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Studies on quinones. Part 41: Synthesis and cytotoxicity of isoquinoline-containing polycyclic quinones☆

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Abstract—In the search for new potentially anticancer drugs, isoquinolinequinone-containing polycyclic compounds have been designed and synthesized through highly regiocontrolled cycloaddition reactions of methyl 1,3-dimethyl-5,8-dioxo-5,8-dihydroisoquinoline-4-carboxylate with polarized 1,3-dienes and a thiazole-*o*-quinodimethane. The new *N*-heterocyclic quinones were tested on normal human fibroblasts and four distinct human cancer cell lines. Two of the evaluated compounds displayed significant in vitro activity (IC_{50}: 0.44–5.9 μM) comparable to that of the reference drug etoposide.

1. Introduction

The quinones occupy an important place among the different classes of antitumor agents. The biological processes involved with the antitumoral activity of quinones are DNA intercalation, bioreductive alkylation of biomolecules, and generation of oxy radicals through redox cycling.[2–6] The aza- and diaza-anthraquinones represent an important class of antitumor agents that exhibit promising in vitro and in vivo activity on several tumor cell lines.[7–11] The antitumor activities of these agents seem to be mediated by DNA intercalation and redox cycling processes which are improved by the basic and electron-withdrawing properties of the *N*-heterocyclic ring.[10]

The Diels–Alder reaction is a well-established method to provide access to aza- and diaza-quinones;[9–15] however, the application of this synthetic strategy to construct 2- and 1,6-diaza-9,10-anthraquinones has limitations, mainly due to the existing synthetic methods for isoquinolinequinones and to the moderate regioselectivity of the cycloaddition.[16,17] As part of our research program aimed at the synthesis of biologically active quinones,[18–22] we became interested in the synthesis and antitumor evaluation of carbo- and heterocyclic fused isoquinolinequinones through regiocontrolled Diels–Alder reactions of functionalized isoquinolinequinones. We now wish to report our initial results on highly regiocontrolled cycloadditions of isoquinolinequinone **3** with polarized 1,3-dienes and a thiazole-*o*-quinodimethane. We also report the in vitro evaluation of some of the new fused isoquinolinequinones against normal fibroblast and four tumor cell lines.

2. Results and discussion

2.1. Chemistry

Substituted isoquinolinequinone **3** was selected as a suitable precursor for the preparation of fused isoquinolinequinones due to the following facts: (a) accessibility from commercially available starting products, (b)

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potentially high regioselectivity in cycloaddition reactions with polarized 1,3-dienes according to predictions based on frontier molecular orbital (FMO) theory,[23] and (c) possibility of introducing further functionalization through the substituents of the heterocyclic ring.

Quinone **3** was prepared using a similar procedure to that reported by Allen and Weiss[24] for the ethyl analogue. Reaction of 2-acetyl-l,4-benzoquinone **1** with methyl 3-aminocrotonate in dichloromethane at room temperature afforded dihydroxyisoquinoline **2** in excellent yield (97%). We attempted to prepare quinone **3** by oxidation of **2** with ferric chloride in methanol according to the method reported for the ethyl analogue;[24] however, the treatment provided **3** in poor yield (26%). Better conversion of **2** to **3** was achieved by using manganese dioxide in dichloromethane,[25] producing quinone **3** in 76% yield (Scheme 1). Interestingly, quinone **3** was prepared in 74% yield in a single operation from 2,5-dihydroxyacetophenone, 1 equiv methyl 3-aminocrotonate, and 4 equiv of silver(II) oxide.

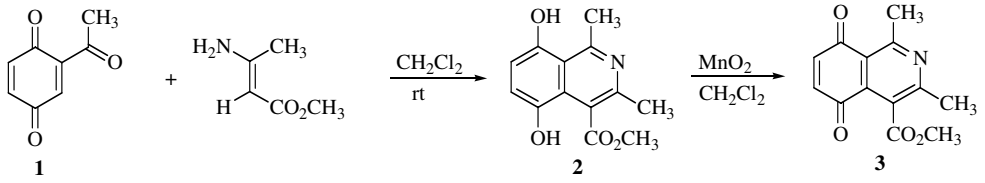
The Diels–Alder reaction of quinone **3** with 1-(*E*)-trimethylsilyloxy-1,3-butadiene led to an 8:1 mixture of cycloadducts **4** + **5** (evaluated by ^{1}H NMR). The major regioisomer was purified by column chromatography and characterized as **4** according to their ^{1}H- and ^{13}C NMR data.

Assignment of the regiochemistry of cycloadduct **4** was established by 2D-NMR experiments (HMBC, 400 MHz) that displayed $^3J_{C,H}$ and $^4J_{C,H}$ couplings for the carbonyl at C-10 (δ 196.3) with the protons at C-9 (δ 2.10; 3.10) and the protons of the methyl group at C-1 (δ 2.88), respectively.

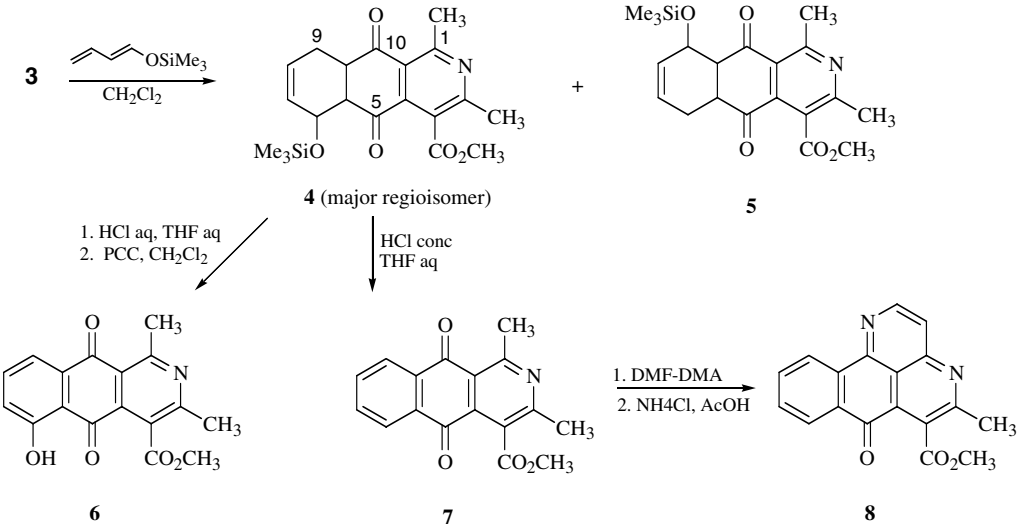
Adduct **4** was then submitted to aromatization. Hydrolysis of the silyloxy group of **4** with 5% hydrochloric acid at room temperature, followed by oxidation of the alcohol intermediate with pyridinium chlorochromate, afforded hydroxybenzo[*g*]isoquinolinequinone **6** in 94% total yield. On the other hand, the reaction of adduct **4** with concentrated hydrochloric acid at room temperature provided benzo[*g*]isoquinolinequinone **7** in 70% yield (Scheme 2).

The presence of the methyl group at C-1 in **7** allowed its conversion to naphthonaphthyridine **8** by using the one-pot annelation procedure reported by Bracher.[26] Several attempts were made to induce heterocyclization of **7** with dimethylformamide diethyl acetal (DMF-DEA), but in all these trials only complex reaction mixtures were obtained. Nevertheless, under the annelation conditions reported by Jackson et al.,[27] compound **8** was detected (^{1}H NMR) and isolated, albeit in poor yield (20%), from a complex reaction mixture.

In order to study the synthesis of 1,6-diaza-anthraquinones from isoquinolinequinone **3**, the following azadienes: methacrolein *N*,*N*-dimethylhydrazone **9**, crotonaldehyde *N*,*N*-dimethylhydrazone **10**, and 1-cyclohexencarbaldehyde *N*,*N*-dimethylhydrazone **11**



Scheme 1. Synthesis of isoquinoline-5,8-quinone **3**.



Scheme 2. Synthesis of heterocyclic quinones **6**–**8** via Diels–Alder reactions from **3**.

were prepared according to previously reported procedures.[28,29] The reaction of **3** with azadienes **9**–**11** was carried out at room temperature in acetonitrile containing acetic anhydride for trapping the dimethylamine which is usually formed in these cycloaddition reactions by elimination from the initial cycloadducts.

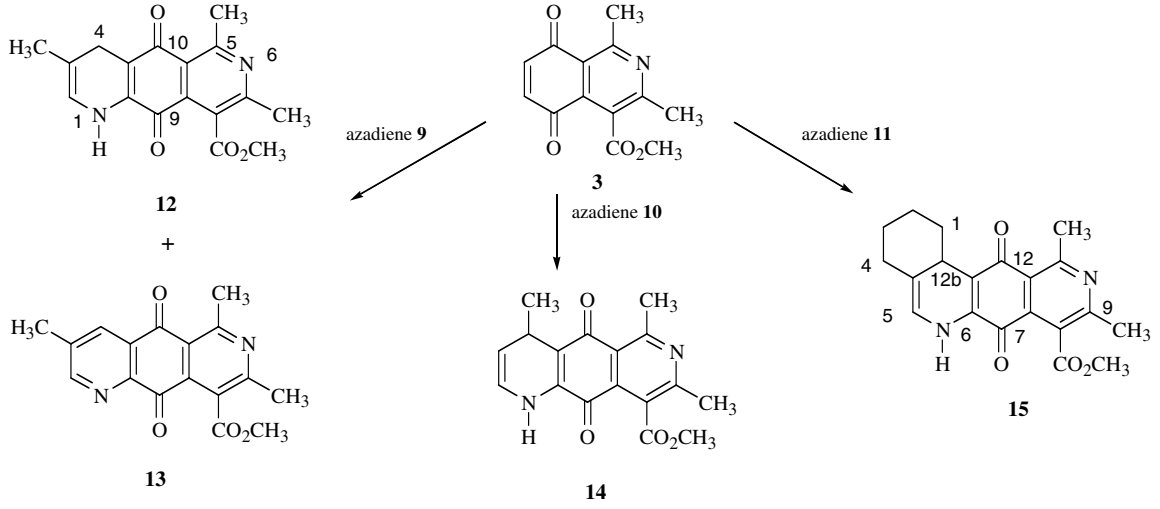
The reaction of **3** with diene **9** afforded diaza-anthraquinones **12** (37%) and **13** (36%). In the case of the reaction of **3** with azadienes **10** and **11**, diaza-anthraquinones **14** and **15** were isolated in 95% and 70% yield, respectively (Scheme 2). It is noteworthy that no regioisomers were detected in the reactions of quinone **3** with azadienes **9**–**11**, indicating that these cycloadditions are highly regiocontrolled. It should be pointed out that compounds **12**, **14**, and **15** were quite stable on standing on air, but they underwent air oxidation on the thin layer chromatography support (Scheme 3).

The regiochemistry of compounds **12**, **14**, and **15** was established by analysis of their HMBC spectra. In compounds **12** and **14**, long-range couplings between the carbon at C-10 and the protons of the methyl group at C-5 and the protons at C-4 were detected. Concerning compound **15**, correlations between the carbon at C-12 (δ 183.5) and the protons at 12b (^3J; δ 2.97) and in the methyl group at C-11 (^4J; δ 3.67) were observed.

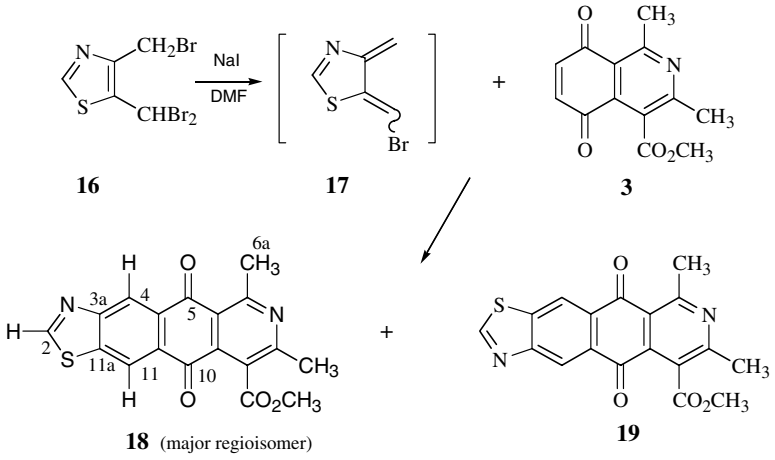
We studied the reactivity of quinone **3** with *o*-quinodimethane **17** in order to obtain hybrid compounds containing the biologically relevant isoquinolinequinone and benzothiazole moieties.[19] Treatment of 4-(bromomethyl)-5-(dibromomethyl)thiazole **16** with sodium iodide in DMF afforded 4-methylene-5-(bromomethylene)-4,5-dihydrothiazole **17**[19] which was trapped in situ with dienophile **3**.

The reaction yielded a 3:1 mixture of the tetracyclic quinones **18** + **19**, with **18** as the major regioisomer. We were unable to separate regioisomers **18** and **19** by flash chromatography and preparative TLC (Scheme 4).

The structure of regioisomers was determined by analysis of the ^{1}H- and ^{13}C NMR spectra of the mixture



Scheme 3. Synthesis of diaza-anthraquinones **12**–**15** via Diels–Alder reactions from **3**.



Scheme 4. Formation of heterocycles **18** and **19** through cycloaddition reaction of **3** with *o*-quinodimethane **17**.

of **18** + **19**. The HMBC experiments indicate that in the major regioisomer **18** the H-2 proton of the benzothiazole ring (δ 9.32) has two ^3J couplings with C-3a (δ 157.6) and C-11a (δ 140.0), with the former appearing as a doublet typical of a cross peak through the nitrogen atom of the thiazole ring (^3J C-3a/H-2 = 16 Hz), while the latter shows an unresolved singlet indicating <5 Hz for ^3J C-11a/H-2. The ^3J coupling of C-11a (δ 140.0) with H-4 (δ 9.01) and the ^3J and ^4J couplings of C-5 (δ 183.3) with H-4 (δ 9.01) and the H-6a protons (δ 3.16), established the relative location of the heterocyclic rings on the 1,4-naphthoquinone nucleus of **18**.

The cycloaddition reactions described here were analyzed in terms of frontier molecular orbital (FMO) theory. Previous work on the regiochemistry of Diels–Alder reactions with 1-azadienes **9**–**11**[28] and 1-(*E*)-trimethylsilyloxybutadiene[30] showed that the largest HOMO eigenvector coefficients are located at the 4-position of the 1,3-diene system. Theoretical calculations of the LUMO eigenvector coefficients of dienophile **3** showed that the largest coefficient is located at the 7-position (0.3949 for C-7 and 0.3142 for C-6).[31] This theoretical prediction agrees with the empirical results where the Diels–Alder adducts, precursors of compounds **5** and **12**–**15**, were formed through the more favorable FMO interactions.

It has been reported that cycloaddition of azadiene **10** with the proper isoquinoline-5,8-quinone afforded a mixture of the two possible regioisomers with rather moderate regioselectivity.[16] More recently, Brahic et al.[17] confirmed the regiochemistry of this cycloaddition which is in agreement with that predicted by FMO theory. Control of the regioselectivity was ascribed to the major LUMO coefficient at the 7-position (0.3233) compared with that at the 6-position (0.3159). Based on these precedents, the high regioselectivity of the cycloaddition of isoquinolinequinone **3** with azadienes **9**–**11** may be attributed to the large difference between the LUMO coefficient of quinone **3** (0.3949 − 0.3142 = 0.807)[31] compared with that of the isoquinolinequinone proper (0.3233 − 0.3159 = 0.0740). The significant difference between the LUMO eigenvector coefficients of the carbon atoms of the quinone double bond can be ascribed to the electron-withdrawing effect of the CO_2Me group at the 4-position in quinone **3**.

Regarding the regiochemistry of the cycloaddition of quinone **3** with *o*-quinodimethane, it was the opposite of that predicted by FMO theory. In fact, calculations of the *o*-quinodimethane **17**[19] indicate that the largest HOMO eigenvector coefficient is located at the carbon bearing the bromine atom (0.441 for =CHBr and 0.354 for $=CH_2$), but the major regioisomer **18** was generated via the less favorable cycloadduct. It is interesting to note that the reaction of *o*-quinodimethane **17** with benzofuran- and benzothiophenequinones proceeds with low regioselectivity.[19] These facts are probably related to the biradicaloid character[32,33] of the thermally generated *o*-quinodimethane **15**.

2.2. Biology

The in vitro cytotoxic evaluation of the tested isoquinoline derivatives **2**, **3**, **6**, **7**, and **12**–**15** against normal human MRC-5 lung fibroblasts and human AGS gastric adenocarcinoma cell lines, HL-60 leukemia cells, SK-MES-1lung cancer cells, and J82 bladder carcinoma cells is shown in Table 1, compared to that of etoposide as the reference drug. The results are expressed as IC_{50}-values, that is, as the micromolar concentration of a compound that achieves 50% cellular growth reduction after 72 h of drug exposure.

The results for the redox couple **2/3** show similar cytotoxic activity against the four tested cancer cell lines, while hydroquinone form **2** is approximately four times more active against normal fibroblasts than quinone **3**.

Comparison of the cytotoxic activity of the fused isoquinolinequinones **6**, **7**, and **12**–**15** shows that the members containing a pyridine or dihydropyridine ring fused to the quinone system **12**–**15** (entries 5–8) have higher cytotoxic activity than that shown by the carbocyclic analogues **6** and **7** (entries 3 and 4).

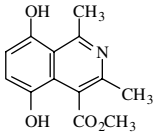
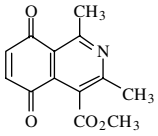
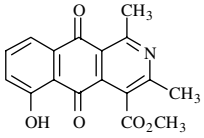
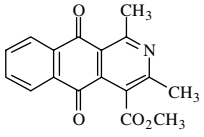
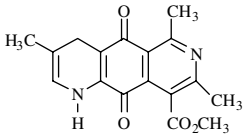
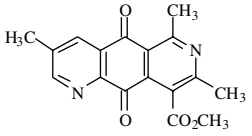
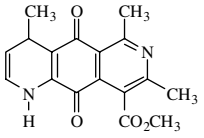
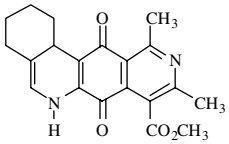
It should be noted that within the group of *N*-heterocyclic quinones, **12**–**15**, compounds **12** and **13** (entries 6 and 7) exhibited the highest toxicity against all the cell lines, with IC_{50}-values comparable to those shown by the reference drug etoposide. Although the number of 1,6-diaza-anthraquinones tested was limited, it appears from this study that the cytotoxicity potency depends on the location of the alkyl substituents in either the C-3 or C-4 positions (entries 5 and 7).

The pronounced cytotoxic effects of the 1,6-diaza-anthraquinones **12**–**15** with respect to its carbocyclic analogues can be attributed in part to the basic and electron-withdrawing effect of the *N*-heterocyclic rings, which improve biomolecular interactions and redox cycling processes.

3. Conclusions

In summary, we have developed the synthesis of a variety of polycyclic quinones by means of highly regiocontrolled cycloaddition reactions of isoquinolinequinone **3** with carbo- and aza-1,3-dienes, and *o*-quinodimethane **17**. Cycloaddition of **3** with the polarized diene 1-(*E*)-trimethylsilyloxybutadiene proceeds with high regiocontrol, in agreement with the prediction of FMO theory, to yield benzo[*g*]isoquinoline **4**. Cycloaddition of **3** with 1-azadienes **9**–**11** provides regiospecific access to 1,6-diaza-anthra-5,10-quinones **12**–**14** and pyrido[3,4-*b*]phenanthridine **15**, and the observed regiochemistries agree with those predicted by FMO theory. High regiocontrolled formation of thiadiaza-cyclopenta[*b*]anthraquinones **18** + **19** were obtained by cycloaddition reaction of **3** with the thiazole-*o*-quinodimethane **17**. The isoquinolinequinone derivatives prepared in this study, which expressed in vitro cytotoxic activity against human normal fibroblasts and the human cancer cell lines AGS, HL-60, SK-MES-1, and J82, represent a

Table 1. Cytotoxic activity of isoquinolines against MRC-5 fibroblasts and tumor cell lines

Compound[a]	No.	(IC_{50}, μM)[b]					Entry
		Cell lines					
		MRC-5	AGS	SK-MES-1	J82	HL-60	
	**2**	12.6 ± 0.51	5.5 ± 0.32	20.3 ± 0.98	21.4 ± 1.1	11.0 ± 0.65	1
	**3**	54.5 ± 2.23	2.1 ± 0.12	14.7 ± 0.74	17.5 ± 0.84	13.7 ± 0.66	2
	**6**	>100	25.6 ± 1.27	51.7 ± 2.46	15.4 ± 0.88	>100	3
	**7**	94.1 ± 4.71	35.3 ± 1.82	42.5 ± 2.33	10.1 ± 0.69	>100	4
	**12**	3.1 ± 0.17	0.80 ± 0.04	0.82 ± 0.03	1.1 ± 0.06	2.9 ± 0.17	5
	**13**	3.0 ± 0.12	0.83 ± 0.05	0.44 ± 0.03	0.83 ± 0.04	5.9 ± 0.26	6
	**14**	11.2 ± 0.64	7.2 ± 0.42	5.6 ± 0.22	4.4 ± 0.31	6.6 ± 0.38	7
	**15**	15.8 ± 0.75	6.2 ± 0.32	22.6 ± 1.54	7.8 ± 0.53	21.3 ± 0.94	8
Etoposide	—	3.9 ± 0.21	0.36 ± 0.02	2.5 ± 0.15	2.8 ± 0.18	0.80 ± 0.04	11

[a] All compounds were quite stable in DMSO solution.
[b] Values are means ± standard error of the mean.

valuable advance in the search for novel anticancer drugs.

4. Experimental

4.1. Chemical synthesis

All reagents were of reagent grade commercial quality and were used without further purification. Melting points were determined on a Köfler hot-stage apparatus and are uncorrected. ^{1}H NMR spectra were measured in $CDCl_3$ on Bruker AM-200 and AM-400 instruments. Chemical shifts are expressed in parts per million downfield relative to tetramethylsilane (TMS, δ scale) and 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 and HMBC) and DEPT were used for signal assignment. IR spectra were recorded in KBr and frequencies are in cm^{-1}. Chemical shifts are reported in parts per million (δ) downfield from TMS, and J-values are given in hertz. Merck silica gel 60 (70–230 mesh) and TLC aluminum foil 60 F254 were used for preparative column and analytical TLC, respec-

tively. The azadienes methacrolein *N*,*N*-dimethylhydrazone **9**, crotonaldehyde *N*,*N*-dimethylhydrazone **10**, and 1-cyclohexencarbaldehyde *N*,*N*-dimethylhydrazone **11** were prepared according to previously reported procedures.[28,29]

4.1.1. Methyl 5,8-dihydroxy-1,3-dimethylisoquinoline-4-carboxylate (2). A solution of 2-acetyl-1,4-benzoquinone **1** (800 mg, 5.33 mmol) in dichloromethane (30 mL) was added dropwise to a stirred solution of methyl aminocrotonate (680 mg, 5.91 mmol) in dichloromethane (30 mL) and the mixture was left at rt overnight. The reaction mixture was evaporated under reduced pressure and the residue was cooled overnight at −20 °C in the presence of a 9:1 mixture of petroleum ether–dichloromethane (10 mL). The solid was triturated, filtered, and washed with petroleum ether to yield crude isoquinoline **2** (1.28 g, 97%). An analytical sample of **2** was prepared by recrystallization from ethyl acetate, mp: 278–280 °C; IR (KBr) ν_{max} 3229 (O–H), 1690 (C=O); ^{1}H NMR (DMSO-d_6, 200 MHz): δ 2.37 (s, 3H, Me), 2.97 (s, 3H, Me), 3.80 (s, 3H, CO_2Me), 6.78 (d, 1H, J = 7.5 Hz, 6-H or 7-H), 6.84 (d, 1H, J = 7.5 Hz, 7-H or 6-H), 9.66 (s, 1H, OH), 9.85 (s, 1H, OH); ^{13}C NMR (50 MHz): δ 21.2, 28.2, 51.8, 110.7, 114.3, 117.1, 119.0, 125.2, 143.1, 144.7, 148.4, 157.7, 170.2. Anal Calcd for $C_{13}H_{13}NO_4$: C, 63.67; H, 4.52; N, 5.71. Found: C, 63.97; H, 4.32; N, 5.68.

4.1.2. Methyl 1,3-dimethyl-5,8-dioxo-5,8-dihydroisoquinoline-4-carboxylate (3). *Method A.* A solution of compound **2** (230 mg, 9.3 mmol), iron(III) chloride hexahydrate (460 mg, 1.70 mmol) in methanol (10 mL) was left at rt for 3 h. The mixture was diluted with water (50 mL) and extracted with ethyl acetate (3× 25 mL). The extract was dried over $MgSO_4$, filtered, and evaporated under reduced pressure. The residue was extracted with hot petroleum ether and the filtrate was evaporated to give quinone **3** (60 mg, 26%) as yellow crystals, mp: 120–122 °C (hexane); IR (Nujol, cm^{-1}): ν_{max} 1728 (CO_2Me), 1670 and 1664 (C=O quinone); ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.62 (s, 3H, 3-Me), 2.97 (s, 3H, 1-Me), 4.03 (s, 3H, CO_2Me), 6.96 (s, 2H, 6- and 7-H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 23.2, 26.1, 53.5, 121.0, 124.9, 135.9, 137.0, 140.7, 160.6, 161.6, 168.9, 184.6, 185.5; Anal. Calcd for $C_{13}H_{11}O_4N$: C, 63.67; H, 4.52; N, 5.71. Found: C, 64.06; H, 4.67; N, 5.52.

Method B. A suspension of isoquinoline **2** (360 mg, 1.45 mmol), manganese dioxide (2.5 g, 28.7 mmol), magnesium sulfate (1 g), and dichloromethane (20 mL) was magnetically stirred for 50 min. The mixture was filtered, the solids were washed with dichloromethane, and the filtrate was evaporated under reduced pressure. The residue was chromatographed on silica gel (9:1 dichloromethane–ethyl acetate) to yield pure quinone **3** (270 mg, 76%).

Method C. A suspension of 2,5-dihydroxyacetophenone (152 mg, 1.0 mmol), silver(II) oxide (510 mg, 2.2 mmol), and $MgSO_4$ (1 g) in dichloromethane (25 mL) was stirred for 1 h. Silver(II) oxide (2.2 mmol) was added to the mixture and the stirring was continued for 90 min. The mixture was filtered and the solvent was removed to yield crude quinone **3** (231 mg, 94%). The crude product was chromatographed as in method B, to yield pure **3** (182 mg, 74%).

4.1.3. Methyl 5,5a,6,9,9a,10-hexahydro-6-trimethylsilyloxy-1,3-dimethyl-5,10-dioxobenzo[*g*]isoquinoline carboxylate (4). A mixture of quinone **3** (202 mg, 0.82 mmol), (*E*)-trimethylsilyloxybutadiene (660 mg, 4.65 mmol) in dichloromethane (5 mL) was left overnight at rt. The mixture was evaporated under reduced pressure and the residue was cooled overnight in the presence of *n*-hexane (10 mL). The solid was triturated, filtered, washed with *n*-hexane, and chromatographed on silica gel (dichloromethane) to give adduct **4** (240 mg; 0.62 mmol, 76%) as white crystals mp 110–112 °C (hexane); IR (KBr): ν_{max} 1727 (C=O ester), 1703 (C=O ketone); ^{1}H NMR ($CDCl_3$, 200 MHz): δ −0.28 (s, 6H, $SiMe_3$) 2.10 (m, 1H, 9-H), 2.58 (s, 3H, 3-Me), 2.88 (s, 3H, 1-Me), 3.10 (m, 1H, 9-H′), 3.25–3.24 (m, 2H, 5a- and 9a-H), 3.99 (s, 3H, CO_2CH_3), 4.40 (m, 1H, 6-H), 5.70–6.00 (m, 2H, 7- and 8-H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ −0.065 (Me_3Si), 23.2, 23.4, 25.8, 44.1, 53.5, 53.9, 65.9, 124.7, 127.1, 127.4, 129.5, 140.4, 158.9, 160.3, 169.8, 196.3 (C-10), 198.7.

4.1.4. Methyl 5,10-dihydro-3,4-dimethyl-5,10-dioxo-6-hydroxybenzo[*g*]isoquinoline carboxylate (6). A solution of adduct **6** (102 mg, 0.265 mmol), aqueous THF (90%, 6 mL), and hydrochloric acid (5%, 1.4 mL) was left at rt for 1 h. The mixture was diluted with water (50 mL), extracted with dichloromethane (20 mL), and the organic layer was washed with water (2× 10 mL). The dry extract was evaporated and the solvents were removed under reduced pressure. The white solid residue was dissolved in dichloromethane (10 mL) and the solution was added to a stirred suspension of pyridinium chlorochromate (473 mg, 2.2 mmol) and sodium acetate (205 mg, 2.5 mmol) in dichloromethane (10 mL). The resulting red-brown mixture was stirred for 2 h and then chromatographed on silica gel (95:5 CH_2Cl_2–AcOEt) to give pure compound **6** (78 mg, 95%) as orange crystals, mp: 228–229 °C; IR (KBr): ν_{max} 3449 (O–H), 1736 (C=O ester), 1672, 1639 (C=O quinone); ^{1}H NMR ($CDCl_3$, 200 MHz): δ 2.65 (s, 3H, 3-Me), 3.07 (s, 3H, 4-Me), 4.07 (s, 3H, CO_2Me), 7.29 (m, 1H, 7-H), 7,78 (m, 2H, 8- and 9-H), 12.65 (s, 1H, OH); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 22.6, 26.7, 53.2, 115.44, 119.8, 122.3, 124.0, 125.7, 133.7, 136.0, 150.2, 160.1, 162.3, 162.4, 166.7, 182.7, 187.0; Anal. Calcd for $C_{17}H_{13}NO_5$: C, 65.59; H, 4.21; N, 4.50. Found: C, 64.92; H, 3.99; N, 4.31.

4.1.5. Methyl 5,10-dihydro-1,3-dimethyl-5,10-dioxobenzo[*g*]isoquinoline-4-carboxylate (7). A mixture of quinone **3** (202 mg; 130.7 mg, 0.53 mmol), (*E*)-trimethylsilyloxybutadiene (660 mg; 420 mg, 2.96 mmol) in dichloromethane (15 mL) was left overnight at rt. The mixture was evaporated under reduced pressure and the residue was cooled overnight in the presence of *n*-hexane. The adduct of **4** + **5** that precipitates as a white solid (240 mg; 159.4 mg) was dissolved in THF (10 mL) containing two drops of concentrated hydrochloric acid,

and the mixture was left at rt for 2 h. It was then diluted with water, neutralized with sodium hydrogencarbonate, and extracted with dichloromethane (2× 15 mL). The organic extract was dried over $MgSO_4$ and evaporated under reduced pressure. The residue was purified by column chromatography (98:2 CH_2Cl_2–AcOEt) to give pure **7** (110 mg, 0.37 mmol, 70%) as pale yellow crystals mp 220–221 °C (ethanol); IR (Nujol, cm^{-1}): ν_{max} 1734 (CO_2Me), 1679 and 1668 (C=O quinone); ^1H NMR (200 MHz, $CDCl_3$): δ 2.66 (s, 3H, 3-Me), 3.10 (s, 3H, 1-Me), 4.09 (s, 3H, CO_2Me), 7.76–7.90 (m, 2H, 7- and 8-H), 8.18–8.30 (m, 2H, 6- and 9-H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 22.8, 26.6, 53.12, 122.3, 125.1, 127.1, 127.4, 132.1, 132.9, 134.0, 135.2, 137.3, 159.9, 162.2, 169.1, 182.7, 183.4; Anal. Calcd for $C_{17}H_{13}O_4N$: C, 69.15; H, 4.44; N, 4.74. Found: C, 68.98; H, 4.32; N, 4.70.

4.1.6. Methyl 4-methyl-7-oxo-7*H*-naphtho[3,2,1-*ij*][2,6]naphthyridine-6-carboxylate (13). A solution of quinone **7** (43.2 mg, 0.14 mmol) in DMF (3 mL) was heated under nitrogen to 120 °C for 30 min. Then, DMF-DMA (0.2 mL, 1.16 mmol) was added and the mixture was heated to 110 °C overnight in a nitrogen atmosphere. Ammonium chloride (1 g, 18 mmol) and acetic acid (3 mL) were added and the mixture was refluxed for 24 h. The reaction mixture was poured into dichloromethane (20 mL) and the solution was washed with sodium hydrogencarbonate solution, water and dried over $MgSO_4$. The solvent was removed under reduced pressure and the extract was chromatographed on silica gel (95:5 CH_2Cl_2-AcOEt). Compound **7** was isolated (5.7 mg) as the less polar substance and tetracycle **8** as the more polar substance (7.7 mg, 20%). Further preparative TLC afforded an analytically pure sample of compound **8** as a deep yellow solid, mp: 240–242 °C; ^1H NMR (200 MHz, $CDCl_3$): δ 2.85 (s, 3H, 4-Me), 4.15 (s, 3H, CO_2Me), 7.66 (t, with fine coupling, J = 7.8 Hz, 9-H), 7.86 (t, with fine coupling, 1H, J = 7.8 Hz, 10-H), 7.89 (d, 1H, J = 5.7 Hz, 3-H), 8.31 (d, 1H, J = 7.8 Hz, 11-H), 8.84 (d, 1H, J = 7.8 Hz, 8-H), 8.95 (d, 1H, J = 5.7 Hz, 2-H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 23.5, 53.4, 115.4, 121.4, 125.4, 126.0, 126.2, 130.6, 131.0, 131.3, 135.45, 136.0, 148.4, 150.1, 150.2, 161.5, 166.7, 182.8; Anal. Calcd for $C_{18}H_{12}O_3N_2$: 304.084244. Found: 304.085342.

4.1.7. Cycloaddition of 3 with methacrolein *N*,*N*-dimethylhydrazone (9). A solution of quinone **3** (145 mg, 0.59 mmol) in acetonitrile (5 mL) was added dropwise to a stirred solution of methacrolein dimethylhydrazone (74 mg, 0.66 mmol) and acetic anhydride (0.5 mL) in acetonitrile (10 ml). The solution was left for 1 day at room temperature and then was evaporated under reduced pressure. The residue was left in an open flask for 4 days and chromatographed on silica gel. Elution with 9:1 dichloromethane–ethyl acetate yielded methyl 3,5,7-trimethyl-9,10-dioxo-1,4,9,10-tetrahydro-1,6-diaza-anthracene-8-carboxylate **12** (67 mg, 37%) as stable blue crystals, mp: 162–163 °C; IR (Nujol, cm^{-1}): ν_{max} 3403 (NH), 1730 (C=O ester), 1670 and 1612 (C=O quinone); ^1H NMR (200 MHz, $CDCl_3$): δ 1.67 (s, 3H, 8-Me), 2.58 (s, 3H, 3-Me), 3.00 (s, 3H, 1-Me), 3.22 (s, 2H, 9-H), 4.00 (s, 3H, CO_2Me), 5.89 (m, 1H, 7-H), 6.37 (br s, 1H, H-N); ^{13}C NMR (50 MHz, $CDCl_3$): δ 20.4, 22.50, 26.0, 26.0, 53.0, 112.4,114.2, 117.6, 120.9, 124.0, 134.1, 136.7, 158.1, 160.3, 179.9, 183.5; Anal. Calcd for $C_{17}H_{16}O_4N_2$: C, 65.38; H, 5.16; N, 8.97. Found: C, 65.26; H, 5.10; N, 8.67.

Further elution with 9:1 dichloromethane–ethyl acetate gave methyl 3,5,7-trimethyl-9,10-dioxo-9,10-dihydro-1,6-diaza-anthracene-8-carboxylate **13** (58 mg, 36%) as pale yellow crystals mp 168–169 °C; IR (Nujol, cm^{-1}): ν_{max} 1735 (CO_2Me), 1688, 1665 (C=O quinone); ^1H NMR (200 MHz, $CDCl_3$): δ 2.58 (s, 3H, 8-Me), 2.67 (s, 3H, 3-Me), 3.07 (s, 3H, 1-Me), 4.07 (s, 3H, CO_2Me), 8.38 (m, 1H, 9-H), 8.91 (d, 1H, J = 1.7 Hz, 7-H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 19.5, 23.3, 26.9, 53.7, 122.0, 125.9, 131.1, 135.5, 137.5, 140.5, 145.8, 156.5, 161.0, 162.6, 168.9, 181.4, 183.6; Anal. Calcd for $C_{17}H_{14}O_4N_2$: C, 65.80; H, 4.55; N, 9.03. Found: C, 65.96; H, 4.42; N, 8.83.

4.1.8. Cycloaddition of 3 with crotonaldehyde *N*,*N*-dimethylhydrazone (10). A solution of quinone **3** (207 mg, 0.85 mmol) in acetonitrile (5 mL) was added dropwise to a stirred solution of the azadiene (145 mg, 1.3 mmol), acetic anhydride (1 mL, 10 mmol) in acetonitrile (10 ml). The solution was left for 4 h at room temperature and then evaporated under reduced pressure. The residue was left in an open flask for 4 days and purified by column chromatography on silica gel (8:2 petroleum ether–ethyl acetate) to furnish methyl 4,5,7-trimethyl-9,10-dioxo-1,4,9,10-tetrahydro-1,6-diaza-anthracene-8-carboxylate **14** (245 mg, 94%) as stable violet crystals, mp: 200–202 °C. IR (Nujol, cm^{-1}): ν_{max} 3379 (C=O ester), 1660 and 1626 (C=O quinone); ^1H NMR (200 MHz, $CDCl_3$): δ 1.17 (d, J = 6.5 Hz, 3H, 4-Me), 2.66 (s, 3H, 7-Me), 2.99 (s, 3H, 5-Me), 4.00 (s, 3H, CO_2Me), 4.95 (m, 1H, 3-H), 6.14 (m, 1H, 3-H), 6.57 (m, 1H, 2-H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 22.5, 23.9, 25.9, 26.1, 53.0, 109.1, 116.1, 121.0, 122.5, 124.0, 134.1, 136.4, 156.2, 160.5, 168.7, 180.0, 183.6; Anal. Calcd for $C_{17}H_{16}O_4N_2$: C, 65.38; H, 5.16; N, 8.97. Found: C, 65.21; H, 4.97; N, 8.88.

4.1.9. Cycloaddition of quinone 3 with 2-cyclohexenecarbaldehyde *N*,*N*-dimethylhydrazone (11). A solution of quinone **3** (150 mg, 0.61 mmol) in acetonitrile (10 mL) was added dropwise to a stirred solution of 2-cyclohexenecarbaldehyde *N*,*N*-dimethylhydrazone (112 mg, 0.73 mmol) and acetic anhydride (0.5 mL) in acetonitrile (10 ml). The solution was left for one week at room temperature, and compound **15**, which precipitated as a blue solid, was isolated by filtration and purified by column chromatography over silica gel (6:4 petroleum ether–AcOEt) to give pure methyl 1,2,3,4,6,7,12,12b-octahydro-9,11-dimethyl-7,12-dioxopyrido[3,4-*b*]phenanthridine-8-carboxylate **15** (150 mg, 70%) as stable blue crystals, mp: 190–191 °C: IR (Nujol, cm^{-1}): ν_{max} 3400 (N–H), 1726 (CO_2CH_3), 1670 (C=O quinone); ^1H NMR (200 MHz, $CDCl_3$): δ 2.10–2.40 (m, 8H, 1-, 2-, 3- and 4-H), 2.55 (s, 3H, 11-Me), 2.97 (s, 3H, 9-Me), 3.67 (dd, 1H, J = 3 and 11 Hz, 12b-H), 3.98 (s, 3H, CO_2CH_3), 5.80 (d, 1H, J = 3.5 Hz, 5-H), 6.34 (br s, 1H, NH); ^{13}C NMR (50 MHz, $CDCl_3$): δ 22.5, 26.0,

27.2, 28.9, 32.8, 34.8, 36.7, 53.0, 114.5, 114.8, 121.3, 123.4, 123.8, 134.0, 157.9, 160.3, 168.8, 180.4, 183.5 (C-12); Anal. Calcd for $C_{20}H_{20}N_2O_4$: C, 68.17; H, 5.72; N, 7.95. Found: C, 68.14; H, 5.80; N, 8.03.

4.1.10. Cycloaddition of 3 with *o*-quinodimethane (17). A solution of 4-(bromomethyl)-5-(dibromomethyl)-thiazole (85 mg, 0.24 mmol) in DMF (0.5 mL) was added dropwise to a stirred solution of quinone **3** (51 mg, 0.20 mmol), NaI (183 mg, 1.22 mmol) in DMF (1.5 mL) at 55 °C and the mixture was kept at the same temperature for 2 h. The mixture was diluted with water (10 mL) followed by addition of a 10% aqueous solution of sodium bisulfite. The resulting solution was extracted with ethyl acetate (3× 20 mL) and the organic extract was washed with water and dried over $MgSO_4$. Evaporation of the solvent followed by column chromatography (dichloromethane) of the crude product yielded a 4:1 mixture of methyl 6,8-dimethyl-5,10-dioxo-5,10-dihydro-1-thia-3,7-diaza-cyclopenta[*b*]anthracene-9-carboxylate and methyl 7,9-dimethyl-5,10-dioxo-5,10-dihydro-1-thia-3,8-diaza-cyclopenta[*b*]anthracene-6-carboxylate (26.5 mg, 0.0753 mmol, 47%) as a pale yellow solid, mp: 210–214 °C (dichloromethane). IR (Nujol, cm^{-1}): ν_{max} 1730 (C=O ester), 1677 (C=O quinone); ^1H NMR ($CDCl_3$, 200 MHz) δ 2.85 (s, 3H, 5-Me), 4.15 (s, 3H, CO_2CH_3), 7.66 (t, 1H, J = 7.7), ms: *e/z* (%): 352 (38), 321 (100), 319 (48), 293 (36).

4.2. Anticancer 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), HL-60 human leukemia cells (CCL-240), SK-MES-1 human lung cancer cells (HTB-58), and J82 human bladder carcinoma cells (HTB-1). Cells were grown in the following media: MRC-5, SK-MES-1, and J82 in MEM, AGS cells in Ham F-12, and HL-60 in RPMI. The MEM medium contained 2 mM L-glutamine, 1 mM sodium pyruvate, and 1.5 g/L sodium bicarbonate. Ham F-12 was supplemented with 2 mM L-glutamine and 1.5 g/L sodium bicarbonate. RPMI contained 1 mM sodium pyruvate and 2 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% 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.[34] The compounds were dissolved in DMSO (1% final concentration) and complete medium. Untreated cells were used as controls. Each experiment was carried out in sextuplicate

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