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Aminoindazole PDK1 Inhibitors: A Case Study in Fragment-Based Drug Discovery

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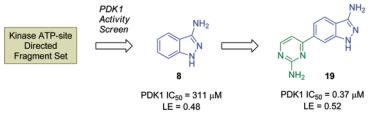
ABSTRACT Fragment screening of phosphoinositide-dependent kinase-1 (PDK1) in a biochemical kinase assay afforded hits that were characterized and prioritized based on ligand efficiency and binding interactions with PDK1 as determined by NMR. Subsequent crystallography and follow-up screening led to the discovery of aminoindazole **19**, a potent leadlike PDK1 inhibitor with high ligand efficiency. Well-defined structure−activity relationships and protein crystallography provide a basis for further elaboration and optimization of **19** as a PDK1 inhibitor.

KEYWORDS Aminoindazole PDK1 inhibitors, fragment-based drug discovery, ligand efficiency, binding interactions



Fragment-based drug discovery (FBDD) is widely recognized as an alternative to high-throughput screening for hit identification.[1−3] Success in implementing FBDD to deliver clinical candidates supports FBDD as a valuable and proven strategy in medicinal chemistry.[4,5] Endeavors in FBDD begin with screening collections of small, low molecular weight molecules (generally <250 MW) for inhibition or binding to a therapeutic target. This offers a broad coverage of chemical space, high hit rates, and quality starting points for hit-to-lead campaigns. Using principles of FBDD and ligand efficiency (LE), the medicinal chemist can accurately define structure−activity relationships (SAR) and measure progress during lead optimization.[6] Once a hit is confirmed in a FBDD campaign, the medicinal chemist is confronted with fragment expansion or evolution, a difficult but necessary step to bridge the gap from fragment to leadlike molecule.[7] This report is an account of our success using a FBDD approach to identify and progress fragment inhibitors of phosphoinositide-dependent kinase-1 (PDK1), an integral component of the PI3K/AKT/mTOR pathway, which is one of the most commonly deregulated signaling pathways across all cancers.[8−11]

The composition of the fragment library used in the PDK1 screen was biased toward molecules with donor and/or acceptor motifs embedded in an aromatic ring, which could fill a flat lipophilic pocket and engage the kinase hinge through hydrogen-bonding interactions, similar to the adenine ring of ATP.[12] The assembled library consisted of 1065 fragments originating from our proprietary compound collection and external sources. We began our screen for PDK1 inhibitors using a biochemical kinase assay at high fragment concentration (400 μM).[13] We subsequently selected 193 compounds for IC_{50} determination based on percent inhibition (≥60%) and chemical purity (≥98%),[14] resulting in 89 compounds with IC_{50} < 400 μM. A set of 36 compounds was selected for further evaluation based on LE (LE > 0.40) and tractability.[15,16] Tractability was a subjective measure and was influenced by structural novelty, ease of synthesis, and the ability to introduce substituents in multiple vectors for optimization. Representative examples are listed in Table 1.

Saturation transfer difference (STD) experiments[17] were used to confirm an interaction of the hits with PDK1. This allowed us to identify false positives and compounds not binding specifically to PDK1 in the biochemical assay. By using this biophysical analytical method, we were able to eliminate nearly half of the hits based on results indicating weak or no interaction with PDK1 (Table 1).

Prior to obtaining NMR results, we performed soaking experiments with a selection of fragment hits (**1**−**3**, **8**, **11**, **12**, and **17**) to ascertain their propensity to displace ATP from preformed PDK1-ATP crystals.[18] Only three of the fragments (**8**, **11**, and **17**) produced quality data sets with well-defined density maps. A fourth compound (**12**) did show density, but it was too ambiguous to interpret. As shown in Figure 1, the fragments exhibit hydrogen bonding to the A162 and S160 hinge residues of PDK1 in the adenine pocket. Consistent with our hit analysis, all three fragment compounds shown were also positively identified by STD to interact with PDK1. Furthermore, the highly ligand efficient pyrazole fragments (**1**, **2**, and **3**) that were determined to not directly interact significantly with PDK1 by STD were not

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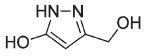
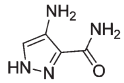
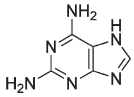
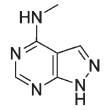
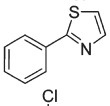
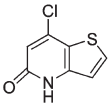
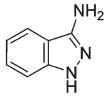
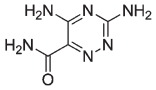
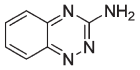
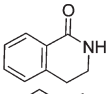
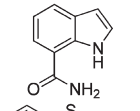
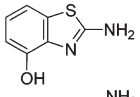
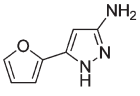
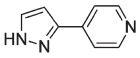
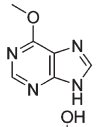
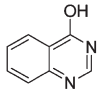
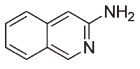
Table 1. PDK1 Fragment Screen Hits and Data Summary

	Entry	MW	PDK1 IC_{50} (μM)[a]	LE[b]	NMR (STD)[c]
	1	114.1	344	0.59	-
	2	126.1	200	0.56	-
	3	123.2	244	0.55	-
	4	150.1	50.8	0.53	+
	5	149.2	90.8	0.50	+
	6	161.2	95.1	0.50	-
	7	185.6	102	0.50	+
	8	133.2	311	0.48	+
	9	154.1	145	0.48	-
	10	146.2	160	0.47	+
	11	147.2	169	0.47	+/-
	12	160.2	97.5	0.46	+
	13	166.2	256	0.45	+/-
	14	149.2	313	0.44	+/-
	15	145.2	326	0.43	+
	16	150.1	340	0.43	+
	17	146.2	345	0.43	+
	18	144.2	376	0.43	+

[a] The PDK1 kinase assay was performed with PDK1 (52−556) enzyme and biotinylated AKT3 (135−479) as substrate. [b] LE defined as $\Delta G/N_{\text{nonhydrogen atoms}}$, where $\Delta G \approx -RT \ln IC_{50}$. [c] STD (+ = STD observed, − = no STD observed, and ± = weak STD observed).

successful at providing cocrystals with PDK1. These data substantiate the importance of using biophysical methods for characterizing hits from biochemical fragment screens, which can dramatically improve the X-ray success rate and confidence level of hit identification.

Concurrently, we carried out substructure searches based on our hits as a means to quickly identify analogues for SAR exploration. Hit substructure searching was performed with a focus on identifying low MW compounds (< 300 MW) that were elaborated versions of the actual fragments. This led to the discovery of aminopyrimidine **19**, which is a leadlike derivative of the aminoindazole fragment hit **8** (see Table 2). With the addition of one heterocyclic ring, the potency increased from 311 μM in **8** to 0.37 μM in **19**. Moreover, the LE was not affected, suggesting that additional productive interactions with PDK1 were introduced with the added MW.

Indeed, an X-ray structure of **19** with PDK1 (Figure 2)[18] revealed that the aminopyrimidine ring engaged in a tight network of hydrogen bonding with the protein. The pyrimidine ring nitrogens act as acceptors for the catalytic residue K111 and T222 at the floor of the binding pocket, while the amino group of the pyrimidine is a hydrogen bond donor to catalytic residue E130. The overall arrangement and presentation of the aminopyrimidine functionality are consistent with the high LE of compound **19** and all components of the heterocycle contributing to its potent PDK1 inhibition.

To further understand the importance of the aminopyrimidine ring, we systematically prepared analogues **20**−**25** and evaluated their activity in the PDK1 kinase assay. As shown in Table 2, replacement of the aminopyrimidine ring nitrogen that participates in hydrogen bonding with T222 (compound **21**) led to a loss of potency (IC_{50} = 7.28 μM). Similarly, loss of the amino group of the pyrimidine (**20**) caused a modest decrease in potency (IC_{50} = 2.67 μM). A large decrease in potency was observed when the aminopyrimidine ring nitrogen interacting with K111 was replaced with a carbon (**22**, IC_{50} = 13.6 μM). Loss of two or more aminopyrimidine nitrogen components (compounds **23**−**25**) resulted in significantly less active compounds. Taken together, these data indicate that the ring nitrogen involved in the hydrogen-bonding interaction with K111 represents a critical binding element. Overall, the cumulative data complement the X-ray structural information and confirm that each nitrogen functionality of the aminopyrimidine positively contributes to PDK1 binding and inhibition of kinase activity.

With **19** established as a genuine and attractive leadlike PDK1 inhibitor, we turned our attention to kinase selectivity. Despite its simple structure, we were pleased by the overall kinase selectivity profile exhibited by **19** (Table 3). Reasonable selectivity was even achieved within the AGC kinase family between PDK1 and some of the other members (e.g., AKT1, SGK1, and, to a lesser extent, p70S6K).[19] This selectivity profile is encouraging for an early lead in a kinase inhibitor program. Moreover, it highlights particular kinases that could pose specificity challenges during lead optimization.

In this FBDD case study of PDK1, we described the identification and characterization of aminoindazole fragment hit **8** from a kinase-biased library of ~1K fragments. Biochemical screening, combined with the use of appropriate filters and orthogonal

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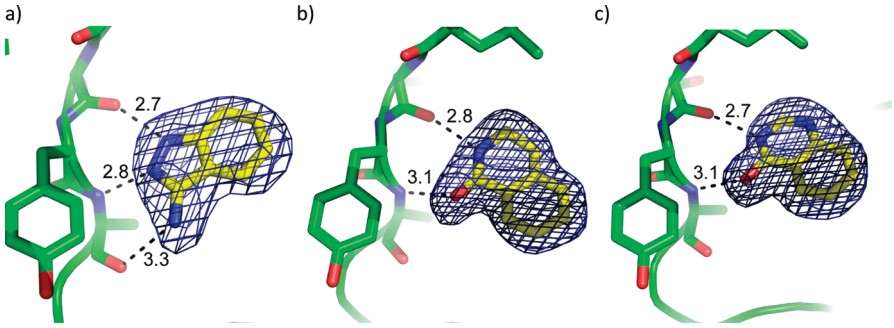
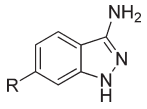
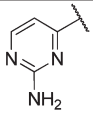
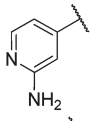
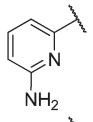
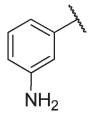


Figure 1. X-ray structures of fragment hits **8** (a), **11** (b), and **17** (c) bound to PDK1. Key hydrogen-bonding interactions with the kinase hinge residues are indicated with dashed lines and annotated with distances (Å).

Table 2. PDK1 Inhibitors Derived from 3-Aminoindazole Fragment **8**



R	Entry	MW	PDK1 IC_{50} (μM)[a]	LE[b]
	**19**	226.2	0.37	0.52
	**20**[c]	211.2	2.67	0.48
	**21**[d]	225.3	7.28	0.41
	**22**	225.3	13.6	0.39
	**23**	224.3	21.2	0.38
	**24**	210.2	>25.1	N/A
	**25**[d]	210.2	13.2	0.42

[a] The PDK1 kinase assay was performed with full-length PDK1 enzyme and biotinylated PDKtide as the substrate. [b] LE defined as $\Delta G/N_{\text{nonhydrogen atoms}}$, where $\Delta G \approx -RT \ln IC_{50}$. [c] Trifluoroacetate salt. [d] Hydrochloride salt.

biophysical methods, proved to be a viable method to discover valid, high-efficiency PDK1 fragment hits. Additional screening of selected leadlike molecules related to **8** within our compound collection led to the discovery of the highly ligand efficient aminopyrimidine-aminoindazole compound **19**. This underscores the importance of compound collection mining as an

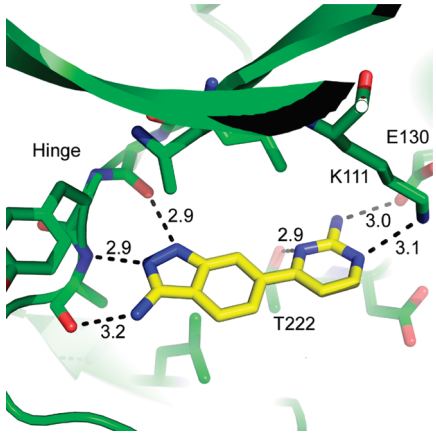
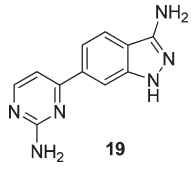


Figure 2. X-ray structure of compound **19** bound to PDK1, showing the hydrogen-bonding interactions.

Table 3. Kinase Selectivity Data for Compound **19**



19

kinase	IC_{50} (μM)	kinase	IC_{50} (μM)
PDK1	0.37	INS	> 10
AKT1	> 10	JAK3	0.99
ASK1	5.69	JNK1	> 10
Aurora A	0.39	p70S6K	6.34
Aurora B	3.48	PI3Kα	> 10
CDK2	0.28	SGK1	> 10
CHK1	> 10	SYK	4.00
IGF1R	> 10	VEGFR2	0.84
IKK1	0.10		

effective method to progress a fragment to a leadlike molecule. Subsequent X-ray structure determination of **19** with PDK1 revealed that the aminopyrimidine engaged in a favorable hydrogen bond network with catalytic residues, supporting the SAR and binding contributions of the aminopyrimidine heterocycle. In addition, the crystallographic data provided a

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basis for further structure-based optimization of PDK1 activity and kinase selectivity. The continuation of this research, including potency optimization and refinement of compound **19** to enhance cellular activity,[20] will be reported in a future publication.

SUPPORTING INFORMATION AVAILABLE Protocols for PDK1 assays, NMR experiments and protein crystallography, and experimental procedures and characterization of compounds **19**−**25**. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

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(9) Alessi, D. R.; James, S. R.; Downes, C. P.; Holmes, A. B.; Gaffney, P. R. J.; Reese, C. B.; Cohen, P. Characterization of a 3-phosphoinositide-dependent protein kinase which phosphorylates and activates protein kinase Bα. *Curr. Biol.* **1997,** *7*, 261–269.
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(13) See the Supporting Information for PDK1 assay details.
(14) The chemical purity of the screening samples was determined by high-throughput LC-MS analysis.
(15) Hopkins, A. L.; Colin, R.; Alex, A. Ligand efficiency: A useful metric for lead selection. *Drug. Discovery Today* **2004,** *9*, 430–431.
(16) Reitz, A. B.; Smith, G. R.; Tounge, B. A.; Reynolds, C. H. Hit Triage Using Efficiency Indices after Screening of Compound Libraries in Drug Discovery. *Curr. Top. Med. Chem.* **2009,** *9*, 1718–1724.
(17) Mayer, M.; Meyer, B. Characterization of ligand binding by saturation transfer difference NMR spectroscopy. *Angew. Chem., Int. Ed. Engl.* **1999,** *38*, 1784–1788.
(18) X-ray data were submitted to the Protein Data Bank (PDB codes: **8**, 1NUS; **11**, 3NUU; **17**, 3NUY; and **19**, 3NUN). See the Supporting Information for PDK1 crystallography details.
(19) Pearce, L. R.; Komander, D.; Alessi, D. R. The nuts and bolts of AGC protein kinases. *Nat. Rev. Mol. Cell Biol.* **2010,** *11*, 9–22.
(20) No inhibition of AKT phosphorylation at T308 was observed in HCC1954 cells when treated with compound **19** at concentrations up to 30 μM.

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