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Original article

Studies on quinones. Part 47. Synthesis of novel phenylaminophenanthridinequinones as potential antitumor agents☆

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ABSTRACT

In our search for potential anticancer agents, a series of 8- and 9-phenylamino-3,4-tetrahydro-phenanthridine-1,7,10(2*H*)-triones with substituent variations at 6-, 8- and 9-positions were prepared using a highly efficient sequence involving: a) solar photoacylation reactions of benzoquinone with arylaldehydes, b) one-pot procedure for the synthesis of 3,4-dihydrophenanthridine-1,7,10(2*H*)-trione intermediates from acylhydroquinones and c) highly regiocontrolled acid-induced amination reaction of phenanthridinequinones with phenylamines. The members of this series were *in vitro* evaluated using the MTT colorimetric method against one normal cell line and three human cancer cell lines. The SAR analysis indicates that the location of nitrogen substituents on the quinone nucleus, the presence of methyl, phenyl, furyl and thienyl groups at the 6-position and the aromatization of the angular cycloaliphatic ring of the phenylamino-3,4-tetrahydrophenanthridine-1,7,10(2*H*)-trione pharmacophore play key roles in the antitumor activity.

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1. Introduction

There are several anticancer agents that contain the quinoid moiety in their structures. Because of the presence of this electroactive unit, these compounds can undergo a biochemical reduction by one or two electrons that are catalyzed by flavoenzymes in the organism using NADPH as an electron donor. This process leads to semiquinone radical intermediates and subsequent reactions with oxygen, all of which are believed to be responsible for most of the drug activity [2–4].

Among the broad variety of *N*-heterocyclic quinones with anticancer activity there are examples of naturally occurring aminoquinones containing the isoquinolinequinone scaffold such as cribrostatin 3 [5], caulibugulone A [6] and mansouramycin C [7] (Fig. 1).

Based on this structural feature we are developing a research program directed to the synthesis and antitumor evaluation of new *N*-heterocyclic quinones substituted with alkylamino- and arylamino groups on the quinone ring. In the frame of this objective we have reported a high yield synthesis of substituted aminoisoquinoline-5,8-quinones, by acid-induced amination reaction of a substituted isoquinolinequinone with alkyl- and arylamines [8]. The screening of these compounds on cancer cell lines demonstrates that these aminoisoquinolinequinones express *in vitro* cytotoxic activity against gastric, lung and bladder cancer cell lines. Also, the quantitative structure-activity relationship (QSAR) analysis of the arylaminoisoquinolinequinones reveals that the half wave potential and the lipophilicity are important parameter determining the antitumoral activity on gastric adenocarcinoma and bladder carcinoma cells. We have also reported the synthesis of a variety of aminopyrimido[4,5-*c*]isoquinolinequinone derivatives using acid-induced nucleophilic substitution reactions of pyrimido[4,5-*c*]isoquinolinequinones with amines [1,9]. The antitumor evaluation of these aminoquinones and their precursors on cancer cells indicates, that the insertion of the nitrogen substituents on the quinone ring of the aminopyrimido[4,5-*c*]isoquinolinequinone pharmacophore increases the cytotoxic potency in almost all the evaluated cell lines. The structure-activity relationship (SAR) study reveal 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 [9].

☆ For Part 46 of this series see Ref. [1].

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[1] In memorial to Dr. Jaime A. Rodríguez.

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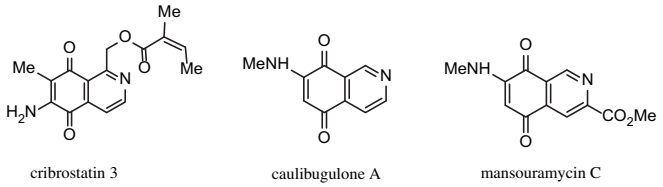


Fig. 1. Structures of naturally occurring isoquinolinequinones with antitumor activity.

Recently we have described preliminary results on the reaction of phenanthridine-7,10-quinones **3a** and **3b** with amines that provides a regioselective access to 8- and 9-amino-3,4-tetrahydrophenanthridine-1,7,10(2*H*)-triones derivatives and the significant antitumor activity observed on some of these members [10]. These results, which suggest that derivatives of this aminoquinone scaffold might be good candidates as antitumor compounds, encouraged us to explore the scope of the amination reaction of various substituted aminophenanthridine-7,10-quinones and the SAR analysis of the series.

During the preparation of this manuscript, the synthesis and cytotoxic evaluation of a number of anilinophenanthridinequinones, prepared from the proper phenanthridine-7,10-quinone, have appeared. The reported aminoquinones display potent cytotoxic activity on breast (MCF-7), lung (NCI-H460, A549), brain (SF268), prostate (DU145), and epothilone-resistant ovarian (A8) cancer cell lines [11].

Herein, we wish to report full details on the access to a broad variety of 8- and 9-phenylamino-3,4-tetrahydrophenanthridine-1,7,10(2*H*)-triones and the *in vitro* antitumor evaluation against normal human lung fibroblasts MRC-5 and three human tumor cells: AGS gastric adenocarcinoma, SK-MES-1 lung, and J82 bladder carcinoma.

2. Chemistry

Phenanthridinequinones **3a**–**e** and a variety of aromatic amines were selected as precursors of the designed phenylaminophenanthridinequinones. Compounds **3a**–**b** were prepared from the commercially available 2,5-dihydroxybenzaldehyde **1a**; 2,5-dihydroxyacetophenone **1b** and 3-aminocyclohex-2-en-1-one **2** by using a previously one-pot procedure [12]. Quinones **3c**–**e** were obtained from acylhydroquinones **1c**–**e** and **2**, employing the above mentioned one-pot procedure (Scheme 1). The access to the precursors **1c**–**e** was performed by solar-chemical photo-Friedel-Crafts acylation of 1,4-benzoquinone with benzaldehyde, furan-2-carbaldehyde and thiophene-3-carbaldehyde, according to a recently reported procedure [13] (Scheme 1).

Recent results on the reaction of phenanthridine-7,10-quinones **3a** and **3b** with aniline indicate that **3a** reacts with aniline in ethanol at room temperature to yield a 40:60 mixture of regioisomers **4a** and **4b** in moderate yield (Scheme 2). The presence of $CeCl_3.7H_2O$ in this reaction induces a change of the regioselectivity and improves the yield of the amination reaction (Table 1). In the case of the reaction of **3b** with aniline, the aminoquinone **10** was isolated in low yield; however the use of the $CeCl_3 \cdot 7H_2O$ catalyst improves the yield of the amination reaction (Table 1) and does not change the regioselectivity (Scheme 2) [10].

In order to explore the scope of the amination reaction, phenanthridinequinones **3a**–**e** were reacted with a variety of phenylamines under the above acid-induced condition and the results are summarized in Table 1. We tried to prepare members of the series of phenylamino-3,4-tetrahydrophenanthridine-1,7,10(2*H*)-triones containing electron-withdrawing substituents in the anilino group by acid-induced amination reaction of phenanthridinequinones **3a** and **3b** with *p*-nitroaniline and 4-aminoacetophenone. However, no reaction of these weak nucleophiles with the quinones **3a** and **3b** were observed even after long period of reflux of the reaction mixtures.

The structure of the new compounds was established on the basis of their nuclear magnetic resonance (^{1}H NMR, ^{13}C NMR, 2D NMR) and high resolution mass spectra (HRMS). The position of the nitrogen substituent in these aminoquinones was determined by means of HMBC experiments. For example, the location of the nitrogen group at C-8 in compound **4a** was deduced by the $^3J_{C,H}$ couplings between the carbon at C-7 (δ 181.42 ppm), with the protons at C-6 (δ 9.26), C-9 (δ 6.43) and that of NH group (δ 7.41). In the case of aminoquinone **10**, the location of the nitrogen substituent at C-8 was established by $^3J_{C,H}$ coupling between the carbon at C-7 (δ 181.58) with the proton at C-9 (δ 6.38), the proton of the NH group (δ 7.69), and by $^4J_{C,H}$ coupling with the protons of the methyl group at C-6 (δ 2.94).

The results show that the amination reaction of quinone **3a** provides access to a mixture of aminophenanthridinequinones containing the nitrogen substituent at 8- and 9-position. According to the ratio between the regioisomers, the reaction of **3a** with the amines proceeds with regioselective preference to give the 8-substituted regioisomer as the main product. Concerning the 6-substituted-aminoquinones **3b**–**e**, the results demonstrate that the amination reaction proceed in high yields and under a regiospecific manner to give the corresponding aminoquinones substituted at 8-position.

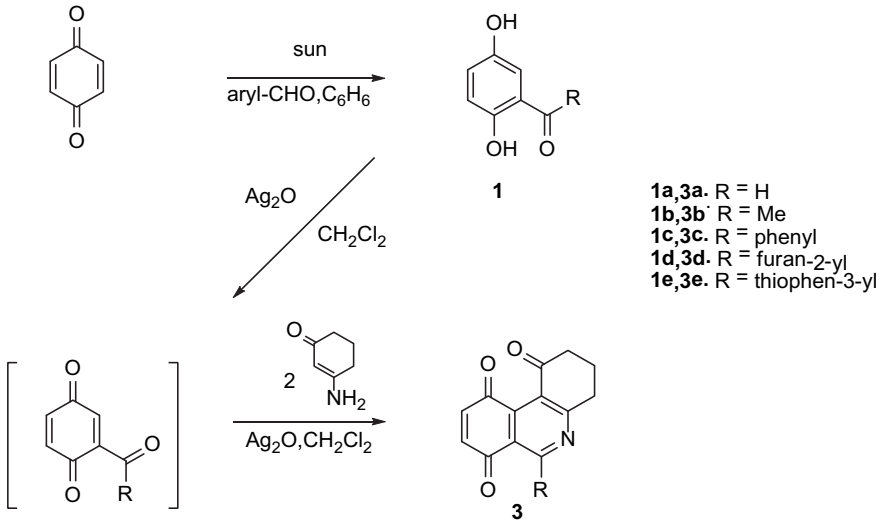
On the basis of the results reported by Pratt [14] on the catalytic action of a Lewis acid, such as cerium chloride, to promote regioselective 6-amination reactions of quinoline-5,8-quinone, we assume that the effect of the $CeCl_3 \cdot 7H_2O$ catalyst to induce a favorable attack of the amines at C-8 in quinones **3a**–**e** 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. The regiospecificity of the substitution reaction on quinones **3b**–**e** can be explained assuming stereoelectronic interactions between the substituent at C-6 with the C-7 carbonyl group. These factors probable affect the electrophilicity of the C-9 atom and the attack of the nucleophiles occurs exclusively at C-8.

The mixtures of the regioisomers arising from the amination reaction of quinone **3a** were purified by column chromatography and the proportion between the isomers was evaluated by ^{1}H NMR, using the signals of the quinone protons. Pure samples of the regioisomers, for characterization and biological evaluation, were obtained by column chromatography of the corresponding mixtures. Attempts to isolated pure samples of regioisomers **5a** and **5b** by chromatography were unsuccessful.

In order to have evidences on the influence of the angular cyclohexanone ring of the phenylamino-3,4-tetrahydro-phenanthridine-1,7,10(2*H*)-trione pharmacophore on the cytotoxic activity, we attempted the preparation of compounds **19** and **20** by aromatization of the corresponding regioisomers **7a** and **7b**. The access to these phenanthridinquinones was achieved by reaction of **7a** and **7b** with $Pd(AcO)_2$ in refluxing acetic acid. Compounds **19** and **20** were isolated in 69 and 63% yields, respectively (Scheme 3).

3. Biological results and discussion

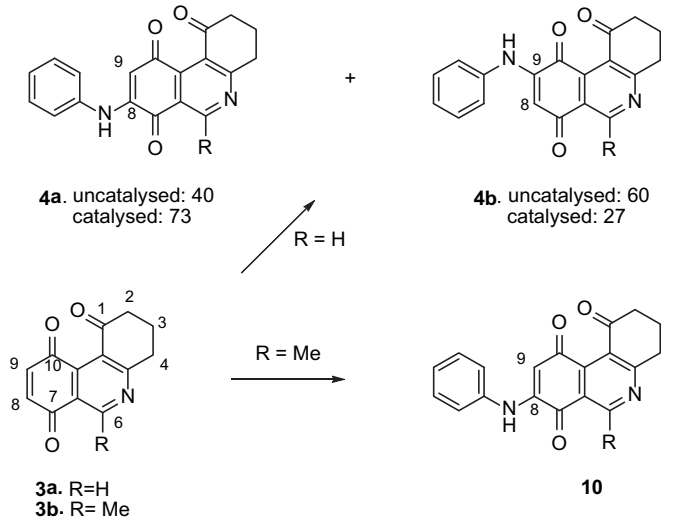
The phenylaminoquinones **4a** + **4b**, **4a**, **4b**, **5a** + **5b**, **6a**, **6b**, **7a**, **7b**, **8a**, **8b**, **9a**, **9b**, **10**–**18** and the parent phenanthridinequinones **3a**–**e** were evaluated for *in vitro* anticancer activity against normal



Scheme 1. Synthesis of phenanthridine-7,10-quinones.

human lung fibroblasts MRC-5 and three human tumor cells: AGS gastric adenocarcinoma, SK-MES-1 lung, and J82 bladder carcinoma, in 72-h drug exposure assays. The cytotoxicity of the compounds was measured using a conventional microculture tetrazolium reduction assay [15–17]. The broad variety of the synthesized compounds was designed in order to gain insight upon the influence of phenylamino groups at the quinone nucleus and also the presence of methyl, phenyl, furyl and thienyl groups at the 6-position of the phenylamino-3,4-tetrahydro-phenanthridine-1,7,10(2*H*)-trione pharmacophore on the biological activity. The cytotoxic and antitumor activities are summarized in Table 2.

The initial SAR analysis was focused on the effects of insertion of the anilino group at the quinone nucleus of compounds **3a**–**e**. The data of Table 2 indicate that, in all cases except **7b**, the insertion of the nitrogen substituent, as in **4a**, **10**, **16**, **17** and **18**, dramatically increases the cytotoxic activity on all the evaluated cell lines, compared to those of their precursors. It is noteworthy that the presence of the anilino group is remarkable on the biological activity in terms of the antitumor activity on gastric adenocarcinoma cells (IC_{50}: 0.23–0.58 μM) and on lung cancer cells (IC_{50}: 1.3–3.1 μM), comparable to that exhibited by the anticancer



Scheme 2. Reactions of **3a** and **3b** with aniline.

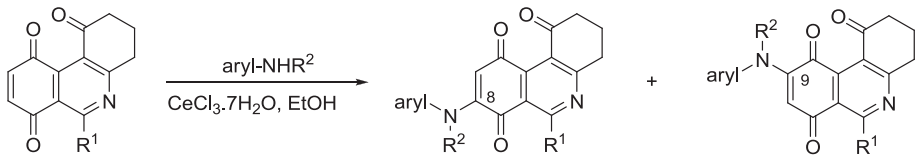
etoposide drug (IC_{50}: AGS: 0.36 μM; SK-MES-1: 2.8 μM), which was included in the assays.

Comparison of the IC_{50} values for the mixture **4a** + **4b** with that for each isomer **4a** and **4b** indicates that **4a** has a greater cytotoxic potency than **4b**, and no synergism occurs between the regioisomers. Similarly, the screening of the couples of regioisomers **6a**/**6b**, **7a**/**7b**, **and 9a**/**b** shows that compounds **6a**, **7a** and **9a** are more cytotoxic than their corresponding regioisomers **6b**, **7b** and **9b**. It can be seen that this difference is remarkable for regioisomers **7a** and **7b**, in particular on gastric cancer cells where the former is 100 times more cytotoxic than the latter. In the case of regioisomers **8a** and **8b**, no significant differences were observed for their cytotoxic activities on the cell lines. These results reveal that phenylamino groups at the 8-position of the phenylamino-3,4-tetrahydro-phenanthridine-1,7,10(2*H*)-trione pharmacophore lead to derivatives with enhanced cytotoxicity respect to the corresponding regioisomers at the 9-position.

Considering that the cytotoxic activity of 1,4-quinones depends largely on their hydrophobic, steric and electronic properties and that hydrophobic and steric properties of the regioisomers of each couple are closely similar, we attributed the difference of the biological activity between the regioisomers of the couples **4a**/**4b**, **6a**/**6b**, **7a**/**7b** and **9a**/**b** to their redox capability. On the basis of this assumption and on precedents on the relationships between redox potentials and antitumor activity of quinones [8,18–21], we decided to evaluate the redox potentials of the regioisomers **4a**/**4b**, **6a**/**6b**, **7a**/**7b** and **9a**/**b** by cyclic voltammetry.

The redox potentials of these compounds were measured by cyclic voltammetry in acetonitrile as solvent, at room temperature, using a platinum electrode and 0.1 M tetraethylammonium tetrafluoroborate as the supporting electrolyte [8]. Well-defined quasi-reversible waves, the cathodic peak related to the reduction of quinone, and the anodic one due to its reoxidation, were observed for the compounds. The voltammograms were run in the potential range 0.0–2.0 V versus non-aqueous Ag/Ag^{+}. The first half-wave potential values, $E^{I}_{1/2}$, evaluated from the voltammograms obtained at a sweep rate of 100 mV s^{-1}, are summarized in Table 3. The $E^{I}_{1/2}$ values for the first electron, which is related with the formation of the semiquinone radical anion, are in the potential range −492 to −399 mV [22]. The data of Table 3 indicate that the insertion of phenylamino substituents at the quinone ring in precursor **3a** induces the displacement of the half wave potential towards more negative values and the magnitude of this effect

Table 1
Reaction of quinone **3a**–**e** with arylamines catalyzed with $CeCl_3 \cdot 7H_2O$.

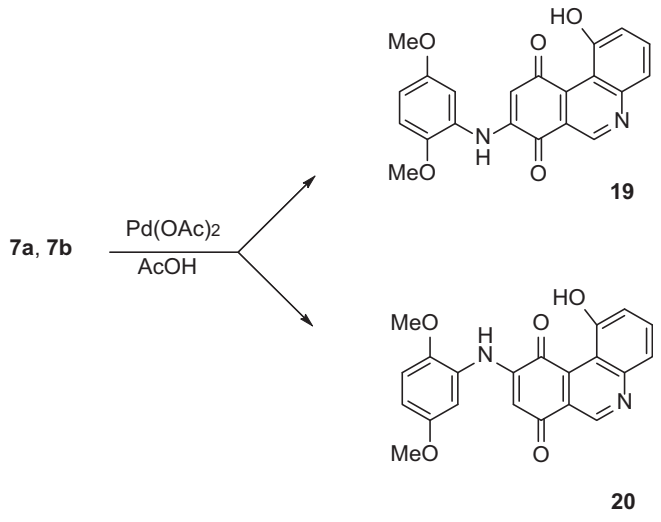


R^1	R^2	Aryl	Time (h)	Products	Yield[a]	*a*/*b*[b]
H	H	Ph	1:40	**4a** + **4b**	88	73/27
H	H	4-HO-Ph	2:20	**5a** + **5b**	68	73/27
H	H	4-MeO-Ph	1:30	**6a** + **6b**	86	73/27
H	H	2,5-$(OMe)_2$-Ph	2	**7a** + **7b**	62	74/26
H	Me	Ph	8	**8a** + **8b**	55	68/32
H	Et	Ph	24	**9a** + **9b**	49	71/29
Me	H	Ph	1:00	**10**	96	100
Me	H	4-HO-Ph	2:50	**11**	55	100
Me	H	4-MeO-Ph	0:53	**12**	79	100
Me	H	4-F-Ph	0:20	**13**	91	100
Me	H	2-MeO-Ph	0:30	**14**	73	100
Me	H	2,5-$(MeO)_2$-Ph	2:40	**15**	48	100
Ph	H	Ph	6:13	**16**	69	100
Furan-2-yl	H	Ph	8:10	**17**	55	100
Thiophen-3-yl	H	Ph	1:30	**18**	45	100

[a] Isolated yields.
[b] Determined by ^1H NMR analysis.

depends on the nature and location of the nitrogen substituents. Comparison of the first half wave potentials between the regioisomers of each couple, reveals that the reduction for the isomers substituted at the 8-position (**4a**, **6a**, **7a** and **9a**) occurs at more negative $E^I_{1/2}$ potentials than that of the corresponding 9-substituted isomer. According to reported precedents on the electronic effect of substituents in 1,4-naphthoquinones on $E^I_{1/2}$ potentials [23], it can be deduced that, in the couples of regioisomers, the nitrogen substituent located at the 8-position has a greater electron-donor capacity than at the 9-position.

Compound **4a** and its phenyl-substituted analogues **6a** and **7a**, showed high cytotoxic activity (range 0.23–2.8 μM) on the cancer cells, whereas compounds **8a** and **9a**, resulting from the replacement of the amino proton in **4a** by an alkyl group, showed moderate activity (range 4.4–15.2 μM). Regarding the regioisomers substituted at the 9-position, it was observed a similar decreasing effect on the cytotoxic activity by substitution of the amino proton in **4b** by a methyl or ethyl group, as in **8b** and **9b**. According to precedents reported by Aguilar–Martínez on 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 [23], the effects of the *N*-alkylanilino substituents in **8a** and **9a** on the biological activity could be related with a weak conjugation degree between the donor and acceptor fragments due to molecular planarity inhibition by steric hindrance induced by the *N*-alkyl groups.



Scheme 3. Preparation of phenenthridinequinones **19** and **20**.

Comparison of IC_{50} values of compounds **4a**, **6a** and **7a** with those of their analogs **10**, **12** and **15** reveals that insertion of a methyl group at the 6-position of the phenylamino-3,4-tetrahydrophenanthridine-1,7,10(2*H*)-trione pharmacophore induces a decrease of the cytotoxic activity on lung fibroblasts and bladder cancer cell lines.

Comparison of the cytotoxic potency of **19** and **20** with their corresponding precursors **7a** and **7b** demonstrate that the replacement of the angular cyclohexenone ring by a fused phenolic ring in phenylaminophenanthridinetriones **7a** and **7b** enhances the cytotoxic potency against all of the tested lines. Evaluation of **19** and **20** also indicates that regioisomer **19**, containing the phenylaminosubstituent at the 8-position, exhibits a higher cytotoxic potency (IC_{50} values: MRC-5 = 0.53 μM; AGS = 0.18 μM; SK-MES-1 = 0.63 μM; J82 = 1.4 μM) than that of its regioisomer **20** (IC_{50} values: MRC-5 = 17.2 μM; AGS = 19.8 μM; SK-MES-1 = 13.5 μM), except on the bladder carcinoma cell line (J82 = 0.61 μM). It is worth to note that although compound **19** exhibits high cytotoxic potency, compound **20** emerges as a promising lead compound as antitumor agent on bladder carcinoma due to the IC_{50} and selective index (SI = IC_{50} fibroblasts/IC_{50} cancer cells) values (IC_{50} = 0.61 μM; SI = 28).

Analysis of the data of Table 2 indicates that, in general, cytotoxicity was observed in all cancer cell lines, but that AGS and

Table 2
Cytotoxic activity of 8- and 9- arylamino-3,4-tetrahydrophenanthridine-1,7,10(2*H*)-triones and their precursors.

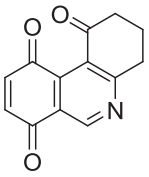
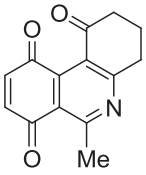
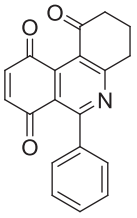
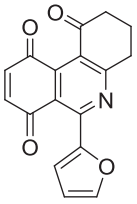
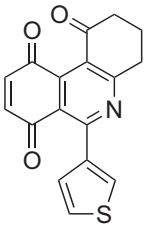
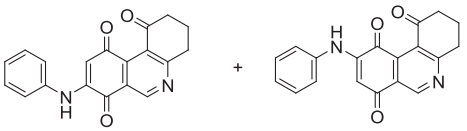
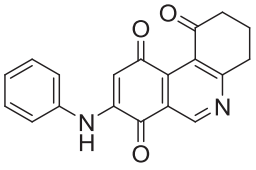
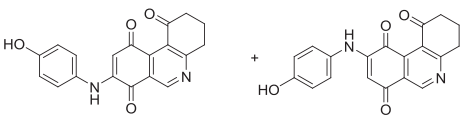
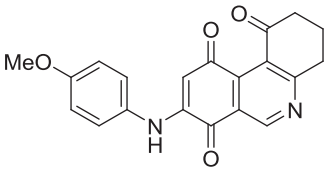
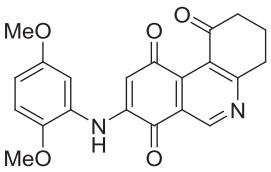
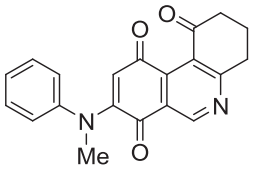
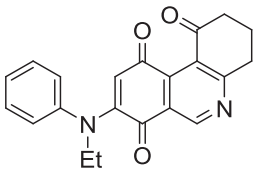
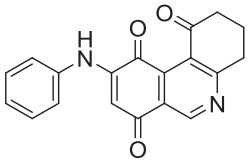
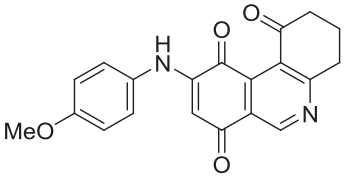
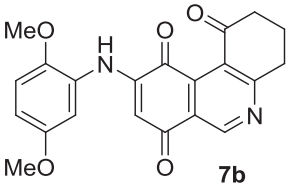
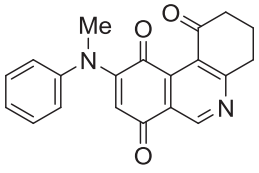
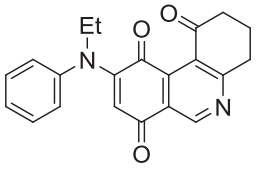
N°	Structure	$IC_{50} \pm SEM$[a] (μM)			
		MRC-5[b]	AGS[c]	SK-MES-1[d]	J82[e]
3a		20.2 ± 1.1	19.4 ± 1.0	25.7 ± 1.7	15.8 ± 0.7
3b		20.5 ± 0.9	22 ± 1.3	3.5 ± 0.2	5.6 ± 0.3
3c		31.2 ± 1.9	13.9 ± 1.1	73.0 ± 4.4	57.6 ± 4.0
3d		59.0 ± 3.5	68.0 ± 3.4	96.9 ± 7.7	80.4 ± 6.4
3e		71 ± 4.9	37.1 ± 2.2	76.7 ± 3.8	>100
(4a + 4b)[f]		4.4 ± 0.2	0.65 ± 0.03	1.8 ± 0.1	6.7 ± 0.3
4a		1.4 ± 0.1	0.23 ± 0.01	1.3 ± 0.1	2.1 ± 0.1
(5a + 5b)[f]		2.3 ± 0.1	0.49 ± 0.02	2.7 ± 0.1	1.8 ± 0.1

Table 2 (*continued*)

N°	Structure	$IC_{50} \pm SEM$[a] (μM)			
		MRC-5[b]	AGS[c]	SK-MES-1[d]	J82[e]
6a		2.6 ± 0.1	0.47 ± 0.02	2.0 ± 0.1	2.8 ± 0.1
7a		2.6 ± 0.1	0.32 ± 0.01	1.8 ± 0.1	2.5 ± 0.1
8a		7.5 ± 0.4	4.7 ± 0.2	10.6 ± 0.6	12.9 ± 0.8
9a		8.6 ± 0.4	4.4 ± 0.2	11.7 ± 0.8	15.2 ± 1.1
4b		5.9 ± 0.4	3.9 ± 0.1	7.5 ± 0.4	8.1 ± 0.4
6b		4.8 ± 0.3	2.2 ± 0.1	3.7 ± 0.2	4.0 ± 0.2
7b	 **7b**	46.4 ± 2.9	32.9 ± 2.0	21.3 ± 1.1	56.7 ± 2.8
8b		7.3 ± 0.4	8.3 ± 0.4	8.7 ± 0.3	8.4 ± 0.4
9b		19.6 ± 1.0	17.1 ± 0.9	17.7 ± 0.9	19.5 ± 1.2

(*continued on next page*)

Table 2 (*continued*)

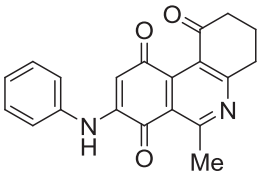
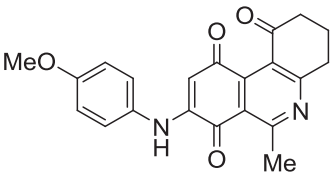
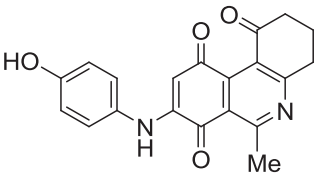
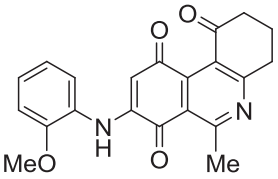
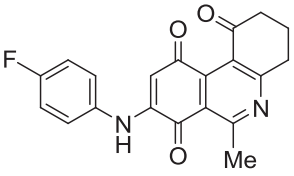
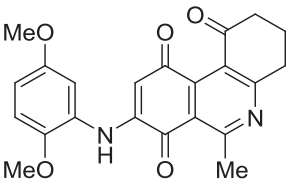
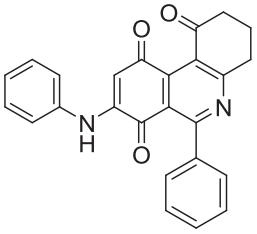
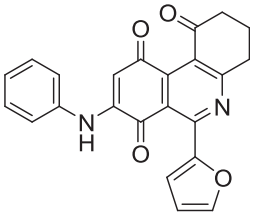
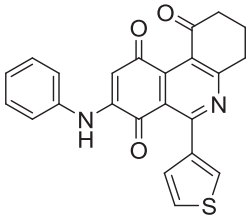
N°	Structure	$IC_{50} \pm SEM$[a] (μM)			
		MRC-5[b]	AGS[c]	SK-MES-1[d]	J82[e]
10		2.0 ± 0.1	0.65 ± 0.03	5.7 ± 0.3	4.2 ± 0.3
11		5.2 ± 0.2	1.2 ± 0.1	4.1 ± 0.2	3.0 ± 0.21
12		2.6 ± 0.1	1.2 ± 0.1	3.2 ± 0.2	1.9 ± 0.1
13		1.6 ± 0.1	0.22 ± 0.01	4.3 ± 0.2	3.7 ± 0.2
14		3.0 ± 0.2	0.79 ± 0.03	3.3 ± 0.2	3.8 ± 0.2
15		5.0 ± 0.2	1.4 ± 0.1	21.7 ± 1.3	7.1 ± 1.0
16		2.7 ± 0.1	0.33 ± 0.02	2.8 ± 0.2	2.9 ± 0.2
17		2.7 ± 0.2	0.58 ± 0.03	3.1 ± 0.2	2.4 ± 0.1

Table 2 (*continued*)

N°	Structure	$IC_{50} \pm SEM$[a] (μM)			
		MRC-5[b]	AGS[c]	SK-MES-1[d]	J82[e]
18		2.8 ± 0.2	0.35 ± 0.02	2.9 ± 0.2	2.5 ± 0.1
Etoposide	–	3.9 ± 0.21	0.36 ± 0.15	2.8 ± 0.18	0.80 ± 0.04

[a] Data represent 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] **4a/4b** = 73/27; **5a/5b** = 73/27.

SK-MES-1 cell lines appear to be more sensitive to the compounds overall. Among the compounds evaluated in the *in vitro* screen and collected in Table 2, the members **4a**, **7a**, **16** and **18** (Fig. 2) were selected for this study as the more significant antitumor members on gastric adenocarcinoma and lung cancer cell lines, according to their IC_{50} and SI values (~6.0–8.0). Compound **20** was also selected and was considered as a promising lead compound due to its high potency and selective index on bladder carcinoma cell line. Further ways to prepare new analogs of compounds **4a**, **7a**, **16**, **18** and **20** are under study.

4. Conclusions

We have developed the regiospecific synthesis of a variety of phenylamino-3,4-tetrahydrophenanthridine-1,7,10(2*H*)-trione 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 insertion of a phenylamino group into the quinone nucleus of compounds **3a**–**e** increases the cytotoxic potency, with respect to their precursors, in almost all the evaluated cell lines. From the current investigation, structure activity relationships of the phenylaminoquinone members demonstrate that phenylamino substituents at the 8-position of the quinone ring show increased antitumor activity than those at the 9-position. The effect of such substitution is more significant in enhancing the antitumor activity for those members unsubstituted at the 6-position and substituted with phenyl, furyl and thienyl groups. The 8-arylamino-3,4-tetrahydrophenanthridine-1,7,10(2*H*)-triones **4a**, **7a**, **16**, and **18** exhibit interesting antitumor activity and selective index on gastric cancer cells, comparable to that of etoposide. The replacement of the angular cyclohexanone ring in compounds **7a** and **7b** by a fused phenolic ring, as in compound **19** and **20**, improves the cytotoxic potency on some of the tested cell lines. This substitution is highly relevant on regioisomer **7b** because the resulting analog **20** is endowed with high antitumor potency and selective index on bladder carcinoma cells.

Given the high incidence of undesirable side-effects induced by the majority of current anticancer drugs and by considering the selective index of aminoquinones **4a**, **7a**, **16**, **18** and, in particular, that of phenylaminophenanthridinequinone **20**, they appear as promising and interesting leads, endowed of potential anticancer activity. These results prompt us to design and synthesize more new 6-aryl-substituted 8-phenylaminophenanthridinequinones in order to discover more active and selective anticancer agents.

Table 3
First half-wave potentials and cytotoxic activity of 8- and 9- arylamino-3,4-tetrahydrophenanthridine-1,7,10(2*H*)-triones.

N°	$-E^{I}_{1/2}$ (mV)	$IC_{50} \pm SEM$ (μM)			
		MRC-5	AGS	SK-MES-1	J82
3a	367	20.2 ± 1.1	19.4 ± 1.0	25.7 ± 1.7	15.8 ± 0.7
4a	452	1.4 ± 0.1	0.23 ± 0.01	1.3 ± 0.1	2.1 ± 0.1
4b	430	5.9 ± 0.4	3.9 ± 0.1	7.5 ± 0.4	8.1 ± 0.4
6a	492	2.6 ± 0.1	0.47 ± 0.02	2.0 ± 0.1	2.8 ± 0.1
6b	460	4.8 ± 0.3	2.2 ± 0.1	3.7 ± 0.2	4.0 ± 0.2
7a	440	2.6 ± 0.1	0.32 ± 0.01	1.8 ± 0.1	2.5 ± 0.1
7b	420	46.4 ± 2.9	32.9 ± 2.0	21.3 ± 1.1	56.7 ± 2.8
9a	451	8.6 ± 0.4	4.4 ± 0.2	11.7 ± 0.8	15.2 ± 1.1
9b	399	19.6 ± 1.0	17.1 ± 0.9	17.7 ± 0.9	19.5 ± 1.2

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. ^{1}H NMR spectra were recorded on Bruker AM-200 and AM-400 instruments in deuterochloroform ($CDCl_3$). ^{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 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. Silica gel Merck 60 (70–230 mesh) was used for preparative column chromatography, and TLC aluminum foil $60F_{254}$ for analytical TLC.

Anhydrous acetonitrile (99.8%) for electrochemical evaluations was obtained from Sigma-Aldrich. Quinones **3a** and **3b** were prepared according to a previously reported procedure [11] and acylhydroquinones were prepared by using a recently reported solar photoacylation procedure [12].

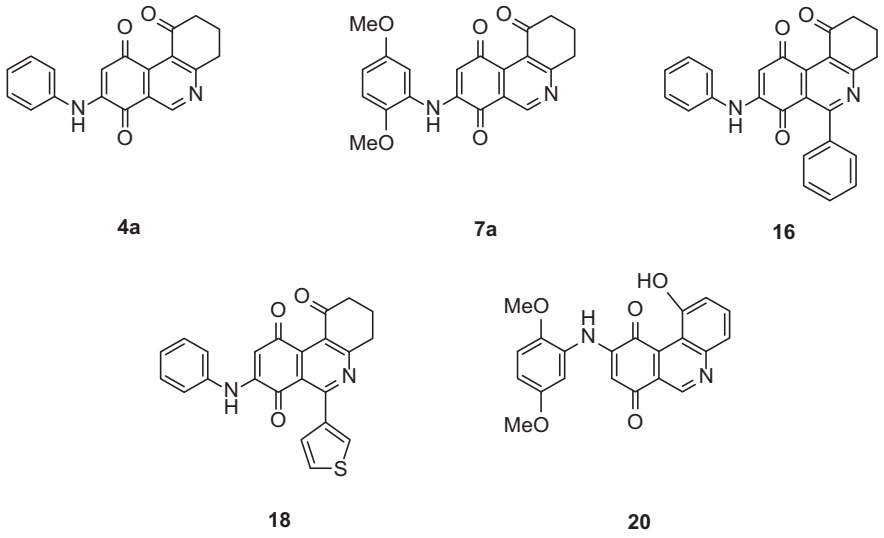


Fig. 2. Structures of selected quinones as the more significant antitumor members.

5.2. General procedure for the synthesis of acylhydroquinones ***1c–e*** [12]

A 100 mL benzene solution of 1,4-benzoquinone (1 mmol) and the required aldehyde (7.5 mmol), was placed into the outer jacket of a Liebig condenser type. The solution was bubbled with nitrogen (2 min), sealed with a septum and then irradiated for six days (total illumination time of 30 h), under solar radiation conditions in the range 800–1100 Watts/m^2 (January–March) The solvent was evaporated under reduced pressure and the residue was chromatographed on silica gel (3:1 petroleum ether/ethyl acetate). The starting aldehyde and the solvent were recovered and employed in the next batches.

The solar photolysis were performed at the Canchones Experimental Center in Iquique/Chile (latitude 20°26′43.80″ S, 990 m above sea level), which is located in the Atacama desert.

5.2.1. 2,5-Dihydroxybenzophenone (***1c***)

Prepared from 1,4-benzoquinone and benzaldehyde (91%), orange solid, mp 121–123 °C (lit. [24]: 125–126 °C), IR (KBr): cm^{-1} 3456 (OH), 1687 (C=O), 1225 (C–O). ^{1}H NMR (200 MHz, $CDCl_3$): δ 6.91 (m, 2H, 4′- + 6′-H), 6.96 (s, 1H, 6-H), 7.04 (m, 2H, 5′- or 3′-H and 2′-H), 8.18 (d, 1H, J = 6.9 Hz, 3- or 4-H), 8.06 (d, 1H, J = 6.9 Hz, 4- or 3-H), 7.12 (m, 2H, 3′- or 5′-H + 5-OH), 11.34 (bs, 1H, 2-OH). ^{13}C NMR (50 MHz, $CDCl_3$): δ 119.6, 119.9, 123.4, 125.5, 129.1, 129.5, 129.7, 130.4, 130.6, 132.7, 138.9, 149.9, 206.1.

5.2.2. (2,5-dihydroxyphenyl)(furan-2-yl)methanone (***1d***)

Prepared from 1,4-benzoquinone and furan-2-carbaldehyde (88%) orange solid, mp, 125.5–126.5 °C. IR (KBr): cm^{-1} 3330 (O–H), 1562 (C=O). ^{1}H NMR (200 MHz, acetone-d_6): δ 6.66 (s, 1H, 6-H), 6.80 (dd, 1H, J = 3.6, 2.7 Hz, 5′- or 4′-H), 7.12 (d, 1H, J = 8.3 Hz, 4- or 3-H), 7.79 (d, 1H, J = 8.3 Hz, 3- or 4-H), 7.49 (m, 1H, 3′-H), 8.01 (dd, 1H, J = 3.6, 2.7 Hz, 4′- or 5′-H), 8.21 (bs, 1H, 5-OH), 11.45 (s, 1H, 2-OH). ^{13}C NMR (50 MHz, acetone-d_6): δ 113.3, 116.5, 116.7, 119.4, 121.8, 125.3, 148.8, 150.2, 152.7, 157.2, 185.1. Anal. Calcd for $C_{11}H_8O_4$: C, 64.71; H, 3.95; found: C, 64.80; H, 3.85.

5.2.3. (2,5-Dihydroxyphenyl)(thiophen-3-yl)methanone (***1e***)

Prepared from 1,4-benzoquinone and thiophene-3-carbaldehyde (87%), orange solid, mp 135–136 °C. IR (KBr): cm^{-1} 3334 (O–H), 1581(C=O). ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.30 (s, 1H, 6-H), 7.49 (d, 1H, J = 3.0 Hz, 5′- or 4′-H), 7.71 (d, 1H, J = 8.2 Hz, 4- or 3-H), 7.85 (d, 1H, J = 8.2 Hz, 3- or 4-H), 7.89 (m, 1H, 4′- or 5′-H), 8.32 (s, 1H, 2′-H), 8.38 (s, 1H, 5-OH), 11.74 (s, 1H, 2-OH). ^{13}C NMR (50 MHz, $CDCl_3$): δ 116.9, 118.5, 122.1, 124.4, 125.9, 128.1, 132.1, 140.1, 148.7, 156.0, 193.6. Anal. Calcd for $C_{11}H_8O_3S$: C, 59.99; H, 3.66; S, 14.56; found: C, 59.93; H, 3.65; S, 14.17.

5.3. General procedure for the preparation of 8- and 9-phenylamino-3,4-dihydrophenanthridine-1,7,10(2H)-triones

A suspension of quinone **3a** (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 and the organic layer was washed with water (3 × 15 mL). The dried extract was evaporated under reduced pressure and the residue was column chromatographed (10:90 AcOEt/petroleum ether) to yield the mixture of regioisomers. These were analysed by ^{1}H NMR to evaluate the proportion between the 8- and 9-phenylaminophenanthridinequinone derivatives. Column chromatography of the mixture, using CH_2Cl_2 as eluent, provided pure samples of the regioisomers.

5.3.1. 8- and 9-Phenylamino-3,4-dihydrophenanthridine-1,7,10(2H)-trione (***4a***, ***4b***) [10]

The mixture of regioisomers was prepared from **3a** and aniline (1:40 h, 88%); red solid; isomers proportion: 73:27 = **4a**:**4b**. Compound **4a** (less polar): red solid, mp 179–182 °C; IR (KBr): cm^{-1} 3441 (N–H), 1668 (C=O), 1610, 1566 (C=O quinone). ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.21 (q, J = 6.5 Hz, 2H, 3-H), 2.89 (t, J = 6.5 Hz, 2H, 2-H), 3.13 (t, J = 6.5 Hz, 2H, 4-H), 6.43 (s, 1H, 9-H), 7.23 (m, 3H, arom.), 7.25 (m, 2H, arom.), 7.41 (s, 1H, NH), 9.24 (s, 1H, 6-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 21.37, 33.40, 39.13, 104.99, 122.67, 124.25 (2C), 126.09, 128.90 (2C), 129.84, 136.92, 140.45, 143.74, 149.66, 169.79, 180.80, 181.42, 198.00; HRMS (M^+): m/z calcd for $C_{19}H_{14}N_2O_3$: 318.10044; found: 318.10012.

Compound **4b**: red solid, mp 182–184 °C; IR (KBr): cm^{-1} 3447 (N–H), 1703 (C=O), 1607, 1590 (C=O quinone). ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.22 (q, J = 6.5 Hz, 2H, 3-H), 2.89 (t, J = 6.5 Hz, 2H, 2-H), 3.18 (t, J = 6.5 Hz, 2H, 4-H), 6.31 (s, 1H, 8-H), 7.23 (m, 3H, arom.),

7.25 (m, 2H, arom.), 7.41 (s, 1H, NH), 9.33 (s, 1H, 6-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 21.50, 33.39, 39.26, 102.24, 122.67, 124.25 (2C), 126.25, 128.89 (2C), 129.83, 136.93, 140.44, 143.75, 150.75, 167.65, 180.79, 181.97, 197.62. HRMS (M^+): *m*/*z* calcd for $C_{19}H_{14}N_2O_3$: 318.10044; found: 318.10005.

5.3.2. 8- and 9-(4-Hydroxyphenylamino)-3,4-dihydrophenanthridine-1,7,10(2H)-trione (**5a**,**b**)

The mixture of regioisomers was prepared from **3a** and *p*-hydroxyaniline (2:20 h, 68%), purple solid, proportion of regioisomers: 73:27 = **5a**:**5b**. ^{1}H NMR (400 MHz, acetone-d_6): δ 2.22 (q, *J* = 6.4 Hz, 2H, 3-H), 2.90 (t, *J* = 6.4 Hz, 2H, 2-H), 3.12 (t, *J* = 6.4 Hz, 2H, 4-H), 5.96 (s, 0.27H, 8-H), 6,04 (s, 0.73H, 9-H), 6,95 (d, *J* = 8,4 Hz, 2H, arom), 7.25 (d, *J* = 8.4 Hz, 2H, arom), 8.06 (s, 1H, OH), 8.51 (s, 1H, NH), 9.15 (s, 0.27H, 6-H); 9,17 (s, 0.73H, 6-H).

5.3.3. 8- and 9-(4-Methoxyphenylamino)-3,4-dihydrophenanthridine-1,7,10(2H)-trione (**6a**, **6b**)

The mixture of regioisomers was prepared from **3a** and *p*-anisidine, (1:30 h, 86%); purple solid, isomers proportion: 73:27 = **6a**:**6b**. Compound **6a** (less polar): purple solid mp 162.5–164.5 °C; IR (KBr): cm^{-1} 3448 (N–H), 1674 (C=O), 1611, 1598 (C=O quinone). ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.21 (q, *J* = 6.6 Hz, 2H, 3-H), 2.87 (t, *J* = 6.6 Hz, 2H, 2-H), 3.11 (t, *J* = 6.6 Hz, 2H, 4-H), 3.80 (s, 3H, OMe), 6.22 (s, 1H, 9-H), 6.92 (d, *J* = 8.8 Hz, 2H, 2′- and 6′-H), 7.16 (d, *J* = 8.8 Hz, 2H, 3′- and 5′-H), 7.40 (s, 1H, NH), 9.20 (s, 1H, 6-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 21.35, 33.35, 39.13, 55.59, 104.13, 115.04 (2C), 124.33, 124.81 (2C), 128.96, 129.48, 140.63, 144.60, 149.53, 157.97, 169.69, 180.87, 181.16, 198.15. HRMS (M^+): *m*/*z* calcd for $C_{20}H_{16}N_2O_4$: 348.1110; found: 348. 1107.

Compound **6b**: purple solid, mp 168–169 °C; IR (KBr): cm^{-1} 3442 (N–H), 1703 (C=O), 1599, 1510 (C=O quinone). ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.24 (q, *J* = 6.8 Hz, 2H, 3-H), 2.91 (t, *J* = 6.8 Hz, 2H, 2-H), 3.14 (t, *J* = 6.8 Hz, 2H, 4-H), 3.83 (s, 3H, OMe), 6.15 (s, 1H, 8-H), 6.97 (d, *J* = 8.9 Hz, 2H, 2′- and 6′-H), 7.16 (d, *J* = 8.9 Hz, 2H, 3′- y 5′-H), 7.42 (s, 1H, NH), 9.35 (s, 1H, 6-H). HRMS (M^+): *m*/*z* calcd for $C_{20}H_{16}N_2O_4$: 348.1110; found: 348.1102.

5.3.4. 8- and 9-(2,5-Dimethoxyphenylamino)-3,4-dihydrophenanthridine-1,7,10(2H)-trione (**7a**,**b**)

The mixture of regioisomers was prepared from **3a** and 2,5-dimethoxyaniline, (2 h, 62%), purple solid, isomers proportion: 74:26 = **7a**:**b**. Compound **7a** (less polar): purple solid, mp 179–181 °C; IR (KBr): cm^{-1} 3449 (N–H), 1705 (C=O), 1625, 1593 (C=O quinone). ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.27 (q, *J* = 6.6 Hz, 2H, 3-H), 2.90 (t, *J* = 6.5 Hz, 2H, 2-H), 3.16 (t, *J* = 6.5 Hz, 2H, 4-H), 3.80 (s, 3H, OMe), 3.88 (s, 3H, OMe), 6.56 (s, 1H, 9-H), 6.68 (dd, *J* = 8.5; 3.4 Hz, 1H, 4′-H), 6.88 (d, *J* = 8.5 Hz, 1H, 3′-H), 6.98 (d, *J* = 3.4 Hz, 1H, 6′-H), 7.96 (s, 1H, NH), 9.27 (s, 1H, 6-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 21.38, 33.40, 39.16, 55.97, 56.29, 105.50, 107.68, 110.01, 111.97, 127.06, 140.49 (2C), 142.63, 145.42 (2C), 149.74, 153.86, 169.66, 180.75, 181.42, 198.05. HRMS (M^+): *m*/*z* calcd for $C_{21}H_{18}N_2O_5$: 378.12155; found: 378.12067.

Compound **7b**: purple solid; mp 175–177 °C; IR (KBr): cm^{-1} 3329 (N–H), 1687 (C=O), 1638, 1598 (C=O quinone). ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.28 (q, *J* = 6.6 Hz, 2H, 3-H), 2.93 (t, *J* = 6.6 Hz, 2H, 2-H), 3.21 (t, *J* = 6.6 Hz, 2H, 4-H), 3.80 (s, 3H, OMe); 3.86 (s, 3H, OMe), 6.49 (s, 1H, 8-H), 6.70 (dd, *J* = 8.9, 3.0 Hz, 1H, 4′-H), 6.87 (d, *J* = 8.9 Hz, 1H, 3′-H), 6.98 (d, *J* = 3.0 Hz, 1H, 6′-H), 7.89 (s, 1H, NH), 9.37 (s, 1H, 6-H). HRMS (M^+): *m*/*z* calcd for $C_{21}H_{18}N_2O_5$: 378.1215; found: 378.1206.

5.3.5. 8- y 9-(N-Methylphenylamino)-3,4-dihydrophenanthridine-1,7,10(2H)-trione (**8a**, **8b**) [10]

The mixture of regioisomers was prepared from **3a** and *N*-methylaniline, (8 h, 55%), red solid, isomers proportion: 68:32 = **8a**:**8b**. Compound **8a** (less polar): red solid, mp 152–153 °C; IR (KBr): cm^{-1} 3447 (N–H), 1686 (C=O), 1561, 1546 (C=O quinone). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.23 (q, *J* = 6.5 Hz, 2H, 3-H), 2.90 (t, *J* = 6.5 Hz, 2H, 2-H), 3.13 (t, *J* = 6.5 Hz, 2H, 4-H), 3.42 (s, 3H, NMe), 6.11 (s, 1H, 9-H), 7.13 (m, 2H, arom), 7.31 (m, 1H, arom), 7.40 (m, 2H, arom), 9.04 (s, 1H, 6-H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 21.24, 33.27, 39.20, 43.16, 60.40, 111.70, 125.41 (2C), 126.93, 127.92, 129.79 (2C), 139.73, 147.17, 150.07, 150.60, 168.75, 180.68, 180.96, 198.01. HRMS (M^+): *m*/*z* calculated for $C_{20}H_{16}N_2O_3$: 332.11609; found: 332.11569.

Compound **8b**: red solid mp 133–135 °C; IR (KBr): cm^{-1} 3448 (N–H), 1691 (C=O), 1627, 1577 (C=O quinone); ^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.26 (q, *J* = 6.4 Hz, 2H, 3-H), 2.78 (t, *J* = 6.4 Hz, 2H, 2-H), 3.20 (t, *J* = 6.4 Hz, 2H, 4-H), 3.50 (s, 3H, NMe), 5.85 (s, 1H, 8-H), 7.26 (m, 3H, arom), 7.48 (m, 2H, arom), 9.27 (s, 1H, 6-H); HRMS (M^+): *m*/*z* calcd for $C_{20}H_{16}N_2O_3$: 332.11609; found: 332.11572.

5.3.6. 8- and 9-(N-Ethylphenylamino)-3,4-dihydrophenanthridine-1,7,10(2H)-trione (**9a**, **9b**)

The mixture of isomers was prepared from **3a** and *N*-ethylaniline, (24 h, 49%), isomers proportion: 71:29 = **9a**:**9b**. Compound **9a** (less polar) was isolated as orange oil. IR (KBr): cm^{-1} 3430 (N–H); 1683 (C=O); 1561, 1545 (C=O quinone). ^{1}H NMR (400 MHz, $CDCl_3$): δ: 1.28 (t, *J* = 6.1 Hz, 3H, $CH_2\underline{CH_3}$), 2.23 (q, *J* = 6.5 Hz, 2H, 3-H), 2.90 (t, *J* = 6.5 Hz, 2H, 2-H), 3.12 (t, *J* = 6.5 Hz, 2H, 4-H), 3.86 (t, *J* = 6.1 Hz, 3H, Me), 4.11 (q, *J* = 6.1 Hz, 2H, $\underline{CH_2}CH_3$), 6.04 (s, 1H, 9-H), 7.11 (m, 2H, arom), 7.33 (m, 1H, arom), 7.42 (m, 2H, arom), 9.05 (s, 1H, 6-H). ^{13}C NMR (400 MHz, $CDCl_3$): δ 12.21, 14.22, 21.45, 29.71, 33.26, 39.22, 49.79, 60.40, 110.93, 126.36 (2C), 127.19, 129.93 (2C), 145.14, 150.01, 150.18, 168.66, 180.88, 181.11, 198.06. HRMS (M^+): *m*/*z* calcd for $C_{21}H_{18}N_2O_3$: 346.13174; found: 346.13153.

Compound **9b** was isolated as orange oil. IR (KBr): cm^{-1} 3448 (N–H), 1695 (C=O), 1600, 1567 (C=O quinone). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.25 (t, *J* = 6.7 Hz, 3H, $CH_2\underline{CH_3}$), 2.24 (q, *J* = 6.5 Hz, 2H, 3-H); 2.80 (t, *J* = 6.5 Hz, 2H, 2-H); 3.20 (t, *J* = 6.5 Hz, 2H, 4-H); 3.64 (t, *J* = 6.7 Hz, 3H, Me); 3.92 (q, *J* = 6.7 Hz, 2H, $\underline{CH_2}CH_3$); 5.70 (s, 1H, 8-H); 7.15 (m, 2H, arom); 7.36 (m, 1H, arom); 7.49 (m, 2H, arom); 9.25 (s, 1H, 6-H). HRMS (M^+): *m*/*z* calcd for $C_{21}H_{18}N_2O_3$: 346.13174; found: 346.13114.

5.4. General procedure for the preparation of the 6-substituted 8-amino-6-methyl-3,4-dihydrophenanthridine-1,7,10(2H)-triones

A suspension of quinone **3b**–**e** (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/water and the organic layer was washed with water (3 × 15 mL). The dried extract was evaporated under reduced pressure and the residue was column chromatographed (10:90 AcOEt/petroleum ether) to yield the corresponding substituted aminophenanthridinequinone.

5.4.1. 8-(Phenylamino)-6-methyl-3,4-dihydrophenanthridine-1,7,10(2H)-trione (**10**) [10]

Prepared from **3b** and aniline, (1 h, 96%), red solid, mp 180-183.5 °C; IR (KBr): cm^{-1} 3442 (N–H), 1696 (C=O), 1591, 1564 (C=O quinone). ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.23 (q, *J* = 6.4 Hz, 2H, 3-H), 2.89 (t, *J* = 6.4 Hz, 2H, 2-H), 2.94 (s, 3H, Me), 3.07 (t, *J* = 6.4 Hz, 2H, 4-H), 6.38 (s, 1H, 9-H), 7.23 (m, 3H, arom), 7.41 (m, 2H, arom), 7.69 (s, 1H, NH). ^{13}C NMR (400 MHz, $CDCl_3$): δ 21.49, 26.46, 33.30, 39.21, 103.45, 122.56, 122.67, 125.95, 128.20, 129.83 (2C), 137.23, 143.53 (2C), 144.78, 162.38, 167.75, 181.58, 182.07, 198.65. HRMS (M^+): *m*/*z* calcd for $C_{20}H_{16}N_2O_3$: 332.11609; found: 332.11539.

*5.4.2. 8-(4-Hydroxyphenylamino)-6-methyl-3,4-dihydrophenanthridine-1,7,10(2H)-trione (**11**)*

Prepared from **6** and *p*-hydroxyaniline, (2:30 h, 55%), purple solid, mp 215.5–217.5 °C; IR (KBr): cm^{-1} 3331 (N–H), 1681 (C=O), 1598, 1517 (C=O quinone). ^{1}H NMR (400 MHz, DMSO): δ 2.22 (q, $J = 6.4$ Hz, 2H, 3-H), 2.88 (t, $J = 6.4$ Hz, 2H, 2-H), 2.90 (s, 3H, Me), 3.07 (t, $J = 6.4$ Hz, 2H, 4-H), 6.16 (s, 1H, 9-H), 6.89 (d, $J = 8.4$ Hz, 2H, 3′- and 5′-H), 7.08 (d, $J = 8.4$ Hz, 2H, 2′- and 6′-H), 7.74 (s, 1H, NH), 9.03 (s, 1H, OH). ^{13}C NMR (400 MHz, DMSO): δ 21.35, 26.35, 29.66, 33.13, 102.13, 116.51 (2C), 122.69, 125.07 (2C), 128.18, 128.36, 143.78, 146.07, 155.89, 162.12, 167.42, 181.57, 181.76, 198.84. HRMS (M^+): *m*/*z* calcd for $C_{20}H_{16}N_2O_4$: 348.11102; found: 348.11028.

*5.4.3. 8-(4-Methoxyphenylamino)-6-methyl-3,4-dihydrophenanthridine-1,7,10(2H)-trione (**12**) [10]*

Prepared from **3b** and *p*-anisidine, (53 min, 79%); purple solid, mp 130–131.5 °C; IR (KBr): cm^{-1} 3442 (N–H), 1674 (C=O), 1560, 1519 (C=O quinone). ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.22 (q, $J = 6.8$ Hz, 2H, 3-H), 2.89 (t, $J = 6.8$ Hz, 2H, 2-H), 2.94 (s, 3H, Me), 3.06 (t, $J = 6.8$ Hz, 2H, 4-H), 3.82 (s, 3H, OMe), 6.19 (s, 1H, 9-H), 6.92 (d, $J = 9.0$ Hz, 2H, 2′-and 6′-H), 7,16 (d, $J = 9.0$ Hz, 2H, 3′- and 5′-H), 7.56 (s, 1H, NH). ^{13}C NMR (400 MHz, $CDCl_3$): δ 21.49, 26.44, 33.29, 39.23, 55.67, 102.62, 115.04 (2C), 122.66, 124.82 (2C), 128.31, 129.82, 143.75, 145.63, 157.90, 162.29, 167.67, 181.70, 181.79, 198.80. HRMS (M^+): *m*/*z* calcd for $C_{21}H_{18}N_2O_4$: 362.12665; found: 362.12587.

*5.4.4. 8-(2-Methoxyphenylamino)-6-methyl-3.4-dihydrophenanthridine-1.7.10(2H)-trione (**13**)*

Prepared from **3b** and *o*-anisidine (30 min, 73%), purple solid, mp 170–172 °C; IR (KBr): cm^{-1} 3448 (N–H). 1672 (C=O) 1619, 1591 (C=O quinone). ^{1}H NMR (400 MHz. $CDCl_3$): δ 2.24 (q. $J = 6.4$ Hz. 2H, 3-H), 2.89 (t, $J = 6.4$ Hz, 2H, 2-H), 2.98 (s, 3H, Me), 3.06 (t, $J = 6.4$ Hz, 2H, 4-H), 3.92 (s, 3H, OMe), 6.47 (s, 1H, 9-H), 6.95 (d, $J = 8.4$ Hz, 1H, 6′-H), 7.00 (m. 1H, 5′-H), 7.14 (dd, $J = 7.6$; 8.4 Hz, 1H, 4′-H), 7.37 (d. $J = 7.6$ Hz, 1H, 3′-H), 8.04 (s, 1H, NH). ^{13}C NMR (400 MHz, $CDCl_3$): δ 21.48, 26.47, 33.27, 39.21, 55.91, 103.64, 111.29, 120.95, 121.00, 122.66, 125.80, 126.64, 128.12, 143.59, 143.84, 151.15, 162.38, 167.59, 181.64, 182.05, 198.59. HRMS (M^+): *m*/*z* calcd for $C_{21}H_{18}N_2O_4$: 362.12665; found: 362.12555.

*5.4.5. 8-(4-Fluorophenylamino)-6-methyl-3,4-dihydrophenanthridine-1,7,10(2H)-trione (**14**)*

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 $NaHCO_3$ and then extracted with ethyl acetate (2 × 15 mL). The organic extract was dried over $NaSO_4$, filtered and evaporated under reduced pressure to yield crude *p*-fluoroaniline. Quinone **3b** was reacted with *p*-fluoraniline under the standard conditions (20 min, 40%) to give compound **14** (91%), red solid, mp 121.5–123 °C; IR (KBr): cm^{-1} 3341 (N–H), 1678 (C=O), 1614, 1594 (C=O quinone). ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.24 (q, $J = 6.4$ Hz, 2H, 3-H), 2.89 (t, $J = 6.4$ Hz, 2H, 2-H), 2.97 (s, 3H, Me), 3.07 (t, $J = 6.4$ Hz, 2H, 4-H), 6.23 (s, 1H, 9-H), 7.12 (m, 2H, arom), 7.22 (m, 2H, arom), 7.54 (s, 1H, NH). ^{13}C NMR (400 MHz, $CDCl_3$): δ 21.55, 26.55, 29.88, 33.38, 39.29, 103.25, 116.28, 117.02, 122.65, 125.10, 125.18, 128.33, 133.15, 143.61, 145.39, 162.57, 167.93, 181.59, 182.15, 198.79. HRMS (M^+): *m*/*z* calcd for $C_{20}H_{15}FN_2O_3$: 350.10666; found: 350.10692.

*5.4.6. 8-(2,5-Dimethoxyphenylamino)-6-methyl-3,4-dihydrophenanthridine-1,7,10(2H)-trione (**15**)*

Prepared from **3b** and 2,5-dimethoxyaniline, (2:40 h, 48%), purple solid, mp 165–167.5 °C; IR (KBr): cm^{-1} 3401 (N–H), 1674 (C=O), 1626, 1556 (C=O quinone). ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.25 (q, $J = 6.8$ Hz, 2H, 3-H), 2.90 (t, $J = 6.8$ Hz, 2H, 2-H), 2.99 (s, 3H, Me), 3.07 (t, $J = 6.8$ Hz, 2H, 4-H), 3.79 (s, 3H, OMe), 3.87 (s, 3H, OMe), 6.53 (s, 1H, 9-H), 6.65 (dd, $J = 8.8$, 2.8 Hz, 1H, 4′-H), 6.87 (d, $J = 2.8$ Hz, 1H, 6′-H), 6.97 (d, $J = 8.8$ Hz, 1H, 3′-H), 8.09 (s, 1H, NH). ^{13}C NMR (400 MHz, $CDCl_3$): δ 14.39, 21.57, 26.60, 33.40, 39.32, 56.13, 56.45, 104.11, 107.64, 109.94, 112.06, 122.77, 127.43, 128.24, 143.68, 145.47, 154.01, 162.59, 167.75, 181.72, 182.23, 198.75. HRMS (M^+): *m*/*z* calcd for $C_{22}H_{20}N_2O_5$: 392.13721; found: 392.13642.

*5.4.7. 6-Phenyl-8-phenylamino-3,4-dihydrophenanthridine-1,7,10(2H)-trione (**16**)*

Prepared from quinone **3c** and aniline (6:15 h, 69%), red solid, mp 193.5–195 °C. IR (KBr): cm^{-1} 3442 (N–H), 1682 (C=O), 1592, 1559 (C=O quinone). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.26 (q, $J = 6.4$ Hz, 3-H), 2.94 (t, $J = 6.4$ Hz, 2H, 2-H), 3.14 (t, $J = 6.4$ Hz, 2H, 4-H), 6.46 (s, 1H, 9-H), 7.21 (m, 3H, arom), 7.40 (m, 2H, arom), 7.48 (m, 6H, NH y arom). ^{13}C NMR (400 MHz, $CDCl_3$) δ: 21.57, 33.48, 39.32, 103.80, 122.59 (2C), 126.06, 128.36 (2C), 128.59 (2C), 128.73, 129.34, 129.95 (2C), 137.21, 140.11, 144.23, 144.79, 161.98, 167.82, 171.28, 180.49, 181.83, 198.60.HRMS (M^+): *m*/*z*: calcd for $C_{25}H_{18}N_2O_3$: 394.13174; found: 394.13074.

*5.4.8. 8-Phenylamino-6-(2-furyl)-3,4-dihydrophenanthridine-1,7,10(2H)-trione (**17**)*

Prepared from quinone **3d** (8:10 h, 55%) and aniline, red solid, mp 166–167 °C. IR (KBr): cm^{-1} 3448 (N–H), 1683 (C=O), 1590, 1557 (C=O quinone). ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.18 (q, $J = 6.8$ Hz, 2H, 3-H), 2.85 (t, $J = 6.8$ Hz, 2H, 2-H), 3.05 (t, $J = 6.8$ Hz, 2H, 4-H), 6.38 (s, 1H, 9-H), 6.57 (m, 1H, –CH furyl), 7.15 (m, 3H, arom), 7.19 (s, 1H, –CH furyl), 7.35 (m, 2H, arom), 7.50 (s, 1H, NH), 7.57 (s, 1H, CH furyl). ^{13}C NMR (400 MHz, $CDCl_3$) δ: 21.49, 33.48, 39.32, 103.59, 112.31, 115.06, 115.28, 122.20, 122.71 (2C), 126.15, 128.32, 129.97 (2C), 137.21, 144.66, 145.17, 149.41, 151.90, 167.92, 179.89, 181.71, 198.39. HRMS (M^+): *m*/*z* calcd for $C_{23}H_{16}N_2O_4$: 384.11101; found: 384.11051.

*5.4.9. 8-Phenylamino-6-(3-thiophen-3-yl)-3,4-dihydrophenanthridine-1,7,10(2H)-trione (**18**)*

Prepared from quinone **3c** and aniline **39** (1:30 h, 45%), red solid mp 168–170 °C. IR (KBr): cm^{-1} 3443 (N–H), 1682 (C=O), 1614, 1591 (C=O quinone). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.24 (q, $J = 6.4$ Hz, 2H, 3-H), 2.92 (t, $J = 6.4$ Hz, 2H, 2-H), 3.11 (t, $J = 6.4$ Hz, 2H, 4-H), 6.45 (s, 1H, 9-H), 7.22 (m, 3H, phenyl), 7.25 (s, 1H, thienyl), 7.39 (m, 3H, phenyl and thienyl), 7.52 (s, 1H, NH), 7.72 (m, 1H, thiophenyl). ^{13}C NMR (400 MHz, $CDCl_3$) δ: 21.56, 33.51, 39.34, 103.73, 122.47, 122.61(2C), 125.13 (2C), 126.14, 127.59, 128.59, 128.71, 129.99,137.22 , 140.54, 144.54, 144.90, 156.51, 168.02, 180.52, 181.93, 198.60. HRMS (M^+): *m*/*z* calcd for $C_{23}H_{16}N_2O_3S$: 400.08816; found: 400.08753.

*5.5. 8-(2,5-Dimethoxyphenylamino)-1-hydroxyphenanthridine-7,10-diona (**19**)*

A suspension of aminoquinone **7a** (46.7 mg), $Pd(OAc)_2$ (52 mg) and glacial acetic acid (5 mL) was heated to reflux for 17 h. The reaction mixture was cooled, neutralized with solid sodium hydrogencarbonate and filtered. The filtrate was diluted with water (23 mL) and then extracted with ethyl acetate (2 × 15 mL). The organic extract was washed with water (2 × 15 mL), dried over sodium sulfate, filtered and evaporated under reduced pressure. The residue was chromatographed on silicagel (CH_2Cl_2) to give quinone **19** (32 mg, 69%), as violet solid, mp 200–202 °C. IR (KBr): cm^{-1} 3443 (N–H), 1650, 1580 (C=O quinone). ^{1}H RMN (400 MHz, CDCl3): δ 3.83 (s, 3H, OMe), 3.91 (s, 3H, OMe), 6.69 (s, 1H, 9-H), 6.74 (dd, $J = 8.8$, 2.8 Hz, 1H, 4′-H), 6.92 (d, $J = 8.8$ Hz, 1H, 3′-H), 7.02 (d,

$J = 2.8$ Hz, 1H, 6′-H), 7.23 (m, 1H, 3-H), 7.72 (m, 1H, 2-H), 7.81 (m, 1H, 4-H); 8.30 (s, 1H, NH); 9.57 (s, 1H, 6-H), 13.66 (s, 1H, −OH). ^{13}C RMN (400 MHz, $CDCl_3$): δ 55.97, 56.32, 104.80, 108.30, 110.71, 112.08, 114.18, 116.39, 121.50, 121.75, 126.35 (2C), 134.69, 142.50, 145.75, 146.97, 153.86, 153.91, 156.33, 181.17, 188.45. HRMS (M^+): *m*/*z* calcd for $C_{21}H_{16}N_2O_5$: 380.13722; found: 380.13719.

5.6. 9-(2,5-Dimethoxyphenylamino)-1-hydroxyphenanthridine-7,10-dione (**20**)

According to the procedure for the preparation of compound **19**, quinone **20** was synthesized in 63% from **7b** (61.4 mg) and $Pd(OAc)_2$ (68 mg) after 19 h reflux. Compound **20** was isolated by column chromatography as a pure violet solid, mp 206–207 °C. IR (KBr): cm^{-1} 3399 (N–H), 1620, 1566 (C=O quinone). ^{1}H RMN (200 MHz, $CDCl_3$): δ 3.83 (s, 3H, OMe), 3.91 (s, 3H, OMe), 6.66 (s, 1H, 8-H), 6.73 (dd, $J = 9.0$, 3.4 Hz, 1H, 4′-H), 6.90 (d, $J = 9.0$ Hz, 1H, 3′-H), 7.02 (d, $J = 3.5$ Hz, 1H, 6′-H), 7.26 (m, 1H, 3-H), 7.77 (m, 1H, 2-H), 7.81 (m, 1H, 4-H), 8.29 (s, 1H, NH), 9.56 (s, 1H, 6-H), 13.61 (s, 1H, OH). HRMS (M^+): *m*/*z* calcd for $C_{21}H_{16}N_2O_5$: 380.13722; found: 380.13710.

5.7. 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), 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 the medium was changed every two days. Cells were grown in the following media: MRC-5, SKMES-1, and J82 in Eagle's minimal essential medium (EMEM) and AGS cells in Ham F-12. The EMEM 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 ug/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 lM during 3 days and finally the MTT reduction (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) 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.

5.8. Electrochemical measurements [8]

Cyclic voltammograms of compounds were obtained on a Bioanalytical Sytem BAS CV-50W 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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References

[1] D. Vásquez, J.A. Rodríguez, C. Theoduloz, P. Buc Calderon, J.A. Valderrama, Eur. J. Med. Chem. 45 (2010) 5234–5242.
[2] A.J. Lin, L.A. Cosby, C.W. Shansky, A.C. Sartorelli, J. Med. Chem. 15 (1972) 1247–1252.
[3] I. Wilson, P. Wardman, T.S. Lin, A.C. Sartorelli, J. Med. Chem. 29 (1986) 1381–1384.
[4] P.L. Gutierrez, Front. Biosci. 5 (2000) 629–638.
[5] G.R. Pettit, J.C. Knight, J.C. Collins, D.L. Herald, R.K. Pettit, M.R. Boyd, V.G. Young, J. Nat. Prod. 63 (2000) 793–798.
[6] D.J. Milanowski, K.R. Gustafson, J.A. Kelley, J.B. McMahon, J. Nat. Prod. 67 (2004) 70–73.
[7] U.W. Hawas, M. Shaaban, K.A. Shaaban, M. Speitling, A. Maier, G. Kelter, H.H. Fiebig, M. Meiners, E. Helmke, H. Laatsh, J. Nat. Prod. 72 (2009) 2120–2124.
[8] J.A. Valderrama, A. Ibacache, V. Arancibia, J.A. Rodríguez, C. Theoduloz, Bioorg. Med. Chem. 17 (2009) 2894–2901.
[9] D. Vásquez, J.A. Rodríguez, C. Theoduloz, J. Verrax, P. Buc Calderon, J.A. Valderrama, Bioorg. Med. Chem. Lett. 19 (2009) 5060–5062.
[10] J.A. Valderrama, A. Ibacache, Tetrahedron Lett. 50 (2009) 4361–4363.
[11] P.H. Bernardo, J.K. Khanijou, T.H. Lam, J.C. Tong, C.L.L. Chai, Tetrahedron Lett. 52 (2011) 92–94.
[12] (a) J.A. Valderrama, M.F. González, P. Colonelli, D. Vásquez, Synletters (2006) 2777–2780;
(b) J.A. Valderrama, P. Colonelli, D. Vásquez, M.F. González, J.A. Rodríguez, C. Theoduloz, Bioorg. Med. Chem. 16 (2008) 10172–10181.
[13] J. Benites, D. Rios, P. Díaz, J.A. Valderrama, Tetrahedron Lett. 52 (2011) 609–611.
[14] Y.T. Pratt, J. Org. Chem. 27 (1962) 3905–3910.
[15] 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, Cancer Res. 48 (1988) 589–601.
[16] A.A. van de Loosdrecht, R.H.J. Beelen, G.J. Ossenkoppele, M.G. Broekhoven, M.M.A.C. Langenhuijsen, J. Immunol. Methods 174 (1994) 311–320.
[17] D.A. Scudiero, R.H. Shoemaker, K.D. Paull, A. Monks, S. Tierney, T.H. Nofziger, M.J. Currens, D. Seniff, M.R. Boyd, Cancer Res. 48 (1988) 4827–4833.
[18] R.P. Verma, Anti-cancer Agents Med. Chem. 6 (2006) 489–499.
[19] J. Koyama, K. Tagahara, T. Osakai, Y. Tsujino, S. Tsurumi, H. Nishino, H. Tokuda, Cancer Lett. 115 (1997) 179–183.
[20] J. Koyama, I. Morita, N. Kobayashi, T. Osakai, H. Hotta, J. Takayasu, H. Nishino, H. Tokuda, Cancer Lett. 201 (2003) 25–30.
[21] J. Koyama, I. Morita, K. Tagahara, T. Osakai, H. Hotta, M.X. Yang, T. Mukainaka, H. Nishino, H. Tokuda, Chem. Pharm. Bull. 49 (2001) 1214–1216.
[22] F.C. de Abreu, P.A. de Ferraz, M.O.F. Goulart, J. Braz. Chem. Soc. 13 (2002) 19–35.
[23] M. Aguilar-Martinez, G. Cuevas, M. Jimenez-Estrada, I. González, B. Lotina-Hennsen, N. Macias-Ruvalcaba, J. Org. Chem. 64 (1999) 3684–3694.
[24] M.T. Bogert, H.P. Howells, J. Am. Chem. Soc. 52 (1930) 837–850.