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

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Supplementary material:

1. Chemistry. General. NMR spectra were performed on a Varian-400 (^{1}H, 400 MHz; ^{13}C, 100 MHz), chemical shifts δ are in ppm, and the following abbreviations are used: singlet (s), doublet (d), triplet (t), quadruplet (q), doublet of doublet (dd), multiplet (m), broad (br). Mass spectra (ESI) were determined on a Waters LCMS MicromassZQ apparatus. Chromatographic purifications were performed by Teledyn Combiflash Rf chromatography. Reactions were monitored by TLC using fluorescent silica gel plates (60 F254 from Merck) or Waters LCMS MicromassZQ apparatus. The purity of all tested compounds was established to be >96% by Waters LCMS MicromassZQ apparatus.

4-((3-(3-(Trifluoromethoxy)phenyl)pyrazolo[1,5-a]pyrimidin-5-yl)amino) -*trans*-cyclohexanol (**9**).

Synthesis of 3-(dimethylamino)-2-(3-(trifluoromethoxy)phenyl)acrylonitrile.

A mixture of 3-trifluoromethoxyphenylacetonitrile (2.5 grams, 12.43 mmol), DIPEA (0.321 grams, 2.48 mmol), and dimethyl formamide dimethyl acetal (20 mL) were heated at reflux for 4 h. On cooling, the reaction was partitioned between EtOAc and saturated aqueous NH_4Cl solution. The aqueous phase was extracted with ethyl acetate and the combined organic phase washed with brine and concentrated in vacuo. The crude product was purified by using Combiflash chromatography (ethyl acetate/hexane, 0-10%) on silica gel (24 grams) to give pure product (2.3 grams, 8.98 mmol, 72% yield.).

Synthesis of 4-(3-(trifluoromethoxy)phenyl)-1*H*-pyrazol-5-amine.

A mixture of acrylnitrile (2.0 g, 7.81 mmol), hydrazine hydrate (4.53 grams, 39.0 mmol), and glacial acetaic acid (2.34 grams, 39.0 mmol) and ethanol (20 mL) were heated at reflux for 16 h. On cooling, the reaction was diluted with water, extracted with AcOEt and the combined organic phases were washed with brine and concerated in vacuo (1.90 grams, 7.84 mmol, 100% yield.). ^{1}H-NMR ($CDCl_3$/400 MHz): δ 7.64 (m, 1H), 7.42 (m, 4H), 7.06 (d, J = 7.6 Hz, 1H). MS (ES^+, m/z): $(M+H)^+$: 244.3.

Synthesis of 3-(3-(trifluoromethoxy)phenyl)pyrazolo[1,5-a]pyrimidin-5(4*H*)-one.

A mixture of pyrazole (0.8 grams, 3.29 mmol), 1,3-dimethyluracil (0.51 grams, 3.62 mmol), and dry EtOH 10 mL were treated dropwise with sodium ethoxide (0.29 grams, 4.28 mmol) and on completion of addition the reaction was heated at reflux for 16 h. On cooling the reaction was concentrated in vacuo and the residue added to ice, neutralized with acetic acid and the resulting precipitate filtered, washed with water and dried to give the product (0.4 grams, 1.36 mmol, 41% yield.). No chromatography was needed for this step.^{1}H-NMR ($CDCl_3$/400 MHz): δ 8.42 (d, J = 8.0 Hz, 1H), 8.09 (s, 1H), 7.62 (d, J = 7.6 Hz, 1H), 7.52 (m, 2H), 7.20 (d, J = 8.8 Hz, 1H), 6.16 (d, J = 7.6 Hz, 1H).

Synthesis of 5-chloro-3-(3-(trifluoromethoxy)phenyl)pyrazolo[1,5-a]pyrimidine.

A mixture of pyrazolepyrimidinone (0.5 grams, 1.69 mmol) was treated with $POCl_3$ 10 mL and the mixture was heated at reflux for overnight. On cooling, after removing the solvent under rotovapor, the reaction was poured onto ice, cautiously made basic with saturated aqueous $NaHCO_3$ solution and extracted with EtOAc. The combined organic phases were washed with brine and concentrated in vacuo to give the product (0.3 grams, 0.96 mmol, 56% yield.).^{1}H-NMR ($CDCl_3$/400 MHz): δ 8.56 (d, J = 7.6 Hz, 1H), 8.39 (s, 1H), 7.92 (d, J = 7.6 Hz, 1H), 7.85 (m, 2H), 7.45 (t, J = 8.0 Hz, 1H), 7.12 (d, J = 8.4 Hz, 1H), 6.85 (d, J = 7.2 Hz, 1H). MS (ES^+, m/z): $(M+H)^+$: 314.3.

Synthesis of 4-((3-(3-(trifluoromethoxy)phenyl)pyrazolo[1,5-a]pyrimidin-5-yl)amino)cyclohexanol.

A solution of 5-chloro-3-(3-(trifluoromethoxy)phenyl)pyrazolo[1,5-a]pyrimidine (0.03 grams, 0.096 mmol) and *trans*-4-aminocyclohexanol (0.017 grams, 0.143 mmol) in iso-propanol (5 mL) was added DIPEA (0.025 grams, 0.191 mmol) and the mixture was heated at 150^{o}C for 12 h under microwave irradiation. The resulting dark brown solution was cooled down and was

concentrated under reduced pressure. The solid was further purified by using combiflash chromatography (12 g column), eluent: 0-6% CH_3OH/DCM and obtained product (0.020 grams, 0.051 mmol, 53% yield.).^{1}H-NMR (CD_3OD/400 MHz): δ 8.22 (s, 1H), 8.18 (m, 2H), 7.80 (d, *J* = 8.0 Hz, 1H), 7.34 (t, *J* = 8.4 Hz, 1H), 6.96 (m, 1H), 6.17 (d, *J* = 7.6 Hz, 1H), 3.90 (m, 1H), 3.58 (m, 1H), 2.18 (m, 2H), 2.00 (m, 2H), 1.38 (m, 4H). MS (ES^+, m/z): $(M+H)^+$: 393.5. LC-MS purity of compound **9** was found to be >96%.

Compounds **10**-**14** were prepared using similar procedures as in compound **9**.

1-Methyl-4-((3-(3-(trifluoromethoxy)phenyl)pyrazolo[1,5-a]pyrimidin-5-yl)amino) -*trans*-cyclohexanol (**10**).

A solution of 5-chloro-3-(3-(trifluoromethoxy)phenyl)pyrazolo[1,5-a]pyrimidine (0.2 grams, 0.638 mmol) and *trans*-4-amino-1-methylcyclohexanol (0.115 grams, 0.893 mmol) in iso-propanol (5 mL) was added DIPEA (0.165 grams, 1.275 mmol) and the mixture was heated at 150^{o}C for 12 h under microwave irradiation. The resulting dark brown solution was cooled down and was concentrated under reduced pressure. The solid was further purified by using combiflash chromatography (12 g column), eluent: 0-6% CH_3OH/DCM and obtained product (0.120 grams, 0.295 mmol, 46% yield.). ^{1}H-NMR (CD_3OD/400 MHz): δ 8.25 (m, 3H), 7.95 (d, *J* = 8.0 Hz, 1H), 7.40 (t, *J* = 8.0 Hz, 1H), 6.99 (d, *J* = 7.6 Hz, 1H), 6.29 (d, *J* = 7.6 Hz, 1H), 4.10 (m, 1H), 2.11 (m, 2H), 1.74 (m, 2H), 1.62 (m, 4H), 1.28 (s, 3H). MS (ES^+, m/z): $(M+H)^+$: 407.5. LC-MS purity of compound **10** was found to be >96%.

4-((3-(3-(Trifluoromethoxy)phenyl)pyrazolo[1,5-a]pyrimidin-5-yl)amino) -*trans*-cyclohexyl)propan-2-ol (**11**).

A solution of 5-chloro-3-(3-(trifluoromethoxy)phenyl)pyrazolo[1,5-a]pyrimidine (0.2 grams, 0.638 mmol) and *trans*-4-aminocyclohexylpropan-2-ol (0.10 grams, 0.638 mmol) in iso-propanol (5 mL) was added DIPEA (0.165 grams, 1.275 mmol) and the mixture was heated at 150^{o}C for 12 h under microwave irradiation. The resulting dark brown solution was cooled down and was concentrated under reduced pressure. The solid was further purified by using combiflash chromatography (12 g column), eluent: 0-6% CH_3OH/DCM and obtained product (0.140 grams, 0.322 mmol, 51% yield.). ^{1}H-NMR (CD_3OD/400 MHz): δ 8.38 (s, 1H), 8.22 (m, 2H), 7.78 (d, *J* = 8.0 Hz, 1H), 7.38 (t, *J* = 8.0 Hz, 1H), 6.97 (d, *J* = 7.6 Hz, 1H), 6.22 (d, *J* = 7.6 Hz, 1H), 3.90 (m, 1H), 2.25 (m, 2H), 1.97 (m, 2H), 2.00 (m, 2H), 1.37 (m, 1H), 1.30 (m, 4H), 1.16 (s, 6H). MS (ES^+, m/z): $(M+H)^+$: 435 .5. LC-MS purity of compound **11** was found to be >96%.

4-(*trans*-Methoxycyclohexyl)-3-(3-(trifluoromethoxy)phenyl)pyrazolo[1,5-a]pyrimidin-5-amine (**12**).

A solution of 5-chloro-3-(3-(trifluoromethoxy)phenyl)pyrazolo[1,5-a]pyrimidine (0.2 grams, 0.638 mmol) and *trans*-4-methoxycyclohexanamine (0.165 grams, 1.275 mmol) in iso-propanol (5 mL) was added DIPEA (0.165 grams, 1.275 mmol) and the mixture was heated at 150^{o}C for 12 h under microwave irradiation. The resulting dark brown solution was cooled down and was concentrated under reduced pressure. The solid was further purified by using combiflash chromatography (12 g column), eluent: 0-6% CH_3OH/DCM and obtained product (0.210 grams, 0.517 mmol, 81% yield.). ^{1}H-NMR (CD_3OD/400 MHz): δ 8.30 (m, 1H), 8.22 (m, 2H), 7.82 (d, *J* = 8.0 Hz, 1H), 7.38 (t, *J* = 8.0 Hz, 1H), 6.38 (m, 1H), 6.22 (d, *J* = 7.6 Hz, 1H), 3.95 (m, 1H), 3.26 (m, 1H), 2.22 (m, 2H), 2.14 (m, 2H), 1.38 (m, 4H). MS (ES^+, m/z): $(M+H)^+$: 407.5. LC-MS purity of compound **12** was found to be >96%.

N-(Tetrahydro-2*H*-pyran-4-yl)-3-(3-(trifluoromethoxy)phenyl)pyrazolo[1,5-a]pyrimidin-5-amine (**13**).

^{1}H-NMR ($CDCl_3$/400 MHz): δ 8.32 (s, 1H), 8.29 (m, 2H), 7.83 (d, *J* = 8.0 Hz, 1H), 7.41 (t, *J* = 8.0 Hz, 1H), 7.02 (d, *J* = 8.0 Hz, 1H), 6.29 (d, *J* = 8.0 Hz, 1H), 4.24 (m, 1H), 4.02 (m, 2H), 3.60 (t, *J* = 12.0 Hz, 2H), 2.12 (m, 2H), 1.62 (m, 2H). MS (ES^+, m/z): $(M+H)^+$: 379.4. LC-MS purity of compound **13** was found to be >96%.

4-(((3-(3-(Trifluoromethoxy)phenyl)pyrazolo[1,5-a]pyrimidin-5-yl)amino)methyl)tetrahydro-2H-thiopyran 1,1-dioxide (**14**).

A solution of 5-chloro-3-(3-(trifluoromethoxy)phenyl)pyrazolo[1,5-a]pyrimidine (0.2 grams, 0.638 mmol) and 4-(aminomethyl)tetrahydro-2*H*-thiopyran 1,1-dioxide (0.104 grams, 0.638 mmol) in iso-propanol (5 mL) was added DIPEA (0.165 grams, 1.275 mmol) and the mixture was heated at 150^{o}C for 12 h under microwave irradiation. The resulting dark brown solution was cooled down and was concentrated under reduced pressure. The solid was further purified by using combiflash chromatography (12 g column), eluent: 0-6% CH_3OH/DCM and obtained

product (0.080 grams, 0.182 mmol, 29% yield.). ^{1}H-NMR (CD_3OD/400 MHz): δ 8.24 (m, 2H), 8.21 (s, 1H), 7.70 (d, *J* = 8.0 Hz, 1H), 7.38 (d, *J* = 8.0 Hz, 1H), 7.23 (m, 1H), 6.07 (d, *J* = 7.2 Hz, 1H), 3.51 (t, *J* = 6.4 Hz, 2H), 3.06 (m, 2H), 3.00 (m, 2H), 2.22 (m, 3H), 1.96 (m, 2H). MS (ES^+, m/z): $(M+H)^+$: 441.4. LC-MS purity of compound **14** was found to be >96%.

N-((1-Methylpiperidin-4-yl)methyl)-3-(3-(trifluoromethoxy)phenyl)pyrazolo[1,5-a]pyrimidin-5-amine (**1**).

A solution of 5-chloro-3-(3-(trifluoromethoxy)phenyl)pyrazolo[1,5-a]pyrimidine (0.2 grams, 0.638 mmol) and (1-methylpiperidin-4-yl)methanamine (0.082 grams, 0.638 mmol) in iso-propanol (5 mL) was added DIPEA (0.165 grams, 1.275 mmol) and the mixture was heated at 150^{o}C for 12 h under microwave irradiation. The resulting dark brown solution was cooled down and was concentrated under reduced pressure. The solid was further purified by using combiflash chromatography (12 g column), eluent: 0-6% CH_3OH/DCM and obtained product (0.160 grams, 0.395 mmol, 62% yield.). ^{1}H-NMR (CD_3OD/400 MHz): δ 8.26 (m, 3H), 7.86 (d, *J* = 8.0 Hz, 1H), 7.37 (m, 1H), 6.99 (d, *J* = 8.0 Hz, 1H), 6.28 (d, *J* = 7.2 Hz, 1H), 3.41 (d, *J* = 6.0 Hz, 2H), 2.80 (d, *J* = 12.0 Hz, 1H), 2.30 (s, 3H), 2.11 (t, *J* = 12.0 Hz, 1H), 1.89 (m, 3H), 1.44 (m, 2H), 1.29 (m, 2H). MS (ES^+, m/z): $(M+H)^+$: 391.4. LC-MS purity of compound **1** was found to be >96%.

N-(3-(3-(Trifluoromethoxy)phenyl)pyrazolo[1,5-a]pyrimidin-5-yl)cyclohexane-1,4-*trans*-diamine (**7**).

A solution of 5-chloro-3-(3-(trifluoromethoxy)phenyl)pyrazolo[1,5-a]pyrimidine (0.15 grams, 0.568 mmol) and *trans*-cyclohexane-1,4-diamine (0.130 grams, 1.136 mmol) in iso-propanol (5 mL) was added DIPEA (0.294 grams, 2.272 mmol) and the mixture was heated at 150^{o}C for 12 h under microwave irradiation. The resulting dark brown solution was cooled down and was concentrated under reduced pressure. The solid was further purified by using combiflash chromatography (12 g column), eluent: 0-6% CH_3OH/DCM and obtained product (0.075 grams, 0.219 mmol, 39% yield.). ^{1}H-NMR (CD_3OD/400 MHz): δ 8.24 (m, 3H), 7.87 (d, *J* = 7.6 Hz, 1H), 7.39 (t, *J* = 8.0 Hz, 1H), 6.99 (d, *J* = 8.0 Hz, 1H), 6.24 (d, *J* = 7.6 Hz, 1H), 3.95 (m, 1H),

2.86 (m, 1H), 2.24 (m, 2H), 1.8 (m, 2H), 1.37 (t, J = 9.2 Hz, 4H). MS (ES^+, m/z): $(M+H)^+$: 392.4. LC-MS purity of compound **7** was found to be >96%.

(*S*)-1-(3-(3-(Trifluoromethoxy)phenyl)pyrazolo[1,5-a]pyrimidin-5-yl)piperidin-3-amine (**8**). ^{1}H-NMR (CD_3OD/400 MHz): δ 8.32 (d, J = 8.0 Hz, 1H), 8.24 (m, 1H), 8.07 (m, 1H), 7.85 (d, J = 5.6 Hz, 1H), 7.38 (t, J = 8.0 Hz, 1H), 6.95 (d, J = 7.6 Hz, 1H), 6.61 (d, J = 7.6 Hz, 1H), 4.22 (m, 2H), 3.17 (m, 2H), 2.91 (m, 1H), 2.10 (m, 1H), 1.80 (m, 1H), 1.60 (m, 1H), 1.50 (m, 1H). MS (ES^+, m/z): $(M+H)^+$: 378.5. LC-MS purity of compound **8** was found to be >96%.

Synthesis of Compound **9a**, 4-((3-(3-(Trifluoromethyl)phenyl)pyrazolo[1,5-a]pyrimidin-5-yl)amino) -*trans*-cyclohexanol.

A mixture of 2-(3-(trifluoromethyl)phenyl)acetonitrile (4.55 g, 24.58 mmol), 1,1-dimethoxy-*N*,*N*-dimethylmethanamine (13.10 ml, 98 mmol), and N^1,N^1,N^2,N^2-tetramethylethane-1,2-diamine (0.737 ml, 4.92 mmol) were heated to reflux for 5h. After cooling to RT, the mixture was partitioned between saturated aqueous NH_4Cl and EtOAc and extracted three times with EtOAc. The combined organic phases were washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*. After absorbing on celite, the compound was purified by combiflash chromatography (40g silica, 10% to 70% EtOAc/hexanes) to give pure 3-(dimethylamino)-2-(3-(trifluoromethyl)phenyl)acrylonitrile (2.93g, 50% yield). ^{1}H NMR (400 MHz, $CDCl_3$): 7.48 (m, 2H), 7.41-7.34 (m, 2H), 6.94 (s, 1H), 3.25 (s, 6H).

To a mixture of 3-(dimethylamino)-2-(3-(trifluoromethyl)phenyl)acrylonitrile (2.93 g, 12.20 mmol) in ethanol (35 ml) was added hydrazine hydrate (3.80 ml, 122 mmol) and acetic acid (6.98 ml, 122 mmol). Upon to reflux; a solution formed. After 5 hours, 0.25 mL of hydrazine hydrate was added and the reaction refluxed an additional 15 hours. The volatiles were removed *in vacuo*, and the residue partitioned between EtOAc and water. After extracting three times with EtOAc, the combined organic phases were washed twice with saturated aqueous $NaHCO_3$, dried over Na_2SO_4, and concentrated *in vacuo* to give pure 4-(3-(trifluoromethyl)phenyl)-1*H*-pyrazol-5-amine (2.7g, 97% yield) as a light yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$): 7.69 (m, 1H), 7.63 (m, 1H), 7.58 (s, 1H), 7.50 (m, 2H), 5.78 (br s, 3H).

To a mixture of 4-(3-(trifluoromethyl)phenyl)-1*H*-pyrazol-5-amine (2.7 g, 11.88 mmol) and 1,3-dimethylpyrimidine-2,4(1*H*,3*H*)-dione (1.999 g, 14.26 mmol) in ethanol (35 ml) was added

sodium ethoxide (1.132 g, 16.64 mmol). After heating to reflux 16 hours under Argon, the reaction was cooled to RT and the volatiles were removed *in vacuo*. The mixture was diluted with 30 mL H_2O and acidified to *p*H=4 with AcOH. The resulting solid was collected by vacuum filtration. The solid was taken up in a 4:1 DCM/2-propanol solution, washed with water, dried over Na_2SO_4, and evaporated under vacuuo rotovaced to give pure 3-(3-(trifluoromethyl)phenyl)pyrazolo[1,5-a]pyrimidin-5(4*H*)-one (2.07 g, 7.41 mmol, 62.4% yield). ^{1}H NMR (400 MHz, DMSO-d_6): 12.38 (br s, 1H), 8.62 (d, 1H), 8.38 (s, 1H), 8.05 (m, 2H), 7.60 (m, 2H), 6.20 (m, 1H).

A mixture of 3-(3-(trifluoromethyl)phenyl)pyrazolo[1,5-a]pyrimidin-5(4*H*)-one (2.07 g, 7.41 mmol) and $POCl_3$ (6.91 ml, 74.1 mmol) was heated to 107ºC. The heating was continued for 16 hours. The reaction mixture was cooled to RT and the $POCl_3$ removed *in vacuo* to give a waxy solid. The solid was triturated multiple times with Et_2O and the Et_2O layers were combined and rotovaced. The resulting residue was taken up in DCM and washed with saturated aqueous $NaHCO_3$, dried over Na_2SO_4 and concentrated *in vacuo* to give 5-chloro-3-(3-(trifluoromethyl)phenyl)pyrazolo[1,5-a]pyrimidine (1.47g, 67% yield) as a brown solid. ^{1}H NMR (400 MHz, $CDCl_3$): 8.60 (d, 1H), 8.47 (s, 1H), 8.24 (d, 1H), 8.19 (s, 1H), 7.55 (m, 2H), 6.87 (d, 1H).

A mixture of 5-chloro-3-(3-(trifluoromethyl)phenyl)pyrazolo[1,5-a]pyrimidine (100 mg, 0.336 mmol), 4-aminocyclohexanol (58.0 mg, 0.504 mmol), and DIEA (0.117 ml, 0.672 mmol) in 2-propanol (3 ml) was irradiated to 135ºC for 14h in a Biotage microwave. After cooling, the mixture was diluted with saturated aqueous $NaHCO_3$, and extracted three times with EtOAc. The combined extracts were washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. After absorbing on celite, the compound was purified by combiflash chromatogrphy (12g silica, 1/5/4 MeOH/EtOAc/hex) to give *trans*-4-((3-(3-(trifluoromethyl)phenyl)pyrazolo[1,5-a]pyrimidin-5-yl)amino)cyclohexanol (101 mg, 80% yield) as a white solid. ^{1}H NMR (400 MHz, CD_3OD): 8.72 (s, 1H), 8.29 (s, 1H), 8.50 (d, 1H), 8.09 (d, 1H), 7.50 (t, 1H), 7.39 (d, 1H), 6.25 (d, 1H), 3.98 (m, 1H), 3.62 (m, 1H), 2.23 (m, 2H), 2.03 (m, 2H), 1.55-1.32 (m, 4H). MS (ES^+, m/z): $(M+H)^+$: 377.5. LC-MS purity of compound **9a** was found to be >96%.

Compounds **10a**-**14a** were prepared using similar procedures as in compound **9a**.

1-Methyl-4-((3-(3-(trifluoromethyl)phenyl)pyrazolo[1,5-a]pyrimidin-5-yl)amino) -*trans*-cyclohexanol (**10a**) .

A mixture of 5-chloro-3-(3-(trifluoromethyl)phenyl)pyrazolo[1,5-a]pyrimidine (100 mg, 0.336 mmol), 4-aminocyclohexanol (58.0 mg, 0.504 mmol), and DIEA (0.117 ml, 0.672 mmol) in 2-propanol (3 ml) was irradiated to 135ºC for 14h in a Biotage microwave. After cooling, the mixture was diluted with saturated aqueous $NaHCO_3$, and extracted three times with EtOAc. The combined extracts were washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. After absorbing on celite, the compound was purified by combiflash chromatogrphy (12g silica, 1/5/4 MeOH/EtOAc/hex) to give *trans*-4-((3-(3-(trifluoromethyl)phenyl)pyrazolo[1,5-a]pyrimidin-5-yl)amino)cyclohexanol (101 mg, 80% yield) as a white solid. ^{1}H NMR (400 MHz, CD_3OD): 8.69 (s, 1H), 8.29 (s, 1H), 8.25 (d, 1H), 8.11 (d, 1H), 7.30 (t, 1H), 7.39 (d, 1H), 6.31 (d, 1H), 4.09 (m, 1H), 2.15 (m, 2H), 1.79-1.52 (m, 6H), 1.30 (s, 3H). MS (ES^+, m/z): $(M+H)^+$: 391.5. LC-MS purity of compound **10a** was found to be >96%.

4-((3-(3-(Trifluoromethyl)phenyl)pyrazolo[1,5-a]pyrimidin-5-yl)amino) -*trans*-cyclohexyl)propan-2-ol (**11a**).

^{1}H NMR (400 MHz, CD_3OD): 8.78 (s, 1H), 8.18 (s, 1H), 8.24 (d, 1H), 8.05 (d, 1H), 7.49 (t, 1H), 7.38 (d, 1H), 6.25 (d, 1H), 3.95 (m, 1H), 2.28 (m, 2H), 1.95 (m, 2H), 1.32 (m, 5H), 1.20 (s, 6H). MS (ES^+, m/z): $(M+H)^+$: 419.5. LC-MS purity of compound **11a** was found to be >96%.

4-(*trans*-Methoxycyclohexyl)-3-(3-(trifluoromethyl)phenyl)pyrazolo[1,5-a]pyrimidin-5-amine (**12a**).

^{1}H NMR (400 MHz, DMSO-d6): 8.76 (s, 1H), 8.49 (d, 1H), 8.45 (s, 1H), 8.13 (d, 1H), 7.66 (d, 1H), 7.56 (t, 1H), 7.42 (d, 1H), 6.30 (d, 1H), 3.75 (m, 1H), 3.27 (s, 3H), 3.19 (m. 1H), 2.10 (m, 4H), 1.30 (m, 4H). MS (ES^+, m/z): $(M+H)^+$: 391.5. LC-MS purity of compound **12a** was found to be >96%.

N-(Tetrahydro-2*H*-pyran-4-yl)-3-(3-(trifluoromethyl)phenyl)pyrazolo[1,5-a]pyrimidin-5-amine (**13a**).

^{1}H NMR (400 MHz, DMSO-d6): 8.73 (s, 1H), 8.52 (d, 1H), 8.47 (s, 1H), 8.15 (d, 1H), 7.77 (d, 1H), 7.55 (t, 1H), 7.43 (d, 1H), 6.33 (d, 1H), 4.08 (m, 1H), 3.93 (m, 2H), 3.43 (m, 2H), 2.05 (m,

2H), 1.53 (m, 2H). MS (ES^+, m/z): $(M+H)^+$: 363.5. LC-MS purity of compound **13a** was found to be >96%.

4-(((3-(3-(Trifluoromethyl)phenyl)pyrazolo[1,5-a]pyrimidin-5-yl)amino)methyl)tetrahydro-2*H*-thiopyran 1,1-dioxide (**14a**).
^{1}H NMR (400 MHz, CD_3OD): 8.66 (s, 1H), 8.31 (s, 1H), 8.29 (s, 1H), 8.12 (d, 1H), 7.52 (t, 1H), 7.40 (d, 1H), 6.31 (d, 1H), 3.50 (d, 2H), 3.18 (m, 4H), 2.21 (m, 3H), 1.88 (m, 2H). MS (ES^+, m/z): $(M+H)^+$: 425.5. LC-MS purity of compound **14a** was found to be >96%.

4-(3-(3-(Trifluoromethyl)phenyl)pyrazolo[1,5-a]pyrimidin-5-yl)thiomorpholine 1,1-dioxide (**15a**).
^{1}H NMR (400 MHz, DMSO-d6): 8.82 (d, 1H), 8.61 (s, 1H), 8.48 (m, 1H), 8.28 (d, 1H), 7.61 (t, 1H), 7.48 (d, 1H), 6.98 (d, 1H), 4.22 (m, 4H), 3.28 (m, 4H). MS (ES^+, m/z): $(M+H)^+$: 397.4. LC-MS purity of compound **15a** was found to be >96%.

4-((3-(3-Chlorophenyl)pyrazolo[1,5-a]pyrimidin-5-yl)amino) -trans-cyclohexanol (**9b**).
Synthesis of 3-(dimethylamino)-2-(3-chlorophenyl)acrylonitrile.
A mixture of 3-chlorophenylacetonitrile (5 grams, 33.0 mmol), N^1,N^1,N^2,N^2-tetramethyl-ethane-1,2-diamine (0.767 grams, 6.60 mmol), and dimethyl formamide dimethyl acetal (20 mL) were heated at reflux for 4 h. On cooling, the reaction was partitioned between EtOAc and saturated aqueous NH_4Cl solution. The aqueous phase was extracted with ethyl acetate and the combined organic phases were washed with brine and concentrated *in vacuo*. The crude product was purified by combiflash chromatography (ethyl acetate/hexane, 0-10%) on silica gel (24 grams) to give pure product (6.8 grams, 33.0 mmol, 100% yield.).^{1}H-NMR ($CDCl_3$/400 MHz): δ 7.31 (m, 2H), 7.22 (m, 2H), 7.08 (m, 1H), 3.31 (s, 3H), 3.30 (s, 3H).

Synthesis of 4-(3-chlorophenyl)-1*H*-pyrazol-5-amine
A mixture of acrylnitrile (5.0 g, 24.19 mmol), hydrazine hydrate (7.75 grams, 242 mmol), and glacial acetic acid (14.53 grams, 242 mmol) and ethanol (20 mL) were heated at reflux for 16 h. On cooling, the reaction was diluted with water, extracted with AcOEt and the combined organic phases were washed with brine and concentrated *in vacuo* (4.0 grams, 20.66 mmol, 85% yield.).

^{1}H-NMR ($CDCl_3$/400 MHz): δ 7.63 (m, 1H), 7.52 (m, 1H), 7.42 (d, *J* = 8.0 Hz, 1H), 7.33 (t, *J* = 8.0 Hz, 1H), 7.17 (d, *J* = 8.0 Hz, 1H). MS (ES^+, m/z): $(M+H)^+$: 194.3.

Synthesis of 3-(3-chlorophenyl)pyrazolo[1,5-a]pyrimidin-5(4H)-one

A mixture of pyrazole (4.0 grams, 20.66 mmol), 1,3-dimethyluracil (3.47 grams, 24.79 mmol), and dry EtOH 10 mL were treated dropwise with sodium ethoxide (1.97 grams, 28.9 mmol) and on completion of addition the reaction was heated at reflux for 16 h. On cooling the reaction was concentrated *in vacuo* and the residue added to ice, neutralized with acetic acid and the resulting precipitate filtered, washed with water and dried to give the product (4.0 grams, 16.28 mmol, 79% yield.). No chromatography was needed for this step. ^{1}H-NMR ($CDCl_3$/400 MHz): δ 8.38 (d, *J* = 8.4 Hz, 1H), 8.04 (s, 1H), 7.65 (s, 1H), 7.65 (s, 1H), 7.51 (m, 1H), 7.40 (t, *J* = 8.0 Hz, 1H), 7.27 (d, *J* = 8.0 Hz, 1H), 6.12 (d, *J* = 7.6 Hz, 1H). MS (ES^+, m/z): $(M+H)^+$: 246.3.

Synthesis of 5-chloro-3-(3-chlorophenyl)pyrazolo[1,5-a]pyrimidine.

A mixture of pyrazolepyrimidinone (4.0 grams, 16.28 mmol) was treated with $POCl_3$ 10 mL and the mixture was heated at reflux for overnight. On cooling, after remove the solvent under rotovapor, the reaction was poured onto ice, cautiously made basic with saturated aqueous NaHCO3 solution and extracted with EtOAc. The combined organic phases were washed with brine and concentrated *in vacuo* to give the product (0.65 grams, 2.46 mmol, 15% yield.). ^{1}H-NMR ($CDCl_3$/400 MHz): δ 8.89 (d, *J* = 7.4 Hz, 1H), 8.61 (s, 1H), 8.10 (m, 1H), 7.96 (d, *J* = 7.6 Hz, 1H), 7.40 (t, *J* = 8.0 Hz, 1H), 7.26 (d, *J* = 8.0 Hz, 1H), 7.06 (d, *J* = 7.2 Hz, 1H). MS (ES^+, m/z): $(M)^+$: 264.3.

Synthesis of 4-((3-(3-chlorophenyl)pyrazolo[1,5-a]pyrimidin-5-yl)amino)cyclohexanol

A solution of 3-chloro-5-chloropyrazolo[1,5-a]pyrimidine (0.150 grams, 0.568 mmol) and *trans*-4-aminocyclohexanol (0.131 grams, 1.14 mmol) in iso-propanol (5 mL) was added DIPEA (0.177 grams, 2.272 mmol) and the mixture was heated at 150^{o}C for 12 h under microwave irradiation. The resulting dark brown solution was cooled down and was concentrated under reduced pressure. The solid was further purified by using combiflash chromatography (12 g column), eluent: 0-6% CH_3OH/DCM and obtained product (0.100 grams, 0.292 mmol, 52% yield.).^{1}H-NMR (CD_3OD/400 MHz): δ 8.41 (s, 1H), 8.24 (d, *J* = 8.0 Hz, 2H), 7.80 (d, *J* = 8.0 Hz, 1H), 7.30 (t, *J* = 8.4 Hz, 1H), 7.10 (m, 1H), 6.25 (d, *J* = 7.6 Hz, 1H), 3.95 (m, 1H), 3.63 (m, 1H), 2.26 (m, 2H), 2.04 (m, 2H), 1.55 (m, 2H), 1.39 (m, 2H). MS (ES^+, m/z): $(M+H)^+$: 343.5. LC-MS purity of compound **9b** was found to be >96%.

Compounds **7b**-**15b** were prepared using similar procedures as in Compound **9b**.

N-(3-(3-Chlorophenyl)pyrazolo[1,5-a]pyrimidin-5-yl)cyclohexane-1,4-*trans*-diamine (**7b**).

A mixture of 5-chloro-3-(3-chlorophenyl)pyrazolo[1,5-a]pyrimidine (0.150 g, 0.568 mmol), *trans*-cyclohexane-1,4-diamine (0.130 g, 1.136 mmol), and DIPEA (0.294 g, 2.276 mmol) in 2-propanol (3 ml) was irradiated to 150ºC for 14h in a Biotage microwave. After cooling, the mixture was diluted with saturated aqueous $NaHCO_3$, and extracted three times with EtOAc. The combined extracts were washed with brine, dried over Na_2SO_4 and concentrated in vacuo. After absorbing on celite, the compound was purified by combiflash chromatography (12g silica, 0-15% methanol/DCM) to give *N*-(3-(3-Chlorophenyl)pyrazolo[1,5-a]pyrimidin-5-yl)cyclohexane-1,4-*trans*-diamine (0.075 g, 0.219 mmol, 39% yield) as a white solid. ^{1}H-NMR (CD_3OD/400 MHz): δ 8.37 (s, 1H), 8.23 (m, 2H), 7.80 (d, *J* = 8.0 Hz, 1H), 7.28 (t, *J* = 7.6 Hz, 1H), 7.09 (d, *J* = 7.6 Hz, 1H), 6.23 (d, *J* = 7.2 Hz, 1H), 3.90 (m, 1H), 2.75 (m, 1H), 2.27 (m, 2H), 2.05 (m, 2H), 1.36 (m, 4H). MS (ES^+, m/z): $(M+H)^+$: 325.5. LC-MS purity of compound **7b** was found to be >96%.

(*S*)-1-(3-(3-Chlorophenyl)pyrazolo[1,5-a]pyrimidin-5-yl)piperidin-3-amine (**8b**).

A mixture of 5-chloro-3-(3-chlorophenyl)pyrazolo[1,5-a]pyrimidine (100 mg, 0.379 mmol), (*S*)-piperidin-3-amine (76 mg, 0.757 mmol), and DIPEA (0.158 ml, 1.515 mmol) in 2-propanol (3 ml) was irradiated to 150ºC for 14h in a Biotage microwave. After cooling, the mixture was diluted with saturated aqueous $NaHCO_3$, and extracted three times with EtOAc. The combined extracts were washed with brine, dried over Na_2SO_4 and concentrated in vacuo. After absorbing on celite, the compound was purified by combiflash chromatography (12g silica, 0-15% methanol/DCM) to give (*S*)-1-(3-(3-chlorophenyl)pyrazolo[1,5-a]pyrimidin-5-yl)piperidin-3-amine (35 mg, 28% yield) as a white solid. ^{1}H-NMR (CD_3OD/400 MHz): 8.47 (d, *J* = 8.0 Hz, 1H), 8.29 (s, 1H), 8.03 (s, 1H), 7.92 (m, 1H), 7.33 (t, *J* = 8.0 Hz, 1H), 7.13 (d, *J* = 8.0 Hz, 1H), 6.73 (d, *J* = 8.0 Hz, 1H), 4.27 (m, 1H), 4.02 (m, 1H), 3.50 (m, 1H), 3.40 (m, 1H), 2.20 (m, 1H), 1.90 (m, 1H), 1.80 (m, 2H), 1.36 (m, 1H). MS (ES^+, m/z): $(M+H)^+$: 328.5. LC-MS purity of compound **8b** was found to be >96%.

4-((3-(3-Chlorophenyl)pyrazolo[1,5-a]pyrimidin-5-yl)amino)-1-methyl-*trans*-cyclohexanol (**10b**).

^{1}H-NMR (CD_3OD/400 MHz): δ 8.36 (s, 1H), 8.23 (m, 2H), 7.84 (d, *J* = 8.0 Hz, 1H), 7.30 (t, *J* = 8.0 Hz, 1H), 7.11 (d, *J* = 7.6 Hz, 1H), 6.29 (d, *J* = 7.6 Hz, 1H), 4.02 (m, 1H), 2.20 (m, 2H), 1.75 (m, 4H), 1.50 (m, 2H), 1.29 (s, 6H). MS (ES^+, m/z): $(M+H)^+$: 357.5. LC-MS purity of compound **10b** was found to be >96%.

4-((3-(3-Chlorophenyl)pyrazolo[1,5-a]pyrimidin-5-yl)amino) -*trans*-cyclohexyl)propan-2-ol (**11b**).

^{1}H-NMR (CD_3OD/400 MHz): δ 8.35 (s, 1H), 8.12 (m, 2H), 7.72 (d, *J* = 8.0 Hz, 1H), 7.27 (t, *J* = 8.0 Hz, 1H), 7.09 (d, *J* = 7.6 Hz, 1H), 6.20 (d, *J* = 7.6 Hz, 1H), 3.95 (m, 1H), 2.30 (m, 2H), 1.94 (m, 2H), 1.32 (m, 4H), 1.50 (s, 6H). MS (ES^+, m/z): $(M+H)^+$: 385.5. LC-MS purity of compound **11b** was found to be >96%.

3-((3-Chlorophenyl)-*N*-4-methoxy-*trans*-cyclohexyl)pyrazolo[1,5-a]pyrimidin-5-amine (**12b**).

A solution of 3-chloro-5-chloropyrazolo[1,5-a]pyrimidine (0.920 grams, 3.48 mmol) and *trans*-(4-aminocyclohexyl)propan-2-ol (0.822 grams, 5.23 mmol) in iso-propanol (5 mL) was added DIPEA (1.089 grams, 13.93 mmol) and the mixture was heated at 150^{o}C for 12 h under microwave irradiation. The resulting dark brown solution was cooled down and was concentrated under reduced pressure. The solid was further purified by using combiflash chromatography (40 g column), eluent: 0-6% CH_3OH/DCM and obtained product (0.520 grams, 1.351 mmol, 39% yield.). ^{1}H-NMR (CD_3OD/400 MHz): δ 8.40 (s, 1H), 8.23 (m, 2H), 7.80 (d, *J* = 8.0 Hz, 1H), 7.29 (t, *J* = 8.0 Hz, 1H), 7.05 (d, *J* = 8.0 Hz, 1H), 6.22 (d, *J* = 7.6 Hz, 1H), 3.95 (m, 1H), 3.30 (m, 1H), 2.25 (m, 2H), 2.15 (m, 2H), 1.45 (m, 4H). MS (ES^+, m/z): $(M+H)^+$: 357.5. LC-MS purity of compound **12b** was found to be >96%.

3-(3-Chlorophenyl)-*N*-(tetrahydro-2*H*-pyran-4-yl)pyrazolo[1,5-a]pyrimidin-5-amine (**13b**).

^{1}H-NMR ($CDCl_3$/400 MHz): δ 8.32 (s, 1H), 8.15 (m, 2H), 7.72 (d, *J* = 8.0 Hz, 1H), 7.26 (t, *J* = 8.0 Hz, 1H), 7.08 (d, *J* = 8.0 Hz, 1H), 6.23 (d, *J* = 8.0 Hz, 1H), 4.20 (m, 1H), 4.00 (m, 2H), 3.62

(t, J = 12.0 Hz, 2H), 2.17 (m, 2H), 1.62 (m, 2H). MS (ES^+, m/z): $(M+H)^+$: 329.5. LC-MS purity of compound **13b** was found to be >96%.

4-(((3-(3-Chlorophenyl)pyrazolo[1,5-a]pyrimidin-5-yl)amino)methyl)tetrahydro-2*H*-thiopyran 1,1-dioxide (**14b**).
^{1}H-NMR (CD_3OD/400 MHz): δ 8.35 (s, 1H), 8.28 (d, J = 8.0 Hz, 1H), 8.24 (s, 1H), 7.81 (d, J = 8.0 Hz, 1H), 7.31 (t, J = 7.2 Hz, 1H), 7.11 (d, J = 7.2 Hz, 1H), 6.29 (d, J = 7.2 Hz, 1H), 3.46 (m, 2H), 3.10 (m, 4H), 2.27 (m, 3H), 1.89 (m, 2H). MS (ES^+, m/z): $(M+H)^+$: 391.4. LC-MS purity of compound **14b** was found to be >96%.

4-((3-(3-Chlorophenyl)pyrazolo[1,5-a]pyrimidin-5-yl)amino)cyclohexanone (**16b**).
A mixture of 5-chloro-3-(3-chlorophenyl)pyrazolo[1,5-a]pyrimidine (100 mg, 0.379 mmol), 4-aminocyclohexanone (43 mg, 0.379 mmol), and DIPEA (0.158 ml, 1.515 mmol) in 2-propanol (3 ml) was irradiated to 150ºC for 14h in a Biotage microwave. After cooling, the mixture was diluted with saturated aqueous $NaHCO_3$, and extracted three times with EtOAc. The combined extracts were washed with brine, dried over Na_2SO_4 and concentrated in vacuo. After absorbing on celite, the compound was purified by combiflash chromatography (12g silica, 0-15% methanol/DCM) to give 4-((3-(3-chlorophenyl)pyrazolo[1,5-a]pyrimidin-5-yl)amino)cyclohexanone (30 mg, 24% yield) as a white solid. ^{1}H-NMR (CD_3OD/400 MHz): 8.35 (s, 1H), 8.26 (d, J = 7.6 Hz, 1H), 8.21 (d, J = 10.4 Hz, 1H), 7.79 (m, 1H), 7.08 (m, 1H), 6.26 (m, 1H), 4.40 (m, 1H), 2.62 (m, 2H), 2.47 (m, 2H), 2.00 (m, 1H), 1.84 (m, 2H), 1.65 (m, 1H). MS (ES^+, m/z): $(M+H)^+$: 341.4. LC-MS purity of compound **16b** was found to be >96%.

2.1. Pim-1 and Pim-2 Kinase Inhibition Assay.

Pim-1 and Pim-2 Kinase-Glo assays using ATP were used to determine the biochemical activity of the inhibitors. The activity of Pim-1and Pim-2 was measured using a luciferase-luciferin based ATP detection reagent (The Kinase-Glo Assay Kit, Promega, Inc., Madison, WI) to quantify ATP depletion resulting from kinase-catalyzed phosphoryl transfer to a peptide substrate. The amount of ATP remaining in the solution after the kinase reaction served as a substrate for the luciferase to catalyze luciferin to oxyluciferin plus one photon of light. Thus, the luminescent

signal read by the Luminoskan Ascent Instrument (Thermo Electron Corp., Milford, MA) correlated with the amount of ATP present after the kinase reaction and inversely correlated with the amount of kinase acitivity. These assays were set up in duplicate 50µL volumes in white, flat bottom 96 well plates. Inhibitors were added to the solution of 1X kinase buffer, 10µM ATP, 100µM Pim-1(Pim-2)-specific substrate, 50ng of active Pim-1 (Pim-2) enzyme, and water in serial dilutions ranging from micromolar to nanomolar concentrations. This solution was incubated at 30 degrees Celsius at 360rpm for two hours. Following the incubation, 50µL of Kinase-Glo reagent was added to each well, including all positive and negative control wells, and incubated at room temperature for 15 minutes. The plate was then read by the Luminoskan Ascent instrument and the results displayed with the Ascent Software version 2.6. The IC_{50} values were calculated for each inhibitor tested.

2.2. In Vitro Antiproliferative Assay.

Briefly, cells were seeded into 96-well, tissue-culture treated, opaque white plates (Thermo Electron, Vantaa, Finland), at between 5000 and 10000 cells per well, depending on the speed of cell proliferation, in 100 µL of appropriate growth medium (determined by the ATCC). Cells were then exposed to the appropriate concentration of drug or an equal amount of DMSO (drug diluent) and allowed to grow in its presence for 96 hours. Following this, 100 µL of Cell-Titer-Glo reagent (Promega, Inc., Madison, WI) was added to each well. Plates were then shaken for 2 minutes at room temperature to allow for cell lysis and incubated for 10 minutes at room temperature to stabilize the luminescent signal. Similar to the Kinase-Glo assay reagent from Promega, this reagent contained both luciferase enzyme and its substrate luciferin. Luciferase, activated by ATP in the cell lysate, catalyzes the conversion of luciferin to oxyluciferin, a reaction which produces light. The amount of light produced was proportionated to the amount of ATP in the cell lysate, which is itself proportional to the cell number and provides an index of cellular proliferation.

2.3. Western blotting assay.

Western blot assay was performed to detect specific inhibition of Pim-1 enzyme in cell culture. Cells which have been treated with a potential Pim-1 inhibitor were lysed with a buffer specific

for the isolation and preservation of proteins (1% Nonidet P-40, 150mM NaCl, 50mM Tris pH 8.0, 5mM EDTA, 1:500 Protease Inhibitor Cocktail III [Calbiochem], 100mM NaF, 100mM Sodium Orthovanadate). The protein concentration in these lysates was then quantified using the BCA Protein Assay Kit (Pierce). Known amounts of protein, e.g. 10 µg, are loaded onto 12% SDS-polyacrylamide gels and are subjected to reducing, denaturing SDS-PAGE. Electrophoresed proteins were transferred to a nitrocellulose membrane, which is then probed with antibodies to p-21 and phospho (Thr 145) p-21. As Threonine-145 of the p-21 protein is a substrate for Pim-1, measuring the amount of phosphorylation at this site in treated cells should provide a means by which to evaluate the efficacy of Pim-1 inhibitors.

2.4. *h*ERG assay.

Compounds were tested for effects on *h*ERG potassium channels by an automated patch clamp method (QPatchHTX) at WuXi AppTec (Shanghai, China). Chinese hamster ovary cells stably expressing hERG potassium channels from Aviva Bioscicences were tested with compound at six concentrations, 3-fold dilution starting at 30 µM with a final DMSO concentration of 0.15%, and compared to vehicle (negative) control and Amitriptyline (positive) controls. Percent of control (vehicle) values were calculated in duplicate for each concentration of drug, and curve-fitting and IC_{50} calculations were performed by QPatch Assay Software.

2.5. Clonogenic cell survival assay.

UM-UC-3 and HSC-3 cells were seeded at 300 cells/well in a 12-well plate and treated with the indicated concentration of compound **11b** (5 nM, 14 nM, 40 nM, 120 nM, 370 nM, 1.1 µM, 3.3 µM, 10 µM, 30 µM) or DMSO for 24 hours after seeding. After compound treatment, cells were incubated in drug-free cell culture medium for 10 days at 37°C in 5% CO_2. Subsequently, the cells were fixed with 4% paraformaldehyde in PBS, washed twice with PBS, and stained with a crystal violet solution (1% crystal violet, 10% ethanol in water). Stained cells were washed twice with water, and imaged after drying on a GelCount colony counter (Oxford Optronix Ltd., Oxford, UK). Total staining intensity per well was determined by lysis of cells with 200 µL of Triton X-100 lysis buffer (1% Triton X-100, 50 mM Tris-HCl pH 7.4, 150 mM NaCl, 1 mM EDTA). Lysates (100 µL) from each well were transferred to a clear 96-well plate and

absorbance at 560 nm was determined on an Envision microplate reader. IC_{50} values were determined using GraphPad Prism software.

2.6. Kinase selectivity of compound 11b against a panel of 119 oncogenic kinases.

The kinase selectivity of compound **11b** was investigated by screening it against a panel of 119 oncogenic kinases (oncoKP, Reaction Biology Corporation, PA, USA) at a single concentration (1 μM).

Reference compounds and their IC_{50}s against a panel of 119 oncogenic kinases.

Kinase	IC50 (nM) Staurosporine*	IC50 (nM) Alternate Control cpd*.	Alternate compound ID
ABL1	32.86		
ABL2/ARG	9.28		
AKT1	1.03		
AKT2	5.55		
AKT3	1.07		
Aurora A	< 1.0		
Aurora B	1.39		
Aurora C	< 1.0		
AXL	5.09		
BRAF	ND	90.32	GW5074
BRK	133.20		
BTK	11.31		
CDK1/cyclin B	1.51		
CDK2/cyclin A	< 1.0		

CDK4/cyclin D1	4.98		
CDK5/p25	1.07		
CDK6/cyclin D1	1.16		
CDK7/cyclin H	296.70		
CDK9/cyclin T1	9.53		
CHK1	< 1.0		
CHK2	1.81		
CK1epsilon	ND	449.60	D4476
CK2a	ND	234.00	GW5074
c-Kit	16.58		
c-MET	138.90		
COT1/MAP3K8	ND	10150.00	Ro-31-8220
c-Src	1.06		
CTK/MATK	445.90		
DAPK1	2.11		
DNA-PK	ND	257.50	LY294002
EEF2K	ND	8333.00	NH125
EGFR	63.67		
EPHA1	58.21		
EPHA2	42.19		
EPHA3	14.26		
EPHA4	15.54		
EPHA5	33.64		
EPHA7	22.65		

EPHA8	98.37		
EPHB1	34.05		
EPHB2	155.30		
EPHB3	1279.00		
EPHB4	89.02		
ERBB2/HER2	210.40		
ERBB4/HER4	213.00		
FAK/PTK2	4.47		
FER	< 1.0		
FES/FPS	< 1.0		
FGFR1	7.14		
FGFR2	1.64		
FGFR3	5.13		
FGFR4	66.23		
FGR	< 1.0		
FLT1/VEGFR1	3.33		
FLT3	< 1.0		
FLT4/VEGFR3	1.13		
FMS	< 1.0		
FYN	< 1.0		
Haspin	3.45		
HCK	< 1.0		
HGK/MAP4K4	< 1.0		
HIPK1	ND	6932.00	Ro-31-8220

HIPK2	699.60		
HIPK3	2484.00		
HIPK4	496.30		
IGF1R	51.47		
IKKa/CHUK	90.07		
IKKb/IKBKB	251.30		
IKKe/IKBKE	< 1.0		
JAK1	< 1.0		
JAK2	< 1.0		
JAK3	< 1.0		
JNK1	15620.00		
JNK2	11940.00		
JNK3	ND	4164.00	JNKi VIII
KDR/VEGFR2	5.75		
LCK	< 1.0		
LKB1	30.91		
LYN	3.16		
MEK1	3.27		
MEK2	40.62		
MRCKa/CDC42BPA	2.53		
MRCKb/CDC42BPB	1.77		
MST4	1.06		
MUSK	61.95		
NEK1	4.49		

P38a/MAPK14	ND	22.89	SB202190
P38b/MAPK11	ND	78.76	SB202190
P38d/MAPK13	92.99		
P38g	150.90		
p70S6K/RPS6KB1	< 1.0		
PAK4	1.18		
PDGFRa	< 1.0		
PDGFRb	1.14		
PIM1	1.03		
PIM2	15.95		
PKCa	< 1.0		
PKCb1	1.71		
PKCd	< 1.0		
PKCepsilon	< 1.0		
PKCeta	< 1.0		
PKCtheta	< 1.0		
PLK1	162.30		
PYK2	3.34		
RAF1	ND	9.03	GW5074
RET	1.71		
ROCK1	< 1.0		
RON/MST1R	118.70		
ROS/ROS1	< 1.0		
SGK1	2.76		

SYK	< 1.0		
TGFBR2	> 20000		
TIE2/TEK	23.05		
TNIK	< 1.0		
TRKA	< 1.0		
TRKB	< 1.0		
TRKC	< 1.0		
TYRO3/SKY	3.97		
YES/YES1	< 1.0		

* Values represent the average of 2 separate experiments.

11b	
kinase	Activity*
ABL1	76.54
ABL2/ARG	83.97
AKT1	95.34
AKT2	90.30
AKT3	91.86
Aurora A	83.98
Aurora B	84.99
Aurora C	91.90
AXL	67.49

BRAF	110.28
BRK	52.25
BTK	81.54
CDK1/cyclin B	73.77
CDK2/cyclin A	74.96
CDK4/cyclin D1	87.00
CDK5/p25	87.59
CDK6/cyclin D1	97.80
CDK7/cyclin H	80.37
CDK9/cyclin T1	70.78
CHK1	91.63
CHK2	102.08
CK1epsilon	90.20
CK2a	25.47
c-Kit	54.35
c-MET	91.71
COT1/MAP3K8	89.49
c-Src	41.45
CTK/MATK	96.53
DAPK1	57.45
DNA-PK	85.70
EEF2K	84.93
EGFR	101.09
EPHA1	90.62

EPHA2	102.09
EPHA3	108.22
EPHA4	98.00
EPHA5	107.83
EPHA7	89.99
EPHA8	91.50
EPHB1	96.41
EPHB2	103.05
EPHB3	104.62
EPHB4	92.66
ERBB2/HER2	103.96
ERBB4/HER4	92.55
FAK/PTK2	91.48
FER	80.25
FES/FPS	109.21
FGFR1	94.80
FGFR2	95.46
FGFR3	96.13
FGFR4	106.69
FGR	53.38
FLT1/VEGFR1	94.80
FLT3	6.30
FLT4/VEGFR3	93.14
FMS	35.30

FYN	32.85
Haspin	10.57
HCK	61.71
HGK/MAP4K4	74.84
HIPK1	87.41
HIPK2	29.11
HIPK3	54.22
HIPK4	16.93
IGF1R	98.04
IKKa/CHUK	82.03
IKKb/IKBKB	104.82
IKKe/IKBKE	82.07
JAK1	90.64
JAK2	34.94
JAK3	69.90
JNK1	94.48
JNK2	98.75
JNK3	97.50
KDR/VEGFR2	91.97
LCK	10.38
LKB1	99.02
LYN	76.93
MEK1	98.58
MEK2	104.98

MRCKa/CDC42BPA	100.20
MRCKb/CDC42BPB	93.29
MST4	138.39
MUSK	15.00
NEK1	71.29
P38a/MAPK14	96.48
P38b/MAPK11	94.13
P38d/MAPK13	102.65
P38g	101.00
p70S6K/RPS6KB1	100.59
PAK4	79.72
PDGFRa	77.52
PDGFRb	66.17
PIM1	1.97
PIM2	29.60
PKCa	74.12
PKCb1	92.03
PKCd	95.19
PKCepsilon	79.55
PKCeta	99.88
PKCtheta	98.98
PLK1	102.12
PYK2	86.38
RAF1	92.20

RET	77.23
ROCK1	103.52
RON/MST1R	91.29
ROS/ROS1	56.69
SGK1	78.22
SYK	88.74
TGFBR2	61.98
TIE2/TEK	99.58
TNIK	75.04
TRKA	16.07
TRKB	6.63
TRKC	3.84
TYRO3/SKY	94.45
YES/YES1	23.76

* Values represent the average of 2 separate experiments.

	11b	
	kinase	Activity*
1	PIM1	1.965154
2	TRKC	3.840984
3	FLT3	6.295302
4	TRKB	6.632169
5	LCK	10.37995

6	Haspin	10.57093
7	MUSK	14.99514
8	TRKA	16.06705
9	HIPK4	16.9267
10	YES/YES1	23.76084
11	CK2a	25.47173
12	HIPK2	29.10777
13	PIM2	29.6005
14	FYN	32.85065
15	JAK2	34.93882
16	FMS	35.29637
17	c-Src	41.44842
18	BRK	52.2481
19	FGR	53.38278
20	HIPK3	54.21787
21	c-Kit	54.35329
22	ROS/ROS1	56.68826
23	DAPK1	57.4512
24	HCK	61.7054
25	TGFBR2	61.98027
26	PDGFRb	66.17142
27	AXL	67.48917
28	JAK3	69.90114
29	CDK9/cyclin T1	70.77933

30	NEK1	71.29039
31	CDK1/cyclin B	73.76878
32	PKCa	74.12231
33	HGK/MAP4K4	74.83763
34	CDK2/cyclin A	74.96454
35	TNIK	75.03711
36	ABL1	76.53915
37	LYN	76.93333
38	RET	77.23456
39	PDGFRa	77.523
40	SGK1	78.21783
41	PKCepsilon	79.55311
42	PAK4	79.71943
43	FER	80.2534
44	CDK7/cyclin H	80.37326
45	BTK	81.53723
46	IKKa/CHUK	82.03436
47	IKKe/IKBKE	82.06968
48	ABL2/ARG	83.96575
49	Aurora A	83.98188
50	EEF2K	84.92741
51	Aurora B	84.98927
52	DNA-PK	85.70344
53	PYK2	86.38275

54	CDK4/cyclin D1	87.00265
55	HIPK1	87.40891
56	CDK5/p25	87.58962
57	SYK	88.74032
58	COT1/MAP3K8	89.48868
59	EPHA7	89.98776
60	CK1epsilon	90.19615
61	AKT2	90.30307
62	EPHA1	90.62266
63	JAK1	90.63747
64	RON/MST1R	91.28522
65	FAK/PTK2	91.47893
66	EPHA8	91.49511
67	CHK1	91.62749
68	c-MET	91.71235
69	AKT3	91.85688
70	Aurora C	91.8998
71	KDR/VEGFR2	91.96723
72	PKCb1	92.03178
73	RAF1	92.19887
74	ERBB4/HER4	92.5531
75	EPHB4	92.6608
76	FLT4/VEGFR3	93.13995
77	MRCKb/CDC42BPB	93.28655

78	P38b/MAPK11	94.13054
79	TYRO3/SKY	94.45096
80	JNK1	94.47617
81	FGFR1	94.79572
82	FLT1/VEGFR1	94.79602
83	PKCd	95.19088
84	AKT1	95.33915
85	FGFR2	95.46037
86	FGFR3	96.13037
87	EPHB1	96.41357
88	P38a/MAPK14	96.47615
89	CTK/MATK	96.52623
90	JNK3	97.49793
91	CDK6/cyclin D1	97.79737
92	EPHA4	98.00282
93	IGF1R	98.03697
94	MEK1	98.58226
95	JNK2	98.75095
96	PKCtheta	98.97511
97	LKB1	99.02296
98	TIE2/TEK	99.5798
99	PKCeta	99.88336
100	MRCKa/CDC42BPA	100.1999
101	p70S6K/RPS6KB1	100.588

102	P38g	101.0034
103	EGFR	101.0856
104	CHK2	102.0755
105	EPHA2	102.0937
106	PLK1	102.1241
107	P38d/MAPK13	102.6485
108	EPHB2	103.0477
109	ROCK1	103.5244
110	ERBB2/HER2	103.9569
111	EPHB3	104.6244
112	IKKb/IKBKB	104.8158
113	MEK2	104.9842
114	FGFR4	106.6886
115	EPHA5	107.8302
116	EPHA3	108.2156
117	FES/FPS	109.2117
118	BRAF	110.2817
119	MST4	138.3896

* Values represent the average of 2 separate experiments.

Compound **11b** was tested against 4 PI3K isoforms.

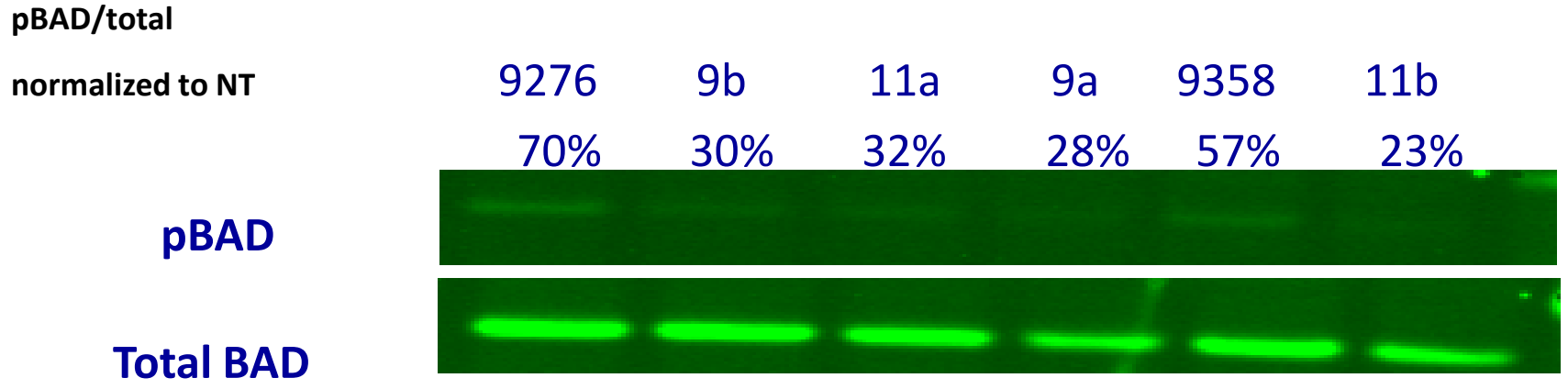
Compound **11b** was tested in a 10-dose IC_{50} with 3-fold serial dilution starting at 10 μM. Control Compound, PI-103, was tested in 10-dose IC_{50} with 3-fold serial dilution starting at 10 μM. Reactions were carried out at 10 μM ATP. HTRF assay format was used for PI3Ks. Data pages include raw data, % Enzyme activity (relative to DMSO controls), and curve fits.

IC_{50} Summary:

	Compound ID IC_{50} (nM)	
Kinase:	**11b**	**PI-103**
PI3Kalpha	146.80	2.49
PI3Kbeta	1048.00	5.66
PI3Kgamma	66.75	38.97
PI3Kdelta	356.90	11.73

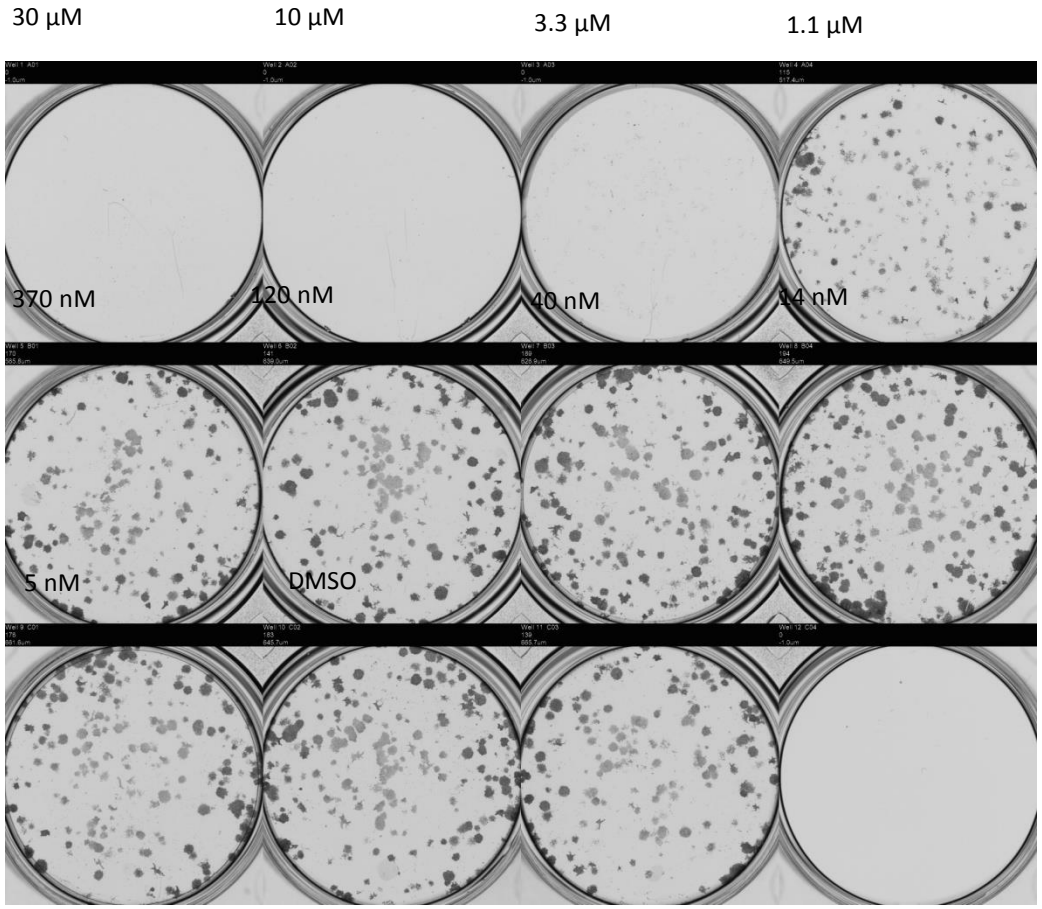
Supplementary material Figure 1.
Selected compounds inhibit BAD phosphorylation in bladder cancer cell lines.

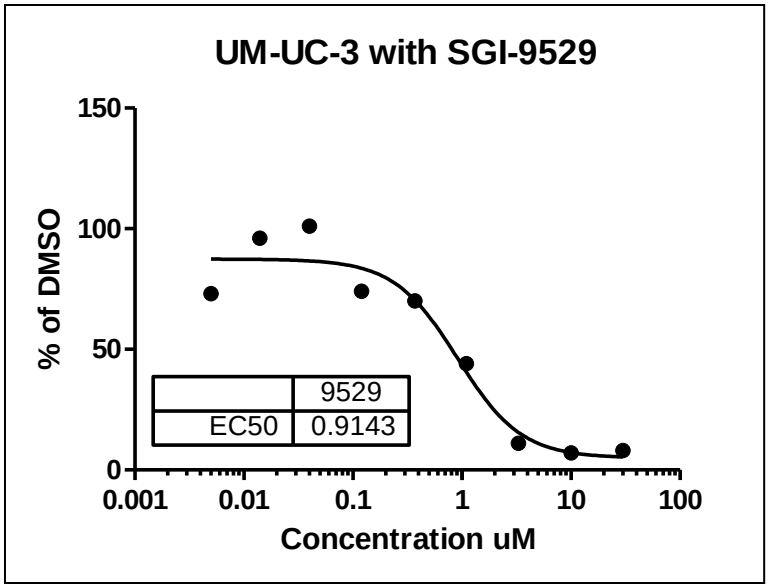
RPMI-8226 cells treated with 1μM drug for 45 min



Supplementary material Figure 2A.
Compound **11b** inhibited and reduced the growth of UM-UC-3 cells in 2-dimensional colony formation assay.

UM-UC-3 with compound **11b**.





Supplementary material Figure 2B.
Compound **11b** inhibited and reduced the growth of HSC3 cells in 2-dimensional colony formation assay.

HSC3 with compound 11b.