Synthesis and Evaluation of 5-(3-(Pyrazin-2-yl)benzylidene)thiazolidine-2,4-dione Derivatives as Pan–Pim Kinases Inhibitors

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Received April 25, 2014; accepted June 10, 2014; advance publication released online June 20, 2014

Pim kinases play a key role in the regulation of signaling pathways including proliferation, migration, and metabolism and are a potential target for cancer therapy. A series of 5-benzylidenethiazolidine-2,4-diones were synthesized as pim kinase inhibitors. The structure–activity relationships (SAR) of the analogues in inhibiting *in vitro* pim kinase activity as well as the proliferation of leukemia cell lines were examined. SAR studies indicated that a hydroxyl group at the 2-position of the benzene ring of 5-benzylidenethiazolidine-2,4-dione plays an important role in the inhibitory activity against all three pim kinases and replacement with a pyrazinyl group at the 5-position of the benzene ring of 5-benzylidenethiazolidine-2,4-dione improved activity significantly. The compounds exerted anti-proliferative activity against the three leukemia cell lines we tested. The most potent compound, 5i, had an EC_{50} value of 0.8 μM in the MV4-11 cell line. The result of kinase profiling indicated that compound 5i was highly selective for pim-kinases.

Key words Pim-1; Pim-2; Pim-3; 5-benzylidenethiazolidine-2,4-dione; pan–pim kinase inhibitor

The proviral integration site for Moloney murine leukemia virus (*pim*) family of serine/threonine kinases are composed of three highly homologous isoforms, Pim-1, Pim-2, and Pim-3, belonging to the group of calcium/calmodulin-regulated kinases (CAMK).[1] Pim kinases play a key role in regulation of signaling pathways *via* Janus kinase (JAK)/signal transducer and activator of transcription (STAT) pathway including proliferation, migration, and metabolism.[2] The pim kinases are rather weak oncogenes when expressed as transgenes. However, their oncogenic potential increases significantly when co-expressed with a strong oncogene such as c-Myc which is a transcription factor that is important in cell growth and differentiation.[3–6] These studies demonstrate strong cooperation between pim kinases and c-Myc in lymphomagenesis. Increasing evidence shows that Pim-1 and Pim-2 kinases are overexpressed not only in hematologic malignancies such as multiple myeloma, lymphomas, and leukemia but also in solid tumors such as prostate cancer while Pim-3 overexpression enhances solid tumors including those of the pancreatic, prostate, colon and others.[1,7] Therefore, the pim kinases are a potential target for cancer therapy. Furthermore, no severe side effects can be expected for inhibiting all three pim kinases because triple pim knockout mice are shown to be viable and fertile.[8]

Several X-ray crystal structures of Pim-1 kinase have been reported over the past decade, including many structures complexed with inhibitors. The Protein Data Bank (PDB) contains more than 90 structures of Pim-1, but only one structure of Pim-2 kinase. No crystal structure has been reported for Pim-3 kinase to date. All reported structures provide insight into the important binding elements. Most notably, there is a proline residue (Pro123 in Pim-1) and a single-residue insertion (Glu for Pim-1 and Pim-2, Met for Pim-3) following the proline residue in the hinge region, which differentiate pim kinases from other kinases. Due to a unique consensus sequence ERPXPX motif that is present only in the pim kinase family, the hydrogen bonding interaction is lacking between the hinge backbone and the adenine group of natural substrate ATP. The Pim-1 structures bound with either ATP or its analogues show a salt bridge between Lys67 and the phosphate group. The Lys67 also forms a salt bridge network with Glu89 in the alpha-C helix and the Asp186 of the DFG motif.[9] Thus, based on the available structural information, the initial potent lead structures can be further optimized.

5-Benzylidenethiazolidine-2,4-dione was identified by Pim-1 high throughput screening (HTS). Further derivatization by introduction of various substituents in the benzene ring yielded potent compounds with double-digit nanomolar IC_{50} values for Pim-1 kinase[10] (compound **A** in Fig. 1). Interestingly, a clinical candidate AZD1208, a highly selective and potent pan-pim kinase inhibitor, also has a 5-benzylidenethiazolidine-2,4-dione scaffold.[11] In addition, 3-(3-pyrazin-2-yl)-benzylidenethiazolidine-2,4-dione (**B**) was found as a Pim-1 kinase inhibitor using fragment-based drug design.[12]

X-Ray crystallographic analysis of Pim-1 kinase in com-

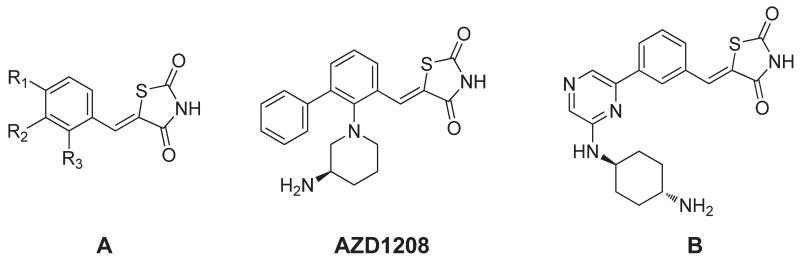

Fig. 1. Structures of Pim Kinase Inhibitors Using 5-Benzylidenethiazolidine-2,4-dione Core Structure

The authors declare no conflict of interest.

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plex with compound **B** suggested that an additional hydrogen bonding interaction with conserved Glu (Glu121 in Pim-1) in the hinge region of the pim kinase is possible by introducing a hydrogen bond donor on a benzene ring of 5-benzylidenethiazolidine-2,4-dione. We also speculated that a more potent inhibitor could be obtained if *trans*-1,4-diaminocyclohexane substituent on a pyrazine ring of compound **B** were replaced with other aminoalkyl groups, which could effectively improve interactions with the enzyme. This prompted us to design and synthesize a series of 5-benzylidenethiazolidine-2,4-dione analogues to explore the effects of both hydroxyl group on a benzene ring and various substituents on a pyrazine on the inhibitory activity of compound in order to find the potent and selective pan–pim kinase inhibitors.

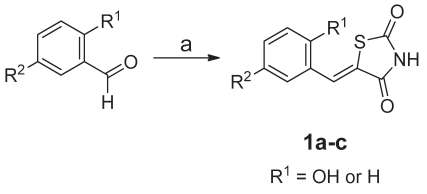
Results and Discussion

Chemistry Compounds were synthesized according to the reported procedures with some modifications as shown in Charts 1 and 2.[10,11,13] Substituted pyrazine **3** was synthesized from the reaction of 2,6-dichloropyrazine with either amine or alcohol and was attached to the 3-position of benzaldehyde or 5-position of 2-hydroxybenzaldehyde using Suzuki cross coupling reaction under microwave irradiation. The intermediate **2** used in the Suzuki reaction was synthesized by Miyaura borylation.[14] Thiazolindine-2,4-dione was reacted with the corresponding aldehyde to give a 5-benzylidenethiazolidine-2,4-dione analogue *via* Knoevenagel condensation.

Biological Evaluation To explore possible hydrogen bonding opportunities between 5-benzylidenethiazolidine-2,4-dione and pim kinase, a hydroxyl group (R^1) was introduced at 2-position of the benzene ring, resulting in improved inhibitory potency by an order of magnitude against Pim-1 kinase (Table 1). However, the expedient effect of the hydroxyl group disappeared when a bromo group at *para* to the hydroxyl group in the benzene ring was replaced with a nitro group. The comparative measurement of the hydrogen-bond donating ability of various OH groups had been represented as hydrogen-bond acidity (pK_{AHY}).[15] The observed activity drop of compound **1c** could be explained partially by the low hydrogen-bond acidity of 4-nitrophenol which is 3.48 while that of 4-bromophenol is 2.66.

Taken together, these results may suggest that the hydroxyl group at 2-position of the benzene ring makes a hydrogen bonding interaction with Pim-1 as a hydrogen bond donor and contributes to improve the binding affinity.

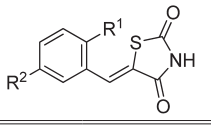
When a bromo group was replaced with a variety of pyrazine analogues, the potencies of inhibitors were much improved by about two orders of magnitude (Table 2). Further optimization of a pyrazine ring with both a hydrophobic cycloalkyl and a hydrophilic aminoalkyl moiety ultimately yielded compounds with subnanomolar IC_{50}s for Pim-1, which is comparable to compound **B**. The similar effect of substituents on potency was also observed for Pim-2 and Pim-3. In general, there is a tendency to improve inhibitory activities against all Pim-1, Pim-2, and Pim-3 kinases with the presence of hydroxyl group (R_1) in the benzene ring. It appears that the



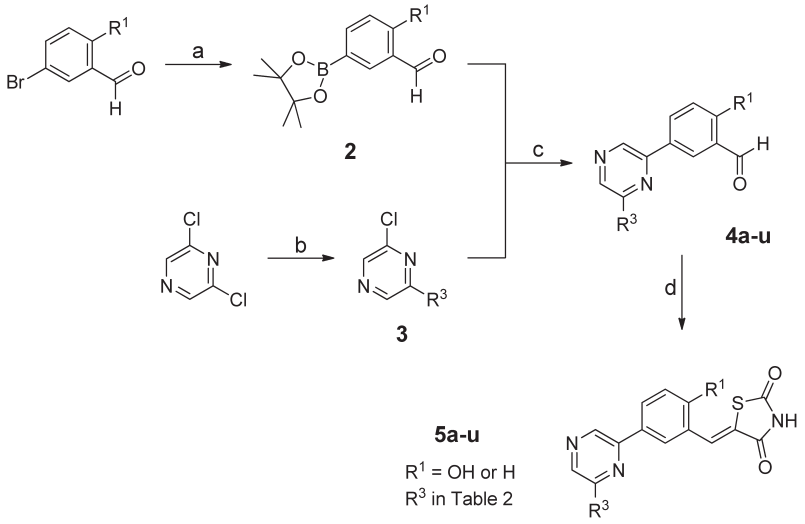
(a) Thiazolidine-2,4-dione, piperidine, acetic acid, toluene, microwave.

Chart 1. Reagents and Experimental Conditions

Table 1. Enzyme Inhibitory Activity of Compounds against Pim-1 Kinase



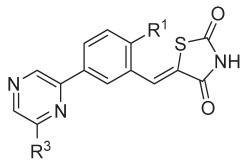
	R^1	R^2	IC_{50} (μM)
1a	H	Br	0.22
1b	OH	Br	0.028
1c	OH	NO_2	0.20

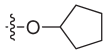
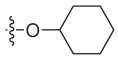
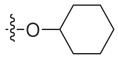
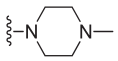
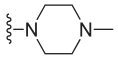
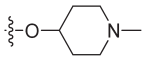
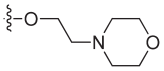


(a) Bis(pinacolato)diboron, $PdCl_2$(dppf), KOAc, 1,4-dioxane, microwave, (b) NaH, aminoalcohol, THF, microwave or aminoalkyamine, K_2CO_3, DMF, RT, (c) $PdCl_2(PPh_3)_2$, 2 M K_2CO_3, 1,4-dioxane : EtOH (2 : 3), microwave, (d) thiazolidine-2,4-dione, piperidine, acetic acid, toluene, microwave.

Chart 2. Reagents and Experimental Conditions

Table 2. Enzyme Inhibitory Activity of Compounds against Pim-1, Pim-2, and Pim-3 Kinase



	R^1	R^3	IC_{50} (μM)*		
			Pim-1	Pim-2	Pim-3
5a	OH		0.0009	0.0094	0.0010
5b	OH		0.0036	0.0143	0.0012
5c	H		0.0055	0.0965	0.0025
5d	OH	$-O(CH_2)_2NMe_2$	<0.0005**	0.0022	<0.0005**
5e	H	$-O(CH_2)_2NMe_2$	0.0053	0.0326	0.0038
5f	OH	$-O(CH_2)_3NMe_2$	<0.0005**	0.0017	<0.0005**
5g	H	$-O(CH_2)_3NMe_2$	0.0025	0.0168	<0.0005**
5h	OH	$-NH(CH_2)_2NMe_2$	<0.0005**	0.0018	<0.0005**
5i	H	$-NH(CH_2)_2NMe_2$	<0.0005**	0.0057	0.0006
5j	OH	$-NH(CH_2)_3NMe_2$	<0.0005**	0.0021	0.0006
5k	H	$-NH(CH_2)_3NMe_2$	0.0058	0.0105	0.0013
5l	OH		<0.0005**	0.0158	<0.0005**
5m	H		0.0018	0.0131	<0.0005**
5n	OH	$-O(CH_2)_2NEt_2$	<0.0005**	0.0022	<0.0005**
5o	OH	$-O(CH_2)_3NEt_2$	<0.0005**	0.0021	<0.0005**
5p	OH		<0.0005**	0.0028	<0.0005**
5q	OH		0.0009	0.0089	<0.0005**
5r	OH	$-NH(CH_2)_2NEt_2$	<0.0005**	0.0028	<0.0005**
5s	OH	$-NH(CH_2)_3NEt_2$	<0.0005**	0.0016	<0.0005**
5t	OH	$-N(Me)(CH_2)_2NMe_2$	<0.0005**	0.0089	<0.0005**
5u	OH	$-N(Me)(CH_2)_2NEt_2$	<0.0005**	0.0326	0.0038

*Values are average of results obtained by two independent experiments. **Lower than assay limit.

improved inhibitory activity gained by the hydroxyl group is even greater when pyrazine is substituted with an aminoalkyl group. All synthesized 5-benzylidenethiazolidine-2,4-dione derivatives, having both hydroxyl group at 2-position and aminoalkyl substituted pyrazine at 5-position, showed very strong potencies against Pim-1 kinase, reaching the lowest limit for IC_{50} determination. Although Pim-2 inhibition was a little weaker than IC_{50} values determined for Pim-1 or Pim-3, many compounds exhibited single digit nanomolar IC_{50} values for Pim-2.

In order to address inhibition of pim kinases in a cellular system, we further studied the effect of compounds on cancer cell growth inhibition in three leukemia cell lines, K562, MV4-11 and Jurkat. As shown in Fig. 2, multiple compounds were found to be more effective growth inhibitors for MV4-11 than either K562 or Jurkat and showed a dose dependency. Compound **5d** was about an order of magnitude more potent than **5e** against all three recombinant enzymes but was far inferior to **5e** in inhibition of cell proliferation. Compounds **5h** and **5i** showed similar enzymatic activity but **5i** inhibits cell proliferation more potently (Fig. 2D). Alteration of molecular properties such as increase in topological surface area value but decrease in $c\log p$ value by the hydroxyl group may attribute partially to the inconsistence between enzymatic activity and cellular effect.

Four compounds were selected for their EC_{50} value determination (Table 3). Compound **5i** showed the highest anti-proliferative activity against all three cell lines. As expected, compound **5i** was more potent for MV4-11 cell line by an order of magnitude than others, and showed an EC_{50} value of 0.8 μM.

The selectivity of synthesized compound over other 14 kinases was evaluated using compound **5i** as a representative compound (Table 4). Compound **5i** did not show any significant inhibition at 1 μM concentration against a panel of diverse

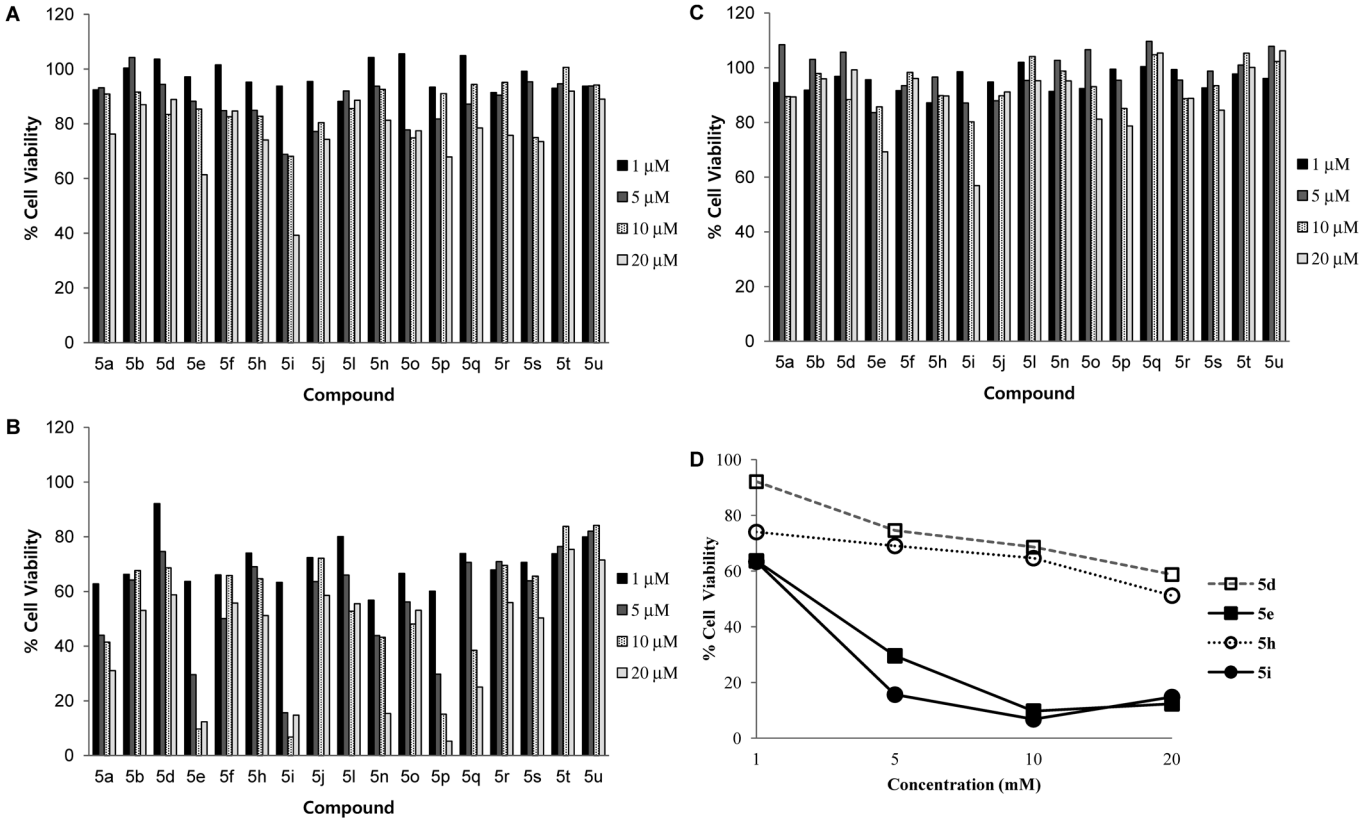


Fig. 2. Dose-Dependent Cell Growth Inhibitory Effects of Synthesized Compounds

(A) K562 cell line, (B) MV4-11 cell line, (C) Jurkat cell line, (D) MV4-11 cell line with selected compounds, **5d**, **5e**, **5h**, and **5i**.

Table 3. Anti-proliferative Activities of Compounds **5e**, **5i**, **5n**, and **5p**

	Cell viability EC_{50} (μM)*		
	Jurkat	K562	MV4-11
5e	>30	>30	2.0±1.3
5i	19.0±4.0	13.0±9.8	0.8±0.2
5n	>30	>30	11.4±1.7
5p	>30	>30	2.5±1.5

EC_{50} values in μM. *Values are average of three independent experiments±S.D.

kinases. Since JAK2[16] and Flt-3[17] regulate the expression of pim kinases in leukemia, the selectivity over these kinases is important for the evaluation of the anti-proliferative activity of pim kinase inhibitors.[18] It is noteworthy that compound **5i** showed less than 5% inhibition against both JAK2 and Flt-3 kinases at 1 μM concentration, indicating that the anti-proliferative effect was likely due to inhibition of pim kinases.

Conclusion

We have synthesized a series of 5-benzylidenethiazolidine-2,4-diones and evaluated their activities as pan–pim kinase inhibitors. Structure–activity studies of these pim inhibitors resulted in several analogues with IC_{50} values ranging from subnanomolar to low single-digit nanomolar and with excellent kinase selectivity against a panel of kinases. Importantly, multiple compounds exhibited anti-proliferative activity in MV4-11 cells with EC_{50} values in the submicromolar to low single-digit micromolar range.

Experimental

Chemistry All reactions were performed using commercially available reagents and solvents without further purification under proper conditions as stated. CEM Discover BenchMate had been used for microwave assisted reactions. Reaction completion was monitored on E. Merck silica gel F254 TLC plates. Purifications of synthesized compounds were performed by flash column chromatography using Merck Silica Gel 60 (230–400 mesh). Synthesized compounds were characterized by ^{1}H- and ^{13}C-NMR on a Bruker AVANCE 400 (^{1}H: 400 MHz, ^{13}C: 100 MHz) spectrometer and chemical shift δ were measured in ppm with tetramethylsilane (TMS) as reference standard. Mass spectra were obtained using Waters ACQUITY UPLC, Micromass Quattro micro™ API.

Preparation of Borylated Compound 2-Hydroxy-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzaldehyde: To 1,4-dioxane (2 mL) in a microwave reaction vessel were added the corresponding aldehyde (0.20 g, 9.9 mmol), bis(pinacolato)diboron (0.28 g, 1.1 mmol), potassium acetate (0.29 g, 3.0 mmol), and (1,1′-bis(diphenylphosphino)ferrocene)-dichloropalladium(II) (0.022 g, 0.030 mmol). The reaction mixture was heated in the microwave reactor at power 100 W and 120°C for 10 min. After solvent was removed *in vacuo*, the residue was treated with dichloromethane. The organic layer was dried over anhydrous sodium sulfate, filtered and removed *in vacuo*. The residue was purified by flash column chromatography over silica gel (hexane : ethyl acetate, 12 : 1) The product was obtained in 76% yield; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 11.24 (1H, s), 9.92 (1H, s), 8.05 (1H, d, J=1.6 Hz), 7.95 (1H, dd, J=8.4, 1.6 Hz), 6.98 (1H, d, J=8.4 Hz), 1.35 (12H, s).

Table 4. The Effect of Compound **5i** on Enzyme Activity of a Panel of 14 Protein Kinases

Enzyme	Type of kinase	% Activity*
Aurora-A	Serine–threonine	103
CDK2/cyclinA	Serine–threonine	95
cSRC	Nonreceptor tyrosine kinase	101
Flt3	Receptor tyrosine kinase	95
GSK3β	Serine–threonine	90
IRAK4	Serine–threonine	84
JAK2	Nonreceptor tyrosine kinase	110
JNK3	Serine–threonine	88
KDR	Receptor tyrosine kinase	78
MAPK2	Serine–threonine	92
Met	Receptor tyrosine kinase	78
Plk1	Serine–threonine	99
SAPK2a	Serine–threonine	90
TAK1	Serine–threonine	120

Percentage activity of kinase at 1 μM concentration of **5i**. *Results were obtained from the KinaseProfiler™ project of Merck Millipore.

3-(4,4,5,5-Tetramethyl-1,3,2-dioxaborolan-2-yl)benzaldehyde: Obtained 78% yield; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 10.05 (1H, s), 8.31 (1H, s), 8.07–8.05 (1H, m), 7.98 (1H, td, J=8.0, 1.6 Hz), 7.53 (1H, t, J=7.4 Hz), 1.37 (12H, s).

Preparation of 2-Alkoxy-6-chloropyrazine 2-Chloro-6-(cyclopentyloxy)pyrazine: To tetrahydrofuran (THF) (1 mL) in a microwave reaction vessel were added cyclopentanol (0.067 mL, 0.74 mmol) and NaH (0.040 g, 1.68 mmol). After the reaction mixture was stirred at room temperature for 10 min, 2,6-dichloropyrazine (0.10 g, 0.67 mmol) was added and the resulting reaction mixture was heated in the microwave reactor at power 100 W and 50°C for 10 min. After removal of solvent *in vacuo*, the residue was treated with ethyl acetate and water mixture. Organic layer was collected and dried over anhydrous sodium sulfate. Removal of solvent *in vacuo* gave 2-chloro-6-(cyclopentyloxy)pyrazine 0.148 g in quantitative yield; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.09 (1H, s), 8.05 (1H, s), 5.40 (1H, sept, J=2.9 Hz), 1.98–1.95 (2H, m), 1.84–1.75 (4H, m), 1.69–1.61 (2H, m).

2-Chloro-6-(cyclohexyloxy)pyrazine: Obtained quantitatively; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.08 (1H, s), 8.06 (1H, s), 5.04 (1H, hept, J=4.4 Hz), 2.01–1.97 (2H, m), 1.80–1.77 (2H, m), 1.60–1.30 (6H, m).

2-(6-Chloropyrazin-2-yloxy)-*N*,*N*-dimethylethanamine: Obtained in 99% yield; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.19 (1H, s), 8.14 (1H, s), 4.44 (2H, t, J=5.4 Hz), 2.74 (2H, t, J=5.4 Hz), 2.34 (6H, s).

2-(6-Chloropyrazin-2-yloxy)-*N*,*N*-diethylethanamine: Obtained in 95% yield; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.14 (1H, s), 8.13 (1H, s), 4.41 (2H, t, J=6.0 Hz), 2.86 (2H, t, J=6.0 Hz), 2.63 (4H, q, J=7.2 Hz), 1.06 (6H, t, J=7.2 Hz).

3-(6-Chloropyrazin-2-yloxy)-*N*,*N*-dimethylpropan-1-amine: Obtained in 77% yield; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.13 (1H, s), 8.12 (1H, s), 4.39 (2H, t, J=6.4 Hz), 2.44 (2H, t, J=7.4 Hz), 2.26 (6H, s), 1.97 (2H, sext, J=5.5 Hz).

3-(6-Chloropyrazin-2-yloxy)-*N*,*N*-diethylpropan-1-amine: Obtained in 88% yield; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.12 (1H, s), 8.10 (1H, s), 4.37 (2H, t, J=6.4 Hz), 2.64–2.54 (6H, m), 1.95 (2H, quint, J=6.9 Hz), 1.04 (6H, t, J=7.2 Hz).

2-Chloro-6-(1-methylpiperidin-4-yloxy)pyrazine: Obtained quantitatively; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.11 (1H, s), 8.10 (1H, s), 5.07 (1H, quint, J=4.0 Hz), 2.70 (2H, br), 2.08 (5H, br), 2.08–2.05 (2H, m), 1.91–1.85 (2H, m).

4-(2-(6-Chloropyrazin-2-yloxy)ethyl)morpholine: Obtained in 72% yield; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.15 (1H, s), 8.14 (1H, s), 4.48 (2H, t, J=5.6 Hz), 3.71 (4H, t, J=4.8 Hz), 2.79 (2H, t, J=5.6 Hz), 2.56 (4H, t, J=4.4 Hz).

Preparation of 2-Amino-6-chloropyrazine N^1-(6-Chloropyrazin-2-yl)-N^2,N^2-dimethylethane-1,2-diamine: To the *N*,*N*-dimethylformamide (DMF) (4 mL) solution of *N*,*N*-dimethylethylenediamine (95 mL, 0.87 mmol) was added K_2CO_3 (0.19 g, 1.3 mmol). After the reaction mixture was stirred at room temperature (RT) for 30 min, 2,6-dichloropyrazine (0.10 g, 0.67 mmol) was added and the resulting reaction mixture was further stirred at RT for 12 h. After removal of solvent *in vacuo*, the residue was treated with dichoromethane. Insoluble impurities were removed by filtration. Removal of solvent *in vacuo* gave the product 0.095 g in 71% yield; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.77 (1H, s), 7.74 (1H, s), 5.69 (1H, br), 3.39 (2H, q, J=5.6 Hz), 2.54 (2H, t, J=5.8 Hz), 2.26 (6H, s).

N^1-(6-Chloropyrazin-2-yl)-N^2,N^2-diethylethane-1,2-diamine: Obtained in 63% yield; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.77 (1H, s), 7.75 (1H, s), 5.66 (1H, br), 3.36 (2H, q, J=5.6 Hz), 2.66 (2H, t, J=6.0 Hz), 2.56 (4H, q, J=7.0 Hz), 1.02 (6H, t, J=7.0 Hz).

N^1-(6-Chloropyrazin-2-yl)-N^3,N^3-dimethylpropane-1,3-diamine: Obtained in 95% yield; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.72 (1H, s), 7.71 (1H, s), 6.49 (1H, br), 3.42 (2H, q, J=6.0 Hz), 2.43 (2H, t, J=6.4 Hz), 2.26 (6H, s), 1.77 (2H, quint, J=6.3 Hz).

N^1-(6-Chloropyrazin-2-yl)-N^3,N^3-diethylpropane-1,3-diamine: Obtained in 69% yield; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.62 (2H, s), 7.14 (1H, br), 3.37 (2H, q, J=5.6 Hz), 2.51 (2H, t, J=6.0 Hz), 2.47 (4H, q, J=7.1 Hz), 1.69 (2H, quint, J=6.1 Hz), 0.99 (6H, t, J=7.0 Hz).

N^1-(6-Chloropyrazin-2-yl)-N^1,N^2,N^2-trimethylethane-1,2-diamine: Obtained in 84% yield; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.86 (1H, s), 7.74 (1H, s), 3.64 (2H, t, J=7.2 Hz), 3.11 (3H, s), 2.50 (2H, t, J=7.1 Hz), 2.29 (6H, s).

N^1-(6-Chloropyrazin-2-yl)-N^2,N^2-diethyl-N^1-methylethane-1,2-diamine: Obtained in 93% yield; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.85 (1H, s), 7.73 (1H, s), 3.60 (2H, t, J=7.2 Hz), 3.11 (3H, s), 2.62 (2H, t, J=7.2 Hz), 2.56 (4H, q, J=7.1 Hz), 1.02 (6H, t, J=7.2 Hz).

2-Chloro-6-(4-methylpiperazin-1-yl)pyrazine: Obtained in 80% yield; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.98 (1H, s), 7.80 (1H, s), 3.63 (4H, t, J=5.0 Hz), 2.51 (4H, t, J=5.2 Hz), 2.35 (3H, s).

General Procedure for Suzuki Coupling (4a to 4u) A microwave tube filled with compound **2** (0.35 mmol), alkoxy heteroaromatic chloride or amine heteroaromatic chloride (0.38 mmol), bis(triphenylphosphine) palladium(II) dichloride (0.010 mmol), 2 M potassium carbonate (1.7 mmol) in dioxane : ethanol (2 : 3) was heated in the microwave reactor at power 100 W and 120°C for 10 min. After solvent was removed *in vacuo*, the residue was treated with ethyl acetate and washed with water. The obtained organic layer was dried over anhydrous sodium sulfate, and concentrated *in vacuo*. The residue was purified by flash column chromatography over silica gel.

3-(6-Cyclohexyloxypyrazin-2-yl)benzaldehyde (**4c**): Obtained

52% yield; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 10.12 (1H, s), 8.61 (1H, s), 8.49–8.48 (1H, m), 8.29–8.26 (1H, m), 8.16 (1H, s), 7.97–7.95 (1H, m), 7.67 (1H, t, J=7.8 Hz), 5.25–5.18 (1H, m), 2.10–2.02 (2H, m), 1.86–1.83 (2H, m), 1.68–1.51 (6H, m).

3-(6-(2-(Dimethylamino)ethoxy)pyrazin-2-yl)benzaldehyde (**4e**): Obtained 52% yield; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 10.12 (1H, s), 8.66 (1H, s), 8.52 (1H, d, J=1.2 Hz), 8.31–8.28 (1H, m), 8.27 (1H, s), 7.96 (1H, td, J=7.6, 1.4 Hz), 7.67 (1H, t, J=7.6 Hz), 4.59 (2H, t, J=5.6 Hz), 2.81 (2H, t, J=5.8 Hz), 2.39 (6H, s).

3-(6-(3-(Dimethylamino)propoxy)pyrazin-2-yl)benzaldehyde (**4g**): Used for the next reaction for synthesis of **5g** without further purification.

3-(6-((2-(Dimethylamino)ethyl)amino)pyrazin-2-yl)-benzaldehyde (**4i**): Obtained 74% yield; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 10.00 (1H, s), 8.41 (1H, s), 8.21 (1H, s), 8.16 (1H, d, J=7.2 Hz), 7.83–7.81 (2H, m), 7.54–7.50 (1H, m), 5.52 (1H, br), 3.47 (2H, t, J=5.4 Hz), 2.54 (2H, t, J=5.8 Hz), 2.23 (6H, s).

3-(6-((3-(Dimethylamino)propyl)amino)pyrazin-2-yl)-benzaldehyde (**4k**): Obtained 54% yield; ^{1}H-NMR (400 MHz, $CDCl_3$+CD_3OD) δ: 9.99 (1H, s), 8.40 (1H, s), 8.17 (1H, d, J=7.6 Hz), 8.13 (1H, s), 7.83 (1H, d, J=7.6 Hz), 7.76 (1H, s), 7.54 (1H, t, J=7.6 Hz), 3.43 (2H, t, J=6.4 Hz), 2.45 (2H, t, J=7.2 Hz), 2.25 (6H, s), 1.81 (2H, quint, J=6.8 Hz).

3-(6-(4-Methylpiperazin-1-yl)pyrazin-2-yl)benzaldehyde (**4m**): Used for the next reaction for synthesis of **5m** without further purification.

5-(6-Cyclopentyloxypyrazin-2-yl)-2-hydroxybenzaldehyde (**4a**): Obtained 74% yield; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 11.19 (1H, s), 10.03 (1H, s), 8.53 (1H, s), 8.25 (1H, d, J=2.4 Hz), 8.21 (1H, dd, J=8.8, 2.4 Hz), 8.09 (1H, s), 7.11 (1H, d, J=8.8 Hz), 5.53 (1H, sext, J=3.2 Hz), 2.10–2.04 (2H, m), 1.90–1.82 (4H, m), 1.70–1.66 (2H, m).

5-(6-Cyclohexyloxypyrazin-2-yl)-2-hydroxybenzaldehyde (**4b**): Obtained 55% yield; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 11.18 (1H, s), 10.02 (1H, s), 8.52 (1H, s), 8.22 (1H, d, J=2.4 Hz), 8.18 (1H, dd, J=8.8, 2.4 Hz), 8.10 (1H, s), 7.12 (1H, d, J=8.8 Hz), 5.19 (1H, sext, J=4.3 Hz), 2.09–2.04 (2H, m), 1.86–1.83 (2H, m), 1.67–1.50 (6H, m).

5-(6-(2-(Dimethylamino)ethoxy)pyrazin-2-yl)-2-hydroxybenzaldehyde (**4d**): Obtained 72% yield; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 10.05 (1H, s), 8.59 (1H, s), 8.29 (1H, d, J=2.4 Hz), 8.21 (1H, dd, J=8.8, 2.0 Hz), 8.18 (1H, s), 7.13 (1H, d, J=8.8 Hz), 4.64 (2H, t, J=5.4 Hz), 2.98 (2H, t, J=5.6 Hz), 2.49 (6H, s).

5-(6-(2-(Diethylamino)ethoxy)pyrazin-2-yl)-2-hydroxybenzaldehyde (**4n**): Obtained 71% yield; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 10.04 (1H, s), 8.57 (1H, s), 8.28 (1H, d, J=2.0 Hz), 8.20 (1H, dd, J=8.8, 2.0 Hz), 8.17 (1H, s), 7.11 (1H, d, J=8.8 Hz), 4.55 (2H, t, J=6.2 Hz), 2.95 (2H, t, J=6.2 Hz), 2.71 (4H, q, J=7.1 Hz), 1.11 (6H, t, J=7.2 Hz).

5-(6-(3-(Dimethylamino)propoxy)pyrazin-2-yl)-2-hydroxybenzaldehyde (**4f**): Obtained 83% yield; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 10.06 (1H, s), 8.58 (1H, s), 8.27 (1H, d, J=2.0 Hz), 8.19 (1H, dd, J=8.8 Hz, J=2.4 Hz), 8.15 (1H, s), 7.12 (1H, d, J=8.8 Hz), 4.53 (2H, t, J=6.0 Hz), 2.97 (2H, t, J=7.8 Hz), 2.61 (6H, s), 2.25–2.23 (2H, m).

5-(6-(3-(Diethylamino)propoxy)pyrazin-2-yl)-2-hydroxybenzaldehyde (**4o**): Obtained 92% yield; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 10.07 (1H, s), 8.56 (1H, s), 8.27 (1H, d, J=2.0 Hz), 8.18 (1H, dd, J=8.8, 2.0 Hz), 8.14 (1H, s), 7.11 (1H, d, J=8.8 Hz), 4.51 (2H, t, J=6.2 Hz), 2.90 (2H, t, J=7.8 Hz), 2.83 (4H, q, J=7.2 Hz), 2.19–2.12 (2H, m), 1.18 (6H, t, J=7.2 Hz).

5-(6-((2-(Dimethylamino)ethyl)amino)pyrazin-2-yl)-2-hydroxybenzaldehyde (**4h**): Obtained 31% yield; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 10.04 (1H, s), 8.27 (1H, d, J=2.0 Hz), 8.19 (1H, dd, J=8.4, 2.0 Hz), 8.17 (1H, s), 7.81 (1H, s), 7.09 (1H, d, J=8.8 Hz), 3.64 (2H, t, J=6.2 Hz), 2.73 (2H, t, J=6.0 Hz), 2.40 (6H, s).

5-(6-((2-(Diethylamino)ethyl)amino)pyrazin-2-yl)-2-hydroxybenzaldehyde (**4r**): Obtained 60% yield; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 10.01 (1H, s), 8.24 (1H, d, J=2.0 Hz), 8.22 (1H, s), 8.17 (1H, dd, J=8.8, 2.4 Hz), 7.84 (1H, s), 7.07 (1H, d, J=8.4 Hz), 5.58 (1H, br), 3.51 (2H, q, J=5.7 Hz), 2.75 (2H, t, J=6.2 Hz), 2.63 (4H, q, J=7.2 Hz), 1.07 (6H, t, J=7.2 Hz).

5-(6-((3-(Dimethylamino)propyl)amino)pyrazin-2-yl)-2-hydroxybenzaldehyde (**4j**): Obtained 95% yield; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 10.02 (1H, s), 8.22 (1H, d, J=2.0 Hz), 8.17–8.15 (2H, m), 7.86 (1H, s), 7.08 (1H, d, J=8.4 Hz), 3.59 (2H, t, J=6.2 Hz), 3.00 (2H, t, J=7.4 Hz), 2.67 (6H, s), 2.08 (2H, quint, J=6.8 Hz).

5-(6-((3-(Diethylamino)propyl)amino)pyrazin-2-yl-2-hydroxybenzaldehyde (**4s**): Obtained 87% yield; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 9.94 (1H, s), 8.13 (1H, d, J=2.4 Hz), 8.08 (1H, s), 8.05 (1H, dd, J=8.8, 2.0 Hz), 7.80 (1H, s), 6.97 (1H, d, J=8.8 Hz), 3.48 (2H, t, J=6.2 Hz), 2.85 (2H, t, J=7.2 Hz), 2.80 (4H, q, J=7.3 Hz), 1.97–1.91 (2H, br), 1.11 (6H, t, J=7.2 Hz).

5-(6-((2-(Dimethylamino)ethyl)(methyl)amino)pyrazin-2-yl)-2-hydroxybenzaldehyde (**4t**): Obtained 60% yield; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 9.99 (1H, s), 8.25–8.18 (3H, m), 7.95 (1H, s), 7.07 (1H, d, J=8.4 Hz), 3.80 (2H, t, J=7.2 Hz), 3.19 (3H, s), 2.61 (2H, t, J=7.0 Hz), 2.36 (6H, s).

5-(6-((2-(Diethylamino)ethyl)(methyl)amino)pyrazin-2-yl)-2-hydroxybenzaldehyde (**4u**): Obtained 61% yield; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 9.99 (1H, s), 8.24 (2H, br), 8.18 (1H, d, J=8.8 Hz), 7.95 (1H, s), 7.06 (1H, d, J=8.8 Hz), 3.77 (2H, t, J=7.4 Hz), 3.19 (3H, s), 2.72 (2H, t, J=7.4 Hz), 2.65 (4H, q, J=7.1 Hz), 1.07 (6H, t, J=7.0 Hz).

2-Hydroxy-5-(6-(4-methylpiperazin-1-yl)pyrazin-2-yl)-benzaldehyde (**4l**): Obtained 57% yield; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 10.01 (1H, s), 8.29 (1H, s), 8.21 (1H, d, J=2.0 Hz), 8.17 (1H, dd, J=8.8, 2.0 Hz), 8.07 (1H, s), 7.08 (1H, d, J=8.4 Hz), 3.72 (4H, t, J=5.0 Hz), 2.57 (4H, t, J=5.2 Hz), 2.38 (3H, s).

2-Hydroxy-5-(6-((1-methylpiperidin-4-yl)oxy)pyrazin-2-yl)-benzaldehyde (**4p**): Obtained 61% yield; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 10.04 (1H, s), 8.55 (1H, s), 8.21 (1H, d, J=2.4 Hz), 8.18 (1H, dd, J=8.8, 2.4 Hz), 8.14 (1H, s), 7.12 (1H, d, J=8.8 Hz), 5.30–5.25 (1H, m), 2.77 (2H, br), 2.46 (2H, br), 2.37 (3H, s), 2.17–2.12 (2H, m), 2.01–1.95 (2H, m).

2-Hydroxy-5-(6-(2-morpholinoethoxy)pyrazin-2-yl)-benzaldehyde (**4q**): Obtained 77% yield; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 10.04 (1H, s), 8.57 (1H, s), 8.28 (1H, d, J=2.0 Hz), 8.21 (1H, dd, J=8.8, 2.0 Hz), 8.16 (1H, s), 7.12 (1H, d, J=8.8 Hz), 4.62 (2H, t, J=5.6 Hz), 3.77 (4H, t, J=4.6 Hz), 2.90 (2H, t, J=5.6 Hz), 2.64 (4H, t, J=4.4 Hz).

Preparation of 5-Benzilidenethiazolidine-2,4-diones (5a to 5u) To toluene (2 mL) in a microwave reaction vessel were added compound **3** (0.179 mmol), thiazolidine-2,4-dione (0.197 mmol), piperidine (0.143 mmol), and acetic acid

(0.143 mmol). The reaction mixture was heated in the microwave reactor at power 100 W and 120°C for 10 min. The precipitate thus formed was collected by filtration. Consecutive washing with DCM (5 mL×3), H_2O (5 mL×3), and MeOH (5 mL×3) followed by drying gave the product as solid.

5-(3-(6-Cyclohexyloxypyrazin-2-yl)benzylidene)-thiazolidine-2,4-dione (**5c**): Obtained as a pale yellow solid in 13% yield; ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 8.85 (1H, s), 8.38 (1H, s), 8.24 (1H, s), 8.20 (1H, d, J=8.0 Hz), 7.86 (1H, s), 7.74 (1H, d, J=8.0 Hz), 7.66 (1H, t, J=7.6 Hz), 5.22 (1H, quint, J=4.6 Hz), 2.12–2.09 (2H, m), 1.81–1.78 (2H, m), 1.62–1.31 (6H, m). ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 168.54, 158.92, 146.97, 136.81, 135.12, 134.31, 133.30, 132.84, 131.24, 130.46, 128.40, 127.14, 125.25, 74.23, 31.71, 25.51, 24.14. Electrospray ionization-mass spectra (ESI-MS) m/z: 382 $(M+H)^+$.

5-(3-(6-(2-(Dimethylamino)ethoxy)pyrazin-2-yl)-benzylidene)thiazolidine-2,4-dione (**5e**): Obtained as a pale yellow solid in 26% yield; ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 8.90 (1H, s), 8.37 (1H, s), 8.32 (1H, s), 8.14 (1H, d, J=6.8 Hz), 7.69–7.61 (3H, m), 4.71 (2H, t, J=5.2 Hz), 3.21 (2H, t, J=5.0 Hz), 2.60 (6H, s). ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 176.14, 172.35, 158.95, 147.58, 136.52, 135.87, 134.58, 134.07, 131.88, 131.12, 130.15, 128.15, 127.38, 126.52, 62.34, 56.79, 44.55. ESI-MS m/z: 371 $(M+H)^+$.

5-(3-(6-(3-Dimethylamino)propoxy)pyrazin-2-yl)-benzylidene)thiazolidine-2,4-dione (**5g**): Obtained as a pale yellow solid in 17% yield; ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 8.87 (1H, s), 8.36 (1H, s), 8.28 (1H, s), 8.10 (1H, d, J=8.8 Hz), 7.65–7.58 (2H, m), 7.51 (1H, s), 4.56 (2H, t, J=6.2 Hz), 3.06 (2H, t, J=7.4 Hz), 2.66 (6H, s), 2.16 (2H, quint, J=6.9 Hz). ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 179.38, 174.09, 159.21, 147.67, 136.40, 136.33, 134.41, 133.75, 130.99, 129.96, 127.83, 126.79, 124.61, 63.86, 54.94, 43.53, 24.91. ESI-MS m/z: 385 $(M+H)^+$.

5-(3-(6-((2-(Dimethylamino)ethyl)amino)pyrazin-2-yl)-benzylidene)thiazolidine-2,4-dione (**5i**): Obtained as a pale yellow solid in 62% yield; ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 8.36 (1H, s), 8.31 (1H, s), 8.03 (1H, d, J=7.6 Hz), 7.99 (1H, s), 7.63–7.55 (3H, m), 7.42 (1H, t, J=5.4 Hz), 3.73 (2H, q, J=5.7 Hz), 3.13 (2H, t, J=6.0 Hz), 2.68 (6H, s). ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 178.30, 173.71, 154.35, 147.81, 137.81, 135.88, 133.24, 132.98, 130.71, 129.82, 128.61, 127.56, 126.90, 125.79, 56.81, 43.89, 36.75. ESI-MS m/z: 370 $(M+H)^+$.

5-(3-(6-((3-(Dimethylamino)propyl)amino)pyrazin-2-yl)-benzylidene(thiazolidine-2,4-dione (**5k**): Obtained as a pale yellow solid in 26% yield; ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 8.37 (1H, s), 8.33 (1H, s), 7.99 (1H, d, J=7.6 Hz), 7.93 (1H, s), 7.59 (1H, d, J=8.0 Hz), 7.54 (1H, t, J=7.6 Hz), 7.48 (1H, s), 7.34 (1H, t, J=5.6 Hz), 3.50 (2H, q, J=6.3 Hz), 2.99 (2H, t, J=7.8 Hz), 2.65 (6H, s), 1.96 (2H, quint, J=7.3 Hz). ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 180.31, 174.73, 154.54, 147.83, 137.73, 136.24, 134.78, 132.90, 130.84, 129.68, 128.07, 127.19, 126.36, 124.36, 55.88, 43.42, 38.01, 25.22. ESI-MS m/z: 384 $(M+H)^+$.

5-(3-(6-(4-Methylpiperazin-1-yl)pyrazin-2-yl)benzylidene)-thiazolidine-2,4-dione (**5m**): Obtained as a pale yellow solid in 20% yield; ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 8.52 (1H, s), 8.36 (1H, s), 8.33 (1H, s), 8.11 (1H, d, J=7.2 Hz), 7.74 (1H, s), 7.65–7.60 (2H, m), 3.78 (4H, br), 2.73 (4H, br), 2.43 (3H, s). ^{13}C-NMR (100 MHz, DMSO-d_6+$CDCl_3$) δ: 172.70, 170.74, 153.96, 147.34, 137.59, 134.98, 130.96, 130.08, 129.73, 128.97, 128.63, 128.16, 127.76, 53.97, 45.26, 43.59. ESI-MS m/z: 382 $(M+H)^+$.

5-(5-(6-Cyclopentyloxypyrazin-2-yl)-2-hydroxy-benzylidene)thiazolidine-2,4-dione (**5a**): Obtained as a yellow solid in 54% yield; ^{1}H-NMR (400 MHz, DMSO-d_6+CD_3OD) δ: 8.67 (1H, s), 8.21 (1H, d, J=2.0 Hz), 8.10 (1H, s), 8.06–8.03 (2H, m), 7.05 (1H, d, J=8.4 Hz), 5.49 (1H, sext, J=4.0 Hz), 2.12–2.11 (2H, m), 1.78–1.76 (4H, m), 1.66–1.64 (2H, m). ^{13}C-NMR (100 MHz, DMSO-d_6+CD_3OD) δ: 168.41, 167.97, 159.09, 147.25, 133.55, 132.13, 130.40, 127.41, 127.11, 126.42, 123.18, 120.91, 116.82, 78.39, 32.60, 23.64. ESI-MS m/z: 384 $(M+H)^+$.

5-(5-(6-Cyclohexyloxypyrazin-2-yl)-2-hydroxybenzylidene)-thiazolidine-2,4-dione (**5b**): Obtained as a yellow solid in 81% yield; ^{1}H-NMR (400 MHz, DMSO-d_6+CD_3OD) δ: 8.68 (1H, s), 8.22 (1H, d, J=2.0 Hz), 8.10 (1H, s), 8.07–8.04 (2H, m), 7.06 (1H, d, J=8.8 Hz), 5.19 (1H, br), 2.11–2.09 (2H, m), 1.79–1.77 (2H, m), 1.61–1.28 (6H, m). ^{13}C-NMR (100 MHz, DMSO-d_6+CD_3OD) δ: 168.29, 167.94, 158.80, 147.18, 133.65, 132.23, 130.37, 127.36, 127.10, 126.26, 123.06, 120.93, 116.86, 74.00, 31.85, 25.55, 24.30. ESI-MS m/z: 398 $(M+H)^+$.

5-(5-(6-(2-(Dimethylamino)ethoxy)pyrazin-2-yl)-2-hydroxybenzylidene)thiazolidine-2,4-dione (**5d**): Obtained as an orange colored solid in 42% yield; ^{1}H-NMR (400 MHz, DMSO-d_6+CD_3OD) δ: 8.63 (1H, s), 8.22 (1H, d, J=1.6 Hz), 8.12 (1H, s), 8.07 (1H, s), 7.79 (1H, d, J=8.4 Hz), 7.08 (1H, d, J=8.4 Hz), 4.59 (2H, t, J=5.6 Hz), 2.83 (2H, t, J=5.8 Hz), 2.35 (6H, s). ^{13}C-NMR (100 MHz, DMSO-d_6+CD_3OD) δ: 174.65, 171.84, 158.88, 147.81, 133.05, 132.87, 129.42, 128.87, 127.16, 126.96, 122.49, 122.29, 116.65, 62.44, 57.01, 44.76. ESI-MS m/z: 387 $(M+H)^+$.

5-(5-(6-(2-(Diethylamino)ethoxy)pyrazin-2-yl)-2-hydroxybenzylidene)thiazolidine-2,4-dione (**5n**): Obtained as an orange colored solid in 41% yield; ^{1}H-NMR (400 MHz, DMSO-d_6+$CDCl_3$) δ: 8.74 (1H, s), 8.28 (1H, d, J=2.4 Hz), 8.19 (1H, s), 8.02 (1H, dd, J=8.8, 2.4 Hz), 7.89 (1H, s), 7.06 (1H, d, J=8.4 Hz), 4.64 (2H, t, J=5.6 Hz), 3.20 (2H, m), 2.86 (4H, m), 1.09 (6H, t, J=7.2 Hz). ^{13}C-NMR (100 MHz, DMSO-d_6+$CDCl_3$) δ: 174.96, 171.98, 158.99, 158.94, 147.84, 133.02, 132.86, 129.36, 129.09, 127.16, 126.92, 122.37, 116.64, 62.65, 50.94, 47.51, 10.92. ESI-MS m/z: 415 $(M+H)^+$.

5-(5-(6-(3-Dimethylamino)propoxy)pyrazin-2-yl)-2-hydroxybenzylidene)thiazolidine-2,4-dione (**5f**): Obtained as an orange colored solid in 49% yield; ^{1}H-NMR (400 MHz, DMSO-d_6+$CDCl_3$) δ: 8.72 (1H, s), 8.34 (1H, d, J=2.0 Hz), 8.17 (1H, s), 7.98 (1H, dd, J=8.4, 2.0 Hz), 7.82 (1H, s), 7.03 (1H, d, J=8.4 Hz), 4.54 (2H, t, J=6.4 Hz), 2.96 (2H, t, J=7.6 Hz), 2.59 (6H, s), 2.13 (2H, quint, J=7.0 Hz). ^{13}C-NMR (100 MHz, DMSO-d_6+$CDCl_3$) δ: 173.26, 172.49, 159.09, 158.88, 147.91, 132.83, 132.56, 130.67, 128.85, 127.15, 126.85, 122.70, 121.16, 116.51, 63.78, 55.09, 43.87, 25.37. ESI-MS m/z: 401 $(M+H)^+$.

5-(5-(6-(3-Diethylaminopropoxy)pyrazin-2-yl)-2-hydroxybenzylidene)thiazolidine-2,4-dione (**5o**): Obtained as a red orange colored solid in 37% yield; ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 8.69 (1H, s), 8.34 (1H, s), 8.15 (1H, s), 7.96–7.94 (1H, m), 7.80 (1H, s), 7.02 (1H, d, J=8.4 Hz), 4.56 (2H, t, J=6.2 Hz), 3.17–3.15 (2H, m), 3.06 (4H, q, J=7.2 Hz), 2.19–2.18 (2H, m), 1.16 (6H, t, J=7.0 Hz). ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 174.22, 172.47, 159.03, 158.65, 147.96, 132.75, 132.58, 131.68, 128.59, 127.12, 126.83, 122.86, 120.41, 116.32, 63.48, 46.73, 24.14, 21.22, 9.31. ESI-MS m/z: 429 $(M+H)^+$.

5-(5-(6-((2-Dimethylamino)ethyl)amino)pyrazin-2-yl)-2-

hydroxybenzylidene)thiazolidine-2,4-dione (**5h**): Obtained as a yellow solid in 29% yield; ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 8.25 (1H, d, J=2.0 Hz), 8.22 (H, s), 7.89 (1H, dd, J=8.4, 2.0 Hz), 7.87 (1H, s), 7.85 (1H, s), 7.21 (1H, t, J=5.4 Hz), 7.00 (1H, d, J=8.8 Hz), 3.65 (2H, q, J=6.0 Hz), 2.96 (2H, t, J=6.0 Hz), 2.55 (6H, s). ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 158.35, 154.28, 147.96, 131.85, 128.81, 128.32, 127.43, 126.79, 122.35, 116.36, 57.34, 44.37, 37.18. ESI-MS m/z: 386 $(M+H)^+$.

5-(5-(6-((2-Diethylamino)ethyl)amino)pyrazin-2-yl)-2-hydroxybenzylidene)thiazolidine-2,4-dione (**5r**): Obtained as a blackish yellow solid in 29% yield; ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 8.26 (1H, d, J=2.0 Hz), 8.22 (1H, s), 7.90–7.88 (2H, m), 7.84 (1H, s), 7.22 (1H, t, J=5.4 Hz), 7.01 (1H, d, J=8.4 Hz), 3.66 (2H, q, J=5.9 Hz), 3.08 (2H, t, J=6.4 Hz), 2.95 (4H, q, J=7.1 Hz), 1.10 (6H, t, J=7.0 Hz). ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 173.06, 158.32, 154.30, 148.09, 131.85, 128.75, 128.30, 127.62, 126.80, 122.52, 116.35, 50.93, 47.21, 36.87, 10.28. ESI-MS m/z: 414 $(M+H)^+$.

5-(5-(6-((3-Dimethylamino)propyl)amino)pyrazin-2-yl)-2-hydroxybenzylidene)thiazolidine-2,4-dione (**5j**): Obtained as a pale yellow solid in 22% yield; ^{1}H-NMR (400 MHz, DMSO-d_6+CD_3OD) δ: 8.39 (1H, d, J=2.0 Hz), 8.19 (1H, s), 7.85 (1H, dd, J=8.4, 2.0 Hz), 7.81 (1H, s), 7.78 (1H, s), 7.23 (1H, t, J=5.6 Hz), 6.98 (1H, d, J=8.4 Hz), 3.49 (2H, t, J=6.8 Hz), 2.94 (2H, t, J=7.8 Hz), 2.61 (6H, s), 1.97–1.89 (2H, m). ^{13}C-NMR (100 MHz, DMSO-d_6+CD_3OD) δ: 158.02, 154.32, 148.01, 131.45, 128.24, 127.97, 126.96, 126.86, 122.84, 116.04, 55.98, 43.55, 37.87, 25.61. ESI-MS m/z: 400 $(M+H)^+$.

5-(5-(6-((3-Diethylamino)propyl)amino)pyrazin-2-yl)-2-hydroxybenzylidene)thiazolidine-2,4-dione (**5s**): Obtained as a yellow solid in 16% yield; ^{1}H-NMR (400 MHz, DMSO-d_6+CD_3OD) δ: 8.40 (1H, s), 8.20 (1H, s), 7.86–7.82 (2H, m), 7.77 (1H, s), 7.26 (1H, br), 6.98 (1H, d, J=8.4 Hz), 3.52 (2H, t, J=6.2 Hz), 3.09–2.99 (6H, m), 1.97–1.91 (2H, m), 1.13 (6H, t, J=7.0 Hz). ^{13}C-NMR (100 MHz, DMSO-d_6+CD_3OD) δ: 172.46, 158.00, 154.35, 154.29, 148.04, 131.42, 128.17, 127.83, 127.03, 126.86, 122.98, 116.00, 49.68, 46.80, 24.57, 21.38, 9.46. ESI-MS m/z: 428 $(M+H)^+$.

5-(5-(6-((2-(Dimethylamino)ethyl)(methyl)amino)pyrazin-2-yl)-2-hydroxy-benzylidene)thiazolidine-2,4-dione (**5t**): Obtained as a blackish yellow solid in 67% yield; ^{1}H-NMR (400 MHz, DMSO-d_6+CD_3OD) δ: 8.31 (1H, s), 8.23 (1H, d, J=2.0 Hz), 8.05 (1H, s), 7.92 (1H, dd, J=8.4, 2.0 Hz), 7.87 (1H, s), 7.03 (1H, d, J=8.4 Hz), 3.89 (2H, t, J=6.6 Hz), 3.15 (3H, s), 2.94 (2H, t, J=6.6 Hz), 2.52 (6H, s). ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 175.49, 172.32, 158.52, 153.53, 147.82, 129.31, 129.11, 128.32, 128.25, 127.57, 126.84, 122.30, 122.24, 116.46, 55.89, 46.01, 44.95, 36.17. ESI-MS m/z: 400 $(M+H)^+$.

5-(5-(6-((2-(Diethylamino)ethyl)(methyl)amino)pyrazin-2-yl)-2-hydroxy-benzylidene)thiazolidine-2,4-dione (**5u**): Obtained as a yellow solid in 76% yield; ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 8.31 (1H, s), 8.23 (1H, d, J=2.0 Hz), 8.06 (1H, s), 7.93 (1H, dd, J=8.4, 2.0 Hz), 7.88 (1H, s), 7.03 (1H, d, J=8.8 Hz), 3.86 (2H, t, J=6.6 Hz), 3.17 (3H, s), 2.95 (2H, t, J=6.6 Hz), 2.83 (4H, q, J=7.2 Hz), 1.02 (6H, t, J=7.0 Hz). ^{13}C-NMR (100 MHz, DMSO-d_6+$CDCl_3$) δ: 172.14, 158.49, 153.53, 147.86, 129.20, 128.41, 128.30, 127.63, 126.91, 122.50, 122.25, 116.45, 49.44, 47.27, 46.11, 36.42, 10.90. ESI-MS m/z: 428 $(M+H)^+$.

5-(2-Hydroxy-5-(6-(4-methylpiperazin-1-yl)pyrazin-2-yl)-benzylidene)thiazolidine-2,4-dione (**5l**): Obtained as a yellow solid in 82% yield; ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 8.44 (1H, s), 8.30 (1H, s), 8.27 (1H, d, J=2.0 Hz), 8.05–8.02 (2H, m), 7.10 (1H, d, J=8.4 Hz), 3.80 (4H, br), 2.71 (4H, t, J=4.6 Hz), 2.44 (3H, s). ^{13}C-NMR (100 MHz, DMSO-d_6+$CDCl_3$) δ: 171.88, 170.50, 158.78, 153.97, 147.50, 129.73, 129.65, 128.63, 128.07, 127.08, 126.28, 124.45, 121.54, 116.66, 54.23, 45.67, 43.93. ESI-MS m/z: 398 $(M+H)^+$.

5-(2-Hydroxy-5-(6-((1-methyl-piperidin-4-yl)oxy)pyrazin-2-yl)benzylidene)thiazolidine-2,4-dione (**5p**): Obtained as a red orange solid in 36% yield; ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 8.75 (1H, s), 8.36 (1H, s), 8.16 (1H, s), 8.02 (1H, d, J=8.4 Hz), 7.88 (1H, s), 7.05 (1H, d, J=8.8 Hz), 5.39 (1H, br), 3.21–3.19 (2H, m), 2.93–2.90 (2H, m), 2.62 (3H, s), 2.27–2.24 (2H, m), 1.99–1.96 (2H, m). ^{13}C-NMR (100 MHz, DMSO-d_6+$CDCl_3$) δ: 172.45, 158.46, 157.90, 147.18, 132.70, 132.10, 129.76, 128.36, 126.63, 126.27, 122.12, 120.73, 116.06, 51.83, 43.60, 28.88, 21.04. ESI-MS m/z: 413 $(M+H)^+$.

5-(2-Hydroxy-5-(6-(2-morpholinoethoxy)pyrazin-2-yl)-benzylidene)thiazolidine-2,4-dione (**5q**): Obtained as a yellow solid in 24% yield; ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 11.04 (1H, br), 8.73 (1H, s), 8.22 (1H, d, J=2.0 Hz), 8.19 (1H, s), 8.08 (1H, dd, J=8.8, 2.0 Hz), 8.02 (1H, s), 7.08 (1H, d, J=8.4 Hz), 4.59 (2H, t, J=6.0 Hz), 3.58 (4H, t, J=4.6 Hz), 2.81 (2H, t, J=6.0 Hz), 2.51 (4H, br). ^{13}C-NMR (100 MHz, DMSO-d_6+$CDCl_3$) δ: 168.85, 159.26, 159.17, 147.36, 133.29, 132.65, 130.41, 127.25, 127.21, 126.08, 124.06, 121.17, 116.94, 66.49, 63.21, 57.17, 53.99. ESI-MS m/z: 429 $(M+H)^+$.

Biology. Biochemical Assay The activity of Pim-1, -2, and -3 kinases was measured as follow using a fluorescence polarization assay method. Enzyme, substrate, and ATP were prepared in assay buffer consisting of 10 mM Tris–HCl (pH 7.2), 10 mM $MgCl_2$, 0.05% NaN_3, 0.01% Triton X-100 and 2 mM dithiothreitol (DTT). The pim kinase assays were performed in 384-well black flat bottom polystyrene plates. Test inhibitors were serially diluted 1 : 3 in 100% dimethyl sulfoxide (DMSO) for the 10-point dose–response. The DMSO dilutions were further diluted in the assay buffer, and 2.5 μL of this solution was then added to the assay plate to give a final starting assay concentration of 10 μM in 1% DMSO. A 2.5 μL Pim-1 (1 nM) or Pim-2 (1 nM) or Pim-3 (1 nM) was added and pre-incubated for 20 min. A 5 μL volume of a mixture of ATP (the final concentrations of ATP were 30 μM, 5 μM, and 20 μM for Pim-1, Pim-2, and Pim-3, respectively) and 100 nM 5-FAM-labeled BAD peptide was added to the assay plate. Reactions were run at room temperature for 90 min and stopped by addition of IMAP binding reagent (Molecular Devices, solution containing 75% Buffer A: 25% Buffer B and a 1 in 600 dilution of beads) to each well. After incubation for a 2 h at room temperature, the fluorescence polarization was measured in an excitation wavelength of 485 nm and an emission wavelength of 530 nm on an Infinity F200 plate reader (Tecan). The data were then fitted to a 4-parameter logistic equation shown below and the IC_{50} value for each compound was calculated using GraphPad Prism (GraphPad Software, Inc., La Jolla, U.S.A.).

$$Y = \text{bottom} + \frac{(\text{top} - \text{bottom})}{1 + 10^{(\log \text{IC}_{50} - X) \cdot \text{hillslope}}}$$

Cell Culture Conditions and Viability Assays MV4-11 (human acute myelocytic leukemia cell line) cells obtained from the American Type Culture Collection (ATCC, Manas-

sas, VA, U.S.A.) were cultured in Iscove's modified Dulbecco's medium (IMDM, ATCC) consisting of 10% fetal bovine serum (FBS, Gibco-BRL, Grand Island, NY, U.S.A.), 100 U/mL penicillin and 100 g/mL streptomycin and maintained 37°C in a 5% CO_2 humidified atmosphere. K562 (human erythromyeloblastoid leukemia cell line) and Jurkat clone E6-1 (human acute T cell leukemia) cells were purchased from Korean Cell Line Bank (KCBL, Seoul, Korea). K562 and Jurkat cells were cultured under the same conditions in RPMI 1640 medium (Gibco-BRL), supplemented with 10% heat inactivated FBS, 100 U/mL penicillin and 100 μg/mL streptomycin. Cell viability was measured using the CellTiter 96® AQ_{UES} One Solution Cell Proliferation Assay (MTS) purchased from Promega (Madison, WI, U.S.A.). MV4-11 and Jurkat cells were seeded into 96-well plates at 200000 cells per well. K562 cells were seeded at 10000 cells per well. The next day, test inhibitors at indicated concentrations were added. The cells were incubated for 24 h at 37°C, and then analyzed by adding 20 μL of MTS solution to each well, and the plates were incubated at 37°C for 4 h. The absorbance of each well was measured at 490 nm on a microplate reader (SPECTRA max 340PC, Molecular Devices, Sunnyvale, CA, U.S.A.).

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