Study of Protein Ligand Interactions of Histamine Inhibitors by Computational Approaches

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بسم الله الرحمن الرحيم

In the name of Allah Most Gracious and Most Beneficial

Department of Bioinformatics and Biotechnology

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FINAL APPROVAL

It is certificate that we have read the thesis submitted by Ms. Javeria Irum and it is our judgment that this project is of sufficient standard to warrant its acceptance by the International Islamic University, Islamabad for the M.S Degree in Bioinformatics

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DECLARATION

I hereby declare that the work presented in the following thesis is my own effort, except where otherwise acknowledged and that the thesis is my own composition. No part of the thesis has been previously presented for any other degree.

Javeria Irum

"Your Lord is Allah who created the Heavens and the Earth in six days"

Al-A'raf (7:54)

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LIST OF ABBREVIATIONS

2D: Two dimension

3D: Three Dimension

A^0: Angstrom

ALA: Alanine

ARG: Arginine

ASP: Aspartic Acid

CAAD: Computer-aided drug design

COMFA: Comparative Molecular Field Analysis

COMSIA: Comparative Molecular Similarity Indices Analysis

FDA: Food and Drug Administration

HBA: Hydrogen Bond Acceptor

HBD: Hydrogen bond Donor

HNMT: Histamine methyltransferase

HOMO: Highest Occupied Molecular Orbital

HTS: High Throughput Screening

IgE: Immunoglobulin E

GLY: Glycine

IC_{50}: Inhibitory Concentration

ILE: Isoleucine

Log P: Partition Coefficient

LUMO: Lowest Unoccupied Molecular Orbital

MEP : Molecular electrostatic potential

MOE: Molecular Orbital Environment

MLP : Molecular lipophilicity potential

NMR : Nuclear magnetic resonance

PDB: Protein Data Bank

Phe: Phenylalanine

QSAR: Quantitative Structure Activity Relationship

SAM: S-adenosyl-L-methionine

SOMFA: Self organized molecular field analysis

Tyr: Tyrosine

Val: Valine

VMD: Visual Molecular Dynamics

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Lastly I plead to Allah (SWT) that may He grants me with true success in all the fields in both the worlds, this and the hereafter and shower His blessings upon me.

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Javeria Irum

ABSTRACT

The rational development of pharmaceutical drugs is one major intention of structural computational biology. Recent advances in drug discovery have created new and powerful technologies that have a prominent bioinformatic component. These technologies significantly use bioinformatics for analysis of their output. The development of antihistamine drugs is a thriving area of research. The present work strives to find out the robust and definite ligands as antihistamine compounds by using different approaches like pharmacophore generation, molecular docking and quantitative structure activity relationship studies. Pharmacophore perception is done using the knowledge of structure of novel drugs. On a set of 40 compounds of indene, benzothiophene, 5-lipoxygenase, 2-aminobenzimidazole and 2-(piperidin-3-yl)-1Hbenzimidazoles, ligand based pharmacophore modeling is carried out. All of these show five common features i.e. hydrogen bond acceptor, hydrogen bond donor, hydrophobic unit, positive ionizable and aromatic ring. To evaluate the likeliness of the compounds as orally active drugs Lipinski's Rule of Five is calculated. Molecular docking studies were carried out for lead optimization using AutoDock Vina. The target protein selected for this purpose is histamine methyltransferase, PDB code: 1JQD. To analyze the binding interactions of the active confirmations of the ligands and the target protein Visual Molecular Dynamics tool (VMD) is used. The lead compound identified showed strong ligand-protein interactions which comprise three hydrogen bonds, three hydrophobic interactions, two Vander wall and two ionic interactions. Lead compound also has low IC_{50} value i.e. 0.32 μM. Five analogues of the lead compound were docked in order to predict their affinity and bioactivity.

A quantitative structure activity relationship (QSAR) study has been made on a set of 2-(piperidin-3-yl)-1H benzimidazoles compounds in order to explore the probable relationships between electronic structural properties and binding affinity. Some electronic and steric descriptors were calculated using HyperChem and Chem Draw software. On the basis of these studies some new compounds are pinpointed for antihistamine activity.

INTRODUCTION

INTRODUCTION

The rational development of pharmaceutical drugs is one major purpose of structural computational biology. Recent advances in drug discovery have created new and powerful technologies that have a prominent bioinformatic component. The development of antihistamine drugs is a thriving area of research. Antihistamines are the drugs which are used against the immune system when the immune system is over reacting. They work in a number of different ways and against different medical conditions. Most commonly antihistamines are used against allergic conditions. When the body is exposed to any foreign pathogen histamine is released which binds to its receptors causing a series of reaction which causes an increase in blood flow to the area, and the discharge of other chemicals that add to the allergic response. Antihistamines work by blocking histamine receptors.

The number of reported cases of allergic disorders has increased considerably during the past quarter of a century. Over the past 20 to 30 years the prevalence of diseases associated with atopy has increased in many parts of the world. The surveys that have depicted an increase in the abundance of asthma have also depicted an increases in the prevalence of many other allergic diseases, such as hay fever and eczema. In the United Kingdom, samples of children born in 1946, 1958, and 1970 have shown a marked increase 5.1%, 7.3%, and 12.2% respectively in the prevalence of eczema as reported by the mother in children aged under 5. In 1960's a dramatic increase in mortality caused by allergic diseases (mainly asthma) in many countries was noticed. There are an estimated 25 to 30 million allergy patients in Pakistan. While in the

developed countries including the United States, allergy is the sixth leading cause of chronic diseases, statistics for developing countries are just as dismal. In Pakistan, the prevalence of allergic diseases is 10 to 18 per cent which is a substantial increase over the past 10 years. In heavily industrialized and polluted areas, prevalence is feared to double or even triple in the next 10 years.

A large number of antihistamine drugs are being prepared and are available in the market. A large number of them are approved by the U.S Food and Drug Administration (FDA). There are two types of antihistamine drugs, the older group, H_1 histamines and the newer group, H_2 antagonists. The H1 class has sedating effects and H2 is non-sedating. All medicines have possible side effects. Side effects caused by antihistamines could worsen conditions such as enlarged prostate gland, obstruction of the gut, glaucoma, retention of urine, and because of this reason they should be used with caution. The H1 antihistamines cause drowsiness and may consequently affect the ability to drive or operate machinery securely. Side effects from antihistamines are more probable to occur in children and the aged, and some of the most widespread are constipation, drowsiness, confusion, blurred vision, headache, dry mouth, dizziness, difficulty passing urine etc. There is a desperate need to develop new drugs with lower side effects so this study was done in order to find novel drug like molecules as antihistamine compounds.

The present work strives to find out the robust and definite ligands as antihistamine compounds by using different approaches like pharmacophore generation, molecular docking and quantitative structure activity relationship studies. Histamine can bind to different proteins apart from histamine receptors. The target protein used for this study is histamine methyltransferase, PDB code: 1JQD retrieved from the Protein Data

Bank. Pharmacophore perception is done using the knowledge of structure of novel drugs for the design of lead compound. The presented pharmacophore model identifies significant binding features of the ligands and possibly will present direction for the rational design to find out fresh drugs. To evaluate the likeliness of the compounds as orally active drugs Lipinski's Rule of Five is also calculated. Molecular docking studies were carried out for lead optimization and prediction of bound conformations. The binding interactions of the active confirmations of the ligands and the target protein were also analyzed. A quantitative structure activity relationship (QSAR) study has been made in order to explore the probable relationships between electronic structural properties and binding affinity.

To comprehend the mechanism of action of a variety of antihistamine drugs used extensively in clinical practice for many of diseases, unearthing the modes of binding and recognition capabilities in diverse proteins has enormous implications. Due to the lack of explicit sequence and structural signatures at the histamine binding sites, the antihistamine compounds frequently designed to imitate histamine are probable to bind to these proteins at divergent extent and possibly show a relationship with dissimilar side-effect profiles exhibited by them. The specific temperament of the residues in the individual binding sites pin points the substitutions that would be tolerated or not on the histamine template. This analytical study consequently lays a scaffold for the design of explicit, effective and competent antihistamines with low side effects.

LITERATURE REVIEW

REVIEW OF LITERATURE

The diagnosis and curing of diseases is an art and it will remain to be. The reason behind this is that human being is a multifaceted organism. There are many aspects in curing and diagnosing diseases basically comprising biological, physiological, sociological, psychological and spiritual ones. The peculiarity between healing illness and curing disease is overwhelmingly evident. The basic objective of therapy is to guide the biochemical circuitry of the living organisms to a healthy state.

The allergic diseases are literally widespread over many parts of the world and entail all ethnic groups with allergic rhinitis, bronchial asthma, eczema and conjunctivitis being the commonest demonstrations (M.Y. Noori, S.M. Hasnain, and M.A. Waqar 2007). Allergies can fluctuate from being just a annoyance, troublesome to hazardous. Allergy is an immune system disorder which is a type of hypersensitivity. The term allergy was first defined in 1906 by an Austrian pediatrician, Von Pirquet in describing a changed or altered reaction in the body (von Pirquet C 1906). He attributed to both immunity that was advantageous and to the detrimental hypersensitivity as "allergy." The word allergy is consequential from two Greek words "allos," which means dissimilar or changed and "ergos," which mean work or action.

Allergic reactions occur because of allergens which are harmless environmental substances. These reactions maybe acquired, predictable or rapid. As soon as these allergens get nearer with specific antigens in our blood, it triggers a response and histamine is released which causes several allergic symptoms such as itchy eyes, runny nose, hives and general inflammation to more brutal reactions such as diarrhea, vomiting,

trouble breathing, quickened heart rate and even perhaps a loss of consciousness due to a fall in the blood pressure. This is called anaphylaxis shock. The inflammatory response results in increase in permeability of vessels which causes the blood fluids to enter the area which results in swelling. So an allergic reaction also acknowledged as a hypersensitivity reaction is a reaction triggered by the immune system in response to a foreign substance (Goran Tošić. 2004).

The dominance of allergic diseases is quite far above the ground in Pakistan. The occurrence of breathlessness is 15.2%, at the same time the investigated cases of asthma are 9.5%. Correspondingly the occurrence of allergic rhinitis is 34.3% and the incidence of those having allergic rhinitis in addition to wheezing episodes is 8% according to a study made in 2007 (M.Y. Noori, S.M. Hasnain, and M.A. Waqar 2007). The most frightening thing is that these numbers persist to boost at a swift rate. This data reflects the dominance of clinically diagnosed, frequently established allergic conditions.

Allergy belongs to one of the four types of hypersensitivity and that is type I or Immediate hypersensitivity. It is basically symbolized by unwarranted activation of some white blood cells which are known as mast cells and basophils by an antibody known as IgE which results in a severe inflammatory response shown in figure 1.1. These reactions involve vasodilatation, smooth-muscle contractions, and mucous-gland secretions. The clinical presentations of type I immune reactions are called atopy (Goran Tošić. 2004). People can be allergic to almost everything but the most common allergic reactions include hives, eczema, hay fever, food allergies, asthma attacks and reaction to the poison of vicious insects for instance wasps and bees (Kay AB 2000).

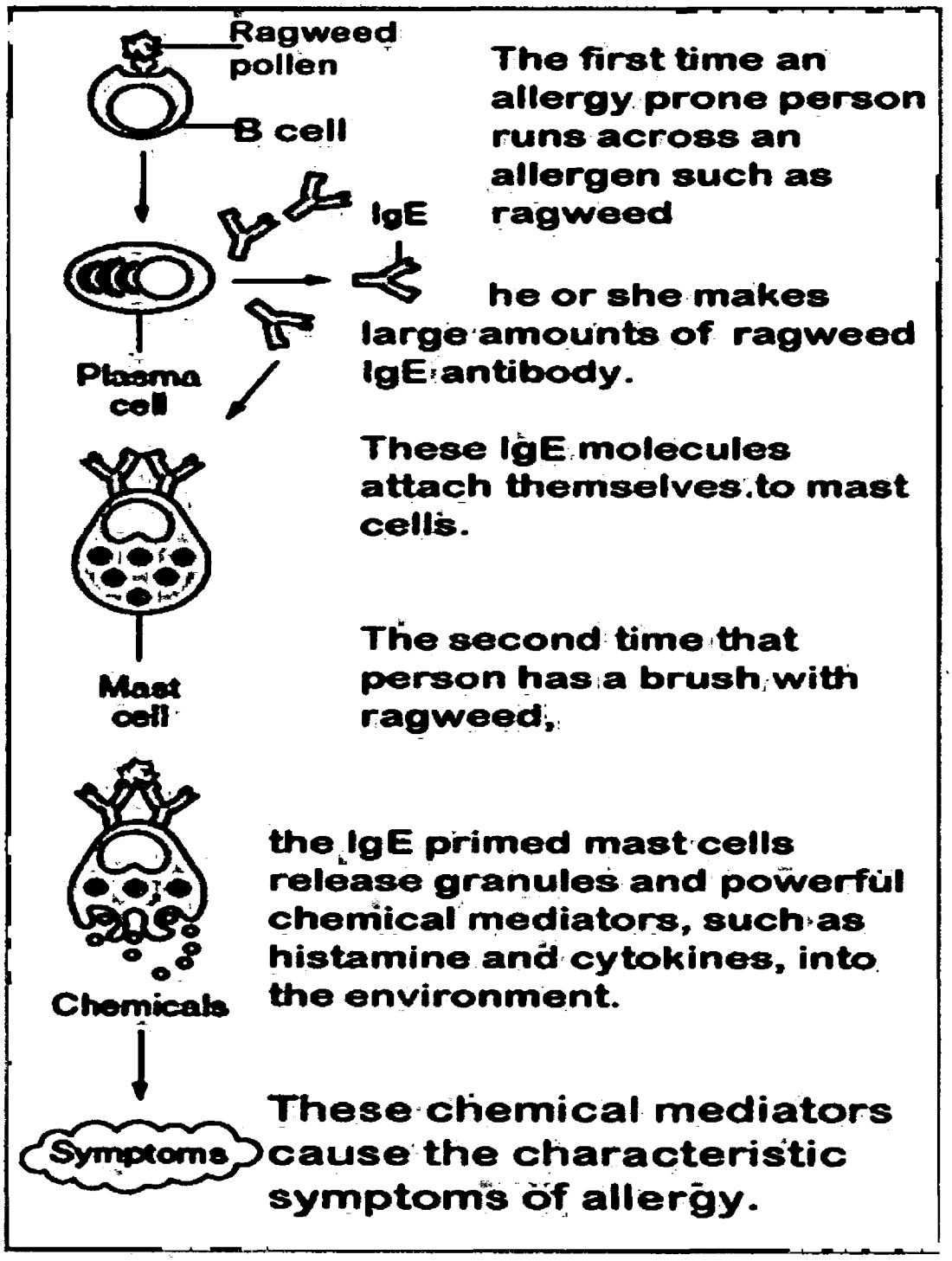


Figure 1.1: Role of mast cells in the progress of allergy

Immunoglobulin E is a type of antibody which is related with type I hypersensitivity (Gould H et al. 2003). It is solitary found in mammals and plays a significant role in allergy. IgE found on the surface of mast cells and basophils, evokes an immune response by their binding to Fc receptors. They are also found on macrophages, eosinophils, monocytes, and platelets in humans. Recent research has shown that the production of IgE can occur locally in the nasal mucosa (Takhar P et al. 2005).

As regards 40% of people experience various types of allergies and they're predominantly awful throughout fall and spring, at what time pollens and molds are towering. However allergies aren't just about a scratchy throat and runny nose. In actual fact, allergies are caused by a response in the immune system. The causes of allergies are divided into two categories (Grammatikos AP 2008) which are inscribed in Table 1.1.

Mild allergies akin to hay fever are widespread in human population and cause symptoms like itchiness, runny nose, and allergic conjunctivitis. In some people arduous allergies to environmental or dietary allergens or to medication may result in hazardous anaphylactic reactions. Some characteristic symptoms of allergy are described in Table 1.2.

The pathophysiology of allergic reactions can be divided into two phases, acute response and the late phase response. Acute response occurs immediately after exposure to an allergen. This phase then progresses into a late phase reaction which prolongs the symptoms of the response and result in tissue damage. Exposure to an allergen causes the mast cells and basophills to activate which then undergo a process called as degranulation explained in figure 1.2.

Table 1.1: Causes of allergy

Host factors	**Environmental factors**
Heredity	Adaptations in introduction to infectious diseases throughout early in childhood
Gender	Environmental pollution
Age	Dietary changes
Race	Allergen levels

Table 1.2: Characteristic symptoms of allergy

Affected organ	**Symptom**
Nose	swelling of the nasal mucosa (allergic rhinitis)
Sinuses	Allergic sinusitis
Eyes	redness and itching of the conjunctiva (allergic conjunctivitis)
Airways	Sneezing, coughing, bronchoconstriction, wheezing and dyspnea, sometimes outright attacks of asthma, in severe cases the airway constricts due to swelling known as laryngeal edema
Ears	feeling of fullness, possibly pain, and impaired hearing due to the lack of eustachian tube drainage.
Skin	Rashes such as eczema and hives (urticaria)

In this process mast cells release histamine and other inflammatory chemical mediators from their granules into the surrounding tissue which cause several systemic effects like vasodilation, mucous secretion, nerve stimulation and smooth muscle contraction. This results in rhinorrhea, itchiness, dyspnea, and anaphylaxis. The symptoms can be system-wide (classical anaphylaxis), or localized to particular body systems depending on the individual, allergen, and mode of introduction for e.g asthma is localized to the respiratory system and eczema is localized to the dermis (Janeway, Charles; et al 2001).

Histamine (2-[4-imidazolyl] ethylamine) was revealed in 1910 by Dale and Laidlaw (Dale HD, Laidlaw PD 1910). Histamine is an organic nitrogen compound formed by the body as a component of usual defenses and is involved in local immune responses. It is also a neurotransmitter which is essential for our brain cells to correspond appropriately (Marieb, E. 2001). It acts as a moderator in allergic reactions, administers stomach acid production and is indispensable to modulate sleep. It is composed of histidine residues as the name shows. It was acknowledged as a moderator of anaphylactic reactions in 1932 (Steinhoff M., et al. 2004). When a foreign pathogen enters the body, histamine is produced by basophils and the mast cells which are found in the connective tissue in close proximity and triggers an inflammatory response. It boosts the amiability of the capillaries to white blood cells and other different proteins to keep the foreign substances in the infected tissues (Di Giuseppe, M., et al. 2003). The blood fluids (containing leukocytes, which take part in immune reactions) arrive in the area resulting in inflammation.

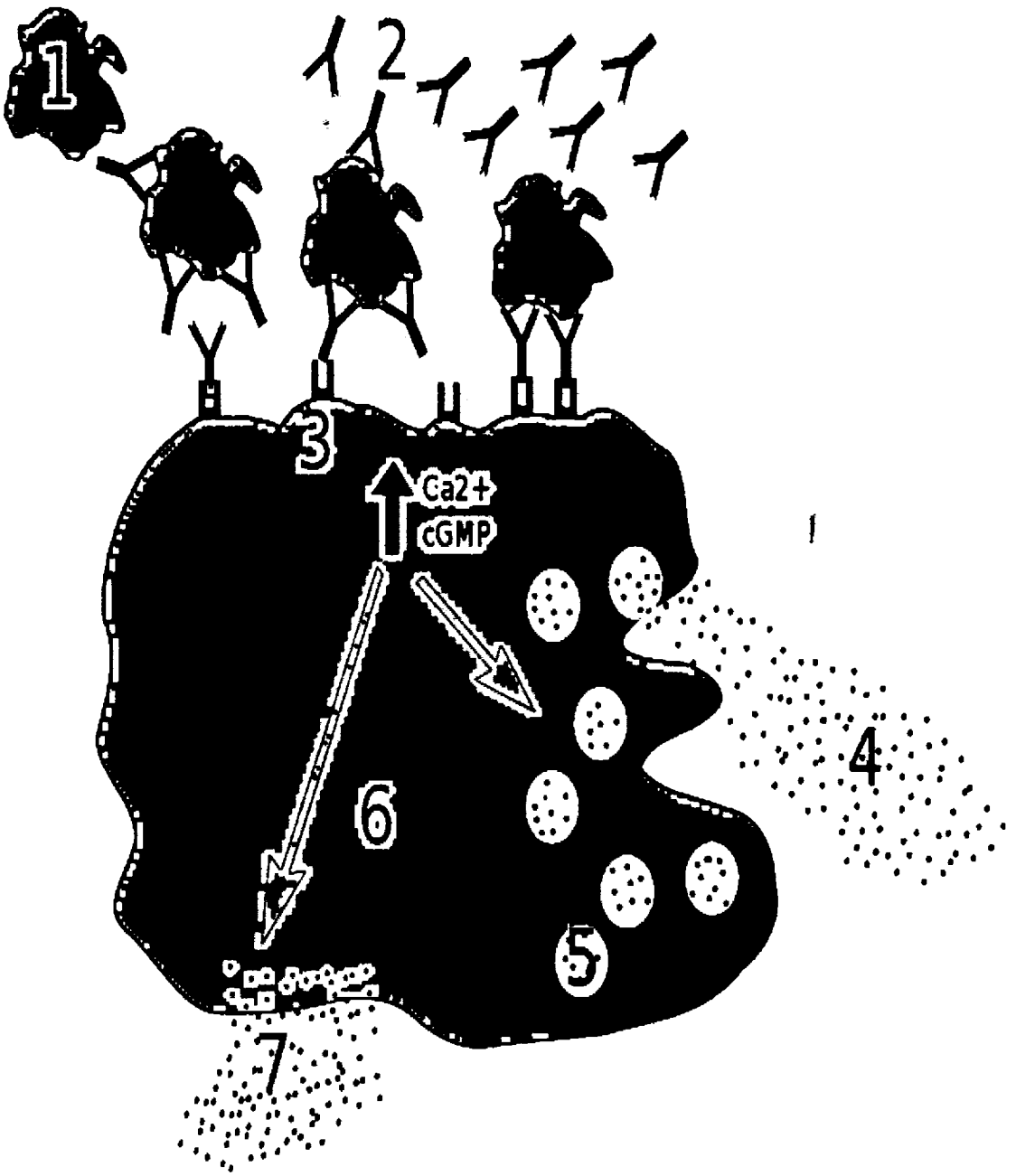


Figure 1.2: Degranulation process in allergy.1 - antigen 2 - IgE antibody 3 - FcεRI receptor; 4 - preformed mediators (histamine, proteases, chemokines, heparine) 5 - granules 6 - mast cell 7 - newly formed mediators (prostaglandins, leukotrienes, thromboxanes)

This is because of the capability of histamine to provoke phosphorylation of an intercellular adhesion protein (called (VE)-cadherin) which is found on vascular endothelial cells (Andriopoulou., et al. 1999). It also causes constriction of smooth muscles for e.g. in asthma therefore the muscles neighboring the airways tighten causing shortness of breath and probably complete tracheal-closure, which is a critical situation.

Histamine is also naturally present in certain foods. An enzyme called diamine oxidase breaks histamine that is present in different foods so it does not shows an effect. The people who have low level of this enzyme suffer from allergy like symptoms when they eat histamine rich food. Children are at highest risk of death from food allergy (Sampson., et al. 1992). Because of the switch of histamine from histidine in flawed food, like fish, food poisioning can occur.

By binding to four receptors [H1R, H2R, H3R, and H4R] histamine exhibit its special effects on target cells in a variety of tissues and these all are G protein-coupled receptors containing seven transmembrane helices (Laura Maintz and Natalija Novak 2007). Even though these receptors belong to the same family there is a well observed difference in their receptor distribution, ligand binding, functions and signaling pathways Table 1.3. Histamine also has suppressive effects which help to protect against the vulnerability to drug sensitization, stress, convulsion, ischemic lesions, denervation supersensitivity (Yanai, K., & Tashiro, M. 2007). The mechanisms by which memories and learning are forgotten is controlled by histamine (Alvarez, E. O 2009).

Table 1.3: Molecular pharmacology profile of Histamine receptor subtypes

	hH_1	**hH_2**	**hH_3**	**hH_4**
Chromosomal gene location	3p25	5q35.2	20q13.33	18q11.2
Amino acids	487	359	445	390
Isoforms			+	+
G-protein coupling	$G_0/_{11}$	G_5	G_1/G_0	G_1/G_0
Principal signal transduction	PLC ↑ Ca^{+2} ↑	cAMP ↑	cAMP ↓ Ca^{+2} ↑ MAPK ↑	cAMP ↓ Ca^{+2} ↑ MAPK ↑
Tissues	Lung, brain, vessels	Heart, stomach, brain	Neurons (CNS, PNS)	Mast cells, eosinophills
Physiological relevance	Contraction of smooth muscles, food intake, sleep-wake regulation	Gastric acid secretion	Sleep, food intake	Chemo taxis
Pathophysiological relevance	Allergic reaction	Gastric ulcer	Cognitive impairment, seizure metabolic syndrome	Inflammation, immune reaction

A lot of proteins particularly distinguish histamine, together with those in the pathways of histidine and histamine metabolism (W. Lorenz., et al. 1983). Apart from the histamine receptors, histamine can bind particularly to at least ten different proteins (S. Goto, Y. Okuno., et al. 2002). Histamine methyltransferase (HNMT) is a protein involved in the metabolism of histamine (V. Badireenath Konkimalla1 and Nagasuma Chandra 2003). Due to the ability of HNMT to methylate histamine, it plays a very important role in histamine biotransformation (R.M. Weinshilboum., et al, 1999). Methylated histamine is inactive when bound to its receptor so methylation is an important step in histamine inactivation. Histamine is inactivated by HNMT by the process of transfer of a methyl group from the S-adenosyl-L-methionine (SAM) or AdoMet to the nitrogen atom of the

imidazole ring of histamine. For the termination of neurotransmitter actions of histamine in the brain, N-methylation is the main process (Schwartz et al., 1991). HNMT and diamine oxidase metabolise histamine in mammals (Weinshilboum, R.M., et al, 1999). HNMT is a 292 amino acids long protein, found majorly in erythrocytes, liver, kidney with ample expression in prostrate, colon, spleen, ovary and spinal cord and minor level expression in placenta, brain, heart, lungs, small intestine cells and stomach (Preuss, C.V., et al., 1998). It is not present in plants, invertebrates and microorganisms. It is encoded by a single gene which is located on chromosome no 2q22.1 (Brown DD, Tomchick R, Axelrod J 1959). It has two domains, a larger conserved SAM binding fold and a smaller histamine binding domain as shown in figure 1.3.

The larger domain consist of seven beta stranded sheets flanked by three alpha helices on each side. The two coextending strands at the carboxy-terminus as well as some insertions specific to HNMT form the binding site. Hydrogen bonding is present amidst the amino group of histamine and the hydroxyl group of Tyr-147, however van der Waals interactions are formed by the the imidazole ring with the aromatic side chains around it (V. Badireenath Konkimalla1 and Nagasuma Chandra 2003). Two variants of HNMT are present in homosapeins, HNMT Thr105 and HNMT Ile105 (John R. Horton., et al. 2001). There is a 5-fold individual dissimilarity in the level of HNMT action as shown by the biochemical genetic studies of HNMT activity in human RBC which is due to the result of common genetic polymorphism (Scott et al., 1988; Price et al., 1993).

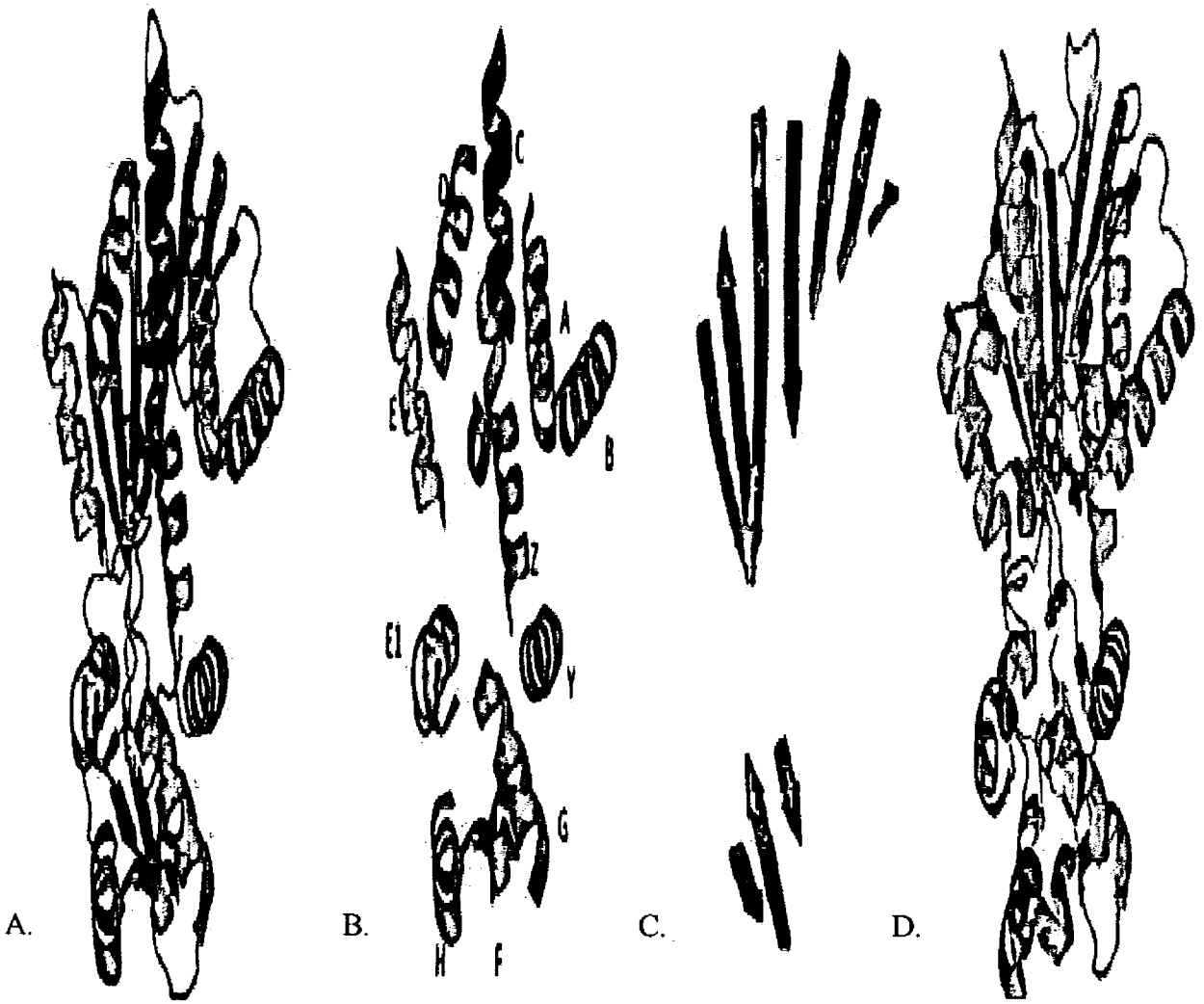


Figure 1.3: Ribbon Diagram of Histamine N Methyltransferase. (A) Diagram of HNMT showing the two domains, MTases, large and the subdomain (B) The alpha helices in HNMT labeled with alphabets (C) The Beta strands labeled number. (D) HNMT with Histamine and S-S-Adenosyl Homocysteine.

There might be a possible role for distinctive alteration in histamine metabohsm due to this polymorphism in the pathophysiology of diseases such as allergy, asthma, peptic ulcer disease, and neuropsychiatric illness (Charles V. Preuss, Mary C. Scott, et al, 1998).

The different receptor subtypes of histamine have been an important target of drugs for many decades (Holger Stark 2007). Different antihistamic drugs treat many physiological disorders by changing the mechanism of one of the histamine receptors (D.S. Pearlman 1976; J.J. Oppenheimer, T.B. Casale 2002). Antihistamines introduced in 1940's are now among the most commonly used medications (Simons, 1988). There are two typoikes of antihistamine drugs, H1 and H2 antagonists (J. Flo´rez., et al, 1997). The first H1 receptor antagonists were discovered by Bovet and Staub in 1937 (Bovet D, Staub A. 1937). The US Food and Drug Administration (FDA) categorize H1 antihistamines as sedating which enter the brain and cause drowsiness and H2 antihistamines as non-sedating. The sedating antihistamines blocks H1 and cholinergic receptors in neural tissues of the central nervous system resulting in reduced physical and mental function (B.A. Wroblewski, J. Head Trauma Rehabil. 1998). Due to this H1 antihistamines are occasionally brought into use as mesmerizing agents in the treatment of insomnia. Because of their extensive utilization, these drugs are normally implicated in accidental or deliberate poising (Taglialatela et al., 2000). Also they are used to treat symptoms of recurring and perennial allergic rhinitis and chronic urticaria, and they can also be used as a supplementary treatment for anaphylaxis (Howarth PH 1999). H2 antihistamines are generally used as antiulcer drugs as they decrease gastric acid production.

A large number of antihistaminic drugs are approved by FDA and are available in the market in different forms like nasal, topical, tablets, capsules and liquid form. These include brompheniramine, promethazine, hydroxyzine, ceterizine, loratadine, mizolastine, astemizole, terfenadine etc. The antihistamines such as loratadine, terfenadine and astemizole belong to the non-sedating class (Alain Didier., et al. 2000). Due to the risk of cardiac arrhythmia, terfenadine was superseded by fexofenadine in the 1990s. Fexofenadine is used to treat seasonal allergies and chronic idiopathic urticaria but its side effects include menstrual cramping, diarrhea, nausea, stomach problems, fatigue, drowsiness, back or muscle pain and headache. Loratadine is given for colds as well as allergies, but it has probable side-effects of insomnia, nervousness, and anxiety. Simillarly mizolastine side effects include dry throat and mouth. Apart from their sedative adverse effects, exuberance of H1 antihistamines can cause serious side effects, which may include convulsions, seizures and worsening of epileptic symptoms (Hestand and Teske, 1977; Magera et al., 1981). The most characteristic side effects of these drugs include blurred vision, headache, drowsiness, dizziness, constipation, difficulty passing urine, dry mouth, dry eyes and confusion (Corren J., et al, 2000). Clearly there is a need to produce new, more effective drugs with low side effects.

Recent progress in the disciplines of molecular biology, cell biology and biochemistry, assisted by the advancements in proteomics and genomics, are producing a great number of fresh biological targets that may be browbeaten for therapeutic involvement. For the past few years the reverberating message is that the discovery and successive expansion of a new-fangled therapeutic molecule involves more than a decade of investigation and nearly 800 million to 1 billion dollars (Bain, W. 2004, Kola, I. and

Landis, J. 2004). This number includes the cost of thousands of failures. Only one compound receives approval out of every 5000 to 10,000 compounds that enters the research and development pipeline. Still regrettably there is no assurance that the designer corporation will regain its research investment. There are also cases where the adverse effects of a drug are only apparent when it has been administered to a large number of populations. To meet the challenges of an ideal drug not only a competent method of drug development is required but also accomplishment of strategies to avoid probable failure is also desperately needed (Pramik, M.J. 1989).

In the late 1980s the influx of supercomputers at pharmaceutical companies heralded the era of rational drug design (Pramik, M.J. 1989). The swift recognition of fresh therapeutic agents for definite disease states is conceivably one of the utmost steps in health care that has occurred in the 21st century. The finding of the 3-dimensional (3D) structures of biological target molecules have become permissible by the advances in structural biology by means of the procedure of nuclear magnetic resonance (NMR) or X-ray crystallography, through more than 16,000 3D structures at present existing in the Protein Data Bank. To smooth the progress of the unearthing of innovative therapeutic agents, rational drug design techniques in amalgamation with structural biology propose enormous prospective.

Rational Drug Design has turned out to be an entrenched discipline in pharmaceutical research. It is the creative process of discovering new medications based on the understanding of biological target (Madsen, Ulf; Krogsgaard-Larsen, Povl; Liljefors, Tommy. 2002). It makes use of computational chemistry with the aim to discover or study drugs and their associated biologically active molecules. The basic

intention is to trim down the number of targets for a good quality drug that have to be subjected to expensive and time-consuming synthesis (Roberta Galeazzi. 2009). Computer-aided drug design (CADD) techniques can bring into play the knowledge in the 3D structures of biological target molecules. It can be used to be acquainted with chemicals with a lofty prospective for binding to the biological target molecules. The chosen compounds are attributed to as lead compounds. These may subsequently be subjected to further structural optimization by way of CADD, novel organic synthetic methods and structural biology to acquire compounds with enhanced actions. Together the optimized analogs of the lead compounds and they themselves symbolize chemical entities with a soaring likelihood of being developed into therapeutic agents and, consequently, they are of a great significance to pharmaceutical companies. The sophisticated methods developed in this field amalgamated with the increased effectiveness of the new computer generation are the tools for the scientist to discover the conformational variability and properties of a huge number of potentially active molecules and their interaction with each other or with their biological target (Roberta Galeazzi. 2009).

Drug designing can be separated into two foremost types; the structure based drug design and the ligand based drug design. Ligand-based design methods take advantage of the fact that ligands which are comparable to an active ligand are expected to be more active than random ligands. Ligand-based approaches on the whole consider two- or three-dimensional chemistry, electrostatic, shape, and interaction points (e.g., pharmacophore points) to evaluate likeness. Structure-based drug design is a swiftly mounting research field. In this numerous achievements have been reported in current

years. It relies on the understanding of 3D arrangement of biological target accomplished all the way through procedures such as X-ray crystallography and NMR studies (Leach, Andrew R.; Harren Jhoti 2007). Structure based study has led to the perception of 'druggability'. This is used to illustrate proteins that attain protein folds that favour interactions with drug-like chemical compounds (Hopkins and Groom, 2002; Keller et al., 2006; Orth et al., 2004; Russ and Lampel, 2005). The structure based drug design process is shown in figure 1.4.

Paul Ehrlich first defined the word pharmacophore in 1909 as a molecular scaffold that caries the indispensable characteristics accountable for a drug's biological activity. A pharmacophore is a definite; three dimensional plot of biological features widespread to all vigorous conformations of a combination of ligands which reveal a scrupulous action. It is a refinement of the functional attributes of ligands.

Pharmacophore modeling is a potent method to swiftly identify new potential drugs. For the abundant therapeutically relevant drug targets with uncertain active site geometries, pharmacophore modeling provides a valuable mechanism for virtual screening. It is a spatial arrangement of functional groups indispensable for biological activity and a blueprint that emerges from a set of molecules with a universal biological activity. Pharmacophores are theoretical motives for the design of drugs. A pharmacophore can be used as a representation for the design of additional molecules that can carry out the identical action just once it is extracted from a set of ligands.

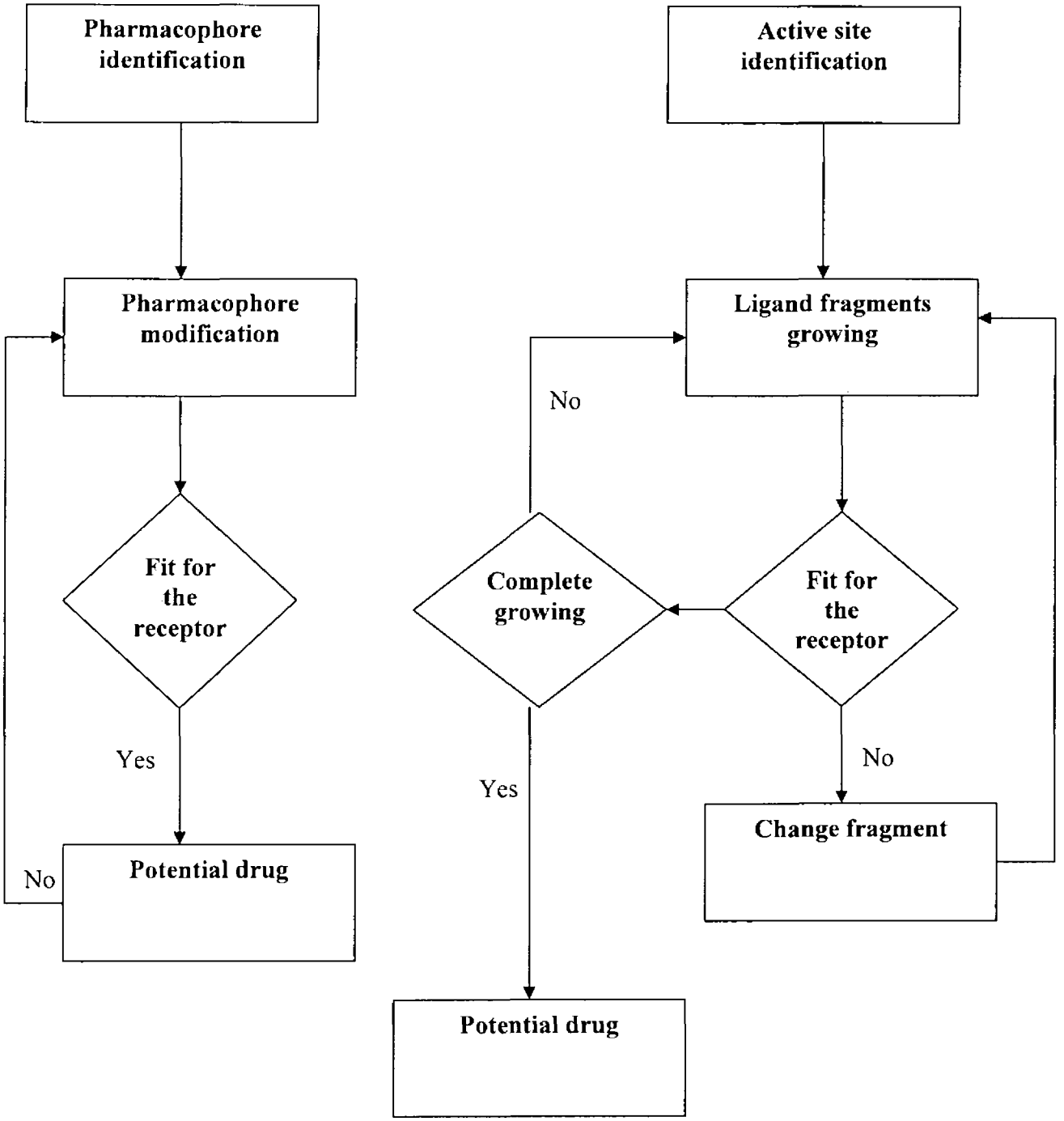


Figure 1.4: Structure based drug design process

The problem of pharmacophore identification is to spawn the pharmacophore from structural data describing ligands and their interaction with the receptor. The pharmacophore features include hydrophobic, aromatic, a hydrogen bond acceptor (HBA), a hydrogen bond donor (HBD), positive ionizable, or negative ionizable. These attributes have to match different chemical groups with analogous properties, for identifying novel ligands. Recent pharmacophore models can be classified into two categories that are receptor-based pharmacophores and ligand-based pharmacophores. For a receptor with a known three-dimensional structure, receptor-based pharmacophores have been studied which are based on the famous concept of a key for the lock (Brooijmans, N. and Kuntz, I. D 2003). On the contrary, for the many proteins whose three-dimensional structures have not been known, ligand-based pharmacophore models are still useful (Martin, Y. C.; Bures, M. G et al., 1993). Conventionally, ligand-based pharmacophore models are computed by extracting common features amid three-dimensional structures of compounds which are known to interact with a target protein (GÄuner, O. F. 2000). To achieve affinity, the ligand should be both sterically and electronically complementary to the receptor binding action.

Automated programmes for pharmacophore development emerged including DISCO by Yvvonne Martin, HipHop by Barnum and coworkers, GASP by Jones and Willett, LigandScout (Wolber, Langer et al., 2005), Catalyst (Guner, O.; Clement, O.; Kurogi, Y, 2004) and MOE. Several examples of predictive pharmacophore model generation approaches include Apex 3D by Golender and Vorpagel, COMFA by Cramer at el. and Hypogen by Teig,Greene and Sprague. Several automated prgrames are on their

way to explore the pharmacophores. They rely on the steric, electronic and hydrophobic properties of the chemical moities.

LigandScout 3.0 is a fully integrated podium for accurate virtual screening based on 3D chemical feature pharmacophore models (Wolber, Langer et al., 2005). It offers flawless workflows, starting both from ligand and structure based pharmacophore modeling, and includes novel high performance alignment algorithms for excellent prediction quality with incomparable screening speed. It also includes a user-friendly screening analysis tools.

For the rational design of novel bioactive compounds, the 3D pharmacophore and receptor illustrations will be used as tools which have higher affinity for the target receptors. A comparable examination of the 3D pharmacophore and receptor illustrations will also make it attainable to design drugs which are selective for one or more subtypes of the receptors. In this manner the time taking drug designing process may be made precised.

In drug discovery process virtual screening techniques are becoming progressively more popular. A well-liked method for virtual screening is molecular docking (G. M. Morris and M. Lim-Wilby, 2008). It screens small-molecule structures from large databases and docks them into the protein binding site (G. Schneider and H.J. Bohm, 2002). There are millions of chemical compounds accessible for docking. The virtual drug screening method can take momentum by eliminating compounds that are improbable to demonstrate high binding affinity from the screening set (Alisa Wilantho., et al, 2008). The arrangement of the intermolecular multifaceted shaped between two or more molecules can be foretold by molecular docking.

For molecular docking diverse search algorithms have been examined. The results specify that quite a lot of different approaches are efficient and give reasonable performance (Westhead DR, Clark DE, Murray CW 1997, McMartin C, Bohacek, RS 1997). Molecular docking can be alienated into two different problems. The search algorithm is supposed to create an optimum number of configurations that comprise the experimentally determined binding modes. Scoring functions are used to assess these configurations to differentiate the experimental binding modes from all other modes investigated through the searching algorithm. Some common searching algorithms comprise Monte Carlo methods, molecular dynamics, fragment-based methods, genetic algorithms, distance geometry methods, point complementary methods, systematic searches and Tabu searches (McMartin C, Bohacek, RS 1997).

Existing docking procedures make use of the scoring functions in one of two ways. The foremost technique makes use of the full scoring function to grade a protein-ligand conformation. Search algorithm is then used to modify the system, and the similar scoring function is once more applied to order the fresh structure. A two stage scoring function is used in the substitute method. To direct the search a reduced function is used and an additional meticulous one is then applied to grade the consequential structures. Various general scoring functions are empirical free energy scoring functions, knowledge-based potential of mean force and force field methods. Quite a lot of computer programs for molecular docking have been exemplified within the previous years (Clark DE, Westhead DR 1996). The initial computer-assisted approach to the sighting of ligands for a specified binding site was the program DOCK (UCSF, CA, USA) (Ewing TJA, Kuntz ID 1997). Other most commonly used programs are GRID

(University of Oxford, UK), AutoDock, FlexX (GMD, Germany), GOLD (Genetic Optimization for Ligand Docking; Cambridge Crystallographic Data Centre, UK), LigandFit, Sybyl, Glide, Hex and MOE.

To offer a programmed system "AutoDock" was developed for anticipating the interaction of ligands with biomacromolecular targets. It makes use of Monte Carlo simulated annealing and Lamarckian genetic algorithm to generate a set of probable conformations. AutoDock look for for the most excellent ways to fit a ligand molecule into a receptor. It comes out with a docking log file that has a comprehensive record of the docking (Morris et al., 1996). It is a priceless implementation in the x-ray structure determination procedure itself. AutoDock can help out to constrict the conformational possibilities and facilitate to recognize a good quality structure if given the electron density for a ligand. The convenience of analyzing a huge search space and a vigorous energy evaluation are brought together. This is demonstrated to be a prevailing approach to the dilemma of docking a flexible substrate into the binding site of a static protein.

Quantitative structure-activity relationship (QSAR) prototype first found its way into the practice of agro chemistry, pharmaceutical chemistry, industrial, environmental chemistry, toxicology, and eventually most facets of chemistry for almost 40 years (C. Hansch and A. Leo, 1979). Quantitative structure activity relationship (QSAR) is a mathematical association amid a biological (ecological, toxicological or pharmacological) activity of a molecular system and its chemical and geometric characteristics. QSAR attempts to come across a constant connection among biological activity and molecular properties, with the intention that these can be used to appraise the activity of new compounds. These studies basically depend on the supposition that the structure of a

molecule (i.e. its steric, geometric, and electronic properties) have got to enclose the characteristics liable for its chemical, biological and physical properties, and on the ability to symbolize the chemical by one, or more, numerical descriptors.

The ultimate QSAR should: (a) consider an ample number of molecules for adequate statistical representation, (b) have a extensive range of quantified end-point potency for regression models or adequate distribution of molecules in each class (i.e. active and inactive) for classification models, (c) be pertinent for unswerving predictions of new chemicals and (d) permit to acquire mechanistic information on the modeled end-point. QSAR relates drugs potency or toxicity with a range of molecular descriptors. It employs electronic, hydrophobic, structural and topological parameters of the compounds (C. Hansch. 1969). Some of the electronic descriptors include the common Hammett constants (σ, $\sigma+$, $\sigma-$), HOMO (highest occupied molecular orbital), LUMO (lowest unoccupied molecular orbital), dipole moment, heat of formation, total energy and binding energy. Few of the steric descriptors are surface area, volume, molecular weight and molar refractivity. The first steric parameter used in QSAR studies was Taft's E_S constant (R. W. Taft in M. S. Newman, Ed. 1956). It is elementary that suitable descriptors be engaged, to attain a noteworthy correlation, whether the descriptors are empirical, theoretical, or derived from eagerly accessible investigational characteristics of the structures.

There are two major advantages of the QSAR study: (a) the compounds and their diverse fragments and substituents can be directly characterized on the basis of their molecular structure only and (b) the anticipated mechanism of action can be directly accounted for in terms of the chemical reactivity of the compounds under study (Cocchi,

M.; Menziani, M. C. 1992). Once a correlation between structure and activity is found, any number of compounds, including those not yet synthesized, can be readily screened on the computer in order to opt for the structures with the properties desired. It is then possible to select the most promising compounds to synthesize and test in the laboratory. Thus, the QSAR approach conserves resources and accelerates the process of development of new molecules for use as drugs, materials, additives, or for any other purpose.

Different programs are specifically developed for calculating QSAR the most important of which is comparative molecular field analysis (CoMFA). It is one of the recognized 3D-QSAR descriptors which has been used frequently to produce the three dimensional models to point out the regions that have an effect on biological activity with a change in the chemical substitution (Huang, M.; Yang, D.Y.; Shang, Z.; Zou, J.; Yu, Q. 2002). The primary advantage of it is that it can forecast the biological activity of the molecules and it also represents the relationship among electrostatic characteristics and biological activity in the form of a contour map. Others include self organized molecular field analysis (SOMFA), molecular electrostatic potential (MEP), molecular lipophilicity potential (MLP), GRID, HyperChem, DRAGON and ChemDraw.

MATERIALS AND METHODS

MATERIALS AND METHODS

2.1 Preparation of ligand structures

The ligands used in this study comprise different groups of indene (Bin-Feng Li, Wilna J. Moree et al., 2010), benzothiophene (Wilna J. Moree, Florence Jovic et al., 2010), 2-aminobenzimidazole (Timothy Coon, Wilna J. Moree et al., 2009) and 2-(piperidin-3 yl)-1Hbenzimidazoles (Karine Lavrador-Erb, Satheesh Babu Ravula et al., 2010). The compounds were drawn in ChemDraw Ultra 8.0. The total data set consists of forty compounds including four standard compounds (Jean-Marie Nicolas et al., 1999). The compounds were then converted to Protein Data Bank (pdb) format using Chem3D Ultra 8.0. The structures of the ligands are shown in Table 2.1.

2.2 Pharmacophore generation

Pharmacophore model is generated using the software LigandScout (version 3.0). It allows swift and obvious derivation of 3D chemical feature-based pharmacophores from structural data of macromolecule ligand complexes in a fully programmed and expedient way. It offers flawless workflows, starting both from ligand and structure based pharmacophore modeling, and includes novel high performance alignment algorithms for outstanding prediction quality with exceptional screening speed (Wolber, Langer et al., 2005).

Ligand based pharmacophore model was created for the dataset of forty compounds using default settings. The pharmacophore for each group of compounds has been generated and the distance among the pharmacophoric features of the compounds has been calculated using software Visual Molecular Dynamics (VMD). VMD is a

molecular visualization program for displaying, animating, and analyzing large biomolecular systems using 3-D graphics and built-in scripting (Humphrey, W., Dalke, A. and Schulten, K., 1996). VMD can simultaneously display any number of structures using a wide variety of rendering styles and coloring methods. Molecules are displayed as one or more "representations," in which each representation embodies a particular rendering method and coloring scheme for a selected subset of atoms. Superimposition of the pharmacophoric features has been done in order to get the common pharmacophore for antihistamine inhibitors. The distances among the pharmacophoric features of the common and distinctive pharmacophore were then calculated.

2.3 Protein structure

Histamine methyltransferase (HNMT) was selected as the target protein as it plays a central role in histamine biotransformation. It inactivates histamine by transferring a methyl group from the S-adenosyl-L-methionine to the nitrogen atom of the imidazole ring of histamine. The arrangement and structure of human HNMT has been determined in recent times (1JQD) and also the binding site of histamine is recognized.

2.4 Molecular Docking

AutoDock 4, a modern docking program was used for docking calculations. AutoDock (Morris et al., 1996) is a group of automated docking tools. It is intended to foretell how small molecules, such as substrates or drug candidates, bind to a receptor of known 3D structure. A new module of AutoDock is used i.e. AutoDock Vina. It is a new open-source program for molecular docking, drug discovery and virtual screening, offering multi-core capability, high performance and enhanced accuracy and ease of use. The target protein which is in pdb format is loaded in AutoDock 4 and converted into

pdbqt format. For this purpose the protein is loaded in Auto Dock and hydrogens were added to all atoms to ensure that their valences are completed. To save the protein in pdbqt format the macromolecule from the grid tab was chosen and then saved in the required format. Then the grid parameter file was created which tells AutoDock 4 which receptor to compute the potentials around, the types of maps to compute and the location and extent of those maps. From the grid box the x, y and z dimensions were noted which are set to 50x50x50 A° grid points and 0.375 A° grid point spacing. Also the x, y, z center values are noted and written in the grid parameter file. This file is then saved and used for the docking of ligands. AutoDock 4 must be run in the directory where the macromolecule in pdbqt format, ligand, and grid parameter file are to be found. Ligand is then opened in AutoDock 4 and all active bonds are made non rotatable by choosing the torsions from the torsion tree. After done with it the ligand is then saved in pdbqt format in the same directory where the input files are saved. Then from the command prompt docking is initiated. Docking simulations were performed using the Lamarckian genetic algorithm with a maximum of 250000 energy evaluations with an output file named as log file in the same directory. From the output file we can get the affinity values in kcal/mol. All the remaining ligands were docked in the similar way.

2.5 Calculation of binding interactions

After the protein ligand docking, the binding interactions were calculated using Visual Molecular Dynamics (VMD). The active confirmation of each ligand obtained after docking was then copied from the pdbqt file and pasted in the protein pdb file in place of heteroatom. This file was then given as input to VMD for interaction calculations. The interactions between the ligand and the active site of the target protein

were calculated within 5 A° region. Four types of interactions were considered including ionic, hydrophobic, hydrogen bonding and Vander wall interactions. Only those interactions are recorded which are less than 5 A°.

2.6 Lead compound identification

After calculating the binding interactions and taking into consideration the bioactivity, the most active compound was identified and considered as the lead compound.

2.7 Analog preparation

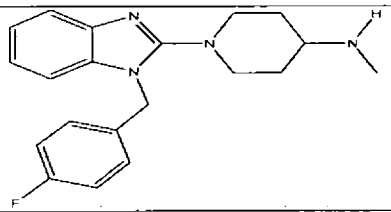
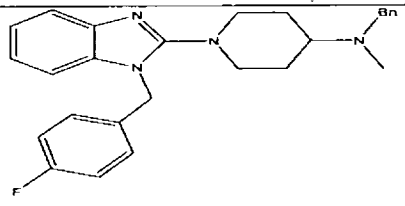
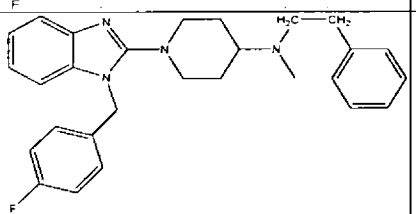
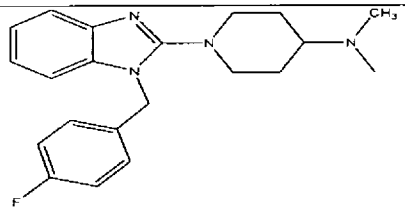
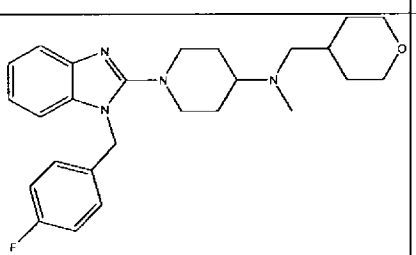
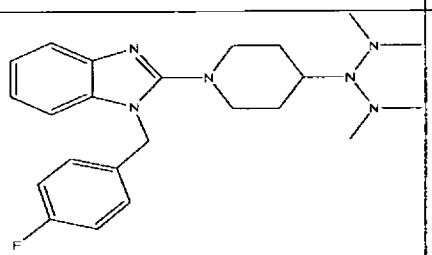
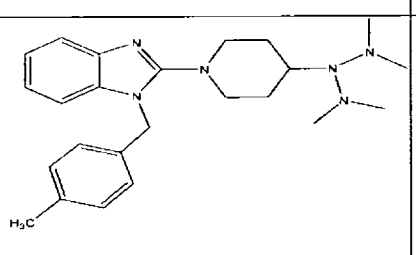
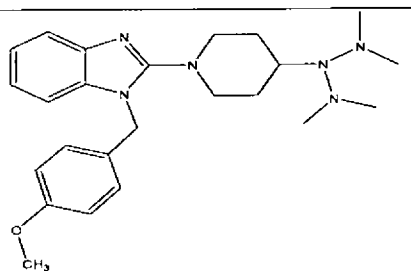
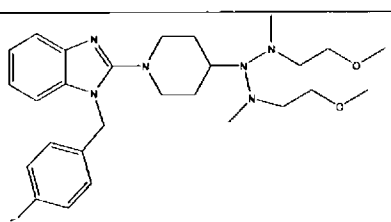
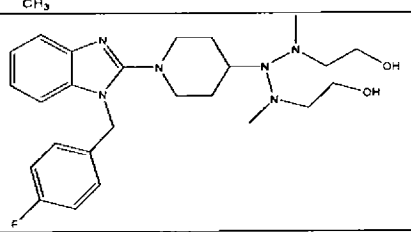
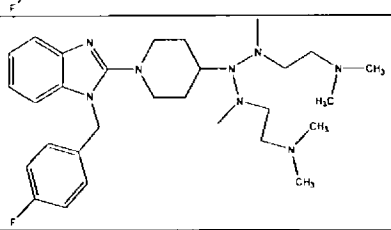
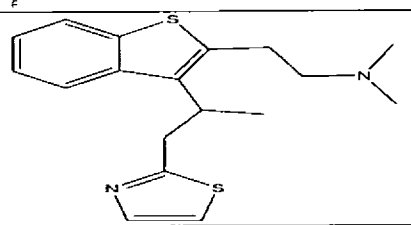
By studying the lead compound five different analogs of the lead were prepared. Docking of these analogs was done by the same procedure as mentioned above and also the binding interactions of these analogs were calculated after docking within 5 A° region.

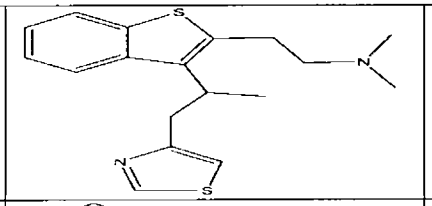
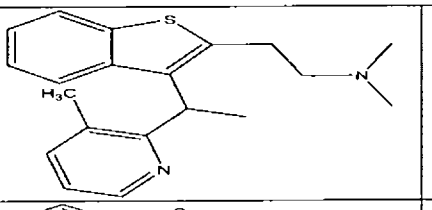
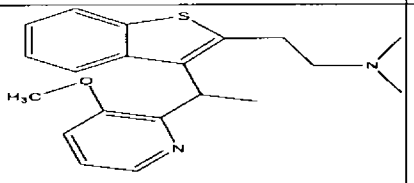
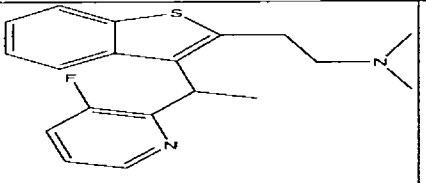
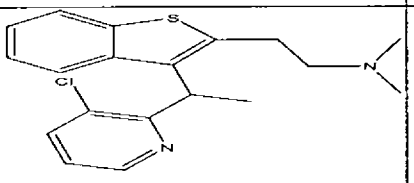
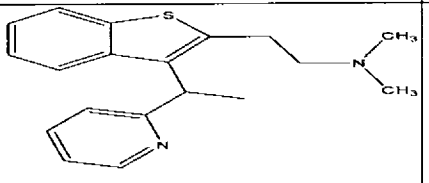
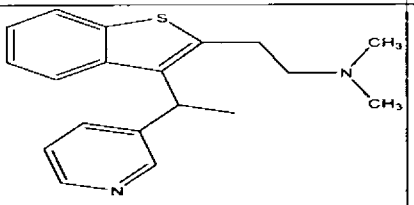
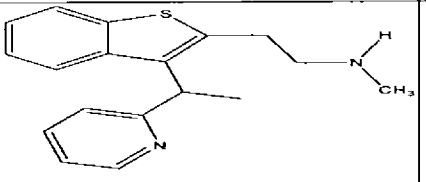
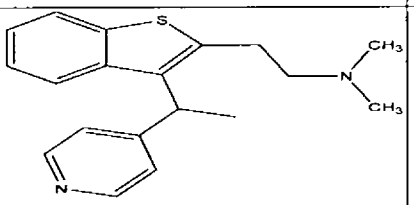
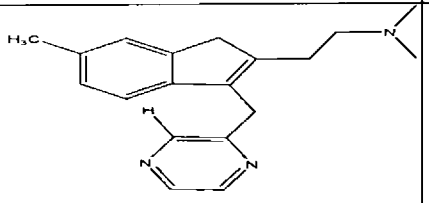
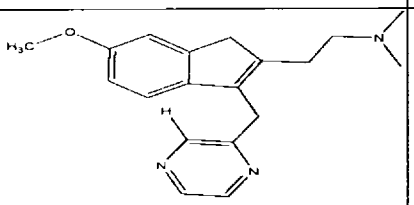
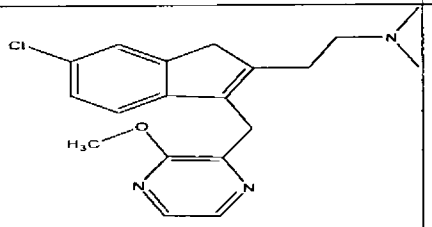
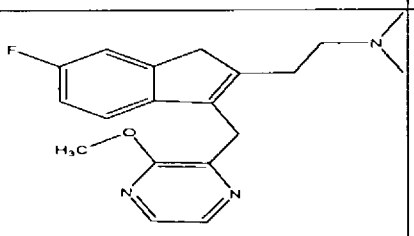
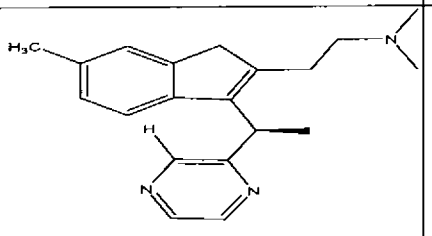
2.8 Quantitative Structure Activity Relationship (QSAR)

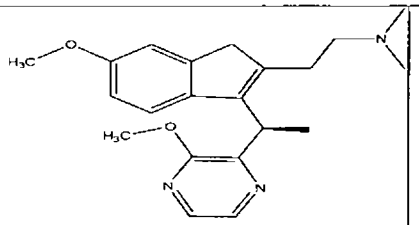
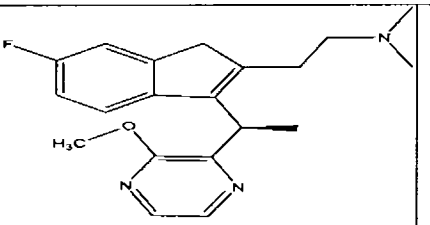
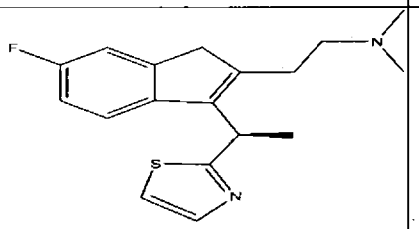
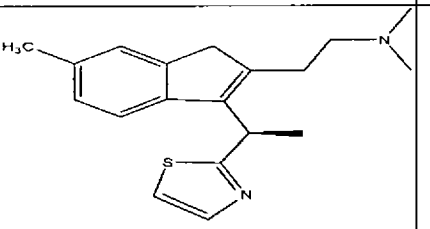
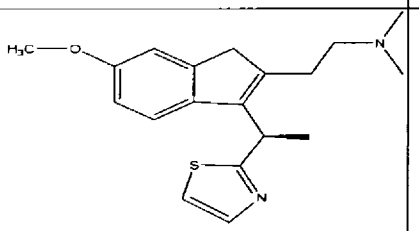
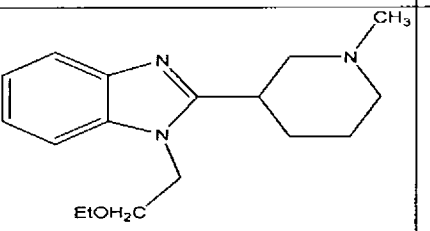
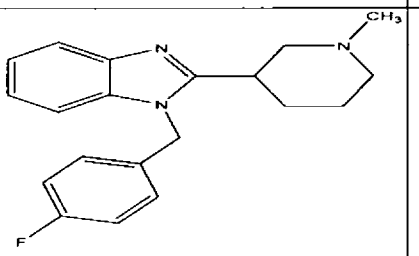
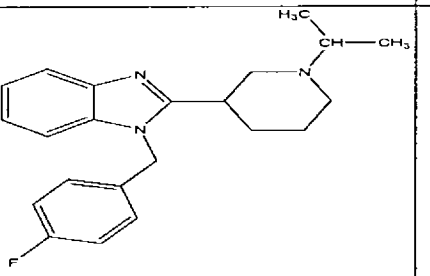
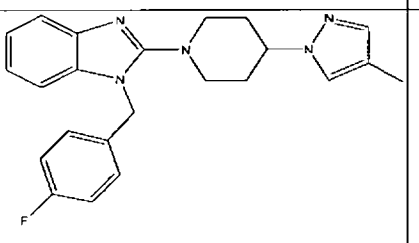
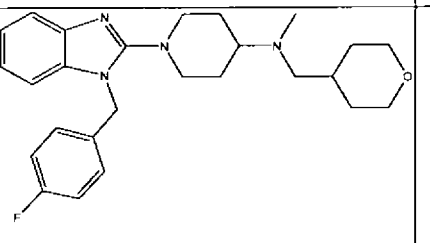
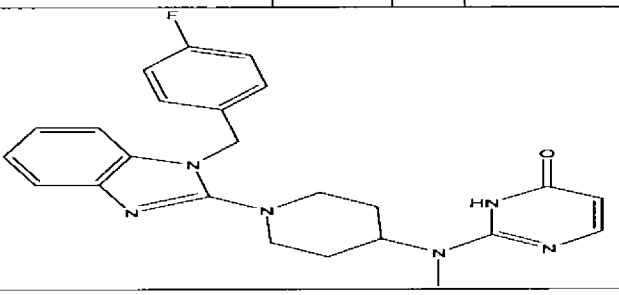
A quantitative structure activity relationship has been established on a set of 20 compounds of 2-(piperidin-3-yl)-1H benzimidazoles group. A number of steric and electronic parameters were calculated using HyperChem (Hyper cube, Inc., Florida, USA, 2007) and Chem Draw softwares. Table 2.2 represents the structures used in this study.

The flowchart of various steps of methodology is shown in figure 2.1 and the summary of tools used is shown in table 2.3.

Table 2.1: Chemical structures of ligands used in the present investigation and their IC_{50} values

No.	Structure	IC_{50} (μM)	No.	Structure	IC_{50} (μM)
1.		6.664	2.		0.805
3.		0.502	4.		12.798
5.		6.360	6.		12.798
7.		7.210	8.		6.072
9.		4.444	10.		35.150
11.		0.776	12.		0.856

13.		4.926	14.		5.5
15.		9.5	16.		4.0
17.		1.5	18.		3.3
19.		2.0	20.		9.8
21.		0.85	22.		6.6
23.		5.3	24.		0.69
25.		0.32	26.		22

27.		1.9	28.		6.4
29.		1.0	30.		1.4
31.		5.6	32.		6.6
33.		2.3	34.		0.618
35.		5.4	36.		11.0
Mizolastine					0.118

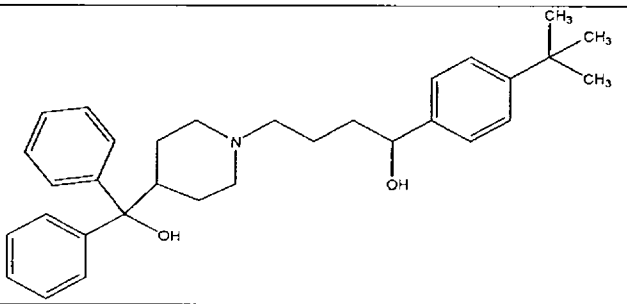
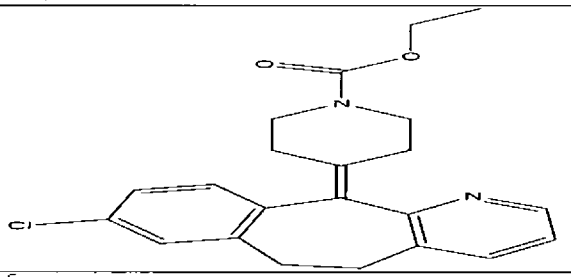
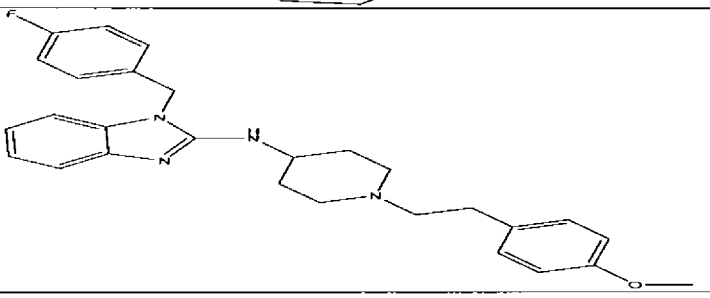
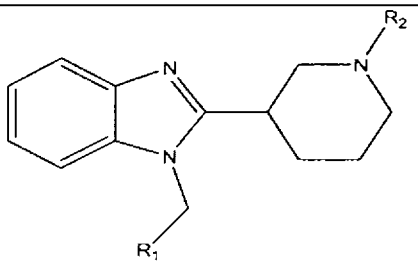
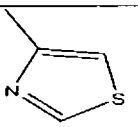
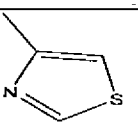
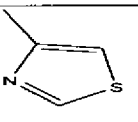
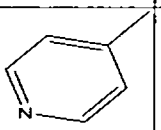
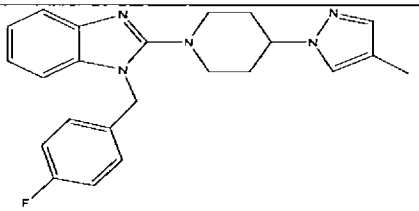
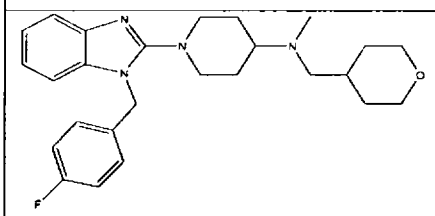
Terfenadine		0.018
Loratadine		0.015
Astemizole		0.036

Table 2.2: Chemical structures of 2-(piperidin-3-yl)-1H benzimidazoles used in the present investigation and their IC_{50} values

Basic structure	No.	R^1	R^2	IC_{50}
	1	p-F-Ph	Me	3065
	2	p-MeOPh	Me	465
	3	CH_2OEt	Me	1556
	4		Me	15989
	5	p-F-Ph	$CH(CH_3)_2$	618
	6	p-F-Ph	Cyclohexyl	195
	7	p-MeOPh	$CH(CH_3)_2$	717
	8	p-MeOPh	Cyclohexyl	122
	9	p-MeOPh	Tetrahydropyran-4-yl	1901
	10	CH_2OEt	Cyclohexyl	526
	11	CH_2OEt	Tetrahydropyran-4-yl	4393
	12		Cyclohexyl	480
	13		Tetrahydropyran-4-yl	6453
	14	p-F-Ph	Me	2.3

	15	p-F-Ph	Me	2.8
	16	CH_2OEt	Me	6.6
	17		Me	53.3
	18	CH_2OEt	Tetrahydropyran-4-yl	32.2
	19	-	-	5.4
	20	-	-	11.0

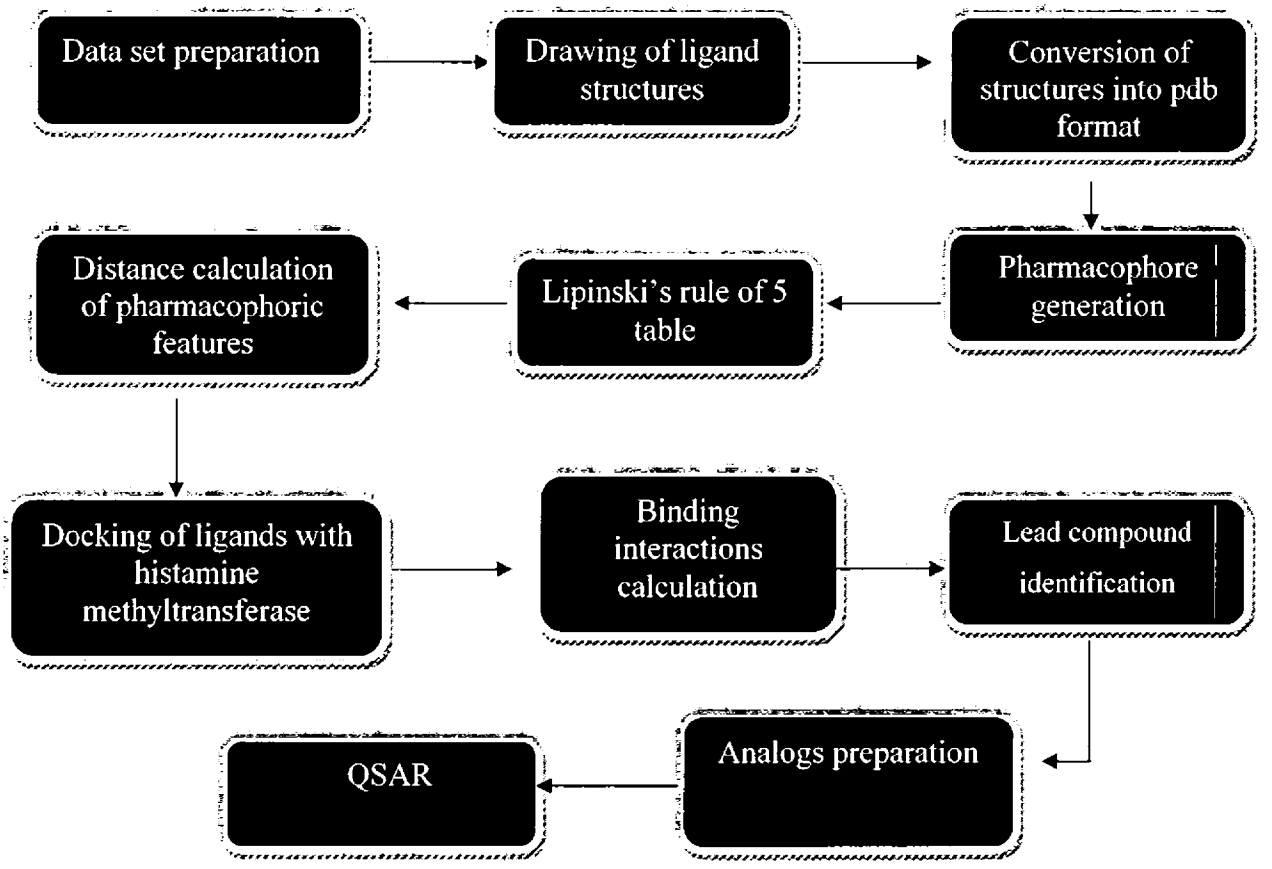


Fig. 2.1: Flow chart of various steps of methodology

S.No.	Tools	Output
1	VMD	Visualizer, interactions calculation
2	AutoDock Vina	Ligand Docking
3	LigandScout	Pharmacophore generation
4	Chem Draw	Drawing of structures
5	HyperChem	QSAR

Table 2.3: Summary of Tools used

RESULTS AND DISCUSSION

Results and Discussion

3.1 Pharmacophore modeling

In this study a 3D pharmacophore model is developed in order to support the unearthing of type specific and effective antihistamine inhibitors for the treatment of allergy related diseases. The dataset as shown in Table 2.1 from earlier experiments and literature cited previously was selected, in order to find out the spatial arrangement of the functional groups that confers drug activity towards the receptor i.e. histamine methyltransferase.

The pharmacophore of human histamine H1 receptor has been reported which consist of five essential features i.e. hydrogen bond acceptor, ring aromatic, positive ionizable and two hydrophobic functions (Keun Woo Lee et al., 2010). In the current study the pharmacophore generated for the present data set by Ligand Scout depicted five chemical features i.e. hydrophobic, hydrogen bond acceptors, hydrogen bond donors, aromatic rings and positive ionizable functions. The pharmacophore generated for the selected group of compounds exhibit uniformity in the above mentioned features. The representative pharmacophore of each group of compounds is shown in figure 3.1-3.4 and that of a standard drug is shown in figure 3.5.

These figures show the 3D and 2D views of the pharmacophore. Different features are shown in the 3D view and are also labeled in the 2D view. Hydrogen bond acceptors are shown in red, hydrogen bond donors in green, hydrophobic features in yellow, aromatic features in blue rings and positive ionizable in blue.

Figure 3.1: Pharmacophore model of compound no 32 showing the 2D and 3D view

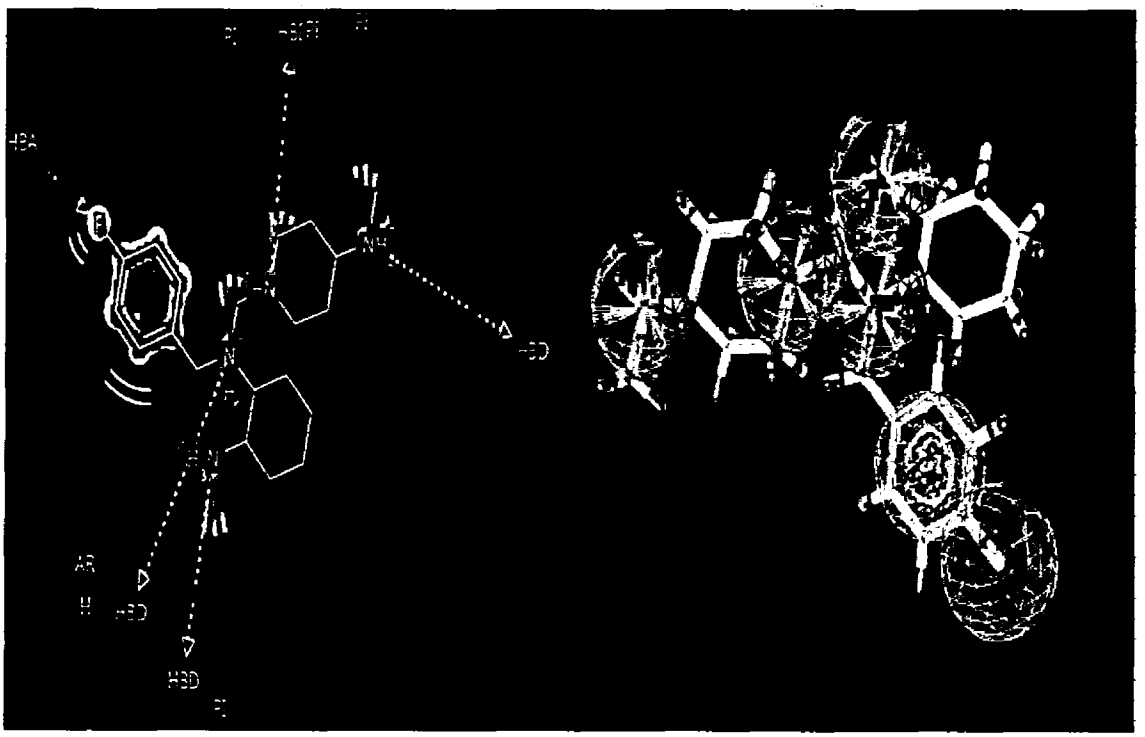


Figure 3.2: Pharmacophore model of compound no 1 showing the 2D and 3D view

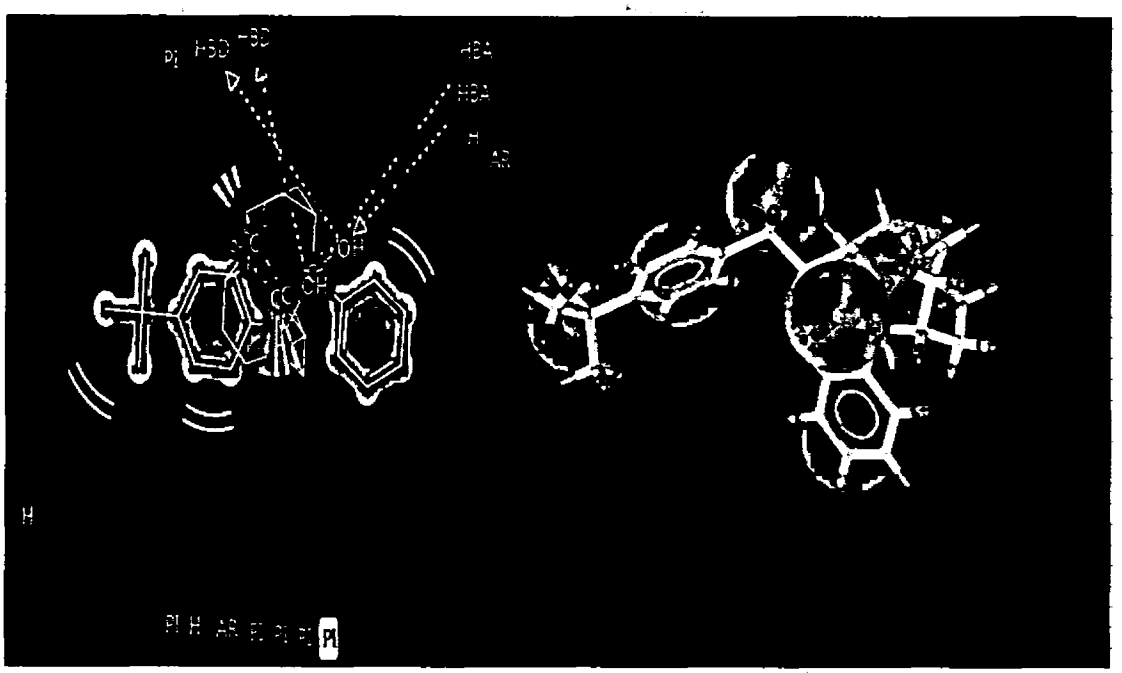


Figure 3.3: Pharmacophore model of terfenadine showing the 2D and 3D view

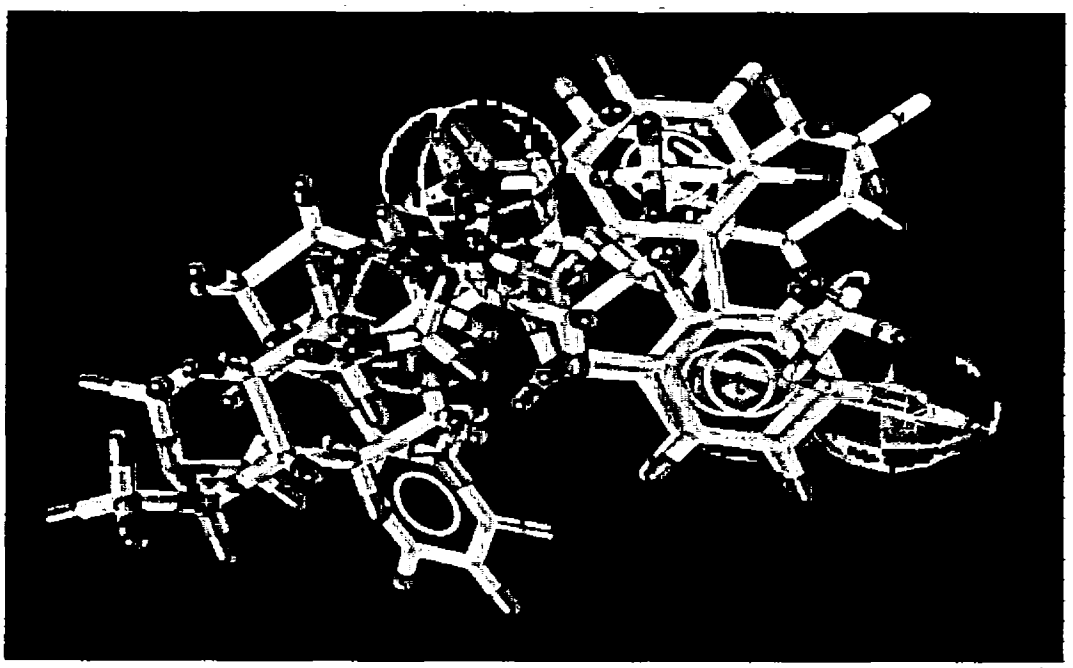


Figure 3.4: Aligned features of all ligands generated by Ligand Scout

The pharmacophore of all the compounds showed five common features. The similar features of all the compounds were then superimposed and merged into a single pharmacophore as shown in figure 3.6. The pharmacophoric features of each group of ligands are shown in table 3.1 where + sign shows the presence of the respective features.

Compounds	Hydrogen bond donor	Hydrogen bond acceptor	Ring aromatic	Hydrophobic	Positive ionizable
2-(piperidin-3-yl)-1Hbenzimidazoles	+	+	+	+	+
2-aminobenzimidazole	+	+	+	+	+
Indene	+	+	+	+	+
Benzothiophene	+	+	+	+	+

Table 3.1: Pharmacophoric features of each group of ligands

Keun Woo Lee et al. (2010) presented the pharmacophore of human histamine H1 receptor showing hydrogen bond acceptor, ring aromatic, positive ionizable and two hydrophobic functions but the pharmacophore model from this study showed an extra feature i.e. hydrogen bond donor which has improved the pharmacophore model.

Class of compounds	Hyd/Ar-HBA	HBA-HBD	HBD-Hyd/Ar
2-(piperidin-3-yl)-1Hbenzimidazoles	2.35-2.37	4.56-6.32	4.32-5.78
2-aminobenzimidazole	2.37-2.40	6.32-6.35	4.14-4.89
Benzothiophene	2.30-2.94	4.96-6.95	4.40-5.04
Indene	2.43-2.70	5.74-7.56	5.83-6.35

Table 3.2: Pharmacophoric triangle distances of each group of ligands measured in A°

The distance measured between the common pharmacophore features of each group of compounds is shown in table 3.2. The distance is measured in A° unit using VMD software. Table 3.2 shows a range of distance from minimum to maximum between hydrophobic and hydrogen bond acceptor, hydrogen bond acceptor and hydrogen bond donor and hydrogen bond donor and hydrophobic.

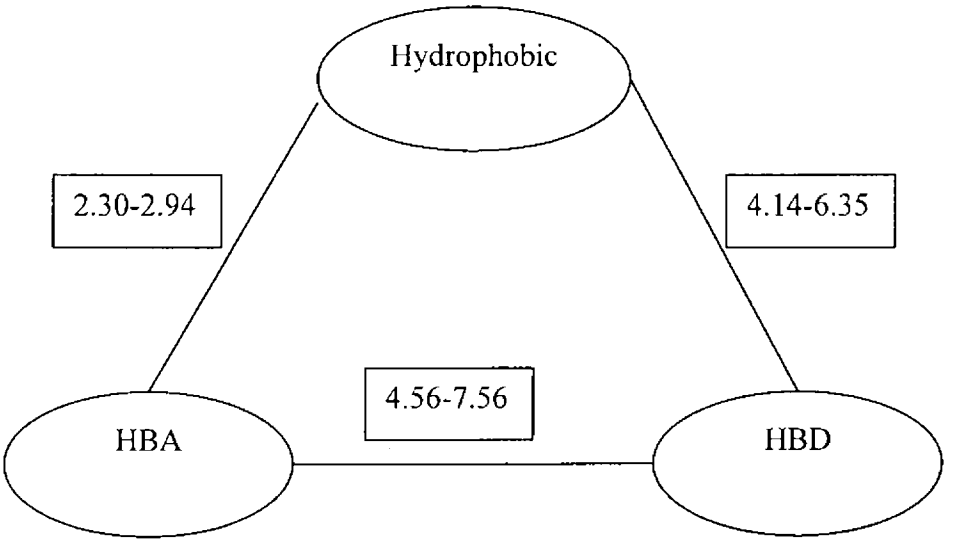


Figure 3.5: Distance range among common pharmacophoric features measured in A°

The distances among the common pharmacophoric features in the predicted model are shown in figure 3.5. The distance between hydrophobic and hydrogen bond donor range from 4.14 to 6.35 A°, between hydrophobic and hydrogen bond acceptor it is from 2.30 to 2.94 A° and between hydrogen bond acceptor and hydrogen bond donor it is 4.56 to 7.56 A°.

The common pharmacophore predicted for four groups of anti histamine inhibitors consist of one aromatic ring (blue circles), two hydrophobic functions (yellow circles), HBA (red circles), two positive ionizable features (blue arrows) and three HBD (green circles) as shown in figure 3.4.

3.2 Docking of prospective antihistamine inhibitors

AutoDock Vina was used for the docking of ligands. AutoDock Vina is a new-fangled open-source program for drug discovery, molecular docking and virtual screening, contributing multi-core capability, high performance and enhanced accuracy and ease of use. Different group of ligands used in this study were docked to the active site of histamine methyltransferase (HNMT) pdb id 1JQD. Histamine methyltransferase (HNMT) plays a central role in histamine biotransformation due to its ability to methylate histamine. The structure of HNMT in ribbon form is shown in figure 3.8. HNMT comprises two chains: chain A and chain B as shown in figure 3.6.

The training set which is shown in table 2.3 is used for docking calculations with HNMT. Polar hydrogen atoms were added and rotatable bonds were defined. In this study grid box of 50x50x50 A° was used from AutoDock 4. Defining grid box settings saves a lot of CPU time and also limits the search space. The grid resolution (spacing) is taken as default i.e. 0.375 A°. The results of docking take account of the docked structures, the energies of the docked structures and their similarities to each other. Different numbers of confirmations are obtained for each docked structure and they are ranked according to their binding affinity which is shown in kcal/mole. The different docked structures obtained after docking are in pdbqt format. A log file is generated in which the affinities are ranked for each confirmation in descending order. The lesser the energy, better is its confirmation so for that reason best confirmation i.e. least energy was selected.

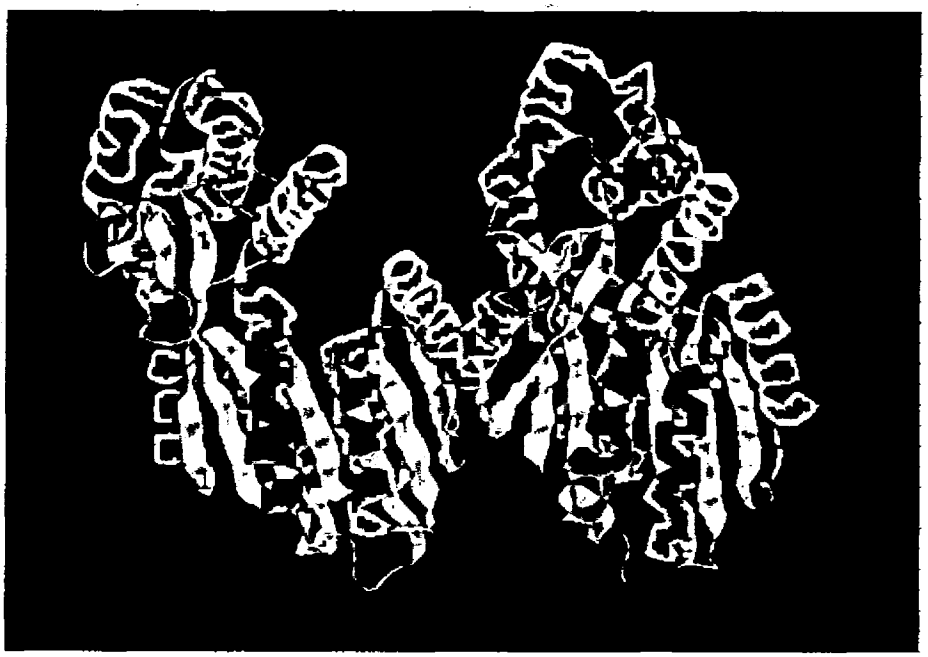


Figure 3.6: Ribbon style of HNMT shown and colored according to the structure

3.2.1 Active Site of HNMT

The active site of a protein is a pocket in the protein that contains the bound ligand. On the basis of docking of the data set and the standard antihistamine drugs with the protein, the active site of histamine methyltransferase was investigated. The amino acids within 5°A were identified as the active site amino acids. Table 3.3 shows the list of amino acids within 5°A region. The study revealed that Ser 91, Tyr 147, Tyr 15, Phe 9, Glu 93, Gln 94, Val 16 are major determinants of binding. Figure 3.7 shows the residues of the active site.

3.2.3 Docking of the standard drugs

Some of the standard drugs like mizolastine, astemizole, loratadine and terfenadine were used for docking with the protein. These drugs are docked with in the active site of HNMT using the same procedure as discussed earlier by using Auto Dock Vina. After docking, the binding interactions were calculated within 5°A region of the protein. This shows the ability of the ligand to bind to the protein that how strongly it binds to the protein. The output file of docking contains one log file in which all the obtained confirmations are ranked according to their energy values. The best configuration of each ligand-receptor was selected on the basis of energy values which is the first one inscribed. Subsequent to the detailed anâlysis of the docked drugs with the protein, it is shown that all the drugs have the same amino acids which are present in the active site of HNMT. Figure 3.8 shows the hydrogen, hydrophobic and Vander wall interactions of the standard drug mizolastine.

Table 3.3: Amino acids within 5°A radius around the ligands. The presence or absence of the amino acid is shown with (+) or (-) signs.

Compound	Phe 9	Leu 8	His 12	Glu 93	Ser 91	Val 16	Gln 94	Phe 19	Lys 38	Lys 112	Glu 28	Glu 34	Tyr 15
1	+	+	+	+	+	+	+	+	-	-	-	-	+
2	+	+	+	+	+	+	+	+	-	-	-	-	+
3	+	-	+	+	+	+	+	-	-	-	-	-	+
4	+	-	+	+	+	+	+	-	-	-	-	-	+
5	-	-	-	+	+	+	+	+	-	-	-	-	+
6	-	-	-	-	-	-	-	-	-	-	-	-	-
7	+	-	+	+	+	+	+	+	-	-	-	-	+
8	-	-	-	-	-	-	-	-	-	-	-	-	-
9	-	-	-	-	-	-	-	-	-	-	-	-	-
10	-	-	-	-	-	-	-	-	-	+	-	+	-
11	-	-	-	-	-	-	-	-	+	+	-	+	-
12	-	-	-	-	-	-	-	-	-	+	-	-	-
13	-	-	-	-	-	-	-	-	-	+	-	-	-
14	-	-	-	-	-	-	-	-	-	+	-	-	-
15	-	-	-	-	-	-	-	-	-	+	-	-	-
16	-	-	+	+	+	+	+	-	-	-	-	-	+
17	+	+	+	+	+	+	+	+	-	-	-	-	+
18	+	+	+	+	+	+	-	-	-	-	-	-	+
19	+	-	+	+	+	+	+	+	-	-	-	-	+
20	-	-	-	-	-	-	-	+	-	-	+	-	-
21	+	+	+	+	+	+	-	-	-	-	-	-	+
22	-	-	-	-	-	-	-	+	-	-	+	-	-
23	-	-	-	-	-	-	-	+	-	-	+	-	-
24	-	-	+	+	+	+	+	+	-	-	-	-	+
25	+	-	-	+	+	+	+	+	-	-	-	-	+
26	+	-	+	+	+	+	+	+	-	-	-	-	+
27	+	+	+	+	+	+	+	+	-	-	-	-	+
28	-	-	-	-	-	-	-	-	-	-	-	-	-
29	+	-	+	+	+	+	+	+	-	-	-	-	+
30	-	-	-	-	-	+	-	+	-	-	+	-	-
31	-	-	-	-	-	-	-	+	-	-	+	-	-
32	-	-	-	-	-	-	-	+	-	-	+	-	-
33	-	-	-	-	-	-	-	+	-	-	+	-	-
34	-	-	-	-	-	-	-	+	-	-	+	-	-
35	+	-	+	+	+	+	+	+	-	-	-	-	+
Astemizole	-	-	-	-	-	-	-	-	-	-	-	-	-
Loratadine	+	-	+	+	+	+	+	+	-	-	-	-	+
Mizolastine	-	-	+	+	+	+	+	+	-	-	-	-	+
Terfenadine	-	-	-	-	-	+	-	+	-	-	+	-	-

Compound	Asn 110	Glu 109	Leu 108	Val 102	Val 111	Phe 113	Thr 105	Trp 115	Ala 103	Ser 176	Asp 219	Leu 221
1	-	-	-	-	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-	-	-	-	-
3	-	-	-	-	-	-	-	-	-	-	-	-
4	-	-	-	-	-	-	-	-	-	-	-	-
5	-	-	-	-	-	-	-	-	-	-	-	-
6	+	+	+	+	+	+	+	+	+	-	+	+
7	-	-	-	-	-	-	-	-	-	-	-	-
8	-	-	-	-	-	-	-	-	-	-	-	-
9	-	+	-	+	-	-	-	-	+	-	+	+
10	-	+	+	+	+	+	+	+	+	-	+	+
11	-	+	+	-	+	-	-	-	+	-	+	+
12	-	+	+	-	+	-	-	-	+	+	+	+
13	-	+	-	+	+	-	+	+	+	-	+	+
14	-	+	+	-	+	+	+	+	+	-	+	+
15	-	+	+	+	+	+	+	+	+	-	+	+
16	+	+	+	+	+	+	+	+	+	-	+	+
17	-	-	-	-	-	-	-	-	-	-	-	-
18	-	-	-	-	-	-	-	-	-	-	-	-
19	-	-	-	-	-	-	-	-	-	-	-	-
20	-	-	-	-	-	-	-	-	-	-	-	-
21	-	-	-	-	-	-	-	-	-	-	-	-
22	-	-	-	-	-	-	-	-	-	-	-	-
23	-	-	-	-	-	-	-	-	-	-	-	-
24	-	-	-	-	-	-	-	-	-	-	-	-
25	-	-	-	-	-	-	-	-	-	-	-	-
26	-	-	-	-	-	-	-	-	-	-	-	-
27	-	-	-	-	-	-	-	-	-	-	-	-
28	-	-	-	-	-	-	-	-	-	-	-	-
29	-	-	-	+	-	-	+	+	+	-	+	+
30	-	-	-	-	-	-	-	-	-	-	-	-
31	-	-	-	-	-	-	-	-	-	-	-	-
32	-	-	-	-	-	-	-	-	-	-	-	-
33	-	-	-	-	-	-	-	-	-	-	-	-
34	-	-	-	-	-	-	-	-	-	-	-	-
35	-	-	-	-	-	-	-	-	-	-	-	-
36	-	-	-	-	-	-	-	-	-	-	-	-
Astemizole	-	-	-	-	-	-	-	-	+	-	-	+
Loratadine	-	-	-	-	-	-	-	-	-	-	-	-
Mizolastine	-	-	-	-	-	-	-	-	-	-	-	-
Terfenadine	-	-	-	-	-	-	-	-	-	-	-	-

Compound	Lys 99	Thr 27	Ser 26	His 25	Asn 24	Leu 23	His 29	Glu 65	Gln 33	Gln 30	Ser 106	Lys 39	Tyr 218
1	-	-	-	-	-	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-	-	-	-	-	-
3	-	-	-	-	-	-	-	-	-	-	-	-	-
4	-	-	-	-	-	-	-	-	-	-	-	-	-
5	-	-	-	-	-	-	-	-	-	-	-	-	-
6	-	-	-	-	-	-	-	-	-	-	-	+	-
7	-	-	-	-	-	-	-	-	-	-	-	-	-
8	-	+	+	+	+	+	+	+	+	+	-	-	-
9	-	-	-	-	-	-	-	-	-	-	+	+	+
10	-	-	-	-	-	-	-	-	-	-	-	+	-
11	+	-	-	-	-	-	-	-	-	-	+	+	+
12	-	-	-	-	-	-	-	-	-	-	-	+	-
13	+	-	-	-	-	-	-	-	-	-	+	+	+
14	-	-	-	-	-	-	-	-	-	-	+	+	+
15	+	-	-	-	-	-	-	-	-	-	-	+	+
16	-	-	-	-	-	-	-	-	-	-	-	+	+
17	-	-	-	-	-	-	-	-	-	-	-	-	-
18	-	-	-	-	-	-	-	-	-	-	-	-	-
19	-	-	-	-	-	-	-	-	-	-	-	-	-
20	-	-	-	-	-	-	-	-	-	-	-	-	-
21	-	-	-	-	+	+	+	+	-	-	-	-	-
22	-	-	-	-	-	-	-	-	-	-	-	-	-
23	-	-	-	-	+	+	+	+	-	-	-	-	-
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27	-	-	-	-	-	-	-	-	-	-	-	-	-
28	-	-	-	-	-	-	-	-	-	-	-	-	-
29	+	-	-	-	-	-	-	-	-	-	-	-	-
30	-	-	-	-	-	-	-	-	-	-	-	-	-
31	-	-	-	-	-	+	+	+	-	-	-	-	-
32	-	-	-	-	+	+	+	+	-	-	-	-	-
33	-	-	+	-	+	+	+	+	-	-	-	-	-
34	-	-	-	-	+	+	+	+	-	-	-	-	-
35	-	-	-	-	+	+	+	+	-	-	-	-	-
36	-	-	-	-	-	-	-	-	-	-	-	-	-
Astemizole	-	-	-	-	-	-	-	-	-	-	-	-	-
Loratadine	-	-	-	-	-	-	-	-	-	-	-	-	-
Mizolastine	-	-	-	-	-	-	-	-	-	-	-	-	-
Terfenadine	-	-	-	-	+	+	+	+	-	-	-	-	-

Compound	Met 32	Ala 273	Ile 66	Ala 92	Asn 282	Glu 100	Lys 274	Lys 104	Pro 269	Gln 143	Ser 272	Gln 69	Glu 270
1	-	-	-	-	-	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-	-	-	-	-	-
3	-	-	-	-	-	-	-	-	-	-	-	-	-
4	-	-	-	-	-	-	-	-	-	-	-	-	-
5	-	-	-	-	-	-	-	-	-	-	-	-	-
6	-	-	-	-	-	-	-	-	-	-	-	-	-
7	-	-	-	-	-	-	-	-	-	-	-	-	-
8	-	-	-	-	-	-	-	-	-	-	-	+	-
9	-	-	-	-	-	-	-	-	-	-	-	-	-
10	-	-	-	-	-	-	-	-	-	-	-	-	-
11	-	-	-	-	-	-	-	-	-	-	-	-	-
12	-	-	-	-	-	-	-	-	-	-	-	-	-
13	-	-	-	-	-	-	-	-	-	-	-	-	-
14	-	-	-	-	-	-	-	-	-	-	-	-	-
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16	-	-	-	-	-	-	-	-	-	-	-	-	-
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31	+	-	+	-	-	-	-	-	-	-	-	-	-
32	+	-	+	-	-	-	-	-	-	-	-	-	-
33	+	-	+	-	-	-	-	-	-	-	-	-	-
34	+	-	-	-	-	-	-	-	-	+	-	-	-
35	+	-	-	-	-	-	-	-	-	+	-	-	-
36	-	-	-	+	-	-	-	-	-	-	-	-	-
Astemizole	-	+	-	-	+	+	+	+	+	-	+	-	+
Loratadine	-	-	-	-	-	-	-	-	-	-	-	-	-
Mizolastine	-	-	-	+	-	-	-	-	-	-	-	-	-
Terfenadine	+	-	+	-	-	-	-	-	-	-	-	-	-

Compound	Arg 20	Ala 63	Thr 284	Cys 217	Leu 220	Leu 195	Tyr 147	Lys 97
1	-	-	-	-	-	-	+	-
2	-	-	-	-	-	-	+	-
3	-	-	-	-	-	-	-	-
4	-	-	-	-	-	-	-	-
5	-	-	-	-	-	-	+	+
6	-	-	-	-	+	-	-	-
7	-	-	-	-	-	-	-	+
8	-	-	-	-	-	-	-	-
9	-	-	-	+	+	-	-	-
10	-	-	+	-	+	-	-	-
11	-	-	-	-	+	-	-	-
12	-	-	+	-	+	-	-	-
13	-	-	-	-	+	-	-	-
14	-	-	+	+	+	-	-	-
15	-	-	-	-	+	-	-	-
16	-	-	+	-	+	-	-	-
17	-	-	-	-	-	-	+	-
18	-	-	-	-	-	-	-	-
19	-	-	-	-	-	+	+	-
20	-	-	-	-	-	-	-	-
21	-	-	-	-	-	-	-	-
22	-	-	-	-	-	+	-	-
23	-	-	-	-	-	-	-	-
24	-	-	-	-	-	-	+	+
25	-	-	-	-	-	-	+	-
26	-	-	-	-	-	+	+	-
27	-	-	-	-	-	-	-	-
28	-	-	-	-	-	-	-	-
29	-	-	+	-	+	-	+	-
30	-	-	-	-	-	-	-	-
31	+	-	-	-	-	-	-	-
32	+	-	-	-	-	-	-	-
33	-	-	-	-	-	-	-	+
34	-	-	-	-	-	-	-	+
35	-	-	-	-	-	-	-	-
36	-	-	-	-	-	+	-	-
Astemizole	-	-	-	-	-	-	-	-
Loratadine	-	-	-	-	-	+	+	-
Mizolastine	-	-	-	-	-	-	-	+
Terfenadine	+	+	-	-	-	-	-	-

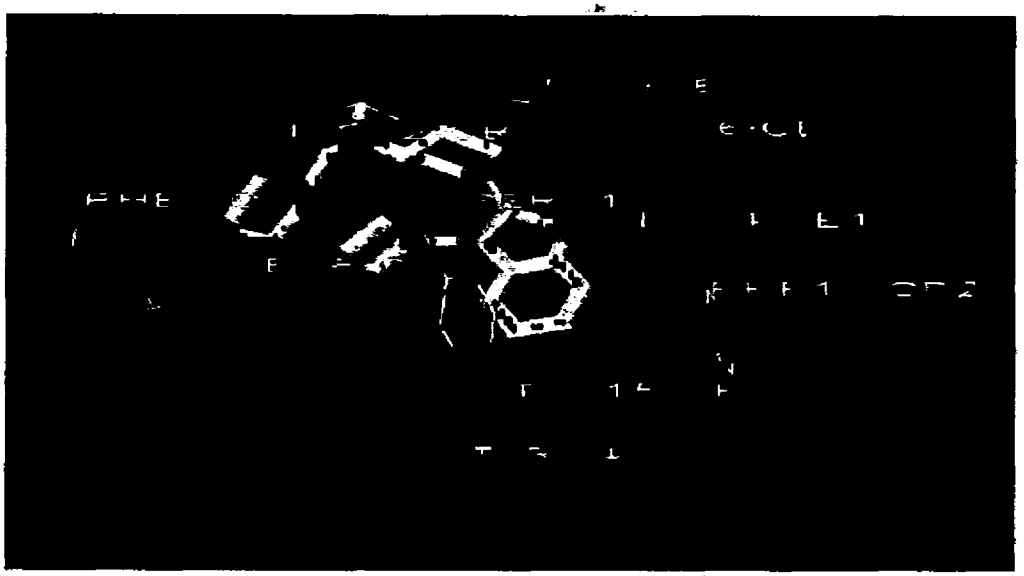


Figure 3.7: Active site of HNMT showing the residues within 5°A

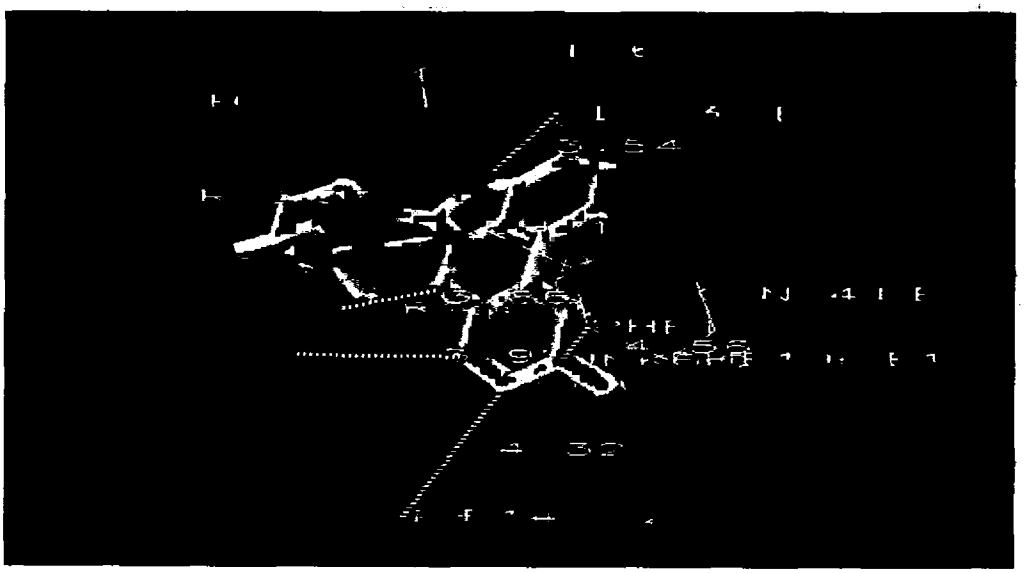


Figure 3.8: Binding interactions of Mizolastine showing hydrogen, hydrophobic and Vander wall interactions.

The above figure shows the binding interactions of the standard drug mizolastine within the active site of HNMT. It consisted of two hydrogen bonds i.e. nitrogen of the ligand binds with two oxygen of Glu 93 of the protein with the distance of 3.54 and 4.56. In the same way the aromatic ring of the ligand interact with Tyr 15 with a distance of 4.28 and with Tyr 147 with a distance of 4.32 and showed Vander wall interactions. Mizolastine also shows six hydrophobic interactions with Tyr 147, Val 16, Tyr 15 and Phe 19 of the protein having 4.32, 3.26, 4.28, 3.31, 3.48 and 3.66 distances respectively. Likewise the remaining standard drugs show similar type of binding interactions with the protein.

3.2.4 Docking of ligands of the data set

The ligands selected as data set were also docked in the active site of HNMT in the same way. After docking Auto Dock Vina outputs a log file in which energies of all generated confirmations are ranked in ascending order. The energy values are measured in unit of kcal/mol and are listed in table 3.4. The files created after docking are used to measure different types of interactions with the help of VMD software. VMD is basically a molecular visualization program for displaying, animating, and analyzing large biomolecular systems using 3-D graphics and built-in scripting. The different interactions calculated were hydrogen bonding, ionic bonding, Vander wall interactions and hydrophobic interactions. These are shown in table 3.5 in detail. Figure 3.9 shows the different interactions of compound no 25. On the basis of these interactions and energies a lead compound was chosen. From table 3.4 it is apparent that compound no 2 shows the lowest binding energy (-7.6 kcal/mol) and for this reason has more binding affinity than any other compound.

Table 3.4: Binding energies of ligands used in the study with their IC_{50} values and logP

Compound	Class	Affinity (kcal/mol)	IC_{50}	Log p
1	2-(piperidin-3-yl)-1Hbenzimidazoles	-6.8	5.4	4.44
2		-7.6	11.0	4.76
3		-6.5	0.618	4.77
4		-6.3	2.3	4.12
5		-6.4	6.6	2.41
6	2-aminobenzimidazole	-6.6	6.664	3.77
7		-7.5	0.805	5.88
8		-5.9	0.502	6.16
9		-6.5	12.798	4.15
10		-6.8	12.798	4.06
11		-6.8	6.36	4.44
12		-6.6	7.21	4.39
13		-6.5	6.072	3.78
14		-6.5	4.444	3.91
15		-6.4	35.15	3.54
16		-6.8	6.134	4.06
17	Benzothiophene	-5.0	0.85	4.2
18		-5.3	4.926	5.03
19		-5.3	3.3	5.04
20		-6.2	2.0	4.43
21		-5.6	9.8	4.71
22		-5.2	0.856	5.12
23		-5.7	5.5	4.56
24		-5.7	9.5	4.2
25		-5.5	4.0	4.98
26		-5.3	1.5	4.18
27	Indene	-5.8	6.6	2.4
28		-5.8	5.3	1.79
29		-6.0	0.69	3.06
30		-6.0	0.32	2.66
31		-5.4	22	2.8
32		-5.6	1.9	2.77
33		-6.0	6.4	3.06
34		-5.9	1.0	4.02
35		-6.0	1.4	4.65
36		-5.2	5.6	3.74
Astemizole	Standard	-7.4	36	5.25
Loratadine		-6.6	15	4.4
Mizolastine		-7.2	118	4.1
Terfenadine		-7.7	18	6.96

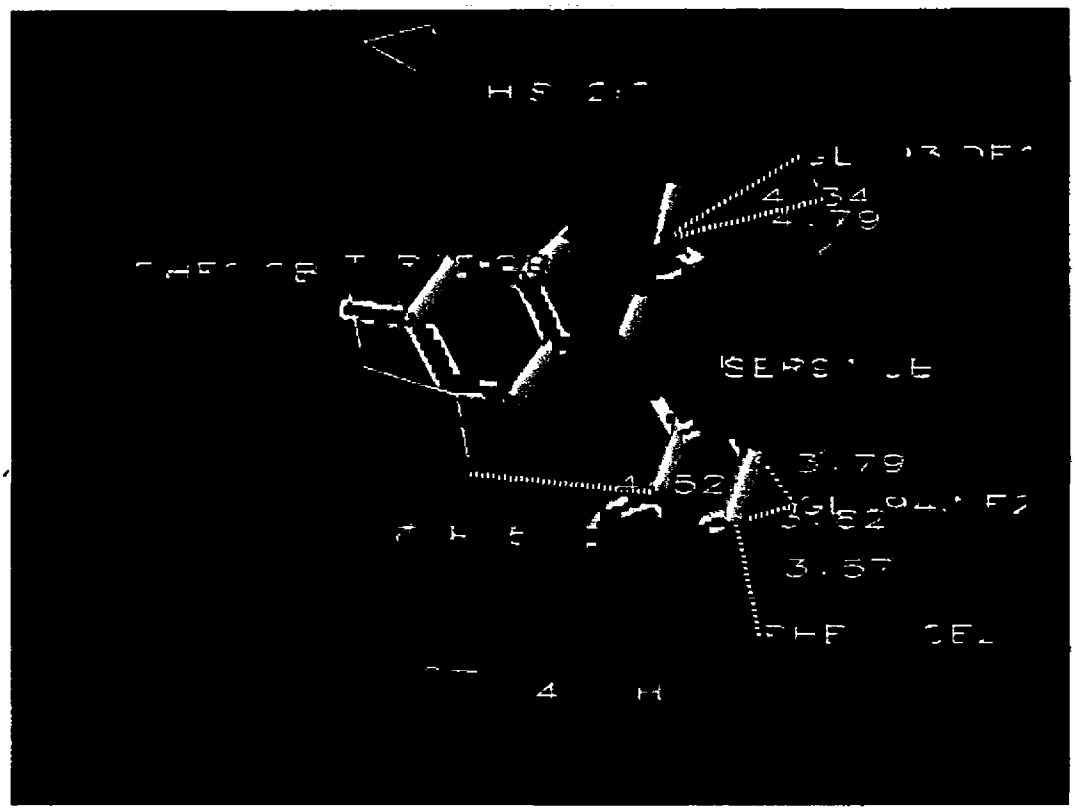


Figure 3.9: Binding interactions of compound no 25 showing hydrogen, hydrophobic and Vander wall interactions.

	Hydrogen bonding	Ionic bonding
		2-aminob
1	Glu 109-N [3.17]	-
	Asp 219-N [4.58]	
	Thr 105-N [4.74]	
	Val 111-H [4.77]	
2	Ser 91-N [3.07]	Glu 93-N [4.34]
	Glu 93-N [4.34]	Glu 93-N [4.14]
	Glu 93-N [4.91]	
	Glu 93-N [4.14]	
3	Asn 24-N [4.65]	-
4	Ser 106-N [4.79]	-
5	Glu 109-N [4.41]	Glu 109-N [4.41]
6	Thr 105-N [4.81]	-
7	Asp 219-N [3.45]	-

8	Ser 106-N [4.27]	-
	Glu 109-N [4.73]	
9	Glu 109-N [3.34]	-
	Thr 105-N [4.78]	
	Lys 39-O [4.50]	
	Asp 219-N [4.52]	
10	Glu 109-N [4.71]	Lys 112-H [3.59]
	Glu 109-O [4.70]	Lys 112-O [4.36]
	Asp 219-N [4.92]	
11	Thr 105-N [4.81]	-
	Glu 109-N [4.20]	
	Glu 109-N [3.30]	
	Asp 219-N [4.45]	

Benzo

12	Ser 91-N [3.19]	-
13	Ser 91-S [4.61]	-
	Gln 94-S [3.84]	
	Tyr 15-N [3.74]	

	Tyr 15-N [4.86]	
14	Ser 91-N [4.49]	-
15	Gln 94-S [4.90]	-
	Tyr 15-N [4.18]	
	Tyr 147-N [4.63]	
16	-	-
17	Ser 91-N [4.43]	-
18	-	-
19	-	-
20	Gln 94-N [3.49]	-
21	Glu 93-N [4.25]	Glu 93-N [4.25]
	Gln 94-N [3.26]	

			In
22	Glu 93-N	[3.46]	Glu 93-N [3.46]
	Glu 93-N	[3.71]	Glu 93-N [3.71]
23	Ser 91-N	[3.02]	-
	Glu 94-O	[3.07]	
24	Asp 219-N	[4.74]	Asp 219- [4.74]
	Asp 219-N	[4.68]	Asp 219- [4.68]
25	Glu 93-N	[4.79]	Glu 93-N [4.79]
	Glu 93-N	[4.34]	Glu 93-N [4.34]
	Gln 94-N	[3.82]	
	Gln 94-N	[3.79]	
26	Glu 65-N	[4.82]	Glu 65-N [4.82]
27	Glu 65-N	[4.32]	-
28	-		-
29	-		-

30	-	-
31	Ser 91-N [4.91]	-
	Gln 94-O [2.96]	
		2-(piperidin-3-yl
32	Ser 91-N [3.41]	-
	Glu 93-N [4.58]	
	Gln 94-N [4.13]	
	Glu 93-N [4.76]	
33	Ser 91-N [4.63]	-
	Gln 94-F [3.14]	
34	Ser 91-N [4.64]	-
	Gln 94-F [3.16]	
35	Ser 91-N [4.66]	-
	Gln 94-N [3.74]	
	Glu 93-N [3.77]	
	Glu 93-N [4.65]	
36	Ser 91-N [4.60]	-
	Ser 91-N [4.97]	

Table 3.5: Binding interactions of training set co

From table 3.5 it was noted that compound no 1, 9, 11, 13 and 25 show good quality hydrogen, ionic, hydrophobic and Vander wall interactions with the amino acids within 5°A region. Exhaustive 3D analyses of the docked site of these compounds indicate that they have nearly same residues within 5°A region. The best configuration of each ligand-receptor complex was selected on the basis of energy comparison. After the detailed 3D analysis of each docked confirmation it was observed that amino acids Ser 91, Tyr 147, Tyr 15, Phe 9, Glu 93, Gln 94, Val 16, Ala 103 and Phe 19 were observed the most in binding interactions. It was noted that Ser 91, Glu 93 and Gln 94 were found to play an important role in hydrogen bonding. Likewise Tyr 15, Phe 9, Tyr 147 and Phe 19 were important in hydrophobic and Vander wall interactions.

3.3 Lipinski's Rule of Five

Lipinski's Rule of Five was calculated to evaluate the drug likeliness so that it can be determined that whether these ligands have the ability to make a good orally active drug. Table 3.6 shows the rule of five calculated for the mentioned group of compounds.

3.4 Lead compound identification

After listing the energies and binding interactions of all the ligands, lead compound identification was done by choosing the possible active compounds. As mentioned earlier compounds 1, 9, 11, 13 and 25 showed good interactions with the amino acids in the active site, compound no 25 was chosen as the lead compound on the basis of low logP and largest number of interactions. Figure 3.9 shows the different interactions of compound no 25. It has four hydrogen bonds with distances 4.79, 4.34, 3.82 and 3.79. Likewise it has two Vander wall interactions with distances 3.57, 4.52 and

three hydrophobic interactions with distances 4.52, 3.94 and 3.57. Two ionic interactions are also showed by this compound with Glu 93 with distances 4.79 and 4.34.

3.4.1 Analogues of Lead Compound

On the basis of binding interactions and the biological activity of the ligands, compound no 25 has been chosen as the lead compound as described earlier. After selecting the lead compound, its analogues were made so that all the possible active compounds can be prepared which can be proposed to be used as potent antihistamine inhibitors. The analogues prepared are shown in table 3.7 with their IUPAC names which were obtained using the software ChemDraw. The analogues were prepared by changing the functional groups in order to get an active compound on the basis of efficacy. After the analogues were prepared, they were docked within the active site of histamine methyltransferase. Docking is done using the same procedure as discussed before and the best obtained confirmation was chosen and saved. The binding interactions of the docked analogues with the protein were then calculated using VMD software. Four types of binding interactions were calculated i.e. ionic bonding, hydrophobic, Vander wall interactions and hydrogen bonding. Table 3.8 shows the binding interactions of the analogues with amino acids present within 5 A° region.

According to table 3.8 analogues 3 and 4 shows the highest number of interactions. Analogue 3 shows two ionic bonding, eight hydrogen bonding, two Vander wall and three hydrophobic interactions. Similarly analogue 4 shows two ionic bonding, eight hydrogen bonding, two Vander wall and four hydrophobic interactions. In analogue 4 the hydrophobicity is increased so the number of hydrophobic interactions also increases as shown in table 3.8.

Table 3.6: Lipinski's Rule of Five calculated for the mentioned group of compounds

Compound	Class	Molecular weight	Log P	HBA	HBD
1	2-(piperidin-3-yl)-1Hbenzimidazoles	450.687	4.44	2	4
2		402.582	4.76	2	3
3		362.557	4.77	1	3
4		336.519	4.12	1	3
5		294.463	2.41	1	3
6	2-aminobenzimidazole	352.542	3.77	1	4
7		438.635	5.88	1	3
8		452.662	6.16	1	3
9		366.569	4.15	1	4
10		395.611	4.06	1	4
11		446.655	4.44	2	3
12		391.648	4.39	0	4
13		407.647	3.78	1	4
14		439.664	3.91	2	4
15		425.637	3.54	2	5
16		453.715	4.06	1	5
17	benzothiophene	319.535	4.2	2	1
18		339.588	5.03	2	1
19		333.562	5.04	2	1
20		345.529	4.43	3	1
21		337.525	4.71	3	1
22		353.980	5.12	2	1
23		319.535	4.56	2	1
24		319.535	4.2	2	1
25		339.588	4.98	2	1
26		305.508	4.18	2	1
27	indene	304.502	2.4	2	1
28		310.421	1.79	3	1
29		354.946	3.06	3	1
30		338.491	2.66	4	1
31		308.449	2.8	2	1
32		354.474	2.77	4	1
33		342.438	3.06	4	1
34		317.450	4.02	2	1
35		313.487	4.65	1	1
36		329.486	3.74	2	1
Astemizole	Standard	454.549	5.25	2	2
Loratadine		371.932	4.4	2	1
Mizolastine		433.511	4.1	3	3
Terfenadine		464.629	6.96	2	2

Table 3.7: Analogues of lead compound with their IUPAC names

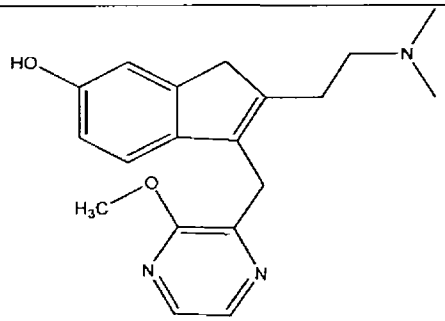
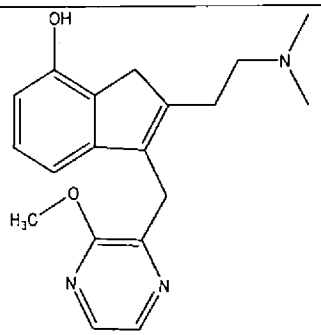
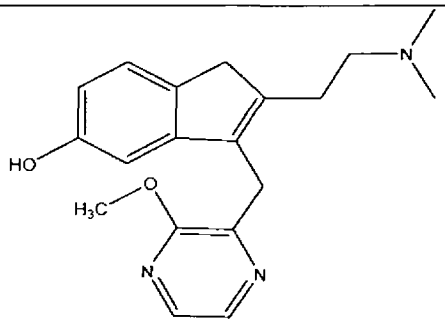
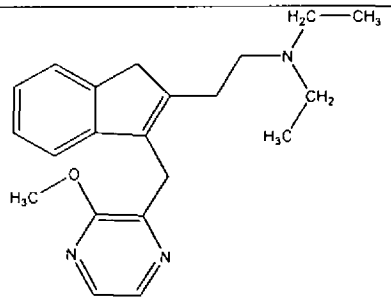
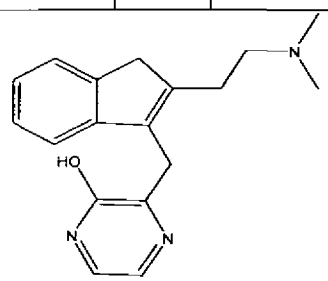
No.	Structure and IUPAC names	No.	Structure and IUPAC names
1.	 2-(2-(dimethylamino)ethyl)-1-((3-methoxypyrazin-2-yl)methyl)-3*H*-inden-5-ol	2.	 2-(2-(dimethylamino)ethyl)-1-((3-methoxypyrazin-2-yl)methyl)-3*H*-inden-4-ol
3.	 2-(2-(dimethylamino)ethyl)-3-((3-methoxypyrazin-2-yl)methyl)-1*H*-inden-5-ol	4.	 *N,N*-diethyl-2-(3-((3-methoxypyrazin-2-yl)methyl)-1*H*-inden-2-yl)ethanamine
5.	 3-((2-(2-(dimethylamino)ethyl)-3*H*-inden-1-yl)methyl)pyrazin-2-ol		

Table 3.8: Binding interactions of analogues with amino acids present within 5 A° region

	Ionic bonding	Hydrogen bonding	Vander wall	Hydrophobic
1	-	Ser 91(OG)-N [4.50]	-	Tyr 15(CE2)-C [3.63]
		Ser 91(OG)-N [4.79]		
		Glu 93(OE1)-N [4.42]		
		Glu 93(OE1)-N [4.84]		
		Glu 93(OE2)-N [4.04]		
		Glu 93(OE2)-N [4.89]		
2	-	Ser 91(OG)-N [4.85]	-	Val 16(CG2)-Ar [4.57]
		Ser 91(OG)-N [4.48]		
		Glu 93(OE1)-N [4.49]		
		Glu 93(OE1)-N [4.62]		
		Glu 93(OE2)-N [3.96]		
3	Glu 93(OE1)-N[4.23]	Ser 91(OG)-N [4.35]	Phe 19(CD2)-Ar [3.95]	Phe 19(CD2)-Ar [3.93]
	Glu 93(OE2)-N[4.57]	Gln 94(NE2)-N [3.78]	Tyr 15(CE2)-Ar [4.45]	Tyr 15(CE2)-C [3.58]
		Gln 94(NE2)-N [3.87]		Val 16(CG2)-C [4.14]
		Gln 94(NE2)-O [4.83]		
		Tyr 15(OH)-O [4.88]		
		Tyr 147(OH)-O [4.95]		
		Glu 93(OE1)-N [4.23]		
		Glu 93(OE2)-N [4.57]		
4	Glu 93(OE1)-N[4.31]	Ser 91(OG)-N [4.29]	Phe 19(CD2)-Ar [3.95]	Phe 19(CD2)-Ar [3.95]
	Glu 93(OE2)-N[4.75]	Tyr 15(OH)-O [4.91]	Tyr 15(CE2)-Ar [4.48]	Tyr 15(CE2)-Ar [4.48]
		Tyr 147(OH)-O [4.98]		Tyr 15(CD2)-C [3.52]
		Gln 94(NE2)-N [3.78]		Val 16(CG2)-C [4.26]
		Gln 94(NE2)-N [3.84]		
		Gln 94(NE2)-O [4.78]		
		Glu 93(OE1)-N [4.31]		
		Glu 93(OE2)-N [4.75]		
5	Glu 93(OE1)-N[4.36]	Ser 91(OG)-N [4.31]	Phe 19(CD2)-Ar [4.04]	Phe 19(CD2)-Ar [4.04]
	Glu 93(OE2)-N[4.79]	Tyr 15(OH)-O [4.99]	Tyr 15(CE2)-Ar [4.49]	Tyr 15(CE2)-Ar [4.49]
		Gln 94(NE2)-N [3.75]		Tyr 15(CD2)-C [3.53]
		Gln 94(NE2)-O [4.89]		Val 16(CG2)-C [4.27]

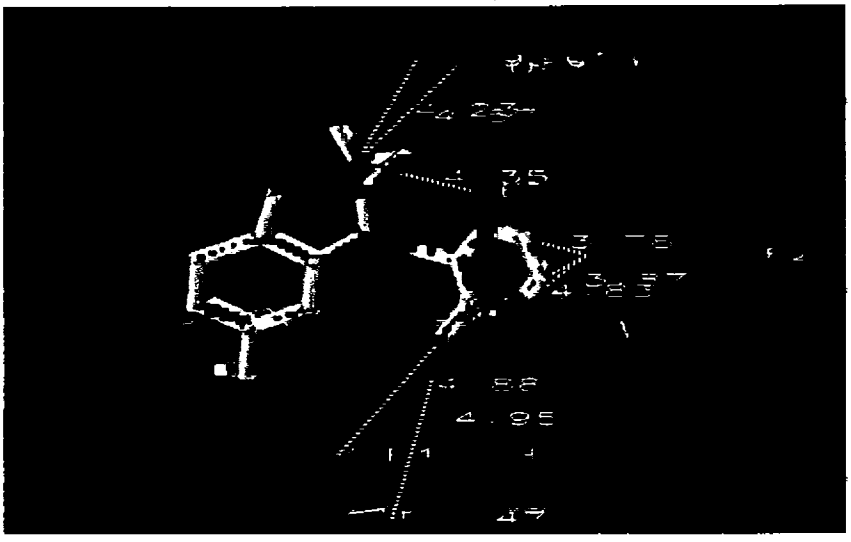


Figure 3.10: Illustration for the predicted hydrogen bonding of analogue 3 with amino acids within the active site of histamine methyltransferase in 5 A° region

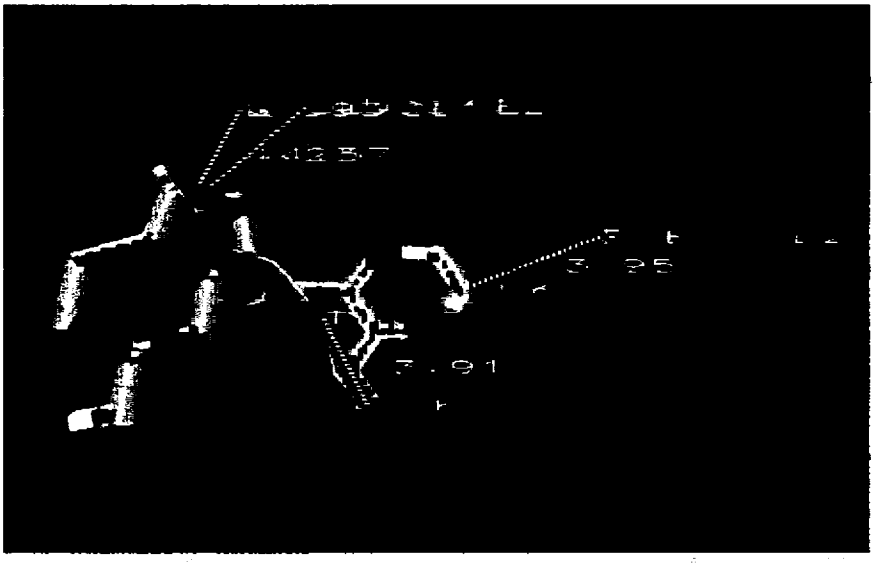


Figure 3.11: Illustration for the predicted hydrophobic and ionic bonding of analogue 3 with amino acids within the active site of histamine methyltransferase in 5 A° region

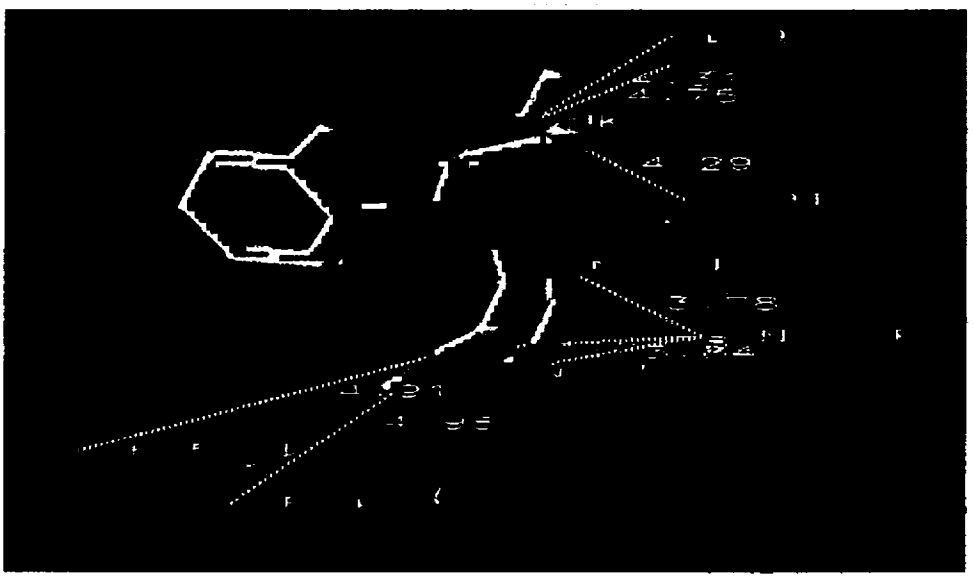


Figure 3.12: Illustration for the predicted hydrogen bonding of analogue 4 with amino acids within the active site of histamine methyltransferase in 5 A° region

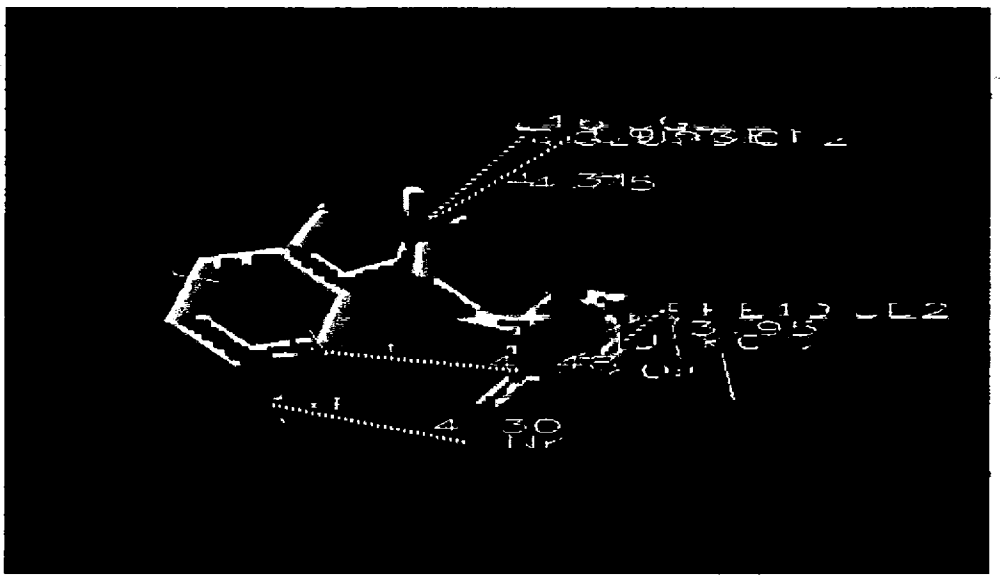


Figure 3.13: Illustration for the predicted hydrophobic and ionic bonding of analogue 4 with amino acids within the active site of histamine methyltransferase in 5 A° region

Figure 3.10 shows the hydrogen bonding of analogue 3 with amino acids within the active site of histamine methyltransferase in 5 A°region. The amino acids SER 91, GLN 94, TYR 15 and GLU 93 show hydrogen bonding with the protein. Likewise the amino acids GLU 93 shows ionic bonding and PHE 19, VAL 16 and TYR 15 show hydrophobic and Vander wall interactions as shown in figure 3.11 for analogue 3. Similar amino acids show hydrogen bonding, hydrophobic and ionic interactions with analogue 4 as shown in figure 3.12 and 3.13 respectively.

3.5 Quantitative Structure Activity Relationship (QSAR)

In order to show the correlation of the biological activity with the physiochemical properties a QSAR model was built. QSAR attempts to find unswerving association between molecular properties and biological activity, so that these set of laws can be used to assess the activity of new compounds. When a relationship between structure and activity is found, any number of compounds, together with those which are not yet synthesized, can be actively screened on the computer in order to choose the structures with the desirable properties. It is very helpful in the structural optimization of drugs and has become possible to select the most potential compounds after screening on the computer to synthesize and test in the laboratory. Therefore the QSAR approach conserves resources and speeds up the process of development and growth of new molecules for the use as drugs. A large number of molecular and chemical parameters characterizing the shape, reactivity and binding properties of a complete molecule in addition to molecular substituents has been reported to be liable for their molecular interactions. To develop a QSAR model, a set of descriptors was chosen which are implicit to influence whether a given compound will succeed or fail in binding to a given target. A number of steric and electronic parameters were selected to deduce the quantitative structure activity relationship. The electronic descriptors used are LUMO energy (E_{LUMO}), HOMO energy (E_{HOMO}) and total energy. The steric descriptors used in the study are log P, molar refractivity and heat of formation. The values of the above mentioned descriptors were calculated using the softwares HyperChem and ChemDraw. The values of the calculated steric and electronic parameters are shown in table 3.9.

Table 3.9: Data set of selected electronic and steric descriptors

No.	Molar refractivity (cm^3/mol)	LogP	Total energy (kcal/mol)	E_{HOMO} (kcal/mol)	E_{LUMO} (kcal/mol)	IC_{50} (μM)	Heat of formation (kcal/mol)
1.	94.05	4.12	1206.453451	-9.537636	1.863013	3.065	92758.11782
2.	100.89	3.83	33144.45944	-39.777942	10.542854	0.465	124806.0054
3.	84.87	2.41	13240.84839	-29.630275	12.912108	1.556	93074.28325
4.	95.58	3.46	21614.38442	-9.610758	7.174366	15.989	105379.653
5.	103.54	4.77	29198.38641	-0.106589	1.836622	0.618	127923.727
6.	119.83	5.99	50238.71959	-3.312836	0.611323	0.195	162681.6022
7.	110.38	4.49	33206.78731	-28.854968	27.394081	0.717	132042.0094
8.	126.68	5.71	-29964.1569	-0.660935	0.520639	0.122	82588.60723
9.	119.03	3.62	44200.05913	-37.363732	6.155098	1.901	156928.2882
10.	110.66	4.28	48687.78545	-13.801765	29.068206	0.526	149412.4385
11.	103	2.2	23530.38283	-1.388257	5.01263	4.393	124430.5008
12.	121.37	5.33	60666.93575	-50.610362	6.042351	0.48	165323.4226
13.	113.72	3.25	16036.47056	-5.380155	1.105895	6.453	120868.4223
14.	94.05	4.12	1206.453451	-9.537636	1.863013	2.3	92758.11782
15.	94.05	4.12	9122.96761	-8.265905	3.015954	2.8	100674.632
16.	84.87	2.41	13240.84839	-29.630275	12.912108	6.6	93074.28325
17.	92.12	2.62	-16393.7780	-3.069573	13.953356	53.3	65840.58668
18.	103	2.2	21027.62608	-1.53492	1.823228	32.2	121927.7441
19.	114.65	4.76	32032.92857	-2.758313	12.19717	5.4	142646.9484
20.	126.82	4.44	69321.34378	-58.97472	15.945121	11	194209.0035

Using these results graphs were made of the steric and electronic descriptors as independent variable against the biological activity as dependent variable in order to get the value of regression coefficient. These plots are made to know either they demonstrate good correlation of activity with the parameters or not. The regression coefficients of steric descriptors i.e. log P and molar refractivity is 0.2416 and 0.0832 respectively. Like wise the value of regression coefficient of electronic descriptors i.e. total energy, heat of formation, E_{LUMO} and E_{HOMO} are 0.1088, 0.6058, 0.0036 and 0.6017 respectively. These results show that there is no correlation between some descriptors like molar refractivity, log P, total energy and E_{LUMO}. But some descriptors like E_{HOMO} and heat of formation show correlation with the biological activity as their values are greater than 0.6.

The plot of steric descriptors log P and molar refractivity against the biological activity are shown in figure 3.14 and figure 3.15 respectively. Whereas the plot of electronic descriptors of HOMO energy and heat of formation are shown in figure 3.16 and figure 3.17 respectively. As it is evident from the plots that the steric descriptors show no correlation with the biological activity whereas the electronic descriptors have shown clear correlation as the value of regression coefficient for heat of formation and HOMO energy are greater than 0.6 means direct correlation is present between these descriptors and the biological activity of the compounds.

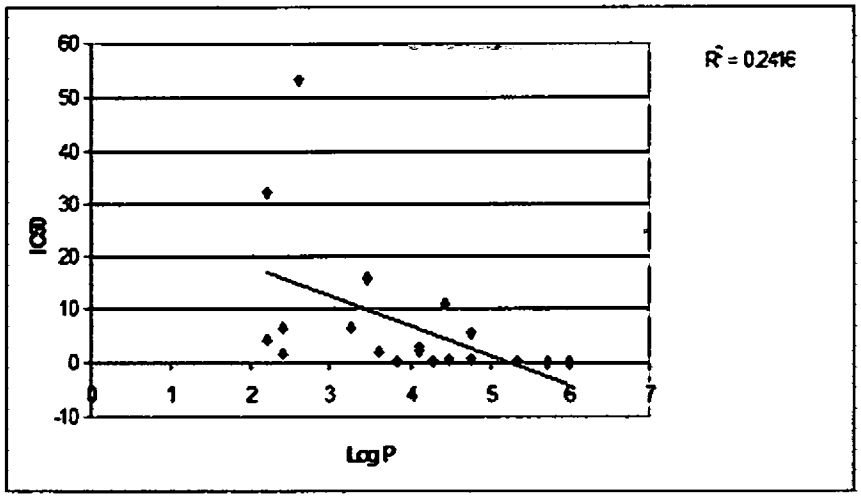


Figure 3.14: Plot of biological activity (IC_{50}) and log P

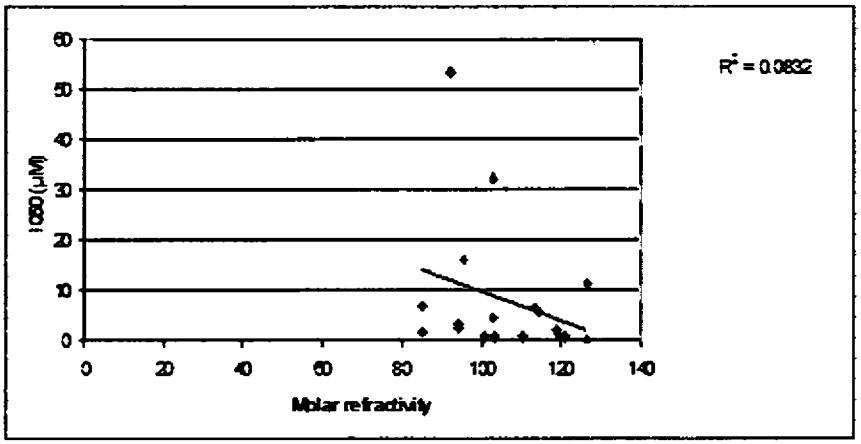


Figure 3.15: Plot of biological activity (IC_{50}) and molar refractivity

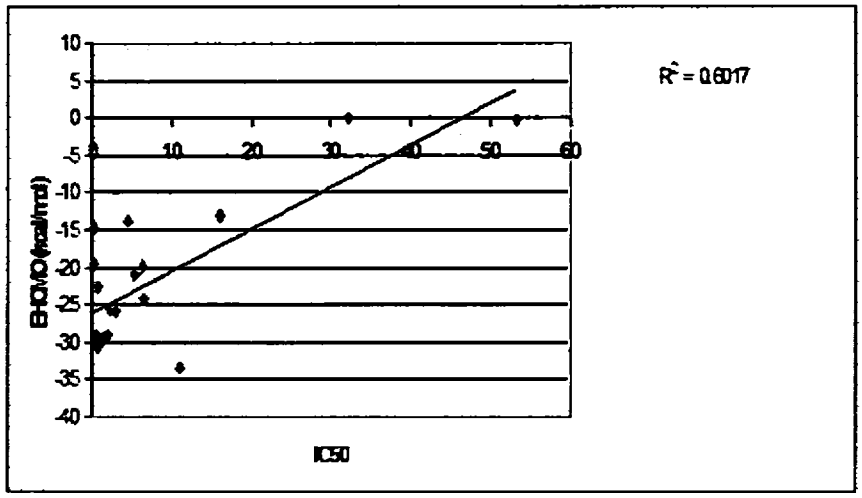


Figure 3.16: Plot of biological activity (IC_{50}) and HOMO energy

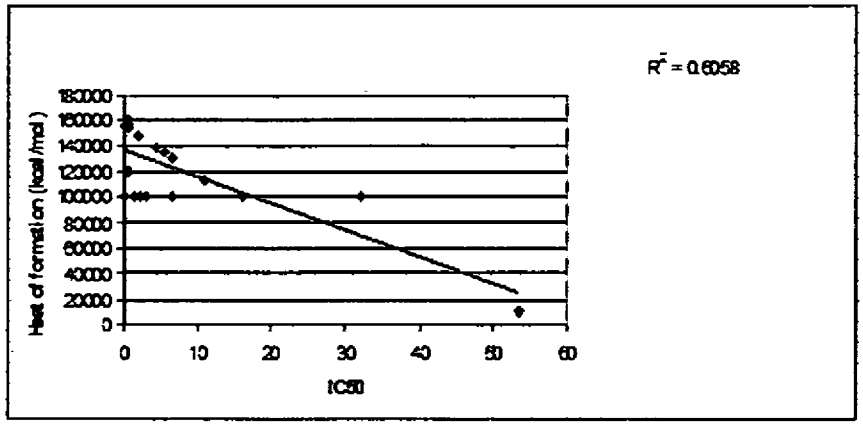


Figure 3.17: Plot of biological activity (IC_{50}) and heat of formation

Conclusion and Future Enhancements

Current study include pharmacophore identification, binding interactions calculation, docking, lead compound identification and analogue preparation of the lead compound on the group of antihistamine inhibitors.

The pharmacophore identified for this group of compounds can be very useful for the drug designing process. The lead compounds identified together with its analogues are projected for clinical trials in order to bring an improved and enhanced drug to the market with low side effects and better efficacy. As the drugs currently available for allergy as anti -histamines have many side effects.

The results of docking can give the scientists an idea that where the drug binds in HNMT and which amino acids are important in binding interactions.

The QSAR done on a group of 2-(piperidin-3-yl)-1H benzimidazoles will also help the researchers to have an idea of the diverse descriptors identified and also they can get an idea of the activity of these compounds which can give assistance to them in drug development.

Like wise there is a need to develop tools for the identification of lead compound so that manual work can be minimized.

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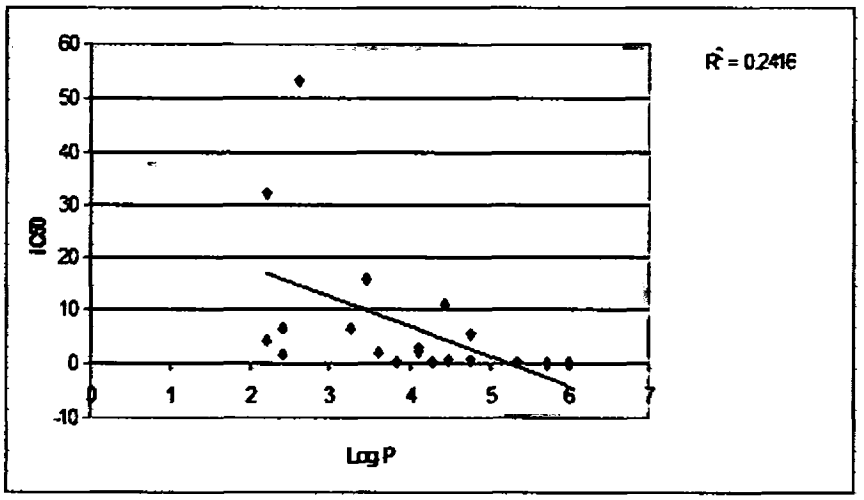

Figure 3.14: Plot of biological activity (IC_{50}) and log P

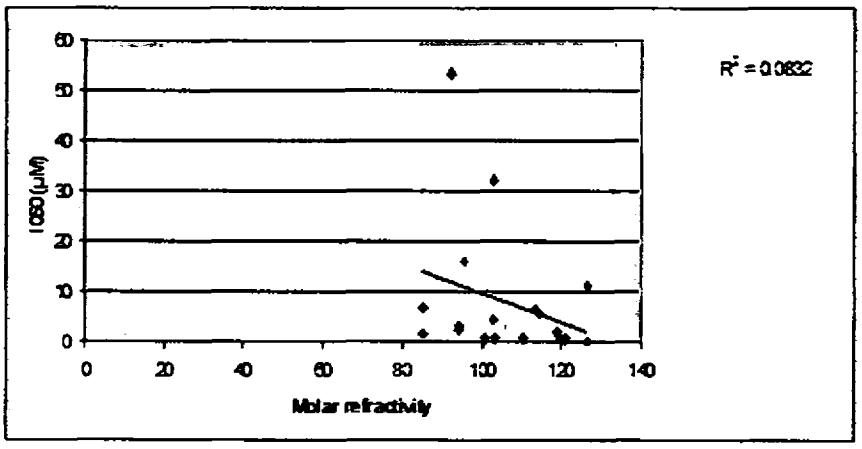

Figure 3.15: Plot of biological activity (IC_{50}) and molar refractivity

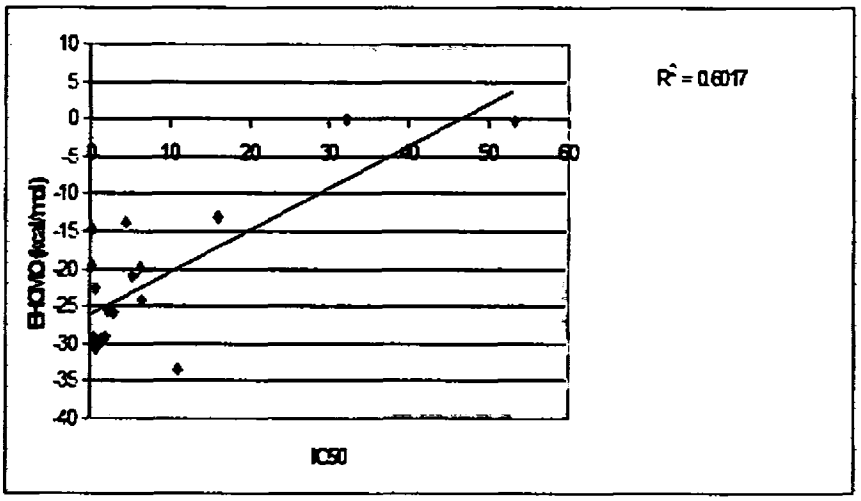

Figure 3.16: Plot of biological activity (IC_{50}) and HOMO energy

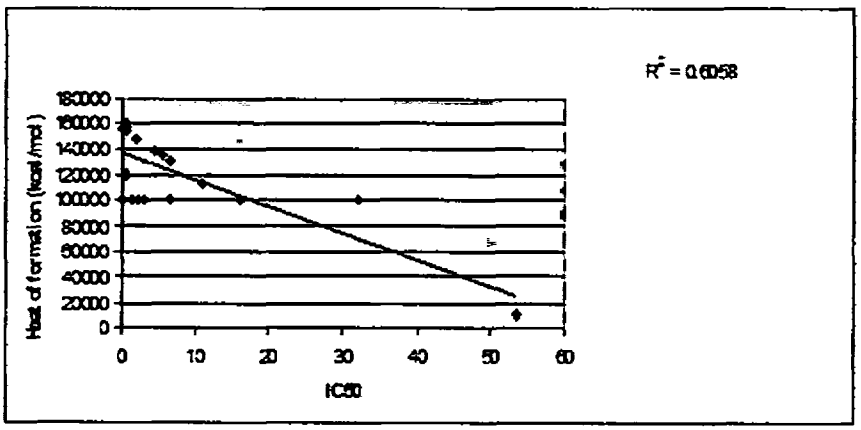

Figure 3.17: Plot of biological activity (IC_{50}) and heat of formation

Conclusion and Future Enhancements

Current study include pharmacophore identification, binding interactions calculation, docking, lead compound identification and analogue preparation of the lead compound on the group of antihistamine inhibitors.

The pharmacophore identified for this group of compounds can be very useful for the drug designing process. The lead compounds identified together with its analogues are projected for clinical trials in order to bring an improved and enhanced drug to the market with low side effects and better efficacy. As the drugs currently available for allergy as anti -histamines have many side effects.

The results of docking can give the scientists an idea that where the drug binds in HNMT and which amino acids are important in binding interactions.

The QSAR done on a group of 2-(piperidin-3-yl)-1H benzimidazoles will also help the researchers to have an idea of the diverse descriptors identified and also they can get an idea of the activity of these compounds which can give assistance to them in drug development.

Like wise there is a need to develop tools for the identification of lead compound so that manual work can be minimized.

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