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Indolinone based phosphoinositide-dependent kinase-1 (PDK1) inhibitors. Part 2: Optimization of BX-517

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Abstract—Based on the lead compound **BX-517**, a series of C-4′ substituted indolinones have been synthesized and evaluated for PDK1 inhibition. Modification at C-4′ of the pyrrole afforded potent compounds (**7b** and **7d**) with improved solubility and ADME properties. In this letter, we describe the synthesis, selectivity profile, and pharmacokinetic data of selected compounds.

The phosphoinositide-dependent kinase-1 (PDK1) is a Ser/Thr kinase that is a key activator of a number of protein kinases in the AGC kinase super-family that play important roles in the progression of cancer, such as Akt, S6 kinase, and protein kinase C.[1a,b] The best characterized cellular substrate for PDK1 is Akt.[2] PDK1 activates each of the three isoforms of Akt (AKT1, AKT2, and AKT3) through phosphorylation of a regulatory Threonine residue within the activation loop (e.g., on Thr308 of AKT1). This Thr residue functions in Akt and other AGC super-family kinases to control access of substrates to the active site.[1a,b] Binding of the phosphoinositide 3-kinase (PI3K) products PtdIns-3,4-P_2 or PtdIns-3,4,5-P_3 to the pleckstrin homology domains of Akt and PDK1, recruits these proteins to the plasma membrane and is necessary for Akt activation. A large number of studies implicate Akt in cancer progression through its role in the promotion of tumor cell growth, metabolism, survival, epithelial to mesenchymal transition (EMT), and angiogenesis.[3] S6 kinase activity also depends on several modifications downstream of PDK1.[1a] As in the case of Akt, PDK1 phosphorylates a critical regulatory site on the activation loop of S6 kinase (Thr229) that is required for catalytic activity. In addition, PDK1-mediated activation of Akt results in phosphorylation of Thr389 on S6 kinase, which is also required for activation. S6 kinase promotes growth and survival of cancer cells and is a key target of rapamycin analogs (e.g., CCI-779/temsirolimus) which are currently being tested clinically as anticancer agents.[4]

The PI3K/PDK1/Akt/S6 kinase signaling pathway is commonly elevated in tumors through the constitutive activation or over-expression of a variety of upstream signaling molecules such as growth factor receptors (e.g., ErbB2, EGFR, c-Met), Src, Ras, or through the elevated expression of PI3K and AKT proteins.[3] The pathway is also activated through the frequent loss of the tumor suppressor PTEN/MMAC1, whose D-3 inositol phosphatase activity removes the phosphorylation products of PI3K.[5] PDK1 has also been implicated in the activation of other potential cancer targets, such as protein kinase C.[1a,b] The role of PDK1 in several key signaling pathways important in the progression of cancer led us to investigate the use of PDK1 inhibitors as anticancer agents.

In the preceding paper, we described our discovery of indoline based inhibitors of PDK1 and their optimization to yield **BX-517**. **BX-517** is a potent and selective inhibitor of PDK1 that binds to the ATP binding pocket of the protein. Furthermore, **BX-517** blocks activation of Akt in tumor cells. Herein, we describe additional

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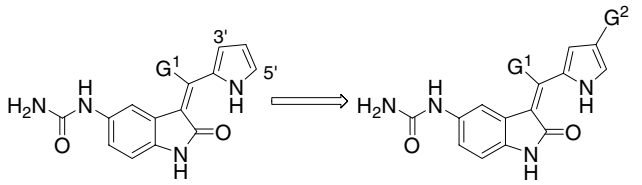


Figure 1. Optimization plan.

optimization of **BX-517**, focused on addressing ADME and solubility liabilities of the compound. This effort led to a series of novel and potent PDK1 inhibitors showing superior physicochemical properties compared to **BX-517**.

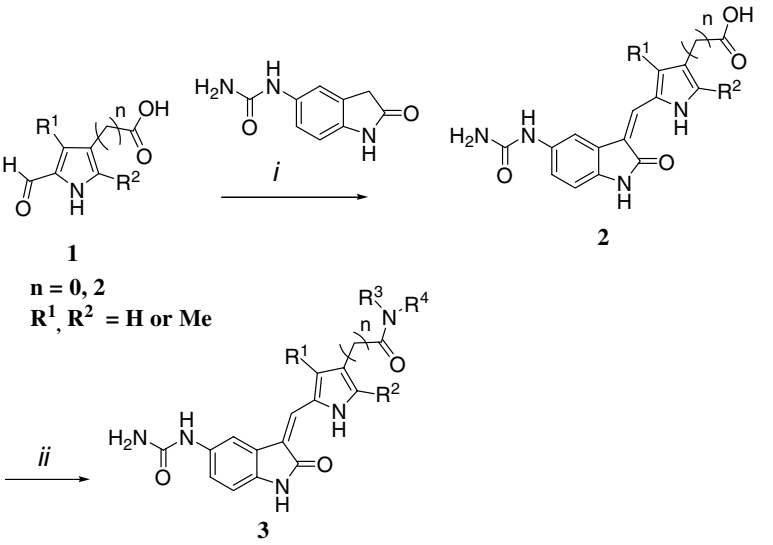
Despite the high enzymatic potency of **BX-517**, it possessed poor solubility and pharmacokinetic properties that prohibited further development (Fig. 1). Examination of the structure of **BX-517** co-crystallized with PDK1 suggests that substitution at the 4-position of the pyrrole ring would be tolerated as it points toward the solvent. To improve the solubility we introduced hydrophilic groups onto the 4-position of pyrrole ring. We first incorporated a carboxylic acid which was further elaborated into amides. Another approach to improve physicochemical properties was to synthesize heteroaryl and aryl linked solubilizing groups. Finally, inhibitors possessing a 4-aminomethyl pyrrole were synthesized and derivatized via amide bond formation.

Syntheses of the compounds having a carboxylate at the 4-position of the pyrrole are shown in Scheme 1. Carboxylic acids **1** were either prepared according to published procedures[6] or purchased. Compounds **2a**–**2d** in Table 1 were prepared by condensation of the appropriate carboxylic acids with 5-ureidoindolin-2-one. The subsequent amide formation afforded compounds **3a**–**3f** (Table 1).

Synthesis of aryl, heteroaryl, and substituted aryl analogs (**7a**–**7l**) is shown in Scheme 2. 4-Bromo-2-formylpyrrole (**4**) was protected[7] with toluenesulfonyl chloride and subsequent Suzuki coupling[8] with boronic acids gave intermediate **6**. Condensation with 5-ureidoindolin-2-one under basic conditions afforded intermediate **7**. Reaction of **7c** with amines under standard conditions for amide formation gave compounds **7d** and **7e**. Demethylation of analog **7g** using BBr_3 furnished analog **7f**.

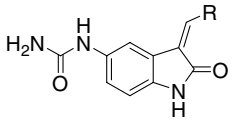
The analogs (**13**–**22**) derived from 4-aminomethyl pyrroles and bearing a methyl substituent on the olefin were prepared as described in Scheme 3. 2-Acetylpyrrole-4-carbonitrile (**8**)[9] was reduced and protected using Boc anhydride in one pot. Condensation of **9** with 5-nitroindolin-2-one under basic conditions followed by reduction of the nitro group with Tin chloride provided **11**. Urea formation from **11** with trimethylsilyl isocyanate and subsequent cleavage of the Boc group with HCl afforded **13**. Acylation of intermediate **13** with acetyl chloride gave analog **14**. Coupling of **13** with pyridine carboxylic acids in presence of EDC afforded analogs **15** and **16**. Coupling with substituted acids and subsequent deprotection under standard conditions furnished analogs **17**–**22**.

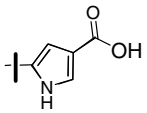
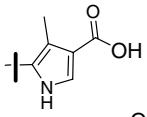
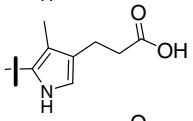
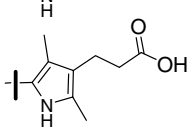
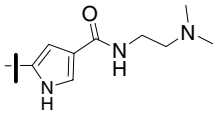
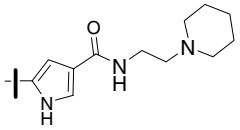
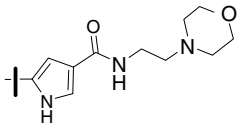
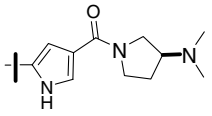
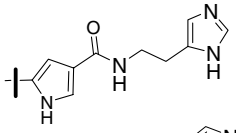
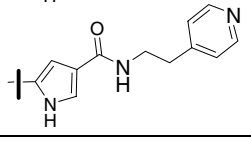
The in vitro data for acid substituted analogs and their corresponding amides are shown in Table 1. While the analogs showed potency in the enzyme assay within about 2-fold of the parent compound, **BX-201** (IC_{50} = 20 nM), they all displayed lower cell based activity. At the highest dose tested (10 μM) none of the analogs were active, and therefore, were at least 10-fold less potent than **BX-517** in cells (IC_{50} = 0.1–1.0 μM). Indeed, some of the analogs slightly stimulated the cell based assay, which can occur through apparent feedback activation of the AKT signaling pathway.[10] We observed such stimulatory effects in PC-3 cells to be a property



Scheme 1. Reagents and conditions: (i) cat. piperidine, ethanol, reflux (40–80%); (ii) amine, HATU (60–75%).

Table 1. Activity of PDK-1 inhibitors



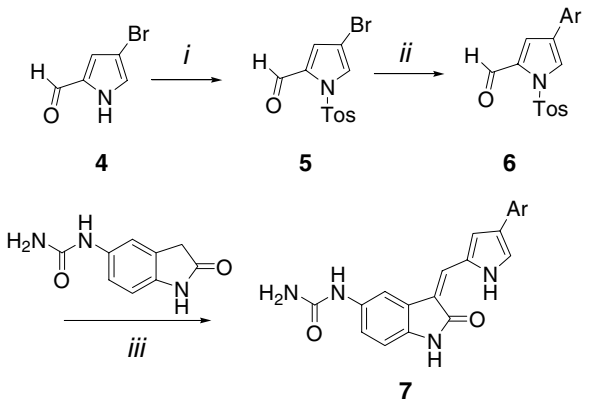
Compound	R	cAKT2[11] IC_{50} (nM)	P-AKT in PC-3 cells[12] IC_{50} (μM)
BX-201		20	3–10
2a		9	>10[a]
2b		9	No inhibition
2c		39	ND
2d		57	>10 (26% inhibition at 10 μM)
3a		27	(62% inhibition at 10 μM)
3b		11	1.0
3c		29	No inhibition
3d		31	No inhibition
3e		14	No inhibition[a]
3f		17	No inhibition[a]

For assay descriptions see Refs. 11 and 12.
[a] Showed some stimulation at highest dose (10 μM).

of some weak PDK1 inhibitors. While introduction of a carboxylic acid group at C-4′ (**2a**) or a methyl group at C-3′ did not affect enzyme potency, compounds with a propionic acid side chain at C-4′ and mono- or cdimethyl substitution at C-3′ (**2c**) and C-5′ (**2d**) were 4- to 6-fold less potent than **2a** which had weak activity in cells.

Amidation of analog **2a** either maintained or reduced potency (**3a**–**3f**) but reduced cellular activity in all cases tried.

In vitro potency of aryl and heteroaryl analogs is shown in Table 2. Phenyl (**7a**) substitution at C-4′ was tolerated as were substituted phenyl compounds. Substituents at



Scheme 2. Reagents and conditions: (i) LDA, toluenesulfonyl chloride, THF, −5 °C (40%); (ii) $ArB(OH)_2$, $Pd(PPh_3)_4$ NaO^tBu, toluene (40–80%); (iii) catalytic piperidine, ethanol, 110 °C (55–85%).

the 3-position of the phenyl ring (**7a**) such as acid (**7c**), methoxy (**7g**), fluoro (**7h**), and methyl (**7i**) afforded similar potency to the unsubstituted phenyl compound. The 3-pyridyl analog (**7b**) was highly potent with good cellular activity (8 nM, and 0.10–1.0 μM, respectively). Amide analogs **7d** and **7e** were also potent PDK1 inhibitors with good cellular activity. The hydroxy and methoxy analogs (**7f** and **7g**) were very potent in cells, but showed high clearance when dosed in rats (data not shown).

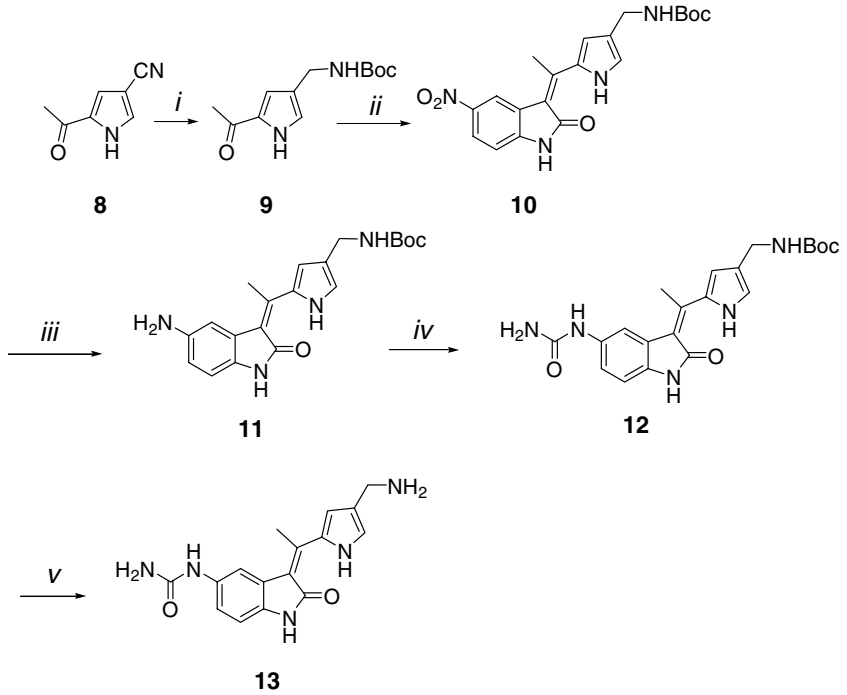
A further strategy to improve solubility was installing an aminomethyl group at the C-4′ of the pyrrole which could serve as a linker to attach water solubilizing groups (Table 3). Although the aminomethyl compound (**13**) and its analogs (**14**–**22**) were all highly potent in the in vitro cAKT assay, the cellular activity of these compounds was lower than that **BX-517** or **7b** and **7d**. Compound **17** displays a solubility similar to **7d**.

An X-ray structure of **7e** bound to PDK1 was obtained during optimization (Fig. 2). The inhibitor forms hydrogen bonds to the backbone C=O of Ser160 (2.8 Å), the NH of Ala162 (2.8 Å) , the C=O of Ala162 (3.0 Å), and the side chains of Lys111 (2.8 Å) and Thr222 (2.8 Å). The hydrogen bonds to Ser160 and Ala162 mimic the interaction of adenosine with the hinge region of PDK1. The hydrogen bond to Thr222 (3.0 Å) and the hydrophobic interaction with Tyr170 (4.0 Å) are sequence specific interactions that augment the selectivity of the compound.

Selectivity of key analogs was assessed against a highly structurally related kinase, protein kinase A (PKA).

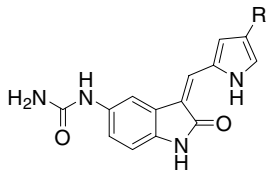
BX-517 displayed good selectivity (320-fold), while compounds **7b**, **7d**, **7e**, and **16** had variable degrees of selectivity against PKA (2-, 78-, 35-, and 21-fold, respectively). These and other data indicated that, among the analogs we tested, **BX-517** was the most selective.

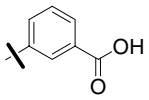
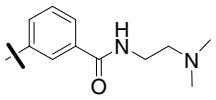
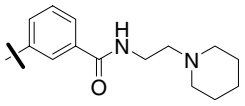
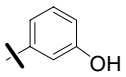
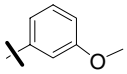
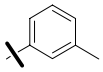
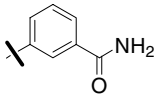
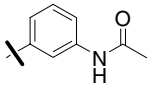
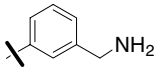
Some of the major issues with further developing **BX-517** as an antitumor agent were its poor pharmaceutical properties including short half-life, low metabolic stability, and poor solubility. As shown in Table 4, selected analogs show longer half-life, better solubility, and



Scheme 3. Reagents and conditions: (i) Pd/C, methanol, $(Boc)_2O$, H_2, (40PSI, 73%); (ii) 5-nitro indolin-2-one, catalytic piperidine, ethanol, 110 °C (20–35%); (iii) $SnCl_4$, pyridine, ethyl acetate, reflux (65%); (iv) $(CH_3)_3SiNCO$, THF, DMF (58%); (v) 4 N HCl in dioxane (80%).

Table 2. Activity of PDK-1 inhibitors



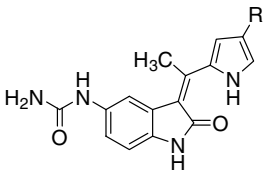
Compound	R	cAKT2 IC_{50} (nM)	P-AKT in PC-3 cells IC_{50} (μM)
BX-201	H	20	3–10
7a		21	ND
7b		8	0.10–1.0
7c		45	>10 (34% inhibition at 10 μM)
7d		4	0.10–1.0
7e		4	1.0
7f		6	0.02–2.0
7g		19	0.01–0.1
7h		51	ND
7i		37	ND
7j		5	>1.0[a]
7k		8	1.0–1.0
7l		3	(65% inhibition at 10 μM)

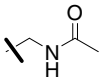
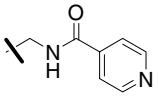
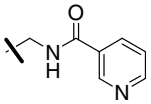
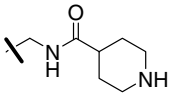
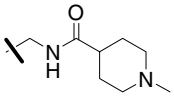
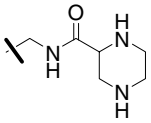
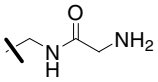
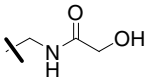
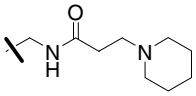
[a] Phosphorylation was stimulated at high doses.

better metabolic stability (except for **7d**) compared to **BX-517**. Plasma clearance and volume of distribution were comparable, however.

Based on their superior overall profile in enzyme and cellular assays and their improved PK properties, we further assessed the in vitro efficacy of compounds **7b** and **7d** by evaluating their effect on MDA-468 breast cancer cell growth in soft agar (transformation-dependent growth) and on tissue culture plastic. Compound **7b** blocked the growth of tumor cells in soft agar with an estimated IC_{50} of 1 μM, while not blocking growth on tissue culture plastic at concentrations below 10 μM. Compound **7d** showed a similar pattern, blocking growth of MDA-468 cells in soft agar with an estimated IC_{50} of 0.3 μM, and not blocking growth on plastic at concentrations below 10 μM. These data are consistent with a significant role of PDK1/Akt signaling in growth and/or survival of tumor cells under certain conditions, such as anchorage-independent growth on soft agar. Whether this activity is sufficient to block tumor progression or

Table 3. Activity of PDK-1 inhibitors



Compound	R	cAKT2 IC_{50} (nM)	P-AKT in PC-3 cells IC_{50} (μM)
BX-517	H	5	0.1–1.0
13		10	ND
14		8	1.0
15		5	(29% inhibition at 10 μM)
16		10	1.0–10.0
17		6	(33% inhibition at 10 μM)
18		4	(28% inhibition at 10 μM)
19		5	No inhibition
20		5	No inhibition[a]
21		10	1.0–0.0
22		3	1.0–10.0

[a] Phosphorylation was stimulated at high doses.

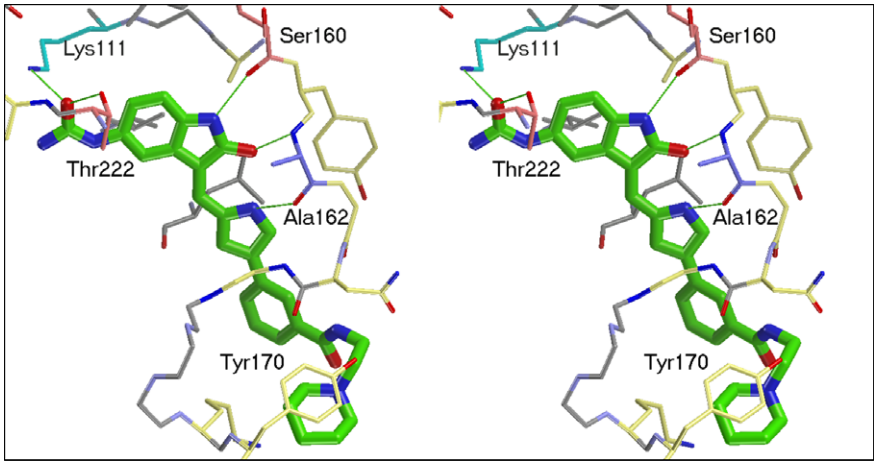


Figure 2. A divergent stereo view of the crystal structure of compound **7e** bound to PDK1, PDB entry 2PE2.

Table 4. PK profile of select PDK-1 inhibitors in rat

Compound	$t_{1/2}$[a] (h)	Cl[a] (mL/min/kg)	Vss[a] L/kg	hLM stability (% remaining 1 h)	Solubility (mg/L in PBS)
BX-517	0.4	30.8	0.9	27	2
7b	1.6	37.1	3.8	82	29
7d	3.7	41.8	3.6	30	13
16	2.0	22.0	0.8	80	nd
17	2.4	8.8	0.8	nd	17

[a] 5 mg/kg iv.

metastasis in animals or humans is a key question for future evaluation.

In summary, we optimized the pharmaceutical properties of **BX-517** by exploring substitution at the C-4′ position of the pyrrole. Carboxylic acids and amides had improved solubility but decreased cellular potency. 3-Pyridyl substitution afforded a compound (**7b**) with improved pharmacokinetic properties. Compound **7d** showed excellent half-life and good solubility but poor in vitro liver microsome stability. Compounds **16** and **17** also had better pharmacokinetic properties compared to **BX-517**. Discovery of these compounds will provide tools for further evaluation of the role of the PDK1/Akt signaling in the treatment of various cancers and the development of new therapeutic agents.

References and notes

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11. *cAKT2 (^{33}P-SPA)*. The coupled assay can detect inhibitors of AKT2 activation, as well as direct inhibition of PDK1 or AKT2. In active AKT2 is activated in situ by incubating with PDK1 and PtdIns-3,4-P2 in presence of compound, ^{33}P-ATP, and a biotinylated peptide substrate for AKT2. The peptide is captured on streptavidin-coated SPA beads for detection.
12. *P-AKT in PC 3-cells*. Inhibition of Akt phosphorylation in cells is measured by incubating compounds with cells, followed by lysis and standard immunoblotting techniques using phosphospecific antibodies to Akt P-Thr 308.