Hydroxylated-Cpd 5: Possible 'better' Arylator on Cell Growth Inhibition

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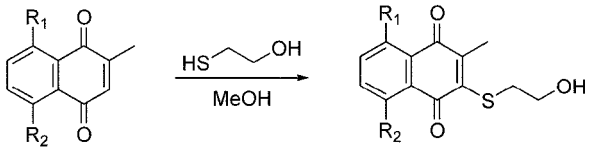
Received April 30, 2004

Key Words : Cdc25 phosphatase, Naphthoquinone, Cell growth inhibition, Reduction potential

As important cell cycle regulators, Cdc25s are key Cdk activating proteins and act by dephosphorylation of conserved Cdk residues. Since Cdc25 protein was first found as the twenty-fifth protein to be related to the cell division cycle,[1] three different members, Cdc25A, -B, and -C were identified in humans[2] and several splice variants of Cdc25 proteins have also been reported.[3] The evidence that Cdc25A and -B are overexpressed and likely important for the growth of different types of human cancer,[4] has stimulated the search for Cdc25 inhibitors. However the literature on Cdc25 inhibition is in its infancy and inhibitor design strategies are just now emerging.[5]

Recently, several 1,4-naphthoquinones have proven to be effective at inhibiting Cdc25, including vitamin K_3.[6] Among them, vitamin K derivative, Cpd 5 (2-(2-mercaptoethanol)-3-methyl-1,4-naphthoquinone) was found to be one of the most growth inhibitors *in vitro* of various tumor cell lines in the range of 9-30 μM,[6c] and markedly less active against PTP1B and other dual specificity phoaphatases, VHR and MKP-1.[7] Previously, we also demonstrated that the 1,4-naphthoquinone derivatives with the hydroxy group at C-5 and/or C-8 of the benzene ring was more active than vitamin K_3 on Cdc25A inhibition.[6b] Therefore, in the current study, we synthesized mono- and dihydroxylated Cpd 5 derivatives **2** and **3** by addition of β-mercaptoethanol to the commercially available naphthoquinones in methanol. To examine the effects of these compounds on the growth of Hep3B cells *in vitro*, cells were cultured with several concentrations of Cpd 5 or hydroxylated Cpd 5 derivatives and a growth curve was drawn from the DNA amounts of each cell sample.



Cpd 5 (1), R_1 = H, R_2 = H
2, R_1 = H, R_2 = OH
3, R_1 = OH, R_2 = OH

As shown in Figure 1, the IC_{50} values for monohydroxy and dihydroxy Cpd 5 were found to be 3 μM and 1 μM, respectively, showing them to be more potent growth inhibitors than the parent Cpd 5.

It has been reported that Cdc25A regulates endogenous ERK phosphorylation status in cells.[8] Therefore, we also measured the amount on Western blots of lysates from

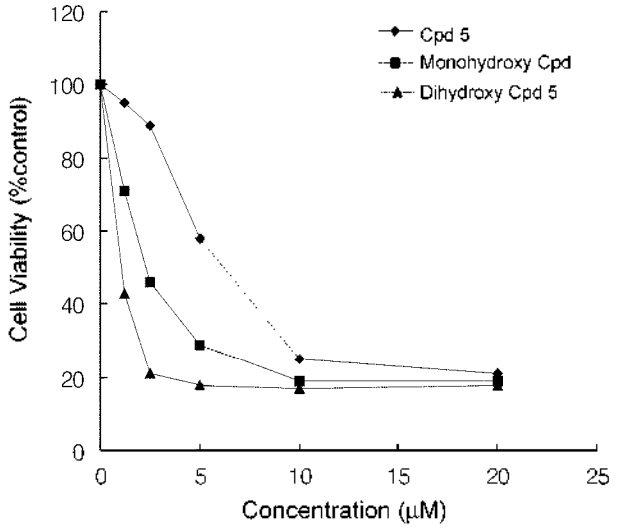


Figure 1. Monolayer cell growth was assayed after cells were plated at 5×10^4 cells/well on 6-well culture plates. After 24 h, the medium was replaced with a medium containing Cpd 5 or hydoxylated Cpd 5 at various concentrations. After treatment for 3 days, cells were trypsinized and suspended in 1 mL of phosphate-buffered saline with 5% calf serum. Absorbance at 660 nm was measured spectrophotometrically. Control experiments demonstrated a linear correlation between Hep3B cell density and absorbance at 660 nm.

treated cells, using phospho-ERK antibody. As shown in Figure 2, phospho-ERK was induced after treatment with each of the compounds on Hep3B cells, while the ERK protein levels remained constant, indicating that the increase of ERK phosphorylation occurred at growth inhibitory doses and dihydroxy Cpd 5 (**3**) was the most potent inhibitor against Cdc25A.

Although most Cdc25 inhibitors with the quinone moiety

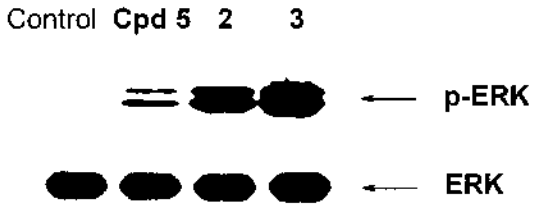


Figure 2. Effects of Cpd 5, monohydroxy Cpd 5 (**2**) and dihydroxy Cpd 5 (**3**) on ERK phosphorylation. Cells were treated with these compounds at 15 μM for 24 h. The cells were lysed and whole cell proteins (40 μg/lane) were resolved by 10% SDS-PAGE. Western blotting was performed with anti-phospho-ERK.

Table 1. HOMO and LUMO Orbital Energies and One-Electron Reduction Potentials For Quinones

	HOMO (eV)	LUMO (eV)	Potential (mV)
Cpd 5	-8.6491	-1.4826	-161
Dihydroxy Cpd 5	-8.6216	-1.5579	-91

have been reported to act by sulfhydryl arylation at the quinone nucleus, the redox properties can also generate toxic oxygen species,[9] which may cause toxicity to normal tissues and thus reduce their therapeutic attractiveness.[10] Regarding oxidative stress of quinones, the single electron reduction enzymes initiates redox cycling and oxidative stress,[11] and the relative one-electron reduction potentials of quinones control the position of the equilibrium defining futile cycling:[12]

$$Q^{\cdot-} + O_2 \rightleftharpoons Q + O_2^{\cdot-}$$

Since the equilibrium constant K is approximately $10^{\Delta E/0.06}$, where ΔE = one-electron reduction potential of oxygen (-0.155 V) - one-electron reduction potential of quinone in volts,[13] the superoxide formation will be increasingly favored at smaller reduction potentials of quinone. Recently, we determined that the potential for the one-electron reduction of quinones can simply be determined the electronic properties of the quinone system through theoretical calculation of LUMO energies using the semi-empirical AM1 method.[14] We have extended the investigation to dihydroxy Cpd 5 from the calculation of its LUMO energy by the AM1 method, resulting in the value of -1.5579 eV and $E_{1/2}$ = -91 mV, which indicates that dihydroxy Cpd 5 is better arylator of cysteine-containing proteins than Cpd 5. Compared with Cpd 5, the higher one electron reduction potential for dihydroxy Cpd 5 may be explained by internal hydrogen bonding in the dihydoxy naphthoquinone contributes to stabilization of the semiquinone, probably as a result of increased delocalization due to exchange of the hydroxyl hydrogen between neighboring oxygen atoms.

The frequency of overexpression has focused increasing attention on Cdc25 phosphatases as potential targets for cancer therapy. Despite success in development of quinones as Cdc25 inhibitors, the redox properties of the quinones can generate toxic oxygen species, resulting in the loss of selectivity of growth inhibitory effects on tumor compared to normal cells. In this study, it demonstrated the possibility that modification by addition of appropriate substituents to the quinone could be a key in achieving better electronical character, as well as better potency.

Acknowledgement. This work was supported by Chung-Ang University (2002).

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