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Fluorinated quinoid inhibitor: possible 'pure' arylator predicted by the simple theoretical calculation

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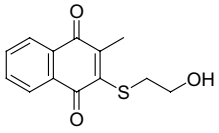
Abstract—We report on the fluorinated form of Cpd 5 as a cell growth inhibitor. This compound is 3-fold more potent than the parent Cpd 5 and is predicted, using the semi-empirical AM1 method to be only an arylator of cysteine-containing proteins, without generating reactive oxygen species.

Protein phosphatases Cdc25A and Cdc25B are overexpressed in various human malignancies,[1] which makes them attractive drug targets for anticancer therapies. Among the Cdc25 inhibitors,[2] several quinoid compounds,[3] including Compound 5 (Cpd 5, or 2-(2-mercaptoethanol)-3-methyl-1,4-naphthoquinone), have proven to be effective at inhibiting Cdc25 phosphatases.[3a] Although most quinones have been reported to inhibit Cdc25 by sulfhydryl arylation at the quinone nucleus, the redox properties of the quinones can also generate toxic oxygen species,[4] which may cause toxicity to normal tissues and thus reduce their therapeutic attractiveness.[5]



Cpd 5 (1)

Regarding oxidative stress of quinones, the single electron reduction of quinones by enzymes such as NADH-cytochrome P450 oxidoreductase, NADH-cytochrome b_5 oxidoreductase, and NADH-ubiquinone oxidoreductase, initiates redox cycling and oxidative stress.[6] And then the relative one-electron reduction potentials of quinones control the position of the equilibrium defining 'futile cycling'.[7]

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

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,[8] the superoxide formation will be increasingly favored at smaller reduction potentials of quinone. For example, 1,4-benzoquinone (BQ) is known to produce cytotoxicity in many cells by only arylation with protein thiols,[9] while 2,3-dimethoxy-1,4-naphthoquinone exerts its toxicity only via oxidative radical generation.[10] As shown in Table 1, the reduction potentials of 1,4-benzoquinone and 2,3-dimethoxy-1,4-naphthoquinone are +99 and −240 mV,[11] respectively, and the redox chemistry of quinones relates closely to biological observation. Therefore, if the reduction potential of an interesting quinone can be provided, it is possible to easily prepare a selective Cdc25 inactivator (arylator) without free radical damage to cells by reactive oxygen species.

It has been reported that the half-wave potential ($E_{1/2}$) for the first reduction step of quinones is correlated with

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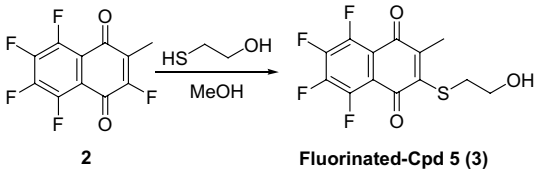
Table 1. HOMO and LUMO orbital energies and one-electron reduction potentials for quinones

	HOMO (eV)	LUMO (eV)	Potential (mV)
1,4-Benzoquinone	−11.0036	−1.7268	99
Methyl-1,4-benzoquinone	−10.8657	−1.6558	23
2,5-Dimethyl-1,4-benzoquinone	−10.8161	−1.5855	−66
2,3-Dimethyl-1,4-benzoquionone	−10.4723	−1.5958	−74
2,6-Dimethyl-1,4-benzoquinone	−10.8056	−1.5920	−80
Trimethyl-1,4-benzoquinone	−10.4251	−1.5279	−165
Vitamin K_1	−9.5599	−1.3925	−170
2-Methyl-1,4-naphthoquinone	−10.2066	−1.4720	−203
2,3-Dimethyl-1,4-naphthoquinone	−10.1769	−1.4166	−240
2,3-Dimethoxy-1,4-naphthoquinone	−9.0808	−1.4753	−240
Cpd 5	−8.6491	−1.4826	−161
Fluorinated Cpd 5	−8.9565	−2.1215	436

the unoccupied molecular orbital of lower energy (LUMO).[12] In this study, in order to estimate the one-electron reduction potential of a novel quinone analog from the values of LUMO energies of the corresponding compound, we first calculated the LUMO energy level of quinones, having the known redox potentials,[11] by means of the semi-empirical AM1 method. As shown in Figure 1, with the exception of vitamin K_1, which possesses the long phytyl side chain, plotting the LUMO energy level (E_{LUMO}) against the experimental reduction potentials of quinones resulted in a linear correlation (Eq. (1)).[13] This confirmed that the potential for the one-electron reduction of quinones can be calculated from the LUMO energy.

$$E = -936.34 \times E_{LUMO} - 1550.2 \quad (R^2 = 0.9945) \quad (1)$$

We previously reported that a synthetic K vitamin analog, Cpd 5 (**1**), was a potent inhibitor of Hep3B hepatoma cell growth.[3a] Later, it was shown that the thiol-antioxidants glutathione and *N*-acetyl-L-cysteine antagonized Cpd 5-induced Cdk4 tyrosine phosphorylation, whereas the nonthiol-antioxidants catalase and superoxide dismutase did not.[14] These results suggested that Hep3B cell growth inhibition by these K vitamin analogs may be related to inactivation of Cdc25A. However, since the antioxidants catalase and SOD cannot penetrate easily into the cell membrane, it still remains to be determined whether Cpd 5 can produce reactive oxygen species in cells. We have extended the investigation to fluorinated Cpd 5, because the reduction potential for fluorinated Cpd 5 is expected to be higher than that for Cpd 5, due to the inductive effects of the electronegative fluorine atoms, preventing superoxide generation.



2 **Fluorinated-Cpd 5 (3)**

On the basis of the relationship between $E_{1/2}$ and LUMO energies, we performed the calculation of its LUMO energy by the AM1 method, resulting in the value of −2.1215 eV and $E_{1/2} = 436$ mV by use of Eq. (1). Even though the fluorine substituents are not directly conjugated with the quinone ring, the substitution effect of the benzene ring on the electrochemical reduction of the naphthoquinone was evident. Given the much higher reduction potential of fluorinated Cpd 5 in comparison to other quinones as well as to Cpd 5, we also synthesized fluorinated Cpd 5 by addition of β-mercaptoethanol to the commercially available pentafluoronaphthoquinone **2** in methanol, and examined the effects of this compound on the growth of Hep3B cells in vitro. Cells were cultured with several concentrations of Cpd 5 or fluorinated Cpd 5 (0, 1.25, 2.5, 5, 10, 20 μM) and a growth curve was drawn from the DNA amounts of each cell sample. As shown in Figure 2, the IC_{50} value for fluorinated Cpd 5 was found to be 2 μM, showing it to be a 3-fold more potent growth inhibitor than the parent Cpd 5.

We recently demonstrated that Cpd 5 specifically inhibited the dephosphorylating actions of Cdc25A protein

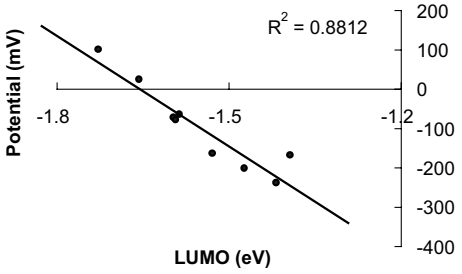


Figure 1. Linear correlation between the LUMO orbital energy and one-electron reduction potential. Labels according to Table 1.

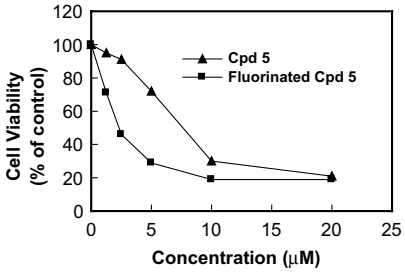


Figure 2. Monolayer cell growth was assayed after cells were plated at 5×10^4 cells/well on six-well culture plates. After 24 h, the medium was replaced with a medium containing Cpd 5 or fluorinated Cpd 5 at various concentrations. After treatment for 3 days, cells were trypsinized and suspended in 1 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.

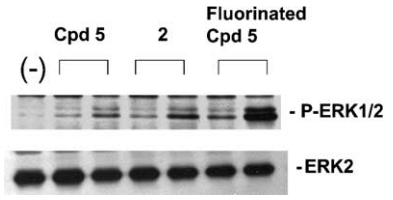


Figure 3. Effects of Cpd 5 and fluorinated Cpd 5 on ERK phosphorylation. Cells were treated with these compounds at 7.5 and 15 μM for 24 h. The cells were lysed and whole cell proteins (40 μg/lane) were resolved by 10% SDS-PAGE. Western blotting was performed with antiphospho-ERK.

on target phospho-ERK and we showed that Cdc25A regulates endogenous ERK phosphorylation status in cells.[15] This observation provided a method to quickly and quantitatively probe for Cdc25 inhibition in intact cells and an index to determine the degree of growth. Therefore, to assess induction of ERK phosphorylation by these compounds on cell lines, we measured the amount on Western blots of lysates from treated cells, using phospho-ERK antibody. As shown in Figure 3, phospho-ERK was induced after treatment with each of the compounds on Hep3B cells. The increase of ERK phosphorylation induced by the compounds was dose dependent and occurred at growth inhibitory doses. Moreover, fluorinated Cpd 5 was the more potent.

Since quinone radicals usually rapidly disproportionate to hydroquinone and quinone, the potential for reduction by a single electron to form a radical is measured by specialized electrochemical techniques such as rapid-scan cyclic voltametry, or by measuring spectrophotometrically the equilibrium constants of electron-transfer equilibria with a redox indicator. However, we simply determined the electronic properties of the quinone system through theoretical calculation using the semi-empirical AM1 method. As a result, fluorinated Cpd 5 was found to be a Hep3B cell growth inhibitor and proposed as a pure arylator.

Acknowledgements

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