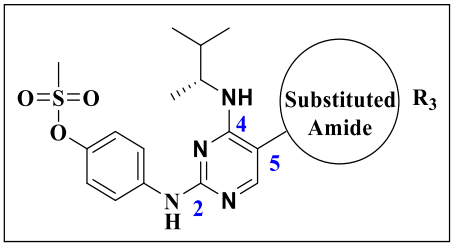
4. Design and synthesis of compounds modified at 5th position of Diamino pyrimidine



Modification at 4th position of pyrimidine ring of **41k** (IC_{50}: 9.5 nM) gave compound **48l** with IC_{50}: 1.7 nM which is much better compare to cerdulatinib (IC_{50}: 8.0 nM). Further, in this Series-3, changes were carried out at the C5 position of **48l**. **Chapter-4** is divided in two parts. In part 1, synthesis of pyrimidine derivatives with the modification at the 5th position were depicted. As shown in **Scheme-11** (compounds **54a-m**, **55**, **56**) total 14 compounds were prepared in this Series. All the compounds were characterized, using various spectroscopic techniques like ^{1}H NMR, ESI-MS and UPLC / HPLC. In part 2, *in-vitro* JAK3 inhibitory activity data of compounds **54a-m**, **55** and **56** are presented.

4.1. Chemistry

4.1.1. Materials and methods

All reagents used were obtained from Sigma Aldrich and were used without further purification. Solvents were purchased from a commercial source and used after distilling or drying according to the known methods. All the air and/or moisture sensitive reactions were carried out in dry solvents, under the Nitrogen (inert) atmosphere. Melting points were

recorded in open glass capillaries, using a scientific melting point apparatus (Mettler Toledo, Switzerland) and are uncorrected.

The ^{1}H NMR spectra were recorded on a Brucker Avance-300 (300 MHz) or Bruker Avance-400 (400MHz) spectrometer, Switzerland. The chemical shift (δ) are reported in parts per million (ppm) relative to TMS (tetramethylsilane), either in $CDCl_3$ or DMSO- d_6. Signal multiplicities are represented as s (singlet), d (doublet), dd (doublet of doublet), t (triplet), q (quartet), bs (broad singlet), and m (multiple). ^{13}C NMR spectra were recorded on Bruker Avance-400 at 100 MHz either in $CDCl_3$ or DMSO-d_6.

Mass spectra (ESI-MS) were obtained on Shimadzu LCMS 2010-A spectrometer, Japan. Elemental analysis were carried out, using a perkin-Elmer 2400 CHN analyzer, UK. HPLC analysis were carried out at λmax 220nm, using column ODS C-18, 150nm*4.6nm*4μ on AGILENT 1100, Germany. UPLC analysis were carried out at λmax 220nm, using column YMC-Triart C18 (100*2.0mm) on Water acquity UPLC, Europe (Austria).

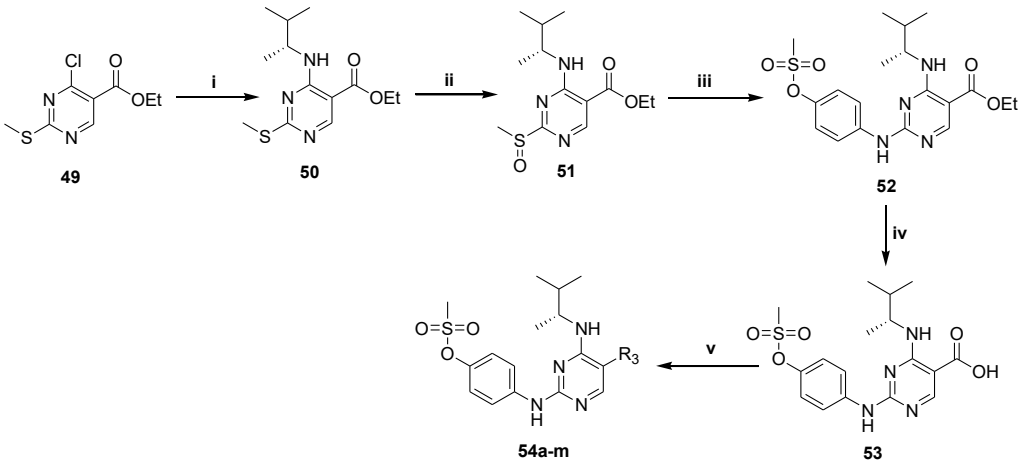
Progress of the reactions was monitor by TLC, using precoated TLC plates (E. Merck Kiesegel 60 F254, Germany) and the spots were visualized by UV and/or iodine vapors. The chromatographic purification was performed on silica gel (230-400 mesh). Few compounds directly used for the next step without purification and analysis.

4.1.2. General procedure for the synthesis of compounds 54a-m

Synthesis of pyrimidine derivatives with the modification at the 5th position was carried out as depicted in the **Scheme-11**. Treatment of commercially available ethyl 4-chloro-2-(methylthio) pyrimidine-5-

carboxylate (**49**) with (*R*)-3-methylbutan-2-amine gives 4-benzylamino carboxylate (**50**). Compound **50** converted to reactive methyl sulfinyl derivate (**51**), using *m*-CPBA, followed by treatment with 4-aminophenylmethanesulfonate gives an intermediate **52**. Hydrolysis of ester leads to acid moiety (**53**). Compound **53** was converted into amide (**54a-m**) by making active ester, using HOBt and EDC.HCl, followed by reaction with different amine.

Scheme-11



Reagents and conditions: (i) (R)-3-methylbutan-2-amine, DIPEA, Dioxane, 30 ^{o}C, 6hr; (ii) Dioxane:$CHCl_3$ (1:1), m-CPBA, 20 mins, 10% sodium metabisulfite; (iii) NMP, PTSA, (4-aminophenyl)methanesulfonate, 120 ^{o}C, 1hr, 75%; (iv) THF, LiOH, water, 30 ^{o}C, 6hr; (v) EDC.HCl, HOBt, amine, DMF, 30 ^{o}C, 2hr.

Stepwise experimental procedure for the synthesis of compounds **50** to **53** and **54a-m**.

Step I: Preparation of ethyl (*R*)-4-((3-methylbutan-2-yl)amino)-2-(methylthio)pyrimidine-5-carboxylate (**50**):

Ethyl 4-chloro-2-(methylthio)pyrimidine 5-carboxylate **49** (14.0 g, 60.2 mmol) was dissolved in dioxane (120 mL) and DIPEA (11.56 mL, 66.2 mmol) was added to the reaction mixture at 0°C. Then after addition of (*R*)-3-methylbutan-2-amine (5.24 g, 60.2 mmol) the

reaction mixture was stirred at 0°C for 30 mins and then 6 hr, at RT. After completion of reaction, the mixture was diluted with water and the compound was extracted with EtOAc. The organic layer was dried over Na_2SO_4, filtered and concentrated under vacuum to get the desired (*R*)-4-((3-methylbutan-2-yl)amino)-2-(methylthio)pyrimidine-5-carboxylate (**50**), as a white solid (Yield: 15.0 g, 88 %, Purity by UPLC: 90%).

^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 0.87 – 0.92 (m, 6H), 1.07 – 1.10 (m, 3H), 1.21 – 1.25 (m, 3H), 1.79 – 1.83 (m, 1H), 2.49 (s, 3H), 4.09 – 4.19 (m, 3H), 8.52 (s, 1H); ESI-MS: Exact mass = 283.1354, m/z $[M+H]^+$ peak at 284.9.

Step II: Preparation of ethyl 4-(((*R*)-3-methylbutan-2-yl)amino)-2-(methylsulfinyl)pyrimidine-5-carboxylate (**51**):

(*R*)-4-((3-methylbutan-2-yl)amino)-2-(methylthio)pyrimidine-5 carboxylate **50** (13 g, 45.9 mmol) was dissolved in dioxane and $CHCl_3$ (1:1) 200 mL. The reaction mixture was cooled up to -2°C and treated with *m*-CPBA 60% (19.78 g, 68.8 mmol) and it was stirred for 20 mins, at 0°C. After completion of reaction, the reaction mixture was quenched with 10% aq. $Na_2S_2O_5$ and organic layer was extracted with DCM and washed with aq. 10 % $NaHCO_3$. The organic layer was dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by column chromatography on silica gel (230-400 mesh) to get ethyl 4-(((*R*)-3-methylbutan-2-yl)amino)-2-(methylsulfinyl)pyrimidine-5-carboxylate (**51**), as white a solid (Yield: 10.3 g, 75 %) which was further used in the next step without purification.

Step III: Preparation of ethyl (*R*)-4-((3-methylbutan-2-yl)amino)-2-((4-((methylsulfonyl)oxy)phenyl)amino)pyrimidine-5-carboxylate (**52**):

Ethyl 4-(((R)-3-methylbutan-2-yl)amino)-2-(methylsulfinyl)pyrimidine-5-carboxylate **51** (9.0 g, 30.1 mmol) and PTSA (5.69 g, 33.1 mmol), in NMP (85 mL) was added (4-aminophenyl)methanesulfonate (5.63 g, 30.1 mmol), at RT. The reaction mixture was heated at 110°C for 1 hr. After completion of reaction, the mixture was diluted with water and the compound was extracted with EtOAc. The organic layer was dried over Na_2SO_4, filtered and concentrated under vacuum to get the crude product. The crude product was purified by flash chromatography over silica gel (230-400 mesh) with 2% MeOH/$CHCl_3$ to get the ethyl (R)-4-((3-methylbutan-2-yl)amino)-2-((4-((methylsulfonyl)oxy)phenyl) amino)pyrimidine-5-carboxylate **52** (Yield: 9.14 g, 72%, Purity by UPLC: 90%).

^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 0.88 – 0.95 (m, 6H), 1.15 – 1.16 (m, 3H), 1.30 (t, *J* = 7.2 Hz, 3H), 1.90 - 1.93 (m, 1H), 3.34 (s, 3H), 4.20 - 4.21 (m, 1H), 4.22 - 4.26 (m, 2H), 7.28 (d, *J* = 8.8 Hz, 2H), 7.84 – 7.88 (m, 2H), 8.21 (s, 1H), 8.58 (s, 1H), 9.98 (s, 1H); ESI-MS: Exact mass = 422.1624, m/z $[M+H]^+$ peak at 423.1.

Step IV: Preparation of (*R*)-4-((3-methylbutan-2-yl)amino)-2-((4-((methylsulfonyl)oxy)phenyl)amino)pyrimidine-5-carboxylic acid (**53**):

Ethyl (*R*)-4-((3-methylbutan-2-yl)amino)-2-((4-((methylsulfonyl) oxy) phenyl) amino)pyrimidine-5-carboxylate **52** (8.0 g, 18.93 mmol) was dissolved in THF (100 mL). Then $LiOH.H_2O$ (2.26 g, 95.0 mmol, dissolved in 4.0 mL water) added to reaction mixture and stirred for 6

hr at 30°C. After completion of reaction, the mixture was concentrated by rotary evaporation to remove excess of THF. The mixture was acidified with dilute HCl (pH ~ 5), to get white precipitate. The solid compound was filtered, washed with water and dried under vacuum to get (*R*)-4-((3-methylbutan-2-yl)amino)-2-((4-((methyl sulfonyl)oxy) phenyl)amino) pyrimidine-5-carboxylic acid (**53**), as a white solid (Yield: 6.12 g, 80%, Purity by UPLC: 90%).

^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 0.92 – 0.95 (m, 6H), 1.05 – 1.16 (m, 3H), 1.83 – 1.90 (m, 1H), 3.31 (s, 3H), 4.06 - 4.10 (m, 1H), 7.22 – 7.29 (m, 2H), 7.86 (d, *J* = 9.2 Hz, 2H), 8.44 (s, 1H), 9.50 (s, 1H); ESI-MS: Exact mass = 394.1311, m/z $[M-H]^+$ peak at 392.9.

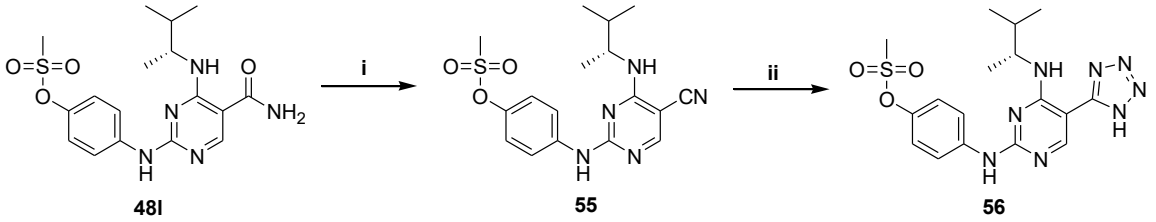
Step VI: General procedure for the synthesis of compound **54a-m**:

(*R*)-4-((3-methylbutan-2-yl)amino)-2-((4-((methylsulfonyl)oxy) phenyl) amino)pyrimidine-5-carboxylic acid **53** (1.0 eq) was dissolved in DMF under the Nitrogen (inert) atmosphere and treated with HOBt (1.0 eq), EDC.HCl (2.0 eq) and DIPEA (2.5 eq), at RT. After stirring for 1 hr, amine (1.0 eq, structure of various amine listed in section 4.1.4.) was added, at 0°C, and it was stirred further for 1 hr, at RT. The reaction mixture was quenched with water and the compound was extracted with EtOAc. The organic layer was dried over Na_2SO_4, filtered and concentrated under vacuum to get the crude product. The crude product was purified by flash chromatography over silica gel (230-400 mesh) with 2% MeOH/$CHCl_3$ to provide the desired title compounds (**54a-m**).

4.1.3. Procedure for the synthesis of 55 and 56

The synthesis of compounds **55** and **56** was carried out as shown in

Scheme-12



Reagents and conditions: (i) TFAA, TEA, DCM, 30 ^{o}C, 2 hr; (ii) NaN_3, NH_4Cl, DMF, 120 ^{o}C, 16 hr

Step I: Preparation of (*R*)-4-((5-cyano-4-((3-methylbutan-2-yl)amino)pyrimidin-2-yl)amino)phenylmethanesulfonate (**55**):

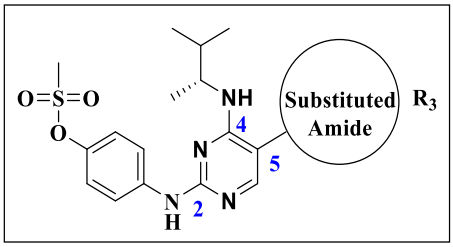
The solution of (*R*)-4-((5-carbamoyl-4-((3-methylbutan-2-yl) amino) pyrimidin-2-yl)amino)phenylmethanesulfonate **48l** (2 g, 5.08 mmol) and TEA (1.7 mL, 12.20 mmol) in DCM (30 mL) was added to trifluoroacetic anhydride (TFAA; 0.862 mL, 6.10 mmol), at 0-5^{o}C. The mixture was stirred for 2 hr at RT. After completion of reaction, the mixture was quenched with water and the organic compound was extracted with DCM. The organic layer was dried over Na_2SO_4 and the solvent was removed under reduced pressure to afford (*R*)-4-((5-cyano-4-((3-methylbutan-2-yl)amino)pyrimidin-2-yl) amino)phenylmethanesulfonate as a white solid (Yield: 1.52 g, 80%, Purity by HPLC: 90%). **[93]**

Step II: Preparation of (*R*)-4-((4-((3-methylbutan-2-yl)amino)-5-(1*H*-tetrazol-5-yl)pyrimidin-2-yl)amino)phenyl methanesulfonate (**56**):

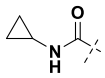
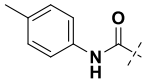
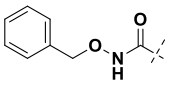
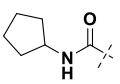
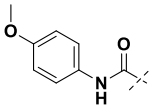
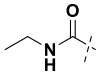
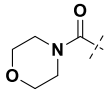
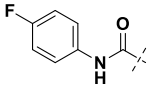
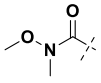
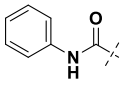
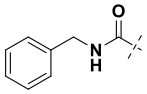
A Solution of (*R*)-4-((5-cyano-4-((3-methylbutan-2-yl)amino)pyrimidin-2-

yl)amino)phenylmethanesulfonate **55** (2.0 g, 5.33 mmol) in DMF (15 mL) was treated with NH_4Cl (0.57 g, 10.65 mmol), at 0°C. The mixture was slowly treated with NaN_3 (0.693 g, 10.65 mmol), at 0°C and then heated to 120°C for 16 hr. After completion of reaction, the mixture was cooled to RT, than the mixture was quenched with ice - water and extracted with EtOAc. The combine organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel (230-400 mesh) to afford (*R*)-4-((4-((3-methylbutan-2-yl)amino)-5-(1*H*-tetrazol-5-yl) pyrimidin-2-yl) amino) phenyl methanesulfonate, as a white solid (Yield: 1.56 g, 70%, Purity by UPLC: 98.21%). **[94]**

4.1.4. List of R_3 substituents at 5th position of pyrimidine derivatives



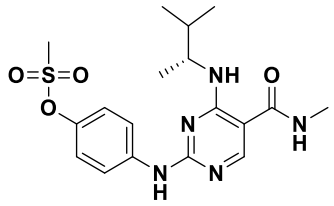
Substituent of compounds **54a-m**, **55** and **56** are listed below.

Compound	R_3	Compound	R_3	Compound	R_3	Compound	R_3
54a		54e		54i		54m	
54b		54f		54j		55	
54c		54g		54k		56	
54d		54h		54l			

Using above synthetic procedure, total 14 compounds were reported as **54a-m**, **55** and **56**. All the compounds were prepared in good yield. These compounds were characterized using suitable spectroscopic techniques and spectral data was found to be confirmed with the structure assigned. The detailed spectral data of **54a-m**, **55** and **56** are listed below.

4.1.5. Spectral Data of compound 54a-m, 55 and 56:

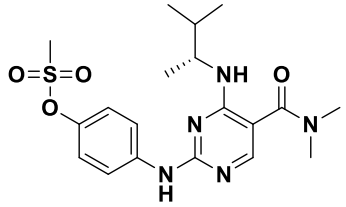
4.1.5.1. (*R*)-4-((4-((3-methylbutan-2-yl)amino)-5-(methyl carba moyl)pyrimidin-2-yl)amino) phenyl methane sulfonate (54a):



MP: 210-211°C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 0.90 (d, *J* = 6.6 Hz, 3H), 0.98 (d, *J* = 4.8 Hz, 3H), 1.15 (d, *J* = 6.8 Hz, 3H), 1.82 - 1.87 (m, 1H), 3.02 (s, 3H), 3.33 (s, 3H), 4.12 – 4.20 (m, 1H), 7.30 - 7.32 (m, 2H), 7.86 - 7.89 (m, 2H), 8.08 - 8.09 (m, 1H), 8.50 (s, 1H), 9.10 (d, *J* = 8.4 Hz, 1H), 9.64 (s, 1H); Purity (HPLC): 98.12%; ESI-MS: Exact mass = 407.1627, m/z $[M]^+$ peak at 407.6; Analysis (CHNS): Calculated for $C_{18}H_{25}N_5O_4S$: C, 53.06%; H, 6.18%; N, 17.19%; S, 7.87% ; Found: C, 53.25%; H, 6.33%; N, 17.28%; S, 7.92%.

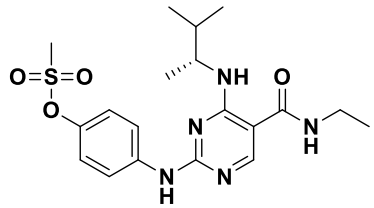
4.1.5.2. (*R*)-4-((5-(dimethylcarbamoyl)-4-((3-methylbutan-2-yl)

amino)pyrimidin-2-yl)amino)phenyl methane sulfonate (54b):



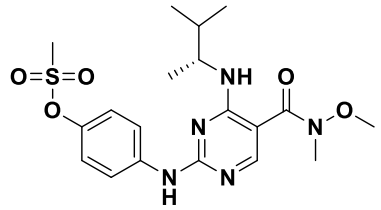
MP: 228-229°C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 0.92 – 0.98 (m, 6H), 1.02 (d, *J* = 4.6 Hz, 3H), 1.83 - 1.89 (m, 1H), 3.14 (s, 6H), 3.34 (s, 3H), 4.10 – 4.25 (m, 1H), 7.30 – 7.33 (m, 2H), 7.80 – 7.92 (m, 2H), 8.52 (s, 1H), 9.12 (d, *J* = 8.4 Hz, 1H), 9.62 (s, 1H); Purity (UPLC): 96.52%; ESI-MS: Exact mass = 421.1784, m/z $[M+H]^+$ peak at 422.2; Analysis (CHNS): Calculated for $C_{19}H_{27}N_5O_4S$: C, 54.14%; H, 6.46%; N, 16.62%; S, 7.61% ; Found: C, 54.05%; H, 6.35%; N, 16.58%; S, 7.56%.

4.1.5.3. (*R*)-4-((5-(ethylcarbamoyl)-4-((3-methylbutan-2-yl) amino)pyrimidin-2-yl)amino)phenyl methanesulfonate (54c):



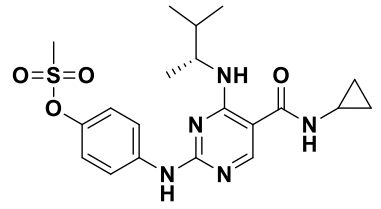
MP: 230-231°C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 0.95 (m, 6H), 0.96 (d, *J* = 4.6 Hz, 3H), 1.15 (t, *J* = 7.4 Hz, 3H), 1.85 - 1.89 (m, 1H), 3.34 (s, 3H), 4.15 – 4.20 (m, 1H), 4.25 - 4.28 (m, 2H), 7.34 - 7.40 (m, 2H), 7.80 -7.81 (m, 2H), 8.12 (m, 1H), 8.55 (s, 1H), 9.30 (d, *J* = 8.2 Hz, 1H), 9.61 (s, 1H); Purity (HPLC): 96.90%; ESI-MS: Exact mass = 421.1784, m/z $[M]^+$ peak at 421.4; Analysis (CHNS): Calculated for $C_{19}H_{27}N_5O_4S$: C, 54.14%; H, 6.46%; N, 16.62%; S, 7.61% ; Found: C, 54.05%; H, 6.35%; N, 16.58%; S, 7.56%.

4.1.5.4. (*R*)-4-((5-(methoxy(methyl)carbamoyl)-4-((3-methyl butan-2-yl)amino)pyrimidin-2-yl)amino)phenylmethane sulfonate (54d):



MP: 241-242°C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 0.88 (d, *J* = 6.8 Hz, 3H), 0.92 (d, *J* = 4.8 Hz, 3H), 1.13 (d, *J* = 6.4 Hz, 3H), 1.87 - 1.88 (m, 1H), 3.23 (s, 3H), 3.34 (s, 3H), 3.61 (s, 3H), 4.07 – 4.08 (m, 1H), 7.25 - 7.27 (m, 2H), 7.84 – 7.86 (m, 2H), 8.02 (d, *J* = 7.6 Hz, 1H), 8.45 (s, 1H), 9.68 (s, 1H); Purity (UPLC): 97.39%; ESI-MS: Exact mass = 437.1733, m/z $[M+H]^+$ peak at 438.0; Analysis (CHNS): Calculated for $C_{19}H_{27}N_5O_5S$: C, 52.16%; H, 6.22%; N, 16.01%; S, 7.33% ; Found: C, 52.26%; H, 6.40%; N, 16.23%; S, 7.41%.

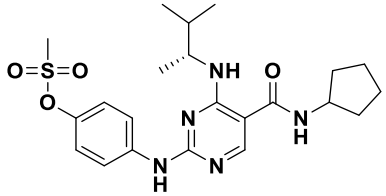
4.1.5.5. (*R*)-4-((5-(cyclopropylcarbamoyl)-4-((3-methylbutan-2-yl) amino)pyrimidin-2-yl)amino)phenyl methane sulfonate (54e):



MP: 249-250°C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 0.50 - 0.56 (m, 2H), 0.64 - 0.67 (m, 2H), 0.89 (d, *J* = 6.8 Hz, 3H), 0.96 (d, *J* = 6.8 Hz, 3H), 1.13 (d, *J* = 6.8 Hz, 3H), 1.88 - 1.89 (m ,1H), 2.73 - 2.77 (m, 1H), 3.33 (s, 3H), 4.07 - 4.09 (m, 1H), 7.23 – 7.26 (m, 2H), 7.84

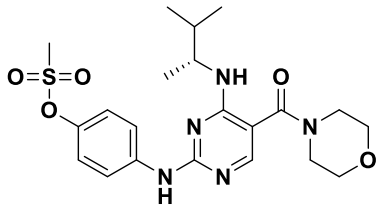
– 7.86 (m, 2H), 8.25 (d, *J* = 3.6 Hz, 1H), 8.46 (s, 1H), 9.15 (d, *J* = 8.8 Hz, 1H), 9.65 (s, 1H); Purity (HPLC): 99.02%; ESI-MS: Exact mass = 433.1784, m/z $[M+H]^+$ peak at 434.0; Analysis (CHNS): Calculated for $C_{20}H_{27}N_5O_4S$: C, 55.41%; H, 6.28%; N, 16.15%; S, 7.40% ; Found: C, 55.53%; H, 6.34%; N, 16.27%; S, 7.55%.

4.1.5.6. (*R*)-4-((5-(cyclopentylcarbamoyl)-4-((3-methylbutan-2-yl) amino)pyrimidin-2-yl)amino)phenyl methane sulfonate (54f):



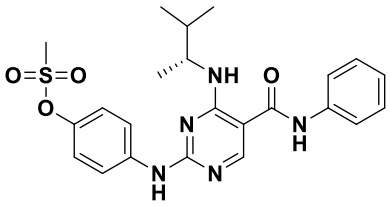
MP: 258-259°C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 0.88 (d, *J* = 6.4 Hz, 3H), 0.94 (d, *J* = 6.6 Hz, 3H), 1.10 (d, *J* = 6.8 Hz, 3H), 1.59 – 1.65 (m, 4H), 1.73 -1.81 (m, 4H), 1.86 - 1.88 (m, 1H), 3.12 – 3.18 (m, 1H), 3.34 (s, 3H), 4.10 - 4.12 (m, 1H), 7.20 – 7.25 (m, 2H), 7.80 – 7.86 (m, 2H), 8.25 (d, *J* = 3.4 Hz, 1H), 8.48 (s, 1H), 9.20 (d, *J* = 8.6 Hz, 1H), 9.65 (s, 1H); Purity (UPLC): 98.66%; ESI-MS: Exact mass = 461.2097, m/z $[M+H]^+$ peak at 462.2; Analysis (CHNS): Calculated for $C_{22}H_{31}N_5O_4S$: C, 57.25%; H, 6.77%; N, 15.17%; S, 6.95% ; Found: C, 57.14%; H, 6.58%; N, 15.09%; S, 6.82%.

4.1.5.7. (*R*)-4-((4-((3-methylbutan-2-yl)amino)-5-(morpholine-4-carbonyl)pyrimidin-2-yl)amino) phenylmethane sulfonate (54g):



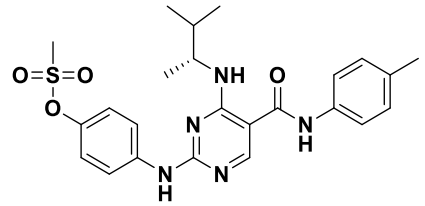
MP: 246-247°C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 0.88 – 0.95 (m, 6H), 1.12 (d, *J* = 6.4 Hz, 3H), 1.85 – 1.90 (s, 1H), 3.33 (s, 3H), 3.52 – 3.57 (m, 4H), 3.60 – 3.62 (m, 4H), 4.05 – 4.10 (m, 1H), 7.11 (d, *J* = 8.4 Hz, 1H), 7.23 -7.25 (m, 2H), 7.82 – 7.85 (m, 2H), 7.98 (s, 1H), 9.55 (s, 1H); Purity (HPLC): 97.57%; ESI-MS: Exact mass = 463.1889, m/z $[M]^+$ peak at 462.9; Analysis (CHNS): Calculated for $C_{21}H_{29}N_5O_5S$: C, 54.41%; H, 6.31%; N, 15.11%; S, 6.92% ; Found: C, 54.20%; H, 6.14%; N, 15.09%; S, 6.82%.

4.1.5.8. (*R*)-4-((4-((3-methylbutan-2-yl) amino)-5-(phenyl carba moyl) pyrimidin-2-yl)amino)phenyl methane sulfonate (54h):



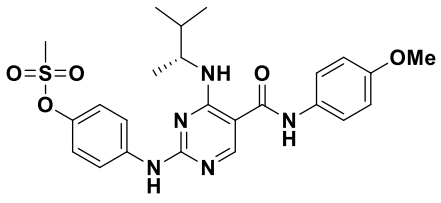
MP: 258-259°C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 0.88 (d, *J* = 6.8 Hz, 3H), 0.92 (d, *J* = 4.6 Hz, 3H), 1.15 (d, *J* = 6.8 Hz, 3H), 1.84 – 1.88 (m, 1H), 3.34 (s, 3H), 4.15 – 4.18 (m, 1H), 7.30 – 7.32 (m, 2H), 7.40 – 7.45 (m, 5H), 7.86 (d, *J* = 6.8 Hz, 2H), 8.51 (s, 1H), 9.29 (d, *J* = 8.4 Hz, 1H), 9.69 (s, 1H), 9.95 (s, 1H); Purity (HPLC): 96.92%; ESI-MS: Exact mass = 469.5768, m/z $[M+H]^+$ peak at 470.1.; Analysis (CHNS): Calculated for $C_{23}H_{27}N_5O_4S$: C, 58.83%; H, 5.80%; N, 14.92%; S, 6.83% ; Found: C, 58.63%; H, 5.71%; N, 14.86%; S, 6.74%.

4.1.5.9. (*R*)-4-((4-((3-methylbutan-2-yl) amino)-5-(4-methylphenyl carbamoyl) pyrimidin-2-yl)amino)phenyl methane sulfonate (54i):



MP: 264-265°C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 0.84 - 0.94 (m, 6H), 1.19 (d, *J* = 6.6 Hz, 3H), 1.80 – 1.84 (m, 1H), 2.50 (s, 3H), 3.33 (s, 3H), 4.12 – 4.17 (m, 1H), 7.34 (d, *J* = 9.2 Hz, 2H), 7.40 (d, *J* = 8.6 Hz, 2H), 7.60 (d, *J* = 8.8 Hz, 2H), 7.86 (d, *J* = 9.2 Hz, 2H), 8.50 (s, 1H), 9.30 (d, *J* = 8.6 Hz, 1H), 9.80 (s, 1H), 10.2 (s, 1H); Purity (UPLC): 98.80%; ESI-MS: Exact mass = 483.1940, m/z $[M]^+$ peak at 483.5; Analysis (CHNS): Calculated for $C_{24}H_{29}N_5O_4S$: C, 59.61%; H, 6.04%; N, 14.48%; S, 6.63% ; Found: C, 59.50%; H, 6.12%; N, 14.64%; S, 6.77%.

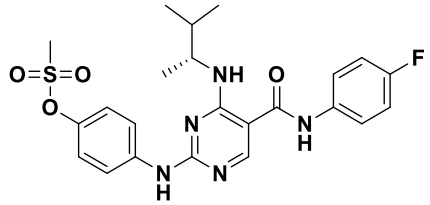
4.1.5.10. (*R*)-4-((4-((3-methylbutan-2-yl) amino)-5-(4-methoxyphenyl carbamoyl) pyrimidin-2-yl)amino)phenyl methane sulfonate (54j):



MP: 270-271°C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 0.82 - 0.90 (m, 6H), 1.20 (d, *J* = 6.4 Hz, 3H), 1.89 – 1.20 (m, 1H), 3.33 (s, 3H), 3.82

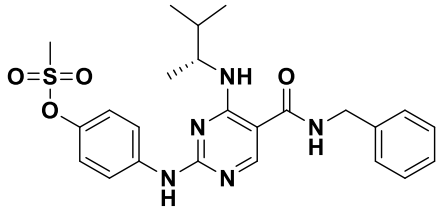
(s, 3H), 4.15 – 4.20 (m, 1H), 7.35 - 7.42 (m, 4H), 7.65 (d, *J* = 8.6 Hz, 2H), 7.88 (d, *J* = 9.2 Hz, 2H), 8.52 (s, 1H), 9.12 (d, *J* = 8.6 Hz, 1H), 9.87 (s, 1H), 10.68 (s, 1H); Purity (HPLC): 97.40%; ESI-MS: Exact mass = 499.1889, m/z $[M]^+$ peak at 499.61; Analysis (CHNS): Calculated for $C_{24}H_{29}N_5O_5S$: C, 57.70%; H, 5.85%; N, 14.02%; S, 6.42% ; Found: C, 57.56%; H, 5.70%; N, 14.00%; S, 6.35%.

4.1.5.11. (*R*)-4-((4-((3-methylbutan-2-yl) amino)-5-(4-fluorophenyl carbamoyl) pyrimidin-2-yl)amino)phenyl methane sulfonate (54k):



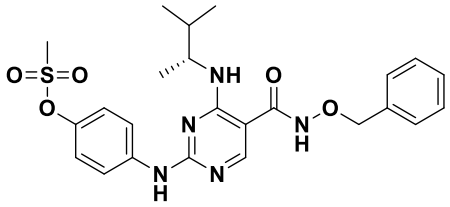
MP: 275-276°C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 0.84 (d, *J* = 6.8 Hz, 3H), 0.98 (d, *J* = 6.8 Hz, 3H), 1.20 (d, *J* = 6.8 Hz, 3H), 1.89 – 1.90 (m, 1H), 3.34 (s, 3H), 4.20 – 4.22 (m, 1H), 7.30 (d, *J* = 9.4 Hz, 2H), 7.45 (d, *J* = 8.8 Hz, 2H), 7.70 (d, *J* = 8.6 Hz, 2H), 7.89 (d, *J* = 9.2 Hz, 2H), 8.45 (s, 1H), 9.24 (d, *J* = 8.4 Hz, 1H), 9.91 (s, 1H), 10.40 (s, 1H); Purity (UPLC): 98.45%; ESI-MS: Exact mass = 487.1690, m/z $[M]^+$ peak at 487.3; Analysis (CHNS): Calculated for $C_{23}H_{26}N_5O_4SF$: C, 56.66%; H, 5.38%; N, 14.36%; S, 6.58% ; Found: C, 56.75%; H, 5.55%; N, 14.32%; S, 6.68%.

4.1.5.12. (*R*)-4-((4-((3-methylbutan-2-yl) amino)-5-(benzyl carbamoyl) pyrimidin-2-yl)amino)phenyl methane sulfonate (54l):



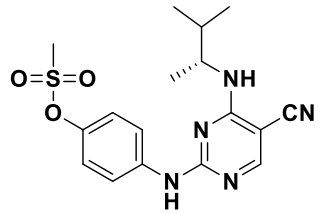
MP: 270-271°C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 0.90 – 0.94 (m, 6H), 1.18 (d, *J* = 6.4 Hz, 3H), 1.82 – 1.86 (m, 1H), 3.33 (s, 3H), 4.10 – 4.12 (m, 1H), 4.56 (s, 2H), 7.25 – 7.27 (m, 2H), 7.38 – 7.43 (m, 5H), 7.80 (d, *J* = 6.4 Hz, 2H), 8.40 (s, 1H), 9.45 (m, 1H), 9.68 (s, 1H), 10.42 (s, 1H); Purity (UPLC): 98.45%; ESI-MS: Exact mass = 483.1940, m/z $[M]^+$ peak at 483.4; Analysis (CHNS): Calculated for $C_{24}H_{29}N_5O_4S$: C, 56.66%; H, 5.38%; N, 14.36%; S, 6.58% ; Found: C, 56.75%; H, 5.55%; N, 14.32%; S, 6.68%.

4.1.5.13. (*R*)-4-((4-((3-methylbutan-2-yl) amino)-5-(benzyloxy carbamoyl) pyrimidin-2-yl)amino)phenyl methane sulfonate (54m):



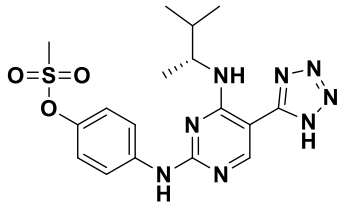
MP: 278-279°C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 0.89 (d, *J* = 6.4 Hz, 3H), 0.95 (d, *J* = 6.8 Hz, 3H), 1.14 (d, *J* = 6.8 Hz, 3H), 1.85 – 1.87 (m, 1H), 3.34 (s, 3H), 4.06 – 4.08 (m, 1H), 4.88 (s, 2H), 7.26 (d, *J* = 9.2 Hz, 2H), 7.36 – 7.45 (m, 5H), 7.84 (d, *J* = 6.8 Hz, 2H), 8.31 (s, 1H), 8.68 (d, *J* = 8.4 Hz, 1H), 9.71 (s, 1H), 11.55 (s, 1H); Purity (UPLC): 98.45%; ESI-MS: Exact mass = 499.1889, m/z $[M+H]^+$ peak at 500.1; Analysis (CHNS): Calculated for $C_{24}H_{29}N_5O_5S$: C, 57.70%; H, 5.85%; N, 14.02%; S, 6.42% ; Found: C, 57.62%; H, 5.70%; N, 13.98%; S, 6.34%.

4.1.5.14. (*R*)-4-((5-cyano-4-((3-methylbutan-2-yl) amino) pyrimidin-2-yl)amino)phenyl methanesulfonate (55):



MP: 264-265°C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 0.90 (d, *J* = 6.6 Hz, 3H), 1.12 (d, *J* = 6.4 Hz, 3H), 1.28 (d, *J* = 6.8 Hz, 3H), 1.94 – 1.98 (m, 1H), 3.32 (s, 3H), 4.10 – 4.12 (m, 1H), 7.30 (d, *J* = 9.2 Hz, 2H), 7.89 (d, *J* = 9.2 Hz, 2H), 8.70 (s, 1H), 9.30 (d, *J* = 8.6 Hz, 1H), 9.90 (s, 1H); Purity (HPLC): 97.99%; ESI-MS: Exact mass = 375.1365, m/z $[M]^+$ peak at 375.8; Analysis (CHNS): Calculated for $C_{17}H_{21}N_5O_3S$: C, 54.39%; H, 5.64%; N, 18.65%; S, 8.54% ; Found: C, 54.48%; H, 5.75%; N, 18.78%; S, 8.62%.

4.1.5.16. (*R*)-4-((5-(1*H*-tetrazol-5-yl)-4-((3-methylbutan-2-yl) amino) pyrimidin-2-yl)amino)phenyl methanesulfonate (56):



MP: 264-265°C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 0.94 (d, *J* = 6.4 Hz, 3H), 1.00 (d, *J* = 6.8 Hz, 3H), 1.22 (d, *J* = 6.4 Hz, 3H), 1.95 – 1.99 (m, 1H), 3.32 (s, 3H), 4.31 – 4.33 (m, 1H), 7.28 (d, *J* = 9.2 Hz, 2H), 7.88 (d, *J* = 9.2 Hz, 2H), 8.58 - 8.60 (m, 1H), 8.65 (s, 1H), 9.73 (s, 1H); Purity (UPLC): 98.21%; ESI-MS: Exact mass = 418.1536, m/z

$[M+H]^+$ peak at 419.1; Analysis (CHNS): Calculated for $C_{17}H_{22}N_8O_3S$: C, 48.79%; H, 5.30%; N, 26.78%; S, 7.66%; Found: C, 48.88%; H, 5.43%; N, 26.92%; S, 7.74%.

From Series-3, all the 14 compounds (**54a-m**, **55** and **56**) were subjected for *in-vitro* JAK3 inhibitory activity screening. Detailed assay protocol and JAK3 inhibitory activity data described in the next section.

4.2. *In-vitro* JAK3 Inhibitory activity data of pyrimidine derivatives modified at 5th position

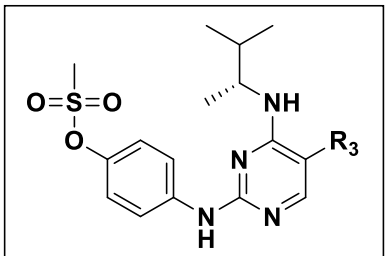
Determination of JAK3 assay

In-vitro screening of compounds **54a-m**, **55** and **56** were carried out, using a fluorogenic substrate assay.

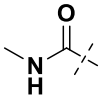
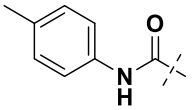
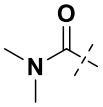
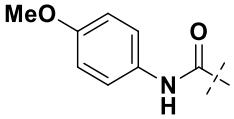
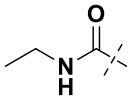
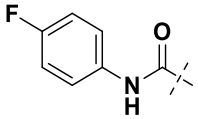
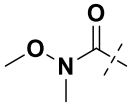
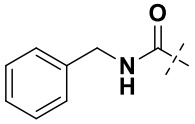
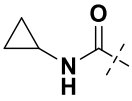
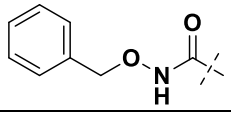
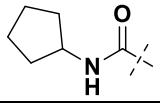
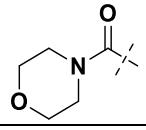
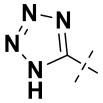
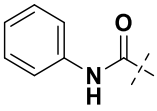
Human JAK1, JAK2, and JAK3 kinase domains were purchased from Carna Biosciences, Inc. (Kobe, Japan), and the assay was performed, using a streptavidin-coated 96-well plate. The reaction mixture contained 15 mM Tris-HCl (pH 7.5), 0.01% Tween 20, 2 mM DTT, 10 mM $MgCl_2$, 250 nM Biotin-Lyn-Substrate-2 (Peptide Institute, Inc., Osaka, Japan) and ATP. The final concentrations of ATP was 8 μM for JAK3 enzyme. The test compounds were dissolved in DMSO and the reaction was initiated by adding the kinase domain, followed by incubation at room temperature for 1 hr. Kinase activity was measured as the rate of phosphorylation of BiotinLyn-Substrate-2, using HRP-conjugated anti-phosphotyrosine antibody (HRP-PY-20; Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) with a phosphotyrosine-specific ELISA. All experiments were performed in duplicate. The IC_{50} value of all compounds was calculated,

using linear regression analysis. **[92]** The IC_{50} values are mentioned below in **Table 5**.

Table 5. *Effect of substituent at 5th position of Pyrimidine moiety on JAK3 inhibitory activity (In-vitro).*



54a-m, 55, 56

Comp.	R_3	JAK-3 IC_{50} (nM)[a]	Comp.	R_3	JAK-3 IC_{50} (nM)[a]
54a		39	**54i**		140
54b		65	**54j**		170
54c		49	**54k**		102
54d		70	**54l**		258
54e		90	**54m**		300
54f		110	**55**		250
54g		81	**56**		189
54h		108	**Cerdulatinib**		8
			Tofacitinib		1.6

[a]All the data are shown as the mean for at least two experiments. [a]JAK3 inhibition (IC_{50}) determination, using *in-vitro* Fluorogenic substrate assays Kit from Millipore.

Results and Discussion

In the third Series (**Table 5**), mainly modifications were carried out at the C5-position of pyrimidine in compound **48l**. As listed in the **Table 5** compounds **54a-m**, **55** and **56** were prepared.

Compound **54a** *N*-methylamide (methylated **48l**, IC_{50}: 39 nM) displayed moderate JAK3 inhibitory activity. Compound **54b** (*N*, *N*-dimethylamide, IC_{50}: 65 nM) showed moderate potency. Whereas compound **54c** (*N*-ethylamide, IC_{50}: 49 nM) displayed better potency than compound **54b**. Compound **54d** (*N*-methyl,*N*-methoxyamide, IC_{50}: 70 nM) was found to be less active among all the alkyl substituted aminocarbonyl compounds.

Introduction of cyclo alkyl ring, compound **54e** (*N*-Cyclopropylamide, IC_{50}: 90 nM) leads to a less potent compound. Compound **54f** (*N*-cyclopentylamide, IC_{50}: 110 nM) showed poor JAK3 inhibitory activity. However, compound **54g** morpholine (both the hydrogen atoms of carbamoyl group replaced by a cyclic system, IC_{50}: 81 nM) showed less potency.

Replacement of a cyclo alkyl ring with a bulky aromatic system, compound **54h** (*N*-phenylamide, IC_{50}: 108 nM) showed less activity. *para*-Substituted compound **54i** (*N*-*p*-tolylamide, IC_{50}: 140 nM) displayed poor JAK3 inhibitory activity. Whereas compound **54j** (*N*-*p*-methoxyphenylamide, IC_{50}: 170 nM) showed ten-fold less potency than compound **48l**. In contrast, compound **54k** (*N*-*p*-fluorophenylamide, IC_{50}: 102 nM) displayed better potency than compound **54j**.

Introduction of methyl linker, compond **54l** (*N*-benzylamide, IC_{50}: 258 nM) showed complete loss of activity. Compound **54m** (*N*-benzyloxyamide, IC_{50}: 300 nM) displayed weakest JAK3 inhibitory activity in this Series-3.

Replacement of aminocarbonyl group by cyano group, compound **55** (IC_{50}: 250 nM) showed poor activity. Introduction of a tetrazole group at the C5 position, compound **56** (IC_{50}: 189 nM) leads to an inactive compound.

4.3. Conclusion

Substitution of the aminocarbonyl group by alkyl substituent leads to moderate potency. Introduction of electronegative methoxy group also leads to moderate activity. The cyclo alkyl substituent displayed less activity may be due to ring size expansion. Bulky, electron donating and withdrawing group both lead to inactive compounds. Non polar cyno and polar tetrazole substituent also displayed loss of activity. Thus, modifications at the 5^{th} position of compound **48l** overall showed no improvement in the JAK3 inhibitory activity. Thus, aminocarbonyl group substituent at the 5^{th} position appears to be essential for JAK3 inhibitory activity.