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International Journal of Pharmaceutical Research & Allied Sciences, 2023, 12(1):59-65
https://doi.org/10.51847/PTuP5sCHCD

Research Article

ISSN : 2277-3657
CODEN(USA) : IJPRPM

In-silico Prediction of Maximum Binding Affinity of Disease-Modifying Antirheumatic Drugs with Homo sapiens Acrosomal Protein SP-10

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ABSTRACT

The increasing population has prompted scientists to explore novel technologies to manage health concerns. Multidrug therapies occupy a prominent position in disease management. Among all the pharmaceuticals, Disease-Modifying Anti-Rheumatic Drugs (DMARDs) captivated global researchers to accomplish the needs of Rheumatoid Arthritis (RA) management. Researchers have documented side effects associated with medications in addition to the therapeutic effects. The issue of infertility is one of the most concerning side effects of the drug. The purpose of this research was to determine the in-silico interaction potential of DMARDs such as Hydroxychloroquine, Leflunomide, Methotrexate, Tofacitinib, Baricitinib, and Upadacitinib with the Homo sapiens acrosomal protein SP-10. The SP-10 protein encoded by the ACRV1 gene is speculated to play an essential role in the binding of egg to sperm during fertilization. A maximum binding affinity of -5.1 kcal/mol was observed for Methotrexate among all drugs that interacted with SP-10 protein structure. The obtained in-silico interaction analysis data can be used for the generation of in-vitro and in-vivo assessment data, which are essential for dealing with fertility-related concerns.

Key words: *Infertility, Methotrexate, Binding affinity, Molecular docking studies*

INTRODUCTION

With the advent of science and technology, researchers are exploring millions of naturally derived and synthetic compounds for the betterment of human life [1, 2]. According to the markets and markets statistics report, the investment in global drug discovery is expected to surge from USD 11.1 billion to USD 21.4 billion by 2025 [3-5]. Multidrug therapy offers several advantages in disease management [6, 7]. However, prescribing more than one drug together may cause side effects including headache, fever, dizziness, skin rashes, nausea, vomiting, diarrhea, and drowsiness to the patients [8-10].

Among all the therapeutics, Disease-Modifying Anti-Rheumatic Drugs (DMARDs) gain the attention of researchers because of the steep rise in Rheumatoid Arthritis (RA) worldwide [11, 12]. RA is a chronic and highly destructive autoimmune disease characterized by symmetrical joint pain and swelling [13, 14]. Recent findings stated that DMARDs such as Hydroxychloroquine, Leflunomide, Methotrexate, Tofacitinib, Baricitinib, and Upadacitinib used in RA treatment also showed drastic effects on fertility [15-27]. However, a few clinical reviews and case studies support the side effects of DMARDs, and the interactions of these medicines with fertility-related biomolecules are not clearly understood.

To obtain the necessary lead information for conducting *in-vitro* and *in-vivo* studies, in the present study I assessed the *in-silico* interaction of DMARDs with the acrosomal protein SP-10 of *Homo sapiens*, which plays a vital role in the binding of eggs and sperm to facilitate fertilization [28-30].

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MATERIALS AND METHODS

Preparation of protein

Before molecular docking, the *Homo sapiens* acrosomal protein SP-10 protein sequence with the accession number AAB28238.2 was retrieved from National Centre for Biotechnology Information (NCBI). A three-dimensional structure was predicted for the obtained sequenceby template-based modeling using the GalaxyTBM server [31]. To obtain thebinding pockets and Grid parameters (X,Y, and Z attributes) that are essential for docking studies, the predicted structure was subjected to the ProBiS web server (http://probis.cmm.ki.si/) analysis [32]. The protein was prepared for molecular docking studies by using BIOVIA Discovery Studio software and AutoDockTools-1.5.6 [33].

Preparation of ligand

The chemical structures of disease-modifying anti-rheumatic drugs such as Hydroxychloroquine (3652), Leflunomide (3899), Methotrexate (126941), Tofacitinib (9926791), Baricitinib (44205240), and Upadacitinib (58557659) were retrieved from PubChem database site in .sdf formats and converted to .pdb format by using OpenBabelGUI chemical toolbox [34]. Whenpreparing, Gasteiger charges were added to the ligands using AutoDockTools-1.5.6. This software gives information about the rotatable bonds for ligands. Also, it helps for convertingprotein, ligands structure of the PDB format into the PDBQT (Protein Data Bank, Partial Charge(Q), & Atom Type (T)) formats, which are essential for finding binding affinity in Autodock Vina [35].

Molecular docking

Molecular docking studies were conducted to obtain the maximum binding affinity of DMARDssuch as Hydroxychloroquine, Leflunomide, Methotrexate, Tofacitinib, Baricitinib, and Upadacitinibwith SP-10 protein using Autodock vina [35]. By using the BIOVIA Discovery Studio SP-10 protein amino acids residues interactions with the ligands were visualized [33].

RESULTS AND DISCUSSION

Preparation of protein

To assess the binding efficiency of DMARDswith*Homo sapiens* acrosomal protein SP-10, the protein was screened for binding sites that are essential for the ligand interaction by using the ProBiS server. The best binding site showing a 1.89 confidence score was selected out of 10 binding sites. The grid parameters, x =162.78, y =19.53, and z =99.238629 coordinates essential for the docking were obtained for the protein using BIOVIA Discovery Studio. When preparingprotein, polar hydrogens were added, and water molecules and hetero atoms were removed from the protein crystal structure to prevent unwanted interactions while docking. The prepared protein was saved in pdbqt format.

Ligand preparation

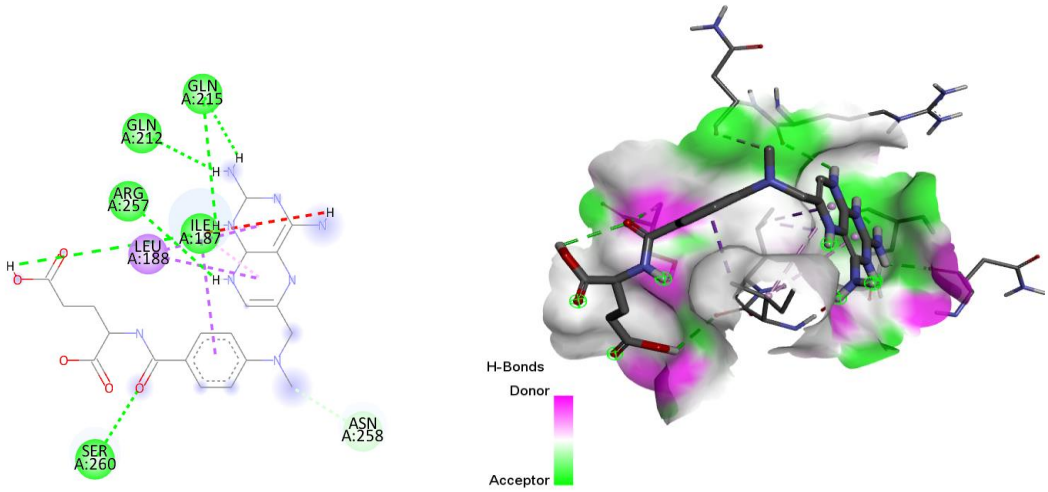
Before molecular docking, the ligands were prepared using AutoDock.While preparing Baricitinib the following parameters were observed, 16 non-polar hydrogens, 9 aromatic carbons, 5 rotatable bonds, and TORSDOF of 5, for Hydroxychloroquine, 24 non-polar hydrogens, 9 aromatic carbons,10 rotatable bonds, and TORSDOF of 10, followed by Leflunomide, 8 non-polar hydrogens, 9 aromatic carbons, 4 rotatable bonds, and TORSDOF of 3, Methotrexate 15 non-polar hydrogens, 12 aromatic carbons, 14 rotatable bonds and TORSDOF of 13, Tofacitinib 19 non-polar hydrogens, 6 aromatic carbons, 4 rotatable bonds and TORSDOF of 3 and for Upadacitinib 17 non-polar hydrogens, 8 aromatic carbons, 6 rotatable bonds, and TORSDOF of 4 other than gasteiger charges.

Molecular docking analysis

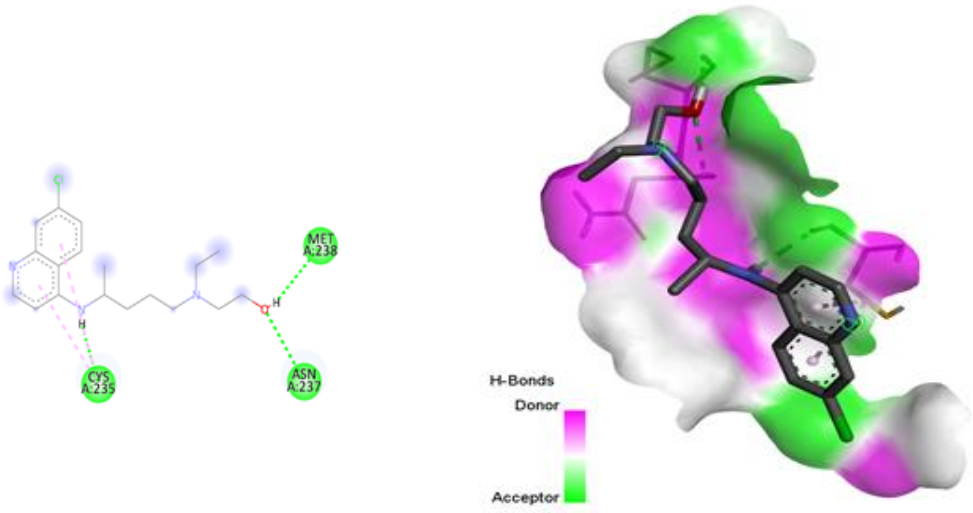
Molecular docking is essential for predicting the binding affinity between the ligands and protein. To investigate the possible interaction of DMARDs such as Hydroxychloroquine, Leflunomide, Methotrexate, Tofacitinib, Baricitinib, and Upadacitinib against *Homo sapiens* acrosomal protein SP-10 structure using molecular docking were analyzed. The results of binding affinity and amino acid residues involved in the hydrogen bond formationwere curated in **Table 1 and Figure 1**.

Table 1. Docking scores and the amino acid residues involved in H bond formation between the ligands and SP-10 protein structure

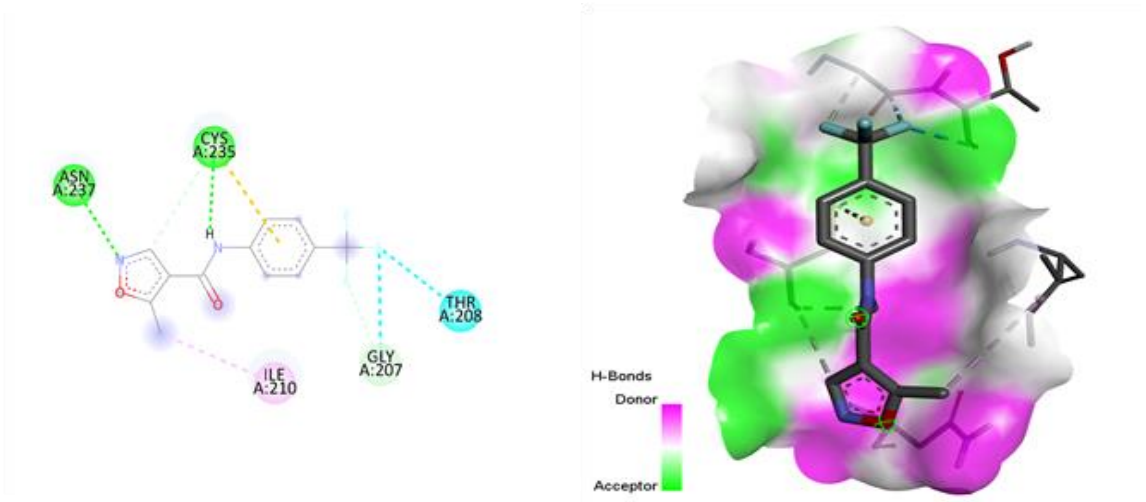
Ligands	*Homo sapiens* acrosomal protein SP-10	
	Binding affinity (kcal/mol)	**Amino acid Residues involved in H bond formation**
Methotrexate	-5.1	ILE187, GLN212, GLN215, ARG 257, SER 260
Hydroxychloroquine	-4.2	CYS 235, ASN237, MET238
Leflunomide	-4.8	CYS 235, ASN237
Tofacitinib	-4.6	CYS 235
Baricitinib	-4.9	GLN215, CYS 235, ASN237
Upadacitinib	-4.8	GLU236



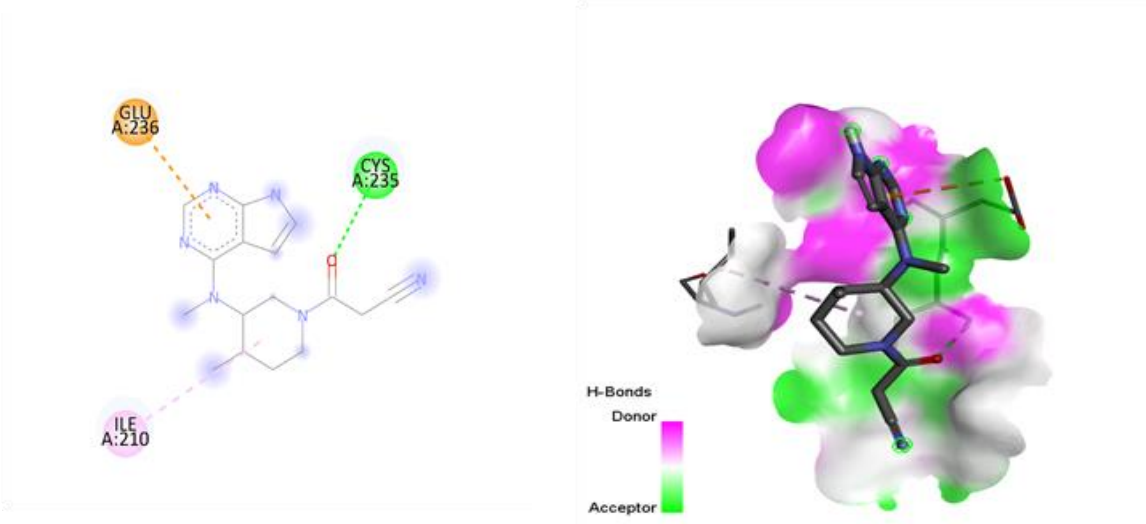
a) Methotrexate



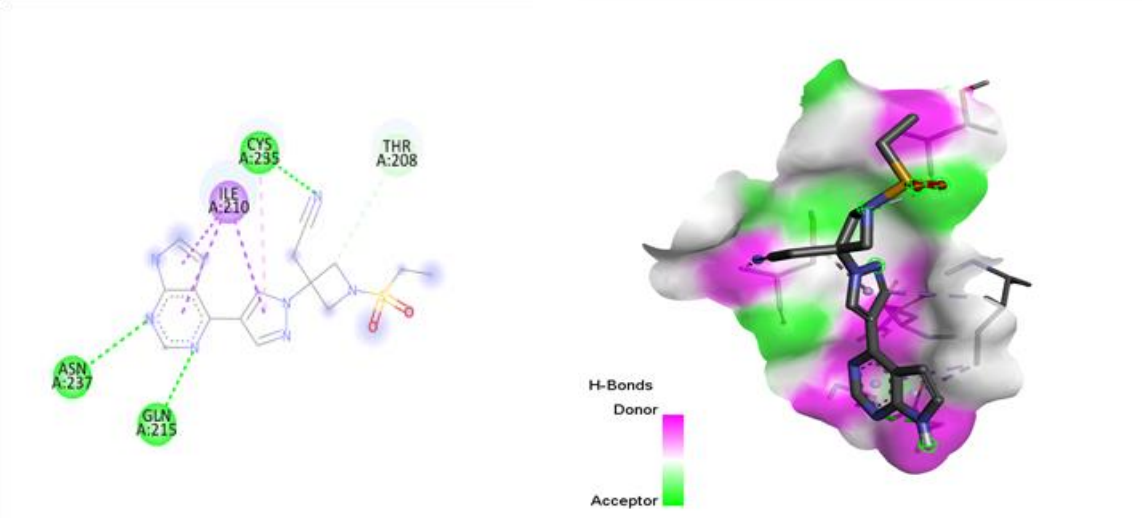
b) Hydroxychloroquine



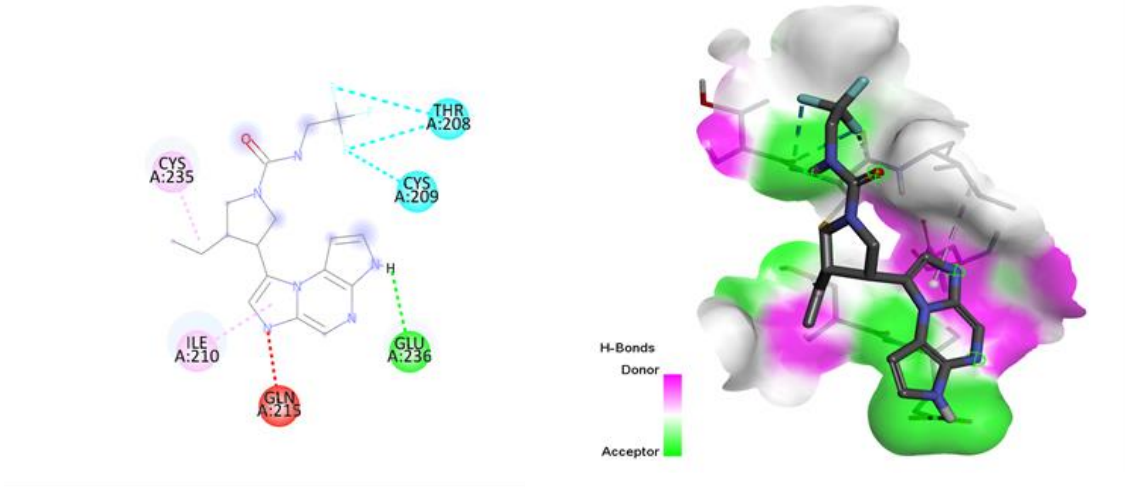
c) Leflunomide



d) Tofacitinib



e) Baricitinib



f) Upadacitinib

Figure 1. Amino acid Residues 2D interaction with the ligands. a) Methotrexate, b) Hydroxychloroquine, c) Leflunomide, d) Tofacitinib, e) Baricitinib, f) Upadacitinib): Green color denotes the H bond interaction between amino acid residues and ligand.

CONCLUSION

Through the *in-silico* analysis, Methotrexate showed the highest binding affinity -5.1 compared toBaricitinib-4.9 followed by Leflunomide -4.8, Upadacitinib-4.8, Tofacitinib-4.6, Hydroxychloroquine -4.2 kcal/mol. The interactions of Hydroxychloroquine, Leflunomide, Tofacitinib, and Baricitinib were compared with one another, in that common hydrogen bond formation withthe Cys235 amino acid residue of SP-10 protein was noticed. Among the six drugs, Methotrexate exhibited the maximum binding affinity with SP-10 protein. To my knowledge, this is the first *in-silico* analysis to report DMARDs and SP-10 protein interaction. This data will hopefully aid researchers in addressing male fertility concerns through *in-vitro* and *in-vivo* studies using methotrexate.

ACKNOWLEDGMENTS : The author would like to thank the HOD Life Sciences, VC, Pro VC, and Christ Deemed to be University research cell for providing the necessary facilities to carry out this work.

CONFLICT OF INTEREST : None

FINANCIAL SUPPORT : None

ETHICS STATEMENT : None

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