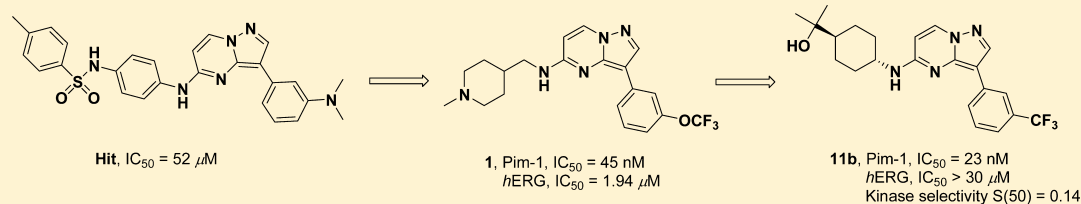


Synthesis and Biological Evaluation of Pyrazolo[1,5-*a*]pyrimidine Compounds as Potent and Selective Pim-1 Inhibitors

Yong Xu,* Benjamin G. Brenning, Steven G. Kultgen, Jason M. Foulks, Adrienne Clifford, Shuping Lai, Ashley Chan, Shannon Merx, Michael V. McCullar, Steven B. Kanner, and Koc-Kan Ho

Astex Pharmaceuticals, Inc., 4140 Dublin Boulevard, Suite 200, Dublin, California 94568 United States

Supporting Information



ABSTRACT: Pim-1 has emerged as an attractive target for developing therapeutic agents for treating disorders involving abnormal cell growth, especially cancers. Herein we present lead optimization, chemical synthesis and biological evaluation of pyrazolo[1,5-*a*]pyrimidine compounds as potent and selective inhibitors of Pim-1 starting from a hit from virtual screening. These pyrazolo[1,5-*a*]pyrimidine compounds strongly inhibited Pim-1 and Flt-3 kinases. Selected compounds suppressed both the phosphorylation of BAD protein in a cell-based assay and 2-dimensional colony formation in a clonogenic cell survival assay at submicromolar potency, suggesting that cellular activity was mediated through inhibition of Pim-1. Moreover, these Pim-1 inhibitors did not show significant *h*ERG inhibition at 30 μM concentration. The lead compound proved to be highly selective against a panel of 119 oncogenic kinases, indicating it had an improved safety profile compared with the first generation Pim-1 inhibitor SGI-1776.

KEYWORDS: Pyrazolo[1,5-*a*]pyrimidine, Pim-1 inhibitor, BAD phosphorylation, 2-dimensional colony formation, *h*ERG inhibition, kinase selectivity

The Pim (provirus insertion site of Moloney murine leukemia virus) genes represent a small family of proto-oncogenes that are encoded by three different serine/threonine protein kinases, Pim-1, Pim-2, and Pim-3, which are highly conserved in vertebrates.^{1,2} Unlike many other protein kinases, Pim kinases do not have a regulatory domain and therefore do not require upstream phosphorylation for activation and are constitutively active once transcribed. The induction of Pim kinase activity is largely regulated at the transcriptional and translational levels. The expression of Pim kinases is relevant for growth factor-induced cell survival and proliferation and is induced by a range of cells; Pim kinases regulate numerous oncogenic processes including cancer cell survival, cytokines, growth factors, and mitogenic stimuli in a large variety of cell types, particularly in cancer apoptosis, migration, and tumorigenesis.^{3,4} As the first discovered and identified Pim kinase, Pim-1 is frequently overexpressed in a wide range of human tumors, including hematological malignancies such as lymphomas and leukemias,^{5,6} and solid tumors, including prostate, bladder, and oral cancers.⁷

The therapeutic potential of Pim-1 inhibition has been demonstrated by siRNA and small molecule inhibitors. Silencing of the Pim-1 gene with siRNA resulted in decreased cell proliferation, inhibition of cell cycle progression, and induction of apoptosis in the PC-3 prostate cancer cell line. In addition, the intratumoral injection of Pim-1 siRNA in nude

mice dramatically suppressed PC-3 tumor progression.⁸ Inhibitors of Pim-1 kinase have recently been reviewed in the literature,³ including both the promiscuous kinase inhibitors and various selective Pim-1 inhibitors. Moreover, the lack of any overt phenotypes in Pim-1 knockout mice indicated that a Pim-1 inhibitor may have a low toxicity profile.² Given the strong correlation of Pim-1 activity and its roles in tumor cell survival and proliferation, Pim-1 kinase is an attractive target for developing therapeutic agents effective for treating disorders involving abnormal cell growth, especially malignancies.

As a continuing effort to discover novel molecules as potent and selective Pim-1 inhibitors, here we describe the synthesis and evaluation of pyrazolo[1,5-*a*]pyrimidine compounds as novel inhibitors of Pim-1 kinase. We employed a virtual screening strategy for the generation and identification of hit compounds as Pim-1 kinase inhibitors. Initial virtual screening of a large number of library compounds identified a pyrazolo[1,5-*a*]pyrimidine as one type of early Pim program hit.⁹ As shown in Scheme 1, this initial pyrazolo[1,5-*a*]pyrimidine hit compound possessed 52 μM potency against

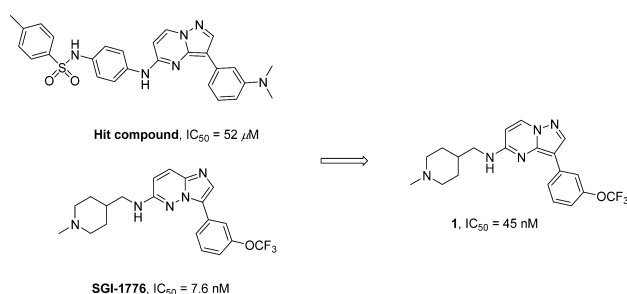
Special Issue: New Frontiers in Kinases

Received: July 25, 2014

Accepted: October 22, 2014

Published: October 22, 2014

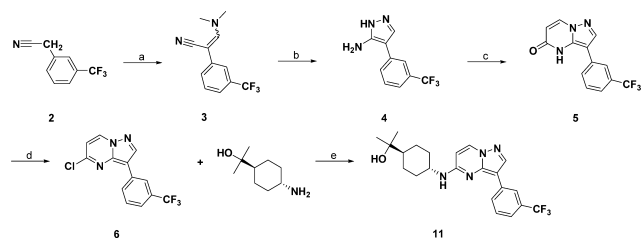
Scheme 1. Generation and Identification of Pyrazolo[1,5-*a*]pyrimidine Hit Compound 1



Pim-1. However, the pyrazolo[1,5-*a*]pyrimidine chemotype was of particular interest to us as the core structure is different from the imidazo[1,2-*b*]pyridazine chemotype compounds (SGI-1776, etc.), which were identified as selective and potent Pim-1 inhibitors, but with significant *h*ERG and CYP inhibition.^{10–13} Starting from this initial hit compound and the imidazo[1,2-*b*]pyridazine candidate SGI-1776, we combined the pyrazolo[1,5-*a*]pyrimidine core with the substituents at the 3,5-positions of SGI-1776 and generated compound **1**, which had an IC_{50} = 45 nM for Pim-1 kinase. On the basis of the structure of compound **1**, we conducted systematic modifications around the pyrazolo[1,5-*a*]pyrimidine core to improve in vitro potency against Pim-1, as well as other critical physicochemical properties.

The synthetic route utilized to build pyrazolo[1,5-*a*]pyrimidines with aryl substitution at the 3-position and a halogen leaving group in the 5-position was a cascade cyclization. Specifically, aryl-substituted acetonitrile (Scheme 2) was treated with *N,N*-dimethylformamide dimethyl acetal to

Scheme 2. Synthesis of Pyrazolo[1,5-*a*]pyrimidine Compounds^a



^aReagents and conditions: (a) *N,N*-dimethylformamide dimethyl acetal, reflux, 4 h, 50%; (b) N_2H_4 , HOAc, ethanol, reflux, 16 h, 97%; (c) *N*-methyl uracil, C_2H_5ONa , ethanol, reflux, 3 h, 62%; (d) $POCl_3$, reflux, 3 h, 67%; (e) DIPEA, *i*PrOH, 130 °C, microwave irradiation, 16 h, 80%.

give the corresponding 3-(dimethylamino)-2-(phenyl)acrylonitrile. The 4-phenyl-1*H*-pyrazol-5-amine was prepared almost quantitatively by the treatment of acrylonitrile **3** with hydrazine and glacial acetic acid in ethanol.¹⁴ The cyclization of 4-phenyl-1*H*-pyrazol-5-amine with *N*-methyl uracil as a masked Michael acceptor in ethanol in the presence of sodium ethoxide provided the key building block 3-phenylpyrazolo[1,5-*a*]pyrimidinone **5** in good yield.¹⁵ Chlorination of the pyrimidinone **5** using $POCl_3$ without solvent gave penult compound 5-chloro-3-phenylpyrazolo[1,5-*a*]pyrimidine, which was used for synthesis of final compounds with different amino groups on the 5-position of 3-phenylpyrazolo[1,5-*a*]pyrimidine. The final amination reaction of *trans*-4-aminocyclohexanol with the 5-

position chloride on 5-chloropyrazolo[1,5-*a*]pyrimidine **6** proceeded selectively with good yield under basic conditions. Despite the presence of a secondary alcohol in *trans*-4-aminocyclohexanol, the amination reaction was highly selective, and no *O*-linked product was detected and separated.¹⁶ The nuclear magnetic resonance (NMR) and mass spectrometry (MS) spectrum unambiguously confirmed the structure of an *N*-linked compound.

Given that the new scaffold pyrazolo[1,5-*a*]pyrimidine (compound **1**) was active against Pim-1 with an IC_{50} = 45 nM, we investigated the optimization of the substituents at the 3- and 5-positions. According to our previous structure–activity relationship (SAR) information, the 5-position of the substituent was critical for potency, particularly the hydrogen bonding interaction with residues Asp-128 and Asp-131 in Pim-1. To maintain this key hydrogen bonding interaction, we replaced the initial (1-methylpiperidin-4-yl)methanamine with various oxygen and nitrogen containing moieties as shown in Table 1, including *trans*-1,4-diaminocyclohexane. Considering that basic amine moieties may induce strong *h*ERG inhibition (observed in compound SGI-1776), we also explored various neutral functional groups at the terminal position of the 5-position substituent, including hydroxyl, ether, and sulfone functional groups. As shown in Table 1, the amino compound **7**

Table 1. SAR of 5-Substituents on Pyrazolo[1,5-*a*]pyrimidine

| Comp. No. | R ¹ | Pim-1 | Pim-2 |
|-----------|----------------|----------------------------------|----------------------------------|
| | | IC ₅₀ nM ^a | IC ₅₀ nM ^a |
| 1 | | 45 | 1491 |
| 7 | | 5 | 100 |
| 8 | | 132 | 987 |
| 9 | | 27 | 269 |
| 10 | | 197 | 2040 |
| 11 | | 204 | 4090 |
| 12 | | 378 | 3620 |
| 13 | | 633 | 3730 |
| 14 | | 148 | 8160 |

^aValues represent the average of at least two separate experiments, and the standard deviation is less than 10% of the mean.

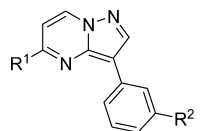
exhibited the most potent Pim-1 inhibition compared with hydroxyl compounds **9**–**11**, ether compounds **12** and **13**, or sulfone compound **14**. Compounds with a hydroxyl group (**9**–**11**) displayed potent inhibition of Pim-1. Ether compounds **12** and **13** and sulfone compound **14**, which do not have an active proton, showed slightly reduced potency on Pim-1. In addition, the ketone compound **16b**, was also active on Pim-1 and Pim-2, which exhibited similar potency compared with ether and sulfone compounds. The SAR of the presence of an active proton enhances Pim-1 inhibition, consistent with the modeling result.

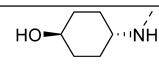
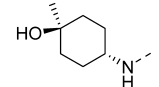
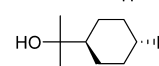
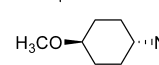
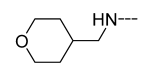
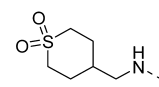
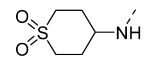
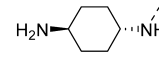
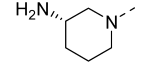
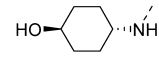
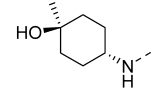
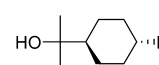
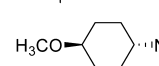
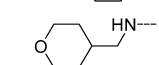
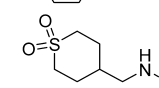
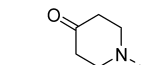
The 3-substituent aromatic ring bearing a meta-position electron-withdrawing group was essential to maintain potency, according to the previous SAR information during the discovery of SGI-1776. Hence, we investigated $-\text{OCF}_3$, $-\text{CF}_3$, and $-\text{Cl}$ at this position. As shown in Tables 2 and 3, all compounds had nanomolar IC_{50} activity on Pim-1. Specifically, compounds with $-\text{CF}_3$ and $-\text{Cl}$ substituents had similar Pim-1 inhibition but had improved Pim-1 inhibition compared to compounds with $-\text{OCF}_3$. It was noteworthy that compound **15a**, which has a shorter chain compared with **14a**, lost potency completely, suggesting that the length of the linker is crucial to maintain hydrogen bonding with Asp-128 and Asp-131 in Pim-1 and potency. The inactivity of compound **15a** also suggested that the 5-position substituent was more important than the 3-position substituent in the pyrazolo[1,5-*a*]pyrimidine for Pim-1 inhibition. Indeed, our analysis of fragment activity also supported this observation. As shown in Scheme 3, the fragment 3-bromo-5-chloropyrazolo[1,5-*a*]pyrimidine **17** was inactive against Pim-1 completely, the 3-substituted 3-aryl-5-chloropyrazolo[1,5-*a*]pyrimidine **18** exhibited 5 μM IC_{50} activity, while the 5-substituted fragment **19** achieved 294 nM IC_{50} , and the 3,5-disubstituted compound **9** had a 27 nM IC_{50} . Apparently, installation of a *trans*-4-aminocyclohexanol group at the 5-position of pyrazolo[1,5-*a*]pyrimidine increased the potency 100-fold, while the introduction of an aryl group at the 3-position only increased the potency 10-fold.

We also tested all compounds against the Pim-2 kinase. All compounds showed relatively weak inhibition of Pim-2 compared with Pim-1, where most of them had low micromolar IC_{50} values against Pim-2, indicating that these pyrazolo[1,5-*a*]pyrimidine compounds were selective between Pim-1 and Pim-2.

Subsequently, we evaluated selected compounds for their effect on *h*ERG potassium channels by using an automated patch clamp assay (QPatch^{HTX}). As expected, the amino compound **1**, which has a basic tertiary amino group, exhibited strong *h*ERG inhibition with an $\text{IC}_{50} = 1.94 \mu\text{M}$. Compounds with neutral terminal hydroxyl moieties, *trans*-4-aminocyclohexanol (compounds **9** and **9a**) and 2-(*trans*-4-aminocyclohexyl)propan-2-ol (compounds **11a** and **11b**), did not show observable *h*ERG inhibition at 30 μM . This demonstrated that the potent *h*ERG inhibition of both compound **1** and SGI-1776 were induced by the terminal basic moieties, either the primary amino group or the tertiary amino group.¹⁷ Removing the basic moiety completely addressed the *h*ERG inhibition in this series of compounds. We also tested selected compounds for Flt-3 IC_{50} and the data are shown in Table 3. These selected pyrazolo[1,5-*a*]pyrimidine compounds strongly inhibited both Pim-1 and Flt-3 as dual inhibitors; however, these compounds (**9**, **9a**, **11a**, and **11b**) were 2–15-fold more potent against Pim-1 than Flt-3.

Table 2. SAR of Substituents on Pyrazolo[1,5-*a*]pyrimidine



| Comp. No. | R ¹ | R ² | Pim-1 IC ₅₀ nM ^a | Pim-2 IC ₅₀ nM ^a |
|------------|--|----------------|--|--|
| 9a |  | $-\text{CF}_3$ | 25 | 282 |
| 10a |  | $-\text{CF}_3$ | 54 | 639 |
| 11a |  | $-\text{CF}_3$ | 17 | 525 |
| 12a |  | $-\text{CF}_3$ | 66 | 4660 |
| 13a |  | $-\text{CF}_3$ | 149 | 1970 |
| 14a |  | $-\text{CF}_3$ | 111 | 6020 |
| 15a |  | $-\text{CF}_3$ | NA ^b | NA ^b |
| 7b |  | $-\text{Cl}$ | 24 | 732 |
| 8b |  | $-\text{Cl}$ | 94 | 2005 |
| 9b |  | $-\text{Cl}$ | 237 | 506 |
| 10b |  | $-\text{Cl}$ | 378 | 1260 |
| 11b |  | $-\text{Cl}$ | 23 | 228 |
| 12b |  | $-\text{Cl}$ | 68 | 1020 |
| 13b |  | $-\text{Cl}$ | 186 | 1640 |
| 14b |  | $-\text{Cl}$ | 164 | 2070 |
| 16b |  | $-\text{Cl}$ | 168 | 564 |

^aValues represent the average of at least two separate experiments, and the standard deviation is less than 10% of the mean. ^bNA, not active.

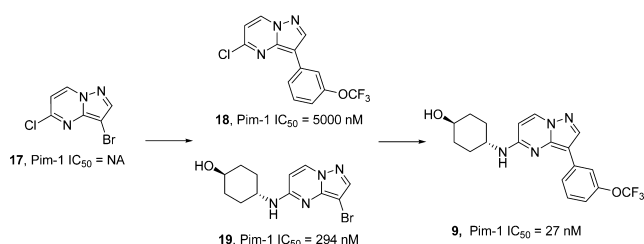
The cellular potency of the pyrazolo[1,5-*a*]pyrimidine compounds was evaluated by measuring their effects on baseline phosphorylation of BAD (BCL-2 antagonist of cell death) protein on serine 112, a known substrate of Pim-1. All 4 tested compounds (**9a/b** and **11a/b**) showed 68–77% inhibition of BAD phosphorylation (normalized to nontreated cells, see Supporting Information) at 1 μM concentration for 45 min (see Supporting Information Figure 1). This demonstrated that these tested compounds exhibited potent Pim-1 cellular activity (sub- μM IC_{50}).

Our recent studies demonstrated that Pim-1 mRNA was significantly reduced using independent *sh*RNAs targeting Pim-

Table 3. *h*ERG IC₅₀ and Flt-3 IC₅₀ values of selected compounds

| Comp. No. | <i>h</i> ERG IC ₅₀ , μM ^a | Flt-3 IC ₅₀ , nM ^a |
|------------|---|--|
| 1 | 1.9 | ND ^b |
| SGI-1776 | <1.0 | ND ^b |
| 9 | >30 | 157 |
| 9a | >30 | 53 |
| 11a | >30 | 271 |
| 11b | >30 | 125 |

*h*ERG IC₅₀ was determined by WuXi AppTec using the QPatch^{HTX} functional *h*ERG assay as described in the experimental section. The Flt-3 IC₅₀ of reference compound staurosporine was less than 1 nM. ^aValues represent the average of at least two separate experiments, and the standard deviation is less than 10% of the mean. ^bND, not determined.

Scheme 3. Analysis of Fragment Activity

1 compared to the nontarget *sh*RNA control in UM-UC-3 epithelial bladder carcinoma cells.¹⁸ Further, Pim-1 protein was reduced using Pim-1 *sh*RNA compared to nontarget *sh*RNA, and UM-UC-3 2-dimensional colony growth was markedly reduced with Pim-1 knockdown.¹⁸ In this study, we also evaluated compound **11b**'s cytostatic effect on colony growth of UM-UC-3 and HSC-3 (human oral squamous carcinoma cells) cells. The clonogenic cell survival assay reflects long-term cytostatic effects caused by anticancer agents and is considered a standard for measuring long-term cell viability since it reveals multiple modes of cell death or arrest.¹⁹ The clonogenic cell survival data (Supporting Information Figure 2) indicated that compound **11b** inhibited and reduced the growth of both UM-UC-3 and HSC-3 with EC₅₀ values of 0.914 and 0.600 μM, respectively, further confirming that the cellular activity of compound **11b** was mediated through inhibition of Pim-1.

Given that most kinase inhibitors interact with the hinge motif within the ATP pocket, which is highly conserved between members of this family, the kinase selectivity profile of any kinase inhibitor is particularly important for predicting and interpreting the effects of the inhibitor in both research and clinical settings. The lack of kinase selectivity is one of the major hurdles to discover and develop a kinase inhibitor with an acceptable therapeutic window and minimal side effects.^{20–22} Therefore, the kinase selectivity of compound **11b** was investigated by screening it against a panel of 119 oncogenic kinases (oncoKP, Reaction Biology Corporation, PA, USA) at a single concentration (1 μM). It was observed that Pim-1 was the most potently inhibited kinase, with inhibition of >98% at 1 μM. The only other kinase with more than 95% inhibition at 1 μM concentration of **11b** was TRKC (96% inhibition at 1 μM). In addition, compound **11b** only inhibited three kinases (TRKC, Flt-3, and TRKB) with more than 90% inhibition and 16 kinases with 50% inhibition at 1 μM concentration. The overall selectivity was determined by calculating the selectivity score for this molecule ($S(50) = 0.14$,

Table 4). (The complete kinase selectivity profiling is available in the Supporting Information.) We conclude that the

Table 4. Kinase Selectivity Profile of Compound **11b**

| selectivity score $S(50)^a$ | 0.14 |
|----------------------------------|---|
| >95% inhibition at 1.0 μM | Pim-1, TRKC |
| 95% to >80% inhibition at 1.0 μM | Flt-3, TRKB, LCK, Haspin, MUSK, TRKA, HIPK4 |
| 80% to >50% inhibition at 1.0 μM | YES1, CK2a, HIPK2, Pim-2, FYN, JAK2, FMS, c-Src |

^a $S(50)$ indicates the ratio of kinases in a panel (119 total) inhibited by >50% at 1 μM concentration.

pyrazolo[1,5-*a*]pyrimidine core interacts selectively with Pim-1. Although the scaffold pyrazolo[1,5-*a*]pyrimidine has been reported as a Pim-1 inhibitor in the literature,^{23–25} herein we demonstrated that the pyrazolo[1,5-*a*]pyrimidine scaffold was a highly selective Pim-1 inhibitor against a comprehensive kinase panel (119 oncogenic kinases). In addition, this is the first report that the *h*ERG issue has been successfully addressed without compromise of potency for Pim-1 inhibition, which could be useful to guide *h*ERG optimization for another series of Pim-1 inhibitors, as well as other kinase inhibitors.

In conclusion, we present the chemical synthesis and SAR studies of novel pyrazolo[1,5-*a*]pyrimidine compounds as selective and potent Pim-1 and Flt-3 kinase inhibitors. A convenient synthesis of the 3-aryl-5-amino-pyrazolo[1,5-*a*]pyrimidine scaffold was achieved to readily access diversely substituted analogues. Most of the tested pyrazolo[1,5-*a*]pyrimidine compounds exhibited nanomolar inhibitory activity of Pim-1. In addition, four compounds (**9a/b** and **11a/b**) showed strong inhibition of BAD phosphorylation in a cell-based assay at 1 μM concentration, suggesting the potent cellular activity may be induced through the inhibition of Pim-1. Compound **11b** also potently inhibited 2D cell colony formation in clonogenic cell survival assays mimicking Pim-1 knockdown by *sh*RNA, further confirming the cellular activity of this compound was mediated through inhibition of Pim-1. Finally, we determined that the pyrazolo[1,5-*a*]pyrimidine compounds not only have a pharmaceutically acceptable *h*ERG profile (>30 μM) but also possess an excellent kinase selectivity profile, critical properties for the development of a therapeutic kinase inhibitor. The *in vivo* efficacy studies in tumor xenografts using this series of Pim-1 inhibitors will be disclosed separately.

ASSOCIATED CONTENT**S** Supporting Information

Experimental procedures for chemical synthesis and biological evaluation, spectra, and kinase selectivity profile. This material is available free of charge via the Internet at <http://pubs.acs.org>.

AUTHOR INFORMATION**Corresponding Author**

*Fax: 925-560-0101. E-mail: yxu22@hotmail.com.

Notes

The authors declare no competing financial interest.

ABBREVIATIONS

Pim, provirus insertion site of Moloney murine leukemia virus; BAD, Bcl-2-associated death promoter; *h*ERG, the human ether-à-go-go-related gene

■ REFERENCES

- (1) Cuyper, H. T.; Selten, G.; Quint, W.; Zijlstra, M.; Maandag, E. R.; Boelens, W.; Van Wezenbeek, P.; Melief, C.; Berns, A. Murine leukemia virus-induced T-cell lymphomagenesis: integration of proviruses in a distinct chromosomal region. *Cell* **1984**, *37*, 141–150.
- (2) Mikkers, H.; Nawijn, M.; Allen, J.; Brouwers, C.; Verhoeven, E.; Jonkers, J.; Berns, A. Mice deficient for All PIM kinases display reduced body size and impaired responses to hematopoietic growth factors. *Mol. Cell. Biol.* **2004**, *24*, 6104–6115.
- (3) Anizon, F.; Shtil, A. A.; Danilenko, V. N.; Moreau, P. Fighting tumor cell survival: advances in the design and evaluation of pim inhibitors. *Curr. Med. Chem.* **2010**, *17*, 4114–4133.
- (4) Swords, R.; Kelly, K.; Carew, J.; Nawrocki, S.; Mahalingam, D.; Sarantopoulos, J.; Bearss, D.; Giles, F. The Pim kinases: new targets for drug development. *Curr. Drug Targets* **2011**, *12*, 2059–2066.
- (5) Amson, R.; Sigaux, F.; Przedborski, S.; Flandrin, G.; Givol, D.; Telerman, A. The human protooncogene product p33pim is expressed during fetal hematopoiesis and in diverse leukemias. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 8857–8861.
- (6) Hsi, E. D.; Jung, S.-H.; Lai, R.; Johnson, J. L.; Cook, J. R.; Jones, D.; Devos, S.; Cheson, B. D.; Damon, L. E.; Said, J. Ki67 and PIM1 expression predict outcome in mantle cell lymphoma treated with high dose therapy, stem cell transplantation and rituximab: a cancer and leukemia group B 59909 correlative science study. *Leuk. Lymphoma* **2008**, *49*, 2081–2090.
- (7) Xu, Y.; Zhang, T.; Tang, H.; Zhang, S.; Liu, M.; Ren, D.; Niu, Y. Overexpression of PIM-1 is a potential biomarker in prostate carcinoma. *J. Surg. Oncol.* **2005**, *92*, 326–330.
- (8) Zhang, T.; Zhang, X.; Ding, K.; Yang, K.; Zhang, Z.; Xu, Y. PIM-1 gene RNA interference induces growth inhibition and apoptosis of prostate cancer cells and suppresses tumor progression in vivo. *J. Surg. Oncol.* **2010**, *101*, 513–519.
- (9) Xu, Y.; Brenning, B. G.; Kultgen, S. G.; Liu, X.; Saunders, M.; Ho, K. Heterocyclic Protein Kinase Inhibitors. WO 2013013188 A1.
- (10) Cervantes-Gomez, F.; Chen, L. S.; Orłowski, R. Z.; Gandhi, V. Biological effects of the Pim kinase inhibitor, SGI-1776, in multiple myeloma. *Clin. Lymphoma Myeloma Leuk.* **2013**, *13* (Suppl 2), S317–329.
- (11) Chen, L. S.; Redkar, S.; Bearss, D.; Wierda, W. G.; Gandhi, V. Pim kinase inhibitor, SGI-1776, induces apoptosis in chronic lymphocytic leukemia cells. *Blood* **2009**, *114*, 4150–4157.
- (12) Hospital, M.-A.; Green, A. S.; Lacombe, C.; Mayeux, P.; Bouscary, D.; Tamburini, J. The FLT3 and Pim kinases inhibitor SGI-1776 preferentially target FLT3-ITD AML cells. *Blood* **2012**, *119*, 1791–1792.
- (13) Yang, Q.; Chen, L. S.; Neelapu, S. S.; Miranda, R. N.; Medeiros, L. J.; Gandhi, V. Transcription and translation are primary targets of Pim kinase inhibitor SGI-1776 in mantle cell lymphoma. *Blood* **2012**, *120*, 3491–3500.
- (14) Gregg, B. T.; Tymoshenko, D. O.; Razzano, D. A.; Johnson, M. R. Pyrazolo[1,5-a]pyrimidines. identification of the privileged structure and combinatorial synthesis of 3-(hetero)arylpyrazolo[1,5-a]-pyrimidine-6-carboxamides. *J. Comb. Chem.* **2007**, *9*, 507–512.
- (15) Gavrin, L. K.; Lee, A.; Provencher, B. A.; Masefski, W. W.; Huhn, S. D.; Ciszewski, G. M.; Cole, D. C.; McKew, J. C. Synthesis of pyrazolo[1,5- α]pyrimidinone regioisomers. *J. Org. Chem.* **2007**, *72*, 1043–1046.
- (16) Shimizu, H.; Tanaka, S.; Toki, T.; Yasumatsu, I.; Akimoto, T.; Morishita, K.; Yamasaki, T.; Yasukochi, T.; Iimura, S. Discovery of imidazo[1,2-b]pyridazine derivatives as IKK β inhibitors. Part 1: Hit-to-lead study and structure-activity relationship. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 5113–5118.
- (17) Vaz, R. J.; Li, Y.; Rampe, D. Human ether-a-go-go related gene (HERG): A chemist's perspective. *Prog. Med. Chem.* **2005**, *43*, 1–18.
- (18) Foulks, J. M.; Carpenter, K. J.; Luo, B.; Xu, Y.; Senina, A.; Nix, R.; Chan, A.; Clifford, A.; Wilkes, M.; Vollmer, D.; Brenning, B.; Merx, S.; Lai, S.; McCullar, M. V.; Ho, K. K.; Albertson, D. J.; Call, L. T.; Bearss, J. J.; Tripp, S.; Liu, T.; Stephens, B. J.; Mollard, A.; Warner, S. L.; Bearss, D. J.; Kanner, S. B. A small molecule inhibitor of PIM kinases as a potential treatment for urothelial carcinomas. *Neoplasia* **2014**, *16*, 403–412.
- (19) Roper, P. R.; Drewinko, B. Comparison of in vitro methods to determine drug-induced cell lethality. *Cancer Res.* **1976**, *36*, 2182–2188.
- (20) Anastassiadis, T.; Deacon, S. W.; Devarajan, K.; Ma, H.; Peterson, J. R. Comprehensive assay of kinase catalytic activity reveals features of kinase inhibitor selectivity. *Nat. Biotechnol.* **2011**, *29*, 1039–1045.
- (21) Davis, M. I.; Hunt, J. P.; Herrgard, S.; Ciceri, P.; Wodicka, L. M.; Pallares, G.; Hocker, M.; Treiber, D. K.; Zarrinkar, P. P. Comprehensive analysis of kinase inhibitor selectivity. *Nat. Biotechnol.* **2011**, *29*, 1046–1051.
- (22) Smyth, L. A.; Collins, I. Measuring and interpreting the selectivity of protein kinase inhibitors. *J. Chem. Biol.* **2009**, *2*, 131–151.
- (23) Bullock, A. N.; Debreczeni, J. E.; Fedorov, O. Y.; Nelson, A.; Marsden, B. D.; Knapp, S. Structural basis of inhibitor specificity of the human protooncogene proviral insertion site in moloney murine leukemia virus (PIM-1) kinase. *J. Med. Chem.* **2005**, *48*, 7604–7614.
- (24) Wang, X.; Magnuson, S.; Pastor, R.; Fan, E.; Hu, H.; Tsui, V.; Deng, W.; Murray, J.; Steffek, M.; Wallweber, H.; Moffat, J.; Drummond, J.; Chan, G.; Harstad, E.; Ebens, A. J. Discovery of novel pyrazolo[1,5-a]pyrimidines as potent pan-Pim inhibitors by structure- and property-based drug design. *Bioorg. Med. Chem. Lett.* **2013**, *23*, 3149–3153.
- (25) Dwyer, M. P.; Keertikar, K.; Paruch, K.; Alvarez, C.; Labroli, M.; Poker, C.; Fischmann, T. O.; Mayer-Ezell, R.; Bond, R.; Wang, Y.; Azevedo, R.; Guzi, T. J. Discovery of pyrazolo[1,5-a]pyrimidine-based Pim inhibitors: A template-based approach. *Bioorg. Med. Chem. Lett.* **2013**, *23*, 6178–6182.