

A New Cytotoxic Naphthoquinone and Other Chemical Constituents of *Sinningia reitzii*

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Phytochemical study of *Sinningia reitzii* (Gesneriaceae) led to the isolation of three new naphthoquinones, 6,7-dimethoxydunnione, 7-hydroxy-6-methoxydunnione, and 5-hydroxy-6,7-dimethoxydunnione from the tubers, together with four known compounds, 7-hydroxydunnione, 8-hydroxydunnione, 5-hydroxy-6,7-dimethoxy- α -dunnione, and 8-hydroxydehydrodunnione. Aerial parts furnished five known compounds, 5-hydroxy-6,7-dimethoxydunnione, 6,8-dihydroxy-7-methoxy-2-*O*-methyldunnione, loureirin B, jacaranone, and methyl 4-hydroxyphenylacetate. Compounds 8-hydroxydunnione, 5-hydroxy-6,7-dimethoxy- α -dunnione, 8-hydroxydehydrodunnione, 5-hydroxy-6,7-dimethoxydunnione and 6,8-dihydroxy-7-methoxy-2-*O*-methyldunnione had been previously reported in the tubers of *S. reitzii*. Density functional theory was used to assign the absolute configuration of compounds 6,7-dimethoxydunnione, 7-hydroxy-6-methoxydunnione, 5-hydroxy-6,7-dimethoxydunnione, 7-hydroxydunnione, 8-hydroxydunnione, 5-hydroxy-6,7-dimethoxy- α -dunnione. Compounds 6,7-dimethoxydunnione, 8-hydroxydunnione, 5-hydroxy-6,7-dimethoxy- α -dunnione and 8-hydroxydehydrodunnione were evaluated for cytotoxicity against PC3 (prostate), SKMEL 103 (melanoma), and HeLa (cervix) human cancer, and 3T3 fibroblast cell lines, using the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) assay. Compound 6,7-dimethoxydunnione displays cytotoxic activity against all the tumor cell lines tested (half maximal inhibitory concentration, IC₅₀, 4.47-26.2 μ mol L⁻¹), and was inactive against 3T3 fibroblasts (IC₅₀ > 100 μ mol L⁻¹). Compounds 8-hydroxydunnione, 5-hydroxy-6,7-dimethoxy- α -dunnione and 8-hydroxydehydrodunnione were inactive against all tested cell lines.

Keywords: Gesneriaceae, *Sinningia reitzii*, naphthoquinones, cytotoxic activity

Introduction

The family Gesneriaceae (150 gen/ 3000 spp) comprises herbs, lianas and shrubs, distributed in the tropics around the world. Several species are used as ornamental plants due their attractive flowers. *Aeschynanthus* spp. (“basket plant”, “lipstick plant”), *Columnnea* spp., *Nematanthus* spp. (both known as “goldfish plant”), *Saintpaulia ionantha* (“African violet”), and *Sinningia speciosa* (“gloxinia”) are widely cultivated species. The chemical profile of Gesneriaceae includes flavonoids, terpenoids, phenolic glycosides, phenyl propanoids, quinones and essential oils. The production of caffeoyl phenylethanoid glycosides together with the lack of iridoids is considered characteristic of the family.¹

Gesneriaceae is represented in Brazil by 28 genera and 221 species. *Sinningia* is a neotropical genus comprising ca. 70 species distributed from Southern Mexico to Northern Argentina. This is the major genus of Gesneriaceae in Brazil, followed by *Nematanthus* (32 spp) and *Besleria* (21 spp).^{2,3}

S. reitzii (Hoehne) L. E. Skog is a perennial subshrub, with numerous small tubers, endemic to Brazil, where it is found in the States of São Paulo, Paraná and Santa Catarina.³ Several *Sinningia* species, including *S. reitzii*, are known as “cachimbo” in Santa Catarina.⁴ No uses for *S. reitzii* were found in traditional medicine. Eight naphthoquinones were previously reported from the tubers of this plant, including one with anti-inflammatory and antinociceptive activities.⁵ The isolation of additional compounds from tubers, the first study of aerial parts, and the cytotoxic evaluation of four naphthoquinones are reported herein.

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Tubers of *S. reitzii* yielded three new naphthoquinones (**1-3**) and four known compounds (**4-7**), while aerial parts furnished five known compounds (**8-12**). All isolated compounds were analyzed by 1D and 2D nuclear magnetic resonance (NMR) spectroscopy, and the data were compared to the literature. Thus, the known compounds were identified as 7-hydroxydunnione (**4**),⁶ 8-hydroxydunnione (**5**),⁶ 5-hydroxy-6,7-dimethoxy- α -dunnione (**6**),⁵ 8-hydroxydehydrodunnione (**7**),⁵ 5-hydroxy-6,7-dimethoxydunnione (**8**),⁵ 6,8-dihydroxy-7-methoxy-2-*O*-methyl dunnione (**9**),⁷ loureirin B (**10**),⁸ jacaranone (**11**),⁹ and methyl 4-hydroxyphenylacetate (**12**)¹⁰ (Figure 1). Compounds **5-9** had been previously reported in the tubers.⁵ The cytotoxic activity of compounds **1** and **5-7** against human tumor cell lines was evaluated.

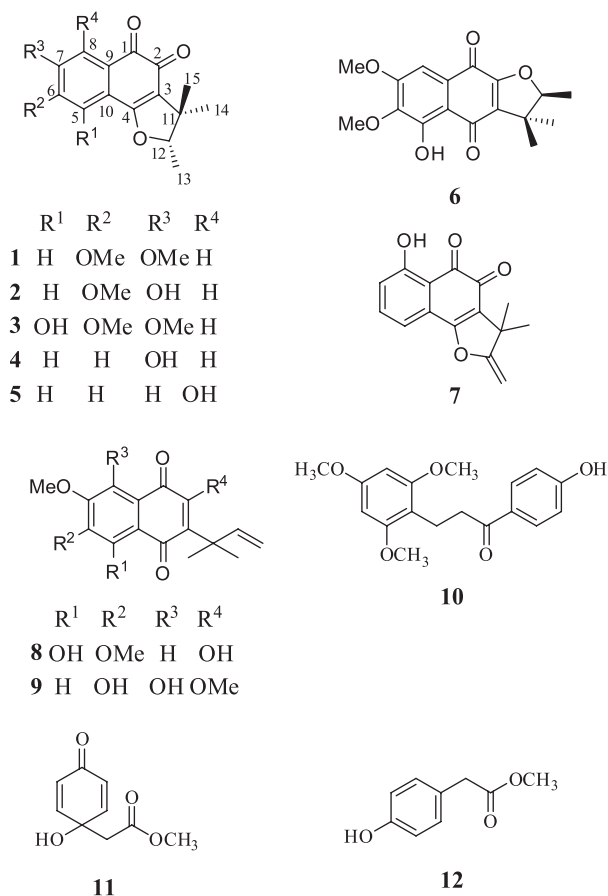


Figure 1. Chemical structures of the compounds **1-12**.

Experimental

General experimental procedures

Optical rotations were measured in CHCl₃ on a Rudolph Research polarimeter. The UV spectrum was obtained in MeOH on a Shimadzu UV-2401PC spectrophotometer.

Optical density ($\lambda = 570$ nm) was measured using a Synergy 2 spectrophotometer (Bio-Tek). One-dimensional (¹H, ¹³C) and two-dimensional (gradient selected heteronuclear multiple bond coherence (gHMBC), gradient selected heteronuclear single quantum coherence (gHSQC)) NMR spectra were recorded on Bruker spectrometers (AC 200, Avance 400 and/or Avance 600), observing ¹H at 200, 400 or 600 MHz and ¹³C at 50, 100 or 150 MHz. CDCl₃ or CD₃OD were used as solvents, and the chemical shifts are given in ppm (δ) using tetramethylsilane (TMS) as internal reference, with coupling constants (*J*) in Hz. High-resolution electrospray ionization mass spectrometry (HRESIMS) data were obtained on a Micromass ESI Q-TOF mass spectrometer. Geometry optimization and density functional theory calculations on the electronic structure of the compounds employed B3LYP functional having Los Alamos ECP as basis set as implemented in Gaussian09 suite program.^{11,12} Theoretical optical activities were calculated after geometry optimization.¹³ High performance liquid chromatography (HPLC) separations were performed in a Waters apparatus equipped with photodiode array (PDA) detector, and a semi-preparative Nucleosil 100-5 C₁₈ column (250 × 10 mm). The mobile phase was acetonitrile:water 80:20 (isocratic elution), applied for 15 min. The flow rate was 2.8 mL min⁻¹ at room temperature. Silica gel (Merck, 230-400 mesh) was used for column chromatographic separations (CC), while pre-coated silica gel plates (Macherey-Nagel) were used for thin layer chromatography (TLC) and preparative TLC (PTLC). Compounds were visualized by exposure under UV_{254/366} light and spraying with 5% (v/v) H₂SO₄ in ethanol solution, followed by heating on a hot plate.

Plant material

Specimens of *Sinningia reitzii* (Hoehne) L. E. Skog were collected from a natural population in Jundiá do Sul, Paraná State, Brazil (23°26'45" S, 50°15'33" W) in March 2010. The plant was identified by Clarice B. Poliquesi, who deposited a voucher specimen in the herbarium of Museu Botânico Municipal (MBM 253150). The plants were cultivated in Curitiba, Paraná State, Brazil (25°26'34.85" S, 49°14'22.58" W). Botanical material for phytochemical study was harvested from the cultivated plants in August 2012 (tubers, sample A) and April 2015 (aerial parts and tubers, sample B).

Extraction and isolation

Dried and powdered tubers (sample A, 30.9 g; sample B 46.6 g) were extracted with hexanes (HEX, mixture of

isomers), CH₂Cl₂, EtOAc and EtOH, successively (50 mL of each solvent for 10 g of material, three times each solvent) at room temperature. The solvent was removed at reduced pressure using a rotatory evaporator, yielding the extracts in HEX (A = 0.30 g; B = 0.34 g), CH₂Cl₂ (A = 0.27 g; B = 0.28 g); EtOAc (A = 0.25 g; B = 0.26 g), and EtOH (A = 1.03 g; B = 1.22 g). Extracts from sample A were kept at -4 °C until fractionation. After TLC analyses, HEX and CH₂Cl₂ extracts from both samples were pooled, and an aliquot (0.14 g) was reserved. The remaining extract (1.05 g) was submitted to CC (HEX:EtOAc 95:5, 9:1, 8:2 and 0:1) to give 20 fractions (F₁₋₂₀) after TLC analyses. Compounds **7** (10.2 mg), **6** (10.9 mg), and **5** (13.6 mg) were obtained from F₄ (127.7 mg, eluted with HEX:EtOAc 9:1) after PTLC (CH₂Cl₂:MeOH 99:1). Fraction F₁₆ (44.0 mg, eluted with HEX:EtOAc 8:2) yielded **1** (6.6 mg) after PTLC (HEX:EtOAc 1:1). Fraction F₁₉ (163.1 mg) was submitted to CC (CH₂Cl₂:EtOAc 95:5, 9:1, 8:2, 7:3 and 0:1) to give eight subfractions (F_{19,1-8}) after TLC analyses. Subfraction F_{19,2} (12.3 mg, eluted with CH₂Cl₂:EtOAc 95:5) yielded **1** (1.7 mg) and **3** (6.1 mg) after PTLC (CH₂Cl₂:EtOAc 95:5). Subfraction F_{19,3} (eluted with CH₂Cl₂:EtOAc 95:5) yielded **3** (4.3 mg). Subfraction F_{19,6} (eluted with CH₂Cl₂:EtOAc 9:1) yielded **2** (5.2 mg). Subfraction F_{19,7} (15.9 mg, eluted with CH₂Cl₂:EtOAc 8:2), yielded **4** (3.7 mg) after preparative TLC (HEX:EtOAc 1:1).

Dried and powdered aerial parts (29.6 g) were extracted with HEX, CH₂Cl₂, EtOAc and EtOH as was described for the tubers, yielding the respective extracts after solvent removal. The HEX extract (0.162 g) was submitted to CC (HEX:CH₂Cl₂ 8:2, 1:1 and 0:1) to give eight fractions (FH₁₋₈), after TLC analyses. Fraction FH₄ (20.3 mg, eluted with HEX:CH₂Cl₂ 1:1) yielded **8** (2.6 mg), after PTLC (CH₂Cl₂). Fraction FH₆ (10.3 mg, eluted with CH₂Cl₂) yielded **9** (0.8 mg), after PTLC (CH₂Cl₂:acetone 95:5). The CH₂Cl₂ extract (0.209 g) was submitted to CC (CH₂Cl₂:EtOAc 1:0, 99:1, 8:2, 0:1) to give five fractions (FD₁₋₅). Fraction FD₂ (27.9 g, eluted with CH₂Cl₂) yielded **9** (3.2 mg) after repeated PTLC (CH₂Cl₂). Fraction FD₄ (24.9 mg, eluted with CH₂Cl₂:EtOAc 8:2) yielded **10** (2.1 mg) after PTLC (CH₂Cl₂:MeOH 99:1). The EtOAc extract (0.240 g) was submitted to CC (CH₂Cl₂:MeOH 99:1, 95:5, 9:1, 0:1) to give eight fractions (FA₁₋₈). Fraction FA₄ (47.4 mg, eluted with CH₂Cl₂:MeOH 99:1) yielded a mixture (8.5 mg), which was separated by HPLC to give **11** (t_R 7.8 min, 2.7 mg) and **12** (t_R 8.1 min, 3.2 mg).

Cytotoxic activity assay

The cytotoxic activity of compounds **1** and **5-7** was evaluated against the human tumor cell lines PC-3

(prostate), SKMEL 103 (melanoma), HeLa (epitheloid cervix carcinoma), and the non-cancer cell line 3T3 (fibroblast), all from the American Type Culture Collection (ATCC). The assays were done as previously described.^{14,15} The cells were distributed in 96-well plates (100 µL cells per well), and exposed to various concentrations of the tested compounds (0.25, 2.5, 25.0 and 250 µg mL⁻¹) in dimethyl sulfoxide (DMSO) (0.1%) at 37 °C, with 5% of CO₂, for 48 h. The final concentration of DMSO did not affect cell viability. Doxorubicin (0.025, 0.25, 2.5 and 25 µg mL⁻¹) was used as the positive control. The proliferation of tumor cells was quantified by the ability of living cells to reduce the yellow dye 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) to a blue formazan product. At the end of 48 h incubation, the medium was replaced by fresh medium containing 0.5 mg mL⁻¹ of MTT. Three hours later, the formazan product was dissolved in DMSO, and the optical density was measured by spectrophotometry at 570 nm. The experiments were carried at least in triplicate and the concentration needed to achieve 50% inhibition of cell viability (IC₅₀) was calculated in µmol L⁻¹ by nonlinear regression using the GraphPad program.¹⁶

6,7-Dimethoxydunnione (**1**)

Dark purple solid; experimental [α]_D²⁰ -228 (c 0.27, CHCl₃), calculated [α]_D -268; UV-Vis (MeOH) λ_{max} / nm (log ε) 214 (3.99), 280 (3.95), 332 (3.32); IR (KBr) ν_{max} / cm⁻¹ 2922 and 2840 (C-H), 1640 (C=O), 1600, 1560, and 1520 (C=C, aromatic), 1270, 1161 and 1022 (C-O); for ¹H and ¹³C NMR data, see Table 1; HRESIMS *m/z*, calcd. for C₁₇H₁₉O₅ [M + H]⁺: 303.12299, found: 303.12349.

7-Hydroxy-6-methoxydunnione (**2**)

Purple solid; experimental [α]_D²⁰ -79 (c 1.0, CHCl₃), calculated [α]_D -224; UV-Vis (MeOH) λ_{max} / nm (log ε) 211 (4.07), 277 (4.01), 283 (4.02), 316 (3.62); IR (KBr) ν_{max} / cm⁻¹ 3539 (OH, free), 3431 (OH, associated), 2916 and 2848 (C-H), 1629 (C=O), 1607, 1576, and 1529 (C-C, aromatic), 1274 (C-O); for ¹H and ¹³C NMR data, see Table 1; HRESIMS *m/z*, calcd. for C₁₆H₁₇O₅ [M + H]⁺: 289.10808, found: 289.10760.

5-Hydroxy-6,7-dimethoxydunnione (**3**)

Dark red solid; experimental [α]_D²⁰ -146 (c 0.21, CHCl₃), calculated [α]_D -440; UV-Vis (MeOH) λ_{max} / nm (log ε) 212 (4.30), 267 (4.03), 300 (3.91); IR (KBr) ν_{max} / cm⁻¹ 3421 (OH), 2958, 2927 and 2848 (C-H), 1643 (C=O), 1604 and 1585 (C=C, aromatic), 1260 (C-O); for ¹H and ¹³C NMR data, see Table 2; HRESIMS *m/z*, calcd. for C₁₇H₁₉O₆ [M + H]⁺: 319.11847, found: 319.11816.

Results and Discussion

Compound **1** was isolated as dark purple solid. HRESIMS in positive mode displays an ion at m/z 303.12299 $[M + H]^+$, indicating the molecular formula $C_{17}H_{18}O_5$, which is consistent with nine indices of hydrogen deficiency. In the 1H NMR data, signals of two *para*-coupled aromatic protons, (δ_H 7.07 and 7.55), two singlets of methoxy groups (δ_H 3.97 and 4.01), and the typical signals of a 2,3-dihydro-2,3,3-trimethylfuran group (quartet at δ_H 4.63, two singlets at δ_H 1.25 and 1.44, and a doublet at δ_H 1.47) were observed (Table 1). The ^{13}C NMR data showed 17 peaks, including two of carbonyl groups at δ_C 175.8 and 180.5. These data indicate a 1,2-naphthoquinone type dunnione.⁶ Analysis of HSQC and HMBC spectra confirmed the structure **1** through the following cross-peaks: H-5 (δ_H 7.07) with carbons at δ_C 125.0 (C-9), 151.5 (C-7), and 168.2 (C-4); H-8 (δ_H 7.55) with carbons at δ_C 122.5 (C-10), 153.9 (C-6), and 180.5 (C-1); and H-14 (δ_H 1.25) with carbons at δ_C 25.8 (C-15), 44.1 (C-11), 92.9 (C-12), and 121.4 (C-3) (Table 1). Therefore, compound **1** was identified as 6,7-dimethoxydunnione (Figure 1).

Compound **2** was isolated as purple solid and has the molecular formula $C_{16}H_{16}O_5$, corresponding to nine indices of hydrogen deficiency, which was deduced from an ion at m/z 289.10808 $[M + H]^+$ in HRESIMS. The

1H NMR data of **2** (Table 1) were very similar to those of compound **1**, showing signals for two aromatic protons in *para* relationship (δ_H 7.07 and 7.59), one singlet of a methoxy group (δ_H 4.03), and the signals for a 2,3-dihydro-2,3,3-trimethylfuran group. In the HMBC spectrum the methoxy group (δ_H 4.03) and H-8 (δ_H 7.59) showed cross-peaks with a carbon at δ_C 151.1, which must be C-6. These and remaining correlations in the HSQC and HMBC spectra (Table 1) led to identification of **2** as 7-hydroxy-6-methoxydunnione (Figure 1).

Compound **3**, a dark red solid, had the molecular formula $C_{17}H_{18}O_6$, with nine indices of hydrogen deficiency, as deduced from an ion at m/z 319.1184 $[M + H]^+$ in the HRESIMS. The 1H NMR data (Table 2) showed signals for one hydroxy group (δ_H 7.68), one aromatic proton (δ_H 7.36), two methoxy groups (δ_H 3.96 and 3.97), and the signals for a 2,3-dihydro-2,3,3-trimethylfuran group. The ^{13}C NMR spectrum showed 17 signals, including three that were typical of C-1 (δ_C 180.5), C-2 (δ_C 175.3), and C-4 (δ_C 168.1) in the dunnione framework.⁶ Analysis of HSQC and HMBC data (Table 2) led to the structure **3** (Figure 1). The aromatic proton (δ_H 7.36) was located in C-8 due to cross-peaks of this signal in the HMBC spectrum with C-1 (δ_C 180.5), C-6 (δ_C 142.3), and C-10 (δ_C 106.9). On the other hand, the position of hydroxy group in C-5 was determined from cross-peaks of the signal at δ_H 7.69 with

Table 1. NMR data (CDCl₃) for the compounds **1** (600 MHz) and **2** (400 MHz)

Position	1			2		
	δ_H (J in Hz)	δ_C	HMBC	δ_H (J in Hz)	δ_C	HMBC
1		180.5			180.5	
2		175.8			175.8	
3		121.4			121.4	
4		168.2			168.3	
5	7.07 (1H, s)	106.7	4, 7, 9	7.07 (1H, s)	106.5	4, 7, 9
6		153.9			151.1	
7		151.5			148.5	
8	7.55 (1H, s)	111.9	1, 6, 10	7.59 (1H, s)	116.2	1, 6, 10
9		125.0			125.8	
10		122.5			121.8	
11		44.1			44.1	
12	4.63 (1H, q, 6.6)	92.9	14, 15	4.62 (1H, q, 6.7)	92.8	14, 15
13	1.47 (3H, d, 6.6)	14.6	11, 12	1.46 (3H, d, 6.7)	14.6	11, 12
14	1.25 (3H, s)	20.4	3, 11, 12, 15	1.25 (3H, s)	20.4	3, 11, 12, 15
15	1.44 (3H, s)	25.8	3, 11, 12, 14	1.43 (3H, s)	25.8	3, 11, 12, 14
6-OCH ₃	4.01 (3H, s)	56.5	6	4.03 (3H, s)	56.6	6
7-OCH ₃	3.97 (3H, s)	56.4				7

HMBC: heteronuclear multiple bond coherence.

C-5 (δ_c 148.9), C-6 and C-10. Therefore, compound **3** was identified as 5-hydroxy-6,7-dimethoxydunnione.

In order to determine the absolute configuration of compounds **1-6**, the optical rotations were calculated for the isomers using density functional theory (DFT). The results indicate that *S* isomers are levorotatory, in agreement with previous X-ray monocrystal diffraction studies with a dunnione derivative.¹⁷ As the experimental optical rotations of compounds **1-6** were negative, their absolute configuration was assigned as 12*S* (Figure 1). Chiral natural products are generally biosynthesized as one pure enantiomer. However, naphthoquinones have been isolated with various optical purity degree. Compound **4** was previously isolated optically pure (optical rotation +350), while **5** was obtained only as racemic mixture.⁶ In the present work, compound **4** showed $[\alpha]_D -23$, indicating a mixture of enantiomers, with predominance of the levorotatory isomer (53:47). The optical purity of the remaining compounds was not experimentally determined. The high optical rotation of **1** ($[\alpha]_D -228$), near from value calculated by DFT ($[\alpha]_D -268$) suggest that it is enantiomerically pure. On the other hand, the optical rotations of **2** ($[\alpha]_D -79$), **3** ($[\alpha]_D -146$), **5** ($[\alpha]_D -78$), and **6** ($[\alpha]_D -13$) were relatively low in comparison with calculated values ($[\alpha]_D > -200$), suggesting that these compounds were isolated as scalemic mixtures.

Table 2. NMR data (400 MHz, CDCl₃) for **3**

Position	δ_H (J in Hz)	δ_c	HMBC
1		180.5	
2		175.3	
3		121.2	
4		168.1	
5		148.9	
6		142.3	
7		155.7	
8	7.36 (1H, s)	108.5	1, 6, 10
9		126.5	
10		106.9	
11		43.0	
12	4.75 (1H, q, 6.7)	94.4	14, 15
13	1.51 (3H, d, 6.7)	14.7	11, 12
14	1.27 (3H, s)	20.3	3, 11, 12, 15
15	1.44 (3H, s)	25.6	3, 11, 12, 14
5-OH	7.68 (1H, s)		5, 6, 10
6-OCH ₃	3.97 (3H, s)	61.1	6
7-OCH ₃	3.96 (3H, s)	56.5	7

HMBC: heteronuclear multiple bond coherence.

Considering that naphthoquinones frequently display cytotoxic activity,¹⁸ compounds **1** and **5-7**, which were obtained in sufficient quantity and purity, were assayed *in vitro* against three human tumor cell lines (PC3, SKMel 103 and HeLa) and the 3T3 (fibroblast) cell line. Compound **1** exhibited strong cytotoxicity against HeLa and PC3 cell lines ($IC_{50} < 10 \mu\text{mol L}^{-1}$), and a smaller activity against SKMEL 103 ($IC_{50} 26.2 \mu\text{mol L}^{-1}$). Compound **1** was more active against HeLa cells ($IC_{50} 4.47 \mu\text{mol L}^{-1}$) than the standard drug doxorubicin ($IC_{50} 14.2 \mu\text{mol L}^{-1}$); it was also less toxic towards the non-cancer cell line 3T3 ($IC_{50} 157 \mu\text{mol L}^{-1}$) in comparison to doxorubicin ($IC_{50} 27.9 \mu\text{mol L}^{-1}$). Compounds **5-7** were considered inactive against all tested cells since they showed $IC_{50} > 50 \mu\text{mol L}^{-1}$ (Table 3). Strong cytotoxic activity had previously been reported for a racemic mixture of **5** against four tumor cell lines (average growth inhibition of 50%, $GI_{50} 0.54-5.2 \mu\text{mol L}^{-1}$), including HeLa cells ($GI_{50} 1.9 \mu\text{mol L}^{-1}$), at roughly the same compound concentrations.¹⁹ This striking difference in cytotoxicity between the two studies may be a consequence of the different methods employed for estimating cell viability. The cytotoxicity of other α -dunnione derivatives (similar, but not identical, to compound **6**) has also been evaluated previously, but no clear pattern of structure-activity relationship was found.¹⁹ Anti-inflammatory and antinociceptive activities were previously reported for compound **7**.⁵ The small number of assayed compounds does not allow generalizations about structure-activity relationship; however, a comparison of the structures of the active compound (**1**) to the inactive compounds (**5-7**) suggests that the absence of substituents in C-5 and C-8 may be important for activity.

Table 3. Cytotoxic activity of compounds **1** and **5-7**

Cell line	IC_{50} / ($\mu\text{mol L}^{-1}$)				Doxo ^a
	1	5	6	7	
PC3	7.77	> 969	> 786	> 972	0.40
SKMEL	26.2	> 969	> 786	> 972	8.29
HeLa	4.47	> 969	398	972	14.2
3T3	157	> 969	> 786	241	27.9

Average of three analyses. The coefficients of variation obtained were $\leq 5\%$. IC_{50} : half maximal inhibitory concentration; Doxo: Doxorubicin. ^aDoxorubicin was used as positive control.

Conclusions

Less polar extracts of *S. reitzii* are characterized by the presence of several prenylated naphthoquinones, mainly dunnione-type. This compound class has been previously

reported in other *Sinningia* species, such as *S. allagophylla*,²⁰ *S. canescens*,²¹ *S. leucotricha*,²¹ and *S. hatschbachii*,²² and can be considered as typical of *Sinningia*. Many biological activities are associated with naphthoquinones, including antibacterial, cytotoxic, antitumoral, insecticidal, and anti-inflammatory.¹⁸ Accordingly, a new naphthoquinone, reported in this work, displays selective cytotoxic activity against three human tumor cell lines.

Supplementary Information

Supplementary information (spectra of compounds **1-12**, and data of known compounds **4-12**) is available free of charge at <http://jbcs.sbq.org.br> as PDF file.

Acknowledgments

We are grateful to CAPES (Finance Code 001), FAEPEX-UNICAMP, FAPESP (processes: 15/03726-8 and 17/03598-5), and CNPq for scholarships and financial support, and to Clarisse B. Poliquesi, from Museu Botânico Municipal de Curitiba, for the collection and identification of the plant material.

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Submitted: December 9, 2018

Published: April 4, 2019

