Chemosensitization of a Multidrug-Resistant *Leishmania tropica* Line by New Sesquiterpenes from *Maytenus magellanica* and *Maytenus chubutensis*

María L. Kennedy,[†,‡] Fernando Cortés-Selva,[§,‡] José M. Pérez-Victoria,[§] Ignacio A. Jiménez,[†] Antonio G. González,[†] Orlando M. Muñoz,[||] Francisco Gamarro,[§] Santiago Castanys,*[,§] and Angel G. Ravelo*[,†]

Instituto Universitario de Bio-Orgánica "Antonio González", Universidad de La Laguna, Avenida Astrofísico Francisco Sánchez, 2, 38206 La Laguna, Tenerife, Spain, Instituto de Parasitología y Biomedicina "López-Neyra", Consejo Superior de Investigaciones Científicas, Granada, Spain, and Facultad de Ciencias, Universidad de Chile, Casilla 653, Santiago de Chile, Chile

Received June 27, 2001

Parasite resistance to drugs has emerged as a major problem in current medicine, and therefore, there is great clinical interest in developing compounds that overcome these resistances. In an intensive study of South American medicinal plants, herein we report the isolation, structure elucidation, and biological activity of dihydro-β-agarofuran sesquiterpenes from the roots of *Maytenus magellanica* (**1**−**14**) and *M. chubutensis* (**14**−**17**). This type of natural products may be considered as *privileged structures*. The structures of 10 new compounds, **1**, **3**, **6**−**9**, and **12**−**15**, were determined by means of ^{1}H and ^{13}C NMR spectroscopic studies, including homonuclear (COSY and ROESY) and heteronuclear correlation experiments (HMQC and HMBC). The absolute configurations of eight hetero- and homochromophoric compounds, **1**, **3**, **6**−**9**, **12**, and **13**, were determined by means of CD studies. Fourteen compounds, **1**−**3** and **6**−**16**, have been tested on a multidrug-resistant *Leishmania tropica* line overexpressing a P-glycoprotein-like transporter to determine their ability to revert the resistance phenotype and to modulate intracellular drug accumulation. From this series, **1**, **2**, **3**, **14**, and **15** showed potent activity, **1** being the most active compound. The structure−activity relationships of the different compounds are discussed.

Introduction

The species of the Celastraceae family have a long history in traditional medicine.[1,2] The leaves and roots of different species of *Maytenus* are used in folk medicine around the world.[1] The stem bark of *M. senegalensis*[3] is used in Sudan for the treatment of tumors, dysentery, and snake bites, whereas *M. ilicifolia* has been used for anticancer and contraception in South America[4] and for the treatment of gastric ulcers, dyspepsia, and other intestinal disorders.[5,6] As part of an intensive investigation into biologically active metabolites from species of this family, we had previously reported quinone-methide[7,8] and dimeric triterpenes[9] showing antimicrobial and cytotoxic activities. On the other hand, sesquiterpene esters, based on the dihydro-β-agarofuran [5,11-epoxy-5β,10α-eudesman-4-(14)-ene] skeleton, are chemotaxonomic indicators of the family,[10] and they have attracted a great deal of interest because of their immunosuppressive,[11] cytotoxic,[12] insect-antifeedant, and insecticidal activities.[13] Recently, they have shown anti-HIV,[14] reversal multidrug resistance (MDR) phenotype,[15,16] and antitumor-promoting activities.[17] These data along with their structural characteristics make dihydro-β-agarofuran sesquiterpenes to be considered as *privileged structures*.[18]

Protozoan parasites are responsible for some of the most important and prevalent diseases of human and domestic animals, threatening the lives of nearly one-quarter of the human population. World Health Organization statistics show that with a 42-fold increase in the past 15 years leishmaniasis has become the second worldwide cause of death among parasitic diseases,[19] mainly because of the appearance of drug-resistance mechanisms. The MDR phenotype due to the increased expression of P-glycoprotein (Pgp)-like transporters has been characterized in tumor cells[20,21] and protozoan parasites,[22] including *Plasmodium*[23] and *Leishmania* spp.[24−26] Pgp belongs to the ABC (ATP−binding−cassette) superfamily of transporters and exports a wide range of drugs from the cell, decreasing their intracellular concentration and preventing their cytotoxic activity. Structural analysis of Pgp shows two homologous halves, each composed of a cytosolic nucleotide-binding domain whose ATPase activity provides the energy needed for the active export of cytotoxic compounds and a transmembrane domain, involved in drug efflux. Mammalian Pgp can be inhibited in vitro by reversal agents, also called chemosensitizers or modulators, such as verapamil and cyclosporin A, which compete with drug binding to the transmembrane domains.[27] However, most of these modulators are also pumped substrates and therefore require high concentrations for effective inhibition. These concentrations produce undesirable side effects that hamper their clinical use. In addition, these classical modulators of drug efflux in mammalian cancer cells only poorly sensitize the MDR phenotype in *Leishmania* parasites.[24,26,28] Thus, new

* To whom correspondence should be addressed. For S.C.: phone, 34-958-805185; fax, 34-958-203323; e-mail, castanys@ipb.csic.es. For A.G.R.: phone, 34-922-318576; fax, 34-922-318571; e-mail, agravelo@ull.es.
† Universidad de La Laguna.
‡ Both authors contribute equally to this paper.
§ Consejo Superior de Investigaciones Científicas.
|| Universidad de Chile.

10.1021/jm010970c CCC: $20.00
Published on Web 11/22/2001

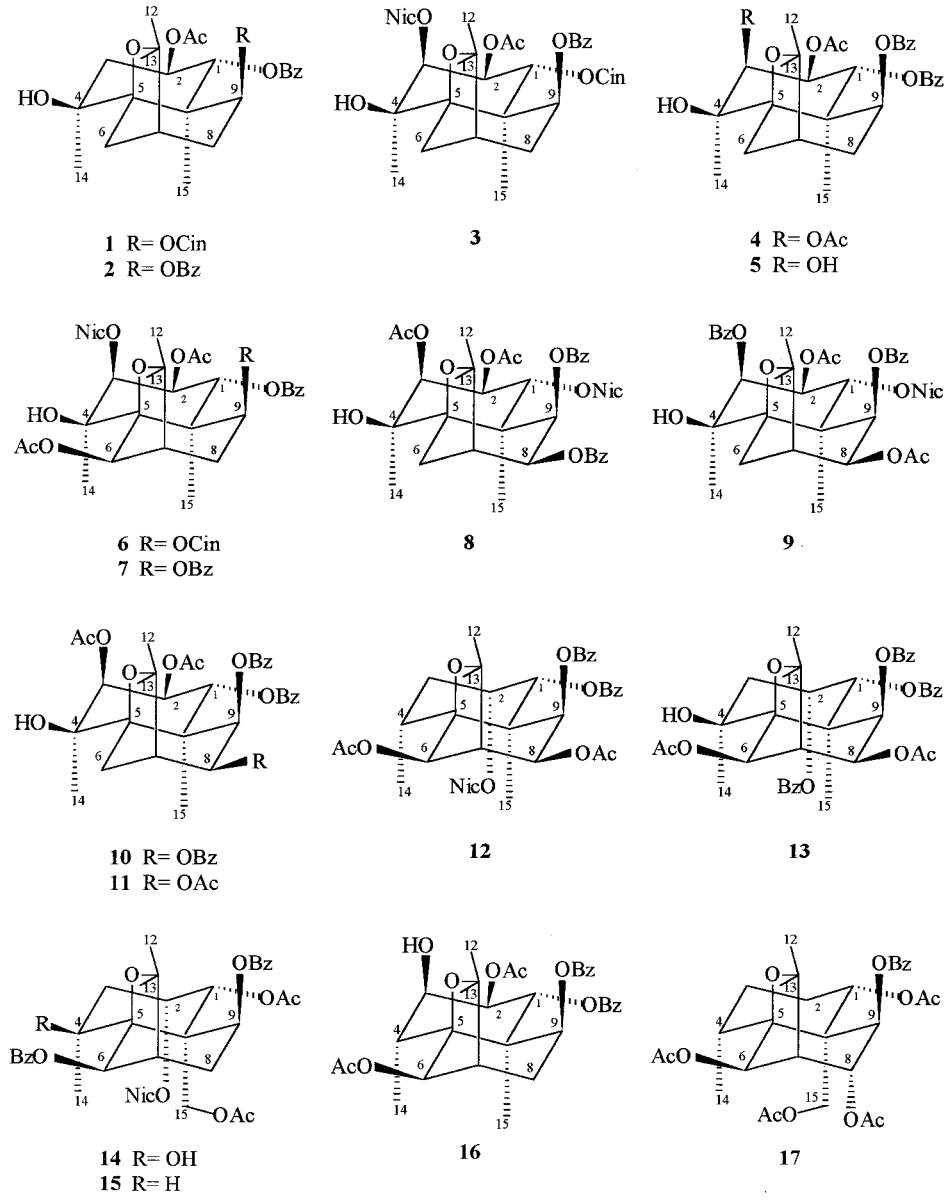

Figure 1. Sesquiterpenes from *Maytenus magellanica* and *M. chubutensis*.

classes of more specific, nontransported inhibitors of Pgp-like transporters with lower host toxicity need to be developed.

Dihydro-*β*-agarofuran sesquiterpenes are particularly promising modulators of the MDR phenotype in eukaryotic organisms. Interestingly, we have recently reported that a number of dihydro-*β*-agarofuran sesquiterpene derivatives from *Crossopetalum tonduzii* constituted efficient modulators of *Leishmania* MDR reverting significantly the Pgp-like transporter mediated daunomycin (DNM) resistance.[15] Besides, dihydro-*β*-agarofuran sesquiterpene derivatives isolated from the roots of *Celastrus orbiculatus* partially or completely reversed the MDR phenotype in cancer cells.[16]

This paper reports on the phytochemical analysis of the root bark extracts of the Chilean genus *Maytenus* (Celastraceae) (*M. chubutensis* and *M. magellanica*) and describes the isolation of 10 new sesquiterpenes (**1**, **3**, **6**−**9**, and **12**−**15**) with a dihydro-*β*-agarofuran skeleton. Their structures were elucidated by means of ^{1}H and ^{13}C NMR spectroscopic studies, including homonuclear (correlation spectroscopy (COSY) and rotating-frame Overhauser enhancement spectroscopy (ROESY)) and heteronuclear correlation experiments (heteronuclear multiple-quantum coherence (HMQC) and heteronuclear multiple-bond correlation (HMBC)). The absolute configurations of the eight compounds **1**, **3**, **6**−**9**, **12**, and **3** were determined by application of the circular dichroism (CD) exciton chirality method. The known compounds **2**,[29] **4**,[30] **5**,[30] **10**,[29] **11**,[31] **16**,[31] and **17**[32] were identified by spectroscopic methods and comparison with authentic samples or reported data. Fourteen compounds **1**−**3** and **6**−**16** have been tested on a MDR *Leishmania tropica* line overexpressing a Pgp-like transporter to determine their ability to revert the resistance phenotype and to modulate intracellular drug accumulation.

Results and Discussion

Repeated chromatography of the *n*-hexane/Et_2O (1:1) extract of the root bark of *M. magellanica* on Sephadex LH-20 and silica gel afforded, in addition to the known compounds **2**, **4**, **5**, **10**, and **11**, the new compounds **1**, **3**, **6**−**9**, and **12**−**14** (Figure 1), and

Table 1. ^{1}H NMR ($CDCl_3$) Chemical Shift δ and J (Hz) Values (in Parentheses) of Compounds **1**, **3**, **6**−**9**, and **12**−**15**

compd	H-1	H-2	H-3	H-6	H-7	H-8	H-9	H-15
1	5.94 d (10.5)	5.14 dt (4.8, 10.5)	2.07 m[b]	2.07 m[b] 1.80 d (12.4)	2.07 m[b]	2.07 m[b] 2.42 m	4.79 d (5.3)	1.45 s[b]
3	6.43 d (11.2)	5.41 dd (2.9, 11.2)	5.52 d (2.9)	2.16 m[b] 1.96 d (12.4)	2.12 m	2.16 m[b] 2.46 m	4.89 d (5.0)	1.55 s
6	6.41 d (10.9)	5.39 dd (2.8, 10.9)	5.40 d (2.8)	5.59 s	2.21 m[b]	2.21 m[b] 2.46 m	4.84 d (5.6)	1.60 s
7	6.42 d (11.2)	5.38 dd (2.8, 11.2)	5.41 d (2.8)	5.65 s	2.25 m[b]	2.47 m 2.25 m[b]	5.06 d (5.6)	1.59 s
8	6.35 d (10.8)	5.33 dd (2.9, 10.8)	5.31 d (2.9)	2.15 d (12.9) 2.67 dd (12.9,4.1)	2.46 t (4.1)	5.77 dd (4.1, 5.8)	5.51 d (5.8)	1.64 s
9[a]	6.78 d (11.2)	5.52 dd (2.7, 11.2)	5.86 d (2.7)	1.27 d (13.0) 2.08 dd (13.0,4.9)	1.76 m[b]	5.41 dd (4.4, 5.9)	5.60 d (5.9)	1.23 s
12	5.94 d (3.6)	6.04 m	2.43 m[b] 2.62 m[b]	5.52 s	2.55 d (2.7)	5.71 dd (2.7, 6.3)	5.36 d (6.3)	1.80 s
13	5.82 d (2.8)	5.93 m	2.30 dd_{AB} (3.6, 15.4)	5.73 s[b]	2.48 d (3.0)	5.73 dd[b] (3.0, 4.7)	5.38 d (4.7)	1.85 s
14	5.76 d (3.5)	5.86 m	2.22 m[b]	6.35 s	2.36 t (2.9)	2.69 m 2.22 m[b]	5.56 d (7.1)	4.51, 5.25 d_{AB} (12.8)
15	5.89 d (3.5)	5.93 m	2.15 m 2.65 m[b]	6.28 s	2.43 t (2.7)	2.65 m[b] 2.31 m[b]	5.53 d (7.2)	4.44, 5.34 d_{AB} (12.5)

[a] C_6D_6. [b] Overlapping signals.

Table 2. ^{13}C NMR ($CDCl_3$) Chemical Shift δ Values[a] of Compounds **1**, **3**, **6**−**9**, and **12**−**15**

carbon	**1**	**3**	**6**	**7**	**8**	**9**[b]	**12**	**13**	**14**	**15**
C-1	73.0 d	67.9 d	67.6 d	67.7 d	67.5 d	67.9 d	71.7 d	70.7 d	69.7 d[c]	71.4 d
C-2	69.4 d	69.0 d	67.7 d	68.6 d	68.8 d	72.2 d	71.6 d	69.9 d	68.9 d	71.0 d
C-3	42.7 t	77.0 d	77.5 d	77.6 d	75.6 d	77.0 d	31.1 t	42.3 t	42.4 t	31.0 t
C-4	70.6 s	70.4 s	70.8 s	70.6 s	69.9 s	70.3 s	33.6 d	69.6 s	69.7 s[c]	33.3 d
C-5	90.0 s	90.1 s	91.5 s	91.5 s	89.4 s	89.8 s	89.4 s	91.3 s	91.1 s	89.4 s
C-6	31.5 t	32.6 t	79.8 d	79.7 d	32.7 t	32.2 t	77.2 d	77.2 d	78.6 d	78.3 d
C-7	43.4 d	42.6 d	48.4 d	48.4 d	48.6 d	47.7 d	53.8 d	54.0 d	49.1 d	48.9 d
C-8	31.0 t	30.9 t	31.9 t	30.9 t	71.1 d	69.3 d	68.6 d	68.3 d	34.7 t	35.0 t
C-9	73.4 d	73.1 d	72.4 d	72.4 d	71.9 d	69.5 d	72.4 d	72.1 d	70.4 d	69.3 d
C-10	48.3 s	47.7 s	51.2 s	51.2 s	47.7 s	48.4 s	48.7 s	50.3 s	54.9 s	53.2 s
C-11	83.9 s	84.2 s	85.4 s	85.5 s	84.2 s	84.3 s	83.3 s	85.1 s	84.9 s	82.9 s
C-12	29.7 q	29.6 q	29.9 q	29.8 q	30.9 q	30.5 q	31.2 q	30.2 q	29.5 q	30.6 q
C-13	24.9 q	24.4 q	26.0 q	26.2 q	25.5 q	25.3 q	25.7 q	26.4 q	25.7 q	26.0 q
C-14	24.3 q	23.7 q	23.6 q	23.4 q	23.9 q	23.1 q	25.5 q	25.5 q	25.2 q	18.3 q
C-15	19.9 q	19.9 q	20.6 q	20.4 q	20.5 q	19.3 q	20.6 q	22.0 q	65.9 t	65.9 t

[a] Data are based on DEPT, HMQC, and HMBC experiments. [b] C_6D_6. [c] Overlapping signals.

repeated chromatography of the *n*-hexane/Et_2O (1:1) extract of the root bark of *M. chubutensis* on silica gel and Sephadex LH-20 afforded the new compounds **14** and **15** and two known compounds **16** and **17** (Figure 1).

Compound **1** has the molecular formula $C_{33}H_{38}O_8$, as determined by HRMS. The IR spectrum showed absorption bands for a hydroxyl group at 3541 cm^{-1} and esther groups at 1735 and 1708 cm^{-1}. The MS and HRMS exhibited peaks consistent with losses of acetic acid (*m*/*z* 502 $[M - HOAc]^+$), benzoic acid (*m*/*z* 440 $[M - PhCO_2H]^+$), and cinnamic acid (*m*/*z* 414 $[M - PhCH{=}CHCO_2H]^+$) units. This was confirmed by the ^{1}H NMR spectrum, which also indicated the presence of signals for one acetyl group at δ 1.83 (3H, s), 12 protons in the aromatic region for one benzoyl and one cinnamoyl group at δ 6.46 (1H, d, J = 16.0 Hz), 7.29 (2H, m), 7.40 (3H, m), 7.46 (2H, m), 7.56 (2H, m), and 7.78 (2H, m), which were confirmed by ^{13}C NMR. When **1** was treated with acetic anhydride in pyridine, compound **1** was unaltered, a fact that together with the presence of a singlet at δ 2.86 in the ^{1}H NMR spectrum, interchangeable with D_2O, confirmed the presence of a tertiary hydroxyl group. The ring protons could be unequivocally assigned from its homonuclear COSY and chemical shift correlated spectral data. In its ^{1}H NMR spectrum (Table 1) were also observed an ABX_2 system of two methine and one methylene protons with signals at δ 5.94 (1H, d, J_{AB} = 10.5 Hz), 5.14 (1H, dt, J_{AB} = 10.5 Hz, J_{BX} = 4.8), and 2.07 (2H, m), assignable to protons H-1, H-2, and H-3, respectively. These data are only compatible with substitution at C-1 and C-2 in this type of sesquiterpene with a H-1ax, H-2ax stereochemistry and another methine proton at δ 4.79 (1H, d, J = 5.3 Hz) assigned to H-9. A tertiary methyl at δ 1.39 binding to a quaternary carbon at δ 70.6 in the ^{13}C NMR spectrum (Table 2) and two angular methyls were also observed. All these data indicate that compound **1** is a tetrasubstituted dihydro-β-agarofuran sesquiterpene. The relative stereochemistry of **1** was established on the basis of the coupling constants and confirmed by a ROESY experiment (Figure 2), showing nuclear Overhauser effects among H-14, H-2, and H-15 and between H-15 and H-9. The chemicals shifts for the carbons attached to protons were assigned according to a 2D heteronuclear COSY and the already known proton shift. Because the quaternary carbons could not be assigned by this method, the locations of the hydroxy and ester functions were determined by an HMBC experiment (Table 3), showing a three-bond correlation between the carboxyl signal of the acetate group at δ 170.4 with the ^{1}H signal at 5.14 (H-2); the carboxyl signal of the cinnamate group at δ 166.1 was correlated with the ^{1}H signal at δ 4.79 (H-9), and finally the carboxyl signal of

Table 3. Three-Bond $^1H-^{13}C$ Coupling (HMBC) in Compounds **1**, **3**, **6**−**9**, and **12**−**15**

compd	H-1	H-2	H-3	H-6	H-8	H-9	H-15
1	C-2,[a] C-9, C-10,[a] OBz	C-1[a] OAc				C-5, C-7, C-8,[a] C-10,[a] OCin	
3	C-9, C-10,[a] C-15, OCin	OAc	C-1, C-4,[a] C-5, ONic			C-5, C-10[a] C-15,[a] OBz	
6	C-2,[a] C-10,[a] C-15, OBz	C-4 OAc	C-4,[a] C-5, C-14, ONic	C-5,[a] C-8, C-10, C-11, OAc		C-5, C-7, C-10,[a] OCin	
7	C-2,[a] C-10,[a] C-15, OBz	C-1[a]	C-1, C-4,[a] C-5, ONic	C-10, C-11, OAc		C-5, C-10,[a] C-15, OCin	
8	C-2,[a] C-10,[a] C-15, ONic	C-1[a] OAc	C-1, C-4,[a] C-5, OAc		C-11, OBz	C-5, C-7, C-15, C-10,[a] OBz	
9	C-2,[a] C-10,[a] ONic	C-1[a] OAc	C-2, C-4,[a] C-5, OBz		C-11	C-5, C-10,[a] C-15, OBz	
12	C-10,[a] C-15, OBz			C-5,[a] C-10, C-11, OAc	C-11, OAc	C-5, C-7, C-15, C-10,[a] OBz	
13	C-2,[a] C-10,[a] C-15, OBz	C-4		C-5,[a] C-8, C-10, C-11, OAc	C-10, C-11, OAc	C-7, C-10,[a] C-15, OBz	
14	C-9, C-10,[a] C-15, OAc	C-10		C-5,[a] C-8, C-10, C-11, OBz		C-5, C-7, C-15, C-10,[a] OBz	C-5, C-9 OAc
15	C-10,[a] C-15	C-10		C-5,[a] C-7, C-8, C-10, C-11, OBz		C-5, C-10,[a] C-15, OBz	C-5, C-9 OAc

[a] Two bond coupling enhancement observed.

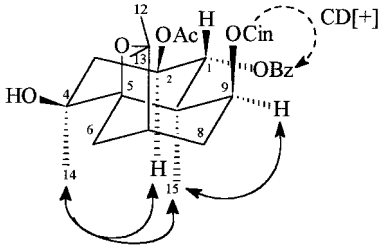

Figure 2. NOE (solid line) and CD exciton couplings (dashed line) for compound **1**.

the benzoate group at δ 165.3 was correlated with the ^1H signal at δ 5.94 (H-1). The absolute configuration of compound **1** was determined from its CD spectrum.

Compound **3** was isolated as a colorless lacquer with the molecular formula $C_{41}H_{43}NO_{12}$ (HRMS). The ^1H and ^{13}C NMR data of **3** (Tables 1 and 2) indicate it to be 1,2,3,4,9-pentasubstituted-β-dihydroagarofuran sesquiterpene with one tertiary hydroxyl, one acetate, one benzoate, one cinnamate, and one nicotinate group. In its ^1H NMR spectrum an ABX system was also observed with signals at δ 6.43 (1H, d, J_{AB} = 11.2 Hz), 5.52 (1H, d, J_{BX} = 2.9 Hz), and 5.41 (1H, dd, J_{AB} = 11.2 Hz, J_{BX} = 2.9 Hz), assignable to protons H-1, H-3, and H-2 respectively, and a signal at δ 4.89 (1H, d, J = 5.0 Hz), assigned to the proton H-9. The relative stereochemistry was determined on the basis of the coupling constants and the nuclear Overhauser effects (NOE) from the ROESY spectrum. The regiosubstitution was established by HMBC experiment (Table 3) in a way similar to that for **1**, which allowed placement of the cinnamate, acetate, nicotinate, and benzoate groups at C-1, C-2, C-3, and C-9, respectively. The absolute stereochemistry was assigned by means of CD studies.

The structure and absolute configuration of compounds **6**−**9** were elucidated by spectral methods in a study of their IR, UV, and ^1H and ^{13}C NMR data (Tables 1 and 2), and 2D experiments showed that the compounds were pentasubstituted 4β-hydroxy-dihydro-β-agarofuran sesquiterpenes, which were located at positions 1, 2, 3, 6, and 9 for **6** and **7** and at positions 1, 2, 3, 8, and 9 for **8** and **9**. An HMBC experiment (Table 3) established the regiosubstitution partners; the relative stereochemistry was resolved by analysis of coupling constants and confirmed by ROESY experiments, and finally the absolute configuration was established by analysis of their CD data.

Table 4. Circular Dichroism Data of Compounds **1**, **3**, **6**−**9**, **12**, and **13** (MeCN)

compd	λ_{ext}, nm ($\Delta\epsilon$)	λ_{ext}, nm ($\Delta\epsilon$)	$\Delta\epsilon = 0$
1	227.8 (−18.1)	270.7 (+20.3)	242.6
3	225.9 (−14.1)	262.6 (+4.3)	243.3
6	226.2 (−9.6)	277.5 (+1.6)	248.7
7	225.2 (−6.5)	238.9 (+3.9)	233.4
8		236.2 (+12.1)	
9	225.2 (−12.1)	237.5 (+8.2)	232.0
12	221.1 (−0.6)	238.7 (+7.1)	224.8
13	221.5 (−6.3)	236.9 (+25.7)	226.0

The structures of compounds **12**−**14** were elucidated by spectral methods, including HREIMS, IR, UV, and ^1H and ^{13}C NMR spectroscopy, and by 2D $^1H-^1H$ and $^1H-^{13}C$ experiments (Tables 1−3). On the other hand, the spectroscopic data of compound **15** and a comparison with those of **14** allowed us to determine its structure, while CD studies allowed the absolute stereochemistry of compounds **12** and **13** to be established.

The absolute configurations were resolved by the dibenzoate chirality method, an extension of the circular dichroism exciton chirality method.[30,33] The dihedral angle between the different chromophores (benzoate, and/or cinnamate, and/or nicotinate) were calculated from J values and molecular mechanics calculations using the PC model.[34] Heterochromophoric **1**, **3**, **6**−**9**, and **12** and homochromophoric **13** compounds were therefore considered suitable for a CD study, showing a Davydof-type split curve (Table 4), while compounds **14** and **15**, which had three chromophores located at C-2 (ONic), C-6 (OBz), and C-9 (OBz), were not suitable for CD because the opposite 2,6 and 2,9 pairwise interactions canceled each other and the 6,9 pairwise interaction was almost coplanar.[35]

The dihedral angle between the two chromophores (benzoate and cinnamate) was approximately 150° for **1**; its CD spectrum showed a strong split curve (Figure 1 and Table 4) with extremes at the right-hand-band wavelength, i.e., the first Cotton effect at 270.7 nm ($\Delta\epsilon$ = +20.3) and the second at 227.8 nm ($\Delta\epsilon$ = −18.1),

Table 5. Effect of Sesquiterpenes on Cytotoxicity of DNM in a MDR *L. Tropica* Line

	growth inhibition[a] (%)					
	30 μM		15 μM		7.5 μM	
compd	wild-type	DNM-R150	wild-type	DNM-R150	wild-type	DNM-R150
1	7.5 ± 7.5	94.3 ± 3.5	7.3 ± 8.9	92.8 ± 3.6	0.0 ± 0.0	88.2 ± 6.7
2	13.3 ± 14.6	94.0 ± 2.6	4.1 ± 5.8	89.7 ± 7.5	5.3 ± 7.4	82.0 ± 9.2
3	7.5 ± 7.3	94.7 ± 2.2	3.7 ± 4.7	90.3 ± 7.2	2.5 ± 3.5	82.0 ± 1.7
6	1.9 ± 2.0	45.0 ± 10.0	1.5 ± 2.1	13.5 ± 3.5	1.5 ± 2.1	7.3 ± 4.4
7	3.7 ± 2.9	69.5 ± 0.7	0.7 ± 0.9	30.7 ± 1.1	2.2 ± 3.1	10.0 ± 4.3
8	11.0 ± 7.6	89.0 ± 0.0	6.0 ± 2.8	54.0 ± 6.5	0.5 ± 0.7	23.9 ± 5.7
9	19.7 ± 4.1	69.7 ± 10.1	12.9 ± 5.4	44.0 ± 15.6	0.0 ± 0.0	21.4 ± 9.4
10	20.5 ± 7.2	93.0 ± 1.4	15.8 ± 6.8	79.0 ± 8.5	0.5 ± 0.7	48.7 ± 9.0
11	23.0 ± 2.0	90.0 ± 4.2	14.4 ± 20.4	65.0 ± 6.4	1.7 ± 2.3	39.0 ± 8.4
12	13.3 ± 13.0	94.5 ± 2.1	13.0 ± 2.8	79.3 ± 10.7	1.7 ± 1.0	42.2 ± 9.8
13	16.2 ± 1.9	92.0 ± 2.8	19.7 ± 6.6	82.2 ± 12.1	9.7 ± 2.3	60.3 ± 10.2
14	45.0 ± 10.0	94.8 ± 3.6	25.8 ± 1.7	91.3 ± 7.0	8.2 ± 8.2	80.7 ± 9.0
15	35.0 ± 10.0	93.7 ± 3.5	27.5 ± 7.7	87.9 ± 9.9	11.5 ± 0.7	83.7 ± 6.1
16	11.7 ± 3.7	77.5 ± 11.3	3.5 ± 4.9	54.0 ± 14.5	0.0 ± 0.0	32.0 ± 11.6

[a] Wild-type and MDR (DNM-R150) parasites were exposed to 30, 15, and 7.5 μM different sesquiterpenes in the absence and presence of 150 μM DNM, respectively. The results are expressed as a percentage of growth inhibition relative to control growth in the absence of sesquiterpene. The data shown are the average of three independent experiments ± SD.

defining the absolute configuration of **1** as (1*R*,2*R*,4*S*,5*R*, 7*S*,9*S*,10*R*)-2-acetoxy-1-benzoyloxy-9-cinnamoyloxy-4-hydroxydihydro-β-agarofuran.

Compounds **3**, **6**, **7**, and **9** had three chromophore groups located at C-1, C-3, and C-9. An analysis of the dihedral angle showed that the opposite 1,3 and 1,9 pairwise interactions added to each other but did not cancel because of the different intensities of the different chromophores. On the other hand, the 3,9 pairwise interaction was almost coplanar.[30] Furthermore, compounds **3** and **6** showed a split curve very similar to that of **1** (Table 4), with a first positive and second negative Cotton effect around 270 and 226 nm, respectively, the weak value of $\Delta\epsilon$ for **3** and **6** with respect to **1** being due to the presence of an additional chromophore on C-3. These data allowed us to determine their absolute configurations of compounds **3** and **6**. On the other hand, an analysis of the CD spectra of compounds **7** and **9**, showing a Davydof-type split curve (Table 4), led to the assignment of their absolute configurations.

Compounds **8** and **9** have the same molecular formula, the main differences being the regiosubstitution patterns. Compound **8** had a nicotinate unit at C-1 and two benzoate units at C-8 and C-9; its CD spectra (Table 4) showed a first Cotton effect at 236.3 nm ($\Delta\epsilon = +12.1$), but we could not observe the second Cotton effect because of the opposite pairwise interactions between the three chromophores together with the strong positive absorption overlaying background ellipticity,[33] which destroyed the second component of the split curve. The absolute configuration of **8** was established as (1*R*,2*S*,3*S*, 4*S*,5*R*,7*S*,8*S*,9*R*,10*R*)-2,3-diacetoxy-8,9-dibenzoyloxy-1-nicotinoiloxy-4-hydroxydihydro-β-agarofuran.

The CD spectra of **12** and **13** (Table 4) showed split curves with a first positive and a second negative Cotton effect due to the couplings of the C-9β with C-1α and C-1α with C-2α, supporting the absolute configuration as that ascribed to both compounds.

The new compounds have the basic polyhydroxy skeletons of 3,6-dideoxymagellanol (**1**),[30] 6-deoxymagellanol (**3**),[30] magellanol (**6** and **7**),[30] isomagellanol (**8** and **9**),[29] 2α-hydroxy-8-*epi*-celapanol (**12**),[29] 2α,4β-dihydroxy-8-*epi*-celapanol (**13**),[29] 3-deoxymaytol (**14**),[36] and 3,4-dideoxymaytol (**15**).[36]

Agarofuran sesquiterpenes are new promising MDR modulators in eukaryotic organisms.[15,16] The reversal effects of 15 dihydro-β-agarofuran sesquiterpenes in a MDR *L. tropica* (*L. tropica)* line grown in the presence of daunomycin (DNM) were studied by using an MTT-based assay. Their intrinsic parasite cytotoxicity was determined by using the same concentration of modulators in the parental wild-type parasites. Table 5 shows that after 72 h of incubation of MDR parasites in the presence of 150 μM DNM with increasing amounts of sesquiterpenes, a concentration-dependent growth inhibition (GI) was observed compared with control cells, grown with the same DNM concentration but in the absence of modulator. The chemosensitization to 150 μM DNM was very efficient for most of the sesquiterpenes tested, but the effective concentration varied between each one. In this form, 7.5 μM of **1**−**3**, **14**, and **15** produce more than 80% GI, requiring a double concentration (15 μM) of **10**, **12**, and **13** and around a 4-fold concentration (30 μM) of **8**, **11**, and **16** to obtain similar reversal effects. This effect is not due to an intrinsic cytotoxicity of sesquiterpenes, as deduced for the low growth inhibition produced by these compounds in the parental wild-type line (Table 5), except for compounds **14** and **15** that at 30 μM produce 45% and 35% GI in the wild-type line, respectively.

DNM resistance in the MDR *L. tropica* line is related to a decreased intracellular drug accumulation mainly due to the Pgp-like transporter overexpression.[24] To analyze if the reversal effect observed by some sesquiterpenes correlated with an increased drug accumulation, as a consequence of the Pgp inhibition, we studied by laser flow cytometry their effect on calcein (CAL) accumulation. Calcein acetoxymethyl ester (Cal-AM) is a highly lipophilic, nonfluorescent, mammalian Pgp substrate that rapidly penetrates the plasma membrane of cells. By cleavage of the ester bonds, intracellular esterases transform the dye into a hydrophilic and intensively fluorescent free acid form (Cal) that cannot be transported by Pgp. This property makes Cal-AM an excellent compound for studying the modulation of mammalian Pgp function,[37] and it has also been recently used to study ABC-like transporter activities in different *Leishmania* lines.[38] Flow cytometry analysis of intra-

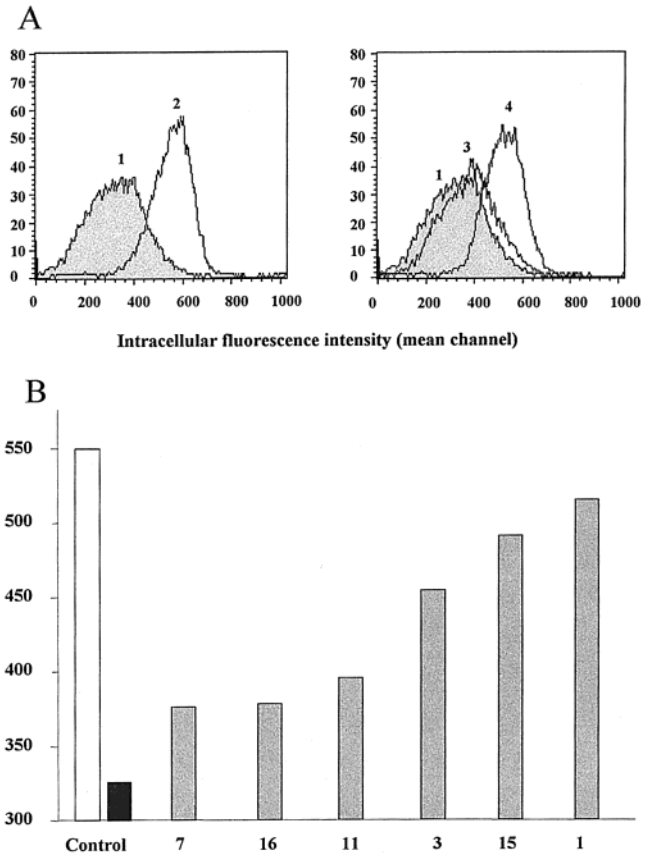

Figure 3. Differential modulation by sesquiterpenes of CAL accumulation in a resistant *L. tropica* line. Fluorescence intensity histograms were obtained by flow cytometry after incubation for 1 h at 28 °C with 2 μM CAL-AM in the presence or absence of 5 μM sesquiterpenes. A total of 10 000 cells were counted for each histogram. Experiments were repeated three times and gave essentially the same profiles as the ones shown here. (A) Profiles 1 and 2 correspond to fluorescence histograms of resistant and wild-type parasites, respectively, incubated in the absence of sesquiterpenes. Profiles 3 and 4 correspond to fluorescence histograms of resistant parasites incubated in the presence of 5 μM sesquiterpenes **7** and **1**, respectively. (B) Wild-type (white bar) and resistant (black bar) parasites incubated with 2 μM CAL-AM are used as control of CAL accumulation. Resistant parasites (gray bars) are incubated with 2 μM CAL-AM in the presence of 5 μM of different sesquiterpenes (**7**, **16**, **11**, **3**, **15**, and **1**).

cellular Cal accumulation (Figure 3A) demonstrated that, as expected, the resistant line accumulated a significantly lower amount of dye (Profile 1), expressed as the mean fluorescence channel ($m = 324$), than the wild-type line ($m = 550$) (profile 2). Co-incubation of the resistant parasites with 5 μM of the most active sesquiterpene **1** resulted in a significant shift of the peak of fluorescence distribution to the right, almost to the control level ($m = 514$) (profile 4). This reversal effect was a consequence of an increased CAL accumulation probably due to Pgp-like transporter inhibition. The same concentration of a sesquiterpene that gave a moderate reversal effect, **7**, only produced a slight increase in the intracellular dye ($m = 376$) (profile 3). Indeed, Figure 3B shows that different sesquiterpenes with distinct reversal efficiencies restored the dye accumulation in the MDR *Leishmania* line with an order of efficiency similar to that obtained with the DNM chemosensitization experiments: **1** > **15** ≥ **3** > **11** > **16** ≥ **7**, with no significant effects in the wild-type line (not shown). These results indicate that the measurement of CAL accumulation may be used to test the reversal effects of possible modulators of the Pgp-mediated MDR phenotype in *Leishmania*.

Preliminary structure−activity relationships from these natural sesquiterpenes revealed the following trends. The substituent at the C-6 position proved to be essential for the reversal activity because the presence of protons at C-6 produces a 10-fold higher chemosensitization with respect to the presence in the same position of an ester group (**3** versus **6**). On the other hand, the substituent at C-8 proved to be significant for the potency of the activity, **3** being 2- and 4-fold more active than **11** and **8**, respectively. In addition, the substituent at C-3 exhibited a slight decrease in the activity (**1** versus **3**), and the substituent at C-1 also modified the activity, being that **10** is 2-fold more active than **8**. Finally, the often tertiary hydroxy group at C-4β does not seem to be important for optimal reversal activity because its presence or absence does not produce significant modification of the activity (**14** versus **15**). These and previous results[15] suggest that the size of the substituent could strongly affect the reversal activity of the compound; the presence of additional ester groups on the basic polyhydroxy skeletons has probably made the compounds too bulky to bind at the active site, being detrimental to the reversal activity associated with these sesquiterpenes.

Dihydro-β-agarofuran sesquiterpenes have proven to be a very interesting class of natural products with a high reversal effect of the MDR phenotype mediated by Pgp-like transporters. Further efforts to elucidate the specific target of these compounds in the Pgp-like transporter, as well as to determine if they are transported, are underway for a better knowledge of their mechanism of action.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer 241 automatic polarimeter, and the [α_D] are given in 10^{-1} deg cm^2 g^{-1}. IR (film) spectra were recorded on a Bruker IFS 55 spectrophotometer, and UV spectra were collected in absolute EtOH on a JASCO V-560. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AMX-500, a Bruker Avance 400, or a Bruker Avance 300 spectrometer. EIMS and HREIMS were recorded on a Micromass Autospec spectrometer. Purification was performed using silica gel (particle size 40−63 μM, Merck and HPTLC-Platten-Sil 20 UV$_{254}$, Panreac), and Sephadex LH-20 (Pharmacia) and was monitored by TLC 1500/LS 25 Schleicher and Schüell foils. Compounds used for CD were purified by HPLC using a semipreparative μ-porasil column column (Waters, 10 μM, 19 mm × 25 cm) and were eluted with a mixture of *n*-hexane/EtOAc (1:1).

Plant Material, Extraction, and Isolation. *M. magellanica* was collected in December 1993 in the Novena Región in the Temuca province on the slopes of the Osorno volcano in Chile and was identified by Prof. Sebastián Teyller, and a voucher specimen is on file at the Facultad de Ciencias (93-5342-A), Universidad de Chile, Santiago. The root bark of the plant (390 g) was extracted with *n*-hexane/Et$_2$O (1:1) in a Soxhlet apparatus. The extract (7.8 g) was chromatographed on Sephadex LH-20 (*n*-hexane/CHCl$_3$/MeOH, 2:1:1) to afford 55 fractions. Fractions 28−40 after chromatography over silica gel (*n*-hexane/AcOEt of increasing polarity), Sephadex LH-20 (*n*-hexane-CHCl$_3$-MeOH, 2:1:1), and preparative HPTLC (HPTLC-Platten-Sil 20 UV$_{254}$) gave rise to the new compounds **1** (9.3 mg), **3** (10.7 mg), **6** (3.1 mg), **7** (1.9 mg), **8** (12.3 mg), **9**

(20.6 mg), **12** (1.9 mg), **13** (6 mg), and **14** (2.2 mg) and the known compounds **2**, **4**, **5**, **10**, and **11**.

M. chubutensis was collected in December 1994 in the Séptima Región in the Talca province, Chile, and was identified by Prof. José San Martín, and a voucher specimen is lodged with the Facultad de Ciencias (93-5200), Universidad de Chile, Santiago. Air-dried, chopped root bark of the plant (390 g) was extracted with *n*-hexane/Et_2O (1:1) in a Soxhlet apparatus. The extract (7.8 g) was chromatographed on silica gel using gradients of *n*-hexane/AcOEt as eluent to afford 180 fractions. Fractions 144−160 (900 mg) after chromatography over Sephadex LH-20 (*n*-hexane/$CHCl_3$/MeOH, 2:1:1) and preparative HPTLC (HPTLC-Platten-Sil 20 UV_{254}) gave rise to the new compounds **14** (12 mg) and **15** (6 mg) and the known compounds **16** and **17**.

(1*R*,2*R*,4*S*,5*R*,7*S*,9*S*,10*R*)-2-Acetoxy-1-benzoyloxy-9-cinnamoyloxy-4-hydroxydihydro-*β*-agarofuran (1). Colorless lacquer; $[\alpha]^{25}_D$ +14.8° (*c* 0.93, $CHCl_3$); UV λ_{max} (EtOH) 275, 223, 218 nm; IR γ_{max} (film) 3541, 2956, 2922, 1735, 1708, 1638, 1450, 1271, 1166, 756, 712 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.33 (3H, s), 1.39 (3H, s), 1.45 (3H, s), 1.83 (3H, s), 2.86 (1H, s), 6.46 (1H, d, J = 16.0 Hz), 7.29 (2H, m), 7.40 (3H, m), 7.46 (2H, m), 7.56 (2H, m), 7.78 (2H, m), for other signals, see Table 1; ^{13}C NMR ($CDCl_3$) δ 20.9 (q), 118.7 (d), 128.2 (4 × d), 128.7 (2 × d), 129.3 (2 × d), 130.1 (s, d), 132.8 (d), 134.7 (s), 144.6 (d), 165.3 (s), 166.1 (s), 170.4 (s), for other signals, see Table 2; MS (EI) *m*/*z* (%) 562 (M^+, 10), 547 (2), 502 (1), 440 (1), 414 (3), 399 (15), 372 (4), 339 (3), 249 (6), 232 (5), 217 (4), 203 (3), 188 (7), 147 (6), 131 (88), 105 (100), 77 (25), 57 (2). HRMS (EI) *m*/*z* Calcd for $C_{33}H_{38}O_8$: 562.256 67. Found: 562.259 03.

(1*R*,2*S*,3*S*,4*S*,5*R*,7*R*,9*S*,10*R*)-2-Acetoxy-9-benzoyloxy-1-cinnamoyloxy-3-nicotinoyloxy-4-hydroxydihydro-*β*-agarofuran (3). Colorless lacquer; $[\alpha]^{25}_D$ +33.2° (*c* 1.07, $CHCl_3$); UV λ_{max} (EtOH) 272, 223 nm; IR γ_{max} (film) 3532, 2966, 2932, 1732, 1282, 1263, 1026, 755, 710 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.34 (3H,s), 1.53 (3H, s), 1.61 (3H, s), 1.77 (3H, s), 3.41 (1H, s), 6.40 (1H, d, J = 16.0 Hz), 7.28 (4H, m), 7.38 (2H, m), 7.49 (4H, m), 7.98 (2H, m), 8.56 (1H, d, J = 8.0 Hz), 8.90 (1H, d, J = 3.3 Hz), 8.60 (1H, s), for other signals, see Table 1; ^{13}C NMR ($CDCl_3$) δ 20.6 (q), 118.0 (d), 123.4 (d), 126.2 (s), 128.7 (4 × d), 129.1 (2 × d), 129.2 (s), 129.5 (2 × d), 130.3 (d), 132.9 (d), 134.5 (s), 137.5 (d), 145.1 (d), 151.5 (d), 153.5 (d), 164.4 (s), 164.8 (s), 166.0 (s), 170.4 (s), for other signals, see Table 2; MS (EI) *m*/*z* (%) 683 (M^+, 4), 668 (11), 642 (4), 573 (3), 538 (11), 520 (5), 202 (2), 149 (21), 131 (40), 105 (100), 97 (13), 77 (17). HRMS (EI) *m*/*z* Calcd for $C_{39}H_{41}NO_{10}$: 683.273 05. Found: 683.273 24.

(1*R*,2*S*,3*S*,4*S*,5*S*,6*R*,7*R*,9*S*,10*R*)-2,6-Diacetoxy-1-benzoyloxy-9-cinnamoyloxy-3-nicotinoyloxy-4-hydroxydihydro-*β*-agarofuran (6). Colorless lacquer; $[\alpha]^{25}_D$ +9.7° (*c* 0.31, $CHCl_3$); UV λ_{max} (EtOH) 271, 223 nm; IR γ_{max} (film) 3531, 2924, 2854, 1735, 1459, 1236, 1107, 1026, 711 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.52 (3H, s), 1.57 (3H, s), 1.62 (3H, s), 1.75 (3H, s), 2.11 (3H, s), 3.56 (1H, s), 6.35 (1H, d, J = 16.0 Hz), 7.25 (2H, m), 7.46 (5H, m), 7.50 (3H, m), 7.69 (2H, m), 8.53 (1H, d, J = 7.9 Hz), 8.88 (1H, d, J = 3.9 Hz), 9.58 (1H, s), for other signals, see Table 1; ^{13}C NMR ($CDCl_3$) δ 21.5 (q), 22.7 (q), 117.9 (d), 123.5 (d), 126.2 (s), 128.3 (4 × d), 128.8 (2 × d), 129.3 (2 × d), 129.6 (s), 130.3 (d), 133.1 (d), 134.4 (s), 137.6 (d), 145.6 (d), 151.6 (d), 153.7 (d), 164.5 (s), 164.9 (s), 165.8 (s), 170.3 (s), 170.4 (s), for other signals, see Table 2; MS (EI) *m*/*z* (%) 741 (M^+, 12), 700 (15), 610 (4), 596 (24), 578 (7), 326 (6), 131 (100), 124 (16), 105 (29), 97 (26), 71 (28), 57 (36). HRMS (EI) *m*/*z* Calcd for $C_{41}H_{43}NO_{12}$: 741.285 23. Found: 741.282 47.

(1*R*,2*S*,3*S*,4*S*,5*S*,6*R*,7*R*,9*S*,10*R*)-2,6-Diacetoxy-1,9-dibenzoyloxy-3-nicotinoyloxy-4-hydroxydihydro-*β*-agarofuran (7). Colorless lacquer; $[\alpha]^{25}_D$ −5.3° (*c* 0.19, $CHCl_3$); UV λ_{max} (EtOH) 264, 225 nm; IR γ_{max} (film) 3350, 2955, 2856, 1736, 1451, 1368, 1284, 1236, 1111, 709 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.53 (3H, s), 1.62 (3H, s), 1.65 (3H, s), 1.71 (3H, s), 2.12 (3H, s), 3.66 (1H, s), 7.28 (3H, m), 7.44 (3H, m), 7.52 (3H, m), 7.99 (2H, m), 8.58 (1H, d, J = 7.8 Hz), 8.92 (1H, d, J = 3.9 Hz), 9.66 (1H, s), for other signals, see Table 1; ^{13}C NMR ($CDCl_3$) δ 21.4 (q), 22.6 (q), 123.4 (d), 126.1 (s), 128.0 (2 × d), 128.2 (2 × d), 129.1 (2 × d), 129.3 (s), 129.4 (s), 130.1 (2 × d), 132.9 (d), 133.1 (d), 137.4 (d), 151.5 (d), 153.7 (d), 163.5 (s), 164.4 (s), 164.5 (s), 169.5 (s), 170.2 (s), for other signals, see Table 2; MS (EI) *m*/*z* (%) 715 (M^+, 8), 700 (16), 594 (3), 578 (7), 536 (4), 139 (6), 124 (13), 105 (100), 91 (20), 71 (24), 57 (39). HRMS (EI) *m*/*z* Calcd for $C_{39}H_{41}NO_{12}$: 715.262 88. Found: 715.264 65.

(1*R*,2*S*,3*S*,4*S*,5*R*,7*S*,8*S*,9*R*,10*R*)-2,3-Diacetoxy-8,9-dibenzoyloxy-1-nicotinoiloxy-4-hydroxydihydro-*β*-agarofuran (8). Colorless lacquer; $[\alpha]^{25}_D$ +27.6° (*c* 1.23, $CHCl_3$); UV λ_{max} (EtOH) 264, 226 nm; IR γ_{max} (film) 3528, 2969, 1733, 1591, 1451, 1277, 1116, 1067, 1026, 755, 709 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.39 (3H, s), 1.52 (3H, s), 1.73 (3H, s), 1.75 (3H, s), 2.32 (3H, s), 3.42 (1H, s), 7.28 (3H, m), 7.45 (3H, m), 7.58 (3H, m), 7.96 (3H, m), 8.67 (1H, d, J = 4.4 Hz), 8.89 (1H, s), for other signals, see Table 1; ^{13}C NMR ($CDCl_3$) δ 20.1 (q), 21.0 (q), 123.1 (d), 125.4 (s), 128.0 (2 × d), 128.2 (2 × d), 129.2 (2 × d), 129.5 (s), 129.7 (s), 130.3 (2 × d), 132.9 (d), 133.2 (d), 137.0 (d), 150.8 (d), 153.5 (s), 164.0 (s), 164.7 (s), 165.7 (s), 170.3 (2 × s), for other signals, see Table 2; MS (EI) *m*/*z* (%) 715 (M^+, 2), 700 (30), 578 (24), 536 (3), 476 (2), 372 (1), 294 (2), 228 (3), 131 (4), 124 (15), 105 (100), 77 (11). HRMS (EI) *m*/*z* Calcd for $C_{39}H_{41}NO_{12}$: 715.284 01. Found: 715.2832.

(1*R*,2*S*,3*S*,4*S*,5*R*,7*S*,8*S*,9*R*,10*R*)-2,8-Diacetoxy-3,9-dibenzoyloxy-1-nicotinoyloxy-4-hydroxydihydro-*β*-agarofuran (9). Colorless lacquer; $[\alpha]^{25}_D$ +12.8° (*c* 0.21, $CHCl_3$); UV λ_{max} (EtOH) 271, 226 nm; IR γ_{max} (film) 3535, 2960, 2928, 1734, 1591, 1367, 1282, 1117, 1026, 754, 708 cm^{-1}; ^1H NMR (C_6D_6) δ 1.04 (3H, s), 1.12 (3H, s), 1.45 (3H, s), 1.58 (3H, s), 1.75 (3H, s), 3.43 (1H, s), 6.99 (3H, m), 6.78 (2H, m), 7.33 (1H, m), 7.52 (2H, m), 7.81 (2H, m), 8.40 (3H, m), 10.1 (1H, s), for other signals, see Table 1; ^{13}C NMR ($CDCl_3$) δ 20.0 (q), 20.8 (q), 123.5 (d), 126.1 (s), 128.2 (2 × d), 128.3 (2 × d), 129.2 (2 × d), 129.4 (s), 129.8 (s), 130.8 (2 × d), 133.0 (d), 133.1 (d), 135.7 (d), 151.5 (d), 153.9 (d), 164.3 (s), 164.5 (s), 165.6 (s), 169.7 (s), 170.4 (s), for other signals, see Table 2; MS (EI) *m*/*z* (%) 715 (M^+, 1), 700 (23), 578 (11), 536 (2), 354 (1), 294 (1), 231 (1), 189 (2), 131 (4), 124 (9), 105 (100), 77 (12). HRMS (EI) *m*/*z* Calcd for $C_{39}H_{41}NO_{12}$: 715.262 88. Found: 715.264 08.

(1*R*,2*S*,4*R*,5*S*,6*R*,7*R*,8*S*,9*R*,10*S*)-6,8-Diacetoxy-1,9-dibenzoyloxy-2-nicotinoyloxydihydro-*β*-agarofuran (12). Colorless lacquer; $[\alpha]^{25}_D$ +31.0° (*c* 0.19, $CHCl_3$); UV λ_{max} (EtOH) 264, 229 nm; IR γ_{max} (film) 2924, 2860, 1728, 1272, 1220, 1125, 1107, 711 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.30 (3H, d, J = 7.7 Hz), 1.52 (3H, s), 1.64 (3H, s), 1.84 (3H, s), 2.16 (3H, s), 2.62 (1H, m), 7.25 (2H, m), 7.26 (2H, m), 7.37 (3H, m), 7.43 (2H, m), 7.46 (2H, m), 7.82 (2H, m), 8.14 (1H, d, J = 8.1 Hz), 8.76 (1H, d, J = 3.9 Hz), 9.11 (1H, d; J = 0.7 Hz), for other signals, see Table 1; ^{13}C NMR ($CDCl_3$) δ 20.9 (q), 21.2 (q), 123.4 (d), 126.0 (s), 127.9 (2 × d), 128.0 (2 × d), 128.4 (s), 128.9 (s), 129.2 (2 × d), 130.0 (2 × d), 132.7 (d), 133.1 (d), 137.0 (d), 150.0 (d), 153.5 (d), 164.9 (s), 165.1 (s), 165.3 (s), 169.2 (s), 169.6 (s), for other signals, see Table 2; MS (EI) *m*/*z* (%) 699 (M^+, 3), 684 (12), 657 (19), 536 (2), 520 (3), 124 (12), 105 (100), 77 (10). HRMS (EI) *m*/*z* Calcd for $C_{39}H_{41}NO_{11}$: 699.267 96. Found: 699.270 32.

(1*R*,2*S*,4*S*,5*S*,6*R*,7*R*,8*S*,9*R*,10*S*)-6,8-Diacetoxy-1,2,9-tribenzolyoxy-4-hydroxydihydro-*β*-agarofuran (13). Colorless lacquer; $[\alpha]^{25}_D$ +9.7° (*c* 0.31, $CHCl_3$); UV λ_{max} (EtOH) 274, 231 nm; IR γ_{max} (film) 3545, 2959, 2924, 1727, 1451, 1265, 1110, 1027, 801, 756, 709 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.60 (3H, s), 1.61 (3H, s), 1.70 (3H, s), 1.84 (3H, s), 2.17 (3H, s), 2.98 (1H, s), 7.19 (2H, m), 7.45 (7H, m), 7.54 (2H, m), 7.83 (2H, m), 7.89 (2H, m), for other signals, see Table 1; ^{13}C NMR ($CDCl_3$) δ 20.6 (q), 21.5 (q), 127.9 (2 × d), 128.1 (2 × d), 128.6 (2 × d), 129.0 (s), 129.3 (2 × d, s), 129.4 (2 × d), 129.8 (s), 130.1 (2 × d), 132.7 (d), 133.1 (d), 133.2 (d), 165.0 (s), 165.2 (s), 165.6 (s), 169.1 (s), 169.9 (s), for other signals, see Table 2; MS (EI) *m*/*z* (%) 592 (M^+ − 122, 1), 532 (15), 472 (1), 419 (1), 335 (2), 246 (2), 202 (22), 151 (1), 105 (100), 77 (9). HRMS (EI) *m*/*z* Calcd for $C_{33}H_{36}O_{10}$: 592.230 85. Found: 592.228 79.

1α,15-Diacetoxy-6*β*,9*β*-dibenzoyloxy-2α-nicotinoyloxy-4*β*-hydroxydihydro-*β*-agarofuran (14). Colorless lacquer; $[\alpha]^{25}_D$ +15.4° (*c* 0.22, $CHCl_3$); UV λ_{max} (EtOH) 273, 229 nm; IR γ_{max} (film) 3555, 2955, 2925, 2854, 1747, 1718, 1591, 1452, 1274, 1106, 1024, 758, 713 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.57 (6H, s), 1.59 (3H, s), 1.62 (3H, s), 2.26 (4H, m), 2.71 (1H, m), 3.13

(1H, s), 7.47 (5H, m), 7.59 (2H, m), 8.07 (2H, m), 8.18 (2H, m), 8.40 (1H, d, J = 7.9 Hz), 8.80 (1H, s), 9.30 (1H, s), for other signals, see Table 1; ^{13}C NMR: ($CDCl_3$) δ 20.3 (q), 21.2 (q), 123.6 (d), 125.3 (s), 128.4 (2 × d), 128.6 (2 × d), 128.9 (s), 129.7 (s), 130.1 (4 × d), 133.4 (d), 133.6 (d), 137.2 (d), 151.1 (d), 153.8 (d), 164.4 (s), 165.2 (s), 166.2 (s), 169.2 (s), 170.8 (s), for other signals, see Table 2; MS (EI) *m/z* (%) 715 (M^+, 4), 700 (4), 595 (2), 470 (19), 275 (5), 228 (9), 124 (14), 105 (100), 77 (10). HRMS (EI) *m/z* Calcd for $C_{39}H_{41}NO_{12}$: 715.262 88. Found: 715.255 19.

1α,15-Diacetoxy-6β,9β-dibenzoyloxy-2α-nicotinoyloxydihydro-β-agarofuran (15). Colorless lacquer; $[\alpha]^{25}_D$ +70.0° (*c* 0.39, $CHCl_3$); UV λ_{max} (EtOH) 264, 229 nm; IR γ_{max} (film) 2954, 2924, 2850, 1747, 1720, 1590, 1451, 1272, 1106, 1025, 757, 712 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.87 (3H, d, J = 6.8 Hz), 1.52 (6H, s), 1.53 (3H, s), 2.10 (3H, m), 2.34 (3H, s), 7.49 (5H, m), 7.60 (2H, m), 8.06 (4H, m), 8.41 (1H, d, J = 8.0 Hz), 8.79 (1H, s), 9.29 (1H, s), for other signals, see Table 1; ^{13}C NMR ($CDCl_3$) δ 20.3 (q), 21.2 (q), 123.5 (d), 125.4 (s), 128.3 (2 × d), 128.7 (2 × d), 129.0 (s), 129.5 (2 × d), 129.8 (s), 130.1 (2 × d), 133.5 (d), 133.6 (d), 137.4 (d), 151.3 (d), 153.3 (d), 164.6 (s), 165.3 (s), 165.6 (s), 169.3 (s), 170.9 (s), for other signals, see Table 2; MS (EI) *m/z* (%) 699 (M^+, 22), 684 (14), 228 (5), 174 (4), 124 (13), 105 (100), 77 (8). HRMS (EI) *m/z* Calcd for $C_{39}H_{41}NO_{11}$: 699.267 96. Found: 699.267 85.

Biological Assays. 1. Parasite Culture. The wild-type *L. tropica* LRC strain was a clone obtained by agar plating.[39] An *L. tropica* line highly resistant to DNM (DNM-R150) was maintained in the presence of 150 μM DNM and used as previously described.[24] This resistant line had an MDR phenotype similar to that of tumor cells, with a cross-resistance profile to several drugs and an overexpressed drug−efflux Pgp-like transporter.[24] Promastigote forms were grown at 28 °C in RPMI 1640-modified medium (Gibco)[40] and supplemented with 20% heat-inactivated fetal bovine serum (Gibco).

2. DNM Chemosensitization Experiments. The viability of parasites in the presence of the different sesquiterpenes was analyzed by an MTT-based assay.[41,42] The screening was performed in flat-bottomed 96-well plastic plates maintained at 28 °C. Promastigote forms from a logarithmic phase culture were suspended in fresh medium to yield 6 × 10^6 cells/mL. Each well was filled with 50 μL of the parasite suspension (3 × 10^5 cells). Stock solutions of sesquiterpenes dissolved in DMSO were diluted directly in the culture medium at suitable concentrations, and 50 μL was added to each well. The final DMSO content did not exceed 0.3%, which had no effect on parasite growth. To assess the chemosensitizing activity of sesquiterpenes, promastigotes of *L. tropica* MDR line were exposed to both DNM (150 μM) and sesquiterpenes. To determine the intrinsic toxicity of the sesquiterpenes, the wild-type *L. tropica* lines were exposed to the sesquiterpenes in the absence of DNM. After 72 h of incubation at 28 °C, the viability of promastigotes was determined by the colorimetric MTT assay. A total of 10 μL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (5 mg/mL in PBS) was added to each well, and plates were incubated for an additional period of 4 h. Water-insoluble formazan crystals were dissolved by adding 100 μL of 20% SDS, and absorbance was read at 540 nm using a microplate reader (Beckman Biomek 2000). Cell survival was determined by dividing the absorbance at a given sesquiterpene concentration by the absorbance of control cells. The results are expressed as percent growth inhibition (GI).

3. Reversion of Calcein Accumulation in a MDR *L. tropica* Line Overexpressing a Pgp-like Transporter. The accumulation of CAL-AM fluorescent dye in wild-type and resistant *Leishmania* lines was estimated by flow cytometry using a Becton Dickinson FacScan, as described for mammalian cells[37] and for *Leishmania* spp.,[38] with some modifications. Briefly, parasites were incubated with 2 μM CAL-AM (Molecular Probes Europe BV, The Netherlands) for 1 h at 28 °C in HPMI/glucose buffer (10 mM HEPES, 120 mM NaCl, 5 mM Na_2HPO_4, 0.4 mM $MgCl_2$, 0.04 mM $CaCl_2$, 10 mM $NaHCO_3$, 10 mM glucose, 5 mM KCl, pH 7.4) in the presence or in the absence of different concentrations of sesquiterpenes. Parasites were then extensively washed, resuspended in cold phosphate-buffered saline (PBS: 1.2 mM KH_2PO_4, 8.1 mM Na_2HPO_4, 130 mM NaCl, 2.6 mM KCl adjusted to pH 7.4), and immediately analyzed. Cells were gated on the basis of size and complexity to eliminate dead cells and debris from the analysis. Quantification of intracellular fluorescence was carried out by scanning the emission between 515 and 545 nm (FL-1) using the Cell Quest software application.

Acknowledgment. This study was supported by the Spanish Grants CICYT-FEDER IFD97-0747-C04-03 (A.G. and S.C.), PPQ2000-1655-C02-02 (F.G.), PB98-0115 (S.C.) PB96-1033 and PB97-1039 (F.G.), BQU2000-0870-CO2-01 (I.A.J.), Gobierno Autónomo de Canarias, and ITC. We thank Pilar Navarro for her help in parasite culture and Prof. J. T. Vázquez for carrying out the CD experiments. We also acknowledge Pharmacia & Upjohn (Barcelona, Spain) for providing the daunomycin used in this study. M.L.K. thanks the A.E.C.I. for a fellowship (Becas MUTIS). F.C.S. was the recipient of a fellowship (B.E.F.I. exp 00/9068) from the Fondo de Investigación Sanitaria, Instituto de Salud Carlos III, Ministerio de Sanidad y Consumo, Spain.

Supporting Information Available: IR, UV, MS, HRMS, and NMR spectra for the new compounds described, and the CD spectra of compounds **1**, **3**, **6**−**9**, **12**, and **13**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) González, A. G.; Bazzocchi, I. L.; Moujir, L. M.; Jiménez, I. A. Ethnobotanical Uses of Celastraceae. Bioactive Metabolites. In *Studies in Natural Products Chemistry*, *Bioactive Natural Products (Part D)*; Elsevier Science Publisher: Amsterdam, 2000; Vol. 23, pp 649−738.

(2) Muñoz, O.; Peñaloza, A.; González, A. G.; Ravelo, A. G.; Crespo, A.; Bazzocchi, I. L.; Alvarenga, N. L. Celastraceae, Bioactive Metabolites. In *Studies in Natural Products*; Elsevier Science Publisher: Amsterdam, 1996; Vol. 18, pp 739−785.

(3) Hussein, G.; Nakamura, N.; Meselhy, R. M.; Hattori, M. Phenolics from *Maytenus senegalensis*. *Phytochemistry* **1999**, *50*, 689−694.

(4) Simoés, C. M. O.; Mentz, A. L.; Schenkel, P. E.; Irgang, E. B.; Stehmann, R. J. Plantas da Medicina Popular do Rio Grande do Sul. In *Universidade Federal do Rio Grande do Sul*: do Rio Grande do Sul: Brazil, 1986.

(5) Souza-Formigoni, M. L. O.; Oliveira, M. G. M.; Monteiro, M. G.; Gomes da Silveira-Filho, N.; Braz, S.; Carlini, E. A. Antiulcerogenic effects of two *Maytenus* species in laboratory animals. *J. Ethnopharmacol.* **1991**, *34*, 21−27.

(6) Oliveira, M. G. M.; Monteiro, M. G.; Macaúbas, C.; Barbosa, V. P.; Carlini, E. A. Pharmacologic and toxicologic effects of two *Maytenus* species in laboratory animals. *J. Ethnopharmacol.* **1991**, *34*, 29−41.

(7) Moujir, L. M.; Navarro-Gutiérrez, A. M.; González, A. G.; Ravelo, A. G.; Luis, J. G. Netzahualcoyone mode of actino. *Antimicrob. Agents Chemother.* **1991**, *35*, 211−213.

(8) González, A. G.; Alvarenga, N. L.; Ravelo, A. G.; Bazzocchi, I. L.; Ferro, E. A.; Navarro, A. G.; Moujir, L. M. Scutione, a new bioactive norquinonemethide triterpene from *Maytenus scutiodes* (Celastraceae). *Bioorg. Med. Chem.* **1996**, *4*, 815−820.

(9) González, A. G.; Alvarenga, N. L.; Estévez-Braun, A.; Ravelo, A. G.; Bazzocchi, I. L.; Moujir, L. M. Structure and absolute configuration of triterpene dimers from *Maytenus scutioides*. *Tetrahedron* **1996**, *52*, 9597−9608.

(10) Brüning, R.; Wagner, H. Übersicht über die celastraceen−inholtsstoffe: chemie, chemotaxonomie, biosynthese, pharmakologie. *Phytochemistry* **1978**, *17*, 1821−1858.

(11) Zheng, Y. L.; Xu, Y.; Lin, J. F. Immunosuppresive effects of wilfortrine and euonine. *Acta Pharm. Sin.* **1989**, *24*, 568−572.

(12) Kuo, Y.; King, M.; Chen, G.; Chen, H.; Chen, C.; Chen, L.; Lee, K. Two new macrolide sesquiterpene pyridine alkaloids from *Maytenus emarginata*: emarginatine G and the cytotoxic emarginate F. *J. Nat. Prod.* **1994**, *57*, 263−269.

(13) González, A. G.; Jiménez, I. A.; Ravelo, A. G.; Coll, J.; González, J. A.; Lloria, J. Antifeedant activity of sesquiterpenes from Celastraceae. *Biochem. Syst.. Ecol.* **1997**, *25*, 513−519.
(14) Duan, H.; Takaishi, Y.; Bando, M.; Kido, M.; Imakura, Y.; Lee, K. Novel sesquiterpene esters with alkaloid and monoterpene and related compounds from *Tripterygium hypoglaucum*: a new class of potent anti-HIV agents. *Tetrahedron Lett.* **1999**, *40*, 2969−2972.
(15) Pérez-Victoria, J. M.; Tincusi, B. M.; Jiménez, I. A.; Bazzocchi, I. L.; Gupta, M. P.; Castanys, S.; Gamarro, F.; Ravelo, A. G. New natural sesquiterpenes as modulators of daunomycin resistance in a multidrug-resistant *Leishmania tropica* line. *J. Med. Chem*, **1999**, *42*, 4388−4393.
(16) Kim, S. E.; Ho Kim, Y.; Lee, J. J. New sesquiterpene ester from *Celastrus orbiculatus* reversing multidrug resistance in cancer cells. *J. Nat. Prod.* **1998**, *61*, 108−111.
(17) González, A. G.; Tincusi, B. M.; Bazzocchi, I. L.; Tokuda, H.; Nishino, H.; Konoshima, T.; Jiménez, I. A.; Ravelo, A. G. Antitumor promoting effects of sesquiterpenes from *Maytenus cuzcoina* (Celastraceae). *Bioorg. Med. Chem.* **2000**, *8*, 1773−1778.
(18) Nicolaou, K. C.; Pfefferkorn, J. A.; Roecker, A. J.; Cao, G. Q.; Barluenga, S.; Mitchell, H. J. Natural product-like combinatorial libraries based on privileged structures. 1. General principles and solid-phase synthesis of benzopyrans. *J. Am. Chem. Soc.* **2000**, *122*, 9939−9953 and references therein.
(19) Hirst, S. I.; Stapley, L. A. Parasitology: the dawn of a new millennium. *Parasitol. Today* **2000**, *16*, 1−3.
(20) Ambudkar, S. V.; Dey, S.; Hrycyna, C. A.; Ramachandra, M.; Pastan, I.; Gottesman, M. Biochemical, cellular and pharmacological aspects of the multidrug transporter. *Annu. Rev. Pharmacol. Toxicol.* **1999**, *39*, 361−398.
(21) Endicott, J. A.; Ling, V. The biochemistry of P-glycoprotein-mediated multidrug resistance. *Annu. Rev. Biochem.* **1989**, *58*, 137−171.
(22) Ullman, B. Multidrug Resistance and P-glycoproteines in Parasitic Protozoa. *J. Bioenerg. Biomembr.* **1995**, *27*, 77−84.
(23) Wilson, C. M.; Serrano, A. E.; Wasley, A.; Bogenschutz, M. P.; Shankar, A. H.; Wirth, D. F. Amplification of a gene related to the mammalian mdr genes in drug resistance *Plasmodium falciparum*. *Science* **1989**, *244*, 1184−1186.
(24) Chiquero, M. J.; Pérez-Victoria, J. M.; O'Valle, F.; Gonzalez-Ros, J. M.; del Moral, R.; Ferragut, J. A.; Castanys, S.; Gamarro, F. Altered drug membrane permeability in a multidrug-resistant *Leishmania tropica* line. *Biochem. Pharmacol.* **1998**, *55*, 131−139.
(25) Chow, L. M. C.; Wong, A. K. C.; Ullman, B.; Wirth, D. F. Cloning and functional analysis of an extrachromosomally amplified multidrug resistance-like gene in *Leishmania enrietti*. *Mol. Biochem. Pharmacol.* **1993**, *60*, 195−208.
(26) Henderson, D. M.; Sifri, C. D.; Rodgers, M.; Wirth, D. F.; Hendrickson, N.; Ullman, B. Multidrug resistance in *Leishmania donovani* is conferred by amplification of a gene homologous to the mammalian mdr1 gene. *Mol. Cell. Biol.* **1992**, *12*, 2855−2865.
(27) Ford, J. M.; Hait, W. N. Pharmacology of drugs that alter multidrug resistance in cancer. *Pharmacol. Rev.* **1990**, *42*, 155−199.
(28) Pérez-Victoria, J. M.; Chiquero, M. J.; Conseil, G.; Dayan, G.; Di Pietro, A.; Barron, D.; Castanys, S.; Gamarro, F. Correlation between the affinity of flavonoids binding to cytosolic site of *Leishmania tropica* multidrug transporter and their efficiency to revert parasite resistance to daunomycin. *Biochemistry* **1999**, *38*, 1736−1743.
(29) González, A. G.; Núñez, M. P.; Jiménez, I. A.; Ravelo, A. G.; Bazzocchi, I. L. Minor sesquiterpenes from *Maytenus magellanica*. *J. Nat. Prod.* **1993**, *56*, 2114−2119.
(30) González, A. G.; Núñez, M. P.; Ravelo, A. G.; Sazatornil, J. A.; Vázquez, J. T.; Bazzocchi, I. L.; Morales, E. Q.; Muñoz, O. M. Structural elucidation and absolute configuration of novel β-agarofuran (epoxyeudesmene) sesquiterpenes from *Maytenus magellanica* (Celastraceae). *J. Chem. Soc., Perkin Trans. 1* **1992**, 1437−1441.
(31) González, A. G.; Núñez, M. P.; Bazzocchi, I. L.; Ravelo, A. G.; Jiménez, I. A. Sesquiterpenes from *Maytenus magellanica* (Celastraceae). *Nat. Prod. Lett.* **1993**, *2*, 163−170.
(32) Muñoz, O. M.; González, A. G.; Ravelo, A. G.; Luis, J. G.; Vázquez, J. T.; Núñez, M. P.; Jiménez, I. A. Sesquiterpenes esters from *Maytenus distica* (Celastraceae). *Phytochemistry* **1990**, *29*, 3225−3228.
(33) Harada, N.; Nakanishi, K. *Circular Dichroism Spectroscopy Exciton Coupling in Organic Stereochemistry*; University Science Books: Mill Valley, CA, 1983.
(34) PC Model from Serena Software, P.O. Box 3076, Bloomington, IN 47402-3076.
(35) Tincusi, B. M.; Jiménez, I. A.; Ravelo, A. G.; Missico, R. New sesquiterpenes from *Crossopetalum tonduzii*. *J. Nat. Prod.* **1998**, *61*, 1520−1523.
(36) Kupchan, S. M.; Smith, R. M. Maytoline, maytine, and maytolidine, novel nicotinoyl sesquiterpene alkaloids from *Maytenus serrata*. *J. Org. Chem.* **1977**, *42*, 115−118.
(37) Hollo, Z.; Homolya, L.; Davis, C. W.; Sarkadi, B. Calcein accumulation as a fluorometric functional assay of the multidrug transporter. *Biochim. Biophys. Acta* **1994**, *1191*, 384−388.
(38) Essodaigui M.; Frezard, F.; Moreira, E. S.; Dagger, F.; Garnier-Suillerot, A. Energy-dependent efflux from Leishmania promastigotes of substrates of the mammalian multidrug resistance pumps. *Mol. Biochem. Parasitol.* **1999**, *100*, 73−84.
(39) Iovannisci, D. M.; Ullman, B. High efficiency plating method for Leishmania promastigotes in semi-defined or completely-defined medium. *J. Parasitol.* **1983**, *69*, 633−636.
(40) Jackson, P. R.; Lawrie, J. M.; Stiteler, J. M.; Hawkins, D. W.; Wohlhieter, J. A.; Rowtin, E. D. Detection and characterization of Leishmania species and strains from mammals and vectors by hybridization and restriction endonuclease digestion of kinetoplast DNA. *Vet. Parasitol.* **1986**, *20*, 195−215.
(41) Mosmann, T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J. Immunol. Methods* **1983**, *65*, 55−63.
(42) Alfieri, S. C.; Zilberfarb, V.; Rabinovitch, M. Destruction of Leishmania mexicana amazonensis amastigotes by leucine methyl ester: protection by other amino acid esters. *Parasitology* **1987**, *95*, 31−41.

JM010970C