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1,3,4-Oxadiazole-2(3*H*)-thione as a New Scaffold for Pim Kinase Inhibitors

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ABSTRACT

Pim kinases are important targets for cancer therapies because they are mainly responsible for cancer metastasis and overall therapeutic treatment responses. Because of their unusual structural feature in the hinge region of the ATP-binding site, new binding motifs have been discovered and used for the development of Pim kinases inhibitors. The results of a screening of 5-membered heteroaromatic compounds and the effects of structural modifications on the inhibition of Pim kinases' activities showed the potential scaffold for Pim inhibitors. 1,3,4-Oxadiazole-2(3*H*)-thione was found as a new scaffold for Pim kinase inhibitors.

Key words : Pim-1 kinase, Pim-2 kinase, Pim-3 kinase, 1,3,4-oxadiazole-2(3*H*)-thione, Inhibitor

Introduction

Pim kinases, Pim-1, Pim-2, and Pim-3, are members of a family of serine/threonine kinases that regulate the cellular signaling pathways involved in cancer development and progression [1]. Pim kinases are overexpressed in hematologic cancers and phosphorylate downstream substrates which contribute to tumor growth and survival [2]. According to the crystal structures of Pim-1 and Pim-2, the Pim kinase family has a unique proline (Pro123 in Pim-1) residue in the hinge region and lacks a hydrogen bond donor which makes one hydrogen bond interaction with ATP. Therefore Pim kinases can make only one hydrogen bond to the adenine of ATP, which is a distinct character of Pim kinases compared with other serine/threonine kinases [3,4]. Most of the reported Pim kinase inhibitors utilize interaction with the amino residue of lysine (Lys67 in Pim-1) in the ATP binding site as an alternative driving force for the binding (Table 1). Therefore, it would be possible to design highly selective inhibitors for this drug target. As a process of developing selective Pim kinase inhibitors, a new binding motif which interacts with the e-amino residue of lysine was investigated using 5-membered heteroaromatic compounds.

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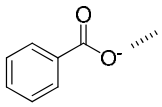
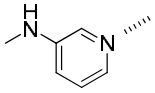
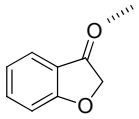
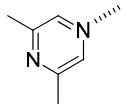
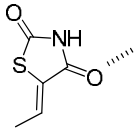
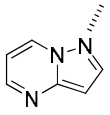
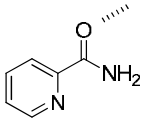
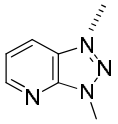
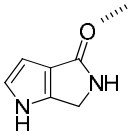
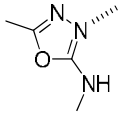
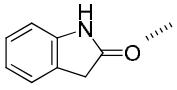
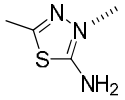
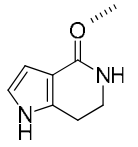
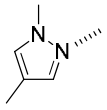
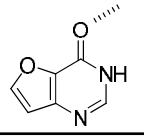
Experimental

Materials and Instruments

^{1}H- NMR and ^{13}C-NMR spectra were recorded on a Bruker Spectrospin 400 (400 MHz) or Jeol ECA 500 (500 MHz) spectrometer. Chemical shifts (δ, ppm) are reported in ppm using tetramethylsilane as an internal standard. Mass spectra were obtained using Waters ACQUITY UPLC, Micromass Quattro micro™ API. Microwave assisted reactions were performed with CEM Discover BenchMate. TLC was performed on E. Merck silica gel 60 F254 plates (0.25 mm). Silica gel column chromatography was performed using Merck silica gel 60

Table 1. Structures of the lysine binding motifs of the reported Pim kinase inhibitors

Functional group	Structure	Ref.	Functional group	Structure	Ref.
Carboxylate		[5]	Pyridine		[6-8]
Ketone		[9,10]	Pyrazine		[11-14]
Thiazolidine-2,4-dione		[15,16]	Pyrazolopyrimidine		[17]
Amide		[18]	Triazolopyridine		[19]
		[20]	1,3,4-Oxadiazol-2-amine		[13]
		[21]	1,3,4-Thiadiazol-2-amine		[22]
		[23]	Pyrazole		[24]
		[25]			

(230∼400 mesh). Unless otherwise noted, all starting materials were obtained from commercially available sources and they were used without further purification. All reactions were performed under nitrogen atmosphere.

Synthesis

3-Bromobenzohydrazide (1)

A microwave vessel was filled with ethyl 3-bromobenzoate (0.44 mmol, 0.10 g), hydrazine hydrate (2.6 mmol, 0.081 mL) and 1 mL ethanol (EtOH). The reaction mixture was irradiated for 10 min at 120°C by applying 100 W. The solvent was removed in vacuo. After the residue was treated with ethyl acetate (EA), the residue was purified by column chromatography over silica gel (n-hexane (Hex) : EA, 1 : 2) to yield 0.034 g (32%) of the title compound. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.90 (s, 1H), 7.98 (s, 1H), 7.81 (d, J = 7.6 Hz, 1H), 7.65 (d, J = 8.0 Hz, 1H), 7.38 (t, J = 8.0 Hz, 1H), 4.48 (s, 2H).

5-(3-Bromophenyl)-1,3,4-oxadiazole-2(3*H*)-thione (2)

To the EtOH (5 mL) solution of compound **1** (0.16 mmol, 0.034 g) were added potassium hydroxide (KOH) (0.19 mmol, 0.011 g) and carbon disulfide (CS_2) (0.40 mmol, 0.024 mL), the resulting mixture was stirred for 30 min at 0°C. After stir-

ring for 1 h at room temperature, the mixture was refluxed for 2 h. After removal of solvent in vacuo, the residue was treated with 2 M hydrochloric acid (HCl) solution until the pH of the solution became 2. The product was extracted with EA from the acidic solution and was washed with water and brine. The organic layer was dried over anhydrous $MgSO_4$ and the solvent was removed in vacuo. The residue was purified by column chromatography over silica gel (dichloromethane (DCM) : methanol (MeOH), 95 : 5) to yield 0.020 g (49%) of the title compound. ^{1}H NMR (400 MHz, DMSO-d_6): δ 14.79 (s, 1H), 7.97 (s, 1H), 7.86 (d, J = 8.0 Hz, 1H), 7.78 (d, J = 8.4 Hz, 1H), 7.52 (t, J = 8.0 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 178.02, 159.71, 135.44, 132.20, 128.87, 125.68, 125.19, 122.91; ESI-MS m/z: 257 $(M+H)^+$.

Isobutyl 3-bromobenzimidate hydrochloride (3)

3-Bromobenzonitrile (0.55 mmol, 0.1 g) was added to the mixture of 1.0 mL isobutanol and 1.0 mL chloroform ($CHCl_3$). To the solution was added acetyl chloride (2.8 mmol, 0.195 mL), and the mixture was stirred for 2 h at 30°C. After removal of solvent in vacuo, the residue was extracted with DCM. Removal of solvent in vacuo gave 0.093 g (58%) of the title compound.

3-Bromobenzimidohydrazide (4)

To the 1 mL acetonitrile (AcCN) solution of compound **3** (0.32 mmol, 0.093 g) was added hydrazine hydrate (0.59 mmol, 0.018 mL), and the resulting mixture was stirred for 4 h at 0°C. After removal of solvent in vacuo, the residue was treated with 0.1 M sodium hydroxide (NaOH) solution until the pH of the solution became 13. The product was extracted with EA from the basic solution and was washed with water and brine. The organic layer was dried over anhydrous $MgSO_4$ and the solvent was removed in vacuo. Removal of solvent in vacuo gave 0.094 g (72%) of the title compound.

^{1}H NMR (400 MHz, DMSO-d_6): δ 8.05 (t, J = 1.6 Hz, 1.68 Hz, 1H), 7.8 (d, J = 7.96 Hz, 1H), 7.58 (dd, J = 6.96 Hz, 1H), 7.34 (t, J = 7.96 Hz, 7.88 Hz, 1H), 6.49 (s, 1H), 2.00 (s, 3H).

5-(3-Bromophenyl)-1,3,4-thiadiazole-2(3*H*)-thione (5)

To the 1 mL EtOH solution of compound **4** (0.44 mmol, 0.094 g) was added carbon disulfide (CS_2) (1.32 mmol, 0.080 mL), and the resulting mixture was stirred for 30 min at 0°C and then 1 h at room temperature. After removal of solvent in vacuo, the residue was extracted with DCM. Removal of solvent in vacuo gave 0.056 g (47%) of the title compound. ^{1}H NMR (400 MHz, DMSO-d_6): δ 14.84 (s, 1H), 7.92 (s, 1H), 7.77 (d, J = 1.84 Hz, 1H), 7.75 (d, J = 1.88 Hz, 1H), 7.49 (t, J = 7.92 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 188.23, 143.21, 134.13, 132.91, 129.72, 128.01, 123.84; ESI-MS m/z: 273 $(M+H)^+$.

(Z)-3-Bromo-*N*'-hydroxybenzimidamide (6)

The mixture of hydroxylamine hydrochloride (5.49 mmol, 0.382 g), potassium carbonate (8.24 mmol, 1.14 g), and 50 mL MeOH was stirred for 30 min. Then 3-bromobenzonitrile (2.75 mmol, 0.500 g) was added to the mixture and it was refluxed for 12 h. After removal of solvent in vacuo, the residue was dissolved in DCM. The resulting solution was washed with 10% citric acid and was dried over anhydrous Na_2SO_4. The removal of solvent in vacuo gave the title compound quantitatively. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.82 (s, 1H), 7.84 (s, 1H), 7.69 (d, J = 7.6 Hz, 1H), 7.57 (d, J = 7.6 Hz, 1H), 7.34 (t, J = 7.6 Hz, 1H), 5.93 (s, 2H).

3-(3-Bromophenyl)-1,2,4-oxadiazol-5(4*H*)-one (7)

To the 10 mL dioxane solution of compound **6** (0.2 mmol, 0.05 g) were added carbonyl diimidazole (CDI) (0.35 mmol, 0.057 g), and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (0.26 mmol, 0.039 g). After refluxing 2 h, the solvent was removed in vacuo. After the residue was dissolved in DCM, the resulting solution was washed with 10% citric acid and brine. The organic layer was dried over anhydrous Na_2SO_4 and the solvent was removed in vacuo. The residue was purified by column chromatography over silica gel (DCM : MeOH, 95 : 5) to yield 0.049 g (85%) of the title compound. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.9 (s, 1H), 7.98 (m, 2H), 7.55 (t, J = 8 Hz, 1H); ESI-MS m/z: 241 $(M+H)^+$.

Ethyl 3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl) benzoate (8)

A microwave vessel was filled with Ethyl 3-bromobenzoate (1.0 mmol, 0.20 g), bis(pinacolato)diboron (1.1 mmol, 0.24 g), potassium acetate (KOAc) (3.0 mmol, 0.26 g), (1,1'-bis(diphenylphosphino) ferrocene)dichloropalladium(II) ($PdCl_2$(dppf)) (0.030 mmol, 0.020 g) and 2.4 mL of dioxane : EtOH (5 : 1) mixture. The reaction mixture was irradiated for 10 min at 120°C by applying 100 W. The solvent was removed in vacuo. After the residue was treated with dichloromethane, it was filter with aid of celite. The filtrate was collected and solvent was removed in vacuo. The residue was purified by column chromatography over silica gel (Hex : EA, 5 : 1) to yield 0.24 g (99%) of the title compound. ^{1}H NMR (500 MHz, $CDCl_3$): δ

8.46 (s, 1H), 8.14 (d, J = 7.5 Hz, 1H), 7.98 (d, J = 7.5 Hz, 1H), 7.45 (t, J = 7.5 Hz, 1H), 4.39 (q, J = 7.0 Hz, 2H), 1.40 (t, J = 7.5 Hz, 3H), 1.36 (s, 12H).

2-((6-Chloropyrazin-2-yl)oxy)-N,N-dimethylethanamine (9)

A microwave vessel was filled with 2-(N,N-Dimethylamino)ethanol (0.81 mmol, 0.081 mL), sodium hydride (NaH) (2.0 mmol, 0.048 g) and 1 mL tetrahydrofuran (THF) and the mixture was stirred for 10 min. After addition of 2,6-dichloropyrazine (0.67 mmol, 0.10 g), the reaction mixture was irradiated for 10 min at 50°C by applying 100 W. The solvent was removed in vacuo. After the residue was treated with EA, the resulting solution was washed with water and brine. The organic layer was dried over anhydrous $MgSO_4$. The removal of solvent in vacuo gave 0.049 g (89%) of the title compound. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.19 (s, 1H), 8.14 (s, 1H), 4.44 (t, J = 5.4 Hz, 2H), 2.74 (t, J = 5.4 Hz, 2H), 2.34 (s, 6H).

Ethyl 3-(6-(2-(dimethylamino)ethoxy)pyrazin-2-yl)benzoate (10)

A microwave vessel was filled with compound **8** (0.72 mmol, 0.20 g), compound **9** (0.87 mmol, 0.18 g), tetrakis(triphenylphosphine)palladium ($Pd(PPh_3)_4$) (0.022 mmol, 0.025 g), 2.0 M Na_2CO_3 aqueous solution (0.36 mmol, 1.8 mL), and 1.0 mL ethylene glycol dimethyl ether (DME). The reaction mixture was irradiated for 10 min at 110°C by applying 100 W. The solvent was removed in vacuo. After the residue was treated with dichloromethane, it was filter with aid of celite. The filtrate was collected and solvent was removed in vacuo. The residue was purified by column chromatography over silica gel (DCM : MeOH, 97 : 3) to yield 0.20 g (87%) of the title compound. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.67 (s, 1H), 8.65 (s, 1H), 8.25 (s, 1H), 8.22 (d, J = 8.0 Hz, 1H), 8.13 (d, J = 7.6 Hz, 1H), 7.57 (t, J = 7.6 Hz, 1H), 4.58 (t, J = 5.6 Hz, 2H), 4.44 (q, J = 7.2 Hz, 2H), 2.80 (t, J = 5.6 Hz, 2H), 2.38 (s, 6H), 1.44 (t, J = 7.2 Hz, 3H).

3-(6-(2-(Dimethylamino)ethoxy)pyrazin-2-yl)benzohydrazide (11)

A microwave vessel was filled with compound **10** (0.16 mmol, 0.050 g), hydrazine hydrate (1.6 mmol, 0.050 mL) and 1 mL ethanol (EtOH). The reaction mixture was irradiated for 10 min at 120°C by applying 100 W. The solvent was removed in vacuo. The residue was purified by column chromatography over silica gel ($CHCl_3$: MeOH : NH_4OH, 100 : 10 : 1) to yield 0.078 g (82%) of the title compound. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.94 (s, 1H), 8.83 (s, 1H), 8.53 (s, 1H), 8.24 (d, J = 6.8 Hz, 1H), 8.22 (s, 1H), 7.92 (d, J = 6.4 Hz, 1H), 7.58 (t, J = 7.6 Hz, 1H), 4.53 (t, J = 5.6 Hz, 4H), 2.70 (t, J = 5.6 Hz, 2H), 2.24 (s, 6H).

5-(3-(6-(2-(Dimethylamino)ethoxy)pyrazin-2-yl)phenyl)-1,3,4-oxadiazole-2(3*H*)-thione (12)

To the EtOH (3 mL) solution of compound **11** (0.26 mmol, 0.078 g) were added KOH (0.31 mmol, 0.017 g) and CS_2 (0.65 mmol, 0.039 mL), the resulting mixture was stirred for 30 min at 0°C. After stirring for 1 h at room temperature, the mixture was refluxed for 1 h. After removal of solvent in vacuo, the residue was treated with 2 M hydrochloric acid (HCl) solution until the pH of solution became 2. Then the acidic solution was basified with 2 M NaOH solution until the pH of solution became 14. After removal of solvent in vacuo, the residue was treated with DCM : MeOH (90 : 10) mixture and filtered with aid of celite. After removal of solvent from the filtrate, the residue was purified by column chromatography over silica gel ($CHCl_3$: MeOH : NH_4OH, 80 : 10 : 1) to yield 0.030 g (34%) of the title compound. ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.79 (s, 1H), 8.45 (s, 1H), 8.22 (s, 1H), 8.12 (d, J = 8.5 Hz, 1H), 7.84 (d, J = 8.5 Hz, 1H), 7.59 (t, J = 8.0 Hz, 1H), 4.49 (t, J = 5.5 Hz, 2H), 2.67 (t, J = 6.0 Hz, 2H), 2.20 (s, 6H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 180.52, 174.28, 161.25, 159.51, 147.69, 136.91, 134.60, 133.68, 130.27, 128.18, 126.63, 123.33, 64.07, 57.73, 45.80, 23.12; ESI-MS m/z: 344 $(M+H)^+$.

5-(3-(6-(2-(Dimethylamino)ethoxy)pyrazin-2-yl)phenyl)-1,3,4-thiadiazole-2(3*H*)-thione (13)

To the EtOH (2 mL) solution of compound **11** (0.17 mmol, 0.050 g) were added KOH (0.25 mmol, 0.014 g) and CS_2 (0.33 mmol, 0.020 mL), the resulting mixture was stirred for 30 min at 0°C. After stirring for 24 h at room temperature, the solvent was removed in vacuo. To the residue was added sulfuric acid (1.68 mmol, 0.088 mL) by dropwise and the mixture was stirred 10 min at −5°C. After addition of a small amount of ice, the acidic solution was basified with 2 M NaOH solution until the pH of solution became 14. After removal of solvent in vacuo, the residue was treated with DCM : MeOH (90 : 10) mixture and filtered with aid of celite. After removal of solvent from the filtrate, the residue was purified by column chromatography over silica gel ($CHCl_3$: MeOH : NH_4OH, 85 : 15 : 1.5) to yield 0.032 g (54%) of the title compound. ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.40 (s, 1H), 8.37 (s, 1H), 8.05 (s, 1H), 7.85 (d, J = 7.5 Hz, 1H), 7.65 (d, J = 7.5 Hz, 1H), 7.58 (m, 1H), 4.11 (t,

J = 7.1 Hz, 2H), 2.82 (s, 6H), 2.76 (t, J = 7.1 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 187.22, 162.24, 150.70, 143.71, 139.97, 133.18, 131.14, 130.16, 129.88, 129.60, 129.23, 126.31, 66.26, 60.56, 47.02; ESI-MS m/z: 360 $(M+H)^+$.

3-(4,4,5,5-Tetramethyl-1,3,2-dioxaborolan-2-yl)benzonitrile (14)

A microwave vessel was filled with 3-bromobenzonitrile (1.37 mmol, 0.250 g), bis(pinacolato)diboron (1.51 mmol, 0.384 g), KOAc (4.12 mmol, 0.404 g), $PdCl_2$(dppf)) (0.041 mmol, 0.030 g), and 3 mL of dioxane. The reaction mixture was irradiated for 10 min at 120°C by applying 100 W. The solvent was removed in vacuo. After the residue was treated with dichloromethane, it was filter with aid of celite. The filtrate was collected and solvent was removed in vacuo to give 0.20 g (64%) of the title compound. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.03 (d, J = 7.5 Hz, 1H), 7.92 (d, J = 7.5 Hz, 1H), 7.69 (s, 1H), 7.63 (t, J = 7.5 Hz, 1H), 1.29 (s, 12H).

3-(6-(2-(Dimethylamino)ethoxy)pyrazin-2-yl)benzonitrile (15)

A microwave vessel was filled with compound **14** (0.87 mmol, 0.20 g), compound **9** (0.87 mmol, 0.18 g), $PdCl_2(PPh_3)_2$ (0.026 mmol, 0.018 g), 2.0 M K_2CO_3 aqueous solution (0.26 mmol, 1.3 mL), and 2.4 mL of dioxane : EtOH (5 : 1) mixture. The reaction mixture was irradiated for 10 min at 120°C by applying 100 W. The solvent was removed in vacuo. After the residue was treated with DCM : MeOH (95 : 5) mixture, it was filter with aid of celite. The filtrate was collected and solvent was removed in vacuo. The residue was purified by column chromatography over silica gel (DCM : MeOH, 95 : 5) to yield 0.15 g (64%) of the title compound. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.61 (s, 1H), 8.36 (s, 1H), 8.29 (s, 1H), 8.23 (d, J = 8 Hz, 1H), 7.74 (d, J = 8 Hz, 1H), 7.61 (t, J = 8 Hz, 1H), 4.56 (t, J = 6 Hz, 2H), 2.81 (t, J = 6 Hz, 2H), 2.39 (s, 6H).

(Z)-3-(6-(2-(Dimethylamino)ethoxy)pyrazin-2-yl)-N'-hydroxybenzimidamide (16)

The mixture of hydroxylamine hydrochloride (1.1 mmol, 0.078 g), K_2CO_3 (1.4 mmol, 0.19 g), and 20 mL MeOH was stirred for 20 min. The mixture was added compound **15** (0.56 mmol, 0.15 g) and refluxed for 10 h. After removal of solvent in vacuo, the residue was treated with DCM : MeOH (95 : 5) mixture and filtered. The filtrate was collected and the solvent mixture was removed in vacuo. The residue was purified by column chromatography over silica gel ($CHCl_3$: MeOH : NH_4OH, 100 : 10 : 1) to yield 0.084 g (50%) of the title compound. ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.77 (s, 1H), 8.86 (s, 1H), 8.39 (s, 1H), 8.28 (s, 1H), 8.13 (d, J = 8 Hz, 1H), 7.80 (d, J = 8 Hz, 1H), 7.54 (t, J = 8 Hz, 1H), 5.99 (s, 2H), 4.53 (t, J = 6 Hz, 2H), 3.39 (s, 6H), 2.69 (t, J = 6 Hz, 2H).

3-(3-(6-(2-(Dimethylamino)ethoxy)pyrazin-2-yl)phenyl)-1,2,4-oxadiazol-5(4*H*)-one (17)

To the 10 mL *N*,*N*-dimethylformamide (DMF) solution of compound **16** (0.022 mmol, 0.065 g) were added carbonyl diimidazole (CDI) (0.32 mmol, 0.052 g) and K_2CO_3 (0.24 mmol, 0.033 g). After stirring 15 h at 80°C, the solvent was removed in vacuo. The residue was purified by column chromatography over silica gel (DCM : MeOH, 90 : 10) to yield 0.046 g (66%) of the title compound. ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.87 (s, 1H), 8.54 (s, 1H), 8.31 (s, 1H), 8.23 (d, J = 8 Hz, 1H), 7.91 (d, J = 8 Hz, 1H), 7.63 (t, J = 8.0 Hz, 1H), 4.67 (t, J = 6 Hz, 2H), 3.18 (t, J = 6 Hz, 2H), 2.59 (s, 6H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 162.25, 159.51, 150.69, 139.96, 133.68, 130.27, 128.90, 128.01, 126.38, 123.33, 64.07, 59.73, 45.80; ESI-MS m/z: 328 $(M+H)^+$.

Assay Method

The activity of Pim-1, Pim-2, and Pim-3 kinases was measured using a fluorescence polarization assay method [26].

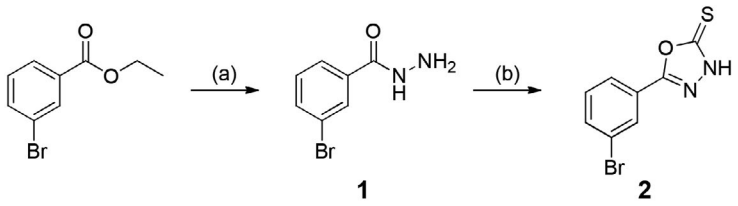
Statistical Analysis

Statistical analysis was conducted using SPSS 25 software. One-way analysis of variance (ANOVA) was performed to identify significant differences. The Duncan test was used to determine the difference between groups ($P < 0.05$).

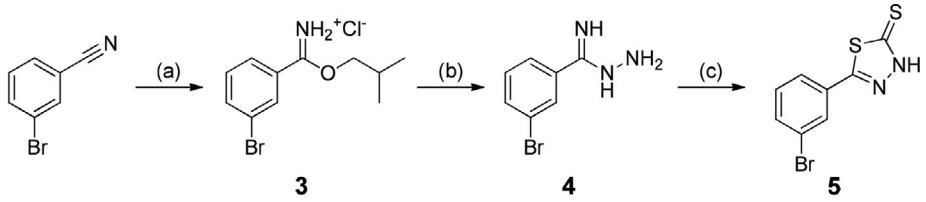
Results and Discussion

Various heteroaromatic rings with acidic characters are used as carboxylic acid bioisosteres in the field of medicinal chemistry. Examples of these heteroaromatics are tetrazole, 1,2,4-oxadiazol-5-one, 1,2,4-oxadiazol-5-thione, 1,3,4-oxadiazole-2-thione and 1,2,3,5-oxathiadiazol-2-oxide rings. Among these heterocycles, 1,2,4-oxadiazol-5(4*H*)-one with a pK_a value of 5.3 [27], 1,3,4-oxadiazole-2(3*H*)-thione with a pK_a value of 4.4 [28], and 1,3,4-thiadiazole-2(3*H*)-thione with a pK_a value of 4.98 [29] were chosen to find a new binding motif as expected to interact with the lysine residue of Pim kinases.

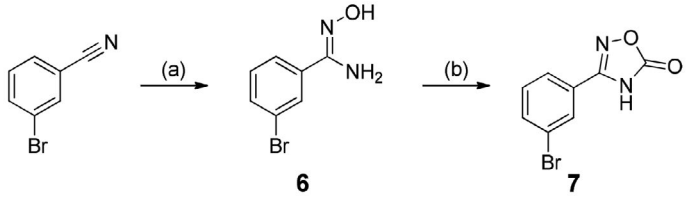
Compound **2** was synthesized from ethyl 3-bromobenzoate



Scheme 1. *Reagents and experimental conditions*: (a) hydrazine hydrate, ethanol, microwave; (b) KOH, CS_2, EtOH, reflux.

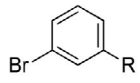


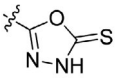
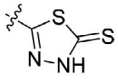
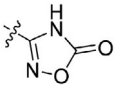
Scheme 2. *Reagents and experimental conditions*: (a) *i*-BuOH, AcCl, $CHCl_3$; (b) $H_2NNH_2 \cdot H_2O$, AcCN; (c) CS_2, EtOH, reflux.



Scheme 3. *Reagents and experimental conditions*: (a) $HONH_2 \cdot HCl$, K_2CO_3, MeOH; (b) CDI, DBU, dioxane, reflux.

Table 2. Enzyme inhibitory activities of synthesized compounds against Pim kinases

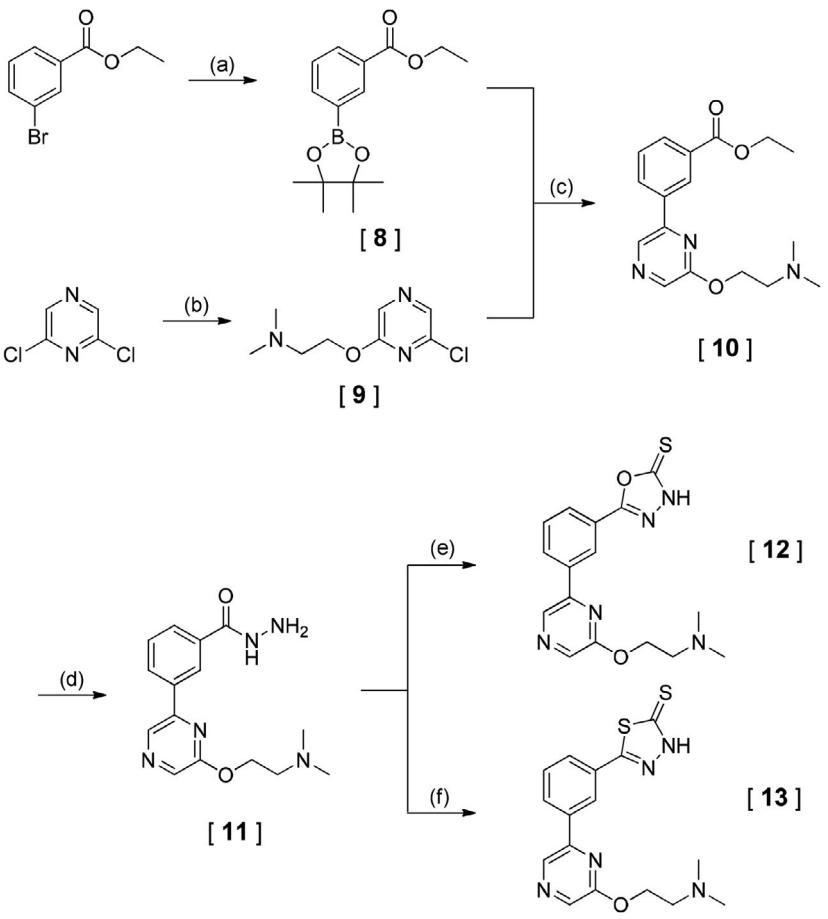


	R		IC_{50} (μM)		
			Pim-1	Pim-2	Pim-3
2	1,3,4-oxadiazole-2(3*H*)-thione		2.2±0.4*	>10	>10
5	1,3,4-thiadiazole-2(3*H*)-thione		>10	>10	>10
7	1,2,4-oxadiazol-5(4*H*)-one		5.4±0.4*	>10	8.4±0.2

* $p < 0.05$

as shown in scheme 1. After the conversion of the functional group from ester to hydrazide using hydrazine, a cyclization reaction with carbon disulfide under basic condition gave 1,3,4-oxadiazole-2(3*H*)-thione.

Compound **5** was synthesized from 3-bromobenzonitrile as shown in scheme 2. The cyano group was converted to benz-



Scheme 4. *Reagents and experimental conditions*: (a) bis(pinacolato)diboron, $PdCl_2(dppf)$, KOAc, 1,4-dioxane : ethanol (5 : 1), microwave; (b) NaH, 2-(dimethylamino)ethanol, THF, microwave; (c) $PdCl_2(PPh_3)_2$, 2M Na_2CO_3, DME, microwave; (d) $H_2NNH_2 \cdot H_2O$, EtOH, microwave; (e) CS_2, KOH, EtOH, reflux; (f) CS_2, KOH, EtOH, then H_2SO_4.

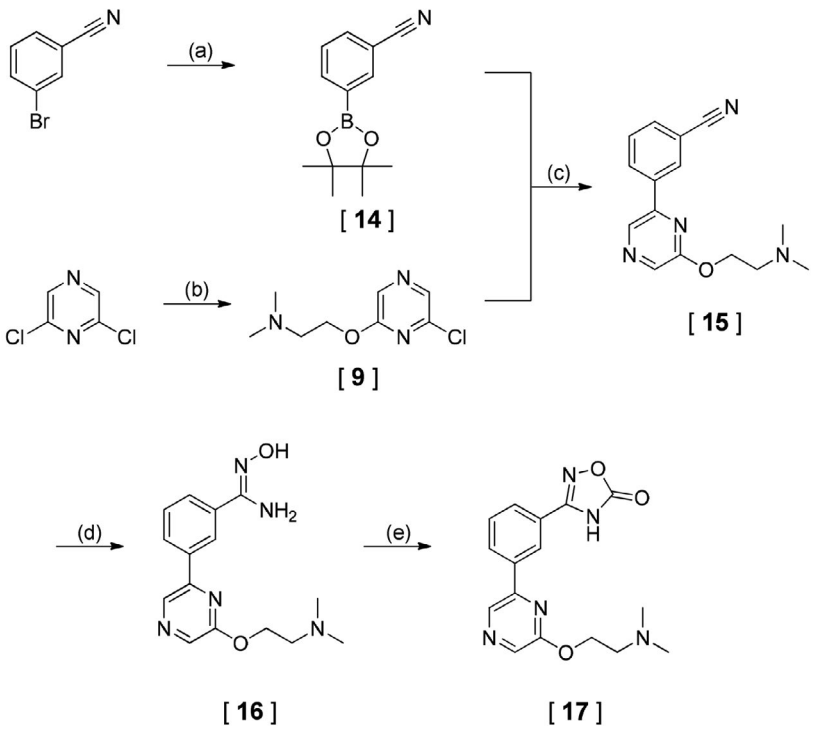
imidate, **3**, which was further modified to benzimidohydrazide, **4**. A cyclization reaction with carbon disulfide gave 1,3,4-thiadiazole-2(3*H*)-thione.

Compound **7** was synthesized from 3-bromobenzonitrile as shown in scheme 3. After the conversion of a functional group from nitrile to *N*-hydroxybenzimidamide, **6**, a cyclization reaction gave 1,2,4-oxadiazol-5(4*H*)-one.

Compound **12** and **13** were synthesized as shown in scheme 4. A palladium-catalyzed borylation at the position 3 of ethyl 3-bromobenzoate was carried out with bis(pinacolato)diboron. A Suzuki coupling reaction between compounds **8** and **9** gave a compound **10**. After the conversion of ester to hydrazide group using hydrazine, a cyclization reaction at different reaction conditions gave 5-membered heteroaromatic rings, 1,3,4-oxadiazole-2(3*H*)-thione, **12**, and 1,3,4-thiadiazole-2(3*H*)-thione, **13**.

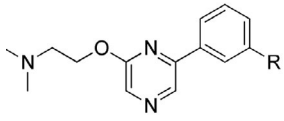
Compound **17** was synthesized as shown in scheme 5. A palladium-catalyzed borylation at the position 3 of 3-bromobenzonitrile was carried out with bis(pinacolato)diboron. Suzuki coupling reaction between compounds **14** and **9** gave a compound **15**. After the conversion of cyano group to N'-hydroxybenzimidamide group using hydroxylamine, a cyclization reaction a 5-membered heteroaromatic ring, 1,2,4-oxadiazol-5(4*H*)-one, **17**.

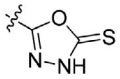
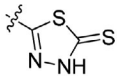
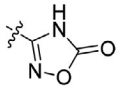
As a first step, three benzene analogs were synthesized by replacing an H atom of the benzene ring with one of the each heteroaromatic ring. The effects of those compounds on the activity of Pim kinases are shown in Table 2. Two compounds, **2** and **7**, showed the inhibitory activities against Pim-1 kinase with a single digit micromolar IC_{50} values. 1,3,4-Oxadiazole-2(3*H*)-thione derivative, **2**, was about twice potent for Pim-1 than 1,2,4-oxadiazol-5(4*H*)-one derivatives, **7**. However, they did not show any inhibitory activities against Pim-2 kinase up to 10 μM concentration. 1,3,4-Thiadiazole-2(3*H*)-thione deriv-



Scheme 5. *Reagents and experimental conditions*: (a) bis(pinacolato)diboron, $PdCl_2$(dppf), KOAc, dioxane, microwave; (b) NaH, 2-(dimethylamino)ethanol, THF, microwave; (c) $PdCl_2(PPh_3)_2$, 2M Na_2CO_3, dioxane : EtOH (5 : 1), microwave; (d) $HONH_2 \cdot HCl$, K_2CO_3, MeOH; (e) CDI, K_2CO_3, DMF, 80°C.

Table 3. Enzyme inhibitory activities of synthesized compounds against Pim kinases



	R		IC_{50} (μM)		
			Pim-1	Pim-2	Pim-3
12	1,3,4-oxadiazole-2(3*H*)-thione		0.5 ± 0.009*	1.6 ± 0.04	0.6 ± 0.15*
13	1,3,4-thiadiazole-2(3*H*)-thione		4.9 ± 0.3*	> 10	2.3 ± 0.2*
17	1,2,4-oxadiazol-5(4*H*)-one		1.3 ± 0.03*	> 10	1.0 ± 0.05*

* $p < 0.05$

atives, **5**, did not show inhibitory activities against all three Pim kinases

According to the above data, two heteroaromatic rings are playing important roles for the binding of compounds to Pim enzymes. To support the findings, the effects of structural modifications on the inhibitory activity were studied. Additional binding motifs such as hydrophobic group and hydrogen bond donor group were attached to the compounds **2, 5, 7**. The effects of the substitutions of a bromo group with 2-(dimethylamino)ethoxy)pyrazine at the 3 position of benzene ring are

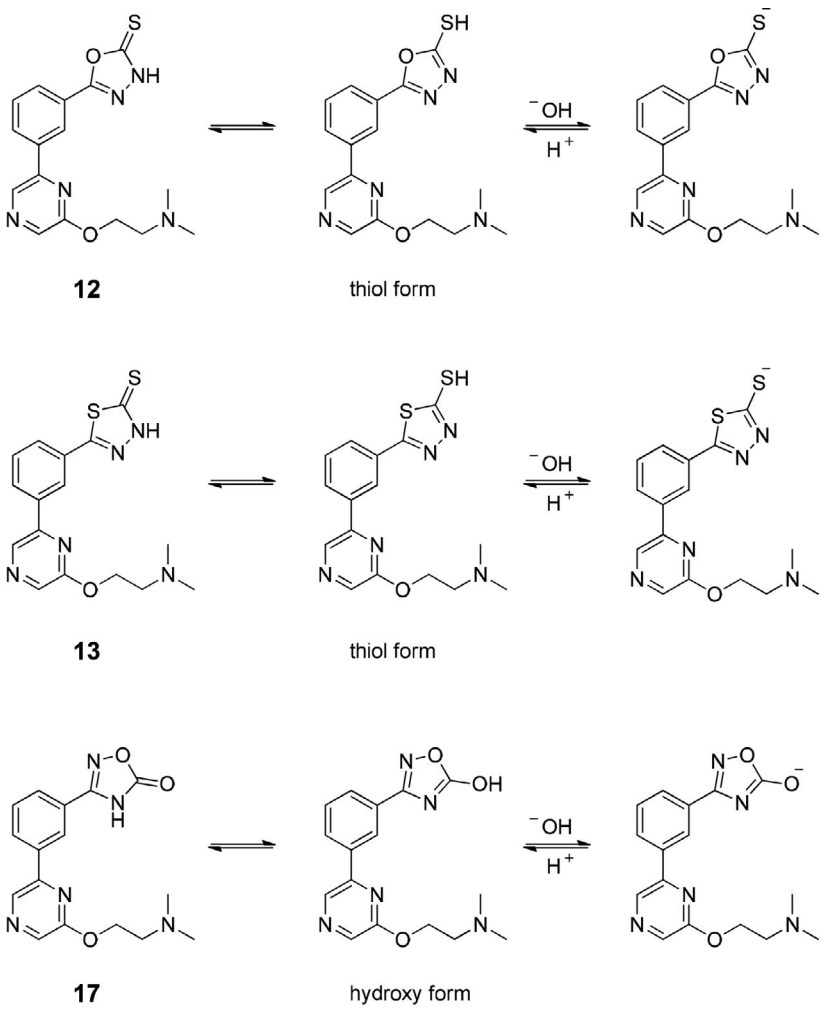


Fig. 1. Equilibria between thione and thiol, and carbonyl and hydroxyl group. Acid-base equilibria of sulfhydryl and hydroxyl group.

summarized in Table 3.

An introduction of additional binding motifs to 1,3,4-oxadiazole-2(3*H*)-thione and 1,2,4-oxadiazol-5(4*H*)-one rings (**12** and **17**, respectively) improved the potency of compounds more than 3 times against both Pim-1 and Pim-3 kinases. For the 1,3,4-thiadiazole-2(3*H*)-thione rings, the addition of binding motifs, **13**, resulted in a potency increase for both Pim-1 and Pim-3 kinases. The most interesting result is the potency of compound **12** against Pim-2 kinase. The inhibitory activity of 1,3,4-oxadiazole-2(3*H*)-thione derivative was enhanced to a single digit micromolar IC_{50} value against Pim-2 kinase. Also, it showed submicromolar IC_{50} values for Pim-1 and Pim-3 kinases.

The binding modes of three compounds were studied using a molecular modeling program as shown in Figs. 2 and 3 [30,31]. Because three heteroaromatic rings were known to have a week acidic character, the docking studies were performed with either their sulfhydryl or hydroxyl form which is the conjugate acid forms of deprotonated forms (Fig. 1).

All three heteroaromatic rings were involved in the hydrogen bonding interactions with the amino residue of lysine 67 of Pim-1 kinase. However, it was found that the atoms of heteroaromatic rings which were involved in the hydrogen bonding interactions and the binding orientations of compounds inside the ATP binding pocket of Pim-1 kinase are different each other. The ε-amino residue of lysine 67 made two hydrogen bond interactions with two nitrogen atoms in 1,3,4-oxadiazole-2(3*H*)-thione ring of compound **12** while it interacted only with a sulfhydryl sulfur atom in 1,3,4-thiadiazole-2(3*H*)-thione ring of compound **13**. In case of 1,2,4-oxadiazol-5(4*H*)-one ring of compound **17**, an oxygen atom of the ring was engaged in the hydrogen bonding interaction. These different

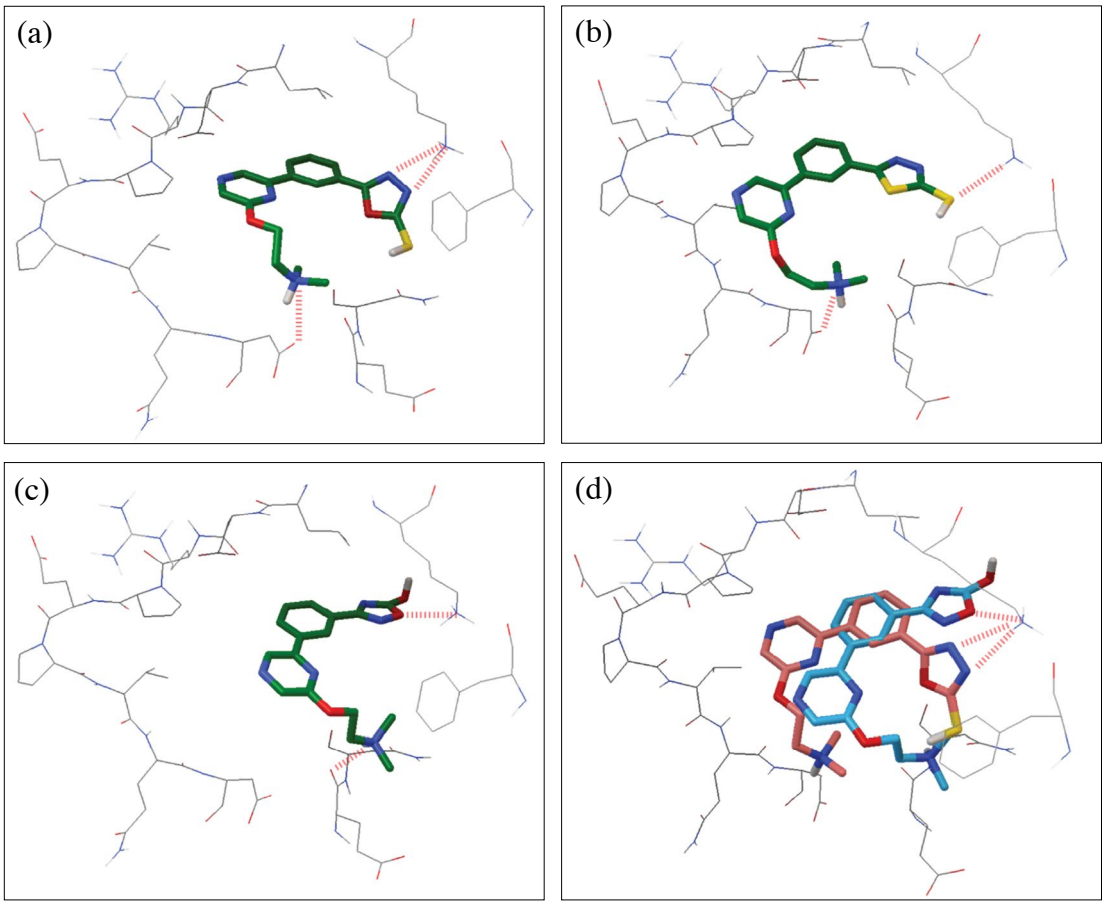


Fig. 2. Suggested binding modes of the selected compounds to Pim-1 kinase (PDB code: 5DWR): (a) binding mode of compound **12**, two nitrogen atoms of oxadiazole ring interact with Lys67; (b) binding mode of compound **13**, a sulfur atom of thiol interacts with Lys67; (c) binding mode of compound **17**, an oxygen atom of oxadiazole ring interacts with Lys67; (d) overlap of compound **12** (brown) with compound **17** (blue).

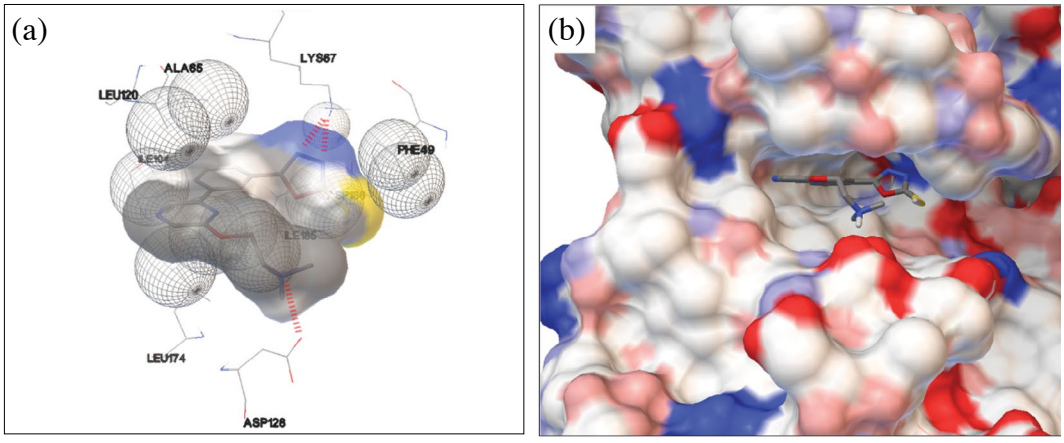


Fig. 3. Suggested binding interactions of compound **12** with Pim-1 kinase (PDB code: 5DWR): (a) compound **12** bound to the ATP binding pocket using 3 hydrogen bonding (red dots) and multiple hydrophobic interactions (meshed balls); (b) compound **12** within to the ATP binding pocket of Pim-1 kinase.

patterns of hydrogen bonding interactions were accompanied with the variation of their binding orientation within the ATP binding pocket. Compound **13** was oriented toward solvent exposed region while compound **12** was located in the inner

pocket. Compound **17** went further into the ATP binding pocket with the sacrifice of hydrophobic interactions with backbone residues of the pocket. The potency differences between compounds could be explained in part with these discrepancies.

The more detailed interactions between compound **12** and Pim-1 enzyme are shown in Fig. 3. Compound **12** bound to the pocket using three hydrogen bonding interactions; two hydrogen bonds between oxadiazole and the ε-amino residue of Lys67 and one hydrogen bond between the amino group of compound **12** with the carboxylate residue of Asp128. Also, it made hydrophobic interactions with hydrophobic residues such as Ala65, Ile104, Leu120, Leu174, Ile185, Asp186, and Phe49.

Conclusion

Compounds with three different heteroaromatic rings were synthesized and evaluated for their potential as a new scaffold for the development of Pim kinase inhibitors. Compounds containing either 1,2,4-oxadiazol-5(4*H*)-one, 1,3,4-oxadiazole-2(3*H*)-thione or 1,3,4-thiadiazole-2(3*H*)-thione ring showed the reasonable inhibitory activity against all three Pim-1, 2, 3 kinases. The molecular modeling studies using the Pim-1 kinase crystal structure showed those three heteroaromatic rings were involved in the hydrogen bonding interaction with lys67 in the ATP binding pocket. Compound **12**, 1,3,4-oxadiazole-2(3*H*)-thione derivative, showed a single digit micromolar IC_{50} value against Pim-2 while it showed submicromolar IC_{50} values for Pim-1 and Pim-3 kinases. Compound **12** was bound to the ATP binding pocket of Pim-1 kinase with both hydrogen bonding and hydrophobic interactions. These results suggest that 1,3,4-oxadiazole-2(3*H*)-thione can be a promising scaffold for the development of Pim kinase inhibitors.

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