Small molecule drugs for the treatment of rheumatoid arthritis

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Abstract. Rheumatoid arthritis (RA) is a chronic autoimmune disease that primarily affects the joints but can also involve other parts of the body. In 2019, an estimated 18 million people across the globe were living with rheumatoid arthritis (RA). Among these individuals, approximately 13 million experienced varying degrees of severity in their RA symptoms. Rheumatoid arthritis (RA) is a multifaceted systemic autoimmune disorder affected by both genetic predisposition and environmental factors, leading to inflammation in the synovial tissue and dysregulation of the immune system. This dysregulation is driven by abnormal cytokines and immune cells, which significantly contribute to the pathogenesis and advancement of the disease. Presently, treatments for RA mainly concentrate on relieving symptoms and achieving disease remission through the use of disease-modifying antirheumatic drugs (DMARDs). These DMARDs encompass conventional DMARDs (cDMARDs) like methotrexate and targeted DMARDs such as JAK inhibitors. Biologic DMARDs (bDMARDs) have also been used but have many limitations. This review explored the different signaling pathways crucial for RA development, as well as the small-molecule drugs that specifically target these pathways.

Keywords: Rheumatoid arthritis, small molecule drugs, Signaling pathways.

1. Introduction

Rheumatoid arthritis (RA), characterized by synovial inflammation, manifests with early symptoms such as joint tenderness, swelling, morning stiffness, and a general feeling of malaise [1]. The development of RA is influenced by various factors, with genetic predisposition accounting for about 60% of cases and frequently influenced by environmental factors. Notably, specific single nucleotide polymorphisms (SNPs) within HLA-DRB1 alleles, particularly DRB101, DRB104, and DQ8, exert a significant influence. However, the exact mechanism triggering the onset of the disease is still poorly understood, potentially encompassing both pathogenic and non-pathogenic triggers such as infections, microvascular damage, and microtrauma, which results in the emergence of auto-reactive immune cells, and ultimately culminates in a loss of tolerance [2]. Moreover, dysregulated factors such as cytokines and immune cells tend to accumulate in synovial sites, thereby contributing to the distinctive destructive effects associated with rheumatoid arthritis (RA) [3].

Currently, the small molecule drugs for RA primarily focus on symptoms alleviation and achieving disease remission. Disease-modifying antirheumatic drugs (DMARDs) constitute a class of drugs that designed to suppress the immune system or specific immune pathways. There are two main categories of DMARDs: Synthetic DMARDs and Biological DMARDs. Synthetic DMARDs can be further categorized into two groups: Conventional DMARDs (cDMARDs) and target DMARDs. The former one is used to treat autoimmune diseases like methotrexate, and the later one is small-molecule chemicals inhibiting specific signaling pathways that are important for RA, such as the JAK inhibitor baricitinib. Biologic disease-modifying antirheumatic drugs (DMARDs) are a class of medications used to treat autoimmune diseases, particularly rheumatoid arthritis (RA), but they are also used in other autoimmune conditions. Unlike conventional DMARDs, which have a more general mechanism of action and broadly suppress the immune system, biologic DMARDs target specific molecules or pathways involved in the immune response. However, the clinical use of these biological DMARDs is limited due to their large molecular size, leading to higher costs and restrictions in drug delivery methods. Therefore, the primary focus for the future development of RA drugs is directed towards small-molecule drugs. These compounds hold significant promise as they offer more feasible approaches to treatment.
The pathogenesis of RA involves a complex network of signaling pathways and interactions within the immune system, including the JAK signaling pathway, the PI3K signaling pathway, the SYK signaling pathway, the cAMP pathway, etc. (Figure 1). In this review, I provided a comprehensive overview of several important signaling pathways that play a crucial role in the development of RA and the small-molecule drugs that target these pathways presently employed for the treatment of RA. Furthermore, this review can be a valuable point of reference for researchers actively involved in the exploration and advancement of innovative pharmaceutical interventions within this area of study.

**Figure 1.** Four important signaling pathways for RA.

SYK signaling pathway, PI3K signaling pathway, JAK signaling pathway, and PDE4 signaling pathways play important roles in RA development. Related cytokines are widely discovered as targets of RA medicine.

2. **Janus Kinase (JAK) Inhibitors**

The Janus family of kinases (JAKs) plays an important role in the signaling pathway of the immune response. JAKs are enzymes that have a crucial role in intracellular signaling pathways associated with both type I and type II cytokine receptors. There are several JAK family members, including JAK1, JAK2, JAK3, and TYK2, which are associated with different cytokine receptor subtypes. When a cytokine binds to its receptor, it induces a conformational change in the receptor, leading to the activation of JAKs associated with the receptor. Once activated, JAKs phosphorylate specific tyrosine residues on the receptor itself, creating docking sites for downstream signaling molecules. Then phosphorylated receptors serve as binding sites for signal transducer and activator of transcription (STAT) proteins. After that, the JAKs phosphorylate STAT proteins, leading to their activation. These activated STAT proteins dimerize, move into the cell nucleus, and bind to specific DNA sequences, thus regulating the transcription (gene expression) of various target genes [4]. JAK inhibitors work by blocking the activity of JAK enzymes. By inhibiting JAKs, these drugs interrupt the downstream signaling of cytokines and growth factors that involves the JAK-STAT pathway. Most JAK inhibitor drugs inhibit a combination of two members of JAK.

Tofacitinib, commonly known as Xeljanz, is a small-molecule inhibitor of JAK developed by Pfizer and approved by the FDA in November 2012 for RA treatment. Tofacitinib functions as a JAK1 and JAK3 inhibitor, which causes the inhibition of the common gamma chain family of cytokines, including IL-2, IL-4, and IL9, etc., ultimately reducing the activation and proliferation of immune cells, particularly T cells. This can dampen the overall immune response and inflammatory
processes [5]. During placebo-controlled trials, it was observed that individuals diagnosed with rheumatoid arthritis (RA) administered tofacitinib at either 5 mg or 10 mg twice daily exhibited enhanced ACR20 responses within a span of 2 weeks. Additionally, the patients exhibited notably more substantial enhancements in physical function when contrasted with the placebo group[4,6]. Nevertheless, tofacitinib treatment comes with safety concerns related to an increased risk of infection, which is linked to its mechanism of action. The inhibition of JAK1 and JAK3 activity can make the immune system more vulnerable to infections due to the suppression of T- and B-lymphocyte functions [6].

Baricitinib, marketed under the brand name Olumiant by Incyte Corp., received approval from the European Commission (EC) in February 2017 for the treatment of rheumatoid arthritis (RA) in adult patients. This medicine was approved by the FDA in May 2018 and marketed by Eli Lilly and Co. with the same name [7]. Baricitinib functions as an inhibitor of both JAK 1 and JAK 2 enzymes, resulting in the attenuation of the immune response and inflammation [8]. However, due to its immunosuppressive effects, baricitinib can lead to certain side effects, including elevated LDL cholesterol levels, upper respiratory tract infections, and nausea [8].

In 2019, the FDA approved Upadacitinib, and marketed as Rinvoq, for the treatment of moderate to severe rheumatoid arthritis (RA). Upadacitinib is a compound characterized by a unique molecular structure, and it is designed to specifically target JAK1. It is primarily indicated for the treatment of moderate to severe rheumatoid arthritis (RA) in adults who have not responded adequately to conventional disease-modifying antirheumatic drugs (DMARDs) or other biologic DMARDs. It can be used as monotherapy or in combination with other RA medications [9].

3. Phosphodiesterase 4 (PDE4) Inhibitors

Phosphodiesterase-4 (PDE4) is an enzyme central to the control of cyclic adenosine monophosphate (cAMP), a crucial molecule participating in intracellular signaling pathways. The interactions orchestrated by PDE4 lead to the assembly of cAMP signalosomes, which wield substantial influence over a multitude of cellular processes. These include the regulation of cell proliferation, cell differentiation, and immune responses [10]. Inhibiting PDE4 leads to the accumulation of cAMP, which activates pathways involving PKA, cyclic nucleotide-gated ion channels, and Epac1/2. The increased levels of cyclic adenosine monophosphate (cAMP) can modulate a variety of cellular processes, including those related to immune responses and inflammation. Activation of Protein Kinase A (PKA) can indeed have a significant impact on the regulation of cytokines, leading to a reduction in inflammatory cytokines and an increase in anti-inflammatory cytokines [11].

Apremilast, an orally administered inhibitor of PDE4, was developed by Celgene Corporation and received FDA approval in 2014 for the treatment of psoriatic arthritis (PsA). This drug is a medication that is used in the treatment of rheumatoid arthritis (RA) and other autoimmune conditions [12]. However, adverse effects may occur after the treatment with apremilast. During the treatment period, any occurrence of undesired or medically significant weight loss should be promptly assessed. Moreover, Apremilast is not recommended for use during pregnancy due to the potential risks it poses, including the possibility of miscarriages and other pregnancy-related complications [13].

4. Bruton's Tyrosine Kinase (BTK) Inhibitors

BTK, an enzyme predominantly present in B cells and other immune cells. It plays a crucial role in relaying signals from the pre-B cell receptor, formed post successful rearrangement of immunoglobulin heavy chains. This receptor is pivotal for B cell development and maturation. BTK's engagement in this process is imperative for the appropriate progress of B cells and the immune response.
In rheumatoid arthritis (RA), B cells play a role in the production of autoantibodies such as rheumatoid factor (RF) and anti-cyclic citrullinated peptide (anti-CCP) antibodies. These antibodies participate in the immune response against the host tissues and lead to the creation of immune complexes that can induce inflammation and damage to joints. [14]. Currently, numerous BTK inhibitors like spebrutinib, acalabrutinib, fenebrutinib, evobrutinib, and tirabrutinib are undergoing clinical trials [15].

Spebrutinib (CC-292) is a small molecule drug that operates by inhibiting BTK activity through a process of irreversible covalent binding. By inhibiting BTK activity, this drug holds promise for the treatment of RA and other autoimmune disorders mediated by B cells. In clinical trials, the results suggest that spebrutinib treatment leads to significant changes in BTK occupancy, B cell subtypes, and B cell ratios, but the ACR 20 response doesn’t have significant differences with the placebo group. Moreover, Spebrutinib demonstrates favorable performance in terms of adverse effects. This medicine is generally well-tolerated, since most treatment-emergent adverse events (TEAEs) are classified as mild or moderate in severity [16].

Acalabrutinib, an FDA-approved medicine, has been developed by AstraZeneca for the treatment of different forms of non-Hodgkin lymphoma, such as mantle cell lymphoma (MCL) and chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL). In the clinical trial, this drug is used in combination with Methotrexate (MTX), but it did not yield a significant clinical response [17].

Fenebrutinib (GDC-0853) is a noncovalent, oral, highly selective, and reversible inhibitor of BTK. The efficacy of fenebrutinib in RA was evaluated in an ANDES trial. In this trial, the scores of ACR 50 reflect a meaningful improvement in clinical response for the patients in the fenebrutinib group, and this drug works for the patients who failed to respond to MTX and TNF inhibitor therapies [18]. Furthermore, fenebrutinib prompted changes in biomarkers linked to B cells and myeloid cells, offering evidence that bolsters its dual mechanism of action.

Tirabrutinib (Velexbru) is an orally administered small molecule inhibitor of BTK, currently being developed through a collaboration between Ono Pharmaceutical and Gilead Sciences. This inhibitor is to be utilized in the therapeutic management of autoimmune diseases and hematological malignancies. Tirabrutinib functions by creating an irreversible and covalent connection with BTK, specifically targeting B cells. This interaction effectively suppresses irregular B cell receptor signaling, rendering it suitable for addressing both B cell-related cancers and autoimmune conditions [19]. The overall effectiveness of tirabrutinib in treating rheumatoid arthritis (RA) is still under investigation. However, the ACR 20 scores reveal a significant distinction between the participants receiving tirabrutinib and those in the placebo group. Therefore, additional research and studies are essential to establish the clinical effectiveness of tirabrutinib more definitively.

Evobrutinib is an orally administered BTK inhibitor known for its high selectivity and covalent binding mechanism. It is undergoing investigation as a potential treatment for multiple sclerosis (MS) [21]. This drug was also subjected to a clinical trial that assessed both its safety profile and its effectiveness in participants with RA who were already receiving a stable dose of MTX. The outcomes of clinical trials indicate that treatment with evobrutinib does not result in a substantial difference in the American College of Rheumatology (ACR) scores. However, there is a notable change in the Disease Activity Score (DAS), suggesting the potential utility of evobrutinib as a therapy for rheumatoid arthritis (RA) in individuals who do not respond to methotrexate (MTX) in clinical practice. The trial also imply that if evobrutinib is administered at daily doses of 25 mg, 75 mg, or 50 mg twice daily, the associated side effects do not pose significant risks [20].

For the current BTK inhibitors, discrepancies exist between preclinical and clinical results. The sample size of the clinical trial and the limitations of the animal model caused problems in analyzing the response. Besides, the irreversible BTK inhibitors have the safety risk of binding to other similar sites and increase the risk of off-target effects. Furthermore, research indicates that BTK inhibitor therapy is more likely to be effective when administered in the early stages of RA [22]. Currently, optimizing the dosing and exploring combination therapies involving BTK inhibitors is anticipated to enhance the efficacy of this medication.
5. Syk (Spleen tyrosine kinase) inhibitors

Spleen tyrosine kinase (Syk) is a cytoplasmic tyrosine kinase with a molecular weight of 72 kDa. It belongs to the Syk family of non-receptor-type protein tyrosine kinases (PTKs) and comprises two SRC homology 2 (SH2) domains along with a kinase domain. It plays a pivotal role in mediating B cell receptor (BCR) signaling. In rheumatoid arthritis (RA), autoreactive B cells play a significant role in the production of autoantibodies and the development of immune complexes, both of which contribute to joint inflammation. Syk is also involved in signaling pathways activated by Fcγ receptors (FcγRs), which are present on the immune cells’ surface and participate in immune response activation. The dysregulation of FcγR signaling is associated with RA [23]. In this condition, immune complexes comprising autoantibodies and self-antigens can activate Syk signaling in immune cells, ultimately resulting in joint inflammation and tissue damage [24]. By inhibiting Syk, it becomes possible to reduce B cell activation and antibody production. This action can help mitigate the autoimmune response and decrease the production of autoantibodies. Additionally, Syk inhibitors may exert anti-inflammatory effects by suppressing immune cell activation and the production of cytokines, both of which are significant contributors to joint inflammation in RA [23]. Fostamatinib is an oral medication approved by the FDA in 2018 for the treatment of immune thrombocytopenia (ITP) when a prior treatment for ITP has not worked well enough. This medication falls into the category of spleen tyrosine kinase (Syk) inhibitors [25]. Given the role of Syk in mediating autoimmune responses, fostamatinib is indeed considered a potential treatment option for various autoimmune diseases, including RA. By inhibiting Syk, fostamatinib may help modulate the abnormal immune responses seen in these conditions, offering a therapeutic approach for managing autoimmune diseases. A phase II trial showed that when the fostamatinib group is compared with the placebo group, there is a significant difference in ACR20 responses, and another study failed to prove the difference in ACR20 responses but showed improved symptoms through magnetic resonance imaging (MRI) images [25]. Compared with some small-molecule therapies mentioned above, the largest difference in Syk inhibitor therapy is the efficacy of this drug, which shows a significant difference with the placebo group, while other therapies still need further studies for this aspect.

6. PI3K (phosphatidylinositol 3 kinase) inhibitor

The PI3K (phosphatidylinositol 3 kinase)-AKT (also known as PKB) pathway is an intracellular signaling pathway responsible for regulating processes such as proliferation, metabolism, angiogenesis, and cell survival in response to external signals. It can be activated by various receptors found on immune cells, including the T cell receptor (TCR) and B cell receptor (BCR). Within this pathway, PI3Ks phosphorylate phosphatidylinositol lipids, generating second messengers that, in turn, activate downstream signaling pathways[26]. The PI3K pathway plays a crucial role in the activation and migration of immune cells, the production of inflammatory cytokines, and the survival of activated immune cells. Dysregulation of PI3K signaling can contribute to the persistent state of inflammation observed in conditions like RA[27]. However, most PI3K inhibitors are still in preclinical trials, some of them will be introduced in the following part. Celastrol, a naturally occurring compound derived from the root bark of the traditional Chinese medicinal plant TwHF, serves as a PI3K inhibitor that is currently in the pre-clinical trial stage. This substance exhibits a wide array of physiological and pharmacological effects, including antibacterial, anti-inflammatory, antioxidant, pro-apoptotic, and anti-cancer properties. In experiments involving mouse models, it was observed that administering celastrol significantly alleviated arthritis symptoms(p>0.05), with a marked distinction seen between the group receiving celastrol and the control group. Importantly, celastrol did not exhibit short-term toxicity in mice [28]. Moreover, ELISA test results indicated that the celastrol-treated group exhibited reduced levels of pro-inflammatory cytokines, including TNF-α and IL-1β, in comparison to the group with induced CIA (Collagen-Induced Arthritis). Through the analysis of cytokine levels in the celastrol-treated group, the MTX-treated group, and the CIA model
group, it was ascertained that celastrol protects against joint damage in CIA mice by enhancing autophagy while suppressing the PI3K/AKT/mTOR pathway [28].

AS-605240, which is chemically known as 5-quinoxalin-6-ylmethylene-thiazolidine-2,4-dione, is a lipid kinase inhibitor that exhibits strong inhibitory effects on PI3Kγ [18]. This compound functions by creating a salt bridge interaction between the nitrogen atom in the thiazolidinedione group and the side chain of Lys833 [29]. Furthermore, the nitrogen in the quinoxaline ring of AS-605240 can form a hydrogen bond with the main-chain nitrogen of Val882. Through these interactions, AS-605240 effectively binds to the ATP-binding pocket of PI3Kγ, thereby inhibiting its enzymatic activity. The administration of AS-605240 resulted in the inhibition of neutrophil recruitment to joints in vivo in mice with PI3Kγ deficiency. This suggests that blocking PI3Kγ may have anti-inflammatory effects by limiting the migration of neutrophils to inflamed joints, which can be relevant in conditions characterized by excessive inflammation, such as certain autoimmune diseases or inflammatory arthritis.

PBT-6, chemically referred to as 6-((4-pyridinyl)-2-benothiazolamine, is an innovative inhibitor of PI3K2γ. The findings from preclinical trials demonstrate that PBT-6 exhibits a high degree of specificity in its ability to inhibit PI3K2γ effectively [30]. This trial further reveals that PBT-6 exerts a substantial inhibitory effect on the phosphorylation of AKT and mTOR, both of which play pivotal roles in the PI3K/AKT pathway. Additionally, the impact of PBT-6 on the migration of macrophages also was investigated. In vitro assessments indicate that PBT-6 effectively curtails the migration of macrophages towards synovial fibroblasts. It is noteworthy, however, that this inhibitory effect may be counteracted by regulating CCL2 inhibition. Moreover, the test results, as demonstrated in the study, reveal that when administered to mice afflicted with Collagen-Induced Arthritis (CIA), PBT-6 treatment leads to noteworthy reductions in synovial inflammation, cartilage deterioration, and bone damage [30]. These encouraging findings strongly indicate that PBT-6 treatment shows considerable potential for mitigating the adverse effects associated with CIA.

7. Conclusion

In summary, the main targets of current DMARDs are JAK, PDE4, BTK, and Syk pathways. Many companies have developed useful DMARDs as first line treatment or supplementary treatment. The small molecule drugs targeting PI3K pathways are all still in preclinical trials and several years still need for the development of these drugs. For now, many preclinical small molecule drugs still stay in preclinical stage because of the difference between animal model and human situation, and the limited time for trials. The development of these small molecule drugs targeting specific pathways will bring a more efficient treatment with less side effect and lower cost.

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