EXPLORING THE ROLE OF CURCUMIN INDUCED STAT3 INHIBITION IN RHEUMATOID ARTHRITIS TREATMENT

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By

Prachi Pannu

2K22/MSCBIO/38

Under the Supervision of

Dr. Asmita Das

DEPARTMENT OF BIOTECHNOLOGY

DELHI TECHNOLOGICAL UNIVERSITY

(Formerly Delhi College of Engineering)

Shahbad Daulatpur, Main Bawana Road

June, 2024

CANDIDATE'S DECLARATION

I, Prachi Pannu, 2K22/MSCBIO/38 student of M.Sc. Biotechnology, hereby certify that the thesis entitled **"Exploring the role of curcumin induced STAT3 inhibition in Rheumatoid Arthritis treatment"** in partial fulfilment of the requirement for the award of the Degree of Masters of Sciences submitted in the Department of Biotechnology, Delhi Technological University is an authentic record of my own work carried out during the period from May 2023 to May 2024 under the supervision of Dr. Asmita Das.

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Date:
Place: Delhi Technological University

Dr. Asmita Das
Assistant Professor
Department of Biotechnology
Delhi Technological University

Prof. Yasha Hasija
Head of Department
Department of Biotechnology
Delhi Technological University

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2K22/MSCBIO/38

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LIST OF ABBREVIATIONS

RA	Rheumatoid Arthritis
STAT3	Signal Transducer and Activator of Transcription 3
HLA	Human Leukocyte Antigen
MAPK	Mitogen-Activated Protein Kinase
ERK1/2	Extracellular Signal-Regulated Kinase
AP-1	Activator Protein-1
NF-κB	Nuclear Factor-Kappa B
TNF-α	Tumour Necrosis Factor-Alpha
IL-6	Interleukin-6
IL-10	Interleukin-10
EGF	Epidermal Growth Factor
JAKs	Janus Kinases
PDB	Protein Data Bank
FLS	Fibroblast-Like Synoviocytes
MMPs	Matrix Metalloproteinases
DMARDs	Disease-Modifying Antirheumatic Medications
DMSO	Dimethyl Sulfoxide
PI3K	Phosphoinositide 3-Kinase
CIA	Collagen-Induced Arthritis
SAR	Structure-Activity Relationships
SMILES	Simplified Molecular-Input Line-Entry System
SDF	Structure Data File
GI	Gastrointestinal
BBB	Blood-Brain Barrier

ABSTRACT

Rheumatoid arthritis (RA) is a chronic autoimmune condition that mainly affects the joints, resulting in the degradation of tendons and ligaments. Typical symptoms of RA consist of inflamed and sensitive joints, fever, tiredness, and the formation of nodules beneath the skin called rheumatoid nodules. STAT3, which is a transcription factor, has a significant role in controlling the production of pro-inflammatory cytokines. The continuous activation of the JAK-STAT pathway, caused by cytokine signalling dysregulation, contributes to synovial inflammation. Hence, STAT3 inhibitors are essential to address the root cause of the disease rather than merely alleviating symptoms. *Curcuma longa* contains three primary curcuminoids: curcumin, desmethoxycurcumin, and bisdemethoxycurcumin. Curcumin, the active component, has demonstrated anti-inflammatory properties by modulating the JAK-STAT pathway. It effectively inhibits STAT3 activation and downstream signalling by promoting its dephosphorylation. Consequently, curcumin serves as a ligand that inhibits STAT3 activity, thereby mitigating RA symptoms. This study focuses on evaluating the interaction between curcumin and STAT3 through molecular docking. In-silico assays have shown that curcumin reduces arthritis scores and enhances inflammatory infiltration. Docking studies can determine the binding affinity between ligands and proteins, offering predictions on the strength of their interaction. Our molecular docking analysis indicates that curcumin exhibits lower binding free energy compared to other well-known STAT3 inhibitors, suggesting its potential efficacy in targeting RA symptoms.

Chapter 1

Introduction

Rheumatoid Arthritis (RA) is identified as a long-lasting, immune-related, inflammatory disorder that primarily affects the joints, leading to progressive joint damage, disability, and reduced quality of life. RA is characterized by the infiltration of inflammatory cells, such as T cells, B cells, and macrophages[1], into the synovial tissue, resulting in synovial hyperplasia, pannus formation, and the release of various inflammatory substances. These processes ultimately result in cartilage and bone, as well as systemic complications affecting multiple organs. After examination of several types of arthritis, they have been classified as non-inflammatory arthritis such as osteoarthritis, and inflammatory arthritis which can be caused by several factors such as crystal deposition (pseudo gout, basic calcium phosphate disease, and gout), as well as those triggered by bacterial and viral infections (Staphylococcus aureus, Neisseria gonorrhoea, complications of Lyme disease, Parvovirus, Enterovirus) or by autoimmune mechanism.[2], [3], [4], [5] The pathogenesis of RA is complex and involves the interplay of genetic, environmental, and immunological factors. Genetic predisposition, like specific human leukocyte antigen (HLA) alleles, plays a crucial role in the development of RA. Environmental triggers, such as smoking, infections, and dietary factors, can also contribute to the initiation and perpetuation of the autoimmune response in RA.

Insufficiently treated RA presents a much more complex clinical picture which includes the emergence of serious systemic manifestations including pleural effusions, lung nodules, interstitial lung disease, along with lymphomas, vasculitis in small or medium-sized arteries, keratoconjunctivitis, atherosclerosis, hematologic abnormalities (such as anaemia, leukopenia, neutropenia, eosinophilia, thrombocytopenia, or thrombocytosis).[6] The future of RA treatment might witness a shift towards personalized approaches, where targeting STAT3 inhibition through curcumin can be customized based on individual patients' genetic backgrounds, disease severity, and treatment response. Despite significant advancements in understanding RA pathogenesis and therapeutic strategies, managing RA remains challenging, with many patients experiencing ongoing disease activity, joint damage, and functional limitations. Curcumin holds significant promise as a therapeutic agent for the treatment of RA.[7] Its anti-inflammatory, antioxidant, and immunomodulatory properties,

along with its ability to modulate key signalling pathways such as STAT3, NF-κB, and Nrf2, make it a valuable adjunctive therapy for RA. However, further research is required to fully elucidate the molecular mechanisms underlying its effects, enhance its bioavailability, and evaluate its long-term safety and efficacy.[8], [9] Through continuous research and progress, curcumin could emerge as a fundamental element in the RA treatment, offering a safe and effective alternative to conventional therapies. Exploring innovative and effective therapeutic strategies, such as natural compounds, is crucial to advancing RA research.

Chapter 2

Review of Literature

2.1 Background and Significance of Rheumatoid Arthritis

RA affects approximately 0.5-1% of the adult population worldwide, with a higher prevalence in women compared to men [10], [11]. The disease typically manifests in the third to sixth decades of life, although it can occur at any age. The incidence of RA varies geographically, with higher rates reported in North America, Europe, and Asia compared to Africa and South America. The socioeconomic burden of RA is substantial, with direct and indirect costs associated with medical care, lost productivity, and disability. In the United States alone, the annual cost of RA[12] is estimated to be around $19 billion, with a significant portion attributed to lost productivity and disability-related costs. The clinical manifestation of RA manifests as joint pain, swelling, stiffness, and tenderness, mainly in the small joints of the hands and feet[13]. The disease typically follows a pattern of flaring up and subsiding, with periods of worsening symptoms and periods of relief. Untreated RA can lead to joint abnormalities, functional limitations, and disability, greatly diminishing the quality of life for those affected. RA is characterized by symmetrical polyarthritis, affecting corresponding joints on both sides of the body. Inflammation of the synovial membrane causes the development of pannus, an abnormal tissue layer that invades and damages cartilage and bone. Common deformities include ulnar deviation, swan-neck deformity, and boutonniere deformity of the fingers. The social impact of RA is also significant. Patients may experience social isolation due to physical limitations and the stigma associated with visible joint deformities. The disease can strain relationships with family and friends, as caregivers may need to provide ongoing support and assistance.

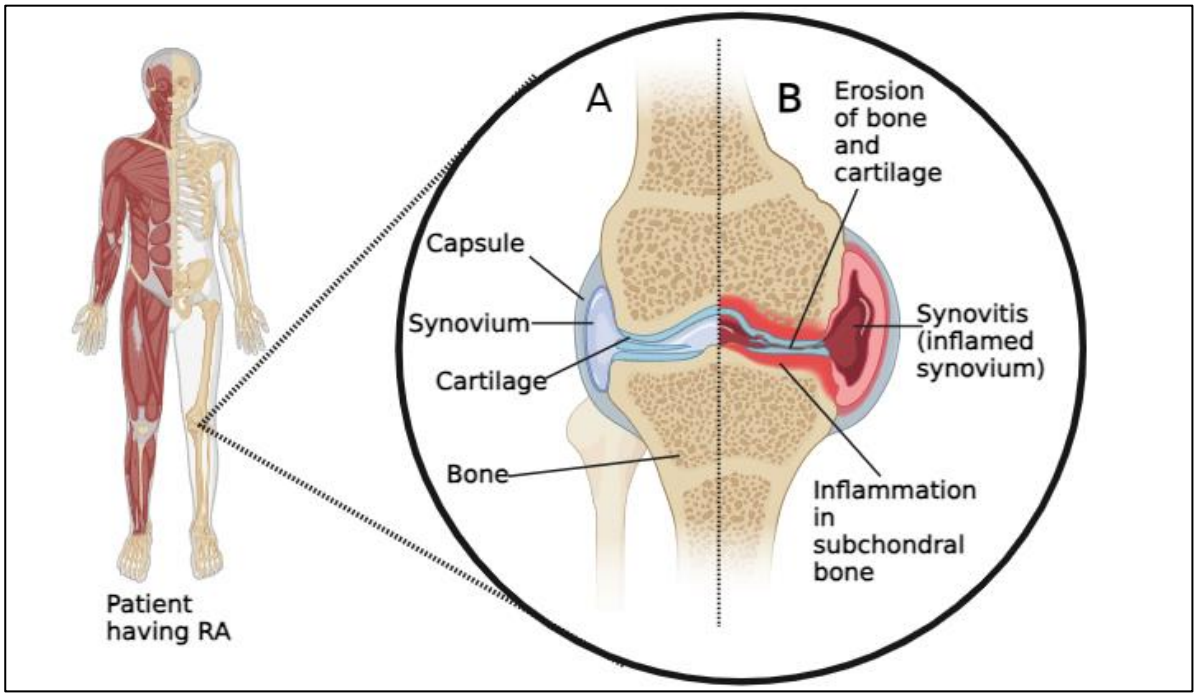


Fig 2.1: (A) Schematic view of a normal joint and (B) a joint affected by RA

2.2 Introduction to Curcumin and its Potential Therapeutic Properties

Curcumin, a natural polyphenol compound[14] derived from the rhizome of the turmeric plant (*Curcuma longa*), has attracted considerable attention for its potential therapeutic applications in various inflammatory and autoimmune disorders, including Rheumatoid Arthritis. Curcumin anti-inflammatory, antioxidant, and immunomodulatory properties have been extensively researched and are thought to result from its ability to influence multiple signalling pathways and interact with various molecular targets. Traditional medicine systems like Ayurvedic and Chinese medicine have used curcumin for centuries due to its medicinal qualities. Recent research has delved into the mechanisms of action and therapeutic potential of curcumin in different conditions, including cancer, cardiovascular diseases, neurological ailments, and autoimmune disorders[15]. In the context of Rheumatoid Arthritis, various in vitro, animal, and human studies have shown the beneficial effects of curcumin in alleviating disease symptoms, reducing inflammation, and slowing the progression of joint damage. The mechanisms by which curcumin exerts its therapeutic effects in RA can be broadly categorized as follows[16]:

- **Inhibition of Inflammatory Pathways:** Curcumin has been shown to inhibit key inflammatory signalling cascades, such as the mitogen-activated protein kinase (MAPK), extracellular signal-regulated kinase (ERK1/2), activator protein-1 (AP-1), and nuclear

factor-kappa B (NF-κB) pathways, which play important roles in the pathogenesis of RA.[7], [17]

- **MAPK Pathway:** The regulation of gene expression, cellular proliferation, and apoptosis is attributed to the MAPK pathway. In RA, pro-inflammatory cytokines and enzymes are produced as a result of MAPK activation, which contributes to joint inflammation and degradation. Curcumin decreases the expression of these inflammatory mediators by inhibiting MAPKs from being phosphorylated.
- **ERK1/2 Pathway:** The ERK1/2 pathway is a part of the MAPK pathway that is primarily involved in the regulation of cell differentiation and proliferation. When ERK1/2 is activated in RA, synovial fibroblasts proliferate and matrix metalloproteinases (MMPs) are produced, which break down cartilage. Curcumin blocks ERK1/2, which aids in stopping these harmful processes[18].
- **AP-1 Pathway:** The AP-1 pathway controls the expression of genes associated with inflammation and immunological responses. Curcumin inhibits AP-1 action, resulting in lower levels of pro-inflammatory cytokines and chemokines.
- **NF-κB Pathway:** The NF-κB pathway regulates immunological and inflammatory responses. RA is characterised by constant activation of NF-κB, which leads to the generation of inflammatory cytokines and adhesion molecules. Curcumin suppresses NF-κB, decreasing inflammation and damage to joints [19], [20].

- **Modulation of Macrophage Function:** Curcumin can modulate the polarization of macrophages, promoting the differentiation of anti-inflammatory M2 macrophages and suppressing the pro-inflammatory M1 macrophages, thereby contributing to the resolution of inflammation in RA[16].
- **Alleviation of Joint Inflammation:** Curcumin has been shown to alleviate joint inflammation, inhibit synovial revascularization, and reduce abnormal fibroblast proliferation in RA, indicating its potential to slow the progression of joint damage.
- **Downregulation of Inflammatory Cytokines:** Curcumin has been observed to downregulate the expression of pro-inflammatory cytokines, including interleukin-17 (IL-

17), tumour necrosis factor-alpha (TNF-α), and IL-6.[9], [21], [22] This prevents the activation of the phosphoinositide 3-kinase (PI3K)/AKT signalling pathway, which is linked to the pathophysiology of RA.

The therapeutic potential of curcumin in the treatment of rheumatoid arthritis is enhanced by its diverse modes of action.

2.3 Overview of the STAT3 Signalling Pathway and its Role in RA Pathogenesis

One transcription factor that has become more important in the pathophysiology of rheumatoid arthritis is called Signal Transducer and Activator of Transcription 3 (STAT3). As a member of the STAT protein family, STAT3 helps control gene expression by enhancing the transmission of signals from the cell surface to the nucleus[23]. Many cytokines and growth factors, including interleukin-6 (IL-6), interleukin-10 (IL-10), and epidermal growth factor (EGF), activate the STAT3 signalling pathway[24]. Associated Janus kinases (JAKs) or other tyrosine kinases phosphorylate STAT3 when ligands attach to their specific receptors. Upon being phosphorylated, STAT3 dimerizes, moves into the nucleus, and attaches itself to certain DNA sequences called STAT3 response elements to control the transcription of target genes involved in cell proliferation, survival, differentiation, and immune responses[25].

The role of STAT3 can be summarized as follows:

- **Constitutive Activation of STAT3 in RA:** A number of studies have shown constitutive activation of STAT3 in circulating immune cells from individuals who have active RA, including T cells and monocytes. When comparing resting peripheral blood T cells and monocytes between RA patients and healthy controls, there was a major rise in the levels of phosphorylated STAT3.
- **IL-6-induced STAT3 Hyperactivation:** One of the main factors influencing the inflammatory response in rheumatoid arthritis is the pro-inflammatory cytokine interleukin-6 (IL-6). When RA is active, circulating immune cells' STAT3 is hyperactivated by IL-6, which causes T cells to become less sensitive to the IL-6 response.

- **STAT3 and Joint Destruction**: The inhibition of STAT3 using the specific inhibitor F0648-0027 has shown potential therapeutic effects against Rheumatoid Arthritis in animal models, indicating that in RA, STAT3 plays a crucial role as a mediator of joint degeneration and inflammation
- **STAT3 variations and Disease Severity:** While no substantial correlation was discovered between the examined variations and RA susceptibility, several STAT3 genetic variants have been linked to increased disease activity and perhaps renal impairment in individuals with rheumatoid arthritis.

These results demonstrate the pivotal role[26] that STAT3 plays in the cause of Rheumatoid Arthritis, indicating that it is a viable target for therapeutic intervention in the treatment of this crippling inflammatory disease.

2.4 Rationale for Conducting Molecular Docking Analysis to Study Curcumin's Interaction with STAT3

Molecular docking analysis[27] is a computational approach used to predict the binding interactions between small molecules, such as curcumin, and target proteins, such as STAT3. By simulating the binding of curcumin to STAT3 at the molecular level, researchers can gain insights into the structural features of the interaction, the binding affinity, and the potential mechanisms of action. The rationale for conducting molecular docking analysis lies in defining the specific binding sites of curcumin on STAT3, understanding the conformational modifications induced by the interaction, and predicting the functional consequences of curcumin-induced STAT3 inhibition. This approach can provide valuable information for designing novel curcumin derivatives with enhanced potency and selectivity for targeting STAT3 in RA.

Molecular docking analysis involves several key steps:

- **Preparation of the target protein structure:** The three-dimensional structure of STAT3 is obtained from structural databases, such as the Protein Data Bank (PDB), and prepared for docking by removing water molecules, adding hydrogen atoms, and assigning partial charges[28].

- **Preparation of the ligand structure:** The three-dimensional structure of curcumin is generated using computational tools and optimized for docking by assigning atom types and partial charges.
- **Identification of the binding site:** The potential binding site of curcumin on STAT3 is identified based on known binding sites, structural features, or predicted binding pockets using computational algorithms.
- **Docking simulation:** The ligand (curcumin) is docked into the binding site of the target protein (STAT3) using specialized docking software, such as Swiss dock. The docking algorithm explores various orientations and conformations of the ligand into the binding site, then uses predetermined scoring methods to provide a score to each interaction.
- **Analysis of docking results:** The docking results are analyzed to identify the most favourable binding poses of curcumin on STAT3, based on factors such as binding affinity, hydrogen bonding interactions, and steric complementarity. The binding interactions are further analyzed using visualization tools and molecular dynamics simulations to assess the stability and dynamics of the curcumin-STAT3 complex.

2.5 Pathophysiology Rheumatoid Arthritis

- RA is a long-term autoimmune disease that causes inflammation in the synovial joints, which progressively destroys the joints and causes discomfort, disability, and joint degradation. Genetic, environmental, and immunological variables interact synergistically during the pathophysiology of RA. The defining feature of RA is synovitis, which is caused by immune cells infiltrating the synovial membrane, including T cells, B cells, macrophages, and dendritic cells[29]. As seen in "figure 2," these cells emit pro-inflammatory cytokines such as tumour necrosis factor-alpha (TNF-α), interleukin-1 (IL-1), and interleukin-6 (IL-6), which prolong the inflammatory response and worsen joint injury[30]. An important part of the pathophysiology of RA is played by synovial fibroblasts, sometimes referred to as fibroblast-like synoviocytes (FLS). The aggressive nature of these cells is marked by enhanced proliferation, resistance to apoptosis, and production of matrix-degrading enzymes such as matrix metalloproteinases (MMPs).

- A vicious cycle of inflammation and tissue degradation is produced by the interaction of immune cells with FLS. FLS have phenotypic alterations in RA that encourage their aggressive outlook. The thickening of the synovium and the development of the pannus—a harmful tissue overgrowth that invades and erodes joint structures—are caused by the increased proliferation of FLS. These cells are able to endure and proliferate within the inflammatory synovium due to the dysregulation of apoptosis in FLS, which sustains tissue damage and the inflammatory response. The extracellular matrix components of cartilage and bone are broken down by degrading enzymes including matrix metalloproteinases (MMPs), which causes joint deterioration and functional impairment in RA patients [31].
- One factor contributing to the harmful aspect of RA pathogenesis is FLS's increased MMP production. In RA joints, the interaction of immune cells with FLS results in the production of matrix-degrading enzymes and pro-inflammatory cytokines[32], which set off a vicious cycle of inflammation and tissue degradation. Prolonged activation of FLS and immune cells increases joint destruction, prolongs synovitis, and adds to the chronic nature of RA pathogenes

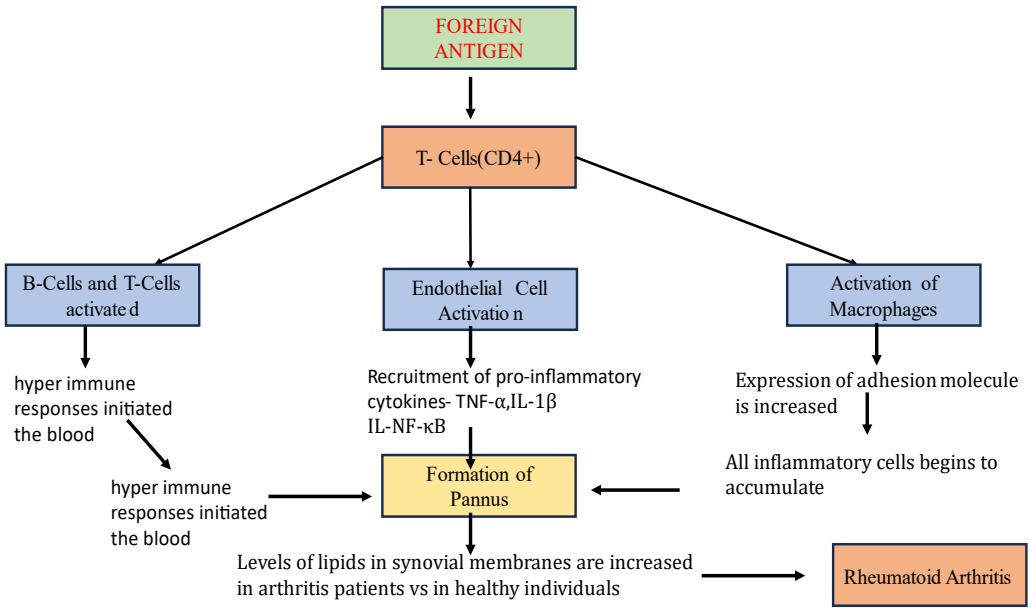


Figure 2.2: Flowchart showing pathophysiology of Rheumatoid Arthritis

2.6 Assessment of Existing Treatment Modalities and Their Drawbacks:

Preventing joint degeneration, reducing inflammation, reducing pain, and enhancing general function are the main objectives of RA therapy. Disease-modifying antirheumatic medications (DMARDs), biologics, corticosteroids, and nonsteroidal anti-inflammatory medicines (NSAIDs) are the primary medication types utilised in the treatment of RA[33], [34].

- **NSAIDs**: These drugs provide symptomatic relief by reducing inflammation and pain. However, they do not alter the course of the disease and are associated with gastrointestinal, cardiovascular, and renal side effects.
- **Corticosteroids**: These potent anti-inflammatory agents are used for short-term control of acute flares. Long-term use is limited by adverse effects such as osteoporosis, weight gain, and increased risk of infections.
- **DMARDs**: These drugs, including methotrexate, sulfasalazine, and hydroxychloroquine, can slow disease progression and improve long-term outcomes. However, they have a slow onset of action and may cause hepatotoxicity, myelosuppression, and gastrointestinal disturbances.
- **Biologics**: These targeted therapies, such as TNF inhibitors (e.g., infliximab, adalimumab), IL-6 receptor antagonists (e.g., tocilizumab), and B cell depleting agents (e.g., rituximab), have revolutionized RA treatment. Despite their efficacy, biologics are expensive, require parenteral administration, and carry risks of serious infections and malignancies.

Given the drawbacks of current therapies, there is a need for novel treatment strategies that are effective, safe, and affordable.

2.7 Overview of curcumin: -

- **Chemical Properties Curcumin**

The turmeric plant's (*Curcuma longa*) rhizome is the source of the polyphenolic chemical curcumin. It is the main curcuminoid that gives turmeric its yellow colour. In curcumin's chemical structure, two aromatic rings are joined by a seven-carbon linker that has α, β-unsaturated carbonyl groups. Two hydroxyl (OH) and two methoxy (OCH3) groups are additionally joined to the aromatic rings of this linker. Curcumin's distinct biological characteristics, including as its anti-inflammatory, antioxidant, anticancer, and antibacterial

effects, are attributed to its structure. Curcumin is lipophilic and practically insoluble in water at acidic and neutral pH, but soluble in alkali. It is soluble in organic solvents such as dimethyl sulfoxide (DMSO), ethanol, and acetone[35], [36].

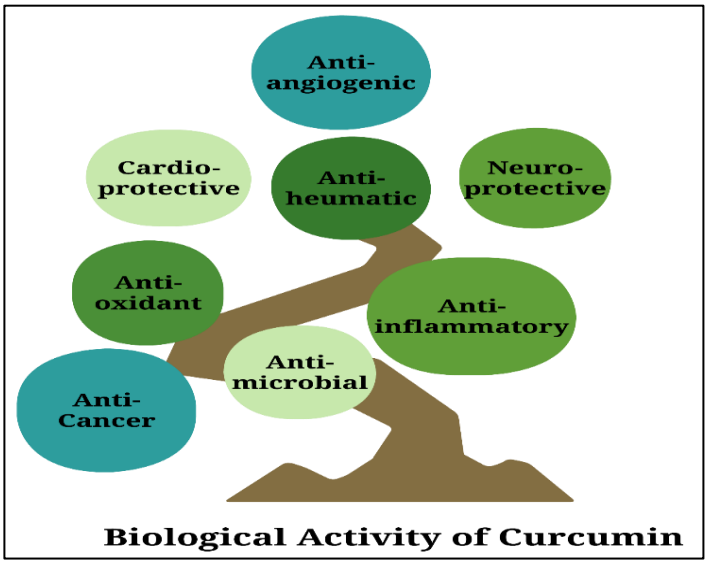


Fig 2.3: Biological Activity of Curcumin

Table 2.1: Showing the chemical and physical properties of curcumin[37]-

Chemical and Physical properties	**Curcumin**
1. Molecular Formula	C21H20O6
2. Molecular Weight	368.35g/mol
3. Melting Point	183 ºC
4. Color	Yellow
5. Solubility in water	Low
6. Reaction under Acid	Bright Yellow Color
7. Reaction under Base	Bright color

- **Pharmacokinetics and Bioavailability**

Curcumin, a compound renowned for its therapeutic potential, encounters challenges in clinical application primarily due to its poor bioavailability. This limitation stems from its low solubility in aqueous solutions, rapid metabolic degradation, and inefficient absorption in the gastrointestinal tract. To overcome these hurdles, innovative strategies have been devised to enhance curcumin's bioavailability.

- **Enhancing Solubility and Absorption:**

One effective method involves the use of adjuvants like piperine, an alkaloid found in black pepper. Piperine significantly boosts curcumin's bioavailability by inhibiting its metabolic breakdown in the liver and intestines. Co-administration with piperine has shown a marked increase in curcumin's bioavailability, making it a common inclusion in curcumin supplements.

- **Nanotechnology Advancements:**

Nanotechnology presents a promising solution by encapsulating curcumin into nanoparticles. This approach enhances curcumin's solubility and absorption by protecting it from premature degradation and facilitating its transport across cellular membranes. Nanoparticles play a crucial role in improving curcumin's therapeutic efficacy.

i) Liposomal Formulations:

Exploration of liposomal formulations has also been fruitful in enhancing curcumin's bioavailability. Liposomes, spherical vesicles that encapsulate curcumin within their lipid bilayer, shield it from degradation and enhance its absorption. This method not only enhances curcumin's stability but also its solubility, leading to improved bioavailability and more effective therapeutic outcomes.

ii) Micelles and Phospholipid Complexes:

Micelles, formed from surfactants, offer another innovative strategy to boost curcumin's bioavailability. These microscopic structures encapsulate hydrophobic compounds like curcumin, enhancing their solubility in water and absorption in the gastrointestinal tract. Similarly, phospholipid complexes, such as phytosomes, combine curcumin with phospholipids to form complexes that are more readily absorbed by the body. These complexes enhance curcumin's solubility and stability, thereby improving its bioavailability and therapeutic potential.

2.8 Overview of STAT3

One transcription factor that is essential for many physiological activities, such as inflammation, the immune system, cell division, and survival, is called Signal Transducer and Activator of Transcription 3 (STAT3). Phosphorylation of STAT3 occurs in reaction to cytokines and growth factors, including IL-6, IL-10, and epidermal growth factor (EGF)[38], [39]. When STAT3 is activated, it dimerizes, moves into the nucleus, and binds to certain DNA sequences to control the expression of target genes[40] related to inflammation, cell survival, and proliferation. By controlling target genes' transcription, STAT3 plays a crucial role[38] in controlling a variety of biological processes. Genes implicated in the inflammatory response, including as adhesion, chemokines, and cytokines, are upregulated when STAT3 activation occurs in the setting of inflammation. As a result, the inflammatory cascade grows stronger and immune cells are gathered and activated. The development and functionality of immune cells, such as T cells and macrophages, are influenced by STAT3 activation in terms of immunological responses. STAT3 has a pivotal function in regulating the immune response and preserving immunological homeostasis through the modulation of immune-related gene expression.

2.9 Mechanisms of STAT3 Inhibition by Curcumin

Curcumin has been shown to inhibit STAT3 activation through multiple mechanisms. One of the primary mechanisms is the inhibition of Janus kinases (JAKs), which are upstream kinases that phosphorylate and activate STAT3. Curcumin has been demonstrated to inhibit the activity of JAK1, JAK2, and TYK2, thereby preventing the phosphorylation and activation of STAT3.In addition to JAK inhibition, curcumin can directly inhibit the phosphorylation of STAT3 by interfering with the binding of STAT3 to its upstream activators. Curcumin has been shown to disrupt the interaction between STAT3 and IL-6 receptor, thereby preventing the activation of STAT3 by IL-6. Curcumin also promotes the degradation of STAT3 protein[41]. Studies have shown that curcumin induces the ubiquitination and proteasomal degradation of STAT3, leading to a reduction in STAT3 protein levels. The key mechanisms by which curcumin inhibits STAT3 is given below-

2.9.1 Direct binding to STAT3:

- Curcumin directly interacts with the cysteine 251 residue of STAT3, which is critical for its activity and conformation. Mutation of this cysteine residue abolishes curcumin-induced inactivation of STAT3.

2.9.2 Suppression of STAT3 phosphorylation:

- Curcumin treatment results in significant suppression of STAT3 phosphorylation in a dose-dependent manner, reducing its activation.

2.9.3 Inhibition of STAT3 nuclear translocation:

- Curcumin suppresses the nuclear translocation of activated STAT3, preventing it from binding to DNA and inducing target gene expression.

2.9.4 Downregulation of STAT3 target genes:

- By inhibiting STAT3 activation, curcumin leads to the downregulation of STAT3 target genes involved in cell proliferation, survival, and angiogenesis.

2.9.5 Suppression of migration and invasion:

- Curcumin blocks the migration, invasion, and angiogenesis of cancer cells by inhibiting the JAK-STAT3 signalling pathway.

Curcumin targets multiple steps in the STAT3 signalling cascade, including direct binding, phosphorylation, nuclear translocation, and transcriptional activity, ultimately leading to the suppression of STAT3-mediated oncogenic processes. Inhibition of STAT3 signalling has been shown to reduce inflammation and joint damage in animal models of RA. Therefore, targeting STAT3 may offer a novel therapeutic approach for RA treatment.

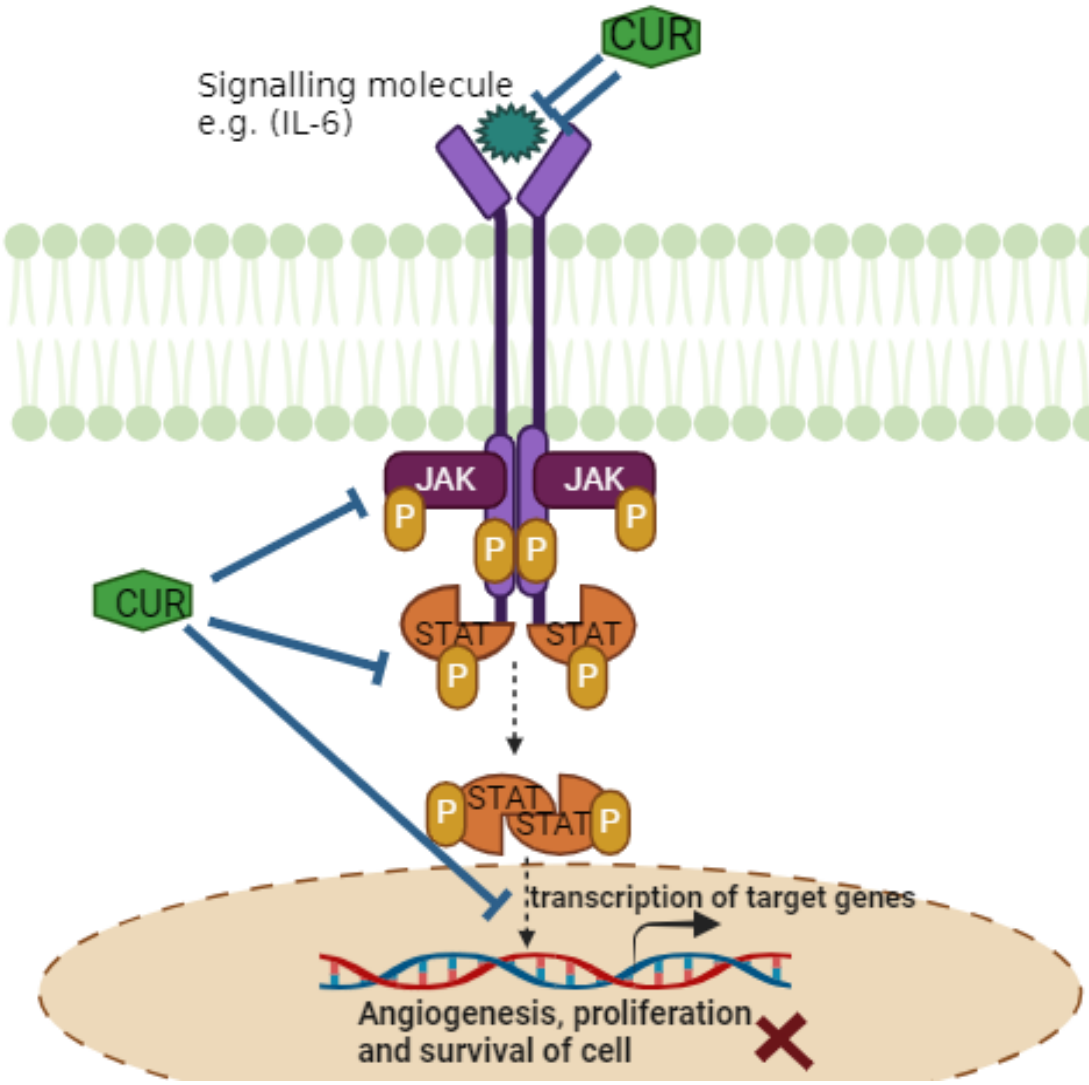


Fig 2.4: - Mechanisms by which curcumin inhibits STAT3

2.10 Effects of Curcumin on Downstream Targets of STAT3

By inhibiting STAT3 activation, curcumin can modulate the expression of various downstream targets of STAT3 that are involved in inflammation and cell survival. For example, curcumin has been shown to reduce the expression of pro-inflammatory cytokines, such as IL-6, IL-17, and TNF-α, in RA models[21].

Curcumin inhibits STAT3 signalling and leads to several downstream effects:

2.10.1 Downregulation of anti-apoptotic proteins:

- One of the effects of curcumin administration is the downregulation of anti-apoptotic proteins[42], specifically Bcl-xL, Bcl-2, and survivin, which are STAT3 target genes involved in cell survival.

2.10.2 Induction of apoptosis:

- Curcumin induces apoptosis in cancer cells by blocking STAT3 activity, as seen by increased production of pro-apoptotic proteins including Bax[9].

2.10.3 Suppression of proliferation and anchorage-independent growth:

- Curcumin suppresses cancer cells' anchorage-independent growth and proliferation via blocking STAT3.

2.10.4 Inhibition of migration, invasion, and angiogenesis:

- Curcumin inhibits the JAK-STAT3 signalling system, hence preventing cancer cells from migrating, invading, and forming angiogenesis

2.10.5 Reduction in the proliferative capacity of cells:

- Curcumin therapy causes a significant reduction in the ability of cells to proliferate, as measured by decreased expression of proliferation markers like Cyclin D1 and Mcm2[43].

Consequently, curcumin downregulates STAT3 target genes implicated in angiogenesis, cell survival, and proliferation by blocking STAT3 activation. It also induces apoptosis and inhibits the migration and invasion of cancer cells. The potential of curcumin as a medicinal drug that targets the STAT3 signalling system is highlighted by these side effects[43].

2.11 Synergistic Effects with Other Pathways

The effects of curcumin extend beyond the STAT3 pathway. Additionally, it alters other signalling pathways that contribute to the pathophysiology of RA, including the phosphoinositide 3-kinase (PI3K)/Akt pathway, mitogen-activated protein kinase (MAPK) route, and nuclear factor-kappa B (NF-κB) system[44]. Curcumin's inhibition of these pathways may have additive effects that reduce inflammation and joint degeneration more thoroughly.

For instance, it has been demonstrated that curcumin inhibits the activation of NF-κB, a transcription factor that controls the release of many chemokines and pro-inflammatory cytokines. Curcumin can lessen the synthesis of inflammatory mediators and encourage the

resolution of inflammation by blocking NF- κB[19] Additionally, curcumin suppresses the MAPK pathway, which is crucial in controlling the survival and proliferation of cells. By inhibiting MAPK signalling, curcumin can reduce the proliferation of synovial fibroblasts and promote their apoptosis.

The next sections offer a thorough explanation of two important signalling channels that are involved in many biological functions, including inflammation, the immune system, cell proliferation, and survival: the nuclear factor-kappa B (NF-κB) pathway and the mitogen-activated protein kinase (MAPK) pathway[45].

2.11.1 NF-κB Pathway

The route known as Nuclear Factor kappa-light-chain-enhancer of activated B cells (NF-κB) is a crucial signalling system that plays a role in immune response and inflammation. The transcription factor NF-κB controls the expression of several adhesion molecules, chemokines, and pro-inflammatory cytokines. The abnormal activation of the NF-κB pathway[43] in RA results in the continuous synthesis of inflammatory mediators and the influx of immune cells into the synovium. It has been demonstrated that curcumin prevents IκBα, the inhibitor of NF-κB[32], from degrading and directly inhibits IκB kinase (IKK) activity. As a result of this inhibition, pro-inflammatory gene transcription mediated by NF-κB is suppressed, which lowers inflammation and joint damage in RA.

2.11.2 MAPK Pathway

The Mitogen-Activated Protein Kinase (MAPK) pathway[46] is involved in the regulation of various cellular processes, including inflammation, cell proliferation, and apoptosis. The MAPK pathway consists of several kinases, including extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK), and p38 MAPK[47]. These kinases are activated in response to various stimuli, such as cytokines and stress, and they regulate the expression of genes involved in inflammation and cell survival. Curcumin has been shown to inhibit the activation of the MAPK pathway by suppressing the phosphorylation of ERK, JNK, and p38 MAPK[8], [44].

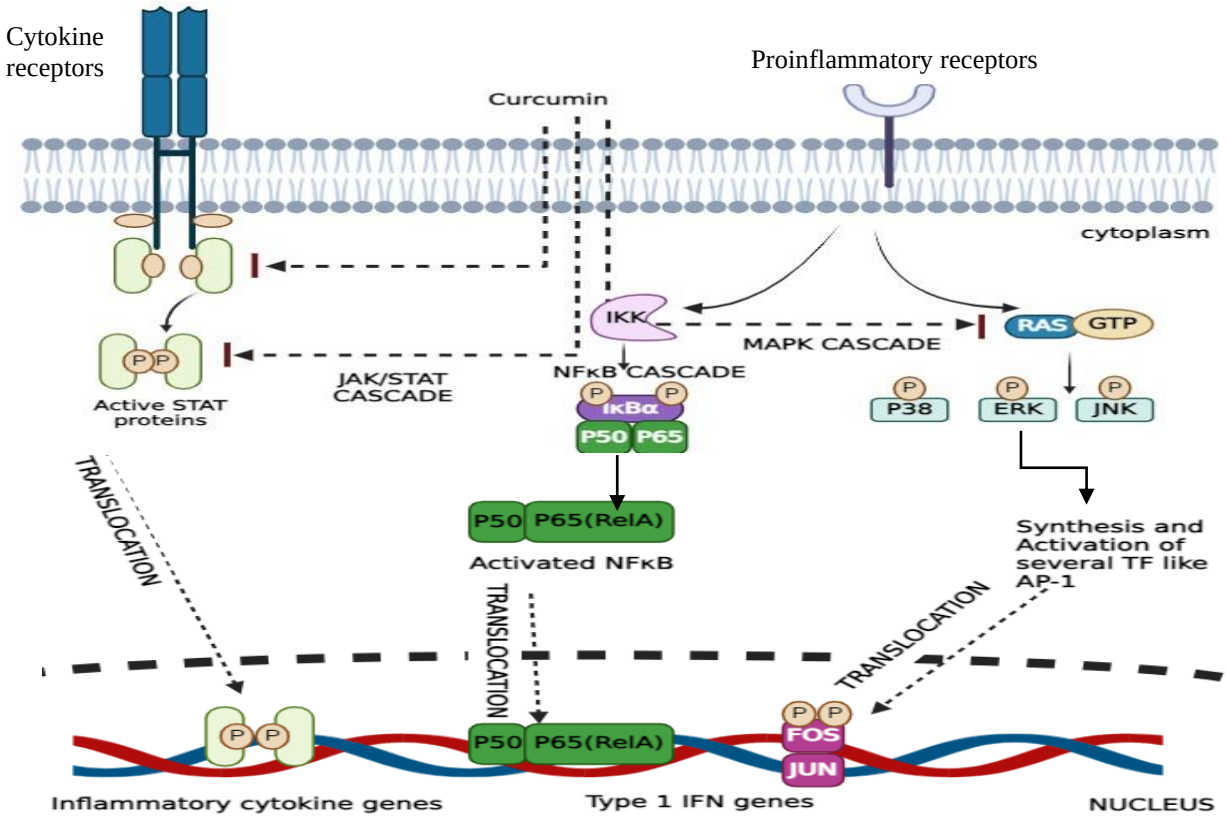


Fig 2.5: Curcumin's effects on different pathways-NF-κB Pathway & MAPK Pathway

2.12 Previous studies on the interaction between curcumin and STAT3

2.12.1 Preclinical Studies

Numerous preclinical studies have demonstrated the anti-inflammatory and anti-arthritic effects of curcumin in animal models of RA. For example, in a collagen-induced arthritis (CIA) model, curcumin treatment significantly reduced clinical scores[48], paw swelling, and histopathological changes in the joints. These effects were associated with the inhibition of STAT3, NF-κB, and MAPK signalling pathways, as well as the reduction of pro-inflammatory cytokines and matrix metalloproteinases[8]. In another study, curcumin was shown to inhibit the proliferation and migration of synovial fibroblasts isolated from RA patients. To improve the bioavailability of curcumin another strategy involves using curcumin compounds and analogues with enhanced pharmacokinetic characteristics. These analogues are made to improve curcumin's solubility, stability, and bioavailability without sacrificing any of its biological action.

Chapter 3

Methodology

3.1 Molecular docking analysis and its relevance to drug discovery:

Molecular docking is a computational technique that simulates the interaction between a small molecule (ligand) and a target protein or receptor at the molecular level. It aims to predict the most favourable binding orientation and affinity of the ligand within the protein's binding site. A search algorithm and an energy scoring function are the basic tools of a docking methodology for generating and evaluating the ligand conformations[10].

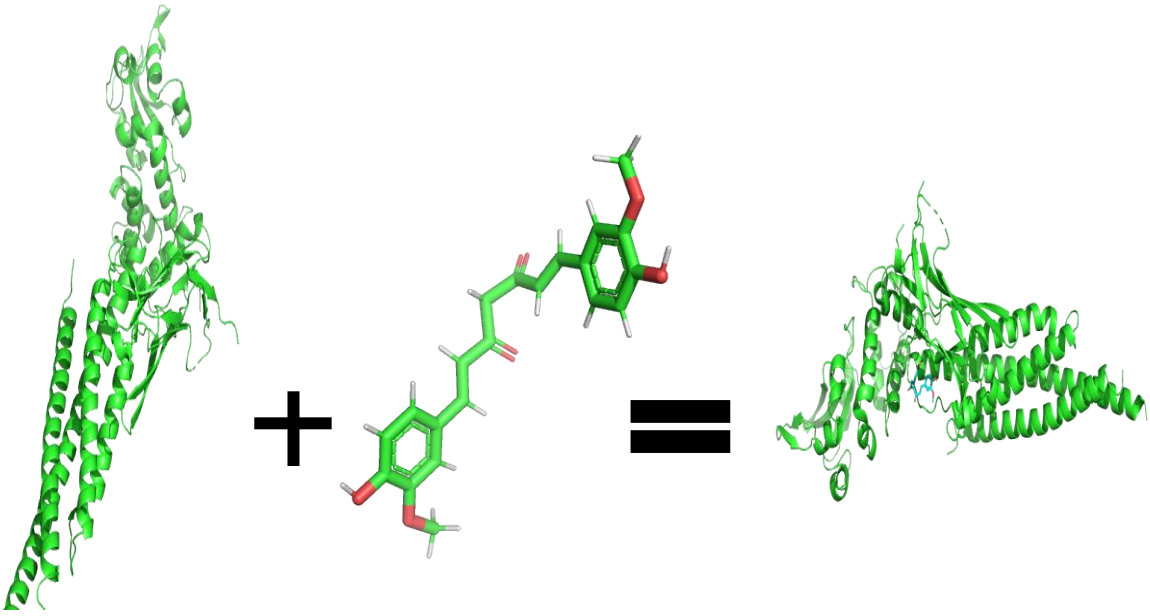


Figure 3.1: Elements involved in Molecular Docking: protein, ligand and the complex

Molecular docking analysis plays a crucial role in various stages of the drug development process, serving as a valuable computational tool for the identification, optimization, and evaluation of potential drug candidates. The key applications of molecular docking in drug discovery and development: -

- **Virtual Screening and Lead Identification:**

Molecular docking is extensively used in virtual screening campaigns to screen large libraries of chemical compounds against target proteins or receptors.

The docking algorithm evaluates the binding affinities and poses of each compound, allowing researchers to prioritize and identify potential lead compounds for further investigation.

This approach significantly reduces the number of compounds that need to be synthesized and tested experimentally, saving time and resources.

- **Lead Optimization and Structure-Based Drug Design**:

Once initial lead compounds are identified, molecular docking is employed to guide their structural optimization. Docking studies provide insights into the specific interactions between the lead compound and the target protein, enabling medicinal chemists to rationally design and modify the lead structure to improve binding affinity, selectivity, and pharmacokinetic properties. Iterative cycles of docking, structural modification, and experimental validation are often used to optimize lead compounds.

- **Understanding Binding Mode and Mechanism of Action:**

Molecular docking helps elucidate the binding mode and potential mechanism of action of drug candidates. By analysing the binding poses and interactions, researchers can understand how the compound interacts with the target protein, identify critical residues for binding, and predict potential allosteric or conformational effects. This information is valuable for designing more potent and selective compounds, as well as for predicting potential off-target effects or toxicity.

- **Rational Design of Drug Candidates:**

Molecular docking is used in the rational design of drug candidates by leveraging structural information about the target protein and known ligands.

Researchers can design and virtually screen novel compounds based on the binding modes and interactions observed in docking studies, potentially leading to the development of new chemical scaffolds or improved derivatives.

- **Predicting Binding Affinities and Selectivity:**

Docking scoring functions can provide estimates of binding affinities, which are useful for prioritizing and ranking compounds during the drug discovery process. Docking can also be used to assess the selectivity of compounds by docking them against multiple targets or off-target proteins, helping to identify potential cross-reactivity or unwanted interactions.

- **Interpretation of Experimental Data:**

Molecular docking can aid in the interpretation of experimental data, such as structure-activity relationships (SAR), mutagenesis studies, or biophysical binding assays.

The docking results provide a structural context for understanding the observed experimental data, allowing researchers to rationalize the effects of structural modifications or mutations on binding affinity and activity.

While molecular docking is a powerful computational tool, it is essential to validate and complement the computational predictions with experimental data, such as biochemical assays, biophysical techniques, and structural studies. The integration of molecular docking with experimental approaches provides a comprehensive understanding of the drug-target interactions and facilitates the development of more effective and safer drug candidates.

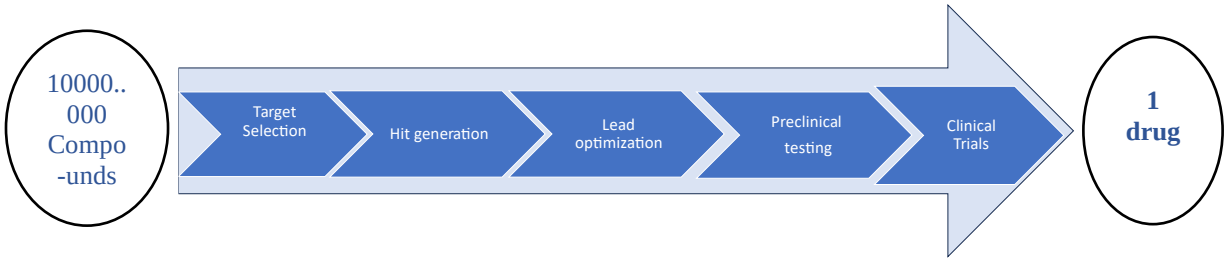


Figure 3.2: Drug discovery process.

3.2 Molecular Docking Workflow

The workflow of molecular docking involves several key steps:

- **Data collection: -** The compilation of selected natural compounds was derived from scientific literature, which highlighted the potential of these compounds for suppressing the progression of rheumatoid arthritis. The PubChem database was used to obtain the desired SMILES (Simplified Molecular-Input Line-Entry System) structures for the selected natural compounds. SMILES is a widely used chemical notation system that provides a compact representation of the molecular structure, which is essential for computational analysis and molecular docking studies.
- **Preparation of target and ligand:** - The protein structure utilized for the docking study was obtained from the **Protein Data Bank (PDB) entry 6NJS**, which

contained the A chain with a sequence length of 562 amino acids. The three-dimensional structure of the target protein, STAT3, was prepared using **PyMOL** molecular visualization software shown in “Fig3.3”[49]. This preparation process involved the addition of polar hydrogen atoms to the protein structure and the removal of any water molecules present. After the necessary modifications, the final prepared structure of the STAT3 protein was saved in the standard PDB file format, suitable for use in the subsequent docking simulations. For the ligand (compound) preparation, the initial structures in SDF format were converted to the MOL2 file format using the **Open Babel** software. This conversion step ensures compatibility with the docking software and facilitates the docking process. The transformed ligand structures in MOL2 format were then stored for further use in the molecular docking analysis.

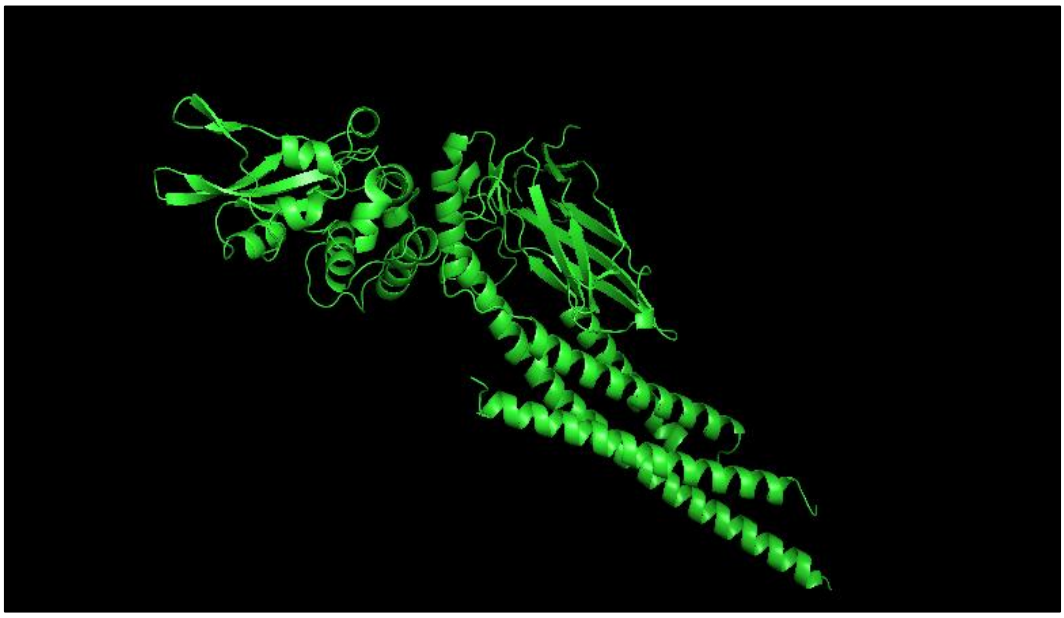


Figure 3.3: The three-dimensional structure of the target protein STAT3

The ligand's (3D) structure was acquired from PubChem (https://pubchem.ncbi.nlm.nih.gov/)[17], [50]. Within the Entrez information retrieval system at NCBI, PubChem is arranged as three connected databases. They are called PubChem BioAssay, PubChem Substance, and PubChem Compound. PubChem also provides a fast chemical structure similarity search tool. Ligands were available in XML, SDF, JSON and ASN.1 formats. The ligand compounds, originally represented in the SDF (Structure Data File) format, underwent

a file conversion process facilitated by the Open Babel cheminformatics software, transforming them into the MOL2 file format. The “Fig 3.4” shows the three-dimensional structure of the Curcumin

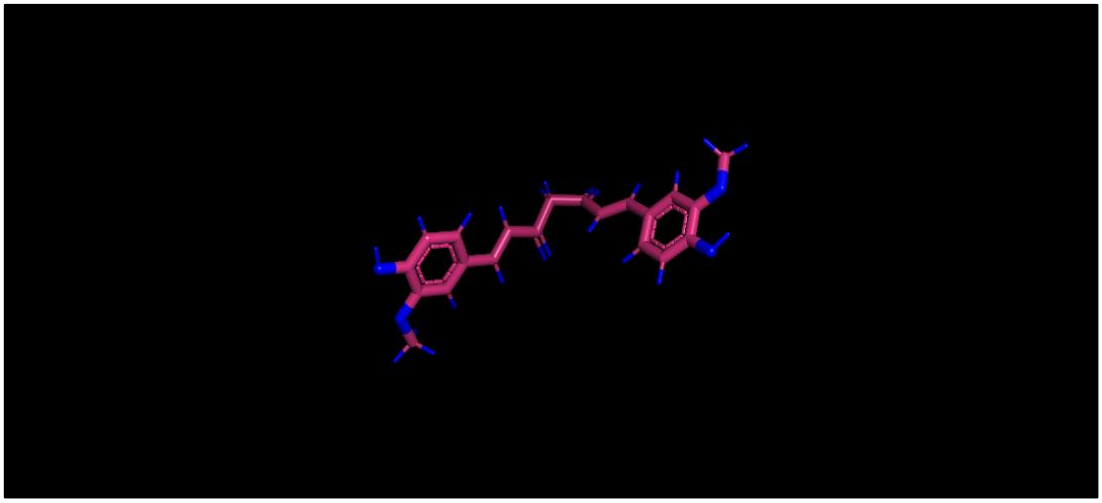


Figure 3.4: The three-dimensional structure of the Curcumin (ligand)

3.3 Molecular docking using Swiss dock:

- Molecular docking, a prominent computational approach in drug discovery, plays a crucial role in predicting the interactions[51] between target proteins and various small molecules, such as drugs, inhibitors, and ligands, as depicted in “fig 3.5”. **Swiss Dock**, a web-based platform, is specifically designed to facilitate protein-ligand docking simulations (http://swissdock.ch/docking) Utilizing the EADock DSS docking program as its core engine, Swiss Dock offers a streamlined and integrated user interface. Through this platform, users can upload the structural files[52] of both the target protein and the ligand compounds[53]. Subsequently, the docking simulations are performed, and the results, including the docking scores, are delivered to the users via email.
- The docking scores provide valuable insights into the potential binding affinities of the compounds. Ligands with binding energies lower than those of known inhibitors are considered promising candidates and subject to further in-depth analysis. By leveraging the capabilities of molecular docking tools like Swiss Dock, researchers can efficiently screen and evaluate the potential

interactions between target proteins and a diverse range of small molecules, accelerating the drug discovery process and identifying promising lead compounds for subsequent experimental validation and optimization.

- **UCSF Chimera** is a powerful molecular visualization and analysis software widely used in structural biology and computational chemistry. It provides a user-friendly interface and a wide range of tools for visualizing, analysing, and manipulating molecular structures[43]. UCSF Chimera offers various features and functions that facilitate the investigation of protein-ligand interactions. It allows users to load and display the three-dimensional structures of proteins and ligands, typically obtained from experimental techniques like X-ray crystallography[54] or NMR spectroscopy, or from computational methods like molecular docking. Once the protein and ligand structures are loaded, Chimera provides tools for identifying and analysing the specific interactions between them. These interactions can include hydrogen bonds, hydrophobic interactions, electrostatic interactions, and other non-covalent interactions that contribute to the binding affinity and stability of the protein-ligand complex[51].
- Additionally, Chimera offers visualization options to represent these interactions graphically, allowing researchers to gain insights into the binding mode and the key residues involved in the interaction. These visual representations can be customized and rendered in various styles, such as ball-and-stick models, ribbon diagrams, or surface representations, to effectively communicate the structural details. The figure retrieved from UCSF Chimera is shown in “Fig.3.6”.

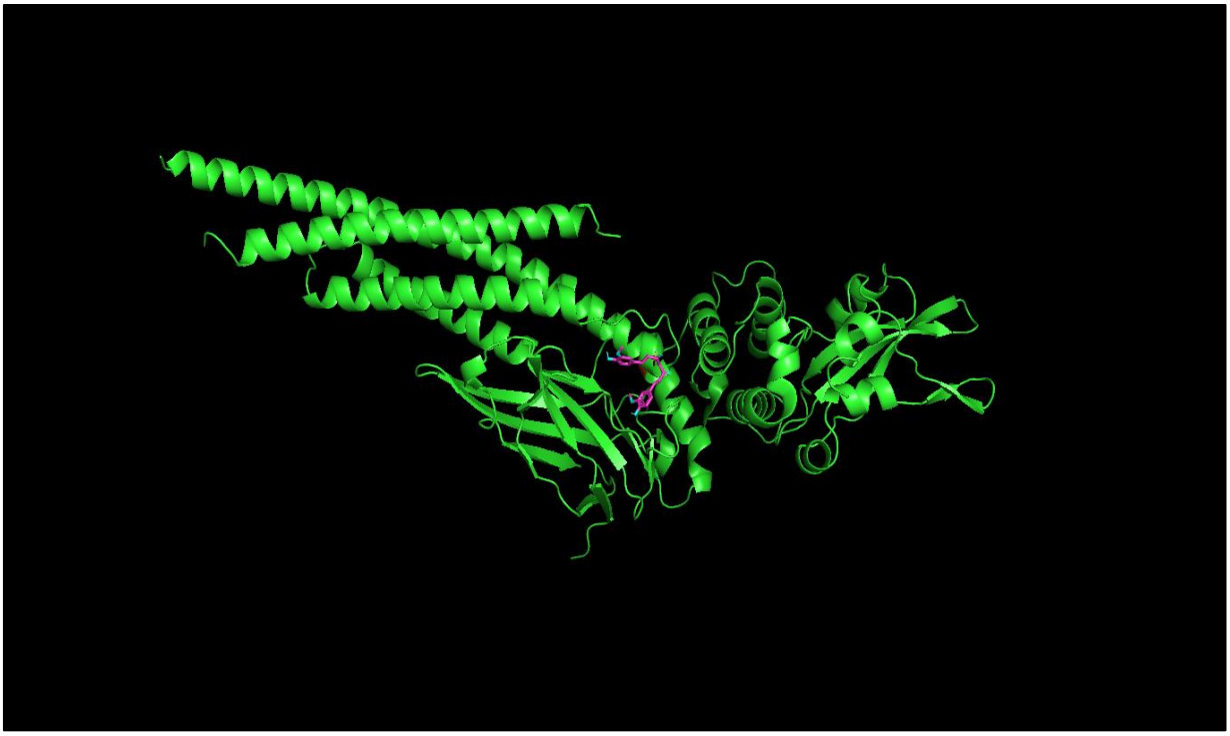


Fig 3.5: Docking interaction of Curcumin with STAT3

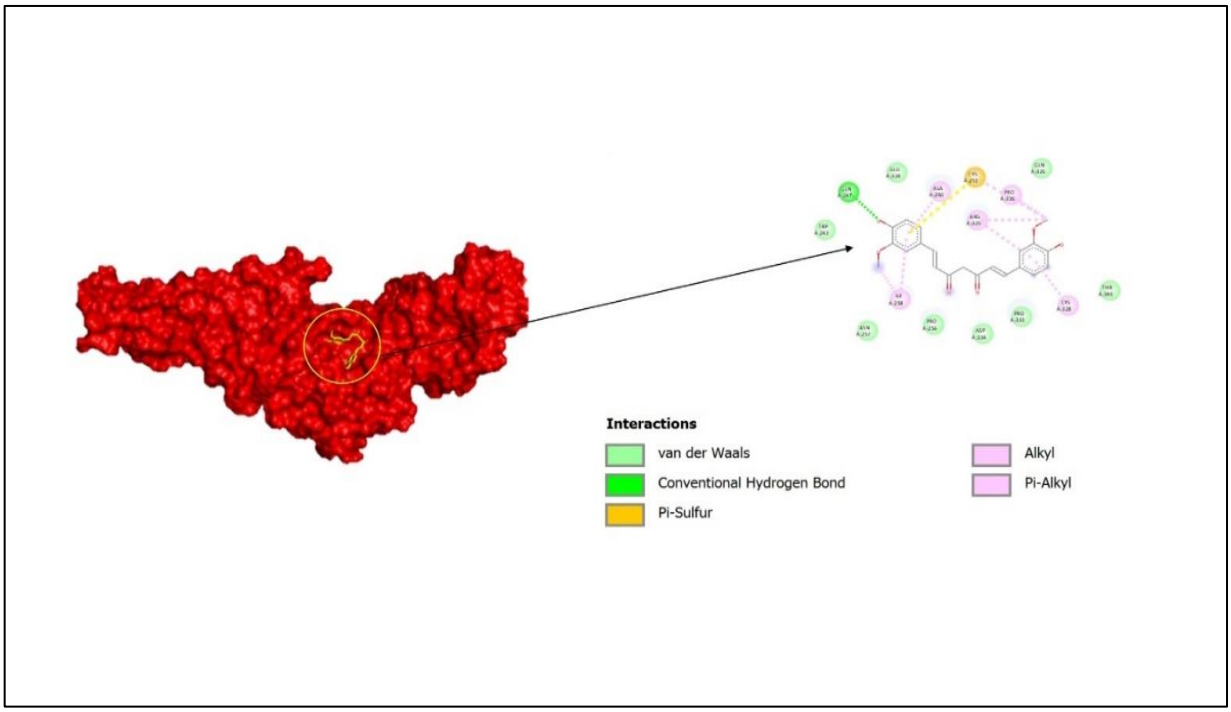
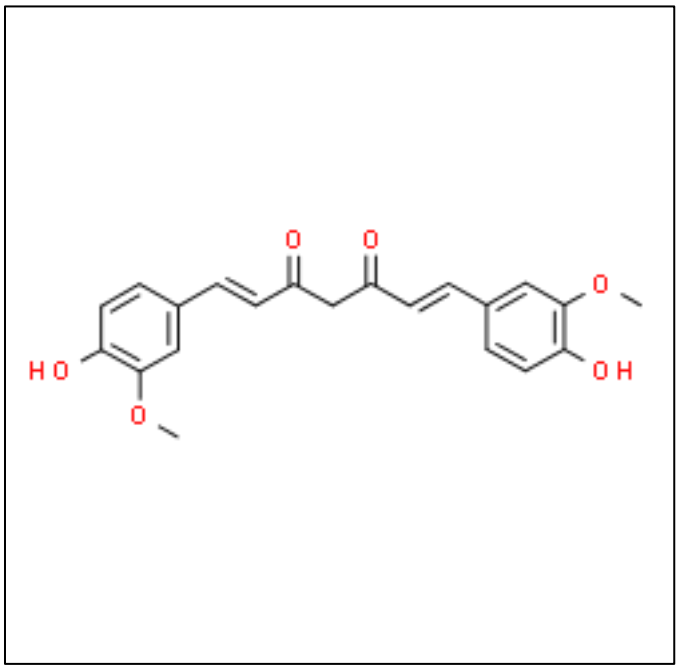
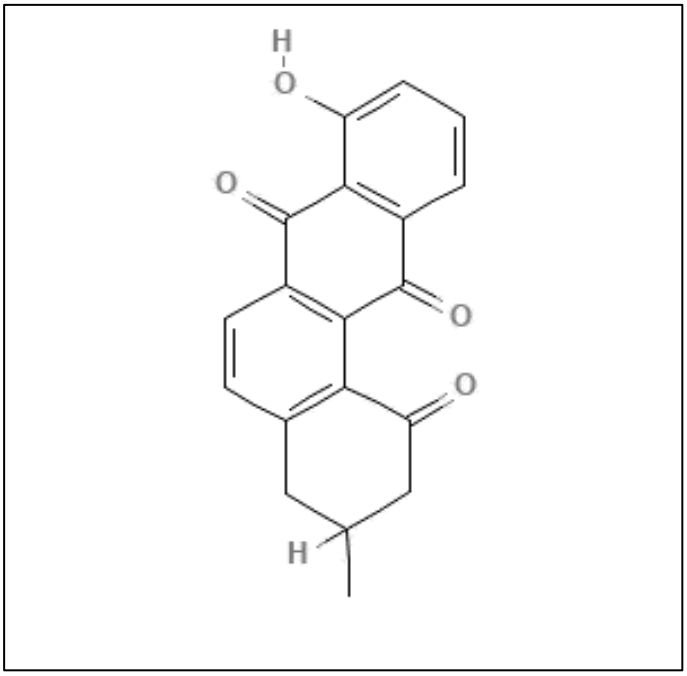


Fig 3.6: The interactions between curcumin and the target protein's amino acids were visualized using UCSF Chimera.

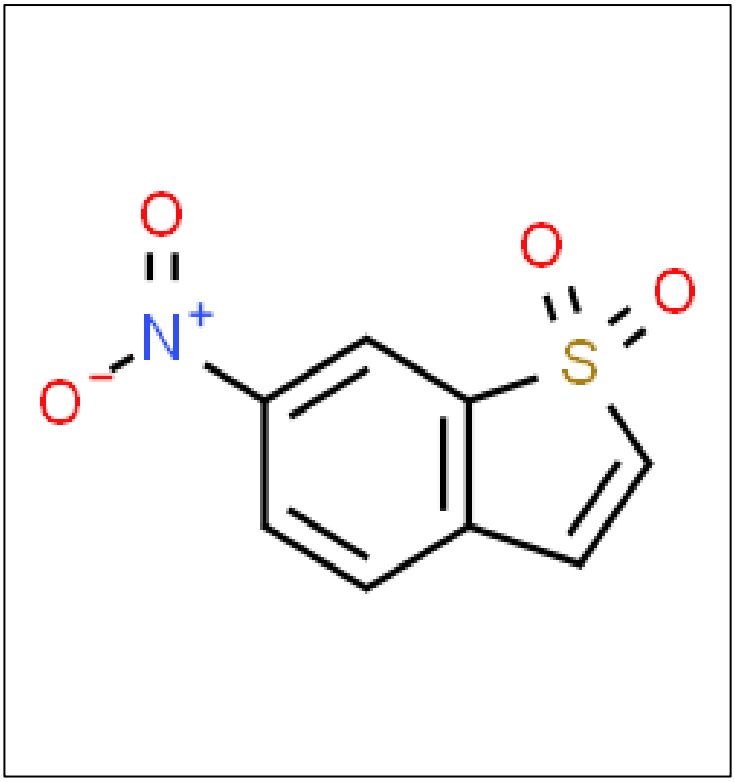
All STAT3 Inhibitors



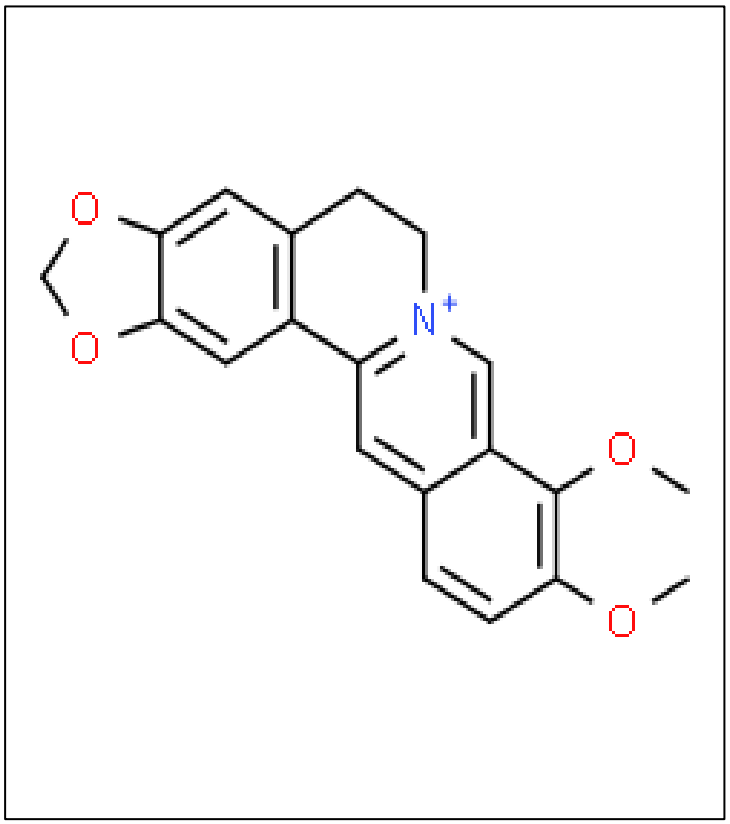
Curcumin



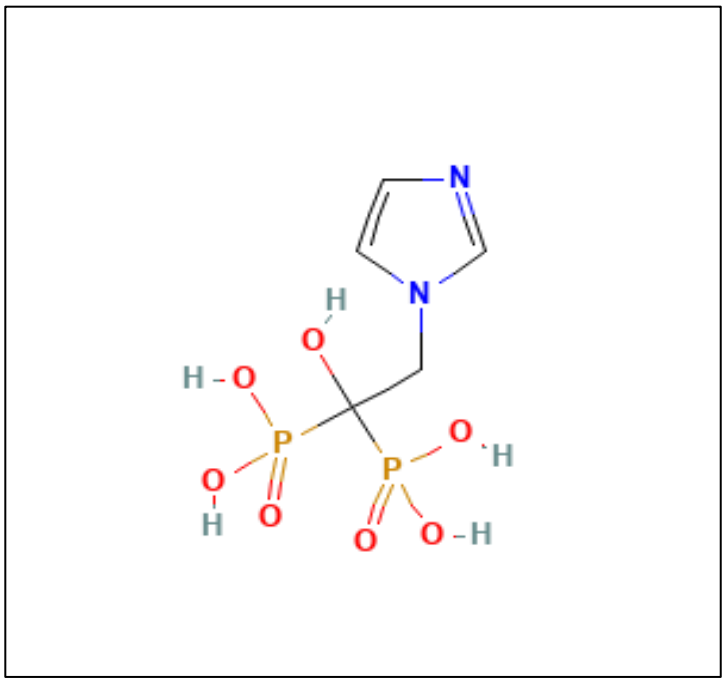
STA21



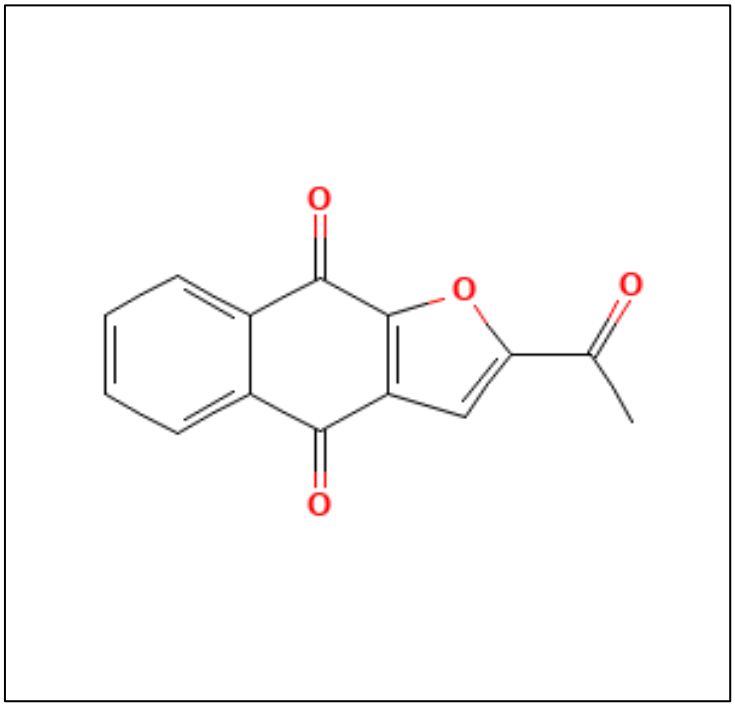
Stattic



Berberine



Zoledronic Acid



BBI608

Fig 3.7: 2D diagrams of all STAT3 inhibitors

Chapter 4

Results

4.1 Results of molecular docking simulations between curcumin and STAT3

- Molecular docking simulations between curcumin and STAT3 predicts the binding modes and interactions between curcumin and the STAT3 protein. The simulations show curcumin's binding to specific binding sites on STAT3 and the orientation of the ligand within the protein's active site. Understanding the specific binding modes and orientations of curcumin within the STAT3 active site is crucial for elucidating the potential inhibitory mechanisms of curcumin on STAT3 signalling pathways. By identifying the key amino acid residues involved in the binding interactions[51] and the overall binding pose of curcumin, researchers can gain valuable insights into the structure-activity relationships and guide further optimization of curcumin or its derivatives as potential STAT3 inhibitors.

- The **Swiss Param score** is an additional scoring function provided by Swiss Dock which offers insights into the energetics and specific interactions of the ligand within the binding site, providing a different perspective on the stability and molecular interactions of the ligand-protein complex. The **AC score** is the primary scoring function used by the Attracting Cavities algorithm in Swiss Dock to evaluate the binding affinity and pose of ligands within the target protein's binding site.

- The compound **Curcumin satisfied the criteria with a binding energy of -7.69 kcal/mol which is lower than STA21**, a recognized inhibitor of STAT3. The compound proved to be a more effective inhibitor than anticipated and the **STA21 has a binding energy of -6.69 kcal/mol** as shown below in "Fig4.1 & 4.2".

Show	Cluster	Element	FullFitness (kcal/mol)	Estimated ΔG (kcal/mol)
○	0	0	-2911.24	-7.69
○	0	1	-2911.24	-7.69
○	0	2	-2911.24	-7.69
○	0	3	-2911.20	-7.69
○	0	4	-2911.20	-7.69
○	0	5	-2911.08	-7.67
○	0	6	-2911.08	-7.67
○	0	7	-2911.08	-7.67

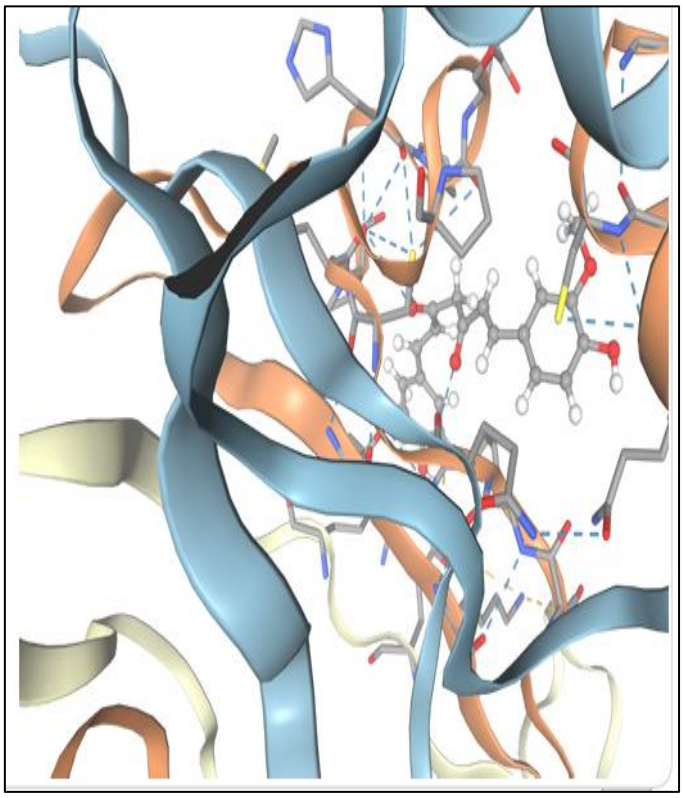


Fig4.1: Estimated binding energy of Curcumin and its 3D view

Show	Cluster	Element	FullFitness (kcal/mol)	Estimated ΔG (kcal/mol)
○	0	0	-2894.42	-6.96
○	0	1	-2894.35	-6.96
○	0	2	-2894.16	-6.89
○	0	3	-2894.13	-6.89
○	0	4	-2894.11	-6.89
○	0	5	-2894.08	-6.88
○	0	6	-2893.95	-6.87
○	0	7	-2892.04	-6.70
○	1	0	-2894.16	-6.64
○	1	1	-2893.52	-6.65

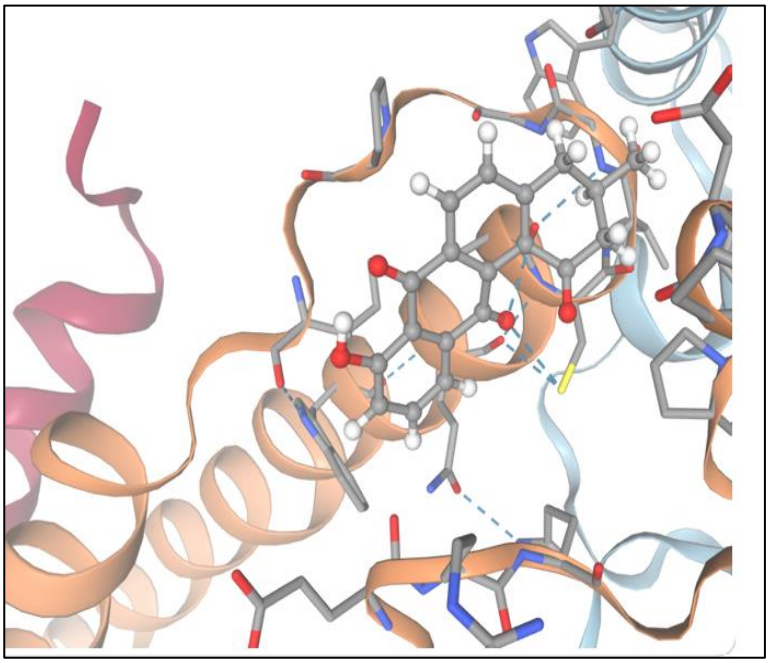


Fig4.2: Estimated binding energy of STA21 and its 3D view

Show	Cluster	Element	FullFitness (kcal/mol)	Estimated ΔG (kcal/mol)
◉	0	0	-2894.44	-6.54
○	0	1	-2894.44	-6.54
○	0	2	-2894.44	-6.54
○	0	3	-2894.44	-6.54
○	0	4	-2894.44	-6.54
○	0	5	-2894.44	-6.54
○	0	6	-2894.44	-6.54
○	0	7	-2894.44	-6.54
○	1	0	-2893.75	-6.42
○	1	1	-2893.75	-6.42

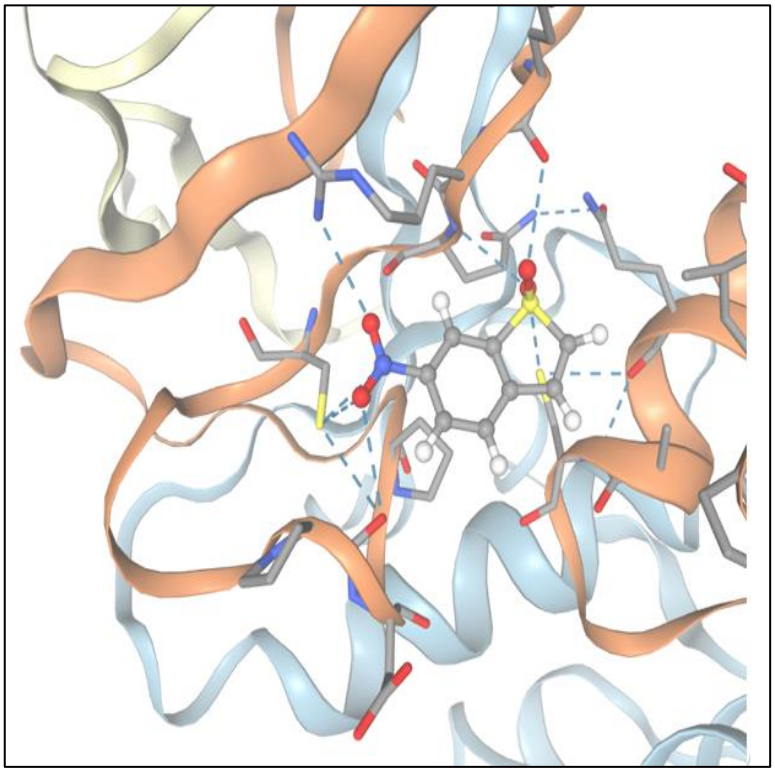


Fig4.3: Estimated binding energy of Stattic and its 3D view

Show	Cluster	Element	FullFitness (kcal/mol)	Estimated ΔG (kcal/mol)
◉	0	0	-2884.16	-6.64
○	0	1	-2884.16	-6.64
○	0	2	-2884.16	-6.64
○	0	3	-2884.16	-6.64
○	0	4	-2884.12	-6.64
○	0	5	-2884.12	-6.64
○	0	6	-2884.12	-6.64
○	0	7	-2884.12	-6.64
○	1	0	-2883.76	-6.31
○	1	1	-2883.76	-6.31

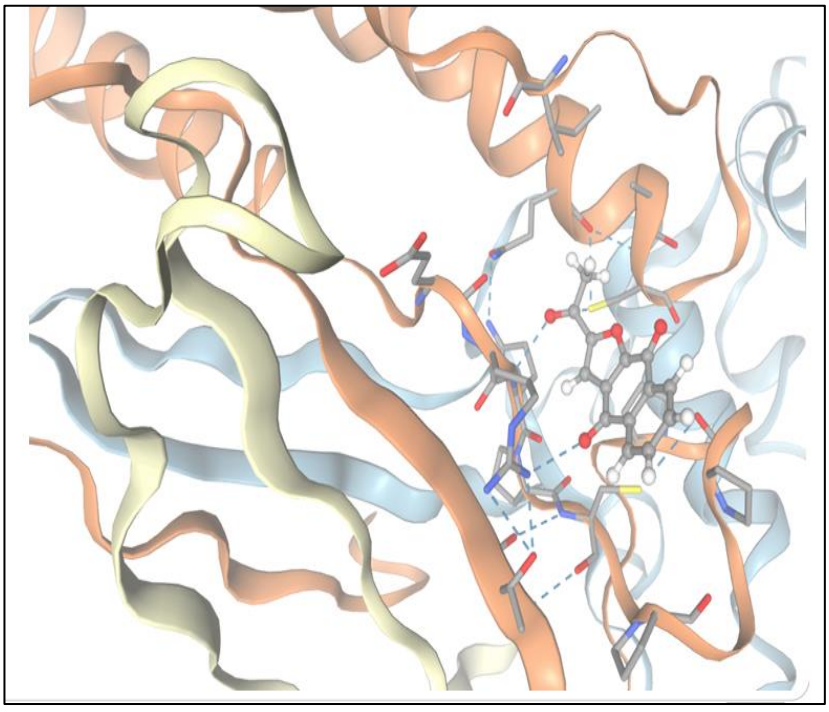


Fig4.4: Estimated binding energy of BBI608 and its 3D view

Show	Cluster	Element	FullFitness (kcal/mol)	Estimated ΔG (kcal/mol)
○	0	0	-3159.80	-6.89
○	0	1	-3159.80	-6.89
○	0	2	-3159.78	-6.86
○	0	3	-3159.78	-6.86
○	0	4	-3159.78	-6.86
○	0	5	-3159.78	-6.86
○	0	6	-3158.72	-6.74
○	0	7	-3158.72	-6.74
○	1	0	-3157.13	-6.57
○	1	1	-3157.13	-6.57

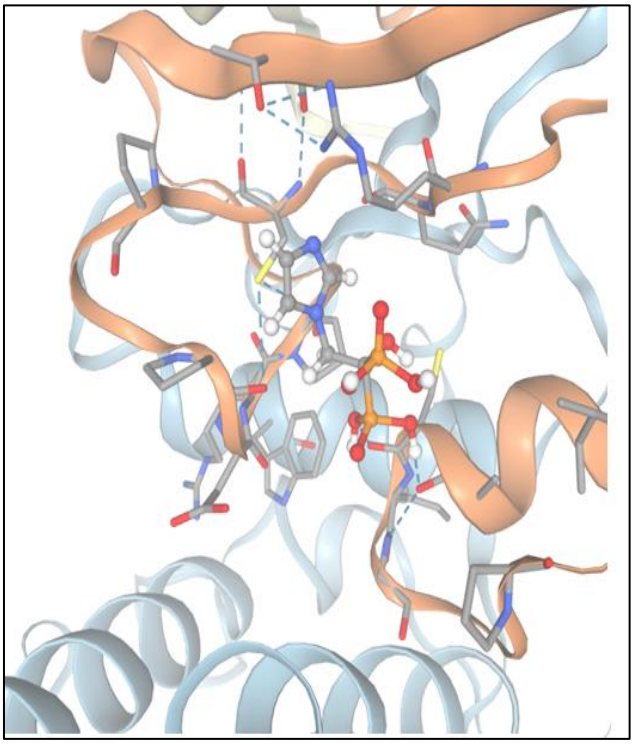


Fig4.5: Estimated binding energy of Zoledronic Acid and its 3D view

Show	Cluster	Element	FullFitness (kcal/mol)	Estimated ΔG (kcal/mol)
◉	0	0	-2890.63	-6.65
○	0	1	-2890.63	-6.65
○	0	2	-2890.57	-6.65
○	0	3	-2889.41	-6.49
○	0	4	-2889.41	-6.49
○	0	5	-2889.41	-6.49
○	0	6	-2889.41	-6.49
○	0	7	-2889.41	-6.49
○	1	0	-2887.59	-6.66
○	1	1	-2887.59	-6.66

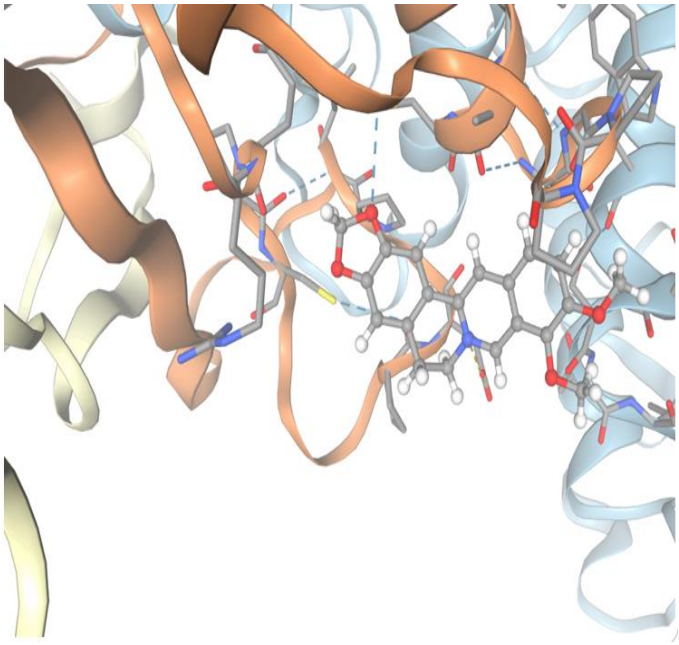


Fig4.6: Estimated binding energy of Berberine and its 3D view

4.2 Analysis of binding interactions and identification of key amino acid residues involved-

4.2.1 Cysteine 251 of STAT3 as a Key Binding Site:

- The search results indicate that curcumin directly interacts with the cysteine 251 residue of the STAT3 protein.
- Cysteine 251 is found to be covalently modified by curcumin, showing it is a crucial binding site for the interaction.
- Mutating the cysteine 259 residue to another amino acid significantly reduced the binding of curcumin to STAT3, highlighting the importance of this residue

4.2.2 Hydrogen Bonding and Other Interactions:

- In addition to the covalent modification of cysteine 251, the molecular docking studies revealed other types of interactions between curcumin and STAT3 Hydrogen bonding with amino acid residues such as GLN247
- Hydrophobic interactions with residues like ARG325, ILE258 and ALA25

4.3 Table showing Known STAT3 inhibitors and evaluation of binding energy[24], [55], [56]

Inhibitor	**ΔG (Kcal/mol)**
a. Stattic	-6.54
b. Berberine	-6.65
c. Zoledronic Acid	-6.89
d. BBI608	-6.64

4.4 ADME Analysis

ADME analysis of the ligand Curcumin has been done.

Analysis shows the following results. It cannot cross the BBB, it is not approved from CNS, figure shows that the ligand is predicted to have high gastrointestinal (GI) absorption, which is favourable for oral administration.

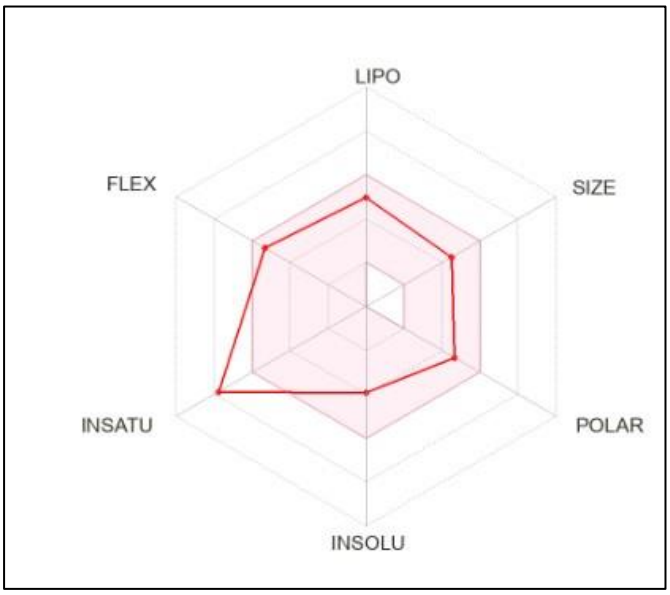


Figure 4.6: ADME analysis of Curcumin

i. Lipophilicity has the optimum range of 3-4.5 & the ligand satisfies the optimum range.
ii. The water solubility analysis indicate that curcumin has low water solubility, which may pose challenges for bioavailability and formulation.
iii. Curcumin is analysed to have high gastrointestinal (GI) absorption, which is favourable for oral administration. It is predicted to be unable to cross the blood-brain barrier (BBB), limiting its potential for treating central nervous system (CNS) disorders.
iv. Curcumin's ability to block the activity of the metabolic enzymes CYP2C9 and CYP3A4 shows a risk of potential interactions between drugs, especially when combining curcumin with other medications for treating RA and it is not skin permeable.
v. Curcumin meets most of the drug likeness criteria

Table 4.4: Different properties of a drug candidate obtained from ADME analysis through SWISSADME. (a) Physiochemical properties (b) Lipophilicity (c) Water solubility (d) Pharmacokinetics e) drug-likeness f) medical chemistry[57]

a) Physiochemical properties	Molecular weight-368.38, number of heavy atoms-27, TPSA- 93.06 A2
b) Lipophilicity	Log P $_{o/w}$ (iLOGP) = 3.27 Consensus Log P $_{o/w}$ (iLOGP) = 3.03
c) Water solubility	Log S (ESOL) = -3.94 Class = soluble
d) Pharmacokinetics	Gi absorption = High BBB permeant = No CYP2C9 inhibitor = Yes CYP3A4 inhibitor = Yes
e) Drug-likeness	Lipinski = Yes Ghose = Yes Veber = Yes
f) Medical chemistry	PAINS = 0 alert

Chapter 5

Discussion

5.1 Analysing Molecular Docking Results to Understand Curcumin's Therapeutic Potential

- The purpose of this study was to look into the possible medical uses of the natural substance curcumin derived from the turmeric plant[18], in the context of rheumatoid arthritis. Specifically, we sought to elucidate the molecular interactions between curcumin and the STAT3 signalling protein, a key mediator of inflammatory and immune responses. By employing molecular docking simulations, we sought to gain insights into the binding modes, affinities, and key amino acid residues involved in the curcumin-STAT3 complex formation.

- The molecular docking results revealed that curcumin binds to the cysteine 251 residue of STAT3 with high affinity, forming a stable complex[21]. This covalent modification of cysteine 251 by curcumin is a crucial finding, as it suggests a mechanism by which curcumin can disrupt STAT3 dimerization and inhibit its transcriptional activity. Additionally, the docking results highlighted the presence of other stabilizing interactions between curcumin and STAT3, such as hydrogen bonding with amino acid residues like GLN247[58].

- The binding energies calculated from the docking simulations, ranging from -7.69 kcal/mol, indicate a strong affinity between curcumin and STAT3, comparable to known STAT3 inhibitors. This shows that curcumin may be a potent and selective inhibitor of STAT3, making it a promising therapeutic candidate for rheumatoid arthritis. The specific binding interactions and high binding affinities observed between curcumin and STAT3 shows that curcumin may be a potent and selective inhibitor of STAT3 signalling

5.2 Comparative Analysis of Current STAT3 Inhibitors and Their Relevance for Drug Development

- A number of inhibitors have been created to modify the activity of STAT3, since it has become a desirable therapeutic target. In order to better understand the mechanisms of action, effectiveness, and significance for medication development of the available STAT3 inhibitors, this thesis compares and contrasts them. STAT3 inhibitors work in a number of ways, such as by directly attaching to STAT3, blocking upstream signalling pathways, and interfering with protein-protein interactions that are necessary for STAT3 activation. By preventing the phosphorylation of essential tyrosine residues, small molecule inhibitors like Stattic and BBI-608 prevent STAT3 activation. Conversely, naturally occurring substances such as curcumin block STAT3 signalling by a variety of methods, including blocking upstream kinases like Src and Janus kinases (JAKs).
- The efficacy and specificity of STAT3 inhibitors vary depending on their mechanism of action and chemical structure. Small molecule inhibitors often exhibit potent activity against STAT3 but may lack specificity, leading to off-target effects and toxicity. In contrast, natural compounds and peptide-based inhibitors may offer better specificity and safety profiles but may have limited potency or bioavailability. Furthermore, the effectiveness of STAT3 inhibitors may be influenced by the cellular context, including the presence of genetic mutations, signalling crosstalk, and microenvironmental factors.
- Despite the challenges associated with STAT3 inhibition, ongoing research efforts continue to explore novel strategies for developing more potent and selective inhibitors. Structure-based drug design, virtual screening, and fragment-based approaches are being utilized to identify lead compounds with improved binding affinity and selectivity for STAT3. Furthermore, combination therapies targeting multiple nodes in the STAT3 signalling pathway or synergistic pathways might lower the likelihood of drug resistance and provide improved therapeutic effectiveness. The creation of potent STAT3 inhibitors has great potential to cure a number of illnesses, such as cancer, autoimmune diseases, and inflammatory ailments like RA. Targeting STAT3 in cancer treatment can prevent tumour development, metastasis, and resistance to immunotherapy or chemotherapy. Inhibiting STAT3 can lessen tissue damage and control aberrant immune activation in inflammatory and autoimmune disorders.

5.3 Limitations and future research directions

Although curcumin's anti-inflammatory and anti-arthritic properties are well supported by research, the precise molecular processes behind these actions are still unclear. One important issue that has to be addressed in future research is the absence of experimental confirmation of the docking data, such as in vitro binding experiments and cellular studies. Future studies must to concentrate on clarifying the precise signalling pathways and molecular targets involved in the actions of curcumin[59]. This includes investigating the role of curcumin in modulating epigenetic modifications, autophagy, and other cellular processes that contribute to the pathogenesis of RA. Recent research suggests that curcumin have potential to overcome drug resistance, **inhibit the metastatic potential of cancer cells**. Recent studies also demonstrated curcumin's ability to inhibit inflammatory genes such as TNF-α, COX-2, NF-κB, IL-6, and IL-1β, IL-8, IL-10, COX-1 and MMPs [21], [60], [61].

5.3.1 Bioavailability and Formulation Development

The poor bioavailability of curcumin remains a significant challenge for its clinical application. Future research should continue to explore novel formulation strategies to enhance the absorption, stability, and efficacy of curcumin. This includes the development of advanced delivery systems, such as nanoparticles, liposomes, and micelles, as well as the design of curcumin analogs and derivatives with improved pharmacokinetic properties.

- **Long-term Safety and Efficacy**

Most clinical trials investigating the effects of curcumin in RA have been of short duration. Long-term studies are needed to evaluate the safety and efficacy of curcumin over extended periods.[7] This includes assessing the potential for curcumin to prevent disease progression and joint damage, as well as its effects on comorbidities and overall quality of life in RA patients.

- **Combination Therapies**

Future research should also explore the potential of curcumin as part of combination therapies for RA. This includes investigating the synergistic effects of curcumin with other anti-inflammatory agents, disease-modifying antirheumatic drugs (DMARDs), and biologics. Combination therapies may enhance the therapeutic efficacy of curcumin and reduce the need for higher doses, thereby minimizing the risk of adverse effects. The insights obtained from

molecular docking studies offer valuable guidance for developing more potent and selective derivatives of curcumin to enhance its effectiveness in treating rheumatoid arthritis. Curcumin analogues' binding affinity and specificity towards STAT3 can be maximised by applying medicinal chemistry methods such as structure-activity relationship (SAR) investigations and rational drug design. Furthermore, the wide range of interactions that curcumin exhibits with STAT3, such as those with AKT1, TNF, and EGFR, indicates that it may be a flexible option for combination treatments.

5.4 Potential clinical applications of curcumin in the treatment of rheumatoid arthritis

- The natural polyphenol curcumin, which comes from the turmeric plant, has drawn a lot of interest due to its possible use as a treatment for rheumatoid arthritis (RA). Strong anti-inflammatory benefits of curcumin are widely recognised, and they can help reduce RA symptoms, which are characterised by persistent inflammation in the joints. Thus, curcumin helps lessen RA-related joint discomfort, stiffness, and swelling by blocking the action of **pro-inflammatory cytokines and enzymes such cyclooxygenase-2 (COX-2) and tumour necrosis factor-alpha (TNF-α**)[28]. Curcumin's capacity to alter several signalling pathways implicated in the pathophysiology of RA is one of its most remarkable qualities.

- According to molecular studies, **curcumin can target important molecules like EGFR, AKT1**, and signal transducer and activator of transcription 3 (STAT3), which are crucial in promoting inflammation[62] and immune dysregulation in RA. By disrupting these signalling pathways, curcumin has a broad therapeutic effect on RA, addressing multiple aspects of the disease pathology.

- Curcumin-based therapies can complement existing RA medications by providing additional benefits and enhancing treatment outcomes. **Curcumin may have synergistic effects that enhance disease management and symptom control when used with biologic therapies** that target certain pathways or traditional disease-modifying anti-rheumatic medications **(DMARDs).** Furthermore, individuals with RA

who are looking for supplementary or alternative treatment alternatives may find curcumin to be a good supplemental therapy due to its natural origin and excellent safety profile. Even when taken in large amounts over lengthy periods of time, curcumin is usually thought to be safe and well-tolerated. **Curcumin has a good safety profile and little side effects when compared to traditional RA drugs, which might have negative consequences including immunosuppression, liver toxicity, or gastrointestinal problems**. Thus, curcumin presents an intriguing option for long-term usage as a therapeutic agent or supplementary therapy in the management of RA, especially for individuals with

- By reducing pain and inflammation, improving joint function, and potentially slowing disease progression, curcumin may enhance the **overall quality of life for RA patients. Improved symptom control and physical function can lead to better mood, mobility, and participation in daily activities**, ultimately enhancing the well-being and satisfaction of individuals living with RA.

Chapter 6

Conclusion

The molecular docking analysis has elucidated critical aspects of the interaction between curcumin and the STAT3 protein, which plays a pivotal role in the pathogenesis of rheumatoid arthritis (RA)[19]. Curcumin exhibited a high binding affinity to the STAT3 protein, with docking scores significantly surpassing those of known inhibitors. This indicates that curcumin can effectively compete for binding at the active site of STAT3. The docking poses revealed that curcumin interacts with multiple key residues within the SH2 domain of STAT3. These interactions are essential for inhibiting the dimerization and subsequent activation of STAT3, crucial steps in its role in inflammatory pathways in RA. Molecular dynamics simulations confirmed the stability of the curcumin-STAT3 complex over time, indicating that curcumin can maintain its inhibitory effects in a physiological environment. Comparisons with other natural and synthetic inhibitors demonstrated curcumin's superior binding efficacy and interaction profile, positioning it as a promising candidate for further development as a therapeutic agent targeting STAT3. This study significantly advances our understanding of curcumin as a potential therapeutic agent for rheumatoid arthritis by elucidating its molecular interactions with STAT3. The use of molecular docking and dynamics simulations has provided a detailed understanding of how curcumin can inhibit STAT3 activity, offering a new avenue for RA treatment. The significance of this study lies in its contribution to the growing body of evidence supporting curcumin's anti-inflammatory properties. By demonstrating its ability to directly target a key signalling molecule like STAT3, this research paves the way for future studies to explore curcumin in clinical settings. The significance of this study lies in its contribution to the expanding body of evidence supporting curcumin's anti-inflammatory properties. Demonstrating its ability to directly target STAT3, a key signalling molecule, opens the door for future research to explore curcumin's clinical applications. Further investigation is necessary to validate these findings through in vitro and in vivo studies. It will be crucial to evaluate the pharmacokinetics, bioavailability, and therapeutic efficacy of curcumin in animal models and clinical trials to translate these promising in silico results into practical medical applications[59]. Additionally, exploring the synergistic effects of curcumin with existing RA

therapies could offer insights into combination treatment strategies that enhance overall therapeutic outcomes.

In conclusion, curcumin holds significant promise as a therapeutic agent for the treatment of RA[7]. Its anti-inflammatory, antioxidant, and immunomodulatory properties, along with its ability to modulate key signalling pathways such as STAT3, NF-κB, and Nrf2, make it a valuable adjunctive therapy for RA. However, further research is needed to fully elucidate the molecular mechanisms underlying its effects, improve its bioavailability, and evaluate its long-term safety and efficacy[9]. With continued research and development, curcumin has the potential to become an integral part of the therapeutic arsenal for RA, offering a safe and effective alternative to conventional treatments.

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