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The optimization of aminooxadiazoles as orally active inhibitors of Cdc7

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ABSTRACT

A series of aminooxadiazoles was optimized for inhibition of Cdc7. Early lead isoquinoline **1** suffered from modest cell potency (cellular IC_{50} = 0.71 μM measuring pMCM2), low selectivity against structurally related kinases, and high IV clearance in rats (CL = 18 L/h/kg). Extensive optimization resulted in azaindole **26** (Cdc7 IC_{50} = 1.1 nM, pMCM2 IC_{50} = 32 nM) that demonstrated robust lowering of pMCM2 in a mouse pharmacodynamic (PD) model when dosed orally. Modifications to improve the pharmacokinetic profile of this series were guided by trapping experiments with glutathione in rat hepatocytes.

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Cell division cycle 7 (Cdc7) is a serine/threonine protein kinase that plays a pivotal role in the initiation of DNA replication.[1] During the S phase of DNA replication, Cdc7 is activated by binding to the regulatory subunit Dbf4.[2] The Cdc7/Dbf4 complex phosphorylates one or more minichromosome maintenance complex (MCM2-7) proteins leading to unwinding of double stranded DNA. The essential role that Cdc7 plays in S phase entry and DNA replication has been studied extensively in tumor cells. Cdc7 is overexpressed in some human tumor cell lines, such as breast, lung, and colon cancers.[3–5] Small interfering RNA (siRNA) knockdown of Cdc7 results in p53 independent apoptotic cell death in tumor cell lines.[6] In the same paper,[6] siRNA knockdown of Cdc7 in normal cells was shown to arrest growth reversibly and not cause cell death, suggesting that normal tissue might be spared during cancer treatment with a Cdc7 inhibitor. Furthermore, others have shown that small molecule Cdc7 inhibitors slow the growth of human tumor cell lines in mouse xenograft models.[7–9] These results spurred interest in the drug discovery community to develop a small molecule inhibitor of Cdc7 for use as a single agent or in combination with chemotherapy.[10–17]

We recently disclosed a series of *N*-substituted azaindoles[18] and trisubstituted thiazoles[19] as potent inhibitors of Cdc7. Isoquinoline **1** (Fig. 1) emerged as an early lead from a parallel effort to discover new chemotypes. The chemical matter leading to **1** was identified through high throughput screening of analogs from our protein kinase B (PKB) program.[20] Compounds were evaluated in an enzymatic assay that measured inhibition of Cdc7/Dbf4 biochemical activity and a cellular assay in HCT-116 cells that measured phosphorylation of MCM2 at serine 53.[21] Although **1** displayed good potency in the Cdc7 assay[21] (IC_{50} = 17 nM), it had several deficiencies that were the focus of SAR efforts. First, potency in the cellular pMCM2 assay[21] (IC_{50} = 0.71 μM) was modest. Second, the rat IV pharmacokinetics (PK) of **1** was very poor with an exceptionally high clearance (CL = 18 L/h/kg) and a very short mean residence time (MRT) of 0.2 h. We chose to first examine replacements for the isoquinoline, without changing the aminooxadiazole core and benzyl amine, with the goal of improving potency and PK. The

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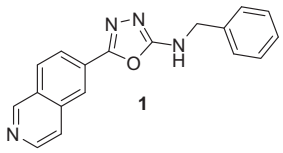


1

Cdc7 IC_{50} = 0.017 μM
pMCM2 IC_{50} = 0.71 μM
CDK2 IC_{50} = 0.35 μM

Rat PK IV (2 mg/kg)
CL = 18 L/h/kg
MRT = 0.2 h
Vss = 4.3 L/kg

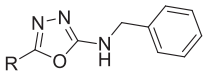
Figure 1. Early lead 1.

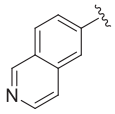
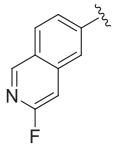
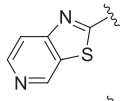
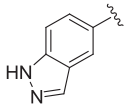
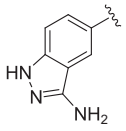
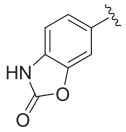
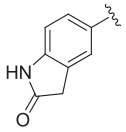
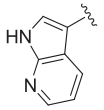
benzyl amine portion would be addressed subsequently while retaining the central aminooxadiazole core.

At the time of this work, no structures of the Cdc7 protein bound to an inhibitor were reported.[22] This complicated efforts to design novel molecules and was especially challenging for improving selectivity over the structurally related cyclin-dependent kinases such as CDK1, CDK2, and CDK9.[21] However, the nature of the binding in the hinge region of kinases is well established and the isoquinoline of **1** was assumed to bind in a similar manner.[23] Thus, novel hinge binders were designed to satisfy canonical kinase interactions and potentially improve binding with the protein. Fluoro isoquinoline[24] **2** (Table 1) had potency similar to **1**, but diminished cellular activity. The lower intrinsic clearance of **2** relative to **1** in both rat and human liver microsomes (RLM/HLM) suggested that the isoquinoline may contribute to the high clearance of **1**. This result led us to examine additional analogs that did not include an isoquinoline as the hinge binder. Although the 5,6-ring analog thiazolopyridine **3** was less potent than **1** and **2**, the 6,5-ring compounds (**4**–**7**) proved to be more active. Indazoles **4** and **5** had potency and selectivity profiles similar to **1**, but improved microsomal stability in the case of **5**. Azaindole **8** possessed potency and CDK2 selectivity comparable to **1** and **5**, but microsomal stability was superior to **1** and similar to **5**. Of the hinge binders examined, the azaindole provided the best balance of potency, selectivity, and microsomal stability. Analogs **4**–**8** potentially offer an additional site for H-bonding in the hinge region. Similar interactions, with the surrogate protein GSK3β, were reported for structurally related Cdc7 inhibitors.[10]

Efforts to further improve azaindole **8** focused on modifications to the benzylic amine (Table 2). A fluoro group was used to probe the effect of substitution on the phenyl ring. The potency of **8**–**11** was within 4-fold in the Cdc7 assay and 3-fold in the pMCM2

Table 1
SAR of *N*-benzyl 2-aminooxadiazole hinge binders



Compound	R	Cdc7 IC_{50} (μM)[a]	pMCM2 IC_{50} (μM)[a]	CDK2 IC_{50} (μM)[a]	HLM/RLM CL_{int} (μL/min/mg)[b]
1		0.017 ± 0.02	0.71 ± 0.4	0.35 ± 0.07	120/166
2		0.010 ± 0.003	3.2 ± 0.8	6.8 ± 4	49/56
3		2.3 ± 2	>50[b]	19 ± 6	23/66
4		0.020 ± 0.02	0.73 ± 0.7	0.17 ± 0.02	98/402
5		0.011 ± 0.004	1.2 ± 0.4	0.23 ± 0.009	26/74
6		0.59 ± 0.2	>50[b]	47 ± 7	69/30
7		0.77 ± 0.7	>50[b]	33 ± 9	<14/<14
8		0.024 ± 0.02	0.78 ± 0.3	0.15 ± 0.1	31/40

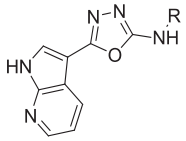
[a] Unless indicated otherwise, data represent an average of at least three determinations ±SD.
[b] Data is the result of one determination.

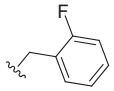
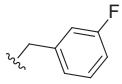
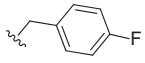
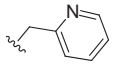
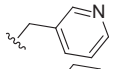
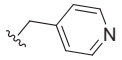
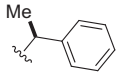
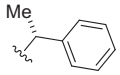
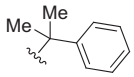
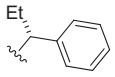
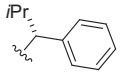
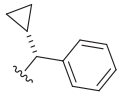
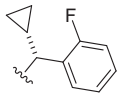
assay. The *ortho*-fluoro isomer **9** displayed increased selectivity over CDK2 (17-fold selective vs 4- to 6-fold selective for **8**, **10**, and **11**). Pyridines **12**–**14** had similar Cdc7 and pMCM2 potency, albeit inferior to **8**–**11**, and enhanced microsomal stability while **12** had the greatest selectivity against CDK2. Substitution at the benzylic carbon was also examined. Both α-methyl benzyl amine enantiomers **15** and **16** were prepared. Despite similar Cdc7 potency, **16** was 7-fold more active in the pMCM2 cellular assay and more selective against CDK2. The *gem*-dimethyl analog **17** was more active against CDK2 (IC_{50} = 7 nM) than Cdc7 (IC_{50} = 0.12 μM). More sterically demanding substituents at the α-carbon such as ethyl (**18**), isopropyl (**19**), and cyclopropyl (**20**) increased potency and selectivity relative to methyl (**16**). The *ortho*-fluoro cyclopropyl analog **21** exhibited excellent Cdc7 and pMCM2 potency and selectivity against CDK2.

The moderate microsomal turnover of **21** translated into an in vivo clearance (CL) of 2.0 L/h/kg (Table 4). Although pyridines **12**–**14** offered improved microsomal stability, introduction of the nitrogen heteroatom reduced Cdc7 and pMCM2 potency relative to **8**. In an effort to identify sites of metabolism within the scaffold, compound **16** was chosen as a representative azaindole for additional studies. As shown in Figure 2, incubation of **16** with rat hepatocytes yielded glutathione (GSH) adduct **16a** as the major metabolite. The GSH-adduct was located in the azaindole/oxadiazole region of **16a**. Therefore, in addition to incorporating a pyridine within the benzylic amine side-chain, blocking metabolism within the azaindole by introducing a heteroatom and/or fluorination could potentially reduce metabolism.

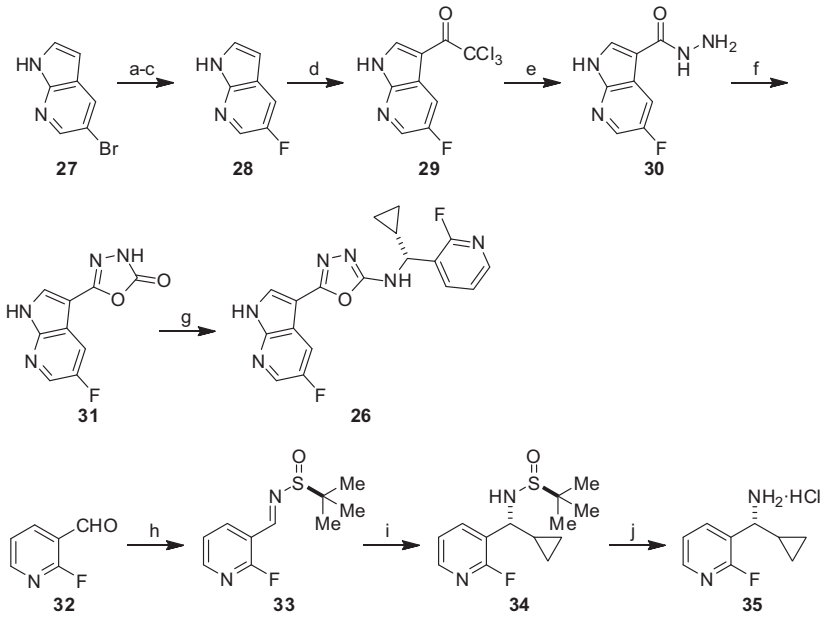
Compounds **22**–**26** were designed to introduce a heteroatom and/or fluorination into both the side-chain and azaindole groups in order to improve metabolism and solubility. In addition to the moderate clearance of **21**, its solubility in simulated intestinal fluid (SIF) was very low. Compounds **21**–**23** and **26** had promising Cdc7 cell activity and selectivity profiles against CDK1, CDK2, and CDK9;

Table 2
SAR of azaindole benzylic amines



Compound	R	Cdc7 IC_{50}[a] (μM)	pMCM2 IC_{50}[a] (μM)	CDK2 IC_{50}[a] (μM)	HLM/RLM CL_{int}[b] (μL/min/mg)
9		0.035 ± 0.03	1.6 ± 0.3	0.58 ± 0.3	32/46
10		0.010 ± 0.009	0.58 ± 0.3	0.061 ± 0.01	54/71
11		0.025 ± 0.02	0.69 ± 0.4	0.098 ± 0.02	47/58
12		0.17 ± 0.2	13 ± 2	2.5 ± 0.5	<14/14
13		0.19 ± 0.2	7.1 ± 3	0.61 ± 0.1	<14/30
14		0.11 ± 0.1	12 ± 4	0.35 ± 0.07	<14/37
15		0.035 ± 0.04	2.6 ± 1	0.098 ± 0.02	15/17
16		0.032 ± 0.03	0.37 ± 0.2	0.31 ± 0.04	64/25
17		0.12 ± 0.07	6.2 ± 5	0.007 ± 0.003	30/35
18		0.004 ± 0.003	0.15 ± 0.02	1.7 ± 0.8	70/29
19		0.002 ± 0.0005	0.14 ± 0.06	1.2 ± 0.3	66/51
20		0.001 ± 0.0006	0.071 ± 0.04	1.6 ± 0.4	56/31
21		0.0009 ± 0.0007	0.020 ± 0.009	0.24 ± 0.06	59/63

[a] Data represent an average of at least three determinations ±SD.
[b] Data is the result of one determination.



Scheme 1. Synthesis of **26**. (a) NaH, TIPSCl, THF, rt, 91% yield; (b) *n*-BuLi, $(PhSO_2)_2NF$, -78 °C to rt, 59% yield; (c) TBAF, THF, rt, 98% yield; (d) $AlCl_3$, $ClCOCCl_3$, CH_2Cl_2, 40 °C; (e) H_2NNH_2, MeOH, 60 °C, 80% yield (2 steps); (f) CDI, iPr_2NEt, DMF, 60 °C, 41% yield; (g) **35**, BOP (benzotriazole-1-yl-oxy-tris-(dimethylamino)-phosphonium hexafluorophosphate), iPr_2NEt, DMF, rt, 41% yield; (h) (*S*)-(-)-2-methyl-2-propane-sulfinamide, $Ti(OEt)_4$, THF, rt, 95% yield; (i) cyclopropylmagnesium bromide, CH_2Cl_2, -78 °C, 68% yield; (j) HCl, MeOH, rt, 95% yield.

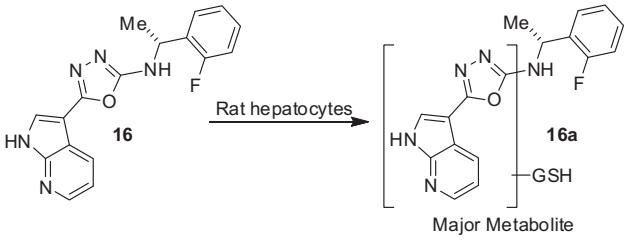


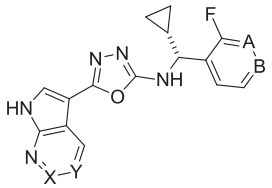
Figure 2. Rat hepatocyte and trapping results with **16**.

therefore, these were evaluated in additional in vitro and in vivo studies.

The addition of a nitrogen atom increased the solubility of analogs **22**, **23**, and **26** relative to **21** (Table 4). The rat PK of **21**–**23** and **26** were determined and despite encouraging microsomal stability (Table 3), only **26** had CL < 2 L/h/kg along with acceptable oral bioavailability (Table 4). The pMCM2 potency for **21**–**23** and **26** were within 3-fold of one another, and the cellular viability in HCT-116 cells[21] was within 6-fold, therefore these parameters were not significant differentiators. All four compounds were dosed orally in a mouse pharmacodynamic (PD) model[21] at 100 mg/kg. The levels of pMCM2 and associated free drug concentrations were measured at 6 and 16 h. All four compounds lowered pMCM2 at 6 and 16 h relative to the vehicle control group. Both **23** and **26** sustained greater levels of pMCM2 reduction and unbound plasma concentrations at 16 h than **21** and **22**.[25]

The synthesis of **26** is shown in Scheme 1. A similar route was used to prepare the analogs in Tables 1–3. Commercially available 5-bromoazaindole **27** was converted to fluoro intermediate **28** via a three-step protection-lithiation-fluorination sequence. Acylation of **28** with trichloroacetyl chloride and $AlCl_3$ provided **29** which

Table 3
SAR of α-cyclopropyl azaindole analogs



Compound	A	B	X	Y	Cdc7 IC_{50} (μM)[a]	pMCM2 IC_{50} (μM)[a]	CDK2 IC_{50} (μM)[a]	CDK1 IC_{50} (μM)[a]	CDK9 IC_{50} (μM)[a]	HLM/RLM CL_{int} (μL/min/mg)[c]
21	CH	CH	CH	CH	0.00085 ± 0.0007	0.020 ± 0.009	0.24 ± 0.06	1.6 ± 0.6	0.031 ± 0.02	59/63
22	**N**	CH	CH	CH	0.00069 ± 0.0004	0.051 ± 0.01	0.49 ± 0.03	7.3[b] ± 0.5	0.12 ± 0.08	21/80
23	CH	**N**	CH	CH	0.0018 ± 0.0009	0.060 ± 0.03	1.9 ± 0.4	>50[c]	0.94 ± 0.8	27/45
24	CH	CH	CH	**N**	0.0015 ± 0.0005	0.13 ± 0.05	0.75 ± 0.5	>50[c]	0.88 ± 0.8	<14/34
25	**N**	CH	**CF**	CH	0.0039 ± 0.002	0.25 ± 0.2	4.0 ± 3	>50[c]	2.4 ± 0.5	<14/413
26	**N**	CH	CH	**CF**	0.0011 ± 0.0005	0.032 ± 0.01	0.67 ± 0.2	6.6[b] ± 1	0.46 ± 0.3	<14/20

[a] Unless indicated otherwise, data represent an average of at least three determinations.
[b] Data is the result of two determinations.
[c] Data is the result of one determination ±SD.

Table 4
In vivo PK and PD data for 21-**23** and **26**.

Compound	SIF Solubility (mg/mL)	Rat PK					Cell Viability IC_{50}[a] (μM)	Mouse PK/PD 100 mg/kg[b]	
		IV/PO Dose (mg/kg)	CL (L/h/kg)	MRT (h)	Vss (L/kg)	%F		% pMCM2 Remaining at 6/16 h[d]	Unbound Plasma Concentration at 6/16 h[c] (μM)
21	0.005	2/5	2.0	2.7	5.3	35	0.88	38/46	0.15/0.06
22	0.20	1/5	4.5	1.3	5.9	41	1.8	40/41	1.3/0.07
23	0.20	2/2	4.5	1.4	6.1	14	5.7	62/24	2.0/0.45
26	0.16	1/5	1.7	1.9	3.3	50	2.4	46/30	0.61/0.30

[a] HCT-116 cells, 4 d.
[b] NCR/nude female mice, $n = 3$ animals,% pMCM2 remaining is relative to vehicle control group.
[c] Calculated from total concentration and percent free fraction in mouse plasma.
[d] All values were statistically significant ($p < 0.05$) by one-way analysis of variance (ANOVA) followed by Dunnett's test.

was converted to acyl hydrazine **30** in 80% overall yield. Acyl hydrazine **30** was converted to the key intermediate **31** with CDI in modest yield. The oxadiazole ring in **26** was formed by BOP-mediated coupling of **35** with **31**.[26] Amine **35** was prepared as shown in Scheme 1 using the Ellman sulfinamide chemistry.[27,28] Aldehyde **32** was condensed with the *S* enantiomer of the *tert*-butanesulfinamide to give **33** in excellent yield. Addition of cyclopropylmagnesium bromide gave a 7:3 ratio of diastereomers and isomer **34** was isolated in 68% yield. The sulfinamide was then cleaved with HCl to give **35**.

In summary, we have extensively optimized a series of aminooxadiazoles for potency, selectivity, and in vivo PK starting from lead isoquinoline **1**. The isoquinoline hinge binder in **1** was replaced with an azaindole which improved microsomal stability without compromising the in vitro potency and selectivity profile. Modifying the benzylic amine portion enhanced the in vitro potency and selectivity. Trapping experiments with glutathione in rat hepatocytes provided insight for structural modifications within the azaindole to potentially improve metabolic stability. Azaindole **26** (Cdc7 IC_{50} = 1.1 nM, pMCM2 IC_{50} = 32 nM) demonstrated robust lowering of pMCM2 at both 6 and 16 h. Additional details of the in vivo efficacy of **26** will be reported in a future publication.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2013.09.055.

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