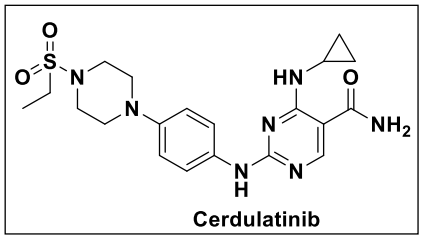
6. Overall summary and Future plans

Overall summary of current investigation

Inflammation is a defense mechanism of the body. Acute and chronic inflammatory conditions, such as Rheumatoid arthritis (RA), affects >1% of the adult population worldwide. RA is an autoimmune disorder in which our immune system attacks our body's own tissues. This creates inflammation thereby the inside of joints (the synovium) get thicken, leading to swelling and pain in the joints. Current medications for RA mainly include NSAIDs, Corticosteroids, DMARDs and Biologic agents. The first of a new kind of DMARDs, JAK inhibitors called Tofacitinib, was approved in 2012, discovered by the Pfizer. For the treatment of RA, biologic agents were also developed but most of them as injected. These biological drugs increase the risk of infections. The high cost and associated immunological adverse effects of existing medications available for RA treatment have triggered discovery of small molecules based therapy. Till today very few small molecules like Tofacitinib is available for the treatment of RA. Common adverse events associated with the Tofacitinib include nasopharyngitis, diarrhea, headache, infection, malignancies, lymphoma, increase blood pressure, abnormal blood test, cold symptoms, night sweat and risk of blood clots.

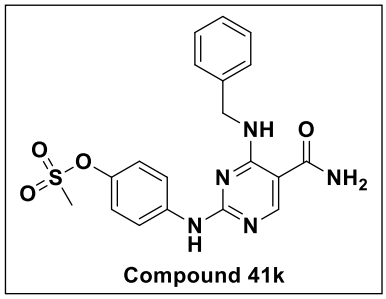
Currently, there is an unmet need to develop safe, cost-effective and efficacious small molecules as JAK3 selective inhibitors. To address these issues, we have designed a novel series of JAKs inhibitors to develop next generation therapies for the treatment of RA.

The JAK family of enzymes (JAK1, JAK2, JAK3 and tyrosine kinase 2 (TYK2)) are cytoplasmic protein tyrosine kinase, associated with various cytokines-mediated signal transduction pathways. JAK transduce cytokine mediated signals via the JAK-STAT (Janus kinase-signal transducers and activators of transcription) pathway. JAK3 is expressed in lymphoid cells, drives pro-inflammatory signaling cascades, inducing cytokine expression in the synovial fibroblasts, activated monocytes and macrophages. JAK3 pairs with the JAK1, and it is involved in common gamma chain (γc) cytokine (IL-2, IL-4, IL-7, IL-9, IL-15 and IL-21) signaling pathways, which play important role in the T-cell differentiation, proliferation and survival. Selective JAK3 inhibition only deters common gamma (γc) chain receptors signaling and spares JAK1 dependent immunoregulatory cytokines (IL-10, IL-27 and IL-35). Thus, JAK3 selective inhibitors are likely to offer a better efficacy to the safety ratio in the clinic for the treatment of chronic inflammatory disorders.



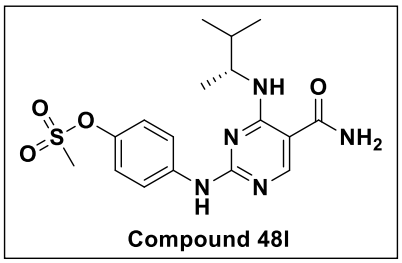
Diaminopyrimidine (DAP) represents a promising pharmacophore. We selected Cerdulatinib (having DAP moiety as a key pharmacophore) to design JAK3 selective inhibitor. Initial modifications were carried out at the C2 position of pyrimidine ring (at R_1 position) in Cerdulatinib, with C4 cyclopropyl of Cerdulatinib altered with a benzyl group. Various intermediates have been synthesized in Series-1.

Synthesis of compounds **41a-r** was accomplished in four steps from readily available ethyl 4-chloro-2-(methylthio)pyrimidine 5-carboxylate. Compounds **41a-r** were prepared in good yield (60-80%) under the mild reaction conditions. This series gave four compounds **41a**, **41h**, **41j** and **41k** which were found to be 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) and 5-fold less potent compared to Tofacitinib (IC_{50}: 1.6 nM). Compound **41k** was selected for further modifications at the 4th position.



Compound 41k

In the second series, changes were carried out at the C4-position of the **41k**. Synthesis of compounds **48a-v** was accomplished in five steps from readily available ethyl 4-hydroxy-2-(methylthio)pyrimidine-5-carboxylate. Compounds **48a-v** were prepared in good yield (60-80%) under the mild reaction conditions. Modification at 4th position of pyrimidine ring of selected compound (**41k** IC_{50} : 9.5 nM) gave compound **48l** with IC_{50}: 1.7 nM, which was found to be much better compared to Cerdulatinib (IC_{50}: 8.0 nM) and it showed JAK3 inhibitory activity as similar as Tofacitinib (IC_{50}: 1.7 nM).



Compound 48l

Further, in the third series, changes were carried out at the C5 position of **48l** which leads to compounds **54a-m**, **55** and **56**. Synthesis of compounds **54a-m**, **55** and **56** was accomplished in five steps, starting from readily available ethyl 4-chloro-2-(methylthio)pyrimidine 5-carboxylate. Compounds **54a-m**, **55** and **56** were prepared in good yield (60-80%) under the mild reaction conditions. Modification at the 5^{th} position of compound **48l** resulted in loss of activity when the aminocarbonyl group was substituted with other groups, which indicates that the aminocarbonyl group is essential for JAK3 inhibitory activity.

Overall, in this research work, total 54 NCEs have been synthesized. In the first series, 18 (**41a-r**) NCEs have been synthesized. In the second series, 22 (**48a-v**) NCEs have been synthesized. In the third series, 14 (**54a-m**, **55** and **56**) NCEs have been synthesized.

Overall, these three series lead to the identification of compound **48l**, which showed the highest potency (IC_{50}: 1.7 nM) among all the synthesized compounds. Therefore, compound **48l** was selected for detailed biological evaluation. **48l** showed greater JAK3 selectivity over other kinase tested. When assessed in the PBMC assay, **48l** showed preferential inhibition of JAK3 over JAK1 in the JAK/STAT pathway. **48l**

showed 75 to 80% plasma protein binding and less than 10% metabolism at 30 minutes, in the liver microsomal metabolic stability study. **48l** was also found to be devoid of CYP and hERG liabilities.

48l showed higher AUC, extended $t_{1/2}$, good oral bioavailability and low clearance compared to the standard. **48l** showed improved isoform selectivity in the biochemical assay. Compound **48l** selectively inhibits JAK3 cytokine signaling in the primary cell, which translates to the promising efficacy in animal models (AIA and CIA) of RA. In the repeat dose acute toxicity study, **48l** showed no adverse changes related to gross pathology, clinical signs and liver toxicity. In docking studies **48l** showed additional interaction with Cys909 through a hydrogen bond between NH of Cys909 with oxygen of methyl sulfonate group, which was not observed with the Cerdulatinib. An additional interaction of **48l** with Cys909 might contribute towards its potent and selective JAK3 inhibitory activity, indicating that the new class of JAK3 selective inhibitor could be a viable therapeutic option for the treatment of RA.

Future plans

Compound **48l** showed excellent JAK3 inhibitory activities (*in-vitro*, *ex-vivo and in-vivo)*, The PK and safety profile of **48l** was found to be satisfactory with respect to the standard compound and therefore **48l** represents a promising candidate for further exploration. Future work includes some additional pre-clinical studies before it can be taken for clinical development. Compound **48l** should be subjected for chronic efficacy studies and for long term toxicological evolution, along with its PK profiling in higher animals such as dog or monkey. If all the results of biological studies will be satisfactory, then we will proceed with the IND enabling studies of compound **48l** as a novel,

potent, selective JAK3 inhibitor for the safe and effective treatment of inflammatory diseases such as RA.