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Designing and Development of Potent Drug Inhibitor to IL13RA2 in Asthma Disease

KEYWORDS: Target molecule, IL-13Rα2, Drug molecule, Receptor-Ligand docking

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ABSTRACT *Asthma is a chronic (long-term) lung disease that inflames and narrows the airways. Asthma causes recurring periods of wheezing (a whistling sound when you breathe), chest tightness, shortness of breath, and coughing. Asthma affects people of all ages, but it most often starts during childhood.*

IL-13 receptor (IL-13R) is a heterodimer composed of the IL-13Rα1 chain (IL-13Rα1) and the IL-4Rα chain (IL-4Rα), both IL-13 and IL-4 activate STAT6 and STAT3. IL-13Rα2 chain (IL-13Rα2) binds to IL-13 with high affinity and it does not contain the Box1 motif which is an important domain for intracellular signaling. IL13Rα2 was the target protein because it does not associate with signaling molecules and instead acts as a "decoy" receptor.

The studies were performed on IL13Rα2 to inhibit the protein that expressed during the causes for asthma disease. The potential drug with IUPAC name "5-[2-(tert-butylamino)-1-hydroxyethyl]-2-(hydroxymethyl)" was designed successfully in inhibit the actions of IL13Rα2 protein.

INTRODUCTION

Asthma (AZ-ma) is a chronic (long-term) lung disease that inflames and narrows the airways. Allergic diseases, such as allergic rhinitis, allergic conjunctivitis, food allergy, atopic dermatitis, and asthma are complex genetic diseases with major environmental influences that occur in a developmental context. Asthma is characterized by airway hyper responsiveness (AHR) and inflammation, as well as underlying structural changes to the airways. Current treatment regimens are effective in controlling asthma in the majority of patients. Yet, nearly 30% of patients in the Gaining Optimal Asthma controL (GOAL) study failed to maintain asthma control despite regular use of high-dose fluticasone and salmeterol. (Bateman ED et al. 2004)

Even though a pure genetic basis of asthma appears unlikely, several chromosomal regions and loci showing linkage to, and association with, airway inflammation, asthma and asthma-linked phenotypes have recently been identified. (A.N. Aggarwal et al. 2005)

Interleukin 13 (IL-13) is a protein that in humans is encoded by the IL13 gene. (Minty A et al.1993). IL-13 is cytokine secreted by many cell types, but especially T helper type 2 (Th2) cells that is a mediator of allergic inflammation and disease. (Wynn TA, 2003). The IL-13 receptor (IL-13R) is a heterodimer composed of the IL-13Rα1 chain (IL-13Rα1) and the IL-4Rα chain (IL-4Rα), and it also acts as type II IL-4R. IL-13 binds to IL-13Rα1 first with low affinity (Kd=2-10nM), and then recruits IL-4Rα to the complex, generating a high-affinity receptor (Kd=0.03-0.4 nM). Heterodimerization of the IL-13R by IL-13 causes activation of the JAK/STAT and phosphatidylinositol-3 (PI-3) kinase pathways inside the cells as well as IL-4. In the JAK/STAT pathway, both TYK2 and JAK1 are first activated, followed by activation of STAT6 and STAT3. Because both IL-13 andIL-4 activate STAT6, these two cytokines exert similar biological activities. STAT6 is a transcription factor, critical for both the IL-13 and IL-4 signals, as described later. The STAT3-STAT1 pathway has been implicated, together with-STAT6, in the induction of 15-lipoxygenase in human monocytes. Insulin receptor substrate (IRS) is a linker protein for PI-3 kinase to associate with tyrosine-phosphorylated IL-4Rα. IRS-2 is involved in Th2 cell differentiation. There is another IL-13 binding unit, the IL-13Rα2 chain (IL-13Rα2), which binds to IL-13 with high affinity (0.25-1.2 nM). However, as the intracellular domain of IL-13Rα2 is short, and does not contain the Box1 motif, an important domain for intracellular signaling, IL-13Rα2does not associate with signaling molecules, acting instead as a "decoy" receptor. (Kenji Izuharaet al.2007)

MATERIALS AND METHODS

Disease identification was performed by doing data mining from various journal papers and articles, after selecting asthma as disease, the pathway studies were carried using KEGG database. The target gene and protein involved were found out from Genecards, PDB and various journal papers.

The FASTA format of protein was retrieved and Sequence validation was done through NCBI, KEGG and UniPort/ SWISS-PORT databases. The structural validation was performed using various databases like CPH model, HH pred and Geno 3D for tertiary structure. Phylogenetic analysis was performed using SDSC Biological Workbench for Clustal W.

Protein structure was downloaded from PDB to Accelrys Discovery Studio (ADS) and natural ligand was found out to design the ligand library using ACD chemsketch. Further high-through-put screening of ligand molecule were performed using ADS for parameters such as TOPKET, and ADMIT. Force field was applied on both receptor protein and screened ligand molecule which was followed by energy minimization of the same using ADS. Docking was performed on receptor and ligand molecule through LibDoc using ADS and docking was also performed using HEX. Finally, Pharmacophore analysis was carried out using LigandScout.

RESULTS

a) Protein Sequence analyses

From the three standard databases (NCBI, KEGG and UniPort) we infer that the amino acid sequence was found similar in all and we conclude that IL13Rα2 protein consists of 380 amino acids as given bellow.

MAFVCLAIGCLYTFLISTTFGCTSSSDTEIKVNPPQDFEIVDP-GYLGYLYLQWQPPLSLDHFKECTVEYELKYRNIGSETWKTIIT-KNLHYKDGFDLNKGIEAKIHTLLPWQCTNGSEVQSSWAETT-YWISPQGIPETKVQDMDCVYYNWQYLLCSWKPGIGVLLDT-NYNLFYWYEGLDHALQCVDYIKADGQNIGCRFPYLEASDYK-DFYICVNGSSENKPIRSSYFTFQLQNIVKPLPPVYLTFTRESS-CEIKLKWSIPLGPIPARCFDYEIEIREDD

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TTLVTATVENETYTLKTTNETRQLCFVVRSKVNIYCSDDGI-WSEWSDKQCWEGEDLSKKTLLRFWLPFGFILILVIFVTGLLL-RKPNTYPKMIPEFFCDT

b) Protein Structure analyses
Protein structure analysis was performed for tertiary structure for different databases and the results are as follows:

i] CPH model
CPH model tools used in analysis of tertiary structure of the protein showed PDB ids related to the query sequence and the results obtained are as follows

PDB ID	Chain	Score	E value
3LB6	D	557	1e-158
3LB6	C	464	1e-130

Summary for the best molecule:

- **Query Mw: 44176 (380 aa)**

- Score: 758.0 bits
- Identity: 93.7 %
- Template= 3LB6.D
- Id= 93.7
- Query length= 380
- Model length= 303
- Coverage= 79.7
- Model Mw= 35496
- Method= 'PDB Blast'

ii] HH pred
HH pred results obtained for protein tertiary structure showed PDB ids related to the query sequence obtained as follows

- **3lb6_C IL-13, interleukin-13 receptor subunit alpha-2;**
- cytokine,
- decoy receptor,
- glycoprotein; HET: MLY: NAG
- 3.05A {Homo sapiens} PDB: 3lb6 _D*
- Probability=100.00
- E-value=3.6e-59
- Score=423.28
- Aligned coils=380
- Identities=99%
- Similarity=1.671
- Sum probs=0.0

iii] Geno3D
Geno3D results obtained for protein tertiary structure are as follows

TEMPLATE	E value	FIRST	LAST	ID
pdb3lb6D-0	1.000000e-114	29	331	93.000000
pdb3lb6C-0	2.000000e-99	31	327	83.000000

Summary of the templates

- **pdb3lb6D**

- Score = 416 bits (1069),
- Expect = e-114,
- Method: Composition-based stats.
- Identities = 284/303 (93%),
- Positives = 284/303 (93%)

From the above protein structural analysis for tertiary structure we infer that IL13Rα2 protein structure has the PDB id of 3LB6 and can be downloaded from prot ein database (PDB).

c) Phylogenetic analysis
Phylogenetic analysis was performed using SDSC Biological Workbench and the results obtained for Cluster W are as follows

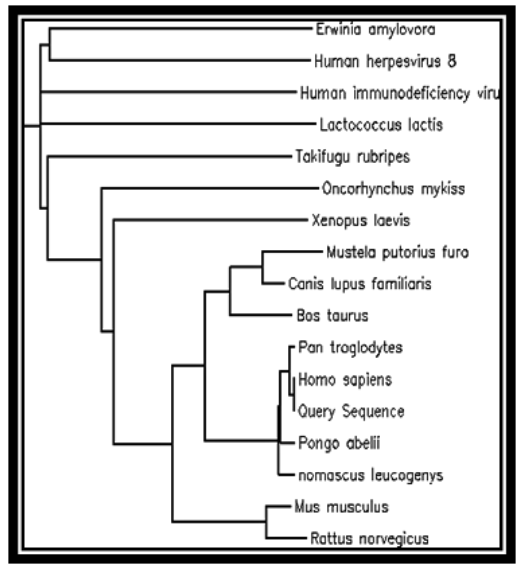

Fig 1: Cluster W result

From Fig 1 we can infer that Clustal W is a widely used for multiple sequence alignment and from the above rooted tree we can observe that query sequence belongs to Homo sapiens. The closest species related to query sequence is Pan troglodytes followed by Pongoabelili. The branching shows mutations and the changes occurred. The farthest species to the query was Laxtococcuslactis.

d) Optimization of lead molecule
2-Acetamido-1, 5-anhydro-2-deoxyhexitol was the natural ligand and parent drug molecule. The daughter molecules were designed using chemsketch software to form a library of ligands.

i] TOPKET
TOPKET was a part of toxicity prediction performed on Accelrys Discovery Studio (ADS). Various models like NTP Carcinogenicity Call (Male Rat) (v3.2), FDA Carcinogenicity Female Rat Single vs

Mult (v3.1), Developmental Toxicity Potential (DTP) (v3.1), Rat Oral LD50 (v3.1), Skin Irritation (v6.1) and Aerobic Biodegradability (v6.1) were selected for TOPKET examination of ligand molecules. The results of TOPKET are as follows

Mol	NTP	FDA	DTP	LD50	Skin Irr	Aerob Bio
3	0.034	0.000	0.999	2.078	0.000	0.999
4	0.000	0.136	0.134	1.605	0.000	0.000
6	0.000	0.248	1.000	1.379	0.000	0.000
7	0.044	0.000	1.000	2.429	0.900	0.011
22	0.004	0.000	1.000	2.429	0.901	0.011
25	0.049	0.999	1.000	2.399	0.055	0.014
27	0.000	1.000	0.992	1.719	0.000	1.000

Table 1: Toxicity TOPKET prediction for ligand molecule

From Table 1 we can observe that the ligand molecule to pass the TOPKET prediction it should be non-carcinogenic.

ii] ADMET
ADMET tests were conducted on Accelrys Discovery Studio (ADS) and the tests were performed on the molecules that had passed TOPKET prediction and the results of ADMET are as follows

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Mol	BBM	BBB level	Ab Level	Solubility	S. Level	Hepatoto	H. Probab	CYP2D6	C. Probob	PPB level	AlogP98	Unkno A.	PSA 2D	Result
3	-	4	1	1.222	5	0	0.052	0	0.405	0	-1.576	0	101.487	Pass
4	-	4	2	1.605	5	0	0.033	0	0.069	0	-2.043	0	101.487	Week
6	-	4	1	1.237	5	0	0.059	0	0.069	0	-1.351	0	92.557	Pass
7	-	4	1	1.287	5	0	0.046	0	0.069	0	-1.698	0	92.557	Pass
22	-1.054	3	0	-0.698	4	0	0.205	0	0.089	0	0.941	0	75.256	Best
25	-	4	1	0.87	5	0	0.072	0	0.168	0	-1.305	0	92.029	Pass
27	-1.16	3	0	-0.9	4	0	0.231	0	0.118	0	0.999	0	83.099	Best

Table 2: ADMET result sheet

From table 2 we can observe that this model predicts blood-brain penetration (blood brain barrier, BBB) after oral administration. This model also contains a quantitative linear regression model for the prediction of blood-brain penetration, as well as 95% and 99% confidence ellipses in the ADMET_PSA_2D, ADMET_AlogP98 plane. The molecules with hepatotoxicity and adsorption levels of zero or less than 2 were selected for energy minimization.

The result for best molecule i.e. mol 22 is shown bellow

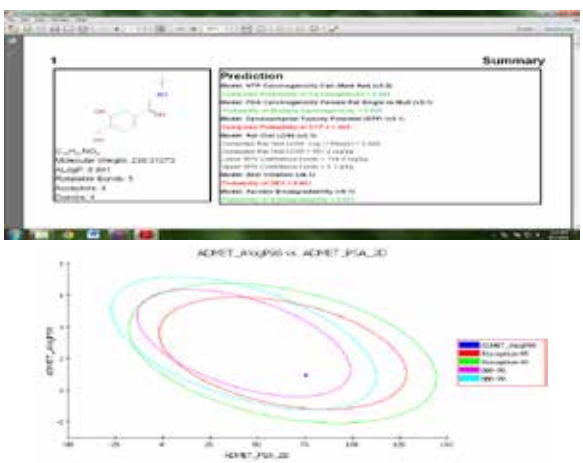


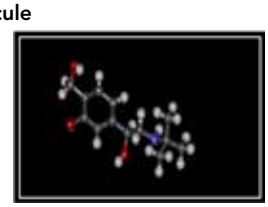
Fig 2: Graph plotted between ADMET AlogP98 v/s ADMET PSA 2D

From the above fig 2 we can observe that the ligand molecule (blue dot) was in between all the concentric circles. More the blue dot in centre (near 50) better is the ligand molecule.

e) Energy minimization

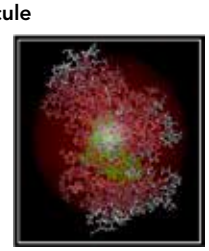
Energy minimization was performed on Accelrys Discovery Studio (ADS) to stabilize both receptor and ligand molecule for appropriate docking to take place.

i] Ligand molecule



Energy minimizations on ligand molecule were carried out by applying charm forcefield and conjugated gradient algorithm was used for minimization. The ligand was minimized at 600 cycles with minimization energy of 40.01293kcal/mol.

ii] Receptor molecule



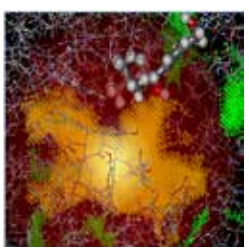
Energy minimization on receptor molecule was carried out by applying charm forcefield and conjugated gradient algorithm was used for minimization. The protein receptor was minimized at 1600 cycles with minimization energy of -47648.84568kcal/mol.

f) Docking

Docking is a method which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex. Knowledge of the preferred orientation in turn may be used to predict the strength of association or binding affinity between two molecules using for example scoring functions. Receptor-Ligand docking was performed using LibDoc on Accelrys Discovery Studio (ADS) and docking was also performed on HEX.

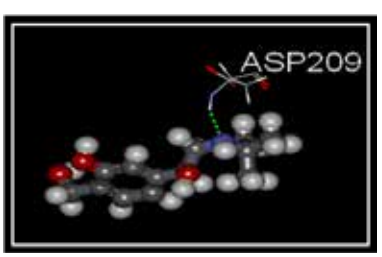
i] Using LibDoc on Accelrys Discovery Studio

Docking was performed with energy minimized 3LB6 receptor and energy minimized designed lead molecule named 5-[2-(tert-butylamino)-1-hydroxyethyl]-2-(hydroxymethyl) phenol. The results of docking are as follows



When ligand molecules were docked with receptor 3LB6 using LibDoc 4 poses were obtained, 4thpose with least LibDoc score value was selected and observations showed that, the ligand molecule had intermolecular H-bond with ASP209 (aspartic acid).

ii] Using HEX



The results for Docking performed using the Hetero Control menu panel on energy minimized 3LB6 receptor and energy minimized designed lead molecule named 5-[2-(tert-butylamino)-1-hydroxyethyl]-2-(hydroxymethyl) phenol on HEX are as follows

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We observe that receptor-ligand docking using hex yield Etot. Etot is the total calculated interaction energy of the system. Lesser the Etot more stable is the complex. Thus we obtain Emin = -119.12, Emax = -90.89

g) Pharmacophore analysis
A pharmacophore is an abstract description of molecular features which are necessary for molecular recognition of a ligand by a biological macromolecule. These pharmacophoric points may be located on the ligand itself or may be projected points presumed to be located in the receptor.

Ligand scout was used in checking ligand molecule receptor pocket and cavity information. The results are shown bellow

Fig 3: Pharmacophore results

From the above fig 3 we observe there was one hydrogen bond acceptor in the ligand molecule and the ligand molecule was binding in the cavity of the receptor at the site by accepting a hydrogen bond and thus inhibiting the protein to express.

DISCUSSION
Allergic diseases, such as allergic rhinitis, allergic conjunctivitis, food allergy, atopic dermatitis, and asthma are complex genetic diseases with major environmental influences that occur in a developmental context. It is still unclear why allergy and the subsequent development of allergic diseases occur in some children but not others.

There were two major findings in this research investigation. The first finding was that the potential protein target was identified in different cancers and, the second finding is that the best drug candidate was identified.

The studies and the causes for the disease were conducted though docking and pharmacophore studies on IL13Rα2 protein. Ligand for the protein was the daughter molecule designed from referring natural ligand i.e. 2-Acetamido-1, 5-anhydro-2-deoxyhexitol. Docking studies were successfully performed by binding the ligand at the binding site of the receptor and thus inhibiting the protein to express that causes asthma. The drug was constructed by modifying it to have less toxic effect and more efficient binding. Daughter Molecule 22 passed through all the necessary requirements such as ADMET and TOPKAT tests. The IUPAC name of designed drug was "5-[2-(tert-butylamino)-1-hydroxyethyl]-2-(hydroxymethyl)". It can be used as the potential drug helpful in curing asthma.

Further studies must be performed for invitro and invivo analysis in wet lab and the drug molecule can be used as the potential drug helpful in curing asthma. This drug can be made available commercially only after passing through different phases of clinical tests and FDA approval.

CONCLUSION
Computer-aided drug design uses computational chemistry to discover, enhance, or study drugs and related biologically active molecules. The most fundamental goal was to predict whether a given molecule will bind to a target and if so how strongly. Molecular mechanics or molecular dynamics are most often used to predict the conformation of the small molecule and to model conformational changes in the biological target that may occur when the small molecule binds to it.

By targeting IL13RA2 protein we can stop it from associating with signaling molecules and inhibiting it to act as a "decoy" receptor. The potential drug with IUPAC name "5-[2-(tert-butylamino)-1-hydroxyethyl]-2-(hydroxymethyl)" was designed successfully to inhibit the actions of IL13Rα2 protein and eventually stopping the hazards effect of Asthma disease.

Finally, through Insilco studies we conclude that the lead molecule 5-[2-(tert-butylamino)-1-hydroxyethyl]-2-(hydroxymethyl) was designed which form Potential inhibitors of Asthma.

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