

ISOLATION OF CYTOTOXIC NEOLIGNANS FROM *Saururus cernuus* L. (SAURURACEAE) USING IONIC LIQUID IN THE MICROWAVE ASSISTED EXTRACTION (MAE)Juliana R. Brito<sup>a</sup>, Fernanda F. Camilo<sup>a</sup>, Carlos R. Figueiredo<sup>b</sup>, Ricardo A. Azevedo<sup>b</sup>, Paulete Romoff<sup>c</sup>, Fátima O. S. Buturi<sup>d</sup>, Oriana A. Fávero<sup>e</sup>, João Henrique G. Lago<sup>f,\*</sup> and Edgard A. Ferreira<sup>d,#</sup><sup>a</sup>Instituto de Ciências Ambientais, Químicas e Farmacêuticas, Universidade Federal de São Paulo, 09972-270 Diadema – SP, Brasil<sup>b</sup>Departamento de Microbiologia, Imunologia e Parasitologia, Universidade Federal de São Paulo, 04021-001 São Paulo – SP, Brasil<sup>c</sup>Escola de Engenharia, Universidade Presbiteriana Mackenzie, 01302-907 São Paulo – SP, Brasil<sup>d</sup>Faculdade de Ciências Biológicas e da Saúde, Universidade São Judas Tadeu, 03166-000 São Paulo – SP, Brasil<sup>e</sup>Centro de Ciências Biológicas e da Saúde, Universidade Presbiteriana Mackenzie, 01302-907 São Paulo – SP, Brasil<sup>f</sup>Centro de Ciências Naturais e Humanas, Universidade Federal do ABC, 09210-580 Santo André – SP, Brasil

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In the present work, dried leaves of *Saururus cernuus* (Saururaceae) were subjected to extraction using an ionic liquid (1-butyl-3-methylimidazolium bromide – BMImBr) in the microwave assisted extraction (MAE). The obtained extract was partitioned using *n*-hexane and cytotoxicity activity of this organic phase against murine melanoma cell line (B16F10-Nex2) was evaluated *in vitro*. Since this extract displayed activity (100% of cell death at 200 µg mL<sup>-1</sup>) it was subjected to a bioactivity-guided fractionation to afford four related neolignans: *threo*-austrobailignan-5 (**1**), *threo*-austrobailignan-6 (**2**), *threo*-dihydroguaiaretic acid (**3**) and saucermetin (**4**). Their chemical structures were established based on NMR and MS spectral analysis. Among the isolated neolignans, compound **2** exhibited the highest cytotoxic activity against HeLa (human cervical melanoma) cells with IC<sub>50</sub> of 28.3 ± 3.9 µg mL<sup>-1</sup> (86 ± 12 µmol L<sup>-1</sup>). Furthermore, compounds **2** and **3** exhibited the highest cytotoxic activity against A2058 (human melanoma) cells with IC<sub>50</sub> of 44.3 ± 4.2 µg mL<sup>-1</sup> (135 ± 13 µmol L<sup>-1</sup>) and 41.5 ± 7.5 µg mL<sup>-1</sup> (126 ± 23 µmol L<sup>-1</sup>), respectively, similar to positive control cisplatin (IC<sub>50</sub> = 43.2 ± 3.2 µg mL<sup>-1</sup> or 144 ± 11 µmol L<sup>-1</sup>). Otherwise, compound **4** was inactive (IC<sub>50</sub> > 100 µg mL<sup>-1</sup> or > 300 µmol L<sup>-1</sup>). The obtained results provide important data for the selection of bioactive neolignans with promising cytotoxic potential using a simple and fast method employing a green solvent as 1-butyl-3-methylimidazolium bromide (BMImBr).

Keywords: Saururaceae, *Saururus cernuus*, neolignans, cytotoxic activity, ionic liquids.

## INTRODUCTION

Saururaceae is formed by four genera and six species of herbaceous plants distributed in Asia and North America.<sup>1</sup> The genus *Saururus* is composed exclusively of two species – *S. cernuus* and *S. chinensis* – which are chemically composed by flavonoids, alkaloids, terpenoids, steroids and mainly several subclasses of lignans/neolignans including tetrahydrofuran, dibenzylbutane, dibenzocyclooctene derivatives.<sup>2-10</sup> Among these lignoids, several compounds have showed biological properties such as anti-inflammatory,<sup>11</sup> antitumor,<sup>12</sup> antiviral,<sup>9</sup> and hepatoprotective effects.<sup>13</sup> *S. cernuus*, popularly known as lizard's tail, is distributed in southeastern of United States<sup>14</sup> and it is found in Brazil as an exotic species and it has been used in folk medicine for the treatment of inflammation, fever, edema and antitumor.<sup>14-16</sup> Previous studies reported the tumor specific cytotoxicity of manassantins A and B as well as 4-O-demethylmanassantin B.<sup>16-19</sup>

The extraction procedures of bioactive compounds from natural sources frequently employed toxic organic solvents in large amounts, leading to a significant environmental impact.<sup>20</sup> Based in this aspect our research group developed a simple and efficient method of extraction using ionic liquids in aqueous solvent assisted by microwave.<sup>21</sup> In continuation with these studies, the leaves of *S. cernuus* were subjected to extraction using aqueous 1-butyl-3-methylimidazolium bromide (BMImBr) under microwave assisted extraction (MAE) and the obtained material was extracted with *n*-hexane. As this organic phase displayed *in vitro* activity against

tumorigenic cell lines of murine melanoma (B16F10) cells it was subjected to a bioactivity-guided fractionation to afford the isolation of *threo*-austrobailignan-5 (**1**), *threo*-austrobailignan-6 (**2**) and *threo*-dihydroguaiaretic acid (**3**) as well as an inactive related compound saucermetin (**4**).

## RESULTS AND DISCUSSION

After extraction of dried leaves from *S. cernuus* using aqueous 1-butyl-3-methylimidazolium bromide (BMImBr) under MAE during 10 min at 60°C, the obtained material was filtered and extracted using *n*-hexane. This organic phase displayed cytotoxic effects against B16F10 (murine melanoma) tumor cell line (100% of cell death at 200 µg mL<sup>-1</sup>) and was subjected to a bioactivity-guided chromatographic fractionation to afford four related known neolignans: *threo*-austrobailignan-5 (**1**), *threo*-austrobailignan-6 (**2**), *threo*-dihydroguaiaretic acid (**3**) and saucermetin (**4**), as showed in Figure 1.

Identification of bioactive isolated compounds was carried out by analysis of their spectral data, especially NMR and MS. Compounds **1-3** were characterized as dibenzylbutane neolignans by analysis of their <sup>1</sup>H NMR spectra, in which were observed typical signals of a 1,2,4-trissubstituted aromatic ring at δ 6.51-6.61 (d, *J* = 1.5 Hz, H-2), 6.68-6.81 (d, *J* = 7.8 Hz, H-5) and δ 6.50-6.58 (dd, *J* = 7.8 and 1.5 Hz, H-6), two peaks assigned to diastereotopic benzylic hydrogens (H-7) at approximately δ 2.5 (dd, *J* = 13.5 and 6.6 Hz) and 2.3 (dd, *J* = 13.5 and 8.1 Hz), one signal attributed to methyl group (H-9) at δ 0.79-0.82 (d, *J* = 6.6 Hz) and one multiplet at δ 1.72-1.75 assigned to H-8. The <sup>1</sup>H NMR spectrum

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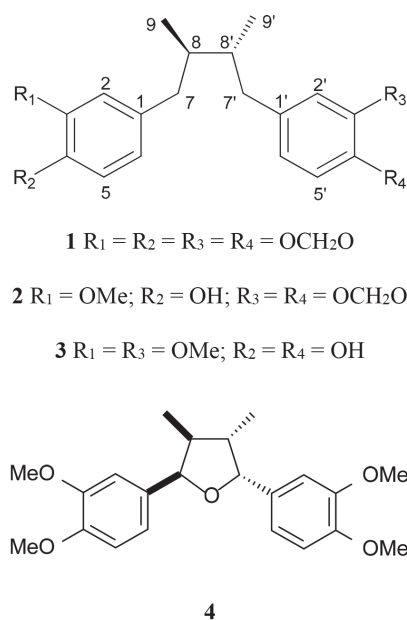


Figure 1. Chemical structures of neolignans **1-4** isolated from *S. cernuus*

of **1** and **2** showed, respectively, singlets at  $\delta$  5.86 (4H) and 5.90 (2H) assigned to methylenedioxy groups. In the case of compound **2**, two additional singlets at  $\delta$  3.83 and 5.50 were assigned to hydrogens of methoxyl and hydroxyl groups, respectively. An intense singlet at  $\delta$  3.80 (6H) was observed in the <sup>1</sup>H NMR spectrum of **3**, which was attributed to hydrogens of two methoxyl groups linked to aromatic ring. In addition, the <sup>13</sup>C NMR spectra of **1-3** confirmed the occurrence of dibenzylbutane neolignans derivatives due to the presence of signals at  $\delta$  107-148, attributed to aromatic carbons C-1 to C-6 and C-1' to C-6', and peaks of C<sub>3</sub> unit at  $\delta$  41.1 (C-7/C-7'), 37.5-39.2 (C-8/C-8'), and  $\delta$  13.9 (C-9/C-9'). Finally, based on chemical shifts of C-7/C-7', C-8/C-8' and C-9/C-9' and comparison with data described in the literature,<sup>22,23</sup> the isolated neolignans **1-3** displayed a *threo* configuration between methyl groups. LREIMS of **1-3** displayed peaks in the spectra that corresponded to molecular ions at  $m/z$  326, 328 and 330 consistent, respectively, with molecular masses C<sub>20</sub>H<sub>22</sub>O<sub>4</sub>, C<sub>20</sub>H<sub>24</sub>O<sub>4</sub> and C<sub>20</sub>H<sub>26</sub>O<sub>4</sub>. Finally, comparison of NMR and MS data with that reported in the literature identified *threo*-austrobailignan-5 (**1**),<sup>23-25</sup> *threo*-austrobailignan-6 (**2**)<sup>26</sup> and *threo*-dihydroguaiaretic acid (**3**).<sup>27</sup> The <sup>1</sup>H NMR spectrum of compound **4** displayed signals of aromatic hydrogens at  $\delta$  6.85 (br s) which were assigned to H-2/H-2', H-5/H-5' and H-6/H-6'. This peak associated to those at  $\delta$  5.45 (d,  $J$  = 5.3 Hz, H-7/H-7') and  $\delta$  0.69 (d,  $J$  = 6.8 Hz) suggested the occurrence of tetrahydrofuran neolignan containing a *cis-trans-cis* stereochemistry in the tetrahydrofuran ring.<sup>28</sup> One intense singlet at  $\delta$  3.87 (12H) was assigned to four equivalent methoxyl groups

at C-3/C-3' and C-4/C-4'. The <sup>13</sup>C NMR spectrum of **4** showed the peaks assigned to aromatic carbons C-1/C-1' to C-6/C-6' at range  $\delta$  148.6-109.6, to oximethine carbon (C-7/C-7') at  $\delta$  83.7 and to methyl carbons (C-9/C-9') at  $\delta$  14.7. Additionally, the presence of methoxyl groups was confirmed by the presence of an intense peak at  $\delta$  55.9. LRESIMS of **4** displayed a *quasi*-molecular ion peak at  $m/z$  395 [M + Na]<sup>+</sup> and 411 [M + K]<sup>+</sup> consistent with molecular mass C<sub>22</sub>H<sub>28</sub>O<sub>5</sub>. Finally, comparison of the NMR data of **4** with those reported in the literature<sup>29</sup> allowed the identification of saucermetin.

Based on the IC<sub>50</sub> values (Table 1), compound **1** displayed reduced cytotoxic activity to all tested cells while the related neolignan **2** exhibited higher potential against HeLa cells with IC<sub>50</sub> of 28.3 ± 3.9 μg mL<sup>-1</sup> (86 ± 12 μmol L<sup>-1</sup>), similar as observed to positive control cisplatin (IC<sub>50</sub> = 20.6 ± 1.5 μg mL<sup>-1</sup> or 69 ± 5 μmol L<sup>-1</sup>). Additionally, this compound showed potent cytotoxicity against B16F10 cell (IC<sub>50</sub> = 32.8 ± 2.1 μg mL<sup>-1</sup> or 100 ± 6 μmol L<sup>-1</sup>), which was stronger than cisplatin (IC<sub>50</sub> = 53.1 ± 4.2 μg mL<sup>-1</sup> or 177 ± 14 μmol L<sup>-1</sup>). Furthermore, compounds **2** and **3** exhibited cytotoxic activity against A2058 cells with IC<sub>50</sub> of 44.3 ± 4.2 μg mL<sup>-1</sup> (135 ± 13 μmol L<sup>-1</sup>) and 41.5 ± 7.5 μg mL<sup>-1</sup> (126 ± 23 μmol L<sup>-1</sup>), respectively, similarly to cisplatin (IC<sub>50</sub> = 43.2 ± 3.2 μg mL<sup>-1</sup> or 144 ± 11 μmol L<sup>-1</sup>). Both compounds were less cytotoxic against the non-tumorigenic cell line (T75), since the IC<sub>50</sub> values remained higher in comparison with tumor cell lines with IC<sub>50</sub> values of 53.2 ± 3.6 μg mL<sup>-1</sup> (162 ± 11 μmol L<sup>-1</sup>) for compound **2** and 66.7 ± 8.5 μg mL<sup>-1</sup> (202 ± 26 μmol L<sup>-1</sup>) for compound **3**. Otherwise, compound **4** was inactive since calculated IC<sub>50</sub> values were higher than 100 μg mL<sup>-1</sup> (> 300 μmol L<sup>-1</sup>) to all tested cells. Due to the structural similarities between compounds **1-3**, it is interesting to observe that the presence of methylenedioxy group in both aromatic rings, as in the case of compound **1**, cause a reduction in the cytotoxic potential. Otherwise, it was observed an increasing of activity to compounds **2** and **3** in which methoxyl and hydroxyl groups are linked directly in the aromatic ring. This effect could be justified, at least in part, to reduction of levels of reactive oxygen species caused by the presence of phenolic group, as observed to tetrahydrofuran neolignan **4** and related derivatives isolated from *Nectandra leucantha* (Lauraceae).<sup>30</sup>

Previous studies showed that related compounds *erythro*-austrobailignan-6 and *meso*-dihydroguaiaretic acid, isolated from *S. chinensis*,<sup>31</sup> displayed cytotoxicity against HT-29 (human colon adenocarcinoma), MCF-7 (human breast adenocarcinoma) and HepG2 (human liver hepatoblastoma). Furthermore, compound **3** (*threo*-dihydroguaiaretic acid) was previously evaluated against HL-60 and HeLa cell lines with IC<sub>50</sub> values of 25.7 ± 0.9 and 21.7 ± 4.1 μmol L<sup>-1</sup>, respectively.<sup>32</sup> However, this is the first report describing the cytotoxic activity of compounds **1-3** as well as their selectivity to tumorigenic and non-tumorigenic cell lines. Based in the obtained results, further studies aiming determination of the mechanism of action of bioactive compounds **2** and **3** must be

Table 1. Cytotoxic effects of compounds **1-4** isolated from leaves of *S. cernuus* and positive control cisplatin against tumorigenic and non-tumorigenic cell lines

	IC <sub>50</sub> / μg mL <sup>-1</sup> (μmol L <sup>-1</sup> )				
	B16F10	A2058	HeLa	MCF-7	T75
1	97.5 ± 9.4 (299 ± 29)	>100 (> 300)	90.9 ± 11.4 (279 ± 35)	> 100 (> 300)	> 100 (> 300)
2	32.8 ± 2.1 (100 ± 6)	44.3 ± 4.2 (135 ± 13)	28.3 ± 3.9 (86 ± 12)	46.3 ± 1.9 (141 ± 6)	53.2 ± 3.6 (162 ± 11)
3	52.6 ± 6.6 (159 ± 20)	41.5 ± 7.5 (126 ± 23)	45.5 ± 12.7 (138 ± 38)	36.3 ± 3.5 (110 ± 11)	66.7 ± 8.5 (202 ± 26)
4	> 100 (> 300)	> 100 (> 300)	> 100 (> 300)	> 100 (> 300)	> 100 (> 300)
Cisplatin	53.1 ± 4.2 (177 ± 14)	43.2 ± 3.2 (144 ± 11)	20.6 ± 1.5 (69 ± 5)	21.1 ± 1.4 (70 ± 5)	> 100 (> 300)

B16F10, murine melanoma; A2058, human melanoma; HeLa, human cervical carcinoma; MCF-7, breast adenocarcinoma tumorigenic cells and T75, human fibroblast non-tumorigenic cell.

performed in order to eventually propose the use of these neolignans as antitumor agents.

## CONCLUSIONS

In the present work, the leaves of *S. cernuus* were subjected to a microwave-assisted extraction (MAE) procedure using an ionic liquid aqueous solution (1-butyl-3-methylimidazolium bromide, BMImBr). After partition with *n*-hexane a bioactive organic phase was obtained and was subjected to a bioactivity-guided procedure. This approach afforded three cytotoxic (**1-3**) and one inactive (**4**) neolignans. Isolated compounds were identified by analysis of the NMR and MS spectral data and comparison with those described in the literature. Compound **1** displayed reduced toxicity while **2** exhibited cytotoxic activity against HeLa cells similar as observed to positive control cisplatin. Similarly, compounds **2** and **3** exhibited cytotoxicity against A2058 cells. This study reports, at first time, the use of a simple and fast method employing a green solvent as 1-butyl-3-methylimidazolium bromide (BMImBr) to *S. cernuus* species and the obtained results provide important data for the selection of bioactive neolignans with cytotoxic potential.

## EXPERIMENTAL SECTION

### General experimental procedures

Silica gel (Merck, 230-400 mesh) was used for the column chromatographic (CC) separation, while silica gel 60 PF<sub>254</sub> (Merck) was used for analytical thin-layer chromatography (TLC). <sup>1</sup>H and <sup>13</sup>C nuclear magnetic resonance (NMR) spectra were recorded at 300 and 75 MHz, respectively, in a Bruker Ultrashield 300 Avance III spectrometer. CDCl<sub>3</sub> (Aldrich) was the solvent and the residual resonance peaks at δ<sub>H</sub> 7.26 (<sup>1</sup>H) and δ<sub>C</sub> 77.2 (<sup>13</sup>C) were used as internal standard. LRESIMS and LREIMS (70 eV) spectra were recorded, respectively, using a Platform II-Micromass Spectrometer (quadrupole) and INCOS 50 Finnigan-Mat (quadrupole) mass spectrometers. Microwave assisted extraction (MAE) experiments were performed with a MAS-I microwave oven (2450 MHz, Sineo Microwave Chemistry Technology Company, Shanghai, China) with a maximum delivered power of 1000 W. The temperature was monitored by an infrared probe inside the microwave oven.

### Plant material

Plant material was obtained from a local producer of ornamental plants in the city of Suzano, São Paulo State. The botanical identification was made by Profs. Dr. Fátima O. de Souza Buturi and Dr. Oriana A. Fávero and the voucher specimen (E. A. Ferreira – 001) was deposited at SPF Herbarium of the Institute of Biosciences of the University of São Paulo.

### Extraction and isolation

1-butyl-3-methylimidazolium bromide (BMImBr) was prepared as previously described in the literature.<sup>21</sup> Dried and powdered leaves (5.0 g) of *S. cernuus* were extracted by microwave-assisted extraction (MAE) with 20 mL of mixture containing H<sub>2</sub>O:BMImBr 1:1 (v/v) during 10 min at 60 °C. After this procedure, the solution was filtered, extracted using *n*-hexane (3 × 30 mL) and dried over Na<sub>2</sub>SO<sub>4</sub>. After distillation of the solvent under reduced pressure, were obtained 215 mg of *n*-hexane phase. Part of this material (200 mg) was fractionated over silica gel 60 column chromatography eluted with increasing amounts of EtOAc in *n*-hexane to afford

five fractions (A-E), in which bioactivity was detected on fractions A (30 mg), C (10 mg) and D (33 mg). Fractions A and C were individually purified using silica gel 60 column chromatography eluted with hexane:EtOAc 9:1 to afford, respectively, compounds **1** (9.2 mg) and **2** (2.5 mg). Fraction D was subjected to silica gel column chromatography eluted with increasing amounts of EtOAc in *n*-hexane to give compounds **3** (5.1 mg) and **4** (2.6 mg).

*Threo-austrobailignan-5 (1)*. Pale yellow oil. <sup>1</sup>H NMR (δ, 300 MHz, CDCl<sub>3</sub>): 6.56 (d, *J* = 1.5 Hz, H2/H2'); 6.68 (d, *J* = 7.8 Hz, H5/H5'); 6.50 (dd, *J* = 7.8 and 1.5 Hz, H6/H6'); 2.31 (dd, *J* = 13.5 and 8.1 Hz, H7a/H7a'); 2.53 (dd, *J* = 13.5 and 6.6 Hz, H7b/H7b'); 1.72 (m, H8/H8'); 0.79 (d, *J* = 6.6 Hz, H9/H9'); 5.85 (s, OCH<sub>2</sub>O). <sup>13</sup>C NMR (δ, 75 MHz, CDCl<sub>3</sub>): 147.5 (C3/C3'); 145.5 (C4/C4'); 135.4 (C1/C1'); 121.8 (C6/C6'); 109.7 (C2/C2'); 107.9 (C5/C5'); 100.7 (OCH<sub>2</sub>O); 41.2 (C7/C7'); 38.2 (C8/C8'); 13.9 (C9/C9'). LREIMS (70 eV) *m/z* (rel. int.) [*M*<sup>+</sup>] 326 (24), 255 (2), 239 (1), 183 (5), 149 (6), 135 (100), 105 (10), 77 (18).

*Threo-austrobailignan-6 (2)*. Pale yellow oil. <sup>1</sup>H-NMR (δ, 300 MHz, CDCl<sub>3</sub>): 6.61 (d, *J* = 1.5 Hz, H2/H2'); 6.81 (d, *J* = 7.8 Hz, H5); 6.72 (d, *J* = 7.8 Hz, H5'); 6.56 (dd, *J* = 7.8 and 1.5 Hz, H6); 6.60 (dd, *J* = 7.8 and 1.5 Hz, H6'); 2.54 (dd, *J* = 13.5 and 6.6 Hz, H7a/H7a'); 2.26 (ddd, *J* = 13.5 and 8.1 Hz, H7b/H7b'); 1.75-1.66 (m, H8/H8'); 0.81 (d, *J* = 6.3 Hz, H9/H9'); 5.90 (s, OCH<sub>2</sub>O); 3.83 (s, OMe); 5.50 (s, OH). <sup>13</sup>C NMR (δ, 75 MHz, CDCl<sub>3</sub>): 147.4 (C3); 146.3 (C3'); 145.4 (C4); 143.6 (C4'); 135.5 (C1); 133.5 (C1'); 121.7 (C6/C6'); 113.9 (C5'); 111.3 (C2); 109.3 (C5); 107.4 (C2); 100.7 (OCH<sub>2</sub>O); 55.8 (OMe); 39.2 (C7); 39.0 (C7'); 41.1 (C8 and C8'); 13.9 (C9); 13.8 (C9'). LREIMS (70 eV) *m/z* (rel. int.) [*M*<sup>+</sup>] 328 (27), 207 (1), 192 (2), 149 (1), 137 (100), 135 (67), 122 (13), 105 (10), 77 (18).

*Threo-dihydroguaiaretic acid (3)*. White amorphous solid. <sup>1</sup>H NMR (δ, 300 MHz, CDCl<sub>3</sub>): 6.51 (d, *J* = 1.5 Hz, H2/H2'); 6.79 (d, *J* = 7.8 Hz, H5/H5'); 6.58 (dd, *J* = 7.8 and 1.5 Hz, H6/H6'); 2.55 (dd, *J* = 13.5 and 6.6 Hz, H7a/H7a'); 2.30 (dd, *J* = 13.5 and 8.1 Hz, H7b/H7b'); 1.73 (m, H8/H8'); 0.82 (d, *J* = 6.7 Hz, H9/H9'); 3.80 (s, OMe). <sup>13</sup>C NMR (δ, 75 MHz, CDCl<sub>3</sub>): 146.3 (C3/C3'); 143.5 (C4/C4'); 133.6 (C1/C1'); 113.8 (C5/C5'); 121.6 (C6/C6'); 111.3 (C2/C2'); 55.8 (OMe); 41.1 (C7/C7'); 37.5 (C8/C8'); 13.9 (C9/C9'). LREIMS (70 eV) *m/z* (rel. int.) [*M*<sup>+</sup>] 330 (18), 207 (1), 192 (2), 151 (1), 137 (100), 122 (17), 94 (8), 77 (1).

*Saucernetin (4)*: White amorphous solid. <sup>1</sup>H NMR (δ, 300 MHz, CDCl<sub>3</sub>): 6.85 (br s, H2/H2', H5/H5' and H6/H6'); 5.45 (d, *J* = 5.3 Hz, H7/H7'); 3.87 (s, OMe), 2.27 (m, H8/H8'); 0.69 (d, *J* = 6.8 Hz, H9/H9'). <sup>13</sup>C NMR (δ, 75 MHz, CDCl<sub>3</sub>): 148.7 (C3/C3'); 147.9 (C4/C4'); 133.9 (C1/C1'); 118.5 (C5/C5'); 110.8 (C2/C2'); 109.6 (C6/C6'); 83.7 (C7/C7'); 55.9 (OMe); 44.0 (C8/C8'); 14.7 (C9/C9'). LRESIMS (positive mode) *m/z* 395 [*M* + Na]<sup>+</sup> and 411 [*M* + K]<sup>+</sup>.

### Cell culture

The following tumorigenic cell lines were used: B16F10 - murine melanoma, A2058 - human melanoma, HeLa - human cervical carcinoma, MCF-7 - human breast adenocarcinoma and non-tumorigenic cell (T75 - human fibroblast). Tumor cells were cultured at 37 °C in a humid atmosphere containing 5% CO<sub>2</sub> using RPMI 1640 medium (Invitrogen, Carlsbad, CA). The T75 cell line was cultured in DMEM medium (Invitrogen, Carlsbad, CA) supplemented with 10 mmol L<sup>-1</sup> 2-hydroxyethylpiperazine-N2 ethanesulfonic acid (Hepes) (Sigma, St. Louis, MO), 24 mmol L<sup>-1</sup> sodium bicarbonate

(Sigma), 40 mg mL<sup>-1</sup> gentamicide (Schering-Plow, São Paulo), pH 7.2 and 10% fetal bovine serum (FBS - Invitrogen).

### Cell viability assay

For the cytotoxicity assays, 1x10<sup>4</sup> viable cells were incubated in 96 wells plate in 100 µL of complete media. After 8 h of inoculation, the studied cells were treated with isolated compounds **1-4** from *S. cernuum* in concentrations of 100, 75, 50, 25 and 12.5 µg mL<sup>-1</sup>, or fresh medium for the control cells. After 24 h, 10 µL of MTT solution (5 mg mL<sup>-1</sup>) was added to each well, and the plate was incubated for 3 h at 37 °C. Thus, 100 µL of a 10% SDS/0.01M HCl solution was added and incubated for 4 h at 37 °C. Readings of the absorbance were performed at 570 nm with a reference filter at λ = 650 nm using a plate reader (SpectraMax®-M2e, Molecular Devices, Sunnyvale, CA). Cell viability was expressed as percentage values comparing with viable cells at the negative control treatments.<sup>33,34</sup> All experiments were performed in triplicates.

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