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Thieno[3,2-*d*]pyrimidin-4(3*H*)-one derivatives as PDK1 inhibitors discovered by fragment-based screening

Angeline C.-H. Lee [†], Pondy Murugappan Ramanujulu [†], Anders Poulsen, Meredith Williams, Stéphanie Blanchard, Diana M. Ma, Zahid Bonday, Kay Lin Goh, Kee Chuan Goh, Miah Kiat Goh, Jeanette Wood, Brian W. Dymock *

*S*BIO PTE LTD, 1 Science Park Road, #05-09 The Capricorn, Singapore Science Park II, Singapore 117528, Singapore*

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ABSTRACT

Ligand efficient fragments binding to PDK1 were identified by an NMR fragment-based screening approach. Computational modeling of the fragments bound to the active site led to the design and synthesis of a series of novel 6,7-disubstituted thienopyrimidin-4-one compounds, with low micromolar inhibitory activity against PDK1 in a biochemical enzyme assay.

3-Phosphoinositide dependent protein kinase-1 (PDK1) is crucial for the activation of a series of AGC family kinases and as a result plays an important role in a number of cellular signaling pathways. Significantly, it is involved in the PI3K/Akt pathway disregulation which, either through upregulation or mutation, has been implicated in the development and progression of a range of cancers. Acting downstream of PI3K, PDK1 activates Akt resulting in a range of signaling events linked to cell growth, survival and proliferation. As a result, the search for inhibitors of PDK1 has attracted much attention as potential therapeutic agents for the treatment of cancer as well as other diseases.[1–4]

Fragment-based drug design has become an important and powerful tool for the discovery of new drug leads.[5–9] Fragment screening involves the use of technologies which can detect weak, but specific, binding of low molecular weight (100–300 Da) ligands to a protein. Once the core binding motifs have been identified they may be elaborated in a variety of ways, most commonly by addition of new substituents to improve potency. Due to the weak binding constants of the fragments, conventional screening methods are not always suitable to detect them. Among other methods, NMR spectroscopy has been used broadly to study fragment binding due to its high sensitivity of detection within the affinity range of fragments (100 μM–10 mM).[10,11]

In this case, NMR screening was carried out on a carefully selected focused library of commercially available compounds with a view to identifying suitable fragments that bind to the PDK1 enzyme. This work was carried out as part of a wider program searching for orally available PDK1 inhibitors, for example compound **1** which was moderately potent against PDK1, active in prostate cell line PC3 and 65% orally bioavailable in mice (Fig. 1).[12]

Each screened fragment was selected from commercial and in-house sources with potential to bind to the kinase hinge region. A 500 MHz cryoprobe equipped NMR instrument was used for the primary screen, employing a Water-LOGSY (Water-Ligand observed via Gradient Spectroscopy) pulse sequence.[7] The fragments were screened as mixtures of 6 or 7 compounds in a single NMR experiment. Compound mixtures were selected so that the diagnostic signals did not overlap. In this way, the library could be screened more rapidly, in some cases, even identifying multiple hits within one NMR experiment.

Through a series of Water-LOGSY experiments, we identified several suitable fragments which then underwent a competition screen with the addition of **1**, an ATP competitive ligand. This competition experiment identified fragments which bound to the ATP site of the kinase. These confirmed competitive fragment hits were then tested in a PDK1 ATPase biochemical assay.[13] The 10 preferred hits are shown in Table 1 with ligand efficiency (LE)[14] and IC_{50}

* Corresponding author at present address: National University of Singapore, Singapore. Tel./fax: +65 66012495.
E-mail address: phadbw@nus.edu.sg (B.W. Dymock).
† These authors contributed equally to this work.

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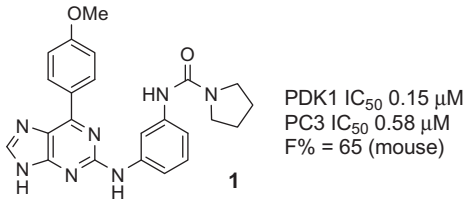
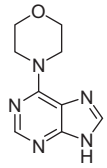
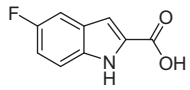
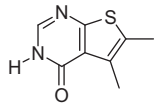
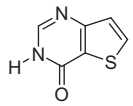
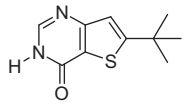
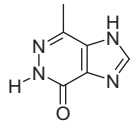
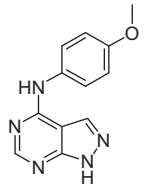
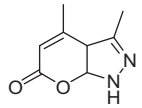
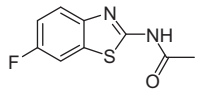
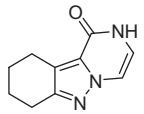


Figure 1. Orally bioavailable purine series.

data. A range of structural types are represented all with LE >0.30 and IC_{50}s in the range 50 μM–1 mM.

Selection of the preferred hits to take forward into synthetic analog studies is a critical decision. Purines **1** and **7** were rejected on the basis of the extensive prior art and anticipated selectivity challenges. Indole carboxylic acid **2** was not favoured for similar reasons as well as a concern that the acid moiety would be a required function. Assessment of fragment binding modes via fragment docking studies[15] contributed to our prioritization decisions. Most compounds had an identifiable donor-acceptor motif which inspired confidence in the postulated binding mode (see Fig. 2). Fused pyrazole lactone **8** has a very good LE but we were unsure of the stability of the lactone and wanted to avoid modification to the core scaffold in the first instance. Benzthiazole **9** has the potential to undergo deacetylation to the potentially toxic amino-thiazole so it was not selected for initial work. To a great extent these selections are based on the quality of the hits, if faced with a number of attractive scaffolds the criteria for selection can be tightened. The remaining hits, thienopyrimidinones, **3**–**5**, pyrazolopyrazinone

Table 1
Competitive fragment hits

Compound	Chemical structure	MW	LE[a]	PDK1 IC_{50} (μM)[13]
1		205	0.38	92
2		179	0.38	297
3		180	0.31	828
4		152	0.42	492
5		208	0.39	55
6		150	0.43	472
7		241	0.34	61
8		166	0.41	302
9		210	0.31	402
10		189	0.36	273

[a] LE = ΔG/N, where *N* = number of heavy (non-hydrogen) atoms, units kcal mol^{-1} per heavy atom.

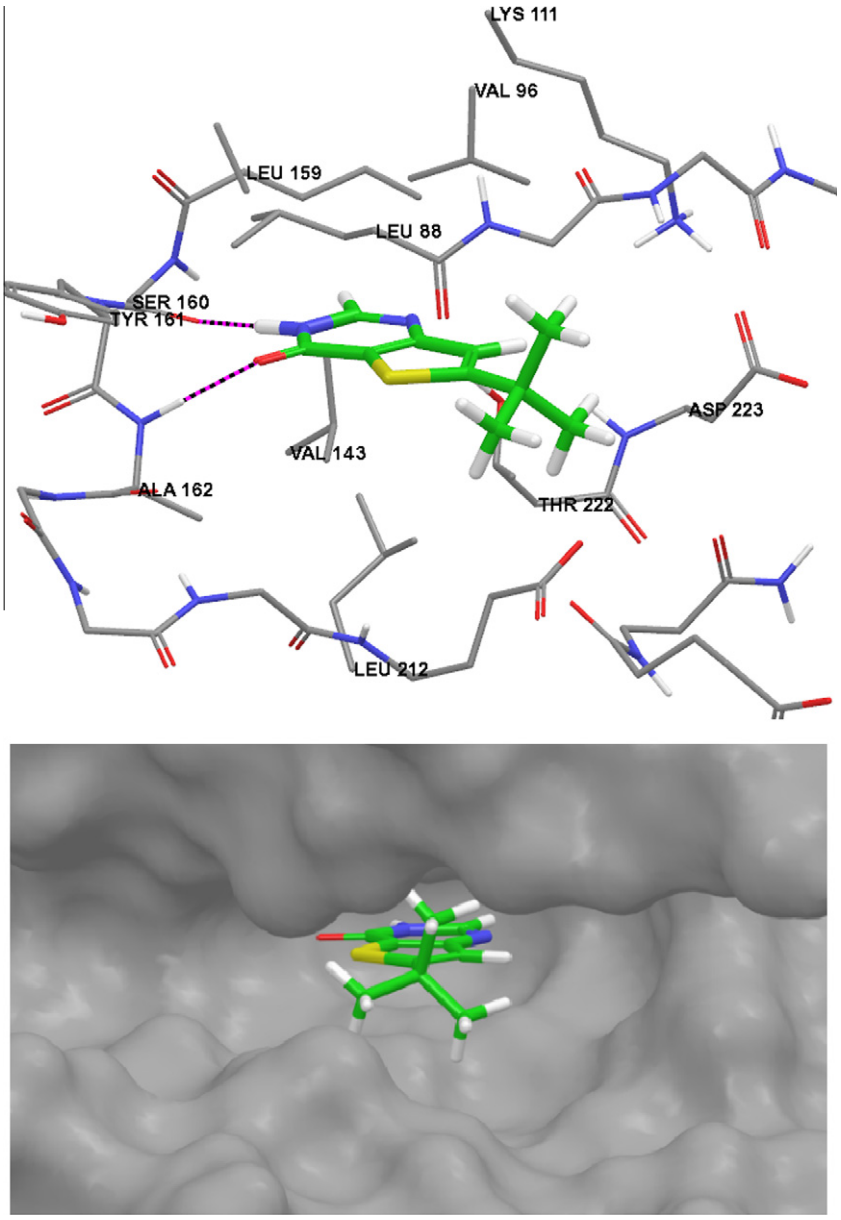


Figure 2. Glide docking of **5**.[15] Top: Fragment **5** (thick tube with green carbon) docked into the PDK1 ATP-binding site (thin tube with gray carbon). Hydrogen bonds are shown as purple/black dashed lines. The aromatic core of the scaffold has hydrophobic contacts to Leu212, Thr222 and Val143 in the bottom of the binding site, Leu159 the gatekeeper residue as well as Leu88, Val96 and Ala109 in the top of the binding site. Bottom: Fragment **5** docked into PDK1 which is shown as a grey surface. There is space for large or bulky substituents on the thiophene carbons (R^1 and R^2, Table 2).

6 and pyrazole **10** had similar binding modes through the pyrimidinone and were all attractive starting points. A balance of structural novelty, synthetic feasibility, potency and high ligand efficiency prompted us to select the thieno[3,2-*d*]pyrimidin-4(3*H*)-ones **4** and **5** as our first-choice start points for medicinal chemistry and we now report their initial evolution to single-digit micromolar leads.

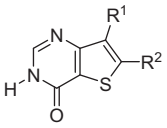
Sparse literature reports increased our confidence that fragments **4** and **5** were quite novel hence more likely to be developed into novel structural types.

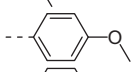
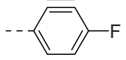
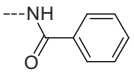
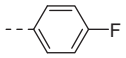
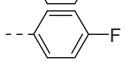
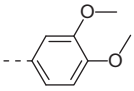
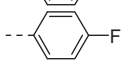
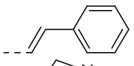
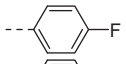
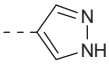
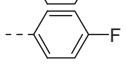
Initial synthetic efforts were focused on adding functionalities to the thiophene carbons. Synthetic targets were selected with the aid of computational docking using the reported X-ray structure of PDK1.[15] With reference to Figure 2, the docking clearly shows that the pyrimidinone motif is forming key hydrogen bonds to the hinge region of the kinase. Opportunities for growth of the compound are greatest from the thiophene carbons. Table 2 shows a selection of mono- and di-substituted derivatives of **4** and **5** prepared on the basis of the binding mode analysis.

The preparation of the core scaffold **4** was carried out in two steps according to the literature.[16] Compounds **14** and **15** are commercially available. Methyl derivative **16** was prepared according to the literature.[16] Nitration of **4** gave **11** (Scheme 1), which, following reduction of the nitro group, gave amine **12** in good yield. Further modifications of this amine, such as reductive amination and acylation, were performed to produce novel analogs **17**, **18** and **19** (Table 2), which exhibited improved activity against PDK1 (IC_{50} = 154, 109 and 95 μM, respectively).

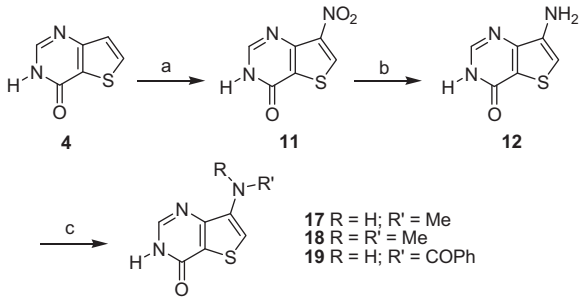
Intermediate **12** can be further derivatized through halogenation to yield **13**,[16] which was subsequently used as a core intermediate for Suzuki coupling with various boronic acids or esters (Scheme 2). Compound **20**, prepared via Suzuki coupling using

Table 2
Synthesised thienopyrimidin-4-one derivatives



Compound	R^1	R^2	MW	cLog*P*[a]	PDK1 IC_{50} (μM)[11]	LE[b]
4	H	H	152	0.12	492	0.42
5	H		208	1.95	55	0.42
14	H		258	2.16	86	0.31
15	H		246	2.37	122	0.32
16	CH_3	H	166	0.62	461	0.42
17		H	181	0.52	154	0.36
18		H	195	0.80	109	0.42
19		H	271	1.40	95	0.29
20	Ph	H	228	2.01	15	0.41
22	CH_3		260	2.56	106	0.30
23	Ph		322	3.65	33	0.27
24			382	3.33	11	0.25
25			348	5.06	39	0.24
26			312	2.00	5.2	0.33

[a] Calculated using ChemDraw Ultra v8.0.
[b] LE = ΔG/N, where *N* = number of heavy (non-hydrogen) atoms, units kcal mol^{-1} per heavy atom.



Reagents and reaction conditions: a) conc. HNO_3, conc. H_2SO_4, 100 °C, 91%. b) Fe powder, NH_4Cl, EtOH, H_2O, 80 °C, 78%. c) HCHO (aq), $Na(OAc)_3BH$, CH_2Cl_2, 31% (**17**) and 55% (**18**); PhCOCl, Et_3N, CH_2Cl_2, 89% (**19**).

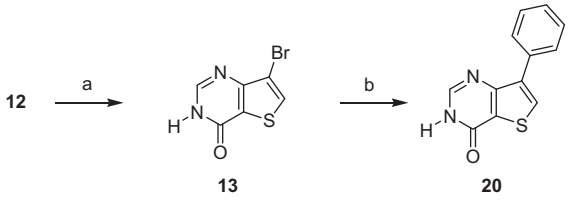
Scheme 1. Preparation of R^1 nitrogen analogs.

phenyl boronic acid, exhibited a marked increase in inhibitory activity against PDK1 with an IC_{50} of 15 μM. On the other hand, fragment hit **5** with a *tert*-butyl group at R^2 also showed good PDK1 activity. Hence, it was logical to next explore functionalization of both positions of the thiophene ring (Table 2).

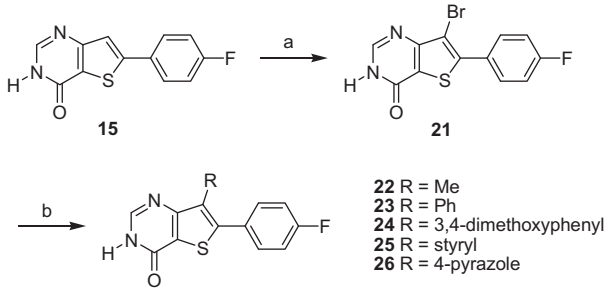
Compound **15** was used as a starting point for accessing di-substituted ring systems. Bromination can be achieved in the presence of bromine and acetic acid at room temperature (Scheme 1). Intermediate **21** was used to generate a series of di-substituted analogs, **22**–**26** (Scheme 2).

Table 2 depicts PDK1 enzyme potency for the key fragments **4** and **5** and synthesized compounds along with their molecular weight, calculated Log*P* and LE. Modification of R^2 with the bulky lipophilic *tert*-butyl in the fragment hit **5** realized nearly an order of magnitude increase in potency with complete retention of LE. Although aromatic ring bearing **14** and **15** were also more potent they suffered from a lower LE due to their increase in MW for modest potency gains. Initial R^1 modifications with small groups or the large benzamide (**16**–**19**) were only slightly more active. However phenyl derivative **20** enjoyed a >30-fold potency increase over **4** while completely maintaining LE. Unfortunately this discovery at R^1 is not additive with R^2: when the phenyl at R^1 is combined with 4-fluorophenyl at R^2 the potency dropped slightly with a concomitant significant drop in LE due to the higher MW. Reducing the size of R^1 back to methyl (**22**) did not provide any benefit and increasing the size of R^1 in combination with R^2 only served to increase MW, cLog*P* and decrease LE. However pyrazole **26** exhibited the best potency of this series with PDK1 IC_{50} of 5.2 μM, acceptable cLog*P* of 2.0, MW 312 and LE of 0.33.

We have shown that elaboration of a low molecular weight fragment resulted in an improved potency from 492 μM in **4** to 5.2 μM in lead compound **26**. Further work will focus on understanding the interplay between R^1 and R^2 while aiming to drive down potency, maintaining LE above 0.3 and assessing enzyme selectivity and cellular potency.



Reagents and reaction conditions: a) i) $NaNO_2$, HBr, 0 °C. ii) CuBr, HBr, 90 °C, 52%. b) Phenylboronic acid, Pd(dppf)Cl_2:CH_2Cl_2, aq. Na_2CO_3, PhMe/MeOH, 100 °C, 63%.



Reagents and reaction conditions: a) Br_2, acetic acid, 89%. b) R-boronic acid, Pd(dppf)Cl_2:CH_2Cl_2, aq. Na_2CO_3, PhMe/MeOH, 100 °C, 56-91%.

Scheme 2. Preparation of R^1 phenyl analog.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.04.080.

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13. *PDK1 assay:* The kinase reactions were initiated by adding 25 μL of 2× substrate solution (2 μM biotin–casein, 4 μM ATP, 10 μCi/ml [γ-33P] ATP in 1× 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 2× enzyme solution (100 nM PDK1 in 1× 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.
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