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N-substituted azaindoles as potent inhibitors of Cdc7 kinase

Marian C. Bryan [a], James R. Falsey [a], Mike Frohn [b], Andreas Reichelt [b], Guomin Yao [b], Michael D. Bartberger [b], Julie M. Bailis [c], Leeanne Zalameda [d], Tisha San Miguel [d], Elizabeth M. Doherty [a], John G. Allen [b,*]

[a] Medicinal Chemistry Research Technologies, Therapeutic Discovery, Amgen Inc., One Amgen Center Dr., Thousand Oaks, CA 91320, USA
[b] Therapeutic Discovery, Amgen Inc., One Amgen Center Dr., Thousand Oaks, CA 91320, USA
[c] Oncology Research, Amgen Inc., 1120 Veterans Blvd., South San Francisco, CA 94080, USA
[d] Bioassay and Profiling, Therapeutic Discovery, Amgen Inc., One Amgen Center Dr., Thousand Oaks, CA 91320, USA

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ABSTRACT

Cdc7 kinase is responsible for the initiation and regulation of DNA replication and has been proposed as a target for cancer therapy. We have identified a class of Cdc7 inhibitors based on a substituted indole core. Synthesis of focused indole and azaindole analogs yielded potent and selective 5-azaindole Cdc7 inhibitors with improved intrinsic metabolic stability (ie **36**). In parallel, quantum mechanical conformational analysis helped to rationalize SAR observations, led to a proposal of the preferred binding conformation in the absence of co-crystallography data, and allowed the design of 7-azaindole **37** as a second lead in this series.

Cell division cycle 7 (Cdc7) is a conserved serine/threonine kinase that, along with cyclin-dependent kinase (CDK), is responsible for the initiation of DNA replication during S-phase.[1–6] At replication origins, phosphorylation of minichromosome maintenance complex (MCM) by Cdc7 leads to the unwinding of double stranded DNA by the DNA helicase. This step is essential for initiation of DNA replication in mammalian cells. In addition to initiation of replication, Cdc7 is also involved in S-phase checkpoint regulation and the maintenance of S-phase genome stability.[7,8]

Given its key role in DNA replication, it is unsurprising that heightened expression of Cdc7 is found in many cancers including leukemia, lymphoma, colorectal and gastric carcinomas, primary breast tumor, colon and lung cancer.[1,9–11] Increased Cdc7 expression also correlates with genomic instability, reduced disease-free survival, and accelerated cell cycle progression.[9] In breast cancer, dysregulation of Cdc7 is associated with the development of an aggressive malignant phenotype while in diffuse large cell lymphoma, increased Cdc7 activity is associated with a poor clinical outcome.[4,9,12] Increased interest in targeting Cdc7 has also been driven by the potential for a wide therapeutic window. For example, it has been found that depletion of Cdc7 leads to p53-independent apoptosis in cancer cells,[13] and to cell cycle arrest without loss of viability in normal cells.[3,4,7–9,13,14] Such alternate outcomes may allow for treatments with limited toxicity even in self-renewing, high-turnover tissue. Taken together these findings suggest that Cdc7 is an attractive target for therapeutic intervention in a variety of cancers.[4,7,8] Others have reported the investigation of small molecule inhibitors of Cdc7,[3,14–20] including a recent disclosure by our group of a series of thiazole-based inhibitors of Cdc7.[21]

A high-throughput screen of the Amgen Screening Collection identified 6-cyanoindole **2**, which had good physical properties, was potent against Cdc7 (IC_{50} = 75 nM) and exhibited excellent selectivity over functionally related CDK2 (IC_{50} = 16,500 nM). Although CDK2 itself is dispensable in cancer cell progression, CDK2 selectivity serves as a marker for off-target effects such as CDK1 activity which could lead to increased toxicity towards normal cells.[22,23] Efforts to establish the structure–activity relationship (SAR) around the indole core of **2** were initiated.

The core, N1, C3 and C6 substitution were rapidly explored following the concise syntheses shown in Scheme 1. Nucleophilic aromatic substitution (SNAr) of the commercially available indole amines (**1**) under basic conditions provided N1-substituted indole analogs **2** through **7**, **9** through **24**, and **37**. Alternately, copper-catalyzed N-arylation of 1*H*-indole-6-carbonitrile with 4-chloropyrimidine was required for the generation of pyrimidine **8**. The indole core was further differentiated at position C3. Molecules **26** through **36** were generated from **1b** in three steps. Iodination under basic conditions provided iodide **25** regiospecifically. Arylation using the SNAr conditions described above followed by Suzuki

* Corresponding author.
E-mail address: johallen@amgen.com (J.G. Allen).

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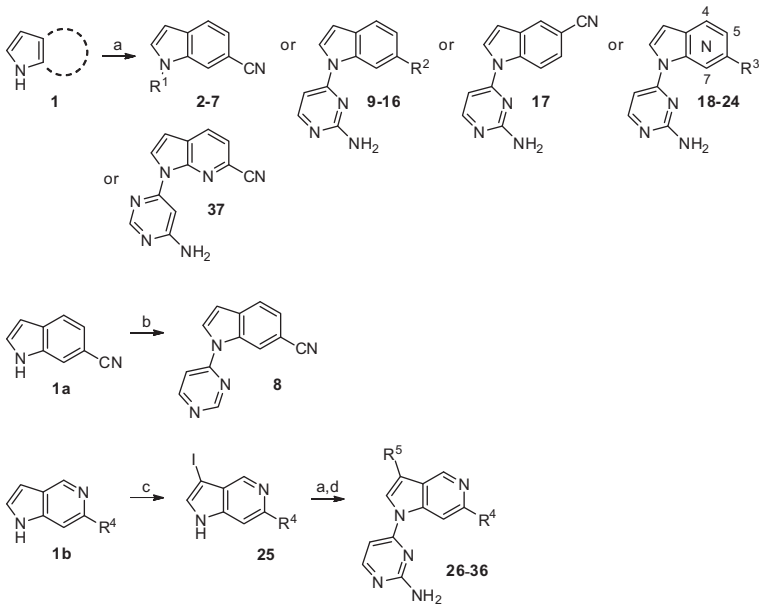


Scheme 1. General synthesis of substituted indoles and azaindoles. Reagents and conditions: (a) R^1Cl, base (Cs_2CO_3, K_2CO_3 or NaH), DMSO, Δ (2–92%); (b) CuI, 4-chloropyrimidine, K_3PO_4, (*1R,2R*)-4-cyclohexene-1,2-diamine (10%); (c) I_2, KOH (87%); (d) $Pd(PPh_3)_4$, K_2CO_3, $R^5B(OH)_2$, toluene:EtOH:H_2O (6:3:2), 100 °C (5–49%).

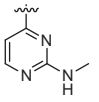
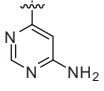
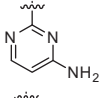
coupling of the iodide with various boronic acids provided the indoles functionalized at N1, C3 and C6. These routes allowed three regions of interest to be modified in parallel. With the substituted indoles in hand, we examined their potency against Cdc7 and selectivity over CDK2.

Biological testing of indole core analogs indicated that minimal modification to the aminopyrimidine was tolerated (Table 1). Methylation of the primary amine (**3**, IC_{50} = 0.025 μM) showed modest improvement in Cdc7 potency without significantly impacting selectivity over CDK2 (IC_{50} = 2.7 μM). Removal of one of the two pyrimidine nitrogens (**4**, **5**) was poorly tolerated, as was rearrangement of the pyrimidine ring (**6**, **7**), although it was noted that inhibition of CDK2 was reduced. Significant loss in potency for Cdc7 was also seen with removal of the primary amine (**8**). These findings highlighted the importance of R^1, presumably binding to the hinge region of the protein, for potency and possibly for selectivity. Our attention then turned to substitution of the C6 position of the indole.

Removal of the nitrile to give unsubstituted indole **9** resulted in a >50× loss in potency relative to **2** (Table 2). Halides such as F, Cl, and Br were well tolerated at C6 with chloride **11** equipotent to nitrile **2** (IC_{50} = 0.066 μM), though an order of magnitude less selective for CDK2 (220× vs 56×). Nitroindole **13** was also equipotent with nitrile **2** and highly selective against CDK2 but was not pursued due to potential metabolic liabilities. Substitution of C6 with either a trifluoromethyl (**14**) or a hydroxyl (**15**) resulted in low micromolar activity for Cdc7 while substituting alkyne (**16**) for nitrile (**2**) resulted in a compound that was an order of magnitude less potent. The regioisomer of **2**, C5 nitrile **17**, was also significantly less active.

Although compound **2** had good potency against Cdc7, it was found to have poor microsomal stability, particularly in human liver microsomes. Introduction of nitrogen to the phenyl ring core

Table 1
Hinge binder SAR

No.	R^1	Cdc7 ± SD[a] (μM)	CDK2 ± SD[a] (μM)
2		0.075 ± 0.065	16.5 ± 3.3
3		0.025 ± 0.026	2.7 ± 1.5
4		10.4 ± 2.7	45 ± 21
5		9.0 ± 2.7	39 ± 21
6		2.7 ± 1.7	>83[b]
7		8.8 ± 5.5	>29
8		1.5 ± 0.4	>83[b]

[a] Values are the mean of at least three determinations.
[b] Values are the result of one determination.

Table 2
R^2 indole SAR

No.	R^2	Cdc7 ± SD[a] (μM)	CDK2 ± SD[a] (μM)
2	CN	0.075 ± 0.065	16.5 ± 3.3
9	H	4.7 ± 1.1	28.5 ± 8.2
10	F	0.156 ± 0.039	34 ± 21
11	Cl	0.066 ± 0.014	3.7 ± 1.7
12	Br	0.35 ± 0.20	8.2 ± 4.3
13	NO_2	0.082 ± 0.033	>30.6
14	CF_3	1.1 ± 0.8	17 ± 11
15	OH	3.3 ± 1.4	5.0 ± 1.2
16	CCH	0.76 ± 0.26	10 ± 2.0
17	—	8.5 ± 5.2	>29.1

[a] Values are the mean of at least three determinations.

was investigated in order to address potential metabolic instability.[24] As shown in Table 3, indole **9** exhibited high intrinsic clearance in both human and rat liver microsomes (HLM/RLM), similarly to **2**. Insertion of nitrogen at position 7 (**18**) was poorly tolerated by Cdc7 and did not improve metabolic stability. 6-Azaindole **19** and 5-azaindole **20** showed equal improvements in Cdc7 potency relative to indole **9** and similar intrinsic clearance, though affinities for CDK2 differed (IC_{50} = 45.1 μM vs IC_{50} = 3.7 μM, respectively). The final heterocycle tested in this series, 4-azaindole **21**, was modestly less potent but exhibited a 6× improvement in metabolic stability relative to indole **9** as measured by RLM.

Efforts to improve the potency of the azaindoles through reintroduction of C6 substitution while maintaining the improved metabolic stability from the 5-nitrogen were then attempted. C6 nitrile **22** did not improve potency compared to unsubstituted azaindole **20**, although metabolic stability was similar. C6 Chloride **23** was 3× more potent for Cdc7 and selectivity over CDK2 increased from 3× to >100× while modestly impacting intrinsic clearance. Nitrile substitution of 4-azaindole **21** to yield **24** did not significantly improve potency. 5-Azaindole compounds **20**, **22** and **23** provided an attractive balance of potency, selectivity and metabolic stability relative to **9**.

In order to recover potency in the more metabolically stable 5-azaindole compounds, we considered the SAR of the related thiazole series.[21] Overlaying thiazole carboxamide **25** with azaindole (Table 4) suggested C3 aryl substitution would be tolerated. Accordingly, the SAR surrounding C3 was investigated starting with 5-azaindole **20** (Table 4). Phenyl substitution of C3 (**26**) gave a 9× improvement in potency for Cdc7 relative to 5-azaindole **20**. 3-Pyridyl **27** and 4-pyridyl **28** had comparable potency to phenyl **26**, and **27** showed a small improvement in CDK2 selectivity. 3-Pyrazole **29** was only slightly more potent than the unsubstituted indole with complete loss of CDK2 selectivity. 3-Furan **30** and 4-(3,6-dihydro-2*H*-pyran) **31** gave comparable activity to C3-phenyl azaindole **26** but again lacked selectivity over CDK2. The first significant improvement in potency over compound **26** was seen when chlorinated phenyl rings were introduced. 2-Chlorophenyl **32** (IC_{50} = 0.011 μM) improved activity an order of magnitude compared to phenyl **26**, while chloro substitution at C3 (**33**) and C4 (**34**) of the appended phenyl ring were less impactful. Interestingly, compound **33** showed excellent selectivity over CDK2 relative to **26** (>500× vs 3×). 3-(2-Chloropyridyl) (**35**), was equipotent to chlorophenyl **32** with improved selectivity relative to **27** (44× vs 7×).

We next attempted to combine SAR findings around the pyrimidine, indole phenyl ring, and C3 in order to identify molecules with improved potency, selectivity, and metabolic stability. Azaindole **24** had modest potency for Cdc7 (IC_{50} = 0.36 μM) but had excellent selectivity over CDK2 (>100×). Chloropyridine **35** had only moderate selectivity (44×) but was very potent (IC_{50} = 0.007 μM). Both molecules had low to moderate intrinsic clearance in HLM/RLM assays. Combination of these SAR features gave pyridinyl azaindole **36** (Table 5), which was slightly less potent for Cdc7 than **35** (IC_{50} = 0.030 μM), but was highly selective over CDK2 (>2700×), and maintained low intrinsic clearance. Also shown in Table 5 is cell-based potency data determined in HCT116 cells for the phosphorylation of the proximal substrate MCM2.[21] These data indicate a fairly consistent 100× loss in potency for these compounds in the cellular assay versus the biochemical assay.

Quantum mechanical conformational analysis of the compounds in Table 6 provides a clear rationale for the observed SAR trends in this series. It was initially speculated that the loss in potency of **18** versus **9** may have been due to the hinge binder pyrimidine being oriented out of the preferred binding conformation due to intramolecular azaindole N/pyrimidine N repulsion. Figure 1 depicts the predicted (B3LYP/6-31G*) dihedral profile about the rotatable C–N bond of **2**, **5**, **9** and **18**. Compounds **2** and **9** exist as a pair of near-coplanar minima (ca. ~20° and ~160°, *syn* and *anti*, respectively) of similar energy (Δ*E* = 0.3 kcal/mol) Conversely, **18** is predicted to exist only in *anti* (180°) orientation, due to N–N repulsion rendering its *syn*-like form thermally inaccessible (Δ*E* >6 kcal/mol at the ~30° local minimum).[25] Comparison of the potency of **18** versus **9** suggests the *anti* binding mode is disfavored by approximately 5×.

The relatively weaker activity of **5** is also satisfactorily explained on the basis of conformational effects. Due to C–H/C–H repulsion, **5** exists as a pair of nonplanar (ca. 40° and 140°) minima, with adoption of either planar conformation disfavored on the order of 4 kcal/mol. This may in part explain the >100× loss in potency for **5** versus **2**.

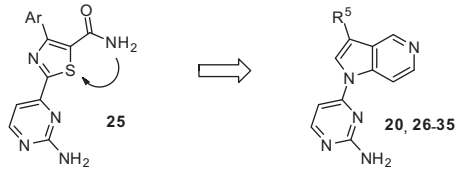
In an effort parallel to the SAR work above, the singleton compound, 7-azaindole **37** (also shown in Fig. 1) was designed based not only on its predicted planar conformation such as for **2** and **9,** but with the added advantage of existing solely in the *syn* form, thus doubling the population of the more potently binding conformer. The 8× improvement of **37** over **2** suggests the pyrimidine N-1 forms additional favorable interactions with the protein compared to **2**. Although functional group contacts with the protein cannot be ignored, it can be inferred from the potency differences

Table 3
Nitrogen heterocycle SAR

No.	N	R^3	Cdc7 ± SD[a] (μM)	CDK2 ± SD[a] (μM)	HLM[c]	RLM[c]
2	Indole	CN	0.075 ± 0.065	16.5 ± 3.3	430	124
9	Indole	R^2 = H	4.7 ± 1.1	28.5 ± 8.2	387	375
18	7-N	H	26.7 ± 4.9	42 ± 18	338	282
19	6-N	—	0.93 ± 0.82	45.1 ± 3.8	134	64
20	5-N	H	0.98 ± 0.65	3.7 ± 0.5	62	101
21	4-N	H	12.6 ± 2.8	21 ± 7.4	60	50
22	5-N	CN	1.24 ± 0.28	>44	94	49
23	5-N	Cl	0.36 ± 0.23	38 ± 12	104	134
24	4-N	CN	5.3 ± 1.3	>83[b]	108	64

[a] Values are the mean of at least three determinations.
[b] Value is the result of one determination.
[c] μl/min/mg.

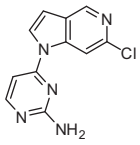
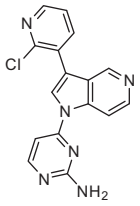
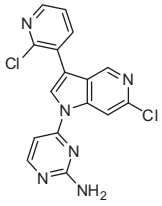
Table 4
R^5 5-azaindole SAR



No.	R^5	Cdc7 ± SD[a] (μM)	CDK2 ± SD[a] (μM)
20	H	0.98 ± 0.65	3.7 ± 0.5
26	Ph	0.104 ± 0.005	0.31 ± 0.03
27	3-Pyridyl	0.16 ± 0.05	1.1 ± 0.29
28	4-Pyridyl	0.25 ± 0.09	0.16 ± 0.06
29	3-Pyrazole	0.58 ± 0.29	0.55 ± 0.05
30	3-Furan	0.12 ± 0.01	0.10 ± 0.05
31	4-(3,6-Dihydro-2*H*-pyran)	0.16 ± 0.03	0.16 ± 0.02
32	2-Chlorophenyl	0.011 ± 0.002	0.11 ± 0.01
33	3-Chlorophenyl	0.16 ± 0.02	>83[b]
34	4-Chlorophenyl	0.33 ± 0.23	0.06 ± 0.13
35	3-(2-Chloropyridyl)	0.007 ± 0.008	0.31 ± 0.04

[a] Values are the mean of at least three determinations.
[b] Value is the mean of one determination.

Table 5
Comparison of enzyme and cellular potency, selectivity, and metabolic stability of compounds **24**, **35**, and **36**

No.	Structure	Cdc7[a] ± SD(μM)	CDK2[a] ± SD(μM)	HLM[c]	RLM[c]	pMCM2 ± SD[a] (μM)
24		0.36 ± 0.23	38 ± 12	104	134	>50[b]
35		0.007 ± 0.008	0.31 ± 0.04	21	72	0.85 ± 0.27
36		0.03 ± 0.02	>83[b]	30	102	2.3 ± 3.1

[a] Values are the mean of at least three determinations.
[b] Value is the result of one determination.
[c] μl/min/mg.

Table 6
Comparison compounds for conformational analysis

No.	Cdc7 ± SD[a] (μM)	CDK2 ± SD[a] (μM)	HLM[c]	RLM[c]	pMCM2 ± SD[a] (μM)
2	0.075 ± 0.065	16.5 ± 3.3	430	124	5.0 ± 2.9
5	9.0 ± 2.7	39 ± 21	201	122	>50[b]
9	4.7 ± 1.1	28.5 ± 8.2	387	375	>50[b]
18	26.7 ± 4.9	42 ± 18	338	282	>50[b]
37	0.009 ± 0.003[b]	>83[b]	34	46	1.37 ± 0.17[b]

[a] Values are the mean of at least three determinations.
[b] Value is the mean of two determinations.
[c] μl/min/mg.

observed for **9** versus **18**, **2** versus **5** and **37** versus **18**, along with the conformational profile depicted in Figure 1 that this class of indole and azaindole compounds binds potently to Cdc7 in an approximately coplanar *syn*, C-shaped orientation. Gratifyingly, in addition to good potency, **37** also possessed excellent CDK2 selectivity (cf. **6**) with low microsomal clearance. The reason for the excellent CDK2 selectivity of **37** was not determined. Similar to the compounds in Table 5, **37** had a significant loss in potency in the cellular assay (~150×).

In summary, structure–activity relationship studies around initial hit **2** defined the scope of the SAR with respect to potency for Cdc7 and selectivity over functionally related CDK2. Introduction of heteroatoms in the indole core provided azaindoles that showed increased intrinsic metabolic stability, although with a loss of Cdc7 potency. Potency was recovered via modifications to C3 and C6 of the azaindole core while metabolic stability was maintained. An alternate solution to the initial loss of potency of the azaindoles was discovered following conformational analysis of the SAR. This study also allowed us to propose the preferred binding orientation of this class of compounds in the absence of a Cdc7 crystal structure, and highlights the use of rational conformational control as an avenue for increasing activity independent of protein contacts. Although 5-azaindole (**36**) and 7-azaindole (**37**) achieved good metabolic stability and potency for Cdc7, and excellent selectivity over CDK2, cellular potency remained poor and was similar to screening compound **2**.

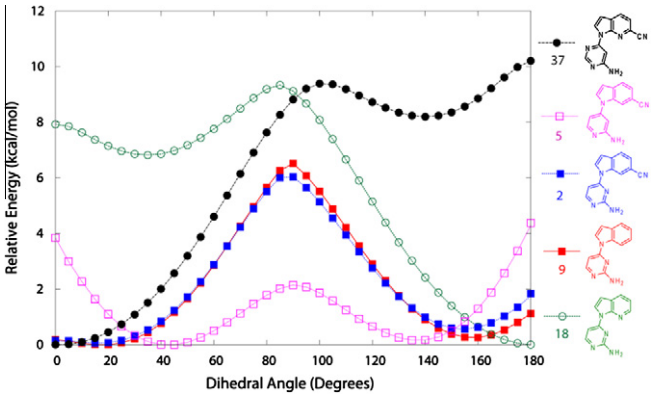


Figure 1. Dihedral profile for compounds **2**, **5**, **9**, **18** and **37**, with 0° (*syn*) conformations depicted to the right.

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