Discovery of Dibenzo[*c*,*f*][2,7]naphthyridines as Potent and Selective 3-Phosphoinositide-Dependent Kinase-1 Inhibitors

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Abstract: With high-throughput screening, substituted dibenzo[*c*,*f*][2,7]-naphthyridine **1** was identified as a novel potent and selective phosphoinositide-dependent kinase-1 (PDK-1) inhibitor. Various regions of the lead molecule were explored to understand the SAR requirement for this scaffold. The crystal structure of **1** with kinase domain of PDK-1 confirmed the binding in the active site. The key interaction of the molecule with the active site residues, observed SAR, and the biological profile are discussed in detail.

Binding of insulin and growth factors to their cell surface receptors activates phosphotidylinositol-3-kinase (PI3K[a]), which phosphorylates phosphatidylinositol 4,5-bisphosphate at the D3 position to generate phosphatidylinositol 3,4,5-trisphosphate (PIP3). PI3K and PIP3 mediate activation of a subgroup of AGC kinases involved in inhibition of apoptosis, promotion of cell growth and proliferation, glucose uptake and storage, and amino acids storage.[1] These AGC kinases including different isoforms of protein kinase B (PKB, also called Akt),[2] p70 ribosomal S6 kinase (S6K),[3] serum- and glucocorticoid-induced protein kinase (SGK),[4] and atypical isoforms of protein kinase C (PKC)[5] all require 3-phosphoinositide-dependent kinase-1 (PDK-1) for full activation.[5] Thus, PDK-1 is central to insulin and growth factor stimulated signal transduction.

A significant number of cancers have mutations in genes encoding PI3K and their regulators or PTEN,[6] a phosphatase that removes the phosphate from the D3 position of PIP3. These cancers presumably have elevated PIP3 levels. Because PIP3 contributes to the activation of AGC kinases and AGC kinases require PDK1 for activation, an inhibitor of PDK1 would likely abrogate the growth and proliferation of many cancers or cause cancer cell apoptosis. We are particularly interested in PDK1 because it is a relatively unexplored kinase and not many potent, selective inhibitors of PDK1 have been reported.[7−12]

To identify potent selective inhibitors of PDK-1 kinase, a homogeneous in vitro assay based on a LANCE format was developed for high-throughput screening. In this screening assay, PDK1 phosphorylates inactive SGK1 kinase on its activation loop, thereby activating SGK1. The activated SGK1 phosphorylates a biotinylated GSK3 peptide. The phosphorylated GSK3 peptide is detected using Eu-labeled phosphospecific antibody. Those compounds that showed the largest differential inhibition of PDK1 and SGK1 were subjected to an ELISA assay in which direct inhibition of SGK1 phosphorylation was measured. From these assays dibenzo[*c*,*f*][2,7]naphthyridine **1** (Figure 1) was identified as a potent inhibitor of PDK-1 with an IC_{50} of 0.06 μM.

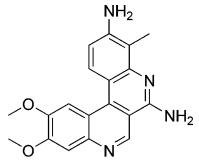

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Figure 1. PDK-1 inhibitor from high-throughput screening.

Compound **1** inhibited the growth of a variety of tumor cell lines as shown in Table 1. This compound was found to be selective when tested against a panel of kinases including SGK1, AKT, S6K, CDK4, IKK, Src, TPL2, MEK, PKA, P38, MK2, and CAMKII (IC_{50} > 10 μM). Employing fluorescence spectroscopy techniques, single-digit nanomolar K_D was determined from the changes in the endogenous tryptophan fluorescence of the enzyme upon inhibitor binding, at the emission and excitation wavelengths of 340 and 295 nm, respectively. From these experiments the stoichiometry of binding was found to be a 1:1 ratio indicative of specific binding.

As shown in Scheme 1, reaction of chloroquinoline **2**[13] with 2,6-diaminotoluene **3** in ethoxyethanol was optimized using design of experiments (DoE)[14] to provide 10,11-dimethoxy-4-methyldibenzo[*c*,*f*][2,7]naphthyridine-3,6-diamine **1**. Analogues **11** and **12**, with different groups in the C10 and C11 positions, were prepared in a similar fashion starting with appropriately substituted chloroquinolines[15] in the place of **2**. To synthesize analogue **6** devoid of the amino moieties, cyanoquinoline **2** was reduced to aldehyde **4** and reacted with boronic acid **5** as shown in Scheme 2. Des-methyl analogue **7** was prepared as shown in Scheme 1 employing 1,3-diaminobenzene in the final step.

Analogues **8**−**10**, where the C3 amino group was varied, were prepared by diazotization of **1** followed by reaction of the diazonium salt with appropriate reagents as shown in Scheme 3. Water solubilizing groups were introduced in the C10 position by replacing the fluoro group in **12** by various amines or alkoxides to afford **13**−**19** as shown in Scheme 4.

Initial SAR studies were focused on determining the importance of the C3, C6 diamino substituents on the dibenzonaphthyridine scaffold (Table 2). Removal of both amino groups and C4 methyl as in analogue **6** rendered the molecule 100-fold less active, indicating the importance of these substituents. Retaining both amino groups and removing just the C4 methyl group **7** led to a 10-fold loss of potency, indicating that the amino groups played a more crucial role than the C4 methyl group. At this point we set out to explore if one of the amino groups is more important than the other for the kinase activity. Toward that end analogue **8**, which retained the C6 amino group and C4 methyl group but is devoid of the C3 amino moiety, was prepared. It showed only a 4-fold decrease in potency, indicating that the C6 amino group is more important for the kinase activity than the C3 amino group. This was further confirmed by synthesizing **9** with a C3 hydroxyl group, which was equipotent to the original amino compound **1**. However,

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[a] Abbreviations: PI3K, phosphotidylinositol-3-kinase; PIP3, phosphatidylinositol 3,4,5-trisphosphate; PKB, protein kinase B; S6K, p70 ribosomal S6 kinase; SGK, serum- and glucocorticoid-induced protein kinase; PKC, protein kinase C; PDK-1, 3-phosphoinositide-dependent kinase-1; PTEN, phosphatase and tensin homologue; GSK, glycogen synthase kinase-3.

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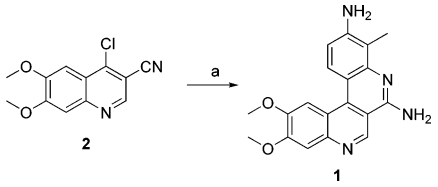
Table 1. Tumor Growth Inhibition Data for Compound **1**

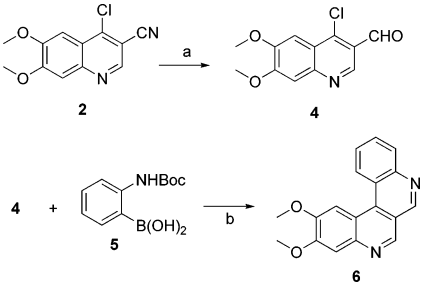
tumor cell lines	IC_{50} (μM)[16]
M49-5	0.35
HCT116	3.6
DU145	2.53
SW620	3.9
K-562	0.48
PC-3mm2	4.3
MDA-MB-468	1.55

Scheme 1[a]



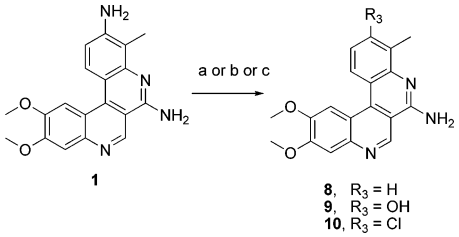
[a] Reagents: (a) 2,6-Diaminotoluene **3**, ethoxyethanol, reflux, 15 h.

Scheme 2[a]



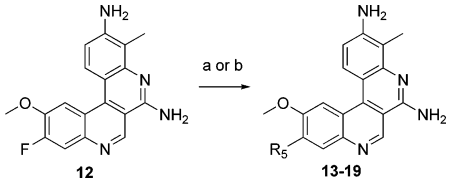
[a] Reagents: (a) DIBAL−H, CH_2Cl_2, −78 °C to room temp, 2 h; (b) $Pd(Ph_3P)_4$, 2 M Na_2CO_3, DME, 80 °C, 18 h.

Scheme 3[a]



[a] Reagents: (a) $NaNO_2$, HOAc/HCl, 0 °C, H_3PO_2, room temp, 6 h; (b) $NaNO_2$, HOAc/HCl, 0 °C, CuCl, 100 °C, 1 h; (c) $NaNO_2$, HOAc/HCl, 0 °C, 20% aqueous H_2SO_4, toluene, room temp, 4 h.

Scheme 4[a]

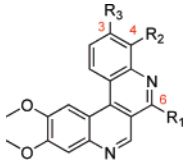


[a] Reagents: (a) amines, NMP, $\mu\lambda$, 200 °C, 3 h; (b) alcohols, NaH, DMF, 70 °C, 2 h.

analogue **10**, which has a lipophilic chloro group, was found to be 40-fold less active.

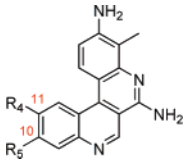
In contrast to the SAR discussed above, SAR requirements for C10 and C11 positions were found to be much less rigid. Dimethoxy groups could be replaced with a diethoxy group (**11**) without much loss in potency. Even replacing the C10 methoxy by fluoro (**12**) retained modest activity. On the basis of this observation, the C10 position was chosen to introduce some bulky groups with the basic amine side chain to improve the aqueous solubility of the molecule. As shown in Table 3, a number of amine and ether substituents were introduced. While most of the groups were tolerated, direct comparison of analogue pairs **13**−**17** and **16**−**18** indicates a slight preference for the ether substituent at the C10 position compared to an amino group. However, within the substituents the length of the linker did not have any significant influence on the activity (**13** vs **14** or **15** vs **16**). An extended lipophilic ether group in that position as in analogue **19** was much less favorable.

Table 2. PDK-1 Activity of Dibenzo[*c*,*f*][2,7]naphthyridine-3,6-diamine: Optimization of Diamino Region



compd	R_1	R_2	R_3	IC_{50} (μM)[16]
1	NH_2	CH_3	NH_2	0.06
6	H	H	H	7.45
7	NH_2	H	NH_2	0.67
8	NH_2	CH_3	H	0.26
9	NH_2	CH_3	OH	0.04
10	NH_2	CH_3	Cl	2.12

Table 3. PDK-1 Activity of Dibenzo[*c*,*f*][2,7]naphthyridine-3,6-diamine: Optimization with Water Solubilizing Groups



compd	R_4	R_5	IC_{50} (μM)[16]
1	$-OCH_3$	$-OCH_3$	0.06
11	$-OCH_2CH_3$	$-OCH_2CH_3$	0.27[a]
12	$-OCH_3$	−F	0.58
13	$-OCH_3$	$-NHCH_2CH_2$(*N*-morpholino)	0.30
14	$-OCH_3$	$-NHCH_2CH_2CH_2$(*N*-morpholino)	0.29
15	$-OCH_3$	$-NHCH_2CH_2$(*N*-CH_3)$_2$	2.49
16	$-OCH_3$	$-NHCH_2CH_2CH_2$(*N*-CH_3)$_2$	0.97
17	$-OCH_3$	$-OCH_2CH_2$(*N*-morpholino)	0.06
18	$-OCH_3$	$-OCH_2CH_2CH_2$(*N*-CH_3)$_2$	0.20
19	$-OCH_3$	$-OCH_2CH_2CH(CH_3)_2$	1.18

[a] Data from single experiment carried out in duplicate.

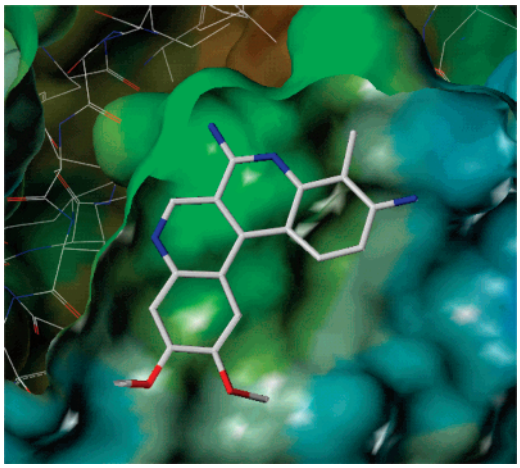


Figure 2. X-ray structure of **1** in complex with PDK-1 in the ATP binding site.

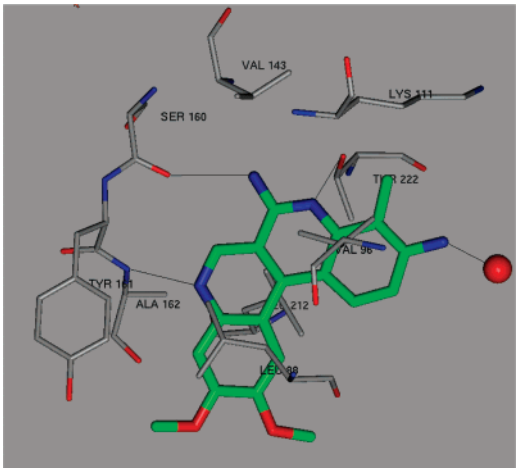


Figure 3. Atomic interactions of **1** with PDK-1. Polar interactions closer than 3.2 Å are shown as solid lines. Compound **1** carbons are colored green.

The crystal structure of **1** and PDK-1 complex was determined by soaking of preformed ATP bound PDK-1 crystals.[17] With a resolution of 2.7 Å, the compound was seen to bind in the active site (Figure 2) with the molecule being oriented such that the dimethoxyl groups are partially exposed to solvent. In the active site, **1** binds to the hinge via a pair of hydrogen bonds: N8 nitrogen to the NH of Ala162 and the C6-NH_2 group to the backbone carbonyl oxygen of Ser160. A third hydrogen bond was also observed from the N5 nitrogen of the scaffold to the side chain of Thr222. The dibenzonaphthyridine ring is sandwiched between the isopropyl group of Leu212 underneath and the isopropyl group of Leu88 above, forming strong van der Waals interactions. In the crystal structure, the C3-NH_2 group interacts with the protein through a bound water molecule. The key interactions are highlighted in Figure 3.

The SAR trend described above was readily supported by the determined X-ray structure. C3- and C6-amino groups make essential contacts to the protein. Removal of them therefore diminishes the activity (Table 2). For **9** where the C3-amino group was replaced with a hydroxyl group, the activity is comparable to that of **1**. A hydroxyl group at this position has similar hydrogen-bonding capabilities as that of an amino group. In the crystal structure, the distances from C4-methyl to side chains of Val96 and Lys111 are 4.1 and 4.6 Å, respectively. The C4-methyl group makes van der Waals interactions with Val96 and Lys111, contributing to the binding of **1**. Therefore, removing this methyl group (**7**) resulted in a 10-fold loss in activity. With the structure, it is also easy to understand why the SAR requirements for C10 and C11 are less rigid as summarized in Table 2. The C10- and C11-dimethyl groups are partially exposed to solvent. Thus, various substitutents can be tolerated at C10 and C11, and these positions are ideal for incorporating water-solubilizing groups to improve the physical properties of the series.

In summary, we have identified a novel series of dibenzo[*c*,*f*][2,7]naphthyridines as potent and selective PDK-1 inhibitors. Importantly, these compounds bind in the active site of the kinase as determined by the protein complex X-ray structure. Dibenzo[*c*,*f*][2,7]naphthyridines represent a novel kinase template with a good SAR trend as explained by the key interactions with the protein.

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Supporting Information Available: Experimental details for the biological assay and the synthetic procedures; HPLC traces for some compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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(16) Unless indicated, data are reported as the average of two or more experiments (each run in duplicate).
(17) PDB code: 2R7B.

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