Novel 3-Methyl-4-(*O*-substituted-oximino)-pyrazolin-5-ones as a Potent Inhibitors of Cdc25B Phosphatase

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Cdc25 dual-specificity phosphatases are known as important regulators in cell division cycle.[1] In human cells, three isoforms have been identified, cdc25A, B, and C, among them cdc25B acts as a checkpoint of G2/M progression in cell division cycle.[2] Cdc25A and B are potential oncogenes due to their overexpression in various human carcinomas (breast, lung, colorectal, gastric, prostate, head and neck, ovary, lymphomas, and melanomas) and tumor cell lines.[3] In addition, transgenic mice overexpressing cdc25B in the mammary and saliva glands develop hyperplasia or show increased susceptibility to chemically-induced mammary tumors.[4] Recently, some cdc25 inhibitors such as quinones,[5] quinolines,[6] norcardiones,[7] and other compounds[8] have been reported, of which the most potent inhibitors were quinones. However, the quinines reported were irreversible inhibitors with little selectivity for cdc25B.

High throughput screening of 25,000 small molecule library from Korea Research Institute of Chemical Technology for cdc25B inhibitory activity gave two active compounds of 3-methyl-4-oximinopyrazolin-5-one scaffold. Based on the hit scaffold, we synthesized 14 compounds by known procedure (Scheme 1).[9]

Therefore, the compounds **1-14** were synthesized and purified by silica gel column chromatography to afford orange to red colored crystalline solids. All the compounds were evaluated for the cdc25B inhibitory activity in microplate assay system and the results are summarized in Table 1.

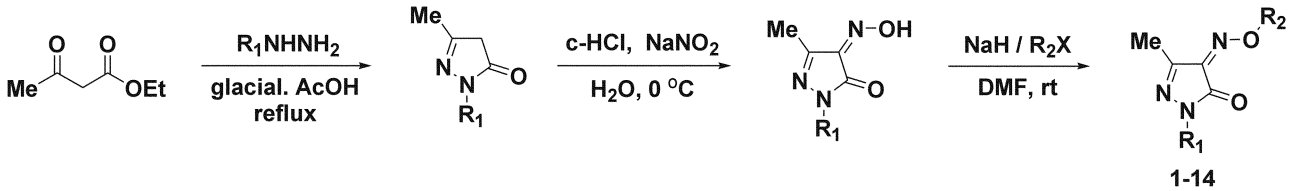
All the compounds synthesized showed inhibitory activity against cdc25B. 3-Methyl-4-(*O*-methyl-oximino)-1-phenyl-pyrazolin-5-one (**1**) and 1,3-dimethyl-4-(*O*-propargyl-oximino)-pyrazolin-5-one (**12**) exhibited the best results, and their IC_{50} was 6.13 μM and 9.17 μM respectively. However, the activity was decreased when phenyl group at 1-position was modified to bigger aromatic groups. We examined the isozyme selectivity of **1** and **12** for various human phosphatases and interestingly, we found these compounds had good selectivity not only for the other human phosphatases but also for cdc25A, the isoform of cdc25B as shown in Table 2. In contrast, most the compounds reported as cdc25 inhibitors did not have selectivity between cdc25B and cdc25A.

Also, we examined the cytotoxicities of **1** and **12** on five

Table 1. Cdc25B inhibition of Compounds **1-14** in Enzyme Assay*

Compd. No.	R_1	R_2	% Inhibition (20 μM)	IC_{50} (μM)
1	phenyl	methyl	85.01	6.13
2	phenyl	n-propyl	55.34	13.02
3	phenyl	isopropyl	31.17	
4	phenyl	propargyl	26.63	29.19
5	phenyl		25.39	33.46
6	phenyl		43.66	17.92
7	3-tolyl	n-propyl	34.35	24.22
8	4-methoxyphenyl	methyl	28.93	27.75
9	2-chlorophenyl	allyl	18.00	
10	2,4-dichlorophenyl	methyl	26.44	20.48
11	2,4-dichlorophenyl	n-propyl	11.72	
12	methyl	propargyl	87.45	9.17
13	methyl		43.81	20.89
14	methyl	methanesulfonyl	44.58	17.43

*Enzyme: cdc25B 0.2 μg. Condition: final volume 200 μL, 60 min incubation at rt. pH 8.5. Substrate: 20 μM FDP. Vehicle: DMSO. Compound concentration: 20 μM.



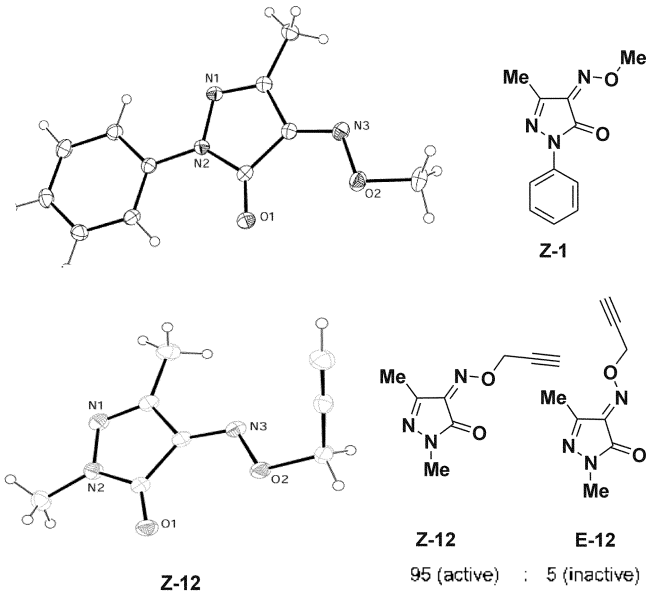
Scheme 1

Table 2. Isozyme Selectivity of 1 and **12** for various Human Phosphatases

Isozyme / Compd. No.	Cdc25B	Cdc25A	PTP-1B	CD45	VHR	YOP	PP1
1	6.13	>>100	28.40	>>100	>100	>>100	>>50
12	9.17	>>100	>>100	>>100	>100	>>100	>>50

Table 3. Cytotoxicities of 1 and **12** on various Human Tumor Cells (ED_{50}; μg/mL)

Compd. No.	A549	SK-OV-3	SK-MEL-2	XF498	HCT15
1	5	10	8	8	3
12	3	7	8	10	7



Scheme 2

human tumor cell lines, and the results were summarized in Table 3. **1** and **2** exhibited potent cytotoxicities on the tested human tumor cells at 3-10 μg/mL (ED_{50}).

During the systhesis of 3-methyl-4-oximinopyrazolin-5-ones (**1-14**), 1-phenyl or substituted-phenyl derivatives (**1-11**) gave single products in the step of nitrosation and *O*-akylation process, while 1-methyl derivatives (**12-14**) gave small amount of isomers with the similar ^{1}H NMR spectra. Z-Form and E-form of oximino double bonds were considered as the possible conformational isomers and their structures were depicted in Scheme 2. We separated the isomers of **Z-12** and **E-12** whose ratio was 95 : 5, and the structure of major compound (**Z-12**) was confirmed by X-ray crystallography. The cdc25B inhibitory activity of the minor isomer, **E-12** was found inactive. The structure of **1** was also proved to be **Z**-form. From these results, we concluded the nitrosation of pyrazolin-5-ones mostly afforded **Z**-form oximes at 4-position and only **Z**-forms of 3-methyl-4-oximinopyrazolin-5-ones (**1-14**) has inhibitory activity for cdc25B. In addition, 3-methyl-(*O*-substituted-oximino)-pyrazolin-5-one derivatives (**1-14**) are thought to be a good lead scaffold for developing a new anticancer drug based on the inhibition of cdc25B phoaphatase.

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