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Discovery of a novel class of 2-aminopyrimidines as CDK1 and CDK2 inhibitors

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ABSTRACT

A series of new 2-(2-aminopyrimidin-4-yl)phenol derivatives were synthesized as potential antitumor compounds. Substitution with pyrrolidine-3,4-diol at the 4-position of phenol provided potent inhibitory activity against CDK1 and CDK2. X-ray crystal structural studies were performed to account for the effect of the substituent on both the enzymatic and cell growth inhibitory activities.

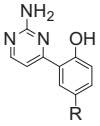
The cell division cycle is regulated by cyclin dependent kinases (CDKs). CDKs are serine/threonine kinases that are regulated precisely by the binding of their regulatory subunits. CDKs are activated by cyclin binding and phosphorylation, and deactivated by either removal of the cyclin or binding with CDK inhibitors (CDKIs) such as Cip/Kip and INK families. Cell cycle deregulation is frequently accompanied by anomalous CDK activity in numerous human cancers, caused by abnormally high expression of cyclin and/or downregulation of CDKIs.[1–3] This suggests that CDKs are attractive pharmacological targets for the treatment of cancer.[4] The majority of drug discovery efforts have focused on the invention of ATP competitive inhibitors.[5]

2-Aminopyrimidines have been widely used as pharmacophores for drug discovery. Among those compounds having potent anticancer activity and CDK inhibitory activity,[6–9] 2-(2-aminopyrimidin-4-yl)phenols showed very potent enzyme inhibitory activity and anti-proliferative activity.[8] As a part of our search for CDK inhibitors, a 2-(2-aminopyrimidin-4-yl)phenol scaffold was chosen and structural variations were performed. This report summarizes the synthesis of these compounds and the effect of structural variations on both enzymatic and cellular activities.

First, the search for new substituents at the 4-position of phenol was tried. Introduction of cyclic amines reduces the inhibitory activity drastically against both CDK1 and CDK2 (Table 1). However, substitution of nitrogen with oxygen improved the potency up to the submicromolar range (compounds **3** and **4**). The introduction of pyrrolidine-3,4-diol (compound **8**) provided very strong inhibitory activity against both CDK1 and CDK2. With our finding that pyrrolidine-3,4-diol had this effect, further modifications were performed at the 6-position of the pyrimidine ring of 2-(2-aminopyrimidin-4-yl)phenol.

The synthetic route for the preparation of (3*S*,4*S*)-1-(3-(2-aminopyrimidin-4-yl)-4-hydroxyphenyl)pyrrolidine-3,4-diol is shown in Scheme 1.

Table 1
CDK inhibitory activities of 2-(aminopyrimidin-4-yl)phenol derivatives[a]



Compds	R	IC_{50}[b] (μM)	
		CDK1	CDK2
1	Piperidin-1-yl	>10	>10
2	4-Ethylpiperazin-1-yl	7.94	>10
3	Morpholino	2.24	0.79
4	1,1-Dioxidothiomorpholino	7.5	3.98
5	4-Oxopiperidin-1-yl	>10	>10
6	4-Hydroxypiperidin-1-yl	10.0	3.76
7	3-Hydroxypyrrolidin-1-yl	2.99	2.11
8	(3*S*,4*S*)-3,4-Dihydroxypyrrolidin-1-yl	0.015	0.021

[a] The CDK inhibitory assays were performed as described in Ref. 10. [ATP] = 100 μM.
[b] The values displayed correspond to the mean average of three experiments.

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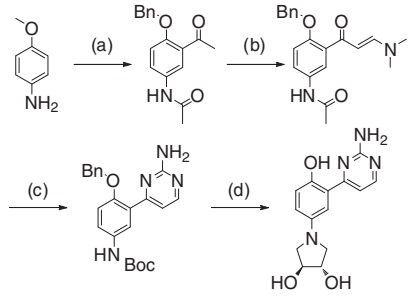
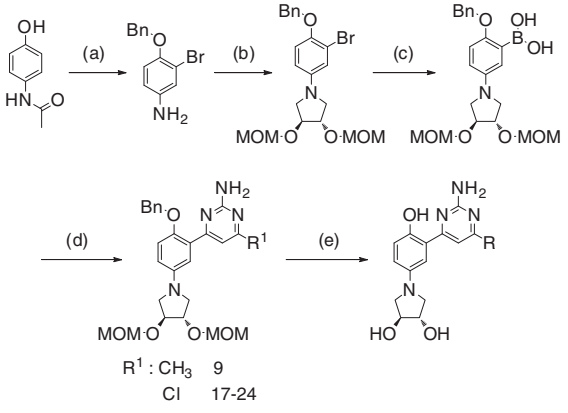


Scheme 1. Synthesis of (3*S*,4*S*)-1-(3-(2-aminopyrimidin-4-yl)-4-hydroxyphenyl)pyrrolidine-3,4-diol **8**. Reagents and conditions: (a) (i) Ac_2O, TEA, DCM, 89%; (ii) $AlCl_3$, CS_2, AcCl, CH_3CN, 85%; (iii) BnBr, K_2CO_3, DMF, 98%; (b) DMFDEA, 98%; (c) (i) Boc_2O, DMAP, TEA, 77%; (ii) 2 N NaOH, 96%; (iii) NH_2CNHNH_2, NaOEt, 98%; (d) (i) TFA, DCM, 80%; (ii) (2*R*,3*R*)-$ICH_2CH(OMOM)CH(OMOM)CH_2I$, TEA, *n*-BuOH, 44%; (iii) BBr_3, DCM, 89%.

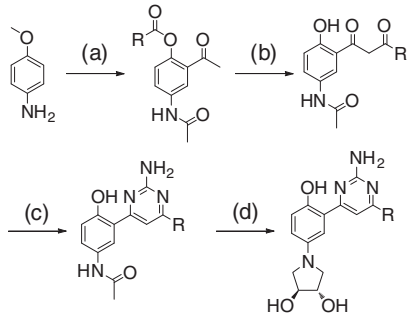
After an acetyl group was introduced through Fries rearrangement, aminopyrimidine was synthesized via reaction with *N*, *N*-dimethylformamide diethylacetal followed by reaction with guanidine. Formation of pyrrolidine was performed with MOM-protected 2,3-dihydroxy-1,4-diiodobutane, which was prepared from diethyl L-tartarate.[11] Modifications of the substituent at the 6-position of 2-aminopyrmidine were performed via two methods, as shown in Schemes 2 and 3. Sukuki coupling with 2-amino-4,6-dichloropyrmidine followed by reaction with corresponding amines provided amine substituted compounds (**17**–**24**) as shown in Scheme 2.

Alkyl groups were introduced at the 6-position of 2-aminopyrmidine, as shown in Scheme 3. The acylated 2-hydroxyacetophenone, upon treatment with sodium hydride in the presence of HMPA, underwent the Baker–Venkataraman rearrangement to give the diketone. Reaction with guanidine provided the 4,6-disubstituted 2-aminopyrimidine.

The inhibitory activity of the compounds was dependent on the substituent at the 6-position of the 2-aminopyrimidine (Table 2). Short alkyl chains were well tolerated, while long alkyl chains gave about one order of magnitude lower potency against both CDK1 and CDK2 (**9**, **10** vs **12**). Cycloalkyl groups also showed good potency, except for cyclopropyl which showed a drastic drop in inhibitory activity (**13** vs **14**–**16**). The cycloheptyl group showed the most potent anti-proliferative activity against three cancer cell lines. Introduction of amino substituents reduced the inhibitory activity about 10-fold (**17**–**22**). However, amino groups substituted with cycloalkyl groups restored the activity, making it similar to that seen for alkyl groups (**23**, **24**).



Scheme 2. Synthesis of substituted (3*S*,4*S*)-1-(3-(2-aminopyrimidin-4-yl)-4-hydroxyphenyl)pyrrolidine-3,4-diol. Reagents and conditions: (a) (i) Br_2, AcOH, 89%; (ii) BnBr, K_2CO_3, 99%; (iii) Boc_2O, DMAP, TEA, 95%; (iv) 2 N NaOH, 93%; (v) TFA, DCM, 93%; (b) (2*R*,3*R*)-$ICH_2CH(OMOM)CH(OMOM)CH_2I$, TEA, *n*-BuOH, 30%; (c) *n*-BuLi, $(CH_3O)_3B$, THF, 24%; (d) $Pd(PPh_3)_4$, K_2CO_3, DMF, 2-amino-4,6-dichloropyrimidine, 40% or 4-chloro-6-methylpyrimidin-2-amine, 86%; (e) (i) R^3R^4NH, DMF, 13–80% for **17**–**24**; (ii) HBr, AcOH, 26–97%.



Scheme 3. Synthesis of substituted (3*S*,4*S*)-1-(3-(2-aminopyrimidin-4-yl)-4-hydroxyphenyl)pyrrolidine-3,4-diol. Reagents and conditions: (a) (i) AC_2O, TEA, DCM, 89%; (ii) $AlCl_3$, CS_2, AcCl, CH_3CN, 85%; (iii) RCOCl, TEA, THF, 22–96%; (b) NaH, HMPA, THF, 23–80%; (c) $NH_2CNHNH_2{\cdot}HCl$, KOBu-*t*, *t*-BuOH, 27–49%; (d) (i) BnBr, K_2CO_3, DMF, 88–98%; (ii) Boc_2O, DMAP, TEA, then 2 N NaOH, 66–79%; (iii) TFA, DCM, 95–98%; (iv) (2*R*,3*R*)-$ICH_2CH(OMOM)CH(OMOM)CH_2I$, *i*-$Pr_2NEt$, *n*-BuOH, 21–68%; (v) BBr_3, DCM, 43–87%.

Table 2
Classical complement inhibition, cytotoxicity, and apoptosis assay results for compounds **1–5** and **9–19**[a]

	R	IC_{50} (μM)		Cytotoxicity[b] (μM)		
		CDK1	CDK2	HCT116	A549	EJ
8	H	0.015	0.021	2.5	5.5	4.0
9	Me	0.020	0.042	0.4	2.0	0.9
10	Et	0.031	0.050	1.0	3.0	1.5
11	*i*-Pr	0.094	0.068	0.9	1.8	1.5
12	*n*-Hex	0.26	0.45	0.5	0.7	0.8
13	Cyclopropyl	>10	>10	nd	nd	nd
14	Cyclopentyl	0.023	0.042	0.4	2.0	0.9
15	Cyclohexyl	0.045	0.052	0.8	1.2	1.2
16	Cycloheptyl	0.031	0.090	0.3	0.6	0.7
17	3-Piperidinyl	0.22	0.36	>10	>10	>10
18	4-Piperidinyl	0.34	0.20	>10	nd	nd
19	Dimethylamino	0.28	0.25	>10	nd	nd
20	Morpholino	1.0	0.94	>10	nd	nd
21	4-Methylpiperazin-1-yl	0.42	0.30	>10	nd	nd
22	2-Morpholinoethylamino	0.79	0.45	>10	nd	nd
23	Cyclopentylamino	0.019	0.015	3.0	7.0	4.5
24	Cyclohexylamino	0.020	0.060	0.9	1.5	2.0

[a] The values displayed correspond to the mean average of three experiments (nd = not determined)

[b] Exponentially growing cells were treated with test compounds at various concentrations for 48 h, and then the cell numbers were measured. The compound concentration with 50% growth inhibition activity was determined.[12]

X-ray structures showed that the substitutions at the 6-position of the 2-aminopyrimidine ring cause the compounds to rotate in the ATP-binding pocket and the extents of rotation are dependent on the nature of the substitutions (Fig. 1).[13]

The substitutions at the 6-position of the pyrimidine are located in a pocket formed by the side chains of Gln85, Asp86 and Lys89. The size of this pocket limits the size of the substituent, as observed in the structure–activity relationship. Compound **15**, with its bulky cyclohexyl group rotates further toward the hinge region, compared to compound **11** with the isopropyl group. Despite these significant movements, the 2-aminopyrimidine core continues to hydrogen bond with the hinge region. Another interesting feature is the orientation of pyrrolidine-3,4-diol. Without the substituent at the 6-position, it is in the same plane as the phenol ring. However, in the presence of the substituent, the pyrrolidine-3,4-diol

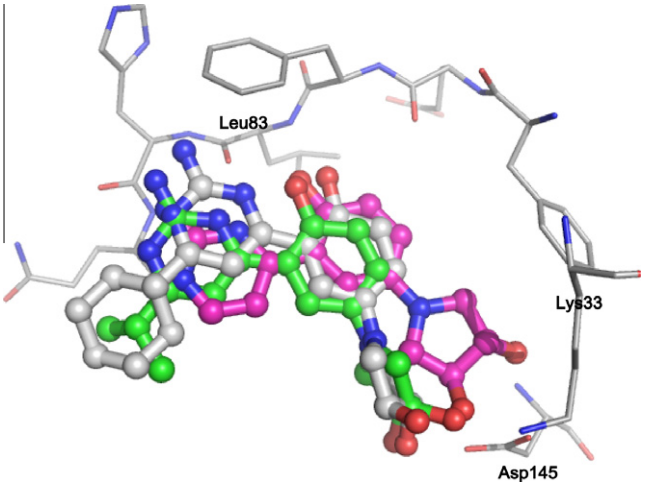


Figure 1. Comparison of the X-ray structures of compounds **8** (purple), **11** (green) and **15** (grey) in the ATP pocket of CDK2.

is rotated about 90° to make it perpendicular to the plane of the phenol ring. In both orientations, the pyrrolidine-3,4-diol makes similar interactions with Lys33 and Asp145.

The *C* log *P* value of the compound can be one factor that affects both enzymatic and cell growth inhibition.[14–16] Compounds having a low inhibitory activity against both enzymes and cells are substituted with piperidine, piperazine, and morpholine groups which provide low *C* log *P* values (−0.12, −0.12, 0.03 and 0.44 for **20**, **18**, **17** and **21,** respectively).[17] Introduction of *n*-hexyl and cycloheptyl groups increased the *C* log *P* values to 2.8 and provided improved cell growth inhibition. The result that compound **12** showed better cellular activity than **9** and **10** even though **12** was one order of magnitude less potent for both CDK1 and CDK2 supports this speculation. Compounds having short alkyl chains did not show correlation between enzymatic and cellular inhibitory activity. It can be partially accounted by the offsetting effect of the binding mode alteration and the lipophilicity change.

In summary, a series of new 2-(2-aminopyrimidin-4-yl)phenol derivatives were synthesized as potential antitumor compounds. Pyrrolidine-3,4-diol was found as a new substituent for 2-(2-aminopyrimidin-4-yl)phenol scaffolds. A series of (3*S*,4*S*)-1-(3-(2-aminopyrimidin-4-yl)-4-hydroxyphenyl)pyrrolidine-3,4-diols was synthesized and found to be decent CDK1 and CDK2 inhibitors.

Acknowledgments

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11. Procedure for the synthesis of (3*S*,4*S*)-pyrrolidine-3,4-diol:
A mixture of diethyl L-tartarate (50.0 g, 0.242 mol), MOMCl (46.0 ml, 0.605 mol), and DIPEA (127.0 mL, 0.726 mol) in DCM was stirred for 10 h at rt. The solvent was removed under reduced pressure, and the remaining residue was dissolved in EA. The organic layer was washed with water and brine, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel using Hex/EA (1:1, v/v) as eluent to give (2*R*,3*R*)-diethyl 2,3-bis(methoxymethoxy)succinate in a yield of 99%. Conventional reduction of ester using $LiAlH_4$ provided (2*S*,3*S*)-2,3-bis(methoxymethoxy)butane-1,4-diol in a yield of 98%. 2,3-Bis(methoxymethoxy)butane-1,4-diol (50.0 g, 0.237 mol) in 200 mL THF was added by dropwise to a mixture of PPh_3 (137 g, 0.522 mol), I_2 (133 g, 0.522 mol), and imidazole (72 g, 1.0 mol) in 1.3 L THF. After stirring for 1 h at rt, the solvent was removed under reduced pressure, and the remaining residue was dissolved in EA. The organic layer was washed with water and brine, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel using Hex/EA (1:1, v/v) as eluent to give (5*R*,6*R*)-5,6-bis(iodomethyl)-2,4,7,9-tetraoxadecane in a yield of 45%. ^{1}H NMR(400 MHz, $CDCl_3$) δ (ppm) 3.30 (2H, m), 3.38 (2H, m), 3.43 (6H, s), 3.99 (2H, m), 4.75 (4H, m); ESI MS(*m*/*e*) = 430 [M+1].
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