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Substituted aminobenzimidazole pyrimidines as cyclin-dependent kinase inhibitors

Sharad Verma,[a,*] Dhanapalan Nagarathnam,[a] Jianxing Shao,[a] Lei Zhang,[a] Jin Zhao,[a] Yamin Wang,[a] Tindy Li,[a] Eric Mull,[a] Istvan Enyedy,[a] Chunguang Wang,[a] Qingming Zhu,[a] Martha Altieri,[b] Jerold Jordan,[b] Thu-Thi-Anh Dang[b] and Sanjeeva Reddy[b]

[a]*Department of Chemistry Research, Bayer Research Center, 400 Morgan Lane, West Haven, CT 06516, USA*
[b]*Department of Cancer Research, Bayer Research Center, 400 Morgan Lane, West Haven, CT 06516, USA*

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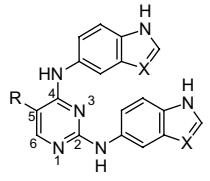
Abstract—A series of aminobenzimidazole-substituted pyrimidines were synthesized and evaluated for biochemical activity against CDK1. A high-speed parallel synthesis approach enabled the identification of a potent lead series having improved potency in the CDK1 assay (IC_{50} < 10 nM). Cell cycle analysis showed that the compounds induced a G2/M block. Docking studies were carried out with a CDK1 homology model, and provide a rationale for the observed activities.

The cyclin-dependent kinases (CDKs) represent a family of serine–threonine protein kinases, which control cell cycle progression in proliferating eukaryotic cells.[1] CDK activity is dependent on the presence of cyclin partners, whose levels are regulated in a sequential manner to ensure that the phases of the cell cycle proceed in the correct order.[2] Perturbations in CDK function and consequent loss of cell cycle regulation have been directly linked to the molecular pathology of cancer.[3] As a result of the strong association between the CDKs and cancer biology, efforts have been aimed towards the development of CDK inhibitors for the treatment of cancer.[4]

The pyrimidine scaffold is found in several kinase inhibitor classes,[5] and the use of 2,4,5-trisubstituted pyrimidines as inhibitors of CDKs reported.[6] In this context, the design, synthesis, and testing of a number of kinase biased analogs was executed, and the bis-2,4-aminobenzimidazole pyrimidines **1a** and **1b** were identified as potent inhibitors of CDK1 (Fig. 1). It quickly became apparent that the presence of an aminobenzimidazole group at either the C-2 and/or C-4 positions is critical for CDK1 inhibitory activity. For example, replacement of the aminobenzimidazoles at C-2 and C-4 with an aminoindole results in IC_{50} > 1000 nM. Furthermore, it was observed that trifluoromethyl or bromo groups were optimal as C-5 substituents, while replacement with hydrogen or fluorine resulted in 200- and 10-fold decreases in potency, respectively.



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X = N	X = C
a) R = CF_3, IC_{50} = 10 nM	e) R = Br, IC_{50} > 1,000 nM
b) R = Br, IC_{50} = 14 nM	f) R = CF_3, IC_{50} > 1,000 nM
c) R = F, IC_{50} = 430 nM	g) R = F, IC_{50} > 1,000 nM
d) R = H, IC_{50} > 1,000 nM	h) R = H, IC_{50} > 1,000 nM

Figure 1. Aminobenzimidazole substituted pyrimidine based CDK1 inhibitors.

CDK1 inhibition is further exemplified in a cell functional assay by compound **1a**, which displays a G2/M cell cycle arrest in proliferating cells (Fig. 2). When MDA-MB-231 cells were treated with 3 μM of compound **1a** for 24 h, the cells showed a complete block in the G2/M phase of the cell cycle.[4c]

* Corresponding author. Tel.: +1 203 812 6574; fax: +1 203 812 3655; e-mail: sharad.verma.b@bayer.com

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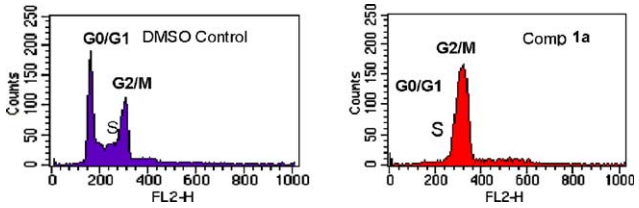


Figure 2. G2/M phase cell cycle arrest of **1a** in MDA-MB-231 cells.

By virtue of their observed activity and potential for efficient analoging, compounds **1a** and **1b** were selected as leads for further optimization. Preparation of substituted pyrimidines was generally straightforward, as described in Figure 3. Modification of either the C-2 or C-4 positions of the lead structure core **1** entailed the use of a two-step synthetic protocol. Treatment of commercially available dichloropyrimidines of type **2** with 5-aminobenzimidazole and sodium carbonate in ethanol at room temperature for 16 h affords C-4 substituted pyrimidines of type **3**.[7] Subsequent coupling of arylamines at the C-2 position was then accomplished by heating chloropyrimidine **3** with the corresponding arylamine in *n*-BuOH under acidic conditions at 110 °C for 12 h, to afford compounds of type **4** (route A). Likewise, reversal of these steps as shown in route B furnished analogs having an aminobenzimidazole group attached at C-2, as in compound **6**. It should be noted that preparation of the lead analogs **1a** and **1b** could be accomplished in one step by heating of chloropyrimidine **2** with 2 equiv of 5-aminobenzimidazole in *n*-BuOH at 110 °C for 12 h. For the preparation of analogs containing an aminobenzimidazole group at the C-2 position wherein X = CF_3, route C was utilized. This method first involved conversion of 5-aminobenzimidazole to its corresponding bis–Boc derivative **8**, obtained as a mixture of regioisomers with respect to *N*-Boc substitution on the imidazole ring. Subsequent coupling of compound **8** with dichloropyrimidine **2** (X = 5-CF_3) occurs regioselectively at the C-2 position to afford chloropyrimidine **9**.[7] Treatment of chloropyrimidine **9** with arylamines as above followed by deprotection furnished analogs of type **6**. Likewise, the *N*-linker could be replaced with either O or S by treatment of chloropyrimidine **9** with the appropriate phenols or thiols.

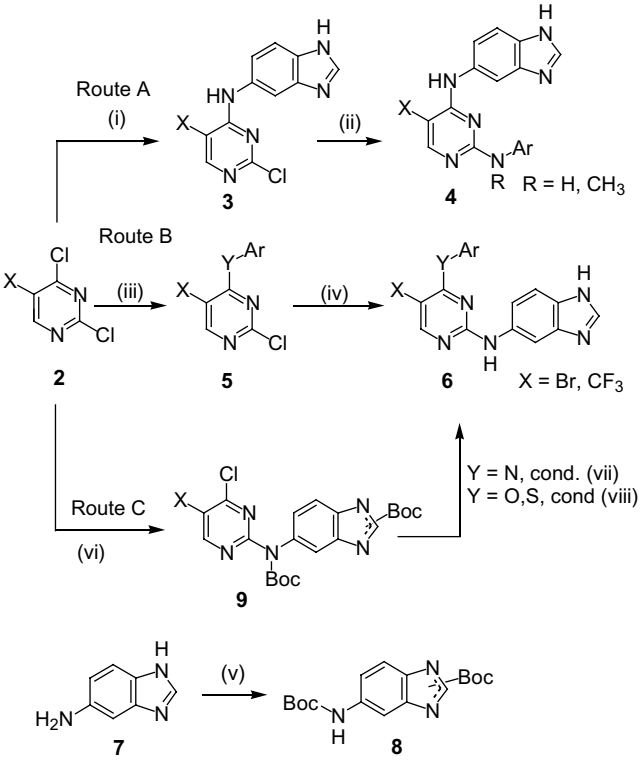
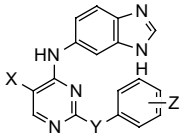


Figure 3. Reagents and conditions: (i) 5-aminobenzimidazole, Na_2CO_3, EtOH, rt, 16 h, 68%; (ii) Ar–NHR, *n*-BuOH, cat. HCl, 110 °C, 12 h, 20–60%; (iii) Ar–NHR, Na_2CO_3, EtOH, rt, 16 h, 85–99%; (iv) 5-aminobenzimidazole, *n*-BuOH, 110 °C, 12 h, 20–60%; (v) $(Boc)_2O$, THF, Et_3N, rt, 16 h, 62%; (vi) *n*-BuLi, THF, −78 °C to rt, compound **8**, 24 h, 55%; (vii) (1) Ar–NH_2, *n*-BuOH, cat. HCl, 110 °C, 12 h, (2) TFA, 45–55% overall; (viii) (1) K_2CO_3, CH_3CN, Ar–OH or Ar–SH, (2) TFA, 35–55% overall.

First, we replaced the aminobenzimidazole group at the C-2 position with anilines while retaining the aminobenzimidazole group at the C-4 position of the pyrimidine ring, and the results are summarized in Table 1. Analogs containing a substituted aniline at the C-2 position of the pyrimidine displayed good activity in the CDK1 biochemical assay, as IC_{50} values <50 nM were routinely observed.[8] Overall, substitution at the 3- or 4-position of the aniline ring is preferred, as placement of a substituent at the 2-position results in a loss in potency (e.g., **15**). The greatest potency was exhibited by analogs bearing anilines containing a sulfonamide group such as compound **10**, which displays a 100-fold improvement in potency compared to lead compound **1a**. This result is consistent with docking studies, as shown in Figure 4.[9] In our binding model, the C-2 NH group comes in contact with the hinge region at Leu83 through two hydrogen bonds, an interaction that would be determined to be crucial for activity.[10] The enhanced activity displayed by compound **10** results from the interaction of the sulfonamide group with Lys89,

Table 1. Activities for C-2 modified analogs



Compound	X	Y	Z	CDK1 IC_{50} (nM)
1a	CF_3	NH	3,4-NCNH	10
10	CF_3	NH	4-SO_2NH_2	0.1
11	CF_3	NH	4-Br	11
12	CF_3	NH	3-Cl	22
13	CF_3	NCH_3	3-Cl	>1000
14	Br	NH	3-Cl	9
15	CF_3	NH	2-Cl	>1000
16	CF_3	NH	3-CH_3	15
17	CF_3	NH	4-CF_3	15
18	Br	NH	4-*O*-(3-Pyridyl)	248

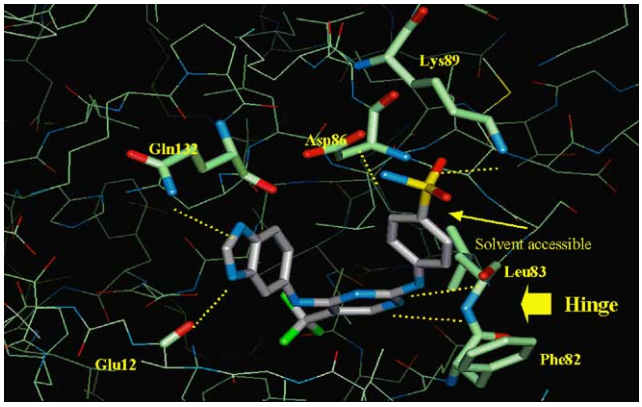


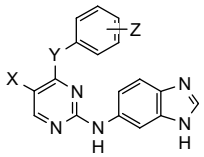
Figure 4. Docking of compound **10** with CDK1.

Asp86, and solvent. Furthermore, the CF_3 group occupies a hydrophobic region defined by Phe80, Val64, Leu135, and Ala145. The benzimidazole fragment forms a hydrogen bond with Gln132 and with the main-chain carbonyl of Glu12.

In order to validate the importance of the C-2 NH group, compound **13** which has an *N*-methyl group substituted at C-2 was synthesized and tested. As anticipated, compound **13** was found to be inactive. The inability of this analog to hydrogen bond in the hinge region, crucial for this class of inhibitors, is reasoned to account for this observation (Fig. 5). Likewise, analogs having a sterically encumbering group substituted to the aniline were found to be less potent (e.g., **18**). These results would suggest that potency for the C-2 modified series is limited to those groups (i.e., benzimidazole, anilines), which are capable of adopting an appropriate hydrogen-bonding pattern for hinge binding.

We then replaced the aminobenzimidazole group at the C-4 position while retaining the aminobenzimidazole group at the C-2 position of the pyrimidine ring, and the results are summarized in Table 2. The C-4 position of the molecule tolerated modification to a greater extent than the C-2 position, as a highly diverse group of compounds were observed to be potent. The main reason for this difference as suggested by docking studies is that the benzimidazole fragment attached at C-2 can adopt a hydrogen bonding pattern similar to that of **10** (Fig. 4). Unlike the C-2 modified series, C-4 bound

Table 2. Activities for C-4 modified analogs



Compound	X	Y	Z	CDK1 IC_{50} (nM)
1a	CF_3	NH	3,4-NCNH	10
19	CF_3	NH	2-Cl	0.9
20	CF_3	NH	3-Cl	3
21	CF_3	NH	4-Cl	4
22	Br	NH	4-F	4
23	CF_3	NH	4-OCH_3	8
24	Br	NH	3,4-OCH_2O	9
25	Br	NH	4-*O*-(3-Pyridyl)	34
26	Br	$NHCH_2$	2,3-Dichloro	126
27	CF_3	O	2-CH_3	4
28	CF_3	S	2-CH_3	135
29	CF_3	O	4-F	32
30	CF_3	O	4-Cl	252
31	CF_3	O	4-Br	562
32	CF_3	O	4-CF_3	>1000
33	CF_3	O	3-OCH_3	160
34	CF_3	S	3-OCH_3	>1000

anilines tolerated substitution at the 2-position of the ring (e.g., **19**). Among the groups at the C-4 position which were also found to demonstrate potency in the biochemical assay were anilines bearing groups which are sterically encumbering (e.g., **24**–**25**). Groups having an *N*-methylene linker (e.g., **26**) displayed activity in the CDK1 biochemical assay as well. Compounds having an O-linker at C-4 were also tolerated, and observed to be more potent than those having an S-linker (e.g., **27** vs **28**, **33** vs **34**). Substitution of the *ortho* position of the phenyl ether ring is tolerated, as well as for the *meta* and *para* positions.

Compound **35** was made with the intention of providing an analog that would be isosteric with compound **26**, and was observed to be potent in the biochemical assay. Modeling of compound **35** suggests two hydrogen bonds between the benzimidazole fragment and Asp86 and

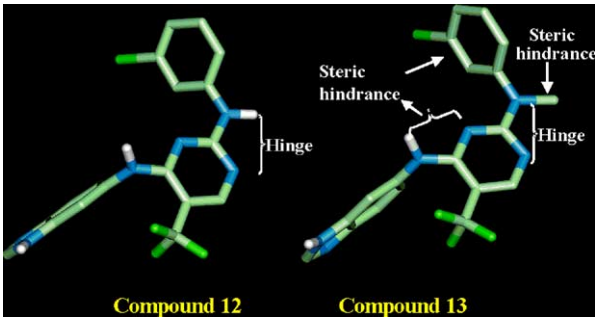


Figure 5. Inability of compound **13** to bind to the hinge region.

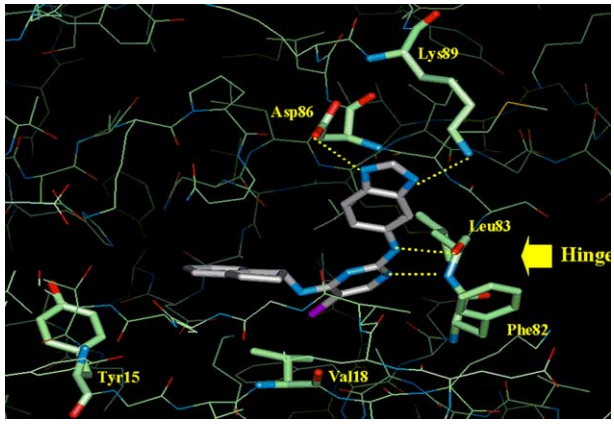


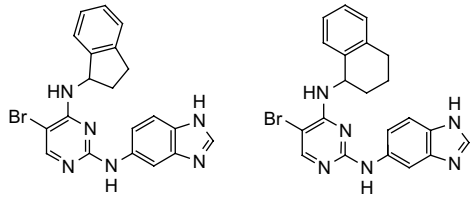
Figure 6. Docking of compound **35** with CDK1.

Lys89, while the phenyl from the benzimidazole group is between Phe82 and Leu135 (Fig. 6). The 5-Br group binds in the same hydrophobic region as the CF_3 group in compound **10**. Likewise, hydrophobic groups at the 4-position on the pyrimidine ring come in contact with Val118. Longer side chains such as in compound **35** can also potentially interact with Tyr15.

However, increasing the size of the five-membered ring of the aminoindane group of compound **35** to the corresponding tetrahydronaphthalene of compound **36**, resulted in a drop in potency (Fig. 7). A similar result was observed for compounds **29**–**32**, wherein potency decreases as the size of the *para*-substituent on the C-4 substituted aniline increases (Table 2). These results would suggest that although larger groups are tolerated with respect to C-4 substitution of the molecule, there are spatial limits. One possible explanation could be that the increasingly larger groups at C-4 disrupt the stabilizing hydrophobic interaction between the 5-Br (or CF_3) group and Val118, thereby resulting in reduced potency.

Interestingly, replacement of the substituted phenyl groups with alkyl groups within the O-linked series was well tolerated in the CDK1 assay (e.g., **37**–**40**, Table 3). Analogs within this series were generally tested below 100 nM, with some less than 5 nM (e.g., **39**). Thus, there is room to further expand this series with respect to potency and structural diversity.

A subset of compounds from these studies were tested against CDK2, CDK4, and other kinases (e.g., CK2, ETK, PDGFR, p38, ERK1), and the results are summarized in Tables 4 and 5. The compounds tested showed

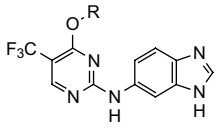


35 CDK_1 IC_{50} = 34 nM

36 CDK_1 IC_{50} = 392 nM

Figure 7. Biochemical potencies of compound **35** and **36**.

Table 3. Activities for C-4 O-linked analogs



Compound	R	CDK_1 IC_{50} (nM)
37	*O*-Ethyl	77
38	*O*-Isopropyl	25
39	*O*-Isobutyl	2
40	*O*-Cyclohexyl	43

Table 4. Activities against other kinases

Compound	IC_{50} (nM)					
	CDK_1	CK2	ETK	PDGFR	p38	ERK1
1a	10	89	400	110	>1000	300
11	11	91	1200	190	>1000	750
19	0.9	510	>1000	290	>1000	>1000
23	8	11	230	33	>1000	>1000

Table 5. Activities against other CDKs

Compound	IC_{50} (nM)		
	CDK1	CDK2	CDK4
1a	10	19	260
11	11	73	260
19	0.9	20	29
23	8	31	57

specificity for the CDKs in comparison to the other kinases, with a preference for CDK1.

In summary, a modular, high-speed route to aminobenzimidazole-substituted pyrimidines and their biological evaluation in the CDK1 biochemical assay has been described. The G2/M cell cycle block displayed by compound **1a** exemplifies the anti-proliferative properties for the compounds reported herein. Docking studies utilizing a CDK1 homology model provides a rationale for the observed activities. Potency in the C-2 modified series is limited to those groups, which are capable of adopting a critical hydrogen bonding pattern to the hinge region, for example, aminobenzimidazoles and anilines. The C-4 position can tolerate a greater number of groups without loss of activity. The presence of a trifluoromethyl or bromo group at C-5 is preferred, while replacements with fluorine or hydrogen result in loss of activity. Further pharmacological evaluation of selected compounds from these series will be reported in due course.

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7. Regiochemical assignments were confirmed by 2D NMR studies. Substitution of dichloropyrimidine **2** occurs regioselectively at C-4 when X = Br. When X = CF_3, a minor amount of C-2 substituted regioisomer is observed (3:1, in favor of the C-4 isomer), while couplings with compound **7** occur specifically at the C-2 position.
8. CDK1 assay was performed according to the manufacturer's protocol (New England Biolabs) with the minor modification of incubating the reaction for 2 h at room temperature. Indurubin 3-monoxime (IC_{50} = 180 nM) was used as a reference inhibitor of CDK1 activity.
9. The structure of human CDK1 was built through homology modeling using MODELLER[11] in INSIGHTII. The template used for building the model was the structure of CDK2 in complex with 4-[(6-amino-4-pyrimidinyl)amino]benzene sulfonamide.[12] The template has 65% sequence identity, 78% sequence similarity and 3% gaps when aligned with the CDK1 sequence. Compounds **10**, **13**, and **35** were docked into CDK1 using GLIDE.[13] The obtained protein–ligand complex was optimized using 500 ps molecular dynamics simulation using TIP3P water for solvation. The simulation was done using CHARMM[14] with constant dielectric of 1, temperature of 300 K, and 1 fs time step.
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