Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2017, 9(10):186-192

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Identification of Natural Compounds as Possible Anti-Allergic Drugs using Molecular Docking Analysis

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ABSTRACT

Allergic diseases have been increasing all over the world, especially allergic respiratory diseases whose incidence is rising at an alarming. Less side effects and low cost of natural resources open new avenues for the treatment of various diseases including allergy and also using computational approaches minimizes experimental time in drug design. Therefore, this study aimed to target C-Chemokine receptor 3 (CCR3) as potential therapeutic target for Allergic respiratory diseases because it mediates the chemotactic response to binding of several chemokines which are highly expressed in the airways of asthmatic patients. The homology model of the target protein was built using MODELLER 9v16 and validated by Ramachandran plot. The modeled structure was virtually screened against natural product database by means of molecular docking approaches. Ligands with low binding affinity were further studied for their pharmacokinetics and drug-likeness properties. Those that are non-substrate to P-gp, inhibitors to CYP450 and with good drug-likeness properties are selected as the hits compounds. Binding analysis of the hits compounds and CCR3 was carried out using Autodock 4.2. Therefore, Ligands with good binding energy and pharmacokinetic properties are recommended to establish ideal lead candidates for the treatment of allergic airway diseases.

Keywords: AllergyC-Chemokine receptor; Natural; Modelling; Docking; Pharmacokinetics; Drug likeness

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INTRODUCTION

Rising of allergic respiratory diseases among different group of people has been observed. Causes of these diseases can be explained by the presence of biologic aeroallergens such as Pollen and House Dust Mites (HDM) which are able to stimulate the sensitization and symptoms of these diseases. Sensitization to different clinical symptoms and severity of the diseases; rhinitis, asthma and rhinitis with/without asthma may be caused by different types of aeroallergens [1]. Asthma is one of the threatening respiratory diseases affecting both children and adult and usually characterized by chronic airway inflammation. It is defined by the history of respiratory symptoms such as wheeze, shortness of breath, chest tightness and cough that vary over time and in intensity, together with variable airflow obstruction [2]. Chemokine receptors CCR3 is preferentially expressed by Th2 cells, mast cells, and eosinophils, all of which are involved in the pathogenesis of allergic diseases. Chemokines are associated with homeostatic cell migration and host defence, excessive production of chemokines has been implicated in the inflammatory components of many clinically important diseases including asthma [1,3-5]. Chemokine receptor CCR3 is expressed predominantly on eosinophils and mediates the chemotactic response to binding of several chemokines, among them, the three eotaxins (CCL11, CCL24, and CCL26) exhibit the highest specificity for CCR3. In addition, their expression levels frequently increase in allergic inflammatory sites. There is increased expression of these chemokines in the airways of asthmatic individuals [6]. The interactions of eotaxin, RANTES and MCP-1 with CCR3 (CD193) are responsible for the recruitment of basophils, eosinophils and mast cells [7,8] had pointed out that eotaxin-rich Th2 promoting pro-angiogenic progenitor cells interact with the lung vascular endothelium to initiate

angiogenesis and consequently eosinophilic airway inflammation. CCR3 was found to be highly expressed on sub mucosal endothelial cells in patients and murine model of asthma [8]. Antagonizing the chemokine receptor CCR3 is of great advantage because they may block the combination of chemokines that act through the same receptor in sequence [9].

Several CCR3 antagonists have been developed for clinical studies in asthma, although so far all have failed for toxicological reasons. Consequently, antagonizing the CCR3 with small natural molecule is of interest as a possible therapeutic approach for the treatment of allergic asthma with fewer side effects. Here we screened a large natural database based on highest binding affinity to our target protein (CCR3), pharmacokinetic and drug-likeness properties using computational approaches. This can help in the selection of the optimal drug and avoid adverse drug reactions in clinical stage. Therefore applying computational methods accelerate various steps of drug designing and reduce the time as well as overall cost.

EXPERIMENTAL SECTION

Comparative Modelling

The protein sequence of CCR3 (376 residues) was obtained from the NCBI (National Center for Biotechnology Information) with the accession number NP_847898.1 and saved in FASTA format for comparative modelling. The sequence was then subjected to protein-protein Basic Local Alignment Search Tool (BLASTp) against the Protein Data Bank for search of suitable template. The alignment of our protein target sequence and the template (4MBS) was generated using the Align2d command (it takes into account structural information from the template when constructing an alignment) in MODELLER 9v16 [10]. Among the five 3D structures developed, the one with low Discrete Optimized Protein Energy (DOPE) was chosen and further subjected for validation using PROCHECK [11] to determine the stereochemical quality of the protein structure by analysing residue-by-residue geometry and overall structure geometry. Superimposition, percentage similarity and RMSD calculations of the template (4MBS) and the predicted model of our protein target (CCR3) was performed using Chimera [12].

Binding Site Prediction

Binding site of the CCR3 model was predicted by submitting the sequence to the COACH server. This server generate a 3D model from the submitted primary sequence using I-TASSER then using the COACH Algorithm the ligand-binding site prediction will be determine [13].

Virtual Screening and Molecular Docking

After modelling the 3D structure of our target protein (CCR3) we adopt a structure-based VS technique for drug design. Firstly, the Zinc Natural Product database (Zinc is not commercial) was virtually screened against the model structure of CCR3 using Autodock vina [14] in PyrX virtual screening tool [15]. File format conversion and preparation like energy minimization of the ligands using mmFF94 force field optimization algorithm conjugate gradients at 200 total numbers of steps and generating the pdbqt files was performed in Open Babel tool [16]. The default Vina search space with a dimension angstrom of x: 25, y: 25 and z: 25 were not changed at the protein centre dimension of x: 158.876, y: 113.986 and z: 37.6045. The set of natural compounds with the lowest binding affinity were selected as compounds that can bind to our model protein. They were further submitted to the SwissAdme [17] for pharmacokinetics and drug-likeness properties. Therefore we filtered out the compounds that have the following pharmacokinetic properties; gastro-intestinal absorption, P-gp substrate, inhibition of CYP450 and drug-likeness. The resulting compounds were lastly docked to the binding site of our target protein (CCR3) using Autodock 4.2 [18] visualization and hydrogen bonding analysis and 2D ligand receptor diagrams were performed using UCSF Chimera [19-25] and Maestro (Schrodinger USA).

Ligand Interaction Analysis

Ligand interactions of the docked hits compounds and known anti-asthmatic drugs were analyzed with the aid of 2D protein–ligand interaction diagrams (schrodinger) to compare the interactions of the target protein (CCR3) with hit ligands and known anti-asthmatic drugs.

RESULTS AND DISCUSSION

Comparative Modelling and Validation of Model

Crystal structure of the CCR5 Chemokine receptor (PDB ID: 4MBS-A) was selected as the most appropriate template for our target sequence over the similar structures because it has the highest identity sequence of 46% and

less *E*-value of 1e-110. Among the five 3D structures developed, the one with lower DOPE (Discrete Optimized Protein Energy) score -44803.85547 was chosen because this was a standard scoring function in MODELLER.

The structural superimposition of the template (4MBS) and target protein CCR3 has shown similarity in the 3D structures of both the template and the model (Figure 1). The RMSD calculated using the Chimera reveals that the overall RMSD between template and the model built was 0.656 Å (Tables 1 and 2). As indicated by the Ramachandran statistics (Table 3) 93.6% of the residues in the model structure were within the most favoured regions, 5.3% of the residues were in the Additional allowed regions, 1.2% of the residues were in the generously allowed regions and 0.0% of the residues were in the disallowed region and a usual score of all the G-factors. Based on these validations, the predicted model of CCR3 structure was adopted for further analysis (Figure 2).

Binding Site Prediction

The residues found to be functional active sites of CCR3 determined by COACH server is shown in the table below (Table 3). Residues with highest confidence score (C-score) of 0.21 and cluster size of 84 were selected for our binding residue. Among the consensus binding residues, the first residue number 111 was chosen for our grid dimension (X:162.649, Y:109.513, and Z:24.732) in docking simulation (Figure 3).

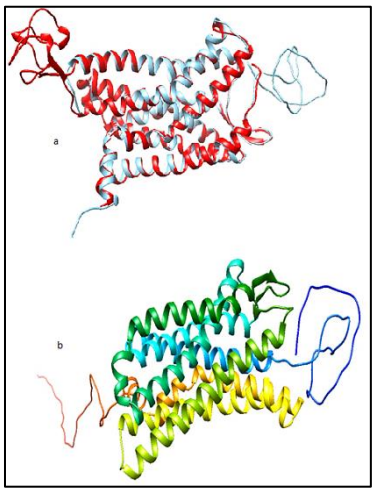

Figure 1: (a) Superimposition of model structure CCR3 (red) and template PDB ID: 4MBS (cyan) with RMSD=0.656 Å (b) predicted 3D-dimensional structure of CCR3

Table 1: Superposition evaluations between template (4MBS) and CCR3

Parameters	Score
Sequence alignment score	1084.6
RMSD between 285 pruned atom pairs	1.751 Å
Overall RMSD	0.656 Å
SDM (cutoff 5.0)	14.379
Q-score	0.634
Sequence lengths	346 (4mbs) vs. 376 (ccr3)
4mbs.pdb, chain A vs. CCR3.pdb	43.88% seq. Identity

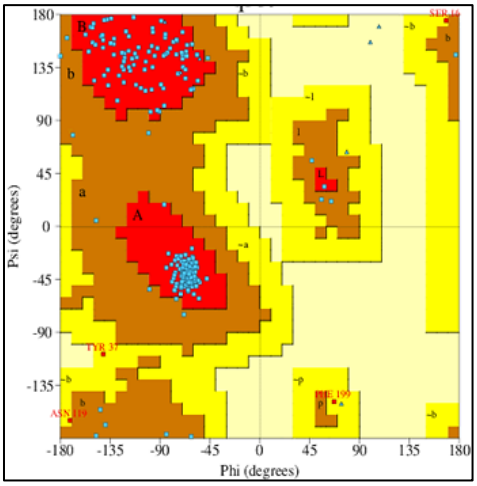

Figure 2: Ramachandran map showing distribution of residues for CCR

Table 2: Ramachandran plot statistics

Regions	Residues	Percentage
Most favoured regions (A,B,L)	320	93.60%
Additional regions allowed (a,b,l,p)	18	5.30%
Generously allowed regions (~a,~b,~l,~p)	4	1.20%
Disallowed regions (XX)	0	0.00%
Non-glycine and non-proline residues	342	100.00%
End-residues (excl. Gly and Pro)	2	
Glycine residues	17	
Proline residues	15	
Total number of residues	376	
G-Factors	**Score**	**Average Score**
Dihedral angles:-		
Phi-psi distribution	0.34	0.05
Chi1-chi2 distribution	-0.25	
Chi1 only	0.13	
Chi3 and chi4	0.32	
Omega	-0.13	
Main chain covalent forces:-		
Main chain bond lengths	-0.12	-0.16
Main chain bond angles	-0.19	
OVERALL AVERAGE		-0.03

Table 3: Binding sites of C-Chemokine receptor 3 (CCR3)

Rank	C-score	Cluster size	PDB hit	Lig name	Download complex	Consensus Binding Residues
1	0.21	84	4ea3A	0NN	Rep, Mult	1,11,13,11,34,13,51,38,00,00,00,00,00,00,00,00,00,00,00,00,000
2	0.08	29	4mbsA	MRV	Rep, Mult	6,21,11,11,41,34,13,51,00,00,00,00,00,00,00,00,00,00,00,00,00,00,00,00,000
3	0.07	34	4dklA	MPG	Rep, Mult	9596172175179
4	0.05	27	3zpqB	2CV	Rep, Mult	2,40,24,42,53,25,62,59,00,00,00,000
5	0.04	23	4ea3B	0NN	Rep, Mult	11,11,14,13,11,34,13,50,00,00,00,00,00,00,00,00,00,00,00,00,00,00,000
6	0.04	21	3oe8B	ITD	Rep, Mult	1,11,11,41,30,13,42,01,00,00,00,00,00,00,000
7	0.02	12	4ldeA	1WV	Rep, Mult	62,65,11,53,05,309
8	0.01	5	2y03A	2CV	Rep, Mult	2,36,23,92,63,266
9	0.01	9	2y02B	2CV	Rep, Mult	1,36,17,31,76,17,91,80,000
10	0.01	4	3nyaA	CLR	Rep, Mult	77,95,99,102

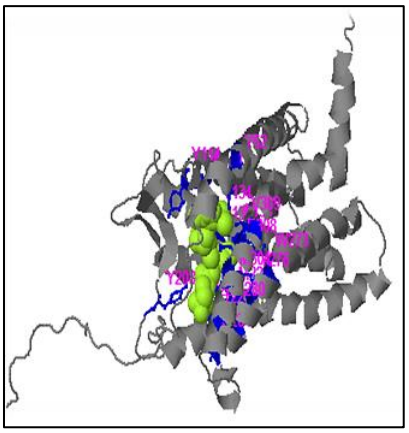

Figure 3: Functional active site of CCR3

Virtual Screening based on Binding Score and Pharmacokinetic Properties

The docking studies of the ZINC Natural Products in PyRx virtual screening tool shows many Ligands that binds with our protein target at different conformations. Among which 532 Ligands with a highest binding energy ranges from -11.0 to -7.90 kcal/mol were filtered for pharmacokinetics and drug-likeness analysis in SwissADME [17] server, eight Ligands with high binding energy and passed the pharmacokinetics and drug-likeness analysis with high gastro-intestinal absorption, non-substrate to P-glycoprotein, inhibitors to some CYP450 (Table 4) were retained as the hits Ligands. It is of great importance in drug design to screened compounds that are non-substrate to P-gp at early stage to avoid drug-drug interactions. Likewise drug-likeness in drug design assesses qualitatively the chance for a molecule to become an oral drug with respect to bioavailability. A drug's effectiveness against a disease depends not only on its interaction with receptors but also its pharmacokinetics properties.

Table 4: Pharmacokinetics and drug-likeness properties of the hits Ligands

Ligands	GI absorption	P-gp substrate	CYP450 inhibitor	Lipinski	Ghose	Veber	Egan	Muegge
1	High	No	CYP1A2, CYP2C19, CYP2C9, CYP3A4	Yes	Yes	Yes	Yes	Yes
2	High	No	CYP1A2, CYP2C19, CYP2C9, CYP3A4	Yes	Yes	Yes	Yes	No
3	High	No	CYP2C19, CYP2C9, CYP3A4	Yes	Yes	Yes	Yes	No
4	High	No	CYP2C19, CYP2C9	Yes	Yes	Yes	Yes	Yes
5	High	No	CYP2C19, CYP2C9, CYP3A4	Yes	Yes	Yes	Yes	No
6	High	No	CYP1A2,CYP2C19, CYP2C9, CYP3A4	Yes	Yes	Yes	Yes	Yes
7	High	No	CYP2C9, CYP3A4	Yes	Yes	Yes	Yes	No
8	High	No	CYP1A2, CYP2C19, CYP2C9, CYP3A4	Yes	Yes	Yes	Yes	Yes

Molecular Docking

All Ligands shows a good binding affinity to the protein target, ligand one having the highest binding energy (-10.01) and ligand 3 has the highest number of hydrogen bonds (Table 5). The interacting residues are the consensus binding residues determined by CORCH binding site prediction server (Figure 4).

Table 5: Molecular docking analysis and ligand protein interactions of the hits ligands

Ligands	Binding energy (kcal/mol)	Inhibition constant (*Ki*)	Bond distance	Interacting residues
Ligand 1	-10.01	45.93nM	2.081Å	Tyr 134, Cys204
Ligand 2	-9.9	55.19nM	2.313 Å	Cys204
Ligand 3	-9.83	61.89nM	1.852 Å	Ser205, Tyr62, Cys204, Tyr114
Ligand 4	-9.78	67.33nM	1.932 Å	Glu308
Ligand 5	-9.57	96.08nM	2.148 Å	Ser205
Ligand 6	-9.33	144.02nM	2.112 Å	Cys204, Tyr114
Ligand 7	-9.31	150.2nM	1.986 Å	Cys204, Tyr114
Ligand 8	-9.09	217.55nM	2.173 Å	Tyr114

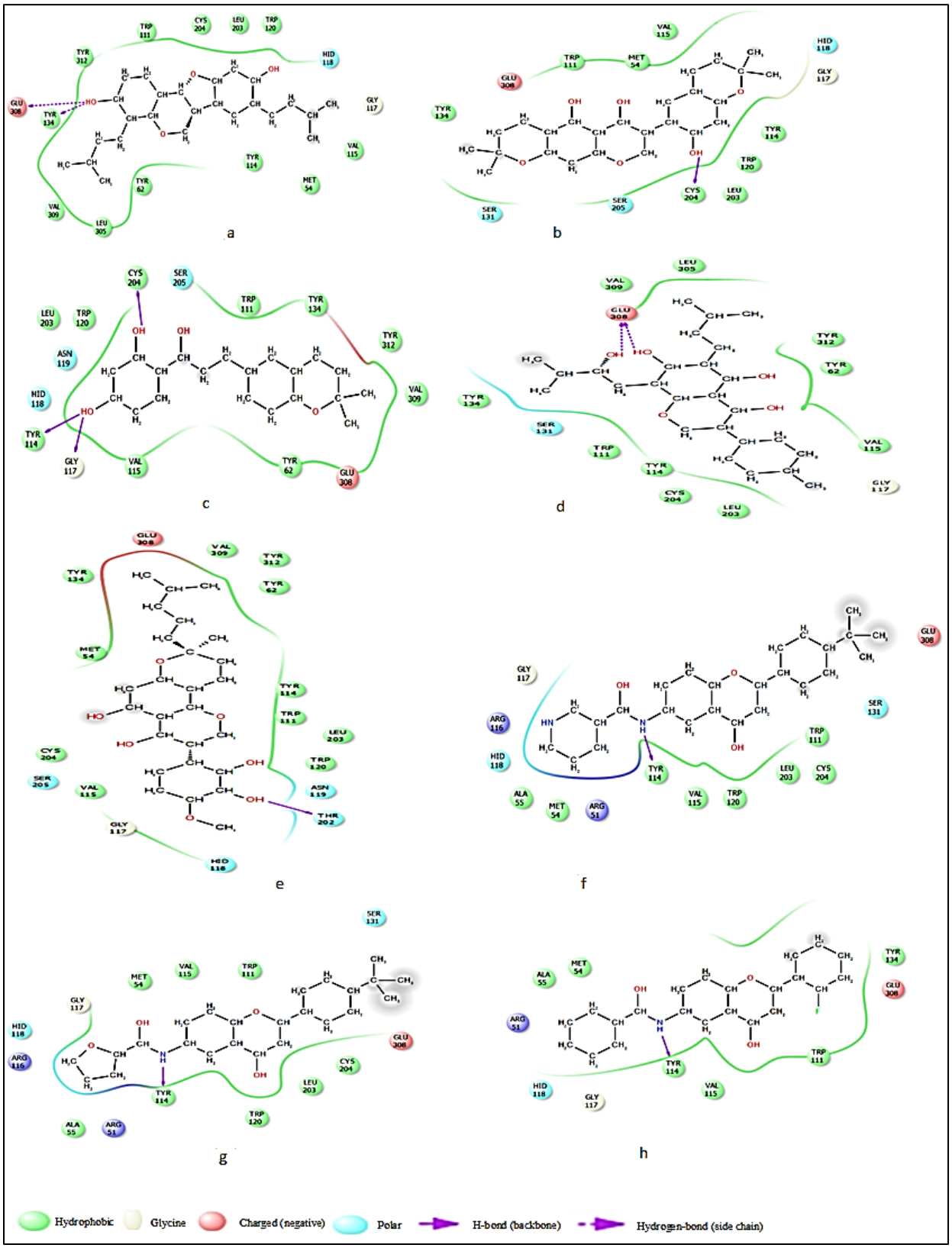

Figure 4: (a-h) 2D diagram of the protein-ligand interaction between CCR3 and natural ligands from zinc natural product database

CONCLUSION

Blocking the CCR3 as a therapeutic target for the treatment of allergic respiratory diseases is of great interest. Determination of 3D structure of our protein target (CCR3) by comparative modelling helps us to understand it function in ligand binding. Exploring the natural compounds for search of ideal candidates by virtual screening methods using computational analysis reduces side effects, cost and time in drug discovery. Screening of the hits Ligands based on pharmacokinetic properties is of utmost important because this will reduce the failure of most drugs at the clinical stage. Therefore this study reveals five potential natural compounds that have good pharmacokinetic properties and binding energy to CCR3, hence suggested as possible drugs for the treatment of allergic respiratory diseases after undergoing further researches.

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