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Cytotoxicity of synthesized 1,4-naphthoquinone analogues on selected human cancer cell lines

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ABSTRACT

In an effort to establish new candidates with enhanced anticancer activity of 5-hydroxy-7-methyl-1,4-naphthoquinone scaffold (7-methyljuglone) previously isolated from the root extract of *Euclea natalensis*, a series of 7-methyljuglone derivatives have been synthesized and assessed for cytotoxicity on selected human cancer lines. These compounds were screened in vitro for anticancer activity on MCF-7, HeLa, SNO and DU145 human cancer cell lines by MTT assay. Most of them exhibited significant toxicity on cancer cell lines with lower IC_{50} values. The most potent derivative (**19**) exhibited the toxicity on HeLa and DU145 cell lines with IC_{50} value of 5.3 and 6.8 μM followed by compound (**5**) with IC_{50} value of 10.1 and 9.3 μM, respectively. Structure–activity relationship reveals that the fluoro substituents at position C-8 while hydroxyl substituents at C-2 and C-5 positions played an important role in toxicity.

1. Introduction

Quinone scaffolds are widespread in nature and present in many drugs which are used clinically in the therapy of solid cancer. The cytotoxic effects of these quinones are mainly due to the inhibition of DNA topoisomerase-II.[1,2] Naphthoquinones are widely distributed in plants, fungi and some animals, and most of these are found to exhibit an interesting range of pharmacological properties. 1,4-Naphthoquinone is an important example of quinone family and is used as a raw material in pharmaceutical industries. 1,4-Naphthoquinone and its derivatives exhibited several interesting and different biological responses including antibacterial,[3] antifungal,[4,5] anti-inflammatory, antiplatelet, antiallergic,[6] antithrombotic,[7] antiviral,[8] anticancer,[9–11] apoptosis,[12] lipoxygenase,[13,14] radical scavenging[15] and anti-ringworm[16] activities. Recently, 1,4-naphthoquinone derivatives were proved to be human DNA topoisomerase I and II inhibitors.[2] The presence of two carbonyl groups in naphthoquinones that have the ability to accept one or two electrons to form the corresponding radical anion or di-anion species and their acid-base properties is responsible for the various biological activities.[17,18] In another study, it was found that the presence of hydroxy groups at 5 and 8 positions, which facilitate the tautomerism in the structure of 1,4-naphthoquinone, reduced the electrophilicity of the naphthoquinone ring.

Cancer is the second leading cause of death worldwide despite a major endeavour of research and development in academia and pharmaceutical industry to search for new anticancer agents.[19–21] Although major advances have been made by researchers but the medical need is still largely unmet due to many factors; among which are the lack of selectivity of conventional drugs leading to toxicity, the metastatic spreading, and multi-drug resistance.[22–27] Novel and selective anticancer agents are urgently required due to problems associated with currently available anticancer drugs. The plant *Euclea natalensis* (A. DC.) is used traditionally for the treatment of cancer in South Africa.[28,29] The metabolite 7-methyljuglone (7-MJ) previously isolated from this plant[3], exhibited anticancer activity. A series of derivatives of 7-MJ were synthesized. Previous studies have shown that mono or dihydroxy substitution (at C-2 or C-5 and C-8 positions in the aromatic ring) of naphthoquinones resulted in higher toxicity as compared to the parent 1,4-naphthoquinone due to increased efficiency of redox cycling.[3] The introduction of a fluorine atom into antibiotic quinolones has been shown to enhance their activity. In view of these previous observations, it was decided to synthesize 7-MJ derivatives in order to establish structure–activity relationship. This paper

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reports the synthesis and cytotoxicity of a series of 5-hydroxy-, 5-alkoxy- and 5-acetoxy-8-substituted-naphthoquinones on selected human cancer cell lines. The mechanism of action was established by cell cycle analysis and structure activity relationship has also been discussed.

2. Result and discussion

2.1. Synthesis of 1,4-naphthoquinone derivatives

The synthesis of 7-MJ (**1**) and its derivatives **2**–**19** (Table 1) has been done by the same method that was previously used for the synthesis in our laboratory.[3]

2.2. Cytotoxicity

This is the first study that discloses the effects of 7-MJ derivatives on four cancer cell lines, cell cycle, apoptosis and caspase 3/7. To determine the fifty percent inhibitory concentration (IC_{50}) values of the compounds, the four adherent cancer cells were treated with several concentrations. All assays were carried out in triplicate. The cytotoxicity results (Table 1) revealed that most of the tested compounds exhibited good activity, mainly against prostate (DU-145), cervical (HeLa) and breast (MCF-7) cell lines while being less active against the oesophageal cancer cell line. Out of 18 derivatives, compounds **2**–**6** and **19** were found to be potent inhibitors of the growth of HeLa, DU-145 and MCF-7 cancer cell lines at very low concentrations.

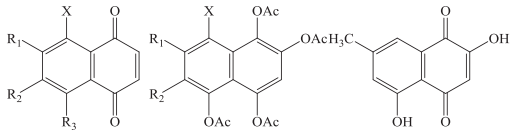
The IC_{50} values showed (Table 1) that the compounds **2**–**15** and **19** exhibited very good anticancer activity against all the cell lines. The derivative **19** was the most significant one against cell lines (HeLa: IC_{50} 5.3 μM and DU-145: IC_{50} 6.8 μM), followed by compounds **5** (DU-145: IC_{50} 9.3 μM and SNO: IC_{50} 9.4 μM) and **4** (HeLa: IC_{50} 10.2 μM and DU-145: IC_{50} 15.4 μM); while the parent compound **1** (7-MJ) was found to be less active than most of the derivatives. The compound **5** also exhibited the toxicity against MCF-7 and HeLa cell lines with IC_{50} values of 10.0 and 10.1 μM, respectively (Comparable to Cisplatin, IC_{50} 10.0 μM).

The introduction of a hydroxyl group at position C-2 in **19** resulted in a sixfold increase in the activity as compared to the parent compound **1**. The presence of halogen substituent at C-8 also increased the activity. The cytotoxicity of **6** was found to be greater than **1**; this clearly indicated that the change of position of -CH_3 group from C-7 to C-6, enhanced the activity. The weak activity of derivatives **7**–**15** (5-Alkoxy or 5-Acetoxy) indicated that the presence of 5- hydroxyl group is necessary for the activity. When the carbonyl group of quinone was converted to acetate, the activity was reduced significantly. Hence, the weakest activity of the tetra-acetate derivatives **16**–**18** (1,2,4,5-tetraacetate) revealed that the quinone moiety is highly required for the development of new significant leads. Out of all the derivatives, on the basis of their activity, compounds **1**–**6** and **19** were taken up for further investigation.

2.3. Cytotoxicity on human macrophages (U937) and peripheral blood mononuclear cells (PBMCs)

Based on the cytotoxicity data of a few derivatives on most of the cell lines, compounds **1**–**6** and **19** were further tested on peripheral blood mononuclear cells (PBMCs) and on human macrophages. The comparative toxicity on PBMCs (Table 2, Fig. 1) suggested that compounds **4** and **19** were the least toxic, whilst the other compounds including **1** were found to be more toxic then the other derivatives at the lower concentrations tested. Compounds **4** (IC_{50}: 188.7 μM) and **19** (IC_{50}: 54.0 μM) were not toxic to the PBMCs, suggesting their actions to be specific for tumour

Table 1
List of synthesized 1,4-naphthoquinone derivatives and their cytotoxicity against human cancer cell lines



1-15 16-18 19

	Substituents on naphthoquinone scaffold				Cytotoxicity of derivatives on selected cancer cell lines (IC_{50}[e] values are given in μM)			
	X	R_1	R_2	R_3	MCF-7[a]	HeLa[b]	SNO[c]	DU 145[d]
5-Hydroxy-7-methyl-1,4-naphthoquinone (7-MJ) (**1**)	H	Me	H	OH	27.2	66.6	81.4	11.9
8-Chloro-5-hydroxy-7-methyl-1,4-naphthoquinone (**2**)	Cl	Me	H	OH	15.7	13.5	17.2	29.5
8-Bromo-5-hydroxy-7-methyl-1,4-naphthoquinone (**3**)	Br	Me	H	OH	15.5	11.3	14.9	46.3
8-Fluoro-5-hydroxy-7-methyl-1,4-naphthoquinone (**4**)	F	Me	H	OH	20.3	10.2	18.9	15.4
8-Chloro-5-hydroxy-6-methyl-1,4-naphthoquinone (**5**)	Cl	H	Me	OH	10.0	10.1	9.4	9.3
5-Hydroxy-6-methyl-1,4-naphthoquinone (**6**)	H	H	Me	OH	15.3	16.4	19.0	15.4
8-Chloro-5-methoxy-7-methyl-1,4-naphthoquinone (**7**)	Cl	Me	H	OMe	20.6	19.1	24.6	26.6
8-Chloro-5-ethoxy-7-methyl-1,4-naphthoquinone (**8**)	Cl	Me	H	OEt	40.0	19.0	44.2	25.4
5-Acetoxy-8-chloro-7-methyl-1,4-naphthoquinone (**9**)	Cl	Me	H	OAc	37.9	26.2	28.8	25.5
5-Acetoxy-7-methyl-1,4-naphthoquinone (**10**)	H	Me	H	OAc	14.7	17.2	28.1	24.1
5-Methoxy-7-methyl-1,4-naphthoquinone (**11**)	H	Me	H	OMe	15.3	21.1	22.5	30.7
5-Ethoxy-7-methyl-1,4-naphthoquinone (**12**)	H	Me	H	OEt	23.7	29.7	25.3	15.0
8-Chloro-5-methoxy-6-methyl-1,4-naphthoquinone (**13**)	Cl	H	Me	OMe	16.2	41.5	44.9	36.5
8-Chloro-5-ethoxy-6-methyl-1,4-naphthoquinone (**14**)	Cl	H	Me	OEt	32.2	33.2	37.3	26.2
5-Acetoxy-8-chloro-6-methyl-1,4-naphthoquinone (**15**)	Cl	H	Me	OAc	18.7	21.8	28.6	12.5
8-Chloro-7-methylnaphthalene-1,2,4,5-tetra-*O*-acetate (**16**)	Cl	Me	H	—	51.1	52.9	56.9	59.6
7-Methylnaphthalene-1,2,4,5-tetra-*O*-acetate (**17**)	H	Me	H	—	82.0	22.4	25.0	64.6
8-Chloro-6-methylnaphthalene-1,2,4,5-tetra-*O*-acetate (**18**)	Cl	H	Me	—	57.5	27.9	53.5	81.6
2,5-Dihydroxy-7-methyl-1,4-naphthoquinone (**19**)	—	—	—	—	14.6	5.3	23.2	6.8
Doxorubicin	—	—	—	—	0.66	0.01	0.01	0.01
Cisplatin	—	—	—	—	10.0	10.0	—	—

[a] MCF-7–Breast adenocarcinoma.
[b] HeLa–Cervical epithelial carcinoma.
[c] SNO–Oesophageal carcinoma.
[d] DU145–Prostate epithelial carcinoma.
[e] IC_{50}–fifty percent inhibitory concentration