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In-Silico Molecular Docking Approach for the Study of Anti-asthmatic Activity of Compounds from *Streptomyces* sp. VITSTK7

Pratibha Sanjenbam, Mohankumar Thenmozhi and Krishnan Kannabiran*

Division of Biomolecules and Genetics, School of Biosciences and Technology, VIT University, Vellore - 632014, India.

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The present study describes the *in-silico* molecular docking of three compounds (ligands) i) Cyclopentanepropanoic acid, 3,5-bis(acetyloxy)-2-[3-(methoxyimino) octyl], methyl ester ($C_{22}H_{37}NO_7$), ii) 5-Azidomethyl-3-(2-ethoxycarbonyl-ethyl)-4-ethoxy-carbonylmethyl-1H-pyrrole-2-carboxylic acid ($C_{17}H_{24}N_4O_6$) and iii) Akuammilan-16-carboxylic acid, 17-(acetyloxy)-10-methoxy, methyl ester ($C_{24}H_{28}N_2O_5$) extracted from *Streptomyces* sp.VITSTK7 with interleukin-13, one of the key pro-inflammatory cytokine of asthma, and the PDB ID is 1IJZ. Patch dock online tool was used to dock these three compounds with interleukin-13. All the three ligands $C_{22}H_{37}NO_7$, $C_{17}H_{24}N_4O_6$ and $C_{24}H_{28}N_2O_5$ showed the binding energy of -159.59, -208.41 and -93.56 kcal/mol respectively. Hence, it could be used as a drug for asthma.

Key words: Active sites, Asthma, Interleukin-13, Patch dock, *Streptomyces* sp.VITSTK7.

Asthma is a chronic inflammatory disorder of airway. The prevalence of asthma is increasing day by day in India. There are 300 million people affected worldwide by asthma. It is a classical disorder of the airway hyperresponses, originating from a T-cell imbalance leading to molecular inflammation[1]. In bronchial asthma, various mediators induce the infiltration of mast cells, eosinophils and Th2 lymphocytes into lesions with downstream mediators, resulting in classical asthmatic phenotypes, such as mucous over-production, airway hyper-responsiveness, and submucosal thickness[2].

* To whom all correspondence should be addressed.
Tel.: +91 4162202473; Fax +91 4162243092;
E-mail: kkb.biomol@gmail.com

In particular, helper T type 2 (Th2) cells (a subset of T cells) are believed to play a central role in initiating and orchestrating the asthmatic airway inflammatory response[3]. Bronchial asthma is a complex disorder that is thought to arise as a result of aberrant T-lymphocyte responses to non-infectious environmental antigens. In particular, the symptoms of asthma are closely associated with the presence of activated T-helper 2 cell (Th2) cytokine-producing cells interleukin (IL)-4, IL-5, IL-9, and IL-13 in the airway wall. Although each of the Th2 cytokines likely contributes to the overall immune response directed against environmental antigens, a substantial body of evidence points to a singular role for IL-13 in the regulation of the allergic diathesis[4].

Reactive oxygen species (ROS) are pivotal in triggering particular degenerative diseases in cells[5]. To expedite the reduction of radicals, antioxidants are required for specific

degenerative diseases, such as asthma and chronic obstructive pulmonary disorders[6]. ROS can attack random cells. Chronic asthma-related disorders arise, in cases where the radicals attack bronchoalveolar cells[5,6]. The antioxidant activity of the isolate *Streptomyces* sp.VITSTK7 was already reported[7].

Patch dock is aimed at finding docking transformations that yield good molecular shape and complementarities. Such transformations, when applied, induce both wide interface areas and small amounts of steric clashes. A wide interface is ensured to include several matched local features of the docked molecules that have complementary characteristics. Then, complementary patches are matched in order to generate candidate transformations. Each candidate transformation is further evaluated by a scoring function that considers both geometric fit and atomic desolvation energy[8]. Finally, an RMSD (root mean square deviation) clustering is applied to the candidate solutions to discard redundant solutions. The main reason behind Patch Dock's high efficiency is its fast transformational search, which is driven by local feature matching rather than brute force searching of the six-dimensional transformation space. It further speeds up the computational processing time by utilizing advanced data structures and spatial pattern detection techniques, such as geometric hashing and pose clustering that were originally developed in the field of computer vision[9]. In this study we report anti-asthmatic activity of three compounds extracted from *Streptomyces* sp.VITSTK7 by demonstrating its interaction with interleukin-13 by *in-silico* molecular docking studies.

MATERIALS AND METHODS

Ligands

Streptomyces sp.VITSTK7 was isolated from Pondicherry coast, India and subjected to extraction of secondary metabolites. The extraction process yielded three antifungal active compounds. The compounds are i) Cyclopentanepropanoic acid, 3,5-bis(acetyloxy)-2-[3-(methoxyimino) octyl], methyl ester ($C_{22}H_{37}NO_7$), ii) 5-Azidomethyl-3-(2-ethoxycarbonyl-ethyl)-4-ethoxycarbonylmethyl-1H-pyrrole-2-carboxylic acid,($C_{17}H_{24}N_4O_6$) and iii) Akuammilan-16-carboxylic acid, 17-(acetyloxy)-10-methoxy, methyl ester ($C_{24}H_{28}N_2O_5$).

The structures of the compounds were identified using Chemspider 2.0 (online tool for chemical database) to copy the canonical SMILES format of these compounds. SMILES (Simplified Molecular-Input Line-Entry System) are a specification in form of a line notation for describing the structure of chemical molecules. Using CORINA 2.64 (online tool for generation of 3D structure) the copied canonical SMILES were pasted to get the pdb format file with 3D structure of the compound. CORINA matured through a series of versions during the past decades and has become the recognized world-wide gold standard in industry and academia to generate 3D molecular models of high quality. Currently, CORINA is used by Symyx, NCI/NIH and most major pharmaceutical and chemical companies to convert their 2D structures into 3D. The structures were downloaded for docking studies (Fig. 1A-C).

Receptors

The structure of the protein interleukin-13 with the PDB ID was retrieved from the PDB-Protein Data Bank (Fig. 2).The PDB is a repository for the 3-D structural data of large biological molecules, such as proteins and nucleic acids. After obtaining the structure from PDB, the potential active sites of protein interleukin-13 were searched using Q-site Finder (http://www.modelling.leeds.ac.uk/qsitefinder/) online tool. It is a new method for ligand binding site prediction.

Docking

For docking studies we used geometry based molecular docking that is patch dock technique beta 1.3 version available as online under the URL http://bioinfo3d.cs.tau.ac.il/PatchDock/. The input files are larger molecule that is receptor protein as interleukin-13 and the smaller molecules that are ligands as $C_{22}H_{37}NO_7$, $C_{17}H_{24}N_4O_6$ and $C_{24}H_{28}N_2O_5$ respectively in the order followed by e-mail ID and clustering RMSD are uploaded. RMSD is a positive number that specifies the radius of the RMSD clustering in angstroms. This value is used in the final clustering stage of the algorithm. It ensures that the distance between any two output solutions will be at least the specified clustering RMSD value. The default value for this parameter is 4^{o}A. The next input is

complex type. Patch Dock has different sets of parameters, optimized for different types of complexes. If this field is not specified, the program will use a default configuration. For this study we set the complex as protein-small ligand docking. So that the algorithm uses a parameter set optimized for small-size molecules. Finally the docking was carried out by submitting the form.

RESULTS AND DISCUSSION

The focus of molecular docking is to simulate the molecular recognition process by computationally to achieve an optimized conformation for the receptor and ligand and relative orientation between the receptor and ligand such that the free energy of the overall system is minimized.

Q Site finder

The receptor interleukin-13 has 696 non polar hydrogen, 65 aromatic carbons and 451 rotatable bonds. It has 2995 atoms with 6803 bonds. It has one chain with 11 polymers contains the 133 groups.

The potential active sites of interleukin-13 were analyzed and found 10 target sites. Site 1 contains 169 residues in it as a target and the volume of site is 279 cubic angstroms. Site 2 contains 154 residues and the total volume of the site is 182 cubic angstroms. Site 3 contains 129 residues with the volume of 122 cubic angstroms. Target site 4 contains 118 residues and the volume of this site is 116 cubic angstroms. The target site 5 contains 115 residues with the volume of 101 cubic angstroms. Site 6 has 85 residues and it occupies 62 cubic angstroms. Site 7 contains 59 residues and occupies 53 cubic angstroms. Site 8 contains 69 residues and the volume contains 48

Table 1. Potential active sites (Target sites) of interleukin-13

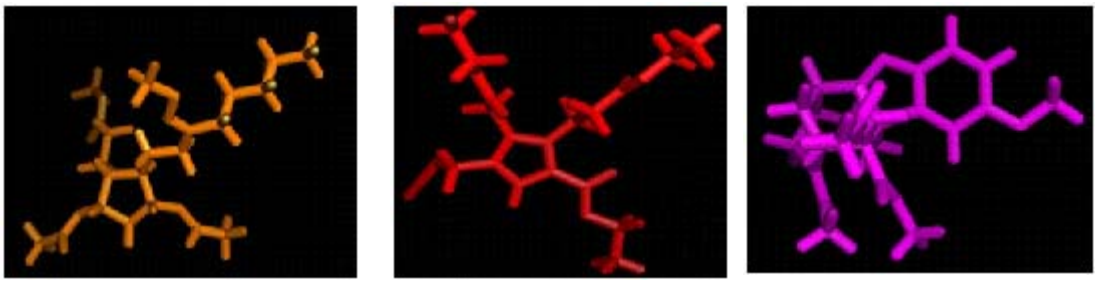
Sites	Residues	Volume in Cubic angstrom
1	169	279
2	154	182
3	129	122
4	118	116
5	115	101
6	85	62
7	59	53
8	69	48
9	55	45
10	77	45

Table 2. Molecular docking results of compounds extracted from *Streptomyces* sp.VITSTK7 with interleukin-13

Receptor	Compound	Score	Area	ACE
Interleukin-13 (1IJZ)	$C_{22}H_{37}NO_7$	4738	600.4	-159.59
Interleukin-13 (1IJZ)	$C_{17}H_{24}N_4O_6$	4122	535.4	-208.41
Interleukin-13 (1IJZ)	$C_{24}H_{28}N_2O_5$	3698	441	-93.56

Table 3. Summary of molecular docking results of compounds identified in the extract of *Streptomyces* sp.VITSTK7 with interleukin-13

Receptor	Compound	Docking report number	Binding amino acid	Number of atoms	Sequence number	Binding position	Chain	Length of interaction	Target Site No.
Interleukin-13	$C_{22}H_{37}NO_7$	2	ASN	25	113	HD21	A	2.1	9
			ALA	10	9	HB1	A	1.6	4
Interleukin-13	$C_{17}H_{24}N_4O_6$	12	VAL	26	75	O	A	2.8	2
			CYS	26	57	H	A	2.3	0
			SER	27	55	HG	A	0.8	0
Interleukin-13	$C_{24}H_{28}N_2O_5$	2	SER	11	68	OG	A	2	2



(A) (B) (C)

Fig. 1. Structure of ligands A) $C_{22}H_{37}NO_7$ B) $C_{17}H_{24}N_4O_6$ and C) $C_{24}H_{28}N_2O_5$

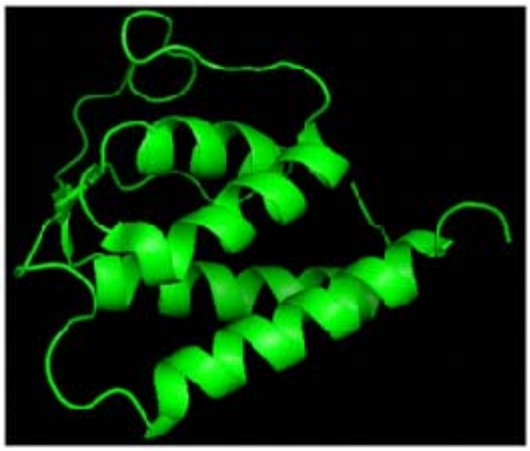


Fig. 2. Structure of interleukin-13 (PDB ID: 1IJZ)

cubic angstroms. Site 9 has 55 residues which occupy 45 cubic angstroms and the target site 10 has 77 residues which occupy 45 cubic angstroms (Table 1).

Docking

The output file of the docking was received through email, which showed all possible docking score between the receptor and ligands (Figure 3). Scoring is important as it gives the number of possible intermolecular interactions such as hydrogen bonds and hydrophobic contacts. Based on this score, possible complexes were arranged in ascending order. Along with the score, area, ACE (Atomic contact energy),

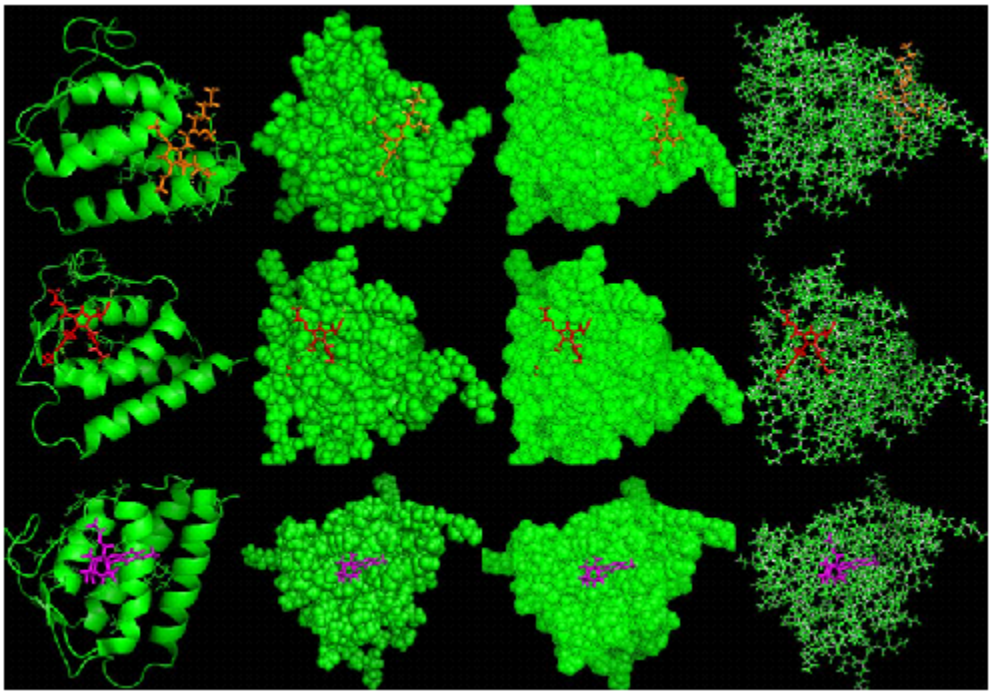
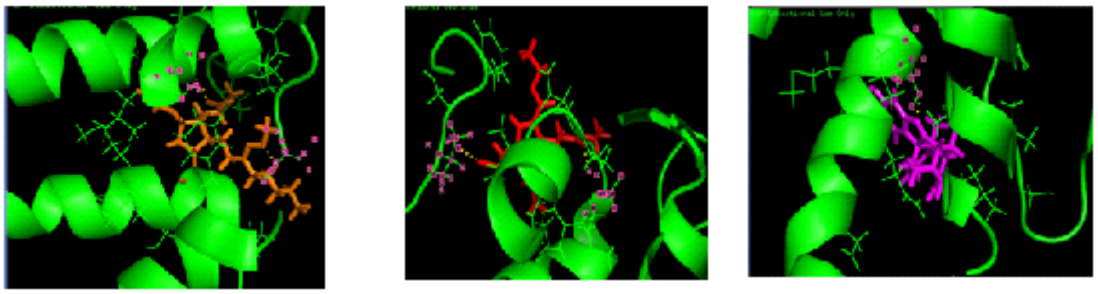


Fig. 3. Schematic representations of docked image of three ligands with interleukin-13



(A) (B) (C)

Fig. 4. Docking of ligands with interleukin-13A) $C_{22}H_{37}NO_7$ with 2 polar contacts, B) $C_{17}H_{24}N_4O_6$ with 3 polar contacts and C) $C_{24}H_{28}N_2O_5$ with one polar contact

transformation, and the pdb file of the complex was also given with the report (Table 2).

The ligand $C_{22}H_{37}NO_7$ docked with interleukin 13 showed possible docking structure in pdb format and was viewed by Pymol. The second report of the docked results showed least binding energy with two polar contacts (Figure 4A). The observed score is 4738 with ACE value of -159.59. There are two different contacts was found with asparagine with 25 atoms and alanine with 10 atoms. These amino acids were positioned in 113rd and 9th code of the sequence and the binding position is HD21 and HB1 of A chain. These two positions were listed in target sites of interleukin-13. It is proved that the binding occurred at target site. The lengths of interactions are 2.1°A and 1.6°A (Table 3).

The ligand $C_{17}H_{24}N_4O_6$ docked with interleukin-13 and 12th report was found be having least atomic contact energy with three polar contacts (Figure 4B). The observed score is 4122 with ACE value of-208.41. Three different contacts were found with valine containing 26 atoms, cysteine containing 26 atoms and serine containing 27 atoms. These amino acids were positioned in 75th, 57th and 55th code of the sequence and the binding position is O, H and HG of chain A. The length of the polar contact with O is 2.8°A. This O position alone was listed in target site. The other two sites are not considered as target sites.

The ligand $C_{24}H_{28}N_2O_5$ docked with interleukin-13 and the second report was found to be having least atomic energy with one polar contact (Figure 4C). The observed score is 3698 with the ACE value of -93.56. The contact was found with serine amino acid containing 11 atoms. This amino acid was positioned in 68th code of the sequence and the binding position is OG of A chain. The length of the interaction with OG is 2°A. This position was listed in target site of interleukin-13. All the amino acids which are involved in the interaction were found to be in conserved region. It is confirmed protein blast: (http://www.ncbi.nlm.nih.gov/BLAST/Blast.cgi? PAGE = Proteins).

In the present study we investigated the interaction of compounds extracted from *Streptomyces* sp.VITSTK7 with interleukin-13 by *in-Silico* molecular docking approach. These three compounds were found to be very active and interacted with target protein of asthma. Earlier reports showed that the anti-asthmatic activity of novel compound 3-substitued Phenyl-2-(furan-2-yl)-4H- Chromen-4-ones proved against interleukin-13 by in-*Silico* molecular docking studies[10]. Interleukin (IL)-13 is an important T-helper type 2 (Th2) cytokine involved in the inflammation of asthmatic airways and has been proved to be a target for antiasthmatic activity[11].

Summary

In this study the interaction of compounds i) Cyclopentanepropanoic acid, 3,5-bis(acetyloxy)-2-[3-(methoxyimino) octyl], methyl ester ($C_{22}H_{37}NO_7$), ii) 5-Azidomethyl-3-(2-ethoxycarbonyl-ethyl)-4-ethoxycarbonylmethyl-1H-pyrrole-2-carboxylic acid ($C_{17}H_{24}N_4O_6$) and iii) Akuammilan-16-carboxylic acid, 17-(acetyloxy)-10-methoxy, methyl ester ($C_{24}H_{28}N_2O_5$) with interleukin-13 was predicted with the help of *in-silico* molecular docking studies. *In vivo* studies are needed to establish the anti-asthmatic activity of *Streptomyces* sp.VITSTK7 extracted compounds.

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