Sesquiterpene Lactones from *Anthemis melanolepis* and Their Antibacterial and Cytotoxic Activities. Prediction of Their Pharmacokinetic Profile

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Nine sesquiterpene lactones, anthemin A (**1**), 1α-hydroxydeacetylirinol-4α,5β-epoxide (**2**), anthemin C (**3**), tatridin A (**4**), 1-*epi*-tatridin B (**5**), anthemin B (**6**), 6-deacetyl-β-cyclopyrethrosin (**7**), elegalactone A (**8**), and 1β,4α,6α-trihydroxyeudesm-11-en-8α-12-olide (**9**), were isolated from the aerial parts of *A. melanolepis* in addition to eight known flavonoids and three phenolic acids. Compounds **1**, **3**, and **6** are new natural products. The structures of the compounds were deduced by spectroscopic methods. The in vitro antimicrobial potential of the isolated sesquiterpene lactones against four Gram-positive and five Gram-negative bacteria and one fungus was evaluated using the microdilution method, and their in vitro cytotoxic activity was determined against a panel of human tumor cell lines. Furthermore, the pharmacokinetic profile of the sesquiterpene lactones was investigated using computational methods.

The genus *Anthemis* (Anthemideae−Asteraceae) is widely distributed over Europe, especially around the Mediterranean, in west, southwest, and central Asia, and in North Africa, and comprises about 150 species. Preparations from several of these are used as aromatic herbal medicines, insecticides, and dyes. About 35 species are distributed in Greece, among which 14 are endemic.[1−3]

Anthemis melanolepis Boiss. (Asteraceae) is a procumbent, subglabrous annual species, which grows on the islands of Rhodes and Crete of Greece and in Syria, Lebanon, Palestine, and Cyprus.[4] Characteristic constituents of *Anthemis* species, many of which are used in folk medicine, are elemanolides, eudesmanolides, germacranolides, and guaianolides.[2,3] As a continuation of our research on Greek *Anthemis* species,[5−7] we now report on the chemical profile as well as the antibacterial and the cytotoxic/cytostatic activities of sesquiterpene lactones isolated from *A. melanolepis*.

A lipophilic extract of the aerial parts of *A. melanolepis* afforded three new (anthemins A−C, compounds **1**, **3**, and **6**) and six known sesquiterpene lactones, **2**,[8] **4**,[9] **5**,[10] **7**,[11,12] **8**,[12,13] and **9**,[14] eight known flavonoids, quercetin (**10**), apigenin (**11**), luteolin (**12**), 7,4′ di-*O*-methylapigenin (**13**), dihydrokaempferol (**14**), 5,7,3′-trihydroxy-3,6,4′-trimethoxyflavonol (**15**), naringenin (**16**), and eriodictyol (**17**), and three phenolic acids, 3-hydroxy-4-methoxybenzoic acid (**18**), *p*-anisic acid (**19**), and protocatechuic acid (**20**).

Compounds **1**, **3**, and **6** are new natural products. The structures of the compounds were established by means of 1D and 2D NMR, MS, and UV and, for the flavonoids,[15] spectroscopic/spectrometric analyses. Moreover, complete ^{13}C NMR assignments are presented for 1α-hydroxydeacetylirinol-4α,5β-epoxide (**2**) and elegalactone A (**8**). In addition, the antimicrobial and cytotoxic activities of compounds **2**−**9** were evaluated, while the epoxide **1** was not tested due to rapid decomposition.

Compound **1** showed in its mass spectrum a pseudomolecular ion $[M - H]^-$ at *m/z* 279, compatible with the molecular formula $C_{15}H_{20}O_5$. The IR spectrum afforded absorption bands typical of hydroxy (3397.5 cm^{-1}) and carbonyl groups 1748.4 (C=O, γ-lactone).

A combination of ^{1}H NMR, COSY, and HSQC data revealed that compound **1** was an 8,12-germacranolide with an exocyclic methylene group at C-10. In the COSY spectrum three major spin systems were observed: H-7/H-13a, H-13b (spin system A), H-5/H-6/H-7/H-8/H-9 (spin system B), and H-1/H-2/H-3 (spin system C). HSQC data were useful for the determination of the nature of the corresponding carbons. H-7 showed the typical allylic coupling with the exocyclic olefinic C-13 methylene protons of sesquiterpene lactones. It was also coupled with the vicinal H-6 (δ_H 3.80) and H-8 (δ_H 4.09). From the downfield shift of C-8 (δ_C 84.4) it was evident that the lactonization occurred at this carbon. In the COSY spectrum H-8 correlated with two methylene protons, which were assigned to H-9a and H-9b (δ_H 2.85, d, J = 12.2 Hz and 2.51, t, J = 11.8 Hz, respectively), while H-6 coupled with the methine H-5 (δ_H 3.39, br s) associated with the oxygenated C-5 (δ_C 70.1). The remaining proton at δ_H 4.64 (br s) was attached to the oxygenated C-1 (δ_C 82.2) and was assigned to H-1. It showed correlations with H-2a and H-2b, which in turn coupled with H-3a and H-3b. From the above data, it was evident that compound **1** was a germacranolide-type sesquiterpene lactone. Correlations between H-1 and the olefinic geminal protons at δ_H 5.04 and 4.98 permitted the location of the exocyclic methylene at C-10. These olefinic protons showed ^4J correlation with H-9a and H-9b, indicating the linkage of spin systems A and B through C-10. In this way, the oxygenation at C-1 along with the allylic nature of H-1 explained the particular deshielding of H-1, when compared to oxymethine H-8, H-6, and H-5. Finally, from the downfield shift of the tertiary methyl protons (δ_H 1.42), it was evident that the methyl group was attached to the 4,5-epoxide ring.

The coupling constants were indicative of the relative configuration of compound **1**, suggesting that H-6/H-7, H-7/H-8, and H-8/H-9a have a *trans* diaxial orientation and therefore a *trans* attachment of the α-methylene-γ-lactone ring to the germacrane skeleton, while the small coupling constant between H-5/H-6 suggested that these protons are *cis*-oriented. As **1** was very rapidly decomposed, a NOESY experiment was not feasible. However, according to the literature, we suggest that C-15 should be α-oriented, since it appears at δ > 20.0, while in the cases of

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β-orientation it should resonate at $\delta < 20.0$.[5,6,13,16] In addition, a remarkable difference in the ^{1}H NMR spectrum compared to the previously isolated C-1 diastereomer from *Tanacetum polycephalum*[8] has been observed supporting this assumption (H-1 at δ_H 4.64, m vs 4.23, br dd).

Compound **3** showed in its HR-ESI mass spectrum a pseudomolecular ion $[M + H]^+$ at *m/z* 263.1288, compatible with the molecular formula $C_{15}H_{18}O_4$. The IR spectrum afforded absorption bands typical of hydroxy (3398.1 cm^{-1}) and carbonyl groups [1729.8 (C=O, C-1), 1759.2 (C=O, γ-lactone)]. A combination of ^{1}H NMR, COSY, and HSQC data revealed that compound **3** was a 8,12-germacranolide with an exocyclic methylene group at C-10. ^{1}H NMR analysis of **3** in combination with HSQC data provided evidence for the presence of one tertiary methyl group (δ_C 17.2), three aliphatic methylene (δ_C 35.7, 36.1 and 39.6), two olefinic methylene (δ_C 123.7 and 126.1), two oxygenated methine (δ_C 69.7 and 76.7), one aliphatic methine (δ_C 50.9), and one olefinic methine carbon (δ_C 130.8).

In the COSY spectrum two spin systems were observed: H-7/H-13a, H-13b (spin system A) and H-5/H-6/H-7/H-8/H-9 (spin system B). H-7 showed the typical allylic coupling with the exocyclic olefinic C-13 methylene protons of sesquiterpene lactones and was coupled with H-6 (δ_H 4.14) and H-8 (δ_H 3.93) attached to oxygenated C-6 (δ_C 69.7) and C-8 (δ_C 76.7). From the downfield shift of C-8 it was evident that the lactonization occurred at that carbon. In the COSY spectrum, H-8 correlated with H-9b (2.14, dd, J = 10.5, 12.8 Hz), while H-6 coupled with H-5 (δ_H 5.05, br d, J = 10.0 Hz). Coupling constants together with a NOESY experiment enabled the establishment of the relative configuration of compound **3**. The coupling constants between H-7, H-5 (J = 10.0 Hz) and H-6 (J = 9.8 Hz) indicated a *trans* diaxial orientation of H-5, H-6, and H-7. NOE association between H-6/H-7/CH_3-15 and H-8/H-9b indicated their cofacial orientation. HMBC cross-peaks of CH_2-14 with C-1 at δ_C 206.8 gave evidence for the presence of a carbonyl group at C-1.

Compound **6** showed in its HR-ESI mass spectrum a pseudomolecular ion $[M + H]^+$ at *m/z* 265.1443, compatible with the molecular formula $C_{15}H_{20}O_4$. The IR spectrum afforded absorption bands typical of hydroxy (3397.4 cm^{-1}) and carbonyl groups [1773.5 (C=O, γ-lactone)]. ^{1}H NMR analysis of **6** in combination with HSQC data provided evidence for the presence of one tertiary methyl (δ_C 21.7), three aliphatic methylene (δ_C 30.9, 38.1, and 38.8), two olefinic methylene (δ_C 112.8 and 123.6), three oxygenated methine (δ_C 68.3, 78.0, and 78.2), and two aliphatic methine (δ_C 55.7 and 58.5) carbons.

The ^{1}H NMR spectra (Table 1) of compound **6** showed typical signals that suggested a eudesmane framework.[15] COSY experiments enabled the assignment of H-7 (δ_H 2.62) according to its allylic coupling to H-13a (δ_H 6.17) and H-13b (δ_H 5.97).

In the COSY spectrum, H-7 showed correlations with H-6 (δ_H 4.22) and H-8 (δ_H 3.96). From the HSQC experiment, it was evident that H-8 was attached to an oxygenated carbon at δ_C 78.2 (C-8), suggesting the lactonization site. The COSY spectrum displayed the following correlations: H-6 with H-5 (δ_H 2.20); H-8 with H-9a and H-9b (δ_H 2.29, 1.99, respectively). A second spin system was also evident: H-1 (δ_H 3.90) coupled with H-2a and H-2b (δ_H 2.48 and 1.58, respectively), which coupled with H-3a and H-3b (δ_H 2.72 and 2.41, respectively). Finally, two geminal olefinic protons at δ_H 5.32 (br s) and 5.01 (br s) were assigned to H-15a and H-15b, and the planar structure was completed as shown in Figure 1. Although compound **6** has the same gross structure as **7** and **8**, differences in the ^{1}H and ^{13}C NMR spectra suggested a different relative configuration at some of the stereogenic centers. 1D and 2D spectra were measured under the same experimental conditions, and data are presented in Table 2.

Coupling constants together with NOESY data permitted the establishment of the relative configuration of compound **6**. The coupling constants between H-7 (J = 11.1 Hz), H-8 (J = 11.2 Hz) and H-6 (J = 9.9 Hz), as well as H-5 and H-6 (J = 9.9 Hz), indicated a *trans* diaxial orientation of H-5, H-6, H-7, and H-8 and therefore a *trans* attachment of the α-methylene-γ-lactone ring to the eudesmane skeleton. Furthermore, NOE associations between H-1/CH_3-10 and H-8/H-6 indicated their cofacial β-nature. The above data along with the strong differences in the ^{1}H NMR chemical shifts when compared to those of **7** and **8** suggested a different orientation of the C-1 hydroxy group. The main differences were the downfield shifts of H-1 (δ_H 3.90 vs δ_H 3.56 in **7** and δ_H 3.77 in **8**), H-2a (δ_H 2.48 vs δ_H 1.87 in **7** and δ_H δ 1.82 in **8**), H-5 (δ_H 2.20 vs δ_H 1.90 in **7** and δ_H 2.02 in **8**), and H-14 (δ_H 1.11 vs δ_H 0.80 in **7** and δ_H 1.03 in **8**) and the upfield shift for H-9 (δ_H 2.29 vs δ_H 2.49 in **7** and δ_H 2.78 in **8**). In addition, C-1 appears deshielded at δ_C 78.0 vs 70.2 in **8**, while C-14 appears deshielded at δ_C 21.7 vs 13.8 in **7** (Table 2).

Table 1. ^{1}H NMR and ^{13}C NMR Spectroscopic Data for **1** (400 MHz, $CDCl_3$) and **3** (1D: 400 MHz, $CDCl_3$; 2D: 600 MHz, methanol-d_4)

	1		**3**	
position	δ_H (J in Hz)	δ_C[a]	δ_H (J in Hz)	δ_C[a]
1	4.64 m	82.2		206.8
2a	2.21 m	28.9	3.27 dd (5.6, 11.1)	36.1
2b	1.72 m		2.54 m	
3a	2.11 m	33.6	2.53 m	35.7
3b	1.69 m		2.39 m	
4				134.8
5	3.39 br s	70.1	5.05 br d (10.0)	130.8
6	3.80 dd (2.0, 9.8)	71.3	4.14 t (9.8)	69.7
7	3.99 ddd (2.9, 6.0, 9.9)	49.7	2.72 m	50.9
8	4.09 ddd (2.0, 6.4, 10.3)	84.4	3.93 m	76.7
9a	2.85 d (12.2)	43.6	3.39 dd (1.8, 12.6)	39.6
9b	2.51 t (11.8)		2.14 dd (10.5, 12.8)	
10				144.2
11				136.4
12				171.6
13a (pro-*Z*)	6.34 d (2.4)	125.8	6.35 dd (1.3, 2.9)	126.1
13b (pro-*E*)	6.14 d (2.1)		6.14 dd (1.3, 2.7)	
14a	5.04 br s	113.7	5.81 br s	123.7
14b	4.98 br s		5.77 d (1.8)	
15	1.42 s	30.8	1.64 s	17.2

[a] Carbon resonances were assigned by HSQC spectra for compound **1**; HMBC spectra have not been measured, since **1** decomposed rapidly.

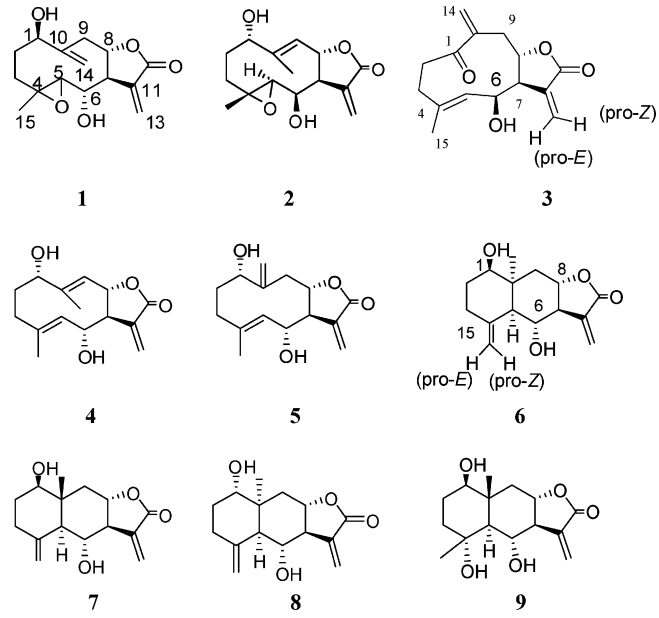

Figure 1. Structures of the isolated sesquiterpene lactones.

Table 2. ^{1}H NMR and ^{13}C NMR Spectroscopic Data (400 MHz, $CDCl_3$) for Compound **6** and ^{13}C NMR Spectroscopic Data for Compounds **2** and **8**

	6		**2**	**8**
position	δ_H (J in Hz)	δ_C[a]	δ_C	δ_C
1	3.90 dd (2.3, 9.4)	78.0	66.9	70.2
2a	2.21 m	30.9	30.8	32.7
2b	1.58 m			
3a	2.72 m	38.8	34.2	31.6
3b	2.41 m			
4			61.8	145.4
5	2.20 d (9.8)	58.5	70.0	64.5
6	4.22 t (9.9)	68.3	71.7	69.2
7	2.62 dt (2.4, 11.1)	55.7	50.6	55.8
8	3.96 ddd (1.2, 4.1, 11.2)	78.2	74.6	78.5
9a	2.29 dd (3.8, 12.2)	38.1	126.9	39.1
9b	1.99 t (11.8)			
10			145.8	42.0
11			151.0	140.6
12			172.1	173.1
13a (pro-*Z*)	6.17 d (2.4)	123.6	122.8	120.5
13b (pro-*E*)	5.97 d (2.7)			
14	1.11 s	21.7	16.2	22.4
15a (pro-*Z*)	5.32 br s	112.8	17.6	115.4
15b (pro-*E*)	5.01 br s			

[a] Carbon resonances were assigned by 2D spectra for compound **6**; missing ^{13}C signals were not observed, due to low amount.

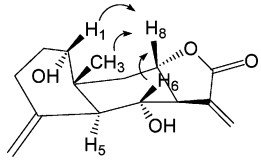

Figure 2. Key NOESY correlations for compound **6**.

The antimicrobial and cytotoxic activities of compounds tested are presented in Tables 3 and 4, respectively. All sesquiterpene lactones were found to show inhibitory effect against almost all bacteria tested, except for *Proteus mirabilis*, against which all compounds investigated were inactive. These results are similar to previous studies concerning related compounds isolated from other *Anthemis* species.[5] Concerning their cytostatic/cytotoxic activity, all compounds had one α,β-unsaturated carbonyl group, which seems to be important for cytotoxicity.[17] 6-Deacetyl-β-cyclopyrethrosine (**7**) was the only compound that was found to show cytotoxic potential. These results are in accordance with previous studies on related sesquiterpene lactones.[18]

In our previous publications, the predicted pharmacokinetic profile of some sesquiterpene lactones possessing an α-methylene-γ-lactone group[19,20] was reported. Herein, we have applied the same computational method, VolSurf,[21] on the isolated sesquiterpene lactones. VolSurf is specifically designed to produce descriptors related to pharmacokinetic properties, starting from 3D molecular field maps. In the standard procedure, GRID interaction fields[22] are calculated around the target molecules. The basic concept of VolSurf is to compress the information present in 3D grid maps into a 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,[21] protein binding,[23] blood−brain barrier (BBB) permeation,[24] drug−water solubility,[25] drug−DMSO solubility, metabolic stability,[26] hERG (human ether-a-go-go related gene) inhibition,[27] and volume of distribution.

The sesquiterpene lactones were projected on the precalculated models of VolSurf: Caco-2 cell absorption, plasmatic protein binding, blood−brain barrier passage, and thermodynamic solubility. It is observed that the pharmacokinetic profile of the examined sesquiterpene lactones is similar to those reported previously.[19,20] In particular, from the plots (Figure 3a−d) it is predicted that the studied compounds cannot be transported across the intestinal epithelium, and they cannot cross the blood−brain barrier. They also have a low affinity for the plasma-protein and moderate affinity for water.

Experimental Section

General Experimental Procedures. Optical rotation values were measured at 20 °C in $CHCl_3$ (Uvasol) on a Perkin-Elmer 341 polarimeter. IR spectra were obtained on a Perkin-Elmer Paragon 500 FT-IR spectrophotometer. The 1D and 2D NMR spectra were recorded using Bruker DRX 400, Varian 600 MHz, and Bruker AC 200 spectrometers. Chemical shifts are reported in δ values. COSY, HSQC, HMBC, and NOESY were performed using standard Bruker microprograms. Mass spectral data were recorded on an ESI-Micromass Quattro LC triple quadrupole mass spectrometer and on a double sector JEOL JMS-AX505HA mass spectrometer and were provided by the University of Notre Dame, Department of Chemistry and Biochemistry, South Bend, IN. VLC[28] was performed on silica gel (Merck; 43−63 μm) and column chromatography on silica gel 60H SDS (40−63 μm) using solvent mixtures indicated in each case. Reversed-phase chromatography was carried out on a CE 1100 liquid chromatography system. Preparative HPLC was performed using a C_{18} 25 cm × 10 mm Techsil 10 column. Fractionations were monitored by TLC silica gel 60 F-254, Merck Art. 5554; cellulose, Merck Art. 5716; RP 18 F-254, Merck, Art. 5685 with visualization under UV (254 and 365 nm) and with anisaldehyde−sulfuric acid reagent on silica gel and Neu's reagent on cellulose.[29]

Plant Material. The aerial parts of *A. melanolepis* were collected at Rethimnon/Herakleion, Crete, in June 2002. The plant was authenticated by Dr. Z. Kypriotakis (Technological Education Institute, School of Agricultural Production), and a voucher specimen was deposited in the Herbarium of the Technological Education Institute, School of Agricultural Production, Laboratory of Taxonomy and Management of Wild Flora, Crete (Kypriotakis 8607).

Extraction and Isolation. The fresh aerial parts of *A. melanolepis* (0.36 kg) were finely ground and extracted at room temperature with cyclohexane−Et_2O−MeOH (1:1:1; extract A) and MeOH−H_2O (1:1; extract B), successively. Extract A was washed with brine, the aqueous layer re-extracted with EtOAc, and the organic layer dried with Na_2SO_4 and concentrated under reduced pressure. The residue (9.9 g) was fractionated by VLC on silica gel (10.0 × 5.0 cm), using cyclohexane−EtOAc−Me_2CO mixtures of increasing polarity as eluents, to give nine fractions of 500 mL each (fractions A−I). Fraction C (cyclohexane−EtOAc, 50:50; 649.0 mg) was subjected to column chromatography on silica gel (2.7 × 16.0 cm; CH_2Cl_2−EtOAc, 10:0 to 0:10, and EtOAc−MeOH, 10:0 to 0:10), to give 12 fractions that led to the isolation of **15** (9.3 mg, fraction 7). Fraction 8 (37.7 mg) of the latter CC was subjected to RPHPLC (MeOH−H_2O, 1:1, 2 mL/min) and gave **1** (1.2 mg) and **16** (3.1 mg); t_R 26.1 and 61.5 min, respectively. Further purification of fraction 9 (79.6 mg) by RPHPLC (MeOH−H_2O, 3:2, 2 mL/min) gave **14** (4.2 mg); t_R 14.3 min. Fraction 10 (53.4 mg) was subjected to RPHPLC (MeOH−H_2O, 1:1, 2 mL/min) to give **18** (1.0 mg); t_R 6.6 min. Fraction D (cyclohexane−EtOAc, 25:75; 861.0 mg) was subjected to column chromatography on silica gel (2.7 × 17.0 cm; CH_2Cl_2−EtOAc, 10:0 to 0:10, and EtOAc−MeOH, 10:0 to 0:10), to give 21 fractions that led to the isolation of **3** (3.2 mg), **6** (1.6 mg), and **7** (37.2 mg). Further purification of fraction 15 (55.5 mg) with RPHPLC (MeOH−H_2O, 9:11, 2 mL/min) gave **19** (1.6 mg), **7** (17.5 mg), **8** (8.5 mg), and **10** (7.0 mg); t_R 11.6, 14.4, 16.0, and 48.9 min, respectively. Fraction 17 (67.3 mg) was subjected to RPHPLC (MeOH−H_2O, 9:11, 2 mL/min) and gave **2** (3.4 mg), **4** (15.0 mg), and **7** (3.9 mg); t_R 8.7, 13.0, and 18.0 min, respectively. Fraction 18 (60.7 mg) was subjected to RPHPLC (MeOH−H_2O, 42:58, 2 mL/min) to give **20** (1.1 mg), **5** (4.6 mg), and **4** (3.5 mg); t_R 5.6, 16.4, and 28.2 min, respectively. Further purification of fraction 19 (80.1 mg) by RPHPLC (MeOH−H_2O, 1:1, 2 mL/min) gave **2** (2.5 mg); t_R 9.6 min. Further column chromatography of fraction 21 on Sephadex LH-20 yielded **17**. Fraction E (EtOAc, 100%; 861.0 mg) was subjected to column chromatography on silica gel (2.7 × 13.5 cm; CH_2Cl_2−EtOAc,

Table 3. Minimum Inhibitory Concentration (MIC, μg/mL) of Compounds **2**−**5** and **7**−**9**[a,b,c]

	B. cereus	*M. luteus*	*Staph. aureus*	*A. tumefaciens*	*E. coli*	*Ps. aeruginosa*	*Ps. tolaasii*	*S. enteritidis*	*S. lutea*	*C. albicans*
2	12.5	12.5	1.25	25.0	25.0	50.0	25.0	25.0	6.25	
3	12.5	12.5	1.25						25.0	
4	6.25	6.25	0.625	25.0	25.0	25.0	25.0	25.0	6.25	25.0
5	6.25	6.25	0.625	25.0	25.0	25.0	25.0	25.0	6.25	25.0
7	6.25	6.25	0.625	25.0	25.0	25.0	25.0	25.0	6.25	25.0
8		50.0	50.0							
9	12.5	12.5	1.25	25.0	25.0	50.0	25.0	25.0	6.25	
streptomycin	50.0	50.0		100.0	100.0		200.0	200.0	50.0	
bifonazole										200.0

[a] All compounds were inactive against *Proteus mirabilis*. [b] **1**: not tested, since it decomposed rapidly; **6**: not tested due to low amount. [c] For list of organisms and protocols used, see Experimental Section.

Table 4. GI_{50}, TGI, and LC_{50} Data (μM) of Compounds **2**−**9** and Vinblastine[a,b,c]

	6			**7**			**8**			**9**			vinblastine		
cell line	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}
SF268	52.3	80.3	*d*	42.7	*d*	*d*	76.0	*d*	*d*	40.3	*d*	*d*	<0.01	1.64	2.48
MCF7	*d*	*d*	*d*	52.3	64.2	85.8	*d*	*d*	*d*	*d*	*d*	*d*	<0.01	1.60	2.50
H460	*d*	*d*	*d*	69.0	*d*	*d*	*d*	*d*	*d*	*d*	*d*	*d*	<0.01	0.35	2.10

[a] The values represent the means of three independent experiments run in triplicate. SD never exceeded 15% of the mean value. [b] Compound **1** was not tested, since it rapidly decomposed. Compounds **2**, **3**, **4**, and **5** were inactive against all tested cancer cell lines. [c] For list of cell lines and protocols used, see Experimental Section. [d] Not active at concentrations as high as 100 μM, which was the maximum concentration tested.

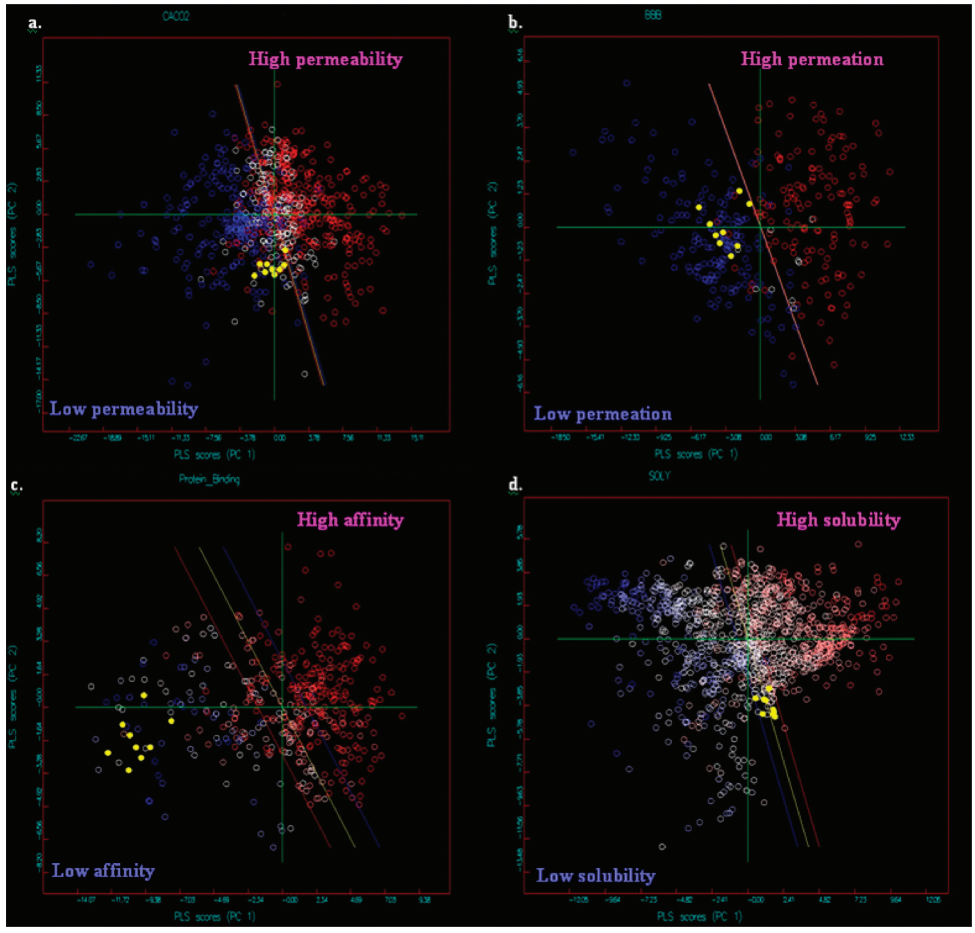

Figure 3. Projection of the sesquiterpene lactones (yellow circles) on the precalculated models of VolSurf: (a) Caco-2, (b) BBB, (c) protein binding, and (d) solubility.

10:0 to 0:10, and EtOAc−MeOH, 10:0 to 0:10) and led to the isolation of **9** (24.8 mg).

Extract B (36.3 g) was fractionated by reversed VLC on silica gel (10.0 × 5.0 cm), using MeOH−H_2O mixtures of decreasing polarity as eluents, to give 12 fractions of 500 mL each (fractions A′−L′). Fraction G′ (H_2O−MeOH, 65:35; 887.2 mg) was subjected to column chromatography on Sephadex LH-20 and gave **11** (2.0 mg) and **12** (2.8 mg). Fraction H′ (H_2O−MeOH, 55:45; 1.032 g) was subjected to repeated column chromatographies on silica gel and Sephadex LH-to give the flavonoid **11**, while fraction I′ (H_2O−MeOH, 40:60; 411.4 mg) was subjected to column chromatography on Sephadex LH-20 to give **13** (2.6 mg).

1 (anthemin A): colorless oil; $[\alpha]^{20}_D$ −3.0 (*c* 0.10, MeOH); IR (film) ν_{max} 3397, 2911, 2840, 1748 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; ESIMS (neg.) *m*/*z* 279 (calcd for $C_{15}H_{20}O_5$ 280.1311).

3 (anthemin C): colorless oil; $[\alpha]^{20}_D$ +8.6 (*c* 0.07, MeOH); IR (film) ν_{max} 3398, 2926, 2840, 1759, 1729 cm^{-1}; ^{1}H and ^{13}C NMR data,

see Table 1; HR-ESIMS *m/z* $[M + H]^+$ 263.1288 (calcd for $C_{15}H_{18}O_4$ 262.1205).

6 (anthemin B): yellowish oil; $[\alpha]^{20}_D$ +4.2 (*c* 0.10, MeOH); IR (film) ν_{max} 3397, 2921, 1773, 1703; ^1H and ^{13}C NMR data, see Table 2; HR-ESI *m/z* $[M + H]^+$ 265.1443 (calcd for $C_{15}H_{20}O_4$ 264.1362).

Bioassays. The following Gram-negative bacteria were used: *Escherichia coli* (ATCC 35218), *Proteus mirabilis* (clinical isolates), *Agrobacterium tumefaciens* (A281), *Pseudomonas aeruginosa* (clinical isolates), *Pseudomonas tolaasii* (isolated from *Agaricus bisporus*), *Salmonella enteritidis* (ATCC 13076). The following Gram-positive bacteria were used: *Staphylococcus aureus* (ATCC 6538), *Micrococcus luteus* (clinical isolates), *Sarcina lutea* (ATCC 9341), *Bacillus cereus* (clinical isolates). One yeast was tested: *Candida albicans* (clinical isolates).

Compounds were dissolved at 10 mg/mL with DMSO and diluted with the nutrient medium to a concentration of 1000 μg/mL. Final concentrations of 50.0, 25.0, 12.5, and 6.25 μg/mL were used. The proportion of DMSO never exceeded 1% in the medium.[30]

In order to obtain quantitative data, a modified microdilution technique was used.[31,32] MIC determination was performed by a serial dilution technique using 96-well microtiter plates. Bacterial species were cultured overnight at 37 °C in TSB medium (Oxoid-CM129). Suspensions contained $\sim 10^9$ cells/mL. 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. *C. albicans* was maintained on Sabouraud agar (SDA), and the cultures were stored at +4 °C and subcultured once a month.[33] The inocula were stored at +4 °C for further use. Dilutions of the inocula were cultured on TSA (Oxoid-CM 131) for the bacteria and on LB for *C. albicans* to verify the absence of contamination and to check the validity of the inoculum. The plates containing the bacteria were incubated for 48 h at 37 °C, while that of *C. albicans* for 72 h at 28 °C. DMSO was used as a control, while streptomycin for bacteria and bifonazole for *C. albicans* were used as positive controls.

Clinical isolates were directly isolated from patients at the Centre for Preventive Medicine, Military Medicinal Academy, Department of Microbiology, Belgrade, Serbia.

The cytotoxic activity of compounds **2**−**9** was tested against the following cell lines: SF268 (CNS), MCF7 (breast), and H460 (non-small-cell lung cancer) and determined by the SRB assay, as previously described.[34] The data represent the mean of three experiments in triplicate and were analyzed using a two-tailed Student's *t* test. The following parameters were determined through our own customized software: GI_{50}, TGI, and LC_{50}.[35] Briefly, GI_{50} is the concentration where $100 \times (T - T_0)/(C - T_0) = 50$ and measures the growth-inhibitory potency of the tested compound. TGI is the concentration of the test compound where $100 \times (T - T_0)/(C - T_0) = 0$ and measures the cytostatic effect of the compound. T is the optical density of the test well after a 48 h period of exposure to the test compound; T_0 is the optical density of the cell population at the time zero (when the compound is added), and C is the optical density of the control well, where cells were incubated for 48 h, and represents the cytotoxic activity of the compound. LC_{50} is the concentration of the test compound where $(T/T - T_0) \times 100 = -50$.

Computational Methods

The sesquiterpene lactones were generated using the SYBYL molecular modeling package,[36] and their energies were minimized using the Powell method with a convergent criterion provided by the Tripos force field.[37] 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, hydrophobic (DRY) and H-bonding carbonyl (O) to generate the 3D interaction energies and a Grid space of 0.5 Å. The Gview molecular graphic system (www.moldiscovery.com) was used to visualize the projection of our molecules on the models.

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Supporting Information Available: This material is available free of charge via the Internet at http://pubs.acs.org.

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