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Discovery of imidazopyridazines as potent Pim-1/2 kinase inhibitors

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ABSTRACT

High levels of Pim expression have been implicated in several hematopoietic and solid tumor cancers, suggesting that inhibition of Pim signaling could provide patients with therapeutic benefit. Herein, we describe our progress towards this goal using a screening hit (*rac*-**1**) as a starting point. Modification of the indazole ring resulted in the discovery of a series of imidazopyridazine-based Pim inhibitors exemplified by compound **22m**, which was found to be a subnanomolar inhibitor of the Pim-1 and Pim-2 isoforms (IC_{50} values of 0.024 nM and 0.095 nM, respectively) and to potently inhibit the phosphorylation of BAD in a cell line that expresses high levels of all Pim isoforms, KMS-12-BM (IC_{50} = 28 nM). Profiling of Pim-1 and Pim-2 expression levels in a panel of multiple myeloma cell lines and correlation of these data with the potency of compound **22m** in a proliferation assay suggests that Pim-2 inhibition would be advantageous for this indication.

Proviral **I**ntegration site of **M**oloney (Pim) murine leukemia virus kinases are constitutively active serine/threonine kinases that are involved in cell survival and proliferation as well as a number of other signal transduction pathways.[1–3] The Pim-1, -2 and -3 isoforms share a high level of sequence homology and appear largely redundant in function. High levels of Pim expression have been implicated as oncogenic drivers in hematologic and solid malignancies[4] including multiple myeloma,[5] acute myeloid leukemia, prostate cancer, and gastric and liver carcinomas.[6] These findings, in conjunction with the observation that the triple knockout of Pim proteins has a mild phenotype, suggest that inhibition of Pim signaling by a pan–Pim inhibitor could provide patients with therapeutic benefit.[3]

All three Pim isoforms share the unique feature among kinases of having a proline residue in the hinge, which results in only one hydrogen bond interaction with ATP.[7] This property can be exploited to derive selectivity over other kinases. However, among the Pim isoforms, the $K_{M,ATP}$ for Pim-2 is 136-fold higher affinity than that for Pim-1[8] which makes the design of Pim inhibitors with potent cellular activities in pan-Pim expressing cell lines very challenging. Pim directly phosphorylates Bcl-2-associated death promoter (BAD) which becomes sequestered in pBAD-(14-3-3) protein heterodimers. Inhibition of Pim-mediated BAD phosphorylation is expected to lead to high levels of free BAD which can bind to the antiapoptotic Bcl-2 protein, causing release of the proapoptotic Bax and BAK.[9] Phosphorylation of BAD at the serine 112 site is a non-redundant function of the Pim kinases.[10] To evaluate the cellular potency of novel Pim inhibitors, a multiple myeloma cell line, KMS-12-BM,[11] was used to develop a cellular assay to advance compounds in this study using the inhibition of phosphorylation of BAD at the serine 112 site (pBAD(s112)) as a gauge for activity.[12]

The development of Pim inhibitors[13] has been very active in recent years resulting in several clinical compounds including: SGI-1776,[14] AZD-1208[15] and PIM447.[16] Herein, we describe our progress towards the development of a novel class of Pim-1/2 inhibitors based on an imidazopyridazine scaffold. In previous

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communications, we described our efforts in the development of Pim inhibitors based on aminothiadiazole **2**,[17] aminooxadiazole **3**,[18] quinazolinone-pyrrolopyrrolones **4**,[19] and macrocyclic quinoxaline-pyrrolo-dihydropiperidinone scaffold **5**.[20] Although several of these compounds possessed attractive cellular activities, limited solubility and/or undesirable pharmacokinetic properties precluded their further development. *rac*-**1** represented a previously identified lead from a high-throughput screening campaign and resulted in the discovery of indazole **6**.[21] The basic amine present in indazole **6** was beneficial for the simulated intestinal fluids (SIF) solubility of these Pim inhibitors and this favorable property could enable improved exposures in high dose toxicology studies. However, its presence likely contributed to a large shift in enzyme to cellular potency resulting in a compound that was >1 μM in the KMS-12 pBAD cellular assay (Fig. 1).

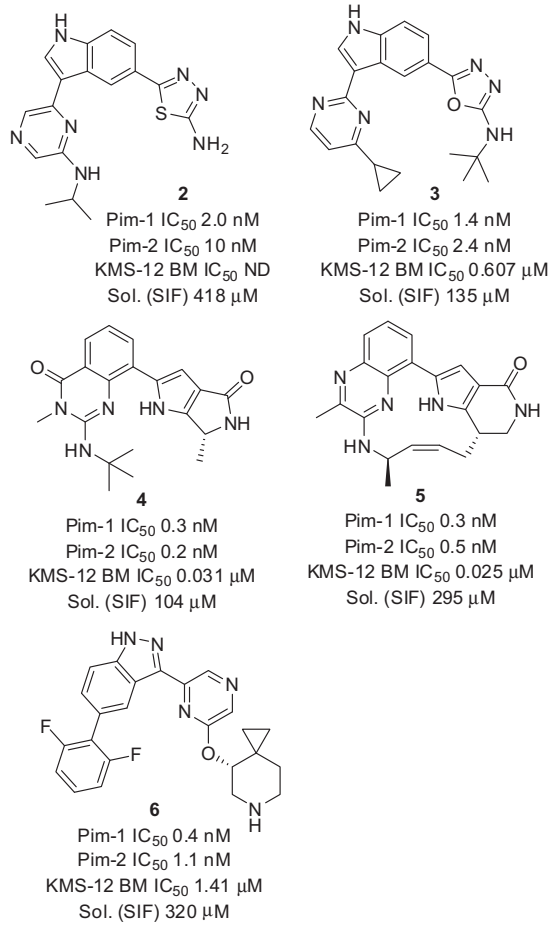

Figure 1. Recent literature reports of Pim inhibitors from Amgen.

A previously reported X-ray co-crystal structure of (*rac*)-**1** in the ATP-binding pocket of Pim-1 protein revealed that the (*S*)-isomer of the 3-aminopiperidine was the preferred enantiomer for binding (Fig. 2). In addition, this compound showed three strong electrostatic interactions that likely contributed to its potency including: (1) engagement of the indazole N–H in a hydrogen bonding interaction with the carbonyl of Glu121 residue (3.0 Å) of the hinge region; (2) formation of a hydrogen bond with the nitrogen of the pyrazine and the catalytic Lys67 (3.2 Å); (3) strong hydrogen bonding interactions of the amino group in the 3-position of the piperidine ring with the Asp186 and Asn172 residues, at 3.2 Å and 3.3 Å, respectively. Inclusion of the protein surface of the binding pocket suggested that additional van der Waals interactions could be obtained by further exploiting hydrophobic dimples on the roof of the binding pocket.

Molecular modeling guided modification of *rac*-**1**[21] led us to prepare compound **7** bearing an amide tethered to the 3-position of an aza-indole. Compound **7** possessed similar Pim-1 potency (Pim-1, IC_{50} = 65 nM), however, this modification led to a 100-fold decrease in its Pim-2 potency (Pim-2, IC_{50} = 314 nM). This scaffold is similar to recently disclosed Pim inhibitors from Novartis **9**[16,22] Biogen **10**[23] and Genentech **11**.[24] Removal of the amide carbonyl and modification of the nitrogen substitution pattern of the bicyclic ring yielded a novel 7-amino-imidazopyridazine core represented by **8** (Fig. 3).[25] This ring system was designed to favor co-planarity of the core while maintaining key interactions for Pim binding.

As a starting point for investigation of the new scaffold **8**, we chose to introduce a benzene ring (Ar = benzene) to append the imidazopyridazine scaffold and to present the basic aminopiperidine motif to the acidic patch of residues in the ATP binding pocket in the Pim-1 protein (compound **8a**). This compound had micromolar activity in the cell (KMS-12 pBAD IC_{50} = 3.4 μM). Introduction of a 3-pyridyl ring (compound **8b**) resulted in subnanomolar potency in the Pim-1 and Pim-2 enzymatic assays and a modest submicromolar activity in the KMS-12-BM cellular assay (IC_{50} = 403 nM). The introduction of other heterocyclic motifs indicated that the positioning and presence of the 3-pyridyl nitrogen was critical for activity. For example, placement of the nitrogen in this heterocycle in the 4-position (compound **8c**) was >1 μM in the Pim-1 and Pim-2 enzymatic assays. Introduction of an additional nitrogen to the 3-pyridyl ring in the 5-position (compound

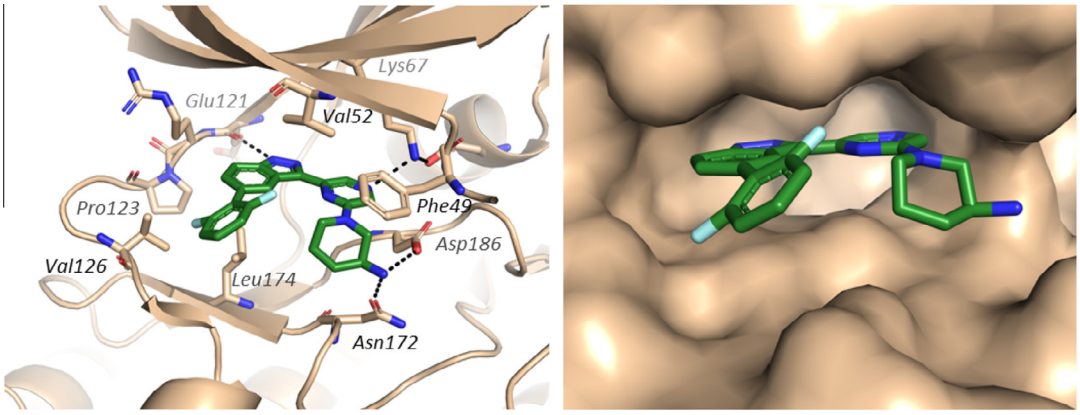

Figure 2. X-ray of compound (*S*)-**1** bound in the ATP binding site of unphosphorylated Pim-1 protein determined to 3.0 Å resolution. PDB code: 4RPV.[21] Dashed lines indicate hydrogen bonds. For the inhibitor, C: green; N: blue; F: light blue.