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Structure-based design of 3-aryl-6-amino-triazolo[4,3-*b*]pyridazine inhibitors of Pim-1 kinase

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ABSTRACT

A series of substituted 3-aryl-6-amino-triazolo[4,3-*b*]pyridazines were identified as highly selective inhibitors of Pim-1 kinase. Initial exploration identified compound **24** as a potent, selective inhibitor, limited in its utility by poor solubility and permeability. Understanding the unusual ATP-binding site of the Pim kinases and X-ray crystallographic data on compound **24** led to design improvements in this class of inhibitor. This resulted in compound **29**, a selective, soluble and permeable inhibitor of Pim-1.

The Pim kinases are a small sub-family of three Ser/Thr protein kinases within the CAMK (calmodulin-dependent protein kinase-related) family, comprising Pim-1, -2 and -3.[1] The Pim kinases are relatively small enzymes (ca. 34 kDa) containing a kinase domain, but no other recognizable domain structures, hence regulation is controlled at the transcriptional and translational levels.[2] Homology between the Pim kinases is high: Pim-2 and Pim-3 are 61% and 71% identical in sequence to Pim-1, respectively. Pim-1 is widely expressed, however overexpression has been observed in a range of human hematopoietic malignancies, such as leukemias and lymphomas[3] as well as in prostate cancers.[4] Additionally, Pim-1 expression is up-regulated in response to a wide variety of cytokine and growth factor stimuli signaling events.[1] Pim-1 knockout mice show no obvious phenotype, suggesting redundancy in the Pim signaling pathways.[5] Pim-1,2,3-triple knockout mice are viable, but are significantly smaller in size than wild-type, indicating impairment of growth factor signaling.[6] In vitro assays also show a dramatic reduction in growth-factor dependent hematopoiesis in colony-forming assays[6] in triple knockout bone marrow cells. Furthermore, mice overexpressing Pim-1 show an increased susceptibility to chemical and radiation induced tumorigenesis.[2] Taken together this information suggests that the Pim kinases may be appropriate targets for cancer intervention.

Recent publications have described the unusual structural features of the Pim-1 kinase domain.[7] Most notably, the hinge region presents two features of interest: an insertion residue as well as a proline residue (Pro123), which combine to form an ATP- and inhibitor-binding region quite distinct from other protein kinases. In a typical protein Ser/Thr or Tyr kinase, ATP forms two hydrogen bonds with the hinge region: one as donor from the adenine NH_2 to a hinge carbonyl, and a second as an acceptor contact between the adenine N-1 and a backbone NH of the kinase. In the Pim family this residue is proline so the same H-bonding interaction cannot exist. The insertion and the proline together cause the hinge region to expand and create greater space for inhibitor occupancy.

Several recent reports have disclosed inhibitors of Pim-1[8–14]: exemplary compounds are illustrated in Figure 1. Bullock et al.[13] have reported imidazo[1,2-*b*]pyrimidine (e.g., compound **6**) and similar pyrazolo[1,5-*a*]pyrimidine (e.g., compound **7**) inhibitors of Pim-1 kinase. Here we report our discovery of triazolo[4,3-*b*]pyridazine-based inhibitors and development of this series as soluble, potent and selective Pim-1 inhibitors.

To identify inhibitors of Pim-1 kinase we undertook a high throughput screen of our compound collection and identified triazolo[4,3-*b*]pyridazine compound **8** as an ATP-competitive Pim-1 inhibitor, with K_i = 320 nM. This inhibitor was sufficiently distinct from other hit classes to warrant further inspection.

To further explore this scaffold we prepared a small library of analogs according to the method shown in Scheme 1.

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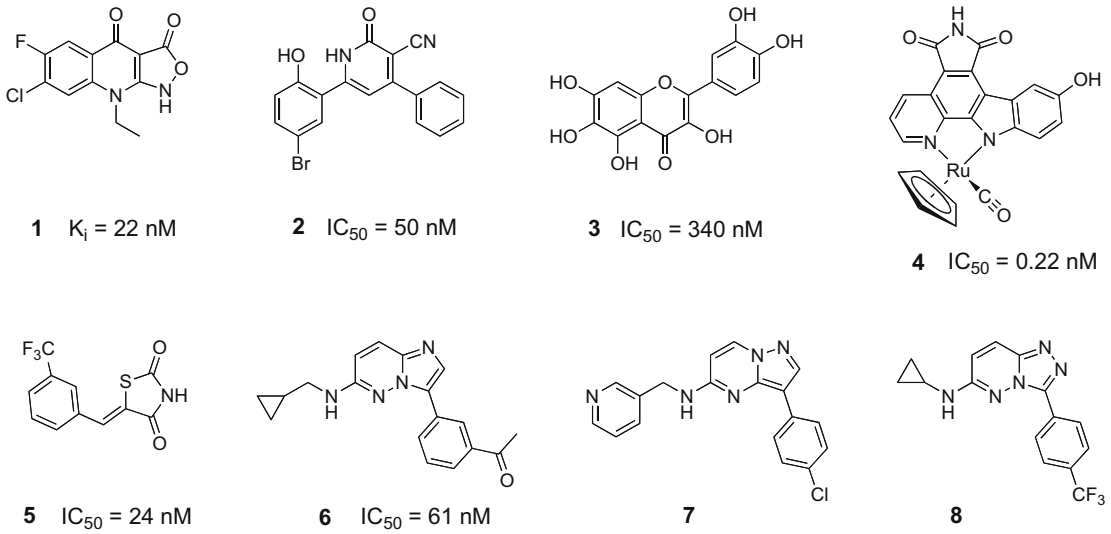
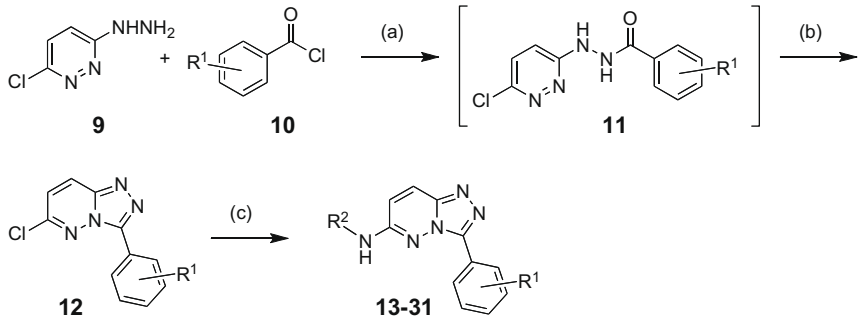


Figure 1. Inhibitors of Pim-1. inhibition data from sources.

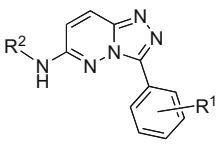


Scheme 1. Synthesis of N-substituted-3-aryl-[1,2,4]triazolo[4,3-*b*]pyridazin-6-amines. Reagents and conditions: (a) MeCN, rt, 2 h; (b) $POCl_3$, MeCN, 80 °C, 16 h (49%, R = *m*-CF_3); (c) R^2–NH_2, 70 °C, 4 h (11–66%).

In this scheme, substituted aroyl chlorides (**10**) and 3-chloro-6-hydrazinylpyridazine (**9**) were combined in MeCN and mixed for 2 h at RT. After confirming the conversion to the acylhydrazine **11** by HPLC, $POCl_3$ was added to effect the ring closure by heating to 80 °C. Compound **12** was treated with neat amine at 70 °C to convert to the desired products. Yields were highly dependent on the particular amine and products were isolated by preparative HPLC (>95% purity).

A brief evaluation of both N-substituents and phenyl ring substituents revealed interesting trends in Pim-1 inhibition summarized in Table 1. *ortho*-Aromatic substituents (compounds **14**, **16**, **19**) were significantly less potent than the screening hit (**8**), as was the unsubstituted ring system (**13**). By moving the CF_3 group from the *para* position of the initial hit (**8**), to the *meta* position (**15**) an 18-fold improvement in inhibition was obtained. For the 6-amino substituent (R^2 in Table 1), extension of the cyclopropyl group with a methylene insertion (**22**), analogous to published compound **6**, did not improve inhibitory activity. Replacement of the *N*-cyclopropyl with a cyclohexyl group afforded the most potent inhibitor of this initial series, **24**. An X-ray crystal structure of **24** bound to Pim-1 was obtained[14] and revealed an unusual binding mode consistent with that of compound **6** previously reported by Pogacic et al.[13b] In the structure of **24** bound to Pim-1 shown in Figure 2, the *m*-CF_3-phenyl ring makes contact with the hinge Glu121 by way of a bifurcated H-bond between the aromatic protons on C-5 and C-6 and the backbone carbonyl of Glu121.[16] This observation helps to explain the weaker activity of the *ortho*-substituted compounds, as *ortho* substitution disrupts this interaction by causing the phenyl ring to twist significantly out

Table 1
N-Substituted-3-aryl-[1,2,4]triazolo[4,3-*b*]pyridazin-6-amine inhibitors of Pim-1



Compound	R^1	R^2	PIM-1, K_i (nM)[15]
13	H	cPr	1800
14	2-CF_3	cPr	>3300
15	3-CF_3	cPr	18
8	4-CF_3	cPr	320
16	2-F	cPr	>3300
17	3-F	cPr	430
18	4-F	cPr	210
19	2-OMe	cPr	>3300
20	3-OMe	cPr	410
21	4-OMe	cPr	980
22	3-CF_3	$cPrCH_2$	50
23	3-CF_3	cBu	54
24	3-CF_3	cHex	11

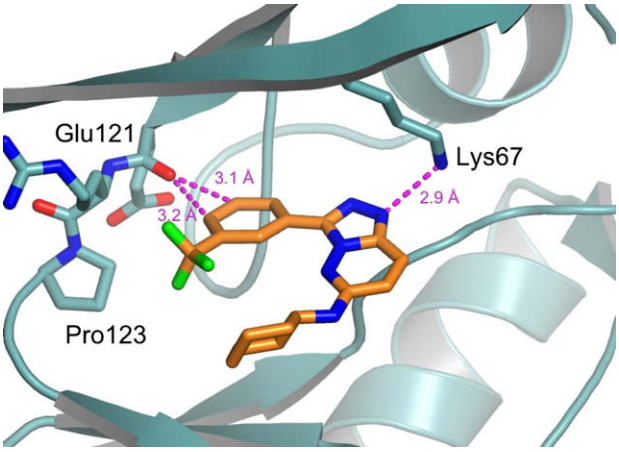


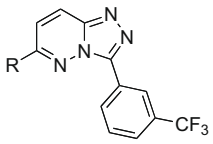
Figure 2. Crystal structure of compound **24** bound to Pim-1.

of plane. The superiority of the *m*-CF_3 relative to *p*-CF_3 is clear from the structure. The *m*-CF_3 creates hydrophobic interaction with the hinge Pro123, while the *p*-CF_3 group disrupts the interaction with the hinge. The bicyclic triazolo[4,3-*b*]pyridazine ring system forms an H-bond between the triazole N-1 and the catalytic lysine (Lys 67). These structural observations are consistent with those previously reported for compound **6** bound to Pim-1. The cyclohexylamino group attached at position-6 of the core bicyclic heterocycle lies within the active site and points outwards to solvent.

Although compound **24** was found to be a potent and selective Pim-1 kinase inhibitor, its physical properties limit its utility. Solubility at pH7.4 was less than 0.3 μM, while *c* log *P* and *c* log $D_{7.4}$ are 4.9 and 5.0, respectively. To improve upon the physical characteristics, solubility-enhancing features were designed into a subsequent library of analogs, prepared according to the method shown in Scheme 1. Data for these compounds are presented in Table 2.

As shown in Table 2, introduction of solubility-enhancing features, such as ethers, basic amines or hydroxyl groups into the cyclohexyl ring N-substituent all serve to significantly improve solubility and calculated physical properties, without compromising Pim-1 kinase inhibition activity. The most significant improvements in pH7.4 solubility were obtained by substituting 3- or 4-piperidyl rings in place of the cyclohexyl ring. Compound **27** showed >200 μM solubility at pH 7.4, while maintaining low nanomolar potency. The most potent inhibitor, compound **29**, contains a trans-4-hydroxycyclohexylamine substituent and inhibits Pim-1 kinase below the limit of detection of our assay (<5 nM). In addition to the improvements in physical properties, enhanced cellular permeability was also observed for compounds **27** and **29**, relative to compound **24**, as measured by MDCK permeability assay.[18]

Table 2
Pim-1 inhibition and physical properties



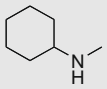
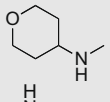
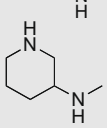
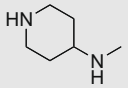
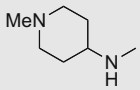
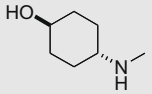
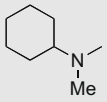
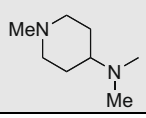
Compound	R	PIM-1 K_i, (nM)[15]	*c* log *P*	*c* log $D_{7.4}$	Solubility (μM)[17]	MDCK permeability[18] A–B/B–A
24		11	4.9	5.0	<0.3	0.6/0.2
25		44	2.5	3.2	46	NA
26		94	3.1	1.1	>200	NA
27		21	2.5	0.2	>200	1.4/13.2
28		160	2.9	1.6	>200	NA
29		<5	2.8	3.9	170	35.3/49.0
30		49	5.1	5.7	<0.3	NA
31		100	3.1	2.4	>200	NA

Table 3
Kinase selectivity profile of compounds **27** and **29**

Kinase	**27** K_i (nM)	**29** K_i (nM)	Kinase	**27** K_i (nM)	**29** K_i (nM)
Pim-1	21	<5	JNK3	>4000	>4000
Aur2	800	NA	KDR	>4000	>4000
CDK2	>4000	3100	MAP3K7	NA	>4000
COT	NA	>4000	MET	>4000	>4000
ERK2	NA	>4000	p38α	>4000	>4000
Flt3	1400	2300	PKA	>4000	>4000
GSK3b	2900	230	PKCθ	>4000	1800
IRAK4	>4000	2300	Plk1	>4000	>4000
ITK	>4000	>4000	ROCK	>4000	>4000
JAK2	>4000	2400	Src	>4000	>4000
JAK3	>4000	2300	Syk	>4000	>4000

Compounds **27** and **29** were further screened against a panel of kinases and showed a high level of selectivity (Table 3).

In summary we have developed a series of highly selective and potent Pim-1 kinase inhibitors that derive selectivity from an unorthodox binding mode to the unique hinge region of Pim-1. X-ray crystallographic data on an advanced molecule allowed for further refinement in the physicochemical property profile of this series resulting in compounds with excellent solubility and improved cellular permeability.

Acknowledgments

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