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Synthesis of 2-amino-4-(7-azaindol-3-yl)pyrimidines as cyclin dependent kinase 1 (CDK1) inhibitors

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Abstract—A novel series of 2-amino-4-(7-azaindol-3-yl)pyrimidines was discovered as cyclin dependent kinase 1 (CDK1) inhibitors. The core structure was synthesized via Pd(II) catalyzed coupling reaction. A number of analogues showed good potency for CDK1 and exhibited cellular antiproliferation activity. The structure–activity relationship is described.
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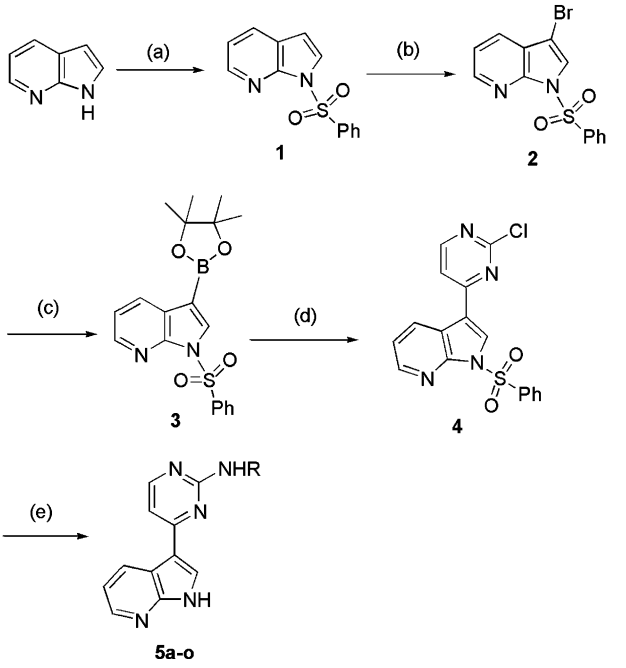
Cyclin dependent kinases (CDKs) are a family of structurally homologous serine/threonine kinases consisted of a catalytic subunit bound to an activating cyclin molecule. They are emerging as valuable anticancer molecular targets due to the observation that CDK regulators are frequently altered and overexpressed in malignancies.[1] CDKs and cyclins play essential roles in governing the eukaryotic cell cycle.[2] For example, CDK1/cyclin B regulates transition from G2 to M phase; while CDKs 4 and 6 coupled to cyclin D govern progression from G1 phase; and CDK2/cyclin A or E controls transition to S phase and progression through S phase. Intensive HTS screening and CDK crystal structure-based drug design efforts have generated a large number of scaffolds as CDK inhibitors. This tremendous research has also made it possible to design inhibitors with selectivity for particular CDKs. Currently two CDK2 selective inhibitors are in Phase I clinical trial, including 2-aminothiazole derivative BMS-387032 (SNS-032)[3] and the purine analogue (*R*)-roscovitine (CYC-202).[4]

Our efforts to develop small molecular ATP-competitive CDK inhibitors as cancer therapeutics have resulted in the discovery of 2-amino-4-aryl-5-chloropyrimidine analogues as antiangiogenic cyclin dependent kinase 1 inhibitors.[5] We now report the discovery of a related series of CDK1 inhibitors, 2-amino-4-(7-azaindol-3-yl)pyrimidines, obtained through structure-based analogue synthesis and optimization. Here, we describe the chemistry, structure–activity relationship (SAR) study, and biological characterization of these 2-amino-4-(7-azaindol-3-yl) pyrimidines.

The analogue synthesis began with protection of 7-azaindole, as shown in Scheme 1. The benzenesulfonyl protected compound **1** was treated with *N*-bromosuccin-



Scheme 1. Synthesis of 2-amino-4-(7-azaindol-3-yl)pyrimidines **5a–o**. Reagents: (a) benzenesulfonyl chloride, Et_3N, THF, 90%; (b) NBS, THF, 86%; (c) bis(pinacolato)diboron, $Pd(II)(dppf)_2Cl_2$, KOAc, THF, 92%; (d) 2,4-dichloropyrimidine, $Pd(PPh_3)_4$, K_2CO_3, DME, 85%; (e) RNH_2, 2-methoxyethanol, 80–95%.

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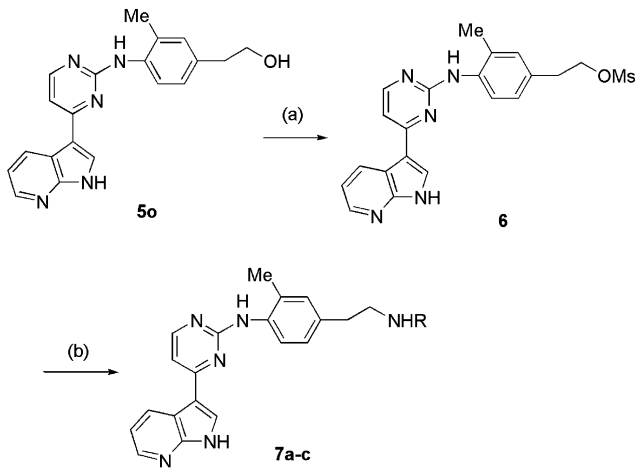
imide to generate bromide **2**, which was converted to boronate **3** using bis(pinacolato) diboron in the presence of $Pd(II)Cl_2(dppf)$.[6] It is noteworthy that NH protection with the benzenesulfonyl group was critical because the reactions with unprotected 7-azaindole or BOC protected 7-azaindole failed to generate the desired product. Coupling of compound **3** with 2,4-dichloropyrimidine using $Pd(0)(PPh_3)_4$ produced biaryl intermediate **4**. This reaction proceeded with superior regioselectivity and high yield. Next, the chloride of compound **4** was displaced with various amines to provide the desired analogues **5a**–**o**. Two equiv of amine was used in this step because cleavage of the benzenesulfonyl group also consumed one equivalent of amine reagent.

Solubilizing amino side chains were installed using the chemistry shown in Scheme 2. The hydroxyl compound **5o** was reacted with methanesulfonyl chloride to generate mesylate **6**, which was then treated with various amines to give the desired derivatives **7a**–**c**.

All analogues were tested for kinase and cellular antiproliferative activity.[7] CDK1 activity was measured using CDK1 in complex with cyclin B to phosphorylate a histone-H1 biotinylated peptide substrate. Inhibition of CDK1 activity was measured by observing a reduced amount of ^{33}P-γ-ATP incorporated into the immobilized substrate in a Flashplate assay format. In the cell proliferation assay, the HeLa (cervical carcinoma) cell line was used. The IC_{50} for inhibition of cell proliferation was determined by quantifying the incorporation of ^{14}C-thymidine into cellular DNA. The data are shown in Table 1. Compound **5a** with an unsubstituted phenyl group showed moderate activity for CDK1 with an IC_{50} of 54 nM. Addition of an extra group at C-2 position of the phenyl ring generated mixed results. For example, a hydroxyl group (**5b**) was detrimental to kinase inhibition, while methoxy (**5c**), fluoro (**5d**), trifluoromethyl (**5e**) or ethyl group (**5f**) had little effect on potency. On the other hand, chloro (**5g**), bromo (**5h**) or methyl (**5i**) group at C-2 position was beneficial to activity. Compound **5i** had an IC_{50} of 3 nM for CDK1. However, shifting methyl group to C-3 or C-4 position of the phenyl ring reduced kinase binding by more than 10-fold. Compounds **5j** and **5k** had double-digit nM IC_{50s} for CDK1. Adding aminoalkyl or hydroxyalkyl side chain to C-4 position of the 2-methylphenyl ring generated positive effect on both CDK1 and HeLa cell potency. The enhanced cellular activities for analogues **7a–c** could be resulted from their improved solubility and cellular permeability. Attaching cyclohexyl (**5l**) or aminocyclohexyl (**5n**) to the pyrimidine ring also generated potent compounds, though an extra methylene linkage between cyclohexyl and the NH group seemed to be harmful to potency.



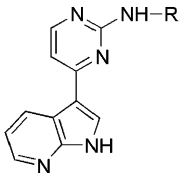
Scheme 2. Synthesis of analogues **7a**–**c** with amino side chains. Reagents: (a) methanesulfonyl chloride, Et_3N, CH_2Cl_2, 95%; (b) RNH_2, DMF, 80–90%.

The NH of the 7-azaindolyl ring was derivatized with several groups in order to assess its role in CDK1 binding. Using the chemistry shown in Scheme 3, compound **5l** was treated with KO*t*-Bu, then various electrophiles were added to produce the corresponding products **8a**–**d**. Alkylation of the NH with methyl or dimethylaminoethyl group resulted in a near complete loss of potency. As shown in Table 2, compounds **8a** and **8d** had an IC_{50} of >10 μM for CDK1. Acetylation or methanesulfonylation also reduced potency, though to a lesser extent. Therefore, an unsubstituted NH is crucial for both CDK1 and cellular antiproliferation activity.

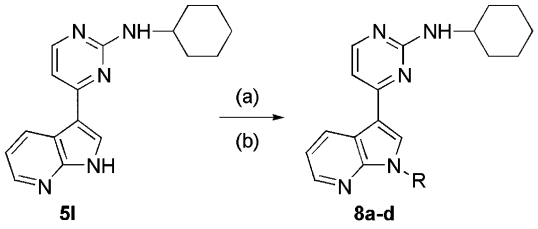
A methyl group was added to C-2 position of the 7-azaindolyl ring using the chemistry shown in Scheme 4. Compound **4** was treated with LDA at −78 °C, followed by addition of methyl iodide to afford compound **9**. Next the chloride was displaced with *trans*-1,4-cyclohexanediamine to generate diamine **10**. In contrast to the facile removal of the *N*-benzenesulfonyl group observed in the synthesis of compounds **5a**–**o**, the adjacent C-2 methyl group caused the *N*-benzenesulfonyl group to be stable under the hot aminolysis conditions. Therefore, an extra deprotecting step with potassium carbonate was used to produce final product **11**. Based on the SAR shown by two closely related series imidazo[1,2-*a*]pyridine and imidazo[1,2-*a*]pyridazine,[8] it was expected that introduction of a methyl group to the C-2 position would be tolerated. However, the results for compound **11** showed a 140-fold reduction for CDK1 inhibition compared to compound **5n**, which indicates that there is a significant difference in SAR between our series and the two series reported previously from AstraZeneca group.[8]

To evaluate the kinase selectivity of this series, selected compounds **5i** and **5l** were screened against a panel of 100 kinases with 2 μM of ATP used. At the concentration of 1 μM, both compounds displayed >50% inhibition of 61 kinases in the panel, and >80% inhibition of 33 kinases. Strong inhibition for other CDK family members (including CDK2/cyclinA, CDK2/cyclinE, CDK 3/cyclinE, CDK5/p35 and CDK6/cyclinD3) was observed. In addition, many other kinases implicated in cancer and other diseases were strongly inhibited, indicating these compounds are promiscuous kinase inhibitors. The selectivity data highlight a commom issue: generating selective CDK inhibitors in order to

Table 1. Enzymatic and cellular activity for selected compounds



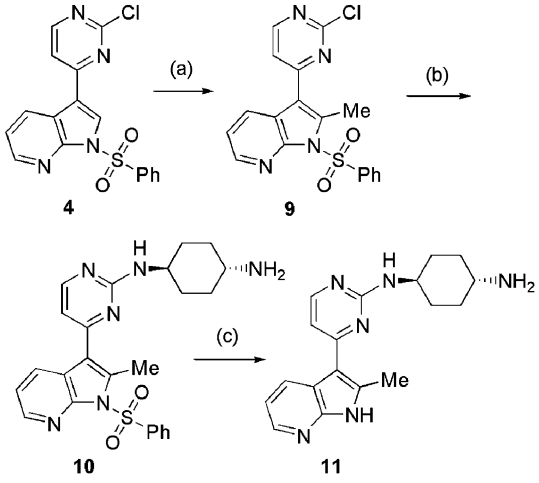
Compound	R	CDK1 IC_{50} (μM)	HeLa prolif. IC_{50} (μM)
5a	Phenyl	0.054	0.382
5b	2-OH–phenyl	0.139	0.955
5c	2-CH_3O–phenyl	0.044	0.350
5d	2-F–phenyl	0.046	0.092
5e	2-CF_3–phenyl	0.056	0.392
5f	2-CH_3CH_2–phenyl	0.068	0.292
5g	2-Cl–phenyl	0.009	0.140
5h	2-Br–phenyl	0.013	0.095
5i	2-CH_3–phenyl	0.003	0.028
5j	3-CH_3–phenyl	0.057	0.290
5k	4-CH_3–phenyl	0.040	0.220
5l	Cyclohexyl	0.014	0.031
5m	Cyclohexyl–CH_2	0.151	0.368
5n	*trans*-4-NH_2–cyclohexyl	0.002	0.001
5o	4-HO(CH_2CH_2)–2-CH_3–phenyl	0.0006	0.011
7a	4-[Morpholin-4-yl-(CH_2)$_2$]–2-CH_3–phenyl	0.0019	0.003
7b	4-[Piperidin-1-yl-(CH_2)$_2$]–2-CH_3–phenyl	0.001	0.003
7c	4-[Pyrrolidin-1-yl-(CH_2)$_2$]–2-CH_3–phenyl	0.0006	0.003



Scheme 3. Synthesis of analogues **8a**–**d**. Reagents: (a) KO-*t*-Bu, THF; (b) methyl iodide for **8a**; acetic anhydride for **8b**; methanesulfonyl chloride for **8c**; 2-dimethylaminoethyl chloride hydrochloride salt for **8d**.

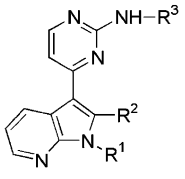
reduce off-target side effects still remains as a challenge, as evidenced by many other CDK scaffolds.[9]

In summary, a novel series of 2-amino-4-(7-azaindol-3-yl)pyrimidines was discovered as cyclin dependent kinase 1 (CDK1) inhibitors. The core structure was



Scheme 4. Synthesis of analogue **11**. Reagents: (a) LDA, MeI, THF, 25%; (b) *trans*-1,4-cyclohexanediamine, 2-methoxyethanol, 50%; (c) K_2CO_3, MeOH, 50%.

Table 2. Enzymatic and cellular activity for selected compounds



Compound	R^1	R^2	R^3	CDK1 IC_{50} (μM)	HeLa prolif. IC_{50} (μM)
8a	Methyl	H	Cyclohexyl	10.0	52.1
8b	Acetyl	H	Cyclohexyl	0.031	0.023
8c	Methanesulfonyl	H	Cyclohexyl	0.128	0.393
8d	2-Dimethylaminoethyl	H	Cyclohexyl	10.4	8.9
11	H	CH_3	*trans*-4-Aminocyclohexyl	0.28	0.13

synthesized via Pd(II) catalyzed coupling reaction. With unsubstituted NH of the 7-azaindolyl group and methyl group at C-2 position of the phenyl ring, excellent kinase and cell activity was achieved. By adding aminoalkyl and hydroxyalkyl side chains to C-4 position of the 2-methylphenyl group, both CDK1 and cell potency were enhanced. Future work for this series will be mainly focused on improving its kinase selectivity for CDK1.

Acknowledgments

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