Cytotoxic Activity of Semi-Synthetic Derivatives of Elatol and Isoobtusol

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Abstract: In the present study, the in vitro cytotoxic effects of six semi-synthetic derivatives of elatol (1) and isoobtusol (2) were investigated. Chemical modifications were performed on the hydroxyl groups aiming to get derivatives of different polarity, namely the hemisuccinate, carbamate and sulfamate. The structural elucidation of the new derivatives was based on detailed NMR and MS spectroscopic analyses. The in vitro cytotoxicity of compounds 1 to 8 was evaluated against A459 and RD tumor cells with CC50 values ranging from 4.93 to 41.53 µM. These results suggest that the structural modifications performed on both compounds could be considered a good strategy to obtain more active derivatives.

Keywords: elatol; isoobtusol; sesquiterpenes; synthesis; cytotoxic activity
1. Introduction

The oceanic environment has been a vast source of natural products, yielding a wide range of bioactive compounds with diverse mechanisms of action [1]. The genus Laurencia (order Ceremiales, family Rhodomelaceae) is a rich source of halogenated secondary metabolites, predominantly sesquiterpenes, diterpenes, and C15 non-terpenoids, chemically diverse compounds with great therapeutic potential [2].

Elatol (1) (Figure 1), a halogenated chamigrane sesquiterpene, was isolated for the first time from Laurencia elata by Sims et al. [3]. Several species of Laurencia produce this sesquiterpene as a major secondary metabolite [4–7], especially Laurencia microcladia from which elatol was obtained with the high yield of ca. 10% (w/w) from the ethanolic extract of the alga [8]. This compound has displayed antifeedant [9], antifouling [10], antibacterial [6,11], antifungal [12], antiparasitic [13,14] and cytotoxic activity against HeLa and Hep-2 human carcinoma cell lines [15]. A recent investigation [16] showed that elatol caused in vitro an increase in cell numbers at the G1 and the sub-G1 phases, indicating apoptosis induction; and was able to reduce tumor growth in vivo in C57Bl6 mice inoculated with B16F10 cells.

Isoobtusol (2) (Figure 1) belongs to the same structural class of elatol and was described for the first time from Laurencia obtusa by González et al. [17]. It showed strong antimicrobial activity against several strains, which included human pathogens [6,12].

In spite of this promising pharmacological profile, the high lipophilicity of elatol and isoobtusol and their consequent low aqueous solubility are limiting aspects for further studies. In order to address these issues, chemical modifications were performed on the hydroxyl groups aiming to get analogues with different polarity. With this objective, six compounds were synthetized by reaction with chlorosulfonyl isocyanate and succinic anhydride to obtain the corresponding carbamates (3 and 6), sulfamates (5 and 8) and hemissuccinates (4 and 7). Such derivatives have been shown to improve not only the water solubility, but in some cases also the cytotoxic activity of the compounds, as its amphiphilic character is supposed to enhance absorption in biological systems [18,19]. In this way, these compounds were evaluated for in vitro cytotoxic activity against lung (A549) and embryo rhabdomyosarcome (RD) tumor cells, and a preliminary structure-activity relationship (SAR) is presented.
2. Results and Discussion

2.1. Chemistry

Six derivatives of elatol (1) and isoobtusol (2) were synthesized by the routes described in Schemes 1 and 2, and their cytotoxic activity was evaluated.

**Scheme 1.** Synthesis of elatol derivatives (3), (4) and (5).

![Scheme 1](image)

The carbamate derivative (3) was prepared by treatment of (1) with chlorosulfonyl isocyanate as described previously by Bandyopadhyay et al. [20]. Compound (4) was prepared by the reaction of (1)
with succinic anhydride in presence of DMAP, pyridine and CH₂Cl₂. Compound (5) was obtained by reaction with chlorosulfonyl isocyanate in the presence of formic acid and DMA [21]. Compounds (6), (7) and (8) were obtained from (2) according to the same methods used for the preparation of compounds (3), (4) and (5), respectively. The compounds were purified by silica gel column chromatography with hexane/ethyl acetate as eluent and their structures confirmed by IR, ¹H-NMR, ¹³C-NMR, 2D-NMR and MS.

2.2. Cytotoxic Activity

To quantify the cytotoxic effects of elatol (1), isoobtusol (2) and their synthetic derivatives (3) to (8), the CC₅₀ value of each compound was measured (CC₅₀ is defined as the concentration that reduced cell growth by 50% after 48 h). Table 1 shows the CC₅₀ values obtained for the tested compounds against human non-small cell lung tumor (A549) and human embryo rhabdomyosarcome (RD) cells.

| Compound | A549 [CC₅₀ (μM)] | RD [CC₅₀ (μM)] |
|----------|-----------------|----------------|
| 1        | 7.56 ± 0.19     | 11.22 ± 1.63   |
| 2        | 14.24 ± 3.43    | 6.24 ± 1.11    |
| 3        | 21.93 ± 1.27    | 13.26 ± 0.76   |
| 4        | 39.05 ± 4.81    | 21.23 ± 7.16   |
| 5        | >100            | 41.53 ± 0.36   |
| 6        | 39.57 ± 2.07    | 23.83 ± 5.28   |
| 7        | 10.74 ± 2.52    | 4.93 ± 0.52    |
| 8        | 23.85 ± 5.21    | 20.48 ± 1.44   |
| Paclitaxel | 0.260 ± 0.027  | 0.025 ± 0.004  |

Values represent the mean ± standard deviations of three independent experiments; * Cytotoxicity was determined by MTT assay on each human tested cancer cell line.

Elatol (1) and isoobtusol (2) exhibited significant cytotoxicity against the tested human tumor cell lines with CC₅₀ values ranging from 6.24 to 14.24 μM. Elatol was approximately twice more active against A549 cells, while, on the other hand, isoobtusol was approximately twice more active against RD cells. In relation to the elatol derivatives (4) and (5) and the isoobtusol derivatives (6) and (8), the modifications on the hydroxyl group at C-9 lead to a reduction in the observed cytotoxicity. Carbamate derivatives (3) and (6) were approximately three times less active than the original compounds against A549 cells, while derivative (3) was just slightly less active than elatol itself towards RD cells. An increase of the cytotoxic activity was observed for compound (7), which is the hemisuccinate of isoobtusol, since it was more cytotoxic than its precursor against A549 cells (CC₅₀ = 10.74 μM) and also against RD cells (CC₅₀ = 4.93 μM). It is interesting to note that the hemisuccinate derivative of isoobtusol (7) and isoobutusol itself (2) showed similar profiles being more active against RD than to A549 cells. Nevertheless, the hemisuccinate derivative of elatol (4) as well the sulfamate derivatives (5) and (8) were less active than the original compounds to both cell lines. These findings suggest that the substituent at C-9 plays an important role for the cytotoxic activity against these cell lines, and the best results were observed with the free hydroxyl or the hemisuccinate group.
3. Experimental

3.1. General

NMR spectra were recorded in CDCl₃ or CD₃OD at 500.13 and 125.13 MHz for ¹H and ¹³C, respectively, on a Bruker Avance 2500 MHz NMR spectrometer with TMS or the signal of residual non-deuterated solvent as internal standard. High-resolution ESI (ESI-HR-MS) mass spectra were recorded on a Bruker-Daltronics MicroTOF-Q II mass spectrometer. IR spectra were obtained on a Shimadzu Prestige 2 instrument by using KBr pellets. Column chromatography was performed using silica gel (200–300 mesh, Merck®). TLC was carried out using Kieselgel 60 F₂₅₄ (Merck® aluminum support plates). All solvents were of analytical grade and were purchased from Nuclear® Chemical Company (Milwaukee, Wisconsin, USA).

3.2. Algal Material

Laurencia microcladia was collected by hand in March 2010 at the lower intertidal zone of Praia da Sepultura (27°07′54″S and 48°31′40″W), Santa Catarina, Southern Brazilian coast. Voucher samples are kept at the Herbarium of the Department of Botany, Federal University of Santa Catarina (FLOR 14516–14520).

Fresh material of L. microcladia (1 kg) was exhaustively extracted with ethanol at room temperature for 3 days (three times). The concentrated extract (5 g) was partitioned with ethyl acetate and water, affording 2 g of aqueous fraction and 3 g of ethyl acetate fraction. This latter was fractionated as previously described by Lhullier et al. [8] to yield pure compounds 1 (500 mg) and 2 (400 mg).

3.3. Synthesis

3.3.1. Elatol 9-Carbamate (3)

To a solution of 1 (100 mg, 0.3 mmol) in CHCl₃ (0.4 mL) chlorosulfonyl isocyanate was added dropwise (0.08 mL, 0.9 mmol) at 20 °C under nitrogen atmosphere with vigorous stirring. The reaction mixture was left overnight during 16 h, then, a solution of THF/H₂O (1/1 v/v, 2 mL) was added, followed by a saturated solution of NaHCO₃ (1 mL). The obtained suspension was stirred for an additional 2 h at 20 °C and then diluted with chloroform (10 mL). The organic phase was separated and washed with H₂O (2 × 10 mL), dried over anhydrous sodium sulphate and evaporated under reduced pressure. The resulting crude product was purified by column chromatography on silica gel using hexane/EtOAc (8:2) and afforded 80 mg (71%) of carbamate 3 as a white solid. Mp: 79–81 °C; IR (KBr): 3470, 3240, 1705, 1600, 1385, 1334, 1078, 1055 cm⁻¹, ¹H NMR (500 MHz, CDCl₃): δ = 1.04 (3H, s, H-12), 1.10 (3H, s, H-13), 1.66 (1H, m, H-5α), 1.70 (3H, s, H-15), 1.78 (1H, m, H-4α), 1.81 (1H, m, H-5β), 1.97 (1H, m, H-4β), 2.36 (1H, d, J = 17.3 Hz, H-1β), 2.51 (1H, dd, J = 15.0/2.8 Hz, H-8α), 2.60 (1H, d, J = 15.0 Hz, H-8β), 2.61 (1H, d, J = 17.3 Hz, H-1α), 4.53 (1H, d, J = 3.4 Hz, H-10), 4.77 (1H, s, H-14β), 5.04 (1H, s, H-14α), 5.15 (1H, dd, J = 6.3/3.4 Hz, H-9). ¹³C NMR (126 MHz, CDCl₃): δ = 19.6 (C-15), 20.4 (C-12), 24.4 (C-13), 25.7 (C-5), 29.4 (C-4), 37.1 (C-8), 38.8 (C-1), 43.6 (C-11), 49.2 (C-6), 63.4 (C-10), 74.5 (C-9), 116.0 (C-14), 124.2 (C-2), 128.3 (C-3), 140.8
(C-7), 156.0 (C-1’); ESI-MS m/z 398.0496 [M + Na]^+ (calcd for C_{16}H_{25}BrClINaO_2, 398.0493), 400.0482 [M + 2 + Na]^+ (calcd. 400.0471), 402.0444 [M + 4 + Na]^+ (calcd. 402.0449), observed isotopic pattern (398/400/402): 79/100/22, (theoretical: 76/100/25).

3.3.2. Elatol 9-Hemisuccinate (4)

To a solution of 1 (50 mg, 0.15 mmol) in CH_2Cl_2 (1 mL) and pyridine (0.1 mL), succinic anhydride (150 mg, 1.5 mmol) and a catalytic amount of DMAP were added with stirring at 20 °C. After 24 h, the mixture was diluted with CH_2Cl_2 (10 mL) and washed with HCl 1 N (2 × 10 mL). The organic phase was dried with anhydrous sodium sulfate and evaporated under reduced pressure. The resulting crude product was purified by flash column chromatography on silica gel using hexane/EtOAc (8:2), to give the hemisuccinate 4 (35 mg, 69.8% yield) as yellow oil. IR (KBr): 3100, 1730, 1160 cm^{-1}; ^1H NMR (500 MHz, CDCl_3): δ = 1.06 (3H, s, H-12), 1.10 (3H, s, H-13), 1.65 (1H, m, H-5α), 1.70 (3H, s, H-15), 1.80 (1H, m, H-4α), 1.81 (1H, m, H-5β), 1.97 (1H, m, H-4β), 2.36 (1H, d, J = 17.3 Hz, H-1β), 2.43 (1H, dd, J = 15.0/2.7 Hz, H-8α), 2.61 (1H, d, J = 15.0 Hz, H-8β), 2.62 (1H, d, J = 17.3 Hz, H-1α), 2.66 (2H, m, H-3’), 2.71 (2H, m, H-2’), 4.53 (1H, d, J = 3.4 Hz, H-10), 4.76 (1H, s, H-14β), 4.99 (1H, s, H-14α), 5.28 (1H, dd, J = 6.3/3.4 Hz, H-9). ^13C NMR (126 MHz, CDCl_3) δ = 19.4 (C-15), 20.2 (C-12), 24.2 (C-13), 25.6 (C-5), 28.8 (C-2’), 29.1 (C-3’), 29.4 (C-4), 36.6 (C-8), 38.6 (C-1), 43.3 (C-11), 48.9 (C-6), 62.8 (C-10), 74.1 (C-9), 116.0 (C-14), 124.0 (C-2), 128.1 (C-3), 140.3 (C-7), 171.1 (C-1’), 177.4 (C-4’); ESI-MS m/z 455.0591 [M + Na]^+ (calcd for C_{19}H_{26}BrClINaO_4, 455.0595), 457.0580 [M + 2 + Na]^+ (calcd. 457.0574), 459.0557 [M + 4 + Na]^+ (calcd. 459.0555), observed isotopic pattern (455/457/459): 73/100/24, (theoretical: 76/100/26).

3.3.3. Elatol 9-Sulfamate (5)

Formic acid (0.03 mL, 0.9 mmol) was added dropwise to chlorosulfonyl isocyanate (0.08 mL, 0.9 mmol) at 0 °C with rapid stirring. Gas evolution was observed during the addition process. The resulting viscous suspension was stirred for 18 h at room temperature. The reaction mixture was cooled to 0 °C, DMA (0.2 mL) was added, and the solution was stirred for 5 min. A solution of 1 (0.3 mmol, 100 mg) in DMA (0.5 mL) was added dropwise, and the reaction was allowed to warm to 20 °C over a 1 h period. The reaction was quenched by the successive addition of EtOAc (10 mL) and brine (5 mL). The mixture was poured on EtOAc (20 mL) and water (10 mL), the organic phase was collected, and the aqueous layer was extracted with EtOAc (20 mL). The combined organic extracts were washed with brine (2 × 10 mL), dried over Na_2SO_4 and concentrated under reduced pressure. Purification of the residue by flash column chromatography on silica gel using hexane/EtOAc (7:3) gave the sulfamidate 5 (30.5 mg, 24% yield) as a white solid. Mp: 199–202 °C; IR (KBr): 3333, 3265, 1557, 1350, 1196, 1140, 900 cm^{-1}; ^1H NMR (500 MHz, CDCl_3): δ = 1.02 (3H, s, H-12), 1.10 (3H, s, H-13), 1.65 (1H, m, H-5α), 1.71 (3H, s, H-15), 1.79 (1H, m, H-5β), 1.80 (1H, m, H-4α), 1.98 (1H, m, H-4β), 2.36 (1H, d, J = 17.3 Hz, H-1β), 2.63 (1H, d, J = 15.0 Hz, H-8α), 2.60 (1H, d, J = 17.3 Hz, H-1α), 2.86 (1H, dd, J = 15.0/2.8 Hz, H-8β), 4.55 (1H, d, J = 3.0 Hz, H-10), 4.84 (1H, s, H-14β), 4.90 (1H, dd, J = 6.0/3.0 Hz, H-9), 5.17 (1H, s, H-14α); ^13C NMR (126 MHz, CDCl_3): δ = 19.4 (C-15), 20.1 (C-12), 24.3 (C-13), 25.6 (C-5), 29.4 (C-4), 37.1 (C-8), 38.6 (C-1), 43.6 (C-11), 49.4 (C-6), 62.6 (C-10), 82.2 (C-9), 117.1 (C-14), 124.0 (C-2), 128.2 (C-3), 139.2 (C-7); ESI-MS m/z 429.0587
[M + NH₄]⁺ (calcd for C₁₅H₂₇BrClN₂O₅S, 429.0608), 431.0575 [M + 2 + NH₄]⁺ (calcd. 431.0587), 433.0565 [M + 4 + NH₄]⁺ (calcd. 433.0563), observed isotopic pattern (429/431/433): 73/100/28, (theoretical: 73/100/29).

3.3.4. Isoobuttsol 9-Carbamate (6)

Compound 6 was similarly prepared, according to the procedure of 3. To a solution of 2 (100 mg, 0.24 mmol) in CHCl₃ (0.4 mL) chlorosulfonyl isocyanate (0.06 mL, 0.74 mmol) was added dropwise at 20 °C under nitrogen atmosphere with vigorous stirring. The reaction mixture was left overnight during 16 h, and then a solution of THF/H₂O (1/1 v/v, 1.5 mL) was added, followed by a saturated solution of NaHCO₃ (0.7 mL). The obtained suspension was stirred for an additional 2 h at 20 °C and then diluted with chloroform (10 mL). The organic phase was separated and washed with H₂O (2 × 10 mL), dried over anhydrous sodium sulphate and evaporated under reduced pressure. The resulting crude product was purified by column chromatography on silica gel using hexane/EtOAc (8:2) afforded 62.4 mg (57% yield) of carbamate 6 as white solid. Mp: 158–160 °C; IR (KBr): 3472, 3241, 1728, 1604, 1324, 1328, 1064 cm⁻¹; ¹H NMR (500 MHz, CDCl₃): δ = 1.11 (3H, s, H-13), 1.32 (3H, s, H-12), 1.80 (1H, ddd, J = 14.0/6.5/3.5 Hz, H-4α), 1.85 (1H, m, H-5α), 1.92 (3H, s, H-15), 2.06 (1H, td, J = 14.0/3.5 Hz, H-4β), 2.24 (1H, m, H-5β), 2.41 (1H, dd, J = 12.2/3.4 Hz, H-8α), 2.82 (1H, dd, J = 15/3.7 Hz, H-1β), 2.92 (1H, t, J = 12.2 Hz, H-8β), 3.17 (1H, d, J = 15 Hz, H-1α), 4.45 (1H, m, H-2), 4.46 (1H, dd, J = 3.4/1.9 Hz, H-10), 4.73 (1H, m, H-9), 4.98 (1H, s, H-14), 5.21 (1H, s, H-14); ¹³C NMR (126 MHz, CDCl₃): δ = 24.6 (C-12), 25.1 (C-13), 25.6 (C-4), 33.1 (C-15), 33.3 (C-5), 34.0 (C-1), 35.4 (C-8), 43.7 (C-6), 44.1 (C-11), 65.2 (C-2), 67.3 (C-10), 71.1 (C-3), 72.0 (C-9), 114.6 (C-14), 146.9 (C-7), 155.6 (C-1′); ESI-MS m/z 477.9773 [M + Na]⁺ (calcd for C₁₆H₂₄Br₂ClNNaO₂, 477.9754), 479.9736 [M + 2 + Na]⁺ (calcd. 479.9733), 481.9727 [M + 4 + Na]⁺ (calcd. 481.9712), 483.9684 [M + 6 + Na]⁺ (calcd. 483.9692), observed isotopic pattern (477/479/481/483): 43/100/69/13, (theoretical: 44/100/70/14).

3.3.5. Isoobuttsol 9-Hemisuccinate (7)

Compound 7 was prepared in a similar way as 4. To a solution of 2 (50 mg, 0.12 mmol) in CH₂Cl₂ (1 mL) and pyridine (0.1 mL) and succinic anhydride (121 mg, 1.2 mmol) and a catalytic amount of DMAP were added with stirring at 20 °C. After 24 h, the mixture was diluted with CH₂Cl₂ (10 mL) and washed with HCl 1 N (2 × 10 mL). The organic phase was dried with anhydrous sodium sulfate and evaporated under reduced pressure. The resulting crude product was purified by flash column chromatography on silica gel using hexane/EtOAc (8:2), to give the hemisuccinate 7 (31 mg, 62% yield) as white solid. Mp: 116–118 °C; IR (KBr): 3100, 1738, 1184 cm⁻¹; ¹H NMR (500 MHz CDCl₃:MeOD, 2:1): δ = 5.21 (1H, s, H-14α), 5.0 (1H, s, H-14β), 4.85 (1H, m, H-9), 4.46 (1H, br s, H-2), 4.42 (1H, dd, J = 3.4/1.9 Hz, H-10), 3.16 (1H, d, J = 15 Hz, H-1α), 2.95 (1H, t, J = 12.2 Hz, H-8β), 2.81 (1H, dd, J = 15/3.7 Hz, H-1β), 2.67 (2H, overlapped, H-3′), 2.62 (2H, overlapped, H-2′), 2.41 (1H, m, H-8α), 2.24 (1H, m, H-5β), 2.07 (1H, td, J = 14.0/3.5 Hz, H-4β), 1.85 (1H, m, H-5α), 1.82 (1H, m, H-4α), 1.31 (3H, s, H-12), 1.11 (3H, s, H-13); ¹³C NMR (126 MHz, CDCl₃:MeOD, 2:1): δ = 174.7 (C-4′), 172.0 (C-1′), 146.8 (C-7), 114.9 (C-14), 72.0 (C-9), 71.2 (C-3), 66.3 (C-10), 65.3 (C-2), 43.9 (C-6), 35.2 (C-8), 34.1 (C-1), 33.4 (C-5), 33.1 (C-15), 29.0 (C-3′), 28.9 (C-2′), 25.7 (C-4), 25.1 (C-13),
24.6 (C-12); ESI-MS m/z 530.0295 [M + NH₄]⁺ (calcd for C₁₂H₁₃Br₂ClNO₄, 530.0303), 532.0276 [M + 2 + NH₄]⁺ (calcd. 532.0282), 534.0260 [M + 4 + NH₄]⁺ (calcd. 534.0262), 536.0175 [M + 6 + NH₄]⁺ (calcd. 536.0244), observed isotopic pattern (530/532/534/536): 42/100/74/11, (theoretical: 43/100/72/15).

3.3.6. Isoobtusol 9-Sulfamate (8)

Compound 8 was prepared according to the procedure of 5. Formic acid (0.03 mL, 0.73 mmol) was added dropwise to chlorosulfonyl isocyanate (0.06 mL, 0.73 mmol) at 0 °C with rapid stirring. Gas evolution was observed during the addition process. The resulting viscous suspension was stirred for 18 h at room temperature. The reaction mixture was cooled to 0 °C, DMA (0.2 mL) was added, and the solution was stirred for 5 min. A solution of 2 (100 mg, 0.24 mmol) in DMA (0.3 mL) was added dropwise, and the reaction was allowed to warm to 20 °C over a 1 h period. The reaction was quenched by the successive addition of EtOAc (10 mL) and brine (5 mL). The mixture was poured dropwise, and the reaction was allowed to warm to 20 °C over a 1 h period. The reaction was quenched by the successive addition of EtOAc (10 mL) and brine (5 mL). The mixture was poured on EtOAc (20 mL) and water (10 mL), the organic phase was collected, and the aqueous layer was extracted with EtOAc (20 mL). The combined organic extracts were washed with brine (2 × 10 mL), dried over Na₂SO₄ and concentrated under reduced pressure. Purification of the residue by flash column chromatography on silica gel using hexanes/EtOAc (7:3) gave the sulfamidate 8 (15.0 mg, 13% yield) as a white solid. Mp: 143–145 °C; IR (KBr): 3410, 3282, 1520, 1366, 1182, 1167, 933 cm⁻¹; ¹H NMR (500 MHz, CDCl₃): δ = 5.26 (1H, s, H-14α), 5.03 (1H, s, H-14β), 4.60 (1H, m, H-9), 4.45 (1H, m, H-2), 4.44 (1H, dd, J = 4.0/2.0 Hz, H-10), 3.16 (1H, d, J = 15 Hz, H-1α), 3.04 (1H, t, J = 12.5 Hz, H-8β), 2.82 (1H, dd, J = 15.6/3.2 Hz, H-1β), 2.58 (1H, dd, J = 12.5/2.0 Hz, H-8α), 2.23 (1H, m, H-5β), 2.07 (1H, dt, J = 14.0/2.0 Hz, H-4β), 1.86 (1H, m, H-5α), 1.80 (1H, m, H-4α), 1.34 (3H, s, H-12), 1.10 (3H, s, H-13); ¹³C NMR (126 MHz, CDCl₃): δ = 146.5 (C-7), 115.5 (C-14), 77.9 (C-9), 70.9 (C-3), 66.3 (C-10), 65.2 (C-2), 44.0 (C-6), 36.2 (C-8), 34.1 (C-1), 33.3 (C-5), 33.1 (C-15), 25.7 (C-4), 25.0 (C-13), 24.7 (C-12); ESI-MS m/z 513.9422 [M + Na]⁺ (calcd for C₁₅H₂₆Br₂CINaO₅S, 513.9424), 515.9419 [M + 2 + Na]⁺ (calcd. 515.9403), 517.9378 [M + 4 + Na]⁺ (calcd. 517.9381), 519.9355 [M + 6 + Na]⁺ (calcd. 519.9358), observed isotopic pattern (513/515/517/519): 73/100/28/2, (theoretical: 73/100/29/2).

3.4. Cell Lines

The human embryo rhabdomyosarcoma cells (RD) were obtained from Adolfo Lutz Institute, São Paulo, SP, Brazil. The human non-small cell lung cancer (A549 cells) were kindly provided by Dr. Rosina Gironès from the Microbiology Department of the University of Barcelona, Spain.

3.5. MTT Assay

RD and A549 cells were grown in minimal essential medium (MEM, Cultilab, São Paulo, Brazil). Both cell lines were supplemented with 10% fetal bovine serum, and 100 U/mL penicillin G, 100 μg/mL streptomycin, and 25 μg/mL amphotericin B (Gibco, São Paulo, Brazil). Cell cultures were kept in tissue culture flasks in a humidified atmosphere of 5% CO₂ at 37 °C. The effect of the samples treatment on proliferation of RD and A549 cells was measured by the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay.
5-diphenyl tetrazolium bromide] assay [22]. Approximately 104 cells were plated per well in 96-well plates and treated with different concentrations of each sample. After 48 h at 37 °C, the medium was removed, 50 μL of MTT reagent (1 mg/mL) were added to each well, and cells were further incubated at 37 °C for more 4 h. The MTT solution was removed, 100 μL of dimethyl sulfoxide (Nuclear, Brazil) were added to each well to dissolve formazan crystals, and the plates were gently shaken, whereby crystals were completely dissolved. The absorbances were read on a multiwell spectrophotometer (Tecan, Grödig, Austria) at 540 nm. The 50% cytotoxic concentration (CC 50) of each sample was defined as the concentration that reduced cell viability by 50% when compared to untreated controls. Paclitaxel (0 to 10 μM, Glenmark, Brazil) was used as positive control (purity > 98%).

3.6. Statistical Analysis

The mean ± standard deviations are representative of three independent experiments. For determination of CC 50 values non-linear regressions of concentration-response curves were used.

4. Conclusions

The preparation and characterization of new derivatives of elatol and isoobtusol was described as well as their in vitro inhibitory effects on two human tumor cell lines growth. The obtained results will stimulate the introduction of further structural modifications in these natural products in order to enhance these effects.

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