Preparation, Spectral Characterization and Anticancer Potential of Cinnamic Esters

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Cinnamic acid and its derivatives show a remarkable variety of biological activities and are often studied in search of the development of new and highly effective drugs. This work aims to synthesize, characterize and evaluate the cytotoxic activity of esters derived from cinnamic acid. Eighteen esters were synthesized through Steglich’s esterification, of which eleven were not reported in the literature. All compounds were fully characterized by Fourier transform infrared spectroscopy (FTIR), nuclear magnetic resonance (1H and 13C NMR) and high-resolution mass spectrometry (HRMS) data. The cytotoxic activity of esters obtained was evaluated using four human tumor cell lines: SNB-19 (astrocytoma), HCT-116 (colon carcinoma, human), PC3 (prostate) and HL60 (promyelocytic leukemia) through the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium (MTT) colorimetric assay. These studies showed that the compound 3-methoxybenzyl (E)-3-(4-methoxyphenyl)acrylate (12) is the most potent against HCT-116, PC3 and SNB-19 cells, with the lowest half maximal inhibitory concentration (IC50) value of 16.2 μM in the HCT-116 strain. The derivatives were obtained in good yields (76.6-95%), except for compounds 5-isopropyl-2-methylphenyl (E)-3-(3-hydroxy-4-methoxyphenyl)acrylate (17) (18.6%) and 2-isopropyl-5-methylphenyl (E)-3-(3-hydroxy-4-methoxyphenyl)acrylate (18) (15.5%).

Keywords: cinnamic esters, cytotoxicity, spectral data, Steglich esterification

Introduction

Among the countless diseases that affect humanity, cancer is that one that affects millions of people, being the second cause of death worldwide, with an estimated number of 9.6 million in the year of 2018. Treatment of the disease includes chemotherapy, which uses drugs that destroy cancer cells. Several chemotherapeutic agents are used such as doxorubicin, epirubicin and cyclophosphamide, among many others. However, these chemical agents often cause serious side effects. In this context, numerous researches around the world are related to the development of new drugs to fighting cancer, many of them related to works involving derivatives of natural products. In addition, cinnamic acid and similar such as acids caffeic and ferulic, are important nutrients present in human food. Several food-stuffs (coffee, chocolate, almonds, among others) that are part of the diet of many people are potentially rich of this type of constituents.

There are reports in the literature on the cytotoxic activity of cinnamic acid (1a) and some of its analogs: acid p-methoxy cinnamic (2a), ferulic acid (3a), isoferulic acid (4a), p-hydroxycinnamic acid (5a) and caffeic acid (6a), Figure 1, against some cancer cell lines: MCF-7 (breast carcinoma), PC3 (prostate) and SW480 (human colon). It is worth mentioning that 1a has attracted the attention of researchers for a long time, due to its anti-cancer properties. Research has shown that synthetic derivatives of phenylpropanoid acids have several biological activities:
Preparation, Spectral Characterization and Anticancer Potential of Cinnamic Esters

J. Braz. Chem. Soc.

1932

Figure 1. Compounds belonging to the family of phenylpropanoid acids.

Figure 2. Reaction mechanism of Steglich esterification.

carbon to attack the hydroxyl group of alcohol. After deprotonation, precipitation of dicyclohexylurea (DCU) occurs with formation of the ester (Figure 2). 19

In order to evaluate the anticancer activity of cinnamates, the present work describes the synthesis via esterification of Steglich and characterization of esters of cinnamic acid, p-methoxycinnamic acid and ferulic acid. The cytotoxic activity of esters (1-18) was assessed using four human tumor cell lines: SNB-19 (astrocytoma), HCT-116 (colon carcinoma, human), PC3 (prostate) and HL60 (promyelocytic leukemia), in addition to of a healthy L929 cell (murine fibroblast).

Experimental

Chemistry

The reagents acetic anhydride, triethylamine and dihydrocarvenol were obtained from Vetec (Caxias do Sul, Brazil). Additionally, potassium bromide (KBr), deuterochloroform (CDCl3), doxorubicin hydrochloride, 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT), cinnamic acid, 4-methoxy-cinnamic acid, 3-methoxy-4-hydroxy-cinnamic acid, (S)-(−)-perillyl alcohol, carvacrol, thymol, 5-indanol, 6-hydroxy-1H-isocromen-1-one, vanillin, N,N′-dicyclohexylcarbodiimide (DCC) and 4-(N,N′-dimethylamino)pyridine (DMAP), were obtained from Sigma-Aldrich Corporation (Saint Louis, USA). The solvents dichloromethane, dimethyl sulfoxide (DMSO), hexane and ethyl acetate, were obtained from Synth (Diadema, Brazil). Nuclear magnetic resonance
(NMR) spectra were recorded on a Bruker Avance DPX-300 NMR spectrometer (300 MHz for $^1$H and 75 MHz for $^{13}$C) (Washington, USA) using CDCl$_3$ solutions, and all chemical shifts reported in ppm ($\delta$ units) with residual CHCl$_3$ ($\delta$ 7.27) as internal standard for $^1$H NMR and the central peak of the triplet ($\delta$ 7.23) of CDCl$_3$ for $^{13}$C NMR. Infrared (IR) spectra were taken as KBr pellets, on PerkinElmer spectrophotometer, model FTIR SPECTRUM (Ontario, Canada). High-resolution mass spectrometry (HRMS) were obtained on XEVO TQ-D triple quadrupole mass spectrometer coupled to a MassLynxTM software (Santa Clara, USA); samples were introduced into the system by direct infusion, being ionized by electrospray operating in positive ion mode [ESI(+)]. Flash chromatography columns were performed using silica gel 60 (0.040-0.063 mm) purchased from Merck (Darmstadt, Germany), adapted to the pressure system with an Omron NE-C 801 compressor (São Paulo, Brazil); reactions were monitored by analytical thin layer chromatography (TLC) utilizing aluminium silica gel 60 F254 precoated 0.25 nm plates, from the same manufacturer, with visualization under UV light (254 nm). Melting points were determined in Mettler Toledo digital micro determination equipment and are uncorrected (Ohio, USA). All yields reported refer to isolated yields.

General procedure for the synthesis of compounds 1-8

In separate experiments, cinnamic acid (1a) (60.0 mg, 0.44 mmol, 1 equiv) in dichloromethane solution (5 mL) under stirring was mixed with N,N'-dicyclohexylcarbodiimide (DCC) (90.7 mg, 0.44 mmol, 1.1 equiv) and 4-(N,N'-dimethylamino)pyridine (DMAP) (48.80 mg, 0.44 mmol, 1 equiv). In each mixture, the corresponding alcohol/phenol (0.44, 1.1 equiv) was added, followed by stirring of the resulting solutions at room temperature for 24 h (Scheme 1). At the end of each reaction, the solutions were filtered and concentrated under reduced pressure. The crude products were purified on silica gel chromatographic columns eluted with 9:1 hexane/EtOAc.

General procedure for the synthesis of compounds 9-16

In separate experiments, p-methoxycinnamic acid (2a) (60.0 mg, 0.33 mmol, 1 equiv) in dichloromethane solution (7 mL) under stirring was mixed with N,N'-dicyclohexylcarbodiimide (DCC) (69.5 mg, 0.33 mmol, 1.1 equiv) and 4-(N,N'-dimethylamino)pyridine (DMAP) (40.90 mg, 0.33 mmol, 1 equiv). In each mixture, the corresponding alcohol or phenol (0.33 mmol, 1.1 equiv)
was added, followed by stirring of the resulting solutions at room temperature for 24 h (Scheme 2). At the end of each reaction, the solutions were filtered and concentrated under reduced pressure. The crude products were purified on silica gel chromatographic columns eluted with 8:2 hexane/EtOAc.

General procedure for the synthesis of compounds 17 and 18

4-(N,N'-Dimethylamino)pyridine (DMAP) (53.7 mg, 0.44 mmol, 1 equiv) and acetic anhydride (Ac₂O) (415 µL, 4.4 mmol, 10 equiv) in dichloromethane (3 mL) were mixed and stirred by 5 min at room temperature. Then, ferulic acid (3a) (87.1 mg, 0.44 mmol, 1 equiv) and triethylamine (Et₃N) (30.5 µL, 0.22 mmol, 0.5 equiv) were added and the mixture was stirred for another 30 min. Next, the solution was concentrated under reduced pressure. The crude product was precipitated by adding 5 mL of ice water and filtered under vacuum. The 3b product was obtained with 86.3% (91.3 mg) yield. Then compound 3b (96 mg, 0.40 mmol, 1 equiv) was added to a stirred solution in dichloromethane (5 mL), DCC (90.6 mg, 0.44 mmol, 1.1 equiv) and DMAP (48.8 mg, 0.44 mmol, 1 equiv). Soon after, appropriate phenol (0.44 mmol, 1.1 equiv) was added and the mixture was stirred for 8 h at room temperature (Scheme 3). At the end of the reaction, the residue was concentrated under reduced pressure. The crude product was purified by column chromatography with silica gel, eluting with hexane/EtOAc (9:1 v/v), obtaining the products 17 and 18.

2,3-Dihydro-1H-inden-5-yl cinnamate (1)

Following the general procedure, and using 5-indanol (59 mg, 0.44 mmol), compound 1 was obtained as white solid in 95% yield; mp 79-81 °C; IR (KBr) ν/cm⁻¹ 3062, 2929, 2845, 1728, 1631, 1309, 1143, 989, 871, 763, 680; ¹H NMR (300 MHz, CDCl₃) δ 7.88 (d, 1H, J 16.0 Hz, H-7), 7.60 (m, 2H, H-2, H-6), 7.43 (m, 3H, H-3, H-4, H-5), 7.24 (d, 1H, J 8.1 Hz, H-17), 7.03 (brs, 1H, H-11), 6.92 (dd, 1H, J 8.0, 2.1 Hz, H-18), 6.65 (d, 1H, J 16.0 Hz, H-8), 2.93 (brq, 4H, 2H-13, 2H-15), 2.21 (brq, 2H, 2H-14); ¹³C NMR (75 MHz, CDCl₃) δ 166.0 (C-9), 149.5 (C-10), 146.4 (C-7), 145.9 (C-12), 141.9 (C-16), 134.5 (C-1), 130.8 (C-4), 129.1 (C-2, C-6), 128.4 (C-3, C-5), 125.0 (C-17), 119.3 (C-18), 117.8 (C-11)*, 117.7 (C-8)*, 33.2 (C-13), 32.5 (C-15), 25.9 (C-14), *exangeable assignments; HRMS m/z, calcd. for C₁₈H₁₆O₂ [M + H]^+: 265.1150, found: 265.1922.

1-Oxo-1H-isochromen-6-yl cinnamate (2)

Following the general procedure, and using 6-hydroxy-1H-isocromen-1-one (71.3 mg, 0.44 mmol), compound 2 was obtained as white solid in 91.4% yield; mp 150-152 °C;

![Scheme 2](image_url)

Scheme 2. Synthesis of β-methoxyccinnamic acid derivatives; alcoholic/phenolic reagents, DCC, DMAP, CH₂Cl₂, reflux, 6 h.
IR (KBr) v / cm⁻¹ 3080, 3053, 1728, 1629, 1307, 1263, 1132, 856, 759; ¹H NMR (300 MHz, CDCl₃) δ 7.91 (d, 1H, J 16.0 Hz, H-7), 7.72 (d, 1H, J 9.6 Hz, H-14), 7.65-7.58 (m, 2H, H-2, H-6), 7.52 (d, 1H, J 8.4 Hz, H-17), 7.48-7.42 (m, 3H, H-3, H-4, H-5), 7.22 (d, 1H, J 1.6 Hz, H-11), 7.16 (dd, 1H, J 8.4, 2.0 Hz, H-18), 6.64 (d, 1H, J 16.0 Hz, H-8), 6.41 (d, 1H, J 9.6 Hz, H-13); ¹³C NMR (75 MHz, CDCl₃) δ 164.7 (C-9), 160.4 (C-15), 154.7 (C-10), 153.4 (C-12), 147.7 (C-7), 142.9 (C-14), 133.9 (C-1), 131.1 (C-4), 129.1 (C-2, C-6), 128.6 (C-17), 128.5 (C-3, C-5), 118.5 (C-8), 116.7 (C-16), 116.5 (C-11)*, 116.1 (C-18)*, 118.5 (C-18), 110.5 (C-13), *exchangeable assignments; HRMS m/z, calcd. for C₁₉H₂₀O₂ [M + H]⁺: 293.0814, found: 293.0818.

3-Methoxyphenyl cinnamate (4)

Following the general procedure, and using 3-methoxybenzyl alcohol (61 μL, 0.44 mmol), compound 4 was obtained as yellow liquid in 83.5% yield; IR (KBr) v / cm⁻¹ 2954, 2835, 1712, 1635, 1600, 1490, 1450, 1311, 1269, 1163, 797, 767, 686; ¹H NMR (300 MHz, CDCl₃) δ 7.70 (d, 1H, J 16.0 Hz, H-7), 7.49 (m, 2H, H-2, H-6), 7.35 (m, 3H, H-3, H-4, H-5), 7.26 (t, 1H, J 7.9 Hz, H-15), 6.96 (brd, 1H, J 8.2 Hz, H-16), 6.93 (brs, 1H, H-12), 6.85 (dd, 1H, J 8.2, 2.3 Hz, H-14), 6.48 (d, 1H, J 16.0 Hz, H-8), 5.20 (s, 2H, 2H-10), 3.79 (s, 3H, CH₃O-H-17); ¹³C NMR (75 MHz, CDCl₃) δ 166.9 (C-9), 160.0 (C-13), 145.4 (C-7), 137.7 (C-11), 134.5 (C-1), 130.5 (C-4), 129.8 (C-15), 129.1 (C-2, C-6), 128.3 (C-3, C-5), 120.6 (C-16), 118.0 (C-8), 114.0 (C-14)*, 113.9* (C-12), 66.4 (C-10), 55.4 (C₉H₀O-17), *exchangeable assignments; HRMS m/z, calcd. for C₁₉H₁₄O₂ [M + H]⁺: 295.0663. Data are in agreement with those previously reported.¹⁷

2-Isopropyl-5-methylphenyl cinnamate (5)

Following the general procedure, and using thymol (66 mg, 0.44 mmol), compound 5 was obtained as yellow viscous liquid in 97% yield; IR (KBr) v / cm⁻¹ 3059, 2962, 2926, 2868, 1726, 1639, 1506, 1448, 1309, 1244, 1161, 981, 765; ¹H NMR (300 MHz, CDCl₃) δ 7.91 (d, 1H, J 16.0 Hz, H-7), 7.63 (m, 2H, H-2, H-6), 7.45 (m, 3H, H-3, H-4, H-5), 7.26 (d, 1H, J 8.0 Hz, H-12), 7.21 (brd, 1H, J 7.9 Hz, H-13), 6.92 (brs, 1H, H-15), 6.70 (d, 1H, J 16.0 Hz, H-8), 3.07 (q, 1H, J 6.0 Hz, H-6), 2.36 (s, 3H, 3H-19), 1.24 (d, 6H, J 6.0 Hz, 3H-17, 3H-18); ¹³C NMR (75 MHz, CDCl₃) δ 165.9 (C-9), 148.1 (C-10), 146.6 (C-7), 137.3 (C-14), 136.7 (C-11), 134.4 (C-1), 130.8 (C-4), 129.1 (C-2, C-6), 128.5 (C-3, C-5), 127.3 (C-12), 126.6 (C-13), 123.0 (C-15), 117.5 (C-8), 27.3 (C-16), 23.2 (C-17, C-18), 21.0 (C-19); HRMS m/z, calcd. for C₁₉H₁₄O₂ [M + H]⁺: 281.1542, found: 281.1563. Data are in agreement with those previously reported.²⁰

5-Isopropyl-2-methylphenyl cinnamate (6)

Following the general procedure, and using carvacrol (64 μL, 0.44 mmol), compound 6 was obtained as white solid in 93.6% yield; mp 45-46 °C; IR (KBr) v / cm⁻¹ 2954, 2835, 1712, 1635, 1600, 1490, 1450, 1311, 1269, 1163, 797, 767, 686; ¹H NMR (300 MHz, CDCl₃) δ 7.70 (d, 1H, J 16.0 Hz, H-7), 7.49 (m, 2H, H-2, H-6), 7.35 (m, 3H, H-3, H-4, H-5), 7.26 (t, 1H, J 7.9 Hz, H-15), 6.96 (brd, 1H, J 8.2 Hz, H-16), 6.93 (brs, 1H, H-12), 6.85 (dd, 1H, J 8.2, 2.3 Hz, H-14), 6.48 (d, 1H, J 16.0 Hz, H-8), 5.20 (s, 2H, 2H-10), 3.79 (s, 3H, CH₃O-H-17); ¹³C NMR (75 MHz, CDCl₃) δ 166.9 (C-9), 160.0 (C-13), 145.4 (C-7), 137.7 (C-11), 134.5 (C-1), 130.5 (C-4), 129.8 (C-15), 129.1 (C-2, C-6), 128.3 (C-3, C-5), 120.6 (C-16), 118.0 (C-8), 114.0 (C-14)*, 113.9* (C-12), 66.4 (C-10), 55.4 (C₉H₀O-17), *exchangeable assignments; HRMS m/z, calcd. for C₁₉H₁₄O₂ [M + H]⁺: 295.0663. Data are in agreement with those previously reported.¹⁷

Scheme 3. Synthesis of ferulic acid derivatives; (a) Et₃N, DMAP, Ac₂O, CH₂Cl₂, rt, 30 min; (b) phenolic reagent, DCC, DMAP, CH₂Cl₂, rt, 8 h.
Preparation, Spectral Characterization and Anticancer Potential of Cinnamic Esters

J. Braz. Chem. Soc.

2,3-Dihydro-1H-inden-5-yl (E)-3-(4-methoxyphenyl)acrylate (9)

Following the general procedure, and using 5-indanol (44.3 mg, 0.33 mmol), compound 9 was obtained as amorphous white solid in 76.6% yield; mp 74.0-76 °C; IR (KBr) ν / cm⁻¹ 3008, 2935, 2841, 2112, 2042, 1894, 1716, 1629, 1600, 1510, 1479, 1249, 1145, 993, 823, 549, 414; 1H NMR (300 MHz, CDCl₃) δ 7.83 (d, 1H, J 15.9 Hz, H-7), 7.55 (d, 2H, J 8.7 Hz, H-2, H-6), 7.23 (d, 1H, J 8.8 Hz, H-17), 7.02 (bs, 1H, J 11.1 Hz), 6.95 (d, 2H, J 8.8 Hz, H-3, H-5), 6.91* (dd, 1H, J 8.7, 2.5 Hz, H-18), 6.51 (d, 1H, J 15.9 Hz, H-8), 3.87 (s, 3H, CH₃O-1a), 2.92 (q, 4H, 2H-12, 2H-15), 2.12 (m, 2H, 2H-14); 13C NMR (75 MHz, CDCl₃) δ 166.3 (C-9), 161.8 (C-4), 149.6 (C-10), 146.1 (C-7), 145.9 (C-12), 141.7 (C-16), 130.1 (C-2, C-6), 127.2 (C-1), 124.9 (C-17), 119.4 (C-18), 117.9 (C-11), 115.1 (C-8), 114.6 (C-3, C-5), 55.6 (CH₃O-1a), 33.2 (C-13)*, 32.5 (C-15)*, 25.9 (C-14), *these data were not obtained by 2D ¹H/¹³C correlated spectroscopy (COSY) spectrum; assignments exchangeable; HRMS m/z, calcd. for C₁₉H₁₄O₅ [M + H]⁺: 295.1455, found: 295.1334.

1-Oxo-1H-isocromen-6-yl (E)-3-(4-methoxyphenyl)acrylate (10)

Following the general procedure, and using 6-hydroxy-1H-isocromen-1-one (53.5 mg, 0.33 mmol), compound 10 was obtained as amorphous white solid in 89.9% yield; mp > 200 °C; IR (KBr) ν / cm⁻¹ 3072, 2933, 1735, 1622, 1602, 1514, 1255, 1139, 1120, 995, 821; 1H NMR (300 MHz, CDCl₃) δ 7.87 (d, 1H, J 15.9 Hz, H-7), 7.72 (d, 1H, J 9.6 Hz, H-14), 7.56 (d, 2H, J 8.7 Hz, H-3, H-5), 7.52 (d, 1H, J 8.5 Hz, H-17), 7.21 (d, 1H, J 2.0 Hz, H-11), 7.14 (dd, 1H, J 8.4, 2.1 Hz, H-18), 6.96 (d, 2H, J 8.7 Hz, H-2, H-6), 6.49 (d, 1H, J 15.9 Hz, H-8), 6.41 (d, 1H, J 9.6 Hz, H-13), 3.92 (s, 3H, CH₃O-1a); ¹³C NMR (75 MHz, CDCl₃) δ 165.2 (C-9), 162.2 (C-15), 166.0 (C-1), 154.9 (C-10), 153.7 (C-12), 147.6 (C-7), 143.1 (C-14), 130.4 (C-3, C-5), 128.7 (C-17), 126.8 (C-8), 118.7 (C-18), 116.7 (C-16), 116.1 (C-8), 114.7 (C-2, C-6), 113.9 (C-11), 110.7 (C-13), 55.6 (CH₃O-1a); HRMS m/z, calcd. for C₁₉H₁₄O₅ [M + H]⁺: 323.0890, found: 323.0919.

rac-(2S)-2-Methyl-5-(prop-1-en-2-yl)cyclohexyl cinnamate (8)

Following the general procedure, and using rac-dihydrocarveol (63 μL, 0.44 mmol), compound 8 was obtained as translucent liquid in 79.5% yield; IR (KBr) ν / cm⁻¹ 3066, 2929, 2858, 1708, 1637, 1452, 1307, 1172, 1006, 766; 1H NMR (300 MHz, CDCl₃) δ 7.70 (d, 1H, J 16.0 Hz, H-7), 7.54 (m, 2H, H-2, H-6), 7.39 (m, 3H, H-3, H-4, H-5), 6.46 (d, 1H, J 16.0 Hz, H-8), 4.71 (brs, 2H, 2H-17), 4.64 (td, 1H, J 10.7, 4.1 Hz, H-10), 2.15 (m, 2H, 2H-14, H-15), 1.85 (m, 1H, H-15*), 1.75 (m, 1H, H-11), 1.64 (m, 1H, H-13), 1.65 (s, 3H, 3H-18), 1.38-1.19 (m, 3H, 2H-12, H-13*), 0.97 (d, 3H, J 6.0 Hz, H-19); ¹³C NMR (75 MHz, CDCl₃) δ 166.9 (C-9), 149.1 (C-16), 144.6 (C-7), 134.7 (C-1), 130.3 (C-4), 129.0 (C-2, C-6), 128.2 (C-3, C-5), 118.8 (C-8), 109.0 (C-17), 78.5 (C-10), 43.9 (C-11), 37.5 (C-14), 37.2 (C-15), 33.3 (C-12), 31.1 (C-13), 21.1 (C-18), 18.5 (C-19); HRMS m/z, calcd. for C₁₉H₂₃O₂ [M + H]⁺: 285.1855, found: 284.9321.

rac-(2S)-2-Methyl-5-(prop-1-en-2-yl)cyclohexyl cinnamate (8)

Following the general procedure, and using (S)-(+)-perillyl alcohol (48 μL, 0.33 mmol), compound 11 was obtained as yellow liquid in 81.3% yield; IR (KBr) ν / cm⁻¹ 3074, 2926, 2827, 1710, 1635, 1606, 1438, 1253, 1159, 827, 518; 1H NMR (300 MHz, CDCl₃) δ 7.67 (d, 1H, J 16.0 Hz, H-7), 7.49 (d, 2H, J 8.7 Hz, H-2, H-6), 6.91 (d, 2H, J 8.7 Hz, H-3, H-5), 6.35 (d, 1H, J 16.0 Hz, H-8), 3.14 (s, 3H, 3H-18), 1.38-1.19 (m, 3H, 2H-12, H-13*), 0.97 (d, 3H, J 6.0 Hz, H-19); ¹³C NMR (75 MHz, CDCl₃) δ 166.9 (C-9), 149.1 (C-16), 144.6 (C-7), 134.7 (C-1), 130.3 (C-4), 129.0 (C-2, C-6), 128.2 (C-3, C-5), 118.8 (C-8), 109.0 (C-17), 78.5 (C-10), 43.9 (C-11), 37.5 (C-14), 37.2 (C-15), 33.3 (C-12), 31.1 (C-13), 21.1 (C-18), 18.5 (C-19); HRMS m/z, calcd. for C₁₉H₂₃O₂ [M + H]⁺: 285.1855, found: 284.9321.
Following the general procedure, and using thymol (46 µL, 0.33 mmol), compound 12 was obtained as yellow liquid in 88.7% yield; IR (KBr) ν/cm⁻¹ 2935, 2835, 1710, 1631, 1602, 1512, 1460, 1253, 1157, 1029, 829; ¹H NMR (300 MHz, CDCl₃) δ 7.70 (d, 1H, J 16.0 Hz, H-7), 7.49 (d, 2H, J 8.7 Hz, H-2, H-6), 7.29 (t, 1H, J 9.0 Hz, H-15), 7.02 (brs, 1H, H-12), 6.97-6.90 (m, 2H, H-16, H-14), 6.92 (d, 2H, J 8.7 Hz, H-3, H-5), 6.37 (d, 1H, J 16.0 Hz, H-8), 5.23 (s, 2H, 2H-10), 3.84 (s, 3H, CH₃O-1a), 3.84 (s, 3H, CH₃O-17); ¹³C NMR (75 MHz, CDCl₃) δ 167.3 (C-9), 161.6 (C-4), 159.9 (C-13), 145.1 (C-7), 137.9 (C-11), 129.9 (C-2, C-6)*, 129.8 (C-15)*, 127.3 (C-1), 120.6 (C-16), 115.5 (C-8), 114.5 (C-3, C-5), 113.9 (C-14)**, 113.8 (C-12)**, 66.2 (C-10), 55.6 (CH₃O-1a), 55.5 (CH₂O-17), *exchangeable assignments, **exchangeable assignments; HRMS m/z, calcld. for C₁₉H₂₄O₃ [M + H]⁺: 299.1316, found: 299.1285.

Following the general procedure, and using vanillin (50.2 mg, 0.33 mmol), compound 15 was obtained as yellow crystalline solid in 93.8% yield; mp 87-89 °C; IR (KBr) ν/cm⁻¹ 3051, 2958, 2927, 2860, 1722, 1506, 1507, 1478, 1328, 1257, 1134, 1026, 833, 526; ¹H NMR (300 MHz, CDCl₃) δ 9.97 (s, 1H, H-16), 7.86 (d, 1H, J 15.9 Hz, H-7), 7.56 (d, 2H, J 8.5 Hz, H-2, H-6), 7.53 (brs, 1H, H-12), 7.51 (brd, 1H, J 8.5 Hz, H-14), 7.31 (d, 1H, J 8.5 Hz, H-15), 6.95 (d, 2H, J 8.5 Hz, H-3, H-5), 6.53 (d, 1H, J 15.9 Hz, H-8), 3.92 (s, 3H, CH₃O-1a), 3.86 (s, 3H, CH₃O-17); ¹³C NMR (75 MHz, CDCl₃) δ 191.1 (C-16), 164.6 (C-9), 161.9 (C-4), 152.2 (C-10), 147.1 (C-7), 145.2 (C-11), 135.2 (C-13), 130.2 (C-2, C-6), 126.8 (C-1), 124.8 (C-14), 123.6 (C-15), 114.5 (C-3, C-5), 113.7 (C-8), 110.9 (C-12), 56.1 (CH₂O-1a), 55.4 (CH₂O-17); HRMS m/z, calcld. for C₁₉H₂₄O₃ [M + H]⁺: 313.1085, found: 313.1076.

Following the general procedure, and using rac-carvacrol (48 µL, 0.33 mmol), compound 14 was obtained as amorphous white solid in 86% yield; mp 75-77 °C; IR (KBr) ν/cm⁻¹ 3051, 2958, 2927, 2866, 1722, 1633, 1600, 1510, 1460, 1313, 1255, 1138, 825, 528; ¹H NMR (300 MHz, CDCl₃) δ 7.86 (d, 1H, J 15.9 Hz, H-7), 7.56 (d, 2H, J 8.7 Hz, H-2, H-6), 7.18 (d, 1H, J 7.8 Hz, H-12), 7.06 (brd, 1H, H-13), 6.97 (brs, 1H, H-15), 6.95 (d, 2H, J 8.7 Hz, H-3, H-5), 6.55 (d, 1H, J 15.9 Hz, H-8), 3.87 (s, 3H, CH₃O-1a), 2.91 (q, 1H, J 6.0 Hz, H-16), 2.19 (s, 3H, 3H-19), 1.26 (d, 6H, J 6.0 Hz, 3H-17, 3H-18); ¹³C NMR (75 MHz, CDCl₃) δ 165.7 (C-9), 161.9 (C-4), 149.6 (C-10), 148.2 (C-14), 146.3 (C-7), 131.1 (C-12), 130.2 (C-2, C-6), 127.6 (C-11)*, 127.2 (C-1)*, 124.2 (C-13), 120.1 (C-15), 114.8 (C-8), 114.6 (C-3, C-5), 55.6 (CH₂O-1a), 33.7 (C-16), 24.1 (C-17, C-18), 16.1 (C-19), *exchangeable assignments; HRMS m/z, calcld. for C₂₀H₂₄O₃ [M + H]⁺: 311.1650, found: 311.1647.

Following the general procedure, and using thymol (49.6 mg, 0.33 mmol), compound 13 was obtained as white solid 79.1% yield; mp 82-84 °C; IR (KBr) ν/cm⁻¹ 2955, 2927, 2852, 2117, 1722, 1635, 1602, 1512, 1456, 1259, 1139, 833, 526; ¹H NMR (300 MHz, CDCl₃) δ 7.85 (d, 1H, J 15.9 Hz, H-7), 7.56 (d, 2H, J 8.7 Hz, H-2, H-6), 7.26 (d, 1H, J 7.9 Hz, H-12), 7.05 (brd, 1H, J 7.7 Hz, H-13), 6.96 (d, 2H, J 8.6 Hz, H-3, H-5), 6.90 (brs, 1H, H-15), 6.54 (d, 1H, J 15.9 Hz, H-8), 3.87 (s, 3H, CH₃O-1a), 3.05 (q, 1H, J 6.0 Hz, H-16), 2.34 (s, 3H, 3H-19), 1.22 (d, 6H, J 6.0 Hz, 3H-17, 3H-18); ¹³C NMR (75 MHz, CDCl₃) δ 166.2 (C-9), 161.9 (C-4), 148.2 (C-11), 146.3 (C-7), 137.4 (C-14), 136.7 (C-11), 130.2 (C-2, C-6), 126.7 (C-1), 127.2 (C-12), 126.7 (C-13), 123.1 (C-15), 114.9 (C-8), 114.6 (C-3, C-5), 55.6 (CH₂O-1a), 27.3 (C-16), 23.2 (C-17, C-18), 21.0 (C-19); HRMS m/z, calcld. for C₂₀H₂₄O₃ [M + H]⁺: 311.1650, found: 311.1647. Data are in agreement with those previously reported.²⁰
Preparation, Spectral Characterization and Anticancer Potential of Cinnamic Esters

J. Braz. Chem. Soc.

C20 H 26 O 3 \([\text{M + H}]^+\): 327.1597, found: 327.1596.

127.3 (C-1), 116.1 (C-8), 114.3 (C-3, C-5), 108.8 (C-17), 161.3 (C-4), 149.0 (C-17), 144.2 (C-7), 129.7 (C-2, C-6), 127.3 (C-1), 116.1 (C-8), 114.3 (C-3, C-5), 108.8 (C-17), 78.1 (C-10), 55.39 (CH2O-1a), 43.7 (C-11), 37.4 (C-14)*, 37.0 (C-15)*, 33.2 (C-12), 30.9 (C-13), 20.9 (C-19), 18.3 (C-18); HRMS m/z, assignments, *exchangeable assignments; HRMS

27.3 (C-16), 23.3 (C-17, C-18), 21.0 (C-19), *exchangeable assignments; HRMS m/z, assignments, **exchangeable assignments; HRMS

115.0 (C-8), 114.8 (C-5), 113.2 (C-2), 56.2 (CH3O-1a), 147.1 (C-3), 146.7 (C-7), 131.1 (C-12), 127.6 (C-11), 126.9 (C-1), 124.6 (C-13), 123.6 (C-6), 120.1 (C-15), 115.0 (C-8), 114.8 (C-5), 113.2 (C-2), 56.2 (CH3O-1a), 33.8 (C-16), 29.9 (C-18), 24.1 (C-17), 16.1 (C-19), *exchangeable assignments; HRMS m/z, calcd. for C20H26O3 [M + H]+: 315.1962, found: 315.1960.

5-Isopropyl-2-methylphenyl (E)-3-(3-hydroxy-4-methoxyphenyl)acrylate (17)

Following the general procedure, and using carvacrol (64.0 μL, 0.44 mmol), compound 17 was obtained as yellow pasty liquid 18.6% yield; IR (KBr) ν / cm-1: 3425, 2960, 2852, 1720, 1631, 1591, 1512, 1269, 1232, 1134; 1H NMR (300 MHz, CDCl3) δ 7.83 (d, 1H, J 15.9 Hz, H-7), 7.17 (d, 1H, J 8.1 Hz, H-12), 7.15 (brd, 1H, H-6), 7.12 (s, 1H, H-2), 7.03 (brd, 1H, H-13), 6.98 (d, 1H, J 8.1 Hz, H-5), 6.95 (s, 1H, H-15), 6.53 (d, 1H, J 15.9 Hz, H-8), 3.97 (s, 3H, CH3O-1a), 2.93-2.81 (sex, 1H, J 6.0 Hz, H-16), 2.19 (s, 3H, 3H-19), 1.26 (d, 6H, J 6.1 Hz, 3H-17, 3H-18); 13C NMR (75 MHz, CDCl3) δ 166.8 (C-9), 149.0 (C-10), 148.5 (C-4)*, 148.3 (C-14)*, 147.1 (C-3), 146.7 (C-7), 131.1 (C-12), 127.6 (C-11), 126.9 (C-1), 124.6 (C-13), 123.6 (C-6), 120.1 (C-15), 115.0 (C-8), 114.8 (C-5), 113.2 (C-2), 56.2 (CH3O-1a), 33.8 (C-16), 29.9 (C-18), 24.1 (C-17), 16.1 (C-19), *exchangeable assignments; HRMS m/z, calcd. for C20H26O3 [M + H]+: 327.1597, found: 327.1596.

2-Isopropyl-5-methylphenyl (E)-3-(3-hydroxy-4-methoxyphenyl)acrylate (18)

Following the general procedure, and using thymol (66.0 μL, 0.44 mmol), compound 18 was obtained as yellow pasty liquid 15.5% yield; IR (KBr) ν / cm-1: 3425, 2960, 2852, 1720, 1631, 1591, 1512, 1269, 1232, 1134; 1H NMR (300 MHz, CDCl3) δ 7.82 (d, 1H, J 15.9 Hz, H-7), 7.23 (d, 1H, J 7.9 Hz, H-12), 7.15 (brd, 1H, J 8.2 Hz, H-6), 7.12 (brs, 1H, H-2), 7.06 (brd, 1H, J 8.2 Hz, H-13), 6.97 (d, 1H, J 8.1 Hz, H-5), 6.89 (brs, 1H, H-15), 6.52 (d, 1H, J 15.9 Hz, H-8), 3.96 (s, 3H, CH3O-1a), 3.12-2.98 (epi, 1H, J 6.1 Hz, H-16), 2.34 (s, 3H, 3H-19), 1.22 (d, 6H, J 6.0 Hz, 3H-17, 3H-18); 13C NMR (75 MHz, CDCl3) δ 166.2 (C-9), 148.5 (C-4)*, 148.0 (C-10)*, 147.1 (C-3), 146.7 (C-7), 137.4 (C-14), 136.7 (C-11), 127.2 (C-12), 126.9 (C-1), 126.6 (C-13), 123.6 (C-6)**, 123.1 (C-15)**, 115.0 (C-8), 114.8 (C-5), 110.7 (C-2), 56.2 (CH3O-1a), 27.3 (C-16), 23.5 (C-17, C-18), 21.0 (C-19), *exchangeable assignments; HRMS m/z, calcd. for C20H26O3 [M + H]+: 327.1576, found: 327.1596.

The structure of compound 6 is reported in the literature, but it does not report spectroscopic data.

Cytotoxicity

The cytotoxic activity of cinnamates was evaluated in SNB-19 (astrocytoma), HCT-116 (colon carcinoma), PC3 (prostate carcinoma), HL60 (promyelocytic leukemia), which were obtained from the National Cancer Institute (Washington, USA). All cells were cultured in Roswell Park Memorial Institute (RPMI) 1640, except for L929, which was cultivated in Dulbecco’s Modified Eagle Medium (DMEM), obtained from the Rio de Janeiro Cell Bank (BCRJ) (Rio de Janeiro, Brazil). All cell culture experiments were performed at 37 °C. Cells were supplemented with 10% fetal calf serum and 1% of antibiotics, in a 5% CO2 humidified atmosphere. The L929 cell line was used to evaluate the selectivity of the extracts and these assays, the anticancer drug doxorubicin was used as positive control.

Cytotoxicity assays were carried out essentially according the MTT colorimetric method [3-(4,5-dimethyl-thiazolyl)-2,5-diphenyl-2H-tetrazolium]. The compounds were tested at 25 μg mL^-1 in four lines tumor cells for initial screening; the half maximal inhibitory concentration (IC50) was determined for samples that showed positive results (growth inhibition > 70%) in at least one cell line. The cells were plated in 96-well plates at the following concentrations: HCT-116/L929: 0.7 × 106 cells mL^-1; SNB-19/PC3: 0.1 × 106 cells mL^-1; HL60: 0.3 × 106 cells mL^-1. The cells were treated with the substances for 72 h. At the end of the treatment, the plates were centrifuged and the supernatant removed. Then, 100 μL of MTT solution (0.5 μg mL^-1) were added and incubated for 3 h. After incubation, the MTT solution was removed, and the precipitated formazan was dissolved with 100 μL of dimethyl sulfoxide (DMSO). The absorbances were read using a plate spectrophotometer (Multimode Detector, DTX 880, Beckman Coulter) provided by Analytical Instruments (Golden Valley, USA) at 595 nm.

Statistical analysis

All experiments were performed in duplicate and repeated three times. For samples that showed > 70% inhibitory, activity the selectivity index (SI) was calculated. The calculation of this index corresponds to the division between the IC50 value of each test compound in the non-tumor cell line L929 and the IC50 value of each compound in the tumor cell line (SI = neoplastic cells IC50/L929 IC50). The results obtained were analyzed using the GraphPad
Gonçalves et al. reported and exhibited antimicrobial activity, but there were no compounds with published biological studies.

Triethylamine (Et₃N) and DMAP gave the corresponding esters after acetylation with acetic anhydride in the presence of a catalyst. In turn, 3-methoxy-4-hydroxy-cinnamic acid, which was then esterified via Sterlich to give 3b, showed promising cytotoxicity, with > 70% inhibition of cell proliferation in at least one of the lines tested. Only those with an inhibition percentage above 70% were evaluated for the mean inhibitory concentration (IC₅₀ = concentration causing a 50% inhibition) (Table 1).

Initially, the esters were screened using a program from the National Cancer Institute (NCI), which easily allows a qualitative or semi-quantitative analysis to determine cytotoxicity. An intensity scale was used to assess the cytotoxic potential of products, according to the following results: 1-50% (low or medium), 50-75% (moderate) and 75-100% (high). According to this program, of the eighteen synthesized compounds, eleven exhibited cytotoxic potential with cell growth inhibition above 50% and six with 75-100% inhibition (high activity). Thus, based on the initial screening, compounds 6, 8, 12, 14, 15 and 18 showed promising cytotoxicity, with > 70% inhibition of cells proliferation in at least one of the lines tested. Only those with an inhibition percentage above 70% were evaluated for the mean inhibitory concentration (IC₅₀ = concentration causing a 50% inhibition) (Table 1).

As summarized in Table 1, the six compounds demonstrated a general (non specific) cytotoxic response. For example, the intensity of the response demonstrated by compound 6 (IC₅₀ 23.2 μM) was similar to that demonstrated by 8 (IC₅₀ 25.2 μM), however, on different cell lines, that is, HCT-116 and HL60, respectively. In the 2a-compounds series, 12 (IC₅₀ 16.2 μM) demonstrated a cytotoxic response greater than 14 (IC₅₀ 29.3 μM) against the HCT-116 strain. The highest intensity of response was shown by compound 18 against HTC-116 cells with an IC₅₀ of 15.4 μM. All compounds showed higher IC₅₀ values with respect to doxorubicin in all strains. Regarding the cytotoxicity of the compounds against the non-tumor cell line (L929), the samples showed IC₅₀ > 77 μM, demonstrating that the samples have low cytotoxicity against non-tumor cell lines. With the exception of the compound 6, which showed inhibitory activity against L929 cells with IC₅₀ values of 32.5 μM.
Table 1. IC₅₀ values of compounds 6, 8, 12, 14, 15 and 18 in tumor and non-tumor cell lines with a 95% confidence interval

| Compound | HL60 IC₅₀/μM | SNB-19 IC₅₀/μM | HCT-116 IC₅₀/μM | PC3 IC₅₀/μM | L929 IC₅₀/μM |
|----------|--------------|----------------|------------------|-------------|-------------|
| 6        | > 89         | 38.3 ± 11.3    | 23.2 ± 3.5       | 49.0 ± 5.9  | 32.5 ± 21.4 |
| 8        | 25.2 ± 3.0   | > 88           | > 88             | > 88        | > 88        |
| 12       | > 84         | 42.1 ± 14.2    | 16.2 ± 6.3       | 41.9 ± 7.9  | > 84        |
| 14       | > 80         | > 80           | > 80             | > 80        | > 80        |
| 15       | 79.8 ± 16.4  | > 80           | > 80             | > 80        | > 80        |
| 18       | > 77         | > 77           | 15.38 ± 1.6      | 67.4 ± 18.1 | > 77        |
| DOX      | 0.01 ± 0.009 | 3.8 ± 0.6      | 0.35 ± 0.09      | 1.4 ± 0.3   | 3.2 ± 0.27  |

*aInhibition of proliferation did not exceed 50% at the highest concentration tested; b doxorubicin was used as a positive control. IC₅₀: half maximal inhibitory concentration; HL60: promyelocytic leukemia; SNB-19: astrocytoma, HCT-116: colon carcinoma, human; PC3: prostate; L929: cell murine fibroblast.

Table 2. Values of the selectivity index (SI) of the tested compounds. SI was calculated for each compound using the formula: SI = IC₅₀ normal cells/IC₅₀ of the respective cancer cells

| Compound | SI HL60 | SI SNB-19 | SI HCT-116 | SI PC3 |
|----------|---------|-----------|------------|--------|
| 6        | -       | 0.84      | 1.4        | 0.7    |
| 8        | -       | -         | -          | -      |
| 12       | -       | -         | -          | -      |
| 14       | -       | -         | -          | -      |
| 15       | -       | -         | -          | -      |
| 18       | -       | -         | -          | -      |

*aSelectivity index could not be calculated in the tested strains because the substances did not present IC₅₀ calculable for the non-tumoral strain (IC₅₀ > 77 μM). IC₅₀, half maximal inhibitory concentration; HL60: promyelocytic leukemia; SNB-19: astrocytoma, HCT-116: colon carcinoma, human; PC3: prostate.

The comparison between the activity in relation to neoplastic cell lines and normal cells (L929) was made to calculate the selectivity index (SI), as an indication of the potential of using the compounds for future clinical tests. Ideally, the drug should only kill patient cancer cells without significantly affecting healthy cells. SI is considered significant when it has a value greater than or equal to 2.0, that is, this value means that the compound has activity twice in the lineages of neoplastic cells than in normal cells. For the compounds 6, 8, 12, 14, 15 and 18 the SI was calculated, which can be seen in Table 2.

For all strains tested, SI values were > 2 showing that the compounds are selective between neoplastic and normal cells. Combining SI with antiproliferative activity, the substances become candidates for drugs for future studies. However, a notable exception was the compound 6, with SI values of 0.84, 1.4 and 0.7 for the SNB-19, HCT-116 and PC3 strains, mutually. It is important to note that the compound 6 showed a high toxicity, preferentially inhibiting normal cells than neoplastic cells SNB-19 and PC3.

It is important to remember that the influence of α,β-unsaturated part of cinnamic acid and its derivatives relative to biological activity, was studied when comparing compounds (E)-3-(3,4-dihydroxyphenyl)acrylate (α,β-unsaturated) and 3-methyl-(3,4-dihydroxyphenyl)propanoate (α,β-saturated). It was observed that the compound α,β-unsaturated contributes positively to the action against breast cancer cells (T-47D) and colon (WiDr), with IC₅₀ values 64 and 59 μM, respectively, while the saturated compound is inactive. On the other hand, in their studies Sova et al. compared the inhibition effect in relation to the phenol/alcohol part of the ester. The (E)-phenyl cinnamate derivative inhibited the growth of HeLa (cervical adenocarcinoma), K562 (myeloid leukemia), Fem-x (malignant melanoma) and MCF-7 (breast cancer) cell lines, with an IC₅₀ of 75.6 ± 12, 52.6 ± 3, 69.0 ± 4 and 58.6 ± 4 μM, respectively, presenting cytotoxic effects superior to the (E)-cyclohexyl cinnamate derivative, with IC₅₀ >180 μM in all cells.

Analyzing the results from the point of view of the structures of the products, there was not a sufficiently coherent answer, however, allows some considerations. For example, 7 showed toxicity < 50% on the NCI scale, while its analog 15, with a methoxy group in the para position on the cinnamate part and with toxicity > 50% on the same scale, exhibited very low IC₅₀ against all cell lines. In another example, 4 exhibited toxicity < 50% on the NCI scale, while its 12 analogue, with a methoxy group in the para position in the cinnamate part, affected SNB-19 and PC3 cells growth with IC₅₀ values of 42.1 and 41.9 μM, respectively, and showed a potent antiproliferative effect against HTC-116 cells with an IC₅₀ value of 16 μM. Finally, 18 showed remarkable toxicity to HTC-116 cells...
of new bioactive compounds, more effective against cancer. Studies involving cinnamic acids and analgesics have shown that inhibition targets in several cancer cell lines occur through the inhibitory action on the deoxyribonucleic acid (DNA) synthesis of growing cells. In general, the data indicate that cinnamates inhibit cell growth by selective induction of cell death and cycle disruption. Thus, the inhibitory action of compounds that showed activity in front of cancer cells in this work, was also suspected of involving inhibition in the synthesis of DNA and guaranteeing an interruption of the cell cycle.

Conclusions

In this study, eighteen esters were obtained through the Stiglich esterification. The MTT test shows that the activities of compounds with an aromatic ring in the cinnamoyl fraction are more active than cyclohexyl. In comparison between the esters obtained, this study showed that the compound 12 is the most potent against HCT-116, PC3 and SNB-19 cells, with the lowest IC_{50} value of 16.2 μM in the HCT-116 strain. The compound 18 also has a low IC_{50} value in HCT-116 (15.38 μM). The compound 8 was the only one that showed the highest cytotoxicity in HL60 (IC_{50} = 25.2 μM). The compounds 8, 12 and 18 showed selectivity against normal cells (L929). According to some examples observed, there was an apparent increase in biological activity with increased conjugation in the cinnamate fraction, provided by electron donating substituents, such as methoxy and hydroxyl groups. This research indicates that the tested cinnamic acid derivatives present good initial performance for the development of candidates for antineoplastic drugs, bringing new perspectives for the structurally modified natural substances under study, contributing to the knowledge and elaboration of new bioactive compounds, more effective against cancer.

Supplementary Information

Supplementary information (1H and 13C NMR, IR, HRMS and potential for cell inhibition) is available free of charge at http://jbcs.sbq.org.br, as PDF file.

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Author Contributions

R. O. G. was responsible for project administration, conceptualization, analysis, investigation, methodology and writing original draft; I. F. F. for responsible for the characterization experiments; M. F. S. S. for cytotoxicity experimental methodology; C. Ó. P. to supervise and conceptualization cytotoxicity experiments; G. J. Z. for investigation; D. Z. for conceptualization, project administration, writing-review and editing; T. L. G. L. for conceptualization, project administration; writing-review and editing; F. J. Q. M. analyzed the experimental results, revised the manuscript, writing-review and editing.

References

1. World Health Organization (WHO); https://www.who.int/en/news-room/fact-sheets/detail/cancer, accessed on February 11, 2020.
2. Ali, I.; Rahis-ud-din; Saleem, K.; Aboul-Enein, H. Y.; Rather, A.; Cancer Ther. 2011, 8, 6.
3. Yuan, H.; Ma, Q.; Ye, L.; Piao, G.; Molecules 2016, 21, 559.
4. Stierle, A.; J. Nat. Prod. 2018, 81, 1125.
5. Kumar, N.; Pruthi, V.; Biotechnol. Rep. 2014, 4, 86.
6. Adisakwattana, S.; Nutrients 2017, 2, 16.
7. Mancuso, C.; Santangelo, R.; Food Chem. Toxicol. 2014, 65, 185.
8. Filho, A. C. V. A.; Rodrigues, P. A. S.; Benjamin, S. R.; Paim, R. T. T.; Holanda, M. O.; Silva, J. Y. G.; Milo, T. S.; Vieira, I. G. P.; Queiroz, M. G. R.; Guedes, M. I. F.; Environ. Toxicol. Pharmacol. 2017, 56, 198.
9. Rodrigues, P. A. S.; Guedes, F. I.; Marques, M. M. M.; Silva, I. N. G.; Vieira, I. G. P.; Int. J. Pharm. Pharm. Sci. 2016, 4, 115.
10. Gielbel, J. M.; Serbian, I.; Loesche, A.; Csuk, R.; Bioorg. Chem. 2019, 90, 103058.
11. Sova, M.; Mini-Rev. Med. Chem. 2012, 12, 749.
12. Malheiro, J. F.; Maillard, J. Y.; Borges, F.; Simões, M.; Int. Biodeterior. Biodegrad. 2019, 141, 71.
13. Seck, R.; Mansaly, M.; Gassama, A.; Cavé, C.; Cojean, S.; J. Chem. Pharm. Res. 2018, 10, 1, available at https://www.jocpr.com/articles/synthesis-and-antimalarial-activity-of-cinnamic-acid-derivatives.pdf, accessed in June 2021.
14. Lima, T. C.; Ferreira, A. R.; Silva, D. F.; Lima, E. O.; de Sousa, D. P.; Nat. Prod. Res. 2018, 32, 572.
15. Xu, C. C.; Deng, T.; Fan, M. L.; Lv, W. B.; Liu, J. H.: Yu, B. Y.; Eur. J. Med. Chem. 2016, 107, 192.
16. Chu, F.; Zhang, W.; Guo, W.; Wang, Z.; Yang, Y.; Zhang, X.; Fang, K.; Yan, M.; Wang, P.; Lei, H.; Molecules 2018, 23, 322.
17. Lutjen, A. B.; Quirk, M. A.; Barbera, A. M.; Kolonko, E. M.; Bioorg. Med. Chem. 2018, 26, 5291.
18. Shirahata, T.; Nagai, T.; Hirata, N.; Yokoyama, M.; Katsumi, T.; Konishi, N.; Nishino, T.; Makino, K.; Yamada, H.; Kaji, E.; Kiyohara, H.; Kobayashi, Y.; *Bioorg. Med. Chem.* 2017, 25, 1747.

19. Neises, B.; Steglich, W.; *Angew. Chem., Int. Ed.* 1978, 17, 522.

20. Tharamak, S.; Yooboon, T.; Pengsook, A.; Ratwatthananon, A.; Kumrungsee, N.; Builangpoti, V.; Pluempanupat, W.; *Pest Manage. Sci.* 2020, 76, 928.

21. Nikumbh, V. P.; Tare, V. S.; Mahulikar, P. P.; *J. Sci. Ind. Res.* 2003, 62, 1086.

22. Foote, P. A.; *J. Am. Pharm. Assoc.* 1928, 17, 958.

23. Dikusar, E. A.; Kozlov, N. G.; *J. Org. Chem.* 2005, 41, 992.

24. Tawata, S.; Taira, S.; Kobamoto, N.; Zhu, J.; Ishihara, M.; Toyama, S.; *Biosci., Biotechnol., Biochem.* 1996, 60, 909.

25. Mosmann, T.; *J. Immunol. Methods* 1983, 65, 55.

26. Lima, B. A. V.; Corrêa, R. S.; Graminha, A. E.; Varela Jr., J. J. G.; da Silva, A. B. F.; Ellena, J.; Silva, T. E. M.; Batista, A. A.; *J. Braz. Chem. Soc.* 2020, 31, 1352.

27. David, C. C.; Lins, A. C. S.; Silva, T. M. S.; Campos, J. F.; Silva, T. G.; Militão, G. C. G.; Camara, C. A.; *J. Braz. Chem. Soc.* 2019, 30, 8.

28. Hostettmann, K.; *Methods in Plant Biochemistry*; Academic Press: London, 1991.

29. GraphPad Prism, version 5.0; GraphPad Software, Inc., San Diego, USA, 2007.

30. Silverstein, R. M.; Webstewr, F. X.; Kiemle, D. J.; *Identificação de Compostos Orgânicos*; LTC Editora: Rio de Janeiro, 2006.

31. Skehan, P.; Storeng, R.; Scudiero, D.; Monks, A.; Mcmahon, J.; Vistica, D.; Warren, J. T.; Bokehsh, H.; Kenney, S.; Boyd, M. R.; *J. Natl. Cancer Inst.* 1990, 82, 1107.

32. Guedes, J. A. C.; Filho, E. G. A.; Rodrigues, T. H. S.; Silva, M. F. S.; Souza, F. V. D.; Alexandre, L. M. S.; Alves, R. E.; Canuto, K. M.; Brito, E. S.; Pessoa, C. Ó.; Nascimento, R. F.; Zocolo, G. J.; *Ind. Crops Prod.* 2018, 124, 466.

33. Souza, L. G. D. S.; Almeida, M. C. S.; Lemos, T. L. G.; Ribeiro, P. R. V.; Brito, E. S.; Silva, V. L. M.; Silva, A. M. S.; Braz-Filho, R.; Costa, J. G. M.; Rodrigues, F. F. G.; Barreto, F. S.; Moraes, M. O.; *Bioorg. Med. Chem. Lett.* 2016, 26, 435.

34. Reta, G. F.; Tonn, C. E.; Ríos-Luci, C.; León, L. G.; Pérez-Roth, E.; Padrón, J. M.; Donadel, O. J.; *Nat. Prod. Commun.* 2012, 10, 1341.

35. Sova, M.; Zizak, Z.; Stankovic, J.; Prijatelj, M.; Turk, S.; Juranic, Z.; Mlinaric-Rascan, I.; Gobec, S.; *Med. Chem.* 2013, 9, 633.

36. Niero, E. L. O.; Machado-Santelli, G. M.; *J. Exp. Clin. Cancer Res.* 2013, 32, 31.

37. Imai, M.; Yokoe, H.; Tsubuki, M.; Takahashi, N.; *Biol. Pharm. Bull.* 2019, 42, 1134.

38. Almeer, R. S.; Areb, A. M.; Husseine, R. A.; Othman, M. S.; Abdel, M. A. E.; *Anticancer Agents Med. Chem.* 2018, 19, 356.

39. Uesawa, Y.; Sakagami, H.; Okudaira, N.; Toda, K.; Takao, K.; Kagaya, H.; Sugita, Y.; *Anticancer Res.* 2018, 38, 817.

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