

CHEMICAL COMPOSITION AND ANTI-INFLAMMATORY ACTIVITY OF *Roldana platanifolia*Amira Arciniegas^a, Ana L. Pérez-Castorena^{a,*}, Antonio Nieto-Camacho^a, Jhon Ironzi Maldonado^a, José Luis Villaseñor^b and Alfonso Romo de Vivar^a^aInstituto de Química, Universidad Nacional Autónoma de México, Circuito Exterior, Ciudad Universitaria, Coyoacán 04510, D.F., México^bInstituto de Biología, Universidad Nacional Autónoma de México, Circuito Exterior, Ciudad Universitaria, Coyoacán 04510, D.F., México

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The chemical study of *Roldana platanifolia* led to the isolation of β -caryophyllene, five eremophilanolides, chlorogenic acid, and a mixture of β -sitosterol-stigmasterol, β -sitosteryl glucopyranoside, and sucrose. The anti-inflammatory activities of the extracts and isolated products were tested using the 12-O-tetradecanoylphorbol-13-acetate (TPA) model of induced acute inflammation. The acetone and methanol extracts showed dose dependent activities (ID₅₀ 0.21 and 0.32 mg/ear, respectively), while none of the isolated compounds exhibited relevant edema inhibition. The active extracts were also evaluated with the myeloperoxidase assay technique (MPO) to determine their ability to prevent neutrophil infiltration. Results showed that the anti-inflammatory activity was related to the compound's ability to inhibit pro-inflammatory mediators such as neutrophils.

Keywords: *Roldana platanifolia*; Senecioneae; Tussilaginatae; Eremophilanolides.

INTRODUCTION

The species of the genus *Roldana* (Asteraceae, Senecioneae, Tussilaginatae) are perennial herbs which may grow to shrubs, in the temperate forests between 1000 to 4000 m elevation. They are distributed from southern Arizona and New Mexico to Panama.^{1,2} Previous chemical studies on 12 of the 48 species grouped into the genus *Roldana* show that they are source mainly of sesquiterpenes of eremophilane type,^{3,4} although some of them contain also oplopanes,⁵ plastoquinones,⁶ and flavonoids.⁷ Pyrrolizidine alkaloids have not been isolated so far in spite of their close taxonomic relation with the genus *Senecio*. Some species of *Roldana* are used in Mexican folk medicine, thus, *R. ehrenbergiana* is employed against leprosy⁸ and *R. sessilifolia* is included in the "cachani complex", which is a mixture of plants traditionally used to treat female sterility, hemorrhoids, and rheumatic pains.⁹ In addition, bioactive metabolites with anti-oxidant, anti-inflammatory,⁶ insecticidal,¹⁰ and antifungal properties⁷ have been reported in *Roldana* species. As part of an ongoing project on species of Senecioneae, this paper describes the first chemical study of *Roldana platanifolia*, as well as, the anti-inflammatory activity of extracts and isolated compounds tested on the 12-O-tetradecanoylphorbol-13-acetate (TPA) model of induced acute inflammation. The active acetonic and methanolic extracts were also tested on the myeloperoxidase (MPO) assay to determine their effect on neutrophil recruitment.

RESULTS AND DISCUSSION

The chemical study of the aerial parts of *Roldana platanifolia* afforded β -caryophyllene 8*R*,9*R*-oxide (**1**), tsoongianolides A, B, and D (**2-4**), ligularenolide (**5**), 2-oxoerenophil-1(10),7(11),8(9)-trien-12,8-olide (**6**), chlorogenic acid (**7**), mixture of β -sitosterol (**8**)-stigmasterol (**9**), β -sitosteryl glucopyranoside (**10**), and sucrose (Figure 1). Structures of the isolated products were determined by spectroscopic analyses and comparison with reported data.

Compounds **2-4** showed the same molecular ion at *m/z* 248 in the EIMS experiment. Their IR spectra were similar and showed absorption bands for hydroxyl and unsaturated γ -lactone groups. These three isomeric compounds showed fifteen signals in their ¹³C NMR spectra (Table 1S) and characteristic resonances of eremophilanolide: a secondary, a tertiary, and a vinylic methyl groups in the ¹H NMR spectra (Table 2S). Signals at δ 5.51, 5.46, and 5.60 for **2**, **3** and **4**, respectively, were assigned to their respective H-9 vinylic protons.

Compounds **2** and **3** showed ¹³C NMR resonances at δ 73.4 and 74.0, assigned to C-10 by their interactions with H-9, H₃-14 and H₂-1, in the respective HMBC experiments, suggesting tertiary hydroxyl groups at this position and an epimeric relation between them. The chemical shift of CH₃-14 at δ 0.84 in compound **2** determined its *trans* decaline structure, and in compound **3** the *cis* annelation was deduced by the CH₃-14 resonance at δ 1.06.¹¹ In compound **4**, C-10 resonated as vinylic carbon at δ 136.5 and C-8 as *hemi*-ketal carbon at δ 102.9. The hydroxyl group at C-8 should be α -oriented since CH₃-15 resonates at lower field (δ 1.00, d, *J* = 7.0 Hz) than CH₃-14 (δ 0.83 s).¹² Based on the above, compounds **2-4** were identified as tsoongianolides A, B and D previously isolated from *Senecio tsoongianus*.¹³ On the bases of 2D MNR spectroscopy, some signals of compounds **2-4** have been reassigned.

Compound **5** showed a molecular ion at *m/z* 230 in the EIMS. The ¹H NMR spectrum of **5** exhibited (Table 2S), as for compounds **2-4**, an eremophilanolide structure pattern. The resonances of two vinylic protons observed at δ 5.79 (t, *J* = 4.0 Hz) and 5.94 (s) were assigned to H-1 and H-9, respectively, on the basis of 2D NMR experiments. The ¹³C NMR spectrum (Table 1S) exhibited the signals of three methyls, three methylenes, three methynes, and six non hydrogenated carbon atoms, consistent with structure **5**. The spectroscopic data and physical constants of **5** are in good agreement with those of ligularenolide previously isolated from *Liguaria* species.¹³⁻¹⁵ Compound **6** showed a *quasi*-molecular ion at *m/z* 245 [M+H]⁺ in DART-MS. The IR spectrum exhibited absorptions of conjugated γ lactone and ketone groups (1777, 1663, and 1633 cm⁻¹). The ¹³C NMR spectrum showed (Table 1S), besides the characteristic signals of eremophilanolide skeleton, that of a ketone carbonyl at δ 197.8 assigned to C-2 by the

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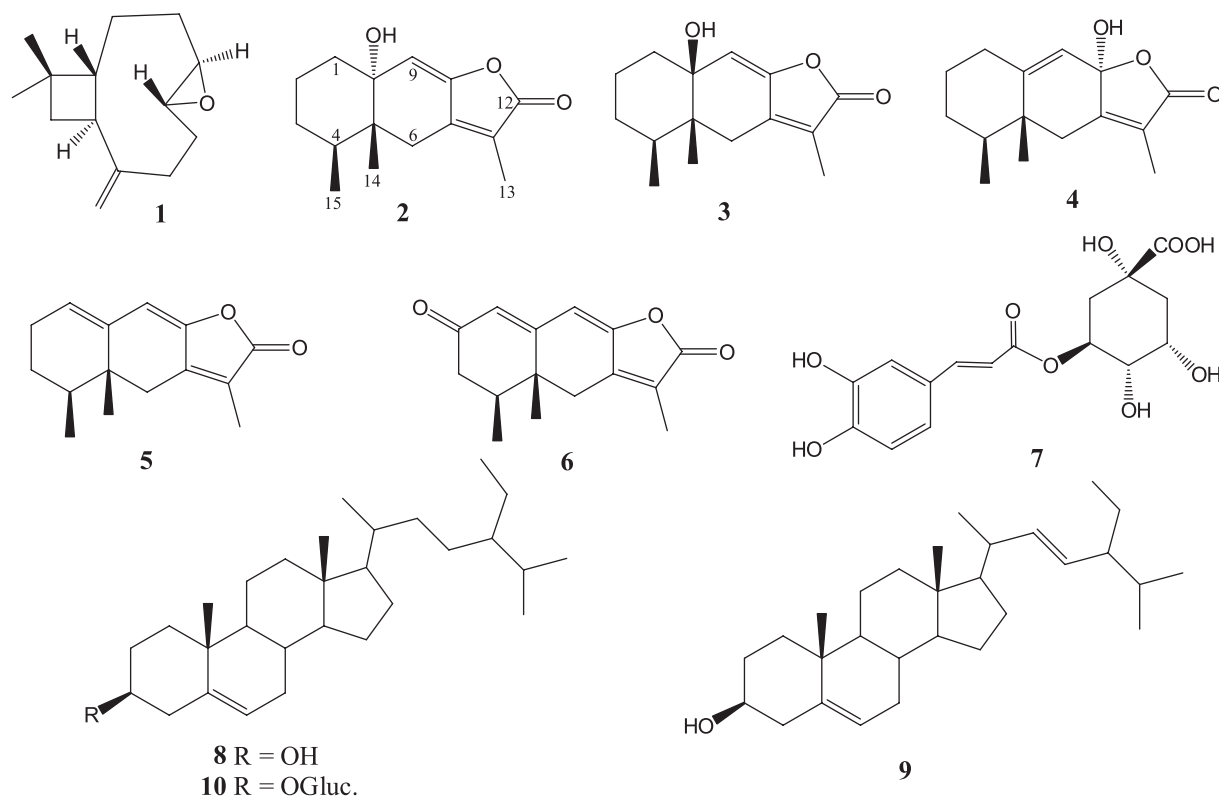


Figure 1. Compounds isolated from *Roldana platanifolia*

cross peaks between this carbon and H-1, H-3 and H-4 in the HMBC experiment. Compound **6** spectroscopic features were in agreement with 2-oxoerenuphil-1(10),7(11),8(9)-trien-12,8-olide, previously isolated from *Ligularia platyglossa*.^{15,16}

β -Caryophyllene 8*R*,9*R*-oxide (**1**),¹⁷ chlorogenic acid (**7**),¹⁸ mixture β -sitosterol¹⁹ (**8**) – stigmasterol²⁰ (**9**), β -sitosteryl glucopyranoside²¹ (**10**), and sucrose were characterized by their spectroscopic data and additionally compared with authentic samples.

Based on the utilization in traditional medicine of *Roldana* species to alleviate rheumatic pains,⁹ and since anti-inflammatory agents have been isolated from species of this genus,⁶ extracts and isolated compounds of *R. platanifolia* were evaluated on the TPA model of induced acute inflammation. In a primary screening with 1 mg / ear (Table 1) the acetone and methanol extracts had a strong anti-inflammatory activity (89.34 and 82.52%, respectively, of edema inhibition) similar to that of the reference compound indomethacin. Among the isolated compounds only tsoongianolide D showed a mild anti-inflammatory effect (43.36% of edema inhibition) and was the most active. The weak activity of chlorogenic acid (21.85%) was in agreement with reported data on TPA model,²² even though its anti-inflammatory action has been reported in the carrageenan-induced inflammation model²³ and its antiarthritis properties demonstrated.²⁴

In the dose-response evaluation (Table 2) the acetonic and methanolic extracts showed dose dependent activities with ID_{50} 0.21 and 0.32 mg / ear, respectively, and were less actives than indomethacin (0.08 mg / ear).

Topical application of TPA induce cell infiltration with release of mediators that increase vascular permeability and promote neutrophil influx.²⁵ MPO activity is well correlated with the number of neutrophils in inflamed regions^{26,27} and is used as biological marker for tissue content of polymorphonuclear leucocytes. In order to determine the influence of neutrophil accumulation in the anti-inflammatory response of the acetonic and methanolic extracts, the MPO activity test was evaluated showing that they attenuated in a dose dependent

Table 1. Effect of extracts and isolated compounds from *R. platanifolia* on TPA-induced mouse edema

Sample	Edema (mg)	Inhibition (%)
Hexanic extract ^{a,c}	11.00 ± 0.44*	29.64
Acetonic extract ^{a,c}	1.67 ± 0.23**	89.34**
Methanolic extract ^{a,c}	2.73 ± 0.90**	82.52**
β -caryophyllene 8 <i>R</i> ,9 <i>R</i> -oxide (1) ^{b,d}	9.63 ± 0.63	19.72
tsoongianolide A (2) ^{b,e}	8.78 ± 0.48*	26.88*
tsoongianolide B (3) ^{b,e}	10.08 ± 1.09*	24.25*
tsoongianolide D (4) ^{b,e}	7.53 ± 0.46**	43.36**
ligularenolide (5) ^{b,e}	11.18 ± 0.68	15.98
2-Oxoerenuphil-1(10),7(11),8(9)-trien-12,8-olide (6) ^{b,e}	9.35 ± 1.42*	24.90*
chlorogenic acid (7) ^{b,f}	10.97 ± 1.31	21.85
β -sitosteryl glucopyranoside (10) ^{b,f}	12.73 ± 0.30	9.26
Indomethacin ^{b,g}	1.99 ± 0.69*	83.73*

Dose: ^a1 mg/ear, ^b1 μ mol/ear. Each value represents the mean of three animals \pm standard error. Control: ^cCHCl₃ 15.63 \pm 1.50, ^dacetone-CH₂Cl₂ 1:1, 12.00 \pm 1.50, ^eCHCl₃ 13.30 \pm 0.68, ^facetone:CHCl₃:DMSO 9:9:2. ^gacetone, 13.53 \pm 104. Results were analysed by the *t* Student test. **p* ≤ 0.05. ***p* ≤ 0.01.

manner the activity of MPO (Table 3), indicating that edema inhibition in the active extracts is associated with the inhibition of infiltrated neutrophils in the ear biopsy.

CONCLUSIONS

The chemistry of *R. platanifolia* is similar to that of the species of the genus studied so far. Isolation of tsoongianolides A, B, and D (**2-4**), previously isolated from *Senecio tsoongianus* and ligularenolides

Table 2. Dose-response evaluation of acetic and methanolic extracts on TPA-induced mouse edema

Sample	Dose (mg/ear)	Edema (mg)	Inhibition (%)	IC ₅₀ (mg/ear)
TPA		14.50 ± 0.34		
Acetic extract	0.032	12.93 ± 0.65	10.80 ± 4.50	0.21
	0.1	10.48 ± 1.28*	27.70 ± 8.82	r = 0.98
	0.32	7.00 ± 1.56**	51.72 ± 10.78	
	1.0	2.20 ± 0.27**	84.83 ± 1.88	
TPA		13.73 ± 0.42		
Methanolic extract	0.032	11.38 ± 0.94	17.11 ± 6.88	0.32
	0.1	9.97 ± 0.80**	27.43 ± 5.79	r = 0.87
	0.32	8.60 ± 0.71**	37.38 ± 5.18	
	1.0	2.22 ± 0.43**	83.86 ± 3.14	
TPA		14.75 ± 1.13		
Indomethacin	0.011	12.78 ± 1.21	13.36 ± 8.22	0.08
	0.036	10.74 ± 1.13*	27.19 ± 7.69	r = 0.98
	0.111	5.62 ± 0.89**	61.90 ± 6.00	
	0.358	2.88 ± 0.73**	78.76 ± 4.97	

Each value represents the mean of six animals ± standard error. Results were analysed using Dunnett's test, values of *p<0.05 and **p<0.01 were considered significant.

Table 3. Dose-response evaluation of acetic and methanolic extracts on MPO activity in the TPA-treated mouse ear

Sample	Dose (mg/ear)	OD 450 nm	MPO inhibition (%)
Basal		0.081 ± 0.018	
TPA		0.369 ± 0.004	
Acetic extract	0.032	0.311 ± 0.050	15.59 ± 13.65
	0.1	0.143 ± 0.024**	61.30 ± 6.45**
	0.32	0.124 ± 0.018**	66.42 ± 4.89**
	1	0.112 ± 0.019**	69.77 ± 5.28**
Basal		0.084 ± 0.023	
TPA		0.373 ± 0.032	
Methanolic extract	0.032	0.197 ± 0.027	47.03 ± 7.15
	0.1	0.210 ± 0.039**	43.73 ± 10.52**
	0.32	0.143 ± 0.025**	61.68 ± 6.67**
	1	0.077 ± 0.012**	79.30 ± 3.17**
Basal		0.013 ± 0.004	
TPA		0.202 ± 0.055	
Indomethacin	0.011	0.104 ± 0.017	48.51 ± 8.48
	0.036	0.087 ± 0.034*	56.93 ± 16.91*
	0.111	0.039 ± 0.012**	80.69 ± 6.20**
	0.358	0.018 ± 0.006**	91.08 ± 2.96**

Each value represents the average of 6 animals ± standard error. Results were analyzed using Dunnett's test, values of p<0.5 (*) and p<0.01 (**) were considered significant.

4 and **5** reported in *Ligularia* species is not surprising since the genus *Ligularia*, which is distributed in the warm regions of Asia and Europe, is classified in Tussilaginatae subtribe as *Roldana*.²⁸ The genus *Roldana* has been segregated from the genus *Senecio* and in both the presence of sesquiterpenoids has been established, so far their main chemotaxonomic difference is the absence of pyrrolizidine alkaloids in *Roldana*.³ On the other hand, the anti-inflammatory activity of the acetic and methanolic extracts is related to their capacity to inhibit neutrophil infiltration and the weak anti-inflammatory

effects of the isolated compounds suggest the presence of other anti-inflammatory agents in methanol and acetone extracts, or a synergetic activity among its components.

EXPERIMENTAL

General

Melting points were determined using a Fisher Jones melting point apparatus and are uncorrected. IR spectra were recorded on a Nicolet Magna-IR 750 spectrometer. Optical rotations were determined on a Perkin-Elmer 343 polarimeter. EIMS data were determined on a JEOL JMS-AX505HA mass spectrometer at 70 eV, and for DART-MS a JEOL AccuTOF JMS-T100LC DART was used. 1D and 2D NMR spectra were obtained on a Bruker Avance III 400 MHz or on a Varian-Unity Inova 500 MHz spectrometer with tetramethylsilane (TMS) as internal standard. Vacuum column chromatography (VCC) was performed using Silica gel 60 G (Merck, Darmstadt, Germany). Flash column chromatography (FCC) was run using Silica gel 60 (230-400 Macherey-Nagel, Germany). Sephadex column was achieved with Sephadex LH 20 (Amersham Pharmacia Biotech AB, Sweden). Preparative TLC was carried out on Silica gel GF₂₅₄ (Macherey-Nagel), layer thickness 1.0 or 2.0 mm.

Plant material

Roldana platanifolia (Benth.) H. Rob. & Bretell was collected at Llano de la Cantimplora, Tlalpan, D. F., México, in November 2005. A voucher specimen was deposited at the Herbarium del Instituto de Biología, UNAM, México (MEXU 1156425).

Extraction and isolation

Dried and ground aerial parts (569 g) of *R. platanifolia* were extracted successively with hexane, acetone and methanol. Solvents were removed at reduced pressure to obtain the corresponding extracts which gave negative tests with the alkaloids Dragendorff reagent. The hexane extract (11 g) was fractionated by VCC eluted with hexane-Me₂CO gradient system. Fractions eluted with hexane afforded mixture A, those obtained with hexane-Me₂CO 19:1 produced mixture B and the hexane-Me₂CO 9:1 eluates gave mixture C. Purification of mixture A (350 mg) by FCC eluted with hexane-EtOAc 49:1 afforded after preparative TLC (benzene-EtOAc 49:1) β-caryophyllene 8R,9R-oxide (**1**, 8 mg).¹⁷ B gave a mixture of β-sitosterol (**8**)¹⁹ and stigmaterol (**9**)²⁰ (123 mg) whose mother liquors (222 mg) were further purified by FCC (hexane-Me₂CO 4:1) followed of preparative TLC (hexane-Me₂CO 9:1, x 2) to obtain tsoongianolide A (**2**, 8 mg)¹³ and tsoongianolide B (**3**, 12 mg).¹³ Purification of mixture C (681 mg) by FCC eluted with hexane-Me₂CO 4:1 followed of preparative TLC (benzene-EtOAc 4:1) produced 20 mg of **3**. The acetone extract (16 g) was purified by VCC using hexane-EtOAc mixtures of increasing polarity. The hexane eluates afforded mixture D; mixtures E, F, and G were obtained with hexane-EtOAc 19:1, and mixture H with hexane-EtOAc 9:1. Mixture D (408 mg) was purified using a VCC eluted with hexane to obtain a mixture of **8** - **9** (20 mg). Mixture E afforded by recrystallization (hexane-EtOAc) ligularenolide (**5**, 58 mg).¹³⁻¹⁵ Mother liquors of **5** (624 mg) were purified by FCC eluted with hexane-EtOAc 19:1 to obtain 85 mg of **5**. Mixture F (960 mg) was subjected to FCC (benzene-EtOAc 9:1) to obtain compound **3** (17 mg) and tsoongianolide D (**4**, 80 mg).¹³ Mixture G (180 mg) was purified by preparative TLC (hexane-EtOAc 3:1) to obtain compound **4** (35 mg). Mixture H (655 mg) produced by VCC (hexane-EtOAc 9:1) compound **4** (32 mg) and 2-oxoerenophil-1(10),7(11),8(9)-trien-12,8-olide (**6**, 11 mg).^{15,16}

The methanol extract (25 g) was fractionated by VCC eluting with EtOAc-MeOH mixtures of increasing polarity to produce 350 mg of sucrose, β -sitosteryl glucopyranoside (**10**, 180 mg)²¹ and mixture I (1.2 g) from the EtOAc-MeOH 3:2 eluates. Mixture I (950 mg) was purified through a sephadex LH-20 column eluted with H₂O-MeOH 1:1 to obtain chlorogenic acid (**7**, 45 mg).¹⁸

Tsoongianolide A (**2**): colorless oil; $[\alpha]_{25}^{+15}$ (c 0.12, CHCl₃); IR (CHCl₃) ν_{\max} / cm⁻¹: 3390, 1762, 1651; ¹H NMR (CDCl₃, 400 MHz) see Table 2S; ¹³C NMR (CDCl₃, 100 MHz) see Table 1S; EIMS *m/z* 248 [M]⁺ (45), 230 (85), 178 (100).

Tsoongianolide B (**3**): colorless needles; mp 104-106 °C; $[\alpha]_{25}^{+107}$ (c 0.13; CHCl₃); IR (CHCl₃) ν_{\max} / cm⁻¹ 3596, 1768, 1655; ¹H NMR (CDCl₃, 500 MHz) see Table 2S; ¹³C NMR (CDCl₃, 125 MHz) see Table 1S; EIMS *m/z* 249 [M+H]⁺ (55), 230 (100).

Tsoongianolide D (**4**): colorless needles; mp 201-203 °C; $[\alpha]_{25}^{-250}$ (c 0.17, CHCl₃); IR (CHCl₃) ν_{\max} / cm⁻¹ 3315, 1742; ¹H NMR (CDCl₃, 500 MHz) see Table 2S; ¹³C NMR (CDCl₃, 125 MHz) see Table 1S; EIMS *m/z* 244 [M-CH₂COOH]⁺ (100), 229 (75), 215 (70);

Ligularenolide (**5**): light yellow prisms; mp 134-136 °C; $[\alpha]_{25}^{-331}$ (c 0.16, CHCl₃); IR (CHCl₃) ν_{\max} / cm⁻¹ 1754, 1645; ¹H NMR (CDCl₃, 500 MHz) see Table 2S; ¹³C NMR (CDCl₃, 125 MHz) see Table 1S; EIMS *m/z* 230 [M]⁺ (100), 215 (90).

2-Oxoerenophil-1(10),7(11),8(9)-trien-12,8-olide (**6**): light yellow prisms; mp 147-149 °C; $[\alpha]_{25}^{-541}$ (c 0.16, CHCl₃); IR (CHCl₃) ν_{\max} / cm⁻¹ 1777, 1663, 1633; ¹H NMR (CDCl₃, 500 MHz) see Table 2S; ¹³C NMR (CDCl₃, 125 MHz) see Table 1S; DART-MS *m/z* 245 [M+H]⁺.

Evaluation of the anti-inflammatory activity

Animals

Male NIH mice weighing 25-30 g were maintained under standard laboratory conditions in the animal house (temperature 27 ± 1 °C) with a 12/12 h light-dark cycle, according with the Mexican official norm MON-062-Z00-1999. They were fed laboratory diet and water *ad libitum*.

TPA-induced edema model

The TPA-induced ear edema assay in mice was performed as previously reported²⁹ (Tables 1 and 2).

Myeloperoxidase assay

Tissue MPO activity was measured using an adapted method of Bradley *et al.*²⁶ and Suzuki *et al.*²⁷ as previously reported³⁰ (Table 3).

Statistical analysis

All data were represented as percentage mean ± standard error of mean (SEM). The statistical analysis was done by means of Student's t-test, whereas analysis of variance ANOVA followed by Dunnett test were used to compare several groups with a control. P values p ≤ 0.05 and p ≤ 0.01 were considered to be significant.

SUPPLEMENTARY MATERIAL

Tables 1S and 2S, ¹H and ¹³C NMR of isolated compounds and 2D NMR spectra of compounds **2-6** are freely available at <http://www.quimicanova.sbq.org.br> in PDF.

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