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Research Article

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Synthesis and Antiproliferative Evaluation of New Aminoisoquinolinequinones Derived from *p*-Phenylenediamine, Benzidine and Dapsone

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Abstract

Insertion of aryl monoamines into the isoquinoline-5,8-quinone core, to produce arylaminoisoquinolinequinones, strongly enhanced the cytotoxic activity of the scaffold. As a contribution to build new potential cytotoxic members of this series the behavior of aryl diamines to act as mono and/or bis nucleophiles against isoquinoline-5,8-quinones was evaluated. The experiments performed with the aryl diamines: p-phenylenediamine, benzidine and dapsone, and a number of isoquinolinequinones, with or without the presence of a Lewis catalyst, indicated that monoamination, is the preferred reaction to yield the respectives arylaminoisoquinolinequinones. The structures of the new products were established and their voltammetric properties were evaluated. Screening of the aminoquinones on a panel of three cancer cells showed moderate to high antiproliferative activities. Among the members of the series, eight compound stand out for their high potencies and selective index on the tested cancer cell lines, compared to those of the etoposide and taxol cancer drugs.

Keywords: Isoquinolinequinones; Aryl diamine; Antiproliferative activity; Cancer cells; Selectivity index

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INTRODUCTION

1,4-Naphthoquinone possesing arylamino groups bonded to the 2-position and some of their azaquinone-analogues have been the subject of study in the last years because of their potential as anticancer agents against a number of human cancer cell lines [1-5]. The common feature of these aminoquinones to target cancer cells has been attributed to their abilities to produce oxidative stress via generation of reactive oxygen species (ROS) [6-13]. Our search of the literature reveals that the biological studies on aminoquinones are focused mainly on members prepared by reaction of 1,4-naphthoquinones with aryl monoamines. Precedents on the reaction of 1,4-naphthoquinones with aryl diamines such as *p*-phenylenediamine and dapsone yield the respectives monoamination products and no bis-amination products, where the amine acts as bidentate nucleophile, has been reported [14].

As a continuation of our research on the synthesis of cytotoxic arylaminoisoquinolinequinones [15-17], we were interested to evaluate the nucleophilic reactivity of diaryl diamines towards isoquinolinequinones aimed to construct twin-drug type bivalent phenylaminoquinones as potential cytotoxic agents on cancer cells. Herein we wish to report results on the amination reaction of isoquinolinequinones 2a-d with the aryldiamines: *p*-phenylenediamine 3, benzidine 4 and dapsone 5 (Figure 1) and the antiproliferative evaluation of the resulting aminoquinones against a murine fibroblast and on a panel of three cancer cell lines.

EXPERIMENTAL SECTION

General

All reagents were commercially available reagent grade and were used without further purification. Melting points were determined on a Stuart Scientific SMP10 apparatus and are uncorrected. ^{1}H-NMR spectra were recorded on Bruker AM-400 instrument in dimethyl sulfoxide (DMSO). ^{13}C-NMR spectra were obtained in DMSO 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. HRMS data for all final compounds were obtained using an Exactive plus 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 TLC. Compounds 2a, b and d were prepared according to a previously reported procedure [18].

Chemistry

4-acetyl-1,3-dimethylisoquinoline-5,8-dione (2c):

A suspension of 2,5-dihydroxyacetophenone (160.1 mg, 1.05 mmol), 4-aminopent-3-en-2-one (104.0 mg, 1.05 mmol), Ag_2O (487.0 mg, 2.10 mmol) and $MgSO_4$ (0.5 g) in CH_2Cl_2 (25 mL) was stirred at rt for 4 h. The mixture was filtered, the solids were washed with CH_2Cl_2 and the solvent removed under reduced pressure. The residue were column cromatographed over silica gel (90:10 CH_2Cl_2/AcOEt) to yield 2c (142.4 mg, 0.63 mmoles, 60%) as yellow solid, mp 151-152°C; IR νmax 1705 (C=O acetyl), 1667 and 1572 (C=O quinone); ^{1}H NMR (400 MHz, DMSO) δ 2.54 (s, 3H, 3-Me), 2.57 (s, 3H, 1-Me), 2.96 (s, 3H, COMe, 6.97 (s, 2H, 6-H and 7H); ^{13}C NMR (100 MHz, DMSO) δ 204.1, 185.8, 185.7, 161.1, 159.0, 141.1, 136.8, 135.7, 133.5, 121.1, 31.4, 26.1, 23.2; HRMS $[M+H]^+$: calcd for $C_{13}H_{11}NO_3$: 229.07389; found: 229.0714.

General procedure for the synthesis of arylaminoisoquinolinequinones 6a-d, 7a-d and 8a-d:

Suspensions of isoquinolinquinones 2a-d (1 equiv.), the required aryl diamines (2 equiv.), $CeCl_3 \times 7H_2O$ (0.05 equiv.) and ethanol (25 mL) were left with stirring at rt after completion of the reaction as indicated by TLC. The solvents were removed under reduced pressure and the residues were column chromatographed over silica gel (90:10 CH_2Cl_2/AcOEt) to yield the corresponding arylaminoisoquinolinequinones 6a-d, 7a-d and 8a-d.

Methyl 7-(4-aminophenylamino)-1,3-dimethyl-5,8-dioxo-5,8-dihydroisoquinoline-4-carboxylate (6a):

Prepared from quinone 2a (100.3 mg, 0.41 mmol), *p*-phenylenediamine (88.8 mg, 0.82 mmol) and $CeCl_3 \times 7H_2O$ in ethanol (20 mL) after stirring for 1.5 h. Isolated as purple solid (97%, 139.1 mg, 0.40 mmol), mp 271-272°C; IR νmax 3452 (N-H), 3353 and 3314 (N-H), 1721 (C=O ester), 1676 and 1648 (C=O quinone); ^{1}H NMR (400 MHz, DMSO) δ 2.46 (s, 3H, 3-Me), 2.87 (s, 3H, 1-Me), 3.83 (s, 3H, CO_2Me), 5.29 (s, 2H, NH_2), 5.81 (s, 1H, 6-H), 6.61 (d, *J* = 8.6 Hz, 2H), 7.00 (d, *J* = 8.6 Hz, 2H), 9.34 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO) δ 181.6, 179.3, 168.6, 159.6, 159.2, 147.9, 147.4, 137.9, 125.5, 125.3 (2C), 124.2, 120.7, 114.1 (2C), 99.4, 52.5, 25.7, 22.3; HRMS $[M+H]^+$: calcd for $C_{19}H_{17}N_3O_4$: 351.12191; found: 351.1286.

Methyl 7-(4´-aminophenylamino)-3-methyl-5,8-dioxo-5,8-dihydroisoquinoline-4-carboxylate (6b):

Prepared from quinone 2b (84.1 mg, 0.36 mmol), *p*-phenylenediamine (78.7 mg, 0.73 mmol) and $CeCl_3 \times 7H_2O$ in ethanol (20 mL) after stirring for 10 h. Isolated as purple solid (61%, 75.0 mg, 0.22 mmol), mp 253-254°C; IR νmax 3450 (N-H), 3354 and 3308(N-H), 1718 (C=O ester), 1649 and 1590 (C=O quinone); ^{1}H NMR (400 MHz, DMSO) δ 2.53 (s, 3H, Me), 3.87 (s, 3H, CO_2Me), 5.37 (s, 2H, NH_2), 5.88 (s, 1H, 6-H), 6.64 (d, *J* = 8.6 Hz, 2H), 7.03 (d, *J* = 8.7 Hz, 2H), 9.11 (s, 1H, NH), 9.37 (s, 1H, 1-H); ^{13}C NMR (100 MHz, DMSO) δ180.5, 179.3, 168.2, 161.0, 147.4, 147.2, 147.1, 146.9, 135.7, 125.4 (2C), 125.0, 122.5, 114.2 (2C), 100.7, 52.6, 22.3 ; HRMS $[M+H]^+$: calcd for $C_{18}H_{15}N_3O_4$: 337.10626; found: 337.1026.

4-acetyl-7-(4-aminophenylamino)-1,3-dimethylisoquinoline-5,8-dione (6c):

Prepared from quinone 2c (109.7 mg, 0.48 mmol), *p*-phenylenediamine (103.6 mg, 0.96 mmol) and $CeCl_3 \times 7H_2O$ in ethanol (20 mL) after stirring for for 2h. Isolated as purple solid (82%, 132.2 mg, 0.39 mmol), mp 250-251°C; IR νmax 3441 (N-H), 3351 and 3292 (N-H), 1684 (C=O ester), 1597 and 1557 (C=O quinone); ^{1}H NMR (400 MHz, DMSO) δ , 2.37 (s, 3H, 3-Me), 2.41 (s, 3H, 1-Me), 2.85 (s, 3H, COMe), 5.28 (s, 2H, NH_2), 5.79 (s, 1H, 6-H), 6.60 (d, *J* = 8.7 Hz, 2H), 6.98 (d, *J* = 8.7 Hz, 2H), 9.34 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO) δ 203.2, 181.7, 180.4, 158.9, 158.2, 148.1, 147.4, 137.9, 132.7, 125.5 (2C), 125.4, 121.0, 114.1 (2C), 99.1, 31.2, 25.5, 22.4; HRMS [M+H]+: calcd for $C_{19}H_{17}N_3O_3$: 335.12699; found: 335.1332.

4-acetyl-7-(4-aminophenylamino)-3-methylisoquinoline-5,8-dione (6d):
Prepared from quinone 2d (73.1 mg, 0.34 mmol), *p*-phenylenediamine (73.5 mg, 0.68 mmol) and $CeCl_3 \times 7H_2O$ in ethanol (20 mL) after stirring for 3 h. Isolated as purple solid (77%, 84.0 mg, 0.26 mmol), mp 222-224°C; IR νmax 3465 (N-H), 3360 and 3309 (N-H), 1696 (C=O ester), 1619 and 1568 (C=O quinone); ^{1}H NMR (400 MHz, DMSO) δ 2.49 (s, 3H, 3-Me), 3.34 (s, 3H, COMe), 5.30 (s, 2H, NH_2), 5.87 (s, 1H, 6-H), 6.62 (dd, *J* = 8.5 Hz, 2H), 7.02 (dd, *J* = 8.5, 2H), 9.05 (s, 1H,1-H), 9.40 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO) δ 203.5, 180.1, 180.5, 160.0, 147.4, 146.8, 135.7, 133.5, 125.5, 125.4(2C), 125.3, 122.6, 114.1(2C), 100.4, 30.7, 22.4; HRMS $[M+H]^+$: calcd for $C_{18}H_{15}N_3O_3$: 321.11134; found: 321.1186.

Methyl 7-(4-aminobiphenylamino)-1,3-dimethyl-5,8-dioxo-5,8-dihydroisoquinoline-4-carboxylate (7a):
Prepared from quinone 2a (100.9 mg, 0.41 mmol), benzidine (151.9 mg, 0.83 mmol) and $CeCl_3 \times 7H_2O$ in ethanol (20 mL) after stirring for 5 h. Isolated as purple solid (64%, 156.4 mg, 0.37 mmol), mp 296-297°C; IR νmax 3458 (N-H), 3361 and 3309 (N-H), 1721 (C=O ester), 1678 and 1646 (C=O quinone); ^{1}H NMR (400 MHz, DMSO) δ 2.50 (s, 3H, 3-Me), 2.91 (s, 3H, 1-Me), 3.86 (s, 3H, CO_2Me), 5.26 (s, 2H, NH_2), 6.10 (s, 1H, 6-H), 6.64 (d, *J* = 8.6 Hz, 2H), 7.39 (t, *J* = 7.7 Hz, 4H), 7.62 (d, *J* = 8.6 Hz, 2H), 9.56 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO) δ 181.4, 180.2, 168.5, 159.7, 159.2, 148.6, 147.1, 138.1, 137.4, 135.3, 127.1 (2C), 126.4, 126.0 (2C), 124.1 (2C), 120.7, 114.3 (2C), 100.7, 52.6, 25.7, 22.3; HRMS $[M+H]^+$: calcd for $C_{25}H_{21}N_3O_4$: 427.15321; found: 427.1599.

Methyl 7-(4-aminophenylamino)-3-methyl-5,8-dioxo-5,8-dihydroisoquinoline-4-carboxylate (7b):
Prepared from quinone 2b (72.5 mg, 0.31 mmol), benzidine (114.1 mg, 0.62 mmol) and $CeCl_3 \times 7H_2O$ in ethanol (20 mL) after stirring for 14 h. Isolated as purple solid (54%, 70 mg, 0.17 mmol), mp 276-277°C; IR νmax 3462 (N-H), 3365 and 3310 (N-H), 1718 (C=O ester), 1647 and 1567 (C=O quinone); ^{1}H NMR (400 MHz, DMSO) δ 2.54 (s, 3H, Me), 3.89 (s, 3H, CO_2Me), 5.30 (s, 2H, NH_2), 6.13 (s, 1H, 6-H), 6.65 (d, *J* = 8.6 Hz, 2H), 7.44 (m, 4H), 7.61 (d, *J* = 8.6 Hz, 2H), 9.14 (s, 1H, 1-H), 9.59 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO) δ 180.3, 180.1, 168.1, 161.0, 148.4, 147.5, 146.3, 138.1, 135.3, 135.1, 127.1(2C), 126.5, 125.9(2C), 124.9, 124.1(2C), 122.5, 114.3(2C), 102.0, 52.7, 22.4; HRMS $[M+H]^+$: calcd for $C_{24}H_{19}N_3O_4$: 413.13756; found: 413.1443.

4-acetyl-7-(4-aminobiphenylamino)-1,3-dimethylisoquinoline-5,8-dione (7c):
Prepared from quinone 2c (40.3 mg, 0.18 mmol), benzidine (64.8 mg, 0.35 mmol) and $CeCl_3 \times 7H_2O$ in ethanol (20 mL) after stirring for 2.5 h. Isolated as purple solid (80%, 57.8 mg, 0.14 mmol), mp 262-263°C; IR νmax 3450 (N-H), 3369 and 3308 (N-H), 1679 (C=O ester), 1645 and 1555 (C=O quinone); ^{1}H NMR (400 MHz, DMSO) δ 2.39 (s, 3H, 3-Me), 2.41 (s, 3H, 1-Me), 2.86 (s, 3H, COMe), 5.27 (s, 2H, NH_2), 6.06 (s, 1H, 6-H), 6.62 (d, *J* = 8.4 Hz, 2H), 7.35 (dd, *J* = 7.6 Hz, 4H), 7.58 (d, *J* = 8.4 Hz, 2H), 9.56 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO) δ 203.2, 181.5, 181.2, 159.0, 158.2, 148.6, 147.1, 138.1, 137.3, 135.2, 132.6, 127.1(2C), 126.4, 125.9(2C), 124.1(2C), 120.5, 114.2(2C), 100.5, 31.1, 25.5, 22.3; HRMS $[M+H]^{+:}$ calcd for $C_{25}H_{21}N_3O_3$: 411.15839; found: 411.1649.

4-acetyl-7-(4-aminobiphenylamino)-3-methylisoquinoline-5,8-dione (7d):
Prepared from quinone 2d (128.5 mg, 0.32 mmol), benzidine (117.82 mg, 0.64 mmol) and $CeCl_3 \times 7H_2O$ in ethanol (20 mL) after stirring for 16 h. Isolated as purple solid (68%), mp 266-268°C; IR νmax 3455 (N-H), 3346 and 3313(N-H), 1686 (C=O ester), 1620 and 1555 (C=O quinone); ^{1}H NMR (400 MHz, DMSO) δ 2.44 (s, 3H, 3-Me), 3.34 (s, 3H, COMe), 5.24 (s, 2H, NH_2), 6.12 (s, 1H,6-H), 6.62 (d, *J* = 8.5 Hz, 2H), 7.36 (dd, *J* = 8.6 Hz, 4H), 7.59 (d, *J* = 8.5 Hz, 2H), 9.08 (s, 1H, 1-H), 9.60 (s, 1H, 1-H); ^{13}C NMR (100 MHz, DMSO) δ 204.3, 182.1, 181.4, 160.9, 149.3, 147.8, 147.2, 139.0, 136.1, 135.9, 134.2, 127.9(2C), 127.3, 126.8(2C), 125.0(2C), 123.3, 115.1(2C), 102.6, 32.0, 23.3; HRMS $[M+H]^+$: calcd for $C_{24}H_{19}N_3O_3$: 397.14264; found: 397.1498.

Methyl-7-(4-(4-aminophenylsulfonyl)phenylamino)-1,3-dimethyl-5,8-dioxo-5,8-dihydroisoquinoline-4-carboxylate (8a):
Prepared from quinone 2a (93.1 mg, 0.38 mmol), dapsone (189.5 mg, 0.76 mmol) and $CeCl_3 \times 7H_2O$ in ethanol (20 mL) after stirring for 6 days. Isolated as purple solid (54%, 100.2 mg, 0.20 mmol), mp 254-255°C; IR νmax 3498 (N-H), 3392 and 3302 (N-H), 1729 (C=O ester), 1676 and 1628 (C=O quinone); ^{1}H NMR (400 MHz, DMSO) δ 2.43 (s, 3H, 3-Me), 2.84 (s, 3H, 1-Me), 3.81 (s, 3H, CO_2Me), 6.14 (s, 2H, NH_2), 6.24 (s, 1H, 6-H), 6.60 (d, *J* = 8.6 Hz, 2H), 7.52 (dd, *J* = 8.8 Hz, 4H), 7.82 (d, *J* = 8.6 Hz, 2H), 9.64 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO) δ 181.5, 168.7, 160.2, 159.7, 154.1, 146.4, 142.3, 139.3, 137.2, 129.8 (2C), 128.4 (2C), 125.9, 124.4, 123.6 (2C), 121.0, 113.5 (2C), 103.4, 52.9, 26.0, 22.7; HRMS $[M+H]^+$: calcd for $C_{25}H_{21}N_3O_6S$: 491.11511; found:491.1211.

Methyl-7-(4-(4-aminophenylsulfonyl)phenylamino)-3-methyl-5,8-dioxo-5,8-dihydroisoquinoline-4-carboxylate (8b):
Prepared from quinone 2b (92.4 mg, 0.40 mmol), dapsone (198.5 mg, 0.80 mmol) and $CeCl_3 \times 7H_2O$ in ethanol (20 mL) after stirring for 24 h. The solvent was removed under reduced pressure. Isolated as purple solid (49%, 93 mg, 0.19 mmol), mp 219-220°C; IR νmax 3450 (N-H), 3353 and 3304 (N-H), 1718 (C=O ester), 1650 and 1590 (C=O quinone); ^{1}H NMR (400 MHz, DMSO) δ 2.51 (s, 3H, Me), 3.89 (s, 3H, CO_2Me), 6.18 (s, 2H, NH_2), 6.32 (s, 1H, 6-H), 6.63 (d, *J* = 8.4 Hz, 2H), 7.55 (dd, *J* = 7.8 Hz, 4H), 7.87 (d, *J* = 8.1 Hz, 2H), 9.15 (s, 1H, 1-H), 9.73 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO) δ 181.1, 180.1, 167.9, 161.1, 153.7, 147.7, 145.4, 141.8, 139.0, 134.8, 129.4 (2C), 128.0 (2C), 125.4, 124.8, 123.3 (2C), 122.4, 113.1 (2C), 104.3, 52.8, 22.4. ; HRMS $[M+H]^+$: calcd for $C_{24}H_{19}N_3O_6S$: 477.09946; found: 477.1062.

4-acetyl-7-(4-(4-aminophenylsulfonyl)phenylamino)-1,3-dimethylisoquinoline-5,8-dione (8c):
Prepared from quinone **2c** (97.5 mg, 0.43 mmol), dapsone (211.3 mg, 0.85 mmol) and $CeCl_3 \times 7H_2O$ in ethanol (20 mL) after stirring for 7 days. Isolated as purple solid (45%, 92.8 mg, 0.20 mmol), mp 223-224°C; IR νmax 3468 (N-H), 3364 and 3319 (N-H), 1698 (C=O ester), 1625 and 1565 (C=O quinone); ^{1}H NMR (400 MHz, DMSO) δ 2.39 (s, 3H, 3-Me), 2.41 (s, 3H, 1-Me), 2.85 (s, 3H, COMe), 6.18 (s, 2H, NH_2), 6.25 (s, 1H, 6-H), 6.61 (d, *J* = 8.8 Hz, 2H), 7.54 (dd, *J* = 8.8, 4H), 7.84 (d, *J* = 8.8 Hz, 2H), 9.70 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO) δ 203.2, 182.2, 181.2, 159.1, 158.3, 153.7, 146.2, 141.9, 139.0, 136.8, 132.5, 129.5(2C), 128.0(2C), 125.5, 123.3(2C), 120.5, 113.1(2C), 102.7, 31.1, 25.5, 22.4; HRMS $[M+H]^+$: calcd for $C_{25}H_{21}N_3O_5S$: 475.12019; found: 475.1268.

4-acetyl-7-(4-(4-aminophenylsulfonyl)phenylamino)-1,3-dimethylisoquinoline-5,8-dione (8d):
Prepared from quinone **2d** (67.6 mg, 0.31 mmol), dapsone (153.8 mg, 0.62 mmol) and $CeCl_3 \times 7H_2O$ in ethanol (20 mL) after stirring for 24h. Isolated as purple solid (60%, 87 mg, 0.19 mmol), mp 214-215°C; IR νmax 3452 (N-H), 3362 and 3306 (N-H), 1698 (C=O ester), 1624 and 1562 (C=O quinone); ^{1}H NMR (400 MHz, DMSO) δ 2.46 (s, 3H, 3-Me), 2.50 (s, 3H, COMe), 6.18 (s, 2H, NH_2), 6.32 (s, 1H,6-H), 6.64 (d, *J* = 8.8 Hz, 2H), 7.57 (dd, *J* = 8.8, 2.1 Hz, 4H), 7.87 (d, *J* = 8.8 Hz, 2H), 9.11 (s, 1H, 1-H), 9.73 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO) δ 203.5, 182.2, 180.2, 160.0, 153.7, 147.1, 145.5, 141.8, 139.1, 134.7, 133.3, 129.5(2C), 128.0(2C), 125.5, 123.3(2C), 122.5, 113.1(2C), 104.1, 31.0, 22.3; HRMS $[M+H]^+$: calcd for $C_{24}H_{19}N_3O_5S$: 461.10454; found: 461.0979.

Cell Growth Inhibition Assay
The cell lines used in this work included MDA-MB-231 human breast adenocarcinoma cells, Caco-2 human colorectal adenocarcinoma cells, B16-F10 mouse melanoma cells and MEF primary mouse embryonic fibroblast. Cells were grown in DMEM high glucose medium (Mediatech, Manassas, VA) supplemented with 10% (MDA-MB-231, Caco-2 and B16-F10) or 15% (MEF) heat-inactivated fetal bovine serum (HyClone laboratories), 100 IU/mL penicillin and 100 µg/mL streptomycin and maintained at 37°C in a 5% CO_2 humidified atmosphere. For the experiments, a total of 5.000 cells/well were seeded onto a flat-bottomed 96-well plate in 200 µL final volume. 6h after seeding, the cells were incubated with the medium containing the compounds at concentrations ranging from 0 up to 100 µM dissolved in DMSO (0.1% final concentration) during 72 h. The concentrations used to calculate the IC_{50} values were: 100.0, 30.0, 10.0, 3.0, 1.0, 0.3, 0.1, 0.01 and 0.0 µM. Untreated cells (medium containing 0.1% DMSO) were used as controls**.** At the end of the incubation, cell viability was measured using CyQuant® Direct Cell Proliferation Assay Kit (Life Technologies) in accordance with manufacturer's instruction. Briefly, 100 µL of culture medium containing the compounds under evaluation was removed from each well and replaced by 2X Detection Reagent. Cells were incubated for 1 h and fluorescence emission was measured at 535 nm with excitation at 480 nm in a microplate reader (Infinite 200 PRO, Tecan). At least four independent experiments were performed for each concentration. Each result was 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 curve (variable slope) generated using GraphPad Prisma 6.0 software.

RESULTS AND DISCUSSION

Chemistry
Isoquinolinequinones 2a-d selected for the study, were synthesized from 2,5-dihydroxy benzaldehyde, 2,5-dihydroxyacetophenone and enaminones 1a,b according to a previously reported procedure [18]. To evaluate the nucleophilic behaviour of the diaryl amines 3, 4 and 5 towards quinones 2a-d, the reactivity of amine 3 with quinone 2a was studied, as reaction model.

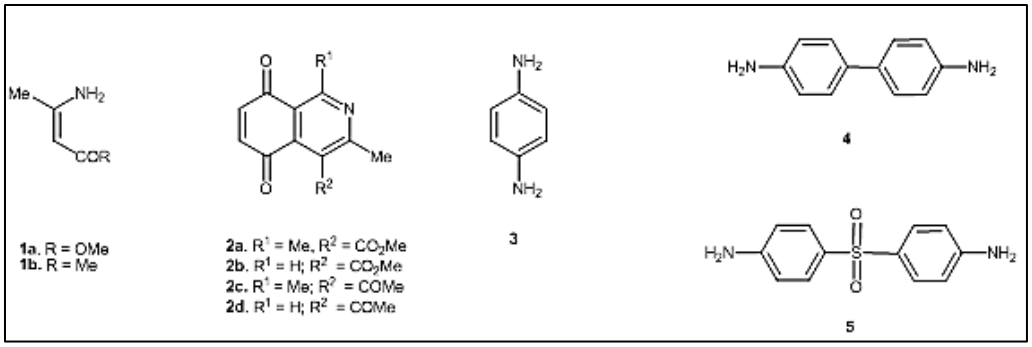
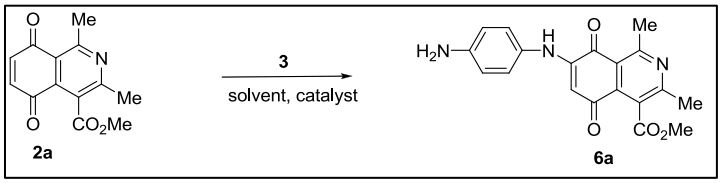


Figure 1: Structure of isoquinolinequinone and aryldiamine precursors

The assays were run in ethanol or acetonitrile, at room temperature, using 5% molar of cerium chloride heptahydrate or indium bromide as Lewis acid catalysts and the results are summarized in Table 1. The trials, carried out using excess of diamine 3 over quinone 2a (entries 1 and 2), were designed to evaluate the potential reactivity of 3 to act as a *bis* nucleophile. In both cases, complex mixtures of decomposition products were produced as evidenced by TLC. Pure samples of compound 6a were isolated in low yields from the mixtures. With regard to the remaining experiments employing equimolar amount of the reactants (entry 3) and excess of the diamine 3 over quinone 2a (entries 4-7) it can be deduced that the efficiency of the monoamination reaction of 2a with amine 3 to yield 6a is sensitive to the nature of the solvent, the presence and nature of the Lewis acid and the excess of the arylamine diamine 3. Indeed, the monoamination reaction of 2a with amine 3 under the experimental conditions described in entry 4 is optimal to prepare compound 6a. It is necessary to point out that the assay carried out in the absence of a Lewis acid (entry 6) yield compound 6a in 95% yield but after 48 h. The reactions of benzidine 4 and dapsone 5 with four equivalents of 2a were examined. As in the case of the reaction of *p*-phenylenediamine 3 with four equivalents of 2a, complex mixtures of decomposition products were produced.

Table 1: Reaction assays of *p*-phenylenediamine 3 with isoquinolinequinone 2a



Entry	2a/3 ratio	Solvent	Time (h)	Yield%[a]
1	04:01	EtOH	48	4[b]
2	02:01	EtOH	24	23[b]
3	01:01	EtOH	22	72[b]
4	01:02	EtOH	2	97[b]
5	01:02	EtOH	5	94[c]
6	01:02	EtOH	48	95[d]
7	01:02	MeCN	96	75[b]
8	01:04	EtOH	24	61[b]

[a]Isolated by column chromatography. [b]$CeCl_3 \times 7H_2O$. [c]$InBr_3$; [d]In the absence of a Lewis acid

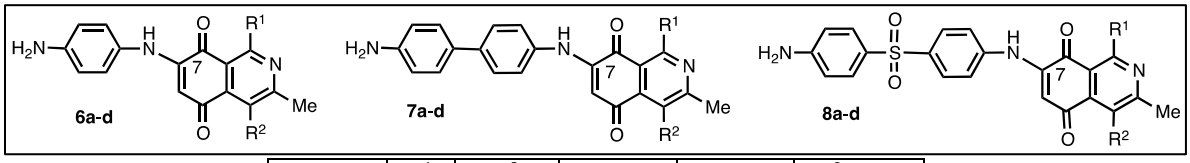
On the basis of the above results, the reactions of diamines 3-5 with quinones 2a-d were performed under the optimal experimental condition determined for the reaction of 2a with *p*-phenylenediamine 3 (Table 1, entry 4). The results summarized in Table 2 indicate that the reaction give rise the corresponding monoamination products 6a-d, 7a-d and 8a-d in moderate to good yields. The structures of the new compounds were established by IR, ^{1}H-, ^{13}C-NMR and high resolution mass spectra (HRMS). The location of the arylmino groups at C-7 in compounds 6-8 was established through HMBC experiments. The behavior of isoquinolinequinones 2a-d to undergo regiospecific substitution with the aryl diamines 3-5 has been previously observed in reactions of isoquinolinequinones with aryl monoamines and this was attributed to the electron-withdrawing effect of the heterocyclic nitrogen atom [19-21].

Electrochemical Results

Based on precedents on the influence of the donor-acceptor properties of phenylaminoquinones on the cytotoxic activities on cancer cells [15-19], the redox properties of aminoquinones 6a-d, 7a-d and 8a-d were evaluated by cyclic voltammetry at room temperature, using a platinum electrode and DMF/tetrabutylammonium perchlorate (0.1

M) [22,23]. The voltammograms were run in the potentials range 0.0-2.0 V, at a sweep rate of 50 mVs-1, as $E_{1/2} = (E_{pa}+E_{pb})/2$, where E_{pa} correspond to anodic and cathodic peak potentials, respectively. Two quasi-reversible waves were observed for compounds 6a-d, 7a-d and 8a-d in the negative region of the cyclic voltammograms, which proceeded in two one-electron diffusion stages (Table 2).

Table 2: Monoarylamination products from the reaction of diamines 3, 4, 5 with quinones 2a-d



Product	R^1	R^2	Time (h)	Yield%[a]	$-E^I_{1/2}$(mV)
6a	Me	CO_2Me	1.5	97	650
6b	H	CO_2Me	10	61	618
6c	Me	COMe	2	82	850
6d	H	COMe	3	77	770
7a	Me	CO_2Me	5	64	631
7b	H	CO_2Me	14	54	539
7c	Me	COMe	2.5	80	775
7d	H	COMe	16	68	768
8a	Me	CO_2Me	142	54	582
8b	H	CO_2Me	24	49	501
8c	Me	COMe	166	45	773
8d	H	COMe	24	60	765

[a]Isolated by column chromatography

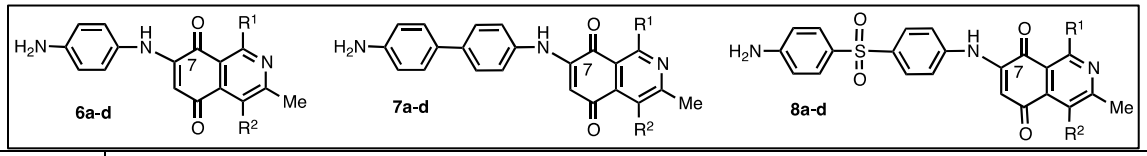
Inspection of the $-E^I_{1/2}$ values of quinones 6, 7, and 8 revealed that the arylamino substituents possess different electron-donor capabilities. In terms of the mean $E_I^{1/2}$ values ($ME^I_{1/2}$), it was observed that compounds 6 ($ME^I_{1/2}$ = -722 mV) appeared at a more negative value, followed by compounds 7 ($ME^I_{1/2}$ = -678 mV) and, compounds 8 ($ME^I_{1/2}$ = -655 mV), at less negative value. Therefore, we can conclude that the electron-donor effect of the aryl diamino substituents decrease in the following order: H_2N-Ph-NH> H_2N-Ph-Ph-NH>H_2N-PhSO_2PhNH. An interesting effect of the carbonyl substituent at the 4-position of the new compounds was observed. Comparison of the $E^I_{1/2}$ values of compounds 6a and 6b, containing the methoxycarbonyl group, to those of their corresponding acetyl analogues 6c and 6d indicated stronger donor-acceptor interactions (more negative $E^I_{1/2}$ values) for the acetyl than methoxycarbonyl derivatives. Similarly, the acetyl derivatives 7c, 7d and 8c, 8d displayed stronger donor-acceptor interactions compared to their corresponding methoxycarbonyl analogues 7a, 7b and 8a, 8b.

***In vitro* Antiproliferative Activity of Aryldiaminoisoquinolinequinones**

The arylaminoisoquinolinequinones 6a-d, 7a-d and 8a-d were evaluated for their *in vitro* antiproliferative activity against primary mouse embryo fibroblasts cell line MEF and three cancer cells lines: MDA-MB-21 human breast adenocarcinoma, B16-F10 murine metastatic melanoma and Caco-2 human colorectal adencarcinoma cells, in 72 h drugs exposure assays. The cytotoxic activity of the new compounds was measured using a conventional fluorescence assay (Cyquant direct Cell Proliferation assays) [24,25]. The antiproliferative activities of quinones 6a-d, 7a-d and 8a-d are expressed in terms of IC_{50} (µM) and collected in Table 3. Etoposide and taxol used clinically as anticancer agents were taken as positive controls.

The data in Table 3 showed moderate to high activities for the members of the new series. A significant number of members of the series such as 6b, 6c, 6d, 7b, 8a, 8b, 8c and 8d showed higher potencies and selectivities on cancer cells than etoposide, in terms of their mean IC_{50} values (0.53-8.69 µM) and selectivity index (SI: 1.11-2.27) than this cancer drug (mean IC_{50}: 10.35 µM; SI: 0.18). Indeed, compound 8b highlights within the series by its potency (mean IC_{50}: 0.71 µM) comparable to that of taxol (mean IC_{50}: 0.75 µM) but 5.5-fold safer than this cancer drug. Comparison of the IC_{50} values of compounds 6-8 with their corresponding half-wave potentials $E^I_{1/2}$ reveals no correlation between these parameters. Therefore, it seems probable that other biological action mechanism, in addition to a redox-cycling process, are involved in the antiproliferative activity. Further studies to shed some light on the antiproliferative activity mechanism of the aryladiaminoisoquinolinquinones are currently under study.

Table 3: Cytotoxic activity of 7-aryldiaminoisoquinolinequinones 6a-d, 7a-d and 8a-d



N°	IC_{50} ± SEM (μM)[a]							
	R^1	R^2	MEF[b]	MDA-MB-21[c]	B16-F10[d]	Caco-2[e]	Mean IC_{50}	SI[f]
6a	Me	CO_2Me	82.85 ± 0.35	> 350	68.89 ± 1.5	119.60 ± 4.59	NC[g]	NC[g]
6b	H	CO_2Me	2.49 ± 0.15	1.79 ± 0.12	1.45 ± 0.08	1.20 ± 0.04	1.48	1.68
6c	Me	COMe	7.76 ± 0.27	8.69 ± 0.56	4.58 ± 0.11	7.76 ± 0.26	7.01	1.11
6d	H	COMe	6.59 ± 0.16	5.04 ± 0.44	5.03 ± 0.13	5.51 ± 0.30	5.19	1.27
7a	Me	CO_2Me	54.83 ± 0.31	39.98 ± 6.97	76.32 ± 1.8	120.80 ± 7.26	79.03	0.69
7b	H	CO_2Me	8.58 ± 0.46	2.17 ± 0.17	3.64 ± 0.1	5.13 ± 0.16	3.65	2.36
7c	Me	COMe	118.90 ± 3.36	28.2 ± 1.11	72.52 ± 2.5	312.30 ± 14.9	137.67	0.86
7d	H	COMe	18.16 ± 0.07	35.13 ± 0.66	5.44 ± 0.28	1.43 ± 0.07	14	1.3
8a	Me	CO_2Me	4.82 ± 0.30	6.77 ± 0.66	2.53 ± 0.16	2.26 ± 0.15	3.85	1.25
8b	H	CO_2Me	1.97 ± 0.28	0.73 ± 0.06	0.53 ± 0.04	0.87 ± 0.05	0.71	2.77
8c	Me	COMe	4.29 ± 0.21	1.37 ± 0.11	3.52 ± 0.15	2.02 ± 0.06	2.3	1.87
8d	H	COMe	2.04 ± 0.09	1.38 ± 0.19	1.92 ± 0.03	0.91 ± 0.02	1.4	1.46
Taxol	-	-	0.35 ± 0.06	1.16 ± 0.11	0.35 ± 0.06	0.75 ± 0.04	0.75	0.47
Etoposide	-	-	1.87 ± 0.09	5.34 ± 0.12	1.87 ± 0.09	23.85 ± 0.15	10.35	0.18

[a]Data represent mean average values for six independent determinations. [b]Normal mouse embryo fibroblasts cell line. [c]Human breast adenocarcinoma cell line. [d]Murine metastatic melanoma cell line. [e]Human colorectal adenocarcinoma cell line; [f]Mean selectivity index= IC_{50} values for fibroblast cells/ IC_{50} values tumor for cells. [g]NC = not calculated because the IC_{50} value for breast adenocarcinoma cell line was higher than 350 μM

CONCLUSION

The aim of this work was to evaluate the nucleophilic reactivity of the aryl diamines: *p*-phenylenediamine, benzidine and dapsone with some isoquinolinequinone derivatives. Based on the experimental results, using *p*-phenylenediamine and isoquinolinequine 2a as model reaction, it was concluded that the monoamination reaction is the prefered course reaction between these reactants to produce the respective arylaminoisoquinolinequinone 6a. By using the optimal reaction conditions arising from the experiments, a new series of the monoamination products 6a-d; 7a-d and 8a-d in yields ranging from 45-97% was prepared. Based on half-wave potential values of the members of the series it was concluded that the electron-donor capacity of the arylamino groups decrease as follow: H_2N-Ph-NH> H_2N-Ph-Ph-NH>H_2N-PhSO_2PhNH. Antiproliferative evaluation of the members of the series against normal mouse embryo fibroblasts cell line MEF and three cancer cells lines: MDA-MB-21 human breast adenocarcinoma, B16-F10 Murine metastatic melanoma and Caco-2 Human intestinal carcinoma cells showed moderate to high potencies. The arylaminoisoquinolinequinone 8b, derived from dapsone and isoquinolinquinone 2b exhibit significant antiproliferative activity on cancer cells, with high potencies and selective index compared with the etoposide and taxol cancer drugs. Studies on synthesis of bivalent phenylaminoisoquinolinequinones by using symmetrical aryldiamines connected through aliphatic spacers are currently under study.

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