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Exploring the Therapeutic Potential of Boswellia serrata Derivatives in Arthritis: A Molecular Docking Study

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Abstract

Docking is the process to understand interactions between any two molecules. It includes using multiple software and the pre-processing the protein and ligands according to need. Computationally we can screen multiple ligands whether they can be used as potential drug or drug targets in curing a disease. In this paper we'll discuss about the *Boswellia serrata* and its oleo gum resin extracts. Its extract has many components like diterpenoids, tripernoids and different kind of Boswellic acids. While going through the pathway of Rheumatoid Arthritis and Osteoarthritis, we took some protein candidates and docked them against the known compounds and Boswellic acids of oleo resin gum. Multiple docking scores were generated and observing them we can say that it can be a potential drug against arthritic activities. It has the potential to block many proteins of the arthritis and reducing inflammation and relief pain. The docking scores were not that good but if the compounds are modified or developed as candidate then multiple ways can be found to treat or even cure some cases of arthritis. Though, the reason and solid proof is still unknown for osteoarthritis but the symptoms can be treated by using the oleo gum resin of *Boswellia serrata*

Keywords : Boswellia serrata, boswellic acids, arthritis, osteoarthritis, rheumatoid arthritis, molecular docking, structural bioinformatics,

Introduction

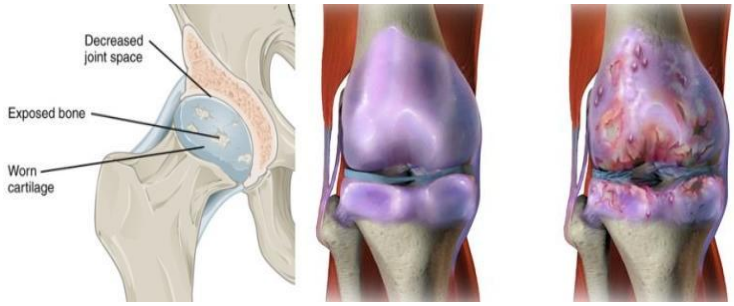
Inflammation in joint means "Arthritis". Meeting point of two bones is called a Joint and any kind of disorder/malfunctioning in it can cause severe pain and problems. Every one of five human have some problem in their joints.[1] In some forms it can even affect our organs, connective tissues like skin.
There are several types known till date but cause is unknown. Majorly it causes:

- As we age
- More in women population
- Over-weight causing excess weight on joints like knees
- Joint injuries
- Different types of bacterial, fungal or viral infections that can trigger inflammation.

Mainly two types of Arthritis are found namely Osteoarthritis and Rheumatoid Arthritis.

Osteoarthritis (OA)

With over 300 million cases worldwide[2], osteoarthritis has a complex pathophysiology. Due to wear and tear of articular cartilage, it causes degradation of joints resulting in abnormal joint function. It destabilizes the synthesis and degradation of ECM (extracellular matrix), subchondral bone and chondrocytes of articular cartilage. It can be triggered by many reasons like genetic, metabolism or any kind of injury/trauma. Changes in both matrix and the cells cause the softening, ulcer formation, fiber formation (fibrillation) and degradation of articular cartilage. Further characteristics like joint pain, movement retardation, crepitus, inflammation of various degree and sometimes effusion followed by osteophytes and subchondral cysts



Diagrammatic representation of OA

Pathogenesis of OA

Osteoarthritis mainly affects articular cartilage but after damage, the deterioration of cartilage is also catalyzed by neuromuscular apparatuses, ligaments, subchondral bones, etc. Type 2 and other proteoglycans and chondrocytes are the main components of articular cartilage. Subchondral bone and synovial fluid supply the nutrition to chondrocytes by blood diffusion.

Up to 10-20% of cartilage collagen is made up of types 3, 4, 9, 10 and 11 along with maintaining the stability of type 2 collagen as type 9 and 11 helps in maintaining the fibril radius. Proteoglycan aggrecan retain water for the conditions of load and during unload, it releases to maintain the elastic property of cartilage. It helps on cartilage to work as shock absorber in the diarthrodial joint. Any kind of malfunctioning can lead to osteoarthritis.

Mononuclear cells, chondrocytes and lining of synovial cells produce interleukins-1(IL-1) which stimulates the production of neutral MMPs which degrades the cartilage. The formation of components of cartilage depends on several growth factors like TGF-beta and IGF-1. Also the chondrocytes extracted from OA patients were found to have nitric oxide, prostaglandins, TNF and IL which are involved in cartilage degradation.[3]

With aging, the balance between degradation and repair of cartilage starts shifting but the reason of this shift is still not known. Due to any trauma, that caused micro fractures or injury leads to the overwhelming of wear particles in the joints even macrophages will be not enough to engulf them all. These become the cause of inflammation. Further, after the breakdown of collagen & proteoglycan, breakdown particles are engulfed by synovial macrophages initiates of pro-inflammatory cytokines. Prostaglandin synthesis is increased by IL-1 and TNF causing further more inflammation.

Role of LOX is still not clear. LTB4 and leukotriene C4 also noted to be high in OA patients but not in the chondrocytes. LTB4 also known to initiate IL-1B formation in synovial cells.

Synovitis is also thought to be linked with structural damage. COMP (cartilage oligomeric protein), component of ECM formed by the activated synovial

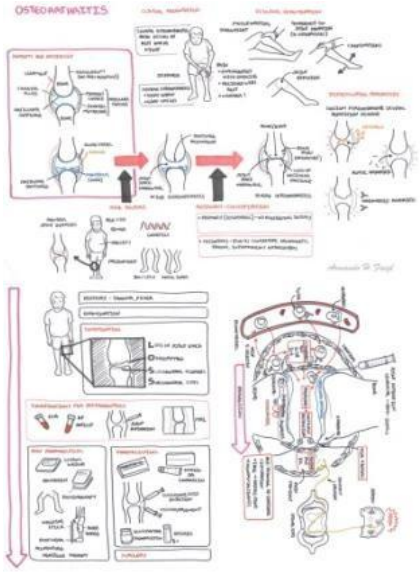
affects IL1 initiated suppression of the glycosaminoglycan & collagen formation continues IL-1 expression calls can be a reason of synovitis. Also, high levels of CRP (C - reactive protein) are supposed to give radiological damage in knee OA. Higher levels of hyaluronic acid were also observed in inflammation.

The presence of NF-kb (nuclear factor kb) and nitric oxide in osteoarthritic synovium and synovial fluid respectively are expression factors in osteoarthritic joints. NO of MMPs and inhibits the production of IL-1 receptor antagonist. Treating NO may be a treatment of OA in future.

Apoptosis plays an important role in this disease. Excess of NO in osteoarthritis has been proved to cause chondrocyte apoptosis.

One of the main risk factor of OA is age. Another is point mutation in gene of young patients type 2 procollagen along with chondroplasia, which shows that defect in matrix articular collagen is the reason for OA. A type of PM known isspondyloepiphyseal dysplasia whose patients suffer from premature OA.

Trauma is also a factor for OA with periarticular injuries of cruciate and collateral knee ligaments. Occupational injuries in the fields of coal mining, farmers, construction site labors and jackhammers and longtime weight exercise or ex athletes may increase the risk of OA in women.



Obesity is also involved in knee **OA. Lacking vitamin D further progresses OA.**

Pathophysiology

Clinical presentation

Pain is the main complaint of OA patient. Most common joint involvement affected is distal and proximal joints. Feel of pain appears in motion of that particular joint and decreases with rest. As cartilage lack nerves, the pain felt is from the subchondral bone, capsule of joint, synovium, ligaments & muscles. Morning stiffness in OA patients lasts around twenty minutes. Gelling (transient stiffness) can lasts for hours. In OA advancement, Heberden's nodes and Bouchard's nodes are formed after 45 mostly in women.

Hip OA can be more seen in men causes' painful gait. Internal flexion rotation and extension, flexion and external flexion rotation are predominantly inhibited.

Knee OA covers single compartment of knee and loss of lateral joint spaces causing genu valgus or 'knock knee'. It can further causes "bow legs" and pain while going upstairs in patellofemoral movement.

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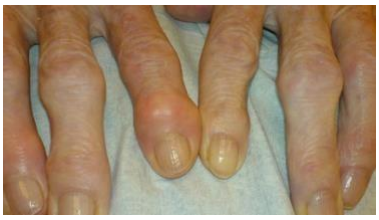


Figure: patient with distal and proximal OA of hand

Diagnosis

X ray is considered as the gold standard for OA. Synovial fluid is analyzed with a cell count <10,000 WBC per millimeter cube. Otherwise MRI is also done for the better evaluation.[4]

Treatment

Osteoarthritis can't be reversed, but treatments can reduce pain and help you move better.

Medications

Medications that can help relieve osteoarthritis symptoms, primarily pain, include Acetaminophen, Nonsteroidal anti-inflammatory drugs (NSAIDs), Duloxetine (Cymbalta).

Rheumatoid Arthritis (RA)

It is an autoimmune disease causing inflammation followed by synovitis which further accompanied by extra articular organs like interstitial pneumonia, pain, swelling, stiffness in many joints and high fever. Usually women at their 30s and 50s get affected by it.

Pathology

The most susceptible gene known for causing rheumatoid arthritis is HLA-DBR1 (human leucocyte antigen D-related B1 gene) in patients with nucleotide polymorphism. It includes PTPN22, cytotoxic T-Lymphocyte antigen-4(CTLA-4), STAT4, CCL21, PADI4 genes. Presence of anti-CPP bodies has been found very dominant in causing cartilage destruction. Also environmental factors like smoking, gingivitis, can be reason for the modulation of epigenome and demethylation of DNA & histone. Inside the synovial tissue, accumulation of auto reactive T and B cells takes place.

After the breakdown of self-tolerance, auto reactive T cells get activated and stimulate B cells further inducing the formation of autoantibodies. Tissues having angiogenesis and vasodilation, lymphocyte accumulation are characteristics of synovitis. Lymphoid follicle or germinal center structures are formed by the accumulation of T and B cells during tissue inflammation.

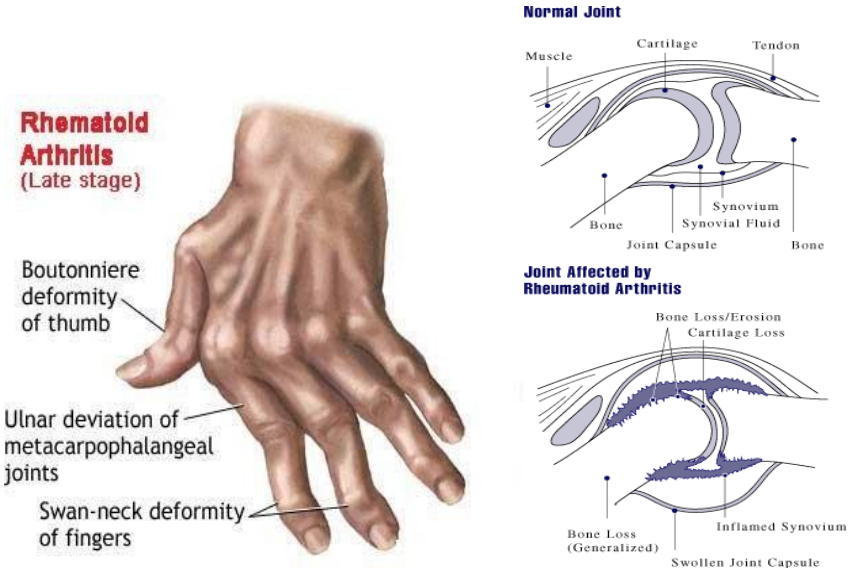
Large quantity of inflammatory cytokines is produced like TNF, IL-1 and IL6 in synovitis lesion. Even low fever, malaise, extra articular organ involvement like keratoconjunctivitis sicca, interstitial pneumonia is often observed. Further MMPs are released by cytokine stimulated synoviocytes. Degradation and absorption of cartilage is done by these enzymes. Receptor activator RANKL is also expressed by synoviovytes and lymphocytes which further induces the activation of osteoclasts. Bones are destroyed and absorbed by multinucleated osteoclasts causing destruction of joints majorly at contact points.[3]

Clinical features

It is characterized by morning stiffness and pain and swelling. Difficulty in movement of fingers after waking up in the morning and forming fist . Limited mobility and swelling. Mostly these appear in fingers and toes (metatarsophalangeal, metatarsophalangeal joints), knees and feet, hands and elbows, spine, etc. Short breaths, numbness in limbs, rheumatoid nodules on extensor surface.

Visually, swelling and accumulating synovial fluid, redness and hot flashes. As it progresses, joint deformation patters are observed. Deformities like buttonhole, swan neck in the fingers, etc. Numbness and inflammation to tendons,

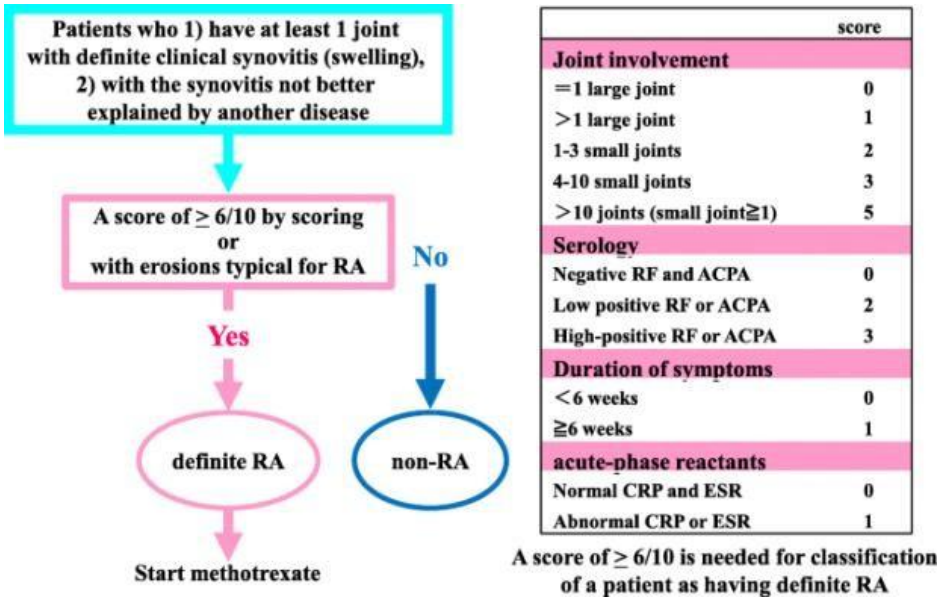
development of carpal tunnel syndrome followed by swelling in wrist.



Rheumatoid Arthritis of hands

Diagnosis

Initially treating with DMARDs, RA is classified into different classes. First step is exclusion of diseases like OA, spondyloarthritis, are excluded. Secondly, four time score of arthritis, serologic test results, duration of diseases, acute phase reaction, are weighed and added. Score of 6 or higher, from a total of 10, is referred as RA. Now treatment with DMARDs is initiated. DAS28 score (Disease activity score) is calculated on the basis of tenderness or swelling of 28 joints. SDAI and CDAI are also widely used. Also, RA is accompanied by lungs disorder which is diagnosed by CT scan. In around 30% cases of the 70% patients diagnosed, they're assessed having interstitial pneumonia.



Diagnosis flow

Treatment

The evaluation is done by using DAS28 SDAI and CDAI scores of patient. Then, a conventional DMARD called methotrexate is used. If no improvement is seen within three months, full dosage is given. If still the goal is not achieved, JAK (Janus kinase) inhibitors, biological DMARD is recommended. Also glucocorticoids for temporary time span are given up to three months to relieve pain and swelling.

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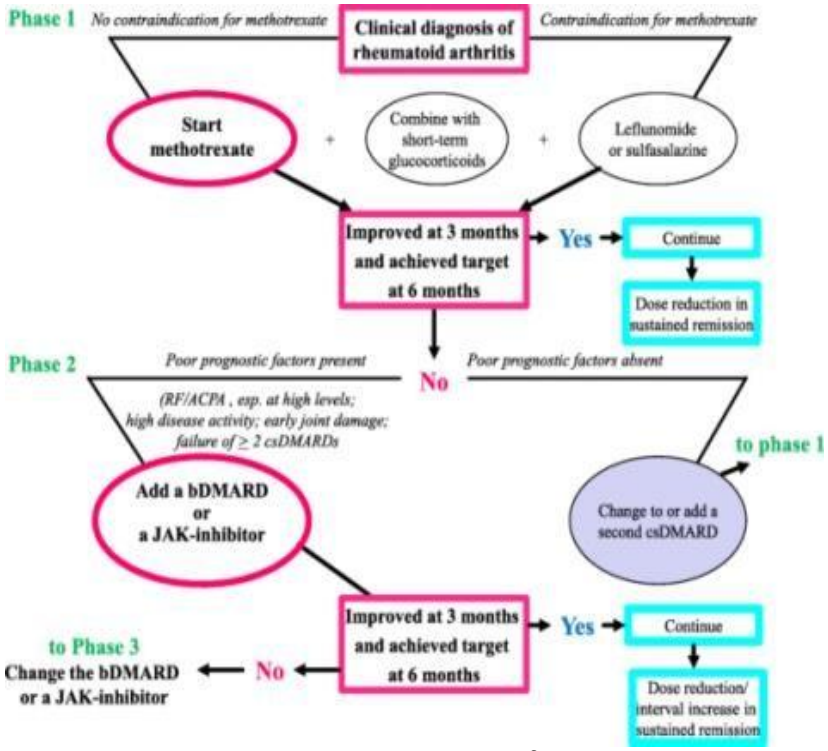


Treatment workflow[6]

Boswellia serrata

Boswellia serrata, well known for its anti-inflammatory and anti-arthritic properties contains mainly Boswellic acid (BA). Its trade name is known as Shallaki and raw product as 'salaigugal'. Many kind of boswellic acids are known and belong to pentacyclic triterpenes. BA like alpha-BA, beta-BA have been tested to inhibit 5-LOX, which plays a main role in leukotriene biosynthesis in inflammation disorders and human leukocyte elastase. So, the docking is done using chemical structures/compounds of BA against selected proteins from the pathway of OA and RA.[4]

Boswellia sap has been utilized as incense in strict and social ceremonies and medication since days of yore. Bosvelliserrata (Salai/Salai), an individual from the family Burseraceae (class Bosvellia), is a tolerably huge extended tree that fills in the bone-dry piles of India, North Africa and the Middle East. Oleo elastic sap is cut from the center of the tree, put away in an exceptionally made bamboo container to eliminate the oil content, to fortify the tar. In the wake of preparing, the gum is assessed by its taste, shading and size. In India, Andhra Pradesh, Gujarat, Madhya Pradesh, Uttarakhand and Chattisgarh are the primary wellsprings of Boswellia Serrata. It is likewise known by different names around there. Oleo gums contain 30-60% tar, 5-10% fundamental oils, dissolvable in natural solvents, and the rest are polysaccharides. Boswellia serrata elastic concentrate has been generally utilized in people medication for quite a long time to treat different persistent incendiary illnesses. Boswellia serrata tar contains monoterpenes, diterpenes, triterpenes, tetracyclic triterpenic acids and four fundamental pentacyclictriterpenic acids, ie. β-boswellic corrosive, liable for restraining mitigating chemicals.

Geological features

Boswellia serrata is a traditional plant which is also used in traditional and cultural practices, have moderate to large sized branches from family Burseraceae (Boswellia genus). It grows in dry Indian mountain regions, Northern Africa and middle of eastern areas. An incision is made on the tree trunk and placed in bamboo baskets for further removal of its oil and then solidified resin. After the processing of resin, it is given grades on the basis of its flavor, size, shape and color. The main sources of this extract are Madhya Pradesh, Andhra Pradesh, Chhattisgarh, Gujarat and Jharkhand.
More than 2 species known in the genus *Boswellia* and mostly are from the areas of Arabia, Africa and India.

Components

The resin of oleo gum has resin 30-60 percent, essential oils 5-10 percent and rest is polysaccharide. The resin contains monoterpenes, diterpenes, triterpenes, tetrracyclictriterpenic acid, and four ore pentacyclictriterpenic acids like alpha-BA, 11-keto-beta-BA and acetyl-11-keto-BA involved in pro inflammatory enzymes.
They are also reported to inhibit 5-HETE and LTB-4 which are pro inflammatory enzymes. Leukotrienes are generated by 5-LOX which further initiates free radicle damage, Ca discolation, adhesion of cell and migration of inflammation inducing cells. In some studies it was also found that BA inhibit leukotriene synthesis by 5-LOX or COX, but did not

alter the 12-LOX & COX activity neither inhibit peroxidation of arachidonic acid by Fe or ascorbate. BA has also showed inhibition activity of HLE (Human Leukocyte elastase). HLE is responsible for mucus secretion which can cause cystic fibrous.

It was also studies that in rats and their mice, 25 to 46 percent inhibition effective against paqoedema. Also, 45-67 percent anti-arthritic properties in same type of dosages. It was found to be effective against both, adjuvant and established arthritis. Also antipyretic without ulcerogenic effects were shown by it. Also, when BA form serrata tested on Papaya Latex Model, inhibited inflammation by 35 percent.[4]

Also, in PBMCs (human peripheral blood mononuclear cells) and macrophages of mouse the anti-inflammatory and TNF-alpha inhibition activity was found. Incensol acetate from the B. resins inhibit NF-kappa B activation. Also A- 11-keto-beta-BA was shown to inhibits growth of prostate tumor by suppressing VEGF-2 mediated angiogenesis.

Proteins selected from the pathway of arthritis

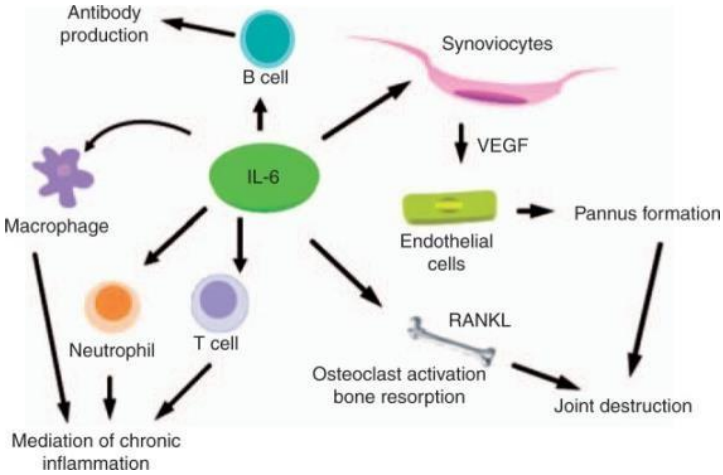
- **HumanInterleukin-6**

HIL-6 is classified as cytokine having PDB ID 1ALU.[9]It has multiple activities in human body along withconstructing IL-6 model while binding to IL-6 receptor. It has a very important role in pathophysiology in RA. Arthritis has a very complex network of cytokines with cytokines available in blood as well as synovial joints. Among them, one is IL-6 which is responsible for the production of auto-antibodies and B-cell maturation. In animal models, it was found that IL-6 plays an important role in Th17 pro-inflammatory lymphocytes.

It has 26-kDa glycoprotein and the gene is in chromosome seven. Many cells produces IL-6 like T and B cells, monocytes, osteoblast cells and some mesangial and tumor cells. Its family is IL-6 cytokine family which need cell surface gp130 for activation of cells for their cytokine receptor. IL-6 also activates cells through two ways i.e. membrane and soluble receptors. It was observed that increased levels of IL-6 and sIL-6 are directly related (proportional) to the risk of destruction in joint of a RA patient.

Role of IL-6

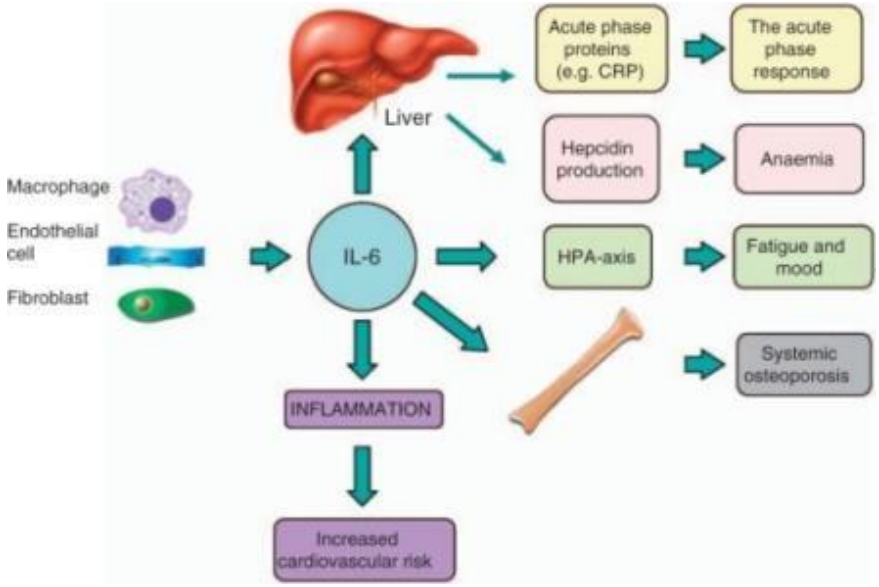
B cells are stimulated by IL-6 and then get differentiated into plasma cells and produces Ig (immunoglobulins). IL-6 also initiates differentiation of B cells and further B cell antibody production. Migration of neutrophils causes inflammation. As it enters, these neutrophils releases protein cleaving, proteolytic enzymes and reactivation of oxygen which further causes distruction of tissue and damages joint in RA. IL-6 also shifts the acute inflammation to chronic by increasing the accumulation of monocytes. These neutrophils also release sIL-6Rwhen they reach where the inflammation is present. This trans-signaling supports monocyte-specific chemokines produced by endothelial cells. IL-6 also induces inflammation by altering VEGF levels in RA. VEGF induces the proliferation of endothelial cells and promotes vascular permeability and mediation of the inflammation. Increased levels of VEGF are a sign of RA.



Inflammation pathway induced by IL-6

Osteoclasts are the main cells involved in erosion of joints and inflammation. IL-6 promotes the accumulation of osteoclasts by altering on HSC. Expression of RANKL is increased by IL-6 in the presence of sIL-6R and downgrades expression of RANK and resorption of bone is induced which further decreases by the osteoclasts inhibitors concluding that sIL-6R trans-sig. induces osteoclast genesis. IL-6 also involved in degradation to articular cartilage in rheumatoid arthritis as sIL-6R & IL-6 inhibit the proteoglycan formation in the culture plate of HAC taken from the patients having RA.

Proteinases like MMPs are synthesized by cells of synovial linings, subliming fibroblasts & macrophages which further target the ECM. It explains the relation of MMPs and articular degradation. The patients having RA, show IL-6 and CRP related to proMMP-3 and IL6 activity



IL-6 systemic effect

IL-6 affect lipid metabolism by inducing synthesis of liver fatty acids and lipolysis of adipose tissue. IL-6 and CRP are linked to increased heart risks. IL-6 increases the formation of cholesterol and decreases its absorption simultaneously.
Also, anti-IL-6R monoclonal antibody like Tocilizumab was selected in clinical trials in animal models. There are also several risks associated with the blockage of IL-6 like, infection increased liver transaminases, decreasing neutrophils and increased lipids.[10]

- **Matrix metalloproteinase 9(MMP9)**

1L6J is the PDB ID of MMP9.[9] MMPs are basically a huge group of enzymes which has role in degradation of matrix. From them, gelatinases family if produced in over quantity in patients joint having rheumatoid arthritis. as it gives a degenerates the ECM, gelatinases it is believed that it plays a crucial role in degradation of cartilage an progression of disease.
MMPs are basically a large group of endopeptidases (Zinc dependent) and have the ability to degrade many components of ECM. If these are overexpressed, then it can cause arthritis, tumor invasions and atherosclerosis and metastasis. The way of action and roles of every MMPs are yet to define.
Among these MMPs, MMP-2 and MMP-9 are majorly important in ECM degradation and denaturing off collagenases. These enzymes are responsible for the digestion of Type I collagen and aggrecan which are components of collagen. In RA, the synovial fluid taken has been found to have increased level of MMP-2 and MMP-9. MMP-9 which is derived from neutrophils and macrophages is believed to play crucial role on movement of these cells when inflammation occurs.
Also, more recent studies have shown that MMPs plays an important role in suppressing inflammation by biologically degrade active molecules like GFR and cytokines. Thus, importance of enzymes in RA is debatable.[11]
A research was done to analyze the role of levels of pro-MMP-3, 8 and 9 MMPs. In alpha-2 macroglobulin complexes and how much it promotes the joint destruction. It was found that in the time of two years, pro-MMP-8 and 9 levels were decreased significantly. And alpha-2M/MMP complex levels were lowered in the patients having mild symptoms only.

- **Cyclooxygenase (COX-2)**

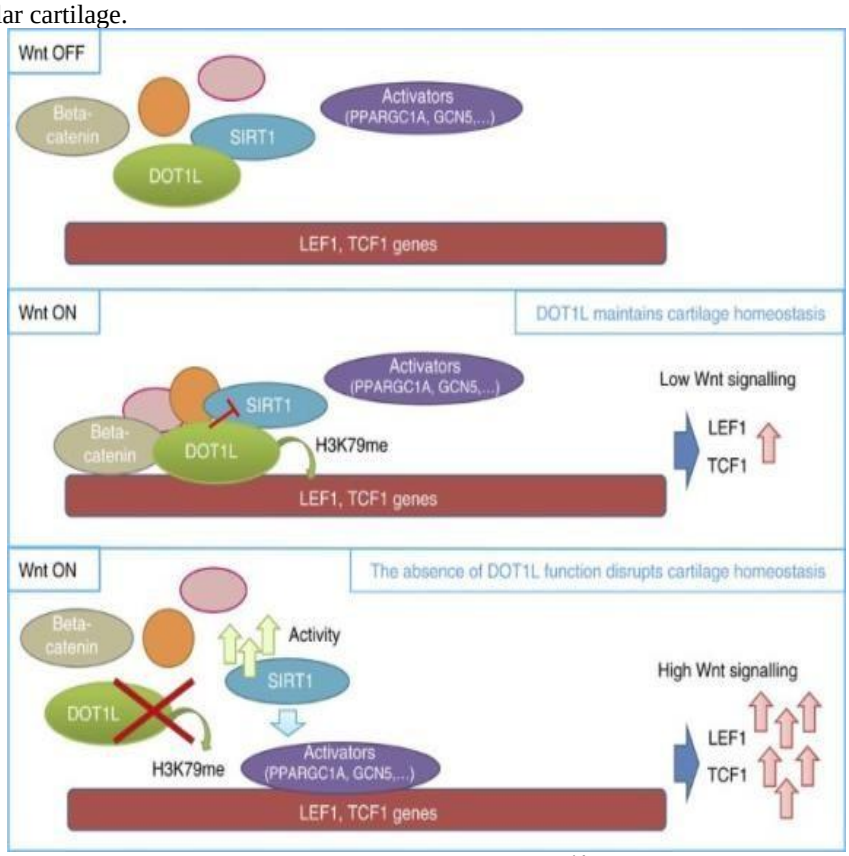
Pain is the main cause why patients consult doctors in arthritis so efficiently handling pain is the main therapeutic target in arthritis. Analgesics are usually used to treat pain but in arthritis, it becomes difficult to deal with it using analgesics. So NSAIDS (Non-Steroidal Anti Inflammatory Drugs) are used as substitute and added to the therapy of analgesics. As NSAIDS are used to deal pain, they also leave toxic effects on the patient which sometimes may result in death. So, COX-2 inhibitors can be very efficient and less toxic in treatment of arthritis. These are supposed to inhibit only COX-2 isoenzymes. A very huge amount of isoenzymes are produced during inflammation activity and produce prostaglandins biosynthetically and other inflammatory and pain sensitizers. As these inhibitors do not alter the activity of COX-1

isoenzymes so they are less toxic. So, NSAIDS provide equally efficient way to inhibit pain but also having additional upper gastrointestinal safety. These have also shown much efficiency in placebo effects.[12] Recent studies have also shown that COX-2 can be a mediator in angiogenesis and increases the effect of rheumatoid arthritis. It was observed that in the cells of RA synovium and microvascular ECs, angiogenesis mediation was doneby COX-2. Rofecoxib which is very selective COX-2 inhibitor plays an important role in altering dermal microvascular ECs of human and inhibit their chemotactic ability. Pretreatment with rofecoxib altered and inhibit the ability of forming tubes by CM of Rheumatoid Arthritis synovial fibroblasts. A 16 hour treatment from rofecoxib of synovial fibroblasts was then stimulated with IL-1beta. And CM induced comparatively less HMVEC tube production in comparison to CM from treated RA fibroblasts.[13]

- **DOT1L (3QOW)**

DOT1L is an enzyme which plays an important role in histone methylation and protects cartilage. If its production is halted or altered, it could result in the change in cartilage production or maintenance. It is basically involved in histone methylation and protects the cartilage. Loss of normal functioning of DOT1L alters the chondrocytes in vitro and can be a cause for osteoarthritis.

Articular cartilage is composed of articular chondrocytes which is involved in production of ECM made up of type-2 collagen fibers. In the studies done by Silvia[14], H2K79 methylation was altered and inhibited EPZ-5657 in chondrocytes of articular cartilage. It was also observed that DOT1L can also limit the Wnt signaling pathway so that it can maintain cartilage homeostasis. DOT1l also acts as a negative and direct regulator of Wnt genes in chondrocytes. The further in-depth study showed that silencing of the SIRT1 was blocked and upregulation of the LEF1 and TCF1 genes after the inhibition of DOT1L gene. In mice models it was also found that loss of DOT1l gene can cause severe retardation in growth of the articular cartilage.



***Role of DOT1L in cartilage*[14]**

- **Bone Morphogenetic Protein(1LXI)**

BMP signaling pathway is found to be active in arthritic cases which are collagen induced and partly TNF-alpha independent. It was observed that the blocking of TNF-alpha expression in the articular cartilage can increase the anabolic pathways. BMPs are part of TGF-beta superfamily which is structurally similar to GDF (Growth and differentiating factor). It shows pleotropic effects in many cell types like that of cell differentiation, adhesion, apoptosis, and etc. Many types of BMPs were shown inside the synovium of a rheumatoid arthritis patient but function and targets cells were not clear much. It was also observed that BMPs can show chondroprotective properties in many animal models of R A. By activating the BMP signaling pathways using P-SMADS using mouse model BMP2 is present in

only synovial lining and articular cartilage whereas BMP7 increased expression affected the joint. It was concluded from a study that expression of BMP is independent of TNF. Upregulation of BMP7 was observed after anti-TNF treatment. It usually inhibits the altering of bone destruction.
BMP plays an important role in RA. Increases level of BMP6 and 2 in joint synovium of a RA patient is related to the synoviocytes apoptosis. BMP4 and 5 were found lowered in the RA patient's synovium. Overall, after treatment the levels of BMP signaling were lowered.[15]

- **GDF-5 (Growth and differentiatingfactor-5)**

PDB id of GDF-5 is 3EVS. GDF 5 is a member of beta-family (Transforming Growth Factor). Its role is mainly in development of a joint in the embryogenic stage and has the ability to regenerate the cartilage in mature animals. During microarray analysis of the experiment done by C.P. Bramlage it was observed that the patients that were not given immunomodulating drugs, there was decrease in the levels of GDF5. Also, cell migration was lowered due to GDF-5 of macrophages. It was very clear from this study that GDF-5 which is expressed in fibroblasts of synovium may be a key component in inhibiting macrophage infiltration. Glucocorticoids were shown to be playing conflicting role if they are suppressed by inflammation and putative repair mechanism.
It can be found in mature tissues and linings of cell. The spatial temporal role pattern of GDF-5 is a proof that it plays an essential role in bone formation and cartilage. GDF-5's signaling mechanism is initiated by its binding to type 1 and 2 receptors, which further is involved in the biochemical processed.[16]

- **MMP-13(3ZXH)**

It was found that collagen MMP-13 and 9 both are crucial and plays an important roles in RA and OA. Both of these MMPs are rate limiting in the process of collagen degradation. MMP-1 is synthesized by synovial cells that are the cell lines of a joint. Also, MMP-13 is produced by chondrocytes which are present in cartilage. It was also found that MMP-13 degrades proteoglycan molecules, many aggrecans, further destructing the matrix of joints. Other MMPs like MMP2, 3 and 9 are found to be elevated in cartilage degradation and they also degrade non-collagen parts or components of a joint. If the effective inhibitors can be designed to inhibit MMP activity that can cure connective tissues destruction of the joints. Many biomarkers which can relate to the articular cartilage degradation in early days of OA includes many enzymes that can degrade matrixes like MMPs and metalloproteins, thrombospondin type-1 motifs, etc. among these, MMP13 is found to be over expressed in joints of an OA patient. Its role is also to degrade ECM (extracellular matrix). Also, OA increase seems to be inhibited by the MMP-13 knockout mice by shielding the cartilage from the effects of proteoglycan. One more type of protein which interacts with MMP-13 by activating the hidden form of it. Pro-MMP-activatorsAs there are 24 types of genes in MMP family and from them, 23 types of proteins which are structurally similar to zinc-dependent endopeptidases which is responsible for degrading parts of ECM and basement membrane.[17]

- **Fibroblast Growth Factor 18(FGF-18)**

FOG is a member of family FGF. Among them, FGF18 is supposed to initiate proliferation in mouse model osteoblasts. In the following studies it was observed that FGF-18 induced doses to increase the thickness of cartilage in the plateau of tibia as a result of new cartilage initiation at articular surface and periphery of joint.
FGF18 plays a crucial role in skeletal growth and the development of body. In mice models it was seen that mice that lack FGF19 were showing malformations in bones and cartilage. The closure of calvarials was reported to be delay and increase in size of proliferation and zones of hypertrophism in the long growth bones growth and development. FGF also reported to activate the IIIc variant of FGFR2 and 3 also receptors that plays major roles in cartilage and bones. It also negatively regulates differentiation and proliferation of chondrocytes. Also, efficacy was taken into consideration whether it can play an important role in cartilage recovery or not. It was observed and taken onto consideration by intra articular injection from the start of 21sst day after making meniscal tear. FGF18 can induce an increased and dose dependent scores in medial tibia plateau. So, we can say that it is an anabolic agent which can show to facilitate repair of cartilage in animal in vivo model.[18]

- **15-Lipoxygenase**

LOX is an enzyme family which is involved in incorporating oxygen at different positions inside the unsaturated FA (Fatty acids). PDB id of 15-lypoxigenase is 4NRE. It plays an important role in multiple pathological processes. It is well known to cause anti-inflammatory functions and properties. From the mentioned study it was observed that 15-LOX 1 and 2 are expressed in articular chondrocytes. There are 3 main type of LOXs named on the basis of carbon position of the arachidonic acid oxygenation i.e. 5, 12 and 15-LOX.
15-LOX along-with its metabolites can exhibit anti-inflammatory actions. But, it is less known about its action or role in human cartilage or bone but may play a critical role in inhibiting the destruction of cartilage and joints. The treatment with 13-HODE and 15-HETE which are main products of 15-lypoxigenase 1 and 2, can suppress and induce the expression of IL-1Beta and MMP-1 and 13 respectively. It can be concluded from above studies that these can be chondroprotective and negatively regulate MMP-1 and 13 expressions. It was also shown that 15-LOX metabolites

were showing anti-inflammatory activities. There are many factors controlling its expression like IL-4 and IL-13 which can increase it. Histone acetylation can increase its expression and DNA methylation can decrease.[19]

- **Frizzled Related Protein(FRZB)**

Study was done to observe whether lacking FRZB i.e. frizzled related protein, an antagonist of Wnt pathway, can alter degradation of cartilage by expressing MMPs from the chondrocytes. Things which were observed in these studies were IL-1-beta orientated MMP induction is altered and increased in FRZB chondrocytes. IL-1-beta increased and load-initiated catabolism FRZB chondrocytes with canonical Wnt/beta-catenin signalling. Concluding the experiment we can say that responsiveness is enhanced to mechanical stress when FRZB is not present and involvement of canonical wnt/beta signalling pathway. Basically due to cracks in matrix molecules causes cartilage breakdown in response to mechanical stress and causing inflammation of some degree. Our outcomes propose that FrzB protectively affects MMP enlistment in mouse chondrocytes. The outcomes show the double part of the Vnt signal in ligament homeostasis, so a controlled measure of the Wnt flagging framework is needed to keep up articular ligament, yet the abundance is hurtful. Further examination is expected to decipher the severe control of the Wnt flagging framework in OA, particularly the separation of London OA cells into hypertrophy.

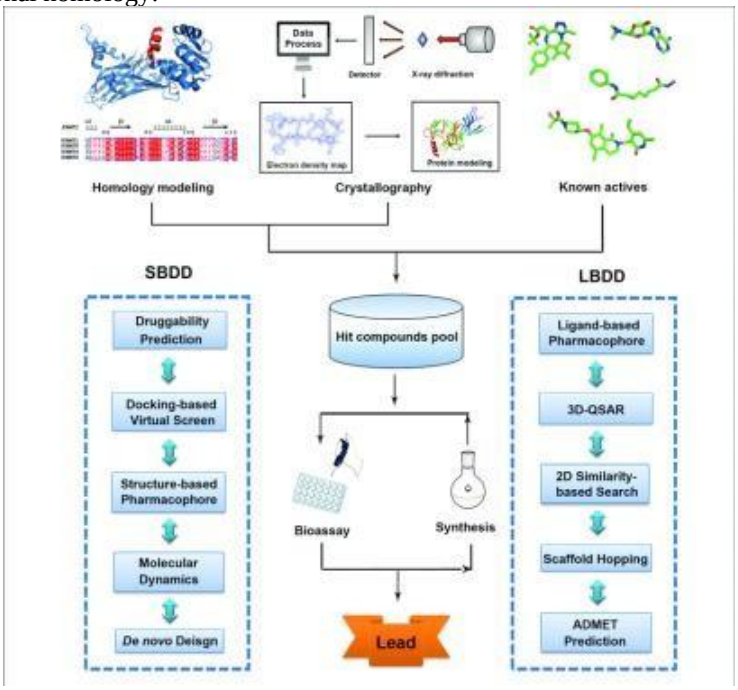
Our outcomes add proof that the Vnt/β-catenin administrative pathway is essential for the systems prompting exorbitant ligament corruption catabolism in OA. In any case, the FrzB-subordinate boundary we noticed may incorporate both normal and unusual Wnt signal, on the grounds that FrzB is a bidirectional inhibitor. Little is thought about the part of the strange Wnt pathway in the improvement of articular ligament homeostasis. Late information propose that overabundance Wnt-5a may animate development of the develop ligament framework sporadically, while adding to the ordinary separation of ligament advancement.[20]

Molecular Docking

Sub-atomic combination, structure-based virtual examining (SBVS), Molecular elements (MD) is quite possibly the most generally utilized SBDD techniques because of its far reaching use Examination of sub-atomic acknowledgment occasions like restricting energy, sub-atomic interactions, and so on. Due to conformational changes, a reasonable way to deal with drug configuration includes the utilization of a bioactive specialist little sub-atomic libraries is finished. It addresses the exceptional substance variety of these libraries.

Structure-Based Drug Design (SBDD)

Understanding the standards by which atomic ligands perceive and collaborate with each other. Macromolecules are significant in the improvement of drug research (R&D). SBDD alludes to the efficient utilization of primary information (for instance, macromolecular targets called receptors), which are generally acquired by displaying exploratory or computational homology.



Workflow of SBDD

After determination of the ligand-receptor complex, the data on biological activity are correlated with the data of structural information. Thus, the SBDD process begins with new steps to include them. Molecular modification with the potential to increase the interaction of the new molecular ligands that connect to each other.

Flexibility of target receptors is an important aspect to consider during modeling otherwise many changes could occur and alter the results.

Molecular docking is very frequently used method while performing SBDD as it is able to predict the conformation with higher degree of accuracy. Its first algorithm was developed in 1980s and it became an essential tool in the procedure of drug designing and discovery. There are basically two steps needed for the conformations i.e. 1. Find potential binding sites by searching conformational spaces around the molecule. 2. Predicting the interaction energies precisely and binding conformations.

Conformational search

Many structural parameters of ligand like of torsional and rotational degrees of rotations are modified and staged. Many systematic and structural algorithms are used to finish this task to find the conformational search stage. After multiple search and cycles, it finds the minimum energy model for the further binding analysis. It is also capable in finding the local and global minima. This further ensures that the result does not get stuck in the local minima. It basically uses genetic algorithm and is successful in docking software like AutoDock and Gold.

As an initial step, the calculation scrambles everything underlying boundaries of the underlying construction on the chromosome, addressed by a vector. The beginning of an irregular inquiry calculation from this chromosome creates an underlying populace of chromosomes covering a wide space of the energy scene. It is assessed that this populace is more adjusted. Chromosomes (i.e., those with the most reduced energy esteems) were chosen as tests for age the following populace. This method lessens the normal chromosome energy, which is characterized as moving ideal primary highlights starting with one populace then onto the next, in this way lessening it considered the space of conformation. After a sensible, GA mode is switched and number of patterns of conformational search assessment measures joined into a conformity (chromosome) as per the world's base energy.

Virtual Screening

The most suitable compounds are selected form the desired database computationally and evaluated further. These are further divided into two types: 1. Ligand based virtual screening and 2) Structure Based Virtual Screening LBVS depends on the investigation of sub-atomic descriptors made out of known mixtures dynamic. When all is said in done, a bunch of common qualities of an unpredictable arrangement is resolved, and these are later utilized as a sub-atomic channel. These data set separating strategies are utilized to choose compounds for trial assessment and diminish the synthetic territory to be concentrated in additional periods of testing.

A few uninhibitedly accessible programming bundles precisely gauge atomic descriptors. These projects are helpful for foreseeing potential properties of natural movement, for example, dissolvability it is the atomic volume in the condition of protonation.

As a rule, there are a few designs of this receptor. In the event that an objective is accessible with Apo and holo-enzymes, both ought to be considered in the SBVS system. Conformational changes, because of the communication with the bonding molecule, the underlying arrangement is a significant detail that requires thought of the decision of the most proper design. Then, the picked structure goes through a few sub-atomic bend study systems to appropriately plan. So, the cooking strategy comprises of adding hydrogen particles, eliminating water atoms (with the exception of) potential communications of go between, confirmation of conditions for right protonation and tautomerization of buildups of the association point by figuring incomplete charges. Another significant advance is to make a bundle of little sub-atomic mixtures. SBVS broadly utilizes information bases that gather in one spot, countless compound providers, and an enormous assortment of substance information. These intricate assortments commonly go about asan intelligent interface for predefined substance channels to look for complex subgroups. Information base connections are generally put away as straight records like III SM, SMART and InChI, which are then changed into three- dimensional atomic constructions. Changing over the first records requires an exact meaning of stereochemistry, the condition of ionization of the fractionalcharge.

KSAR examination is a ligand-based computation

System for depicting the quantitative connection between complex organic movement ы its physio-compound properties or on the other hand underlying attributes, which is a serious step forward of the period for the plan of normal medications. Since the remedial worth of HDAC is has been prepared throughout the long term, exceptionally solid HDACs have been distinguished and unmistakably far reaching KSAR considers have been directed utilizing diverse information types. In 2004, we created KSAR Models dependent on HDAC is dependent on hydrochloric corrosive were found genuinely critical connection of charge appropriation ы hydrophobicity, mathematical state of a compound and its relativity Contraception for PC-3 cell lines. From that point forward, the quantity of KSAR demonstrating considers has expanded emotional speed and main KSAR study utilized for virtual screening has been accounted for

Tang ل accomplices. In view of the confirmed KSAR models, the creators inspected the library.
About 9.5 million mixtures were found for four to four new frameworks that chose to boycott HDAC. In 2012, Xiang ل and his associates built up a drug store 3D-KSAR models on imidazole inhibitors. The outcomes prompted the revelation of 27 inhibitors associated with repressing HDAC2 action. Accordingly, a few gatherings utilized KSAR work process displaying to anticipate the suitability and selectivity of HDAC is. In 2014, Kandakatla et al. directed ligand-based 3D-KSAR drug displaying, distinguished eight stun compounds from Maibridge and NCI data sets as potential HDAC2 inhibitors. Atomic docking programs use assessment capacities to appraise the limiting energy of anticipated ligand-receptor intricacies. Energy motions because of the arrangement of the ligand-receptor structure gives the limiting consistent (Kd) Free Gibbs free energy (ΔGL). Obligatory energy estimate is made considering the most significant physico-synthetic marvels Ligand-receptor restricting, including intermolecular cooperations, processing endropic impacts. Thusly, the higher the quantity of assessed physicochemical boundaries, the higher the exactness of the assessment works. Notwithstanding, the assessed esteem expansions in relation to the quantity of factors engaged with the capacity, which is an inconvenience that diminishes the efficiency of the dosing calculation. Preferably, productive assessment capacities should offer a harmony between "speed" precision, which is a significant perspective when working with huge chain chains. Profit capacities are characterized into the accompanying three gatherings: experimental, information based electric field-based capacities. The power based assessment capacities gauge the limiting energy by adding the commitments of the connected (tractable bowing, precise twisting, bipolar change) and random conditions (electrostatic van der Waals cooperations) to the absolute center capacity. This sort of assessment work utilizes the stomach muscle initio strategy to ascertain the energy related with every part.
work utilizing the conditions of traditional mechanics. The primary restriction of power based techniques is incorrectness in assessing atropic ventures. This weakness is because of the absence of a sensible actual model to portray this wonder. Besides, the dissolvable is clearly not noticed, which makes it hard to assess the retention energy. Observational assessment capacities are another sort of assessment technique. Each capacity of the term portrays one kind of actual occasion that is associated with the arrangement of a ligand-receptor complex. These incorporate hydrogen holding, ionic-hypolar connections, just as desalination and endropic impacts. The initial phase in the advancement of experimental capacity since 2015, 20 13391 particles are through known protein-ligand edifices The obligatory relationship is utilized as a preparation set to play out different direct relapse breaks down. At that point, the weight constants created by the measurable model are utilized as coefficients that manage the conditions. Equity the detriment of exact assessment capacities is their reliance on the exactness of the information used to build up the model. In any case, because of the effortlessness of the energy terms utilized, exact capacities are quicker than power based techniques. Surflex and FlexX are broadly utilized in atomic docking applications utilizing experimental assessment capacities. A third methodology used to appraise ligand receptor restricting energy is information based evaluation capacities. The strategy utilizes two fold energy possibilities got from known ligand-receptor buildings to acquire an overall capacity. These possibilities are based on the discovery recurrence of two distinct particles at a given distance in an underlying data set. The kinds of collaborations saw in the information base are ordered and weighted by their recurrence. The last grade is given as the amount of these individual cooperations. Since information put together capacities don't depend with respect to compulsory connection (exact techniques) or fundamental estimations (power field strategies), they offer the correct harmony between exactness, speed, etc. Each acquiring capacity has its ideals and impediments. Subsequently, the synchronous utilization of various techniques of the unit is progressively utilized as a way to accomplish concurred results. This can be useful in light of the fact that it consolidates the benefits and limits the hindrances every strategy. Instances of understanding assessment capacities are MultiScore, X-Cscore, GFscore, SCS, SeleX-CS and CONSENSUS-DOCK [65–70]. Most port projects can effectively foresee association arrangements at the objective area, which can be affirmed by contrasting the anticipated edifices and the relating crystallographic information. Nonetheless, most projects don't replicate the supreme energy of the ligand-receptor complex connection with adequate understanding. Debasement and endropic impacts, like corruption and endropic impacts, are instances of the test of conquering current port calculations. Malignancy, diabetes and irresistible, cardiovascular, gastrointestinal-neurological sicknesses. Ongoing reports propose that around 33% of the chemical modulators presently sold are covalent. Inhibitors. Covalent ligands work irreversibly to prohibit their objectives. thusly, rebuilding of repressed natural capacity includes re-amalgamation of the objective protein. Typically covalent Inhibitors are profoundly connected with their atomic targets, prompting a delayed pharmacological reaction and thus requiring less incessant use. Known weaknesses of covalent medications, like harmfulness, absence of explicitness,and high reactivity, have driven most innovative work projects to keep away from such mixtures. This idea has been modified and it has as of late been declared that interest in covalent inhibitors has expanded. Therefore, different systems have been created to move toward the limiting of covalent little atom inhibitors. Covalent landing calculations plan to contemplate the energy scene accessible to the alliance when it is covalently bound to the receptor, just as to appraise the limiting energy of the collaboration. Regardless of the new enactment of covalent medications, Molecular displaying strategies created to tackle covalent dosing issues are not as cutting edge as those for noncovalent dosing.
Restricting of covalent medications has a few contrasts with noncovalent atomic connections, particularly comparable to the thermodynamics of restricting. Current atomic mechanics (MM) calculations can precisely anticipate noncovalent

restricting occasions. In any case, the development of covalent bonds didn't sufficiently move toward these techniques. The issue of making a covalent bond can be appropriately tackled by quantum-mechanical techniques (KM) fit for considering the entire reaction component. Adaptability of the objective site is conceivable, however is frequently dismissed because of sub-atomic curve contemplations. During the time spent sub-atomic acknowledgment, chemical receptors can go through conformational changes. Sometimes, these underlying adjustments are little. The association is situated at a junction with little portability. Something else, a few proteins go through huge conformational changes that may incorporate components of auxiliary construction. Such issues with Fleck can be settled utilizing procedures like MD. Correspondence typically settles a subset of a few potential receptor changes by migrating them balance towards insignificant energy structures. In such cases, MD recreations can create elective conformational states relating to those ligand-guided designs. When not? There are precious stone designs that are appropriate for a specific atomic objective (i.e., structures with blocked off or inadequately characterized restricting destinations), and MD can be utilized to frame various reasonable circular segment structures. As needs be, potential conformational states are inspected dependent on accessible crystallographic information, in view of reenactments. MD can be additionally used to survey the soundness of the proposed sub-atomic docking ligand-receptor complex. At the point when the MD-initiated ligand compliance strays more than the given RMSD esteem from the relating port arrangement, the anticipated ligand-receptor complex can be considered flimsy. Sub-atomic elements utilizes Newtonian conditions of movement, as portrayed in old style mechanics, to decide the position and speed of every iota in every framework considered. Accordingly, the way of J. The transient advancement of the ligand-receptor complex can be contemplated. At first, molecules are given a unique construction to imitate the temperature and pressing factor of the genuine framework. From the estimation of the powers following up on every molecule, it is feasible to decide the position and speed of every one of these iotas. These estimations are played out a few times until the sub-atomic pathways are incorporated throughout some undefined time frame. The powers following up on the framework are dictated by the atomic communication possibilities, which are normally defined by quantum compound computations or exploratory information. This arrangement of boundaries (power field) decides the commitment of each sort of association to the general capacity. STER, AR ARM and GROMOS can be recognized among the different accessible power fields since they are broadly utilized in displaying sub-atomicelements.

Methodology

Ligand and Target Protein Selection

A systematic approach was adopted to identify potential therapeutic targets for arthritis. Various plants with reported anti-arthritic properties were reviewed, leading to the selection of Boswellia Serrata due to its oleo gum resin's rich content of diterpenoids, tripernoids, and boswellic acids. These compounds were identified from existing literature, and their chemical structures were retrieved from online chemical databases like PubChem[5]

Protein targets involved in arthritis pathogenesis, including osteoarthritis (OA) and rheumatoid arthritis (RA), were selected based on their biological relevance. Target proteins such as Interleukin-6 (IL-6, PDB ID: 1ALU), Matrix Metalloproteinase-9 (MMP-9, PDB ID: 1L6J), and Frizzled-Related Protein (FRZB, modeled using homology modeling) were included. Protein structures were retrieved from the Protein Data Bank (PDB) with a focus on high-resolution, non-mutated structures[6]

Ligand Characterization

The chemical structures of boswellic acids and related compounds were extracted from the literature and chemical databases. Ligands were characterized based on:

- Molecular weight
- Hydrogen bond donors (HBD)
- Hydrogen bond acceptors (HBA)
- Protonation states
- LogP values

These parameters were assessed using Discovery Studio to confirm that the ligands adhered to Lipinski's Rule of Five for drug-likeness. A virtual compound library of Boswellia Serrata derivatives was created for subsequent docking studies.[8]

Protein Target Selection and Classification

Target proteins involved in osteoarthritis (OA) and rheumatoid arthritis (RA) were selected from the Protein Data Bank (PDB) and UniProt. Proteins were chosen based on their relevance to arthritis pathogenesis and their involvement in inflammatory pathways. A summary table was created, including:

- Gene names
- PDB IDs

- Functional descriptions
- Protein classification

Proteins with the best resolution and non-mutated structures were prioritized. However, for FRZB (Frizzled-Related Protein), no structural data was available, so homology modeling was employed.

Homology Modeling
Homology modeling was performed for FRZB using Modeller[9]:

1. Template Selection and Initial Alignment:
   - Templates were identified using BLAST and PSI-BLAST against the PDB database.
   - The sequence alignment showed 96% similarity with Mus musculus (PDB ID: 1IJX). The template was chosen based on the E-value and sequence coverage. [7]

2. Backbone Generation:
   - The protein backbone was constructed using Modeller's automated pipeline. Templates were used to predict missing segments in the protein structure.

3. Loop Modeling:
   - Conformational changes in loop regions were addressed using Monte Carlo simulations and molecular dynamics (MD). Loops were refined for optimal stability.
   - Surface rings and helices were adjusted to align with the Ramachandran plot.

4. Side Chain Modeling:
   - Side chain orientations were modeled using rotamer libraries. Hydrophobic core residues were optimized, while surface residues were refined for minimal steric clashes.

5. Model Validation:
   - The stereochemical quality of the models was assessed using Ramachandran plots, ensuring >95% of residues were in allowed regions.
   - Energy minimization was conducted to reduce structural errors and improve model accuracy.

Protein Pre-Processing
Proteins were pre-processed using Chimera and Discovery Studio. Steps included[10]:
1. Removal of Unnecessary Components:
   - Water molecules, metal ions (e.g., calcium and iron), and other crystallization agents (e.g., glycerol, sulfate ions) were removed.
   - Ligands co-crystallized with proteins were retained for binding site prediction.

2. Hydrogen Addition and Charge Assignment:
   - Hydrogens were added to protein structures based on protonation states using the Amber ff99SB force field.
   - Gasteiger charges were assigned to residues to prepare proteins for docking.

3. Binding Site Identification:
   - Active sites were identified using Discovery Studio and validated using structural data from the PDB.

4. Validation with Ramachandran Plot:
   - Ramachandran plots were used to assess the stereochemical quality of the protein structures. Proteins with <95% of residues in allowed regions underwent energy minimization using Discovery Studio.

Ligand Pre-Processing
Ligands derived from Boswellia serrata were processed using the following steps:

1. Structure Retrieval:
   - Chemical structures of boswellic acids, diterpenoids, and tripernoids were obtained from PubChem and other literature sources.

2. Structure Optimization:
   - Ligand structures were energy-minimized using the steepest descent algorithm with 400-500 steps to achieve a

stable conformation.
- Functional groups and stereochemical properties were verified and adjusted as needed.

3. Format Conversion:
- Ligand structures were saved in .mol2 format, which retains molecular charge information required for docking.

4. Validation of Ligands:
- The ligands were evaluated for their drug-likeness using Lipinski's Rule of Five and other chemical descriptors, including:
  - Molecular weight <500 Da
  - HBD ≤5
  - HBA ≤10
  - LogP ≤5

Molecular Docking Protocol
Molecular docking was performed using AutoDock v4.2 to evaluate the interaction of Boswellia serrata compounds with target proteins.

1. Docking Setup:
- The docking protocol was based on a structure-based drug design (SBDD) approach.
- A grid box was defined around the active site of each protein. Grid dimensions were set to 60 x 60 x 60 $Å^3$, centered on the binding pocket residues.

2. Docking Parameters:
- The genetic algorithm was used for docking with the following settings:
- Population size: 150
- Maximum number of energy evaluations: 2,500,000
- Number of docking runs per ligand: 10

3. Docking Execution:
- Ligands were docked into the active sites of the proteins, and binding poses were scored based on Gibbs free energy (ΔG). Lower docking scores indicated stronger binding affinities.

4. Post-Docking Analysis:
- The top-scoring poses were analyzed for hydrogen bonding, hydrophobic interactions, and other molecular interactions using Discovery Studio.

Validation of Docking Results
Docking results were validated to ensure reliability and accuracy:

1. Re-Docking of Co-Crystallized Ligands:
- To confirm the docking protocol's accuracy, co-crystallized ligands were re-docked into their respective proteins, and RMSD values were calculated. RMSD <2.0 Å indicated successful re-docking.

2.Energy Minimization of Complexes:
- Protein-ligand complexes were energy-minimized to resolve steric clashes and refine binding poses.

3. Binding Site Consistency:
- Binding interactions were compared with known active site residues reported in the literature to confirm consistency.

Energy Minimization
Energy minimization is a critical step to refine the structure of both proteins and ligands, ensuring that they are in a stable conformation before docking. It reduces steric clashes and optimizes bond lengths, angles, and torsions.

Protein Energy Minimization
1. Preliminary Preparation:
- Protein structures retrieved from the Protein Data Bank (PDB) often contain crystallization artifacts such as water molecules, metal ions, and non-standard residues like glycerol (GOL) or sulfate ions. These extraneous components

were removed using Chimera.
- Hydrogens were added to the protein structures to stabilize protonation states based on physiological pH (7.4).
- Amber ff99SB, a widely used force field for proteins, was applied to assign atomic charges and parameterize the structure. [7]

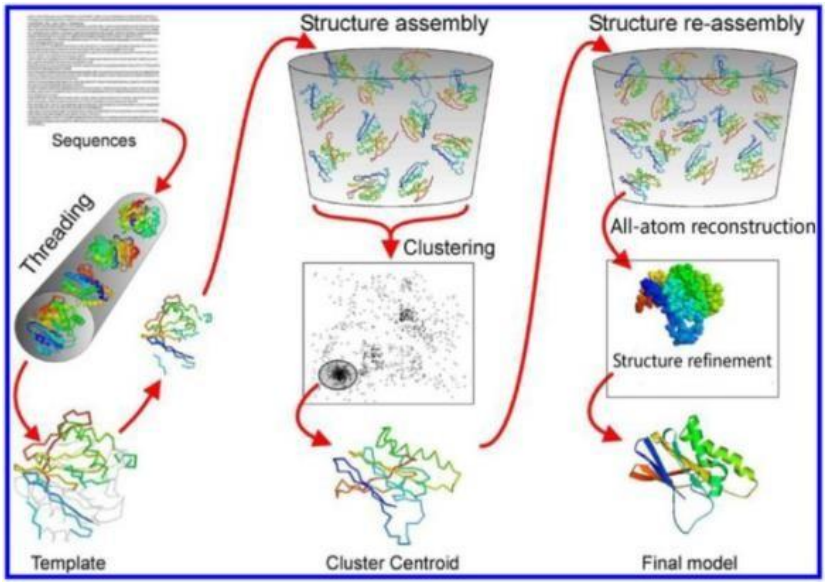
2. Minimization Process:
- Energy minimization was performed using the steepest descent algorithm followed by conjugate gradient steps in Discovery Studio.
- The steepest descent algorithm corrected major steric clashes during the initial 400-500 iterations. Subsequently, the conjugate gradient algorithm refined the structure to convergence by reducing the energy gradient to near zero.

3. Evaluation of Results:
- Post-minimization, the structural quality of the proteins was validated using Ramachandran plots. Residues in disallowed regions were carefully reviewed, and further minimization was performed if >5% of residues fell outside allowed regions.
- Minimization ensured a balance between electrostatic and van der Waals interactions, leading to a more reliable protein conformation for docking.

Ligand Energy Minimization
1. Ligand Optimization:
- Ligands retrieved from PubChem or other databases were initially processed to confirm stereochemistry and bond assignments.
- Hydrogen atoms were added, and charges were assigned using the Gasteiger method, which is compatible with most docking programs.

2.Algorithm Application:
- Ligands were minimized using the steepest descent method in Discovery Studio to resolve steric clashes and optimize molecular geometry.
- Iterations were stopped when the Root Mean Square Gradient (RMSG) reached <0.01 kcal/mol/Å², indicating convergence to a local energy minimum.

3. Validation:
- The minimized ligands were visually inspected for irregularities in bond lengths, angles, or torsions.
- Any outliers were corrected manually or re-minimized for consistency.



Grid Box Generation
The grid box defines the docking search space by enclosing the binding site of the target protein. Accurate grid box dimensions are essential to ensure proper sampling of ligand conformations.

Identification of Binding Site

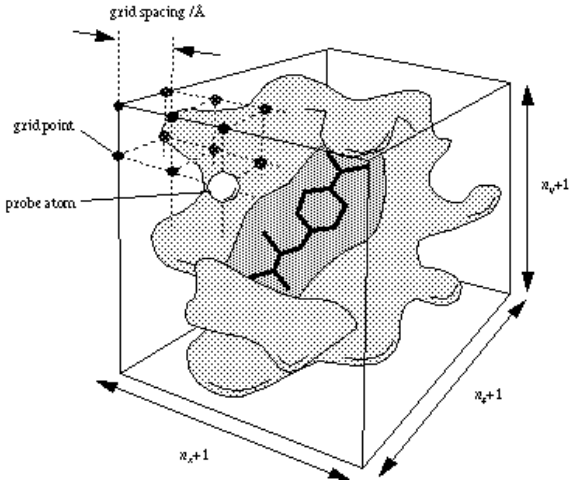
1. Active Site Localization:
   - Known binding sites were identified from co-crystallized ligands or structural data in the PDB files.
   - If no co-crystallized ligands were available, active sites were predicted using Discovery Studio based on pocket volume and residue interactions.

2. Definition of Center Coordinates:
   - The geometric center of the binding pocket was calculated using the coordinates of key active site residues.
   - For example, if a ligand interacted with residues Asp189, Ser195, and His57, their Cartesian coordinates were averaged to define the center.

Grid Dimensions Setup

1. Configuration:
   - The grid box was manually configured using a text editor (xyz.config file) or AutoGrid 3.0. Key parameters included[7]:
   - *Center Coordinates (x, y, z):* Defined based on active site residues.
   - *Dimensions (Length x Width x Height):* Typically set to 60 x 60 x 60 Å³ to encompass the binding pocket and nearby residues, ensuring sufficient space for ligand flexibility.

2. Energy Maps Calculation:
   - AutoGrid calculated the atomic contributions of each grid point based on electrostatic, van der Waals, and hydrogen bonding energies.
   - Atomic-specific affinity maps were generated for carbon, oxygen, nitrogen, and hydrogen atoms to model ligand-protein interactions accurately.



Validation of Grid Box

1. Visual Inspection:
   - The grid box was visualized in Auto Dock Tools to ensure proper alignment with the binding pocket.
   - Adjustments were made if key residues or the ligand's active groups were outside the grid boundaries.

2. Consistency Check:
   - The grid box setup was validated by re-docking co-crystallized ligands into the same binding site. RMSD values <2.0 Å confirmed the box's reliability.

3. Hydration and Stability:
   - The impact of the solvent environment was considered by ensuring that key residues forming hydrogen bonds or ionic interactions were included within the box.

Structure-Based Drug Design (SBDD) Approach

A structure-based drug design (SBDD) workflow was implemented to predict potential inhibitors of arthritis-related target proteins. The process involved the following steps[11]:

1. Conformational Search
- Ligand conformations were explored using systematic and stochastic algorithms to identify low-energy configurations.
- Tools such as AutoDock were employed to perform a conformational search, ensuring the ligand's flexibility during docking.

2. Virtual Screening:
- A virtual compound library of Boswellia serrata compounds was screened against selected target proteins.
- Ligand-based virtual screening (LBVS) and structure-based virtual screening (SBVS) methods were used to refine the list of potential candidates.
- LBVS involved analyzing molecular descriptors, while SBVS was based on docking scores and binding affinities.

3. Binding Energy Estimation:
- Binding energies were calculated to estimate the strength of interactions between ligands and proteins.
- Scoring functions accounted for intermolecular forces, entropic effects, and solvent interactions.

4. Energy Functions:
- Empirical, force-field, and knowledge-based scoring functions were used to predict ligand-receptor binding energy.
- Evaluations included hydrogen bonding, van der Waals interactions, and electrostatic forces.[ ]

Homology Modeling Validation and Optimization
1. Validation of Homology Models:
- Homology models were validated using Ramachandran plots to ensure structural reliability.
- >95% of residues in allowed regions were set as the threshold for model acceptance.

2. Loop Refinement and Energy Reduction:
- Loops with significant conformational variations were optimized using Monte Carlo simulations.
- Energy functions were applied to find the most stable conformations.

3. Side Chain Orientation:
- Rotamers were adjusted to optimize side chain orientations using a rotamer library derived from high-resolution crystal structures.
- Hydrophobic and electrostatic interactions were minimized to improve accuracy.

4. 3D Distribution and Energy Minimization:
- Energy calculations ensured that bond lengths and angles remained within normal ranges.
- Deviations were corrected to reduce potential model errors.

Pre-Processing Steps for Docking
1. Protein and Ligand Preparation:
- Tools in Discovery Studio were used to add hydrogens and assign charges to proteins.
- Ligands were processed by calculating protonation states and optimizing molecular geometry.

2. Ramachandran Plot Analysis:
- Proteins were validated using Ramachandran plots. Structures with a high percentage of residues in disallowed regions underwent further refinement.

3. Active Site Identification:
- Binding sites were predicted using tools in Discovery Studio. Known co-crystallized ligands or structural residues identified in the literature guided active site localization.

4. Grid Configuration:
- Grid dimensions for docking were centered on active site residues. Coordinates for the grid box were manually defined, and boundary limits ensured that the entire binding pocket was encompassed.

Docking Procedure
1. Binding Site Exploration:
- Discovery Studio identified conformational spaces and predicted potential binding sites.
- Essential ions like calcium and other crystallization components (e.g., glycerol and sulfate) were excluded.

2. Docking Execution:
- AutoDock v4.2 performed the docking simulation.
- Multiple iterations ensured reproducibility, with a focus on identifying the best ligand-protein interactions.

3. Analysis of Docking Results:
- Top docking poses were selected based on binding scores.
- Hydrogen bonding, hydrophobic interactions, and π-π stacking were visualized using Discovery Studio.

4. Comparative Analysis:
- Ligand interactions were compared across multiple targets to identify the most promising candidates for further in vitro and in vivo validation.

Result

Docking Results

Molecular docking was performed to evaluate the binding affinities of Boswellic acid derivatives with key target proteins involved in the pathogenesis of arthritis, specifically Rheumatoid Arthritis (RA) and Osteoarthritis (OA). The docking results, summarized in Table 1, include docking scores (binding affinities) for the top-scoring compounds against the selected target proteins.

Protein: IL-6 (PDB ID: 1ALU)

The docking results for IL-6 revealed a binding energy of *-6.3 kcal/mol*, indicating moderate binding affinity between Boswellic acid derivatives and the protein. Eight active torsions were identified in the ligand, with key binding interactions observed between the following atoms:

1. O_3 and C_16
2. O_4 and C_20
3. C_6 and C_13
4. C_8 and C_15
5. C_11 and C_17
6. C_13 and C_18
7. C_13 and C_19
8. C_24 and C_25

These interactions suggest that the Boswellic acid derivatives may inhibit IL-6-mediated inflammatory pathways in arthritis.

Protein: MMP-9 (PDB ID: 1L6J)

For MMP-9, the docking analysis yielded a higher binding energy of *-8.6 kcal/mol*, indicating a stronger interaction with Boswellic acid derivatives. Similar to IL-6, eight active torsions were identified, and the critical binding interactions included:

1. O_3 and C_12
2. O_4 and C_13
3. C_5 and C_10
4. C_6 and C_17
5. C_10 and C_20
6. C_10 and C_21
7. C_11 and C_19
8. C_22 and C_24

These results highlight the potential of Boswellic acid derivatives in targeting MMP-9, which is involved in cartilage degradation and extracellular matrix remodeling in arthritis.[12]

Protein-Specific Insights

1. IL-6 (1ALU): As a cytokine involved in chronic inflammation, the interaction with Boswellic acid derivatives provides insights into the inhibition of IL-6 signaling pathways. The moderate binding energy suggests that structural modifications to the derivatives may enhance their therapeutic potential.
2. MMP-9 (1L6J):The higher binding affinity suggests Boswellic acid derivatives as promising candidates for inhibiting MMP-9-mediated matrix degradation in arthritis.

Overall Docking Analysis

The docking results provide evidence for the potential therapeutic efficacy of Boswellic acid derivatives in arthritis treatment. While the docking scores indicate binding affinity, further optimization and validation through in vitro and in vivo studies are necessary to confirm these findings.

Figures and Tables

Table 1: Docking Scores and Binding Interactions of Boswellic Acid Derivatives with Target Proteins

Protein (PDBID)	Docking Score (kcal/mol)	Key Interactions
IL-6 (1ALU)	-6.3	O_3–C_16, O_4–C_20, C_6–C_13, etc.
MMP-9 (1L6J)	-8.6	O_3–C_12, O_4–C_13, C_5–C_10, etc.

Figures illustrating the docking poses and molecular interactions are provided in Supplementary Material.

Discussion

In the present study, molecular docking was employed to evaluate the interactions between Boswellia serrata derivatives and key protein targets implicated in arthritis, including Interleukin-6 (IL-6) and Matrix Metalloproteinase-9 (MMP-9). The results revealed binding energies of –6.3 kcal/mol for IL-6 and –8.6 kcal/mol for MMP-9, suggesting that these compounds can effectively interact with, and potentially inhibit, critical inflammatory and matrix-degrading processes. These findings are in line with earlier reports demonstrating that boswellic acids modulate inflammatory pathways by inhibiting cytokine production and enzymatic activity [3],[12]. Additionally, the incorporation of homology modeling for proteins lacking resolved structures (e.g., FRZB) further validates the utility of computational approaches in broadening our understanding of potential molecular targets in arthritis [9].

Despite the promising outcomes, several limitations must be acknowledged. The computational approach, while cost-effective and time-efficient, relies on static docking models that do not fully capture the dynamic nature of protein-ligand interactions under physiological conditions. Moreover, the study's limited ligand library and simplified solvent models may not completely reflect the in vivo environment, thereby necessitating further refinement [13.14]. Importantly, the lack of experimental validation—such as in vitro or in vivo assays—restricts the immediate translational potential of these findings. Future studies should incorporate molecular dynamics simulations to better understand binding kinetics and stability, expand the chemical diversity of the ligand library, and undertake experimental evaluations to substantiate the computational predictions. Such integrated methodologies have been shown to enhance the reliability of docking studies and could ultimately facilitate the development of novel anti-arthritic therapies [15],[16].

Conclusion

The study explored the therapeutic potential of Boswellia serrata oleo gum resin and its derivatives against key proteins involved in the pathogenesis of arthritis, specifically Rheumatoid Arthritis (RA) and Osteoarthritis (OA). Molecular docking studies revealed that Boswellic acid derivatives exhibit moderate to high binding affinities with target proteins such as Interleukin-6 (IL-6) and Matrix Metalloproteinase-9 (MMP-9). These interactions highlight the potential of Boswellic acids in inhibiting inflammatory pathways and cartilage degradation, which are central to arthritis progression.

While the docking scores demonstrated promising therapeutic potential, they indicate the need for structural optimization of these compounds to enhance their activity further. Additionally, the integration of homology modeling and virtual screening provided a robust framework for understanding ligand-receptor interactions, laying a foundation for future drug design.

In conclusion, this study underscores the anti-inflammatory and cartilage-protective properties of Boswellia serrata. However, further in vitro and in vivo studies are imperative to validate these findings and establish their clinical relevance in arthritis treatment.[11] Optimizing Boswellic acid derivatives could lead to the development of novel therapeutic agents capable of managing or potentially curing arthritis.

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