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Article

Synthesis, Characterization and *in vitro* Anticancer Activity of Novel 8,4'-Oxyneolignan Analogues

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Neolignans are a class of natural products with a wide range of biological effects. These substances are of great synthetic and biological interest, especially in searching for novel anticancer agents. In this paper, we report the synthesis of a new subclass of 8,4'-oxyneolignan analogues (β-ketoethers and β-ketoesters) and their cell viability assay on twenty four different cancer cells, among leukemias and carcinomas. Three compounds inhibited the growth of most human cancer cells. 2-Oxo-2-phenylethyl(2*E*)-3-[4-(2-oxo-2-phenylethoxy) phenyl]prop-2-enoate showed an antiproliferative activity superior to doxorubicin for U-87, U-138 MG and H1299 cell types and (*E*)-2-oxo-2-phenylethyl 3-(3-methoxy-4-(2-oxo-2-phenylethoxy)phenyl)acrylate was found to be very selective, demonstrating a growth inhibition of 92.0% against KG-1 cells. Furthermore, 1-oxo-1-phenylpropan-2-yl cinnamate exhibited significant inhibition activity in a range of 52.2 to 91.2% against twelve kinds of leukemia cell lines, revealing excellent results and very comparable to the reference drug.

Keywords: neolignans, antiproliferative activity, MTT assay, β-ketoester, β-ketoether

Introduction

Cancer is a generic term for a large group of diseases that can affect any part of the body and remains a leading cause of death worldwide. It is considered a public health problem according to the World Health Organization, and many efforts have been made towards its prevention and cure. Treatment usually involves a series of interventions, and approximately 90% of tumors can be treated with antiblastic drugs,[1,2] which makes chemotherapy the most used treatment. However, these drugs are non-selective and are toxic to healthy tissues, especially those of rapid cell proliferation.[2-5] As a consequence, there is an urgent need for novel and effective drugs that act against cancer.

Several substances have been thoroughly studied for their biological activities and a very promising class are the neolignans, which are substances derived from the oxidative coupling of allyl and/or propenyl phenols and are normally found in plants of the *Myristicacea* family.[6-8] Neolignans have a wide range of biological effects such as antioxidant,[9-14] antibacterial,[15-17] anti-inflammatory,[12,18-21] antifungal,[22,23] anti-leishmanial,[24-28] anti-trypanosomastid,[27,28] and anticancer activity,[13,14,29,30] among others.[31-34] Therefore, this type of natural product is of great synthetic and biological interest, especially in searching for novel anticancer agents. In this paper, we report the synthesis of new 8,4'-oxyneolignan analogues and the cell viability assays for different neoplasms, among leukemias and carcinomas.

Experimental

Chemistry

All reagents were purchased from commercial suppliers and were used as received, unless otherwise specified. Reactions were monitored by thin layer chromatography

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(TLC) using aluminum plates from Merck (silica gel 60 F_{254}). Melting points (mp) were determined using a PFM II apparatus (model 382). Proton (^{1}H) and carbon (^{13}C) nuclear magnetic resonance (NMR) spectra were recorded on the following spectrometers: Bruker AC 250/P, Varian Mercury Plus 300 MHz, Bruker Avance 600 MHz or Varian Inova 500 MHz. Chemical shifts are reported in ppm (δ) with values relative to TMS used as internal standard. High-resolution mass spectra (HRMS) were recorded on a VG AutoSpec High Resolution Mass Spectrometer (Micromass Company) or on a triple TOF 5600+ High Resolution Mass Spectrometer (AB Sciex) with internal calibration and direct solution (1 ppm in methanol).

General procedure for the synthesis of 8,4'-oxyneolignan analogues: β-ketoesters and β-ketoethers

A solution of 1.02 equivalent of phenols (or 0.51 equivalent of cinnamic acid derivatives) and 1.80 equivalent of anhydrous K_2CO_3 in anhydrous butanone (4.5 mL of solvent *per* mmol of phenol or cinnamic acid derivative) was stirred for 15 min at room temperature. After this period, a solution of 1.00 equivalent of α-bromoketone in anhydrous butanone (1.5 mL of solvent *per* mmol of ketone) was added dropwise and the mixture was stirred under reflux for 24 h. The solution was cooled to room temperature, filtered, and the residue washed with $CHCl_3$. The solution was concentrated in vacuum (to remove butanone), diluted with H_2O, and extracted with $CHCl_3$ (3×). The organic extracts were combined, washed with water, 5% NaOH solution, brine, dried over Na_2SO_4 and then filtered and concentrated in vacuum. The reaction products were purified by crystallization or column chromatography.

2-(4-Nitro-phenoxy)-1-phenyl-ethanone (**3a**)

Obtained according to the general procedure from 1.50 g (10.76 mmol) of 4-nitrophenol, 2.10 g (10.55 mmol) of phenacyl bromide, 2.62 g (19.00 mmol) of K_2CO_3 and purified by recrystallization from acetone/hexane (84% yield). The product obtained was an orange crystalline solid (mp 148-150 °C). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.20 (d, *J* 9.0 Hz, 2H, Ar-H), 7.99 (d, *J* 7.2 Hz, 2H, Ar-H), 7.66 (t, *J* 7.2 Hz, 1H, Ar-H), 7.54 (t, *J* 9.0 Hz, 2H, Ar-H), 6.99 (d, *J* 9.0 Hz, 2H, Ar-H), 5.43 (s, 2H, CH_2); ^{13}C NMR (150 MHz, $CDCl_3$) δ 192.8, 163.0, 142.1, 134.4, 134.0, 129.1, 128.0, 125.9, 114.8, 70.6. HREIMS *m/z* 280.0581 $[M + Na]^+$ (calcd. for $C_{14}H_{11}NNaO_4^+$, 280.0580).

2-(3,5-Dichlorophenoxy)-1-phenylethanone (**3b**)

Obtained according to the general procedure from 0.83 g (5.07 mmol) of 2,4-dichlorophenol, 1.00 g (5.02 mmol) of phenacyl bromide and 1.25 g (9.04 mmol) of K_2CO_3 and purified by recrystallization from acetone/hexane (42% yield). The product obtained was a light brown crystalline solid (mp 74-75 °C). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.00 (d, *J* 7.2 Hz, 2H, Ar-H), 7.63 (t, *J* 7.2 Hz, 1H, Ar-H), 7.50 (t, *J* 7.2 Hz, 2H, Ar-H), 7.38 (d, *J* 3.0 Hz, 1H), 7.13 (dd, J_1 9.0 Hz, J_2 3.0 Hz, 1H, Ar-H), 6.78 (d, *J* 9.0 Hz, 1H, Ar-H), 5.34 (s, 2H, CH_2); ^{13}C NMR (150 MHz, $CDCl_3$) δ 193.7, 152.7, 134.3, 134.3, 130.4, 129.0, 128.3, 127.7, 127.0, 124.3, 115.1, 72.1. HREIMS *m/z* 302.9949 $[M + Na]^+$ (calcd. for $C_{14}H_{10}Cl_2NaO_2^+$, 302.9951).

2-(4-Nitro-phenoxy)-1-phenyl-propan-1-one (**3c**)

Obtained according to the general procedure from 0.55 g (3.93 mmol) of 4-nitrophenol, 0.82 g (3.85 mmol) of 2-bromopropiophenone and 0.96 g (6.93 mmol) of K_2CO_3 and purified by recrystallization from methanol (91% yield). The product obtained was a white crystalline solid (mp 78-80 °C). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.14 (d, *J* 9.0 Hz, 2H, Ar-H), 8.04 (d, *J* 7.8 Hz, 2H, Ar-H), 7.63 (t, *J* 7.8 Hz, 1H, Ar-H), 7.51 (t, *J* 7.8 Hz, 2H, Ar-H), 6.90 (d, *J* 9.0 Hz, 2H, Ar-H), 5.63 (q, *J* 6.6 Hz, 1H, CH), 1.78 (d, *J* 6.6 Hz, 3H, CH_3); ^{13}C NMR (150 MHz, $CDCl_3$) δ 197.2, 162.4, 142.0, 134.2, 133.7, 129.0, 128.7, 126.0, 115.0, 76.9, 18.8. HREIMS *m/z* 294.0737 $[M + Na]^+$ (calcd. for $C_{15}H_{13}NNaO_4^+$, 294.0737).

2-Pentachlorophenyloxy-1-phenyl-ethanone (**3d**)

Obtained according to the general procedure from 0.68 g (2.56 mmol) of pentachlorophenol, 0.50 g (2.51 mmol) of phenacyl bromide and 0.62 g (4.52 mmol) of K_2CO_3 and purified by recrystallization from methanol/CH_2Cl_2 (100% yield). The product obtained was a white crystalline solid (mp 125-127 °C). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.97 (d, *J* 7.2 Hz, 2H, Ar-H), 7.63 (t, *J* 7.2 Hz, 1H, Ar-H), 7.51 (t, *J* 7.2 Hz, 2H, Ar-H), 5.30 (s, 2H, CH_2); ^{13}C NMR (150 MHz, $CDCl_3$) δ 192.1, 151.2, 134.3, 134.2, 132.2, 130.2, 129.1, 128.3, 128.2, 74.8. HREIMS *m/z* 406.8745 $[M + Na]^+$ (calcd. for $C_{14}H_7Cl_5NaO_2^+$, 406.8752).

1-Phenyl-2-*m*-tolyloxy-ethanone (**3e**)

Obtained according to the general procedure from 0.28 g (2.56 mmol) of *m*-cresol, 0.50 g (2.51 mmol) of phenacyl bromide and 0.62 g (4.52 mmol) of K_2CO_3 and purified by recrystallization from methanol (69% yield). The product obtained was a yellow crystalline solid (mp 70-72 °C). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.01 (d, *J* 7.8 Hz, 2H, Ar-H), 7.61 (t, *J* 7.8 Hz, 1H, Ar-H), 7.50 (t, *J* 7.8 Hz, 2H, Ar-H), 7.16 (t, *J* 7.8 Hz, 1H, Ar-H), 6.80 (d, *J* 7.8 Hz, 1H, Ar-H), 6.78 (s, 1H, Ar-H), 6.74 (dd, J_1 7.8 Hz, J_2 1.8 Hz, 1H, Ar-H), 5.24 (s,

2H, CH_2), 2.32 (s, 3H, CH_3); ^{13}C NMR (150 MHz, $CDCl_3$) δ 194.7, 158.1, 139.7, 134.7, 133.8, 129.3, 128.8, 128.2, 122.5, 115.7, 111.6, 70.8, 21.5. HREIMS *m/z* 249.0891 $[M + Na]^+$ (calcd. for $C_{15}H_{14}NaO_2^+$, 249.0886).

2-(3,5-Dichlorophenoxy)-1-phenylpropan-1-one (**3f**)

Obtained according to the general procedure from 0.39 g (2.40 mmol) of 2,4-dichlorophenol, 0.50 g (2.35 mmol) of 2-bromopropiophenone and 0.58 g (4.23 mmol) of K_2CO_3 and purified by column chromatography in hexane/acetate (80:20) (54% yield). The product was obtained as a colorless oil. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.08 (d, *J* 7.2 Hz, 2H, Ar-H), 7.59 (t, *J* 7.2 Hz, 1H, Ar-H), 7.47 (t, *J* 7.2 Hz, 2H, Ar-H), 7.35 (d, *J* 2.6 Hz, 1H, Ar-H), 7.06 (dd, J_1 8.8 Hz, J_2 2.6 Hz, 1H, Ar-H), 6.72 (d, *J* 8.8 Hz, 1H, Ar-H), 5.40 (q, *J* 6.8 Hz, 1H, CH), 1.78 (d, *J* 8.8 Hz, 3H, CH_3); ^{13}C NMR (150 MHz, $CDCl_3$) δ 194.7, 158.1, 139.7, 134.7, 133.8, 129.3, 128.8, 128.2, 122.5, 115.7, 111.61, 70.8, 21.5. HREIMS *m/z* 317.0115 $[M + Na]^+$ (calcd. for $C_{15}H_{12}Cl_2NaO_2^+$, 317.0107).

2-Pentachlorophenyloxy-1-phenyl-propan-1-one (**3g**)

Obtained according to the general procedure from 0.64 g (2.40 mmol) of pentachlorophenol, 0.50 g (2.35 mmol) of 2-bromopropiophenone and 0.58 g (4.23 mmol) of K_2CO_3 and purified by recrystallization from methanol (66% yield). The product obtained was a light brown crystalline solid (mp 111-112 °C). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.04 (d, *J* 7.2 Hz, 2H, Ar-H), 7.61 (t, *J* 7.2 Hz, 1H, Ar-H), 7.50 (t, *J* 7.2 Hz, 2H, Ar-H), 5.70 (q, *J* 7.0 Hz, 1H, CH), 1.68 (d, *J* 7.0 Hz, 3H, CH_3); ^{13}C NMR (150 MHz, $CDCl_3$) δ 196.3, 150.7, 134.5, 133.9, 132.2, 129.7, 129.1, 128.9, 128.4, 82.1, 18.8. HREIMS *m/z* 420.8903 $[M + Na]^+$ (calcd. for $C_{15}H_9Cl_5NaO_2^+$, 420.8908).

1-Phenyl-2-*m*-tolyloxy-propan-1-one (**3h**)

Obtained according to the general procedure from 0.26 g (2.40 mmol) of *m*-cresol, 0.50 g (2.35 mmol) of 2-bromopropiophenone and 0.58 g (4.23 mmol) of K_2CO_3 and purified by recrystallization from methanol (54% yield). The product obtained was a light brown crystalline solid (mp 109-111 °C). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.07 (d, *J* 7.8 Hz, 2H, Ar-H), 7.57 (t, *J* 7.8 Hz, 2H, Ar-H), 7.46 (t, *J* 7.8 Hz, 2H, Ar-H), 7.10 (t, *J* 7.9 Hz, 1H, Ar-H), 6.75 (d, *J* 7.9 Hz, 1H, Ar-H), 7.10 (t, *J* 7.9 Hz, 1H, Ar-H), 6.75 (d, *J* 7.9 Hz, 1H, Ar-H), 6.72 (s,1H, Ar-H), 6.65 (dd, J_1 7.9 Hz, J_2 2.4 Hz, 1H, Ar-H), 5.45 (q, *J* 7.0 Hz, 1H, CH), 2.27 (s, 3H, CH_3), 1.69 (d, *J* 7.0 Hz, 3H, CH_3); ^{13}C NMR (150 MHz, $CDCl_3$) δ 199.2, 157.6, 139.8, 134.4, 133.7, 129.4, 129.0, 128.9, 122.5, 116.3, 112.0, 76.7, 21.6, 18.8. HREIMS *m/z* 263.1051 $[M + Na]^+$ (calcd. for $C_{16}H_{16}NaO_2^+$, 263.1043).

1-(4-Bromophenyl)-2-(3,5-dichlorophenoxy)ethanone (**3i**)

Obtained according to the general procedure from 0.30 g (1.84 mmol) of 2,4-dichlorophenol, 0.50 g (1.80 mmol) of 2,4'-dibromoacetophenone and 0.45 g (3.24 mmol) of K_2CO_3 and purified by recrystallization from methanol/CH_2Cl_2 (39% yield). The product obtained was a white crystalline solid (mp 99-101 °C). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.88 (d, *J* 8.4 Hz, 2H, Ar-H), 7.65 (d, *J* 9.0 Hz, 2H, Ar-H), 7.39 (d, *J* 2.4 Hz, 1H, Ar-H), 7.14 (dd, J_1 9.0 Hz, J_2 2.4 Hz, 1H, Ar-H), 6.78 (d, *J* 9.0 Hz, 1H, Ar-H), 5.25 (s, 2H, CH_2); ^{13}C NMR (150 MHz, $CDCl_3$) δ 193.1, 152.4, 132.9, 132.2, 130.4, 129.8, 129.5, 127.6, 127.2, 124.2, 114.9, 72.1. HREIMS *m/z* 380.9042 $[M + Na]^+$ (calcd. for $C_{14}H_9BrCl_2NaO_2^+$, 380.9056).

1-(4-Bromophenyl)-2-(*m*-tolyloxy)ethanone (**3j**)

Obtained according to the general procedure from 0.10 g (0.92 mmol) of *m*-cresol, 0.25 g (0.90 mmol) of 2,4'-dibromoacetophenone and 0.23 g (1.62 mmol) of K_2CO_3 and purified by recrystallization from methanol (69% yield). The product obtained was a light brown crystalline solid (mp 91-93 °C). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.88 (d, *J* 8.4 Hz, 2H, Ar-H), 7.63 (d, *J* 8.4 Hz, 2H, Ar-H), 7.16 (t, *J* 8.0 Hz, 1H, Ar-H), 6.81 (d, *J* 8.0 Hz, 1H, Ar-H), 6.76 (s, 1H, Ar-H), 6.72 (d, *J* 8.0 Hz, 1H, Ar-H), 5.17 (s, 2H, CH_2), 2.32 (s, 3H, CH_3); ^{13}C NMR (150 MHz, $CDCl_3$) δ 194.1, 157.9, 139.8, 133.4, 132.1, 129.8, 129.3, 129.1, 122.7, 115.6, 111.5, 70.9, 21.5. HREIMS *m/z* 326.9997 $[M + Na]^+$ (calcd. for $C_{15}H_{13}BrNaO_2^+$, 326.9992).

2-Oxo-2-phenylethyl(2*E*)-3-[4-(2-oxo-2-phenylethoxy) phenyl]prop-2-enoate (**4a**)

Obtained according to the general procedure from 0.21 g (1.28 mmol) of *p*-hydroxycinnamic acid, 0.50 g (2.51 mmol) of phenacyl bromide and 0.62 g (4.52 mmol) of K_2CO_3 and purified by recrystallization from acetone (55% yield). The product obtained was a colorless crystalline solid (mp 145-147 °C). ^{1}H NMR (250 MHz, DMSO-d_6) δ 8.03 (d, *J* 8.8 Hz, 2H, Ar-H), 8.00 (d, *J* 8.8 Hz, 2H, Ar-H), 7.70 (t, *J* 8.8 Hz, 4H, Ar-H; d, *J* 15.8 Hz, 1H, CH), 7.50-7.63 (m, 4H, Ar-H), 7.04 (d, *J* 8.5 Hz, 2H, Ar-H), 6.65 (d, *J* 15.8 Hz, 1H, CH), 5.68 (s, 2H, CH_2), 5.60 (s, 2H, CH_2); ^{13}C NMR (62.5 MHz, DMSO-d_6) δ 194.2, 193.0, 165.9, 160.0, 145.1, 134.3, 134.0, 133.9, 130.2, 128.9, 128.9, 127.9, 127.8, 126.9, 115.1, 114.8, 70.2, 66.4. HREIMS *m/z* 401.1389 $[M + H]^+$ (calcd. for $C_{25}H_{21}O_5^+$, 401.1384).

(*E*)-2-Oxo-2-phenylethyl 3-(3-methoxy-4-(2-oxo-2-phenyl-ethoxy)phenyl)acrylate (**4b**)

Obtained according to the general procedure from 0.25 g (1.28 mmol) of ferulic acid, 0.50 g (2.51 mmol) of

phenacyl bromide and 0.57 g (4.52 mmol) of K_2CO_3 and purified by recrystallization from methanol (74% yield). The product obtained was a white solid (mp 135-137 °C). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.01 (d, *J* 7.3 Hz, 2H, Ar-H), 7.96 (d, *J* 7.3 Hz, 2H, Ar-H), 7.73 (d, *J* 16.1 Hz, 1H, CH), 7.62 (td, J_1 7.3 Hz, J_2 3.6 Hz, 2H, Ar-H), 7.50 (td, J_1 7.8 Hz, J_2 2 Hz, 4H, Ar-H), 7.12 (d, *J* 1.8 Hz, 1H, Ar-H), 7.06 (dd, J_1 8.4 Hz, J_2 1.8 Hz, 1H, Ar-H), 6.80 (d, *J* 8.4 Hz, 1H, Ar-H), 6.48 (d, *J* 16.1 Hz, 1H, CH), 5.47 (s, 2H, CH_2), 5.40 (s, 2H, CH_2), 3.93 (s, 3H, OCH_3); ^{13}C NMR (150 MHz, $CDCl_3$) δ 193.8, 192.4, 166.4, 149.8, 149.7, 145.8, 134.4, 134.4, 134.0, 133.9, 128.9, 128.6, 128.1, 127.8, 122.5, 115.3, 113.9, 110.8, 71.6, 66.0, 56.0. HREIMS *m/z* 453.1307 $[M + Na]^+$ (calcd. for $C_{26}H_{22}NaO_6^+$, 453.1309).

(*E*)-1-(4-Methoxyphenyl)-1-oxopropan-2-yl 3-(4-((1-(4-methoxyphenyl)-1-oxopropan-2-yl)oxy)phenyl) acrylate (**4c**)

Obtained according to the general procedure from 0.10 g (0.53 mmol) of *p*-hydroxycinnamic acid, 0.25 g (1.03 mmol) of 4-methoxy-8-bromopropiophenone and 0.26 g (1.85 mmol) of K_2CO_3 and purified by recrystallization from methanol (71% yield). The product obtained was a colorless crystalline solid (mp 142-144 °C). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.12 (d, *J* 8.8 Hz, 2H, Ar-H), 8.05 (d, *J* 8.8 Hz, 2H, Ar-H), 7.70 (d, *J* 8.8 Hz, 2H, Ar-H), 7.64 (d, *J* 16.0 Hz, 1H, CH), 7.19-7.08 (m, 4H, Ar-H), 6.94 (d, *J* 8.8 Hz, 2H, Ar-H), 6.61 (d, *J* 16.0 Hz, 1H, CH), 6.16-6.06 (m, 2H, CH), 3.91 (s, 3H, OCH_3), 3.90 (s, 3H, OCH_3), 1.59 (d, *J* 7.0 Hz, 3H, CH_3), 1.52 (d, *J* 7.0 Hz, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 196.1, 195.1, 165.8, 163.8, 163.6, 159.2, 144.9, 130.9, 130.8, 130.3, 126.9, 126.7, 126.7, 115.3, 115.0, 114.3, 114.2, 74.3, 71.2, 55.7, 18.5, 17.2. HREIMS *m/z* 489.1941 $[M + H]^+$ (calcd. for $C_{29}H_{29}O_7^+$, 489.1942).

3-[3-(2-Oxo-2-phenyl-ethoxy)-phenyl]-acrylic acid (**5a**)

Obtained according to the general procedure from 0.42 g (2.56 mmol) 3-hydroxycinnamic acid, 1.00 g (5.02 mmol) of phenacyl bromide and 1.24 g (9.00 mmol) of K_2CO_3 and purified by recrystallization from methanol (76% yield). The product obtained was a white crystalline solid (mp 139-141 °C). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.02 (d, *J* 7.3 Hz, 2H, Ar-H), 7.96 (d, *J* 7.3 Hz, 2H, Ar-H), 7.76 (d, *J* 16.0 Hz, 1H, CH), 7.59-7.68 (m, 2H, Ar-H), 7.48-7.56 (m, 4H, Ar-H), 7.32 (t, *J* 8.0 Hz, 1H, Ar-H), 7.19 (d, *J* 8.0 Hz, 1H, Ar-H), 7.12 (s, 1H, Ar-H), 7.00 (dd, J_1 8.0 Hz, J_2 2.2 Hz, 1H, Ar-H), 6.57 (d, *J* 16.0 Hz, 1H, CH), 5.47 (s, 2H, CH_2), 5.32 (s, 2H, CH_2); ^{13}C NMR (150 MHz, $CDCl_3$) δ 194.1, 192.4, 166.3, 158.5, 145.9, 135.9, 134.6, 134.4, 134.1, 134.0, 130.2, 129.0, 129.0, 128.2, 128.0, 122.0, 117.7, 117.3, 114.2, 70.9, 66.3. HREIMS *m/z* 423.1208 $[M + Na]^+$ (calcd. for $C_{25}H_{20}NaO_5^+$, 423.1203).

(*E*)-2-(4-Nitrophenyl)-2-oxoethyl 3-(3-(2-(4-nitrophenyl)-2-oxoethoxy)phenyl)acrylate (**5b**)

Obtained according to the general procedure from 0.09 g (0.52 mmol) of 3-hydroxycinnamic acid, 0.25 g (1.02 mmol) of *p*-nitrophenacyl bromide and 0.25 g (1.84 mmol) of K_2CO_3 and purified by recrystallization from methanol (45% yield). The product obtained was a light brown crystalline solid (mp 101-103 °C). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.20-8.14 (m, 2H, Ar-H), 7.80-7.73 (m, 2H, Ar-H), 7.71-7.62 (m, 2H), 7.56-7.46 (m, 3H, Ar-H), 7.26 (t, *J* 7.8 Hz, 1H, Ar-H), 7.09 (d, *J* 7.7 Hz, 1H, Ar-H), 6.85 (s, 1H, Ar-H), 6.82 (d, *J* 8.4 Hz, 1H, Ar-H), 6.28 (d, *J* 16.1 Hz, 1H, CH), 5.12 (s, 2H, CH_2), 4.91 (s, 2H, CH_2); ^{13}C NMR (150 MHz, $CDCl_3$) δ 198.9, 197.4, 165.6, 157.5, 147.3, 146.3, 145.8, 135.5, 134.7, 134.4, 134.4, 134.2, 131.5, 131.4, 130.1, 128.8, 128.8, 124.0, 123.8, 122.2, 117.0, 116.8, 114.1, 72.0, 67.6. HREIMS *m/z* 513.0901 $[M + Na]^+$ (calcd. for $C_{25}H_{18}N_2NaO_9^+$, 513.0905).

2-Oxo-2-phenylethyl cinnamate (**6a**)

Obtained according to the general procedure from 0.38 g (2.56 mmol) of cinnamic acid, 0.50 g (2.51 mmol) of phenacyl bromide and 0.62 g (4.52 mmol) of K_2CO_3 and purified by recrystallization from acetone (86% yield). The product obtained was a colorless crystalline solid (mp 141-143 °C). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.96 (d, *J* 7.2 Hz, 2H, Ar-H), 7.81 (d, *J* 16.1 Hz, 1H, CH), 7.62 (t, *J* 7.2 Hz, 1H, Ar-H), 7.55 (s, 2H, Ar-H), 7.50 (t, *J* 7.2 Hz, 2H, Ar-H), 7.40 (s, 3H, Ar-H), 6.60 (d, *J* 16.1 Hz, 1H, CH), 5.80 (s, 2H, CH_2); ^{13}C NMR (150 MHz, $CDCl_3$) δ 192.4, 166.4, 146.3, 134.5, 134.4, 134.0, 130.7, 129.1, 129.0, 128.4, 128.0, 117.2, 66.3. HREIMS *m/z* 289.0836 $[M + Na]^+$ (calcd. for $C_{17}H_{14}NaO_3^+$, 289.0836).

2-(4-Nitrophenyl)-2-oxoethyl cinnamate (**6b**)

Obtained according to the general procedure from 0.38 g (2.59 mmol) of cinnamic acid, 0.62 g (2.54 mmol) of 4-nitrophenacyl bromide and 0.63 g (4.56 mmol) of K_2CO_3 and purified by recrystallization from acetone/hexane (75% yield). The product obtained was a light yellow crystalline solid (mp 157-158 °C). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.36 (d, *J* 8.8 Hz, 2H, Ar-H), 8.12 (d, *J* 8.8 Hz, 2H, Ar-H), 7.81 (d, *J* 16.0 Hz, 1H, CH), 7.52-7.63 (m, 2H, Ar-H), 7.36-7.45 (m, 3H, Ar-H), 6.58 (d, *J* 16.0 Hz, 1H, CH), 5.46 (s, 2H, CH_2); ^{13}C NMR (150 MHz, $CDCl_3$) δ 191.3, 166.1, 150.7, 146.7, 138.8, 134.1, 130.7, 129.0, 129.0, 128.3, 127.9, 124.1, 116.4, 66.2. HREIMS *m/z* 334.0696 $[M + Na]^+$ (calcd. for $C_{17}H_{13}NNaO_5^+$, 334.0686).

1-Oxo-1-phenylpropan-2-yl cinnamate (**6c**)

Obtained according to the general procedure from 0.36 g (2.40 mmol) of cinnamic acid, 0.50 g (2.35 mmol) of 2-bromopropiophenone and 0.58 g (4.23 mmol) of K_2CO_3 and purified by recrystallization from methanol (57% yield). The product obtained was a white crystalline solid (mp 70-71 °C). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.99 (d, *J* 7.2 Hz, 2H, Ar-H), 7.74 (d, *J* 16.2 Hz, 1H, CH), 7.59 (t, *J* 7.2 Hz, 1H, Ar-H), 7.51-7.55 (m, 2H, Ar-H), 7.49 (t, *J* 7.2 Hz, 2H, Ar-H), 7.37-7.40 (m, 3H, Ar-H), 6.54 (d, *J* 16.2 Hz, 1H, CH), 6.12 (q, *J* 7.0 Hz, 1H, CH), 1.61 (d, *J* 7.0 Hz, 3H, CH_3); ^{13}C NMR (150 MHz, $CDCl_3$) δ 196.9, 166.2, 145.9, 134.5, 134.3, 133.5, 130.5, 128.9, 128.8, 128.5, 128.2, 117.2, 71.4, 17.2. HREIMS *m/z* 303.0996 $[M + Na]^+$ (calcd. for $C_{18}H_{16}NaO_3^+$, 303.0992).

2-(4-Chlorophenyl)-2-oxoethyl cinnamate (**6d**)

Obtained according to the general procedure from 0.34 g (2.32 mmol) of cinnamic acid, 0.53 g (2.27 mmol) of 2-bromo-4'-chloroacetophenone and 0.57 g (4.10 mmol) of K_2CO_3 and purified by recrystallization from acetone (59% yield). The product obtained was a white crystalline solid (mp 128-130 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.89 (d, *J* 8.6 Hz, 2H, Ar-H), 7.80 (d, *J* 16.0 Hz, 1H, CH), 7.52-7.56 (m, 2H, Ar-H), 7.46 (d, *J* 8.6 Hz, 2H, Ar-H), 7.38-7.41 (m, 3H, Ar-H), 6.58 (d, *J* 16.0 Hz, 1H, CH), 5.42 (s, 2H, CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ 191.2, 166.1, 146.2, 140.3, 134.1, 132.5, 130.5, 129.2, 128.9, 128.5, 128.2, 116.7, 65.9. HREIMS *m/z* 323.0451 $[M + Na]^+$ (calcd. for $C_{17}H_{13}ClNaO_3^+$, 323.0446).

(*E*)-2-Oxo-2-phenylethyl-3-(benzo[*d*][1,3]dioxol-5-yl) acrylate (**6e**)

Obtained according to the general procedure from 0.49 g (2.56 mmol) of 3,4-(methylenedioxy)cinnamic acid, 0.50 g (2.51 mmol) of phenacyl bromide and 0.59 g (4.52 mmol) of K_2CO_3 and purified by recrystallization from acetone/hexane (69% yield). The product obtained was a colorless crystalline solid (mp 144-146 °C). ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.95 (d, *J* 7.4 Hz, 2H, Ar-H), 7.71 (d, *J* 16.0 Hz, 1H, CH), 7.61 (t, *J* 7.4 Hz, 1H, Ar-H), 7.49 (t, *J* 7.4 Hz, 2H, Ar-H), 7.06-7.02 (m, 2H, Ar-H), 6.81 (d, *J* 7.9 Hz, 1H, Ar-H), 6.42 (d, *J* 16.0 Hz, 1H, CH), 6.01 (s, 2H, OCH_2O), 5.46 (s, 2H, CH_2); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 192.4, 166.4, 149.8, 148.3, 145.8, 134.3, 133.8, 129.2, 128.8, 127.8, 124.7, 114.8, 108.5, 106.6, 101.6, 66.0. HREIMS *m/z* 349.0478 $[M + K]^+$ (calcd. for $C_{18}H_{14}KO_5^+$, 349.0473).

(*E*)-2-(4-Chlorophenyl)-2-oxoethyl-3-(3,4,5 trimethoxy-phenyl)acrylate (**6f**)

Obtained according to the general procedure from 1.04 g (4.37 mmol) of 3,4,5-trimethoxycinnamic acid, 1.00 g (4.28 mmol) of 2-bromo-4'-chloroacetophenone and 1.09 g (7.70 mmol) of K_2CO_3 and purified by recrystallization from acetone (45% yield). The product obtained was a white crystalline solid (mp 117-119 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.90 (d, *J* 8.0 Hz, 2H, Ar-H), 7.71 (d, *J* 16.0 Hz, 1H, CH), 7.48-7.46 (m, 2H, Ar-H), 6.78 (s, 2H, Ar-H), 6.50 (d, *J* 16.0 Hz, 1H, CH), 5.44 (s, 2H, CH_2), 3.89 (s, 9H, OCH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 191.2, 166.1, 153.3, 146.2, 140.3, 140.3, 132.5, 129.6, 129.1, 115.9, 105.4, 65.8, 60.9, 56.1. HREIMS *m/z* 391.0948 $[M + H]^+$ (calcd. for $C_{20}H_{20}ClO_6^+$, 391.0943).

(*E*)-Methyl-3-(3-methoxy-4-(2-oxo-2-phenylethoxy) phenyl) acrylate (**7a**)

Obtained according to the general procedure from 0.75 g (3.59 mmol) of methyl ferulate, 0.70 g (3.52 mmol) of phenacyl bromide and 0.88 g (6.34 mmol) of K_2CO_3 and purified by recrystallization from ethanol (53% yield). The product obtained was a colorless crystalline solid (mp 105-107 °C). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.00 (d, *J* 8.0 Hz, 2H, Ar-H), 7.63-7.59 (m, 2H, Ar-H/CH), 7.50 (t, *J* 8.0 Hz, 2H, Ar-H), 7.07 (s, 1H, Ar-H), 7.02 (d, *J* 8.0 Hz, 1H, Ar-H), 6.77 (d, *J* 8.0 Hz, 1H, Ar-H), 6.31 (d, *J* 16.0 Hz, 1H, CH), 5.41 (s, 2H, CH_2), 3.92 (s, 3H, OCH_3), 3.79 (s, 3H, OCH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 193.7, 167.5, 149.5, 149.3, 144.5, 134.2, 133.9, 128.8, 128.5, 127.9, 122.0, 115.9, 113.6, 110.4, 71.3, 55.9, 51.6. HREIMS *m/z* 327.1233 $[M + H]^+$ (calcd. for $C_{19}H_{19}O_5^+$, 327.1227).

3-{4-[2-(4-Bromo-phenyl)-2-oxo-ethoxy]-3-methoxy-phenyl}-acrylic acid methyl ester (**7b**)

Obtained according to the general procedure from 0.19 g (0.92 mmol) of methyl ferulate**,** 0.25 g (0.90 mmol) of 2,4'-dibromoacetophenone and 0.22 g (1.62 mmol) of K_2CO_3 and purified by recrystallization from acetone/CH_2Cl_2 (68% yield). The product obtained was a light yellow crystalline solid (mp 160-162 °C). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.88 (d, *J* 8.4 Hz, 2H, Ar-H), 7.64 (d, *J* 8.4 Hz, 2H, Ar-H), 7.61 (d, *J* 16.2 Hz, 1H, CH), 7.07 (d, *J* 1.8 Hz, 1H, Ar-H), 7.03 (dd, J_1 8.4 Hz, J_2 1.8 Hz, 1H, Ar-H), 6.79 (d, *J* 8.4 Hz, 1H, Ar-H), 6.31 (d, *J* 16.2 Hz, 1H, CH), 5.31 (s, 2H, CH_2), 3.91 (s, 3H, CH_3), 3.79 (s, 3H, CH_3); ^{13}C NMR (150 MHz, $CDCl_3$) δ 193.3, 167.5, 149.8, 149.2, 144.4, 133.1, 132.2, 129.7, 129.2, 128.9, 122.0, 116.3, 114.1, 110.7, 71.8, 56.0, 51.6. HREIMS *m/z* 427.0158 $[M + Na]^+$ (calcd. for $C_{19}H_{17}BrNaO_5^+$, 427.0152).

(*E*)-Methyl 3-(3-((1-oxo-1-phenylpropan-2-yl)oxy)phenyl) acrylate (**8**)

Obtained according to the general procedure from

0.22 g (1.22 mmol) of methyl 3-(3-hydroxyphenyl)acrylate, 0.25 g (1.20 mmol) of 2-bromopropiophenone and 0.30 g (2.16 mmol) of K_2CO_3 and purified by recrystallization from acetone/CH_2Cl_2 (88% yield). The product obtained was a light yellow crystalline solid (mp 115-117 °C). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.06 (d, *J* 7.2 Hz, 2H, Ar-H), 7.63-7.53 (m, 1H, Ar-H/CH), 7.48 (t, *J* 7.2 Hz, 2H, Ar-H), 7.24 (t, *J* 7.8 Hz, 1H, Ar-H), 7.10 (d, *J* 7.8 Hz, 1H, Ar-H), 7.02 (s, 1H, Ar-H), 6.87 (dd, J_1 7.8 Hz, J_2 1.8 Hz, 1H, Ar-H), 6.34 (d, *J* 15.6 Hz, 1H, CH), 5.50 (q, *J* 7.2 Hz, 1H, CH), 3.79 (s, 3H, OCH_3), 1.73 (d, *J* 7.2 Hz, 3H, CH_3); ^{13}C NMR (150 MHz, $CDCl_3$) δ 198.5, 167.2, 157.8, 144.4, 135.9, 134.1, 133.8, 130.0, 128.8, 128.8, 121.3, 118.3, 116.9, 114.7, 76.7, 51.7, 18.7. HREIMS *m/z* 333.1099 $[M + Na]^+$ (calcd. for $C_{19}H_{18}NaO_4^+$, 333.1098).

2-Oxo-2-phenylethyl-4-(2-oxo-2-phenylethoxy)benzoate (**9**)

Obtained according to the general procedure from 0.35 g (2.56 mmol) *p*-hydroxybenzoic acid, 0.50 g (2.51 mmol) of phenacyl bromide and 0.62 g (4.52 mmol) of K_2CO_3 and purified by recrystallization from ethanol (41% yield). The product obtained was a white crystalline solid (mp 146-148 °C). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.07 (d, *J* 8.9 Hz, 2H, Ar-H), 7.98 (d, *J* 8.5 Hz, 2H, Ar-H), 7.94 (d, *J* 8.5 Hz, 2H, Ar-H), 7.46-7.52 (m, 4H, Ar-H), 6.97 (d, *J* 8.9 Hz, 2H, Ar-H), 5.54 (s, 2H, CH_2), 5.37 (s, 2H, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ 193.6, 192.3, 165.5, 162.0, 134.4, 134.3, 134.1, 133.8, 132.1, 128.9, 128.9, 128.1, 127.8, 122.7, 114.5, 70.5, 66.3. HREIMS *m/z* 397.1052 $[M + Na]^+$ (calcd. for $C_{23}H_{18}NaO_5^+$, 397.1047).

Biology

Cell culture and treatment

Human myeloid leukemia (KG-1, K-562, HL-60, NB4), human burkit lymphoma (RAMOS, RAJI), human lymphoid T leukemia (JURKAT, CEM, MOLT-4), human lymphoid B leukemia (NALM-6, SUP-B15, RS4;11), human prostatic adenocarcinoma (PC3, LNCaP), human ovarian carcinoma (NCI/ADR), human malignant neoplasm cervix uteri (HeLa), human breast adenocarcinoma (MCF-7), human osteosarcoma (HOS, U-2 0S, MG-63), human glioblastoma-astrocytoma, epithelial-like (U-87 MG), human glioblastoma cell lines (U-138 MG) and human non-small cell lung cancer (NCI-H1299) were cultured in RPMI medium supplemented with 10% fetal calf serum (Gibco 16000-044), 1% penicillin (10,000 IU mL^{-1}) and streptomycin 10 mg mL^{-1} (15,070 Gibco) and exposed to concentration of drugs (10 μM) in DMSO (0.1%) and maintained at 37 °C in 95% humidified atmosphere, containing 5% CO_2.

In vitro cell viability assay - MTT assay

All steps in this assay were automated in Liquid Handling Workstation epMotion® 5070 (Eppendorf, Vaudaux, Schonenbuch, Switzerland). Cells were distributed in 96 wells (100 μL cells $well^{-1}$) and incubated for 48 h, before addition of test compounds. Cells were then exposed to the compounds at a concentration of 10 μM. After 24 h of exposure at 37 °C, cell viability was determined by colorimetric MTT[35] (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide) based on the metabolic ability of active cells to convert the yellow MTT reagent into a blue insoluble salt (formazan), which is spectrophotometrically measured. Then, the amount of formazan produced was dissolved in solution containing 150 μL of isopropanol and optical density was read by a spectrophotometer at 570 nm (Bio-Tek Power Wave XS). Absorbance of wells containing the compounds and those with cells in control (cells treated with vehicle, 0.1% DMSO) were compared to estimate the cell viability. The results were expressed as inhibition percentage relative to control (considered as 100%) and doxorubicin was used as a reference drug. Compound **6c** was also evaluated for cytotoxicity against human leukemia cells, using the MTT method. Doxorubicin was used as positive control. All assays were performed in triplicate and mean ± standard deviation (SD) values were used to estimate cell viability.

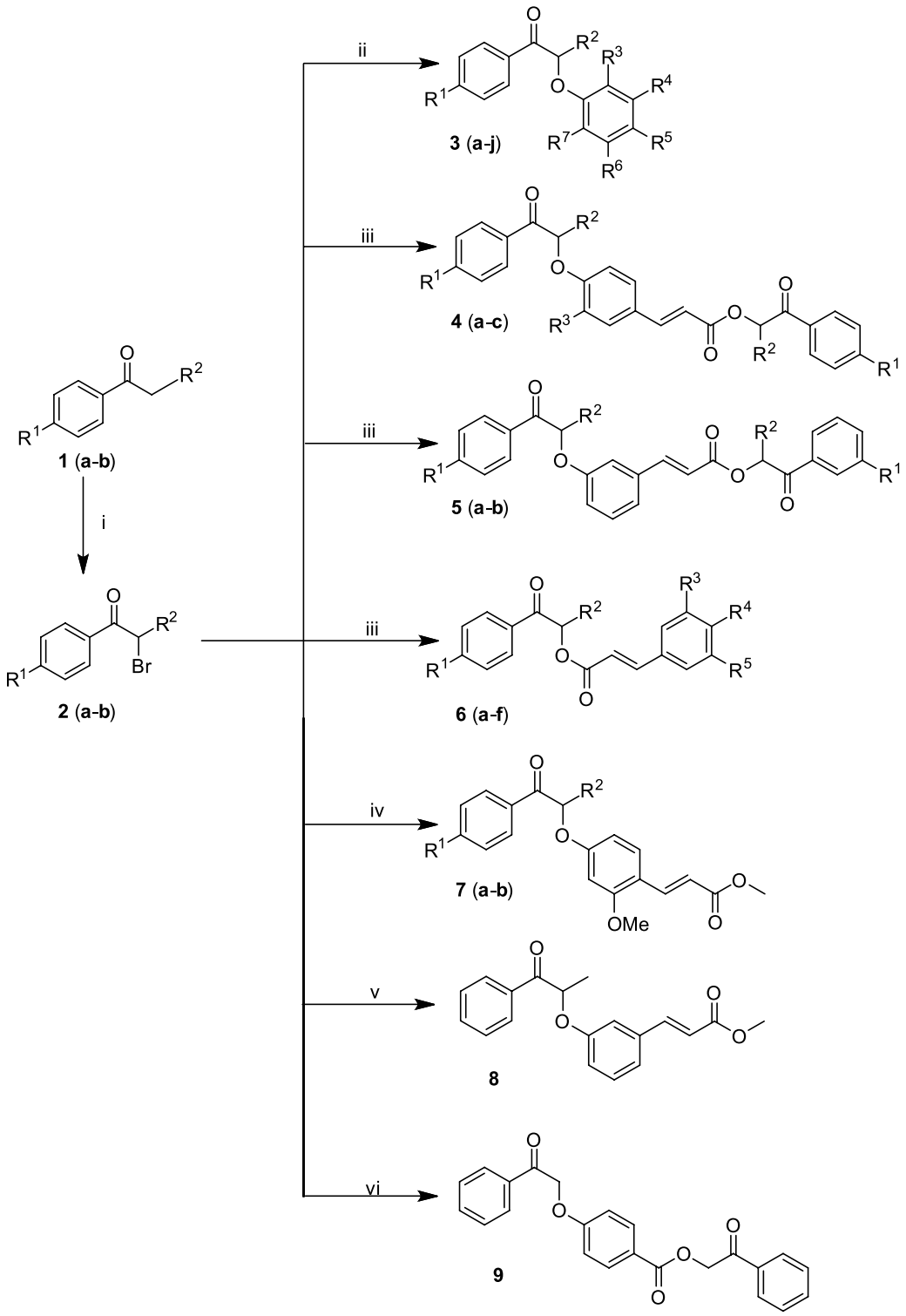
Results and Discussion

Chemistry

In this work, 25 oxyneolignan analogues (β-ketoethers or β-ketoesters) were synthesized and the synthetic strategies for their preparation are summarized in Scheme 1. The β-ketoethers and β-ketoesters analogues **3a**-**3j**, **4a**-**4c**, **5a**-**5b**, **6a**-**6f**, **7a**-**7b**, **8** and **9** (Table 1) were obtained following a procedure described by Barata *et al.*[26]

Initially, the α-bromoketone intermediates **2a-2b** were prepared according to a known procedure,[26] then, without any purification due to their lacrimogenic property, were reacted with an *in situ* generated phenoxy and/or carboxylate ion. These condensation reactions were carried out in the presence of K_2CO_3 and butanone as solvent furnishing the products in yields ranging from 40 to 100%. The phenolic/carboxylic compounds were used in an excess of 2% instead of using excess of α-bromoketone. During isolation, the volume of solvent was reduced to 1/3 of the initial volume before work-up, since the slight solubility of the solvents in water hampers the isolation procedure.

In order to obtain compounds **7a-7b** and **8**, previous esterification of the cinnamic acid derivatives with



Scheme 1. Synthesis of 8,4'-oxyneolignan analogues. Reagents and conditions: (*i*) Br_2, $CHCl_3$ r.t. 2 h; (*ii*) ArOH, K_2CO_3, butanone or MeCN, 80 °C, 12 h; (*iii*) cinnamic acids, K_2CO_3, butanone or MeCN, 80 °C; (*iv*) methyl ferulate, butanone or MeCN, 80 °C, 12 h; (*v*) methyl 3-hydroxycinnamate, K_2CO_3, butanone or MeCN, 80 °C; (*vi*) 4-hydroxy-benzoic acid, K_2CO_3, butanone, 80 °C.

methanol and sulfuric acid was mandatory to avoid the competitive nucleophilic displacement of the bromine atom by the carboxylate anions. This was confirmed in the obtention of compounds **4a**-**4c** and **5a**-**5b** by using 2 equivalents of **2a-2b**. As far as we know, this creates a new subclass of 8,4'-oxyneolignan analogues, which we called 8,4'-oxyneolignan cinnamic analogues. Compound **9** was synthesized in a similar fashion.

Table 1. Chemical structures, yields, and melting points (mp) of the synthesized compounds

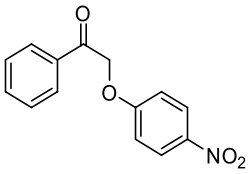
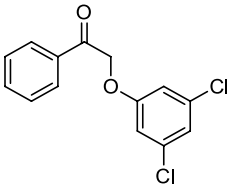
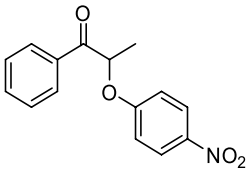
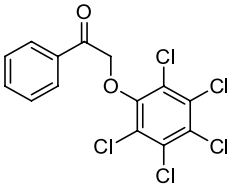
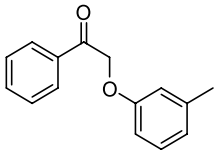
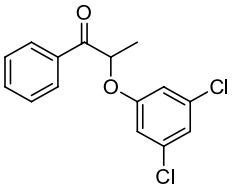
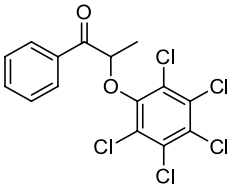
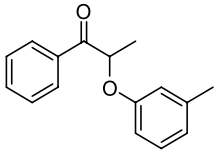
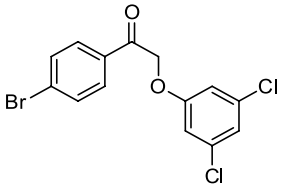
Compound	Structure	Yield / %	mp / °C
3a		84	148-150
3b		42	74-75
3c		91	78-80
3d		100	125-127
3e		69	70-72
3f		54	–
3g		66	111-112
3h		54	109-111
3i		39	99-101

Table 1. Chemical structures, yields, and melting points (mp) of the synthesized compounds (cont.)

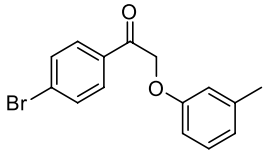
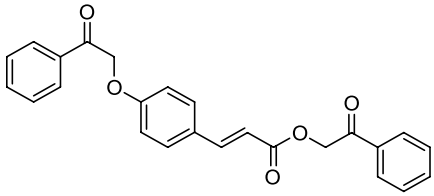
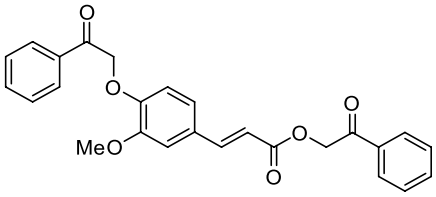
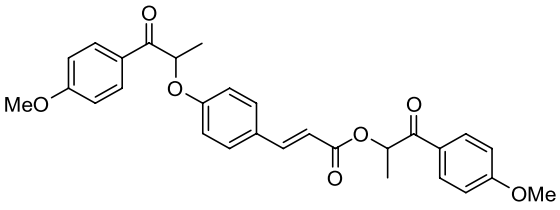
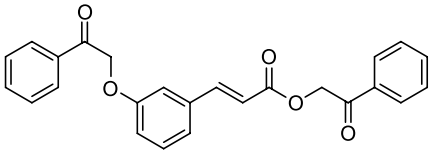
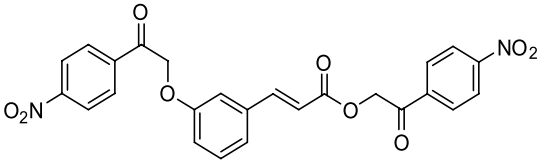
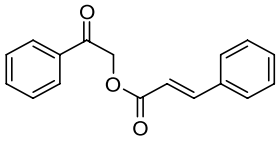
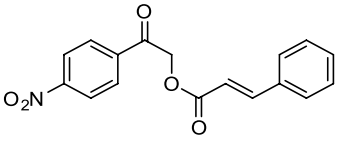
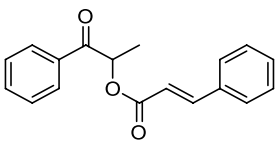
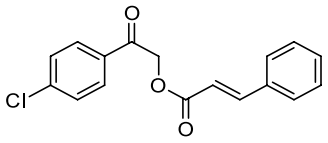
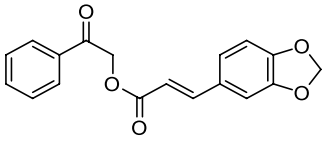
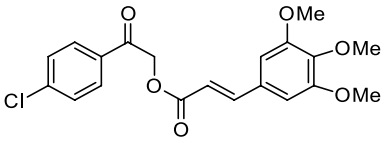
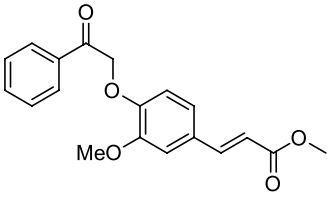
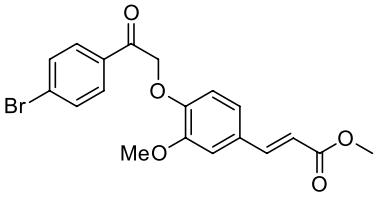
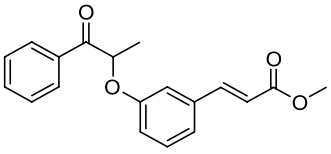
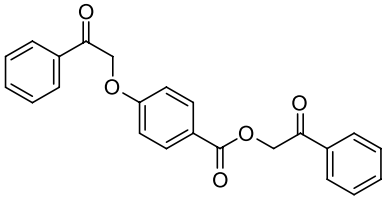
Compound	Structure	Yield / %	mp / °C
3j		69	91-93
4a		55	145-147
4b		74	135-137
4c		71	142-144
5a		76	139-141
5b		45	101-103
6a		86	141-143
6b		75	157-158
6c		57	70-71

Table 1. Chemical structures, yields, and melting points (mp) of the synthesized compounds (cont.)

Compound	Structure	Yield / %	mp / °C
6d		59	128-130
6e		69	144-146
6f		45	117-119
7a		53	105-107
7b		68	160-162
8		88	115-117
9		41	146-148

The ^{1}H NMR spectra of the products show the presence of one peak (compounds **3**, **6**, **7** and **8**) or two peaks (compounds **4**, **5** and **9**) in between 5.12-6.14 ppm, assignable to the CH_2/CH carbinolic protons. These signals confirm the formation of the C–O–C bond and, consequently, the expected products.

All synthesized substances were purified by crystallization and fully characterized by usual spectroscopic methods (melting points, HRMS, ^{1}H and ^{13}C NMR). The chemical structures are described in Table 1.

Biology

To evaluate the antiproliferative activity, the amount of surviving cells at the dose level of 10 μM was measured after 24 h of incubation by the MTT method colorimetric assay[35] and the results were expressed as percentage of inhibition relative to control and compared with the reference drug (doxorubicin). The tests were carried out in triplicate, using doxorubicin as positive control and these data are schematically listed in Table 2.

Table 2. Evaluation of cytotoxicity towards leukemia cells (% inhibition)[a] for all synthesized compounds

entry	Compound	K-562	HL-60	Nalm-6	Ramos
1	**3a**	11.8	15.4	< 5	14.7
2	**3b**	15.6	4.8	5.8	21.0
3	**3c**	20.4	10.9	8.7	< 5
4	**3d**	28.3	26.1	13.4	29.8
5	**3e**	NT	NT	NT	NT
6	**3f**	17.2	1.4	< 5	15.0
7	**3g**	34.4	6.5	< 5	18.6
8	**3h**	13.9	16.9	< 5	10.4
9	**3i**	10.3	8.7	14.1	18.5
10	**3j**	27.4	38.1	< 5	17.6
11	**4a**	34.1	52.1	56.7	48.6
12	**4b**	< 10	57.5	62.0	60.4
13	**4c**	NT	NT	NT	NT
14	**5a**	21.6	< 5	12.4	17.9
15	**5b**	12.2	< 5	8.6	15.7
16	**6a**	39.3	34.1	32.6	43.7
17	**6b**	27.8	7.1	21.7	42.8
18	**6c**	52.2	86.3	73.2	84.6
19	**6d**	< 5	51.4	56.9	61.3
20	**6e**	43.6	33.2	38.5	38.3
21	**6f**	19.9	33.7	34.9	36.9
22	**7a**	NT	NT	NT	NT
23	**7b**	13.7	16.3	< 5	9.9
24	**8**	12.8	14.8	9.9	21.7
25	**9**	47.0	66.7	51.6	54.1
26	doxorubicin	71.1	97.7	65.4	81.3

[a]Inhibition percentages measured at a single concentration of 10 μM. NT: not tested.

The antiproliferative screening results show that five compounds presented a promising antiproliferative activity (≥ 50% of cell inhibition) against the leukemia cell lines. The other analogues were less active or completely inactive at the dose of 10 μM. Compounds **4a**, **4b**, **6d** and **9** (entries 11, 12, 19 and 25, respectively) demonstrate similar inhibition of growth proliferation against HL-60, Ramos and Nalm-6 (48.6-66.7%). The same substances did not reveal a good inhibition level against K-562 cells. The best compound of the series was **6c** (entry 18) inhibiting cell proliferation over 50% against four leukemia cell lines and very comparable to the positive control used in this test (entry 26). For Nalm-6 and Ramos cells, the percentage of inhibition was even superior to doxorubicin inhibition, presenting 73.2 and 84.6%, respectively.

Compounds which presented significant activity profile against leukemia cells (**4a**, **4b**, **6c**, **6d**, **9**) were selected to be evaluated for their activity on cell proliferation on other nineteen different kinds of human neoplasms, among tumors and leukemia cell lines. These tests were performed by using the MTT method, as previously mentioned. The cells used in this evaluation are listed in the Experimental section and the obtained results are shown on Tables 3-4 and Figures 1-2.

Table 3. Evaluation of antiproliferative activity towards tumor cells (% inhibition at 10 μM) for compounds **4a**, **4b**, **6c**, **6d** and **9**

Tumor	Antiproliferative activity / (% inhibition at 10 μM)					
	4a	**4b**	**6c**	**6d**	**9**	Doxorubicin
PC3	82.9	67.3	57.1	47.1	47.5	85.1
LNCaP	64.3	67.6	74.9	50.6	50.0	86.0
NCI/ADR	76.2	56.5	60.1	42.6	58.4	94.0
MCF-7	39.7	35.2	59.0	31.2	36.5	72.1
HeLa	61.3	71.1	36.3	51.4	33.7	93.7
U-87	72.2	34.2	43.4	32.1	50.0	59.0
U-138 MG	75.7	5.4	29.8	27.0	53.0	73.2
HOS	76.3	66.6	68.3	65.0	27.5	91.5
U-2 OS	21.2	52.0	41.9	35.2	20.9	90.5
MG-63	71.7	59.4	70.2	36.9	54.0	74.5
H1299	73.4	24.8	15.0	23.1	35.5	65.4

Table 4. Evaluation of cytotoxicity towards leukemia cells (% inhibition at 10 μM) for compounds **4a**, **4b**, **6c**, **6d** and **9**

Leukemia cell	Cytotoxicity / (% inhibition at 10 μM)					
	4a	**4b**	**6c**	**6d**	**9**	Doxorubicin
KG-1	55.1	92.0	89.7	34.0	< 5	86.9
NB4	33.2	48.9	81.4	35.2	45.0	88.2
RAJI	19.6	37.9	65.5	46.3	37.1	90.8
JURKAT	56.8	62.2	91.2	51.9	40.8	97.5
CEM	52.4	61.7	91.1	45.6	57.8	98.4
MOLT-4	14.1	41.5	87.1	34.0	62.0	90.0
SUP-B15	48.5	57.4	64.4	51.7	56.4	89.4
RS4;11	53.0	40.5	89.6	35.8	50.5	95.2

As can be seen from Table 3 and Figures 1-2, all compounds exhibited some degree of activity against the eleven tumor cells used in this assay. β-Ketoester **6c** presented a good antiproliferative activity for tumors, specially LNCaP (74.9%), HOS (68.3%) and MG-63 (70.2%), whereas compound **6d** showed an inhibition rate greater than 50% only for LNCaP (50.6%), HeLa (51.4%) and HOS (65.0%) tumors. The oxyneolignan analogue **9** demonstrated a considerable reduction of cell growth on four tumor cells: LNCaP (50.0%), NCI/ADR (58.4%), U-138 MG (53.0%) and MG-63 (54.0%).

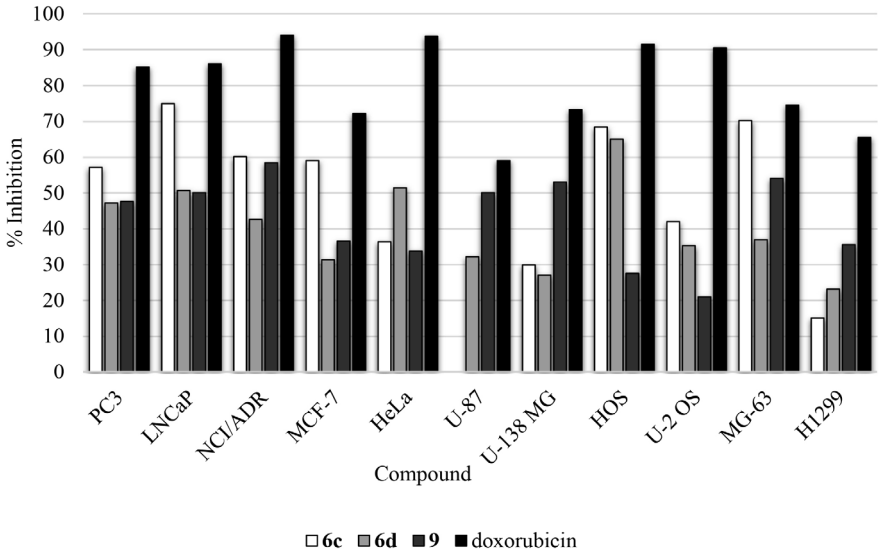


Figure 1. MTT assay for human tumor cell lines of compounds **6c**, **6d** and **9**.

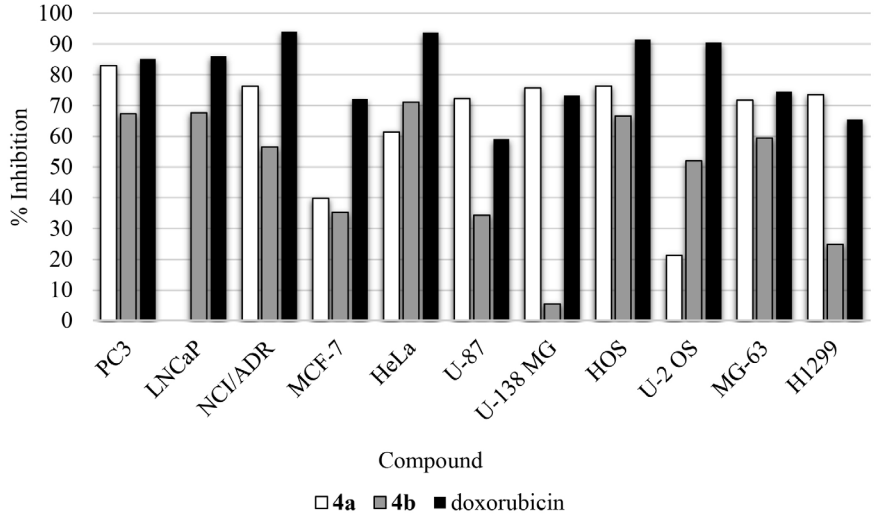


Figure 2. MTT assay for human tumor cell lines of compounds **4a** and **4b**.

8,4'-Oxyneolignan cinnamic analogues **4a** and **4b** were shown to be active against most cells, exhibiting a good inhibition profile. The most active compound was **4a**, showing a significant inhibition rate of cell growth against PC3 (82.9%), MG-63 (71.7%), U-87 (72.2%), U-138 MG (75.7%) and H1299 (73.4%) tumors. In these last three cell lines results, the activity of the oxyneolignan **4a** was even superior to that of doxorubicin, the positive control used in this trial.

The evaluation of compounds **4a**, **4b**, **6c**, **6d** and **9** on leukemic cells are expressed in Table 4 and Figures 3-4.

Compound **9** was most active against CEM, SUP-B15, MOLT-4 and RS4;11 cells, reducing its growth by 50.5 to 62.0%. Compound **4a** presented levels of cell inhibition against KG-1, JUKART, CEM, RS4;11 around 52.4-56.8%, whereas a great selectivity and stronger antiproliferative activity were observed for compound **4b**, which exhibited 92.0% of inhibition against KG-1 cells.

Compound **6c** was found to be the most promising of the series, presenting antileukemic activity higher than 50% for all cell lines used in this study and superior or comparable results when compared to the reference drug. In the case of KG-1 cells, the extent of inhibition levels of products **4b** and **6c** were even better than doxorubicin, indicating its possible efficacy against this leukemia type.

Considering the results obtained in the antiproliferative tests (Table 4, Figure 4), *in vitro* cytotoxicity assay was used to assess the activity of the most potent compound (**6c**). The analogue was tested against eleven human leukemic cell lines. The assays were carried out in triplicate, and doxorubicin was used as the positive control. The biological endpoint was determined according to the concentration,

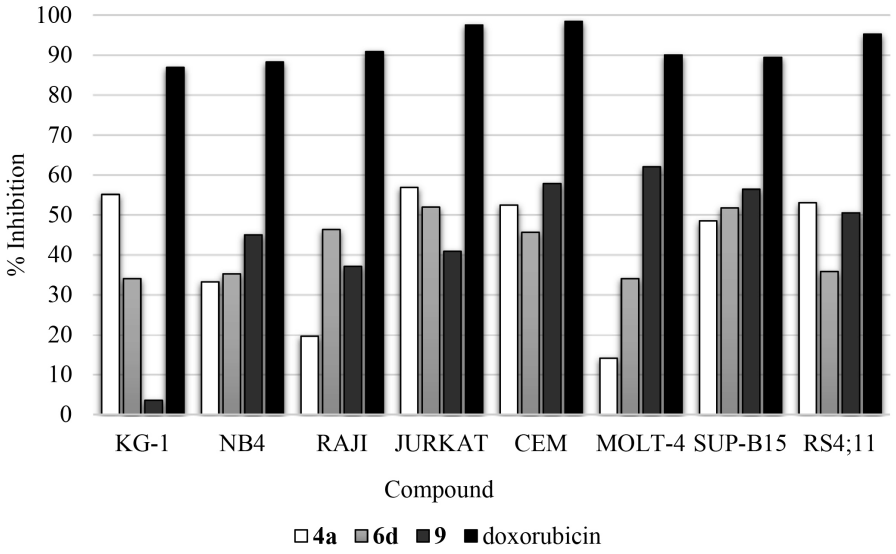


Figure 3. MTT assay for human leukemia cell lines of compounds **4a**, **6d** and **9**.

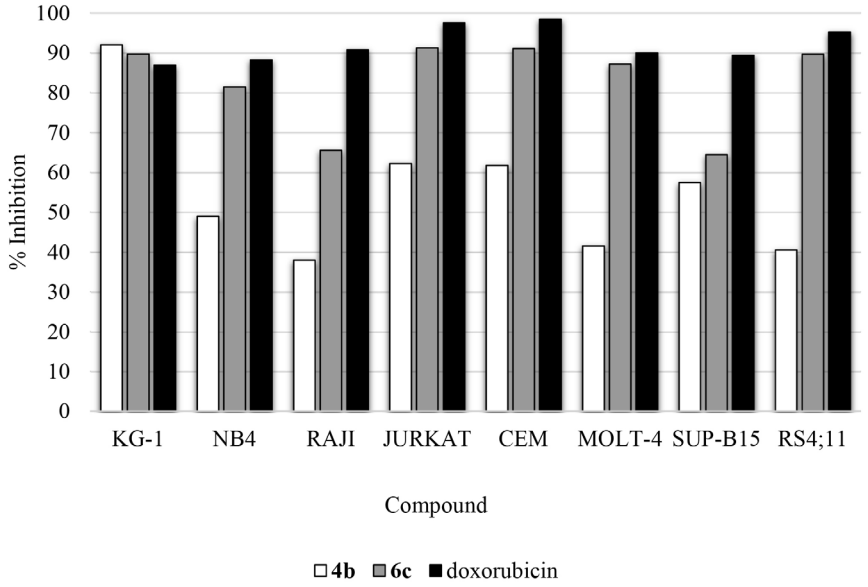


Figure 4. MTT assay for human leukemia cell lines of compounds **4b** and **6c**.

which causes fifty percent of cell growth inhibition (IC_{50}). Compound **6c** showed similar values of IC_{50} against leukemia cells (Table 5), ranging from moderate to good activity, except for K-562 cells, for which the drug showed no activity. Compound **6c** induced better cytotoxic effects on Ramos cells, which presented an IC_{50} of 9.4 μM.

The correlation between the structures of the synthesized compounds and their antiproliferative activities leads to the conclusion that the compounds bearing a cinnamic moiety on its structure possess better potency in MTT assay. The presence of the cinnamic portion in the basic structure of the 8,4'-oxyneolignans and the variation on its position on the aromatic ring affect considerably the antiproliferative activities. When comparing compounds **4a**-**4b** and **5a**-**5b**, **7a**-**7b** and **8** better inhibition showed by compounds **4a** and **4b** against cancer cells could be attributed to its *para* substitution, whereas *meta*-substituted compounds **5a**-**5b** did not show an expressive percentage of inhibition. However, in compounds **7a**-**7b** and **8**, the cinnamate moiety and the absence of an aromatic ring may be responsible for the drastic decrease in activity.

In the 8,4'-oxyneolignans **3a**-**3j**, the effects of substitution on the aromatic rings were not clearly observed. These compounds did not show enhanced activity when comparing substituent groups, positions and side chain length.

Among compounds **6a**-**6f**, as shown in Tables 2 and 3, the effect of alkyl chain substitution in compound **6c** greatly

Table 5. *In vitro* cytotoxicity of compound **6c** against leukemia cell lines

Leukemia cell	Doxorubicin IC_{50} / μM	**6c** IC_{50} / μM
K-562	3.1	> 100
KG-1	0.8	12.0
HL-60	0.5	12.6
NB4	0.4	12.6
Ramos	5.7	9.4
RAJI	1.0	18.5
Nalm-6	0.2	13.5
JURKAT	0.5	14.2
MOLT-4	0.1	14.0
SUP-B15	0.5	33.7
RS4;11	0.1	13.3
CEM	NT	NT

Incubation time = 24 h; IC_{50} >100 μM means not active; NT: not tested.

decreased cell proliferation when compared to compound **6a**. In the same aspect, the presence of a halogen substituent in compound **6d** intensified the cytotoxicity level compared to **6a** and also compared with **6b**, which possesses an electron-withdrawing group at the same position. These results suggest that the electronic characteristics of the substituent groups and the alkyl chain affect the capability of the molecule to interact with the bioactive target increasing/decreasing the antiproliferative activity.

Conclusions

From our study we were able to produce a new subclass of neolignan analogues, the 8,4'-oxyneolignan cinnamic analogues. These compounds were evaluated against a variety of cancer cell lines, among tumors and leukemias. In tumor cells assays we identified that compound **4a** showed an antiproliferative activity superior to doxorubicin for U-87, U-138 MG and H1299 cell types. Compound **6c** exhibited significant inhibition activity in a range of 52.2 to 91.2% against twelve kinds of leukemia cell lines, revealing excellent results and very comparable to the reference drug. In addition, compound **4b** was found to be very selective, demonstrating a growth inhibition of 92.0% against KG-1 cells. These preliminary results suggest that further investigation is needed to elucidate the characteristics underlying the antiproliferative activities of these analogues.

Supplementary Information

The supplementary data of ^{1}H NMR, ^{13}C NMR and HRMS spectra of all compounds synthesized are available free of charge at http://jbcs.org.br as a PDF file.

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