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Review Article

Interleukin-6: A Multifunctional Target Cytokine for Treatment of Rheumatoid Arthritis

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ABSTRACT

Arthritis is commonly used to describe any disorder affecting the joints. Rheumatic disorders typically affect the joints, ligaments, tendons, muscles and bones. Rheumatoid arthritis (RA) is an autoimmune disorder in which the body's own immune system assaults the joints. Cytokines are involved in all stages of the pathogenesis of RA, increasing autoimmunity, maintaining chronic inflammatory synovitis, and causing the destruction of neighboring joint tissue. Interleukins are cytokines that transmit chemical messages between white blood cells. The interleukin (IL)- 6 is a pleiotropic pro-inflammatory cytokine that plays a significant role in RA and its associated conditions. As a primary activator of the production of most acute-phase proteins, IL-6 is involved in acute inflammation. Recently inhibition of IL-6 becomes a primary approach for the treatment of RA. Numerous natural and synthetic compounds have been discovered and reported as IL-6 for RA treatment. This review describes pathophysiology of RA including role of cytokines especially IL-6 role in the progression of RA, paradigms of treatment in RA and lastly reported various chemical moieties reported as IL-6 inhibitors.

INTRODUCTION

Rheumatoid arthritis (RA) is a long-term autoimmune disorder marked by inflammation of the synovial membrane in joints. RA affects about 0.5-1% of the population worldwide and about 0.92% of the adult population in India [1]. Although a disease may manifest at any age, its prevalence rises with age, particularly in the fourth and sixth decades of life. The etiology is not completely known and assumed to be affected by environmental, genetic, and other factors. It has been hypothesized that a genetically susceptible individual is more likely to acquire RA if exposed to certain environmental conditions favorable for the disease. A group of alleles that encode amino acid sequences inside the major histocompatibility complex (MHC) constitute a major genetic risk factor in the human leukocyte antigen (HLA) region called shared epitope (SE). Numerous studies have shown that the presence of SE is associated with high anticitrullinated peptide antibody (ACPA) levels [2]. RA patients are evaluated by the presence of elevated levels of the autoantibodies

rheumatoid factor (RF) and antibodies to ACPAs in the serum. Environmental factors (viral infections, bacterial infections, cigarette smoking, etc.) promote the loss of self-tolerance to self-antigen. Other factors such as lifestyle, hormonal status, pregnancy, age, sex, and stochastic factors are also responsible for the onset of disease. These various elements interact to trigger or advance autoimmunity, ultimately resulting in damage to tissues and inflammation [3].

Key indicators of RA encompass inflammation and tissue damage, initiated by the infiltration of lymphocytes (including B cells, CD4+ helper T cells, and CD8+ cytotoxic T cells), alongside the activation of plasma cells (including mast cells, macrophages, and synovial fibroblasts) to generate pro-inflammatory substances such as IL-6, TNF-α, and IL-1 within the synovium. These substances attract and activate neutrophil granulocytes, which in turn produce various enzymes capable of breaking down cartilage, including metalloproteinases, thus promoting joint degradation. The accumulation of such substances in the joints can also lead to a range of extra-articular manifestations like vasculitis, nodules, and accelerated atherosclerosis [4].

PATHOPHYSIOLOGY OF RA

Rheumatoid arthritis (RA) is characterized by a prolonged and targeted immune reaction directed towards unknown self-antigens. It's an autoimmune reaction where the synovial tissue undergoes inflammation and infiltration by immune cells, leading to tissue damage. This process is associated with increased levels of cytokines present in the fluid within the joint. Various cell types are present in the sub-intimal synovial layer, including T cells, B cells, dendritic cells (DCs), fibroblasts, granulocytes, macrophages, and mast cells. This diversity results in low levels of Th1 cytokines (such as IFN- γ and IL-2) and elevated concentrations of cytokines produced by macrophages and fibroblasts (such as IL-1, IL-6, IL-15, IL-18, GM-CSF, and TNF-α) in the synovial fluid. Cytokine generation is the central basis of the pathogenesis of rheumatoid arthritis.

ROLE OF CYTOKINES

Cytokines are pivotal in various biological processes, influencing cellular growth, proliferation, differentiation, inflammation, tissue repair, and regulation of the immune system. Their role in inflammation and joint destruction is well-reported in the literature[5]. The inflammation characteristic of RA arises from an abundance of pro-inflammatory cytokines (such as IL-1, IL-12, IL-17, IL-18, IL-23, IL-32, and TNF-α) compared to anti-inflammatory cytokines (such as IL-4, IL-10, IL-13, and IL-35). There are a few cytokines that play an ambivalent role such as IL-27 suppresses IL-6 and TGF-β mediated Th17 differentiation and thereby inhibits IL-17, other proinflammatory cytokines, decreasing the influx of monocyte and joint destruction. All cytokines are produced from T cells, B cells,monocytes, macrophages, dendritic cells, fibroblasts, mast cells, and endothelial cells. A high concentration of IL-1β in plasma and synovial fluid (SF) accounts for various parameters, mainly related to the duration of morning stiffness and increases the release of MMPs, PGs, iNOS, PGs, osteoclast activation, and endothelial cell adhesion molecules to a certain extent. The balance between IL-1 and IL-1 receptor antagonist (IL-1Ra) is disrupted in disease. In the synovium and serum of the RA patient IL-6 isa major cytokine, a potent inducer of pro-inflammatory cytokines disturbing the normal physiological balance between pro- and anti-inflammatory mediators. In combination with transforming growth factor (TGF)-β, it promotes Th17 differentiation from CD4+ T cells. These cells and synoviocytes secrete inflammatory mediators, such as interleukin (IL)-6, IL-17, and TNF-α, which sustain inflammation and tissue damage by influencing other cell types within the synovium and periarticular structures [6].

IL-8 is another cytokine produced from fibroblast-like synoviocytes (FLS) and along with IL-6, it contributes to inflammation and joint damage. IL-12 is a heterodimeric cytokine produced by APCs such as monocytes, macrophages, and dendritic cells. Research indicates that both serum and synovial fluid of rheumatoid arthritis (RA) patients exhibit elevated levels of IL-12, which correlate with disease severity. IL-12 promotes the synthesis of other pro-inflammatory cytokines like IL-6, TNF-α, and IL-2. The IL-17 cytokine, predominantly produced by CD4+ Th17 cells, is

associated with increased production of MMP1 and MMP3, along with several pro-inflammatory cytokines including IL-6, TNF-α, IL-1β, and IL-8, contributing to bone degradation.

It also enhances the infiltration of immune cells as well as causes neovascularisation by promoting the growth of blood vessels and migration of endothelial cells and vascular endothelial growth factor (VEGF) into the synovium. IL-18 is an 18-kDa moiety that enhances cell-cell interaction leading to cytokine-facilitated activation of T cells and macrophages. It has been found to increase the severity of arthritis in the CIA study model. Elevated levels of IL-21 have been detected in the synovial tissue and serum of RA patients which causes activation and infiltration of Th17 and B cells in synovial tissue and leads to joint destruction. IL-23 is produced by monocytes, macrophages, and dendritic cells and is involved in the promotion and stabilization of IL-17 as well as osteoclastogenesis. IL-32 induces the production of pro-inflammatory cytokines such as IL-6, IL-1β, and chemokines which promotes osteoclast differentiation [7,8]. IL-33 is generated by macrophages, fibroblasts, and dendritic cells and is responsible for the severity of disease by the production of pro-inflammatory cytokines and chemokines [9]. TNF-α plays a fundamental role by activating cytokines, inducing chemokine expression and endothelial cell adhesion molecules, supporting synovial fibroblasts, promoting angiogenesis, suppressing regulatory T cells, and eliciting pain. TNF-α contributes to the expression of endothelial cell adhesion molecules, the suppression of regulatory T cells, the stimulation of angiogenesis, and the generation of pain. Elevated levels of various pro-inflammatory cytokines have been detected in the serum and synovial fluid of rheumatoid arthritis (RA) patients, underscoring their significant involvement in the progression of the disease. The role of various cytokines in RA is given in Table 1 and Figure 1.

Table 1: Role of cytokines in RA.

Cytokine	Function
IL-1	Synovial fibroblasts produce various cytokines, chemokines, matrix metalloproteinases (MMPs), prostaglandins (PGs), inducible nitric oxide synthase (iNOS), and activate osteoclasts, endothelial cell adhesion molecule expression
IL-6	Increases the production of pro-inflammatory cytokines, triggers the release of reactive oxygen intermediates and proteolytic enzymes, induces the release of MMPs, promotes osteoclast differentiation, and stimulates the production of vascular endothelial growth factor (VEGF).
IL-8	Inflammation and joint damage
IL-12	Promotes production of other pro-inflammatory cytokines
IL-17	Increases the production of MMP1 and 3, somepro-inflammatory cytokines, bone destruction
IL-18	It prompts the synthesis of tumor necrosis factor (TNF), prostaglandin E2 (PGE2), cyclooxygenase-2 (COX-2), chemokines, and angiogenesis. Additionally, it impedes chondrocyte proliferation.
IL-21	It triggers the differentiation of Th17 cells, as well as B cells and plasma cells.
IL-23	Promotion and stabilization of IL-17,osteoclastogenesis
IL-32	Induces production of pro-inflammatory cytokines such as IL-6, IL-1β, chemokines
IL-33	Activate pro-inflammatory cytokines production, chemokines, and PG E2
TNF-α	It promotes the proliferation of collagenase and MMP, enhances the expression of GM-CSF and ICAM-1, stimulates the production of pro-inflammatory cytokines, and facilitates the differentiation of T and B cells.

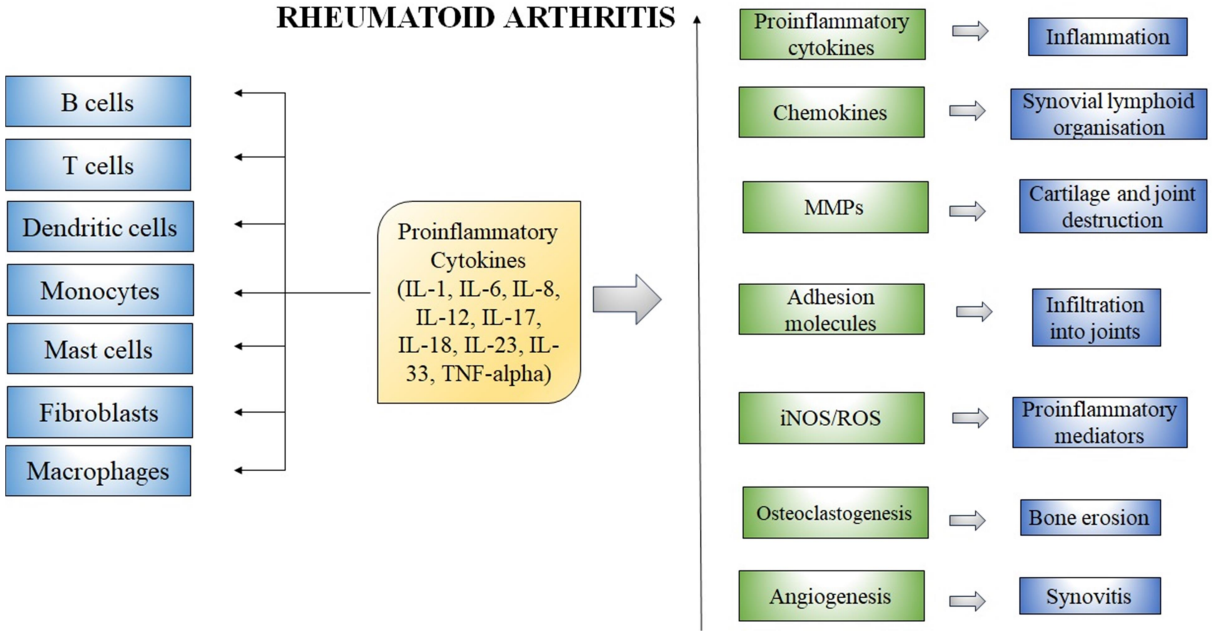


Fig. 1. Role of cytokines in RA.

Il-6 and its Role in the Progression of RA

IL-6 is a glycoprotein weighing approximately 26 kDa and functions as a pleiotropic cytokine. It is generated by various cell types including B cells, T cells, fibroblasts, endothelial cells, monocytes, macrophages, keratinocytes, chondrocytes, and certain tumor cells. Deregulated and persistent production of IL-6 plays a crucial role in the manifestation of key features of rheumatoid arthritis (RA). Elevated levels of IL-6 are commonly found in the synovial fluid and serum of RA patients, and its concentration correlates with disease activity, underscoring its significance in RA pathology.IL-6 contributes to synovitis and joint degradation by promoting neutrophil migration, osteoclast maturation, and the formation of pannus stimulated by VEGF. IL-6, in conjunction with transforming growth factor (TGF)-β, fosters the differentiation of Th17 cells from CD4+ T cells, leading to the secretion of IL-6, IL-17, and TNFα. While IL-6 inhibits TGF-β-induced differentiation of regulatory T cells (Tregs) from CD4+ T cells. Tregs play a pivotal role in maintaining immune homeostasis and preventing autoimmune diseases [10].

The imbalance between Th17 and Treg cells, influenced by IL-6, contributes to the pathogenesis of numerous autoimmune and chronic inflammatory conditions. IL-6 triggers the expression of VEGF in synovial fibroblasts, resulting in significant microvascular permeability. This permeability affects endothelial cells by inducing phosphorylation and internalization of VE-cadherin, a key structural protein. These changes in VE-cadherin ultimately lead to vascular leakage [11]. Moreover, IL-6 stimulates endothelial cells to produce additional IL-6, IL-8, and MCP-1, while also upregulating the expression of intercellular adhesion molecule (ICAM)-1. These actions of IL-6 facilitate increased recruitment of leukocytes and inflammation within the joints.In addition, IL-6 may also mediate many systemic manifestations including acute-phase reaction, anemia, fatigue, and osteoporosis. The role of IL-6 in RA is given in Figure 2.

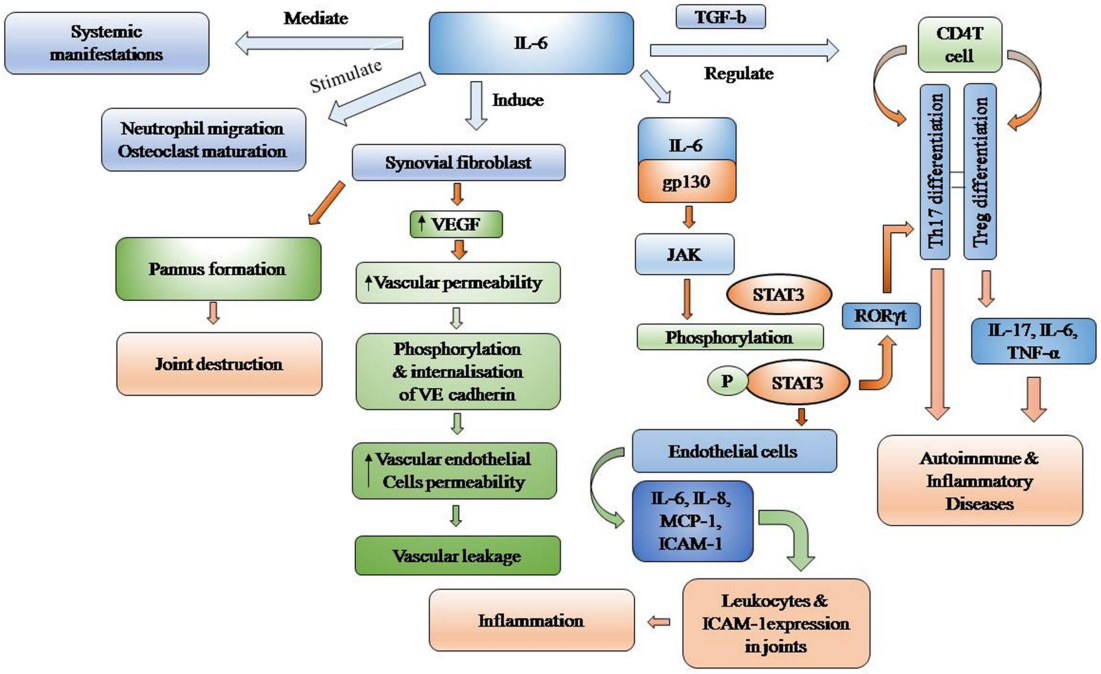


Fig. 2. Role of IL-6 in RA.

The IL-6 receptor comprises two chains: an IL-6-specific receptor (IL-6R) and a signal transducer known as gp130. Both subunits are present in soluble form. In the signaling process, IL-6 initially binds to the transmembrane IL-6R. Subsequently, this complex is associated with the signal-transducing molecule gp130, leading to the initiation of downstream signaling cascades in target cells. These target cells, such as neural cells, smooth muscle cells, and endothelial cells, exclusively express gp130 on their surfaces. The IL-6 signal transduction pathway involves the activation of Janus kinase (JAK) tyrosine kinase family members. Once activated, these kinases induce tyrosine phosphorylation and subsequent activation of signal transducer and activator of transcription (STAT3). Upon phosphorylation, STAT3 forms dimers that migrate to the nucleus, facilitating the transmission of signals from the cell membrane to the nucleus [12]. STAT3 further stimulates the expression of several genes which leads to the pleiotropic activity that includes differentiation of Th17 via upregulation of retinoid orphan receptor (ROR)-γtand osteoclasts, synthesis of acute phase proteins, production of MMPs, synoviocyte proliferation and induction of endothelial cells which produces IL-8 and MCP-1 resulting into local inflammation and thus joint destruction. All of the above makes IL-6 blockade a desirable therapeutic alternative in the treatment of RA.

PARADIGMS OF TREATMENT IN RA

The currently available strategies for the treatment of RA include the use of Disease-modifying antirheumatic drugs (DMARDs- methotrexate, sulphasalazine) alone, or in combination with nonsteroidal anti-inflammatory *drugs* (*NSAIDs- ibuprofen, celecoxib*) and glucocorticoids (*GCs-* beclomethasone, betamethasone, budesonide) that provide symptomatic relief. Currently available treatment for RA is given in Figure 3 [13].

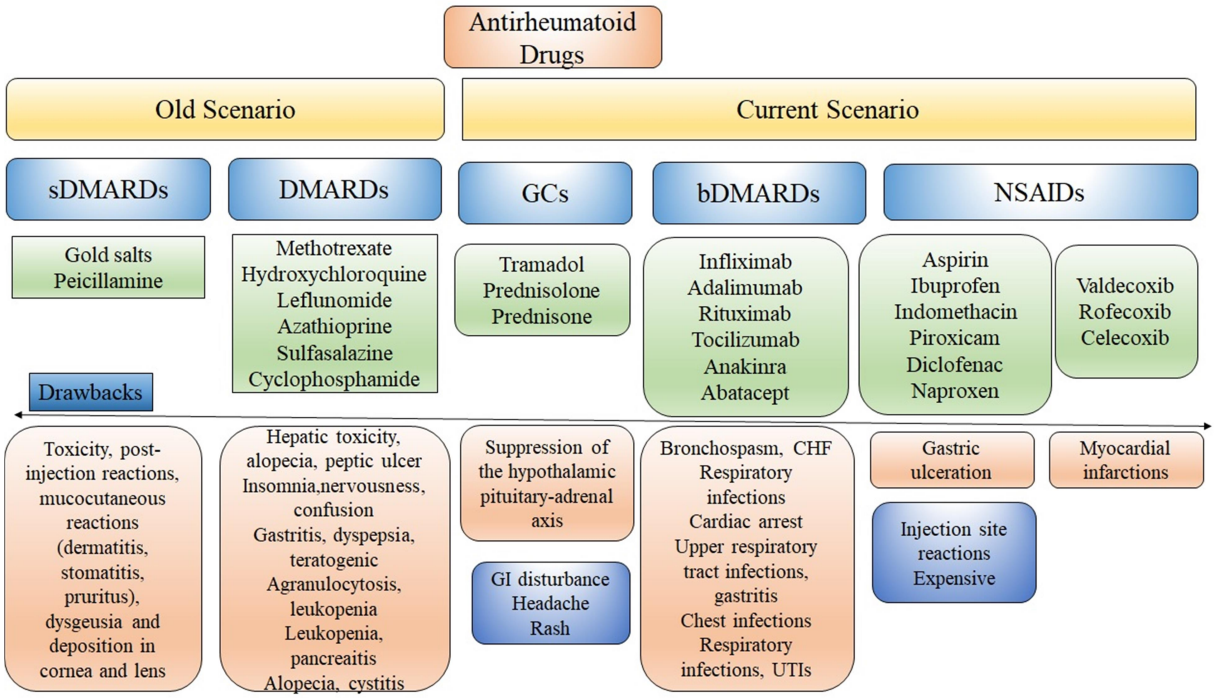


Fig. 3. Current RA treatment strategies and its drawback.

VARIOUS CHEMICAL MOIETIES REPORTED AS IL-6 INHIBITOR

The therapeutic approach of inhibiting IL-6 with antibodies has emerged as an effective and alternative strategy for treating inflammatory conditions that do not respond well to conventional medications. Clinical investigations targeting IL-6 using antibodies such as sirukumab (CNTO136), olokizumab (CP6038), PF-423691, siltuximab (CNTO328), elsilimomab (BE-8), clazakizumab (BMS945429), and MEDI5117, as well as anti-IL-6R antibodies like sarilumab (REGN88) and tocilizumab (Actemra), are either ongoing or completed [14]. The use of antibodies targeting IL-6 has achieved tremendous commercial successes, however, suffers from major drawbacks such as high cost, invasive route of administration, and high rate of immunogenicity. Therefore, the search for small molecules as IL-6 inhibitors is warranted by their superiority in oral absorption and low antigenicity.

(+)-Madindoline A (1), a nontoxic small organic molecule, originally isolated by fermentation of *Streptomyces nitrosporeus* K93- 0711 has displayed potent IL-6/IL-6R blocking properties. Unfortunately, (+)-Madindoline A is produced in a low yield by fermentation and is difficult to synthesize chemically.

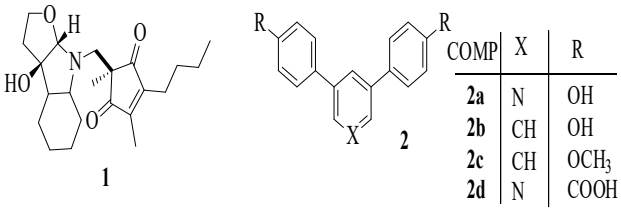


Fig. 4. Structure of (+)-Madindoline A and compounds 2a-2d.

Tagat et al (1995), were the pioneers in developing small molecules for the inhibition of IL-6, a multifunctional cytokine involved in various inflammatory and tumorigenic pathways, synthesized 3,5-diaryl pyridines and meta-terphenyls [15]. Authors achieved this synthesis through nickel and palladium-catalyzed coupling of organometallic reagents to aryl halides, resulting in compounds with IL-6 inhibitory activity. Compounds (2a-2d), screened at a concentration of 10 µM for their ability to inhibit lipopolysaccharide (LPS)-induced IL-6 production in murine myelomonocytic leukemia (WEHI-265.1) cells, demonstrated inhibition rates exceeding 70% (Figure 4). It was reported as the polarity of groups (such as COOH, OCH3, and OH) increased, so did the activity. The IC_{50} values for the most potent compounds, (2a) and (2b)

were reported to be 0.4µM and 1.5µM, respectively. Pyrazolo[3,4-b] pyridines and related structural analogues have been identified as potent inhibitors of interleukin-6 (IL-6). These compounds were synthesized via a one-pot condensation reaction involving 5,6-dihydro-4H-pyran-3-carbaldehyde, 2-formyl-3,4,6-tri-O-methyl-D-glucal, or chromone-3-carbaldehyde with various heteroaromatic amines. Among them, only three compounds, labeled as 3a, 3b, and 3c, exhibited more than 50% inhibition at a concentration of 10µM, with calculated IC_{50} values of 0.2µM, 0.3µM, and 0.16µM, respectively. These findings suggest that the isoxazole-pyridine class of compounds, particularly those with the lowest IC_{50} values, have the potential to serve as a novel class of anti-inflammatory agents with specific IL-6 inhibitory activity [16] (Figure 5).

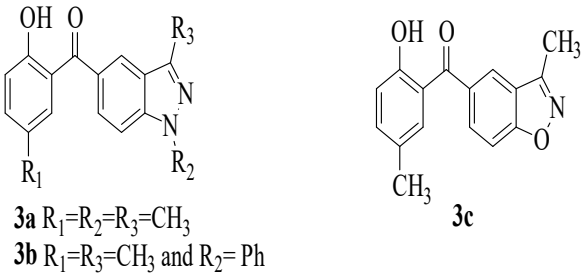


Fig. 5. Structures of Pyrazolo[3,4-b] pyridines and related structural analogues.

Kahlon et al (2009), demonstrated the inhibition of IL-6 *via* NF-κB mediated gene transcription by Imidazole. Initially, a small series of *trans*-imidazole was investigated for its potential inhibitor of IL-6 production [17]. The success of this study led to further exploration of the same nucleus at various positions R_1, R_2, R_3, and R_4 with various groups. Among all the exploration compound 4 has phenyl at R_1 and R_3, benzyl at R_4, and COOEt at R_3 with the lowest IC_{50} value of 0.8. In 2010, a group of nitrogen-containing benzophenone analogues was synthesized through the Mannich reaction and assessed for their ability to inhibit TNF-α and IL-6.Their antioxidant potential was investigated through DPPH (1,1-diphenyl-2-picryl hydrazine) radical scavenging activity and kinetics. The synthesized compounds displayed promising activity against IL-6, with inhibition rates exceeding 80% and 40% at concentrations of 10 and 1 µM, respectively. Compound (5e) demonstrated efficacy as an IL-6 inhibitor, achieving inhibition rates of 97% and 17% at concentrations of 10 and 1 µM, respectively. Structure-activity relationship (SAR) studies revealed that substitution at the 3-position with chlorine (35-54%) or fluorine (30-40%) is more conducive to IL-6 inhibition. Conversely, substitution at the 2-position with fluorine (15-21%) or bromine (9-22%) was found to be less favorable for inhibition (Figure 6).

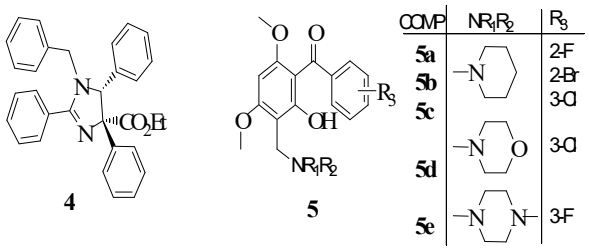


Fig. 6. Structure of imidazole and benzophenone analogues possess inhibition of IL-6.

Further, Bandgar et al (2010) synthesized a combinatorial library of β-chlorovinylchalcones and evaluated their anti-inflammatory activity (IL-6 and TNF-α inhibition). The study revealed promising anti-inflammatory potential by showing 95-97% and 66-67% inhibition of IL-6 and TNF-α respectively at 10µM. Compounds (6a) and (6b) have shown maximum IL-6 inhibitory activity while the remaining compounds i.e. (6c-6g) were moderately active [18, 19] (Figure 7).

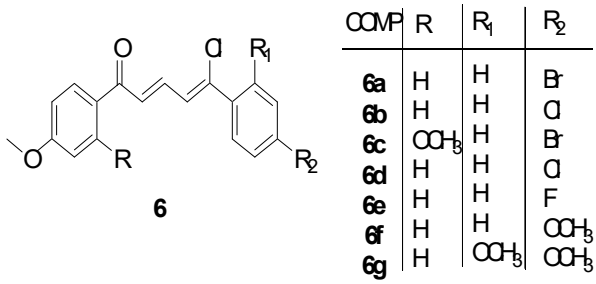


Fig. 7. Structure of β-chlorovinylchalcones possess inhibition of IL-6 and TNF-α.

A small library of pyrrolidinesulphonylaryl and piperiazinesulphonylaryl molecules have been synthesized and evaluated for their ability to inhibit IL-6 signaling

through STAT-3. Various aryl moieties at R_2 contain different electronic groups like OH, OCH_3, NO, Cl, SH, CH_3,NH and hetero rings at R_1 are furanyl, thrienyl, and thiazole. Initially, the molecules tested for all viability using MDB-MB-231 breast (STAT-3 dependent) and A4 (STAT-3 null) cancer cell lines. Compound (7) proved to be a potential STAT-3-specific inhibitor. This compound was progressed into luciferase reported assay in which the cells were treated with oncostatin M to activate STATs-3 signaling *via* the IL-6/gp130 receptor that was shown to selectively inhibit STAT-3 transcriptional activity. The compound (7) was found to be inhibiting STAT-3 with an EC_{50} of 15µM. Pyrrolidinesulphonylaryl molecule (7) was intact with a protein having PDB 1P9M and the interaction energy for the docking was calculated as -8.82 Kcal/mol and -8.25 Kcal/mol for two sites with IL-6 receptor [20]. Variedly substituted chalcones (8) were synthesized by utilizing the Vilsmayer–Hack formylation reaction. The results of IL-6 revealed the elemental effect on the activity i.e. fluoro group increases the potency in comparison to bromo and chloro. Further presence of the chloro group at R_1 also decreased the activity [21].

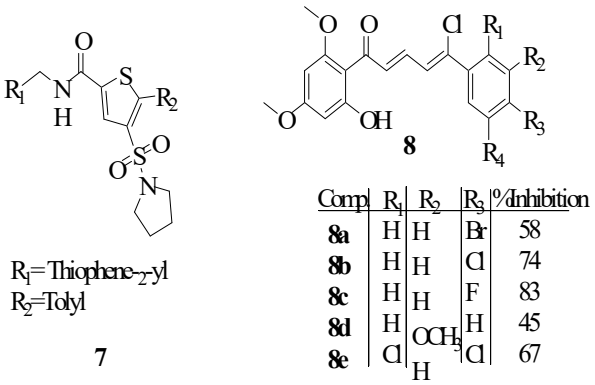


Fig. 8. Structure of Pyrrolidinesulphonylaryl molecule and substituted chalcones.

A series of thalidomide analogues (referred to as compound 9) were synthesized and compared to both thalidomide and its more potent analogue, lenalidomide. Their effectiveness in inhibiting the production of the pro-inflammatory cytokines tumor necrosis factor (TNF)-α and interleukin (IL)-6 was evaluated using LPS-activated peripheral blood mononuclear cells (PBMCs). Thalidomide and the compounds were tested at concentrations of 100, 10, and 1 µM to assess their impact on TNF-α and IL-6 production. While thalidomide was found to inhibit TNF-α production, it did not affect IL-6 levels. Whereas, lenalidomide possesses 2-3 times increased potency for both TNF-α and IL-6. The study reported that compound 9c at 100 µM concentration inhibits TNF-α and IL-6 secretion significantly, but is devoid of effect at lower concentrations i.e. 10 and 1 µM. This signifies that compound (9b) induced a dose-dependent inhibition of TNF-α and IL-6 production as compared to thalidomide. The tricyclic structure and the sulfonyl group can be utilized as potent scaffolds for the development of anti-TNF-α and IL-6 agents [22].

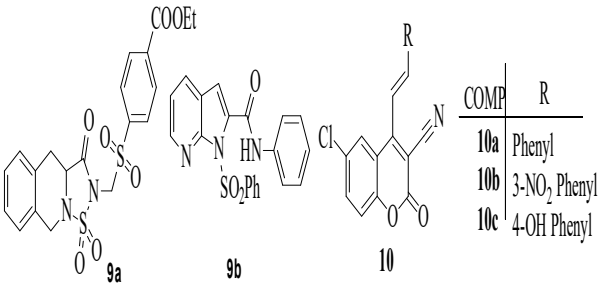
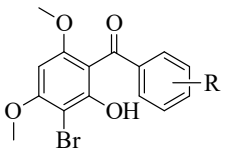


Fig. 9. Structure of thalidomide analogues and 4-styryl-coumarin derivatives.

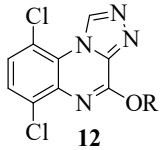
In 2011, Upadhyay et al synthesized a new series of 4-styryl-coumarin derivatives (10) and evaluated them for their anti-inflammatory activity via the inhibition of IL-6 [23]. Among the various compounds substituted by electronic groups at various positions at the phenyl ring, compounds10a, 10b, and 10c exhibited promising activity against IL-6 with 87%, 72%, and 73% at 10µM concentration, respectively. These results revealed that the un-substituted phenyl group (10a) at the 4-position acquires good inhibitory activity while the substituted phenyl group (10b and 10c) weakens the inhibitory activity (Figure 9). Moreover, the orientation of the substituent on the phenyl ring also influences the potency *i.e. para* orientation increases the potency in comparison to *ortho* and *meta.* The size of the substituent should be optimal as bulkiness decreases the potency. A set of benzophenone derivatives (designated as compound 11) was synthesized through Fridel-Craft acylation and subsequently assessed for their anti-inflammatory potential against TNF-α and IL-6 using a lipopolysaccharide (LPS)-induced cytokine production assay. This evaluation utilized human THP-1 cells stimulated with phorbol myristate acetate in vitro.

Cytotoxicity studies were conducted using CCK-8 cells at a concentration of 10μM. All the compounds demonstrated significant activity against IL-6, ranging from 63% to 82% inhibition at the 10μM concentration. The fluoro group at *para* position (11a) having 63% IL-6 inhibitory activity proves itself to be safe in comparison to the compound with methoxy at *para position* (11b) having 82% IL-6 inhibition [24]. In 2012, Guirado et al. developed a series of compounds called 3-alkoxy-5,8-dichloro-2-cyanoquinoxalines by synthesizing them from 5,8-dichloro-2,3-dicyanoquinoxaline and alcohols in the presence of triethylamine [25]. They found that many of these compounds exhibited promising activity as inhibitors of both cytokines, TNF-α and IL-6 while displaying very low levels of cytotoxicity. The result of the study showed that compounds (12a), (12b), (12c), and (12d) exhibit 80%, 100%, 70% and 93% inhibition of IL-6 at 50μM concentration with IC_{50} 35.5, 10, 49.9 and 36.8 respectively. Compounds (12a), (12b), and (12c) act as specific inhibitors of IL-6 without affecting the activity of TNF-α (Figure 10).



11

COMP	R
11a	4-F
11b	4-OCH_3
11c	2-F

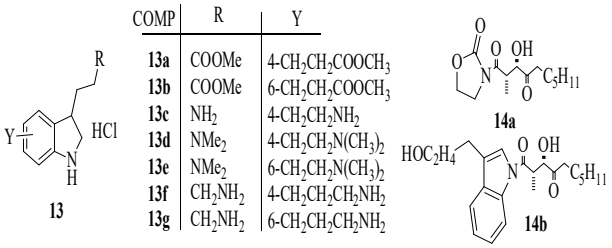


12

COMP	R
12a	$CH_3CH_2CHCH_3$
12b	4-$CH_3OC_6H_4CH_2$
12c	3-$CH_3C_6H_4CH_2$
12d	$CH_2C_6H_4$

Fig. 10. Structure of benzophenone derivatives and compounds 3-alkoxy-5,8-dichloro-2-cyanoquinoxalines.

A study investigated a series of newly synthesized carbamate derivatives of indoline-3 or 1-substituted with propionic ester, primary, and tertiary amine (13a-13g) for their effects on various cytokines (TNF-α and IL-6) in LPS-activated macrophages. The findings indicate that several of these indoline derivatives effectively decreased levels of NO, TNF-α, and IL-6 proteins at concentrations ranging from 1 to 10 pM. Notably, these concentrations were significantly lower—ranging from 1000 to 100 times—than those required for the steroid budesonide [26].



COMP	R	Y
13a	COOMe	4-$CH_2CH_2COOCH_3$
13b	COOMe	6-$CH_2CH_2COOCH_3$
13c	NH_2	4-$CH_2CH_2NH_2$
13d	NMe_2	4-$CH_2CH_2N(CH_3)_2$
13e	NMe_2	6-$CH_2CH_2N(CH_3)_2$
13f	CH_2NH_2	4-$CH_2CH_2CH_2NH_2$
13g	CH_2NH_2	6-$CH_2CH_2CH_2NH_2$

13

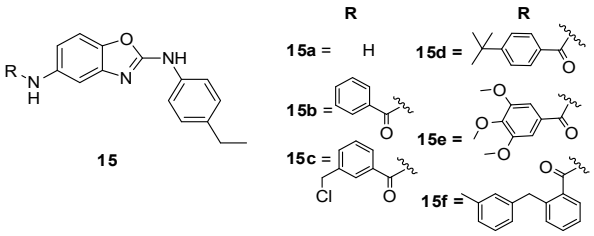
14a

14b

Fig. 11. Structure of carbamate derivatives of indoline-3 and oxazolidinone derivatives.

Singh et al (2016) demonstrated that a series of oxazolidinone (14a) and indole (14b) derivatives act as blockers of IL-6 signaling [27]. They assessed their impact on IL-6-induced luciferase expression in human hepatocarcinoma HepG2 cells transfected with p-STAT3-Luc. Among these compounds, 14a emerged as the most potent IL-6 signaling blocker, with an IC_{50} value of 5.9 μM, surpassing the standard IL-6 inhibitor (+)-Madindoline A (IC_{50} = 21 μM). The mode of action of compound 14 was reported inhibition of IL-6 through the GP130/STAT3 signal transduction mechanism.The computational modeling has been carried out which proved that compound (14a) binds with the G130 D1 domain of the hexameric complex composed of the two trimers in a similar way as that of madindoline.

Kim et al (2017) synthesized a series of benzoxazole derivatives and reported their suppressive effects on IL-6-mediated signalling [28].



15

Fig. 12. Structure of compounds benzoxazole derivatives.

Compounds 15a-15f exhibited significant suppression of IL-6-induced phosphorylation of signal transducer and activator of transcription (STAT)3, ranging from 80% to 90%. Compound 15a demonstrated particularly potent inhibition of IL-6-STAT3 signaling (figure 12).

Many naturally occurring agents have also been explored for their potential for inhibition of various cytokines like TNF-α, IL-6, IL-1, etc. Very few reports exist in the literature

regarding the isolation and preparation of semi-synthetic derivative of the phytoconstituent poses IL-6 inhibitory activity.

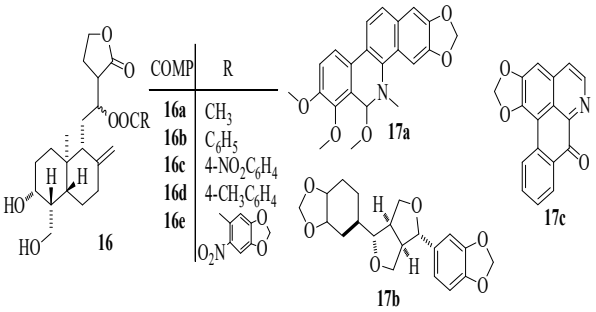


Fig. 13. Structure of semi-synthetic derivative of andrographolide.

Andrographolide, extracted from *Andrographis paniculata* leaves, has been observed to inhibit TNF-α and IL-6 expression in murine macrophages in a concentration-dependent manner. This effect is achieved through the suppression of the ERK1/2 and PI3K-Akt signaling pathways. The semi-synthetic derivative of andrographolide (16a-16e) was prepared and evaluated against LPS-induced IL-6 and TNF-α released in mouse macrophages. The most of compound shoe percentage inhibits in a range of 30-45% whereas as andrographolide was observed maximally potent with 56% inhibit of IL-6 expression. The SAR of the class suggests that the aryl moiety at 12-C (16b, 16c, 16d) showed better inhibitory effects compared to compounds with an alkyl moiety at 12-C (17a) in the expression of both TNF-α and IL-6.Further, the presence of electron electron-withdrawing group in the aromatic ring improved the activity [29]. In 2014, Liu et al isolate angoline (17a) from *Zanthoxylumnitidum* as a potent IL-6/STAT3 signaling pathway inhibitor. They also isolate L-sesamin(17b) and liridenine (17c). Among all these isolated compounds, angoline (17a) showsa potent inhibitory effect on STAT-3 transcription activity with an IC_{50} 11.5 µM, whereas L-sesamin (17b) and liridenine (17c) do not affect IL-6/STAT3 signaling pathway [30] (Figure 13).

Sharma et al (2016) obtained Boswellic acid from *Boswelliaserrata* has been reported to be a potent anti-inflammatory [31].

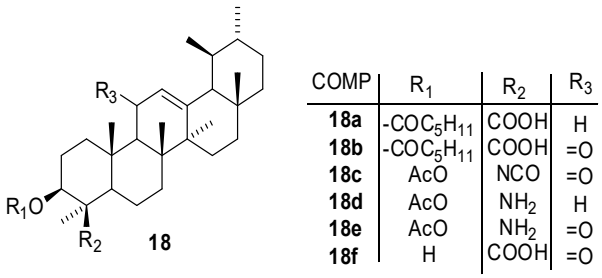


Fig. 14. Structure of acyl analogue of boswellic acid.

They synthetically prepared the acyl analogue of boswellic acid including their epimers and 4-amino analogs used as inhibitors of pro-inflammatory cytokines (TNF-α and IL-6). Compound 18a-18f was proved to be a highly effective inhibitor of IL-6 in an *in-vitro* study and percentage inhibition ranges from 53-69% inhibition at a concentration of 10 µM. Among all these *vivo* studies further evaluated these compounds showed that compound 18f is the most potent inhibitor of IL-6 with 57.5±1.6 % at 10 mg/kg concentration. The SAR reported for the study reveals that the higher acyl homologues show better IL-6 inhibitors in comparison to lower acyl homologues and the replacement of acid with amino group results in an increase in anti-inflammatory activity. The four compounds (19, 20, 21, and 22) with percentage inhibition values ranging from 81.81- 87.26% (pg/ml) were found to be most active against IL-6 [32] (Figure 15). The tetrazole-based COX-2 inhibitors were designed, synthesized and an anti-inflammatory evaluation of PGE2, TNF-α, and IL-6 was carried out by Labib et al (2020), out of five compounds that were tested for IL-6 inhibition the compound (23) was found to be most active with 61.56% inhibition [33]. Three series of substituted phenyl cycloalkyl ureas, phenyl cycloalkyl thioureas, and phenylcycloalkylsquaramides reported that cytokine levels were reduced by more than 70% up to 95% which is higher than the inhibitory effect observed with ibuprofen by the below-shown compounds (24, 25, and 26) [34]. Yoo et al (2022) synthesized a series of benzoxazole derivatives and concluded that the compound (27) was proved effective in the animal model of RA by inhibiting the IL-6/STAT3 signaling pathway and inhibiting the generation of IL-1 [35].

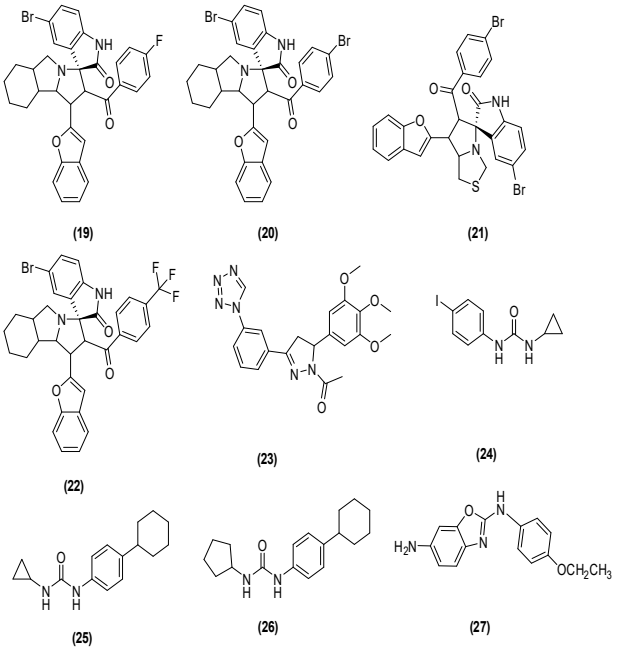


Fig. 15. Structure of compounds (19-27).

Three clinically significant medications approved by the US FDA, Bazedoxifine (28), Raloxifine (29), and Pracinostat (30) were used in the treatment of postmenopausal osteoporosis(28 &29) and breast cancer metastasis (30). Bazedoxifene and Raloxifeneare also reported to specifically suppress the Phosphorylation of STAT3 induced by IL-6 in the signaling pathways involving GP130/JAK/STAT3 [30]. Tian et al (2019) reported Bazedoxifene as a novelIL-6/GP130 inhibitor for the treatment of triple-negative breast cancer. The result of the study showed that bazedoxifene downregulated the P-TAT3,Genes were targeted downstream by the IL-6/GP130 signaling pathway stimulated by IL-6, and so onIL-6/GP130/STAT3 signalling cascade [36]. Raloxifene is a selective estrogen receptor modulator used in the prevention and treatment of osteoporosis.In 2020 Luo et al. reported that Raloxifene also prevents high-fat-induced atherosclerosis by inhibiting the interaction between IL-6 and its receptor subunit GP130 and decreasing IL-6-dependent P-STAT3 [37]. According to recent research,Pracinostat retards breast cancer metastasis by inhibiting the IL-6/STAT3 signaling pathway [38] (Figure 16).

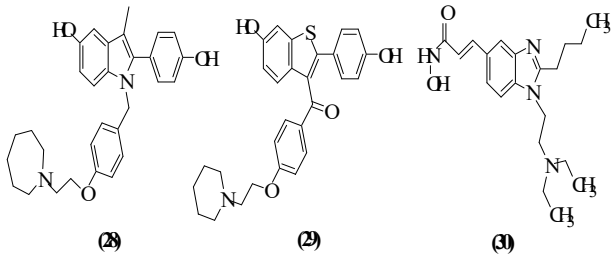


Fig. 16. Structure of compounds (28-30).

CONCLUSION

Inhibition of IL-6 is the primary approach for the treatment of RA. Numerous antibodies such as sirukumab (CNTO136), olokizumab (CP6038), PF-423691, siltuximab (CNTO328), elsilimomab (BE-8), clazakizumab (BMS945429), and MEDI5117, as well as anti-IL-6R antibodies like sarilumab (REGN88) and tocilizumab (Actemra) have been reported to targeting IL-6 for RA treatment. Furthermore, both naural and synthetic compounds have been reported for RA treatment. Natural compounds including (+)-madindoline A, andrographolide, angoline, L-sesamin, liridenine and boswellic acid have beed well reported for IL-6 mediated anti-RA activity. 3,5-diaryl pyridines, meta-terphenyls, pyrazolo[3,4-b]pyridines, β-chlorovinylchalcones, pyrrolidinesulphonylaryls, piperiazinesulphonylaryls, thalidomide analogues, indoline-3 or 1-substituted and benzoxazole derivatives are the synthetic compounds reported by numerous researchers possess anti-RA activity. Bazedoxifine, raloxifine and pracinostat are US FDA approved anti-RA drugs.

ACKNOWLEDGEMENTS

None

CONFLICT OF INTEREST

None

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