A DISSERTATION ON

To Identify a Potential Dual Inhibitor against IL-6R and GP-130 through Computer Aided Drug Designing

SUBMITTED TO THE
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FOR THE
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IN BIOINFORMATICS

BY

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M. Tech Bioinformatics (IV Semester)
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UNDER THE SUPERVISION OF

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DECLARATION FORM

I, **UMME AIMAN**, a student of **M. Tech Bioinformatics** (IInd Year/ IVth Sem), Integral University have completed my six months dissertation work entitled **"To Identify a Potential Dual Inhibitor against IL-6R and GP-130 through Computer Aided Drug Designing"** successfully from **Centre of Bioinformatics & Computational Biology, Biochemistry & Structural Biology Division, CSIR -Central Drug Research Institute, Lucknow** under the able guidance of **Dr. M. I. Siddiqi, Senior Principal Scientist.**

I, hereby, affirm that the work has been done by me in all aspects. I have sincerely prepared this project report and the results reported in this study are genuine and authentic.

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TO WHOMSOEVER IT MAY CONCERN

This is to certify that **Ms. UMME AIMAN** (Enrollment Number 1700100048) has carried out the research work presented in this thesis entitled "To Identify a Potential Dual Inhibitor against IL-6R and GP-130 through Computer Aided Drug Designing" for the award of M.Tech Bioinformatics from Centre of Bioinformatics & Computational Biology, Biochemistry & Structural Biology Division, CSIR – Central Drug Research Institute, Lucknow under my supervision. The thesis embodies results of original work and studies carried out by the student herself and the contents of the thesis do not form the basis for the award of any other degree to the candidate or to anybody else from this or any other University/Institution. The dissertation was a compulsory part of M.Tech Bioinformatics.

I wish her good luck and bright future.

Sincerely,

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I wish her good luck and bright future.

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TABLE CONTENTS

LIST OF ABBREVIATIONS

S.NO.	ABBREVIATIONS	FULL FORM
1.	RA	Rheumatoid Arthritis
2.	IL-6	Interleukin 6
3.	IL-6R	Interleukin 6 Receptor
4.	GP	Glycoprotein
5.	MD	Molecular Dynamics
6.	bb	backbone
7.	Conf.	Conformation
8.	RMSD	Root Mean Square Deviation
9.	RMSF	Root Mean Square Fluctuations
10.	Rg	Radius of Gyration
11.	bb	Backbone
12.	LIG	Ligand
13.	H-Bond	Hydrogen Bonds
14.	SASA	Solvent Accessible Surface Area
15.	Pro	Protein
16.	abs	Antibodies
17.	RF	Rheumatoid Factor
18.	FDA	Food & Drug Administration

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1. INTRODUCTION

Rheumatoid Arthritis is an autoimmune disease pervaded by chronic inflammation influencing the joints, bones, and cartilage leading towards the abundant degrees of osteoarthritis. Moreover, it can also lead to numerous degrees of disability. It's a diversified disease bridge number of disease subsets with likely distinct pathogenic pathways. Despite the fact that the demanding path needs to be determined yet, an interwoven progression is considered for the development of RA [McInnes IB, Schett G 2011]. Initially, the territory-gene interactions generate tolerance loss to self-antigens that involve citrulline reside developed by post-translational modification. On the other hand, the response is generated in T-cells as well as B-cells. Consequently, the determination of inflammatory responses evaluated in joints along with the synovitis is inaugurated and perpetuated by positive feedback loops and initiates systemic disorders. In this whole phenomenon, contribution towards the progression is caused by various cells with their products. A diversified disease, RA needs to be apart into distinct subsets with diverse causes and severity. Conventionally, the subsets have been disjoined on the basis of the existence and absence of auto-abs. The definitive auto-abs in RA are the Rheumatoid factor found by studies and research.

According to the research analysis, ground pathogenic markers against IgG are IgM, IgA RF. In the current scenario, most of the antibodies typically anti-citrullinated-proteins abs are used for determining the clinical subsets of disease with relevant classification in accordance with features in genetic and system risk factors. ACPA & RF markers have been found clinically useful as prognostic markers that are also used to predict more aggressive yet destructive diseases [Matthias Jarlborg, Cem Gabay 2022]. To the extent, that the progression and development of RA are not yet inferred completely, but in spite of that, there are some treatable options used for the therapy that have completely changed the forecasting of the disease. The enlightened disease stages can cause a leading path in introducing loss of functioning and dynamics. The physiological condition by further loss of strength in the immune system plays a vital role that made people concerned [Cross M, Smith E, Hoy D *et al.,* 2014].

Through survey analysis, it was detected that about 1% of the whole world population is affected by the attack of RA. Wherein, approximately 790,000 people were found affected in the Netherlands. The emergence of RA arises usually implicated between the age of 30 and 50 but might occur at any other age [Krishnan, E., Tugwell, P., & Fries, J. F. (2004)]. The percentile of women was found on hype comparatively to men and also those individuals who had lesser socioeconomic resources experienced more emergence in RA. Although the fundamental cause

for RA is still not enlightened up, accurate cures have not yet been discovered yet [McInnes IB, Schett G. 2011]. One of the main dominating risk factors for the

progression of RA is smoking, especially dominates in RF+ and ACPA+ patients. A high chance of risk conceiving of ACPA+ RA can be developed in colloidal interaction between smoking and shared epitope. Transfiguration of bronchial stress likely exposure to silica may demand a hike risk of developing RA with the individuals having susceptibility to HLA DR4 alleles. In accordance with the recent study implies that being overweight together could be a boom for the development of RA. Numerous anti-rheumatic therapeutic drugs are widely available today either DMARD drugs with broad nonspecific immune modulation effects or modern biological substances generated by gene technology that targets a particular molecule or a substance in the immune system [Smolen JS *et al.,* 2010]. Regardless of this, it's still found complicated to find the accurate yet best treatment for person to person right from the initial stage, and still, there are limited effects in approx. 30% of the patient [Guo *et al.,* 2018]. This strategy occurs due to limited knowledge of the imposed mechanism of action of each individual anti-rheumatoid drug in different clinical settings. In particular, this area of interest is moderately restricted due to the complications in scrutinizing the inflammation active site where the inflammation problem occurs. The overview of the causes of RA is given below in the figure.

1.1.The Concatenation of Rheumatoid Arthritis

RA initiates inflammation of the synovial membrane of the joints [Matteson EL *et al.,* 2021]. It usually affects in the area around small fingers, feet, and joints and is mostly bilateral. When cells produced an inappropriate amount of inflammation cells, it damages not only the body tissues but also induced discomfort. The synovial fluid then accumulates and causes swelling in joints along with thickening into the pannus. Over a period of time, the pannus diminished the joint's cartilage leaving behind scar tissue. Later on, these scar tissues within the joints get buckled and paralyzed. The nearby structures of the inflammatory joints along with the origin of muscles are often involved in supporting joint disabilities as well [Tran QH *et al.,* 2022].

The concatenation of rheumatoid arthritis clearly variates from individual to individual [Amaya *et al.,* 2013]. It initiates from ceaseless mild devolution, over an uninterrupted phase of active illness to a progressed long-time illness, disrupting joint damages and interfering with functioning loss. Moreover, disease phylogenetic pattern differs. To very distinct, people concerned with restriction in joint movements [Scott DL, Wolfe F, Huizinga TW 2010]. About 90% of the people complain and are concerned about severe pain and restricted joint movements suffering from this disease. They also own loss of functions. It also imparts mental condition.

Besides that, people often look after themselves as general practicians rarely recognize mental discomfort as fatigue complaints, appetite loss,

minimal energy, stress, and social isolation. Among them, pain and fatigue ness found to be the habitual reported symptoms in patients with RA.

1.2. Pathways Involved in Rheumatoid Arthritis

The signaling pathways and impression of inflammatory cytokines such as TNF, IL-1, IL-17 task as promotor activators of IL-6 [Ding *et al.,* 2023]. Once at the joint, IL-6 has a significant role in the inflammation process, in osteoclast-mediated bone resorption with pannus development, this process helps in the development of IgM and IgG RA factors with citrullinated antibodies [Radu, A. F. & Bungau, S. G. 2021].

Additional pathways also take place in the development of RA which can eventually proceed to downregulation of IL-6. For the occasion, the NF-kB pathway was found as a ruling inflammatory mediator in RA which leads in turn to an increment in IL-6 levels [Liu *et al.,* 2017]. The involvement of IL-6 might have occurred in the development of RA. The study has been conducted and concluded that IL-6 is found in cytokine release syndrome. Inhibiting this cytokine can be proved for a better result [Pesce B *et al.,* 2013]. It's also has been observed for other systemic symptoms related to RA, particularly in the cardiovascular system.

The uplifted serum levels of IL-6 and its receptor were traced in serum and synovial fluid. Other substitute cytokines are currently ongoingly studied further depicting the interactions between adaptive and innate immunities. Among the individuals, the ones who faced tragedy from chronic conditions, abs are prone to be at elevated risk of generating a wide range of dis informalities, focusing on depression [Satu K *et al.,* 1993]. Thus, desiring IL-6 as a target may help in improving health in RA patients [Tanaka *et al.,* 2014]. To be noted, IL-1b & IL-6 are signature proteins in driving inflammation and joint disruptions. Other cytokines involvement such as IL-4 &IL-10 have been advised for better improvemen.

With lots of pleiotropic functions, IL-6 is one of them which is a paramount pro-inflammatory cytokine. RA is an autoimmune disorder and IL-6 proved a signature player of immune activation and inflammation in RA. As a pivotal stimulator of acute-phase protein, there is a huge involvement of IL-6 in acute inflammation. Interleukin (IL)-6 is induced locally in retaliation to an inflammatory stimulus and is able to persuade systemic manifestations at a distance from the inflammation site. It comprises a unique signaling mechanism consisting of trans and cis pathways which expand various cell types responding an IL-6. The classical pathway demands membrane-bound receptor IL-6R, the binding process of this receptor is conducted by the dimerization with another signalling receptor i.e., GP-130.

The conventional approach of seeking small-molecule inhibitors proved troublesome when the investigation's team established that IL-6 signal transduction had been carried out by a hexametric high-affinity complex of IL-6, IL-6R, and glycoprotein 130 (gp130). Regardless of the previously mentioned receptor, the currently marketed medicines that suppress IL-6 have started to become less effective. Drug resistance-related infections, which enhance mortality rates, have been deemed to be the causes of this occurrence. It was still unclear what caused it, though.

Keeping this research analysis in mind, the study aimed, a computer-aided drug design approach to disclose the dual potential inhibitors for IL-6/IL-6R and IL-6/GP130 against RA.

Therefore, the objectives of the study are:

- Library preparation of anti-inflammatory molecules from different databases and literature.
- Virtual screening of collected small molecules against IL-6R and GP-130 through PyRx.
- Validation of top hits (found common against both IL-6R and GP-130) through Molecular docking & MD simulation studies.

2. REVIEW OF LITERATURE

A chronic autoimmune disease RA, was characterized by synovial inflammation, abs production such as RF, ACPA, cartilage, bone destruction, and skeletal disorders highlighting and affecting the inner cavity of the synovial joint except for surfaces of cartilage [Yap, *et al*., 2018]. It was also a heterogenous disease that takes longer steps in spanning the bundle of diseases. The very first deduction of RA was defined by Sir Alfred Garrod in 1959, nonetheless, the typical symptoms had been investigated earlier [Entezami, *et al.,* 2011].

The particular accurate pathogenesis was still found to be a suspicious secret, therefore multiple algorithms were considered for RA development. According to the population-based investigation, the report stated that approx. 0.5- 1 % of adults were in the trap of RA in major developed countries [Silman AJ *et al.,* 2002]. The disease was frequently found 2 -3x times in women compared with men. The age factor rises the prevalence and 60 – 65 years of age was found more targeted [Chauhan K *et al.,* 2023]. In wealthy countries, the incidence ranges from 5 to 50 per 100 000 adults and rises with age. The incidence of RA variation deducted by location. Compared to regions of the developing world, such as sections of the west of Africa, the disease was found more prevalent in northern Europe and North America [Costenbader *et al.,* 2008]. With later life began to accomplish, it appeared that the incidence of RA may be declining nowadays.

A new classification system, adopted by the ACR and European League Against Rheumatism (EULAR) in 2010 [Kay *et al.,* 2012], has been approved to address the requirement for earlier diagnosis and the implementation of efficient disease-suppressing therapy to prevent or decrease the incidence of the undesired sequelae. As opposed to defining the disease by its late-stage symptoms, such as rheumatoid nodules and preexisting erosions, this new categorization system redefined the present paradigm of RA by focusing on markers at earlier stages of the disease that had been linked to persistent and erosive disease [Deane *et al.,* 2020]. The 2010 ACR/EULAR criteria were intended to categorize both early and advanced illnesses mentioned below.

- Joint involvement

  More than 10 joints
- Serology (0-3)

  Negative RF and negative ACPA (0)

  Low positive RF, low positive ACPA (2)

  High positive Rf and ACPA (3)

- Acute phase reaction

  Abnormal CRP

  Duration: More than 6 weeks

Although the precise cause of the exceptionally complex genetic disease RA was still unknown, it was believed that individuals who were genetically susceptible were exposed to certain environmental risk factors [Frisell T *et al.,* 2013]. These molecular processes which foster the development of arthritis were the result of interactions between genetic and environmental risk factors [Hemminki K *et al.,* 2009].

In a recent study, it was discovered that being overweight and smoking were risk factors for getting RA in a group of people who tested positive for autoantibodies [Gregersen PK *et al.,* 1987]. Additionally, there were at present a number of anti-rheumatic remedies available, notably modern biologicals, which were partially native human substances produced through gene technology and target specific molecules in the immune system, as well as the classic disease-modifying anti-rheumatic medicines (DMARD) [Favalli EG *et al.,* 2017], which had a wide non-specific immune modulation impact. Despite this, it was still challenging to determine the appropriate course of action for each patient straight away, and only approximately 30% of patients get complete remissions and significant side effects [Selmi C, Kon E, De Santis M, *et al.,* 2018, Ajeganova, S., & Huizinga, T. (2017)]. This is partially because there has been little understanding of the precise mechanisms of action of each anti-rheumatic medication in various clinical scenarios. This kind of research was somewhat constrained, particularly in RA, because it is challenging to examine the synovium, which is the site of active inflammation [Nistala K, *et al.,* 2010, Kamimura D, *et al.,* 2014].

2.1 Clinical manifestation of RA

The majority of patients initially experience vague symptoms like weakness, fatigue, nebulous musculoskeletal pain, and weight loss; this prodrome could last for weeks or even months [Suresh E. (2004)]. Later, more specific symptoms like morning stiffness, tenderness, and swelling of joints predominated [Visser H *et al.,* 2002].

- Joint manifestation:

  The hand joints were typically afflicted in patients. The distal interphalangeal joints (DIP) were typically spared, while modifications typically affected the wrists, metacarpophalangeal (MCP), proximal interphalangeal (PIP), and metatarsophalangeal (MTP) joints of the fingers, thumbs, and toes. With prolonged inflammation, a number of joint abnormalities might appear, which was typical of chronic and established RA [Heidari B. (2011)].

These alterations were the result of pathologic processes such as muscle imbalance, cartilage loss, and the weakening and deterioration of tendons, ligaments, and tendon sheaths [Heidari B. (2010].

- Extra-articular manifestation:

  Around 40% of RA patients encountered extra-articular signs, and in 13% of those cases, these manifestations were deemed severe [Cojocaru *et al.,* 2010]. According to Sahatçiu-Meka V, Rexhepi S, Manxhuka-Kerliu S. *et al.,* smoking and, and high RF titers indicated risk factors for extraarticular involvement.

Rheumatoid nodules, which could appear whatsoever on the body (even inside organs) but were typically seen in the subcutaneous fatty tissue on pressure sites, affect about 20% of RA patients [Moore CP, Wilkins RF (1997)]. Episcleritis, scleritis, and a condition known as keratoconjunctivitis were three conditions that could affect the eyes [Promelle, V., Goeb, V., & Gueudry, J. (2021)]. Patients experiencing RA could also develop uveitis [Hamideh F *et al.,* 2001]. Patients with RA were usually reported to have generalized osteoporosis. The causes included immobility, a persistent inflammatory condition, and consistent glucocorticoid therapy effects [Shimizu T *et al.,* 2016].

2.2 Pathogenesis of RA

The pathophysiology of RA also lacked an agreed-upon etiology. Contrary to current thinking, RA was a clinical condition that was brought on by a variety of disease subgroups [Geusens P *et al.,* 2006]. These distinct groups resulted in several inflammatory cascades, all of which ultimately contributed to synovitis and the destruction of bone and cartilage [Hirano T *et al.,* 2019]. Ten genetically vulnerable people experienced the activation of T cells due to an unknown environmental stimulus. The interaction between B-lymphocytes and the activated T-lymphocytes resulted in the generation of autoantibodies. TNF alpha, interleukin (IL) 6, and IL 1 were among the cytokines that excessively produced and overexpressed as a result of interactions between activated T cells and macrophages. These cytokines started the growth of fibroblasts and caused inflammatory cells to invade the membrane of the synovial sac [Lubberts E, van den Berg WB (2013)].

Genetically vulnerable people experienced the activation of T cells due to an unknown environmental stimulus [Brzustewicz E., Bryl E (2015)]. Both checkpoints B-cell and T-cell tend to get disrupted in RA patients, which caused an excessive generation of autoreactive mature clueless B-cells. According to a prior study, untreated RA patients had 3.4 times as many autoreactive B-cells in their peripheral blood as healthy individuals [Manca M.L *et al.,* 2017].

A pleiotropic pro-inflammatory cytokine known as interleukin (IL-) 6, RA, and associated comorbidities were significantly influenced by interleukin (IL-) 6.A glycoprotein with a 26 kDa molecular weight and pleiotropic action, IL-6. It was first recognized as B cell differentiation factor (BCDF) or B cell stimulatory factor 2 (BSF-2), a soluble portion generated from T cells that aid in the differentiation of active B cells into cells that produce antibodies. TNF alpha, interleukin (IL) 6, and IL 1 are just a few of the cytokines that have been overproduced and overexpressed as a result of interactions between activated T cells and macrophages [Derksen V.F.A.M. *et al.,* 2017].

These cytokines triggered the growth of fibroblasts and caused inflammatory cells to invade the synovial membrane. The major features of RA happened as a result of interleukin-6 (IL-6) production that was dysregulated and chronic. Autoimmune B-cells, the main underlying cause of RA, were also involved in the production of autoantibodies, T-cell activation, and pro-inflammatory cytokines, all of which contributed to the pathogenesis of RA [Bugatti S *et al.,* 2014]. Although the underlying causes of autoreactive B-cells that attacked host cells were still undetermined, the list of autoantibodies connected to RA was growing. Recent studies have also shown that localized autoreactive B-cells act on pathologically relevant cells in RA, causing immunological dysfunction, inflammation, and fractures in the bones [Schlegel P.M *et al.,* 2013]. These cytokines include TNF-, IL-6, IL-12, IL-23, and IL-1. Numerous inflammatory rheumatic disorders, including RA, have been managed significantly better via IL-6 targeting. NF-B, which was triggered by other proinflammatory cytokines (including IL-1, TNF, and IL-17), and Toll-like receptors (TLRs)-mediated signals [Browne E.P. 2012] constituted two transcription factors that up-regulate the expression of IL-6. The vast majority of leucocytes and stromal cells had the capacity to generate IL-6 in response to signs of cellular stress. By attaching to an 80 kDa transmembrane IL-6 receptor (IL-6R), IL-6 activates its communication mechanism. Target cells' Janus kinase (JAK) gets activated as a result of the complex formed by IL-6 and transmembrane IL-6R binding to IL-6R and forming an association with the signaling protein gp130. The traditional transduction route was the designation for this activation. While gp130 had been expressed in a variety of cells, transmembrane IL-6R only exists in a small subset of cells, including hepatocytes and certain leukocytes [Rose-John S. (2012)]. In serum, a soluble version of IL-6R (sIL-6R) excluded the cytoplasmic portion and bound IL-6 with a similar affinity as transmembrane IL-6R. Additionally, the IL-6 and sIL-6R combination could bind to gp130, activating the signaling cascade. Trans-signaling had been the term for this activity. More and more evidence pointed to the pro-inflammatory aspect of IL-6

trans-signaling, whereas regenerative or anti-inflammatory functions need traditional signaling. IL-6 initially was demonstrated to be a soluble factor synthesized by helper T cells that encouraged the secretion of immunoglobulin by activated B cells [Lesina M *et al.,* 2011], but recent evidence suggested that IL-6 also controled CD4+ T cell development and activation. Furthermore, and perhaps more significantly, IL-6 could increase Th17 cell differentiation in the presence of transforming growth factor (TGF)- via STAT3-mediated activation of retinoid orphan receptor (ROR)t while inhibiting TGF-induced regulatory T cell (Treg) differentiation. Thus, IL-6 encouraged Th17 to outnumber Treg among the effector CD4+ T cell subsets, which was regarded to be a key factor in the emergence of RA and a number of other immune-mediated ailments.

Likewise, it had been established that IL-6 encourages the growth of T follicular helper cells, which release IL-21, another differentiation of B cells factor [Greenhill CJ *et al.,* 2011]. It has been additionally shown that IL-6 has an impact on the local inflammation that damages joints. IL-6 was a substance that synoviocytes were capable of producing, and through the stimulation of the receptor activator of NF-kappa B ligand (RANKL), IL-6 could promote synoviocyte proliferation and osteoclast differentiation. Particularly linked to the IL-6-induced stimulation include the onset of osteoporosis and bone loss. Additionally, increased vascular permeability and angiogenesis in synovial tissue were pathogenic aspects of RA brought on by inadequate VEGF synthesis, which was also brought on by IL-6 in synovial fibroblasts. The systemic inflammatory signs and symptoms of RA include fever, malaise, drowsiness, muscular weakness, and anemia. In RA patients, elevated CRP, hypercoagulability, and hypoalbuminemia were common laboratory findings. These were thought to be IL-6-mediated in the majority of cases [Chow D *et al.,* 2001]. Patients with RA had higher levels of IL-6 in both their serum and synovial fluid. These levels have been linked to the severity of the RA condition, and successful use of DMARDs or TNF inhibitors has been demonstrated to lower serum IL-6 levels. Additionally, a predictive indication for a better clinical outcome was allegedly a decrease in IL-6 levels within the first 12 months of treatment. To the extent, it has recently been demonstrated that a decrease in blood IL-6 levels shortly after TCZ treatment can serve as a predictor of remission status maintenance [Smolen JS *et al.,* 2008]. These results unequivocally demonstrated the pathogenic function of IL-6 in RA. The precise methods that ensured that IL-6 was perpetually over-synthesized in RA and treated with TCZ bringing about a decrease in the intrinsic synthesis of IL-6 are nevertheless unknown.

2.3. IL-6 and Systemic Inflammation

Immunity and systemic inflammation were both profoundly impacted by IL-6. Myeloid cells promptly synthesize and release it in response to numerous danger cues and cytokines including IL-1 and TNF-. Through the bloodstream, IL-6 traveled from the local site of aggravation to important organs and trigger systemic reactions. The acute-phase response was the moniker given to this mechanism [Ohshima S *et al.,* 1998]. Inducing inflammatory systemic responses, IL-6 operates synergistically with other cytokines notably TNF-, IL-1, and IFN. Acute-phase reactions were accompanied by a number of proteins, collectively referred to as the acute-phase proteins, whose plasma concentrations evolve by at least 25% during inflammation. Hepatocytes provide IL-6R and gp130, allowing an instantaneous reaction to IL-6 through the conventional signaling pathway.

In research studies, Acute-phase proteins, including fibrinogen and haptoglobin, contribute to inflammation as well as coagulation and wound healing. Mice missing IL-6 struggled to develop the greatest defenses against infections and stressful conditions while synthesizing acute-phase proteins at a level that is substantially lower [Alonzi T *et al.,* 1998].

A number of physiological and behavioral processes, such as anorexia, somnolence, hematological and metabolic abnormalities, pain, fever, and pain, were also affected by IL-6 and the acute phase response.

The transition from acute inflammation to persistent and adaptive responses was facilitated by IL-6, as was noted in the introduction. IL-6 trans-signaling encouraged the secondary accumulation of monocytes at the site of inflammation, which was the hallmark of chronic inflammation [Akira S, Kishimoto T 1992]. The earliest phases of inflammation were characterized by the influx of neutrophils to the site of damage or infection. These results demonstrated the essential function that IL-6 played in the development of systemic and joint inflammation in RA, and it also contributed to the generation of immunological abnormalities.

2.4 Therapies/ Treatments/ Drugs

Patients with multiple myeloma participated in the first clinical studies, which had mixed results. Later, the chronic inflammatory symptoms of Castleman's illness were successfully treated with tocilizumab, a humanized anti-IL-6R antibody. Tocilizumab and sarilumab, two IL-6R inhibitors, have been approved since the late 2000s for the treatment of RA that was resistant to conventional synthetic disease-modifying anti-rheumatic medications (DMARDs). Suppression of structural damage and an improvement in composite disease activity scores were both correlated with the use of IL-6R inhibitors [Sebba A. 2008]. When implemented as monotherapy,

tocilizumab, and sarilumab outperformed the TNF antagonist adalimumab in controlling symptoms associated with inflammation [Raimondo *et al.,* 2017].

More recently, tocilizumab has been demonstrated to be effective in the treatment of various inflammatory conditions, such as giant cell arteritis, adult-onset Still's disease, and systemic juvenile idiopathic arthritis [Atsushi Ogata *et al.,* 2018]. Greater iron availability come about because of IL-6R inhibition, which mitigates the effects of IL-6 on iron metabolism. In patients with Castleman disease, tocilizumab causes a rapid and long-lasting decrease in hepcidin levels [John D Isaac *et al.,* 2019]. This has also been shown in RA when anemia has improved [Kaye, A. G., & Siegel, R. (2020)]. The reduction of hepcidin levels and the improvement of hemophilia were more effectively achieved by IL-6 inhibition in a cohort of RA patients treated with either tocilizumab or infliximab (a TNF-inhibitor, TNFi). These findings, therefore, imply that the preferred therapy for RA patients is an IL-6R inhibitor.

Treating RA aimed to reduce systemic inflammation and avoid long-term complications that could affect the quality of life and result in disability. A prompt, efficient, and effective therapeutic strategy was required. The following pillars [Kumar, P., & Banik, S. (2013)] served as the foundation for treating RA Drug treatment on the one hand involves

- analgesics and non-steroidal anti-inflammatory drugs (NSAIDs),
- traditional disease-modifying anti-rheumatic drugs (DMARDs),
- biologicals,
- glucocorticoids,
- non-malicious therapy like physiotherapy, occupational therapy and psychological support, and surgical procedures,
- analgesics:

These widely-used drugs served as the foundation for treating RA symptoms like pain. Additionally, NSAIDs were found beneficial for inflammation. It must be made clear that neither pharmaceutical has a beneficial effect on how the condition will progress over time.

DMARDs

DMARDs were a diverse set of medications that can lessen disease activity and had an advantageous impact on the progression of the disease in the context of maintaining joint function and preventing joint degeneration. Assuming there were no contraindications, methotrexate (MTX) was the recommended first-line DMARD. Leflunomide or sulfasalazine might be recommended if MTX was contraindicated [Aletaha D, Smolen JS. 2018].

Due to their adverse consequences, gold, azathioprine, and ciclosporin were currently only occasionally used

Biologicals

Recent studies on the inflammatory and pathogenic aspects of RA have rendered possible the ability to identify and regulate pro-inflammatory cytokines, widening the door to novel therapy strategies. Five TNF inhibitors (TNF-i) possess currently received licensure and these are:

- infliximab,
- adalimumab,
- etanercept,
- certolizumab and
- golimumab

In addition, IL-6R inhibitors and neutralising antibodies aimed at IL-6 specifically have found additional therapeutic uses outside of inflammatory rheumatic illnesses [Yip, R. M. L., & Yim, C. W. (2021)]. For instance, a phase II investigations were testing the IL-6 neutralizing antibody sirukumab on people with major depressive illness.

Tocilizumab, the resulting humanized IL-6R antibody, binds to both the sIL-6R and the mIL-6R and blocks IL-6 signaling by blocking IL-6 from binding to IL-6R11,12. Due to the therapeutic effectiveness of this IL-6R antibody, several IL-6 antibodies (sirukumab, olokizumab, and clazakizumab) had been developed.

Sarilumab, a substitute monoclonal antibody to IL-6R, has been licensed for the treatment of RA. Since tocilizumab was first approved for the diagnosis of RA. It has been shown to be effective and safe. In RA clinical studies, sirukumab, olokizumab, and clazakizumab, three other monoclonal IL-6 antibodies, have also been investigated. In phase III RCTs, sirukumab outperformed a placebo in terms of reducing disease activity, enhancing physical function, and enhancing associated health quality of life, as well as delaying the progression of radiographic disease in patients with RA who had failed to comply with therapy with csDMARDs and biological DMARDs [Mississauga, Ontario; November 28, 2018]. However, sirukumab monotherapy was comparable to but not superior to adalimumab, and efforts to secure regulatory approval for the treatment of RA were abandoned. Olokizumab phase II trials revealed effectiveness for therapy, and clinical trials for phase III are still being conducted. However, clazakizumab's development as a RA medication had also been suspended.

The FDA initially approved tocilizumab, a humanized monoclonal antibody that targets the IL-6 receptor, as an intravenous formulation for the treatment of RA in 2010. Subcutaneous formulation approval followed. The FDA approved the use of sarilumab, a fully human monoclonal antibody against the IL-6 receptor, in 2017 for the treatment of persons with moderately to severely active RA who did not respond well to or were intolerant to a csDMARD. The FDA has placed a boxed warning on tocilizumab and sarilumab with regard to the potential of getting serious infections that might outcome in hospitalisation or perhaps death [Scott LJ 2017]. Active tuberculosis, invasive fungal infections, as well as viral, bacterial, and other diseases brought through opportunistic pathogens, were among the infections which were reported. The majority of individuals who contracted infections were also receiving immunosuppressants such as methotrexate or corticosteroids.

3. MATERIALS AND METHOD

3.1. Retrieval of Target Proteins

The crystal structure of desired proteins IL-6, IL-6R, and GP-130 was retrieved through the complex structure of hexametric complex PDB ID- 1P9M from Research Collaboratory from Structural Bioinformatics Protein Data Bank (RCSB-PDB). According to the works of literature and the research, it was stated that IL-6 and its receptors were significantly involved in RA [Sharma, Y., Kumar, N., & Thakur, D. (2021)]. The main complex structure is shown below.

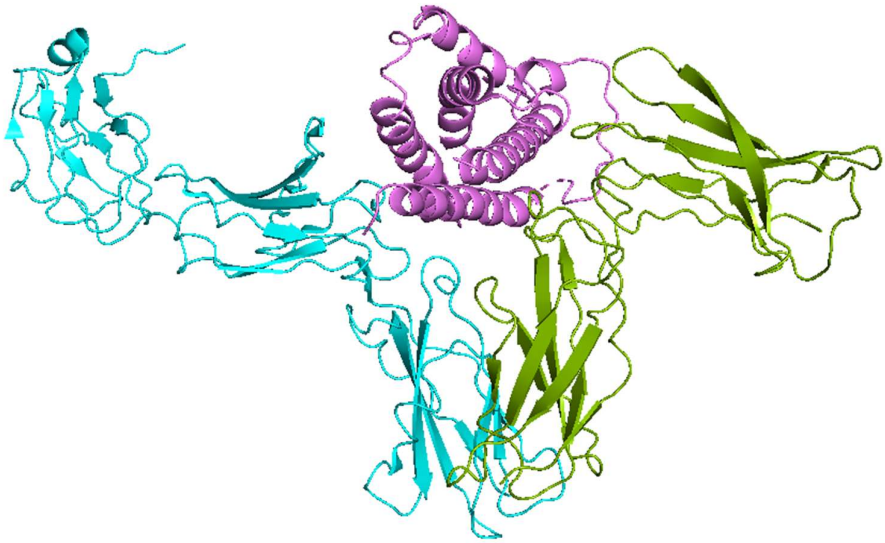


Figure 1: Complex hexameric structure PDB -1P9M

Blue color represents GP-130, Violet color represents IL-6 and green color represents IL-6R protein

The receptor structures i.e., IL-6R & GP- 130 was retrieved from the complex reference structure PDB ID -1P9M.

3.2. Homology Modelling

Structure refinement of targeted protein is essential before proceeding to molecular docking. The whole primary sequence of IL-6 was extracted with the help of UniProtKB. Afterward, the quality insurance validation was performed via Swiss-model for template selection. The template 1ALU was taken as a template. Further validation modeling was performed via MODELLER software. The predicted refined structure was shown below.

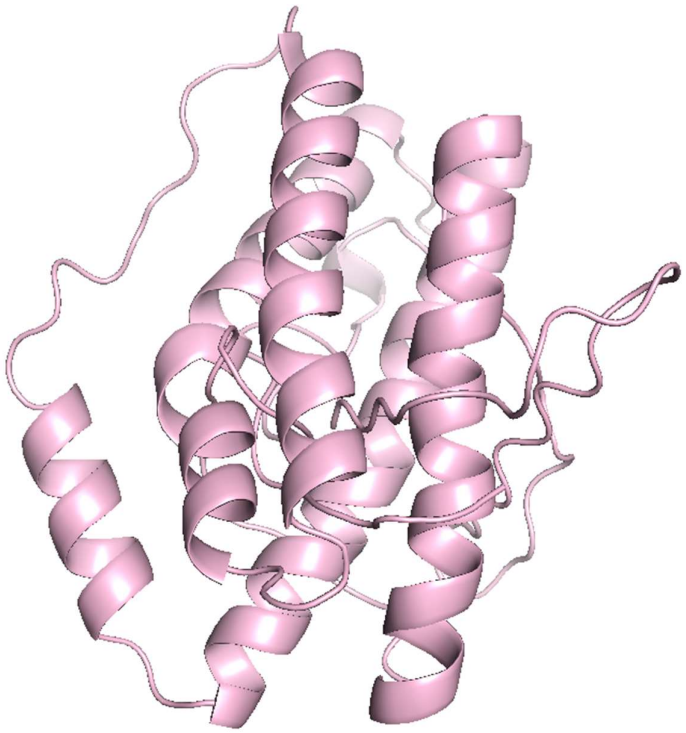


Figure 2: Modelled Structure of IL-6

The structure was visualized with the help of Pymol software.

3.3. Screening of Chemical Compounds

The initial step for computational drug designing is to find novel inhibitors against a targeted protein. The inhibitor compounds against IL-6 have known medicines used in the treatment of R.A diagnosis. Therefore, natural and synthetic derivatives compounds against our target proteins were retrieved through ChEMBL and PubChem databases. The natural compounds were validated from literature sources initially. A total of 2170 compounds were extracted successfully. Ligands selection was extracted using specific keywords which relate to our targeted disease i.e., R.A, such as:

- Anti-inflammatory
- Anti-rheumatoid
- Anti-rheuma
- Rheumatoid arthritis

3.4. Virtual Screening

One of the lead steps toward drug discovery before performing wet-lab experiments is a virtual screening of the compound. This process involves the estimation of the binding affinity of the drug candidate towards a target protein. The virtual screening algorithm helps out in exhibiting the possible binding modes against the active sites of the targeted compounds. For an accurate binding site of the target, the correct residues must be known. With the help of the reported literature's main residues, the investigation for the IL-6, IL-6R & GP-130 was done by the online software HADDOCK 2.4 web server.

3.4.1 Preparation of the Protein

The three-dimensional protein IL-6 was extracted from the structure downloaded from PDB, PDB ID- 1P9M**.** The extraction of the proteins was done via the visualization software Pymol.

The receptor protein was prepared by removing the hetero atoms, water molecules, ligands and adding polar hydrogens. The whole algorithm was done by using the BIOVIA Discovery Studio 2021 software.

3.4.2. Preparation of the Ligand

All ligands/inhibitors were minimized and converted to pdbqt format from SDF format by using PyRx software.

3.4.3. Interaction Study

After all the preparations, PyRx software was selected for the docking environment to recognize the desired inhibitor or ligand in two intervals:

- Docking with the IL-6R or IL-6 alpha receptor
- Docking with the GP-130 Glycoprotein or IL-6 Beta receptor

The top 50 compounds based on the best binding energies were drawn out from both the docking batches (IL-6, GP-130).

From the top 50 compounds, the common inhibitor existing in both docking results was taken. A total of 16 inhibitors were common there in the top 50 compounds.

3.5. Re-docking of Common Compounds/Inhibitors

The top common inhibitors need to be re-docked for further procedure. The re-docking of the compounds was done by the use of AutoDock Suite software.

3.5.1 Preparation of the Protein and Ligand

The receptor protein was arranged by removing the hetero atoms, water molecules, and ligands & integrating polar hydrogens. Then saved it in the pdbqt format.

The ligand was prepared by adding all hydrogens, and detection of roots. Afterward, it was saved in pdbqt format.

3.5.2 Molecular Docking

In order to set the seal on the conformations to fit accurately in the grid box, the box was centralized and set accordingly.

The map files for each type of atom present in the complex were carried out individually for docking. One of the main lead conformational search approaches employed in AutoDock suite is Lamarckian Genetic Algorithm, which was used. The parameter file enforced was processed by using Genetic Algorithm 150 runs, the max number of evaluations was 250000, the max number for a generation was 27000, and the gene mutation was 0.02%. The docking operation was set by default. The output files were generated in Lamarckian Genetic Algorithm (4.2). The same criteria were followed against both receptors docking.

3.6. Molecular Dynamics Simulation

Based on the molecular interactions and analysis of the docking results, the top 5 ranked complexes were pointed out for simulation investigation of Molecular Dynamics. The simulation was accomplished on supercomputers by GROMACS [Hollingsworth, S. A., & Dror, R. O. (2018)].

Force field AMBER99SB.ILDN has been used for all compounds. Each complex was solvated within a cubic box with a TIP3 water model. At a temperature of 300°K, and a pressure of 1.013 bar, NVT & NPT at 100 PS was run. Then, finally with 10,000 PS & at 10 nanoseconds, MD simulation was performed. A simulation interaction diagram was used to scrutinize the MD simulation trajectory.

Several steps were followed for the completion of the MD Simulation. The steps were:

- Preparation of topologies for protein/ligand
  - Topol.top
  - .gro
  - .itp (topologies file formats)
- Preparation of the complex.gro file
  - (An integrated file of protein and ligand)
- Solvation
- Extracting and saving parameter files required for proceeding:
  - npt.mdp,
  - nvt.mdp,
  - ions.mdp,
  - em.mdp,
  - md.mdp
- Energy minimization
- Restraining the ligand
- Thermostats
- NVT equilibrium
- NPT equilibrium
- MD production
- Computation of RMSD Ligand and Protein
- Computation of RMSF
- Computation of SASA
- Computation of RG, and
- Computation of Hydrogen Bonds

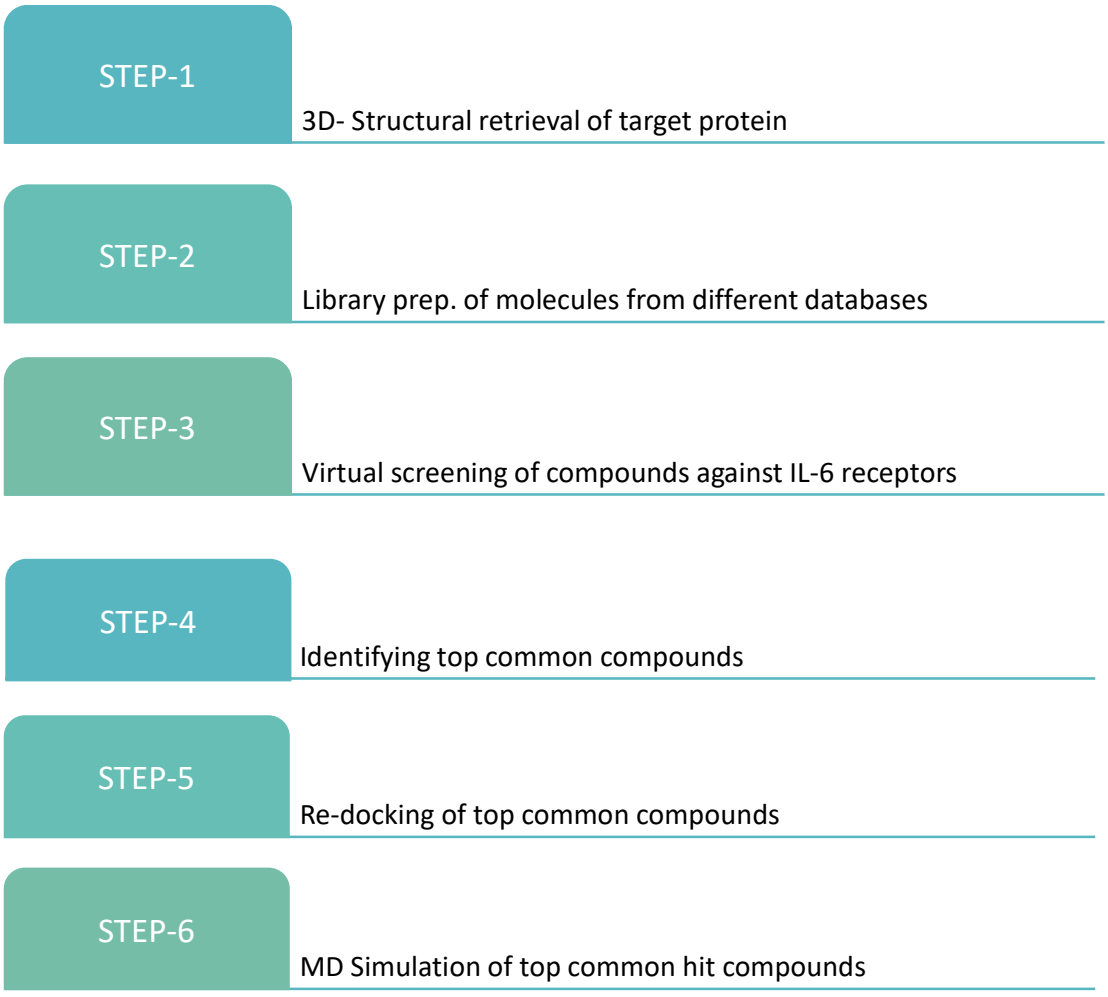


Figure 3: A systematic representation of methodology workflow

4. RESULTS AND DISCUSSION

4.1. Prepared Macromolecule

The PDB ID-1P9M consisted of 3 chains, the targeted molecule and both receptors. The targeted protein was extracted by removing both receptor chains and modeled with MODELLER software, with the templates taken from Swiss-model. After all this protocol is followed, the protein was converted into pdbqt format itself by the PyRx software.

For second-time docking i.e., for common inhibitors, the protein is converted into pdbqt format followed by removing hetero-atoms, and water molecules along with the integration of polar hydrogens by using AutoDock suite software.

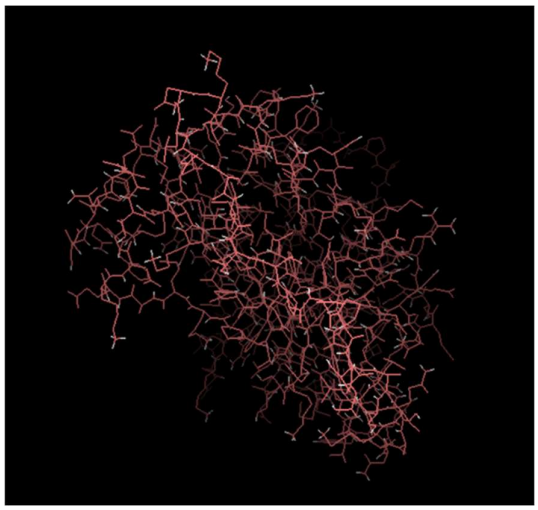


Figure 4: Prepared macromolecule

4.2. Grid Box

A precise grid box for both receptors was prepared carefully covering all the active site residues implicated in cartoon form. The grid box has been shown below in the figure. Grid box coordinates covering all the aspects of the active site are also shown in the table below

Table 1: Grid Box coordinates

Coordinates	IL-6R	GP-130	Spacing
x-Dimension	82	52	0.375
y-Dimension	64	46	
z-Dimension	62	58	
x-Center	-3.511	6.269	
y-Center	-23.979	-11.181	
z-Center	-3.576	0.776	

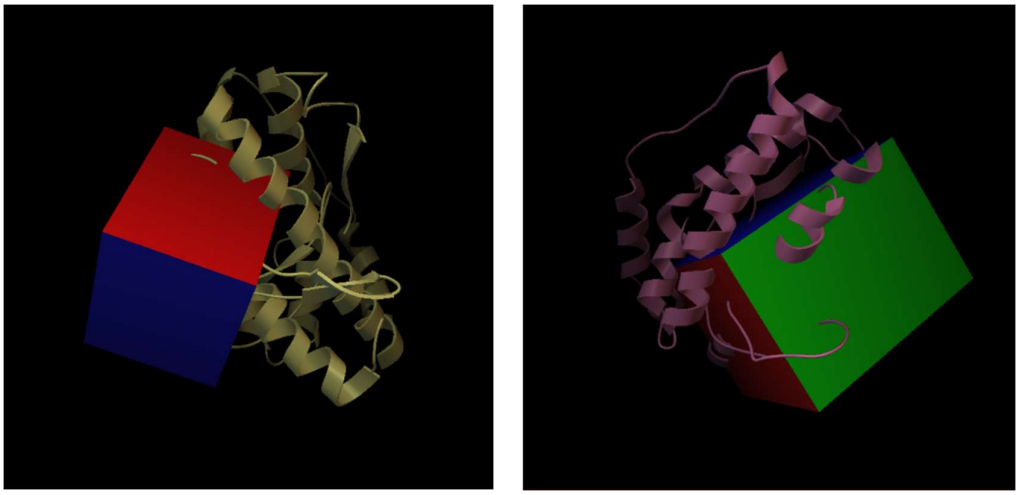


Figure 5: Grid Box (Pale Yellow- GP-130, Mauve-IL-6R)

4.3. Molecular Docking

The molecular docking of the common inhibitor compounds found in both receptors has been extracted from top-50 compounds in terms of top-docked binding energies. Among all the 50 compounds, 16 compounds were found common. The common compounds are docked against both the receptors and their results are listed below in the table.

Table 2: Docking Analysis against GP-130 (- indicates no bond formed)

Compounds	Conformations	B-E	H-B
C1	7	-9.03	-
C2	3	-9.23	-
C3	2	-10.69	1
C4	8	-8.39	1
C5	5	-9.87	1
C6	7	-9.33	2
C7	7	-9.71	1
C8	9	-9.77	-
C9	6	-8.81	-
C10	3	-8.99	-
C11	4	-8.92	1

C12	6	-9.14	-
C13	9	-9.36	-
C14	6	-9.33	-
C15	3	-8.39	-
C16	4	-9.01	-

B. E.: Binding Energy KJ/mol, H-B: Hydrogen Bonds

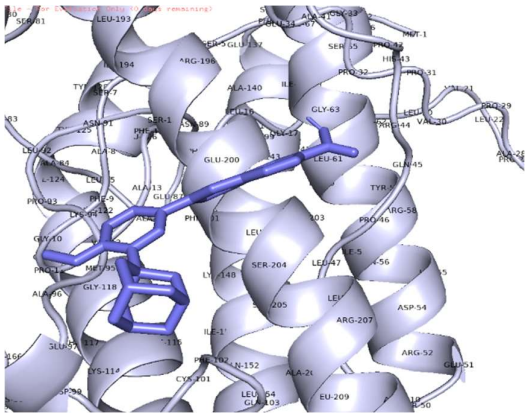
Table 3: Docking Analysis against IL-6R (- indicates no bond formed)

Compounds	Conformations	B-E	H-B
C1	8	-6.33	3
C2	5	-7.06	1
C3	7	-6.37	-
C4	9	-6.33	2
C5	1	-5.71	4
C6	1	-7.63	2
C7	3	-7.35	-
C8	7	-6.81	-
C9	7	-7.83	1
C10	4	-7.47	-
C11	6	-7.8	2
C12	10	-7.44	-
C13	4	-6.49	-
C14	10	-6.93	-
C15	2	-6.45	1
C16	8	-6.24	-

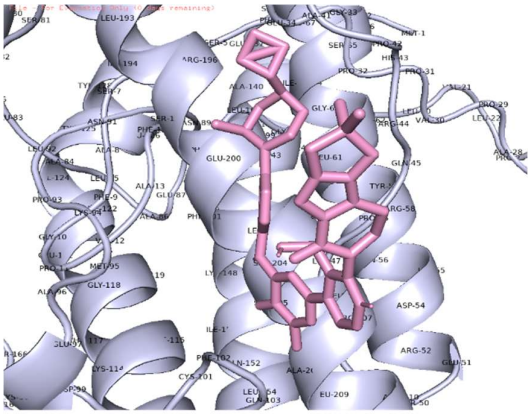
Out of 16 docked compounds against both receptors, we picked the 5 common compounds based on the visual evaluation. These compounds further went through blind docking and then simulation studies for better accuracy. Compound C3, C5, C7, C8 and C13 were chosen.

A)

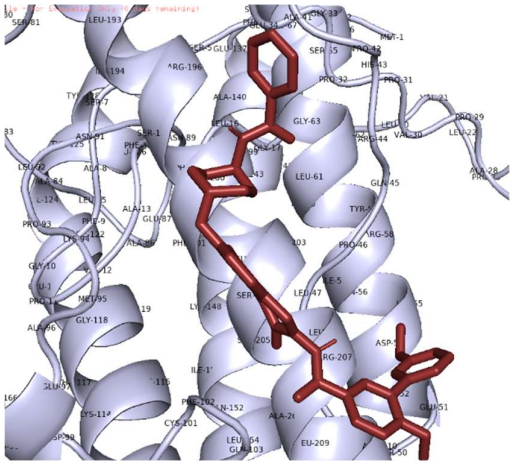
i



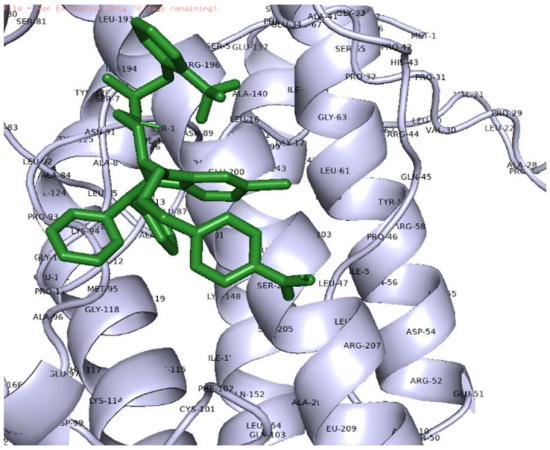
ii



iii



iv



v

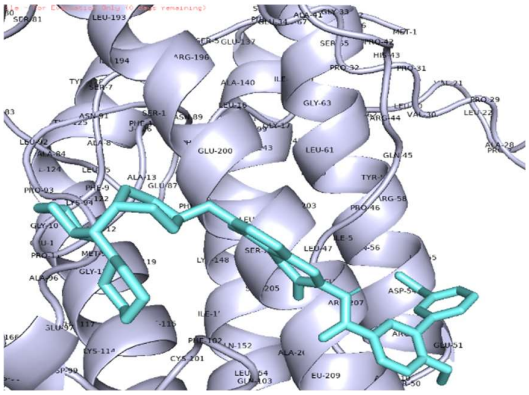


Figure 6A: Interaction diagram of IL-6R

B)

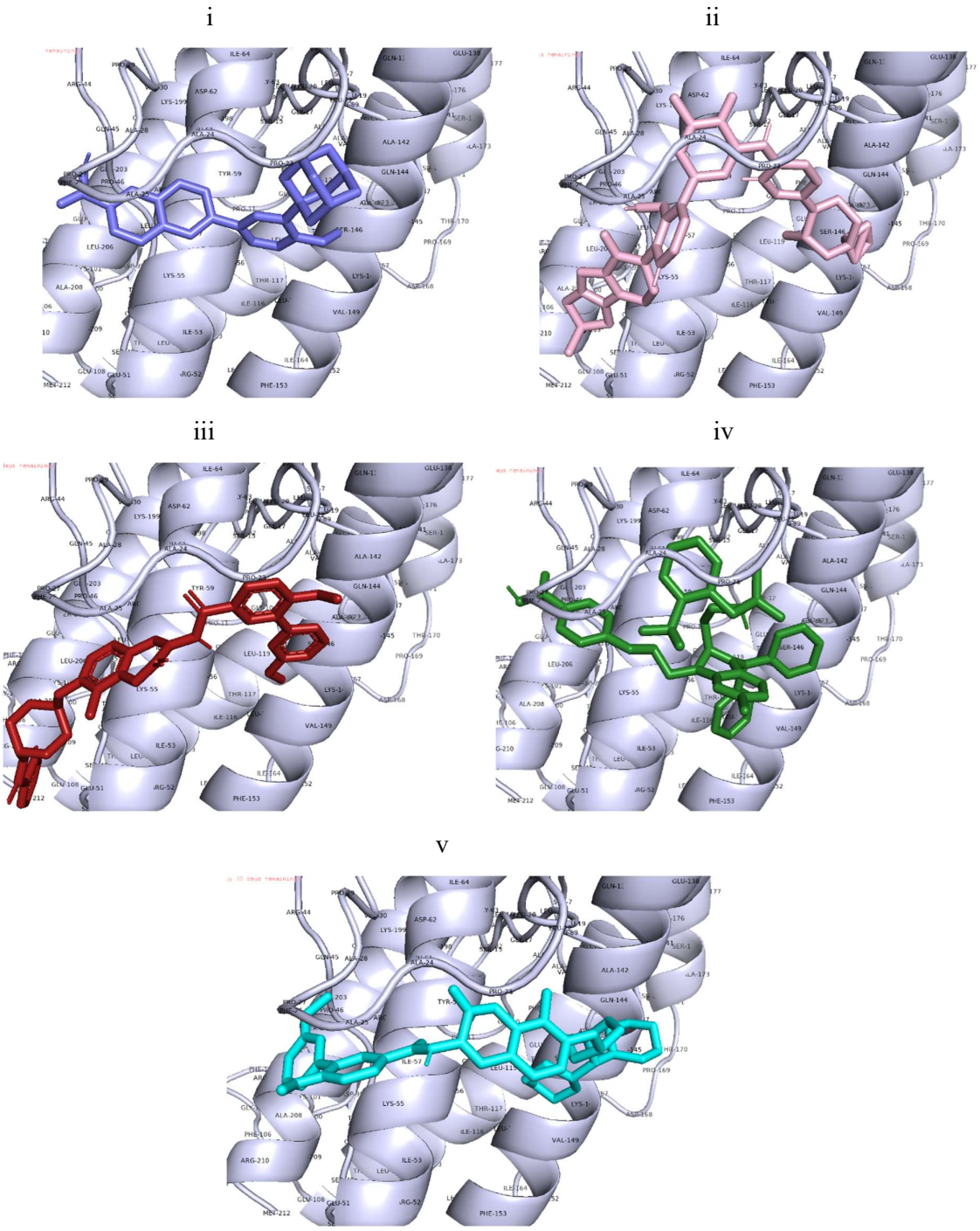


Figure 6B: Interaction diagram of GP-130

The figure 6A describes interaction diagram of IL-6R and figure 6B shows interaction of GP-130 with their best compounds. Label i shows C3, ii shows C5, iii shows C7, iv shows C8, v shows C13. All these compounds were bounded to their pocket residues.

4.4. Blind Docking

The blind docking was also processed by AutoDock suite software targeting the whole protein in which the top 5 compounds with the highest binding energies were taken. The below table shows the blind-docked results.

Table 4: Blind-docked compounds against whole protein

Comp.	Best fit Conf.	B.E	Target Site
C3	10	-8.33	-
C5	2	-9.72	-
C7	3	-10.42	GP-130
C8	3	-6.93	GP-130
C13	6	-7.82	IL-6R

- Represents binding out of the pocket.

Through analysis of blind docking, it was concluded that the two compounds C7 and C8 with the best conformations were found fitted in the binding pocket of GP-130. The best conformation of the compound C13 was found fit in the binding site of the IL-6 alpha receptor i.e., IL-6R. The best conformation of the compound C5, were also found nearby in the active region of IL-6R.

4.5. MD Simulation

At most, docking cannot dispense full insight into the binding mode, steadiness as well as dynamics of proposed ligands. The MD studies were carried out to attain insight into the dynamics of ligand at the binding cavity of the protein & to look over how ligands accommodate the area.

As a matter of fact, we use the GROMACS to run MDS at 10 nanosecond frames. The investigations were plotted out with respect to five criteria:

- ❖ Root Mean Square Deviation
- ❖ Root Mean Square Fluctuation
- ❖ Radius of Gyration
- ❖ Solvent Accessible Surface Area
- ❖ Hydrogen Bonds

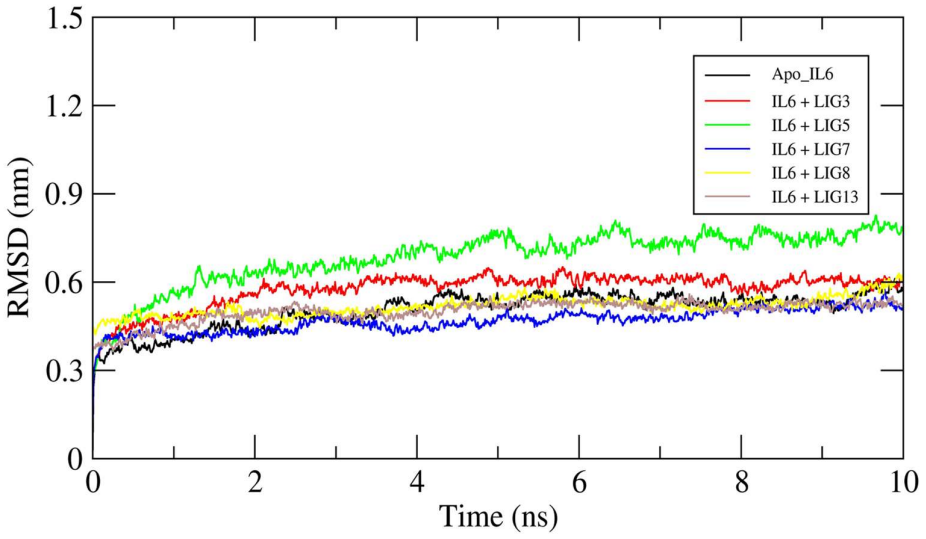
4.5.1 RMSD

RMSD attributes to the phenomenon of deviations that were remarked during the progression of the simulation [Humphrey *et al.,* 1996]. To extend, it describes the steady of the protein as the miniature of the RMSD, greater the stability. In the steady flow inspection, the RMSD was analyzed for the protein backbone and ligand. The graphs of the RMSD protein and RMSD ligand against both receptors have been shown in the figure below.

The stability of the protein backbone and ligand against IL-6R and GP-130 has been studied and shown in the figures. The investigation describes in the case of IL-6R backbone (bb) that ligands/compounds showed stability till last at 10 ns, slightly higher peaks than ref. protein was observed by compounds namely C3, C5 but can still be counted as in good stability. While, in the case of GP-130 bb, C8 showed slight fluctuation initially but was detected stable after 1.5 ns and can be negligible. The RMSD complex protein bb of both receptors has been rendered below 0.9 mm.

Alternatively, the RMSD ligand complex of IL-6R showed overall good stability. On the other hand, the RMSD ligand complex of GP-130, C8 displayed visible fluctuations and raised more than other compounds. The range of RMSD occurred at 0-1.5 mm with a time period of 10 nanoseconds.

A)



B)

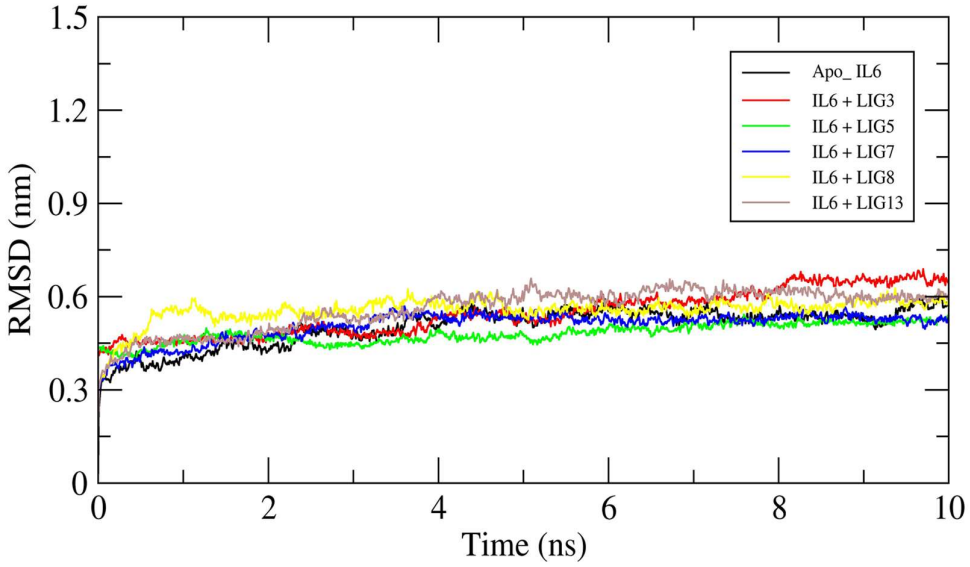


Figure 7: RMSD pro: A) IL-6R, B) GP-130

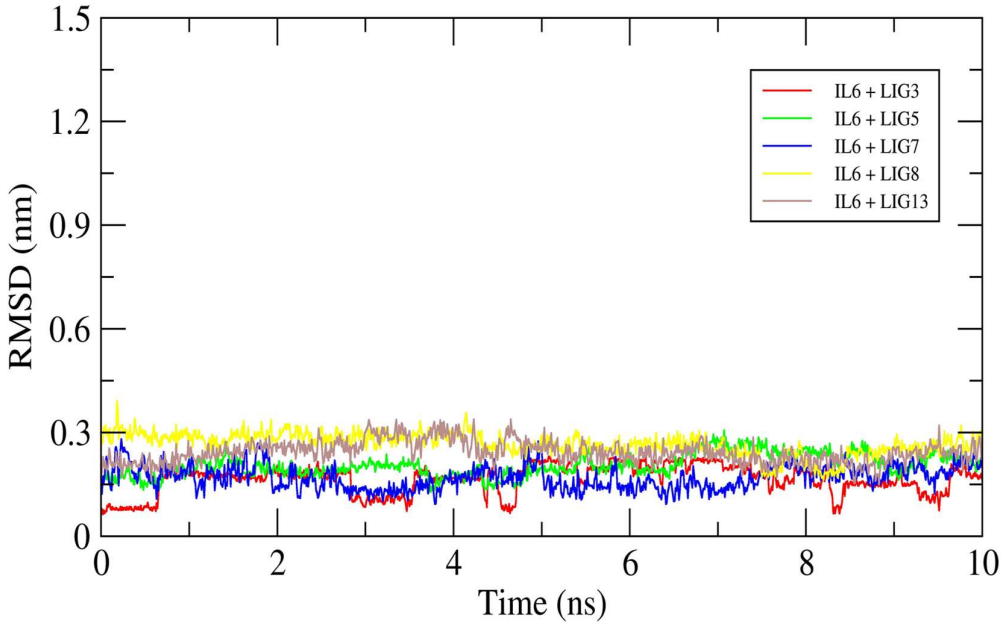
Table 5: RMSD Pro Mean table: IL-6R

	Compounds	Mean (nm)
IL-6R	Apoprotein	0.503 +/- 0.063
	C3	0.575 +/- 0.058
	C5	0.684 +/- 0.094
	C7	0.467 +/- 0.039
	C8	0.517 +/- 0.032
	C13	0.500 +/- 0.036

Table 6: RMSD Pro Mean table: GP-130

	Compounds	Mean (nm)
GP-130	Apoprotein	0.503 +/- 0.063
	C3	0.546 +/- 0.072
	C5	0.480 +/- 0.309
	C7	0.505 +/- 0.047
	C8	0.556 +/- 0.041
	C13	0.559 +/- 0.0679

A)



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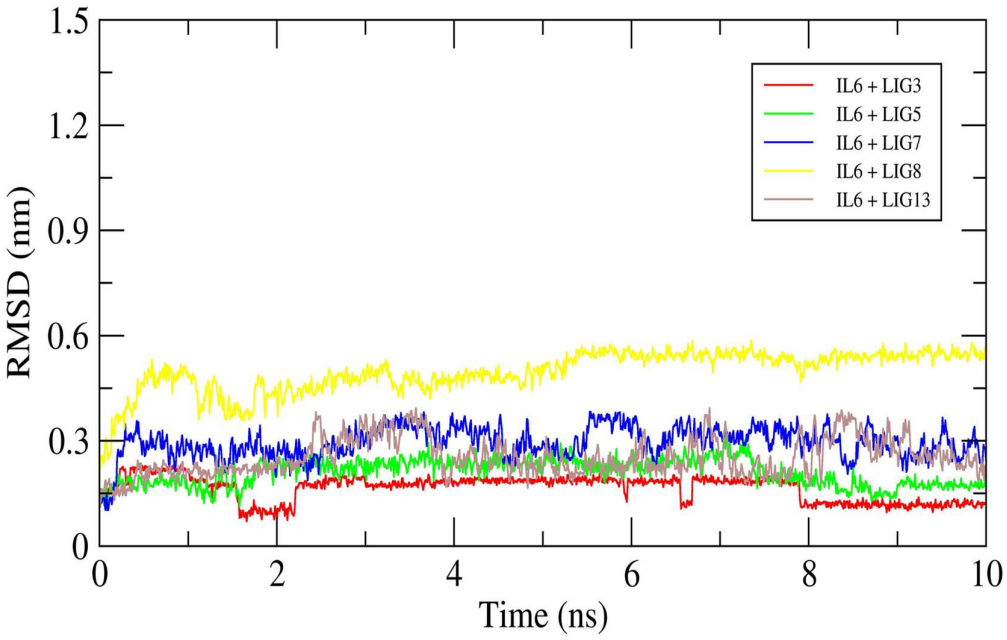


Figure 8: RMSD Ligand, A) IL-6R, B) GP-130

Table 7: RMSD Ligand, IL-6R

	Compounds	Mean (nm)
IL-6R	Apoprotein	-
	C3	0.164 +/- 0.041
	C5	0.205 +/- 0.032
	C7	0.169 +/- 0.034
	C8	0.265 +/- 0.032
	C13	0.243 +/- 0.032

Table 7: RMSD Ligand, GP-130

	Compounds	Mean (nm)
GP-130	Apoprotein	-
	C3	0.165 +/- 0.035
	C5	0.210 +/- 0.037
	C7	0.294 +/- 0.044
	C8	0.496 +/- 0.059
	C13	0.252 +/- 0.054

The Compounds in the tables represent the Ligands presented on the graph.

C3 represents LIG3 in red color

C5 represents LIG5 in green color

C7 represents LIG7 in blue color

C8 represents LIG8 in yellow color

C13 represents LIG13 in brown color.

The present findings infer the super stability of ligand or compound 7 majorly in the backbone or ligand. C3, and C5 also showed good stability with negligible fluctuation. The well-behaved simulation was depicted for these compounds.

4.5.2 RMSF

RMSF plot lights up the knowledge on residue's specific fluctuations that occurred during simulation [Ghahremainian *et al.,* 2022].

The protein bb residues were examined to be below 0.9 nm. Stating its stability supporting RMSD and Rg results. A minor peak was observed initially between the residues 20-30, and a minor fluctuation between 150-160 residues which was also in the ref bb. These residues generally prone to be fluctuation and are not a part of active site regions. Moreover, the residues of the active site were observed stable. The RMSF demonstrating the graphs was figured below in the figure

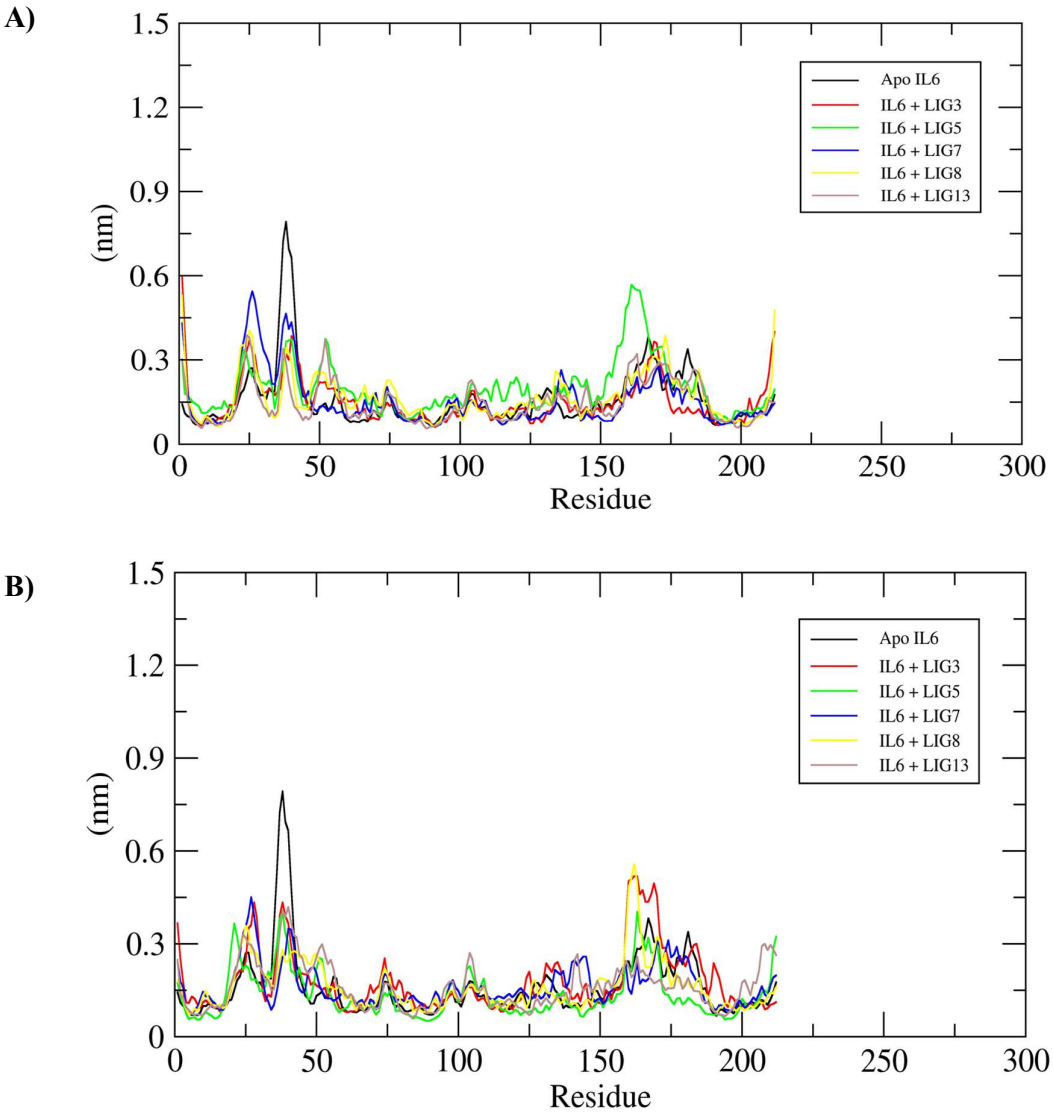


Figure 9: RMSF, A) IL-6R, B) GP-130

Table 9: RMSF IL-6R

	Compounds	Mean (nm)
IL-6R	Apoprotein	0.158 +/- 0.108
	C3	0.151 +/- 0.079
	C5	0.199 +/- 0.93
	C7	0.154 +/- 0.94
	C8	0.166 +/- 0.800
	C13	0.148 +/- 0.735

Table 10: RMSF GP-130

	Compounds	Mean (nm)
GP-130	Apoprotein	0.158 +/- 0.108
	C3	0.178 +/- 0.101
	C5	0.135 +/- 0.764
	C7	0.162 +/- 0.067
	C8	0.161 +/- 0.080
	C13	0.161 +/- 0.073

The Compounds in the tables represent the Ligands presented on the graph.

C3 represents LIG3 in red color, C5 represents LIG5 in green color, C7 represents LIG7 in blue color, C8 represents LIG8 in yellow color, and C13 represents LIG13 in brown color.

The mean table was shown for better analysis. The results indicated that all compounds were stable except C5 compounds which fluctuate initially and between the residue 150 to 175. The initial fluctuation was also found in the apoprotein. However, the last fluctuations that occurred were out of the binding pocket indicating the compound's stability.

\

4.5.3 Radius of Gyration

The Rg demonstrated the compactness of the protein bb during the simulation run. Rg defined as the root mean square average of the distance shattered all over. It simply measures the distance between the center of the mass of protein. Moreover, Rg indicates the overall information of the protein [Christopher Bernett *et al.,* 2023].

The Rg was determined for the protein backbone. For both systems, the Rg was analyzed at the range of 1.67 and 1.83 nm. Determining the compactness of the protein refers to the figure.

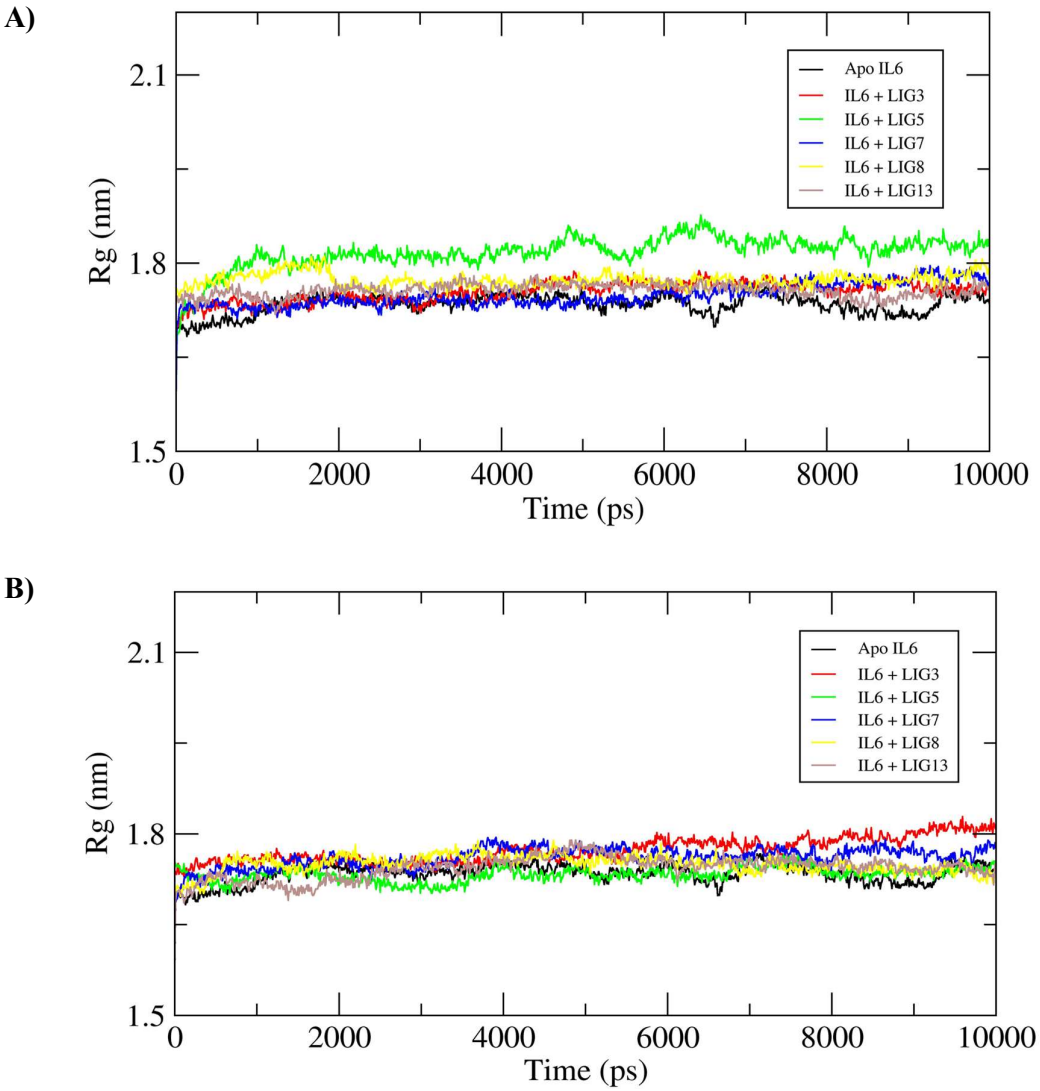


Figure 10: Rg, A) IL-6R, B) GP-130

Table 11: Radius of Gyration IL-6R

	Compounds	Mean (nm)
IL-6R	Apoprotein	1.734 +/- 0.016
	C3	1.753 +/- 0.016
	C5	1.817 +/- 0.026
	C7	1.748 +/- 0.018
	C8	1.773 +/- 0.010
	C13	1.756 +/- 0.010

Table 12: Radius of Gyration GP-130

	Compounds	Mean (nm)
GP-130	Apoprotein	1.734 +/- 0.016
	C3	1.772 +/- 0.020
	C5	1.732 +/- 0.011
	C7	1.760 +/- 0.011
	C8	1.749 +/- 0.015
	C13	1.743 +/- 0.019

The Compounds in the tables represent the Ligands presented on the graph.

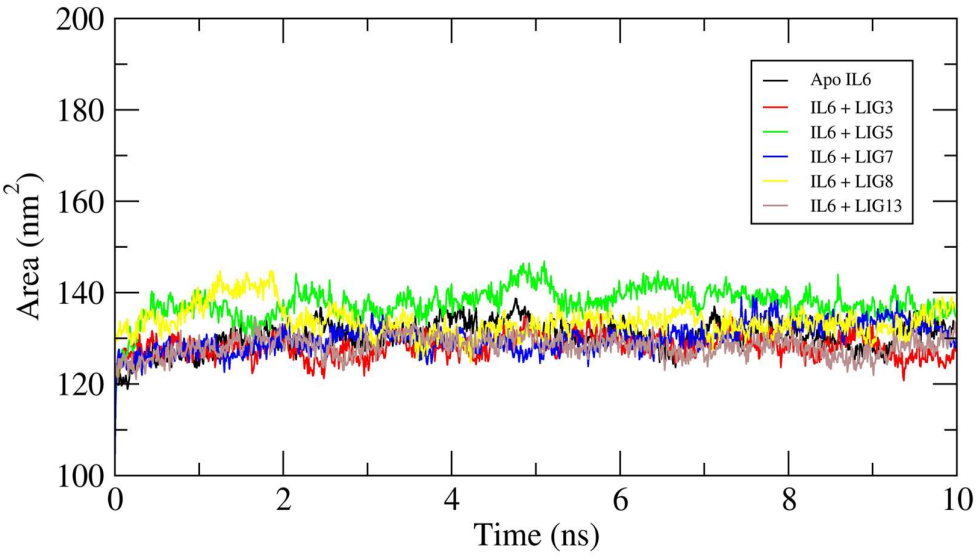
C3 represents LIG3 in red color, C5 represents LIG5 in green color, C7 represents LIG7 in blue color, C8 represents LIG8 in yellow color and C13 represents LIG13 in brown color.

The present investigation findings by Rg analysis appear to ensure the stability of all complexes. Rg fluctuations were depicted for C5 against IL-6R depicting variable fluctuations. The highest average 1.74 nm was noted down for both receptors. C7 and C13 showed the best results comparatively stability in both receptors.

4.5.4. SASA

Solvent accessible surface area is defined as the surface area of protein which interacts with its molecules. SASA contemplates protein stability & folding studies. The traces of SASA values fluctuated around a constant average of 129 nm- 141 nm for C5 and C8. The other compounds exhibited the stability behavior with ref to apoprotein against IL-6R. Interestingly, the compounds showed higher SASA values. While concerning the other target GP-130, a slight fluctuation was observed by C7 from 3.2 ns to 4 ns. C5 showed lower stability with ref protein. C13 showed good stability against both targets. The area values ranged between 100 nm to 200 nm.

A)



B)

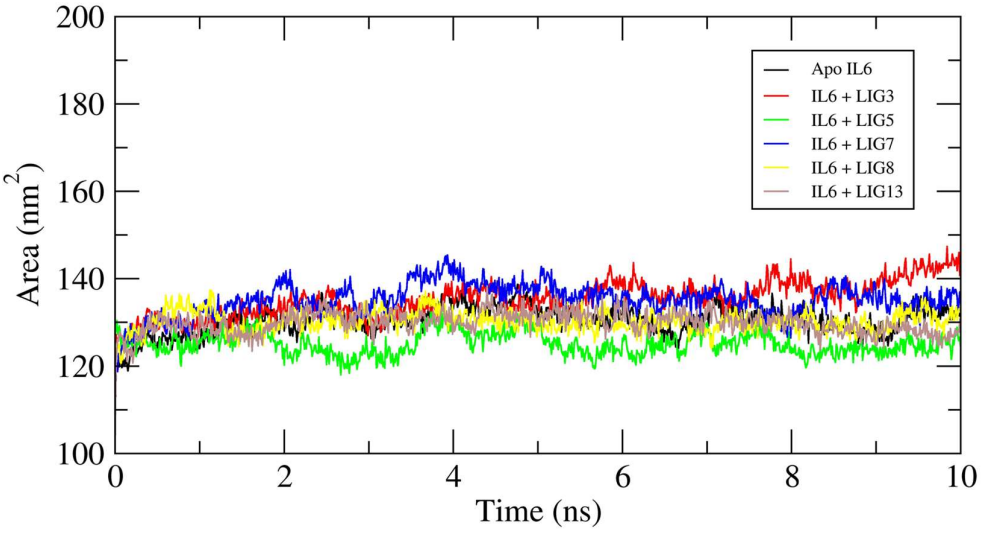


Figure 11: SASA A) IL-6R, B) GP-130

Table 13: SASA IL-6R

	Compounds	Mean (nm)
IL-6R	Apo_protein	130.402 +/- 3.273
	C3	128.235 +/- 2.524
	C5	137.645 +/- 3.491
	C7	130.115 +/- 3.102
	C8	133.709 +/- 3.330
	C13	

Table 14: SASA GP-130

	Compounds	Mean (nm)
GP-130	Apo protein	130.402 +/- 3.273
	C3	135.225 +/- 4.049
	C5	125.153 +/- 2.649
	C7	135.117 +/- 3.820
	C8	130.194 +/- 2.486
	C13	129.878 +/- 2.623

The Compounds in the tables represent the Ligands presented on the graph.

C3 represents LIG3 in red color, C5 represents LIG5 in green color, C7 represents LIG7 in blue color, C8 represents LIG8 in yellow color and C13 represents LIG13 in brown color.

The total area covered was about 120 nm^2- 142 nm^2. For IL-6R, C5 showed higher fluctuations. While the same compounds showed good stability for GP-130. Excitingly, C13 showed good SASA trajectories.

4.5.5. Hydrogen Bonds

The stability of the hydrogen bonds was depicted successfully during MD Simulation studies.

IL-6R: 1 hydrogen bond was formed and analyzed in all compounds except, in C5 3 hydrogen bonds were depicted.

GP-130: 2 hydrogen bonds were apparent in C3 and C8. While C7 and C13 showed 1 HB. Furthermore, 5 HB was detected in C5

A) IL-6R

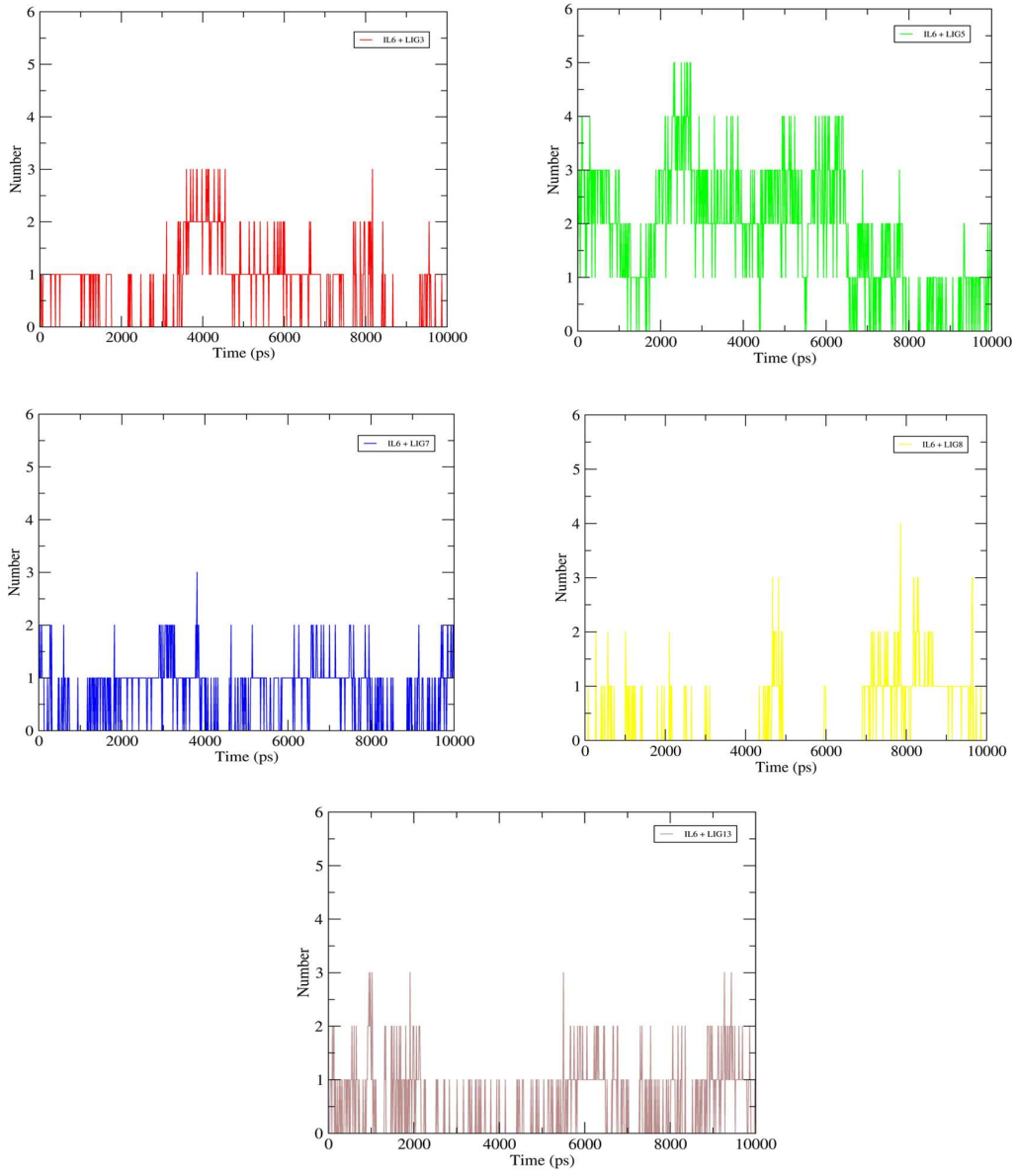


Figure 12: H-Bonds depicted against IL-6R

Red depicts C3, Green shows C5, Blue represents C7, Yellow represents C8 & Brown represents C13

B) GP-130:

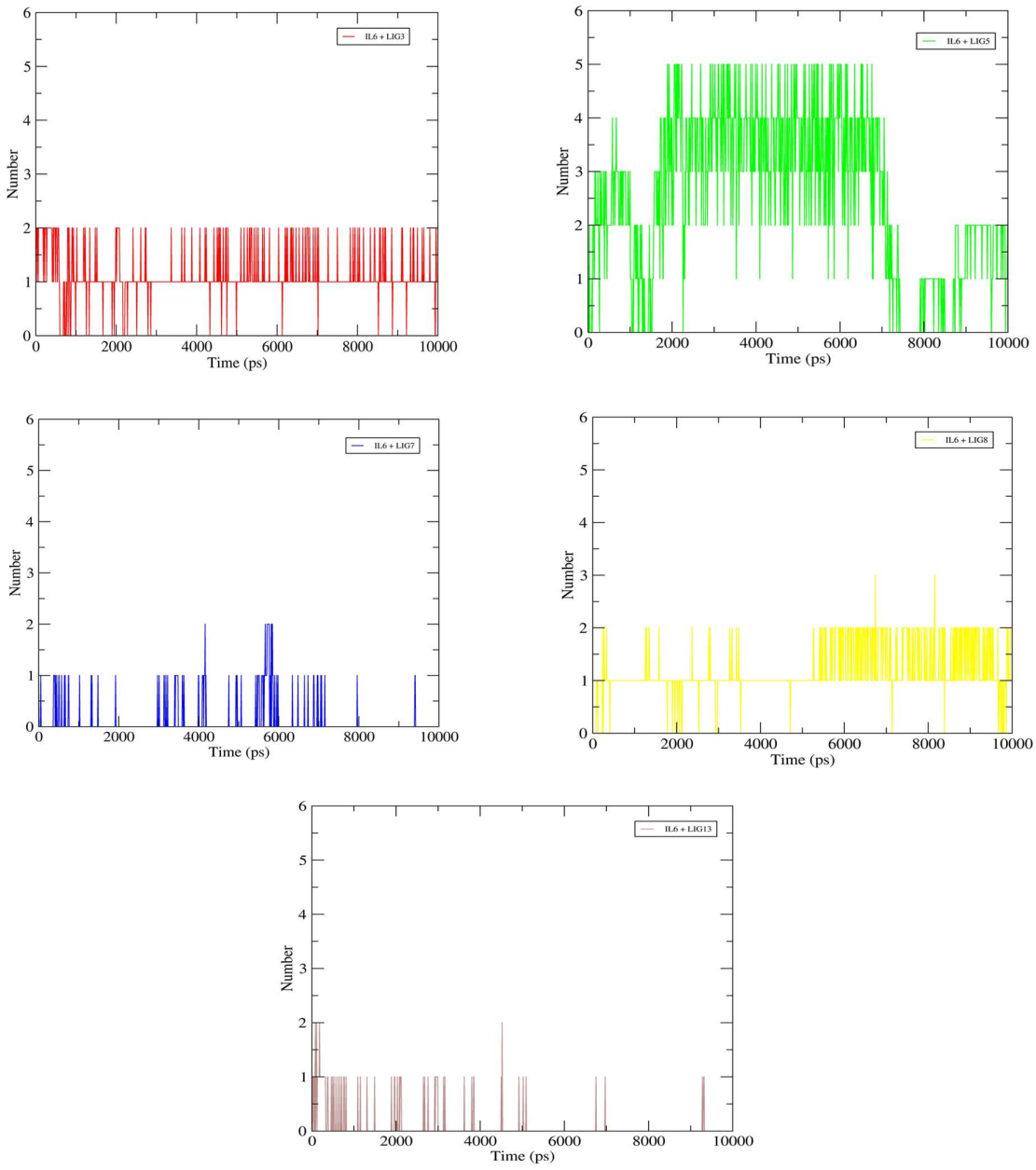


Figure 13: H-bond against GP-130

Red depicts C3, Green shows C5, Blue represents C7, Yellow represents C8 & Brown represents C13

Table 15: H-Bond for IL-6R

	Compounds	Mean (nm)
IL-6R	C3	0.718 +/- 0.728
	C5	1.771 +/- 1.177
	C7	0.659 +/- 0.599
	C8	0.402 +/- 0.631
	C13	0.524 +/- 0.675

Table 16 H-Bond for GP-130

	Compounds	Mean (nm)
GP-130	C3	1.189 +/- 0.430
	C5	2.486 +/- 1.484
	C7	0.109 +/- 0.346
	C8	1.194 +/- 0.486
	C13	0.080 +/- 0.287

The Compounds in the tables represent the Ligands presented on the graph.

C3 represents LIG3 in red color, C5 represents LIG5 in green color, C7 represents LIG7 in blue color, C8 represents LIG8 in yellow color and C13 represents LIG13 in brown color.

The stability of hydrogen bonds of all complexes was depicted across the MDS. The bonds were calculated through the distance trajectories. In both cases, the maximum number of hydrogen bonds was shown by C5.

5. CONCLUSION

The result analyzed in this study stipulates that the two dominant compounds C7 & C13 were found best among all the compounds. Although C5 was also depicted in some trajectories. These compounds can contribute to potential candidates for the development of drug design that can effectively constrain both the IL-6 receptors. The validation of these compounds went through various computational insights such as multiple docking, data analysis, and then MD simulation. These compounds can serve as lead compounds as anti-rheumatic for Rheumatoid Arthritis patients who become drug resistant from existing medicines. Although, its recommended to validate more through *in – vitro* experiments before proceeding with clinical trials.

REFERENCES

Actemra® product monograph. Mississauga, ontario; november 28, 2018.

Ajeganova, s., & huizinga, t. (2017). Sustained remission in rheumatoid arthritis: latest evidence and clinical considerations. *Therapeutic advances in musculoskeletalhdisease*, *9*(10),249–262.

Akira s, kishimoto t 1992. Il-6 and nf-il6 in acute-phase response and viral infection. Immunol rev 127: 25–50

Aletaha d, smolen js. Diagnosis and management of rheumatoid arthritis: a review. Jama. 2018 oct 02;320(13):1360-1372.

Alonzi t, fattori e, lazzaro d, et al. Interleukin 6 is required for the development of collagen-induced arthritis. The journal of experimental medicine. 1998;187(4):461–468.

Amaya-amaya j, rojas-villarraga a, mantilla rd, et al. Rheumatoid arthritis. In: anaya jm, shoenfeld y, rojas-villarraga a, et al., editors. Autoimmunity: from bench to bedside [internet]. Bogota (colombia): el rosario university press; 2013 jul 18. Chapter 24. Arthritis foundation. Rheumatoid arthritis: causes, symptoms, treatments and more. *(https://www.arthritis.org/diseases/rheumatoid-arthritis)* accessed 2/18/2022.

Atsushi ogata and others, il-6 inhibitor for the treatment of rheumatoid arthritis: a comprehensive review, *modern rheumatology*, volume 29, issue 2, 4 march 2019, pages 258–267.

Browne e.p. Regulation of b-cell responses by toll-like receptors. Immunology. 2012;136:370–379.

Brzustewicz e., bryl e. The role of cytokines in the pathogenesis of rheumatoid arthritis—practical and potential application of cytokines as biomarkers and targets of personalized therapy. *Cytokine.* 2015;76:527.

Bugatti s., vitolo b., caporali r., montecucco c., manzo a. B cells in rheumatoid arthritis: from pathogenic players to disease biomarkers. Biomed res. Int. 2014;2014:681678.

Centers for disease control and prevention. Rheumatoid arthritis. (https://www.cdc.gov/arthritis/basics/rheumatoid-arthritis.html) accessed 2/18/2022

Chauhan k, jandu js, brent lh, et al. Rheumatoid arthritis. [updated 2023 may 25]. In: statpearls [internet]. Treasure island (fl): statpearls publishing; 2023 jan-. Available from: https://www.ncbi.nlm.nih.gov/books/nbk441999

Chow d, he x, snow al, rose-john s, garcia kc. Structure of an extracellular gp130 cytokine receptor signaling complex. Science. 2001;291:2150–5.

Cross m, smith e, hoy d, et al. The global burden of rheumatoid arthritis: estimates from the global burden of disease 2010 study. Ann rheum dis. 2014;73:1316–22.

Crowson, c. S., liao, k. P., davis, j. M., 3rd, solomon, d. H., matteson, e. L., knutson, k. L., hlatky, m. A., & gabriel, s. E. (2013). Rheumatoid arthritis and cardiovascular disease. *American heart journal*, *166*(4), 622–628.

Deane, k. D., demoruelle, m. K., kelmenson, l. B., kuhn, k. A., norris, j. M., & holers, v. M. (2017). Genetic and environmental risk factors for rheumatoid arthritis. *Best practice & research. Clinical rheumatology*, *31*(1), 3–18.

Deane, kevin & holers, v. (2020). Rheumatoid arthritis: pathogenesis, prediction and prevention - an emerging paradigm shift. Arthritis & rheumatology. 73. 10.1002/art.41417.

Derksen v.f.a.m., huizinga t.w.j., van der woude d. The role of autoantibodies in the pathophysiology of rheumatoid arthritis. *Semin. Immunopathol.* 2017;39:437–446.

Ding, q., hu, w., wang, r. *Et al*. Signaling pathways in rheumatoid arthritis: implications for targeted therapy. *Sig transduct target ther* **8**, 68 (2023).

Entezami, p., fox, d. A., clapham, p. J., & chung, k. C. (2011). Historical perspective on the etiology of rheumatoid arthritis. *Hand clinics*, *27*(1), 1–10.

Ghahremanian, s., rashidi, m. M., raeisi, k., & toghraie, d. (2022). Molecular dynamics simulation approach for discovering potential inhibitors against sars-cov-2: a structural review. *Journal of molecular liquids*, *354*, 118901

Greenhill cj, rose-john s, lissilaa r, ferlin w, ernst m, hertzog pj. Et al. Il-6 trans-signaling modulates tlr4-dependent inflammatory responses via stat3. Journal of immunology. 2011;186:1199–208.

Guo, q., wang, y., xu, d., nossent, j., pavlos, n. J., & xu, j. (2018). Rheumatoid arthritis: pathological mechanisms and modern pharmacologic therapies. *Bone research*, *6*, 15.

Heidari b. (2011). Rheumatoid arthritis: early diagnosis and treatment outcomes. *Caspian journal of internal medicine*, *2*(1), 161–170.

Heidari b. Undifferentiated arthritis, the associated factors of progressive disease and treatment decision. *Caspian j intern med.* 2010;1:79–88.

Hirano t, akira s, taga t, kishimoto t 1990. Biological and clinical aspects of interleukin 6. *Immunol today* 11: 443–449

Hollingsworth, s. A., & dror, r. O. (2018). Molecular dynamics simulation for all. *Neuron*, *99*(6), 1129–1143.

Humphrey, w., dalke, a., and schulten, k. (1996). Vmd: visual molecular dynamics. J. Mol. Graph. 14, 33–38.

Kamimura d, arima y, hirano t, ogura h, murakami m. Il-6 and inflammatory diseases. In: yoshimoto t, editor. Cytokine frontiers. Tokyo: springer 2014. P. 53–78.

Kay, j., & upchurch, k. S. (2012). Acr/eular 2010 rheumatoid arthritis classification criteria. *Rheumatology (oxford, england)*, *51 suppl 6*, vi5–vi9.

Kaye, a. G., & siegel, r. (2020). The efficacy of il-6 inhibitor tocilizumab in reducing severe covid-19 mortality: a systematic review. *Peerj*, *8*, e10322.

Krishnan, e., tugwell, p., & fries, j. F. (2004). Percentile benchmarks in patients with rheumatoid arthritis: health assessment questionnaire as a quality indicator (qi). *Arthritis research & therapy*, *6*(6), r505–r513

Kumar, p., & banik, s. (2013). Pharmacotherapy options in rheumatoid arthritis. *Clinical medicine insights. Arthritis and musculoskeletal disorders*, *6*, 35–43.

Lesina m, kurkowski mu, ludes k, rose-john s, treiber m, klöppel g. Et al. Stat3/socs3 activation by il-6 trans signaling promotes progression of pancreatic intraepithelial neoplasia and development of pancreatic cancer. Cancer cell. 2011;19:456–69.

Liu, t., zhang, l., joo, d., & sun, s. C. (2017). Nf-κb signaling in inflammation. *Signal transduction and targeted therapy*, *2*, 17023

Lubberts e, van den berg wb. Cytokines in the pathogenesis of rheumatoid arthritis and collagen-induced arthritis. In: madame curie bioscience database [internet]. Austin (tx): landes bioscience; 2000-2013.

Manca m.l., alunno a., d'amato c., bistoni o., puxeddu i., gerli r., migliorini p., pratesi f. Anti-citrullinated peptide antibodies profiling in established rheumatoid arthritis. *Jt. Bone spine.* 2017;85:441–445.

Matteson el, et al. Overview of the systemic and nonarticular manifestations of rheumatoid arthritis. Https://www.uptodate.com/contents/search. Accessed feb. 9, 2021.Matthias jarlborg, cem gabay, systemic effects of il-6 blockade in rheumatoid arthritis beyond the joints, cytokine,volume 149,2022, 1043-4666.

Mcinnes ib, schett g. The pathogenesis of rheumatoid arthritis. *The new england journal of medicine.* 2011;365(23):2205–2219.

Mcinnes ib, schett g. The pathogenesis of rheumatoid arthritis. *The new england journal of medicine.* 2011;365(23):2205–2219.

Moore cp, wilkins rf. The subcutaneous nodule: its significance in the diagnosis of rheumatic disease. Sem arth rheum. 1977;7:63–79.

Nistala k, adams s, cambrook h, et al. Th17 plasticity in human autoimmune arthritis is driven by the inflammatory environment. *Proc natl acad sci u s a.* 2010;107:14751–14756.

Ohshima s, saeki y, mima t, et al. Interleukin 6 plays a key role in the development of antigen-induced arthritis. *Proceedings of the national academy of sciences of the united states of america.* 1998;95(14):8222–8226.

Pesce b, soto l, sabugo f, et al. Effect of interleukin-6 receptor blockade on the balance between regulatory t cells and t helper type 17 cells in rheumatoid arthritis patients. *Clinical & experimental immunology.* 2013;171(3):237–242.

Promelle, v., goeb, v., & gueudry, j. (2021). Rheumatoid arthritis associated episcleritis and scleritis: an update on treatment perspectives. *Journal of clinical medicine*, *10*(10), 2118.

Radu, a. F. & bungau, s. G. Management of rheumatoid arthritis: an overview. *Cells* **10**, 2857 (2021).

Raimondo, m. G., biggioggero, m., crotti, c., becciolini, a., & favalli, e. G. (2017). Profile of sarilumab and its potential in the treatment of rheumatoid arthritis. *Drug design, development and therapy*, *11*, 1593–1603.

Rheumatoid arthritis support network. Ra progression: what are the signs of rheumatoid arthritishprogression? (https://www.rheumatoidarthritis.org/ra/symptoms/progression/) accesse d 2/18/2022.

Rose-john s. (2012). Il-6 trans-signaling via the soluble il-6 receptor: importance for the pro-inflammatory activities of il-6. International journal of biological sciences, 8(9), 1237–1247.

Sahatçiu-meka v, rexhepi s, manxhuka-kerliu s, et al. Extra-articular manifestations of seronegative and seropositive rheumatoid arthritis. *Bosnian journal of basic medical sciences.* 2010;10(1):26–31.

Sato k, tsuchiya m, saldanha j, et al. Reshaping a human antibody to inhibit the interleukin 6-dependent tumor cell growth. *Cancer research.* 1993;53(4):851–856.

Schlegel p.m., steiert i., kötter i., müller c.a. B cell contribute to heterogeneity of il-17 producing cells in rheumatoid arthritis and healthy controls. Plos one. 2013;8:e82580. Doi: 10.1371/journal.pone.0082580.

Scott dl, wolfe f, huizinga tw. Rheumatoid arthritis. Lancet. 2010;376:1094–108.

Scott lj. Sarilumab: first global approval. *Drugs*. 2017;77:705–712.

Sebba a. (2008). Tocilizumab: the first interleukin-6-receptor inhibitor. *American journal of health-system pharmacy : ajhp : official journal of the american society of health-system pharmacists*, *65*(15), 1413–1418.

Selmi c, kon e, de santis m, et al. How advances in personalized medicine will change rheumatology. Per med. 2018;15:75–8.

Sharma, y., kumar, n., & thakur, d. (2021). Interleukin 6 in patients with rheumatoid arthritis. Intechopen. Doi: 10.5772/intechopen.96887. Shimizu t, takahata m, kimura-suda h, et al. Autoimmune arthritis deteriorates bone quantity and quality of periarticular bone in a mouse model of rheumatoid arthritis. Osteoporos int 2016;28:709–18.

Silman aj, pearson je. Epidemiology and genetics of rheumatoid arthritis. *Arthritis res.* 2002;4(suppl. 3):s265–s272.

Smolen js, aletaha d, bijlsma jw, et al. Treating rheumatoid arthritis to target: recommendations of an international task force. *Annals of the rheumatic diseases.* 2010;69(4):631–637.

Smolen js, beaulieu a, rubbert-roth a, et al. Effect of interleukin-6 receptor inhibition with tocilizumab in patients with rheumatoid arthritis (option study): a double-blind, placebo-controlled, randomised trial. *Lancet.* 2008;371:987–997.

Suresh e. (2004). Diagnosis of early rheumatoid arthritis: what the non-specialist needs to know. *Journal of the royal society of medicine*, *97*(9), 421–424.

Tanaka, t., narazaki, m., & kishimoto, t. (2014). Il-6 in inflammation, immunity, and disease. *Cold spring harbor perspectives in biology*, *6*(10), a016295.

Tran q-h, nguyen q-t, vo n-q-h, mai tt, tran t-t-n, tran t-d, et al. (2022) structure-based 3d-pharmacophore modeling to discover novel interleukin 6 inhibitors: an in silico screening, molecular dynamics simulations and binding free energy calculations. Plos one 17(4): e0266632.

Van staa tp, geusens p, bijlsma jw, leufkens hg, cooper c. Clinical assessment of the long-term risk of fracture in patients with rheumatoid arthritis. Arthritis rheum 2006;54:3104–12.

Yap, h. Y., tee, s. Z., wong, m. M., chow, s. K., peh, s. C., & teow, s. Y. (2018). Pathogenic role of immune cells in rheumatoid arthritis: implications in clinical treatment and biomarker development. *Cells*, *7*(10), 161.

Yip, r. M. L., & yim, c. W. (2021). Role of interleukin 6 inhibitors in the management of rheumatoid arthritis. *Journal of clinical rheumatology: practical reports on rheumatic & musculoskeletal diseases*, *27*(8), e516–e524.

Zhang jg, zhang y, owczarek cm, ward ld, moritz rl, simpson rj. Et al. Identification and characterization of two distinct truncated forms of gp130 and a soluble form of leukemia inhibitory factor receptor alpha-chain in normal human urine and plasma. J biol chem. 1998;273:10798–805.

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