

Semisynthesis and Cytotoxic Activities of Novel Oxime Ester Derivatives of the
Diterpene Stemodin

José Augusto C. de Oliveira,^a Carlos José Alves Filho,^a Antônio Marcelo A. Lima,^a
Cláudia do Ó Pessoa,^b Fátima de Cássia E. de Oliveira,^b Fátima N. Miranda,^a
Marcos Carlos de Mattos,^{ib} Maria Conceição F. Oliveira,^{ib} Jair Mafezoli^{ib} *^a and
Francisco Geraldo Barbosa^{ib} *^a

^aDepartamento de Química Orgânica e Inorgânica, Centro de Ciências, Universidade Federal do Ceará,
Campus do Pici, 60021-940 Fortaleza-CE, Brazil

^bDepartamento de Fisiologia e Farmacologia, Universidade Federal do Ceará, 60430-270 Fortaleza-CE, Brazil

Stemodin (**1**) is a diterpene produced by *Stemodia maritima* L. (Scrophulariaceae) and shows antiviral and tumor cell proliferation inhibitory activities. Chemical modifications of **1** were performed to produce the known stemodinone (**2**), two new stemodinone oximes, *Z* (**3**) and *E* (**4**), and seven new oxime esters derivatives (**5**, **6**, **7**, **8**, **9**, **10**, and **11**). All derivatives were evaluated for their cytotoxic activity in HL60 (promyelocytic leukemia), SNB-19 (astrocytoma), HCT-116 (colon carcinoma), and PC3 (prostate) human cancer cell lines, and L929 (healthy murine cells). Oximes **3** and **4** showed lower cytotoxic values than **1** against all cancer cell lines tested. However, esters **9** and **10** exhibited cell growth inhibition percentages higher than **1** against PC3, SNB-19, and HCT-116 cancer cell lines, ranging from 62.96 to 94.27%. Many of these values exceed that presented by doxorubicin (66.05-86.87%). All compounds showed no cytotoxic effect on healthy cells at the tested concentration of 25 µg mL⁻¹.

Keywords: *Stemodia maritima*, stemodin, chemical derivatives, cytotoxicity

Introduction

Diterpenes are an important class of phytochemicals found in different natural sources. Several studies¹⁻⁶ have already been made with these substances, confirming their bioactivities. Stemodin (**1**) is a diterpene found in *Stemodia maritima* L., a small shrub found in northeastern Brazil, mainly in the seacoast.⁷ This natural product had its structural elucidation and absolute configuration established using infrared (IR), nuclear magnetic resonance (NMR), and X-ray spectroscopic techniques.⁸⁻¹⁰

Derivatives obtained by chemical and microbial semisynthesis of **1** are reported in the literature.¹¹⁻²⁰ However, studies exploring the biological potential of this compound and its derivatives are still scarce.¹¹⁻²⁰ Some activities of **1** and its derivatives, such as lipid peroxidation, tumor cell proliferation, and cyclooxygenase inhibition, have been evaluated.¹⁵ According to Russell *et al.*,¹⁵

stemodin (**1**) showed antitumor activity against human gastric (AGS) and colon (HCT-116) cancer cell lines, with viability values of 41.9 and 24.4%, respectively. Some of its derivatives showed inhibition values against cyclooxygenase and tumor cell proliferation higher than stemodin (**1**).

Over the past two decades, targeted drugs based on macromolecules and small molecules have been used and investigated for cancer treatment. In this case, macromolecules such as monoclonal antibodies, polypeptides, antibody-drug conjugates, and nucleic acids show high selectivity. However, their targets are often restricted to the cell surface. Small molecules vary in selectivity and can bind to a variety of extracellular and intracellular targets. Despite the challenges faced in discovering targeted anticancer drugs from small molecules, these have advantages such as pharmacokinetic properties, costs, patient compliance, and transport and storage conditions.²¹⁻²³ Thus, studies that can contribute to discovering targeted drugs from small molecules are still needed.

*e-mail: jmafez@ufc.br, fgerhar@gmail.com

Editor handled this article: Hector Henrique F. Koolen (Associate)



Although searching for promising natural products as anticancer agents is encouraged, there are many challenges in identifying and optimizing these molecules as prototype drugs since these compounds may need adequate physicochemical, pharmacokinetic, and pharmacological properties.²⁴ These properties can be optimized through structural modifications by the semisynthesis of these compounds.^{24,25} According to Schepetkin *et al.*,²⁶ a reasonable approach to preparing cytotoxic agents is introducing an oxime group into a suitable chemical structure. Furthermore, acylated oxime derivatives exhibit cytotoxic or antiproliferative activity against many cancer cell lines.²⁶

Once there are few studies regarding the anticancer properties of **1** and the chemical derivatization effect on the biological activity of this natural product, in this study, we report the semisynthesis and the characterization of ten new derivatives of stemodin (**1**) obtained by conventional organic reactions and the evaluation of their cytotoxic activity against four cancer cell lines (HL60 (promyelocytic leukemia), SNB-19 (astrocytoma), HCT-116 (colon carcinoma), and PC3 (prostate) human cancer cell lines) and healthy cell (L929).

Experimental

All solvents were acquired from commercial suppliers and used without previous treatment. Reactions were monitored by thin layer chromatography (TLC), using aluminum plates (Silica Gel 60 ALUGRAM® SIL G/UV254, Macherey-Nagel, Düren, Germany), and visualization by vanillin acid solution in EtOH followed by heating. Flash column chromatography was carried out on silica gel (220-440 mesh; Sigma-Aldrich, Saint Louis, USA) and eluted with hexane/EtOAc in different proportions (Synth, Diadema, Brazil). Infrared (IR) spectra were recorded on a PerkinElmer Spectrum 100 FT-IR spectrometer (Fremont, USA). Melting points (mp) were determined using an MQAPF-302 apparatus (Microquímica, Palhoça, Brazil). Hydrogen (¹H) and carbon (¹³C) NMR spectra were recorded in CDCl₃ solutions (Sigma-Aldrich, with tetramethylsilane (TMS) as the internal standard) on a Bruker 500 MHz spectrometer (Bremen, Germany). Chemical shifts are expressed in ppm (δ). High-resolution electrospray ionization mass spectra (HR-ESI-MS) were obtained on a Thermo Scientific spectrometer (Waltham, USA) in positive and negative modes.

General procedure for extraction and isolation of stemodin (**1**) and synthesis of its derivatives

The aerial parts of *S. maritima* L. were collected in Pentecoste City (Ceará state, Brazil) in October 2020.

Dry-powdered aerial parts (700 g) were extracted with hexane (3 × 500 mL) at room temperature (rt) for 7 days. The solvent was removed under a vacuum, affording 34 g of extract (EHST), which was subsequently dissolved in hexane and submitted to partition with MeCN/MeOH 1:1. The fraction MeCN/MeOH was concentrated, resulting in 18 g of material (EHST-P). EHST-P was subjected to column chromatography with silica gel (220-440 mesh) as the stationary phase using the eluent hexane/EtOAc (3:7). Twenty fractions containing the natural product were gathered. The solvent was removed, providing 900 mg (5.0% yield) of the stemodin (**1**).^{8,9} Mp 192-195 °C (lit. 196-197 °C).⁸

Semisynthesis of stemodinone (**2**)

To an aliquot of 220 mg (0.71 mmol) of **1** dissolved in 3 mL of CH₂Cl₂ were added 2 mL of Jones reagent. The reaction mixture was stirred at room temperature for 1 h. After that, it was quenched with 25 mL of distilled water. The product was extracted with dichloromethane (5 × 5 mL), and the organic phase was dried over anhydrous Na₂SO₄ and concentrated under a vacuum. Stemodinone (**2**)^{8,9} was purified by column chromatography using hexane/EtOAc (1:2) as eluent, affording a white solid (96.3% yield). Mp 215.4-216.7 °C (lit. 215-216 °C).⁸

Semisynthesis of (Z)-stemodinone oxime (**3**) and (E)-stemodinone oxime (**4**) derivatives

In a 50 mL round bottom flask were added 3 mL of CH₂Cl₂, 248.1 mg (1.795 mmol) of K₂CO₃, 82.3 mg (1.183 mmol) of hydroxylamine hydrochloride and 129.6 mg of **2** (0.425 mmol). The reaction mixture was stirred under reflux at 60 °C for 1 h and quenched with 30 mL of distilled water. The product was extracted with dichloromethane (3 × 10 mL), and the organic phase was dried over anhydrous Na₂SO₄, filtered, and concentrated under reduced pressure. Two substances were obtained and purified by column chromatography using hexane/EtOAc (1:2). Both compounds, **3** (54.4 mg, 40.0% yield) and **4** (47.7 mg, 35.1% yield), were obtained as white solids.

(Z)-Stemodinone oxime (**3**)

IR (KBr) ν / cm⁻¹ 3395, 2902, 1694; mp 112.4-113.5 °C; ¹H NMR (500 MHz, CDCl₃) δ 0.89 (H-19, s, 3H), 0.95 (H-20, s, 3H), 1.01 (H-18, s, 3H), 1.13 (H-17, s, 3H), 1.61 (H-1, d, 1H, *J* 12 Hz), 1.96 (H-3, d, 1H, *J* 13 Hz), 2.05 (H-3, d, 1H, *J* 13 Hz), 3.41 (H-1, d, 1H, *J* 12 Hz); ¹³C NMR (125 MHz, CDCl₃) δ 18.28 (C-20), 22.38 (C-6), 23.17 (C-19), 28.31 (C-11), 28.37 (C-17), 30.46 (C-16),

32.92 (C-12), 34.11 (C-18), 34.73 (C-1), 36.42 (C-7), 36.99 (C-4), 37.73 (C-8), 38.37 (C-15), 42.64 (C-10), 46.26 (C-14), 47.03 (C-3), 48.10 (C-5), 50.17 (C-9), 72.68 (C-13), 159.68 (C-2); ESI m/z , calcd. for $C_{20}H_{33}NNaO_2^+ [M + Na]^+$: 342.2409, found: 342.2402.

(E)-Stemodinone oxime (4)

IR (KBr) ν / cm^{-1} 3395, 2902, 1694; mp 189.5–190.6; ^1H NMR (500 MHz, CDCl_3) δ 0.87 (H-19, s, 3H), 0.92 (H-20, s, 3H), 1.04 (H-18, s, 3H), 1.12 (H-17, s, 3H), 1.53 (H-3, m, 1H), 2.0 (H-1, d, 1H, J 12 Hz), 2.31 (H-1, d, 1H, J 12 Hz), 3.12 (H-3, d, 1H, J 13 Hz); ^{13}C NMR (125 MHz, CDCl_3) δ 18.68 (C-20), 22.52 (C-6), 23.82 (C-19), 28.23 (C-11), 28.35 (C-17), 30.38 (C-16), 33.05 (C-12), 34.03 (C-18), 36.47 (C-7), 37.06 (C-8), 37.64 (C-4), 38.29 (C-15), 39.65 (C-3), 42.29 (C-1), 42.48 (C-10), 46.30 (C-14), 48.08 (C-5), 50.24 (C-9), 72.59 (C-13), 159.36 (C-2); HR-ESI-MS m/z , calcd. for $C_{20}H_{33}NNaO_2^+ [M + Na]^+$: 342.2409, found: 342.2402.

General procedure for the semisynthesis of oxime esters (5–11)

In a flask containing **3** or **4** dissolved in 3 mL of dichloromethane was added 4-(dimethylamino)pyridine (DMAP), the acylating agent, and triethylamine in different proportions. The reaction was stirred at room temperature for 1–3 h, and subsequently, the solvent was removed under reduced pressure. The products were purified by flash column chromatography using variable mixtures of hexane/EtOAc as eluent.

(Z)-Stemodinone O-acetyl oxime (5)

Obtained according to the general procedure from 10.7 mg (0.033 mmol) of **3**, 8.0 mg (0.066 mmol) of DMAP, 6.24 μL (0.066 mmol) of acetic anhydride and 9.0 μL (0.066 mmol) of Et_3N . After 1 h of reaction, the product was purified by column chromatography on silica flash, utilizing the EtOAc/hexane (2:1) as eluent. A white solid (10.0 mg, 82.6% yield) was obtained. IR (KBr) ν / cm^{-1} 3461, 2948, 1759, 1629, 1211; mp 172.2–173.8; ^1H NMR (500 MHz, CDCl_3) δ 0.92 (H-19, s, 3H), 0.94 (H-20, s, 3H), 1.06 (H-18, s, 3H), 1.14 (H-17, s, 3H), 1.85 (H-3, d, 1H, J 12 Hz), 2.04 (H-3, d, 1H, J 12 Hz), 2.15 (H-2', s, 3H), 2.32 (H-1, d, 1H, J 12.5 Hz), 3.29 (H-1, d, 1H, J 12.5 Hz); ^{13}C NMR (125 MHz, CDCl_3) δ 18.39 (C-20), 19.84 (C-2'), 22.32 (C-6), 23.15 (C-19), 28.42 (C-11), 28.44 (C-17), 30.52 (C-16), 32.83 (C-12), 34.19 (C-18), 36.29 (C-7), 37.31 (C-8), 37.61 (C-1), 37.67 (C-4), 38.36 (C-15), 43.32 (C-10), 46.29 (C-14), 46.65 (C-3), 48.10 (C-5), 50.14 (C-9), 72.44 (C-13), 167.38 (C-2), 169.12 (C-1'); HR-ESI-MS

m/z , calcd. for $C_{22}H_{35}NNaO_3^+ [M + Na]^+$: 384.2515, found: 384.2505.

(Z)-Stemodinone O-propionyl oxime (6)

Obtained according to the general procedure from 21.00 mg (0.0626 mmol) of **3**, 7.65 mg (0.0626 mmol) of DMAP, 16.33 μL (0.188 mmol) of propionyl chloride, and 26.18 μL (0.188 mmol) of Et_3N . After 2 h of reaction, the product was purified by flash column chromatography utilizing EtOAc/hexane (2:1) as eluent. A viscous liquid material (23.9 mg, 96.8% yield) was obtained. IR (KBr) ν / cm^{-1} 3466, 2938, 1753, 1635, 1146; ^1H NMR (500 MHz, CDCl_3) δ 0.91 (H-19, s, 3H), 0.92 (H-20, s, 3H), 1.05 (H-18, s, 3H), 1.13 (H-17, s, 3H), 1.20 (H-3', t, J 7.5 Hz, 3H), 1.83, (H-3, d, 1H, J 12 Hz), 2.02 (H-3, d, 1H, J 12 Hz), 2.30 (H-1, d, 1H, J 12.5 Hz), 2.42 (H-2', m, 2H), 3.27 (H-1, d, 1H, J 12.5 Hz); ^{13}C NMR (125 MHz, CDCl_3) δ 9.32 (C-3'), 18.35 (C-20), 22.29 (C-6), 23.11 (C-19), 26.68 (C-2'), 28.32 (C-11), 28.41 (C-17), 30.48 (C-16), 32.80 (C-12), 34.16 (C-18), 36.27 (C-7), 37.25 (C-8), 37.64 (C-1), 37.64 (C-4), 38.33 (C-15), 43.32 (C-10), 46.27 (C-14), 46.67 (C-3), 48.07 (C-5), 50.11 (C-9), 72.43 (C-13), 167.43 (C-2), 172.26 (C-1'); ESI m/z , calcd. for $C_{23}H_{37}NNaO_3^+ [M + Na]^+$: 398.2671, found: 398.2660.

(Z)-Stemodinone O-hexanoyl oxime (7)

Obtained according to the general procedure from 20.00 mg (0.0626 mmol) of **3**, 7.65 mg (0.0626 mmol) of DMAP, 26.28 μL (0.188 mmol) of hexanoyl chloride, and 26.18 μL (0.188 mmol) of Et_3N . After 2 h of reaction, the product was purified by flash column chromatography utilizing EtOAc/hexane (2:1) as eluent. A viscous liquid material (18.2 mg, 56.4% yield) was obtained. IR (KBr) ν / cm^{-1} 3468, 2957, 1745, 1632; ^1H NMR (500 MHz, CDCl_3) δ 0.89 (H-6', m, 3H), 1.27 (H-3', m, 2H), 1.33 (H-4', m, 2H), 1.69 (H-5', m, 2H), 1.85 (H-3, d, 1H, J 12 Hz), 2.04 (H-3, d, 1H, J 12 Hz), 2.33 (H-1, d, 1H, J 12.5 Hz), 2.39 (H-2', t, J 7.4 Hz, 2H), 3.29 (H-1, d, 1H, J 12.5 Hz); ^{13}C NMR (125 MHz, CDCl_3) δ 13.98 (C-6'), 18.34 (C-20), 22.31 (C-6), 22.44 (C-5'), 23.15 (C-19), 24.85 (C-4'), 28.36 (C-11), 28.44 (C-17), 30.50 (C-16), 31.47 (C-3'), 32.84 (C-12), 33.33 (C-2'), 34.18 (C-18), 36.29 (C-7), 37.30 (C-8), 37.63 (C-4), 37.66 (C-1), 38.34 (C-15), 43.31 (C-10), 46.29 (C-14), 46.69 (C-3), 48.10 (C-5), 50.13 (C-9), 72.42 (C-13), 167.42 (C-2), 171.58 (C-1'); HR-ESI-MS m/z , calcd. for $C_{26}H_{43}NNaO_3^+ [M + Na]^+$: 440.3141, found: 440.3130.

(E)-Stemodinone O-acetyl oxime (8)

Obtained according to the general procedure from 15.00 mg (0.053 mmol) of **4**, 12.00 mg (0.094 mmol) of DMAP, 8.88 μL (0.094 mmol) acetic anhydride and

12.80 μL (0.094 mmol) of Et_3N . After 2 h of reaction, the product was purified by flash column chromatography utilizing EtOAc/hexane (2:1) as eluent. A white solid (18.5 mg, 96.2% yield) was obtained. IR (KBr) ν / cm^{-1} 3482, 2935, 1755, 1643, 1211; mp 150.6–151.8 $^{\circ}\text{C}$; ^1H NMR (500 MHz, CDCl_3) δ 0.87 (H-19, s, 3H), 0.97 (H-20, s, 3H), 1.06 (H-18, s, 3H), 1.11 (H-17, s, 3H), 1.72 (H-15, d, 1H, J 6 Hz), 1.82 (H-16, m, 2H), 1.84 (H-16, d, 1H, J 11 Hz), 1.94 (H-8, m, 1H), 1.99 (H-5, m, 1H), 2.13 (H-2', s, 3H), 2.54 (H-1, d, 1H, J 13 Hz), 2.99 (H-3, d, 1H, J 13 Hz); ^{13}C NMR (125 MHz, CDCl_3) δ 18.60 (C20), 19.82 (C-2'), 22.40 (C6), 23.63 (C19), 28.03 (C11), 28.34 (C17), 30.30 (C16), 32.98 (C12), 33.90 (C18), 36.25 (C7), 37.46 (C8), 37.77 (C4), 38.20 (C15), 41.91 (C3), 42.04 (C1), 43.02 (C10), 46.18 (C14), 47.85 (C5), 50.35 (C9), 72.33 (C13), 167.34 (C2), 169.24 (C-1'); HR-ESI-MS m/z , calcd. for $\text{C}_{22}\text{H}_{35}\text{NNaO}_3^+$ $[M + \text{Na}]^+$: 384.2515, found: 384.2501.

(E)-Stemodinone O-propionyl oxime (9)

Obtained according to the general procedure from 15.00 mg (0.053 mmol) of **4**, 5.73 mg (0.0469 mmol) of DMAP, 12.12 μL (0.1407 mmol) of propionyl chloride, and 19.59 μL (0.1407 mmol) of Et_3N . After 3 h of reaction, the product was purified by flash column chromatography utilizing EtOAc/hexane (2:1) as eluent. A white solid (15 mg, 85.1% yield) was obtained. IR (KBr) ν / cm^{-1} 3469, 2958, 1750, 1643; mp 123.4–124.6 $^{\circ}\text{C}$; ^1H NMR (500 MHz, CDCl_3) δ 0.86 (H-19, s, 3H), 0.97 (H-20, s, 3H), 1.06 (H-18, s, 3H), 1.11 (H-17, s, 3H), 1.20 (H-3', t, 3H, J 7.6 Hz), 1.84 (H-16, d, 1H, J 11 Hz), 1.94 (H-8, m, 1H), 1.99 (H-5, t, 1H, J 6 Hz), 2.14 (H-1, d, 1H, J 12.5 Hz), 2.42 (H-2', m, 2H), 2.55 (H-1, dd, 1H, J 13 and 1.7 Hz), 2.98 (H-3, dd, 1H, J 13 and 1.7 Hz); ^{13}C NMR (125 MHz, CDCl_3) δ 9.29 (C-3'), 18.61 (C20), 22.41 (C6), 23.63 (C19), 26.66 (C-2'), 28.04 (C11), 28.35 (C17), 30.31 (C16), 33.00 (C12), 33.91 (C18), 36.26 (C7), 37.47 (C8), 37.79 (C4), 38.21 (C15), 41.93 (C3), 42.07 (C1), 43.06 (C10), 46.20 (C14), 47.87 (C5), 50.37 (C9), 72.34 (C13), 167.39 (C2), 172.38 (C-1'); HR-ESI-MS m/z , calcd. for $\text{C}_{23}\text{H}_{37}\text{NNaO}_3^+$ $[M + \text{Na}]^+$: 398.2671, found: 398.2659.

(E)-Stemodinone O-hexanoyl oxime (10)

Obtained according to the general procedure from 20.00 mg (0.0626 mmol) of **4**, 7.65 mg (0.0626 mmol) of DMAP, 26.25 μL (0.1878 mmol) of hexanoyl chloride, and 26.15 μL (0.1878 mmol) of Et_3N . After 2 h of reaction, the product was purified by flash column chromatography utilizing EtOAc/hexane (1:2) as eluent. A white solid (15.7 mg, 60.1% yield) was obtained. IR (KBr) ν / cm^{-1} 3482, 2953, 1760, 1634; mp 95.9–96.7 $^{\circ}\text{C}$; ^1H NMR (500 MHz, CDCl_3) δ 0.86 (H-19, s, 3H), 0.89 (m, 3H), 0.98 (H-20, s, 3H), 1.07 (H-18, s, 3H), 1.12 (H-17, s, 3H),

1.33 (m, 3H), 1.69 (m, 3H), 1.84 (H-16, d, 1H, J 11 Hz), 1.94 (H-8, m, 1H), 1.99 (H-5, t, 1H, J 6 Hz), 2.14 (H-1, d, 1H, J 12.5 Hz), 2.38 (t, J 7.5 Hz, 2H), 2.55 (H-1, dd, 1H, J 13 and 1.7 Hz), 2.98 (H-3, dd, 1H, J 13 Hz and 1.7 Hz); ^{13}C NMR (125 MHz, CDCl_3) δ 13.99 (C-6'), 18.63 (C20), 22.43 (C6), 23.66 (C19), 24.83 (C-4', C-5'), 28.07 (C11), 28.38 (C17), 30.35 (C16), 31.47 (C-3'), 33.05 (C12), 33.30 (C-2'), 33.94 (C18), 36.29 (C7), 37.51 (C8), 37.78 (C4), 38.24 (C15), 42.01 (C1), 42.10 (C3), 43.07 (C10), 46.24 (C14), 47.91 (C5), 50.40 (C9), 72.34 (C13), 167.39 (C2), 171.69 (C-1'); HR-ESI-MS m/z , calcd. for $\text{C}_{26}\text{H}_{43}\text{NNaO}_3^+$ $[M + \text{Na}]^+$: 440.3141, found: 440.3124.

(E)-Stemodinone O-decanoyl oxime (11)

Obtained according to the general procedure from 12.70 mg (0.0398 mmol) of **4**, 9.72 mg (0.0796 mmol) of DMAP, 16.52 μL (0.076 mmol) of decanoyl chloride, and 16.73 μL (0.1194 mmol) of Et_3N . After 2 h of reaction, the product was purified by flash column chromatography utilizing EtOAc/hexane (2:1) as eluent. A viscous liquid material (17.5 mg, 92.9% yield) was obtained. IR (KBr) ν / cm^{-1} 3436, 2930, 1750, 1639; ^1H NMR (500 MHz, CDCl_3) δ 0.86 (H-19, s, 3H), 0.88 (H-10', m, 3H), 0.92 (H-3', s, 1H), 0.97 (H-20, s, 3H), 1.06 (H-18, s, 3H), 1.12 (H-17, s, 3H), 1.25 (H-9'-H-4', m, 12H), 1.84 (H-16, d, 1H, J 11 Hz), 1.94 (H-8, m, 1H), 1.99 (H-5, t, 1H, J 6 Hz), 2.14 (H-1, d, 1H, J 12.5 Hz), 2.33 (H-3', m, 1H), 2.38 (H-2', m, 2H), 2.55 (H-1, dd, 1H, J 13 and 1.7 Hz), 2.98 (H-3, dd, 1H, J 13 and 1.7 Hz); ^{13}C NMR (125 MHz, CDCl_3) δ 14.19 (C-10'), 18.62 (C20), 22.43 (C6), 22.78 (C-9'), 23.67 (C19), 24.96 (C-8'), 25.15 (C-7'), 28.06 (C11), 28.35 (C17), 29.31 (C-6'), 29.38 (C-5'), 29.55 (C-4'), 30.34 (C16), 31.99 (C-3'), 33.02 (C12), 33.34 (C18), 33.93 (C-2'), 36.28 (C7), 37.50 (C8), 37.78 (C4), 38.23 (C15), 42.00 (C1), 42.09 (C3), 43.06 (C10), 46.23 (C14), 47.90 (C5), 50.30 (C9), 72.38 (C13), 167.39 (C2), 171.70 (C-1'); HR-ESI-MS m/z , calcd. for $\text{C}_{30}\text{H}_{51}\text{NNaO}_3^+$ $[M + \text{Na}]^+$: 496.3767, found: 496.3757.

Cell culture and treatment

Human cancer cell lines promyelocytic leukemia (HL60), astrocytoma (SNB-19), colon carcinoma (HCT-116), and prostate (PC3), provided by the National Cancer Institute in the United States (Frederick, USA), were cultured in RPMI-1640 Medium (Thermo Fisher Scientific, Paisley, UK). Non-tumor cell line L929 (murine fibroblast) obtained from Cell Bank of Rio de Janeiro (Rio de Janeiro, Brazil) was cultured in Dulbecco's Modified Eagle Medium (DMEM, Thermo Fisher Scientific, Paisley, UK). Both mediums were supplemented with 10% fetal calf serum at 37 $^{\circ}\text{C}$ in a 5% CO_2 atmosphere.

In vitro cell viability assay-MTT assay

Cells were plated in 96-well plates (HL60 0.3×10^6 cells mL^{-1} , PC3 and SNB-19 0.1×10^6 cells mL^{-1} , HCT-116 0.7×10^5 cells mL^{-1} *per well*) and incubated for 24 h before addition of the test substances. Firstly, the cells were exposed to derivatives dissolved in DMSO at a single concentration of $25 \mu\text{g mL}^{-1}$ in quadruplicate for 72 h at 37°C in an atmosphere of 5% CO_2 . Subsequently, derivatives that inhibited $\geq 70\%$ were used to calculate the inhibitory concentrations of 50% (IC_{50}) in the tested lines cell. Besides, it evaluated the cytotoxicity of the substances toward a non-tumor cell line L929 (mouse fibroblast) after 72 h exposure. Doxorubicin was used as a positive control ($8.62 \mu\text{g mL}^{-1}$). The control group was subject to the same amount of dimethyl sulfoxide (DMSO). After the time exposition, wells were centrifuged, and the supernatant was replaced by 100 μL of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, Sigma-Aldrich, Saint Louis, USA) solution ($0.5 \mu\text{g mL}^{-1}$). After 3 h of incubation, the quantity of formazan produced was dissolved in 100 μL of pure DMSO, and absorbance was measured by a spectrophotometer at 595 nm (DTX 880 Multi mode Detector, PerkinElmer, Pontyclun, UK). The inhibition percentage was calculated, along the media $\pm$ confidence interval (CI), using the GraphPad Prism program (version 8.0).²⁷

Results and Discussion

In this work, ten derivatives of **1** were produced by chemical transformation (Figure 1). Products **3** and **4** were prepared following a procedure described by Kim *et al.*,²⁸

while products **5-11** were obtained following a procedure described by Almeida *et al.*²⁹

Stemodinone **2**, already reported in literature,⁸ was obtained from the oxidation of **1**. The oximation reaction of **2** produced two diastereoisomeric oximes, **3** (40.0%) and **4** (35.1%), with *Z* and *E* chemical configurations, respectively. Each diastereoisomer was subject to acylation with different acid chlorides to obtain three ester derivatives with *Z* configuration (**5-7**) and four ester derivatives with *E* configuration (**8-11**). To obtain these derivatives, the reaction was performed utilizing a slight excess of acid chloride and ambient temperature (25°C) to perform a selective acylation in the oxime group and avoid acylation in the tertiary hydroxyl located in C-13 present in compounds **3** and **4**.

The chemical structures of derivatives **3** and **4** were confirmed by the absence of the ketone carbonyl carbon signal at 212 ppm, located in C-2 of the stemodinone (**2**), and by the appearance of signals close to 159 ppm (see Figures S2 and S21, in Supplementary Information (SI) section) attributed to the $\text{N}=\text{C}$ groups of oximes. In addition, variations observed in the chemical shifts of carbons and hydrogens neighboring C-2 of the oxidized derivative (**2**) were utilized to attribute the correct stereochemistry of these two oximes produced. Magnetic shielding promoted by the oxime hydroxyl group acts on α -carbons according to its relative positions. When an α -carbon is in *syn* position to the oxime hydroxyl, a diamagnetic shift of approximately 14 ppm is observed, whereas the α -carbon in the *anti*-position undergoes presents shift values around 9 ppm.³⁰

Regarding the esterified derivatives **5-11** obtained from **3** and **4**, we can confirm the chemical transformations by analyses of the ^{13}C NMR spectra. The presence of ester

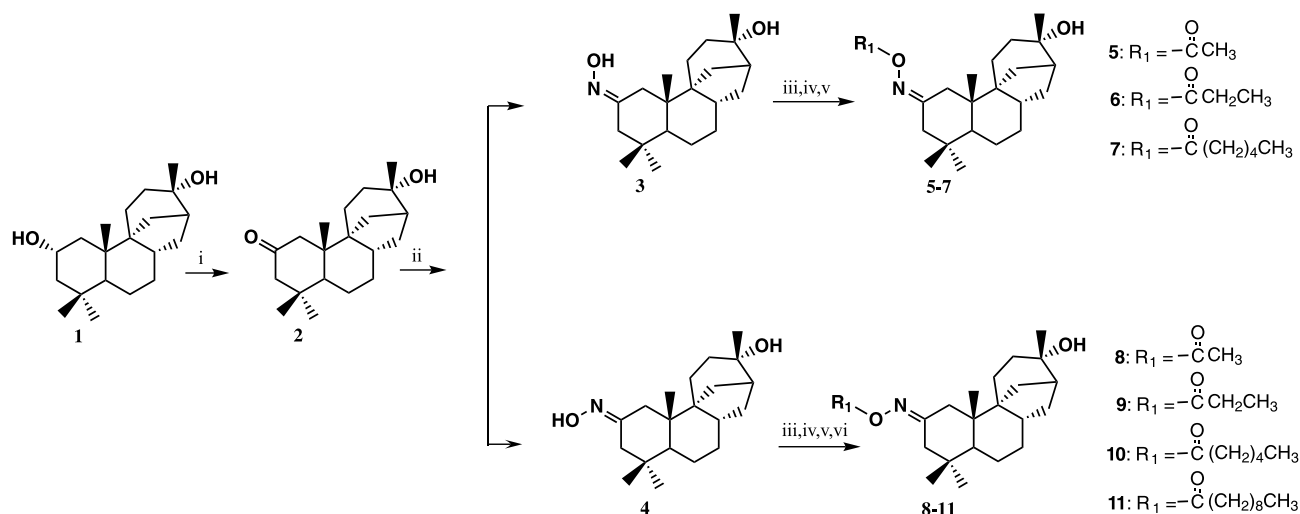


Figure 1. Scheme of semisynthesis of stemodin (**1**) derivatives. Reagents and conditions: (i) chromic acid (Jones reagent), CH_2Cl_2 , r.t., 2 h; (ii) hydroxylamine hydrochloride ($\text{NH}_2\text{OH}\cdot\text{HCl}$), K_2CO_3 , MeOH, reflux, 1 h; (iii) acetic anhydride, Et_3N , DMAP, CH_2Cl_2 , r.t., 1 h for **5** and 2 h for **8**; (iv) propionyl chloride, Et_3N , DMAP, CH_2Cl_2 , r.t., 2 h for **6** and 3 h for **9**; (v) hexanoyl chloride, CH_2Cl_2 , r.t., 2 h; (vi) decanoyl chloride, Et_3N , DMAP, CH_2Cl_2 , r.t., 2 h.

carbonyl carbon was confirmed by the signals around 170 ppm, and the carbon chain by the appearance of methyl and methylene signals which were not present in the begging materials (Figure S6, S11, SI section). In the IR spectra for the compounds, absorptions related to the alcohol hydroxyl group, C=N (oxime group), and C=O (ester carbonyl) were observed. High-resolution mass analysis showed peaks that supported the structure of the compounds (see SI section).

A screening of the cytotoxic potential of stemodin (**1**) and derivatives was carried out using a single concentration of 25 $\mu\text{g mL}^{-1}$, considering the number of cancer cells surviving after 24 h of incubation to the lines PC3, SNB-19, HCT-116, and HL60. The results were expressed as a percentage of inhibition (Table 1). Stemodin (**1**) showed an inhibition percentage against PC3 (87.82%), HCT-116 (87.35%), and HL60 (92.18%), demonstrating the anticancer potential of this natural product.

All semisynthetic compounds present promising antiproliferative activity ($\geq 50\%$ of cell inhibition) against prostate (PC3) and promyelocytic leukemia (HL60). Against the astrocytoma human cancer cell line (SNB-19), only compounds **1**, **9**, and **10** presented values of cell inhibition more significantly than 50%. To the colon carcinoma human cancer cell line (HCT-116), only compound **6** did not show activity above 50%.

The oximes **3** and **4** showed cell inhibition percentages lower than $\geq 50\%$ only to SNB-19, with values of 41.40 and 45.68%, respectively. Both oximes exhibited close values of cytotoxic activity for the tested cells, except for HL60. For this cancer cell line, oximes **3** and **4** showed inhibition of 67.15 and 85.12%, respectively. In addition, the values of antiproliferative activities presented for these two substances were lower than that presented by **1** against all cancer cell lines tested, showing that the presence of the hydroxyl group at C-2 is important for biological activity maintenance.

Among oxime **3** (*Z*) derivatives, compound **5** showed an inhibition level (89.19%) higher than **1** (87.35%) against HCT-116. Similar behavior was observed against HL60 cells for derivatives **5** (90.95%) and **7** (90.06%). Oxime **4** (*E*) derivatives also presented promising antiproliferative activity values. Compounds **9** (87.48%), **10** (92.05%), and **11** (85.01%) showed cell growth inhibition against PC3 tumor cell lines similar to **1**. Against SNB-19 cells, the compounds **9** (62.96%) and **10** (72.22%) presented activity superior to **1**. Derivatives **9** and **10** were very selective for HCT-116 tumor cell lines, giving inhibition values of 93.97 and 94.27%, respectively. For HL60 tumor cell lines, compounds **8** (93.12%), **9** (92.52%), and **10** (91.96%) also exhibited high antiproliferative activities, but these values are very similar to those presented by **1** (92.18%).

Analyzing the values presented by esters derivatives of oximes **3** and **4**, some patterns can be observed when compounds that only differ in the stereochemistry and have the same added carbon chain. The **4** (*E*) ester derivatives showed greater antiproliferative activity compared to the **3** (*Z*) esters against PC3, SNB-19, and HL60, with differences in values varying mainly in SNB-19, which ranged from 23.02 to 46.39% for derivatives of compound **3** and 39.49 to 72.22% for derivatives of compound **4**, suggesting better cytotoxicity for the *E* stereochemistry. For HCT-116, only propanoyl derivatives showed inversion of values, with **5** (*Z*, 89.19%) and **8** (*E*, 79.99%).

Regarding *Z* ester oxime derivatives (**8**, **9**, **10**, and **11**) with different carbon chain lengths, a direct proportionality between the size of the carbon chain added, and cell growth inhibition activity was observed until six carbons (**8**, **9**, and **10**) in all cancer cell lines. The increase in lipophilicity until six carbons in the side chain is supposed to play an important role in the cytotoxic activities. With the increase

Table 1. Evaluation of cytotoxicity forward four human cancer cell lines (percentage of inhibition at 25 $\mu\text{g mL}^{-1}$) for compounds **1** and **3-11**, using MTT assay. Doxorubicin (DOX) positive control at 8.62 $\mu\text{g mL}^{-1}$

Compound	Inhibition ($\pm$ standard deviation) / %			
	PC3	SNB-19	HCT-116	HL60
1	87.82 $\pm$ 1.30	52.48 $\pm$ 11.77	87.35 $\pm$ 0.19	92.18 $\pm$ 0.16
3	77.15 $\pm$ 1.74	41.40 $\pm$ 2.00	83.84 $\pm$ 3.60	67.15 $\pm$ 3.05
5	73.07 $\pm$ 1.17	46.39 $\pm$ 1.47	89.19 $\pm$ 0.53	90.65 $\pm$ 1.61
6	64.66 $\pm$ 2.30	23.02 $\pm$ 6.17	37.32 $\pm$ 1.27	55.62 $\pm$ 4.10
7	72.59 $\pm$ 1.10	32.87 $\pm$ 4.59	83.11 $\pm$ 4.54	90.06 $\pm$ 2.94
4	79.13 $\pm$ 1.17	45.68 $\pm$ 1.53	78.61 $\pm$ 1.04	85.12 $\pm$ 0.40
8	74.90 $\pm$ 0.64	48.66 $\pm$ 3.70	79.99 $\pm$ 1.10	93.12 $\pm$ 0.10
9	87.48 $\pm$ 0.50	62.96 $\pm$ 1.61	93.97 $\pm$ 0.11	92.53 $\pm$ 0.50
10	92.05 $\pm$ 0.43	72.22 $\pm$ 5.43	94.27 $\pm$ 0.28	91.96 $\pm$ 1.02
11	85.01 $\pm$ 1.00	39.49 $\pm$ 3.40	62.08 $\pm$ 3.53	84.56 $\pm$ 4.36
DOX	78.93 $\pm$ 1.35	66.05 $\pm$ 1.07	82.76 $\pm$ 0.28	86.87 $\pm$ 1.94

HL60: promyelocytic leukemia; SNB-19: astrocytoma, HCT-116: colon carcinoma, human; PC3: prostate.

of the side chain to ten carbons (**11**), a decrease in the percentage of inhibition was observed.

For *E* ester oxime derivatives, the same correlations were not observed. With the increase in the side chain from two to three carbons in derivatives **5** and **6**, a decrease in the inhibitory activity in all tumor cells was observed. However, as the side chain was increased to six carbon atoms (**7**), an increase in cell growth inhibition activity was again observed.

Although the derivatives tested at a single concentration (25 µg mL⁻¹) showed selectivity in inhibiting tumor cells, the IC₅₀ values were relatively high compared to the standard compound (doxorubicin). IC₅₀ values were determined for the compounds with the highest tumor cell inhibitory activity (**1**, **5**, **7**, **4**, **8**, **9**, and **10**). These results are presented in Table 2. For PC3 tumor cells, the acylated derivatives of the oxime with stereochemistry *E*, **8** (IC₅₀ = 60.52 µM), **9** (IC₅₀ = 62.39 µM), and **10** (IC₅₀ = 54.52 µM) showed a decrease in IC₅₀ values compared to oxime **4** (IC₅₀ = 75.39 µM). Furthermore, only derivative **10** (IC₅₀ = 59.72 µM) showed some cytotoxicity against SNB-19 tumor cells.

Concerning the HCT-116 tumor cells, the IC₅₀ values of the derivatives revealed an increase in cytotoxicity compared to **1** (IC₅₀ = 51.35 µM). In this case, **5** (IC₅₀ = 50.65 µM) and **6** (IC₅₀ = 50.02 µM) showed a small decrease in IC₅₀, while **4** (IC₅₀ = 35.96 µM), **8** (IC₅₀ = 37.56 µM), **9** (IC₅₀ = 43.64 µM), and **10** (IC₅₀ = 41.85 µM) showed more significant reductions in IC₅₀ values. For HL60 tumor cells, all tested derivatives (**5**, **7**, **4**, **8**, **9**, and **10**) presented IC₅₀ values lower than **1** (IC₅₀ = 57.48 µM). Among them, derivative **10** showed the lowest IC₅₀ (30.17 µM). It is worth highlighting that the IC₅₀ values > 59.86–81.56 µM presented by **1** and derivatives **5**, **7**, **4**, **8**, **9**, and **10** for the healthy cell lines (L929) showed good selectivity in comparison with the standard compound doxorubicin (IC₅₀ = 1.72 µM).

Conclusions

Of the 10 derivatives obtained by chemical transformation of the diterpene stemodin (**1**), nine of them (**3–11**) are being described for the first time in the literature. These derivatives were evaluated for their anticancer activity against four human cancer cell lines (PC3, HCT-116, HL60, and SNB-19) and a healthy murine fibroblast cell line (L929). The natural product stemodin (**1**) showed antiproliferative activities against these cancer cell lines. The chemical modifications (introduction of oxime group and acylation) promoted the selectivity as a function of the stereochemistry (*E*) and of the increase of the side chain up to six carbons. Compound **10** was found to have the best antitumor activity. The IC₅₀ assay results showed reduced activity towards doxorubicin. However, no cytotoxicity in healthy cells was observed for the tested derivatives. These results support the use of **1** as an interesting prototype in the discovery and development of new drugs through novel structural modifications and *in silico* studies, including molecular docking tools.

Supplementary Information

Supplementary information (Figures S1-S45) is available free of charge at <http://jbcs.sbq.org.br> as a PDF file.

Acknowledgments

The authors thank the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for financial support (Finance Code 001-PROEX 23038.000509/2020-82.AUXPE No. 1227/2020), the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for research grant to M. C. F. de Oliveira (process: 310881/2020-0) and M. C. de Mattos (process: 306289/2021-0), the Fundação Cearense

Table 2. IC₅₀ values of compounds **1**, **5**, **7**, **4**, **8**, **9**, and **10** in tumor and non-tumor cell lines

Cell lineage	IC ₅₀ (confidence interval) / µM							Dox
	1	5	7	4	8	9	10	
PC3	67.63 (54.52-83.91)	63.92 (44.92-90.98)	68.82 (51.34-92.00)	75.39 (61.09-93.02)	60.52 (45.47-80.58)	62.39 (49.74-78.24)	54.52 (36.37-81.68)	0.76 (0.59-0.93)
SNB-19	> 81.56	> 69.15	> 59.86	> 78.24	> 69.15	> 66.57	59.72 (49.64-71.88)	0.5 (0.42-0.68)
HCT-116	51.35 (40.03-65.90)	50.65 (33.55-76.40)	50.02 (39.27-63.72)	35.96 (26.91-47.94)	37.56 (23.98-58.83)	43.64 (32.30-58.98)	41.85 (28.52-61.47)	0.21 (0.16-0.29)
HL60	57.48 (51.45-64.24)	47.30 (37.78-59.19)	37.83 (31.29-45.52)	44.88 (37.40-53.86)	40.30 (32.44-50.01)	34.56 (27.35-43.70)	30.17 (25.71-35.44)	0.02 (0.01-0.02)
L929	> 81.56	> 69.15	> 59.86	> 78.24	> 69.15	> 66.57	> 59.86	1.72 (1.58-1.87)

IC₅₀: 50% inhibitory concentration; HL60: promyelocytic leukemia; SNB-19: astrocytoma, HCT-116: colon carcinoma; PC3: prostate; L929: cell murine fibroblast, not tumoral cell; Dox: doxorubicin.

de Apoio ao Desenvolvimento Científico e Tecnológico (FUNCAP) for the academic research sponsorship to J. A. C. de Oliveira (process: BMD-0008-00070.01.73/19), J. C. A. Filho (process: BMD-0008-00070.01.77/20) and A. M. A. Lima (process: BMD-0008-01997.01.18/22). The authors also thank Prof Manuel Andrade Neto (UFC) for collecting the botanic material.

Author Contributions

José Augusto C. de Oliveira was responsible for investigation, data curation, writing original draft; Carlos José A. Filho for investigation, data curation, writing original draft; Antônio Marcelo Alves Lima for investigation, data curation; Fátima de Cássia E. de Oliveira for investigation, data curation; Cláudia do Ó Pessoa for supervision, data curation, writing-review and editing; Fátima M. Nunes for supervision, data curation, writing-review and editing; Marcos C. Mattos for supervision, data curation, writing-review and editing; Maria C. F. Oliveira for supervision, data curation, writing-review and editing; Jair Mafezoli for conceptualization, project administration, funding acquisition, writing-review and editing; Francisco G. Barbosa for conceptualization, project administration, funding acquisition, writing-review and editing.

References

1. Ogawa, K.; Nakamura, S.; Hosokawa, K.; Ishimaru, H.; Saito, N.; Ryu, K.; *J. Nat. Med.* **2018**, *72*, 439. [Crossref]
2. Abreu, L. S.; do Nascimento, Y. M.; Costa, R. D. S.; Guedes, M. L. S.; Souza, B. N. R. F.; Pena, L. J.; Costa, L. J.; Costa, V. C. O.; Scotti, M. T.; Braz-Filho, R.; Barbosa-Filho, J. M.; da Silva, M. S.; Velozo, E. S.; Tavares, J. F.; *J. Nat. Prod.* **2019**, *82*, 272. [Crossref]
3. Zhao, L.; Xiang, K. L.; Liu, R. X.; Xie, Z. P.; Zhang, S. M.; Dai, S. J.; *Bioorg. Chem.* **2020**, *96*, 103651. [Crossref]
4. Gu, C. Z.; Xia, X. M.; Lv, J.; Tan, J. W.; Baerson, S. R.; Pan, Z. Q.; Song, Y. Y.; Zeng, R. S.; *Phytotherapy* **2019**, *136*, 104183. [Crossref]
5. Tani, K.; Kamada, T.; Phan, C. S.; Vairappan, C. S. *Nat. Prod. Res.* **2019**, *33*, 3343. [Crossref]
6. Li, Q. J.; Zhao, C. L.; Ku, C. F.; Zhu, Y.; Zhu, X. J.; Zhang, J. J.; Deyrup, S. T.; Pan, L. T.; Zhang, H. J.; *Bioorg. Chem.* **2020**, *95*, 103512. [Crossref]
7. Teixeira, A. H.; Freire, J. M. D. O.; de Sousa, L. H. T.; Parente, A. T.; de Sousa, N. A.; Arriaga, A. M. C.; Lopes da Silva, F. R.; Melo, I. M.; Castro da Silva, I. I.; Pereira, K. M. A.; Goes, P.; Costa, J. J. D. N.; Cristino-Filho, G.; Pinto, V. D. P. T.; Chaves, H. V.; Bezerra, M. M.; *Front. Physiol.* **2017**, *8*, 988. [Crossref]
8. Manchand, P. S.; White, J. D.; Wright, H.; Clardy, J.; *J. Am. Chem. Soc.* **1973**, *95*, 2705. [Crossref]
9. Hufford, C. D.; *J. Nat. Prod.* **1988**, *51*, 367. [Crossref]
10. Rodrigues, F. E. A.; Lima, J. Q.; de Oliveira, M. C. F.; Vasconcelos, J. N.; Santiago, G. M. P.; Mafezoli, J.; Braz-Filho, R.; Arriaga, A. M. C.; *J. Braz. Chem. Soc.* **2010**, *21*, 1581. [Crossref]
11. Leonelli, F.; Migneco, L. M.; Valletta, A.; Bettolo, R. M.; *Molecules* **2021**, *26*, 2761. [Crossref]
12. Buchanan, G. O.; Reese, P. B.; *Phytochemistry* **2001**, *56*, 141. [Crossref]
13. Martin, G. D. A.; Reynolds, W. F.; Reese, P. B.; *Phytochemistry* **2004**, *65*, 701. [Crossref]
14. Martin, G. D. A.; Reynolds, W. F.; Reese, P. B.; *Phytochemistry* **2005**, *66*, 901. [Crossref]
15. Russell, F. A.; Mulabagal, V.; Thompson, D. R.; Singh-Wilmot, M. A.; Reynolds, W. F.; Nair, M. G.; Langer, V.; Reese, P. B.; *Phytochemistry* **2011**, *72*, 2361. [Crossref]
16. Chen, A. R.; Ruddock, P. L.; Lamm, A. S.; Reynolds, W. F.; Reese, P. B.; *Phytochemistry* **2005**, *66*, 1898. [Crossref]
17. Hanson, J.; Reese, P. B.; Takahashi, J. A.; Wilson, M. R.; *Phytochemistry* **1994**, *36*, 1391. [Crossref]
18. Lamm, A. S.; Reynolds, W. F.; Reese, P. B.; *Phytochemistry* **2006**, *66*, 1088. [Crossref]
19. Hufford, C. D.; Badria, F. A.; Abou-Karam, M.; Shier, W. T.; Rogers, R. D.; *J. Nat. Prod.* **1991**, *54*, 1543. [Crossref]
20. Badria, F. A.; Hufford, C. D.; *Phytochemistry* **1991**, *30*, 2265. [Crossref]
21. Bedard, P. L.; Hyman, D. M.; Davids, M. S.; Siu, L. L.; *Lancet* **2020**, *395*, 1078. [Crossref]
22. Zhong, L.; Li, Y.; Xiong, L.; Wang, W.; Wu, M.; Yuan, T.; Yang, W.; Tian, C.; Miao, Z.; Wang, T.; Yang, S.; *Signal Transduction Targeted Ther.* **2021**, *6*, 201. [Crossref]
23. Newman, D. J.; Cragg, G. M.; *Nat. Prod.* **2020**, *83*, 770. [Crossref]
24. Majhi, S.; Das, D.; *Tetrahedron* **2021**, *78*, 131801. [Crossref]
25. Barnes, E. C.; Kumar, R.; Davis, R. A.; *Nat. Prod. Rep.* **2016**, *33*, 372. [Crossref]
26. Schepetkin, I. A.; Plotnikov, M. B.; Khlebnikov, A. I.; Plotnikova, T. M.; Quinn, M. T.; *Biomolecules* **2021**, *11*, 777. [Crossref]
27. Motulsky, H.; *Software Prism*, version 8.0; GraphPad Software, USA, 1989.
28. Kim, B. R.; Sung, G. H.; Kim, J.; Yoon, Y.; *J. Korean Chem. Soc.* **2013**, *57*, 295. [Crossref]
29. de Almeida, D. K. C.; da Silva, M. R.; de Oliveira, M. C. F.; Mafezoli, J.; de Mattos, M. C.; Moura, A. F.; Moraes Filho, M. O.; Barbosa, F. G.; *Quim. Nova* **2017**, *40*, 1186. [Crossref]
30. Vágvolgyi, M.; Martins, A.; Kulmány, Á.; Zupkó, I.; Gáti, T.; Simon, A.; Tóth, G.; Hunyadi, A.; *Euro. J. Med. Chem.* **2018**, *144*, 730. [Crossref]

Submitted: August 1, 2023

Published online: November 30, 2023