

Sopron University
Kitaibel Pál Doctoral School of Environmental Sciences
Bioenvironmental Sciences

Theses of doctoral (PhD) dissertation

**TARGETING HISTAMINE H4 RECEPTORS IN ALLERGIES
CAUSED BY AIR POLLUTANTS**

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The choice of the subject and the aim of the dissertation

Air pollution is one of the greatest problems that the world is facing today and is increasing with every passing year causing irreparable damage to the earth. It also affects the human health directly and indirectly. Among the various health consequences, allergy is the most common disorder influenced by air pollution. The pollutants act as an allergen causing the allergic disorder. When a susceptible person encounters an allergen, large amounts of IgE will be produced. Subsequent exposure to the allergen cause allergic reaction which depends on the type and amount of the allergen encountered. These IgE binds to its receptor on mast cells which leads to the production of histamine. This histamine activities are mediated by G protein-coupled histamine receptor, designated as H1, H2, H3 and H4. H4 histamine receptor have been identified and are found to be expressed on various immune cells and elicit biological response. This makes Histamine H4 receptor an attractive target for anti-allergic therapy.

Based on this background, the main objectives of this research is to develop a potential lead candidate drug for allergy caused by the air pollutants. To achieve this structure based drug designing was followed.

The main objectives are

1. To collect evidence based information on the major environmental pollutants which that trigger allergic response. It is also intended to determine the involvement of H4R in eliciting this immune response.
2. To develop an antagonist against the immune response elicited. To serve this purpose, a 3D structure model of the receptor H4. The main aim of this section is to generate a high quality structure of the H4R. Since the experimental 3D structure is not available, the structure has to be modelled and the binding site has to be determined.
3. Next a database with all the known small chemical structures has to be built. This allows to choose the best drug lead from a wide range of structures. To achieve this 3 already commercially available ligands were used as a template.
4. To identify the best structure from the databases, molecular docking of the structures to the 3D model was performed. The lead structures identified are made to go through few in silico assays to determine its efficacy.

Materials and methods

The air pollutants used for the discussion of its possible role with Histamine receptors were chosen from <http://www.epa.gov/air/airpollutants.html>. Bibliographies for the relation between the individual pollutants and allergy were searched. The results were then tabulated based on the criteria. A second Table was developed to list the relation between the Pollutant and histamine receptors.

Antagonism of histamine's action at H4R has been the cornerstone of an immense market for pharmacological treatment of Asthma. Five structural models of H4 receptor were generated using I TASSER and were validated by PROCHECK AND ERRAT. From the five models, the best model was chosen based on the results given by the validity tools. The docking site and the amino acids that take part in antagonist binding were analyzed and predicted. Three different databases with similar structures of the known ligands JNJ7777120, Vuf6002 and Thioperamide was constructed.

Based on these, molecular docking of the databases with the receptor model using DISCOVERY STUDIO lead to the identification of the six lead compounds. The six compounds were identified based on the dock score and its ability to form hydrogen bonds with the receptor model. ADMET and Molecular dynamic studies were performed to further understand the efficacy of the identified lead compounds.

The methodologies are categorized in Figure 1.

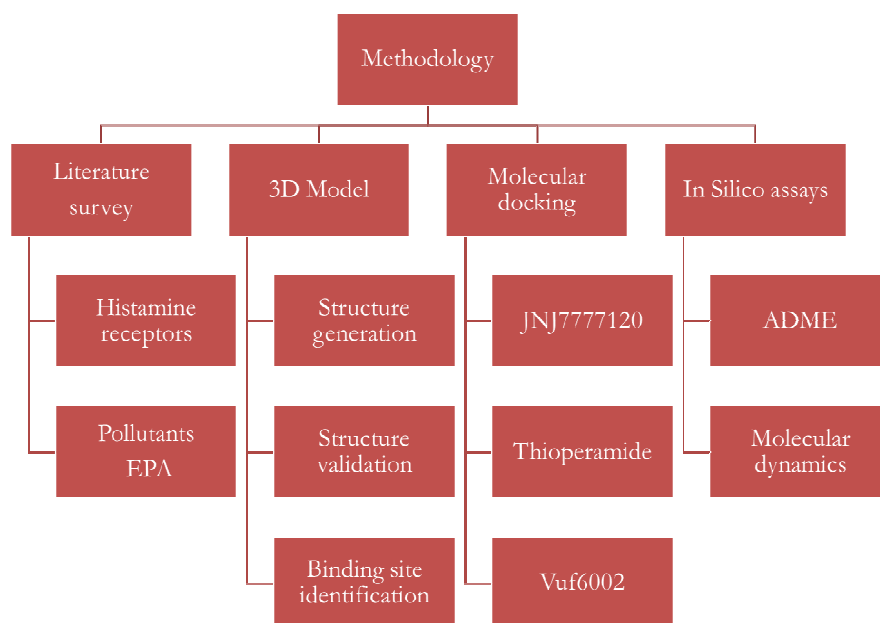


Figure 1 Methodologies

Theses

***THESIS 1:* The evidence based information on the type of pollutants that cause allergy suggest that exposure to air pollutant adversely affects the immune response. This study informs the understanding of the mast cells in mediating allergic mechanism influenced by air pollutants. The role of H4R remains largely unexplored since only very few studies have focused on its role. This insight would possibly mitigate the effect of air pollutant on allergy.**

The evidence based information on the type of pollutants that cause allergy were carried out. The 6 major pollutants causing allergy were established. The mechanisms by which the pollutants mediate allergic diseases have been briefed out and listed. The importance of mast cells in allergy has been further assured through this study. Researches showing pollutants mediating allergy via Histamine receptors are lacking. Though the mRNA expression of H4R receptor in allergies caused by pollen is known, further investigation of the expression of the receptor at protein level could strengthen the research on finding specific antagonist for the receptor.

THESIS 2 The hH4R model developed by I-TASSER had used the human GPCR as its top template. This is the first report where a human GPCR was used as a template as previous studies have employed bovine GPCR.

Previous studies used bovine GPCR as template for predicting the 3D structure of hH4R. This research has utilized human GPCR as the template for modelling the 3D structure of Hh4r. Moreover, in the present study, I-TASSER, a threading alignment tool was employed to predict the 3D structure model of hH4R, whereas previous studies have applied homology based models. Five models were generated based on the input sequence of hH4R (Q9H3N8). I-TASSER output also contained top ten ranks of templates used for the structure prediction. The top template used by ITASSER is the high-resolution crystal structure of human β 2-adrenergic GPCR (PDB ID: 2rh1A). The remaining templates used for threading are the isoforms of human β 2-adrenergic GPCR. It is contradictory to the BLAST search result which predicted that human adrenoceptor (2R4R A) has the close sequence similarity with hH4R compared to human β 2-adrenergic GPCR (2RH1 A). This might give a notion that 2R4R A has the close sequence similarity with the hH4R, while 2RH1 A has the close structural similarity.

THESIS 3 The binding site of the model was determined both computationally and visually The determined binding site 2 retained the key residues that were identified by the previous studies, thus making the model more reliable for further analysis.

The binding site of the model in this study was predominantly based on two major anchoring points at the hH4R binding site Asp94 (3.32) and Glu182 (5.46).

Consequently, ligand fit module in DS studio was employed to identify the binding site. DS LigandFit uses a method based on protein shape searching for cavities or holes. The method employs a cavity detection algorithm for detecting invaginations in the protein as candidate active site regions. It generated 11 active sites. Based on the visualization of the multichannel surfaces in our hH4R model, among the 11 binding sites predicted by the LigandFit, site 2 was found to possess most of the key residues. The H4 receptor model and its binding site is displayed in the Figure 2.

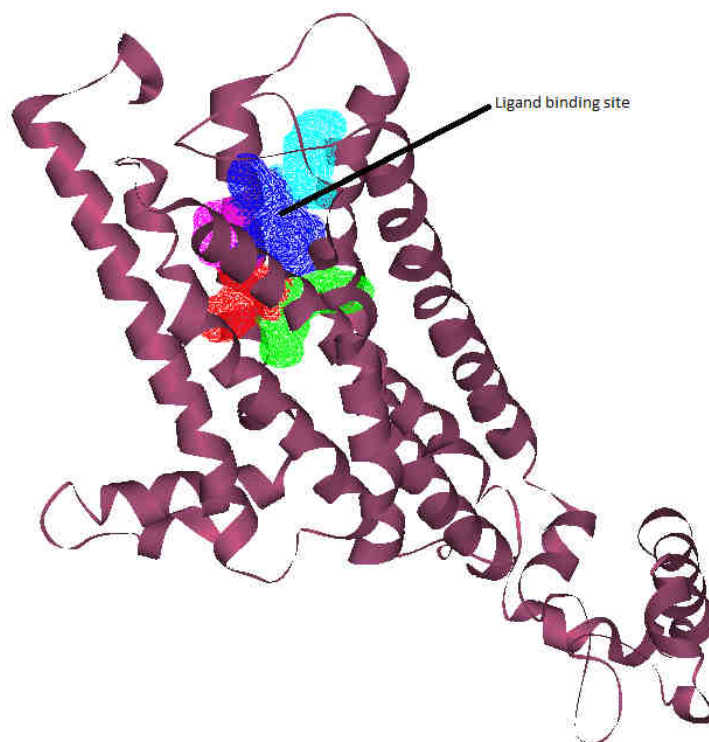


Figure 2: 3D model of H4R and its binding site

***THESIS 4* Three different database of chemical structures similar to JNJ777120, thioperamide and Vuf6002 retrieved from PubChem was built for virtual screening and The six compounds which are identified have not been previously reported by any other research groups.**

Previously JNJ777120 was only used predominantly as a precursor, thioperamide and Vuf are used first for this kind of studies. 150 similar structures of JNJ777120 were generated and were docked onto the binding site of the modelled receptor. Out of the 150 structures, 148 were successfully docked onto the binding site. PubChem retrieved 49 structures which are 90% similar to Thioperamide. All the 49 structures together form a database. The database is then docked onto the binding site of hH4R. Out of 49 structures, 42 successfully docked on the binding sites. In the third set of database containing 90% similar structures of Vuf 6002, 193 compounds in a total of 198 compounds were successfully docked. The compounds were ranked based on the dock score, however out of all these compounds, only six formed hydrogen bond interaction with the receptor. Model and they are considered the lead ligand hits in this study.

The top 6 compounds were identified which formed interaction with the receptor. These compounds are novel and were not previously identified.

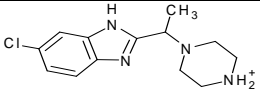
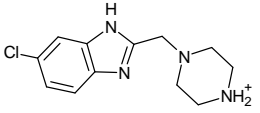
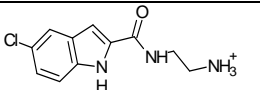
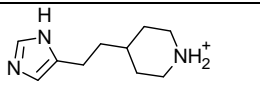
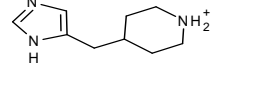
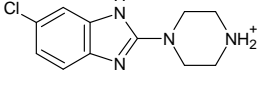
Compound name	Pubchem Id	Structure	Docking score	Hydrogen bonding Interactions		
				Donor	Acceptor	Distance in Å
Compound I	28468621		123.095	N3	Asp94: OD2	2.47
Compound J	28750273		119.56	N3	Asp94: OD2	2.25
Compound A	39732646		115.116	N5	Asp94: OD2	2.18
Compound E	44290805		115.046	N1	Asp94: OD2	2.35
Compound F	25271899		111.136	N1	Asp94: OD2	2.33
Compound K	28470894		105.162	N2	Asp94: OD2	2.29

Table: Top 6 compounds with high dock score and hydrogen bond interaction with ASP 94

Publications

Articles with Impact factor

1. **Fenila Jacob**, Claudina Perez Novo, Claus Bachert, Koen Van Crombruggen.: Purinergic signalling in inflammatory cells: P2 receptor expression, functional effects, and modulation of inflammatory responses. *Purinergic Signalling*. 2013, 9(3): 285-306. IF: 2.639
2. Van Crombruggen K, **Jacob F**, Zhang N, Bachert C.: Damage-associated molecular patterns and their receptors in upper airway pathologies. *Cellular and Molecular Life Sciences*. 2013, 70(22): 4307-21. IF: 5.62
3. **Fenila Christopher**, Elden Berla Thangam, Muthaiyan Xavier Suresh.: A Bioinformatics Search for Selective Histamine H4 Receptor Antagonists through Structure- Based Virtual Screening Strategies. *Chemical Biology and Drug Design*. 2012, 79: 74-759. IF: 2.469
4. L. Muthulakshmi, H. Nellaiah, T. Kathiresan, N. Rajini & **Fenila Christopher**.: Identification and Production of Biofloculants by Enterobacter sp. and Bacillus sp. and their Characterization Studies, Preparative Biochemistry and Biotechnology. 2017 (In press). IF: 0.67

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1. **Fenila Christopher**, Berla Thangam, S.Priya.: Targeting H4 receptor for the development of new antagonist. *4th International symposium on Recent Trends in Macromolecular Structure and Function conducted by Centre of Advanced Study in Crystallography and biophysics*, University of Madras, January 2010.
2. **Fenila Christopher**, Rajini Nagarajan, Winowlin Jappes, Gusztáv Fekete and Karthikeyan Subramanian.: Influence of carbon emission in pollen allergy. *International Conference on Thermal, Energy and Environment (INCOTEE2016)*, Kalasalingam University, Krishnacoil, Kalasalingam University, 2016.
3. **Fenila Christopher**, Gusztáv Fekete, Muthulakshmi L, Senthil Muthu Kumar Thiagamani and Indiradevi M P.: Classification and interaction of air pollutants, 2nd *International Conference on Thermal, Energy and Environment*, conducted by Department of Mechanical Engineering. Kalasalingam University, 2016.
4. **Fenila Christopher**, M. Xavier Suresh, Muthulakshmi L, Indiradevi M P, Gusztav Fekete, Immunological responses caused by air pollutants – A review, *ICAAM 2017*, Kalasalingam University, Krishnacoil, Kalasalingam University, 2017.