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Target ROS to induce apoptosis and cell cycle arrest by 5,7-dimethoxy-1,4-naphthoquinone derivative

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ABSTRACT

The 1,4-naphthoquinone derivatives bearing 5,7-dimethoxyl moiety were designed, synthesized, and tested as the antitumor agents against five human cancer cell lines (A549, Hela, HepG2, NCI-H460 and HL-60). All the compounds are described herein for the first time. The structure-activity relationships indicated that the presence of chlorine atom at the 2-position was crucial for the antiproliferative activity. Further, the electrochemical properties of the representative compounds (**7e**, **8e** and **9e**) were evaluated and a definite correlation between the redox potential and the antiproliferative activity. The most potent compound **9e** displayed significant anti-leukemic activity with IC_{50} value of 3.8 μM in HL-60 cells and weak cytotoxicity with IC_{50} of 40.7 μM in normal cells WI-38. In mechanistic study for **9e**, the increased numbers of apoptotic cells and increased cell population at G2/M phase correlated with ROS generation. Together, our results suggested that the derivatives of 2-chlorine-1,4-naphthoquinone might be the promising candidates for the treatment of promyelocytic leukemia.

Compared with counterparts of normal cells, most tumor cells enhance intracellular levels of reactive oxygen species (ROS) and disturb capacity of antioxidant defense system. When ROS increases to the threshold, it may overwhelm the antioxidant capacity of the cells and trigger cell death. As such, cancer cells are more vulnerable to be killed by ROS-generating agents. Thus, targeting the difference in cellular ROS level could serve as a potential strategy for the development of anticancer drugs with high therapeutic index.[1,2]

The derivatives of 1,4-naphthoquinone have been widely studied by the National cancer institute (NCI) as anticancer drug candidates, which kill cancer cells mainly by the enhancement of ROS level and inhibiting DNA topoisomerase I and II.[3–7] For example, lapachol (**1**, Fig. 1) is a naturally occurring naphthoquinone from the grain of several wooden trees of the Bignoniaceae family, which has demonstrated to promote regression of tumors in approximately 30% of patients, but side effects such as anemia and gastrointestinal problems greatly aggravated the clinical conditions for cancer patients.[8] Over the last few years, the structural modification sites of lapachol were mostly focused on the isoprenyl side chain.[9–12] Especially, the FDA-approved drugs atovaquone[13,14] (**2**, Fig. 1) and buparvaquone[15] (**3**, Fig. 1) are analogues of lapachol, which are used for the treatment of malaria and leishmaniasis, respectively. Recent studies have found that atovaquone is a clinically accessible inhibitor of STAT3 and mitochondrial complex III, including eradicating cancer stem cells and showing anticancer efficacy in both animal models and humans.[13,14]

Here, the target lapachol derivatives were rationally designed as follows: (i) use 2-hydroxy-1,4-naphthoquinone as a core structure; (ii) introduce m-dimethoxyl moiety to benzene ring to increase the structural diversity of 1,4-naphthoquinones, because the most of chemical modifications performed on the quinone ring;[4,16] (iii) substitute the isoprenyl side chain with hydrocarbon tails which vary in the length and bulkiness; (iv) in order to explore the effect of 2-hydoxyl group on antitumor activity, design derivatives of 2-methoxy-1,4-naphtjoquinone and 2-chlorine-1,4-naphthoquinone. All target compounds were evaluated for their in vitro antiproliferative activities against five tumor cell lines and one normal cell line using 5-Fluorouracil (5-FU) as a positive control. The antiproliferative mechanism was evaluated for the most potent compound **9e** by the determinations of ROS production, apoptosis and cell cycle.

The synthesis of target compounds is illustrated in Scheme 1. According to the previous synthetic method,[17] the 3,5-dimethoxyphenylacetate **5** was prepared from 3,5-dimethoxybenzaldehyde **4** as starting material. Compounds **6a-6n** were obtained through acylating **5** with acyl chloride, in dichloromethane

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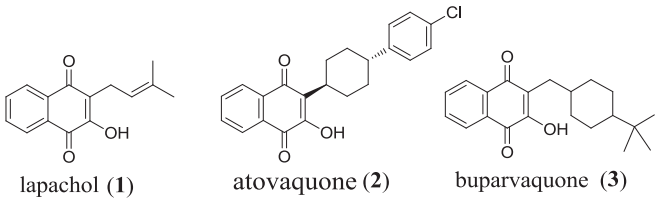
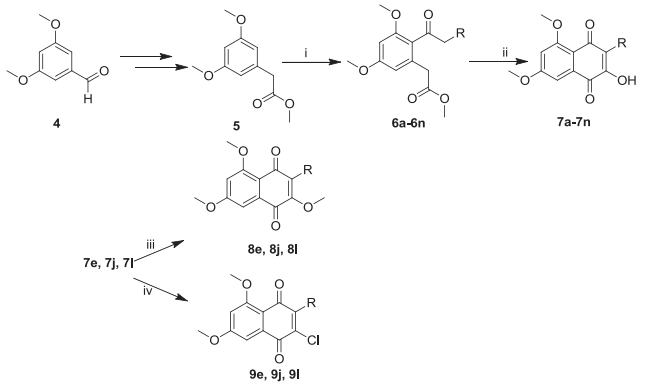


Fig. 1. Chemical structures of lapachol (**1**), atovaquone (**2**) and buparvaquone (**3**).



Compds	R	Compds	R
7a	H	**7k**	
7b		**7l**	
7c		**7m**	
7d		**7n**	
7e		**8e**	
7f		**8j**	
7g		**8l**	
7h		**9e**	
7i		**9j**	
7j		**9l**	

Scheme 1. Reagents and conditions: (i) RCOCl, $AlCl_3$, 0-rt, 2 h; (ii) CH_3ONa, reflux-rt, 12 h; (iii) $(CH_3)_2SO_4$, K_2CO_3, Acetone, rt, 2 h; (iv) Toluene, $SOCl_2$, reflux, 24 h.

containing $AlCl_3$ as the Lewis acid catalyst. The crude products of **6a-6n** were subsequently cyclized in refluxing sodium methoxide to afford naphthoquinones **7a-7n** after aerial oxidation and acidic workup in isolated yields of 28–65%. Additionally, methylation or chlorination of **7e**, **7j**, **7l** on C-2 hydroxyl group gave **8e**, **8j**, **8l** and **9e**, **9j**, **9l**, respectively.

The antiproliferative activities of the 20 compounds were evaluated against five tumor cell lines by MTT method.[18] The human cell lines used were: hepatoma (HepG2), cervical carcinoma (HeLa), lung carcinoma (A549 and NCI-H460), leukemia (HL-60), including the normal cell lines, fetal lung fibroblast cell (WI-38). Cells were treated with compounds for 48 h with the maximum test concentration at 100 μM. The results are summarized in

Table 1
Antiproliferative activity of the targeted compounds against five tumor cell lines.

Compds	IC_{50} (μM)[a]				
	Hela	HepG2	A549	NCI-H460	HL-60
7a	>100	>100	>100	>100	>100
7b	90.2	>100	>100	>100	>100
7c	96.5	>100	>100	>100	>100
7d	>100	>100	78.7	>100	>100
7e	>100	95.9	65.4	>100	>100
7f	>100	90.4	>100	>100	>100
7g	>100	>100	>100	>100	>100
7h	>100	>100	>100	>100	95.5
7i	>100	>100	>100	>100	86.7
7j	79.4	49.9	78.5	86.2	95.8
7k	>100	95.7	>100	>100	96.1
7l	72.8	60.7	82.4	97.5	93.1
7m	>100	73.2	>100	>100	>100
7n	>100	81.4	>100	>100	26.1
8e	52.4	55.9	>100	75.2	61.4
8j	70.2	37.4	>100	43.1	55.9
8l	35.0	47.5	>100	37.1	52.0
9e	22.3	23.1	24.1	38.7	**3.8**
9j	21.2	41.4	63.0	61.0	9.2
9l	29.3	37.1	83.1	>100	15.3
5-FU[b]	30.2	36.6	55.7	8.5	67.2

[a] IC_{50} values were calculated from three independent experiments.
[b] Used as a reference.

Table 1. Firstly, with the elongation of the alkyl side tail at 3-position, the activities of compounds **7a-i** did not increase in a linear fashion. In the case of A549 cells, the compounds **7d** with propyl group and **7e** with butyl group showed the antiproliferative activity with the IC_{50} value of 78.7 and 65.4 μM, respectively. But the other compounds among **7a-i** were inactive to A549 cells. Similar results were observed for **7b** and **7c** against Hela cells, **7e** and **7f** against HepG2 cells, **7 h** and **7i** against HL-60 cells. Hence, the results indicated that the length of side chain displayed selectivity to inhibit the growth of different cancer cell lines. Secondly, in order to investigate the effects of bulkiness of alkyl tail on antitumor activity, cyclohexyl-substituent (**7j**) and phenyl-substituents (**7k-n**) were introduced to 3-position. Compared to the compounds with the straight tail, compounds **7j** (cyclohexyl**)** and **7l** (p-methoxyphenyl) displayed the enhanced antiproliferative activities. Thirdly, to disclose whether the hydroxyl group on 2-position was crucial for the antitumor activity, 2-hydroxyl group was then converted to methoxyl group **(8e**, **8j**, **8l**) and chlorine atom (**9e**, **9j**, **9l**). The IC_{50} values revealed that the presence of 2-chlorine increased the activity more dramatically than 2-methoxyl group. Interestingly, derivatives (**9e**, **9j**, **9l**) exhibited the higher antiproliferative activity against HL-60 cells, with the IC_{50} value of 3.8 μM (**9e**), 9.2 μM (**9j**), and 15.3 μM (**9l**).

Having exhibiting better anti-leukemia activity in HL-60 cells, we further tested the antiproliferative activity of compounds (**9e**, **9j**, **9l**) on myeloid leukemia K562 cells. It was found that compounds (**9e, 9j, 9l**) were more sensitive to HL-60 than K562 cells (Table 2). For **9e**, the activity against HL-60 cells was eight-fold higher than against K562 cells. Moreover, the antiproliferative activity of derivatives (**9e, 9j, 9l**) on normal cells WI-38 was also

Table 2
Antiproliferative activity of compounds (**9e**, **9j** and **9l**) and selectivity index (SI).

Compds	IC_{50}			SI[a]
	HL-60	K562	WI-38	
9e	**3.8**	**32.2**	**40.7**	**10.7**
9j	9.2	29.9	34.2	3.7
9l	15.3	19.2	26.8	1.8

[a] SI = IC_{50} WI-38 normal cells/IC_{50} HL-60 cancer cells.