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Identification of potent 5-pyrimidinyl-2-aminothiazole CDK4, 6 inhibitors with significant selectivity over CDK1, 2, 5, 7, and 9

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Abstract—5-Pyrimidinyl-2-aminothiazole **1** was identified as an inhibitor of cyclin-dependent kinases (CDKs) by a screening of the Merck sample repository. The introduction of a methyl group at the C-5 or C-6 position on the pyrimidine ring, directed toward the gate keeper residue of CDK4 (Phe93), led to significant enhancement of selectivity for CDK4 over other CDKs. Compound **3** exhibited more than 300-fold selectivity for CDK4 over CDK1, 2, 5, 7, and 9. Subsequent improvements in aqueous solubility afforded compound **4**, which is available for further in vivo studies and this compound inhibited pRb phosphorylation and BrdU incorporation in tumor models.

CDKs are a prominent family of protein kinases which plays key roles in regulation of the cell cycle in eukaryotic cells.[1] Orderly cell cycle progression requires activation of CDKs, which is mainly controlled by expression of their activator subunit, cyclin. CDK4 and CDK6 complex with cyclin D, and CDK2 complex with cyclin E or A sequentially phosphorylate retinoblastoma protein (pRb) to facilitate the G1/S progression. CDK4 and CDK6 are closely related proteins with basically indistinguishable biochemical properties. pRb is a negative regulator of transcription factor E2F, with which it forms a complex. When hyperphosphorylated by CDKs, pRb loses its binding activity to E2F and the released E2F activates transcription of genes whose products are critical for the cell cycle progression. CDK activity is also regulated by CDK inhibitory proteins (CDKIs). Among them, INK4 family protein represented by $p16^{INK4a}$ selectively inhibits cyclin D/CDK4 and CDK6 complexes, and induces G1 cell cycle arrest when overexpressed in pRb-positive mammalian cells. Deregulation of the cell cycle is a hallmark of cancer.[2] Indeed, genetic or epigenetic mutations in $p16^{INK4a}$/cyclin D/CDK4 and CDK6/pRb pathway are commonly observed in a variety of many types of human cancers, suggesting CDK4, 6 may be an attractive target for development of anti-cancer drugs.

To date, 13 types of CDKs have been identified in the human genome, and there are some with functions unrelated to cell cycle regulations.[3] CDK7 and CDK9 are components of transcription regulation factor TFIIH and P-TEFb, respectively,[4] and CDK5 plays numerous functions in the nervous system.[5] Thus, for development of anti-cancer agents, a small molecule inhibitor of CDK4, 6 with selectivity over non-cell cycle CDKs would be important to further understanding of biology of CDKs and development for anti-cancer agents. Here, we report the development of novel CDK4, 6 selective inhibitors with a 5-pyrimidinyl-2-aminothiazole scaffold.

We previously reported that the diarylurea class of compounds was identified to be a novel class of CDK4 selective inhibitors.[6] Further characterization of this class of compounds showed limited CDK4 selectivity over some other CDKs, except for CDK1 and 2. We attempted to find new classes and strategies for generating inhibitors with selectivity for CDK4 over other CDKs including CDK5, 7, and 9 as well as 1 and 2. In the first place, we screened the Merck sample repository, and a series of compounds with a 5-pyrimidinyl-2-aminothiazole scaffold was identified to show potent inhibitory activities against all CDKs tested. Structure–activity relationships (SARs) obtained during screening indicated that the cyclohexyloxy group at the

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C-2 position on the pyrimidine ring is an important component of the compounds' potent inhibitory activity against CDK4.

We investigated the docking study of compound **1** with the ATP-binding pocket of a CDK4 model (Fig. 1). As a result of this study, it seemed that the gate keeper[7] and the solvent accessible site[6] were accessible from compound **1**. The gate keeper residue is generally accepted as the target site for influencing selectivity in protein kinases. Also as reported previously, non-conserved residue, Thr102 in CDK4, was found to be the key residue for selectivity of CDK4 over other CDKs, especially CDK1 and 2. Therefore, we decided to introduce substituents at the C-5 and C-6 positions on the pyrimidine ring, directed toward the gate keeper and the C-5 position on the pyridine ring, directed toward the solvent accessible site.

The results of the gate keeper-directed modification are shown in Table 1. It was concluded from the docking study that the space available between the pyrimidine ring and Phe93 gate keeper residue was limited (Fig. 1); therefore, a methyl group was introduced at either the C-5 or the C-6 position on the pyrimidine ring. Surprisingly, these gate site-directed modifications were found to greatly reduce the inhibitory activity against other CDKs than CDK4, while CDK4 inhibitory activity was retained. In particular, the C6-methyl derivative **3** showed more than 300-fold selectivity for CDK4 over other CDKs (Table 1).

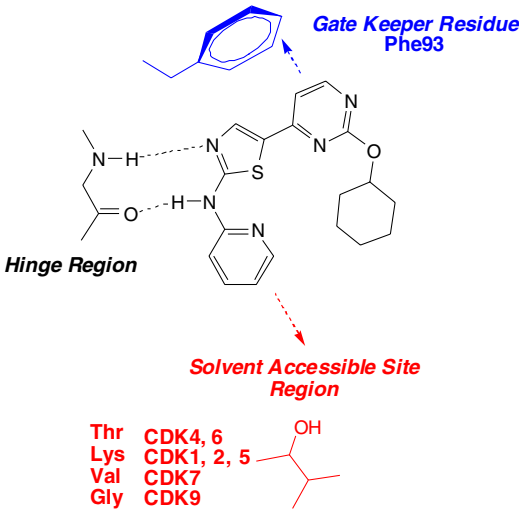
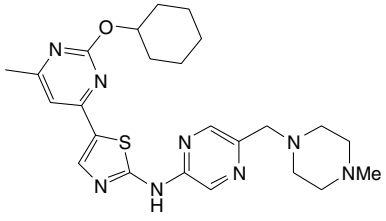


Figure 1. Schematic representation of predicted binding mode of compound **1** in CDK4 model.

Selective inhibitors of CDK4 have been reported in the literatures[8]; however, there are few reports of showing the inhibitory activity against non-cell cycle-related CDKs, such as CDK5, 7, and 9 among current selective CDK4 inhibitors. To our knowledge, this is the first identification of an inhibitor that displays high selectivity for CDK4 over CDK1, 2, 5, 7, and 9. In the course of medicinal chemistry efforts to generate compounds with adequate solubility, compound **3** led us to identify compound **4**[9] (Table 2) which has an amino alkyl group on the pyrazine ring. The compound **4** also exhibited significant selectivity for CDK4, 6 over other CDKs and showed sufficient aqueous solubility for intravenous administration. Compound **4** potently inhibited more than 80% of pRb phosphorylation at Ser780 residue, which is specifically phosphorylated by CDK4, 6, and consequently roughly 90% of cells were arrested at G1 in Eol-1 human eosinophilic leukemia cells at 300 nM

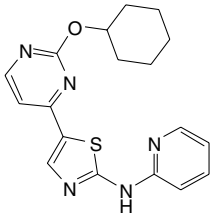
Table 2. CDK4/6 selective inhibition by compound **4**



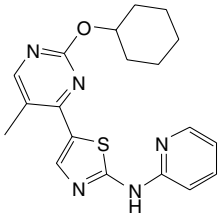
4

Inhibitory activity (IC_{50}, nM)						
CDK4	CDK6	CDK1	CDK2	CDK5	CDK7	CDK9
9.2	7.8	600	1700	3000	530	2500

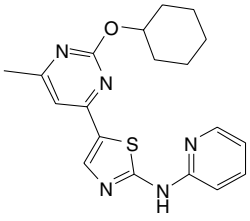
Table 1. Structures and enzyme inhibitory activities of 5-pyrimidinyl-2-aminothiazole-based compounds



1

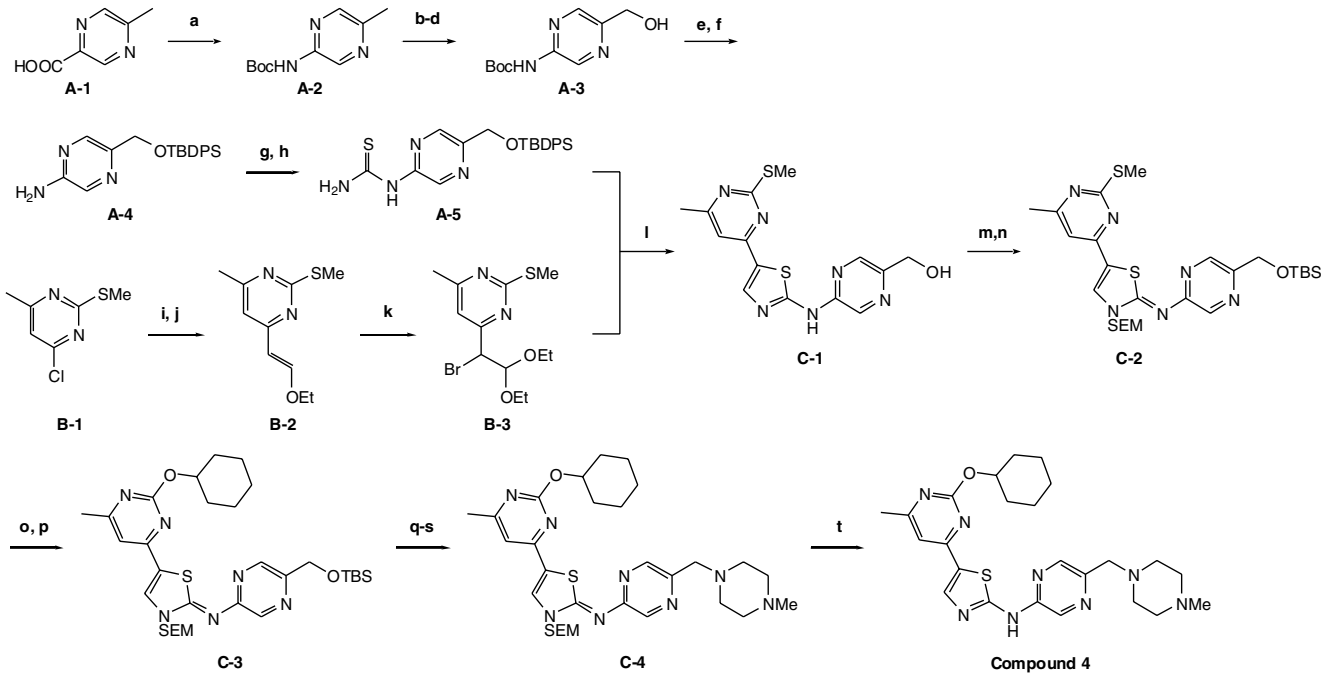


2



3

Compound	Inhibitory activity (IC_{50}, nM)					
	CDK4	CDK1	CDK2	CDK5	CDK7	CDK9
1	4.2	24	14	34	20	2.5
2	13	>1000	270	>10000	190	26
3	34	>10000	>10000	>10000	>10000	>10000



Scheme 1. Synthesis of compound **4**. Reagents and conditions: (a) DPPA, Et_3N, 3 h, 100 °C, dioxane, ^tBuOH, 88%; (b) NBS, AIBN, 12 h, 100 °C, CCl_4; (c) KOAc, 18-crown-6-ether, 1 h, rt, MeCN; (d) 3 N NaOH aq, 12 h, rt, MeOH, 29% in 3 steps; (e) TBDPSCl, imidazole, 12 h, rt, DMF, 99% ; (f) TFA, 5 h, rt, $CHCl_3$, 92%; (g) BzNCS, rt, 2 h, THF, 96%; (h) K_2CO_3, 45 °C, 5.5 h, THF/MeOH/H_2O, 99%; (i) ethyl ethynyl ether, $BH_3 \cdot THF$, rt, 4 h, THF; (j) 3 N NaOH aq, PPh_3, $Pd(OAc)_2$, rt, 6 h, THF, 88% in 2 steps; (k) NBS, rt, 0.5 h, EtOH, 80%; (l) *p*-TsOH · H_2O, 90 °C, 12 h, EtOH/H_2O; (m) TBSCl, imidazole, 3 h, rt, DMF; (n) SEMCl, EtN^iPr_2, 1 h, 0 °C, DMF, 27% in 3 steps; (o) *m*CPBA, 1.5 h, 0 °C, $CHCl_3$, 99%; (p) NaH, cyclohexyl alcohol, rt, 0.5 h, DMF; (q) 1 M TBAF in THF, 1 h, rt, THF, 40% in 2 steps; (r) MsCl, EtN^iPr_2, 1 h, 0 °C, $CHCl_3$; (s) *N*-methylpiperazine, K_2CO_3, 1 h, 70 °C, $CHCl_3$; (t) 4 N HCl in dioxane, 12 h, rt, MeOH, 45% in 3 steps.

in vitro. In addition, this compound inhibited pRb phosphorylation and BrdU incorporation by 99% and 91%, respectively, in Eol-1 xenograft tumor models in nude rats when administered via constant intravenous infusion for 24 h at a plasma concentration of 510 nM (manuscript in preparation).

The synthetic route for preparation of compound **4** is illustrated in Scheme 1. Reaction of the thiourea **A-5**, which was prepared from 5-methylpyrazine-2-carboxylic acid **A-1** in 8 steps, with 2-bromo-diethyl acetal **B-3** gave 5-pyrimidinyl-2-aminothiazole **C-1**. Next, the methylthio group was oxidized with *m*CPBA, followed by introduction of a cyclohexyloxy group to give **C-3**. *N*-Methylpiperazine was then introduced via the mesylation reaction. Finally, deprotection of SEM group provided compound **4** as a HCl salt.

In conclusion, we used compound screening and medicinal chemistry techniques, supported by molecular modeling, to identify potent CDK4 inhibitors **3** and **4** which show high selectivity over other CDK family kinases. These compounds are based on a novel 5-pyrimidinyl-2-aminothiazole scaffold. Introduction of a methyl group at the C-6 position of the pyrimidine ring in this series was found to greatly enhance CDK4 selectivity over other CDK family kinases. Compound **4**, which showed significant selectivity for CDK4, 6 over non-cell cycle CDKs, showed mechanism-based inhibition of CDK4 in cultured cells and in vivo. These results suggest that compound **4** may be useful in drug development and for further elucidation of CDK biology. Further SAR analysis of the analogues and molecular modeling studies to clarify the mechanism of their CDK4, 6 selective inhibitions and biological profiles of compound **4** will be reported elsewhere in due course.

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9. ^{1}H NMR(400 MHz, DMSO-d_6) δ [ppm] 8.63 (s, 1H), 8.57 (s, 1H), 8.41 (s, 1H), 7.47 (s, 1H), 5.02–4.93 (m, 1H), 4.47 (br s, 2H), 3.64 (br s, 4H), 3.40 (br s, 4H), 2.81 (s, 3H), 2.39 (s, 3H), 2.05–1.95 (m, 2H), 1.81–1.70 (m, 2H), 1.61–1.22 (m, 6H). MS (ESI+): *m/z* 481 $(M+H)^{+}$.