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Functional Structure/Activity Relationships

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Easy access to alkoxy, amino, carbamoyl, hydroxy and thiol derivatives of sesquiterpene lactones and evaluation of their bioactivity on parasitic weeds

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ABSTRACT

It has been hypothesized that the α-methylene-γ-lactone moiety of sesquiterpene lactones is a key unit for their bioactivity. As a consequence, modifications of these compounds have been focused on this fragment. In the work reported here, two sesquiterpene lactones, namely dehydrocostuslactone and β-cyclocostunolide, a eudesmanolide obtained by controlled cyclization of costunolide, were chosen for modification by Michael addition at C-13. On applying this reaction to both compounds it was possible to introduce the functional groups alkoxy, amino, carbamoyl, hydroxy and thiol to give products in good to high yields depending on the base and solvent employed. In particular, the introduction of a thiol group at C-13 in both compounds was achieved with outstanding yields (>90%) and this is unprecedented for these sesquiterpene lactones. The bioactivities of the products were evaluated on etiolated wheat coleoptile elongation and germination of seeds of parasitic weeds, with significant activity observed on *Orobanche cumana* and *Phelipanche ramosa*. The structure-activity relationships are discussed.

Keywords: sesquiterpene lactone, etiolated wheat coleoptiles, seed germination, dehydrocostuslactone, costunolide, Michael addition.

INTRODUCTION

Sunflower (*Helianthus annuus*) is rich in natural products and it exudes a wide variety of sesquiterpenes.[1,2] In particular, the sesquiterpene lactones dehydrocostuslactone (**1**) and costunolide (**2**) are bioactive compounds exuded by the roots of this plant[3] and they are also produced in higher quantities in the roots of *Saussurea costus* (Falc.) Lipsch, along with other bioactive compounds for chemical defense.[4–6] Moreover, compounds **1** and **2** are germination elicitors for *Orobanche cumana* Wallr, which is a highly specific parasitic weed to sunflower.[7,8]

It is widely believed that the key structural feature for the specific parasitic recognition of sesquiterpene lactones is the α-methylene-γ-lactone moiety.[7] In this respect, the methylene could react with a receptor nucleophile through a Michael addition reaction. For this reason, the aforementioned fragment has been identified as a key feature of parasitic weed seed-germination elicitors.[9] Nevertheless, sesquiterpene lactones that do not contain this unsaturation, such as 13-hydroxy-dehydrocostuslactone, have been found to actively promote germination of *O. cumana* seeds.[10,11] Regarding eudesmanolides, compounds lacking that unsaturation are also active eliciting germination of parasitic weeds. For instance, no significant differences were observed in the germination profiles of *Phelipanche ramosa* seeds either tested with dihydrosantamarine or anhydrojudaicin, when compared with the profiles of their unsaturated counterparts santamarine and 11,13-dehydroanhydrojudaicin.[12] Other examples are the bioactive 11,13-saturated eudesmanolides erivanin and isoerivanin, with antibacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa*.[13]

As part of a project to identify new germination stimulants of parasitic weed seeds based on natural products, we selected dehydrocostuslactone (**1**) and β-cyclocostunolide (**3**) (figures 1 and 2), a eudesmanolide derived from costunolide (**2**), to perform derivatization of the methylene in the lactone fragment (at position C-13). The aim was to introduce different functional groups and to test the bioactivities of the resulting compounds. Important aspects for these procedures were good yields and easy access to the target compounds. The effects of the changes on the bioactivity were evaluated.

The α-methylene-γ-lactone fragment is a good Michael acceptor and we carried out this reaction with different bases and nucleophiles to synthesize hydroxy-, thiol-, carbamoyl-, amino-, and alkoxy 13-derivatives of **1** and **3** (compounds **4–16**, figures 1 and 2). The procedure described herein is also compared with the previously described hydroxylation of **1**.[11]

MATERIALS AND METHODS

General Experimental Procedures. The main objective of this study was the synthesis of different derivatives of the sesquiterpene lactones dehydrocostuslactone (**1**) and costunolide (**2**) by the introduction of new functional groups at C-13. The two starting materials were chosen due to the good yields achieved on isolating the compounds from a *Saussurea costus* root extract by column chromatography, with a mixture of *n*-hexane:EtOAc (95:5) used as eluent. Compound **1** was used directly to obtain derivatives whereas **2** was first isomerized to eudesmanolide lactones α-, β- and γ-cyclocostunolide, with β-cyclocostunolide (**3**) employed to synthesize the derivatives as this was the major product of the cyclization of costunolide. New functional groups introduced at C-13 were hydroxyl (**5** and **7**), thiol (**9** and **11**), amino (**12**), carbamoyl (**13**) and alkoxy (**14**–**16**). The bioactivities of the products were evaluated in two bioassays: the growth of etiolated wheat coleoptiles and stimulatory activity for the germination of seeds of three species of the problematic parasitic weed broomrape.

The purity of each compound was assessed by ^{1}H NMR spectroscopy prior to the bioactivity tests. The structural determination of all compounds was carried out by combining different 1D- (^{1}H, ^{13}C) and 2D-NMR (^{1}H-^{1}H COSY, NOESY, ^{1}H-^{13}C HSQC and HMBC) experiments along with specific rotation, UV, FTIR and, in particular, MS to determine the molecular formulae. ^{1}H NMR and ^{13}C NMR spectra were recorded on Agilent spectrometers at 400 and 500 MHz using $CDCl_3$ (MagniSolv™, Merck) as solvent. The residual solvent peaks were used as internal reference (δ 7.26 ppm in ^{1}H and δ 77.0 ppm in ^{13}C NMR for $CDCl_3$). COSY, HSQC, HMBC and NOESY experiments were performed using Varian vnmrj microprograms. Exact masses were measured on a UPLC-QTOF ESI (Waters Synapt G2, Manchester, UK) high-resolution mass spectrometer (HRTOFESIMS). Mass spectra were recorded in the negative- or

positive-ion mode in the range *m*/*z* 100–2000, with a mass resolution of 20,000 and an acceleration voltage of 0.7 kV. FTIR spectra were obtained on Perkin-Elmer Spectrum TWO IR spectrophotometer. Major absorptions in the infrared are given as wavenumbers ῡ in cm^{-1}. Optical rotations were measured in $CHCl_3$ on a JASCO P-2000 polarimeter.

Column chromatography (CC) was performed on silica gel (Merck, Geduran® Si 60, 0.063–0.200 mm) and C_{18}-reversed phase silica gel (Sigma-Aldrich, C_{18} phase 90 A pore size). The reagents and solvents were supplied by either Sigma-Aldrich Co. (St. Louis, Missouri), Merck (Darmstadt, Germany) or Alfa Aesar (Ward Hill, Massachusetts).

Synthesis of derivatives. The starting materials **1** and **2** were isolated from a natural source, i.e., a *Saussurea costus* root extract, according to the previously described procedure.[4] Compound **3** was obtained by cyclization of **2** with *p*-toluenesulfonic acid according to the reported procedure with some modifications (figure 3).[14] Compound **2** (99 mg, 0.426 mmol) was dissolved in CH_2Cl_2 (5 mL) and *p*-toluenesulfonic acid (20 mg, 0.116 mmol) was added to the stirred solution at room temperature. The mixture was stirred for 4 hours and the reaction was quenched with saturated aqueous $NaHCO_3$ (25 mL) and extracted three times with CH_2Cl_2 (25 mL). The combined organic layers were washed with brine (50 mL), dried over anhydrous Na_2SO_4 and the solvent was evaporated under vacuum to obtain a crude product, which was purified by column chromatography with a gradient of *n*-hexane:EtOAc 1:0–17:3 to give three products: **3** (51 mg, 52%), **17** (34 mg, 34%) and **18** (6 mg, 6%). These compounds were identified as β-, α- and γ-costunolide, respectively, by comparing their NMR data with available literature data (the ^{1}H NMR spectrum of **3** included in the supporting information for comparison).[14–16]

Synthesis of alcohols 5 and 7. This synthesis was carried out in two steps as shown in figures 1 and 2 for compounds **1** and **3**. The procedure for compound **5** is described as a representative example. Compound **1** (200 mg, 0.868 mmol) was dissolved in 4-methoxybenzyl alcohol (2 mL, 15.62 mmol). 1,8-Diazabicyclo[5.4.0]undec-7-ene (DBU, 1.35 mL, 8.85 mmol) was added and the mixture was stirred for 2 days at room temperature. The mixture was diluted with EtOAc (75 mL) and washed with brine (2 × 20

mL), dried over anhydrous Na_2SO_4 and the solvent was evaporated under vacuum. The crude product was purified by column chromatography using *n*-hexane/EtOAc 4:1 as eluent to give compound **4** (200 mg, 63%), traces of its epimer, and unreacted **1** (60 mg, 30%).

In order to obtain the alcohol, the oxidation was carried out according to a procedure similar to that reported in the literature.[17] The ether **4** (699 mg, 1.90 mmol) was dissolved in a round-bottomed flask containing CH_2Cl_2 (17 mL) at 0 ºC and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ, 646 mg, 2.85 mmol) and deionized H_2O (850 µL, 5% v/v) were added to the mixture. This mixture was vigorously stirred for 6 hours at 0 ºC and then diluted with CH_2Cl_2, dried over $MgSO_4$ and the solvent was evaporated under vacuum. The crude product was purified by column chromatography using *n*-hexane/EtOAc 3:2 as eluent to give compound **5** (406 mg, 86%) in an overall yield of 54%.

The same procedure was employed to convert **3** (31 mg, 0.133 mmol) into **6** (22 mg, 46%), and **6** (108 mg, 0.292 mmol) into **7** (47 mg, 62%), with a global yield of 29%.

Synthesis of thiols 9 and 11. As shown in figures 1 and 2, compound **1** (63 mg, 0.274 mmol) was dissolved in MeOH (2 mL) at 0 ºC and a solution of the thiol (57 µL, 0.408 mmol) in MeOH (2 mL) was added at 0 ºC. Et_3N (107 µL, 0.761 mmol) was added. The mixture was stirred at 0 ºC for 30 minutes and then allowed to warm up to rt and stirred for a total reaction time of 4 hours. The mixture was diluted with EtOAc and the MeOH solvent was evaporated under vacuum. The residue was purified by column chromatography with a gradient of *n*-hexane:EtOAc from 1:0–17:3 to give **8** (76 mg, 72%) and traces of its epimer at C-11.

Thiol **9** was obtained by the oxidation procedure with DDQ as described above. Compound **8** (76 mg, 0.198 mmol) was dissolved in CH_2Cl_2 (2 mL) and the solution was treated with DDQ (67 mg, 0.295 mmol) and H_2O (100 µL). The mixture was allowed to warm up and to rt and, after a total reaction time of 24 h, the crude product was purified by column chromatography with *n*-hexane:EtOAc as eluent in a gradient of 1:0–4:1. Compound **9** (52 mg, 99%) was obtained and the global yield was 71% for the two steps.

This two-step procedure was successfully applied to **3** (53 mg, 0.228 mmol) to give the thioether **10** in quantitative yield (88 mg, 100%). Similarly, compound **10** (49 mg, 0.127 mmol) was successfully oxidized to obtain **11** in good yield (32 mg, 94%).

Synthesis of amine 12 and amide 13. Two possible paths were explored to obtain amine **12** and amide **13** (figures 1 and 2) with the aim of obtaining a higher yield and selectivity for the former or the latter.

Procedure A. Synthesis of amides at position C-13. Compound **1** (100 mg, 0.434 mmol) was dissolved in formamide (5 mL, 125 mmol) with the assistance of an ultrasound bath at rt. After complete dissolution of **1**, DBU (1 mL, 10 mmol) was added and the mixture was stirred at rt for 6 days and quenched with Sorensen buffer solution (pH 7, 10 mL). The resulting mixture was extracted with EtOAc (5 × 20 mL) and the combined organic layers were washed with brine (2 × 50 mL), dried over anhydrous Na_2SO_4 and the solvent was evaporated under vacuum. Purification of the residue by column chromatography using a gradient of *n*-hexane:EtOAc 3:2–3:2 as eluent yielded **13** (66 mg, 56%), traces of its epimer at C-11 and **12** as a minor product (8 mg, 7%).

Procedure B. Synthesis of amines at position C-13. Compound **1** (200 mg, 0.868 mmol) was dissolved in tetrahydrofuran (THF, 10 mL). Formamide (800 μL, 20 mmol), *n*-Bu_4NBr (56 mg, 0.174 mmol) and 2 M NaOH (10 mL, 20 mmol) were added. The mixture was stirred and heated at 70 ºC for 24 h. Sorensen buffer solution (pH 7, 10 mL) was added to quench the reaction. Brine (20 mL) was added to the mixture and the product was extracted with EtOAc (8 × 20 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and the solvent was evaporated under vacuum. The residue was subjected to column chromatography using a gradient of *n*-hexane:EtOAc 1:0–3:7 as eluent to give **12** (159 mg, 74%), traces of its epimer at C-11 and **13** as a minor product (17 mg, 7%).

Synthesis of methoxy derivatives 15 and 16. The procedure described in the literature[18] was applied to compound **3** but with a longer reaction time and a higher amount of MeONa per mL of MeOH. Specifically, **3** (47 mg, 0.202 mmol) was dissolved in MeOH (5 mL), MeONa (33 mg, 0.611 mmol) was

added and the mixture was stirred at rt for 48 h. The MeOH was evaporated under vacuum almost to dryness. The mixture was diluted with EtOAc (50 mL) and brine (50 mL) was added. After extraction with EtOAc (3 × 50 mL), the organic layers were combined, dried over anhydrous Na_2SO_4 and the solvent was evaporated under vacuum. Purification of the residue by column chromatography using mixtures of *n*-hexane:EtOAc 1:0–4:1 as eluent yielded **15** (25 mg, 47%), its epimer **16** (5 mg, 9%) and unreacted **3** (11 mg, 23%).

Experimental data for the new compounds. Compounds **5** and **14** were previously reported in the literature and were identified by comparing their spectroscopic data with those available.[11,18] The ^{1}H and ^{13}C NMR spectra of these compounds are included in the supporting information for completeness. Data for the new compounds are listed below.

(3*R*,3a*S*,6a*R*,9a*R*,9b*S*)-3-{[(4-methoxybenzyl)oxy]methyl}-6,9-dimethylenedecahydroazu-leno[4,5-*b*]furan-2(9b*H*)-one (4). A colorless oil, the spectroscopic data are as follows: HRMS, *m*/*z* calcd for $C_{23}H_{28}O_4H$ 369.2066 $[M + H]^+$, found 369.2069; IR $\tilde{\nu}_{max}$ 3080, 2930, 2860, 1771 (lactone C=O), 1641 (C=C), 1612, 1586, 1513 cm^{-1}; $[\alpha]^{20}_D = -9.0°$ (*c* = 0.03); UV λ_{max} (MeOH) 274 nm (ε 1680), 226 nm (ε 13460), 202 nm (ε 28720). ^{1}H NMR (500 MHz, $CDCl_3$, δ, ppm): 2.88 (dt, *J* = 8.2, 4.2 Hz, 1H, H-1), 1.86 (dddd, *J* = 13.4, 8.7, 4.2, 5.6 Hz, 1H, H-2α), 1.93 (dt, *J* = 13.4, 8.7 Hz, 1H, H-2β), 2.51 (m, 2H, H-3), 2.82 (t, *J* = 9.3 Hz, 1H, H-5), 3.93 (t, *J* = 9.3 Hz, 1H, H-6), 2.42 (dd, *J* = 11.6, 4.5 Hz, 1H, H-7), 2.13 (ddt, *J* = 12.0, 5.2, 1.5 Hz, 1H, H-8α), 1.29 (ddd, *J* = 12.0, 5.1, 1.6 Hz, 1H, H-8β), 2.03 (dt, *J* = 13.2, 5.2 Hz, 1H, H-9α), 2.44 (dt, *J* = 13.2, 5.2 Hz, 1H, H-9β), 2.39 (ddd, *J* = 11.6, 4.6, 3.6 Hz, 1H, H-11), 3.76 (dd, *J* = 9.8, 4.6 Hz, 1H, H-13α), 3.69 (dd, *J* = 9.8, 3.6 Hz, 1H, H-13β), 4.86 (s, 1H, H-14), 4.76 (s, 1H, H-14′), 5.20 (d, *J* = 2.3 Hz, 1H, H-15), 5.04 (s, 1H, H-15′), 4.52 (d, *J* = 11.8 Hz, 1H, H-1′a), 4.45 (d, *J* = 11.8 Hz, 1H, H-1′b), 7.25 (d, *J* = 8.7 Hz, 2H, H-3′, H-7′), 6.88 (d, *J* = 8.7 Hz, 2H, H-4′, H-6′), 3.81 (s, 3H, O$\underline{C}H_3$). ^{13}C NMR (125 MHz, $CDCl_3$, δ, ppm): 47.0 (C-1), 30.2 (C-2), 32.6 (C-3), 151.7 (C-4), 51.8 (C-5), 85.5 (C-6), 44.4 (C-7), 32.5 (C-8), 37.7 (C-9), 150.0 (C-10), 48.0 (C-11), 176.1 (C-12), 66.3 (C-13), 111.7 (C-14), 109.1 (C-15), 73.1 (C-1′), 130.1 (C-2′), 129.2 (C3′, C-7′), 113.8 (C-4′, C-6′), 159.2 (C-5′), 55.3 (O$\underline{C}H_3$).

(3*R*,3a*S*,5a*R*,9a*S*,9b*S*)-3-{[(4-methoxybenzyl)oxy]methyl}-5a-methyl-9-methylenedecahydronaphthol[1,2-*b*]furan-2(3*H*)-one (6). A colorless oil, the spectroscopic data are as follows: HRMS, *m*/*z* calcd for $C_{23}H_{29}O_4$ 369.2066 $[M-H]^-$, found 369.2085; IR $\tilde{\nu}_{max}$ 2929, 1757 (lactone C=O), 1612 (C=C), 1516, 1456 cm^{-1}; $[\alpha]^{20}_D$ = +52° (*c* = 0.1); UV λ_{max} (MeOH) 256 nm (ε 12580). ^{1}H NMR (500 MHz, $CDCl_3$, δ, ppm): 1.46 (dt, *J* = 12.9, 2.6 Hz, 1H, H-1a), 1.37 (m, 1H, H-1b), 1.62 (m, 2H, H-2), 2.31 (m, 1H, H-3a), 1.99 (dt, *J* = 12.6, 6.8 Hz, 1H, H-3b), 2.15 (d, *J* = 10.8 Hz, 1H, H-5), 3.98 (t, *J* = 10.8 Hz, 1H, H-6), 2.05 (ddd, *J* = 12.7, 10.8, 3.6 Hz, 1H, H-7), 1.88 (m, 1H, H-8a), 1.54 (m, 1H, H-8b), 1.57 (m, 1H, H-9a), 1.36 (m, 1H, H-9b), 2.53 (ddd, *J* = 12.7, 5.8, 3.7 Hz, 1H, H-11), 3.78 (dd, *J* = 9.9, 3.7 Hz, 1H, H-13β), 3.70 (d, *J* = 9.9, 5.8 Hz, 1H, H-13α), 0.84 (s, 3H, H-14), 4.91 (s, 1H, H-15), 4.74 (s, 1H, H-15′), 4.49 (d, *J* = 11.7 Hz, 1H, H-1′a), 4.44 (d, *J* = 11.7 Hz, 1H, H-1′b), 7.23 (d, *J* = 8.7 Hz, 2H, H-3′, H-7′), 6.87 (d, *J* = 8.7 Hz, 2H, H-4′, H-6′), 3.80 (s, 3H, OC<u>H</u>$_3$). ^{13}C NMR (125 MHz, $CDCl_3$, δ, ppm): 41.7 (C-1), 22.8 (C-2), 35.9 (C-3), 144.7 (C-4), 54.4 (C-5), 79.9 (C-6), 48.3 (C-7), 23.5 (C-8), 39.9 (C-9), 38.3 (C-10), 46.9 (C-11), 176.7 (C-12), 66.7 (C-13), 18.0 (C-14), 108.7 (C-15), 73.0 (C-1′), 130.0 (C-2′), 129.2 (C3′, C-7′), 113.7 (C-4′, C-6′), 159.1 (C-5′), 55.2 (O<u>C</u>H$_3$).

(3*R*,3a*S*,5a*R*,9a*S*,9b*S*)-3-(hydroxymethyl)-5a-methyl-9-methylenedecahydronaphtho[1,2-*b*]furan-2(3*H*)-one (7). A colorless solid with m.p. 140–141 ºC, the spectroscopic data are as follows: HRMS, *m*/*z* calcd for $C_{15}H_{21}O_3$ 249.1491 $[M-H]^-$, found 249.1514; IR $\tilde{\nu}_{max}$ 3450 (O–H), 2926, 1765 (lactone C=O) cm^{-1}; $[\alpha]^{20}_D$ = +128° (*c* = 0.01). ^{1}H NMR (500 MHz, $CDCl_3$, δ, ppm): 1.47 (ddt, *J* = 12.9, 3.6, 1.3 Hz, 1H, H-1a), 1.37 (m, 1H, H-1b), 1.60 (m, 2H, H-2), 2.32 (m, 1H, H-3a), 1.99 (m, 1H, H-3b), 2.16 (d, *J* = 10.9 Hz, 1H, H-5), 4.05 (t, *J* = 10.8 Hz, 1H, H-6), 2.02 (m, 1H, H-7), 1.85 (m, 1H, H-8a), 1.58 (m, 1H, H-8b), 1.58 (m, 1H, H-9a), 1.36 (m, 1H, H-9b), 2.52 (ddd, *J* = 12.9, 5.6, 4.2 Hz, 1H, H-11), 3.96 (dd, *J* = 11.6, 4.2 Hz, 1H, H-13β), 3.77 (dd, *J* = 11.6, 5.7 Hz, 1H, H-13α), 0.85 (s, 3H, H-14), 4.91 (d, *J* = 1.5 Hz, 1H, H-15), 4.73 (d, *J* = 1.5 Hz, 1H, H-15′). ^{13}C NMR (125 MHz, $CDCl_3$, δ, ppm): 41.7 (C-1), 22.8 (C-2), 35.9 (C-3), 144.7 (C-4), 54.4 (C-5), 80.4 (C-6), 46.8 (C-7), 23.2 (C-8), 39.8 (C-9), 38.4 (C-10), 48.6 (C-11), 178.3 (C-12), 59.5 (C-13), 18.0 (C-14), 108.8 (C-15).

(3*S*,3a*S*,6a*R*,9a*R*,9b*S*)-3-{[(4-methoxybenzyl)thio]methyl}-6,9-dimethylenedecahydroazule-no[4,5-*b*]furan-2(3*H*)-one (8). A colorless oil, the spectroscopic data are as follows: HRMS, *m*/*z* calcd for $C_{23}H_{28}O_3SH$ 385.1837 $[M + H]^+$, found 385.1835; IR $\tilde{\nu}_{max}$ 2923, 1769 (lactone C=O), 1640, 1609, 1511 cm^{-1}; $[\alpha]^{20}_D = -1.5°$ (*c* = 0.3); UV λ_{max} (MeOH) 230 nm (ε 5910). ^1H NMR (400 MHz, $CDCl_3$, δ, ppm): 2.84 (dt, *J* = 8.2, 4.4 Hz, 1H, H-1), 1.86 (dddd, *J* = 13.4, 8.5, 6.0, 4.4 Hz, 1H, H-2α), 1.92 (m, 1H, H-2β), 2.49 (m, 2H, H-3), 2.77 (t, *J* = 9.3 Hz, 1H, H-5), 3.89 (t, *J* = 9.3 Hz, 1H, H-6), 2.26 (dq, *J* = 11.4, 3.2 Hz, 1H, H-7), 2.13 (ddt, *J* = 12.4, 5.0, 3.8 Hz, 1H, H-8α), 1.24 (dd, *J* = 11.4, 5.1 Hz, 1H, H-8β), 1.98 (m, 1H, H-9α), 2.42 (m, 1H, H-9β), 2.40 (m, 1H, H-11), 2.79 (d, *J* = 4.7 Hz, 2H, H-13), 4.84 (s, 1H, H-14), 4.75 (s, 1H, H-14′), 5.17 (d, *J* = 1.9 Hz, 1H, H-15), 5.02 (d, *J* = 1.9 Hz, 1H, H-15′), 3.71 (d, *J* = 2.2 Hz, 2H, H-1′), 7.24 (d, *J* = 8.7 Hz, 2H, H-3′, H-7′), 6.84 (d, *J* = 8.7 Hz, 2H, H-4′, H-6′), 3.78 (s, 3H, O$C\underline{H}_3$). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 47.0 (C-1), 30.1 (C-2), 32.5 (C-3), 151.6 (C-4), 51.8 (C-5), 85.3 (C-6), 46.4 (C-7), 32.7 (C-8), 37.5 (C-9), 149.8 (C-10), 47.3 (C-11), 176.5 (C-12), 30.0 (C-13), 111.8 (C-14), 109.2 (C-15), 37.1 (C-1′), 130.0 (C-2′), 130.0 (C3′, C-7′), 113.9 (C-4′, C-6′), 158.7 (C-5′), 55.3 (O$\underline{C}H_3$).

(3*S*,3a*S*,6a*R*,9a*R*,9b*S*)-3-(mercaptomethyl)-6,9-dimethylenedecahydroazuleno[4,5-*b*]furan-2(3*H*)-one (9). A pale-yellow oil, the spectroscopic data are as follows: HRMS, *m*/*z* calcd for $C_{15}H_{20}O_2S$ 263.1106 $[M - H]^-$, found 263.1108; IR $\tilde{\nu}_{max}$ 3453, 2927, 1768 (lactone C=O), 1640, 1441 cm^{-1}; $[\alpha]^{20}_D = -50°$ (*c* = 0.3). ^1H NMR (400 MHz, $CDCl_3$, δ, ppm): 2.89 (dt, *J* = 8.0, 4.5 Hz, 1H, H-1), 1.86 (m, 1H, H-2α), 1.93 (m, 1H, H-2β), 2.50 (m, 2H, H-3), 2.81 (t, *J* = 9.1 Hz, 1H, H-5), 3.97 (t, *J* = 9.4 Hz, 1H, H-6), 2.37 (ddd, *J* = 11.5, 9.4, 3.2 Hz, 1H, H-7), 2.28 (ddt, *J* = 12.4, 4.6, 3.9 Hz, 1H, H-8α), 1.35 (dd, *J* = 11.5, 5.1 Hz, 1H, H-8β), 2.06 (dt, *J* = 11.9, 5.2 Hz, 1H, H-9α), 2.45 (*J* = 12.9, 4.6 Hz, 1H, H-9β), 2.66 (ddd, *J* = 11.5, 5.6, 4.9 Hz, 1H, H-11), 3.07 (dd, *J* = 13.9, 5.6 Hz, 1H, H-13α), 3.16 (dd, *J* = 13.9, 4.9 Hz, 1H, H-13β), 4.87 (s, 1H, H-14), 4.77 (s, 1H, H-14′), 5.17 (d, *J* = 1.9 Hz, 1H, H-15), 5.04 (d, *J* = 1.9 Hz, 1H, H-15′). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 47.1 (C-1), 30.1 (C-2), 32.5 (C-3), 151.5 (C-4), 51.9 (C-5), 85.3 (C-6), 46.1 (C-7), 32.6 (C-8), 37.3 (C-9), 149.6 (C-10), 46.5 (C-11), 176.3 (C-12), 36.8 (C-13), 112.0 (C-14), 109.2 (C-15).

(3*S*,3a*S*,5a*R*,9a*S*,9b*S*)-3-{[(4-methoxybenzyl)thio]methyl}-5a-methyl-9-methylenedecahydronaphtho[1,2-*b*]furan-2(3*H*)-one (10). A colorless solid with m.p. 110–111 ºC, the spectroscopic data are as follows: HRMS, *m*/*z* calcd for $C_{23}H_{30}O_3SH$ 387.1994 $[M + H]^+$, found 387.2000; IR $\tilde{\nu}_{max}$ 2928, 1773 (lactone C=O), 1650, 1609, 1511, cm^{-1}; $[\alpha]^{20}_D$ = +63° (*c* = 0.05); UV λ_{max} (MeOH) 229 nm (ε 7790). ^1H NMR (400 MHz, $CDCl_3$, δ, ppm): 1.46 (dt, *J* = 11.8, 3.1 Hz, 1H, H-1a), 1.38 (dd, *J* = 12.1, 6.0 Hz, 1H, H-1b), 1.62 (dt, *J* = 11.3, 4.0 Hz 2H, H-2), 2.31 (m, 1H, H-3a), 1.99 (m, 1H, H-3b), 2.12 (d, *J* = 10.8 Hz, 1H, H-5), 3.95 (t, *J* = 10.6 Hz, 1H, H-6), 1.92 (ddd, *J* = 12.2, 10.6, 3.4 Hz, 1H, H-7), 1.96 (dt, *J* = 6.3, 2.2 Hz, 1H, H-8a), 1.51 (m, 1H, H-8b), 1.55 (m, 1H, H-9a), 1.32 (dd, *J* = 12.1, 4.0 Hz, 1H, H-9b), 2.49 (ddd, *J* = 12.4, 7.4, 4.2 Hz, 1H, H-11), 2.91 (dd, *J* = 13.5, 4.2 Hz, 1H, H-13β), 2.69 (dd, *J* = 13.5, 7.4 Hz, 1H, H-13α), 0.83 (s, 3H, H-14), 4.91 (d, *J* = 1.4, 1H, H-15), 4.72 (d, *J* = 1.4, 1H, H-15′), 3.72 (s, 2H, H-1′), 7.24 (d, *J* = 8.7 Hz, 2H, H-3′, H-7′), 6.85 (d, *J* = 8.7 Hz, 2H, H-4′, H-6′), 3.80 (s, 3H, O$\underline{C}H_3$). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 41.7 (C-1), 22.9 (C-2), 36.0 (C-3), 144.7 (C-4), 54.4 (C-5), 79.7 (C-6), 50.5 (C-7), 23.8 (C-8), 39.9 (C-9), 38.3 (C-10), 46.4 (C-11), 177.1 (C-12), 30.1 (C-13), 18.0 (C-14), 108.8 (C-15), 37.0 (C-1′), 131.3 (C-2′), 130.0 (C3′, C-7′), 114.0 (C-4′, C-6′), 158.7 (C-5′), 55.3 (O$\underline{C}H_3$).

(3*S*,3a*S*,5a*R*,9a*S*,9b*S*)-3-{[(4-methoxybenzyl)thio]methyl}-5a-methyl-9-methylenedecahydronaphtho[1,2-*b*]furan-2(3*H*)-one (11). A colorless oil, the spectroscopic data are as follows: HRMS, *m*/*z* calcd for $C_{15}H_{22}O_2SH$ 267.1419 $[M + H]^+$, found 267.1417; IR $\tilde{\nu}_{max}$ 2928, 1774 (lactone C=O), 1650 cm^{-1}; $[\alpha]^{20}_D$ = +146° (*c* = 0.2). ^1H NMR (400 MHz, $CDCl_3$, δ, ppm): 1.45 (dt, *J* = 13.2, 2.7 Hz, 1H, H-1a), 1.38 (m, 1H, H-1b), 1.62 (dt, *J* = 10.5, 3.6 Hz 2H, H-2), 2.31 (bd, *J* = 13.2 Hz, 1H, H-3a), 1.99 (m, 1H, H-3b), 2.14 (d, *J* = 10.9 Hz, 1H, H-5), 4.03 (t, *J* = 10.6 Hz, 1H, H-6), 1.97 (ddd, *J* = 12.2, 10.6, 3.5 Hz, 1H, H-7), 2.06 (dt, *J* = 6.6, 3.2 Hz, 1H, H-8a), 1.61 (m, 1H, H-8b), 1.57 (m, 1H, H-9a), 1.35 (dd, *J* = 13.3, 3.5 Hz, 1H, H-9b), 2.79 (ddd, *J* = 12.5, 7.2, 4.2 Hz, 1H, H-11), 3.24 (dd, *J* = 13.8, 4.3 Hz, 1H, H-13β), 2.91 (dd, *J* = 13.8, 7.3 Hz, 1H, H-13α), 0.85 (s, 3H, H-14), 4.91 (s, 1H, H-15), 4.72 (s, 1H, H-15′). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 41.7 (C-1), 22.8 (C-2), 35.9 (C-3), 144.6 (C-4), 54.4 (C-5), 79.7 (C-6), 50.4

(C-7), 23.8 (C-8), 39.9 (C-9), 38.3 (C-10), 45.7 (C-11), 176.9 (C-12), 36.9 (C-13), 18.0 (C-14), 108.8 (C-15).

(3*R*,3a*S*,6a*R*,9a*R*,9b*S*)-3-(aminomethyl)-6,9-dimethylenedecahydroazuleno[4,5-*b*]furan-2(9b*H*)-one (12). A colorless solid with m.p. 111–121 ºC, the spectroscopic data are as follows: HRMS, *m*/*z* calcd for $C_{15}H_{21}NO_2H$ 248.1651 $[M + H]^+$, found 248.1655; IR $\tilde{\nu}_{max}$ 2925, 2854, 1767 (lactone C=O), 1640 (C=C), 1455 cm^{-1}; $[\alpha]^{20}_D$ = +4.7° (*c* = 0.5). ^{1}H NMR (500 MHz, $CDCl_3$, δ, ppm): 2.90 (dt, *J* = 8.2, 4.1 Hz, 1H, H-1), 1.86 (dt, *J* = 13.4, 4.9 Hz, 1H, H-2α), 1.94 (ddt, *J* = 13.4, 8.1, 1.4 Hz, 1H, H-2β), 2.52 (m, 2H, H-3), 2.79 (t, *J* = 9.4 Hz, 1H, H-5), 3.95 (t, *J* = 9.4 Hz, 1H, H-6), 2.33 (m, 1H, H-7), 2.12 (m, 1H, H-8α), 1.33 (ddd, *J* = 11.6, 5.4, 4.5 Hz, 1H, H-8β), 2.05 (dt, *J* = 12.5, 5.4 Hz, 1H, H-9α), 2.48 (m, 1H, H-9β), 2.33 (dt, *J* = 5.4, 3.7 Hz, 1H, H-11), 2.88 (dd, *J* = 12.7, 5.4 Hz, 1H, H-13α), 3.00 (dd, *J* = 12.7, 3.7 Hz, 1H, H-13β), 4.87 (s, 1H, H-14), 4.76 (s, 1H, H-14′), 5.19 (d, *J* = 1.8 Hz, 1H, H-15), 5.04 (d, *J* = 1.8 Hz, 1H, H-15′). ^{13}C NMR (125 MHz, $CDCl_3$, δ, ppm): 47.0 (C-1), 30.2 (d, C-2), 32.6 (C-3), 151.8 (C-4), 52.1 (C-5), 85.8 (C-6), 44.6 (C-7), 32.5 (C-8), 37.7 (C-9), 149.9 (C-10), 47.8 (C-11), 177.7 (C-12), 47.4 (C-13), 111.8 (C-14), 109.0 (C-15).

***N*-{[(3*R*,3a*S*,6a*R*,9a*R*,9b*S*)-6,9-dimethylene-2-oxododecahydroazuleno[4,5-*b*]furan-3-yl]methyl}formamide (13).** A colorless solid with m.p. 111–121 ºC, the spectroscopic data are as follows: HRMS, *m*/*z* calcd for $C_{15}H_{21}NO_2H$ 248.1651 $[M + H]^+$, found 248.1655; IR $\tilde{\nu}_{max}$ 2925, 2854, 1767 (lactone C=O), 1640 (C=C), 1455 cm^{-1}; $[\alpha]^{20}_D$ = +4.7° (*c* = 0.5). ^{1}H NMR (500 MHz, $CDCl_3$, δ, ppm): 2.87 (dt, *J* = 8.3, 4.5 Hz, 1H, H-1), 1.86 (dt, *J* = 13.4, 4.5 Hz, 1H, H-2α), 1.94 (ddt, *J* = 13.4, 9.2, 1.5 Hz, 1H, H-2β), 2.53 (m, 2H, H-3), 2.79 (t, *J* = 9.5 Hz, 1H, H-5), 3.99 (t, *J* = 9.5 Hz, 1H, H-6), 2.26 (ddd, *J* = 13.0, 9.5, 3.7 Hz, 1H, H-7), 2.12 (dt, *J* = 11.7, 3.3 Hz, 1H, H-8α), 1.34 (ddd, *J* = 11.7, 4.9, 1.4 Hz, 1H, H-8β), 2.05 (dt, *J* = 12.4, 4.9 Hz, 1H, H-9α), 2.48 (dt, *J* = 12.4, 5.2 Hz, 1H, H-9β), 2.38 (dd, *J* = 13.0, 6.6, 3.9 Hz, 1H, H-11), 3.48 (dd, *J* = 14.0, 6.6 Hz, 1H, H-13α), 3.73 (dd, *J* = 14.0, 3.9 Hz, 1H, H-13β), 4.89 (s, 1H, H-14), 4.78 (s, 1H, H-14′), 5.16 (d, *J* = 2.0 Hz, 1H, H-15), 5.05 (d, *J* = 2.0 Hz, 1H, H-15′), 6.42 (bs, 1H, NH), 8.22 (s, 1H, CHO). ^{13}C NMR (125 MHz, $CDCl_3$, δ, ppm): 47.0 (C-1), 30.1 (d, C-2), 32.5 (C-3), 151.5 (C-4), 51.7 (C-

5), 86.1 (C-6), 45.0 (C-7), 32.3 (C-8), 37.5 (C-9), 149.6 (C-10), 47.7 (C-11), 177.4 (C-12), 35.1 (C-13), 112.1 (C-14), 109.2 (C-15), 161.4 (C-16).

(3*R*,3a*S*,5a*R*,9a*S*,9b*S*)-3-(methoxymethyl)-5a-methyl-9-methylenedecahydronaphtho[1,2-*b*]furan-2(3*H*)-one (15). A colorless solid with m.p. 66–71 ºC, the spectroscopic data are as follows: HRMS, *m*/*z* calcd for $C_{16}H_{23}O_3$ 263.1647 $[M - H]^-$, found 263.1653; IR $\tilde{\nu}_{max}$ 2929, 1782 (lactone C=O), 1650, 1458 cm^{-1}; $[\alpha]^{20}_D$ = +70° (*c* = 0.2). ^1H NMR (400 MHz, $CDCl_3$, δ, ppm): 1.43 (dt, *J* = 13.0, 3.5 Hz, 1H, H-1a), 1.37 (m, 1H, H-1b), 1.62 (dt, *J* = 10.5, 3.4 Hz, 2H, H-2), 2.31 (bd, *J* = 13.2 Hz, 1H, H-3a), 1.99 (m, 1H, H-3b), 2.15 (d, *J* = 10.9 Hz, 1H, H-5), 3.99 (t, *J* = 10.7 Hz, 1H, H-6), 2.03 (ddt, *J* = 12.5, 10.8, 3.6 Hz, 1H, H-7), 1.93 (m, 1H, H-8a), 1.55 (m, 1H, H-8b), 1.57 (m, 1H, H-9a), 1.38 (m, 1H, H-9b), 2.51 (ddd, *J* = 12.7, 5.7, 3.8 Hz, 1H, H-11), 3.72 (dd, *J* = 9.9, 3.8 Hz, 1H, H-13β), 3.63 (d, *J* = 9.9, 5.7 Hz, 1H, H-13α), 0.85 (s, 3H, H-14), 4.91 (d, *J* = 1.4, 1H, H-15), 4.74 (d, *J* = 1.4 Hz, 1H, H-15′), 3.36 (s, 3H, $OC\underline{H}_3$). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 41.8 (C-1), 22.9 (C-2), 36.0 (C-3), 144.7 (C-4), 54.5 (C-5), 79.9 (C-6), 48.3 (C-7), 23.6 (C-8), 40.0 (C-9), 38.4 (C-10), 46.9 (C-11), 176.6 (C-12), 69.6 (C-13), 18.0 (C-14), 108.8 (C-15), 59.2 ($O\underline{C}H_3$).

(3*S*,3a*S*,5a*R*,9a*S*,9b*S*)-3-(methoxymethyl)-5a-methyl-9-methylenedecahydronaphtho[1,2-*b*]furan-2(3*H*)-one (16). A colorless oil, the spectroscopic data are as follows: HRMS, *m*/*z* calcd for $C_{16}H_{24}O_3H$ 265.1804 $[M + H]^+$, found 265.1810; IR $\tilde{\nu}_{max}$ 2928, 1773 (lactone C=O), 1649, 1458 cm^{-1}; $[\alpha]^{20}_D$ = +82° (*c* = 0.03). ^1H NMR (400 MHz, $CDCl_3$, δ, ppm): 1.44 (dt, *J* = 12.5, 2.8 Hz, 1H, H-1a), 1.31 (t, *J* = 7.2 Hz, 1H, H-1b), 1.61 (m, 2H, H-2), 2.31 (bd, *J* = 13.2 Hz, 1H, H-3a), 1.99 (m, 1H, H-3b), 2.08 (d, *J* = 11.0 Hz, 1H, H-5), 4.41 (t, *J* = 11.0 Hz, 1H, H-6), 2.15 (ddt, *J* = 13.4, 8.5, 5.1 Hz, 1H, H-7), 1.76 (dd, *J* = 9.3, 3.5 Hz, 1H, H-8a), 1.73 (dd, *J* = 9.5, 3.2 Hz, 1H, H-8b), 1.60 (dt, *J* = 11.2, 3.6 Hz, 1H, H-9a), 1.35 (m, 1H, H-9b), 2.64 (ddd, *J* = 8.2, 4.9, 3.2 Hz, 1H, H-11), 3.78 (dd, *J* = 9.6, 4.9 Hz, 1H, H-13β), 3.62 (d, *J* = 9.6, 3.2 Hz, 1H, H-13α), 0.84 (s, 3H, H-14), 4.90 (d, *J* = 1.5, 1H, H-15), 4.78 (d, *J* = 1.5 Hz, 1H, H-15′), 3.35 (s, 3H, $OC\underline{H}_3$). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 41.8 (C-1), 22.9 (C-2), 36.0 (C-3), 144.9 (C-4), 55.5 (C-5),

81.0 (C-6), 47.7 (C-7), 20.7 (C-8), 40.3 (C-9), 38.5 (C-10), 45.2 (C-11), 178.0 (C-12), 69.6 (C-13), 18.1 (C-14), 109.0 (C-15), 59.1 ($O\underline{C}H_3$).

Bioassays. The isolated compound **1** and the synthetic products **3–16** were tested in triplicate in the etiolated wheat (*Triticum aestivum* L.) coleoptile and the broomrape seed germination bioassays (on three species: *Orobanche cumana*, *Orobanche crenata* and *Phelipanche ramosa*) at the concentration ranges of 10^3–10 μM and 100–0.1 μM, respectively. The positive controls were the synthetic herbicide Logran® for the etiolated coleoptile bioassay and the synthetic compound GR24 for the broomrape bioassay. Negative controls were also included as specified in the experimental procedure. The procedures for performing these bioassays were previously reported in the literature[4,19–22] and are included in the supporting information for reference. The results are shown in figures 4 and 5. The etiolated wheat coleoptile bioassay is used to perform a quick evaluation (24 h) of the phytotoxic activity of bioactive compounds. The results are expressed as percentage difference against the negative control, where positive values represent stimulation of coleoptile growth and negative values represent inhibition.

Seeds for the etiolated wheat coleoptile bioassay were kindly supplied by Fitó (Barcelona, Spain) and seeds of *O. cumana*, *O. crenata* and *P. ramosa* were provided by the researcher Maurizio Vurro (Institute of Sciences of Food Production, National Council of Research, Bari, Italy).

Calculation of EC_{50}, IC_{50} and cLog *P*. The compounds that gave minimum activities of 50% and were active at more than one concentration were statistically analyzed for their EC_{50} using the GraphPad Prism v.5.00 software package (GraphPad Software, Inc., San Diego, USA). The bioactivity data were fitted to a sigmoidal dose-response model with constant slope. The results of this analysis are shown in table 1. The cLog *P* values were obtained by means of the appropriate tool in Chem Draw Professional v.17.1 (Perkin Elmer, Waltham, USA).

RESULTS AND DISCUSSION

The objective of the present study was the functionalization at C-13 of sesquiterpene lactones **1** and **3**, which contain an α-methylene-γ-lactone group. The synthesis started with the introduction of hydroxyl groups.

Synthesis of hydroxy-derivatives. A method for obtaining hydroxy-derivatives of **1** has been described previously and this involves the use of $CaCO_3$ and hexamethylphosphoramide at 90 ºC with a reaction time of 5 days.[11] However, the lack of selectivity for the monohydroxylated compounds and the low yields motivated us to use a different route to that reported in the literature. Inspired by the procedure reported by Pietras et al. in their patent for parthenin,[23] a two-step procedure involving Michael addition followed by oxidation with 2,3-Dichloro-5,6-dicyano-1,4-benzoquinone (figures 1 and 2) was employed.

Michael addition of 4-methoxybenzyl alcohol catalyzed with 1,8-Diazabicyclo[5.4.0]undec-7-ene provided ethers **4** and **6** from **1** and **3**, respectively. In addition, traces of the epimers of **4** and **6** were identified in the crudes ^{1}H NMR but were not isolated. The incorporation of a benzyl group in compounds **4** and **6** was evidenced by NMR experiments. The most noticeable change was the disappearance of two signals corresponding to the protons of the double bond at C-13 when compared with the starting material. The presence of the new signal for H-11 (δ 2.39 and 2.53 for **4** and **6**, respectively) and the signals for H-13 at higher field (δ 3.76 and 3.69 for **4**; δ 3.78 and 3.70 for **6**) was also observed. In addition, the presence of the benzyl group was evidenced by two characteristic doublets in the aromatic part of the spectra at δ 7.25–6.84 (^{1}H NMR). These signals corresponded to two protons each and correlated in HSQC experiments with two signals at δ 130–114 (^{13}C NMR). Further clear evidence for the incorporation of the benzyl group was the presence of a singlet at δ 3.80 (^{1}H NMR), which corresponds to the 3 protons of the methoxy group, and a signal at δ 55.3 (for compound **4**) and 55.2 (for compound **6**) in the ^{13}C NMR spectrum.

In the second step, ethers **4** and **6** were oxidized to the target alcohols **5** and **7**, respectively. The disappearance of the aromatic proton signals and the methoxy group singlet from the spectra confirmed the success of the oxidation with DDQ. Furthermore, the signals corresponding to H-13 were shifted to lower

337 field, from δ 3.78–3.69 to 3.99–3.75, while in the case of the ^{13}C NMR spectra, the C-13 signal moved to
338 higher field (from δ 66.3 for **4** to 59.6 for **5**; and from δ 66.7 for **6** to 59.5 for **7**).
339 The stereochemistry of the compounds was deduced from the coupling constants between protons
340 H-11 and H-7 (*J* between 11.4 and 12.9 Hz) and was confirmed by NOESY experiments that showed effect
341 between H-6 and H-11 in compounds **4–7**. The configuration was confirmed as 11*R*.
342 **Synthesis of thiol derivatives.** Good results were obtained for lactones **1** and **3** in providing
343 hydroxyl derivatives, with global yields of 54% and 29% for isomers **5** and **7**, respectively. A similar
344 procedure to obtain the thiol group in the lactones in C-13 (figures 1 and 2) was carried out. As in the
345 previous procedure, 4-methoxybenzyl mercaptan was used in the two-step synthesis of the corresponding
346 thiols (**8** and **10**).
347 The first step has already been reported in the literature for α-santonin,[24] but the oxidation of the
348 thioether to the thiol was not reported. Interestingly, in the literature procedure MeOH was the solvent of
349 choice for the conjugate addition at low temperature. However, in our work, the use of MeOH as solvent
350 in the hydroxylation route led to a mixture of compounds that included **14** as the major product. In
351 particular, when the conditions used to obtain **8** were employed to give **4** by replacing the benzyl mercaptan
352 with benzyl alcohol, compound **14** was obtained as the major product and half of the substrate was
353 recovered. For instance, 12 eq each of Et_3N and benzyl alcohol in MeOH yielded 21% of **14** and 51% of **1**.
354 The isolation of **14** was confirmed by the presence of two relevant signals, namely a singlet at δ 3.35 (^{1}H
355 NMR), corresponding to 3 protons, and a signal at 59.3 (^{13}C NMR), both corresponding to a methoxy group.
356 A decrease in the number of equivalents of benzyl alcohol employed in the synthesis of **4** and the
357 use of a non-nucleophilic solvent led to a decrease in the yield. In order to maximize the yields, the
358 minimum quantity of benzyl alcohol required to dissolve the lactone was used without another solvent.
359 Lower yields of **4** were also obtained on using temperatures higher than 25 ºC.
360 The higher nucleophilicity of the mercaptan can explain the observed preference over the alkoxy
361 compound **14** in this reaction. As an additional remark, the higher reactivity of the sulfur-containing

362 compounds can also explain the higher global yields obtained for **9** and **11** (71% and 94%, respectively).
363 The amount of base was a critical factor for the yield in the first step and the use of 3 eq of Et_3N instead of
364 1.5 eq led to an increase in the yield of **10** from 82 to 99%. On the other hand, a change in the solvent from
365 MeOH to THF while using 1.5 eq of Et_3N led to a decrease in the yield from 82% to 49%.
366 The incorporation of a benzyl group in **8** and **10** and the subsequent oxidation to thiols **9** and **11**
367 was confirmed by the same NMR signals discussed for the elucidation of **4** and **6**. In the case of the thiols,
368 signals corresponding to H-13 moved to lower field, i.e., from δ 2.91–2.69 to 3.24–2.91. In the case of the
369 ^{13}C NMR spectra, the C-13 signal moved to lower field (from δ 30.0 for **8** to 36.8 for **9**; from δ 30.1 for **10**
370 to 36.9 for **11**). The 11*R* configuration at C-13 of **8–11** was confirmed by NOESY experiments as an NOE
371 effect was observed between H-7 and H-11.
372 **Synthesis of methoxy derivatives.** The alkylation of compound **1** has previously been described
373 in the literature using 0.1% MeONa in MeOH with high yields.[18] When this procedure was applied to
374 compound **3**, the yield of **15** was lower (49%) than that described for **14** (88%), even on using a higher
375 concentration of MeONa (about 0.7% instead of 0.1%) and a longer reaction time (48 h instead of 8 h).
376 Compound **16**, the epimer at C-11, was also obtained in 9% yield. The NMR spectra of the alkoxy
377 derivatives **15** and **16** contained signals corresponding to a methoxy group: a singlet for three protons in
378 the ^1H NMR spectrum and a signal in the ^{13}C NMR spectrum (δ 3.36 and 59.2 in **15**; and δ 3.35 and 59.1
379 in **16**). The stereochemistries of these compounds at C-11 were deduced from NOE experiments.
380 **Synthesis of amino-derivatives.** Given the high susceptibility of **1** to Michael addition with
381 different nucleophilic solvents such as EtOH, MeOH and dimethylformamide in basic media, we considered
382 the possibility of introducing an amino group at C-13. Compound **1** was reacted with DBU and formamide
383 at rt and TLC indicated that the amide **13** was formed shortly after addition of the base. After a longer
384 reaction time (24 h) a new compound was observed with a higher R_F by TLC. The new compound was
385 identified as amine **12**, which is supposed to have been originated from **13** with the release of formic acid.
386 Since these conditions (procedure A, figure 3) gave **12** in low yields, we designed another experiment

(procedure B) in which aqueous 2 M NaOH was used as the base instead of DBU and THF was employed as solvent at 70 ºC. In this experiment compound **12** was obtained selectively in 74% yield, possibly by the transformation of **13**.

Regarding amide **13**, the signal for the protons at C-13 moved to higher field after transformation to amine **12** (δ 3.73 and 3.48 to δ 3.00 and 2.88), while the carbon signal was shifted downfield (δ 35.1 to 47.4). The clearest evidence for the presence of an amide in **13** was the presence of two specific singlets in the ^{1}H NMR spectrum at δ 8.22 and δ 6.42, which correspond to the protons of the carbonyl and the nitrogen, respectively. In the ^{13}C NMR spectrum another indicator for the presence of an amide was the carbonyl signal at δ 161.4, which was not observed for amine **12**.

The procedures outlined above were applied to compounds **2** and **3** (figures 2 and 3). Although we were successful in synthesizing compounds **7** and **11** from **3**, we could not obtain the corresponding amino derivatives of **3**. The hydroxy-, thiol- or amino derivatives of **2** could not be obtained and complex mixtures of compounds were obtained according to TLC.

In the case of **3**, both path A and B in the aminal synthesis failed and complex mixtures of compounds were obtained. The procedure was changed by combining different quantities of formamide, solvents (DMF, THF, acetone, MeOH) and bases (DBU, Et_3N, NaOH) but all attempts ultimately led to the degradation of **3**, with a major undesired product identified by ^{1}H NMR spectroscopy as the ester of the lactone (**19**, figure 2) when 3 eq formamide, 3 eq Et_3N and MeOH as solvent were used. Relative positions of H-6 and H-7 in **19** were assigned by using NOESY experiments, as well as calculating the coupling constants which were similar to those of the structurally similar starting material **3** (^{1}H, ^{13}C NMR and NOESY 1D avaialable in the supporting information).

After several attempts, it was concluded that the nature of the sesquiterpene backbone could have a marked effect on the reactivity of the substrate under the different experimental conditions. Therefore, the guaiane-type (**1**) would presumably be more robust to different conditions and this would be followed

411 by the eudesmane-type (**3**) and finally the germacrane-type (**2**), which was highly sensitive to the
412 conditions.

413 **Etiolated wheat coleoptile bioassay.** A quick analysis of the graph in figure 4 shows that data
414 follow a dose-dependent response. The natural products dehydrocostuslactone (**1**) and β-cyclocostunolide
415 (**3**) strongly inhibit coleoptile elongation and their bioactivity profile is similar to that of the synthetic
416 herbicide Logran® (positive control). Amongst all of the tested compounds, the closest bioactivity profiles
417 to the natural products were obtained for **13**–**16**, followed by **5**–**7**, whereas **8** and **12** had low activity and
418 **9**–**11** did not show any inhibitory activity. On considering the IC_{50} values (table 1) it can be seen that the
419 compounds with a guaiane-type backbone generally have lower values than the eudesmane-type
420 compounds.

421 At a first level, by forming pairs with the same functional group at C-13 and according to the
422 bioactivity profiles, the compounds can be ranked into three groups:

423 - High activity: **1** and **3** (starting materials), **13** (amide), **14** and **15**/**16** (methyl ethers).

424 - Moderate activity: **4** and **6** (benzyl ethers) and **5** and **7** (hydroxyls).

425 **-** Low activity: **8** and **10** (thioethers), **9** and **11** (thiols) and **12** (amine).

426 Data from table 1 was used for a regression of cLog *P* vs Log ($1/IC_{50}$) (figure 5), where the most
427 active compounds were located close to the maximum of the curve.

428 On considering the ethers, the data indicate that the presence of a methyl ether (**14**, **15**, **16**),
429 regardless of the stereochemistry (**15** vs. **16**), does not lead to a significant loss of phytotoxicity, but the
430 addition of a methoxybenzyl ether (**4** and **6**) does lower the bioactivity. Compounds **4** and **6** have a cLog *P*
431 close to 4 (table 1), whereas for compounds **14–16** the values are closer to 2. A higher lipophilicity is
432 generally associated with higher bioactivity, but it is recommended that Log*P* is below 4 in order to facilitate
433 transport in the phloem.[25] As a consequence, the higher lipophilicity of the benzyl ethers **4** and **6** leads to
434 decrease in their activity. Regarding the stereochemistry, the bioactivity profiles of the epimers **15** and **16**
435 are similar – as are their IC_{50} values, which are of the same order of magnitude but slightly lower for **16**

436 (50.6 vs. 24.1). Therefore, the stereochemistry at C13 is not critical for the activity although it does have a
437 slight effect on bioactivity.

438 Alcohols **5** and **7** are more polar than their methoxybenzyl ethers (**4** and **6**, respectively), with cLog
439 *P* values closer to 2, and their activity at the highest concentration is higher. However, the activity for **5** and
440 **7** decreases more rapidly with concentration and, consequently, their IC_{50} values are higher than those of
441 the benzyl ethers (157 μM vs. 30 μM and 358 μM vs. 149 μM, respectively).

442 In the third group, the thioethers (**8** and **10**) and the thiols (**9** and **11**) have the weakest activity
443 profiles, with slight inhibitory activity in the case of the thioether **8** and a growth stimulatory activity in the
444 cases of thioether **10** and the thiols. Once again, the lipophilicity of the compounds could play an important
445 role, since the thioethers have cLog *P* values close to 5 and therefore do not follow Tice's rule.[26] However,
446 both thiols have cLog *P* values closer to 3 and they also show poor activity.

447 Factors other than Log *P* could play a role in the activity and these include electronic interactions,
448 since alcohols **5** and **7** showed moderate activity as they have an oxygen instead of a sulfur. In contrast to
449 the above, compounds **12** and **13** showed completely different behavior in the bioassay. The amine **12** and
450 amide **13** have similar cLog *P* values (1.67 and 1.43, respectively), but the amine did not show significant
451 activity while the amide (**13**) has a low IC_{50} of 120 μM. Once again, electronic interactions may explain the
452 high activity of this compound rather than the lipophilicity. In addition, the possible decomposition of the
453 amide **13** to amine **12** and the release of formic acid may contribute to the high activity of this compound.
454 The phytotoxic effect of formic acid has been reported previously on lettuce seed germination and it inhibits
455 growth by 100% after 36 hours incubation at 1400 ppm[27] and barley seedling root extension by 20% at 5
456 mM.[28]

457 **Broomrape seed germination bioassays.** The results of these assays are represented graphically
458 in figure 6 as % of seeds germinated. Only the positive control GR24 stimulated the germination of *O.*
459 *crenata* (not shown), while the germination of the other two species (*O. cumana* and *P. ramosa*) was
460 triggered differently depending on the backbone structure and functionalization of the assayed compounds.

In general, eudesmane-derived products promoted germination of *O. cumana* significantly and guaiane-derived products promoted germination of *P. ramosa*. Surprisingly, results in literature show that an opposite tendency is found with other eudesmane derivatives[12] or guaianolide compounds,[11] by only introducing new hydroxyls[12] or changing unsaturation degree.[11] Therefore, substitution at C13 highly affect promotion of parasitic weed seed germination.

The results presented herein indicate that, guaiane-type products **4**, **5**, **9** and **12**–**16** showed strong or moderate activity on *P. ramosa* seeds, while eudesmane compounds **3**, **6** and **7** were best on *O. cumana*. Only a proportion of the synthesized compounds stimulated germination of both species significantly, namely **1**, **3**, **9** and **14**, while the rest were significantly active only on one of the species. The most versatile compounds in this bioassay were the natural products **1** and **3**, which were at least moderately active at all concentrations tested in both species.

It is noteworthy that some products are active on one of the species but inactive on the other. Since compound **1**, the starting material for the guaiane derivatives, is a specific germination elicitor for *O. cumana*,[7] it was expected that the corresponding guaiane products would show promising activity profiles. However, this was only the case at highest concentration tested for thioether **8**, thiol **9** and methyl ethers **14** and **15**, with a loss in activity observed when the molecule contained the methoxybenzyl ether or hydroxyl moiety at C-13 (**4** and **5**). Nevertheless, the eudesmane analogs (**6** and **7**) of these inactive guaianes were the most active products on *O. cumana*. This finding highlights the huge relevance of the skeleton for stimulatory activity, for which not only substituents at C-13 are important factors. It is interesting to note that, while ether **15** showed weak activity at the highest concentration, its epimer **16** was not active at all. This trend was also observed for *P. ramosa*, where **15** was slightly more active than **16** at all concentrations tested, thus hinting that there is some selectivity for that stereochemistry by the C11–C13 bond.

In general, *P. ramosa* is less specific than *O. cumana*. The results obtained for *P. ramosa* show opposite trends to those found for *O. cumana*. Eudesmane derivatives **6** and **7** were inactive on *P. ramosa*, whereas their analogous guaiane compounds **4** and **5** show excellent stimulation profiles. The reason for

this preference, which depends on the species, is unclear. Compounds **9**–**13** and **16**, which were not active on *O. cumana*, presented significant stimulatory activity at the two highest concentrations tested.

It is interesting to note that some compounds were more active at lower concentrations, reaching a concentration at which they showed inhibition of germination. That is the case for **5** and **13** on *P. ramosa*. It has been observed previously[7,29,30] that high concentrations of **1**, a potent stimulator of *O. cumana*, have a negative effect on seed germination. The compounds used here are derivatives of natural products that show activity on broomrape seed germination and it is therefore not surprising that similar behavior was observed.

Another aspect that is worth highlighting is the phytotoxic activity of these compounds in the etiolated wheat coleoptile bioassay. The case of **13** is particularly interesting as this has a markedly lower phytotoxic activity at the lowest concentrations (30 and 10 μM) and reaches its peak germinatory activity at 1 μM on *P. ramosa*, a concentration at which inhibitory activity was not observed in the coleoptile bioassay. The same phenomenon was observed with **5**, which loses its phytotoxic activity at 10 μM and reaches its maximum stimulatory activity at 1 μM – also on *P. ramosa*. There is no clear correlation between the two bioassays, which have different targets, but it would be interesting to explore in the future the possible interactions between the phytotoxic activity of sesquiterpene lactones at certain ranges of concentrations and their stimulatory activity on other species.

In conclusion, novel and previously reported procedures were used for the synthesis of derivatives, with good yields for hydroxylation, thiolation and amination, especially for thiolation with yields close to quantitative. These procedures could be applied to other compounds that contain α,β-unsaturated carbonyls.

Regarding bioassays, the most active derivatives on etiolated wheat coleoptiles were the 13-methoxy eudesmanes **15** and **16**. In the case of parasitic weeds, the most active compound was the 13-hydroxy eudesmane **7** on *O. cumana* and both the 13-hydroxy guaiane **5** and the 13-carbamoyl guaiane **13** on *P. ramosa.* The bioactivity data for the compounds synthesized here, may offer clues to better understand better the processes involved in the interactions between chemicals and plants.

In the next stage of our project we plan to obtain strigolactone analogs, preferably eudesmane systems, using the procedures described here as a first step and to test their activity on *O. cumana.* A butenolide fragment will be added in the second step with the ultimate aim being the synthesis of new parasitic weed germination stimulants as alternatives for parasitic weed management.

SUPPORTING INFORMATION DESCRIPTION

Supporting Information Available: [description of bioassays, ^{1}H and ^{13}CNMR of synthesized compounds]. This material is available free of charge via Internet at http://pubs.acs.org

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Notes

The authors declare no competing financial interest.

REFERENCES

(1) Macías, F. A.; Oliva, R. M.; Varela, R. M.; Torres, A.; Molinillo, J. M. G. Allelochemicals from sunflower leaves cv. Peredovick. *Phytochemistry* **1999**, *52*, 613–621.

(2) Macías, F. A.; Varela, R. M.; Torres, A.; Molinillo, J. M. G. New bioactive plant heliannuols from cultivar sunflower leaves. *J. Nat. Prod.* **1999**, *62*, 1636–1639.

(3) Raupp, F. M.; Spring, O. New sesquiterpene lactones from sunflower root exudate as germination stimulants for *Orobanche cumana*. *J. Agric. Food Chem.* **2013**, *61*, 10481–10487.

(4) Cárdenas, D. M.; Cala, A.; Molinillo, J. M. G.; Macías, F. A. Preparation and phytotoxicity study of lappalone from dehydrocostuslactone. *Phytochem. Lett.* **2017**, *20*, 66–72.

(5) Sun, C.-M.; Syu, W.-J.; Don, M.-J.; Lu, J.-J.; Lee, G.-H. Cytotoxic sesquiterpene lactones from the root of *Saussurea lappa*. *J. Nat. Prod.* **2003**, *66*, 1175–1180.

(6) Duan, J.-A.; Hou, P.; Tang, Y.; Liu, P.; Su, S.; Liu, H. A new sesquiterpene and other constituents from *Saussurea lappa* root. *Nat. Prod. Commun.* **2010**, *5*, 1531–1534.

(7) Joel, D. M.; Chaudhuri, S. K.; Plakhine, D.; Ziadna, H.; Steffens, J. C. Dehydrocostus lactone is exuded from sunflower roots and stimulates germination of the root parasite *Orobanche cumana*. *Phytochemistry* **2011**, *72*, 624–634.

(8) de Luque, A. P.; Galindo, J. C. G.; Macias, F. A.; Jorrin, J. Sunflower sesquiterpene lactone models induce *Orobanche cumana* seed germination. *Phytochemistry* **2000**, *53*, 45–50.

(9) Galindo, J. C. G.; de Luque, A. P.; Jorrín, J.; Macías, F. A. SAR studies of sesquiterpene lactones as *Orobanche cumana* seed germination stimulants. *J. Agric. Food Chem.* **2002**, *50*, 1911–1917.

(10) Macías, F. A.; García-Díaz, M. D.; Pérez-de-Luque, A.; Rubiales, D.; Galindo, J. C. G. New chemical clues for broomrape-sunflower host-parasite interactions: synthesis of guaianestrigolactones. *J. Agric. Food Chem.* **2009**, *57*, 5853–5864.

(11) Macias, F. A.; García-Díaz, M. D.; Massanet, G. M.; Gomez-Madero, J. F.; Fronczek, F. R.; Galindo, J. C. G. An easy access to bioactive 13-hydroxylated and 11,13-dihydroxylated sesquiterpene lactones (SLs) through Michael addition of a nucleophilic hydroxyl group. *Tetrahedron* **2008**, *64*, 10996–11006.

(12) Zorrilla, J. G.; Rial, C; Varela, R. M.; Molinillo, J. M. G.; Macías, F. A. Facile synthesis of anhydrojudaicin and 11,13-dehydroanhydrojudaicin, two eudesmanolide-skeleton lactones with potential allelopathic activity. *Phytochem. Lett.*, **2019**, *31*, 229–236.

(13) Talbi, M.; Ainane, T.; Boriky, D.; Bennani, L.; Blaghen, M; Elkouali, M. Antibacterial activity of eudesmanolide compounds isolated from medicinal plant *Artemisia herba-alba*. *J. Mater. Environ.*

Sci. **2015**, *6*, 2125–2128.

(14) Azarken, R.; Guerra, F. M.; Moreno-Dorado, F. J.; Jorge, Z. D.; Massanet, G. M. Substituent effects in the transannular cyclizations of germacranes. Synthesis of 6-epi-costunolide and five natural steiractinolides. *Tetrahedron* **2008**, *64*, 10896–10905.

(15) Kraut, L.; Mues, R.; Sim-Sim, M. Sesquiterpene lactones and 3-benzylphthalides from *Frullania muscicola*. *Phytochemistry* **1994**, *37*, 1337–1346.

(16) Kulkarni, G. H.; Kelkar, G. R.; Bhattacharyya, S. C. Terpenoids-LV. Cyclocostunolides. *Tetrahedron* **1964**, *20*, 2639–2645.

(17) Kumar, P.; Cherian, S. K.; Jain, R.; Show, K. Chemoselective deprotection of *N*-allylic amines using DDQ. *Tetrahedron Lett.* **2014**, *55*, 7172–7176.

(18) Matsuda, H.; Kageura, T.; Inoue, Y.; Morikawa, T.; Yoshikawa, M. Absolute stereostructures and syntheses of saussureamines A, B, C, D and E, amino acid-sesquiterpene conjugates with gastroprotective effect, from the roots of Saussurea lappa. *Tetrahedron* **2000**, *56*, 7763–7777.

(19) Cala, A.; Masi, M.; Cimmino, A.; Molinillo, J.; Macias, F.; Evidente, A. (+)-epi-Epoformin, a phytotoxic fungal cyclohexenepoxide: structure activity relationships. *Molecules* **2018**, *23*, 1529.

(20) Cala, A.; Molinillo, J. M. G.; Fernández-Aparicio, M.; Ayuso, J.; Álvarez, J. A.; Rubiales, D.; Macías, F. A. Complexation of sesquiterpene lactones with cyclodextrins: synthesis and effects on their activities on parasitic weeds. *Org. Biomol. Chem.* **2017**, *15*, 6500–6510.

(21) Cala, A.; Ghooray, K.; Fernández-Aparicio, M.; Molinillo, J. M.; Galindo, J. C.; Rubiales, D.; Macías, F. A. Phthalimide-derived strigolactone mimics as germinating agents for seeds of parasitic weeds. *Pest Manag. Sci.* **2016**, *72*, 2069–2081.

(22) Pereira, R. G.; Cala, A.; Fernández-Aparicio, M.; Molinillo, J. M. G.; Boaventura, M. A. D.; Macías, F. A. Gibberellic and kaurenoic hybrid strigolactone mimics for seed germination of parasitic weeds. *Pest Manag. Sci.* **2017**, *73*, 2529–2537.

(23) Pietras, R., Jung, M., Marquez-Garban, D. and Deng, G. Compositions and methods for treating

cancer. U.S. Patent 9,266,901 B2, October 2, 2014.

(24) Khazir, J.; Riley, D. L.; Chashoo, G.; Mir, B. A.; Liles, D.; Islam, M. A.; Singh, S. K.; Vishwakarma, R. A.; Pilcher, L. A. Design, synthesis and anticancer activity of Michael-type thiol adducts of α-santonin analogue with exocyclic methylene. *Eur. J. Med. Chem.* **2015**, *101*, 769–779.

(25) Macías, F. A.; Velasco, R. F.; Castellano, D.; Galindo, J. C. G. Application of Hansch's model to guaianolide ester derivatives: a quantitative structure-activity relationship study. *J. Agric. Food Chem.* **2005**, *53*, 3530–3539.

(26) Tice, C. M. Selecting the right compounds for screening: does Lipinski's Rule of 5 for pharmaceuticals apply to agrochemicals? *Pest Manag. Sci.* **2001**, *57*, 3–16.

(27) Manios, V. I.; Tsikalas, P. E.; Siminis, H. I.; Verdonck, O. Phytotoxicity of olive tree leaf compost in relation to the organic acid concentration. *Biol. Wastes* **1989**, *27*, 307–317.

(28) Lynch, J. M. Effects of organic acids on the germination of seeds and growth of seedlings. *Plant. Cell Environ.* **1980**, *3*, 255–259.

(29) Raupp, F. M.; Spring, O. New sesquiterpene lactones from sunflower root exudate as germination stimulants for *Orobanche cumana*. *J. Agric. Food Chem.* **2013**, *61*, 10481–10487.

(30) Ueno, K.; Furumoto, T.; Umeda, S.; Mizutani, M.; Takikawa, H.; Batchvarova, R.; Sugimoto, Y. Heliolactone, a non-sesquiterpene lactone germination stimulant for root parasitic weeds from sunflower. *Phytochemistry* **2014**, *108*, 122–128.

Figure Captions

Figure 1. Reactions for the synthesis of compounds **4**, **5**, **8**, **9**, **12**–**14**.

Figure 2. Reactions for the synthesis of compounds **6**, **7**, **10**, **11**, **15**, **16**, **19**.

Figure 3. Synthesis of compounds **3**, **17** and **18** by intramolecular cyclization of **2** with *p*-toluensulfonic acid.

Figure 4. Results for the etiolated wheat coleoptile bioassay for compounds **1**, **3**–**16** and the positive control Logran®, where standard deviation is represented in lines over the bars. Positive values represent stimulation of coleoptile growth against the negative control and negative values represent inhibition of growth.

Figure 5. Graph of cLog *P* vs Log ($1/IC_{50}$) regression of inhibitor compounds in the etiolated wheat coleoptile bioassay. Compounds showing stimulation effects and which activity values were null or lower than 50% are not included. Derivative **7** did not fit any correlation and was not included in the regression.

Figure 6. Results of the broomrape seed germination bioassays for compounds **1**, **3**–**16**, and the positive control GR24. For each concentration in the broomrape bioassay * indicates differences of each compound compared with the negative control (water) assessed by Dunnett's test at the 0.05 level. Most active compounds are marked in red.

Tables

Table 1. IC_{50} and EC_{50} values of compounds **1** and **4**–**16** in μM. Only those compounds with activities higher than 50% and active at more than one concentration were analyzed for their IC_{50} or EC_{50} (< or >: value out of tested range, n.a.: not active, +: low stimulatory activity, *: value at which the inhibitory or stimulatory activity was 50%).

	cLog *P*	*Etiolated wheat coleoptiles*	*O. cumana*	*P. ramosa*
		IC_{50} (μM)	EC_{50} (μM)	EC_{50} (μM)
1	2.79	28.8	288	<0.1
3	3.27	45.2	7.31	<0.1
4	4.02	30*	n.a.	1*
5	1.59	157	n.a.	<0.1
6	4.50	149	0.1*	n.a.
7	2.07	358	0.1*	n.a.
8	4.86	>1000	>100	n.a.
9	2.68	n.a.	>100	10*
10	5.34	+	n.a.	>100
11	3.16	+	n.a.	100*
12	1.67	>1000	n.a.	1*
13	1.43	120	n.a.	<0.1
14	2.35	77.6	100*	>100
15	2.83	50.6	>100	84.4
16	2.83	24.1	n.a.	>100
Logran®	-	30.2	-	-
GR24	-	-	0.915	<0.1

Figures and Artwork

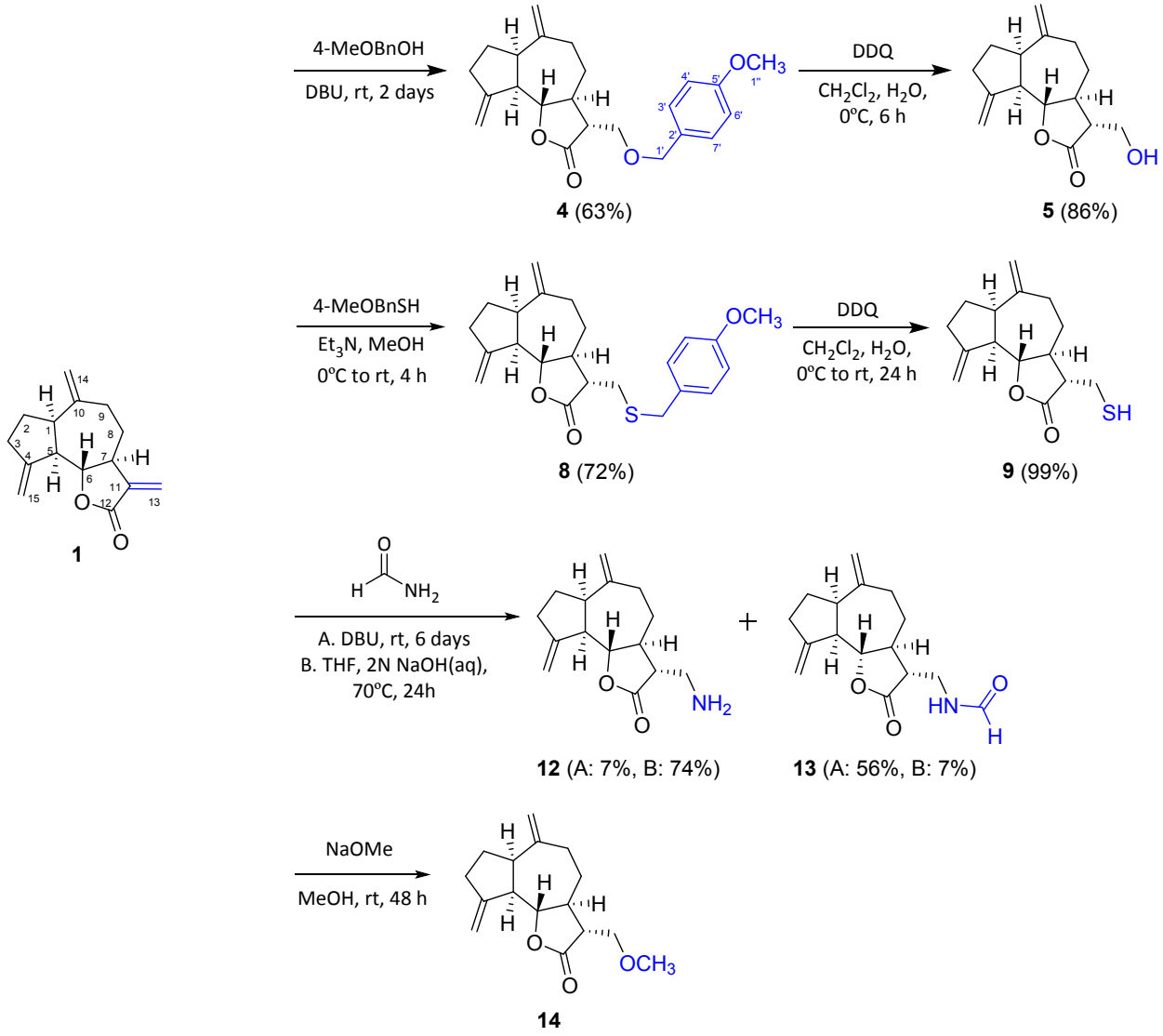


Figure 1

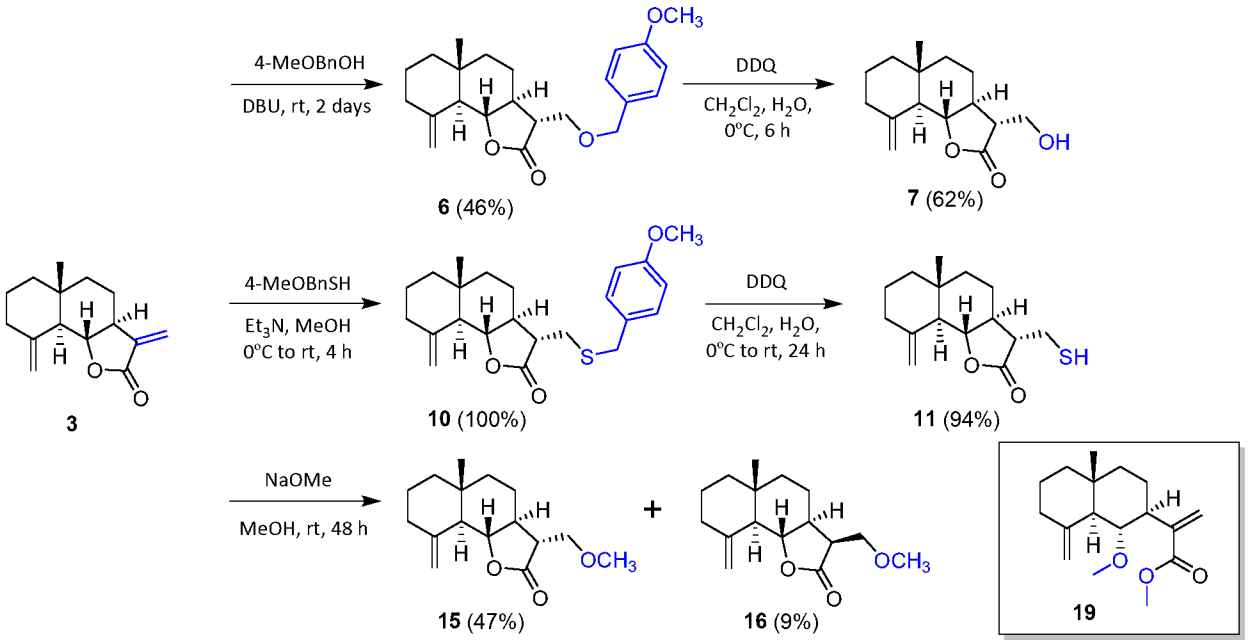


Figure 2

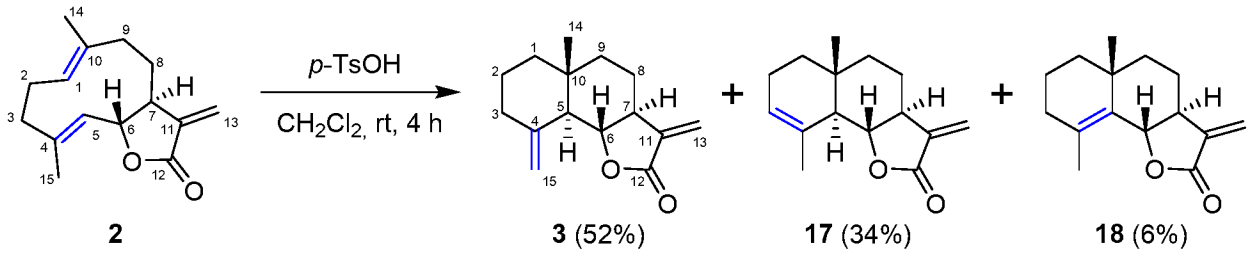


Figure 3

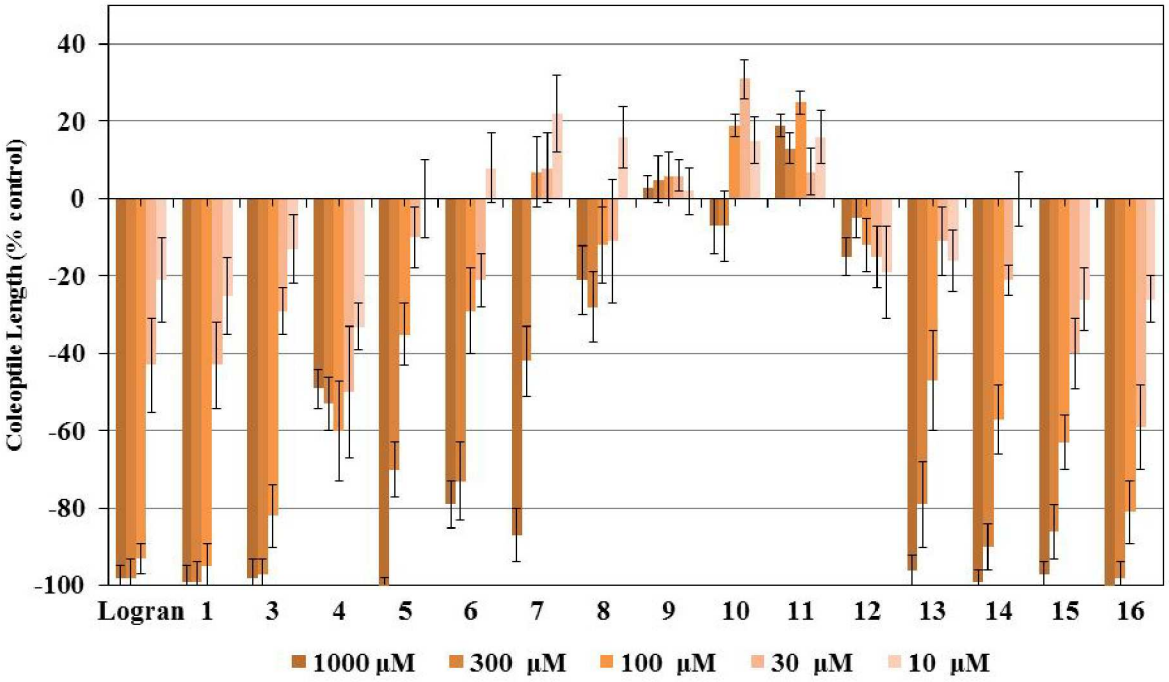


Figure 4

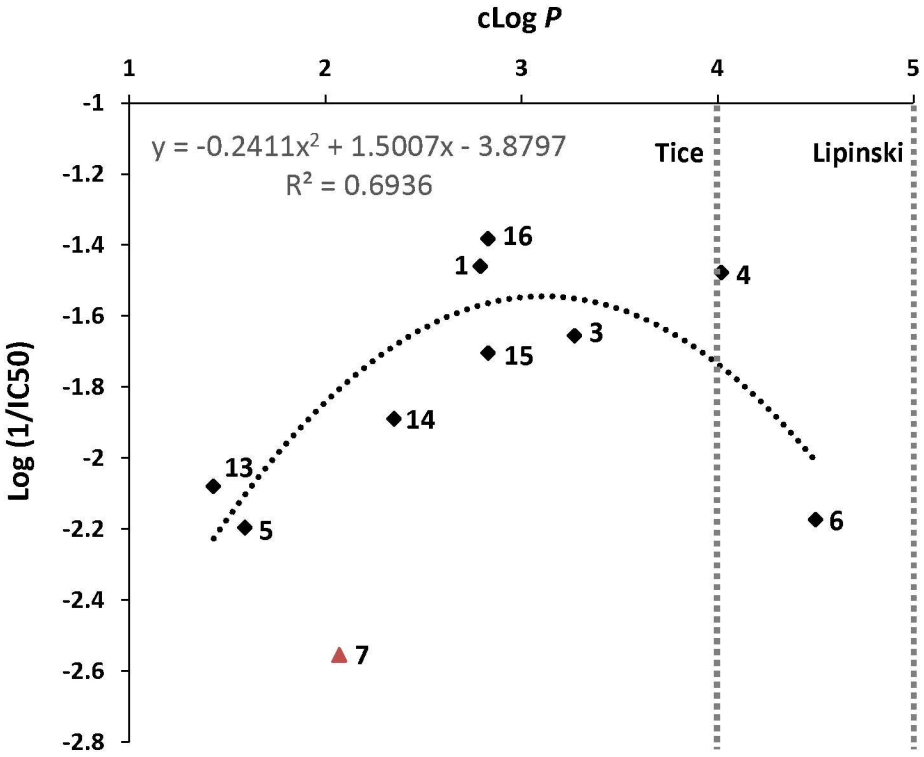


Figure 5

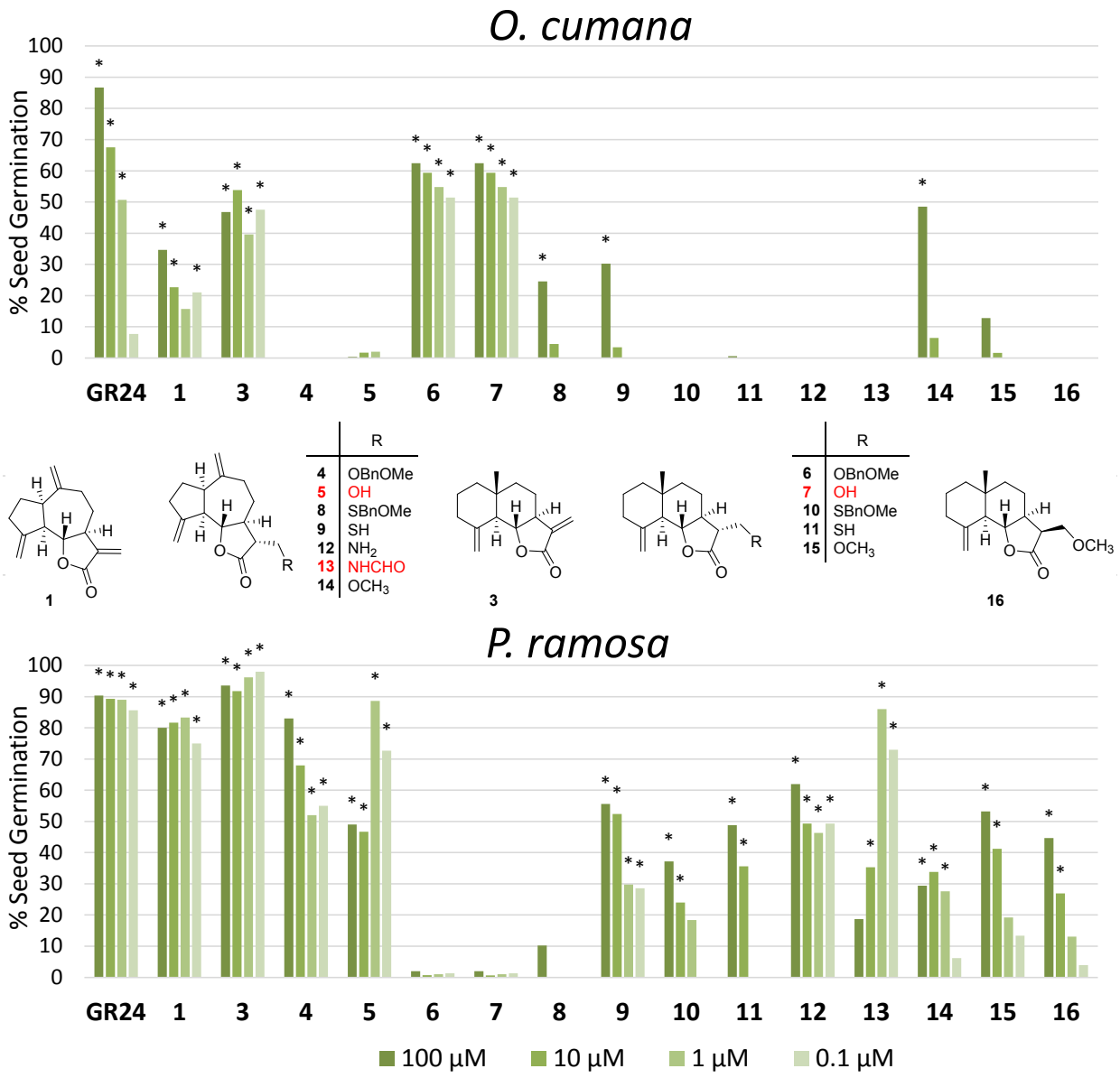
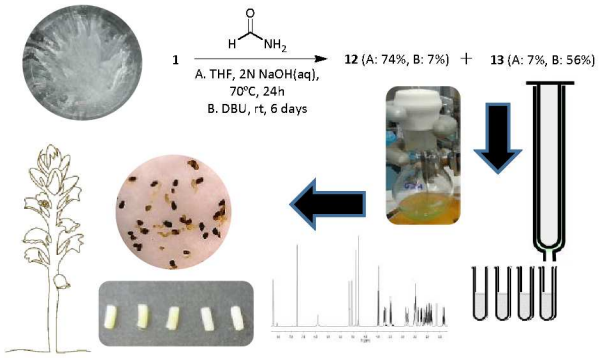

O. cumana
% Seed Germination
100
90
80
70
60
50
40
30
20
10
0
GR24 1 3 4 5 6 7 8 9 10 11 12 13 14 15 16
R
4 OBnOMe
5 OH
8 SBnOMe
9 SH
12 NH2
13 NHCHO
14 OCH3
R
6 OBnOMe
7 OH
10 SBnOMe
11 SH
15 OCH3
OCH3
1
3
16
P. ramosa
% Seed Germination
GR24 1 3 4 5 6 7 8 9 10 11 12 13 14 15 16
100 µM
10 µM
1 µM
0.1 µM

Figure 6

GRAPHIC FOR TABLE OF CONTENTS



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