Synthesis and antitumor evaluation of 8-phenylaminopyrimido[4,5-c]isoquinolinequinones

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ABSTRACT

A series of 8-phenylaminopyrimido[4,5-c]isoquinoline-7,10-quinone derivatives were prepared by regio-selective amination reaction of pyrimido[4,5-c]isoquinoline-7,10-quinones with arylamines in the presence of a Lewis acid catalyst. Preliminary evaluation of the members of the series against cancer cell lines and assays of activation of their cytotoxic activity on K562 cells with ascorbic acid are reported.

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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 this last 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, recently described by Hanahan and Weinberg.[1] Among several strategies capable of activating cancer cell death, the induction of an oxidative stress represents an interesting approach. Indeed, given that the formation of reactive oxygen species (ROS) is associated to the carcinogenic process, cancer cells have a compromised redox equilibrium, which renders them sensitive to an oxidant injury. In addition, cancer cells are usually deficient in antioxidant enzymes. Hence, our group and several other laboratories have developed a particular approach by exposing selectively cancer cells to an oxidative stress generated during a redox cycling between ascorbate (vitamin C) and menadione.[2–10]

The molecular framework of several naturally occurring antitumoral agents contains the aminoquinone scaffold as the key structural component, (e.g., mitomycin C, cribrostatin 3 and streptonigrin).[11,12] This structural array has stimulated the synthesis of novel lead compounds that exhibit significant cytotoxicity on human cancer cell lines.[13–16]

In a previous work we have developed a high yield synthesis of 7-substituted aminoisoquinoline-5,8-quinones 2, using acid-induced substitution reactions of isoquinolinequinone 1 with alkyl- and arylamines.[17] These aminoquinones expressed in vitro cytotoxic activity against gastric, lung and bladder cancer cell lines. The SAR analysis of substituted arylaminoquinones 2 (R^1 = H, R^2 = Ar) reveals that the half wave potential is an important parameter determining the antitumoral activity on gastric adenocarcinoma and bladder carcinoma cells.

We have recently reported that phenylaminoquinones 4a and 4b, prepared by amination of quinone 3, displays high antitumor activity against gastric and lung cancer cells compared to that of precursor 3. This observation reveals that introduction of an anilino donor group into the 8- or 9-position of compound 3
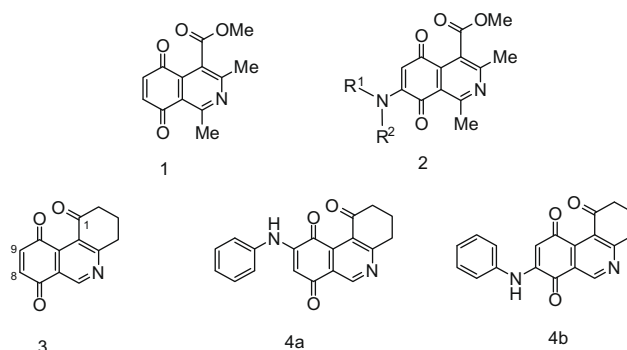
enhances the antitumor activity of the phenanthridinequinone chromophore.[18]

Apparently, the presence of donor arylamino groups into the isoquinoline- and phenanthridinequinone chromophores of **1** and **3** modify their redox potentials thus affecting the abilities of arylaminoquinones to participate as redox-cyclers, inducing cytotoxic activity on cancer cells. The interest to extend our studies on the design of new aminoquinone derivatives for antitumor evaluation led us to synthesize novel prototypes based on the pyrimidoisoquinolinequinone chromophore due to its presence in antitumor compounds.[19] Herein, we wish to report the regioselective access to 8-phenylaminopyrimido[4,5-c]isoquinoline-7,10-quinone, the antitumor evaluation against two cancer cell lines and preliminary evidence on the biological mechanism involved in the cytotoxicity against K562 cells.

Pyrimidoisoquinolinequinone **5** and **6** were prepared according to a recently reported procedure.[19] Firstly, we explored the reaction of **5** with aniline in ethanol at room temperature. The reaction went to completion in 24 h to give a 67:33 mixture of regioisomers **7a** and **7b** (Scheme 1). Surprisingly, when quinone **5** was allowed to react in ethanol in the presence of 5 mmol % of $CeCl_3 \cdot 7H_2O$, the reaction was clean and fast (2 h) to give **7a** with a yield of 99% as the sole regioisomer (Table 1). These results clearly demonstrate that the Lewis acid catalyst improves the amination yield and induces regiospecific formation of the substitution product **7a**.

Then we analyzed the reactivity of quinone **6** with aniline in ethanol at room temperature. In this case the result was totally different to that observed for quinone **5**. In fact, the reaction yields, after 24 h, aminoquinone **8** as the sole regioisomer in 87% yield. The addition of 5 mmol % of $CeCl_3 \cdot 7H_2O$ increases the reaction rate and the yield and does not change the sense of the regioselectivity. The structure of compounds **7a**[20] and **8**[21] was determined through 2D-NMR (HMBC, HMQC) experiments. The regioselectivity of the substitution reaction on quinone **6** can be explained assuming steric and electron-donor interactions between the methyl and C-7 carbonyl groups. These factors probably affect the electrophilicity of C-9 and the attack of the nucleophiles occurs β to the C-10 carbonyl group.

Based on the aforementioned results, a variety of 8-substituted aminopyrimido[4,5-c]isoquinolinequinones were regioselectively prepared by the reaction of quinones **5** and **6** with substituted anilines in the presence of 5 mmol % of $CeCl_3 \cdot 7H_2O$. The corresponding aminoquinones were isolated in high yields (Table 1) and no regioisomers were detected in the reaction mixtures.
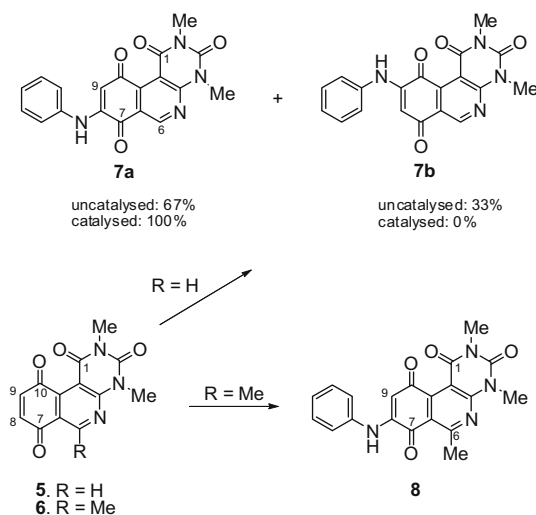
Table 1
Preparation of aminopyrimido[4,5-c]isoquinolinequinones from quinones **5** and **6**
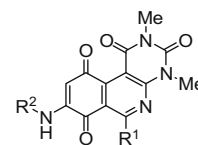
Product	R^1	R^2	Yield^a
7a	H	Ph–	99
9	H	4-MeOPh–	99
10	H	4-HOPh–	40
11	H	4-FPh–	91
8	Me	Ph–	99
12	Me	4-MeOPh–	99
13	Me	4-HOPh–	46
14	Me	4-FPh–	72

^{a} Isolated yields.

The results of the experiments under catalysed conditions demonstrate that the C-10 carbonyl group in quinones **5** and **6** exerts a higher activation than the C-7 carbonyl group into the corresponding π-enone system, favoring the attack of the nucleophile at the 8-position. The effect of the catalyst to promote the attack of the nucleophile at the 8-position in **5** and **6** may be ascribed to the coordination of the cerium ion with the heterocyclic nitrogen atom and/or the carbonyl group at the 10-position. The coordination strongly enhances the electro-withdrawing capacity of the carbonyl group at the 10-position, which is transferred to the 8-position, leading to preferential C-8 substitution via nucleophilic attack of the amines.

The newly synthesized aminoquinones were evaluated for in vitro antitumor activity against normal MRC-5 human lung fibroblasts, AGS gastric adenocarcinoma cells and SK-MES-1 lung cancer cells lines in 72-h drug exposure assays. The cytotoxicity of the compounds was measured using a conventional microculture tetrazolium reduction assay.[22] The average IC$_{50}$ values are shown in Table 2.

The data on Table 2 indicate that the insertion of 4-R-phenylamino groups into the 8-position of quinones **5** and **6** increases the cytotoxic activity on the normal and cancer cell lines in comparison to their precursors.

The IC$_{50}$ values of compounds **7a–14** show that the 4-R-phenylamino groups at the 8-position and the methyl group at the 6-position have influence on their antitumor activities. Indeed, in the group of aminoquinones prepared from **5**, compounds **9** and **11** have a similar profile of activity as compared to **7a**. However, compound **9** has the best selectivity, since it is cytotoxic towards

Scheme 1. Reaction of quinones **5** and **6** with aniline.

Table 2
In vitro antitumor activity of aminopyrimido[4,5-c]isoquinoline-7,10-quinones and their precursors

Aminoquinone	(IC$_{50}$ ± SEM)^a (μM)		
	MRC-5	AGS	SK-MES-1
5	72.8 ± 3.4	82.1 ± 4.4	67.5 ± 3.6
7a	9.2 ± 0.6	2.5 ± 0.2	7.2 ± 0.6
9	20.5 ± 1.2	2.8 ± 0.2	6.5 ± 0.5
10	47.4 ± 3.8	15.5 ± 0.9	55.9 ± 2.8
11	6.4 ± 0.3	3.0 ± 0.2	5.6 ± 0.4
6	67.1 ± 3.5	50.3 ± 2.7	70.2 ± 3.3
8	4.6 ± 0.3	1.0 ± 0.1	2.9 ± 0.2
12	49.6 ± 2.5	5.5 ± 0.3	16.0 ± 0.8
13	17.4 ± 1.0	3.3 ± 0.3	3.9 ± 0.2
14	4.0 ± 0.2	1.0 ± 0.1	2.6 ± 0.2

^{a} Data represent mean values for six independent determinations.

the two cancer cell lines but it shows low activity in non transformed human fibroblasts (MRC-5 cells). Its structural aminoquinone analogue in the series of compounds prepared from **6**, namely compound **12**, has the lowest activity in both healthy and tumor cells. In this series, a similar kind of selectivity is observed with compound **13**. Its structural analogue, compound **10**, has the lowest activity in the series of quinones **5**.

These findings reveal that the cytotoxicity of compounds **7a–14** is related to the electronic nature of the 4-R-phenylamino group, which could be due to the redox ability of these quinonoid compounds to act as a redox-cycler. Apparently, the donor effect of the 4-hydroxyphenylamino- and 4-methoxyphenylamino substituents, as in **9**, **10**, **12**, **13**, decreases the redox ability of the quinone moiety with respect to the parent compounds **7a** and **8**, thus lowering their cytotoxic capacities.

In order to obtain evidence on the redox ability of these quinones on cell survival we tested them in the absence and presence of vitamin C. The experimental model was the release of LDH (a well-known end point of cell death) by incubating human erythromyeloblastoid leukemia (K562) cells for 24 h with quinones (at 5 μM) either in the absence or in the presence of vitamin C (2 mM). The results of the assays are shown in Figure 1.

The data in Figure 1 shows that no particular cytotoxicity is observed when K562 cells were incubated with the quinones alone (LDH leakage in control values = 14.0%). The addition of vitamin C enhances the cytotoxicity of all quinones, whatever the series, including their precursors **5** and **6** (LDH leakage in control values = 13.1%). Only compound **12**, which was the least active in the MTT reduction test, does not show any sign of redox cycling activity even at 20 μM (data not shown). In the series of compounds **7a–11**, prepared from quinone **5**, no effect of the substituents at the 4-position of the phenyl group on the cytotoxicity was observed in the absence of vitamin C. Indeed, compounds **7a** and **9** have the highest activity when they are associated to vitamin C (Fig. 1). Among the members of the series of quinones prepared from **6**, compound **13** appears as the most active and with the best selectivity as shown by the MTT test (Table 2).

Comparison of the cytotoxicity between the members of groups **7a**–**11** and **8**–**14** reveals that the former are better redox-cyclers than the latter. Assuming that the cytotoxicity of the tested quinones proceeds via an oxidative stress mediated by vitamin C, the methyl group at the 6-position in the pyrimido[4,5-c]isoquinoline-7,10-quinone chromophore should have influence on the stability of the semiquinone radical intermediate involved in the ROS generation.

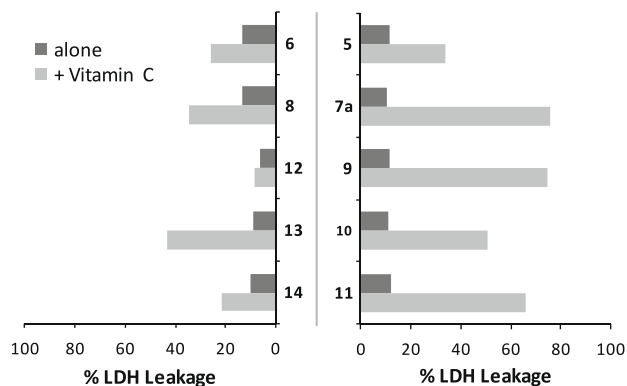In conclusion, we have prepared a variety of 8-phenylaminopyrimido[4,5-c]isoquinoline-7,10-quinones, **7a–14**, in high yields, by reaction of quinones **5** and **6** with phenylamines in the presence of $CeCl_3 \cdot 7H_2O$. The cytotoxic evaluation on normal MRC-5 human lung fibroblasts, AGS gastric adenocarcinoma cells and SK-MES-1 lung cancer cells lines demonstrates that biological activity is related to the donor–acceptor capacity of the 4-R-phenylamino group, which apparently modulates the redox ability of the quinone nucleus to act as redox-cycler. The cytotoxic activity of almost all the new compounds (except **12**) against K562 cells, is enhanced in the presence of vitamin C compared to the cytotoxic activity observed in the absence of vitamin C. The synthesis of a broad variety of 8-substituted aminopyrimido[4,5-c]isoquinoline-7,10-quinones for both electrochemical studies and antitumor evaluation on representative cancer cell lines is currently in progress in our laboratory. In addition, since ROS formation (during ascorbate-driven quinone redox cycling) plays a key role in cancer cell death, the mechanisms conditioning cell death (apoptosis–necrosis) will be further investigated.

Acknowledgment

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20. *Compound* **7a**: red solid, mp 236–237 °C; IR (KBr): ν_{max} 3320 (N–H), 1726 (C=O), 1716 (C=O) 1676 and 1666 (C=O quinone); ^{1}H NMR (400 MHz, CDCl$_3$): δ 3.50 (s, 3H, 2-Me), 3.77 (s, 3H, 4-Me), 6.52 (s, 1H, 9-H), 7.25 (m, 3H, 2′-, 4′- and 6′-H), 7.44 (m, 2H, 3′- and 5′-H), 7.49 (s, 1H, N–H), 9.30 (s, 1H, 6-H); ^{13}C NMR (100 MHz, CDCl$_3$): δ 29.2, 30.6, 105.7, 107.1, 121.4, 122.4 (2C), 125.9, 129.8 (2C), 136.9, 143.4, 145.5, 150.9, 152.3, 155.2, 158.3, 179.5, 180.9. The HMBC spectrum of **7a** shows ${}^{3}J_{C,H}$ coupling of the C-7 carbon (δ 179.5 ppm) with the protons at: δ 6.52, 7.49 and 9.30 ppm. Anal. Calcd for C$_{19}$H$_{14}$N$_4$O$_4$: C, 62.98; H, 3.89; N, 15.46. Found: C, 62.88; H, 3.52; N, 15.32.
21. *Compound* **8**: red solid, mp 221.5–222 °C; IR (KBr): ν_{max} 3334 (N–H), 1721 (C=O), 1716 (C=O) 1682 and 1667 (C=O quinone); ^{1}H NMR (400 MHz, CDCl$_3$): δ 3.05 (s, 3H, 6-Me), 3.51 (s, 3H, 4-Me), 3.78 (s, 3H, 2-Me), 6.51 (s, 1H, 9-H), 7.28 (m, 3H, 2′-, 4′- and 6′-H), 7.47 (m, 2H, 3′- and 5′-H), 7.65 (s, 1H, N–H); ^{13}C NMR (100 MHz, CDCl$_3$): 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. The HMBC spectrum of **8** shows ${}^{3}J_{C,H}$ coupling of the C-7 carbon (δ 180.2 ppm) with the protons at: δ 6.51, 7.47 ppm and ${}^{4}J_{C,H}$ coupling with the protons of the methyl group at δ 3.05 ppm. Anal. Calcd for C$_{20}$H$_{16}$N$_4$O$_4$: C, 63.82; H, 4.28; N, 14.89. Found: C, 63.77; H, 3.99; N, 14.93.
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Figure 1. Cytotoxic activity of aminopyrimido[4,5-c]isoquinolinequinones (at 5 μM) and redox modulation by vitamin C (2 mM).