



Original article

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ABSTRACT

In the search of structure–activity relationship studies and to explore the antitumor effect associated with the pyrimidoisoquinolinequinone scaffold, several diversily substituted 8-aminopyrimido[4,5-c]isoquinolinequinones were regioselectively synthesized. Variation in the structure of the nitrogen substituent bonded to the 8-position of the pyrimidoisoquinolinequinone system led to a set of alkylamino-, phenylamino- and alkylphenylamino derivatives. The cytotoxic activity of the aminoquinone derivatives was evaluated *in vitro* using the MTT colorimetric method against one normal cell line (MRC-5 lung fibroblasts) and four human cancer cell lines (AGS human gastric adenocarcinoma; SK-MES-1 human lung cancer cells, and J82 human bladder carcinoma; HL-60 human leukemia) in 72-h drug exposure assays. Among the series, five compounds exhibited interesting antitumor activity against AGS human gastric adenocarcinoma and human lung cancer cells. The SAR studies revealed that both the nature of the nitrogen substituent into the quinone ring and the methyl group at the 6-position play key roles in the antitumor activity.

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1. Introduction

Cancer, second cause of mortality in the world, is characterized by a deregulation of the cell cycle, which results in a progressive loss of the cellular differentiation and a non-controlled cellular growth. Despite the progress achieved in medicine during century, cancer is still a leading life threatening pathology. Therefore, there is an increasing need for new therapies, especially those that are based on current knowledge of cancer biology as well as that taking advantage of the cancer cells phenotype, described by Hanahan and Weinberg [2].

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 [3,4]. Because of the complex structure of most antitumor quinonoid compounds it is often difficult to separate the contributions of chemical reactivity and the different pathways of metabolism to overall biological activity. 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 give its extra electron to molecular oxygen to give 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 comproportionation 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 [5–8].

The molecular framework of several naturally occurring antitumor agents contains an aminoquinonoid moiety as the key

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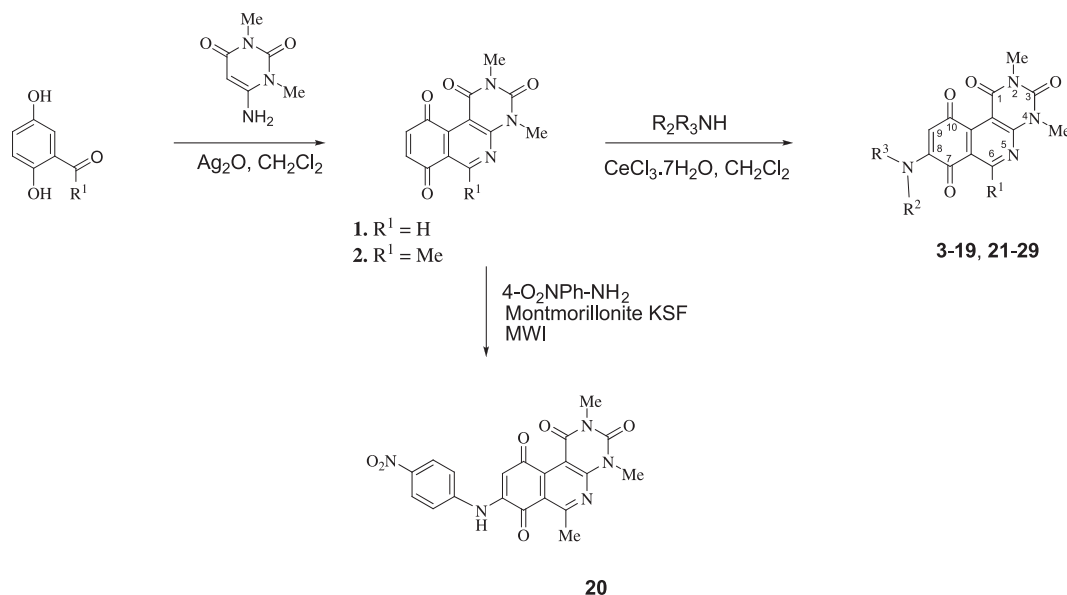
structural component (e.g. mitomycin C, cribrostatin 3, streptonigrin), [9–11]. This structural array has stimulated the synthesis of novel compounds endowed with cytotoxic activity on human cancer cell lines [1,12–14].

In a previous work we have reported that substituted 7-aminoisoquinoline-5,8-quinones display antitumor activity against several cell lines [1]. The QSAR analysis reveals that the half-wave potential of 7-phenylaminoisoquinoline-5,8-quinones is an important parameter determining the antitumoral activity on AGS gastric adenocarcinoma and J82 bladder carcinoma cell lines. Recently, we have reported preliminary results on the preparation and antitumor evaluation of a number of aminopyrimido[4,5-*c*]isoquinoline-7,10-quinones [15]. The results suggest that derivatives of this aminoquinone scaffold might be good candidates as antitumor compounds and prompted us to explore the SAR of aminopyrimido[4,5-*c*]isoquinoline-7,10-quinones. Herein, we wish to report full details on the regioselective synthesis of a broad variety of 8-aminopyrimido[4,5-*c*]isoquinoline-7,10-quinone derivatives and the *in vitro* antitumor evaluation on normal human lung fibroblasts MRC-5 and four human tumor cells: AGS gastric adenocarcinoma, SK-MES-1 lung, J82 bladder carcinoma and HL-60 leukemia human cancer cell lines.

2. Results and discussion

2.1. Chemistry

The synthesis of the aminopyrimido[4,5-*c*]isoquinolinequinones was achieved by amination reaction of quinones **1** and **2** with a variety of primary and secondary amines in ethanol in the presence of 5% mol of $\text{CeCl}_3 \cdot 7\text{H}_2\text{O}$ and under aerobic conditions (Scheme 1). The nucleophilic substitution reaction takes place at room temperature to give, under regiospecific manner, the corresponding aminoquinones in moderate to good yields. The low yield formation of aminoquinones **15** and **29** probably is due to the fact that 1-adamantylamine has a low nucleophilicity due to steric hindrance around the nitrogen atom. Since the nucleophilic substitution on quinone **2** with *p*-nitroaniline was unsuccessful, the nitrophenyl derivative **20** was prepared, albeit in low yields, by microwave irradiation (MWI) of the precursors loaded on the acid clay montmorillonite KSF (Scheme 1). Table 1 summarizes the results of the preparation of the 8-aminopyrimidoisoquinolinequinone derivatives **3–29**.



Scheme 1. Synthesis of 8-aminopyrimido[4,5-*c*]isoquinolinequinone derivatives.

The structures of the new compounds **3–29** were established on the basis of their nuclear magnetic resonance (^1H NMR, ^{13}C NMR, 2D NMR) spectra and elemental analyses. The location of the nitrogen substituent at 8-position in compounds **3–15** was determined by means of HMBC experiments. For example, aminoquinone **3** displayed $^3J_{\text{C,H}}$ couplings between the carbon at C-7 (δ 179.5), the proton at C-6 (δ 9.30), the proton at C-9 (δ 6.52) and the proton of the N–H group (δ 7.49). In the case of aminoquinones that lack of the amino proton, such as compound **10**, the location of the nitrogen substituent was established by $^3J_{\text{C,H}}$ coupling between the carbon at C-7 (δ 179.4) and the protons at C-6 (δ 9.07), and the proton at C-9 (δ 6.17), together with the $^4J_{\text{C,H}}$ coupling between the carbon at C-9 (δ 111.9) and the methyl protons (δ 3.43) of the nitrogen substituent. The observed NOE enhancement between the methyl protons (δ 3.43) and the proton at C-9 (δ 6.52) supported the assignment.

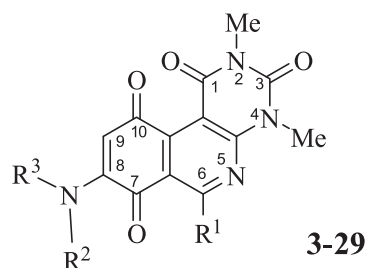
Regarding the position of the nitrogen substituent in compounds **16–29**, this was deduced by the $^3J_{\text{C,H}}$ coupling of C-7 with the proton at C-9, the proton of the amino group, together with the $^4J_{\text{C,H}}$ coupling of C-7 with the protons of the methyl group at C-6. The location of the nitrogen substituent in compounds **24**, **25** and **27** was deduced in a similar manner to that mentioned for compound **10**.

On the basis of results reported by Pratt [16] on the catalytic action of a Lewis acid, such as cerium chloride, to promote regioselective 6-amination reactions of quinoline-5,8-quinone and its further applications on the regioselective synthesis of amino-*N*-heterocyclic quinones [1,17–21], we assume that the effect of the catalyst $\text{CeCl}_3 \cdot 7\text{H}_2\text{O}$ to induce the attack of the amines at the 8-position in quinones **1** and **2** may be ascribed to coordination of the cerium ion to the heterocyclic nitrogen atom and/or the carbonyl group at the C-10. The coordination strongly enhances the electron-withdrawing capacity of the carbonyl group at the C-10, which is transferred to the 8-position, leading to preferential C-8 substitution *via* nucleophilic attack by the amines.

2.2. Cytotoxic activities

The newly synthesized 8-aminopyrimidoisoquinolinequinone derivatives **3–13**, **15–29** and their precursors **1** and **2** were evaluated for *in vitro* anticancer activity against normal human lung

Table 1
Substituent types and yield of the synthesized pyrimidoisoquinolinquinones **3–29**.



R ³ R ² N	Compound	R ¹	Yield (%)	Compound	R ¹	Yield (%)
	3	H	99	16	Me	99
	4	H	40	17	Me	46
	5	H	99	18	Me	99
	6	H	91	19	Me	72
	–	–	–	20	Me	39
	7	H	95	21	Me	95
	8	H	74	22	Me	65
	9	H	81	23	Me	79
	10	H	61	24	Me	63
	11	H	55	25	Me	58
	12	H	75	26	Me	75
	13	H	74	27	Me	78

Table 1 (continued)

R ³ R ² N	Compound	R ¹	Yield (%)	Compound	R ¹	Yield (%)
	14	H	49	28	Me	65
	15	H	6	29	Me	16

fibroblasts MRC-5 and four human tumor cells: AGS gastric adenocarcinoma, SK-MES-1 lung, J82 bladder carcinoma and HL-60 leukemia in 72-h drug exposure assays. The cytotoxicity of the compounds was measured using a conventional microculture tetrazolium reduction assay [22–24]. The average IC₅₀ values (μM) are collected in Table 2. The broad variety of the synthesized compounds was designed in order to gain insight upon the effects of the substituted amino group at C-8 and the methyl group at C-6 of the pyrimidoisoquinolinquinones on the biological activity. The cytotoxic and antitumor activities are summarized in Table 2.

The initial structure–activity relationship (SAR) focused on the effects of insertion of phenyl-, phenylalkyl- and alkylamino groups at the 8-position in precursors **1** and **2**. The data of Table 2 indicate that insertion on quinone **1** leads to a variety of aminoquinones (**3**, **5–15**) with enhanced cytotoxic and antitumor activity compared to that of their precursor **1**, except for compound **4**. A similar effect is observed in quinone **2** where the insertion of substituted amino groups at the 8-position increases the biological activity respect to their precursor, except for compounds **21**, **23** and **25**. It is noteworthy that the presence of nitrogen substituents on the biological activity is remarkable in terms of the antitumor activity on gastric adenocarcinoma cells, particularly for compounds **16**, **19** and **20** which are 82-fold more active than precursor **2**.

Next, the SAR analysis was focused on the effects of the methyl and nitrogen substituent into the pyrimidoisoquinolinquinone chromophore. The data of Table 2 indicate that insertion of a methyl group at the 6-position of the phenylaminoquinones **3**, **4** and **6**, as in **16**, **17** and **19**, induces an increase of the cytotoxic and antitumor activity. On the contrary, introduction of a methyl group at the 6-position in phenylaminoquinones **5**, as in **18**, induces a decrease of the biological activity. These effects produced by the methyl group in such position, could be attributed to its electronic and/or lipophilic properties, which modify the ability of the pharmacophore to interact with the biological targets involved in the cancer cell death. Comparison of the biological activity of the phenylaminoquinone **16** to that of its 6-methyl substituted analogues **17–25** indicates that insertion in **16**, of the electron-donor substituents OH and OMe at *para*-position, induces a decrease of the cytotoxic and antitumor activity. In the case of the electron-withdrawing substituents located at *para*-position, such as F and NO₂, no significant effect on the biological activity is detected. These facts clearly demonstrate the influence on the biological activity of the donor–acceptor properties of the nitrogen substituent into the pyrimidoisoquinolinquinone pharmacophore.

As can be seen in Table 2, insertion in **16** of a methoxy group at the *ortho*-position, as in **21** and **23**, or replacement of the amino proton by an ethyl group, as in **25**, produces a decrease of cytotoxic and antitumor activity. Taking into account precedents reported by Aguilar-Martínez [25] concerning the effect of substituents in the aniline ring on the conjugation degree of the nitrogen lone pair with the quinone system in anilino-1,4-naphthoquinones, the effects of substituents in **21**, **23** and **25** on the biological activity could be related with a low conjugation degree between the donor

Table 2
Cytotoxic activity of aminopyrimidoisoquinolinquinone derivatives and their precursors.

N°	IC ₅₀ ± SEM ^a (μM)				
	MRC-5 ^b	AGS ^c	SK-MES-1 ^d	J82 ^e	HL-60 ^f
1	39.9 ± 2.2	75.2 ± 3.9	>100	95.3 ± 4.4	43.6 ± 2.5
3	9.2 ± 0.6	2.5 ± 0.2	7.2 ± 0.6	10.2 ± 0.6	9.3 ± 0.6
4	47.4 ± 3.8	15.5 ± 0.9	55.9 ± 2.8	41.3 ± 2.5	>100
5	20.5 ± 1.2	2.8 ± 0.2	6.5 ± 0.5	15.6 ± 0.9	15.8 ± 1.1
6	6.4 ± 0.3	3.0 ± 0.2	5.6 ± 0.4	9.4 ± 0.5	7.6 ± 0.6
7	8.2 ± 0.6	4.0 ± 0.3	5.7 ± 0.3	13.4 ± 0.7	47.5 ± 2.9
8	16.6 ± 1.2	5.2 ± 0.4	17.3 ± 0.9	21.3 ± 1.7	7.6 ± 0.5
9	30.9 ± 1.5	9.1 ± 0.5	30.9 ± 1.5	43.7 ± 2.2	>100
10	4.3 ± 0.2	2.1 ± 0.1	9.3 ± 0.7	14.0 ± 0.7	20.8 ± 1.7
11	7.8 ± 0.5	3.9 ± 0.3	8.5 ± 0.7	8.9 ± 0.9	12.9 ± 0.9
12	8.4 ± 0.4	2.5 ± 0.2	6.3 ± 0.3	11.0 ± 0.7	9.7 ± 0.8
13	7.9 ± 0.4	4.6 ± 0.3	3.3 ± 0.3	8.8 ± 0.6	4.6 ± 0.2
15	6.1 ± 0.4	2.0 ± 0.1	4.5 ± 0.4	9.3 ± 0.6	9.5 ± 0.8
2	72.8 ± 3.4	82.1 ± 4.4	67.5 ± 3.6	78.6 ± 3.9	31.5 ± 1.7
16	4.6 ± 0.3	1.0 ± 0.1	2.9 ± 0.2	3.8 ± 0.2	5.7 ± 0.4
17	17.4 ± 1.0	3.3 ± 0.3	3.9 ± 0.2	7.0 ± 0.4	11.6 ± 0.9
18	49.6 ± 2.5	5.5 ± 0.3	16.0 ± 0.8	24.2 ± 1.2	>100
19	4.0 ± 0.2	1.0 ± 0.1	2.6 ± 0.2	7.7 ± 0.5	5.6 ± 0.5
20	5.9 ± 0.3	1.0 ± 0.1	3.2 ± 0.3	5.0 ± 0.4	2.9 ± 0.2
21	>100	>100	>100	>100	>100
22	6.2 ± 0.4	1.9 ± 0.1	2.3 ± 0.1	6.9 ± 0.5	20.9 ± 1.3
23	>100	31.7 ± 1.9	>100	>100	>100
24	12.1 ± 0.7	4.7 ± 0.2	27.3 ± 1.6	29.2	>100
25	87.7 ± 4.4	19.2 ± 1.3	83.9 ± 4.2	>100	>100
26	19.9 ± 1.4	5.2 ± 0.4	15.2 ± 1.0	17.9 ± 0.9	19.7 ± 1.6
27	4.8 ± 0.2	3.9 ± 0.3	6.4 ± 0.3	17.3 ± 1.2	33.8 ± 1.7
28	11.8 ± 5.9	2.2 ± 0.1	15.6 ± 1.1	14.1 ± 0.7	29.9 ± 2.4
29	25.6 ± 2.0	4.2 ± 0.2	16.1 ± 0.8	26.8 ± 1.3	20.8 ± 1.5
Etoposide	3.9 ± 0.21	0.36 ± 0.02	2.5 ± 0.15	2.8 ± 0.18	0.80 ± 0.04

^a Data represent mean average values for six independent determinations.

^b Normal human lung fibroblasts cells.

^c Human gastric adenocarcinoma cell line.

^d Human lung cancer cell line.

^e Human bladder carcinoma cell line.

^f Human leukemia cell line.

and acceptor fragments due to the molecular planarity inhibition by steric hindrance.

It is noteworthy that insertion of a fluorine atom at the *ortho*-position in compounds **3** and **16**, as in **8** and **22**, induces a decrease of the cytotoxic and antitumor activity respect to their precursors. However, no significant influence on these biological properties was observed when a fluorine atom is substituted at the *para*-position in compounds **3** and **16**, as in **6** and **19**. These results demonstrate that the *ortho*-fluorophenylamino group exerts a greater influence on the antitumor activity than that of its *para*-isomers.

Regarding the effect of the structure of the alkylamino group at 8-position in the pyrimidoisoquinolinquinone chromophore, in particular *n*-butyl- and adamantyl- substituents (compounds **12/26** and **15/29**), no significant differences on the biological activity were observed. These facts suggest that the structure of the alkylamino group, in these compounds, does not significantly influence on the cytotoxic activity. It should be noted that the introduction of a methyl group in compounds **12**, **13** and **15** leads to the analogues **26**, **27** and **29** with lower activity.

Analysis of the data in Table 2 indicates that, in general, cytotoxicity was observed in all the cancer cell lines but that AGS and SK-MES-1 cell lines appeared to be more sensitive to the compounds overall. Aminoquinones **3**, **5**, **6**, **12**, **15**, **16**, **19**, **20** and **22** exhibit interesting antitumor properties on gastric adenocarcinoma and lung cancer cells, expressed by their IC₅₀ values and selectivity index (IC₅₀ fibroblasts/IC₅₀ cancer cells) (Figs. 1 and 2).

Compounds **16**, **19**, **20** and **22** have antitumor activity and selectivity index on lung cancer cells at levels comparable to those obtained for the antitumor drug etoposide, which was included in

the assays. Among the compounds evaluated in the *in vitro* screen, derivatives **5**, **16**, **19**, **20** and **22** (Fig. 3) were selected for this study as the more significant antitumor members. Although compound **5** exhibited less antitumor activity than compounds **16**, **19**, **20** and **22**, it was included into the selected group since it showed the highest selectivity index for AGS and SK-MES-1 cell lines.

No linear correlation could be found between the IC₅₀ of the evaluated phenylaminoquinones with any single variable such as electronic, lipophilic or steric parameters (data not shown). Therefore, a combination of these molecular descriptors could explain the differences on the antitumor activity.

3. Conclusion

We have developed the regiospecific synthesis of a variety of 8-aminopyrimido[4,5-*c*]isoquinolinequinone derivatives. The majority of the new aminoquinones expressed *in vitro* cytotoxic activity against normal human lung fibroblasts (MRC-5) and on gastric adenocarcinoma (AGS), lung cancer (SK-MES-1), and bladder carcinoma (J82) cell lines. The substitution of phenylamino- and alkylamino groups into the 8-position of quinones **1** and **2** increases the cytotoxic potency, with respect to their precursors, in almost all the evaluated cell lines. This substitution effect on the biological activity is remarkable in the antitumor activity on gastric adenocarcinoma cells, for some of the aminoquinones prepared from **2**, which were 82-fold more active than the precursor.

From the current investigation, structure–activity relationships of the phenylaminoquinone members suggest substituents at the *ortho* or *para*-position of the phenyl ring will determine the

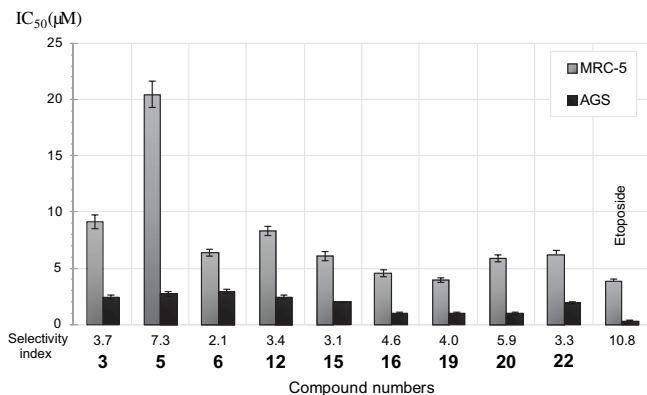


Fig. 1. Comparative MTT assay for MRC-5 and AGS cell lines.

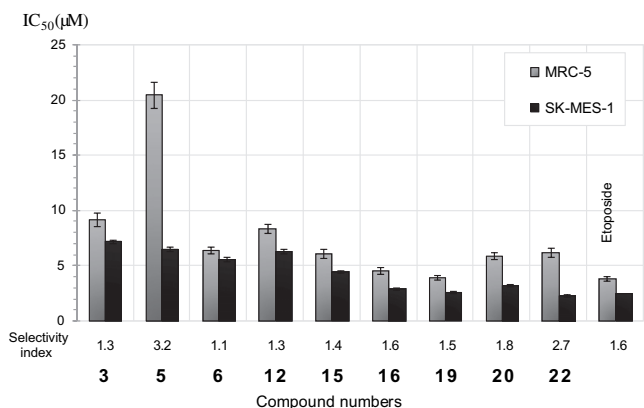


Fig. 2. Comparative MTT assay for MRC-5 and SK-MES-1.

antitumor activity. The effect of such substitutions were more significant in enhancing the antitumor activity for the 6-methyl substituted 8-phenylaminopyrimido[4,5-*c*]isoquinolinequinones than those of the 6-unsubstituted analogs.

Pyrimido[4,5-*c*]isoquinolinequinone derivatives **5**, **16**, **19**, **20** and **22** exhibited interesting antitumor activity and selective index comparable to that of reference drug etoposide. Given the high incidence of undesirable side-effects induced by the majority of currently anticancer drugs and by considering the selective index of aminoquinone **5**, this compound appeared as promising and

interesting lead, endowed of potential anticancer activity. These results prompt us to design and synthesize more new aminopyrimido[4,5-*c*]isoquinolinequinone derivatives, structurally related to **5**, in order to discover new, more active and selective anticancer agents.

4. Experimental

4.1. Chemistry

All reagents were commercially available reagent grade and were used without further purification. Melting points were determined on a Stuart Scientific SMP3 apparatus and are uncorrected. ¹H NMR spectra were recorded on Bruker AM-200 and AM-400 instruments in deuteriochloroform (CDCl₃). ¹³C NMR spectra were obtained in CDCl₃ at 50 and 100 MHz. 2D NMR techniques (COSY, HMBC, NOESY) and DEPT were used for signal assignment. Chemical shifts are expressed in ppm downfield relative to tetramethylsilane (TMS, δ scale), and the coupling constants (*J*) are reported in Hertz. The elemental analyses were performed in a Fison SA, model EA-1108 apparatus. HRMS were obtained on a Thermo Finnigan spectrometer, model MAT 95XP. The reaction induced by microwave irradiation was performed in a 10 mL sealed tube with a single mode cavity Discover Microwave apparatus. Silica gel Merck 60 (70–230 mesh) was used for preparative column chromatography, and TLC aluminum foil 60F₂₅₄ for analytical TLC. Quinones **1** and **2** were prepared according to a previously reported procedure [26].

4.2. General procedure for the synthesis of 8-aminopyrimidoisoquinolinequinone derivatives

A suspension of quinone **1** (40 mg, 0.1476 mmol) or **2** (50 mg, 0.1753 mmol), the required amine (2 equiv.), CeCl₃·7H₂O (5% mmol respect to **1** or **2**), and ethanol (5 mL) was left with stirring at room temperature until completion of the reaction as indicated by TLC. The reaction mixture was evaporated under reduced pressure and the residue was column chromatographed (1:1:2 AcOEt/CH₂Cl₂/light petroleum) to yield the corresponding aminoquinone (Table 1).

4.2.1. 2,4-Dimethyl-2H, 4H-8-(phenylamino)pyrimido[4,5-*c*]isoquinoline-1,3,7,10-tetraone (**3**)

Prepared from **1** and aniline (3 h, 99%): red solid, mp 236–237 °C; ¹H NMR (400 MHz, CDCl₃): δ 3.50 (s, 3H, 2-NMe), 3.77 (s, 3H, 4-NMe), 6.52 (s, 1H, 9-H), 7.25 (m, 3H, 2'-, 4'- and 6'-H), 7.44 (t, 2H, *J* = 7.9 Hz, 3'- and 5'-H), 7.49 (s, 1H, N-H), 9.30 (s, 1H, 6-H); ¹³C NMR (100 MHz,

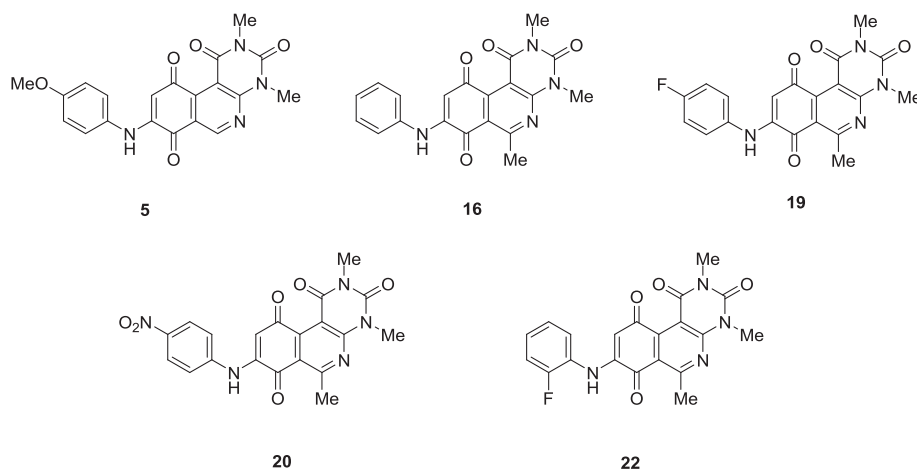


Fig. 3. Structure of the selected as the more significant antitumor members.

CDCl₃): δ 29.2, 30.6, 105.7, 107.1, 121.4, 122.4, 125.9, 129.8, 136.9, 143.4, 145.5, 150.9, 152.3, 155.2, 158.3, 179.5, 180.9. Anal. Calcd. for C₁₉H₁₄N₄O₄: C, 62.98; H, 3.89; N, 15.46. Found: C, 62.88; H, 3.52; N, 15.32.

4.2.2. 2,4-Dimethyl-2H, 4H-8-(4'-hydroxyphenylamino)pyrimido[4,5-c]isoquinoline-1,3,7,10-tetraone (**4**)

Prepared from **1** and *p*-hydroxyaniline (6 h, 40%); violet solid, mp 305–306 °C; ¹H NMR (400 MHz, DMSO-*d*₆): δ 3.27 (s, 3H, 2-NMe), 3.59 (s, 3H, 4-NMe), 5.93 (s, 1H, 9-H), 6.83 (d, 2H, *J* = 8.8 Hz, 2'- and 6'-H), 7.18 (d, 2H, *J* = 8.8 Hz, 3'- and 5'-H), 9.18 (s, 1H, 6-H), 9.20 (s, 1H, N-H), 9.63 (s, 1H, O-H); ¹³C NMR (100 MHz, DMSO-*d*₆): δ 29.1, 30.6, 103.1, 107.4, 116.4, 122.1(2C), 125.9, 129.3(2C), 137.4, 145.4, 146.4, 151.2, 155.3, 155.9, 158.5, 179.8, 180.1. Anal. Calcd. for C₁₉H₁₄N₄O₅: C, 60.32; H, 3.73; N, 14.81. Found: C, 60.11; H, 3.83; N, 15.01.

4.2.3. 2,4-Dimethyl-2H, 4H-8-(4'-methoxyphenylamino)pyrimido[4,5-c]isoquinoline-1,3,7,10-tetraone (**5**)

Prepared from **1** and *p*-anisidine (3 h, 99%); red solid mp 236.5–237.5 °C; ¹H NMR (400 MHz, CDCl₃): δ 3.49 (s, 3H, 2-NMe), 3.79 (s, 3H, 4-NMe), 3.85 (s, 3H, 4'-OMe), 6.33 (s, 1H, 9-H), 6.96 (d, 2H, *J* = 8.8 Hz, 3'- and 5'-H), 7.19 (d, 2H, *J* = 8.8 Hz, 2'- and 6'-H), 7.36 (s, 1H, N-H), 9.28 (s, 1H, 6-H); ¹³C NMR (100 MHz, CDCl₃): δ 29.2, 30.5, 55.5, 104.7, 107.1, 115.0(2C), 121.5, 124.6(2C), 129.5, 145.7, 150.9, 152.2, 155.2, 157.9, 158.3, 179.6, 180.7. Anal. Calcd. for C₂₀H₁₆N₄O₅: C, 61.22; H, 4.11; N, 14.28. Found: C, 60.74; H, 3.84; N, 14.10.

4.2.4. 2,4-Dimethyl-2H, 4H-8-(4'-fluorophenylamino)pyrimido[4,5-c]isoquinoline-1,3,7,10-tetraone (**6**)

A suspension of *p*-fluoronitrobenzene (141.1 mg, 1 mmol), iron powder (1 g, 17.9 mmol) and a 1:1:1 mixture of water/ethanol/acetic acid (45 mL) was stirred for 1 h at 50–60 °C. The mixture was neutralized with NaHCO₃ and then extracted with ethyl acetate (2 × 15 mL). The organic extract was dried over Na₂SO₄, filtered and evaporated under reduced pressure to yield crude *p*-fluoroaniline. Quinone **1** (40 mg, 0.1476 mmol) was reacted with *p*-fluoroaniline under the standard conditions (3 h, rt) to give compound **6** (91%), as red solid, mp 260–265 °C ¹H NMR (400 MHz, CDCl₃): δ 3.49 (s, 3H, 4-NMe), 3.77 (s, 3H, 2-NMe), 6.35 (s, 1H, 9-H), 7.15 (m, 2H, 2'- and 6'-H), 7.25 (m, 2H, 3'- and 5'-H), 7.42 (s, 1H, N-H), 9.29 (s, 1H, 6-H). ¹³C NMR (100 MHz, CDCl₃): δ 29.3, 30.7, 106.4, 107.4, 116.9 (d, 2C, 3'- and 5'-C, *J* = 22.7 Hz), 124.8 (d, 2C, 2'- and 6'-C, *J* = 8.2 Hz), 133.4 (d, 1C, 4'-C, *J* = 2.7 Hz), 147.4, 150.7, 152.6, 155.8, 156.5, 158.6, 160.6, 179.4, 180.4. Anal. Calcd. for C₁₉H₁₃FN₄O₄: C, 60.00; H, 3.45; N, 14.73. Found: C, 60.04; H, 3.34; N, 14.60.

4.2.5. 2,4-Dimethyl-2H, 4H-8-(2'-methoxyphenylamino)pyrimido[4,5-c]isoquinoline-1,3,7,10-tetraone (**7**)

Prepared from **1** and *o*-anisidine (5 h, 95%); red solid mp >300 (d); ¹H NMR (400 MHz, CDCl₃): δ 3.47 (s, 3H, 4-NMe), 3.74 (s, 3H, 2-NMe), 3.90 (s, 3H, 2'-OMe), 6.55 (s, 1H, 9-H), 6.98 (m, 2H, 4'- and 5'-H), 7.14 (m, 1H, 6'-H), 7.38 (m, 1H, 3'-H), 7.89 (m, 1H, N-H), 9.27 (s, 1H, 6-H); ¹³C NMR (100 MHz, CDCl₃): δ 29.2, 30.7, 55.6, 104.4, 107.0, 110.6, 114.9, 118.7, 121.3, 121.3, 136.5, 146.5, 147.5, 150.6, 152.4, 155.1, 156.4, 158.4, 179.8, 180.8. Anal. Calcd. for C₂₀H₁₆N₄O₅: C, 61.22; H, 4.11; N, 14.28. Found: C, 60.20; H, 3.88; N, 14.22.

4.2.6. 2,4-Dimethyl-2H, 4H-8-(2'-fluorophenylamino)pyrimido[4,5-c]isoquinoline-1,3,7,10-tetraone (**8**)

Quinone **1** (40 mg; 0.1476 mmol) was reacted with *o*-fluoroaniline [prepared from *o*-fluoronitrobenzene (141.1 mg, 1 mmol) and iron powder (1 g, 17.9 mmol)] under the standard conditions (6 h, rt) to give compound **8** (74%), red solid mp 207–208 °C; ¹H NMR (400 MHz, CDCl₃): δ 3.49 (s, 3H, 4-NMe), 3.77 (s, 3H, 2-NMe), 6.42 (s, 1H, 9-H), 7.23 (m, 3H, 4', 5'- and 6'-H), 7.44 (m, 1H, 3'-H),

7.49 (s, 1H, N-H), 9.31 (s, 1H, 6-H); ¹³C NMR (100 MHz, CDCl₃): δ 29.2, 30.8, 106.1, 107.1, 116.5 (d, 1C, *J* = 19.7 Hz, 3'-C), 121.2, 123.3, 124.9 (d, 1C, *J* = 3.5 Hz, 6'-C), 125.5 (d, 1C, *J* = 11.6 Hz, 1'-C), 126.6 (d, 1C, *J* = 7.5 Hz, 4'-C), 146.8, 151.4, 152.6, 155.1 (d, 1C, *J* = 248.3 Hz, 2'-C), 155.6, 155.6, 158.5, 179.7, 180.6. Anal. Calcd. for C₁₉H₁₃N₄O₄: C, 60.00; H, 3.45; N, 14.73. Found: C, 59.12; H, 3.19; N, 14.27.

4.2.7. 2,4-Dimethyl-2H, 4H-8-(2',5'-dimethoxyphenylamino)pyrimido[4,5-c]isoquinoline-1,3,7,10-tetraone (**9**)

Prepared from **1** to 2,5-dimethoxyaniline (6 h, 81%); violet solid mp 260–261 °C; ¹H NMR (400 MHz, CDCl₃): δ 3.49 (s, 3H, 4-NMe), 3.76 (s, 3H, 2-NMe), 3.80 (s, 3H, OMe), 3.88 (s, 3H, OMe), 6.62 (s, 1H, 9-H), 6.67 (dd, 1H, *J* = 8.9, 2.7 Hz, 4'-H), 6.88 (d, 1H *J* = 8.9 Hz, 3'-H), 6.97 (d, 1H, *J* = 2.7 Hz, 6'-H), 7.96 (s, 1H, N-H), 9.27 (s, 1H, 6-H); ¹³C NMR (100 MHz, CDCl₃): δ 29.4, 30.8, 56.1, 56.5, 106.4, 107.3, 107.5, 110.1, 112.2, 121.7, 127.4, 142.5, 145.4, 145.7, 151.1, 152.6, 154.1, 155.4, 158.5, 179.7, 181.1. Anal. Calcd. for C₂₁H₁₈N₄O₆: C, 59.71; H, 4.30; N, 13.26. Found: C, 59.42; H, 4.08; N, 13.33.

4.2.8. 2,4-Dimethyl-2H, 4H-8-(*N*-methylphenylamino)pyrimido[4,5-c]isoquinoline-1,3,7,10-tetraone (**10**)

Prepared from **1** and *N*-methylaniline (16 h, 61%); red solid mp 185–185.5 °C ¹H NMR (400 MHz, CDCl₃): δ 3.43 (s, 3H, Me), 3.50 (s, 3H, 4-NMe), 3.74 (s, 3H, 2-NMe), 6.17 (s, 1H, 9-H), 7.12 (d, 2H, 2'- and 6'-H), 7.32 (t, 1H, 4'-H), 7.42 (t, 2H, 3'- and 5'-H), 9.07 (s, 1H, 6-H); ¹³C NMR (100 MHz, CDCl₃): δ 29.2, 30.6, 43.1, 106.3, 111.9, 123.0, 125.4(2C), 126.9, 129.8(2C), 144.6, 147.2, 150.4, 151.0, 152.5, 154.7, 158.7, 179.4, 180.5. Anal. Calcd. for C₂₀H₁₆N₄O₄: C, 63.82; H, 4.28; N, 14.89. Found: C, 63.45; H, 3.98; N, 14.67.

4.2.9. 2,4-Dimethyl-2H, 4H-8-(*N*-ethylphenylamino)pyrimido[4,5-c]isoquinoline-1,3,7,10-tetraone (**11**)

Prepared from **1** and *N*-ethylaniline (16 h, 55%); red solid mp 212–213 °C ¹H NMR (400 MHz, CDCl₃): δ 1.28 (t, 3H, *J* = 7.09 Hz, CH₃CH₂), 3.49 (s, 3H, 4-NMe), 3.74 (s, 3H, 2-NMe), 3.87 (q, 2H, *J* = 7.09 Hz, CH₃CH₂), 6.09 (s, 1H, 9-H), 7.11 (m, 2H, 2'- and 6'-H), 7.34 (m, 1H, 4'-H), 7.43 (m, 2H, 3'- and 5'-H), 9.07 (s, 1H, 6-H); ¹³C NMR (100 MHz, CDCl₃): δ 11.7, 29.3, 30.6, 41.0, 106.4, 112.1, 113.1 (2C), 117.5, 122.8, 129.3(2C), 147.2, 149.3, 150.5, 151.1, 152.6, 155.5, 158.5, 179.5, 180.4. Anal. Calcd. for C₂₁H₁₈N₄O₄: C, 64.61; H, 4.65; N, 14.35. Found: C, 64.87; H, 4.72; N, 14.66.

4.2.10. 2,4-Dimethyl-2H, 4H-8-(*n*-butylamino)pyrimido[4,5-c]isoquinoline-1,3,7,10-tetraone (**12**)

Prepared from **1** and *n*-butylamine (3 h, 75%); red solid mp 188–189 °C ¹H NMR (400 MHz, CDCl₃): δ 0.99 (t, 3H, *J* = 7.4 Hz, CH₃C₃H₇), 1.45 (m, 2H, CH₃CH₂C₂H₄), 1.70 (m, 2H, C₂H₅CH₂CH₂), 3.20 (m, 2H, CH₂C₃H₇), 3.50 (s, 3H, 4-NMe), 3.77 (s, 3H, 2-NMe), 5.69 (s, 1H, 9-H), 5.82 (s, 1H, N-H), 9.39 (s, 1H, 6-H); ¹³C NMR (100 MHz, CDCl₃): δ 13.7, 20.2, 29.3, 30.4, 30.6, 42.5, 103.4, 107.4, 116.4, 144.2, 150.7, 151.3, 151.5, 154.5, 158.4, 180.2, 180.4. Anal. Calcd. for C₁₇H₁₈N₄O₄: C, 59.64; H, 5.30; N, 16.37. Found: C, 59.78; H, 5.45; N, 15.98.

4.2.11. 2,4-Dimethyl-2H, 4H-8-(4-morpholino)pyrimido[4,5-c]isoquinoline-1,3,7,10-tetraone (**13**)

Prepared from **1** and morpholine (4 h, 74%); red solid mp 198–199 °C ¹H NMR (400 MHz, CDCl₃): δ 3.49 (s, 3H, 4-NMe), 3.56 (m, 4H, 3'- and 5'-H), 3.75 (s, 3H, 2-NMe), 3.86 (m, 4H, 2'- and 6'-H), 6.10 (s, 1H, 9-H), 9.20 (s, 1H, 6-H); ¹³C NMR (100 MHz, CDCl₃): δ 29.1, 30.5, 48.8(2C), 66.3(2C), 106.2, 112.0, 123.1, 144.1, 150.9, 151.5, 152.6, 154.6, 158.3, 180.3, 180.4. Anal. Calcd. for C₁₇H₁₆N₄O₅: C, 57.30; H, 4.53; N, 15.72. Found: C, 57.02; H, 4.32; N, 15.55.

4.2.12. 2,4-Dimethyl-2H, 4H-8-(cyclohexylamino)pyrimido[4,5-*c*]isoquinoline-1,3,7,10-tetraone (**14**)

Prepared from **1** and cyclohexylamine (4 h, 49%): red solid mp 178–179 °C ¹H NMR (400 MHz, CDCl₃): δ 1.33–2.05 (m, 10H, 5 × CH₂), 3.30 (m, 1H, 1'-H), 3.46 (s, 3H, 4-NMe), 3.72 (s, 3H, 2-NMe), 5.80 (s, 1H, 9-H), 5.88 (d, 1H, *J* = 5.9 Hz, N-H), 9.15 (s, 1H, 6-H); ¹³C NMR (100 MHz, CDCl₃): δ 24.4, 25.3, 29.4, 30.8, 32.0, 51.7, 104.6, 107.3, 121.3, 143.4, 145.5, 150.9, 153.4, 155.3, 158.4, 180.2, 180.4. Anal. Calcd. for C₁₉H₂₀N₄O₄: C, 61.95; H, 5.47; N, 15.21. Found: C, 61.77; H, 5.31; N, 15.03.

4.2.13. 2,4-Dimethyl-2H, 4H-8-(1-adamantylamino)pyrimido[4,5-*c*]isoquinoline-1,3,7,10-tetraone (**15**)

Prepared from **1** and 1-adamantylamine (24 h, 6%): red solid mp 110–111 °C ¹H NMR (400 MHz, CDCl₃): δ 1.73 (m, 6H, adamantyl), 2.03 (m, 6H, adamantyl), 2.19 (m, 3H, adamantyl), 3.49 (s, 3H, 4-NMe), 3.74 (s, 3H, 2-NMe), 5.81 (s, 1H, N-H), 6.14 (s, 1H, 9-H), 9.19 (s, 1H, 6-H); ¹³C NMR (100 MHz, CDCl₃): δ 29.3, 29.3, 30.7, 36.2, 41.2, 52.8, 104.9, 107.5, 121.5, 144.3, 145.7, 150.7, 152.9, 155.4, 158.4, 180.3, 180.4. Anal. Calcd. for C₂₃H₂₄N₄O₄: C, 65.70; H, 5.75; N, 13.33. Found: C, 65.54, H, 5.77, N, 13.27.

4.2.14. 2,4,6-Trimethyl-2H, 4H-8-(phenylamino)pyrimido[4,5-*c*]isoquinoline-1,3,7,10-tetraone (**16**)

Prepared from **2** and aniline (2 h, 99%): red solid mp 222–223 °C; ¹H NMR (400 MHz, CDCl₃): δ 3.05 (s, 3H, 6-Me), 3.51 (s, 3H, 2-NMe), 3.78 (s, 3H, 4-NMe), 6.51 (s, 1H, 9-H), 7.28 (m, 3H, 2'-, 4'- and 6'-H), 7.47 (t, 2H, *J* = 8.0 Hz, 3'- and 5'-H), 7.65 (s, 1H, N-H). ¹³C NMR (100 MHz, CDCl₃): δ 27.0, 29.1, 30.2, 103.9, 106.2, 119.9, 122.3(2C), 125.7, 129.8(2C), 137.2, 144.5, 148.9, 151.2, 153.1, 158.6, 165.6, 180.2, 182.0. Anal. Calcd. for C₂₀H₁₆N₄O₄: C, 63.82; H, 4.28; N, 14.89. Found: C, 63.77; H, 3.99; N, 14.93.

4.2.15. 2,4,6-Trimethyl-2H, 4H-8-(4'-hydroxyphenylamino)pyrimido[4,5-*c*]isoquinoline-1,3,7,10-tetraone (**17**)

Prepared from **2** and *p*-hydroxyaniline (6 h, 46%): red solid mp 281–281.5 °C; ¹H NMR (400 MHz, DMSO-*d*₆): δ 2.91 (s, 3H, 6-Me), 3.25 (s, 3H, 2-NMe), 3.57 (s, 3H, 4-NMe), 5.87 (s, 1H, 9-H), 6.96 (d, 2H, *J* = 8.9 Hz, 2'- and 6'-H), 7.19 (d, 2H, *J* = 8.9, 3'- and 5'-H), 9.13 (s, 1H, N-H), 9.57 (s, 1H, O-H); ¹³C NMR (100 MHz, DMSO-*d*₆): δ 27.0, 29.2, 30.2, 103.0, 106.6, 120.1, 122.1(2C), 125.9, 129.3(2C), 137.4, 145.9, 148.5, 151.2, 153.0, 158.6, 165.6, 180.2, 181.6. Anal. Calcd. for C₂₀H₁₆N₄O₅: C, 61.22; H, 4.11; N, 14.28. Found: C, 61.58; H, 4.60; N, 14.61.

4.2.16. 2,4,6-Trimethyl-2H, 4H-8-(4'-methoxyphenylamino)pyrimido[4,5-*c*]isoquinoline-1,3,7,10-tetraone (**18**)

Prepared from **2** and *p*-anisidine (3 h, 99%): red solid mp 242–243 °C; ¹H NMR (400 MHz, CDCl₃): δ 3.00 (s, 3H, 6-Me), 3.47 (s, 3H, 2-NMe), 3.74 (s, 3H, 4-NMe), 3.84 (s, 3H, OMe), 6.27 (s, 1H, 9-H), 6.94 (d, 2H, *J* = 8.3 Hz, 2'- and 6'-H), 7.18 (d, 2H, *J* = 8.3 Hz, 3'- and 5'-H), 7.47 (s, 1H, N-H); ¹³C NMR (100 MHz, CDCl₃): δ 26.9, 29.1, 30.2, 55.6, 103.0, 106.3, 115.0(2C), 120.0, 124.5(2C), 129.8, 145.4, 149.2, 151.2, 153.0, 157.8, 158.6, 165.6, 180.3, 181.7. Anal. Calcd. for C₂₁H₁₈N₄O₅: C, 62.06; H, 4.46; N, 13.79. Found: C, 62.17; H, 4.53; N, 13.87.

4.2.17. 2,4,6-Trimethyl-2H, 4H-8-(4'-fluorophenylamino)pyrimido[4,5-*c*]isoquinoline-1,3,7,10-tetraone (**19**)

Quinone **2** (50 mg, 0.1753 mmol) was reacted with *p*-fluoroaniline [prepared from *p*-fluoronitrobenzene (141.1 mg, 1 mmol) and iron powder (1 g, 17.9 mmol)] to give **22** (3 h, 72%), red solid mp 216–217 °C; ¹H NMR (400 MHz, CDCl₃): δ 3.00 (s, 3H, 6-Me), 3.47 (s, 3H, 4-NMe), 3.74 (s, 3H, 2-NMe), 6.30 (s, 1H, 9-H), 7.13 (m, 2H, 2'- and 6'-H), 7.24 (m, 2H, 3'- and 5'-H), 7.50 (s, 1H, N-H). ¹³C NMR (100 MHz, CDCl₃): δ 26.9, 29.1, 30.2, 103.7, 106.3, 116.9 (d, 1C,

J = 22.9 Hz, 3'- and 5'-C), 119.8, 124.8 (d, 1C, *J* = 8.3 Hz, 2'- and 6'-C), 133.3 (d, 1C, 4'-C, *J* = 2.7 Hz), 145.2, 147.2, 150.9, 152.7, 158.6, 160.6 (d, 1C, *J* = 247 Hz, 4'-C), 165.5, 180.0, 181.8. Anal. Calcd. for C₂₀H₁₅FN₄O₄: C, 60.91; H, 3.83; N, 14.21. Found: C, 60.72; H, 3.95; N, 13.37.

4.2.18. 2,4,6-Trimethyl-2H, 4H-8-(4'-nitrophenylamino)pyrimido[4,5-*c*]isoquinoline-1,3,7,10-tetraone (**20**)

A mixture of *p*-nitroaniline (40.7 mg, 0.3506 mmol), quinone **2** (50 mg, 0.1753 mmol) and montmorillonite KSF (0.5 g) into a 10 mL microwave vial was irradiated at 100 °C and 100 W for 5 min. The mixture was thoroughly washed with CH₂Cl₂, the extract dried over Na₂SO₄ and evaporated under reduced pressure. The residue was column chromatographed (1:1:7 CH₂Cl₂/ethyl acetate/light petroleum) to give **20** (22%): orange solid mp 269–270 °C; ¹H NMR (400 MHz, DMSO-*d*₆): δ 2.93 (s, 3H, 6-Me), 3.27 (s, 3H, 4-NMe), 3.58 (s, 3H, 2-NMe), 6.57 (s, 1H, 9-H), 7.66 (d, 2H, *J* = 9.1 Hz, 2'- and 6'-H), 8.24 (d, 2H, *J* = 9.1 Hz, 3'- and 5'-H), 9.70 (s, 1H, N-H); ¹³C NMR (100 MHz, DMSO-*d*₆): δ 26.5, 28.5, 29.8, 105.4, 106.4, 119.7, 121.4 (2C), 125.1(2C), 142.4, 144.3, 145.0, 146.9, 150.8, 152.5, 158.0, 164.3, 179.5, 181.5. Anal. Calcd. for C₂₀H₁₅N₅O₆: C, 57.01; H, 3.59; N, 16.62. Found: C, 56.76; H, 3.21; N, 17.01. HRMS (*M*⁺): *m/z* calcd for C₂₀H₁₅N₅O₆ [*M*⁺]: 421.10223; found: 421.09972.

4.2.19. 2,4,6-Trimethyl-2H, 4H-8-(2'-methoxyphenylamino)pyrimido[4,5-*c*]isoquinoline-1,3,7,10-tetraone (**21**)

Prepared from **2** and *o*-anisidine (6 h, 95%): red solid mp 266–267 °C; ¹H NMR (400 MHz, CDCl₃): δ 3.03 (s, 3H, 6-Me), 3.48 (s, 3H, 4-NMe), 3.75 (s, 3H, 2-NMe), 3.92 (s, 3H, OMe), 6.55 (s, 1H, 9-H), 7.01 (m, 2H, 4'- and 5'-H), 7.16 (m, 1H, 6'-H), 7.41 (m, 1H, 3'-H), 8.06 (s, 1H, N-H). ¹³C NMR (100 MHz, CDCl₃): δ 27.0, 29.1, 30.1, 55.7, 102.9, 106.7, 110.5, 114.9, 118.6, 119.9, 121.4, 136.3, 145.3, 147.5, 149.3, 151.4, 153.2, 158.3, 165.2, 180.4, 180.8. Anal. Calcd. for C₂₁H₁₈N₄O₅: C, 62.06; H, 4.46; N, 13.79. Found: C, 61.46; H, 4.11; N, 13.48.

4.2.20. 2,4,6-Trimethyl-2H, 4H-8-(2'-fluorophenylamino)pyrimido[4,5-*c*]isoquinoline-1,3,7,10-tetraone (**22**)

Quinone **2** (50 mg, 0.1753 mmol) was reacted with *o*-fluoroaniline [prepared from *o*-fluoronitrobenzene (141.1 mg, 1 mmol) and iron powder (1 g, 17.9 mmol)] to give **22** (3 h, 65%): red solid mp 211–212 °C; ¹H NMR (400 MHz, CDCl₃): δ 3.03 (s, 3H, 6-Me), 3.48 (s, 3H, 4-NMe), 3.75 (s, 3H, 2-NMe), 6.40 (s, 1H, 9-H), 7.21 (m, 3H, 4'-, 5'- and 6'-H), 7.44 (m, 1H, 3'-H), 7.61 (s, 1H, N-H); ¹³C NMR (100 MHz, CDCl₃): δ 26.9, 29.2, 30.1, 103.9, 107.2, 116.5 (d, 1C, *J* = 19.2 Hz, 3'-C), 120.1, 123.3, 124.9 (d, 1C, *J* = 3.7 Hz, 6'-C), 125.5 (d, 1C, *J* = 11.7 Hz, 1'-C), 126.6 (d, 1C, *J* = 7.6 Hz, 4'-C); 145.4, 149.5, 151.5, 153.4, 155.1 (d, 1C, *J* = 248.1 Hz, 2'-C), 158.4, 165.4, 180.1, 180.4. Anal. Calcd. for C₂₀H₁₅FN₄O₄: C, 60.91; H, 3.83; N, 14.21. Found: C, 61.23; H, 4.01; N, 14.33.

4.2.21. 2,4,6-Trimethyl-2H, 4H-8-(2',5'-dimethoxyphenylamino)pyrimido[4,5-*c*]isoquinoline-1,3,7,10-tetraone (**23**)

Prepared from **2** to 2,5-dimethoxyaniline (6 h, 79%): red solid mp 255–256 °C; ¹H NMR (400 MHz, CDCl₃): δ 3.02 (s, 3H, 6-Me), 3.48 (s, 3H, 4-NMe), 3.75 (s, 3H, 2-NMe), 3.80 (s, 3H, OMe), 3.89 (s, 3H, OMe), 6.59 (s, 1H, 9-H), 6.66 (dd, 1H, *J* = 8.9, 2.6 Hz, 6'-H), 6.88 (d, 1H, *J* = 8.9 Hz, 3'-H), 6.99 (d, 1H, *J* = 2.6 Hz, 4'-H), 8.1 (s, 1H, N-H). ¹³C NMR (100 MHz, CDCl₃): δ 27.0, 29.1, 30.2, 56.0, 56.3, 104.4, 106.2, 107.1, 109.8, 112.0, 127.4, 143.4, 145.2, 148.9, 151.2, 153.0, 153.9, 158.6, 166.0, 180.2, 182.0. Anal. Calcd. for C₂₂H₂₀N₄O₆: C, 60.55; H, 4.62; N, 12.84. Found: C, 60.19; H, 4.49; N, 12.56.

4.2.22. 2,4,6-Trimethyl-2H, 4H-8-(*N*-methylphenylamino)pyrimido[4,5-*c*]isoquinoline-1,3,7,10-tetraone (**24**)

Prepared from **2** and *N*-methylaniline (16 h, 63%): red solid mp 182.5–183.5 °C (d). ¹H NMR (400 MHz, CDCl₃): δ 2.70 (s, 3H, 6-Me),

3.41 (s, 3H, NMe), 3.48 (s, 3H, 4-NMe), 3.72 (s, 3H, 2-NMe), 6.10 (s, 1H, 9-H), 7.11 (d, 2H, $J = 7.6$ Hz, 2'- and 6'-H), 7.27 (m, 1H, 4'-H), 7.42 (t, 2H, $J = 7.8$ Hz, 3'- and 5'-H); ^{13}C NMR (100 MHz, CDCl_3): δ 25.9, 29.0, 30.1, 42.2, 105.0, 108.6, 121.8, 125.1(2C), 126.5, 129.7(2C), 147.1, 147.4, 151.3, 152.3, 154.0, 158.6, 164.4, 181.2, 181.4. Anal. Calcd. for $\text{C}_{21}\text{H}_{18}\text{N}_4\text{O}_4$: C, 64.61; H, 4.65; N, 14.35. Found: C, 64.31; H, 4.64; N, 14.03.

4.2.23. 2,4,6-Trimethyl-2H, 4H-8-(*N*-ethylphenylamino)pyrimido[4,5-*c*]isoquinoline-1,3,7,10-tetraone (**25**)

Prepared from **3** and *N*-ethylaniline (16 h, 58%): red solid mp 205–206 °C ^1H NMR (400 MHz, CDCl_3): δ 1.28 (t, 3H, $J = 7.10$ Hz, CH_3CH_2), 2.70 (s, 3H, 6-Me), 3.48 (s, 3H, 4-NMe), 3.72 (s, 3H, 2-NMe), 3.83 (q, 2H, $J = 7.10$ Hz, CH_3CH_2), 6.04 (s, 1H, 9-H), 7.07 (d, 2H, $J = 7.5$ Hz, 2'- and 6'-H), 7.29 (m, 1H, 4'-H), 7.39 (t, 2H, $J = 7.7$ Hz, 3'- and 5'-H). ^{13}C NMR (100 MHz, CDCl_3): δ 11.8, 26.1, 29.0, 30.2, 41.2, 105.2, 108.3, 112.9(2C), 117.3, 121.5, 129.5(2 \times), 147.3, 148.5, 148.8, 151.3, 152.5, 158.7, 165.4, 180.8, 181.5. Anal. Calcd. for $\text{C}_{22}\text{H}_{20}\text{N}_4\text{O}_4$: C, 65.34; H, 4.98; N, 13.85. Found: C, 65.48; H, 4.68; N, 13.92.

4.2.24. 2,4,6-Trimethyl-2H, 4H-8-(*n*-butylamino)pyrimido[4,5-*c*]isoquinoline-1,3,7,10-tetraone (**26**)

Prepared from **2** and *n*-butylamine (2 h, 75%): red solid mp 146–147 °C ^1H NMR (400 MHz, CDCl_3): δ 0.98 (t, 3H, $J = 7.4$ Hz, $\text{CH}_3\text{C}_3\text{H}_7$), 1.44 (m, 2H, $\text{CH}_3\text{CH}_2\text{C}_2\text{H}_4$), 1.68 (m, 2H, $\text{C}_2\text{H}_5\text{CH}_2\text{CH}_2$), 2.95 (s, 3H, 6-Me), 3.18 (m, 2H, $\text{CH}_2\text{C}_3\text{H}_7$), 3.47 (s, 3H, 4-NMe), 3.73 (s, 3H, 2-NMe), 5.78 (s, 1H, 9-H), 5.91 (s, 1H, N-H); ^{13}C NMR (100 MHz, CDCl_3): δ 13.7, 20.2, 26.8, 29.1, 30.2, 30.3, 42.4, 100.7, 106.4, 120.2, 147.9, 149.6, 151.3, 152.9, 158.8, 185.3, 180.2, 180.8. Anal. Calcd. for $\text{C}_{18}\text{H}_{20}\text{N}_4\text{O}_4$: C, 60.66; H, 5.66; N, 15.72. Found: C, 60.74; H, 5.54; N, 15.34.

4.2.25. 2,4,6-Trimethyl-2H, 4H-8-(4-morpholino)pyrimido[4,5-*c*]isoquinoline-1,3,7,10-tetraone (**27**)

Prepared from **2** and morpholine (3 h, 78%): red solid mp 196–197 °C; ^1H NMR (400 MHz, CDCl_3): δ 2.90 (s, 3H, 6-Me), 3.46 (s, 3H, 4-NMe), 3.49 (m, 4H, 3'- and 5'-H), 3.73 (s, 3H, 2-NMe), 3.86 (m, 4H, 2'- and 6'-H), 6.01 (s, 1H, 9-H); ^{13}C NMR (100 MHz, CDCl_3): δ 26.5, 29.2, 30.3, 48.8(2C), 66.6(2C), 105.2, 108.5, 121.9, 147.3, 151.4, 152.6, 154.5, 158.7, 164.9, 181.6, 181.8. Anal. Calcd. for $\text{C}_{18}\text{H}_{18}\text{N}_4\text{O}_5$: C, 58.37; H, 4.90; N, 15.13. Found: C, 57.76; H, 4.85; N, 14.52.

4.2.26. 2,4,6-Trimethyl-2H, 4H-8-(cyclohexylamino)pyrimido[4,5-*c*]isoquinoline-1,3,7,10-tetraone (**28**)

Prepared from **2** and cyclohexylamine (6 h, 65%): red solid mp 84–85 °C ^1H NMR (400 MHz, CDCl_3): δ 1.36–2.03 (m, 10H, $5\times\text{CH}_2$), 2.94 (s, 3H, 6-Me), 3.29 (m, 1H, 1'-H), 3.46 (s, 3H, 4-NMe), 3.72 (s, 3H, 2-NMe), 5.79 (s, 1H, 9-H), 5.88 (d, 1H, $J = 5.9$ Hz; N-H); ^{13}C NMR (100 MHz, CDCl_3): δ 24.6, 25.7, 26.7, 29.1, 30.2, 31.9, 51.4, 101.3, 106.6, 120.4, 145.6, 150.3, 151.7, 153.0, 158.7, 165.3, 181.3, 181.8. Anal. Calcd. for $\text{C}_{19}\text{H}_{20}\text{N}_4\text{O}_4$: C, 61.95; H, 5.47; N, 15.21. Found: C, 61.97; H, 5.55, N, 15.29.

4.2.27. 2,4,6-Trimethyl-2H, 4H-8-(adamantylamino)pyrimido[4,5-*c*]isoquinoline-1,3,7,10-tetraone (**29**)

Prepared from **2** and 1-adamantylamine (24 h, 16%): red solid mp 244–245 °C (d). ^1H NMR (400 MHz, CDCl_3): δ 1.72 (m, 6H, adamantyl), 2.02 (m, 6H, adamantyl), 2.17 (m, 3H, adamantyl), 2.94 (s, 3H, 6-Me), 3.47 (s, 3H, 4-NMe), 3.72 (s, 3H, 2-NMe), 5.92 (s, 1H, N-H), 6.09 (s, 1H, 9-H). ^{13}C NMR (100 MHz, CDCl_3): δ 26.8, 29.1, 29.3, 30.3, 36.1, 41.1, 52.8, 102.9, 106.1, 120.2, 145.1, 149.3, 151.3, 152.9, 158.7, 165.3, 180.3, 180.8. Anal. Calcd. for $\text{C}_{24}\text{H}_{26}\text{N}_4\text{O}_4$: C, 66.34; H, 6.03, N, 12.89. Found: C, 65.56; H, 5.87; N, 12.50.

4.3. Anticancer assay

The cell lines used in this work were obtained from the American Type Culture Collection (ATCC, Manassas, 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%, as 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, AGS cells in Ham F-12; and HL-60 in RPM1. The MEM medium contained 2 mM L-glutamine, 1 mM sodium pyruvate, and 1.5 g/L sodium hydrogencarbonate. Ham F-12 was supplemented with 2 mM L-glutamine and 1.5 g/L sodium hydrogencarbonate. RPM1 contained 1 mM sodium pyruvate and 2 g/L sodium hydrogencarbonate. All media were supplemented with 10% heat-inactivated FBS, 100 IU/mL penicillin, and 100 $\mu\text{g}/\text{mL}$ 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. 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.

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References

- [1] J.A. Valderrama, J.A. Ibacache, V. Arancibia, J. Rodriguez, C. Theoduloz, Studies on quinones. Part 45: novel 7-aminoisoquinoline-5,8-quinone derivatives with anti-tumor properties on cancer cell lines, *Bioorg. Med. Chem.* 17 (2009) 2894–2901.
- [2] D. Hanahan, R.A. Weinberg, The hallmarks of cancer, *Cell* 100 (2000) 57–70.
- [3] M.O. Foye, *Cancer Chemotherapeutic Agents* (1995) Washington D.C.
- [4] R. Martinez, L. Chacon-Garcia, The search of DNA-intercalators as antitumoral drugs: what it worked and what did not work, *Curr. Med. Chem.* 12 (2005) 127–151.
- [5] I. Wilson, P. Wardman, T.S. Lin, A.C. Sartorelli, One-electron reduction of 2- and 6-methyl-1,4-naphthoquinone bioreductive alkylating agents, *J. Med. Chem.* 29 (1986) 1381–1384.
- [6] R.B. Silverman, *The Organic Chemistry of Drug Design and Drug Action*. Academic Press, New York, 1992.
- [7] T.J. Monks, R.P. Hanzlik, G.M. Cohen, D. Ross, D.G. Graham, Quinone chemistry and toxicity, *Toxicol. Appl. Pharmacol.* 112 (1992) 2–16.
- [8] P.L. Gutierrez, The metabolism of quinone-containing alkylating agents: free radical production and measurement, *Front. Biosci.* 5 (2000) D629–D638.
- [9] R.H. Thomson, *Naturally Occurring Quinones III Recent Advances*. Chapman and Hall, London, 1987.
- [10] G.R. Pettit, J.C. Knight, J.C. Collins, D.L. Herald, R.K. Pettit, M.R. Boyd, V.G. Young, Antineoplastic agents 430. Isolation and structure of cribrostatins 3, 4, and 5 from the republic of maldives cribrochalina species, *J. Nat. Prod.* 63 (2000) 793–798.
- [11] K.V. Rao, J.W. Beach, Streptonigrin and related compounds. 5. Synthesis and evaluation of some isoquinoline analogues, *J. Med. Chem.* 34 (1991) 1871–1879.
- [12] C.K. Ryu, I.K. Lee, S.H. Jung, C.O. Lee, Synthesis and cytotoxic activities of 6-chloro-7-arylamino-5,8-isoquinolinediones, *Bioorg. Med. Chem. Lett.* 9 (1999) 1075–1080.
- [13] K.H. Chung, S.Y. Hong, H.J. You, R.E. Park, C.K. Ryu, Synthesis and biological evaluation of 5-arylamino-1H-benzod[*j*]imidazole-4,7-diones as inhibitor of endothelial cell proliferation, *Bioorg. Med. Chem.* 14 (2006) 5795–5801.
- [14] M. Das Sarma, R. Ghosh, A. Patra, B. Hazra, Synthesis of novel aminoquinonoid analogues of diospyrin and evaluation of their inhibitory activity against murine and human cancer cells, *Eur. J. Med. Chem.* 43 (2008) 1878–1888.

- [15] D. Vásquez, J.A. Rodríguez, C. Theoduloz, J. Verrax, P.B. Calderon, J.A. Valderrama, Synthesis and antitumor evaluation of 8-phenylaminopyrimido[4,5-c]isoquinolinequinones, *Bioorg. Med. Chem. Lett.* 19 (2009) 5060–5062.
- [16] Y.T. Pratt, Quinolinequinones. VI. Reactions with aromatic amines, *J. Org. Chem.* 27 (1962) 3905–3910.
- [17] E.Y. Yoon, H.Y. Choi, K.J. Shin, K.H. Yoo, D.Y. Chi, D.J. Kim, The regioselectivity in the reaction of 6,7-dihaloquinoline-5,8-diones with amine nucleophiles in various solvents, *Tetrahedron Lett.* 41 (2000) 7475–7480.
- [18] A. Defant, G. Guella, I. Mancini, Regioselectivity in the Multi-component synthesis of indolizinoquinoline-5,12-dione derivatives, *Eur. J. Org. Chem.* 2006 (2006) 4201–4210.
- [19] K. Yoshida, M. Ishiguro, H. Honda, M. Yamamoto, Y. Kubo, Regioselective 6-amination and 6-arylation of 5,8-quinolinedione promoted by metal ions, *Bull. Chem. Soc. Jpn.* 61 (1988) 4335–4340.
- [20] J.A. Valderrama, J.A. Ibacache, Regiochemical control in the amination reaction of phenanthridine-7,10-quinones, *Tetrahedron Lett.* 50 (2009) 4361–4363.
- [21] B.J. Mulchin, C.G. Newton, J.W. Baty, C.H. Grasso, W.J. Martin, M.C. Walton, E.M. Dangerfield, C.H. Plunkett, M.V. Berridge, J.L. Harper, M.S. Timmer, B.L. Stocker, The anti-cancer, anti-inflammatory and tuberculostatic activities of a series of 6,7-substituted-5,8-quinolinequinones, *Bioorg. Med. Chem.* 18 (2010) 3238–3251.
- [22] M.C. Alley, D.A. Scudiero, A. Monks, M.L. Hursey, M.J. Czerwinski, D.L. Fine, B.J. Abbott, J.G. Mayo, R.H. Shoemaker, M.R. Boyd, Feasibility of drug screening with panels of human tumor cell lines using a microculture tetrazolium assay, *Cancer Res.* 48 (1988) 589–601.
- [23] A.A. van de Loosdrecht, R.H. Beelen, G.J. Ossenkoppele, M.G. Broekhoven, M.M. Langenhuijsen, A tetrazolium-based colorimetric MTT assay to quantitate human monocyte mediated cytotoxicity against leukemic cells from cell lines and patients with acute myeloid leukemia, *J. Immunol. Methods* 174 (1994) 311–320.
- [24] D.A. Scudiero, R.H. Shoemaker, K.D. Paull, A. Monks, S. Tierney, T.H. Nofziger, M.J. Currens, D. Seniff, M.R. Boyd, Evaluation of a soluble tetrazolium/formazan assay for cell growth and drug sensitivity in culture using human and other tumor cell lines, *Cancer Res.* 48 (1988) 4827–4833.
- [25] M. Aguilar-Martinez, G. Cuevas, M. Jimenez-Estrada, I. Gonzalez, B. Lotina-Hennsen, N. Macias-Ruvalcaba, An experimental and theoretical study of the substituent effects on the redox properties of 2-[(R-phenyl)amine]-1,4-naphthalenediones in acetonitrile, *J. Org. Chem.* 64 (1999) 3684–3694.
- [26] J.A. Valderrama, P. Colonelli, D. Vasquez, M.F. Gonzalez, J.A. Rodriguez, C. Theoduloz, Studies on quinones. Part 44: novel angucyclinone *N*-heterocyclic analogues endowed with antitumoral activity, *Bioorg. Med. Chem.* 16 (2008) 10172–10181.