ANTIPROLIFERATIVE ACTIVITY OF NEW 6-BROMINE DERIVATIVES OF 7-ANILINO-1-ARYLISOQUINOLINEQUINONES

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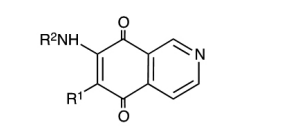
ABSTRACT

A variety of 6-bromine-containing 7-anilino-1-arylisoquinolinequinones **2a-g** were synthesized to evaluate their half-wave potentials and *in vitro* antiproliferative activity on gastric and leukemia cancer cell lines. The new compounds displayed significant IC50 values in the range: 1.31 to 11.04 μM. The structure activity relationship analysis of the new series suggest that the antiproliferative activity is dependent, in part, on the push-pull electronic effects of the nitrogen and bromine substituents inserted into the redox fragment of the 1-arylisoquinolinequinone scaffold. Linear regression analysis provided satisfactory relationships between the log IC50 and ClogP values for the AGS gastric cancer cell line.

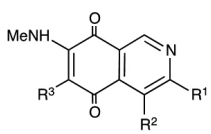
Keywords: Isoquinolinequinones; half-wave potentials; MTT assay; antiproliferative activity

INTRODUCTION

Quinones are a important naturally occurring compounds that are widely distributed in nature, play vital roles in the biochemistry of living cells and have diverse biological activities such as antitumoral, antibacterial, antifungal and antimalarial [1-4]. The isoquinoline-5,8-quinone scaffold appears in a number of naturally occurring cytotoxic compounds such as caulibugulones A-D and mansouramysin A-D (Figure 1) [5-10]. Caulibugulones A–D, evaluated for antitumor activity against the murine IC-2WT cell line exhibit high potency. In these assays, the A–C members displayed significant cytotoxicity againts the tested cell line (IC50: 0.22 to 0.34 μg/mL) [8]. These data indicate that the insertion of nitrogen and halogen atoms in the quinone nucleus moiety have influence on the cytotoxicity of the isoquinoline-5,8-quinone scaffold. Hence, this structural array has stimulated the synthesis of novel aminoisoquinoline-5,8-quinones mainly directed to extend the spectrum of biological activity on cancer cells [9-16].



caulibugulone A: R^1 = H, R^2 = Me
caulibugulone B: R^1 = Br, R^2 = Me
caulibugulone C: R^1 = Cl, R^2 = Me
caulibugulone D: R^1 = H, R^2 = CH_2CH_2OH



mansouramycin A: R^1 = R^2 = Me, R^3 = H
mansouramycin B: R^1 = Me, R^2 = H, R^3 = Cl
mansouramycin C: R^1 = CO_2Me, R^2 = R^3 = H

Figure 1. Structures of caulibugulones A-D and mansouramycins A-C.

In a previous work we reported that a number of anilinoisoquinolinequinone exhibit interesting antiproliferative activity against human gastric adenocarcinoma, human lung and human bladder carcinoma cancer cells [17]. On the basis of this background information we decided to design a new series of isoquinoline-5,8-quinone containing aniline and bromine substituents in the quinone ring to evaluate the combined electronic effects (push pull) of these groups on the redox and antiproliferative properties of the scaffold.

EXPERIMENTAL

General

All reagents were commercially available reagent grade and were used without further purification. Melting points were determined on a Stuart Scientific SMP3 apparatus and are uncorrected. ^{1}H-NMR spectra were recorded on Bruker AM-400 instrument in deuterochloroform ($CDCl_3$). ^{13}C-NMR spectra were obtained in $CDCl_3$ at 100 MHz. Chemical shifts are expressed in ppm downfield relative to tetramethylsilane and the coupling constants (*J*) are reported in Hertz. HRMS data for all final compounds were obtained using a LTQ-Orbitrap mass spectrometer (Thermo-Fisher Scientific, MA 02454, USA) 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 aluminium foil $60F_{254}$ for analytical TLC. Compounds **1a-g** were prepared according to a previously reported procedure [17].

Chemistry

Synthesis of compounds **2a-g**. *General procedure:* A solution of 1-aryl-6-aminoisoquinolinequinone **1a-g** (1 mmol), *N*-bromosuccinimide (NBS) (1 mmol) and methanol (15 mL) was left with stirring at rt and, once the reaction is completed (1:30-5:30 hrs), the solvent was removed under reduced pressure and the residue was column chromatographed over silica gel (CH_2Cl_2/AcOEt 90:10) to yield the corresponding bromoisoquinolinequinone **2a-g.**

Methyl 6-bromo-3-methyl-5,8-dioxo-1-phenyl-7-(phenylamino)-5,8-dihydroisoquino-line-4-carboxylate (**2a**). Prepared from **1a** and NBS (3:00 h, 84% yield): dark red solid, mp 160-162 ºC; IR ν_{max} 3296 (N-H), 1728 (C=O ester), 1681 (C=O quinone); ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.69 (s, 3H, Me), 4.07 (s, 3H, CO_2Me), 7.05 (m, 2H, arom), 7.34 (m, 3H, arom), 7.47 (s, 5H, phenyl), 7.81 (s, 1H, NH); ^{13}C NMR (100 MHz): δ 23.08, 53.5, 105.0, 119.0, 124.9 (2C), 126.3, 126.3, 126.6, 128.4, 128.6, 128.7, 129.3 (2C), 136.7, 137.1, 139.6, 145.1, 161.1, 161.5, 163.4, 168.6, 175.4, 178.6; HRMS (M^+): *m/z* calcd for $C_{24}H_{17}N_2O_4Br$: 477.03717; found: 477.04204.

Methyl 6-bromo-7-(4'-methoxyphenyl)amino-3-methyl-5,8-dioxo-1-phenyl-5,8-dihydro-isoquinoline-4-carboxylate (**2b**). Prepared from **1b** and NBS (3:00 h, 88% yield): dark red solid, mp 196-197 ºC; IR ν_{max} 3285 (N-H), 1732 (C=O ester), 1682 (C=O quinone); ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.68 (s, 3H, Me), 3.81 (s, 3H, OMe), 4.06 (s, 3H, CO_2Me), 6.84 (d, 2H, *J* = 8.4 Hz, 2´- and 6´-H), 7.00 (d, 2H, *J* = 8.4 Hz, 3´- and 5´-H), 7.45 (s, 5H, phenyl), 7.79 (s, 1H, NH); ^{13}C NMR (100 MHz): δ 23.1, 53.1, 55.5, 103.5, 113.5 (2C), 119.4 (2C), 126.9, 128.5 (2C), 128.6, 129.3 (2C), 137.3, 139.2, 145.1, 146.8, 158.5, 161.1, 161.5, 168.5, 175.3, 178.6, 178.9; HRMS (M^+): *m/z* calcd for $C_{25}H_{19}N_2O_5Br$: 507.04773; found: 507.39777.

Methyl 6-bromo-3-methyl-5,8-dioxo-7-(phenylamino)-1-(thiophen-2-yl)-5,8-dihydro-isoquinoline-4-carboxylate (**2c**). Prepared from **1c** and NBS (2:00 h, 95% yield): dark red solid, mp 149-151ºC; IR ν_{max} 3200 (N-H), 1713 (C=O ester), 1678 (C=O quinone); ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.55 (s, 3H, Me), 3.96 (s, 3H, CO_2Me), 7.00 (m, 3H, arom), 7.18 (m, 1H, thienyl), 7.29 (m, 2H, arom), 7.45 (m, 1H, thienyl), 7.62 (m, 1H, thienyl), 7.81 (s, 1H, NH); ^{13}C NMR (100 MHz): δ 23.0, 53.3, 104.9, 118.4, 124.9, 125.8, 126.5, 127.6, 128.8,

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130.7, 131.1, 131.9, 137.1, 141.5, 145.9, 153.0, 161.1, 168.4, 177.3, 178.6; HRMS (M^+): *m/z* calcd for $C_{22}H_{15}N_2O_4BrS$: 482.99154; found: 482.99859.

Methyl 6-bromo-7-(4'-methoxyphenyl)amino 3-methyl-5,8-dioxo-1-(thiophen-2-yl)-5,8-dihydroisoquinoline-4-carboxylate (**2d**). Prepared from **1d** and NBS (1:30 h, 75% yield): dark red solid, mp 188.1-190.5 ºC; IR ν_{max} 3423 (N-H), 1729 (C=O ester), 1609 (C=O quinone); ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.63 (s, 3H, Me), 3.82 (s, 3H, OMe), 4.04 (s, 3H, CO_2Me), 6.87 (d, 2H, *J* = 8.6 Hz, 2´- and 6´-H), 7.04 (d, 2H, *J* = 8.6 Hz, 3´- and 5´-H), 7.10 (m, 1H, thienyl), 7.54 (m, 1H, thienyl), 7.69 (m, 1H, thienyl), 7.89 (s, 1H, NH); ^{13}C NMR (100 MHz): δ 23.0, 53.1, 55.5, 114.0, 118.4, 125.8, 126.9, 127.6, 129.7, 130.7, 131.0, 137.9, 141.5, 145.9, 152.9, 158.5, 161.1, 168.5, 175.1, 178.6; HRMS (M^+): *m/z* calcd for $C_{23}H_{17}N_2O_5BrS$: 513.00415; found: 513.01134.

Methyl 6-bromo-1-(furan-2-yl)-3-methyl-5,8-dioxo-7-(phenylamino)-5,8-dihydroiso-quinoline-4-carboxylate (**2e**). Prepared from **1e** and NBS (3:30 h, 96% yield): dark red solid, mp 176-178 ºC; IR ν_{max} 3300 (N-H), 1731 (C=O ester), 1683 (C=O quinone); ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.65 (s, 3H, Me), 4.05 (s, 3H, CO_2Me), 6.56 (m, 1H, furyl), 7.13 (m, 3H, arom), 7.17 (m, 1H, furyl), 7.37 (m, 2H, arom), 7.51 (m, 1H, furyl), 7.73 (s, 1H, NH); ^{13}C NMR (100 MHz): δ 23.0, 53.3, 105.9, 112.2, 114.4, 119.5, 124.6, 125.6, 126.0, 126.4, 128.8, 136.9, 137.5, 144.8, 146.8, 148.2, 151.6, 161.1, 168.4, 171.2, 175.0, 177.9; HRMS (M^+): *m/z* calcd for $C_{22}H_{15}N_2O_5Br$: 467.01643; found: 467.67317.

Methyl 6-bromo-1-(furan-2-yl)-7-(4'-methoxyphenyl)amino)-3-methyl-5,8-dioxo-5,8-dihydroisoquinoline-4-carboxylate (**2f**). Prepared from **1f** and NBS (4:00 h, 73% yield): dark red solid, mp 183-184 ºC; IR ν_{max} 3299 (N-H), 1732 (C=O ester), 1609 (C=O quinone); ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.64 (s, 3H, Me), 3.82 (s, 3H, OMe), 4.04 (s, 3H, CO_2Me), 6.57 (m, 1H, furyl), 6.88 (d, 2H, *J* = 8.4 Hz, 2´- and 6´-H), 7.07 (d, 2H, *J* = 8.4 Hz, 3´- and 5´-H), 7.17 (m, 1H, furyl), 7.52 (m, 1H, furyl), 7.73 (s, 1H, NH); ^{13}C NMR (100 MHz): δ 23.0, 53.3, 55.6, 104.2, 112.1, 114.0, 114.4, 119.4, 125.8, 126.6, 130.2, 137.1, 144.8, 145.6, 148.3, 151.7, 158.4, 161.2, 168.4, 174.9, 178.0; HRMS (M^+): *m/z* calcd for $C_{23}H_{17}N_2O_6Br$: 497.02700; found: 497.45511.

4-acetyl-6-bromo-3-methyl-7-(phenylamino)-1-(thiophen-2-yl) isoquinoline-5,8-dione (**2g**). Prepared from **1g** and NBS (5:30 h, 92% yield): dark red solid, mp 178-180 ºC; IR ν_{max} 3314 (N-H), 1730 (C=O ester), 1666 and 1630 (C=O quinone); ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.59 (s, 3H, Me), 2.76 (s, 3H, CO_2Me), 7.11 (m, 1H, thienyl), 7.13 (m, 3H, arom), 7.38 (m, 2H, arom), 7.56 (m, 1H, thienyl), 7.73 (m, 1H, thienyl), 7.97 (s, 1H, NH); ^{13}C NMR (100 MHz): δ 21.9, 29.6, 101.6, 118.0, 120.8, 122.7, 127.8, 128.3, 128.5, 128.9, 129.3, 139.7, 142.6, 142.9, 156.5, 161.7, 173.9, 177.9, 178.6, 199.8 ; HRMS (M^+): *m/z* calcd for $C_{22}H_{15}N_2O_3BrS$: 466.99867; found: 466.00594.

Electrochemical Meassurements

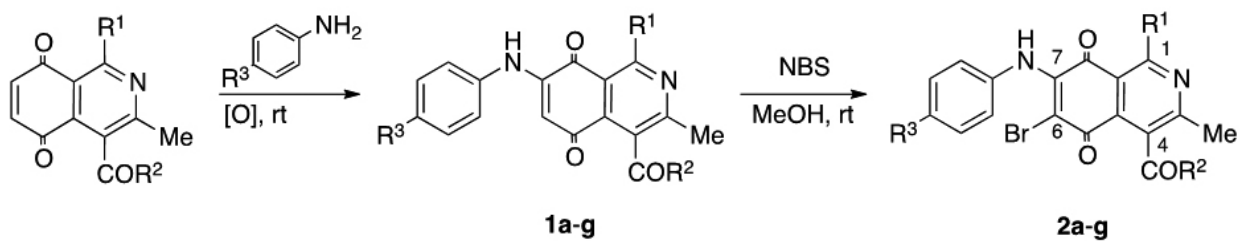
Cyclic voltammograms of compounds were obtained on a Bioanalytical Sytem BAS CV-50W electrochemical analyzer. A small capacity measuring cell was equipped with a platinum disc as working electrode, an Ag/10 nM Ag (MeCN) reference electrode for non aqueous solvent, with a platinum wire auxiliary electrode,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

Cell Growth Inhibition 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), and HL-60 promyelocytic leukemia cells (CCL-240). 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 in Eagle minimal essential medium (EMEM), AGS cells in Ham F-12, and HL-60 in suspension in RPM1. 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. RPM1 medium containing 1mM sodium pyruvate and 2.0 g/L sodium bicarbonate. All media were supplemented with 10% heat-inactivated FBS, 100 IU/mL penicillin and 100 µg/mL streptomycin in a humidified incubator with 5% CO2 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 IC50 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. The culture medium containing the compounds under evaluation, was removed from each well by means by vacuum aspiration before adding the MTT solution. 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 (ELx800, 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 IC50 values were graphically obtained from the dose-response curves. The IC50 value was obtained adjusting the dose-response curve to a sigmoidal model (a + (b − a)/1 + 10(x − c)), where c = log IC50.

RESULTS AND DISCUSSION

The synthetic route to the target bromine isoquinoline-5,8-quinones **2a-g** is shown in Scheme 1. The required 7-anilino-isoquinoline-5,8-quinone precursors **1a-g** were prepare from the respectives isoquinolinequinones according to previously reported procedures [17].



Scheme 1. Synthesis of 7-anilino-1-aryl-6-bromoisoquinolinequinones **2a-g**

The treatment of compounds **1a-g** with *N*-bromosuccinimide (NBS) was conducted in methanol at room temperature and monitored by TLC. The reaction proceeded cleanly to give the corresponding bromine compounds **2a-g** in good yields (Figure 2). The structures of the new compounds **2a-g** were established on the basis of their nuclear magnetic resonance (^{1}H NMR, ^{13}C NMR) and high resolution mass spectra (HRMS).

The redox potentials of the synthesized compounds **2a-g** were measured by cyclic voltammetry in acetonitrile at room temperature, using a platinum electrode and 0.1 M tetraethylammonium tetrafluoroborate as the supporting electrolyte. The voltammograms were run in the potential range 0.0–2.0 V versus non-aqueous Ag/Ag+ [18]. The first half-wave potential values, $E^I_{1/2}$, evaluated from the voltammograms obtained at a sweep rate of 100 mV s^{-1}. The $E^I_{1/2}$ values for the first electron, which are related with the formation of the semiquinone radical anion [19, 20], are in the potential range −407 to −485 mV (Figure 2; Table 1).

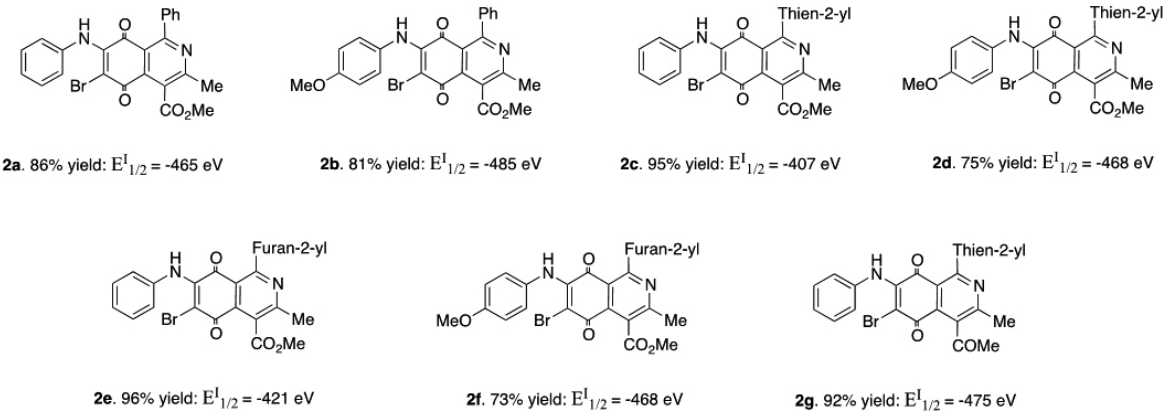


Figure 2. Yields and half-wave potential ($E^{I}_{1/2}$) values of bromoisoquinolinequinones **2a-g**

The $E^{I}_{1/2}$ values of compounds **2a-g** and precursors **1a-g** [17] are showed in Figure 3 and Table 1. Analysis of the data indicate that the insertion of bromine into the scaffold of compounds **1a-g**, as in 2a-g, induces the displacement of the half-wave potentials of the precursors **1a-**g ($E^{I}_{1/2}$: −560 to −465 mV), towards more positive values in the products 2a-g ($E^{I}_{1/2}$: -407 to -485 mV). This fact can be attributed to the inductive effect of the bromine group which enhanced the redox ability of the quinone nucleus of the scaffolds.

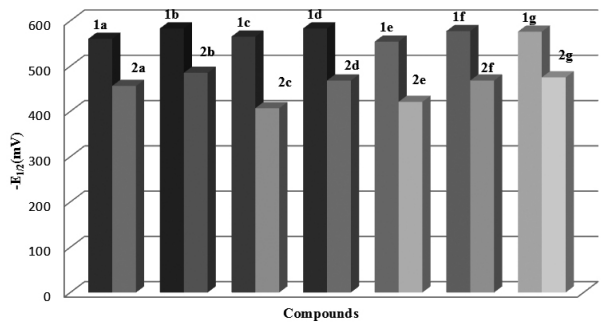


Figure 3. Comparative -$E^{I}_{1/2}$ values of compounds **1a-g** and **2a-g**

The compounds **2a–g** were evaluated for their *in vitro* antiproliferative activity against normal human lung fibroblast MRC-5 and two human cancer cells lines: AGS gastric adenocarcinoma and HL-60 promyelocytic leukemia cells, in 72 h drugs exposure assays. The antiproliferative activity of the new compounds was measured using conventional microculture tetrazolium reduction assays [21–23]. The antiproliferative activity of the new quinones are expressed in terms of IC_{50} (μM) and collected in Table 1. Etoposide, a clinically used anticancer agent, was taken as a positive control.

[a] The IC50 and $E^{I}_{1/2}$ values were taken from ref [17]. [b]Data represent mean average values for six independent determinations; [c] Normal human lung fibroblasts cell line; [d] Human gastric adenocarcinoma cell line; [e] Promyelocytic leukemia cell line; [f] Determined by the ChemBioDraw Ultra 11.0 software.

The screening showed that compounds **2a-g** exhibit significant antitumor activity in the range IC_{50}: 1.31-11.04 μM. Comparison of the IC_{50} values of compounds **2a–g** indicates that **2a**, **2c** and **2e** are the more potent members of the series on AGS and HL-60 cell lines. The data in Table 3 indicate that compound **2e** exhibit significant activity on the HL-60 cell line (IC_{50}: 1.92 μM) at similar level to that of etoposide (IC_{50}: 2.23 μM).

In Table 1 appeared the IC_{50} reported values of precursors **1a-g** [17], that have been included together with those of the bromination products **2a-g**, to highlight the differences in the antiproliferative activity as consequence of the insertion of the bromine atom into the scaffolds. We observed that, in almost all cases, the insertion of bromine atoms at the C-6 position of scaffolds enhanced the antiproliferative activity on safe and cancer cell lines. This insertion effect is particulary noticeable on the scaffold of **1b** and **1g**, as in compounds **2b** and **2g**. Therefore, it can be concluded that the bromination insertion is relevant to improve the antiproliferative activity of the isoquinolinequinone scaffold.

Table 1. IC_{50}, $E^{I}_{1/2}$ and ClogP values of **1a-g**[a] and **2a-g**

N°	IC_{50} ± SEM (μM) [b]			$-E^{I}_{1/2}$ (mV)	ClogP[f]
	MRC-5[c]	AGS[d]	HL-60[e]		
1a	5.91 ± 0.36	2.52 ± 0.17	4.39 ± 0.26	560	2.84
1b	> 100	> 100	> 100	583	2.71
1c	9.89 ± 0.51	4.24 ± 0.21	5.19 ± 0.31	565	2.82
1d	9.19 ± 0.53	3.28 ± 0.13	10.26 ± 0.09	583	2.69
1e	4.72 ± 0.29	1.79 ± 0.11	5.00 ± 0.35	554	1.45
1f	4.58 ± 0.35	1.83 ± 0.11	8.04 ± 0.49	577	1.33
1g	> 100	> 100	> 100	576	2.31
2a	1.84 ± 0.42	1.67 ± 0.23	6.08 ± 0.26	465	3.15
2b	5.65 ± 0.32	2.57 ± 0.17	8.28 ± 0.49	485	3.02
2c	1.73 ± 0.09	1.31 ± 0.06	2.61 ± 0.13	407	3.13
2d	10.40 ± 0.51	2.94 ± 0.21	11.04 ± 0.54	468	3.00
2e	1.71 ± 0.06	2.01 ± 0.08	1.92 ± 0.02	421	1.76
2f	5.39 ± 0.41	3.51 ± 0.22	3.09 ± 0.24	468	1.64
2g	5.13 ± 0.35	7.13 ± 0.43	5.35 ± 0.37	475	2.62
Etoposide	0.33 ± 0.02	0.58 ± 0.02	2.23 ± 0.09	-	-

Taken into account that the lipophilicity is an important parameter related with the biological activity of quinonoid compounds [24,25], the ClogP descriptors of compounds **2a-g** were calculated using the AM1 semiempirical method (ChemBioDraw Ultra 11.0 sofware). Relationships between the of logIC50 and ClogP values were obtained for the AGS gastric adenocarcinoma and HL-60 leukemia cell lines according to regression Eqs. 1 and 2, respectively. Indeed, a better correlation was obtained between log IC_{50} and logP values for the AGS cell line.

$$\log IC50 = -1.054(ClopP)^2 + 4.934(ClogP) - 4.893, \quad n = 7,\ R^2 = 0.94 \quad (1)$$

$$\log IC50 = -0.385(ClopP)^2 + 2.106(ClogP) - 2.072, \quad n=7,\ R^2 = 0.46 \quad (2)$$

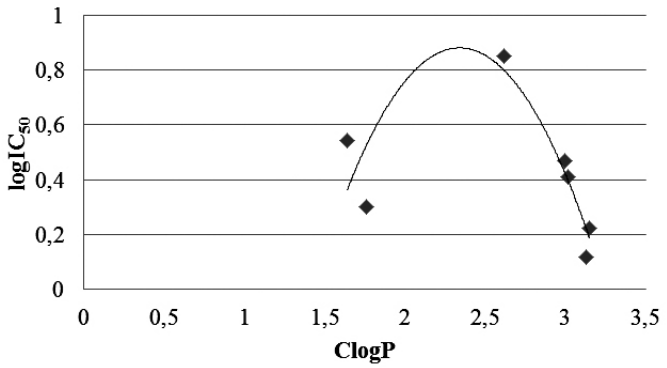


Figure 4. Correlation between ClogP vs log IC_{50} of **2a-2g** for the AGS cell line

The graph indicates that for those compounds **2a-g** with lipophilicity values within the range: 1.64 to 3.13, it should be expected the greater antiproliferative activities.

CONCLUSION

In summary, we have synthesized a variety of 1-aryl-7-anilino-6-bromoisoquinolinequinones **2a-g** in good yields. The results of the biological screening show that the majority of the members of the series express *in vitro* antiproliferative activity against normal human lung fibroblasts (MRC-5), gastric adenocarcinoma (AGS), and human leukemia cells (HL-60) cell lines. Compounds **2a**, **2c** and **2e** were selected as the most active members of the new series. Compound **2e** exhibited the highest antiproliferative activity on HL-60 cell lines (IC50: 1.92 μM) comparable to that of etoposide (IC50: 2.23 μM). Biological comparative effects as function of the nature of the substituents reveals that the insertion of bromine atoms in the 6-position of the quinone ring of the isoquinolinequinone scaffolds is an important factor on the antiproliferative activity. A good correlation is observed between log IC_{50} and ClogP values for the gastric cancer cell line.

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