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Synthesis and antiproliferative evaluation of 8-anilino-6-arylphenanthridinequinones

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ABSTRACT

A number of 8-anilino-6-aryl-3,4-dihydrophenanthridine-1,7,10(2H)-triones were synthesized to measure their half-wave potentials and assessed for their in vitro antiproliferative activity on a panel of a non-tumor fibroblast and four human-derived tumor cell lines, using the MTT assay. The SAR analysis indicates that the antiproliferative activity is strongly dependent on the electron-donor and inductive effects of methoxy and bromine substituents, inserted into the 8-anilino-6-arylphenanthridinequinone scaffold. Among the series, three members showed significant antiproliferative activity (0.58-5.78 µM).

Keywords: phenanthridinequinones; halogenation; half-wave potential; cancer cells; antiproliferative activity.

INTRODUCTION

Compounds with quinone cores are ubiquitous in nature and are known by their various physiological activities as antibiotic and anti-cancer agents [1-2]. Quinones form the second large class of antitumor agents approved for clinical use and several other antitumor members are currently in different stages of clinical and preclinical development. Among the more efficient agents it could be mentioned the anthracycline-glycosides, e.g. daunorubicin, doxorubicin, aclacinomycin A; the N-heterocyclic quinones, e.g. mitomycin C and the iminoquinone actinomycin D. The potency of the quinones on cancer cell growth inhibition is related to their capacity to undergo biochemical reduction by one or two electrons, which are catalyzed by flavoenzymes in the organism using NADPH as an electron donor. This process leads to semiquinone radical intermediates and subsequent reactions with oxygen, all of which are believed to be responsible for most of the drug activity [3-6]. Among the broad variety of N-heterocyclic quinones with anti-cancer activity are the naturally occurring aminoquinones: cribrostain 3 [7], lavendamycin [8], and calothrixin B [9]. As part of an ongoing research program on potential anticancer alkyl- and arylamino-1,4-quinones, we have reported, in the last few years, the synthesis and antiproliferative activity of diverse series of N-heterocyclic-containing aminoquinones. In this regard, the study on 3, 4-dihydrophenanthridine-1,7,10(*2H*)-trione series reveals significant *in vitro* antiproliferative activities at comparable levels to those of etoposide, a clinically used anticancer agent [10]. The SAR analysis of phenylaminoquinones, structurally related with the 8-anilino-6-aryl-3,4-dihydrophenanthridine-1,7,10(*2H*)-trione series, indicates that insertion of substituents at the nitrogen donor group and halogen atoms in the acceptor quinone nucleus, induces significant changes on the anti-cancer activity [11]. Based on these precedents and looking to get structure-activity insights on the 8-anilino-6-arylphenanthridinequinones series, new analogues were designed to evaluate their in vitro antiproliferative activity on cancer cells. The goal of this study is to evaluate the insertion effects of a methoxy group at the anilino group

and/or a bromine atom in the electroactive fragment, on the antiproliferative activity of the 8-anilino-6-arylphenanthridinequinone scaffold.

EXPERIMENTAL SECTION

Chemistry: General

All the solvents and reagents were purchased from different companies such as Aldrich and Merck and were used as supplied. Melting points were determined on a Stuart Scientific SMP3 apparatus and are uncorrected. The IR spectra were recorded on an FT Bruker spectrophotometer using KBr disks, and the wave numbers are given in cm-1.^{1}H NMR spectra was recorded on Bruker AM-400 instrument in deuterochloroform ($CDCl_3$). ^{13}C NMR spectra were obtained in $CDCl_3$ at 100 MHz. Bidimensional NMR techniques and DEPT were used for signal assignment. Chemical shifts are expressed in ppm downfield relative to tetramethylsilane and the coupling constants (*J*) are reported in Hertz. The HRMS spectra were obtained on a Thermo Finnigan spectrometer, model MAT 95XP and LTQ-Orbitrap mass spectrometer (Thermo-Fisher Scientific) with the analysis performed using an APCI source operated in positive mode. Silica gel Merck 60 (70–230 mesh) was used for preparative column chromatography and TLC aluminum foil 60F254 for analytical TL.

General procedure for the synthesis of 6-aryl-3,4-dihydrophenanthridine-1,7,10(*2H*)-triones **2a-c**.

A solution of 1 (1 mmol), 3-aminocyclohex-2-en-1-one (1 mmol), Ag_2O (2 mmol), anhydrous magnesium sulfate (0.5 g) and dichloromethane (25 mL), was left with stirring at rt after completion of the reaction as indicated by TLC. The mixture was filtered, the solids were washed with dichloromethane, and the filtrate was evaporated under reduced pressure. The residue was chromatographed on silica gel (9:1 dichloromethane–ethyl acetate) to yield the corresponding pure phenanthridinequinone **2**.

6-Phenyl-3,4-dihydrophenanthridine-1,7,10(*2H*)-trione (**2a**). Prepared from **1a** and 3-aminocyclohex-2-en-1-one; (4:00 h, 43% yield): orange solid, m.p. 169-170°C; IR(KBr) ν/cm^{-1} 3499 (N-H), 1699 (C=O ester), 1676 and 1550 (C=O quinone); ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.30 (q, 2H, *J* 6.8 Hz, 3-H), 2.92 (t, 2H, *J* 6.8 Hz, 2-H), 3.19 (t, 2H, *J* 6.8 Hz, 4-H), 6.82 (d, 1H, *J* 10.4 Hz, 9H), 7.07 (d, 1H, *J* 10.4 Hz, 8-H), 7.46 (m, 5H, phenyl); ^{13}C NMR (100 MHz): δ 21.6, 33.5, 39.3, 124.1, 127.4, 128.4 (2C), 128.9, 129.6 (2C), 138.3, 138.7, 139.6, 142.2, 162.6, 167.1, 183.7, 184.4, 197.6; HRMS $[M+H]^+$ *m/z*, observed: 303.08871for $C_{19}H_{13}NO_3$; requieres: 303.08954.

6-(Thiophen-2-yl)-3,4-dihydrophenanthridine-1,7,10(*2H*)-trione (**2b**). Prepared from **1b** and 3-aminocyclohex-2-en-1-one; (3:30 h, 89% yield): orange solid, m.p. 176-178°C; IR(KBr) ν/cm^{-1} (N-H), 1697 (C=O ester), 1668 and 1545 (C=O quinone); ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.18 (q, 2H, *J* 6.0 Hz, 3-H), 2.81 (t, 2H, *J* 6.0 Hz, 2-H), 3.06 (t, 2H, *J* 6.0 Hz, 4-H), 6.83 (d, 1H, *J* 10.2 Hz, 9H), 6.98 (d, 1H, *J* 10.2 Hz, 8-H), 7.06 (m, 1H, thienyl), 7.50 (m, 1H, thienyl), 7.66 (m, 1H, thienyl); ^{13}C NMR (100 MHz): δ 21.5, 33.3, 39.3, 123.0, 126.6, 128.0, 131.4, 132.0, 137.9, 139.1, 141.7, 143.2, 154.4, 166.8, 184.0, 184.5, 197.4; HRMS $[M+H]^+$ *m/z*, observed: 309.04525 for $C_{17}H_{11}NO_3S$; requieres: 309.04597.

6-(Furan-2-yl)-3,4-dihydrophenanthridine-1,7,10(*2H*)-trione (**2c**). Prepared from **1c** and 3-aminocyclohex-2-en-1-one; (3:00 h, 48% yield): yellow solid, m.p. 167-169°C; IR(KBr) ν/cm^{-1} (N-H), 1694 (C=O ester), 1613 and 1552 (C=O quinone); ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.19 (q, 2H, *J* 6.4 Hz, 3-H), 2.82 (t, 2H, *J* 6.4 Hz, 2-H), 3.09 (t, 2H, *J* 6.4 Hz, 4-H), 6.54 (m, 1H, furyl), 6.85 (d, 1H, *J* 10.4 Hz, 9-H), 6.98 (d, 1H, *J* 10.4 Hz, 8H), 7.19 (m, 1H, furyl), 7.56 (m, 1H, furyl); ^{13}C NMR (100 MHz): δ 21.6, 33.5, 39.3, 112.4, 115.7, 123.5, 126.8, 138.0, 138.9, 142.4, 145.6, 149.9, 151.6, 167.2, 183.3, 184.1, 197.3; HRMS $[M+H]^+$ *m/z*, observed: 293.06769 for $C_{17}H_{11}NO_4$; requieres: 293.06879.

Synthesis of 8-phenylamino-6-aryl-3,4-dihydrophenanthridine-1,7,10(*2H*)-triones **3a-f**. General procedure.

A suspension of quinone **2** (1 mmol), the required amine (2 mmol), $CeCl_3x7H_2O$ (0.05 mmol), and ethanol (20 mL) was left with stirring at rt after completion of the reaction as indicated by TLC. The reaction mixture was partitioned between chloroform/water, the organic extract was washed with water (2x15 mL), dried over Na_2SO_4, and evaporated under reduced pressure. The residue was column chromatographed over silica gel (dichloromethane/ethyl acetate 90:10) to yield the corresponding 8-arylamino-6-arylphenanthridinequinone.

8-(4'-Methoxypheny)amino-6-phenyl-3,4-dihydrophenanthridine-1,7,10(*2H*)-trione (**3b**). Prepared from **2a** and *p*-anisidine; (2:55 h, 67% yield): purple solid, m.p. 200-202°C; IR(KBr) ν/cm^{-1} 3405 (N-H), 1678 (C=O ester), 1620 and 1598 (C=O quinone); ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.26 (q, 2H, *J* 6.4 Hz, 3-H), 2.94 (t, 2H, *J* 6.4 Hz, 2-H), 3.14 (t, 2H, *J* 6.4 Hz, 4-H), 3.82 (s, 3H, OMe), 6.29 (s, 1H, 9-H), 6.92 (d, 2H, *J* 8.7 Hz, 2´- and 6´-H), 7.12 (d, 2H, *J* 8.7 Hz, 3´- and 5´-H), 7.35 (s, 1H, NH), 7.49 (m, 5H, phenyl); ^{13}C NMR (100 MHz): δ 21.4, 33.2, 39.1, 55.5, 102.7, 114.7, 114.9, 116.4, 122.4, 124.4 (2C), 128.2 (2C), 128.6 (2C), 129.1, 129.5 (2C), 139.9, 144.2, 157.8, 161.7, 167.6,

180.3, 181.4, 198.7; HRMS $[M+H]^+$ *m/z*, observed: 424.14224 for $C_{26}H_{20}N_2O_4$; requieres: 424.14231.

8-Phenylamino-6-(thiophen-2-yl)-3,4-dihydrophenanthridine-1,7,10(*2H*)-trione (**3c**). Prepared from **2b** and aniline; (2:30 h, 68% yield): dark purple solid, m.p. 168-170°C; IR(KBr) ν/cm^{-1} 3445 (N-H), 1680 (C=O ester), 1615 and 1590 (C=O quinone); ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.24 (q, 2H, *J* 6.3 Hz, 3-H), 2.91 (t, 2H, *J* 6.9 Hz, 2-H), 3.12 (t, 2H, *J* 6.3 Hz, 4-H), 6.43 (s, 1H, 9-H), 6.63 (m, 1H, thienyl), 7.25 (m, 4H, phenyl and thienyl), 7.41 (m, 2H, phenyl and thienyl), 7.60 (s, 1H, NH), 7.62 (m, 1H, thienyl); ^{13}C NMR (100 MHz): δ 21.2, 33.2, 39.0, 103.3, 121.9, 122.4 (2C), 125.8, 128.0, 129.0, 129.2, 129.7 (2C), 136.9, 144.4, 144.9, 149.1, 151.6, 167.6, 171.1, 179.6, 181.4, 198.1; HRMS $[M+H]^+$ *m/z*, observed: 401.11250 for $C_{23}H_{16}N_2O_3S$; requieres: 401.09152.

8-(4'-Methoxyphenyl)amino)-6-(thiophen-2-yl)-3,4-dihydrophenanthridine-1,7,10(*2H*)-trione (**3d**). Prepared from **2b** and *p*-anisidine; (2:05 h, 71% yield): dark purple solid, m.p. 166-168°C; IR(KBr) ν/cm^{-1} 3440 (N-H), 1680 (C=O ester), 1610 and 1590 (C=O quinone); ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.20 (q, 2H, *J* 6.3 Hz, 3-H), 2.87 (t, 2H, *J* 6.9 Hz, 2-H), 3.05 (t, 2H, *J* 6.3 Hz, 4-H), 3.80 (s, 3H, OMe), 6.22 (s, 1H, 9-H), 6.62 (d, 2H, *J* 8.8 Hz, 2´-and 6´-H), 6.72 (d, 2H, *J* 8.8 Hz, 3´- and 5´-H), 7.12 (m, 1H, thienyl), 7.52 (s, 1H, NH), 7.56 (m, 1H, thienyl), 7.72 (m, 1H, thienyl); ^{13}C NMR (100 MHz): δ 21.2, 32.9, 39.0, 55.4, 102.8, 122.0 (2C), 121.5 (2C), 122.7, 124.9, 127.9, 128.4, 130.0, 131.7, 140.4, 142.2, 145.8, 146.2, 158.2, 167.7, 180.6, 181.8, 198.9; HRMS $[M+H]^+$ *m/z*, observed: 431.10484 for $C_{24}H_{18}N_2O_4S$; requieres: 431.10208.

6-(Furan-2-yl)-8-(4'-methoxyphenyl)amino-3,4-dihydrophenanthridine-1,7,10(*2H*)-trione (**3f**). Prepared from **2c** and *p*-anisidine; (2:30 h, 80% yield): dark purple solid, m.p. 170-172°C; IR(KBr) ν/cm^{-1} 3320 (N-H), 1690 (C=O ester), 1610 and 1550 (C=O quinone); ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.24 (q, 2H, *J* 6.3 Hz, 3-H), 2.91 (t, 2H, *J* 6.9 Hz, 2-H), 3.12 (t, 2H, *J* 6.3 Hz, 4-H), 3.83 (s, 3H, OMe), 6.26 (s, 1H, 9-H), 6.63 (m, 1H, furyl), 6.94 (d, 2H, *J* 8.9 Hz, 2´- and 6´-H), 7.18 (d, 2H, *J* 8.9 Hz, 3´- and 5´-H), 7.22 (m, 1H, furyl), 7.44 (s, 1H, NH), 7.64 (m, 1H, furyl); ^{13}C NMR (100 MHz): δ 21.3, 33.2, 39.1, 55.5, 102.5, 112.1, 114.7, 114.9 (2C), 122.1, 124.6 (2C), 128.2, 129.6, 144.6, 144.9, 145.8, 149.1, 151.7, 157.8, 167.6, 179.8, 181.2, 198.3; HRMS $[M+H]^+$ *m/z*, observed: 415.12770 for $C_{24}H_{18}N_2O_5$, requieres: 415.12493.

General procedure for the synthesis of 8-anilino-9-bromo-6-aryl-3,4-dihydrophenanthridine-1,7,10(*2H*)-triones **4a-f**. A solution of the 8-arylamino-6-aryphenanthridinequinone (1 mmol), N-bromosuccinimide (NBS) (1 mmol) and methanol (20 mL) was left with stirring at rt after completion of the reaction as indicated by TLC. The solvent was removed under reduced pressure and the residue was column cromatographed over silica gel (90:10 CH_2Cl_2/EtOAc) to yield the corresponding 8-arylamino-7-bromophenanthridinequinone.

9-Bromo-6-phenyl-8-phenylamino-3,4-dihydrophenanthridine-1,7,10(*2H*)-trione (**4a**). Prepared from **3a** and NBS; (4:15 h, 65% yield): red solid, m.p. 171-172ºC; IR(KBr) ν/cm^{-1} 3317 (N-H), 1730 (C=O ester), 1690 and 1636 (C=O quinone); ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.28 (q, 2H, *J* 6.0 Hz, 3-H), 2.94 (t, 2H, *J* 6.0 Hz, 2-H), 3.16 (t, 2H, *J* 6.3Hz, 4-H), 7.22 (m, 2H, phenyl), 7.35 (m, 3H, phenyl), 7.47 (m, 5H, phenyl), 7.71 (s, 1H, NH); ^{13}C NMR (100 MHz): δ 21.3, 33.3, 38.9, 105.5, 121.7, 124.2, 126.0, 128.2, 128.5, 128.6, 128.7, 129.1, 129.4, 136.8, 139.3, 143.2, 144.9, 153.5, 156.8, 161.9, 167.5, 171.1, 175.4, 178.3, 197.9; HRMS $[M+H]^+$ *m/z*, observed: 473.04827 for $C_{25}H_{17}BrN_2O_3$, requieres: 473.31808.

9-Bromo-8-(4'-methoxyphenyl)amino-6-phenyl-3,4-dihydrophenanthridine-1,7,10(*2H*)-trione (**4b**). Prepared from **3b** and NBS; (10 h, 60% yield): red solid, m.p. 174-176ºC; IR(KBr) ν/cm^{-1} 3295 (N-H), 1730 (C=O ester), 1678 and 1710 (C=O quinone); ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.28 (q, 2H, *J* 6.0 Hz, 3-H), 2.94 (t, 2H, *J* 6.0 Hz, 2-H), 3.15 (t, 2H, *J* 6.0 Hz, 4-H), 3.82 (3H, OMe), 6.86 (d, 2H, *J* 7.1 Hz, 2´- and 6´-H), 7.02 (d, 2H, *J* 7.1 Hz, 3´- and 5´-H), 7.48 (m, 5H, phenyl), 7.68 (s, 1H, NH); ^{13}C NMR (100 MHz): δ 21.4, 33.2, 39.1, 55.7, 105.7, 114.9 (2C), 116.4, 122.5, 124.4 (2C), 128.2, 128.2, 128.6, 129.1, 129.5, 139.9, 139.3, 144.2, 145.3, 157.8, 161.7, 167.5, 180.4, 181.3, 196.6; HRMS $[M+H]^+$ *m/z*, observed: 505.08898 for $C_{26}H_{19}BrN_2O_4$; requieres: : 504.05077.

9-Bromo-8-(phenylamino)-6-(thiophen-2-yl)-3,4-dihydrophenanthridine-1,7,10(*2H*)-trione (**4c**). Prepared from **3c** and NBS; (1:39 h, 78% yield): red solid, m.p. 177-179ºC; IR(KBr) ν/cm^{-1} 3320 (N-H), 1728 (C=O ester), 1690 and 1630 (C=O quinone); ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.17 (q, 2H, *J* 6.9 Hz, 3-H), 2.83 (t, 2H, *J* 6.9 Hz, 2-H), 3.05 (t, 2H, *J* 6.9 Hz, 4-H), 6.51 (m, 1H, thienyl), 7.07 (m, 1H, phenyl), 7.17 (m, 2H, phenyl), 7.30 (m,1H, phenyl), 7.40 (m, 1H, thieny), 7.48 (m, 2H, thienyl and NH); ^{13}C NMR (100 MHz): δ 21.2, 33.2, 39.0, 106.5, 115.4, 121.3 (2C), 122.5, 125.8, 128.7 (2C), 129.8, 131.8, 137.5, 142.6, 145.2, 146.4, 148.9, 151.4, 167.3, 175.0, 177.7, 197.6; HRMS $[M+H]^+$ *m/z*, observed: 479.00475 for $C_{23}H_{15}BrN_2O_3S$; requieres: : 479.34580.

9-Bromo-8-(4'-methoxyphenyl)amino)-6-(thiophen-2yl)-3,4-dihydrophenanthridine-1,7,10(*2H*)-trione (**4d**). Prepared from **3d** and NBS; (3 h, 73% yield): red solid, m.p. 180-182ºC; IR(KBr) ν/cm^{-1} (N-H), 1720 (C=O ester),

1696 and 1630 (C=O quinone); ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.24 (q, 2H, *J* 6.9 Hz, 3-H), 2.90 (t, 2H, *J* 6.9 Hz, 2-H), 3.10 (t, 2H, *J* 6.9 Hz, 4-H), 3.84 (s, 3H, OMe), 6.89 (d, 2H, *J* 8.8 Hz, 2´- and 6´-H), 7.07 (d, 2H, *J* 8.7 Hz, 3´- and 5´-H), 7.12 (m, 1H, thienyl), 7.58 (m, 1H, thienyl), 7.70 (m, 1H, thienyl), 7.76 (s, 1H, NH); ^{13}C NMR (100 MHz): δ 21.2, 33.1, 39.0, 55.5, 114.0 (2C), 126.2 (2C), 127.7, 128.0, 128.1, 129.7, 129.9, 131.2, 131.5, 139.1, 141.4, 145.8, 153.7, 158.0, 167.2, 175.4, 178.3, 197.8. HRMS $[M+H]^+$ *m/z*, observed: 511.01264 for $C_{24}H_{17}BrN_2O_4S$; requieres: 510.00719.

9-Bromo-6-(furan-2-yl)-8-(phenylamino)-3,4-dihydrophenanthridine-1,7,10(*2H*)-trione (**4e**). Prepared from **3e** and NBS; (2:50 h, 74% yield): dark purple solid, m.p.167-169°C; IR(KBr) ν/cm^{-1} 3250 (N-H), 1690 (C=O ester), 1620 and 1590 (C=O quinone); ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.23 (q, 2H, *J* 6.9 Hz, 3-H), 2.88 (t, 2H, *J* 6.9 Hz, 2-H), 3.08 (t, 2H, *J* 8.9 Hz, 4-H), 7.08 (m, 3H, phenyl), 6.25 (m, 1H, furyl), 7.36 (m, 2H, phenyl), 7.55 (m, 1H, furyl), 7.70 (m, 1H, furyl), 7.77 (s, 1H, NH); ^{13}C NMR (100 MHz): δ 21.2, 33.0, 38.9, 105.3, 120.6, 124.1 (2C), 125.9, 127.7, 127.8, 128.7 (2C), 131.3, 131.6, 137.1, 141.3, 144.14, 145.9, 153.6, 167.2, 175.4, 178.3, 197.7; HRMS $[M+H]^+$ *m/z*, observed: 464.01056 for $C_{23}H_{15}BrN_2O_4$; requieres: 464.01947.

9-Bromo-6-(furan-2-yl)-8-(4'-methoxyphenyl)amino-3,4-dihydrophenanthridine-1,7,10(*2H*)-trione (**4f**). Prepared from **3f** and NBS; (3:15 h, 72% yield): dark purple solid, m.p. 173-175°C; IR(KBr) ν/cm^{-1} 3300 (N-H), 1690 (C=O ester), 1610 and 1590 (C=O quinone); ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.26 (q, 2H, *J* 6.9 Hz, 3-H), 2.91 (t, 2H, *J* 6.9 Hz, 2-H), 3.12 (t, 2H, *J* 6.9 Hz, 4-H), 3.84 (s, 3H, OMe), 6.59 (m, 1H, furyl), 6.89 (d, 2H, *J* 9.1 Hz, 2´- and 6´-H), 7.09 (d, 2H, *J* 8.8 Hz, 3´- and 5´-H), 7.22 (m, 1H, furyl), 7.56 (m, 1H, furyl), 7.59 (s, 1H, NH); ^{13}C NMR (100 MHz): δ 21.2, 33.2, 39.0, 55.5, 104.7, 112.2, 113.9, 115.2 (2C), 121.6, 126.0, 127.9 (2C), 130.3, 143.0, 145.1, 146.3, 151.4, 158.0, 160.7, 167.3, 174.9, 177.8, 197.7; HRMS $[M+H]^+$ m/z, observed: 493.03764 for $C_{24}H_{17}BrN_2O_5$; requieres: 493.03208.

Electrochemical measurements

Cyclic voltammograms of compounds were obtained on a Bioanalytical Sytem BAS CV-50W electrochemical analyzer. A small capacity measuring cell was equipped with a platinum disc as working electrode, an Ag/10 nM Ag (MeCN) reference electrode for non aqueous solvent, with a platinum wire auxiliary electrode, a mechanical mini-stirrer, and a capillary to supply an inert argon atmosphere. A 0.1 M solution of tetrabutylammonium tetrafluoroborate in acetonitrile was used as supporting electrolyte.

Antiproliferative assay

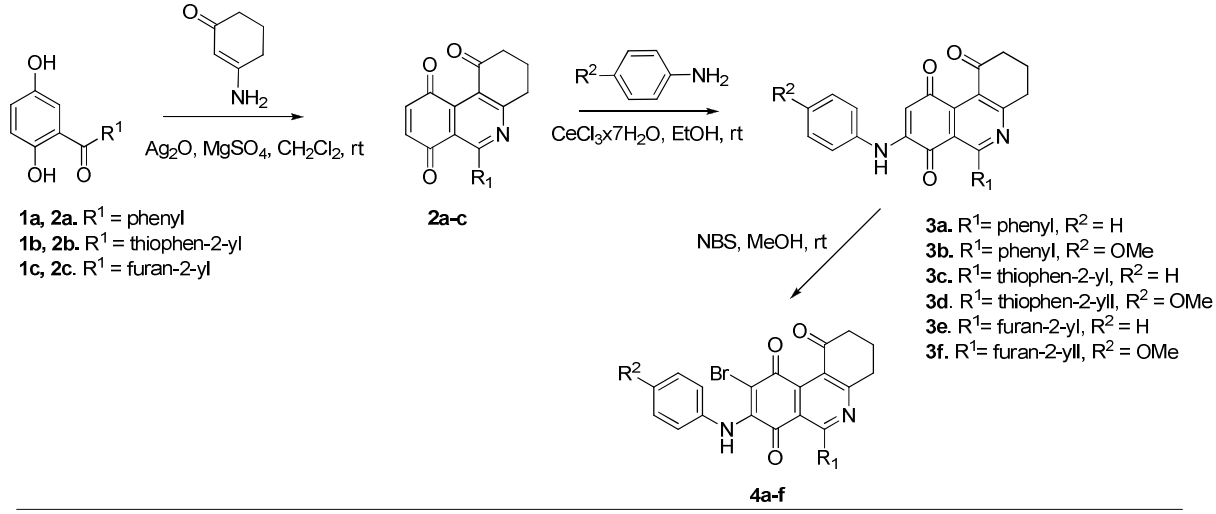
The cell lines used in this work were obtained from the American Type Culture Collection (ATCC. Manasas, VA, USA). They included MRC-5 normal human lung fibroblasts (CCL-171), AGS human gastric adenocarcinoma cells (CRL-1739), SK-MES-1 human lung cancer cells (HTB-58) and J82 human bladder carcinoma cells (HTB-1). After the arrival of the cells, they were proliferated in the corresponding culture medium as suggested by the ATCC. The cells were stored in medium containing 10% glycerol in liquid nitrogen. The viability of the cells after thawing was higher than 90%, as assessed by trypan blue exclusion test. Cells were sub-cultured once a week and the medium was changed every two days. Cells were grown in the following media: MRC-5, SKMES-1, and J82 in Eagle's minimal essential medium (EMEM) and AGS cells in Ham F-12. The EMEM medium contained 2 mM L-glutamine, 1 mM sodium pyruvate and 1.5 g/L sodium hydrogen carbonate. Ham F-12 was supplemented with 2 mM L-glutamine and 1.5 g/L sodium hydrogen carbonate. All media were supplemented with 10% heat-inactivated FBS, 100 IU/mL penicillin and 100 µg/mL streptomycin in a humidified incubator with 5% CO_2 in air at 37°C. For the experiments, cells were plated at a density of 50,000 cells/mL in 96-well plates. One day after seeding, the cells were treated with the medium containing the compounds at concentrations ranging from 0 up to 100 µM during 3 days. The concentrations used to calculate the IC_{50} values were: 100, 50, 25, 12.5, 6.25, 3.125, 1.56, 0.78, 0.39, 0.195 and 0.00 µM. The compounds were dissolved in DMSO (1% final concentration) and complete medium. Untreated cells (medium containing 1% DMSO) were used as controls. At the end of the incubation, the MTT reduction (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay was carried out to determine cell viability. The final concentration of MTT was 1 mg/mL. MTT metabolite was dissolved adding 100 µL of ethanol (acidified with HCl). The plates were shaken for 10 min and the absorbance was measured at 550 nm using a Universal Microplate Reader (ELX 800, Bio-Tek Instruments Inc., Winnoski, VT, USA). Six replicates for each concentration were used and the values were averaged. The results were transformed to percentage of controls and the IC_{50} values were graphically obtained from the dose-response curves. The IC_{50} value was obtained adjusting the dose-response curve to a sigmoidal model $(a + (b - a)/ 1 + 10(x-c))$, where $c = \log IC_{50}$.

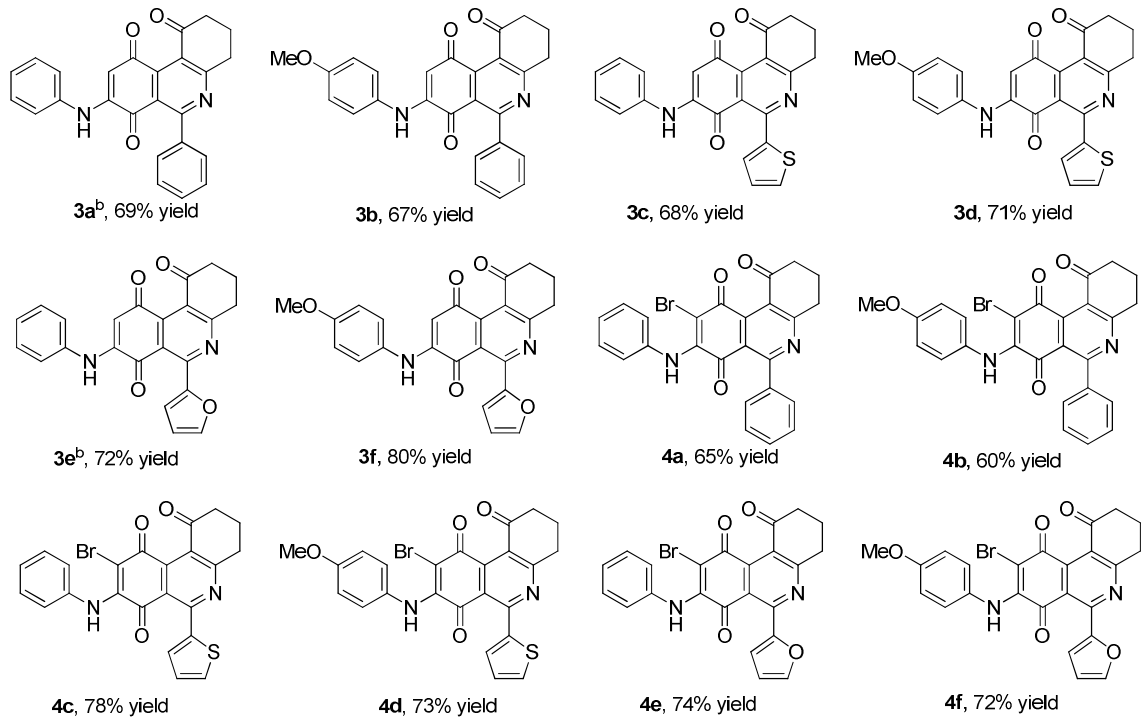
RESULTS AND DISCUSSION

Chemistry: The synthetic route to the target, 8-anilino-6-aryl-3,4 dihydrophenanthridine-1,7,10(*2H*)-triones **3a-f** and **4a-f**, is shown in Scheme 1. Acylhydroquinones **1a-c** and 3-aminocyclohexen-2-en-1-one were employed to prepare

the required phenanthridinequinone precursors **2a-c**. The synthesis of **1a-c** was carried out by solar photo-Friedel-Crafts acylation of 1,4-benzoquinone with the corresponding arylcarbaldehyde, according to a previously reported procedure [11]. The reaction of **1a-c** with 3-aminocyclohex-2-en-1-one in the presence of silver (I) oxide provides access to quinones **2a-c**, which by further reactions with aniline and *p*-anisidine, in the presence of $CeCl_3x7H_2O$, produced compounds **3a-f** (Scheme 1, See Supporting information File 1 for full experimental data).

The preparation of the bromine derivatives **4a-f** was accomplished by reaction of quinones **3a-f** with *N*-bromosuccinimide in methanol at room temperature. The preparation of the bromine derivatives **4a-f** was accomplished by reaction of quinones **3a-f** with *N*-bromosuccinimide in methanol at room temperature (scheme 1). The structures of the new compounds were established on the basis of their nuclear magnetic resonance (^{1}H-NMR, ^{13}C-NMR, 2D-NMR) and high resolution mass spectra (HRMS). The location of the arylamino group at C-8 in **3a-f** compounds was unequivocally settled by means of HMBC experiments (See Supporting information File 1 for full experimental data).





Scheme 1: Synthesis of 8-anilino-6-aryl-3,4-dihydrophenanthridine-1,7,10(*2H*)-triones 3 and 4.[b]Reported in reference [10]

Electrochemical results: The redox potentials of the synthesized compounds **3a-f** and **4a-f** were measured by cyclic voltammetry in acetonitrile at room temperature, using a platinum electrode and 0.1 M tetraethylamonium tetrafluoroborate as the supporting electrolyte [12]. The voltammograms were run in the potential range 0.0–2.0 V versus non-aqueous Ag/Ag+. Well-defined quasi-reversible waves were observed for the compounds, the cathodic

peak related to the reduction of quinone, and the anodic one due to its re-oxidation. The first half-wave potential values, $E^{I}_{1/2}$, evaluated from the voltammograms, obtained at a sweep rate of 100 mV s^{-1}, are shown in Table 1 and figure 1. The $E^{I}_{1/2}$ values for the first electron, which are related with the formation of the semiquinone radical anion [13,14], are in the potential range −413 to −796 mV. The data in Figure 1 show that the insertion of a methoxy group induces cathodic shifts of the scaffolds (**3a**, **3c**, **3e**: -471 to -745 mV), while the insertion of a bromine atom causes anodic shifts of the scaffolds (**3b**, **3d**, **3f**: -484 to -796 mV) to less negative values (**4a**, **4c**, **4e**: -493 to -462 mV). The insertion effects are attributed to the electron-releasing and inductive properties of the substituents. From a comparison of the magnitudes of the cathodic and anodic shift it can be concluded that the bromine insertion causes significant changes in the redox capacity of the thienyl- and furyl-containing scaffolds **3c** and **3e**. Regarding the insertion of both methoxy and bromine substituents into the **3a**, **3c** and **3e** scaffolds, as in compounds **4b**, **4d** and **4f**, anodic shifts were observed but in less magnitude than that for the bromine insertion.

Table 1: IC_{50}, $E^{I}_{1/2}$ and ClogP values of phenanthridinequinones 3 and 4

	IC_{50} ± SEM(μM)[a]					
N°	**MRC-5[b]**	**AGS[c]**	**HL-60[d]**	**SK-MES-1[e]**	**J82[f]**	**$-E^{I}_{1/2}$[g] (mV)**
3a	2.8±0.2	0.35±0.02	1.8±0.1	2.9±0.2	2.5±0.2	471
3b	>100	2.44±0.12	2.61±0.35	>100	>100	484
3c	3.0±0.13	0.57±0.03	3.2±0.13	1.89±0.12	2.8±0.15	584
3d	0.65±0.03	2.49±0.86	12.9±1.03	3.32±0.27	4.59±0.21	602
3e	0.27±0.1	0.58±0.02	2.1±0.1	3.1±0.2	2.9±1.5	745
3f	3.13±0.22	5.87±0.47	3.13±0.22	3.9±0.27	3.72±0.31	796
4a	3.43±0.21	2.68±0.18	1.33±0.06	34.2±2.29	14.8±1.1	462
4b	4.03±0.56	2.07±0.02	1.98±0.24	5.78±0.40	4.78±0.36	467
4c	18.35±1.22	15.59±1.09	11.94±0.96	18.81±1.85	20.08±1.9	428
4d	5.04±0.35	10.45±1.15	9.41±0.67	9.01±0.50	9.32±0.56	522
4e	2.71±0.13	2.69±0.19	1.36±0.05	4.54±0.37	4.59±0.36	493
4f	4.05±0.32	9.12±0.56	4.80±0.39	5.83±0.35	7.08±0.56	539
Eto.	0.33 ± 0.02	0.58 ± 0.02	2.23 ± 0.09	1.83 ± 0.09	3.49 ± 0.16	-

[a]Data represent mean average values for six independent determinations.
[b]Normal human lung fibroblasts cell line.
[c]Human gastric adenocarcinoma cell line.[d] Promyelocytic leukemia cell line.
[e]Human lung carcinoma cell line, [g]$E^{I}_{1/2}$ = half wave potential; values expressed in mV.

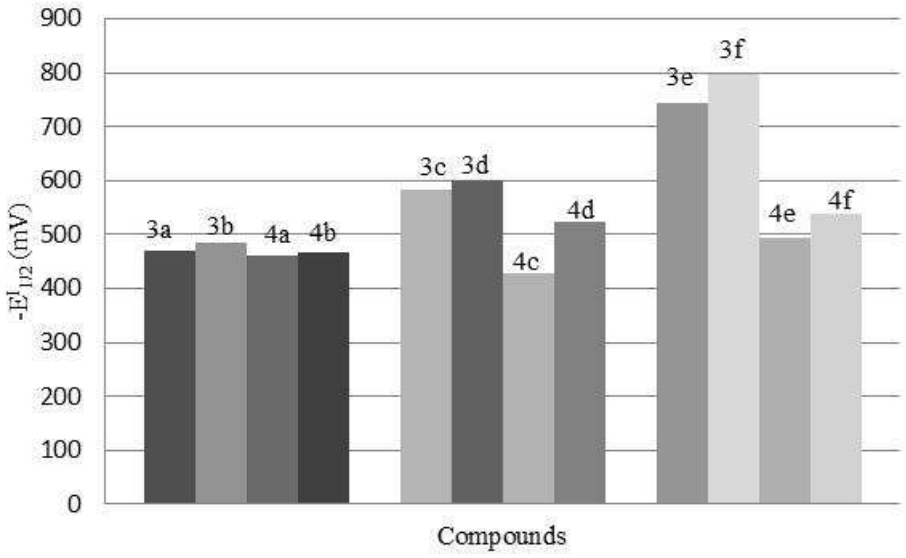


Figure 1: $E^{I}_{1/2}$ values of methoxy and bromine derivatives of parent compounds 3a, 3c and 3e

Anticancer activity: The 8-anilino-6-aryl-3,4-dihydrophenanthridine-1,7,10-(*2H*)-triones **3** and **4** were evaluated for their *in vitro* antiproliferative activity against normal human lung fibroblasts MRC-5 and four human tumor cells: AGS gastric adenocarcinoma, HL-60 promyelocytic leukemia, SK-MES-1 lung and J82 bladder carcinoma cells in 72-h drug exposure assays. The antiproliferative activity of the compounds was measured using a conventional microculture tetrazolium reduction assay [15-17]. Etoposide, a clinically used anticancer agent, was taken as a positive control.

The data set out in Table 1 reveals that, in general, the compounds **3b**, **3c**, **3d**, **3f** and **4a-f** exhibit moderate to high antiproliferative activities on cancer cells, with IC_{50} values ranging from 0.57 to 34.2 μM, with the exception of compound **3b** on lung and bladder cell lines. Within the series, compounds **3c**, **4b** and **4e** exhibited higher antiproliferative potencies (0.58-5.78 μM). Compound **3b** stand out for their antiproliferative activity on MRC-5, AGS and HL-60 cell lines (IC_{50} >100, 2.44 and 2.61 μM) and high selective index (> 40).

Analysis of the relationship between the structure of the phenanthridinquinones **3** and **4** and the antiproliferative activity reveals three major points. First, the insertion of the electron-releasing methoxy group at the 4-position of the nitrogen donor group of the **3a**, **3c** and **3e** scaffolds, induces a decreasing effect on the antiproliferative activity since the analogs **3b**, **3d** and **3f** were less potent than their corresponding parent compounds in both normal and cancer cells. The second major point, which results from the comparison of the IC_{50} values for cancer cells of compounds **4a**, **4c** and **4e**, is that the insertion of an electron-withdrawing bromine atom at the quinone nucleus of the parent compounds **3a**, **3c** and **3e** induces a decreasing effect on the antiproliferative activity, except for **4a** on AGS and **4e** on AGS and HL-60 cell lines. Important decreasing effect of bromine insertion on the antiproliferative activity is observed for the thienyl-containing parent compound **3c**, as in **4c**.

The third point concerns on the push-pull effect induced by the insertion of both methoxy and bromine groups. In this case, a similar profile to the bromine insertion, in terms of decreasing effects of the antiproliferative activity, was observed, being more significant on the thienyl-containing scaffold **3c**, as in **4d**.

CONCLUSION

In conclusion, we have synthesized a variety of new 8-anilino-6-aryl-3,4-dihydrophenanthridine-1,7,10(*2H*)-trione derivatives **3** and **4**. The first half-wave potentials were measured and the *in vitro* anticancer activity were assessed on a panel of four cancer cell lines, including non-tumor fibroblast and four human-derived tumor cell lines, using the MTT assay. The SAR analysis indicates that the antiproliferative activity is dependent on the electron-donor and inductive effects of the methoxy and bromine substituents, inserted into the 8-anilino-6-arylphenanthridinequinone scaffold. Within the series, compounds **3c**, **4b** and **4e** showed significant antiproliferative potencies (0.58-5.78 μM). Even when the analogue **3b** (IC_{50} = 2.44-100 μM) has less antiproliferative activity than the parent compound **3a** (0.35-2.9 μM), it showed high selectivity index (> 40) against gastric and leukemia cancer cell lines.

Acknowledgements
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REFERENCES

[1] R.H Thompson. *Naturally Occurring Quinones III*; Recent Advances Chapman and Hall, London, **1987**.
[2] K.W Wellington. *Understanding cancer and the anticancer activities of naphthoquinones–a review*; RSA Advances 5, **2015**.
[3] C Avendaño; C Menéndez. *J. Medicinal Chemistry of Anticancer Drugs*, 2th ed.; Elsevier BV, Oxford, **2008.**
[4] PL Gutierrez. *Front. Biosci*. **2000**, 5, 629.
[5] AJ Lin; LA Cosby; CW Shansky; AC Sartorelli. *J. Med. Chem*. **1972**, 15, 1247.
[6] I Wilson; P Wardman; TS Lin; AC Sartorelli. *J. Med. Chem*. **1986**, 29, 1381.
[7] GR Pettit; JC Knight; JC Collins; DL Herald; RK Pettit; MR Boyd; VG Young. *J. Nat. Prod*. **2000**, 63, 793.
[8] DM Balitz; JA Bush; WT Bradner; TW Doyle; FA O'Herron; DE Nettleton. *J. Antibiot*. **1982**, 35, 259.
[9] RW Rickards; JM Rothschild; AJ Willis; NM de Chazal; J Kirk; K Kirk; KJ Saliba; GD Smith. *Tetrahedron*. **1999**, 55, 13513.
[10] JA Valderrama; JA Ibacache; JA Rodriguez; C Theoduloz; J Benites. *Eur. J. Med. Chem.* **2011**, 46, 3398.
[11] J Benites; D Ríos; P Díaz; JA Valderrama. *Tetrahedron Lett*. **2011**, 52, 609.
[12] Y Prieto; M Muñoz; V Arancibia; M Valderrama; FJ Lahoz; ML Martín. *Polyhedron*. **2007**, 26, 5527.
[13] FC De Abreu; PA de Ferraz; MOF Goulart. *J. Braz. Chem. Soc*. **2002**, 13,19.
[14] M Aguilar-Martinez; G Cuevas; M Jimenez-Estrada; I González; B Lotina-Hennsen; N Macias-Ruvalcaba. *J. Org. Chem*. **1999**, 64, 3684.
[15] MC Alley; DA Scudiero; A Monks; ML Hursey; MJ Czerwinski; DL Fine; BJ Abbott; JG Mayo; RH Shoemaker; MR Boyd. *Cancer Res*. **1988**, 48, 589.
[16] DA Scudiero; RH Shoemaker; KD Paull; A Monks; S Tierney; TH Nofziger; MJ Currens; D Seniff; MR Boyd. *Cancer Res*. **1988**, 48, 4827.

[17] AA Van de Loosdrecht; RH Beelen; GJ Ossenkoppele; MG Broekhoven; MM Langenhuijsen. *J. Immunol. Methods*. **1994**, 174, 311.