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RESEARCH ARTICLE

Homology Modeling of Inactive Ubiquitin Thioesterase FAM105A and Molecular Docking Studies of Benzimidazole Derivatives

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Abstract: Homology modeling and molecular docking studies were performed to explore structural features and binding mechanism of synthesized benzimidazole derivatives as ubiquitin inhibitors. A homology modeling procedure was employed to construct a 3D model of ubiquitin protein by using MODELLER 9.15. For this procedure, the x-ray crystal structure of Gumby/fam105b in Complex with linear Di-ubiquitin (PDB ID: 4KSL) at 2.83 Å resolution was used as template. The predicted model was analyzed by PROCHECK. The 3D structure of predicted model shows 93.9% of amino acids in most favored region. The predicted model was then used for molecular docking studies by using Autodock 4.2. All the synthesized benzimidazole derivatives show good binding energy and interactions with the modelled protein.

Keywords: Benzimidazole, Homology modeling, Modeller 9.15, Molecular docking studies, Procheck

Introduction

Ubiquitination is the process of classifying a target protein with ubiquitin[1]. The process of ubiquitination is mediated by three enzymes 1. ubiquitin activating enzyme (E1), 2. ubiquitinconjugating enzyme (E2) and 3. ubiquitin ligase (E3).The most importantfunction of E1 ubiquitin activating enzyme is to catalyze the adenylation of ubiquitin at the expense of one ATP molecule[2]. Ubiquitination play an important role in cellular process, which include cell-cycle progression, endocytosis and trafficking, andimmune-signal transduction[3]. Activation enzyme of Ubiquitin E1 activates the 76-amino acid residue ubiquitin polypeptide by forming a thioester bond. The thioester bond forms in between its catalytic cysteine and the C terminus of ubiquitin in an ATP-dependent manner[4]. Through a transthiolation reaction Ubiquitin is transferred to the activate site cysteine in ubiquitin-conjugating enzyme E2. In eukaryotes it is highly conserved but absent in bacteria.In the fused heterocyclic moieties benzimidazole nucleus plays an important pharmacophore with

unique chemical and biological properties[5-8]. They have been found to possess various biological activities like analgesic, antihistaminic, anti-inflammatory, antispasmodic, analgesic, antimicrobial, antitumor,antiproliferative, anti-HIV-RT, antiulcer, anti-tubercular,anti-cancer and anti-fungal[9-12] anti-inflammatory[13], proton pump inhibitors[14,15]and cycloxygenase inhibitor activities[16-20]. Some of the benzimidazole derivatives are involved in medicinal treatment such as infertility, epilepsy and diabetic deseases[21,22].

In the present study, MODELLER 9.15 was used to generate 3D model of Inactive ubiquitin thioesterase FAM105A (uniprot accessionnumber: Q9NUU6) protein from human. Gumby/fam105b in Complex with linear Di-ubiquitin (PDB ID: 4KSL), is used as a template for model build up. Validation of model was performed by PROCHECK program. Active site prediction was performed by using 3D ligandsite, an online active site prediction tool and molecular docking study was performed using AutoDock 4.2.

Experimental

The amino acid sequence of Homosapiens Inactive ubiquitin thioesterase FAM105A (Accession No.Q9NUU6) was retrieved from the UniProtKB database (http://www.uniprot.org/)[23]. A BLAST[24] (Basic Local Alignment Search Tool) search was performed to select the template and resulted with the best match Crystal Structure of Gumby/fam105b In Complex with Linear Di-ubiquitin (PDB ID: 4KSL (Chain A)) (http://blast.ncbi.nlm.nih.gov/Blast.cgi)[3] with 41% similarity having a resolution of 2.83 Å making it an excellent template. The three dimensional structure was generated using Modeller 9.15[25]. The final validation of the model was performed using PROCHECK[24] for Ramachandran plot. The RMSD (root mean square deviation) was calculated by superimposing (4KSL) over the generated model to access the accuracy and reliability of the generated model using SPDBV[26] by selecting the main chain atom (*i.e*. the backbone atoms of alpha carbon).

MODELLER 9.15 was then used to gain satisfactory models; an automated approach to homology modeling by satisfaction of spatial restrains[27]. Initially, both the query and template were aligned by using clustalX. After manually modifying the alignment input file in MODELLER 9.15 to match the query and templatesequence, 20 models were generated. After generating files least modeler objective function value containing PDB was selected to validate the model. These models were then checked in detail for the protein structure stereochemistry by using PROCHECK[28], which generates Ramachandran plot and comprehensive residue by residue listing facilitates, the in depth assessment of Psi/Phi angles and the backbone conformation of the models.

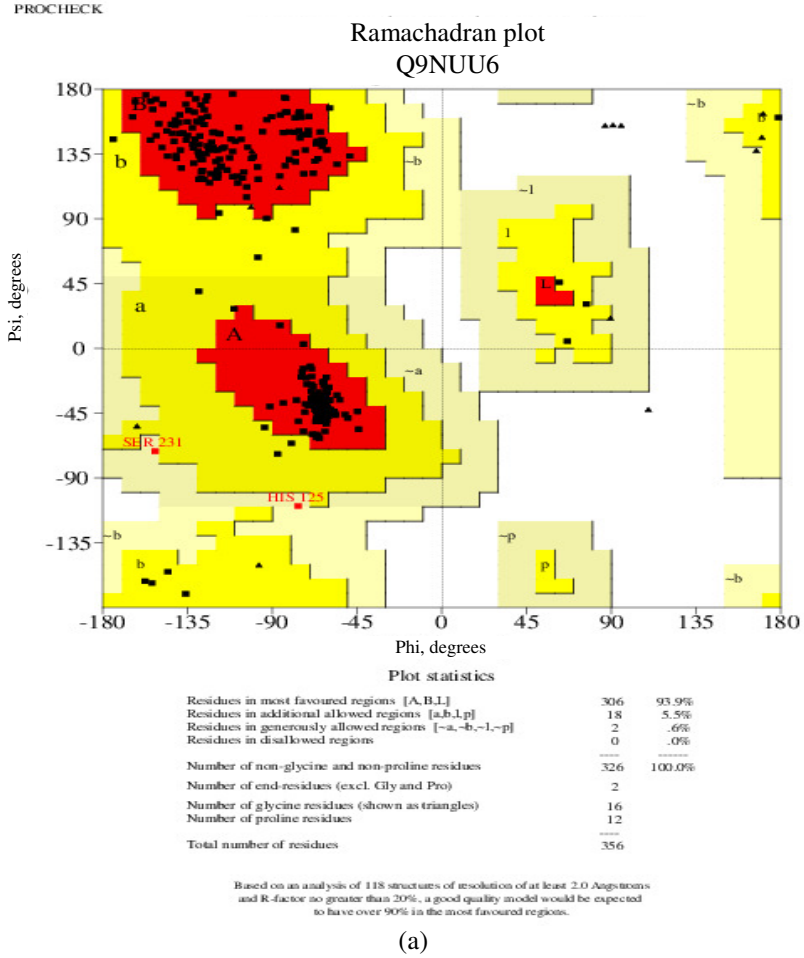
Docking protocol

The five synthesized Benzimidazole derivatives were sketched in sybyl6.7 and saved it into .mol2 format. Then the molecules were minimized using Triposforcefield, Gasteiger-Huckel charges were added and used convergence criterion of 0.005 kcal/mol Å. Molecular Docking study was performed to all the synthesized molecules separately by using AutoDock 4.2 program, using the Lamarckian Genetic Algorithm (LGA) and implemented empirical free energy function[29]. Initially, the modelled protein was loaded and polar hydrogen were added. The molecule was loaded and set conformations and saved it in PDBQT format and then saved generated PDB file to PDBQT format. The grid maps were selected and calculated using AutoGrid[30]. For all dockings, a grid map with 60×60×60 points and also used a grid-point spacing of 0.375 Å was applied.Coordinates of x, y, z was set as -42.267, 33.699 and 12.982 respectively. For all docking parameters, default values were used.

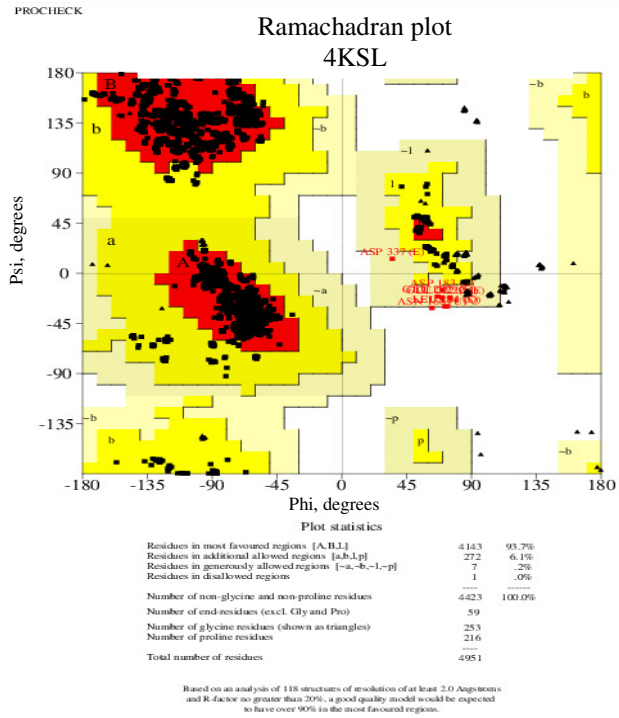
Results and Discussion

Homologymodelling and model evaluation

Thepresent study reports that the template protein (PDB ID: 4KSL) having high degree of homology with Q9N996 protein was used as a template with good atomic resolution of its crystal structure. The target sequence of Inactive ubiquitin thioesterase FAM105A having 356 amino acid residues was retrieved from the uniprot protein sequence database with Accession No. Q9NUU6. PDB Id-4KSL was identified and selected as template using BLAST having 41% identity. The structure was modelled using modeller 9.15. The generated structurewas validated using Protein Structure and by PROCHECK. The model shows 93.9% of amino acid residues in core region, 5.5% of amino acid residues in additionally allowed region, 0.6% of amino acid residues in generously favored region. There is no amino acid present in disallowed region. Both target and template molecules shows nearly same amino acid residues in most favored region that is query sequence shows 93.7% in most favored region and template molecule contains 93.7% in most favored region. Ramachandran plot and Secondary structure of the modelled protein is shown in Figure 1 and Figure 2 respectively.



(a)



(b)

Figure 1. Ramachandran plot analysis of the backbone dihedral angles PSI (Ψ) and PHI (ϕ) of (a) the generated model and (b) the template model 4KSL chain A.

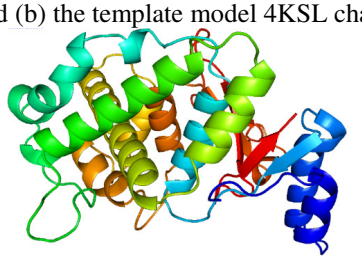


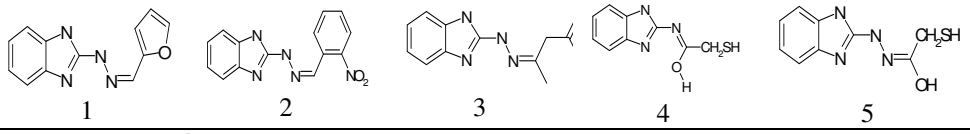
Figure 2. Secondary structure of the predicted model

Molecular docking results

Molecular docking is the most widely used method for the calculation of protein–ligand interactions. Docking is a most efficient technique to predict the potential ligand binding sites on the whole protein. To explore the predictability as well as the characteristics of the binding pocket of the modelled model and to make the rational design of novel and more selective antagonists of inactive ubiquitin thioesterase FAM105A. Molecular docking was carried out on developed inactive ubiquitin thioesterase FAM105A binding pocket using a set of Inactive ubiquitin thioesterase FAM105A antagonists shown in Table 1. The 10 docking conformations for each molecule was generated. Autodock 4.2 also uses free energy binding assessment to assign the best binding conformation. Energies estimated by Autodock are described by intermolecular energy (including Van der Waals, hydrogen bonding and electrostatic energies), internal energy, and torsional free energy.

The hydrogen bond interaction and electrostatic interaction between the receptor and ligand is the most important, because it can allocate the strength of binding and the exact position of the ligand in the active site. Structures of molecules are given in Table 1.

Table 1. Synthesized benzimidazole derivatives used for molecular docking



1 2 3 4 5

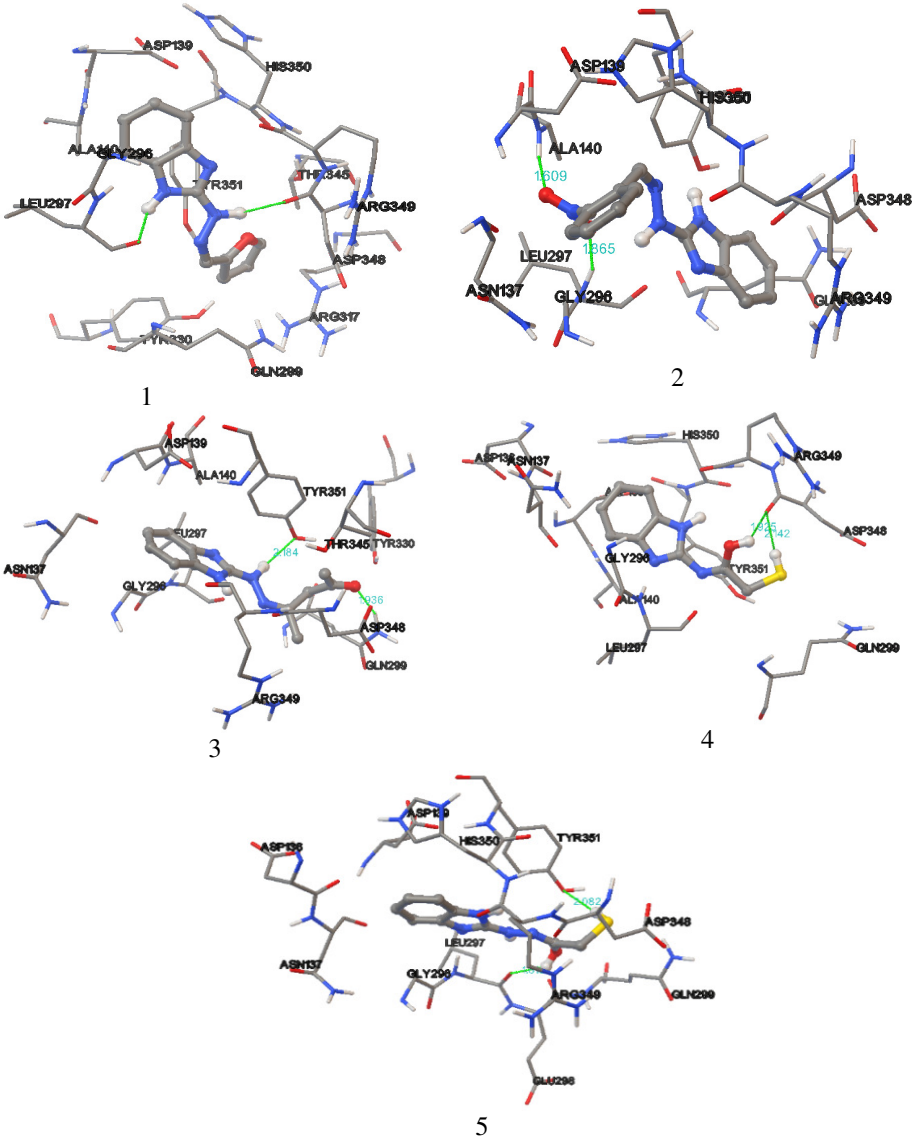


Figure 3. Docking pose of the compounds 1-5 in the active site of Inactive ubiquitin thioesterase FAM105A

Molecular docking studies were carried out for five synthesized Benzimidazole derivatives against Inactive ubiquitin thioesterase FAM105A. The binding energy, inhibition constant, hydrogen bond forming residues and interacting residues of all the five synthesized derivatives when docked with Inactive ubiquitin thioesterase FAM105A is as given in Table 2. The binding energy for all the molecules range from -5.69 to -7.39 kcal/mol. Compound 2having highest binding energy of -7.39 kcal/mol. This compound had shown two interactions with the Ala140, Leu297 as shown in Figure 3 thus indicating that Inactive ubiquitin thioesterase FAM105A has lowest affinity towards compound 2. Compound one and compound four interacts with Asp348 and compound 3 and compound 5 interacts with Tyr351.

Table 2. Binding energy and predicted contacting residues of Benzimidazole derivatives that interact with modelled protein of Inactive ubiquitin thioesterase FAM105A

C. No	Interacting amino acids	Grid X-Y-Z coordinates	Binding energy ΔG (kcal/Mol)	Dissociation constant (kI)
1	Leu297, Asp348	-42.267, 33.699, -12.982	-6.57	15.29µM
2	Ala140, Leu297	-42.267, 33.699, -12.982	-7.39	3.8µM
3	Gln299, Tyr351	-42.267, 33.699, -12.982	-6.56	15.51µM
4	Asp348	-42.267, 33.699, -12.982	-5.69	67.72µM
5	Glu298, Tyr351	-42.267, 33.699, -12.982	-6.08	34.7µM

Conclusion

We had synthesized Benzimidazole derivatives in present studyand evaluated for ubiquitone inhibition. The 3D structure of Q9NUU6 of Human wasgenerated using Modeller 9.15. The generated model assessment was revealed that the model is reliable and is aquality model with stable energies. Additionally the molecular docking studies were performed to all the compoundsinto thebinding cavity of Q9NUU6, which showed favorable interactions with all the compounds. Second compound shows highest binding energy of -7.39 kcal/mol. Except fourth compound all the other compounds shows two interactions. Hence we conclude that all these synthesized compounds couldbe a potential lead molecule.

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