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Discovery and evaluation of 3,5-disubstituted indole derivatives as Pim kinase inhibitors

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ABSTRACT

Pim kinases are promising therapeutic targets for the treatment of hematological cancers. A potent Pim kinase inhibitor **7f,** derived from meridianin C, was further optimized by the replacement of 2-aminopyrimidine with substituted benzene. The optimization of the C-3 and C-5 positions of indole yielded compound **43** with improved cellular potency and high selectivity against a panel of 14 different kinases.

Pim proteins, which comprise three homologous forms, Pim-1, Pim-2, and Pim-3, are members of a family of serine/threonine kinases that regulate various signaling pathways involved in cancer development and progression.[1] Pim kinases are frequently activated in both hematologic and solid cancers and phosphorylate numerous downstream substrates that are thought to contribute to tumor growth and survival.[2] Although Pim isoforms have different expression levels and distinct roles, there is compelling evidence of compensatory mechanism for all three *Pim* genes in cancer.[3–7] Therefore, efforts to develop a pan-Pim kinase inhibitor targeting all three isoforms are ongoing across many research institutes and in the pharmaceutical industry.

Hydrophobic interactions with the residues in the periphery of the hinge region of Pim kinase are considered one of the key factors for the tight binding of ligand.[8,9] For example, the substitution of Val126 in Pim-1 by Ala122 in Pim-2 in the binding pocket is believed to contribute to the low km value of Pim-2. Previously, we reported a novel series of meridianin C derivatives with strong inhibitory activities against Pim-1 and Pim-3 kinases.[10] However, their cell growth inhibitory activities were not as potent as their enzymatic inhibitory activities. As shown by the modeling study, the 2-aminopyrimidine ring of the meridianin C derivatives can form H-bond interactions, but the ring does not interact with hydrophobic residues within the hinge region. To improve the cellular potency of the meridianin C derivatives, we then attempted to substitute the 2-aminopyrimidine with a phenyl moiety, which could accommodate various functional groups to provide other interactions with the enzyme and improve the physicochemical properties of the compounds.

Since the preliminary experiments (**meridianin C** vs. **1**; **7a** vs. **2**; **7f** vs. **3** in Table 1) showed that 2-aminopyrimidine could be replaced by a phenyl group with appropriate substituents, further variations of the substituents and their positions on the phenyl ring were tested to identify the optimal replacement for the 2-aminopyrimidine.

The optimization efforts were started with the modification of the phenyl moieties, while the substituent at the C-5 position of indole was fixed with a 6-(2-(dimethylamino)ethyl)aminopyrazin-2-yl group. Analogs were synthesized according to Scheme 1. The iodination at the C-3 position of 5-bromoindole was conducted by using *N*-iodosuccinimide formed *in situ*, followed by *N*-1 protection with *p*-toluenesulfonyl chloride to yield compound **5**. The reaction of compound **5** with the aryl boronic esters **6** yielded compound **7**. Miyaura borylation at the C-5 position of compound **7** and Suzuki coupling with Het-Cl **9** afforded compound **10**. The cleavage of Ts and Boc under basic and acidic conditions, respectively, generated compound **11**.

The Pim kinase inhibitory activities of the monosubstituted analogs are shown in Table 2. The electronic properties of the substituents on the phenyl ring did not result in any meaningful effect on the inhibitory activity of the compound. However, the inhibitory activity depended on the position of the substituent, especially for Pim-2 kinase. The amino group showed a preference for the *para* position over the *meta* position in all three kinases (**15** vs. **16**). In addition, the fluoro group showed a high preference for the *ortho* position over the *para* and *meta* positions (**23**, **24**, and **25**, respectively). Three substituents, specifically the trifluoromethyl, trifluoromethoxy, and phenyl groups (**13**, **21**, and **26**, respectively), at the *para* position had lower activity for Pim-3 kinase,

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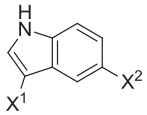
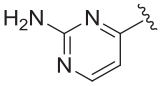
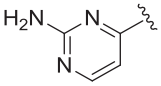
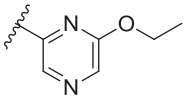
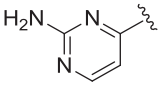
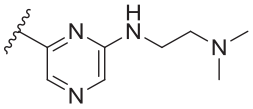
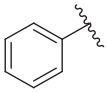
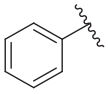
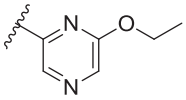
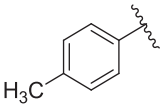
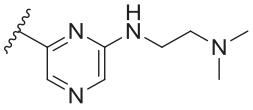
Table 1
Effect of substituent variation on C-3 and C-5 position of indole ring.



	X^1	X^2	IC_{50} (μM)		
			Pim-1	Pim-2	Pim-3
Merid C			1.0	ND[a]	ND
7a[b]			0.05	0.74	0.05
7f[b]			0.003	0.11	0.007
1			> 10	ND	ND
2			> 10	> 10	> 10
3			0.019	1.8	0.033

[a] Not determined.
[b] Ref. 10.

by an order of magnitude.

The cell growth inhibitory activities of the compounds were screened with the MV4-11 cell line (Fig. 1). There was no clear correlation between enzyme inhibitory activities and cell growth inhibitory activities. The trifluoromethyl and phenyl groups at either the *meta* or the *para* position were not potent kinase inhibitors, but they intensely inhibited cell growth at 10 μM concentration. In contrast, the *p*-methansulfonyl and *p*-sulfonamide groups (**27** and **28**, respectively) strongly inhibited both Pim kinases and cell growth.

Next, the effect of the substituent at position 6 of pyrazine was studied using either a *p*-trifluoromethylphenyl or an *o*-fluorophenyl group at position 3 of indole (Table 3). The activity of the compound was affected by the type of attached atom and the length of carbon linkage between the pyrazine and aminoalkyl groups. As suggested previously,[10] the H-bond interaction between the amino group at position 2 of pyrazine and the carbonyl group of Asn172 in Pim-1 increased the inhibitory activity by approximately five-fold (**13** vs. **30** or **35**). However, the H-bonding was insignificant when the inhibitory activity was low, owing to the non-optimal length of the carbon linkage (**33** vs. **31** or **34**). It was found that either homopiperazine or 3-aminopiperidine (**38** and **39**, respectively) could be attached to position 6 of pyrazine instead of the aminoalkyl chain.

On the basis of the above results, the Pim inhibitors were further optimized by combining two substituents on the phenyl group (Table 4). The combinations of CN and NH_2 (*meta* and *para* position, respectively, **43** vs. **15** and **18**) or F and NH_2 (*ortho* and *para* positions, respectively, **48** vs. **15** and **25**) enhanced the synergistic inhibitory activity for all three Pim isoforms (Table 4). However, the combination of F and CF_3 at either the *meta* or *para* position (**42** vs. **13** and **24**, **41** vs. **14** and **23**) had a detrimental effect. The acetyl group resulted in the best inhibitory activity for all Pim kinases among the substituents at position 5 (**46** vs. **44** and **45**).

Docking studies revealed that 3,5-disubstituted indole compounds bind in the ATP pocket of Pim-1 using two hydrogen bonds (Fig. 2), which are formed between the indole moiety and the carbonyl group of Glu121 and between the pyrazine moiety and Lys67.[11–13] The phenyl group at position 3 of the indole is packed against the side chain of Leu44 and Val126. This additional interaction explains partly the selectivity of the compound for Pim-1 over Pim-2, which has Ala122 instead of Val126. In the compound **42**, the CF_3 group in the *para* position of the phenyl ring is in close proximity to the backbone carbonyl group of Leu44 and hinders the binding of the compound.

The growth of the MV4-11 cell line was studied after treatment with the compounds (Table 5) that were selected based on an initial screening (Fig. 3). Compound **43** potently inhibited cell growth, with an IC_{50} value of 0.8 μM, which is comparable with the values reported for the Pim kinase inhibitors in the literature (Pim447 and AZD1208, 0.13 μM and 0.9 μM, respectively).[14,15] The high cellular potency of SGI1776 results in part from its ability to inhibit both Pim and Flt3 kinases.[16] In addition, compound **43** showed good selectivity for MV4-11 over HepG2 cells, as shown by FACS analysis of the HepG2 cell line (Table 5).

A kinome profiling study of compound **43** showed that it had a high degree of selectivity against 14 protein kinases, regardless of serine-