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4-(1*H*-Indazol-5-yl)-6-phenylpyrimidin-2(1*H*)-one analogs as potent CDC7 inhibitors

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ABSTRACT

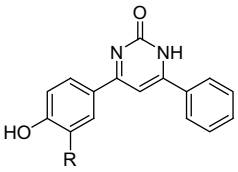
A series of 4-(4-hydroxyphenyl)-6-phenylpyrimidin-2(1*H*)-ones were identified by HTS as inhibitors of CDC7. Molecular modeling and medicinal chemistry techniques were employed to explore the SAR for this series with a focus on removing potential metabolic liabilities and improving cellular potency.

The serine/threonine kinase CDC7 plays an essential role in initiation of DNA replication in eukaryotic cells.[1] After assembly of the pre-replication complex to the replication origin, the CDC7 kinase phosphorylates MCM (minichromosome maintenance) proteins and allows for recruitment of CDC45 and DNA polymerase thereby initiating DNA replication.[2] CDC7 requires association with one of its cofactors, ASK (also known as DBF4) or ASKL1 (also known as Drf1), for kinase activation.[3–6] CDC7 deficient mice die between day 3.5 and 6.5 indicating that CDC7 is essential for early embryonic development.[7] Conditional knock-down of CDC7 in mouse embryonic stem (ES) cell lines (CDC7−/−tg) revealed immediate inhibition of cell proliferation, rapid cessation of DNA synthesis, and arrest in S phase progression.[7] CDC7 has been implicated in DNA damage checkpoint signaling in response to Etoposide treatment or DNA single strand breaks.[8] A role for CDC7 in DNA damage response is supported by the observation that CDC7 depleted mouse ES cells accumulate RAD51 foci in the nucleus.[7] Deletion of CDC7 in yeast results in hypersensitivity to hydroxyurea treatment.[9]

Recently, CDC7 has emerged as an attractive target for cancer therapy. Depletion of CDC7 using siRNA oligonucleotides results in induction of apoptosis in cancer cell lines while normal dermal fibroblast cells are spared.[10] Further, CDC7-mediated phosphorylation sites on MCM2, MCM4, and MCM6 in tumor cells have been identified, but the functional relevance of those sites remains to be determined.[3,11–13]

High-throughput screening (HTS) yielded the pyrimidin-2(1*H*)-one compounds (Fig. 1, **1** and **2**) as potent inhibitors of CDC7. These compounds, however, contained a phenolic moiety, which we felt could constitute a metabolic liability for the series. Analysis of in vitro incubation with mouse microsomes did, in fact, show a short half-life ($t_{1/2}$ 4 min). Furthermore, glucuronidation was the major metabolite as determined by CLND. In addition, these compounds lacked the ability to inhibit cellular proliferation.[14] We approached these issues using a ligand-based pharmacophore model[15] to design molecules to remove the phenol, retain in vitro potency and improve cellular inhibition.

Compound **2** (Table 1), which has a methyl group *ortho* to the hydroxyl showed a 10-fold improvement in enzymatic affinity compared to **1**, which lacked the methyl group. This increase in po-



1 R = H 0.03 μM
2 R = CH_3 0.003 μM

Figure 1. CDC7 HTS phenol pyrimidin-2(1*H*)-one hits and IC_{50} values.

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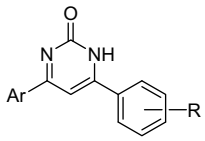
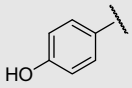
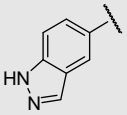
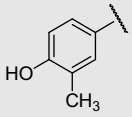
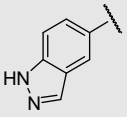
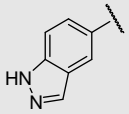
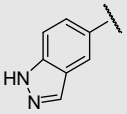
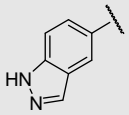
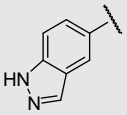
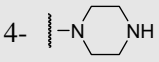
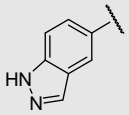
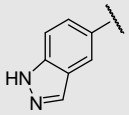
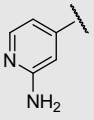
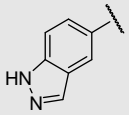
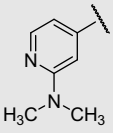
Table 1
Structure–activity relationship of the pyrimidin-2(1*H*)-one series for CDC7



Compound	Ar	R	CDC7 DBF4 IC_{50} (μM)	Compound	Ar	R	CDC7 DBF4 IC_{50} (μM)
1		—	0.03	**8**		3-OBn	0.006
2		—	0.003	**9**		4-OBn	0.35
3		—	0.01	**10**		4-Et	0.10
4		2-F	0.01	**11**			0.04
5		3-F	0.15	**12**		3-Cl	>13
6		3-Cl	0.005	**13**		2,4-OCH_3	1.0
7		3-CF_3	0.11	**14**		2,4-OCH_3	7.3

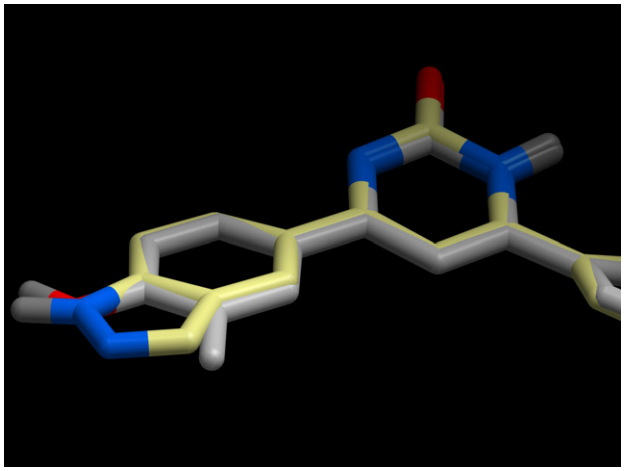


Figure 2. Overlap of **2** (gray) and **6** (light brown) in their phenol and indazole moieties, respectively.

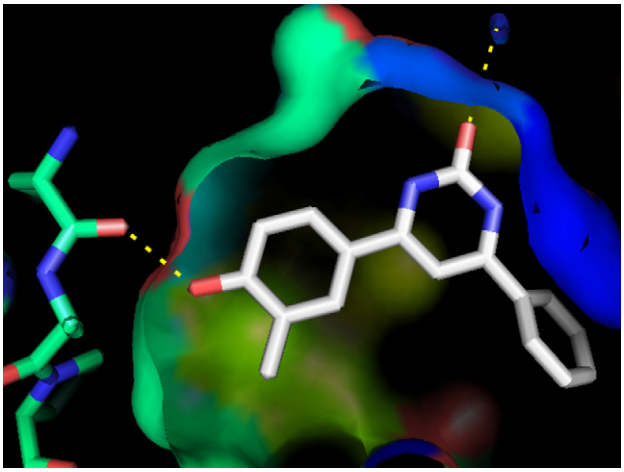
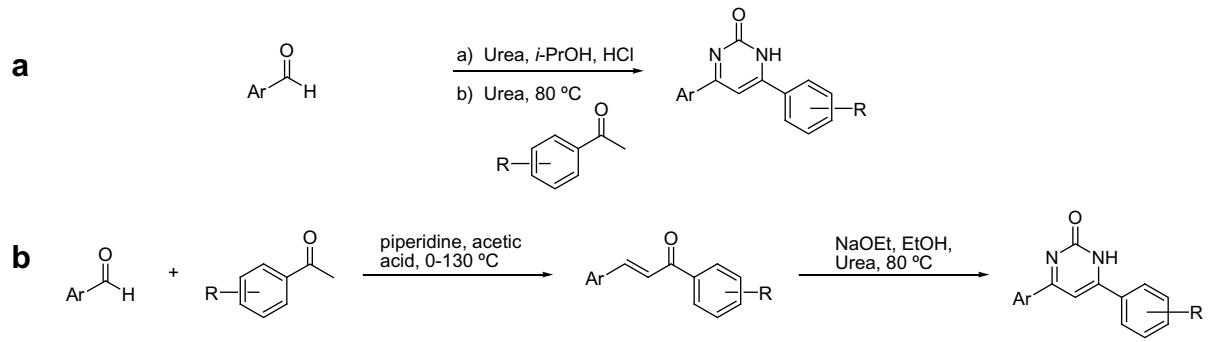


Figure 3. The binding site in a crystal structure of **2** in PIM-1 (PDB ID: 3DCV).



Scheme 1. Synthetic methods for pyrimidin-2(1*H*)-one series.

tency may be partially due to the fact that the *ortho*-methyl group induces a preference for a single hydroxyl group conformation and preorganizes the group for binding to CDC7, presumably in the hinge region. Quantum mechanical conformational analysis suggests that the hydroxyl hydrogen preferably points away from the methyl group. In evaluating this scaffold with the intention of replacing the hydroxyl group with another group which would make similar interactions and have less propensity for rapid metabolism, the indazole moiety was suggested as a phenol isostere. Modeling confirmed that the indazole of **6** overlaid well with the phenol (**2**, Fig. 2), and the required tautomer was preferred by quantum mechanical modeling.[16] Furthermore, the preferred indazole tautomer and preferred phenol conformation of **2** provided overlap of the hydrogen bond donors groups.

The indazole analog **3** was then synthesized and found to retain similar affinity as the phenols (**1** and **2**). A small set of analogs with a 4-indazole moiety was then synthesized with varying substituents on the 6-phenyl ring (compounds **4**–**11** in Table 1). Compound **4**, which has a fluorine at the *ortho* position of the phenyl ring, exhibited identical potency as unsubstituted analog **3** while substitution at the *meta* position with a fluorine (**5**) or a trifluoromethyl group (**7**) led to a significant decrease in in vitro activity. Interestingly though, a *meta*-chloro substituent (**6**) did not lead to a reduction in enzymatic potency. Finally, the large, hydrophobic 3-benzyloxy analog (**8**) was also potent against CDC7. The corresponding *para*-substituted benzyloxy analog (**9**), however, led to a nearly 60-fold decrease in CDC7 affinity compared to **8**. A hydrophobic group such as ethyl at the *para* position (**10**) was also less potent while the 4-piperazinyl analog (**11**) gave an IC_{50} of 0.04 μM against CDC7 suggesting that the *para* position groups extend to a hydrophilic part of the protein or to solvent.

Finally, to test the validity of the ligand-based pharmacophore model, several pyridine analogs were synthesized where the phenol was replaced by a pyridine or substituted pyridine as in compounds **12**–**14**. Modeling suggested that the pyridine nitrogen acceptor would be one bond length too short for optimal hydrogen binding to the hinge. The in vitro data supported this hypothesis as the unsubstituted pyridine (**12**) did not inhibit CDC7, and the dimethylaminopyridine **14** exhibited poor affinity. In addition, the aminopyridine (**13**) gave slight inhibition suggesting the necessity of a hydrogen bond donor.

Subsequently, a crystal structure of **2** in complex with PIM-1 (Proviral Insertion of Maloney kinase)[17] was obtained (Fig. 3). While the details of binding interactions are likely to be different in PIM-1 than in CDC7—as **2** is 50-fold less potent against PIM-1 than CDC7, and the indazole analogs of **2** are even weaker inhibitors of PIM-1—the PIM-1 co-crystal structure supported the role of the phenol as the hinge binding group. The co-crystal structure also supported the details used in the modeling in that the preferred pyrimidinone tautomer as drawn in Figure 2 and overall conformation of the compound, where the phenol and pyrimidinone are coplanar while the pendant phenyl is out of plane relative to the rest of the molecule, were predicted. These details are compatible with the CDC7 binding site shape and with interactions generally observed in kinases.

Although the 4-(1*H*-indazol-5-yl)-6-phenylpyrimidin-2(1*H*)-one analogs showed striking affinity for CDC7 in vitro, only compound **6** inhibited cellular growth (A549 lung carcinoma GI_{50} 0.6 μM). Evaluation of this series in CaCO-2 cells indicates both permeability and efflux as issues for this series. Compound **6**, the only compound to show inhibition of cellular growth, exhibited the highest permeability and lowest efflux ratio (BA/AB) for the series.[18] In addition, the efflux ratios for the series of compounds correlated well with the cellular potency observed. Furthermore, assessment of microsomal stability indicates that the indazole moiety did not improve the half-life for this series indicating that another portion of the molecule is also prone to metabolism.

The pyrimidin-2(1*H*)-one analogs described herein, were synthesized either by a modified Biginelli reaction[19] (Scheme 1a) or by condensation of a chalcone with urea under basic conditions[20] (Scheme 1b). Despite the moderate yields described in the literature, the yields obtained for the pyrimidin-2(1*H*)-ones presented in this letter were typically less than 5% regardless of the method used.

In conclusion, from the initial HTS hits, the 4-(1*H*-indazol-5-yl)-6-phenylpyrimidin-2(1*H*)-ones, a novel series of compounds potent against the kinase CDC7, were designed and synthesized. While cellular potency was obtained in only one example, further modifications could be envisioned which might increase cell permeability and thus improve cellular proliferation.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.07.061.

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14. GI_{50} data in the A549 human lung carcinoma cell line is >10 μM for both compounds **1** and **2**.
15. There is no reported CDC7 crystal structure, and the closest kinases have low homology of 30% for the purpose of building homology models for docking.
16. Jaguar version 6.0 (Schrodinger, LLC, Portland, Oregon, 2005) was used for geometry optimization at the B3LYP/6-31G* level of theory in the presence of Jaguar's continuum water model.
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