1. Introduction

1.1. Inflammation

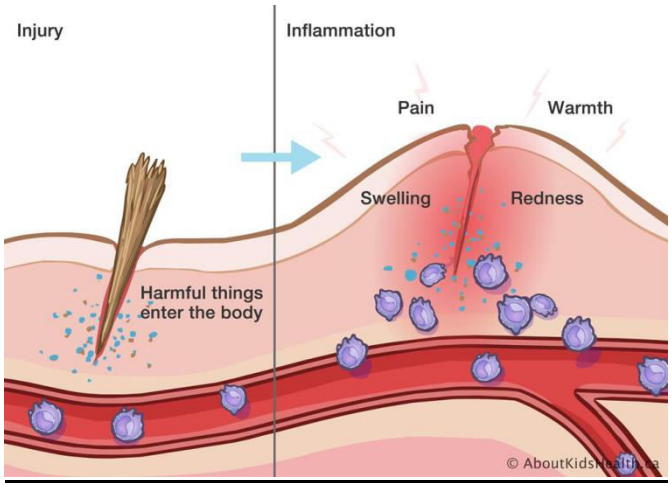


Figure 1. Inflammation

Inflammation (**Figure 1**) is a defence mechanism of a body. **[1]** Which is a complex biological response of the body tissues to harmful stimuli, such as pathogens, damaged cells, or irritants. It acts as a protective response, involving immune cells, blood vessels, and molecular mediators. When inflammation occurs, the chemicals from white blood cells (WBC) release into the blood or affected tissues. This release of chemicals increases the blood flow to the area of injury or infected area and may result in redness and warmth. Some of the chemicals cause fluid to leak into tissues, resulting in swelling. The inflammatory process may stimulate nerves and cause pain sensation. **[2, 3]**

1.1.1. Causes of Inflammation

Many factors can lead to inflammation, such as: infective agents, like bacteria, viruses and their toxins; fungi and parasites. Immunological agents like cell-mediated and antigen antibody reactions. Physical agents

like heat, cold, radiation and mechanical trauma. Chemical agents like organic and inorganic poisons. Inert materials such as foreign bodies. **[4]**

1.1.2. Symptoms of Inflammation

Symptoms of inflammation includes **[5]**:

- Rubor (redness)
- Tumor (swelling)
- Calor (heat)
- Dolor (pain)
- Loss of function

1.1.3. Classification of inflammation

Inflammation can be classified into two categories: Acute and Chronic inflammation. Chronic inflammation can be again classified into several diseases as shown in the **Figure 2**. Acute versus chronic inflammation are distinguished by the duration and the type of infiltrating inflammatory cells. **[6]**

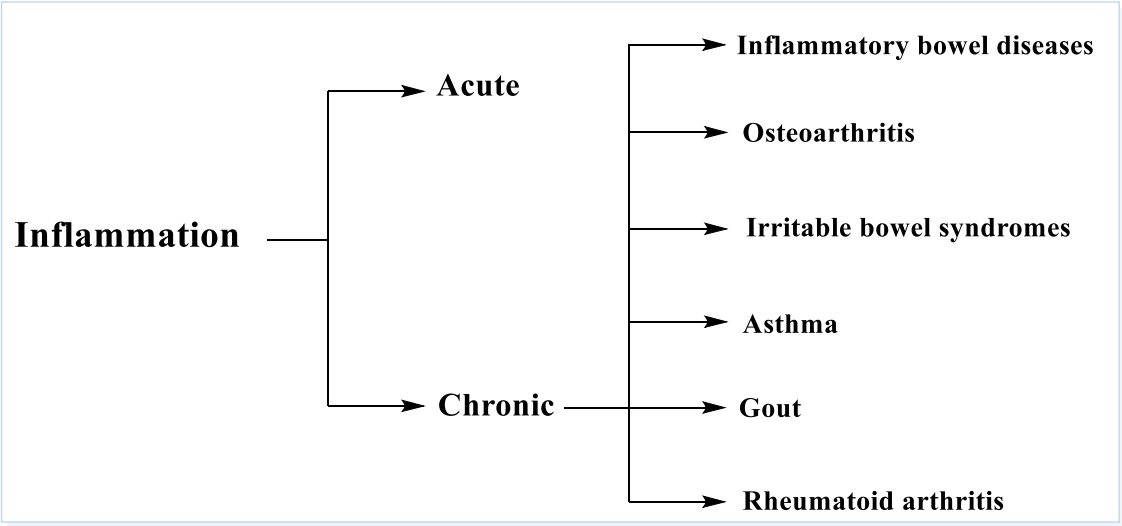


Figure 2. Classification of Chronic inflammation

1.1.3.1. Acute inflammation:

Acute inflammation starts in seconds to minutes, after tissue injury. The damage may be purely physical, or it may involve the activation of an immune response. It starts by exudation of fluids and plasma proteins and involves migration of leukocytes, particularly neutrophils into the injured area. An acute inflammatory response is a defence mechanism aimed to kill bacteria, virus and parasites. Many 1^{st} and 2^{nd} generation NSAIDs (nonsteroidal anti-inflammatory drugs) and steroids are available in the market for the treatment of acute inflammation such as Paracetamol, Aspirin, Diclofenac, Ibuprofen, Indomethacin, Rofecoxib, Celecoxib, Parecoxib, Etoricoxib, Prednisone, Dexamethasone etc. **[7]**

1.1.3.2. Chronic inflammation:

Chronic inflammation is an inflammatory reaction that lasts for months to years. Historically, it is characterized by the presence of lymphocytes and macrophages, resulting in fibrosis and tissue necrosis. Long term chronic inflammation lead to the development of a degenerative illness such as Irritable bowel diseases (IBD), Irritable bowel syndromes (IBS), Osteoarthritis (OA), Asthma, Gout and Rheumatoid arthritis (RA) that are connected with immunological changes. There are numerous inflammatory diseases of unknown etiology which are distinguished simply by signs. A few chronic inflammations are the result of self-replicating parasite like bacterium, virus or neoplasm. The inflammation may additionally end up greater complex because of persisting harmful agents or their degraded products. Some of the chronic inflammatory diseases are described in detail, in the next section. **[8]**

(a)Inflammatory Bowel Disease (IBD):

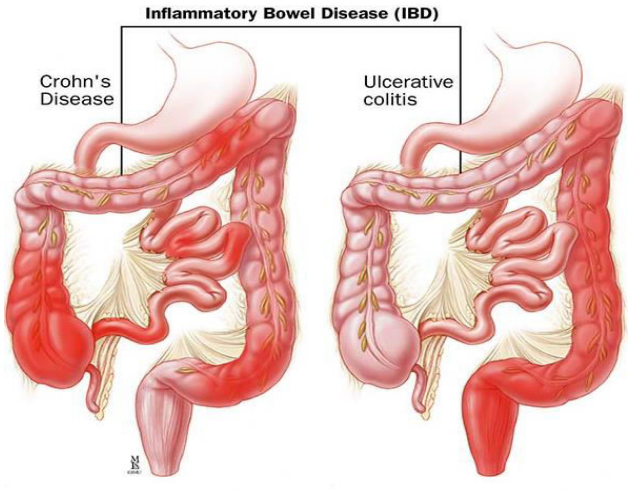


Figure 3. Inflammatory Bowel Disease

Inflammatory Bowel Disease (**Figure 3**) is a general term used to describe chronic disorder involving inflammation of the digestive track. **[9]** Types of IBD include: Crohn's disease (CD) and Ulcerative colitis (UC). CD and UC exist with extra intestinal manifestations, which include liver problems, arthritis, skin manifestations and eye troubles, in different proportions. Mainly interleukin (IL) is involved as a pro-inflammatory mechanisms for IBD. The medications of IBD includes antibiotics, such as Amoxicillin, Minocycline, Tetracycline, Ciprofloxacin, etc. There are some new therapies under clinical development for the treatment of IBD, such as, Phosphodiesterase-IV (PDE-IV) inhibitors, Interleukin-17 (IL-17) Antagonist, Angiotensin Converting Enzyme -2 (ACE-2) inhibitors, Hydrogen Sulfide-based Derivative (HSD) Mesalazine, etc. Globally, UC contributes around 0.1-16 case per 100,000 person-years and the prevalence of CD is around 396 case per 100,000 person-years. **[10, 11]**

(b)Osteoarthritis (OA):

Figure 4. Osteoarthritis

Osteoarthritis (**Figure 4**) is a disorder of synovial joints, a joint where two bones come together. **[12]** The ends of these bones are covered with protective tissue called cartilage. In OA, cartilage breaks down, causing the bones within the joint to rub together. Pain, stiffness, tenderness, loss of flexibility, grating sensation, bone spurs, swelling, etc., are some of the common symptoms of OA. Causes of OA are age, gender, obesity, joint injury, joint abnormalities, genetic factors, weather, diet etc. The first drug recommended for the OA is Acetaminophen. For people who do not respond to Acetaminophen, a NSAIDs is regularly prescribed at the lowest effective dose. Examples of NSAIDs include Aspirin, Ibuprofen, Naproxen Sodium, Diclofenac, Celebrex etc. Corticosteroids like Prednisone, Dexamethasone, Methylprednisolone, Triamcinolone etc., are also used for OA. In case of severe OA, doctors recommend an opioid medication like Codeine, Hydrocodone, Oxycodone etc. There are some new therapies under clinical development for the treatment of OA, which includes LTB4 (Leukotriene B4) antagonist, dual inhibitor of 5-LOX (5-Lipoxygenase) and COX-2 (Cyclooxygenase-2), MMP's (Matrix metalloproteinase)

inhibitors, collagenase inhibitors, BK2 (Bradykinin-2) antagonist, IL-17 antagonist etc. 15% worldwide population is affected by OA. **[13, 14]**

(c)Irritable Bowel Syndrome (IBS):

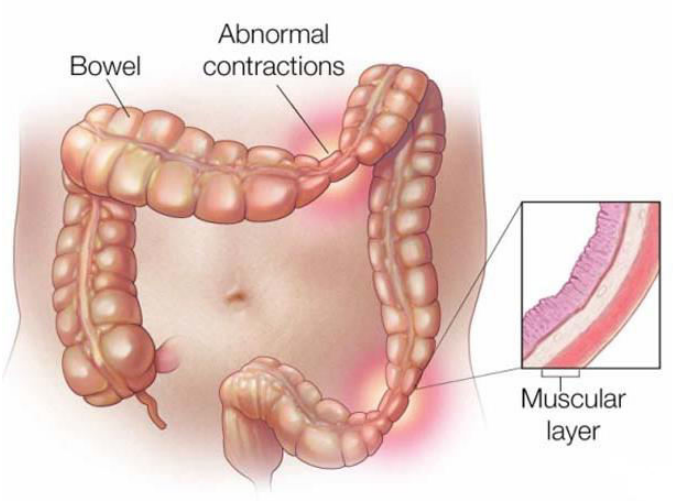


Figure 5. Irritable bowel syndrome

Irritable bowel syndrome (IBS) as shown in **Figure 5** is a set of symptoms that occur together, with repeated ache in the stomach and changes in the bowel movement, which lead to diarrhea, constipation, or both. **[15]** If the muscles of the colon do not contract in the right way, the contents inside the colon do not move correctly, resulting in abdominal pain, cramps, a sense of incomplete stool movement, diarrhea, gas, food intolerance etc. Causes of IBS are uncontrolled muscle contraction in the intestine, imbalance in the nervous system, severe infection, changes in the bacterial microflora in the gut etc. Symptoms of the IBS are triggered by food, stress and hormones. The treatment for IBS mainly includes Dicyclomine, Amitriptyline, Diphenoxylate, Rifaximine, Eluxadoline, Linaclotide, Lubiprostone, Tegaserod etc. There are some new therapies under clinical development for the treatment of IBS which includes KOR (Kappa opioid receptor) agonist , Tryptophan hydroxylase inhibitor, SK

(Sphingosine Kinase) inhibitor, DOR (Delta opioid receptor) agonist, ß3-Adrenoceptor agonist, 5-HT1A (5- Hydroxytryptamine receptor 1A) agonist and 5-HT3 (5-Hydroxytryptamine3) antagonist etc. Worldwide it is calculated that 10-15% of the population has IBS. Most people with IBS are below the age of 50. Approximately, 1 in 3 IBS patients are male. **[16, 17]**

(d)Asthma

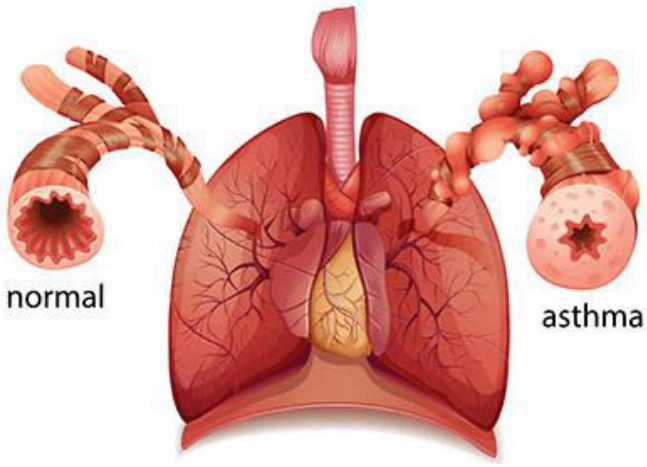


Figure 6. Asthma

Asthma (**Figure 6**) is a chronic inflammatory disease of the airways. In the case of asthma, the inner walls of the airways, known as the bronchial tubes, become swollen or inflamed. **[18]** This swelling or inflammation makes the airways extremely sensitive and increases their susceptibility to allergic reactions. In an allergic reaction, the airways swell, and muscle tissue around the airways tighten, making it harder for air to move in and out of the lungs. Symptoms of asthma include cough, wheezing, chest tightness, shortness of breath, rapid breathing, tiredness etc. Causes of asthma are environmental factors such as, air pollutant, smoking, dust, pets, chemicals etc., and genetic factors. Asthma is mainly classified as acute or chronic based on the frequency of symptoms, forced expiratory volume per 1 second (FEV1)

and peak expiratory flow rate. Sign and symptoms are often worse at night, in the early morning hours or in response to exercise or cold air. Medications for asthma mainly include Salbutamol, Theophylline, Cromolyn sodium and Ipratropium etc. There are some new therapies under clinical development for the treatment of asthma, which includes, CRTH2 (Chemoattractant receptor-homologous molecule expressed on T_H2 cells) antagonist, CCR4 (Chemokine receptor 4) inhibitors, PDE-IV (Phosphodiesterase-IV) inhibitors, IL-17 antagonist, 5-LOX inhibitors, LTA-4 (Leukotriene A4) inhibitors etc. Asthma affects estimated 300 million people worldwide. **[19]**

(e)Gout

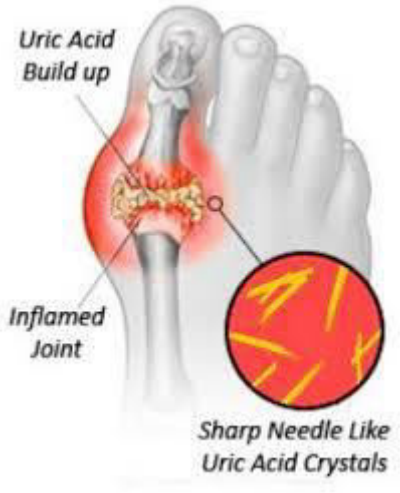


Figure 7. Gout

People suffering from Gout (**Figure 7**) have an excessive amount of uric acid in their blood. **[20]** Uric acid is a breakdown product of purines. An abnormality in handling uric acid and crystallization of uric acid in joints can cause severe pain. Symptom of gout are intense joint pain, warmth, swelling, reddish discoloration, tenderness etc. Risk factors which can increase the amount of uric acid in the blood are age, gender, genetics, diet, medication, alcohol,

obesity, sodas, bypass surgery and other health conditions. Existing medications for gout include Allopurinol, Colchicine, Febuxostat, Indomethacin, Lesinurad, Pegloticase, Probenecids and Steroids etc. There are some new therapies under clinical development for the treatment of Gout which include uricosuric agent, URAT-1 (Urate transporter 1) inhibitor, XO (Xanthine oxidase) inhibitor etc. In the general population, the occurrence of gout varies globally from 0.1% to approximately 10%, which accounts for 0.3 to 6 cases per 1000 person-year. **[21]**

(f)Rheumatoid Arthritis (RA)

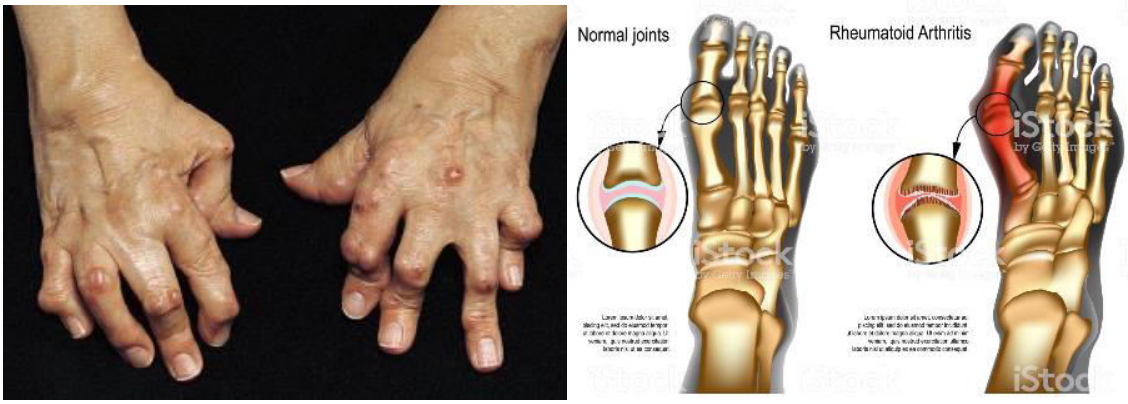


Figure 8. Rheumatoid arthritis

Rheumatoid arthritis (**Figure 8**) is an autoimmune disorder in which our immune system, which normally protects our body from the attack of foreign substances like bacteria and viruses, mistakenly attacks our body's own tissues. **[22]** This creates inflammation, which leads to joints (the synovium) thickness, there by causes swelling and pain in the joints. The severe RA, can harm cartilage, elastic tissues that are present at the end of the bones in the joints, as well as the bones itself. Over a period, there is loss of cartilage and the distance between the bones becomes smaller in the joints as shown in the **Figure 9**. **[23]** Slowly, mobility of joints loses and deformation of joints can also occur. **[24]**

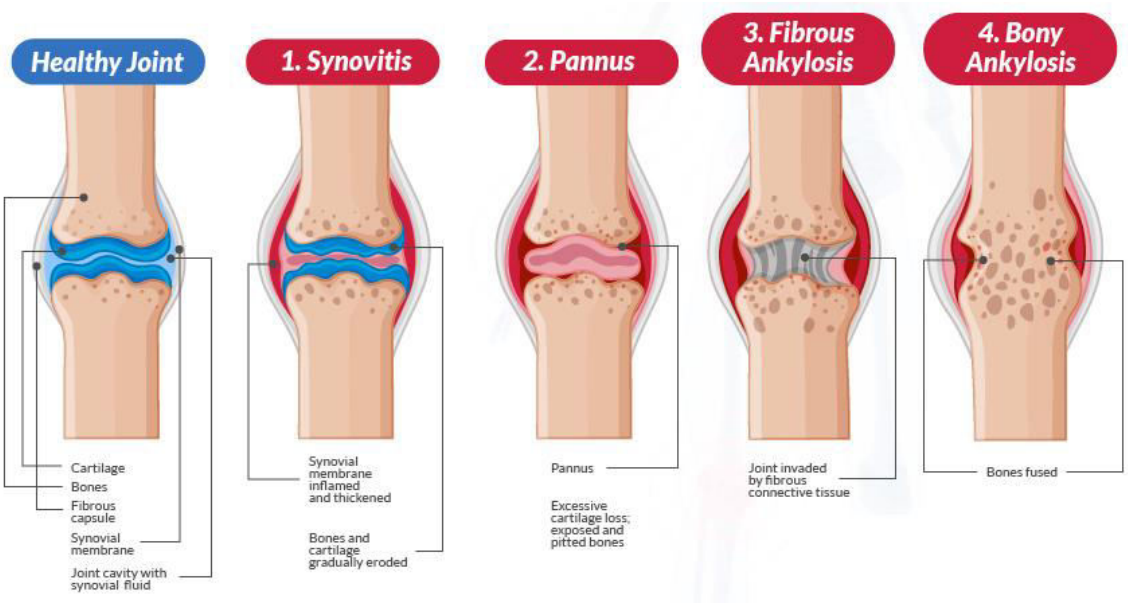


Figure 9. Process of RA

Symptoms of RA includes joint pain (like knees, hands, feet), swollen joints, limping, loss of range of motion, tender joints, loss of joint function, stiff joints, fatigue, joint redness, joint warmth, joint deformity etc. **[25]** Causes of RA are gender, age, family history, smoking, obesity, environmental exposures etc. 1% of the world population is affected by RA. **[26]** Medication for RA mainly includes NSAIDs like Ibuprofen and Naproxen sodium. Corticosteroids like Prednisone. Diseases modifying anti-rheumatic drugs (DMARDs) like Methotrexate, Leflunomide, Hydroxychloroquine and Sulfasalazine. Biologic agents include Abatacept, Adalimumab, Anakinra, Certolizumab, Tocilizumab, Rituximab, Etanercept, Infliximab, Golimumab etc. The first of a new kind of DMARDs, Tofacitinib (JAK inhibitor) was approved in 2012, discovered by the Pfizer. There are some new therapies under clinical development for the treatment of RA, which includes, P38 MAP (P38 Mitogen-activated protein) kinase, TACE (Tumour necrosis factor-alpha converting enzyme) inhibitors, LTB4 antagonist, JAK inhibitors, SYK (Spleen tyrosine kinase) inhibitors etc. **[27]**

In the treatment of RA, biologic agents are injected or given by infusion. These biological drugs also increase the risk of infections. The high cost and associated immunological adverse effects have triggered discovery of small molecules for the treatment of RA. Till today very few small molecules like Tofacitinib are available for the treatment of RA.

Common adverse events with Tofacitinib included nasopharyngitis, diarrhea, headache, infection (pneumonia, cellulitis, herpes zoster, upper respiratory track infection, and urinary track infection), malignancies, lymphoma, increase blood pressure, abnormal blood test, cold symptoms, night sweat, risk of blood clots etc. **[28]**

Current therapies for the treatment of RA

Currently, various new therapies are available for the treatment of RA. Current therapies and their side effects are listed in **Table 1**. **[29]**

Table 1*. New therapies for the treatment of RA*

Sr. No.	Class	Drugs name	Side effects
1.	Disease-modifying anti-rheumatic drugs (DMARDs)	Methotrexate, Leflunomide, Hydroxychloroquine, Sulfasalazine	Sickness, loss of appetite, a sore mouth, diarrhea, headaches, hair loss
2	Biological treatments	Etanercept , Infliximab	Skin reactions at the site of the injections, infections, feeling sick, a high temperature, headaches

3.	JAK inhibitors	Tofacitinib, Baricitinib	Nasopharyngitis, Diarrhea, Infection, Lymphoma, Risk of blood clots
4.	Non-steroidal anti-inflammatory drugs (NSAIDs)	Ibuprofen, Diclofenac	Heartburn, abdominal pain, gas, vomiting, constipation
5.	Steroids	Prednisone, Dexamethasone, Triamcinolone	Weight gain, osteoporosis muscle weakness, thinning of the skin
6.	Surgery	---	---
7.	Arthroscopy (remove inflamed joint tissue)	---	---
8.	Joint replacement	---	---

Among various therapies listed in **Table 1**, small molecule based JAK inhibitors appear to be a promising therapy. In the next section JAK enzyme and its role in the RA described.

1.2. Janus Kinase (JAK)

Janus kinase is also known as Jakinibs. JAK is a family of intracellular, non-receptor tyrosine kinase that transduce cytokine mediated signals via the JAK-STAT (Janus kinase-signal transducers and activators of transcription) pathway. They were originally called "just another kinase" 1 and 2, but eventually published as "Janus kinase". The name is taken from the two-faced Roman god of the beginning and end. JAK has two phosphate-transferring domains, which are nearly identical. One domain shows the kinase activity, while the other negatively controls the kinase activity of the first. JAKs are a family of cytoplasmic protein tyrosine kinases with four known members [JAK1, JAK2, JAK3 and TYK2 (Tyrosine kinase 2)]. Due to the unique role of JAKs, it has emerged as one of the most validated

and potential target for the treatment of autoimmune disorders. **[30, 31]**

1.2.1. Structure of JAK protein and STAT protein

- **JAK protein**

In the human, JAK1 gene is located on chromosome 1p31.3, JAK2 is on 9p24, JAK3 and TYK2 genes are clustered together on chromosome 19p13.1 and 19p13.2, respectively. The three-dimensional structure of the JAKs is large proteins of more than 1100 amino acids, with apparent molecular masses of 120-140 kDa (Kilo dalton). **[32]**

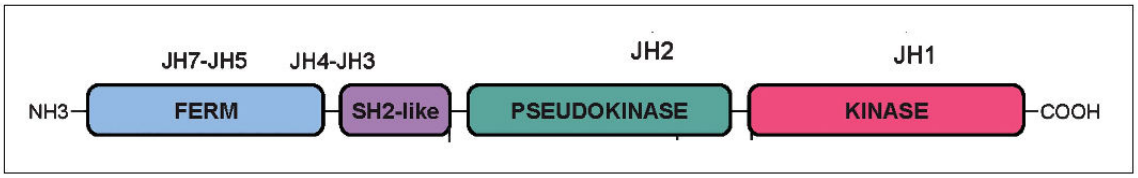


Figure 10. Domain structure of JAKs

From the first structure of JAK, seven different JAK homology regions (JHs) have been identified (JH1-JH7), numbered from the carboxyl to the amino terminus (**Figure 10**). **[33]** The enzymatically active kinase domain (JH1) present at the carboxyl terminus has all the features of a typical eukaryotic tyrosine kinase domain. This domain is closely associated with the kinase domain of the epidermal growth factor receptor family tyrosine kinase, indicating that the JAK family may have emerged from this large family of protein kinases. Next to the JH1 domain is the catalytically inactive pseudo-kinase domain or kinase like domain (JH2), which is a unique feature of JAK proteins unlike other protein tyrosine kinases. Even though there is a

lack of catalytic activity, the pseudo-kinase domain is necessary for the suppression of basal activity of tyrosine kinase and for cytokine-inducible activation of signal transduction. The amino terminus contains SH2 (Src homology2) domain (JH3-JH4) and FERM (F for 4.1 protein, E for ezrin, R for radixin and M for moesin) domain (JH6-JH7). The FERM domain is 300 amino acids in length and is involved in mediating interactions with the trans membrane proteins such as cytokine receptors. JAKs seem to be vital in controlling cell-surface expression of the cognate receptors. Also, the FERM domain binds the kinase domain and positively controls catalytic activity. **[34-36]**

- **STAT protein**

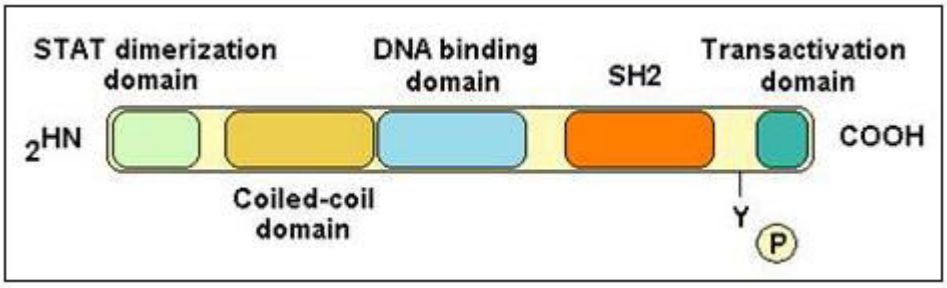


Figure 11. Structure of STAT protein

The Signal Transducer and Activators of Transcription (STAT) family was first discovered in 1990 as key proteins in cytokine signaling. STAT proteins are intracellular transcription factors that mediate many aspects of cellular immunity, proliferation, apoptosis and differentiation. They are typically activated by membrane receptor-associated JAK. The STAT family has seven family members. They are STAT1, STAT2, STAT3, STAT4, STAT5 (STAT5A and STAT5B), and STAT6. All seven STAT proteins share a common structural motif consisting of a N-terminal domain followed by a coiled-coil, DNA-binding, linker, Src homology2 (SH2), and C-terminal trans activation

domain as shown in **Figure 11**. **[37]** Both the N-terminal and SH2 domains mediate homo or hetero dimer formation, while the coiled-coil domain functions partially as a nuclear localization signal (NLS). Transcriptional activity and DNA association are determined by the trans activation and DNA-binding domains, respectively. **[38-40]**

1.2.2. Binding of JAK proteins with different cytokine

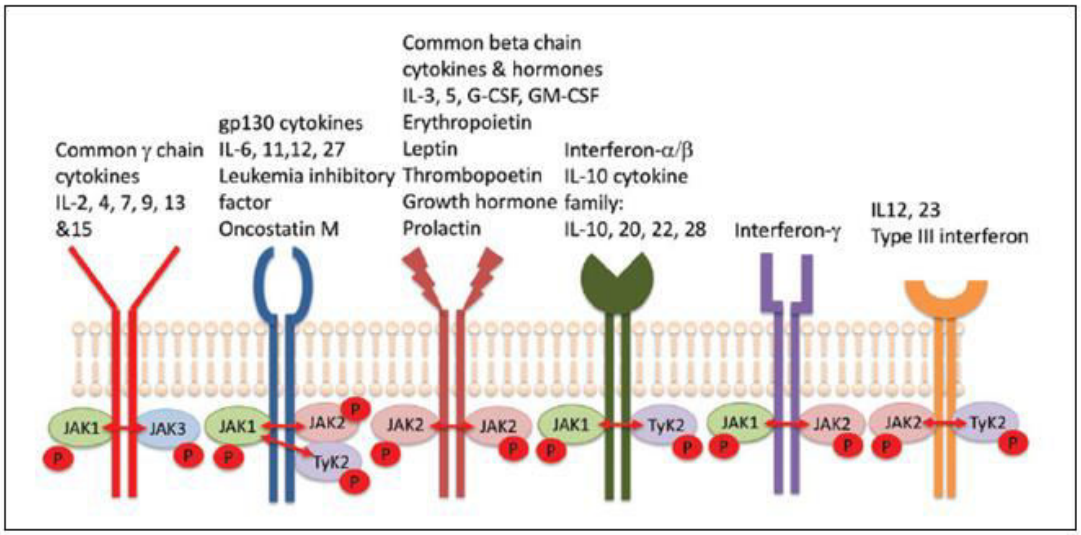


Figure 12. Cytokine signaling via JAK isoforms and their inhibitors

Each JAK protein can bind to many cytokine receptors, but some particularity exists as shown in the **Figure 12**. **[41]** A large variety of cytokines are dependent on JAK1, including a family that use a common γc chain, which include interleukin IL-2, IL-4, IL-7, IL-9, IL-15 and IL-21. These cytokines are also dependent on JAK3 because JAK3 binds γc. JAK1 is also important for other families that uses the common receptor subunit gp 130 [IL-6, IL-11, oncostatin M, leukemia inhibitory factor (LIF), ciliary neurotrophic factor (CNF)] as well as granulocyte colony-stimulating factor (G-CSF) and interferons (IFNs). JAK2 is very important for hormone-like cytokines such as, growth

hormone (GH), prolactin (PRL), erythropoietin (EPO). Thrombopoietin (TPO) and the family of cytokines that signal through the IL-3 receptor [IL-3, IL-5 and granulocyte-macrophage colony-stimulating factor (GM-CSF)]. JAK2 is also important for cytokines that use the gp130 receptors and for some IFNs. TYK2 is essential for IL-6, IL-11, IL-12, IL-23, IL-27, and IL-10 cytokine family, OSM (Oncostatin M), LIF (Leukemia inhibitory factor), CNF, G-CSF and IFNs. **[42, 43]**

1.2.3. The clinical significance for targeting JAK3

All the JAK family members are good therapeutic targets in different cases. Clinically, JAK1 inhibition induces undesirable secondary pharmacodynamics effects such as cholesterol and liver enzyme elevation. JAK2 mediates signaling via hematopoietic cytokines such as erythropoietin (EPO), thus dose-limiting tolerability and safety issues such as anaemia are being associated with the JAK2 inhibition. However, JAK3 has very restricted function, so specifically interfering with JAK3 function would be a good strategy for developing a novel class of anti-inflammatory agents or anti-cancer drugs. Among all the family members of JAK, expression level of JAK3 is the highest in hematopoietic cells. Selective JAK3 inhibitions only deter common gamma chain receptors signaling and spare JAK1 dependent immune regulatory 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. **[44-46]**

1.2.4. Mechanism of action of JAK-STAT pathway

JAK-STAT signaling is important for many developmental and homeostatic processes, including haematopoiesis, immune cell development, stem cell maintenance, organismal growth, and mammary gland development. Cytokines are usually used for signaling between cells of the immune system. Cytokine-induced signal transduction cascades are often direct pathways to the nucleus for switching on sets of genes.

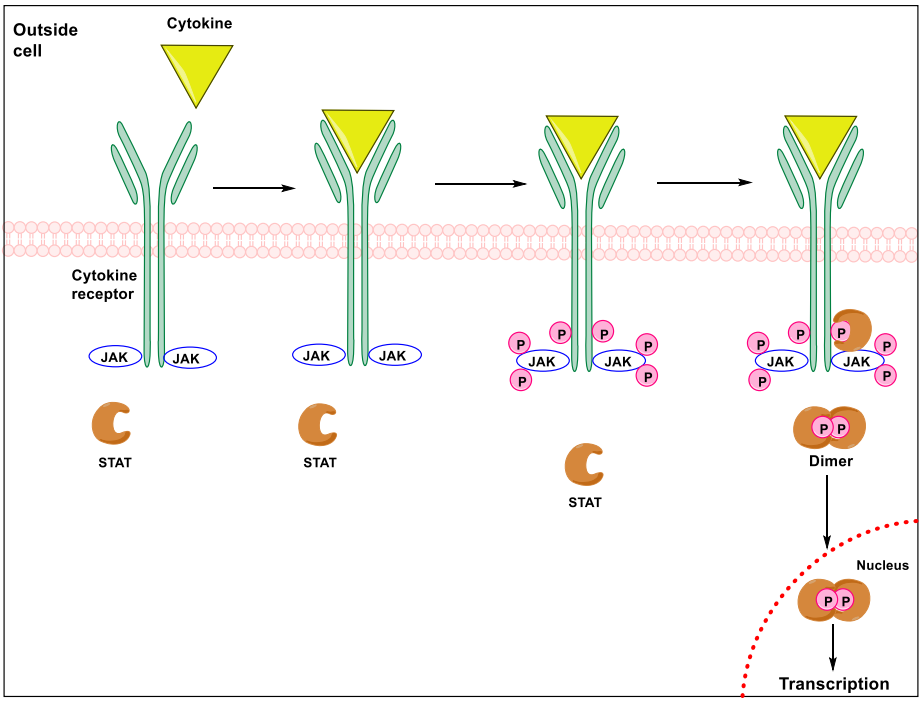


Figure 13. Mechanism of JAK-STAT pathway

JAK-STAT signaling pathway is made up of three main proteins as shown in **Figure 13**:

- *Cell-surface receptors,*
- *JAKs,*
- *STATs.*

The binding of different ligands (yellow triangle), generally cytokines, like interferons and interleukins, to cell-surface receptors, causes JAKs to add phosphate (pink circle) to the receptor. Then two

STAT proteins bind to the phosphates (STATs phosphorylation by JAKs). STATs are latent transcription factors that reside within the cytoplasm until activated. STATs bear a conserved tyrosine residue near the C-terminus that is phosphorylated by JAKs. This phospho tyrosine allows the dimerization of STATs through interaction with a conserved SH2 domain. The dimer enters the nucleus by a mechanism that is dependent on importin α-5 (also known as nucleoprotein interaction 1) and the Ran nuclear import pathway. Dimerized STATs bind with specific regulatory sequences to activate or repress transcription of target genes. Thus, JAK-STAT signaling pathways provide a direct mechanism to translate an extracellular signal into a transcriptional response. **[47-49]**

1.2.5. Regulation of JAK-STAT pathway

There are various mechanisms that cells have for regulating the amount of signal occurs. Three major groups of proteins that cells use to regulate this signaling pathway are protein inhibitors of activated STAT (PIAS), protein tyrosine phosphatases (PTPs) and suppressors of cytokine signaling (SOCS). **[50]**

- PIAS

These proteins add a marker, known as small ubiquitin-like modifier (SUMO), onto other proteins like JAKs and STATs, modifying their function.

A) Adding a SUMO group to STAT protein can block their phosphorylation, which prevents STAT entering the nucleus.

B) Histone deacetylase (HDAC) recruitment can remove acetyl modifications on histones, lowering gene expression.

C) PIAS can also prevent STAT binding to DNA. **[51]**

- PTPs

Adding phosphate groups on tyrosine is an important part of JAK-STAT signaling pathway function, removing this phosphate group can inhibit signaling. PTPs are tyrosine phosphate, so are able to remove this phosphate and prevent signaling. **[52]**

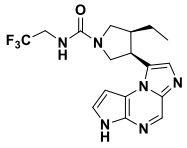
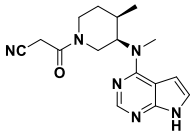
- SOCS

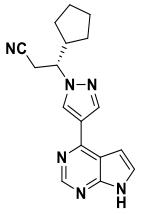
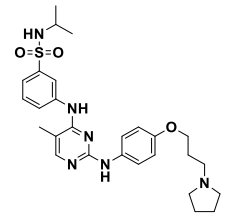
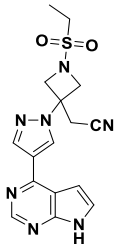
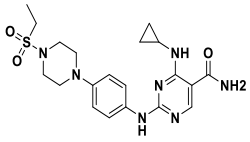
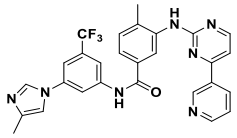
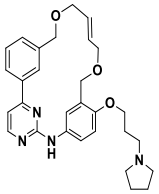
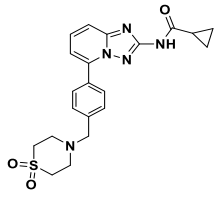
SOCS can interact with numerous proteins to form a protein complex, and this complex can cause the breakdown of JAKs and the receptors themselves, therefore inhibiting JAK-STAT signaling. **[53]**

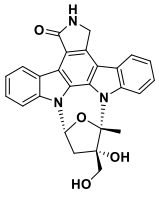
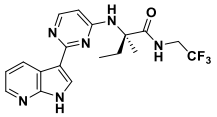
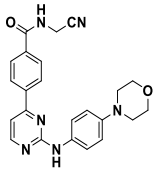
1.2.6. JAK inhibitors

Few JAK inhibitors are listed below in **Table 2** Some of them are launched and some are in various phases of clinical trials.

Table 2. *List of JAK Inhibitor in clinical or in clinical development*

Sr. No	Selectivity	Generic Name	Development Phase	Indication	Company
1	JAK-1	Upadacitinib 	Launched	Rheumatoid arthritis	AbbVie
2	Pan/JAK	Tofacitinib 	Launched	Psoriasis, Rheumatoid arthritis	Pfizer

3	JAK-1,2	Ruxolitinib 	Launched	Myelofibrosis	Incyte corp
4	JAK-2	Fedratinib 	Launched	Myelofibrosis	Sanofi
5	JAK-1,2	Baricitinib 	Launched	Psoriasis, Rheumatoid arthritis	Eli Lilly
6	PAN SYK/JAK	Cerdulatinib 	Launched	Leukaemia, Lymphoma	Portola Pharmaceuticals
7	JAK-2	Gandotinib 	Phase II	Myelofibrosis	Eli Lilly
8	JAK-2	Pacritinib 	Phase III	Lymphoproliferative disorders, Myelofibrosis	S*BIO
9	JAK-1	Filgotinib 	Phase III	Rheumatoid arthritis	Galapagos

10	JAK-2	Lestaurtinib 	Phase II	Acute myeloid leukaemia	Cephalon
11	JAK-3	Decernotinib 	Phase II	Rheumatoid arthritis	Vertex Pharmaceuticals
12	JAK-1,2	Momelotinib 	Phase III	Myeloproliferative disorders	Cytopia

The most advanced Upadacitinib was approved for RA in the United States and in the European Union in 2019. It was discovered by the biotech company AbbVie. Upadacitinib (ABT-494) is a selective JAK1 inhibitor. It is 74-fold selective for JAK1 over JAK2 (and 58-fold selective for JAK1 over JAK3. It is 52% bound to human plasma proteins. The recommended oral dose is 15 mg once daily with or without food. The common side effects are upper respiratory tract infections, nausea, cough and fever. **[54, 55]**

PAN-JAK inhibitor Tofacitinib was discovered by Pfizer and has been approved for the treatment of RA in 2012. Tofacitinib, a JAK inhibitor showed excellent potency against JAK with the IC_{50} of 1.6 nM, 4.2 nM and 3.2 nM against JAK3, JAK2 and JAK1 respectively. Bioavailability of Tofacitinib is 74% and protein binding is 40%. Elimination half-life of Tofacitinib is 3 hr. The recommended dose of Tofacitinib is one tablet (5 mg) by mouth, twice daily, with or without

food. But it has some side effects and limitations as described in earlier. It is also a very expensive medicine. **[56, 57]**

Ruxolitinib (INCB018424, INC424) from Incyte corp is use for the treatment of Myelofibrosis. Ruxolitinib is the selective JAK1/2 inhibitor with the IC_{50} of 3.3 nM/2.8 nM and >130-fold selectivity for JAK1/2 against JAK3. Its bioavailabity is 95% and protein binding is 97%. Elimination half-life of Ruxolitinib is 3 hr. Minimum dose of Ruxolitinib is 5 mg orally, twice a day and maximum dose of 25 mg orally twice a day. Its side effects include anaemia, headache, balance impairment, labyrinthitis, neutropenia, vertigo, orthostatic dizziness. **[58, 59]**

Fedratinib (SAR302503) was discovered by TargeGen and Sanofi-Aventis; it was approved by the FDA on 16 August 2013 for the treatment of myelofibrosis. Fedrarinib is a selective JAK2 inhibitor with the IC_{50} of 3.0 nM. IC_{50} of JAK1 is 105 nM and JAK3 is 1000 nM. Bioavailability of Fedratinib ranges from 19 to 37%. Fedratinib is ≥ 92% protein bound in plasma. Half-life of fedratinib is 41 hours and elimination half-life is 114 hr. The Recommended dosage of Fedratinib is 400 mg orally, once daily, with or without food. Its side effects include low blood counts, weight gain, nausea, vomiting, diarrhea, loss of appetite etc. **[60, 61]**

Baricitinib (dual JAK1 and JAK2 inhibitor, approved in Europe, 2018) was discovered by Incyte and Eli Lilly for moderate to severe RA treatment. Baricitinib is a JAK1 and JAK2 inhibitor with the IC_{50} of 5.9 nM and 5.4 nM respectively. JAK3 IC_{50} of Baricitinib is greater than 400 nM. Bioavailability is 79% and protein binding is 50%. Elimination

half-life of Baricitinib is 12.5 hr. The recommended dose of Baricitinib is 2 mg once a day, orally, with or without food. Adverse events of Baricitinib are upper respiratory track infection, hypercholesterolemia, herpes zoster, herpes simplex, urinary track infections and gastroenteritis. **[62, 63]**

The reversible ATP (Adenosine triphosphate)-competitive PAN SYK/JAK inhibitor, Cerdulatinib (PRT062070 from Portola Pharmaceuticals) was approved by the FDA in September 2018 for peripheral T-cell lymphoma. Cerdulatinib is a PAN SYK/JAK inhibitor with the IC_{50} of 12 nM, 6.0 nM and 8.0 nM against JAK1, JAK2 and JAK3 respectively. Its side effects included lipase increase, neutropenia, amylase increase and diarrhea. **[64, 65]**

Gandotinib (LY-2784544) is developed by Eli Lilly for the treatment of cancer. It is a potent JAK2 inhibitor with IC_{50} of 3 nM. It is in phase-II trials. **[66]**

Pacritinib (SB1518) is developed by S*BIO for the treatment of myelofibrosis. It is a macrocyclic JAK2 inhibitor with the IC_{50} of 23 nM. It is in phase-III trials. Oral bioavailability of Pacritinib is 24%. **[67]**

Filgotinib (GLPG 0634) was developed by Galapagos. It is a selective JAK1 inhibitor with the IC_{50} of 10 nM, 28 nM, and 810 nM for JAK1, JAK2 and JAK3 respectively. Its protein binding is approximately 55-59%. Elimination half-life of Filgotinib is 6 hr. Maximum 200 mg once a day is being tested in the Phase-III programs. Its side effects are nausea, upper respiratory tract infection, urinary tract infection, dizziness etc. **[68, 69]**

Lestaurtinib (CEP-701) is a JAK2 inhibitor developed by Cephalon for the treatment of acute myeloid leukaemia. It is under Phase-II clinical trials. **[70]**

Decernotinib is a JAK3 inhibitor discovered by Vertex Pharmaceuticals for the treatment of Rheumatoid arthritis. It is under Phase-II clinical trials. **[71]**

Momelotinib (CYT-387) discovered by Cytopia, currently under Phase-III trials for myelofibrosis treatment. Momelotinib is a dual JAK1 and JAK2 inhibitor with the IC_{50} of 2.7 nM and 4.5 nM respectively. IC_{50} of JAK3 is 332 nM. The oral dose of Momelotinib is 400 mg once daily or 150 mg twice daily. Its side effects are diarrhea, peripheral neuropathy, thrombocytopenia etc. **[72]**

In general, various pharmacophore (pyridine/pyrimidine) showed JAK inhibitory activity. The potency and JAK isoform selectivity can be modulated by changing central core or side chain substituents. Among all these inhibitors some of the pyrimidine based JAK inhibitors are shown below **Figure 14**.

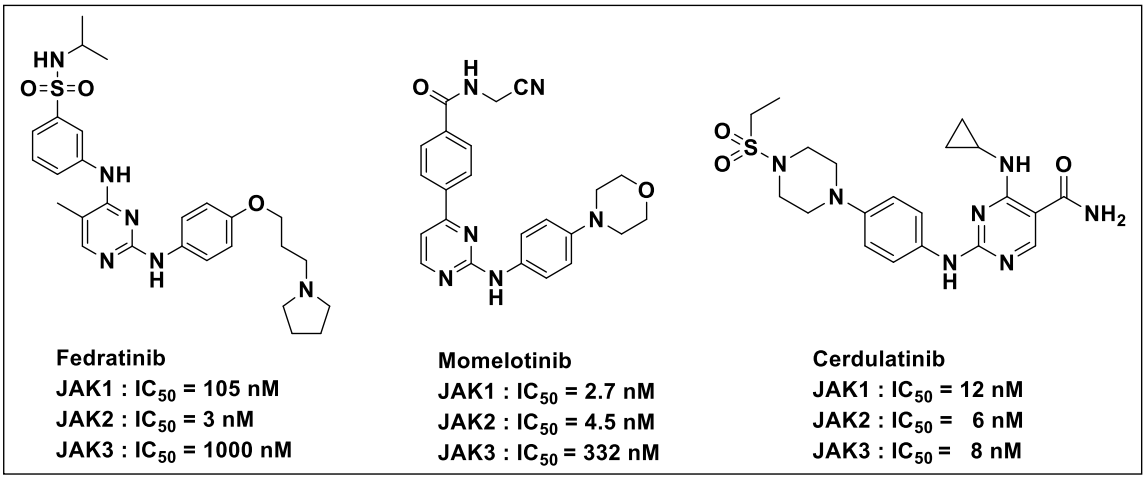


Figure 14. Structures of Pyrimidine based JAK inhibitors

To develop JAK3 selective inhibitor, we decided to work on pyrimidine-based scaffold. Diamino pyrimidine (DAP) based Fedratinib is use for the treatment of myeofibrosis. It is a JAK2 selective inhibitor with the IC_{50} of 3.0 nM. IC_{50} of JAK1 is 105 nM and JAK3 is 1000 nM. 2-Amino pyrimidine based Momelotinib is also used for the treatment of myeofibrosis. It is a dual JAK1 and JAK2 inhibitor with the IC_{50} of 2.7 nM and 4.5 nM respectively. IC_{50} of JAK3 is 332 nM. DAP based Cerdulatinib is used for the peripheral T-cell lymphoma. It is a reversible ATP (Adenosine triphosphate)-competitive PAN SYK/JAK inhibitor with the IC_{50} of 12 nM, 6.0 nM and 8.0 nM against JAK1, JAK2 and JAK3 respectively. From this data we can say that selectivity is possible in DAP. **[73-75]**

Currently, NSAIDs, DMARD, Steroid and biological agents are available for the treatment of RA. Till today a few small molecules like Tofacitinib is available for the treatment of RA. But Tofacitinib also has many side effects and limitations.

Presently there is non-availability of safe, cost-effective and efficacious small molecule based JAK inhibitors. To address these issues, in the next section, we have designed novel Series of JAK inhibitors to develop next generation therapies for the treatment of RA.

1.3. Objectives

Our objective was to design a novel, orally active, selective JAK3 inhibitor as an anti-inflammatory agent for the treatment of RA. As described above, iso enzyme selectivity (particularly JAK3) can be achieved by modulating core or substituent. Among various pharmacophore, we select DAP based Cerdulatinib as reference molecule. Our current research work involves:

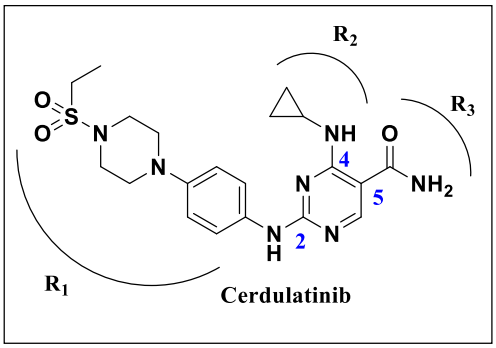
a) Design of DAP based JAK3 selective inhibitor (molecular modeling)
b) Synthesis and characterization of molecules
c) *In-vitro* JAK3 inhibitory activity study
d) Selectivity against isoenzymes
e) Pharmacokinetic study
f) *In-vivo* study
g) Profiling study
h) Safety pharmacology study

1.4. Design strategy

Knowing the potential side effects associated with the PAN JAKs isoforms inhibition, recently, more efforts are directed towards the development of isoform selective inhibitors, particularly JAK3 selective

inhibitors (Ex: Decernotinib, Momelotinib and Ritlecitinib), for the effective treatment of autoimmune disorders, such as RA.

In this regard, we aim to discover novel, potent and orally bioavailable JAK3 selective inhibitors, mainly by favouring the suitable interactions of the designed molecules, in the specificity and binding pocket, to achieve JAK3 selectivity. Considering the strong interaction of pyrimidine moiety with the ATP binding pocket of JAK enzymes as a starting point, structural modifications were carried out in the dual SYK (Spleen tyrosine kinase)/JAK inhibitor, Cerdulatinib to improve the JAK3 selectivity. Three sets of compounds were designed. Initial modifications on the C2 position of pyrimidine ring (at R_1 position) in Cerdulatinib, with C4 cyclopropyl of Cerdulatinib altered with benzyl group, led to the single digit nM potent compounds, with moderate isoform selectivity. In the second set changes were carried out on the C4-position (at R_2 position) to improve isoform selectivity and in vivo profile. And then modifications were carried out on the C5-position (at R_3 position) of pyrimidine ring to understand structure activity relationship (SAR).



Cerdulatinib

Herein, we report, design, synthesis and biological evaluation of novel 2, 4-diaminopyrimidine-5-carboxamides based JAK3 selective inhibitors.

In the next section (**Chapter-2**), detailed synthesis, protocol including *in-vitro* JAK3 inhibitory activity screening has been described for the sereis-1, wherein modification were carried out on the 2nd position of DAP.