side of the ring as H-3, and β protons on the opposite side.

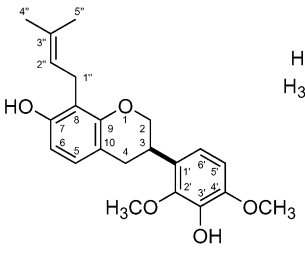
Table 2. 100 MHz ^{13}C NMR Spectral Data for Isoflavans **4**−**6** from *Smirnowia iranica* ($CDCl_3$)

carbon	**4**	**5**	**6**
C-2	70.56	70.57	70.62
C-3	31.67	31.71	31.81
C-4	32.04	31.63	31.68
C-5	127.56	129.15	125.83
C-6	108.12	108.70	103.93
C-7	153.70	151.94	155.02
C-8	114.39	109.92	117.06
C-9	152.33	149.71	147.65
C-10	114.39	114.35	114.77
C-1′	127.75	127.53	127.50
C-2′	145.37	145.36	145.36
C-3′	138.70	138.70	138.72
C-4′	146.62	146.67	146.72
C-5′	106.54	106.52	106.52
C-6′	117.00	116.99	117.06
C-1″	22.38	116.93	103.73
C-2″	122.13	128.97	143.75
C-3″	134.30	75.60	
C-4″	25.81	27.51[a]	
C-5″	17.87	27.83[a]	
2′-CH_3	61.07	61.04	61.04
4′-CH_3	56.24	56.23	56.25

[a] These assignments may be interchanged.

provided by a HMBC cross-peak between H-1′ and C-6. The phenylpropenes **2** and **3** have not been described prior to this work, although their *O*-methyl derivatives are known.[20−22]

The remaining three compounds isolated from *S. iranica* roots had two aromatic rings each, according to ^{13}C NMR spectra, and a $-CH_2-CH-CH_2-$ spin system apparent in ^{1}H NMR and COSY spectra, characteristic of isoflavans. The structure of the major isoflavan was established as **4**. While the presence of a *C*-prenyl moiety, two methoxy groups, two hydroxy groups, and two *ortho*-coupled aromatic proton pairs were readily apparent in the ^{1}H and ^{13}C NMR spectra, the elucidation of their relative positions posed a considerable problem. This was due to a coincidence of two quaternary carbon resonances and chemical exchange between the two phenolic protons, which resulted in transferred NOEs in the NOESY spectrum of **4**. However, one of the two *ortho*-coupled proton pairs could be assigned to H-5 and H-6 from a small coupling between H-4 and H-5 (J < 1 Hz) observed in a resolution-enhanced spectrum, which showed that the A-ring substituents are placed at C-7 and C-8. HMBC cross-peaks from H-1″ to C-7, C-8, and C-9 placed the *C*-prenyl group at C-8. The C-9 resonance was unequivocally identified by HMBC connectivities to H-2, H-4, and H-5, and the C-7 resonance by connectivities to H-5 and H-6. Since the methoxy group with only one *ortho* substituent (δ 56.24) showed NOE to H-5′, this group was placed at C-4′, and hence one of the hydroxy groups was attached to C-7. The other hydroxy group was placed at C-3′ on the basis of three HMBC cross-peaks: to C-2′ and C-4′ (identified by correlation to methoxy hydrogens), and to C-3′, the signal of which (δ 138.70, affected by two *ortho* oxygen functions) was considerably upfield relative to those of C-2′ and C-4′ (respectively δ 145.37 and 146.62, each affected by only one *ortho* oxygen function), as generally observed in 1,2,3-trioxygenated benzenes.[18,19,23,24]



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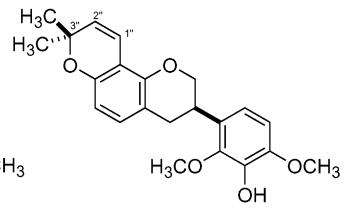

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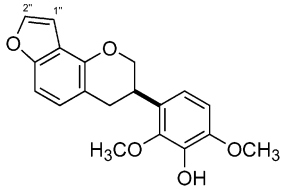

6

The remaining isoflavans **5** and **6** differed by alterations of the prenyl side chain, their structures being elucidated similarly as described for **4**. A characteristic feature of the ^{1}H NMR spectra of **5** and **6** was five-bond couplings

Table 3. Inhibition of Promastigote and Amastigote Stages of *Leishmania* Species in Vitro and Cytotoxicity against Macrophage-like RAW Cells

	IC_{50}, μM					
compound	*L. donovani* promastigotes	*L. infantum* promastigotes	*L. major* promastigotes	*L. donovani* amastigotes	*L. infantum* amastigotes	RAW cell cytotoxicity
1	97 ± 5	74 ± 1	72 ± 1	>800	>800	256 ± 14
2	395 ± 21	267 ± 11	372 ± 3	>800	>800	281 ± 19
3	232 ± 18	198 ± 11	387 ± 20	589 ± 17	>800	125 ± 16
4	6.9 ± 0.9	9.2 ± 1.1	7.9 ± 0.9	99 ± 8	109 ± 9	218 ± 11
5	25.3 ± 1.3	14.2 ± 0.6	23.7 ± 1.8	77 ± 5	89 ± 12	172 ± 13
6	22.9 ± 2.1	11.3 ± 0.4	17.4 ± 1.4	112 ± 6	81 ± 4	193 ± 12
amphotericin B	0.02 ± 0.02	0.02 ± 0.01	0.03 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	
pentostam				8.2 ± 0.5	8.4 ± 0.5	

between H-6 and H-1″ ($J_{6,1''} \leq 0.7$ Hz) along a zigzag path. ^{1}H and ^{13}C NMR spectral data for **4**−**6** are collected in Tables 1 and 2. A summary of 2D NMR connectivities is included in the Supporting Information. Isoflavans **4** and **6** are new natural products, whereas compound **5** has previously been isolated from *Glycyrrhiza aspera* (Leguminoseae) and named glyasperin H.[25] The stereochemistry of the latter at C-3 was established to be *R* from a positive Cotton effect at 277 nm.[25] The compound **5** isolated from *S. iranica* had the same sign of optical rotation ($[\alpha]_D$ +15.5°) as that from *G. aspera*[25] ($[\alpha]_D$ +8.0°). This shows that the configuration of both compounds is *R*, although the material isolated from *G. aspera* might not have been optically pure, as is sometimes observed with isoflavans.[26] Since **5** and **6** are presumed to be biosynthetic derivatives of **4**, the stereochemistry of all three isoflavans from *S. iranica* is formulated as *R*. Compound **4** is the 8-prenyl derivative of (*R*)-mucronulatol.[26−28] A trivial name smiranicin is suggested for **6**. The molecular formulas of all new compounds were confirmed by high-resolution MS.

Results of evaluation of toxicity of **1**−**6** against various species of *Leishmania* parasites are summarized in Table 3. Extracellular as well as intracellular stages of the parasite were included in the study. In their mammalian hosts, protozoa of the genus *Leishmania* are obligate intracellular parasites of the monocyte-macrophage system. Inside their host cells, they reside and multiply within parasitophorous vacuoles. Following phagocytosis, *Leishmania* parasites transform from the promastigote, i.e., flagellated form that is found in the gut of the insect vector and can also be maintained in axenic cell culture, into amastigotes. The intracellular localization of the pathogen may pose specific problems for drugs, which have to pass the host cell membrane or be otherwise internalized by the host cell. The intracellular efficacy of a drug depends on its pathway and rate of uptake, its resistance to intracellular degradation, intracellular trafficking, and host cell toxicity. The in vitro assays used here, which cover both life stages of several *Leishmania* species, give a better prediction of toxicity as well as of preliminary kinetics in the host cells, allowing rapid evaluation of the compounds tested in a more general way, than in an assay employing promastigote cultures alone.

The activities of **1**−**3** against various strains of *Leishmania* promastigotes were rather low, the quinone **1** being the most active (Table 3). Leishmanicidal activity of **4**−**6** was of interest, because compounds with two aromatic rings connected by a carbon spacer, such as chalcones and aurones, exhibit significant leishmanicidal effects.[29−36] Activities of **4**−**6** determined for promastigotes of various *Leishmania* species and expressed as IC_{50} values were in the low micromolar range (Table 3). Thus, the potency of the isoflavans **4**−**6** against the infective promastigote forms is higher than or similar to what has been observed with many natural products.[31,33,37−39] For example, the IC_{50} value of a recently isolated antiprotozoal agent from plants having two aromatic rings, licochalcone A, was reported to be 13 ± 1 μM.[32] However, the isoflavans **4**−**6** are considerably less potent against promastigotes than the reference compound amphotericin B (Table 3) and less potent than many aurones and chalcones studied so far.[35,36]

Table 4. Toxicity against *Plasmodium falciparum* 3D7 Strain

compound	IC_{50}, μM
1	>200
2	>200
3	>200
4	10.3 ± 1.9
5	33.3 ± 9.8
6	57.6 ± 12.3
chloroquine	78.0 ± 18.2 nM

The activities of **4**−**6** against the intracellular *Leishmania* stages were considerably lower than those against promastigotes (Table 3). This may reflect a lower intrinsic activity or a low intracellular concentration of the compounds achieved during the assay conditions. A lower in vitro toxicity against the amastigotes as compared to the promastigotes has been generally observed with aurones and chalcones,[35,36] but numerous exceptions, including licochalcone A, are known.[29,33,35,36] The cytotoxicity of **4**−**6** determined with host cells was low (Table 3), but the selectivity index with respect to the intracellular stages is only about 2.

Since chalcones[40−43] and aurones[44] also exhibit antiplasmodial activity, activities of **4**−**6** against a chloroquine-sensitive *Plasmodium falciparum* strain 3D7 were determined. However, only a weak toxicity was observed, the isoflavan **4** being the most active (Table 4). Recently it was observed that some compounds apparently inhibit growth of the *Plasmodium* parasites cultivated inside erythrocytes indirectly, i.e., through modification of erythrocyte membrane and not by effects on the parasite itself.[45,46] After the infection of erythrocytes, the parasite modifies the structure of the erythrocyte membrane as it grows, changing its permeability and establishing new protein structures within the membrane.[47,48] These changes result in morphological alterations and changes of rheological properties of the erythrocytes.[47,48] It can be anticipated that incorporation of a chemical into the erythrocyte membrane interferes with these processes, which could result in unfavorable growth conditions and hence apparent antiplasmodial activity in vitro, even in the absence of a direct interaction of the chemical with a specific molecular target belonging to the parasite itself. Compounds exhibiting a *Plasmodium* growth inhibitory effect resulting from general, nonspecific interaction with the erythrocyte lipid bilayer have hardly any interest as potential drugs.[45] Since incorporation of chemicals into the erythrocyte mem-

brane usually results in changes of membrane curvature, care should be exercised if a potential drug causes microscopically observable changes of erythrocyte membrane curvature at a concentration close to its IC_{50} value.[45,46] It was therefore of interest to determine whether **4**−**6** affect the shape of the erythrocyte membrane. When nonparasitized erythrocytes were incubated with **4** under conditions identical with those used in the *Plasmodium* toxicity assay, no microscopically observable changes of erythrocyte shape could be detected at concentrations up to 67 μM. Similarly, no effects on erythrocyte morphology were seen with **5** and **6** at concentrations comparable to their IC_{50} values (Table 4). However, at higher concentrations (130−300 μM) the compounds caused the erythrocyte membrane to leak, and formation of ghost cells and echinocytes was observed. Thus, at high concentrations the compounds affect the erythrocyte membrane, but there is no evidence of membrane modifications at the inhibitory concentrations (i.e., concentration close to IC_{50} values), as previously observed with other compounds.[45,46] This is especially true for the most potent compound, **4**, which is therefore concluded to have a genuine antiparasitic effect. On the other hand, RAW cell toxicity observed at very high concentrations (Table 3) is likely to be due to membrane effects.

In conclusion, the present work established *S. iranica* as a source of novel phenylpropanoids and novel isoflavans, the latter possessing significant promastigocidal effects and very little host cell toxicity. The isoflavan **4** also exhibits antiplasmodial activity. The isoflavan skeleton thus represents a preliminary, alternative template structure for studies of structure−activity relationships of antiprotozoal activity. Isoflavans with appropriate substituent pattern may be a new class of potent leishmanicidal and antiplasmodial agents.

Experimental Section

General Experimental Procedures. Optical rotations were measured using a Perkin-Elmer 241 polarimeter. NMR spectra were obtained at 25 °C on a Bruker AMX 400 spectrometer (proton frequency 400.13 MHz) in chloroform-*d*, using TMS as internal standard. NOESY spectra were obtained with mixing times of 500−800 ms. HMBC spectra were optimized for $J_{C,H} = 7$ Hz. ^{1}H,^{13}C chemical shift correlations were obtained using polarization transfer with BIRD decoupling. High-resolution mass measurements for exact mass determination were carried out using a Bruker APEX III Fourier transform mass spectrometer equipped with a 7 T superconducting magnet and an external electrospray ion source. The spectra were externally calibrated with a capillary skimmer dissociation spectrum of LHRH (luteinizing hormone releasing hormone). The samples were introduced into the electrospray ion source using a 250 μL syringe with a syringe pump flow of 2 μL/min. VLC separations were performed on Merck silica gel 60H for TLC. HPLC separations were performed on a chromatograph consisting of a Gynkotek P 580 pump, Rheodyne 7725 injector, Shimadzu SPD-10AV spectrophotometric detector operating at 254 nm, and a recorder, using a 25 cm × 1.6 cm i.d. column packed with Lichrosorb Si-60 (7 μm). Melting points were determined in capillaries and are uncorrected.

Plant Material. Roots of *Smirnowia iranica* H. Sabeti (Persian name: dom gavi) were collected at the altitude of 900−950 m in the Kashan District, Isfahan, Iran, in August 1996; the plant was identified by K. Bagherzadeh and M. Majid. A voucher specimen (number 11054) was deposited in the herbarium of the Isfahan Research Centre of Natural Resources and Animal Science, Isfahan, Iran.

Extraction and Isolation. Air-dried and powdered roots of *S. iranica* (560 g) were extracted with 4 × 4 L of EtOAc at room temperature. The extract was evaporated, and the residue (23.4 g) was dissolved in 20 mL of EtOAc and applied to a VLC column of silica gel (8 cm × 10 cm i.d.). The column was eluted with 1.5 L of toluene to give 5.5 g of a residue, which was further fractionated by VLC (4 cm × 4 cm i.d. column). Elution with toluene gave 3.3 g of a residue. A portion of the latter fraction (0.4 g) was resolved by preparative HPLC using 6 mL/min of heptane−EtOH, 95:5, to give (in order of elution) 20 mg of pure **1**, 310 mg of a mixture of **2**, **3**, and **4**, 40 mg of crude **5**, and 70 mg of pure **6**. Repeated HPLC with 10 mL/min of heptane−EtOH, 99:1 or 99.5:0.5, as the mobile phase gave 19 mg of **2**, 45 mg of **3**, 19 mg of **4**, and 11 mg of **5** as pure compounds.

2,3,5-Trimethoxy-6-(1-propenyl)-2,5-cyclohexadiene-1,4-dione (1): red resin; ^{1}H NMR ($CDCl_3$) δ 1.92 (3H, dd, J = 6.9 and 1.7 Hz, CH_3), 3.98, 3.99, 4.00 (each 3H, s, OCH_3), 6.36 (1H, dq, J = 16.0 and 1.7 Hz, H-1′), 6.87 (1H, dq, J = 16.0 and 6.9 Hz, H-2′); ^{13}C NMR ($CDCl_3$) δ 20.52 (CH_3), 61.03, 61.26, and 61.32 (OCH_3), 119.31 (C-1′), 124.50 (C-6), 138.56 (C-2′), 143.08, 144.04, and 152.14 (C-2, C-3, and C-5), 180.52 and 183.99 (C-1 and C-4); HRFTMS *m/z* 239.09208 (MH^+), $C_{12}H_{15}O_5^+$ requires 239.09140.

2,3-Dimethoxy-5-(1-propenyl)phenol (2): colorless resin; ^{1}H NMR ($CDCl_3$) δ 1.86 (3H, dd, J = 6.5 and 1.7 Hz, CH_3), 3.87 (3H, s, 3-OCH_3), 3.88 (3H, s, 2-OCH_3), 5.71 (1H, s, OH), 6.13 (1H, dq, J = 15.7 and 6.5 Hz, H-2′), 6.28 (1H, dq, J = 15.7 and 1.7 Hz, H-1′), 6.44 (1H, d, J = 1.9 Hz, H-4), 6.60 (1H, J = 1.9 Hz, H-6); ^{13}C NMR ($CDCl_3$) δ 18.35 (CH_3), 55.80 (3-OCH_3), 61.02 (2-OCH_3), 101.91 (C-4), 105.45 (C-6), 125.44 (C-2′), 130.68 (C-1′), 134.29 (C-5), 134.63 (C-2), 149.24 (C-1), 152.31 (C-3); HRFTMS *m/z* 195.10159 (MH^+) and 217.08342 (MNa^+), $C_{11}H_{15}O_3^+$ requires 195.10157, $C_{11}H_{14}O_3Na^+$ requires 217.08342.

2,3,6-Trimethoxy-5-(1-propenyl)phenol (3): yellowish resin; ^{1}H NMR ($CDCl_3$) δ 1.90 (3H, dd, J = 6.6 and 1.7 Hz, CH_3), 3.79 (3H, s, 6-OCH_3), 3.85 (3H, s, 3-OCH_3), 3.90 (3H, s, 2-OCH_3), 5.70 (1H, s, OH), 6.18 (1H, dq, J = 15.8 and 6.5 Hz, H-2′), 6.49 (1H, s, H-4), 6.61 (1H, dq, J = 15.8 and 1.7 Hz, H-1′); ^{13}C NMR ($CDCl_3$) δ 18.75 (CH_3), 56.00 (3-OCH_3), 61.04 (2-OCH_3), 61.21 (6-OCH_3), 99.67 (C-4), 124.96 (C-1′), 126.15 (C-5), 126.37 (C-2′), 135.66 (C-2), 139.02 (C-6), 142.59 (C-1), 149.04 (C-3); HRFTMS *m/z* 225.11208 (MH^+) and 247.09343 (MNa^+), $C_{12}H_{17}O_4^+$ requires 225.11214, $C_{12}H_{16}O_3Na^+$ requires 247.09408.

(*R*)-8-Prenylmucronulatol [(*R*)-3,4-Dihydro-3-(3-hydroxy-2,4-dimethoxyphenyl)-8-(3-methyl-2-butenyl)-2*H*-1-benzopyran-7-ol] (4): colorless crystals (from ethanol−heptane); mp 61−63 °C; $[\alpha]_D^{25}$ +21.9° (*c* 0.5, $CHCl_3$); HRFTMS *m/z* 371.18533 (MH^+) and 393.16732 (MNa^+), $C_{22}H_{27}O_5^+$ requires 371.18533, $C_{12}H_{26}O_5Na^+$ requires 393.16725.

Glyasperin H [(*R*)-3-[3,4-Dihydro-8,8-dimethyl-2*H*,8*H*-benzo[1,2-*b*:3,4-*b*′]dipyran-3-yl]-2,6-dimethoxyphenol] (5): colorless crystals (from ethanol−heptane); mp 56−58 °C, lit.[25] mp 58−60 °C; $[\alpha]_D^{25}$ +15.5° (*c* 0.3, $CHCl_3$), lit.[25] +8.0° (*c* 0.1, $CHCl_3$).

Smiranicin [(*R*)-3-[3,4-Dihydro-2*H*-furo[2,3-*h*]-1-benzopyran-3-yl]-2,6-dimethoxyphenol] (6): yellowish solid; $[\alpha]_D^{25}$ −37.6° (*c* 0.2, $CHCl_3$); HRFTMS *m/z* 327.12279 (MH^+) and 349.10474 (MNa^+), $C_{19}H_{19}O_5^+$ requires 327.12270, $C_{19}H_{18}O_5Na^+$ requires 349.10464.

Assay for *Leishmania* Promastigote Toxicity. *Leishmania donovani* strain LV9,[49] *L. infantum* strain D.SCH,[50] and *L. major* strain LV39[49] promastigotes cryopreserved in liquid nitrogen were used. The promastigotes were exposed to test compounds during a 96 h period, and viability of the parasites was determined using the MTT assay essentially as described elsewhere.[44]

Assay for *Leishmania* Amastigote Toxicity. The assay was performed with amastigotes grown in murine monocyte-macrophage RAW 264.7 cells[51] similarly as described previously.[52] Briefly, the RAW cells were infected in vitro with *L. donovani* or *L. infantum* promastigotes in stationary growth phase, seeded in 96-well flat-bottom microtiter plates at 1 × 10^5 cells per well in 100 μL of RPMI medium supplemented with 10% of fetal calf serum, and incubated for 24 h at 37 °C

to allow internalized *Leishmania* parasites to transform into the amastigote form. Test compounds were added in 100 μL of the medium. Each concentration was tested in duplicate. The plates were incubated at 37 °C for 72 h, and the cells were washed and then lysed with 100 μL of lysis medium (containing 0.008% SDS). Macrophage disintegration was monitored with an inverted microscope. After more than 95% of the host cells were lysed, 150 μL of post-lysis medium (*Leishmania* growth medium containing all supplements at 1.6-fold concentration[44,52]) was added, and the plate incubated at 25 °C for 3−4 days to allow viable parasites to transform to promastigotes, the amount of which was determined using the MTT assay.

Assay for RAW Cell Cytotoxicity. Noninfected RAW 264.7 cells were exposed to test compounds for 48 h in parallel to the assay for intracellular leishmanicidal activity, and the amount of viable cells was determined using MTT as previously described.[44,52]

Assay for Antiplasmodial Activity. The assay was performed using chloroquine-sensitive *Plasmodium falciparum* 3D7 strain essentially as previously described.[45,53]

Assay for Erythrocyte Membrane Transformation. The test compounds **4**−**6** (1−300 μM) were incubated with nonparasitized erythrocytes for 48 h exactly as in the assay for the antiplasmodial activity. After the incubation period, 20 μL samples were spread on microscope slides, allowed to dry, fixed with methanol, stained with Giemsa, and examined microscopically.[45] The results are described in the text.

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Supporting Information Available: 2D NMR connectivities (COSY, NOESY, HMBC) for **4**−**6**. This material is available free of charge via the Internet at http://pubs.acs.org.

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