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Research Article

# IDENTIFICATION OF ANTIRHEUMATOID ARTHRITIS LIGANDS USING *IN SILICO* ANALYSIS

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**ABSTRACT**

**Objective:** The purpose of the research was to implement molecular docking simulation to investigate the anti-inflammatory capability of against thioesterase-2, an essential component of the inflammatory reaction in humans.

**Methods:** After obtaining three-dimensional structures of arthritis ligand and thioesterase-2 protein from IUPHAR/BPS Guide to PHARMACOLOGY, PUBCHEM, and RCSB PDB databases server, ligands with suitable absorption, distribution, metabolism, and excretion (ADME) properties were docked against thioesterase-2 protein using PyRx 0.8 and AutoDock tools 1.5.7.

**Results:** Indomethacin showed better binding affinity to thioesterase-2.

**Conclusion:** In light of its promising binding affinities with thioesterase-2, indomethacin could be taken into consideration as a prospective pharmacological agent for thioesterase-2 inhibition, according to molecular docking simulation and SWISS ADME evaluation.

**Keywords:** Thioesterase, Ligands, Thioesterase-2 inhibitor, Molecular docking.

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## INTRODUCTION

An autoimmune condition termed rheumatoid arthritis can impair your entire body, causing joint soreness, swelling, and impairment. As rheumatoid arthritis impacts both sides of the body, if one arm or leg experiences joint deterioration, the other arm or leg will very definitely experience the same impairment as the other. Rheumatoid arthritis is divided into three distinct stages of succession: Inflammation with pain, acute inflammatory synovial membrane, and eventually bone and cartilage damage exacerbating joint inflammation with pain. It is strongly correlated to the autoimmune response that is stimulated by diverse genetic, epigenetic, and some external factors [1,2]. However, the underlying etiology or predisposing of rheumatoid arthritis is yet undetermined. As part of the inflammatory progression in rheumatoid arthritis, the immune system delivers antibodies to the interior of your joints. These antibodies destroy the synovial cells that coat the tissues around your joints, which cause inflammation.

Substances that might harm adjacent bones, ligaments, muscles, and ligaments are generated during this phase. In the absence of treatment for rheumatoid arthritis, the joint would deteriorate, lose its form, and ultimately fracture. Knees, hands, and wrists are typically affected by rheumatoid arthritis. Long-lasting or persistent pain, uncontrollable shaking (loss of balance), and disfigurement can all be driven by tissue damage [3].

According to statistics, 1% of people worldwide suffer from rheumatoid arthritis [16]. It is an aggressive inflammatory condition that progresses to joint degeneration until the inflammation is reduced or eliminated and may go into relapse without medication. It is highly suggested to begin therapy following the diagnosis. Multiple rheumatoid joint pain medications can alleviate knee pain, stiffness, and inflammation. The optimized medical care includes drug therapy and non-drug methods.

According to estimations, individuals with rheumatoid arthritis have an increased death rate of 70%, which can be attributed to circulatory disorders in up to 50% of occurrences [5,6]. These individuals are also at an elevated risk of cardiac episodes. It has been noted that people with prolonged duration of illness have an estimated myocardial infarction risk that is 3 times higher [11]. A highly compacted, highly oxidizable, and powerfully atherogenic low-density lipoprotein particle is linked to the dyslipidemia seen in rheumatoid arthritis, which is characterized by low levels of total and HDL cholesterol and high levels of triglycerides [12]. In the current research, the molecular docking and pharmacokinetic studies were implemented to identify potential anti-arthritis drug candidates from IUPHAR/BPS Guide to PHARMACOLOGY server database to address thioesterase-2 proteins.

## METHODS

### Preparation of target protein

Thioesterase-2 protein file was obtained from RCSB PDB server (PDB ID: 4XJV) and was utilized for docking studies (Fig. 1). The structure was resolved using X-ray diffraction method. Stereochemical properties and the secondary structure were validated by generating the Ramachandran plot using Z lab (Fig. 2) [6].

### Selection of Ligand as potential ligands

After researching several literatures, the plant-sourced active ingredient for "rheumatoid arthritis" was retrieved from the IUPHAR/BPS Guide to PHARMACOLOGY server database [7]. To identify possible compounds, a library of 14 ligands was first constructed for virtual screening. All of the ligands' 3D structures (Fig. 1) were retrieved from the PUBCHEM database in SDF format, which was then transformed into PDB format by the ONLINE SMILE TRANSLATOR AND STRUCTURE FILE GENERATOR site. The PUBCHEM open chemistry database server was used to retrieve physiochemical characteristics and to analyse the drug-likeness properties of the candidate ligand molecules.

### Pharmacological properties

Employing Lipinski's Rule of Five (Table 2), which essentially assesses a chemical compound's "drug ability" to be deployed as an orally active drug in humans, the drug likeness of the possible ligands was computed [8]. Before a chemical is used as a medicine in the humans, it

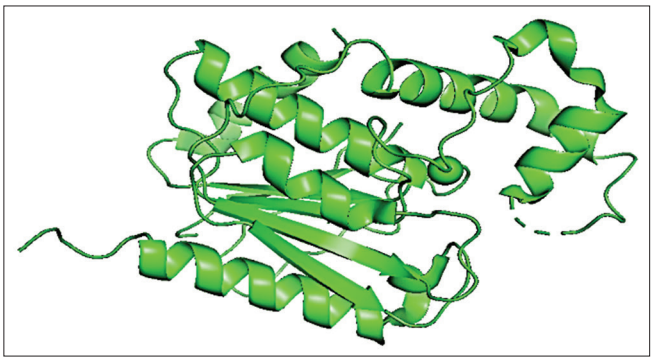


**Fig. 1: Protein structure (4XJV) visualized with PyMOL molecular graphics system 2.5.2**

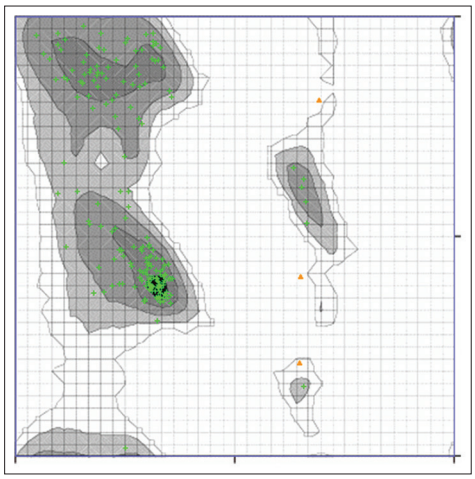


**Fig. 2: Stereochemical property analyzing using Ramachandran plot using Z lab server black, dark gray, gray, and light gray represent highly preferred conformation (Delta ≥ -2). White with black grid represent preferred confirmation (-2> Delta ≥ -4). White with gray grid represent questionable confirmation (Delta <-4). Highly preferred observation (98.421%) shown as green crosses, preferred observation (1.579%) shown as BROWN triangle as questionable observation shown as RED circle (0.000%)**

must meet certain requirements of pharmacokinetic qualities, including molecular weight, hydrophilicity, lipophilicity, polar surface area, hydrogen bonding, and charge. It also includes the body's absorption, distribution, metabolism, and excretion (ADME). To determine how closely these 14 ligands resembled drugs, Lipinski filter, and SWISS ADME analyses were performed. After this stage, compounds with less plausible stereochemical characteristics were removed. Molecular weight ≤500, hydrogen bond donor ≤5, hydrogen bond acceptor ≤15, and octanol-water partition coefficient (logP) ≤5 were the parameters employed for Lipinski filter examination. SWISS ADME, a common drug discovery tool, was used to evaluate the ADME features of small molecules as well as their pharmacokinetic and medicinal chemistry compatibility [10].

**Ramachandran PLOT**
Screening of ligands for molecular docking.

Employing PyRx, a virtual screening program used in computational drug discovery [8], ligands identified from the SWISS ADME investigation were further assessed according to their binding affinity toward the thioesterase-2 protein target.

**Molecular docking studies**
Molecular docking study was performed to predict the interaction of the selected ligands with thioesterase-2 receptor protein target using AutoDock tools 1.5.7 software. Experiments were carried out with default parameters to get the accurate result. Entire docking process was performed on a Windows 10 Workstation with Intel(R) Core (TM) i3-7100 CPU at 3.90 GHz 3.91 GHz processor and 8 GB RAM.

Utilizing the graphical interface of the AutoDock tools, PDBQT files for ligands and proteins were constructed. These files comprise atomic partial charges and atom kinds, grid boxes, and grid parameter files. To prepare the protein, molecules were removed, polar hydrogen was added, Kollman charges, fragmental volumes, and solvation parameters were added, and the protein was then saved in PDBQT format [9]. The protein was embedded into a three-dimensional grid box using the autogrid process to create the grid map. Grid center was set at dimensions, grid size was set to 126 × 126 × 126 xyz points, and grid spacing was set to 0.603. (x, y, and z). AutoDock affinity grids, electrostatic potentials, and desolvation potentials are monitored for each type of atom in the ligands. Subsequently, employing grid values from the AutoDock simulation, the energetics of a specific ligand configuration are assessed. Lamarckian genetic algorithm was used for docking, with 10 runs, and the best configuration was chosen from the cluster RMSD table. To facilitate additional research, the conformations with the lowest binding energies were taken from the DLG data and positioned alongside the protein.

**Analysis of docking results**
The selected ligand's conformation with the lowest binding energy was selected from the DLG data and evaluated using AutoDock tools. The ONLINE SMILE TRANSLATOR AND STRUCTURE FILE GENERATOR server was used to convert the PDBQT format of the protein-ligand docked complexes into the PDB format. The complexes were then seen using PyMOL 2.5.2.

## RESULTS AND DISCUSSION

**Pre-docking procedures**
The protein (PDB ID: 4XVJ) was prepared by removing the heteroatoms and non-structural water molecules (Fig. 1) and the purified protein was subjected to Ramachandran Plot analysis (Fig. 2) which revealed that all the amino residues demonstrated preferred torsions and orientation.

**Docked picture of protein and ligand**
The ligand with best binding with the target protein was visualized to identify the molecular interactions with that of the amino acids in the binding pocket (Fig. 4).

### *Ligand library generation*
Physiochemical properties of all 14 ligands obtained from PUBCHEM database all are listed in Table 1.

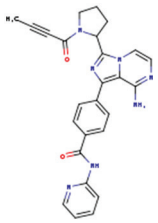
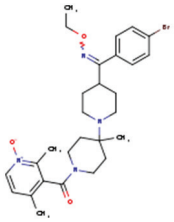
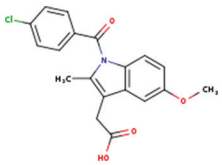
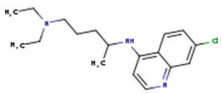
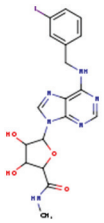
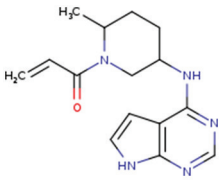
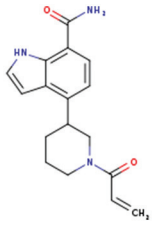
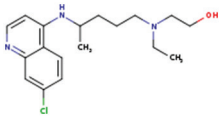
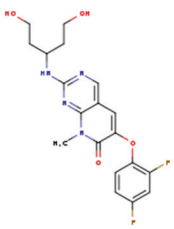
### *Analysis of drug likeness*
Ligands with no more than 2 Lipinski violations were then selected for further screening the result are summarized in Table 1.

### *Screening of ligands using PyRx*
Ligands which were selected after Lipinski filter analysis were further screened based on their binding energies with thioesterase-2 receptor protein using PyRx and presented in Table 2.

Finally, seven ligands showing binding affinity <7 kcal/mol and no more than 2 Lipinski violations (indomethacin, branebrutinib, piclidenoson, elsubrutinib, tamatinib, maraviroc, and pamapimod) were chosen for the molecular docking studies with AutoDock tools 1.5.7.

**Binding energies of ligands**
From the results of molecular docking analysis, it is evident that the ligands Maraviroc, Indomethacin, Piclidenoson, (Table 2) demonstrated significantly better binding among the 14 ligand and the energy associated with the binding I documented in Table 3.

**Table 1: Lipinski filter analysis**

| Ligands | PUBCHEM ID | Molecular structure | Molecular refractivity | H-bond donors | H-bond acceptor | MLOGP | Lipinski violations |
|---|---|---|---|---|---|---|---|
| Maraviroc | 3002977 |  | 136.51 | 2 | 5 | 1.52 | 0 |
| Ancriviroc | 9574343 |  | 156.61 | 0 | 5 | 3.94 | 1 |
| Indomethacin | 3715 |  | 96.12 | 1 | 4 | 3.30 | 0 |
| Chloroquine | 2719 |  | 97.41 | 1 | 2 | 3.20 | 0 |
| Piclidenoson | 123683 |  | 111.43 | 4 | 7 | -0.58 | 1 |
| Ritlecitinib | 118115473 |  | 86.07 | 2 | 3 | 1.25 | 0 |
| Elsubrutinib | 117773770 |  | 89.43 | 2 | 2 | 1.40 | 0 |
| Hydroxychloroquine | 3652 |  | 98.57 | 2 | 3 | 2.35 | 0 |
| Pamapimod | 16220188 |  | 102.35 | 3 | 8 | 1.74 | 0 |

*(Contd...)*

**Table 1:** ***(Continued)***

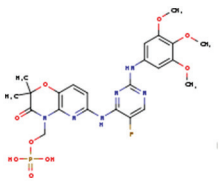
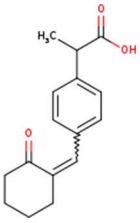
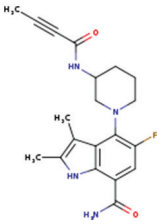
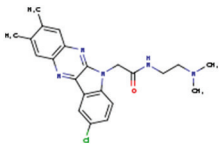
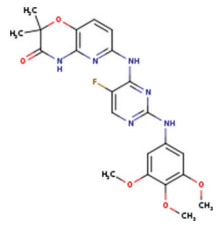
| Ligands | PUBCHEM ID | Molecular structure | Molecular refractivity | H-bond donors | H-bond acceptor | MLOGP | Lipinski violations |
|---|---|---|---|---|---|---|---|
| Fostamatinib | 11671467 |  | 141.79 | 4 | 13 | 0.33 | 2 |
| Pelubiprofen | 5282203 |  | 75.01 | 1 | 3 | 2.46 | 0 |
| Branebrutinib | 121293929 |  | 106.12 | 3 | 3 | 1.93 | 0 |
| Rabeximod | 56841552 |  | 118.68 | 1 | 4 | 2.79 | 0 |
| Tamatinib | 11213558 |  | 124.82 | 3 | 9 | 1.56 | 1 |

**Table 2: Molecular docking results**

| S. No. | PUBCHEM ID | Ligands | Energy | Binding affinity (KCAL/MOL) |
|---|---|---|---|---|
| 1 | 3002977 | Maraviroc | 754.48 | −8.9 |
| 2 | 9574343 | Ancriviroc | 651.5 | −7.7 |
| 3 | 3715 | Indomethacin | 591.21 | −8.6 |
| 4 | 2719 | Chloroquine | 262.53 | −6.3 |
| 5 | 123683 | Piclidenoson | 626.36 | −8.3 |
| 6 | 11213558 | Tamatinib | 482.96 | −8.1 |
| 7 | 118115473 | Ritlecitinib | 424.29 | −7.5 |
| 8 | 117773770 | Elsubrutinib | 431.78 | −7.9 |
| 9 | 3652 | Hydroxychloroquine | 280.61 | −6.2 |
| 10 | 16220188 | Pamapimod | 372.61 | −7.9 |
| 11 | 11671467 | Fostamatinib | 784.07 | −7.6 |
| 12 | 5282203 | Pelubiprofen | 363.59 | −7.4 |
| 13 | 121293929 | Branebrutinib | 763.58 | −7.8 |
| 14 | 56841552 | Rabeximod | 513.31 | −7.6 |

**Docking results**

Molecular docking studies of thioesterase-2 protein were performed using AutoDock tools 1.5.7 with these seven ligands using Lamarckian genetic algorithm. Among the 10 configurations, the best configuration was showed and selected from cluster RMSD table for further analysis.

Molecular docking result is presented in Table 4.

On the basis of binding energies, one ligand is showing that binding energies could be promising drug candidate against thioesterase-2 target. The one ligand is Indomethacin with binding energies

**Table 3: Binding energies and molecular docking result of ligands**

| S. No. | Ligands | Cluster RMSD | Reference RMSD | Binding energy after docking with 4XJV (kcal/mol) |
|---|---|---|---|---|
| 1 | Indomethacin | 0.00 | 37.47 | −169000000 |
| 2 | Branebrutinib | 0.00 | 38.90 | −90293584 |
| 3 | Piclidenoson | 0.00 | 21.99 | −7.55 |
| 4 | Elsubrutinib | 0.00 | 34.73 | −7.1 |
| 5 | Tamatinib | 0.00 | 29.35 | −6.71 |
| 6 | Maraviroc | 0.00 | 32.58 | −6.59 |
| 7 | Pamapimod | 0.00 | 35.34 | −5.34 |

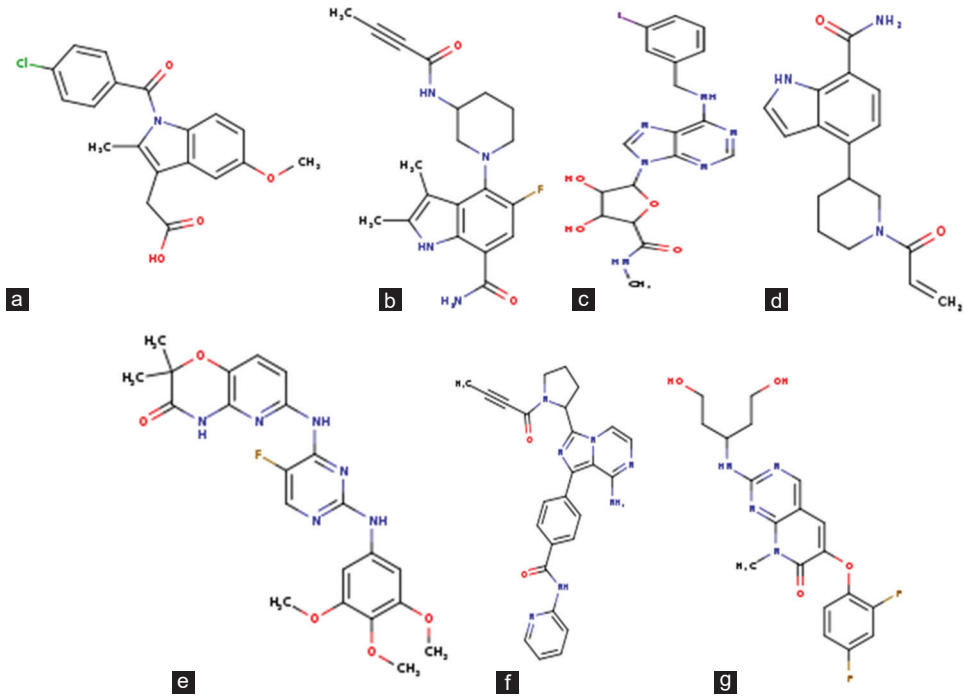



**Fig. 3: Molecular structure of selected ligands, (a) indomethacin, (b) branebrutinib, (c) piclidenoson, (d) elsubrutinib, (e) tamatinib, (f) maraviroc, and (g) pamapimod**

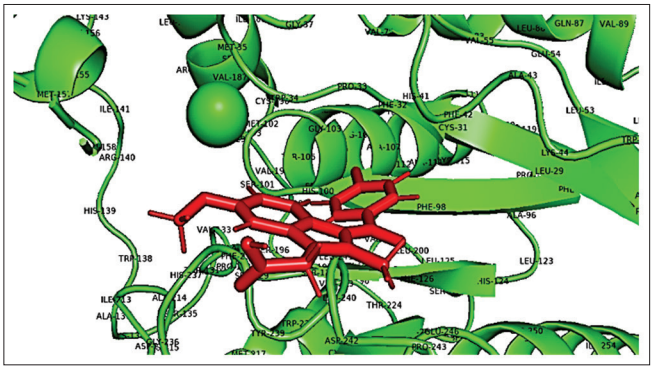


**Fig. 4: Receptor-ligand interaction to indomethacin with thioesterase-2-binding site**

(−169000000). Structure of indomethacin ligand along with the thioesterase drug is shown in Fig. 3.

A non-selective cyclooxygenase blocker from the nonsteroidal anti-inflammatory medication (NSAID) family, indomethacin is a synthetic organic substance. Branebrutinib is a covalently, irrevocable inhibitor of the TEC family non-receptor tyrosine kinase. Bruton's tyrosine kinase (BTK), branebrutinib is a chemically synthesized chemical (BTK), is a preliminary lead for the therapy of rheumatoid arthritis. The potential for translating piclidenoson anti-inflammatory effects into the clinic is being studied. Piclidenoson is a selective agonist of the adenosine A3 receptor. Elsubrutinib could be employed to investigate inflammatory diseases. Fostamatinib is a pro-drug of the spleen tyrosine kinase inhibitor tamatinib, which is used to treat rheumatoid arthritis [13] and primary chronic adult immunological thrombocytopenia. The synthetic organic chemical family of maraviroc is a small molecule antagonist of the C-C chemokine receptor type 5 expressed on T cells. Pamapimod may be used to treat autoimmune illnesses including rheumatoid arthritis [15].

## DISCUSSION

Being a chronic autoimmune disorder, rheumatoid arthritis is characterized by reddish joints, swelling, and destruction to the cartilage and bones that may potentially progress to multiorgan failure [17].

The antiarthritic capability of an Ayurveda medication was proved by the nearly 60% of the population who search for an alternative herbal treatment for the long-term cure of rheumatoid arthritis [17,18]. Comparing two ligands, certain autoimmune illnesses are treated or prevented using hydroxychloroquine (lupus and rheumatoid arthritis). It is a member of the class of medications known as antirheumatoid class modifiers. It can alleviate lupus-related skin issues and reduce arthritis pain and swelling [19].

Indomethacin is used to treat rheumatoid arthritis, gout, bursitis, tendonitis, and other musculoskeletal pain, edema, and stiffness. It is also used to treat pain caused by a number of other conditions.

This substance is categorized as a NSAID. It functions by preventing your body from producing specific natural compounds that induce inflammation [20].

Indomethacin has been identified as a viable medication for the management of rheumatoid arthritis with thioesterase-2 through *in silico* studies, which was the primary focus of our exploration into possibly useful novel compounds from ligands with adequate biological efficacy and minimal toxicity.

## CONCLUSION

The findings of this research indicate that indomethacin exhibits the best binding energy compared to other ligands, while the remaining six molecules (branebrutinib, piclidenoson, elsubrutinib, tamatinib, maraviroc, and pamapimod) bind to thioesterase-2 but never exhibit the best binding as those of thioesterase, making them potential drug candidates against tioesterase-2 target. In addition, Ramachandran plot analysis and SWISS ADME analysis of pharmacokinetic features of the substances approved for human consumption using Lipinski's filter (RULE OF FIVE) further support our observations. It is crucial to leverage the properties of natural chemicals and investigate the possibilities of using them as possible medications because commercially available "Thioesterase" impart some adverse effects to our system. Although the natural ligands' binding affinity may need to be increased before they can be used as drug candidates, this comprehensive examination may spur further investigations on the creation of plausible derivatives from the natural ligands to the targets encountered in arthritis that can ultimately replace the presently offered, side effect producing commercial medications.

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