Sesterterpenes as TTL Inhibitors. First Insight of Structure-Activity Relationships and Discovery of New Lead.

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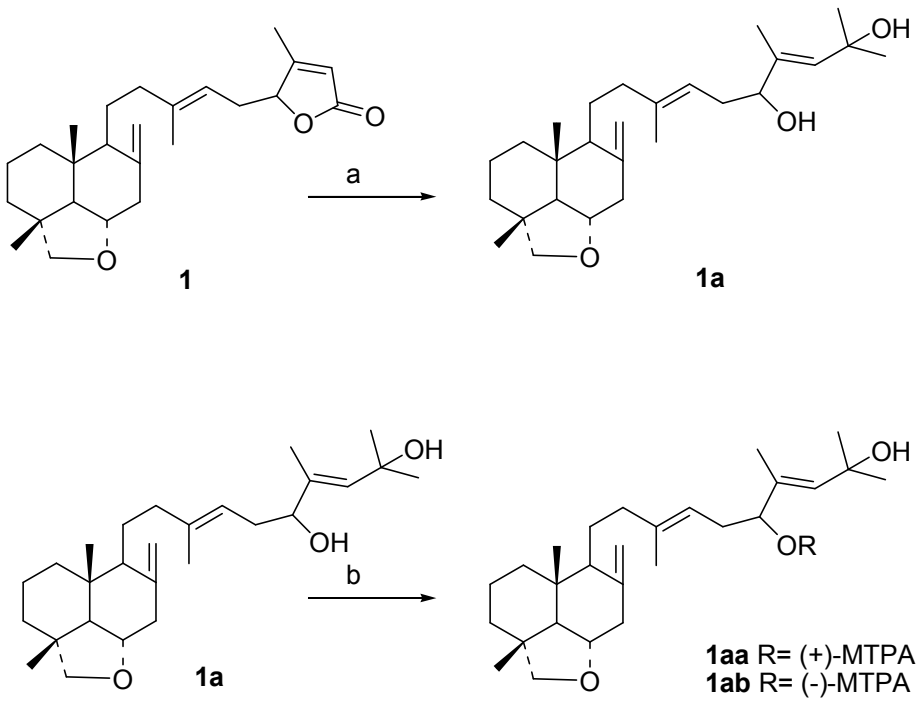
Table of Contents

SI1. Stereochemistry of Compound 1

Due to the anisotropic effect of the benzene ring, negative $\Delta\delta$ (=δ(-)-δ(+)) values were obtained for the signals ascribable to protons attached to the carbons at C-18 (-0.05) and C-20 (-0.03) of (+)-MTPA and (-)-MTPA esters of compound **1**a, while positive $\Delta\delta$ values were obtained for the signals due to the protons at C-15 (+0.04, +0.03 respectively), C-14 (+0.09), and C-21 (+0.03).
Compound 1a: Colorless amorphous powder. ESIMS *m/z* 439 $[M+Na]^+$, $C_{27}H_{44}O_3$. ^1H NMR data (CD_3OD, 600 MHz) δ 1.30 (2H, m, H-11), 1.35 (3H, s, Me at C-19), 1.39 (3H, s, Me at C-19), 1.70 (3H, br s, Me-21), 1.78 (3H, br s, Me-20), 1.99 (1H, ddd, *J* = 12.4, 4.5, 5.5 Hz, H-12b), 2.02 (1H, ddd, *J* = 12.4, 11.0, 4.5 Hz, H-12a), 2.28 (1H, ddd, *J* = 13.0, 5.5, 3.0 Hz, H-12a), 2.52 (1H, ddd, *J* = 13.0, 7.5, 3.0 Hz, H-12a), 4.00 (

Scheme 1[a]



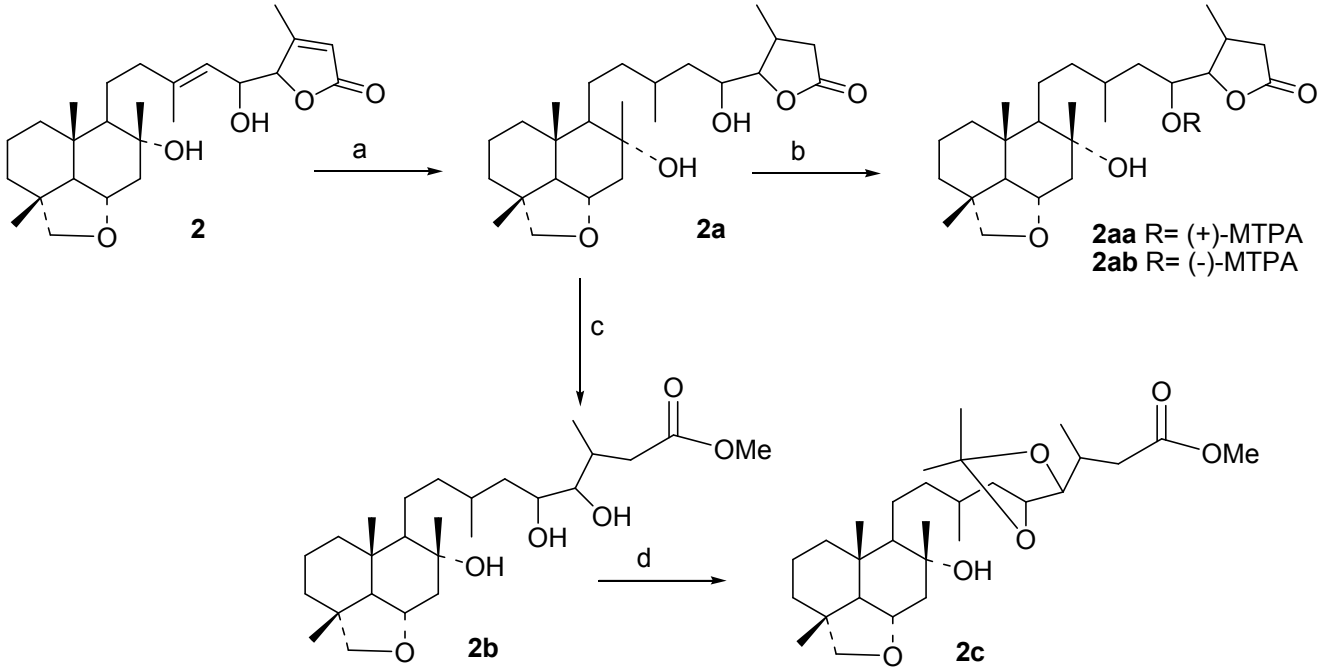
[a]Reagents and conditions: (a) methyl lithium, diethyl ether, 0°C; (b) (+)- (MTPA) or (-)- (MTPA), DCC, DMAP, 25°C.

SI2. Stereochemistry of Compound 2

Comparison of relevant ^1H NMR chemical shift differences between the *R*- and *S*-MTPA esters [H-14a (-0.05), H-14b (-0.03), H-13 (-0.02), H-21 (-0.02), H-18a (+0.07), H-18b (+0.05), H-16 (+0.05), H-17 (+0.02)] showed that the ^1H NMR signals associate with the lactone ring system in the (R)-MTPA ester were shifted upfield relative to those of the (S)-MTPA, and the opposite was true for the resonances of the H-13, H-14, H-21. This is a proof for the C-15 (S)-configuration of **2**.
Compound 2a: Colorless amorphous powder. ESIMS *m/z* 445 $[M+Na]^+$, $C_{25}H_{42}O_5$. ^1H NMR data

(CD_3OD, 600 MHz) δ 0.98 (3H, d, *J* =6.5 Hz, Me-20), 1.00 (3H, d, *J* =6.0 Hz, Me-21), 1.38 (1H, m, H-12a), 1.40 (2H, m, H-14), 1.43 (1H, m, H-12b), 1.65 (1H, m, H-13), 2.28 (1H, dd, d, *J* =10.0, 5.5 Hz, H-18), 2.56 (1H, m, H-17), 2.70 (1H, dd, d, *J* =10.0, 7.0 Hz, H-18), 3.91 (1H, dd, d, *J* =8.0, 4.0 Hz, H-15), 4.36 (1H, br t, *J* =8.2 Hz, H-16).

Scheme 2[a]



[a]Reagents and conditions: (a) H_2/Pd,EtOH; (b) (+)- (MTPA) or (-)- (MTPA), DCC, DMAP, 25°C; (c) KOH 60°C, CH_2N_2, Et_2O 0°C; (d) 2,2-dimethoxypropane, THF, p-TsOH 25°C

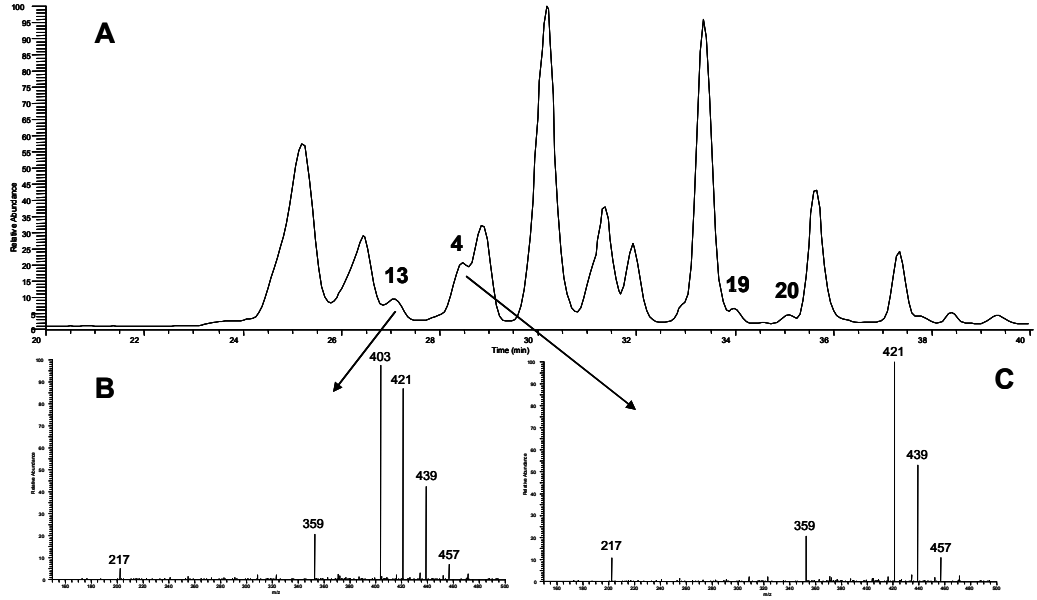
SI3. Extraction and isolation

The dried aerial parts of *S. dominica* (180 g) were powdered and exhaustively extracted using hexane (3.8 g), chloroform (7.2 g), chloroform-methanol (3.5 g), methanol (6 g), by ASE 2000.
Part of $CHCl_3$-MeOH residue (3.0 g) was submitted to chromatographic separation on a Sephadex LH-20 column, using MeOH as mobile phase; fractions were collected, analysed by TLC on silica 60 F_{254} gel-coated glass sheets with and $CHCl_3$-MeOH-H_2O (40:9:1), $CHCl_3$-MeOH (9:1), and grouped to obtain eight fractions (1-8). Fraction 3 (420 mg) was purified by preliminary SPE followed by RP-HPLC with MeOH-H_2O (67:33) as mobile phase (flow rate 2.0 ml min^{-1}) to afford pure compounds **2** (21.0 mg, t_R = 20 min), **3** (4.0 mg, t_R = 21 min), **5** (5.0 mg, t_R = 33 min), **6** (10.0 mg, t_R = 45 min), **7** (30 mg, t_R = 12 min), **8** (9 mg, t_R = 26 min), **13** (24.0 mg, t_R = 8 min), **14** (6.0 mg, t_R =16 min). Fraction 4 (72 mg) was chromatographed over RP-HPLC with MeOH-H_2O (67:33) as mobile phase (flow rate 2.0 ml min^{-1}) to yield pure compounds **2** (19 mg, t_R = 20 min), **3** (2.5 mg, t_R =21 min), **4** (3.5 mg, t_R = 24 min), **7** (13.0 mg, t_R = 12 min), **8** (3.0 mg, t_R = 26 min), and **13** (8.0 mg, t_R = 8 min). Fraction 5 (60 mg) was subjected to RP-HPLC with MeOH-H_2O (3:2)

as eluent (flow rate 2.0 ml min^{-1}) to yield pure compounds **3** (4.0 mg, t_R = 20 min), **4** (2.0 mg, t_R = 33 min), and **10** (15 mg, t_R = 22 min). Fraction 7 contained pure rosmarinic acid (12 mg).
Part of chloroform extract (6.0 g) was subjected to column chromatography using silica gel and eluting with $CHCl_3$ followed by increasing concentrations of MeOH in $CHCl_3$ (between 1% and 70%). Fractions of 50 ml were collected, analysed by TLC (silica gel plates, in $CHCl_3$ or mixtures $CHCl_3$-MeOH 99:1, 98:2, 97:3, 9:1, 4:1; $CHCl_3$-MeOH-H_2O 40:9:1), and grouped into 20 fractions (A-V). Fraction V contained pure rosmarinic acid (7 mg). Fraction F (40 mg) was subjected to RP-HPLC with MeOH-H_2O (33:22) as eluent (flow rate 2.0 ml min^{-1}) to give pure compounds **3** (3.0 mg, t_R = 19 min), and **5** (6 mg, t_R = 30 min). Fraction G (45 mg) was subjected to RP-HPLC with MeOH-H_2O (7:3) as mobile phase (flow rate 2.0 ml min^{-1}) to yield compounds **3** (15.0 mg, t_R = 12 min), **4** (11.0 mg, t_R = 23 min), **5** (8.0 mg, t_R = 35 min), and **11** (3.0 mg, t_R = 52 min). Fraction H (95 mg) was purified by RP-HPLC using MeOH-H_2O (34:16) as mobile phase (flow rate 2.0 ml min^{-1}) to give compounds **3** (3.0 mg, t_R = 20 min), **4** (5.0 mg, t_R = 20 min), **6** (3.0 mg, t_R = 36 min). Fraction M (99 mg) was subjected to RP-HPLC with MeOH-H_2O (3:2) as eluent (flow rate 2.0 ml min^{-1}) to give pure compounds **1** (9.0 mg, t_R = 45 min), **8** (10.0 mg, t_R = 30 min), **9** (3.5 mg, t_R = 27 min), **12** (6.5 mg, t_R = 18 min), **13** (15.0 mg, t_R = 25 min). Fraction O (99 mg) was purified by preliminary SPE followed by RP-HPLC with MeOH-H_2O (3:2) (flow rate 2.0 ml min^{-1}) to give pure compounds **1** (22.0 mg, t_R = 45 min), **2** (16.0 mg, t_R = 42 min), **13** (3.0 mg, t_R = 25 min), **16** (4.0 mg, t_R = 32 min), **18** (18.5 mg, t_R = 68 min). Fraction P (214 mg) was subjected to RP-HPLC using MeOH-H_2O (58:42) (flow rate 2.0 ml min^{-1}) to give pure compounds **2** (14. 0 mg, t_R = 45 min), **10** (2.0 mg, t_R = 23 min), **13** (9.0 mg, t_R = 27 min), **17** (10.0 mg, t_R = 21 min), **19** (15.0 mg, t_R = 27 min), **20** (12.0 mg, t_R = 35 min), **21** (16.0 mg, t_R = 32 min), and **22** (10.0 mg, t_R = 18 min). Fraction Q (312 mg) was subjected to RP-HPLC with MeOH-H_2O (58:42) as mobile phase (flow rate 2.0 ml min^{-1}) to give pure compounds **7** (35.0 mg, t_R = 33 min), **10** (3.0 mg, t_R = 22 min), and **13** (4.0 mg, t_R = 28 min). Fraction R (250 mg) was subjected to RP-HPLC with MeOH-H_2O (58:42) (flow rate 2.0 ml min^{-1}) to give pure compounds **7** (22.0 mg, t_R = 34 min), **9** (4.0 mg, t_R = 38 min), **10** (4.0 mg, t_R = 23 min), **13** (3.0 mg, t_R = 27 min), **15** (4.0 mg, t_R = 52 min). Fraction S (72 mg) was subjected to RP-HPLC using MeOH-H_2O (55:45) as mobile phase (flow rate 2.0 ml min^{-1}) to give pure compounds **7** (15.0 mg, t_R = 53 min), **13** (3.0 mg, t_R = 40 min), **22** (9 mg, t_R = 24 min). Fraction T (110 mg) was subjected to RP-HPLC using MeOH-H_2O (55:45) as eluent (flow rate 2.0 ml min^{-1}) to give pure compounds **7** (4.0 mg, t_R = 53 min), **23** (14 mg, t_R = 24 min), **24** (11 mg, t_R = 47 min).

SI4. LC/MS^n analysis of crude acetone extract of *S. dominica*

To confirm the co-presence of compounds **13** and **4**, and **19** and **20**, the crude acetone extract of *S dominica* underwent LC/MS^n analysis. MS and MS/MS spectra of peaks at 27.2 min, 28.6 min, 34.0 min and 35.1 min allowed us to identify them as compounds **13**, **4**, **19** and **20**, respectively LC/MS^n analysis was performed in positive ion mode on a ThermoFinnigan LCQ Deca XP Max ion-trap mass spectrometer equipped with Xcalibur software. Chreomatographic separation was achieved using a Luna C_{18} (150x2 mm, Phenomenex, USA) column and 0.1% TFA in H_2O (solvent A) and 0.1% TFA in CH_3CN (solvent B) mobile phase. A 50 min linear gradient from 35 to 85 % of solvent B was used.



LC/MS^n analysis of acetone crude extract of *S. dominica*: Total ion current chromatogram (A). MS^2 spectra of ion at m/z 457 detected at 27.2 min (B). MS^2 spectra of ion at m/z 457 detected at 28.6 min (C).

SI5. Cytoxicity of Compound 11