Bioorganic & Medicinal Chemistry 20 (2012) 4155–4163

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Synthesis of Leubethanol derivatives and evaluation against *Mycobacterium tuberculosis*

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ARTICLE INFO

Article history:
Received 27 January 2012
Revised 22 April 2012
Accepted 27 April 2012
Available online 4 May 2012

Keywords:
Leubethanol
SAR
Mycobacterium tuberculosis
MABA
Cytotoxicity

ABSTRACT

Twenty-five derivatives of the natural diterpene leubethanol, including several potential pro-drugs, with changes in the functionality of the aliphatic chain or modifications of the phenolic group, were synthesized and tested in vitro by the MABA technique for their activity against the H37Rv strain of *Mycobacterium tuberculosis*. Several compounds showed antimycobacterial potencies similar to that of the lead compound and two of them displayed higher selectivity indexes.

1. Introduction

Tuberculosis (TB), mainly caused by *Mycobacterium tuberculosis* (*MTB*), has been widespread since ancient times and remains a worldwide major health problem. According to the latest report of World Health Organization on tuberculosis,[1] 9.4 million new cases were reported in 2009, 14 million prevalent cases, 1.3 million deaths among HIV-negative people and 0.38 million deaths among HIV-positive people. Most cases occurred in the Southeast Asian, African and Western Pacific regions (35%, 30% and 20%, respectively). An estimated 11–13% of incident cases were HIV-positive; the African Region accounted for 80% of these cases approximately.

Mycobacteria are able to survive in a latent state in infected individuals, thereby serving as a reservoir, waiting for the opportunistic reactivation.[2] It is estimated that there are around 2 billion people, almost one-third of the world's population, infected with latent or active *MTB*.[3] An additional contributing factor is the continued emergence of new MDR-*MTB* strains,[4] often associated to poor compliance to treatments and, since 2006, to the officially recognized appearance of *MTB* strains with extended resistance (XDR-*MTB*),[5] now spread to more than 58 countries.[6] Furthermore, TB treatment protocols are prolonged up to six or more months, complex and rather expensive, and most times end in the poor patient compliance.

Due to those facts mentioned above, new anti-TB drugs and better therapeutic strategies against TB are urgently and permanently needed. The new drug candidates should shorten the standard regimens, being effective enough against MDR-TB. Though several compounds are currently in advanced phases of clinical assay, for some 40 years no new compounds have been brought into the market for TB treatment. However, in recent years, an emphasized research activity for the development of new TB drugs has been being produced. Some compounds are presently in clinical development, while others are being investigated pre-clinically, in an attempt to discover new molecules for a target-based treatment of TB.[7]

Waksman and co-workers[8] recently reported on the structure assignment and antimycobacterial activity of leubethanol (**1**, Fig. 1), a natural serrulatane diterpenoid isolated from the methanol extract of *Leucophyllum frutescens* (Fam.: Scrophulariaceae). The compound displayed a certain activity (MIC = 25.1 μM) against the H37Rv strain, sensitive to the first-line anti-TB agents, with similar potency (MIC = 21.9 μM), against the MDR-*MTB* strain CIBIN/UMF15:99, a clinical isolate that had resulted resistant to all five first-line anti-TB drugs: streptomycin, isoniazid, rifampin, ethambutol, and pyrazinamide (SIREP-resistance). The present study was intended to explore the possibility of obtaining better antimycobacterial compounds through modification of the leubethanol structure. Thus, several derivatives of leubethanol with changes in the functionality of the side-chain along with several phenolic ethers and esters (Fig. 1) were prepared and evaluated against *MTB*.

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http://dx.doi.org/10.1016/j.bmc.2012.04.059

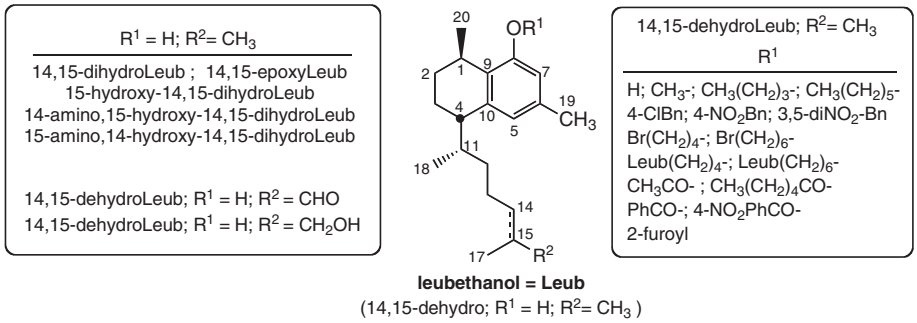


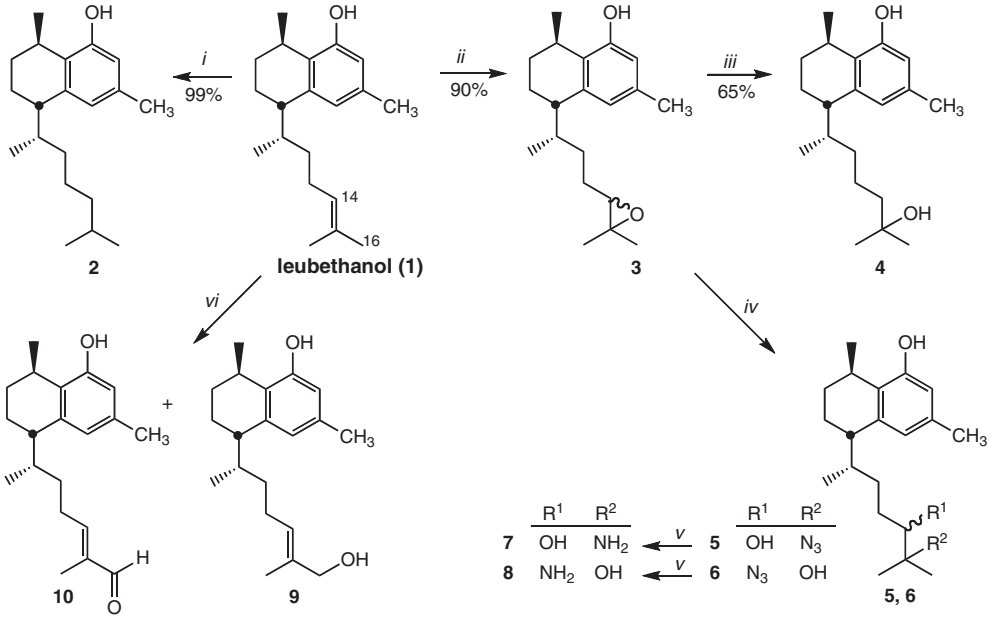
Figure 1. Global structure and variants of leubethanol derivatives.

2. Chemistry

Aiming to investigate the influence of how the structural changes could affect the antimycobacterial activity of leubethanol derivatives, the two main parts of the molecule, the olefinic bond in the side-chain and the phenolic fragment, were taken into consideration. Changes at the side chain included hydrogenation, epoxidation followed by oxirane aperture and allylic oxidation (Scheme 1). Hydrogenation of **1** at ambient temperature and pressure in the presence of Pd-C gave dihydroleubethanol **2** almost quantitatively. The treatment of **1** with MCPBA gave the mixture of the epimeric oxiranes **3a**,**b**, whose reduction with lithium aluminum hydride led to the tertiary alcohol **4**. The mixture of epoxides **3a,b** was used to prepare some β-aminoalcohol derivatives, for which, in accordance with our previous experience on lipidic aminoalcohols and diamines,[9] good antimycobacterial activities were expected. When the oxirane mixture **3a,b** was treated with sodium azide, the nucleophilic attack occurred at both C-14 and C-15 positions leading to the four possible hydroxyazides, which were chromatographed and characterized as mixtures **5a,b** (14-OH,15-N_3) and **6a,b** (14-N_3,15-OH). These azide mixtures were reduced separately with $NaBH_4$/MeOH in the presence of a catalytic amount of Pd-C, to give mixtures of the corresponding β-aminoalcohols **7a,b** and **8a,b**, respectively. We finally treated leubethanol with selenium dioxide to obtain the allylic alcohol **9** and the α,β-unsaturated aldehyde **10**.

Relating to the phenolic function, its masking through the formation of ethers and esters was considered opportune taking into account the antimycobacterial results found by Rodriguez and Ramirez,[10] for some natural products closely related to leubethanol. Erogorgiane, another natural serrulatane diterpenoid without any phenolic hydroxyl in its structure, was reported to inhibit up to 96% of *MTB* growth at a 46.2 μM concentration, while its 7-hydroxyl derivative attained a 77% inhibition at a similar concentration. These findings suggested that the 8-hydroxyl group of leubethanol would not be an essential function for the antimycobacterial activity. Therefore, several types of ethers and esters with different chemical-metabolic stability or with expected bioactivity significance were considered for the blocking of the phenolic group at C-8, also aiming to introduce some extra fragments on the leubethanol structure that could promote additional interactions with the mycobacterial target and lead to more efficacious compounds.

One group of derivatives was constituted by simple alkyl ethers with variable chain size (methyl, *n*-butyl, *n*-hexyl), for which a considerable stability was expected and by haloalkyl derivatives (4-bromobutyl and 6-bromohexyl), which would be able to alkylate



i) H_2/Pd-C, EtOH; *ii)* MCPBA, $CHCl_3$; *iii)* $LiAlH_4$, THF; *iv)* NaN_3 /EtOH, 24h at 70ºC; *v)* THF/Pd-C; $NaBH_4$/MeOH; *vi)* SeO_2/ EtOH

Scheme 1. Synthesis of leubethanol derivatives modified at the side chain.

irreversibly any nucleophilic function close to the target biomolecule in the mycobacteria. Another group of ethers were of benzylic nature (4-chloro, 4-nitro and 3,5-dinitro), chemically and metabolically less stable, whose syntheses were proposed on the basis of an observed enhancement of the antimycobacterial activity caused by the introduction of a benzyl group in a series of aminoalcohol derivatives previously obtained by us.[9] Furthermore, it would be expected that the incorporation of a *m*-dinitrophenyl substituent could act in two different ways. On one hand it would add to leubethanol its intrinsic antimicrobial toxicity, and additionally due to its reported character of potential antigenic response inducer, it could also act as an immuno-killer promoting death of the *MTB*-infected cell by attracting antibodies to it.[11]

The hydroxyl group was transformed into alkyl and benzyl ethers in presence of potassium carbonate by reaction with the appropriate iodinated/brominated alkyl or arylalkyl reagent to provide compounds **11a** to **11f** in medium to good yields (Scheme 2). Interestingly the reaction of **1** with 3,5-dinitrobenzyl bromide, in addition to the expected ether **11f**, led to compound **12** in 15% yield. The HRMS of compound **12** denoted a molecular weight of 466.3596, in agreement with the molecular formula $C_{27}H_{34}N_2O_5$. Its IR spectrum showed the absorption of the free hydroxyl group at 3400 cm^{-1}, while its ^1H NMR spectrum, in addition to the aromatic signals associated to the *m*-dinitrobenzyl group, showed only one signal in the aromatic region (δ 6.54 ppm), correlating with a methine signal at δ 134.8 ppm in the ^{13}C NMR spectrum. The identity of compound **12** was ascertained on the light of several 1D- and 2D- $^1H/^{13}C$ NMR experiments and particularly of NOE-difference experiments. Thus, irradiation of the signal for the benzylic protons (δ 4.14 ppm) generated NOE enhancements on the signals associated to those 2′,6′-aromatic protons (δ 8.13 ppm) of the dinitrobenzyl group, to the methyl singlet Me-19 (δ 2.05 ppm), to the methyl doublet Me-18 (δ 0.80 ppm) and to the methine multiplet CH-11 (δ 2.64 ppm). The last two effects can only occur if the dinitrobenzyl fragment is attached to position C-5 and would support the unexpected structure proposed for compound **12** in Scheme 2. The treatment of **1** with sodium hydride followed by reaction with 1,4-dibromobutane and 1,6-dibromohexane allowed us to obtain the bromoethers **11g** and **11i**, in addition to the pseudo-dimeric diethers **11h** and **11j**.

Leubethanol was also treated with acetic anhydride in pyridine to provide the acetate **11k**, while other ester derivatives **11m** to **11p** were prepared in good yield by treatment with sodium hydride followed by the appropriate acyl chloride.

3. Bioactivity

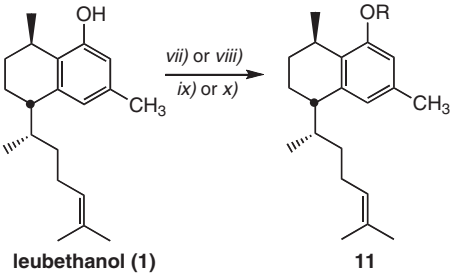
The anti-*MTB* activity was assessed in vitro against the H37Rv strain (ATTC 27294),[12] susceptible to all SIREP anti-TB drugs, according to a modified Microplate Alamar Blue Assay (MABA).[13] Ethambutol (EMB) was used as the reference drug and the assays were performed by triplicate independent experiments. Cytotoxicity on Vero cells[14] was also measured for those compounds with an appreciable in vitro anti-*MTB* effect.

4. Results and discussion

Those MIC values found for the compounds represented in Schemes 1 and 2 are shown in Tables 1 and 2, respectively. MIC and CC_{50} values are expressed in μM units, rather than in μg/mL units, most commonly found in the literature related with this type of evaluations, to provide more adequate comparisons of antimycobacterial potencies. It is also relevant to note that MIC values in tables refer to the total killing of the cultured mycobacteria, whereas CC_{50} values refer to only 50% killing of Vero cells, thus real SI values should actually be higher than those calculated and shown in Tables 1 and 2.

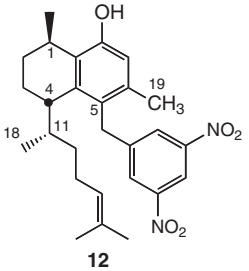
As it can be observed in Table 1 the hydrogenation of the side-chain double bond slightly decreased the activity and the toxicity of compound **2**, whereas the introduction of oxygen or nitrogen containing functions in the C14–C15 fragment (compounds **3–8**), decreased the antimycobacterial activity by a factor of three times or more. Furthermore, no improvement of cytotoxicity on Vero cells was observed for these compounds. As can also be observed for compounds **9** and **10**, the introduction at C-16 of an allylic hydroxyl group or a conjugated aldehyde, failed to improve the antimycobacterial/cytotoxicity properties of the natural compound leubethanol.

Related to the results observed for leubethanol ethers **11a–11j** to and esters **11k–11p**, with modifications of the phenolic hydroxyl



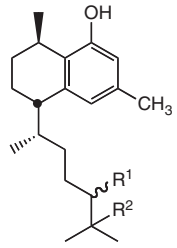
R	
CH_3-	**11a**
$CH_3(CH_2)_3$-	**11b**
$CH_3(CH_2)_5$-	**11c**
Cl-C6H4-CH_2-	**11d**
O_2N-C6H4-CH_2-	**11e**
3,5-$(O_2N)_2$-C6H3-CH_2-	**11f**
$Br(CH_2)_4$-	**11g**
Leub$O(CH_2)_4$-	**11h**
$Br(CH_2)_6$-	**11i**
Leub$O(CH_2)_6$-	**11j**

R	
CH_3CO-	**11k**
$CH_3(CH_2)_4CO$	**11m**
C6H5-CO-	**11n**
O_2N-C6H4-CO-	**11o**
2-furyl-CO-	**11p**



Scheme 2. Synthesis of ethers, esters and pseudo-dimeric leubethanol derivatives.

Table 1
Antimycobacterial activity, cytotoxicity and selectivity indexes of leubethanol derivatives modified at the side chain



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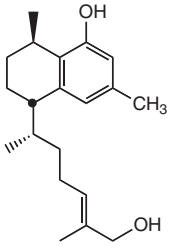

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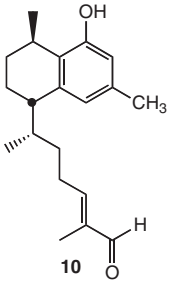

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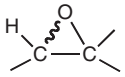
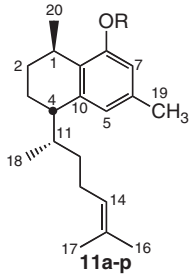
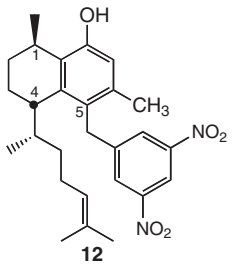
Compd	R^1	R^2	*MTB* $H_{37}Rv$ MIC (μM)	Vero cells CC_{50} (μM)	Selectivity index (SI)
Leub	Δ^{14}		**25.1**	**123**	**4.9**
2	H	H	**30.3**	**192**	**6.3**
3			**103**	**661**	**6.4**
4	H	OH	200	nd	–
5	OH	N_3	181	231	1.3
6	N_3	OH	90	127	1.4
7	OH	NH_2	98	141	1.4
8	NH_2	OH	98	158	1.6
9	—	—	**25.8**	**132**	**5.1**
10	—	—	**25.8**	**116**	**4.5**
EMB			**7.5**	**>500**	**>67**

MIC and CC_{50}: rounded media values of three experiments; nd = no determined.
SI: selectivity index calculated by the equation: SI = CC_{50} (Vero)/MIC $H_{37}Rv$).
–: not calculated; EMB = ethambutol.

Table 2
Antimycobacterial activity, cytotoxicity and selectivity indexes of ethers, esters, pseudo-dimeric ethers and compound **12**



11a-p



12

Compd	R	*MTB* $H_{37}Rv$ MIC (μM)	Vero cells CC_{50} (μM)	Selectivity index (SI)
Leub	H	**25.1**	**123**	**4.9**
11a	CH_3–	>200	nd	–
11b	$CH_3(CH_2)_3$–	>200	nd	–
11c	$CH_3(CH_2)_5$–	>200	nd	–
11d	4-ClBn	>200	nd	–
11e	4-NO_2Bn	>200	nd	
11f	3,5-diNO_2Bn	>200	nd	–
11g	$Br(CH_2)_4$–	>200	nd	–
11h	Leub-O–$(CH_2)_4$–	200	nd	–
11i	$Br(CH_2)_6$–	>200	nd	–
11j	Leub-O–$(CH_2)_6$–	190	nd	–
11k	CH_3CO–	**48.7**	**>1100**	**>22.6**
11m	$CH_3(CH_2)_4CO$–	>200	nd	–
11n	PhCO	>200	nd	–
11o	4-NO_2PhCO	>200	nd	–
11p		>200	nd	–
12	—	**17.2**	**>770**	**>45**
EMB		**7.5**	**>500**	**>67**

MIC and CC_{50}: rounded media values of three experiments; nd = no determined.
Selectivity index calculated by the equation: SI = CC_{50} (Vero)/MIC $H_{37}Rv$).
–: Not calculated; EMB = ethambutol.

leubethanol (Table 2), practically all the derivatives resulted inactive and only the acetate **11k** retained approximately one half of the antimycobacterial activity of leubethanol (MIC: 48.7 μM vs 25.1 μM, respectively), that could be associated either to its intrinsic antimycobacterial ability or to its potential pro-drug character and the possible hydrolysis during the in vitro evaluation period. Regarding its cytotoxicity, it is interesting to note that compound **11k** showed a fair decrease in cytotoxicity (CC_{50}: 1100 μM) with respect to leubethanol (CC_{50}: 123 μM), which leads to a higher selectivity index (SI: 22.6 for **11k** vs 4.9 for leubethanol). These results, indicate the convenience of the presence of the free phenolic group at C-8 of the serrulatane skeleton, in contrast to the findings of Rodriguez and Ramirez[10] for the hydrocarbon erogorgiane and its 7-hydroxyderivative. Significantly, and to confirm the anterior statement, the dinitrobenzyl derivative **12**, while maintaining the free hydroxyl group, showed a slightly increase of the antimycobacterial potency (17.2 μM for **12** vs 25.1 μM for leubethanol) and in spite of containing two nitro groups, resulted around one order of magnitude less cytotoxic than the parent compound (SI = 45 for **12** vs 4.9 for leubethanol).

In summary, the results found in this research and, in particular, those SI calculated for compounds **11k** and **12**, could support the continuation of further research focused on establishing the mechanism of action and the target molecule or the affected pathway in the mycobacteria, previous to carry out an eventual structure optimization of the side-chain functionality through preparation and evaluation of a larger number of substances, oriented to define a good candidate for in vivo assays.

5. Experimental section

5.1. General

All commercial chemicals and solvents used were reagent grade. Flash column chromatography was done using Merck Silica Gel 60 (0.04–0.063 mm). Reactions were monitored by TLC using Merck 60F_{254} silica gel plates. Compounds were detected visually under UV irradiation (254 nm) and by spraying with sulfuric acid and phosphomolybdic acid reagents followed by heating at 100 °C. ^{1}H NMR and ^{13}C NMR spectra were obtained with a Brucker AC 200 spectrometer (200 and 50.3 MHz, respectively). Chemical shifts were recorded in parts per million (ppm, δ) and were reported relative to the solvent peak or TMS. High resolution mass spectra (HRMS) were measured with a QSTAR XL quadrupole time-of-flight mass spectrometer, by direct injection on the sample dissolved in MeOH and Ionization voltage of 5500 V. Infrared (IR) spectra were measured on a Nicolet Impact 410 spectrophotometer. The absorbances were measured with a Bio-Rad Benchmark model microplate reader. Middlebrook 7H9 broth medium was obtained from Difco®, resazurin from Probiotek®, and 0.45 μm pore size, 13 mm diameter PTFE from Millipore Millex®. Isoniazid, rifampicin, penicillin, streptomycin, and MTT were obtained from Sigma® and fetal bovine serum from Hyclone®.

5.1.1. Leubethanol isolation

Dry grounded root bark (2.0 kg) of *Leucophyllum frutescens* was extracted with methanol and the extract (146 g) fractionated by silica gel liquid chromatography using a gradient of *n*-hexane and ethyl acetate (AcOEt) as eluents. Fraction hexane/AcOEt (20:1, 56 g) was fractionated in a reverse phase Lobar RP-18 column using aqueous methanol 80%, 90% and 100%. Methanol 100% fraction was chromatographed in parallel on silica flash columns and eluted with hexane/AcOEt (95:5) to obtain 8.5 g (5.9% from MeOH extract) of the serrulatane leubethanol.

5.1.1.1. Leubethanol (1). Yellow oil. IR (ν): 3463, 2953, 2923, 2866, 1618, 1576, 838 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$): δ ppm = 1.01 (d, J = 6.8 Hz, 3H), 1.23 (d, J = 7.0 Hz, 3H), 1.10–1.31 (m, 2H), 1.50–1.97 (m, 5H), 1.59 (s, 3H), 1.69 (s, 3H), 1.82–2.01 (m, 2H), 2.27 (s, 3H), 2.61 (m, 1H), 3.09 (m, 1H) 4.84 (br s, OH), 5.03 (t, J = 6.4 Hz, 1H), 6.44 (s, 1H), 6.61 (s, 1H); ^{13}C NMR (50.3 MHz, $CDCl_3$) δ (ppm) 17.7 (CH_3), 18.8 (CH_3), 19.5 (CH_2), 21.3 (CH_3), 21.3 (CH_3), 25.8 (CH_3), 26.3 (CH_2), 26.7 (CH), 27.6 (CH_2), 33.5 (CH_2), 38.5 (CH), 42.5 (CH), 113.3 (CH), 122.6 (CH), 125.0 (CH), 126.3 (C), 131.2 (C), 135.2 (C), 141.1 (C), 153.1 (C). HRMS (ESI) for $C_{20}H_{30}O$: calcd 286.2297; found: 286.2391.

5.1.2. Synthesis of compound 2

To a solution of leubethanol (57 mg, 0.20 mmol) in 2 mL of absolute ethanol a catalytic amount of Pd/C was added and the mixture maintained under hydrogen atmosphere, at room temperature and stirred for 3 h. The crude mixture was filtered in a filter plate, washed with ethyl acetate and the organic layer concentrated under vacuum. The residue was purified by silica flash chromatography using hexane/ethyl acetate (95:5) as eluent to give compound **2**.

5.1.2.1. 14,15-Dihydroleubethanol (2). Yield: 57 mg (99% after chromatography); yellow oil. ^1H NMR (200 MHz, $CDCl_3$) δ (ppm) 0.86 (d, J = 6.5 Hz, 6H), 0.97 (d, J = 6.8 Hz, 3H), 1.10–1.30 (m, 2H), 1.23 (d, J = 7.0 Hz, 3H) 1.50–2.03 (m, 8H), 2.27 (s, 3H), 2.58 (m, 1H), 3.08 (m, 1H), 4.87 (br s, OH), 6.45 (s, 1H), 6.61 (s, 1H); ^{13}CNMR (50.3 MHz, $CDCl_3$) δ (ppm) 19.0 (CH_3), 19.6 (CH_2), 21.2 (CH_3), 21.3 (CH_3), 22.6 (CH_3), 22.9 (CH_3), 25.6 (CH_2), 26.7 (CH), 27.4 (CH_2), 28.0 (CH), 33.8 (CH_2), 34.2 (CH_2), 38.5 (CH), 42.5 (CH), 113.3 (CH); 122.8 (CH), 126.3 (C), 135.1 (C), 141.2 (C), 153.1 (C). HRMS (ESI) for $C_{20}H_{32}O$: calcd 288.2453; found: 288.6235.

5.1.3. Synthesis of compound 3

To a cooled solution of leubethanol (229 mg, 0.8 mmol) in 2 mL of $CHCl_3$, solutions of MCPBA (0.9 mmol) in 2 mL $CHCl_3$ and $NaHCO_3$ (0.9 mmol) in 1 mL $CHCl_3$ were added under stirring on ice bath for 15 min. The reaction was maintained with stirring at room temperature for 12 h. The crude mixture was washed with a 40% solution of $NaHSO_3$ and the solvent removed under vacuum. The resulting solid was dissolved in ethyl acetate, washed with 5% $NaHCO_3$, water and dried over Na_2SO_4. The product was purified by silica flash chromatography using hexane/ethyl acetate (8:2) as eluent to give compound **3** as mixture of α and β epoxides.

5.1.3.1. 14,15-Epoxyleubethanol (3a,b). Yield: 217 mg (90% after chromatography); yellow oil. ^1H NMR (200 MHz, $CDCl_3$) δ (ppm), 0.98 (d, J = 6.8 Hz, 3H), 1.1–1.5 (m, 4H), 1.20 (d, J = 6.8 Hz, 3H), 1.23 (s, 3H), 1.29 (s, 3H), 1.70–1.98 (m, 5H), 2.23 (s, 3H), 2.62 (m, 1H), 3.01 (m, 1H), 3.12 (m, 1H), 5.30 (br s, OH), 6.44 (s, 1H), 6.57 (s, 1H); ^{13}C NMR (50.3 MHz, $CDCl_3$) δ (ppm) 18.8 ($2 \times CH_3$), 19.2/19.3, (CH_2), 21.2 ($2 \times CH_3$), 24.9 (CH_3), 26.6 (CH), 27.3 (CH_2), 27.5/27.6 (CH_2), 30.0/30.2 (CH_2), 38.4/38.5 (CH), 42.4 (CH), 58.6/59.0 (C), 64.8/65.1 (CH), 113.4 (CH), 122.1/122.3 (CH), 126.4 (C), 135.1 (C), 140.6/140.7 (C), 153.4 (C). HRMS (ESI) for $C_{20}H_{30}O_2$: calcd 302.2246; found: 302.4126.

5.1.4. Synthesis of compound 4

To a stirring solution of **3** (40 mg, 0.13 mmol) in 2 mL of dry THF, $LiAlH_4$ (0.26 mmol) were added and maintained for 6 h at room temperature. Then, a solution of water-saturated ether was added and the resulting white solid filter off. The organic layer was concentrated under vacuum and the solid dissolved with ether, washed with 10% $NaHCO_3$, 2 N HCl and water. The product was purified by silica flash chromatography using hexane/ethyl acetate (85:15) as eluent to provide compound **4**.

5.1.4.1. 15-Hydroxy-14,15-dihydroleubethanol (4). Yield: 26 mg (66% after chromatography); yellow oil. IR (ν): 3378, 2953, 2930, 1579, 1459, 1258 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$) δ (ppm), 0.96 (d, J = 6.8 Hz, 3H), 1.10–1.30 (m, 2H), 1.18 (s, $2 \times 3H$), 1.20 (d, J = 6.8 Hz, 3H), 1.31–1.51 (m, 2H), 1.55–2.03 (m, 7H), 2.24 (s, 3H), 2.57 (m, 1H), 3.05 (m, 1H), 5.08 (br s, OH), 6.42 (s, 1H), 6.57 (s, 1H); ^{13}C NMR (50.3 MHz, $CDCl_3$) δ (ppm) 19.0 (CH_3), 19.3 (CH_2), 21.2 ($2 \times CH_3$), 22.5 (CH_2), 26.6 (CH), 27.4 (CH_2), 29.2 (CH_3), 29.3 (CH_3), 34.0 (CH_2), 38.6 (CH), 42.4 (CH), 44.1 (CH_2), 71.3 (C), 113.3 (CH), 122.6 (CH), 126.4 (C), 135.1 (C), 140.9 (C), 153.1(C). HRMS (ESI) for $C_{20}H_{32}O_2$: calcd 304.2402; found: 304.4506.

5.1.5. Synthesis of compounds 5 and 6

To a stirring solution of **3a**,**b** (0.29 mmol) in 3 mL ethanol, NaN_3 (2.9 mmol) and NH_4Cl (3.5 mmol) were added and the mixture maintained for 24 h at 70 °C. The ethanol was removed under vacuum, and the solid dissolved in ethyl acetate. The organic layer was washed with water and then dried over Na_2SO_4. Elimination of the organic solvent gave a crude mixture of compounds **5a**,**b** and **6a**,**b** that were purified by silica flash chromatography using hexane/ethyl acetate (9:1) as solvent.

5.1.5.1. 15-Azo,14-hydroxy-14,15-dihydroleubethanol (5a,b). Yield: 42 mg (42% after chromatography); yellow oil. IR (ν): 3378, 2953, 2930, 2105, 1579, 1258, 1062, 843 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$) δ (ppm) 0.95 (d, J = 6.8 Hz, 3H), 0.99 (d, J = 6.8 Hz, 3H), 1.05–1.15 (m, 2H), 1.20 (d, J = 6.8 Hz, $2 \times 3H$), 1.23 (s, $2 \times 3H$), 1.24 (s, $2 \times 3H$), 1.47–1.54 (m, 4H), 1.62–1.95 (m, 12H), 2.24 (s, $2 \times 3H$), 2.60 (m, $2 \times 1H$), 3.05 (m, $2 \times 1H$), 3.22 (m, $2 \times 1H$), 4.83 (br s, $2 \times OH$), 6.42 (s, $2 \times 1H$), 6.56 (s, $2 \times 1H$); ^{13}C NMR (50.3 MHz, $CDCl_3$) δ (ppm) 18.9 (CH_3), 19.0 (CH_2), 19.3 (CH_3), 19.6 (CH_2), 21.2 ($6 \times CH_3$), 22.8 ($2 \times CH_3$), 26.6 ($2 \times CH$), 27.3 (CH_2), 27.4 (CH_2), 29.3 (CH_2), 29.6 (CH_2), 29.9 (CH_2), 30.9 (CH_2), 38.6 (CH), 38.8 (CH), 42.3 ($2 \times CH$), 65.4 ($2 \times C$), 77.4 (CH), 77.8 (CH), 113.4 ($2 \times CH$), 122.3 (CH), 122.4 (CH), 126.2 (C), 126.5 (C), 135.2 (C), 135.3 (C), 140.7 (C), 140.9 (C), 153.2 ($2 \times C$). HRMS (ESI) for $C_{20}H_{31}N_3O_2$: calcd 345.2416; found 345.4791.

5.1.5.2. 14-Azo,15-hydroxy-14,15-dihydroleubethanol (6a,b). Yield: 38 mg (38%); yellow oil. ^1H NMR (200 MHz, $CDCl_3$) δ (ppm) 0.96 (d, J = 6.9 Hz, 3H), 0.99 (d, J = 6.9 Hz, 3H), 1.16 (s, 3H), 1.17 (s, 3H), 1.19 (s, $2 \times 3H$), 1.20 (d, J = 6.8 Hz, $2 \times 3H$), 2.24 (s, $2 \times 3H$), 1.32–1.95 (m, 18H), 2.60 (m, $2 \times 1H$), 3.05 (m, $4 \times 1H$), 4.81 (br s, $2 \times OH$), 6.43 (s, $2 \times 1H$), 6.58 (s, $2 \times 1H$); ^{13}C NMR (50.3 MHz, $CDCl_3$) δ (ppm) 18.9 (CH_3), 19.1 (CH_2), 19.4 (CH_3), 19.7 (CH_2), 21.2 ($4 \times CH_3$), 25.3 ($2 \times CH_3$), 26.5 ($2 \times CH$), 26.6 ($2 \times CH_3$), 27.3 ($2 \times CH_2$), 28.2 ($2 \times CH_2$), 30.9 (CH_2), 31.7 (CH_2), 38.6 ($2 \times CH$), 42.2 ($2 \times CH$), 73.6 (CH), 73.2 (CH), 73.8 ($2 \times C$), 113.5 ($2 \times CH$), 122.5 (CH), 122.6 (CH), 126.2 (C), 126.5 (C), 135.2 ($2 \times C$), 140.6 ($2 \times C$), 153.1 ($2 \times C$). HRMS (ESI) for $C_{20}H_{31}N_3O_2$: calcd 345.2416; found 345.3954.

5.1.6. Procedure for the synthesis of compounds 7 and 8

To a stirring solution of **5a**,**b** (0.12 mmol), $NaBH_4$ (0.36 mmol) and a catalytic amount of Pd/C in 1 mL of dry THF, dry methanol (4 mL) drop by drop was added. The mixture was maintained at room temperature for 12 h. The reaction product was filtered over celite, washed with ethyl acetate and the organic solvents removed under vacuum. The resulting solid was dissolved in ethyl acetate and washed with water. Elimination of the ethyl acetate gave a

crude that was purified by silica flash chromatography using dichloromethane/methanol (95:5) as eluent.

5.1.6.1. 15-Amino,14-hydroxy-14,15-dihydroleubethanol (7a,b). Yield: 26 mg (68%); yellow oil. ^{1}H NMR (200 MHz, $CDCl_3 + CD_3OD$) δ (ppm) 0.88 (d, J = 6.8 Hz, 3H), 0.98 (d, J = 6.8 Hz, 3H), 1.30–1.95 (m, 18H), 1.15 (d, J = 6.8 Hz, 2 × 3H), 1.34 (s, 2 × 3H), 1.40 (s, 2 × 3H), 2.19 (s, 2 × 3H), 2.58 (m, 2 × 1H), 3.03 (m, 2 × 1H), 3.38 (m, 2 × 1H), 6.44 (s, 2 × 1H), 6.52 (s, 2 × 1H); ^{13}C NMR (50.3 MHz, $CDCl_3 + CD_3OD$) δ (ppm) 22.8 (2 × CH_3), 22.9 (2 × CH_3), 23.0 (2 × CH_2), 24.6 (4 × CH_3), 26.7 (2 × CH_3), 30.4 (2 × CH), 31.2 (2 × CH_2), 33.4 (2 × CH_2), 34.2 (CH_2), 34.9 (CH_2), 42.8 (CH), 43.1 (CH), 46.2 (2 × CH), 61.6 (2 × C), 78.9 (CH), 79.3 (CH), 116.7 (CH), 116.7 (CH), 125.2 (CH), 125.3 (CH), 130.8 (2 × C), 138.7 (2 × C), 144.1 (2 × C), 158.2 (2 × C). HRMS (ESI) for $C_{20}H_{33}NO_2$: calcd 319.4815; found 319.4932.

Reduction of azides **6a**,**b** were performed similar to compounds **5a**,**b**. To a stirring solution of **6a**,**b** (0.11 mmol), $NaBH_4$ (0.33 mmol) and a catalytic amount of Pd/C in 1 mL of dry THF, dry methanol (4 mL) drop by drop was added. The mixture was maintained at room temperature for 12 h. The crude was purified by silica flash chromatography using dichloromethane/methanol (95:5) as eluent.

5.1.6.2. 14-Amino,15-hydroxy-14,15-dihydroleubethanol (8a,b). Yield 15%: yellow oil. ^{1}H NMR (200 MHz, $CDCl_3$) δ (ppm) 0.96 (d, J = 6.8 Hz, 2 × 3H), 1.02 (s, 2 × 3H), 1.16 (s, 2 × 3H), 1.18 (d, J = 6.8 Hz, 2 × 3H), 1.42–1.98 (m, 16H), 2.21 (s, 2 × 3H), 2.43 (m, 2 × 1H), 2.59 (m, 2 × 1H), 3.07 (m, 4 × 1H), 3.44 (br s, NH_2 + OH), 6.42 (s, 2 × 1H), 6.54 (s, 2 × 1H); ^{13}C NMR (50.3 MHz, $CDCl_3$) δ (ppm) 18.9 (CH_3), 18.9 (CH_3), 19.4 (2 × CH_2), 21.2 (4 × CH_3), 23.0 (2 × CH_3), 26.6 (2 × CH), 27.3 CH_3), 27.5 CH_3), 27.5 (2 × CH_2), 29.7 (CH_2), 30.2 (CH_2), 31.3 (CH_2), 31.4 (CH_2), 38.6 (CH), 39.2 (CH), 42.1 (2 × C), 60.2 (CH), 61.4 (CH), 71.7 (2 × C), 113.5 (C), 113.7 (C), 122.0 (CH), 122.4 (CH), 126.7 (2 × C), 135.2 (C), 135.4 (C), 140.6 (C), 140.8 (C), 153.4 (2 × C). HRMS (ESI) for $C_{20}H_{33}NO_2$: calcd 319.4815; found 319.45017.

5.1.7. Synthesis of compounds 9 and 10

To a stirring solution of leubethanol (200 mg, 0.70 mmol) in 2 mL dry ethanol, SeO_2 (87 mg, 0.78 mmol) was added and the mixture maintained at room temperature for 1 h. The solvent was removed under vacuum and the solid mixture dissolved in ethyl acetate, washed with brine and the organic layer dried over Na_2SO_4. Elimination of the solvent gave a crude (200 mg) that was purified by silica flash chromatography using *n*-hexane/ethyl acetate (9:1) as eluent, to separate 15 mg of starting material, 129 mg (0.43 mmol, 61%) of the allylic alcohol (**9**) and 56 mg (0.19 mmol, 27%) of the α,β-unsaturated aldehyde (**10**).

5.1.7.1. 16-Hydroxy leubethanol (9). Yellow oil. ^{1}H NMR (200 MHz, $CDCl_3$) δ (ppm) 0.98 (d, J = 6.8 Hz, 3H), 1.12–1.52 (m, 4H), 1.20 (d, J = 6.8 Hz, 3H), 1.62 (s, 3H), 1.72–1.95 (m, 7H), 2.24 (s, 3H), 2.58 (m, 1H), 3.07 (m, 1H), 3.98 (s, 2H), 5.15 (br s, OH), 5.29 (1H, t, J = 6.5 Hz), 6.42 (s, 1H), 6.57 (s, 1H); ^{13}C NMR (50.3 MHz, $CDCl_3$) δ (ppm) 13.7 (CH_3), 18.8 (CH_3), 19.4(CH_2), 21.2 (2 × CH_3), 26.0 (CH_2), 26.6 (CH), 27.5 (CH_2), 33.1 (CH_2), 38.3 (CH), 42.4 (CH), 69.2 (CH_2), 113.3 (CH), 122.4 (CH), 128.6 (C), 127.0 (CH), 134.4 (C), 135.1 (C), 140.9 (C), 153.3 (C). HRMS (ESI) for $C_{20}H_{30}O_2$: calcd 302.4510; found 302.5312.

5.1.7.2. 16-Oxoleubethanol (10). Yellow oil. IR (ν): 3402, 2951, 2868, 1703, 1675, 1580, 1248, 1059, 841 cm^{-1}. ^{1}H NMR (200 MHz, $CDCl_3$) δ (ppm) 1.04 (d, J = 6.8 Hz, 3H, 1.21 (d, J = 6.8 Hz, 3H), 1.24–1.58 (m, 3H), 1.69–1.97 (m, 4H), 1.70 (s, 3H), 2.12–2.38 (m, 2H), 2.24 (s, 3H), 2.63 (m, 1H), 3.09 (m, 1H), 4.82 (br s, OH), 6.35 (1H, t, J = 7.9 Hz) 6.45 (s, 1H), 6.57 (s, 1H), 9.34 (s, 1H); ^{13}C NMR (50.3 MHz, $CDCl_3$) δ (ppm) 9.2 (CH_3), 18.8 (CH_3), 19.0 (CH_2), 21.2 (2 × CH_3), 26.6 (CH), 27.6 (2 × CH_3), 31.9 (CH_2), 38.6 (CH), 42.1 (CH), 113.4 (CH), 122.1 (CH), 126.5 (C), 135.4 (C), 139.3 (C), 140.5 (C), 153.3 (C), 155.9 (CH), 195.7 (CH). HRMS (ESI) for $C_{20}H_{28}O_2$: calcd 300.4351; found 300.5863.

5.1.8. General procedure for the preparation of 11a–11f

To a stirring solution of leubethanol (86 mg, 0.30 mmol) in 2 mL of dry acetone, K_2CO_3 (0.80 mmol) was added. The solution was stirred for 30 min and the corresponding alkyl halide (1 equiv) was added and maintained for 6 h at room temperature. The crude mixture was extracted with ethyl acetate, washed with water and the organic layer dried over Na_2SO_4. The resulting products, ethers **11a**–**11f** were purified by silica flash chromatography using hexane/ethyl acetate (9:1) as eluent. Compound **12** resulted as a secondary product in the reaction to obtain **11f**.

5.1.8.1. Leubethanol methyl ether (11a). Yield: 63 mg (70% after chromatography); yellow oil. IR (ν_{max}): 2952, 2925, 1610, 1578, 1271, 832 cm^{-1}. ^{1}H NMR (200 MHz, $CDCl_3$) δ (ppm) 1.02 (d, J = 6.8 Hz, 3H), 1.10–1.31 (m, 2H), 1.19 (d, J = 6.8 Hz, 3H), 1.50–1.97 (m, 5H), 1.60 (s, 3H), 1.71 (s, 3H), 1.82–2.01 (m, 2H), 2.35 (s, 3H), 2.61 (m, 1H), 3.18 (m, 1H), 3.85 (s, 3H), 5.04 (t, J = 6.5 Hz, 1H), 6.55 (s, 1H), 6.65 (s, 1H); ^{13}C NMR (50.3 MHz, $CDCl_3$) δ (ppm) 17.7 (CH_3), 18.9 (CH_3), 19.5 (CH_2), 21.5 (CH_3), 21.7 (CH_3), 25.8 (CH_3), 26.4 (CH_2), 26.6 (CH), 27.6 (CH_2), 33.5 (CH_2), 38.0 (CH), 42.5 (CH), 55.1 (CH_3), 108.4 (CH), 122.2 (CH), 125.1 (CH), 128.7 (C), 131.1 (C), 134.7 (C), 140.5 (C), 157.1 (C). HRMS (ESI) for $C_{21}H_{32}O$: calcd 300.2453 found: 300.3572.

5.1.8.2. Leubethanol *n*-butyl ether (11b). Yield: 73 mg (71% after chromatography); yellow oil. ^{1}H NMR (200 MHz, $CDCl_3$) δ (ppm) 0.91 (t, J = 6.6 Hz, 3H), 0.98 (d, J = 6.8 Hz, 3H), 1.18 (d, J = 6.8 Hz, 3H), 1.20–1.99 (m, 13H), 1.58 (s, 3H), 1.68 (s, 3H), 2.29 (s, 3H), 2.58 (m, 1H), 3.15 (m, 1H), 3.92 (t, J = 6.6 Hz, 2H), 4.98 (t, J = 6.5 Hz, 1H), 6.48 (s, 1H), 6.60 (s, 1H); ^{13}CNMR (50.3 MHz, $CDCl_3$) δ (ppm) 17.7 (CH_3), 18.8 (CH_3), 19.2 (CH_3), 19.5 (CH_2), 21.4 (CH_3), 21.5 (CH_3), 25.8 (CH_2), 26.2 (CH_2), 26.3 (CH_3), 26.6 (CH), 27.6 (CH_2), 31.4 (CH_2), 33.5 (CH_2), 38.3 (CH), 42.5 (CH), 67.5 (CH_2), 109.2 (CH), 122.2 (CH), 125.1 (CH), 128.8 (C), 131.0 (C), 134.6 (C), 140.5 (C), 156.5 (C). HRMS (ESI) for $C_{24}H_{38}O$: calcd 342.2923 found: 342.3072.

5.1.8.3. Leubethanol *n*-hexyl ether (11c). Yield: 74 mg (66% after chromatography); yellow oil. ^{1}H NMR (200 MHz, $CDCl_3$) δ (ppm) 0.92 (t, J = 6.8 Hz, 3H), 0.97 (d, J = 6.8 Hz, 3H), 1.16 (d, J = 6.8 Hz, 3H), 1.19–1.97 (m, 17H), 1.57 (s, 3H), 1.66 (s, 3H), 2.30 (s, 3H), 2.56 (m, 1H), 3.17 (m, 1H), 3.93 (t, J = 6.5 Hz, 2H), 4.97 (t, J = 6.5 Hz, 1H), 6.48 (s, 1H), 6.59 (s, 1H); ^{13}C NMR (50.3 MHz, $CDCl_3$) δ (ppm) 17.7 (CH_3), 18.8 (CH_3), 19.1 (CH_3), 19.5 (CH_2), 21.5 (CH_3), 21.6 (CH_3), 25.7 (CH_2), 26.0 (CH_2), 26.3 (CH_3), 26.6 (CH), 27.6 (CH_2), 29.5 (CH_2), 30.9 (CH_2), 31.6 (CH_2), 33.5 (CH_2), 38.0 (CH), 42.4 (CH), 67.5 (CH_2), 109.0 (CH), 121.9 (CH), 125.1 (CH), 128.7 (C), 131.1 (C), 134.6 (C), 140.4 (C), 156.6 (C). HRMS (ESI) for $C_{26}H_{42}O$: calcd 370.3236 found: 370.3559.

5.1.8.4. Leubethanol 4-chlorobenzyl ether (11d). Yield: 121 mg (98% after chromatography); yellow oil. ^{1}H NMR (200 MHz, $CDCl_3$) δ (ppm) 1.02 (d, J = 6.8 Hz, 3H), 1.10–1.30 (m, 2H), 1.23 (d, J = 6.8 Hz, 3H), 1.50–2.03 (m, 7H), 1.61 (s, 3H), 1.71 (s, 3H), 2.35 (s, 3H), 2.63 (m, 1H), 3.25 (m, 1H), 5.05 (t, J = 6.4 Hz, 1H), 5.06 (s, 2H), 6.60 (s, 1H), 6.70 (s, 1H), 7.38 (d, J = 7.0 Hz, 2H), 7.66 (d, J = 7.0 Hz, 2H); ^{13}CNMR (50.3 MHz, $CDCl_3$) δ (ppm) 17.7 (CH_3), 18.9 (CH_3), 19.4 (CH_2), 21.6 (2 × CH_3), 25.8 (CH_3), 26.4

(CH_2), 26.7 (CH), 27.5 (CH_2), 33.5 (CH_2), 38.1 (CH), 42.5 (CH), 69.0 (CH_2), 109.6 (CH), 122.8 (CH), 125.1 (CH), 128.4 (2 × CH), 128.7 (2 × CH), 129.0 (C), 131.2 (C), 133.4 (C), 134.8 (C), 136.4 (C), 140.8 (C), 156.0 (C). HRMS (ESI) for $C_{27}H_{35}ClO$: calcd 410.2376 found: 410.3746.

5.1.8.5. Leubethanol 4-nitrobenzyl ether (11e). Yield: 52 mg (41% after chromatography); yellow oil. ^1H NMR (200 MHz, $CDCl_3$) δ (ppm), 0.98 (d, J = 6.8 Hz, 3H), 1.21 (d, J = 6.8 Hz, 3H), 1.1–1.4 (m, 2H), 1.51–2.07 (m, 7H), 1.56 (s, 3H), 1.66 (s, 3H), 2.30 (s, 3H), 2.60 (m, 1H), 3.25 (m, 1H), 4.99 (t, J = 6.5 Hz, 1H), 5.17 (s, 2H), 6.53 (s, 1H), 6.67 (s, 1H), 7.64 (d, J = 8.6, 2H), 8.27 (d, J = 8.6 Hz, 2H); ^{13}C NMR (50.3 MHz, $CDCl_3$) δ (ppm) 17.7 (CH_3), 18.8 (CH_3), 19.3 (CH_2), 21.6 (2 × CH_3), 25.8 (CH_3), 26.3 (CH_2), 26.7 (CH), 27.4 (CH_2), 33.5 (CH_2), 38.1 (CH), 42.4 (CH), 68.5 (CH_2), 109.5 (CH), 123.1 (CH), 123.8 (2 × CH), 125.1 (CH), 127.3 (2 × CH), 129.0 (C), 131.2 (C), 134.9 (C), 141.1 (C), 145.4 (C), 147.6 (C), 155.6 (C). HRMS (ESI) for $C_{27}H_{35}NO_3$: calcd 421.2617 found: 421.3359.

5.1.8.6. Leubethanol 3,5-dinitrobenzyl ether (11f). Yield: 52 mg (36% after chromatography); yellow oil. IR (ν): 2926, 2868, 1609, 1544, 1343, 1269, 875 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$) δ (ppm) 0.98 (d, J = 6.8 Hz, 3H), 1.10–1.30 (m, 2H), 1.23 (d, J = 6.8 Hz, 3H), 1.50–2.03 (m, 7H), 1.60 (s, 3H), 1.65 (s, 3H), 2.31 (s, 3H), 2.60 (m, 1H), 3.28 (m, 1H), 4.98 (t, J = 6.4 Hz, 1H), 5.26 (s, 2H), 6.54 (s, 1H), 6.70 (s, 1H), 8.70 (d, J = 1.0 Hz, 2H), 9.02 (d, J = 1.0 Hz, 1H); ^{13}C NMR (50.3 MHz, $CDCl_3$) δ (ppm) 17.7 (CH_3), 18.8 (CH_3), 19.3 (CH_2), 21.6 (2 × CH_3), 25.8 (CH_3), 26.3 (CH_2), 26.8 (CH), 27.3 (CH_2), 33.5 (CH_2), 38.1 (CH), 42.4 (CH), 67.1 (CH_2), 109.1 (CH), 118.1 (CH), 123.7 (CH), 124.9 (CH), 126.8 (2 × CH), 129.1 (C), 131.3 (C), 135.0 (C), 141.4 (C), 142.6 (C), 148.8 (2 × C), 155.0 (C). HRMS (ESI) for $C_{27}H_{34}N_2O_5$: calcd 466.2468 found: 466.4132.

5.1.8.7. Leubethanol 5-(3,5-dinitrobenzyl) (12). Yield: 21 mg (15% after chromatography); yellow oil. IR (ν): 3400, 2926, 2873, 1591, 1541, 1452, 1344, 1241 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$) δ (ppm), 0.80 (d, J = 6.8 Hz, 3H), 1.1–1.2 (m, 2H), 1.24 (d, J = 7.2 Hz, 3H), 1.35 (m, 2H), 1.5–1.7 (m, 4H), 1.52 (s, 3H), 1.64 (s, 3H), 1.88 (m, 1H), 2.05 (s, 3H), 2.64 (m, 1H), 3.20 (m, 1H), 4.09 (d, J = 17.1 Hz, 1H), 4.25 (d, J = 17.2 Hz, 1H), 4.76 (br s, OH), 4.92 (t, J = 6.5 Hz, 1H), 6.54 (s, 1H), 8.13 (d, J = 2.1, 2H), 8.34 (d, J = 2.1 Hz, 1H); ^{13}C NMR (50.3 MHz, $CDCl_3$) δ (ppm) 17.7 (CH_3), 18.1 (CH_3), 20.2 (CH_2), 20.5 (CH_3), 22.4 (CH_3), 25.4 (CH_2), 25.8 (CH_3), 26.7 (CH_2), 26.8 (CH), 34.4 (CH_2), 35.2 (CH_2), 38.5 (CH), 39.9 (CH), 115.9 (CH), 116.6 (C), 124.5 (CH), 127.9 (2 × CH), 131.6 (2 × C), 134.8 (CH), 134.8 (C), 139.7 (C), 146.2 (C), 148.6 (2 × C), 154.2 (C). HRMS (ESI) for $C_{27}H_{34}N_2O_5$: calcd 466.2468; found: 466.3596.

5.1.9. General procedure for the preparation of 11g–11j

To a stirring solution of leubethanol (0.25 mmol) in 2 mL of dry acetone, NaH (0.25 mmol) was added. Then, after 30 min 0.12 mmol of Br-(CH_2)$_n$-Br (n = 4 or 6) was added and maintained for 6 h at room temperature. The crude mixture was extracted with ethyl acetate, washed with water and the organic layer dried over Na_2SO_4. The resulting products were purified by silica flash chromatography using hexane/toluene (9:1) as eluent.

5.1.9.1. Leubethanol 4-bromobutyl ether (11g). Yield: 22 mg (20% after chromatography); yellow oil. ^1H NMR (200 MHz, $CDCl_3$) δ (ppm) 0.97 (d, J = 6.8 Hz, 3H), 1.10–1.30 (m, 2H), 1.14 (d, J = 6.8 Hz, 3H), 1.50–2.07 (m, 11H), 1.56 (s, 3H), 1.66 (s, 3H), 2.21 (s, 3H), 2.57 (m, 1H), 3.14 (m, 1H), 3.52 (t, J = 6.5 Hz, 2H), 3.98 (t, J = 6.5 Hz, 2H), 4.99 (t, J = 6.5 Hz, 1H), 6.47 (s, 1H), 6.60 (s, 1H); ^{13}C NMR (50.3 MHz, $CDCl_3$) δ (ppm) 17.7 (CH_3), 18.8 (CH_3), 19.4 (CH_2), 21.6 (2 × CH_3), 25.8 (CH_3), 26.3 (CH_2), 26.6 (CH), 27.5 (CH_2), 28.8 (CH_2), 29.8 (CH_2), 33.5 (CH_2), 33.8 (CH_2), 38.1 (CH), 42.4 (CH), 66.4 (CH_2), 109.0 (CH), 122.3 (CH), 125.1 (CH), 128.7 (C), 131.2 (C), 134.7 (C), 140.6 (C), 156.2 (C). HRMS (ESI) for $C_{24}H_{37}BrO$: calcd 420.2028; found: 420.2231.

*5.1.9.1.1. Compound **11h**.* Yield: 56 mg (36% after chromatography); yellow oil. ^1H NMR (200 MHz, $CDCl_3$) δ (ppm) 0.97 (d, J = 6.8 Hz, 2 × 3H), 1.15 (d, J = 6.8 Hz, 2 × 3H), 1.30–2.09 (m, 2 × 9H), 1.56 (s, 2 × 3H), 1.66 (s, 2 × 3H), 2.02 (m, 2 × 2H), 2.29 (s, 2 × 3H), 2.56 (m, 2 × 1H), 3.16 (m, 2 × 1H), 3.95 (t, J = 6.8 Hz, 2 × 2H), 5.00 (t, J = 6.5 Hz, 2 × 1H), 6.49 (s, 2 × 1H), 6.59 (s, 2 × 1H); ^{13}C NMR (50.3 MHz, $CDCl_3$) δ (ppm) 17.8 (2 × CH_3), 18.8 (2 × CH_3), 19.4 (2 × CH_2), 21.6 (2 × CH_3), 21.7 (2 × CH_3), 25.8 (2 × CH_3), 26.1 (2 × CH_2), 26.3 (2 × CH_2), 26.6 (2 × CH), 27.6 (2 × CH_2), 33.5 (2 × CH_2), 38.0 (2 × CH), 42.5 (2 × CH), 67.1 (2 × CH_2), 109.0 (2 × CH), 122.0 (2 × CH), 125.1 (2 × CH), 128.7 (2 × C), 131.2 (2 × C), 134.7 (2 × C), 140.5 (2 × C), 156.5 (2 × C). HRMS (ESI) for $C_{44}H_{66}O_2$: calcd 626.5063; found: 626.6128.

5.1.9.2. Leubethanol 6-bromohexyl ether (11i). Yield: 31 mg (27% after chromatography); yellow oil. ^1H NMR (200 MHz, $CDCl_3$) δ (ppm) 0.97 (d, J = 6.8 Hz, 3H), 1.10–1.30 (m, 2H), 1.15 (d, J = 6.8 Hz, 3H), 1.50–2.07 (m, 7H), 1.56 (s, 3H), 1.66 (s, 3H), 1.88 (m, 8H), 2.30 (s, 3H), 2.57 (m, 1H), 3.15 (m, 1H), 3.43 (t, J = 6.8 Hz, 2H), 3.96 (t, J = 6.8 Hz, 2H), 5.00 (t, J = 6.5 Hz, 1H), 6.48 (s, 1H), 6.60 (s, 1H); ^{13}C NMR (50.3 MHz, $CDCl_3$) δ (ppm) 17.7 (CH_3), 18.8 (CH_3), 19.5 (CH_2), 21.6 (CH_3), 21.7 (CH_3), 25.6 (CH_2), 25.8 (CH_3), 26.4 (CH_2), 26.6 (CH), 27.6 (CH_2), 28.0 (CH_2), 29.4 (CH_2), 32.8 (CH_2), 33.5 (CH_2), 33.9 (CH_2), 38.0 (CH), 42.4 (CH), 67.2 (CH_2), 109.0 (CH), 122.1 (CH), 125.1 (CH), 128.7 (C), 131.1 (C), 134.7 (C), 140.5 (C), 156.4 (C). HRMS (ESI) for $C_{26}H_{41}BrO$: calcd 448.2341; found: 448.5296.

*5.1.9.2.1. Compound **11j**.* Yield: 74 mg (45% after chromatography); yellow oil. ^1H NMR (200 MHz, $CDCl_3$) δ (ppm) 0.97 (d, J = 6.8 Hz, 2 × 3H), 1.10–1.30 (m, 2 × 2H), 1.15 (d, J = 6.8 Hz, 2 × 3H), 1.50–2.09 (m, 2 × 7H), 1.56 (s, 2 × 3H), 1.66 (s, 2 × 3H), 1.88 (m, 8H), 2.29 (s, 2 × 3H), 2.56 (m, 2 × 1H), 3.16 (m, 2 × 1H), 3.95 (t, J = 6.8 Hz, 2 × 2H), 5.00 (t, J = 6.5 Hz, 2 × 1H), 6.49 (s, 2 × 1H), 6.59 (s, 2 × 1H); ^{13}C NMR (50.3 MHz, $CDCl_3$) δ (ppm) 17.8 (2 × CH_3), 18.8 (2 × CH_3), 19.4 (2 × CH_2), 21.6 (2 × CH_3), 21.7 (2 × CH_3), 25.8 (2 × CH_3), 26.1 (2 × CH_2), 26.3 (2 × CH_2), 26.6 (2 × CH), 27.6 (2 × CH_2), 29.5 (2 × CH_2), 33.5 (2 × CH_2), 38.0 (2 × CH), 42.5 (2 × CH), 67.4 (2 × CH_2), 109.0 (2 × CH), 122.0 (2 × CH), 125.1 (2 × CH), 128.7 (2 × C), 131.2 (2 × C), 134.7 (2 × C), 140.5 (2 × C), 156.5 (2 × C). HRMS (ESI) for $C_{46}H_{70}O_2$: calcd 656.5533; found: 656.9119.

5.1.10. Preparation of 11k

To a cooled solution of leubethanol (0.25 mmol) in 1 mL of pyridine, 1 mL of acetic anhydride was added and the mixture stirred for 12 h on an ice bath. The product was extracted with ethyl acetate, washed with 2 N HCl, with water to pH 7 and dried over Na_2SO_4. The solvent was removed under vacuum and the crude mixture purified by silica flash chromatography using hexane/ethyl acetate (9:1) to obtain compound **11k**.

5.1.10.1. Leubethanyl acetate (11k). Yield: 70 mg (85% after chromatography); yellow oil. IR (ν): 2954, 2926, 1765, 1619, 1571, 1207 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$) δ (ppm) 0.99 (d, J = 6.8 Hz, 3H), 1.10–1.31 (m, 2H), 1.15 (d, J = 6.8 Hz, 3H), 1.50–2.07 (m, 4H), 1.58 (s, 3H), 1.68 (s, 3H), 1.82–2.01 (m, 3H), 2.31 (s, 3H), 2.32 (s, 3H), 2.61 (m, 1H), 2.96 (m, 1H), 5.00 (t, J = 6.4 Hz, 1H), 6.71 (s, 1H), 6.89 (s, 1H); $^{13}CNMR$ (50.3 MHz, $CDCl_3$) δ (ppm) 17.7 (CH_3), 18.8 (CH_3), 19.3 (CH_2), 21.2 (3 × CH_3), 25.8 (CH_3), 26.3 (CH_2), 27.2 (CH), 27.5 (CH_2), 33.3 (CH_2), 38.0 (CH), 42.4 (CH), 120.3 (CH), 124.9 (CH), 127.6 (CH), 131.2 (C), 131.6 (C), 135.1 (C),

141.2 (C), 148.7 (C), 169.8 (CO). HRMS (ESI) for $C_{22}H_{32}O_2$: calcd 328.2402; found: 328.2591.

5.1.11. General procedure for the preparation of 11m–11p

To a stirring solution of leubethanol (0.20 mmol) in 2 mL of dry ethyl ether, NaH (0.20 mmol) was added. Then, after 30 min 0.20 mmol of the correspondent acyl halide was added and the mixture maintained for 8 h at room temperature. The product was extracted with ethyl acetate, washed with water and the organic layer dried over Na_2SO_4. The solvent was removed under vacuum and the crude mixture purified by silica flash chromatography using hexane/ethyl acetate (9:1) as eluent, to obtain esters **11m–11p**.

5.1.11.1. Leubethanyl hexanoate (11m). Yield: 53 mg (69% after chromatography); yellow oil. ^{1}H NMR (200 MHz, $CDCl_3$) δ (ppm) 0.93 (t, J = 7.1 Hz, 3H), 0.98 (d, J = 6.8 Hz, 3H), 1.10–1.30 (m, 2H), 1.14 (d, J = 6.8 Hz, 3H), 1.33 (m, 4H), 1.50–1.97 (m, 5H), 1.57 (s, 3H), 1.67 (s, 3H), 1.80–2.10 (m, 6H), 2.30 (s, 3H), 2.57 (t, J = 7.2 Hz, 2H), 2.61 (m, 1H), 2.94 (m, 1H), 5.00 (t, J = 6.4 Hz, 1H), 6.68 (s, 1H), 6.86 (s, 1H); ^{13}CNMR (50.3 MHz, $CDCl_3$) δ (ppm) 14.1 (CH_3), 17.7 (CH_3), 18.8 (CH_3), 19.3 (CH_2), 21.1 (CH_3), 21.8 (CH_3), 22.6 (CH_2), 25.1 (CH_2), 25.8 (CH_3), 26.3 (CH_2), 27.3 (CH_2), 27.3 (CH), 29.0 (CH_2), 31.6 (CH_2), 33.3 (CH_2), 34.6 (CH_2), 38.0 (CH), 42.3 (CH), 120.4 (CH), 124.9 (CH), 127.6 (CH), 130.6 (C), 131.6 (C), 135.1 (C), 141.2 (C), 148.7 (C), 172.5 (CO). HRMS (ESI) calcd for $C_{27}H_{42}O_2$ 398.3185; found: 398.6275.

5.1.11.2. Leubethanyl benzoate (11n). Yield: 63 mg (80% after chromatography); yellow oil. ^{1}H NMR (200 MHz, $CDCl_3$) δ (ppm), 1.03 (d, J = 6.8 Hz, 3H), 1.21 (d, J = 6.6 Hz, 3H), 1.1–1.2 (m, 2H), 1.50–2.0 (m, 7H), 1.61 (s, 3H), 1.70 (s, 3H), 2.36 (s, 3H), 2.67 (m, 1H), 3.07 (m, 1H), 5.00 (t, J = 6.5 Hz, 1H), 6.88 (s, 1H), 6.95 (s, 1H), 7.54 (dd, J = 7.6; 7.5 Hz, 2H), 7.64 (dd, J = 7.5; 1.1 Hz, 1H), 8.36 (dd, J = 7.6; 1.1 Hz, 2H); ^{13}CNMR (50.3 MHz, $CDCl_3$) δ (ppm) 17.7 (CH_3), 18.8 (CH_3), 19.4 (CH_2), 21.2 (CH_3), 22.1 (CH_3), 25.8 (CH_3), 26.3 (CH_2), 27.3 (CH_2), 27.3 (CH), 33.4 (CH_2), 38.1 (CH), 40.4 (CH), 120.5 (CH), 125.0 (CH), 127.9 (CH), 128.7 (2 × CH), 130.1 (2 × CH), 131.3 (2 × C), 131.9 (C), 133.5 (CH), 135.2 (C), 141.3 (C), 148.9 (C), 165.3 (CO). HRMS (ESI) calcd for $C_{27}H_{34}O_2$ 390.2559; found: 390.6541.

5.1.11.3. Leubethanyl 4-nitrobenzoate (11o). Yield: 40 mg (46% after chromatography); yellow oil. IR (ν): 2959, 2926, 1741, 1609, 1530, 1348, 1250, 716 cm^{-1}. ^{1}H NMR (200 MHz, $CDCl_3$) δ (ppm), 1.00 (d, J = 6.5 Hz, 3H), 1.16 (d, J = 6.8 Hz, 3H), 1.1–1.3 (m, 2H), 1.50–2.03 (m, 7H), 1.57 (s, 3H), 1.67 (s, 3H), 2.34 (s, 3H), 2.65 (m, 1H), 2.98 (m, 1H), 5.00 (t, J = 6.5 Hz, 1H), 6.85 (s, 1H), 6.95 (s, 1H), 8.34 (br s, 4H); ^{13}C NMR (50.3 MHz, $CDCl_3$) δ (ppm) 17.7 (CH_3), 18.8 (CH_3), 19.2 (CH_2), 21.2 (CH_3), 22.1 (CH_3), 25.8 (CH_3), 26.3 (CH_2), 26.3 (CH), 27.3 (CH_2), 33.3 (CH_2), 38.1 (CH), 42.3 (CH), 120.1 (CH), 123.8 (2 × CH), 124.9 (CH), 128.2 (CH), 131.2 (2 × CH), 131.6 (2 × C), 135.4 (2 × C), 141.6 (C), 148.5 (C), 150.9 (C), 163.5 (CO). HRMS (ESI) calcd for $C_{27}H_{33}NO_2$ 435.2410; found: 435.2619.

5.1.11.4. Leubethanyl 2-furoate (11p). Yield: 62 mg (85% after chromatography); yellow oil. ^{1}H NMR (200 MHz, $CDCl_3$) δ (ppm), 1.01 (d, J = 6.8 Hz, 3H), 1.18 (d, J = 6.8 Hz, 3H), 1.11–1.32 (m, 2H), 1.50–2.09 (m, 7H), 1.59 (s, 3H), 1.69 (s, 3H), 2.34 (s, 3H), 2.65 (m, 1H), 3.05 (m, 1H), 5.02 (t, J = 6.5 Hz, 1H), 6.61 (dd, J = 3.4; 1.6 Hz, 1H), 6.66 (s, 1H); 6.93 (s, 1H), 7.38 (br d, J = 3.4 Hz, 1H), 7.69 (dd, J = 1.6; 0.8 Hz, 1H); ^{13}C NMR (50.3 MHz, $CDCl_3$) δ (ppm) 17.7 (CH_3), 18.8 (CH_3), 19.3 (CH_2), 21.2 (CH_3), 22.0 (CH_3), 25.8 (CH_3), 26.3 (CH_2), 27.3 (CH), 27.4 (CH_2), 33.3 (CH_2), 38.1 (CH), 42.4 (CH), 112.2 (CH), 119.0 (CH), 120.3 (CH), 124.9 (CH), 127.9 (CH), 131.3 (C), 131.9 (C), 135.2 (C), 141.3 (C), 144.4 (C), 147.1 (CH), 148.1 (C), 157.2 (C). HRMS (ESI) calcd for $C_{25}H_{32}O_3$ 380.2351; found: 380.3568.

5.2. Biochemical studies

The antimycobacterial activity was assessed against *M. tuberculosis* H37Rv ATTC 27294 susceptible to all five first-line anti-TB drugs (streptomycin, isoniazid, rifampin, ethambutol, and pyrazinamide) in a modified Microplate Assay Blue Alamar.[11,12] The compounds for *M. tuberculosis* bioassays were prepared at a concentration of 1 mg/mL in 2.5% DMSO in Middlebrook 7H9 (Becton Dickinson and Co., Sparks MD, USA) broth. All solutions were sterilized by filtration using 13 mm diameter PTFE acrodiscs (0.22 μm pore size, Millipore Co., Bedford, MA, USA): The concentrations for organic compounds used ranged form 100 μg/mL to 0.78 μg/mL, results are reported as minimal inhibition concentration (MIC). Ethambutol (EMB) was used as positive control. All biological assays were developed at least by triplicate.

5.3. Cytotoxicity assay

Cytotoxicity was determined according to the MTT method[13] using African green monkey kidney epithelial cells (Vero cells) grown in RPMI-1640 medium supplemented with penicillin (100 units/mL), streptomycin (100 μg/mL), and fetal bovine serum (10%) at 37 °C under 5% CO_2. In the confluence, 4000 cells per well was applied to a 96-well microtitre plate. After incubation for 24 h (37 °C, 5% CO_2), 100 μL of a solution containing concentrations ranging from 500 to 0.5 μg/mL of the compound under evaluation was added and incubated for 48 h. 25 μL of MTT (4 mg/mL in PBS) was added to each well and incubated for another 3 h. The solution in each well was removed and DMSO (200 μL) was then added to each well. The absorbance was recorded on a microplate reader at a wavelength of 575 nm. The CC_{50} value was calculated by linear regression as the concentration of the compound inhibiting 50% cellular viability.

Acknowledgements

Collaborative research performed under the auspices of Mexican Network (PROMED) and CYTED-X.11: PIBATUB. R.E. thanks the research contract financed by ISCIII-RICET. B.A. thanks the Ph.D. fellowship received from CONACYT, Mexico.

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13. *Assay:* The modified Microplate Alamar Blue Assay (MABA) was used to determine the anti-*MTB* activity. Summarily, solutions of compounds (2 mg/mL, DMSO), standard drug, EMB.HCl (2 mg/mL, H_2O), were sterilised by filtration (PTFE acrodiscs, 0.22 μm pore size, Millipore Co., Bedford, MA, USA). Std. drugs were divided in 0.5 mL aliquots, and stored at −70 °C until used, while the compounds were assayed immediately. Deionised sterile water (200 μL) was dispensed in the perimeter wells to diminish evaporation. The inner wells were divided in lanes of 6 to perform the assays. 100 μL of a 1:50 bacterial suspension (having about 6×10^6 CFU/mL, Mc Farland std. 1) in Middlebrock 7H9-OADC enriched broth (Becton Dickinson and Co., Sparks, MD, USA), were added to each testing well. In the 6-well lanes, compound and standard drug solutions were two-step serially diluted with 7H9-OADC medium (100 μL per well). Evaluations were performed on dilutions within the ranges: 1.0–32 μg/mL (EMB). A blank (100 μL of medium), and a positive mycobacterial growth control (100 μL of 1:50 bacterial suspension) were included. After incubation (37 °C for 5–7 days in 5% CO_2 atmosphere, into plastic O_2 permeable sealed bag), 32 μL of freshly prepared 20:12 mixture Alamar Blue plus 10% Tween-80 (v/v), were added to each well. The plates were re-incubated at 37 °C for 24 h. Pink and blue colors indicated mycobacterial growth or its absence, respectively. The MIC value for a tested compound, was assigned to that of the first blue well. Destruction of mycobacteria in the first blue well was confirmed microscopically.
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