Comparative Docking Study of Azole Derivatives on Toll-Like Receptor 1

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Abstract: - Molecular docking study of newly synthesized azole derivatives was performed through Swiss Dock online tool. Various supportive drug design tools like Marvin Sketch for drawing ligand molecules and Discovery Studio Visualizer for preparing protein molecules were also played important role during docking study. In docking study azole derivatives were used as ligand whereas Toll-like receptors which is a part of innate immunity were used as receptor for ligand. The principle/theory behind this docking study is that the various toll-like receptors generate non-specific immune response against various pathogenic microbes when they are activated by any type of suitable ligand molecules. File related to crystallographic structure of TLR1 (PDB ID: 6nih) were downloaded in the form of .pdb from Protein Data Bank (https://www.rcsb.org/) online database. Ligand file prepared in the form of. mol2 format by Marvin Sketch. Based on lowest negative docking score value azole derivative T1 showed best result, which is comparable to the standard drug compound Ciprofloxacin whose docking score is predicted to be -9.64. This docking study will help differentiate preexisting molecule and newly designed molecule based on in-silico study. The relationship between docking score and biological activity can also be established in the future.

Key Words: —*Docking score, receptor, ligand, toll-like receptor, non-covalent interaction.*

I. INTRODUCTION

Nowadays human beings are suffering from many diseases due to various reasons (Figure 1) such as wrong diet, daily routine, and pollution. To avoid diseases, all people nowadays are consuming medicines indiscriminately. Initially, the drugs are effective, but over time, the drug resistance is slowly developing in the microbes. This is a serious problem, so we also have to keep searching for new medicines for microbes from time to time. In the olden times, this was a very laborious task and it took 10-15 years for each drug to be made and it was not necessary that whatever molecule was developed should work as per the wish. There was a high probability of failure.

Manuscript revised April 28, 2022; accepted April 29, 2022.
Date of publication May 02, 2022.

This paper available online at www.ijprse.com

ISSN (Online): 2582-7898; SJIF: 5.59

This entire drug development process consumes a lot of time, money, and labor. Now with time, resources are also increasing for drug discovery and advancement is also happening in technology. Earlier, biological study could be done only after drug synthesis in the form of in-vivo and in-vitro; techniques that take place inside of a living organism and those that take place outside of a living organism. Nowadays in the age of computer a revolutionary system has emerged in the field of in-silico method biological study. Using this system saves time, labor, and money. The term "in-silico" is a contemporary term that refers to computer-assisted testing. The origins of the phrase "in silico" are unclear, with various scholars claiming credit for its creation [1].

Many topics of research are becoming increasingly important in the modern era. Apart from its uses, researchers' interest in pharmaceutical applications has developed. Proteins play a significant role in numerous in-vitro and in-vivo investigations to better understand drug action. Docking programs are used in a variety of applications, including protein engineering and drug design [2].

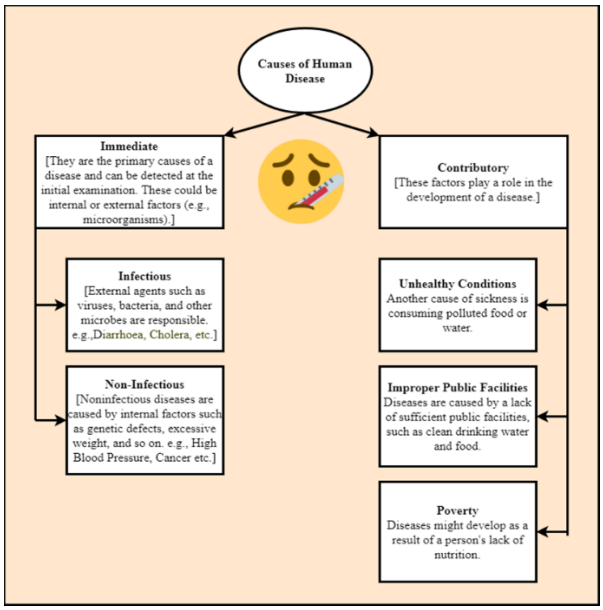


Fig.1. Causes of Human Disease

A Protein is any of a group of nitrogenous organic compounds with big molecules made up of one or more long chains of amino acids that are important components of all living creatures, particularly as structural components of bodily tissues like muscle, hair, and skin, as well as enzymes and antibodies. Cellular receptors are signal-receiving proteins that can be found within or on the surface of a cell. Chemical signal occurs when a ligand interacts to a protein receptor in normal physiology. The ligand is a chemical messenger produced by one cell to communicate with another cell. The binding has a biological effect, which can show as a variety of alterations in the cell, such as changes in gene transcription or translation, as well as changes in cell shape. In most cases, a single ligand will bind to a single receptor and induce a physiological response. There are numerous forms of cellular signaling, each of which is dependent on a particular set of ligands and receptors [3]. Similarly, there is a protein receptor in our body, which is part of our in innate immunity, known as Toll-like receptors (TLR1-TLR10) (Table 1). Pattern recognition receptors recognize pathogen-associated molecular patterns (PAMPs) and endogenous damage-associated molecular patterns (DAMPs) by pattern recognition receptors (PRRs). The toll-like receptors (TLRs) are the most ancient class of PRRs discovered, with the broadest range of pathogen recognition. TLRs are type I transmembrane proteins with three structural domains: a leucine-rich repeats (LRRs) motif, a transmembrane domain, and a Toll/IL-1 receptor (TIR) domain in the cytoplasm. The TIR domain interacts with signal transduction adaptors and starts signaling, whereas the LRRs motif is important for pathogen identification [4]. Azole derivatives may act on components of the innate immune system aiding in the body's natural defense against the infecting pathogens. The innate immune response has physical barriers that provide protection from the environment which include the skin and mucus membranes of the respiratory, gastrointestinal, and genito-urinary tracts. Once microbes have invaded these barriers, which encounter a multitude of innate defenses that include phagocytes, natural killer cells, T cells, B cells, and endothelial cells [5].

Table.1. Various TLR Receptors and Its Description

TLRs	Exogenous Ligand	Signal Adaptor	Production	References
TLR1	Bacteria: triacyl-lipopeptides	MyD88	Proinflammatory cytokines	6, 7
TLR2	Bacteria: peptidoglycan, lipoproteins, LTA Fungi: zymosan	MyD88/ TIRAP	Proinflammatory cytokines	8, 9
TLR3	Viruses: dsRNA	TRIF	Proinflammatory cytokines, type IENS	10
TLR4	Bacteria: LPS Viruses: RSV fusion protein Fungi: mannan Protozoa: Glycoinositol phospholipids	MyD88/ TIRAP/ TRAM/ TRIF	Proinflammatory cytokines, type I IFNS	11
TLR5	Bacteria: flagellin	MyD88	Proinflammatory cytokines	12, 13
TLR6	Gram+ bacteria: peptidoglycan, lipopeptides and lipoproteins Mycoplasma: Diacyl lipopeptides Fungi: zymosan	MyD88	Proinflammatory cytokines	14
TLR7	Viruses: SSRNA	MyD88	Proinflammatory cytokines, type I IFNS	15
TLR8	Viruses: SSRNA	MyD88	Proinflammatory cytokines, type I IFNS	16, 17, 18
TLR9	Bacteria: CpG DNA Viruses: CpG DNA Protozoa: CpG DNA, haemozoin	MyD88	Proinflammatory cytokines, type I IFNS	18, 19, 20
TLR10 (Unconfirmed)	dsRNA	MyD88	Induce the production of proinflammatory cytokines	21

1.1. Receptor-Ligand Complex

Receptors are proteins that are present both inside and outside of cells. They are in-charge of almost every biochemical activity that occurs in our body. Ligands are chemicals that bind to a receptor. Ligands can either activate (agonists) or deactivate (antagonists) receptors by binding to them.A receptor–ligand complex is a receptor–ligand complex that forms as a result of molecular recognition between receptor proteins that interact with other ligand molecules.

1.2. Docking

When the 3D structure of the target protein is available, molecular docking (Figure 2) is one of the most commonly

used virtual screening approaches. This approach was able to estimate the ligand–protein binding affinity as well as the structure of the protein–ligand complex, which is essential information for lead optimization [22]. In this docking study, SwissDock online docking platform was used which is based on the docking program EADock DSS, which has the following phases in its algorithm:

- It creates any binding modes in a box (local docking) or in the vicinity of all target cavities (blind docking).
- It calculates their CHARMM energies on a grid at the same time.
- It uses FACTS to assess and cluster the binding modes with the most favorable energies.
- The most advantageous clusters may be seen and downloaded from the internet.

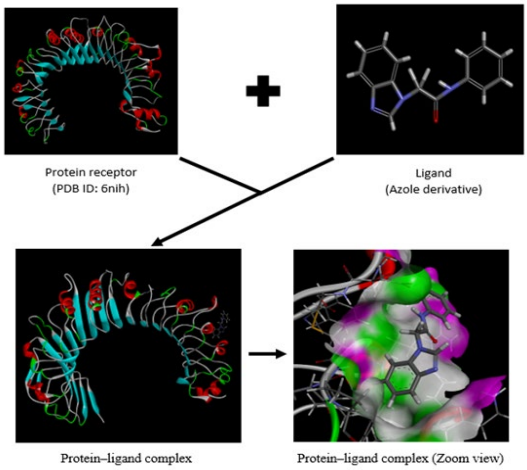


Fig.2. Docking: Protein Ligand Interaction and Formation of Protein-Ligand Complex

II. MATERIALS AND METHOD

2.1. Materials

By using in-silico computational method docking study was performed on online SwissDock tool. The configuration of system at which docking study performed was running on 2.0 GHz AMD A8-6410 APU with Radeon R5 Graphics, 4 GB RAM, 256 GB SSD memory and 64-Bit Window operating system. The webpage address of SwissDock was http://www.swissdock.ch/docking. For offline chemical structure drawing MarvinSketch was used. Discovery Studio Visualizer software were used for target preparation.

2.2. Methodology

2.2.1. Protein Selection and Preparation: The crystallographic structure of protein receptor TLR1 (PDB ID: 6nih) was selected for docking study after literature survey from the online RCSB Protein Data Bank (https://www.rcsb.org/). The user has 2 options to prepare the target. If the user has proper knowledge of how to prepare proteins, then target preparation can be done using other softwares like MGL Tools, Discovery Studio Visualizer etc. Discovery Studio Visualizer (DSV) is very important software in drug designing. By opening the .pdb file of the protein in the DSV software, all water molecules and ligand molecules are removed from the protein structure. After that, polar hydrogen is added to the protein structure. Finally save the protein file in .pdb format. For users who are not familiar with 3D structure files of protein, SwissDock provides some pdb records on its server. From where the user can select the protein he needs. After selecting the protein, the user can perform the study by selecting the required chain present in the protein for docking.

2.2.2. Ligand Selection and Preparation: Structure of five-azole derivative ligand molecules (B1, B2, B3, B4 and B5) was prepared after literature survey. There are two methods available for ligand preparation. In first method, prepare the various ligand on anyone offline tools like Chemdraw, Chemsketch, Marvin tools and save the file in ".mol2" format then open the file in online SwissDock. The rest of the desired modification in ligand molecule will be done by the SwissDock platform itself after submitting the molecules.

In second method, there are already some online platforms, such as Zinc, from where the. mol file can be downloaded. Then the prepared ligand has to be saved in .pdb format using MGL tools for ligand preparation. Since the SwissDock online docking tool does not support the .pdb ligand file, another file conversion software will be needed. For this, using the Open Babel GUI software, convert the .pdb ligand file to. mol2. Now our ligand molecule is ready for docking.

2.2.3. Docking Score Prediction: After performing the target protein and ligand preparation, visited the SwissDock website (http://www.swissdock.ch/docking) and the pointwise steps were followed to find the docking score as given in figure.3. The protein file is uploaded to the "Target Selection" option by visiting the website. If the file size is more than 5MB, the file will not be uploaded due to the limitation. In the target selection option, options related to the PDB code, protein name, sequence or URL related to the target protein are also

given. Such users who have problems with target preparation can directly make target selection through these means.

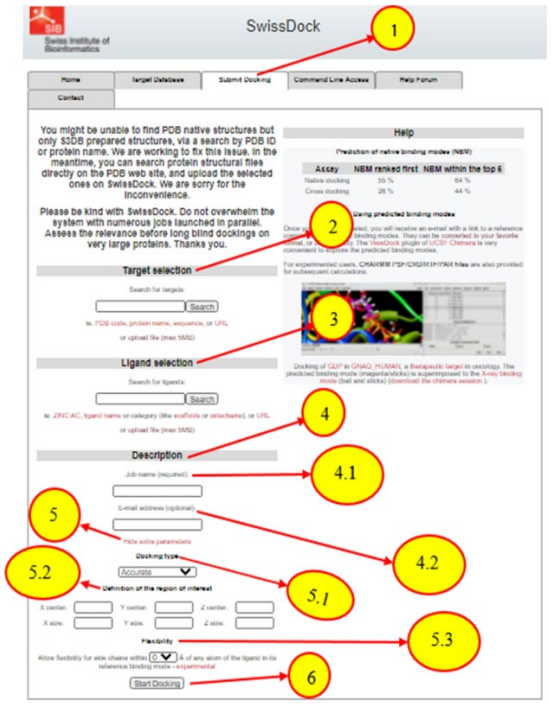


Fig.3. Swissdock (An Online Docking Server for Docking Study)

Like the target selection option, the second option is that of "Ligand selection". In this also, the size limit of the uploaded file is 5MB only. In these also, the prepared ligand file was uploaded. In this way, the pre-existing ligand file can be searched via ZINC AC, legend name or URL.

After selecting the target and ligand file, some description has to be given in "Description" option. Job name and e-mail address have to be given in the description. When we have to know the docking scores of a lot of proteins and ligands, the job name helps us to differentiate them. When the docking job is completed on the SwissDock server, the related link is sent to the given e-mail, which we can see by visiting the given link.

After the description section, "Show extra parameters" option is given below the e-mail address. After tapping on this option, we can define three other parameters. The first is the "Docking Type" in which we can increase or decrease the time of our docking process.

However, the thing to note is that the faster the docking process, the more the accuracy decreases. The second parameter is "Definition of the region of interest" through which we can define the dimensions of X, Y, and Z three on the basis of both center and size to form a grid box. The advantage of creating a grid box is that we can study the docking of the ligand to a specific region of the protein. The third parameter is that of "Flexibility". In general, both the protein and the ligand are considered rigid entities, but for the detail study purpose, we can also define the ligand flexibility by making some changes to the flexibility option.

When all the files related to the protein and ligand have been uploaded and the description has been completed, on clicking “Start Docking” the corresponding job is sent to the SwissDock server for docking analysis.

When all the files related to the protein and ligand have been uploaded and the description has been completed, on clicking "Start Docking" the corresponding job is sent to the SwissDock server for docking analysis. After this, the request submission message from SwissDock comes in the e-mail. It usually takes a few minutes to a few hours for the Docking score to be generated. The time taken to generate the result depends on the size of the protein and ligand structure and on the busyness of the online server. As soon as the docking score is generated, a job termination message is received in the e-mail in which a link is given. From the given link we can get the docking score related output file. The docking score of all the compounds was obtained by adopting the same method. A complete procedure for docking study is given in the figure 4.

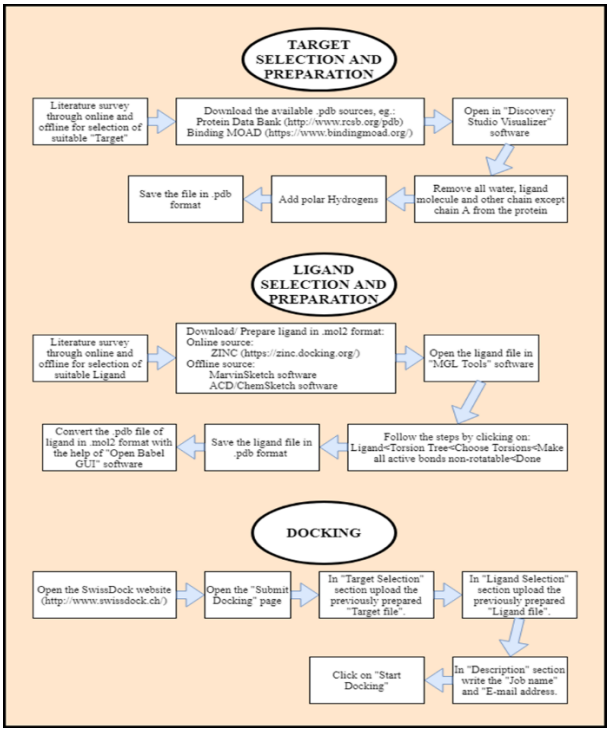
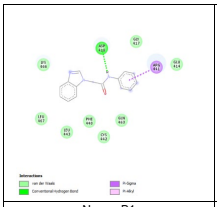
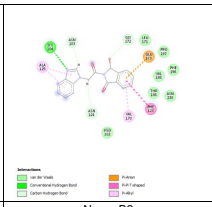
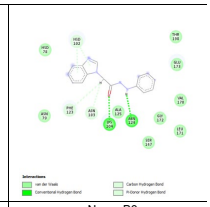
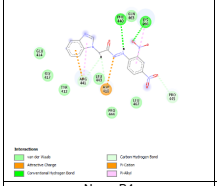
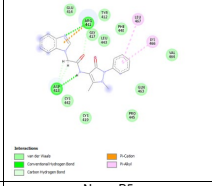
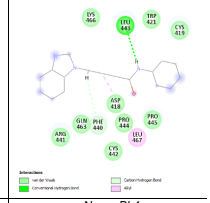
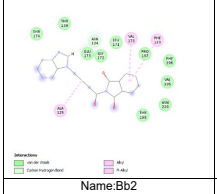
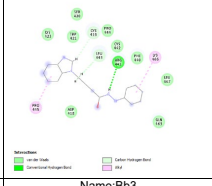
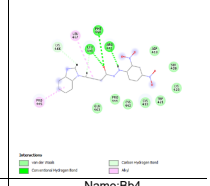
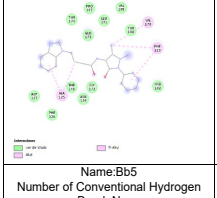
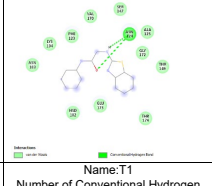
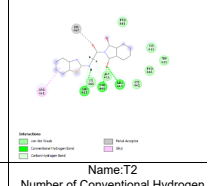


Fig.4. A Flowchart for Docking Study

III. RESULT AND DISCUSSION

Docking studies of all 10 azole compounds were performed on the SwissDock online server. The best score was recognized out of several docking scores obtained from the server. The file received from the server was then opened on Discovery Studio Visualizer (DSV) software for further detailed study of the best docking position. The "target.pdb" file as well as the required ".crd" file of the ligand was opened in the DSV software. The ligand structure of the .crd file was shown to interact with the target receptor (A chain of TLR1). "Show 2D diagram" was activated after activating ligand, interacting atom, pocket atom, ligand interactions options respectively in the "view interactions" section of DSV software, which gave us the 2D structure of the receptor-ligand complex. The table 2 gives the complete picture of 2D structure of each complex.

Table.2. 2d Structure of Various Receptor-Ligand Complex

 Name: B1 Number of Conventional Hydrogen Bond: 1 (2.08Å) Docking Score: -7.52	 Name:B2 Number of Conventional Hydrogen Bond: 1 (2.71Å) Docking Score: -6.85	 Name:B3 Number of Conventional Hydrogen Bond: 2 (2.22Å& 2.14Å) Docking Score: -7.10
 Name:B4 Number of Conventional Hydrogen Bond: (2.45Å & 2.15Å) Docking Score: -7.80	 Name:B5 Number of Conventional Hydrogen Bond: 2 (2.40Å & 1.92Å) Docking Score: -7.86	 Name:Bb1 Number of Conventional Hydrogen Bond: 1 (2.07Å) Docking Score: -7.16
 Name:Bb2 Number of Conventional Hydrogen Bond: None Docking Score: -6.74	 Name:Bb3 Number of Conventional Hydrogen Bond: 1 (2.04Å) Docking Score: -7.03	 Name:Bb4 Number of Conventional Hydrogen Bond: 3 (2.48Å, 3.23Å &1.89Å) Docking Score: -7.65
 Name:Bb5 Number of Conventional Hydrogen Bond: None Docking Score: -7.56	 Name:T1 Number of Conventional Hydrogen Bond: 2 (2.08Å &3.08Å) Docking Score: -7.90	 Name:T2 Number of Conventional Hydrogen Bond: 3 (2.88Å, 3.23Å &2.19Å) Docking Score: -7.33

In the center of this 2D structure is the ligand compound in the form of a stick model arranged in the pocket of the receptor. The pocket of the receptor is formed by chains of several amino acids linked together. In this 2D structure, amino acids are represented as color balls. All these amino acids present in the pocket interact with the ligand, through which the receptor-ligand complex is formed. The table shows several types of non-covalent interactions between the receptor-ligand, including hydrogen bond (conventional hydrogen bond, etc.), van der waals, electrostatic (pi-cation), hydrophobic (pi-alkyl, pi-pi t-shaped etc.) are prominent. All these interactions are present in every 2D image in the form of color blocks. All these interactions play an important role in stabilizing the receptor-ligand complex.

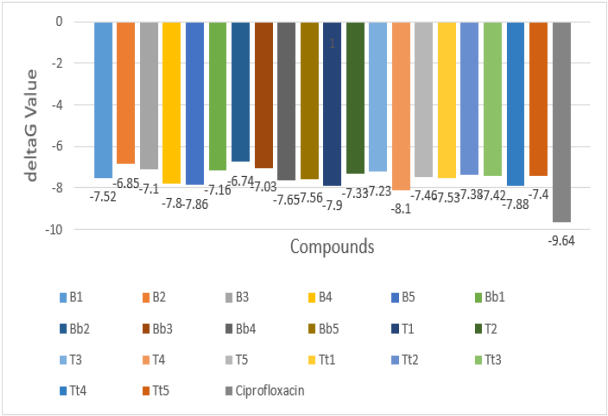


Fig.5. Docking Score Analysis Chart

The column chart (Figure 5) is given in which the deltaG value is given, this gives the docking score. Designed ligand T4 has the highest negative score out of 20 azole derivatives. The T4 ligand molecule in the represented 2D structure is attached to the receptor's amino acid (ASN124) via a conventional hydrogen bond. ASN124 amino acid is forming 1 conventional hydrogen bond with the Hydrogen atom of N-H group with bond length of 2.33Å. Generally, the length of hydrogen bond below 3Å is considered as good. The highest negative score represents the highest score. This suggests that the ligand T4 has the highest binding affinity to the receptor, so it can be assumed that this compound will exhibit the highest biological activity among the 20 designed ligand compound but there is also a twenty first compound in the chart that is already present in the market in the form of ciprofloxacin. We have taken it here as the standard compound. However, all of the test compounds here have a docking score lower than ciprofloxacin which is predicted as -9.64.

IV. CONCLUSION

Some new azole derivatives were designed and their docking studies were performed on chain A of the TLR1 receptor (PDB ID: 6nih). Through docking score, it was found that compound T4 has the most negative docking score, which is -8.1. For this reason, its binding affinity with the receptor will also be high and biological activity will be likely to be displayed best. Compound Bb2 had the lowest docking score, which is -6.74. Because of this, its biological activity will be less likely to be better. One thing to be remembered from this entire docking study is that ciprofloxacin, which is already present in the market, was taken as the standard ligand compound in this study. Whose docking score is predicted to be -9.64, which is much better than the designed ligand molecule. Apart from this, non-covalent interaction between receptor and ligand were also identified. Throughout this study, we also used several software as part of an in-silico study, such as MarvinSketch, MGL Tool, Discovery Studio Visualizer, O:pen babel, Chimera and the online docking server SwissDock. This also makes us understand that for this type of detailed study, we need a group of software. The detailed in-silico study answers many questions, which helps us to identify the best possible drug-like compound. This saves labor, time and money.

Acknowledgement:

We thank and express our gratitude for the invaluable support of the faculties and staffs of Goel Institute of Pharmacy and Sciences. We are grateful to Er. Mahesh Goel, Managing Director, Goel Group of Institutions for providing moral support and encouragement.

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