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ORIGINAL ARTICLE

Design, synthesis, and antitumor evaluation of novel anthraquinone derivatives

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Abstract

N-alkylated and *O*-alkylated anthraquinone derivatives with structures analogous to mitoxantrone were synthesized, characterized, and evaluated for their cytotoxic properties against three tumor cell lines (Human Breast Adenocarcinoma *MCF-7*, Human Cervical Adenocarcinoma *HeLa*, and Human Glioblastoma *M059J*) and a normal cell line (human lung fibroblasts *GM-07492A*). A structure-activity relationship study was carried out to verify the influence of lipophilic chain size on the biological activity of these compounds. The results indicated promising candidates for antineoplastic agents for the cancers evaluated, since these compounds showed significant selectivity and high cytotoxic potential for cancer cells, rather than mitoxantrone, the compound of which is already used in anticancer therapy.

Keywords Synthesis · Natural products · Anthraquinones · Mitoxantrone · Cytotoxicity

Introduction

The biological control of cancer remains a medical and scientific challenge. The inherent diversity of cancers leads the resistance and relapse of therapy, making the drugs unable to eliminate effectively all malignant cells (Lytle et al. 2018).

Among the numerous compounds with recognized anticancer potential, we highlight mitoxantrone (MTX), an anthraquinone frequently used as an antineoplastic agent effective in the treatment of several malignant neoplasms. It is indicated for chemotherapy in patients with cancers such as breast, ovarian, prostate, leukemia, lymphomas, and sarcomas (Damiani et al. 2016; Varadwaj et al. 2010) and in the treatment of active forms of multiple sclerosis (Neuhaus et al. 2006).

Although the MTX shows promising results for patients, its use has been limited due to its cardiotoxicity and induction of drug resistance (Salustiano et al. 2016). In addition, its action on the cells of the immune system is not selective, triggering a series of serious side effects, such as increased infections, rashes, and ulcers (Jones et al. 2010).

Anthraquinone derivatives analogous to MTX appear as an alternative, in the search to minimize undesirable pharmacological effects and increase their biological activity. Analogs containing alkyl groups, epoxides, halohydrins, *N*-alkylated, and *O*-alkylated compounds and aminoalcohols are described with potent pharmacological properties such as antitumor, antibacterial (Kumar et al. 2011; Johnson et al. 1997; Ulrich et al. 1998; Krapcho et al. 1986) immunosuppressive (Alves et al. 2012), and anti-inflammatory (Alves et al. 2013). In this context, some authors such as Huang et al. (2004) and Kumar et al. (2011) reported the in vitro cytotoxic evaluation and synthesis of MTX lipophilic analogs by varying the size of the lateral carbon chain and introducing alkylated and amino groups (Kumar et al. 2011; Huang et al. 2004). The compounds obtained showed better inhibitory activity against the tumor cell lines evaluated than MTX. According to the authors, the promising result observed for this series of compounds can be attributed to the lipophilicity of the substituents, which would

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have increased the affinity for the cell membrane facilitating absorption and penetration into the cell.

Corrêa et al. (2013) reported the synthesis of mono and disubstituted anthraquinone derivatives containing different side chains and evaluated their effect on the production of IL-1β, TNF-α, and nitric oxide (NO) by RAW264.7 cells. These compounds had a greater potential for inhibition of NO, TNF-α, and IL-1β production at low concentrations and lower cytotoxicity than MTX with reliable cell viability. Studies developed by Alves et al. (2012) have shown efficacy in in vivo assays of a lipophilic anthraquinone derivative in the treatment of experimental autoimmune encephalomyelitis. Through the study, the percentage of inflammatory cells IL-17, IFN-γ, IL-12p40, IL-6, TGF-β, CCL5, and CCL20 in the spinal cord was observed with a significant improvement in the clinical picture of the disease. In addition, in vitro studies demonstrated low cytotoxicity compared with MTX for most of the concentrations tested (Alves et al. 2012).

Different studies also show that MTX analogs, which did not have hydroxyl groups attached to the anthraquinone ring (positions 5 and 8 in the MTX structure), would be less cardiotoxic than the corresponding hydroxylates, presenting high antitumor activity (Kumar et al. 2011; Krapcho et al. 1986; Shcekotikhin et al. 2009).

Given the pharmacological potential of anthraquinone derivatives of MTX and the influence of carbonic chain size on cytotoxic activity, the present study describes the preparation, characterization and in vitro cytotoxic evaluation of two series of *N*-alkylated and *O*-alkylated analogs from anthraquinone derivatives.

Materials and methods

Chemistry

The reagents used in the experiments were all commercially available. The melting points were obtained in a digital apparatus MQAPF-Microchemistry. The FTIR spectra obtained in the infrared region were recorded in a Bruker ALPHAFT-IR MB102 spectrometer, in the region of 4000–400 cm^{-1}, using KBr pelletis or attenuated total reflectance technique. The analysis were done at the Department of Chemistry of Federal University of Juiz de Fora (UFJF). ^{1}H-NMR (500 MHz) and ^{13}C NMR (125 MHz) spectra data, HMQC and COSY were obtained on a BRUKER AVANCE III 500 MHz spectrometer (Chemical shifts (δ) were reported in parts per million (ppm) referenced to the reference of the appropriate residual solvent peak with the abbreviations *s* (singlet), *d* (doublet), *t* (triplet), *dd* (doublet of doublets), *m* (multiplet), internal reference was used tetramethylsilane; *J* were given in Hertz (Hz)). The progress of the reactions and the purity analysis of the compounds were monitored by silica thin-layer chromatography (TLC) using Kiesegel aluminum plates (chromate sheets 60F254). For silica column chromatography, silica gel 60G (70–230 mesh ASTM) was used. Visible and ultraviolet light was used for the disclosure of the compounds. Solvents used for the synthesis and purification of the compounds were Alphatec, Neon, Synth, Exodus, Vetec, Biotec. Further purification was carried out by distillation of the Exodus brand hexane solvent.

Synthesis of anthraquinone derivatives 2a–2j and 4a–4j

To a solution of the epoxide intermediate **1b** (0.30 mmol) or **3a** (0.60 mmol) in 3.0 mL of tetrahydrofuran (THF) was added excess (2.4 mmol, 8 equivalents to **2a–2j**; 1.8 mmol, 3 equivalents to **4a–4f** and 3.6 mmol, 6 equivalents to **4g–4j**) of the aliphatic, cyclic, or aromatic commercial amines. The reaction mixture was maintained under magnetic stirring and refluxing of THF for 18–72 h. The reactions development was monitored by TLC (eluent: CH_2Cl_2/MeOH 97:3 v/v). After the completion of the reactions, the anthraquinones **2a–g** (blue solids) and **4a–g** (yellow solids) were obtained through induced precipitation, adding drops of treated hexane to the solution until the solution is cloudy. The supernatant was removed and the solids formed were filtered and washed with cold distilled hexane and recrystallized from a mixture of dichloromethane and hexane. Compounds **2h–j** (blue solids) and **4h–j** (orange solids) were purified by column chromatography through silica gel and CH_2Cl_2/MeOH as eluent.

Cytotoxic assessment from anthraquinone derivatives

Culture conditions of the cell lines

The cell lines were used in the present study after the 4th passage. The normal human cell line (lung fibroblasts, GM07492A) (courtesy of Mutagenesis Laboratory of the Sao Paulo State University, Brazil) was included to evaluate the possible cytotoxicity and selective activity. The cancer cell lines used were human breast adenocarcinoma (MCF-7) (courtesy of Mutagenesis Laboratory, Department of Biological Sciences, Sao Paulo State University, Brazil), human cervical adenocarcinoma (HeLa), and human glioblastoma (MO59J) obtained from the Cell Bank of the Federal University of Rio de Janeiro. The different cell lines were maintained as monolayers in plastic culture flasks (25 cm^2) containing HAM-F10 plus DMEM (1:1; Sigma-Aldrich) supplemented with 10% fetal bovine serum

(Nutricell, Campinas, Brazil), 2.38 mg/mL Hepes (Sigma-Aldrich) and antibiotics (0.01 mg/mL streptomycin and 0.005 mg/mL penicillin; Sigma-Aldrich). The cells were incubated at 37 °C in a humidified 5% CO_2 atmosphere.

Cytotoxicity assay

The cytotoxic effects from the samples were determined by monitoring the growth of untreated and treated cells using the Cell Proliferation Kit (an XTT-based colorimetric assay, Roche, Mannheim, Germany) after 24 h of incubation. The samples were dissolved in dimethylsulfoxide (1%; Sigma-Aldrich) and complete medium. For the experiments, 10^4 cells were plated on to 96-well microplates and incubated for 24 h. After incubation, the culture medium was removed and the cells were washed with phosphate-buffered saline and exposed to 100 µL HAM-F10 culture medium without phenol red. At designated time points, 25 µL of XTT were added to each well and the microplates were incubated for 17 h at 37 °C to allow the formation of an orange formazan dye product by metabolically active cells. Absorbance was read spectrophotometrically in an ELISA reader (Asys UVM 340/Microwin 2000 (Biochrom, Holliston, MA, USA)) at a wavelength of 450 nm and at a reference wavelength of 620 nm. The experiments were performed in triplicate. The assays were carried out at University of Franca-SP, Brazil.

Calculation of the selectivity index (SI)

The SI was calculated by dividing the IC_{50} value (50% cell growth inhibition) of the isolated compound obtained for GM07492A cells by the IC_{50} value obtained for the cancer cell line (Alves et al. 2012). Only values >1 were considered selective.

Statistical analysis

Cytotoxicity was assessed using the IC_{50} value as a response parameter, which was calculated with the GraphPad Prism program by plotting cell survival against the respective concentrations of the samples tested. One-way ANOVA plus Tukey test was used for the comparison of means ($p < 0.05$).

SI evaluates the potential use of the compounds synthesized in clinical trials from the selectivity between the different cells lines studied. In cytotoxicity assay, it is desirable that the SI be >5 (Suffness and Pezzuto 1990).

Results and discussion

Synthesis from MTX anthraquinone derivatives

The 1,4-diaminoanthraquinone was treated with epichlorohydrin in the presence of acetic acid and then, under basic condition, converted to epoxide **1b** (Johnson et al. 1997). Intermediate **1b** was then epoxy ring opened by commercial amines as nucleophiles leading to the formation of compounds **2a–j**, which were obtained as blue solids in yields of 22–73% (Scheme 1).

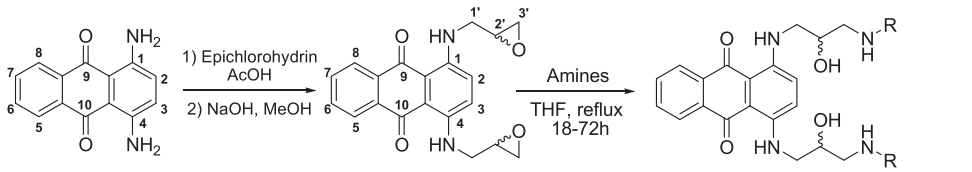
For the synthesis of *O*-alkylated anthraquinone derivatives, 1,4-dihydroxyanthraquinone **3** was subjected to the reaction with epichlorohydrin in dimethylformamide and K_2CO_3 anhydrous powder (oven dried at 400 °C for 3 h). The reaction mixture was taken to the microwave (Power: 150 W) for 1 h leading to the formation of the intermediate **3a**. Intermediate **3a** underwent epoxide ring opening using aliphatic, cyclic, and aromatic commercial nucleophiles, leading to the formation of compounds **4a–j** (Scheme 2), which were obtained as solids by precipitation in yields ranging from 3 to 57%. Anthraquinone derivatives were obtained in low-to-moderate yields mainly due to the difficulty of purification from the compounds by analytical techniques used.

The structures from all the compounds obtained were confirmed by FTIR and NMR spectroscopy.

Experimental section

1,4-bis(2′, 3′-epoxypropylamino)anthracene-9,10-dione (1b)

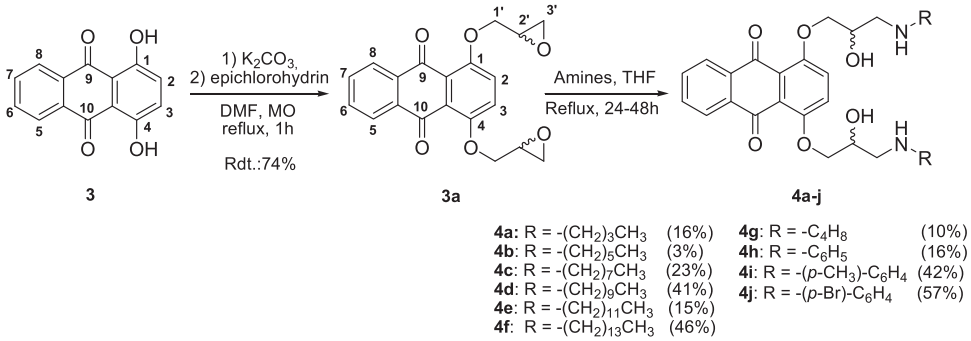
Dark blue solid: Yield: 71%; mp: 189.0–190.0 °C; *IR* (*KBr*) ν_{max} 3417, 3060, 2994W, 2923, 2858, 1594, 1572, 1526,



2a: R = -$(CH_2)_3CH_3$ (53%)
2b: R = -$(CH_2)_5CH_3$ (35%)
2c: R = -$(CH_2)_7CH_3$ (49%)
2d: R = -$(CH_2)_9CH_3$ (50%)
2e: R = -$(CH_2)_{11}CH_3$ (62%)
2f: R = -$(CH_2)_{13}CH_3$ (32%)
2g: R = -C_4H_8 (72%)
2h: R = -C_6H_5 (54%)
2i: R = -(*p*-CH_3)-C_6H_4 (22%)
2j: R = -(*p*-Br)-C_6H_4 (73%)

Scheme 1 Synthesis from anthraquinone derivatives **2a–j**

Scheme 2 Synthesis from anthraquinone derivatives **3a** and **4a–j**



1285 cm^{-1}; *¹H-NMR (500 MHz, $CDCl_3$, ppm)*: δ 10.75 (2H, *t*, *J* = 5.9 Hz, N*H*); 8.32 (2H, *dd*, *J* = 5.9, 3.3 Hz, H-5, H-8); 7.70 (2H, *dd*, *J* = 5.9, 3.3 Hz, H-6, H-7); 7.31 (2H, *s*, H-2, H-3); 3.76 (2H, *ddd*, *J* = 15.0, 6.1, 3.4 Hz, H-1′a); 3.56 (2H, *ddd*, *J* = 15.0, 6.1, 4.9 Hz, H-1′b); 3.24–3.27 (2H, *m*, H-2′); 2.86 (2H, *dd*, *J* = 4.7, 4.0 Hz, H-3′a); 2.73 (2H, *dd*, *J* = 4.8, 2.7 Hz, H-3′b); *¹³C NMR (125 MHz, $CDCl_3$, ppm):* δ 183.3 (C, C-9, C-10), 146.3 (C, C-1, C-4); 134.5 (C, C-11, C-12), 132.5 (CH, C-6, C-7), 126.3 (CH, C-5, C-8), 123.7 (CH, C-2, C-3), 110.6 (C, C-13, C-14), 51.4 (CH, C-2′); 45.2 (CH, C-3′); 44.1 (CH_2, C-1′).

1,4-bis(3′-butylamino-2′-hydroxypropylamino)anthracene-9,10-dione (2a)

Dark blue solid; Yield: 53%; mp: 109.0–112.0 °C; *IR* (*KBr*) ν_{max} 3391, 2955, 2929, 2851, 1572, 1520, 1263 cm^{-1}; *¹H-NMR (500 MHz, $CDCl_3$, ppm):* δ 8.01–7.93 (2H, *m*, H-5, H-8), 7.55–7.38 (2H, *m*, H-6, H-7), 6.85-6.47 (2H, sl, H-2, H-3), 4.14–3.93 (2H, *s*, H-2′), 3.52–3.02 (4H, m, H-1′a, H-1′b), 2.86–2.42 (8H, m, H-3′a, H-3′b, H-4′), 1.55–1.23 (8H, *m*, H-5′, H-6′), 0.95–0.82 (6H, *m*, H-7′); *¹³C NMR (125 MHz, $CDCl_3$, ppm):* δ 181.9 (C, C-9, C-10), 146.3 (C, C-1, C-4), 134.2, 132.0 (CH, C-6, C-7), 126.0 (CH, C-5, C-8), 123.4 (CH, C-2, C-3), 109.8, 68.8 (C-H, C-2′), 53.2 (CH_2, C-3′), 49.8 (CH_2, C-4′), 47.3 (CH_2, C-1′), 32.4, 20.6, 14.1 (CH_3, C-7′).

1,4-bis(3′-hexylamino-2′-hydroxypropylamino)anthracene-9,10-dione (2b)

Dark blue solid; Yield: 35%; mp: 65.5–66.5 °C; *IR* (*KBr*) ν_{max} 3396, 3284, 2928, 2856, 1578, 1551, 1525, 1261 cm^{-1}; *¹H-NMR (500 MHz, $CDCl_3$, ppm):* δ 10.66 (2H, t, *J* = 5.4 Hz, N*H*), 7.99 (2H, *dd*, *J* = 5.9, 3.3 Hz, H-5, H-8), 7.55 (2H, *ddd*, *J* = 5.9, 3.3, 0.7 Hz, H-6, H-7), 7.26 (2H, *s*, H-2, H-3), 4.06 (2H, *s*, H-2′), 3.33–3.21 (4H, *m*, H-1′a, H-1′b), 2.82–2.61 (8H, *m*, H-3′a, H-3′b, H-4′), 1.56–1.24 (16H, *m*, H-5′–8′), 0.88 (6H, *t*, *J* = 6.9 Hz, H-9′); *¹³C NMR (125 MHz, $CDCl_3$, ppm):* δ 181.5 (C, C-9, C-10), 146.2 (C, C-1, C-4), 134.1, 131.9 (CH, C-6, C-7), 126.0 (CH, C-5, C-8), 123.2 (CH, C-2, C-3), 109.7, 68.8 (CH, C-2′), 53.4 (CH_2, C-3′), 50.2 (CH_2, C-4′), 47.4, (CH_2, C-1′), 31.9, 30.1, 27.2, 22.8, 14.2 (CH_3, C-9′).

1,4-bis(2′-hydroxy-3′-(octylamino)propylamino)anthracene-9,10-dione (2c)

Dark blue solid; Yield: 49%; mp: 78.7–81.6 °C; *IR* (*KBr*) ν_{max} 3314, 2918, 2848, 1575, 1521, 1467, 1260 cm^{-1}; *¹H-NMR (500 MHz, $CDCl_3$, ppm):* δ 10.66 (2H, *t*, *J* = 5.4 Hz, N*H*), 7.99 (2H, *s*, H-5, H-8), 7.55 (2H, *m*, H-6, H-7), 6.89 (2H, *d*, *J* = 2.6 Hz, H-2, H-3), 4.01 (2H, *s*, H-2′), 3.32–3.17 (4H, *m*, H-1′a, H-1′b), 2.77–2.56 (8H, *m*, H-3′a, H-3′b, H-4′), 1.52–1.15- (24H, *m*, H-5′-10′), 0.82 (6H, *t*, *J* = 6.9 Hz, H-11′). *¹³C NMR (125 MHz, $CDCl_3$, ppm):* δ 181.7 (C, C-9, C-10), 146.2 (C, C-1, C-4), 134.1, 132.0 (CH, C-6, C-7), 126.0 (CH, C-5, C-6), 123.2 (CH, C-2, C-3), 109.8, 68.7 (CH, C-2′), 53.4 (CH_2, C-3′), 50.2 (CH_2, C-4′), 47.4, 47.1 (CH_2, C-1′), 32.0, 30.2, 29.7, 29.4, 27.5, 22.8, 14.2 (CH_3, C-11′).

1,4-bis(3′-decylamino-2′-hydroxypropylamino)anthracene-9,10-dione (2d)

Dark blue solid; Yield: 50%; mp: 79.7–82.3 °C; *IR* (*KBr*) ν_{max} 3326, 2924, 2846, 1578, 1520, 1266 cm^{-1}; *¹H-NMR (500 MHz, $CDCl_3$, ppm):* δ 10.74–10.72 (2H, m, N*H*), 8.10–8.07 (2H, *m*, H-5, H-8), 7.59 (2H, *ddd*, *J* = 5.9, 3.3, 1.7, H-6, H-7), 7.00 (2H, *d*, *J* = 5.3 Hz, H-2, H-3), 4.73 (2H, *s*, N*H*), 4.05 (2H, *s*, H-2′), 3.42–3.27 (4H, *m*, H-1′a, H-1′b), 2.84–2.61 (8H, *m*, H-3′a, H-3′b, H-4′), 1.90 (*s*, OH), 1.55–1.22 (32H, *m*, H-5′-12′); 0.87 (6H, *t*, *J* = 6,9 Hz, H-13′); *¹³C NMR (125 MHz, $CDCl_3$, ppm):* δ 181.9 (C, C-9, C-10), 146.3 (C, C-1, C-4), 134.2, 132.1 (CH, C-6, C-7), 126.1 (CH, C-5, C-6), 123.4 (CH, C-2, C-3), 109.9, 68.8, 68.7 (CH, C-2′), 53.4 (CH_2, C-3′), 50.1(CH_2, C-4′), 47.3, 47.1 (CH_2, C-1′), 32.1, 30.3, 29.8, 29.5, 27.5, 22.8, 14.2 (CH_3, 13′).

1,4-bis(3′-dodecylamino-2′-hydroxypropylamino) anthracene-9,10-dione (2e)

Dark blue solid; Yield: 62%; mp: 63.2–64.3 °C; *IR* (*KBr*) ν_{max} 3314, 2917, 2846, 1578, 1520, 1266 cm^{-1}; *¹H-NMR*

(500 MHz, $CDCl_3$, ppm): δ 10.69 (2H, *s*, N*H*), 8.06–8.02 (2H, *m*, H-5, H-8), 7.59–7.55 (2H, *m*, H-6, H-7), 6.99 (2H, *d*, $J = 4.9$ Hz, H-2, H-3), 4.06 (2H, *s*, H-2′), 3.40–3.26 (4H, *m*, H-1′a, H-1′b), 2.84–2.62 (8H, *m*, H-3′a, H-3′b, H-4′), 1.56–1.22 (40H, *m*, H-5′-14′); 0.87 (6H, *t*, $J = 6{,}9$ Hz, H-15′); *^{13}C NMR (125 MHz, $CDCl_3$, ppm):* δ 181.8 (C, C-9, C-10), 146.2 (C, C-1, C-4), 134.2, 132.0 (CH, C-6, C-7), 126.1 (CH, C-5, C-6), 123.2 (CH, C-2, C-3), 109.8, 68.7, 68.7 (CH, C-2′), 53.4 (CH_2, C-3′), 50.2 (CH_2, C-4′), 47.1 (CH_2, C-1′), 42.4, 34.0, 32.1, 30.2, 29.8, 29.7, 29.5, 27.5, 27.0, 22.8, 14.3 (CH_3, 15′).

1,4-bis(2′-hydroxy-3′-(tetradecylamino)propylamino) anthracene-9,10-dione (2f)

Dark blue solid; Yield: 32%; mp: 76.0–77.6 °C; *IR* (*KBr*) ν_{max} 3384, 3293, 2923, 2851, 1577, 1539, 1259 cm^{-1}; *^{1}H-NMR (500 MHz, $CDCl_3$, ppm):* δ 10.61 (2H, *s*, N*H*), 7,94–7,93 (2H, *m*, H-5, H-8), 7.52–7.51 (2H, *m*, H-6, H-7), 6.77 (2H, *s*, H-2, H-3), 4.06 (2H, *s*, H-2′), 3.33–3.15 (4H, *m*, H-1′a, H-1′b), 2.82–2.62 (8H, *m*, H-3′a, H-3′b, H-4′), 1.58–1.18 (48H, *m*, H-5′-16′); 0.87 (6H, *t*, $J = 6{,}9$ Hz, H-17′); *^{13}C NMR (125 MHz, $CDCl_3$, ppm):* δ 181.3 (C, C-9, C-10), 146.1 (C, C-1, C-4), 134.0, 131.8 (CH, C-6, C-7), 125.9 (CH, C-5, C-6), 123.1 (CH, C-2, C-3), 109.6, 68.7, 68.6 (CH, C-2′), 53.5 (CH_2, C-3′), 50.2 (CH_2, C-4′), 47.4 (CH_2, C-1′), 47.2, 32.1, 30.1, 29.8, 29.5, 27.5, 22.8, 14.2 (CH_3, C-17′).

1,4-bis(2′-hydroxy-3′-(pyrrolidino) propylamino) anthracene-9,10-dione (2g)

Dark blue solid; Yield: 72%; mp: 54.0–57.0 °C; *IR* (*KBr*) ν_{max} 3469, 3366, 3248, 2956, 2813, 1591, 1573, 1268 cm^{-1}; *^{1}H-NMR (500 MHz, $CDCl_3$, ppm):* δ 10.92 (2H, *t*, $J =$ 5.6 Hz, N*H*), 8.32 (2H, *ddd*, $J = 5.8$, 3.3, 0.6 Hz, H-5, H-8), 7.66 (2H, *dd*, $J = 5.9$, 3.3 Hz H-6, H-7), 7.29 (2H, *s*, H-2, H-3), 4.03–3.98 (2H, *m*, H-2′), 3.53–3.42 (4H, *m*, H-1′a, H-1′b), 2.81 (2H, *dd*, $J = 12.0$, 10.0 Hz, H-3′a, H-3′b), 2.72–2.68 (4H, *m*, H-4′, H-7′), 2.54–2.51 (4H, *m*, H-4′, H-7′), 2,49 (2H, *dd*, $J = 12.0$, 3.5 Hz, H-3′a, H-3′b), 1.80–1.78 (8H, *m*, H-5′, H-6′); *^{13}C NMR (125 MHz, $CDCl_3$, ppm):* δ 182.6 (C, C-9, C-10), 146.5 (C, C-1, C-4), 134.6, 132.1, 126.2, 123.8, 110.2, 67.9 (CH, C-2′), 59.7 (CH_2, C-3′), 54.2 (CH_2, C-4′, C-7′), 46.9 (CH_2, C-1′), 23.8 (CH_2, C-5′, C-6′).

1,4-bis(3′-phenylamino-2′-hydroxypropylamino) anthracene-9,10-dione (2h)

Dark blue solid; Yield: 54%; mp: 184.0–186.0 °C; *IR* (*KBr*) ν_{max} 3378, 2930, 2852, 1604, 1571, 1513, 1261 cm^{-1}; *^{1}H-NMR (500 MHz*, Acetone-*d6, ppm):* δ 10.96 (2H, t, $J =$ 5.5 Hz, N*H*), 8.23 (2H, *dd*, $J = 5.9$, 3.3 Hz, H-5, H-8), 7.67 (2H, *dd*, $J = 5.9$, 3.3 Hz, H-6, H-7), 7.35 (2H, *s*, H-2, H-3), 7.01 (4H, *dd*, $J = 8.5$, 7.3 Hz, H-6′, H-8′), 6.62 (4H, *d*, $J =$ 7.7 Hz, H-5′, H-9′), 6.50 (2H, *tt*, $J = 7.3$, 1.0 Hz, H-7′), 4.54 (2H, *d*, $J = 4.9$ Hz, N*H*), 4.12–4.06 (2H, *m*, H-2′), 3.67–3.62 (2H, *m*, H-1′a), 3.49–3.44 (2H, *m*, H-1′b), 3.34–3.30 (2H, *m*, H-3′a), 3.22–3.17 (2H, *m*, H-3′b); *^{13}C NMR (125 MHz*, Acetone-*d6, ppm):* δ 182.3 (C, C-9, C-10), 149.7 (C, C-4′), 147.2 (C, C-1, C-4), 135.3, 132.7 (CH, C-6, C-7), 129.7 (CH, C-6′, C-8′), 126.6 (CH, C-5, C-8), 124.8 (CH, C-2, C-3), 117.3 (CH, C-7′), 113.4 (CH, C-5′, C-9′), 110.2, 69.6 (CH, C-2′), 48.4 (CH_2, C-3′), 47.4 (CH_2, C-1′).

1,4-bis(2′-hydroxy-3′-(4-methylphenylamino)propylamino) anthracene-9,10-dione (2i)

Dark blue solid; Yield: 22%; mp: 171.0–173.0 °C; *IR* (*KBr*) ν_{max} 3377, 2915, 2856, 1578, 1525, 1255 cm^{-1}; *^{1}H-NMR (500 MHz*, Acetone-*d6, ppm):* δ 11.03 (2H, t, $J =$ 5.8 Hz, N*H*), 8.30 (2H, *dd*, $J = 5.9$, 3.3 Hz, H-5, H-8), 7.75 (2H, *dd*, $J = 5.9$, 3.3 Hz, H-6, H-7), 7.43 (2H, *s*, H-2, H-3), 6.91 (*d*, 4H, $J = 7{,}9$ Hz, H-6′, H-8′), 6.61 (4H, *d*, $J = 8.4$ Hz, H-5′, H-9′), 4.18–4.13 (2H, *m*, H-2′), 3.74-3.69 (2H, *m*, H-1′a), 3.56–3.51 (2H, *m*, H-1′b), 3.39–3.34 (2H, *m*, H-3′a), 3.27–3.22 (2H, *m*, H-3′b), 2.16 (6H, *s*, H-10′); *^{13}C NMR (125 MHz*, Acetone-*d6, ppm):* δ 182.4 (C, C-9, C-10), 147.6 (C, C-1, C-4), 147.2 (C, C-4′), 135.4, 132.8 (CH, C-6, C-8), 130,3 (CH, C-6′, C-8′), 126.7 (CH, C-5, C-8), 126.2, 124.9 (CH, C-2, C-3), 113.8 (CH, C-5′, C-9′), 110.4, 69.8 (CH, C-2′), 48.8 (CH_2, C-3′), 47.5 (CH_2, C-1′), 20.4 (pCH_3, C-10′).

1,4-bis(3′-(4-bromophenylamino)-2′-hydroxypropylamino) anthracene-9,10-dione (2j)

Dark blue solid; Yield: 73%; mp: 189.0–192.0 °C; *IR* (*KBr*) ν_{max} 3377, 2923, 2851, 1597, 1571, 1500, 1265, 1025 cm^{-1}; *^{1}H-NMR (500 MHz*, Acetone-*d6, ppm):* δ 11.04 (2H, *s*, N*H*), 8.33 (2H, *dd*, $J = 5.5$, 3.3 Hz, H-5, H-8), 7.77 (2H, *dd*, $J = 5.7$, 3.1 Hz, H-6, H-7), 7.45 (2H, *s*, H-2, H-3), 7.22 (*d*, 4H, $J = 8.5$ Hz, H-6′, H-8′), 6.69 (4H, *d*, $J = 8.5$ Hz, H-5′, H-9′), 4.57 (2H, *d*, $J = 3.9$ Hz, N*H*), 4.18 (2H, *s*, H-2′), 3.76–3.71 (2H, *m*, H-1′a), 3.59–3.35 (2H, *m*, H-1′b), 3.44–3.39 (2H, *m*, H-3′a), 3.31–3.25 (2H, *m*, H-3′b); *^{13}C NMR (125 MHz, $CDCl_3$, ppm):* δ 182.5 (C, C-9, C-10), 149.2 (C, C-4′), 147.2 (C, C-1, C-4), 135.4, 132.9, 132.5, 126.7, 124.9, 115.3 (CH, C-5′, C-9′), 110.4, 108.2, 69.6 (CH, C-2′), 48.4 (CH_2, C-3′), 47.4 (CH_2, C-1′).

1,4-bis(2′,3′-epoxy-methoxy)anthracene-9,10-dione (3a)

Yellow solid; Yield: 74%; mp: 159.2–159.9 °C; *IR* (*KBr*) ν_{max} 3073, 3008, 2930, 2865, 1670, 1591, 1564, 1278 cm^{-1};

^{1}H-NMR (500 MHz, $CDCl_3$, ppm): δ 8.16 (2H, *dd*, $J = 5.8$, 3.3 Hz, H-5, H-8); 7.71 (2H, *dd*, $J = 5.9$, 3.3 Hz, H-6, H-7); 7.37 (2H, *s*, H-2, H-3); 4.44 (2H, *ddd*, $J = 11.0$, 2.7, 1.4 Hz, H-1′a), 4.15 (2H, *ddd*, $J = 11.0$, 5.0, 1.1 Hz, H-1′b); 3.49–3.47 (2H, *m*, H-2′); 3.03 (2H, *dd*, $J = 5.1$, 2.7 Hz, H-3′ a); 2.96 (2H, *dd*, $J = 4.9$, 4.1 Hz, H-3′b); *^{13}C NMR (125 MHz, $CDCl_3$, ppm):* δ 183.0 (C, C-9, C-10), 153.7 (C, C-1, C-4) 134.1, 133,4 (CH, C-6, C-7), 126.5 (CH, C-5, C-8), 124.0, 123.3 (CH, C-2, C-3), 70.8 (CH_2, C-1′); 50.3 (C-H, C-2′), 44.7 (CH_2, C-3′).

1,4-bis[(3′-butylamino-2′-hydroxy)propoxy]anthracene-9,10-dione (4a)

Yellow solid; Yield: 16%; mp: 85.0–87.2 °C; *IR (KBr)* ν_{max} 3372, 2956, 2930, 2872, 1667, 1591, 1564, 1244 cm^{-1}; *^{1}H-NMR (500 MHz, $CDCl_3$, ppm):* δ 8.16 (2H, *ddd*, $J = 5.8$, 3.3, 1.1 Hz, H-5, H-8); 7.73 (2H, *dd*, $J = 5.8$, 3.3 Hz, H-6, H-7); 7.35 (2H, *s*, H-2, H-3); 4.30 (2H, *ddd*, $J = 9.0$, 4.3, 3.3 Hz, H-1′a), 4.21–4.16 (2H, *m*, H-2′), 4.11–4.07 (2H, *m*, H-1′b), 2.89–2.88 (4H, *m*, H-3′), 2.68 (4H, *m*, H-4′), 1.55–1.49 (4H, *m*, H-5′), 1.38 (4H, sex, $J = 7.5$ Hz, H-6′), 0.93 (6H, *t*, $J =$ 7,2 Hz, H-7′); *^{13}C NMR (125 MHz, $CDCl_3$, ppm):* δ 183.7 (C, C-9, C-10), 154.4 (C, C-1, C-4), 134.2, 133.8 (CH, C-6, C-7), 126.9 (CH, C-5, C-8), 123.4, 74.3 (CH_2, C-1′), 68.5 (CH, C-2′), 64.4 (CH_2, C-3′), 51.8, 50.0 (CH_2, C-4′), 32.4 (CH_2, C-5′), 20.6 (CH2, C-6′), 14.2 (CH3, C-7′).

1,4-bis(3′-hexylamino-2′-hydroxypropoxy)anthracene-9,10-dione (4b)

Yellow solid; Yield: 3%; mp: 90.0–93.0 °C; *IR (KBr)* ν_{max} 3339, 2929, 2864, 1665, 1593, 1567, 1252 cm^{-1}; *^{1}H-NMR (500 MHz, $CDCl_3$, ppm):* δ 8.16–8.00 (2H, *m*, H-5, H-8), 7.74–7.53 (2H, *m*, H-6, H-7), 7.33 (2H, *s*, H-2, H-3), 3.96–4.32 (8H, *m*, H-), 2.97–2.56 (8H, *m*), 1.63–1.19 (12H, *m*, H-6′, H-7′, H-8′), 0.89–0.82 (6H, *m*, H-9′).

1,4-bis[(2′-hydroxy-3′-octylamino)propoxy]anthracene-9,10-dione (4c)

Yellow solid; Yield: 23%; mp: 74.0–77.0 °C; *IR (KBr)* ν_{max} 3411, 2923, 2845, 1669, 1593, 1561, 1249 cm^{-1}; *^{1}H-NMR (500 MHz, $CDCl_3$, ppm):* δ 8.13 (2H, *dd*, $J = 5.3$, 3.1 Hz, H-5, H-8), 7.70 (2H, *dd*, $J = 5.7$, 3.3 Hz, H-6, H-7), 7.31 (2H, *s*, H-2, H-3), 4.35–4.01 (10H, *m*, H-1′a, H-1′b, H-3′a, H-3′ b), 2.67–2.95 (4H, *m*, H-4′), 1.62–1.19 (24H, *m*, H-5′-10′), 0.87–0.85 (6H, *m*, H-11′); *^{13}C NMR (125 MHz, $CDCl_3$, ppm):* δ 183,6 (C, C-9, C-10), 154.3 (C, C-1, C-4), 134.1, 133.8 (CH, C-5, C-8), 126.8 (CH, C-5, C-8), 123.2, 74.2 (CH_2, C-1′), 68.2 (C-H, C-2′), 51.7 (CH_2, C-3′), 50.2 (CH_2, C-4′), 32.0, 29.9, 29.7, 29.5, 29.4, 27.5, 22.8, 14.2 (CH_3, C-11′).

1,4-bis[(3′-decylamino-2′-hydroxy)propoxy]anthracene-9,10-dione (4d)

Yellow solid; Yield: 41%; mp: 76.0–78.3 °C; *IR (KBr)* ν_{max} 3416, 2923, 2851, 1665, 1593, 1564, 1252 cm^{-1}; *^{1}H-NMR (500 MHz, $CDCl_3$, ppm):* δ 8.10 (2H, *dd*, $J = 5.7$, 3.4 Hz, H-5, H-8), 7.68 (2H, *dd*, $J = 5.7$, 3.3 Hz, H-6, H-7), 7.30 (2H, *s*, H-2, H-3), 4.26–4.07 (6H, *m*, H-1′a, H-1′b, H-2′), 2.93–2.91 (4H, *m*, H-3′a, H-3′b), 2,71–2,68 (4H, *m*, H-4′); 1.56–1.24 (32H, *m*, H-5′-12′), 0.87–0.85 (6H, *t*, $J = 6$,9 Hz, H-13′); *^{13}C NMR (125 MHz, $CDCl_3$, ppm):* δ 183,5 (C, C-9, C-10), 154.2 (C, C-1, C-4), 134.1, 133.7 (CH, C-5, C-8), 126.8 (CH, C-5, C-8), 123.2, 123.0, 74.1 (CH_2, C-1′), 68.2 (C-H, C-2′), 51.8 (CH_2, C-3′), 50.2 (CH_2, C-4′), 32.0, 29.9, 29.7, 29.4, 27.4, 22.8, 14.2 (CH_3, C-13′).

1,4-bis[(3′-dodecylamino-2′-hydroxy)propoxy]anthracene-9,10-dione (4e)

Oil; Yield: 15%; *IR (KBr)* ν_{max} 3385, 2924, 2846, 1666, 1591, 1562, 1247 cm^{-1}; *^{1}H-NMR (500 MHz, $CDCl_3$, ppm):* δ 8.09–7.97 (2H, m, H-5, H-8), 7.67–7.54 (2H, *m*, H-6, H-7), 7.27 (2H, *s*, H-2, H-3), 4.26–3.99 (6H, *m*, H-1′a, H-1′b, H-2′), 2.95–2.53 (8H, *m*, H-3′a, H-3′b, H-4′), 1.62–1.14 (40H, *m*, H-5′-14′), 0.85 (6H, *t*, $J = 6$,9 Hz, H-15′); *^{13}C NMR (125 MHz, $CDCl_3$, ppm):* δ 183,4 (C, C-9, C-10), 154.2 (C, C-1, C-4); 134.0, 133.7 (CH, C-5, C-8), 126.8 (CH, C-5, C-8), 123.1, 123.0, 74.1 (CH_2, C-1′), 68.2 (C-H, C-2′), 51.8 (CH_2, C-3′), 50.2 (CH_2, C-4′), 32.0, 29.7, 29.4, 27.4, 22.8, 14.2 (CH_3, C-15′).

1,4-bis[2′-hydroxy-3′-(tetradecylamino)propoxy] anthracene-9,10-dione (4f)

Yellow solid; Yield: 46%; mp: 66.0–68.0 °C; *IR (KBr)* ν_{max} 3378, 2924, 2846, 1666, 1594, 1562, 1244 cm^{-1}; *^{1}H-NMR (500 MHz, $CDCl_3$, ppm):* δ 8.13–7.99 (2H, *m*, H-5, H-8), 7.69–7.56 (2H, *m*, H-6, H-7), 7.29 (2H, *s*, H-2, H-3), 4.28–3.98 (6H, *m*, H-1′a, H-1′b, H-2′), 2.91–2.58 (8H, *m*, H-3′a, H-3′b, H-4′), 1.58-1.17 (48H, *m*, H-5′-16′), 0.86 (6H, *t*, $J = 6$,9 Hz, H-17′); *^{13}C NMR (125 MHz, $CDCl_3$, ppm):* δ 183,5 (C, C-9, C-10), 154.3 (C, C-1, C-4), 134.1, 133.7 (CH, C-5, C-8), 126.8 (CH, C-5, C-8), 123.2, 74.1 (CH_2, C-1′), 68.4 (C-H, C-2′), 51.8 (CH_2, C-3′), 50.3 (CH_2, C-4′), 32.0, 30.1, 29.8, 29.5, 27.5, 22.8, 14.2 (CH_3, C-17′).

1,4-bis[(2′-hydroxy-3′-pyrrolidino)propoxy]anthracene-9,10-dione (4g)

Yellow solid; Yield: 10%; mp: 41.6–42.8 °C; *IR (KBr)* ν_{max} 3404, 2968, 2878, 2806, 1663, 1590, 1564, 1240 cm^{-1}; *^{1}H-NMR (500 MHz, $CDCl_3$, ppm):* δ 8.16 (2H, *dd*, $J = 5.8$, 3.3 Hz, H-5, H-8), 7.71 (2H, *dd*, $J = 5.8$, 3.3 Hz, H-6, H-7),

7.36 (2H, *s*, H-2, H-3), 4.29 (2H, dt, $J = 9.0$, 3.5 Hz, H-1′a), 4.24–4.19 (2H, *m*, H-2′), 4.09–4.05 (2H, *m*, H-1′a), 2.80 (2H, *ddd*, $J = 12.0$, 7.5, 1.1 Hz, H-3′a), 2.73 (2H, *ddd*, $J =$ 12.0, 5.7, 1.0 Hz, H-3′b), 2.67–2.61 (8H, *m*, H-4′-7′), 1.80 (8H, qui, $J = 3.4$ Hz, H-5′, H-6′); *^{13}C NMR (125 MHz, $CDCl_3$, ppm):* δ 183,6 (C, C-9, C-10), 154.4 (C, C-1, C-4); 134.2, 133.7, 126.9, 126.8, 123.4, 123.3, 74.0 (CH_2, C-1′), 68.2 (C-H, C-2′), 58.6 (CH_2, C-3′), 54.7 (CH_2, C-4′, C-7′), 23.7 (CH_2, C-5′, C-6′).

1,4-bis[(3′-phenylamino-2′-hydroxy)propoxy]anthracene-9,10-dione (4h)

Yellow solid; Yield: 16%; mp: 91.0–94.0 °C; *IR* (*KBr*) ν_{max} 3365, 2929, 2871, 1663, 1600, 1565, 1250 cm^{-1}; *^{1}H-NMR (500 MHz, $CDCl_3$, ppm):* δ 8.19 (2H, *dd*, $J = 5.7$, 3.3 Hz H-5, H-8), 7.75 (2H, *dd*, $J = 5.8$, 3.3 Hz, H-6, H-7), 7.31 (2H, *s*, H-2, H-3), 7.20 (4H, *t*, $J = 7.9$ Hz, H-6′, H-8′), 6.75–6.72 (6H, *m*, H-5′, H-7′, H-9′), 4.58 (2H, *m*, N*H*), 4.35–4.31 (4H, *m*, H-1′a, H-1′b), 4.20–4.16 (2H, *m*, H-2′), 3.50 (2H, *dd*, $J = 13.0$, 4.7 Hz, H-3′a), 3.38 (*dd*, 2H, $J = 13.0$, 5.3 Hz, H-3′b); *^{13}C NMR (125 MHz, $CDCl_3$, ppm):* δ 183,7 (C, C-9, C-10), 154.4 (C, C-1, C-4), 148.5 (C, C-4′), 134.1, 134.0, 129.4, 127.0, 123.4, 123.3, 117.9, 113.4, 73.9 (CH_2, C-1′), 68.3 (C-H, C-2′), 46.3 (CH_2, C-3′).

1,4-bis[{2′-hydroxy-3′-(4-methylphenylamino)}propoxy] anthracene-9,10-dione (4i)

Orange solid; Yield: 42%; mp: 124.0–126.0 °C; *IR* (*KBr*) ν_{max} 3351, 2917, 2868, 1663, 1619, 1590, 1564, 1243 cm^{-1}; *^{1}H-NMR (500 MHz, $CDCl_3$, ppm):* δ 8.19 (2H, sl, H-5, H-8), 7.75 (2H, sl, H-6, H-7), 7.31 (2H, *s*, H-2, H-3), 7.01 (4H, *d*, $J = 7.3$ Hz, H-6′, H-8′), 6.66 (4H, *d*, $J = 7.4$ Hz, H-5′, H-9′), 4.32 (4H, *s*, H-1′a, H-1′b), 4.17 (2H, sl, H-2′), 3.50–3.44 (2H, *m*, H-3′a), 3.38–3.32 (2H, *m*, H-3′b), 2.25 (6H, *s*, H-10′); *^{13}C NMR (125 MHz, $CDCl_3$, ppm):* δ 183,7 (C, C-9, C-10), 154.4 (C, C-1, C-4); 146.2 (C, C-4′), 134.1, 134.0, 129.9, 127.2, 127.0, 123.4, 123.3, 113.6, 73.9 (CH_2, C-1′), 68.4 (C-H, C-2′), 46.7 (CH_2, C-3′), 20.5 (*p*CH_3, C-10′).

1,4-bis[(3′-(4-bromophenylamino)-2′-hydroxy)propoxy] anthracene-9,10-dione (4j)

Orange solid; Yield: 57%; mp: 128.7–130.7 °C; *IR* (*KBr*) ν_{max} 3346, 2929, 2878, 1661, 1592, 1561, 1247, 1051 cm^{-1}; *^{1}H-NMR (500 MHz*, Acetone-*d6, ppm):* δ 8.21 (2H, *dd*, $J =$ 5.8, 3.3 Hz, H-5′, H-8), 7.89 (2H, *dd*, $J = 5.8$, 3.3 Hz, H-6, H-7), 7.62 (2H, *s*, H-2, H-3), 7.24 (4H, *d*, $J = 8.9$ Hz, H-6′, H-8′), 6.79 (4H, *d*, $J = 8.9$ Hz, H-5′, H-9′), 4.31–4.25 (6H, *m*, H-1′a, H-1′b, H-2′), 3.58–3.53 (2H, *m*, H-3′a), 3.42–3.34 (2H, *m*, H-3′b); *^{13}C NMR (125 MHz*, Acetone-*d6, ppm):* δ 183,9 (C, C-9, C-10), 154.7 (C, C-1, C-4); 149.4 (C, C-4′), 135.1, 134.6, 132.4, 127.2, 123.9, 123.5, 115.3, 108.0, 73.9 (CH_2, C-1′), 68.8 (C-H, C-2′), 47.7 (CH_2, C-3′).

In vitro cytotoxicity and structure-activity relationship analysis

From cell viability assays at different concentrations of the synthesized compounds, were obtained the SI and IC_{50}.

The cytotoxicity of the synthesized compounds was evaluated against three tumor cell lines (MCF-7, HeLa, and M059J) and a normal human cell line (GM07492A).

The IC_{50} values obtained of the synthesized compounds and the MTX are shown in Table 1. The results indicated that the most compounds showed potent anticancer activity against cancer cell lines. Among the *N*-alkylated derivatives, compounds such as **1b**, **2a**, **2b**, **2c**, **2d**, **2g**, and **2h** had better antiproliferative activity than MTX. Especially the compound **2b** had the highest cytotoxicity against the three cancer cells with IC_{50} values 13.6, 14.1, and 14.8 μM to MCF-7, HeLa, and MO59J, respectively.

According to the structure-activity relationship study, an increase in cytotoxic activity was observed from the introduction of four carbon atoms in the side chain, and the same behavior was observed for compounds bearing 6, 8, and 10 carbon atoms in the side chain. Compounds with higher carbon chains such as **2e** and **2f**, containing 12 and 14 carbon atoms, did not show significant improvement in cytotoxic activity. This factor may be related to the larger size of the carbon chain, which would have caused a greater steric hindrance.

The influence of the cyclic and aromatic carbon chains on the cytotoxic activity of the compounds was analyzed. Among the compounds, the pyrrolidine derivative **2g** showed cytotoxic activity comparable with that MTX and greater selectivity for tumor cells with IC_{50} values of 153.9 μM (SI = 2.36) and 169.3 μM (SI = 2.14) for MCF-7 cell line, respectively. With the introduction of donor substituents and electron withdrawals into compounds **2i** and **2j**, no significant cytotoxic action was observed for the tumor cell lines evaluated.

Among the *O*-alkylated derivatives, compounds **3a, 4a, 4b, 4c, 4d, 4e, 4f, 4j** were more active than MTX having much lower IC_{50}. The compound **3a** (LogP = 3.35) showed potent cytotoxic activity with IC_{50} values lower than that MTX and its nitrogenous analog **1b**, in addition to presenting considerable selectivity for all tumor cells tested (MCF-7, IS = 2.57, HeLa, SI = 1.75, M059J, SI = 2.26). With the introduction of lipophilic carbon chains into epoxide **3a**, the same behavior of nitrogenous analogs was observed, with compounds **4b** (MCF-7 $IC_{50} = 28.6$ μM, HeLa $IC_{50} = 32.3$ μM, M059J $IC_{50} = 53.4$ μM, HeLa $IC_{50} = 27.8$ μM, M059J $IC_{50} = 27.3$ μM) and **4d** (MCF-7 $IC_{50} =$ 33.3 μM; HeLa $IC_{50} = 23.4$ μM, M059J $IC_{50} = 26.1$ μM)

Table 1 Inhibition concentration of 50% and selectivity index (SI) from compounds *N*-alkylated 1a–b, 2a–j, *O*-alkylated 3a, 4a–j and MTX (positive control) against different cell lines

Compound	logP[a]	Concentration (μM)						
		GM07492A	MCF-7		HeLa		M059J	
		IC_{50} (μM)	IC_{50} (μM)	SI	IC_{50} (μM)	SI	IC_{50} (μM)	SI
MTX	0.36	141.1 ± 7.2	146.3 ± 3.5	–	146.3 ± 3.5	–	150.0 ± 8.3	–
1b	2.95	57.4 ± 5.2	107.6 ± 9.1	–	115.6 ± 3.4	–	120.8 ± 12.6	–
2a	3.74	165.9 ± 5.9	109.9 ± 5.0[b]	1.51	94.2 ± 6.6[b]	1.76	142.3 ± 0.0[b]	1.16
2b	5.76	10.3 ± 0.6	13.6 ± 0.1	–	14.1 ± 0.4	–	14.8 ± 1.5	–
2c	7.79	15.5 ± 0.3	38.0 ± 3.3	–	28.9 ± 1.5	–	29.7 ± 1.3	–
2d	9.03	29.2 ± 0.2	47.2 ± 7.8	–	56.2 ± 0.0	–	40.3 ± 0.4	–
2e	9.57	394.3 ± 14.5	533.6 ± 15.0	–	566.1 ± 2.0	–	360.0 ± 16.9	–
2f	9.92	236.5 ± 5.9	228.9 ± 0.9	1.03	438.2 ± 18.0	–	269.1 ± 0.9	–
2g	2.86	362.6 ± 17.5	153.9 ± 9.4[b]	2.36	169.3 ± 11.3[b]	2.14	298.5 ± 12.3[b]	1.21
2h	4.96	777.2 ± 59.9	442.8 ± 9.9[b]	1.75	545.7 ± 28.2[b]	1.42	664.5 ± 30.8[b]	1.17
2i	5.86	81.8 ± 3.3	298.5 ± 11.2	–	578.6 ± 84.1	–	459.8 ± 8,4	–
2j	6.58	410.6 ± 28.5	908.4 ± 113.8	–	640.8 ± 24.2	–	680.3 ± 6.1	–
3a	3.35	164.8 ± 10.8	64.0 ± 0.9[b]	2.57	93.9 ± 3.3[b]	1.75	72.9 ± 7.5[b]	2.26
4a	4.14	72.2 ± 2.3	94.4 ± 0.6	–	98.9 ± 0.2	–	92.4 ± 5.1	–
4b	6.16	53.6 ± 6.2	28.6 ± 1.1[b]	1.87	32.3 ± 1.7[b]	1.66	53.4 ± 0.3	–
4c	8.17	27.9 ± 5.0	24.5 ± 2.3	1.14	27.8 ± 1.1	–	27.3 ± 2.9	1.02
4d	9.16	24.5 ± 0.2	33.3 ± 1.5	–	23.4 ± 1.7	1.05	26.1 ± 2.6	–
4e	9.65	77.0 ± 5.0	108.5 ± 17.7	–	126.4 ± 3.0	–	124.3 ± 8.1	–
4f	9.98	118.9 ± 10.7	84.5 ± 6.7[b]	1.41	90.4 ± 2.9[b]	1.31	104.1 ± 6.6	1.14
4g	3.26	157.3 ± 5.5	224.2 ± 2.9	–	324.9 ± 18.7	–	209.3 ± 7.0	–
4h	5.36	294.8 ± 24.4	958.9 ± 90.3	–	>742.7	–	>742.7	–
4i	6.26	228.8 ± 7.2	312.6 ± 4.5	–	672.5 ± 0.4	–	306.1 ± 3,2	–
4j	6.98	30.6 ± 0.2	30.6 ± 0.1	–	36.9 ± 3.1	–	37.5 ± 2.9	–

The results in μM are expressed as mean ± standard deviation

GM07492A human lung fibroblasts, *MCF-7* human breast adenocarcinoma, *HeLa* human cervical adenocarcinoma, *M059J* human glioblastoma, IC_{50} dose that inhibits 50% of cell growth (μM), *SI* selectivity index

[a]Calculated at <http://www.molinspiration.com/cgi-bin/properties>

[b]Significantly different from GM07492A at *0.05, **0.01, and ***0.001

revealing greater cytotoxic activity against all tumor cell lines.

The study of the structure-activity relationship showed that increased lipophilicity provided an increase in the cytotoxic activity with low IC_{50} values, especially the compound **4b** (LogP = 6.16), which showed selectivity for HeLa (SI = 1.66) and MCF-7 (SI = 1.87), indicating good influence of the lipophilic substituents on the activity of these compounds. Other factors besides the partition coefficient (LogP) of compound **4b** and other compounds that presented significant selectivity may be involved in the evaluation of the biological activity, since the most active compound of the series **3a** was more selective, with a partition coefficient smaller. Compound **4f** (14 carbon atoms in the side chain) showed improved selectivity although with a higher IC_{50} value than the analogs bearing 8, 10, and 12 carbon atoms in the side chain. One of the factors that may have affected the improvement of its cytotoxic activity is a possible conformation adopted by the molecule and its target site. Compound **4f** showed a high partition coefficient (LogP = 9.98) as the other compounds compared, indicating high hydrophobicity and affinity for lipophilic membranes (Gareth 2010).

Anthraquinone aromatic derivatives **4g**, **4h**, and **4i** did not present significant cytotoxic activities. Derivative **4j**, containing an electron withdrawing group in the aromatic ring, showed modest cytotoxicity for the tumor cell lines evaluated, with low IC50 values (MCF-7 IC50 = 30.6 μM; HeLa IC50 = 36.9 μM; M059J IC50 = 37.5 μM) and comparable with the alkylated derivatives **4b–d**, however they did not show selectivity. The presence of a phenyl group (**4h**) considerably decreased the toxicity (IC_{50} = 958.9) of

MTX ($IC_{50} = 146.3$) to the MCF-7 tumor cell line. However, the presence of an electron donor group on the aromatic ring such as methyl group (**4i**) increased up to 3 times the cytotoxicity ($IC_{50} = 312.6$) as compared with its phenyl derivative ($IC_{50} = 958.9$) and a withdrawing group in the aromatic ring as bromine (**4j**) increased up to 30 times the cytotoxicity ($IC_{50} = 30.6$) relative to the derivative only substituted with the phenyl group ($IC_{50} = 958.9$).

These results also indicated that the presence of epoxide group increased selectivity of compounds for the tumor cell lines evaluated MCF-7 (SI = 2.57) and MO59J (SI = 2.26) and the derivative **2b** showed high cytotoxicity against the all cancer cell line evaluated, with IC_{50} values <15 μM. However, in normal fibroblasts, it shows IC_{50} at 10.3 μM.

Conclusion

The structural modification of bioactive natural products constitutes an effective and promising method for obtaining of pharmacologically active compounds or to optimize the activity of natural active molecules in order to increase therapeutic potency, selectivity, and confer lower toxicity. In this study, two series of MTX-anthraquinone analogous were obtained and evaluated for their cytotoxic potential against MCF-7, HeLa, and M059J tumor cell lines and a normal cell line from human lung fibroblasts GM07492A, whose results showed an improvement, even if modest, in the antiproliferative activity of these compounds in relation to MTX, through the introduction of different sizes of carbon chain. The results obtained through the in vitro cytotoxicity bioassays showed that the introduction of carbon chains with different sizes potentiated the cytotoxic activity against the tumor cells tested, evidencing the importance of the alkyl chain in the therapeutic potential and nature of the pharmacological effect of the molecule for these types of cancer. The introduction of aromatic substituents with electron withdrawing groups as halogens significantly enhanced the toxicity of the compounds. The potent and selective activity of semisynthetic anthraquinones against cancer cell lines reveals that the compound may represent the scaffold for the design of compounds with anticancer properties and encourage the development of further compounds for further studies in vivo.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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