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Studies on quinones. Part 45: Novel 7-aminoisoquinoline-5,8-quinone derivatives with antitumor properties on cancer cell lines ☆

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ABSTRACT

A variety of 7-aminoisoquinoline-5,8-quinone derivatives were prepared from 2,5-dihydroxyacetophenone, methyl aminocrotonate, and the corresponding amines, through a highly efficient three-step sequence. The members of this series were tested on normal human fibroblasts and on a panel of three human cancer cell lines and their redox properties were determined by cyclic voltammetry in acetonitrile. Both the cytotoxicity and antitumor activity of 7-phenylaminoisoquinoline-5,8-quinone derivatives showed correlation with their half wave potentials and lipophilicities.

1. Introduction

Quinones are widespread in nature and are present in many drugs such as anthracyclines, daunorubicin, doxorubicin, mitomycin, mitoxantrones, and saintopin, which are used clinically in the therapy of solid cancers. The cytotoxic effects of these quinones are mainly due to the inhibition of DNA topoisomerase-II.[2,3]

The quinoid anticancer agents undergo enzymatic reduction via one or two electrons to give the corresponding semiquinone radical or hydroquinone. Under aerobic conditions the semiquinone radical anion can yield its extra electron to molecular oxygen to yield the parent quinone and superoxide radical anion. This reaction sequence initiated by bioreduction of the quinone followed by oxidation with dioxygen of the radical anion intermediate is known as redox-cycling, and it continues until the system becomes anaerobic. The hydroquinone formed via a two-electron reduction, depending upon its stability, can be excreted by the organism in a detoxification pathway or can undergo a comproportianation reaction with the parent quinone to yield the semiquinone radical anion. Both the semiquinone and the superoxide radical anion can generate the hydroxyl radical, which is the cause of DNA strand breaks.[4–7]

The molecular framework of several naturally occurring antitumoral agents contains an aminoquinonoid moiety as the key structural component, (e.g., streptonigrin **1**, mitomycin C **2**, cribrostratin 3 **3**, Fig. 1).[8,9] This structural array has stimulated the synthesis of novel lead compounds that exhibited significant cytotoxicity on human cancer cell lines.[10–13]

In a recent paper, we noticed that compounds **5** and **6**, prepared from substituted isoquinolinequinone **4** (Fig. 2), showed high antitumor activity against representative cell tumor lines (0.8–5.9 μM) compared to that of isoquinolinequinone precursor **4** (2.1–17.5 μM).[14]

These 2,5-diaza-anthraquinones displayed antitumor potencies comparable to that etoposide, an antineoplastic agent, evaluated under the same conditions. Apparently, the nitrogen substituent bonded to the quinone nucleus exerts influence on the redox properties of the quinone moiety improving the cytotoxic activity. Taking into account these observations and precedents on the influence of the electronic and lipophilic characteristics of the substituents in substituted 1,4-naphthoquinones on the biological activity,[15–18] we were interested in the synthesis and antitumor evaluation of a variety of 7-aminoisoquinoline-5,8-quinones having a range of electronic and lipophilic characteristics. Since the cytotoxic activity of quinoid compounds is related to the redox properties of the electroactive quinone moiety, the effects of nitrogen substituents on the isoquinolinquinone system could provide clues on the biological mechanism of their biological activity.

2. Chemistry

Isoquinolinequinone **4** was selected as a suitable precursor for the preparation of aminoisoquinoline-5,8-quinone derivatives due to its accessibility from commercially available starting

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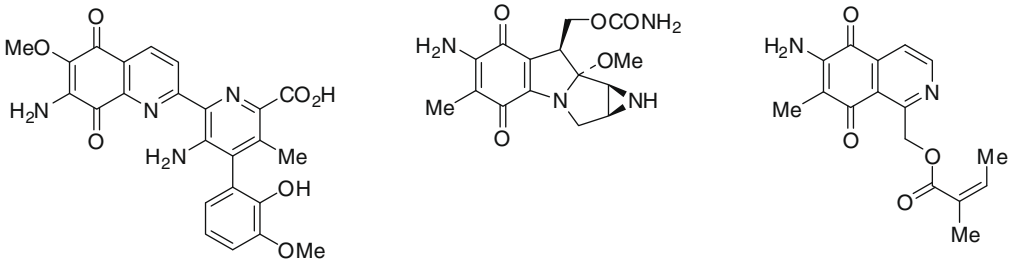


Figure 1. Structure of streptonigrin (**1**), mitomycin C (**2**) and cribrostatin 3 (**3**).

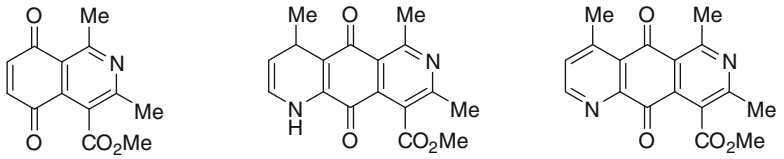


Figure 2. Structure of anti-tumoral diaza-anthraquinones **5** and **6** prepared from **4**.

products and to its high regioselectivity in substitution reactions with nucleophiles according to LUMO energies predicted by frontier molecular orbital (FMO) theory.[14] This compound was prepared in 74% yield from 2,5-dihydroxyacetophenone and methyl 3-aminocrotonate using our previously reported one pot procedure.[14] First we explored the reaction of isoquinolinquinone **4** with aniline. After considerable experimentation in different solvents (ethanol, dichloromethane and acetonitrile) and Lewis acid catalysts ($CeCl_3{\cdot}7H_2O$, $InBr_3$, $FeCl_3$), the reaction was performed by treating 1 equiv of **4** with 2 equiv of aniline, and 0.5 equiv of $CeCl_3{\cdot}7H_2O$ in ethanol for 4 h at room temperature. Under these conditions, anilinoquinone **7** was isolated in 93% yield (Scheme 1).

The structure of **7** was assigned on the basis of the HMQC/HMBC experiments. The HMBC spectrum of **7** shows $^3J_{C,H}$ and $^4J_{C,H}$ coupling of the C-8 carbon (δ 181.6 ppm) with the proton at C-6 (δ 6.36 ppm) and the protons of the methyl group at C-1 (δ 3.00 ppm), respectively.

It is noteworthy that no regioisomers were detected in the reaction assays of quinone **4** with aniline either in presence or absence of the $CeCl_3{\cdot}7H_2O$, thus indicating that the arylamination reaction is totally regiocontrolled. The control of the regioselectivity could be ascribed to the large difference between the LUMO coefficients of the C-6 and C-7 carbon atoms of quinone **4** (C-6 = 0.3142 and C-7 = 0.3949 eV),[14] which is probably enhanced by coordination of the catalyst with the nitrogen atom and/or the carbonyl oxygen at the 5-position of the isoquinoline system.[19,20]

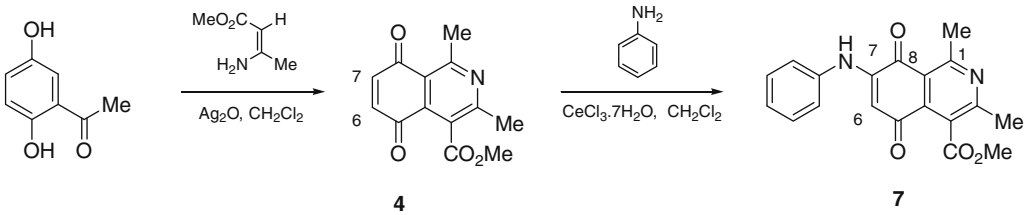
Isoquinolinequinone **4** was reacted with a variety of aryl- and alkylamines under the aforementioned conditions to yield the corresponding substitution products **8**–**17** in good to excellent yields. Aminoisoquinolinequinone **18** was prepared in 45% yield by reaction of **4** with sodium azide in acetic acid. Table 1 summarizes the results of the preparation of the amino isoquinolinequinone derivatives.

All new compounds were fully characterized on the basis of their ^1H, ^{13}C NMR spectra and high resolution mass spectra. Regarding the location of the amino group in products **8**–**18**, they were established by analysis of their corresponding HMBC spectra. In all cases long-range couplings between the carbon at C-8, the vinyl proton at C-6, and the protons of the methyl group at C-1 were observed.

3. Biological results and discussion

The newly synthesized 7-aminoisoquinolinequinone derivatives **7**–**18** were evaluated for in vitro anticancer activity against human normal cells: MRC-5 human lung fibroblasts and three human tumor cells: AGS gastric adenocarcinoma, SK-MES-1 lung cancer, and J82 bladder carcinoma, in 72 h drug exposure MTT assays. The cytotoxicity of the compounds was measured using a conventional microculture tetrazolium assay.[21] As indicated in Table 2, the tested compounds showed moderate to good activity in the in vitro antitumor screening expressed by the IC_{50} values.

The data of Table 2 indicate that substitution of one of the amino-protons in the parent compound **18** by phenyl- or 4-R-phenyl groups, as in **7–10**, increases the cytotoxic activity on the normal and almost all the cancer cell lines. On the other hand, the substi-



Scheme 1. Preparation of 7-anilinoisoquinolinequinone **7**.

Table 1
Preparation of 7-aminoisoquinolinequinone derivatives **7–18**

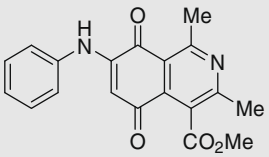
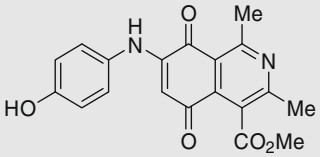
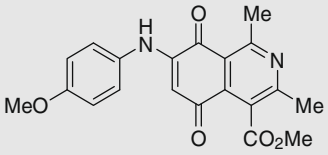
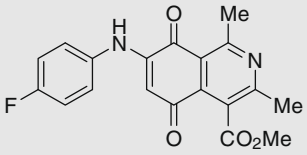
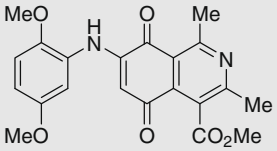
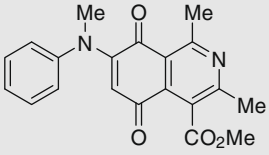
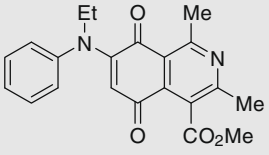
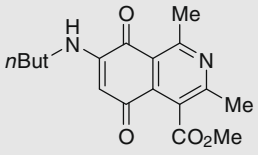
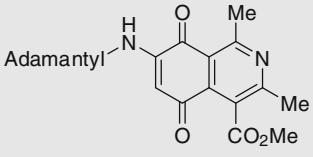
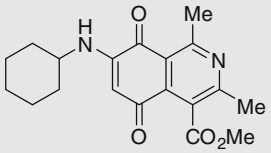
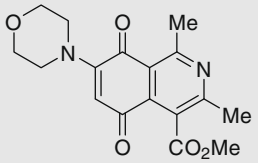
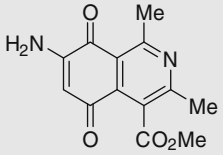
No.	Structure	Yield[a] (%)
7		93
8		91
9		89
10		84
11		90
12		92
13		30
14		83
15		6

Table 1 (*continued*)

No.	Structure	Yield[a] (%)
16		68
17		97
18		45

[a] Isolated by column chromatography.

tution of one of the amino-protons in **18** by alkyl groups, as in **14–17**, decreases the antitumor activity against all of the tested cancer cell lines.

Comparison of the IC_{50} values for phenylaminoquinone **7** and its *N*-alkyl derivatives **10** and **11** shows that substitution of the amino-proton in **7** by an alkyl group decreases the cytotoxic activity. On the other hand, comparison of the IC_{50} values of the arylaminoisoquinolinequinones **7–10** indicates that the substituents located on the 4-position of the arylamino group have a relatively significant influence on their antitumor activities.

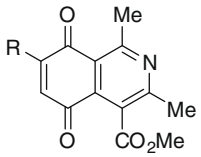
It is noteworthy that the introduction of methoxy groups into the 2,5-positions of **7**, as in arylaminoisoquinolinequinone **11**, induces a remarkable decreasing cytotoxic effect on the MRC-5 and AGS cell lines and a suppression of the antitumor activity on the SK-MES-1 and J82 cell lines. These findings, along with the observed antitumor activity of compound **9**, suggest that the cytotoxic activity of the arylaminoisoquinolinequinone chromophore depends on the location of methoxy substituents on the phenyl ring.

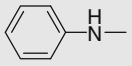
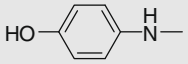
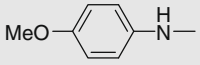
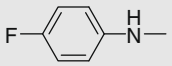
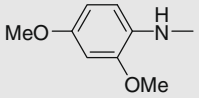
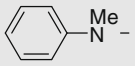
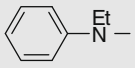
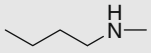
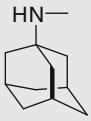
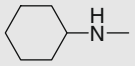
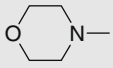
Replacement of the amino-proton in phenylaminoquinone **7** by an alkyl group, as in **12** and **13**, induces a slight modification of the antitumor activity in the case of a methyl group, but a significant decrease of the biological activity for the ethyl group. Apparently, the size of the alkyl group bonded to the nitrogen atom of the phenylamino group has an influence on the biological activity.

Comparison of the IC_{50} values for the aminoisoquinolinequinone derivatives **14–17** shows that the presence of an alkylamino group, as in **14–16**, is more relevant on the antitumor activity of the pharmacophore than that of an dialkylamino group as in **17**.

It is well-known that the cytostatic and antimicrobial activity of numerous quinones emerges from their ability to act as potent inhibitors of electron transport, as uncouplers of oxidative phosphorylation, as intercalating agents in the DNA double helix, as bioreductive alkylating agents of biomolecules, and as producers of reactive oxygen radicals, by redox cycling under aerobic conditions. In all these cases the mechanism of action in vivo requires bioreduction of the quinones as the first activating step.[22–24] Therefore, those molecular properties that provide proof of the reduction capability of quinonoid compounds could provide valuable information on the mechanism of biological activity and aid

Table 2
Cytotoxic activity, half wave potential ($E_{1/2}$), and lipophilicity (log*P*) of aminoisoquinolinequinone derivatives **7–18**



No.	R	MRC-5[b]	AGS[c]	IC_{50} ± SEM[a] (μM)				
				SK-MES-1[d]	J82[e]	$E^{I}_{1/2}$ (mV)	$E^{II}_{1/2}$ (mV)	Log*P*
7		5.6 ± 0.3	2.1 ± 0.2	4.2 ± 0.3	5.8 ± 0.2	592	1172	1.443
8		8.3 ± 0.4	1.3 ± 0.1	4.2 ± 0.2	4.4 ± 0.2	603	953	1.054
9		9.0 ± 0.4	2.3 ± 0.2	7.2 ± 0.4	7.4 ± 0.5	622	1220	1.317
10		3.7 ± 0.3	2.2 ± 0.1	3.9 ± 0.2	4.2 ± 0.3	646	1234	1.602
11		31.3 ± 1.9	19.9 ± 1.2	>100	>100	573	1164	1.190
12		8.8 ± 0.5	3.3 ± 0.2	8.7 ± 0.5	5.2 ± 0.2	588	1034	2.232
13		17.9 ± 0.8	8.1 ± 0.5	11.3 ± 0.7	14.8 ± 0.8	418	1074	2.570
14		13.3 ± 0.7	6.8 ± 0.3	7.8 ± 0.4	10.7 ± 0.6	687	1339	1.020
15		7.7 ± 0.5	4.6 ± 0.3	9.5 ± 0.5	12.1 ± 0.7	374	1225	1.607
16		16.6 ± 0.9	10.5 ± 0.6	18.1 ± 1.0	14.9 ± 0.9	722	1221	1.330
17		24.1 ± 1.1	9.6 ± 0.5	28.7 ± 1.6	30.8 ± 1.7	647	1245	−0.243
18	H_2N—	10.1 ± 0.7	4.2 ± 0.3	5.9 ± 0.4	5.9 ± 0.3	982	1278	−741
—	Etoposide	3.9 ± 0.2	0.36 ± 0.02	2.5 ± 0.2	2.8 ± 0.2	—	—	

[a] Data represent mean average values for six independent determinations.
[b] Human lung fibroblasts cells.
[c] Human gastric adenocarcinoma cell line.
[d] Human lung cancer cell line.
[e] Human bladder carcinoma cell line.

in designing new molecules with greater biological activity. Based on this general precedent and those reported on the effects of substituents on the redox properties of 2(4-*R*-phenylamino)-1,4-naphthoquinones,[14] a voltammetric study of compounds **7**–**18** was made in order to explain their biological activity through the electronic effect of the substituents on the aminoquinone chromophore.

Electrochemical results. The redox potentials of the compounds were measured by cyclic voltammetry in acetonitrile as a solvent at room temperature, using a platinum electrode and 0.1 M tetraethylamonium tetrafluoroborate as the supporting electrolyte. The voltammograms were run in the potential range 0.0–2.0 V versus non-aqueous Ag/Ag^+.

Figure 3 shows the typical redox behaviour of the aminoisoquinolinequinone derivatives, such as **11**, that proceeded in two one-electron diffusion stages: $AQ + e = AQ^{\cdot -}$ and $AQ^{\cdot -} + e = AQ^{2-}$.

The first wave is due to the addition of an electron to the quinone (AQ) to give a semiquinone radical anion ($AQ^{\cdot -}$) which by a subsequent addition of a second electron produces a hydroquinone dianion (AQ^{2-}). It is interesting to note that formation of the semiquinone radical anion and its reaction with dioxygen to yield the superoxide anion are crucial features required in the antitumoral mechanism of drugs by redox cycling.

The half-wave potential values, $E_{1/2}$, for both waves were evaluated from the voltammograms obtained at a sweep rate of 100 mV s^{-1}, as $E_{1/2} = (E_{pa} + E_{pc})/2$, where E_{pa} and E_{pc} correspond to anodic and cathodic peak potentials, respectively. Table 2 shows the half-wave potential values $E_{1/2}$ for both waves. The $E^{I}_{1/2}$ values for the first electron, corresponding to the formation of the semiquinone radical anion, are in the potential range −374 to −982 mV. For the second one-electron transfer that produces the corresponding dianion, the $E^{II}_{1/2}$ values fall in the range −603 to −1339 mV.

Since arylaminoisoquinolinequinone derivatives **7**–**13** showed higher antitumor activity than the alkyl derivatives **13**–**17**, we focused our attention on the electrochemical behavior of these compounds in order to explain their biological activity through the electronic effect of the substituents on the electroactive quinone moiety. The data for these compounds indicate that the introduction of electron-donor groups (F, OH and OMe) into the phenyl substituent of compound **7**, as in **8**–**10**, displaced the $E^{I}_{1/2}$ potentials for the first reduction wave towards a more negative region with respect to **7**. On the contrary, the insertion of two methoxy groups in **6** as well as in **9**, and the replacement of the amino hydrogen atom by alkyl substituents, as in **12** and **13**, produced a less negative $E^{I}_{1/2}$ for the corresponding AQ with respect to the parent compound **7**.

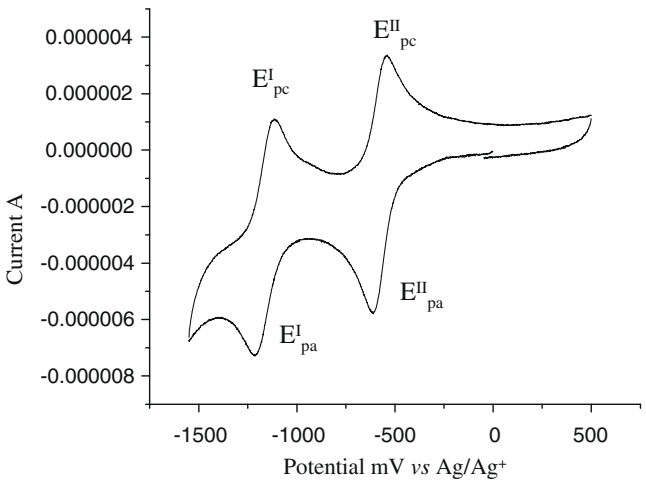


Figure 3. Cyclic voltammogram of compound **11** in 0.1 M Et_4NBF_4/acetonitrile obtained in Pt electrode, scan rate 100 mV/s. The cathodic (E^{I}pc, E^{II}pc) and anodic (E^{I}pa, E^{II}pa) peak potentials are indicated in the figure.

According to the results of the electrochemical study, the electron capability of the quinone nucleus in compounds **7**–**13**, evaluated as a function of their half-wave potentials for wave I ($E^{I}_{1/2}$), the electron donor effect of the substituents decreases in the following order: 4-F > 4-OH > 4-OMe > H > *N*-Me > 2,5-(OMe) > *N*-Et.

Linear regression analysis provided satisfactory relationships between the log IC_{50} values and $E^{I}_{1/2}$ for the AGS gastric adenocarcinoma and J82 bladder carcinoma cell lines (Eqs. 1 and 2). In these regression equations *n* is the number of the test compounds and *r* is the correlation coefficient. Compound **11** was not included in this analysis because its IC_{50} values in two of the tested cell lines lie at concentrations greater than 100 μM.

$$2 = 0.826 \log IC_{50} = 0.0029(E_{1/2}) + 2.1307 \quad n = 6\ r^2 = 0.826 \tag{1}$$

$$2 = 0.833 \log IC_{50} = 0.0022(E_{1/2}) + 2.0900 \quad n = 6\ r^2 = 0.833 \tag{2}$$

The relationship between log IC_{50} and $E^{I}_{1/2}$, which corresponds to a regression line, indicates that for the studied aminoquinones, the more negative the $E^{I}_{1/2}$ the stronger the anti-tumor promoting effect on the AGS and J82 cell lines. This behaviour agrees with reported precedents on the relationship between redox potentials and inhibitory effects of 2-aza-anthraquinones on Epstein-Barr virus early antigen (EBV-EA) activation.[25,26]

As lipophilicity is an important parameter affecting the biological activity of quinonoid compounds,[15,27] the log*P* descriptors of arylaminoisoquinolinquinone derivatives **7**–**13** were calculated using the AM1 semiempirical method. Apparent relationships between the values of log IC_{50} and log*P* were obtained for the AGS gastric adenocarcinoma and J82 bladder carcinoma cell lines according to regression Eqs. 3 and 4, respectively.

$$n = 6 \log IC_{50} = 0.5421 \log P - 0.3706 \quad n = 6\ r^2 = 0.711 \tag{3}$$

$$n = 6 \log IC_{50} = 0.4382(\log P)^2 - 1.0739 \log P + 1.3651$$
$$n = 6\ r^2 = 0.738 \tag{4}$$

4. Conclusion

The aim of this work was to test 7-aminoisoquinoline-5,8-quinone derivatives with substituents having a range of electronic and lipophilic properties, in order to help elucidate the mechanism of their antitumor action. We have developed a high yield synthesis of 7-arylamino- and 7-alkylaminoisoquinoline-5,8-quinones **7**–**17** using acid-induced substitution reactions of isoquinolinequinone **4** with alkyl- and arylamines. Compounds **7**–**17** and 7-aminoisoquinolinquinone **18**, prepared by addition of hydrazoic acid to quinone **4**, expressed in vitro cytotoxic activity against human lung fibroblasts (MRC-5), gastric adenocarcinoma (AGS), lung cancer (SK-MES-1), and bladder carcinoma (J82) cell lines. The analysis of the screening shows that replacement of the amino-proton of the parent compound 7-aminoisoquinoline-5,8-quinone **18** by aryl- or alkyl groups induces significant changes in the cytotoxic activity due to the nature of the substituents. The 7-phenylaminoisoquinolinquinones **7**–**10**, **12**, and **13** exhibited higher antitumoral activity than that of the 7-alkylaminoisoquinolinquinone **14**–**17**.

The electrochemical study of the electron capacity of the quinone nucleus in compounds **7**–**13**, evaluated as a function of their half wave potentials $E_{1/2}$, shows that the electron donor effect of

the substituents decrease in the following order: 4-F > 4-OH > 4-OMe > H > *N*-Me > 2,5-(OMe) > *N*-Et.

The QSAR analysis of the data revealed that for the evaluated phenylaminoisoquinolinquinones **7**–**10**, **12** and **13**, the first reduction potential $E_{1/2}$ is an important parameter determining the antitumoral activity on AGS gastric adenocarcinoma and J82 bladder carcinoma cell lines.

5. Experimental

5.1. Chemical synthesis

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. The IR spectra were recorded on an FT Bruker spectrophotometer using KBr disks, and the wave numbers are given in cm^{-1}. ^1H NMR spectra were run on Bruker AM-200 and AM-400 instruments in deuterochloroform ($CDCl_3$). Chemical shifts are expressed in ppm downfield relative to tetramethylsilane (TMS, δ scale), and the coupling constants (J) are reported in Hertz. ^{13}C NMR spectra were obtained in $CDCl_3$ at 50 and 100 MHz. 2D NMR techniques (COSY, HMBC) and DEPT were used for signal assignment. Chemical shifts are reported in δ ppm downfield fromTMS, and J-values are given in Hertz. HRMS were obtained on a Thermo Finnigan spectrometer, model MAT 95XP. Silica gel Merck 60 (70–230 mesh) was used for preparative column chromatography, and TLC aluminum foil 60F254 for analytical TLC. Acetonitrile anhydrous (99.8%) for electrochemical evaluations was obtained from Sigma–Aldrich.

5.2. General procedure for the synthesis of 7-aminoisoquinolinequinone derivatives 7–17

A suspension of quinone **4** (1 mmol), the required amine (2 mmol), $CeCl_3{\cdot}7H_2O$ (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 and water, the organic extract was washed with water (2×15 mL), dried over Na_2SO_4, and evaporated under reduced pressure. The residue was column chromatographed over silica gel (petroleum ether/ethyl acetate 90:10) to yield the corresponding aminoisoquinolinequinone derivative (Table 1).

5.2.1. Methyl 7-phenylamino-1,3-dimethyl-5,8-dioxo-5,8-dihydroisoquinoline-4-carboxylate (7)

Prepared from **4** and aniline (3 h, 93% yield): red solid, mp 202–203.5 °C; IR: ν_{max} 1736 (C=O ester), 1627 and 1625 (C=O quinone); ^1H NMR (400 MHz): δ 2.60 (s, 3H, 3-Me), 3.20 (s, 3H, 1-Me), 4.00 (s, 3H, CO_2Me), 6.41 (s, 1H, 6-H), 7.30 (m, 3H, arom.), 7.50 (m, 2H, arom.), 7.70 (s, 1H, NH); ^{13}C NMR (100 MHz): δ 23.0, 26.2, 53.1, 102.4, 119.9, 122.9 (2C), 125.2, 126.3 (2C), 129.8, 136.9, 137.8, 145.5, 160.2, 161.5, 169.1, 181.5, 181.6.

HRMS (M+): *m/z* calcd for $C_{19}H_{16}N_2O_4$: 336.11101; found: 336.11093.

5.2.2. Methyl 7-(4-hydroxyphenyl)amino-1,3-dimethyl-5,8-dioxo-5,8-dihydroisoquinoline-4-carboxylate (8)

Prepared from **4** and 4-aminophenol (5 h, 91% yield): purple solid, mp 233–235 °C; IR: ν_{max} 3339 (NH), 3300 OH), 1732 (C=O ester), 1678 and1600, (C=O quinone); ^1H NMR (400 MHz): δ 2.57 (s, 3H, 3-Me), 3.01 (s, 3H, 1-Me), 3.95 (s, 3H, CO_2Me), 6.03 (s, 1H, CH), 6.88 (d, 2H, J = 8.5 Hz, 3′- and 5′-H), 7.09 (d, 2H, J = 8.5 Hz, 2′- and 6′-H), 8.56 (s, 1H, NH); 9.22 (s, 1H, OH); ^{13}C NMR (100 MHz): δ 22.50, 25.73, 52.46, 100.55, 116.09 (2C), 119.99, 120.1, 124.66, 125.33 (C2), 128.12, 137.90, 147.21, 155.90, 160.18, 168.88, 180.34, 181.58.

HRMS (M+): *m/z* calcd for $C_{19}H_{16}N_2O_5$: 352.10592; found: 352.10593.

5.2.3. Methyl 7-(4-methoxyphenyl)amino-1,3-dimethyl-5,8-dioxo-5,8-dihydroisoquinoline-4-carboxylate (9)

Prepared from **4** and *p*-anisidine (4 h, 89% yield): violet solid, mp 166–167 °C; IR: ν_{max} 3436 NH, 1733 (C=O ester), 1676 and1604 (C=O quinone); ^1H NMR (400 MHz): δ 2.64 (s, 3H, 3-Me), 3.01 (s, 3H, 1-Me), 3.86 (s, 3H, OMe), 4.03 (s, 3H, CO_2Me), 6.19 (s, 1H, 6-H), 6.99 (d, 2H, 2′- and 6′-H), 7.18 (d, 2H, 3′- and 5′-H), 7.64 (s, 1H, NH) ^{13}C NMR (100 MHz): δ 22.93, 26.08, 52.97, 55.58, 101.62, 115.04 (2C), 119.97, 124.9 (2C), 125.17, 129.44, 137.99, 146.29, 158.09, 160.86, 161.21, 169.20, 181.17, 181.72.

HRMS (M+): *m/z* calcd for $C_{20}H_{18}N_2O_5$: 366.12157; found:366.12149.

5.2.4. Methyl 7-(4-fluorophenyl)amino-1,3-dimethyl-5,8-dioxo-5,8-dihydroisoquinoline-4-carboxylate (10)

Prepared from **4** and 4-fluoroaniline (24 h, 84% yield): orange solid, mp 161–163 °C; IR: ν_{max} 1720 (C=O ester), 1672 and 1614 (C=O quinone); ^1H NMR (400 MHz): δ 2.61 (s, 3H, 3-Me), δ 2.97 (s, 3H, 1-Me), 4.00 (s, 3H, CO_2Me), 6.17 (s, 1H, CH), 7.12 (m, 2H, arom.), 7.24 (m, 2H, arom.), 7.65 (s, 1H, NH); ^{13}C NMR (100 MHz): δ 23.11, 26.24, 53.16, 102.26, 116.84, 117.06, 120.00, 125.31, 125.51, 132.93, 132.96, 137.83, 146.17, 159.61, 161.13, 162.07, 169.22, 181.56, 181.62.

HRMS (M+): *m/z* calcd for $C_{19}H_{15}FN_2O_4$: 354.10156; found: 354.10281.

5.2.5. Methyl 7-(2,5-dimethoxyphenyl)amino-1,3-dimethyl-5,8-dioxo-5,8-dihydroisoquinoline-4-carboxylate (11)

Prepared from **4** and 2,5-dimethoxyaniline (19 h, 90% yield): purple solid, mp 205.5–207 °C; IR: ν_{max} 3454 (NH), 1725 (C=O ester), 1678 and 1633 (C=O quinone); ^1H NMR (400 MHz): δ 2.58 (s, 3H, 3-Me), 2.96 (s, 3H, 1-Me), 3.74 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 3.98 (s, 3H, CO_2Me), 6.43 (s, 1H, 6-H), 6.66 (dd, 1H, J = 8.5; 3.0 Hz, 4′-H), δ 6.84 (d, 1H, J = 3.0 Hz, 6′-H), δ 6.91 (d, 1H, J_{orto} = 8,5 Hz, 3′-H), 8.12 (s, 1H, NH); ^{13}C NMR (100 MHz): δ 22.67, 26.11, 53.00, 55.89, 56.25, 102.91, 107.95, 110.07, 111.94, 119.99, 125.07, 126.96, 137.77, 144.21, 145.58, 153.78, 160.97, 161.10, 169.18, 181.48, 181.59.

HRMS (M+): *m/z* calcd for $C_{21}H_{20}N_2O_6$: 396.13214; found: 396.13377.

5.2.6. Methyl 7-(methylphenylamino)-1,3-dimethyl-5,8-dioxo-5,8-dihydroisoquinoline-4-carboxylate (12)

Prepared from **4** and *N*-methylaniline (9:25 h, 92% yield): dark orange oil; IR (neat) ν_{max}: 1735 (C=O ester), 1634 and 1633 (C=O quinone); ^1H NMR (400 MHz): δ 2.55 (s, 3H, 3-Me), 2.65 (s, 3H, 1-Me), 3.39 (s, 3H, NMe), 3.97 (s, 3H, CO_2Me), 5.97 (s, 1H, 6-H), 7.00–7.35 (m, 5H, arom.); ^{13}C NMR (100 MHz): δ 22.67, 23.90, 24.89, 42.76, 53.00, 109.03, 122.60, 124.31, 125.19 (2C), 126.86, 129.77 (2C), 137.13, 147.10, 154.07, 159.76, 169.16, 181.01, 182.92.

HRMS (M+): *m/z* calcd for $C_{20}H_{18}N_2O_4$: 350.12666; found: 350.12607.

5.2.7. Methyl 7-(ethylphenylamino)-1,3-dimethyl-5,8-dioxo-5,8-dihydroisoquinoline-4-carboxylate (13)

Prepared from **4** and *N*-ethylaniline (47 h, 30% yield): dark orange solid, mp 124–125.5; IR: ν_{max} 1731 (CO_2Me), 1681 and 1627 (C=O); ^1H NMR (400 MHz): δ 1.21 (t, 3H, J = 5.0 Hz, Me), 2.58 (s, 3H, 3-Me), 2.69 (s, 3H, 1-Me), 3.87 (q, 2H, J = 5.0 Hz, CH_2), 4.00 (s, 3H, CO_2Me), 5.94 (s, 1H, 6-H), 7.10–7.41 (m, 5H, arom.).^{13}C NMR (100 MHz): δ 12.20, 14.22, 22.68, 24.83,

29.71, 49.56, 52.97, 108.32, 122.81, 124.23, 126.08, 127.11, 129.80, 137.16, 145.12, 153.75, 159.56, 159.64, 169.20, 180.93, 180.43.

HRMS (M+): *m/z* calcd for $C_{21}H_{20}N_2O_4$: 364.14227; found: 364.14217.

5.2.8. Methyl 7-(*n*-butylamino)-1,3-dimethyl-5,8-dioxo-5,8-dihydroisoquinoline-4-carboxylate (14)

Prepared from **4** and *n*-butylamine (2:20 h, 83% yield): orange solid, mp 97–99 °C; IR: ν_{max} 3378 (NH), 1735 (CO_2Me), 1677 and 1610 (C=O); ^{1}H NMR (400 MHz): δ 0.94 (t, 3H, J = 7.0 Hz, Me), 1.40 (m, 2H, CH_2), 1.62 (m, 2H, CH_2), 2.55 (s, 3H, 3-Me), 2.88 (s, 3H, 1-Me), 3.14 (t, 2H, J = 7.0 Hz, CH_2), 3.97 (s, 3H, CO_2Me), 5.66 (s, 1H, NH), 6.09 (s, 1H, 6-H); ^{13}C NMR (100 MHz): δ 14.11, 20.14, 22.83, 25.91, 30.15, 42.38, 52.87, 100.00, 120.05, 125.28, 138.35, 148.44, 160.63, 160.94, 169.35, 180.09, 181.41.

HRMS (M+): *m/z* calcd for $C_{17}H_{20}N_2O_4$: 316.14231; found: 316.14207.

5.2.9. Methyl 7-(1-adamantylamino)-1,3-dimethyl-5,8-dioxo-5,8-dihydroisoquinoline-4-carboxylate (15)

Prepared from **4** and 1-adamantylamine (7 d, 6% yield): orange oil; IR (neat): ν_{max}: 3388 (NH), 1735 (CO_2Me), 1634 and 1676 (C=O quinone); ^{1}H NMR (400 MHz): δ *2.14–1.23* (m, 15H, adamantyl), 2.60 (s, 3H, 3-Me), 2.95 (s, 3H, 1-Me), 4.00 (s, 3H, CO_2Me), 6.01 (s, H, 6-H), 6.93 (s, H, NH).

HRMS (M+): *m/z* calcd for $C_{17}H_{20}N_2O_4$: 394.18927; found: 394.18903.

5.2.10. Methyl 7-cyclohexylamino-1,3-dimethyl-5,8-dioxo-5,8-dihydroisoquinoline-4-carboxylate (16)

Prepared from **4** and cyclohexylamine (3 h, 68% yield): orange oil; IR: ν_{max} 3388 (NH), 1735 (CO_2Me), 1634 and 1676 (C=O); ^{1}H NMR (400 MHz): δ 1.40 (m, 6H, 3 × CH_2), 1.66 (m, 1H, CH), 1.78 (m, 2H, CH_2), 2.01 (m, 2H, CH_2), 2.59 (s, 3H, 3-Me), 2.93 (s, 3H, 1-Me), 4.00 (s, 3H, CO_2Me), 5.73 (s, 1H, 6-H), 6.05 (s, 1H, NH); ^{13}C NMR (100 MHz): δ 23.03, 24.60 (2C), 25.55 (2C), 26.15, 31.94, 51.45, 53.08, 100.07, 120.32, 125.46, 138.57, 147.35, 160.80, 161.16, 169.54, 180.29, 181.85.

HRMS (M+): *m/z* calcd for $C_{19}H_{22}N_2O_4$: 342.15796; found: 342.15808.

5.2.11. Methyl 7-[4-morpholino)-1,3-dimethyl-5,8-dioxo-5,8-dihydroisoquinoline-4-carboxylate (17)

Prepared from **4** and morpholine (2:50 h, 97% yield): solid, mp 191–193 °C; IR: ν_{max} 1735 (CO_2Me),1633 and 1681 (C=O quinone), ^{1}H NMR (400 MHz): δ 2.58 (s, 3H, 3-Me), 2.87 (s, 3H, 1-Me), 3.52 (t, 4H, J = 5 Hz, 2- and 6-H′), 3.87 (t, 4H, J = 5 Hz, 3- and 5-H′), 4.00 (s, 3H, CO_2Me), 5.93 (s, 1H, 6-H). ^{13}C NMR (100 MHz): δ 22.87, 25.49, 49.19 (2C), 53.15, 66.55 (2C), 108.69, 122.77, 124.46, 136.92, 155.01, 159.99, 160.20, 169.20, 181.26, 183.41.

HRMS (M+): *m/z* calcd for $C_{17}H_{18}N_2O_5$: 330.12158; found: 330.12228.

5.2.12. Methyl 7-amino-1,3-dimethyl-5,8-dioxo-5,8-dihydroisoquinoline-4-carboxylate (18)

A solution of sodium azide (1.2 mmol) in water (5 mL) was added to a hot solution (~40 °C) of **4** (1 mmol) in glacial acetic acid (10 mL) and the solution was stirred for 2 d to rt and then poured on water. The resulting solid was filtered and purified by silica gel column chromatography (petroleum ether/AcOEt 80:20) to give **18** (140 mg, 45%) as a red solid, mp 213–214; IR: ν_{max} 3427 (NH_2), 1735 (CO_2Me), 1682 and 1629 (C=O), ^{1}H NMR (400 MHz): δ 2.57 (s, 3H, 3-Me), 2.94 (s, 3H, 1-Me), 4.01 (s, 3H, CO_2Me), 6.00 (s, 1H, 6-H), 6.37 (br s, 2H, NH_2). ^{13}C NMR (100 MHz): δ 22.71, 25.79, 52.77, 102.89, 120.05, 125.03, 138.18, 150.04, 160.32, 160.51, 169.31, 180.72, 181.66.

HRMS (M+): *m/z* calcd for $C_{13}H_{12}N_2O_4$: 260.07971; found: 260.07978.

5.3. Anticancer assay[21]

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% assessed by trypan blue exclusion test. Cells were sub-cultured once a week and medium was changed every two days. Cells were grown in the following media: MRC-5, SK-MES-1, and J82 in MEM, and AGS cells in Ham F-12. The MEM medium contained 2 mM L-glutamine, 1 mM sodium pyruvate, and 1.5 g/L sodium hydogencarbonate. Ham F-12 was supplemented with 2 mM L-glutamine and 1.5 g/L sodium hydrogencarbonate. 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, and finally the MTT reduction assay was carried out. The final concentration of MTT was 1 mg/mL. The compounds were dissolved in DMSO (1% final concentration) and complete medium. Untreated cells (medium containing 1% DMSO) were used as controls. Each experiment was carried out in sextuplicate.

5.4. Electrochemical measurements[28]

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

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