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Graphical Abstract

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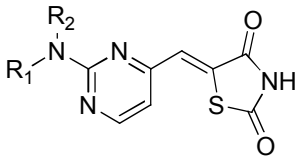

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A versatile synthesis of novel pan-PIM kinase inhibitors with initial SAR study

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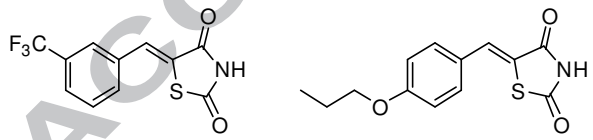
ABSTRACT

Herein, we describe the versatile synthesis of (Z)-5-((2-aminopyrimidin-4-yl)methylene)thiazolidine-2,4-dione inhibitors (**1**) of the PIM family of kinases. This chemistry strategy was a key element in the multi-variable optimization program with the goal of identifying high quality leads for the development of a treatment for cancer.

The three Pim kinases (PIM1, PIM2 and PIM3) are a small family of serine/threonine kinases regulating several signaling pathways that are fundamental to cancer development and progression[1]. Over-expression of the PIM kinases has been reported in several hematological and solid tumors[2]. Novel small molecule pan-PIM inhibitors developed by AstraZeneca (AZD-1208)[3] and Novartis (LGH-447)[4] have entered Phase I/2 clinical trials, validating these very attractive targets for pharmaceutical development in the treatment of cancer. In fact, Novartis has described PIM2 as critical for multiple myeloma cell survival. However, the PIMs do exhibit redundant activity, which in part has led to the fact that all known PIM inhibitors show activity against the entire family.



2 **3**

Figure 1. Known benzylidenethiazolidine-2,4-dione PIM inhibitors.

A number of unique chemical structures have demonstrated the ability to inhibit PIM kinase enzymatic activity. The known benzylidenethiazolidine-2,4-diones,[5] **2** and **3** (Figure 1) were among the early classes of potent PIM inhibitors to be described, which exhibited micromolar IC50's against the family of PIM isoforms.

Our design efforts centered around utilizing the thiazolidine-2,4-dione component as a key element in a novel chemical scaffold. One thought was to investigate heteroaromatic moieties attached to the thiazolidine and to achieve this in a modular approach that would facilitate hit-to-lead efforts on active molecules. A pyrimidine replacement of the substituted benzene ring was of keen interest since aminopyrimidines have been identified by a number of groups to provide potent, selective, and orally available small molecule inhibitors of various kinases[6]. A literature search identified a process that described the synthesis of a suitably elaborated pyrimidine system, compound **6**[7]. The synthesis, detailed in Figure 2, started with the condensation of the readily available 1,1-dimethoxy-4-dimethylaminobut-3-en-2-one **4** and thiourea. The resulting substituted pyrimidine could then be methylated to provide intermediate **5**. After acid catalyzed removal of the dimethyl acetal, the aldehyde **6** was condensed with thiazolidine-2,4-dione affording the novel intermediate **7,** exclusively as the Z-isomer. Finally, mild oxidation conditions using oxone were developed to cleanly provide the library template **8**, which served as a versatile starting point for the development of a general experimental procedure for the displacement of the methylsulfoxide leaving group[8]. The thermal conditions allowed for the use of both primary and secondary amines. In addition, amines containing free hydroxyl groups and heterocycles also performed well in the displacement. However, anilines and heteroaromatic amino groups were not effective in the displacement reaction under the general procedure affording this new class of molecules.

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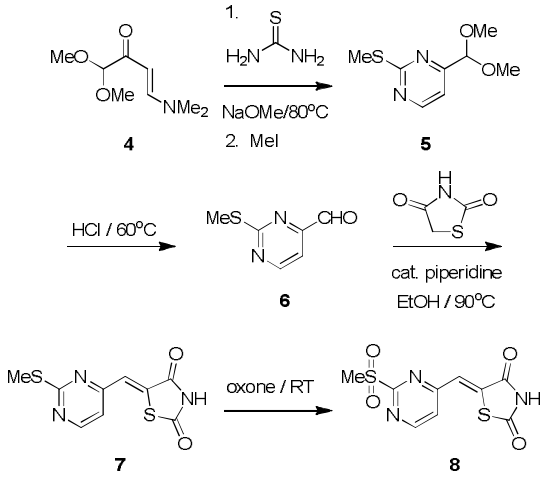
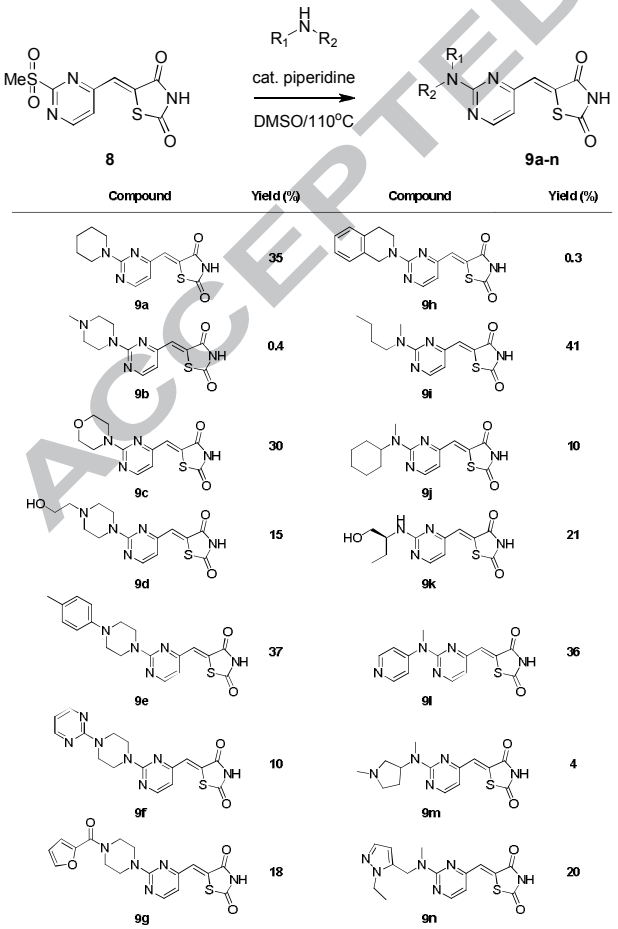
Figure 2. Synthesis of pyrimidine library template.



Each new compound, described in Figure 3, was purified using reverse phase HPLC (yields not optimized) and characterized by LCMS and/or NMR. This initial set of analogs was screened against the PIM kinases (isoforms 1, 2, & 3) using a standard radiometric assay, which identified a number of micromolar inhibitors. The general procedure was then utilized to synthesize more than 120 analogs using commercially available amines to further explore the chemical series. This data led to a more detailed knowledge of the SAR (Structure Activity Relationship). The pattern of activity generated an interest in better understanding the impact of incorporating a wider range of potential binding elements to increase potency and selectivity. One approach involved using mono-protected diamines, which could provide terminal basic NH groups.

Figure 3. Library synthesis of 2-aminopyrimidine analogs.



To this end, an initial set of analogs were designed to incorporate a diamine component into the molecule. A representative set of these analogs are described in Table 1 along with PIM inhibition (IC50s) and permeability data (PAMPA assay). Incorporation of an end terminal NH moiety dramatically increased the enzymatic inhibition by comparison of the IC50 data against PIM1, PIM2 and PIM3 between compound **10** and **11** as well as IC50 data of compound **13** versus **14** in Table 1. However, introduction of the third NH group in the molecule (**16** vs **13**) or methylation of the NH group at the alpha-position of the pyrimidine (**12** vs **11**) has no significant affect on the inhibitory activity. We believe that the terminal NH group at this length and position in compound **13** is likely necessary to form a hydrogen bond with an enzyme residue of the target resulting in the observed activity increase. In addition, when the terminal basic NH group was replaced with an amide group (comparing **17** with **13**), the IC50 data was substantially decreased by about 10-fold indicating that basicity of the terminal NH group plays an important role in the enzymatic activity.

Unfortunately, the most active compound **13** in Table 1 has very poor permeability as measured in PAMPA assay[9], which was not desired for achieving good cell-based potency or oral bioavailability in vivo. Encouraged by the much improved permeability of compound **15**, which included a fused hydrophobic benzene ring into the diamine linker, as compared with **13** in Table 1 (74.1 vs 0.4), we started to explore the incorporation of aromatic groups attached to the end of the molecule for increasing hydrophobicity while retaining the basic NH moiety at the same position as that in compound **13**.

Table 1. PIM kinase inhibition via diamine analogs.

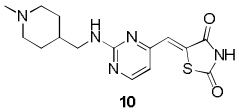
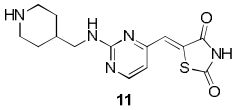
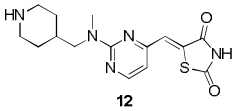
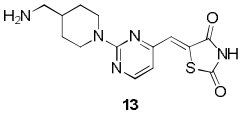
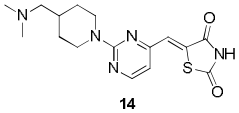
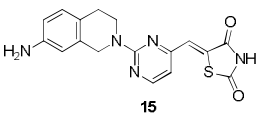
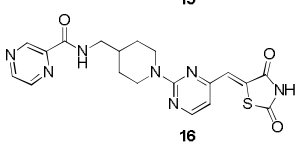
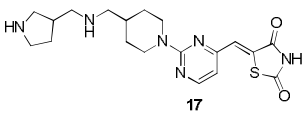
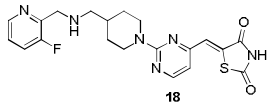
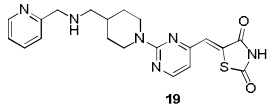
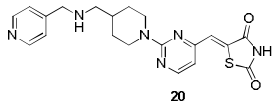
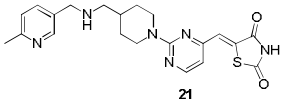
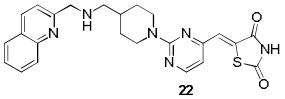
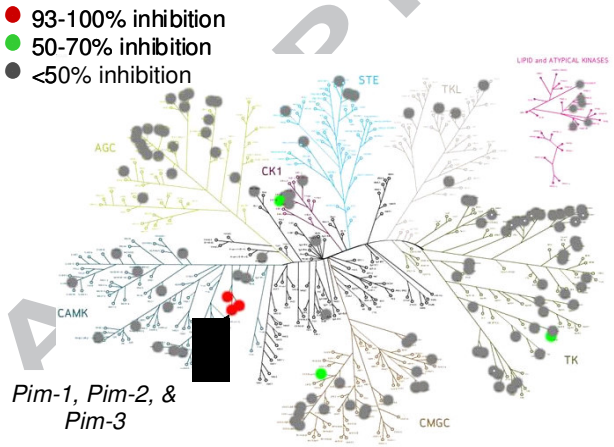
Compound	PAMPA	PIM1 IC50 nM	PIM2 IC50 nM	PIM3 IC50 nM
 10	8.9	628	228	310
 11	8.2	258	90	31
 12	3.5	246	98	171
 13	0.4	45	47	27
 14	0.4	>1,000	>1,000	>1,000
 15	74.1	97	89	419
 16	39.8	475	146	249
 17	39.8	475	146	249

Table 2. PIM kinase inhibition via modified diamine analogs.

Structure	PAMPA	PIM1 IC50 nM	PIM2 IC50 nM	PIM3 IC50 nM
 18	95.1	146	105	44
 19	49.9	157	142	46
 20	58.6	96	87	22
 21	196.6	58	75	16
 22	113	13	15	15

A focused set of diamine analogs were designed and synthesized to address the need to improve both permeability and PIM kinase inhibition (Table 2). A newly developed and general reductive amination procedure[10] was utilized to provide all of the analogs **18-22**, which introduce a heteroaromatic group but maintain the basic nature of the NH. These analogs all have both enhanced permeability and increased PIM inhibition. The best compound **22** was determined to have a Ki of 7.7 nM against PIM1. Compound **22** also demonstrated a clear selectivity profile across a panel of 150 oncology relevant kinases[11] (Figure 4) when screened at 0.5 uM.

Figure 4. Kinase selectivity panel for compound **22**.



In addition, **22** also exhibited potent IC50's against a number of cancer cell lines in prostate, leukemia, and multiple myeloma (Table 3). An in vivo rat PK study, dosing the animals both i. v. and orally with 5 mg/kg of compound **22**, demonstrated a plasma half-life ($T_{1/2}$) of 3.1 h with very good oral bioavailability (F = 49.9%).

Table 3. Cell proliferation assay data for compound **22**.

Cell Line	IC50 (uM)	Cancer
DU145	3.7	Prostate
LNCaP	5.9	Prostate
K562	6.4	Leukemia
RPMI-8226	6.9	Myeloma
NCI-H929	5.6	Myeloma

In conclusion, we have identified a novel class of (Z)-5-((2-aminopyrimidin-4-yl)methylene)thiazolidine-2,4-diones as potent and selective inhibitors of the PIM family of kinases. The PIM kinases have been implicated as critical to cancer cell survival and proliferation confirming them as attractive biological targets for pharmaceutical drug development. This report demonstrates the ability to optimize initially identified active molecules within this series to provide lead structures with enhanced PIM kinase inhibition and other pharmaceutical properties. Selected compounds such as **22** have exhibited a high degree of selectivity for inhibition of the PIM kinases with the ability to inhibit cancer cell proliferation in both hematologic malignancies and solid tumors. A comprehensive description of the lead optimization effort to afford a targeted drug candidate for the treatment of cancer will be disclosed in due course.

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8. **General Displacement Procedure:** 2 dram round bottomed vials were charged with (Z)-5-((2-(methylsulfonyl)pyrimidin-4-yl)methylene)thiazolidine-2,4-dione (25 mg, 0.0877 mmol) prepared according to the general procedure, DMSO (1 mL, 0.08 M), diisopropylethylamine (50 µL, 0.288 mmol, 3.2 equiv.), and the appropriate amine (0.0877 mmol, 1.0 equiv.). The reaction mixture was heated to 110°C and shaken for 24 h. The solvent was removed under reduced pressure (genvac HT-4) and the crude residues were purified using reverse phase HPLC (MS-triggered fraction collection) with an acetonitrile/water gradient and trifluoroacetic acid as a modifier. The pure fractions were then concentrated under reduced pressure (Genevac HT-4). **General De-Protection Procedure:** The crude protected products were prepared using the General Displacement Procedure and were then treated with 2 mL DCE and 500 uL of TFA and shaken for 24 h. The solvent was removed under reduced pressure (Genevac HT-4) and the crude residues were purified using reverse phase HPLC (MS-triggered fraction collection) with an acetonitrile/water or methanol/water gradient and trifluoroacetic acid as a modifier. The pure fractions were then concentrated under reduced pressure (Genevac HT-4).
9. PAMPA assay kit: BD Biosciences Gentest™ pre-coated PAMPA plate system, catalog number 353015. Parallel Artificial Membrane Permeability Assays (PAMPA) conditions: The target concentration in the assay was 200 µM, prepared by diluting (50-fold) the 10 mM stock solution of each compound in DMSO into PBS, pH 7.4. The final DMSO concentration was 2%. The 200 µM solutions were added, 300 µL, to wells in the donor plate. The receiver plate, which contained 200 µL of PBS, pH 7.4 per well, was placed in the donor plate and the assembly was incubated for 5 hours at ambient temperature. At the end of the incubation period the plates were separated and the compound concentrations in each solution were determined by LC/MS/MS. The assay was performed in triplicate. Dexamethasone and verapamil were used as reference compounds.
10. **General Reductive Amination Procedure 1:** A 2-dram round bottomed vial was charged with the crude amine/TFA salt intermediate prepared using the general displacement procedure followed by the general TFA de-protection procedure (0.115 mmol), DCE (2 mL), DIPEA (6 eq. 0.690 mmol), DMF (1 mL), the aldehyde (1 equiv., 0.115 mmol), and the reaction mixture was shaken for 1 h at RT. The reaction mixture was then treated with $NaBH(OAc)_3$ (2.5 equiv., 0.230 mmol) and the reaction was shaken 16 h at RT. The reaction mixture was then diluted with DCE (2 mL) and $NaHCO_3$ (2 mL). The aqueous layer was back extracted with DCE (2 x 2 mL) and the combined organic layer was concentrated under reduced pressure (Genevac HT-4) and the crude residue was purified using reverse phase HPLC (MS-triggered fraction collection) with an acetonitrile/water or methanol/water gradient and triflouroacetic acid as the modifier. The pure fractions were then concentrated under reduced pressure (Genevac HT-4) to afford the pure products as the TFA salt.
11. EMD Millipore Kinase Profiler™ Services, www.emdmillipore.com. Assay conditions: **Pim-1 (h):** Pim-1 (h) is incubated with 8 mM MOPS pH 7.0, 0.2 mM EDTA, 100 µM KKRNRTLTV, 10 mM MgAcetate and [γ-33P-ATP] (specific activity approx. 500 cpm/pmol, concentration as required). The reaction is initiated by the addition of the MgATP mix. After incubation for 40 minutes at room temperature, the reaction is stopped by the addition of 3% phosphoric acid solution. 10 µL of the reaction is then spotted onto a P30 filtermat and washed three times for 5 minutes in 75 mM phosphoric acid and once in methanol prior to drying and scintillation counting. **Pim-2 (h):** Pim-2 (h) is incubated with 8 mM MOPS pH 7.0, 0.2 mM EDTA, 300 µM RSRHSSYPAGT, 10 mM MgAcetate and [γ-33P-ATP] (specific activity approx. 500 cpm/pmol, concentration as required). The reaction is initiated by the addition of the MgATP mix. After incubation for 40 minutes at room temperature, the reaction is stopped by the addition of 3% phosphoric acid solution. 10 µL of the reaction is then spotted onto a P30 filtermat and washed three times for 5 minutes in 75 mM phosphoric acid and once in methanol prior to drying and scintillation counting. **Pim-3 (h):** Pim-3 (h) is incubated with 8 mM MOPS pH 7.0, 0.2 mM EDTA, 0.1% Triton X-100, 300 µM RSRHSSYPAGT, 10 mM MgAcetate and [γ-33P-ATP] (specific activity approx. 500 cpm/pmol, concentration as required). The reaction is initiated by the addition of the MgATP mix. After incubation for 40 minutes at room temperature, the reaction is stopped by the addition of 3% phosphoric acid solution. 10 µL of the reaction is then spotted onto a P30 filtermat and washed three times for 5 minutes in 75 mM phosphoric acid and once in methanol prior to drying and scintillation counting.