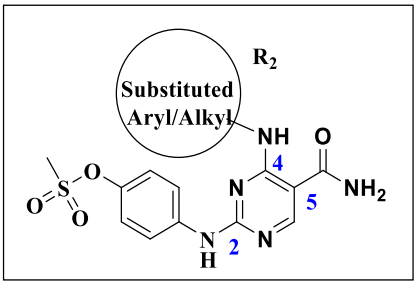
3. Design and synthesis of compounds modified at 4th position of Diamino pyrimidine



As described in the **Chapter-2**, modification at 2nd the position of cerdulatinib (C4 cyclopropyl of Cerdulatinib altered with benzyl group) led to the single digit nM potent compound **41k** (IC_{50}: 9.5 nM). Therefor, in the second Series changes were carried out at C4 position of the **41k**. **Chapter-3** is divided in two parts. In part 1, synthesis of pyrimidine derivatives with modifications at 4th position were described as shown in the **Scheme-10**. Total 20 compounds were prepared in this Series as **48a-v**. All the compounds were characterized, using various spectroscopic techniques like ^{1}H, ^{13}C NMR, ESI-MS and UPLC / HPLC. In part 2, *in-vitro* JAK inhibitory activity data of compounds **48a-v** was described.

3.1. Chemistry

3.1.1. 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).

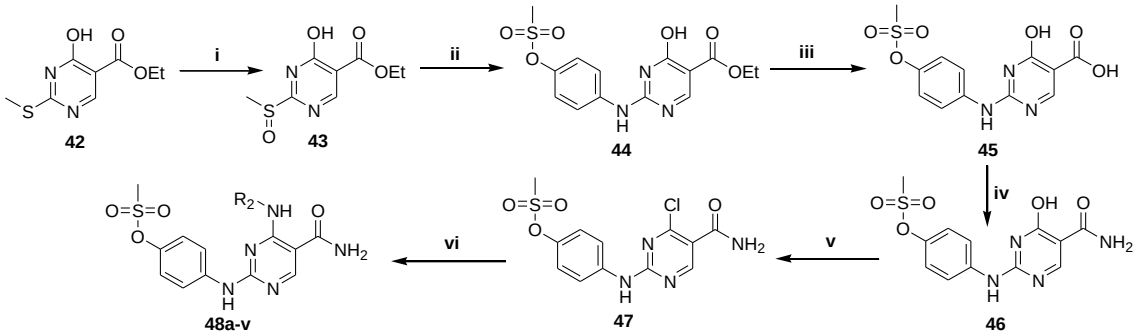
Progress of the reactions was monitor by TLC, using precoated TLC plates (E. Merck Kiesegel 60 F254, Germany) and the spots were visualized by UV and/or iodine vapors. The chromatographic purification was performed on silica gel (230-400 mesh). Few compounds directly used for the next step without purification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

3.1.2. General procedure for the synthesis of compounds 48a-v

As depicted in **Scheme-10**, for the synthesis of **48a-v**, starting material substituted ethyl 4-hydroxy pyrimidine-5-carboxylate (**42**) was first converted to reactive methyl sulfinyl derivate (**43**), using *m*-CPBA followed by treatment with (4-aminophenyl)methanesulfonate

gave intermediate ethyl 4-hydroxy-2-((4-((methylsulfonyl)oxy) phenyl)amino) pyrimidine -5-carboxylate **44**, which was hydrolysed to corresponding acid (**45**), using $LiOH.H_2O$ as a base. Acid functionality of **45** converted to corresponding amide (**46**), using aq. ammonia. The phenolic hydroxyl group of **46** was transformed into a reactive chloro group (**47**), using $POCl_3$, followed by reaction with substituted amines, furnished compounds **48a-v**. **[91]**

Scheme-10



Reagents and conditions: (i) Dioxane:$CHCl_3$ (1:1), m-CPBA, 20 mins, 10% sodium metabisulfate; (ii) NMP, PTSA, (4-aminophenyl)methanesulfonate, 120 ^oC, 1hr, 75%; (iii) THF, LiOH, water, 30 ^oC, 6hr; (iv) EDC.HCl, HOBt, aq NH_3, DMF, 30 ^oC, 2hr; (v) $POCl_3$, DIPEA, Toluene. 110 ^oC, 2hr; (vi) R_2-NH_2, DIPEA, Dioxane, 30 ^oC, 6hr

Stepwise experimental procedure for the synthesis of compounds **43** to **47** and **48a-v**.

Procedure for steps I and II : Preparation of ethyl 4-hydroxy-2-((4-((methylsulfonyl)oxy) phenyl)amino) pyrimidine -5-carboxylate (**44**)

Step I: Ethyl 4-hydroxy-2-(methylthio)pyrimidine-5-carboxylate **42** (10 g, 46.7 mmol) was dissolved in dioxane and $CHCl_3$ (1:1) 260 mL. The reaction mixture was cooled up to -2°C and treated with *m*-CPBA 60% (20.14 g, 70.0 mmol) and it was stirred for 20 mins at 0°C. After completion of the reaction, the 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 to obtain ethyl 4-hydroxy-2-(methyl sulfinyl)pyrimidine-5-carboxylate, as a white solid. (Yield: 8.6 g, 80 %), which was further used in the next step without purification.

Step II: Ethyl 4-hydroxy-2-(methylsulfinyl)pyrimidine-5-carboxylate **43** (8.6 g, 37.4 mmol) and PTSA (7.82 g, 41.1 mmol), in NMP (80 mL) was added (4-aminophenyl)methanesulfonate (6.99 g, 37.4 mmol), at RT. The reaction mixture was heated at 100-110°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 afford the crude product. Crude product was purified by flash chromatography over silica gel (230-400 mesh) with 2% MeOH/$CHCl_3$ to get the ethyl 4-hydroxy-2-((4-((methylsulfonyl) oxy)phenyl)amino) pyrimidine-5-carboxylate **44** (Yiald: 9.9 g, 75%, Purity by UPLC: 96.60%).

^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 1.26 (t, J = 7.0 Hz, 3H), 3.48 (s, 3H), 4.20 (q, J_1 = 6.8 Hz, J_2 = 7.2 Hz, 2H), 6.57 (d, J = 9.2 Hz, 2H), 7.34 (d, J = 8.8 Hz, 2H), 8.49 (s, 1H), 9.58 (s, 1H), 11.25 (s, 1H); ESI-MS: Exact mass = 353.0682, m/z $[M]^+$ peak at 353.9.

Step III: Preparation of 4-hydroxy-2-((4-((methylsulfonyl)oxy) phenyl)amino)pyrimidine-5-carboxylic acid (**45**)

Ethyl 4-hydroxy-2-((4-((methylsulfonyl)oxy)phenyl)amino)pyrimidine-

5-carboxylate **44** (9.0 g, 25.5 mmol) was dissolved in THF (90 mL). $LiOH.H_2O$ 3.05 g (127 mmol) was dissolved in 5 mL water and added to the reaction and stirred for 6 hr at 30°C. After completion of reaction, the reaction mixture was concentrated by rotary evaporation to remove excess THF. The mixture was acidified with dilute HCl (pH ~ 5), to get white precipitate. The solid compound filtered, washed with water and dried under vacuum to afford 4-hydroxy-2-((4-((methylsulfonyl)oxy)phenyl)amino)pyrimidine-5-carboxylic acid **45**, as a white solid. (Yield: 6.63 g, 80%, Purity by UPLC: 97.89%).

^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 3.38 (s, 3H), 7.37 (d, J = 8.8 Hz, 2H), 7.67 (d, J = 8.8 Hz, 2H), 8.51 (s, 1H), 9.85 (s, 1H); ESI-MS: Exact mass = 325.0369, m/z $[M]^+$ peak at 325.9.

Step IV: Preparation of 4-((5-carbamoyl-4-hydroxypyrimidin-2-yl)amino)phenyl methanesulfonate (**46**)

4-Hydroxy-2-((4-((methylsulfonyl)oxy)phenyl)amino)pyrimidine-5-carboxylic acid **45** (6.0 g, 18.44 mmol) was dissolved in DMF (50 mL) under Nitrogen (inert) atmosphere and treated with HOBt (2.82 g, 18.44 mmol), EDC.HCl (7.07 g, 36.9 mmol), at RT. After stirring for 1 hr aq. ammonia (20 mL) was added at 0°C, and it was stirred for a further 1 hr, at RT. The reaction mixture was quenched by ice - water to get white precipitate. The solid compound was isolated by filtration, washed with water and dried under reduced pressure to afford 4-((5-carbamoyl-4-hydroxypyrimidin-2-yl)amino)phenyl methanesulfonate **46** (Yield: 4.19 g, 70%, Purity by UPLC: 83%).

^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 3.33 (s, 3H), 7.21 (d, J = 8.8 Hz,

2H), 7.87 (d, *J* = 8.8 Hz, 2H), 8.37 (s, 1H), 9.33 (s, 1H); ESI-MS: Exact mass = 324.0528, m/z $[M-H]^+$ peak at 323.8.

Step V: Preparation of 4-((5-carbamoyl-4-chloropyrimidin-2-yl)amino)phenyl methanesulfonate (**47**)

To a solution of 4-((5-carbamoyl-4-hydroxypyrimidin-2-yl)amino) phenyl methanesulfonate **46** (4.0 g, 12.33 mmol), in toluene (50 mL) was added DIPEA (2.6 mL, 14.8 mmol) and phosphorus oxychloride ($POCl_3$; 2.3 mL, 24.67 mmol) drop wise, at 0-5°C. The reaction mixture was refluxed for 2 hr. After completion of reaction, the reaction mixture was cooled to RT, quenched by ice-water to get white precipitate. The precipitated compound was filtered and washed with cold water to get 4-((5-carbamoyl-4-chloropyrimidin-2-yl)amino) phenyl methane sulfonate **47** (Yield: 2.96 g, 70%, Purity by UPLC: 80%).

^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 3.37 (s, 3H), 7.33 – 7.38 (m, 2H), 7.78 – 7.81 (m, 2H), 8.92 (s, 1H), 10.70 (s, 1H); ESI-MS: Exact mass = 342.0190, m/z $[M+H]^+$ peak at 343.8.

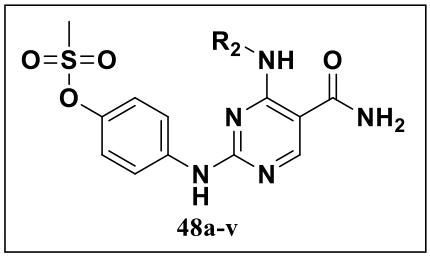
Step VI: General procedure for the synthesis of compound **48a-v**:

4-((5-carbamoyl-4-chloropyrimidin-2-yl)amino)phenyl methane sulfonate **47** (1.0 eq) was dissolved in dioxane and DIPEA (1.1 eq) was added to the reaction mixture at 0°C followed by the addition of different amines (1.0 eq, structure of various amine listed in section 3.1.3.). 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 in EtOAc. The organic layer

was dried over Na_2SO_4, filtered and concentrated under vacuum to afford the crude product. Crude product was purified by flash chromatography over silica gel (230-400 mesh) with 2% MeOH/$CHCl_3$ to provide the desired title product (**48a-v**).

3.1.3. List of R_2 substituents at 4th position of pyrimidine derivatives

Substituent of compounds **48a-v** are listed below.



48a-v

Compound	R_2
48a	H
48b	
48c	
48d	
48e	
48f	

Compound	R_2
48g	
48h	F_3C
48i	
48j	
48k	Racemic

Compound	R_2
48l	R-isomer
48m	S-isomer
48n	NC, R-isomer
48o	HO, R-isomer
48p	H_2N, O, R-isomer

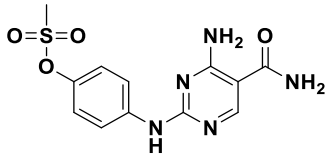
Compound	R_2
48q	
48r	F
48s	F
48t	F
48u	MeO
48v	

Following above 6 steps synthetic procedure, total 20 compounds **48a-v** 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 **48a-v** are listed below.

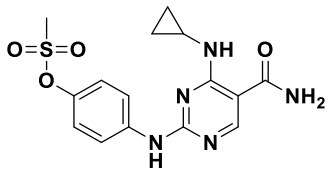
3.1.4. Spectral Data of compounds 48a-v:

3.1.4.1. 4-((4-Amino-5-carbamoylpyrimidin-2-yl)amino)phenyl methanesulfonate (48a):



MP: 150-151°C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 3.33 (s, 3H), 7.22 (dd, *J* = 2.0 Hz, 2H), 7.89 (d, *J* = 9.2 Hz, 2H), 8.54 (s, 1H), 9.58 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ ppm: 37.4, 100.1, 120.8, 122.3, 140.2, 142.9, 155.1, 158.2, 160.5, 161.8, 169.3 (carbonyl carbon); Purity (UPLC): 98.35%; ESI-MS: Exact mass = 323.0688, m/z $[M]^+$ peak at 323.8; Analysis (CHNS): Calculated for $C_{12}H_{13}N_5O_4S$: C, 44.58%; H, 4.05%; N, 21.66%; S, 9.92% Found: C, 45.08%; H, 4.44%; N, 21.70%; S, 9.98%.

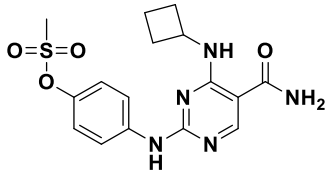
3.1.4.2. 4-((5-Carbamoyl-4-(cyclopropylamino)pyrimidin-2-yl) amino)phenyl methanesulfonate (48b):



MP: 158-159°C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 0.54 (t, *J* = 3.0 Hz, 2H), 0.84 (t, *J* = 3.4 Hz, 2H), 2.66 - 2.67 (m, 1H), 3.32 (s, 3H), 7.26 (d, *J* = 9.2 Hz, 2H), 8.02 (d, *J* = 8.8 Hz, 2H), 8.53 (s, 1H), 9.22

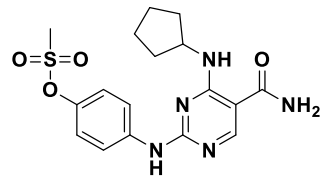
(s, 1H), 9.75 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ ppm 7.5, 25.0, 37.1, 100.5, 121.0, 122.5, 140.5, 144.0, 158.2, 160.4, 161.8, 169.4 (carbonyl carbon); Purity (HPLC): 95.26%; ESI-MS: Exact mass = 363.1001, m/z $[M+H]^+$ peak at 364.0; Analysis (CHNS): Calculated for $C_{15}H_{17}N_5O_4S$: C, 49.58%; H, 4.72%; N, 19.27%; S, 8.82% Found: C, 49.85%; H, 4.66%; N, 19.40%; S, 8.90%.

3.1.4.3. 4-((5-Carbamoyl-4-(cyclobutylamino)pyrimidin-2-yl) amino)phenyl methanesulfonate (48c):



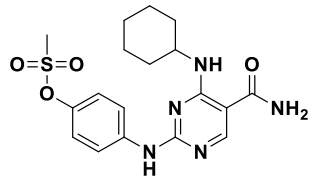
MP: 162-163°C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 1.25 (m, 2H), 1.78 (m, 2H), 2.37 (m, 2H), 3.33 (s, 3H), 4.51- 4.55 (m, 1H), 7.28 (d, *J* = 9.2 Hz, 2H), 7.87 (d, *J* = 9.2 Hz, 2H), 8.53 (s, 1H), 9.30 (d, *J* = 7.2 Hz, 1H), 9.67 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ ppm: 12.5, 30.2, 37.3, 51.2, 100.8, 121.5, 122.3, 140.1, 144.5, 158.8, 160.9, 161.7, 169.3 (carbonyl carbon); Purity (UPLC): 98.55%; ESI-MS: Exact mass = 377.1158, m/z $[M]^+$ peak at 377.5; Analysis (CHNS): Calculated for $C_{16}H_{19}N_5O_4S$: C, 50.92%; H, 5.07%; N, 18.56%; S, 8.49% Found: C, 50.85%; H, 4.98%; N, 18.30%; S, 8.35%.

3.1.4.4. 4-((5-Carbamoyl-4-(cyclopentylamino)pyrimidin-2yl) amino)phenylmethanesulfonate (48d):



MP: 162-163°C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 1.46 - 149 (m, 2H), 1.50 - 173 (m, 4H), 1.98 - 2.06 (m, 2H), 3.32 (s, 3H), 4.30 - 4.38 (m, 1H), 7.25 (d, *J* = 9.2 Hz, 2H), 7.88 (d, *J* = 9.2 Hz, 2H), 8.52 (s, 1H), 9.20 (d, *J* = 7.2 Hz, 1H), 9.65 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ ppm: 23.8, 33.2, 37.5, 52.0, 100.2, 120.4, 122.6, 140.1, 143.5, 157.5, 160.2, 161.4, 169.5 (carbonyl carbon); Purity (UPLC): 98.51%; ESI-MS: Exact mass = 391.1314, m/z $[M+H]^+$ peak at 392.0; Analysis (CHNS): Calculated for $C_{17}H_{21}N_5O_4S$: C, 52.16%; H, 5.41%; N, 17.89%; S, 8.19% Found: C, 52.21%; H, 5.50%; N, 17.93%; S, 8.25%.

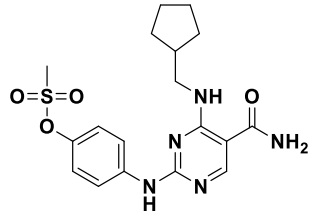
3.1.4.5. 4-((5-Carbamoyl-4-(cyclohexylamino)pyrimidin-2-yl) amino)phenylmethanesulfonate (48e):



MP: 170-171°C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 1.24 - 1.31 (m, 3H), 1.35 - 1.44 (m, 2H), 1.62 - 1.59 (m, 1H), 1.70 - 1.73 (m, 2H), 1.95 - 1.98 (m, 2H), 3.33 (s, 3H), 3.93 - 3.94 (m, 1H), 7.22 – 7.26 (m, 2H), 7.85 -7.88 (m, 2H), 8.52 (s, 1H), 9.17 (d, *J* = 7.2 Hz, 1H), 9.66 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ ppm: 24.8, 25.7, 32.6, 37.5, 49.0, 100.0, 120.3, 122.6, 140.1, 143.5, 157.7, 160.3, 161.0, 169.6 (carbonyl carbon); Purity (UPLC): 98.22%; ESI-MS: Exact mass = 405.1471, m/z $[M+H]^+$ peak at 406.0; Analysis (CHNS): Calculated for $C_{18}H_{23}N_5O_4S$: C, 53.32%; H, 5.72%; N, 17.27%; S, 8.25% Found: C, 53.41%; H, 5.80%; N, 17.33%; S, 8.30%.

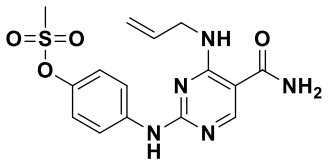
3.1.4.6. 4-((5-Carbamoyl-4-((cyclopentylmethyl)amino)pyri

midin-2-yl)amino)phenyl methanesulfonate (48f):



MP: 169-170°C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 1.18 - 1.22 (m, 2H), 1.53 - 155 (m, 2H), 1.56 - 160 (m, 2H), 1.62 - 1.75 (m, 2H), 1.98 - 2.00 (m, 1H), 3.51 (s, 5H), 7.28 (d, *J* = 8.8 Hz, 2H), 7.85 (d, *J* = 8.0 Hz, 2H), 8.51 (s, 1H), 9.40 (s, 1H), 9.78 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ ppm: 25.2, 30.3, 37.5, 45.3, 100.5, 120.9, 122.8, 139.4, 143.9, 158.9, 159.4, 161.8, 169.1 (carbonyl carbon); Purity (UPLC): 96.55%; ESI-MS: Exact mass = 405.1471, m/z $[M+H]^+$ peak at 406.3; Analysis (CHNS): Calculated for $C_{18}H_{23}N_5O_4S$: C, 53.32%; H, 5.72%; N, 17.27%; S, 7.91% Found: C, 53.20%; H, 5.65%; N, 17.21%; S, 7.75%.

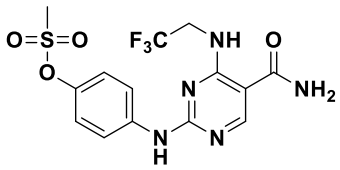
3.1.4.7. 4-((4-(allylamino)-5-carbamoylpyrimidin-2-yl)amino) phenyl methanesulfonate (48g):



MP: 154-155°C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 3.30 (s, 3H), 4.11 (t, *J* = 5.2 Hz, 2H), 5.11 – 5.14 (m, 1H), 5.18 – 5.23 (m, 1H), 5.95 – 6.02 (m, 1H), 7.25 (d, *J* = 9.2 Hz, 2H), 7.85 (d, *J* = 8.8 Hz, 2H), 8.55 (s, 1H), 9.28 (d, *J* = 5.6 Hz, 1H), 9.68 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ ppm: 20.9, 37.7, 49.1, 102.2, 111.0 121.2, 122.7, 131.1, 143.2, 161.5, 167.5, 169.9 (carbonyl carbon); Purity

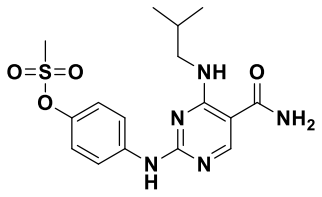
(UPLC): 97.20%; ESI-MS: Exact mass = 363.1001, m/z $[M+H]^+$ peak at 364.0; Analysis (CHNS): Calculated for $C_{15}H_{17}N_5O_4S$: C, 49.58%; H, 4.72%; N, 19.27%; S, 8.82% Found: C, 49.81%; H, 4.82%; N, 19.34%; S, 8.92%.

3.1.4.8. 4-((5-carbamoyl-4-((2,2,2-trifluoroethyl)amino)pyrimidin-2-yl)amino)phenyl methanesulfonate (48h):



MP: 161-162°C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 3.33 (s, 3H), 4.35 – 4.39 (m, 2H), 7.25 – 7.27 (m, 2H), 7.79 – 7.82 (m, 2H), 8.63 (s, 1H), 9.53 (t, J = 8.0 Hz, 1H), 9.82 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ ppm: 37.6, 61.0, 102.4, 121.4, 122.3, 128.5, 131.4, 145.1, 162.5, 167.4, 169.7 (carbonyl carbon); Purity (UPLC): 98.89%; ESI-MS: Exact mass = 405.0719, m/z $[M-H]^+$ peak at 403.9; Analysis (CHNS): Calculated for $C_{14}H_{14}N_5O_4SF_3$: C, 41.48%; H, 3.48%; N, 17.28%; S, 7.91% Found: C, 41.42%; H, 3.44%; N, 17.30%; S, 7.88%.

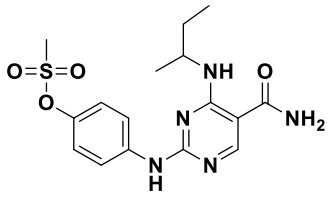
3.1.4.9. 4-((5-Carbamoyl-4-(isobutylamino)pyrimidin-2-yl) amino) phenylmethanesulfonate (48i):



MP: 170-171°C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 0. 93 (d, J = 6.8 Hz, 6H), 3.16 (m, 2H), 3.33 (s, 3H), 4.10 - 4.15 (m, 1H), 7.25 (d, J =

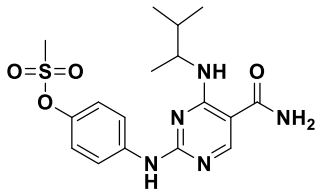
9.2 Hz, 2H), 7.87 (d, *J* = 9.2 Hz, 2H), 8.53 (s, 1H), 9.38 (s, 1H), 9.66 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ ppm: 21.3, 29.9, 37.4, 49.2, 100.1, 121.2, 122.0, 139.6, 144.0, 159.0, 159.9, 161.9, 169.4 (carbonyl carbon); Purity (HPLC): 98.89%; ESI-MS: Exact mass = 379.1314, m/z $[M]^+$ peak at 379.6; Analysis (CHNS): Calculated for $C_{16}H_{21}N_5O_4S$: C, 50.65%; H, 5.58%; N, 18.46%; S, 8.45% Found: C, 50.55%; H, 5.42%; N, 18.34%; S, 8.36%.

3.1.4.10. 4-((4-(sec-butylamino)-5-carbamoylpyrimidin-2-yl) amino) phenyl methanesulfonate (48j):



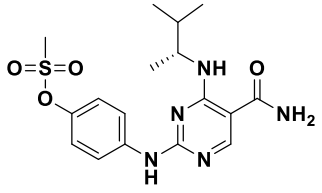
MP: 175-176°C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 0.91 (t, *J* = 7.6 Hz, 3H), 1.19 (d, *J* = 6.4 Hz, 3H), 1.51 – 1.60 (m, 2H), 3.32 (s, 3H), 4.06 - 4.10 (m, 1H), 7.24 – 7.27 (m, 2H), 7.84 -7.87 (m, 2H), 8.52 (s, 1H), 9.12 (d, *J* = 8.0 Hz, 1H), 9.64 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ ppm: 10.2, 20.5, 29.8, 37.4, 53.2, 102.3, 121.5, 122.3, 131.2, 145.4, 162.5, 167.7, 169.7 (carbonyl carbon); Purity (UPLC): 98.50%; ESI-MS: Exact mass = 379.1314, m/z $[M+H]^+$ peak at 380.1; Analysis (CHNS): Calculated for $C_{16}H_{21}N_5O_4S$: C, 50.65%; H, 5.58%; N, 18.46%; S, 8.45% Found: C, 50.69%; H, 5.64%; N, 18.51%; S, 8.50%.

3.1.4.11. 4-((5-Carbamoyl-4-((3-methylbutan-2-yl)amino)pyri midin-2-yl)amino)phenyl methanesulfonate (48k):



MP: 180-181°C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 0.90 (d, *J* = 6.8 Hz, 3H), 0.94 (d, *J* = 6.8 Hz, 3H), 1.13 (d, *J* = 6.8 Hz, 3H), 1.85 - 1.90 (m, 1H), 3.34 (s, 3H), 4.06 - 4.11 (m, 1H), 7.23 - 7.27 (m, 2H), 7.84 – 7.88 (m, 2H), 8.53 (s, 1H), 9.27 (d, *J* = 8.4 Hz, 1H), 9.65 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ ppm: 16.6, 18.0, 18.4, 31.9, 37.1, 50.1, 99.5, 119.9, 122.2, 139.6, 143.1, 157.1, 159.8, 161.0, 169.2 (carbonyl carbon); Purity (UPLC): 98.72%; ESI-MS: Exact mass = 393.1471, m/z $[M+H]^+$ peak at 394.2; Analysis (CHNS): Calculated for $C_{17}H_{23}N_5O_4S$: C, 51.89%; H, 5.89%; N, 17.80%; S, 8.15% Found: C, 51.74%; H, 5.79%; N, 17.55%; S, 8.10%.

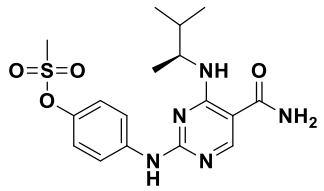
3.1.4.12. (*R*)-4-((5-Carbamoyl-4-((3-methylbutan-2-yl)amino) pyrimidin-2-yl)amino)phenyl methanesulfonate (48l):



MP: 199-200°C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 0.90 (d, *J* = 6.8 Hz, 3H), 0.94 (d, *J* = 6.8 Hz, 3H), 1.13 (d, *J* = 6.4 Hz, 3H,), 1.84 - 1.92 (m ,1H), 3.34 (s, 3H), 4.05 - 4.12 (m ,1H), 7.26 (d, *J* = 9.2 Hz, 2H), 7.87 (d, *J* = 9.2 Hz, 1H), 8.54 (s, 1H), 9.28 (d, *J* = 8.4 Hz, 1H), 9.64 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ ppm: 17.1, 18.4, 18.8, 32.4, 37.5, 50.6, 100.0, 120.3, 122.6, 140.0, 143.5, 157.7, 160.3, 161.5, 169.7 (carbonyl carbon); Purity (HPLC): 99.00%; ESI-MS: Exact mass = 393.1471, m/z $[M+H]^+$ peak at 394.1; Analysis (CHNS):

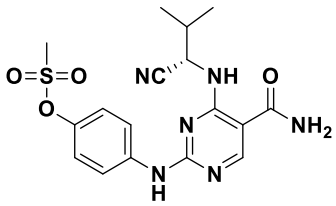
Calculated for $C_{17}H_{23}N_5O_4S$: C, 51.89%; H, 5.89%; N, 17.80%; S, 8.15% Found: C, 51.92%; H, 5.93%; N, 17.85%; S, 8.20%.

3.1.4.13. (*S*)-4-((5-Carbamoyl-4-((3-methylbutan-2-yl)amino) pyrimidin-2-yl)amino)phenyl methanesulfonate (48m):



MP: 225-226°C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 0.88 (d, *J* = 6.8 Hz, 3H), 0.94 (d, *J* = 6.8 Hz, 3H), 1.12 (d, *J* = 6.8 Hz, 3H), 1.85 - 1.89 (m, 1H), 3.33 (s, 3H), 4.06 - 4.11 (m, 1H), 7.23 – 7.27 (m, 2H) 7.84 - 7.87 (m, 2H), 8.52 (s, 1H), 9.27 (d, *J* = 8.4 Hz, 1H), 9.64 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ ppm: 16.9, 18.1, 18.6, 32.1, 37.3, 50.2, 100.1, 120.1, 122.3, 140.1, 143.4, 157.5, 160.0, 161.2, 169.5 (carbonyl carbon); Purity (HPLC): 97.19%; ESI-MS: Exact mass = 393.1471, m/z $[M+H]^+$ peak at 394.2; Analysis (CHNS): Calculated for $C_{17}H_{23}N_5O_4S$: C, 51.89%; H, 5.89%; N, 17.80%; S, 8.15% Found: C, 51.96%; H, 5.95%; N, 17.90%; S, 8.23%.

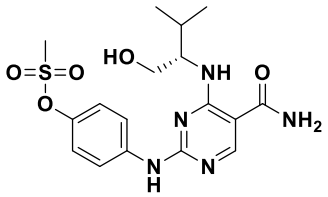
3.1.4.14. (*R*)-4-((5-Carbamoyl-4-((1-cyno-2-methylpropyl)amino) pyrimidin-2-yl)amino)phenyl methanesulfonate (48n):



MP: 234-235°C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 1.02 – 1.11 (m, 6H), 2.13 – 2.33 (m, 1H), 3.34 (s, 3H), 4.91 (t, *J* = 6.8 Hz, 1H), 7.27

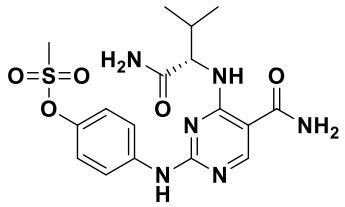
(d, *J* = 8.8 Hz, 2H) 7.37 (s, 1H), 7.85 (d, *J* = 8.8 Hz, 2H), 7.98 (s, 1H), 8.67 (s, 1H), 9.74 (d, *J* = 7.2 Hz, 1H), 9.90 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ ppm: 17.2, 17.5, 27.5, 37.3, 58.5, 102.1, 116.0, 121.1, 121.5, 122.3, 122.8, 131.6, 145.4, 162.0, 167.0, 168.2, 169.8 (carbonyl carbon); Purity (UPLC): 97.98%; ESI-MS: Exact mass = 404.1267, m/z $[M]^+$ peak at 404.8; Analysis (CHNS): Calculated for $C_{17}H_{20}N_6O_4S$: C, 50.49%; H, 4.98%; N, 20.78%; S, 7.93% Found: C, 50.33%; H, 4.81%; N, 20.75%; S, 7.82%.

3.1.4.15. (*R*)-4-((5-Carbamoyl-4-((1-hydroxy-3-methylbutan-2-yl)amino) pyrimidin-2-yl)amino)phenyl methane sulfonate (48o):



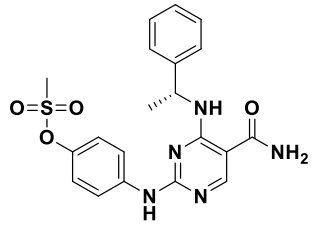
MP: 242-243^{o}C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 0.92 (d, *J* = 6.0 Hz, 6H), 1.98 – 2.06 (m, 1H), 3.32 (s, 3H), 3.46 – 3.50 (m, 1H), 3.52 – 3.56 (m, 1H) 4.03 – 4.06 (m, 1H), 4.75 (t, *J* = 5.2 Hz, 1H), 7.23 - 7.26 (m, 2H), 7.84 – 7.86 (m, 2H), 8.52 (s, 1H), 9.31 (d, *J* = 8.4 Hz, 1H), 9.61 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ ppm: 19.6, 19.8, 37.4, 63.2, 69.6, 102.2, 121.2, 122.3, 131.2, 145.3, 162.4, 166.8, 169.4 (carbonyl carbon); Purity (UPLC): 96.21%; ESI-MS: Exact mass = 409.1420, m/z $[M]^+$ peak at 409.9; Analysis (CHNS): Calculated for $C_{17}H_{23}N_5O_5S$: C, 49.87%; H, 5.66%; N, 17.10%; S, 7.83% Found: C, 49.95%; H, 5.72%; N, 17.22%; S, 7.90%.

3.1.4.16. (*R*)-4-((1-amino-3-methyl-1-oxobutan-2-yl)amino)-5-carbamoylpyrimidin-2-yl)amino)phenyl methane sulfonate (48p):



MP: 255-256°C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 0.94 (d, *J* = 6.4 Hz, 6H), 2.21 – 2.24 (m, 1H), 3.34 (s, 3H), 4.42 (t, *J* = 6.0 Hz, 1H), 7.13 (s, 1H), 7.25 (d, *J* = 9.2 Hz, 2H) 7.52 (s, 1H), 7.85 (d, *J* = 9.2 Hz, 2H), 8.54 (s, 1H), 9.56 (d, *J* = 7.2 Hz, 1H), 9.66 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ ppm: 18.6, 18.8, 30.6, 37.6, 76.5, 102.1, 121.2, 121.4, 122.3, 122.5, 131.4, 162.3, 166.5, 168.2, 169.8 and 176.5 (carbonyl carbons); Purity (UPLC): 96.76%; ESI-MS: Exact mass = 422.1372, m/z $[M+H]^+$ peak at 423.2; Analysis (CHNS): Calculated for $C_{17}H_{22}N_6O_5S$: C, 48.33%; H, 5.25%; N, 19.89%; S, 7.59% Found: C, 48.44%; H, 5.43%; N, 19.96%; S, 7.66%.

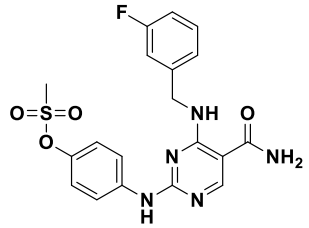
3.1.4.17. (*R*)-4-((5-Carbamoyl-4-((1-phenylethyl)amino)pyrimidin-2-yl)amino)phenyl methanesulfonate (48q):



MP: 260-261°C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 1.48 (d, *J* = 8.2 Hz, 3H), 3.32 (s, 3H), 5.20 - 5.23 (m, 1H), 7.21 - 7.16 (m, 3H), 7.23 - 7.42 (m, 4H), 7.60 - 7.66 (m, 2H), 8.55 (s, 1H), 9.63 (m, 2H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ ppm: 20.9, 37.4, 55.2, 100.4, 121.1, 122.7, 127.1, 127.9, 128.7, 139.7, 140.3, 143.9, 158.5, 160.8, 162.2, 169.8 (carbonyl carbon); Purity (UPLC): 97.61%; ESI-MS: Exact mass = 427.1314, m/z $[M]^+$ peak at 427.8; Analysis (CHNS): Calculated for

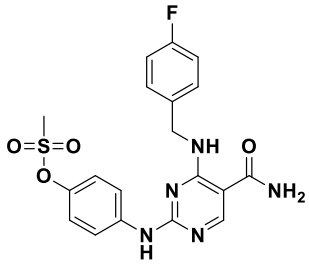
$C_{20}H_{21}N_5O_4S$: C, 56.19%; H, 4.95%; N, 16.38%; S, 7.50% Found: C, 56.10%; H, 4.87%; N, 16.25%; S, 7.40%.

3.1.4.18. 4-((5-Carbamoyl-4-((3-fluorobenzyl)amino)pyrimidin-2-yl) amino)phenyl methanesulfonate (48r):



MP: 266-267°C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 3.33 (s, 3H), 4.67 (d, *J* = 6.0 Hz, 2H), 7.04 - 7.19 (m, 5H), 7.35 - 7.41 (m, 1H), 7.68 (d, *J* = 9.2 Hz, 2H), 8.57 (s, 1H), 9.59 (t, *J* = 5.6 Hz, 2H), 9.68 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ ppm: 37.6, 43.7, 100.3, 112.5, 117.9, 121.1, 122.8, 123.1, 127.0, 139.5, 142.1, 143.8, 158.2, 159.9, 160.9, 162.0, 169.6 (carbonyl carbon); Purity (UPLC): 99.33%; Exact mass = 431.1064, m/z $[M]^+$ peak at 431.8; Analysis (CHNS): Calculated for $C_{19}H_{18}FN_5O_4S$: C, 52.89%; H, 4.21%; N, 16.23%; S, 7.43% Found: C, 52.92%; H, 4.26%; N, 16.25%; S, 7.47%.

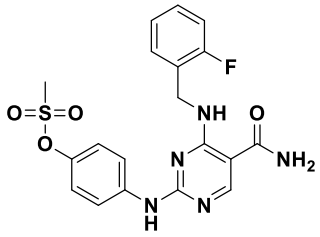
3.1.4.19. 4-((5-Carbamoyl-4-((4-fluorobenzyl)amino)pyrimidin-2-yl) amino)phenyl methanesulfonate (48s):



MP: 266-267°C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 3.32 (s, 3H),

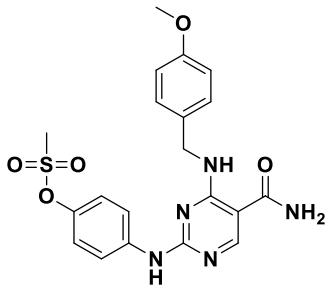
4.67 (d, *J* = 6.0 Hz, 2H), 7.15 - 7.20 (m, 4H), 7.35 - 7.39 (m, 2H), 7.72 (d, *J* = 8.8 Hz, 2H), 8.56 (s, 1H), 9.55 (s, 1H), 9.67 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ ppm: 37.5, 43.4, 100.5, 115.2, 121.0, 122.5, 127.7, 139.2, 140.3, 143.2, 159.4, 160.2, 160.8, 162.3, 169.1 (carbonyl carbon); Purity (UPLC): 98.97%; Exact mass = 431.1064, m/z $[M]^+$ peak at 431.9; Analysis (CHNS): Calculated for $C_{19}H_{18}FN_5O_4S$: C, 52.89%; H, 4.21%; N, 16.23%; S, 7.43% Found: C, 52.95%; H, 4.30%; N, 16.31%; S, 7.50%.

3.1.4.20. 4-((5-Carbamoyl-4-((2-fluorobenzyl)amino)pyrimidin-2-yl) amino)phenyl methanesulfonate (48t):



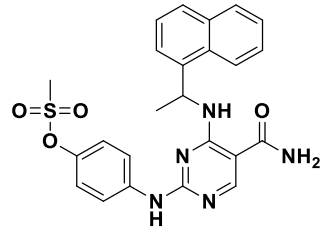
MP: 264-265^{o}C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 3.32 (s, 3H), 4.67 (d, *J* = 6 Hz, 2H), 7.13 - 7.17 (m, 3H), 7.21 - 7.33 (m, 3H), 7.69 (d, *J* = 8.8 Hz, 2H), 8.57 (s, 1H), 9.55 (s, 1H), 9.67 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ ppm: 37.2, 39.3, 100.6, 114.7, 121.4, 122.8, 124.0, 128.1, 129.5, 139.0, 139.2, 143.5, 159.2, 160.5, 161.2, 162.9, 169.7 (carbonyl carbon); Purity (UPLC): 98.48%; Exact mass = 431.1064, m/z $[M]^+$ peak at 431.9; Analysis (CHNS): Calculated for $C_{19}H_{18}FN_5O_4S$: C, 52.89%; H, 4.21%; N, 16.23%; S, 7.43% Found: C, 52.94%; H, 4.27%; N, 16.25%; S, 7.48%.

3.1.4.21. 4-((5-Carbamoyl-4-((4-methoxybenzyl)amino)pyrimidin-2-yl) amino)phenyl methanesulfonate (48u):



^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 3.33 (s, 3H), 3.71 (s, 3H), 4.61 (d, *J* = 5.2 Hz, 2H), 6.90 (d, *J* = 8.4 Hz, 2H), 7.14 -7.37 (m, 4H), 7.77 (d, *J* = 8.8 Hz, 2H), 8.55 (s, 1H), 9.45 (s, 1H), 9.67 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ ppm: 37.5, 42.4, 55.9, 102.2, 114.2, 121.2, 122.5, 130.6, 131.4, 132.5, 145.4, 158.5, 162.5, 166.6, 168.4, 169.5 (carbonyl carbon); Purity (UPLC): 97.45%; Exact mass = 443.1263, m/z $[M+H]^+$ peak at 444.0; Analysis (CHNS): Calculated for $C_{20}H_{21}N_5O_5S$: C, 54.17%; H, 4.77%; N, 15.79%; S, 7.23% Found: C, 54.10%; H, 4.60%; N, 15.68%; S, 7.15%.

3.1.4.22. 4-((5-Carbamoyl-4-((naphthalen-1-ylmethyl)amino) pyrimidin-2-yl)amino)phenyl methanesulfonate (48v):



MP: 272-273^{o}C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 1.67 (d, *J* = 6.8 Hz, 3H), 3.28 (s, 3H), 6.05 (t, *J* = 6.4 Hz, 1H), 6.52 - 6.58 (m, 2H), 7.36 - 7.44 (m, 2H), 7.45 - 7.48 (m, 2H), 7.64 - 7.85 (m, 2H), 7.89 - 7.91 (m, 1H), 8.01 (d, *J* = 8.0 Hz, 1H), 8.27 (d, *J* = 8.4 Hz, 1H), 8.59 (s, 1H), 8.97 (s, 1H), 9.83 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ

ppm: 21.5, 37.6, 57.9, 100.2, 121.8, 122.4, 122.9, 125.8, 126.9, 128.1, 131.0, 139.4, 140.9, 143.5, 159.7, 161.0, 162.7, 169.2 (carbonyl carbon); Purity (UPLC): 97.30%; Exact mass = 477.1471, m/z $[M]^+$ peak at 477.0; Analysis (CHNS): Calculated for $C_{24}H_{23}N_5O_4S$: C, 60.36%; H, 4.85%; N, 14.67%; S, 6.71% Found: C, 60.47%; H, 4.91%; N, 14.74%; S, 6.79%.

All to 20 compounds (**48a-v**) prepared were screened for *in-vitro* JAK3 inhibitory activity. In the next section JAK3 inhibitory activity data described in detailed.

3.2. *In-vitro* JAK3 Inhibitory activity data of pyrimidine derivatives modified at 4th position

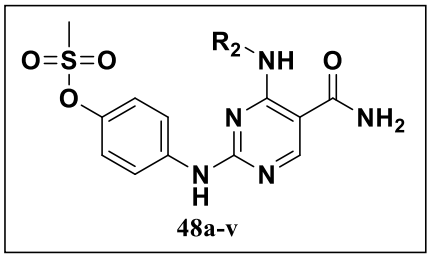
Determination of JAK3 assay

In-vitro screening of compounds **41a-r** was carried out, using a fluorogenic substrate assay.

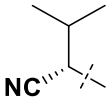
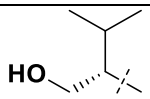
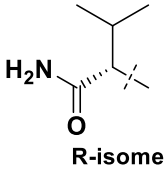
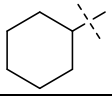
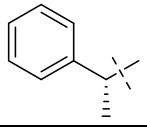
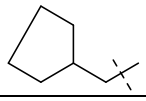
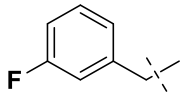
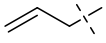
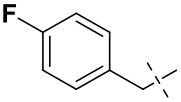
Human JAK1, JAK2, and JAK3 kinase domains were purchased from Carna Biosciences, Inc. (Kobe, Japan), and the assay was performed, using a streptavidin-coated 96-well plate. The reaction mixture contained 15 mM Tris-HCl (pH 7.5), 0.01% Tween 20, 2 mM DTT, 10 mM $MgCl_2$, 250 nM Biotin-Lyn-Substrate-2 (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

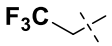
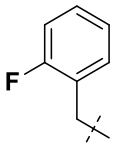
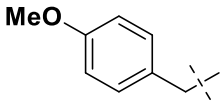
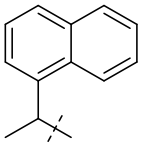
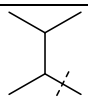
performed in duplicate. The IC_{50} value of all compounds was calculated, using linear regression analysis. **[92]** The IC_{50} values are mentioned below in **Table 4**.

Table 4. *Effect of substituent at 4th position of Pyrimidine moiety on JAK3 inhibitory activity (In-vitro).*



48a-v

Comp.	R_2	JAK-3 IC_{50} (nM)[a]	Comp.	R_2	JAK-3 IC_{50} (nM)[a]
48a	H	300	**48m**	 S-isomer	256
48b		49	**48n**	 NC R-isomer	40
48c		37	**48o**	 HO R-isomer	80
48d		33	**48p**	 H_2N O R-isomer	50
48e		79	**48q**		39
48f		12	**48r**	 F	43.2
48g		150	**48s**	 F	45.4

48h		110	**48t**		49.2
48i		9.8	**48u**		60
48j		25	**48v**		189
48k	 Racemic	20	**Tofacitinib**		8
48l	 R-isomer	1.7	**Cerdulatinib**		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 Series-2 (**Table 4**), mainly modifications were carried out at the C4-position of pyrimidine in **41k**. As listed in **Table 4**, compounds **48a-v** were prepared.

Compound **48a** (debenzylated **41k**, IC_{50}: 300 nM) was found to be less active. In contrast, replacement of the benzyl group with a cycloalkyl group, compound **48b** (cyclopropyl, IC_{50}: 49 nM) showed moderate potency. Compound **48c** (cyclobutyl, IC_{50}: 37 nM) showed moderate potency. Whereas compound **48d** (cyclopentyl, IC_{50}: 33 nM) showed slight improvement in the activity. Compound **48e** (cyclohexyl, IC_{50}: 79 nM) was found to be less active among all the cycloalkyl, also all the cycloalkyl derivatives were found to be less potent compared to **41k**. Compound **48f** (methylcyclopentyl, IC_{50}: 12 nM) showed slight

improvement in the activity. In contrast, compound **48g** with the allyl group showed moderate JAK3 inhibitory activity, with the IC_{50} of 150 nM. Compound **48h** (trifluoroethyl IC_{50}: 110 nM) displayed moderate potency, while **48i** (iso-butane, IC_{50}: 9.8 nM) was found to be equipotent to **41k**. Compound **48j** (sec-butyl, IC_{50} : 25 nM) was found to be 2.5-fold less active than compound **41k**. Racemic compound **48k** (iso-pentane, IC_{50}: 20 nM) showed two-fold less potency compared to **41k**.

Optically pure compound **48l** (*R*-isomer of **48h**) was found to be the five-fold more potent (IC_{50}: 1.7 nM), whereas **48m** (*S*-isomer of **48h**) was found to be less potent (IC_{50}: 256 nM). Compound **48n** (1-cyano-2-methylpropyl, IC_{50}: 40 nM) was found to be four-fold less potent than compound **41k**. Compound **48o** (Hydroxy, IC_{50}: 80 nM) showed moderate activity. Compound **48p** (amide, IC_{50}: 50 nM) was found to be five-fold less potent than compound **41k**. Compound **48q** (phenyl ethyl, IC_{50}: 39 nM) displayed moderate JAK3 inhibitory activity.

Fluoro-substituted benzyl analogues, compound **48r** (3-fluoro, IC_{50}: 43.2 nM), compound **48s** (4-fluoro, IC_{50}: 45.4 nM) and compoud **48t** (2-fluoro, IC_{50}: 49.2 nM) showed moderate activity compared to **41k**. Compound **48u** (4-methoxy, IC_{50}: 60 nM) showed moderate activity, while **48v** (naphthyl, IC_{50}: 189 nM) was found to be less active.

3.3. Conclusion

Further structure-activity relationship (SAR) studies on the C4-position of **41k** resulted in compounds (**48a-v**) with different JAK3 inhibitory activity. Electron donating group (amino), at C4 position, was found to be an inactive compound. Increase in ring size leads to the

decrease in activity, whereas presence of methylene linker showed good potency. Allylic compounds and electron withdrawing group (-CF_3) leads to the poor activity, while the alkyl group at C4 position showed moderate activity. In case of chiral alkyl, racemic alkyl showed moderate activity. When optically pure *S*-isomer of 1,2 dimethylpropyl (**48m**) showed poorer activity, and its *R*-isomer (**48l**, with IC_{50}: 1.7 nM) showed 5-fold improvement in the potency which was found to be even better than Cerdulatinib (IC_{50}: 8.0 nM). The presence of electron withdrawing (cyano and aminocarbonyl) group in the *R*-isomer leads to the moderate activity.

Electron donating hydroxy group also leads to moderarte activity. Bulky group with chirality (**48q**) leads to moderate activity. Electron withdrawing fluoro at *ortho*, *meta* and *para* position showed moderate activity. Electron withdrawing methoxy group at *para* position also leads to moderate activity. Bulky group (**48v**) at C4 position leads to an inactive compound. Modification at 4^{th} position of pyrimidine ring of **41k** (IC_{50}: 9.5 nM) gave compound **48l** with IC_{50}: 1.7 nM, which was found to be better compare to cerdulatinib (IC_{50}: 8.0 nM) and also it showed JAK3 inhibitory activity similar to that of Tofacitinib (IC_{50}: 1.7 nM).

Further to understand the SAR of DAP Series, modification were also carried out at the 5^{th} position of compound **48l**. In the next section, the experimental procedure and *in-vitro* JAK3 inhibitory activity of this Series is described in detail.