Bioorganic & Medicinal Chemistry Letters 22 (2012) 2880–2884

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

2-Anilino-4-aryl-8*H*-purine derivatives as inhibitors of PDK1

Stéphanie Blanchard *, Chang Kai Soh, Chai Ping Lee, Anders Poulsen, Zahid Bonday, Kay Lin Goh, Kee Chuan Goh, Miah Kiat Goh, Mohammed Khalid Pasha, Haishan Wang, Meredith Williams, Jeanette M. Wood, Kantharaj Ethirajulu, Brian W. Dymock

*S*BIO Pte. Ltd, 1 Science Park Road, #05-09 The Capricorn, Singapore Science Park II, Singapore 117528, Singapore*

ARTICLE INFO

Article history:
Received 6 November 2011
Revised 16 February 2012
Accepted 20 February 2012
Available online 27 February 2012

Keywords:
3-Phosphoinositide-dependent kinase 1 (PDK1)
PI3K
Akt
CDK2
4-Aryl-purine

ABSTRACT

A series of 2-anilino substituted 4-aryl-8*H*-purines were prepared as potent inhibitors of PDK1, a serine-threonine kinase thought to play a role in the PI3K/Akt signaling pathway, a key mediator of cancer cell growth, survival and tumorigenesis. The synthesis, SAR and ADME properties of this series of compounds are discussed culminating in the discovery of compound **6** which possessed sub-micromolar cell proliferation activity and 65% oral bioavailability in mice.

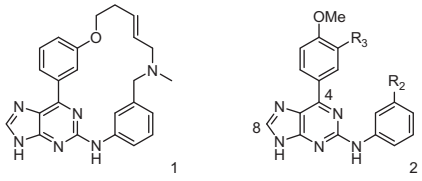
© 2012 Elsevier Ltd. All rights reserved.

Mutations or altered expression of most of the components of the PI3K/Akt pathway have been strongly implicated in oncogenesis. Gene amplification of p110 occurs in some cases of human ovarian cancer,[1] and amplification of Akt is found in ovarian, breast, and colon cancer.[1–3] In addition, activating mutations in p85 have been identified in ovarian and colon cancer.[1,2,4] PDK1 (3-phosphoinositide-dependent kinase 1) is a 63 kDa Ser/Thr kinase over-expressed in the majority of human cancer cell lines and in the tissue specimens of breast, prostate, stomach and pancreatic cancer patients.[5–7] Expression of PDK1 alone was sufficient to transform mouse mammary epithelial cells, a process believed to depend on PDK1-mediated activation of protein kinase C rather than activation of Akt1.[8] PDK1 has been shown to serve as a master regulator of a group of protein kinases known as the AGC kinase super-family which play important roles in the progression of cancer, such as activation of Akt, S6 kinase (S6K, SGK, and PKA), and protein kinase C (PKC). Most importantly PTEN, a phosphatase upstream of PDK1, has been identified as a major tumor suppressor in humans and loss-of-function mutations in the PTEN gene are extremely common among sporadic glioblastomas, melanomas, prostate cancers, and endometrial carcinomas, and a significant percentage of breast tumors, lung cancers, and lymphomas also bear PTEN mutations.[9] Thus, through a variety of mechanisms, a high percentage of human cancers possess activated PI3K signaling.

PDK1 presents an attractive anti-cancer target because it is located downstream of PI3K in the signaling pathway.[10] Thus, PDK1 inhibition, might avoid possible adverse effects resulting from inhibiting PI3K which is upstream of a wide range of signaling cascades.

Considerable effort has been expended in the search for inhibitors of PDK1, most of which target the ATP binding site.[10] Advanced programs have led to new PDK1 inhibitors recently entering the clinic.[11] However these compounds often inhibit many kinases and in many cases are not optimal for oral dosing. We sought to develop a small molecule selective PDK1 inhibitor suitable for oral administration.

Our strategy was to carry out a virtual screen of our in-house chemical libraries and test a subset of hit compounds in an isolated ATPase enzyme assay. In parallel, we set out to screen our fragment library by NMR with follow-up of competitive binders in the ATP-



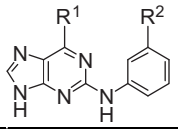
1 2

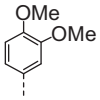
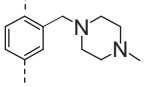
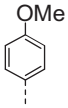
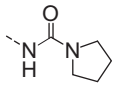
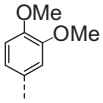
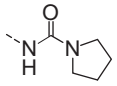
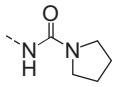
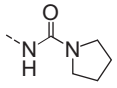
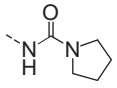
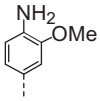
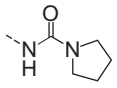
Figure 1. PDK1 hits discovered by focused screening.

* Corresponding author.
E-mail address: stephanie_blanchard@sbio.com (S. Blanchard).

0960-894X/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2012.02.058

Table 1
2-Anilinophenyl and 4-aryl groups of **2** produced via Scheme 1



Compound	R^1	R^2	PDK1 IC_{50} (μM)[15]	CDK2 IC_{50} (μM)[16]	Solubility (μg/ml)*
3		Cl	0.97	>10	N.T.
4		Cl	1.6	>10	N.T.
5		Cl	0.26	>10	N.T.
6			0.15	>10	25
7			0.17	>10	N.T.
8			0.28	>10	23
9			1.2	N.T.	13
10			0.4	>10	29
11			0.41	>10	65

* MTS dilution based turbidimetric assay using DMSO stock solution; N.T. = not tested.

ase assay.[12] From our initial hits we identified several different classes of compounds that were optimized using structural models of the hit structures docked into the PDK1 active site. This focused screening approach resulted in the discovery of compound **1** (Fig. 1) as a potent inhibitor of PDK1 with an IC_{50} of 0.39 μM in our ATPase assay.

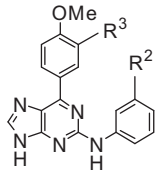
Macrocycle **1** was originally synthesized as part of a cyclin-dependent protein kinase (CDK) inhibitor program where it had been shown to inhibit CDK2 at low micromolar concentrations. In order to identify potentially selective PDK1 inhibitors it was necessary to deconstruct **1** to eliminate the linker and basic centre which were known to be responsible for the unwanted CDK activity. The subsequent hit to lead process was heavily influenced by prior art in this congested area of kinase inhibitors and led to the design of novel 4-aryl purines (**2**), where A, B or D are either carbon or nitrogen (Fig. 1).

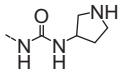
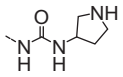
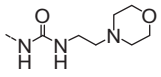
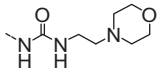
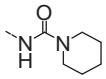
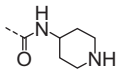
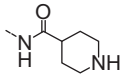
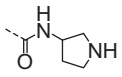
Herein, we disclose our optimization strategy applied to the 4-aryl-purine scaffold leading to potent PDK1 inhibitors with good physicochemical and pharmacological properties and devoid of CDK activity. A counter-screen against CDK2 was introduced at an early stage of our screening cascade to ensure this unwanted activity was not re-introduced.

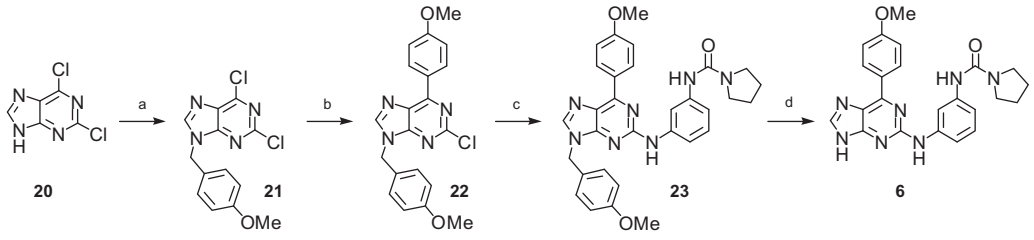
It was clear from initial SAR, and supported by modeling studies, that substitutions on either 9-N or C-8 were not tolerated so attention focused on the 2-position where *meta*-substituted anilines were preferred. Hence the strategy focused on development of 2-anilinophenyl side-chains and parallel optimization of the 4-aryl group (R^1). Introduction of various R^1 groups combined with *meta*-substituted aniline groups was the first major area investigated in this series (Table 1).

Aromatic or heteroaromatic moieties at R^1 conferred novelty and encouraging initial potency and selectivity: dimethoxy **3** with IC_{50} of 0.97 μM was also selective over CDK2, a key objective in our target profile; the bulky basic methylpiperazine (**5**) improved target potency while maintaining selectivity. Structure-based design (see Ref. 15) suggested amides or ureas as R^2 substituents which turned out to be beneficial (compare fivefold potency difference between **7** and **3**): methoxy, and a range of other ether substituents (**6**, **7**, **8** and **11**) gave good potency with IC_{50}s at or below 400 nM. Introduction of polar groups in the *para* position, such as phenol moiety **4** or pyridyl **9**, resulted in a significant reduction in potency. *meta* Substituents on phenyl ring R^1, such as benzyl methyl piperazine **5**, generally gave good potency. Similarly, the methoxy group of compound **11** appears to be responsible for its potency, expected to be lower due to the polar amine group in the *para* position, as discussed above. These promising results indicate that *meta* substituents, including larger basic groups, likely to be more soluble, were tolerated in this region of the active site. In general, this first

Table 2
2-[3-Anilinophenyl]-4-[4-methoxyaryl] analogues produced via Scheme 1



Compound	R^3	R^2	PDK1 IC_{50} (μM)[15]	CDK2 IC_{50} (μM)[16]	Solubility (μg/ml)
12	OMe		0.11	>10	175
13	H		0.046	>10	168
14	OMe		0.084	>10	186
15	H		0.075	>10	148
16	H		0.22	N.T.	N.T.
17	OMe		4.5	N.T.	N.T.
18	OMe		0.079	>10	175
19	OMe		3.3	N.T.	171



Scheme 1. Synthesis of PDK1 inhibitor **6**. Reagents and conditions: (a) 1-Chloromethyl-4-methoxy-benzene, K_2CO_3, DMSO, 80 °C (52% yield); (b) 4-methoxyphenylboronic acid,[1,1′-bis(diphenylphosphino)ferrocene]dichloropalladium(II), complex with dichloromethane, K_2CO_3, dioxane/water (75% yield); (c) pyrrolidine-1-carboxylic acid (3-amino-phenyl)-amide, $Pd_2(dba)_3$, NaOtBu, dioxane (35% yield); (d) TFA (53% yield).

series of compounds showed good initial potency towards PDK1 while achieving excellent selectivity over CDK2. To simplify the SAR in moving forward we chose to fix R^1 as a 3,4-dimethoxyphenyl or 4-methoxyphenyl group. To guide the optimization, modeling studies were carried out using published X-ray structures of PDK1.[13,14]

Purines from Tables 1 and 2 were accessible using a straight forward and high yielding procedure: the synthesis of representative PDK1 inhibitor **6** is outlined in Scheme 1 and its docking to the ATP binding site is illustrated in Fig. 2. Commercially available 2,4-dichloropurine **20** was first protected using 1-chloromethyl-4-methoxy-benzene under basic conditions giving **21** followed by a Suzuki coupling to give 4-aryl-purine intermediate **22**. Reaction with pyrrolidine-1-carboxylic acid (3-amino-phenyl)-amide was performed under Buchwald–Hartwig cross coupling conditions. Final deprotection with trifluoroacetic acid yielded compound **6**.

Modification of R^2 was investigated with a view to increasing potency as well as improving the solubility of the compounds. *meta* Substitution was preferred (SAR not shown). Based on the urea analogues **6** and **7**, which gave good potency against PDK1 as well as in cellular assays (described below) whilst also achieving selectivity over CDK2, but with low solubility, we decided to investigate the introduction of various ureas and amides with appended solubility groups (Table 2).

Docking of compound **6** suggests key hydrogen bonds form from the aminopurine with the hinge region (Tyr161, Ala162) and the urea with Leu88. Addition of hydrogen bond donors (**12** and **13**) or a morpholine group on the urea linker (**14** and **15**) rendered the compounds more soluble and enhanced PDK1 potency with IC_{50}s now below 100 nM. Further studies indicated that the basic nitrogen of the pyrrolidine moiety in **13** may form an additional salt bridge to Glu166 explaining its increased potency.

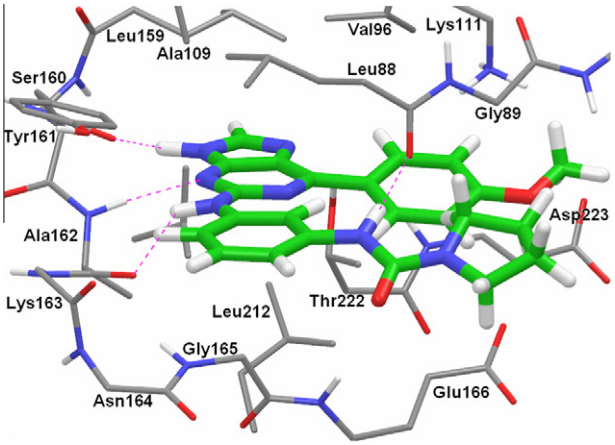


Figure 2. Compound **6** (bold tube with green carbon) docked into the PDK1 ATP-binding site (thin tube with grey carbon). Hydrogen bonds are shown as yellow dashed lines.

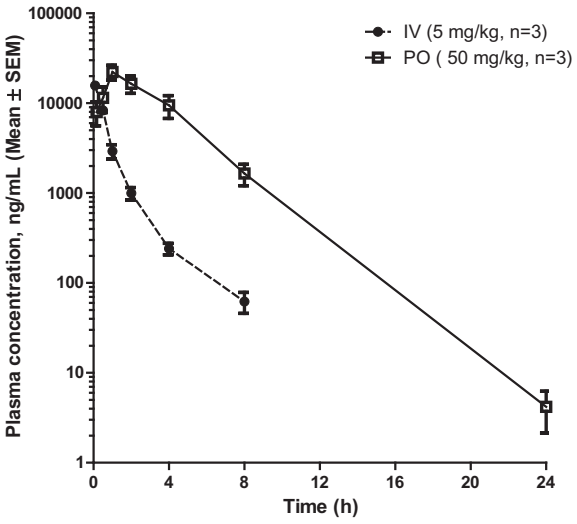


Figure 3. Oral and intravenous pharmacokinetics of compound **6**.

Replacement of the urea linker by an amide (**18**) had little effect on potency whereas reversal of the amide group, as seen in **17** and **19**, results in a dramatic loss of activity for PDK1, probably due to the destruction of the NH—Leu88 H-bond. Comparison of **7** and the ring expanded analogue **16** implies that small steric changes in the urea do not affect the potency. For this second iteration of compounds, although improved potency and solubility were achieved permeability was seriously compromised (data not shown) rendering these compounds useful only as in vitro ligands with negligible utility in cells. In our experience loss of permeability when the number of H-bond donors exceeds three appears to be a particular danger in PDK1 inhibitor design.[11]

Selected compounds were evaluated in cellular proliferation assays, metabolic stability in mouse and human liver microsomes and Caco-2 permeability. All compounds had very good in vitro metabolic stability ($t_{1/2}$) as assessed in human and mouse liver microsomes. Compounds **6** and **18** showed encouraging activity against human tumor cell lines sensitive to the PI3K/Akt pathway, such as the prostate cancer cell line PC3 (Table 3). However an additional hydrogen bond and high polar surface area (128 Å^2) rendered **13** much less active than **6** or **18** in PC3 cells. This was not unexpected from its negligible permeability which serves to explain the poor cellular activity. Although not apparently permeable **18** was still active in PC3 cells perhaps due to active transport, further studies would be required to fully understand this activity. From this data it is clearly preferred to maintain a maximum H-bond donor count of three in these polar molecules and ensure global polar surface area is below 120 Å^2.

Compound **6** was also potent in another prostate cell line, DU145, and in a breast cancer cell line, MDA-MB468, leading us to select it for pharmacokinetic studies in the mouse. The results are represented in Figure 3 and Table 4 and show that **6** has an excellent pharmacokinetic profile in mice. A single oral dose results in rapid exposure with a high C_{max} and good oral bioavailability of 65%.

In conclusion we have designed and synthesized a series of 2-anilino-4-aryl-8*H*-purine derivatives with potent PDK1 in vitro inhibition and a good understanding of the structural elements required for desirable drug-like properties.

Compound **6** represents a promising lead with good ADME properties, cell proliferation inhibition and good oral bioavailability in the mouse. Further studies of PDK1 inhibitors are under investigation to fully elucidate their intracellular mechanism of action.[17]

Table 4
Pharmacokinetic parameters of compound **6**

PK parameters	po (50 mg/kg)	iv (5 mg/kg)
C_{max} (μg/ml)	22	–
T_{max} (h)	1	–
$T_{1/2}$ (h)	1.84	1.48
CL (L/h/kg)	0.63	0.41
Vdz (L/kg)	1.7	0.9
AUC_{0-last} (μg h/ml)	79	12
F %	65	–

References and notes

1. Campbell, I. G.; Russell, S. E.; Choong, D. Y. H.; Montgomery, K. G.; Ciavarella, M. L.; Hooi, C. S. F.; Cristiano, B. E.; Pearson, R. B.; Phillips, W. A. *Cancer Res.* **2004**, *64*, 7678.
2. Levine, D. A.; Bogomolniy, F.; Yee, C. Y.; Lash, A.; Barakat, R. R.; Borgen, P. I.; Boyd, J. *Clin. Cancer Res.* **2005**, *11*, 2875.
3. Vivanco, I.; Sawyers, C. L. *Nat. Rev. Cancer* **2002**, *2*, 489.
4. Courtney, K. D.; Corcoran, R. B.; Engelman, J. A. *J. Clin. Oncol.* **2010**, *28*, 1075.
5. Liang, K.; Lu, Y.; Li, X.; Zeng, X.; Glazer, R. I.; Mills, G. B.; Fan, Z. *Mol. Pharmacol.* **2006**, *70*(3), 1045.
6. Xie, Z.; Yuan, H.; Yin, Y.; Zeng, X.; Bai, R.; Glazer, R. I. *BMC Cancer* **2006**, *6*, 77.
7. Maurer, M.; Su, T.; Saal, L. H.; Koujak, S.; Hopkins, B. D.; Barkley, C. R.; Wu, J.; Nandula, S.; Dutta, B.; Xie, Y.; Chin, Y. R.; Kim, D.-I.; Ferris, J. S.; Gruvberger-Saal, S. K.; Laakso, M.; Wang, X.; Memeo, L.; Rojtman, A.; Matos, T.; Yu, J. S.; Cordon-Cardo, C.; Isola, J.; Terry, M. B.; Toker, A.; Mills, G. B.; Zhao, J. J.; Murty, V. V. V. S.; Hibshoosh, H.; Parsons, R. *Cancer Res.* **2009**, *69*, 6299.
8. Lawlor, M. A.; Mora, A.; Ashby, P. R.; Williams, M. R.; Murray-Tait, V.; Malone, L.; Prescott, A. R.; Lucocq, J. M.; Alessi, D. R. *EMBO J.* **2002**, *21*, 372.
9. Lim, M. A.; Yang, L.; Zheng, Y.; Wu, H.; Dong, L. Q.; Liu, F. *Oncogene* **2004**, *23*, 9348.

Table 3
Cellular, microsomal and physicochemical profile of preferred compounds

Compound	PC3 IC_{50} (μM)	DU145 IC_{50} (μM)	MDA-MB468 IC_{50} (μM)	MLM $t_{1/2}$ (min)	HLM $t_{1/2}$ (min)	P_{APP} ($\times 10^{-6}$ cm/s)	TPSA (Å^2)	H-bond donor count
6	0.58	0.83	1.3	>60	>60	2.07	104	3
13	44	N.T.	N.T.	>60	>60	0	128	5
18	1.07	N.T.	N.T.	>60	N.T.	0	121	4

10. Engelman, J. A. *Nat. Rev. Cancer* **2009**, *9*, 550.
11. Peifer, C.; Alessi, D. R. *ChemMedChem* **1810**, *2008*, 3.
12. Lee, A. C. H.; Murugappan, S. R.; Poulsen, A.; Williams, M.; Blanchard, S.; Ma, D. M.; Bonday, Z.; Goh, K. L.; Goh, K. C.; Goh, M. K.; Wood, J. M.; Dymock, B. W. *Bioorg. Med. Chem. Lett.*, in preparation.
13. All computational chemistry software used is from Schrödinger (http://www.schrodinger.com). The PDK1 and CDK2 X-ray structures (1Z5M and 1AQ1, respectively) were downloaded from the Protein Data Bank (http://www.pdb.org) and prepared with the Protein Preparation Wizard as recommended by Schrodinger. Ligands were prepared with Ligprep and docked into the protein structures using Glide and the XP scoring function with standard settings. PDB entry 1Z5M. Feldman, R. I.; Wu, J. M.; Polokoff, M. A.; Kochanny, M. J.; Dinter, H.; Zhu, D.; Biroc, S. L.; Alicke, B.; Bryant, J.; Yuan, S.; Buckman, B.; Lentz, D.; Ferrer, M.; Whitlow, M.; Adler, M.; Finster, S.; Chang, Z.; Arnaiz, D. O. *J. Biol. Chem.* **2005**, *280*, 19867.
14. Poulsen, A.; Blanchard, S.; Soh, C. K.; Lee, C.; Williams, M.; Wang, H.; Dymock, B. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 305.
15. *PDK1 assay*: The kinase reactions were initiated by adding 25 μL of 2x substrate solution (2 μM biotin–casein, 4 μM ATP, 10 μCi/ml [γ-^{33}P] ATP in 1x assay buffer: 30 mM Hepes pH 7.5, 5 mM $MgCl_2$, 30 mM KCl, 1 mM DTT and 0.2 mg/ml BSA) to 25 μL of 2x enzyme solution (100 nM PDK1 in 1x assay buffer). After 1 h incubation at room temperature, the reaction was terminated with 25 μL of stop buffer (45 mM EDTA, 50 mM ATP, 0.1% Triton-X, 30 mM Hepes pH 7.5) and 60 μL of the stopped reaction was transferred to a 384-well Streptavidin FlashPlate HTS PLUS (Perkin Elmer Cat # SMP410). After minimum 30 min incubation at room temperature, the FlashPlate was washed 3× with 90 μL/well of H_2O/0.02% Tween 20, air-dried and counted on a Wallac MicroBeta (Perkin–Elmer) scintillation counter.
16. CDK2 kinase assay was carried out in 384-well white microtiter plates using the PKLight assay system from Cambrex. For CDK2/Cyclin A assay, the reaction mixture consisted of the following components in 25 μL assay buffer (50 mM Hepes pH 7.5, 10 mM $MgCl_2$, 5 mM $MnCl_2$, 5 mM BGP, 1 mM DTT, 0.1 mM sodium orthovanadate), 1.4 μg/ml of CDK2/Cyclin A complex, 0.5 μM of RbING substrate (Invitrogen, Cat # PV2939) and 0.5 μM of ATP. The reaction was incubated at room temperature for 2 h in each case. Thirteen microliter of PKLight ATP detection reagent was added and the reaction was incubated for 10 min. Luminescence signals were detected on a multi-label plate reader (Victor^2 V 1420, Perkin–Elmer). The analytical software, Prism 4.0 (GraphPad Software Pte Ltd) was used to generate IC_{50} values from the data.
17. Tan, J.; Lee, P. L.; Li, Z.; Jiang, X.; Lim, Y. C.; Hooi, S. C.; Yu, Q. *Cancer cell* **2010**, *18*, 459.