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Dihydro-β-agarofuran sesquiterpenes isolated from *Celastrus vulcanicola* as potential anti-*Mycobacterium tuberculosis* multidrug-resistant agents

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ABSTRACT

In the present study, we report four new dihydro-β-agarofuran sesquiterpenes (**1–4**), which were isolated from the leaves of *Celastrus vulcanicola*, in addition to five derivatives (**5–9**). Their stereostructures were elucidated on the basis of spectroscopic analysis, including 1D and 2D NMR techniques, X-ray studies, chemical correlations and biogenetic means. Compounds **1–9** and the previously reported sesquiterpenes **10–25** have been tested as potential antimycobacterial agents against sensitive and resistant *Mycobacterium tuberculosis* strains. 1α-Acetoxy-6β,9β-dibenzoyloxy-dihydro-β-agarofuran (**20**) exhibited antituberculosis activity against the MDR TB strain with a MIC value of 6.2 μg/mL, comparable to or better than isoniazid or rifampin, two of the best first-line drugs commonly used in the treatment of TB. The structure–activity relationship is discussed.

1. Introduction

It is estimated that there are approximately 9 million new tuberculosis (TB) cases every year. Two major obstacles to global tuberculosis control are due to the high prevalence of HIV among TB patients and the deteriorating socio-economic conditions of the most underprivileged members of society.[1] Although the existing standard regimen is very effective against TB, the length of treatment (6 months), the toxicity and the potential for drug–drug interactions, particularly in the setting of antiretroviral treatment, are factors that highlight the need for new antitubercular drugs.[2,3] In addition, the advent of strains of *Mycobacterium tuberculosis* (MTB) resistant to, at least, the two first-line drugs (MDR TB), isoniazid and rifampin or resistant to three of the six classes of second-line drugs (XDR TB) is of great concern.[4] Thus, there is a great need to develop new therapeutic agents to treat tuberculosis, particularly MDR TB which has severely limited the number of effective treatment options.[5]

The sesquiterpene polyesters with a dihydro-β-agarofuran skeleton are the most widespread and characteristic group of secondary metabolites isolated from Celastraceae family. These compounds have attracted considerable attention from synthetic organic chemists and pharmacologists due to their complex structures and wide range of biological properties, including P-glyprotein dependent multidrug resistance modulation in *Leishmania tropica*[6] and cancer cells.[7] On the basis of these properties sesquiterpenes have been selected as 'privileged structures'.[8] Recently, the antituberculosis activity of this type of metabolites on *M. tuberculosis* sensitive strains has been reported.[9–11]

In the present study, and as a continuation of our research on *Celastrus vulcanicola*,[7,12] we describe the isolation, structure elucidation and bioactivity of four new (**1–4**) sesquiterpenes with a dihydro-β-agarofuran skeleton, in addition to five derivatives (**5–9**). Their structures were elucidated by means of ^{1}H and ^{13}C NMR spectroscopic studies, including homonuclear (COSY and ROESY) and heteronuclear correlation (HSQC and HMBC) experiments. The absolute configurations of these compounds were established by biogenetic means, chemical correlations and X-ray crystallographic analysis. In the search for potential antimycobacterial agents, sesquiterpenes **1–9** and those previously isolated from *C. vulcanicola*, **10–25**,[7,12] were evaluated against the MTB $H_{37}Rv$ and MDR strains.

2. Results and discussion

The dichloromethane extract of the leaves of *C. vulcanicola* was subjected to repeated chromatography on Sephadex LH-20 and silica gel, affording four new sesquiterpenes (**1–4**) (Fig. 1). Compound **1** was isolated as a colorless amorphous solid and showed the

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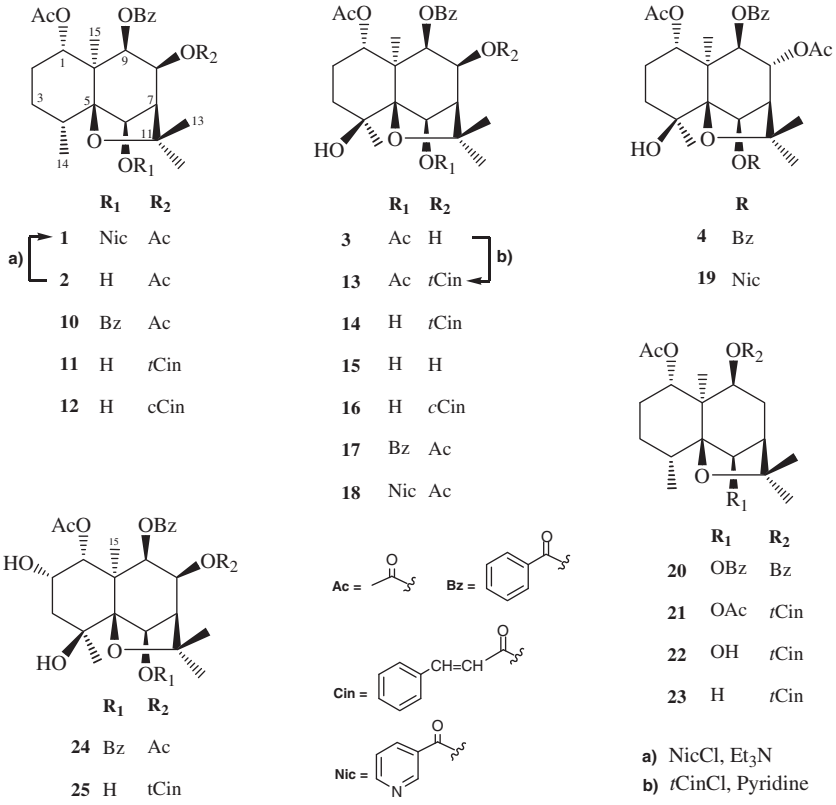


Figure 1. Structure of the natural sesquiterpenes assayed for their anti-*Mycobacterium tuberculosis* activity.

molecular formula $C_{32}H_{37}NO_9$ by EI-HRMS, ^1H and ^{13}C NMR data. The IR spectrum showed an absorption band for ester (1729 cm^{-1}) group. The EI-MS exhibited peaks consistent with loss of methyl (*m/z* 564 [M^+−Me]), acetic acid (*m/z* 519 [$M^+-CH_3CO_2H$]), benzoic acid (*m/z* 458 [M^++1−$PhCO_2H$], *m/z* 105 [PhCO]$^+$) and nicotinic acid (*m/z* 456 [$M^+-C_5H_4NCO_2H$], *m/z* 106 [C_5H_4NCO]$^+$). Its ^1H NMR data (Table 1) included signals for two acetyl groups at δ 1.66 (3H, s) and 1.91 (3H, s) and nine protons in the aromatic region [δ 7.50 (3H, m), 7.60 (1H, m), 8.11 (2H, m), 8.33 (1H, m) 8.85 (1H, m) and 9.26 (1H, s)], corresponding to benzoyl and nicotinoyl groups. In addition, resonances for six methine protons, three forming an ABX system at δ 5.68 (dd, *J* = 3.0, 6.3 Hz, H-8), 5.35 (d, *J* = 6.3 Hz, H-9) and 2.66 (d, *J* = 3.0 Hz, H-7), two oxymethine protons at δ 5.60 (s, H-6) and 5.50 (dd, *J* = 4.0, 11.7 Hz, H-1) and one methine proton at δ 2.43 (m, H-4), were observed. Two methylene systems at δ 1.47, 2.04 (2H, m) and 1.72, 2.27 (2H, m) were assigned to protons H-2, and H-3, respectively. Also singlets from three tertiary methyl groups at δ 1.43, 1.46 and 1.58, and a doublet for one secondary methyl group at δ 1.03 (d, *J* = 7.4 Hz) were observed. All these data were confirmed by the ^{13}C NMR spectrum (Table 1), and the chemicals shifts for the carbons attached to protons were assigned according to a 2D heteronuclear HSQC experiment. These data indicate that **1** is a polyester sesquiterpene with a 1,6,8,9-tetrasubstituted-dihydro-β-agarofuran skeleton.

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

Position	1		2	
	δ_H	δ_C[a]	δ_H	δ_C[a]
1	5.50 dd (4.0, 11.7)	73.0 d	5.45 dd (4.2, 11.8)	73.4 d
2	1.47 m, 2.04 m	22.5 t	1.64 m, 1.91 m	21.4 t
3	1.72 m, 2.27 m	26.5 t	1.45 m, 2.18 m	26.5 t*
4	2.43 m	34.1 d	2.31 m	34.4 d
5		89.6 s		90.7 s
6	5.60 s	78.4 d	4.42 s	76.3 d
7	2.66 d (3.0)	53.6 d	2.40 d (2.7)	55.3 d
8	5.68 dd (3.0, 6.3)	68.9 d	5.41 dd (2.7, 6.2)	69.6 d
9	5.35 d (6.3)	72.3 d	5.23 d (6.2)	72.6 d
10		48.8 s		48.4 s
11		82.7 s		82.6 s
12	1.46 s	31.3 q	1.51 s	31.3 q
13	1.58 s	26.3 q	1.53 s	26.5 q*
14	1.03 d (7.4)	17.2 q	1.18 d (7.3)	17.7 q
15	1.43 s	18.5 q	1.34 s	18.6 q
OAc-1	1.66 s	20.5 q*, 169.7 s	1.63 s	20.6 q, 169.6 s
OAc-8	1.91 s	20.5 q*, 169.0 s	1.90 s	20.5 q, 169.7 s

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

The regiosubstitution of **1** was determined by an HMBC experiment, showing a three-bond correlation between the nicotinoyloxy carbonyl resonance at δ_C 163.9 and the proton at δ_H 5.60 that corresponds to H-6. The attachment of the benzoyloxy group to C-9 was defined by the cross-peak between the carboxyl resonance at δ_C 165.4 and the signal at δ_H 5.35 (H-9), whereas the acetoxy groups were located at positions C-1 and C-8 by correlation of the carboxyl resonances at δ_C 169.7 and δ_C 169.0 with signals assigned to H-1 (δ_H 5.50) and H-8 (δ_H 5.68), respectively. The relative configuration of **1** was established on the basis of the coupling constants and confirmed by 2D homonuclear experiments. Therefore, the coupling constants of H_1–H_2 (J_{1-2} = 4.0, 11.7 Hz), H_6–H_7 (J_{6-7} = 0 Hz) and H_8–H_9 (J_{8-9} = 6.3 Hz), observed in the COSY experiment, indicated an axial orientation for H-1, an equatorial one for H-6 and a *cis*-relationship between H-8 and H-9. The ROESY experiment showed correlations between H-1 and H-3 and between Me-15 and H-6, H-8, H-9 and Me-14 (Fig. 2). Thus, the structure of compound **1** was established as 1α,8β-diacetoxy-9β-benzoyloxy-6β-nicotinoyloxy-dihydro-β-agarofuran. Its absolute configuration

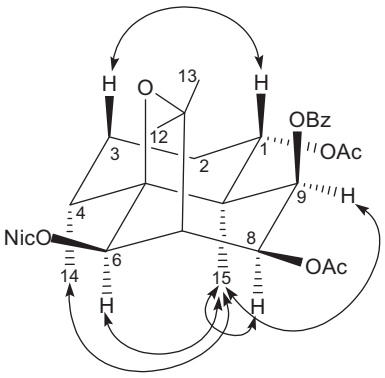


Figure 2. ROE effects for compound **1**.

was assumed based on it having the same polyhydroxy sesquiterpene core as compound **11**, whose absolute stereochemistry has been previously established by dichroism circular.[7]

The EI-HRMS of compound **2** gave a molecular ion at *m*/*z* 474.2261, corresponding to the molecular formula $C_{26}H_{34}O_8$. Its ^1H and ^{13}C NMR data (Table 1) were closely related to those of **1**, except for the absence of the signals assigned to the nicotinoyl group at C-6 and the shift of the signal corresponding to the H-6 proton from δ_H 5.60 in **1** to δ_H 4.42 in **2**. Detailed assignments of the ^1H and ^{13}C NMR data and the relative configuration were determined on the basis of COSY, HSQC, ROESY and HMBC experiments. The absolute configuration of **2** was established by chemical correlation. When compound **2** was treated with nicotinoyl chloride yielded a compound whose spectroscopic data were identical to those of **1**.

Compound **3** was obtained as a colorless lacquer. Its molecular formula was established as $C_{26}H_{34}O_9$ by EI-HRMS and ^{13}C NMR data. The IR absorption bands at 3466 and 1731 cm^{-1} indicated hydroxyl and ester functions, and the UV spectrum revealed the occurrence of aromatic ring absorptions at 224 and 274 nm. This was confirmed by the ^1H and ^{13}C NMR spectra (Table 2), which included signals of five aromatic protons (δ_H 7.48–8.10), two acetate methyl groups (δ_H 1.80, 2.16) and three carboxyl groups at δ_C 169.5, 170.0 and 170.4, assigned to a benzoyl and two acetyl groups. In addition, two signals at δ_H 2.88 (1H, s, OH-4) and 4.35 (1H, s, OH-8) interchangeable with D_2O, indicating the presence of two hydroxyl groups, a methyl group at δ_C 1.34 attached to an oxygen bearing carbon at δ_C 72.4, and three methyls at δ_H 1.55, 1.49 and δ 1.41, were also observed. The regiosubstitution was established by the long-range ^1H–13C HMBC couplings observed between the δ_H 5.36 (dd, J = 4.5, 12.0 Hz, H-1) and 5.72 (s, H-6) proton resonances and the signals of the acetate groups at δ_C 170.0 and 170.4, respectively, whereas the signal at δ_H 5.29 (d, J = 6.2 Hz, H-9) was coupled to the signal of the benzoate group (δ_C 169.5). The observed cross-peaks between H-1 and H-3β and between Me-15 and H-6, H-8, H-9 and Me-14 in a ROESY experiment assigned the relative configuration of **3** as 1α, 4β, 6β, 8β and 9β. The absolute configuration was established as (1*S*,4*S*,5*S*,6*R*,7*R*,8*S*,9*R*,10*S*)-1,6-diacetoxy-9-benzoyloxy-4,8-dihydroxy-dihydro-β-agarofuran through chemical correlation, as treatment of **3** with *trans*-cinnamoyl chloride yielded a compound with spectroscopic data identical to the known compound **13**, whose absolute stereochemistry has been previously established.[7]

Compound **4** had the same molecular formula and substitution patterns as **17**[12] as shown by their spectroscopic data. Thus, careful comparison of their ^1H NMR data (Table 2) indicated the main differences were the chemical shift, multiplicity and coupling constants assigned to the methine protons H-8 (δ_H 5.34 d, $J_{7,8}$ 3.0 Hz in **4** instead of δ_H 5.74 dd, $J_{7,8}$ 2.8 Hz, $J_{8,9}$ 6.3 Hz in **17**) and H-9 (δ_H 5.15 s in **4** instead of δ_H 5.39 d, $J_{8,9}$ 6.3 Hz in **17**). The multiplicity of the H-9 resonance in compound **4** indicated a dihedral angle very close to 90°, which is only possible for a H-8β, H-9α relative stereochemistry in the dihydro-β-agarofuran skeleton. This was confirmed by molecular mechanic analysis[13] and the ROE correlation observed between H-8 and Me-13 in a ROESY experiment. Even so, it should be noted that a complete set of 2D NMR spectra (COSY, HSQC, HMBC) was acquired in order to gain the complete and unambiguous assignment of the ^1H and ^{13}C NMR resonances as listed in Table 2. Taking into account biosynthetic considerations, the absolute configuration of compound **4** can be proposed because the only difference with compound **17**[12] is the epimerization at C-8.

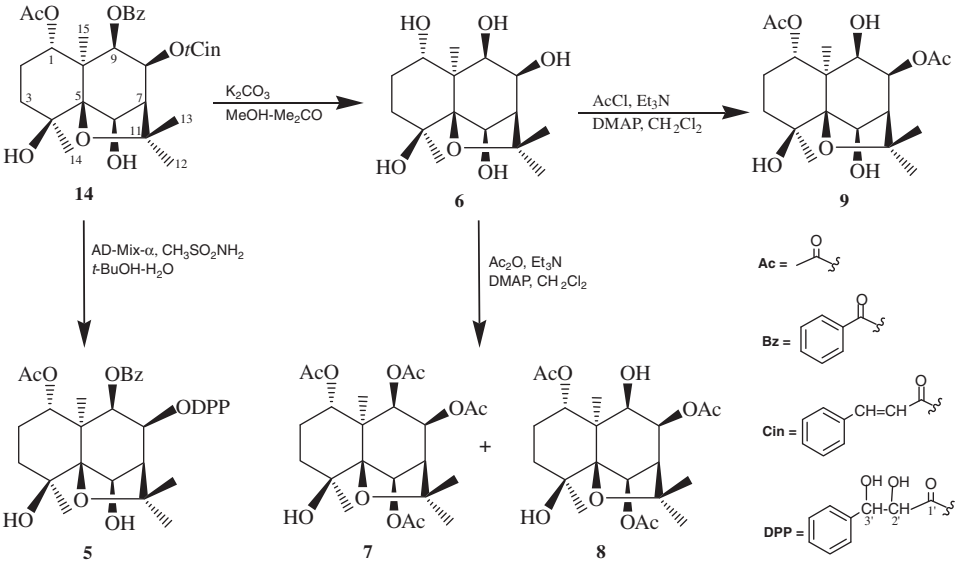
Derivatives **5**–**9** were obtained, partly to provide support for their chemical structures, as well as to learn about the bioactivity changes resulting from altering the parent compound. The natural sesquiterpene **14**[7] was used as the starting material and was transformed into derivative **5** by stereoselective Sharpless dihydroxylation, using AD-mix-α as the oxidant. Furthermore, the absolute configurations at C-2′ and C3′ were assigned as *R* and *S*, respectively, by applying the Sharpless mnemonic device for asymmetric dihydroxylation.[14] The basic hydrolysis of **14** with K_2CO_3 in MeOH–Me_2CO resulted in the formation of polyol **6**. Acetylated derivatives **7**–**9** were prepared by treatment of **6** with acetic anhydride or acetyl chloride (Scheme 1).

The structures of these derivatives were established on the basis of detailed spectroscopic analysis and comparison with those reported for **14**.[7] Furthermore, the structure of **6** was confirmed by a single-crystal X-ray diffraction experiment of a crystal obtained from $CDCl_3$ (Fig. 3). Subsequent structure elucidation and refinement led to an unambiguous determination of the absolute configuration of **6**. Its crystallographic data reveal that the torsion angles obtained correspond to a decalin system with a *trans* union between rings A and B, which adopt a chair conformation, whereas the furan ring presented an envelope conformation as expected. Thus, the stereostructure of this semisynthetic compound was established as (1*S*,4*S*,5*S*,6*R*,7*R*,8*S*,9*R*,10*S*)-1,4,6,8,9-pentahydroxy-dihidro-β-agarofuran, which confirms the absolute configuration of those sesquiterpenes with a 4β,8β-dihydroxycelorbicol polyhydroxy sesquiterpenoid core[15] previously established by CD

Table 2
^1H and ^{13}C NMR (δ, $CDCl_3$, J in Hz in parentheses) data of compounds **3** and **4**

Position	**3**		**4**	
	δ_H	δ_C[a]	δ_H	δ_C[a]
1	5.36 dd (4.5, 12.0)	75.1 d	5.35 dd (3.9, 12.1)	72.4 d
2	1.45 m*, 1.97 m	22.9 t	1.46 m, 1.90 m	22.4 t
3	1.64 m, 1.91 m	38.3 t	1.73 m, 1.98 m	38.5 t
4		72.4 s		70.4 s
5		90.9 s		91.8 s
6	5.72 s	77.8 d	6.07 s	76.4 d
7	2.46 d (3.1)	54.8 d	2.66 d (3.0)	52.8 d
8	4.60 m	70.2 d	5.34 d (3.0)	75.4 d
9	5.29 d (6.2)	75.0 d	5.15 s	76.2 d
10		49.4 s		50.7 s
11		84.5 s		83.4 s
12	1.55 s	30.4 q	1.59 s	29.8 q
13	1.49 s	26.2 q	1.61 s	25.2 q
14	1.34 s	23.7 q	1.42 s	23.7 q
15	1.41 s	19.7 q	1.60 s	19.2 q
OAc-1	1.80 s	21.3q*, 170.0 s	1.64 s	20.5 q, 169.6 s
OAc-6	2.16 s	21.3q*, 170.4 s		
OAc-8			2.33 s	21.0 q, 169.3 s

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



Scheme 1. Preparation of derivatives **5**–**9** from the natural sesquiterpene **14**.

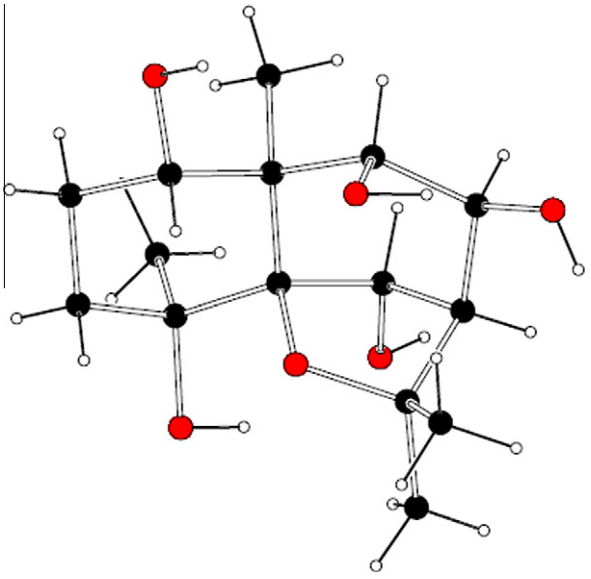


Figure 3. A view of the molecular structure of **6**. The ellipsoids are drawn at the 30% probability level.

studies.[7] This result has a special significance, as to date, there are few examples of the absolute configuration of related sesquiterpenes determined by X-ray, the most recent being those given for boariol[16] and a 3,12-oxygenated dihydro-β-agarofuran.[17] However, this is the first X-ray report of a 4β,8β-dihydroxy-celorbicol-related sesquiterpenoid.

So far, few reports concerning the biological evaluation of dihydro-β-agarofuran sesquiterpenes on *M. tuberculosis* drug-sensitive strains have been published.[9–11] Moreover, there are not any reports on the activity of this type of compounds against drug-resistant *M. tuberculosis* strains.

The biological activity in vitro of the isolated compounds (**1**–**4**) and their derivatives (**5**–**9**), along with the previously isolated sesquiterpenes **10–25**[7,12] (Fig. 1 and Scheme 1), was determined by their ability to inhibit the growth of *M. tuberculosis* (MTB) drug-sensitive and multidrug-resistant (MDR TB) strains using the tetrazolium microplate assay (TEMA) method.[18] This assay determines MIC values as quickly and accurately as the more expensive MABA procedure.[19] The clinically used antituberculosis agents, isoniazid and rifampicin, were used as positive controls. The antituberculosis activity data are shown in Table 3. All the assayed sesquiterpenes were inactive against the MTB $H_{37}R_v$ drug-sensitive strain with MIC values over 25 μg/mL (data not shown). Surprisingly, six of the compounds showed moderate activity against the MDR TB strain, with MIC values of 12.5 μg/mL. Moreover, compound **20** showed the more potent antimycobacterial activity against the resistant strain with a MIC value of 6.2 μg/mL (11.9 μM) comparable or better than the positive controls used, isoniazid (MIC 4 μg/mL, 29.1 μM) and rifampicin (MIC >16.0 μg/mL, >19.4 μM).

In general, we observed some trends on the preliminary structure–activity relationship of this series of sesquiterpenes that seem to be important for high anti-MDR-TB activity in this class of compounds: (a) the overall esterification level of the compound; (b) the presence of two aromatic ester moieties; (c) the regiosubstitution of

Table 3
Anti-*Mycobacterium tuberculosis* activity[a] of compounds **1**–**25**[b]

Compound	MDR-TB		Compound	MDR-TB	
	MIC[c] (μg/mL)	MIC (μM)		MIC[c] (μg/mL)	MIC (μM)
1	25	43.1	15	>25	>60
2	>25	>60	16	>25	>60
3	>25	51.0	17	>25	>60
4	12.5	21.0	18	>25	>60
5	>25	>60	19	>25	>60
6	>25	>60	20	6.2	11.9
7	>25	>60	21	12.5	25.8
8	>25	>60	22	25	56.6
9	>25	>60	23	>25	>60
10	12.5	21.6	24	>25	>60
11	12.5	22.2	25	>25	>60
12	25	44.4	isoniazid[d]	4	29.1
13	12.5	20.1	rifampicin[d]	>16	>19.4
14	12.5	21.6			

[a] Data were means of 3 or 4 replicates.
[b] Compounds **1**–**25** showed a MIC value >25 μg/mL against the sensitive $H_{37}Rv$ strain.
[c] MIC values is the minimum inhibitory concentration of the tested compounds effecting 100% of inhibition.
[d] Isoniazid and rifampicin were used as positive controls, with MICs values against the $H_{37}Rv$ strain of 0.125 and 0.063 μg/mL, respectively.

the molecule. Thus, compounds which contain an ester group on C-6 were more potent than those with an hydroxyl group (**21** vs **22**). Moreover, the presence of a benzoyl group instead of a nicotynoyl group at position C-6 significantly improve the activity, as can be seen in compounds **4** versus **19** or **10** versus **1**, while the absence of functionalization at C-6 causes a loss of activity (**21** vs **23**). In addition, the presence of a hydroxyl group at C-2 produces a decrease in activity, as seen from the lower activity of compound **25** compared to **14**. The substituent at C-8 also proved to be important for the activity. Thus, compounds with a *trans*-cinnamate ester group (**13** and **14**) are more active than those with a dihydroxyphenyl-propanoate (**5**), a *cis*-cinnamate (**16**) or a hydroxyl group (**3**).

We can conclude that compound **20**, with the basic polyhydroxy skeleton of celorbicol,[15] is structurally quite different from known antitubercular agents, and its activity on the MDR-TB strain falls into the range of the clinically used anti-TB drugs, isoniazid and rifampicin. Thus, our results warrant further investigation to improve the observed activity and elucidate the mechanism of the anti-MDR-TB potential of this class of compounds.

3. Experimental

3.1. General procedures

Optical rotations were measured on a Perkin–Elmer 241 automatic polarimeter in $CHCl_3$ at 25 °C and the $[\alpha]_D$ values are given in 10^{-1} deg cm^2 g^{-1}. UV spectra were obtained in absolute EtOH on a JASCO V-560 instrument. IR (film) spectra were measured in $CHCl_3$ on a Bruker IFS 55 spectrophotometer. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectra were recorded on a Bruker Avance 400 spectrometer; chemical shifts were referred to the residual solvent signal ($CDCl_3$: δ_H 7.26, δ_C 77.0); DEPT, COSY, TOCSY (spin lock field 8000 Hz), ROESY (spin lock field 2500 Hz), HSQC and HMBC (optimized for J = 7.7 Hz) experiments were carried out with the pulse sequences given by Bruker. EI-MS and EI-HRMS were recorded on a Micromass Autospec spectrometer. Silica gel 60 (15–40 μm) for column chromatography, silica gel 60 F_{254} for TLC, and nanosilica gel 60 F_{254} for preparative HPTLC were purchased from Macherey-Nagel, and Sephadex LH-20 was obtained from Pharmacia Biotech. The spots were visualized by UV light and heating silica gel plates sprayed with H_2O/H_2SO_4/AcH (1:4:20). All solvents used were analytical grade from Panreac. Reagents were purchased from Sigma-Aldrich and used without further purification.

3.2. Plant material

C. vulcanicola J. Donnell Smith, collected in June 2004 at the Montecristo National Park, province of Santa Ana, El Salvador, was identified by Jorge Monterrosa, and a voucher specimen (J. Monterrosa & R. Carballo 412) is deposited in the Herbarium of Missouri Botanical Garden, USA. The dried leaves (1.5 kg) of *C. vulcanicola* were sliced into chips, extracted with EtOH in a Soxhlet apparatus, and concentrated under reduced pressure. The EtOH extract (311.0 g) was partitioned into CH_2Cl_2/H_2O (1:1, v/v) solution. Removal of the CH_2Cl_2 from the organic fraction under reduced pressure yielded 74.8 g of residue, which was chromatographed by vacuum liquid chromatography (VLC) on a silica gel column chromatography using mixtures of *n*-hexane–EtOAc of increasing polarity as eluent to afford seven fractions (A–G). Fraction D was subjected to column chromatography over Sephadex LH-20 using mixtures of CH_2Cl_2/MeOH (1:1) to provide two subfractions (D1 and D2). Fraction D2 was further purified by silica gel column chromatography, and preparative TLC developed with CH_2Cl_2/Me_2CO (9:1) to yield the new compounds **1** (8.8 mg), **2** (21.1 mg), **3** (12.1 mg) and **4** (6.0 mg).

3.2.1. (1*S*,4*R*,5*S*,6*R*,7*R*,8*S*,9*R*,10*S*)-1,8-Diacetoxy-9-benzoyloxy-6-nicotinoyloxy-dihydro-β-agarofuran (1)

Colorless lacquer; $[\alpha]_D^{25}$ −10.8 (*c* 0.1, $CHCl_3$); UV λ_{max} (EtOH) (log ε) 264 (1.4), 225 (5.1) nm; IR ν_{max} (film) 2923, 2853, 1729, 1456, 1368, 1275, 1222, 1106, 1025, 772, 670 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ OBz, ONic [7.50 (3H, m), 7.60 (1H, m), 8.11 (2H, m), 8.33 (1H, m), 8.85 (1H, m), 9.26 (1H, s)], for other signals, see Table 1; ^{13}C NMR ($CDCl_3$) δ OBz, ONic [123.4 (d) 125.4 (s), 128.1 (2× d), 129.1 (s), 130.0 (2× d), 133.1 (d), 136.9 (d), 150.6 (d), 153.8 (d)], 163.9 (s, ONic-6), 165.4 (s, OBz-9), for other signals, see Table 1; EI-MS *m/z* (%) 579 [M]$^+$ (15), 564 (13), 519 (5), 458 (14), 456 (2), 400 (7), 339 (3), 245 (13), 106 (38), 105 (100), 77 (14); EI-HRMS *m/z* 579.2457 (calcd for $C_{32}H_{37}NO_9$, 579.2468).

3.2.2. (1*S*,4*R*,5*S*,6*R*,7*S*,8*S*,9*R*,10*S*)-1,8-Diacetoxy-9-benzoyloxy-6-hydroxy-dihydro-β-agarofuran (2)

Colorless lacquer; $[\alpha]_D^{25}$ −21.4 (*c* 0.2, $CHCl_3$); UV λ_{max} (EtOH) (log ε) 274 (1.0), 230 (9.8) nm; IR ν_{max} (film) 3504, 2923, 2852, 1721, 1456, 1367, 1279, 1232, 1104, 1028, 960, 712, 670 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ OBz [7.45 (2H, m), 7.56 (1H, m), 8.08 (2H, m)], for other signals, see Table 1; ^{13}C NMR ($CDCl_3$) δ OBz-9 [128.0 (2× d), 129.2 (s), 130.0 (2× d), 133.0 (d), 165.6 (s)], for other signals, see Table 1; EI-MS *m/z* (%) 474 [M]$^+$ (3), 459 (16), 414 (6), 399 (8), 353 (4), 339 (8), 251 (12), 149 (13), 105 (100), 83 (30), 77 (18); EI-HRMS *m/z* 474.2261 (calcd for $C_{26}H_{34}O_8$, 474.2254).

3.2.3. (1*S*,4*S*,5*S*,6*R*,7*R*,8*S*,9*R*,10*S*)-1,6-Diacetoxy-9-benzoyloxy-4,8-dihydroxy-dihydro-β-agarofuran (3)

Colorless lacquer; $[\alpha]_D^{25}$ −7.5 (*c* 0.1, $CHCl_3$); UV λ_{max} (log ε) (EtOH) 274 (0.6), 224 (3.8) nm; IR ν_{max} (film) 3466, 2928, 1731, 1369, 1276, 1234, 1101, 1029, 966, 756, 716 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.88 (1H, s, OH-4), 4.35 (1H, s, OH-8), OBz [7.48 (2H, m), 7.60 (1H, m), 8.10 (2H, m)], for other signals, see Table 2; ^{13}C NMR ($CDCl_3$) δ OBz-9 [128.1 (2× d), 130.0 (s), 130.6 (2× d), 133.4 (d), 169.5 (s)], for other signals, see Table 2; EI-MS *m/z* (%) 475 [M$^+$−15] (6), 457 (3), 430 (3), 353 (17), 293 (4), 251 (4), 233 (5), 202 (10), 166 (8), 105 (100), 77 (15), EI-HRMS *m/z* 475.1958 [M−CH_3]$^+$ (calcd for $C_{25}H_{31}O_9$, 475.1968).

3.2.4. (1*S*,4*S*,5*S*,6*R*,7*R*,8*S*,9*R*,10*S*)-1,8-Diacetoxy-9,6-dibenzoyloxy-4-hydroxy-dihydro-β-agarofuran (4)

Colorless lacquer; $[\alpha]_D^{25}$ −4.9 (*c* 0.4, $CHCl_3$); UV λ_{max} (EtOH) (log ε) 230 (13.9) nm; IR ν_{max} (film) 3562, 2921, 2852, 1741, 1721, 1271, 1232, 1095, 1028, 713 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 3.08 (1H, s, OH-4), OBz [7.49 (4H, m), 7.60 (2H, m), 8.08 (2H, m), 2.22 (2H, m)], for other signals, see Table 2; ^{13}C NMR ($CDCl_3$) δ OBz [128.2 (2× d), 128.4 (2× d), 128.6 (s), 129.8 (s), 129.9 (4× d), 133.0 (d), 133.5 (d)], 164.6 (s, OBz-9), 165.6 (s, OBz-6), for other signals, see Table 2; EI-MS *m/z* (%) 594 [M]$^+$ (9), 579 (1), 534 (5), 472 (3), 457 (2), 384 (1), 270 (5), 167 (1), 149 (4), 105 (100), 77 (11); EI-HRMS *m/z* 594.2437 (calcd for $C_{33}H_{38}O_{10}$, 594.2465).

3.3. Chemical correlations

A mixture of nicotinoyl chloride hydrochloride (50 mg, 0.28 mmol), triethylamine (6 drops), compound **2** (3.5 mg, 0.07 mmol) and a catalytic amount of 4-dimethylamino-pyridine in dry dichloromethane (2 mL) was refluxed for 16 h. The mixture was evaporated to dryness, and the residue was purified by preparative TLC using *n*-hexane/EtOAc (1:1) to give compound **1** (2.0 mg, 47%). Moreover, compound **3** (2.9 mg, 0.06 mmol) was dissolved in dry pyridine (0.5 mL), and *trans*-cinnamoyl chloride (35 mg, 0.23 mmol) and some crystals of 4-dimethylamino-pyridine were added under argon atmosphere. The mixture was heated to 60 °C for 15 h, poured over H_2O and extracted with EtOAc. The organic phases were combined, dried over anhydrous $MgSO_4$ and

evaporated under reduced pressure. The residue was purified on preparative TLC with a mixture of *n*-hexane/EtOAc (1:1) to give compound **13**[7] (1.8 mg, 48%).

3.4. Preparation of 5

AD-mix-α (51 mg) and methanesulfonamide (6.3 mg, 0.07 mmol) were dissolved in a solvent mixture of *t*-BuOH (1.5 mL) and H_2O (1.5 mL). The resulting mixture was stirred at room temperature for 5 min, then it was cooled to 0 °C and a solution of compound **14**[7] (20 mg, 0.04 mmol) in dichloromethane was added. Subsequently, a 10% $Na_2S_2O_3$ solution was added to quench the reaction, and the mixture was extracted with EtOAc. The organic phases were combined, dried ($MgSO_4$) and evaporated. The residue was purified by preparative TLC using *n*-hexane/EtOAc (1:1) to afford **5** (6.3 mg, 25%).

3.4.1. (1*S*,4*S*,5*S*,6*R*,7*S*,8*S*,9*R*,10*S*)-1-Acetoxy-9-benzoyloxy-8-[2*R*,3*S*-dihydroxy-3-phenyl]-propanoil-oxy-4,6-dihydroxy-dihydro-β-agarofuran (5)

Colorless lacquer; $[\alpha]_D^{25}$ −8.8 (*c* 0.5, $CHCl_3$); UV λ_{max} (log ε) (EtOH) 230 (2.0) nm; IR ν_{max} (film) 3351, 2926, 1734, 1453, 1369, 1320, 1280, 1152, 1103, 1037, 984, 772, 717 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.42 (3H, s, Me-15), 1.48 (1H, m, H-2β), 1.55 (6H, s, Me-12, Me-13), 1.62 (3H, s, Me-14), 1.65 (3H, s, OAc-1), 1.74 (1H, m, H-3α), 1.95 (1H, m, H-3β), 2.02 (1H, m, H-2α), 2.40 (1H, d, *J* = 3.0 Hz, H-7), 2.84 (1H, s, OH-3′), 3.05 (1H, s, OH-2′), 3.18 (1H, s, OH-4), 4.09 (1H, m, H-2′), 4.49 (1H, d, *J* = 5.2 Hz, H-6), 4.81 (1H, m, H-3′), 5.13 (1H, d, *J* = 5.2 Hz, OH-6), 5.33 (1H, d, *J* = 6.4 Hz, H-9), 5.38 (1H, dd, *J* = 4.1, 11.9 Hz, H-1), 5.51 (1H, dd, *J* = 3.0, 6.4 Hz, H-8), OBz, ODPP [7.15 (2H, dd, *J* = 1.7, 6.5 Hz), 7.28 (2H, m), 7.51 (3H, m), 7.65 (1H, m), 8.08 (2H, m)]; ^{13}C NMR ($CDCl_3$) δ 19.5 (q, C-15), 20.4 (q, OAc-1), 22.8 (t, C-2), 23.5 (q, C-14), 26.5 (q, C-13), 30.2 (q, C-12), 36.8 (t, C-3), 48.6 (s, C-10), 54.5 (d, C-7), 70.6 (d, C-8), 71.8 (d, C-1), 72.1 (d, C-9), 72.8 (s, C-4), 73.9 (d, C-3′), 74.9 (d, C-2′), 77.9 (d, C-6), 84.4 (s, C-11), 90.7 (s, C-5), ODPP, OBz [125.9 (2× d), 127.8 (d), 128.2 (2× d), 128.3 (2× d), 128.4 (s), 130.0 (2× d), 133.7 (d), 139.3 (s)], 166.1 (s, OBz-9), 169.6 (s, OAc-1), 171.2 (s, C-1′); EI-MS *m/z* (%) 597 [M^+−15] (1), 579 (3), 506 (8), 475 (5), 446 (15), 249 (4), 233 (3), 202 (9), 191 (6), 105 (100), 83 (10), 77 (15); EI-HRMS *m/z* 597.2330 [M−CH_3]$^+$ (calcd for $C_{32}H_{37}O_{11}$, 597.2336).

3.5. Preparation of 6

A mixture of **14** (205 mg, 0.4 mmol) and K_2CO_3 (300 mg) in a solvent mixture of MeOH (20 mL) and acetone (12 mL) was refluxed for 33 h, until TLC showed complete conversion. The mixture was filtered and the solution was concentrated under reduced pressure. The residue was further purified by flash column chromatography on silica gel (2 × 30 cm, 10 g) using *n*-hexane/EtOAc (1:4) to give a crystalline compound, **6** (71.1 mg, 59%).

3.5.1. (1*S*,4*S*,5*S*,6*R*,7*R*,8*S*,9*R*,10*S*)-1,4,6,8,9-Pentahydroxy-dihydro-β-agarofuran (6)

Colorless orthorhombic crystals; $[\alpha]_D^{25}$ −20.1 (*c* 0.4, $CHCl_3$); recrystallization solvent: $CDCl_3$; mp 180.8–181.2 °C; IR ν_{max} (film) 3399, 2924, 2853, 1458, 1389, 1216, 1140, 1039 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.13 (3H, s, Me-15), 1.54 (3H, s, Me-14), 1.56 (3H, s, Me-12), 1.62 (2H, m, H-2), 1.70 (2H, m, H-3), 1.73 (3H, s, Me-13), 2.48 (1H, d, *J* = 3.2 Hz, H-7), 3.07 (1H, d, *J* = 5.4 Hz, OH-9), 3.18 (1H, s, OH-4), 3.71 (1H, t, *J* = 5.4 Hz, H-9), 4.09 (1H, dd, *J* = 3.2, 5.4 Hz, H-8), 4.22 (1H, d, *J* = 5.2 Hz, H-6), 4.37 (1H, dd, *J* = 4.0, 12.0 Hz, H-1), 5.00 (1H, d, *J* = 5.2 Hz, OH-6); ^{13}C NMR ($CDCl_3$) δ 18.2 (c, C-15), 23.6 (c, C-14), 26.2 (t, C-2), 27.0 (c, C-13), 30.5 (c, C-12), 37.5 (t, C-3), 49.0 (s, C-10), 56.7 (d, C-7), 68.9 (d, C-1), 70.4 (d, C-8), 73.0 (s, C-4), 74.9 (d, C-9), 78.1 (d, C-6), 83.9 (s, C-11), 91.4 (s, C-5); EI-MS *m/z* (%) 287 [M^+−15] (100), 233 (11), 205 (10), 191 (10), 166 (32), 151 (21), 137 (12), 125 (19), 109 (27), 98 (39), 85 (38); EI-HRMS *m/z* 287.1505 [M−CH_3]$^+$ (calcd for $C_{14}H_{23}O_6$, 287.1495).

3.6. X-ray crystal structure analysis

Compound **6** crystallizes as a hemihydrate with the oxygen of the water molecule lying on a binary crystallographic axis of symmetry. Intensity data were collected at room temperature on an Enraf-Nonius κCCD diffractometer with Mo Kα radiation (λ = 0.71707 Å). Cell refinement and data reduction were performed with COLLECT[20] and DENZO.[21] The structure was determined by direct methods using SIR97.[22] Refinements were performed with SHELXL-97,[23] using full-matrix least squares with anisotropic displacement parameters for all the non-hydrogen atoms.[24] All the H-atoms were located in successive difference-Fourier synthesis and isotropically refined, with the exception of those corresponding to O4, O5 and the oxygen of the water co-crystallization molecule, which were added as a fixed isotropic contribution. Molecular graphics were computed with PLATON.[25]

X-ray crystal data: $C_{15}H_{26}O_6$. 0.5 (H_2O), Mw = 311.4, orthorhombic, space group $P2_12_12$, $Z = 4$, $a = 10.748(2)$, $b = 17.444(5)$, $c = 7.787(7)$ Å, $V = 1460.0(14)$ Å^3, μ (Mo Kα) = 0.09 mm^{-1}, ρ_{calcd} = 1.42 g cm^{-3}; $S = 1.07$, final R indices: $R_1 = 0.034$ and $R_w = 0.089$ for 1668 observed from 1781 independent and 8031 measured reflections ($\theta_{max} = 27.0$, $I > 2\sigma(I)$ criterion and 291 parameters); maximum and minimum residues are 0.28 and −0.17 e Å^{-3}, respectively.

Crystallographic data (excluding structure factor tables) for the structure reported has been deposited with the Cambridge Crystallographic Data Centre as supplementary publications no. 778506. Copies of the data can be obtained free of charge on application to the Director, CCDC, 12 Union Road, Cambridge CB 1EZ, UK (fax: Int. +44 1223 336 033); e-mail: deposit@ccdc.cam.ac.uk.

3.7. Acetylation of 6

A mixture of acetic anhydride (55 mg, 0.79 mmol), triethylamine (0.3 mL), compound **6** (64.5 mg, 0.21 mmol), and 4-dimethylamino-pyridine (12.0 mg) in dichloromethane (12.0 mL) was stirred at rt for 16 h. The mixture was evaporated to dryness, and the residue was purified by preparative TLC using CH_2Cl_2-Me_2CO (9:1) to give compounds **7** (21.3 mg, 24%) and **8** (15.6 mg, 19%). Furthermore, a mixture of acetyl chloride (0.13 mL, 1.92 mmol), triethylamine (0.13 mL, 0.96 mmol), compound **6** (71.0 mg, 0.24 mmol), and 4-dimethylamino-pyridine (12 mg) in dichloromethane (5.0 mL) was stirred at rt for 24 h. Then an aqueous solution of HCl 0.1 N (2 mL) was added, and the residue was extracted three times with EtOAc (2 mL). The collected organic layers were dried over anhydrous $MgSO_4$ and evaporated under reduced pressure giving a thick oil, which was purified by preparative TLC using *n*-hexane/diethylether (1:3) to yield **9** (46.2 mg, 50%).

3.7.1. (1*S*,4*S*,5*S*,6*R*,7*R*,8*S*,9*R*,10*S*)-1,6,8,9-Tetra-acetoxy-4-hydroxy-dihydro-β-agarofuran (7)

Colorless lacquer; $[\alpha]_D^{25}$ −32.4 (*c* 0.15, $CHCl_3$); IR ν_{max} (film) 3554, 2928, 1739, 1368, 1234, 1175, 1054, 968, 875 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.34 (3H, s, Me-14), 1.38 (3H, s, Me-15), 1.43 (1H, m, H-2α), 1.55 (3H, s, Me-12), 1.65 (1H, m, H-3α), 1.66 (3H, s, Me-13), 1.82 (1H, m, H-3β), 1.84 (1H, m, H-2β), 1.95 (3H, s, OAc-1), 2.00 (3H, s, OAc-8), 2.07 (3H, s, OAc-9), 2.14 (3H, s, OAc-6), 2.41 (1H, d, *J* = 3.0 Hz, H-7), 2.80 (1H, s, OH-4), 5.05 (1H, d, *J* = 6.1 Hz, H-9), 5.29 (1H, dd, *J* = 4.0, 12.1 Hz, H-1), 5.47 (1H, dd, *J* = 3.0, 6.1 Hz, H-8), 5.48 (1H, s, H-6); ^{13}C NMR ($CDCl_3$) δ 19.0 (q,

C-15), 20.5 (q, OAc-8), 20.8 (q, OAc-9), 20.9 (q, OAc-1), 21.2 (q, OAc-6), 23.4 (q, C-14), 23.5 (t, C-2), 25.6 (q, C-13), 30.1 (q, C-12), 38.1 (t, C-3), 49.1 (s, C-10), 53.4 (d, C-7), 68.8 (d, C-8), 70.1 (s, C-4), 71.3 (2× d, C-1, C-9), 77.1 (d, C-6), 84.2 (s, C-11), 91.0 (s, C-5), 168.9 (s, OAc-8), 169.8 (s, OAc-6), 169.9 (s, OAc-9), 170.2 (s, OAc-1); EI-MS *m/z* (%) 470 $[M]^+$ (1), 455 (36), 428 (11), 410 (39), 395 (35), 368 (34), 353 (20), 308 (22), 248 (41), 208 (54), 191 (50), 166 (56), 140 (89), 109 (43), 98 (75), 83 (100); EI-HRMS *m/z* 470.2176 (calcd for $C_{23}H_{34}O_{10}$, 470.2152).

3.7.2. (1*S*,4*S*,5*S*,6*R*,7*R*,8*S*,9*R*,10*S*)-1,6,8-Triacetoxy-4,9-dihydroxy-dihydro-β-agarofuran (8)

Colorless lacquer; $[\alpha]_D^{25}$ −58.0 (*c* 0.8, $CHCl_3$); IR ν_{max} (film) 3535, 2940, 1737, 1368, 1235, 1046, 966 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.34 (6H, s, Me-14, Me-15), 1.56 (3H, s, Me-12), 1.68 (1H, m, H-3α), 1.74 (3H, s, Me-13), 1.78 (1H, m, H-3β), 1.81 (1H, m, H-2α), 1.88 (1H, m, H-2β), 2.04 (3H, s, OAc-1), 2.12 (3H, s, OAc-8), 2.13 (3H, s, OAc-6), 2.49 (1H, d, *J* = 3.0 Hz, H-7), 2.55 (1H, d, *J* = 10.3 Hz, OH-9), 2.63 (1H, s, OH-4), 3.76 (1H, dd, *J* = 5.4, 10.3 Hz, H-9), 5.23 (1H, dd, *J* = 3.0, 5.4 Hz, H-8), 5.31 (1H, dd, *J* = 4.1, 11.9 Hz, H-1), 5.46 (1H, s, H-6); ^{13}C NMR ($CDCl_3$) δ 18.8 (q, C-15), 20.8 (q, OAc-1), 21.1 (q, OAc-6), 21.2 (q, OAc-8), 23.4 (q, C-14), 23.5 (t, C-2), 26.2 (q, C-13), 29.9 (q, C-12), 38.3 (t, C-3), 49.1 (s, C-10), 53.6 (d, C-7), 70.1 (d, C-4), 71.3 (d, C-1), 71.7 (d, C-8), 72.8 (d, C-9), 77.3 (d, C-6), 85.2 (s, C-11), 92.8 (s, C-5), 169.6 (s, OAc-8), 169.8 (s, OAc-6), 170.0 (s, OAc-1); EI-MS *m/z* (%) 413 $[M^+-15]$ (20), 386 (12), 368 (17), 353 (31), 308 (35), 207 (42), 166 (70), 149 (45), 109 (78), 83 (100); EI-HRMS *m/z* 413.1840 (calcd for $C_{20}H_{29}O_9$, 413.1812).

3.7.3. (1*S*,4*S*,5*S*,6*R*,7*S*,8*S*,9*R*,10*S*)-1,8,-Diacetoxy-4,6,9-trihydroxy-dihydro-β-agarofuran (9)

Colorless lacquer; $[\alpha]_D^{25}$ −35.0 (*c* 0.2, $CHCl_3$); IR ν_{max} (film) 3434, 2934, 1728, 1438, 1368, 1244, 1106, 1037, 965 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.24 (3H, s, Me-15), 1.55 (6H, s, Me-13, Me-14), 1.52 (1H, m, H-2α), 1.63 (1H, m, H-3α), 1.73 (3H, s, Me-12), 1.77 (1H, m, H-3β), 1.88 (1H, m, H-2β), 2.02 (3H, s, OAc-1), 2.10 (3H, s, OAc-8), 2.40 (1H, d, *J* = 7.4 Hz, OH-9), 2.49 (1H, d, *J* = 2.9 Hz, H-7), 3.10 (1H, s, OH-4), 3.69 (1H, dd, *J* = 5.6, 7.4 Hz, H-9), 4.39 (1H, d, *J* = 5.4 Hz, H-6), 4.98 (1H, d, *J* = 5.4, OH-6), 5.03 (1H, dd, *J* = 2.9, 5.6 Hz, H-8), 5.34 (1H, dd, *J* = 4.4, 12.0 Hz, H-1); ^{13}C NMR ($CDCl_3$) δ 19.0 (q, C-15), 20.9 (q, OAc-8), 21.1 (q, OAc-1), 23.3 (q, C-14), 23.4 (t, C-2), 27.1 (q, C-13), 30.5 (q, C-12), 36.7 (t, C-3), 48.2 (s, C-10), 54.7 (d, C-7), 71.2 (d, C-1), 72.1 (d, C-8), 72.7 (s, C-4), 73.0 (d, C-9), 77.9 (d, C-6), 85.0 (s, C-11), 92.1 (s, C-5), 169.7 (s, OAc-8), 170.0 (s, OAc-1); EI-MS *m/z* (%) 371 $[M^+-15]$ (40), 353 (6), 311 (23), 308 (31), 293 (24), 233 (20), 207 (22), 191 (23), 156 (27), 109 (100), 98 (94), 83 (76); EI-HRMS *m/z* 371.1710 (calcd for $C_{18}H_{27}O_8$, 371.1706).

3.8. Antimycobacterial activity in vitro

Bioassays were conducted on sensitive $H_{37}Rv$ ATCC 27294 (American Type Culture Collection) and multidrug-resistant (clinical isolate, strain 02TBDM039EP097) *M. tuberculosis* strains. For the preparation of the inoculums, a suspension of MTB was prepared by mixing growth from slants (20–30-d-old) with 100 μL of Tween 80 into 0.2% (BSA; Sigma Chemical Co., St. Louis, MO). Turbidity of the suspension was then adjusted to a McFarland standard No. 1 (3×10^7 CFU/mL) by adding Tween 80 and BSA. The bacterial suspension (300 mL) was further transferred to 7.2 mL of 7H9GC broth (4.7 g of Middlebrook 7 H9 broth base (Difco, Detroit, MI), 20 mL of 10% glycerol, 1 g of Bacto Casitone (Difco), 880 mL of distilled H_2O, 100 mL of OADC (oleic acid, albumin, dextrose, catalase; Remel, Lenexa, KS)). For the bioassay, the compounds were resuspended in DMSO at a concentration of 1 mg/mL (stock soln.). These stock solutions were further diluted with appropriate volumes of 7H9GC broth to yield final concentrations of 0.1–50 μg/mL. Final drug concentration ranges of standard antibiotics used as positive controls were 0.125–32 μg/mL for isoniazid and 0.063–16 μg/mL for rifampin (Sigma Chemical Co., St. Louis, MO). The standard drugs or compounds (100 μL) were mixed in the wells with 100 μL of bacterial inoculum, resulting in a final bacterial concentration of ca. 1.2×10^6 CFU/mL. Solvent (DMSO) was included in every experiment as a negative control. The plates were sealed in plastic bags and then incubated at 37 °C for 5 days. On day 5, 50 μL of the MTT/Tween 80 mixture (1.5 mL of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide; Aldrich Chemical Co., Milwaukee, WI) at a dilution of 1 mg/mL in absolute EtOH and 1.5 mL of 10% Tween 80) was added to the wells, and the plate was incubated at 37 °C for 24 h. After this incubation period, the growth of the microorganism was visualized by the change in colour of the dye from yellow to purple. The tests were carried out in triplicate. MIC value is defined as the lowest drug concentration that prevents the aforementioned change in colour.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2011.02.034.

References and notes

1. World Health Organization, Global Tuberculosis Control: Surveillance, Planning, Financing. Geneva: World Health Organization, 2008. WHO Publication No. WHO/HTM/TB/2008.393.
2. Dye, Ch.; Williams, B. G. *Science* **2010**, *328*, 856.
3. Burman, W. J. *CID* **2010**, *50*, S165.
4. LoBue, P. *Curr. Opin. Infect. Dis.* **2009**, *22*, 167.
5. NIAID, <http://www.niaid.nih.gov/topics/tuberculosis/>.
6. Cortés-Selva, F.; Campillo, M.; Reyes, C. P.; Jiménez, I. A.; Castanys, S.; Bazzocchi, I. L.; Pardo, L.; Gamarro, F.; Ravelo, A. G. *J. Med. Chem.* **2004**, *47*, 576.
7. Torres-Romero, D.; Muñoz-Martínez, F.; Jiménez, I. A.; Castanys, S.; Gamarro, F.; Bazzocchi, I. L. *Org. Biomol. Chem.* **2009**, *7*, 5166.
8. Gao, J. M.; Wu, W. J.; Zhang, J. W.; Konishi, Y. *Nat. Prod. Rep.* **2007**, *24*, 1153.
9. Chen, J.-J.; Chou, T.-H.; Peng, Ch.-F.; Chen, I.-S.; Yang, S.-Z. *J. Nat. Prod.* **2007**, *70*, 202.
10. Chen, J. J.; Yang, C. S.; Peng, C. F.; Chen, I. S.; Miaw, C. L. *J. Nat. Prod.* **2008**, *71*, 1016.
11. Chou, T.-H.; Chen, I.-S.; Peng, C.-F.; Sung, P.-J.; Chen, J.-J. *Chem. Biodiv.* **2008**, *5*, 1412.
12. Torres-Romero, D.; King-Díaz, B.; Jiménez, I. A.; Lotina-Hennsen, B.; Bazzocchi, I. L. *J. Nat. Prod.* **2008**, *71*, 1331.
13. PC Model, version 7.0 with MMX force field, Serena Software, Bloomington, IN.
14. Sharpless, K. B.; Amberg, W.; Bennani, Y. L.; Crispino, G. A.; Hartung, J.; Jeong, K.-S.; Kwong, H.-L.; Morikawa, K.; Wang, Z.-M.; Xu, D.; Zhang, X.-L. *J. Org. Chem.* **1992**, *57*, 2768.
15. Smith, C. R.; Miller, R. W.; Weisleder, D.; Rohwedder, W. K. *J. Org. Chem.* **1976**, *41*, 3264.
16. González, A. G.; Muñoz, O. M.; Ravelo, A. G.; Crespo, A.; Bazzocchi, I. L.; Jiménez, I. A.; Rodríguez-Romero, V. *Tetrahedron Lett.* **1992**, *33*, 1921.
17. Hohmann, J.; Nagy, G.; Guenther, G.; Argay, G.; Kalman, A.; Czira, G. *J. Chem. Soc., Perkin Trans. 1* **1994**, *22*, 3281.
18. Rojas, R.; Caviedes, L.; Aponte, J. C.; Vaisberg, A. J.; Lewis, W. H.; Lamas, G.; Sarasara, C.; Gilman, R. H.; Hammond, G. B. *J. Nat. Prod.* **2006**, *69*, 845.

19. Caviedes, L.; Delgado, J.; Gilman, R. H. *J. Clin. Microbiol.* **2002**, *40*, 1873.
20. Nonius, B.V. COLLECT. Delft, The Netherlands, 2000.
21. Otinowski, Z.; Minor, W. Processing of X-ray Diffraction Data Collected in Oscillation Mode. Methods in Enzymology In Carter, C. W., Jr., Sweet, R. M., Eds., Part A; Macromolecular Crystallography; Academic: New York, 1997; Vol. 276, pp 307–326.
22. Altomare, A.; Cascarano, G.; Giacovazzo, C.; Guagliardi, A.; Moliterni, A. G. G.; Burla, M. C.; Polidori, G.; Camalli, M.; Spagna, R. *SIR97. A Package for Crystal Structure Solution by Direct methods and Refinement*; University of Bari: Italy, 1997.
23. Sheldrick, G. M. SHELXL*97, Program for the Solution of Crystal Structures*; University of Göttingen: Germany, 1997.
24. Farrugia, L. J. *J. Appl. Crystallogr.* **1999**, *32*, 837.
25. Spek, A. L. PLATON; University of Utrecht: The Netherlands, 2003.