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The discovery and optimization of aminooxadiazoles as potent Pim kinase inhibitors.

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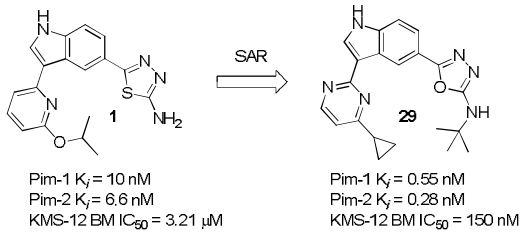
Abstract—High levels of Pim expression have been implicated in several hematopoietic and solid tumor cancers. These findings suggest that inhibition of Pim signaling by a small molecule Pim-1,2 inhibitor could provide patients with therapeutic benefit. Herein, we describe our progress towards this goal starting from the highly Pim-selective indole-thiadiazole compound (**1**), which was derived from a nonselective hit identified in a high throughput screening campaign. Optimization of this compound's potency and its pharmacokinetic properties resulted in the discovery of compound **29**. Cyclopropane **29** was found to exhibit excellent enzymatic potency on the Pim-1 and Pim-2 isoforms (K_i values of 0.55 nM and 0.28 nM, respectively), and found to inhibit the phosphorylation of BAD in the Pim-overexpressing KMS-12 cell line (IC_{50} = 150 nM). This compound had moderate clearance and bioavailability in rat (CL = 2.42 L/kg/h; %F = 24) and exhibited a dose-dependent inhibition of p-BAD in KMS-12 tumor pharmacodynamic (PD) model with an EC_{50} value of 6.74 μM (18 μg/mL) when dosed at 10, 30, 100 and 200 mg/kg P.O. in mice.



Proviral **I**ntegration site of **M**oloney (Pim) murine leukemia virus kinases are serine/threonine kinases that are involved in cell survival and proliferation as well as a number of other signal transduction pathways.[1,2] 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[3] including multiple myeloma,[4] acute myeloid leukemia, prostate cancer, and gastric and liver carcinomas. These findings suggest that inhibition of Pim signaling by a small molecule pan-Pim inhibitor could provide patients with therapeutic benefit since the upregulation of Pim kinases correlates with a poor prognosis.[1] In addition to cancer, Pim kinases have been reported to play a role in several autoimmune diseases.[5]

All three Pim isoforms share the unique feature of being the only enzymes in the kinome with a proline residue in the hinge, which results in only one hydrogen bond interaction with ATP.[6] This unique property can be exploited to derive selectivity over other kinases. In addition, the $K_{M,ATP}$ for Pim-2 is 136-fold lower than that for Pim-1.[7] As a consequence of this phenomenon, Pim inhibitors that have potent cellular activities in pan-Pim expressing cell lines has been very challenging. Pim directly phosphorylates Bcl-2-associated death promoter (BAD), which becomes sequestered in p-BAD-(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.[8] To evaluate the cell potency of novel Pim inhibitors, a multiple myeloma KMS-12 BM cell line[9] with a readout of the inhibition of phosphorylation of BAD (p-BAD) was chosen to interrogate chemical matter in this manuscript.

Numerous research groups have investigated small molecule Pim inhibitors[10,11] which resulted in several clinical compounds such as SGI-1776[12] and AZD-1208[13] (Fig. 1). Herein, we describe our progress towards the development of a novel class of Pim-1,2 inhibitors based on an aminooxadiazole-indole scaffold.

INSERT FIGURE 1 HERE

Compound **1** resulted from an effort to improve Pim potency and kinase off-target selectivity of the indazole-thiazole high-throughput screening (HTS) hit.[14] While compound **1** exhibited a favorable selectivity profile, the Pim potency and oxidative stability of the molecule were seen as major limitiations. Improvements in potency could be achieved upon replacement of the aminothiadiazole

motif with an aminooxadiazole (**2** versus **1,** Table 1).[15] Although rat liver microsomal turnover was modest, rat pharmacokinetics of **2** yielded high clearance with low bioavailability (CL 3.9 (L/kg)/h; %F = 3.5, Table 5). The parent compound, lacking substitution at the 3-position of the indole ring (R = H, **3**), displayed greatly diminished activity in the Pim-1 and Pim-2 enzyme assays (K_i values >1 μM). Removal of the pyridine nitrogen present in compound **2** was also greatly detrimental (**4**). Replacement of the *iso*-propoxy group with a morpholine (compound **5**) resulted in erosion in enzymatic and cellular potency as compared to compound **2** with a small improvement in microsomal stability. Introduction of an additional nitrogen atom into the pyridine ring of compound **2** resulted in compound **6** which was roughly equipotent to compound **2** but whose microsomal stability was somewhat diminished. Replacement of the *iso*-propoxy group with either a difluoropiperidine or a difluoropyrrolidine (compounds **7**-**8**) resulted in an improvement in potency, however, these substitution patterns led to a decline in microsomal stability. Introduction of a 4-cyclopropylpyrimidin-2-yl group (compound **9**) was one substitution that resulted in a modest improvement in Pim-2 enzymatic potency along with a 2-fold gain in cellular potency (IC_{50} = 319 nM) as compared to compound **2**. Furthermore, this substitution pattern also resulted in a favorable improvement in microsomal activity. Rat pharmacokinetics demonstrated a nominal improvement in rat iv clearance (CL 2.9 (L/kg)/h) compared to compound **2**, however, the problem of low bioavailability persisted (%F = 1.2, Table 5). The cyclopropyl group was an important driver of potency as its removal (compound **10**) led to a large decrease in enzyme potency. The positions of the two nitrogen atoms in the 4-cyclopropylpyrimidin-2-yl ring were somewhat important as introduction of a 6-cyclopropylpyrazin-2-yl ring (compound **11**) was slightly inferior to compound **9** both in terms of cellular activity and microsomal activity. Other substitution patterns on the pyrimidine ring were briefly explored (see compounds **12-17**), however, the combination of good cellular potency and microsomal stability did not surpass compound **9**.

INSERT TABLE 1 HERE

We explored replacements for the aminooxadiazole motif in an attempt to further drive cellular potency and improve PK properties. These modifications included triazolopyridine and triazolopyridazine rings (compounds **18-19**, Table 2) which preserved the two hydrogen bond acceptors of the oxadiazole ring. Presumably the introduction of the second nitrogen in compound **19** allowed the compound to adopt a planar conformation and this proved beneficial for the enzyme and cellular potency of this compound. Unfortunately, **19** exhibited higher microsomal turnover compared to compound **9**. Introduction of pyrimidin-4-one or cyclopropylpyrazine[16] motifs were also explored (compounds **20-21**), but these compounds were found to lack sufficient enzyme potency.[17]

INSERT TABLE 2 HERE

In an attempt to remedy the rapid clearance of compounds **2** and **9**, we returned to explore the introduction of various substitutions on the oxadiazole ring. Replacement of the amino-group with three all-carbon-based substitutions with increasing size including a cyclopropyl, *iso*-butyl or a phenyl ring led to a loss in enzymatic potency (compounds **22**-**24,** Table 3). A more promising avenue of exploration resulted from introduction of small alkyl groups to the primary amine of the aminooxadiazole motif thereby preserving a single hydrogen bond donor. In particular, compound **25**, which contains a 1,1,1-trifluoroethyl group, exhibited subnanomolar potency in the Pim-1 and Pim-2 enzyme assays, however, this improvement did not translate into improved cellular potency. Unfortunately, this substitution also led to erosion in microsomal stability. Rat pharmacokinetics of this compound again showed high iv clearance (CL = 2.98 (L/kg)/h) but a promising improvement in bioavailability (%F = 25) when compared to compound **9** (Table 5). Introduction of an *iso*-propyl group (**26**) again gave excellent enzymatic potency in both Pim-1 and Pim-2 enzyme assays but with a smaller cell-shift than observed with the 1,1,1-trifluoroethyl group. This compound also showed similar microsomal stability to compound **9**. Rat pharmacokinetics showed a slight improvement in iv clearance compared to compound **9** with a similar increase in bioavailability (CL 2.33 (L/kg)/h; %F =

20, Table 5) as seen with compound **25**. A cyclopropyl substitution (compound **27**) was found to have decreased microsomal stability and was slightly less potent in the cellular assay. Introduction of additional polarity in an attempt to lower the cLogP of the compound using an oxetane ring (compound **28**) lead to erosion in the enzyme potency, however, the shift in the cellular assay was not a large as was the case with previous compounds. Expanding the size of the substituent on the amino-group to a *tert*-butyl group (**29**) gave a small improvement in microsomal stability compared to compound **26** and a small gain in cellular potency (KMS-12 IC_{50} = 150 nM). The pharmacokinetic properties of this compound were virtually identical to compound **26** (CL 2.43 (L/kg)/h; %F = 19, Table 5). Finally, larger groups such as a 3-fluorophenyl substitution (**30**) were not well tolerated in terms of potency.

INSERT TABLE 3 HERE

In a further attempt to address the poor pharmacokinetic properties of this series, we took two different approaches: introduction of fluorine atoms to protect potential metabolic soft spots, and introduction of polarity to reduce cLogP. We believed that introduction of a 5-fluoro-substitution extending from the 4-cyclopropylpyrimidin-2-yl ring into the solvent exposed portion of the inhibitor should be tolerated and could possibly improve the microsomal stability. Unfortunately, compound **31** bearing this substitution displayed a moderate loss of enzymatic potency as compared to **26** with little improvement on microsomal stability. In an attempt to reduce the cLogP of the comopund, an amide function was introduced at the 5- and 6-positions of the 4-cyclopropylpyrimidin-2-yl ring (compounds **32-33**). The 6-substitution (compound **33**) was preferred in terms of cellular potency, however, this compound had inferior microsomal stability to its regioisomer in the 5-position (**32**). Removal of the cyclopropyl group (compound **34**) resulted in improved microsomal stability but also resulted in a dramatic loss in enzyme potency. Modifications to the indole ring in an attempt to improve PK prompted the synthesis a 6-fluoro-indole[18] analog (compound **35**). This substitution was tolerated in terms of cellular potency and led to a small improvement in rat iv clearance (CL = 2.16 (L/h)/kg), however, this modification was detrimental to oral bioavailability (%F = 6). Introduction of a nitrogen

atom into the indole ring at the 6-position (compound **36**)[19] was beneficial for microsomal stability; however, its introduction ablated cellular potency.

INSERT TABLE 4 HERE

The X-ray co-crystal structure of compound **29** in the Pim-1 protein was obtained (Fig. 2). This structure revealed a number of key interactions with the Pim-1 protein that likely contributed to potency. The N–H of the indole engages in a hydrogen bonding interaction (3.1 Å) with the carbonyl of the Glu121 residue of the linker region of the ATP binding pocket. The benzene ring of the indole sits against the gatekeeper residue Leu120 and potentially gains van der Waals interactions. Additional favorable hydrophobic contacts are observed between the pyrimidine ring and Val126 and Leu174 residues along with the *tert*-butyl group and Ile185. One of the nitrogen atoms in the oxadiazole ring engages in a hydrogen bonding (2.9 Å) interaction with the conserved catalytic Lys67 while the *tert*-butylamino group donates a hydrogen bond to form what is likely a weak hydrogen bonding interaction with the Asp186 residue (3.4 Å). The cyclopropyl group and *tert*-butyl group are facing each other (3.4 Å and 3.7 Å) to give the inhibitor a horseshoe like shape. Finally, the Phe49 residue is displaced from the binding pocket to accommodate the ligand.

INSERT FIGURE 2 HERE

A representative synthesis of this class of Pim inhibitors is depicted in Scheme 1 where compound **29** is prepared in a seven step sequence.[20] Commercially available 1*H*-indole-5-carboxylic acid (**37**) was selectively iodinated at the 3-position in high yield using iodine and potassium hydroxide in DMF to afford the corresponding 3-iodoindole **38**.[21] Protection of the indole with a tosyl group yielded compound **39**. The hydrazide **40** was then prepared using a HOBt/EDAC mediated coupling which was then converted into the desired *tert*-butylaminooxadiazole **41** upon heating with *tert*-butyl-isothiocyanate followed by treatment with EDAC.[22] Transformation of the protected 3-iodoindole **41** into its

corresponding pinacolatoboron reagent was accomplished using standard borylation conditions[23] affording the key intermediate for analoging (compound **42**). A Pd-catalyzed Suzuki coupling with commercially available 2-bromo-4-cyclopropylpyrimidine then afforded the fully elaborated inhibitor **43**.[24] Tosyl group deprotection with 1N NaOH in dioxane afforded compound **29** in moderate yield (55%).[23]

INSERT SCHEME 1 HERE

INSERT TABLE 5 HERE

Based on the best-in-series rat PK properties and cellular potency, compound **29** was selected for further evaluation in an in vivo mouse tumor pharmacodynamic (PD) model. Female CD1 *nu/nu* mice were implanted with KMS-12 BM tumor cells (5 x 10^6 cells) and dosed orally with **29** at 10, 30, 100 and 200 mg/kg. At 3, 6, 12, 24 h time points post dosing, tumor cells and plasma were harvested. Total p-BAD levels in the tumor were measured with a quantitative MSD assay. Compound **29** showed a dose-dependent inhibition of phosphorylation of BAD, indicating that it effectively inhibited Pim in vivo (Fig. 3). The total plasma concentration for 50% tumor PD reduction (EC_{50}) in this experiment was calculated to be 6.74 μM (18 μg/mL).

INSERT FIGURE 3 HERE

The selectivity of compound **29** against a panel of 112 protein and lipid kinases was examined in the DiscoveRX KINOMEscan platform.[25] In this panel, the competitive binding of **29** at 1 μM was measured as a percentage of control (POC). For all of the kinases assayed, competitive binding (POC <35%) by **29** was observed for 7 kinases including GAK (0.3% POC), CLK4, (4.3% POC), CLK1 (11% POC), CLK2 (11% POC), DYRK1B (20% POC), DYRK1A (27% POC) and CSNK2A1 (34% POC).[26] Compound **29** showed moderate permeability and low efflux (19.8 x 10^{-6} cm/s and ER = 1.8 and 2.5, respectively in mouse and rat LLC-PK1 cell line transfected with a MDR1 gene), and exhibited moderate

plasma protein binding (97.7%, 97.8% and 97.4% bound in human, mouse and rat, respectively). The aqueous solubility of compound **29** was quite low: 0.14 mg/mL, 0.001 mg/mL and 0.005 mg/mL in 0.01N HCl, PBS and SIF, respectively.[27]

In conclusion, a structurally novel class of Pim-1,2 inhibitors containing an aminooxadiazole motif has been prepared. From this series, compound **29** was highly selective against 112 other kinases and had superior cellular potency and PK properties over compound **1**. Compound **29** was efficacious in a tumor PD assay in mice with an EC_{50} value of 6.74 μM (18 μg/mL).

Supplementary data X-Ray data for the co-crystal structure of compound **29** in Pim-1 protein is provided.

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17. It should be noted that compound **21** bears some structural resemblance to naturally occurring meridianin C, a marine natural product. See for example: More, K. N.; Jang, H. W.; Lee, J. *Bioorg. Med. Chem. Lett*. **2014**, *24*, 2424-2428.

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19. Compound **36** bears some structural resemblance to meriolin, a marine derived 7-azaindole natural product which is known to inhibit a range of protein kinases. See for example: Walker, S. R.; Czyz, M. L.; Morris, J. C. *Org. Lett*. **2014**, *16*, 708-711 and references cited therein.

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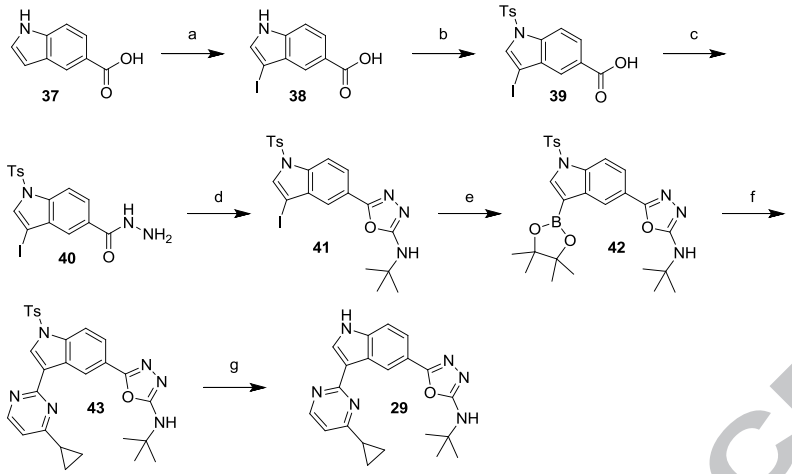
25. For more information see: http://www.discoverx.com/technology/technology-kinomescan.php. The Ambit kinase panel included: Abl, Akt1, Akt2, ALK, AMPK, Aur2, BMPR1A, BRAF, BRK, BTK, CAMK2, CAMK4, CDC7, CDK2, CDK4, CDK8, CDK18, c-KIT, CHK1, CK1δ, CKIIα CHK2, CLK1, CLK2, CLK4, DAPK1, DDR1, DMPK, DYRK1α, DYRK1β, EGFR, EPHA2, EPHA4, EPHB3, Erk1, Erk2, FGFR1, FLT3, FYN, GAK, GRK1, GSK3β, HGK, IGF1R, INSR, IRAK4, IRE1, JAK2, JNK3, KDR, LCK, LIMK1, LYN, MAPK1, MAP2K3, MAP3K1, MAP3K9, MAPK4K1, MAPKAPK2, MARK1, MARK3, MEK5, MET, MNK1b, MSK1, MST2, mTOR, NEK4, NEK6, OXSR1, p38α, p38γ p70S6K, PAK2, PDGFRβ, PI3Kα, PI3Kδ, PIM1, PIM2, PKAα, PKAC2, PKBα, PKBβ, PKC, PKR, PKCβ2, PKD2, PKGα, PLK1, PLK4, PRAKAA1, PRKG1, RAF, RIPK2, ROCK2, RPS6KB1, RSK1, SGK1, SRC, STK33, STK32B, SYK, TAK1, TAO2 TBK1, TIE2, TGFBR1, TRKA, TSSK1β, TYK2, WEE1, YSK4

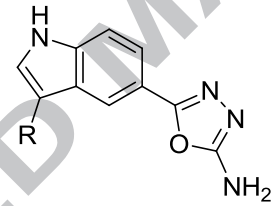
26. For the POC values of the full kinase panel see the Supporting Info.

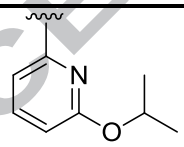
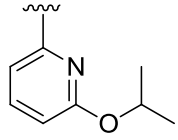
27. Aqueous equilibrium solubility assay was conducted on Symyx Solubility System. 1 mg solid compound was added to each of the three media, equilibrated at room temperature for 48-72 hours, centrifuged and the supernatants were analyzed by HPLC and compared against a standard curve.

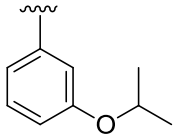
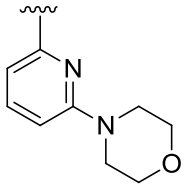
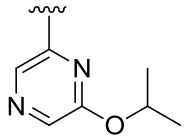
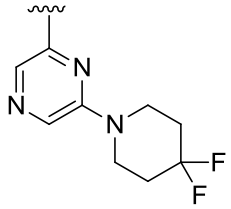
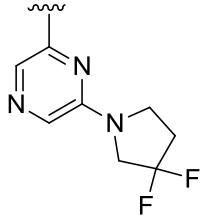
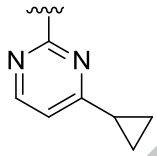
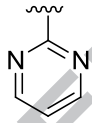
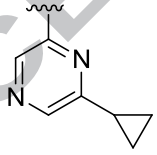
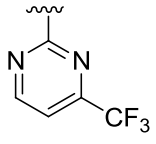
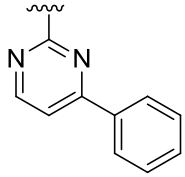


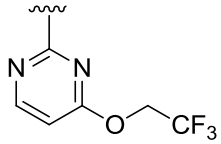
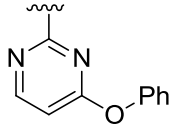
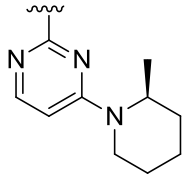
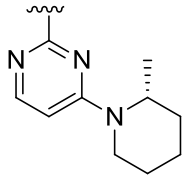
Scheme 1. General scheme for the synthesis of compound **29**. Reagents and conditions: a) I_2 (1.05 equiv.), KOH (2.2 equiv.), DMF, RT, 1 h, 98%; b) NaH (2.5 equiv.), TsCl (1.1 equiv.), DMF, 0 – 10 °C, 30 min; c) NH_2NH_2 (5 equiv.), HOBt (1.2 equiv.), EDAC (1.2 equiv.), DMF, RT, 2 h, 98% (2 steps); d) i) *t*-BuNCS (3 equiv.), THF, 60 °C, 1 h ii) EDAC (1.5 equiv.), DMF, 90 °C, 1 h, 66% (2 steps); e) $(BPin)_2$ (3 equiv.), KOAc (5.0 equiv.), $Pd(dppf)Cl_2$ (15 mol%), DMF, 90 °C, 1 h; f) 2-Bromo-4-cyclopropylpyrimidine (1.15 equiv.), $Pd_2(dba)_3$ (3 mol%), X-Phos (6 mol%), K_3PO_4 (3 equiv.), 5:1 dioxane:water, μwave, 130 °C, 30 min, 56% (2 steps); g) 1N NaOH, dioxane, μwave 100 °C, 15 min, 55%.[1]

Table 1. Enzyme and cellular assay results for compounds arising from modifications to the 3-position of the indole[a]



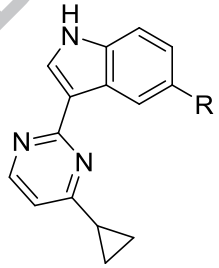
Compd	R	Pim-1 K_i (nM)	Pim-2 K_i (nM)	KMS-12 p-BAD IC_{50} (nM)	RLM/HLM μL/(min*mg)[b]
1[c]		10 ± 2.0	6.6 ± 0.1	3210 ± 1005	85/90
2		1.7 ± 0.23	1.0 ± 0.11	697 ± 54	74/99
3	H	2560	899 ± 83	ND	150/65

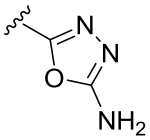
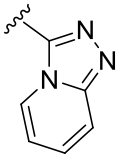
4		55 ± 0.5	116 ± 101	ND	158/>399
5		2.1 ± 0.03	3.4 ± 0.9	1008 ± 187	88/92
6		3.0 ± 0.48	1.2 ± 0.12	468 ± 191	134/182
7		1.6 ± 1.4	1.1 ± 0.92	236 ± 23	147/182
8		0.52 ± 0.042	0.73 ± 0.014	373 ± 18	156/180
9		1.4 ± 0.71	0.27 ± 0.24	319 ± 45	79/16
10		150 ± 58	47 ± 0.67	ND	ND
11		2.0 ± 0.59	1.1 ± 0.30	557 ± 108	124/70
12		31 ± 7.9	8.7 ± 0.98	3280 ± 396	58/40
13		2.1 ± 0.72	0.40 ± 0.17	371 ± 71	160/125

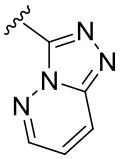
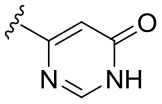
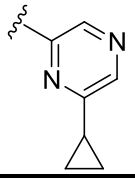
14		2.7 ± 0.94	0.39 ± 0.021	560 ± 35	77/42
15		14.7 ± 3.5	2.2 ± 0.52	1529 ± 71	100/49
16		2.7 ± 1.8	0.15 ± 0.02	160 ± 18	213/332
17		1.1 ± 0.38	0.42 ± 0.31	376 ± 35	614/288

[a] Data represents an average of at least two separate determinations. [b] Single experimental value; estimated clearance from percent parent compound (1 μM) remaining following a 30 min incubation in liver microsomes (0.25 mg/mL) and NADPH (1 mM). [c] This compound contained an aminothiadiazole.

Table 2. Enzyme and cellular assay results for compounds arising from replacement of the aminooxadiazole motif.[a]

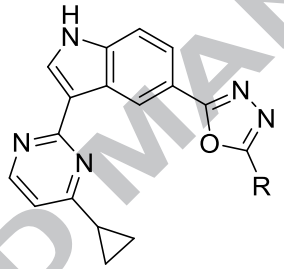


Compd	**R**	**Pim-1 K_i (nM)**	**Pim-2 K_i (nM)**	**KMS-12 p-BAD IC_{50} (nM)**	**RLM/HLM μL/(min*mg)[b]**
9		1.4 ± 0.71	0.27 ± 0.24	319 ± 45	79/16
18		52 ± 9.7	22 ± 7.0	ND	157/195

19		3.0 ± 0.14	1.0 ± 0.13	297 ± 5.7	181/163
20		114 ± 11	60 ± 3.6	ND	140/107
21		32 ± 14	76 ± 47	ND	121/ND

[a] Data represents an average of at least two separate determinations. [b] Single experimental value; estimated clearance from percent parent compound (1 μM) remaining following a 30 min incubation in liver microsomes (0.25 mg/mL) and NADPH (1 mM).

Table 3. Enzyme and cellular assay results for compounds arising from SAR on the oxadiazole ring.[a]

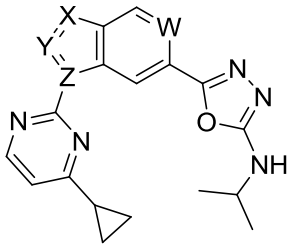


Compd	**R**	**Pim-1 K_i (nM)**	**Pim-2 K_i (nM)**	**KMS-12 p-BAD IC_{50} (nM)**	**RLM/HLM μL/(min*mg)[b]**
9	NH_2	1.4 ± 0.71	0.27 ± 0.24	319 ± 45	79/16
22	*c*-C_3H_5	8.3 ± 3.7	1.4 ± 0.28	18900 ± 5798	257/245
23	*i*-Bu	251 ± 74	177 ± 113	ND	364/251
24	Ph	115 ± 33	355 ± 191	ND	102/58

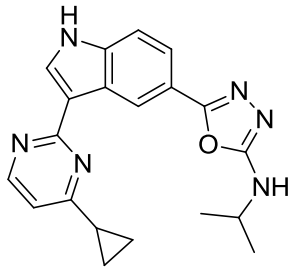
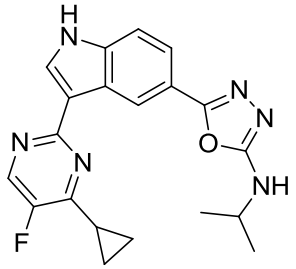
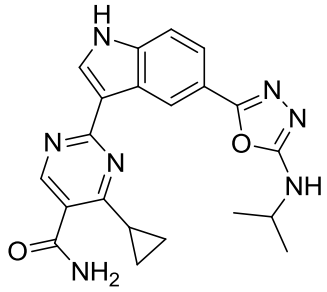
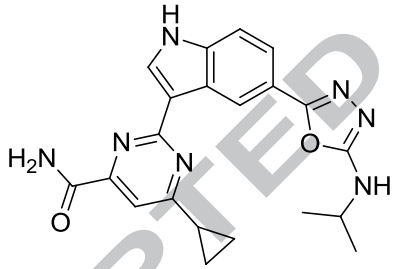
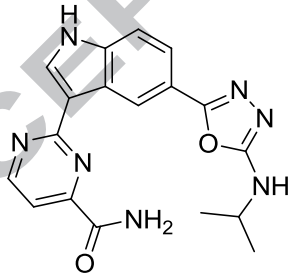
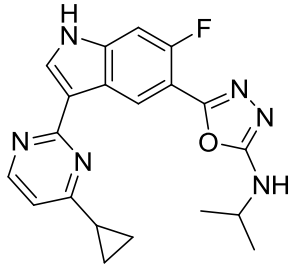
25	NH-CH_2CF_3	0.24 ± 0.008	0.17 ± 0.002	1002 ± 54	162/88
26	NH-*i*-Pr	0.84 ± 0.20	0.30 ± 0.08	207 ± 116	88/87
27	NH-*c*-C_3H_5	1.2 ± 0.36	0.87 ± 0.75	328 ± 54	200/73
28	NH-oxetane	2.2 ± 0.31	0.71 ± 0.26	347 ± 77	ND
29	NH-*t*-Bu	0.55 ± 0.052	0.28 ± 0.072	150 ± 63	59/71
30	NH-3-F-Ph	69 ± 17	38 ± 1.3	ND	102/73

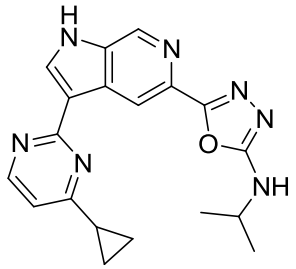
[a] Data represents an average of at least two separate determinations. [b] Single experimental value; estimated clearance from percent parent compound (1 μM) remaining following a 30 min incubation in liver microsomes (0.25 mg/mL) and NADPH (1 mM).

Table 4. Enzyme and cellular assay results for compounds arising from variation of the indole scaffold and peripheral substitutions on the 4-cyclopropylpyrimidin-2-yl ring.[a]



Compd	**Structure**	**Pim-1 K_i (nM)**	**Pim-2 K_i (nM)**	**KMS-12 p-BAD IC_{50} (nM)**	**RLM/HLM μL/(min*mg)[b]**

26		0.84 ± 0.20	0.30 ± 0.08	207 ± 118	88/87
31		1.3 ± 0.42	0.54 ± 0.17	490 ± 46	84/60
32		0.45 ± 0.024	0.34 ± 0.041	673 ± 116	35/37
33		1.6 ± 0.45	0.49 ± 0.072	193 ± 17	177/100
34		54 ± 6.6	14 ± 0.75	ND	57/28
35		1.2 ± 0.091	0.57 ± 0.004	484 ± 21	135/98

36		3.6 ± 1.1	1.7 ± 0.15	>10 000	35/45

[a] Data represents an average of at least two separate determinations. [b] Single experimental value; estimated clearance from percent parent compound (1 μM) remaining following a 30 min incubation in liver microsomes (0.25 mg/mL) and NADPH (1 mM).

Table 5. Pharmacokinetic properties of selected compounds

Compd	iv[b]			po[c]	
	CL ((L/h)/kg)	V_{dss} (L/kg)	$t_{1/2}$ (h)	$AUC_{(0\text{-inf.})}$ (μM*h)	*F* (%)
2	3.90	2.66	1.82	1.64	3.5[d]
9	2.91	4.40	10.2	2.25	1.2[e]
25	2.98	6.34	7.6	1.70	25
26	2.33	2.12	4.13	2.50	20
29	2.43	3.78	4.13	2.28	19
35	2.16	24.9	2.95	2.57	6

[a] Pharmacokinetic parameters following administration in male Sprague Dawley rats; mean values from 3 animals per dosing route. [b] Dosed at 2 mg/kg as a solution in DMSO. [c] po doses were 5 mg/kg in 1% Tween 80, 2% hydroxypropyl methylcellulose (HPMC), 97% water/methanesulfonic acid pH 2.2. [d] po doses were 3 mg/kg. [e] po doses were 2 mg/kg.

[1]. Compound **29** was isolated as a light yellow amorphous solid as its TFA salt. ^{1}H NMR (400 MHz, *DMSO-d*$_6$) δ ppm 11.98 - 12.07 (1 H, m), 8.97 - 9.02 (1 H, m), 8.60 (1 H, d, *J*=5.3 Hz), 8.32 (1 H, d, *J*=2.9 Hz), 7.66 - 7.73 (2 H, m), 7.63 (1 H, d, *J*=8.4 Hz), 7.21 (1 H, d, *J*=5.3 Hz), 2.15 - 2.24 (1 H, m), 1.43 (9 H, s), 1.23 - 1.29 (2 H, m), 1.15 - 1.23 (2 H, m). ^{19}F NMR (377 MHz, *DMSO-d*$_6$) δ ppm -74.99 (1 F, s).

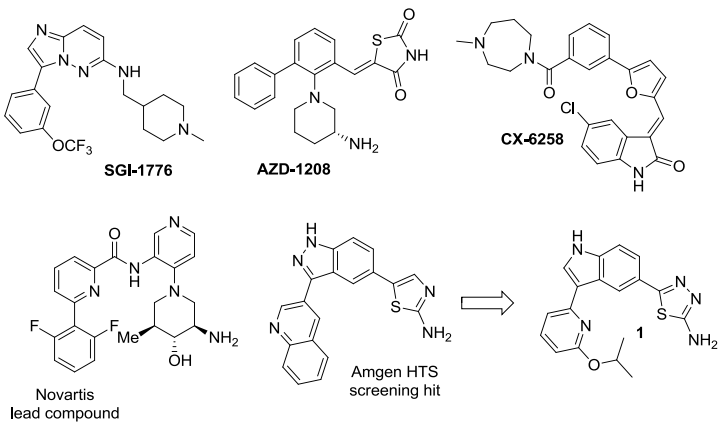


Figure 1. Structures of publicly disclosed Pim inhibitors and Amgen's starting point (compound **1**).

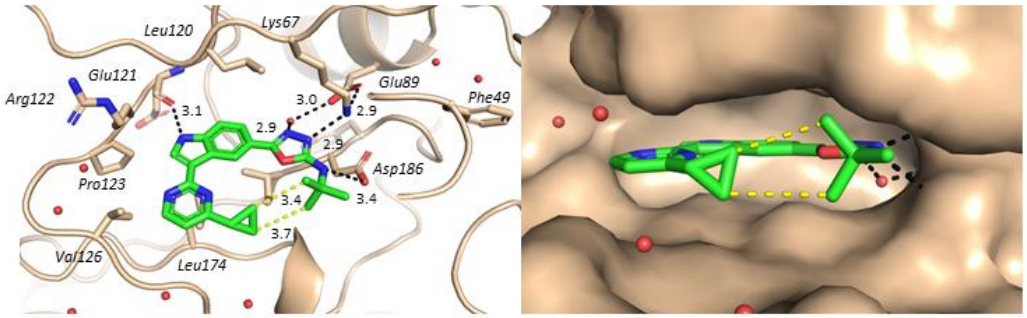


Figure 2. X-ray co-crystal of compound **29** bound in the ATP binding site of unphosphorylated Pim-1 protein determined to 2.7 Å resolution. PDB code: 4TY1. Dashed lines indicate hydrogen bonds (distances are in Angstroms). For the inhibitor, C: green; N: blue; O: red.

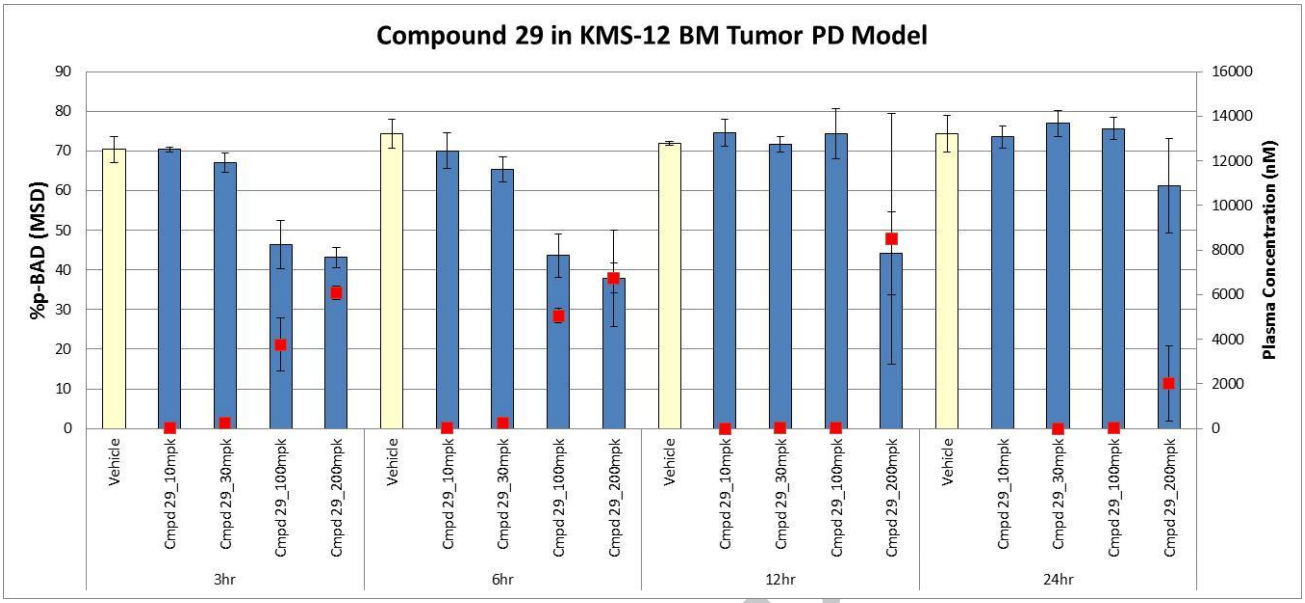


Figure 3. Effect of compound **29** in a KMS-12 tumor pharmacodynamic model measuring the inhibition of phosphorylation of BAD. Oral dose-response study at 4 time points. Statistical significance was evaluated by Dunnett's method. Bars represent the average ± SD (n=3).