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Structure-based design of low-nanomolar PIM kinase inhibitors

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ABSTRACT

PIM kinases are implicated in variety of cancers by promoting cell survival and proliferation and are targets of interest for therapeutic intervention. We have identified a low-nanomolar pan-PIM inhibitor (PIM1/2/3 potency 5:14:2 nM) using structure based modeling. The crystal structure of this compound with PIM1 confirmed the predicted binding mode and protein–ligand interactions except those in the acidic ribose pocket. We show the SAR suggesting the importance of having a hydrogen bond donor in this pocket for inhibiting PIM2; however, this interaction is not important for inhibiting PIM1 or PIM3. In addition, we report the discovery of a new class of PIM inhibitors by using computational *de novo* design tool implemented in MOE software (Chemical Computing Group). These inhibitors have a different interaction profile.

Proviral Integration Moloney (PIM) kinases 1, 2 and 3 are homologous constitutively active serine/threonine kinases in the Jak/Stat pathway.[1,2] PIM kinases are overexpressed in a variety of hematological malignancies[3] as well as solid tumors including pancreatic, prostate, colon,[4–7] and are implicated in cancer cell survival and proliferation and tumor growth.[1,8] Currently the most understood activity is cell survival signaling through the phosphorylation of BAD leading to the release of anti-apoptotic proteins,[9,10] but many other mechanisms have been described.[1,2] PIM kinases are upregulated in most cancers by a mutation of upstream regulators (e.g., Jak2, BCR-Abl, Flt3) or an activation of RTKs or Stats leading to activation of a complex network of signaling pathways.[11–13] As there is evidence of compensatory activation of all three PIMs in lymphomas,[14] as well as suggested additional role of PIM2 in translation regulation,[2,15] a pan-PIM kinase inhibitor is desired.[8,16–18] It would act as a single agent or in a combination with existing therapies (e.g., chemo, PI3K or MAPK pathway inhibitors[19]) to induce apoptosis in PIM-overexpressing lymphomas and solid tumors.

We are presenting the discovery of novel pan-PIM inhibitors through the use of computer-aided drug design. We used the target structural information that we outline below as a guide to design new molecules through computational modeling. Protein Data Bank[20] contained more than 25 structures of PIM-1, including two apo-proteins,[21,22] a few complexes with ATP analogs,[21–23] and many structures with inhibitors. No significant protein conformational change appeared upon binding of most inhibitors.[24–33] Valeant inhibitor (PDB code 2OBJ[24]) was one of a few that caused larger protein movement including some change in glycine rich loop conformation and its tip F49 pointing away from the binding pocket. In all other structures, F49 was oriented towards the binding site and was involved in the stacking interactions with the ligand aromatic groups. There were a few distinct conformations of E89, D186 and D128 side chains as well as the glycine-rich loop, however, they were close in space and maintained same interactions. All three PIMs bound the natural substrate ATP via only one hinge hydrogen bond involving the adenine amino substituent and the E121 backbone carbonyl. P123 of the hinge lacked the hydrogen bond donor functionality normally present in other kinases to interact with the ATP adenine ring nitrogen.[21,22] The presence of the proline at the hinge differentiated PIMs in the kinome as it was present in only two other kinases. The hinge also contained V126 insertion that was absent in other kinases. This insertion changed the hinge conformation enlarging the adjacent binding pocket. Such differences between PIMs and other kinases presented opportunity for developing selective pan-PIM inhibitors.

Most PIM inhibitors found in the PDB formed a hydrogen bond or a salt bridge between one or two ligand acceptors and the catalytic K67.[25–28,30] It replaced the salt bridge between this lysine and the phosphate groups in the ATP structures (e.g., in 2BZK[23]

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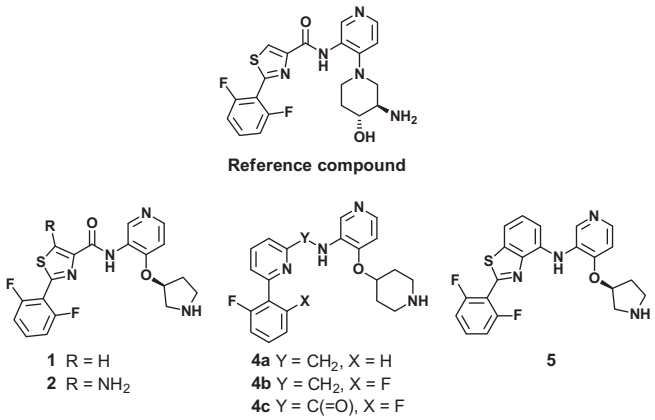

Scheme 1. Initial PIM inhibitors.

structure from the PDB). K67 was part of the salt bridge network involving E89 of the alpha-C helix and D186 of the DFG loop. All PIM protein structures were in the DFG-in conformation. In addition, some inhibitors formed a hydrogen bond with the hinge E121 carbonyl in the similar manner as the ATP. Some also formed a salt bridge with D128 located below the hinge.[23,27,29,30]

In the first part of the Letter we describe structure-based design and exploration of the O-linked series. In the second part we present how we combined a computational *de novo* design method incorporated in the Chemical Computing Group MOE[34] tool with the knowledge of the binding pharmacophore to help identify an unrelated pyrrolopyrimidine inhibitor.

We designed compounds **1** and **2** (Scheme 1) as potential pan-PIM inhibitors by using previously published structures as starting points.[35] Both compounds were predicted to form a hydrogen bond with the catalytic K67 via the pyridine N. The compounds had a positively charged pyrrolidine that we hypothesized might interact with D128 and/or E171. This interaction was present in a fraction of the known PDB complexes and thus we wanted to understand its importance. Docking **1** and **2** (Fig. 1a) confirmed the key interaction between the pyridine nitrogen and the K67 (N...HN distance 2.3 Å). It also suggested that the S isomers made direct interactions to D128 and E171, whereas the R isomers did not. Since the S stereoisomer was 5–20 fold more active (**1S**, PIM1/PIM2/PIM3 biochemical IC_{50} 5:14:2 nM correspondingly; **1R**, 28:360:32 nM), we initially suggested that a direct salt bridge to D128 and E171 might be necessary for tight binding. Like ATP, compound **2** also made a hydrogen bond to the hinge E121 main chain carbonyl via amino group off the thiazole ring (NH...O distance 1.9 Å). In **1**, this hydrogen bond was replaced by favorable but weaker interaction between the negatively charged E121 carbonyl and the positively charged edge of the thiazole ring (distance 3.5 Å), resulting in some loss of activity: compound **2** was more potent at 1:1:1 nM. The amide linker did not make polar interactions to the protein, but was important to achieve low-energy bound conformation through intramolecular hydrogen bonds to the thiazole nitrogen and the ether linker. In addition, the difluorophenyl ring occupied the hydrophobic pocket outlined by L44, L104, P123, R122, V126 and L174.

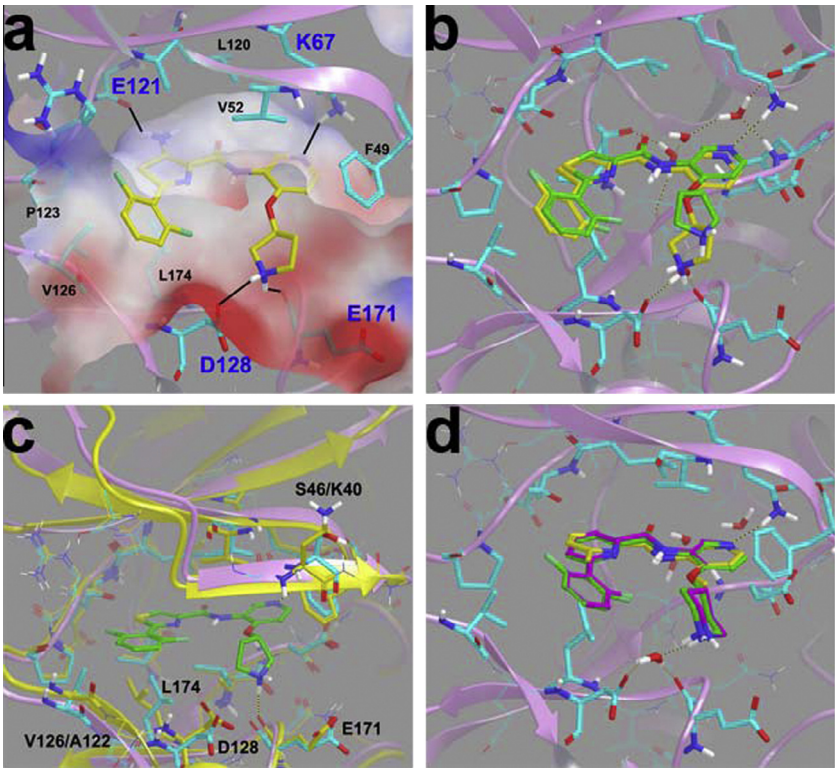

Figure 1. Designed O-linked series compounds. (a) Predicted binding mode of **2** (yellow). The binding site is shown as a surface colored by the electrostatic potential. Positively charged amine is predicted to make direct hydrogen bonds to D128 and E171 (black lines) and to be located in the negatively charged binding site region (red surface color). (b) Overlay of the model (yellow) and X-ray structure (green) of **1S** in PIM1. In the crystal structure, the inhibitor interacts with the water located approximately at the nitrogen of the predicted conformation. This water in turn makes hydrogen bonds to D128 and E171. (c) X-ray structure of **1S** (green) in PIM2 shown superimposed on PIM1 protein. PIM1 and PIM2 proteins are shown as purple and yellow ribbon diagrams, binding site residues shown in cyan and yellow correspondingly. Some protein residues are highlighted. Protein–ligand hydrogen bonds are shown as yellow-black lines. The compound makes direct hydrogen bond to E171. (d) Models of **4a** (magenta) and related compound **4c** (green). **4a** model has low-energy conformation of the linker as it is close to the geometry optimized by QM shown in yellow.