Potential Use of Selective and Nonselective Pim Kinase Inhibitors for Cancer Therapy

Miniperspective

Denis Drygin,[†] Mustapha Haddach,[‡] Fabrice Pierre,[§] and David M. Ryckman*,[†]

[†]Cylene Pharmaceuticals, 5820 Nancy Ridge Drive, Suite 200, San Diego, California 92121, United States
[‡]HTK Corporation, 5218 Rivergrade Road, Irwindale, California 91706, United States
[§]3244 Caminito Eastbluff, Apt 40, La Jolla, California 92037, United States

INTRODUCTION

Targeted agents and personalized medicine for cancer are the topics of much discussion and research in the medical community and pharmaceutical industry. Success in this area began with the first molecular target, the estrogen receptor, and its inhibitors such as tamoxifen (ICI 46474). Subsequently, a number of drugs have been approved that block oncogene induced signal transduction such as imatinib (STI 571)[1] and others that affect proteins that regulate gene function exemplified by Vorinostat (SAHA). Other classes of targeted agents induce cells to undergo apoptosis like bortezomib (PS-341)[2] and a number of commercialized agents with function similar to that of sunitinib (SU 11248)[3] were designed to block angiogenesis. Clearly, drugs designed to inhibit precise molecular targets or specific signaling pathways represent a valid approach for cancer therapeutics.

Particularly, the deregulation of kinase function has emerged as a very active area of research, as it is one of the major mechanisms by which cancer cells avoid normal constraints on growth and proliferation. Additionally, the presence of an ATP-binding pocket makes these proteins easily amenable to medicinal chemistry efforts. In this Miniperspective we will discuss the inhibitors of the Pim kinase family that have been publically disclosed and their potential development as useful therapeutic agents in oncology. The Pim family has also been implicated in inflammation,[4] and a Pim-1 inhibitor was shown to attenuate of allergen-induced airway hyper-responsiveness and inflammation;[5] however, this is beyond the scope of this Miniperspective.

The Pim kinases, Pim-1, -2, and -3, are a class of constitutively active serine/threonine kinases that are highly homologous (60–70%) in their kinase domains and have been implicated in several normal biological process including cell survival, proliferation, differentiation, and apoptosis. However, when these processes become disrupted or hyperactivated, they express several hallmarks of cancer. The name Pim arises from the original *pim-1* gene as the proviral insertion site of the Moloney murine leukemia virus induced T-cell lymphoma.[6] Pims are notably involved in signaling mechanisms associated with tumorigenesis. Mechanistic studies have shown that high expression levels of Pims are associated with hematologic and epithelial cancers in humans; thus, they can serve as potential therapeutic targets to address numerous unmet medical needs.[7]

Several classes of inhibitors have been described in the literature, and yet only two molecules have progressed to the clinic so far.[8,9] It is noteworthy that for a long time, only inhibitors of Pim-1 were available despite the fact that increasing evidence suggests that all family members should be concurrently inhibited for optimal efficacy. For example, it has been shown that simultaneous inhibition of Pim-1 and Pim-2 is necessary to overcome the oncogenic signaling by protein tyrosine kinases in leukemia.[10] Only recently have examples of molecules able to modulate all isoforms (pan-Pim kinase inhibitors) been disclosed, representing one of the most interesting developments in the field. It is our desire to provide here a better understanding of the biology associated with Pim-regulated signaling pathway and to promote additional research on these associated inhibitors to discover and develop compounds that have suitable drug properties and the ability to benefit patients.

The concept of simultaneous inhibition of multiple pathways may have its roots in traditional combination chemotherapy but has applicability in targeted or multitargeted drug development as well. In fact, a significant proportion of the kinase inhibitors approved for cancer therapy today are multikinase targeting agents that are able to simultaneously modulate several biological processes of the disease. Although many of these drugs were not deliberately created for their multitargeting profile, a more rational design of molecules with multiple predefined targets has emerged. In this Miniperspective we will provide some discussion on the potential of the development of Pim kinase inhibitors with dialed in activity and selectivity properties to specifically target kinases known to be involved in cross-compensatory signaling for the disruption of cancer networks.

PIM BIOLOGY

The Pim kinases isoforms are a three-membered collection of constitutively active enzymes belonging to the CAMK (calmodulin dependent kinase) group.[7,10] They are involved in tumorigenesis, cell survival, and regulation of signaling pathways including proliferation, migration, and metabolism (Figure 1). This kinase family is highly homologous with the kinase domains being very closely related in the linker region and the ATP binding site. Pim-1 and Pim-2 kinases have two and three isoforms, respectively, due to the use of alternative translation initiation sites.[7] The functional significance of
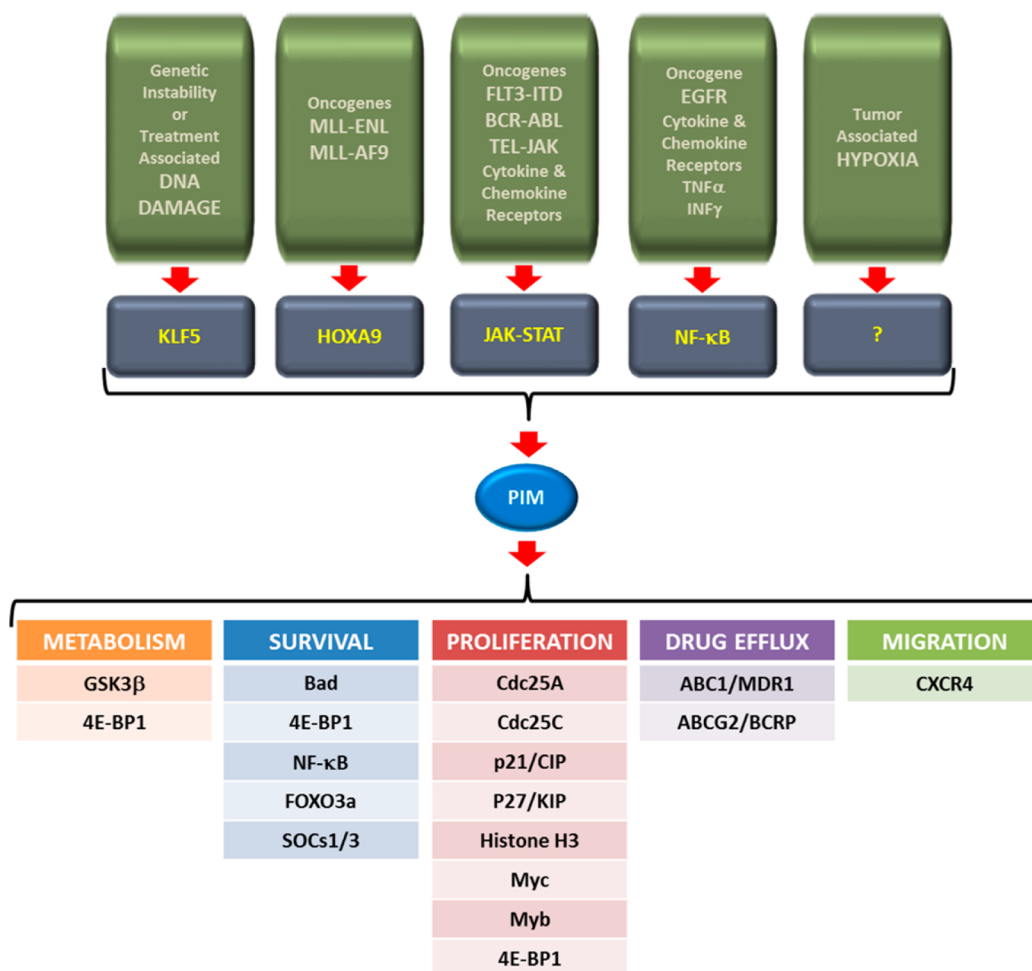
**Figure 1.** Role of Pim kinases in oncogenic signaling.

different isoforms is not yet completely understood; however, certain findings, such as the involvement of nucleus- associated p34, but not p44, isoform of Pim-1 in drug resistance, highlight the potential differences in substrate specificity.[10] Pims are constitutively active Ser/Thr kinases that were shown to be dispensable for embryonic development and survival, as mice deficient in all three kinase are viable, albeit display a significant reduction in body size, indicating that Pim kinases are important for growth.[7,10] All three Pim kinases have several common substrates; however, several targets that are unique to a particular member of the Pim family have been identified, as in case of C-X-C chemokine receptor type 4 (CXCR4) for Pim-1.[7,10]

Pim kinases have short half-lives for both mRNAs and proteins, indicating that their activity is largely regulated on transcription and translation levels. However, in certain instances, such as in chronic myelocytic leukemia (CML) cell line K562, the half-life of Pim-1 protein was found to be significantly increased pointing at the possible regulatory post-translational modification.[10] Alternatively, this observation can be potentially explained by the protection from proteosomal degradation through stabilizing interaction with Hsp90,[11] as K562 cells were shown to have a higher expression of Hsp90 than normal blood cells.[12] While Pim kinases do not require activation for their function, phosphorylation of Pim-1 on Tyr218 by tyrosine kinase ETK was shown to increase Pim-1 kinase activity.[10] The signaling pathways that regulate the

expression of Pims are quite diverse and are cell/tissue type dependent, with transcription factors STATs, NF-κB, and HOXA9 being primarily involved in Pim mRNA synthesis (Figure 1).[10]

Numerous lines of evidence denote Pim kinases as proto-oncogenes. Pim-1 is up-regulated in B-cell non-Hodgkin's lymphoma, and this is generally correlated with poor prognosis. In addition, overexpression of Pim-1 is also noted in acute myeloid leukemia (AML) and nonhematologic cancers such as prostate.[7,10] Pim-2 has aberrant overexpression in several types of lymphomas, leukemias, multiple myeloma, and prostate cancer.[7,10] Pim-3 is implicated in tumors including those of the liver, pancreas, colon, and stomach.[7,10]

The expression of Pim-1 and Pim-2 has been shown to be directly regulated by oncogenes commonly associated with malignant transformation, i.e., Bcr-Abl, Flt3-ITD, JAK2 V617F, MLL-ENL, and MLL-AF9.[10] Furthermore, Pim kinases were shown to be crucial mediators of mitogenic and prosurvival signaling by these oncogenes.[10] Transgenic mice overexpressing Pim-1 under the control of the upstream immunoglobulin enhancer with downstream proviral long-term repeat were shown to develop spontaneous lymphomas, albeit with long latency. Moreover, transgenic mice coexpressing both Eμ-Myc and Eμ-Pim-1 succumb to lymphomas in utero or at birth, suggesting a synthetic lethal consequence of co-up-regulated Myc and Pim-1.[7,10] High levels of Myc are known to induce apoptosis. By up-regulating Pim kinases, cancer cells counteract
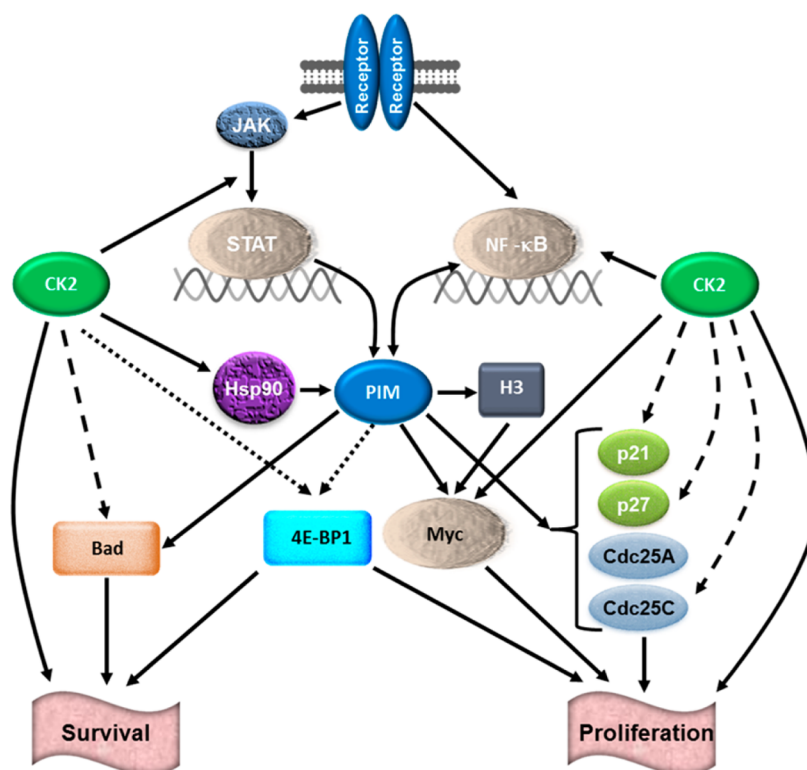
Figure 2. Signaling crosstalk between Pim and CK2 kinases. Solid lines designate direct modulation. Dotted lines designate indirect modulation. Dashed lines designate interaction with unknown function.

this proapoptotic stimuli, as Pim kinases are known to positively regulate the antiapoptotic machinery, in part by phosphorylating and thus inactivating proapoptotic member of Bcl-2 family, Bad.[7,10] Furthermore, Pims were shown to phosphorylate Myc directly, thus inhibiting its degradation.[7,10] Recent studies suggest that phosphorylation of histone H3 by Pim-1, which stimulates RNA polymerase II-driven transcription of Myc target genes, can also play an important role in Pim-1/Myc cooperation.[7,10] Another interesting discovery came from the report that indicated a potential feedback loop by showing that expression of Pim-3 is directly regulated by Myc.[13] In line with its role in apoptosis suppression, Pim-1 was also shown to cooperate in tumorigenesis with overexpression of Bcl-2 or loss of Fas ligand.[10]

In addition, Pim kinases are involved in cell cycle progression facilitating G1/S and G2/M transitions and thus contribute to aberrant cell growth. Accordingly, Pim-1 phosphorylates Cdc25A, which promotes G1/S cell cycle transition.[7,10] Furthermore, cyclin-dependent kinase inhibitors p21/CIP1 and p27/KIP1, which inhibit G1/S cell cycle progression, are deactivated by Pim-1, and this in effect removes the braking mechanism to promote uncontrolled proliferation.[7,10] Also, the G2/M transition is accelerated by inactivation of Cdc25C and phosphorylation of C-TAK1.[7,10] Accompanying increased proliferation, the Pim kinases act as antiapoptotic factors. Increased level of the Pim kinases inactivates the proapoptotic factor Bad, a member of the BH3 family of proteins, predominantly by phosphorylation on Ser 112.[7,10]

Genetic manipulations demonstrated that all three members of the Pim family are capable of compensatory function. Deletion of Pim-1 in Eμ-Myc background leads to the activation of Pim-2, while selective induction of Pim-3 was evident in Eμ-Myc/Pim-1$^{-/-}$/Pim-2$^{-/-}$ mice.[14] These finding

should be considered during the design of specific inhibitors of Pim kinases, as these results highlight the necessity to target all three Pim kinases in order to prevent induction of compensatory mechanisms.

Cancer cells are known to resist treatment through activation of compensatory signaling pathways. One of the well described compensatory interactions is between Pims and Akt, another kinase that is heavily implicated in suppression of apoptosis and in carcinogenesis and that shares the recognition motif: Arg at 5 and 3 positions with the Pim kinases.[15] Numerous phosphoproteomics screens identified multiple substrates that are commonly phosphorylated by both Pims and Akt, with the most frequently cited being Ser112 on Bad.[16] In addition, Pim-2 was shown to regulate translational control in AML by phosphorylating 4E-BP1 on Ser65, a residue traditionally identified as a substrate for mTORC1, a kinase complex that is activated by Akt signaling.[17] Another kinase that is involved in the regulation of pathways affected by Pims is CK2 (Figure 2). Like Pims, CK2 is a constitutively active Ser/Thr kinase that is commonly overexpressed in cancer that was also shown to regulate the stability of Myc protein by direct phosphorylation.[18] While CK2 has a distinct recognition motif compared to Pim kinases and hence does not phosphorylate the same residues as Pims, it has been shown to regulate the same pathways that are known to be linked to carcinogenesis. Like Pim kinases, CK2 plays a prominent role in suppression of apoptosis by increasing the expression and/or stability of antiapoptotic proteins such as Mcl-1 and survivin, as well as through the inhibition of caspase activity.[19] CK2 may also negatively affect the activity of 4E-BP1, potentially through the stimulation of the PI3K-Akt-mTOR pathway,[20] and like Pims may positively regulate NF-kB-driven transcription.[21] Both Pim-1 and CK2 were shown to be activated by hypoxia, and
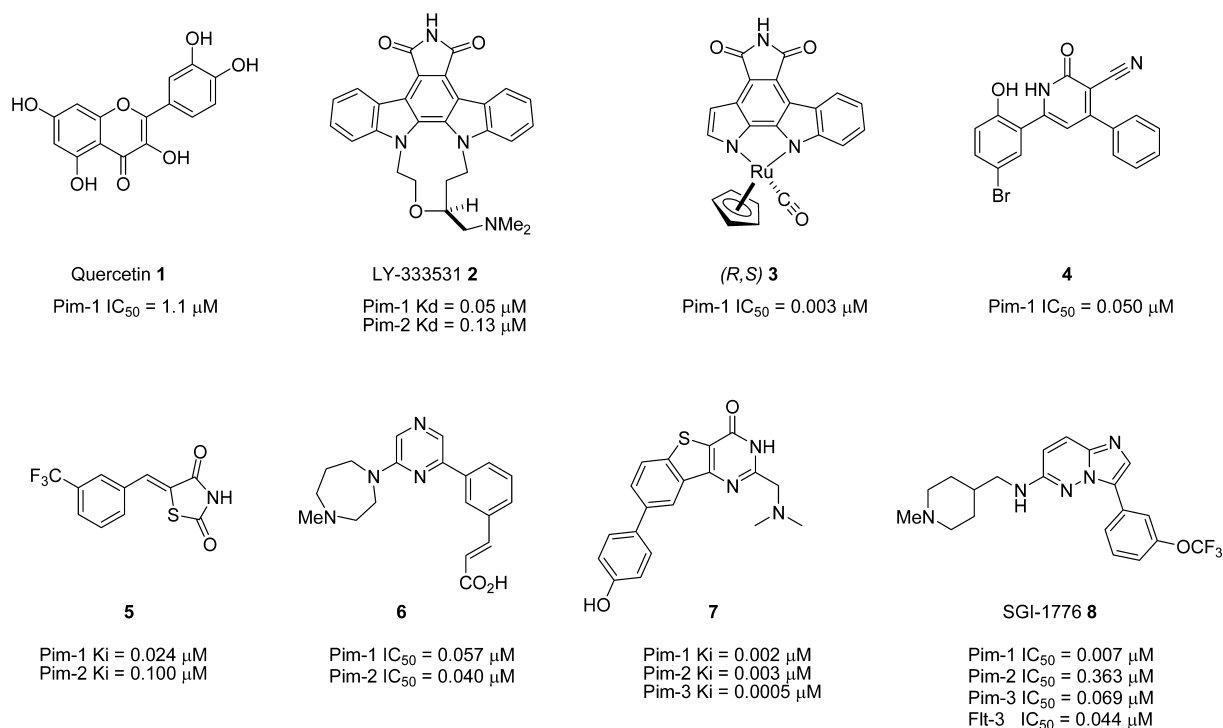
Figure 3. Representative structures of different classes of PIM inhibitors discussed in previous reviews.[36,37]

Quercetin **1**
Pim-1 IC$_{50}$ = 1.1 μM

LY-333531 **2**
Pim-1 Kd = 0.05 μM
Pim-2 Kd = 0.13 μM

(R,S) **3**
Pim-1 IC$_{50}$ = 0.003 μM

4
Pim-1 IC$_{50}$ = 0.050 μM

5
Pim-1 Ki = 0.024 μM
Pim-2 Ki = 0.100 μM

6
Pim-1 IC$_{50}$ = 0.057 μM
Pim-2 IC$_{50}$ = 0.040 μM

7
Pim-1 Ki = 0.002 μM
Pim-2 Ki = 0.003 μM
Pim-3 Ki = 0.0005 μM

SGI-1776 **8**
Pim-1 IC$_{50}$ = 0.007 μM
Pim-2 IC$_{50}$ = 0.363 μM
Pim-3 IC$_{50}$ = 0.069 μM
Flt-3 IC$_{50}$ = 0.044 μM

such activation can be linked to chemoresistance.[22,23] Furthermore, both kinases were shown to promote drug resistance through the activation of members of the ABC transporter family.[24,25] In addition to co-regulating the pathways, CK2 can potentially have an impact on the expression of Pims themselves, as it has been shown to mediate the JAK-STAT signaling transduction[26] and be an important player in the activation of Hsp90 machinery,[27] which is implicated in the stabilization of Pim proteins.[11]

Taking into consideration the potential development of resistance to the inhibition of Pim kinases through activation of compensatory signaling pathways by Akt and/or CK2, one can see a good rationale for combining selective inhibitors that target these pathways individually or a discovery and development of inhibitors with dialed in activity against all of these kinases. While the latter approach is more challenging from the medicinal chemistry perspective, it removes the need for drug–drug interaction studies and allows for a simpler preclinical and clinical development plans.

STRUCTURE OF THE PIM KINASES

Several independent groups have reported the crystal structure of Pim-1 in the presence or absence of their inhibitors.[15,28−31] The structural studies revealed several unique features of Pim-1 that differentiate it from other kinases of known structures. Most notably, the presence of a proline at position 123, critical for ATP binding in other kinases, does not allow the formation of the canonical second hydrogen bond between the hinge backbone and the adenine moiety of ATP, making the Pim kinases family unique in the way they interact with ATP and consequently ATP mimetic inhibitors.[31] In addition, the insertion of an additional residue in the Pim-1 hinge results in structural changes in the hinge region due to the unique hinge sequence ERPEPV. Another important difference between Pim-1 and other kinases is the presence of β-hairpin

in the N-terminal lobe of the protein. Apart from these features, the Pim-1 kinase structure adopts a classic bilobal protein kinase domain architecture with the two-lobe kinase fold separated by a deep, intervening cleft. The N-terminal lobe is composed primarily of β-sheets, whereas the C-terminal lobe is composed primarily of α-helices. The two domains are connected via the hinge region (residues 121−126). The catalytic domain of the Pim-1 kinase covers the region of amino acid positions 38−290 comprising a conserved glycine loop motif at amino acid positions 45−50, a phosphate binding site at amino acid positions 44−52 and 67, and a proton acceptor site at amino acid 167. The replacement of a lysine at position 67 by a methionine results in a kinase inactive Pim-1 mutant.

The crystal structure of Pim-2 has been reported recently and revealed high structural similarity with Pim-1 with several notable changes, such as the absence of the C-terminal αJ helix in Pim-2.[32] Both Pim-1 and Pim-2 assume an active conformation in the presence or absence of ATP. The crystal structures of Pim showed that this constitutive activity is due to an extensive network of hydrophobic interactions and hydrogen bond between the unphosphorylated activation segment and the catalytic loop keeping the ATP pocket open and maintaining its active conformation.

The crystal structure of Pim-3 protein has not yet been reported. However, because of the high sequence similarity between the Pim proteins, it is expected that Pim-3 kinase will have a structure similar to those described for Pim-1 and Pim-2.

While unique architecture of Pim kinases' ATP-binding site allows identification of highly selective Pim inhibitors, there is enough similarity with other kinases of interest for the creation of inhibitors that target several parallel signaling pathways. For example, a comparison of Pim-1/DAPPA complex structure with a structure of CK2/IQA complex revealed a good overlap in the key residues associated with carboxylic moiety of the ligands between Pim-1 and CK2.[33] In line with that, several
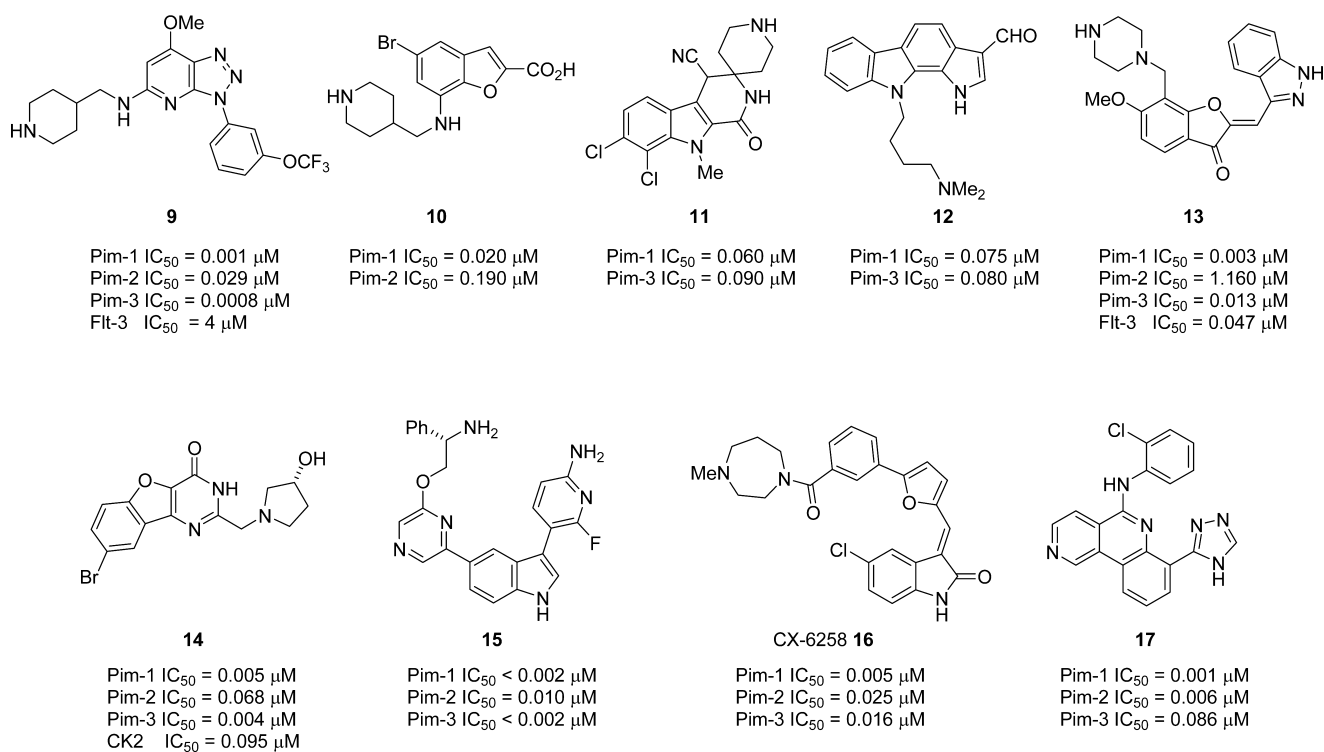
Figure 4. Representative structures of PIM kinase inhibitors published since 2010.

9
Pim-1 IC$_{50}$ = 0.001 μM
Pim-2 IC$_{50}$ = 0.029 μM
Pim-3 IC$_{50}$ = 0.0008 μM
Flt-3 IC$_{50}$ = 4 μM

10
Pim-1 IC$_{50}$ = 0.020 μM
Pim-2 IC$_{50}$ = 0.190 μM

11
Pim-1 IC$_{50}$ = 0.060 μM
Pim-3 IC$_{50}$ = 0.090 μM

12
Pim-1 IC$_{50}$ = 0.075 μM
Pim-3 IC$_{50}$ = 0.080 μM

13
Pim-1 IC$_{50}$ = 0.003 μM
Pim-2 IC$_{50}$ = 1.160 μM
Pim-3 IC$_{50}$ = 0.013 μM
Flt-3 IC$_{50}$ = 0.047 μM

14
Pim-1 IC$_{50}$ = 0.005 μM
Pim-2 IC$_{50}$ = 0.068 μM
Pim-3 IC$_{50}$ = 0.004 μM
CK2   IC$_{50}$ = 0.095 μM

15
Pim-1 IC$_{50}$ < 0.002 μM
Pim-2 IC$_{50}$ = 0.010 μM
Pim-3 IC$_{50}$ < 0.002 μM

CX-6258 16
Pim-1 IC$_{50}$ = 0.005 μM
Pim-2 IC$_{50}$ = 0.025 μM
Pim-3 IC$_{50}$ = 0.016 μM

17
Pim-1 IC$_{50}$ = 0.001 μM
Pim-2 IC$_{50}$ = 0.006 μM
Pim-3 IC$_{50}$ = 0.086 μM

classes of compounds capable of inhibiting both Pims and CK2 have been reported.[33−35]

INHIBITORS OF THE PIM KINASES

The involvement of the Pim kinases in key hallmarks of cancer has triggered a search for small molecule ATP-competitive inhibitors susceptible to lead to new targeted oncology drugs. A large number of these inhibitors (representative structures **1−8** in Figure 3) were discussed in recent, comprehensive reviews.[36,37] Many of the first generation molecules were only inhibitors of the Pim-1 isoform, the Pim-2 isoform being notably more difficult to target likely because of its low K_m for ATP. An effort toward the identification of modulators of the other isoforms has emerged in order to counteract potential compensatory mechanisms arising from the inhibition of only one Pim (vide supra). For example, the dual Pim-1/Pim-2 inhibitor pyrazine **6** was identified by Pim-2 HTS of the corporate compound collection of Boerhinger Ingelheim.[33] The SAR revealed that the carboxylic acid was essential for inhibition of the target, and a series of potent analogues bearing different basic amine side chains on the left-hand side were identified. A kinase selectivity assessment of **6** in a panel of 49 kinases showed that the molecule was remarkably selective, with the notable exception of protein kinase CK2, whereby **6** inhibited the two isoforms CK2α and CK2α′ with IC$_{50}$ values of 663 and 701 nM, respectively. Although the binding mode of **6** to Pim-1 was studied in detail by crystallography, no cellular activities of any analogues were disclosed, limiting their usefulness.

Another promising class of Pim inhibitors is represented by benzo[4,5]thieno[3,2-*d*]pyrimidin-4(3*H*)-one **7**, analogue of a series of potent pan-Pim kinase inhibitors discovered by scientists at Abbott.[38] Starting from a novel HTS hit, this group used structure-based drug design to optimize the series and carry out extensive SAR studies. This class achieved high

potency for the three isoforms concomitant with a remarkable kinase selectivity profile. Several analogues showed cytotoxicity against leukemia cell lines K-562 and MV-4-11 at low micromolar and submicromolar levels. The molecules inhibited the phosphorylation of the Pim target Bad at Ser112 in K562 leukemia and LnCaP prostate cancer cells, strongly indicating that the molecules exerted their antiproliferative effect through the inhibition of the Pim kinases. The PK profile of **7** was promising in mice with a high oral absorption and a reasonable in vitro ADME profile, making these compounds excellent druglike tools to study the biology of the Pim kinases.

N-((1-Methylpiperidin-4-yl)methyl)-3-(3-(trifluoromethoxy)phenyl)imidazo[1,2-*b*]pyridazin-6-amine **8** (SGI-1776) (Figure 3) was the first Pim kinase inhibitor to reach clinical trials, in spite of being a relatively weak inhibitor of Pim-2.[39] Although its inventors identified the imidazo[1,2-*b*]pyridazine core by virtual screening of 1.5 million compounds,[40] this potent Pim inhibitor scaffold had been discovered earlier by others.[15,41] Further scaffold optimization led to **8** as a lead compound that showed dose dependent inhibition of Pim-1 (IC$_{50}$ = 7 nM), Pim-2 (IC$_{50}$ = 363 nM), and Pim-3 (IC$_{50}$ = 69 nM). The molecule was selective in a panel of over 300 kinases and was shown to exhibit inhibitory activity against two other kinases: Flt-3 (IC$_{50}$ = 44 nM) and haspin (IC$_{50}$ = 34 nM).

A number of biological studies of the drug in various oncology contexts have been published. Compound **8** reduced phosphorylation of known Pim substrates involved in cell cycle progression and apoptosis (p21 and Bad), resulting in a cell proliferation inhibition of various prostate cancer cells at low micromolar concentrations.[40] The same authors reported that the drug was able to reduce cell viability in multidrug resistant prostate cancer cell line and was able to resensitize chemo-resistant cells to taxane based therapies. Siu et al.[42] demonstrated that treatment of DU-145 and PC3 prostate

cancer cells with **8** or another specific Pim inhibitor induced up-regulation of the MIG56 gene that encodes a negative regulator of EGFR signaling. These properties resulted in a synergistic effect with gefitinib, suggesting possible combination therapies to increase the efficacy of EFGR kinase inhibitor drugs. In renal cell carcinoma (RCC), **8** treatment decreased phosphorylated and total c-Myc levels and significantly reduced tumor burden in RCC animal models when used in combination with sunitinib.[43]

In the context of leukemia, **8** induced apoptosis in chronic lymphocytic leukemia (CLL) cells in a concentration dependent manner through a mechanism believed to involve Mcl-1 reduction.[39] The drug also exerted a potent cytotoxicity against AML cells and activity in MV-4-11 xenograft models. Drug treatment reduced the phosphorylation of 4E-BP1 (Thr 36/Thr 37) and c-Myc (Ser62) in AML cell lines, and this was correlated with a significant reduction in Mcl-1 levels.[44] However, the increased antiproliferative activity in cells carrying the Flt-3-ITD mutation strongly suggests that the inhibition of Flt-3 by the drug may also play an important role in its cellular effects. In correlation with this hypothesis, a recent publication by Hospital et al.[45] reported the inhibition of Flt-3 autophosphorylation on tyrosine residues in MOLM-14 AML cells treated with **8**, similar to that observed after treatment with the specific Flt-3 inhibitor AC-220. With Flt-3 being known to regulate the expression of Pim kinases in leukemia,[46,47] potent Flt-3 inhibition by a drug will necessarily create ambiguity in the interpretation of the cellular effect of Pim inhibition and as such should probably be avoided to clearly understand the mechanism of a drug. The recent discovery of structurally close inhibitors 1,2,3-triazolo[4,5-b]pyridines, which are cell-active and devoid of potent Flt-3 inhibition (compound **9**, Figure 4),[48] should provide useful tools for differentiating the effect of Pim and Flt-3 inhibition by **8**. A paper by Fathi et al.[49] recently suggested that the simultaneous modulation of Flt-3 and Pim might be beneficial in overcoming the emergence of resistance to Flt-3 inhibition, implying that a dual inhibitor of Flt-3 and Pim such as **8** might still be more beneficial for certain types of cancer. The phase 1 clinical trial of **8** was discontinued in November 2010 because of dose limiting cardiac QTc prolongation,[8] a likely result of the hERG inhibition observed in vivo.[48] This unfortunate withdrawal due to off-target activity leaves the floor open for a new generation of molecules with improved properties.

During the past 2 years, several novel inhibitors of the Pim kinases have emerged from the literature, including compound **9** discussed above (Figure 4). Benzofuran 2-carboxylic acid **10** was discovered by fragment based screening followed by X-ray structure guided optimization.[50] Compound **10** was found to be selective in a 442 kinase panel, and its binding mode to Pim-1 was elucidated by crystallography. Although the molecule has appealing in vitro PK/ADME properties, no cellular activities of the molecule or its analogues were reported. With the aim of discovering very selective inhibitors, Huber et al.[51] prepared several derivatives of a 6,7-dichloroindole scaffold known to bind kinases in an unusual way. Screening of the resulting molecules in a thermal stability shift assay allowed the discovery of 7,8-dichloro-1-oxo-β-carboline **11** which inhibited Pim-1 and Pim-3 with IC$_{50}$ values below 100 nM and displayed good selectivity.[51] This compound showed antiproliferative activity in the low micromolar range against various cell lines and inhibited the phosphorylation of 4E-BP1 in MV-4-11 cells. Compound **12**, which was discovered independently by another

research group,[52] shared some structural similarity with **11** by also bearing an indole ring. However, no cellular activity of the latter molecule was revealed. Finally, optimization of a screening hit led to the discovery of **13**,[53] which showed great activity against Pim-1 (IC$_{50}$ = 3 nM) and Pim-3 (IC$_{50}$ = 13 nM). The activity of this molecule against Pim-2 was remarkably low (IC$_{50}$ = 1.1 μM), although the potent inhibition of Flt-3 (IC$_{50}$ = 47 nM) may account in part for the potent antiproliferative activity of the molecule against MV-4-11 cells, similar to **8**. This compound, however, was reported to have lower hERG inhibition, addressing a major issue of the former clinical candidate.

The molecules discussed below (**14−17**, Figure 4) are recent examples of pan-Pim inhibitors able to potently inhibit the three isoforms of the enzyme. The benzo[4,5] thieno[3,2-d]pyrimidin-4(3H)-ones (**7**, Figure 3) discussed above also belong to this class of molecules. Using structure-based drug design, scientists at Exelixis converged toward a very similar series of molecules in which the sulfur atom of the scaffold was replaced by an oxygen atom. The resulting pan-Pim inhibitors exemplified by **14** (Figure 4) showed good kinase selectivity, cellular potency, and good oral exposure.[54] Interestingly, the molecule displayed some CK2 inhibition, noted also for other Pim inhibitors in this series (vide infra). Scientists from Novartis reported the discovery of **15** and its analogues by structure-guided optimization of an HTS hit.[55] Compound **15** potently modulated the three Pim isoforms and revealed a good kinase selectivity profile.

(E)-5-Chloro-3-((5-(3-(4-methyl-1,4-diazepane-1-carbonyl)-phenyl)furan-2-yl)methylene)indolin-2-one **16** (CX-6258), discovered through the optimization of a screening hit by Haddach et al.,[56] is another example of a publically disclosed pan-Pim inhibitor. The molecule inhibited the three Pim isoforms with IC$_{50}$ values below 25 nM and showed an excellent selectivity profile in a 107-kinase panel. Although the molecule modulated Flt-3 activity in an enzyme assay, it was inactive against this enzyme in a cellular context. Compound **16** caused dose dependent inhibition of the phosphorylation of the prosurvival proteins Bad and 4E-BP1 at their respective Pim specific sites. In vitro, the drug produced synergistic cell killing in combination with doxorubicin and paclitaxel in prostate cancer PC3 cells, validating a potential use of pan-Pim kinases inhibitors in combination with chemotherapy. In vivo, **16** reduced tumor growth in a xenograft model of leukemia (MV-4-11, tumor growth inhibition TGI = 75% at 100 mg/kg) and prostate cancer (PC3, TGI = 51% at 50 mg/kg). A novel class of potent 7-(4H-1,2,4-triazol-3-yl)benzo[c][2,6]naphthyridines exemplified by **17** was discovered by modifying the potent protein kinase CK2 inhibitor 5-(3-chlorophenylamino)benzo-[c][2,6]naphthyridine-8-carboxylic acid (CX-4945).[57] The resulting compounds were very potent inhibitors of the Pim-1 and Pim-2 isoforms and, to a lesser extent, inhibitors of Pim-3. The molecules inhibited cell proliferation of various cancer cell lines at submicromolar concentrations and modulated the phosphorylation of Bad at Ser 112. A certain level of Flt-3 inhibition was present in enzymological assays of the molecules but absent in a cellular context.

Replacing the left pyridine ring in **17** (Figure 4) by various five membered rings such as in thiophene **18** (Figure 5)[35] maintained potent activity against Pim and surprisingly restored inhibition of protein kinase CK2 while suppressing Flt-3 inhibition. The resulting dual inhibitors of CK2 and the Pims displayed antiproliferative activity against several solid and
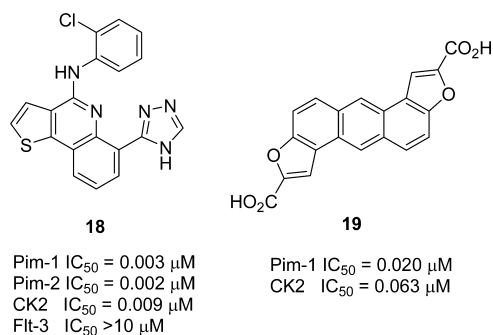
Figure 5. Structures of dual Pim/CK2 inhibitors.

blood cancer cell lines. CK2 and the Pim kinases share a relatively similar ATP binding pocket, allowing the rational design of dual inhibitors of the kinases. This interesting convergence of structure and the signaling crosstalk of the enzymes (see Figure 2) can be exploited for the rational design of dual inhibitors, an approach that was discussed in detail by Lopez-Ramos et al. in a work describing the discovery of dual CK2/Pim inhibitor **19** (Figure 5).[34] Unfortunately this compound was not cell-permeable. Multikinase inhibition creates opportunities for a multiprong targeting of the hallmarks of cancer, and dual inhibitors of CK2/Pim may supplement the arsenal of existing therapeutic oncology agents. However, this innovative concept needs more validation and in particular potent dual CK2/Pim inhibitors with good drug properties, which will allow hypothesis testing in vivo.

Finally, a number of remarkable inhibitors with undisclosed structures were presented in various conference posters. Array Biopharma described a molecule (structure not disclosed) with potent inhibitory properties for Pim-1 (IC_{50} = 0.4 nM) and Pim-3 (IC_{50} = 1.7 nM) and moderate inhibition for Pim-2 (IC_{50} = 81 nM).[58] The compound reduced tumor growth of TEL-JAK2/BaF3 subcutaneous tumors in nude mice at 100 mg/kg (TGI = 54%) and 200 mg/kg (TGI = 75%). Scientists at Genentech reported that their pan-Pim inhibitor GNE-652 (structure not disclosed) had picomolar biochemical potency, an excellent selectivity profile, and favorable ADME properties.[59] The molecule showed excellent in vivo efficacy in combination with the PI3K inhibitor 4-(2-(1H-indazol-4-yl)-6-((4-(methylsulfonyl)piperazin-1-yl)methyl)thieno[3,2-d]-pyrimidin-4-yl)morpholine (GDC-0941). Selvita discovered a novel series of Pim kinase inhibitors of which analogue SEL24-B58 (structure not disclosed; IC_{50} of 31 nM (Pim-1), 154 nM (Pim-2), 182 nM (Pim-3)) showed in vitro synergy with inhibitors for PI3K, mTOR, JAK1/2, and Bcl-2.[60] The molecule was orally efficacious in a xenograft model of AML (MV-4-11) and was well tolerated. Finally, a significant progress in the Pim field was achieved by AstraZeneca scientists who described AZD1208 (structure not disclosed),[61] an orally available, potent, and highly selective pan-Pim kinase inhibitor. The compound reduced the phosphorylation of Bad, 4E-BP1, and p70S6K. AZD12078 suppressed the growth of tumors in AML (MOLM-16 and KG-1a) xenograft models of cancer. AZD12078 is currently investigated in a phase 1 clinical trial in AML patients.[9]

■ CONCLUSION

There is overwhelming evidence that the Pim kinases represent a promising class of drug targets for oncology. Many of the biology and medicinal chemistry studies discussed here strongly

suggest that ATP-competitive pan-Pim kinase inhibitors can be used for the treatment of various disorders such as CML, AML, CLL, B-cell non-Hodkin's lymphoma, multiple myeloma, RCC, and prostate, liver, pancreas, colon, and stomach cancers. Modern understanding of compensatory signaling by different kinases underscores the practical necessity of combining several inhibitors. In that context, some of the studies described above suggest that patients may also benefit from the combination of Pim kinases inhibitors with existing drugs such as modulators of the Akt-PI3K pathways, taxanes, gefitinib, and other inhibitors of the EGFR pathway, sunitinib or doxorubicin among others. As more Pim inhibitors progress into the clinic, the list of possible combination is likely to increase, requiring the design of drugs with profiles with limited potential for drug–drug interactions.

An alternative to combination is the discovery of molecules that possess dialed in activity and selectivity against multiple kinases of interest for the complete blockade of the oncogenic signaling. A prime example of this approach is the design of dual inhibitors of CK2 and Pim. Another potentially beneficial strategy is to design the inhibitors that are capable of co-inhibiting Pim and Akt signaling, as these kinases share a substantial amount of common substrates and were shown to be actively involved in compensatory signaling.[16] The fact that several compounds, such as natural flavonoids or their synthetic derivative LY294002, were shown to have activity against Pim, CK2, and PI3K,[28,62,63] a kinase that regulates Akt signaling, demonstrates the feasibility of this approach. However, a multitargeting approach does not come without a price, as promiscuous kinase inhibitors have been associated with unwanted side effects. Indeed, following the discovery that multitargeted inhibitors sunitinib and sorafenib cause cardio-toxicity,[64] Hasinoff and Patel investigated the connection between the selectivity of kinase inhibitors and their ability to damage cardiomyocytes in vitro. Their findings demonstrated that combined tyrosine kinase and serine–threonine kinase selectivity scores were highly correlative with the myocyte-damaging effects of the kinase inhibitors,[65] highlighting a potential drawback of multitargeting approach. While Pim kinases were not among the 12 kinases that were specifically identified in the screen as being linked to the damage of cardiomyocytes, Pim-1 kinase was shown to play a significant role in the preservation of myocardial structure after myocardial infarction.[66] Pim-3 was also found to be induced by ischemia–reperfusion injury; however, the reports on its ability to protect the myocardial tissue have been mixed.[67,68] In addition to Pim-1, Akt, but interestingly not CK2, was also shown to play an important role in the protection of heart from the injury.[69,70] Because of that, suitable preclinical toxicology studies should be undertaken when administrating agents capable of simultaneous inhibition of Pim and Akt signaling to high-risk cardiovascular patients.

It is surprising that only two Pim inhibitory agents have reached clinical trials and unfortunate that no human proof of concept data were generated because of the early withdrawal of the first drug 8.[8] However, the pace of discovery of novel Pim inhibitor has dramatically accelerated in the past 2 years, and a new generation of potent molecules without hERG inhibition and appropriate pharmacologic profiles will likely generate Pim kinase inhibitors drugs suitable for human cancer therapy.

AUTHOR INFORMATION

Corresponding Author

*Phone: +1 858 875 5114. E-mail: dryckman@cylenepharma. com.

Notes

The authors declare no competing financial interest.

Biographies

Denis Drygin received his B.S. and M.S. degrees in Chemistry from Moscow State University, M.S. and Ph.D. degrees in Biochemistry and Molecular Biology from the University of Massachusetts at Amherst, and his postdoctoral training at Isis Pharmaceuticals, where he worked with Dr. Frank Bennett. Dr. Drygin joined Cylene Pharmaceuticals in 2005 as a Research Scientist and has earned increasing responsibilities for the company's drug mechanism, screening, and in vivo pharmacology operations across all R&D projects, being promoted to Vice President, Biology, in 2012. His discovery and research efforts led to the creation of the first therapeutic agent to directly target RNA polymerase I transcription for the treatment of cancer. Dr. Drygin also played leading roles in Cylene's CK2 and pan-Pim inhibitors R&D programs.

Mustapha Haddach received a Ph.D. in Chemistry from the University of Nice-Sophia Antipolis, Nice, France. Most recently, he directed the medicinal chemistry effort in several oncology programs at Cylene Pharmaceuticals. Prior to that he was the Vice President of Chemistry at Parallax Biosystems, a company he cofounded to develop novel molecular detection platforms. Before Parallax he held positions at Neurocrine Biosciences, working to develop therapies for CNS related diseases such as depression and neurodegenerative disorders. He is a named inventor on 17 patents.

Fabrice Pierre earned his Ph.D. in Chemistry from The University of Rennes, France (1997), with Prof. Claude Moinet. He was awarded a Humboldt Fellowship to pursue postdoctoral studies in asymmetric synthesis with Prof. Dieter Enders in Aachen (RWTH), Germany. After completing a postdoc with Prof. Marco Ciufolini in Lyon, France, he was hired in 2001 by Triad Therapeutics in San Diego, CA, where he worked on multiple drug discovery projects. He joined Cylene Pharmaceuticals in 2004 where he made significant contributions to the creation of the company's oncology pipeline. Dr. Pierre was involved in the discovery of several clinical candidates and is the coauthor of 40 articles and patent applications. He currently works as a contract medicinal chemist for Vertex Pharmaceuticals in San Diego, CA.

David M. Ryckman is Senior Vice President of Chemistry and Pharmaceutical Operations at Cylene Pharmaceuticals, Inc. He earned his B.S. and Ph.D. at the University of California at Los Angeles, working for Professors C. S. Foote, R. V. Stevens, and M. E. Jung on the total synthesis of natural products. Subsequently he undertook postdoctoral studies at Harvard University, MA, in the laboratories of Professor Y. Kishi, studying the conformational analysis of carbon glycosides and their relationship to the parent glycosides. He has worked at both large and small pharmaceutical companies including GlaxoSmithKline, Novartis, and Gensia Laboratories, Inc., where he has received several awards in both research and technical operations. His current professional interests include the discovery and development of new targeted agents for oncology.

ACKNOWLEDGMENTS

ABBREVIATIONS USED

AML, acute myeloid leukemia; CAMK, calmodulin dependent kinase; CLL, chronic lymphocytic leukemia; CML, chronic myelocytic leukemia; CXCR4, C-X-C chemokine receptor type 4; RCC, renal cell carcinoma

REFERENCES

(1) Druker, B. J.; Talpaz, M.; Resta, D. J.; Peng, B.; Buchdunger, E.; Ford, J. M.; Lydon, N. B.; Kantarjian, H.; Capdeville, R.; Ohno-Jones, S.; Sawyers, C. L. Efficacy and safety of a specific inhibitor of the BCR-ABL tyrosine kinase in chronic myeloid leukemia. *N. Engl. J. Med.* **2001**, *344* (14), 1031−1037.

(2) Orlowski, R. Z.; Stinchcombe, T. E.; Mitchell, B. S.; Shea, T. C.; Baldwin, A. S.; Stahl, S.; Adams, J.; Esseltine, D. L.; Elliott, P. J.; Pien, C. S.; Guerciolini, R.; Anderson, J. K.; Depcik-Smith, N. D.; Bhagat, R.; Lehman, M. J.; Novick, S. C.; O'Connor, O. A.; Soignet, S. L. Phase I trial of the proteasome inhibitor PS-341 in patients with refractory hematologic malignancies. *J. Clin. Oncol.* **2002**, *20* (22), 4420−4427.

(3) Shaheen, P. E.; Rini, B. I.; Bukowski, R. M. Clinical activity of sorafenib and sunitinib in renal cell carcinoma refractory to previous vascular endothelial growth factor-targeted therapy: two case reports. *Clin. Genitourin. Cancer* **2006**, *5*, 78−81.

(4) Jackson, L. J.; Pheneger, J. A.; Pheneger, T. J.; Davis, G.; Wright, A. D.; Robinson, J. E.; Allen, S.; Munson, M. C.; Carter, L. L. The role of PIM kinases in human and mouse CD4+ T cell activation and inflammatory bowel disease. *Cell. Immunol.* **2012**, *272*, 200−213.

(5) Shin, Y. S.; Takeda, K.; Shiraishi, Y.; Jia, Y.; Wang, M.; Jackson, L.; Wright, A. D.; Carter, L.; Robinson, J.; Hicken, E.; Gelfand, E. W. Inhibition of Pim1 kinase activation attenuates allergen-induced airway hyperresponsiveness and inflammation. *Am. J. Respir. Cell Mol. Biol.* **2012**, *46*, 488−497.

(6) Cuypers, 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.

(7) Nawijn, M. C.; Alendar, A.; Berns, A. For better or for worse: the role of Pim oncogenes in tumorigenesis. *Nat. Rev. Cancer* **2011**, *11*, 23−34.

(8) Study of SGI-1776, a PIM Kinase Inhibitor, in Subjects with Relapsed/Refractory Leukemias. http://clinicaltrials.gov/ct2/show/NCT01239108.

(9) Safety and Tolerability of AZD1208 and the Pharmacokinetics and Efficacy of AZD1208 in Acute Myelogenous Leukemia (AML) Patients. http://clinicaltrials.gov/ct2/show/NCT01489722.

(10) Brault, L.; Gasser, C.; Bracher, F.; Huber, K.; Knapp, S.; Schwaller, J. PIM serine/threonine kinases in the pathogenesis and therapy of hematologic malignancies and solid cancers. *Haematologica* **2010**, *95*, 1004−1015.

(11) Mizuno, K.; Shirogane, T.; Shinohara, A.; Iwamatsu, A.; Hibi, M.; Hirano, T. Regulation of Pim-1 by Hsp90. *Biochem. Biophys. Res. Commun.* **2001**, *281*, 663−669.

(12) Xiao, K.; Liu, W.; Qu, S.; Sun, H.; Tang, J. Study of heat shock protein HSP90 alpha, HSP70, HSP27 mRNA expression in human acute leukemia cells. *J. Tongji Med. Univ.* **1996**, *16* (4), 212−216.

(13) Forshell, L. P.; Li, Y.; Forshell, T. Z.; Rudelius, M.; Nilsson, L.; Keller, U.; Nilsson, J. The direct Myc target Pim3 cooperates with other Pim kinases in supporting viability of Myc-induced B-cell lymphomas. *Oncotarget* **2011**, *2*, 448−460.

(14) Mikkers, H.; Allen, J.; Knipscheer, P.; Romeijn, L.; Hart, A.; Vink, E.; Berns, A. High-throughput retroviral tagging to identify components of specific signaling pathways in cancer. *Nat. Genet.* **2002**, *32*, 153−159.

(15) Bullock, A. N.; Debreczeni, J.; Amos, A. L.; Knapp, S.; Turk, B. E. Structure and substrate specificity of the Pim-1 kinase. *J. Biol. Chem.* **2005**, *280*, 41675−41682.

(16) Amaravadi, R.; Thompson, C. B. The survival kinases Akt and Pim as potential pharmacological targets. *J. Clin. Invest.* **2005**, *115* (10), 2618−2624.

(17) Tamburini, J.; Green, A. S.; Bardet, V.; Chapuis, N.; Park, S.; Willems, L.; Uzunov, M.; Ifrah, N.; Dreyfus, F.; Lacombe, C.; Mayeux, P.; Bouscary, D. Protein synthesis is resistant to rapamycin and constitutes a promising therapeutic target in acute myeloid leukemia. *Blood* **2009**, *114*, 1618−1627.

(18) Channavajhala, P.; Seldin, D. C. Functional interaction of protein kinase CK2 and c-Myc in lymphomagenesis. *Oncogene* **2002**, *21* (34), 5280−5288.

(19) Ahmad, K. A.; Wang, G.; Unger, G.; Slaton, J.; Ahmed, K. Protein kinase CK2—a key suppressor of apoptosis. *Adv. Enzyme Regul.* **2008**, *48*, 179−187.

(20) Bliesath, J.; Huser, N.; Omori, M.; Bunag, D.; Proffitt, C.; Streiner, N.; Ho, C.; Siddiqui-Jain, A.; O'Brien, S. E.; Lim, J. K.; Ryckman, D. M.; Anderes, K.; Rice, W. G.; Drygin, D. Combined inhibition of EGFR and CK2 augments the attenuation of PI3K-Akt-mTOR signaling and the killing of cancer cells. *Cancer Lett.* **2012**, *322*, 113−118.

(21) Romieu-Mourez, R.; Landesman-Bollag, E.; Seldin, D. C.; Traish, A. M.; Mercurio, F.; Sonenshein, G. E. Roles of IKK kinases and protein kinase CK2 in activation of nuclear factor-kappaB in breast cancer. *Cancer Res.* **2001**, *61* (9), 3810−3818.

(22) Chen, J.; Kobayashi, M.; Darmanin, S.; Qiao, Y.; Gully, C.; Zhao, R.; Yeung, S. C.; Lee, M. H. Pim-1 plays a pivotal role in hypoxia-induced chemoresistance. *Oncogene* **2009**, *28*, 2581−2592.

(23) Mottet, D.; Ruys, S. P.; Demazy, C.; Raes, M.; Michiels, C. Role for casein kinase 2 in the regulation of HIF-1 activity. *Int. J. Cancer* **2005**, *117* (5), 764−774.

(24) Di Maira, G.; Brustolon, F.; Bertacchini, J.; Tosoni, K.; Marmiroli, S.; Pinna, L. A.; Ruzzene, M. Pharmacological inhibition of protein kinase CK2 reverts the multidrug resistance phenotype of a CEM cell line characterized by high CK2 level. *Oncogene* **2007**, *26*, 6915−6926.

(25) Xie, Y.; Xu, K.; Linn, D. E.; Yang, X.; Guo, Z.; Shimelis, H.; Nakanishi, T.; Ross, D. D.; Chen, H.; Fazli, L.; Gleave, M. E.; Qiu, Y. The 44-kDa Pim-1 kinase phosphorylates BCRP/ABCG2 and thereby promotes its multimerization and drug-resistant activity in human prostate cancer cells. *J. Biol. Chem.* **2008**, *283*, 3349−3356.

(26) Zheng, Y.; Qin, H.; Frank, S. J.; Deng, L.; Litchfield, D. W.; Tefferi, A.; Pardanani, A.; Lin, F. T.; Li, J.; Sha, B.; Benveniste, E. N. A CK2-dependent mechanism for activation of the JAK-STAT signaling pathway. *Blood* **2011**, *118*, 156−166.

(27) Miyata, Y. Protein kinase CK2 in health and disease: CK2: the kinase controlling the Hsp90 chaperone machinery. *Cell. Mol. Life Sci.* **2009**, *66* (11−12), 1840−1849.

(28) Jacobs, M. D.; Black, J.; Futer, O.; Swenson, L.; Hare, B.; Fleming, M.; Saxena, K. Pim-1 ligand-bound structures reveal the mechanism of serine/threonine kinase inhibition by LY294002. *J. Biol. Chem.* **2005**, *280*, 13728−13734.

(29) Kumar, A.; Mandiyan, V.; Suzuki, Y.; Zhang, C.; Rice, J.; Tsai, J.; Artis, D. R.; Ibrahim, P.; Bremer, R. Crystal structures of proto-oncogene kinase Pim1: a target of aberrant somatic hypermutations in diffuse large cell lymphoma. *J. Mol. Biol.* **2005**, *348*, 183−193.

(30) Qian, K. C.; Studts, J.; Wang, L.; Barringer, K.; Kronkaitis, A.; Peng, C.; Baptiste, A.; LaFrance, R.; Mische, S.; Farmer, B. Expression, purification, crystallization and preliminary crystallographic analysis of human Pim-1 kinase. *Acta Crystallogr., Sect. F: Struct. Biol. Cryst. Commun.* **2005**, *61*, 96−99.

(31) Qian, K. C.; Wang, L.; Hickey, E. R.; Studts, J.; Barringer, K.; Peng, C.; Kronkaitis, A.; Li, J.; White, A.; Mische, S.; Farmer, B. Structural basis of constitutive activity and a unique nucleotide binding mode of human Pim-1 kinase. *J. Biol. Chem.* **2005**, *280*, 6130−6137.

(32) Bullock, A. N.; Russo, S.; Amos, A.; Pagano, N.; Bregman, H.; Debreczeni, J. E.; Lee, W. H.; von Delft, F.; Meggers, E.; Knapp, S. Crystal structure of the PIM2 kinase in complex with an organo-ruthenium inhibitor. *PLoS One* **2009**, *4* (10), e7112.

(33) Qian, K.; Wang, L.; Cywin, C. L.; Farmer, B. T., 2nd; Hickey, E.; Homon, C.; Jakes, S.; Kashem, M. A.; Lee, G.; Leonard, S.; Li, J.; Magboo, R.; Mao, W.; Pack, E.; Peng, C.; Prokopowicz, A., 3rd; Welzel, M.; Wolak, J.; Morwick, T. Hit to lead account of the discovery of a new class of inhibitors of Pim kinases and crystallographic studies revealing an unusual kinase binding mode. *J. Med. Chem.* **2009**, *52* (7), 1814−1827.

(34) Lopez-Ramos, M.; Prudent, R.; Moucadel, V.; Sautel, C. F.; Barette, C.; Lafanechere, L.; Mouawad, L.; Grierson, D.; Schmidt, F.; Florent, J. C.; Filippakopoulos, P.; Bullock, A. N.; Knapp, S.; Reiser, J. B.; Cochet, C. New potent dual inhibitors of CK2 and Pim kinases: discovery and structural insights. *FASEB J.* **2010**, *24*, 3171−3185.

(35) Pierre, F.; Regan, C. F.; Chevrel, M. C.; Siddiqui-Jain, A.; Macalino, D.; Streiner, N.; Drygin, D.; Haddach, M.; O'Brien, S. E.; Rice, W. G.; Ryckman, D. M. Novel potent dual inhibitors of CK2 and Pim kinases with antiproliferative activity against cancer cells. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 3327−3331.

(36) 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.

(37) Morwick, T. Pim kinase inhibitors: a survey of the patent literature. *Expert Opin. Ther. Pat.* **2010**, *20* (2), 193−212.

(38) Tao, Z. F.; Hasvold, L. A.; Leverson, J. D.; Han, E. K.; Guan, R.; Johnson, E. F.; Stoll, V. S.; Stewart, K. D.; Stamper, G.; Soni, N.; Bouska, J. J.; Luo, Y.; Sowin, T. J.; Lin, N. H.; Giranda, V. S.; Rosenberg, S. H.; Penning, T. D. Discovery of 3*H*-benzo[4,5]thieno-[3,2-*d*]pyrimidin-4-ones as potent, highly selective, and orally bioavailable inhibitors of the human protooncogene proviral insertion site in moloney murine leukemia virus (PIM) kinases. *J. Med. Chem.* **2009**, *52* (21), 6621−6636.

(39) 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.

(40) Mumenthaler, S. M.; Ng, P. Y.; Hodge, A.; Bearss, D.; Berk, G.; Kanekal, S.; Redkar, S.; Taverna, P.; Agus, D. B.; Jain, A. Pharmacologic inhibition of Pim kinases alters prostate cancer cell growth and resensitizes chemoresistant cells to taxanes. *Mol. Cancer Ther.* **2009**, *8*, 2882−2893.

(41) Pogacic, V.; Bullock, A. N.; Fedorov, O.; Filippakopoulos, P.; Gasser, C.; Biondi, A.; Meyer-Monard, S.; Knapp, S.; Schwaller, J. Structural analysis identifies imidazo[1,2-*b*]pyridazines as PIM kinase inhibitors with in vitro antileukemic activity. *Cancer Res.* **2007**, *67*, 6916−6924.

(42) Siu, A.; Virtanen, C.; Jongstra, J. PIM kinase isoform specific regulation of MIG6 expression and EGFR signaling in prostate cancer cells. *Oncotarget* **2011**, *2*, 1134−1144.

(43) Mahalingam, D.; Espitia, C. M.; Medina, E. C.; Esquivel, J. A., 2nd; Kelly, K. R.; Bearss, D.; Choy, G.; Taverna, P.; Carew, J. S.; Giles, F. J.; Nawrocki, S. T. Targeting PIM kinase enhances the activity of sunitinib in renal cell carcinoma. *Br. J. Cancer* **2011**, *105*, 1563−1573.

(44) Chen, L. S.; Redkar, S.; Taverna, P.; Cortes, J. E.; Gandhi, V. Mechanisms of cytotoxicity to Pim kinase inhibitor, SGI-1776, in acute myeloid leukemia. *Blood* **2011**, *118*, 693−702.

(45) 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.

(46) Kim, K. T.; Baird, K.; Ahn, J. Y.; Meltzer, P.; Lilly, M.; Levis, M.; Small, D. Pim-1 is up-regulated by constitutively activated FLT3 and plays a role in FLT3-mediated cell survival. *Blood* **2005**, *105*, 1759−1767.

(47) Kim, K. T.; Levis, M.; Small, D. Constitutively activated FLT3 phosphorylates BAD partially through pim-1. *Br. J. Haematol.* **2006**, *134*, 500−509.

(48) Pastor, J.; Oyarzabal, J.; Saluste, G.; Alvarez, R. M.; Rivero, V.; Ramos, F.; Cendon, E.; Blanco-Aparicio, C.; Ajenjo, N.; Cebria, A.; Albarran, M. I.; Cebrian, D.; Corrionero, A.; Fominaya, J.; Montoya,

G.; Mazzorana, M. Hit to lead evaluation of 1,2,3-triazolo[4,5-b]pyridines as PIM kinase inhibitors. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 1591−1597.

(49) Fathi, A. T.; Arowojolu, O.; Swinnen, I.; Sato, T.; Rajkhowa, T.; Small, D.; Marmsater, F.; Robinson, J. E.; Gross, S. D.; Martinson, M.; Allen, S.; Kallan, N. C.; Levis, M. A potential therapeutic target for FLT3-ITD AML: PIM1 kinase. *Leuk. Res.* **2012**, *36*, 224−231.

(50) Xiang, Y.; Hirth, B.; Asmussen, G.; Biemann, H. P.; Bishop, K. A.; Good, A.; Fitzgerald, M.; Gladysheva, T.; Jain, A.; Jancsics, K.; Liu, J.; Metz, M.; Papoulis, A.; Skerlj, R.; Stepp, J. D.; Wei, R. R. The discovery of novel benzofuran-2-carboxylic acids as potent Pim-1 inhibitors. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 3050−3056.

(51) Huber, K.; Brault, L.; Fedorov, O.; Gasser, C.; Filippakopoulos, P.; Bullock, A. N.; Fabbro, D.; Trappe, J.; Schwaller, J.; Knapp, S.; Bracher, F. 7,8-Dichloro-1-oxo-beta-carbolines as a versatile scaffold for the development of potent and selective kinase inhibitors with unusual binding modes. *J. Med. Chem.* **2012**, *55* (1), 403−413.

(52) Akue-Gedu, R.; Letribot, B.; Saugues, E.; Debiton, E.; Anizon, F.; Moreau, P. Kinase inhibitory potencies and in vitro antiproliferative activities of N-10 substituted pyrrolo[2,3-a]carbazole derivatives. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 3807−3809.

(53) Nakano, H.; Saito, N.; Parker, L.; Tada, Y.; Abe, M.; Tsuganezawa, K.; Yokoyama, S.; Tanaka, A.; Kojima, H.; Okabe, T.; Nagano, T. Rational evolution of a novel type of potent and selective proviral integration site in moloney murine leukemia virus kinase 1 (PIM1) inhibitor from a screening-hit compound. *J. Med. Chem.* **2012**, *55* (11), 5151−5164.

(54) Tsuhako, A. L.; Brown, D. S.; Koltun, E. S.; Aay, N.; Arcalas, A.; Chan, V.; Du, H.; Engst, S.; Franzini, M.; Galan, A.; Huang, P.; Johnston, S.; Kane, B.; Kim, M. H.; Laird, A. D.; Lin, R.; Mock, L.; Ngan, I.; Pack, M.; Stott, G.; Stout, T. J.; Yu, P.; Zaharia, C.; Zhang, W.; Zhou, P.; Nuss, J. M.; Kearney, P. C.; Xu, W. The design, synthesis, and biological evaluation of PIM kinase inhibitors. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 3732−3738.

(55) Nishiguchi, G. A.; Atallah, G.; Bellamacina, C.; Burger, M. T.; Ding, Y.; Feucht, P. H.; Garcia, P. D.; Han, W.; Klivansky, L.; Lindvall, M. Discovery of novel 3,5-disubstituted indole derivatives as potent inhibitors of Pim-1, Pim-2, and Pim-3 protein kinases. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 6366−6369.

(56) Haddach, M.; Michaux, J.; Schwaebe, M. K.; Pierre, F.; O'Brien, S. E.; Borsan, C.; Tran, J.; Rafaelle, N.; Ravula, S.; Drygin, D.; Siddiqui-Jain, A.; Darjania, L.; Stansfield, R.; Proffitt, C.; Macalino, D.; Streiner, N.; Bliesath, J.; Omori, M.; Whitten, J. P.; Anderes, K.; Rice, W. G.; Ryckman, D. M. Discovery of CX-6258. A potent, selective, and orally efficacious pan-Pim kinases inhibitor. *ACS Med. Chem. Lett.* **2012**, *3*, 135−139.

(57) Pierre, F.; Stefan, E.; Nedellec, A. S.; Chevrel, M. C.; Regan, C. F.; Siddiqui-Jain, A.; Macalino, D.; Streiner, N.; Drygin, D.; Haddach, M.; O'Brien, S. E.; Anderes, K.; Ryckman, D. M. 7-(4H-1,2,4-Triazol-3-yl)benzo[c][2,6]naphthyridines: a novel class of Pim kinase inhibitors with potent cell antiproliferative activity. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 6687−6692.

(58) Gross, S. D.; Robinson, J. E.; Allen, S.; Cox, A.; DeWolf, W. E.; Galbraith, S.; Lee, P.; Lemieux, C.; Lyssikatos, J. P.; Marmsater, F.; Martinson, M.; Munson, M. C.; Wallace, R. D.; Woessner, R.; Zuzack, J.; Winkler, J.; Koch, K. Characterization of the Effects of Pim Kinase Inhibition on Multiple Oncogene-Driven Cell Lines. Presented at the 50th Annual Meeting of the American Society of Hematology, San Francisco, CA, 2008.

(59) Ebens, A. J.; Berry, L.; Chen, Y.-H.; Deshumkh, G.; Drummond, G.; Du, C.; Eby, M.; Fitzgerald, K.; Friedman, L. S.; Gould, S. E.; Kenny, J. R.; Maecker, H.; Moffat, J.; Moskalenko, M.; Pacheco, P.; Saadat, A.; Slaga, D.; Sun, L.; Wang, G.; Yang, Y.; Munugalavdla, V. A Selective PIM Kinase Inhibitor Is Highly Active in Multiple Myeloma: The Biology of Single Agent and PI3K/AKT/mTOR Combination Activity. Presented at the 53rd Annual Meeting and Exposition of the American Society of Hematology, San Diego, CA, 2011.

(60) Czardybon, W.; Windak, R.; Krawczynska, K.; Zarebski, A.; Dolata, I.; Salwinska, M.; Surawska, M.; Sabiniarz, A.; Szamborska-Gbur, A.; Prymula, K.; Milik, M.; Kosowska-Wardega, A.; Trebacz, E.; Rsymski, T.; Kowalczyk, P.; Brzozka, K. Development of Selective Pim Kinase Inhibitors for Cancer Treatment. Presented at the 2012 Annual Meeting of the American Association for Cancer Research, Chicago, IL, 2012.

(61) Keeton, E.; McEachem, K.; Alimzhanov, M.; Wang, S.; Cao, Y.; Bao, L.; Palakurthi, S.; Grondine, M.; Chen, Y.; Dillman, K.; Chinnappan, D.; Shen, M.; Dakin, L.; Zheng, X.; Lamb, M.; Wu, A.; Chen, H.; Lyne, P.; Huszar, D. Efficacy and Biomarker Modulation by AZD1208, a Novel, Potent and Selective Pan-Pim Kinase Inhibitor, in Models of Acute Meloid Leukemia. Presented at the 2012 Annual Meeting of the American Association for Cancer Research, Chicaogo, IL, 2012.

(62) Davies, S. P.; Reddy, H.; Caivano, M.; Cohen, P. Specificity and mechanism of action of some commonly used protein kinase inhibitors. *Biochem. J.* **2000**, *351* (Part 1), 95−105.

(63) Vlahos, C. J.; Matter, W. F.; Hui, K. Y.; Brown, R. F. A specific inhibitor of phosphatidylinositol 3-kinase, 2-(4-morpholinyl)-8-phenyl-4H-1-benzopyran-4-one (LY294002). *J. Biol. Chem.* **1994**, *269* (7), 5241−5248.

(64) Cheng, H.; Force, T. Why do kinase inhibitors cause cardiotoxicity and what can be done about it? *Prog. Cardiovasc. Dis.* **2010**, *53*, 114−120.

(65) Hasinoff, B. B.; Patel, D. The lack of target specificity of small molecule anticancer kinase inhibitors is correlated with their ability to damage myocytes in vitro. *Toxicol. Appl. Pharmacol.* **2010**, *249*, 132−139.

(66) Fischer, K. M.; Cottage, C. T.; Wu, W.; Din, S.; Gude, N. A.; Avitabile, D.; Quijada, P.; Collins, B. L.; Fransioli, J.; Sussman, M. A. Enhancement of myocardial regeneration through genetic engineering of cardiac progenitor cells expressing Pim-1 kinase. *Circulation* **2009**, *120*, 2077−2087.

(67) Liu, D.; He, M.; Yi, B.; Guo, W. H.; Que, A. L.; Zhang, J. X. Pim-3 protects against cardiomyocyte apoptosis in anoxia/reoxygenation injury via p38-mediated signal pathway. *Int. J. Biochem. Cell Biol.* **2009**, *41*, 2315−2322.

(68) Zhao, L.; Wang, Y.; Min, X.; Yang, H.; Zhang, P.; Zeng, Q. Ischemia−reperfusion injury up-regulates Pim-3 gene expression in myocardial tissue. *J. Huazhong Univ. Sci. Technol., Med. Sci.* **2010**, *30* (6), 704−708.

(69) Kim, S. O.; Baines, C. P.; Critz, S. D.; Pelech, S. L.; Katz, S.; Downey, J. M.; Cohen, M. V. Ischemia induced activation of heat shock protein 27 kinases and casein kinase 2 in the preconditioned rabbit heart. *Biochem. Cell Biol.* **1999**, *77* (6), 559−567.

(70) Sussman, M. A.; Volkers, M.; Fischer, K.; Bailey, B.; Cottage, C. T.; Din, S.; Gude, N.; Avitabile, D.; Alvarez, R.; Sundararaman, B.; Quijada, P.; Mason, M.; Konstandin, M. H.; Malhowski, A.; Cheng, Z.; Khan, M.; McGregor, M. Myocardial AKT: the omnipresent nexus. *Physiol. Rev.* **2011**, *91*, 1023−1070.