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N-substituted azaindoles as potent inhibitors of Cdc7 kinase

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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

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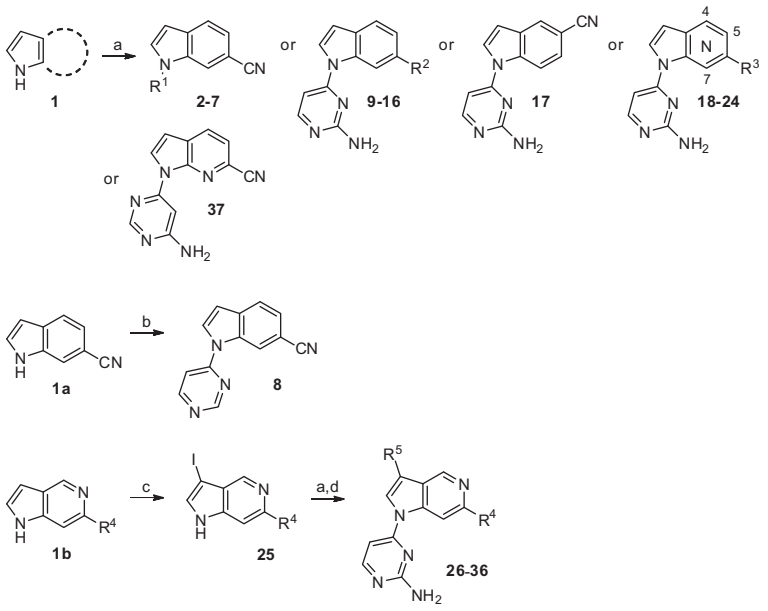


Scheme 1. General synthesis of substituted indoles and azaindoles. Reagents and conditions: (a) R^1Cl, base (Cs_2CO_3, K_2CO_3 or NaH), DMSO, Δ (2–92%); (b) CuI, 4-chloropyrimidine, K_3PO_4, (*1R,2R*)-4-cyclohexene-1,2-diamine (10%); (c) I_2, KOH (87%); (d) $Pd(PPh_3)_4$, K_2CO_3, $R^5B(OH)_2$, toluene:EtOH:H_2O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

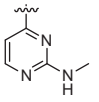
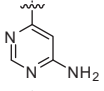
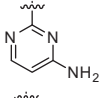
coupling of the iodide with various boronic acids provided the indoles functionalized at N1, C3 and C6. These routes allowed three regions of interest to be modified in parallel. With the substituted indoles in hand, we examined their potency against Cdc7 and selectivity over CDK2.

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.