Benzoic Acid Derivatives from *Piper* Species and Their Antiparasitic Activity

Ninoska Flores,[†,‡] Ignacio A Jiménez,[†] Alberto Giménez,[‡] Grace Ruiz,[‡] David Gutiérrez,[‡] Genevieve Bourdy,[‡] and Isabel L. Bazzocchi*[,†]

Instituto Universitario de Bio-Orgánica "Antonio González", Universidad de La Laguna, Avenida Astrofísico Francisco Sánchez 2, La Laguna, 38206 Tenerife, Canary Islands, Spain, and Instituto de Investigaciones Fármaco Bioquímicas, Facultad de Ciencias Farmacéuticas y Bioquímicas, Universidad Mayor de San Andrés, Avenida Saavedra 2224, Miraflores, La Paz, Bolivia

Received February 14, 2008

Piper glabratum and *P. acutifolium* were analyzed for their content of main secondary constituents, affording nine new benzoic acid derivatives (**1**, **2**, **4**, **5**, **7**, and **10**−**13**), in addition to four known compounds (**3**, **6**, **8**, and **9**). Their structures were elucidated on the basis of spectroscopic data. Riguera ester reactions and optical rotation measurements established the new compounds as racemates. In the search for antiparasitic agents, the compounds were evaluated *in vitro* against the promastigote forms of *Leishmania* spp., *Trypanosoma cruzi*, and *Plasmodium falciparum.* Among the evaluated compounds, methyl 3,4-dihydroxy-5-(3′-methyl-2′-butenyl)benzoate (**7**) exhibited leishmanicidal effect (IC_{50} 13.8−18.5 μg/mL) against the three *Leishmania* strains used, and methyl 3,4-dihydroxy-5-(2-hydroxy-3-methylbutenyl)benzoate (**1**), methyl 4-hydroxy-3-(2-hydroxy-3-methyl-3-butenyl)benzoate (**3**), and methyl 3,4-dihydroxy-5-(3-methyl-2-butenyl) benzoate (**7**) showed significant trypanocidal activity, with IC_{50} values of 16.4, 15.6, and 18.5 μg/mL, respectively.

Parasitic diseases such as leishmaniasis, malaria, and trypanosomiasis have a significant impact in developing countries, affecting hundreds of millions of people and which are the cause of a mortality rate of several million per year.[1] The existing chemotherapy for these diseases is not satisfactory due to its lack of effectiveness and also the toxicity associated with long-term treatments using empirically discovered drugs. Drug resistance and different strain sensitivity to the available drugs are drawbacks for clinically accessible chemotherapy. Therefore, in the absence of vaccines, new chemotherapies are needed urgently to help in the prevention and control of these parasitic diseases.[2]

In spite of the great advances in modern medicine in recent decades, plants still make an important contribution to health care, and natural products have provided the inspiration for most of the active ingredients in medicines.[3] The genus *Piper* is widely distributed in the tropical and subtropical regions of the world, and species belonging to this genus are included in folklore medicine of Latin America.[4] In Bolivia, where resorting to medicinal plants represents a primary health care measure of the native population, the leaves of several species of *Piper* are used for the treatment of parasitic diseases.[5]

Phytochemical investigations of *Piper* species have led to the isolation of several classes of physiologically active compounds,[4] such as flavanones and dihydrochalcones isolated from *P. hostmannianum* with antiplasmodial activity[6] and those isolated from *P. elongatum*[7] and *P. rusbyi*[8] with leishmanicidal activity. Neolignans from *P. regnellii* with trypanocidal activity have also been reported.[9] The evaluation of the essential oil from the leaves of *P. acutifolium* against *Callosobruchus maculates* (a stored grain insect pest) is the only study described for this species.[10]

As part of our research aiming to uncover antiparasitic compounds from Bolivian *Piper* species, we have carried out a phytochemical analysis of the leaves of *Piper glabratum and P. acutifolium*. We now report the isolation and structural elucidation of nine new benzoic acid derivatives (**1**, **2**, **4**, **5**, **7**, and **10**−**13**) by means of ^{1}H and ^{13}C NMR spectroscopic studies, including homonuclear (COSY) and heteronuclear correlation experiments (HSQC and HMBC). In addition, four known compounds were isolated and were identified as methyl 4-hydroxy-3-(2-hydroxy-3-methyl-3-butenyl)benzoate (**3**),[11] methyl 4-hydroxy-3-(methyl-2-butenyl)benzoate (**6**),[12] 4-hydroxy-3,5-bis(3-methyl-2-butenyl)benzoic acid (**8**),[13] and 4-methoxy-3,5-bis(3-methyl-2-butenyl)benzoic acid (**9**),[13] by comparison of their spectroscopic data with reported data. In order to determine the configuration of the stereogenic center of the new compounds, Riguera's method,[14] a modified Mosher ester procedure, was applied to compounds **1** and **10**, which together with the optical rotation measurements established the new compounds as racemates. We have investigated the isolated compounds for their leishmanicidal, trypanocidal, and antiplasmodial activities.

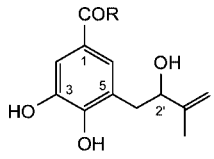
Results and Discussion

Repeated chromatography of the dichloromethane extracts of the leaves of *P. glabratrum* and *P. acutifolium* on Sephadex LH-20 and silica gel yielded nine new prenylated 4-hydroxybenzoic acids (**1**, **2**, **4**, **5**, **7**, and **10**−**13**).

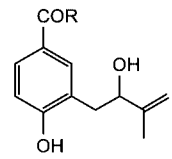
Compound **1** was isolated as a white, amorphous solid and showed the molecular formula $C_{13}H_{16}O_5$ by analysis of its HREIMS spectrum (*m/z* 252.1014, calcd 252.0998). The IR spectrum indicated the presence of hydroxy (3409 cm^{-1}) and carbonyl groups (1692 cm^{-1}) and an aromatic ring (1602 and 770 cm^{-1}). The ^{1}H NMR (Table 1) spectrum exhibited signals for two aromatic protons at δ 7.33 (d, J = 1.9 Hz) and 7.48 (d, J = 1.9 Hz) and two singlets at δ 1.81 and 3.85 assigned to one methyl group attached to a double bond and one methoxy group, respectively. The presence of an oxymethine proton at δ 4.42 (dd, J = 2.1, 8.5 Hz) associated with the singlets at δ 4.88 and 5.99 suggested a hydroxylated isoprene chain with a terminal double bond. The ^{13}C NMR (Table 1) spectrum showed signals for 13 carbon atoms, assigned as one carbomethoxy (δ 167.2), six aromatic carbons (δ 114.9, 122.3, 124.6, 125.2, 145.5, 147.3), two signals corresponding to a double bond at δ 145.9 (s) and 111.5 (t), one oxymethine carbon (δ 78.3), one methoxy (δ 51.9), one aliphatic methylene (δ 37.7), and one methyl (δ 18.1) carbon, suggesting a 3,4,5-trisubstituted benzoic acid derivative with an isoprene chain. The assignments of the ^{1}H and ^{13}C NMR data were based on 2D experiments and comparison with reported data for known prenylated benzoic acid derivatives.[11] The connectivity between the aromatic and aliphatic moieties was revealed by analysis of the HMBC experiment (Figure 1A). Therefore, correlation of signals at δ 7.48 (H-2)/7.33 (H-6) with 167.2 (CO_2Me) indicated that the carboxyl group was attached to C-1. The position of the isoprene side chain on the aromatic ring

* To whom correspondence should be addressed. Tel: +34-922-318594. Fax: +34- 922-318571. E-mail: ilopez@ull.es.
† Universidad de La Laguna.
‡ Universidad Mayor de San Andrés.

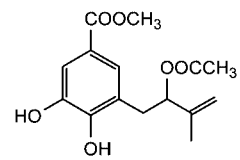
10.1021/np800104p CCC: $40.75
Published on Web 08/20/2008



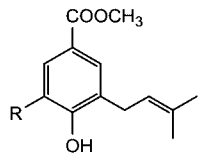
1 R = OCH_3
2 R = OCH_2CH_3



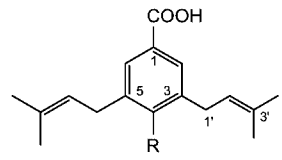
3 R = OCH_3
4 R = OCH_2CH_3



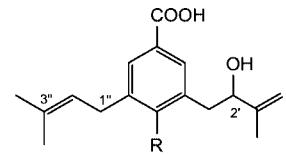
5



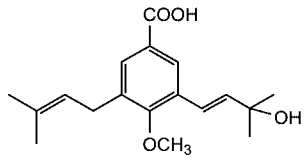
6 R = H
7 R = OH



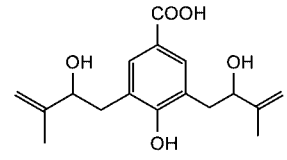
8 R = OH
9 R = OCH_3



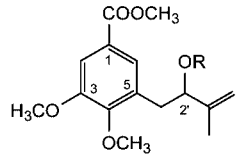
10 R = OH
11 R = OCH_3



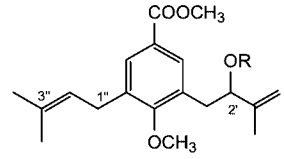
12



13



14 R = H
16 R = (*R*)-MPA



15 R = H
17 R = (*R*)-MPA

was determined by a cross-peak of the signal at δ_H 4.42 (H-2′) and δ_C 125.2 (C-5) and correlation between the signal at δ_H 2.99 (H-1′) and δ_C 124.6 (C-6)/147.3 (C-4). Moreover, the hydroxy group was placed at C-2′ on the side chain, in agreement with the HMBC correlations observed from the proton signals at δ_H 4.88, 5.99 (H-4′), and 1.81 (Me-5′) to the carbon signal at δ 78.3 (C-2′). These data established its structure as methyl 3,4-dihydroxy-5-(2-hydroxy-3-methyl-3-butenyl)benzoate.

The structure of compound **2**, with the molecular formula $C_{14}H_{18}O_5$ (HREIMS), was elucidated by physical methods, including IR, UV, and 1D and 2D NMR data (Table 1). The ^1H and ^{13}C NMR data showed that **2** was related to compound **1**, with the presence of an ethoxy group [δ_H 4.32 (2H, J = 7.2, Hz, H-1″), 1.37 (3H, t, J = 7.2 Hz, H-2″) and δ_C 14.1 (C-2″), 60.4 (C-1″)] instead of a methoxy group as found in **1**. Assignments of the ^1H and ^{13}C NMR signals performed by extended 2D NMR methods confirmed the structure of **2** as ethyl 3,4-dihydroxy-5-(2-hydroxy-3-methyl-3-butenyl)benzoate.

The HREIMS of compound **4** gave a molecular ion at *m*/*z* 250.1196, corresponding to the molecular formula $C_{14}H_{18}O_4$, and it was found to have a similar structure to **2** by comparison of their NMR data (Table 1). The main difference observed was the loss of the oxygenated aromatic carbon signal (δ_C 146.7) and the presence of an additional aromatic methine with signals at δ_H 6.92 and δ_C 116.9. These data and 2D NMR experiments defined the structure of **4** as ethyl 4-hydroxy-3-(2-hydroxy-3-methyl-3-butenyl)benzoate.

Compound **5**, with the molecular formula $C_{15}H_{18}O_6$ (HREIMS), was shown by its IR, UV, and ^1H and ^{13}C NMR data (Table 1) and 2D NMR experiments to be a 3,4,5-trisubstituted benzoic acid derivative with an isoprene side chain. The NMR spectra indicated that **5** is the 2-*O*-acetyl derivative of **1**, as the presence of the *O*-acetyl signals (δ_H 2.10 and δ_C 21.0, 171.9) and a less shielded position of the signal at δ_H 5.20 assignable to H-2′ that appeared in **1** at δ_H 4.42 were the most notable differences. Therefore, the structure of **5** was determined as methyl 3-[2-(acetoxy)-3-methyl-3-butenyl]-4,5-dihydroxybenzoate.

The structure of compound **7** was elucidated by physical methods (Table 1), showing it to be related to **1**, with the absence of the secondary hydroxy group at C-2′ and isomerization of the double bond on the side chain being the major differences. The isoprene chain was confirmed by the presence of signals in the ^1H NMR spectrum at δ 1.78 (6H, s), 3.38 (2H, d, J = 7.2 Hz, H-1′), and 5.32 (1H, t, J = 7.2 Hz, H-2′) and signals in the ^{13}C NMR spectrum at δ 17.9 (C-4′), δ 25.8 (C-5′), 29.2 (C-1′), 121.8 (C-2′), and 134.9 (C-3′). The linkage position of the isoprene side chain to the aromatic ring was determined by an HMBC experiment, in which correlations between the signals of H-2′/C-5 and H-1′/C-6, C-3′ were observed. Moreover, the complete assignment of the NMR data was made by analysis of 2D NMR experiments and comparison with those reported for related compounds,[12] which established the structure of **7** as methyl 3,4-dihydroxy-5-(3-methyl-2-butenyl)benzoate.

Compound **10** was isolated as an amorphous powder. Its molecular formula was established as $C_{17}H_{22}O_4$ by analysis of the HREIMS spectrum (*m*/*z* 290.1537, calcd 290.1518). The IR spectrum indicated the presence of hydroxy (3370 cm^{-1}), carboxylic acid (3520−2670, 1680 cm^{-1}), and aromatic (1601, 776 cm^{-1}) groups. The ^1H NMR (Table 2) spectrum exhibited signals for two aromatic protons at δ 7.68 (d, J = 1.3 Hz) and 7.79 (d, J = 1.3

Hz), characteristic of a 1,3,4,5-tetrasubstituted aromatic ring. Additional signals included three vinyl methyl groups as singlets at δ 1.74, 1.76, and 1.83, two aliphatic methylenes at δ 2.86 (1H, dd, $J = 1.3$, 11.0 Hz), 2.99 (1H, dd, $J = 6.5$, 11.0 Hz), and 3.38 (2H, d, $J = 5.4$ Hz), one oxymethine proton at δ 4.43 (dd, $J = 1.3$, 6.5 Hz), and three vinylic protons, two of them as singlets at δ 4.90 and 5.02 and the other as a triplet at δ 5.34 (2H, $J = 5.4$ Hz), indicating the presence of two double bonds in the side chains. The ^{13}C NMR (Table 2) spectrum exhibited signals for 17 carbon atoms, showing in the low-field region one carboxylate carbon at δ 170.1, six aromatic carbons (δ 121.8, 124.9, 129.5, 130.7, 131.5, and 158.9), signals corresponding to two double-bond carbons (δ 111.2, 121.7, 133.2, and 146.2), and one secondary oxymethine carbon at δ 77.9. These data indicated that compound **10** is a 3,4,5-trisubstituted benzoic acid derivative with two isoprene chains. The connectivity between aromatic and aliphatic moieties was revealed by analysis of the HMBC spectrum (Figure 1B), showing correlations between the signals at δ_H 7.68 (H-2)/7.79 (H-6) and δ_C 170.1 (CO_2H), which indicated that the carboxyl group was attached to C-1. The placements of the two isoprene side chains were based on the long-range $^3J_{H,C}$ correlations, H-1′/C-4, C-6, C-3′ and H-1″/C-2, C-4, C-3″. Analysis of the COSY, HSQC, and HMBC experiments allowed full assignment of the ^{1}H and ^{13}C NMR signals, and the structure of **10** was determined as 4-hydroxy-3-(2-hydroxy-3-methyl-3-butenyl)-5-(3-methyl-2-butenyl)benzoic acid.

Compound **11** was identified as 3-(2-hydroxy-3-methyl-3-butenyl)-4-methoxy-5-(3-methyl-2-butenyl)benzoic acid, since its ^{1}H and ^{13}C NMR data (Table 2) were similar to those of compound **10**. The difference was confined to the presence of the signals at δ_H 3.82, δ_C 60.8 and 170.1, corresponding to a carboxymethyl group on C-1.

Compound **12** exhibited the same molecular formula as **11**. Comparison of their NMR data indicated the modification of one isoprene chain as the most notable difference, revealing the presence of a chain with a *trans* double bond, two methyl groups, and a tertiary hydroxy group. The HMBC experiment showed as the most relevant connectivities on this side chain those of the two vinylic protons at δ_H 6.56 (H-2′)/6.86(H-1′) with δ_C 130.7 (C-3) and correlation of the signals at δ_H 1.46 (Me-4′, Me-5′) with δ_C 71.1 (C-3′). COSY, HSQC, and HMBC experiments permitted the complete assignment of all the protons and carbons as shown in Table 2. This evidence allowed definition of the structure of **12** as 3-[(1*E*)-3-hydroxy-3-methyl-1-butenyl)-4-methoxy-5-(3-methyl-2-butenyl)benzoic acid.

The IR absorption bands (3442, 3494−2776, and 1699 cm^{-1}) of compound **13** indicated the presence of hydroxy and carboxylic acid groups, respectively. Resonances in the ^{1}H and ^{13}C NMR spectra were assigned by the COSY, HSQC, and HMBC experiments as shown in Table 2, which suggested a prenyl 3,4,5-trisubstituted benzoic acid structure for **13**. The NMR signals due to nine hydrogens and 10 carbons were observed in its ^{1}H and ^{13}C NMR spectra, whereas the molecular weight was found to be *m*/*z* 306 by the EIMS spectrum, suggesting that **13** has a symmetric structure. On the basis of these findings the molecular formula was deduced to be $C_{17}H_{22}O_5$ (HREIMS). All the spectral data and connectivity found in the HMBC experiment are compatible only with the structure of 4-hydroxy-3,5-bis(2-hydroxy-3-methyl-3-butenyl)benzoic acid for compound **13**.

The specific rotations ($[\alpha]_D$) of the new compounds **1**, **2**, **4**, **5**, **10**, and **13** were found to be close to 0, which suggested they were racemates. Riguera esters[14] prepared from derivatives **14** and **15** of compounds **1** and **10**, respectively, confirmed this suggestion. Theoretically, the Riguera reaction of an optically pure compound will result in a single ester derivative being formed. Prior to preparing the Riguera ester derivatives, the phenolic hydroxy and carboxylic acid groups of compounds **1** and **10** were protected with a methyl moiety, yielding derivatives **14** and **15**, respectively.

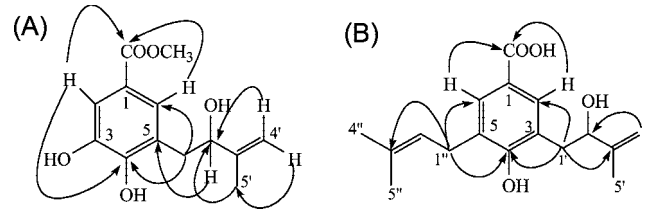


Figure 1. ^{1}H−^{13}C long-range connectivities (HMBC) for compounds **1** (A) and **10** (B).

Treatment of those with (*R*)-(−)-α-methoxyphenylacetic acid (MPA) afforded two monoester derivatives (**16** and **17**, respectively) in approximately 1:1 ratio for each reaction. Thus, **1** and **10** were each confirmed to be an equimolecular racemic mixture. Riguera esterifications have not been performed for **2**, **4**, **5**, **11**, and **13** due to the paucity of material. However, since their structures are closely related to those of **1** and **10**, and their specific rotations were close to 0, it is assumed that these compounds are also racemic mixtures.

In the search for new antiparasitic natural compounds, all the isolated compounds from the two *Piper* species studied, except for compounds **2**, **4**, and **5** due to the small amounts obtained, were investigated for their leishmanicidal, trypanocidal, and antimalarial properties. The results of the evaluation of the compounds against promastigote forms of *Leishmania amazonensis*, *L. braziliensis*, and *L. donovani* (Table 3) showed compound **7** as the most active, with IC_{50} values of 18.2, 13.8, and 18.5 μg/mL, respectively. Compounds **1**, **3**, **6**, **8**, and **10** showed poor leishmanicidal activity (IC_{50} 34.1−89.5 μg/mL), while compounds **9** and **11**−**13** were inactive ($IC_{50} > 100$ μg/mL). Pentamidine was used as positive control (IC_{50} 10 μg/mL). Regarding the trypanocidal activity (Table 3), compounds **1**, **3**, and **7** showed significant activity (IC_{50} 16.4, 15.6, and 18.5 μg/mL, respectively) in comparison with benznidazole, used as positive control (IC_{50} 7.4 μg/mL), while the rest were inactive ($IC_{50} > 20$ μg/mL). The compounds were also evaluated for their antiplasmodial activity against a strain of chloroquine-sensitive *Plasmodium falciparum*, and an $IC_{50} > 5$ μg/mL was considered as inactive. Compounds **6** and **7** exhibited weak activity, as the IC_{50} values were 2.8 and 4.1 μg/mL, respectively (Table 3). Chloroquine was used as positive control ($IC_{50} > 0.1$ μg/mL). Taking into account the results of the biological activities evaluated, a preliminary structure−activity relationship revealed that the prenylated 4-hydroxybenzoic acid derivatives with one side chain (**1**, **3**, **6**, and **7**) were substantially more active as potential antiparasitic agents than those with two side chains (**8**, **9**, **10**, and **11**).

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer 241 automatic polarimeter in $CHCl_3$ at 20 °C, and the $[\alpha]_D$ are given in 10^{-1} deg cm^2 g^{-1}. UV spectra were obtained on a JASCO V-560 spectrophotometer in absolute EtOH. IR (film) spectra were measured on a Bruker IFS 55 spectrophotometer. NMR spectra were recorded on a Bruker Avance 400 NMR spectrometer, and chemical shifts are shown in δ (ppm) with TMS as an internal reference and coupling constants in Hz. EIMS and HREIMS were obtained on a Micromass Autospec spectrometer. Silica gel 60 (particle size 15−40 and 63−200 μm, Machery-Nagel) and Sephadex LH-20 (Pharmacia Biotech) were used for column chromatography, while silica gel 60 F_{254} (Machery-Nagel) was used for analytical and preparative TLC. The spots were visualized by UV light and heating silica gel plates sprayed with H_2O−H_2SO_4−HOAc (1:4:20). All solvents used were analytical grade (Panreac). The optically pure (*R*)-(−)-α-methoxyphenylacetic acid (MPA) was purchased from Sigma, whereas the other reagents were purchased from Aldrich and used without further purification.

Plant Material. Leaves of *P. glabratum* were collected in Ixiamas, Abel Iturralde Province, La Paz, Bolivia, in October 1998. Leaves of *P. acutifolium* were collected in south Yungas Province, La Paz, Bolivia,

Table 1. $^1H-^{13}C$ NMR (δ, $CDCl_3$, J in Hz in parentheses) Data[a] of Compounds **1**, **2**, **4**, **5**, and **7**

	1		**2**		**4**		**5**		**7**	
	δ_H	δ_C[a]	δ_H	δ_C[a]	δ_H	δ_C[a]	δ_H	δ_C[a]	δ_H	δ_C[a]
1		122.3 s		122.4 s		122.1 s		121.4 s		122.2 s
2	7.48 d (1.9)	114.9 d	7.51 d (2.0)	114.6 d	7.75 d (2.1)	133.1 d	7.32 d (1.9)	124.7 d	7.49 d (1.8)	114.5 d
3		145.5 s		146.7 s		125.0 s		122.5 s		143.1 s
4		147.3 s		146.5 s		160.0 s		146.9 s		146.8 s
5		125.2 s		124.9 s	6.92 d (8.4)	116.9 d		143.6 s		127.3 s
6	7.33 d (1.9)	124.6 d	7.35 d (2.0)	124.2 d	7.85 dd (2.1, 8.4)	130.2 d	7.46 d (1.9)	114.2 d	7.43 d (1.8)	123.9 d
1′	2.84 dd (2.1,14.7)	37.7 t	2.85 dd (2.0, 14.4)	37.5 t	2.85 dd (2.2, 14.8)	37.8 t	2.97 d (6.4)	34.2 t	3.38 d (7.2)	29.2 t
	2.99 dd (8.5, 14.7)		3.01 dd (8.8, 14.4)		2.99 dd (8.5, 14.8)					
2′	4.42 dd (2.1, 8.5)	78.3 d	4.44 dd (2.0, 8.8)	78.4 d	4.42 dd (2.2, 8.5)	77.9 d	5.20 t (6.4)	78.1 d	5.32 t (7.2)	121.8 d
3′		145.9 s		145.7 s		145.9 s		140.9 s		134.9 s
4′	4.88 s 5.99 s	111.5 t	4.90 s 5.01 s	111.4 t	4.89 s 5.01 s	111.2 t	4.83 s 4.90 s	114.2 t	1.78 s	17.9 q
5′	1.81 s	18.1 q	1.82 s	17.9 q	1.82 s	18.1 q	1.83 s	17.8 q	1.78 s	25.8 q

[a] Data are based on DEPT, HSQC, and HMBC experiments.

Table 2. $^1H-^{13}C$ NMR (δ, $CDCl_3$, J in Hz in parentheses) Data[a] of Compounds **10**−**13**

	10		**11**		**12**		**13**	
	δ_H	δ_C[a]	δ_H	δ_C[a]	δ_H	δ_C[a]	δ_H	δ_C[a]
1		121.8 s		124.8 s		124.9 s	7.76 s*	121.7 s
2	7.68 d (1.3)	131.5 d	7.83 s	130.9 d*	7.82 d (1.3)	126.9 d		132.4 d*
3		124.9 s		131.9 s		130.7 s		126.9 s*
4		158.9 s		161.1 s		160.1 s		159.6 s
5		129.5 s		135.2 s		135.4 s		126.9 s*
6	7.79 d (1.3)	130.7 d	7.85 s	130.9 d*	8.07 d (1.3)	130.6 d	7.76 s*	132.4 d*
1′	2.86 dd (1.3, 11.0)	37.9 t	2.87 dd (8.6, 13.8)	36.5 t	6.86 d (16.2)	120.4 d	2.93 d (6.5)*	37.6 t*
	2.99 dd (6.5, 11.0)		2.95 dd (4.2, 13.8)					
2′	4.43 dd (1.3, 6.5)	77.9 d	4.35 dd (4.2, 8.6)	75.8 d	6.56 d (16.2)	139.8 d	4.38 d (6.5)*	76.8 d*
3′		146.2 s		146.8 s		71.1 s		146.3 s*
4′	4.90 s 5.02 s	111.2 t	4.87 s 5.01 s	110.9 t	1.46 s*	29.6 q*	4.87 s* 5.00 s*	110.9 t*
5′	1.83 s	17.9 q	1.84 s	17.8 q	1.46 s*	29.6 q*	1.82 s*	17.9 q*
1″	3.38 d (5.4)	28.6 t	3.41 d (7.1)	28.0 t	3.38 d (5.4)	28.1 t	2.93 d (6.5)*	37.6 t*
2″	5.34 t (5.4)	121.7 d	5.29 t (7.1)	121.8 d	5.27 t (5.4)	121.9 d	4.38 d (6.5)*	76.8 d*
3″		133.2 s		133.3 s		133.1 s		146.3 s*
4″	1.74 s	17.5 q	1.75 s	17.7 q	1.74 s*	17.6 q	4.87 s* 5.00 s*	110.9 t*
5″	1.76 s	25.6 q	1.77 s	25.5 q	1.74 s*	25.5 q	1.82 s*	17.9 q*

[a] Data are based on DEPT, HSQC, and HMBC experiments. * Overlapping signals.

Table 3. Antiparasitic Activity of Compounds **1**, **3**, **6**, and **7**−**13** *in Vitro* on *Leishmania* spp., *Trypanosoma cruzi*, and *Plasmodium falciparum*

	$IC_{50} \pm SD$ (μg/mL)[a]				
compound	*L. amazonensis*	*L. braziliensis*	*L. donovani*	*T. cruzi*	*P. falciparum*
1	89.5 ± 0.8	81.9 ± 0.6	81.9 ± 0.6	16.4 ± 0.8	>10
3	78.2 ± 1.4	74.4 ± 0.4	86.9 ± 1.0	15.6 ± 0.7	10.0 ± 0.9
6	74.4 ± 2.7	74.4 ± 1.9	62.5 ± 1.1	>20	2.8 ± 0.6
7	18.2 ± 0.7	13.8 ± 0.5	18.5 ± 0.2	18.5 ± 0.3	4.1 ± 0.5
8	45.3 ± 1.6	34.1 ± 0.5	40.0 ± 1.4	>20	>10
10	67.6 ± 6.4	67.6 ± 3.4	67.3 ± 2.5	>20	>10
12	>100	78.8 ± 2.2	>100	>20	>10
control[b]	10.0 ± 0.7	10.0 ± 0.7	10.0 ± 0.7	7.4 ± 0.5	0.1 ± 0.02

[a] Data are expressed as mean standard deviation of three determinations. [b] Pentamidine, benznidazole, and chloroquine were used as the reference drugs for leishmanicidal, trypanocidal, and antiplasmodial tests, respectively.

in June 1998. Voucher specimens (GB-1877 and GB-1643, respectively) are deposited in the Herbario Nacional de La Paz, Universidad Mayor de San Andrés, La Paz, Bolivia, and identified by Dr. R. Callejas, Herbarium of the Antioquia University, Colombia.

Extraction and Isolation. The dry leaves (200 g) from *P. glabratum* were crushed and extracted with EtOH−H_2O (70:30, v/v) at room temperature for 48 h. Evaporation of the solvent under reduced pressure provided 35 g of crude extract, which was partitioned into a CH_2Cl_2−H_2O (1:1, v/v) solution, yielding the organic (19.2 g) and aqueous (0.9 g) extracts. The organic extract was fractionated by VLC on silica gel with gradient systems of increasing polarity of *n*-hexane−EtOAc (0−100%), yielding eight fractions (1−8). Fraction 4 (1120 mg) was subjected to flash silica gel column chromatography eluted with *n*-hexane containing increasing concentrations of Me_2CO, yielding five fractions (I−V). Fraction II (125 mg) was chromatographed on a silica gel column, using a gradient from *n*-hexane to EtOAc to yield five fractions (A−E). Fraction D (39 mg) was applied to preparative TLC (CH_2Cl_2−Me_2CO 9:1) to yield **3** (16.6 mg) and **4** (2.4 mg). Fraction IV (209 mg) was subjected to gel permeation column chromatography on Sephadex LH-20 (60 × 2 cm), using CH_2Cl_2−MeOH (1:1) as eluent, to yield seven fractions (A−D). Fraction B (42 mg) was subjected to preparative TLC eluted with *n*-hexane−Et_2O (6:4), yielding **1** (15.5 mg), **2** (2.2 mg), and **3** (1.5 mg). Fraction 5 (4.7 g) was subjected to gel permeation column chromatography on Shephadex LH-20 (1.30 × 4 cm), with CH_2Cl_2−MeOH (1:1) as eluent, affording eight fractions (I−VIII). Fraction III (923 mg) was subjected to flash silica gel column chromatography, using a gradient elution from CH_2Cl_2 to Me_2CO, providing five fractions (A−E). Among them, fraction B (116.5 mg) was rechromatographed on a silica gel column, eluted with *n*-hexane containing increasing amounts of CH_2Cl_2 to give **5** (3.2 mg) and **6** (12.9 mg). Compounds **1** (13 mg) and **7** (19.5 mg) were obtained from fraction D (58 mg) after preparative TLC, using CH_2Cl_2−Me_2CO (9:1) as eluent.

Leaves (400 g) of *P. acutifolium* were extracted with EtOH using a Soxhlet apaparatus for 48 h. Evaporation of the solvent under reduced

pressure provided 55 g of crude extract, which was partitioned into a $CH_2Cl_2-H_2O$ (1:1, v/v) solution, yielding the organic (38 g) and aqueous (1.1 g) extracts. The organic extract was fractionated by VLC on silica gel (63−200 mesh, 12 × 15 cm) and eluted with gradient systems of increasing polarity of *n*-hexane to EtOAc to afford nine fractions (1−9). Further flash column chromatography of fraction 5 (3.2 g) on silica gel, using a gradient elution from *n*-hexane to Et_2O, yielded seven fractions (I−VII). Compound **10** (7 mg) was obtained by purification of fraction V (28 mg) after preparative TLC, using *n*-hexane−Me_2CO (8:2) as eluent. Fraction VII (26 mg) was subjected to preparative TLC (*n*-hexane−Et_2O, 3:7) to yield compounds **11** (6.8 mg) and **12** (10.1 mg). Analysis of fraction 7 (1.6 g), which was applied to a flash silica gel column and eluted with CH_2Cl_2 containing increasing volumes of Me_2CO, afforded eight fractions (I−VIII). Fraction VII (117 mg) was subjected to silica gel column chromatography using a gradient mixture of EtOAc in CH_2Cl_2, resulting in four fractions (A−D). Compound **13** (6.7) was obtained from fraction D (30 mg) after preparative TLC, using CH_2Cl_2−EtOAc (7:3) as eluent.

(±)-Methyl 3,4-dihydroxy-5-(2-hydroxy-3-methyl-3-butenyl)benzoate (1): white, amorphous solid; $[\alpha]^{20}_D$ +1.2 (*c* 0.35, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 214 (3.4), 266 (4.5), 298 (4.6) nm; IR (film) ν_{max} 3409, 2953, 1692, 1602, 1439, 1311, 1228, 901, 770 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 3.09 (1H, br s, OH), 3.85 (3H, s, OMe), 5.93 (1H, br s, OH), 9.20 (1H, br s, OH), for other signals, see Table 1. ^{13}C NMR ($CDCl_3$, 125 MHz) δ 51.9 (q, OCH_3), 167.2 (s, $\underline{C}OOCH_3$), for other signals, see Table 1; EIMS *m/z* 252 (M^+, 28), 234 (55), 219 (81), 203 (19), 182 (100), 150 (47), 123 (18); HREIMS *m/z* 252.1014 (calcd for $C_{13}H_{16}O_5$, 252.0998).

(±)-Ethyl 3,4-dihydroxy-5-(2-hydroxy-3-methyl-3-butenyl)benzoate (2): amorphous solid; $[\alpha]^{20}_D$ −4.8 (*c* 0.12, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 218 (4.9), 265 (5.0), 299 (5.1) nm; IR (film) ν_{max} 3433, 2923, 2853, 1712, 1601, 1448, 1303, 1221, 1056, 767 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.37 (3H, t, J = 7.2 Hz, H-2″), 4.32 (2H, q, J = 7.2, Hz, H-1″), for other signals, see Table 1; ^{13}C NMR ($CDCl_3$, 125 MHz) δ 14.1 (q, C-2″), 60.4 (t, C-1″), 166.2 (s, $\underline{C}OOCH_2CH_3$), for other signals, see Table 1; EIMS *m/z* 266 (M^+, 61), 248 (69), 233 (57), 221 (30), 196 (95), 167 (100), 149 (67), 123 (41), 57 (36); HREIMS *m/z* 266.1154 (calcd for $C_{14}H_{18}O_5$, 266.1154).

(±)-Ethyl 4-hydroxy-3-(2-hydroxy-3-methyl-3-butenyl)benzoate (4): white, amorphous solid: $[\alpha]^{20}_D$ +3.9 (*c* 0.2, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 260 (4.5) nm; IR (film) ν_{max} 3409, 2923, 1710, 1690, 1607, 1284, 1180, 1018, 772 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.37 (3H, t, J = 7.1 Hz, H-2″), 4.32 (2H, q, J = 7.1 Hz, H-1″), for other signals, see Table 1; ^{13}C NMR ($CDCl_3$, 125 MHz) δ 14.2 (q, C-2″), 60.4 (t, C-1″), 166.4 (s, $\underline{C}OOCH_2CH_3$), for other signals, see Table 1; EIMS *m/z* 250 M^+, 6), 232 (4), 217 (3), 205 (11), 180 (100), 151 (41), 134 (24), 107 (23), 71 (10); HREIMS *m/z* 250.1196 (calcd for $C_{14}H_{18}O_4$, 250.1205).

(±)-Methyl 3-[2-(acetoxy)-3-methyl-3-butenyl)-4,5-dihydroxybenzoate (5): amorphous solid; $[\alpha]^{20}_D$ −1.4 (*c* 0.2, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 213 (4.4), 265 (4.5), 298 (4.6) nm; IR (film) ν_{max} 3391, 2953, 2926, 1712, 1603, 1441, 1375, 1308, 1233, 1020, 768 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 2.10 (3H, s, H-2″), 3.86 (3H, s, OCH_3), for other signals, see Table 1; ^{13}C NMR ($CDCl_3$, 125 MHz) δ 21.0 (q, C-2″), 51.8 (q, OCH_3), 166.9 (s, $\underline{C}OOCH_3$), 171.9 (s, C-1″), for other signals, see Table 1; EIMS *m/z* 294 (M^+, 2), 234 (78), 219 (100), 203 (21), 182 (34), 174 (51), 157 (43), 129 (21); HREIMS *m/z* 294.1107 (calcd for $C_{15}H_{18}O_6$, 294.1103).

Methyl 3,4-dihydroxy-5-(3-methyl-2-butenyl)benzoate (7): amorphous solid; UV (EtOH) λ_{max} (log ε) 217 (4.3), 266 (4.4), 295 (4.5) nm; IR (film) ν_{max} 3471, 3371, 2923, 1692, 1604, 1440, 1303, 1237, 1010, 768 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 3.83 (3H, s, OCH_3); 5.98 (1H, br s, OH), for other signals, see Table 1; ^{13}C NMR ($CDCl_3$, 125 MHz) δ 52.0 (q, OCH_3), 167.3 (s, $\underline{C}OOCH_3$), for other signals, see Table 1; EIMS *m/z* 236 (M^+, 100), 221 (5), 205 (18), 180 (100), 148 (18); HREIMS *m/z* 236.1034 (calcd for $C_{13}H_{16}O_4$, 236.1049).

(±)-4-Hydroxy-3-(2-hydroxy-3-methyl-3-butenyl)-5-(3-methyl-2-butenyl)benzoic acid (10): amorphous solid; $[\alpha]^{20}_D$ −3.6 (*c* 0.2, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 209 (4.4), 258 (4.5) nm; IR (film) ν_{max} 3520−2670, 3370, 3167, 2924, 2855, 1680, 1601, 1441, 1409, 1286, 1214, 776 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz), see Table 2; ^{13}C NMR ($CDCl_3$, 125 MHz) δ 170.1 (s, COOH), for other signals, see Table 2; EIMS *m/z* 290 (M^+, 5), 272 (29), 257 (45), 217 (38), 199 (100), 173 (55), 159 (39), 91 (50), 69 (53); HREIMS *m/z* 290.1537 (calcd for $C_{17}H_{22}O_4$, 290.1518).

(±)-3-(2-Hydroxy-3-methyl-3-butenyl)-4-methoxy-5-(3-methyl-2-butenyl)benzoic acid (11): white, amorphous solid; $[\alpha]^{20}_D$ −4.7 (*c* 0.2, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 207 (4.5), 242 (4.6) nm; IR (film) ν_{max} 3518−2694, 3469, 2927, 1694, 1603, 1205, 1002, 756 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 3.82 (3H, s, OCH_3), for other signals, see Table 2; ^{13}C NMR ($CDCl_3$, 125 MHz) δ 60.8 (q, OCH_3); 170.1 (s, COOH), for other signals, see Table 2; EIMS *m/z* 304 (M^+, 12), 286 (21), 247 (70), 232 (92), 199 (88), 149 (84), 91 (85), 71 (100); HREIMS *m/z* 304.1608 (calcd for $C_{18}H_{24}O_4$, 304.1596).

3-[(1*E*)-3-Hydroxy-3-methyl-1-butenyl]-4-methoxy-5-(3-methyl-2-butenyl)benzoic acid (12). amorphous solid; $[\alpha]^{20}_D$ +1.0 (*c* 0.1, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 229 (4.1) nm; IR (film) ν_{max} 3829−2790, 3433, 2970, 2928, 1696, 1601, 1380, 1265, 1202, 999, 757 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 3.76 (3H, s, OCH_3), for other signals, see Table 2; ^{13}C NMR ($CDCl_3$, 125 MHz) δ 61.0 (q, OCH_3), 171.2 (s, COOH), for other signals, see Table 2; EIMS *m/z* 304 (M^+, 12), 286 (83), 271 (44), 245 (47), 231 (75), 217 (100), 199 (83), 173 (69), 128 (63), 91 (53), 59 (59); HREIMS *m/z* 304.1675 (calcd for $C_{18}H_{24}O_4$, 304.1675).

(±)-4-Hydroxy-3,5-bis(2-hydroxy-3-methyl-3-butenyl)benzoic acid (13): white, amorphous solid; $[\alpha]^{20}_D$ −5.0 (*c* 0.1, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 213 (4.6), 253 (4.7) nm; IR (film) ν_{max} 3494−2776, 3442, 2925, 2854, 1699, 1605, 1455, 1378, 1292, 1201, 773 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ, see Table 2; ^{13}C NMR ($CDCl_3$, 125 MHz) δ 170.2 (s, COOH), for other signals, see Table 2; EIMS *m/z* 306 (M^+, 5), 288 (7), 270 (14), 236 (48), 218 (100), 203 (32), 173 (24), 130 (18), 69 (57); HREIMS *m/z* 306.1521 (calcd for $C_{17}H_{22}O_5$, 306.1467).

Preparation of Derivatives 14 and 15. To a solution of compound **1** (16 mg) or **10** (7 mg) in acetone (2 mL) were added K_2CO_3 (45 mg) and dimethyl sulfate (0.05 mL), and the reaction was stirred for 72 h at room temperature. The reaction mixture was concentrated to remove the organic solvent. Water (5 mL) was added and the product was extracted using CH_2Cl_2 (3 × 5 mL). The organic layer was dried over $MgSO_4$, filtered, and concentrated to yield an oil, which was purified in preparative TLC (CH_2Cl_2−acetone, 9:1) to afford **14** (12 mg) and **15** (5 mg), respectively.

(±)-Methyl 3,4-dimethoxy-5-(2-hydroxy-3-methyl-3-butenyl)benzoate (14): amorphous solid; $[\alpha]^{20}_D$ +0.4 (*c* 0.9, $CHCl_3$); IR (film) ν_{max} 3459, 2953, 1693, 1602, 1439, 1311, 1228, 901, 772 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.82 (3H, s, H-5′), 2.19 (1H, br s, OH), 2.81 (1H, dd, J = 2.2, 14.7 Hz, H-1′), 2.96 (1H, dd, J = 8.4, 14.7 Hz, H-1′), 3.89 (3H, s, OCH_3), 3.90 (6H, s, 2 × OCH_3), 4.29 (1H, dd, J = 2.2, 8.4 Hz, H-2′), 4.84 (1H, s, H-4′), 4.96 (1H, s, H-4′), 7.49 (1H, s), 7.55 (1H, s); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 17.8 (q, C-5′), 36.6 (t, C-1′), 51.9 (q, OCH_3), 55.6 (q, OCH_3), 60.4 (q, OCH_3), 75.8 (d, C-2′), 110.7 (t, C-4′), 111.8 (d, C-2), 124.6 (d, C-6), 125.2 (s, C-1), 132.0 (s, C-5), 146.8 (s, C-3′), 151.1 (s, C-3), 152.1 (s, C-4), 166.5 (s, $\underline{C}OOMe$); EIMS *m/z* 280 (M^+, 4), 262 (37), 247 (32), 210 (100), 195 (51), 172 (40), 151 (15), 91 (11); HREIMS *m/z* 280.1309 (calcd for $C_{15}H_{20}O_5$, 280.1311).

(±)-Methyl 4-methoxy-3-(2-hydroxy-3-methyl-3-butenyl)-5-(3-methyl-2-butenyl)benzoate (15): amorphous solid; $[\alpha]^{20}_D$ −3.2 (*c* 0.3, $CHCl_3$); IR (film) ν_{max} 3469, 2928, 1692, 1603, 1405, 1002, 775 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.76 (3H, s, H-4″), 1.78 (3H, s, H-5″), 1.85 (3H, s, H-5′), 2.20 (1H, br s, OH), 2.87 (1H, dd, J = 8.6, 13.8 Hz, H-1′), 2.97 (1H, dd, J = 4.2, 13.8 Hz, H-1′), 3.41 (2H, d, J = 7.1 Hz, H-1″), 3.82 (3H, s, OCH_3), 3.90 (3H, s, OCH_3), 4.35 (1H, dd, J = 4.2, 8.6 Hz, H-2′), 4.88 (1H, s, H-4′), 5.03 (1H, s, H-4′), 5.30 (1H, t, J = 7.1 Hz, H-2″), 7.79 (1H, s, H-2), 7.80 (1H, s, H-6); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 17.6 (q, C-4″), 17.8 (q, C-5′), 25.5 (q, C-5″), 28.1 (t, C-1″), 36.5 (t, C-1′), 51.9 (q, OCH_3), 60.8 (q, OCH_3), 75.9 (d, C-2′), 110.9 (t, C-4′), 121.9 (d, C-2″), 123.8 (s, C-1), 130.9 (2 × d, C-2, C-6), 131.9 (s, C-3), 133.3 (s, C-3″), 135.2 (s, C-5), 146.8 (s, C-3′), 161.1 (s, C-4), 167.3 (s, $\underline{C}OOCH_3$); EIMS *m/z* 318 (M^+, 5), 300 (19), 285 (14), 248 (100), 233 (25), 210 (22), 151 (12), 91 (5); HREIMS *m/z* 318.1694 (calcd for $C_{19}H_{26}O_4$, 318.1674).

Preparation of Riguera's Esters. A solution of (*R*)-MPA (8 mg), **14** (6 mg) or **15** (4 mg), DCC (10 mg), and a catalytic amount of DMAP in dry CH_2Cl_2 was stirred at room temperature for 24 h, and the solvent was removed to give a thick oil, which was purified by preparative TLC (*n*-hexane−EtOAc, 6:4) to give **16** (4 mg) and **17** (3 mg), respectively.

(*R*)-α-Methoxy-α-phenylacetyl derivative of 14 (16): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.68 (3H, s, H-5′), 1.78 (3H, s, H-5′), 2.88 (2H, m, H-1′), 2.97 (1H, dd, $J = 4.6$, 13.8 Hz, H-1′), 2.09 (1H, dd, $J = 5.2$, 14.2 Hz, H-1′), 3.28 (3H, s, OCH_3, MPA), 3.36 (3H, s, OCH_3, MPA), 3.86 (3H, s, OCH_3), 3.89 (3H, s, OCH_3), 3.92 (6H, s, $2 \times OCH_3$), 3.93 (3H, s, OCH_3), 3.95 (3H, s, OCH_3), 4.64 (1H, s, H-4′), 4.68 (1H, s, H-4′), 4.70 (1H, s, MPA), 4.72 (1H, s, MPA), 4.87 (1H, s, H-4′), 4.90 (1H, s, H-4′), 5.48 (2H, m, H-2′), 7.26−7.55 (14H, m).

(*R*)-α-Methoxy-α-phenylacetyl derivative of 15 (17): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.60 (3H, s, H-5′), 1.75 (6H, s, H-4″), 1.77 (6H, s, H-5″), 1.78 (3H, s, H-5′), 2.86 (2H, m, H-1′), 2.96 (1H, dd, $J = 4.0$, 13.2 Hz, H-1′), 3.08 (1H, dd, $J = 4.1$, 13.4 Hz, H-1′), 3.29 (3H, s, OCH_3, MPA), 3.35 (3H, s, OCH_3, MPTA), 3.42 (4H, d, $J = 6.9$ Hz, H-1″), 3.80 (3H, s, OCH_3), 3.81 (3H, s, OCH_3), 3.91 (3H, s, OCH_3), 3.92 (3H, s, OCH_3), 4.64 (1H, s, H-4′), 4.67 (1H, s, H-4′), 4.69 (1H, s, MPA), 4.71 (1H, s, MPA), 4.80 (1H, s, H-4′), 4.89 (1H, s, H-4′), 5.31 (2H, t, $J = 6.9$ Hz, H-2″), 5.46 (2H, m, H-2′), 7.25−7.49 (14H, m).

Leishmanicidal Activity. The *in vitro* leishmanicidal activity was evaluated against promastigote forms of *Leishmania braziliensis* 2903, *L. amazonensis* PH8, and *L. donovani* PP75 (all from IBBA, Instituto Boliviano de Biología de Altura), which were grown at 28 °C in stationary culture and were seeded at 1×10^4/100 μL/well in 96-well flat bottom microtriter plates in *Leishmania* medium based on RPMI 1640. Test samples or standard drugs dissolved in DMSO were added at a further 100 μL/well to give a final concentration of 100 μg/mL and serial dilutions thereof.[15] Leishmanicidal activity was expressed as IC_{50} values (the concentration of a compound that caused a 50% reduction in parasite viability). Pentamidine (10 μg/mL) (Sigma-Aldrich) was used as positive control.

Trypanocidal Activity. Epimastigote forms of *Trypanosoma cruzi* Tulahuen strain were cultivated at 26 °C in liver infusion tryptose medium (LIT), supplemented (5%) with heat-inactivated (56 °C for 30 min) fetal calf serum (technically modified from Chataing et al.).[16] Parasites in logarithmic growth phase were distributed in 96-well flat-bottom microtiter plates at a concentration of 5×10^4/mL. Test samples or standard drugs dissolved in DMSO were added at a further 100 μL/well to give a final concentration of 100 μg/mL and serial dilutions thereof.[15] The activity was measured by optic counting with an inverted microscope and comparison with control wells. Benznidazole (7.4 μg/mL) was used as the reference drug for this assay. All assays were carried out in triplicate.

Antiplasmodial Activity. F-32 Tanzania (chloroquine sensitive) strains of *Plasmodium falciparum* (kindly provided by Dr. Fandeur, Pasteur Institute, Cayenne, France) were cultured according to Trager and Jensen[17] on glucose-enriched RPMI 1640 medium, supplemented with 10% human serum at 37 °C. After 24 h of incubation at 37 °C, the medium was replaced by fresh medium supplemented with the compound to be evaluated at three different concentrations (0.1, 1, and 10 μg/mL), and incubation was continued for a further 48 h. On the third day of the test, a blood smear was taken from each well, and parasitemia was calculated for each concentration of sample compared to the control.[18] IC_{50} values were determined graphically by plotting concentrations versus percent inhibition. Chloroquine (0.1 μg/mL) was used as a positive control. All tests were performed in triplicate.

Acknowledgment. This investigation was supported by International Foundation of Sciences (Switzerland, grant F/3408-1) and DGCYT CTQ2006-13376/BQU and PCI-Iberoamérica (A/4954/06, AECI) projects. International collaboration was supported by project X.5 "Búsqueda, Obtención y Evaluación de Nuevos Agentes Antiparasitarios" CYTED (Iberoamerican Program of Science and Technology for Development, Subprogram X).

References and Notes

(1) Pink, R.; Hudson, A.; Mouriès, M. A.; Bendig, M. *Drug Discovery* **2005**, *4*, 727–740.
(2) Liñares, G. E. G.; Ravaschino, E. L.; Rodríguez, J. B. *Curr. Med. Chem.* **2006**, *13*, 335–360.
(3) Harvey, A. L. *Curr. Opin. Chem. Biol.* **2007**, *11*, 480–484179189.
(4) Parmar, V. S.; Jain, S. C.; Bisht, K. S.; Jain, R.; Taneja, P.; Jha, A.; Tyagi, O. D.; Prasad, A. K.; Wengel, J.; Olsen, C. E.; Boll, P. M. *Phytochemistry* **1997**, *4*, 597–673.
(5) Tagboto, S.; Townson, S. *Adv. Parasitol.* **2001**, *50*, 199–295.
(6) Portet, B.; Fabre, N.; Roumy, V.; Gornitzka, H.; Bourdy, G.; Chevalley, S.; Sauvain, M.; Valentin, A.; Moulis, C. *Phytochemistry* **2007**, *68*, 1312–1320.
(7) Hermoso, A.; Jiménez, I. A.; Mamani, Z. A.; Bazzocchi, I. L.; Piñero, J. E.; Ravelo, A. G.; Valladares, B. *Bioorg. Med. Chem.* **2003**, *11*, 3975–3980.
(8) Flores, N.; Cabrera, G.; Jiménez, I. A.; Piñero, J.; Giménez, A.; Bourdy, G.; Cortés-Selva, F.; Bazzocchi, I. L. *Planta Med.* **2007**, *73*, 206–211.
(9) Luize, P. S.; Ueda-Nakamura, T.; Filho, B. P.; Cortéz, G. D.; Nakamura, C. V. *Biol. Pharm. Bull.* **2006**, *10*, 2126–2130.
(10) Lognay, G. C.; Bouxin, P.; Marlier, M.; Haubruge, E.; Gaspar, C.; Rodríguez, A. *J. Essent. Oil Res.* **1996**, *8*, 689–691.
(11) Díaz, D. P.; Arias, C. T.; Joseph-Nathan, P. *Phytochemistry* **1987**, *26*, 809–811.
(12) Pereda-Miranda, R.; Bernard, C. B.; Durst, T.; Arnason, J. T.; Sánchez-Vindas, P.; Poveda, L.; San Roman, L. *J. Nat. Prod.* **1997**, *60*, 282–284.
(13) Orjala, J.; Erdelmeier, C. A. J.; Wright, A. D.; Rali, T.; Sticher, O.; Poveda, L.; San Roman, L. *Planta Med.* **1993**, *59*, 546–551.
(14) Seco, J. M.; Quínoa, E.; Riguera, R. *Chem. Rev.* **2004**, *104*, 17–117.
(15) Deharo, E.; Ruiz, G.; Vargas, F.; Sagua, H.; Ortega, E.; Rojas, A.; Giménez A. *Técnicas de laboratorio para la selección de sustancias antichagas y leishmanicidas*; Prisa Ltda.-CYTED: La Paz, Bolivia, 2004.
(16) Chataing, B.; Concepción, J. L.; Lobatón, R.; Usubillaga, A *Planta Med.* **1998**, *64*, 31–36.
(17) Trager, W.; Jensen, J. B. *Science* **1976**, *193*, 673–675.
(18) Deharo, E.; Gaultiere, Ph.; Muñoz. V.; Sauvain, M. *Técnicas de la boratorio para la selección de sustancias antimaláricas*; CYTED-IRD: La Paz, Bolivia, 2000.

NP800104P