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Article

Synthesis, Antitumor Activity and Docking of 2,3-(Substituted)-1,4-Naphthoquinone Derivatives Containing Nitrogen, Oxygen and Sulfur

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Eleven 2,3-(substituted)-1,4-naphthoquinone derivatives were synthesized in yields ranging from 52-89%. These derivatives were evaluated for their cytotoxic effects on human lungs (H460), triple-negative breast (MDA-MB-231) and ovarian (A2780) cancer cell lines. Compounds **5f** and **8** showed IC_{50} values of 3.048×10^{-5} mol L^{-1} and 4.24×10^{-6} mol L^{-1} for H460; **5c** and **8** showed IC_{50} values of 2.16×10^{-5} mol L^{-1} and 1.60×10^{-5} mol L^{-1} for MDA-MB-231, and **5g** and **8** showed IC_{50} values of 2.68×10^{-6} mol L^{-1} and 3.89×10^{-6} mol L^{-1} for A2780. Additionally, we conducted a docking study with the four most active compounds and the therapeutic targets PI3K and *topoisomerase II* showing the pharmacophoric conformation of these compounds.

Keywords: 1,4-naphthoquinone, antineoplastic activity, *topoisomerase*, PI3K, nucleophilic substitution

Introduction

Notwithstanding, the progress observed in cancer treatments in the past decades, epidemiology data, clearly point to urgent new therapeutic approaches to control the disease. Improvements in the quality of life and overall survival rates of cancer patients strongly rely on the development of novel compounds with promising anticancer activity, such as natural or synthetic substances containing the quinone nuclei. The antineoplastic properties and the mechanism of action of quinone derivatives (Figure 1) have been widely studied and it is known that quinone derivatives can inhibit the activity of *topoisomerase* and *telomerase* through DNA alkylation or intercalation, inhibiting the heat shock protein HSP90.[1] In particular, the anticancer properties of quinone derivatives seem to be mainly due to the induction of oxidative stress caused by reactive oxygen species, such as superoxide, generated from the reduction of the quinone nucleus by cellular reductases.[1] These data show that quinone derivatives can have multiple antineoplastic mechanisms, leading our group to postulate that some of them might, as well as, inhibit the activity of

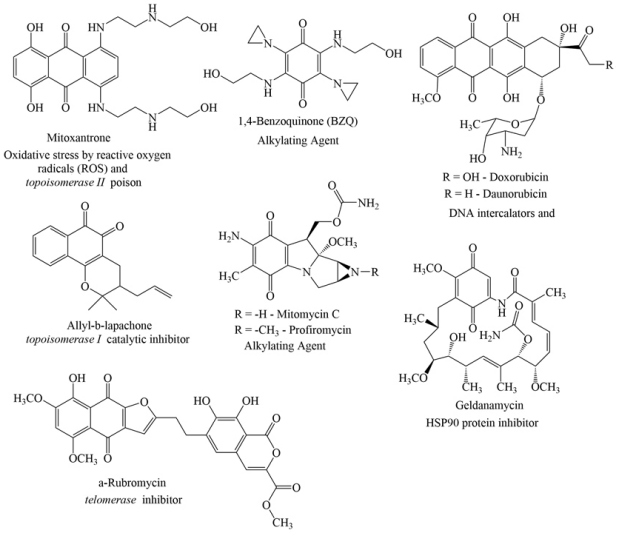

Figure 1. Quinones with antineoplastic activity.

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the phosphatidylinositol 3-kinase (PI3K). This suggestion may open an avenue to use of quinone derivatives as target therapies against PI3K-related pathways, which are frequently regulated in several human cancers, promoting cellular proliferation, differentiation and drug resistant phenotype, among other carcinogenic properties.[2]

It has been established that many of the biological effects of quinone derivatives depend on their 1,4-naphthoquinone pharmacophore group. Such is the case for antitumor, antiproliferative, antibacterial, anti-inflammatory, antimalarial, antiviral, antifungal and antileishmanial compounds.[3] Moreover, the incorporation of nitrogen and sulfur atoms on C_2 and C_3 of the 1,4-naphthoquinone core has led to the formation of compounds with diverse biological activities, including anticancer activity.[3]

Thus, considering the importance of the substitution of C_2 and C_3 of the naphthoquinone core for various biological activities, the literature has provided numerous examples that 2,3-dichloro-1,4-naphthoquinone reacts with nucleophiles to form monosubstituted and disubstituted products and, more commonly, a mixture of both, depending on the softness of the nucleophile used.[4-10] However, reactions carried out with electron donor groups such as amines replace just one of the chlorine atoms due to the increasing of the electron density in the naphthoquinone core.[11] The second replacement happens when the withdrawing electrons effect is imposed on the naphthoquinone ring or a catalyst, normally containing palladium, is employed in the reaction.[2]

Herein, we present the 2,3-(substituted)-1,4-naphthoquinone derivatives containing nitrogen, oxygen and sulfur atoms, obtained from 2,3-dichloro-1,4-naphthoquinone **1**, 2-methoxy-1,4-naphthoquinone **2** and 1,4-naphthoquinone **3** (Figure 2) and the respective *in vitro* biological assays for their potential antiproliferative activity. Importantly, the active antineoplastic substances seem to act by multiple cellular pathways, which is an effective strategy to avoid the occurrence of the chemoresistant phenotype frequently observed in tumor cells.

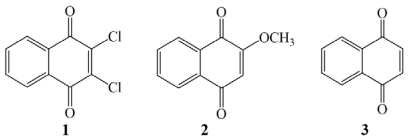


Figure 2. Substrates for synthesis of the desired compounds.

Experimental

General information

All solvents and reagents were commercially purchased and were used without any treatment.

Melting points were determined using Fisatom 430D equipment. Infrared (IR) spectra were recorded on Bomem FTLA2000-102-ABB spectrometer. The ^{1}H nuclear magnetic resonance (NMR) and ^{13}C NMR spectra were obtained on a Vary VNMRS spectrometer model 400 (400 MHz) with tetramethylsilane (TMS) as internal standard. NMR analyses of compounds **5a**-**g**, **6a**-**c** and **8** were performed in chloroform deuterated (99.8%) with 1% (v/v) of TMS and stabilized with silver foil (Cambridge Isotope Laboratories, Inc.).

Mass spectra were recorded on ultra-high resolution and accuracy mass spectrometer (model 9.4 T Solarix, Bruker Daltonics), operated in both ionization modes: positive and negative electrospray ionization with Fourier transform ion clyclotron resonance mass spectrometry, ESI(+) and ESI(−)-FT-ICR MS spectra were acquired with resolving power of m/Dm50% ca. 500000, in which Dm50% is the full peak width at half-maximum peak height of *m/z* 400 and a mass accuracy < 1 ppm. It provides an unambiguous molecular formula assignment for singly charged molecular ions such as $[M - H]^+$ or $[M + H]^-$ and DBE (double bound equivalents) values.

Compounds **5b** and **6b** were partially soluble in most common deuterated solvents used in NMR and accurate ^{13}C NMR spectra could not be obtained for these compounds.

Synthesis of compounds **5a**-**g**

2-Chloro-3-[(pyridin-2-ylmethyl)amino]naphthoquinone (**5a**)

A suspension of 2,3-dichloro-1,4-naphthoquinone **1** (0.2270 g, 1.0 mmol), methanol (10 mL), picolylamine **4a** (155 µL, 1.5 mmol) and triethylamine (153 µL, 1.1 mmol) was stirred for 2 h at room temperature. The product was filtered, washed with cold methanol to obtain **5a** as an orange colored solid (0.2624 g, 88%); melting point (m.p.): 156-157 °C (literature: 126-127 °C);[12] ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.65 (d, 1H, *J* 4.9 Hz, Py-H), 8.15 (dd, 1H, *J* 7.7 Hz, 1.3, H-8), 8.06 (dd, 1H, *J* 7.6 Hz, 1.4, H-5), 7.82 (s, 1H, N-H), 7.75-7.69 (m, 2H, H-6,7), 7.63 (td, 1H, *J* 7.6 Hz, 1.3, Py-H), 7.29 (d, 1H, *J* 7.9 Hz, Py-H), 7.30-7.22 (m, 1H, Py-H), 5.19 (d, 2H, *J* 5.0 Hz, CH_2); ^{13}C NMR (101 MHz, $CDCl_3$) δ 180.57, 176.78, 155.10, 149.04, 144.39, 136.87, 134.77, 132.65, 132.40, 129.96, 126.77, 126.71, 122.66, 121.72, 48.60; EI-FT-ICRMS (M^+) calcd. for $C_{16}H_{11}ClN_2O_2$: 299.0587; found: 299.0583 (DBE = 12).

2-[(3-Aminopropyl)amino]-3-chloronaphthoquinone (**5b**)

A suspension of 2,3-dichloro-1,4-naphthoquinone **1** (0.2270 g, 1.0 mmol), methanol (10 mL), 1,3-diaminopropane **4b** (167 µL, 2 mmol) was stirred for

7 h at room temperature. The product was filtered, washed with cold methanol to give **5b** as a reddish orange solid (0.2356 g, 89%); m.p.: 215 °C (with decomposition); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.14 (dd, 1H, *J* 7.6 Hz, 1.0, H-8), 8.02 (dd, 1H, *J* 7.7 Hz, 1.0, H-5), 7.73 (td, 1H, *J* 7.6 Hz, 1.3, H-6), 7.63 (td, 1H, *J* 7.5 Hz, 1.2, H-7), 6.13 (bs, 1H, N-H), 4.00 (dd, 2H, *J* 13.5 Hz, 6.8, H_2N-C$\underline{H}_2$), 3.72 (dd, 1H, *J* 14.0 Hz, 7.0, HN-C$\underline{H}_2$), 2.91 (t, 1H, *J* 6.4 Hz, HN-C$\underline{H}_2$), 2.12 (dt, 1H, *J* 13.9 Hz, 7.1, CH_2), 1.83 (dt, 1H, *J* 12.9 Hz, 6.6, CH_2); EI-FT-ICRMS (M^+) calcd. for $C_{13}H_{13}ClN_2O_2$: 265.0744; found: 265.0739 (DBE = 8).

2-Chloro-3-((2-hydroxyethyl)amino)naphthalene-1,4-dione (**5c**)

A suspension of 2,3-dichloro-1,4-naphthoquinone **1** (0.2270 g, 1.0 mmol), methanol (10 mL), 2-aminoethanol **4c** (121 µL, 2.0 mmol) was stirred for 24 h at room temperature. The product was filtered, washed with cold water to give **5c** as an orange solid (0.2240 g, 89%); m.p.: 139-140 °C (literature: 147 °C);[13] ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.14 (dd, 1H, *J* 7.7 Hz, 0.9, H-8), 8.03 (dd, 1H, *J* 7.7 Hz, 1.0, H-5), 7.73 (td, 1H, *J* 7.6 Hz, 1.3, H-7), 7.63 (td, 1H, *J* 7.6 Hz, 1.3, H-6), 6.42 (bs, 1H, N-H), 4.06 (dt, 2H, *J* 10.7 Hz, 5.3, C$\underline{H}_2$-OH), 3.93 (dd, 2H, *J* 10.1 Hz, 5.0, C$\underline{H}_2$-NH), 1.81 (bs, 1H, *J* 4.7 Hz, CH_2-O$\underline{H}$); ^{13}C NMR (101 MHz, $CDCl_3$) δ 180.42, 176.86, 144.42, 134.92, 132.58, 132.51, 129.81, 126.84, 126.82, 61.97, 46.57; EI-FT-ICRMS (M^+) calcd. for $C_{12}H_{10}ClNO_3$: 252.0427; found: 252.0419 (DBE = 8); $[M + Na]^+$: 274.0247; found: 274.0238 (DBE = 8).

2-Chloro-3-methoxynaphthalene-1,4-dione (**5d**)

A suspension of 2,3-dichloro-1,4-naphthoquinone **1** (0.2270 g, 1.0 mmol), methanol (10 mL) and triethylamine (153 µL, 1.1 mmol) was stirred for 3 h at room temperature. The product was filtered, washed with cold water to give **5d** as a yellow solid (0.1794 g, 81%); m.p.: 144-147 °C (literature: 140 °C);[14] ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.17-8.12 (m, 1H, H-8), 8.11-8.07 (m, 1H, H-5), 7.79-7.72 (m, 2H, H-6,7), 4.32 (s, 3H, OCH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 179.68, 178.59, 156.76, 134.35, 133.92, 131.03, 130.78, 128.29, 126.97, 126.86, 61.87; EI-FT-ICRMS (M^+) calcd. for $C_{11}H_7ClO_3$; $[M + Na]^+$: 244.9981; found: 244.9972 (DBE = 8).

2,3-Dihydronaphtho[2,3-b][1,4]dithiine-5,10-dione (**5e**)

A suspension of 2,3-dichloro-1,4-naphthoquinone **1** (0.2270 g, 1.0 mmol), methanol (10 mL), 1,2-ethanedithiol **5e** (149 µL, 2.0 mmol) was stirred for 12 h at 50 °C. The solution was allowed to cool and the obtained product was filtered and washed with cold methanol to give **5e** as a dark red solid (0.1522 g, 62%); m.p.: > 230 °C (literature: > 250 °C);[15] ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.07 (dd, 2H, *J* 5.7 Hz, 3.3, H-5,8), 7.69 (dd, 2H, *J* 5.7 Hz, 3.3, H-6,7), 3.30 (s, 4H, S-CH_2); ^{13}C NMR (101 MHz, $CDCl_3$) δ 178.60, 140.74, 133.74, 131.56, 126.90, 26.99; EI-FT-ICRMS (M^+) calcd. for $C_{12}H_8S_2O_2$: 249.0044; found: 249.0037 (DBE = 8); $[M + Na]^+$: 270.9863; found: 270.9856 (DBE = 8).

2-Chloro-3-[(mercaptomethyl)thio]naphthoquinone (**5f**)

A suspension of 2,3-dichloro-1,4-naphthoquinone **1** (0.2270 g, 1.0 mmol), methanol (10 mL), 1,2-ethanedithiol **4e** (210 µL, 2.5 mmol) was stirred for 1 h at room temperature. Then, water was added (15 mL) slowly and stirred for 12 h. The product was filtered, washed with cold water to give **5f** as a purple solid (0.2164 g, 66%); m.p.: 125 °C (with decomposition; literature: 196 °C);[5] ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.22-8.17 (m, 2H, H-5,8), 8.15-8.10 (m, 2H, H-6,7), 3.59-3.42 (m, 4H, S-CH_2), 1.62 (s, 1H, SH); ^{13}C NMR (101 MHz, $CDCl_3$) δ 186.55, 186.44, 135.08, 134.81, 133.55, 131.17, 128.44, 128.42, 75.35, 42.40, 39.84; EI-FT-ICRMS (M^+) calcd. for $C_{12}H_9ClS_2O_2$: 284.9811; found: 284.9804 (DBE = 7).

2,3-Bis[(2-hydroxyethyl)thio]naphthoquinone (**5g**)

A suspension of 2,3-dichloro-1,4-naphthoquinone **1** (0.2270 g, 1.0 mmol), acetone (5 mL), 2-mercaptoethanol **4f** (210 µL, 2.0 mmol) was stirred for 1 h at room temperature. Then, water was added (10 mL) slowly and stirred for 24 h. The product was filtered, washed with cold water to obtain **5g** as an orange solid (0.1614 g, 52%); m.p.: 111-113 °C (literature: 117-118 °C);[16] ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.07 (dd, 2H, *J* 5.7 Hz, 3.3, H-5,8), 7.72 (dd, 2H, *J* 5.7 Hz, 3.3, H-6,7), 3.79 (q, 4H, *J* 4.9 Hz, C$\underline{H}_2$-OH), 3.47-3.37 (m, 4H, S-CH_2), 2.71 (s, 2H, CH_2-O$\underline{H}$); ^{13}C NMR (101 MHz, $CDCl_3$) δ 178.88, 148.99, 133.81, 132.85, 127.18, 61.94, 38.20; EI-FT-ICRMS (M^+) calcd. for $C_{14}H_{14}S_2O_4$: 309.0255; found: 309.0260 (DBE = 7).

Synthesis of compounds **6a-c**

2-[(Pyridin-2-ylmethyl)amino]naphthoquinone (**6a**)

A suspension of 2-methoxy-1,4-naphthoquinone **2** (0.0941 g, 0.5 mmol), methanol (10 mL), picolylamine **4a** (180 µL, 2.0 mmol) and triethylamine (84 µL, 0.6 mmol) was stirred for 24 h at room temperature. Then, water was slowly added (20 mL) and stirred for 24 h. The product was filtered, washed with cold methanol to obtain **6a** as an orange solid (0.1506 g, 58%); m.p.: 146-148 °C (literature: 153-155 °C);[17] ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.63 (dd, 1H, *J* 4.9 Hz, 0.7, Py-H), 8.12-8.07 (m, 2H, H-5,8), 7.78-7.66 (m, 2H, H-6,7), 7.63 (td, 1H, *J* 7.5 Hz, 1.2, Py-H), 7.29 (d, 1H, *J* 8.7 Hz, Py-H), 7.29-7.22 (m, 1H, Py-H), 7.16 (s, 1H, NH),

5.78 (s, 1H, H-4), 4.49 (d, 2H, *J* 5.2 Hz, NH-C$\underline{H}_2$); ^{13}C NMR (101 MHz, $CDCl_3$) δ 183.08, 181.70, 154.51, 149.49, 147.76, 136.91, 134.67, 133.57, 132.04, 130.60, 126.31, 126.17, 122.81, 121.67, 101.74, 47.03; EI-FT-ICRMS (M^+) calcd. for $C_{16}H_{12}N_2O_2$: 265.0977; found: 265.0971 (DBE = 12); $[2M + H]^+$: 529.1876; found: 529.1869 (DBE = 24); $[M + Na]^+$: 287.0796; found: 287.0790 (DBE = 12) and $[2M + Na]^+$: 551.1695; found: 551.1687 (DBE = 24).

2-[(3-Aminopropyl)amino]naphthoquinone (**6b**)

A suspension of 2-methoxy-1,4-naphthoquinone **2** (0.0941 g, 0.5 mmol), methanol (10 mL), 1,3-diaminopropane **4b** (83 µL, 1 mmol) and triethylamine (84 µL, 0.6 mmol) was stirred for 24 h at room temperature. Then, water was slowly added (20 mL) and stirred for 7h. The product was filtered, washed with cold methanol to obtain **6b** as an orange solid (0.1335 g, 58%); m.p.: 124-126 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.10 (dd, 1H, *J* 7.7 Hz, 1.2, H-8), 8.05 (dd, 1H, *J* 7.7 Hz, 1.2, H-5), 7.74 (td, 1H, *J* 7.6 Hz, 1.4, H-6), 7.63 (td, 1H, *J* 7.6 Hz, 1.3, H-7), 5.91 (s, 1H, NH), 5.76 (s, 1H, H-4), 3.35 (dd, 2H, *J* 6.6 Hz, C$\underline{H}_2$-NH$_2$), 2.11 (dt, 2H, *J* 6.9 Hz, NH-C$\underline{H}_2$), 1.25 (s, 2H, CH_2); EI-FT-ICRMS (M^+) calcd. for $C_{13}H_{15}N_2O_2$: 231.1133; found: 231.1118 (DBE = 8).

2-((2-Hydroxyethyl)amino)naphthalene-1,4-dione (**6c**)

A suspension of 2-methoxy-1,4-naphthoquinone **2** (0.1881 g, 1.0 mmol), methanol (10 mL), 2-aminoethanol **4c** (121 µL, 2.0 mmol) was stirred for 32 h at room temperature. The solution was cooled and the product was filtered, washed with cold methanol to obtain **6c** as an orange solid (0.1642 g, 76%); m.p.: 156-157 °C (literature: 146-147 °C);[18] ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08 (ddd, 2H, *J* 18.6 Hz, 7.7, 1.0, H-5,8), 7.73 (td, 1H, *J* 7.6 Hz, 1.3, H-6), 7.62 (td, 1H, *J* 7.6 Hz, 1.3, H-7), 6.22 (s, 1H, NH), 5.77 (s, 1H, H-4), 3.93 (dd, 2H, *J* 10.2 Hz, 5.0, C$\underline{H}_2$-OH), 3.37 (dd, 1H, *J* 10.8 Hz, 5.5, NH-C$\underline{H}_2$), 1.79 (t, 1H, *J* 5.1 Hz, CH_2-O$\underline{H}$); ^{13}C NMR (101 MHz, $CDCl_3$) δ 183.10, 181.73, 148.09, 134.77, 132.06, 126.33, 126.19, 101.16, 77.33, 77.21, 77.01, 76.69, 60.05, 44.39; EI-FT-ICRMS (M^+) calcd. for $C_{12}H_{11}NO_3$; $[M + Na]^+$: 240.0637; found: 240.0628; $[2M + Na]^+$: 457.1376; found: 457.1364.

Synthesis of compound **7**

2-Methoxynaphthalene-1,4-dione (**7**)

2-Hidroxy-1,4-naphthoquinone (1.000 g, 5.7 mmol) was dissolved in methanol (50 mL) containing concentrated chloridric acid (0.8 mL). The reaction mixture was maintained under reflux for 4 h. Then, the solution was allowed to cool and the product was filtered and recrystallized from hot water to obtain **7** as a yellow solid (0.7937g, 73%); m.p.: 179-180 °C (literature: 183 °C);[19] ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.19-8.04 (m, 2H, H-5,8), 7.81-7.68 (m, 2H, H-6,7), 6.19 (s, 1H, H-4), 3.92 (s, 3H, OCH_3).

Synthesis of compound **8**

2-((2-Hydroxyethyl)thio)naphthalene-1,4-dione (**8**)

A solution of 1,4-naphthoquinone **3** (0.0941 g, 0.5 mmol), acetone (2.5 mL) and 2-mercaptoethanol **4f** (70 µL, 1.0 mmol) was stirred for 0.5 h at room temperature. The crude mixture was then purified by chromatography (15-25% ethyl acetate:hexane) to obtain product **8** as a yellow solid (0.0449 g, 77%); m.p.: 124-127 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.14-8.04 (m, 2H, H-5, 8), 7.79-7.66 (m, 2H, H-6,7), 6.71 (s, 1H, H-4), 3.98 (dd, 2H, *J* 10.7 Hz, 5.4, C$\underline{H}_2$-OH), 3.10 (t, 2H, *J* 6.1 Hz, S-CH_2), 2.06 (s, 1H, CH_2-O$\underline{H}$); ^{13}C NMR (101 MHz, $CDCl_3$) δ 182.04, 181.59, 154.21, 134.42, 133.40, 132.09, 131.84, 127.44, 126.92, 126.59, 59.73, 33.26; ESI(+)FT-ICRMS calcd. for $C_{12}H_{10}O_3S$ $[M + Na]^+$: 257.0248; found: 257.0244; $[2M + Na]^+$: 491.0599; found 491.0596.

In vitro antineoplastic activity evaluation

The *in vitro* cytotoxicity activity of the synthesized compounds was carried out against three human cancer cell lines of solid tumors namely H460 (non-small cell lung cancer/large cell lung cancer), A2780 (epithelial ovarian carcinoma) and MDA MB-231(triple negative breast cancer). Cells were cultured in Roswell Park Memorial Institute (RPMI) medium supplemented with 10% of fetal bovine serum (FBS) and antibiotics, at 37 ºC in 5% of CO_2. For dimethyl thiazolyl diphenyl tetrazolium bromide (MTT) assay,[20] the cells were plated in 96-well culture dishes at 7.5×10^4 cell/well, and allowed to recover for 24 h, then, treated for 24 h in a dose-dependent manner with each tested compound at concentrations of 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7} and 10^{-8} mol L^{-1}. After, was used MTT (5 mg mL^{-1}) to evaluate the cellular metabolic viability. The absorbance at 630 nm was measured on a spectrophotometer (MR-96A, Bioclin).

Control treatments were performed with chemotherapy standard. The results are representatives of three independent experiments, mean and standard-deviation of the absorbances were used to calculate cell metabolic viability, and the IC_{50} using PrismaGraphPad version 5.1.

Docking

The docking studies were performed in AutoDock Vina software[21] with the ligand and the enzymes *PI3K*γ

(PDB ID: 1E7U) and *topoisomerase II* (PDB ID: 1QZR). The 3D structures of the compounds were obtained after semi-empirical PM6 optimization.[22] Docking was performed covering an 18 × 10 × 12 Å box size, centered on ligand. After validation of the method with the crystallographic ligands, interaction studies with new compounds were performed.

Results and Discussion

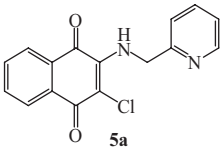
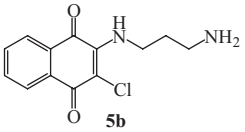
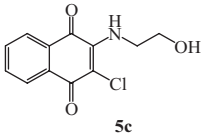
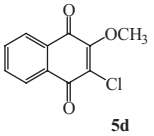
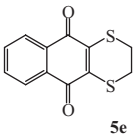
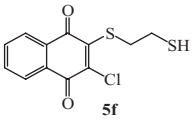
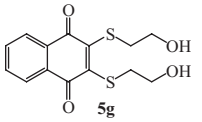
Chemistry

Initially, we carried out monosubstitution of the 2,3-dichloronaphthoquinone **1** with nitrogen nucleophiles (picolylamine **4a**, 1,2-diaminopropane **4b** and 2-aminoethanol **4c**), oxygen nucleophile (methanol **4d**), and sulfur nucleophiles (1,2-ethanedithiol **4e** and 2-mercaptoethanol **4f**) using the various experimental conditions compiled in Table 1.

For the synthesis of compound **5a** (entry 1, Table 1), triethylamine was used with the nucleophile and methanol as the solvent to facilitate the precipitation of the product, which was obtained with high purity and in an excellent yield.[12] The synthesis of **5b** (entry 2, Table 1) followed a similar methodology, except for the absence of triethylamine. Moreover, to the best of our knowledge, the preparation of compound **5b** has not been reported before.

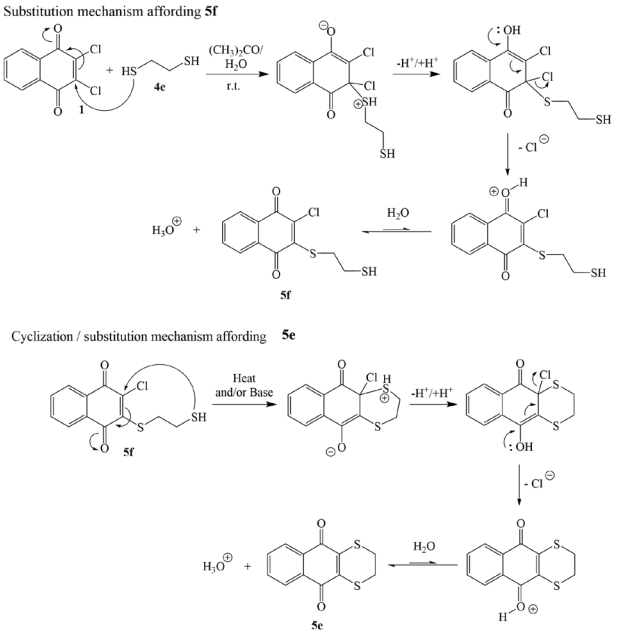
In turn, compounds **5c** (entry 3, Table 1) was synthesized under milder conditions than that previously published and yet resulted in better yields. Compound **5c** was synthesized by Brun *et al.*,[13] reacting 2,3-dichloro-1,4-naphthoquinone, 2-ethanolamine and Et_3N, in diethyl ether solution, obtaining a 46% yields. In the present work, the same product was obtained with a yield of 89% by using methanol as the solvent and excess of nucleophile. The substance **5d** (entry 4, Table 1) was prepared before using

Table 1. Synthesis of substituted naphthoquinone derivatives from 2,3-dichloro-1,4-naphthoquinone **1**

Entry No.	Nucleophile	Solvent	Base	time / h	Product	Yield / %
1	**4a**	CH_3OH	Et_3N	2	 **5a**	88
2	**4b**	CH_3OH	not used	7	 **5b**	89
3	**4c**	CH_3OH	not used	24	 **5c**	89
4	**4d**	CH_3OH	Et_3N	3	 **5d**	81
5	**4e**	CH_3OH	not used	12	 **5e**	62
6	**4e**	$(CH_3)_2CO/ H_2O$	not used	12	 **5f**	66
7	**4f**	$(CH_3)_2CO/ H_2O$	not used	24	 **5g**	52

a solution of 2,3-dichloro-1,4-naphthoquinone and sodium methoxide in methanol, where methoxide acted as the nucleophile.[14] In this work, compound **5d** was synthesized with a yield of 81% using a solution of 2,3-dichloro-1,4-naphthoquinone, methanol and Et_3N, where methanol was the nucleophile and the solvent, simultaneously.

Preparation of the compound **5f** by reacting **1** with 1,2-ethanedithiol **4e** (entry 6, Table 1) was particularly challenging since we failed to reproduce the previously described method of nucleophilic substitution in water.[4] Therefore, we enacted several experimental modifications, including different solvents (water, ethanol, methanol and acetone), variable temperatures (reactions were carried out at room temperature (r.t.) and under reflux, different bases such as K_2CO_3 and Et_3N and reaction times ranging from 1-48 h. However, due to the softness and the high nucleophilicity of **4e**, the disubstituted cyclized product **5e**[15] (entry 5, Table 1) was systematically obtained from an intramolecular nucleophilic substitution (Scheme 1). Heat and the use of the Et_3N base favored generation of the product. The desired compound **5f** was obtained only when the reaction was carried out in a mixture of acetone:water at r.t. with excess nucleophile.



Scheme 1. Substitution mechanisms affording **5e** and **5f**.

In order to obtain the monosubstituted product from **1** and 2-mercaptoethanol **4f**, a reaction was carried out under the same optimized conditions as previously described for the formation of **5f**. However, the disubstituted product **5g** was formed (entry 7, Table 1) from a double intermolecular nucleophilic substitution. No alicyclic product was obtained, which is likely due to the increased hardness and lower nucleophilicity of the hydroxyl group of 2-mercaptoethanol. The monosubstituted product was not obtained by this methodology.

Subsequently, several attempts were made to develop a simple and effective novel methodology that would enable us to obtain the disubstituted compounds from the products **5a** and **5f** using, initially, nucleophiles **4a** and **4e** (Table 2).

Table 2. Tested methods to obtain the disubstituted product from **4a** and **4e**

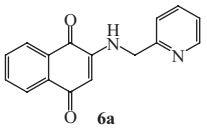
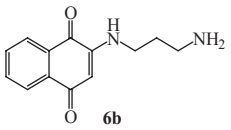
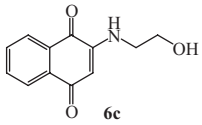
Entry No.	Solvent	Temperature / °C	time / h	Catalyst
1	CH_3OH	r.t.	1	not used
2	CH_3OH	r.t.	24	$AgNO_3$
3	CH_3OH	65	24	$AgNO_3$
4	C_2H_5OH	r.t.	24	$AgNO_3$
5	C_2H_5OH	78	24	$AgNO_3$
6	H_2O	r.t.	24	$AgNO_3$
7	H_2O	50	24	$AgNO_3$
8	CH_3OH/ H_2O	50	24	$AgNO_3$
9	C_2H_5OH / H_2O	50	24	$AgNO_3$
10	H_2O	r.t.	24	$AlCl_3$
11	H_2O	50	24	$AlCl_3$
12	$(H_3C)_2CO$	56	72	$AlCl_3$
13	H_3CCN	r.t.	24	$AlCl_3$
14	H_2O	50 °C	24	$CeCl_3.7H_2O$
15	H_3CCN	r.t.	12	$CeCl_3$/NaI
16	H_3CCN	82 °C	24	$CeCl_3$/NaI

r.t.: room temperature.

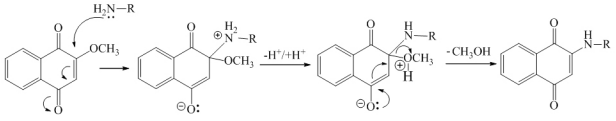
In attempt to render the chlorine atom a better leaving group, we added $AgNO_3$ to the reactional solution, aiming at the complexation of the Ag^+ ions with the chlorine atom (entries 2-9, Table 2);[23] the same rationale applies for the use of $AlCl_3$, a Lewis acid with an affinity for oxygen and halogen atoms (entries 10-13, Table 2). However, the two methodologies proved to be unsatisfactory. Next, $CeCl_3$ was used to impose an electron withdrawing effect on the quinone ring by complexation with the carbonyl group and, thus, increasing the electrophilicity of C_3 and facilitating the nucleophile attack (entry 14, Table 2). Again, an unsatisfactory result was obtained. We next tried to complete the second nucleophilic substitution under Finkelstein conditions using NaI to replace the remaining chlorine by iodine, which is a better leaving group (entries 15 and 16, Table 2). Nonetheless, we did not succeed in obtaining the desired disubstituted products.

To evaluate the importance of the chlorine at the carbon atom C_3, as well as, the nitrogen substituent at the C_2 in the **5a-c** products for antitumor activity, nucleophilic substitution

Table 3. Synthesis of monosubstituted derivatives **6a-c** from 2-methoxy-1,4-naphthoquinone **7**

Entry No.	Nucleophile	Solvent	time / h	Product	Yield / %
1	**4a**	CH_3OH	24	 **6a**	58
2	**4b**	CH_3OH	7	 **6b**	58
3	**4c**	CH_3OH	32	 **6c**	76

reactions at r.t. with 2-methoxy-1,4-naphthoquinone **7** using the **4a** picolylamine, 1,2-diaminopropane **4b** and 2-aminoethanol **4c** nucleophiles, were carried out (Table 3). The compounds **6a**[17] and **6c**[18,24] were obtained in good-to-moderate yields by nucleophilic substitution of the methoxyl group in the 2-methoxy-1,4-naphthoquinone **7** by picolylamine and ethanolamine, respectively (Scheme 2). The compound **6b** was synthesized following the same method, but using 1,3-diaminopropane as the nucleophile and methanol as the solvent, at room temperature. Moreover, to the best of our knowledge, the preparation of compound **6b** has not been reported before.



Scheme 2. Mechanism for nucleophilic substitution on 2-methoxy-1,4-naphthoquinone.

The 2-methoxy-1,4-naphthoquinone **7** was prepared by methylation of 2-hidroxy-1,4-naphthoquinone via heating in methanol and hydrochloric acid, as described in the literature.[19]

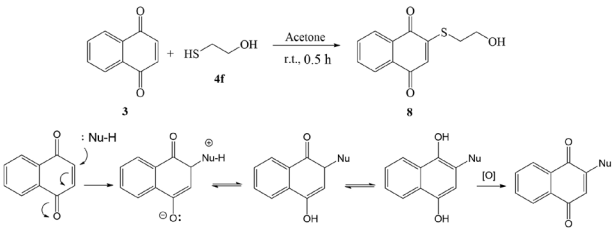
The reaction of 2-methoxy-1,4-naphthoquinone was also tested with the sulfur nucleophiles **4e** and **4f**; however, distinct from the observation with nitrogen nucleophiles, the reactions with sulfur nucleophiles resulted in a complex mixture of products which were difficultly separated and it was not possible to obtain the desired product. A possible undesired reaction in this system is the oxidative Michael addition.

Because we failed to substitute the methoxyl group in **7** by the sulfur nucleophiles **4e** and **4f** to obtain the corresponding monosubstituted product containing the sulfur atom in the position 2 of the naphthoquinone core, we then tried to obtain these products by the oxidative Michael addition.

This methodology, which is well recommended, employs cerium(III), copper(II), nickel(II) or gold(III) salts as Lewis acids to promote the Michael addition type in the naphthoquinone, with moderate-to-good results when primary and secondary aliphatic and aromatic amines were used.[6,24-27]A comprehensive study of the methods was carried out by Lisboa *et al.*,[25] who obtained better yields when $Cu(OAc)_2.H_2O$ and acetic acid was used at 60 °C. Interestingly, recent studies have described simplified methods to carry out the referred reaction without the need for a metal, which promotes the oxidative addition of quinone, but using methanol, ethanol, ethanol:water, or even water only as solvents.[4,14,28-31]

Initially, the oxidative Michael reaction was tested between the 2-mercaptoethanol **4f** and the 1,4-naphthoquinone **3**, using $Cu(OAc)_2.H_2O$ as the oxidant and acetic acid or ethanol as solvent, as described in the literature.[6] However, in both cases, a complex mixture of products was formed, making them difficult to isolate and purify. Subsequently, the reaction was performed using oxygen as the oxidizing agent and water or ethanol as the solvent. The reaction was performed at room temperature and under reflux, but again the desired product could not be obtained.

Only when acetone was used as the solvent, at r.t., compound **8** was obtained in a 77% yield (Scheme 3). The occurrence of oxidative addition in absence of an external oxidizing agent can be explained by the oxidizing ability of 1,4-naphthoquinone, which can assist in the formation of the desired product, as described by Taylor *et al.*,[32] is worth emphasizing that, to the best of our knowledge, compound **8** has never been before described in the literature.



Scheme 3. Synthesis of naphthoquinone derivatives **8**.

The aforementioned optimized oxidative Michael addition was employed with 1,2-ethanedithiol **4e** as the nucleophile; however, the respective product was not obtained. We also tested hydrated copper acetate in acetic acid or water; nonetheless, the strategy was proven to be ineffective.

In vitro antineoplastic activity

Over the past years, efforts of our research group have been devoted to synthesizing and proving the antineoplastic efficacy of various naphthoquinone derivatives in several human cancer cell lines, such as lung cancer H460 and A549, ovarian cancer A2780, triple negative breast cancer MDA-MB-231 and promyelocytic leukemia HL-60, which are representative cancers with poor prognoses.

In the present study, the *in vitro* cytotoxicity of the synthesized compounds **5a**-**5g**, **6a**-**6c** and **8** was assessed, by a standard colorimetric assay (the MTT method)[20] to estimate IC_{50} values, in three human cancer cell lines of solid tumors: H460 (non-small cell lung cancer/large cell lung cancer), A2780 (epithelial ovarian carcinoma) and MDA MB-231 (triple negative breast cancer). For internal experimental controls, the lineages were treated with routinely prescribed chemotherapy agents: etoposide, cisplatin for lung cancer line cell H460, cisplatin and paclitaxel for epithelial ovarian carcinoma A2780, paclitaxel, and doxorubicin for MDA MB-231 (triple negative breast cancer). All cells were treated continuously for 24 h in a dose-dependent fashion. The results are listed in Table 4.

When analyzing the proliferation of the lung cancer cell line H460, we noted that compound **8** was 10 times more potent than the control drugs at inhibiting H460 proliferation. On the other hand, compounds **5f** and **5g** showed cytotoxic activities similar to the controls. Thus, the compound containing a sulfur atom at the C_2 carbon of the naphthoquinone was the most potent drug against the type of lung cancer studied, suggesting that the drug might serve as an alternative, but yet efficient, therapeutic strategy to fight this deadly disease.

For the triple negative breast cancer cell line, MDA MB-231, all tested compounds were less potent than paclitaxel at controlling the cell proliferative activity. Nonetheless, among the tested compounds, substance **8** was the most potent. Of interest, compound **5c**, which contains a nitrogen substituent at the C_2 position of the naphthoquinone, was potent against the proliferation of the cancer at the same order of magnitude as compound **8**. These observations are of remarkable clinical interest since triple negative breast cancer still challenges the field of medicine due to its low overall survival rates. These low rates are caused, in part, by the relapse of resistant clones, despite the initial satisfactory response to chemotherapy, thus leading to an overall low index of survival.[33]

The *in vitro* screening for ovarian cancer A2780 antiproliferation revealed that compounds **8** and **5g** were 10 times more potent than paclitaxel. Other compounds, such as **6a** and **6b**, exhibited activities similar to paclitaxel, but were more potent than cisplatin against the disease. Overall, and as discussed above, these findings are of major clinical interest since ovarian cancer cells are either refractory or acquire resistant phenotypes, accounting for ovarian cancer second position in the ranking of the most frequent causes of deaths related to gynecological cancers in the world.

Interestingly, we noted that when alkyl groups containing sulfur atom are present at C_2 of the carbon atom of the naphthoquinone core, the resulting compounds are more potent against human cancers than compounds

Table 4. IC_{50} values for human lung (H460), triple-negative breast (MDA-MB-231) and ovarian (A2780) cancer cell lines

Compound	IC_{50} / mol L^{-1}		
	H460	MDA-MB-231	A2780
Cisplatin	8.586×10^{-5}	–[a]	4.01×10^{-5}
Etoposide	3.441×10^{-5}	–[a]	–[a]
Doxorubicine	–[a]	1.7×10^{-4}	–[a]
Paclitaxel	–[a]	1×10^{-6}	1.36×10^{-5}
5a	1.960×10^{-4}	–[b]	3.04×10^{-4}
5b	5.008×10^{-4}	8.6×10^{-4}	2.43×10^{-4}
5c	1.293×10^{-4}	2.13×10^{-5}	6.29×10^{-5}
5d	2.292×10^{-4}	6.08×10^{-5}	4.97×10^{-5}
5e	1.861×10^{-4}	1.1×10^{-3}	–[b]
5f	3.048×10^{-5}	1.3×10^{-4}	3.38×10^{-5}
5g	4.410×10^{-5}	4.28×10^{-5}	2.68×10^{-6}
6a	7.595×10^{-4}	9.4×10^{-4}	2.77×10^{-5}
6b	6.635×10^{-4}	7.8×10^{-4}	1.91×10^{-5}
6c	2.334×10^{-4}	–[b]	–[b]
8	4.24×10^{-6}	1.603×10^{-5}	3.89×10^{-6}

[a]Routinely prescribed as chemotherapy agents; [b]no antiproliferative activity.

containing nitrogen atoms. It is worthwhile pointing out that the most active compounds with substituents at the C_2 position of the naphthoquinone core have either –OH or –SH groups in their terminal alkyl chain.

Docking

As previously discussed, PI3K is frequently mutated in human cancers, thus enabling constitutive activation of PI3K-related pathways, as in PI3K/AKT/mTOR pathways. Because the genotype facilitates carcinogenesis and cancer progression,[34] targeting PI3K, as well as, its effect or molecules have attracted the attention of cancer research groups in both academia and the pharmaceutical industry. Indeed, there are a few examples in clinical trials now; nonetheless, many have failed to confer sustained disease remission. Therefore, the generation of novel anti-PI3K molecules is urgent. Recently, the elucidation of the crystal structure of PI3Kγ and their complexes with Wortmannim and LY294002 ligands have facilitated the rational design of new inhibitors of the enzyme. Its crystal structure was obtained from the Protein Data Bank (PDB) under code 1E7U (Figure 3). Docking studies were conducted in order to analyze the conformation and binding energy through the AutoDock Vina software.[21]

Docking studies were also conducted in relation to *topoisomerase II*, another target of great importance in the study and development of new naphthoquinone substances with potential antineoplastic activity.[35,36] The crystal structure of this protein was obtained from the PDB under code 1QZR.

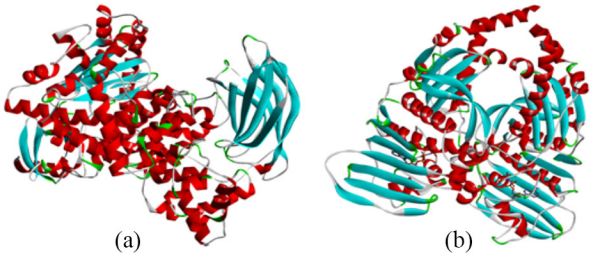


Figure 3. Crystallographic structure representation of PI3K (1E7U) (a) and *topoisomerase II* (1QZR) (b).

PI3K and *topoisomerase II* were prepared with their respective ligands, dexrazoxane and Wortmannim, according to the protocol described in the literature.[21] Grid boxes with dimensions of 18 × 10 × 12 Å for both receptors and coordinates x = 23.426, y = 62.986 and z = 20.716 (*PI3K*) and x = 28.166, y = 33.408 and z = 32.263 (*topoisomerase II*), both centered in the ligand, were constructed to fully cover the active site of the enzymes. Immediately after, the crystallographic ligands were redocked with their receptors in order to validate the efficiency of the docking calculations (Figure 4).

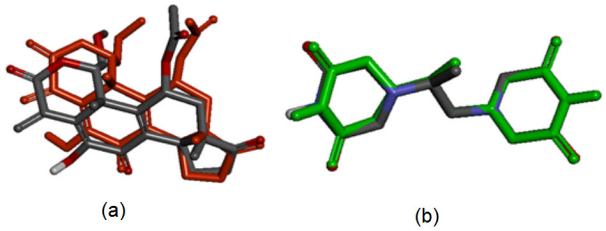


Figure 4. Crystallographic ligands (grey) and redocked ligand. (a): red Wortmannim (1E7U-KWT:PDB) and (b): green dexrazoxane (1QZR-CDX:PDB).

Next, the control drugs Etoposide, a *topoisomerase II* inhibitor, and Wortmannim, a PI3K inhibitor compound described in the literature,[37,38] were superimposed on the binding site of the enzyme, the adenosine triphosphate (ATP) cleft, via the same method. Note that the chemical structure of the ligand was fully optimized using the parametric method 6 (PM6) semi-empirical method.[22] As a result, we obtained the superposition of the ligand in the active site of PI3Kγ by calculating the interaction energy ligand/receptor (Table 5). The same procedures of molecular modeling calculations were used for all naphthoquinone derivatives.

The results obtained for the tested compounds (Table 5) indicated interaction energies close to crystallographic reference structures, especially **5a** and **6a** against PI3Kγ and **6c** against *topoisomerase II*. These results lead us to suppose that the anticancer mechanism of action to these naphthoquinone derivatives occur through PI3K and *topoisomerase II* inhibition, as widely reported previously.[2]

The docking studies are able to identify the best pose of ligands into the binding site. Figure 5 depicts the intermolecular interaction between Wortmannim and **6a** against the binding site of *PI3K* enzyme performing van der Waals interactions with Lys808, Pro810, Trp812, Ile831, Ile879, Glu880, Ile881, Asp950, Phe961, Ile967 amino acids; electrostatic interactions with Met804, Val882, Lys883, Met953, Asp964, Tyr867; and hydrogen bonds with Val882, Lys883, Tyr867, Asp964 amino acids (Figure 6a).

Likewise, the most active compound by docking, **6a**, can complex by van der Waals interactions with Ser806, Pro810, Glu880, Lys833, Ile879, Val882, Met953, Phe961, Ile963 amino acids; electrostatic interactions with Met804, Tyr867, Asp964 amino acids. No hydrogen bonds could be observed in these compounds. However, new derivatives with acceptor hydrogen bond can improve the active by an interaction with Tyr867, similar to Wortmannim. In addition, Figure 6c shows the cluster of all ligands in the

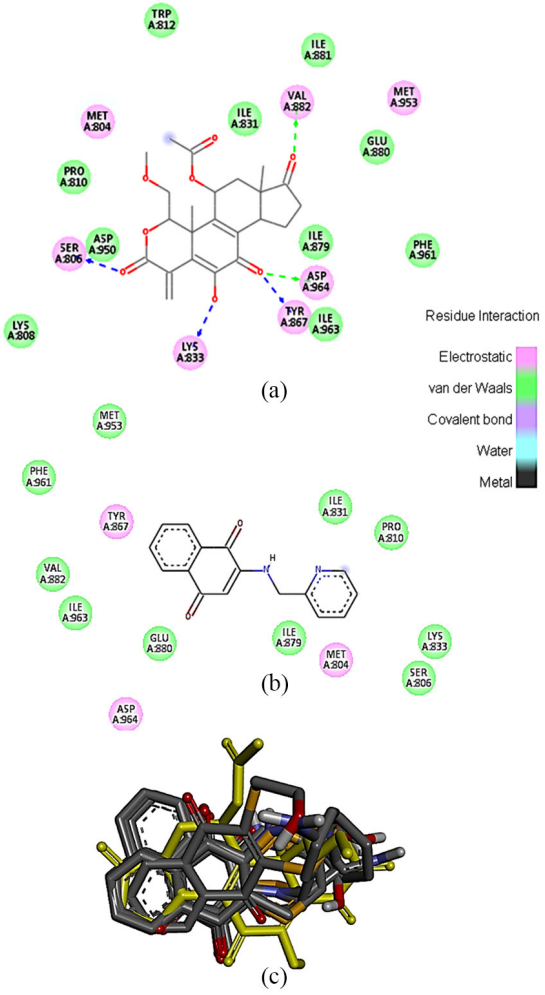


Figure 5. Pharmacophoric conformation of Wortmannim in (a); **6a** in (b) and cluster of all naphthoquinone derivatives in the binding site of *PI3K*, in which Wortmannim is highlighted in yellow in (c).

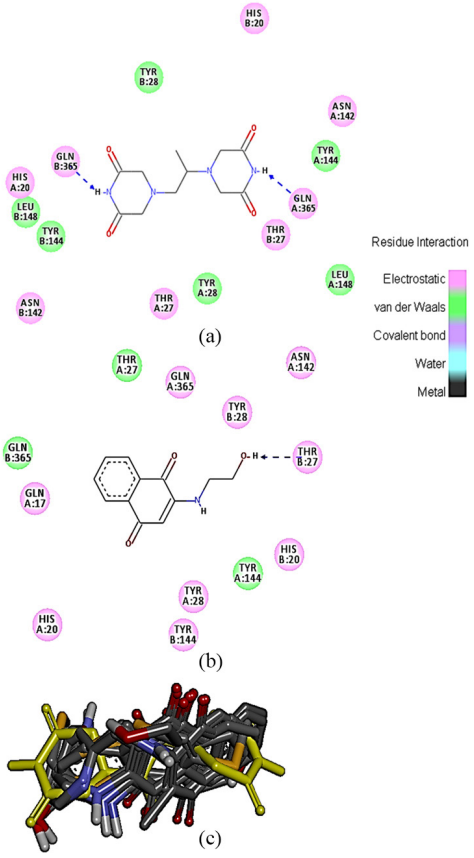


Figure 6. Pharmacophoric conformation of dexrazone in (a); **6c** in (b) and cluster of all naphthoquinone derivatives in the binding site of *topoisomerase II,* in which dexrazone is highlighted in yellow in (c).

Table 5. Interaction energies of compounds **5a**-**5g**, **6a**-**6c**, **8**, Wortmannim, dexrazone and etoposide against respective molecular targets

Compound	Interaction Energy / (kcal mol^{-1})	
	PI3Kγ	*Topoisomerase II*
Wortmannim	–7.7	–
Dexrazone	–	–7.8
Etoposide	–	–8.1
5a	–7.7	–4.0
5b	–6.7	–6.9
5c	–6.5	–7.1
5d	–7.0	–6.6
5e	–7.0	–5.2
5f	–6.5	–4.8
5g	–6.1	–4.8
6a	–7.9	–5.0
6b	–6.8	–7.5
6c	–6.7	–7.8
8	–6.6	–7.2

binding site. As can be seen, the naphthalene complex is very similar to Wortmannim into the binding site of phosphatidylinositol 3-kinase (*PI3K*), suggesting this molecular target as a receptor of naphthoquinones.

Similarly, to *PI3K* enzyme, Figure 6 shows the intermolecular interaction between dexrazone, and **6c**. As can be seen, dexrazone performs van der Waals interactions with Tyr28, Tyr144, Leu148 amino acids; electrostatic interactions with Thr27, His28, Asn142 amino acids; and hydrogen bond with Gln365 amino acid, all for both chains of enzyme (a and b) (Figure 6a). Similarly, compound **6c** can complex through van der Waals interactions with Thr27, Tyr144, Gln365 amino acids; electrostatics interaction with Gln17, His20, Thr27, Tyr28, Asn142; a hydrogen bond can be observed between **6c** and Thr27 (Figure 6b). Finally, Figure 6c shows a cluster formed by dexrazone and naphthoquinones. These results suggest that these compounds can inhibit the *topoisomerase II*.

In addition, the naphthoquinone derivatives **5c**, **5f**, and **8** can highlight the electrostatic interaction between the naphthoquinone carbonyl core and amino acids from the Asp964 residue of the *PI3K* protein (Figure 7).

Another important interaction occurs between –SH and –OH substituents and the C_2 naphthoquinone carbon

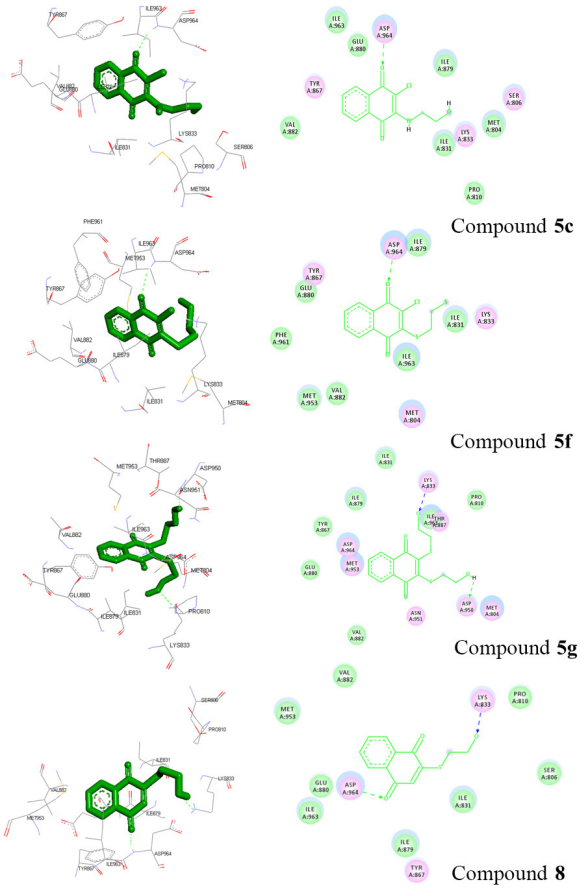


Figure 7. Interaction between PI3Kγ and compounds **5c**, **5f**, **5g** and **8**.

with different amino acid residues, such as the electrostatic interaction with the Lys833 to compound **5c** and the hydrogen bonds with Asp950 and Lys833 with **5g** and **8**, respectively. The other interactions of these compounds are van der Waals interactions with different amino acid residues.

The docking of etoposide against the active site of *topoisomerase II* showed a hydrogen bond between the hydroxyl group and Gly365, electrostatic interactions with the Gly17, Thr27, Tyr28, and van der Waals interactions with Tyr144, as shown in Figure 8.

Naphthoquinone derivatives have in general similar interactions to those presented in the docking etoposide, where the major amino acid residues responsible for this interaction have been Gly365, Gly17, Thr27, Tyr28 and Tyr144 (Figure 9). This similarity likely reflects the interaction energies between naphthoquinone derivatives and etoposide when *topoisomerase II* is the therapeutic target.

Compound **5c** showed two important hydrogen bonds, the amino and hydroxyl groups from the side chain of the substituent (ethanolamine) with the tyrosine amino acid residues glycine 28 and 17. Furthermore, an electrostatic interaction with threonine 27 was observed.

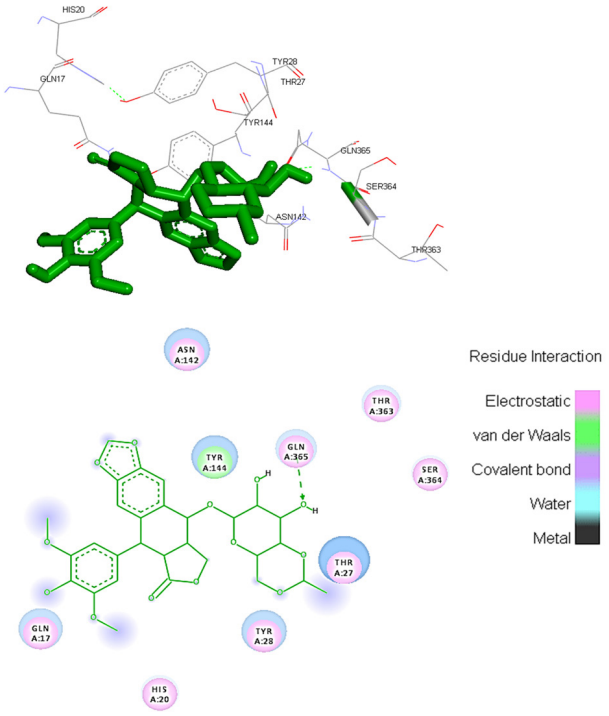


Figure 8. Pharmacophoric conformation of etoposide against the active site of the *topoisomerase II*.

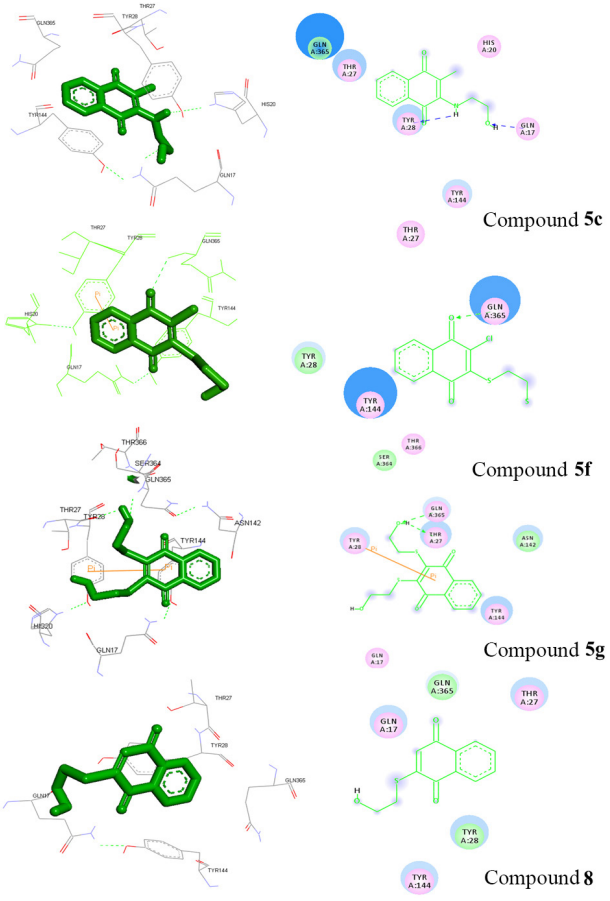


Figure 9. Interactions between *topoisomerase II* and compounds **5c**, **5f**, **5g** and **8**.

Compounds **5f** and **5g** showed the same hydrogen bonds with etoposide described for the amino acid residue Gly365. However, the group that was responsible for this interaction in **5f** (**5g**) was the naphthoquinone carbonyl core (the terminal hydroxyl substituent from mercaptoethanol). Besides these interactions, compound **5g** showed an electrostatic interaction with the amino acid threonine 27. Compound **8** showed two electrostatic interactions with the amino acid residues Gly17 and Tyr144. van der Waals interactions were also observed with Gly365 and Tyr28.

Conclusions

In this work, we have described novel and highly efficient methodologies for preparation of eleven 1,4-naphthoquinone derivatives **5a-g**, **6a-c** and **8** containing substituents in positions 2 and/or 3 in yields ranging from 38% to 89%. The following compounds showed greater *in vitro* antitumor activity: **5f** and **8** against human lung cancer lines H460; **5c** and **8** against triple-negative breast cancer lines MDA-MB-231; **5g** and **8** against ovarian cancer lines A2780. The clinical drugs tested in H460, etoposide and cisplatin; and in A2780, cisplatin and paclitaxel, showed higher values of IC_{50} compared with the results obtained for synthesized novel compounds, thus, pointing to the higher antineoplastic potency of the latter. The structure/activity relationship of these compounds showed that bioisosteric substitution for sulfur atoms at the carbon C_2 in naphthoquinone core, or hydroxyl groups in the substituent chain, are primarily responsible for the biological activity of the compounds. The docking analysis revealed that a possible therapeutic target of these substances are *topoisomerase II* and PI3K. These compounds have shown to be promising drug candidates to combat human cancer.

Supplementary Information

Supplementary data (Figures S1-S36) are available free of charge at http://jbcs.sbq.org.br as PDF file.

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References

1. Avendaño, C.; Menéndez, J. C.; *Medicinal Chemistry of Anticancer Drugs*, Elsevier: Amsterdam, 2008.
2. Gonelli, A.; Mischiati, C.; Guerrini, R.; Voltan, R.; Salvadori, S.; Zauli, G.; *Mini-Rev. Med. Chem.* **2009**, *9*, 498; Kong, D.; Yamori, T.; *Curr. Med. Chem.* **2009**, *16*, 2839; Ihle, N. T.; Powis, G.; *Mol. Aspects Med.* **2010**, *31*, 135; Pinho, B. R.; Sousa, C.; Oliveira, J. M. A.; Valentão, P.; Andrade, P. B.; *Bioactive Compounds: Types, Biological Activities and Health Effects*, Nova Science Publishers: New York, 2012; Cerqueira, E. C.; Netz, P. A.; Canto, V. P.; Pinto, A. C.; Follmer, C.; *Chem. Biol. Drug. Dis.* **2014**, *83*, 401.
3. Niculescu, V. C.; Muresan, N.; Salageanu, A.; Tucureanu, C.; Mrinescu, G.; Chirigiu, L.; Lepadatu, C. J.; *Organom. Chem.* **2012**, *700*, 13; Silva, M. N.; Ferreira, V. F.; Souza, M. C. B. V.; *Quim. Nova* **2003**, *26*, 407; Liu, K. C.; Li, J.; *Mini-Rev. Med. Chem.* **2004**, *4*, 1105; Asche, C.; *Mini-Rev. Med. Chem.* **2005**, *5*, 449; Barbosa, T. P.; Camara, C. A.; Silva, T. S. M.; Martins, R. M.; Pinto, A. C.; Vargas, M. D.; *Bioorg. Med. Chem.* **2005**, *13*, 6464; Teixeira, M. J.; Almeida, Y. M.; Viana, J. R.; Holanda F. J. G.; Rodrigues, T. P.; Prata, J. R. C.; Coelho, L. C. B.; Rao, V. S.; Pompeu, M. M. L.; *Phytother. Res.* **2001**, *15*, 44; Almeida, E. R.; Silva-Filho, A. A. A.; Santos, E. R.; Lopes, C. A. J.; *Ethnopharmacol.* **1990**, *29*, 239; Gafnier, S.; Wolfender, J. L.; Nianga, M.; Stoeckli-Evans, H.; Hostettmann, K.; *Phytochemistry* **1996**, *42*, 1315; Moura, K. C. G.; Emery, F. S.; Neves-Pinto, C.; Pinto, M. C. F. R.; Dantas, A. P.; Salomão, K.; Castro, S. L.; Pinto, A. V.; *J. Braz. Chem. Soc.* **2001**, *12*, 325; Zani, C. L.; Chiari, E.; Krettli, A. U.; Murta, S. M. F.; Cunningham, M. L.; Fairlamb, A. H.; Romanha, A. J.; *Med. Chem.* **1997**, *5*, 2185; Stagliano, K. W.; Emadi, A.; Lu, Z.; Malinakova, H. C.; Twenter, B.; Yu, M.; Holland, L. E.; Rom, A. M.; Harwood, J. S.; Amin, R.; Johnson, A.; Yves, P.; *Bioorg. Med. Chem.* **2006**, *14*, 5651; Kongkathip, B.; Akkarasamiyo, S.; Hasitapan, K.; Sittikul, P.; Boonyalai, N.; Kongkathip, N.; *Eur. J. Med. Chem.* **2013**, *60*, 271; Katoh, T.; Monma, H.; Wakasugi, J.; Narita, K.; Katoh, T.; *Eur. J. Org. Chem.* **2014**, *32*, 7099; Brötz, E.; Herrmann, J.; Wiese, J.; Zinecker, H.; Maier, A.; Kelter, G.; Imhoff, J. F.; Müller, R.; Paululat, T.; *Eur. J. Org. Chem.* **2014**, *24*, 5318; Duchowicz, P. R.; Bennardi, D. O.; Bacelo, D. E.; Bonifazi, E. L.; Rios-Luci, C.; Pádron, J. M.; Burton, G.; Misico, R. I.; *Eur. J. Org. Chem.* **2014**, *24*, 2318.
4. Tandon, V. K.; Maurya, H. K.; *Tetrahedron Lett.* **2009**, *50*, 5896.
5. Tandon, V. K.; Maurya, H. K.; Verma, M. K.; Kumar, R.; Shukla, P. K.; *Eur. J. Med. Chem.* **2010**, *45*, 2418.
6. Benites, J.; Valderrama, J. A.; Bettega, K.; Pedrosa, R. C.; Calderon, P. B.; Verrax, J.; *Eur. J. Med. Chem.* **2010**, *45*, 6052.
7. Tandon, V. K.; Maurya, H. K.; Mishra, N. N.; Shukla, P. K.; *Bioorg. Med. Chem. Lett.* **2011**, *21*, 6398.
8. Tandon, V. K.; Maurya, H. K.; Tripathi, A.; ShivaKeshava, G. B.; Shukla, P. K.; Srivastava, P.; Panda, D.; *Eur. J. Med. Chem.* **2009**, *44*, 1086.
9. Lee, D.; Ko, J. H.; Lee, K.; *Monatsh. Chem.* **2007**, *138*, 741.

10. Ibis, C.; Tuyun, A. F.; Ozsoy-Gunes, Z.; Bahar, H.; Stasevych, M. V.; Musyanovych, R. Y.; Komarovska-Porokhnyavets, O.; Novikov, V.; *Eur. J. Med. Chem.* **2011**, *46*, 5861.
11. Win, T.; Bittner, S.; *Tetrahedron Lett.* **2005**, *46*, 3229.
12. Madhupriya, S.; Elango, K. P.; *Spectrochim. Acta A* **2012**, *97*, 100.
13. Brun, M. P.; Braud, E.; Angotti, D.; Mondésert, O.; Quaranta, M.; Montes, M.; *Bioorg. Med. Chem.* **2005**, *13*, 4871.
14. Sarhan, A. E. A. O.; El-Dean, A. M. K.; Abdel-Monem, M. I.; *Monatsh. Chem.* **1998**, *29*, 205.
15. Tandon, V. K.; Yadav, D. B.; Singh, R. V.; Vaish, M.; Chaturvedi, A. K.; Shukla, P. K.; *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3463.
16. Kulka, M.; *Can. J. Chem.* **1962**, *40*, 1235.
17. Jali, B. R.; Masud, K.; Baruah, J. B.; *Polyhedron* **2013**, *51*, 75.
18. MacGregor, K. A.; Abdel-Hamid, M. K.; Odell, L. R.; Chau, N.; Whiting, A.; Robinson, P. J.; McCluskey, A.; *Eur. J. Med. Chem.* **2014**, *85*, 191.
19. Panichayupakaranant, P.; Reanmongko, W.; *Pharm. Biol.* **2002**, *40*, 429.
20. Mosmann, T. J.; *Immunol. Methods* **1983**, *65*, 55.
21. Trott, O.; Olson, A. J. J.; *Comput. Chem.* **2010**, *31*, 455.
22. Stewart, J. J. P.; *J. Mol. Model.* **2007**, *13*, 1173.
23. Weibel, J. M.; Blanc, A.; Pale, P.; *Chem. Rev.* **2008**, *108*, 3149.
24. Bouffier, L.; Lister, K. E.; Higgins, S. J.; Nichols, R. J.; Doneux, T. H.; *J. Electroanal. Chem.* **2012**, *664*, 80.
25. Lisboa, C. S.; Santos, V. G.; Vaz, B. G.; Lucas, N. C.; Eberlin, M. N.; Garden, S. J.; *J. Org. Chem.* **2011**, *76*, 5264.
26. Pratt, Y. T.; *J. Org. Chem.* **1962**, *27*, 3905.
27. Yoshida, K.; Ishiguro, M.; Honda, H.; Yamamoto, M.; Kubo, Y.; *Bull. Chem. Soc. Jpn.* **1988**, *61*, 4335.
28. Aguilar-Martínez, M.; Cuevas, G.; Jiménez-Estrada, M.; González, I.; Lotina-Hennsen, B.; Macías-Ruvalcaba, N.; *J. Org. Chem.* **1999**, *64*, 3684.
29. Machocho, A. K.; Win, T.; Grinberg, S. Bittner, S.; *Tetrahedron Lett.* **2003**, *44*, 5531.
30. Lawrence, H. R.; Kazi, A.; Luo, Y.; Kendig, R.; Ge, Y.; Jain, S.; Daniel, K.; Santiago, D.; Guida, W. C.; Sebti, S. M.; *Bioorg. Med. Chem.* **2010**, *18*, 5576.
31. Yadav, J. S.; Reddy, B. V. S.; Swamy, T.; Shankar, K. S.; *Monatsh. Chem.* **2008**, *139*, 1317.
32. Taylor, S.; Lam, M.; Pararasa, C.; Brown, J. E. P.; Carmichal, A. R.; Griffiths, H. R.; *Cancer Cell. Int.* **2015**, *1*, 15.
33. Tandon, V. K.; Kumar, S.; Mishra, N. N.; Shukla, P. K.; *Eur. J. Med. Chem.* **2012**, *56*, 375.
34. Cantley, L. C.; *Science* **2002**, *296*, 1655; Mendoza, M. C.; Er, E. E.; Blenis, J.; *Trends Biochem. Sci.* **2011**, *36*, 320.
35. Nitiss, J. L.; *Nature* **2009**, *9*, 338.
36. Jiménez-Alonso, S.; Orellana, H. C.; Estévez-Braun, A.; Ravelo, A. G.; Pérez-Sacau, E.; Machín, F.; *J. Med. Chem.* **2008**, *51*, 6761.
37. Burden, D. A.; Osheroff, N.; *Biochem. Biophys. Acta* **1998**, *1400*, 139; Baldwin, E. L.; Osheroff, N.; *Curr. Med. Chem. Anticancer Agents* **2005**, *5*, 363.
38. Walker, E. H.; Pacold, M. E.; Perisic, O.; Stephens, L.; Hawkins, P. T.; Wymann, M. P.; Williams, R. L.; *Mol. Cell* **2000**, *6*, 909.

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