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ScienceDirect
Bioorganic & Medicinal Chemistry 16 (2008) 3725–3731
Bioorganic & Medicinal Chemistry

A novel sesquiterpene lactone from *Centaurea pullata*: Structure elucidation, antimicrobial activity, and prediction of pharmacokinetic properties

Samah Djeddi,[a] Anastasia Karioti,[a] Marina Sokovic,[b] Catherine Koukoulitsa[c] and Helen Skaltsa[a,*]

[a]*Department of Pharmacognosy and Chemistry of Natural Products, School of Pharmacy, University of Athens, Panepistimiopolis, Zografou, 157 71 Athens, Greece*
[b]*Department of Plant Physiology, Mycological Laboratory, Institute for Biological Research, 29 Novembar 142, 11 000 Belgrade, Serbia*
[c]*Institute of Organic and Pharmaceutical Chemistry, National Hellenic Research Foundation, Vas. Constantinou 48, 11635 Athens, Greece*

Received 7 December 2007; revised 24 January 2008; accepted 30 January 2008
Available online 2 February 2008

Abstract—A novel elemanolide with an α-methyl-γ-lactone moiety, 8α-*O*-(4-hydroxy-2-methylenebutanoyloxy)melitensine, in addition to four known sesquiterpene lactones also bearing the same lactone ring, melitensin, 11β,13 dihydrosalonitenolide, 8α-hydroxy-11β,13-dihydro-4-*epi*-sonchucarpolide, and 8α-hydroxy-11β,13-dihydro-onopordaldehyde have been isolated from the aerial parts of *Centaurea pullata*. The in vitro antibacterial and antifungal activities of the isolated sesquiterpene lactones were tested against six bacteria and eight fungal species, using a microdilution method. All compounds tested showed greater antibacterial and antifungal activities than the positive controls used. Moreover, the pharmacokinetic profile of these compounds was investigated using computational methods.

1. Introduction

The genus *Centaurea* L. (Asteraceae), with nearly 300 species[1] traditionally has been considered problematic taxonomically. *Centaurea pullata* L., known under the common name 'Achbet Ennegar',[2] is a biennial plant belonging to the section Melanoloma,[3,4] with large terminal pink flowers, very variable in height, distributed from Spain to France and North Africa.[5] In Algeria, it is a common edible herb used in the preparation with other plants of a local traditional dish called 'El Hammama'.

Sesquiterpene lactones (SLs) constitute the more distributed natural products in the genus *Centaurea* L., which display a wide spectrum of biological activity such as antimicrobial, antifeedant, cytotoxic, and antifungal.[6–10]

Instead, there is little information available about the bioavailability of these natural compounds. The bioavailability and uptake of parthenolide using Caco-2 cell monolayers as a model of intestinal mucosa was reported in the literature.[11] It was demonstrated a substantial transport of parthenolide by the human intestinal cells, predominately via passive diffusion. Moreover, the interactions of dihydrohelenalin acetate, dihydrohelenalin methacrylate, helenalin isobutyrate, and parthenolide with human serum albumin, plasma and whole blood were investigated.[12] The SLs in the ethanolic preparations showed a lower degree of protein binding.

As a contribution to the knowledge about the pharmacokinetic properties of sesquiterpene lactones it was investigated in our laboratory the pharmacokinetic profile of 22 potent antifungal sesquiterpene lactones

Keywords: *Centaurea pullata*; 8α-*O*-(4-Hydroxy-2-methylenebutanoyloxy)melitensine; Antifungal activity; Antibacterial activity; ADME; VolSurf.
* Corresponding author. Tel./fax: +30 210 7274593; e-mail: skaltsa@pharm.uoa.gr

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doi:10.1016/j.bmc.2008.01.056

possessing an α-methylene-γ-lactone group, isolated from different Greek taxa of *Centaurea* sp., with the use of the computational method VolSurf.[13] From the in silico study it was predicted that the studied compounds exhibited poor pharmacokinetic properties. More precisely, it was predicted a low permeability through Caco-2 cells and a limited aqueous solubility, which suggest that the *per os* administration of these molecules could not be effective. Instead, a topical application would be preferable when applicable. Moreover, it was predicted a low penetration through the blood–brain barrier, which suggests a low toxicity at the central nervous system (CNS) level.

In our efforts to identify novel bioactive sesquiterpene lactones, we have recently reported the isolation from *C. pullata* and the antimicrobial and antifungal activities of five sesquiterpene lactones possessing an α-methyl-γ-lactone moiety: 11β,13-dihydrocnicin (**1**), 11β,13-dihydro-19-desoxycnicin (**2**), 8α-*O*-(4-acetoxy-5-hydroxyangeloyl)-11β,13-dihydrocnicin (**3**), 8α-*O*-(4-hydroxy-2-methylenebutanoyloxy)-11β,13-dihydrosonchucarpolide (**4**), and 8α-*O*-(4-hydroxy-2-methylenebutanoyloxy)-11β,13-dihydro-4-*epi*-sonchucarpolide (**5**) (Fig. 1).[14]

In continuation of our work on the chemical constituents of *C. pullata*, we report herein the isolation and structure elucidation of a novel elemanolide with an α-methyl-γ-lactone moiety, 8α-*O*-(4-hydroxy-2-methylenebutanoyloxy)melitensin (**6**), along with four known sesquiterpene lactones possessing also an α-methyl-γ-lactone moiety: melitensin (**7**),[15] 11β,13 dihydrosalonitenolide (**8**),[16] 8α-hydroxy-11β,13-dihydro-4-*epi*-sonchucarpolide (**9**),[17] and 8α-hydroxy-11β,13-dihydro-onopordaldehyde (**10**) (Fig. 1).[18]

Since the sesquiterpene lactones isolated previously have exhibited more potent antibacterial and antifungal activities than the positive controls used,[14] we thought that it would be interesting to evaluate the antimicrobial potential of the herein described compounds, having similar structure. The obtained biological results were compared to those previously reported on sesquiterpene lactones possessing an α-methyl-γ-lactone moiety instead of an α-methylene-γ-lactone group, which is usually present in sesquiterpene lactones isolated from other *Centaurea* spp.[19–24] Additionally, the interesting finding of a potent antimicrobial activity triggered us to investigate also their pharmacokinetic profile using computational methods.

2. Results and discussion

Compound **6** showed in its HR-ESI-MS a pseudomolecular ion *m*/*z* 387.1777 $[M+Na]^+$, compatible with the molecular formula $C_{20}H_{28}O_6$ [calcd 364.1878 $[M]^+$, 387.1776 $[M+Na]^+$ (Δ: +0.01 mmu)]. The IR spectrum afforded absorption bands at 3600–3300 (OH), 1757 (C=O, γ-lactone, ester).

The ^{1}H NMR spectrum (Table 1) together with the COSY, HSQC, and HMBC data revealed the presence of an elemanolide with an α-methyl-γ-lactone moiety. The signals in the ^{1}H NMR spectrum (Table 1) at δ_H 5.74 (dd, J = 11.0, 17.2 Hz, H-1), 5.03 (d, J = 11.0 Hz, *cis*-coupling, H-2a), 4.98 (d, J = 16.8 Hz, *trans*-coupling, H-2b), 5.39 (br s, H-3a), and 4.96 (br s, H-3b), along with the presence of a quaternary methyl group at δ_H 1.16 (s), allylic to double bond (H-14), supported an elemane framework. The resonance of a secondary methyl group at δ_H 1.22 (d, J = 6.6 Hz) along with the chemical shift of the carbonyl group of the lactone ring (at δ_C 178.1) suggested the presence of an α-methyl-γ-lactone moiety instead of an α-methylene-γ-lactone group, which is usually present in sesquiterpene lactones isolated from other *Centaurea* spp.[6–10,19–24]

This finding was further supported by a COSY experiment where the spin system H-13/H-11/H-7 was observed. As a result of the absence of a 11,13 exocyclic double bond, H-7 appeared shielded at δ_H 2.00 (dd, J = 10.9, 11.8). A COSY experiment on **6** displayed the following spin systems: H-1/H-2a,b (spin system A), H-5/H-6/H-7/H-8/H-9a,b (spin system B), H-7/H-11, H-11/H-13a,b (spin system C), and H-3′a,b/H-4′a,b (spin system D).

Key HMBC correlations between H-3a′, 3b′ (2.56)/C-2′ (136.6); H-5a′, 5b′ (6.25, 5.73)/C-1′ (166.0) and C-3′ (35.0) enabled the identification of the side chain (Fig. 2). Accordingly, compound **1** was assigned as 8α-*O*-(4-hydroxy-2-methylenebutanoyloxy)melitensin.

The antifungal activity of sesquiterpene lactones possessing an α-methylene-γ-lactone group was investigated in our laboratory and it was interestingly evidenced that these compounds are potent antifungal agents.[19–21] In this study, the isolated compounds **6**–**10** were tested against six bacteria and eight fungal species. According to MICs, MBCs, and MFCs values (Tables 2 and 3) it was demonstrated that all investigated compounds exhibited more potent antimicrobial activity than streptomycin (the positive control used), as well as a fungicidal potential higher than miconazole (the commercial fungicide used as a control). Taking in by consideration these biological results and those reported on our previous study regarding the antifungal activity of similar compounds,[14] it can be deduced that sesquiterpene lactones possessing an α-methyl-γ-lactone moiety instead of an α-methylene-γ-lactone group are also potent antimicrobial agents. This is in agreement with the observation that the antimicrobial activity appears to be independent of the presence or absence of an α-methylene-γ-lactone moiety.[25] Other requirements such as molecular accessibility and lipophilicity seem to play an important role for their antifungal activity.[10] Differences in the physiology of the individual fungal species must also be considered.[9] Moreover, the conjugated carbonyl group presented in the side chain should be taken into account. Since, the mechanism of antibacterial action of compounds having unsaturated ketone and lactone functions is by reaction of these groups with the thiol groups in enzymes,[26] the second alkylating site presented in the side chain may be relevant to the biological activity.

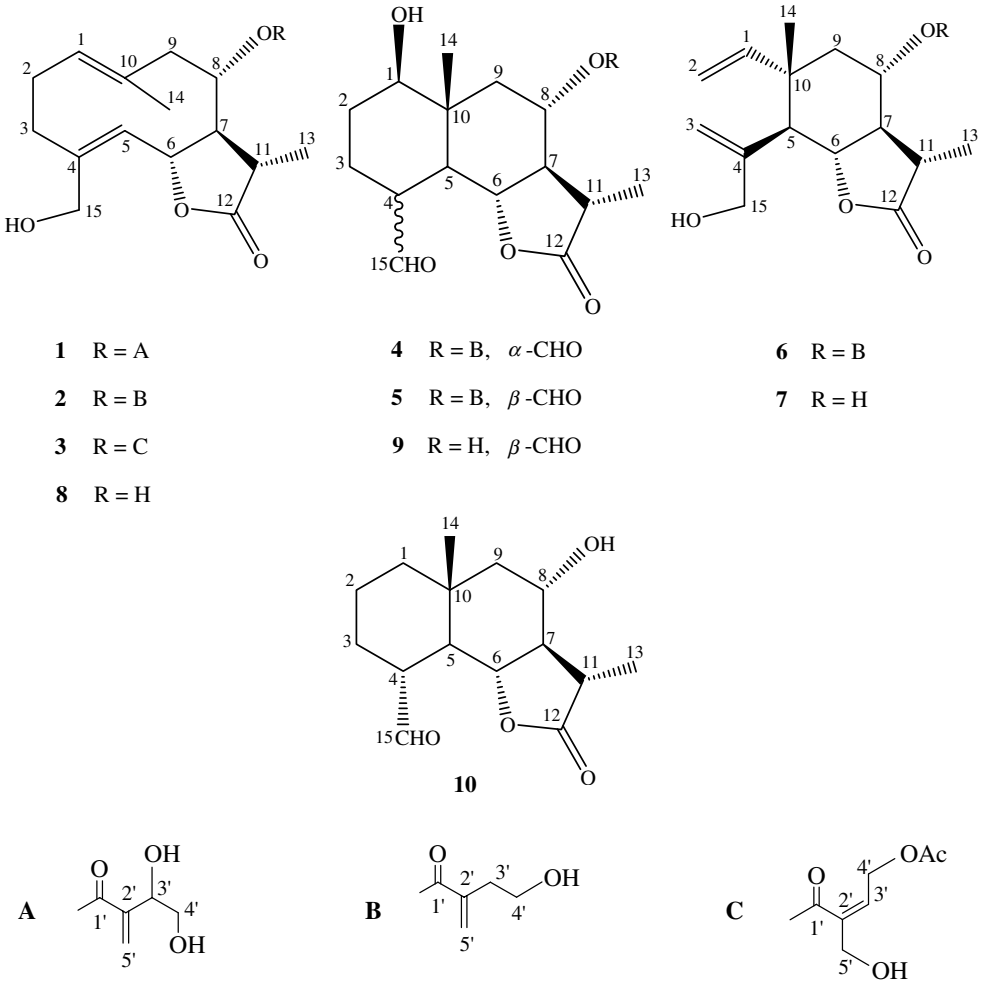

Figure 1. Chemical structure of the sesquiterpene lactones **1**–**5** isolated previously,[14] and the studied compounds **6**–**10**.

The interesting biological finding encouraged us to investigate their pharmacokinetic profile, using the VolSurf procedure.[27] VolSurf is a computational procedure that is specifically designed to produce descriptors related to pharmacokinetic properties, starting from 3D molecular field maps. In the standard procedure, GRID interaction fields[28] are calculated around the target molecules. The basic concept of VolSurf is to compress the information present in 3D grid maps into few 2D numerical descriptors, which are simple to understand and to interpret. The molecular descriptors obtained refer to molecular size and shape, to size and shape of both hydrophilic and hydrophobic regions and to the balance between them. The ADME models included in VolSurf predict Caco-2 cell (human intestinal epithelial cell line derived from a colorectal carcinoma) absorption,[27] protein binding,[29] blood–brain barrier (BBB) permeation,[30] drug–water solubility,[31] drug–DMSO solubility, metabolic stability,[32] hERG (human Ether-a-go-go Related Gene) inhibition[33] and volume of distribution.

In the examined data set was included the sesquiterpene lactones **1**–**5** isolated previously.[14] All molecules **1**–**10** were projected on the pre-calculated models: Caco-2 cell absorption, plasmatic protein binding, blood–brain barrier passage and thermodynamic solubility. From the plots (Fig. 3) it is predicted that the examined compounds cannot be transported across the intestinal epithelium, they have a low affinity to the plasma protein, they cannot cross to the blood–brain barrier and are medium–low aqueous soluble. However, it should be noticed that there are several compounds in the models that cover an empty chemical space. For these molecules the prediction could not be reliable. The values of VolSurf Caco-2/, protein binding/, BBB/, and solubility/prediction for all compounds are presented in Table 4.

As in our previous publication regarding the prediction of the pharmacokinetic profile of 22 antifungal sesquiterpene lactones possessing an α-methylene-γ-lactone group, the present results suggest a non-optimal pharmacokinetic profile. Thus, it can be deduced that the pharmacokinetic profile is not modified by the presence of an exocyclic α-methyl group conjugated to the γ-lactone. This finding seems quite interesting and useful to the design of semi-synthetic derivatives. A modification in the side chain that contains the second alkylating site (the conjugated carbonyl group) could optimize the pharmacokinetic profile.

Table 1. ^{1}H and ^{13}C NMR spectroscopic data for compound **6**[a] (400 MHz, $CDCl_3$)

Position	δ_H (J in Hz)	δ_C, mult.
1	5.74, dd (11.0, 17.2)	145.6
2a	5.03 d (J = 11.0)	112.8
2b	4.98 d (J = 16.8)	
3a	5.39 br s	114.6
3b	4.96 br s	
4	—	144.0
5	2.43 d (J = 11.8)	50.1
6	4.25 dd (J = 11.0, 11.3)	78.2
7	2.00 dd (J = 10.9, 11.8)	55.9
8	5.17 td (J = 3.9, 11.0)	70.4
9a	1.92 dd (J = 3.9, 12.5)	44.8
9b	1.67 dd (J = 11.7, 12.5)	
10	—	41.1
11	2.63 dq (J = 6.6, 12.1)	40.8
12	—	178.1
13	1.22 d (J = 6.6)	13.8
14	1.16 s	18.5
15a	4.07 dd (J = 6.2, 5.8)	67.1
15b	3.96 dd (J = 6.3, 5.7)	
1′	—	166.0
2′	—	136.6
3′a, 3′b	2.56 dd (J = 6.2, 5.8)	35.0
4′a, 4′b	3.75 dd (J = 6.3, 5.7)	61.3
5′a	6.25 br s	127.8
5′b	5.73 br s	

[a] Carbon resonances were assigned by HSQC and HMBC spectra.

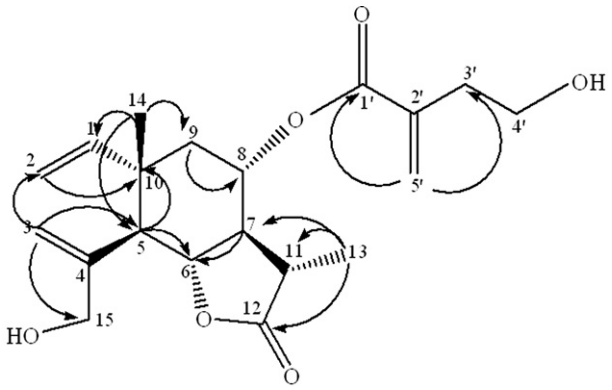

Figure 2. Key HMBC correlations for compound **6**.

3. Experimental

3.1. General experimental procedures

Polarimeter: Perkin-Elmer 341. FT-IR spectrometer: Perkin-Elmer Paragon 500. NMR: the 1D and 2D spectra (400 MHz) were recorded using Bruker DRX 400 and Bruker AC 200 spectrometers. Chemical shifts are reported in δ (ppm) values. COSY, HMQC, HSQC, HMBC, and NOESY (mixing time 950 ms) were performed using standard Bruker microprograms. MS: recorded on a Thermo LTQ Orbitrap (FT-MS^n) (University of Florence, Italy). Vacuum liquid chromatography (VLC): silica gel (Merck; 43–63 μm). Column chromatography: silica gel (SDS; 40–63 μm), gradient elution with the solvent mixtures indicated in each case. HPLC support: preparative HPLC was performed using a C_{18} 25 cm × 10 mm Kromasil column using a JASCO HPLC system equipped with a RI detector. Fractionation was always monitored by TLC silica gel 60 F-254, Merck, Art. 5554 with visualization under UV (254 and 365 nm) and spraying with anisaldehyde–sulfuric acid reagent on silica gel.

3.2. Plant material

Aerial parts of *C. pullata* L. were collected from Chrea mountain in Blida (North Algeria) in April 2006 and authenticated by Mr. Beloued abd El Kader (Agronomic National Institute, Algiers). A voucher specimen has been deposited in the Herbarium of the Department of Biology, Environmental Laboratory, University of Annaba, under the code: Ann-BV 2006/0010.

3.3. Extraction and isolation

The fresh plant material (1.2 kg) was ground finely and extracted at room temperature with cyclohexane/Et_2O/MeOH (1:1:1) and MeOH/H_2O (5:1), successively. The latter extract concentrated under reduced pressure (138.0 g) was prefractionated by VLC on silica gel, using CH_2Cl_2–MeOH mixtures of increasing polarity as eluents to give eleven fractions (A_1–A_{11}). Column chromatography over silica gel of fraction A_2 (6.0 g; eluted with CH_2Cl_2/MeOH, 97:3) afforded 87 fractions combined in 14 groups (B_1–B_{14}). Further purification of group B_6 (22.2 mg; eluted with CH_2Cl_2/MeOH, 97:3) by RP-HPLC (MeOH/H_2O, 1:1) afforded compounds **6** (R_t = 23.51 min, 2.0 mg), and **7** (R_t = 14.32 min, 15.2 mg). Further purification of group B_8 (12.0 mg; eluted with CH_2Cl_2/MeOH, 96:4) by CC over Sephadex LH-20 (MeOH) yielded compound **10** (2.2 mg). Group B_9 (125.5 mg; eluted with CH_2Cl_2/MeOH, 95:5) was subjected to RP-HPLC (MeOH/H_2O, 4:3) and afforded compounds **10** (R_t = 8.02 min, 1.1 mg) and **8** (R_t = 13.37 min, 8.9 mg). Fraction A_3 (6.8 g; eluted with CH_2Cl_2/MeOH, 90:10) was subjected to column chromatography on silica gel, using CH_2Cl_2–EtOAc mixtures of increasing polarity as eluents to give three hundred fractions (C_1–C_{300}). Further purification of combined fractions C_{97}–C_{116} (70.7 mg; eluted with CH_2Cl_2/EtOAc, 95:5) by RP-HPLC (MeOH/H_2O, 1:1) afforded compound **9** (R_t = 10.32 min, 4.4 mg). Combined fractions C_{201}–C_{210} (56.2 mg; eluted with CH_2Cl_2/EtOAc, 70:30) were identified as compound **7**.

3.3.1. 8α-O-(4-Hydroxy-2-methylenebutanoyloxy)melitensin (6). Oil; $[\alpha]_D^{20}$ +15.0 (c 0.10, MeOH); IR (CaF_2) ν_{max} 3600–3300 (OH), 1757 (C=O, γ-lactone, ester) cm^{-1}; ^{1}H NMR and ^{13}C NMR data, see Table 1; HRESIMS m/z 387.1777 $[M+Na]^+$ [calcd for $C_{20}H_{28}O_6$: 364.1878 $[M]^+$, 387.1776 $[M+Na]^+$ (Δ: +0.01 mmu)].

3.4. Biological assays

The following Gram-negative bacteria were used: *Escherichia coli* (ATCC 35210), *Pseudomonas tolaasii* (isolated from *Agaricus bisporus*), *Salmonella enteritidis* (ATCC 13076), and the following Gram-positive

Table 2. Minimum inhibitory and bactericidal concentrations (MICs/MBCs, μmol/mL × 10^{-3}) of compounds **6–10**[a]

Bacteria	MIC MBC **6**	MIC MBC **7**	MIC MBC **8**	MIC MBC **9**	MIC MBC **10**	MIC MBC **Streptomycin**
Bacillus subtilis	0.2 ± 0.02 0.4 ± 0.05	1.5 ± 0.2 2.3 ± 0.1	0.8 ± 0.1 1.1 ± 0.06	0.7 ± 0.07 0.7 ± 0.06	0.3 ± 0.1 0.5 ± 0.1	0.8 ± 0.2 0.8 ± 0.1
Micrococcus flavus	0.4 ± 0.05 0.5 ± 0.06	2.3 ± 0.1 2.3 ± 0.1	1.1 ± 0.2 1.1 ± 0.1	0.7 ± 0.1 0.7 ± 0.1	0.5 ± 0.06 0.7 ± 0.06	0.8 ± 0.1 1.6 ± 0
Staphylococcus epidermidis	0.5 ± 0.02 0.8 ± 0.07	2.3 ± 0.2 2.3 ± 0.1	1.5 ± 0.2 1.9 ± 0.2	1.0 ± 0.1 1.4 ± 0.2	0.7 ± 0.07 1.1 ± 0.06	1.6 ± 0 1.6 ± 0
Escherichia coli	0.4 ± 0.06 0.5 ± 0	2.3 ± 0.2 2.3 ± 0.1	1.5 ± 0.2 1.9 ± 0.05	1.0 ± 0.1 1.4 ± 0.2	0.5 ± 0.1 0.7 ± 0.1	0.8 ± 0.1 1.6 ± 0
Pseudomonas tolaasii	0.5 ± 0.02 0.8 ± 0.07	2.3 ± 0.1 2.3 ± 0.1	0.8 ± 0.06 0.8 ± 0.07	0.7 ± 0.06 0.7 ± 0.06	0.7 ± 0.1 1.1 ± 0.2	1.6 ± 0.1 3.2 ± 0
Salmonella enteritidis	0.2 ± 0 0.4 ± 0.02	2.3 ± 0.2 2.3 ± 0.1	1.1 ± 0.1 1.5 ± 0.1	0.7 ± 0.07 1.0 ± 0	0.3 ± 0.06 0.5 ± 0.07	1.6 ± 0 1.6 ± 0

[a] For list of organisms and protocols used, see Section 3.

Table 3. Minimum inhibitory and fungicidal concentrations (MICs/MFCs μmol/mL × 10^{-3}) of compounds **6–10**[a]

Fungal species	MIC MFC **6**	MIC MFC **7**	MIC MFC **8**	MIC MFC **9**	MIC MFC **10**	MIC MFC **Miconazole**
Alternaria alternata	0.1 ± 0.06 0.1 ± 0.06	0.7 ± 0.06 0.7 ± 0.06	0.7 ± 0.1 0.7 ± 0.2	0.3 ± 0.06 0.3 ± 0.06	0.2 ± 0.06 0.2 ± 0.06	0.4 ± 0.06 2.0 ± 0
Aspergillus flavus	0.1 ± 0.05 0.2 ± 0.06	0.7 ± 0.1 0.7 ± 0.2	0.3 ± 0.06 0.7 ± 0.06	0.3 ± 0.07 0.3 ± 0.06	0.2 ± 0 0.4 ± 0.1	1.0 ± 0.06 4.0 ± 0.1
Aspergillus niger	0.3 ± 0.1 0.3 ± 0.1	0.7 ± 0.1 0.7 ± 0.1	0.7 ± 0.1 0.7 ± 0.1	0.3 ± 0.06 0.3 ± 0.07	0.6 ± 0.06 0.6 ± 0.07	3.0 ± 0.1 8.0 ± 0
Aspergillus ochraceus	0.2 ± 0.06 0.2 ± 0.06	0.7 ± 0.1 0.7 ± 0.2	0.7 ± 0.2 0.7 ± 0.1	0.3 ± 0.06 0.3 ± 0.06	0.4 ± 0.1 0.4 ± 0.1	3.0 ± 0.1 8.0 ± 0
Fusarium tricinctum	0.1 ± 0 0.2 ± 0.06	0.7 ± 0.1 0.7 ± 0.1	0.7 ± 0.2 0.7 ± 0.1	0.3 ± 0 0.3 ± 0	0.2 ± 0.06 0.4 ± 0	0.4 ± 0.07 2.0 ± 0
Penicillium funiculosum	0.2 ± 0.06 0.3 ± 0.07	0.7 ± 0 0.7 ± 0	0.7 ± 0 0.7 ± 0	0.7 ± 0.1 0.7 ± 0.1	0.4 ± 0.06 0.6 ± 0.06	4.0 ± 0 10.0 ± 0
Penicillium ochrachloron	0.2 ± 0.06 0.2 ± 0.07	0.7 ± 0.1 0.7 ± 0.1	0.7 ± 0.1 0.7 ± 0.1	0.3 ± 0.07 0.7 ± 0.1	0.4 ± 0.1 0.4 ± 0.1	4.0 ± 0.1 10.0 ± 0.1
Trichoderma viride	0.2 ± 0. 0.2 ± 0	0.3 ± 0.06 0.7 ± 0.1	0.3 ± 0.07 0.7 ± 0.1	0.7 ± 0 0.7 ± 0	0.4 ± 0 0.4 ± 0	4.0 ± 0 4.0 ± 0

[a] For list of organisms and protocols used, see Section 3.

bacteria: *Bacillus subtilis* (ATCC 10907), *Micrococcus flavus* (ATCC 10240), and *Staphylococcus epidermidis* (ATCC 12228). For the antifungal bioassays, eight fungi were used: *Alternaria alternata* (DSM 2006), *Aspergillus flavus* (ATCC 9643), *Aspergillus niger* (ATCC 6275), *Aspergillus ochraceus* (ATCC 12066), *Fusarium tricinctum* (CBS 514478), *Penicillium funiculosum* (ATCC 36839), *Penicillium ochrochloron* (ATCC 9112) and *Trichoderma viride* (IAM 5061). The organisms were obtained from the Mycological Laboratory, Department of Plant Physiology, Institute for Biological Research 'Siniša Stanković', Belgrade, Serbia.

The micromycetes were maintained on malt agar and the cultures stored at 4 °C and sub-cultured once a month.[34] In order to investigate the antimicrobial activity of the isolated compounds, a modified microdilution technique was used.[35,36] Bacterial species were cultured overnight at 37 °C in Luria broth medium. The spores were washed from the surface of agar plates with sterile 0.85% saline containing 0.1% Tween 80 (v/v). The spore suspension was adjusted with sterile saline to a concentration of approximately 1.0×10^5 in a final volume of 100 μL per well. The inocula were stored at 4 °C for further use. Dilutions of the inocula were cultured on Müller–Hinton agar for bacteria and solid malt agar for fungi to verify the absence of contamination and to check the validity of the inoculum.

Minimum inhibitory concentration (MIC) determinations were performed by a serial dilution technique using 96-well microtiter plates. The compounds investigated

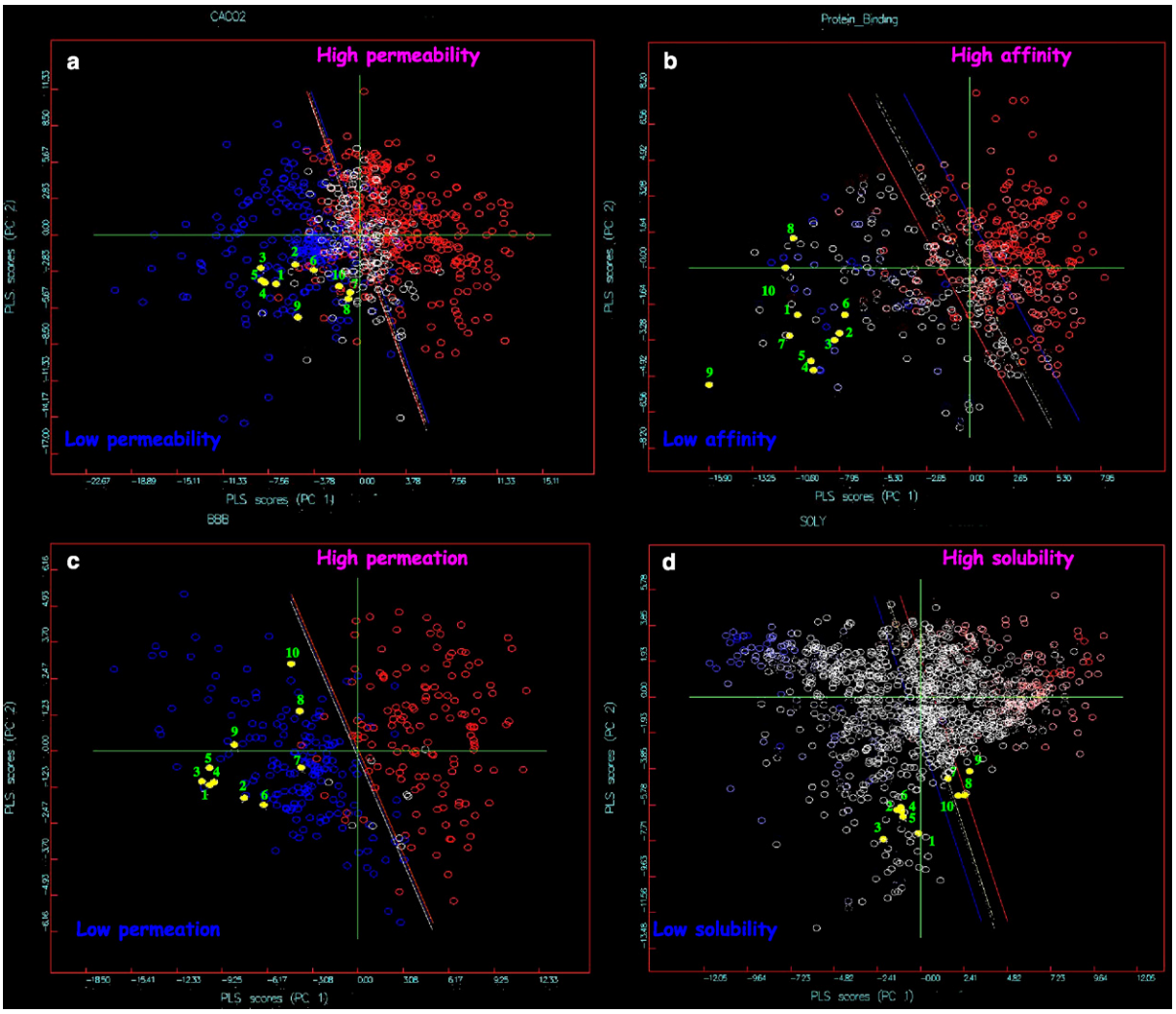

Figure 3. Projection of the sesquiterpene lactones **1**–**10** (yellow color), on the pre-calculated VolSurf models (a) Caco-2, (b) protein binding, (c) blood–brain barrier, and (d) solubility (passive permeation is the basic assumption of Caco-2 and BBB models).

Table 4. VolSurf Caco-2/, protein binding/, BBB/, and solubility/ prediction for all compounds

Compound	Caco-2	Protein binding[a]	BBB	Aqueous solubility[b]
1	−0.78	17.77	−1.60	−3.22
2	−0.53	28.58	−1.33	−3.48
3	−0.88	26.74	−1.66	−3.91
4	−0.89	15.74	−1.55	−3.31
5	−0.91	16.50	−1.53	−3.32
6	−0.13	29.87	−0.45	−2.22
7	−0.09	12.25	−0.66	−2.40
8	−0.37	31.5625	−1.17	−3.44
9	−0.69	−12.16	−1.20	−1.78
10	−0.18	23.07	−0.34	−2.31

[a] The protein binding is expressed as percentage.
[b] The aqueous solubility is expressed in mol/L at 25 °C; for more details see the manual of VolSurf.

were dissolved in DMSO (0.1–1 μg/mL) and added in broth medium (bacteria)/broth Malt medium (fungi) with inoculum. The microplates were incubated for 48 h at 37 °C or 72 h at 28 °C, respectively. The lowest concentrations without visible growth (at the binocular microscope) were defined as MICs.

The minimum bactericidal concentrations (MBCs) and minimum fungicidal concentrations (MFCs) were determined by serial sub-cultivation of a 2 μL into microtiter plates containing 100 μL of broth per well and further incubation for 48 h at 37 °C or 72 h at 28 °C, respectively. The lowest concentration with no visible growth was defined as MBC/MFC, respectively, indicating 99.5% killing of the original inoculum. DMSO was used as a negative control, while streptomycin (0.8–3.2 μmol/mL) and commercial fungicide, miconazole (0.4–10.04 μmol/mL), were used as positive controls. All components were tested in triplicate and MICs and MFCs were presented as mean values ($x \pm$ SD).

3.5. Computational methods

The data set consists of sesquiterpene lactones **1**–**5** isolated previously,[14] and sesquiterpene lactones **6**–**10**, for which their isolation is described in this manuscript. The molecules were generated using SYBYL molecular

modeling package,[37] and their energies were minimized using the Powell method with a convergent criterion provided by the Tripos force field.[38]

Caco-2 cell permeability, plasma protein affinity, BBB permeation and thermodynamic solubility of the studied compounds were predicted using VolSurf (version 4) (www.moldiscovery.com). We used the probe water (OH2), hydrophobic (DRY) and H-bonding carbonyl (O) to generate the 3D interaction energies and a Grid space of 0.5 Å.

Gview molecular graphic system (www.moldiscovery.com) was used in order to visualize the projection of our molecules on the models.

Acknowledgments

The authors thank the Ministry of Education Superior and Scientific Research of Republic of Algeria for a scholarship, Mr. Beloued Abdel Kader (National Institute of Agronomy, Algiers, Algeria) for the identification of the plant, and Dr. Guido Mastrobuoni (Mass Spectrometry Center, University of Florence-CISM) for recording the HRESI mass spectra. The authors are also grateful to Prof. Gabriele Cruciani (Laboratory for Chemometrics, School of Chemistry, University of Perugia, Italy) for kindly providing us the VolSurf program, and to Prof. Thomas Mavromoustakos (Department of Chemistry, University of Athens) for kindly allowing us access to the SYBYL molecular modeling package.

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