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Structure-Dependent Cytotoxic Effects of Eremophilanolide Sesquiterpenes

M. Teresa Agulló-Ortuño[a*], Carmen E. Díaz[b], Azucena González-Coloma[c] and Matías Reina[b]

[a]*Laboratory of Translational Oncology*, *Instituto de Investigación Hospital 12 de Octubre*, *28041 Madrid*, *Spain*
[b]*Instituto de Productos Naturales y Agrobiología*, *CSIC*, *38206 La Laguna*, *Tenerife*, *Spain*
[c]*Instituto de Ciencias Agrarias CSIC*, *28006 Madrid*, *Spain*

agullo@h12o.es

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The aim of this research was to determine the cytotoxic action of sixteen structurally-related eremophilane-type sesquiterpenes, isolated from several species of *Senecio,* against a panel of cancer cell lines. The cytotoxic activities were evaluated by WST-1 test and the IC_{50} values calculated. The investigated compounds exerted dose-dependent cytotoxic actions against selected cancer cell lines and no-tumoral HS5 cell line. The comparative structure-activity relationships demonstrated the importance of C-1, C-6, and C-8 substituents in the molecule. Our results show that eremophilane-type sesquiterpenes may represent an important source of novel potential antitumor agents due to their pronounced cytotoxic actions towards malignant cells.

Keywords: Eremophilanolides, Cytotoxic activity, Structure-activity relationships.

Natural products are a rich source of compounds with promising cancer therapeutic potential, such as vinca alkaloids, taxanes, campothecins, etc. [1]. Sesquiterpenes represent a group of natural compounds with diverse skeletal types. Among them, the eremophilane-type sesquiterpenes, including eremophilanes, furano-eremophilanes, eremophilanolides and secoeremophilanolides, are widely present in several genera of Asteraceae (such as *Cacalia*, *Ligularia*, *Petasites*, *Senecio*) [2] and fungi [3], and are biosynthetically differently from other sesquiterpenes. These compounds have a number of biological activities such as anti-tumor, anti-bacterial and anti-inflammatory among others [2b].

In *Senecio* species, furanoeremophilanes and eremophilanolides are the most frequently isolated sesquiterpene skeletons [2, 4]. As part of our ongoing studies on bioactive sesquiterpenes from *Senecio* species, sixteen structurally-related eremophilanolides have been tested against a panel of tumoral cell lines and their structure activity relationships (SAR) studied.

The cytotoxicity of the test compounds on several tumoral (MCF7, A549, H292, HCT116, HeLa, SKMEL5, and DU145) and non-tumoral (HS5) cell lines compared to that of two usual drugs in clinical practice (cisplatin and paclitaxel) is shown in Table 1.

The eremophilanolides tested here inhibited the celular growth in a dose-dependent manner (Table 1). HS5 and HCT116 cell lines were the most sensitive followed by A549 and SKMEL5 cell lines. H292 cell line was the most resistant. Compound **1** was the most active for all cell lines tested. The cytotoxic effects of compound **2** were also remarkable. In general, the activity of these two compounds was superior to that of cisplatin and paclitaxel.

The cytotoxic activity of eremophilanolides from *Ligularia sp.* [3e, 5] and *Calomeria amaranhoides* [6] has been described. However, this is the first report on the cytotoxic effects of eremophilanolides **1-16** from *Senecio* sp.

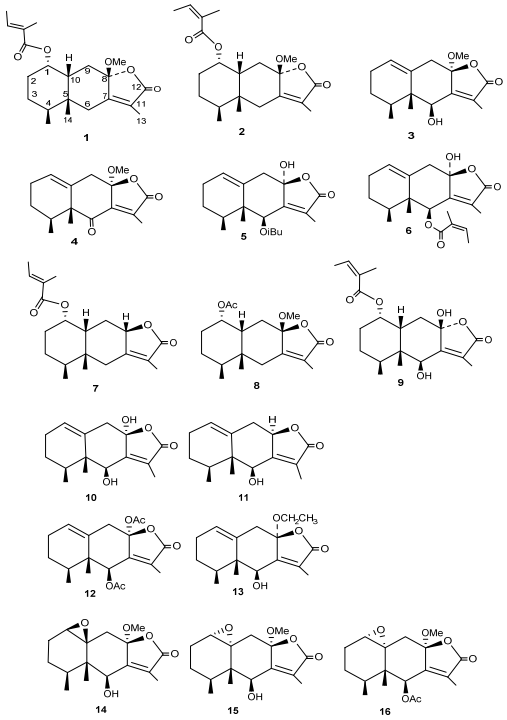

Figure 1: Eremophilanolides from *Senecio* species.

Table 2 shows the SAR of compounds **1-16**. The most active eremophilanolides were found in the 1,8 substituted group and had a β-OMe at C-8 in their moiety (**1**, **2**). Among the 1,10 unsaturated group of terpenes, the presence of an α-OMe at C-8 resulted in the most active compounds (**3**, **4**), followed by **5** and **6** with the bulkiest substituents at C-6. The least active group was the 1,10 epoxide, however it is interesting to note that the activity depended on the epoxide stereochemistry (**14**, β-epox) (Table 2).

Table 1: Cytotoxic effects of several eremophilanolides on cell lines.

Compds	Cell Lines (IC_{50} ± SD, μM)							
	MCF7	A549	H292	HCT116	HeLa	SKMEL5	DU145	HS5
1	3.4±1.5	2.5±1.5	4.3±2.3	3.8±1.2	2.6±1.9	2.7±1.6	3.8±2.3	1.8±1.5
2	>50	43.8±2.3	>50	1.2±1.1	3.1±1.4	5.4±1.9	13.8±1.8	2.8±1.0
3	41.3±1.5	>100	>100	10.4±2.6	>100	10.9±1.4	>100	9.4±2.5
4	>100	8.2±1.1	>100	13.8±1.1	>100	7.8±2.8	>50	>50
5	3.8±1.0	>100	>100	>100	6.9±1.7	>100	>100	>100
6	6.3±2.3	>100	>100	>100	>100	>100	10.9±1.8	>100
7	>100	13.2±1.0	>100	31.9±1.7	>50	>50	>100	32.7±5.4
8	>50	24.7±1.2	>100	>50	>50	>100	>100	>50
9	41.6±4.1	>50	>50	47.0±1.5	>50	>50	>50	11.3±1.2
10	>100	>100	>50	16.0±5.2	>100	>50	>100	14.3±7.3
11	>50	>100	>50	40.2±1.9	>50	10.2±1.0	>50	24.5±4.5
12	>50	>50	>100	>50	20.6±2.9	>100	>100	>100
13	>50	>100	>100	>100	>100	>100	>100	>100
14	>100	3.5±2.5	>50	>100	>100	>100	>100	>50
15	>100	>50	>100	>50	>100	>100	>100	>50
16	>100	>100	>100	>100	>100	>100	>100	>100
CDDP	9.6±2.3	37.0±5.2	19.3±3.4	10.5±3.2	25.8±5.1	8.0±2.0	>50	8.5±1.8
PTX	2.5±0.9	18.0±4.1	5.5±1.5	>50	2.6±1.2	15.1±3.1	>50	35.6±4.1

CDDP: Cisplatin, PTX: Paclitaxel (Taxol®).

IC_{50}: The half maximal inhibitory concentration.

Table 2: Cytotoxic structure-activity relationships (SAR) of *Senecio* eremophilanolides.

Compds	Substituents				Activity Index*	Group
	C-1	C-6	C-8	C-10		
1	α-OTigl	H_2	β-OMe	β-H	8	1,8 substituted
2	α-OAng	H_2	β-OMe	β-H	5	
3	Δ^1	β-OH	α-OMe	Δ^{10}	3	1,10 unsaturated
4	Δ^1	C=O	α-OMe	Δ^{10}	3	
5	Δ^1	β-OiBu	α-OH	Δ^{10}	2	
6	Δ^1	β-OAng	α-OH	Δ^{10}	2	
7	α-OTigl	H_2	β-H	β-H	2	
8	α-OAc	H_2	β-OMe	β-H	1	
9	α-OAng	β-OH	β-OH	β-H	1	
10	Δ^1	β-OH	α-OH	Δ^{10}	1	1,10 unsaturated
11	Δ^1	β-OH	α-H	Δ^{10}	1	
12	Δ^1	β-OAc	α-OAc	Δ^{10}	1	
13	Δ^1	β-OH	α-OEt	Δ^{10}	0	
14	β-epox	β-OH	α-OMe	β-epox	1	1,10 epoxide
15	α-epox	β-OH	α-OMe	α-epox	0	
16	α-epox	β-OAc	α-OMe	α-epox	0	

*Number of cell lines affected by the compound with IC_{50} values within the range of the positive control (Table 1).

Therefore, the substitution pattern at C-1, C-6, and C-8 of eremophilanolides resulted in tumor-selective cytotoxicity against human tumor cell lines. This would be an important biological structural requirement and could provide new rational basis for the design of new antitumor compounds.

In summary, the most interesting eremophilanoides were **1** and **2**, due to their high cytotoxic activity against the panel of tumor cell lines tested. The cytotoxicity of these compounds showed dose- and cell line-dependent effects, with IC_{50} lowers of those of CDDP and PTX. The comparative structure-activity relationships showed the importance of C-1, C-6, and C-8 substituents in the molecule.

Experimental

Compounds: Compounds **3**, **10** and **11** were isolated from *S. magellanicus* [7]; **1**, **2** and **9** from *S. kingii* [8]; **8** *from S. miser* [9] and compound **7** from *S. poepigii* [10]. Compounds **4**, **14**-**16** are derivatives of **3** and **12** is an acetylated derivative of **10** [7]. Compounds **5**, **6** and **13** have been isolated from *S. bollei* collected in Lanzarote (Canary Islands), (Voucher number ORT32501. Centro de Investigaciones y Tecnología Agraria, Tenerife). Dried aerial parts of *S. bollei* (1.6 Kg) were extracted with EtOH in a Soxhlet. The ethanolic extract (122 g, 7.62% yield of dry plant weight) was chromatographed on a silica gel vacuum liquid chromatography (VLC) column using an *n*-hexane-EtOAc-MeOH gradient, to give six fractions. All fractions were further chromatographed on a Si gel column and /or by preparative normal-phase HPLC on a Inertsil Prep-Sil column (25 x 2 i.d.; Gasukuro Kogyo) and Ultrasphere Si (25 x 1 i.d.; Beckman) to yield 6β-angeloyloxy-8α-hydroxyeremophil-1(10),7(11)-dien-8β,12-olide (**13**) (5.4 mg), 6β-angeloyloxy-8α-hydroxyeremophil-1(10),7(11)-dien-8β,12-olide (**5**) (1.8 mg) and 6β-isobutanoiloxy-8α-hydroxy-eremophil-1(10),7(11)-dien-8β,12-olide (**6**) (5.0 mg). The structures of these compounds were determined by comparison of their spectroscopic data with that reported in the literature [11-13].

Cell culture and reagents: Human cell lines, A549 (lung adenocarcinoma), H292 (squamous lung cancer), HCT116 (colorectal carcinoma), and DU145 (prostate cancer) were cultured in RPMI 1640 medium (Lonza Verviers), and MCF-7 (breast adenocarcinoma), HeLa (cervix adenocarcinoma) SK-MEL-5 (malignant melanoma), and HS5 (bone marrow stroma) were cultured in Dulbecco's modified Eagle's medium (DMEM) (Lonza Verviers), supplemented with 10% FBS, 100 U/mL penicillin, 100 U/mL streptomycin, and 2mM glutamine. All cells were grown at 37ºC in a humidified atmosphere with 5% CO2 and were in the logarithmic growth phase at the initiation of the experiments. All lines were obtained from LGC Promochem, SLU-ATCC (Barcelona, Spain).

Stock solutions of compounds were prepared in dimethyl sulfoxide and diluted in fresh medium containing 0.1% FBS before each experiment.

Proliferation assays: Growth inhibition was assessed by using the WST-1 assay (Roche, Mannheim) according to the manufacturer's protocol. Cells diluted in 100 μL/well of cell culture medium were plated in 96-well flat-bottom plates and allowed to attach for 24 hours at 37ºC/5% CO_2. The number of cells per well used in these experiments were as follows: A549, HCT116, and HeLa, 3000 cells; MCF7, H292, SKMEL5, DU145 and HS5, 5000 cells. Test products were added to each well and incubated for another 72 hours. All experimental points were set up in six wells, and all experiments were repeated at least three times. Each value represents the mean of the experiments performed.

Statistical analysis: The median inhibition concentration (IC_{50}) values were calculated using GraphPad Prism 6.0 (GraphPad Software, Inc, La Jolla, CA), using nonlinear curve fitting.

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