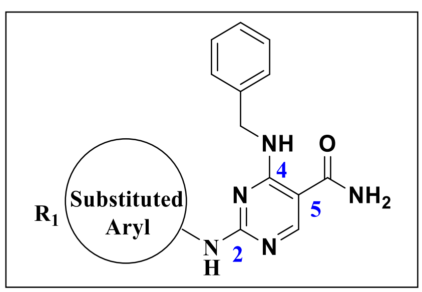
2. Design and synthesis of compounds modified at 2^{nd} position of Damion pyrimidine

As discussed in the **Chapter-1**, an inhibitor of JAK appears to be the most promising therapy for the safe and effective treatment of RA. Knowing the therapeutic potential of these inhibitors, we attempt to design novel Series of molecules. In this research, we present our efforts to develop novel non-covalent inhibitors of the JAK3. It was hypothesized that the non-covalent JAK3 selective inhibitors could be devoid of systemic toxicity. An approach undertaken to overcome the JAK isozymes selectivity was to capitalize on the amino acid sequence differences among the JAKs, more specifically to favour hydrogen bonding interaction with the JAK3 cysteine (Cys-909) residue in the catalytic domain to develop the novel, noncovalent JAK3 selective inhibitors. While designing JAK3 inhibitors, attempts were made to avoid an introduction of the covalent reactive groups (CRGs), in the structural features to circumvent the possibility of a covalent modification of Cys-909. Structural modifications were carried out in the Cerdulatinib (having pyrimidine moiety as a key pharmacophore).



Initial modifications were carried out at the C2 position of pyrimidine ring (at R_1 position) in Cerdulatinib, with C4 cyclopropyl of Cerdulatinib altered with a benzyl group.

2.1. Chemistry

2.1.1. *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 220 nm, using column ODS C-18, 150 nm*4.6 nm*4 μ on AGILENT 1100, Germany. UPLC analysis were carried out at λmax 220nm, using column YMC-Triart C18 (100*2.0 mm) on Water acquity UPLC, Europe (Austria).

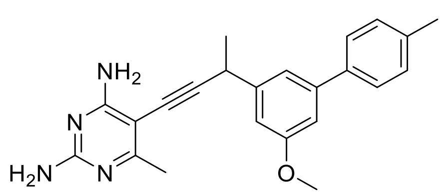
Progress of the reactions was monitor by TLC, using precoated TLC plates (E. Merck Kiesegel 60 F254, Germany) and the spots were visualized by UV and/or iodine vapors. The chromatographic purification was performed on silica gel (230-400 mesh). Few compounds directly used for the next step without purification and analysis. Detailed synthetic procedures and characterization data of all the final compounds and intermediates are described in the next section. However, in the next section initially we highlighted the importance of Diamino pyrimidine (DAP) as a pharmacophore and possible routes for the synthesis of DAP.

2.1.2. Importance of Diaminopyrimidine (DAP) in biological system

2.1.2.1. Biological Importance

Pyrimidine is six member aromatic heterocyclic compound, containing two nitrogen atoms in the ring. Thiamine, cytosine, thymine, uracil etc, having pyrimidine ring are commonly found in nature. DAP is simple pyrimidine derivatives that has two amino groups. DAP acts as a crucial pharmacophore. Here, we report some examples of DAP as key pharmacophore for various biological activities.

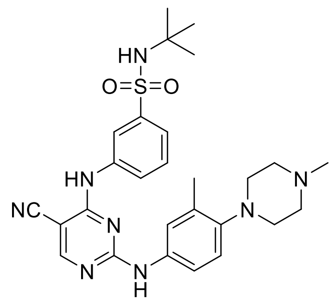
Example:-1



J.L.Paulsen et al., reported antifungal activity of DAP derivative 5-(3-(5-methoxy-4’-methyl-[1,1’-biphenyl]-3-yl)but-1-yn-1-yl)-6-methylpyrimidine-2,4-diamine. The above compound showed highly potent DHFR (Dihydrofolate reductase) inhibitory (*in-vitro*) activity.

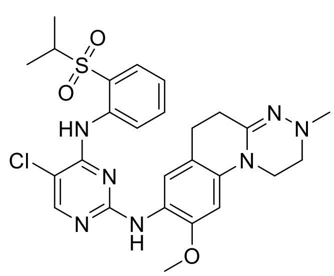
Thus, DAP derivative, example-1, represent novel scaffold as a DHFR inhibitor. **[76]**

Example:-2



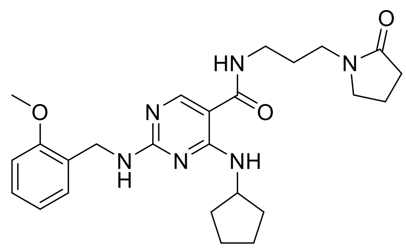
Antimalarial activity was reported by O. Phuangsawai et al., for DAP derivative *N*-(*tert*-butyl)-3-((5-cyno-2-((3-methyl-4-(4-methyl piperazin-1-yl)phenyl)amino)pyrimidin-4-yl) amino) benzenesulfona mide. Example-2 was identified as μM inhibitor (IC_{50}: 4.56 μM, *in-vitro*), with improved cytotoxicity and human kinase selectivity. **[77]**

Example:-3



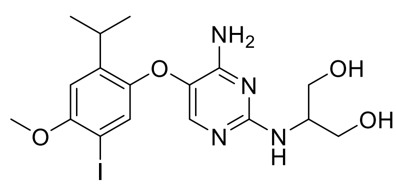
R. Achary et al., reported anti-tumor activity of DAP 5-chloro-N^4-(2-(isopropylsulfonyl)phenyl)-N^2-(9-methoxy-3-methyl-2,3,5,6-tetra hydro 1*H*-[1,2,4]triazino[4,3-a]quinolin-8-yl) pyrimidine-2,4-diamine. The above compound displayed highly potent ALK (Anaplastic lymphoma kinase) inhibitory (*in-vitro*) activity. Thus, DAP derivative example-3 represent a novel scaffold as an ALK inhibitor. **[78]**

Example:-4



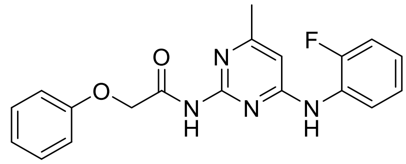
N.A. Powell et al., discovered SYK inhibitor based on DAP 4-(cyclopentylamino)-2-((2-methoxybenzyl)amino)-*N*-(3-(2-oxo pyrro lidin-1-yl)propyl)pyrimidine-5-carboxamide as a potential anti-platelet agent. Example-4 displayed μM *in-vitro* activity. Thus, DAP derivative example-4 represent a novel scaffold as a SYK inhibitor. **[79]**

Example:-5



A. Jahangir et al., reported drug like $P2X_3/P2X_{2/3}$ antagonist of DAP 2-((4-amino-5-(5-iodo-2-isopropyl-4-methoxyphenoxy)pyrimidin-2-yl)amino)propane-1,3-diol. Selectivity testing, *in-vitro* safety assessment and pharmacokinetic evaluation indicates that example-5 can serve as a valuable pharmacophore as dual $P2X_3/P2X_{2/3}$ antagonist. **[80]**

Example:-6



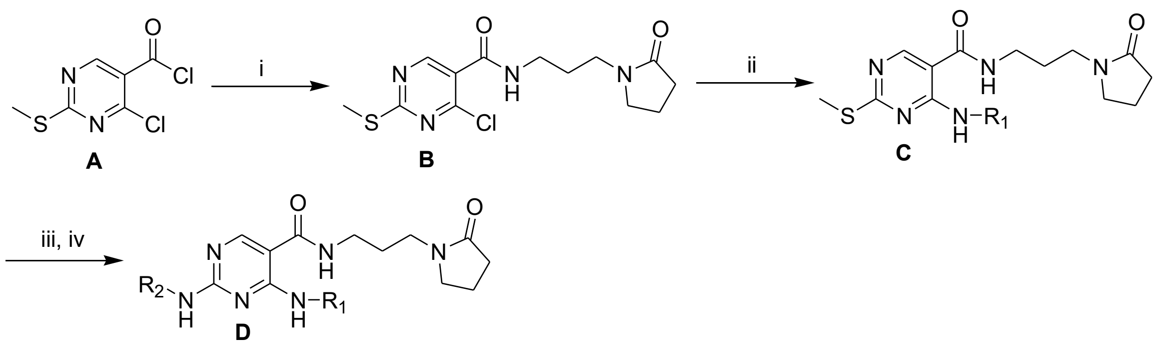
A novel Series of DAP was designed, synthesized and biologically evaluated for their Caspase-1 inhibition by S. Patel et al. Compound *N*-

(4-((2-fluorophenyl)amino)-6-methylpyrimidin-2-yl)-2-phenoxy acetamide displayed µM *in-vitro* activity. Thus, DAP derivative example-6 represent a novel scaffold as Caspase-1 inhibitor. **[81]**

2.1.2.2. Possible routes for the synthesis of DAP derivatives

In general, DAP derivatives can be prepared by various routes. Here, we reported different routes to prepare DAP.

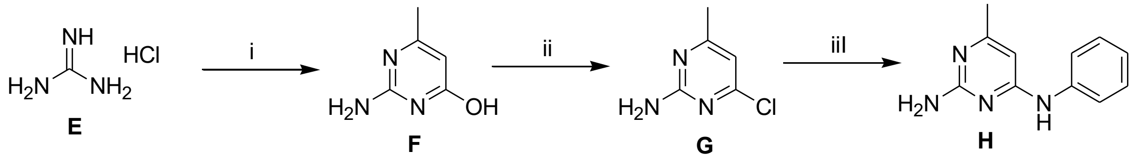
Route:-1



Reagents and conditions: (i) N-(3-Aminopropyl)-butyrolactam, TEA, DMAP, DCM; (ii) R_1NH_2, TEA, ACN, 80 ^oC; (iii) *m*-CPBA, DCM, 0 ^oC; (iv) R_2NH_2, TEA, Dioxane, reflux

Condensation of *N*-(3-aminopropyl)-butyrolactam with compound **A** provided the amide **B**. The chloride of compound **B** was displaced by an amine in hot acetonitrile (ACN) to afford **C**. After oxidation of the thiomethyl group to methyl sulfone, displacement of the sulfone by amine in refluxing dioxane leads to the formation of compound **D**. **[79]**

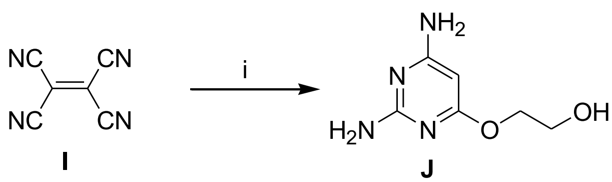
Route:-2



Reagents and conditions: (i) Ethyl 3-oxobutanoate, EtOH, NaOH, 3 hr, rt ; (ii) $POCl_3$, Toluene, 30 - 40 min, 110 ^oC; (iii) Aniline, EtOh, Conc. HCl, 3 - 4 hr, 40 ^oC

The cyclocondensation of compound **E** and ethyl 3-oxobutanoate afforded compound **F**, which was heated with phosphorus oxychloride to get compound **G**. Compound **G** was refluxed with aniline in the presence of catalytic conc. HCl in ethanol to afford compound **H**. **[81]**

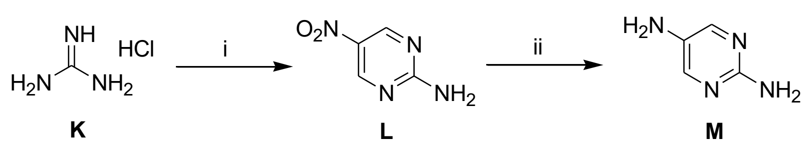
Route:-3



Reagents and conditions: (i) Ethylene glycol, guanidine, urea, MeOH, NaOMe, 4 hr, rt

Route-3 represent a facile route for the one-spot synthesis of pyrimidine ring. Mainly it involves cyclization of compound **I**, with ethylene glycol, guanidine, urea in methanol, in the presence of sodium methoxide. **[82]**

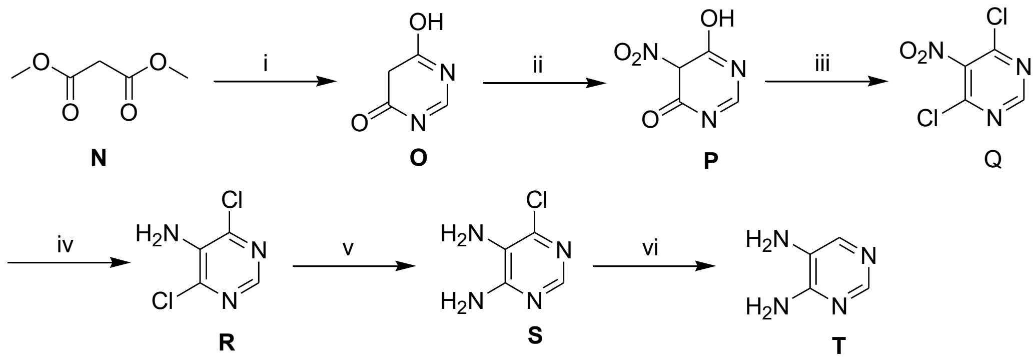
Route:-4



Reagents and conditions: (i) 3-tert-Butylamino-2-nitro-2-propenal, EtOH, TEA, 1 day, 80 ^oC; (ii) H_2, Pd/C, MeOH, overnight, rt

Route-4 represent two-step synthesis of DAP. Synthesis of pyrimidine ring involves cyclization of compound **K**, with 3-*tert*-butylamino-2-propenal in ethanol, followed by reduction of nitro group. **[83, 84]**

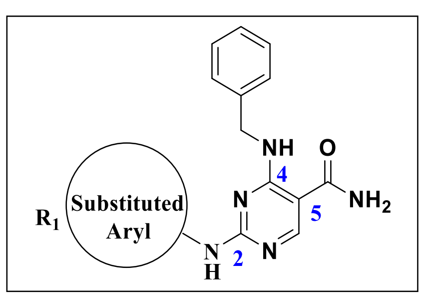
Route:-5



Reagents and conditions: (i) Formamide, Sodium t-butoxide, butanol, 1 hr, 70 ^oC; (ii) TFA, HNO_3, H_2O, 2 hr, rt; (iii) $POCl_3$, DIPEA, Toluene, reflux; (iv) Fe, AcOH, MeOH, 60 ^oC, 3 hr; (v) NH_3, EtOH, 8 hr, rt (vi) H_2, Pd/C, H_2O

As depicted in route-5, DAP can be prepared starting from Dimethyl malonate and formamide to afford compound **O**. Further nitration of compound **O** with nitric acid affords intermediate **P**. Conversion of hydroxy group of compound **P** to chloride (**Q**), followed by reduction of nitro to amino lead to formation of compound **R**. Selectively one of the chlorine of compound **R** was displaced by an amine to afford compound **S**, followed by removal of chloride to yield DAP (**T**). **[85-87]**

As described earlier, in Series-1 modification were carried out at the C2 position of pyrimidine ring and total 18 compounds were prepared. For the synthesis of title compounds **41a-r** in Series-1, we first prepared intermediates as described below.

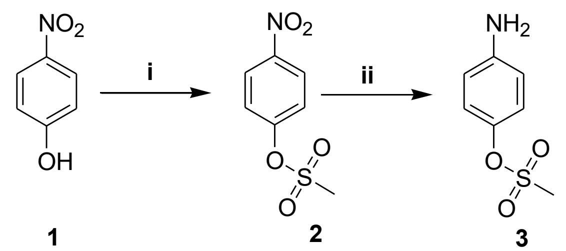


2.1.3. Synthesis of intermediates for R_1 Substitution

Initially, we prepared *para* substituted intermediates as shown below.

2.1.3.1. Synthesis of intermediate for making compound 41k

Scheme-1



Reagents and conditions: (i) CH_3SO_2Cl, TEA, DCM, 30 ^oC, 2 hr; (ii) Fe, NH_4Cl, Ethanol, reflux, 1 hr

Step-I Preparation of 4-nitrophenyl methanesulfonate (**2**)

To the solution of 4-nitrophenol **1** (10 g, 71.9 mmol) and trimethylamine (TEA; 13.48 mL, 97 mmol) in DCM (dichloromethane; 100 mL), methane sulfonyl chloride (CH_3SO_2Cl; 6.88 mL, 88 mmol) was added at 0-5^{o}C. The mixture was stirred for 2 hr at room temperature (RT). After completion of reaction, mixture was quenched in water and the organic layer was extracted with DCM. The organic layer was dried over sodium sulfate (Na_2SO_4) and the solvent was removed under reduced pressure to afford 4-nitrophenyl methane sulfonate, as a yellow solid (Yield: 14 g, 90%, Purity by UPLC: 90%).

^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 3.51 (s, 3H), 7.61 - 7.65 (m, 2H), 8.34 - 8.38 (m, 2H); ESI-MS: Exact mass = 217.0045, m/z $[M]^+$ peak at 217.0.

Step-II Preparation of 4-aminophenyl methane sulfonate (**3**)

The mixture of 4-nitrophenyl methane sulfonate **2** (13 g, 60.4 mmol) and iron metal powder (Fe; 27 g, 483 mmol), in ethanol (EtOH; 150 L) was stirred at RT. A solution of ammonium chloride (NH_4Cl; 4.85 g, 91 mmol) dissolved in water was added, and the resulting reaction mixture was heated to reflux for 1 hr. The reaction mixture was diluted with DCM, filtered through celite, washed with DCM and combined organic extract was washed with water, dried over Na_2SO_4, filtered, and concentrated under vacuum to provide the desired product (Yield: 8.37 g, 74%, Purity by UPLC: 93.21%) as a black solid.

^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 3.23 (s, 3H), 5.25 (s, 2H), 6.56 - 6.60 (m, 2H), 6.96 - 7.00 (m, 2H); ESI-MS: Exact mass = 187.0303, m/z $[M+H]^+$ peak at 188.8.

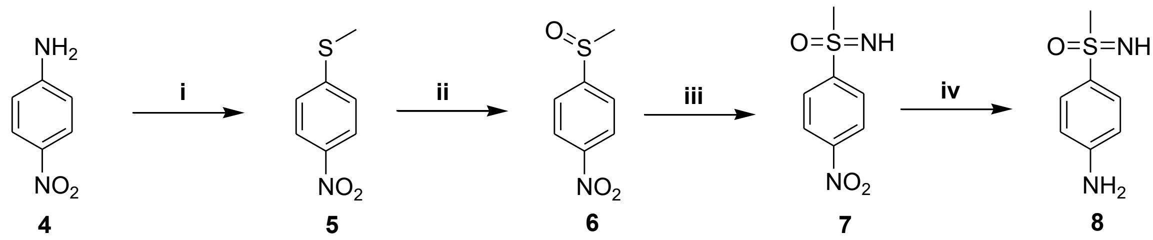
2.1.3.2. Synthesis of intermediate for making compound 41l

Intermediate 4-aminophenyl propane-2-sulfonate **41l** was prepared, using a procedure similar to that described in the preparation of intermediate **41k,** starting from isopropyl sulfonyl chloride instead of methane sulfonyl chloride.

^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 1.38 (d, J = 6.8 Hz, 6H), 3.53 - 3.60 (m, 1H), 5.20 (s, 2H), 6.54 - 6.58 (m, 2H), 6.91 - 6.95 (m, 2H); ESI-MS: Exact mass = 215.0616, m/z $[M\text{-}H]^+$ peak at 214.8.

2.1.3.3. Synthesis of intermediate for making compound 41i

Scheme-2



Reagents and conditions: (i) Dimethyl disulfide, *tert*-Butyl Nitrile, ACN, 40 ^oC, 3 hr; (ii) m-CPBA, $CHCl_3$, -10 ^oC, 20mins; (iii) NaN_3, H_2SO_4, $CHCl_3$, 40 ^oC, 24 hr; (iv) Fe, NH_4Cl, Ethanol, reflux, 1 hr

Step-I Preparation of methyl (4-nitrophenyl)sulfane (**5**)

Tert-butyl nitrite (12.92 mL, 109 mmol) was added to a solution of *p*-nitro aniline **4** (10 g, 72.4 mmol) in ACN (110 mL) at 0-5^oC. The mixture was stirred for 30 mins at RT. After 30 mins, dimethyl disulfide (7.82 mL, 88 mmol) was added to the reaction mixture and the mixture was stirred at 40^oC for 3 hr. After cooling, the reaction mixture was poured in to ice-water and the aqueous phase was extracted with ethyl acetate (EtOAc). The combined organic phases were washed with water, dried over Na_2SO_4, filtered and subsequently concentrated on a rotary evaporator, under reduced pressure. The crude product was purified by column chromatography on silica gel (230-400 mesh) to afford methyl (4-nitrophenyl)sulfane (Yield: 11.02 g, 90%, Purity by HPLC: 93%) as a yellow solid. **[88]**

^1H NMR (DMSO-d_6, 400 MHz) δ ppm: 2.84 (s, 3H), 7.95 - 7.98 (m, 2H), 8.39 - 8.42 (m, 2H); ESI-MS: Exact mass = 169.0197, m/z $[M]^+$ peak at 169.2.

Step-II Preparation of 1-(methylsulfinyl)-4-nitrobenzene (**6**)

To a solution of methyl (4-nitrophenyl)sulfane **5** (10 g, 59.1 mmol) in chloroform ($CHCl_3$; 120 mL) at -10^oC was added *m*-chloroperbenzoic acid (*m*-CPBA 60%; 20.61 g, 59.1 mmol), lot wise. After completion of reaction (around 20 mins), the mixture was quenched with saturated sodium bicarbonate ($NaHCO_3$) solution and the organic layer was extracted by DCM. 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 afford 1-(methylsulfinyl)-4-nitrobenzene, as a pale yellow solid (Yield: 7.66 g, 70%, Purity by UPLC: 90%).

^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 2.81 (s, 3H), 7.80 (d, J = 8.6 Hz, 2H), 8.38 (d, J = 8.7 Hz, 2H); ESI-MS: Exact mass = 185.0147, m/z $[M]^+$ peak at 185.2.

Step-III Preparation of 1-(*S*-methylsulfonimidoyl)-4-nitrobenzene (**7**)

A solution of 1-(methylsulfinyl)-4-nitrobenzene **6** (6.0 g, 32.4 mmol) in $CHCl_3$ (80 mL) was treated with sodium azide (NaN_3; 11.85 g, 130 mmol), at 0^{o}C. The mixture was slowly treated with 9.72 mL (130 mmol) of concentrated sulfuric acid (H_2SO_4) at 0^{o}C and then heated to 40^{o}C, for 24 hr. After completion of reaction, mixture was cooled to RT, reaction mixture was basified by sat $NaHCO_3$ solution and extracted with DCM. 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 1-(*S*-methylsulfonimidoyl)-4-nitro benzene as solid, as a white solid (Yield: 3.89 g, 60%, Purity by HPLC: 86%). **[89]**

^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 3.16 (s, 3H), 4.60 (s, 1H), 8.16 - 8.20 (m, 2H), 8.39 - 8.43 (m, 2H); ESI-MS: Exact mass = 200.0256, m/z $[M]^+$ peak at 200.6.

Step-IV Preparation of 4-(*S*-methylsulfonimidoyl) aniline (**8**)

The reduction of nitro group was carried out by similar procedure as described in the step II for the preparation of 4-aminophenyl methane sulfonate, starting from 1-(*S*-methylsulfonimidoyl)-4-nitrobenzene **7** (3.0 g) as reactant to afford 4-(*S*-methylsulfonimidoyl)aniline (Yield: 1.78 g , 70%, Purity by HPLC: 88%).

^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 2.92 (s, 3H), 3.48 (s, 1H), 5.91 (s, 2H), 6.59 - 6.63 (m, 2H), 7.51 - 7.54 (m, 2H); ESI-MS: Exact mass = 170.0514, m/z $[M]^+$ peak at 170.7.

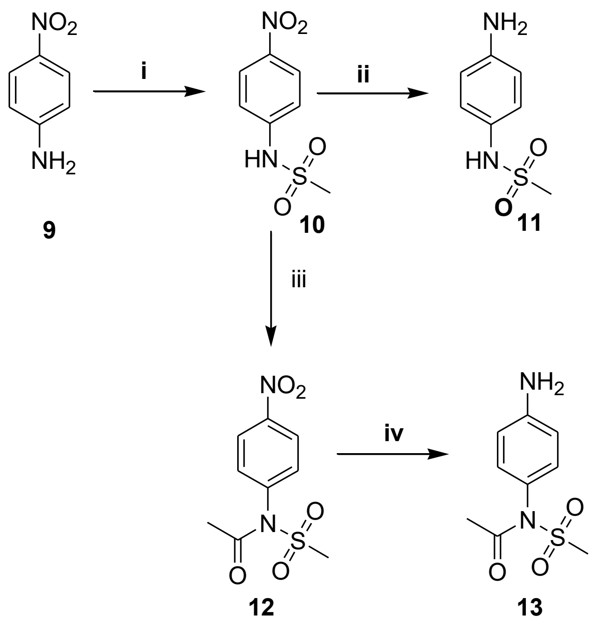
2.1.3.4. Synthesis of intermediate for making compound 41f

Intermediate 3-(*S*-methylsulfonimidoyl) aniline **41f** was prepared, using a procedure similar to that described for the preparation of intermediate **41i**.

^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 2.96 (s, 3H), 3.93 (s, 1H), 5.56 (s, 2H), 6.72 - 7.68 (m, 1H), 7.0 (d, J = 7.3 Hz, 1H), 7.11 (t, J = 2.1 Hz, 1H), 7.19 (t, J = 7.40 Hz, 1H); ESI-MS: Exact mass = 170.0514, m/z $[M]^+$ peak at 170.6.

2.1.3.5. ***Synthesis of intermediate for making compound 41h***

Scheme-3



Reagents and conditions: (i) CH_3SO_2Cl, TEA, DCM, 30 °C, 2 hr; (ii) Fe,NH_4Cl, Ethanol, reflux, 1 hr; (iii) CH_3COCl, TEA, DCM, 30 °C, 2 hr; (iv) Fe,NH_4Cl, Ethanol, reflux, 1 hr

Step-I Preparation of *N*-(4-nitrophenyl) methane sulfonamide (**10**)

4-Nitroaniline **9** (10 g, 72.4 mmol) was dissolved in DCM (100 mL) and treated with TEA (20.18 mL, 145 mmol), at RT. After stirring for 20 mins, methane sulfonyl chloride (6.77 mL, 87 mmol) was added, at 0° C, and it was stirred for 2 hr at RT. After completion of reaction, the reaction mixture was quenched with water and the organic layer was extracted by DCM. The organic layer was dried over Na_2SO_4 and the solvent was removed under reduced pressure to afford *N*-(4-nitrophenyl) methane sulfonamide as a solid (Yield: 14.0 g, 90%, Purity by HPLC: 87%).

^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 3.17 (s, 3H), 7.35 (d, J = 9.4 Hz, 2H), 8.20 (d, J = 9.1 Hz, 2H), 10.69 (s, 1H); ESI-MS: Exact mass = 216.0205, m/z $[M]^+$ peak at 216.3.

Step-II Preparation of *N*-(4-aminophenyl) methane sulfonamide (**11**)

The title compound was prepared by similar procedure to that described in step II for the preparation of 4-aminophenyl methane sulfonate, using *N*-(4-nitrophenyl)methane sulfonamide **10** as reactant (13 g) to afford *N*-(4-aminophenyl)methane sulfonamide (Yield: 8.06 g, 72%, Purity by UPLC: 89%).

^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 2.79 (s, 3H), 5.07 (s, 2H), 6.49-6.53 (m, 2H), 6.86 - 6.90 (m, 2H), 8.90 (s, 1H); ESI-MS: Exact mass = 186.0463, m/z $[M]^+$ peak at 186.7.

Step-III Preparation of *N*-(methylsulfonyl)-*N*-(4-nitrophenyl) acetamide (**12**)

The solution of *N*-(4-nitrophenyl) methane sulfonamide **10** (7.0 g, 32.4 mmol) in DCM (80 mL) was treated with TEA (16.9 mL, 97 mmol), at RT. After stirring for 20 mins, acetyl chloride (CH_3COCl; 3.45 mL, 38.9 mmol) was added at 0° C, and it was stirred for 2 hr, at RT. After completion of reaction, the reaction mixture was quenched by water and the organic layer was extracted with DCM. The organic layer was dried over Na_2SO_4 and the solvent was removed under reduced pressure to afford *N*-(4-aminophenyl)-*N*-(methyl sulfonyl) acetamide, as a solid (Yield: 7.11 g, 85%, Purity by UPLC: 83%).

^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 2.08 (s, 3H), 3.20 (s, 3H), 8.10 - 8.18 (m, 2H), 7.90 - 7.97 (m, 2H); ESI-MS: Exact mass = 258.0310, m/z $[M]^+$ peak at 258.4.

Step-IV Preparation of *N*-(4-aminophenyl)-*N*-(methyl sulfonyl) acetamide (**13**)

The title compound was prepared, using a procedure similar as described in the step II for the preparation of 4-aminophenyl methane sulfonate, starting from *N*-(4-aminophenyl)-*N*-(methyl sulfonyl) acetamide as reactant **12** (6.0 g), to afford *N*-(4-aminophenyl)-*N*-(methyl sulfonyl) acetamide (Yield: 6.61 g, 68%, Purity by UPLC: 83%).

^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 1.86 (s, 3H), 3.42 (s, 3H), 5.47 (s, 2H), 6.55 - 6.58 (m, 2H), 6.98 - 7.02 (m, 2H); ESI-MS: Exact mass = 228.0569, m/z $[M]^+$ peak at 228.9.

2.1.3.6. Synthesis of intermediate for making compound 41e and 41g

Intermediate *N*-(3-aminophenyl) methane sulfonamide **41e** was prepared, using a procedure similar as described for the preparation of intermediate **41h**.

^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 2.92 (s, 3H), 5.14 (s, 2H), 6.27 - 6.30 (m, 1H), 6.32 - 6.35 (m, 1H), 6.44 - 6.45 (m, 1H), 6.90 - 6.94 (m, 1H), 9.36 (s, 1H); ESI-MS: Exact mass = 186.0463, m/z $[M]^+$ peak at 186.7.

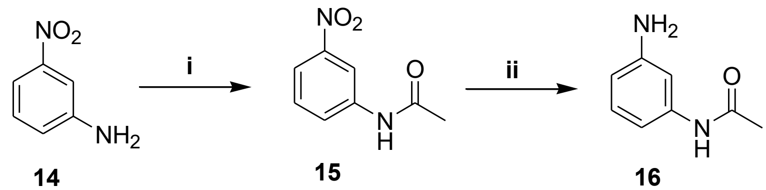
Intermediate *N*-(3-aminophenyl)-*N*-(methylsufonyl)acetamide **41g** was prepared, using a procedure similar as described for the preparation of intermediate **41j**.

^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 1.90 (s, 3H), 3.45 (s, 3H), 5.35 (s, 2H), 6.51 - 6.53 (m, 2H), 6.63 - 6.66 (m, 1H), 7.08 - 7.12 (m, 1H); ESI-MS: Exact mass = 228.0569, m/z $[M]^+$ peak at 228.0.

Then we synthesized *meta* substituted intermediates as shown below.

2.1.3.7. Synthesis of intermediate for making compound 41p

Scheme-4



Reagents and conditions: (i) CH_3COCl, TEA, DCM, 30 ^{o}C, 2 hr; (ii) Fe, NH_4Cl, Ethanol, reflux, 1 hr

Step-I Preparation of *N*-(3-nitrophenyl)acetamide (**15**)

The solution of *m*-nitro aniline **14** (5.0 g, 36.2 mmol) in DCM (50 mL) was treated with TEA (6.05 mL, 43.4 mmol), at RT. After stirring for 20 mins, acetyl chloride (2.83 mL, 39.8 mmol) was added, at 0°C, and it was stirred for 2 hr, at RT. After completion of reaction, the reaction mixture was quenched by water and the organic layer was extracted with DCM. The organic layer was dried over Na_2SO_4 and the solvent was removed under reduced pressure to afford *N*-(3-nitrophenyl)acetamide, as a solid (Yield: 5.87 g, 90%, Purity by HPLC: 80%).

^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 2.10 (s, 3H), 7.59 (t, J = 8.4 Hz, 1H), 7.87 - 7.90 (m, 2H), 8.61 (t, J = 2.0 Hz, 1H), 10.42 (s, 1H); ESI-MS: Exact mass = 180.0535, m/z $[M]^+$ peak at 180.3.

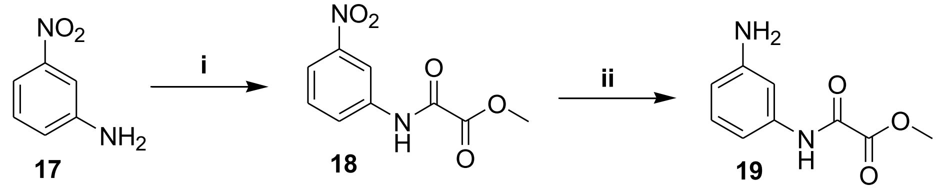
Step-II Preparation of N-(3-aminophenyl)acetamide (**16**)

The reduction of nitro group was carried out by similar procedure as described in step II for the preparation of 4-aminophenyl methanesulfonate, starting from *N*-(3-nitrophenyl)acetamide **15** (4 g) as a reactant to afford *N*-(3-aminophenyl)acetamide (Yield: 2.36 g, 71%, Purity by UPLC: 86%).

^1H NMR (DMSO-d_6, 400 MHz) δ ppm: 1.99 (s, 3H), 5.00 (s, 2H), 6.23 (m, 1H), 6.64 (m, 1H), 6.88 (t, J = 8.0 Hz, 1H), 6.92 (t, J = 2.0 Hz, 1H), 9.58 (s, 1H); ESI-MS: Exact mass = 150.0793, m/z $[M]^+$ peak at 150.4.

2.1.3.8. Synthesis of intermediate for making compound 41b

Scheme-5



Reagents and conditions: (i) $CH_3OCOCOCl$, TEA, DCM, 30 ^oC, 2 hr; (ii) Fe, NH_4Cl, Ethanol, reflux, 1 hr

Step-I Preparation of methyl 2-((3-nitrophenyl) amino)-2-oxoacetate (**18**)

Methyl 2-((3-nitrophenyl)amino)-2-oxoacetate **18** was prepared following the procedure that was described in the step I which was used for the preparation of *N*-(3-nitrophenyl)acetamide, starting from *m*-nitroaniline **17** (5.0 g) as a reactant and methyl 2-chloro-2-oxoacetate instead of acetyl chloride to afford methyl 2-((3-nitrophenyl)amino)-2-oxoacetate (Yield: 6.49 g, 80%, Purity by UPLC: 81%).

^1H NMR (DMSO-d_6, 400 MHz) δ ppm: 3.61 (s, 3H), 7.65 - 7.74 (m, 1H), 7.90 - 7.98 (m, 2H), 8.70 - 8.75 (m, 1H), 10.61 (s, 1H); ESI-MS: Exact mass = 224.0433, m/z $[M]^+$ peak at 224.3.

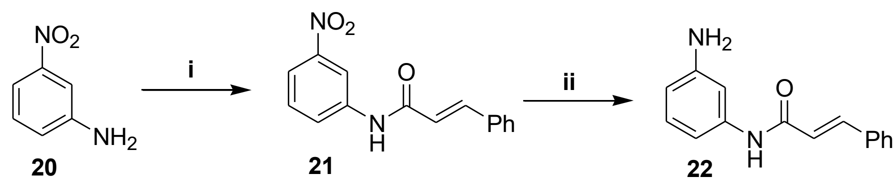
Step-II Preparation of methyl 2-((3-aminophenyl) amino)-2-oxoacetate (**19**)

The reduction of nitro group was carried out by similar procedure as described in step II for the preparation of 4-aminophenyl methane sulfonate, starting from methyl 2-((3-nitrophenyl)amino)-2-oxoacetate **18** (5.0 g) as reactant to afford methyl 2-((3-aminophenyl)amino)-2-oxoacetate (Yield: 3.12 g, 72%, Purity by HPLC: 84%).

^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 3.63 (s, 3H), 5.28 (s, 2H), 6.50 - 6.85 (m, 2H), 7.05 - 7.12 (m, 1H), 7.31 - 7.40 (m, 1H), 10.67 (s, 1H); ESI-MS: Exact mass = 194.0691, m/z $[M]^+$ peak at 194.4.

2.13.9. Synthesis of intermediate for making compound 41d

Scheme-6



Reagents and conditions: (i) PhCH=CHCOOH, EDC.HCl,HOBt, DMF, 30 °C, 2 hr; (ii) Fe, NH_4Cl, Ethanol, reflux, 1 hr

Step-I Preparation of *N*-(3-nitrophenyl)cinnamamide (**20**)

Cinnamic acid (5 g, 36.2 mmol) was dissolved in dimethylformamide (DMF; 40 mL) and treated with hydroxybenzotriazole (HOBt; 6.65 g, 43.4 mmol), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC.HCl; 13.88 g, 72.4 mmol). The mixture was stirred for 2 hr, at RT. After completion of acid activation, *m*-nitro aniline **19** (5.0 g, 36.2 mmol) was added, and the reaction was quenched in water and extracted with EtOAc. The organic layer was dried over Na_2SO_4 and the solvent was removed under reduced pressure to afford the desired title product (Yield: 7.7 g, 80 %, Purity by HPLC: 79%).

^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 7.00 (s, 1H), 7.22 (s, 1H), 7.30 – 7.41 (m, 4H), 7.80 – 7.85 (m, 4H), 8.40 – 8.42 (m, 1H), 9.22 (s, 1H); ESI-MS: Exact mass = 268.0848, m/z $[M]^+$ peak at 268.5.

Step-II Preparation of *N*-(3-aminophenyl)cinnamamide (**21**)

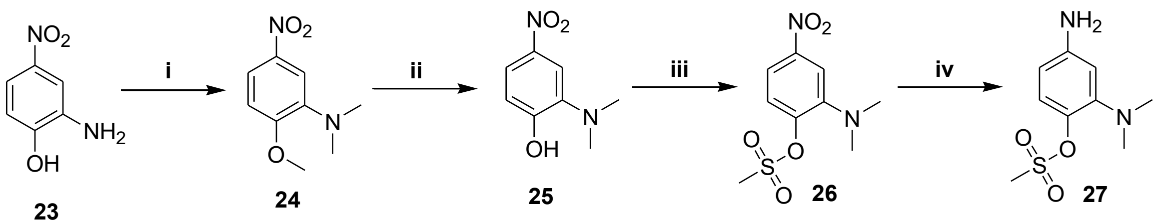
The reduction of nitro group was carried out by similar procedure as described in step II for the preparation of 4-aminophenyl methane sulfonate, starting from *N*-(3-nitrophenyl)cinnamamide **20** (6 g) as reactant to afford *N*-(3-aminophenyl)cinnamamide (Yield: 3.73 g, 70%, Purity by UPLC: 83%).

^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 5.20 (s, 2H), 6.50 – 6.81 (m, 2H), 7.01 (s, 1H), 7.30 (s, 1H), 7.40 – 7.47 (m, 2H), 7.82 – 7.90 (m, 5H), 9.28 (s, 1H); ESI-MS: Exact mass = 238.1106, m/z $[M]^+$ peak at 238.2.

Synthesis of disubstituted intermediates was carried out as described below.

2.1.3.10. Synthesis of intermediate for making compound 41n

Scheme-7



Reagents and conditions: (i) CH_3I, K_2CO_3, DMF, 30 ^oC, 2 hr; (ii) BBr_3, DCM, 30 ^oC, 2 hr; (iii) CH_3SO_2Cl, TEA, DCM, 30 ^oC, 2 hr; (iv) Fe, NH_4Cl, Ethanol, reflux, 1 hr

Step-I Preparation of 2-methoxy-*N*, *N*-dimethyl-5-nitroaniline (**24**)

To the solution of 2-amino-4-nitrophenol **23** (10 g, 64.9 mmol), Potassium carbonate (K_2CO_3; 44. 8 g, 324 mmol) in DMF 90 mL was added methyl iodide (CH_3I; 40.6 mL, 649 mmol), at 0°C, and it was stirred for 2 hr, at RT. After completion of reaction, the reaction mixture was quenched by water and the organic layer was extracted with DCM.

The organic layer was dried over Na_2SO_4 and the solvent was removed under reduced pressure to afford 2-methoxy-*N*, *N*-dimethyl-5-nitroaniline as a solid (Yield: 11.71 g, 92%, Purity by UPLC: 88%).

^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 2.77 (s, 6H), 3.94 (s, 3H), 7.12 (d, *J* = 8.8 Hz, 1H), 7.60 (d, *J* = 2.8 Hz, 1H), 7.85 (dd, *J* = 2.8 Hz, 1H); ESI-MS: Exact mass = 196.0848, m/z $[M]^+$ peak at 196.5.

Step-II Preparation of 2-(dimethylamino)-4-nitrophenol (**25**)

The solution of 2-methoxy-*N*, *N*-dimethyl-5-nitroaniline **24** (10 g, 51 mmol) in DCM (100 mL) was treated with boron tribromide (BBr_3; 14.45 mL, 153 mmol), at 0^0C. Then the reaction mixture was stirred for 2 hr, at RT. After completion of reaction, the reaction mixture was quenched by ice - water and the organic layer was extracted with DCM. The organic layer was dried over Na_2SO_4 and the solvent was removed under reduced pressure to afford 2-(dimethylamino)-4-nitrophenol as a solid (Yield: 7.89 g, 85%, Purity by HPLC: 84%). **[90]**

^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 3.00 (s, 6H), 7.15 - 7.17 (m, 2H), 8.11 (dd, *J* = 2.4 Hz, 1H), 8.32 (s, 1H); ESI-MS: Exact mass = 182.0691, m/z $[M]^+$ peak at 182.4.

Step-III Preparation of 2-(dimethylamino)-4-nitrophenyl methane sulfonate (**26**)

The title compound was prepared by similar procedure as described in step I for the preparation of 4-nitrophenyl methanesulfonate, starting from 2-(dimethylamino)-4-nitrophenol **25** as reactant (7.0 g) to afford 2-(dimethylamino)-4-nitrophenyl methanesulfonate (Yield: 8.8 g, 88%, Purity by UPLC: 80%).

^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 2.88 (s, 6H), 3.48 (s, 3H), 7.51 - 7.55 (m, 2H), 7.75 - 7.80 (m, 1H); ESI-MS: Exact mass = 260.0467, m/z $[M]^+$ peak at 260.6.

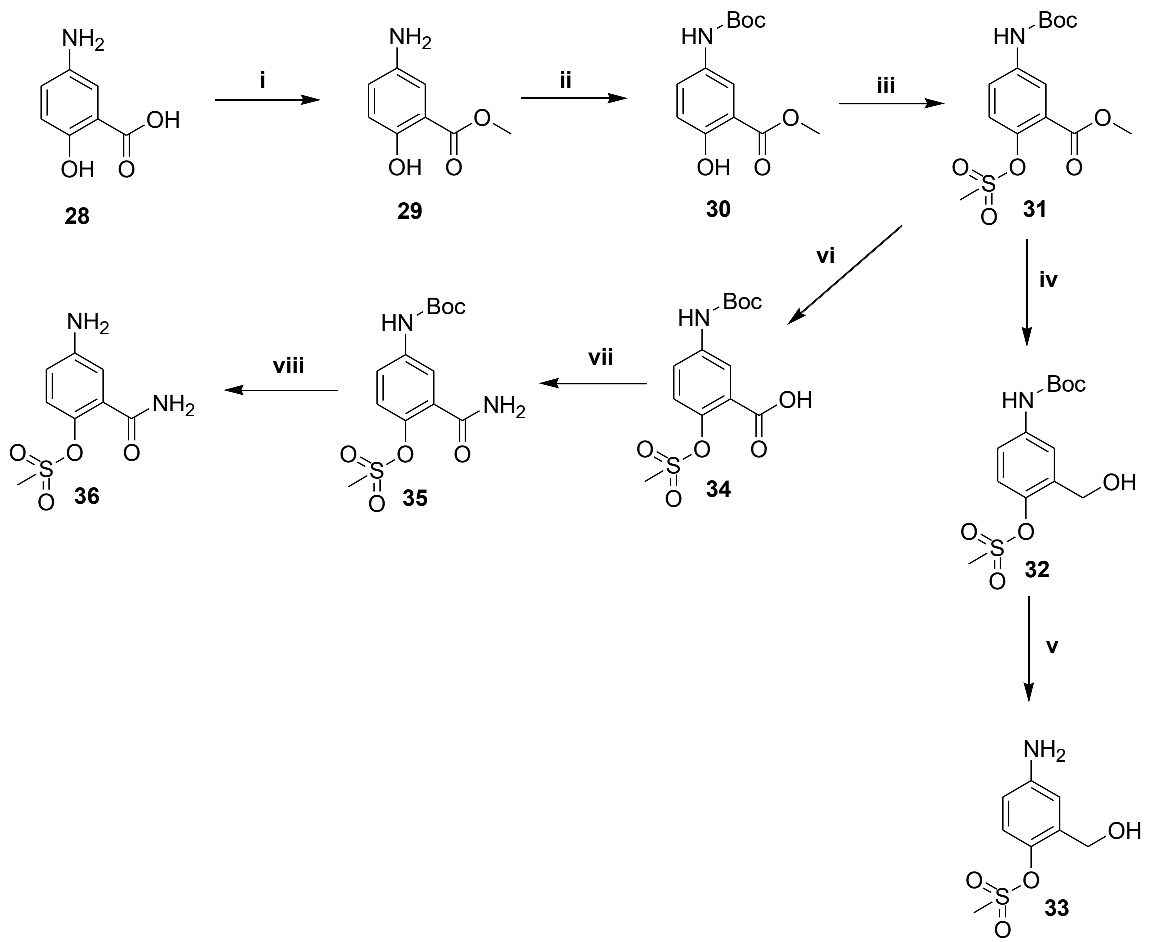
Step-IV Preparation of 4-amino-2-(dimethylamino) phenyl methane sulfonate (**27**)

The reduction of nitro group was carried out by similar procedure as described in step II for the preparation of 4-aminophenyl methanesulfonate, starting from 2-(dimethylamino)-4-nitrophenyl methane sulfonate **26** (7.0 g) as reactant to afford 4-amino-2-(dimethyl amino) phenyl methanesulfonate (Yield: 4.58 g, 74%, Purity by HPLC: 87%).

^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 3.03 (s, 6H), 3.55 (s, 3H), 5.30 (s, 2H), 6.05 (d, J = 1.5 Hz, 1H), 6.25 (d, J = 7.5 Hz, 1H), 6.98 -7.02 (m, 1H); ESI-MS: Exact mass = 230.0725, m/z $[M]^+$ peak at 230.3.

2.1.3.11. Synthesis of intermediate for making compound 41q and 41r

Scheme-8



Reagents and conditions: (i) ClCOCOCl, DMF, MeOH, 60 ^oC, 2 hr; (ii) $(BOC)_2O$, TEA, DCM, 30 ^oC, 4 hr; (iii) CH_3SO_2Cl, TEA, DCM, 30 ^oC, 2 hr; (iv) $NaBH_4$, THF, 30 ^oC, 1 hr; (v) TFA, DCM, 30 ^oC, 6 hr; (vi) $LiOH.H_2O$, THF, 30 ^oC, 5 hr; (vii) EDC.HCl, HOBt, aq NH_3, DMF, 30 ^oC, 2 hr; (viii) TFA, DCM, 30 ^oC, 6 hr

Step-I Preparation of methyl 5 amino-2-hydroxybenzoate (**29**)

As shown in **scheme-8**, to a solution of 5-aminosalicylic acid **28** (30 g, 196 mmol), methanol (MeOH; 230 mL) in DMF (0.1 mL, as catalyst) was added oxalylchloride (ClCOCOCl; 22.32 mL, 255 mmol), at 0-5^{o}C. The reaction mixture was heated at 60^{o}C for 2 hr. After completion of reaction, the mixture was quenched with water and extracted with EtOAc. 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 afford the desired title product (Yield: 26.9 g, 82 %, Purity by UPLC: 77%) as a pale yellow solid.

^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 3.84 (s, 3H), 4.80 (s, 2H), 6.71 (d, J = 8.8 Hz, 1H), 6.80 – 6.83 (m, 1H), 7.00 (d, J = 2.8 Hz, 1H), 9.74 (s, 1H); ESI-MS: Exact mass = 167.0582, m/z $[M]^+$ peak at 167.2.

Step-II Preparation of methyl 5-((*tert*-butoxycarbonyl) amino)-2-hydroxybenzoate (**30**)

TEA (25 mL, 179 mmol) was added drop wise to a solution of 5-amino-2-hydroxybenzoate **29** (25 g, 0.150 mmol) and di-*tert*-butyl dicarbonate (45 mL, 194 mmol) in DCM (250 mL) at RT and stirred for 4 hr. After completion of reaction, the mixture was quenched with water and extracted with DCM. The organic layer was dried over Na_2SO_4 and the solvent was removed under reduced pressure to afford the desired title product (Yield: 28 g, 70 %, Purity by HPLC: 80%).

^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 1.48 (s, 9H), 3.89 (s, 3H), 6.91 (d, J = 8.8 Hz, 1H), 7.46 (dd, J = 2.4 Hz, 1H), 8.02 (s, 1H), 9.30 (s, 1H), 10.20 (s, 1H); ESI-MS: Exact mass = 267.1107, m/z $[M]^+$ peak at 267.5.

Step-III Preparation of methyl 5-((*tert*-butoxycarbonyl) amino)-2-((methylsufonyl) oxy) benzoate (**31**)

The title compound was prepared, using a procedure similar as described in the preparation of 4-aminophenyl methanesulfonate, starting from methyl 5-((*tert*-butoxycarbonyl)amino)-2-hydroxybenzoate **30** (27 g) to afford methyl 5-((*tert*-butoxycarbonyl)amino)-2-((methylsufonyl) oxy) benzoate (Yield: 31.7 g, 91%, Purity by HPLC: 81%).

^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 1.47 (s, 9H), 3.34 (s, 3H), 3.83 (s, 3H), 7.38 (d, J = 9.2 Hz, 1H), 7.66 (dd, J = 2.8 Hz, 1H), 8.08 (d, J = 2.8 Hz, 1H), 9.74 (s, 1H); ESI-MS: Exact mass = 345.0882, m/z $[M]^+$ peak at 345.2.

Step-IV Preparation of 4-((*tert*-butoxycarbonyl) amino)-2-(hydroxymethyl) phenyl methanesulfonate (**32**)

To a solution of methyl 5-((*tert*-butoxycarbonyl) amino)-2-((methylsulfonyl) oxy) benzoate **31** (30 g, 87 mmol), in THF (300 mL) was added sodium borohydride ($NaBH_4$; 3.88 g, 87 mmol) at once and mixture was stirred at RT for 1 hr. After completion of reaction, the mixture was quenched with water and extracted with EtOAc. The organic layer was dried over Na_2SO_4 and the solvent was removed under reduced pressure to afford the desired title product (Yield: 22.6 g, 82 %, Purity by UPLC: 85%).

^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 1.47 (s, 9H), 3.37 (s, 3H), 4.53 (d, J = 6.0 Hz, 2H), 5.27 (t, J = 5.8 Hz, 1H), 7.20 (d, J = 8.8 Hz, 1H), 7.35 (dd, J = 2.4 Hz, 1H), 7.73 (d, J = 2.4 Hz, 1H), 9.49 (s, 1H); Exact mass = 317.0993, m/z $[M]^+$ peak at 317.4.

Step-V Preparation of 4-amino-2-(hydroxymethyl) phenyl methane sulfonate (**33**)

The solution of 4-((*tert*-butoxycarbonyl) amino)-2-(hydroxymethyl) phenyl methanesulfonate **32** (21 g, 66.2 mmol) in DCM (210 mL) was added trifluoro acetic acid (TFA; 51 mL, 662 mmol) at 0^{o}C (dropwise). Then the reaction mixture was stirred at RT for 6 hr. After completion of reaction, the reaction mixture was basified with sat $NaHCO_3$ solution and the aqueous layer was extracted with DCM. The organic layer was dried over Na_2SO_4 and the solvent was removed under reduced pressure to afford the desired title product (Yield: 13.08 g, 91 %, Purity by HPLC: 88%).

^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 3.64 (s, 3H), 4.61 (d, J = 5.6 Hz, 2H), 5.28 (s, 2H), 6.80 - 6.67 (m, 1H), 7.06 (s, 1H), 7.14 – 7.16 (m, 1H); Exact mass = 217.0409, m/z $[M]^+$ peak at 217.4.

Step-VI Preparation of 5-((tert-butoxycarbonyl) amino)-2-((methylsulfonyl) oxy) benzoic acid (**34**)

To a solution of methyl 5-((tert-butoxycarbonyl) amino)-2-((methyl sulfonyl) oxy) benzoate **31** (12 g, 34.7 mmol) in THF:H_2O (2:1, 130 mL) was added lithium hydroxide monohydrate (LiOH.H_2O; 7.29 g, 174 mmol) at RT. Mixture was stirred at the same temperature for 5 hr. After completion of hydrolysis, the reaction mixture was quenched with water and acidified (pH=3-4) by adding acetic acid. The compound was extracted with EtOAc. The organic layer was dried over Na_2SO_4 and the solvent was removed under reduced pressure to afford the desired title product (Yield: 9.21 g, 80 %, Purity by HPLC: 84%).

^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 1.52 (s, 9H), 3.40 (s, 3H), 6.67 - 6.70 (m, 1H), 7.00 – 7.03 (m, 2H), 9.80 (s, 1H); Exact mass = 331.0726, m/z $[M]^+$ peak at 331.4.

Step-VII Preparation of 4-((*tert*-butoxycarbonyl) amino)-2-carbamoyl phenyl methanesulfonate (**35**)

The above acid **34** (8.0 g, 24.14 mmol) was dissolved in DMF (60 mL) and treated with HOBt (4.44 g, 29 mmol) and EDC.HCl (9.26 g, 48.3 mmol) and mixture was stirred for 2 hr at RT. After completion of acid activation, aqueous ammonia was added till the pH of reaction became basic. Then the reaction mixture was quenched with water and extracted with EtOAc. The organic layer was dried over Na_2SO_4 and the solvent was removed under reduced pressure to afford the desired title product (Yield: 6.46 g, 81 %, Purity by UPLC: 90%).

^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 1.48 (s, 9H), 3.33 (s, 3H), 7.29 (d, J = 8.8 Hz, 1H), 7.51 - 7.57 (m, 1H), 7.70 (d, J = 2.8 Hz, 1H), 7.94 (s, 2H), 9.63 (s, 1H); Exact mass = 330.0886, m/z $[M]^+$ peak at 330.4.

Step-VIII Preparation of 4-amino-2-carbamoylphenyl methanesulfonate (**36**)

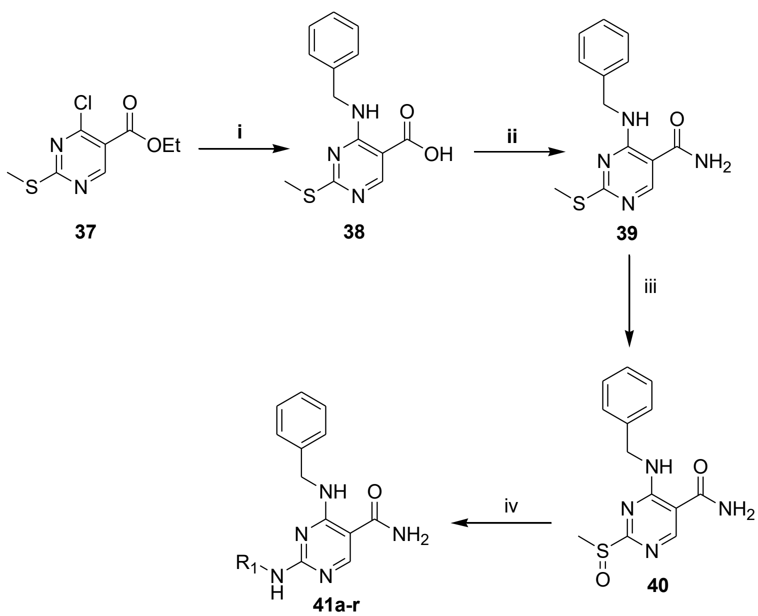
Compound **36** was prepared, using a procedure similar as described in the preparation of 4-amino-2-(hydroxymethyl)phenyl methanesulfonate **33**, starting from 4-((*tert*-butoxycarbonyl)amino)-2-carbamoyl phenyl methanesulfonate (5.0 g) to afford 4-amino-2-carbamoylphenyl methanesulfonate (Yield: 3.14 g, 90%, Purity by HPLC: 92%).

^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 3.40 (s, 3H), 5.30 (s, 2H), 7.30 - 7.40 (m, 1H), 7.50 – 7.54 (m, 2H), 7.68 – 7.71 (m, 1H), 7.80 – 7.92 (m, 1H); Exact mass = 230.0361, m/z $[M]^+$ peak at 230.6.

2.1.4. General procedure of the synthesis of title compounds 41a-r:

Synthesis of 2, 4-diaminopyrimidine-5-carboxamides derivatives (**41a-r**) was carried out as depicted in **Scheme-9**, following the modified literature procedure. **[91]** Treatment of commercially available ethyl 4-chloro-2-(methylthio) pyrimidine-5-carboxylate **37** with benzyl amine gives 4-benzylamino carboxylate, followed by hydrolysis, to get the acid moiety **38**. Compound **38** was converted into amide **39**, using HOBt and EDC.HCl, followed by reaction with aq. ammonia. Compound **39** converted to reactive methyl sulfinyl derivate **40**, using *m*-CPBA, followed by treatment with substituted aryl amines furnished compounds **41a-r**.

Scheme-9



Reagents and conditions: (i) {a} Benzyl amine, DIPEA, Dioxane, 30 ^oC, 4 hr; {b} $LiOH.H_2O$, 30 ^oC, 8 hr; (ii) EDC.HCl, HOBt, aq NH_3, DMF, 30 ^oC, 2 hr; (iii) m-CPBA, Dioxane:$CHCl_3$,-10 ^oC, 20 mins; (iv) R_1-NH_2, PTSA, NMP, 110 ^oC, 1 hr

Detailed stepwise procedure for synthesis of intermediate **38** to **40** and title compounds **41a-r**.

Step I: Preparation of 4-(Benzylamino)-2-(methylthio) pyrimidine-5-carboxylic acid (**38**)

Ethyl 4-chloro-2-(methylthio)pyrimidine 5-carboxylate **37** (13.3 g, 57.2 mmol) was dissolved in dioxane (100 mL) and *N*,*N* diisopropylethylamine (DIPEA; 10.99 mL, 62.9 mmol) was added to the reaction mixture at 0°C followed by the addition of benzyl amine (6.13 g, 57.2 mmol). The reaction mixture was stirred at 0°C for 30 mins, and then 4 hr, at RT. LiOH.H_2O (6.85 g, 286 mmol) was dissolved in 50 mL water and added to the reaction mixture and stirred for 8 hr, at 28°C. After completion of reaction, the reaction mixture was concentrated by rotary evaporation to remove a dioxane, and then acidified with dilute HCl (pH ~ 5) to get white precipitate. The solid compound filtered, washed with water and dried under vacuum to afford the 4-(benzylamino)-2 (methylthio) pyrimidine-5-carboxylic acid **38** as a white solid (Yield: 12.5 g, 79%, Purity by UPLC: 95.04%).

^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 2.38 (s, 3H), 4.68 (d, J = 6.0 Hz, 2H), 7.22 - 7.28 (m, 1H), 7.30 - 7.33 (m, 4H), 8.52 (s, 1H), 9.25 (s, 1H); Exact mass = 275.0728, m/z $[M]^+$ peak at 275.9.

Step II: Preparation of 4-(Benzylamino)-2-(methylthio) pyrimidine-5-carboxamide (**39**)

4-(Benzylamino)-2-(methylthio)pyrimidine-5-carboxylic acid **38** (12 g, 43.6 mmol) was dissolved in DMF (100 mL) under Nitrogen (inert) atmosphere and treated with HOBt (6.68 g, 43.6 mmol) and EDC.HCl (16.68 g, 87 mmol) at RT. After stirring for 1 hr, aq. Ammonia (40 mL) was added at 0°C, and it was stirred for 1 hr at RT. After completion of reaction, the reaction mixture was quenched with ice-water to get white precipitate. The solid compound was isolated by filtration, washed with water and dried under reduced pressure to afford 4-(benzylamino)-2-(methylthio) pyrimidine-5-carboxamide **39**

(Yield: 11.36 g, 95 %, Purity by UPLC: 94.20%).

^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 2.40 (s, 3H), 4.67 (d, J = 6.0 Hz, 2H), 7.23 - 7.28 (m, 1H), 7.31- 7.35 (m, 4H), 7.45 (s, 1H), 8.04 (s, 1H), 8.53 (s, 1H), 9.48 (t, J = 6.0 Hz, 1H); Exact mass = 274.0888, m/z $[M]^+$ peak at 274.8.

Step III: Preparation of 4-(Benzylamino)-2-(methylsulfinyl) pyrimidine-5-carboxamide (**40**)

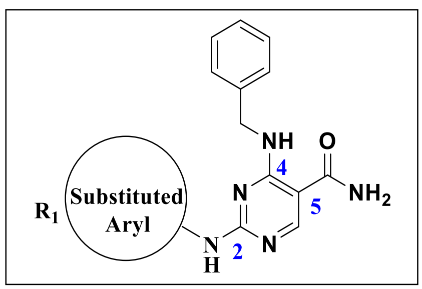
4-(Benzylamino)-2-(methylthio) pyrimidine-5-carboxamide **39** (11 g, 40.1 mmol) was dissolved in dioxane and chloroform (1:1) 280 mL. The reaction mixture was cooled up to -2°C and treated with *m*-CPBA 60% (17.3 g, 60.1 mmol) and was stirred for 20 mins at 0°C. After completion of reaction, the reaction mixture was quenched with 10% aq. sodium metabisulfite ($Na_2S_2O_5$) and organic compound 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 afford 4-(benzylamino)-2-(methylsulfinyl) pyrimidine-5-carboxamide **40** as a white solid (Yield: 9.31 g, 80%, Purity by UPLC: 99.32%).

^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 2.75 (s, 3H), 4.70 (d, J = 6.0 Hz, 2H), 7.24 - 7.31 (m, 1H), 7.33 - 7.38 (s, 4H), 7.74 (s, 1H), 8.28 (s, 1H), 8.76 (s, 1H), 9.60 (t, J = 5.6 Hz, 2H); Exact mass = 290.0837, m/z $[M]^+$ peak at 290.8.

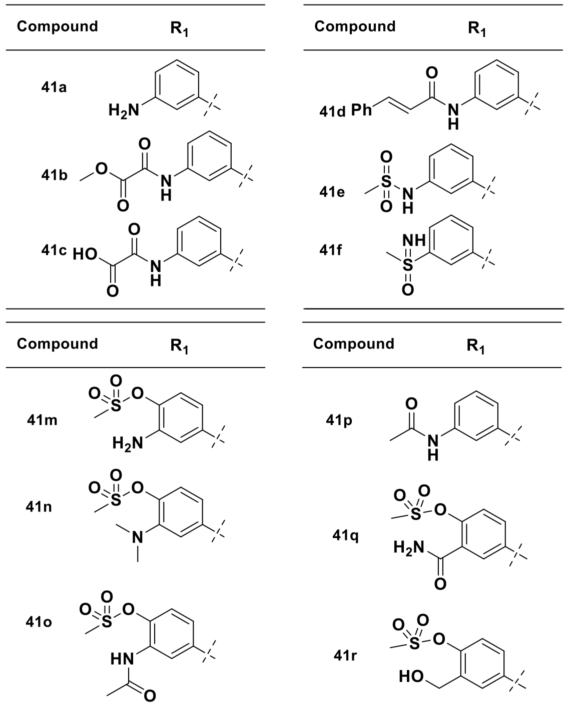
Step IV: General procedure for the synthesis of compound **41a-r.**

To a solution of 4-(benzylamino)-2-(methylsulfinyl) pyrimidine-5-carboxamide **40** (1.0 eq) and PTSA (1.1 eq), in *N*-Methyl-2-pyrrolidone (NMP) was added different substituted aryl amines (1.0 eq) at RT. (Synthesis of these substituted aryl amines were described in the previous section an intermediates for **41a-r**). 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 in EtOAc. The organic layer was dried over Na_2SO_4, filtered and concentrated under vacuum to afford 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 desired title product **41a-r**.

2.1.5. List of R_1 substituents at 2nd position of pyrimidine derivatives



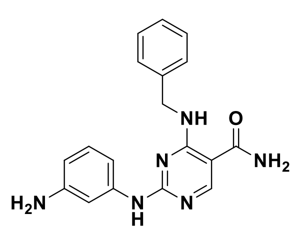
Substituent of compounds **41a-r** are listed below.



In Series-1, total 18 compounds were prepared (**41a-r**) as JAK inhibitors. All the title compounds were prepared in good yield and characterized by various spectroscopic techniques. Assigned structures were found to be confirmed with spectral data. Spectral data of **41a-r** are listed below.

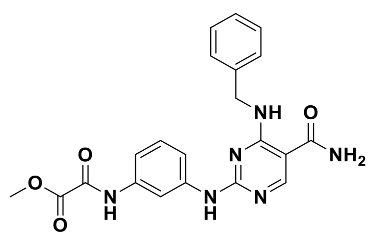
2.1.6. Spectral Data of compound 41a-r:

2.1.6.1. 2-((3-Aminophenyl) amino)-4-(benzylamino) pyrimidine -5-carboxamide (41a):



MP: 220-221°C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 4.67 (d, J = 5.6 Hz, 2H), 6.18 (d, J = 6.8 Hz, 1H), 6.82 - 6.86 (m, 2H), 6.97 (s, 1H), 7.22 - 7.35 (m, 5H), 8.51 (s, 1H), 9.20 (s, 1H), 9.47 (t, J = 6 Hz, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ ppm: 43.9, 100.5, 100.9, 103.2, 107.0, 126.6, 127.0, 128.7, 129.5, 139.9, 142.9, 148.0, 161.8, 165.1, 166.2, 169.6 (carbonyl carbon); Purity (UPLC): 96.1%; Exact mass = 334.1542, m/z $[M]^+$ peak at 334.9; Analysis (CHNS): Calculated for $C_{18}H_{18}N_6O$: C, 64.66%; H, 5.43%; N, 25.13, Found: C, 65.00%; H, 5.60%; N, 25.30%.

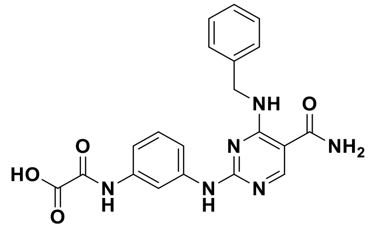
2.1.6.2. Methyl-2-((3-((4-(Benzylamino)-5-carbamoyl pyrimidin-2-yl) amino) phenyl) amino)-2-oxoacetate (41b):



MP: 226-227°C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 3.84 (s, 3H), 4.72 (d, J = 6.0 Hz, 2H), 7.18 - 7.31 (m, 8H), 8.25 (s, 1H), 8.55 (s, 1H), 9.71 (s, 2H), 10.68 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ ppm: 43.8, 51.1, 100.4, 107.8, 111.0, 113.6, 126.5, 127.1, 128.8, 129.6, 137.2, 140.0, 142.7, 155.0 and 162.2 (carbonyl carbons), 164.5, 165.9, 166.8, 169.7 (carbonyl carbon); Purity (UPLC): 97.41%; Exact mass = 420.1546, m/z $[M]^+$ peak at 420.8; Analysis (CHNS): Calculated for $C_{21}H_{21}N_6O_4$: C, 59.99%; H, 4.80%; N, 19.99%, Found: C, 60.12%; H, 4.85%; N, 20.05%.

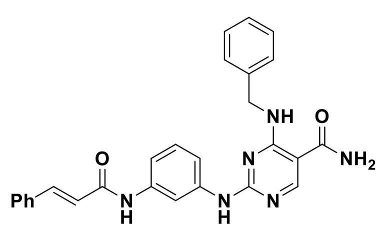
2.1.6.3. 2-((3-((4-(Benzylamino)-5-carbamoylpyrimidin-2-yl)

amino) phenyl) amino)-2-oxoacetic acid (41c):



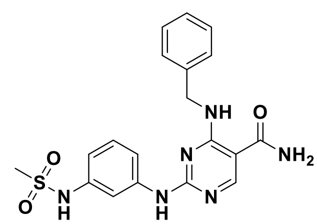
MP: 220-221°C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 4.71 (d, J = 6.0 Hz, 2H), 6.98 - 7.70 (m, 7H), 7.86 - 7.94 (m, 1H), 8.17 (s, 1H), 8.53 (s, 1H), 9.62 (s, 2H), 10.58 (s, 1H). ^{13}C NMR (DMSO-d_6, 100 MHz) δ ppm: 43.8, 100.5, 107.9, 110.9, 113.8, 126.5, 127.1, 128.8, 129.9, 137.8, 140.1, 143.0, 156.9 and 162.0 (carbonyl carbons), 164.1, 166.0, 166.9, 169.5 (carbonyl carbon); Purity (HPLC): 98.91%; Exact mass = 406.1390, m/z $[M]^+$ peak at 406.0; Analysis (CHNS): Calculated for $C_{20}H_{18}N_6O_4$: C, 59.11%; H, 4.46%; N, 20.68%, Found: C, 59.26%; H, 4.40%; N, 20.58%.

2.1.6.4. 4-(Benzylamino)-2-((3-cinnamamidophenyl) amino) pyrimidine-5-carboxamide (41d):



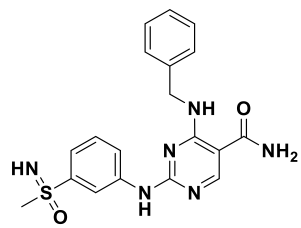
MP: 268-269°C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 4.74 (d, J = 6.0 Hz, 2H), 6.84 (s, 1H), 6.88 (s, 1H), 7.21 - 7.32 (m, 5H), 7.38 - 7.45 (m, 5H), 7.47 - 7.61 (m, 3H), 8.14 (s, 1H), 8.56 (s, 1H), 9.53 (s, 2H), 10.11 (s, 1H). ^{13}C NMR (DMSO-d_6, 100 MHz) δ ppm: 43.5, 100.4, 108.0, 111.2, 113.9, 119.0, 126.7, 127.4, 128.0, 128.4, 128.7, 128.9, 129.6, 135.6, 137.1, 139.8, 142.0, 143.2, 162.2 (carbonyl carbon), 165.8, 166.7, 167.2, 169.8 (carbonyl carbon); Purity (UPLC): 98.05%; Exact mass = 464.1961, m/z $[M]^+$ peak at 464.9; Analysis (CHNS): Calculated for $C_{27}H_{25}N_6O_2$: C, 69.81%; H, 5.21%; N, 18.09%, Found: C, 69.60%; H, 5.11%; N, 18.00%.

2.1.6.5. 4-(benzylamino)-2-((3- (methylsulfonamido) phenyl) amino) pyrimidine-5-carboxamide (41e):



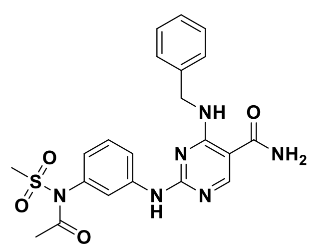
MP: 210-211°C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 3.33 (s, 3H), 4.70 (d, J = 5.6 Hz, 2H), 7.10 - 7.05 (m ,2H), 7.31 - 7.35 (m, 4H), 7.80 - 7.88 (m, 2H), 7.94 (s, 1H), 8.59 (s, 1H), 9.55 (s, 1H), 9.69 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ ppm: 40.0, 44.5, 101.0, 101.5, 107.4, 109.0, 126.5, 127.1, 128.6, 130.0, 138.3, 139.5, 143.0, 161.9, 165.5, 167.0, 169.1 (carbonyl carbon); Purity (HPLC): 97.22%; Exact mass = 412.1318, m/z $[M]^+$ peak at 412.2; Analysis (CHNS): Calculated for $C_{19}H_{20}N_6O_3S$: C, 55.33%; H, 4.89%; N, 20.38%; S, 7.77% Found: C, 55.21%; H, 4.79%; N, 20.25%; S, 7.75%.

2.1.6.6. 4-(Benzylamino)-2-((3-(S-methylsulfonimidoyl) phenyl) amino) pyrimidine-5-carboxamide (41f):



MP: 216-217°C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 2.51 (s, 3H), 4.65 (d, J = 6.0 Hz, 2H), 6.80 - 6.87 (m ,2H), 7.26 - 7.27 (m, 5H), 7.33 - 7.34 (m, 2H), 8.37 (s, 1H), 9.61 (s, 1H), 9.78 (s, 1H). ^{13}C NMR (DMSO-d_6, 100 MHz) δ ppm: 43.8, 44.2, 100.9, 112.2, 114.5, 120.3, 126.8, 127.0, 128.7, 128.9, 140.1, 142.3, 146.8, 162.0, 165.9, 168.2, 169.8 (carbonyl carbon); Purity (UPLC): 96.99%; Exact mass = 396.1368, m/z $[M]^+$ peak at 396.2; Analysis (CHNS): Calculated for $C_{19}H_{20}N_6O_2S$: C, 57.56%; H, 5.08%; N, 21.20%; S, 8.09% Found: C, 57.62%; H, 5.13%; N, 21.30%; S, 8.10%.

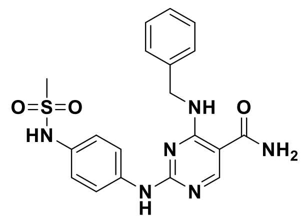
2.1.6.7. 4-(Benzylamino) -2-((3-(N-(methylsulfonyl) aceta mido) phenyl) amino)pyrimidine-5-carboxamide (41g):



MP: 219-220°C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 1.83 (s, 3H), 3.46 (s, 3H), 4.70 (d, J = 6.0 Hz, 2H), 7.03 (d, J = 7.2 Hz, 1H), 7.23 - 7.26 (m, 1H), 7.31 - 7.35 (m, 5H), 7.66 (d, J = 7.2 Hz, 1H), 7.93 (S, 1H), 8.59 (s, 1H), 9.64 (s, 1H), 9.75 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ ppm: 20.0, 43.7, 44.0, 101.2, 108.4, 113.9, 119.9, 126.9, 127.2, 128.6, 129.1, 131.5, 140.0, 142.5, 162.3, 166.2, 166.5, 168.5 and 169.7 (carbonyl carbons); Purity (HPLC): 97.89%; Exact mass =

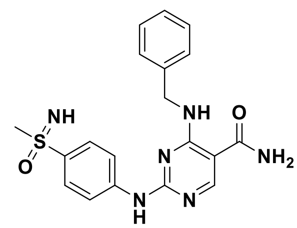
454.1423, m/z $[M+H]^+$ peak at 455.0; Analysis (CHNS): Calculated for $C_{21}H_{22}N_6O_4S$: C, 55.50%; H, 4.88%; N, 18.49%; S, 7.05% Found: C, 55.45%; H, 4.80%; N, 18.39%; S, 7.00%.

2.1.6.8. 4-(Benzylamino)-2-((4-(methylsulfonamido) phenyl) amino) pyrimidine-5-carboxamide (41h):



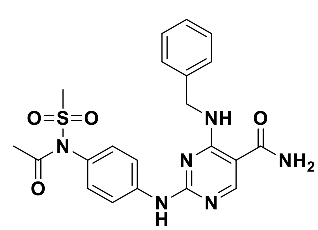
MP: 214-215°C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 2.90 (s, 3H), 4.67 (d, J = 6.0 Hz, 2H), 7.05 (d, J = 9.2 Hz, 2H), 7.32 - 7.33 (m, 5H), 7.58 (d, J = 8.8 Hz, 2H), 8.54 (s, 1H), 9.39 (s, 1H), 9.45 (s, 1H), 9.47 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ ppm: 43.9, 43.6, 100.8, 117.9, 118.6, 126.6, 126.9, 127.8, 128.2, 128.9, 139.8, 162.5, 166.2, 168.5, 169.8 (carbonyl carbon); Purity (HPLC): 98.12%; Exact mass = 412.1318, m/z $[M]^+$ peak at 412.8; Analysis (CHNS): Calculated for $C_{19}H_{20}N_6O_3S$: C, 55.33%; H, 4.89%; N, 20.38%; S, 7.77% Found: C, 55.40%; H, 4.91%; N, 20.45%; S, 7.80%.

2.1.6.9. 4-(Benzylamino)-2-((4-(S-methylsulfonimidoyl) phenyl) amino) pyrimidine-5-carboxamide (41i):



MP: 218-219°C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 2.50 (s, 3H) 4.41 (d, J = 6.0 Hz, 2H), 6.64 (d, J = 8.8 Hz, 2H), 7.22 - 7.32 (m, 5H), 7.54 (d, J = 8.0 Hz, 2H), 8.45 (s, 1H), 9.42 (s, 1H), 9.65 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ ppm: 43.8, 45.2, 101.7, 115.0, 126.2, 126.5, 126.7, 128.0, 136.2, 140.0, 141.3, 162.4, 166.1, 168.8, 169.5 (carbonyl carbon); Purity (UPLC): 97.64%; Exact mass = 396.1368, m/z $[M]^+$ peak at 396.8; Analysis (CHNS): Calculated for $C_{19}H_{20}N_6O_2S$: C, 57.56%; H, 5.08%; N, 21.20%; S, 8.09% Found: C, 57.45%; H, 5.00%; N, 21.00%; S, 7.92%.

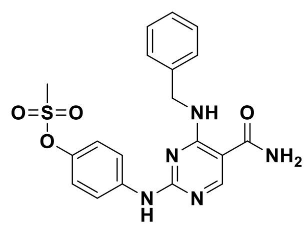
2.1.6.10. 4-(Benzylamino)-2-((4-(N-(methylsulfonyl) aceta mido) phenyl)amino)pyrimidine-5-carboxamide (41j):



MP: 225-226°C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 1.88 (s, 3H), 3.49 (s, 3H), 4.70 (d, J = 6.0 Hz, 2H), 7.22 - 7.31 (m, 5H), 7.33 - 7.36 (m, 2H), 7.78 (d, J = 8.8 Hz, 2H), 8.58 (s, 1H), 9.57 (t, J = 6.0 Hz, 1H), 9.74 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ ppm: 19.5, 42.9, 43.7, 100.5, 117.6, 119.6, 121.8, 126.4, 126.9, 127.9, 139.7, 140.1, 162.8, 166.9, 167.2, 168.8 and 169.2 (carbonyl carbons);

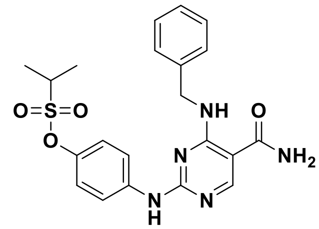
Purity (HPLC): 96.98%; Exact mass = 454.1423, m/z $[M]^+$ peak at 454.9; Analysis (CHNS): Calculated for $C_{21}H_{23}N_6O_4S$: C, 55.50%; H, 4.88%; N, 18.49%; S, 7.05% Found: C, 55.62%; H, 4.94%; N, 18.55%; S, 7.15%.

2.1.6.11. 4-((4-(Benzylamino)-5-carbamoylpyrimidin-2-yl) amino) phenyl methanesulfonate (41k):



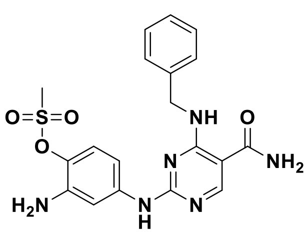
MP: 228-229°C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 3.33 (s, 3H) 4.68 (d, J = 5.6 Hz, 2H), 7.17 - 7.27 (m, 3H), 7.34 - 7.43 (m, 4H), 7.73 (d, J = 8.4 Hz, 2H), 8.56 (s, 1H), 9.57 (s, 1H), 9.66 (s, 1H). ^{13}C NMR (DMSO-d_6, 100 MHz) δ ppm: 37.5, 44.0, 100.5, 120.5, 122.6, 127.4, 127.8, 128.9, 139.8, 143.5, 157.7, 160.2, 161.9, 169.4 (carbonyl carbon); Purity (UPLC): 99.15%; Exact mass = 413.1158, m/z $[M]^+$ peak at 413.9; Analysis (CHNS): Calculated for $C_{19}H_{20}N_5O_4S$: C, 55.20%; H, 4.63%; N, 16.94%; S, 7.75% Found: C, 55.15%; H, 4.60%; N, 16.91%; S, 7.70%.

2.1.6.12. 4-((4-(Benzylamino)-5-carbamoylpyrimidin-2-yl) amino) phenyl propane-2-sulfonate (41l):



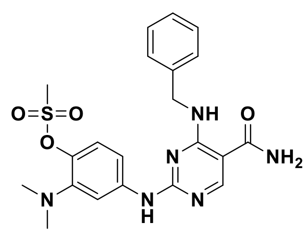
MP: 234-235°C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 1.41 (d, J = 6.8 Hz, 6H), 3.64 - 3.67 (m, 1H), 4.68 (d, J = 6.0 Hz, 2H), 7.13 (d, J = 9.2 Hz, 2H), 7.23 - 7.26 (m, 1H), 7.33 - 7.34 (m, 4H), 7.71 (d, J = 9.2 Hz, 2H), 8.56 (s, 1H), 9.55 (s, 1H), 9.65 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ ppm: 16.0, 43.8, 49.8, 100.7, 121.0, 122.4, 127.3, 127.7, 128.6, 138.9, 142.9, 158.0, 160.9, 162.2, 169.8 (carbonyl carbon); Purity (UPLC): 98.05%; Exact mass = 441.1471, m/z $[M+H]^+$ peak at 442.1; Analysis (CHNS): Calculated for $C_{21}H_{23}N_5O_4S$: C, 57.13%; H, 5.25%; N, 15.86%; S, 7.26% Found: C, 57.20%; H, 5.31%; N, 15.90%; S, 7.32%.

2.1.6.13. 2-Amino-4-((4-(benzylamino)-5-carbamoylpyrimidiyl) amino)phenylmethanesulfonate (41m):



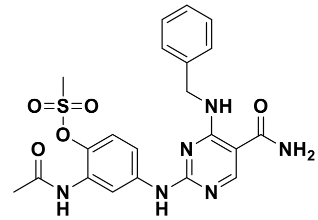
MP: 237-238°C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 3.33 (s, 3H) 4.68 (d, J = 6.0 Hz, 2H), 5.18 (s, 2H), 6.87 - 6.94 (m, 2H), 7.13 - 7.25 (m, 2H), 7.30 - 7.36 (m, 4H), 8.53 (s, 1H), 9.37 (s, 1H), 9.49 (t, J = 5.8 Hz, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ ppm: 37.9, 44.0, 100.1, 107.1, 108.1, 123.2, 127.3, 127.7, 128.8, 130.7, 139.9, 140.0, 141.1, 157.7, 160.4, 161.9, 169.5 (carbonyl carbon); Purity (HPLC): 98.02%; Exact mass = 428.1267, m/z $[M]^+$ peak at 428.8; Analysis (CHNS): Calculated for $C_{19}H_{20}N_6O_4S$: C, 53.26%; H, 4.71%; N, 19.61%; S, 7.48% Found: C, 53.18%; H, 4.63%; N, 19.50%; S, 7.33%.

2.1.6.14. 4-((4-(Benzylamino)-5-carbamoylpyrimidin-2-yl) amino) -2-(dimethylamino) phenyl methanesulfonate (41n):



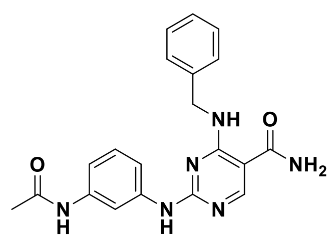
MP: 242-243°C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 2.67 (s, 6H), 3.32 (s, 3H), 4.72 (d, J = 5.6 Hz, 2H), 7.05 (d, J = 8.8 Hz, 1H), 7.23 - 7.29 (m, 2H), 7.33 - 7.36 (m, 4H), 7.57 (s, 1H), 8.57 (s, 1H), 9.55 (s, 2H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ ppm: 37.4, 42.9, 44.2, 100.9, 107.7, 108.4, 127.1, 127.9, 128.5, 130.4, 140.0, 140.2, 143.8, 157.3, 160.7, 161.4, 169.2 (carbonyl carbon); Purity (UPLC): 96.87%; Exact mass = 456.1580, m/z $[M]^+$ peak at 456.9; Analysis (CHNS): Calculated for $C_{21}H_{25}N_6O_4S$: C, 55.25%; H, 5.30%; N, 18.41%; S, 7.02% Found: C, 55.40%; H, 5.39%; N, 18.53%; S, 7.14%.

2.1.6.15. 4-((4-(Benzylamino)-5-carbamoylpyrimidin-2-yl) amino) -2-carbamoylphenyl methanesulfonate (41o):



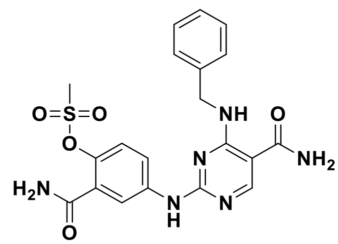
MP: 250-251°C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 2.05 (s, 3H), 3.33 (s, 3H), 4.71 (d, J = 6.0 Hz, 2H), 7.19 - 7.26 (m, 6H), 7.47 (d, J = 7.6 Hz, 1H), 8.31 (s, 1H), 8.57 (s, 1H), 9.50 (s, 1H), 9.54 (s, 1H), 9.67(s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ ppm: 23.8, 38.1, 43.9, 100.7, 109.1, 116.2, 123.1, 127.2, 127.5, 128.8, 131.2, 139.6, 139.8, 142.0, 157.7, 160.3, 161.9, 163.2, 169.4 (carbonyl carbon); Purity (UPLC): 96.67%; Exact mass = 470.1372, m/z $[M]^+$ peak at 470.1; Analysis (CHNS): Calculated for $C_{21}H_{23}N_6O_5S$: C, 53.61%; H, 4.71%; N, 17.86%; S, 6.81% Found: C, 53.68%; H, 4.81%; N, 17.94%; S, 6.85%.

2.1.6.16. 2-acetamido-4-((4-(benzylamino)-5-carbamoyl pyr imidin-2-yl) amino) phenyl methanesulfonate (41p):



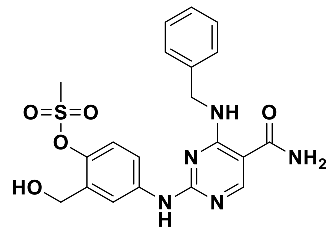
MP: 231-232°C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 2.01 (s, 3H), 4.71 (d, J = 5.7 Hz, 2H), 7.10 - 7.13 (m, 2H), 7.19 - 7.35 (m, 6H), 7.99 (s, 1H), 8.53 (s, 1H), 9.59 (s, 2H), 9.84 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ ppm: 23.9, 42.1, 102.3, 108.2, 111.2, 113.0, 126.4, 128.9, 139.5, 142.5, 162.0, 166.3, 168.6 and 169.9 (carbonyl carbons); Purity (HPLC): 97.00%; Exact mass = 376.1648, m/z $[M+H]^+$ peak at 377.1; Analysis (CHNS): Calculated for $C_{20}H_{20}N_6O_2$: C, 63.82%; H, 5.36%; N, 22.33%; Found: C, 63.95%; H, 5.44%; N, 22.42%

2.1.6.17. 4-((4-(benzylamino)-5-carbamoylpyrimidin-2-yl) amino)-2-carbamoylphenyl methanesulfonate (41q):



MP: 255-256°C; [1]H NMR (DMSO-d_6, 400 MHz) δ ppm: 3.23 (s, 3H), 4.71 (d, J = 5.6 Hz, 2H), 7.24 - 7.26 (m, 2H), 7.30 - 7.33 (m, 4H), 7.59 (s, 1H), 7.74 – 7.78 (m, 2H), 8.04 (d, J = 2.4 Hz, 1H), 8.56 (s, 1H), 9.68 (s, 1H), 9.88 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ ppm: 37.0, 42.6, 102.0, 113.5, 116.5, 120.2, 125.4, 126.5, 128.4, 135.2, 139.6, 144.8, 162.1, 166.7, 168.5 and 169.5 (carbonyl carbons); Purity (UPLC): 98.89%; Exact mass = 456.1216, m/z $[M+H]^+$ peak at 457.0; Analysis (CHNS): Calculated for $C_{20}H_{20}N_6O_5S$: C, 52.62%; H, 4.42%; N, 18.41%; S, 7.02% ; Found: C, 52.55%; H, 4.30%; N, 18.33%; S, 6.80%.

2.1.6.18. 4-((4-(benzylamino)-5-carbamoylpyrimidin-2-yl) amino)-2-(hydroxymethyl) phenyl methanesulfonate (41r):



MP: 265-266°C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 3.40 (s, 3H), 4.53 (d, J = 5.6 Hz, 2H), 4.72 (d, J = 6.0 Hz, 2H), 5.27 (t, J = 5.4 Hz, 1H), 7.16 (d, J = 9.2 Hz, 1H), 7.25 (d, J = 6.8 Hz, 1H), 7.34 (m, 4H), 7.61 (m, 1H), 8.05 (d, J = 2.4 Hz, 1H), 8.56 (s, 1H), 9.53 (s, 1H), 9.68 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ ppm: 37.2, 45.2, 60.0, 102.1, 116.1, 121.1, 126.6, 128.4, 129.8, 135.0, 139.7, 141.5, 162.5, 166.8, 168.4, 169.7 (carbonyl carbon); Purity (UPLC): 99.12%; Exact mass = 443.1263, m/z $[M+H]^+$ peak at 444.1; 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, 4.65%; N, 15.80%; S, 7.20%.

From Series-1, all the 18 compounds (**41a-r**) was subjected to *in-vitro* JAK3 inhibitory activity screening. Detailed assay protocol and JAK3 inhibitory activity data described in the next section.

2.2. *In-vitro* JAK3 Inhibitory activity data of pyrimidine derivatives modified at 2nd position

Determination of JAK3 assay

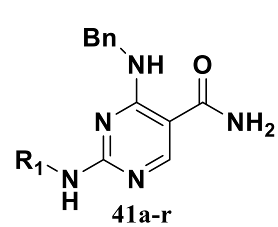
In-vitro screening of compounds **41a-r** was carried out, using a fluorogenic substrate assay.

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]**

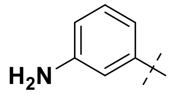
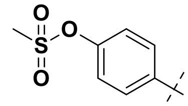
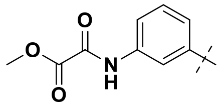
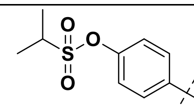
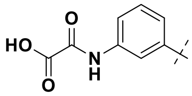
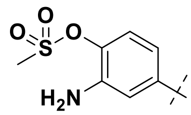
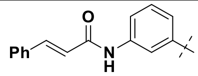
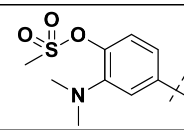
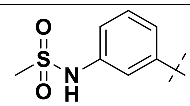
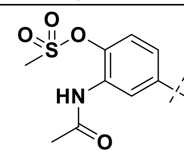
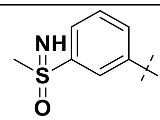
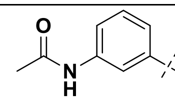
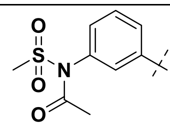
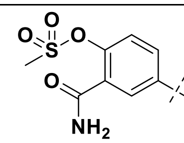
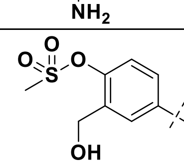
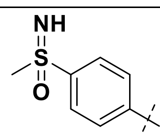
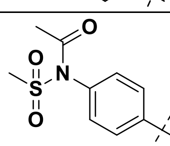
For *in-vitro* JAK3 inhibitory activity screening, Cerdulatinib and Tofacitinib were used as reference molecules. In Series-1 2-substituted diaminopyrimidine-5-carboxamides (**41a-r**) analogues displayed varying degree of JAK3 inhibitory activities. Results are represented in **Table 3**.

Table 3. *Effect of substituent at 2nd position of Pyrimidine moiety on JAK3 inhibitory activity (In-vitro)*



41a-r

Comp.	R_1	JAK-3 IC_{50} (nM)[a]	Comp.	R_1	JAK-3 IC_{50} (nM)[a]

41a		12.6	**41k**		9.5
41b		33.4	**41l**		22.4
41c		53.7	**41m**		56
41d		178	**41n**		66
41e		122	**41o**		89
41f		136	**41p**		100
41g		132	**41q**		50
41h		12.4	**41r**		98
41i		48.4	**Cerdulatinib**		8
41j		10.2	**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

meta-Substituted *N*-phenyl derivatives showed moderate to poor JAK3 inhibitort activity. Compound **41a** (*m*-aniline) with the IC_{50} of 12.6 nM showed moderate JAK3 inhibitory activity. Whereas compound **41b** having an ester group at the *meta* position (oxopropanamide) with the

IC_{50} of 33.4 nM showed three-fold less potency than compound **41a**. Acidic group at *meta* position **41c** (oxoaceticacid, IC_{50}: 53.7 nM) showed less potency than compound **41a** and **41b**.

Introduction of a bulky group at *meta* position **41d** (cinnamamide, IC_{50}: 178 nM) resulted in lowering inhibitory activity. Compound **41e** (methanesulfonamide) with the IC_{50} of 122 nM showed tenfold less potency than compound **41a**. Compound **41f** (sulfoximine, IC_{50}: 136 nM) had less potency. Compound **41g** (sulfonylacetamide) showed poor JAK3 inhibitory activity with the IC_{50} of 132 nM. This data shows that any substitition on $-NH_2$ at the *meta* position leads to less active compounds.

para-Substituted *N*-phenyl derivatives shows better potency than *meta* substituted derivatives. Compounds **41h** and **41i** (positional isomer of **41e** and **41f**) exhibited enhanced JAK3 inhibitory activity (IC_{50}: 56 and 48 nM). In contrast, Compound **41j** (acetyl protection of **41h**) displayed moderate JAK3 activity (IC_{50}: 102 nM). Replacement of the sulfonamide group (**41h**) to methane sulfonate (**41k**, IC_{50}: 9.5 nM) and iso-propyl sulfonate (**41l**, IC_{50}: 22.4 nM) led to higher JAK3 inhibitory activity.

Whereas *ortho*-substitution with the electron donating groups such as amino (**41m**, IC_{50}: 56 nM), di-methylamine (**41n**, IC_{50}: 66 nM) and *N*-acetyl derivative (**41o**: IC_{50}: 89 nM) displayed comparatively weaker JAK3 inhibitory activity. *meta*-Substituted compound **41P** showed less potency with the IC_{50} of 100 nM. However, *ortho*-substituted compounds **41q** (*N*-acetyl, IC_{50}: 50 nM) and **41r** (alcohol, IC_{50}: 98 nM) displayed moderate JAK3 activity.

2.3. Conclusion

In Series-1 modification at C2-position of pyrimidine ring led to an identification of a single digit nM potent JAK3 inhibitor. Electron donating group like amino at *meta* position displayed good JAK3 inhibitory activity. Oxoacetate and oxoacetic acid group at *meta* position showed moderate activity. Bulky group and electron withdrawing group like sulfonamide, sulfoximine at *meta* position were found to be less potent. Electron withdrawing group sulfonamide, sulfonylacetamide and mesylate led to higher JAK3 inhibitory activity. Sulfoximine at the *para* position showed moderate activity. Disubstituted (*para* and *meta*) compounds with mesylate at *para* position and electron donating group like amino, dimethyl amino, methylalcohol group at *meta* position leads to the moderate active compounds. Mesylate at *para* position and elcectron withdrawing group acyl amino, aminocarbonyl at *meta* position displayed moderate activity. Removal of mesylate from the *para* position and presence of acyl amino group at the *meta* position led to an inactive compound.

This Series gave four compounds **41a**, **41h**, **41j** and **41k** which were equipotent as Cerdulatinib. Compound **41k** (methane sulfonate) showed most potent JAK3 inhibitory activity, which was found to be comparable to Cerdulatinib (IC_{50}: 8 nM; **Table 3**) and 5-fold less potent compared to Tofacitinib (IC_{50}: 1.6 nM). Thus, in the Series-1, substitution on *p*-position of phenyl ring, followed by replacement of *N*-acetyl to *O*-sulfonate led to the single digit nM potent compound (**41k**). Compound **41k** was selected for further modification at the 4th position (as described in **Chapter-3**) and detailed biological evaluation (as described in **Chapter-5**).