

## Research Article

# Molecular Modeling Guided Drug Designing for the Therapeutic Treatment of Rheumatoid Arthritis

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Rheumatoid arthritis (RA) is a systemic inflammatory disorder that can cause destructive joint disease, significant disability, and increased mortality. RA is the most frequent of all chronic inflammatory joint diseases, and its prevalence frequency in Pakistan is 1.6 per thousand people. Different cytokines and receptors were involved in the triggering of RA, including interleukin-6 (IL-6), major histocompatibility complex (MHC) antigen human leukocyte (HLA-DR) receptor, and CD20. Several studies illustrated RA as an inherent immune response and triggered due to the “shared epitope.” Therefore, the involvement of all these receptors (IL-6, HLA-DR, and CD20) leads to the neurological, ocular, respiratory, cardiac, skin, and hematological manifestations that have been considered a potential therapeutic target for drug design. Various herbal, natural, and synthetic source inhibitors of interleukin-6 (IL-6), human leukocyte (HLA-DR), and CD20 were studied and reported previously. Reported inhibitors are compared to elucidate the best inhibitor for clinical trials, leading to the orally active drug. In this study, a computer-aided drug designing approach disclosed the potential inhibitors for all receptors based on their distinct binding affinity. Moreover, drug suitability was carried out using Lipinski’s rule by considering the adsorption, distribution, metabolism, and excretion (ADME) of ligands. Results elucidated “calycosin 7-O-glucoside” and “angeliferulate” as putative ligands for IL-6 and HLA-DR, respectively. However, the pharmacokinetic properties (ADMET) revealed angeliferulate as an effete ligand for the biological system compared to calycosin 7-O-glucoside. Based on docking, drug toxicity profiling or pharmacokinetics, and MD simulation stability, this study highlights orally active therapeutic inhibitors to inhibit the activity of pivotal receptors (IL6, HLA-DR, and CD20) of RA in humans. After clinical trials, the resultant inhibitors could be potential therapeutic agents in the drug development against RA.

## 1. Introduction

Rheumatoid arthritis (RA) is considered a systemic inflammatory disorder that can cause significant disability and joint disease and enhance the mortality rate [1]. The persis-

tent reduction in RA mortality has been noticed, but the prevalence has increased the economic burden [2]. RA is an autoimmune chronic inflammatory disease with articular and extra-articular manifestations. Autoimmune disease is a type of connective tissue disease that mostly overlays the

joint and epitenon synovium [3]. RA leads to joint hardness, inflammation, and probable loss of function in any joint lined by a synovial membrane. However, small joints of hands and feet are most frequently damaged [4]. Other extra-articular tissues, including the vasculature, lungs, muscles, skin, and heart, are also affected by RA inflammation [3]. RA patients may suffer an increase in coronary infarction (heart attack), the risk of arterial sclerosis (hardening of the arteries), and stroke [5]. Some other issues associated with RA could include endocarditis, inflammation in the cardiac valve, and fibrosis [6]. The prevalence of RA in Pakistan (1.6 per 1,000 people) is less compared to that in Bahrain (2.0), Djibouti (2.2), Lebanon (2.1), Qatar (1.8), Somalia (1.9), and Tunisia (1.8) [7]. However, the frequency of RA in North America and Europe [8, 9] is comparatively higher than that in Japan [10], China [4], Indonesia, Malaysia, the Philippines, and even rural Africa [11]. The prevalence of RA is relatively constant at 0.5–1% worldwide [12].

A growing number of disease pathways have been discovered and reported [13–16], leading to potential target proteins for drug design [17–20]. Several studies illustrate RA as an inherent immune response [21], which could be genetic due to “shared epitope.” The shared epitope is a motif sequence of five amino acids (70–74 residues) present on the HLA-DR $\beta$  chain [22–24], associated with severe rheumatoid arthritis in most cases [25]. In RA pathogenesis, the interleukin-6 receptor (IL6R) also plays a pivotal role in triggering RA [26]. Since RA mainly attacks synovial articulations and induces systemic inflammation over time, the insights into the pathogenesis of RA have led to the development of B cell-driven therapies as promising new therapeutic targets in autoimmune disorders. Besides autoantibody-secreting cells, B cells may have several important roles in the immuno-pathogenesis of RA by producing proinflammatory cytokines [27–29]. The anti-CD20 rituximab antibody has been used to treat patients with RA-refractory Tumor Necrosis Factor (TNF) [30]. In the pathogenesis of rheumatoid arthritis, many receptors and antigens are involved, and the type of inflammatory response may also be different depending upon the initiation of autoimmune response [31, 32]. Several herbal, natural, and synthetic source inhibitors of interleukin-6 (IL-6), human leukocyte antigen (HLA-DR), and CD20 were studied and reported previously [3, 33–35]. However, a lack of comparison of the available inhibitors can lead to selection bias in a given biological system. To address this gap, previously reported RA inhibitors have been compared to identify the optimal choice molecule that can potentially lead to orally active drugs.

Computer-aided drug designing (CADD) [36, 37] is the most common and widely used method in the modern age for drug discovery [38]. In this study, three receptor proteins (IL-6, HLA-DR, and CD20) are docked in silico to elucidate efficient ligands against these receptors. Molecular docking is aimed at predicting the experimental binding affinities of inhibitors (ligands) within the active site of the target receptor (protein). Thus, molecular docking discloses the interaction of ligand with active sites of pro-

tein, whereas drug-likeness elucidates the potential of ligand for suitability in a biological system [39]. Hence, we predicted the drug-likeness of each ligand parallel to molecular docking analysis for explication of putative orally active ligand against rheumatoid arthritis. Physicochemical and molecular characteristics frequently have contradictory effects on pharmacokinetic and pharmacodynamic processes as well as medication safety. In tandem with target affinity, the current approach in drug discovery is to examine ADMET characteristics. In order to help the medicinal chemist in prioritizing drug candidates, the idea of “drug-likeness” delineates acceptable limits of fundamental features expressed as simple rules of thumb. Absorption, distribution, metabolism, excretion, and toxicity (ADMET) of chemical entities play important roles in drug discovery and development. A good drug candidate should not only be effective against the therapeutic target but also have adequate ADMET characteristics at a therapeutic dosage. During clinical development, ADMET profiling may assist to reduce possible hazards [40]. That is why we finalized therapeutic potential agents based on their ADMET properties as well.

## 2. Materials and Methods

**2.1. Characterization of Target Proteins.** The physicochemical properties of all receptor proteins (IL-6, HLA-DR, and CD20) were predicted using ProtParam online server [41]. ProtParam works on the Edelhoch method [42] to identify weight value, hydropathy values for extinction coefficients, instability index (II), and GRAVY value (grand average of hydropathy value) of proteins.

**2.2. Tertiary Structures of Target Proteins.** The crystal structures of target proteins were retrieved from the Research Collaboratory for Structural Bioinformatics Protein Data Bank (RCSB-PDB) [41]. The literature stated that IL-6, CD20, and HLA-DR are significant proteins involved in RA [12]. After analysis, IL-6 (PDB ID: 1il6) and CD20 (PDB ID: 2osI) were finalized based on high-resolution power and the lowest *R* value (*R* value determines how well the simulated diffraction pattern matches the experimentally observed diffraction pattern). Their tertiary structures are shown in Figure 1.

However, the tertiary structure of HLA-DR of *Homo sapiens* was not available in PDB, which was predicted through homology modeling. The primary sequence of HLA-DR *Homo sapiens* (UniProt ID: P01911) was retrieved from UniProtKB [43]. MODELLER [44] software was used to predict the 3D structure of HLA-DR through homology modeling. Predicted structure validation is necessary before doing docking to check the quality of the structure; therefore, the Ramachandran plot [45] was used for structural stereochemistry validation of our target proteins. The predicted tertiary structure of HLA-DR is shown in Figure 2.

**2.3. Screening of Chemical Inhibitors.** The initial step for pharmacophore designing is to identify the novel inhibitors against target receptors. The chemical compounds against active sites of IL-6 [46], CD20 [30, 47], and HLA-DR [48]

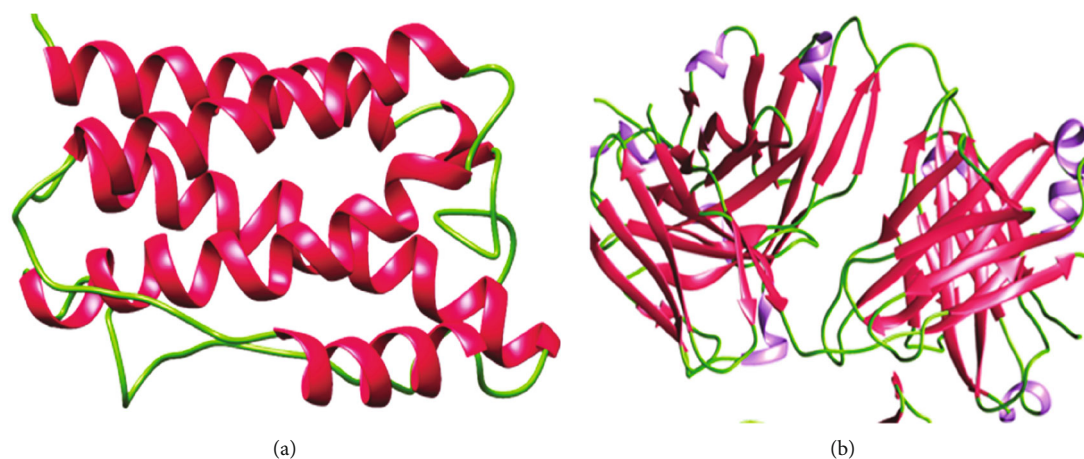


FIGURE 1: Retrieved 3D structure of target proteins: (a) tertiary structure of IL-6 and (b) 3D structure of CD20 antigen with the heavy as well as the light chain.

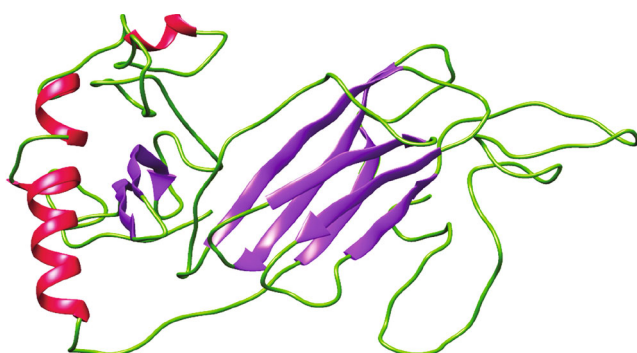


FIGURE 2: Predicted 3D structure of HLA-DR protein visualized by Chimera software.

have known medicines used for RA treatment. Therefore, natural and synthetic derivatives of compounds having inhibitory potential against our target proteins were retrieved, and structures of these ligands were downloaded using PubChem [39, 49, 50]. Ligands selection was based on using keywords of our targets such as “CD20”, “HLA-DR”, and “IL-6” in PubChem, and we selected those chemical entities having biological inhibitory assays against required sites which are helpful in treating RA disease.

PubChem retraces compounds based on their chemical formula and physicochemical properties [49]. In the current study, we mainly focused on three major receptors (HLA-DR, IL-6, and CD20) of RA [12]. Several herbal, synthetic, and animal source inhibitors (ligands) used in the study are listed in Table 1. In addition, X-ray crystalized tertiary structure of antibody “ofatumumab” (PDB ID: 6Y92) was retrieved from the Protein Data Bank (PDB) [51].

**2.4. Active Site Prediction.** InterPro [52, 53], an online web-based tool, was used to investigate the active sites of target proteins for molecular docking against reported ligands.

**2.5. Molecular Docking Analysis of Inhibitors.** Virtual screening (VS) is one of the standard steps for drug discovery before wet-lab experiments. This process involves the

estimation of the binding affinity of the drug candidate towards a target protein. Virtual screening also exhibits possible binding modes against active sites of target proteins. Most prominent drug candidates show promising binding affinity towards target protein and can be screened out using High-Performance Computing (HPC) infrastructure [54]. A molecular operating environment (MOE© 2009.10 version) was selected for molecular docking to identify a putative ligand for receptor inhibition [50, 55]. MOE© prioritizes efficient binding geometries based on *S*-score’s numerical value for docked poses among various available resources listed elsewhere.

Molecular docking [36, 56] analysis was implemented to finalize the hits for pharmacophore designing. For this, the 2D structures were obtained from the PubChem [57] using their respective PubChem ID, and energy minimization was done using the Marvin Desktop Suite, ChemAxon, Hungary [58]. The energy minimized structures were then converted to Microsoft Access Database (MDB) formats and further docked with the target structure of MOE©. Binding energy, molecular interactions (at the active site), and structural confirmation were the prime parameters to finalize the inhibitors. The target proteins (IL-6, CD20, and HLA-DR) were prepared by removing hetero-atoms and adding polar hydrogen and Kollmann charges using MOE©. The average value was found for binding energy as an *S*-score, and all the molecules above the average value (-6 kcal/mol) were chosen for pharmacophore designing [59]. This 3D structure was converted to MDB format, and a total of 30 confirmation poses of each ligand were created against respective receptor proteins. The first ten results (based on pose confirmation and binding energy) were analyzed using MOE© ligand-receptor interaction and pocket map tools.

**2.6. Enrichment Analysis of the Docking Library.** The chemical properties of selected active compounds were determined by enrichment analysis. Useful decoys (DUD) were narrowed down for enrichment analysis based on the chemical properties of compounds, as explained elsewhere [39]. A

TABLE 1: List of reported compounds against IL-6 and human HLA-DR for RA treatment.

Compounds	PubChem ID	Compounds	PubChem ID	Compounds	PubChem ID
Angelicide	5316848	Angelifer	N/A	Bupivacaine	2474
Calycosin 7-O-glucoside	5318267	Capsaicin	1548943	Celecoxib	2662
Chloroquine	2719	Ferulic acid	445858	Formononetin	5280378
Ononin	442813	Senkyunolide G	10013283	Senkyunolide I	11521428
Senkyunolide P	91731751	Valerophenone	66093	Vanillic acid	8468

subgroup of commercialized chemical compounds, 31,200 in number, was randomly selected from a broader dataset. The same molecular weighted DUD distribution was chosen to narrow down the active compounds further and alleviate the notable tendency of the scoring component to support large compounds. The set of decoys was refined by filtering functional groups and cutting out both rotational bonds and molecular weight to guarantee that separation was mainly based on the scoring function. When we combined the randomly chosen subset with 450 receptor-specific ligands and excluded redundant structures, a total of 3100 compounds were obtained and prepared for additional docking investigations.

**2.7. Molecular Property Distribution of Arbitrarily Chosen Compounds.** Before conducting virtual screening, the molecular properties viz polar surface area [60], rotary bonds, molecular weight (MW), and logD of selected compounds against particular targets were already computed. In addition, hydrogen bond acceptors [61] and donors (HBD) were also calculated. This was needed to decide an evident foundational inconsistency between the known active compounds assortment and the arbitrarily picked compounds. However, drug-likeness was applied for the underlying irregular arrangement of the compounds. As a result, no apparent discrepancies were found between known active substances and randomly chosen compounds in the chemical properties around the predefined ranges.

**2.8. Pharmacokinetic Properties and Toxicity of Ligands.** The SwissADME© tool was utilized to predict the drug-likeness of our inhibitors [62]. Different drug-likeness parameters, including excretion, metabolism, distribution, and absorption of the compounds for the biological system, were evaluated [63]. The potential ligands were further assessed based on absorption, distribution, metabolism, excretion, and toxicity (ADMET) to reduce failure in drug discovery [64]. AdmetSAR© is a web-based tool containing information on toxicity, carcinogenicity, and compliance of medicine with Lipinski's regulations. The finalized ligands' SMILES were then posed for toxicity tests in the AdmetSAR© programmer [65].

**2.9. Molecular Dynamic Simulation.** Based on molecular interaction and visual evaluation of docking data, the top-ranked complexes (calycosin 7-O-glucoside-IL6, angeliferulate-HLA-DR, and ofatumumab-CD20) were chosen for the Molecular Dynamic (MD) simulation investigation. The MD simulation was run on a supercomputer cluster

with HPE-DL385 [66], Gen10, AMD EPYC 64 cores, RAM 128 GB, 500 GB SSDs, and NVIDIA Tesla T4 16 GB Accelerator on Schrodinger's Desmond module [61]. A water-soaked solvent solution was used to make predictions. Desmond's system builder tool was used to design the water-soaked solvated system. The TIP3P water model was looked at to see if it may help address the problem. A box with periodic boundary conditions and a buffer distance of at least 10 Å from the protein's outer surface was used to produce the orthorhombic simulation. To neutralise the system, a suitable amount of counterions was introduced. The isosmotic condition of the simulation box was maintained by adding 0.15 M NaCl. A predefined equilibration method was followed before the simulation's production run. At a temperature of 300°K and a pressure of 1.013 bar, the MD simulation was run. The Simulation Interaction Diagram was used to examine the MD simulation trajectory.

### 3. Results and Discussion

**3.1. Characterization of Target Proteins.** Physiochemical properties of HLA-DR were predicted from ProtParam©, an online web-based tool. Results showed that HLA-DR protein has a molecular weight of 29914.10 Daltons and isoelectric pH 7.61. The GRAVY value -0.206 indicates that HLA-DR has hydrophilic nature. The instability index of 46.65 predicted that HLA-DR is a stable protein containing an aliphatic index of 80.86, as shown in Table 2.

IL-6 has a molecular weight of 20980.97 Daltons and isoelectric pH 6.22. The GRAVY value -0.498 indicates that IL-6 has hydrophilic property. The instability index of 59.45 predicted that IL-6 is a stable protein containing an aliphatic index of 84.43, as shown in Table 3.

CD20 has a molecular weight of 33077.32 Daltons and isoelectric pH 5.04. The GRAVY value -0.048 exhibits the hydrophilic nature of IL-6. The instability index of 64.69 predicted IL-6 as a stable protein containing an aliphatic index of 91.62, as shown in Table 4.

**3.2. In Silico Molecular Docking.** Molecular docking of IL-6, HLA-DR, and CD20 target proteins was accomplished by using MOE©. The results verified that adopted inhibitors were inside the active pocket sites of the target proteins with possible interactions. However, some have shown an important binding affinity with active site residues such as calycosin 7-O-glucoside, ononin, angeliferulate, and ofatumumab. An inhibitor with a lower value (high in negative) of free binding energy is supposed to establish strong interaction

TABLE 2: Physiochemical properties of HLA-DR.

Sr. no.	Property	Value
1	Residues	266
2	Total atoms	4178
3	Theoretical pI	7.61
4	Molecular weight	29914.10
5	Aliphatic index	80.86
6	Extinction coefficient*	41285 at Abs 0.1% 1.380, assuming all pairs of Cys residues form cystines
7	Charge	Positive
8	Instability index	46.65
9	Grand average of hydropathicity (GRAVY)	-0.206
10	Chemical formula	$C_{1335}H_{2072}N_{370}O_{389}S_{12}$

TABLE 3: Characteristics of properties of IL-6 predicted by ProtParam.

Sr. no.	Property	Value
1	Residues	185
2	Total atoms	2956
3	Theoretical pI	6.22
4	Molecular weight	20980.97
5	Aliphatic index	84.43
6	Extinction coefficient*	10220 at Abs 0.1% 0.487, assuming all pairs of Cys residues form cystines
7	Charge	Negative
8	Instability index	59.45
9	Grand average of hydropathicity (GRAVY)	-0.498
10	Chemical formula	$C_{917}H_{1487}N_{255}O_{288}S_9$

TABLE 4: Physiochemical properties of Homo sapiens B lymphocyte antigen CD20 protein.

Sr. no.	Property	Value
1	Residues	297
2	Total atoms	4665
3	Theoretical pI	5.04
4	Molecular weight	33077.32
5	Aliphatic index	91.62
6	Extinction coefficient*	27180 at Abs 0.1% 0.822, assuming all pairs of Cys residues form cystines
7	Charge	Positive
8	Instability index	64.69
9	Grand average of hydropathicity (GRAVY)	-0.048
10	Chemical formula	$C_{1481}H_{2349}N_{373}O_{443}S_{19}$

on a specific active site; therefore, the results are ranked based on binding affinity, as shown in Table 5.

We docked 15 synthetic compounds against IL-6 and HLA-DR and one antibody against CD20 antigen. Thus, MOE© generated 200 possible poses with numerous *S*-scores (binding energy score). Out of 200, the top pose of each ligand is enlisted in Table 5. Out of these top poses, calycosin 7-O-glucoside, angeliferulate (*S*-score = -10.9376), and ofatumumab antibody (*S*-score = -10.3737) have shown maximum binding energy affinity (*S*-score = -10.751) against

IL-6, HLA-DR, and CD20, respectively. A threshold for binding energy score was set as -6.0 kcal/mol [59], and the ligand has to follow at least three conditions out of Lipinski's rule of five. Docked poses of high binding affinity ligand-protein complex are shown in Figure 3.

**3.3. Enrichment Analysis.** Enrichment analysis represents the behavior of active DUD and ligands versus inactive ligands and DUD. Two thresholds ( $IC_{50}$  and Lipinski's rule of five) were implemented to differentiate between active and

TABLE 5: Binding affinities of inhibitors against active sites of respective target proteins.

Sr. no.	Ligands	PubChem ID	IL-6	HLA-DR	CD20
			Energy score (kcal/mol)	Energy score (kcal/mol)	Energy score (kcal/mol)
1	Calycosin 7-O-glucoside	5318267	-10.751	-10.0867	
2	Ononin	442813	-10.1685	-9.5494	
3	Ferulic acid	445858	-9.6542	-7.6326	
4	Senkyunolide I	11521428	-9.5790	-8.4334	
5	Chloroquine	2719	-9.413	-9.0577	
6	Angeliferulate	N/A	-9.0491	-10.9376	
7	Celecoxib	2662	-8.9649	-8.5187	
8	Capsaicin	1548943	-8.9621	-9.5142	
9	Formononetin	5280378	-8.3592	-7.7794	
10	Bupivacaine	2474	-8.0281	-7.3768	
11	Angelicide	5316848	-7.3918	-9.1857	-10.3737 (antibody ofatumumab)
12	Senkyunolide P	91731751	-7.3510	-10.9160	
13	Senkyunolide G	10013283	-7.1097	-7.4000	
14	Valerophenone	66093	-6.6092	-6.4327	
15	Vanillic acid	8468	-6.5054	-8.6943	

inactive ligands. Those compounds were considered active in which IC<sub>50</sub> values ranged between 1 and 10  $\mu$ M, while the rest were declared inactive. According to Lipinski's rule of five (LRO5), the chemical compounds violating more than one condition of Lipinski's rule were supposed to be inactive for biological implications and *vice versa*. The graphical representation of enrichment analysis of active and inactive ligands is shown in Figure 4.

**3.4. Drug Liability and Toxicity Evaluation.** The ligand's antagonistic interactions with receptor protein do not ensure the inhibitor is an active oral drug. Consequently, ADME and drug-likeness play a critical role in inspecting the credentials of ligands for biological systems. Egan's BOILED-Egg method and LRO5 were utilized to evaluate ligands' pharmacokinetics. LRO5 relies upon edge upsides of four physicochemical properties: molecular weight (MW)  $\leq$  500 g/mol, lipophilicity  $\leq$  5, hydrogen bond donors (HBD)  $\leq$  5, and hydrogen bond acceptors  $\leq$  10. The radar diagram demonstrates that both selected ligands satisfied the LRO5, which indicates the suitability of these chemical compounds (ligands) for bioavailability Figure 5. However, immunoassay is necessary to cross-check our findings related to the ofatumumab monoclonal antibody.

Calycosin 7-O-glucoside showed human intestinal absorption, obeyed Lipinski's rule, behaved as noncarcinogenic, and led the liability of human ether-a-go-go-related gene (hERG) channel inhibition, estrogen receptor binding (ERB), androgen receptor binding (ARB), and thyroid receptor binding (TRB). These liabilities validate our identity hit (calycosin 7-O-glucoside) as an orally active therapeutic agent against RA. In contrast, angeliferulate did not show human intestinal absorption and exhibited liver toxicity. Calycosin 7-O-glucoside is considered a better therapeutic compound than angeliferulate due to its high binding affinity, good physicochemical properties, and liability with body channels, as shown in Table 6.

There are three general classes of medications typically utilized to treat rheumatoid joint pain: nonsteroidal calming specialists (NSAIDs), corticosteroids, and disease-modifying antirheumatic drugs (DMARDs) [20]. NSAIDs and corticosteroids have a short-span onset of action, while DMARDs have delayed effects, and it can take several weeks to show a clinical impact. DMARDs include methotrexate, sulfasalazine, leflunomide, etanercept, infliximab, adalimumab, certolizumab pegol, golimumab, abatacept, rituximab, tocilizumab, anakinra, and antimalarials. Other immunomodulators are occasionally used, including azathioprine (Imuran) and cyclosporine [67]. Since cartilage harm and hard disintegrations typically happen within the initial two years of the disease, DMARDs are preferred from the beginning and throughout sickness, usually when an analysis is affirmed. Pain-relieving drugs are also used at the start as they are concurrently in diminishing torment until DMARDs produce results [68, 69]. Though DMARDs significantly ameliorate the disease, they have multiple common side effects like abdominal pain, chills or high fever, dizziness, hair loss, headache, light sensitivity, itching, hepatic disorders, and low blood counts. Besides these, dry cough, fever, or difficulty in breathing may result from lung inflammation requiring special care in COPD patients [70].

With all the advancements in DMARD research, there is still a need for a greater understanding of its underlying mechanisms, as by modifying the mechanism of drugs' mode of action towards studying the exact mechanism of disease at molecular levels, we can facilitate the development of new drugs and produce a paradigm shift in treatment [71].

**3.5. Molecular Dynamic Simulation.** Docking alone cannot provide full insight into the binding mode, stability, and dynamics of proposed ligands. As a result, we used the Desmond module of Schrödinger to run MD simulations for different nanosecond frames based on the stability point of

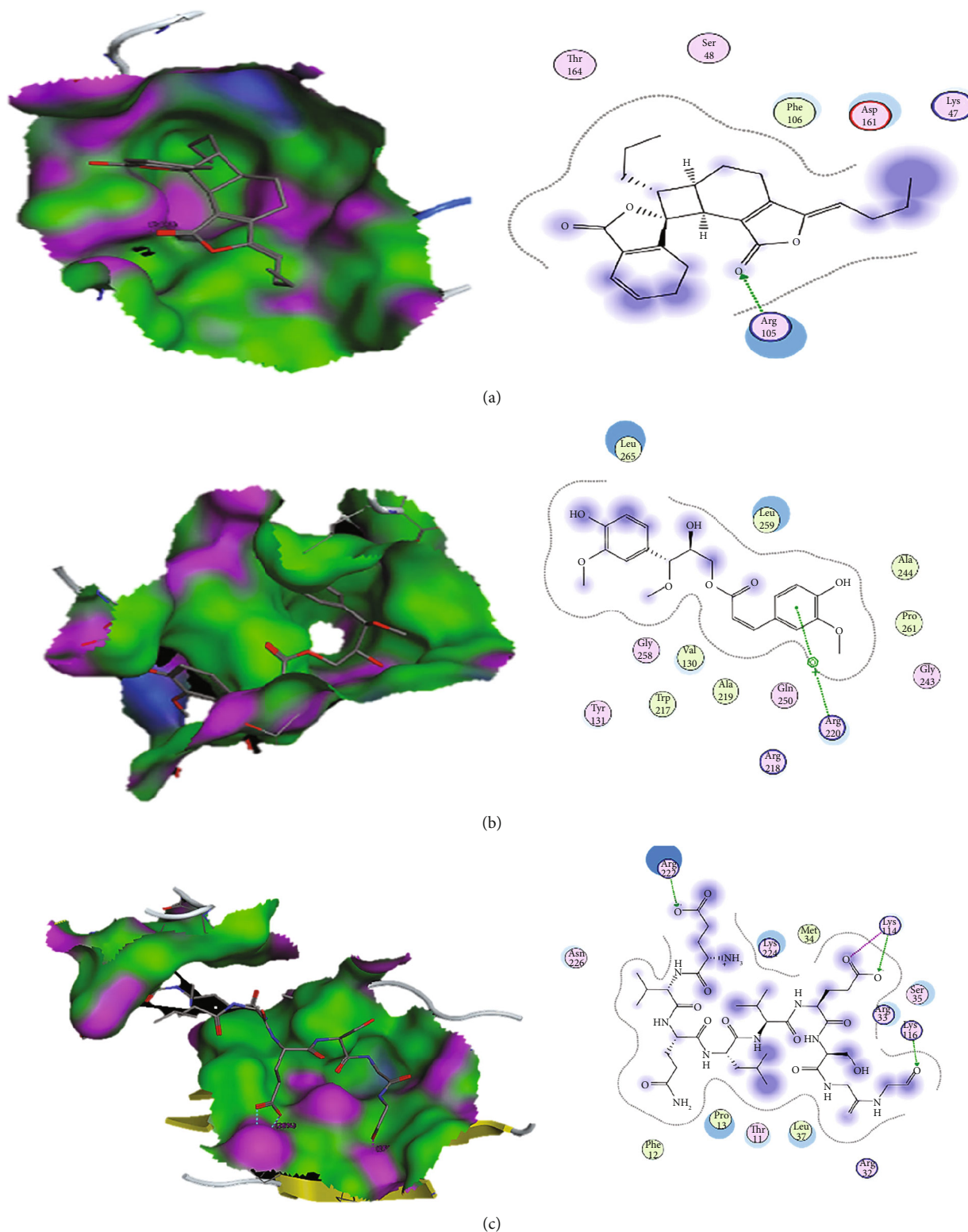


FIGURE 3: 3D and 2D docked ligand-receptor complexes showing inhibition of respective receptors: (a) calycosin 7-O-glucoside is completely covering the active site of IL-6 with  $-10.751$  kcal/mol binding affinity, (b) angeliferulate shows complex intermolecular attractions with binding affinity of  $-10.9376$  kcal/mol to inhibit HLA-DR active site, and (c) the activity of CD20 is inhibited by binding of ofatumumab with  $-10.3737$  kcal/mol binding affinity at the active site.

docked complexes with human HLA-DR, IL-6 and CD20. Based on molecular interaction and visual evaluation of human receptor protein's docking results, the top-ranked

complexes were chosen for the MD simulation investigation. The requirement for this simulation was further supported by the drug toxicity profile (Table 6), since

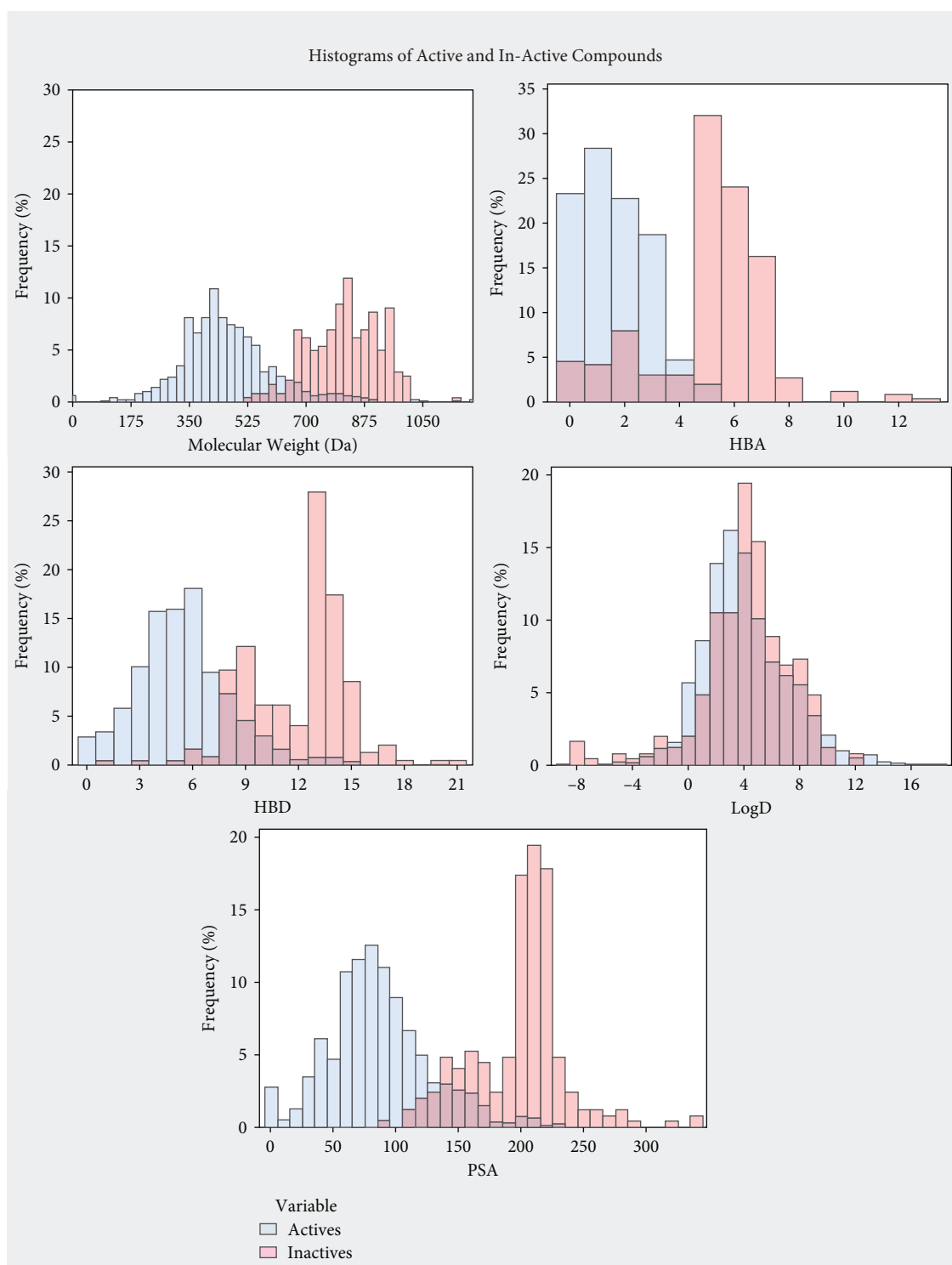


FIGURE 4: ADMET parameter disseminations of ligands (observing+decoys): based on the threshold, light blue ligand bars are active and pink color bars are inactive in benchmarking sets.

calycosin 7-O-glucoside, angeliferulate, and ofatumumab demonstrate drug appropriateness for IL-6, HLA-DR, and CD20, respectively.

The MD simulation revealed that calycosin 7-O-glucoside fits tightly into the binding pocket of human IL-6, as simulation trajectories of both ligand and receptor are

aligned. Human IL-6 and calycosin 7-O-glucoside are making a strong complex and have shown strong structural dynamic stability after docking as complex RMSD is 0.53 Å (Figure 6) throughout 200-nanosecond MD simulation, which is almost a negligible difference in MD simulation trajectory criteria.



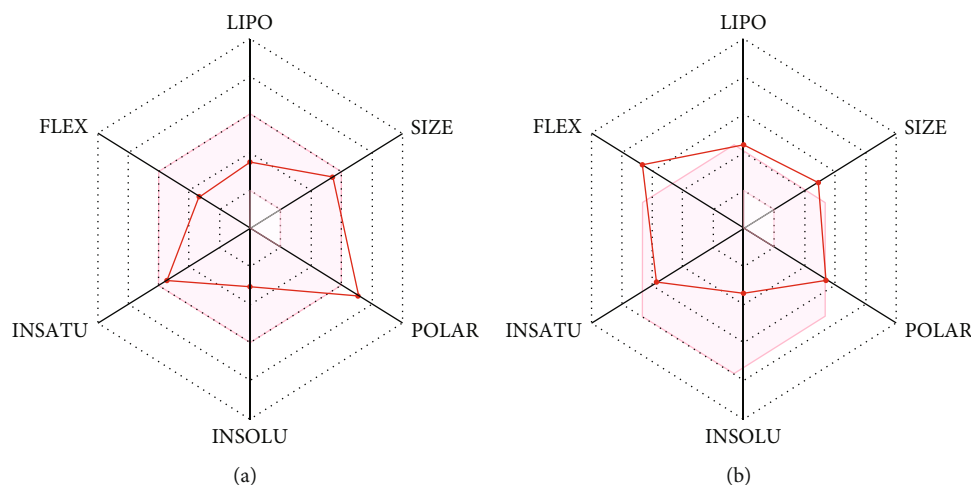


FIGURE 5: Radar diagram representing Lipinski's rule of five: (a) calycosin 7-O-glucoside and (b) angeliferulate both are not violating LRo5 condition and considered active ligands against the biological system.

TABLE 6: Physicochemical properties and drug toxicity liability profiling of identified hits.

No.	Compound	Drug like properties					ERB	ARB	TRB
		HIA	HOB	Carcinogenicity	hERG CI	Hepatotoxicity			
		< 0.7	> 0.5 (50%)		≤ 0.7	≥ 0.8 (80%)			
1	Calycosin 7 - O-glucoside	0.37	0.52	Non-carcinogenic	0.59	0.67	0.77	0.68	0.52
2	Angeliferulate	0.98	0.7	Non-carcinogenic	0.41	0.65	0.78	0.67	0.74

HIA: human intestinal absorption; HOB: human oral bioavailability; ERB: estrogen receptor binding; ARB: androgen receptor binding; TRB: thyroid receptor binding; hERG CI: human ether-a-go-go-related gene channel inhibition; green color: potentially liable with transporter/channel; light green color: liable with transporter/channel; red color: does not show liability with transporters; light red color: slightly shows transporter liability.

The protein-ligand complex combined RMSD during MD simulation remains stable throughout the simulation time frame of 200 ns. From this stability, it is elucidated that with respect to time, this docked complex is structurally stable due to hydrogen bonds.

While the MD simulation RMSD trajectory of HLA-DR and angeliferulate complex has revealed that docked complex is dynamically stable with RMSD value of 1.5 Å to 3.5 Å (RMSD change < 3 Å) throughout a 150-nanosecond time period (Figure 7). The combined RMSD of the protein-ligand complex during this MD simulation also remained stable throughout the simulation time frame of 150 ns as combined trajectories did not show any notable fluctuations (only shown at a short time frame from 82 ns to 86 ns) in trajectory or RMSD change. This stability elucidated that with respect to time, the docked complex is structurally stable after making hydrogen

bonds and other interactions. The combined RMSD of the protein-ligand complex during MD simulation remained stable throughout the simulation time frame of 150 ns.

CD20 and ofatumumab MD simulation RMSD trajectory plot elucidated this docked complex interaction stability throughout 100 ns simulation time with 1000 frames of snapshots. The CD20-ofatumumab complex RMSD plot has shown that the trajectory of RMSD deviates just 1.1 Å from the complex initial position at the docked time (Figure 8), and this deviation is acceptable and reflects the stability of the docked complex with respect to time. The shift in the protein's RMSD of the order of 1.8 Å-2.7 Å during the simulation suggests that the protein underwent moderate conformational change throughout the simulation.

Based on molecular docking, MD modeling, and toxicity profiling, calycosin 7-O-glucoside, angeliferulate, and

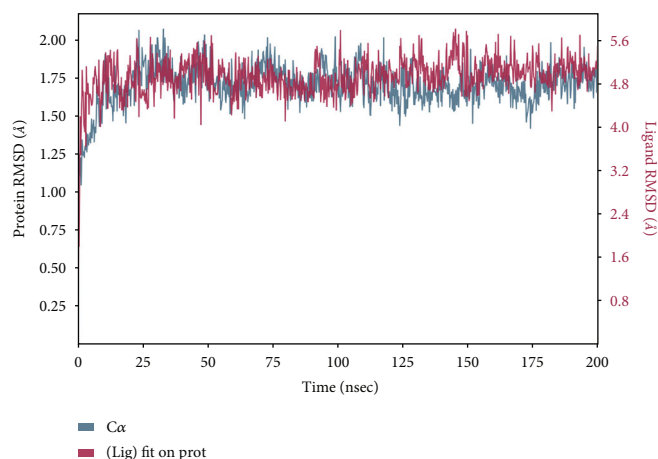


FIGURE 6: RMSD plot for  $C\alpha$  atom ( $\text{\AA}$ ) locations for the initial structure vs. the simulation time (ns) of human IL-6 and calycosin 7-O-glucoside (ligand) for the MD simulation of their interaction complex.

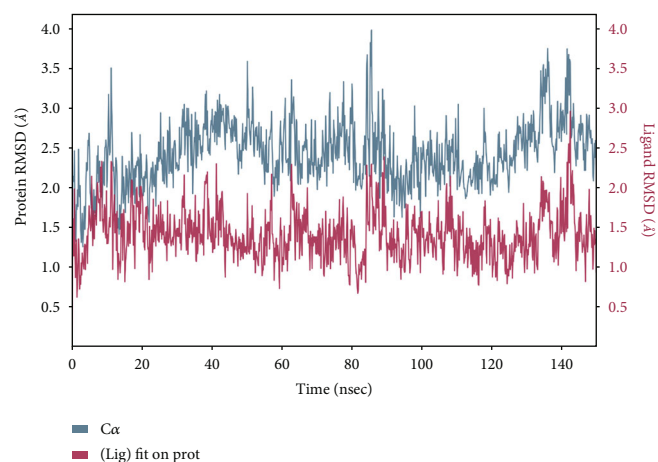


FIGURE 7: RMSD plot for  $C\alpha$  atom ( $\text{\AA}$ ) locations for the initial structure vs. the simulation time (ns) of human HLA-DR and angeliferulate (ligand) for the MD simulation of their interaction complex.

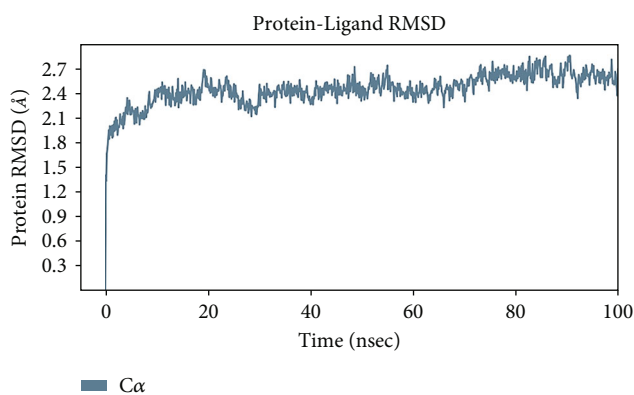


FIGURE 8: RMSD plot for  $C\alpha$  atom ( $\text{\AA}$ ) locations for the initial structure vs. the simulation time (ns) of human CD20 and ofatumumab (ligand) for the MD simulation of their interaction complex.

ofatumumab were discovered to be effective and selective potential agents to treat RA disease. Concerns have been raised about the risk of the usage of these agents; for example, it has been shown to increase the risk of cardiovascular disease and the severity of infections. Recent research suggests, however, that administration of these agents can reduce the incidence of cardiovascular disease and has a neutral infection risk. During clinical studies, it is advised that the other stated medical condition be considered to better examine its potential as a medication against HLA-DR, IL-6, and CD20 targets.

*In silico* comparative analysis is recognized as an initial step for insight into ligand-receptor complexes and drug targets that lead to a better selection process of potential disease inhibitors. Our findings show “calycosin 7-O-glucoside” as a potential orally active drug due to its better pharmacokinetic profile and significantly higher binding affinity against HLA-DR (-10.0867) and IL-6 (-10.751).

## 4. Conclusion

Briefly, combined results of molecular docking, Lipinski's rule of five, and drug toxicity assessment exhibited calycosin 7-O-glucoside ( $S$ -score =  $-10.751$ ) to be of most significance among selected ligands against RA. Angeliferulate ( $S$ -score =  $-10.9376$ ) showed an effective binding score against target proteins but less human intestinal absorption. Moreover, the hepatotoxicity of both compounds predicted adverse effects of these compounds on the liver after long-term usage. In contrast, the monoclonal antibody ofatumumab exhibited potential therapeutic efficacy against receptor (CD20) of RA. In the light of current findings, calycosin 7-O-glucoside has all those characteristics which should be considered during drug discovery and clinical trials.

## Data Availability

The corresponding author will provide all data produced during the current study on a reasonable request.

## Conflicts of Interest

The authors have declared no conflict of interest.

## Authors' Contributions

HAR and AAB conceptualized, designed, interpreted, and supervised the study and contributed to the final draft of the manuscript. SAA and MI performed molecular docking analysis. MH, MU, and QUA carried out drug-likeness methods and interpreted the ligands' pharmacokinetics. MM, MI, SAA, and MH contributed to writing the manuscript in discussion with all other authors.

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## References

- [1] A. Linos, J. W. Worthington, M. O'Fallon, and L. T. Kurland, "The epidemiology of rheumatoid arthritis in Rochester Minnesota: a study of incidence, prevalence, and mortality," *American Journal of Epidemiology*, vol. 111, no. 1, pp. 87–98, 1980.
- [2] N. S. Ab Rajab, M. R. Salleh, M. A. Mohd Yasin et al., "Genetic associations study of rs12745968 and rs4822752 between rheumatoid arthritis and schizophrenia patients," *Asian Journal of Medicine and Biomedicine*, p. 13, 2018.
- [3] S. Shakeel, W. Iffat, A. Qamar et al., "Healthcare Professionals' Compliance with the Standard Management Guidelines towards the Use of Biological Disease-Modifying Anti-Rheumatic Drugs in Rheumatoid Arthritis Patients," *International Journal of Environmental Research and Public Health*, vol. 19, no. 8, p. 4699, 2022.
- [4] L. F. Dans, S. Tankeh-Torres, C. M. Amante, and E. G. Penserga, "The prevalence of rheumatic diseases in a Filipino urban population: a WHO-ILAR COPCORD Study. World Health Organization. International League of Associations for Rheumatology. Community Oriented Programme for the Control of the Rheumatic Diseases," *The Journal of Rheumatology*, vol. 24, no. 9, pp. 1814–1819, 1997.
- [5] F. H. Pilszczek, D. Salina, K. K. H. Poon, et al., "A novel mechanism of rapid nuclear neutrophil extracellular trap formation in response to *Staphylococcus aureus*," *The Journal of Immunology*, vol. 185, no. 12, pp. 7413–7425, 2010.
- [6] N. Zeng, M. Ayyub, H. Sun, X. Wen, P. Xiang, and Z. Gao, "Effects of physical activity on motor skills and cognitive development in early childhood: a systematic review," *BioMed Research International*, vol. 2017, Article ID 2760716, 13 pages, 2017.
- [7] M. Moradi-Lakeh, M. H. Forouzanfar, S. E. Vollset et al., "Burden of musculoskeletal disorders in the eastern Mediterranean region, 1990–2013: findings from the global burden of disease study 2013," *Annals of the Rheumatic Diseases*, vol. 76, no. 8, p. 1365, 2017.
- [8] M. Mijiyawa, "Epidemiology and semiology of rheumatoid arthritis in third world countries," *Revue du Rhumatisme (English Ed.)*, vol. 62, no. 2, pp. 121–126, 1995.
- [9] A. Chopra, J. Patil, V. Billempelly, J. Relwani, H. S. Tandle, and WHO-ILAR COPCORD Study, "Prevalence of rheumatic diseases in a rural population in western India: a WHO-ILAR COPCORD Study," *The Journal of the Association of Physicians of India*, vol. 49, pp. 240–246, 2001.
- [10] A. J. Silman, W. Ollier, S. Holligan et al., "Absence of rheumatoid arthritis in a rural Nigerian population," *The Journal of Rheumatology*, vol. 20, no. 4, pp. 618–622, 1993.
- [11] A. Del Puente, W. C. Knowler, D. J. Pettitt, and P. H. Bennett, "High incidence and prevalence of rheumatoid arthritis in Pima Indians," *American Journal of Epidemiology*, vol. 129, no. 6, pp. 1170–1178, 1989.
- [12] J. S. Smolen, D. Aletaha, M. Koeller, M. H. Weisman, and P. Emery, "New therapies for treatment of rheumatoid arthritis," *The Lancet*, vol. 370, no. 9602, pp. 1861–1874, 2007.
- [13] S. P. Mahamuni, R. D. Khose, F. Menaa, and S. L. Badole, "Therapeutic approaches to drug targets in hyperlipidemia," *Biomedicine*, vol. 2, no. 4, pp. 137–146, 2012.
- [14] T. Yamamoto, W.-C. Hung, T. Takano, and A. Nishiyama, "Genetic nature and virulence of community-associated methicillin-resistant *Staphylococcus aureus*," *Biomedicine*, vol. 3, no. 1, pp. 2–18, 2013.
- [15] Y.-M. Chang, B. K. Velmurugan, W.-W. Kuo et al., "Inhibitory effect of alpinate oxyphyllae fructus extracts on ang ii-induced cardiac pathological remodeling-related pathways in h9c2 cardiomyoblast cells," *Biomedicine*, vol. 3, no. 4, pp. 148–152, 2013.
- [16] Y. M. Leung, K. L. Wong, S. W. Chen et al., "Down-regulation of voltage-gated  $Ca^{2+}$  channels in  $Ca^{2+}$  store-depleted rat insulinoma rlnm5f cells," *Biomedicine*, vol. 3, no. 3, pp. 130–139, 2013.
- [17] V. Janssens and J. Goris, "Protein phosphatase 2a: a highly regulated family of serine/threonine phosphatases implicated in cell growth and signalling," *Biochemical Journal*, vol. 353, no. 3, pp. 417–439, 2001.
- [18] K.-P. Su, "Inflammation in psychopathology of depression: clinical, biological, and therapeutic implications," *Biomedicine*, vol. 2, no. 2, pp. 68–74, 2012.
- [19] M. A. Leissring, E. Malito, S. Hedouin et al., "Designed inhibitors of insulin-degrading enzyme regulate the catabolism and

- activity of insulin,” *PLoS One*, vol. 5, no. 5, article e10504, 2010.
- [20] Y. Okada, D. Wu, G. Trynka et al., “Genetics of rheumatoid arthritis contributes to biology and drug discovery,” *Nature*, vol. 506, no. 7488, pp. 376–381, 2014.
- [21] J. C. Edwards, G. Cambridge, and V. M. Abrahams, “Do self-perpetuating b lymphocytes drive human autoimmune disease?,” *Immunology*, vol. 97, no. 2, pp. 188–196, 1999.
- [22] I. Moreno, A. Valenzuela, A. García, J. Yélamos, B. Sánchez, and W. Hernánz, “Association of the shared epitope with radiological severity of rheumatoid arthritis,” *The Journal of Rheumatology*, vol. 23, no. 1, pp. 6–9, 1996.
- [23] C. Turesson, D. J. Schaid, C. M. Weyand et al., “The impact of HLA-DRB1 genes on extra-articular disease manifestations in rheumatoid arthritis,” *Arthritis Research & Therapy*, vol. 7, no. 6, pp. R1386–R1393, 2005.
- [24] P. K. Gregersen, J. Silver, and R. J. Winchester, “The shared epitope hypothesis. An approach to understanding the molecular genetics of susceptibility to rheumatoid arthritis,” *Arthritis and Rheumatism*, vol. 30, no. 11, pp. 1205–1213, 1987.
- [25] M. A. Gonzalez-Gay, C. Garcia-Porrua, and A. H. Hajeer, “Influence of human leukocyte antigen-drb1 on the susceptibility and severity of rheumatoid arthritis,” *Seminars in Arthritis and Rheumatism*, vol. 31, no. 6, pp. 355–360, 2002.
- [26] G. R. Burmester, E. Feist, and T. Dörner, “Emerging cell and cytokine targets in rheumatoid arthritis,” *Nature Reviews Rheumatology*, vol. 10, no. 2, pp. 77–88, 2014.
- [27] K. S. Rosenthal, K. Mikecz, H. L. Steiner et al., “Rheumatoid arthritis vaccine therapies: perspectives and lessons from therapeutic ligand epitope antigen presentation system vaccines for models of rheumatoid arthritis,” *Expert Review of Vaccines*, vol. 14, no. 6, pp. 891–908, 2015.
- [28] R. Satyam, E. M. Janahi, T. Bhardwaj, P. Somvanshi, S. Haque, and M. Z. Najm, “In silico identification of immunodominant B-cell and T-cell epitopes of non-structural proteins of Usutu virus,” *Microbial Pathogenesis*, vol. 125, pp. 129–143, 2018.
- [29] U. K. Adhikari, M. Tayebi, and M. M. Rahman, “Immunoinformatics approach for epitope-based peptide vaccine design and active site prediction against polyprotein of emerging Oropouche virus,” *Journal of Immunology Research*, vol. 2018, Article ID 6718083, 22 pages, 2018.
- [30] R. Korhonen and E. Moilanen, “Anti-cd20 antibody rituximab in the treatment of rheumatoid arthritis,” *Basic & Clinical Pharmacology & Toxicology*, vol. 106, pp. 13–21, 2009.
- [31] L. A. van de Stadt, M. H. M. T. de Koning, R. J. van de Stadt et al., “Development of the anti-citrullinated protein antibody repertoire prior to the onset of rheumatoid arthritis,” *Arthritis and Rheumatism*, vol. 63, no. 11, pp. 3226–3233, 2011.
- [32] A. Hanyecz, K. Olasz, O. Tarjanyi et al., “Proteoglycan aggregate conducting t cell activation and apoptosis in a murine model of rheumatoid arthritis,” *BioMed Research International*, vol. 2014, Article ID 942148, 13 pages, 2014.
- [33] H. Chen, C. Wang, J. Li et al., “Chinese herbal formula, Huayu Tongbi Fang, attenuates inflammatory proliferation of rat synoviocytes induced by IL-1 $\beta$  by regulating proliferation and differentiation of T lymphocytes,” *Evidence-based Complementary and Alternative Medicine*, vol. 2020, Article ID 1706837, 18 pages, 2020.
- [34] V. H. Morris, S. C. Cruwys, and B. L. Kidd, “Characterisation of capsaicin-induced mechanical hyperalgesia as a marker for altered nociceptive processing in patients with rheumatoid arthritis,” *Pain*, vol. 71, no. 2, pp. 179–186, 1997.
- [35] R. Ziadlou, A. Barbero, I. Martin et al., “Anti-inflammatory and chondroprotective effects of vanillic acid and epimedin c in human osteoarthritic chondrocytes,” *Biomolecules*, vol. 10, no. 6, p. 932, 2020.
- [36] M. Z. Nawaz, Q.-U. Ain, S. Zahid et al., “Physicochemical features and structural analysis of xanthine oxidase as a potential therapeutic target to prevent gout,” *Journal of Radiation Research and Applied Sciences*, vol. 13, no. 1, pp. 616–628, 2020.
- [37] G. Mustafa, H. S. Mahrosh, M. Zafar, S. A. Attique, and R. Arif, “Exploring the antihyperglycemic potential of tetrapeptides devised from admc1 via different receptor proteins inhibition using in silico approaches,” *International Journal of Immunopathology and Pharmacology*, vol. 36, 2022.
- [38] S. A. Attique, M. Hassan, M. Usman et al., “A molecular docking approach to evaluate the pharmacological properties of natural and synthetic treatment candidates for use against hypertension,” *International Journal of Environmental Research and Public Health*, vol. 16, no. 6, p. 923, 2019.
- [39] M. Hassan, A. A. Baig, S. A. Attique et al., “Molecular docking of alpha-enolase to elucidate the promising candidates against streptococcus pneumoniae infection,” *DARU Journal of Pharmaceutical Sciences*, vol. 29, no. 1, pp. 73–84, 2021.
- [40] L. Guan, H. Yang, Y. Cai et al., “Admet-score – a comprehensive scoring function for evaluation of chemical drug-likeness,” *MedChemComm*, vol. 10, no. 1, pp. 148–157, 2019.
- [41] A. Kouranov, L. Xie, J. de la Cruz et al., “The RCSB PDB information portal for structural genomics,” *Nucleic Acids Research*, vol. 34, no. 90001, pp. D302–D305, 2006.
- [42] H. Edelhoch, “Spectroscopic determination of tryptophan and tyrosine in proteins,” *Biochemistry*, vol. 6, no. 7, pp. 1948–1954, 1967.
- [43] E. Boutet, D. Lieberherr, M. Tognolli, M. Schneider, and A. Bairoch, “Uniprotkb/swiss-prot,” in *Plant Bioinformatics: Methods and Protocols*, D. Edwards, Ed., pp. 89–112, Humana Press, Totowa, NJ, USA, 2007.
- [44] N. Eswar, D. Eramian, B. Webb, M.-Y. Shen, and A. Sali, “Protein structure modeling with modeller,” in *Structural Proteomics: High-Throughput Methods*, B. Kobe, M. Guss, and T. Huber, Eds., pp. 145–159, Humana Press, Totowa, NJ, USA, 2008.
- [45] K. Gopalakrishnan, G. Sowmiya, S. S. Sheik, and K. Sekar, “Ramachandran plot on the web (2.0),” *Protein & Peptide Letters*, vol. 14, no. 7, pp. 669–671, 2007.
- [46] G. W. Kim, N. R. Lee, R. H. Pi et al., “IL-6 inhibitors for treatment of rheumatoid arthritis: past, present, and future,” *Archives of Pharmacal Research*, vol. 38, no. 5, pp. 575–584, 2015.
- [47] T. Shaw, J. Quan, and M. C. Totoritis, “B cell therapy for rheumatoid arthritis: the rituximab (anti-cd20) experience,” *Annals of the Rheumatic Diseases*, vol. 62, pp. ii55–ii59, 2003.
- [48] I. E. van der Horst-Bruinsma, H. Visser, J. M. W. Hazes et al., “Hla-dq-associated predisposition to and dominant hla-dr-associated protection against rheumatoid arthritis,” *Human Immunology*, vol. 60, no. 2, pp. 152–158, 1999.
- [49] S. Kim, P. A. Thiessen, E. E. Bolton et al., “Pubchem substance and compound databases,” *Nucleic Acids Research*, vol. 44, no. D1, pp. D1202–D1213, 2016.

- [50] H. A. Alghamdi, S. A. Attique, W. Yan et al., “Repurposing the inhibitors of covid-19 key proteins through molecular docking approach,” *Process Biochemistry*, vol. 110, pp. 216–222, 2021.
- [51] P. W. Rose, A. Prlić, A. Altunkaya et al., “OUP accepted manuscript,” *Nucleic Acids Research*, vol. 45, no. D1, pp. D271–D281, 2016.
- [52] S. Hunter, R. Apweiler, T. K. Attwood et al., “Interpro: the integrative protein signature database,” *Nucleic Acids Research*, vol. 37, no. Database, pp. D211–D215, 2009.
- [53] S. A. Attique, S. A. A. Shah, M. Hassan et al., “A molecular analysis of homo sapiens’ tumor-causing tp53 gene and associated p53 protein via bioinformatics pipeline,” *Malaysian Journal of Human Genetics*, vol. 1, pp. 44–56, 2020.
- [54] M. M. Jaghoori, B. Bleijlevens, and S. D. Olabarriaga, “1001 ways to run autodock vina for virtual screening,” *Journal of Computer-Aided Molecular Design*, vol. 30, no. 3, pp. 237–249, 2016.
- [55] S. Vilar, G. Cozza, and S. Moro, “Medicinal chemistry and the molecular operating environment (MOE): application of QSAR and molecular docking to drug discovery,” *Current Topics in Medicinal Chemistry*, vol. 8, no. 18, pp. 1555–1572, 2008.
- [56] Z. M. Nawaz, A. S. Attique, Qurat-ul-Ain et al., “A nanoinformatics approach to evaluate the pharmacological properties of nanoparticles for the treatment of Alzheimer’s disease,” *Combinatorial Chemistry & High Throughput Screening*, vol. 25, no. 4, pp. 730–737, 2022.
- [57] S. Kim, J. Chen, T. Cheng et al., “PubChem in 2021: new data content and improved web interfaces,” *Nucleic Acids Research*, vol. 49, no. D1, pp. D1388–D1395, 2021.
- [58] D. Gentile, V. Fucchi, A. Rescifina, and P. M. Furneri, “New anti sars-cov-2 targets for quinoline derivatives chloroquine and hydroxychloroquine,” *International Journal of Molecular Sciences*, vol. 21, no. 16, p. 5856, 2020.
- [59] S. Shityakov and C. Förster, “In silico predictive model to determine vector-mediated transport properties for the blood-brain barrier choline transporter,” *Advances and Applications in Bioinformatics and Chemistry*, vol. 7, pp. 23–36, 2014.
- [60] M. Champsaur and L. L. Lanier, “Effect of nkg2d ligand expression on host immune responses,” *Immunological Reviews*, vol. 235, no. 1, pp. 267–285, 2010.
- [61] N. C. Peeri, N. Shrestha, M. S. Rahman et al., “The SARS, MERS and novel coronavirus (COVID-19) epidemics, the newest and biggest global health threats: what lessons have we learned?,” *International Journal of Epidemiology*, vol. 49, no. 3, pp. 717–726, 2020.
- [62] M. Mahanthesh, D. Ranjith, R. Yaligar, R. Jyothi, G. Narappa, and M. Ravi, “Swiss ADME prediction of phytochemicals present in *Butea monosperma* (lam.) Taub,” *Journal of Pharmacognosy and Phytochemistry*, vol. 9, pp. 1799–1809, 2020.
- [63] A. Daina, O. Michielin, and V. Zoete, “SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules,” *Scientific Reports*, vol. 7, no. 1, article 42717, 2017.
- [64] C.-Y. Jia, J.-Y. Li, G.-F. Hao, and G.-F. Yang, “A drug-likeness toolbox facilitates ADMET study in drug discovery,” *Drug Discovery Today*, vol. 25, no. 1, pp. 248–258, 2020.
- [65] H. Yang, L. Sun, W. Li, G. Liu, and Y. Tang, “Corrigendum: *In Silico* prediction of chemical toxicity for drug design using machine learning methods and structural alerts,” *Frontiers in Chemistry*, vol. 6, 2018.
- [66] J. Bhachoo and T. Beuming, “Investigating protein–peptide interactions using the Schrödinger computational suite,” in *Modeling Peptide-Protein Interactions: Methods and Protocols*, O. Schueler-Furman and N. London, Eds., pp. 235–254, Springer, New York, NY, USA, 2017.
- [67] M. Yang, X. Feng, J. Ding, F. Chang, and X. Chen, “Nanotherapeutics relieve rheumatoid arthritis,” *Journal of Controlled Release*, vol. 252, pp. 108–124, 2017.
- [68] J. S. Smolen, R. Landewé, F. C. Breedveld et al., “Eular recommendations for the management of rheumatoid arthritis with synthetic and biological disease-modifying antirheumatic drugs: 2013 update,” *Annals of the Rheumatic Diseases*, vol. 73, no. 3, pp. 492–509, 2014.
- [69] J. S. Smolen, R. Landewé, J. Bijlsma et al., “Eular recommendations for the management of rheumatoid arthritis with synthetic and biological disease-modifying antirheumatic drugs: 2016 update,” *Annals of the Rheumatic Diseases*, vol. 76, no. 6, pp. 960–977, 2017.
- [70] G. Singh, J. F. Fries, C. A. Williams, E. Zatarain, P. Spitz, and D. A. Bloch, “Toxicity profiles of disease modifying antirheumatic drugs in rheumatoid arthritis,” *The Journal of Rheumatology*, vol. 18, no. 2, pp. 188–194, 1991.
- [71] N. J. Olsen and C. M. Stein, “New drugs for rheumatoid arthritis,” *New England Journal of Medicine*, vol. 350, no. 21, pp. 2167–2179, 2004.