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Small-Molecule Inhibitors of PDK1

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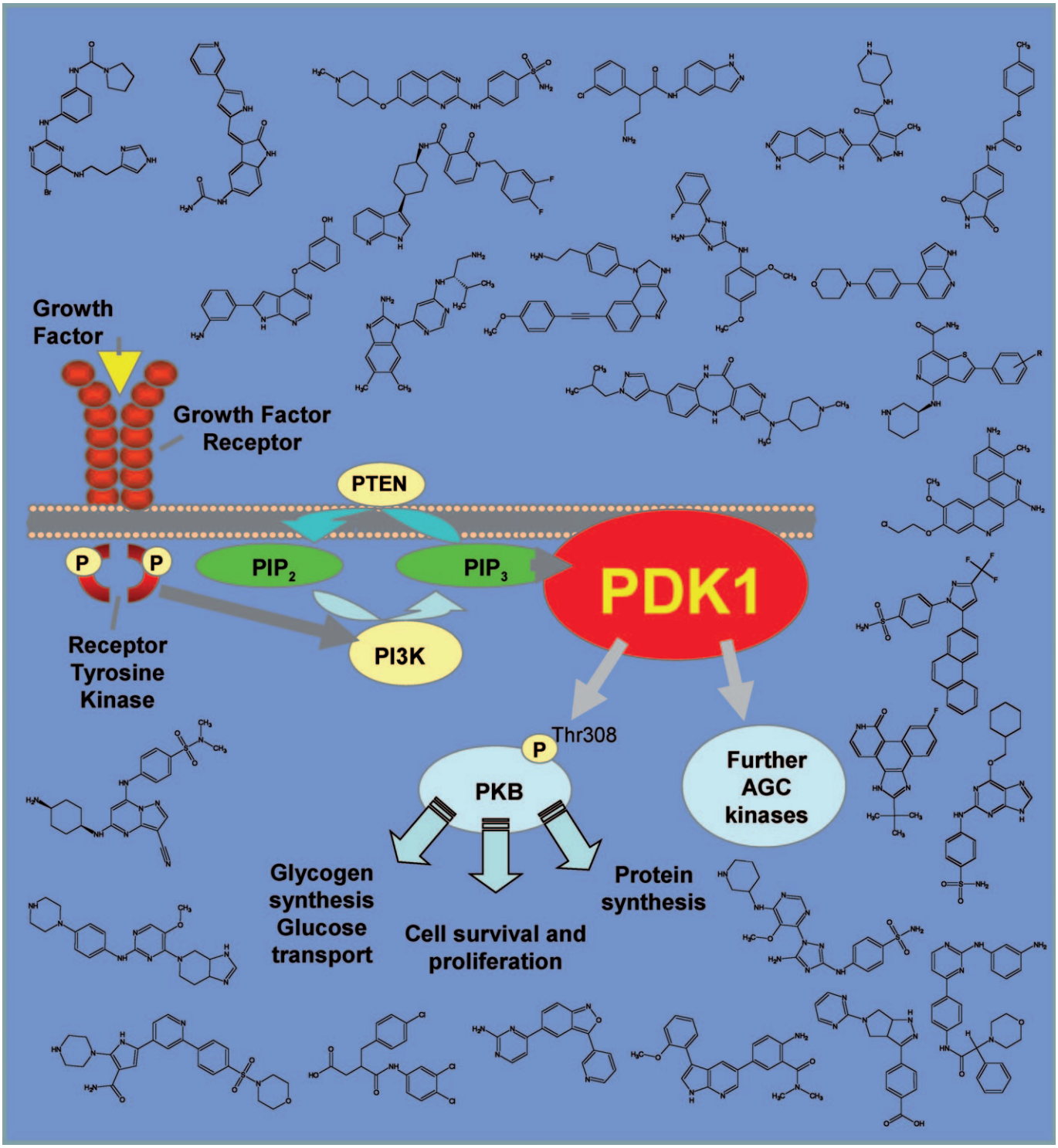

Signal transduction of many growth factors and oncogenes is mediated by 3-phosphoinositide-dependent protein kinase-1 (PDK1), a master regulator of a number of downstream signal protein kinase cascades. Hence, PDK1 represents a convergence point for receptor tyrosine kinase and cytokine-mediated pathways for the regulation of vital cell processes such as cell survival and proliferation. Pathological upregulation of PDK1 signalling due to constitutive growth factor receptor activation and/or PTEN (phosphatase and tensin homologue) mutations significantly triggers downstream signalling, e.g. PKB/Akt, which subsequently promote proliferative events such as tumour invasiveness, angiogenesis, and progression. Consistent with this, a mouse model expressing low levels of PDK1 is protected from tumourigenesis resulting from loss of PTEN. Because more than 50% of all human cancers possess significant overstimulation of the PDK1 signalling pathway, inhibition of this protein kinase by small molecules is predicted to result in effective inhibition of cancer cell proliferation and thus be therapeutically beneficial. Various classes of small-molecule PDK1 inhibitors have been published in patents and papers. Herein we present for the first time a comprehensive collection of small molecules reported to interact with PDK1, and we refer to their biological characterisation in terms of activity and selectivity for PDK1.

Introduction

Protein kinases (PK) comprise 22% of the drugable genome and are currently among the most important targets for drug discovery.[1] PK regulate signal transduction by phosphorylating serine, threonine, and tyrosine residues in key proteins involved in signalling pathways with significant relevance to many diseases such as diabetes and cancer.[2] For cell growth, survival, and tumour angiogenesis, growth factors bind to specific receptors localised at the surface of cell membranes. This activates the phosphoinositide-3 kinase (PI3K), which phosphorylates phosphatidylinositol-4,5-bisphosphate (PtdIns(4,5)P_2, PIP2) to generate the "second messenger" PtdIns(3,4,5)P_3, (PIP3). This factor binds to the PH domain of 3-phosphoinositide-dependent protein kinase-1 (PDK1) and in turn recruits and co-localises the enzyme at the plasma membrane.[3,4] PDK1 plays an important role in insulin and growth factor signalling cascades (Figure 1).[5–8]

Biochemical and genetic studies have shown that PDK1 is present in a single isoform that functions as a master regulator of at least 23 related PK belonging to the AGC kinase family (cAMP-dependent, cGMP-dependent, and PKC). These signal transduction pathways downstream of PDK1 involve a group of related serine/threonine protein kinases such as isoforms of protein kinase B (PKB/Akt), the p70 ribosomal S6 kinase (p70S6K1), the serum- and glucocorticoid-induced protein kinase (SGK), the p90 ribosomal S6 kinase (RSK), and protein kinase C (PKC). These PK mediate the diverse effects that growth factors have on cell proliferation and survival.[7]

Significantly, ~50% of common human tumour types including breast, lung, gastric, prostate, haematological, and ovarian cancers possess mutations in genes that regulate PIP3 production, and this imparts these cancer cells with abnormally high levels of this lipid second messenger.[9,10] As a consequence, PIP3 causes overstimulation of PDK1, and hence constitutive activation of AGC kinases (e.g. PKB/Akt and S6K). This regulation by PDK1 results in multiple effects such as enhanced tumour cell proliferation, reduced apoptosis, and angiogenesis.[11] Conversely, PDK1-mediated downstream signalling of PKB/Akt and S6K cannot be activated by growth factors in mammalian cells or mice that lack PDK1.[12–15] The importance of this pathway in tumourigenesis has been highlighted by the finding that PTEN, the lipid phosphatase that breaks down PIP3 to PIP2 (Figure 1), is frequently mutated in human cancer. It has been demonstrated that cells lacking PTEN (e.g. glioblastoma U87 PTEN$^{-/-}$ cells) possess elevated levels of PIP3, PKB/Akt, and p70S6K1 activity. As a consequence, heterozygous PTEN$^{+/-}$ mice develop a variety of tumours.[16,17] On the other hand, decreasing the expression of PDK1 in PTEN$^{+/-}$ mice significantly protects these animals from developing a wide range of tumours. Therefore, this model mimics mice treated with a powerful PDK1 inhibitor.

In addition, the effects of PDK1 inhibitors on cancer cell growth in vitro and in vivo indicate PDK1 as a valid drug target for clinically effective small-molecule anticancer agents to treat tumours that possess elevated PKB/Akt and S6K activity.[18–20] Furthermore, several X-ray crystal structures of PDK1–ligand complexes have been solved, providing a substantial basis for structure-based inhibitor design. Based on these results, the scientific value and therapeutic potential of powerful PDK1 inhibitors are evident. The number of publications on PDK1 has increased each year since its discovery in 1998, demonstrating the scientific interest in this key PK (Figure 2).

ATP Binding Site of PDK1

Most inhibitors of PDK1 are ATP competitive and target the ATP binding site of PDK1. To date, analysis of 11 small-molecule inhibitor–PDK1 complexes can be found in the Protein Data Bank (PDB), providing substantial structural knowledge in terms of ligand–protein interactions.[21] These data are useful for structure-based approaches and molecular-modelling-aided design of novel compounds. To illustrate the molecular architecture of PDK1 as a drug target, an overview of the ATP–enzyme complex and a closer view into the interactions of LY333531 (**7**)[22] and staurosporine[23] inside the ATP binding pocket of PDK1 are shown in Figure 3.

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Structural analysis of the PDK1–LY333531/staurosporine complexes revealed insight into their particular binding modes, which explain the weak inhibitory activity of maleimide LY333531 ($IC_{50} = 0.75$ μM) relative to the lactam staurosporine ($IC_{50} = 0.006$ μM) towards PDK1. LY333531 displays three hydrogen bonds between the polar groups of the maleimide moieties and the hinge region amino acids of PDK1 (O5···NH Ala162, N6···O Ser160, and O7···OH Thr222). In this case, both the O5···NH Ala162 and N6···O Ser160 H bonds mimic the interactions of ATP with PDK1. In particular, the O7···OH Thr222 H bond causes a shift of the molecule as well as a flip of the PDK1 backbone amino acids Val123/Thr222 resulting in an overall poorer H bond geometry for LY333531 in PDK1, which is not observed in the staurosporine complex with PDK1 (Figure 3). With staurosporine, two key H bond donor/acceptor interactions are formed with Ser160 and Ala162. In contrast to the situation with LY333531, Thr222 is involved in water-mediated interactions to the backbone carbonyl oxygen atom of Val143. Furthermore, a salt bridge is formed from the protonated side chain amine of staurosporine to Glu166. The different binding modes for LY333531 and staurosporine in PDK1 correlate with their biological activity, showing the lactam moiety to be favourable. However, staurosporine is a potent but wildly unselective "pan-kinase" inhibitor,[25] and is therefore unsuitable for therapeutic use. In line with this notion, the specificity and potency of PK inhibitors were considered to be important factors for drug development.[26] Most of the potent PDK1 inhibitors reported to date possess inadequate selectivity for PDK1 or have been assayed only in limited PK panels. Furthermore, translation of their potency obtained in isolated enzyme assays to their cellular activity seems to be difficult in many cases.

Christian Peifer obtained his PhD in 2003 from Prof. Dr. G. Dannhardt in Medicinal Chemistry at the Institute of Pharmacy, Mainz (Germany). He worked on the design, synthesis, analytical characterisation, and biological evaluation of tubulin and VEGFR protein kinase inhibitors as novel antiangiogenic therapeutics. From 2004 to 2008 he worked as scientific assistant in the group of Prof. Dr. S. Laufer at the University of Tübingen. The focus of his research interest is a medicinal chemistry approach for the development and biological characterisation of ATP-competitive kinase inhibitors with optimised selectivity and cellular activity. Christian joined the Alessi research group in March 2008 as a visiting postdoctoral investigator funded by the DFG. In his current project he is working on structure-based optimisation of small-molecule PDK1 inhibitors.

Dario Alessi was born in France, went to school in Brussels, and obtained his BSc in biochemistry at the University of Birmingham (1985–1988). He undertook his PhD project (1988–1991) jointly with Professor Ian Trayer (University of Birmingham, UK) and Dr. David Trentham FRS (National Institute of Medical Research, Mill Hill, London) where he worked on synthesising spin-labelled ATP analogues to study muscle contraction. Between 1991 and 1996 Dario carried out postdoctoral research in the laboratory of Professor Sir Philip Cohen FRS in the MRC Protein Phosphorylation Unit at the University of Dundee. During this period Dario became fascinated by kinases that are regulated by extracellular stimuli such as insulin and growth factors that control all aspects of cell biology. In 1997 Dario was appointed as Programme Leader at the MRC Protein Phosphorylation Unit, where he has since worked on unravelling the molecular details of how protein kinases and other signalling molecules exert their physiological effects. A key focus of Dario's research is to determine ways to exploit the findings of these studies to develop novel treatments for disease.

Small-Molecule Inhibitors of PDK1

ATP-competitive inhibitors of PDK1 that belong to different structural classes possessing highly variable selectivity profiles have been disclosed in papers and patents.

Bisindolylmaleimides, LY333531, LY317615, and UCN-01

Bisindolylmaleimides (BIM, 3,4-di-1*H*-indol-3-yl-1*H*-pyrrole-2,5-dione scaffold **1**, Table 1) have been reported as scaffolds for various ATP-competitive PK inhibitors,[27] among them compounds with activity against PDK1. Complexes of **2**–**5** and **7** with PDK1 showed insight into their particular binding mode in the ATP pocket of PDK1.[22] However, these compounds have been developed primarily as highly potent PKC inhibitors with IC_{50} values in the low nanomolar range,[28] but they have also been shown to block further PK such as MSK1, MAPKAPK1α, S6K1, Chk1, GSK-3β, and AMPK in a panel of 29 kinases.[29]

In 2005 researchers at Eli Lilly & Co. patented compound **6** (LY317615, enzastaurin),[30] which is useful for treating prostate cancer and Akt-mediated diseases. Therein, enzastaurin was characterised as a dual inhibitor of PDK1 ($EC_{50} = 370$ nM) and p70S6K ($EC_{50} < 500$ nM). However, enzastaurin was also reported to be a highly potent PKC inhibitor, and an inhibition profile against 30 kinases showed only modest activity against PDK1 (19% inhibition at 1 μM).[29] Recently, a phase II clinical study was published in which enzastaurin was demonstrated as a PKC inhibitor with therapeutic potential against large B-cell lymphoma.[31] Compared with BIM **2**–**7**, compound **8** (UCN-01) possesses a planar 5*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazol-5-one scaffold (identical to the core of staurosporine) and showed much higher potency against PDK1 ($IC_{50} = 0.006$ μM).[23]

Analysis of the UCN-01–PDK1 complex (Figure 4) relative to BIM–PDK1 complexes revealed, among other things, significantly different poses of the BIM inhibitors, with the maleimide head group rotated and the indole rings tilted in the hydro-

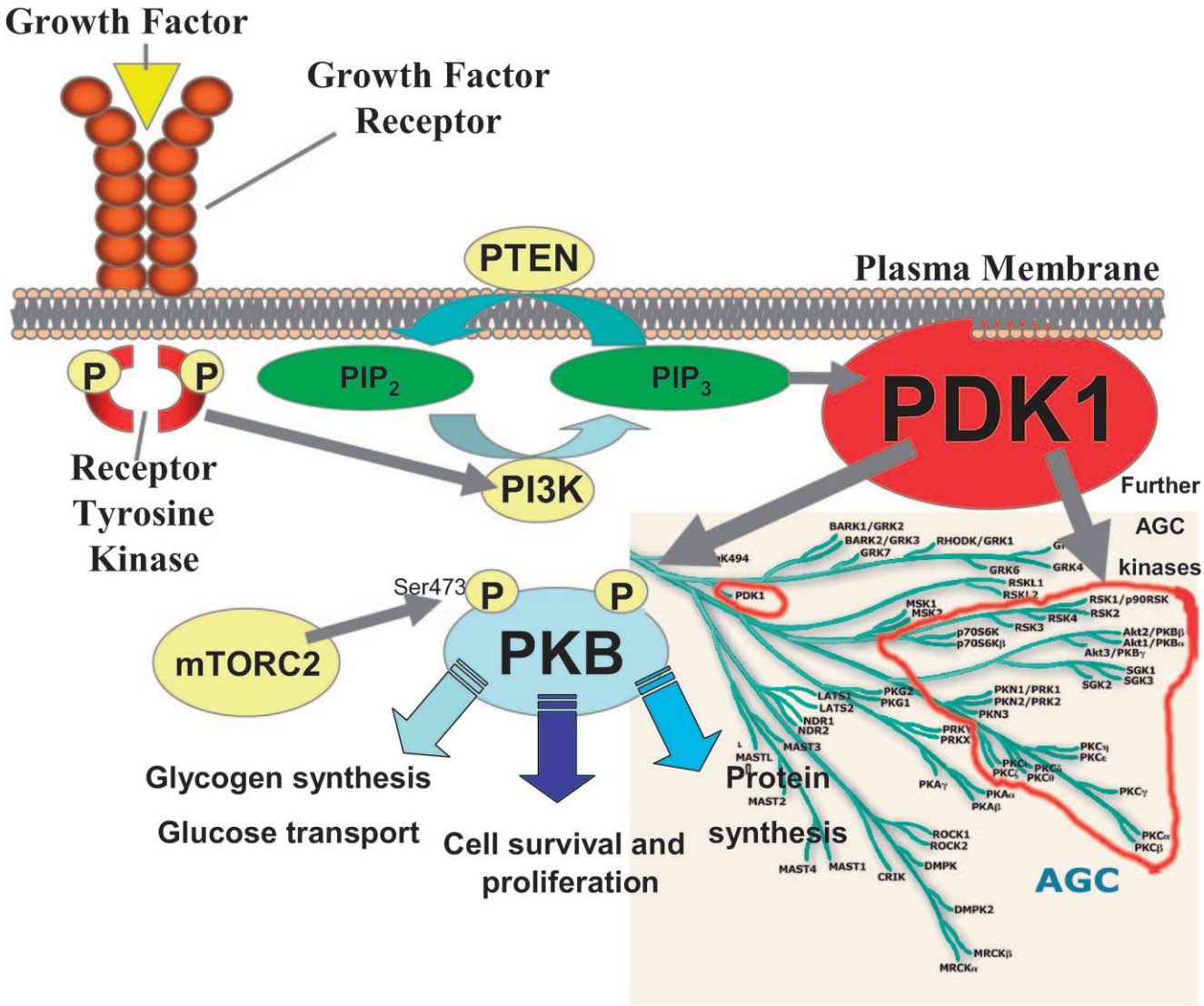


Figure 1. Key role of 3-phosphoinositide-dependent protein kinase-1 (PDK1) in growth factor signalling. PDK1 represents a convergence point for receptor tyrosine kinase and cytokine-mediated pathways for the regulation of vital cell processes such as cell survival and proliferation. Pathological upregulation of PDK1 signalling due to constitutive growth factor receptor activation and/or PTEN mutations results in overstimulation of PKB/Akt and further AGC kinases which subsequently promotes proliferative events such as tumour invasiveness, angiogenesis, and progression. A second critical phosphorylation of PKB/Akt at Ser473 by the mTORC2 complex leads to PKB/Akt activation in cells.

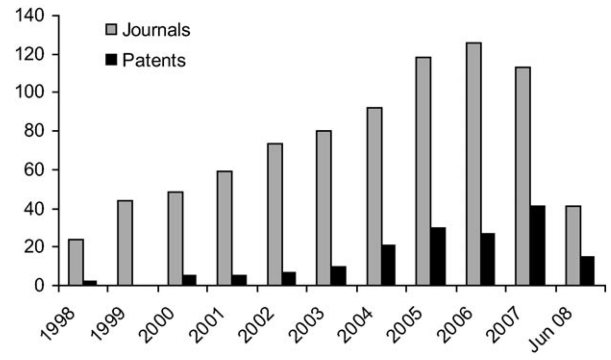


Figure 2. Number of papers and patents published since the discovery of PDK1 in 1998.

phobic pockets (overlay not shown, see also Figure 3). These data suggest a putative favourable energy conformation for planar UCN-01 (and staurosporine) in the ATP binding pocket of PDK1. In line with this notion, a further study comparing ATP binding sites of different PK using a series of conformationally diverse BIM showed that AGC kinases such as PDK1, PKC, MSK1, p70S6K, and MAPKAPK1α were most potently inhibited by BIM that have a compressed conformation.[32]

Substituted thieno[3,2-*c*]pyridine-7-carboxamides

Researchers at AstraZeneca reported in 2006 a series of substituted thieno[3,2-*c*]pyridine-7-carboxamides as PDK1, Chk1, and PAK1/4 inhibitors.[33] Two examples were shown in detail to inhibit PDK1 with sub-micromolar IC_{50} values (Table 2). However, several compounds of this series were demonstrated in the

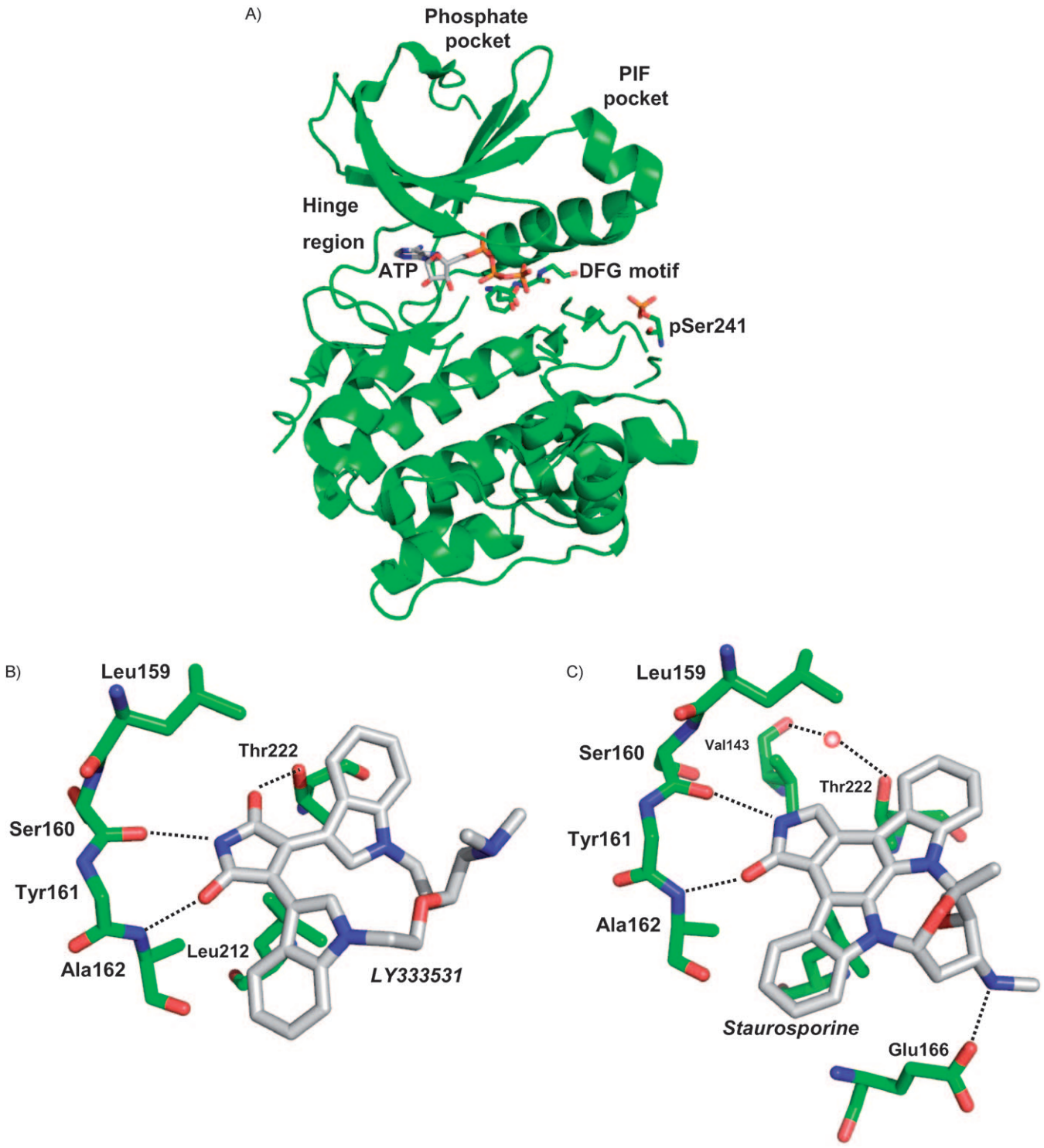


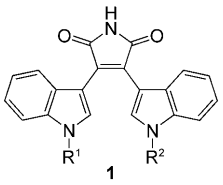
Figure 3. PDK1–ligand structures determined crystallographically. A) Global overview of PDK1 in complex with ATP bound to the hinge region in the catalytic centre with DFG in motif. The PIF pocket and phosphate pocket regulatory domains are shown. PDK1 autophosphorylates at Ser241 in the activation loop.[24] Binding modes of B) LY333531 and C) staurosporine in the ATP binding pocket of PDK1; important protein–ligand interactions are shown.

patent to be low nanomolar Chk1 inhibitors (without PDK1 data). As a measurement of cellular PDK1 inhibition, an assay for T308 phosphorylation on PKB/Akt1 was used, but no detailed results for particular compounds were given.

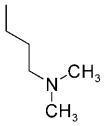
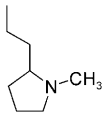
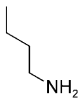
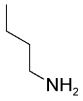
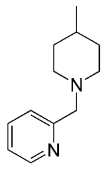
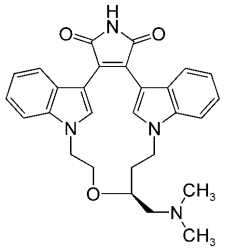
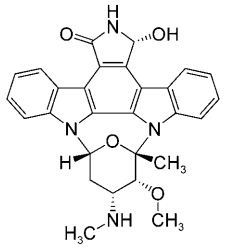
Indolinones

In 2006 researchers at Bayer Schering Pharma AG patented a class of indolinone derivatives as PDK1 inhibitors.[34] These compounds possess the (3*Z*)-3-(1*H*-pyrrol-2-ylmethylene)-1,3-dihydro-2*H*-indol-2-one scaffold (**11**, Table 3), which is also the

Table 1. Examples of BIM derivatives and UCN-01 with biological activity against PDK1.



1

Compound	R^1	R^2	IC_{50} [μM]
2 (BIM-1, GF-109203X)	H		9
3 (BIM-2)	H		14
4 (BIM-3)	H		4
5 (BIM-8, Ro 317549)	Me		1
6 (LY317615, enzastaurin)	Me		0.37[a]
7 (LY333531, ruboxystaurin)			0.75
8 (UCN-01)			0.006

[a] EC_{50} value.

core structure of sunitinib (SU11248), a multi-kinase inhibitor.[35] Unsubstituted core **11** blocked Akt2 activation in the low micromolar range and was initially identified as a lead structure by high-throughput screening using a PDK1-mediated Akt2 activation assay (cAKT2).[36]

Compound **11** was determined to inhibit PDK1 in vitro with an IC_{50} value of 0.52 μM. It also blocked the activation of Akt in tumour cells. In line with the application of **11** as a scaffold in further multi-kinase inhibitors, significant activity against other PK such as VEGFR and CDK4 was found (data not shown). However, **11** was ninefold less potent against the highly homologous PKA (IC_{50} = 16 μM). The optimisation of this lead structure resulted in potent single-digit nanomolar PDK1 inhibitors with selectivity over PKA (e.g. structures **12**–**20**, Table 4). In particular, compound **19** (also referred to as BX-517) was reported to be a highly potent PDK1 inhibitor. BX-517 is 320-fold selective over PKA and 100-fold selective (or better) against a panel of seven Ser/Thr and Tyr kinases (reported as unpublished data in ref. [37]). An X-ray crystallographic analysis of BX-517 in the ATP binding pocket of PDK1 disclosed the binding mode at the molecular level (Figure 5).

Herein the pyrrole–indolinone core is involved in three key H bonds to the hinge region of PDK1. The indolinone nitrogen interacts with the carbonyl group of Ser160, and the indolinone oxygen accepts an H bond from the amide of Ala162. The pyrrole nitrogen addresses an H bond to the carbonyl group of Ala162, although with poor geometry. Notably, this interaction can be formed only by the 3*Z* isomer of BX-517 and not by the 3*E* isomer, showing that the 3*Z* stereochemistry at the double bond of this inhibitor is crucial. The 5-urea group accepts an H bond directly from both the side chains of Lys111 and the hydroxy function of Thr222. In particular, this Thr222 interaction may account for a degree of selectivity for PDK1, as it was also shown to be unique for the binding mode of UCN-01 (**8**, PDB code 1OKZ)[23] in contrast to staurosporine (Figure 3, PDB code 1OKY) in PDK1. Accordingly, a further X-ray crystallographic analysis of compound **20** in the ATP pocket of PDK1 shows the interaction to these residues via the hydroxy group to be indirectly water mediated (not shown, PDB code 1PE0).[36] The greater potency of BX-517 for PDK1 over the closely related PKA may be explained by the bulky gatekeeper Met120 in PKA. This residue is directed into the ATP binding pocket and thereby presumably causes clash-

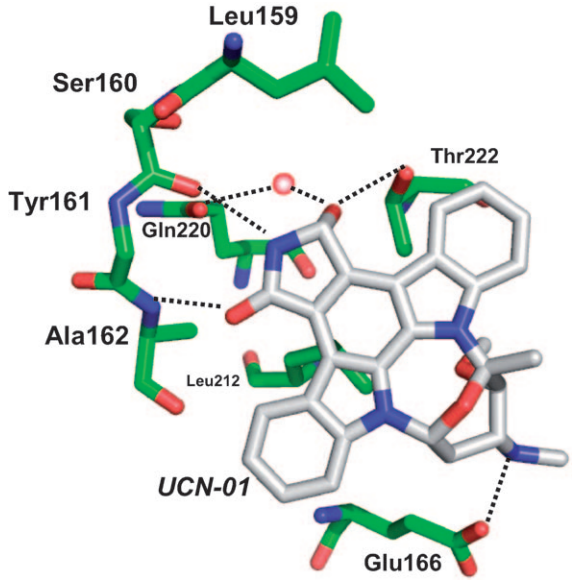


Figure 4. Binding mode of UCN-01 in the ATP binding pocket of PDK1 (PDB code 1OKZ); key residues and H bond interactions are shown.

Table 2. Examples of thieno[3,2-*c*]pyridine-7-carboxamides and their biological activity as PDK1 inhibitors.

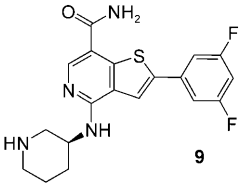
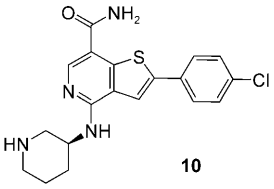
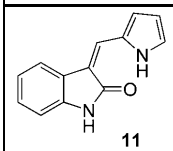
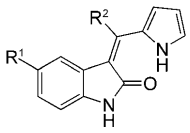
Compound	PK	IC_{50} [μM]
 **9**	PDK1	0.35
	PAK1	0.73
	PAK4	0.14
 **10**	PDK1	0.16
	PAK1	0.60
	PAK4	0.10

Table 3. Biological activity of lead structure (3*Z*)-3-(1*H*-pyrrol-2-ylmethylene)-1,3-dihydro-2*H*-indol-2-one (**11**).

Compound	PK	IC_{50} [μM]
 **11**	PDK1	0.52
	cAkt2	2.3
	PKA	16

es with the urea moiety of the inhibitor, in contrast to the less bulky Leu159 in PDK1 that provides space to accommodate BX-517. In line with this notion, BX-517 showed similar nanomolar activity in the PDK1 assay and in the PDK1-mediated Akt2 activation assay (IC_{50} = 5 nM), and it blocked Akt2 activation in cells with sub-micromolar potency. However, BX-517 has a poor ADME profile that includes a short half-life, low metabolic stability, and poor solubility in aqueous media, thus prohibiting further development.[37]

Table 4. Derivatives of **11** and their biological activity as cAkt2 inhibitors.

	R^1	R^2	IC_{50} [nM]
12	SO_2NH_2	H	290
13	SO_2NH_2	Me	67
14	SO_2NH_2	Et	14
15	SO_2NH_2	Ph	29
16	$NHCONH_2$	H	18
17	$NHCONH_2$	Et	3
18	$NHCONH_2$	4-Pyr	10
19 (BX-517)	$NHCONH_2$	Me	5
20	OH	Me	80

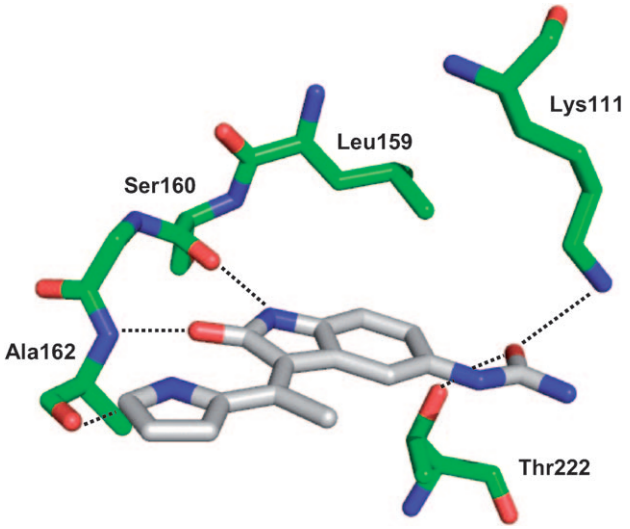
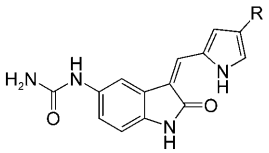


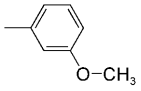
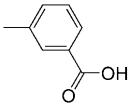
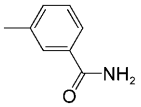
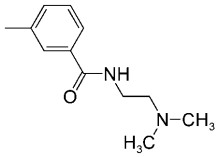
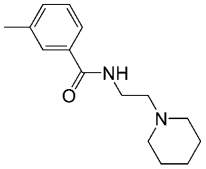
Figure 5. Binding mode of BX-517 in the ATP binding pocket of PDK1 (PDB code 2PE1); key residues and H bond interactions are shown.

The binding mode of BX-517 in PDK1 suggested substitution of the 4′-pyrrole moiety by hydrophilic groups, as the pyrrole moiety is situated in a solvent-accessible site of the ATP binding pocket. This position was therefore used to enhance water solubility and to optimise pharmacokinetic properties. Subsequently, medicinal chemistry efforts led to potent compounds (e.g. **21**–**28**, Table 5) in which **28** showed superior overall profiles in enzyme and cellular assays as well as improved pharmacokinetic properties. However, **28** was only twofold selective for PDK1 over PKA compared with the 320-fold selectivity of lead structure **11**. No further profiling in a protein kinase panel of **28** or related compounds was reported.

The binding mode of **27** has been determined by X-ray crystallographic analysis (Figure 6).[37] Similar to the pose of BX-517, compound **27** forms significant H bonds to Ser160 and Ala162 situated in the hinge region and to the side chains of

Table 5. Examples of second-generation indolinones and their biological activity as Akt2 inhibitors.



	R	cAkt2 IC_{50} [nM]	p-Akt IC_{50} [μM][a]
21	H	20	3–10
22	Ph	21	–
23		19	0.01–0.1
24		45	>10[b]
25		5	>1
26		4	0.1–1.0
27		4	1.0
28		8	0.1–1.0

[a] Determined in PC-3 cells. [b] 34% inhibition at 10 μM.

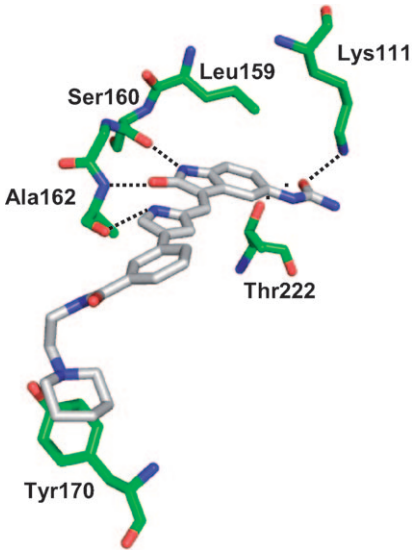


Figure 6. Binding mode of **27** in the ATP binding pocket of PDK1 (PDB code 2PE2); key residues and H bond interactions are shown.

Lys111 and Thr222. Furthermore, a hydrophobic interaction of the piperidine moiety to Tyr170 occurs, which, in combination with the H bond to Thr222, may form PDK1-sequence specific interactions.

Pyridinonyl-PDK1 inhibitors

Researchers at Sunesis Pharmaceuticals/Biogen Idec Inc. recently patented a series of substituted pyridinonyls (1-benzyl-2-oxo-1,2-dihydropyridine-3-carboxamides) as potent PDK1 inhibitors.[38] The concept of these inhibitors typically consists of using a flexible linker to tether 1-(3,4-difluorobenzyl)-2-oxo-1,2-dihydropyridine-3-carboxamide (scaffold **29**) to an H bond donor (HD)/acceptor (HA) moiety that is presumably able to interact with Ala162 and Ser160 of the PDK1 hinge region (Figure 7). While the hinge-binding concept of these inhibitors is in line with conventional ATP-competitive PK inhibitors, the exact role of the pyridinonyl moiety in binding PDK1 remains unclear. Examples of pyridinonyl inhibitors (**30**–**59**)

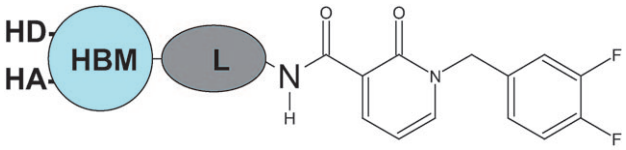
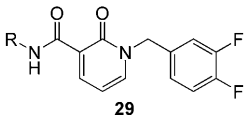


Figure 7. Schematic representation of the concept of pyridinonyl PDK1 inhibitors. Typically 1-(3,4-difluorobenzyl)-2-oxo-1,2-dihydropyridine-3-carboxamide (right) is tethered by a flexible linker (L) to a hinge binding moiety (HBM) bearing vicinal H bond donor (HD) and H bond acceptor (HA) groups. The distance between HD and the pyridinone amide nitrogen atom (highlighted) was reported to be approximately six carbon units (7–8 Å), whereas the distance between HA and the pyridinone amide nitrogen was reported to be eight carbon units (8.5–9.5 Å, excluding HD/HA and amide N, respectively).

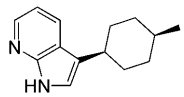
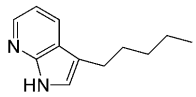
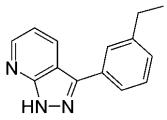
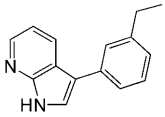
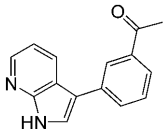
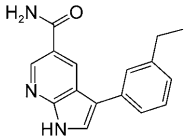
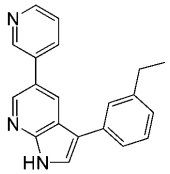
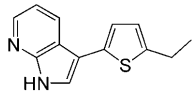
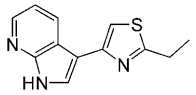
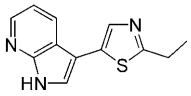
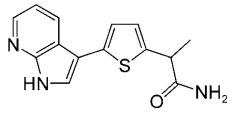
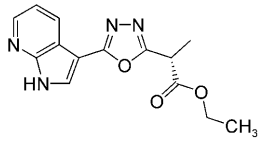
and their biological properties are shown in Table 6. Notably, not all compounds possess HD and HA (e.g. **47** and **48**); these agents are nevertheless potent PDK1 inhibitors.

Interestingly, to determine the biological activity of the compounds in this study, both a phosphorylated and a dephosphorylated PDK1 kinase assay, together with two different cellular techniques for measuring p-Akt (Thr308) phosphorylation were used (Table 6). The potent in vitro p-PDK1 inhibition eli-

Table 6. Examples of 1-(3,4-difluorobenzyl)-2-oxo-1,2-dihydropyridine-3-carboxamides in the inhibition of PDK1 and Akt.



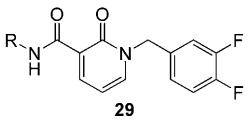
29

Compd	R	IC_{50} [μM] p-PDK1[a]	dp-PDK1[b]	PC-3[c]	Western [%][d]
30		<0.5	<0.5	>5	–
31		<0.5	<0.5	–	–
32		<0.5	–	1–5	–
33		<0.5	–	1–5	–
34		–	<0.5	–	–
35		<0.5	–	<1	–
36		<0.5	–	<1	–
37		<0.5	<0.5	1–5	–
38		<0.5	<0.5	<1	–
39		<0.5	<0.5	–	>75
40		<0.5	<0.5	1–5	–
41		<0.5	–	<1	>75

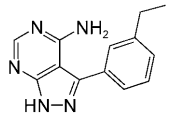
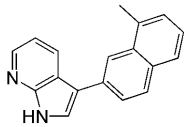
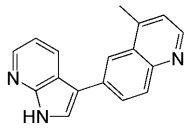
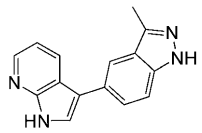
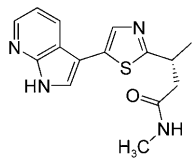
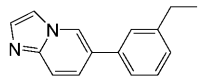
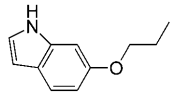
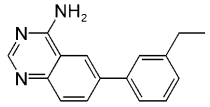
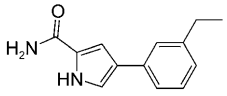
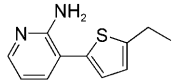
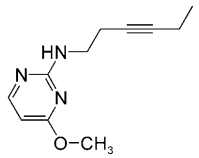
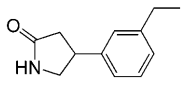
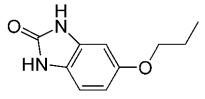
cited by compounds **39**, **41**, **47**, **55**, **56**, and **59** translates into significant decreases in p-Akt (Thr308) phosphorylation in cells detected by Western blotting. However, these compounds show a varied profile in terms of dephosphorylated PDK1 inhibition and p-Akt (Thr308) activity as determined by a phospho-Akt 308 ELISA technique (Meso Scale Discovery, MSD).[39] Notably, highly potent compounds such as **47** possess an HA but not an HD moiety in the hinge-binding part of the molecule. On the other hand, the effective compound **59** has a 4-chlorophenyl substitution instead of the typical 3,4-difluoro pattern in the benzylpyridinonyl moiety, indicating variable SAR for these positions.

It has been reported for targeted compounds that phosphorylation of PKC in the enzyme assay (using PKCtide) was inhibited less than the phosphorylation of Akt (e.g. compound **39**: IC_{50} ratio p-PKCtide/Akt = 71.9). This could result from the compounds affecting Akt substrate binding moieties specifically in PDK1. Because Akt, but not PKC, interacts through the PH domain with PDK1,[40,41] these data suggest that pyridinonyl binding to the enzyme has a significant impact on the PH domain of PDK1 and thus potentially blocks PDK1–Akt binding. A structural analysis of the pyridinonyl ligand–PDK1 interactions would be useful to understand the binding mode at the molecular level. The relative selectivity for Akt is shown in particular for compound **52** (IC_{50} ratio PKCtide/Akt = 51.8) at concentrations of 10 and 30 μM in cellular assays comparing the total blockage of p-Akt (Thr308) phosphorylation against unaffected p-PKCδ (Thr505), p-PKCθ (Thr538), and p-PKCζ (Thr410). In contrast to its potent enzyme inhibition, **52** has an EC_{50} value of

Table 6. (Continued)



29

Compd	R	IC$_{50}$ [μM] p-PDK1[a]	dp-PDK1[b]	PC-3[c]	Western [%][d]
42		<0.5	<0.5	–	–
43		<0.5	–	>5	–
44		<0.5	–	<1	–
45		<0.5	–	1–5	–
46		–	<0.5	–	–
47		<0.5	<0.5	–	>75
48		<10	<10	>5	–
49		<0.5	<0.5	>5	–
50		<0.5	–	1–5	–
51		<0.5	–	1–5	–
52		<0.5	<0.5	–	–
53		<10	<10	–	–
54		<0.5	<0.5	>5	–

>5 μM in cellular assays (Table 6). This may be explained by poor bioavailability of the compound in cellular assays. The relative selectivity to block Akt signalling and simultaneous weaker PKC inhibition may cause fewer side effects and thus result in the clinical benefit of the pyridinonyl compounds. Furthermore, it is remarkable that compounds such as **34** and **46** potently block the unphosphorylated PDK1 but not the phosphorylated, and thus active, form of the enzyme. However, both compounds were ineffective in cellular assays.

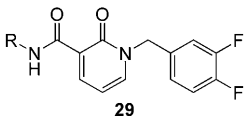
N-Phenylpyrimidin-2-amines

In 2004 researchers at Schering AG patented substituted pyrimidine derivatives as Chk1, PDK1, and Akt inhibitors.[42] From the large number of pyrimidine derivatives shown in the invention, three compounds (BX-320, BX-795, and BX-912) were published in 2005 as novel small-molecule inhibitors of PDK1 (Table 7).[43] These highly potent PDK1 inhibitors share the *N*-(3-{[4-(alkylamino)pyrimidin-2-yl]amino}phenyl)-pyrrolidine-1-carboxamide scaffold (compound **60**).

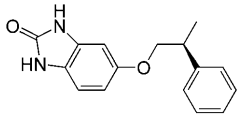
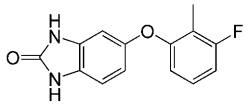
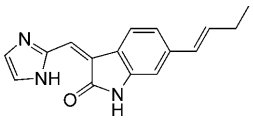
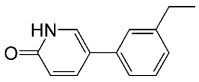
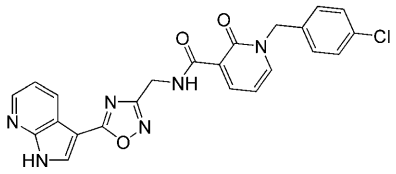
The compounds were initially identified in a coupled assay measuring PDK1- and PIP3-mediated Akt activation, which could detect inhibitors of PDK1, Akt2, or other steps critical for Akt2 activation. It was subsequently found that **61**–**63** were direct ATP-competitive inhibitors of PDK1 and failed to block pre-activated Akt2 activity (IC$_{50}$> 10 μM).

BX-795 (IC$_{50}$=0.3 μM) and BX-320 (IC$_{50}$=1–3 μM) significantly decreased p-Thr308-Akt and p-Thr386-S6K1 levels in, for example, PTEN$^{-/-}$ PC-3 cells (a human prostate carcinoma cell line showing constitutively activated Akt), but no data were reported for BX-912. Interestingly, treat-

Table 6. (Continued)



29

Compd	R	IC_{50} [μM] p-PDK1[a]	dp-PDK1[b]	PC-3[c]	Western [%][d]
55		<0.5	–	<1	>75
56		<0.5	<0.5	–	>75
57		<0.5	–	<1	–
58		<10	<10	–	–
59		<0.5	<0.5	1–5	>75

[a] Inhibition of phosphorylated PDK1. [b] Inhibition of dephosphorylated PDK1. [c] Inhibition of P308 Akt in PC-3 cells using an assay by MSD. [d] Decrease in cellular levels of Akt P308 in PC-3 cells by Western blot analysis.

ment of PC-3 cells with BX compounds also caused a decrease in the levels of p-Ser241-PDK1 autophosphorylation. Selectivity profiles of BX compounds were reported using different kinase panels (such as in Table 8),[26] showing that further kinases were inhibited beyond PDK1.

The structure of BX-320 in the active site of PDK1 was determined (Figure 8).[43] The 2-aminopyrimidine core binds to the hinge residue Ala162 through H bonds, whereas the terminal amide carbonyl oxygen atom of the side chain accepts an H bond with the OH group of Ser94. Although BX-795 (thiophene) and BX-912 (imidazole) possess different functionalities at this position, similar ligand–protein interactions would be plausible with the sulfur in thiophene and sp2-hybridised nitrogen in imidazole, both capable of accepting the H bond from Ser94. Interestingly, thioethers such as in BX-795 have been reported to yield excellent H bond strength, whereas those with carbonyl oxygen (BX-320) or imidazole (BX-912) are slightly weaker. These data correlate with BX-795 having the highest potency (IC_{50} = 11 nM) versus BX-912 (IC_{50} = 26 nM) and BX-320 (IC_{50} = 30 nM) in this series.

4-Heterocycloalkyl-2-aminopyrimidines

Researchers at Boehringer Ingelheim International GmbH recently patented a series of substituted 4-heterocycloalkyl-2-aminopyrimidines (scaffold **64**, Table 9) as potent inhibitors against various PK, particularly against PDK1.[44] Typical examples of this series are shown in Table 10 (derivatives of **65**). The 4-heterocycloalkyl-2-aminopyrimidines were reported to have activity in a recombinant human PDK1 assay in which the majority of compounds had IC_{50} values <0.1 μM, but no specific details were given. The cellular activity of the compounds was determined in the human prostate carcinoma cell line PC-3. In this assay the inhibitors showed EC_{50} values of <5 μM, generally <1 μM.

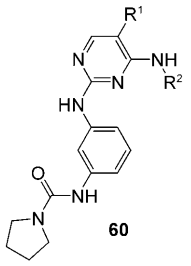
Neither a detailed SAR for PDK1 nor an impact of functionalisation of the 4-heterocycloalkyl-2-aminopyrimidine scaffold **65** on selectivity towards PDK1 is reported. However, in this series the constant 2-aminopyrimidine moiety (Table 9 and Table 10) suggests a vicinal H bond donor (NH)/acceptor (N^1) system interacting with the hinge region, while R^1 and R^3 may address the hydrophobic regions of the ATP pocket. This assumption is based on the binding mode of chemically related **61** (BX-320) in PDK1 (Table 11).[43] The popular 2-aminopyrimidine moiety was reported to be a pharmacophore for frequent kinase hitters.[45] In line with this notion, further PK inhibitors also contain the 2-aminopyrimidine moiety such as the Boehringer Ingelheim compound BI 2536 (pose in ATP binding site of Plk1, PDB code 2RKU), its derivative **66**,[46] **67**,[47] imatinib, and nilotinib (Table 11).[48]

Diazepinones

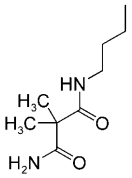
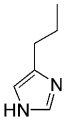
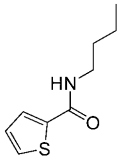
In 2007 researchers at Merck Patent GmbH reported a class of novel diazepinones (2-amino-6,11-dihydro-5*H*-pyrimido[4,5-*b*,1,5]benzodiazepin-5-ones **68**) as PK inhibitors with activity against PDK1, which could be useful for the treatment of cancer, arteriosclerosis, diabetic retinopathy, and inflammation.[49] Examples of this compound series (**69**–**84**) with specified sub-micromolar PDK1 potency are presented in Table 12.

From this study, limited SAR can be deduced based on diazepinone derivatives and their in vitro activity against PDK1. The most potent compound **76** (IC_{50} = 0.11 μM) shows a bulky R^1 residue (1-isobutyl-1*H*-pyrazole-4-yl) at the 8′-substituted position of the 6,11-dihydro-5*H*-pyrimido[4,5-*b*,1,5]benzodiazepin-5-one core. However, at this position relatively small groups also resulted in notable PDK1 inhibition (e.g. -CH_3: **72**

Table 7. Biological activity of BX compounds.



60

Compound	R^1	R^2	IC_{50} [μM] PDK1[a]	Chk1	PC-3
61 (BX-320)	Br		0.030	0.82	1–3
62 (BX-912)	Br		0.026	0.83	–
63 (BX-795)	I		0.011	0.51	0.3

[a] Direct PDK1 assay.

Table 8. Profile of two BX compounds in a panel of nine PK.

Compd	PDK1	MKK1	ERK8	MNK2	Aurora-B	Aurora-C	MARK3	IKKε	TBK1
BX-795[a]	70	50	60	53	83	73	88	79	93
BX-320[b]	66	40	43	28	93	92	64	63	68

[a] Percent inhibition at 0.01 μM. [b] Percent inhibition at 1 μM.

IC_{50} = 0.4 μM; -NH_2: **77** IC_{50} = 0.19 μM). In line with this notion, *N*-dimethyl- (**78** IC_{50} = 0.19 μM) and *N*-urea-substituted derivatives (e.g. **80** IC_{50} = 0.15 μM, **82** IC_{50} = 0.22 μM), or compounds with an amino function at the core 9′-position (**84** IC_{50} = 0.51 μM) inhibit PDK1 in the submicromolar range. This indicates flexible ligand–protein interactions for this site of the molecule. For the series of compounds reported in this study no data of structures bearing any substitution different from hydrogen, either at the ring amide (N^1) or at the ring amine (N^2), are included. Thus it is impossible for those positions of the core to expand on SAR. Furthermore, a possible binding mode of the diazepinone scaffold in the ATP binding pocket of PDK1 may involve the conventional vicinal H bond donor/acceptor system of the exposed N^1 amide (NHC=O) to form classical hinge region interactions. On the other hand, the pair HN^2/N^3 could also function in a similar manner (see also 2-aminopyrimidine compounds in Table 11), although this part of the molecule seems to be sterically more hindered (Figure 9). No kinase selectivity profile or data for cellular activity of diazepinones are given.

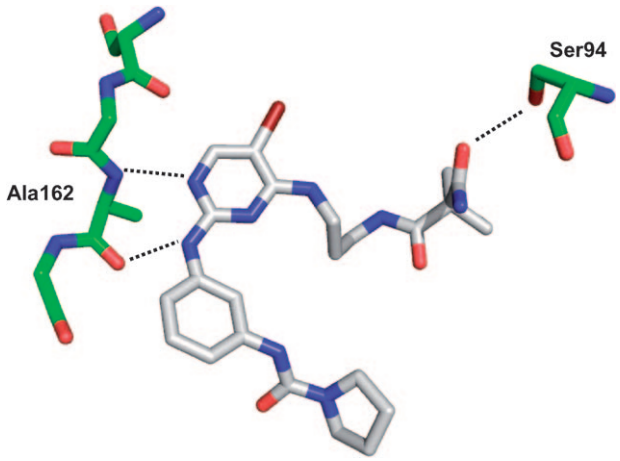
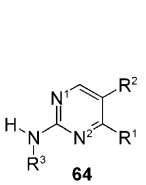


Figure 8. Binding mode of **61** (BX-320) in the ATP binding pocket of PDK1 (PDB code 1Z5M); key residues and H bond interactions are shown.

Tetracyclic Imidazophenanthrenones

In 2007 researchers at Merck & Co. Inc. patented a series of tetracyclic imidazophenanthrenone derivatives (scaffold **85**: 1,6-dihydro-7*H*-benzo[*h*]imidazo[4,5-*f*]isoquinolin-7-ones, Table 13 examples **86**–**94**) as inhibitors of Jak1/2/3, TYK2 (IC_{50} values 0.1 nM–20 μM), and PDK1 (IC_{50} values < 50 μM).[50] The compounds were tested for cellular activity in a CTLL-2 proliferation assay, but no details are reported. The structures in this study are closely related to the pyridone lead **93** reported in 2002 as a low nanomolar ATP-competitive Jak family and TYK2 kinase inhibitor.[51] Compound **93** was tested in a panel of 21

Table 9. Examples of compounds bearing the 4-heterocycloalkyl-2-aminopyrimidine scaffold reported as PDK1 inhibitors.



64

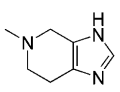
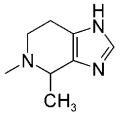
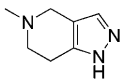
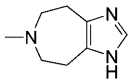
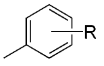
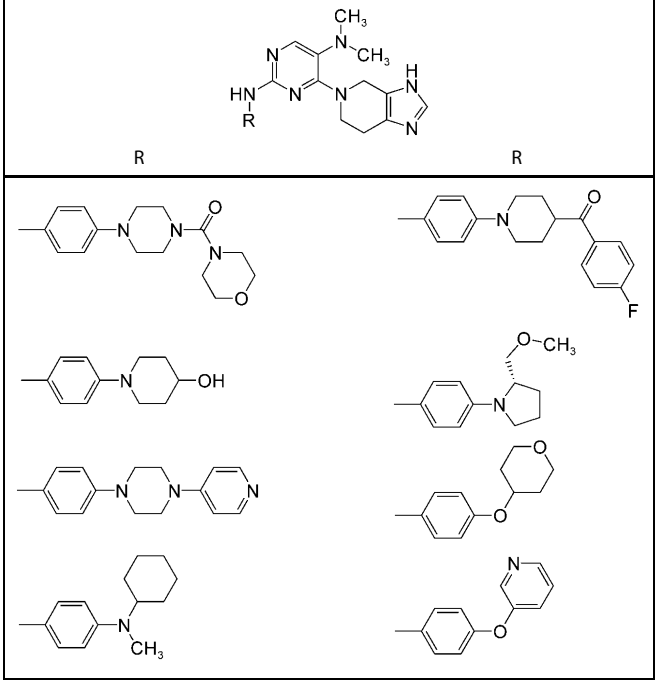
R^1					
R^2	$N(CH_3)_2$	OMe			
R^3					

Table 10. Examples of derivatives of **65** reported as potent PDK1 inhibitors.

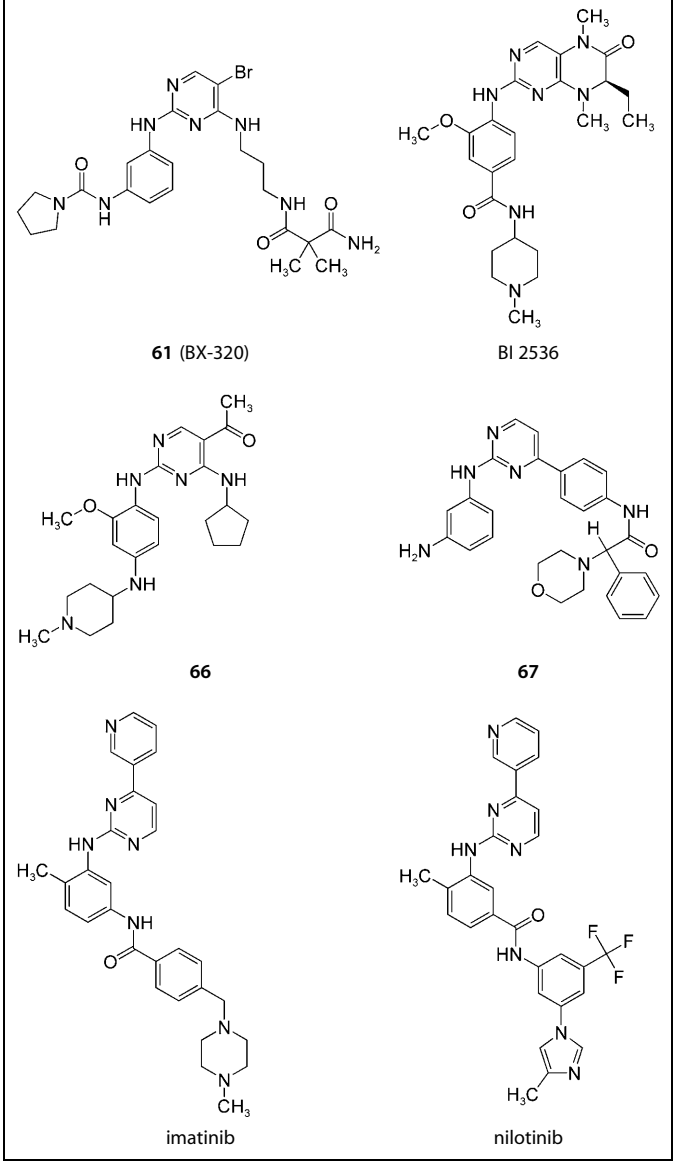


other PK with IC_{50} values ranging from 0.13 μM (Mek) to >10 μM (Raf). However, our in-house data showed **93** to have an IC_{50} value of 200 nM against PDK1. Another compound of this series, **94**, was reported to be a potent Chk1 inhibitor (IC_{50} = 0.11 nM).[52]

Imidazo[4,5-*c*]quinolines

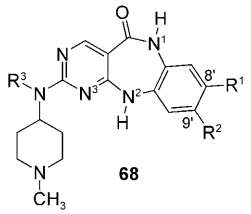
Novartis AG patented in 2005 a class of substituted imidazo-[4,5-*c*]quinolines (**95**, Table 14) as potent PK inhibitors, particularly against PDK1 and PI3K.[53] Further kinases exemplified in this publication were KDR (VEGFR), PDGFR, c-kit, Flt-3, and Flt-4. Recently, in a paper on imidazo[4,5-*c*]quinolines, the authors reported compounds as inhibitors of the PI3K/PKB pathway (representative examples: **96**–**102**; see Table 14).[54] Herein, lead structure **96** is shown to be a potent dual PDK1/PI3K inhibitor while having a strong selectivity profile against other kinases such as PKA and PKB, with 24 and 0% inhibition at 10 μM, respectively. During the course of the medicinal chemistry programme the potentially metabolic weak methylcyano moiety was replaced, leading to compound **100** showing decreased activity against PDK1 but remaining equipotent against PI3K relative to **96**. Further modifications of **96** by changing the imidazole ring to *N*-methylimidazolinone **101** resulted in a complete loss of PDK1 inhibition. Importantly, the compounds were tested in an in vitro PDK1 assay at an ATP concentration of 10 μM. However, **101** retained its activity against PI3K and effectively blocked the cellular activation of PKB. The *N*-methylimidazolinone core can also be found in the dual PI3K/mTOR inhibitor **102** (also referred to as NVP-BEZ235, which recently entered phase I clinical trials).[55]

Table 11. Various protein kinase inhibitors bearing the 2-aminopyrimidine moiety.

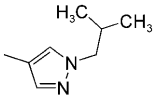
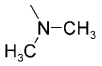
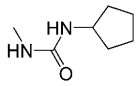
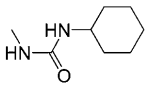
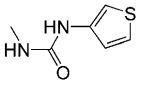
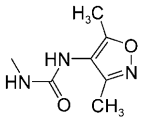


The imidazo[4,5-*c*]quinoline core clearly does not feature an H bond donor capable of interacting with the hinge region. Accordingly, docking studies of **96** in the ATP binding pocket of PDK1 predicted a pose of the compound in which quinoline N^1 (see core structure in Table 14) accepts an H bond from the amide group of Ala162 and thus mimics the original ATP binding at this residue (Figure 10).[54] Furthermore, in the modelled binding mode of **96** the 2′-position of the imidazole ring (bearing R^2) is situated in close proximity (3.4 Å) to the backbone carbonyl group of Leu88 belonging to the P-loop. In agreement with the in vitro PDK1 inhibition, an imidazole 2′-methyl (compounds **97**, **99**) or ethyl (compound **98**) substituent is sterically tolerated, but a carbonyl group (in **101**) results in a strong repulsive interaction.

Table 12. Examples of diazepinone derivatives and their biological activity as PDK1 inhibitors.



68

Compd	R^1	R^2	R^3	IC_{50} [μM][a]
69	OMe	H	Me	0.8
70	OEt	H	Me	0.43
71	OEt	H	H	0.52
72	Me	H	Me	0.4
73	*t*Bu	H	Me	0.4
74	*t*Bu	H	H	0.7
75	Me	H	*i*Pr	6.2
76		H	Me	0.11
77	NH_2	H	Me	0.19
78		H	H	0.19
79		H	Me	0.15
80		H	Me	0.15
81		H	Me	0.16
82		H	Me	0.22
83	Me	Me	H	2.5
84	H	NH_2	Me	0.51

[a] Inhibition of PDK1 in vitro.

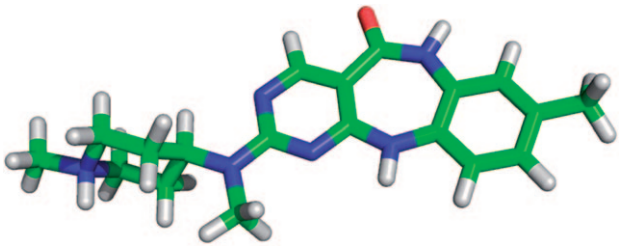
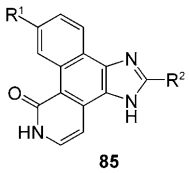


Figure 9. Three-dimensional view of energy-minimised* compound **72** to illustrate the vicinal H bond donor/acceptor system of the exposed amide system which may form hinge region interactions to PDK1. *LigPrep version 2.1, Schrödinger LLC, New York, NY (USA), 2005.

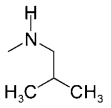
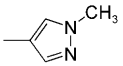
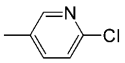
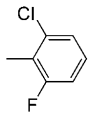
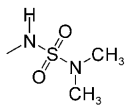
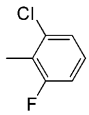
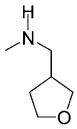
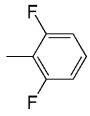
Pyrrole derivatives

Novartis AG patented a series of 4-(1*H*-pyrrol-2-yl)pyridines as inhibitors of PK, in particular MAPKAPK2 and PDK1.[56] Two examples, **103** and **104**, for which biological activity against MAPKAPK2 have been disclosed, are shown in Table 15. However, no further details for PDK1 inhibition can be found.

Table 13. Examples of tetracyclic imidazophenanthrenones reported as PK inhibitors (including PDK1).



85

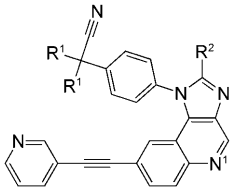
Compd	R^1	R^2
86	Br	
87	Br	Ph
88		
89		
90		
91		
92		
93	F	*t*Bu
94	F	

Quinazolines

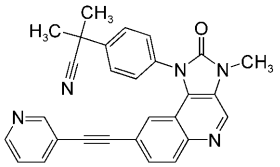
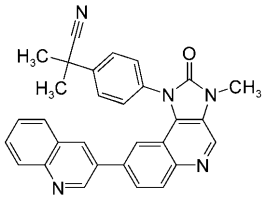
Novartis Vaccines & Diagnostics reported in 2007 on quinazoline derivatives (*N*-arylquinazolin-2-amines, scaffold **105**) as inhibitors of PDK1, CDK1, and CDK2.[57] Biologically active examples **106**–**115** of this compound class with IC_{50} values <5 μM in the PDK1 enzyme assay and EC_{50} values <5 μM in a cell proliferation assay (A2780, PC-3, or PC-3MM cells) are presented in Table 16.

Generally the *N*-arylquinazolin-2-amine scaffold is decorated with a combination of various functionalities including sulfonamides, piperidines, and aromatic moieties. Because no selectivity data concerning detailed IC_{50} values for PDK1 or other kinases were reported, it is not possible to reveal SAR. From a chemical point of view, these structures are closely related to the common concept of substituted 2-aminopyrimidines as PK inhibitors (Table 11). Furthermore, 5-substituted 4-anilinoquinazolines were reported to be potent and selective ErbB-2 receptor tyrosine kinase inhibitors.[58]

Table 14. Examples of imidazo[4,5-*c*]quinolines and their biological activity as PDK1 inhibitors.



95

Compound	R^1	R^2	IC_{50} [nM] PDK1	p-T308-PKB
96	H	H	34	94
97	H	Me	110	77
98	H	Et	327	138
99	Me	H	150	139
100	Me	Me	245	45
101			>25 000	33
102 (NVP-BEZ235)			Dual PI3K/mTOR inhibitor	

through multiple mechanisms such as direct inhibition of PAK ($IC_{50} = 1$ μM).[61] Molecular modelling exercises of **116** in the ATP binding pocket of PDK1 revealed the benzenesulfonamide moiety to be involved in hinge region interactions with Ala162, with the phenanthrene ring situated in a nonpolar region.[59] In contrast, a further docking study reported quite different orientations of this compound in the ATP site of PDK1.[62] However, our research group has not been able to confirm the finding that celecoxib or OSU03012 inhibits PDK1 potently (unpublished observations).

4-Aryl-7-azaindoles

Researchers at OSI Pharmaceuticals reported a series of substituted 4-aryl-7-azaindoles (4-phenyl-1*H*-pyrrolo[2,3-*b*]pyridines, **119**) that inhibit multiple kinases with IC_{50} values <10 μM,

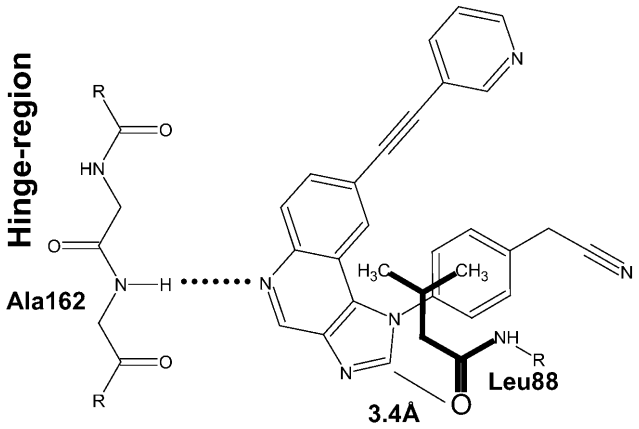
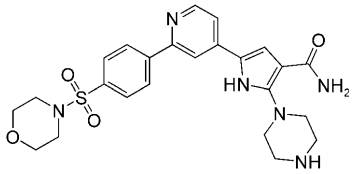
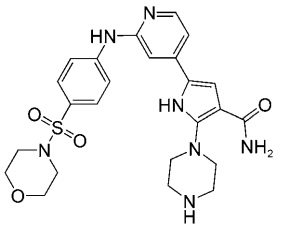


Figure 10. Schematic representation of the modelled binding mode of **96** in the ATP pocket of PDK1.

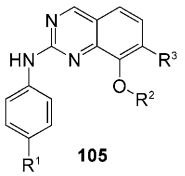
Table 15. Examples of 4-(1*H*-pyrrol-2-yl)pyridines reported as inhibitors of PDK1 and their biological activity against MAPKAPK2.

Compound	Structure	IC_{50} [μM]
103		0.05
104		0.09

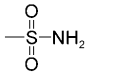
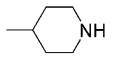
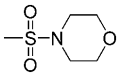
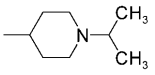
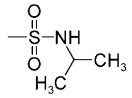
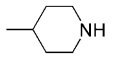
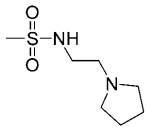
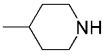
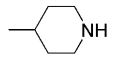
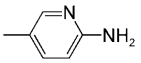
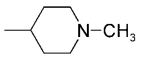
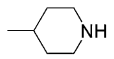
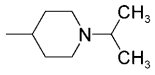
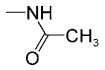
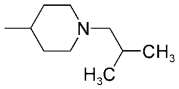
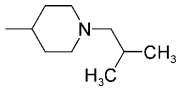
Celecoxib derivatives

Among the COX-2-independent effects of celecoxib are moderate in vitro inhibition of PDK1 and cellular activity in a PC-3 assay (cell death measurement after 24 h exposure, Table 17).[59] Derivatives of celecoxib **116**–**118** have also been shown to inhibit Akt phosphorylation in PC-3 cells in the micromolar range and had downstream effects on BAD, GSK-3β, FoxO1a, p70S6K, and MDM-2. Besides the ability to induce apoptosis, **117** has been shown in further studies[60] to act including the serine/threonine kinases PDK1, Akt, CDK2, IKKb, MEK1, PKN1, PKA, PKC, RSK1, p70S6K, SGK, Aurora-A, and the tyrosine kinases CSF-1R, Ret, KDR, Kit, IGF-1R, Met, EGFR, Alk, and Flt-3.[63] Highly potent inhibition of ROCK (IC_{50}: 5 nM–10 μM) was highlighted. Examples of the 4-aryl-7-azaindoles are shown in Table 18. The design of these compounds includes hinge region interactions by the 7-azaindole core H bond donor/acceptor system. Decoration of this scaffold by

Table 16. Examples of *N*-arylquinazolin-2-amines with IC_{50} values $< 5\ \mu M$ against PDK1.



105

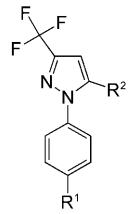
Compd	R^1	R^2	R^3
106			H
107			H
108			H
109			H
110			
111		H	
112			
113			OH
114			H
115			H

various functional moieties provides additional interaction sites. As the ATP site of PK is highly conserved, this concept results in quite undifferentiated inhibition of multiple kinases. Concerning PDK1, no detailed data can be found in terms of enzyme inhibition, selectivity, or cellular activity.

3,5-Diaryl-7-azaindoles

SGX Pharmaceuticals Inc. disclosed 3,5-diaryl-7-azaindole derivatives (3,5-diaryl-1*H*-pyrrolo[2,3-*b*]pyridines, **120**) as kinase inhibitors, and among them some compounds were specified as potent PDK1 inhibitors (**121**–**126** Table 19).[64] Similar to the concept demonstrated for 4-aryl-7-azaindoles (**119**, Table 18) the 7-azaindole scaffold in this series may also address key H bond interactions to the hinge region of PDK1. Furthermore, the most potent compounds **121**–**123** have common H bond donor functional groups at the aromatic systems, indicating

Table 17. Examples of celecoxib derivatives and their biological activity as PDK1 inhibitors.



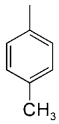
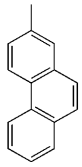
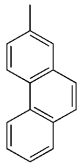
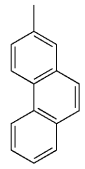
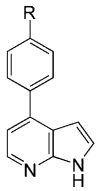
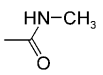
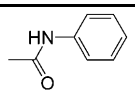
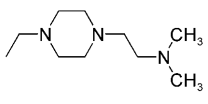
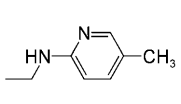
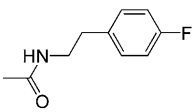
Compound	R^1	R^2	IC_{50} [μM] PDK1	PC-3
celecoxib	SO_2NH_2		48	30
116 (OSU-02067)	SO_2NH_2		9	5
117 (OSU-03012)			5	5
118 (OSU-03013)			2	3

Table 18. Examples of 4-aryl-7-azaindoles as PK inhibitors (including PDK1).

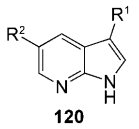


119

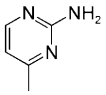
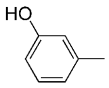
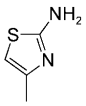
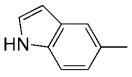
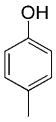
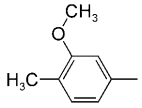
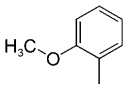
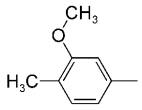
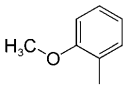
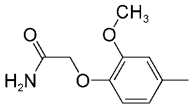
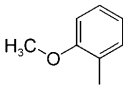
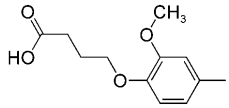
R	R	R
		
		

specific interactions to PDK1. In particular, the 3′-aryl substitution of the 7-azaindole core seems to be critical because a direct comparison between **123** and the less potent **124**

Table 19. Examples of 3,5-diaryl-7-azaindoles reported as PK inhibitors (including PDK1).



120

Compd	R^1	R^2	IC_{50} [μM]
121			<1
122			<1
123			<1
124			1–5
125			1–5
126			1–5

points to a significant ligand–protein interaction of the OH moiety in **123**. Compounds **122** and **126** were reported to further inhibit Aurora-A with an IC_{50} value of <0.5 μM.

Notably, all the PDK1-inhibiting 3,5-diaryl-7-azaindoles in this study (examples presented in Table 19) were also potent inhibitors in an Abl_T315I assay, with IC_{50} values <0.05 μM. In particular, **122** and **123** were shown to be highly active in a cellular Abl_T315I assay ($IC_{50}<0.1$ μM). To illustrate a plausible binding mode of 3,5-diaryl-7-azaindoles in the ATP binding pocket of PDK1, a pose of related compound **127** (PPY-A) in complex with Abl_T315I ($IC_{50}=0.009$ μM, PDB code 2Z60)[65] is shown in Figure 11. However, similar interactions are proposed for the pose of potent 3,5-diaryl-7-azaindoles in PDK1.

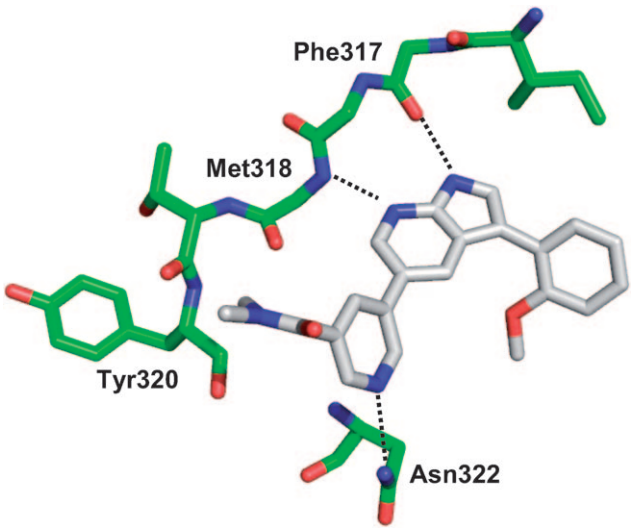
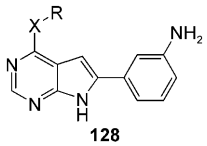
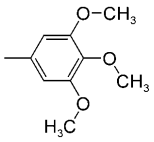
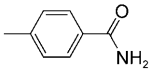
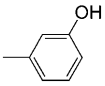


Figure 11. Binding mode of **127** in the ATP binding pocket of Abl_T315I (PDB code 2Z60); key residues and H bond interactions are shown.

Table 20. Examples of pyrrolo[2,3-*d*]pyrimidines as PK inhibitors (including PDK1).



128

Compound	X	R
129	NH	
130	NH	
131	NH	
132	O	

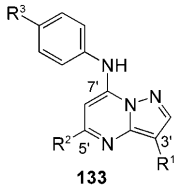
Pyrrolo[2,3-*d*]pyrimidines

In 2004 researchers at the Scripps Research Institute reported an invention of a series of substituted pyrrolo[2,3-*d*]pyrimidines (**128**, Table 20) that inhibit c-Abl, Her-1, Her-2, KDR, Flt-1, Flt-3, Flt-4, c-Raf-1, PDGFR-β, c-Kit, Tek, c-Src, CDK1, FGFR-1, FGFR-2, Fer, MAP3K13, EPHA7, c-Met, and PDK1 at a concentration of 10 μM by >70%.[66] The compounds were further claimed to induce neuronal differentiation in embryonic stem cells. Examples of 6-(3-aminophenyl)-*N*-aryl-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amines **129**–**131** and 3-(4-aryloxy-7*H*-pyrrolo[2,3-*d*]pyrimidin-6-yl)aniline **132** are presented in Table 20. However, these compounds are promiscuous kinase inhibitors and do not specifically target PDK1.

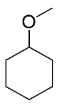
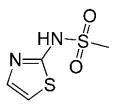
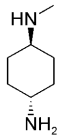
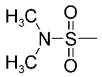
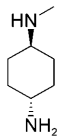
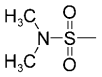
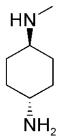
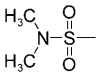
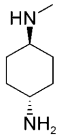
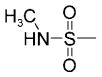
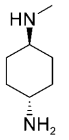
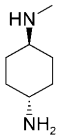
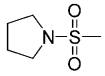
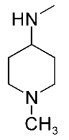
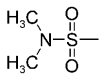
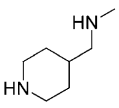
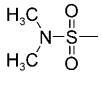
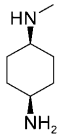
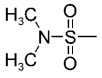
Pyrazolo[1,5-*a*]pyrimidines

In 2004 researchers at Vernalis patented a class of substituted 7-aminopyrazolo[1,5-*a*]pyrimidines (scaffold **133**, Table 21) as PK inhibitors, with potency against CDK2, PDK1, and Chk1.[67] This scaffold originally arose as a hit from an HTS programme and inhibited CDK2 and GSK-3β in the single-digit micromolar range. Thus it was decided to explore SAR for the 3′-, 5′-, and 7′-positions of the pyrazolo[1,5-a]pyrimidine template (see numbering of core **133**, Table 21) for CDK2 inhibition (and inhibition of further kinases). However, those compounds within

Table 21. Examples of 7-aminopyrazolo[1,5-*a*]pyrimidines as PK inhibitors and their biological activity against PDK1, Chk1, and CDK2.



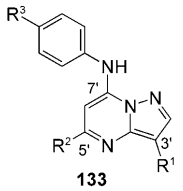
133

Compd	R^1	R^2	R^3	IC_{50} [μM] PDK1	Chk1	CDK2
134	H			24.6	>200	0.59
135	H			3.4	–	0.098
136	Br			0.33	1.27	0.008
137	Et			2.44	2.61	0.07
138	*i*Pr			11	6.6	0.032
139	*i*Pr			4.4	–	0.026
140	*i*Pr			2.0	3.2	0.024
141	*i*Pr			32	19	0.18
142	*i*Pr			2.6	>200	0.68
143	*i*Pr			0.54	2.2	0.98

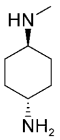
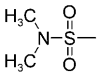
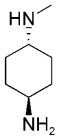
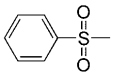
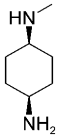
this series notably inhibiting PDK1 share the *N*-phenylpyrazolo[1,5-*a*]pyrimidin-7-amine scaffold **133**.

As can be observed from the SAR of compounds **134**–**146** the PDK1 inhibition seems to be mainly dependent on the 5′-substituent bearing a cyclohexane-1,4-diamine moiety with a terminal unsubstituted amine function. In line with this notion, compounds **134** and **141**, which lack the mentioned structural feature, have diminished activity against PDK1 (and Chk1/CDK2). The most potent compound **144** shows an IC_{50} value of 180 nM against PDK1, while considerable activity is also found against closely related Chk1 (IC_{50} = 1 μM) and CDK2 (IC_{50} = 22 nM). To match possible ligand–protein interactions for PDK1, the binding mode of chemically related *N*-phenylpyrazolo[1,5-*a*]pyrimidin-7-amine in the ATP binding pocket of the AGC kinase CDK2 (**147**: IC_{50} = 0.059 μM) is presented in Figure 12 (PDB code 1Y91).[68] Compound **147** is a stereoisomer of **143** with respect to the stereochemistry of the cyclohexane-1,4-diamine moiety. In the complex of **147** with CDK2, key interactions are formed by the 7-aminopyrazolo-[1,5-*a*]pyrimidine core functioning as an H bond donor/acceptor/donor motif (7-NH, pyrazole-N, and aromatic C2-H, respectively) with the hinge region. Moreover, a significant salt bridge is formed from the primary amine of the ligand to an aspartate residue. Direct comparison of CDK2 inhibition by **147** (IC_{50} = 0.059 μM) with its much weaker stereoisomer **143** (IC_{50} = 0.98 μM) underlies the crucial role of this interaction. However, *N*-phenylpyrazolo[1,5-*a*]pyrimidin-7-amines may bind in a similar way in the ATP pocket of PDK1.

Table 21. (Continued)



133

Compd	R^1	R^2	R^3	IC_{50} [μM] PDK1	Chk1	CDK2
144				0.18	1.03	0.022
145	Br			1.06	0.72	0.016
146	Br			1.20	0.62	0.026

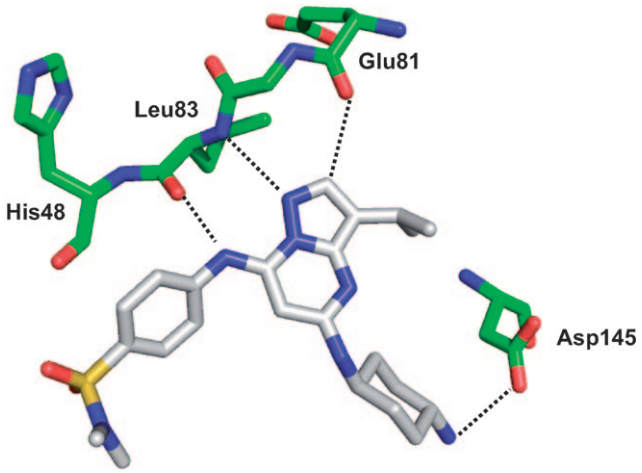


Figure 12. Binding mode of **147** in the ATP binding pocket of CDK2 (PDB code 1Y91); key residues and H bond interactions are shown.

Triazolo[1,5-*a*]pyrimidines

In an additional invention in 2004 Vernalis researchers published a series of triazolo[1,5-*a*]pyrimidines (scaffold **148**, Table 22),[69] which are chemically closely related to the previously reported pyrazolo[1,5-*a*]pyrimidines (**133**, Table 21).[67] Those triazolo[1,5-*a*]pyrimidine compounds share the [1,2,4]triazolo[1,5-*a*]pyrimidin-5-amine scaffold (Table 22) and show notable PDK1 inhibition. Compounds **149**–**151** possess a secondary amine at the 7′-position of the triazolo[1,5-*a*]pyrimidine scaffold (**148**) and therefore may be compared with pyrazolo[1,5-*a*]pyrimidines (**133**, Table 21) in terms of their binding mode in the ATP pocket of PDK1, as presented in Figure 12 for the similar CDK2 inhibitor **147**. In line with this notion, compounds **149**–**151** also inhibit CDK2. Furthermore, compounds **152**–**163** do not possess this NH function and thus lose potency against CDK2. However, compounds **152**–**163** are still active in the low micromolar range against PDK1 and Chk1 (with the exception of **156**), indicating a putative different binding mode in these kinases. A plausible pose may involve the 5′-amine/pyridine nitrogen as an H bond donor/acceptor system in the ATP binding pocket of PDK1, resulting in an inverted orientation of the [1,2,4]triazolo[1,5-*a*]pyrimidin-5-amine core relative to the 7-aminopyrazolo[1,5-*a*]pyrimidine scaffold. Most notably, compound **162** shows significant activity against PDK1 (IC_{50} = 0.25 μM) with 10-fold selectivity over Chk1 (IC_{50} = 3 μM) and 20-fold selectivity over CDK2 (IC_{50} = 48 μM).

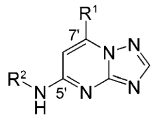
Pyrazolylbenzimidazoles

In 2006 Vernalis reported a series of pyrazole-substituted benzimidazoles (scaffold **165**, Table 23) as PDK1 and Chk1 kinase inhibitors.[70] Whereas the previous Vernalis 7-aminopyrazolo-[1,5-*a*]pyrimidines (**133**, Table 21)[67] mainly target CDK2 and the triazolo[1,5-*a*]pyrimidines (**148**, Table 22)[69] block PDK1 in the micromolar range, the pyrazolylbenzimidazoles of this series show PDK1 inhibition with IC_{50} values in the medium-to-low nanomolar range, with remarkable selectivity over CDK2 (micromolar IC_{50} values). Examples of the highly potent compounds **166**–**181** and their detailed biological activity against PDK1, Chk1, as well as their related kinases Akt-1, PKA, and CDK2 are shown in Table 23. The concept of these PDK1 inhibitors consists of the 5-methyl-1*H*-pyrazole-4-carboxamide core **165**, which is 3′-substituted by a benzimidazole moiety. The 4′-carboxamide side chain bears an *N*-piperidine moiety. The pyrazole core may bind to the hinge region, as is the case for another pyrazole (compound **164**) in complex with Akt2 (Figure 13).[71]

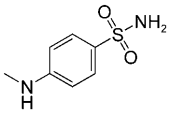
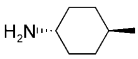
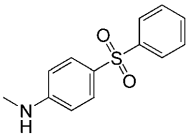
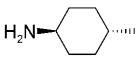
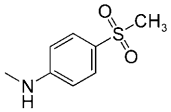
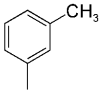
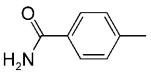
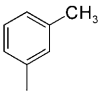
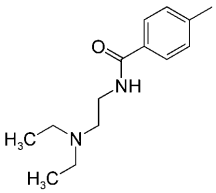
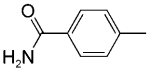
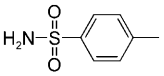
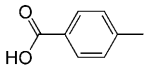
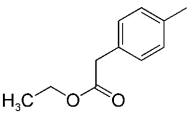
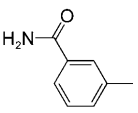
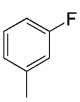
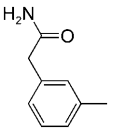
The pyrazolylbenzimidazoles in this study show an almost parallel inhibition of PDK1 and closely related Chk1. The elementary compound **166** (3-(1*H*-benzimidazol-2-yl)-5-methyl-*N*-piperidin-4-yl-1*H*-pyrazole-4-carboxamide) was found to have an IC_{50} value of ~100 nM for both PDK1 and Chk1, with selectivity over Akt-1 (170-fold), PKA (73-fold), and CDK2 (33-fold). More potency was gained from further substitution of the ben-

zimidazole moiety while also addressing the issue of selectivity. Thus, compound **172** has an IC_{50} value of 10 nM against PDK1 and 6 nM for Chk1, while it is quite selective over Akt-1 (2700-fold), PKA (720-fold), and CDK2 (510-fold). Uniformly for the substitution of the core, optimisation towards activity against PDK1 and Chk1 goes almost parallel in this compound series. Interestingly, compound **178** is a single-digit nanomolar Chk1 inhibitor ($IC_{50} = 2$ nM) but is less potent against PDK1 ($IC_{50} =$ 70 nM). It is not reported, however, how the high in vitro potency of these compounds translates into cellular activity.

Table 22. Examples of [1,2,4]triazolo[1,5-*a*]pyrimidin-5-amines and their biological activity as PDK1, Chk1, and CDK2 inhibitors.



148

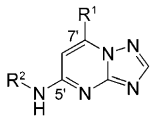
Compd	R^1	R^2	IC_{50} [μM] PDK1	Chk1	CDK2
149			43	93	0.27
150			14	2.2	0.26
151			3.6	59	10.9
152			4.1	15	>200
153			6.1	5.5	>200
154			5.1	9.8	98
155			6.3	1.5	116
156			36	>200	>200
157			2.3	8.8	>200
158			8.1	23.2	>200
159			1.1	3.8	>200

Indazoles

In 2003 Vertex Pharmaceuticals patented a class of indazole derivatives as PK inhibitors, and particular compounds among them bear an *N*-1*H*-indazol-5-aminoacyl core **183** with activity against PDK1.[72] The indazoles are closely related to pyrazolylbenzimidazoles (Table 23)[70] and pyrazoles such as **164** (Figure 13),[71] because these scaffolds share the pyrazole moiety as a key pharmacophore for interacting with the hinge region. Notably, indazoles can be present in two possible tautomeric forms, of which the tautomer shown in **183** is favoured for this series. However, the indazole moiety in closely related compound **182** has also been reported to be a pharmacophore for frequent hitters of kinases.[45]

Examples of indazole inhibitors **184**–**199** with biological activity against PDK1, ROCK, and PKA are presented in Table 24. The PDK1-inhibiting indazoles show the *N*-1*H*-indazole core decorated at the 3′- and 5′-positions. However, SAR cannot be properly stated, as no detailed IC_{50} values were reported. It is remarkable that compounds **184**–**199** are potent dual inhibitors of PDK1 and PKA, whereas compounds **187**–**190** simultaneously show potency against ROCK. The activity against PDK1 furthermore seems to correlate with the presence of a basic primary amine in the side chain of R^2 (Table 24). In line with this

Table 22. (Continued)



148

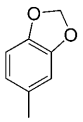
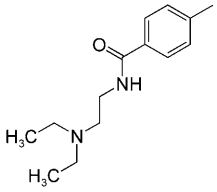
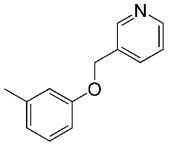
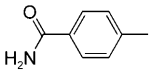
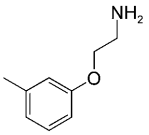
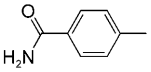
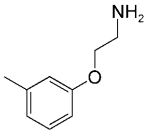
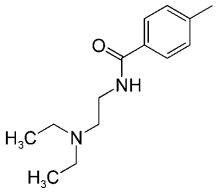
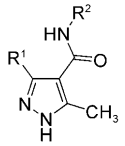
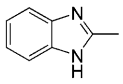
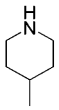
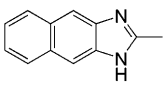
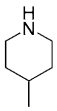
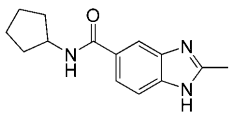
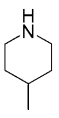
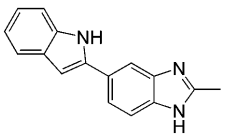
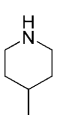
Compd	R^1	R^2	IC_{50} [μM] PDK1	Chk1	CDK2
160			1.8	1.0	> 200
161			1.7	9.9	23.1
162			0.25	3.0	48
163			0.81	1.4	7.9

Table 23. Examples of pyrazole-substituted benzimidazoles and their biological activity as PDK1, Chk1, and Akt-1 inhibitors.



165

Compd	R^1	R^2	IC_{50} [μM] PDK1	Chk1	Akt-1
166			0.11	0.1	17
167			0.06	0.01	> 50
168			0.07	0.043	29
169			0.02	0.012	5.8

notion, **199** lacking the amino function, shows diminished activity against PDK1, ROCK, and PKA. In this study no cell-based activities of indazoles were reported.

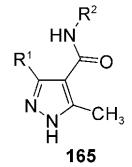
Further PDK1 scaffolds by Vertex

In 2003 Vertex patented various scaffolds as kinase inhibitors (Table 25) with activity against Akt and PDK1, among them pyrazolopyrrole derivatives (such as **200**).[73] Additionally, diaminotriazoles (e.g. **201**)[74] and pyrimidine compounds (e.g. **67**)[47] were claimed as inhibitors of PK such as PDK1. Soon thereafter Vertex also reported benzisoxazoles (e.g. **202**)[75] and phthalimide compounds (e.g. **203**)[76] as unspecific kinase inhibitors but namely including PDK1. In 2006 Vertex patented diaminotriazoles (e.g. **204**)[77] as PK inhibitors, among them compounds with IC_{50} values < 100 nM against PDK1. Furthermore, Vertex reported in 2007 that benzimidazoles **205** can act as inhibitors of Aurora, Flt-3, and PDK1.[78] No particular inhibition data concerning PDK1 was specified for these compound classes. However, structure **205** was reported to be an Aurora-B and Flt-3 inhibitor with an IC_{50} value < 1 μM,[78] and similar compounds have been developed as JAK3 inhibitors.[79]

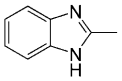
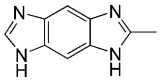
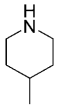
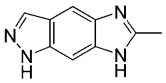
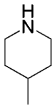
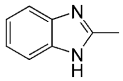
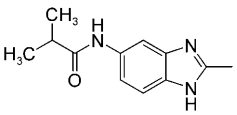
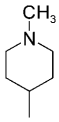
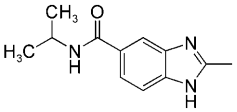
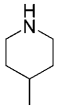
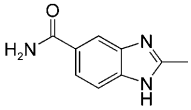
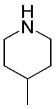
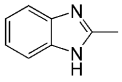
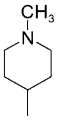
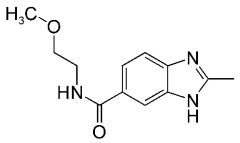
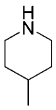
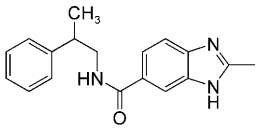
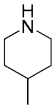
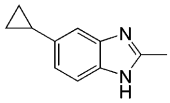
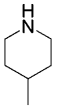
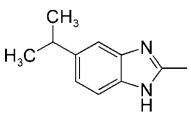
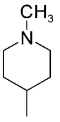
Dibenzo[c,f][2,7]naphthyridines

Researchers at Wyeth patented in 2007 a class of dibenzo[*c*,*f*]-[2,7]naphthyridines (scaffold **206**, Table 27)[80] and in the same year published the discovery of this class of compounds as potent and selective PDK1 inhibitors.[81] Compound **207** was identified by HTS as a lead structure with an IC_{50} value of 0.06 μM against PDK1, and the compound was shown to potently inhibit the

Table 23. (Continued)



165

Compd	R^1	R^2	IC_{50} [μM] PDK1	Chk1	Akt-1
170			0.13	0.21	11
171			0.12	0.09	> 50
172			0.01	0.006	27
173			0.05	0.06	> 50
174			0.07	0.006	> 50
175			0.05	0.04	34
176			0.08	0.07	> 50
177			0.14	0.06	12
178			0.07	0.002	31
179			0.04	0.003	18
180			0.02	0.022	> 10
181			0.04	0.014	> 10

growth of tumour cells (Table 26). Compound **207** was found to be selective for PDK1 in a panel of kinases including SGK1, Akt, S6K, CDK4, IKK, Src, TPL2, MEK, PKA, p38, MK2, and CAMKII ($IC_{50} > 10$ μM). SAR studies (Table 27, **208**–**212**) and X-ray structural analysis of **207** complexed in the ATP binding pocket of PDK1 (PDB code 2R7B) were performed to evaluate interactions of this scaffold with the enzyme (Figure 14). Herein the dibenzo[*c*,*f*]-[2,7]naphthyridine core is sandwiched between Leu212 and Leu88 and binds to the hinge region by key H bonds. Specifically, N8 accepts an H bond from the amide NH of Ala162 while C6-NH_2 addresses an H bond to the carbonyl oxygen of Ser160. A third H bond is established between the N5 nitrogen and the OH group of Thr222 (similar to the PDK1 binding modes of UCN-01, Figure 4, and the pose of BX-517, Figure 5). Most notably the C3-amino function interacts with the protein via a water molecule.

Interestingly, these residues were not specified, whereas this particular water-mediated interaction between **207** and PDK1 seems to be unique among the available ligand–PDK1 structures. However, one could speculate that this water-bridged H bond network may be a major contribution to the excellent PDK1 selectivity of **207** in the kinase profile. Thus, putative structure-based replacement of the water by appropriate functional groups may result in higher potency as well as selectivity of dibenzo[*c*,*f*]-[2,7]naphthyridine derivatives (and other scaffolds).

The physicochemical properties of the poorly water-soluble lead **207** were improved by the introduction of functional groups in the side chain of the rigid dibenzo[*c*,*f*]-[2,7]naphthyridine scaffold

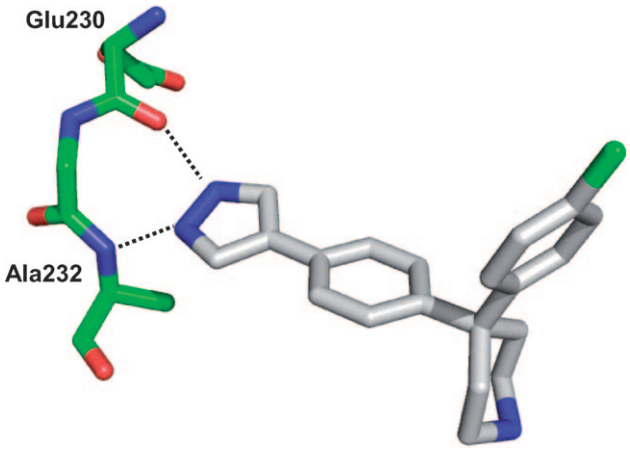


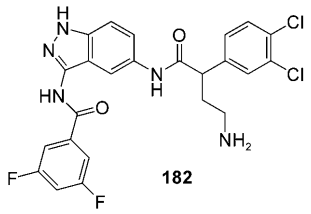
Figure 13. Binding mode of **164** in the ATP binding pocket of Akt2 (PDB code 1UW9); key residues of the hinge region and H bond interactions are shown.

(Table 27, **213**–**220**). Vis-á-vis comparison between **220** (IC_{50} = 0.2 μM, N-derivative) and **221** (IC_{50} = 1.18 μM, C-derivative) indicates a significant impact that a basic nitrogen or an alkyl chloride **222** (IC_{50} = 0.043 μM) situated in the side chain has on activity. Replacement of the 3′-NH_2 function (e.g. **207** IC_{50} = 0.06 μM) by a 3′-methoxy group (**223** IC_{50} = 0.05 μM) was tolerated.

3-Hydroxyanthranilic acid

It has been shown that 3-hydroxyanthranilic acid (HAA, structure highlighted in Figure 15) inhibits autophosphorylation of PDK1 at Ser241 in cellular assays in a dose-dependent manner in a concentration range of 50–200 μM.[82] A modelled binding mode for interactions of HAA in the ATP binding pocket was proposed (Figure 15). Herein, similar to common ligand–PDK1 interactions, H bonds to the hinge region were formed by both the hydroxy and amino function to the carbonyl oxygen of Ser160, whereas the hydroxy oxygen accepts an H bond from Ala162. A third H bond is addressed from the OH group of Thr222 to the carbonyl oxygen atom. Nevertheless, further SAR investigations of the HAA scaffold are necessary to confirm this interesting binding mode.

Table 24. Examples of *N*-1*H*-indazol-5-aminoacyl derivatives and their biological activity as PDK1, ROCK, and PKA inhibitors.



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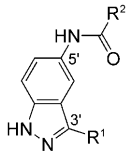
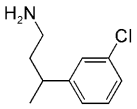
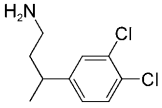
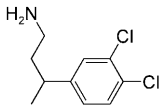
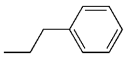
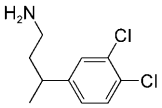
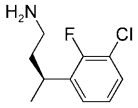
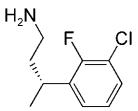
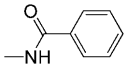
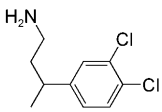


183

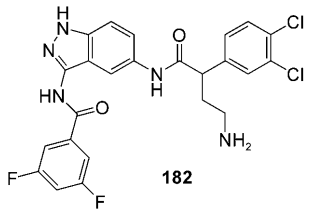
Compd	R^1	R^2	K_i [μM] PDK1	ROCK	PKA
184	H		<1	<1	<1
185	Me		<1	–	<1
186	Ph		<1	–	<1
187			<1	<1	<1
188	Me		<1	<1	<1
189	Me		<1	<1	<1
190			<1	<1	<1

PDK1 activators and modulators

In contrast to the common concepts of ATP-competitive PDK1 inhibitors, studies towards allosteric small-molecule *PDK1 activators* have also been reported.[83,84] These compounds do not target the ATP binding site, but instead target the regulatory hydrophobic motif pocket (HM, also referred to as PIF in the case of PDK1) and may have the ability to activate the enzyme allosterically.

In PDK1, the HM/PIF pocket docks the HM of substrate kinases such as RSK, p70S6K, and SGK only when they are phosphorylated. This interaction not only provides docking for the substrates, but it also activates PDK1, in turn enabling ATP binding and phosphorylation of these substrate kinases.[85,86] Specifically, the model of stabilising allostery between the regulatory PIF domain and the active site in

Table 24. (Continued)



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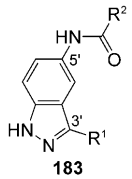
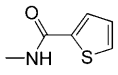
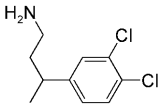
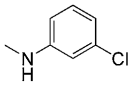
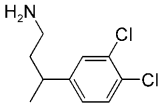
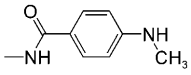
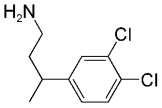
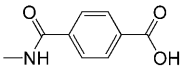
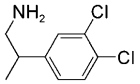
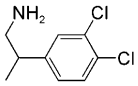
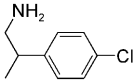
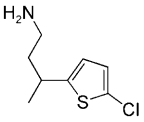
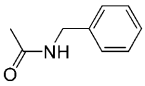
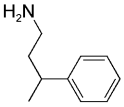
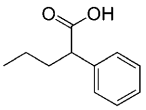


183

Compd	R^1	R^2	K_i [μM] PDK1	ROCK	PKA
191			<1	–	<1
192			<1	–	<1
193			<1	–	<1
194			<1	–	<1
195	Ph		<1	–	<1
196	H		<1	–	<1
197	Me		<1	>3	<1
198			1–3	–	–
199	Me		>3	>3	–

PDK1 involves a process by which Glu130 correctly positions Lys111 as a key active site residue interacting with the α-phosphate of ATP (Figure 16). Thus, the basic idea of such compounds as PDK1 activators consist, on one hand, of a hydrophobic binding in the PIF motif, and on the other hand, mimicking the phosphorylatable substrate residue with an acidic moiety.

In 2004 researchers at Phosphosites GmbH reported small-molecule compounds that are kinase regulators.[83] Subsequently, five compounds that possess the (3-oxo-1,3-diphenylpropyl)thioacetic acid scaffold **224** have been published and were shown to be selective PDK1 modulators (Table 28).[84] Herein, the most potent acid **225** activated PDK1 but had no significant effect on the phosphorylation of S6K1, PKA, and further AGC kinases (including PKBα/Akt1, SGK1, PRK2, and PKCζ; data not shown). Consistent with the hypothesis that the acidic moiety is essential for PDK1 activity (as described above), compound **226** bearing a methyl ester instead of the free carboxyl function was found to be almost inactive. Besides the acidic function, chloride group substitution on the aromatic moieties has a huge impact on the activity, as can be observed for **227**–**229**. Scaffold **224** bears a chiral carbon atom in the pharmacophore moiety, suggesting that either *R* or *S* configuration of these compounds may be biologically active. However, it remains unclear if the racemic mixture or one defined stereoisomer contributes to the activity.

As mentioned above, PDK1/PIF-mediated substrate docking of SGK and S6K is required to trigger their own phosphorylation. However, it is expected that an acidic moiety present in the PIF pocket (instead of the phosphate function in p-SGK1 or p-S6K1 docking to this site) would inhibit their binding and thus activation by PDK1. In line with this hypothesis, specific PDK1-catalyzed phosphorylation of S6K1 and SGK1 was blocked by **225** in an enzyme assay, as well as in HEK293 cells at a concentration of 200 μM.

Taken together, the (3-oxo-1,3-diphenylpropyl)thioacetic acid compounds are simultaneously activators of PDK1 and inhibitors of the PIF substrate kinases S6K1 and SGK1, and can therefore be considered as small-molecule modulators of PDK1. Activation of S6K is induced by nutrients via the mTOR pathway, which triggers negative feedback loops that inhibit the insulin signalling pathway. These compounds could be useful in treating obesity-induced insulin resistance; however, the clinical use of these compounds remains to be evaluated.

Table 25. Examples of compounds (patented by Vertex) that possess different scaffolds as inhibitors of PK, including PDK1.

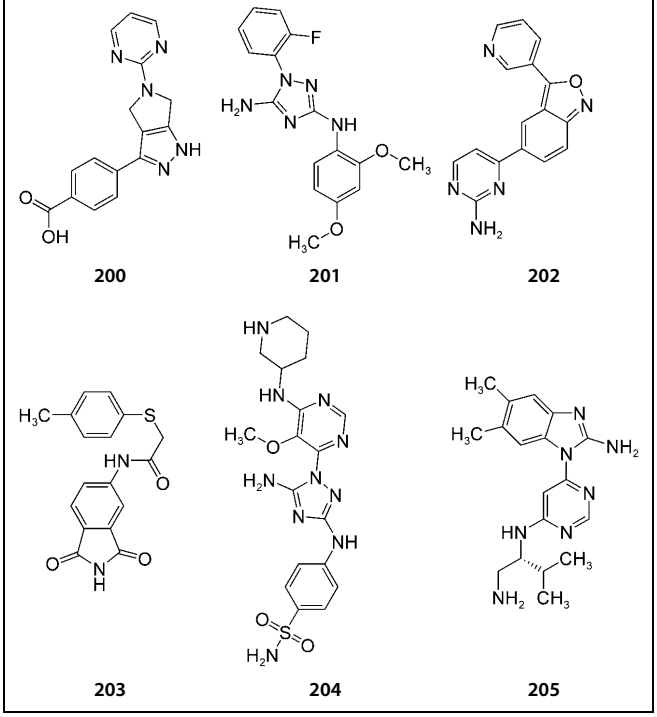


Table 26. Biological activity of **207** (IC_{50} PDK1 = 0.06 μM) in blocking the growth of various tumour cell lines.

Cell line	IC_{50} [μM]
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

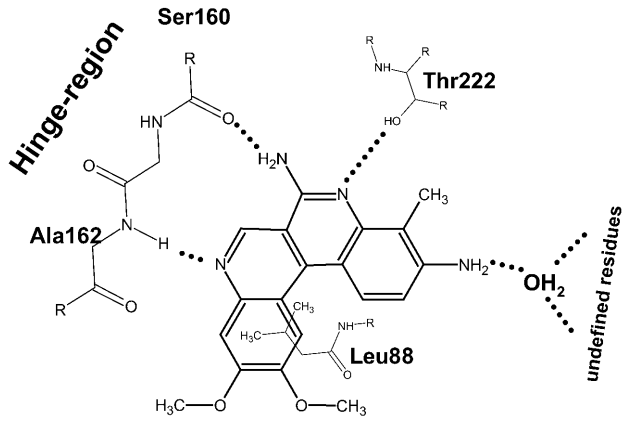


Figure 14. Schematic presentation of the pose of **207** in the ATP binding pocket of PDK1 (PDB code 2R7B, unreleased structure); key residues and H bonds are shown. In particular, the water-mediated interaction to unspecified residues of the protein is thought to contribute mainly to the particular selectivity of dibenzo[c,f][2,7]naphthyridines towards PDK1.

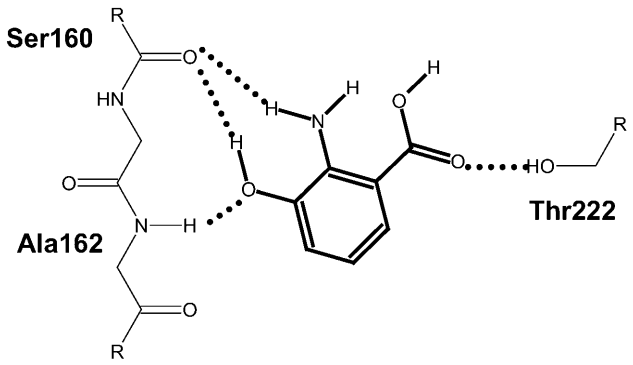


Figure 15. Schematic representation of the proposed HAA (highlighted) binding mode in the ATP binding pocket of PDK1; key residues and H bond interactions are shown.

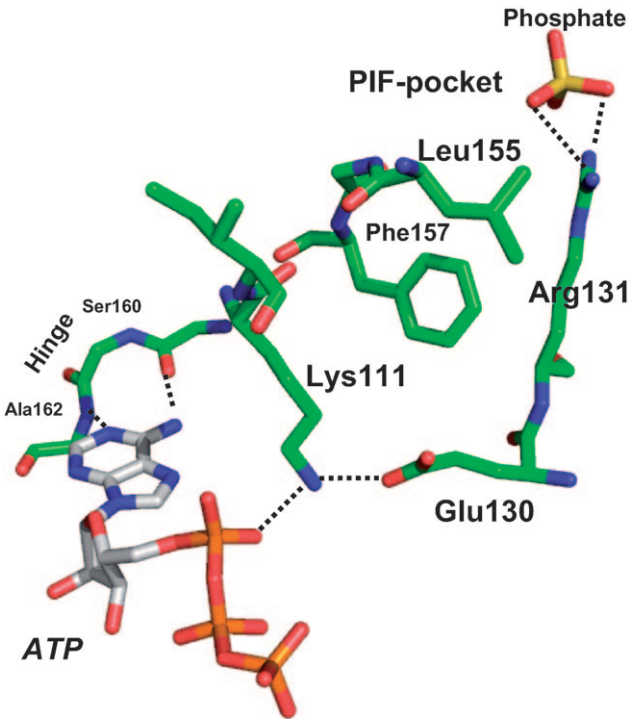
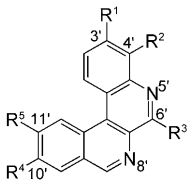


Figure 16. Binding mode of ATP in PDK1 (PDB code 1H1W) and key residues involved in the model of allosteric modulation of activity by small molecules. A phosphate moiety (originally from phosphorylated substrate) binds close to the regulatory PIF pocket and interacts with Arg131, which in turn positions Glu130 correctly to fix the catalytic residue Lys111 involved in ATP binding via the α-phosphate moiety. PDK1-modulating small molecules are thought to bind to the PIF pocket (hydrophobic residues Leu155, lined by Phe157; Val127 and Ile119 are not shown for clarity) and mimic the intrinsic phosphate by an acidic moiety interacting with Arg131. This translates down to the catalytic cascade towards the ATP binding pocket as mentioned above, thereby triggering intrinsic PDK1 activity.

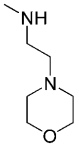
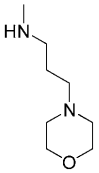
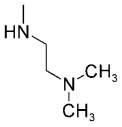
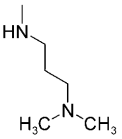
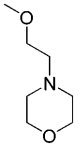
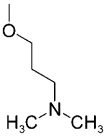
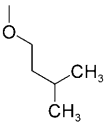
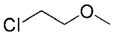
Summary

PDK1 represents a convergence point for a plethora of receptor tyrosine kinase and cytokine-mediated pathways for the regulation of vital cell processes such as cell survival and proliferation. In multiple human tumours, PDK1 is overexpressed and/or PDK1 signalling is upregulated, promoting tumour inva-

Table 27. Examples of dibenzo[*c*,*f*][2,7]naphthyridines and their biological activity as PDK1 inhibitors.

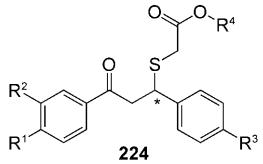


206

Compd	R^1	R^2	R^3	R^4	R^5	IC_{50} [μM]
207	NH_2	Me	NH_2	OMe	OMe	0.06
208	H	H	H	OMe	OMe	7.45
209	NH_2	H	NH_2	OMe	OMe	0.67
210	H	Me	NH_2	OMe	OMe	0.26
211	OH	Me	NH_2	OMe	OMe	0.04
212	Cl	Me	NH_2	OMe	OMe	2.12
213	NH_2	Me	NH_2	OEt	OEt	0.27
214	NH_2	Me	NH_2	F	OMe	0.58
215	NH_2	Me	NH_2		OMe	0.30
216	NH_2	Me	NH_2		OMe	0.29
217	NH_2	Me	NH_2		OMe	2.49
218	NH_2	Me	NH_2		OMe	0.97
219	NH_2	Me	NH_2		OMe	0.06
220	NH_2	Me	NH_2		OMe	0.20
221	NH_2	Me	NH_2		OMe	1.18
222	NH_2	Me	NH_2		OMe	0.04
223	OMe	Me	NH_2	OMe	OMe	0.05

siveness, angiogenesis, and progression. Biological characterisation has advanced PDK1 as a validated drug target, and inhibition of this PK by small molecules has been shown to result in significant inhibition of cancer cell proliferation. Based on the strong biological evidence of PDK1 as a significant cancer drug target, the availability of substantial structural data for structure-based drug design, and extensive medicinal chemistry efforts, few potent and selective small-molecule

Table 28. Examples of (3-oxo-1,3-diphenylpropyl)thioacetic acid derivatives and their biological activity as PDK1 modulators.



224

Compd	R^1	R^2	R^3	R^4	PDK1[a]	S6K1[a]	PKA[a]
225	Cl	H	H	H	234	109	100
226	Cl	H	H	Me	109	109	109
227	Cl	Cl	H	H	223	101	109
228	H	Cl	H	H	110	93	91
229	Cl	Cl	Cl	H	109	105	102

[a] Activation values expressed as percentages at a compound concentration of 20 μM.

PDK1 inhibitors can be found in patents and papers to date. At the molecular level, the permanent autophosphorylation of PDK1 may result in a relatively inflexible conformation of the ATP binding pocket, suggesting that inhibitors with a stiff bioactive conformation prevalently bind to this site. In line with this notion, the majority of published PDK1 inhibitors belong to structurally rigid scaffolds and thus often possess poor physicochemical properties, making it difficult to translate their potent enzymatic inhibition into cellular or in vivo settings. Furthermore, the rigid central pharmacophore that interacts with the hinge region also frequently binds to additional protein kinases, resulting in inadequate selectivity profiles. Herein we summarised a medicinal chemistry view of small molecules that interact with PDK1.

Outlook

Despite PDK1 being a validated drug target, it is not clear that any highly specific and selective PDK1 inhibitors have been disclosed thus far. This is likely to change given the amount of work being done on this enzyme coupled with increased evidence that PDK1 is a critical signalling node in cancer. To guide the design of effective novel PDK1 inhibitors, it would be helpful if more PDK1 complexes with highly potent inhibitors were determined and published. SAR at the individual molecular target should be investigated in greater detail, and beyond mere potency at the isolated enzyme, small molecular agents need to be optimised with regard to parameters such as membrane permeability, protein binding, solubility, oral availability, and toxicity. The challenge for medicinal chemists will be to get away from rigid scaffolds with poor physicochemical properties and low specificity and to develop compounds which translate their potent enzymatic PDK1 inhibition into cellular settings. Given the constitutively active status of PDK1, we also recommend that more screens be undertaken with the Ser241Ala mutant form in order to identify drugs that trap PDK1 in the inactive dephosphorylated state.

Ten years after the discovery of PDK1, a significant body of scientific knowledge has been developed. With increasing experience for PDK1 as an anticancer target it is an exciting and realistic prospect that small molecular PDK1 inhibitors will be advanced into clinical trials and that drugs will emerge from these candidates.

Abbreviations

Abl	Abelson tyrosine kinase
ADME	adsorption, distribution, metabolism, excretion
Alk	anaplastic lymphoma kinase
AMPK	5′-AMP-activated protein kinase
Aurora	member of a family of mitotic serine/threonine kinases
BIM	bisindolylmaleimides
CAMK	Ca^{2+}/calmodulin-dependent protein kinase
CDK	cyclin-dependent kinase
Chk1	checkpoint homologue kinase-1
c-kit	CD117 cytokine receptor
COX	cyclooxygenase
CSF-1R	colony-stimulating factor-1 receptor
EGFR	epidermal growth factor receptor
EPHA	ephrin receptor subfamily
Fer	fps/fes-related tyrosine kinase
FGFR	fibroblast growth factor receptor
Flt	Fms-related tyrosine kinase
GSK	glycogen synthase kinase
HA	H bond acceptor
HAA	3-hydroxyanthranilic acid
HD	H bond donor
Her-2	human epidermal growth factor receptor-2 (also ErbB-2 or ERBB2)
HM	hydrophobic motif
IGF-1R	insulin growth factor-1 receptor
IKKb	inhibitor of κB kinase
JAK	Janus kinase
MAPK	mitogen-activated protein kinase
MAPKAPK1	MAPK-activated protein kinase-1
MAPKAPK2	MAPK-activated protein kinase-2
MEK1	mitogen-activated protein kinase kinase-1
Met	receptor tyrosine kinase, hepatocyte growth factor
MK2	MAPKAPK2
MSD	Meso Scale Discovery
MSK1	mitogen- and stress-activated protein kinase-1
mTOR	mammalian target of rapamycin
p38	p38 MAPK
p70S6K	p70 ribosomal S6 kinase
PAK	P21-activated kinase
PDGFR	platelet-derived growth factor receptor
PDK1	3-phosphoinositide-dependent protein kinase-1
PH	pleckstrin homology
PI3K	phosphoinositide-3 kinase
PIF	PDK1-interacting fragment
PIP2	phosphatidylinositol-4,5-bisphosphate
PIP3	phosphatidylinositol-3,4,5-trisphosphate
PK	protein kinase
PKA	protein kinase A
PKB/Akt	protein kinase B

PKC	protein kinase C
PKN1	protein kinase N1
Plk	polo-like kinase
PRK2	protein kinase C-related kinase-2
PTEN	phosphatase and tensin homologue
Ret	proto-oncogene, encodes a receptor tyrosine kinase
ROCK	Rho-associated coiled-coil-containing protein kinase
RSK	p90 ribosomal S6 kinase
SAR	structure–activity relationship
SGK	serum- and glucocorticoid-induced protein kinase
Tek	tyrosine endothelial kinase
TPL	tumor progression Loci-2, serine/threonine MAP kinase
VEGFR	vascular endothelial growth factor receptor (also KDR)

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Keywords: biological activity · inhibitors · PDK1 · proliferative diseases · selectivity

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