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Original Research Article

Design of dual inhibitors of human TNF-α and IL-6 with potentials for the treatment of rheumatoid arthritis

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Abstract

Purpose: *To design dual inhibitors of tumor necrosis factor α (TNF-α) and interleukin 6 (IL-6) with potentials for the treatment of rheumatoid arthritis (RA).*
Methods: *Tumor necrosis factor α (TNF-α) and IL-6 were investigated as potential drug targets for the treatment of RA. Dual inhibitors targeting both TNF-α and IL-6 were designed simultaneously using molecular docking simulation-based in silico virtual screening technique. National Cancer Institute (NCI) diversity set-II consisting of 1818 diverse ligands were screened against both drug targets in order to identify potential lead molecules on the basis of lowest binding energy.*
Results: *Out of 1818 diverse ligand molecules present in the NCI diversity set-II, five lead molecules were selected based on best binding interactions with both target receptors. The results of toxicity profiling showed that compounds ZINC19701771 and ZINC06576501 lacked major toxicity-associated functional groups linked to mutagenic, tumorigenic, irritant and reproductive effects. However, ZINC03898665 and ZINC05015095 possessed some mutagenic and reproductive effects. Compound ZINC01757986 also showed a high chance of mutagenicity.*
Conclusion: *These results indicate that the two lead molecules (ZINC19701771 and ZINC06576501) that showed reliable physicochemical properties can serve as potential candidates for development of anti-arthritis drug for effective inhibition of human TNF-α and IL-6 receptors.*

Keywords: *Rheumatoid arthritis, Docking, Interleukin-6, Tumor necrosis factor α, Toxicity*

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INTRODUCTION

Rheumatoid arthritis (RA) is an inflammatory disorder characterized by painful inflammation of the joints [1]. Inflammatory proliferating synovium (pannus) is the hallmark of RA, and it causes destruction of joints due to erosions [2]. The immune system is a very complex system which works on the principle of discrimination between self and non-self antigens. In rheumatic disorders, the immune system is unable to perform this discriminatory function, and so attacks synovial and other connective tissues of joints, thereby producing painful inflammation [3]. Rheumatoid arthritis (RA) is more common in females than in males. The incidence of symptomatic osteoarthritis is 9.6 % in men and 18.0 % in women aged 60 years and above.

More than 80 % of osteoarthritic patients have difficulty with walking, while 25 % of them are unable to perform routine activities [4]. Cytokines such as TNF-α and IL-6 play key roles in the initiation and progression of rheumatoid inflammation. The multitude of T-cells present in synovial membrane use these cytokines to promote the proliferation of chondrocytes, macrophages, and osteoclasts, and these in turn stimulate the production of metalloproteinases and other cytotoxins, leading to erosion of bone and cartilage. The aim of this study was to design dual inhibitor(s) of TNF-α and IL-6 with potential for use in the treatment of RA.

METHODS

Molecular docking simulation of human TNF-α

Selection of macromolecule and its preparation

The three-dimensional protein model of TNF-α (pdb id-2AZ5) was downloaded from protein data bank. The receptor protein was prepared for molecular docking simulation by removing ligand and water from the active site, and by addition of polar hydrogens [5-7].

Preparation of ligand for molecular docking

Complexed small inhibitor ligand molecule of TNF-α was prepared for molecular docking simulation by providing rotatable, non-rotatable, and unrotatable bonds in the ligand using AutoDock software [8].

Identification of binding site

Ligand binding site of the human TNF-α was identified using DS visualizer software. The complexed ligand bound in the receptor's active binding site was separated from the complex using chimera software [9,10].

Molecular docking

The binding site of the TNF-α receptor was identified using various protein visualization softwares such as PyMol, and DS visualizer. Parameters of grid box were used for docking. In order to ensure that extended conformations of the ligand fit perfectly within the grid box, the ligand was centralized in the grid box and all the residues involved in binding were covered. Separate map files for each type of atom present in the receptor and ligand (A C HD OA N SA) were prepared using Autogrid utility of the AutoDock suite. These map files were used for carrying out molecular docking simulations. Lamarckian genetic algorithm (LGA) is one of the primary conformational search approaches employed in AutoDock for molecular docking simulation. A trial population was created for various possible conformations, followed by mutation, conformational parameter exchange, and competition was carried out in a manner similar to biological evolution in successive generations for eventual selection of individuals with lowest binding energy. The search for individual conformation with specific local conformational space, and local minima were performed using ''Lamarckian'' aspect. Binding energies of small molecules with macromolecular targets were predicted using semi-empirical force field. The force field allowed for the assimilation of intramolecular energy into the predicted binding energy by evaluating the energetics of both bound and unbound states based on a comprehensive thermodynamic model. The parameter file required for docking of each ligand molecule was prepared using 150 Genetic Algorithm (GA) runs, 250000 maximum numbers of evaluations, 27000 maximum numbers of generations, and 0.02 % of gene mutation [11].

Validation of the docking method

The position and orientation of the ligand obtained after molecular docking represented probable binding patterns of the inhibitors. The various docking parameters were validated by re-docking individually crystallized inhibitor molecule against TNF-α receptor. The molecular docking simulation technique was validated using the following parameters:

Overlay method

In overlay method**,** docked conformation of bound ligand was impeccably overlaid relative to the bioactive conformation of the ligand present in the crystal structure of downloaded protein.

Chemical resemblance

In chemical resemblance, the docked ligand had the same interactions with residues of macromolecule as that present in the downloaded crystallized macromolecule [12].

Molecular docking simulation of IL-6

Selection of macromolecule and its preparation

The three-dimensional protein model of IL-6 (pdb id-1ALU) was downloaded from protein data bank [6]. The receptor protein was prepared by

removing ligand and water from the active site, and by addition of polar hydrogens [6,13].

Preparation of ligand for molecular docking

Interleukin-6 complexed with tartaric acid was prepared for molecular docking simulation by providing rotatable, non-rotatable and unrotatable bonds in the ligand using AutoDock software [8].

Identification of binding site

Ligand binding site of human IL-6 protein was identified using DS visualizer software. The complexed ligand bound to the receptor's active site was separated from the complex using chimera software [9,10].

Molecular docking

The binding site of IL-6 receptor was identified using protein visualization software PyMol. The docking parameters were similar to those used for docking of TNF-α protein [11].

Validation of docking method

The various docking parameters used were validated by re-docking individually crystallized ligand molecule against IL-6 receptor. The molecular docking simulation technique was validated using overlay method and chemical resemblance [12].

Virtual screening

After validation of the docking procedure, molecular docking simulation-based *in silico* virtual screening was performed against NCI diversity set-II containing 1818 diverse ligand molecules [5,10,11].

Analysis of virtual screening results

After performing molecular docking simulation-based virtual screening of NCI diversity set-II, lead molecules were selected based on lowest binding energy in the predefined range of -5 to -15 kcal/mol. The results obtained were evaluated on the basis of hydrophilic and lipophilic interactions between binding residues present in the active ligand binding site of the protein and ligand. The binding affinity of ligand for a particular target was calculated as shown in Equation 1:

$$K_i = e^{[(\Delta G/(RT)]} \quad \ldots\ldots\ldots\ldots \quad 1)$$

where ΔG = change in free energy on binding, R = gas constant and T = temperature in kelvin.

Absorption, distribution, metabolism and excretion (ADME), and toxicity prediction

Physicochemical parameters and toxicity profile of selected lead molecules were evaluated using DataWarrior software. DataWarrior software checked for the presence of major toxic effects such as mutagenicity, tumorigenicity, irritant and reproductive effects in the lead molecules. It also searched for the presence of functional groups responsible for such toxic effects. DataWarrior software was also used to calculate drug-likeness and drug score of selected lead molecule on the basis of their physicochemical properties [14,15].

RESULTS

Selected and prepared macromolecule

Human TNF α bound to small inhibitor molecule (pdb id: 2AZ5) was downloaded from protein data bank. The 2AZ5 protein complex consisted of four identical polypeptide chains, each of 148 amino acids. The three dimensional structure model of TNF α is shown in Figure 1. Chains A and B were retained by removing the remaining polypeptide chains using chimera software. After processing the receptor molecule, it was saved in *pdbqt format using AutoDock software.

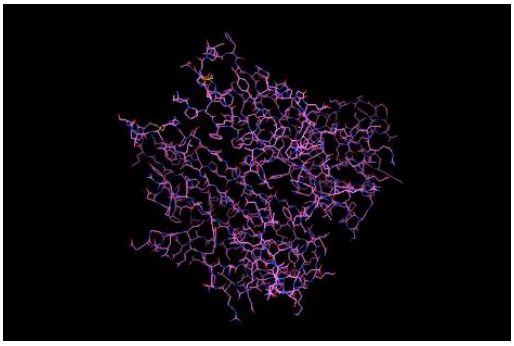


Figure 1: Three-dimensional (3D) structural model of TNF-α obtained from RCSB protein data bank

Ligand prepared for molecular docking

Nine rotatable bonds were present in the ligand molecule, and were kept rotatable. The prepared ligand was saved in *pdbqt format.

Identified binding site and prepared grid box

The amino acid residues Tyr119, Tyr151, Leu57 and Gly121 were involved in active binding of the ligand to TNF-α receptor. An appropriate grid box

was prepared by covering all the macromolecular residues involved in active binding of ligand. The coordinates used for preparation of the grid box are shown in Table 1. The grid box covering the active binding site of macromolecule is shown in Figure 2.

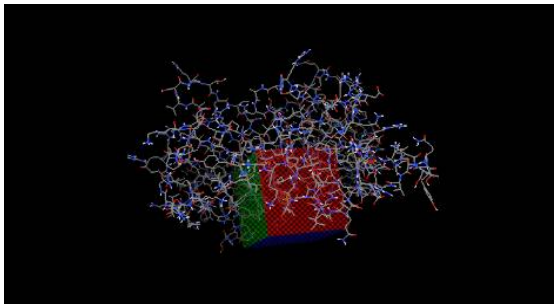


Figure 2: The 3D grid box covering ligand binding site present in human TNF-α receptor

Molecular docking simulation and its validations

The results obtained after molecular docking of ligand bound to human TNF-α receptor are shown in Table 2. The overlaid conformation of the docked ligand with reference to the crystal structure of downloaded protein is shown in Figure 3. The interactions present in the docked conformation with respect to its crystallized structure are shown in Figure 4.

Outcome of molecular docking simulation of human IL-6

Tartaric acid bound to human IL-6 (pdb id: 1ALU) was downloaded from protein data bank. The 1ALU protein complex consisted of a polypeptide chain of 186 amino acids (Figure 5). After processing the receptor molecule, it was saved in *pdbqt format using AutoDock software.

The prepared ligand for molecular docking

Three rotatable bonds were present in the ligand molecule, and they were kept rotatable. The prepared ligand was saved in *pdbqt format.

Identified binding site and prepared grid box

The amino acid residues Gln175, Arg179 and Arg182 were involved in active binding of ligand tartaric acid to human IL-6 receptor. An appropriate grid box was prepared by covering macromolecular residues involved in active binding of ligand to human IL-6 receptor. The coordinates used for preparation of the grid box are shown in Table 3.

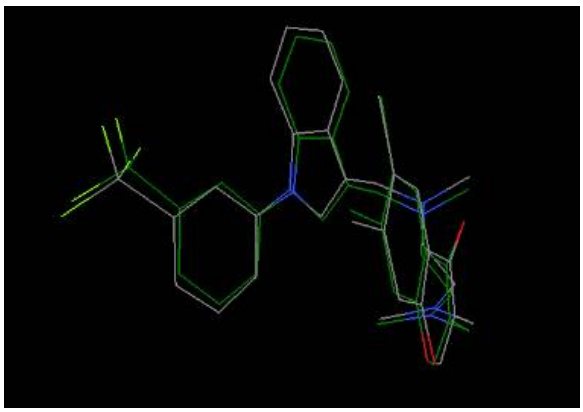


Figure 3: Overlay conformation of the docked ligand with respect to its bioactive conformation

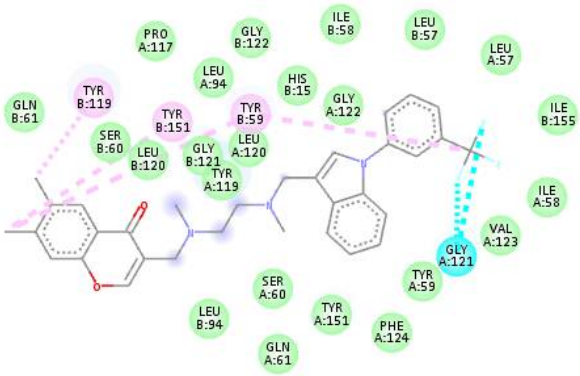


Figure 4: Binding mode and chemical interactions of ligand bound to human TNF-α receptor

Table 1: Coordinates used for preparation of the grid box

Proteins	x-D	y-D	z-D	Spacing (Å)	x center	y center	z center
2AZ5	44	42	44	0.369	-19.163	74.452	33.837

Table 2: Molecular docking results for ligand bound to human TNF-α receptor (2AZ5)

Protein	Interacting residues	Internal validation RMSD	Binding energy (kcal/mol)	Binding affinity (μM)
2AZ5	Tyr119, Tyr151, Leu57 and Gly121	0.61	-7.75	2.07

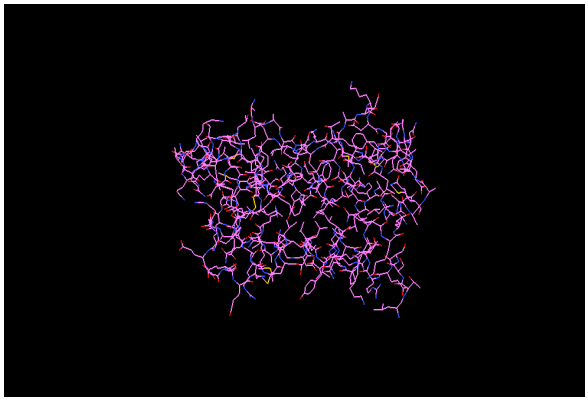


Figure 5: Three-dimensional crystal structure of IL-6

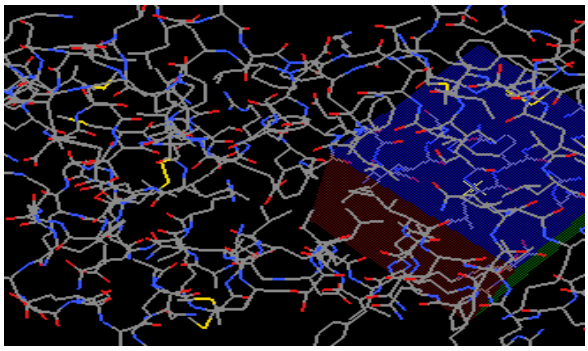


Figure 6: The 3D grid box covering ligand binding site of human IL-6 receptor

Results of molecular docking simulation and their validation

The results obtained after molecular docking of ligand bound to human IL-6 receptor are shown in Table 4. The overlaid conformation of the docked ligand with reference to the crystal structure of downloaded protein is shown in Figure 7. The interactions present in docked conformation with respect to its crystallized structure are shown in Figure 8.

Virtual screening

The results obtained after performing molecular docking simulation-based virtual screening of selected lead molecules against human TNF-α and IL-6 receptors are shown in Table 5.

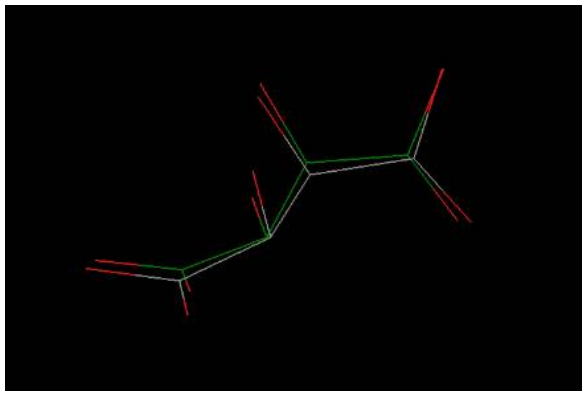


Figure 7: Overlay conformation of the docked ligand with respect to its bioactive conformation

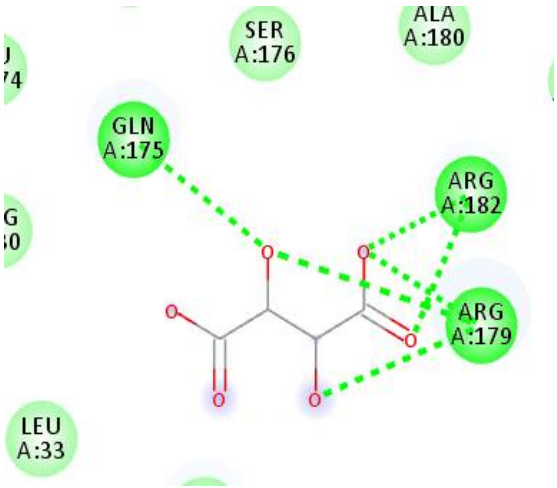


Figure 8: Binding mode and chemical interactions of ligand bound to IL-6 receptor

Physicochemical properties of lead molecules

The five selected lead molecules were assessed for pharmacokinetics profiling using DataWarrior software. Their physicochemical properties were selected based on Lipinski's rule of five and Vebar rule.

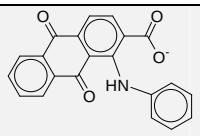
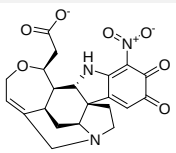
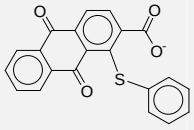
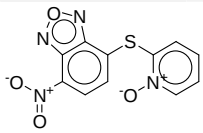
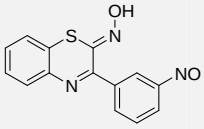
Table 3: Coordinates used for preparation of the grid box

Protein	x-D	y-D	z-D	Spacing (Å)	x center	y center	z center
1ALU	40	40	40	0.364	-7.677	-12.743	0.048

Table 4: Molecular docking results of ligand bound to human TNF-α receptor (2AZ5)

Protein	Interacting residues	Internal validation RMSD	Binding energy (kcal/mol)	Binding affinity (μM)
1ALU	Gln175, Arg179 and Arg182	0.45	-5.65	72.72

Table 5: Virtual screening results

S/N	Name	Structure	1ALU	2AZ5
1	ZINC03898665		-8.61	-7.65
2	ZINC19701771		-8.53	-7.51
3	ZINC05015095		-8.31	-7.98
4	ZINC01757986		-8.00	-7.57
5	ZINC06576501		-7.90	-7.10

The significant physicochemical properties used in this study were calculated partition coefficient (cLogP), topological polar surface area (TPSA), molecular weight, hydrogen bond donor (HBD) and hydrogen bond acceptor (HBA) sites. The physicochemical properties of the five lead molecules for both TNF-α and IL6 receptors are shown in Table 6.

ADME and toxicity profiling of dual inhibitors targeting TNF-α and IL-6

The results of toxicity profiling showed that compounds ZINC19701771 and ZINC06576501 do not possess any functional group responsible for major toxicity such as mutagenic, tumorigenic, irritant and reproductive effects. However, ZINC03898665 and ZINC05015095 possess some mutagenic and reproductive effects. Compound ZINC01757986 also showed high chance of mutagenicity (Table 7).

DISCUSSION

An understanding of the immunopathogenesis of RA, and progress in biopharmaceutical development, have facilitated the introduction of novel immune-modulating therapies the effectiveness achieved with certain agents, particularly the TNF inhibitors, has spurred the development of additional biological agents targeting other components of the dysregulated immune response relevant to the etiology and sustenance of immune-driven systemic inflammation characteristic of RA. Among these other potential targets is IL-6, a cytokine with effects on numerous cell types, including those involved in the pathogenesis of RA. Based on its activities, IL-6 appeared to be a viable target for autoimmune disease. Inhibitors of IL-6 were successful in animal models of autoimmune disease paving the way for subsequent studies in humans. The greatest experience to date has been with tocilizumab, a humanized monoclonal antibody specific for IL-6 receptor (IL-6R). The presence of cytokines such as TNF-α and IL-6 in affected joint tissues reveals their involvement in the pathogenesis of RA.

Table 6: "Lipinski's rule of five" for dual inhibitors targeting TNF-α and IL-6

Compound ID	Mol wt	Clog*P*	TPSA (Å^2)	HBA	HBD
ZINC03898665	342	1.97	86.3	5	1
ZINC19701771	426	-4.233	144.59	10	1
ZINC05015095	359	2.26	99.57	4	0
ZINC01757986	290	1.13	135.5	8	0
ZINC06576501	299	1.46	116.07	6	1

(ClogP = calculated partition coefficient; Mol wt = molecular weight; TPSA = two dimensional polar surface area; HBA = hydrogen bond acceptor; and HBD = hydrogen bond donor)

Table 7: Toxicity profiling for dual inhibitors targeting TNF-α and IL-6

Lead compound	Effects			
	Mutagenic	Tumorigenic	Irritant	Reproductive
ZINC03898665	Low	None	None	High
ZINC19701771	None	None	None	None
ZINC05015095	Low	None	None	High
ZINC01757986	High	None	None	None
ZINC06576501	None	None	None	None

Hence, they serve as potential drug targets for the development of anti-rheumatic drugs. Rheumatoid arthritis therapies that are based on inhibition of a single cytokine such as TNF-α or IL-6, produce clinically meaningful responses in only about half of the treated patients. In this study, *in silico* molecular docking simulation based virtual screening approach was employed to design dual inhibitors of TNF-α and IL-6 in a bid to develop a potential anti-rheumatic drug. The target receptors for TNF-α and IL6 were prepared for molecular docking simulation using AutoDock based MGL tools software. Two chains A and B out of four identical chains having 148 amino acids were retained for experimental studies, while the other two were removed by chimera software. In IL-6, a single polypeptide chain having 186 amino acids was present in the macromolecular complex.

After performing molecular docking simulation based virtual screening of "NCI Diversity set-II" ligand library having 1880 diverse ligand molecules, five lead molecules were selected based on lowest binding energy for both target macromolecules. Osiris Molecular Property Explorer tool was then used to predict their pharmacokinetic properties. Molecular docking simulation-based virtual screening technique is very useful in selecting potential lead compounds. After virtual screening, the five selected lead molecules out of 1818 diverse ligands were further evaluated against human TNF-α and IL-6 receptors for their dual inhibitory effects. They exhibited promising *in silico* results with potent inhibition of both target receptors by showing good binding affinity, good pharmacokinetic properties and absence of any major toxic effects.

Two of the selected lead molecules exhibited good inhibitory effects with excellent pharmacokinetics profiles, and did not show any major toxic effects such as mutagenic, tumorigenic, irritant and reproductive effects. Two of the compounds (ZINC03898665 and ZINC05015095) exhibited some mutagenic and reproductive effects, while ZINC01757986 appeared to have high chance of mutagenicity.

Limitations of the study

In silico computational prediction studies are based on mathematical calculations using physical theoretical rules, and as such there are rare chances of obtaining false positive results. Therefore, experimental validation of predicted computational results is highly recommended before proceeding on clinical trials.

CONCLUSION

The results obtained in this study indicate that the two lead molecules (ZINC19701771 and ZINC06576501) that showed reliable physicochemical properties can serve as potential candidate for development of anti-arthritic drug that can effectively inhibit human TNF-α and IL-6 receptors.

DECLARATIONS

Acknowledgement

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Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

This work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them. The authors contributed equally both in executing the work, and writing of the manuscript. Shu-Qiang Wang, Meng Shi and Lei Fang conceptualized the study. Ying Fan, Meng Shi, Sheneg-Ming Xu, Cong Wang and Zhong-Xiang Yu performed the molecular docking simulations and virtual screening. Pharmacokinetics profiling and toxicity evaluation were performed by Shu-Qiang Wang and Lei Fang.

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