BULLETIN

OF THE

KOREAN CHEMICAL SOCIETY

ISSN 0253-2964 BKCSDE 25(12)
Volume 25, Number 12 December 20, 2004

Communication

Evaluation of Sulfone Analogs of Cpd 5 as Cdc25 Inhibitors

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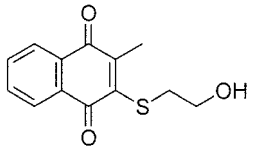
Received August 31, 2004

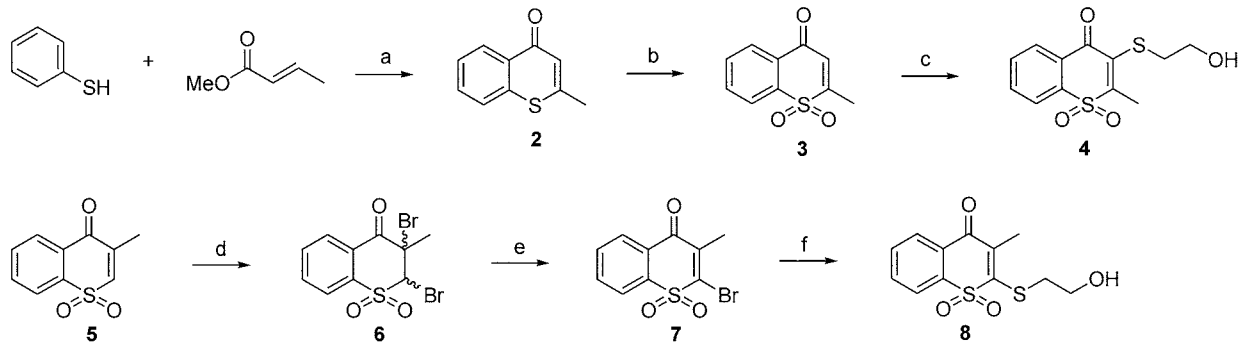
Key Words : Cdc25 phosphatase, Inhibitor, Sulfone

The search for less toxic and more effective anticancer agents has involved a detailed mechanistic investigation of the cell cycle and possible critical enzymes as rational targets. Recently, the observation that Cdc25A and Cdc25B as important cell cycle regulators are proto-oncogenes[1] and are overexpressed in many cancer cells[2] has made them as attractive drug targets. Among the Cdc25 inhibitors,[3] several quinoid compounds,[4] including Compound 5 (Cpd 5, or 2-(2-mercaptoethanol)-3-methyl-1,4-naphthoquinone), was found to be a selective and partial-competitive inhibitor of Cdc25, and was markedly less active against PTP1B, VHR and MKP-1.[4a,5] More recently, computer docking studies of Cpd 5 to Cdc25A showed that the sulfur atom of Cpd 5 is close to the cysteine of the enzyme active site in the best conformation.[6]



Cpd 5 (1)

The redox properties of the naphthoquinones can also generate toxic oxygen species,[7] which may cause toxicity to normal tissues and thus reduce their therapeutic attractiveness.[8] In present study, Cpd 5 analogs with the sulfone as an isostatic group of one of the carbonyl group were synthesized as illustrated in Scheme 1. Acylation of the addition product of thiophenol and methyl crotonate in polyphosphoric acid (PPA) yielded **2**. After oxidation of **2** with H_2O_2 in acetic acid at 100 °C, the resulting **3** was



Scheme 1. Reagents: (a) i. piperdine, ii. PPT, 90%; (b) 30% H_2O_2, acetic acid, 100 °C, 84%; (c) i. Br_2, CH_2Cl_2, ii. triethylamine, β-mercaptoethanol, 76%; (d) Br_2, CH_2Cl_2, 95%; (e) triethylamine, CH_2Cl_2, 93%; (f) β-mercaptoethanol, THF, 92%.

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Table 1. Inhibition of Cdc25A and cell cytotoxicity of Hep3B by sulfone analogues of naphthoquinone

Compounds	IC_{50} (μM)	
	Cdc25A	Hep3B
Cpd 5 (**1**)	5.0 ± 0.4	5.6
3	12.1 ± 0.9	~10
4	7.2 ± 0.6	~10
5	> 20	> 10
7	> 20	> 10
6	2.1 ± 0.2	2.5
8	> 20	> 10

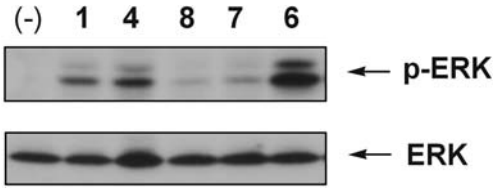

Figure 1. Effects of Cpd 5 and sulfone analogs on ERK phosphorylation. Cells were treated with these compounds at 10 μM for 24 h. The cells were lysed and whole cell proteins (40 μg/lane) were resolved by 10% SDS-PAGE. Western blotting was performed with anti-phospho-ERK.

converted to **4** by bromination, followed by addition of β-mercaptoethanol in the presence of trimethylamine. Compound **8** was also prepared from the readily available **5**[9] by the same procedure of syntheses of compound **4**.

These compounds were then tested for their inhibitory activity against Cdc25A and *in vitro* anti-proliferative activity. Although Cpd 5 is known to inhibit the dephosphorylation of *O*-methyl fluorescein phosphate (OMFP) with an IC_{50} of 0.8 μM,[10] IC_{50} value for the GST-fusion Cdc25A using the substrate *p*-nitrophenylphosphate (*p*-NPP) was 5 μM. Because the choice of substrate can affect the kinetics of enzyme inhibitions, as can the domains of the eynzyme used,[3c] IC_{50} values of the newly synthesized compounds were compared with that of Cpd 5 in the same assay condition. As shown in Table 1, the sulfone analog of Cpd 5 (**4**) exhibited Cdc25A inhibition with reasonable activity (IC_{50} = 7.5 μM), while its regioisomer **8** did not inhibit Cdc25 up to 20 μM. Consistent with enzyme inhibition, **4** showed inhibitory activity of Hep3B cell growth.

We also observed among analogs, the unexpected result that compound **6** had strong inhibition activity against Cdc25A. Since compound **6** can undergo to **7** under mild basic conditions, we tested the possible intermediate **7** against Cdc25A activity. However, the result showed no inhibition, indicating that **6** was the inhibitor of the enzyme. Compound **6** also showed stronger antitumor activity in Hep3B cells than Cpd 5.

It has been reported that Cdc25A regulates endogenous ERK phosphorylation status in cells, providing a method to quickly and quantitatively probe for Cdc25A inhibition in intact cells and also an index to determine the degree of growth inhibition.[11] We measured the amount of phospho-ERK (p-ERK) on Western blots of lysates from treated cells, using phospho-ERK antibody. As shown in Figure 1, phospho-ERK was strongly induced after treatment with compound **6** on Hep3B cells.

We here describe a new type of non-quinone Cdc25 inhibitors. Compound **6** acts with lower IC_{50} values for inhibition of both Cdc25A enzyme and Hep3B cell growth. Currently, we are investigating the detail mechanisms of inhibitory actions with potent **6** against Cdc25 and tumor cells, as well as investing its selectivity against other phosphatases.

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