Notes

Synthesis and Cytotoxicity of Anilinomethyl-1,4-naphthoquinones

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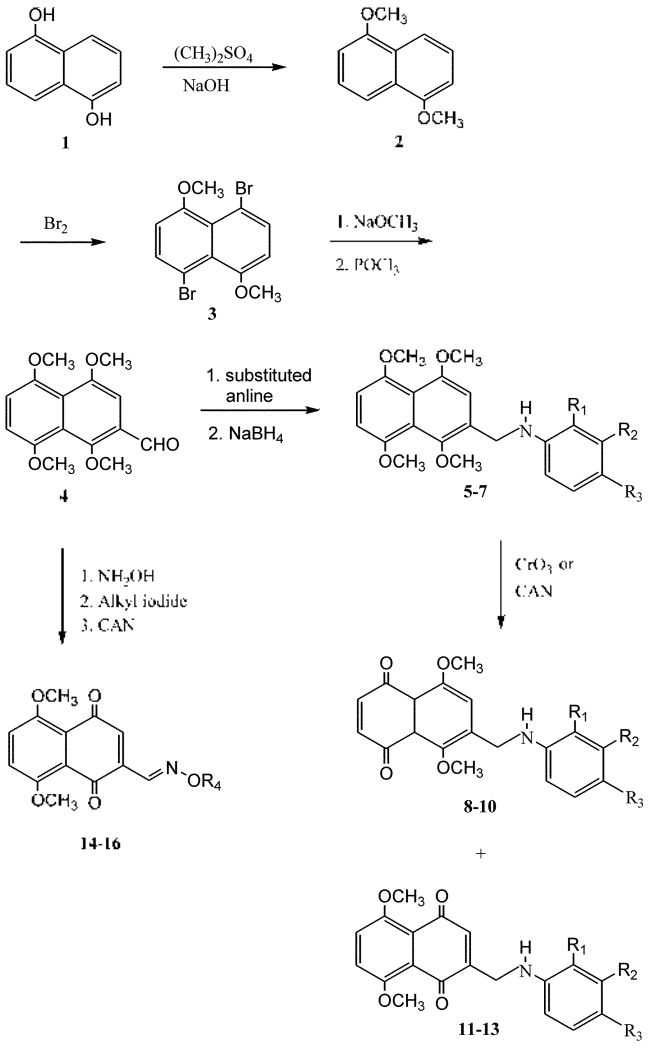
Quinones are widely distributed in nature and many clinically important antitumor drugs containing quinone nucleus, such as anthracyclines, mitoxantrones and saintopin, show the excellent anticancer activity. These anticancer agents are effective inhibitors of DNA topoisomerase, and it is generally known that the cytotoxicity of quinone analogues results from the inhibition of DNA topoisomerase II.[1-4] The quinone analogues can also induce the formation of semiquinone radical, which can transfer an electron to oxygen to produce superoxide. This process is catalyzed by flavoenzymes such as NADPH-cytochrome-*P*-450 reductase. Both superoxide and semiquinone radical anions of naphthoquinone analogues can generate the hydroxyl radical, which is known to cause DNA strand breaks.[5-8] In addition, a number of 1,4-naphthoquinone derivatives have been found to possess powerful pharmacological effects and are also associated with marked antimicrobial and antitumor activities.[9-12] In view of these facts, previously we had synthesized some 1,4-naphthoquinone derivatives with a propenoate substituent functional group and reported their cytotoxicity and antitumor activity.[13] In this study, we report the synthesis of 2-anilinomethyl-1,4-naphthoquinones and 6-anilinomethyl-1,4-naphthoquinones and their cytotoxic activity against cancer L1210, P388, SNU-1, HL-60, A549 and normal Vero cells.

Results and Discussion

2-Formyltetramethoxynaphthalene (**4**) was prepared from 1,5-dihydroxynaphthalene (**1**) through 4-step reactions of methylation (86%), bromination (85%), methoxylation (61%), and formylation (96%).[14] 2-Formyltetramethoxynaphthalene was condensed with substituted anilines at pH 5 and the resulting imine compounds were reduced to 2-anilinonomethyl-1,4,5,8-tetramethoxynaphthalenes (**5-7**) using $LiAlH_4$ or $NaBH_4$ in good yields. We could obtain 6-substituted naphthoquinone derivatives **8-10** with a yield of 80-85% by oxidation with chromium(VI) oxide and 2-substituted naphthoquinone derivatives **11-13** with a yield of 65-80% by oxidation with cerium(IV) ammonium nitrate (CAN) from corresponding 2-anilinomethyl-1,4,5,8-tetramethoxynaphthalenes. Compounds **14-16** were prepared from compound **4** by 3-step reactions of condensation with hydroxyl amine, alkylation and oxidation with CAN (Scheme 1).

The cytotoxicity of naphthoquinone derivatives was measured against cancer cells L1210, P388, SNU-1, HL-60,



Scheme 1

Table 1. Cytotoxicity of 1,4-naphthoquinone derivatives against cancer cell lines

No. of Compd.	R_1	R_2	R_3	R_4	ED_{50} (μg/mL)					
					L1210	P388D_1	HL-60	A549	SNU-1	Vero
8	H	CF_3	H		0.45	0.36	2.24	24.57	9.81	2.84
9	H	CF_3	NO_2		0.61	0.60	1.37	113.74	8.85	3.77
10	CH_3	H	NO_2		0.16	0.21	1.54	20.79	3.44	2.80
11	H	CF_3	H		0.18	0.91	1.00	9.86	4.64	3.40
12	H	CF_3	NO_2		0.19	0.67	0.96	15.24	2.51	8.60
13	CH_3	H	NO_2		0.05	0.13	0.63	3.06	0.48	2.34
14				CH_3	4.97	2.24	1.26	56.42	11.82	41.37
15				C_2H_5	5.10	2.29	2.17	61.18	10.76	45.04
16				C_3H_7	5.28	2.76	2.03	48.06	19.14	51.29
Adriamycin					0.07	0.14	0.64	3.92	2.01	6.26

A549 and normal Vello cells using the MTT colorimetric method.[15] ED_{50} value (μg/mL) was defined as the concentration of compound to produce a 50% reduction in viability relative to the control in three independent experiments. The results were shown in Table 1. It could be recognized that the solid cancer cell line SNU-1 and the normal Vero cell line were more resistant to the synthesized naphthoquinone derivatives, compared to the L1210 (Lymphocytic leukemia) and P388 (Lymphoid neoplasma) cell lines. The ED_{50} values of compounds **8-10** were 0.16 μg/mL-0.45 μg/mL and those of compounds **11-12** were 0.18 μg/mL-0.19 μg/mL against L1210. In the case of compound **13**, which has methyl group at R_1 position, the ED_{50} values against L1210, P388, HL-60 were found to be comparable to the ED_{50} values of adriamycin and were even better against the solid cancer cell line A549 and SNU-1. Interestingly, it was observed that 2-substituted compounds **11-13** exhibited a better inhibitory activity than 6-substituted compounds **8-10** against L1210, P388, SNU-1, HL-60, A549 in vitro. In earlier works, we observed that the cytotoxic activity was dependent upon the location of the substituent groups. In the case of naphthoquinone derivatives with a propenoate substituent functional group at C6 or C2 position, we had shown that 6-substituted derivatives were more effective than the 2-substituted derivatives and the introduction of electronegative fluorine on the benzoyl group increased the cytotoxicity.[13] This result was in accord with the works reported by other researchers and it was said that the C2 or C3 of 6-substituted compounds would be better Michael acceptors than the C3 of 2-substituted compounds and attacked more easily by nucleophiles such as amine or thiol functional groups in the cell.[16] Compounds **14-16** were prepared from compound **4** through the reactions described above. To increase the electrophilicty of C3 of derivatives, alkoxy imino group, which has less steric hindrance and is expected to conjugate with the double bond at C2 position, was introduced to C2 of 1,4-naphthoquinone. However, unexpectedly, the cytotoxicity of compounds **14-16** was lower than that of 2-anilinonomethyl-1,4-naphthoquinones. The obsrved ED_{50} values of compounds **14-16** were 4.97 μg/mL-5.28 μg/mL against L1210 and 1.26 μg/mL-2.03 μg/mL against HL-60. The reason why 2-anilinomethyl substituted derivatives are more effective than 6-anilinomethyl substituted derivatives is not clear yet and further investigations are now in progress.

MTT Assay. Target cancer cells were suspended at 2×10^5 cells/mL in medium (10% fetal bovine serum) containing each concentrations of synthesized naphthoquinone derivatives, vigorously vortexed and then 100 μL aliquots were dispensed into 96-well flat-bottomed microtiter plates using a multichannel pipet. Plates were then incubated at 37 °C for 72 h in 5% CO_2 incubator. MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] was dissolved in PBS at 5 mg/mL and filtered to remove a small amount of insoluble residue present in some batches of MTT. An aliquot of 10 μL of MTT stock solution was added to each well using a multichannel pipette and the plate was incubated at 37 °C for 4 h. To each well 150 μL of 0.01 N HCl solution containing 10% sodium dodecyl sulfate were added to solubilize the MTT. Plates were gently shaken until all crystals were dissolved, and the absorbance at 540 nm was determined with a Microplate Reader (SPECTRA MAX 340). All results were corrected for background absorbance detected in wells without added MTT and a linear relationship was observed between the cell numbers and the absorbance at 540 nm when cells are in the range of 4×10^2 to 4×10^5 per well examined.

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