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. These data indicate that 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 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 antioxidant, 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-2W7 cell line exhibit high potency. In these assays, the A-C members displayed significant cytotoxicity against 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 aminoisouquinoline-5,8-quinones mainly directed to extend the spectrum of biological activity on cancer cells [9-16].

![Figure 1. Structures of caulibugulones A-D and mansouramysins A-C.](image-url)

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. 1H-NMR spectra were recorded on Bruker AM-400 instrument in deuteriochloroform (CDCl3). 13C-NMR spectra were obtained in CDCl3 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, 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 60F254 for analytical TLC.

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 (CH2Cl2/MeOH 90:10) to yield the corresponding bromoisouquinolinequinone 2a-g.

Methyl 6-bromo-3-methyl-5,8-dioxo-1-phenyl-7-(phenylamino)-5,8-dihydroisoquinoline-4-carboxylate (2a). Prepared from 1a and NBS (3:00 h, 84% yield): dark red solid, mp 160-162 ºC; IR νmax 3296 (N-H), 234 (C=O ester), 1728 (C=O quinone); δ 2.69 (s, 3H, Me), 4.07 (s, 3H, OMe), 7.05 (m, 2H, arom), 7.34 (m, 3H, arom), 7.47 (s, 5H, phenyl), 7.81 (s, 1H, NH); 13C NMR (100 MHz): δ 23.0, 53.3, 104.9, 118.4, 124.9, 125.8, 126.5, 127.6, 128.8, 131.5 (3C), 136.7, 137.1, 139.6, 145.1, 161.1, 161.5, 163.4, 168.6, 175.4, 178.6; HRMS (M+): m/z 477.04204.

Methyl 6-bromo-7-(4′-methoxyphenylamino)-3-methyl-5,8-dioxo-1-phenyl-5,8-dihydroisoquinoline-4-carboxylate (2b). Prepared from 1b and NBS (3:00 h, 88% yield): dark red solid, mp 196-197 ºC; IR νmax 3285 (N-H), 1732 (C=O ester), 1682 (C=O quinone); 1H NMR (400 MHz, CDCl3); δ 2.68 (s, 3H, Me), 3.81 (s, 3H, OMe), 4.06 (s, 3H, CO2Me), 7.35 (s, 3H, arom), 7.47 (s, 5H, phenyl), 7.79 (s, 1H, NH); 13C NMR (100 MHz): δ 23.7, 31.9, 55.3, 103.5, 113.5 (2C), 119.4 (2C), 126.5, 129.6, 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 C29H20N2O2Br: 507.04773; found: 507.03977.

Methyl 6-bromo-7-(3′-thiophenylamino)-5,8-dioxo-1-phenyl-5,8-dihydroisoquinoline-4-carboxylate (2c). Prepared from 1c and NBS (2:00 h, 95% yield): dark red solid, mp 149-151 ºC; IR νmax 3200 (N-H), 1713 (C=O ester), 1678 (C=O quinone); 1H NMR (400 MHz, CDCl3); δ 2.55 (s, 3H, Me), 3.96 (s, 3H, CO2Me), 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); 13C NMR (100 MHz): δ 23.0, 53.3, 104.9, 118.4, 124.9, 125.8, 126.5, 127.6, 128.8, 131.5 (3C), 132.9, 135.4, 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 C30H19N2O2SBr: 539.06422; found: 539.06442.
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 calecd for C_{12}H_{14}N_{2}OBrS: 513.00415; found: 513.01134.

Methyl 6-bromo-7-(4'-methoxyphenyl)amino-5,8-dihydroisoquinoline-4-carboxylate (2d). Prepared from 1d and NBS (1:30 h, 75% yield): dark red solid, mp 188.1-190.5°C; IR ν_{max} = 3299 (N-H), 1730 (C=O ester), 1666 and 1565 cm^{-1}.

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 (1H NMR, 13C NMR) and high resolution mass spectra (HRMS).

Electrochemical Measurements

Cyclic voltammograms of compounds were obtained on a Bioanalytical System BAS CV-50W electrochemical analyzer. A small capacity measuring cell was equipped with a platinum disc as working electrode, an Ag/10 mM 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.

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 prepared from the respective 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 (1H NMR, 13C 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 tetrabutylammonium tetrafluoroborate as the supporting electrolyte. The voltammograms were run in the potential range 0.0–2.0 V versus non-aqueous Ag/AgCl [18]. The first half-wave potential values, E_{1/2}, evaluated from the voltammograms obtained at a sweep rate of 100 mV s^{-1}. The E_{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 −0.407 to −0.485 mV (Figure 2; Table 1).

RESULTS AND DISCUSSION
The $E_{1/2}$ values of compounds 2a-g and precursors 1a-g [17] are shown 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 ($E_{1/2}^0$ = -560 to -465 mV), towards more positive values in the products 2a-g ($E_{1/2}^f$ = -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 2. Yields and half-wave potential ($E_{1/2}$) values of bromoisquinolinequinones 2a-g

| N° | IC$_{50}$ (µM) ± SEM | -E$_{1/2}$ (mV) | ClogP* |
|----|----------------------|-----------------|--------|
| MRC-5* | AGS* | HL-60* |
| 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 | - | - |

Table 1. IC$_{50}$, $E_{1/2}$ and ClogP values of 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 cell 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.

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, 2e 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.** Comparative IC$_{50}$ and ClogP values of 1a-g and 2a-g

*The IC50 and $E_{1/2}$ values were taken from ref [17]. *Data represent mean average values for six independent determinations; *Normal human lung fibroblasts cell line; *Human gastric adenocarcinoma cell line; *Promyelocytic leukemia cell line; *Determined by the ChemBioDraw Ultra 1.0 software.

The E$_{1/2}$ values of compounds 2a-g and precursors 1a-g [17] are shown 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 ($E_{1/2}^0$ = -560 to -465 mV), towards more positive values in the products 2a-g ($E_{1/2}^f$ = -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$_{1/2}$ 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 cell 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.

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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 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 IC50 and ClogP values for the gastric cancer cell line.

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