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Identification and structure–activity relationships of substituted pyridones as inhibitors of Pim-1 kinase

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Abstract—A novel series of highly potent substituted pyridone Pim-1 kinase inhibitors is described. Structural requirements for in vitro activity are outlined as well as a complex crystal structure with the most potent Pim-1 inhibitor reported (IC_{50} = 50 nM). A hydrogen bond matrix involving the Pim-1 inhibitor, two water molecules, and the catalytic core, together with a potential weak hydrogen bond between an aromatic hydrogen on the R^1 phenyl ring and a main-chain carbonyl of Pim-1, accounts for the overall potency of this inhibitor.

The *pim-1* proto-oncogene was initially identified as a frequent site of integration for the slowly transforming Maloney murine leukemia virus in murine T cell lymphomas.[1] Pim-1 is an oncogenic serine/threonine kinase that is a member of a family of proteins containing homologues Pim-2 and Pim-3, and is transcriptionally regulated by cytokines, mitogens, and numerous growth factors.[2–4] Elevated expression of Pim kinases has been described predominantly in murine and human leukemias and lymphomas.[5] The enzymatic substrates reported for Pim kinases are diverse (p100,[6] Bad,[7] NFATc1,[8–10] p21/Cip1,[11] Cdc25a,[12] SOCS,[13,14] and others), suggesting that Pim plays a central regulatory role in numerous processes. Indeed, Pim kinases have defined biological roles in cell survival, proliferation, and differentiation,[4] particularly for cells of hematopoietic lineage. Studies using either *pim* transgenic or *pim* knockout mice suggest that overlapping functions exist for Pim-1 and Pim-2, and that the full transforming potential of Pim kinases cannot be realized in vivo without cooperation from other oncogenes.[15–19] Perhaps the most compelling biological roles described for Pim-1 and Pim-2 include the regulation of rapamycin-resistant T cell growth and survival[18] as well as the recently reported impairment of mutant Fms-like tyrosine kinase 3 (FLT3)[10,20] or drug-resistant BCR/ABL-transformed hematopoietic cell survival,[21] upon functional suppression of Pim kinases. From these perspectives, inhibitors of Pim kinases may have immunomodulatory potential alone or in combination with immunosuppressive drugs such as rapamycin, or as an alternative method to treating patients who have developed resistance to small-molecule protein tyrosine kinase inhibitors for cancer.

X-ray crystallographic studies of Pim-1 support a high degree of main chain similarity to other structurally defined serine/threonine kinases, with characteristic fold-hinge-fold domains.[22–24] However, Pim kinases possess a unique proline residue, Pro123, in the hinge region where other hydrophobic amino acids are more typically found, as well as insertion of one or two residues in the hinge region following Pro123. As Pim-1 adopts a unique structure in the hinge region, which acts in part as a boundary for positioning ATP binding, it may be possible to identify highly selective ATP-competitive Pim kinase inhibitors for potential use as anticancer or immunomodulatory agents. Small molecule Pim-1 kinase inhibitors derived from various chemical scaffolds have been previously described.[23–25] None of these inhibitors have progressed to the clinic. Here we report the identification of a novel series of Pim-1 kinase inhibitors based on a substituted pyridone scaffold and outline the potential mechanism by which this type of inhibitor could compete and interfere with Pim-1 ATP utilization.

Keywords: Pim-1 kinase; Pim-1 kinase inhibitors; Pyridone; Fused-ring pyridone; X-ray complex structure; Pim-1; Pim.
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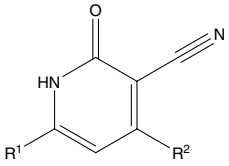
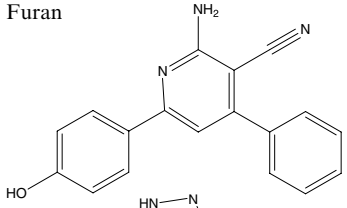
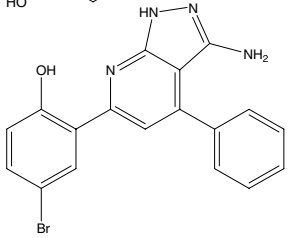
In order to identify alternative scaffolds we screened a compound library of approximately 160,000 molecules using an enzymatic luminescent assay for Pim-1 inhibition.[26] A substantial number of Pim-1-inhibitory hits were identified, many of which had IC_{50} values less than 1 μM. Several potent chemical library hits along with a number of newly synthesized derivatives of these compounds are listed in Table 1. Corresponding IC_{50} values were determined by radioactive filter-plate assay using histone proteins as the substrate for Pim-1 kinase.[27] Compound **1**, a substituted pyridone scaffold, had the lowest IC_{50} at 0.05 μM. This molecule served both as a starting point for SAR chemical syntheses and was used for co-crystallization with Pim-1 protein. A limited number of compounds with highly similar chemical composition were also identified from our library including compounds **4**, **6**, **7**, **8**, and **10**. Although none of these compounds produced IC_{50} values superior to compound **1** they were informative with respect to the SAR. Specifically, modification of R^1 contributed more substantially to the loss of in vitro activity compared to alteration of R^2.

To help comprehend the inhibitory mechanism of action for compound **1**, we took advantage of a highly purified and enzymatically active Pim-1 protein[28] to initiate complex structure studies by X-ray crystallography.[29,30] The structure of Pim-1 co-crystallized with compound **1** was resolved to 2.5 Å. Analysis of the electron density plot for **1** supported the conformation of the complex structure without ambiguity (Fig. 1). Compound **1** appeared to adopt a partially flat conformation with co-planar R^1 phenyl and central pyridone rings, while the R^2 phenyl ring was twisted perpendicularly with respect to the other rings. We contend that the possibility for H-bonding between the 2-OH group on the R^1 phenyl ring and the pyridone N is high and within acceptable H-bonding distance (1.9 Å). This internal H-bonding may serve to fix the conformation of the two rings into a nearly planar relationship.

Compound **1** bound convincingly within the ATP-binding site of Pim-1 suggesting an ATP-competitive inhibitory mechanism (Fig. 2). Perhaps the most significant finding from this complex structure was the prominent interaction of the carbonyl group on the pyridone ring with both the Lys67 side chain (interacts with phosphates from ATP) of Pim-1 together with a hydrogen bond to a water molecule (Wat1) that appeared to play an integral part of a larger H-bonding network in this region. The apparent H-bonding network further consisted of a second conserved water molecule (Wat2) that interacted with both the Pim-1 kinase catalytic residue Phe187 and Wat1 (Fig. 2). Interestingly, the only potential interaction of **1** with the hinge region occurred on the left side of the R^1 phenyl ring where the complex

Table 1. Pyridone-based inhibitors of Pim-1 kinase



Compound	R^1	R^2	IC_{50}[a] (μM)
1	3-Br-6-OH-Ph	Ph	0.05
2	3-Me-6-OH-Ph	Ph	0.34
3	Ph	3-Me-6-OH-Ph	0.37
4	6-OH-Ph	4-OH-Ph	0.43
5	3-Et-6-OH-Ph	3-Cl-Ph	1.03
6	4-OMe-5-Br-Ph	CF_3	1.14
7	Thiophene	Thiophene	0.99
8	CF_3	Thiophene	3.54
9	Furan	CF_3	16.00
10			4.41
11			1.77

[a] Values are means of three experiments.