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New terpenoids from *Maytenus apurimacensis* as MDR reversal agents in the parasite *Leishmania*

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Abstract—Two new sesquiterpenes (**1**–**2**) and one new lupane triterpene (**3**) have been isolated from the roots of *Maytenus apurimacensis*. The novel β-dihydroagarofurans are the first sesquiterpenes with a basic polyhydroxy skeleton of 15-deoxyalatol and 4,15-dideoxyalatol that show high MDR reversing activity in the protozoan parasite *Leishmania tropica*.

1. Introduction

Chemical substances derived from animals, plants, and microbes have been used to treat human disease since the dawn of medicine. The investigation of natural products as a source of novel human therapeutics reached its peak in the Western pharmaceutical industry in the period 1970–1980, which resulted in a pharmaceutical landscape heavily influenced by non-synthetic molecules. Of the 1184 small-molecules New Chemical Entities (NCEs) introduced between 1981 and 2006, roughly half (48%) were natural products, semi-synthetic natural product analogues or synthetic compounds based on natural product pharmacophores.[1] The remarkable chemical diversity encompassed by natural products continues to be of relevance to drug discovery. According to WHO report, leishmaniasis is one of the 10 more important infectious diseases. Despite recent scientific and technological advances, infectious diseases continue to affect poor and marginalized people throughout the world. At least three key factors contribute to the emergence and re-emergence of such diseases: 1-failure to use properly existing tools effectively; 2-failure or non-existence of tools to control the disease, and 3-insufficient knowledge of the disease.[2] Leishmaniasis is the most important emerging and uncontrolled infectious disease and the second cause of death among parasitic diseases, after Malaria. Numerous plant-derived natural products from different structural classes have been investigated as antileishmanial candidates, including various alkaloids, terpenoids, flavonoids, and quinonoids.[3] One of the main problems concerning the control of infectious diseases is the increased resistance to usual drugs. Overexpression of P-glycoprotein (Pgp)-like transporters represents a very efficient mechanism to reduce the intracellular accumulation of drugs in cancer cells and parasitic protozoans, thus conferring a multidrug resistance (MDR) phenotype. Pgps are active pumps belonging to the ATP-binding cassette (ABC) superfamily of proteins. The inhibition of the activity of these proteins represents an interesting way to control drug resistance both in cancer and in infectious diseases. Most conventional mammalian Pgp-MDR modulators are ineffective in the modulation of Pgp activity in the protozoan parasite *Leishmania*.[4] Consequently, there is a necessity to find effective modulators of Pgp-MDR for protozoan parasites.[5] It has been previously described that natural dihydro-β-agarofuran sesquiterpenes from *Celastraceae*

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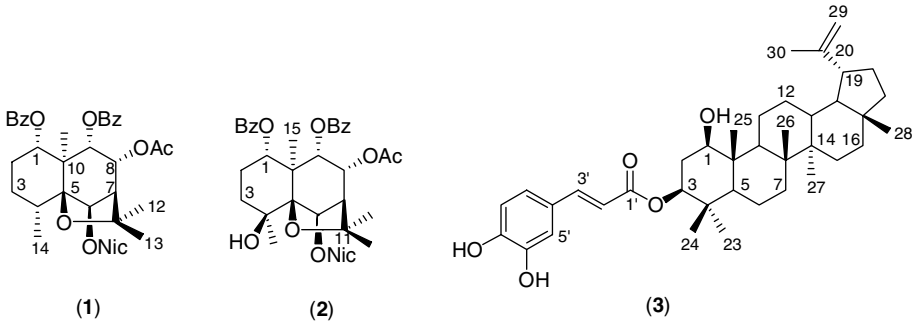

Figure 1. Chemical structures of new terpenoids (**1**–**3**) isolated from *Maytenus apurimacensis*.

plants are potent and specific inhibitors of Pgp and devoid of intrinsic toxicity in the concentration range of Pgp inhibition.[6] As a part of an intensive investigation into active metabolites as reversal agents of the Pgp-dependent MDR phenotype, we have focused our efforts in the characterization of new natural compounds as potential Pgp inhibitors. In this paper, we describe the phytochemical analysis of *n*-hexane–Et_2O extract of the root bark of *Maytenus apurimacensis*, which yielded three new terpenoids; one triterpenoid with lupane structure and two sesquiterpenes that present two new polyhydroxylated skeletons (Fig. 1). *M. apurimacensis* also yielded friedelin,[7] β-sitosterol,[8] baruol,[9] pristimerine,[10] 6′,7′-dihydrocangoronsin αA,[11] 3-epiglochidiol,[12] paniculatadiol,[13] 3β,29-dihidroxy-glut-5-ene,[14] glochidiol,[15] zeylasteral,[16] 6-oxo-pristimerol,[17] tingenone,[18] blepharodol,[19] dispermoquinone,[20] and lupeol.[21] Their structures were elucidated by means of ^1H and ^{13}C NMR spectroscopic studies, including homonuclear (COSY and ROESY) and heteronuclear correlation (HSQC and HMBC) experiments. The absolute configuration of the new sesquiterpenes was determined by CD studies. Furthermore, these sesquiterpenes have been tested on a MDR *Leishmania tropica* line overexpressing a Pgp-like transporter[22] to determine their ability to revert the resistance phenotype.

2. Results and discussion

Repeated chromatography of the *n*-hexane–Et_2O (1:1) extract of the root bark of *M. apurimacensis* on silica gel and Sephadex LH-20 yielded two new dihydro-β-agarofuran sesquiterpenes (**1**–**2**) and one new lupane triterpenoid (**3**).

Compound **1** was isolated as an amorphous white solid with the molecular formula $C_{37}H_{39}NO_9$, established by HR-MS. The IR spectrum showed absorption bands for ester groups (1725 cm^{-1}). The mass spectrum contained fragments attributable to the presence of nicotinate (*m*/*z* 124), benzoate (*m*/*z* 105), and acetate ($M^+ - 60$, *m*/*z* 581) moieties. This was confirmed by the ^1H and ^{13}C NMR spectroscopic data, which included signals for 14 aromatic protons between δ 6.90 and 9.37 for two benzoates and one nicotinate group and one acetate methyl as a singlet at δ 2.17. In its ^1H NMR spectrum also was observed signals assignable to protons on the carbons bearing four secondary ester groups at δ 6.21 bs (H-6), 5.70 d (J = 3.6 Hz, H-9), 5.58 m (2 H, H-1 + H-8), three angular methyls at δ 1.46 (Me-13), 1.62 (Me-12), and 1.67 (Me-15), and one doublet methyl at δ 1.16 (J = 5.4 Hz, Me-14). The ^{13}C NMR and DEPT spectra indicated that **1** contained a skeleton based on 15 carbons: three methyl carbons, two methylene carbons, six methine carbons, and three quaternary carbons. All these data suggested that **1** was a tetrasubstituted-β-dihydroagarofuran sesquiterpene. The locations of the different esters were determined on the basis of ^1H–^{13}C long-range correlations, which indicated that two benzoate esters were located at C-1 and C-9, as well as a nicotinate ester at C-6, and the acetate moiety at C-8. The orientations of H-1, H-6, H-8, and H-9 were determined by analysis of the coupling constants and also by ROESY experiments. The β-orientation for the nicotinate ester was established for the signal singlet at δ 6.21 attributable to H-6 because the dihedral angle of H-6 and H-7 is about 90° and also by the NOE detected between H-6 and Me-15. The doublet at δ 5.70 corresponding to H-9 showed NOE with Me-12, which established its β-disposition and consequently the same orientation for H-8 due to the value of coupling constant (J = 2.3 Hz) characteristic of a *H-9ax–H-8eq* disposition. Concerning the H-1 disposition, the β-stereochemistry was established on the basis of biogenetic considerations[23] and the NOE detected between the multiplet at δ 2.4 (H-4) and the multiplet at δ 5.58 assignable to H-1 and H-8. Its absolute configuration was resolved by the dibenzoate chirality method, an extension of the CD exciton chirality method.[24] The CD spectrum showed a split curve with a first negative Cotton effect at 237.2 nm (Δε − 2.72) and a second positive one at 219.8 nm (Δε + 4.57). The absolute configuration of **1** was accordingly established as (1*S*, 4*S*, 5*S*, 6*R*, 7*R*, 8*S*, 9*R*, 10*R*)-8-acetoxy-1,9-dibenzoyloxy-6-nicotynoyloxy-dihydro-β-agarofuran.

Compound **2** was isolated as an amorphous white solid with the molecular formula $C_{37}H_{39}NO_{10}$, established by HRMS. It presented spectral data similar to those of **1**. The main differences were the presence of Me-14 as singlet instead of a doublet and the lack of the multiplet at δ 2.40 attributable to H-4. These data suggested that compound **2** had the same chemical structure as compound **1** with an additional hydroxyl group located at C-4. The presence of a quaternary carbon at δ 71.1 in the ^{13}C NMR and DEPT spectra confirmed this fact.

The location of all ester groups and also the hydroxyl group was ratified by HMBC experiments. The relative stereochemistry of the hydroxyl group was determined as β because of the NOE detected in its ROESY spectrum between H-6 and Me-14. The CD spectrum of **2** was very similar to those of **1**, it also presented a split curve with a first negative Cotton effect at 239.6 nm ($\Delta\varepsilon - 2.28$) and a second positive Cotton effect at 220.0 nm ($\Delta\varepsilon + 4.35$). All of the above data led to (1*S*, 4*R*, 5*R*, 6*R*, 7*R*, 8*S*, 9*R*, 10*R*)-8-acetoxy-1,9-dibenzoyloxy-4-hydroxy-6-nicotynoyloxy-dihydro- β-agarofuran as the structure for **2**. The new sesquiterpenes have a basic polyhydroxy skeleton of 4,15-dideoxyisoalatol and 15-deoxyisoalatol.[23,25] Compound **3** was isolated as an amorphous white solid, with the molecular formula $C_{39}H_{56}O_5$ (HR-EIMS). The IR spectrum suggested the presence of hydroxyl groups (3404.5 cm^{-1}), aromatic nucleus (1603, 1455 cm^{-1}), ester group (1683 cm^{-1}), and a terminal double bond (2947, 1635.3 and 881.5 cm^{-1}). The ^1H NMR spectrum showed six angular methyls [δ 0.79, 0.86, 0.89, 0.96 × 2, 1.05], an isopropenyl group [δ 1.68 (3H, s), 4.56 (1H, br s), and 4.68 (1H, br s), two oxymethine protons geminal to hydroxyl and ester groups [δ 3.55 (1H, dd, J = 4.9, 10.8 Hz), 4.59 (1H, dd, J = 4.7, 7.8 Hz)], a typical lupene H_β-19 proton signal δ_H (1H, 2.37 m), and signals characteristic of a caffeate moiety [δ 7.54 (1 H, d, J = 15.9 Hz), 7.07 (1 H, bs), 6.98 (1 H, d, J = 8.0 Hz), 6.86 (1 H, d, J = 8.0 Hz), 6.22 (1 H, J = 15.9 Hz)]. All these data indicate that compound **3** is a disubstituted lupane triterpene. Its ^{13}C NMR spectrum revealed 39 carbon signals, and confirmed the existence of two oxygenated methine carbons at δ 77.6 and 78.7 and the presence of a caffeate ester [δ 167.6 (s) C-1′, 146.6 (s) C-7, 145.1 (d) C-3′, 144.0 (s) C-6′, 127.4 (s) C-4′, 122.4 (d) C-9′, 115.7 (d) C-2′, 115.5 (d) C-8′, 114.4 (d) C-5′]. The two sp^2 carbons observed at δ 150.8 (s) and 109.4 (t) ratified the Δ-functionality[20,29]of a lupane skeleton. The positions of the hydroxy and ester were determined on the basis of ^1H–^{13}C long-range correlations. Thus, the location of the ester group at C-3 and the C-1-OH group was established from the following correlations (H-3/C-1′, H-3/C-23, H-3/C-24, H-1/C-25, H-1/C-5, H-1/C-9). The relative stereochemistry of the oxymethine protons at the C-1 and C-3 positions was established as α, on the basis of the coupling constants and confirmed by the NOEs found in the ROESY spectrum. Thus, the NOEs between H-1/H-3, H-3/Me-23, H-3/H-5, and H-1/H-5 indicated an axial orientation for the hydroxyl and the ester groups on the α-face. All of these data and comparison with those found in the literature for 3-epiglochidiol (1β,3β-dihydroxylup-20(29)-ene[12] established the structure of **3** as 1β-hydroxy-3β-caffeatelup-20(29)-ene. There are not many lupane triterpenoids with a caffeate ester. In fact, there are only six examples: 3-(*Z*)-caffeoyllupeol,[26] betulin-3-caffeate,[27]lup-20(29)en-28-al-3β-yl caffeate,[28] betulinic acid-3β-yl caffeate,[29] and 3β,23-dihydroxylup-20(29)-en-28-oic acid 3β-caffeate.[30]

With the antecedent of agarofuran sesquiterpenes as new promising MDR modulators in eukaryotic organisms, we decided to study the reversal effects of the new sesquiterpenes in an MDR *L. tropica* line grown in the presence of daunomycin 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 2 shows that after 72 h of incubation of MDR parasites in the presence of 150 μM daunomycin (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 efficient for both sesquiterpenes, but the effective concentration varied between each one. In this sense, 7 μM of **2** produces 90% GI, requiring around 1.5-fold concentration of **1** 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. Structurally the only difference between both compounds is the presence of a hydroxyl group at C-4, which produces a higher reversal effect. In previous papers we have found that the most active sesquiterpenes present five, six, or seven oxygenated functions in addition to the oxygen atom which belong to the tetrahydrofuran ring. The results commented above on the reversal effect of compounds **1** and **2** indicate us that the series type 4,15-dideoxyisoalatol and 15-deoxyisoalatol constitute a new entry to more potent sesquiterpene derivatives.

3. Experimental section

3.1. Materials and methods

Roots of *M. apurimacensis* were collected in Santuario de Ampay, Huancavelica (Peru), in November 2001, and were identified by the botanist *G. Yarupaitan*. A voucher specimen is on file (No. 3020) with the Herbarium of the Museo de Historia Natural, Lima, Peru.

3.2. Extraction and isolation

Root bark of *M. apurimacensis* (0.2 kg) was extracted with *n*-hexane–Et_2O (1:1) (2 L) in a Soxhlet apparatus for 48 h. Evaporation of the solvent under reduced pressure provided 10 g of a dark extract. This residue was chromatographed on Sephadex LH-20 eluting with *n*-hexanes–$CHCl_3$–MeOH (2:1:1) to afford 56 fractions. Fractions with similar TLC profile were combined and reduced to five fractions (A–E). Each was rechromatographed on silica gel column, using mixtures of *n*-hexane–EtOAc of increasing polarity as eluent. Some of the eluted products were separated by preparative TLC. Friedelin (8.4 mg) and β-sitosterol (19.4 mg) were isolated from fraction A. Fraction B yielded baruol (3.7 mg) and pristimerine (381.4 mg). Fraction C afforded 6′,7′-dihydrocangarosin A (11.6 mg), 3-epiglochidiol (96.2 mg), paniculatadiol (23.2 mg), 3β-29-dihidroxy-glut-5-ene (17.4 mg), glochidiol (10.0 mg), compound **1**(5.6 mg), and compound **2** (13.8 mg). Zeylasteral (77.8 mg), zeylasterone (10.4 mg), and 6-oxopristimerol (37.6 mg) were separated from fraction D. Fraction E yielded tingenone (43.3 mg), blepharodol

(9.7 mg), dispermoquinone (8.0 mg), lupeol (8.3 mg), and compound **3** (9.0 mg).

3.2.1. (1*S*, 4*S*, 5*S*, 6*R*, 7*R*, 8*S*, 9*R*, 10*R*)-8-acetoxy-1,9-dibenzoyloxy-6-nicotynoyloxy-dihydro-β-agarofuran (1). Amorphous white solid; $[\alpha]_D^{20}$ −9.6 (c 1.4, $CHCl_3$); IR (film) ν_{max} 1726, 1590, 1451, 1376, 1323, 1278, 1231, 110.8, 1025.8, 706.9 cm^{-1}. UV (EtOH) λ_{max} (log ε) 263.8 (3.37), 226.4 (4.09). CD λ_{max} (CH_3CN) (Δε) 237.2 (-2.72), 219.8 (+4.57). ESI-MS *m/z* (rel. int): 641 (M^+, 2), 581 (M+-OAc, 3), 520 (10), 414 (5), 245 (10), 228 (6), 245 (12), 228 (5), 124 (12), 105 (100), 77 (13). HR-EIMS 641.2636 (Calcd 641.2625, $C_{37}H_{39}NO_9$). ^1H NMR ($CDCl_3$) δ 9.26 (bs,1H), 8.36 (m,1H), 8.30 (dd, *J* = 1.9, 7.9 Hz, 1H), 7.62 (d, *J* = 7.2 Hz, 3H), 7.44 (m, 1H), 7.32 (m, 1H), 7.27 m (1H), 7.18 (m, 3H), 6.92 (t, 2H), 2.16 (s, 3H, CH_3COO–), for the rest of the signals see Table 1. ^{13}C NMR ($CDCl_3$) δ 169.8 (CH_3COO–), 165.5 (Ph*C*OO–), 164.8 (Ph*C*OO–), 164.3 (Nic*C*OO–), 154.0 (CH), 150.8 (CH), 137.0 (CH), 132.5 (CH), 132.2 (CH), 129.6, C), 129.5 (C), 129.2 (CH), 127.9 (CH), 127.6 (CH), 125.9 (C), 123.7 (CH), 21.0 (CH_3COO–), for the rest of the signals see Table 1.

3.2.2. (*1S*, 4*R*, 5*R*, 6*R*, 7*R*, 8*S*, 9*R*, 10*R*)-8-acetoxy-1,9-dibenzoyloxy-4-hydroxy-6-nicotynoyloxy-dihydro-β-agarofuran (2). Amorphous white solid; $[\alpha]_D^{20}$ −2.3 (c 0.5, $CHCl_3$); IR (film) ν_{max} 2926.5, 1724.9, 1591.6, 1541.2, 1368.8, 1315.0, 1278.8, 1232.0, 1176.2, 1113.1, 1026.1, 959.9 cm^{-1}; UV (EtOH) λ_{max} (log ε) 263.6 (3.6), 226.8 (4.3). CD λ_{max} (CH_3CN) (Δε) 239.6 (−2.28), 220.0 (4.35); EI-MS *m/z* (rel. int): 657 (M^+) (30), 641 (55), 597 (70), 571 (100), 520 (32), 414 (20), 375(40), 271(45), 228 (90); HR-EIMS 657.2551 (Calcd 657.2574, $C_{37}H_{39}NO_{10}$); ^1H NMR ($CDCl_3$, 300 MHz) δ: 9.37 (d, *J* = 1.3 Hz, 1 H), 8.81 (dd,*J* = 1.8, 4.8 Hz, 1H), 8.50 (dd, *J* = 1.8, 6.0 Hz), 7.62 (d, *J* = 8.2 Hz, 2H), 7.60 (d, *J* = 8.2 Hz, 2H), 7.44 (m, 2H), 7.40 (m, 1H), 7.13 (m, 1H), 6.91 (t, *J* = 7.9 Hz, 2H), 2.18 (s, 3H); ^{13}C NMR ($CDCl_3$, 300 MHz) δ: 169.8 (CH_3COO–), 165.4 (C═O), 164.7 (C═O, x 2), 137.5 (CH), 132.6 (CH), 132.4 (CH), 129.4 (C), 129.5 (CH), 129.1 (CH), 128.6 (CH), 128.0 (CH), 127.6 (CH), 125.7 (C), 123.5 (CH), 20.9 (CH_3), for the rest of the signals see Table 1.

3.2.3. 1β-Hydroxy-3β-caffeate lup-20(29)-ene (3). Amorphous white solid; $[\alpha]_D^{20}$ −15.65 (c 0.1, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 331.4 (3.92), 301.6 (3.80), 242.6 (3.73), 216.2 (3.89) nm. IR (film) ν_{max} 3404.5, 2947.8, 2360.2, 2341.5, 1683.3, 1273.8, 1653.3, 1455.5, 1380.7, 1029.1, 881.5 cm^{-1}; EI-MS *m/z* (rel. int): 604 (M^+, 10), 483 (20), 424 (25), 406 (56), 229 (35), 203 (45), 175 (90), 95 (100). HR-EIMS 604.4107 (Calcd 604.4127, $C_{39}H_{56}O_5$); ^1H NMR ($CDCl_3$, 300 MHz) δ: 7.54 (d, *J* = 15.9 Hz, 1H, H-3′), 7.07 (bs, 1H, H-5′), 6.98 (d, *J* = 8.0 Hz, 1H, H-9′), 6.86 (d, *J* = 8.1 Hz, 1H, H-8′), 6.22 (d, *J* = 15.9 Hz, 1H, H-2′), 4.68 (bs, 1H, H-29 a), 4.56 (bs, 1H, H-29 b), 4.59 (dd, *J* = 4.3, 12.2 Hz, 1H, H-3), 3.55 (dd, *J* = 4.9, 10.8 Hz, 1H, H-1), 2.37 (m, 1H, H-19), 1.68 (s, 3H, Me-30), 1.05 (s, 3H, Me-26), 0.96 (s, 6H, Me-25 + Me-27), 0.89 (s, 3H, Me-24), 0.86 (s, 3H, Me-23); ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 167.6 (s, C-1′), 150.8 (s, C-20), 146.6 (s, C-7′), 145.1 (d, C-3′), 144.0 (s, C-6′), 127.4 (s, C-4′), 122.4 (d, C-9′), 115.7 (d, C-2′), 115.5 (d, C-8′), 114.4 (d, C-5′), 109.4 (t, C-29), 78.7 (d, C-1), 77.6 (d, C-3), 53.1 (d, C-5), 51.4 (d, C-9), 48.3 (d, C-18), 48.0 (d, C-19), 43.5 (s, C-10), 42.9 (s, C-17), 41.4 (s, C-8), 40.0 (t, C-22), 38.1(d, C-13), 37.6 (s, C-14), 35.6 (t, C-16), 34.0 (t, C-7), 27.8 (q, C-23), 4.2 (t, C-2), 29.8 (t, C-21), 27.8 (q, C-23), 27.5 (t, C-15), 25.0 (t, C-12), 23.8 (t, C-11), 19.2 (q, C-30), 17.8 (t, C-6), 17.9 (q, C-28), 16.2 (q x 2, C-26 + C-24), 14.4 (q, C-27), 12.0 (q, C-25).

3.3. Biological assays

3.3.1. Parasite culture. The wild-type (WT) *L. tropica* LRC-strain was a clone obtained by agar plating.[22a] A *L. tropica* line highly resistant to DNM (MDR line) was maintained in the presence of 150 μM DNM and used as previously described.[22b] This resistant line had an MDR phenotype similar to tumor cells, with a cross-resistance profile to several drugs and an overexpressed drug-efflux Pgp-like transporter. Promastigote forms were grown at 28 °C in RPMI 1640-modified

Table 1. ^1H NMR ($CDCl_3$, *J* in Hz in parentheses), ^{13}C NMR ($CDCl_3$) data and HMBC correlations of 1 and 2

Position	Compound 1			Compound 2		
	δ_H	δ_C	HMBC	δ_H	δ_C	HMBC
1	5.58 m	78.9 d	C_9,C_{10},C_{15}- O*C*OPh	5.48 dd (4.3, 11.6)	77.7 d	C_9,C_{15}- O*C*OPh
2	1.82 m	22.4 t		1.79 m	24.6 t	
3	1.65 m	26.6 t		1.89 m	38.6 t	
4	2.40 m	34.3 d	C_5,C_{10},C_{14}	—	71.1 s	
5	—	93.3 s		—	92.5 s	
6	6.21 bs	77.0 d	C_5,C_8,C_{11}- O*C*ONi	6.32 bs	77.0 d	C_5,C_7,C_8,C_{10}, C_{11},-O*C*ONi
7	2.69 d (3.0)	52.6 d	C_5,C_6,C_8,C_9	2.68 d (4.9)	52.5 d	C_5,C_6,C_8
8	5.58 m	71.4 d	C_6,C_{10}, -*C*OOPh	5.58 t (4.9)	70.6 d	C_7,C_9,C_{10},-*C*OOPh
9	5.70 d (3.6)	74.3 d	C_1,C_{10},C_{15}- O*C*OCH_3	5.75 d (4.9)	73.9 d	C_1,C_{10},C_{15}-O*C*OCH_3
10	—	49.3 s	—	—	50.2 s	—
11	—	81.8 s	—	—	83.3 s	—
12	1.62 s	30.9 q	C_7,C_{11},C_{13}	1.56 s	29.6 q	C_7,C_{11},C_{13}
13	1.46 s	24.1 q	C_{12}	1.69 s	23.8 q	C_7,C_{11},C_{12}
14	1.16 d (5.4)	16.9 q	C_3,C_4,C_5	1.43 s	23.3 q	C_3,C_4,C_5
15	1.67 s	12.3 q	C_1,C_5,C_9	1.73 s	12.9 q	C_1,C_5,C_9,C_{10}
OAc	2.16 s	21.0 q		2.18 s	20.9 q	

Table 2. Effect of sesquiterpenes on the growth of WT and MDR *L. tropica* lines

Compound	Growth inhibition[a] (%)							
	15 μM		7 μM		3 μM		1 μm	
	WT	MDR	WT	MDR	WT	MDR	WT	MDR
1	2.3 ± 3.2	96.1 ± 2.0	0.8 ± 1.1	59.2 ± 1.3	0.0	21.2 ± 4.0	0.0	8.6 ± 1.3
2	13.6 ± 6.7	98.5 ± 0.9	0.0	90.2 ± 5.4	0.0	62.8 ± 3.0	0.0	15.8 ± 0.6

Wild-type (WT) and MDR *Leishmania* parasites were exposed to 15, 7, 3, and 1 μM of different sesquiterpenes, in the absence or presence of 150 μM DNM, respectively. The results are expressed as percentage of growth inhibition relative to control growth in the absence of sesquiterpenes. The data shown are the average of three independent experiments ± SD.

medium (Gibco), and supplemented with 20% heat-inactivated fetal bovine serum (Gibco).

3.3.2. DNM chemosensitization experiments. The viability of parasites in the presence of the different sesquiterpenes was analyzed by an MTT-based assay, as previously described for *Leishmania*.[6b] 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 the 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. In order to assess the chemosensitizing activity of sesquiterpenes, promastigotes of the *L. tropica* MDR line were exposed to both DNM (150 μM) and sesquiterpenes. To determine the intrinsic toxicity of the sequiterpenes, the WT and MDR *L. tropica* lines were exposed to 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. About 10 mL 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 SDS 20% and absorbance was read at 540 nm using a microplate reader (Beckman Biomek 2000). Cell survival was determined by dividing the absorbance of control cells. The results are expressed as % growth inhibition.

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