Life Science Informatics Publications

Research Journal of Life Sciences, Bioinformatics, Pharmaceutical and Chemical Sciences

Journal Home page http://www.rjlbpcs.com/

RJLBPCS

ISSN 2454-6348

Original Research Article **DOI: 10.26479/2018.0406.14**

MODELING AND DOCKING OF TOLL LIKE RECEPTOR 8 FROM HOMO SAPIENS WITH RUTIN AND KAEMPFEROL

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ABSTRACT: TLR agonists which are targeting TLR8 have been successfully used as therapeutics for melanoma, functioning by recruiting dendritic cells and inducing T-cell responses. A three dimensional model of Toll like receptor 8 from Homo sapiens was generated using 3W3G, as a template with Modeller9v7. With the aid of molecular mechanics and dynamic methods models were generated checked by Procheck and 3D graph. After energy minimization, the 3D structure of Toll like receptor 8 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 8 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 8 are important determinant residues in binding process as they have strong hydrogen bonding with these compounds. These hydrogen bonding interactions play an important role for stability of the complex.

KEYWORDS: Toll like receptor 8, modeling, molecular dynamics, Homo sapiens, docking studies.

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1. INTRODUCTION

Toll Like Receptors are an important immunological component expressed by keratinocytes and melanocytes, which are the main cell types involved in both non-melanoma and melanoma skin cancers. TLRs induce inflammatory responses meant for clearing pathogens, but their activation can

also potentiate chronic inflammation, which can ultimately contribute to skin carcinogenesis. In contrast, TLR agonists, specifically targeting TLR7, 8, and 9, have been successfully used as therapeutics for melanoma and BCC, functioning by recruiting dendritic cells and inducing T-cell responses. It is important to consider local versus systemic applications of TLR therapies and the balance between efficacy and inducing TLR tolerance. TLR3 agonists have been shown to be well-tolerated and effective in both directly killing cancer cells and directing immune responses in melanoma. TLR-targeted therapies may be potential treatment options for large or reoccurring skin tumors that may be difficult to treat with surgery or for other skin tumors that are not responsive to current therapies. Although TLR expression on tumor cells may allow tumors to evade surveillance, TLRs are also considered to be targets for anti-cancer interventions that result in the recognition and ultimate destruction of tumor cells using a tolerant immune system. This idea is further illustrated by the fact that recent studies have demonstrated a dual nature of immune responses in the context of cancer therapies, highlighting the importance of considering conditions, TLR targets, and combinations of immune interventions and TLR ligands [1]. There are studies and case reports that show that 5% imiquimod cream treatment is an effective therapeutic option for actinic keratosis (AK), BCC, Bowen's disease, and lentigo maligna [2-7]. The mechanism of action of imiquimod is through the activation of TLR7 [8], and imiquimod has been approved to treat both premalignant actinic keratoses, and malignant superficial BCC [9]. The mechanism may also involve Th1-response promotion, the recruitment of macrophages, anti-tumor cytotoxic CD8 T cells, and NK cells to the lesion, as well as induce apoptosis of tumor cells [10]. Imiquimod has also been shown to induce IFN-α and IL-12 production, resulting in a heightened immune response [11, 12]. The suggested mechanism for exertion of anti-tumor effects on UVB-induced SCC by imiquimod is through the activation of Th17/Th1 cells as well as cytotoxic T lymphocytes [13], Five percent topical imiquimod has been effective in several clinical trials [14]. The related drug, resiquimod, has been demonstrated as a safe and effective topical intervention for AK and is a potential treatment option for patients who have large patches of AK [15]. Several cancer types including melanoma have been successfully treated with Taxol, CpG, or other TLR ligands [16, 17], PF3512676, a synthetic CpG ODN, uses a TLR9-targeted approach to effectively treat BCC [18], TLR 7 and 8 agonists activate a pro-inflammatory response for SCC treatment [19]. Additionally, IL-1, 6, 8, and 12 modulations along with a promotion of a Th1-response have been shown to exert anti-tumor and antiviral behavior [20]. Previous studies have demonstrated TLR3 agonists to be promising adjuvants for cancer vaccines, especially in regards to their immune stimulatory properties. A recent study has demonstrated that human melanoma cells express TLR3, which in combination with TLR3 agonists, results in tumor cell death via caspase activation when cells are pretreated with cycloheximide or IFN-α, suggesting that TLR3 agonists may be multifunctional adjuvants offering more clinical treatment options. Therefore, TLRs and their signaling pathways may be potential

therapeutic targets to control tumor progression, especially in diseases such as cutaneous malignant melanoma, which is an aggressive tumor that is not effectively managed with current treatments [21]. It is important to note that, especially in the case of TLR7 agonists such as imiquimod and resiquimod, though quite effective when applied topically to AKs and BCCs, systemic therapeutic interventions have not been as successful. This TLR tolerance has previously been demonstrated with TLR4 agonists, which resulted in decreased NF-kB activation [22]. The suggested mechanism for TLR7 tolerance is the diminished capacity for IL-12 secretion as well as IFN-α secretion by plasmacytoid DCs. Recent studies have found that local and systemic TLR-targeted therapies have different modes of action and require further investigation, especially into the timing and dosage of treatments to reach maximum efficacy without inducing TLR tolerance [23].

2.MATERIALS AND METHODS

Domain Identification and Template Search

The Toll like receptor 8 (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) [24] program against PDB (Protein Data bank). Sequence that showed maximum identity with high score and e-value either zero or less negative values were aligned and were used as a reference structure to build 3D models for Toll like receptor 8. The co-ordinates for the structurally conserved regions (SCRs) for Toll like receptor 8were assigned from the template using multiple sequence alignment, based on the Needleman-Wunsch algorithm [25]. Then using the template structure, Toll like receptor 8 structure was developed with MODELLER 9v7 software.

3D model

The initial models of Toll like receptor 8were 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 8 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 [29]. 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 8 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 8was 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.

3.RESULTS AND DISCUSSION

Homology Modeling of Toll likes receptor 8

Toll like receptor 8 from Homo sapiens was collected and submitted for domain identification. The functional domain region was highlighted in the Figure 1, and taken for further studies.

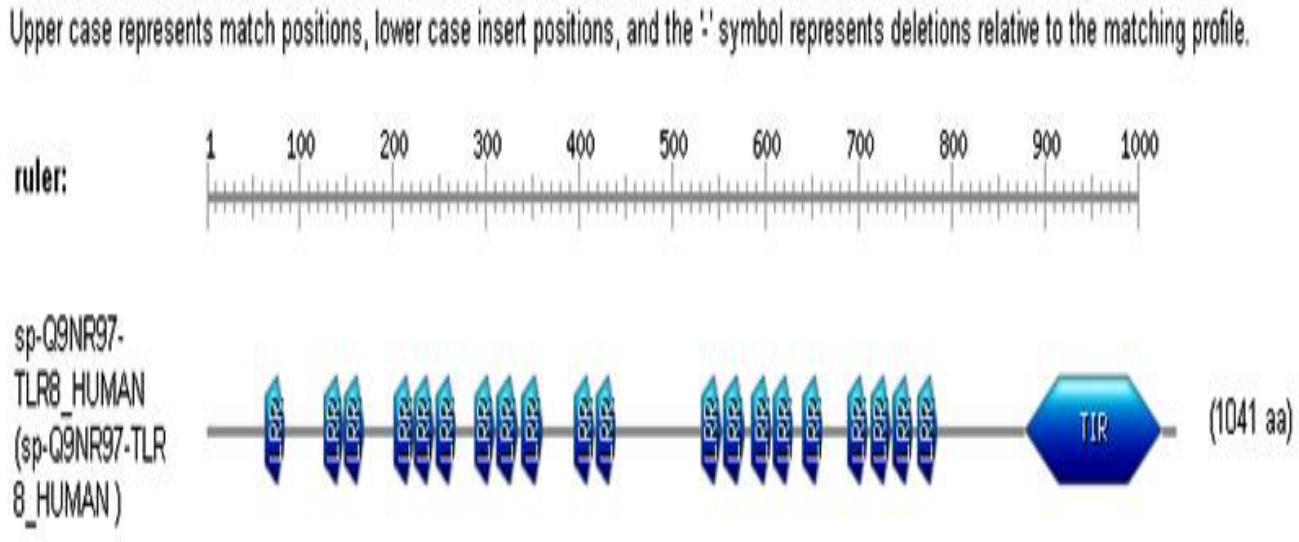


Figure 1: Domain identification in Toll like receptor 8 using Expasy

A high level of sequence identity should guarantee more accurate alignment between the target sequence and template structure. BLAST search against PDB, only 3W3G has a high level of sequence identity 75% with the Toll like receptor 8. 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 3W3G 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 76% identity with Toll like receptor 8 from Homo sapiens.

Sequence Alignment

In the following study, we have chosen 3W3G as a reference structure for modeling Toll like receptor 8 domain. Coordinates from the reference protein (3W3G) 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.

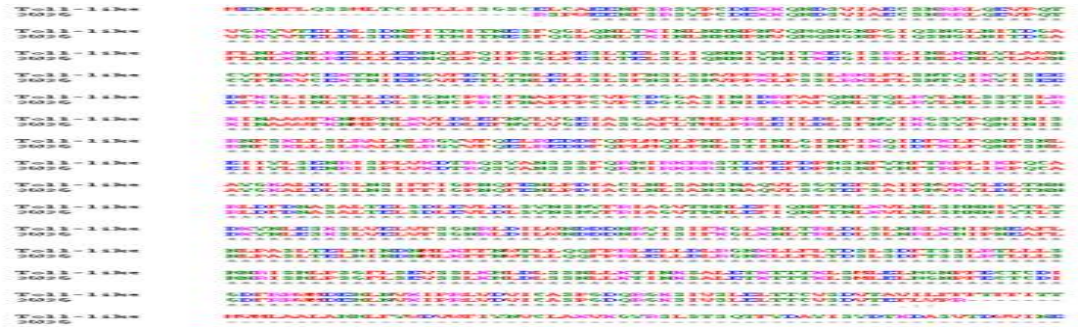


Figure 3: Alignment of Toll like receptor 8 from Homo sapiens with template 3W3G

Homology Modeling

The 3W3G structure was used as the templates for building the 3D models of the Toll like receptor 8 using Modeller9v7. The final stable structure of the Toll like receptor 8 obtained was shown in Figure 4.

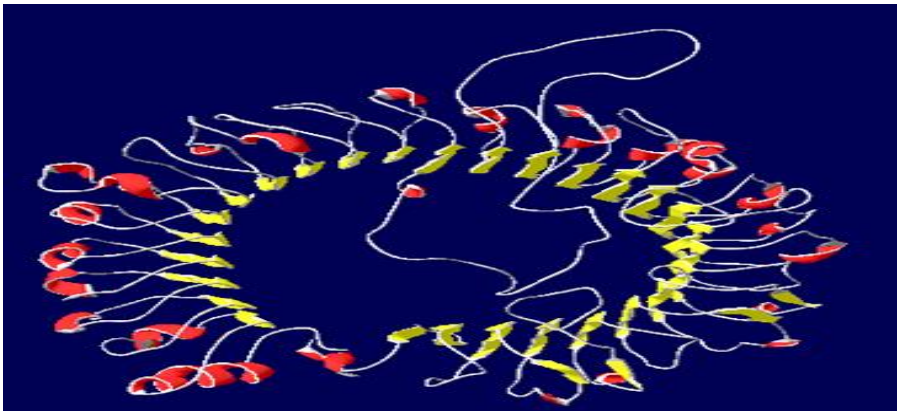


Figure 4: 3D structures of Toll like receptor 8 from Homo sapiens by Modeller9v7.

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

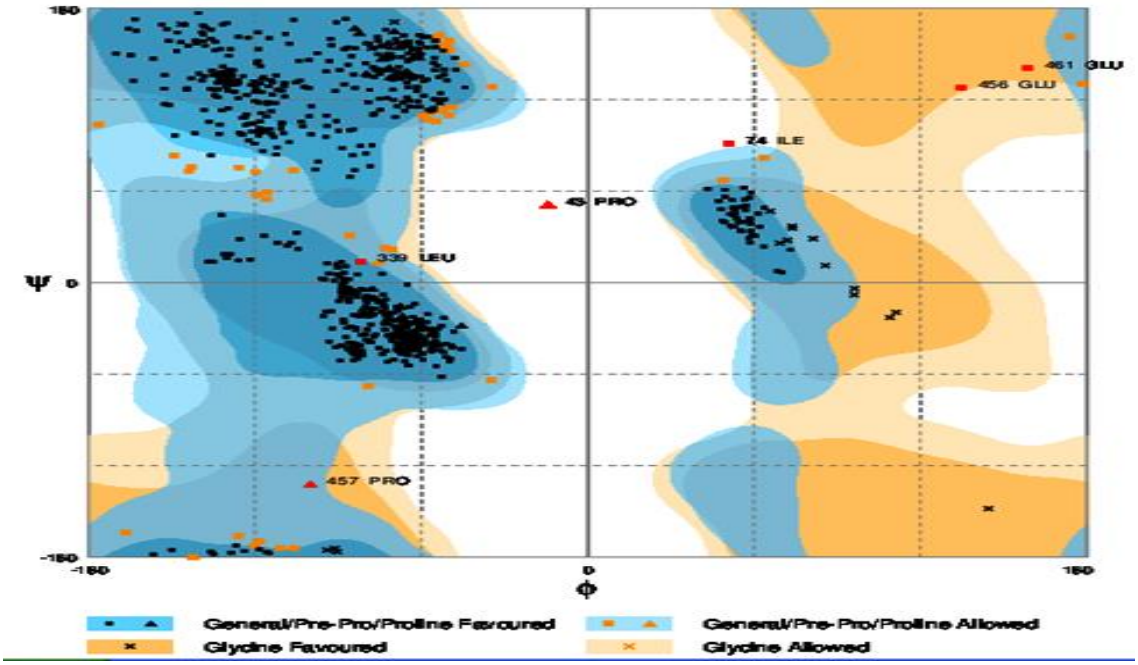


Figure 5: Ramachandran plot of Toll like receptor 8 from Homo sapiens

Number of residues in favored region (~98.0% expected) : 735 (94.4%)

Number of residues in allowed region (~2.0% expected) : 42 (4.6%)

Number of residues in outlier region : 8 (1.0%)

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.

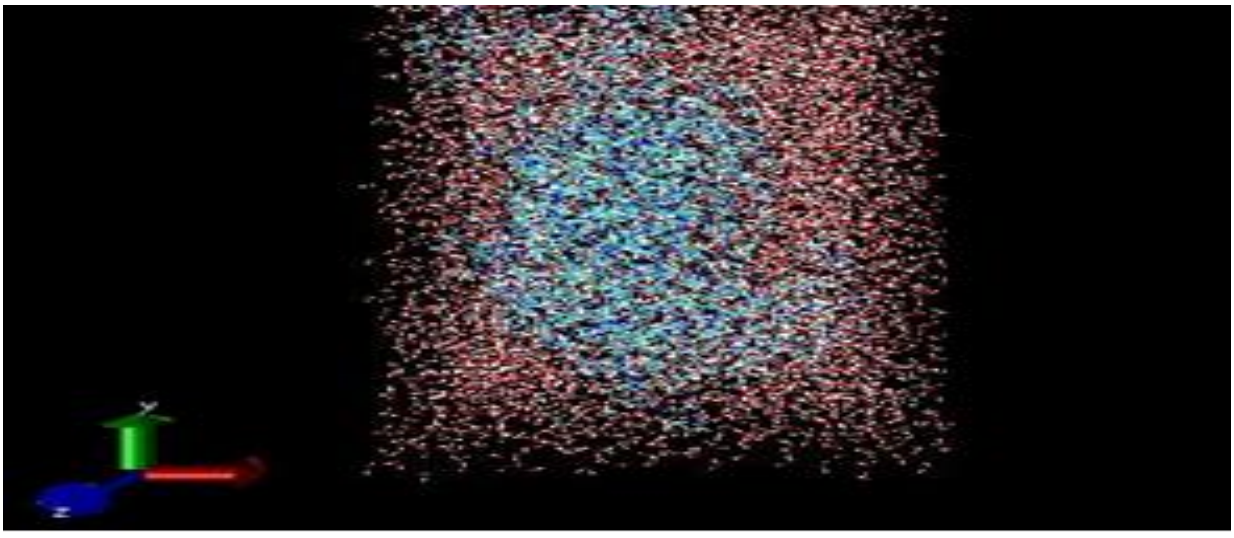


Figure 6: Molecular Dynamics studies of Toll like receptor 8from Homo sapiens

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

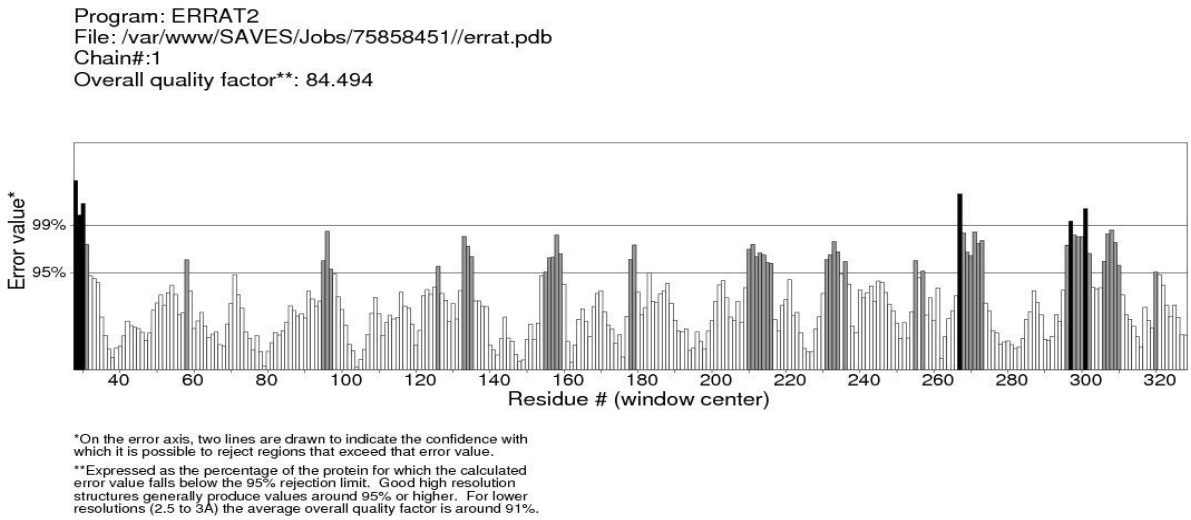


Figure 7: The ERRAT results of Toll like receptor 8 from Homo sapiens; overall quality score indicates residues are reasonably folded

Validation of Toll like receptor 8Domain

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 8 was -1.23 and -0.38 Å. Altogether 99.0 % of the residues of Toll like receptor 8 were in favored and allowed regions. The overall PROCHECK G-factor of Toll like receptor 8 was -0.36 and verify3D environment profile was good (Figure 8).

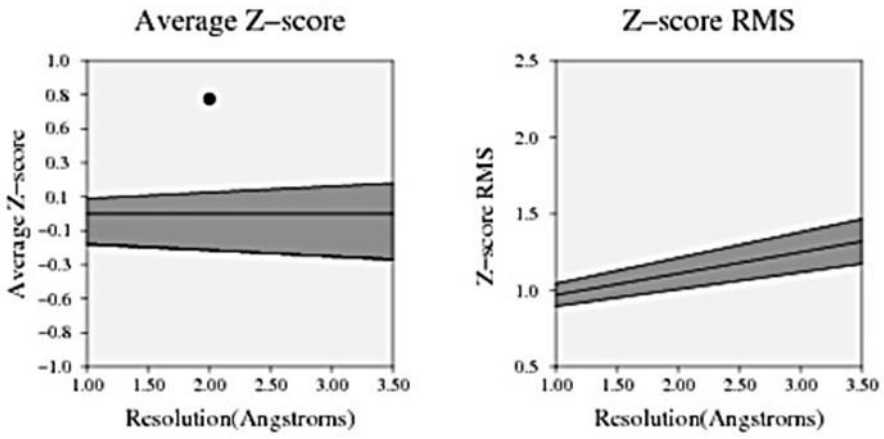


Figure 8: Average Z-score for Toll like receptor 8 from Homo sapiens

Superimposition of 3W3G with Toll like receptor 8 domain

The structural superimposition of 3W3G template and Toll like receptor 8 is shown in Figure 9. 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 8.

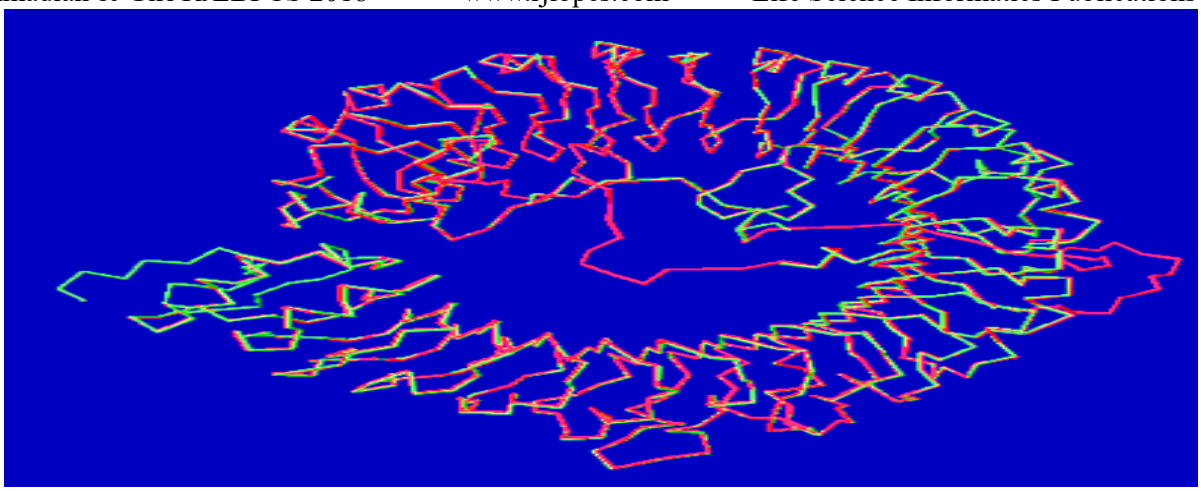


Figure 9: Superimposition Toll like receptor 8 (represented in green color) and 3W3G (represented in red color).

Active Site Identification

After the final model was built, the possible binding sites of Toll like receptor 8 was searched based on the structural comparison of template and the model build and also with CASTp server and was shown in Figure 10. Since, Toll like receptor 8 and the 3W3G 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.

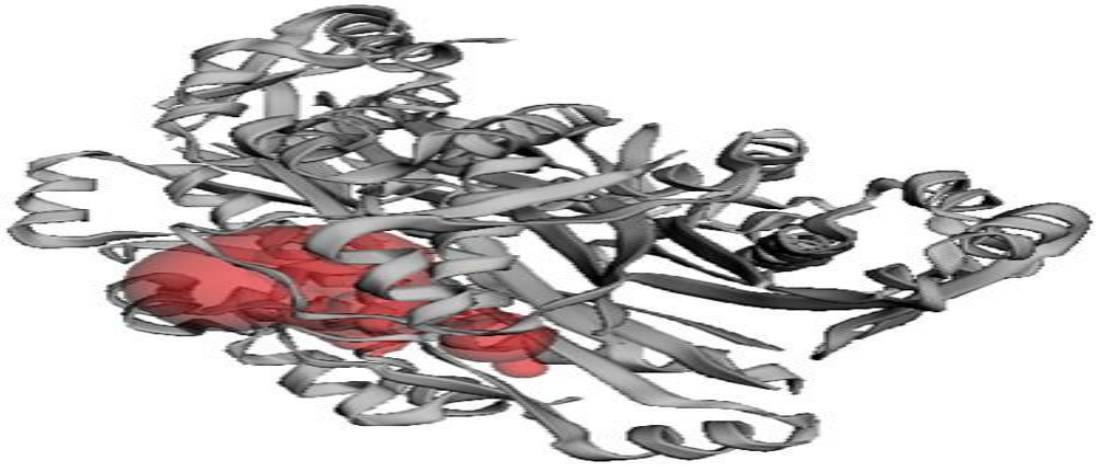
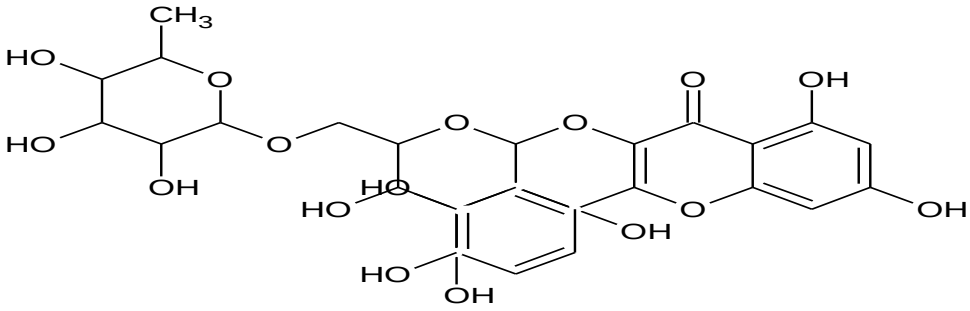
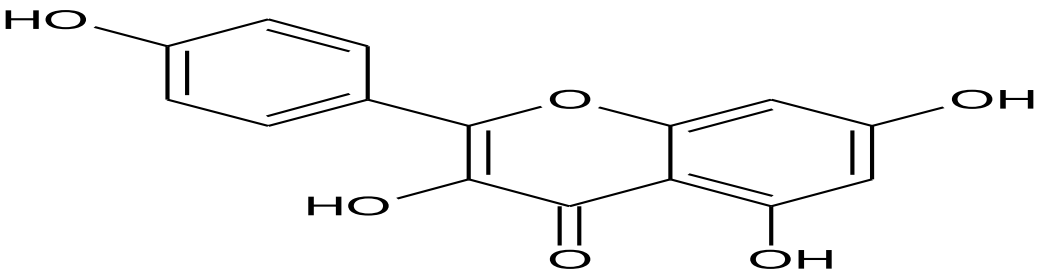


Figure 10: Active site of **Toll like receptor 8 from Homo sapiens**

Structures of the compounds used for inhibition of **Toll like receptor 8**



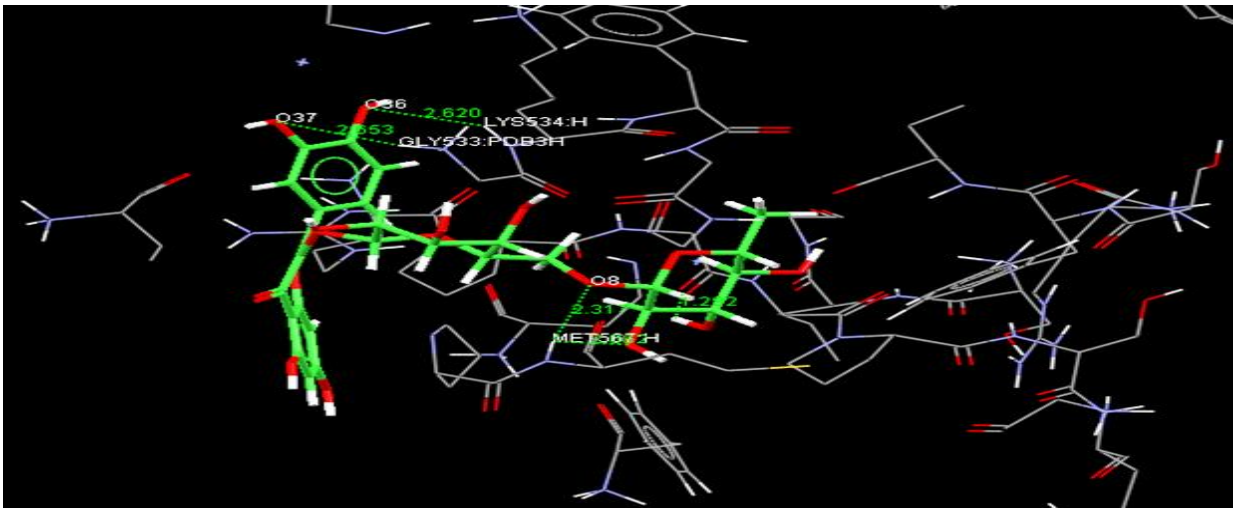
RUTIN



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 8 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 8 was identified from Homo sapiens Figure11.



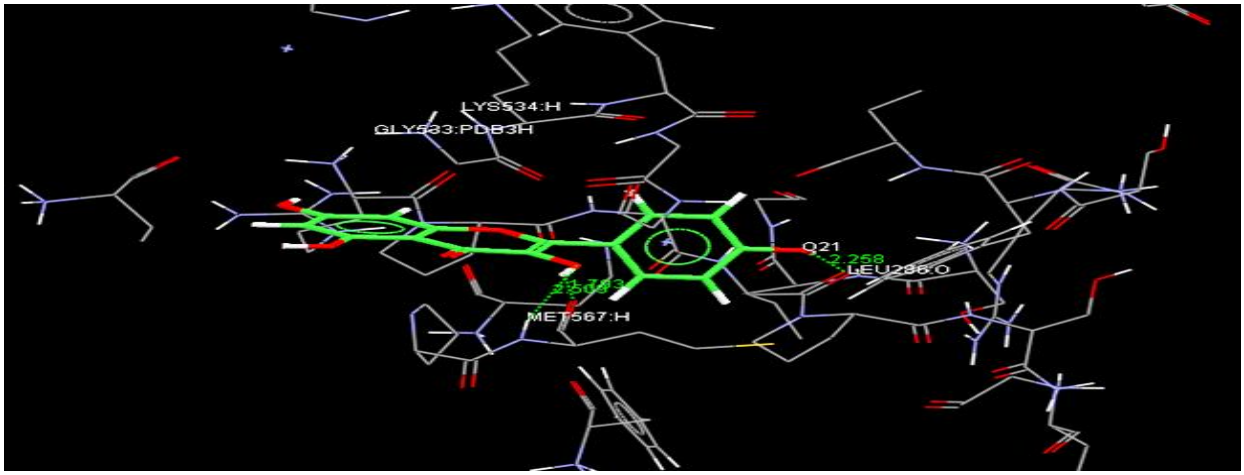


Figure 11: Docking studies of natural compounds with Toll like receptor 8 from Homo sapiens

Fitness	S(hb_ext)	S(vdw_ext)	S(hb_int)	S(int)	Ligand name
45.26	0.71	33.74	0.00	-5.49	kaempferol
44.28	8.17	37.05	0.00	-12.24	Rutin

4. CONCLUSION

In this work, we have constructed 3D model of Toll like receptor 8 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 8was further used for docking with some natural inhibitors. Docking results indicate that conserved amino-acid residues Toll like receptor 8main play 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, MET568, HIS645 in Toll like receptor 8 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.

CONFLICT OF INTEREST

Authors declared that there is no conflict of interest.

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