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New Chalcone Ester Derivatives as Potential Cytotoxic Agents

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Chalcones are a group of molecules with recognized biological potential against many diseases, including cancer. Thus, studies on this structure and derivatives have become an attractive chemical strategy to optimize their observed biological activities. One of the synthetic routes used to obtain chalcone derivatives is esterification using either commercial acid chlorides or carboxylic acids. This work focuses on preparing chalcone derivatives and investigating their biological potential against cancer cells. Compound 3′-hydroxychalcone (**1**) was synthetized by Claisen-Schmidt condensation followed by esterification of the 3′-OH, resulting in eight compounds named **1a–b** and **2a–f**. All structures were confirmed by ^{1}H and ^{13}C NMR and FT-IR, and cytotoxicity was evaluated in the HCT 116 (colon adenocarcinoma), MCF-7 (breast adenocarcinoma), and CCD-18Co (nontumoral colon fibroblasts) cell lines. Chalcone derivatives were generally more active toward the colon cancer cell line, and **1a** and **2b** were selected for IC_{50} determination, presenting IC_{50} values of approximately 10 μM in HCT 116 cells and above 20 μM in both MCF7 and CDC-18-Co cells, suggesting moderate selectivity. Additionally, we tested compounds **1a** and **2b** in combination with doxorubicin, but they did not act synergistically with this anthracycline. In conclusion, considering these compounds obtained by the esterification reaction, **1a** and **2d** showed better results against cytotoxic cells.

1. Introduction

Chalcones and their derivatives comprise a group of structures that present many biological activities in different targets. They are the biogenetic precursors of flavonoids and isoflavonoids, which are abundant in plants. Chemically, the chalcone core is composed of two aromatic rings linked via a three-carbon chain that contains carbon-α and β-unsaturated carbonyl systems. The presence of a double bond in conjugation with a carbonyl group is believed to be responsible for the biological activities of these compounds.[1,2]

Over the past few years, a large volume of research papers and review articles highlighting the significance of chalcone derivatives has been compiled in the literature. Synthetic manipulations of chalcones or their isolation from natural sources have been investigated for the development of new and efficient drugs to treat several diseases, including tumors, antimalarials, tuberculosis, malaria, bacterial infections, etc.[3–7]

Therefore, the chemistry of chalcones is still a long-standing attraction among organic chemists. Several synthetic routes have been reported for skeletal modification to produce new classes of organic compounds. A straightforward strategy would be the esterification of hydroxylated chalcones with acid chlorides in the presence of Et_3N to neutralize the HCl formed in the reaction and pyridines such as DMAP as a catalyst.[8]

Cancer is a major cause of morbidity and mortality worldwide. It is an extremely complex disease characterized by intense and uncontrolled cell growth involving molecular, cellular, and tissue factors that can deteriorate the entire affected organism. Given the complexity of the disease and the limitations of current treatments, the search for new therapeutic resources has become increasingly compelling. Several studies have demonstrated that chalcone derivatives possess biological activity against cancer cells. Examples have been reported showing promising results against cancer.[9–15]

In a previous study, hydroxylated chalcone derivatives were synthesized and screened for chemopreventive effects using human and mouse colon cancer cell lines (SW620 and CT26.WT); and human hepatocytes (HL-7702), both *in vitro* and *in vivo*.[10] In addition, a series of naphthalene-chalcone derivatives were prepared and evaluated as tubulin polymerization inhibitors for the treatment of breast cancer. All compounds were evaluated for their antiproliferative activity against the

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MCF-7 cell line. Most of the compounds displayed potent antiproliferative activity, and one of them demonstrated relatively lower cytotoxicity in a normal cell line (HEK293) than in a tumor cell line.[11] Moreover, indolyl-chalcone derivatives have been synthesized and their anticancer activities have been evaluated both *in vitro* and *in vivo* by utilizing human lung cancer cell line A549, and one of them showed good results, making it a promising lead compound for the future exploration of selective human lung cancer treatment.[12]

Therefore, this work uses 3′-hydroxychalcone as a starting material for the synthesis of esters derived from chalcone, and subsequently, its biological potential was evaluated against colorectal and breast cancer cell lines.

2. Results and Discussion

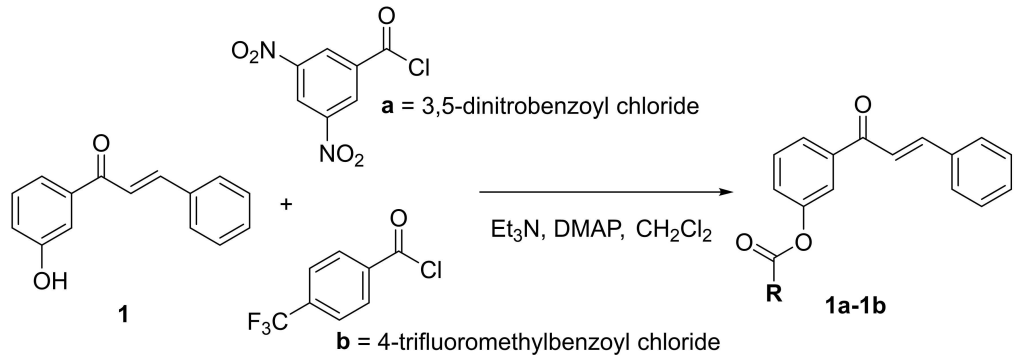
2.1. Chemistry

A total of eight chalcone ester derivatives were produced. Compounds **1a**–**b** were synthesized according to Scheme 1, while compounds **2a**–**f** were obtained as outlined in Scheme 2. Compound **1** was prepared via the Claisen-Schmidt reaction of 3′-hydroxyacetophenone and benzaldehyde in the presence of NaOH ethanolic solution. In all compounds obtained, chemical shifts belonging to the starting material 3′-hydroxychalcone (compound **1**) were observed. In the ^{13}C NMR experiments, esterification could be observed by shielding of the phenolic carbon (δ ranging from 150.6–151.3 ppm) compared to the chemical shift observed for the phenolic carbon present in the starting material at δ 156.7 ppm. Ester carboxyl was observed in all new compounds ranging from δ 161.6–164.2 ppm.

In the ^{1}H-NMR experiment for compound **1a**, a multiplet in δ 9.11–9.14 ppm integrating for three aromatic hydrogens was observed, evidencing the presence of the 1,3,5-trisubstituted ring. Compounds **1b**, **2a**, **2c**, and **2f** present *para*-disubstituted rings, as evidenced by the presence of two doublets, each integrating two hydrogens, each with coupling constants ranging from 8.6 to 9.0 Hz. Furthermore, for compound **2f**, a singlet at δ 3.90 ppm was observed, integrating to three hydrogens, characteristic of the methoxy group in this compound. Compounds **2b** and **2d**-**e** showed 1,3-disubstituted rings that were evidenced by the multiplicities and coupling constants of the aromatic hydrogens in these compounds.

2.2. Cytotoxicity Activity

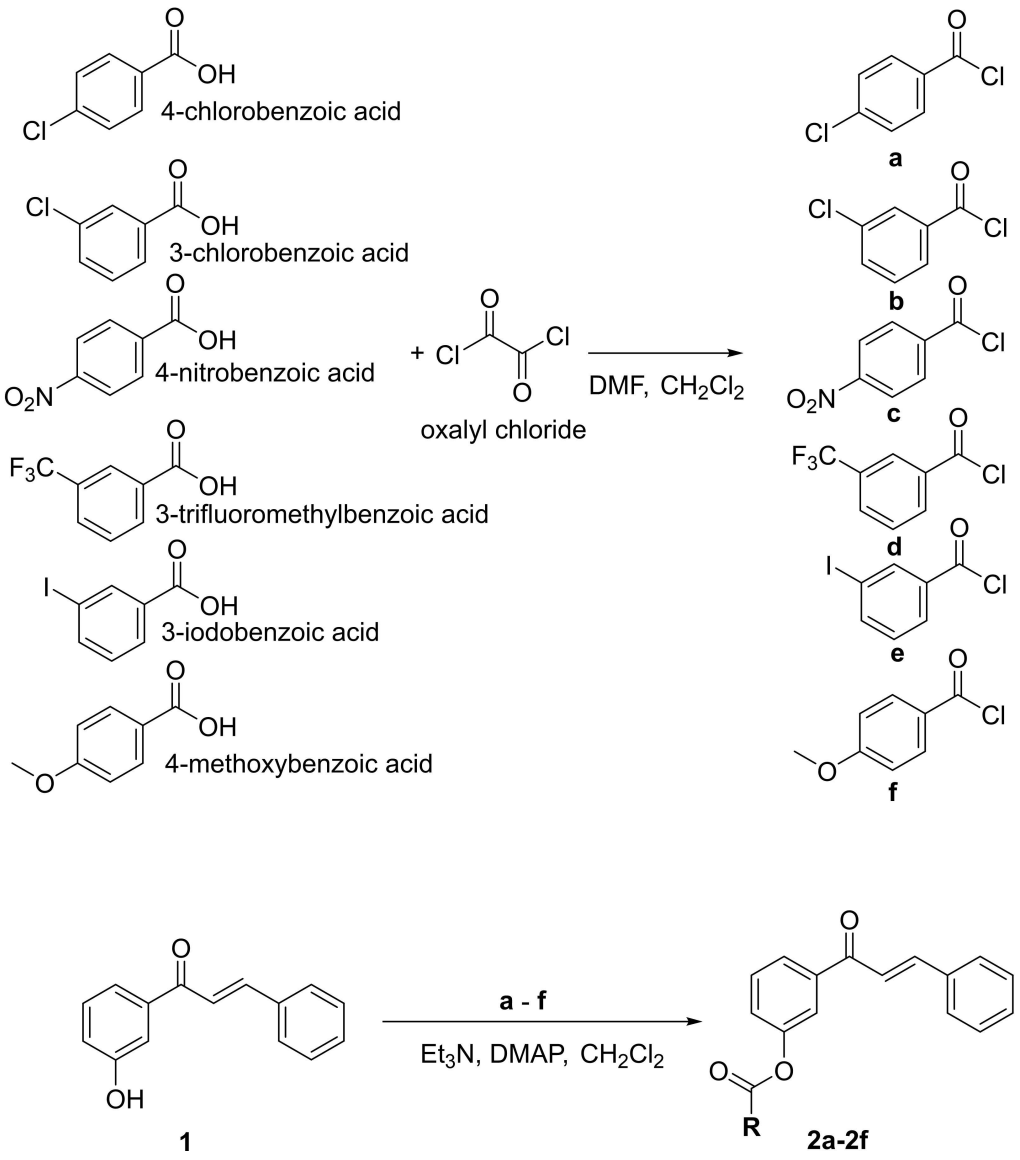
Initially, the cytotoxicity of substances **1a**--**b** and **2a**–**e** was evaluated in two tumor cell lines, HCT 116 (colorectal adenocarcinoma) and MCF-7 (breast adenocarcinoma), at two concentrations, 5 and 50 μM (Table 1). A cytotoxic potential was observed in this group of substances, which presented cell growth inhibition of colorectal adenocarcinoma cells (HCT 116)



Scheme 1. General synthesis of esters derivatives from acid chlorides.

Table 1. Growth inhibition (%) of compounds **1a**–**b** and **2a**–**e** in a colorectal carcinoma cell line (HCT-116) and breast carcinoma cells (MCF7) at concentrations of 5 μM and 50 μM (mean ± SEM from 2 independent experiments in duplicate) after 72 h of incubation.

Compound	HCT 116				MCF7			
	5 μM		50 μM		5 μM		50 μM	
	GI%	SEM	GI%	SEM	GI%	SEM	GI%	SEM
1a	52.6	17.2	98.0	1.3	14.5	3.6	68.6	4.1
1b	25.8	7.6	100.1	0.1	19.2	3.8	51.1	7.7
2a	0.3	0.1	99.7	0.2	14.1	6.2	44.8	10.6
2b	6.7	3.5	99.5	0.2	15.7	0.0	52.6	14.9
2c	33.1	16.2	99.9	0.1	1.9	1.9	62.2	7.5
2d	25.2	8.7	99.8	0.2	3.0	3.7	88.4	2.4
2e	0.00	–	99.8	0.3	0.2	2.2	52.1	5.9



Scheme 2. General synthesis of esters derivatives from carboxylic acid.

of approximately 100% at a concentration of 50 μM (Figure 1; Table 1). MCF-7 was generally less sensitive to the compounds, with a maximum inhibition of 88.4% observed for compound **2d** at 50 μM, followed by 68.6% inhibition for compound **1a** at 50 μM. Doxorubicin (at 5 μM), used as a positive control, completely inhibited the growth of both tested cell lines.

Based on these results, compounds **1a** and **2d** were selected for IC_{50} determination in both cancer cells, HCT 116 and MCF7 (Table 2). Compound **1a** showed IC_{50} values of 10.2 μM in HCT 116 cells and 26.4 μM in MCF-7 cells, whereas **2d** was as active as **1a** in HCT 116 cells (IC_{50} of 10.7 μM), but it was less toxic to MCF7 cells (IC_{50} of 44.8 μM). Indeed, the positive control, doxorubicin, acknowledges a lower sensitivity in MCF-7 cells in comparison to HCT 116 cells, with an IC_{50} value 6.4 times higher for the first cell type (Table 2).

The cytotoxicity of both **1a** and **2d** was further evaluated against the nontumor colon cell line CCD-18-Co to assess their selectivity. As shown in Tables 2, the IC_{50} values for both tested compounds were very similar between MCF7 and CDC-18-Co, suggesting poor selectivity when comparing these two cell lines. Nonetheless, both compounds showed moderate selectivity toward colon cancer cells, while compound **1a** was 2.8 times more active against HCT 116 cells than against nontumor cells, and compound **2d** was 4.5 times more active. According to Franzoni et al.[16] compounds exhibiting $SI > 5$ are considered highly selective, those with $2 < SI < 5$ as moderately selective, and those with $SI < 2$ as displaying poor selectivity. Doxorubicin was highly selective to HCT 116 and moderately selective to MCF7 when compared to CDC-Co.18.

Cancer chemotherapy generally involves the combination of different drugs. Herein, to explore the effect of both compounds **1a** and **2d** in association with doxorubicin, each chalcone was incubated with the anthracycline in serial concentrations, with 20 μM being the highest concentration

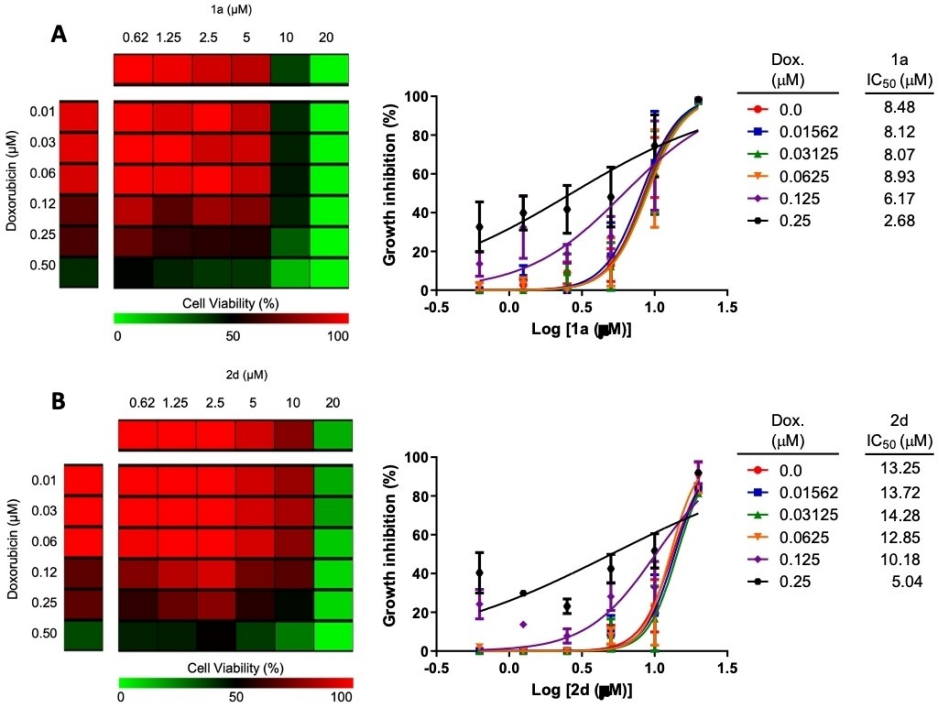


Figure 1. Dose–response curves of compound **1a** (0.625–10 μM) (**A**) and compound **2d** (0.625–10 μM) (**B**) cytotoxicity in combination with doxorubicin (0.015–0.25 μM) after 72 h of treatment in HCT 116 cells. Values are expressed as the mean and standard error of the mean (mean ± SEM) of three independent experiments (n = 3).

Table 2. Half maximal inhibition concentration (IC_{50}) of **1a** and **2b** in HTC 116 (colon adenocarcinoma cells), MCF7 (breast adenocarcinoma cells) and CCD-18-Co (nontumor colon cells). Doxorubicin was used as a positive control. Selectivity index (SI) of compounds **1a** and **2d** and doxorubicin for colorectal carcinoma (HCT 116) and breast adenocarcinoma (MCF-7) compared to nontumor colon cells (CCD-18Co): [IC_{50} of nontumor cells (CCD-18Co)/IC_{50} of tumor cells (HCT 116 or MCF7)].

	HCT 116 IC_{50} (CI95 %) μM	MCF7 IC_{50} (CI95 %) μM	CCD-18-Co IC_{50} (CI95 %) μM	HCT 116 SI	MCF7 SI
1a	10.2 (8.0–13.0)	26.4 (23.4–29.8)	28.6 (23.0–35.4)	2.8	1.1
2d	10.7 (7.4–15.4)	44.8 n.d.	48.2 (36.3–64.0)	4.5	1.1
Doxorubicin	0.2 (0.1–0.2	1.2 (0.9–1.6)	3.0 (1.9–4.8)	15.0	2.5

Data correspond to two independent experiments performed in duplicate. The IC_{50} values and their 95 % confidence intervals (CI95 %) were obtained by nonlinear regression using GraphPad Prism.

used in the combination assay. The assays were conducted in HCT116 cells with 72 h of incubation. Nonetheless, there was no significant synergic effect between the substances and the positive control since a reduction in the IC_{50} values was not observed (Figure 1). These results indicate that there is no crosstalk pathway between the signaling pathways modulated by doxorubicin and compounds **1a** and **2d**.

Materials and Methods

Instruments and Reagents

Solvents with analytical grades and purities higher than 99.5 % were purchased from Synth (São Paulo, SP, Brazil). Dimethylformamide, dichloromethane, and triethylamine were dried following literature procedures.[17,18] Commercial acids (4-nitrobenzoic acid, 3-chlorobenzoic acid, 4-chlorobenzoic acid), commercial acid chlorides (−trifluoromethylbenzoyl chloride, 3,5-dinitrobenzoyl chloride), oxalyl chloride ReagentPlus®, and deuterated solvent ($CDCl_3$) were purchased from Sigma−Aldrich (St. Louis, MO, USA) and used as received. N,N-dimethyl-4-aminopyridine (DMAP) was purchased from Fluka (St. Gallen, Germany) and used as received. 3-trifluoromethylbenzoic acid, 3-iodobenzoic acid, and 4-methoxybenzoic acid[19–21] were prepared following literature procedure and compounds 4-chlorobenzoyl chloride (**a**), 3-chlorobenzoyl chloride

(**b**), 4-nitrobenzoyl chloride (**c**), 3-(trifluoromethyl)benzoyl chloride (**d**), 3-iodobenzoyl chloride (**e**), 4-methoxybenzoyl chloride (**f**) were prepared as described in Scheme 2. Reactions were run under an argon atmosphere using oven-dried glassware. Column chromatography was performed using silica gel with a particle size of 0.063–0.200 mm at the stationary phase and hexane:ethyl acetate at the mobile phase. Nuclear magnetic resonance (NMR) spectra were recorded on a Varian 400 MHz instrument (Palo Alto, USA) using deuterated chloroform ($CDCl_3$) and dimethyl sulfoxide (DMSO-D_6) as solvents and tetramethylsilane (TMS) as internal standard. The chemical shifts (δ) are reported in ppm and *J* values in Hertz (Hz). Infrared spectra were recorded on an Agilent Technologies Cary 630 FTIR (FTIR spectrometer) by Agilent Technologies (Santa Clara, USA) with an attenuated total reflection (ATR) module, a spectral range from 4,000 to 400 cm^{-1}, 16 scans, and 4 cm^{-1} resolution. Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS) analyses were performed with a SOLARIX 9.4 T mass spectrometer (Bruker Daltonics, Bremen, Germany) coupled to an electrospray source (ESI) configured to operate in positive ionization mode (ESI^+); the compound dissolved in methanol (MeOH) from Sigma Aldrich (St. Louis, MO, USA) was injected directly. This provided an unambiguous molecular formula assignment for singly charged molecular ions, such as $[M-H]^-$ or $[M+H]^+$ values.

3.2. Synthetic Procedures

3.2.1. Synthesis of 3′-hydroxychalcone

3′-Hydroxychalcone was synthesized by the Claisen-Schmidt reaction using protocols previously reported by our group[22,23] with minor modifications. In the reaction flask, 3′-hydroxyacetophenone (10 mmol) and benzaldehyde (10.5 mmol) were solubilized in NaOH ethanolic solution (1.0 mol L^{-1}, 30 mL). The reaction mixture was stirred at room temperature. The progress of the reaction was monitored by thin-layer chromatography (TLC) using hexane and ethyl acetate (8.5:1.5) and revealed under UV light (254 and 365 nm). After 72 h, the reaction medium was poured into crushed ice from deionized water and acidified with HCl solution (1.0 mol L^{-1}) at pH 2. The precipitate was filtered, washed with cold deionized water, and dried at room temperature. The crude product was purified by a chromatography column over silica gel and eluted with hexanes and ethyl acetate (9:1), furnishing 3′-hydroxychalcone (44% yield).

Esters Derived from Acid Chlorides

A 10 mL round-bottom flask was charged with 3′-hydroxychalcone (57 mg, 0.25 mmol), Et_3N (66 μL, 0.47 mmol, 1.9 eq.), DMAP (10 mol-%), and CH_2Cl_2 (1 mL). The resulting yellow solution was then cooled to 0 °C, and acid chloride (1.4 eq.) was added dropwise at the same temperature. The reaction mixture was further stirred at room temperature for 3 h and then quenched with H_2O. The aqueous phase was extracted three times with CH_2Cl_2. The combined organic layer was sequentially washed with HCl 0.2 M (2×10 mL), saturated aqueous $NaHCO_3$ (3×10 mL), and brine (1×10 mL). After drying (Na_2SO_4) and removal of solvent under reduced pressure, the crude material was purified either by column chromatography using hexane-EtOAc as the eluent or by recrystallization from hexane-CH_2Cl_2.

Compound 3′-hydroxychalcone (**1**): ^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) 7.81 (d, 1H, J 15.6 Hz, CH) 7.67 (t, 1H, J 1.9 Hz, CH) 7.58–7.63 (m, 2H, 2CH) 7.55 (dt, 1H, J 0.8, 7.8 Hz, CH) 7.50 (d, 1H, J 15.6 Hz, CH) 7.38–7.42 (m, 3H, 3CH) 7.35 (t, 1H, J 7.8 Hz, CH) 7.16 (ddd, 1H, J 0.8, 1.9, 8.2 Hz, CH). ^{13}C NMR (100 MHz, CDCl3): δ (ppm) 191.2 (CO) 156.7 (CO) 145.6 (CH) 139.3 (C) 134.6 (C) 130.7 (CH) 129.9 (CH) 128.9 (2CH) 128.6 (2CH) 121.9 (CH) 120.9 (CH) 120.7 (CH) 115.3 (CH).

Compound *(E)*-3-cinnamoylphenyl-3,5-dinitrobenzoate (**1a**): 18 mg (0.043 mmol, 17%); slightly yellow solid; recrystallization; FT-IR (attenuated total reflection- ATR) ν_{max}/cm^{-1} 3,106, 1,741, 1,662, 1,541, 1,342. ^{1}H-NMR (400 MHz, DMSO-D_6): δ (ppm) 9.14–9.11 (m, 3H, CH) 8.20–8.16 (m, 2H, CH) 7.96 (d, 1H, *J* 15.6 Hz, CH) 7.92–7.88 (m, 2H, CH) 7.80 (d, 1H, *J* 15.6 Hz, CH) 7.74–7.72 (m, 2H, CH) 7.49–7.45 (m, 3H, CH). ^{13}C NMR (100 MHz, DMSO-D_6): δ (ppm) 188.1 (CO) 161.6 (OCO) 150.6 (C) 148.4 (CN) 148.4 (CN) 144.7 (CH) 139.1 (C) 134.5 (C) 132.1 (C) 130.8 (CH) 130.3 (CH) 129.4 (CH) 129.4 (CH) 129.0 (CH) 129.0 (CH) 128.9 (CH) 128.9 (CH) 126.7 (CH) 126.6 (CH) 123.1 (CH) 121.8 (CH) 121.7 (CH). HR-ESI- MS $[M+Na]^+$ Found: 441.06931 Calc. for $C_{22}H_{14}NaN_2O_7^+$ 441.06932; $[2M+Na]^+$ Found: 859.14951 Calc. for $C_{46}H_{28}NaN_4O_{14}^+$ 859.14943.

Compound *(E)*-3-cinnamoylphenyl-4-trifluoromethylbenzoate (**1b**): 55 mg (0.14 mmol, 56%); slightly yellow needles; recrystallization; FT-IR (attenuated total reflection-ATR) ν_{max}/cm^{-1} 1,735, 1,662, 1,323, 1,267, 1,075. ^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) 8.34 (brd, 2H, *J* 7.8 Hz, CH) 7.96 (dt, 1H, *J* 1.5, 7.8 Hz, CH) 7.88–7.86 (m, 1H, CH) 7.82 (d, 1H, *J* 15.6 Hz, CH) 7.80 (m, 2H, CH) 7.67–7.64 (m, 2H, CH) 7.60 (dd, 1H, *J* 7.8, 8.2 Hz, CH) 7.51 (d, 1H, *J* 15.6 Hz, CH) 7.47 (ddd, 1H, *J* 1.2, 2.3, 8.2 Hz, CH) 7.45–7.40 (m, 3H, CH). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 189.3 (CO) 163.8 (OCO) 150.9 (CO) 145.6 (CH) 139.8 (C) 135.4 (C) 135.1 (C) 134.7 (C) 130.8 (CH) 130.8 (CH) 130.6 (CH) 129.9 (CH) 129.0 (CH) 129.0 (CH) 128.5 (CH) 128.5 (CH) 126.2 (CH) 126.0 (CH) 125.7 (CH) 125.6 (CH) 121.7 (CH) 121.6 (CH). HR-ESI- MS $[M+H]^+$ Found: 397.10461 Calc. for $C_{23}H_{16}F_3O_3^+$ 397.10460; $[M+Na]^+$ Found: 419.08652 Calc. for $C_{23}H_{15}NaF_3O_3^+$ 419.08655; $[2M+Na]^+$ Found: 815.18349 Calc. for $C_{44}H_{30}NaF_6O_6^+$ 815.18387.

Esters Derived from Carboxylic Acid

To a solution of aryl carboxylic acid (0.45 mmol, 1.8 eq.) and cat. anhydrous DMF (2 drops) in CH_2Cl_2 (1.5 mL) at 0 °C, oxalyl chloride (52 μL, 0.60 mmol) was added dropwise with vigorous gas evolution. After further stirring the reaction mixture at room temperature for 12 h, excess oxalyl chloride was removed under vacuum. The residue was diluted with dry CH_2Cl_2 (1 mL), and the resulting acid chloride solution was added dropwise to a solution of 3′-hydroxychalcone (56 mg, 0.25 mmol), Et_3N (95 μL, 0.68 mmol, 2.7 eq.), and DMAP (10 mol-%) in CH_2Cl_2 (1 mL) cooled at 0 °C. After stirring for 1–2 h at room temperature, the reaction was performed as described in Scheme 2.

Compound *(E)*-3-cinnamoylphenyl-4-chlorobenzoate (**2a**): 67 mg (0.18 mmol, 72%); slightly yellow solid; purification by column chromatography (hexane-EtOAc; 47:3); FT-IR (attenuated total reflection- ATR) ν_{max}/cm^{-1} 1,725, 1,663, 1,270. ^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) 8.16 (d, 2H, *J* 8.6 Hz, CH) 7.95 (dt, 1H, *J* 1.2; 7.8 Hz, CH) 7.85 (d, 1H, *J* 2.0 Hz, CH) 7.84 (d, 1H, *J* 15.6 Hz, CH) 7.67–7.64 (m, 2H, CH) 7.58 (t, 1H, *J* 7.8 Hz, CH) 7.51 (d, 2H, *J* 8.6 Hz, CH) 7.50 (d, 1H, *J* 15.6 Hz, CH) 7.45 (ddd, 1H, *J* 1.2; 2.0; 7.8 Hz, CH) 7.44—7.41 (m, 3H, CH). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 189.4 (CO) 164.2 (OCO) 151.0 (CO) 145.5 (CH) 140.4 (CCl) 139.8 (C) 134.7 (C) 131.6 (CH) 131.6 (CH) 130.7 (CH) 129.8 (CH) 129.0 (CH) 129.0 (CH) 129.0 (CH) 129.0 (CH) 129.0 (CH) 128.5 (CH) 128.5 (CH) 127.6 (C) 126.1 (CH) 126.1 (CH) 126.1 (CH) 121.8 (CH). HR-ESI-MS $[M+H]^+$ Found: 363.07825 Calc. for $C_{22}H_{16}ClO_3^+$ 363.07825; $[M+Na]^+$ Found: 385.06016 Calc. for $C_{22}H_{15}NaClO_3^+$ 385.06019; $[2M+Na]^+$ Found: 747.13108 Calc. for $C_{44}H_{30}NaCl_2O_6^+$ 747.13116.

Compound *(E)*-3-cinnamoylphenyl-3-chlorobenzoate (**2b**): 70 mg (0.19 mmol, 76%); slightly yellow solid; purification by column chromatography (hexane-EtOAc; 23:2); FT-IR (attenuated total reflection- ATR) ν_{max}/cm^{-1} 1,735, 1,660, 1,221. ^{1}H-NMR (400 MHz,

$CDCl_3$): δ (ppm) 8.21 (brt, 1H, *J* 2.0 Hz, CH) 8.11 (ddd, 1H, *J* 1.2; 2.0; 7.8 Hz, CH) 7.95 (ddd, 1H, *J* 1.2; 2.0; 7.8 Hz, CH) 7.86 (d, 1H, *J* 2.0 Hz, CH) 7.84 (d, 1H, *J* 15.6 Hz, CH) 7.68–7.64 (m, 2H, CH) 7.63 (ddd, 1H, *J* 1.2; 2.0; 8.2 Hz, CH) 7.59 (dd, 1H, J = 7.8; 8.2 Hz, CH) 7.50 (d, 1H, *J* 15.6 Hz, CH) 7.49 (dd, 1H, *J* 7.8; 8.2 Hz, CH) 7.46 (ddd, 1H, *J* 1.2; 2.0; 8.2 Hz, CH) 7.44–7.41 (m, ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 189.3 (CO) 163.8 (OCO) 150.9 (CO) 145.5 (CH) 139.8 (C) 134.7 (C) 133.8 (CH) 130.9 (C) 130.7 (CH) 130.2 (CH) 130.0 (CH) 129.8 (CH) 129.0 (CH) 129.0 (CH) 128.5 (CH) 128.5 (CH) 128.3 (CH) 126.1 (CH) 126.0 (CH) 121.7 (CH) 121.6 (CH). HR-ESI- MS $[M+H]^+$ Found: 363.07822 Calc. for $C_{22}H_{16}ClO_3^+$ 363.07825; $[M+Na]^+$ Found: 385.06016 Calc. for $C_{22}H_{15}NaClO_3^+$ 385.06019; $[2M+Na]^+$ Found: 747.13107 Calc. for $C_{44}H_{30}NaCl_2O_6^+$ 747.13116.

Compound *(E)*-3-cinnamoylphenyl-4-nitrobenzoate (**2c**): 27 mg (0.07 mmol, 28%); slightly yellow solid; recrystallization; FT-IR (attenuated total reflection- ATR) ν_{max}/cm^{-1} 1,731, 1,662, 1,520, 1,273, 1,230. ^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) 8.41 (d, 2H, *J* 8.6 Hz, CH) 8.37 (d, 2H, J 8.6 Hz, CH) 7.97 (ddd, 1H, *J* 1.2; 2.0; 7.8 Hz CH) 7.89 (t, 1H, *J* 2.0 Hz, CH) 7.86 (d, 1H, *J* 15.6 Hz, CH) 7.68–7.64 (m, 2H, CH) 7.61 (dd, 1H, *J* 7.8; 8.2 Hz, CH) 7.51 (d, 1H, *J* 15.6 Hz, CH) 7.48 (ddd, 1H, *J* 1.2; 2.0; 8.2 Hz, CH) 7.45–7.41 (m, 3H, CH). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 189.2 (CO) 163.2 (OCO) 151.0 (CO) 150.8 (CN) 145.7 (CH) 139.9 (C) 134.6 (C) 134.6 (C) 131.4 (CH) 131.4 (CH) 130.8 (CH) 130.0 (CH) 129.0 (CH) 129.0 (CH) 128.5 (CH) 128.5 (CH) 126.4 (CH) 125.8 (CH) 123.7 (CH) 123.7 (CH) 121.6 (CH) 121.6 (CH). HR-ESI- MS $[M+H]^+$ Found: 373.09502 Calc. for $C_{22}H_{16}NO_5^+$ 373.09502; $[M+Na]^+$ Found: 396.08421 Calc. for $C_{22}H_{15}NaNO_5^+$ 396.08424; $[2M+Na]^+$ Found: 769.17917 Calc. for $C_{44}H_{30}NaN_2O_{10}^+$ 769.17926.

Compound *(E)*-3-cinnamoylphenyl-3-trifluoromethylbenzoate (**2d**): 65 mg (0.16 mmol, 64%); slightly yellow solid; recrystallization; FT-IR (attenuated total reflection- ATR) ν_{max}/cm^{-1} 1,735, 1,662, 1,323, 1,267, 1,075. ^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) 8.50 (brs, 1H, CH) 8.42 (brd, 1H, *J* 7.8 Hz, CH) 7.96 (ddd, 1H, *J* 1.5; 2.0; 7.8 Hz, CH) 7.92 (brd, 1H, *J* 7.8 Hz, CH) 7.88 (d, 1H, *J* 2.0 Hz, CH) 7.85 (d, 1H, *J* 15.6 Hz, CH) 7.70 (dd, 1H, *J* 7.8; 8.2 Hz, CH) 7.67–7.64 (m, 2H, CH) 7.60 (dd, 1H, *J* 7.8; 8.2 Hz, CH) 7.51 (d, 1H, *J* 15.6 Hz, CH) 7.48 (ddd, 1H, *J* 1.5; 2.0; 8.2 Hz, CH) 7.44–7.41 (m, 3H, CH). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 189.3 (CO) 163.7 (OCO) 150.9 (CO) 145.6 (CH) 139.8 (C) 134.7 (C) 133.4 (CH) 130.8 (CH) 130.3 (C) 130.1 (CH) 129.9 (CH) 129.4 (CH) 129.0 (CH) 129.0 (CH) 128.5 (CH) 128.5 (CH) 127.1 (CF_3) 127.1 (CH) 126.2 (CH) 126.0 (CH) 121.7 (CH) 121.6 (CH). HR-ESI- MS $[M+H]^+$ Found: 397.10458 Calc. for $C_{23}H_{16}F_3O_3^+$ 397.10460; $[M+Na]^+$: Found: 419.08647 Calc. for $C_{23}H_{15}NaF_3O_3^+$ 419.08655; $[2M+Na]^+$ Found: 815.18382 Calc. for $C_{46}H_{30}NaN_4O_{10}^+$ 815.18387.

Compound *(E)*-3-cinnamoylphenyl-3-iodobenzoate (**2e**): 51 mg (0.11 mmol, 44%); slightly yellow solid; recrystallization. ^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) 8.56 (dd, 1H, *J* 1.2; 2.0 Hz, CH) 8.19 (ddd, 1H, *J* 1.2; 2.0; 8.2 Hz, CH) 7.99 (ddd, 1H, *J* 1.2; 2.0; 7.8 Hz, CH) 7.95 (ddd, 1H, *J* 1.2; 2.0; 8.2 Hz, CH) 7.86 (t, 1H, *J* 2.0 Hz, CH) 7.85 (d, 1H, *J* 15.6 Hz, CH) 7.68–7.64 (m, 2H, CH) 7.57 (dd, 1H, *J* 7.8; 8.2 Hz, CH) 7.51 (d, 1H, *J* 15.6 Hz, CH) 7.45 (ddd, 1H, *J* 1.2; 2.0; 8.2 Hz, CH) 7.44–7.41 (m, 3H, CH) 7.28 (dd, 1H, *J* 7.8 ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 189.3 (CO) 163.5 (OCO) 150.9 (CO) 145.5 (CH) 142.7 (CH) 139.8 (C) 139.0 (CH) 134.7 (C) 131.1 (C) 130.7 (CH) 130.3 (CH) 129.8 (CH) 129.4 (CH) 129.0 (CH) 129.0 (CH) 128.5 (CH) 128.5 (CI) 126.1 (CH) 126.0 (CH) 121.7 (CH) 121.7 (CH). HR-ESI- MS $[M+H]^+$ Found: 455.01382 Calc. for $C_{22}H_{16}IO_3^+$ 455.01386; $[M+Na]^+$: Found: 476.99574 Calc. for $C_{22}H_{15}NaIO_3^+$ 476.99580; $[2M+Na]^+$ Found: 931.00214 Calc. for $C_{44}H_{30}NaIO_6^+$ 931.00239.

Compound *(E)*-3-cinnamoylphenyl 4-methoxybenzoate (**2f**): 49.9 mg (0.14 mmol, 56%), slightly yellow solid; recrystallization. ^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) 8.18 (d, 2H, *J* 9.0 Hz, CH) 7.93 (ddd, 1H, *J* 1.2; 1.5; 7.8 Hz, CH) 7.85 (brd, 1H, *J* 2.0 Hz, CH) 7.84 (d, 1H, *J* 15.6 Hz, CH) 7.67–7.64 (m, 2H, CH) 7.56 (dd, 1H, J 7.8; 8.2 Hz, CH) 7.51 (d, 1H, *J* 15.6 Hz, CH) 7.45 (ddd, 1H, *J* 1.2; 2.0; 8.2 Hz, CH) 7.43–7.41 (m, 3H, CH) 7.0 (d, 2H, *J* 9.0 Hz, CH) 3.90 (s, 3H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 189.5 (CO) 164.7 (C) 164.1 (OCO) 151.3 (CO) 145.3 (CH) 139.6 (C) 134.7 (C) 132.4 (CH) 132.4 (CH) 130.6 (CH) 129.7 (CH) 128.9 (CH) 128.9 (CH) 128.5 (CH) 128.5 (CH) 126.3 (CH) 125.7 (CH) 121.9 (CH) 121.8 (CH) 121.4 (C) 113.9 (CH) 113.9 (CH) 55.2 ($OCCH_3$). HR-ESI-MS $[M+H]^+$ Found: 359.12775 Calc. for $C_{23}H_{19}O_4^+$ 359.12778; $[M+Na]^+$ Found: 381.10967 Calc. for $C_{23}H_{18}NaO_4^+$ 381.10973; $[2M+Na]^+$ Found: 739.23017 Calc. for $C_{46}H_{36}NaO_8^+$ 739.23024.

Cytotoxic Evaluation

HCT 116 (colon adenocarcinoma, ATCC® 247™), MCF7 (breast adenocarcinoma, ATCC® HTB-22™), and CCD-18Co (nontumoral colon fibroblasts, ATCC® CRL-1459™) cells were obtained from the American Type Culture Collection (ATCC). Cell culture conditions were developed using Roswell Park Memorial Institute (RPMI) medium (Thermo Fisher Scientific, San Jose, CA, USA) for HCT 116, Dulbecco's modified Eagle's medium (DMEM) (Thermo Fisher Scientific) for MCF7, and Eagle's minimum essential medium (EMEM) for CCD-18Co (Thermo Fisher Scientific), supplemented with 10% fetal bovine serum (FBS) (Thermo Fisher Scientific) and 1% penicillin−streptomycin (Thermo Fisher Scientific). Cells were kept in a humidified atmosphere with 5% CO_2 at 37 °C. For the methylthiazol tetrazolium (MTT) (Thermo Fisher Scientific) assay,[24] cells were seeded on 96-well plates at a density of 6×10^3 per well with 200 μL culture medium. After 24 h, the samples were tested at different concentrations, 5 μM and 50 μM for screening and concentrations from 0.0064 to 50 μM for the determination of the half inhibitory concentration (IC_{50}) of each compound. After the treatment, the plates were incubated for 72 h. Dimethyl sulfoxide (DMSO; 0.05%) (Synth, Diadema, SP, Brazil) was used as a negative control, and the antineoplastic compound doxorubicin (0.00064–10 μM) (Sigma–Aldrich, St. Louis, MO, USA) was used as a positive control. For combined treatment analysis, HCT 116 cells were treated with graded doses of doxorubicin (0.01, 0.03, 0.06, 0.12, 0.25, or 0.5 μM) and compounds **1a** and **2d** (0.62, 1.25, 2.5, 5, 10, or 20 μM) alone or in combination for 72 h. Data were illustrated using the multiple experiment viewer (MeV) 4.9.0 software. Culture media were replaced by fresh media containing MTT solution (0.5 mg mL^{-1}) 3 h before the end of the experiment. After 3 h, the MTT solution was removed, and the formazan product was solubilized in 150 μL DMSO. The absorbance was obtained at 570 nm. The IC_{50} values and their 95% confidence intervals were estimated by sigmoidal nonlinear regression using GraphPad Prism 6 software.

3. Conclusions

The formation of ester derivatives from chalcones is possible from the esterification reaction. While most of the obtained compounds did not show high cytotoxic potential, compound **2d** showed an interesting IC_{50} in colon cancer cells (HCT 116), being selective against cancer cells when compared to non-tumor cells. Further studies are in progress to characterize their mechanism of action and potential targets in colon cancer cells.

Supporting Information Summary

The following supporting information can be downloaded at https://onlinelibrary.wiley.com/journal/16121880. Figures S1–S53: NMR and FT-ICR MS data of compounds **1**, **1a**–**b**, and **2a**–**f**.

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Author Contributions

R.B.S.: Investigation (supporting), Visualization (supporting); J.R.P.: Investigation (supporting), Visualization (supporting); C.R.S.: Writing original draft (equal); Investigation (supporting); L.R.Q.: Investigation (supporting), Visualization (supporting); L.C.A.: Investigation (supporting), Visualization (supporting); L.V.C.-L.: Investigation (lead), Visualization (supporting), Writing original draft (equal), Funding acquisition (supporting); L.O.R.: Funding acquisition (supporting), Resources (supporting); R.R.K.: Investigation (supporting), Writing original draft (equal), Visualization (supporting); E.F.M.: Investigation (supporting), Writing original draft (equal), Visualization (supporting); W.S.B.: Investigation (lead), Writing original draft (equal), Visualization (supporting), Funding acquisition (lead), Resources (lead). All authors have read and agreed to the published version of the manuscript.

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Conflict of Interests

The authors declare that they have no conflicts of interest regarding the publication of this paper.

Data Availability Statement

The data used to support the findings of this study are available upon reasonable request from the corresponding author.

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