10.13147/SOE.2017.013

10.13147/SOE.2017.013

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Christopher Fenila

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TARGETING HISTAMINE H4 RECEPTORS IN ALLERGIES CAUSED BY AIR POLLUTANTS

HISZTAMIN H4 RECEPTOROK VIZSGÁLATA LÉGSZENNYEZŐ ANYAGOK ÁLTAL KIVÁLTOTT ALLERGIÁS ESETEKBEN

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című értekezés doktori (PhD) fokozat elnyerése érdekében készült a Soproni Egyetem Kitaibel Pál Környezettudományi Doktori Iskolája K1. Biokörnyezettudományi program keretében.

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A jelölt a doktori szigorlaton .....%-ot ért el.
Szombathely, ...

a Szigorlati Bizottság elnöke

Az értekezést bírálóként elfogadásra javaslom (igen / nem)
Első bíráló (Dr. .....) igen / nem

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(aláírás)

A jelölt az értekezés nyilvános vitáján .....%-ot ért el.
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A doktori (PhD) oklevél minősítése .....

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10.13147/SOE.2017.013

NYILATKOZAT

Alulírott Christopher Fenila jelen nyilatkozat aláírásával kijelentem, hogy a *Targeting histamine h4 receptors in allergies caused by air pollutants* című PhD értekezésem önálló munkám, az értekezés készítése során betartottam a szerzői jogról szóló 1999. évi LXXVI. törvény szabályait, valamint a Kitaibel Pál Környezettudományi Doktori Iskola által előírt, a doktori értekezés készítésére vonatkozó szabályokat, különösen a hivatkozások és idézések tekintetében.[1]

Kijelentem továbbá, hogy az értekezés készítése során az önálló kutatómunka kitétel tekintetében témavezetőimet, illetve a programvezetőt nem tévesztettem meg.

Jelen nyilatkozat aláírásával tudomásul veszem, hogy amennyiben bizonyítható, hogy az értekezést nem magam készítettem, vagy az értekezéssel kapcsolatban szerzői jogsértés ténye merül fel, a Soproni Egyetem megtagadja az értekezés befogadását.

Az értekezés befogadásának megtagadása nem érinti a szerzői jogsértés miatti egyéb (polgári jogi, szabálysértési jogi, büntetőjogi) jogkövetkezményeket.

Sopron, 2017…….

……..
doktorjelölt

[1] **1999. évi LXXVI. tv.** 34. § (1) A mű részletét – az átvevő mű jellege és célja által indokolt terjedelemben és az eredetihez híven – a forrás, valamint az ott megjelölt szerző megnevezésével bárki idézheti.

36. § (1) Nyilvánosan tartott előadások és más hasonló művek részletei, valamint politikai beszédek tájékoztatás céljára – a cél által indokolt terjedelemben – szabadon felhasználhatók. Ilyen felhasználás esetén a forrást – a szerző nevével együtt – fel kell tüntetni, hacsak ez lehetetlennek nem bizonyul.

10.13147/SOE.2017.013

It does not; therefore depend on man's desire or effort, but on God's mercy

Romans 9:16

10.13147/SOE.2017.013

10.13147/SOE.2017.013

Abstract

Inhalation of airborne pollutants can result in allergic sensitization. Responses may occur in the upper respiratory tract, the lower respiratory tract or systemically, for example, a febrile response. The mechanisms underlying these responses are not always clear but include production of reaginic antibody, activation of T-lymphocyte subsets, and release of inflammatory mediators. A variety of agents have been associated with elicitation of these reactions including chemical vapors, dusts and particulates, and microbial organisms. As a result of the widespread occurrence of allergens in both indoor and outdoor environments, development of allergy and its treatment management has received close attention. Six common pollutants listed in the EPA website have been used to check for currently available background literature showcasing the role of those pollutants in allergy. However, no studies have been performed to demonstrate the relation of Histamine receptor with the pollutants, except for mRNA expression studies. Evidence-based information about the major pollutants responsible for causing or exacerbating allergic diseases is lacking. Hence in this study we have prioritised the major pollutants and its involvement in allergy.

Histamine has long been known to be the mediator that orchestrates inflammatory and allergic responses acting through H1, H2, H3 and H4 receptors. Recent reports suggest the involvement of H4R in the control of immune cell trafficking and pro-inflammatory responses. This was derived from H4R-mediated histamine-induced activation of eosinophils, increased expression of adhesion molecules and rearrangement of the actin cytoskeleton leading to immune cell migration from bloodstream to sites of inflammation. Consequently, H4 receptors are currently an attractive target for the pharmacological modulation of histamine transferred signals in inflammatory conditions and for the development of therapeutic strategies for allergic conditions. The 3D structure of H4R has not been experimentally elucidated till date. Hence in this study, 3D structure of the Histamine H4 receptor was generated using iTASSER with human β2-adrenergic GPCR as a top template. This is the first Histamine H4 receptor structure model developed where human template is being used. From the five models generated by iTASSER only one model was chosen for further analysis after various validation tests.

Subsequently, the active site of the receptor model was identified using Discovery studio 2 and previous literature as evidence. Among the 11 binding sites predicted by the ligand fit module of Discovery studio, site 2 was found to possess most of the key residues that were identified in previous works. Three databases containing similar structures of the known ligand JNJ7777120, Thioperamide and

10.13147/SOE.2017.013

Vuf6002 were constructed from PubChem database. The molecular docking of the three separate databases onto the binding site of the modelled receptor revealed that 148 of 150 JNJ7777120 analogues, 42 of 49 Thioperamide analogues, 193 in a total of 198 Vuf 6002 analogues successfully docked onto the binding site. Out of these, six compounds with high docking scores were identified (Compound I, J, A, E, F, K). The ADMET properties as analyzed by the module in Discovery studio show that except 2 compounds (Compounds E, F) others have good ADMET properties. Similarly the molecular dynamic simulations which determine the conformational variations of the H4R-ligand complexes clearly show that the compounds are well stabilized at their respective active sites.

Structure based virtual screening has resulted in scaffolds from which new compounds could be developed. The identified structures have to be further analyzed and optimized for interesting chemical structures. The receptor model developed in this study also could serve as a basis for future investigations since it is modelled based on the human GPCR. To conclude, this study has given insights for the development of new antagonists.

10.13147/SOE.2017.013

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10.13147/SOE.2017.013

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10.13147/SOE.2017.013

Contents

10.13147/SOE.2017.013

10.13147/SOE.2017.013

List of figures

10.13147/SOE.2017.013

10.13147/SOE.2017.013

List of tables

10.13147/SOE.2017.013

Abbreviations

ADMET	Absorption Distribution Metabolism, Excretion and Toxcity
AIP4	Atrophin-1-interacting protein 4
APC	Antigen presenting cell
AR	Allergic rhinitis
Arah1	Arachis hypogaea
Betv1	Betula verrucosa
BLAST	BLAST for Basic Local Alignment Search Tool
BLOSSUM	BLOcks SUbstitution Matrix
cAMP	Cyclic adenosine monophosphate
CD4+	Cluster of differentiation 4
CHARMM	Chemistry at HARvard Molecular Mechanics
CO	Carbon monoxide
CO2	Carbon dioxide
COPD	Chronic obstructive pulmonary disease
CXCL12	Chemokine (C-X-C motif) ligand 12
CXCR4	Chemokine (C-X-C motif) receptor 4
DC	Dendritic cell
DNA	Deoxy nucleic acid
EPA	Environmental Protection Agency
FcεRI	Fc region of immunoglobulin E
FEV1	Forced expiratory volume in 1 second
GM-CSF	Granulocyte-macrophage colony-stimulating factor
GPCR	G-protein-coupled receptors
H1R	Histamine 1 receptor
H2R	Histamine 2 receptor
H3R	Histamine 3 receptor
H4R	Histamine 4 receptor
hH4R	human Histamine 4 receptor
HMMTOP	Hidden Markov Model for TOpology prediction
IgE	immunoglobulin E
IL	Interleukin
IP3	Inositol trisphosphate
I-TASSER	Iterative Threading ASSEmbly Refinement
JNK	c-Jun N-terminal kinases
LC-1	Liver-cytosol type 1
MAPKs	Mitogen-activated protein kinases

10.13147/SOE.2017.013

MC	Mast cells
MHC	Major Histocompatibility complex
MoDC	Monocyte derived dendritic cells
mRNA	messenger Ribonucleic acid
NCBI	National Center for Biotechnology Information
NMR	Nuclear Magnetic resonance
NO	Nitric oxide
O3	Ozone
PDB	Protein data bank
Phlp1	Pollen allergens
pKd	dissociation constant
PM10	Particulate matter
PSA	Polar surface area
SBVS	Structure based virtual screening
LVS	Ligand-based virtual screening
SO2	Sulphur dioxide
Th2cells	T helper cells
TM HMM	Trans membrane hidden Markov model
TNF-α	Tumour necrosis factor
TRAP	Traffic associated air pollution
VOC5	Volatile organic chemicals
WHO	World Health Organization

10.13147/SOE.2017.013

Chapter 1

Introduction

10.13147/SOE.2017.013

1.1 General Introduction

Environment can be defined as "the complex of physical, chemical, and biotic factors (such as climate, soil, and living things) that act upon an organism or an ecological community and ultimately determine its form and survival". It therefore includes everything that may directly affect the behaviour of a living organism or species, including light, air, soil, water and other living organisms. For human well-being, we interact with the environment for our benefits. As the population increased, humans started to exploit the environment intensely. This ultimately had led to detrimental effects on the environment which directly affects humans. Pollution is one of the major adverse outcomes in response to the exploitation of the environment. Environmental pollution is popularly grouped into air, water, light, land, noise and thermal pollution. Air is important for sustaining life. Approximately we breathe over 3,000 gallons of air each day. Therefore, clean air is vital for healthy living. Air pollution can damage trees, crops, other plants, lakes, and animals. In addition to damaging the natural environment, air pollution also damages buildings, monuments, and statues. Hence air pollution is a major threat to human health and his ecosystem.

Air pollution has long been recorded from the prehistoric period where soot deposition in the caves were identified by Spengler (Spengler *et al.*, 1983), but air pollution by itself has gained a major concern for health hazards only from the last century. World Health Organisation (WHO) defines air pollution as "contamination of the indoor or outdoor environment by any chemical, physical or biological agent that modifies the natural characteristics of the atmosphere. Household combustion devices, motor vehicles, industrial facilities and forest fires are common sources of air pollution. Pollutants of major public health concern include particulate matter, carbon monoxide, ozone, nitrogen dioxide and sulphur dioxide. Outdoor and indoor air pollution cause respiratory and other diseases, which can be fatal. WHO has estimated a total of 7 million deaths in 2012 as a consequence of air pollution (http://www.who.int/ mediacentre/news/releases/ 2014/air-pollution/en/). This accounts for 12.5 % of the total global human deaths. This data is twofold larger than the previous estimates and confirms that air pollution is now the world's largest single environmental health risk. Some unconfirmed reports say that in the last 20 years the mortality rate due to air pollution is far high compared to the mortality rate from the infectious diseases. The number of deaths caused by both indoor and outdoor pollution has been depicted in Figure 1. In 2010, WHO estimated that more than 6 million people die prematurely every year because of air pollution (Wong, 2013).

The Western Pacific region (WPr) and South East Asian regions (Sear) bear most of the environmental hazard burden with 2.8 and 2.3 million deaths,

10.13147/SOE.2017.013

respectively. Nearly 680,000 deaths occur in Africa, about 400,000 in the Eastern Mediterranean region, 287,000 in Europe and 131,000 in the Americas. Comparing to the low income countries (LMI), high income countries (HI) such as Europe (295,000), Americas (96,000), Western Pacific (68,000) and Eastern Mediterranean region (EMR) (14,000) have lesser number of deaths.

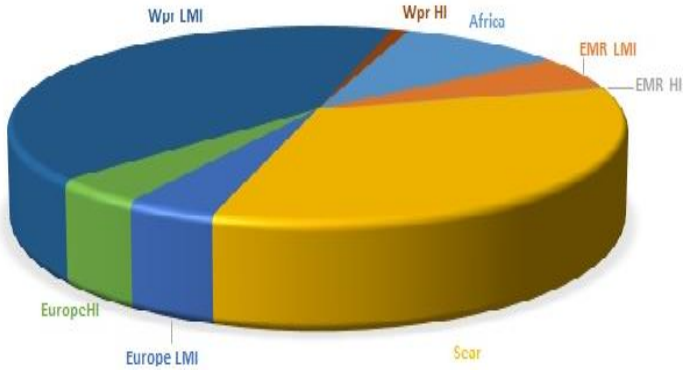

Figure 1 Number of deaths by indoor and outdoor pollution

Based on the chemical structure, air pollutants can be classified into primary and secondary pollutants as categorized in Table 1. Substances that are directly released from the source are primary pollutants while these primary pollutants react to form the precursor of the secondary pollutants (Stern *et al.*, 1973)

Table 1 Source of air pollutants

Source	Pollutants
Primary pollutants	Carbon dioxide, Carbon monoxide, Sulphur dioxide, Nitric oxide
Secondary pollutants	Dioxins

Based on their physical state, air pollutants are further classified as: 1) gaseous pollutants 2) particulate organic pollutants 3) heavy metals 4) particulate matter (Kampa *et al.*, 2008).

With the technological advances, we as a society have disturbed the environment which has ultimately affected the human health. Among the various consequences, allergy is the most common disorder influenced by air pollution. Allergic diseases reduce the quality of life and negatively impact the socio-economic welfare of the society. These air pollutants act allergens to cause several allergic disorders in humans. The prevalence of the allergic diseases has increased in the

10.13147/SOE.2017.013

past three decades with the increased exposure to allergens (Holgate, 2004). Since this increase in prevalence has occurred in this short duration, genetic changes cannot only be the major reason. External environment factors can be attributed as a major contributor to the increase in allergic diseases (Bartra *et al*., 2007).

Any foreign substance when contacted by human body can induce allergy, thereby referred here as allergen and this can be a product of environmental pollution. Though our immune system defends from the alien substance, some of them react differently leading to allergic reactions. When the allergen enters the human body through inhalation, the body produces a certain type of antibody called Immunoglobulin E (IgE). This IgE is very specific in nature which means each pollutant induces specific IgE. A person can be allergic to one pollutant but not necessarily to the other. 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. A wealth of evidence suggest that atmospheric concentrations of pollutants such as ozone (O3), nitric oxides (NO), respirable particulate (PM10) and volatile organic chemicals (VOC5), which result from increased use of liquid petroleum gas or kerosene, may be linked to the increased prevalence of allergic diseases which develop more frequently in urban areas of developed countries (Brauer *et al*., 2007; D'Amato *et al*., 2000). It is estimated that over 20 percent of world population suffers from IgE mediated allergic diseases i.e. allergic asthma, allergic rhinitis, allergic conjunctivitis, atopic eczema/atopic dermatitis and anaphylaxis. Though stringent pollution control measures may reduce the release of pollutants, in industrialised society it is inevitable to find a solution to existing establishment (automobiles, industries) which will continue emitting allergens to the environment. Hence treatment and prevention of the allergic disease has been a major concern. Though these allergic diseases already have established treatment pathways and medications, it gets more challenging with the addition of new type of pollutants to the existing spectrum. This has urged us to target the modus in developing a new drug lead against the allergens from the air pollution. Before going into the therapeutics a clear insight into the molecular mechanism of the development of the allergy is necessary.

Allergies occur when our immune system becomes hypersensitive to particular substances. Various cell molecules and mechanisms are involved in this process of mediating allergy in our body. The IgE produced during the allergic reaction is primarily produced by the plasma cells. This IgE binds to its receptor on mast cells which leads to the production of histamine. Histamine is considered the first allergic mediator implicated in the process of allergy because the levels of histamine is elevated in plasma and tissue when an allergen encounters the human body. The pleiotropic effects of histamine are mediated by different histamine membrane receptors. So far four different sub types of G protein-coupled histamine

10.13147/SOE.2017.013

receptor, designated as H1, H2, H3 and H4 have been identified and are found to be expressed on various immune cells (Hill *et al*., 1997; Hough, 2001).

The H4 receptor is more widely distributed, especially in organs associated with the immune system. It is preferentially expressed in intestinal tissue, spleen, thymus, medullary cells, bone marrow and peripheral hematopoietic cells, including eosinophils, basophils, mast cells, T lymphocytes, leukocytes and dendritic cells. These cell types are primarily involved with the development and continuation of allergic responses. Recent evidences of the *in vivo* and *in vitro* studies using animal models and human biological samples elucidate the role of H4 receptor in histamine-induced chemotaxis of mast cells, eosinophils and other immune cells which are hallmark characters of allergic diseases (Hanuskova *et al*., 2013). Thus these biological functions and the expression pattern indicate a crucial role of H4R in allergy caused by the pollutants. These Histamine H4 receptors have become an attractive target for anti-allergic therapy.

Conventionally, the prevention and management of allergic disorders is fundamental to avoid allergen exposure. Apart from this, several pharmacotherapies like anti-histamines, cortisone, dexamethasone, hydrocortisone, theophylline, cromolyn sodium etc. are prescribed to block the action of allergic mediators. With the increased prospects of H4R, antagonism of histamine's action at H4R has been the key for an immense market for pharmacological treatment. The pathophysiological significance of H4R in inflammatory conditions, such as asthma and allergic disorders, as well as its contribution in acute and chronic inflammation was initially suggested by using the dual H3R/H4R antagonist thioperamide (Hofstra *et al*., 2003) and subsequently by the use of the selective H4R antagonist JNJ-7777120 (Thurmond *et al*., 2004). With the success of the H4R antagonist, more researches are focused on the antagonism of the receptor to identify a competitive lead for the development of drug, thereby providing a remedy to the allergic responses caused by the environmental pollution.

In our attempt to identify a potential antagonist we followed structure based virtual screening. This structure-based method confide on the 3D structure of the target receptor or protein which can be obtained either experimentally or by homology modelling to develop a new drug. The identification of new ligands for a given receptor is necessary. A ligand is a substance or a chemical that binds to the receptor and invokes biological response. Ligand can either be agonist, antagonist or inverse agonist. An agonist binds to a receptor and activates the receptor to produce a biological response. Whereas an agonist causes an action, an antagonist blocks the action of the agonist and an inverse agonist causes an action opposite to that of the agonist. In this study, we aimed to identify a potential antagonist that can block the function of H4R by searching from large databases of 3D structures of small molecules. The structures are tried to fit into the binding pocket of the

10.13147/SOE.2017.013

receptor using fast approximate docking programs. The promising ligand best fits onto the binding site. This method is known as virtual screening. The physical properties of these compounds can be tested using in silico assays. These *in silico* hits or lead structures can serve as a suitable starting point for the development of novel, potent and selective antagonist. Thus the identification of these compounds will open new avenues for the development of a new drug for allergy.

10.13147/SOE.2017.013

1.2 Objectives of the research

The main objectives of this research are to develop a potential lead candidate drug for allergy caused by the air pollutants. As mentioned above a structure based drug designing was followed to achieve this. Some of the main objectives of this research are

1. Though it is known that pollution is an important factor in contributing allergy, there is not much evidence based information on it. There are many pollutants which are hazardous to health but we aim to discuss and identify the environmental pollutants that trigger allergic response. Secondly, it is also intended to determine the involvement of H4R in eliciting this immune response. In order to counteract the effect of histamine released with the intake of allergen, an antagonist against the immune response elicited has to be developed. To serve this purpose, a 3D structure model of the receptor H4, in which the histamine binds, is necessary. 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.

2. Next a database with all the known small structure has to be built which 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 to filter the structures from PubChem and construct 3 separate databases.

3. Our aim is virtual screening the best lead structures from the 3 sets of databases. The dock score and hydrogen bond formation are the parameters used to identify the best hit or lead. Once the lead structure has been identified to be potential to bind the target molecule, they are made to go through few in silico assays to determine its efficacy.

10.13147/SOE.2017.013

Chapter 2

Review of Literature

10.13147/SOE.2017.013

2.1 Definition of Environment

The word "Environment" is originated from French meaning "encircle", therefore in simple terms it means one's own encircling surrounding. Environment can be defined in many ways, some of the commonly used definitions are

1. It is in totality of all social, physical, biological and chemical individually as well as collectively that composes man made surroundings.

2. It refers to sum total conditions which surround man at a given point of space and time.

3. It is the representativeness of the physical component of the earth wherein man is the important factor influencing his environment.

4. Environment is the holistic view of the world as its functions at any time with multitude of special elemental and socio-economic distinguished by quality and attributes of space and mode of behaviour of biotic and abiotic forms (Prof. R. K. Agarwal, 2010).

5. The non-genetic conditions and circumstances that affect person's conduct and health (Sharma *et al.*, 2005).

Thus environment is a very broad concept and it involves everything that affects an organism in its lifetime and includes both abiotic (non-living) and biotic (living) substances. Some components of the environment serve as a resource such as soil, water etc., while others act as a regulatory factor such as temperature, light etc. Different components of the environment are interdependent and interlinked. In general, the environment can be described as the physical surrounding and conditions affecting the lives of people and animals (Chauhan, 2008). Human health, well-being and survival ultimately depend on the health and integrity of the whole environment (Nadakavukaren, 2011). The environment accounts for almost 20% of all deaths in the WHO European Region.

From the beginning humans have been dependent on the world for air, water, food and solar energy which drives the whole complex system of living organisms. This relationship between living things to their environment is called Ecology. In the book "A perspective of environmental pollution" the author terms this study as the study of the first world of man. According to him, the second world of man is his own handicraft with the use of technology. With the start from forest clearance for expansion of pasture and extensive cultivation, human have extensively changed the productivity of the world. As a by-product of the increase in the technology, the waste materials disposed into the environment is substantial increased. This waste can be harmful to the ecological system and humans and are called pollution (Holdgate, 1979).

10.13147/SOE.2017.013

The word pollution is derived from a Latin word "polluere" which means "to soil" or "to defile". It has threatened the well-being of human and other living organisms. Just as weed is a plant out of place, pollutant is a chemical out of place. Oil enclosed in a tanker is not a pollutant but if it is spilled it is a pollutant (Hill, 2010; Hill *et al.*, 1997). Pollution could also be defined as when a substance occurs in a location or organism at higher levels than normal (Rieuwerts, 2015). Pollution could be an end result of any forms of human activity. It can vary from a simple form of human smoking to a breakdown of a huge nuclear power plant. Environmental pollution is commonly classified into air, water, light, land, noise and nuclear pollution. All these pollution types ultimately affect the major life supporting system which includes air, water and soil. Some of the pollution is localized such as cigarette smoking or smoke from a wood fire, but air and water pollution can be transboundary (Rana, 2011). Willerroider (Willerroider, 2003) reported that polar bears which travel long distance for food tend to accumulate high levels of industrial pollutants such as polychlorinated bi phenyls in their body. Therefore major concern has been the air and water pollution. In this study our focus will be on air pollution.

Though air pollution has become a hot topic in the recent times, its prevalence was documented in the 2nd century. In 79 AD Pluinus described the death of his uncle due to volcanic fumes. Beginning from that, various air pollutants have been reported, differing in their chemical composition, reaction properties, emission and persistence in the environment. Air pollution can cause concern in two ways 1) by affecting individual health 2) by disturbing the environmental set up.

2.2 Air pollution affecting Environment

In this section, we discuss on the global effects of the air pollution. Air pollution has been a major player in altering the environmental patterns. The natural greenhouse gases such as CO2 and methane help to prevent the heat entering into the earth atmosphere, thereby providing a good environment for the living beings. However, the increased production of CO2 from the industries has started to slowly reverse the protection provided by these greenhouse gases. These gases have started to increase the temperature of the earth atmosphere thereby leading to global warming (Green, 2011). It has been reported that the global surface temperature has increased by 0.5 °C since 1975 (Hansen *et al.*, 1999; Jones *et al.*, 1999). The global warming of 0.1 - 0.2 °C every decade has resulted in increasing loss of snow cover and Arctic sea ice, frequent occurrence of heavy precipitation, rising sea level, and shifts in the natural ranges of plants and animals. It is estimated that the global average temperature is already approximately 0.8 °C above its preindustrial level, and present atmospheric levels of greenhouse gases will contribute to further warming of 0.5 - 1 °C (MC, 2008). Additionally, the increase in CO2 content also affects the pollination of plants. CO2 is an important component for the

10.13147/SOE.2017.013

photosynthesis; thus increased CO2 exposure in turn increases the pollen production and biomass (Ziska *et al*., 2008; Ziska *et al*., 2011). The increase in pollen indirectly affects the population with allergy and asthma. In Ambrosia, high CO2 increased pollen production by 32% and 55% (Rogers *et al*., 2006; Wayne *et al*., 2002).

Another adverse effect of air pollution is acidification of lakes and rivers which affects the soil and vegetation of the land. Acidification is mainly due to the emission of pollutants such as sulphur di oxide and nitrous oxide. These compounds mix with the water vapour and travel thousands of kilometres and result in acid rain (Kaitala *et al*., 1992; Green, 2011). The acid rain can damage trees, crops, wildlife, lakes and other bodies of water. Those pollutants can also harm fish and other aquatic life.

2.3 Air Pollution and health

Air pollution gained global concern when intense smog with 2000μg of SO2 surrounded London and killed 4000 people in 1952 (Bartra *et al*., 2007). It was reported by Ministry of Health that the people suffering from cardiorespiratory diseases were amenable to the smog. Thus it was concluded that this extravasation of the cardiorespiratory diseases is particularly due to the air pollution. Pollution caused by traffic is a major risk for respiratory diseases. The gaseous and particulate emissions caused by automobiles remains the major cause for the urban pollution. It has been reported that for every 100 km, a car emits a mean value of about 1 kg of pollutants into the atmosphere. Traffic associated air pollution (TRAP) leads to increased incidence of childhood asthma and allergy. Studies by Bowatte *et al* in birth cohort studies have highlighted that increased longitudinal childhood exposure to PM 2.5 and black carbon was associated with increased risk of subsequent asthma in childhood. This early childhood exposure to TRAP was associated with development of asthma across childhood up to 12 years of age (Bowatte *et al*., 2015).

Population-based, cross-sectional studies of metropolitan areas in the United States have also found associations between particulate air pollution and annual mortality rates. In India and China, the increase in PM10 of 10 $\mu g/m^3$ is associated with an increase in mortality of 0.6% in daily all natural cause mortality (Committee. 2010). Air pollution was positively associated with death from lung cancer and cardiopulmonary diseases but not with death from other causes considered together (Dockery *et al*., 1993). Epidemiological studies have revealed that increased air pollution has been a reason for lower lung function and increased susceptibility to Chronic obstructive pulmonary disease (COPD) (Sunyer, 2001). Thus it has been a common thought that air pollution increases the risk of respiratory and other diseases. On the contrary, Bouhys *et al* in 1978 have alleged

10.13147/SOE.2017.013

that higher air pollution has no considerable effects on chronic lung diseases (Bouhuys *et al*., 1978). Pollutants also affect the function of lungs, increase in total lung resistance by an average of 67% and decrease in FEV1 (Forced expiratory volume) by 23 % was evident with inhalation of 1.0 ppm of SO2 for 10 minutes (Koenig *et al*., 1982; Koenig *et al*., 1983). It was later found that exposure of SO2 for 2.5min can lead to bronchoconstriction in asthmatic patients. This action of SO2 can be reverted with the use of albuterol, cromolyn, theophylline, and corticosteroids (Koenig *et al*., 1991).

A study based on daily counts of hospital admissions for 1999-2002 obtained from billing claims of Medicare enrollees clearly showed that the risk of cardiovascular and respiratory hospital admission with the short term exposure of air pollutants (Dominici *et al*., 2006). Air pollution has been thought to be a major reason for aggravation of asthma leading to increased hospital admission and drugs. In controlled experiments, asthmatic patients are found to be more sensitive to SO2. Nielsen and colleagues recently reported an 18% increase in lung cancer incidence for each 5 $\mu g/m^3$ increase in PM2·5 concentration in a longitudinal cohort study (Raaschou-Nielsen *et al*., 2013).

Another health hazard from increased air pollution is allergy. The incidence of allergy and bronchial asthma is elevated in the last decade in the industrial countries. It has been reported that the prevalence of Allergic rhinitis is high in the developed countries affecting 40 % population worldwide (Bousquet *et al*., 1990; Long *et al*., 2002), 23%-30% in Europe (Bachert *et al*., 2006; Bauchau *et al*., 2004) and 12%- 30% in US (Nathan *et al*., 2008). In Hungary 52.5% of patients suffered from seasonal AR and 35.1% from perennial AR (Szilasi *et al*., 2012). In a cross sectional study on Taiwanese children by Hwang *et al*, it was concluded that exposure to pollutants such as SO2, NO2 and Nox increase the risk of allergic rhinitis (Hwang *et al*., 2006). In a longitudinal study performed by Martimoer *et al,* it was predicted that in children who have asthma or had prenatal and early exposure to air pollutants like CO2, SO2, CO, O3 had high sensitization to allergens (Mortimer *et al*., 2008).

Airborne allergens such as ragweed pollen, mold spores, cat dander and dust mites affect the respiratory system, producing classic hay fever-type symptoms of sneezing, runny nose and congestion. Patients with untreated allergies can then often experience sinusitis. If untreated, bacterial or fungal sinus and ear infection may arise in people with immune system weakened by frequent allergic reaction. Untreated allergies make asthma patients sick more often and subject to severe, life-threatening asthma attacks. Hence proper diagnosis and treatment of allergies is important.

10.13147/SOE.2017.013

2.4 Interaction of the pollutant with the immune system

Our immune system encounters the environment in an outstanding manner; however the environmental pollutants interfere with the immune response thereby leading to immunological disorders. Bahadar *et al* in his review have briefed that environmental toxicants can affect the immune system ranging from organ to cellular components. They have also detailed the changes in the immune response to the pollutants based on the composition, structure and function. These changes are illustrated in Figure 2 (Bahadar *et al*., 2015).

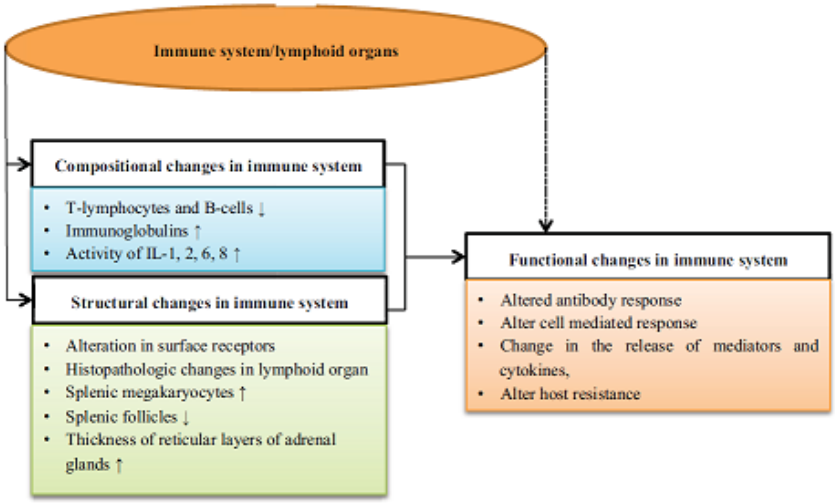

Figure 2 Changes in the immune system in response to pollutants (Bahadar *et al*., 2015)

Immune responses may occur in the upper respiratory tract (rhinitis), the lower respiratory tract (wheeze, bronchospasm) or systemically, for example, a febrile response. The underlying mechanism is still ambiguous. A variety of pollutants have been associated with elicitation of these reactions. As a result of the widespread occurrence of allergy caused by environmental pollution, mechanism in the development of allergy has received close attention. With regard to the above Figure 2, increase in the production of the Immunoglobulin which changes the release of the mediators is the clinical feature of Allergy. In immunological aspect, any particles that elicit the production of IgE antibody are termed allergen which in this study relates to the pollutant. The clinical manifestation of IgE dependent immunological reaction is Allergy whereas Atopy is the genetic tendency to generate IgE response (Holgate *et al*., 2011). The allergic reaction in the nose involves a complex interaction between allergen and multiple effector cells. Allergic reactions consists of two phases 1) Acute phase that typically subsides within 60 minutes 2) Late phase which is developed in 3 to 12 hours by the development of an intense

10.13147/SOE.2017.013

inflammatory reaction (Lemanske, RobertF. *et al.*, 1988). Acute phase is mast cell mediated whereas the late phase is believed to be dependent on the local accumulation and activation of leukocytes, including neutrophils, eosinophils, and basophils (Salvi, Sundeep and Blomberg, Anders and Rudell, Bertil And Kelly, Frank And Sandstram, Thomas And Holgate, Stephen,t. And Frew, 1999; Gleich, 1982; Dolovich *et al.*, 1973). The delayed or late response can be attributed to the chronic allergic diseases (Gleich, 1982).

The steps involved in the process of both acute and late phase allergy caused by pollutants are discussed in the following sections. The overall process of allergy is depicted in Figure 4.

2.4.1 Acute phase allergy

Acute phase is the immediate reaction, taking effect within minutes of allergen (pollutant) provocation, results in the release of mediators that lead to symptoms characteristic of the target organ (Adelman *et al.*, 2012). Following sequence of events happens in the process.

2.4.1.1 Presentation of antigen/pollutant to the immune system

Pollutants can make entry into the respiratory tract as volatile gas (ozone, benzene), liquid droplets (sulphuric acid, nitrogen dioxide), or particulate matter (diesel exhaust, aromatic hydrocarbons). Initial entry of the pollutant into the respiratory system is mediated by coupling of the pollutant with protein or conjugates (Albright *et al.*, 1996). Other allergens such as pollen, dust mites and animal dander are protein in nature with molecular weight of 10 to 20 kDa (Adelman *et al.*, 2012). For example, the pollen particles contain pollenic allergens, high environmental humidity conditions can result in osmotic shock of this pollen particle. This leads to the release of microparticles or paucimicronic particles that contain allergenic proteins. Allergens are mostly lipid binding proteins (e.g. Bet v 1 and homologues, house dust mite group 2 allergens, lipocalins of pets, plant lipid transfer proteins), and some are glycoproteins (e.g. peanut Ara h 1 and grass pollen Phl p 1). After the entry of the allergen, it leads to the reduced epithelium and facilitate the contact of the inhalatory allergens with the network of antigen presenting cells (APC). Cells that act as APCs in the airway include mucosal macrophages, dendritic cells, pulmonary alveolar macrophages, and B cells themselves. These lipid ligands and conjugated glycans have been shown to interact with pathogen recognition receptors such as Toll-like receptors (TLR) and C-type lectins (CTL) on antigen-presenting cells. All of them take up the antigen by the process of endocytosis. Then the antigen is degraded or processed and the linear peptides are presented to the T cells (Figure 3).

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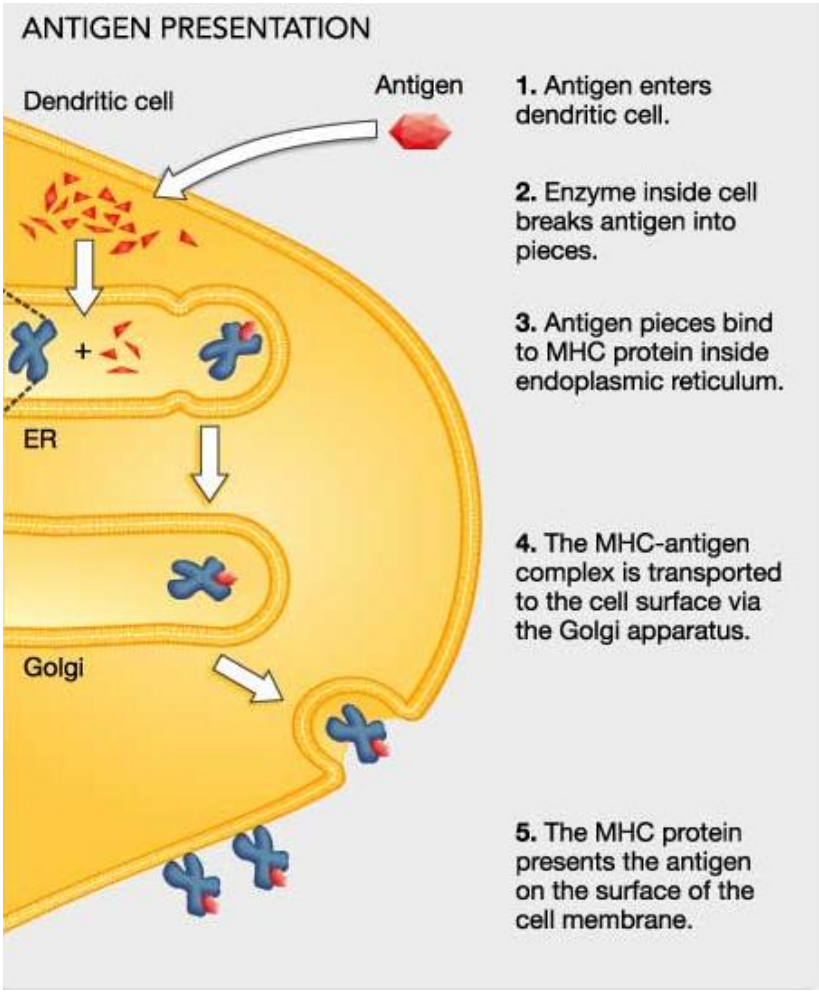

Figure 3 Processing of the antigen (http://www.uic.edu/)

2.4.1.2 Activation of T cells

The processed antigen is co-presented along with Major histocompatibility complex (MHC) class 2 molecules to CD4+T cells (Th2 lymphocytes or Th2 cells), which are the major regulators (Young, 1998). The APC exert their effects through production of interleukins such as IL-4, IL-5, and IL-13. IL-4 that is critical to the development of Th2 cells (Woodfolk, 2007). Next step is the T cell activation for the induction of Th2 cell response which is an important step in the process of allergy (Cezmi A, 2007). There are two known mechanisms for the activation of the Th2 response.

10.13147/SOE.2017.013

1. Dendritic cells (DC) are APC which present the antigen but cannot themselves activate the T cells. It requires additional factors like ligands for TLR to migrate and then activate T cells by increased production of CD40, CD80 and CD86.

2. In addition, there is accumulating evidence that mast cells also modulate DC into Th2 immune response. Caron *et al* have suggested a mechanism for the maintenance of Th2 based responses in allergic disorders. From their study they concluded that histamine released by mast cells of allergic subjects upon contact with the sensitizing allergen, polarizes maturing DC into DC2 through both H1 and H2 receptors. By this polarization of DC2, histamine favours the induction of Th2-biased responses and sensitization to diverse encountered allergens, as observed in atopics (Caron *et al*., 2001).

   Once the T cells are activated by the above mechanism, clonal expansions of the cells occur and begin cognate interaction with the B cells.

2.4.1.2 IgE production

IgE antibodies play a key role in instigating immediate hypersensitivity reactions and contribute to the pathophysiology of a wide range of allergic diseases. As mentioned before, activation of T cells lead to the production of interleukins which in-turn stimulate B cells to produce Ig E. IgE production is carried out in B cells. B cells are developed in the bone marrow and leave as mature B cells, expressing IgM and IgD on their surfaces. These IgM or IgD B cell receptors recognize the antigen specifically and thereby activate the B cell. However, if the mature B cell binds to the processed antigen presented on a T helper (Th) cell, the B cell gets activated which is followed by Ig class switch recombination (Luger *et al*., 2010). The B cells entail class-switch recombination at the immunoglobulin heavy chain locus into the IgE heavy chain (Cε). Furthermore, CD4+ Th2 cells that produces IL-4 also orchestrate this class switching of IgE mediated in B cells.

2.4.1.3 Sensitization and degranulation of mast cells

IgE sensitizes mast cells by binding to their high-affinity Fc receptor (FcεRI) on tissue mast cells or blood basophils. An allergic reaction is initiated when an antigen crosslinks immunoglobulin E (IgE) antibody bound to the Fc receptor (Sutton *et al*., 1993). Subsequently, mast cells degranulate, releasing vaso active amines (mainly histamine), lipid mediators (prostaglandins and cysteinyl leukotrienes), chemokines and other cytokines (Larche *et al*., 2006). TNF- α, GM-CSF, macrophage inflammatory protein-1α, and a number of "T helper (Th) 2-cell type" cytokines, such as interleukins IL-3, IL-4, IL-5, IL-6, IL-9, IL-10, and IL-1 are also released upon mast cell activation (Gould *et al*., 2003). The activities of allergen-activated mast cells are said to "orchestrate the allergic response".

10.13147/SOE.2017.013

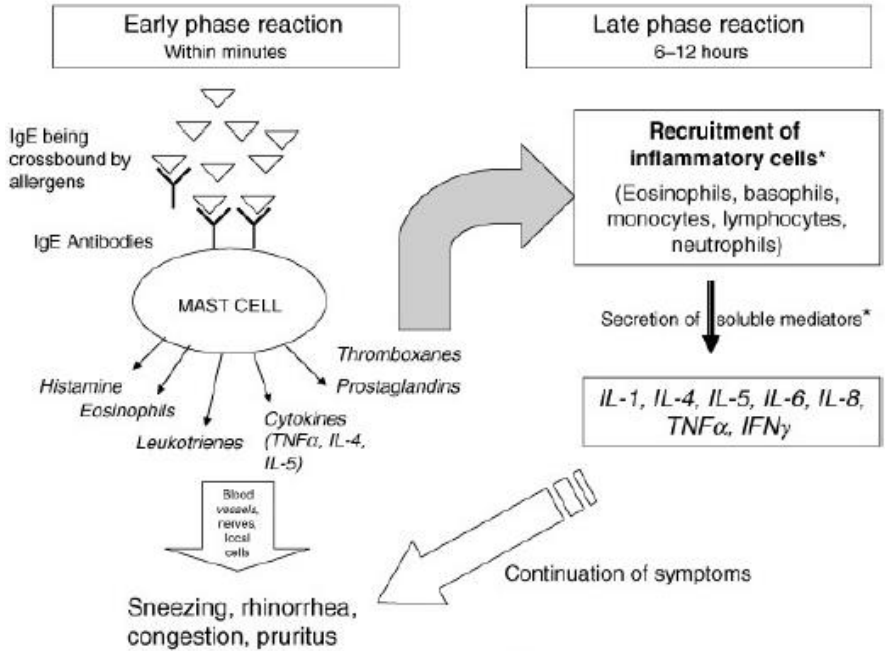

Figure 4 Allergy mechanisms (Derendorf *et al.*, 2008)

2.4.2 Late phase of allergic reaction:

Late phase reactions are sequel to the early phase reactions because in some individuals this initial response evolves slowly into extensive inflammation (Lemanske, Robert. *et al.*, 1988). It lasts longer than the initial phase reaction. It usually peaks between 4th and 8th hour after allergen challenge and subsides after 12h to 24 h. IgE binds to the FcεRI at the surface of dendritic cells (DCs) and monocytes, as well as to the low-affinity receptor for IgE, FcεRII (also known as CD23) present at the surface of B cells. This process increases the uptake of allergen by these antigen presenting cells (APCs) and the subsequent presentation of allergen-derived peptides to specific CD4+ T cells, which drive the late phase of the allergic reaction. A late-phase response associated with the influx of T cells, monocytes, and eosinophils may ensue some hours later (Gould *et al.*, 2003). Mast cell derived mediators and cytokines are responsible for cellular recruitment and development of the late-phase response. The late phase reaction was first noticed by Blakley *et al* around 100 years ago where he found the association of allergen inhalation and asthma after several hours later.

10.13147/SOE.2017.013

2.5 Role of Histamine receptors in allergic diseases

Histamine was initially thought to be obtained from non-human source. Only in the late 1920s it was found to be a natural constituent of human. Riley and West initially determined those mast cells are the storage cabin of histamine. However in their later studies they proved that basophils, platelets and other cells can also accommodate histamine (Riley, 1953). In mammals the histamine amounts from 1 to 100 g-1 in tissues. Early researchers found that it had a stimulant effect on smooth muscle of the gut and respiratory tract, caused vasodepression, stimulated cardiac contractility and induced a shock-like syndrome when introduced into animals. These and other effects are mediated by histamine specific receptors expressed on the respective target cells. Until now four histamine receptors have been characterized and cloned in humans and mice, referred to as histamine H1R, H2R, H3R, and H4R, (Hill, 1990; Liu *et al*., 2001; Nguyen *et al*., 2001; Oda *et al*., 2000). These receptor subtypes display homologies of about 20% within a species and of about 70–95% of a given receptor subtype between humans and mice (Liu *et al*., 2001; Thurmond *et al*., 2008; Hill, 1990). All of the histamine receptors belong to the family of G protein coupled receptors (GPCR). GPCR are heptahelical transmembrane molecules that transduce the extracellular signal by using G-proteins and intracellular second messenger systems. The molecular mechanism of GPCR is depicted in the Figure 5. Binding of an agonist (activating ligand) induces a conformational change in the G protein-coupled receptor (GPCR) to activate it. Activated receptors couple to heterotrimeric G proteins composed of Gα, Gβ and Gγ subunits. Subsequently, the heterotrimeric G proteins dissociate and G protein signaling mediates the generation of second messengers such as cyclic AMP, inositol triphosphate (IP3) and Ca2+. Activated receptors are phosphorylated, primarily in the carboxyl terminus, by GPCR kinases. Phosphorylated receptors recruit β-arrestins, which are multifunctional adaptor proteins that block further G protein–GPCR coupling, potentially through a steric hindrance mechanism (referred to as desensitization). β-arrestins also mediate clathrin-dependent endocytosis of activated GPCRs as well as independent signalling pathways downstream of GPCRs. β-arrestins scaffold mitogen-activated protein kinases (MAPKs; such as extracellular signal-regulated kinase (ERK) and c-Jun N-terminal kinase (JNK)), tyrosine kinases and E3 ubiquitin ligases (such as atrophin-1-interacting protein 4 (AIP4)). The arrows next to cAMP indicate that cAMP levels can go up or down in response to GPCR activation.

10.13147/SOE.2017.013

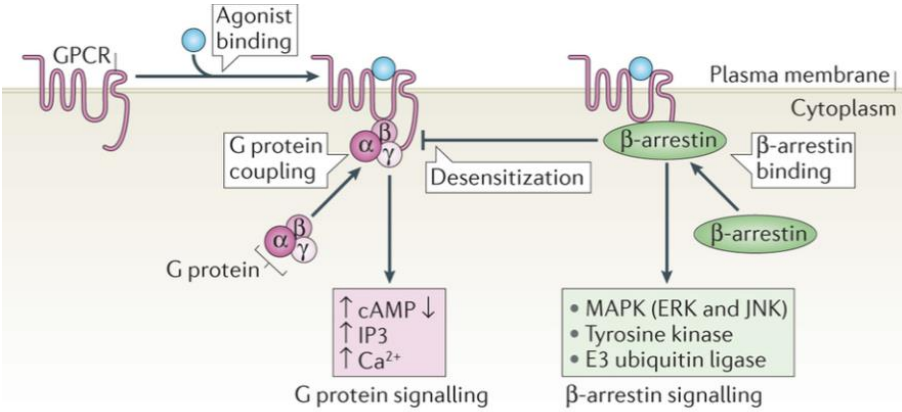

Figure 5 Signalling mechanism of GPCR (Ghosh *et al.*, 2015)

The four Histamine receptor subtypes are distinct in terms of their pharmacology and molecular biology and have been implicated in diverse biological effects. The affinity of histamine binding to different Histamine receptors varies significantly, with Ki values ranging from 5-10 nM for the H3 and H4 receptors to 2-10 mM for the H1 and H2 receptors (Thurmond *et al.*, 2004; Endo, 1982). This difference in affinities of receptors determines the biological effects of histamine upon activation.

H1 receptors are expressed on multiple cell types including endothelial cells and smooth muscle cells, where they mediate vasodilation and bronchoconstriction. Because of its varied role, antihistamine specific to this receptor were developed in 1930s and widely used. Many effects of histamine were not blocked by these antihistamine and this made Ash and Shild propose that a second type of histamine receptor might exist in heart and stomach tissues (Ash *et al.*, 1966). This was later experimentally identified by Black *et al* and was named H2 receptor (Black *et al.*, 1972). In 1980s Schwartz *et al* identified a third class of histamine receptors (H3 receptor) which mainly influences the histamine synthesis and release in CNS neurons.

The discovery of this fourth histamine receptor, and the evidence that it is expressed on many cell types involved in allergic responses, suggested that the H4R play an important role in mediating the histamine effects in asthma and allergic diseases. Hence in this study H4R is focused, thus a detailed description of its role and mechanism in contributing the allergic process is given.

2.5.1 Histamine H4 receptor (H4R)

The H4 receptor which is the latest discovered was not identified using the traditional pharmacological means. It was cloned by several groups independently in 2000 and 2001 (Liu *et al.*, 2001; Oda *et al.*, 2000; Nguyen *et al.*, 2001). The H4R

10.13147/SOE.2017.013

protein shows high level of homology towards H3R. Both H3R and H4R have splice variants and also exhibit species to species specificity. For example, histamine display 25 times higher activity in human H4R than in rat H4R (Timmerman *et al.*, 2009). H4R displayed 40% structural homology and 58% transmembrane homology with the H3R.

2.5.1.1 Expression and activation of H4R

Histamine H4 receptor is a pertussis-toxin-sensitive GPCR predominantly expressed on cells of the immune system, including MCs, monocytes, eosinophils, dendritic cells (DCs), T cells and natural killer cells; in peripheral tissues such as spleen, thymus, colon, blood leukocytes and bone marrow, its expression being induced or altered in response to inflammatory stimuli.

APC

APC which includes monocytes and DC are primary immune cells which aid in the uptake and presentation of the antigen into the system and initiate allergic inflammation. The expression of H4R protein was confirmed in human monocyte derived dendritic cell (MoDC). Furthermore they also showed that H4R mediated histamine induced chemotaxis and calcium mobilization in MoDC (Damaj *et al.*, 2007; Gutzmer *et al.*, 2005), which directly suggest the chemotactic and immunomodulatory effect of Histamine via H4R. IL-12p70 is an interleukin produced by APCs which is important for the elucidation of a Th1-type immune response, whereas the absence of IL-12p70 indicates Th2-type immune responses. This indicates the role of H4R in allergic diseases which are characterized by Th2 mediated responses. Gutzmer *et al* have elucidated that the suppression of IL-12p70 is mediated by H4R. The downstream signal transduction of IL-12p70 follows MAPK and cAMP pathway where cAMP signalling is thought to be induced by H2R. Experiments show that, preincubation of MoDC with U0126, an inhibitor of MEK 1/2 that blocks the phosphorylation of ERK1/2, rescued IL-12p70 suppression via the H4R-mediated signalling but not via the H2R-mediated response (Gutzmer *et al.*, 2005). Taken together we can conclude that H4R plays a vital role in promoting Th2 immune responses, chemotaxis and intermodulation effect thereby mediating allergy.

T cells

In CD4 (+) T cells, H4R expression is present both in mRNA and protein levels. This upregulation is favoured in Th2 environment than in Th1 or naive T cells. Treatment of the cells with H4R specific agonist exhibited the mRNA induction of AP1 and IL-33 (Gutzmer *et al.*, 2009).

10.13147/SOE.2017.013

Mast cells and basophils

Mast cells are important effector cells in allergic diseases. Mast cells bind IgE with IgE receptor, and subsequent contact with antigens triggers IgE receptor cross-linking and the release of preformed mediators, such as serotonin and histamine, and de novo produced mediators, such as prostaglandins and leukotrienes. The release of mediators dictates the signs and symptoms of allergic diseases (Galli *et al.*, 2012). In mouse cells, H4R was found to mediate mast cell migration in response to histamine. This effect can be attributed to the accumulation of mast cell in allergic tissue (Hofstra *et al.*, 2003). In addition, histamine H4 receptor has been reported to mediate mast cell migration toward CXCL12, a constitutive chemokine (ligand of CXCR4 and CXCR7) that is expressed in the skin and airway epithelium and plays a significant role in allergic airway diseases (Godot *et al.*, 2007). Human mast skin cells and tissue mast cells were found to express H4R in 2004 (Lippert *et al.*, 2003). However only recently Jemima *et al* in their study have characterized the functional expression of h4R in human mast cells that leads to the stimulation of Th2 cytokines(IL-5, IL-4 and IL-13) (Jemima *et al.*, 2014).

Basophils, unlike mast cells which reside in the tissue, circulate in the blood and migrate to sites of inflammation. Human basophil expressed H4R (Hofstra *et al.*, 2003). In humans, basophils are the prominent sources of the biologically active Th2-type cytokines IL-4 and IL-13, which cause IgE class switching in B cells (Yanagihara *et al.*, 1997). Recently the interplay between mast cell, basophil and H4R have been elucidated which stresses the importance of the receptor in allergic diseases (Shiraishi *et al.*, 2012).

Eosinophils

Eosinophils are bone marrow-derived granulocytic leukocytes, which reside in tissues, especially in the respiratory and intestinal systems and in the uterus. Eosinophil numbers in the blood stream are relatively low, and the control of eosinophil migration towards the tissues has been attributed to adhesion molecules and chemokines (Tachimoto *et al.*, 2002; Lukacs, 2001). Eosinophils are important effector cells in the late phase allergic response, and they have been implicated in the pathogenesis of allergic disease (Bousquet *et al.*, 1990). Ling *et al* have demonstrated a new mechanism of eosinophil recruitment driven by mast cells via the release of histamine. They conclude that histamine released from mast cells mediates eosinophil chemotaxis, cell shape change and upregulation of adhesion molecules via H4R (Ling *et al.*, 2004; O'Reilly *et al.*, 2002).

10.13147/SOE.2017.013

Neutrophils

It was also demonstrated that H4R antagonists cause a significant inhibition of polymorphonuclear cell influx into the peritoneum or pleural cavity in zymosan-induced neutrophilic inflammation models (Thurmond *et al.*, 2004; Takeshita *et al.*, 2003). H4R blocked adhesion dependent degranulation of neutrophils in response to mast cells (Dib *et al.*, 2014).

Taken all these together, H4R are functionally present in diverse immune cells and mediate biological activities leading to allergy and inflammation. Therefore H4R makes a promising target of drug design for allergic diseases.

2.6 Therapeutic potential of Histamine receptors for allergy

2.6.1 Antihistamines:

The effect of histamine had urged the researchers to find a way to thwart the response. This was first started in Pasteur institute by Boven where he had access to Fourneau's bank of compounds (Bovet *et al.*, 1937). The first compound reported as an antihistamine by Ungar, Parrot and Bovet was the adrenolytic benzodioxan, piperoxan (933F) in 1937, which blocked the effect of histamine on the guinea-pig ileum. This was followed by the search of more antihistamines. After 1945, these antihistamines became widely used in the treatment of various allergic disorders such as hay fever, allergic rhinitis and urticaria (Parsons *et al.*, 2006).

The prevalence rates of allergic diseases such as allergic rhinitis and asthma can be attributed to the increased level of pollutants in the environment and is increasing in many countries. As mentioned earlier, although several mediators are involved in the pathophysiology of allergic diseases, histamine plays a fundamental role, particularly in allergic rhinitis and urticaria. Antihistamines combine with and stabilize the inactive conformation of H1-receptors and thus interfere with the actions of histamine. The antihistamines down regulate the antigen presentation, expression of pro-inflammatory cytokines and cell adhesion molecules and chemotaxis (Simon *et al.*, 2008). Antihistamines provide symptomatic relief of allergic symptoms caused by histamine release. Antihistamines have remained at the forefront of treatment for allergic diseases for many years and are among the most commonly prescribed medicines (van Schoor, 2008). Antihistamines are chemically and functionally classified into 6 types namely; Alkylamines, Piperazines, Piperidines, Ethanolamines, Ethylenediamines, Phenothiazines (Table 2) (van Schoor, 2008; Simon *et al.*, 2008). The antihistamines can be broadly classified into 1) first generation antihistamine 2) second generation and 3) third generation.

10.13147/SOE.2017.013

	Alkylamines	Ethanolamines	Ethylene diamines	Phenothiazines	Piperiadine	Piperiazines
First generation	Brompheniramine, Chlorpheniramine, Dexchlorpheniramine, Pheniramine, Triprolidine	Clemastine, Diphenhydramine, Doxylamine	Antazoline, Mepyramine	Promethazine, Trimeprazine	Azatadine, Cyproheptadine	Buclizine, Cyclizine, Hydroxyzine, Mebhydrolin, Meclizine
Second generation	Acrivastine	Astemizole, Desloratadine, Ebastine, Fexofenadine, Levocabastine, Loratadine, Mizolastine, Terfenadine	Cetirizine, Levocetirizine			

Table 2 Structural classification of H1 antihistamines

10.13147/SOE.2017.013

2.6.1.1 First generation antihistamine

The first generation antihistamines are also called classic antihistamines and they are clinically used from 1940s and 1950s. These antihistamines have the same root as cholinergic muscarinic antagonists, tranquillizers, antipsychotics and antihypertensive agents. As a result they have the following disadvantages (Church *et al.*, 2010).

• They have poor receptor selectivity and often interact with receptors of other biologically active amines thereby causing antimuscarinic, anti-adrenergic and antiserotonin effects.

• They readily cross the blood brain barrier which leads to drowsiness, sedation, and somnolence, fatigue leading to impairment of cognitive function, memory and psychomotor performance.

• In case of overdose, it can ultimately be life threatening.

Some of the first generation antihistamines are Pheniramine, Tripolidine, Antazoline etc. There are 6 structural classes of the first generation of the antihistamines that are listed in Table 2. There were over 40 first generation antihistamine antagonists in market until 1980s.

2.6.1.2 Second generation antihistamines

In-order to improve the selectivity and tolerability of the H1 antagonists and also reduce the side effects of the first generation antihistamines, second generation anti-histamines was developed. The second generation antihistamines gained entry into market in 1981 and the first of its type are terfenadine and astimizole. The second generation had more refined properties than the first generation (Slater *et al.*, 1999) which include

• Improved H1 receptor selectivity,

• Less sedation,

• They exhibited antiallergic property apart from antihistaminic property.

Besides acting on H1R, second generation antihistamines inhibit mast cell activation apart from blocking the release of histamines by mast cells. This makes them exhibit the anti-allergic and anti-inflammatory effects. In addition, the second generation antihistamine also lessens nasal obstruction which is the prominent symptom of late allergic reaction (van Schoor, 2008). Along with the structural classes of the first generation antihistamines, second generation antihistamines are also listed in Table 2.

10.13147/SOE.2017.013

2.6.1.3 Third generation antihistamines

The second generation antihistamines were successful, however some of the drugs such as terfenadine and astemizole were found to cause potentially serious arrhythmias. It was determined that the cardiac toxicity was mainly due to the parent drugs. As active metabolites or enantiomers could account for most of the clinical benefit, the goal for the third generation of antihistamines was to develop therapeutically active metabolites that were devoid of cardiac toxicity. The first of these drugs, fexofenadine (the active metabolite of terfenadine), was approved in July 1996 (Handley *et al.*, 1998). Third-generation antihistamines are developed with the goal of improving clinical efficacy and minimizing side effects. Some of the drugs in this category are fexofenadine, norastemizole, and descarboethoxyloratadine.

.6.2 H4R receptor antagonists

Antihistamines which target the H1R are primarily used for the treatment of atopic allergy and relieve few symptoms of allergy. However the latest H4R were found to arbitrate many functions of allergy which suggest the possibilities of its therapeutic potential in treating allergy (Fung-Leung *et al.*, 2004). Unlike H1 antihistamines, H4R antagonists are not yet used as therapeutic drugs. However many antagonists or inverse agonists have been identified to be promising. There are many antagonists that have been determined as promising leads, however in this section we are going to give importance to JNJ7777120, Vuf6002 and Thioperamide. These antagonists have proven anti-inflammatory properties and are detailed in the below section.

JNJ7777120

It was first reported in 2003 through high throughput screening initiated by Johnson and Johnson. JNJ7777120 (Figure 6) is the first reported neutral antagonist of H4R (Thurmond *et al.*, 2004). It was derived from indole containing hit structure and was converted into JNJ7777120. This ligand is been considered as the reference. This is the first non-imidazole ring ligand discovered. JNJ 7777120 combines an hH4R affinity in the Nano molar range with a selectivity of >1000-fold over other histamine receptors. In mouse, oral administration of JNJ 7777120 had an absolute oral availability of 22 to 100% and a half-life of 1 to 2h depending on the species (Thurmond *et al.*, 2004).

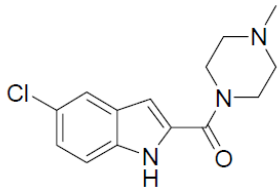

Figure 6 Chemical structure of JNJ7777120

10.13147/SOE.2017.013

Allergic dermatitis improved after the administration of JNJ7777120 (Ohsawa *et al.*, 2012). Oral administration of JNJ7777120 has decreased the symptoms of allergic rhinitis. Furthermore the serum IgE levels and IL-4 showed a marked decrease (Takahashi *et al.*, 2009). In allergic asthma model of guinea pigs, JNJ7777120 inhibited the airway hyperactivity (Riley *et al.*, 2008). The administration of JNJ7777120 (JNJ), showed significant anti-inflammatory effects during sensitization and effector phase. These findings indicate that H4 receptors are also involved in the initial priming of the immune system after allergen challenge (Thurmond *et al.*, 2008). Antigen-induced asthma-like reactions in guinea pigs decreased the levels of LC-1 and increased TNF-α and eicosanoid production. JNJ pre-treatment reduced allergic asthmatic responses and airway inflammation, an effect associated with LC-1 up-regulation (Somma *et al.*, 2013).

Thioperamide

Thioperamide (Figure 7) is a dual antagonist of H3 and H4 receptor and crosses the blood brain barrier. This antagonist was identified in 1987 by the traditional pharmacological approach, consisting of assessing the inhibitory effect of histamine on its own release from depolarized rat brain slice. Thioperamide has shown a significant improvement in symptoms of allergic reaction via induction of regulatory T lymphocytes (Amaral *et al.*, 2011). However in experimental asthmatic mice, thiopermaide offered only partial beneficial effects as compared with JNJ7777120 (Neumann *et al.*, 2013).

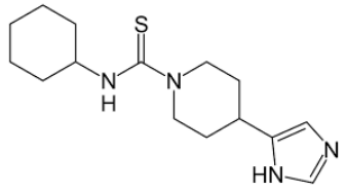

Figure 7 Chemical structure of Thioperamide

Vuf 6002

Vuf 6002 (Figure 8) which is also known as 10191584 is a derivative of JNJ7777120. Similar to JNJ7777120, Vuf 6002 is also a useful tool in characterizing the H4R (Terzioglu *et al.*, 2004). Vuf 6002 has been shown to exhibit anti-inflammatory effect against paw edema and hyperalgesia (Coruzzi *et al.*, 2007). However its role in allergic models and underlying pathway has not been identified yet.

10.13147/SOE.2017.013

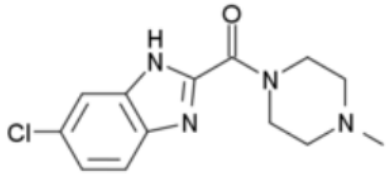

Figure 8 Chemical structure of Vuf 6002

2.7 Structure based virtual screening

The discovery of innovative leads with potential interaction to specific targets is of central importance to the early-stage drug discovery (Cheng *et al.*, 2012). Virtual Screening (VS) refers to the selection of compounds based on their binding from a large set of database. They can be either Structure based virtual screening (SBVS) or Ligand based virtual screening (LVS). If the 3D structure of the target receptor is available SBVS is performed and if the reference ligand with a known bioactive conformation is available LVS is performed (Rahman, 2007). Hence in this study SBVS is preferred than LVS. SBVS encompasses variety of sequential computer phases including target and database preparation, docking and post processing of the identified lead molecule. The steps involved in the process are displayed in Figure 9.

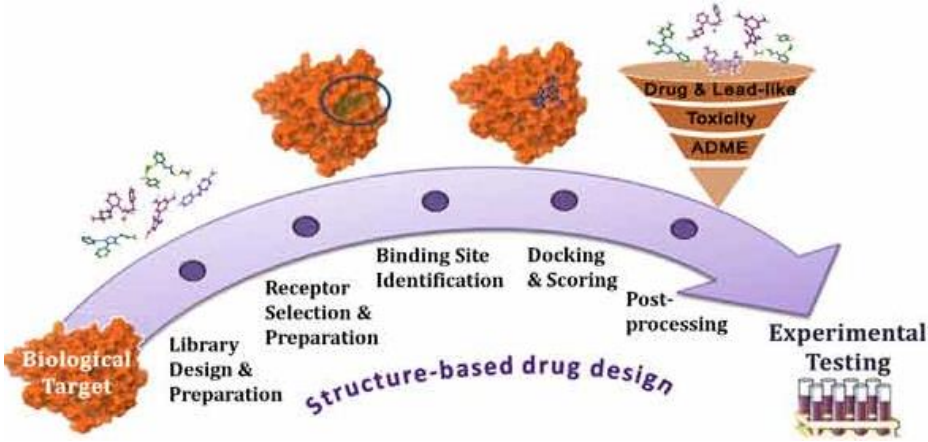

Figure 9 SBVS flowchart (Lionta *et al.*, 2014)

10.13147/SOE.2017.013

2.7.1 SBVS and histamine receptors

A structure-based virtual screening (SBVS) was conducted on a homology model of the human histamine H4 receptor (hH4R). More than 8.7 million 3D structures derived from different vendor databases were investigated by docking to the hH4R binding site. Several novel scaffolds were identified that can be used to develop selective H4 ligands in the future. This is the first SBVS reported on H4R, representing one of the largest virtual screens validated by the biological evaluation of the virtual hits (Kiss *et al*., 2008).

Structure based virtual screening for fragment like ligands of histamine receptors have been performed recently. SBVS studies against β2R-based and H1R-based H4R homology models led to the discovery of different new fragment-like H4R ligand chemotypes. Of the 37 tested compounds, 9 fragments had affinities between 0.14 and 6.3 μM at the H4R (Istyastono *et al*., 2015). Graaf *et al* have developed and validated a customized structure-based virtual fragment screening protocol against the crystal structure of human histamine H1R. Many of the identified fragments were both promising and challenging new starting points for structure-based ligand optimization (de Graaf *et al*., 2011).

10.13147/SOE.2017.013

Chapter 3

Materials and Methods

10.13147/SOE.2017.013

3.1 Description of tools and Software

3.1.1 Basic Local Alignment Search Tool (BLAST)

Sequence alignment is a procedure of comparing two or more proteins or nucleotide sequence for the purpose of identifying similar sequence which may share similar structure and function (Koonin EV, 2003). BLAST is a computational tool to find local similarity between two sequences such as amino acid sequences of proteins or nucleotides of DNA sequence. BLAST analysis is a fundamental way of analysing a gene or protein. It reveals the similarities of a particular sequence in the same species and different species as well. It helps to compare or search for a homologous sequence in a protein or nucleotide database and calculates the significance of matches. The user can select a sequence which is termed as query and perform a sequence alignment with an entire database termed the target. More than tens of millions of sequences are evaluated in a BLAST search from which only closely related sequences are given as output. BLAST was developed by National Centre for Biological Information (NCBI). The results are reported in the form of a ranked list followed by a series of individual sequence alignments, plus various statistics and scores (Altschul *et al*., 1990). The output results will have the following information

- The description/title of matched database sequence,
- The highest alignment score (Max score) from that database sequence,
- The total alignment scores (Total score) from all alignment segments,
- The percentage of query covered by alignment to the database sequence,
- The best (lowest) Expect value (E value) of all alignments,
- The highest percent identity (Max ident) of all query-subject alignments,
- The accession of the matched database sequence.

3.1.2 Transmembrane helix predictors

GPCRs are the gatekeepers and molecular messengers of the cell which transmits signals from inside of the cell to outside. They are membrane bound proteins that span the cell membrane in the form of seven transmembrane helices which are connected by three loops, three on the intracellular side and three on the extracellular side. Hence attempts to separate the GPCR from the membrane will destroy its integrity. Hence, transmembrane protein structure prediction is an important part in determining the integral structure of the protein (Cuthbertson *et al*., June 2005). As discussed previously, hH4R is a G-protein coupled receptor which constitutes 7 transmembrane proteins. In this study, the transmembrane domain of the hH4R was determined by using a series of freely available online webservers. All these tools predicted the transmembrane of proteins from the given

10.13147/SOE.2017.013

amino acid sequence. The principle and the aim of the webservers are further discussed below.

HMMTOP

HMMTOP (Hidden Markov Model for TOpology prediction) is a freely available automatic server which helps in predicting the transmembrane helices and topology of proteins (Tusnady *et al*., 1998). This is based on the principle that the topology of the transmembrane protein is determined by the maximum divergence of the amino acid composition of sequence segments. HMMTOP achieved about 96% average accuracy in predicting transmembrane helices in data sets, and was able to predict the overall topology correctly in the same data sets with an average accuracy of 85% (Carnohan, 2012). The webserver is available at

http://www.enzim.hu/hmmtop/index.php

TM HMM

TMHMM is a membrane protein topology prediction method based on a hidden Markov model. Dynamic programming is commonly used to match a sequence against the model in order to find the most probable match. It has been trained to detect hydrophobic transmembrane helices. It also identifies the individual domains in the membrane both intracellularly and extracellularly (Krogh *et al*., 2001; Sonnhammer *et al*., 1998). TMHMM method indicates that, in cross-validated tests on sets of 83 and 160 proteins with known topology, their method was successful in predicting the entire topology of a protein 85% of the time for both data sets (Carnohan, 2012). TM HMM prediction server is available at

http://www.cbs.dtu.dk/services/TMHMM/.

TM Pred

The TMpred program makes a prediction of membrane-spanning regions and their orientation. The algorithm is based on the statistical analysis of TMbase, a database of naturally occurring transmembrane proteins. The prediction is made using a combination of several weight-matrices for scoring (Hofmann *et al*., 1993). It correctly predicted the overall topologies of 23 out of 24 proteins (96% accuracy), and identified all 135 transmembrane segments from the sample, plus one over prediction (Carnohan, 2012). It can be used from

http://www.ch.embnet.org/software/TMPRED_form.html.

SOSUI

SOSUI distinguishes between membrane and soluble proteins from amino acid sequences, and predicts the transmembrane helices of membrane proteins (Hajat *et al*., 2001). The accuracy of the classification of proteins was 99% and the

10.13147/SOE.2017.013

corresponding value for the transmembrane helix prediction was 97%. SOSUI is available at

http://harrier.nagahama-i-bio.ac.jp/sosui/sosui_submit.html.

3.1.3 Q-site finder

Determination of the location of ligand binding sites on a protein is of fundamental importance for a range of applications including molecular docking, de novo drug design, structural identification and comparison of functional sites. In-order to identify the binding site of hH4R we employed Q-site finder which is freely available at http://www.bioinformatics.leeds.ac.uk/qsitefinder. The program uses the interaction energy between the protein and a simple Van der Waals probe to locate energetically favourable binding sites. Energetically favourable probe sites are clustered according to their spatial proximity and clusters are then ranked according to the sum of interaction energies for sites within each cluster (Laurie *et al.*, 2005).

3.1.4 I-TASSER

I-TASSER (Iterative Threading ASSEmbly Refinement) is a method for predicting three-dimensional structure model of protein molecules from amino acid sequences. It predicts the structure templates from the Protein Data Bank by a technique called fold recognition (or threading). Protein threading, is a method of protein modelling which models proteins based on folds of another protein with known structures, however, both the proteins do not share the same homology. Threading works by using statistical knowledge of the relationship between the structures deposited in the PDB and the sequence of the protein of interest. I-TASSER generates C score for each model generated. C score is a confidence score for estimating the quality of predicted models by I-TASSER. It is calculated based on the significance of threading template alignments and the convergence parameters of the structure assembly simulations. C-score is typically in the range of [-5 to 2], where a C-score of higher value signifies a model with a high confidence and vice-versa (Roy *et al.*, 2010; Zhang, 2008; Yang *et al.*, 2015).

3.1.5 ERRAT

ERRAT is a protein structure verification algorithm that is especially well suited for validating the given 3D structure model. Our 3D structure model of the hH4R developed by I-TASSER was validated using this tool. This program works by analyzing the statistics of non-bonded interactions between different atom types. As a result, single output plot is produced that gives the value of the error function vs position of a 9-residue sliding window. By comparison with statistics from highly refined structures, the error values have been calibrated to give confidence limits. This tool is extremely useful in making decisions about reliability (MacArthur *et al.*, 1994).

10.13147/SOE.2017.013

3.1.6 PROCHECK

PROCHECK is another structure validating tool that was utilized for our hH4R model validation. The PROCHECK suite of programs provides a detailed check on the stereochemistry of a protein structure. The output provides number of plots in PostScript format and a comprehensive residue-by-residue listing. These give an assessment of both the overall quality of the structure, as compared with well-refined structures of the same resolution, and also highlight regions that may need further investigation. The PROCHECK programs are useful for assessing the quality not only of protein structures in the process of being solved, but also of existing structures and those being modelled on known structures (Laskowski *et al.*, 1996; Laskowski *et al.*, 1993).

3.1.7 PubChem

PubChem (http://pubchem.ncbi.nlm.nih.gov) is a public repository for biological properties of small molecules hosted by the US National Institute of Health (NIH). In 2010, the PubChem databases hold records for over 69 million substances (SID) containing 27 million unique chemical structures (or CID records) and 449,401 bioassays (AID). More than 1.8 million of these substances and 1.5 millions of compounds have bioactivity data in at least one of the thousands *in vitro* biochemical and cell-based screening assays, targeting more than 7,000 proteins and genes. The millions of compound records and bioassay data collections provide great opportunities for drug discovery research. They also create a major challenge for scientists for the development of cheminformatics tools and modelling algorithms that are suitable to handle such high volume of PubChem compounds and bioactivity datasets for virtual screening and in silico drug design (Xie, 2010). PubChem offers different services, however we exploited PubChem structure in this research. PubChem Structure Search allows the PubChem Compound Database to be queried by chemical structure or chemical structure pattern. The webserver also offers PubChem Sketcher that allows a query to be drawn manually. The structural query input could be specified by PubChem Compound Identifier (CID), SMILES, SMARTS, InChI, Molecular Formula, or by upload of a supported structure file format. For each structure output, PubChem gives information on its physical and chemical properties (Molecular weight, Hydrophobicity, Molecular formula). The Chemical Structure Search tool allows users to narrow a search to the result from a previous Entrez or chemical structure search or to the set of CIDs uploaded in a file. Optional filters may be applied to limit the search result, based on various properties, such as molecular weight, heavy atom count, presence or absence of stereochemistry, depositor name or category and so on. A query can be exported to an XML file, which allows one to import the query from the XML file and to repeat the search without filling out the search form again (Kim *et al.*, 2015).

10.13147/SOE.2017.013

3.1.8 ChemSketch

ChemSketch is a freeware chemical structure drawing package from Advanced Chemistry Development, Inc. (ACD/Labs). Our study utilized ChemSketch to optimize the ligands retrieved before proceeding into docking. ChemSketch Freeware allows drawing chemical structures including organics, organometallics, polymers, and Markush structure. It also includes features such as 2D and 3D structure cleaning and viewing, functionality for naming structures (fewer than 50 atoms and 3 rings). Some features of ChemSketch are

- Drawing and viewing the structures in 2D and render in 3D to view from any angle
- Drawing reactions and reaction schemes, and calculating the reactant quantities
- Generating structures from InChI and SMILES strings
- Generating IUPAC systematic names for molecules of up to 50 atoms and 3 ring structures
- Predicting logP for individual structures
- Searching structures in the built-in dictionary of over 165,000 systematic, trivial, and trade names

3.1.9 Discovery Studio

Discovery Studio is a comprehensive software suite for analyzing and modelling molecular structures, sequences, and other data of relevance. It contains established gold-standard applications such as Catalyst, MODELER, CHARMm, etc. It is an interactive, visual and integrated software. The user interface is consistent and contemporary. Discovery Studio delivers a comprehensive, scalable portfolio of scientific tools, tailored to support and assist Structure based design strategies from hit discovery through to late-stage lead optimization. Some of the built-in features of Discovery studio are

Preparation of the macromolecule structures for SBD

- Analyzing and preparing 3D structure models (e.g., PDB, X-ray structure, homology model) using MODELER
- Predicting residue ionization states at pH
- Identifying and studying putative ligand binding sites

Preparing ligands

- Cleaning and calculating 3D coordinates
- Generating ligand conformations
- Filtering ligands based on molecular properties, or undesirable groups

10.13147/SOE.2017.013

Hit Identification and optimization

- Performing virtual screening on ligands and fragments using either the CATALYST pharmacophore engine, or the LIBDOCK or CDOCKER docking approaches
- Identifying critical interacting residues using the most comprehensive set of favourable, unfavourable and unsatisfied non-bond monitors on the market
- Profiling and prioritizing the screening hits
- Optimizing the potency and target specificity
- Performing *in situ* lead optimization using classical medicinal chemistry reaction transformations and commercially available reagents
- Scaffold-hopping or performing R-group substitutions *in situ* using molecular fragments derived from commercially available compounds

Additional design tools

- Performing combinatorial library design and optimizing using Pareto optimization, diversity and similarity analysis
- Calculating QSAR, fingerprint, and Quantum Mechanics based descriptors
- Creating advanced statistical models including Bayesian models, MLR (Multiple Linear Regression), PLS (Partial Least Squares), GFA (Genetic Functional Analysis), and NN (Neural Networks)
- Building drug-like and ADME properties
- Minimizing toxicity using TOPKAT
- Optimizing pharmacokinetic profile

3.2 Methodology

3.2.1 Evidence based information

The air pollutants used for the discussion of possible role with Histamine receptors were chosen from http://www.epa.gov/air/airpollutants.html., where EPA stands for US Environmental Protection Agency. The mission of EPA is to protect human health and the environment. The bibliographic search for each pollutant was carried out by Google Scholar search engine. Bibliographies for the relation of the individual pollutants and allergy were searched using the keyword "Pollutant and allergy" and "Pollutant and mast cells". Identification and first screening of the articles were performed using the information available in the title and the abstract. The results were then tabulated based on the criteria. A second Table was developed to list the relation between the Pollutant and histamine receptors. To generate this Table, bibliographic search was performed. The

10.13147/SOE.2017.013

selection criteria of both the searches are that the articles were to be an original research article: abstracts, case reports, ecological studies and letters to the editor were excluded.

3.2.2 Sequence analysis

Determination of amino acid sequence of proteins, the study of the conformation changes of proteins and also the study of the complex molecules with any other non-peptide molecule is is referred as protein sequence analysis. The cellular processes of a living organism are known by the discovery of the structure and function of proteins, thus allowing researcher to develop and design drug targets. The sequencing of the genomes from several organisms, and high-throughput X-ray structure analysis, have brought to the scientific community a large amount of data about the sequences and structures of several thousand proteins. This information can effectively be used for medical and biological research only if one can extract functional insight from it.

The hH4R is a protein sequence consisting of 390 amino acids. The sequence of this protein is analysed or aligned with all the available sequences in protein database to identify sequences which has close similarity. This was performed using BLAST (Altschul *et al.*, 1990). Following are the steps involved in performing BLAST which is followed as in the book "Bioinformatics and Functional Genomics" (Pevsner, 2015).

3.2.2.1 Selecting a BLAST program

There are five different blast programs, which can be distinguished based on the query sequence (DNA or protein) and the type of the subject database. Since our aim is to find a homologous amino acid sequence for hH4R, we chose BLASTp. BLASTp compares an amino acid query sequence against an entire protein sequence database.

3.2.2.2 Specifying the sequence of interest

The initial step is the input of the amino acid sequence. The data can be fed in two ways

1. Copy and paste the amino acid sequence of H4R in FASTA format or
2. Use an accession number(s), gi(s) of hH4R

In this study, FASTA format was given as an input (Figure 10)

10.13147/SOE.2017.013


```
>sp|Q9H3N8|HRH4_HUMAN Histamine H4 receptor OS=Homo sapiens GN=HRH4 PE=1
SV=2
MPDTNSTINLSLSTRVTLAFFMSLVAFAIMLGNALVILAFVVDKNLRHRSSYFFLNLAIS
DFFVGVISIPLYIPHTLFEWDFGKEICVFWLTTDYLLCTASVYNIVLISYDRYLSVSNAV
SYRTQHTGVLKIVTLMVAVWVLAFLVNGPMILVSESWKDEGSECEPGFFSEWYILAITSF
LEFVIPVILVAYFNMNIYWSLWKRDHLSRCQSHPGLTAVSSNICGHSFRGRLSSRRSLSA
STEVPASFHSERQRRKSSLMFSSRTKMNSNTIASKMGSFSQSDSVALHQREHVELLRARR
LAKSLAILLGVFAVCWAPYSLFTIVLSFYSSATGPKSVWYRIAFWLQWFNSFVNPLLYPL
CHKRFQKAFLKIFCIKKQPLPSQHSRSVSS
```

Figure 10 FASTA format of hH4R

Selection of the database

There are seven different databases available to choose for running a BLAST programme depending on the research. In this present study, Protein database (PDB) was entitled to perform BLAST against hH4R. PDB contains information about experimentally-determined structures of proteins, nucleic acids, and complex assemblies.

Selection of parameters

1 Maximum target sequence is set to default value 100.

2 Expect Threshold is set to default value of 10. This setting specifies the statistical significance threshold for reporting matches against database sequences. The default value (10) means that 10 such matches are expected to be found merely by chance, according to the stochastic model of Karlin and Altschul (Karlin *et al.*, 1990).

3 Maximum match in a query: Matches of one region of inters can be obscured by frequent matches to a different region of a protein. This option is useful if many strong matches to one part of a query may prevent BLAST from presenting weaker matches to another part of the query. Hence to limit the number of matches to a query range we set zero.

4 Matrix: A key element in evaluating the quality of a pairwise sequence alignment is the "substitution matrix", which assigns a score for aligning any possible pair of residues. We have used the default matrix BLOSSUM 62.

5 Gap cost: Gap is a space introduced into an alignment to compensate for insertions and deletions in one sequence relative to another. To prevent the accumulation of too many gaps in an alignment, the system is set so that introduction of a gap in the alignment results in the reduction of the alignment score. So too many gaps can reduce the alignment score.

10.13147/SOE.2017.013

6 Compositional adjustments: Amino acid substitution matrices may be adjusted in various ways to compensate for the amino acid compositions of the sequences being compared. The simplest adjustment is to scale all substitution scores by an analytically determined constant, while leaving the gap scores fixed; this procedure is called "composition-based statistics". Conditional compositional score matrix adjustments is selected as default.

7 Filter: Filtering masks portions of the query sequence that have low compositional complexity. Low compositional sequences are frequently occurring sequences with less informational content.

8 MaskFilter (Mask for lookup Table only): BLAST search consists of two phases, finding hits based upon a lookup Table and then extending. This option masks only for purposes of constructing the lookup Table used by BLAST so that no hits are found based upon low-complexity sequence or repeats (if repeat filter is checked). The BLAST extensions are performed without masking and so they can be extended through low-complexity sequence.

9 Mask Lower Case: With this option selected you can cut and paste a FASTA sequence in upper case characters and denote areas you would like filtered with lower case. This allows you to customize what is filtered from the sequence during the comparison to the BLAST databases.

3.2.3 Transmembrane helix Predictions

Prediction of transmembrane helices of membrane proteins provides valuable information about the protein topology when the high resolution structures are not available. This prediction of TM residue contacts can provide crucial constraints for accurately constructing 3D structures of membrane protein (Simakova *et al.*, 2014). To predict the transmembrane helices of hH4R we used four webservers, the steps involved in each tool is described below.

HMMTOP

The HMMTOP method uses a hidden Markov model to find the most probable of all the possible topologies of a protein, which is a prediction and hopefully a match with the experimentally determined topology. Following are settings involved in performing HMMTOP (Figure 11)

The server can handle several file formats including plain text, Fasta and NBRF/PIR, however the hH4R input sequence was given in FASTA format.

The sequence type chosen was single. Hence, prediction was based on the sequence information of the input sequence (hH4R).

10.13147/SOE.2017.013

The mode of prediction preferred in this study is reliable mode. In reliable mode the server makes the Baum-Welch iteration for the submitted sequence(s), i.e. it searches or makes optimization for the best topology. Therefore the results are reliable but more time consuming.

If the localization of some parts of the query protein is known, then this option allows submitting this or these part(s). hH4R had no known information regarding the localization.

The output file can be chosen as either html file or as simple text file based on the preference of the study. The simple text file option is chosen in this study as it is useful for additional processing of the result(s).

Then the sequence is submitted.

Figure 11 HMMTOP

TMHMM

TMHMM (Figure 12) does not have any parameters to be set. The sequence of hH4R is pasted as an input in FASTA format. The results were produced in few minutes.

10.13147/SOE.2017.013

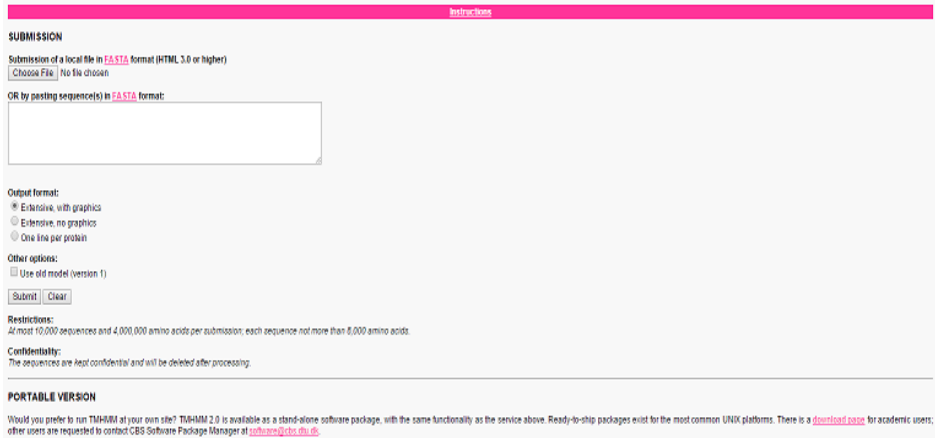

Figure 12 TM HMM

TMpred

TMpred requires the input sequence to be either in plain text or FASTA format (Figure 13). In this study, FASTA format was submitted. The minimal and maximal length of the hydrophobic part of the transmembrane helix is set to default which is 17 and 33 respectively.

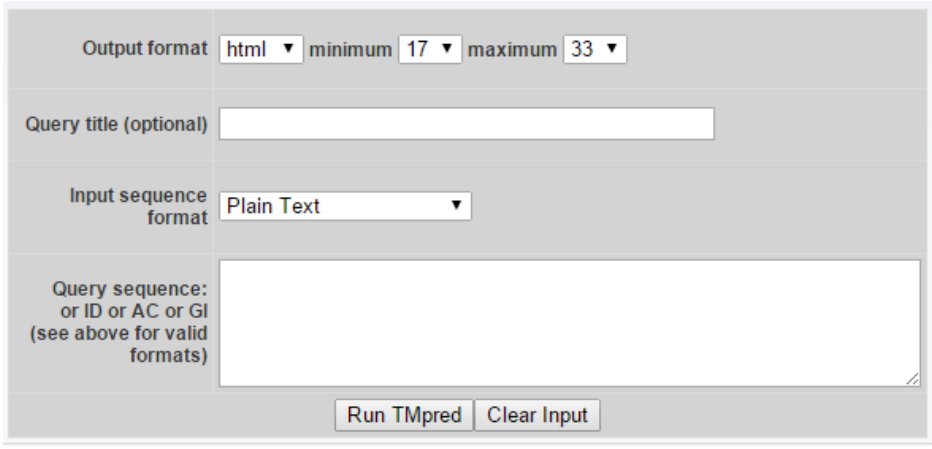

Figure 13 TMPred

10.13147/SOE.2017.013

SOSUI

The input sequence was pasted and allowed to run (Figure 14). The system SOSUI can be accessed online via.

http://www.tuat.ac.jp/mitaku/sosui/.

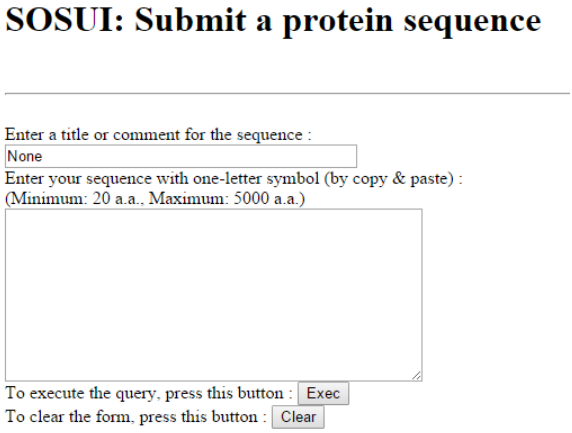

Figure 14 SOSUI

3.2.4 3D Model prediction

The 3D structure of a protein is implicitly related to the function of the protein but it is not always straight forward to infer function from structure. There are cases where proteins with similar structures have different functions and if a protein represents a new fold (i.e. resembles no previously solved structure) it might be hard to assign the function. Nevertheless, a good way to start studying the function for a protein is to determine its 3D structure. There are a number of experimental techniques for three-dimensional structure determination. The classical methods commonly used for globular proteins are X-ray crystallography and nuclear magnetic resonance (NMR) spectroscopy. Although technical progress is made continuously, it is not feasible to experimentally determine structures for every known protein. It would take too much effort both in terms of cost and labour. Therefore computational techniques are being used. Protein structure prediction refers to the effort of generating 3-dimensional models from amino acid sequences using computer algorithms. Prediction of 3-dimensional protein structures from amino acid sequences represents one of the most important problems in computational structural biology (Simakova *et al.*, 2014). The three different methods of protein structure prediction are 1) Ab initio modelling and 2) homology modelling and 3) threading (Wu *et al.*, 2007). In this study we employed threading i.e fold recognition method. A protein fold recognition technique involves incrementally replacing the sequence of a known protein structure with a query sequence of unknown structure. The new "model" structure is evaluated using a

10.13147/SOE.2017.013

simple heuristic measure of protein fold quality. The process is repeated against all known 3D structures until an optimal fit is found (Bowie *et al.*, 1991; Jones *et al.*, 1992; Xu *et al.*, 2000; Zhou *et al.*, 2005). The 3D model of hH4R is predicted using the computational tool I-TASSER. The steps included are as follows:

1. I-TASSER web page is found at http://zhanglab.ccmb.med.umich.edu/I-TASSER.
2. Input the amino acid sequence of hH4R. It can be provided either as FASTA format or can be directly uploaded. I-TASSER server can accept sequences up to 1500 residues.
3. There are specific options such as assign external inter-residue distance restraints add-in an additional template or exclude some template proteins during the structure modelling process. All the options were not checked in our study.
4. To submit the sequence, click on the "Run I-TASSER" button. The browser will be directed to a confirmation page displaying user specified information, job identification (Job ID) number and a link to a webpage where the results will be deposited after completion of job. The link is bookmarked for future reference.

3.2.5 Model Validation

With the growing size of sequence databases and the difficult task of experimentally elucidating the corresponding protein structure using classical techniques like NMR or X-ray crystallography, it is necessary to develop in silico methods in order to predict the protein structure. However, many heuristics and other approximations must be introduced to obtain theoretical models in a reasonable amount of time. Thus, when no close homolog protein structure is known, a computational developed protein model can be very unreliable. Molecular modelling by computational tools can be tricky especially if the distantly related target and template protein share the same fold. That is why it is important to experimentally validate these models (Bhattacharya *et al.*, 2007). All the hH4R models are generated by I-TASSER which is based on fold recognition method. These models were subjected to validation by two independent tests, ERRAT and PROCHECK. The details of these tools were described in the previous section. The pdb format was used to submit the model to the server for analysis. The results were displayed in few minutes in the web page.

10.13147/SOE.2017.013

3.2.6 Binding site prediction

The function of a protein is defined by the interactions it makes with other proteins and ligands. Identification of binding site is an important step in analyzing ligand binding interactions, molecular docking, de novo drug design, structural identification and comparison of functional sites. Computational methods for the detection and characterization of functional sites on proteins have increasingly become an area of interest. This is frequently achieved through functional site detection, which often uses protein evolutionary information or by structural comparisons of functional sites. In addition, functional site detection is important for targeting specific sites in structure-based drug design to assist in the development of therapeutic agents. Virtual screening of ligands against protein structures using docking is widely used for identifying potential lead compounds in the drug design process. In addition de novo drug design can lead to the creation of novel ligands not found in molecular databases. Therefore, it is essential that the ligand binding site is identified prior to either study as both procedures require this information. Furthermore, all methods can be made more efficient by further restricting the search to critical regions (Xie *et al*., 2015)

The amino acid residues in the binding site were predicted with the help of binding pocket detection server tools, such as pocketfinder and Q-site finder (http://www.modelling.leeds.ac.uk/ qsitefinder). In addition to that, the binding pockets of the receptor were also determined by using Accelrys Discovery studio.

3.2.7 Preparation of ligand database

Three different databases each consisting of similar structures of JNJ7777120, Vuf 6002 and thioperamide respectively has to be built to perform docking. The three ligands are subjected to optimization before similar structures are retrieved from PubChem. A step by step procedure is listed below

- The 2-D structure of the three ligands JNJ7777120, thioperamide and Vuf 6002 was drawn with the tools of ChemSketch.
- The structures are then cleaned using the tool “clean”.
- The 2D structures are converted to 3D structures and are saved.
- Then the model and the ligands were subjected to energy minimization using the CHARMm forcefield implemented in the Discovery studio software package.
- Each ligand was given as an input in the PubChem structure search as a structure file, and the output contained similar structure with the Tanimoto score of similarity >0.9 of the ligands in .sdf format.

10.13147/SOE.2017.013

- For JNJ777720, the similarity percentage used was 95%, whereas for the thioperamide and Vuf 6002, the similarity was kept 90%.
- Numerous similar structures for each ligand were obtained. 150 similar structures of JNJ7777120, 49 and 198 similar structures of Thioperamide and Vuf6002 were acquired, respectively. These accounted for three different databases.

3.2.8 Molecular Docking

Molecular docking has become an increasingly important tool for drug discovery. The molecular docking approach can be used to model the interaction between a small molecule and a protein at the atomic level, which allows us to characterize the behaviour of small molecules in the binding site of target proteins as well as to elucidate fundamental biochemical processes. The docking process involves two basic steps: prediction of the ligand conformation as well as its position and orientation within these sites (usually referred to as pose) and assessment of the binding affinity (Ferreira *et al.*, 2015).

Docking calculations were performed by Discovery studio version 2.0. The convergence gradient was set to 0.01 kcal/ - mol and 1000 steps of steepest descent algorithm followed by 50 000 or more steps of conjugate gradient algorithm. A spherical cut-off of 14 was used for non-bonding interactions, and other parameters were set as default. To validate the docking protocol, prior to screening, the known antagonists JNJ7777120, thioperamide, and Vuf6002 have been docked in the ligand-binding site and the results were compared with earlier results. Then the databases were subjected to virtual screening against the receptor model. Ligand fit module in the DISCOVERY STUDIO is used for docking the compound databases (Venkatachalam *et al.*, 2003). The LigandFit docking procedure consists of two major parts: (i) specifying the region of the receptor to use as the binding site for docking; (ii) docking the ligands to the specified site. The steps involved in molecular docking are

- Open the Docking protocol which is in the Parameters Explorer,
- Input the pdb format of the target receptor (hH4R) and input the selected binding site.
- Specify the ligand file into the protocol.
- Select PLP1 which specifies the energy function for docking.
- Click Value Parameter and enter the value 10. This specifies a value up to 10 poses to save for each ligand. Only poses that are distinct based on RMS and energy criteria are saved.

10.13147/SOE.2017.013

- In the parameter value, check the scores for LigScore1, LigScore2, PLP1, PMF. The specified scoring functions will be calculated for each docked ligand pose when the protocol is run.
- Run the docking protocol. This job typically takes several minutes to complete. The status of the job can be monitored in the Jobs Explorer.
- The results can be obtained in the output folder. This simultaneously opens the resulting docked ligand poses of the Docking job into a Table Browser and opens an associated 3D View containing the first docked ligand pose and the protein receptor used for the calculation

The top 10 docked poses were allowed to be saved. The successful poses were evaluated using a set of scoring functions as implemented in Discovery studio program including LigScore1, LigScore2, PLP1, PLP2, and PMF, whereas the candidate ligands in the binding site are prioritized according to the Dock- Score function.

LigScore: 3 descriptors representing (i) the Van der Waals interaction, (ii) the influence of the buried polar surface area between a protein and ligand which involves attractive protein-ligand interactions and (iii) the influence of the buried polar surface area between a protein and ligand involving both attractive and repulsive protein-ligand interactions, grouped into equations.

Pairwise Linear Potential (PLP): fast, simple docking function that has been shown to correlate well with protein-ligand binding affinities (two versions of the PLP function were used: PLP1 and PLP2),

Potential of Mean Force (PMF): statistical analysis approach using 3D structure databases to provide a fast and accurate prediction of protein-ligand binding free energies. The scoring function is defined as the sum of the interaction free energies over all interatomic pairs of the protein-ligand complex.

3.2.9 ADMET predictions

Most of drug candidates fail in clinical trials due to poor Absorption, Distribution, Metabolism, Excretion and Toxicity (ADMET) properties. Thus, an important aspect of drug discovery is to avoid compounds not having drug likeliness and good ADME property. ADMET describe the kinetics of drug exposure to the tissues and pharmacological activity of the compounds (Van de Waterbeemd *et al.*, 2003). ADMET properties of the compounds were tested using ADMET descriptors in Discovery studio. Models of intestinal absorption, blood–brain barrier penetration, cytochrome P450 2D6 inhibition, and hepatotoxicity were tested for our compounds using ADMET descriptor module of Discovery studio. Use the calculated results to eliminate compounds with unfavourable ADMET

10.13147/SOE.2017.013

characteristics and evaluate proposed structural refinements, designed to improve ADMET properties prior to synthesis.

3.2.10 Molecular dynamics

The conformational dynamics of protein molecules is encoded in their structures and is often a critical element of their function. A fundamental appreciation for how proteins work therefore requires an understanding of the connection between three-dimensional structure, and dynamics, which is much more difficult to probe experimentally. Molecular dynamics simulations provide links between structure and dynamics by enabling the exploration of the conformational energy landscape accessible to protein molecules. CHARMm (Chemistry at HARvard Macromolecular Mechanics) is the set of force fields used for molecular dynamics study. A typical molecular dynamics run involves the following basic steps:

Preliminary preparation: A molecular structure with all Cartesian coordinates defined is required for a dynamics simulation. After determining the internal coordinate values of the molecule, total energy as a function of the Cartesian coordinates is computed.

Minimization: All dynamics simulations begin with an initial structure that may be derived from experimental data. Energy minimization is performed on structures prior to dynamics to relax the conformation and remove steric overlap that produces bad contacts. In the absence of an experimental structure, a minimized ideal geometry can be used as a starting point.

Heating: A minimized structure represents the molecule at a temperature close to absolute zero. Heating is accomplished by initially assigning random velocities according to a Gaussian distribution appropriate for that low temperature and then running dynamics. The temperature is gradually increased by assigning greater random velocities to each atom at predetermined time intervals.

Equilibration: Equilibration is achieved by allowing the system to evolve spontaneously for a period of time and integrating the equations of motion until the average temperature and structure remain stable. This is facilitated by periodically reassigning velocities appropriate to the desired temperature. Generally, the procedure is continued until various statistical properties of the system become independent of time.

Production: In the final molecular dynamics simulation, CHARMm takes the equilibrated structure as its starting point. In a typical simulation, the trajectory traces the motions of the molecule through a period of at least 10 picoseconds. Just as with energy minimization, provision is made to update the non-bonded and

10.13147/SOE.2017.013

hydrogen bonded lists periodically. Additional options are available, making the dynamics facility quite flexible.

Quenching: The logical opposite of heating, this optional step takes the molecule from the equilibrated temperature to zero. Quenching is a form of minimization, utilizing molecular dynamics to slowly remove all kinetic energy from the system. Strictly speaking, minimization and heating are not necessary, provided the equilibration process is long enough.

However, these steps can serve as a means to arrive at an equilibrated structure in an effective way. A molecular dynamics run generates a dynamics trajectory consisting of a set of frames of coordinates and velocities that represent the trajectory of the atoms over time. Using trajectory data, the average structure and analyze fluctuations of geometric parameters, thermodynamics properties, and time-dependent processes of the molecule can be computed.

Molecular dynamics (MD) simulations were performed using the simulation module in Discovery studio with the standard CHARMm forcefield parameters. The six top scored receptor–ligand complexes were used for performing MD simulations. Implicit solvent with a distance dependent dielectric constant of 4*r was used, where r denotes distance. Temperature was maintained at 300 K, and 14 cut-off for non-bonding interactions were used. A total of 6 nanoseconds simulations were performed for the six complexes.

10.13147/SOE.2017.013

Chapter 4

Results and discussions

10.13147/SOE.2017.013

4.1 Evidence based information

The objective of this study is to explore the relationships between air pollutant and respiratory allergies. Allergic diseases are characterized by elevated allergen-specific immunoglobulin E (IgE) titres, IgE-dependent activation of mast cells and recruitment of activated eosinophils and T cells to mucosal surfaces (Kay, 2001). The aim of this section is to systematically review the association of allergy, environmental pollutants and histamine receptors.

In this study, we used literature until date for several pollutants to determine its role in allergy. The EPA website provide various information on pollution and pollutants (Koenig, 2012). In this study, full use of the website EPA which provides the information on pollutants was made. EPA categorises pollutants into six common air pollutants also known as "criteria pollutants". Ozone, Particulate Matter, Carbon Monoxide, Nitrogen Oxides, Sulphur Dioxide and Lead are the listed common air pollutants. Exposure to these pollutants is associated with numerous effects on human health, including increased respiratory symptoms, hospitalization for heart or lung diseases, and even premature death.

Based on the listed pollutants, a search on how it mediates Allergy was carried out. The type of the pollutant and its effect on the target population has been tabulated in Table 3

4.1.1 Ozone

Interactions between NOx and hydrocarbons released from traffic and/or industrial sources catalyzed by photochemical reactions lead to the formation of ozone (O3) which is a major component of vehicle-based pollution (Zhang *et al.*, 2014). Ozone exposure has both a priming effect on House dust mite (HDM) allergen induced responses and an intrinsic inflammatory action in the nasal airways of perennially allergic asthmatics (Peden *et al.*, 1995; Peden *et al.*, 1995). This indicates the role of mast cells where evidences have shown the involvement of mast cells in T cell priming following inhaled allergen exposure (Reuter *et al.*, 2010). The exposure also significantly induces mast cells and affects the initiation and maintenance of bronchiolar inflammation and epithelial responses. Taken together, it can be concluded that inhalation of ozone in combination with an IgE-independent mast cell-activating stimulus i.e. HDM leads to the migration of local dendritic cells to the regional lymph nodes and there to an induction of a Th2 response. Th2 response leads to the development of asthma and other allergic inflammatory diseases.

10.13147/SOE.2017.013

4.1.2 Particulate matter

Particulate matter (PM) air pollution is an air-suspended mixture of solid and liquid particles that vary in number, size, shape, surface area, chemical composition, solubility, and origin. PM can be either coarse or fine particles. Coarse particles are often indicated by mass concentrations of particles greater than a 2.5-m cut point. They are derived primarily from suspension or resuspension of dust, soil, or other crystal materials from roads, farming, mining, windstorms, and volcanos. They also include sea salts, pollen, mold, spores, and other plant parts. Fine particles consists of particles with an aerodynamic diameter less than or equal to a 2.5-m cut point. They are derived primarily from direct emissions from combustion processes, such as vehicle use of gasoline and diesel, wood burning, coal burning for power generation, and industrial processes, such as smelters, cement plants, paper mills, and steel mills (Bartra *et al.*, 2007). The most common coarse and fine PM particles are pollen and DEP respectively which are discussed in this study.

DEP

DEPs, are one of the main constituents of urban particulate air pollutants and are associated with allergic respiratory disorders, including asthma and allergic rhinitis (Kelly *et al.*, 2011). A number of studies have shown that DEP enhanced allergic hyper responsiveness (AHR) and allergic airway inflammation. Inhalation of DEP could lead to allergic asthma which is mediated by increased expression of IL-5 (Takano *et al.*, 1998). DEP exposure has also been known to increase the mast cell activation and degranulation process thereby producing histamine (Takano *et al.*, 1998; Dia Sanchez *et al.*, 2000; Salvi, Sundeep and Blomberg, Anders and Rudell, Bertil And Kelly, Frank And Sandstram, Thomas And Holgate, Stephen,t. And Frew, 1999). In a comprehensive manner, DEP exposure might activate not only mast cells which initiate and promote airway inflammation and AHR and also secrete several mast cell-produced mediators that are hallmarks of allergic asthma. In mice, co-exposure of DEP and HDM together exacerbated allergic sensitization (Acciani *et al.*, 2013). Similarly DEP along with pollen increased IgE response (Muranaka *et al.*, 1986). This indicates the adjuvant activity of DEP in eliciting immunological response.

Pollen

Pollen allergens are water-soluble proteins or glycoproteins, which make them readily available biologically, being capable of evoking an IgE antibody-mediated allergic reaction in seconds. Allergenic particles are expelled from the cytoplasm by at least two suggested mechanisms. In the first mechanism, allergens rapidly diffuse when the pollen grain is in direct contact with the mucosa in an isotonic medium, leading to immediate allergic symptoms on the accessible mucosa surfaces such as the conjunctiva and the nose. In the second mechanism a

10.13147/SOE.2017.013

hypotonic medium (such as rain water) allows rapid hydration of the pollen grain which expels allergen-containing inhalable materials that, due to their reduced size, reach lower airways and induce asthma. Thus, allergen release from pollen grains is a prerequisite for its effect in sensitized individuals (TaketomiI *et al*., 2006).

Pollen allergen has been found to stimulate cell activation, accumulation of activated eosinophils and the epithelial migration of mast cells after the administration of pollen allergen (Bentley *et al*., 1992). Currently, environmental pollutants, especially diesel engine exhaust particles, have been considered as significant pollen allergen releasing factors in the air. These particles contain minerals such as silica, iron, aluminium, magnesium, manganese, sulphur, and others. According to Knoxx *et al*, pollen allergens associated with carbon particles from diesel engine fumes (DECP) would concentrate many allergic molecules in a single particle (Knox *et al*., 1997). These findings suggest that the synergistic effects caused by DEPs and pollen allergens might contribute to the major pathways underlying exacerbation of allergic asthma and seasonal allergies.

Nitrogen oxides:

Nitrogen dioxide (NO_2) is a major air pollutant produced by combustion, the main sources being traffic exhaust outdoors and gas appliances indoors. Initially, it has been shown that repeated short exposure to an ambient level of NO2 enhances the airway response to a nonsymptomatic allergen dose. Later it was demonstrated that NO_2 inhalation is injurious to the lung and can augment the degree of allergic airway inflammation and prolong allergen-induced airway hyperresponsiveness in rodent models of asthma (Poynter *et al*., 2005) Moreover, environmental exposure to NO_2 may promote allergen sensitization, resulting in allergic airway disease in response to otherwise innocuous inhaled antigens, even when the inhalation of antigens occurs as much as several days following exposure to NO_2 (Bevelander *et al*., 2007). This allergic sensitization also requires the activation of mast cells which is an important regulatory step for the development of specific T cell responses to the allergen.

The effects of nitrite which is a chemical product of inhaled nitrogen dioxide on mast cell functions were investigated to evaluate the relationship between atmospheric nitrogen dioxide exposure and the development of allergic diseases. High concentrations of nitrite enhanced mast cell histamine release; low concentrations of nitrite did not have significant effects on mast cell functions (Fujimaki *et al*., 1993).

10.13147/SOE.2017.013

Sulphur dioxide:

Sulphur dioxide (SO_2) is one of a group of highly reactive gasses known as "oxides of sulphur." The largest sources of SO_2 emissions are from fossil fuel combustion at power plants and other industrial facilities. Exposure to a combination of sulphur dioxide and nitrogen dioxide in concentrations that could be encountered in heavy traffic enhances the airway response to inhaled allergen (Devalia *et al.*, 1994). The hallmark of allergic inflammation is mucosal esoinophilic infiltration (Ring *et al.*, 2012). SO_2 is found to induce this process of allergic inflammation with increased nasal eosinophil infiltration. This allergic inflammation is an important pathophysiological feature of allergic asthma, which is a chronic airway inflammation that leads to airway obstruction and airway hyperresponsiveness (AHR). This disorder is driven by an unregulated Th2 response to aeroallergens that leads to Th2-type cytokine production in the lung (Dubois *et al.*, 2010). The Th2 response and cytokine production was observed with SO_2 inhalation (A *et al.*, 2008).This clearly indicates that the pollutant SO_2 might lead to allergic inflammation.

Lead:

Lead (Pb) is a metal found naturally in the environment as well as in manufactured products. The major sources of lead emissions have historically been from fuels in on-road motor vehicles (such as cars and trucks) and industrial sources. Lead substantively increased whole blood Pb levels which may promote TH cell dysregulation and alter the availability of key TH1 and TH2 cytokines, effects that could ultimately contribute to development of pulmonary allergic diseases (Hsiao *et al.*, 2011).

10.13147/SOE.2017.013

Table 3 Allergy causing pollutants and its effect

Pollutant	Type	Source	Effect or response	References
Ozone	Human	Mast cell inflammation		Stenfors2010, Kleeberger2001
	Rat mast cell lines		Degranulation of mast cell and PGD2 production	Peden1995
Particulate matter	DEP	Human subjects	Mast cell granulation	Diaz-Sanchez
		Healthy humans	Increase in mast cell number	SALVI1999
Pollen			Mast cell activation, accumulation of activated eosinophils and the epithelial migration of MC	Bentley1992
Nitrogen oxides	NO_2	Human asthmatics	potentiate the specific airway response of patients with mild asthma to inhaled HDM allergen	Devalia1994, Tunnicliffe1994
		Human Bronchial Epithelial Cells	synthesis of proinflammatory cytokines	Devalia1993
	Nirite	peritoneal mast cells (PMC) and intestinal mucosal mast cells	high concentrations of nitrite enhanced mast cell histamine release	Fujimaki1993
Sulphur dioxide	SO_2	Human asthmatics	enhances the airway response to inhaled allergen	Devalia1994
		Mice	Release of Th2-derived cytokines, infiltration of Eosniophils, aggravation of allergic rhinitis	A2008
Lead		Human	TH cell dysregulation, pulmonary allergic diseases.	Hsiao2011

10.13147/SOE.2017.013

Table 4 Pollutant and Histamine receptors

Pollutant	Type of histamine receptor	Source	Effect or response	References	Treatment
Ozone	H3R	Rhesus monkey	HDMA+O3 changed histamine H3 receptor expression in CNS pathways involving lung and nasal afferent nerves	Sekizawa2010	
DEP	H1R	human nasal epithelial cells (HNECs) and human mucosal microvascular endothelial cells (HMMECs)	Increased expression of H1R mRNA, histamine-induced IL-8 and GM-CSF production	TERADA1999	
Pollen	H4R	human blood cells	mRNA expression of the receptor in seasonal AR	Hodge	
	H2R	AR subjects	up-regulation of H2R in Treg cells	Ciebiada2014	
	H1R	Asthmatics		Rafferty1990	Terfanidine
	H1R	patients with a positive skin prick test to grass pollen	allergic rhinitis and conjunctivitis	Howarth1984	Astemizole

In conclusion, all the pollutants listed above form an important constituent in different phases of allergy and allergic diseases. Taken together, these findings clearly demonstrate that mast cells play a vital role in mediating allergic reactions. Therefore, modulation of mast cell activation could be a potential therapeutic strategy for the prevention and treatment of allergic disease. Jemima *et al* have established the functional activation of mast cells by the histamine receptor H4R. This paves the way to know more on the response of H4R towards the environmental pollutants.

Next, a thorough search of literature was performed to identify studies that have determined the response of histamine receptor towards the pollutants. From Table 4, it can be inferred that the presence of all the four receptors have been seen in allergy induced by different pollutants. H4R receptor expression has been studied

10.13147/SOE.2017.013

only recently in human cells to evaluate the effects of atopy and grass pollen season in peripheral blood leukocytes *ex vivo*. However, it has to be noted the expression profile of all the receptor were studied only at mRNA level. The expression levels of the receptor protein when allergy induced by the pollutant has not been explored. H1R antagonist Astemizole and Terfanidine were tested against allergy caused by pollen in the late 1980s. However, the effect of H4R antagonists remains unexplored. Hodge *et al* have alleged that atopy-independent seasonal variation in truncated H4R expression might affect putative negative regulation of full length H4R during high grass pollen season. If verified, this should be considered during the design of drugs targeting H4R to treat allergic inflammation, particularly for seasonal allergic rhinitis.

4.2 3D structure development

To provide evidence for the utility of H4R antagonists in the treatment of allergy caused by pollen, a lead candidate drug has to be developed. The first step in achieving this is developing a 3 dimensional structure of the hH4R. Since hH4R does not have an experimentally determined 3D structure, computational techniques has to be relied. Following are the steps to generate a 3D model using computational tools.

4.2.1 Sequence analysis

Proteins display diverse sequence and structure similarity relationships among themselves. Understanding this similarity relationships of proteins is vital for the designing a model of the protein whose 3D structure is undetermined. In this study, 3D structure of hH4R is determined by using computational tools which requires the knowledge of the sequence similarity of the receptor with other proteins. BLAST is the computational tool used to analyse the given amino acid sequence of hH4R. BLAST listed out a series of sequence that have close similarity with the hH4R sequence. The results are categorised based on different calculations.

Max score = highest alignment score (bit-score) between the query sequence and the database sequence segment.

Total score = sum of alignment scores of all segments from the same database sequence that match the query sequence (calculated over all segments). This score is different from the max score if several parts of the database sequence match different parts of the query sequence.

Query coverage = percent of the query length that is included in the aligned segments. This coverage is calculated over all segments (cf. total score).

E-value = number of alignments expected by chance with a particular score or better. The expect value is the default sorting metric and normally gives the

10.13147/SOE.2017.013

same sorting order as Max score. This aids in judging the level of confidence to on alignment (Madden, 2013).

Based on the above calculations, BLAST generated a Table as shown in Figure 15

Description	Max score	Total score	Query cover	E value	Ident	Accession
Chain A, M3-mt4l Receptor Bound To Tiotropium	137	137	90%	1e-35	27%	4U15_A
Chain A, Structure Of Active Human M2 Muscarinic Acetylcholine Receptor Bound To The Agonist Iperoxo	127	127	89%	1e-32	29%	4MQS_A
Chain A, Crystal Structure Of The Chimeric Protein Of 5-ht1b-bril In Complex With Dihydroergotamine (psi Community Target)	122	122	94%	1e-30	24%	4IAQ_A
Chain A, Crystal Structure Of The Chimeric Protein Of 5-ht1b-bril In Complex With Ergotamine (psi Community Target)	120	120	94%	3e-30	24%	4IAR_A
Chain A, Structure Of The M3 Muscarinic Acetylcholine Receptor Bound To The Antagonist Tiotropium Crystallized With Disulfide-stabilized T4 Lysozyme (dst4l)	109	169	69%	3e-26	34%	4U14_A
Chain A, Structure Of The M3 Muscarinic Acetylcholine Receptor	108	167	68%	1e-25	34%	4DAJ_A
Chain A, Structure Of The Human M2 Muscarinic Acetylcholine Receptor Bound To An Antagonist	99.4	154	67%	1e-22	33%	3UON_A
Chain A, Crystal Structure Of The Human Beta2 Adrenoceptor	96.7	96.7	97%	5e-22	23%	2R4R_A
Chain A, Crystal Structure Of A Methylated Beta2 Adrenergic Receptor- Fab Complex	96.3	96.3	97%	6e-22	23%	3KJ6_A
Chain A, Crystal Structure Of The Human Beta2 Adrenoceptor	95.1	95.1	94%	1e-21	23%	2R4S_A
Chain R, Crystal Structure Of The Beta2 Adrenergic Receptor-Gs Protein Complex	94.4	94.4	94%	1e-20	24%	3SN6_R
Chain A, Crystal Structure Of The Chimeric Protein Of 5-ht2b-bril In Complex With Ergotamine	89.4	89.4	90%	3e-19	23%	4IB4_A
Chain A, Structure Of The Human Dopamine D3 Receptor In Complex With Eticlopride	85.9	140	52%	6e-18	34%	3PBL_A
Chain A, Structure Of The Human Histamine H1 Receptor In Complex With Doxepin	84.3	150	68%	2e-17	26%	3RZE_A
Chain A, Crystal Structure Of The Chimeric Protein Of A2aar-Bril In Complex With Zm241385 At 1.8a Resolution	81.6	81.6	89%	1e-16	22%	4EIY_A
Chain A, Cholesterol Bound Form Of Human Beta2 Adrenergic Receptor	79.3	124	70%	9e-16	28%	3D4S_A
Chain A, Crystal Structure Of Human Adenosine A2a Receptor With An Allosteric Inverse-Agonist Antibody At 2.7 A Resolution	77.0	77.0	89%	2e-15	25%	3VG9_A
Chain A, Structure Of A Nanobody-Stabilized Active State Of The Beta2 Adrenoceptor	78.2	124	71%	2e-15	28%	3POG_A
Chain A, High Resolution Crystal Structure Of Human B2-Adrenergic G Protein- Coupled Receptor	77.8	123	71%	2e-15	28%	2RH1_A

Figure 15 BLAST of hH4R

A list of homologous sequence of hH4R is displayed in the above Figure 15. The first crystal structure whose sequence is identical to hH4R is the crystal structure of Human Beta 2 adreno receptor (2R4R A). It is 23% identical with hH4R and the next sequence in this order during the preparation of the manuscript was the High resolution crystal structure of Human B2 adregenic GPCR (2RH1 A) which is 28% identical. Respective BLAST analysis for 2RH1 A and 2R4RA with hH4R are shown in Figure 16 and Figure 17

In support of this analysis, another study by Levita *et al* also identified beta-2-adrenergic receptor (2RH 1 A) and human adenosine A2A receptor (3 EM1 A) as the top hit by BLAST search of the SWISS-MODEL (Levita *et al*., 2012). During the preparation of both the manuscript, the crystal structure of H1R has not been published. Figure 15 shows the results of BLAST search performed after the generation of the crystal structure of H1R (Shimamura *et al*., 2011). The H1R shared 26% identity with H4R with an e value of 2e-17. This identity percentage is lesser when compared to the identity percentage between 2RH1 A and H4R but the E value remained lower.

10.13147/SOE.2017.013

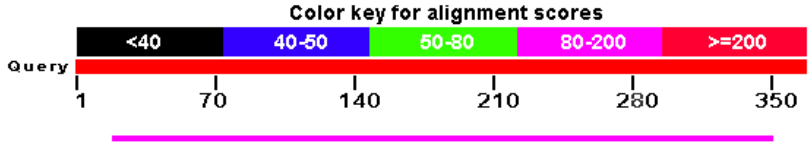

Figure 16 BLAST of 2R4R A against hH4R

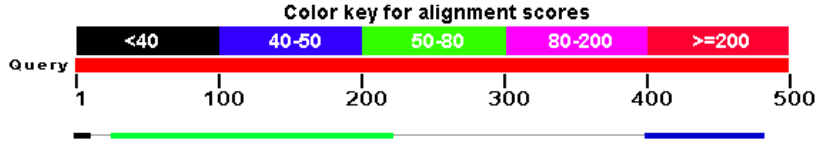

Figure 17 BLAST of 2RH1 A against hH4R

4.2.2 Automated generation of the structures by fold recognition

Experimental determination of protein structure through X-ray crystallography or nuclear magnetic resonance spectroscopy remains a difficult and costly process. Hence dependence on computational methods has greatly increased. The 3D structure of hH4R has not been experimentally predicted yet. This urged researchers to develop their own 3D structure model of hH4R. Since hH4R is a GPCR, the structure was modelled using another known 3D structure of a GPCR as a template. Most of the studies were dependent on the crystal structure of bovine rhodopsin (Kiss *et al.*, 2008; Rahim, 2010). With the advent of the determination of the crystal structure of human GPCR, ß2 adrenegic receptor, researchers have started to develop models of hH4R based on it.

In the present study, I-TASSER, a threading alignment tool was employed to predict the 3D structure model of hH4R. Fold recognition or threading method was used to develop the 3D model since sequence identity was less than 30% (Zhang, 2008). Five models (Figure 18-22) 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 I-TASSER is the high-resolution crystal structure of human ß2-adrenergic GPCR (PDB ID: 2rh1A). The remaining templates used for threading are the isoforms of human ß2-adrenergic GPCR. It is contradictory to the BLAST search result which predicted that human adrenoreceptor (2R4R A) has the close sequence similarity with hH4R compared to human ß2-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.

The output also contained functional annotations on ligand-binding sites, Enzyme Commission numbers, and Gene Ontology terms of the top models. Accuracy of the predictions was also provided based on the confidence score (C-score), which is an estimate of the confidence of structure prediction. C-score is

10.13147/SOE.2017.013

typically in the range -5 to 2; a higher score reflects a model of better quality. In general, models with C-score >-1.5 are considered to have a correct fold.

In the present investigation, Model 1 gained the highest score followed by model 2, which signifies its highest confidence than the other models as shown in

Table 5. Figure 18 to Figure 22 are the 3D structures of hH4R predicted by I-TASSER and are designated as Model 1, Model 2, Model 3, Model 4 and Model 5.

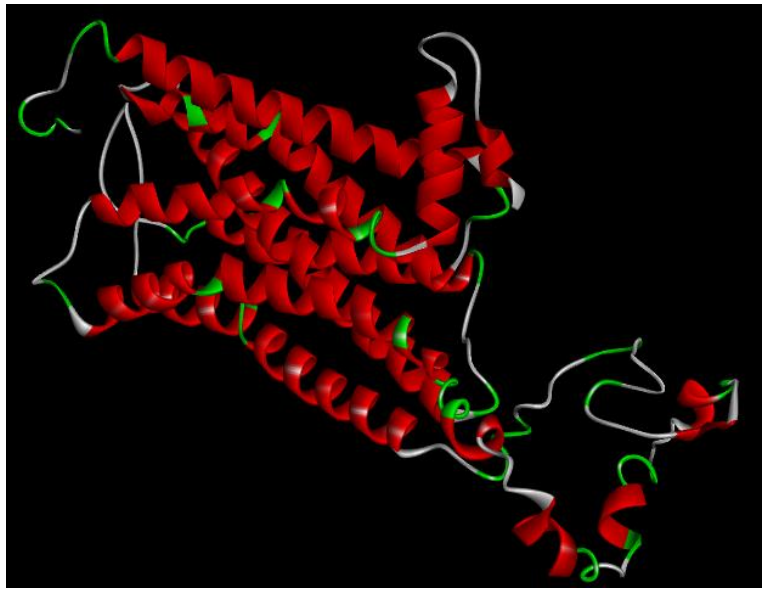

Figure 18 3D Model 1 of hH4R predicted by I-TASSER

10.13147/SOE.2017.013

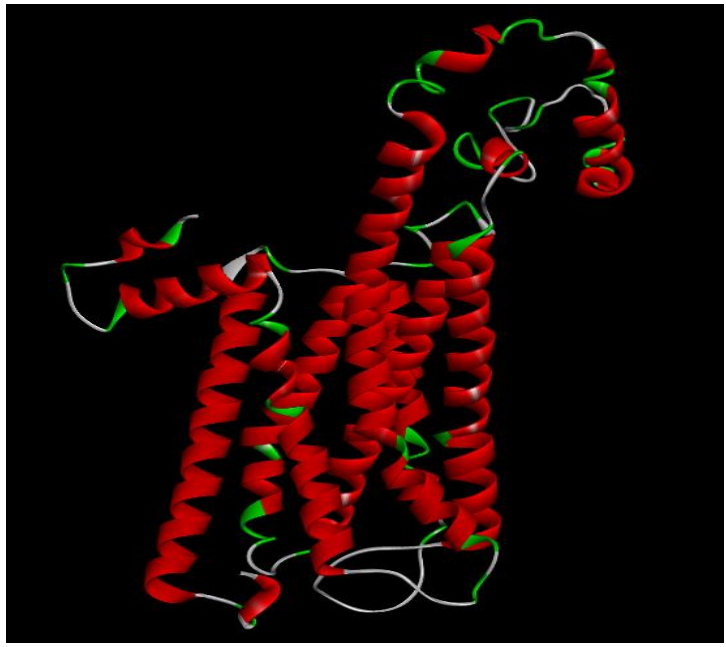

Figure 19 3D Model 2 of hH4R predicted by I-TASSER

Figure 20 3D Model 3 of hH4R predicted by I-TASSER

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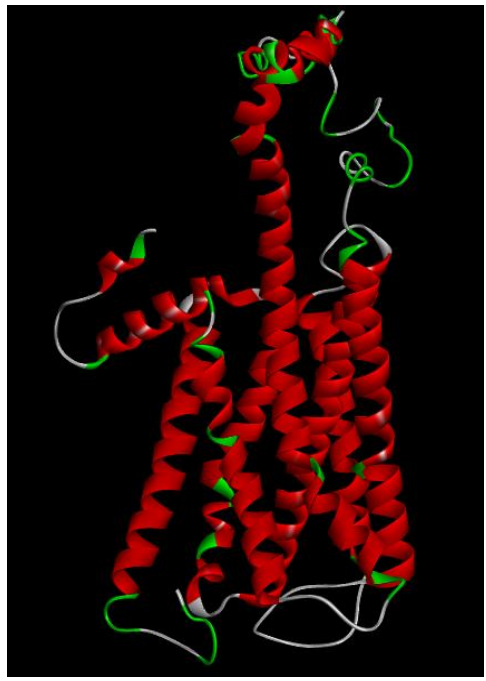


Figure 21 Model 4 of hH4R predicted by I-TASSER

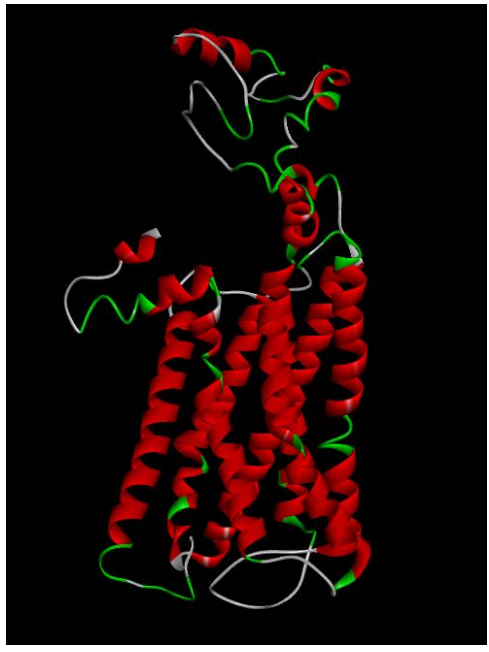


Figure 22 Model 5 of hH4R predicted by I-TASSER

10.13147/SOE.2017.013

Table 5 C-score of the models

Predicted Models	C-score
Model 1	-0.52
Model 2	-0.74
Model 3	-1.84
Model 4	-4.15
Model 5	-5

In the earlier studies, H4R models were generated by Homology modelling, this probably would be the first study to use Threading methodology in modelling. The reasons for selecting this methodology are discussed here. When the sequence identity drops below 30%, termed the "twilight zone," Homology modelling model accuracy sharply decreases because of the lack of a significant structure match and substantial alignment errors. This is because; the models provided by homology modelling are often closer to the template on which the model is based rather than the native structure of the sequence of interest. This has been a significant unsolved problem (Tramontano *et al.*, 2003). Moreover, TASSER often refines the structures closer to native than the templates on which they are based (Zhang *et al.*, 2004; Zhang *et al.*, 2004). Therefore, full-length TASSER models offer substantial advantages over traditional homology modelling methods and are likely to be of greater aid in understanding the ligand and signalling interactions of GPCRs (Zhang *et al.*, 2006). Recently, a hybrid protocol in I-TASSER was proposed to construct GPCR structure models that integrates experimental mutagenesis data with ab initio transmembrane (TM) helix assembly simulations (Zhang *et al.*, 2015)

With the rapid advancements in technologies, the 3D structure of the first histamine receptor was experimentally determined after the completion of the manuscript. Shimamura *et al* in 2012 predicted the crystal structure of H1R complex with doxepin, a first-generation H1R-antagonist (Shimamura *et al.*, 2011). This made the researchers to use H1R as a template for building 3D structure of H4R (Feng *et al.*, 2013; Nijmeijer *et al.*, 2013). Typically, H4R shares high sequence similarity with H3R than H1R. At the protein level, H4R and H3R share 38% sequence identity and 53.6% sequence similarity. While in the trans-membrane domain, they share up to 54% sequence identity. However, H1R only shares ∼20% sequence identity with H3R and ∼23% sequence identity with H4R (de Esch *et al.*, 2005). Furthermore, sequence identity between the H4R and H1R binding site (28%) is only slightly higher than between the H4R and β2R binding site (26%,) (Kooistra *et al.*, 2013). Although the TM fold of the β2R and H1R crystal structure templates are similar, the different EL2 loop conformations (in particular the orientation of F168) results in different H4R models. As a result, the ligands identified in prospective virtual screening studies have similar binding modes in β2R based and H4R models,

10.13147/SOE.2017.013

including H-bond conserved H-bond interactions with D943.32 and E1825.46, but adopt slightly different orientations in the EL2 region (Istyastono *et al.*, 2015).

However with the advent of experimentally determined 3D structure of H1R, the importance of ß2-adrenergic GPCR cannot be neglected. ß2-adrenergic GPCR is an ideal model system because of its agonist role in airway diseases (Brown *et al.*, 2007). Interestingly, H4R models based on β2R and H1R crystal structure templates were equally successful in explaining H4R mutation data, while H1R-based H4R models could better explain ligand SAR than β2R-based H4R models (Schultes *et al.*, 2013). Later in 2015, Istyastono *et al* have revealed that the H4 model based on β2R and H1R differ in their binding pocket structure. Though both modelling templates yield H4R models with good early enrichments, the retrospective virtual screening accuracy of the β2R-based models is higher than the H1R based H4R models (Istyastono *et al.*, 2015). From this it is evident that ß2-adrenergic GPCR is also an efficient template even after the discovery of the crystal structure of H1R.

PROCHECK

PROCHECK computes Ramachandran plot for each model (Figure 23-27). The Ramachandran plot provides an easy way to view the distribution of torsion angles Phi and Psi of a protein structure. It also provides an overview of allowed and disallowed regions of torsion angle values, serving as an important indicator of the quality of protein three-dimensional structures. The plot has three regions:

1. The white regions correspond to conformations where atoms in the polypeptide come closer than the sum of their Van der Waals radii. These regions are sterically disallowed for all amino acids except glycine which is unique because it lacks a side chain.

2. The red regions correspond to conformations with no steric clashes, i.e. these are the allowed regions namely the αa-helical and α-sheet conformations.

3. The yellow areas show the allowed regions if slightly shorter van der Waals radii are used in the calculation, i.e. the atoms are allowed to come a little closer together. This brings out an additional region which corresponds to the left-handed a-helix.

Table 6 shows that the quality of the Ramachandran plot for Model 2 and was found to be better than the other models. The Phi/Psi distribution exposes that 96.2% of the residues in the Model 2 are in the most favoured or allowed regions, which is high when compared to the other models (Model 1: 93.9%, Model 3: 95%, Model 4: 93%, Model 5: 92.8%). This high percentage of Phi/Psi angles in the

10.13147/SOE.2017.013

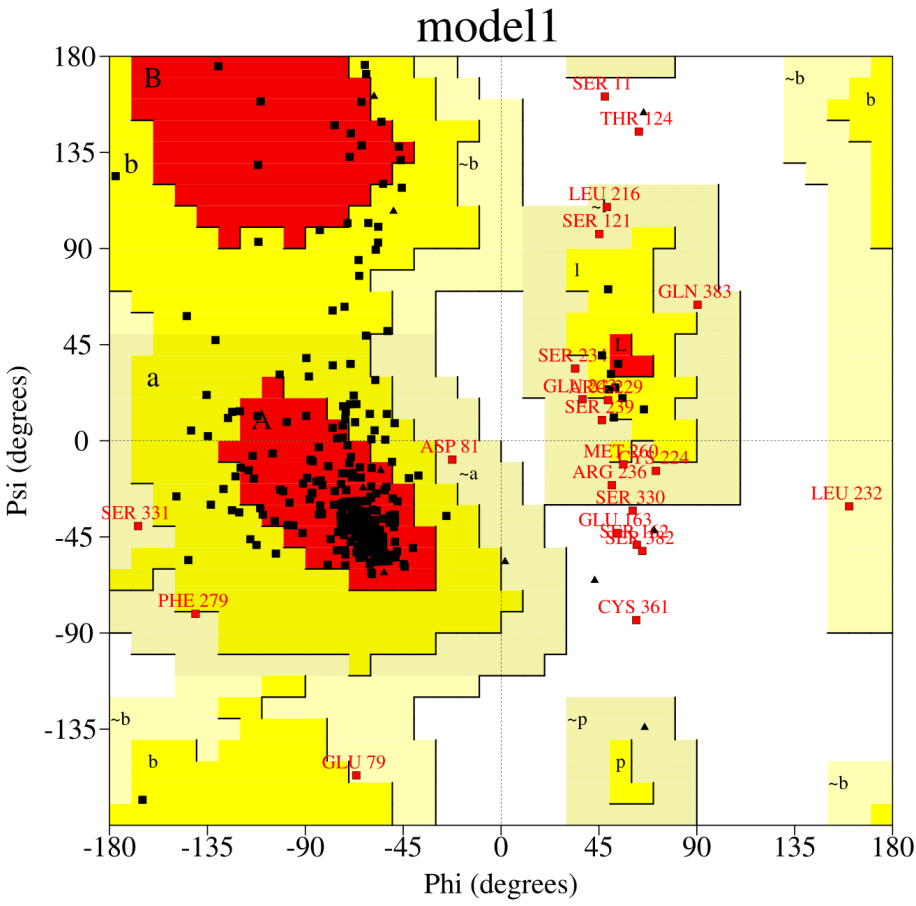
allowed and the disallowed regions suggest model 2 to be superior to the others. The Ramachandran plots of all the models are shown in Figure 23 to Figure 27.

Other studies have also exploited PROCHECK to validate the model generated. H4R model generated by Homology modelling with bovine receptor as template had Phi/Psi distribution that shows 97.5% of the residues in the favourable regions (Kiss *et al.*, 2008), however Model 2 showed only 96.2 % residues in the favoured region. In another study, H4R modelled on 2RH1 A had only 90.9% in favoured region which is less when compared to our output (Levita *et al.*, 2012). Comparison of Model 2 with the other models makes the validation of Model 2 convincing.

Table 6 Main geometric parameters of the model prediction and validation

Structure	Core (%)	Allowed (%)	General (%)	Disallowed (%)
Model 1	76.2	17.7	4.2	1.9
Model 2	77.6	18.6	2.2	1.7
Model 3	72.6	22.4	3.3	1.7
Model 4	72.9	20.2	3.9	3.0
Model 5	69.3	23.5	3.6	3.6

10.13147/SOE.2017.013



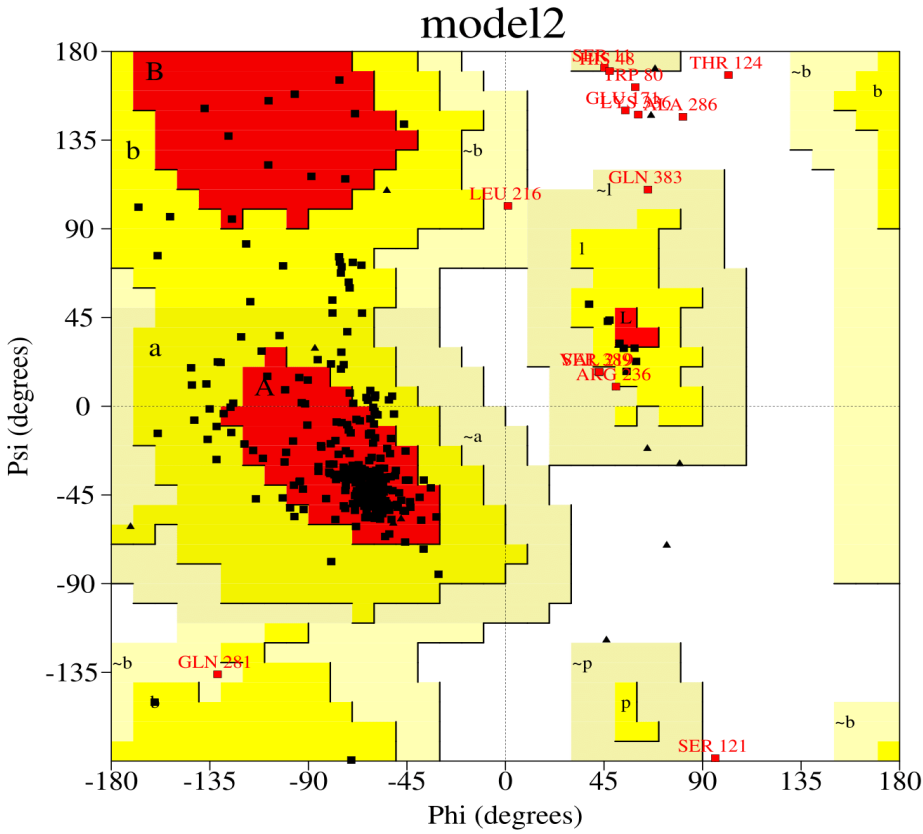
Plot statistics

Residues in most favoured regions [A,B,L]	275	76.2%
Residues in additional allowed regions [a,b,l,p]	64	17.7%
Residues in generously allowed regions [~a,~b,~l,~p]	15	4.2%
Residues in disallowed regions	7	1.9%
Number of non-glycine and non-proline residues	361	100.0%
Number of end-residues (excl. Gly and Pro)	2	
Number of glycine residues (shown as triangles)	13	
Number of proline residues	14	
Total number of residues	390	

Based on an analysis of 118 structures of resolution of at least 2.0 Angstroms and R-factor no greater than 20%, a good quality model would be expected to have over 90% in the most favoured regions.

Figure 23 Ramachandran plot of Model 1

10.13147/SOE.2017.013



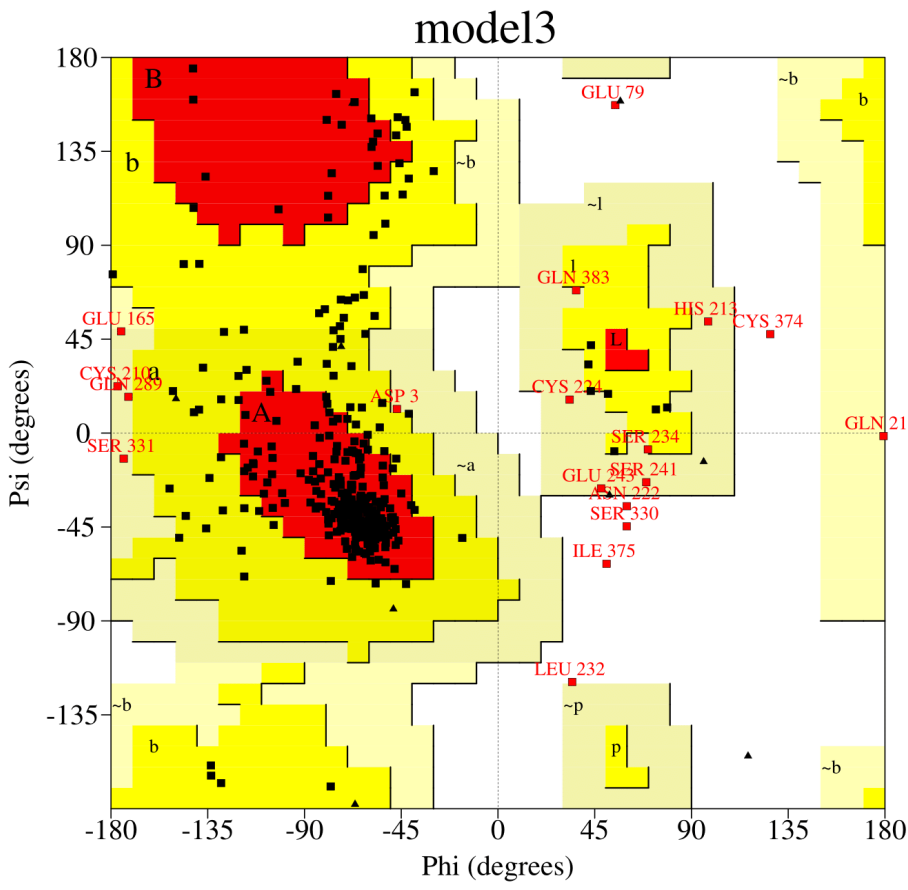
Plot statistics

Residues in most favoured regions [A,B,L]	280	77.6%
Residues in additional allowed regions [a,b,l,p]	67	18.6%
Residues in generously allowed regions [~a,~b,~l,~p]	8	2.2%
Residues in disallowed regions	6	1.7%
Number of non-glycine and non-proline residues	361	100.0%
Number of end-residues (excl. Gly and Pro)	2	
Number of glycine residues (shown as triangles)	13	
Number of proline residues	14	
Total number of residues	390	

Based on an analysis of 118 structures of resolution of at least 2.0 Angstroms and R-factor no greater than 20%, a good quality model would be expected to have over 90% in the most favoured regions.

Figure 24 Ramachandran plot of Model 2

10.13147/SOE.2017.013



Plot statistics

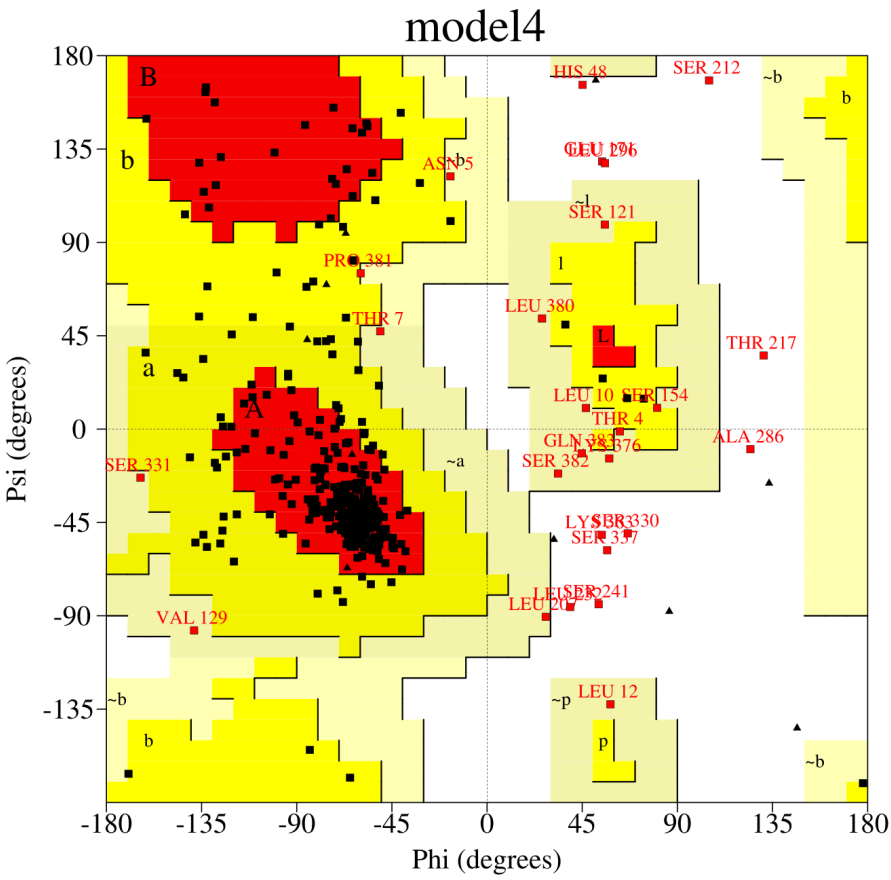
Residues in most favoured regions [A,B,L]	262	72.6%
Residues in additional allowed regions [a,b,l,p]	81	22.4%
Residues in generously allowed regions [~a,~b,~l,~p]	12	3.3%
Residues in disallowed regions	6	1.7%
	----	------
Number of non-glycine and non-proline residues	361	100.0%
Number of end-residues (excl. Gly and Pro)	2	
Number of glycine residues (shown as triangles)	13	
Number of proline residues	14	

Total number of residues	390	

Based on an analysis of 118 structures of resolution of at least 2.0 Angstroms and R-factor no greater than 20%, a good quality model would be expected to have over 90% in the most favoured regions.

Figure 25 Ramachandran plot of Model 3

10.13147/SOE.2017.013



Plot statistics

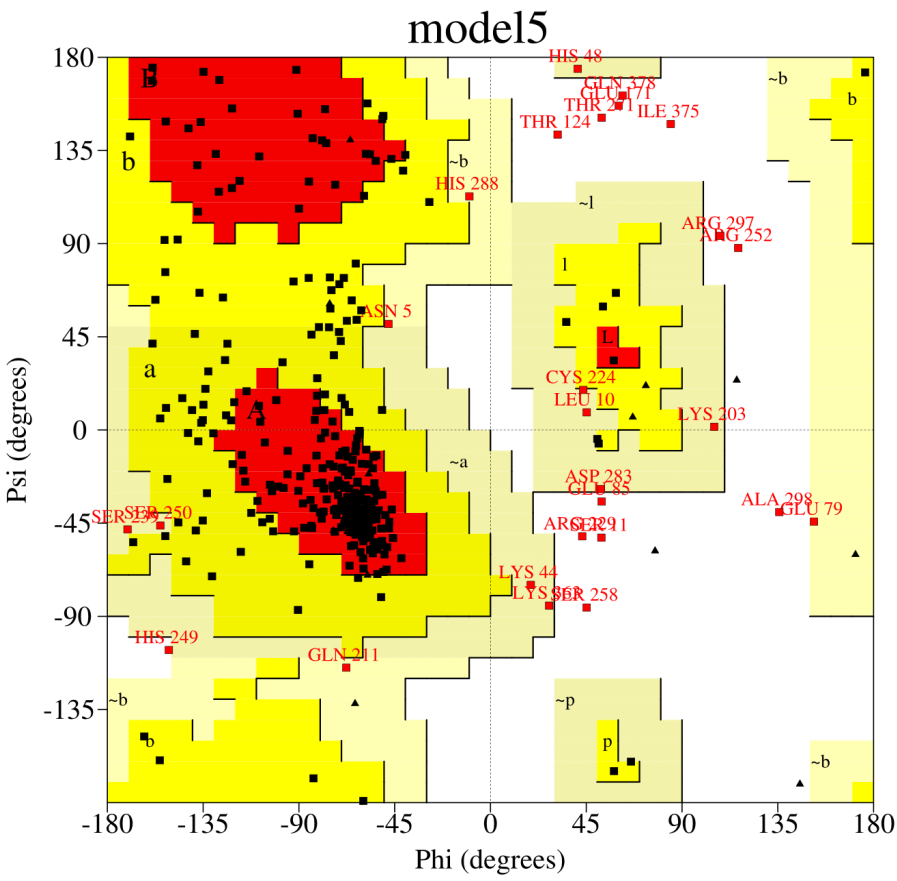
Residues in most favoured regions [A,B,L]	263	72.9%
Residues in additional allowed regions [a,b,l,p]	73	20.2%
Residues in generously allowed regions [~a,~b,~l,~p]	14	3.9%
Residues in disallowed regions	11	3.0%
	----	------
Number of non-glycine and non-proline residues	361	100.0%
Number of end-residues (excl. Gly and Pro)	2	
Number of glycine residues (shown as triangles)	13	
Number of proline residues	14	

Total number of residues	390	

Based on an analysis of 118 structures of resolution of at least 2.0 Angstroms and R-factor no greater than 20%, a good quality model would be expected to have over 90% in the most favoured regions.

Figure 26 Ramachandran plot of Model 4

10.13147/SOE.2017.013



Plot statistics

Residues in most favoured regions [A,B,L]	250	69.3%
Residues in additional allowed regions [a,b,l,p]	85	23.5%
Residues in generously allowed regions [~a,~b,~l,~p]	13	3.6%
Residues in disallowed regions	13	3.6%
	----	------
Number of non-glycine and non-proline residues	361	100.0%
Number of end-residues (excl. Gly and Pro)	2	
Number of glycine residues (shown as triangles)	13	
Number of proline residues	14	

Total number of residues	390	

Based on an analysis of 118 structures of resolution of at least 2.0 Angstroms and R-factor no greater than 20%, a good quality model would be expected to have over 90% in the most favoured regions.

Figure 27 Ramachandran plot of Model 5

10.13147/SOE.2017.013

ERRAT

The second program used for the validation is ERRAT. Good and high resolution structures generally produce values around 95% or higher. For lower resolutions, the average overall quality factor is around 91%. The overall quality factor of model 2 is 96.597%, which is high when compared to other models (Table 7).

Table 7 ERRAT

Model	Quality factor %
Model 1	92.670
Model 2	96.597
Model 3	89.527
Model 4	92.147
Model 5	90.052

4.2.3 Choosing the best model

There are five models and it is required that only one model is used for the further studies. The C-score of I-TASSER describes Model 1 as the best, while the Quality factor from ERRAT and Ramachandran plot from PROCHECK predicts Model 2 as the finest comparing the others. Taken together, Model 2 (Figure 19) has been chosen for further analysis and studies.

The chosen model was subjected to Energy minimization which is also performed in most of the models generated by Homology modelling (Sirci *et al.*, 2012; Engelhardt *et al.*, 2013). In recent studies, further refinements of the generated 3D models are being carried out with new techniques. Molecular dynamics (MD) simulations in an explicit water-membrane environment have been employed in a handful of instances to probe the structure of GPCRs (Grossfield, 2011; Hanin *et al.*, 2005). An equilibrated hH4R structure in a membrane environment was set up to improve the quality of the 3D model of the H4R modelled based on the crystal structure of H1R (Pappalardo. *et al.*, 2014). This use of an explicit bilayer environment is viewed as an enhancement to the model, as it grants access to a detailed view of molecular interactions involving solvent molecules.

4.2.4 Transmembrane topology of Histamine H4 receptor

Transmembrane helices form the integral structure of the protein; therefore its exact location reveals functional annotation and direct functional analysis. The transmembrane topology of the receptor is predicted via different prediction tools such as HMM Top, TM HMM, Tm Pred and SOSUI and the results are consolidated in Table 8. Model 2 was analysed both structurally and

10.13147/SOE.2017.013

visually with Discovery Studio 2 and the amino acid sequences of the transmembrane were recorded. Based on the analysis, TMH1 is present between 16 and 42, TMH2 between 51 and 75, TMH3 between 86 and 121, TMH4 between 130 and 151, TMH5 between 172 and 195, TMH6 between 305 and 329, and TMH7 between 339 and 369. These TM regions fall in accordance with the prediction by the servers. The TM regions are highlighted with the respective amino acids in Figure 28. The transmembrane helices as predicted by Kiss *et al* for H4R is TMH1 15-41, TMH2 52-74 TMH3 88-110, TMH4 132-153, TMH5 174-195, TMH6 301-324 and TMH7 339-360 (Kiss *et al*., 2008). This prediction of TM is closely similar to our predictions except with differences of one or two amino acids. However, prediction of the TMH3 varies with more than 10 amino acids. This might be due to the difference in the template and computational tool used for the prediction by Kiss *et al* which are Bovine rhodopsin and ClustalW respectively.

Though the transmembrane residues have been detected, the membrane environment is lacking in our model. This lack of knowledge might lead some ligands to position partially outside the protein. Hence only ligands which completely fit in the binding site can be chosen as the lead candidate (Kiss *et al*., 2008). However, recent studies have started focussing on the membrane environment and are discussed in the above section. To study the structural integrity of the H4R receptor Paapalardo *et al* performed molecular dynamics stimulation to observe the trans-membrane domain. The seven helices of the trans-membrane domain remained unaltered over the whole simulation. On the contrary, residues spanning from 200 to 290, belonging to flexible domain of the receptor, exhibited a highly dynamic equilibrium between helical, random coil and turn structural motifs. However, residues 35, 75, 110, 150 and 330 remained unstructured (Pappalardo. *et al*., 2014).

Table 8 Prediction of transmembrane regions using various web servers

Transmembrane	HMM Top	TM HMM	TmPred	SOSUI
Helix 1	18–42	20–42	16–42	19–41
Helix 2	51–73	51–73	52–72	53–75
Helix 3	86–109	88–110	91–109	99–121
Helix 4	132–153	130–152	131–151	130–151
Helix 5	174–193	172–194	173–195	173–195
Helix 6	305–327	307–329	306–328	306–328
Helix 7	342–361	339–369	341–362	341–362

10.13147/SOE.2017.013

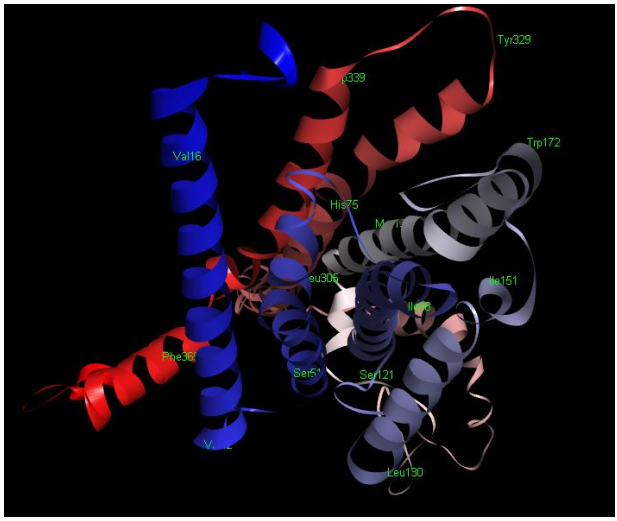


Figure 28 TM of hH4R

4.2.5 Analysis of the binding site

Determining the binding site is crucial for docking and further analysis. Accurate prediction of putative binding sites on the protein surface is helpful for rational drug design on target proteins. The ligand-binding sites were predicted with the help of binding pocket detection server tools such as pocket finder and Q-site finder (http://www.modelling.leeds.ac.uk/qsitefinder). In addition to that, the binding pockets of the receptor were also determined by using Discovery studio.

The binding site of the model in this study was predominantly based on previous literatures that are discussed subsequently. Generally, histamine has two major anchoring points at the hH4R binding site. Site directed mutagenesis have revealed the crucial role of Asp94 (3.32) and Glu182 (5.46) residues of H4R in histamine binding (Shin *et al.*, 2002). Another study of mutagenesis and docking together with ligands such as histamine, clopazine and non-imidazole agonist VUF 8430 in a rhodopsin-based homology model also revealed the interactions in the binding pocket with Asp94 and Glu182 (Jongejan *et al.*, 2008). Later, Kiss *et al* identified that Thr323 also helped in histamine binding, however the mutational data is unavailable (Kiss *et al.*, 2008). Similarly, every hit compound screened by Kiss *et al* formed interactions with either Asp94 or Glu182 (Kiss *et al.*, 2008). In another study, the binding site of the receptor model with 2RH1 A template as predicted by Q-site finder included amino acids Asp94, Tyr95, Glu182, Trp316, Tyr319, and Phe344 (Levita *et al.*, 2012). In regard to the transmembrane, the binding site of hH4R is predominantly formed by residues of TM3, TM5, and TM6 helices (Jaajrt *et al.*, 2008; Buschauer *et al.*, 2015).

10.13147/SOE.2017.013

Other amino acid residues which indulge in distinct roles were studied by mutagenesis. It was also demonstrated that mutations of Thr178 (5.42), Ser179 (5.43), Asn147 (4.57), and Ser320 (6.52) have only a minor effect on histamine binding; on the other hand, mutations of Asn147 (4.57) and Ser320 (6.52) have a significant effect on the hH4R activation process (Jaajrt *et al*., 2008). It has been reported previously that JNJ777120 interacts with Asp94 and Glu182 and forms lipophilic interactions with Val64, Phe312, Trp316, Tyr319, and Trp348 (Kiss *et al*., 2008). Generally, the protonated ethylamine sides and other protonated parts (NH) of the ligands interact with Asp 94 in H4R, while the imidazole NH of agonists interacts with Glu 182 (Feng *et al*., 2013).

This information on the important binding site residues served as valuable evidence in locating the exact binding site in Model 2. Moreover, based on the ligands bound in the crystal structure of human β2 adrenergic GPCR, the binding site residues Phe193, Tyr199, Ser103, Ser204, Asn393, Tyr308, Trp109, Asn312, Trp109, Asn312, Asp103, Val104, Phe289, Phe290, Thr118, Val117, Trp286, and Tyr316 were identified. As the H4R and B2 adrenergic GPCR share structural similarity, the superimposition of these two structures revealed the corresponding amino acid residues in H4R. The corresponding amino acid residues in the model are Phe168, Ile174, Tyr340, Trp90, Phe344, Lys84, Tyr319, Ser320, Glu182, Thr88, Cys98, Trp316, and Trp348.

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 that were cited in the previous paragraph. Hence, site 2 is considered as the best binding site for further docking studies and is depicted in the following Figure (Figure 29).

Though the X-ray crystallographic structure of H1R has been delineated, the determination of the exact binding site of H4R still remains a challenging task. Recently, SAR and mutagenesis studies in combination with docking and MD-simulations were used to elucidate the protein -ligand interactions (Schultes *et al*., 2013). By keeping Asp 94 and Glu 182 as the key residues, the binding modes of different ligand classes in H4R were explained by them. In another study molecular dynamics (MD) simulations were performed for H4R in complex with its compounds. The stable docking mode was identified by this MD studies (Feng *et al*., 2013). In another study, SAR models, protein-based H4R modelling studies, and in silico guided site-directed mutagenesis experiments were combined to identify the molecular determinants that drive H3R/H4R selectivity. This information was used

10.13147/SOE.2017.013

to elucidate the binding modes of clobenpropit and its analogues in the H4R binding pocket (Istyastono *et al.*, 2011). Conclusively, it is not essential that docking of histamine is necessary to identify the binding site, because the antagonists of H4R are competitive and they displace histamine in a competitive manner. Therefore, pharmacological data suggests that any nonimidazole compounds can be used to describe the orthosteric binding site of the H4R (Smits *et al.*, 2006).

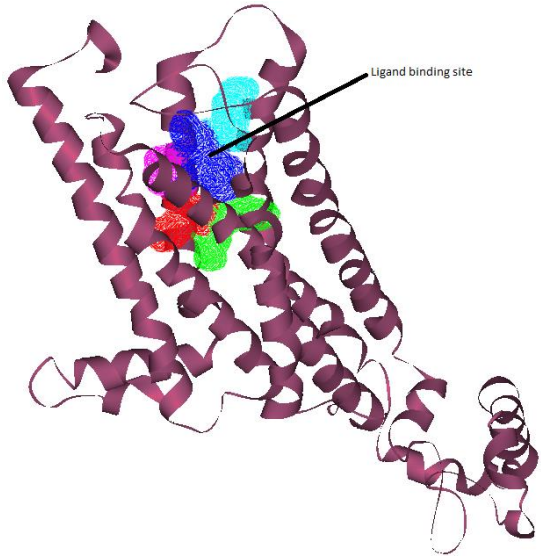


Figure 29 Ligand binding site of hH4R model

4.2.6 Structure based virtual screening

Structure-based drug discovery (SBDD) is becoming an essential tool in assisting fast and cost-efficient lead discovery and optimization. The application of rational, structure-based drug design is proven to be more efficient than the traditional way of drug discovery since it aims to understand the molecular basis of a disease and utilizes the knowledge of the three-dimensional structure of the biological target in the process. Although JNJ-7777120 has been widely used as reference antagonist to investigate the H4R, unfavourable pharmacokinetic properties and questions arising from partial agonist activity with low to moderate intrinsic activity in certain pharmacological models, underline the urgent need for new bioactive compounds. The remarkable feature for the H4R is that rather small structural variations in the ligands may result in largely changed functional properties (Sander *et al.*, 2008). Hence one of the aims of this study is to identify ligands using the known ligands JNJ7777120, thioperamide and Vuf6002 as the starting point. PubChem database was used in this study and it is the largest database of chemical structures and also consists of validation and standardisation

10.13147/SOE.2017.013

protocols for example, the structure is checked for valid atom types, valence checks are performed and functional groups such as nitro groups are converted to a consistent representation (Hersey *et al.*, 2015).

Three different databases of chemical structures similar to JNJ777120, thioperamide and Vuf6002 were built for virtual screening. This database was created with the structures retrieved from PubChem. A similar approach was carried out where databases were prepared based on compounds compiled from the catalogues of various suppliers and then interrogated using the two query compounds (JNJ7777120 and Thioperamide) and a variety of virtual screening techniques. A set of 1177 compounds was initially selected from the results of these searches (Cramp *et al.*, 2010). Other studies have used alternate database for the virtual screening. Kiss *et al* in their large scale virtual screening used ZINC database which provided 8743666 3D structures of small molecules (Kiss *et al.*, 2008). Papplardo *et al* selected randomly 9000 compounds from the ZINC database to represent a set of presumably inactive molecules at hH4R (Pappalardo. *et al.*, 2014). Databases for H4R ligands were also created using in-house fragment library and the ChEMBL database for structure-based and ligand-based (LBVS) virtual screening approaches (Istyastono *et al.*, 2015). A set of 1177 compounds was initially selected from the results of these searches.

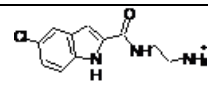
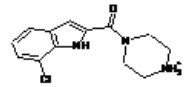
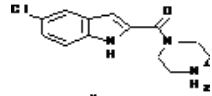
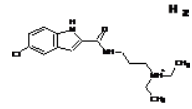
In the present study, the screening of the top hits was based on the following criteria which were followed by Kiss *et al* (Kiss *et al.*, 2008). Structures were inspected visually considering the following criteria: (i) the ligand has to be positioned entirely into the binding site, and (ii) potential H-bond donor group. In addition, interaction(s) with Asp94 (3.32) or Glu182 (5.46) was taken into account as a positive feature.

JNJ7777120 database

This database comprises similar structures of the JNJ7777120 as retrieved from PubChem. As mentioned in the methodology, 150 similar structures of JNJ7777120 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. The top four structures with high dock score are listed in Table 9.

10.13147/SOE.2017.013

Table 9 Top 4 compounds from JNJ777120 database

S.No	Name	PubChem id	Structure	DockScore
1	Compound A	39732646		115.116
2	Compound B	39732686		102.132
3	Compound C	39732675		83.560
4	Compound D	7317615		68.223

IUPAC name of Compound A is 2-[(5-chloro-1H-indole-2-carbonyl) amino] ethylazanium. Input of the canonical SMILES of Compound A in ChemSpider website suggested a close match with the structure "N-(2-Aminoethyl)-5-chloro-1H-indole-2-carboxamide". This structure is an allosteric modulator of cannabinoid type 1 (CB1) receptor which is a GPCR. This indirectly suggests a probable link between compound A and histamine receptor since histamine receptors are GPCR. Furthermore, analysis with Discovery Studio showed that Compound A formed hydrogen bond interaction with Asp94 (2.18) (Figure 30) with the receptor, whereas other compounds did not reveal any interaction.

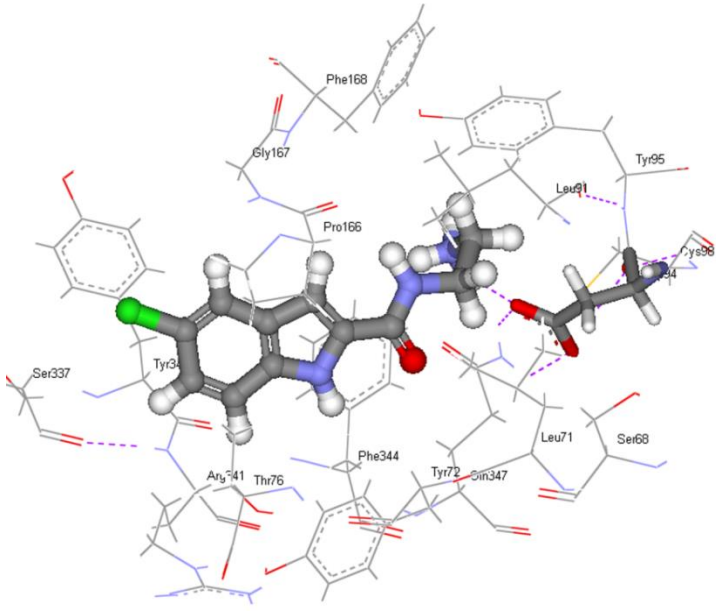


Figure 30 Binding mode of Compound A with the receptor

10.13147/SOE.2017.013

Analysis of the difference in structure between Compound A and JNJ7777120 shows that the former lacks the methyl piperizine moiety. Moreover, compound A has only a slight modification of compound 12 (Figure 31) that was previously reported by Kiss *et al.* (Kiss *et al.*, 2008). The compound 12 identified by them lacked the 5-chloro substituent on the indole ring and contained a simple ethylamine side chain, whereas compound A possessed chlorine substituents on the indole ring. Comparing Compound 12 and JNJ777120, the authors have interpreted that compound 12 lacks the 5-chloro substituent on the indole-ring and consisted a simple ethylamine side chain instead of the 4-methyl-piperazin moiety. This clearly highlights that modifications of the structure of JNJ777120 can lead to the development of more H4R antagonists.

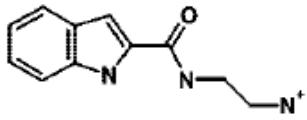
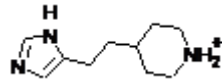
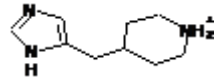
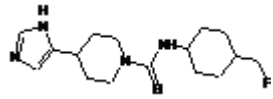
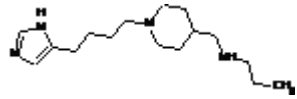


Figure 31 Chemical structure of Compound 12

Thioperamide database

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 to the binding sites. The top structures with top 4 dock score are listed in Table 10

Table 10 Top 4 compounds from Thioperamide database

S.No	Name	PubChem id	Structure	DockScore
1	Compound E	44290805		115.046
2	Compound F	25271899		111.136
3	Compound G	9905325		54.102
4	Compound H	10221295		52.774

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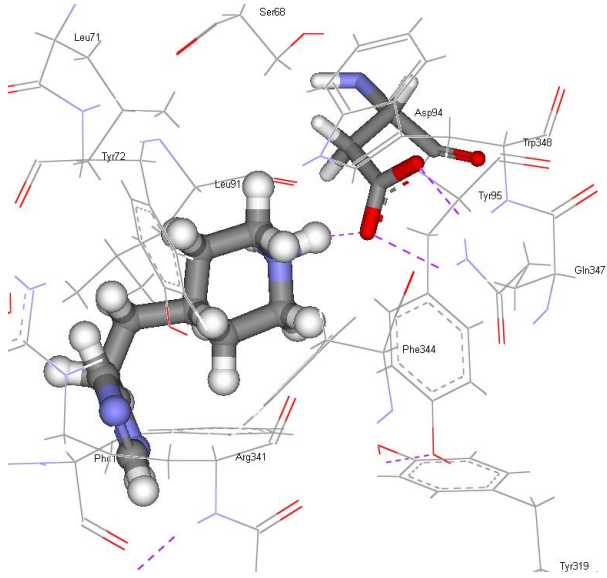


Figure 32 Binding mode of Compound E with the receptor

The structure with highest dock score in Thioperamide database is Compound E. Compound E showed hydrogen bond interactions with Asp94 (2.35) of the receptor model (Figure 32). The IUPAC of Compound E is 4-(2-(1H-Imidazol-5-yl)-ethyl)-piperidinium. With the help of ChemSpider webserver, the parent structure of Compound E was found to be 4-[2-(1H-imidazol-5-yl)ethyl]piperidine. Parent structure is the denotation for a compound consisting of an unbranched chain of skeletal atoms (not necessarily carbon), or consisting of an unsubstituted monocyclic or polycyclic ring system. The identified parent structure exhibits numerous biological activities in GPCR. The related structures of the parent structure 4-[2-(1H-imidazol-5-yl) ethyl]piperidine were explored with ChemSpider. Some of the related compounds that were identified to have biological activities are Immepip, Impentamine, Methimepip and UNII-1P032TC0JJ. The biological functions of each related structure is discussed below.

Immepip (Figure 33) is a selective H3 antagonist (Ishikawa *et al.*, 2010; Vollinga *et al.*, 1994). Experiments in rats have exposed the role of Immepip in attenuating inflammation via activation H3R. Recent evidences have suggested the role of immepip as an agonist to H4R which induced an increase in calcium via the intracellular PLC signalling pathway and TRPV1 (Jian *et al.*, 2016).

10.13147/SOE.2017.013

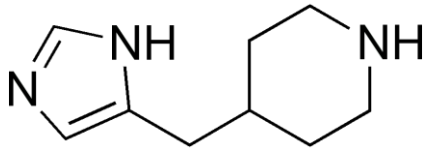


Figure 33 Chemical structure of Immepip

Impentamine is a selective H3 ligand (van der Goot *et al.*, 2000) which was first identified as an antagonist (Figure 34). *In vivo* microdialysis shows that impentamine also acts as an H3 agonist in the rat hypothalamus, inhibiting the basal release of histamine. VUF4904, an impentamine analog with an isopropyl group at the amino group of the side chain, bound with a relatively high affinity (12 nM) and acted as a neutral antagonist in the transfected SK-N-MC cells. These data indicate that ligands, previously identified as H3 antagonists, can cover the whole spectrum of pharmacological activities, ranging from full inverse agonism to agonism.

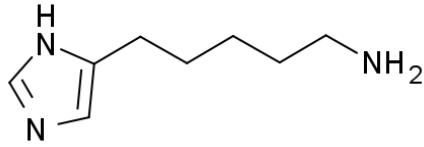


Figure 34 Chemical structure of Impentamine

Methimepip (Figure 35) exhibits high affinity and agonist activity at the human H3R (pK(i) = 9.0 and pEC(50) = 9.5) with a 2000 fold selectivity at the human H3R over the human H4R and more than a 10000 fold selectivity over the human H1R and H2R.

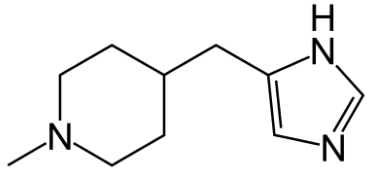


Figure 35 Chemical structure of Methimepip

The effect of Methipep at H4R has been studied but there are no evidences for the effects of immepip and Impentamine on H4R. From the three related structures, it can be conceived that Compound E might exhibit dual selectivity with H3R and H4R. However, experimental studies are required to ascertain it.

Compound F is the structure with second high dock score in Thioperamide database. It forms hydrogen bond interaction with Asp94 (2.33) (Figure 36) Its structure is similar to the Compound E with difference in the position of imidazole and methyl group. Thus, our findings suggest that

10.13147/SOE.2017.013

Compounds E and F can be an effective ligand for both H3R and H4R, but further *in vivo* studies have to be carried out to prove its efficacy.

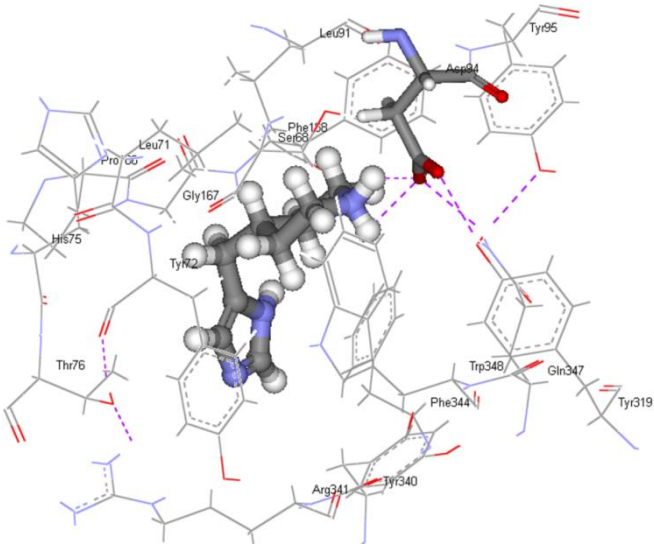


Figure 36 Binding mode of Compound F with the receptor

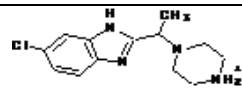
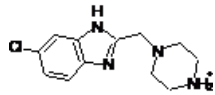
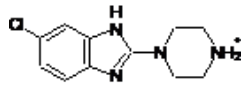
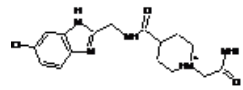
Vuf 6002 database

Vuf 6002 is a derivative of JNJ7777120 and contains benzimidazole moiety. Though oral availability is 27%, the half-life is only 1 hour (Thurmond *et al.*, 2004; Thurmond *et al.*, 2008). Pfizer optimized the structure of Vuf6002 by introducing an amidine moiety but it leads to serious adverse effects. In another approach, Johnson and Johnson used Vuf6002 as a starting point and identified a compound which had excellent selectivity over other histamine receptors (Yu *et al.*, 2010). Taking this approach as base, virtual screening of structures similar to Vuf 6002 has been performed.

In the third set of database containing 90% similar structures of Vuf 6002, 193 compounds in a total of 198 compounds were successfully docked. Top four score of high docking score are listed in Table 11

10.13147/SOE.2017.013

Table 11 Top 4 compounds from Vuf 6002 database

S.No	Name	PubChem id	Structure	DockScore
1	Compound I	28468621		123.095
2	Compound J	28750273		119.560
3	Compound K	28470894		105.162
4	Compound L	28810073		78.479

Hydrogen bond interactions were found with Compounds I, J, and K. These three compounds formed interactions with Asp94 (Figure 37, Figure 38, Figure 39). All the three compounds have the same core benzimidazole structure with modification in the ring structure. Hence, the modification at those positions can lead to the identification of a potent H4R ligand. The nitrogen in the imidazole ring has a possibility of forming hydrogen bond (4.00) with the Phe168. Similarly, there are also Carbon-Carbon (C-C) interaction in the central amino group of the ligand with Leu71 (3.73) and Thr76 (4.86). This attributes to the hydrophobic interlock that could lead to multifold selectivity of the ligand.

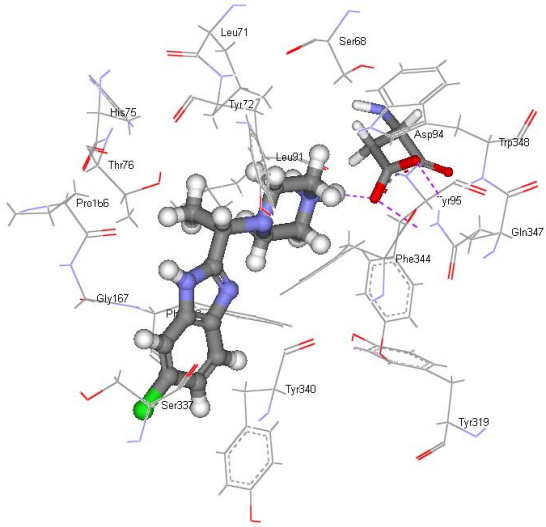


Figure 37 Binding mode of Compound I with the receptor

10.13147/SOE.2017.013

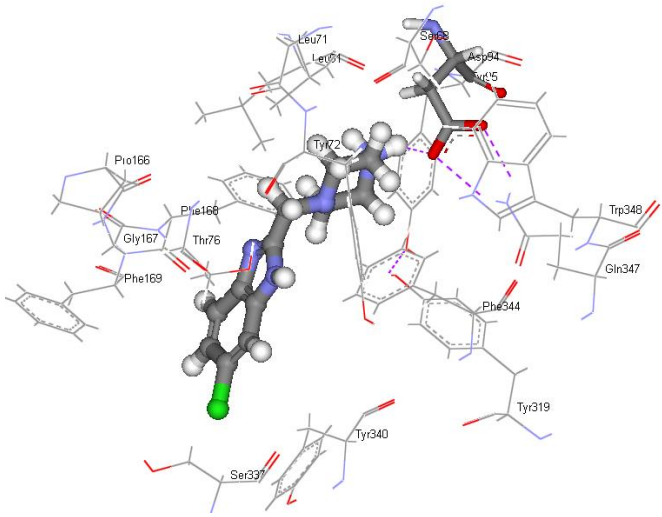


Figure 38 Binding mode of Compound J with the receptor

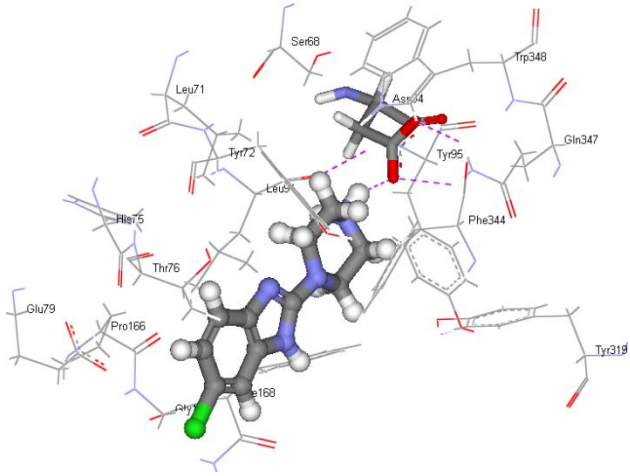


Figure 39 Binding mode of Compound K with the receptor

10.13147/SOE.2017.013

4.3 Lead compounds

Analysis of ligand binding shows that out of hundreds of compounds in all database, six compounds created an interaction with Asp94, preferably a hydrogen bond, situated at reasonably acceptable (Table 12) distance. Hydrogen bonds provide most of the directional interactions that underpin protein folding, protein structure and molecular recognition. A hydrogen bond is formed by the interaction of a hydrogen atom that is covalently bonded to an electronegative atom (donor) with another electronegative atom (acceptor). The importance of Hydrogen bonding between the ligand and the receptor are (Hubbard *et al*., 2001)

- It confers rigidity to the protein structure and specificity to intermolecular interactions.
- The accepted geometry for a hydrogen bond is a distance of less than 2.5 Å (1.9 Å).
- Intramolecular hydrogen bond between the main chain polar groups is required during protein folding.

The six compounds which formed hydrogen bond interactions with the receptor model are the lead ligand hits in this study.

Table 12 Top six compounds of high docking score and their interactions with Asp94

Compound name	PubChem Id	Molecular weight	Dock Score	Hydrogen bonding Interactions		
				Donor	Acceptor	Distance in Å
Compound I	28468621	265.76	123.095	N3	Asp94: OD2	2.47
Compound J	28750273	251.74	119.56	N3	Asp94: OD2	2.25
Compound A	39732646	238.69	115.116	N5	Asp94: OD2	2.18
Compound E	44290805	251.74	115.046	N1	Asp94: OD2	2.35
Compound F	25271899	166.24	111.136	N1	Asp94: OD2	2.33
Compound K	28470894	237.71	105.162	N2	Asp94: OD2	2.29

The Hydrogen bond is an ubiquitous element of the recognition in biological systems. Hydrogen bond requires the desolvation of both donor and acceptor. Hydrogen bonding also plays a major role in stabilizing protein-ligand complexes, in our case hH4R and ligands (Compounds I, J, A, E, F, K). The H-

10.13147/SOE.2017.013

bonding exists between the Asp94 of the hH4R which is the hydrogen accepting sites and the N (3)eH moieties of the Compounds that are potential hydrogen donor groups. Our result underlines the importance of further experimental investigations of the hH4R and ligand complex. Moreover, the H bonds of all the protein ligand complex has donor-acceptor distances between 2.2-2.5 Å which implies the presence of strong and mostly covalent bond. It is postulated that Asp94 interacts in its anionic state, whereas Glu182 interacts in its neutral form. The hypothesis was tested with the point mutations Asp 94 and Glu 182. Mutation at Asp 94 resulted in the absence of binding affinity towards any of the ligands. This is in sharp contrast to the Glu 182 mutant, which discriminates between various ligands (Jongejan *et al.*, 2008). Hence, this indirectly suggests the importance of Asp 94 in ligand binding.

4.4 ADMET prediction

Once a small molecule has been identified as potential lead it must be evaluated before proceeding to further stages. The acceptable toxicity as well as preliminary ADME properties defines the compound as "drug-like" which means an ideal clinical candidate (Kerns *et al.*, 2008). Leads are evaluated for their likelihood to be orally bioavailable. ADMET descriptor in Discovery studio is used to check the bioavailability of the identified compounds. ADMET Descriptors perform computational prediction based solely on the chemical structure of the molecule. In this thesis, ADMET Absorbtion has been determined. This predicts the Human Intestinal Absorption (HIA) after oral administration and reports a classification of absorption level. The method is based on calculations of logP and polar surface area. Molecular polar surface area (PSA) is a very useful parameter for prediction of drug transport properties. Polar surface area is defined as a sum of surfaces of polar atoms (usually oxygens, nitrogens and attached hydrogens) in a molecule. logP is a measure of molecular hydrophobicity. Hydrophobicity affects drug absorption, bioavailability, hydrophobic drug-receptor interactions, metabolism of molecules, as well as their toxicity. LogP has also evolved as a key parameter in studies of the environmental fate of chemicals (Tehrany *et al.*, 2004).

ADMET absorption prediction for all the compounds resulted in a plot of polar surface area (PSA) versus AlogP as shown in Figure 40. Compound E and F are located out of the accepted region of ADMET in the plot which indicates that other compounds have good ADMET properties.

10.13147/SOE.2017.013

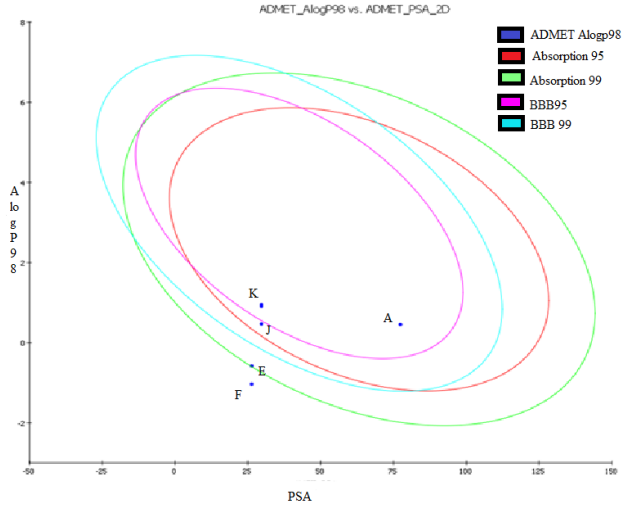


Figure 40 ADMET Plot of polar surface area versus AlogP.

4.5 Molecular dynamics

To ascertain the conformational variations of the H4R-ligand complexes, MD simulations have been carried out for the top scored receptor–ligand complexes. Analysis of the 1 ns MD trajectories for each complex structure reveals that the complexes were well stabilized at the active site. Figure 41 to Figure 46 shows the total energy, kinetic energy, and potential energy profile over the period of simulations during production run. It can be noted that there is not much deviation which indicates that the compounds bind in a better position. Further analyses reveals that the binding modes of the compounds established after the MD simulation are nearly the same as that obtained of molecular docking. The compounds are stabilized by intermolecular hydrogen bonds. The interactions with the residue Asp94 are seen throughout the dynamics trajectory of all the complexes illustrating its pivotal role in ligand binding at the active site (Table 12). Invariably in all the complexes, the interactions with the key residues are consistent with the molecular docking studies and may provide guidance for the rational design of more potent H4R inhibitors.

10.13147/SOE.2017.013

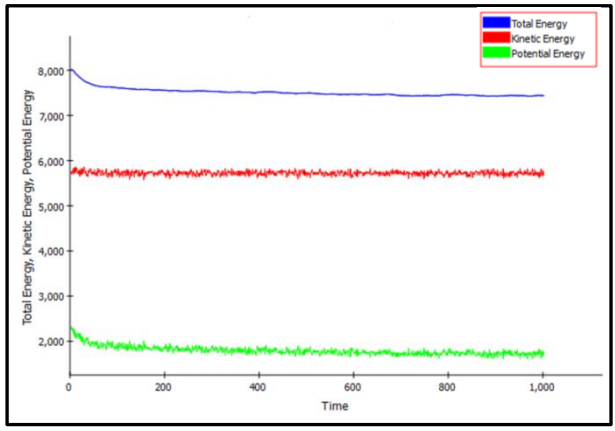


Figure 41 Compound I

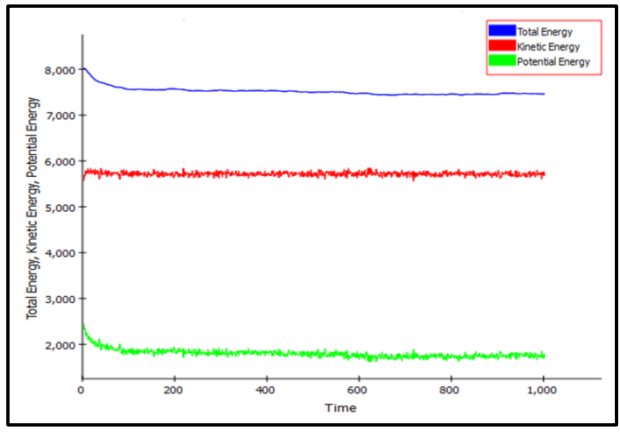


Figure 42 Compound J

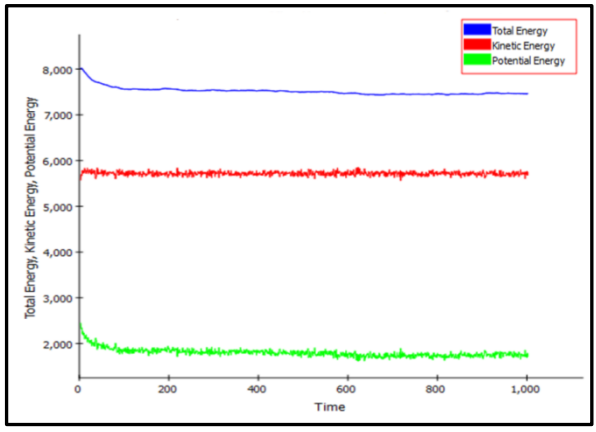


Figure 43 Compound A

10.13147/SOE.2017.013

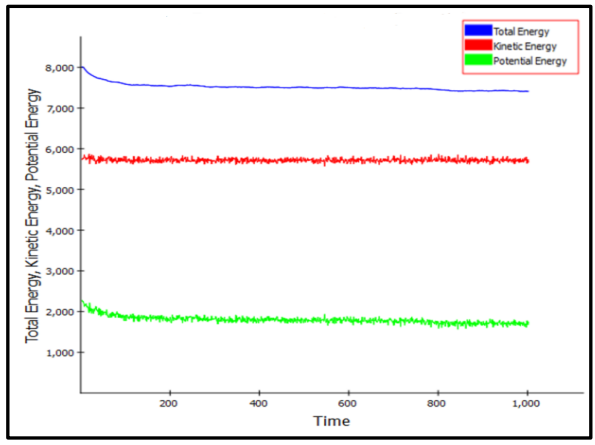


Figure 44 Compound E

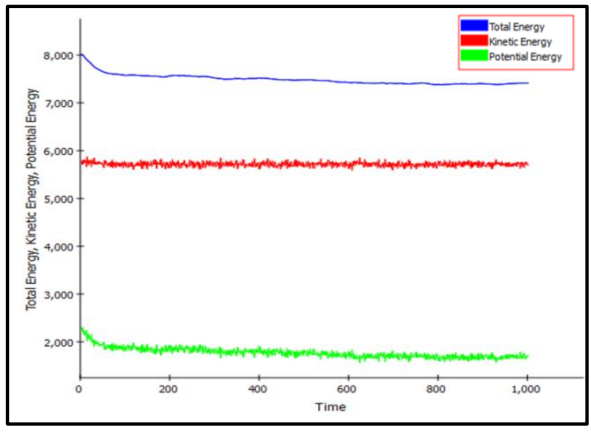


Figure 45 Compound F

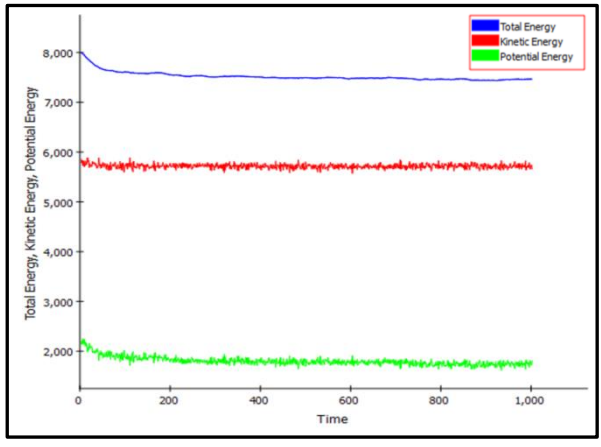


Figure 46 Compound K

10.13147/SOE.2017.013

Chapter 5

Scientific findings

10.13147/SOE.2017.013

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 mechanisms influenced by air pollutants. The role of H4R remains largely unexplored since only very few studies have focussed on its role. This insight would possibly mitigate the effect of air pollutant on allergy.

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.

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.

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.

10.13147/SOE.2017.013

Chapter 6

Conclusions and future research

10.13147/SOE.2017.013

6.1 Conclusions

Environmental pollution is the presence of dangerous unnatural ingredients causing imbalance in the ecosystems and health hazards to human beings and animals. There are many particles, and biological materials in atmosphere with potential health effects. The 6 major pollutants causing allergy were established in this thesis. These pollutants are listed by EPA as dangerous and health hazard. 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 H4R receptor has been found at mRNA level in allergy caused by pollen, knowledge of protein level expression can further enrich the need for antagonist. Since pollen allergy is also associated with the H1R, there are antihistamines available for its treatment. NO2 and SO2 have not been studied yet for its association with H4R. Confirmation of allergy and identification of causative allergens are crucial to manage allergic diseases. Evidence-based information about the major pollutants responsible for causing or exacerbating allergic diseases and asthma is lacking. Hence in this present study we have limited our search to the major pollutants and its involvement in allergy

H4 receptor exhibits a very restricted localization, expression is primarily found in intestinal tissue, spleen, thymus and immune active cells, such as T cells, mast cells, neutrophils and eosinophils. It suggests an important role for the H4 receptor in the regulation of immune function and offers novel therapeutic potentials for histamine receptor ligands in allergic and inflammatory diseases. H4R is an effective candidate in treating diseases associated with chronic pruritus and asthma. This attractive property of H4R urged us to explore its therapeutic potential in treating allergy. We exploited structure-based virtual screening to develop lead compounds which has H4R ligand property. A 3D model of the receptor was generated and its binding site was determined. A series of six potential lead ligands of H4R by virtual screening was ascertained. Molecular docking revealed the hydrogen bond interactions of all the 6 compounds towards H4R model. These 6 compounds could be a potential lead candidate drug in treating allergy.

Allergy is a complex process which involves various biomolecules. The causative agent for allergy from air is also diverse. This study have brought both the framework i.e. allergy particularly allergy stimulated by air pollutants together and have succeeded in bringing a countermeasure by identifying a lead compound in treating allergy caused by pollutants.

10.13147/SOE.2017.013

6.2 Future research

The collected information shows that allergy caused by pollen has been studied for the presence of H4R; however no research has been performed to test the efficacy of the H4R antagonist against pollen allergy. Available antagonist such as JNJ7777120 could be used to determine the molecular mechanism of H4R in allergy caused by Pollen. Given the potential of H4R to modulate the functions of inflammatory cells that are associated with allergy, H4R antagonist may completely block histamine transferred signals in inflammatory conditions. With the use of H4R antagonist for pollen allergy, the detrimental effects of the H1R could also be avoided.

There are over 500 hazardous air pollutants listed by EPA. These chemicals causing health hazard is a known fact but the ones which cause allergy is unknown. Categorising the hazardous chemicals based on their effect on allergy can help in providing specific treatments.

The 3D model generated in this study could be refined with introducing membrane environment and performing molecular dynamics stimulation to determine structural integrity of the H4R receptor.

The results obtained through the computational strategies are conclusive. The top compounds identified could be a kick start for the development of other potential leads. Our results provided novel scaffolds; hence further design could lead to potent and selective H4R antagonist.

The compounds identified in this study can be tested for its *in vitro* efficacy. Radio ligand binding assay could be performed since it gives a clear insight on the selectivity of the ligands towards H4R. Later the compounds could be tested for its efficacy in murine and cell lines to check if it intermediates any of the H4R function. H4R activation mediates the selective recruitment and chemotaxis of inflammatory cells and mediator release leading to allergy. Given the potential of H4R to modulate the functions of inflammatory cells that are associated with allergy, the lead compounds identified must be tested if it can completely block histamine transferred signals via H4R. Once the antagonist is proved to have potential effects, it can be tested for its effect on air pollutants.

Further research on the aforementioned tasks could help widen the knowledge on the pollutants effects on allergy process and also pave way for the development of new drugs.

10.13147/SOE.2017.013

Chapter 7

Summary

10.13147/SOE.2017.013

Environmental pollution is the contamination of the physical and biological components of the earth and atmosphere. Although pollution had existed for an over thousands of years, only with the onset of the industrial revolution it has received attention. Environmental pollutants are constituent parts of the pollution process. They are the actual "executing agents" of environmental pollution. Environmental pollutants have various adverse health effects such as perinatal disorders, infant mortality, respiratory disorders, allergy, malignancies, cardiovascular disorders, and increase in stress oxidative, endothelial dysfunction, mental disorders, and various other harmful effects.

One of the major health hazards affecting large number of population is Allergy. Allergy is caused when the body's own immune system does not recognises the foreign particle such as environmental pollutant. There are hundreds of air pollutants. Nevertheless, pollutants which play a major role have not been detailed. This research has identified the major pollutants which produce response in allergic process. The environmental pollutants were chosen from the air pollutants listed by EPA. The previous literatures of these pollutants were screened to characterize its role in allergy. .

The pollutants entering the body cause allergy in susceptible individuals. Allergy is a disorder of immune system against normally harmless environmental substances known as allergens. The prevalence of allergy and allergic asthma is estimated that over 20 percent of world population suffers from IgE mediated allergic diseases such as allergic asthma, allergic rhinitis, allergic conjunctivitis, atopic eczema/atopic dermatitis and anaphylaxis. Conventionally, the prevention and management of allergic disorders is fundamental to avoid allergen exposure. Apart from this, several pharmacotherapies are prescribed to block the action of allergic mediators like anti-histamines, cortisone, dexamethasone, hydrocortisone, theophylline, cromolyn sodium etc. These drugs are helpful to alleviate the symptoms of allergy.

Histamine has long been known to be the mediator that orchestrates inflammatory and allergic responses acting mainly through Histamine receptors H1R, H2R, H3R and H4R. H1R antagonists also referred to as antihistamines, have long been used to treat allergies, offering symptomatic relief in atopic nasal, conjunctival and skin disease. Recent reports indicate that H4R involvement in the control of immune cell trafficking and pro-inflammatory responses was derived from the H4R-mediated histamine-induced activation of eosinophils, increased expression of adhesion molecules and rearrangement of the actin cytoskeleton leading to immune cell migration from the bloodstream into the sites of inflammation. Consequently, the H4 receptor is currently an attractive target for the

10.13147/SOE.2017.013

pharmacological modulation of histamine transferred signals in inflammatory conditions and for the development of beneficial therapeutic strategies for allergic conditions.

Antagonism of histamine's action at H4R has been the cornerstone of an immense market for pharmacological treatment of Allergy. One of the aims of our study is to develop antagonists for H4histamine receptor using bioinformatics tools. For this, the structural model of H4 receptor was predicted and its docking site was identified. Similar structures of JNJ7777120, Vuf6002 and Thioperamide were retrieved from PubChem database and virtual screening was carried out and the top six compounds with high docking score were identified. The activity of these compounds has to be tested using *in vitro* biological assays.

10.13147/SOE.2017.013

Chapter8

Publications

10.13147/SOE.2017.013

Papers 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 2012,79:749–759 IF:2.469

4. L. Muthulakshmi, H. Nellaiah, T. Kathiresan, N. Rajini & Fenila Christopher, Identification and Production of Bioflocculants by Enterobacter sp. and Bacillus sp. and their Characterization Studies, Preparative Biochemistry and Biotechnology IF 0.67

Conference Proceedings

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 Feketel and Karthikeyan Subramanian, Influence of carbon emission in pollen allergy, International Conference on Thermal, Energy and Environment (INCOTEE2016), Kalasalingam University, Krishnacoil, Kalasalingam University

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

10.13147/SOE.2017.013

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

10.13147/SOE.2017.013

Chapter 9

References

10.13147/SOE.2017.013

A, W., YF, A., and CQ, Z. (2008). Effectsects of sulphur dioxide inhalation on allergic rhinitis in mice. Zhonghua Er Bi Yan Hou Tou Jing Wai Ke Za Zhi., 3(7):509-513.

Acciani, T. H., Brandt, E. B., Khurana Hershey, G. K., and Le Cras, T. D. (2013). Diesel exhaust particle exposure increases severity of allergic asthma in young mice. Clinical and Experimental Allergy, 43(12):1406-1418.

Adelman, D., Casale, T., and Corren, J. (2012). Manual of Allergy and Immunology. Wolters Kluwer Health.Albright, J. F. and Goldstein, R. A. (1996). Airborne pollutants and the immune system. Otolaryngology - Head and Neck Surgery, 114(2):232-238.

Altschul, S. F., Gish, W., Miller, W., Myers, E. W., and Lipman, D. J.(1990). Basic local alignment search tool. Journal of Molecular Biology,215(3):403-410.

Amaral, M. M., Alvarez, C. A., Langellotti, C., Jancic, C., Salamone, G.,Gener, J., and Vermeulen, M. (2011). Thioperamide induces cd4(+)cd25(+) foxp3(+) regulatory t lymphocytes in the lung mucosa of allergic mice through its action on dendritic cells. Journal of Asthma and Allergy, 4:93-102.

Ash, A. S. and Schild, H. O. (1966). Receptors mediating some actions of histamine. British Journal of Pharmacology and Chemotherapy,27(2):427-439.

Bachert, C., Van Cauwenberge, P., Olbrecht, J., and Van Schoor, J.(2006). Prevalence, classication and perception of allergic and nonallergic rhinitis in belgium. Allergy, 61(6):693-698.

Bahadar, H., Maqbool, F., Mostafalou, S., Baeeri, M., Gholami, M., Boroujerdi, E. G., and Abdollahi, M. (2015). The molecular mechanisms of liver and islets of langerhans toxicity by benzene and its metabolite hydroquinone *in vivo* and *in vitro*. Toxicology Mechanisms and Methods, pages 1-9.

Bartra, J., Mullol, J., del Cuvillo, Aband Davila, I., Ferrer, M., Jauregui,I., Montoro, J., Sastre, J., and Valero, A. (2007). Air pollution and allergens. Investig Allergol Clin Immunol, 17(2):3-8.

Bauchau, V. and Durham, S. (2004). Prevalence and rate of diagnosis of allergic rhinitis in Europe. European Respiratory Journal, 24(5):758-764.

Bentley, A. M., Jacobson, M. R., Cumberworth, V., Barkans, J. R., Moqbel, R., Schwartz, L. B., Irani, A.-M. A., Kay, A., and Durham, S. R. (1992). Forty ninth

10.13147/SOE.2017.013

annual meeting immunohistology of the nasal mucosa in seasonal allergic rhinitis: Increases in activated eosinophils and epithelial mast cells. Journal of Allergy and Clinical Immunology,89(4):877-883.

Bevelander, M., Mayette, J., Whittaker, L. A., Paveglio, S. A., Jones, C. C., Robbins, J., Hemenway, D., Shizuo Akira, S. U., and Poynter, M. E. (2007). Nitrogen dioxide promotes allergic sensitization to inhaled antigen. The Journal of Immunology, 179:3680-3688.

Bhattacharya, A., Tejero, R., and Montelione, G. T. (2007). Evaluating protein structures determined by structural genomics consortia. Proteins: Structure, Function, and Bioinformatics, 66(4):778-795.

Black, J. W., Duncan, W. A. M., Durant, C J.and Ganellin, C. R., and Parsons, E. M. (1972). Denition and antagonism of histamine h2 receptors. Nature, 236(5347):385-390.

Bouhuys, A., Beck, G. J., and Schoenberg, J. B. (1978). Do present levels of air pollution outdoors aect respiratory health? Nature, 276(5687):466-471.

Bousquet, J., Chanez, P., Lacoste, J. Y., Barnon, G., Ghavanian, N., Enander, I., Venge, P., Ahlstedt, S., Simony-Lafontaine, J., Godard, P.,and Michel, F.-B. (1990). Eosinophilic inammation in asthma. New England Journal of Medicine, 323(15):1033-1039. PMID: 2215562.

Bovet, D. and A, S. (1937). Action protectrice des ethers phenoliques an cours de lintoxication histaminique. C R Soc Biol Paris, 124:547-549.

Bowatte, G., Lodge, C., Lowe, A. J., Erbas, B., Perret, J., Abramson, M. J., Matheson, M., and Dharmage, S. C. (2015). The influence of childhood trac-related air pollution exposure on asthma, allergy and sensitization: a systematic review and a meta-analysis of birth cohort studies. Allergy, 70(3):245-256.

Bowie, J. U., Lüthy, R., and Eisenberg, D. (1991). A method to identify protein sequences that fold into a known three-dimensional structure. Science, 253(5016):164-170.

Brauer, M., Hoek, G., Smit, H. A., de Jongste, J. C., Gerritsen, J., Postma, D. S., Kerkhof, M., and Brunekreef, B. (2007). Air pollution and development of asthma, allergy and infections in a birth cohort. European Respiratory Journal, 29(5):879-888.

10.13147/SOE.2017.013

Brown, A. D., Bunnage, M. E., Glossop, P. A., James, K., Jones, R., Lane, C. A., Lewthwaite, R. A., Mantell, S., Perros-Huguet, C., Price, D. A., Trevethick, M., and Webster, R. (2007). The discovery of long acting b2-adrenoreceptor agonists. Bioorganic & Medicinal Chemistry Letters, 17(14):4012-4015.

Carnohan, K. (2012). Methods for transmembrane protein topology and alpha helix prediction. Master's thesis.

Caron, G., Delneste, Y., Roelandts, E., Duez, C., Bonnefoy, J.-Y., Pestel, J., and Jeannin, P. (2001). Histamine polarizes human dendritic cells into th2 cell-promoting effector dendritic cells. The Journal of Immun-ology, 167(7):3682-3686.

Cezmi A, A. (2007). Crc desk refrence for allergy and asthma. Technical report, Library of congress cataloging in publication data.Chauhan, B. S. (2008). Environmental studies. University Science press.

Cheng, T., Li, Q., Zhou, Z., Wang, Y., and Bryant, S. H. (2012). Structure based virtual screening for drug discovery a problem centric review. The AAPS Journal, 14(1):133-141.

Church, M. K., Maurer, M., Simons, F. E. R., Bindslev-Jensen, C.,Van Cauwenberge, P., Bousquet, J., Holgate, S. T., and Zuberbier, T.(2010). Risk of first-generation h1-antihistamines: a ga2len position paper. Allergy, 65(4):459-466.

Ciebiada, M., Kasztalska, K., Ciebiada, G., Malgorzata, and Gorski, P. (2014). Histamine type 2 receptor expression on peripheral blood regulatory lymphocytes in patients with allergic rhinitis treated with specific immunotherapy. American Journal of Rhinology nad Allergy, 28:130-135.

Committee., H. I. S. O. (2010). Outdoor air pollution and health in the developing countries of asia: A comprehensive review. technical report, special report 18. Technical report, Health Effects Institute, Boston, MA.

Coruzzi, G., Adami, M., Guaita, E., de Esch, I. J., and Leurs, R. (2007). Antiin-ammatory and antinociceptive e-ects of the selective histamine h4-receptor antagonists jnj7777120 and vuf6002 in a rat model of carrageenan-induced acute in-ammation. European Journal of Phar-macology, 563:240 - 244.

Cramp, S., Dyke, H. J., Higgs, C., Clark, D. E., Gill, M., Savy, P., Jennings, N., Price, S., Lockey, P. M., Norman, D., Porres, S., Wilson, F., Jones, A., Ramsden, N., Mangano, R., Leggate, D., Andersson, M.,and Hale, R. (2010). Identification and

10.13147/SOE.2017.013

hit-to-lead exploration of a novel series of histamine h4 receptor inverse agonists. Bioorganic & Medicinal Chemistry Letters, 20(8):2516-2519.

Cuthbertson, J. M., Doyle, D. A., and Sansom, M. S. (June 2005). Transmembrane helix prediction: a comparative evaluation and analysis. Protein Engineering Design and Selection, 18(6):295-308.

Damaj, B. B., Becerra, C. B., Esber, H. J., Wen, Y., and Maghazachi, A. A. (2007). Functional expression of h4 histamine receptor in human natural killer cells, monocytes, and dendritic cells. The Journal of Immunology, 179(11):7907-7915.

D'Amato, G., Liccardi, G., and D'Amato, M. (2000). Environmental risk factors (outdoor air pollution and climatic changes) and increased trend of respiratory allergy. Journal of investigational allergology & clinical immunology, 10(3):123-128.

David, W., Ganther, B., Stefan, D., Buschauer, and Armin (2015). The extracellular loop 2 (ecl2) of the human histamine h4 receptor substantially contributes to ligand binding and constitutive activity. PLoS ONE, 10(1):e0117185.

de Esch, I. J., Thurmond, R. L., Jongejan, A., and Leurs, R. (2005). The histamine h4 receptor as a new therapeutic target for inflammation. Trends in Pharmacological Sciences, 26(9):462-469.

de Graaf, C., Kooistra, A., Vischer, H., Katritch, V., Kuijer, M., Shiroishi, M., Iwata, S., Shimamura, T., Stevens, R., de Esch, I., and Leurs, R. (2011). Crystal structure based virtual screening for novel fragment like ligands of the human histamine h1 receptor. Journal of medicinal chemistry, 54(23):8195-8206.

Derendorf, H. and Meltzer, E. O. (2008). Molecular and clinical pharmacology of intranasal corticosteroids: clinical and therapeutic implications. Allergy, 63(10):1292-1300.

Devalia, J., Rusznak, C., Herdman, M., Trigg, C., Davies, R., and Tarraf, H. (1994). Effect of nitrogen dioxide and sulphur dioxide on airway response of mild asthmatic patients to allergen inhalation. The Lancet, 344(8938):1668-1671. Originally published as Volume 2, Issue 8938.

Devalia, J. L., Campbell, A. M., Sapsford, R. J., Rusznak, C., Quint, D., Godard, P., Bousquet, J., and Davies, R. J. (1993). Effect of nitrogen dioxide on synthesis of inflammatory cytokines expressed by human bronchial epithelial cells *in vitro*. Am J Respir Cell Mol Biol, 9(3):271-278.

10.13147/SOE.2017.013

Dia Sanchez, D., PenichetGarcia, M., and Saxon, A. (2000). Diesel exhaust particles directly induce activated mast cells to degranulate and increase histamine levels and symptom severity. Journal of Allergy and Clinical Immunology, 106(6):1140-1146

Dib, K., Perecko, T., Jenei, V., McFarlane, C., Comer, D., Brown, V.,Katebe, M., Scheithauer, T., Thurmond, R. L., Chazot, P. L., and Ennis, M. (2014). The histamine h4 receptor is a potent inhibitor of adhesion-dependent degranulation in human neutrophils. Journal of Leukocyte Biology, 96(3):411-418.

Dockery, D. W., Pope, C. A., Xu, X., Spengler, J. D., Ware, J. H., Fay, M. E., Ferris, B. G., and Speizer, F. E. (1993). An association between air pollution and mortality in six u.s. cities. New England Journal of Medicine, 329(24):1753-1759. PMID: 8179653.

Dolovich, J., Hargreave, F., Chalmers, R., Shier, K., Gauldie, J., and Bienenstock, J. (1973). Late cutaneous allergic responses in isolated Ige-dependent reactions. Journal of Allergy and Clinical Immunology,52(1):38-46.

Dominici, F., Peng, R. D., Bell, M. L., Pham, L., McDermott, A., Zeger, S. L., and Samet, J. M. (2006). Fine particulate air pollution and hospital admission for cardiovascular and respiratory diseases. JAMA :the journal of the American Medical Association, 295(10):1127-1134.

Dubois, A., Deruytter, N., Adams, B., Kanda, A., Delbauve, S., Fleury, S., Torres, D., Francois, A., Petein, M., Goldman, M., Dombrowicz, D., and Flamand, V. (2010). Regulation of th2 responses and allergic inflammation through bystander activation of cd8+ t lymphocytes in early life. The Journal of Immunology, 185(2):884-891.

Endo, Y. (1982). Simultaneous induction of histidine and ornithine decarboxylases and changes in their product amines following the injection of escherichia coli lipo polysaccharide into mice. Biochemical Pharmacology, 31:1643-1647.

Engelhardt, H., Schultes, S., de Graaf, C., Nijmeijer, S., Vischer, H. F.,Zuiderveld, O. P., Dobler, J., Stachurski, K., Mayer, M., Arnhof, H., Scharn, D., Haaksma, E. E. J., de Esch, I. J. P., and Leurs, R. (2013). Bispyrimidines as potent histamine h4 receptor ligands: Delineation of structure activity relationships and detailed h4 receptor binding mode. Journal of Medicinal Chemistry, 56(11):4264-4276. PMID: 23668417.

10.13147/SOE.2017.013

Feng, Z., Hou, T., and Li, Y. (2013). Docking and md study of histamine h4r based on the crystal structure of h1r. Journal of Molecular Graphics and Modelling, 39:1 -12.

Ferreira, L. G., dos Santos, R. N., Oliva, G., and Andricopulo, A. D. (2015). Molecular docking and structure-based drug design strategies. Molecules, (20):13384-13421.

Fujimaki, H., Ozawa, M., Bissonnette, E., and Befus, A. (1993). Further studies on the effect of nitrogen dioxide on mast cells: The effect of the metabolite, nitrite. Environmental Research, 61(2):223 -231.

Fung-Leung, W.-P., Thurmond, R. L., Ling, P., and Karlsson, L. (2004). Histamine h4 receptor antagonists: the new antihistamines? Current opinion in investigational drugs (London, England : 2000), 5(11):1174-1183.

Galli, S. J. and Tsai, M. (2012). Ige and mast cells in allergic disease. Nat Med, 18(5):693-704.

Ghosh, E., Kumari, P., Jaiman, D., and Shukla, A. K. (2015). Methodological advances: the unsung heroes of the gpcr structural revolution.Nat Rev Mol Cell Biol, 16(2):69-81.

Gleich, G. J. (1982). The late phase of the immunoglobulin e-mediated reaction: a link between anaphylaxis and common allergic disease? Journal of Allergy and Clinical Immunology, 70(3):160-169.

Godot, V., Arock, M., Garcia, G., Capel, F., Flys, C., Dy, M., Emilie, D., and Humbert, M. (2007). H4 histamine receptor mediates optimal migration of mast cell precursors to cxcl12. Journal of Allergy and Clinical Immunology, 120(4):827-834.

Gould, H. J., Sutton, B. J., Beavil, A. J., Beavil, R. L. M., N. Coker, H. A., Fear, D., and Smurthwaite, L. (2003). The biology of ige and the basis of allergic disease. Annual Review of Immunology, 21(1):579-628.

Green, J. (2011). Reducing pollution and waste. Raintree publishers.

10.13147/SOE.2017.013

Grosseld, A. (2011). Recent progress in the study of g protein-coupled receptors with molecular dynamics computer simulations. Biochimica et Biophysica Acta (BBA) - Biomembranes, 1808(7):1868-1878.

Gutzmer, R., Diestel, C., Mommert, S., Kather, B., Stark, H., Wittmann, M., andWerfel, T. (2005). Histamine h4 receptor stimulation suppresses il-12p70 production and mediates chemotaxis in human monocytederived dendritic cells. The Journal of Immunology, 174(9):5224-5232.

Gutzmer, R., Mommert, S., Gschwandtner, M., Zwingmann, K., Stark, H., and Werfel, T. (2009). The histamine h4 receptor is functionally expressed on th2 cells. Journal of Allergy and Clinical Immunology, 123(3):619 - 625.

Hajat, S., Haines, A., Atkinson, R. W., Bremner, S. A., Anderson, H. R., and Emberlin, J. (2001). Association between air pollution and daily consultations with general practitioners for allergic rhinitis in London, united kingdom. American Journal of Epidemiology, 153(7):707-714.

Handley, D. A., Magnetti, A., and Higgins, A. J. (1998). Therapeutic advantages of third generation antihistamines. Expert Opinion on In- vestigational Drugs, 7(7):1045-1054.

Hanin, J. m., Maigret, B., Tarek, M., Escrieut, C., Fourmy, D., and Chipot, C. (2005). Probing a model of a gpcr/ligand complex in an explicit membrane environment: The human cholecystokinin-1 receptor. Biophysical Journal, 90(4):1232-1240.

Hansen, J., Ruedy, R., Glascoe, J., and Sato, M. (1999). Giss analysis of surface temperature change. Journal of Geophysical Research: Atmospheres, 104(D24):3099731022.

Hanuskova, E. and Plevkova, J. (2013). The role of histamine h4 receptors as a potential targets in allergic rhinitis and asthma. Open Journal of Molecular and Integrative Physiology, 3:6-14.

Hersey, A., Chambers, J., Bellis, L., Bento, A. P., Gaulton, A., and Overington, J. P. (2015). Chemical databases: curation or integration by user dened equivalence? Drug Discovery Today: Technologies, 14:17 - 24. From Chemistry to Biology Database Curation.

Hill, M. (2010). Understanding Environmental Pollution. Cambridge University Press.

10.13147/SOE.2017.013

Hill, S. J. (1990). Distribution, properties, and functional characteristics of three classes of histamine receptor. Pharmacological Reviews, 42(1):45-83.

Hill, S. J., Ganellin, C. R., Timmerman, H., Schwartz, J. C., Shankley, N. P., Young, J. M., Schunack, W., Levi, R., and Haas, H. L. (1997). International union of pharmacology. xiii. classication of histamine receptors. Pharmacological Reviews, 49(3):253-278.

Hodge, E., Chang, W. Y., Selby, K. A., Hall, I. P., and Sayers, I. (2013).Effects of atopy and grass pollen season on histamine h4 receptor expression in human leukocytes. Annals of Allergy, Asthma & Immunology, 111(1):38-44.

Hofmann, K. and Stofel, W. (1993). Tmbase a database of membrane spanning proteins segments. Biological Chemistry, 374:166.

Hofstra, C. L., Desai, P. J., Thurmond, R. L., and Fung-Leung, W.-P.(2003). Histamine h4 receptor mediates chemotaxis and calcium mobilization of mast cells. Journal of Pharmacology and Experimental Therapeutics, 305(3):1212-1221.

Holdgate, M. W. (1979). A perspective of Environmental Pollution. University Press, Cambridge. Holgate, S., Church, M., Broide, D., and Martinez, F. (2011). Allergy.Elsevier Health Sciences UK.

Holgate, S. T. (2004). The epidemic of asthma and allergy. Journal of the Royal Society of Medicine, 97(3):103-110.

Hough, L. B. (2001). Genomics meets histamine receptors: New subtypes, new receptors. Molecular Pharmacology, 59(3):415-419.

Howarth, P., Emanuel, M., and Holgate, S. (1984). Astemizole, a potent histamine h1receptor antagonist: effect in allergic rhinoconjunctivitis, on antigen and histamine induced skin weal responses and relationship to serum levels. British Journal of Clinical Pharmacology, 18(1):1-8.

Hsiao, C.-L., Wu, K.-H., and Wan, K.-S. (2011). Effects of environmental lead exposure on t helper cellspecific cytokines in children. Journal of Immunotoxicology, 8(4):284-287.

Hubbard, R. E. and Kamran Haider, M. (2001). Hydrogen Bonds in Proteins Role and Strength. John Wiley and Sons, Ltd.

10.13147/SOE.2017.013

Hwang, B.-F., Jaakkola, J., Lee, Y.-L., Lin, Y.-C., and Leon Guo, Y.-l. (2006). Relation between air pollution and allergic rhinitis in taiwanese schoolchildren. Respiratory Research, 7(1):23.

ichi Sekizawa, S., Bechtold, A. G., Tham, R. C., Kott, K. S., Hyde, D. M., Joad, J. P., and Bonham, A. C. (2010). House-dust mite allergen and ozone exposure decreases histamine h3 receptors in the brainstem respiratory nuclei. Toxicology and Applied Pharmacology, 247(3):204 - 210.

Ishikawa, M., Watanabe, T., Kudo, T., Yokoyama, F., Yamauchi, M.,Kato, K., Kakui, N., and Sato, Y. (2010). Investigation of the histamine h3 receptor binding site. design and synthesis of hybrid agonists with a lipophilic side chain. Journal of Medicinal Chemistry, 53(17):6445-6456. PMID: 20690643.

Istyastono, E. P., Kooistra, A. J., Vischer, H. F., Kuijer, M., Roumen, L.Nijmeijer, S., Smits, R. A., de Esch, I. J. P., Leurs, R., and de Graaf, C. (2015). Structure based virtual screening for fragment-like ligands of the g protein coupled histamine h4 receptor. Med. Chem. Commun.,6:1003-1017.

Istyastono, E. P., Nijmeijer, S., Lim, H. D., van de Stolpe, A., Roumen,L., Kooistra, A. J., Vischer, H. F., de Esch, I. J. P., Leurs, R., and de Graaf, C. (2011). Molecular determinants of ligand binding modes in the histamine h4 receptor: Linking ligand-based three-dimensional quantitative structure activity relationship (3d-qsar) models to in silicoguided receptor mutagenesis studies. Journal of Medicinal Chemistry, 54(23):8136-8147. PMID: 22003888.

Jaajrt, B., Kiss, R., Viskolcz, B., and Keser, G. M. (2008). Activation mechanism of the human histamine h4 receptor an explicit membrane molecular dynamics simulation study. Journal of Chemical Information and Modeling, 48(6):1199-1210. PMID: 18572901.

Jemima, E. A., Prema, A., and Thangam, E. B. (2014). Functional characterization of histamine h4 receptor on human mast cells. Molecular Immunology, 62(1):19 - 28.

Jian, T., Yang, N., Yang, Y., Zhu, C., Yuan, X., Yu, G., Wang, C., Wang, Z., Shi, H., Tang, M., He, Q., Lan, L., Wu, G., and Tang, Z. (2016). Trpv1 and plc participate in histamine h4 receptor induced itch. Neural Plasticity, 2016:9.

Jones, D. T., Taylort, W. R., and Thornton, J. M. (1992). A new approach to protein fold recognition. Nature, 358(6381):86-89.

10.13147/SOE.2017.013

Jones, P. D., New, M., Parker, D. E., Martin, S., and Rigor, I. G. (1999). Surface air temperature and its changes over the past 150 years. Reviews of Geophysics, 37(2):173-199.

Jongejan, A., Lim, H. D., Smits, R. A., de Esch, I. J. P., Haaksma, E.,and Leurs, R. (2008). Delineation of agonist binding to the human histamine h4 receptor using mutational analysis, homology modeling, and ab initio calculations. Journal of Chemical Information and Modeling, 48(7):1455-1463. PMID: 18553960.

Kaitala, V., Pohjola, M., and Tahvonen, O. (1992). Transboundary air pollution and soil acidification: A dynamic analysis of an acid rain game between inland and the ussr. Environmental and Resource Economics, 2(2):161-181.

Kampa, M. and Castanas, E. (2008). Human health effects of air pollution. Environmental Pollution, 151(2):362 - 367. Proceedings of the 4th International Workshop on Biomonitoring of Atmospheric Pollution (With Emphasis on Trace Elements).

Karlin, S. and Altschul, S. F. (1990). Methods for assessing the statistical significance of molecular sequence features by using general scoring schemes. Proceedings of the National Academy of Sciences of the United States of America, 87(6):2264-2268.

Kay, A. (2001). Allergy and allergic diseases. New England Journal of Medicine, 344(2):109-113. PMID: 11150362.

Kelly, F. J. and Fussell, J. C. (2011). Air pollution and airway disease. Clinical & Experimental Allergy, 41(8):1059-1071.

Kerns, E. H. and Di, L. (2008). Drug like Properties Concepts, Structure Design and Methods from ADME to Toxicity Optimization. Academic Press Inc.

Kim, S., Thiessen, P. A., Bolton, E. E., Chen, J., Fu, G., Gindulyte, A., Han, L., He, J., He, S., Shoemaker, B. A., Wang, J., Yu, B., Zhang, J.,and Bryant, S. H. (2015). Pubchem substance and compound databases.Nucleic Acids Research, 44(Database issue):D1202-D1213.

Kiss, R., BelaKiss, Kacza, A., Szalai, F., Jelinek, I., Laslo, V., Noszal, B.,Falus, A., and Keser, G. M. (2008a). Discovery of novel human histamine h4 receptor ligands by large-scale structure-based virtual screening.Journal of Medicinal Chemistry, 51(11):3145-3153. PMID: 18459760.

10.13147/SOE.2017.013

Kiss, R., Noszall, B., Rakcz, A., Falus, A., Eras, D., and Keser, G. M.(2008b). Binding mode analysis and enrichment studies on homology models of the human histamine h4receptor. European Journal of Medicinal Chemistry, 43(5):1059 -1070.

Kleeberger, S. R., Ohtsuka, Y., Zhang, L.-Y., and Longphre, M. (2001). Airway responses to chronic ozone exposure are partially mediated through mast cells. Journal of Applied Physiology, 90(2):713-723.

Knox, R. B., Suphioglu, C., Taylor, P., Desai, R., Watson, H. C., Peng, J. L., and Bursill, L. A. (1997). Major grass pollen allergen lol p 1 binds to diesel exhaust particles: implications for asthma and air pollution. Clin Exp Allergy, 27(3):246-51.

Koenig, J. (2012). Health Effects of Ambient Air Pollution: How safe is the air we breathe? Springer US.

Koenig, J. Q., M.D., W. E. P., B.S., M. H., and M.D., R. F. (1982). Effects of inhaled sulfur dioxide (so2) on pulmonary function in healthy adolescents: Exposure to so2 alone or so2 + sodium chloride droplet aerosol during rest and exercise. Archives of Environmental Health: An International Journal, 37(1):5-9. PMID: 7059231.

Koenig, J. Q. and Pierson, W. E. (1991). Air pollutants and the respiratory system: Toxicity and pharmacologic interventions. Journal of Toxicology: Clinical Toxicology, 29(3):401411. PMID: 1920573.

Koenig, J. Q., Pierson, W. E., and Horike, M. (1983). The effects of inhaled sulfuric acid on pulmonary function in adolescent asthmatics. Am Rev Respir Dis, 128(2):221-225.

Kooistra, A. J., Kuhne, S., de Esch, I. J. P., Leurs, R., and de Graaf, C.(2013). A structural chemogenomics analysis of aminergic gpcrs: lessons for histamine receptor ligand design. British Journal of Pharmacology, 170(1):101-126.

Koonin EV, G. M. (2003). Sequence - Evolution - Function: Computational Approaches in Comparative Genomics. Kluwer Academic;.

Krogh, A., Larsson, B., von Heijne, G., and Sonnhammer, E. L. (2001).Predicting transmembrane protein topology with a hidden markov model: application to complete genomes1. Journal of Molecular Biology, 305(3):567-580.

10.13147/SOE.2017.013

Larche, M., Akdis, C. A., and Valenta, R. (2006). Immunological mechanisms of allergen-specic immunotherapy. Nat Rev Immunol, 6(10):761-771.

Laskowski, R. A., MacArthur, M. W., Moss, D. S., and Thornton, J. M. (1993). Procheck a program to check the stereochemical quality of protein structures. Journal of Applied Crystallography, 26(2):283291.

Laskowski, R. A., Rullmann, J. A. C., MacArthur, M. W., Kaptein, R., and Thornton, J. M. (1996). Aqua and procheck nmr: Programs for checking the quality of protein structures solved by nmr. Journal of Biomolecular NMR, 8(4):477-486.

Laurie, A. T. R. and Jackson, R. M. (2005). Q-sitefinder an energy based method for the prediction of protein and ligand binding sites. Bioinformatics, 21(9):1908-1916.

Lemanske, RobertF., J. and Kaliner, M. (1988). Late-phase ige-mediated reactions. Journal of Clinical Immunology, 8(1):1-13.

Levita, J., Saptarini, N. M., and Ardiansyah, A. (2012). Generating a 3d structure model of histamine 4 receptor for anti-inflammatory drug design. E3 Journal of Biotechnology and Pharmaceutical Research, 3(2):22-27.

Ling, P., Ngo, K., Nguyen, S., Thurmond, R. L., Edwards, J. P., Karlsson, L., and Fung-Leung, W.-P. (2004). Histamine h(4) receptor mediates eosinophil chemotaxis with cell shape change and adhesion molecule upregulation. British Journal of Pharmacology, 142(1):161-171.

Lionta, E., Spyrou, G., Vassilatis, D. K., and Cournia, Z. (2014). Structure based virtual screening for drug discovery: Principles, applications and recent advances. Current Topics in Medicinal Chemistry, 14(16):1923-1938.

Lippert, U., Artuc, M., Grutzkau, A., Babina, M., Guhl, S., Haase, I., Blaschke, V., Zachmann, K., Knosalla, M., Middel, P., Kruger-Krasagakis, S., and Henz, B. M. (2003). Human skin mast cells express h2 and h4, but not h3 receptors. J Investig Dermatol, 123(1):116-123.

Liu, C., Ma, X.-J., Jiang, X., Wilson, S. J., Hofstra, C. L., Blevitt, J.,Pyati, J., Li, X., Chai, W., Carruthers, N., and Lovenberg, T. W.(2001a). Cloning and pharmacological characterization of a fourth histamine receptor (h4) expressed in bone marrow. Molecular Pharmacology, 59(3):420-426.

10.13147/SOE.2017.013

Liu, C., Wilson, S. J., Kuei, C., and Lovenberg, T. W. (2001b). Comparison of human, mouse, rat, and guinea pig histamine h4 receptors reveals substantial pharmacological species variation. Journal of Pharmacology and Experimental Therapeutics, 299(1):121-130.

Long, A., C, M., D, D., P, C., B, K., and J, L. (2002). Management of allergic and nonallergic rhinitis. evid rep technol assess. Evid Rep Technol Assess (Summ), 54:1-6.

Luger, E. O., Wegmann, M., Achatz, G., Worm, M., Renz, H., and Radbruch, A. (2010). Allergy for a lifetime? Allergology International, 59(1):1 - 8.

Lukacs, N. W. (2001). Role of chemokines in the pathogenesis of asthma. Nat Rev Immunol, 1(2):108-116.

MacArthur, M. W., Laskowski, R. A., and Thornton, J. M. (1994). Knowledge-based validation of protein structure coordinates derived by x-ray crystallography and nmr spectroscopy. Current Opinion in Structural Biology, 4(5):731 - 737.

Madden, T. (2013). The NCBI Handbook [Internet]. 2nd edition. Bethesda (MD): National Center for Biotechnology Information (US).

MC, M. (2008). Prospects for future climate change and the reasons for early action. Journal of air and waste management association, 58(6):735.

Mortimer, K., Neugebauer, R., Lurmann, F., Alcorn, S., Balmes, J., and Tager, I. (2008). Early-lifetime exposure to air pollution and allergic sensitization in children with asthma. Journal of Asthma, 45(10):874-881.

Muranaka, M., Suzuki, S., Koizumi, K., Takafuji, S., Miyamoto, T., Ikemori, R., and Tokiwa, H. (1986). Adjuvant activity of diesel-exhaust particulates for the production of ige antibody in mice. Journal of Allergy and Clinical Immunology, 77(4):616-623.

Nadakavukaren, A. (2011). Our Global Environment: A Health Perspective, Seventh Edition. Waveland Press.

Nathan, R. A., Meltzer, E. O., Derebery, J., Campbell, U. B., Stang, P. E.,Corrao, M. A., Allen, G., and Stanford, R. (2008). The prevalence of nasal symptoms attributed to allergies in the united states: Findings from the burden of rhinitis in an america survey. Allergy and Asthma Proceedings, 29(6):600-608.

10.13147/SOE.2017.013

Neumann, D., Beermann, S., Burhenne, H., Glage, S., Hartwig, C., and Seifert, R. (2013). The dual h3/4r antagonist thioperamide does not fully mimic the effects of the standard h4r antagonist jnj 7777120 in experimental murine asthma. Naunyn-Schmiedeberg's Archives of Pharmacology, 386(11):983-990.

Nguyen, T., Shapiro, D. A., George, S. R., Setola, V., Lee, D. K., Cheng,R., Rauser, L., Lee, S. P., Lynch, K. R., Roth, B. L., and O'Dowd, B. F. (2001). Discovery of a novel member of the histamine receptor family. Molecular Pharmacology, 59(3):427-433.

Nijmeijer, S., Vischer, H. F., Sirci, F., Schultes, S., Engelhardt, H., de Graaf, C., Rosethorne, E. M., Charlton, S. J., and Leurs, R. (2013). Detailed analysis of biased histamine h4 receptor signalling by jnj 7777120 analogues. British Journal of Pharmacology, 170(1):78-88.

Oda, T., Morikawa, N., Saito, Y., Masuho, Y., and Matsumoto, S. (2000). Molecular cloning and characterization of a novel type of histamine receptor preferentially expressed in leukocytes. Journal of Biological Chemistry, 275(47):36781-36786.

Ohsawa, Y. and Hirasawa, N. (2012). The antagonism of histamine h1 and h4 receptors ameliorates chronic allergic dermatitis via anti-pruritic and anti-inflammatory effects in nc/nga mice. Allergy, 67(8):1014-1022.

O'Reilly, M., Alpert, R., Jenkinson, S., Gladue, R. P., Foo, S., Trim, S., Peter, B., Trevethick, M., and Fidock, M. (2002). Identification of a histamine h4 receptor on human eosinophils role in eosinophil chemotaxis. Journal of Receptors and Signal Transduction, 22(1-4):431-448.

Pappalardo., M., Shachaf., N., Basile, L., Milardi, D., Zeidan, M., Raiyn, J., Guccione, S., and Rayan, A. (2014). Sequential application of ligand and structure based modeling approaches to index chemicals for their hh4r antagonism. Plos One, 9(10).

Parsons, M. E. and Ganellin, C. R. (2006). Histamine and its receptors. British Journal of Pharmacology, 147(Suppl 1):S127-S135.

Peden, D. B. and Dailey, L. (1995). Modulation of mast cell functions by *in vitro* ozone exposure. American Journal of Physiology Lung Cellular and Molecular Physiology, 268(6):902-910.

10.13147/SOE.2017.013

Peden, D. B., Setzer, R. W., and Devlin, R. B. (1995). Ozone exposure has both a priming effect on allergen-induced responses and an intrinsic inflammatory action in the nasal airways of perennially allergic asthmatics. Am J Respir Crit Care Med, 151(5):1336-1345.

Pevsner, J. (2015). Bioinformatics and Functional Genomics. Wiley.

Poynter, M. E., Persinger, R. L., Irvin, C. G., Butnor, K. J., van Hirtum, H., Blay, W., Heintz, N. H., Robbins, J., Hemenway, D., Taatjes, D. J.,and Janssen Heininger, Y. (2005). Nitrogen dioxide enhances allergic airway inflammation and hyperresponsiveness in the mouse. American Journal of Physiology Lung Cellular and Molecular Physiology,290(1):L144-L152.

Prof. R. K. Agarwal, P. (2010). Krishna's Environment and Ecology; for B.Tech Ist and IInd semester students of All Engineering Colleges affliated to U.P. Technical University, Lucknow; As per revised syllabus, w.e.f. 2008-09. Krishna Prakashan.

Raaschou-Nielsen, O., Andersen, Z. J., Beelen, R., Samoli, E., Stafoggia, M., Weinmayr, G., Hoffmann, B., Fischer, P., Nieuwenhuijsen, M. J.,Brunekreef, B., Xun, W. W., Katsouyanni, K., Dimakopoulou, K., Sommar, J., Forsberg, B., Modig, L., Oudin, A., Oftedal, B., Schwarze, P. E., Nafstad, P., Faire, U. D., Pedersen, N. L., astenson, C.-G., Fratiglioni, L., Penell, J., Korek, M., Pershagen, G., Eriksen, K. T., SÃºrensen, M.,Tanneland, A., Ellermann, T., Eeftens, M., Peeters, P. H., Meliefste, K., Wang, M., de Mesquita, B. B., Key, T. J., de Hoogh, K., Concin, H., Nagel, G., Vilier, A., Grioni, S., Krogh, V., Tsai, M.-Y., Ricceri, F., Sacerdote, C., Galassi, C., Migliore, E., Ranzi, A., Cesaroni, G., Badaloni, C., Forastiere, F., Tamayo, I., Amiano, P., Dorronsoro, M.,Trichopoulou, A., Bamia, C., Vineis, P., and Hoek, G. (2013). Air pollution and lung cancer incidence in 17 European cohorts: prospective analyses from the European study of cohorts for air pollution effects (escape). The Lancet Oncology, 14(9):813 - 822.

Raferty, P., Jackson, L., Smith, R., and Holgate, S. (1990). Terfenadine, a potent histamine h1-receptor antagonist in the treatment of grass pollen sensitive asthma. British Journal of Clinical Pharmacology, 30(2):229-235.

Rahim, F. (2010). Research article an in silico development of selective inhibitor for histamine receptors. Biotechnology, 9(2):157-163.

Rahman, A. (2007). Frontiers in Drug Design and Discovery. Frontiers in Drug Design and Discovery Series. Bentham Science Publishers.

10.13147/SOE.2017.013

Rana, S. (2011). Environmental Pollution: Health and Toxicology. Alpha Science International Limited.

Reuter, S., Stassen, M., and Taube, C. (2010). Mast cells in allergic asthma and beyond. Yonsei Medical Journal, 51(6):797-807.

Rieuwerts, J. (2015). The Elements of Environmental Pollution. Taylor and Francis.

Riley, J. F. (1953). Histamine in tissue mast cells. Science, 118(3064):332-333.

Riley, J. P. and Dunford, P. J. (2008). Measurement and inhibition of airway hyperreactivity in a guinea pig model of allergic asthma. Immunology, 22.

Ring, J. and Behrendt, H. (2012). New Trends in Allergy V. Springer Berlin Heidelberg.

Rogers, C. A., Wayne, P. M., Macklin, E. A., Muilenberg, M. L., Wagner, C. J., Epstein, P. R., and Bazzaz, F. A. (2006). Interaction of theonset of spring and elevated atmospheric co(2) on ragweed (ambrosia artemisiifolia l.) pollen production. Environmental Health Perspectives,114(6):865-869.

Roy, A., Kucukural, A., and Zhang, Y. (2010). I-tasser: a unified platform for automated protein structure and function prediction. Nature protocols, 5(4):725-738.

Salvi, S., Blomberg, A., Rudell, B., Kelly, F., Sandstram, T., Holgate, S. and Frew, A. (1999). Acute inflammatory responses in the airways and peripheral blood after short-term exposure to diesel exhaust in healthy human volunteers. Am J Respir Crit Care Med, 159(3):702-709.

Sander, K, Kottke, T, Stark, and H (2008). Histamine h3 receptor antagonists go to clinics. Biol Pharm Bull, 31:2163-2181.

Schultes, S., Nijmeijer, S., Engelhardt, H., Kooistra, A. J., Vischer, H. F., de Esch, I. J. P., Haaksma, E. E. J., Leurs, R., and de Graaf, C. (2013). Mapping histamine h4 receptor-ligand binding modes. Med. Chem. Commun., 4:193-204.

Sharma, P. and D, S. (2005). Ecology And Environment. Rastogi Publications.

10.13147/SOE.2017.013

Shimamura, T., Shiroishi, M., Weyand, S., Tsujimoto, H., Winter, G., Katritch, V., Abagyan, R., Cherezov, V., Liu, W., Han, G. W., Kobayashi, T., Stevens, R. C., and Iwata, S. (2011). Structure of the human histamine h(1) receptor complex with doxepin. Nature, 475(7354):65-70.

Shin, N., Coates, E., Murgolo, N. J., Morse, K. L., Bayne, M., Strader, C. D., and Monsma, F. J. (2002). Molecular modeling and site specific mutagenesis of the histamine binding site of the histamine h4 receptor. Molecular Pharmacology, 62(1):38-47.

Shiraishi, Y., Jia, Y., Domenico, Jand Joetham, A., Karasuyama, H., Takeda, K., and Gelfand, E. W. (2012). Sequential engagement of fceri on mast cells and basophil histamine h(4) receptor and fceri in allergic rhinitis. Journal of immunology, 190(2):539-548.

Simakova, M. N. and Simakov, N. N. (2014). Topography prediction of helical transmembrane proteins by a new modification of the sliding window method. BioMed Research International, 2014:9.

Simon, F. E. R. and Simons, K. J. (2008). H(1)antihistamines: Current status and future directions. The World Allergy Organization Journal, 1(9):145-155.

Sirci, F., Istyastono, E. P., Vischer, H. F., Kooistra, A. J., Nijmeijer, S., Kuijer, M., Wijtmans, M., Mannhold, R., Leurs, R., de Esch, I.J. P., and de Graaf, C. (2012). Virtual fragment screening: Discovery of histamine h3 receptor ligands using ligand-based and protein-based molecular fingerprints. Journal of Chemical Information and Modeling, 52(12):3308-3324. PMID: 23140085.

Slater, J., Zechnich, A., and Haxby, D. (1999). Second-generation antihistamines. Drugs, 57(1):31-47.

Smits, R. A., Lim, H. D., Stegink, B., Bakker, R. A., de Esch, I. J. P and Leurs, R. (2006). Characterization of the histamine h4 receptor binding site part 1 synthesis and pharmacological evaluation of dibenzodiazepine derivatives. Journal of Medicinal Chemistry, 49(15):4512-4516. PMID:16854056.

Somma, T., Cinci, L., Formicola, G., Pini, A., Thurmond, R., Ennis, M.,Bani, D., and Masini, E. (2013). A selective antagonist of histamine h4 receptors prevents antigen-induced airway inflammation and bronchoconstriction in guinea pigs: involvement of lipocortin-1. British Journal of Pharmacology, 170(1):200-213.

10.13147/SOE.2017.013

Sonnhammer, E. L. L., Heijne, G. v., and Krogh, A. (1998). A hidden markov model for predicting transmembrane helices in protein sequences. In Proceedings of the 6th International Conference on Intelligent Systems for Molecular Biology, ISMB '98, pages 175-182. AAAI Press.

Spengler, J. and Sexton, K. (1983). Indoor air pollution: a public health perspective. Science, 221(4605):9-17.

Stenfors, N., Bosson, J., Helleday, R., Behndig, A. F., Pourazar, J., Tarnqvist, H., Kelly, F. J., Frew, A. J., Sandstram, T., Mudway, I. S., and Blomberg, A. (2010). Ozone exposure enhances mast-cell inflammation in asthmatic airways despite inhaled corticosteroid therapy. Inhalation Toxicology, 22(2):133-139.

Stern, A. C., Wohlers, H. C., Boubel, R. W., and Lowry, W. P. (1973). Fundamentals of Air pollution. Academic Press Inc.

Sunyer, J. (2001). Urban air pollution and chronic obstructive pulmonary disease: a review. European Respiratory Journal, 17(5):1024-1033.

Sutton, B. J. and Gould, H. J. (1993). The human ige network. Nature,366(6454):421-428.

Szilasi, M., Galfy, G., Fanay, K., Mark, Z., Ranai, Z., Szalai, Z., Szilasi, M. E., Budai, M., Maller, V., Somfay, A., Horvath, I., and Tamasi, L.(2012). A survey of the burden of allergic rhinitis in Hungary from a specialists perspective. Multidisciplinary Respiratory Medicine, 7(1):49- 49.

Tachimoto, H., Ebisawa, M., and Bochner, B. (2002). Cross-talk between integrins and chemokines that influences eosinophil adhesion and migration. Int Arch Allergy Immunol, 128(suppl 1)(Suppl. 1):18-20.

Takahashi, Y., Kagawa, Y., Izawa, K., Ono, R., Akagi, M., and Kamei, C. (2009). Effect of histamine {H4} receptor antagonist on allergic rhinitis in mice. International Immunopharmacology, 9(6):734 - 738. Trends Immusupression, Berlin, 2008.

Takano, H., Ichinose, T., Miyabara, Y., Shibuya, T., Lim, H.-B., Yoshikawa, T., and Sagai, M. (1998). Inhalation of diesel exhaust enhances allergen-related eosinophil recruitment and airway hyperresponsiveness in mice. Toxicology and Applied Pharmacology, 150(2):328 - 337.

10.13147/SOE.2017.013

Takeshita, K., Sakai, K., Bacon, K. B., and Gantner, F. (2003). Critical role of histamine h4 receptor in leukotriene b4 production and mast cell dependent neutrophil recruitment induced by zymosan *in vivo*. Journal of Pharmacology and Experimental Therapeutics, 307(3):1072-1078.

TaketomiI, E. A., SopeleteII, M. C., de Sousa MoreiraIII, P. F., and de Assis Machado VieiraIV, F. (2006). Pollen allergic disease: pollens and its major allergens. Revista Brasileira de Otorrinolaringologia, 72:562 -567.

Tehrany, E. A., Fournier, F., and Desobry, S. (2004). Simple method to calculate octanol water partition coefficient of organic compounds. Journal of Food Engineering, 64(3):315 - 320.

TERADA, HAMANO, MAESAKO, HIRUMA, HOHKI, SUZUKI, ISHIKAWA, and KONNO (1999). Diesel exhaust particulates upregulate histamine receptor mrna and increase histamine-induced il-8 and gmcsf production in nasal epithelial cells and endothelial cells. Clinical & Experimental Allergy, 29(1):52-59.

Terzioglu, N., van Rijn, R. M., Bakker, R. A., Esch, I. J. D., and Leurs, R.(2004). Synthesis and structure activity relationships of indole and benzimidazole piperazines as histamine h4 receptor antagonists. Bioorganic & Medicinal Chemistry Letters, 14(21):5251 - 5256.

Thurmond, R. L., Desai, P. J., Dunford, P. J., Fung-Leung, W.-P., Hofstra, C. L., Jiang, W., Nguyen, S., Riley, J. P., Sun, S., Williams, K. N., Edwards, J. P., and Karlsson, L. (2004). A potent and selective histamine h4 receptor antagonist with anti-inflammatory properties. Journal of Pharmacology and Experimental Therapeutics, 309(1):404-413.

Thurmond, R. L., Gelfand, E. W., and Dunford, P. J. (2008). The role of histamine h1 and h4 receptors in allergic inflammation: the search for new antihistamines. Nat Rev Drug Discov, 7(1):41-53.

Timmerman, H. and van der Goot, H. (2009). Histamine receptors and their ligands: Mechanisms and applications. Encyclopedia of Neuroscience, 4:1149-1166. Tramontano, A. and Morea, V. (2003). Assessment of homology based predictions in casp5. Proteins, 53(S6):352-368.

Tunnicliffe, W., Burge, P., and Ayres, J. (1994). Effect of domestic concentrations of nitrogen dioxide on airway responses to inhaled allergen in asthmatic patients. The Lancet, 344:1733 - 1736.

10.13147/SOE.2017.013

Tusnady, G. E. and Simon, I. (1998). Principles governing amino acid composition of integral membrane proteins application to topology prediction1.Journal of Molecular Biology, 283(2):489 - 506.

van de Waterbeemd, H. and Gifford, E. (2003). Admet in silico modelling towards prediction paradise? Nat Rev Drug Discov, 2(3):192-204.

van der Goot, H. and Timmerman, H. (2000). Selective ligands as tools to study histamine receptors. European Journal of Medicinal Chemistry, 35(1):5 - 20.

van Schoor, J. (2008). Antihistamines: a brief review. SA Pharmaceutical Journal, 75(8).

Venkatachalam, C., Jiang, X., Oldfield, T., and Waldman, M. (2003). Ligand Fit: a novel method for the shape-directed rapid docking of ligands to protein active sites. Journal of Molecular Graphics and Modelling, 21(4):289 - 307.

Vollinga, R. C., de Koning, J. P., Jansen, F. P., Leurs, R., Menge, W. M. P. B., and Timmerman, H. (1994). A new potent and selective histamine h3 receptor agonist, 41himidazol4ylmethylpiperidine. Journal of Medicinal Chemistry, 37(3):332-333. PMID: 8308858.

Wayne, P., Foster, S., Connolly, J., Bazzaz, F., and Epstein, P. (2002).Production of allergenic pollen by ragweed (ambrosia artemisiifolia l.) is increased in co2-enriched atmospheres. Annals of Allergy, Asthma & Immunology, 88(3):279 - 282.

Willerroider, M. (2003). Roaming polar bears reveal arctic role of pollutants.Nature, 426(6962):5-5.

Wong, G. W. K. (2013). Air pollution and health. The Lancet Respiratory Medicine, 2(1):8-9.

Woodfolk, J. A. (2007). T-cell responses to allergens. Journal of Allergy and Clinical Immunology, 119(2):280-294.

Wu, S., Skolnick, J., and Zhang, Y. (2007). Ab initio modeling of small proteins by iterative tasser simulations. BMC Biology, 5(1):1-10.

Xie, X.-Q. (2010). Exploiting pubchem for virtual screening. Expert opinion on drug discovery, 5(12):1205-1220.

10.13147/SOE.2017.013

Xie, Z.-R. and Hwang, M.-J. (2015). Methods for predicting protein and ligand binding sites. In Kukol, A., editor, Molecular Modeling of Proteins, volume 1215 of Methods in Molecular Biology, pages 383-398.Springer New York.

Xu, Y. and Xu, D. (2000). Protein threading using prospect: Design and evaluation. Proteins: Structure, Function, and Bioinformatics, 40(3):343-354.

Yanagihara, Y., Kajiwara, K., Basaki, Y., Ikizawa, K., Ebisawa, M., RA, C., Tachimoto, H., and Saito, H. (1997). Cultured basophils but not cultured mast cells induce human ige synthesis in b cells after immunologic stimulation. Clinical and Experimental Immunology, 111(1):136-143.

Yang, J., Yan, R., Roy, A., Xu, D., Poisson, J., and Zhang, Y. (2015). The i-tasser suite: protein structure and function prediction. Nat Meth, 12(1):7-8.

Young, M. C. (1998). Rhinitis, sinusitis, and polyposis. Allergy Asthma Proc, 19:211-218.

Yu, F, Wolin, L, R., Wei, and J (2010). Pharmacological characterization of oxime agonists of the histamine h4 receptor. J Receptor Ligand Channel Res,, 3:37-49.

Zhang, Yang, DeVries, E, M., Skolnick, and Jeffrey (2006). Structure modelling of all identified g protein coupled receptors in the human genome. PLoS Comput Biol, 2(2):e13.

Zhang, J., Yang, J., Jang, R., and Zhang, Y. (2015). Gpcr-i-tasser: A hybrid approach to g protein-coupled receptor structure modeling and the application to the human genome. Structure, 23(8):1538-1549.

Zhang, Q., Qiu, Z., Chung, K. F., and Huang, S.-K. (2014). Link between environmental air pollution and allergic asthma: East meets west. Journal of Thoracic Disease, 7(1):14-22.

Zhang, Y. (2008). I-tasser server for protein 3d structure prediction. BMC Bioinformatics, 9(1):1-8.

Zhang, Y. and Skolnick, J. (2004a). Automated structure prediction of weakly homologous proteins on a genomic scale. Proceedings of the National Academy of Sciences of the United States of America, 101(20):7594-7599.

10.13147/SOE.2017.013

Zhang, Y. and Skolnick, J. (2004b). Tertiary structure predictions on a comprehensive benchmark of medium to large size proteins. Biophysical Journal, 87(4):2647-2655.

Zhou, H. and Zhou, Y. (2005). Fold recognition by combining sequence profiles derived from evolution and from depth-dependent structural alignment of fragments. Proteins, 58(2):321-328.

Ziska, L., Knowlton, K., Rogers, C., Dalan, D., Tierney, N., Elder, M. A., Filley, W., Shropshire, J., Ford, L. B., Hedberg, C., Fleetwood, P., Hovanky, K. T., Kavanaugh, T., Fulford, G., Vrtis, R. F., Patz, J. A., Portnoy, J., Coates, F., Bielory, L., and Frenz, D. (2011). Recent warming by latitude associated with increased length of ragweed pollen season in central north america. Proceedings of the National Academy of Sciences of the United States of America, 108(10):4248-4251.

Ziska, L. H., Epstein, P. R., and Schlesinger, W. H. (2008). Rising co(2),climate change, and public health: Exploring the links to plant biology. Environmental Health Perspectives, 117(2):155-158.