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Modeling and Docking Studies of Toll Like Receptor 7 From Homo Sapiens with Biosynthetic Compounds Rutin and Kaempferol

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Abstract- Toll-like receptors (TLRs) serve as signaling molecules that identify pathogen-associated molecular patterns (PAMPs) as well as damage-associated molecular patterns (DAMPs), and are expressed by various skin cells including keratinocytes and melanocytes, which are the main cell types involved in both non-melanoma and melanoma skin cancers. A three dimensional model of Toll like receptor 7 from Homo sapiens was generated using 5GMF, as a template with Modeller9v7. With the aid of molecular mechanics and dynamic methods models were generated and checked by Procheck and 3D graph. After energy minimization, the 3D structure of Toll like receptor 7 was compared with a template, and with the aid of the molecular mechanics and molecular dynamics methods, the final models were obtained. Flexible docking studies were performed using a Toll like receptor 7 that is highly expressed with natural inhibitors Rutin and Kaempferol. The results indicated that ARG283, PRO284, LEU286, ILE523, PHE535, MET567, HIS645 in Toll like receptor 7 are important determinant residues in binding process as they have strong hydrogen bonding with these compounds indicating that these are potent inhibitors of toll like receptor 7.

Keywords- Toll like receptor 7, modeling, molecular dynamics, Homo sapiens, docking studies

I. INTRODUCTION

Skin cancer is the most common form of cancer, accounting for nearly half of all cancers in the USA. The most common types of skin cancer are non-melanoma skin cancers (NMSCs): basal cell carcinoma (BCC), which forms in the basal cells, and squamous cell carcinoma (SCC), which forms in the squamous cells. BCCs are rarely fatal, but can be highly disfiguring if allowed to grow. From the most recent estimate, approximately 2.8 million BCCs and 700,000 SCCs are diagnosed annually in the USA [1].

Non-melanoma skin cancer (NMSC) includes BCC and squamous cell carcinoma (SCC). With over 3.5 million new diagnoses annually, NMSC is the most common cancer in the United States. Risk factors for developing NMSC include ultraviolet (UV) light exposure, skin color, sunburns, age, and immunosuppressive status [2]. NMSCs account for over 3,000 deaths each year [3] and also contribute to over $1.4 billion annually for the treatment and management of these skin tumors [4]. Melanoma contributes to approximately 5% of all skin cancer diagnoses, with 76,000 new cases diagnosed in 2012 [5]. Importantly, melanoma leads to over 9,000 deaths annually, which accounts for the majority of skin cancer deaths. Risk factors for melanoma include UV exposure, sunburn, nevi, immunosuppressive status, and family history.

The most common treatments for SCC include excision, Mohs micrographic surgery, and cryosurgery, which when the lesion is detected early and promptly removed, are effective and cause minimal damage [2]. If left untreated, the tumors are able to grow exponentially or metastasize, leading to more invasive procedures. For melanoma, surgical excision is the most common treatment, with recent preferences for Mohs surgery [5]. However, in the case of recurring lesions or lesion patches, surgery may not be an option due to extensively damaged skin or lack of tissue for removing clear margins, resulting in the need for alternative treatment options.

The skin is the largest organ in the body and contains three major cell types, which include melanocytes, Langerhans cells, and keratinocytes. Keratinocytes are the major cell type of the epidermis and provide defense against the environment both as a physical barrier and a key component of the innate immune response [6, 7]. Epidermal keratinocytes, as the outmost environmental barrier, are responsible for the production of antimicrobial peptides [8], which are up-regulated by various stimuli through both the mitogen-

activated protein (MAP) kinase and nuclear factor (NF) kappaB pathways [9]. TLRs are expressed by various skin cells including keratinocytes and melanocytes [10], which are the main cell types involved in both non-melanoma and melanoma skin cancers. Human keratinocytes have been shown to express TLRs 1–6 and 9 [10–14]. Recently, it has been reported that TLR2–5, 7, 9, and 10 are constitutively expressed in human melanocytes [15].

Toll-like receptors serve as signaling molecules that recognize PAMPs, or pathogen-associated molecular patterns, as well as DAMPs and thus, activate the innate immune response through the transcription factor NF-kB [16]. The 10 human TLR family members are characterized by the leucine-rich repeat domain content in both their extracellular region and the intracellular Toll-IL-1 receptor (TIR) domain [17], which can therefore interact with adaptor molecules that contain appropriate adaptor molecules [18].

Toll-like receptors have been proven to be important for both innate immune response specificity [19, 20] as well as for adaptive immune responses such as dendritic cell maturation and co stimulatory molecule expression and the promotion of Th-1 cell-mediated responses through increased production of IL-12 by activated TLRs on dendritic cells [21, 22]. It also has been reported that innate inflammatory responses confined to the epidermis may be affected by TLR expression in human melanocytes [23]. TLRs are initiated in melanocytes, as a result of the inflammatory response to tissue injury, sunburn or skin infection, and constitute a natural defense to recruit innate immune cells.

II. METHODOLOGY

Domain Identification and Template Search

The Toll like receptor 7 (Accession no: Q9NYK1) sequence from Homo sapiens was submitted to Expasy for domain prediction. The predicted domain was searched to find out the related protein structure to be used as a template by the BLAST (Basic Local Alignment Search Tool) program against PDB (Protein Data bank) [24]. Sequence that showed maximum identity with high score and e-value either zero or less negative values were aligned and was used as a reference structure to build a 3D models for Toll like receptor 7. The co-ordinates for the structurally conserved regions (SCRs) for Toll like receptor 7were assigned from the template using multiple sequence alignment, based on the Needleman-Wunsch algorithm [25]. Then using the template structure, Toll like receptor 7 structure was developed with MODELLER 9v7 software.

3D model

The initial models of Toll like receptor 7were built by using homology-modeling method and the MODELLER software; a program for comparative protein structure modeling optimally satisfying spatial restraints derived from the alignment and expressed as probability density functions (pdfs) for the features restrained. The pdfs restrain main-chain N-O distances, C^{α}-C^{α} distances, main-chain and side-chain dihedral angles. The 3D model of the protein was obtained by optimization of the molecular pdf such that the model violates the input restraints as little as possible. The molecular pdf was derived as a set of pdfs restraining individual spatial features of the whole molecule. The optimization procedure was a variable target function method that was applied to the conjugate gradients algorithm positions of all non-hydrogen atoms.

Molecular Dynamics

The structure with least modeler objective function was improved by molecular dynamics and equilibration methods using NAMD 2.5 software for lipids and proteins [26] along with TIP3P model for water [27]. The energy of the structure was optimized with 1,00,000 steps and a cutoff of 12 Å (switching function starting at 10 Å) for van der Waals interactions was assumed. An integration time step of 2 ps was used, permitting a multiple time-stepping algorithm to be employed in which interactions involving covalent bonds were computed every time step, short-range non bonded interactions were computed every two time steps and long-range electrostatic forces were computed every four time steps. The pair list of the non bonded interaction was recalculated every ten time steps with a pair list distance of 13.5 Å. The short-range of non bonded interactions were known as van der Waals and electrostatics interactions between particles within 12 Å. A smoothing objective function was implicated for the van der Waals interactions at a distance of 10 Å. CHARMM27 force-field parameters were used in all simulations for this study [28]. An equilibrated system was simulated for 2ps with a 500 kcal/mol/Å2 restraint on the protein backbone under 1 atm constant pressure and 310 K constant temperature (NPT) and the Langevin damping coefficient was set to 20ps unless otherwise stated.

Structure Validation

Finally structure with least energy having low RMSD (Root Mean Square Deviation) was used for further studies. With this step, the qualities of the predicted models were improved. The final refined structures obtained were analyzed by Ramachandran's plot using PROCHECK (Programs to check the Stereo chemical Quality of Protein Structures) and environment profile using ERRAT graph (Structure Evaluation server). These 3D models were used for active site and for docking of the Ofloxacin to the Proteins [29].

Active site Identification

Active site of Toll like receptor 7 was identified using CASTp server. A new program, CASTp, for automatically locating and measuring protein pockets and cavities, is based on precise computational geometry methods, including alpha

shape and discrete flow theory. CASTp identifies and measures pockets and pocket mouth openings, as well as cavities. The program specifies the atoms lining pockets, pocket openings, and buried cavities; the volume and area of pockets and cavities; and the area and circumference of mouth openings.

Docking method

Docking with GOLD 3.0.1

GOLD (Genetic Optimization of Ligand Docking) a genetic algorithm (GA) based software, mainly utilizes an evolutionary strategy involving 3 genetic operators; cross overs, mutations and migrations [30]. GOLD imports the partial flexibility to proteins and full flexibility to inhibitors. Rutin and Kaempferol were docked into the active sites of Toll like receptor 7 and the interaction of these compounds with the active site residues are thoroughly studied using calculations of molecular mechanics. The parameters used for GA were population size (100), selection pressure (1.1), number of operations (10,000), number of island (1) and niche size. Operator parameters for crossover, mutation and migration were set to 100, 100 and 10 respectively. Default cut off values are, 3.0Å (dH-X) for hydrogen bonds and 6.0Å for van der Waals were employed. The default algorithm speed was selected and the inhibitor binding site in Toll like receptor 7was defined within a 10Åradius with the centroid as HH atom of GLU33 respectively. The number of poses for compounds was set to 100 and early termination was allowed if the top three bound conformations of inhibitors were within 1.5ÅRMSD. After docking, the individual binding poses of these compounds were observed and the interaction with the protein was studied. The best and most energetically favorable conformation of each compound was selected.

GOLD Score fitness function

The four components viz, Protein-ligand hydrogen bond energy (external H-bond); Protein-ligand van der Waals energy (external vdw); Ligand internal van der Waals energy (internal vdw); and Ligand intramolecular hydrogen bond energy (internal- H- bond) were considered for calculating the fitness function of GOLD score. The protein-ligand hydrophobic contact was encouraged by making an empirical correction by multiplying external vdw score with 1.375. The fitness function has been optimized for the prediction of ligand binding positions.

Gold Score = S (hb_ext) + S (vdw_ext) + S (hb_int) + S (vdw_int)

Where,
S (hb_ext) was the protein-ligand hydrogen bond score,
S (vdw_ext) was the protein-ligand van der Waals score,
S (hb_int) was the score from intra molecular hydrogen bond in the ligand
S (vdw_int) was the score from intra molecular strain in the ligand.

III. RESULTS AND DISCUSSION

Homology Modeling of Toll like receptor 7

Domain Identification

Toll like receptor 7 from Homo sapiens was collected and submitted for domain identification. The functional domain region was highlighted in the Fig 1, and taken for further studies.

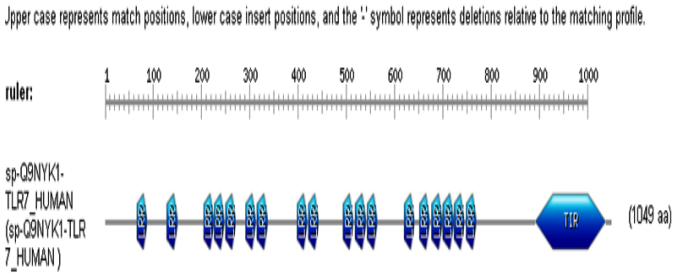


Fig 1: Domain identification in Toll like receptor 7 using Expasy

A high level of sequence identity should guarantee more accurate alignment between the target sequence and template structure. BLAST search against PDB, only 5GMF has a high level of sequence identity 75% with the Toll like receptor 7. Structurally conserved regions (SCRs) for the model and the template were determined by superimposition of the two structures and multiple sequence alignment.

TEMPLATE SELECTION:

Template selection is a process of identifying a suitable protein which shares nearly the same structure of the query protein which doesn't possess the 3D structure. Template selection is very important in comparative protein modeling. Templates can be chosen by various tools such as BLAST, FASTA, Swiss-model, etc. In the case of Blast and Fasta the sequence of protein in Fasta format can be uploaded and the templates can be manually selected by considering the score value and the E value. In the case of Swiss-Model server, it automatically chooses the template and models the protein structure.

BLAST:

A high level of sequence identity should guarantee more accurate alignment between the target sequence and template structure. In the results of BLAST search against PDB, only one-reference protein 5GMF has a high level of sequence identity and the identity of the reference protein.

Template Selection using BLAST:

Figure 2: Blast result with a similar template having 75% identity with Toll like receptor 7 from Homo sapiens.

SEQUENCE ALIGNMENT:

In the following study, we have chosen 5GMF as a reference structure for modeling Toll like receptor 7 domain. Coordinates from the reference protein (5GMF) to the SCRs, structurally variable regions (SVRs), N-termini and C-termini were assigned to the target sequence based on the satisfaction of spatial restraints.

Sequence of the reference structures were extracted from the respective structure files and aligned with the target sequence using the default parameters in ClustalW.

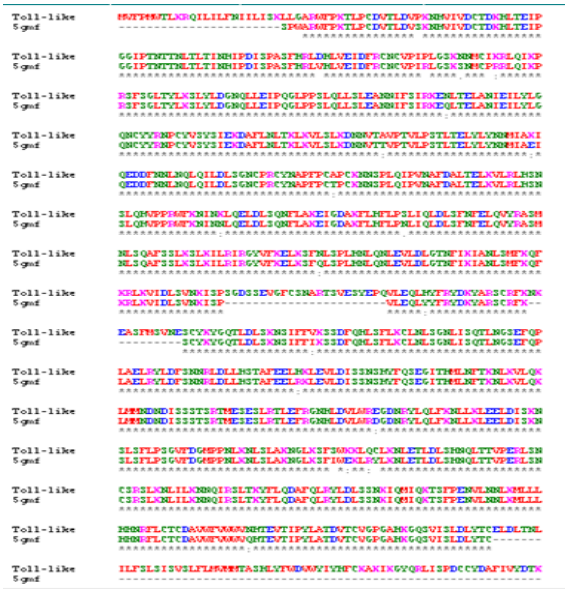


Figure 3: Alignment of Toll like receptor 7 from Homo sapiens with template 5GMF

HOMOLOGY MODELING:

The 5GMF structure was used as the templates for building the 3D models of the Toll like receptor 7 using Modeller9v7. The final stable structure of the Toll like receptor 7 obtained was shown in Figure 4.

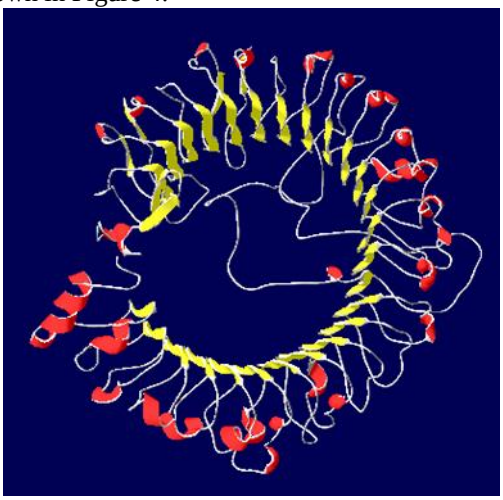
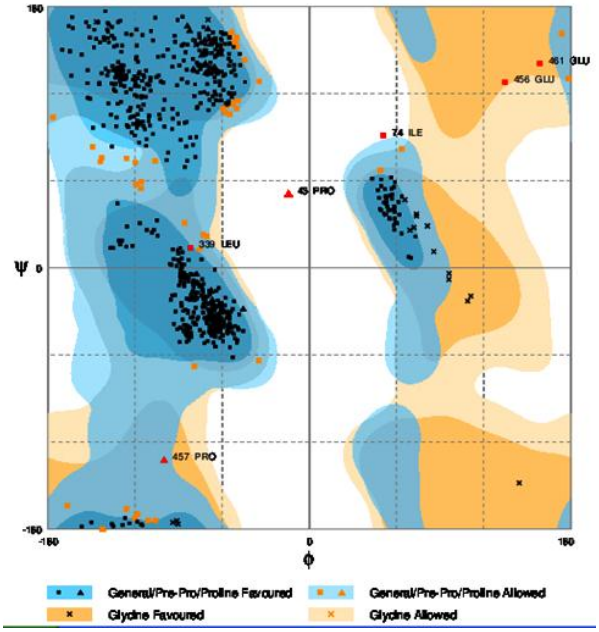


Figure 4: 3D structures of Toll like receptor 7 from Homo sapiens by Modeller9v7.

After the model development, these protein structures were submitted to Ramachandran plot using Rampage server. The favored and allowed regions of amino acids were predicted in Fig 5.



Number of residues in favoured region (~98.0% expected) : 735 (93.6%)
Number of residues in allowed region (~2.0% expected) : 44 (5.6%)
Number of residues in outlier region : 6 (0.8%)

Figure 5: Ramachandran plot of Toll like receptor 7 from Homo sapiens

The structure having the least energy with low RMSD (Root Mean Square Deviation) which was obtained by the NAMD is in Figure 5. The structure having the least energy with low RMSD (Root Mean Square Deviation) which was obtained by the NAMD is in water molecule (TIP3) shown in Figure 6 and 7.

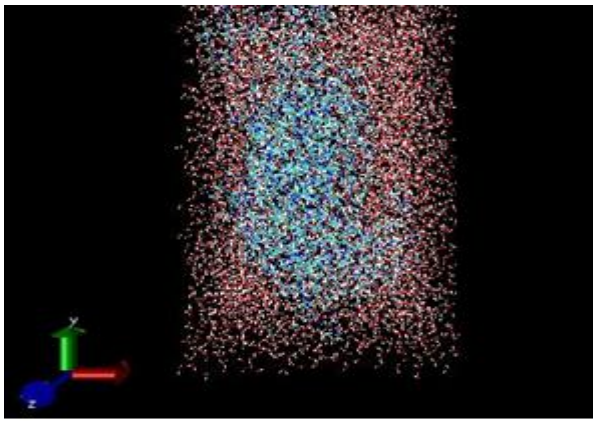


Figure 6: Molecular Dynamics studies of Toll like receptor 7 from Homo sapiens

The final structure was further checked by ERRAT2 and the results have been shown in Figure 7: The overall scores indicates acceptable protein environment.

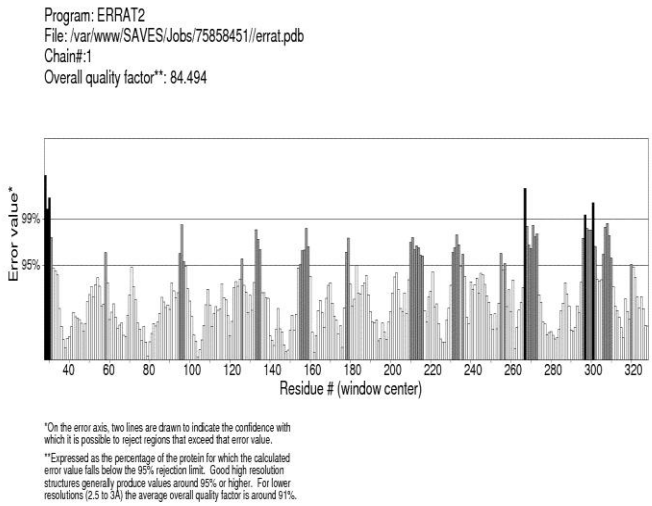


Figure 7: The ERRAT results of Toll like receptor 7from Homo sapiens; overall quality score indicates residues are reasonably folded

Validation of Toll like receptor 7Domain

After the refinement process, validation of the model was carried out using Ramachandran plot calculations computed with the PROCHECK program. The psi and pi distributions of the Ramachandran plots of non-glycine, non-proline residues are summarized in Table 1. The RMSD (Root Mean Square deviation) deviation for covalent bonds and covalent angles relative to the standard dictionary of Toll like receptor 7 was -1.56 and -0.42 Å. Altogether 99.2 % of the residues of Toll like receptor 7 were in favored and allowed regions. The overall PROCHECK G-factor of Toll like receptor 7was -0.44 and verify3D environment profile was good (Fig 8).

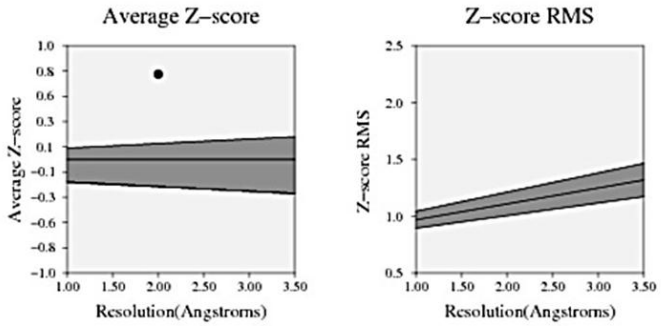


Fig 8: Average Z-score for Toll like receptor 7 from Homo sapiens

Superimposition of 5GMF with Toll like receptor 7 domain

The structural superimposition of 5GMF template and Toll like receptor 7 is shown in Figure 10. The weighted root mean square deviation of trace between the template and final refined models is 0.23A^{o}. This final refined model was used for the identification of active site and for docking of the substrate with the domain Toll like receptor 7.

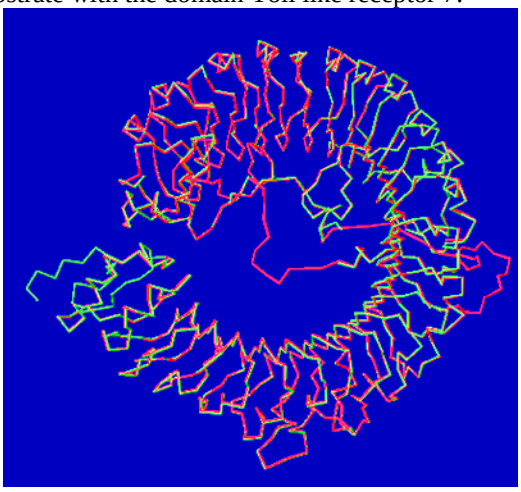


Figure 9: Superimposition Toll like receptor 7 (represented in green color) and 5GMF (represented in red color).

ACTIVE SITE IDENTIFICATION

After the final model was built, the possible binding sites of Toll like receptor 7 was searched based on the structural comparison of template and the model build and also with CASTp server and was shown in Figure 11. Since, Toll like receptor 7 and the 5GMF were well conserved in both sequence and structure; their biological function should be identical. Infact from the structure-structure comparison of template, it was found that secondary structures are highly conserved and the residues, are shown in the figures.

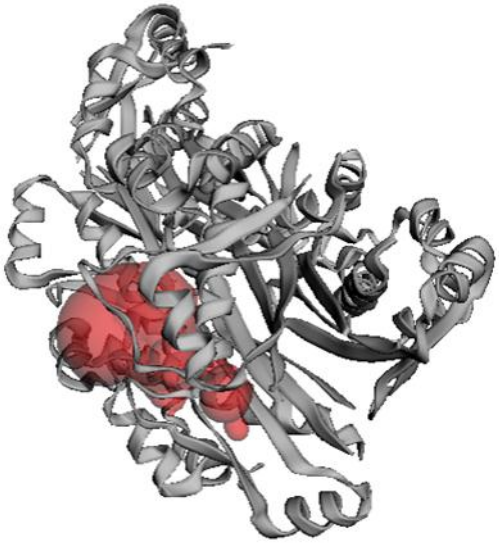
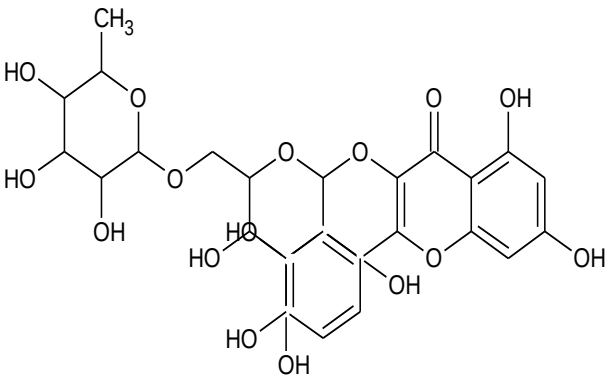
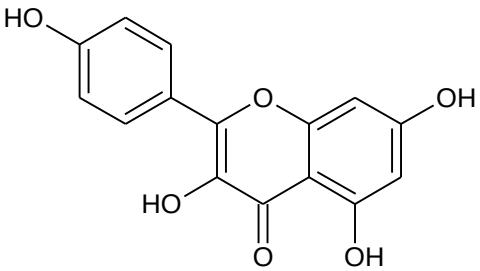


Figure 10: Active site of Toll like receptor 7 from Homo sapiens

Structures of the compounds used for inhibition of **Toll like receptor 7** :



RUTIN



KAEMPFEROL

DOCKING STUDIES

Docking studies were performed to gain insight into the binding conformation of pharmacophore models derived from structural manipulations onto compounds. These compounds were selected based on the criteria of satisfying Lipinski's Rule-of-Five with zero violations for docking onto Toll like receptor 7 model. All docking calculations were carried out using GOLD and the files generated were analyzed for their binding conformations. Analysis was based on Free energy of binding; Lowest docked energy and calculated RMSD values. The total clusters of docking conformations, with the docked the compounds showed positive binding energies. Among all docking conformations, the best predicted binding free energy to the Toll like receptor 7 was identified from Homo sapiens (Fig.11).

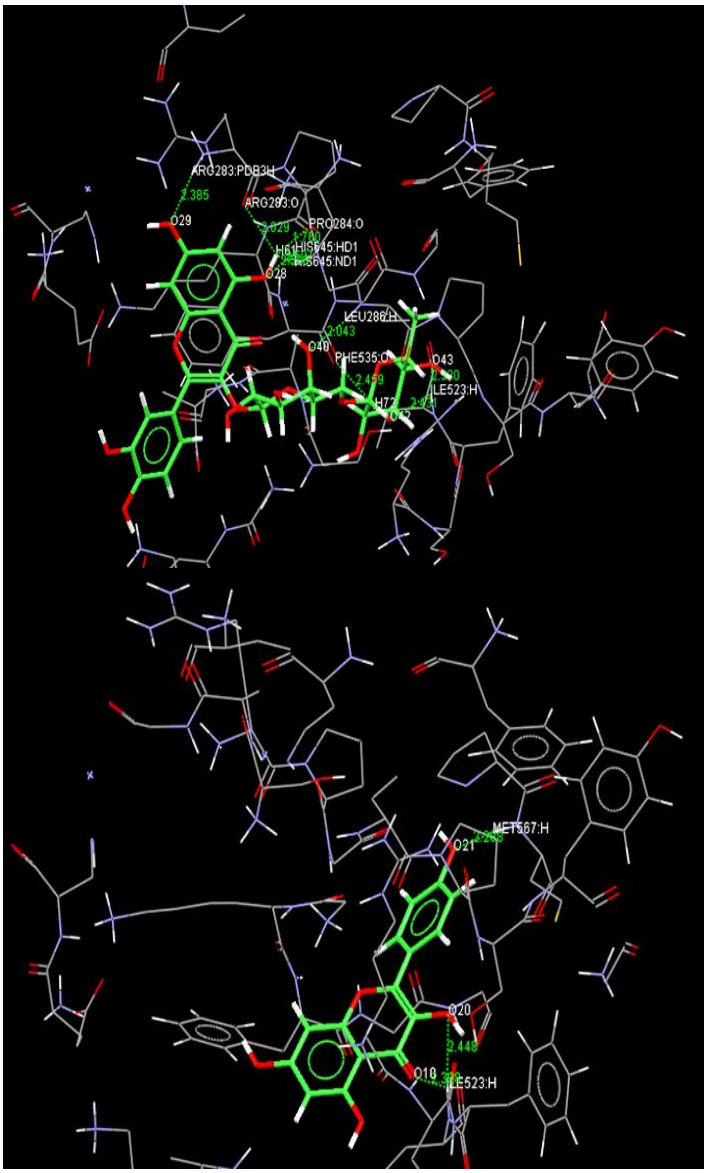


Fig 11: Docking studies of natural compounds with Toll like receptor 7 from Homo sapiens

Ligand name	Fitness	S(hb_ext)	S(vdw_ext)	S(hb_int)	S(int)
kaempferol	41.62	0.71	33.74	0.00	-5.49
rutin	46.86	8.17	37.05	0.00	-12.24

IV. CONCLUSION

In this work, we have constructed 3D model of Toll like receptor 7 domain, using the Homology modeling method and obtained a refined model after energy minimization. The final refined models were further assessed by PROCHECK program, and the results show that these models are reliable. The stable structure of Toll like receptor 7 was further used for docking with natural inhibitors rutin and kaempferol. Docking results indicate that conserved amino-acid residues Toll like receptor 7 main plays an important role in maintaining a functional conformation and are directly involved in donor substrate binding. The interaction between the domain and the compounds proposed in this study are useful for understanding the potential mechanism of domain and the inhibitor binding. As is well known, hydrogen bonds play important role for the structure and function of biological molecules. In this study it was found that ARG283, PRO284, LEU286, ILE523, PHE535, MET567, HIS645 in Toll like receptor 7 are important for strong hydrogen bonding interaction with the inhibitors. To the best of our knowledge these are conserved in this domain and may be important for structural integrity or maintaining the hydrophobicity of the inhibitor-binding pocket.

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