IN-SILICO STUDY OF NOVEL 5HT2A ANTAGONIST FOR ANTIHYPERTENSIVE ACTION

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Abstract

Hypertension occurs when blood pressure gets elevated to 130-139 mmHg(systolic) and 80-89 mmHg(diastolic). The cause of hypertension is the generation of angiotensin II (octapeptide) from angiotensin I (decapeptide) by an angiotensin-converting enzyme (ACE). This angiotensin II is further converted into angiotensin III by an action of an aminopeptidase. This angiotensin III is 2-10 times less potent than angiotensin II which is produced by an action of an angiotensin-converting enzyme (ACE). So, for the treatment of hypertension, it is necessary to target the angiotensin-converting enzyme (ACE). 1.28 billion adults aged 30-79 years worldwide have hypertension. 5ht2a receptor inhibitor are the drug which inhibit the all the actions of 5ht2a receptors. 5ht2a receptors is the most widely expressed postjunctional 5HT receptors located on vascular and visceral smooth muscle,platelates and cerebral neurones especially prefrontal cortex. It mediates most of the direct actions of 5-HT like vasoconstriction, intestinal, uterine and bronchial contraction platelate aggregation and activation of cerebral neurones.the drugs which inhibit 5-HT2a receptors give vasodilation which can be used in hypertension. The ligand i.e. 4-(5H-Dibenzo[a,d]cycloheptene-5-ylidene)-1,4-pyrazine are bind to the maximum amino acids of 5HT2A receptors and shows an interaction with 5HT2a receptor (PDB ID:OAJ). This novel ligand shows Stable binding affinity. This study concluded that, novel ligand 4-(5H-Dibenzo[a,d]cycloheptene-5-ylidene)-1,4-pyrazine might be helpful for the treatment of Hypertension

Keywords: Antihypertension, 5HT2a receptor, Pyrazine, Vasoconstriction

1. INTRODUCTION

1.1 Drug Discovery

In the past most drugs were discovered either by identifying the active ingredient from traditional remedies or by serendipitous discovery. The process of drug discovery involves the identification of lead molecules, synthesis, characterization, screening and assays for therapeutic efficacy. Once compound has shown its value in these tests, it will begin the process of drug development prior to clinical trials.

Pharmaceutical chemistry is the core branch of pharmacy education and research. It can be categorized as synthesis of new drug molecule, its analysis and pharmacological studies. The identification of suitable lead which would forms a focal point around which a group of compound may be built. Search of therapeutically effective safer medicinal agents in treatment of various diseases in continued struggle since ages and lead to new opportunities.

The advances in medicine over past century has changed the way we live, but there remain an enormous number of unmet medical needs of 21st century. The society has been faced with challenges such as new diseases like AIDS, Multi-resistance and adverse effects of drugs. More than 50% of the drugs fail due to poor pharmacokinetic properties and acute toxicity, which necessitates an urgent need for developing new molecules with improved ADME data (absorption, distribution, metabolism and excretion) and fewer side effects[7].

Medicinal chemistry remains a challenging science, which provides profound satisfaction to its practitioners. It intrigues those of us who like to solve problems posed by nature. It verges increasingly on biochemistry and all the physical, genetic and chemical riddles in animal physiology which bear on medicine. The practice of medicinal chemistry is devoted to the discovery and development of new drugs for treating various diseases. Most of the activities in this discipline to design the new synthetic organic compounds, and achieve their increasingly specific pharmacological activites [8].

Today medicinal chemist has been trying to reduce the toxicity and increases the therapeutic efficacy of the existing compounds by the structural modification using computer aided drug design (CADD) and quantitative structural activity relationships (QSAR). Now, with rapid advances in our knowledge of drug designing, organic reaction mechanism, availability of better synthetic organic methods and other high-tech techniques such as NMR, MS, FTIR etc. for their structural elucidation. It is possible to prepare new drug and efficient derivatives of existing drug by structural manipulation and this mode of drug discovery is called as drug repurposing[1].

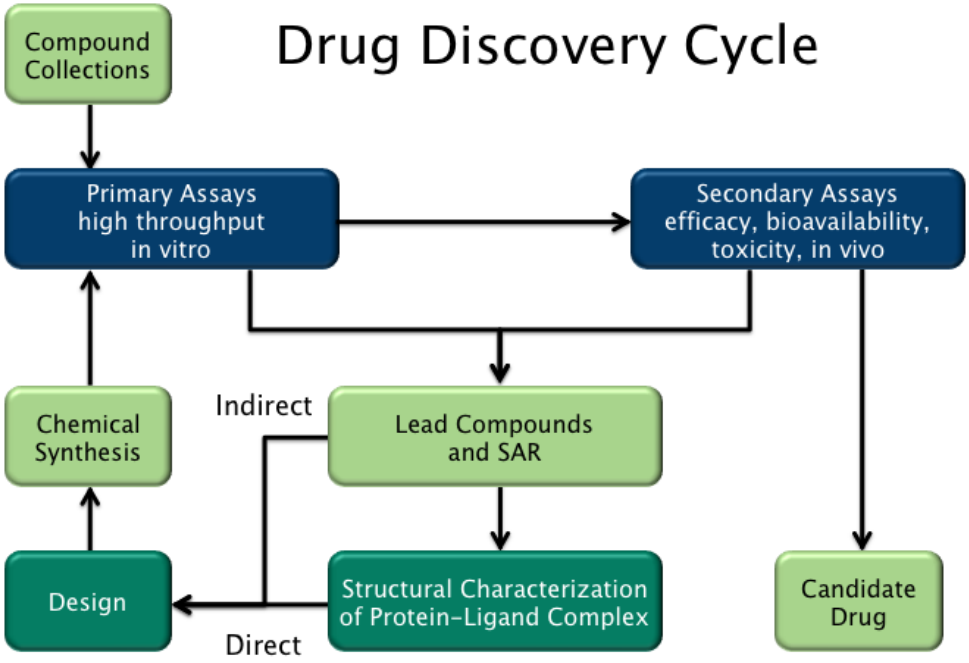

Figure 1 : DRUG DISCOVERY CYCLE

This drug discovery process may help the medicinal chemist to develop the new candidates for treating several disease and disorders. Recently, chemical libraries of synthetic small molecules, natural products or extracts were screened in intact cells or whole organisms to identify substances that had a desirable therapeutic effect in a process known as classical pharmacology. After sequencing of the human genome allowed rapid cloning and synthesis of large quantities of purified proteins, it has become common practice to use high throughput screening of large compounds libraries against isolated biological targets which are hypothesized to be disease-modifying in a process known as reverse pharmacology. Hits from these screens are then tested in cells and then in animals for efficacy. Modern drug discovery involves the identification of screening hits and optimization of those hits to increase the affinity, selectivity (to reduce the potential of side effects), efficacy/potency, metabolic stability (to increase the half-life), and oral bioavailability. Once a compound that fulfills all of these requirements has been identified, the process of drug development continues.

Modern drug discovery is thus usually a capital-intensive process that involves large investments by pharmaceutical industry corporations as well as national governments (who provide grants and loan guarantees). Despite advances in technology and understanding of biological systems, drug discovery is still a lengthy, "expensive, difficult, and inefficient process" with low rate of new therapeutic discovery. In 2010, the research and development cost of each new molecular entity was about US$1.8 billion. In the 21st century, basic discovery research is funded primarily by governments and by philanthropic organizations, while late-stage development is funded primarily by pharmaceutical companies or venture capitalists. To be allowed to come to market, drugs must undergo several successful phases of clinical trials, and pass through a new drug approval process, called the New Drug Application.

Discovering new drugs that may be a commercial success, or a public health success, involves a complex interaction between investors, industry, academia, patent laws, regulatory exclusivity, marketing and the need to balance secrecy with communication. Meanwhile, for disorders whose rarity means that no large commercial success or public health effect can be expected, the orphan drug funding process ensures that people who experience those disorders can have some hope of pharmacotherapeutic advances[2] [3].

1.2 Screening and design

The process of finding a new drug against a chosen target for a particular disease usually involves high-throughput screening (HTS), wherein large libraries of chemicals are tested for their ability to modify the target. For example, if the target is a novel GPCR, compounds will be screened for their ability to inhibit or stimulate that receptor (see antagonist and agonist): if the target is a protein kinase, the chemicals will be tested for their ability to inhibit that kinase.

Another important function of HTS is to show how selective the compounds are for the chosen target, as one wants to find a molecule which will interfere with only the chosen target, but not other, related targets. To this end, other screening runs will be made to see whether the "hits" against the chosen target will interfere with other related targets – this is the process of cross-screening. Cross-screening is important, because the more unrelated targets a compound hits, the more likely that off-target toxicity will occur with that compound once it reaches the clinic.

It is unlikely that a perfect drug candidate will emerge from these early screening runs. One of the first steps is to screen for compounds that are unlikely to be developed into drugs; for example compounds that are hits in almost every assay, classified by medicinal chemists as "pan-assay interference compounds", are removed at this stage, if they were not already removed from the chemical library. It is often observed that several compounds are found to have some degree of activity, and if these compounds share common chemical features, one or more pharmacophores can then be developed. At this point, medicinal chemists will attempt to use structure-activity relationships (SAR) to improve certain features of the lead compound:

- Increase activity against the chosen target
- Reduce activity against unrelated targets
- Improve the drug likeness or ADME properties of the molecule.

This process will require several iterative screening runs, during which, it is hoped, the properties of the new molecular entities will improve, and allow the favoured compounds to go forward to in vitro and in vivo testing for activity in the disease model of choice.

Amongst the physicochemical properties associated with drug absorption include ionization (pKa), and solubility; permeability can be determined by PAMPA and Caco-2. PAMPA is attractive as an early screen due to the low consumption of drug and the low cost compared to tests such as Caco-2, gastrointestinal tract (GIT) and Blood–brain barrier (BBB) with which there is a high correlation.

A range of parameters can be used to assess the quality of a compound, or a series of compounds, as proposed in the Lipinski's Rule of Five. Such parameters include calculated properties such as cLogP to estimate lipophilicity, molecular weight, polar surface area and measured properties, such as potency, in-vitro measurement of enzymatic clearance etc. Some descriptors such as ligand efficiency (LE) and lipophilic efficiency (LiPE) combine such parameters to assess drug likeness. While HTS is a commonly used method for novel drug discovery, it is not the only method. It is often possible to start from a molecule which already has some of the desired properties. Such a molecule might be extracted from a natural product or even be a drug on the market which could be improved upon (so-called "me too" drugs). Other methods, such as virtual high throughput screening, where screening is done using computer-generated models and attempting to "dock" virtual libraries to a target, are also often used.

Another important method for drug discovery is de novo drug design, in which a prediction is made of the sorts of chemicals that might (e.g.) fit into an active site of the target enzyme. For example, virtual screening and computer-aided drug design are often used to identify new chemical moieties that may interact with a target protein.Molecular modelling and molecular dynamics simulations can be used as a guide to improve the potency and properties of new drug leads.

There is also a paradigm shift in the drug discovery community to shift away from HTS, which is expensive and may only cover limited chemical space, to the screening of smaller libraries (maximum a few thousand compounds). These include fragment-based lead discovery and protein-directed dynamic combinatorial chemistry.The ligands in these approaches are usually much smaller, and they bind to the target protein with weaker binding affinity than hits that are identified from HTS. Further modifications through organic synthesis into lead compounds are often required. Such modifications are often guided by protein X-ray crystallography of the protein-fragment complex. The advantages of these approaches are that they allow more efficient screening and the compound library, although small, typically covers a large chemical space when compared to HTS.

Phenotypic screens have also provided new chemical starting points in drug discovery. A variety of models have been used including yeast, zebrafish, worms, immortalized cell lines, primary cell lines, patient-derived cell lines and whole animal models. These screens are designed to find compounds which reverse a disease phenotype such as death, protein aggregation, mutant protein expression, or cell proliferation as examples in a more holistic cell model or organism. Smaller screening sets are often used for these screens, especially when the models are expensive or time-consuming to run. In many cases, the exact mechanism of action of hits from these screens is unknown and may require extensive target deconvolution experiments to ascertain. Once a lead compound series has been established with sufficient target potency and selectivity and favourable drug-like properties, one or two compounds will then be proposed for drug development. The best of these is generally called the lead compound, while the other will be designated as the "backup". These important decisions are generally supported by computational modelling innovations[4] [5]..

1.2 COMPUTER AIDED DRUG DESIGN

Discovery and development of a new drug is generally known as a very complex process which takes a lot of time and resources. So now a day's computer aided drug design approaches are used very widely to increase the efficiency of the drug discovery and development course. Various approaches of CADD are evaluated as promising techniques according to their need, in between all these structure-based drug design and ligand-based drug design approaches are known as very efficient and powerful techniques in drug discovery and development. These both methods can be applied with molecular docking to virtual screening for lead identification and optimization. In the recent times computational tools are widely used in pharmaceutical industries and research areas to improve effectiveness and efficacy of drug discovery and development pipeline. In this article we give an overview of computational approaches, which is inventive process of finding novel leads and aid in the process of drug discovery and development research.

Computational approaches in drug design, discovery and development process gaining very rapid exploration, implementation and admiration. Introducing a new drug in a market is a very complex, risky and costly process in terms of time, money and manpower. Generally it is found that drug discovery and development process takes around 10-14 years and more than 1 billion dollars capital in total[6].

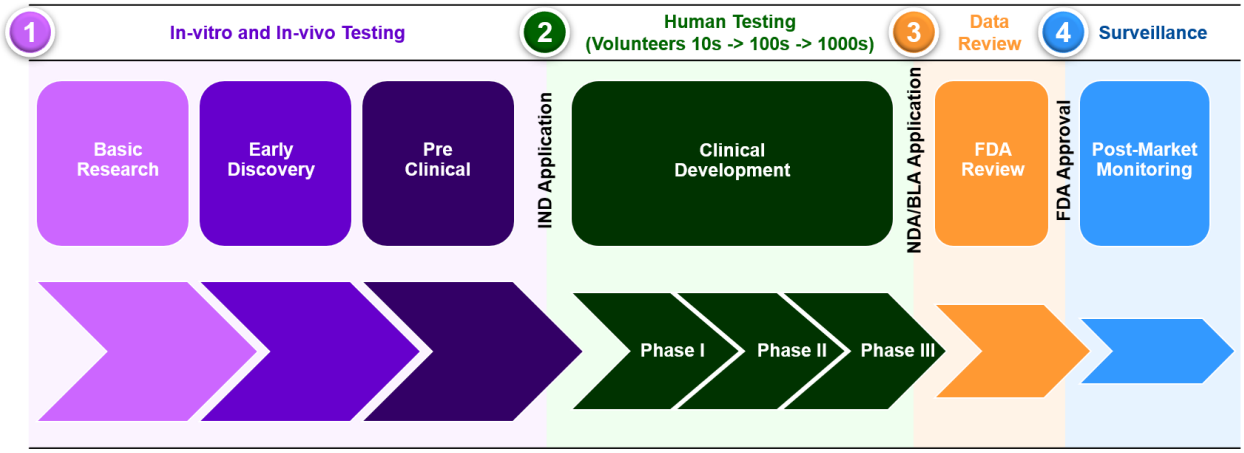

Figure no. 2 :-Traditional Process of Drug Discovery and Development

So for reducing time, cost and risk borne factors computer aided drug design (CADD) method is widely used as a new drug design approach. It has been seen that by the use of CADD approaches we can reduced the cost of drug discovery and development up to 50% CADD consist use of any software program based process for establishing a standard to relate activity to structure.

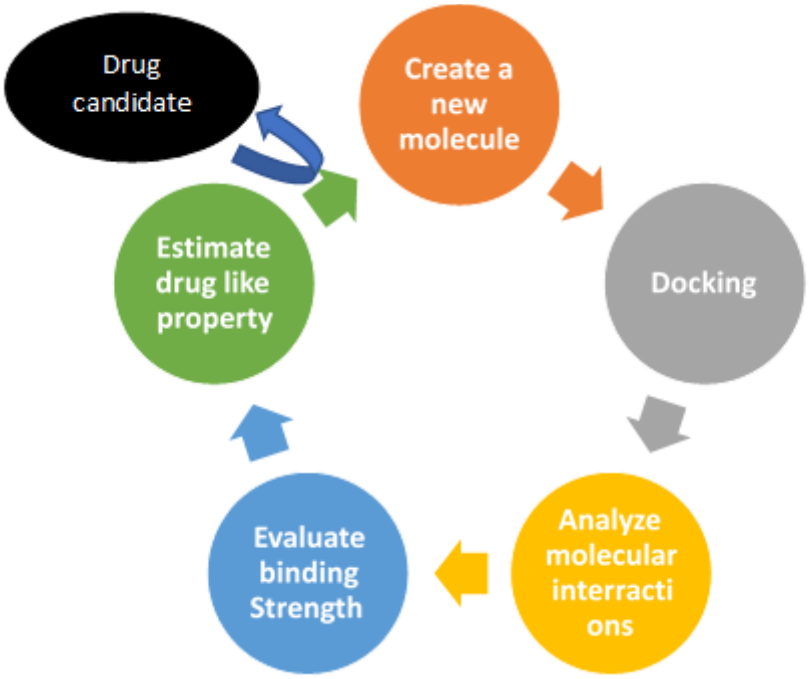

Figure 3:- General Principle for Drug Design through CADD

❖ **Major types of approaches in CADD**

There are mainly two types of approaches for drug design through CADD is the following:

❖ Structure based drug design / direct approach
❖ Ligand based drug design / indirect approach standard to relate activity to structure

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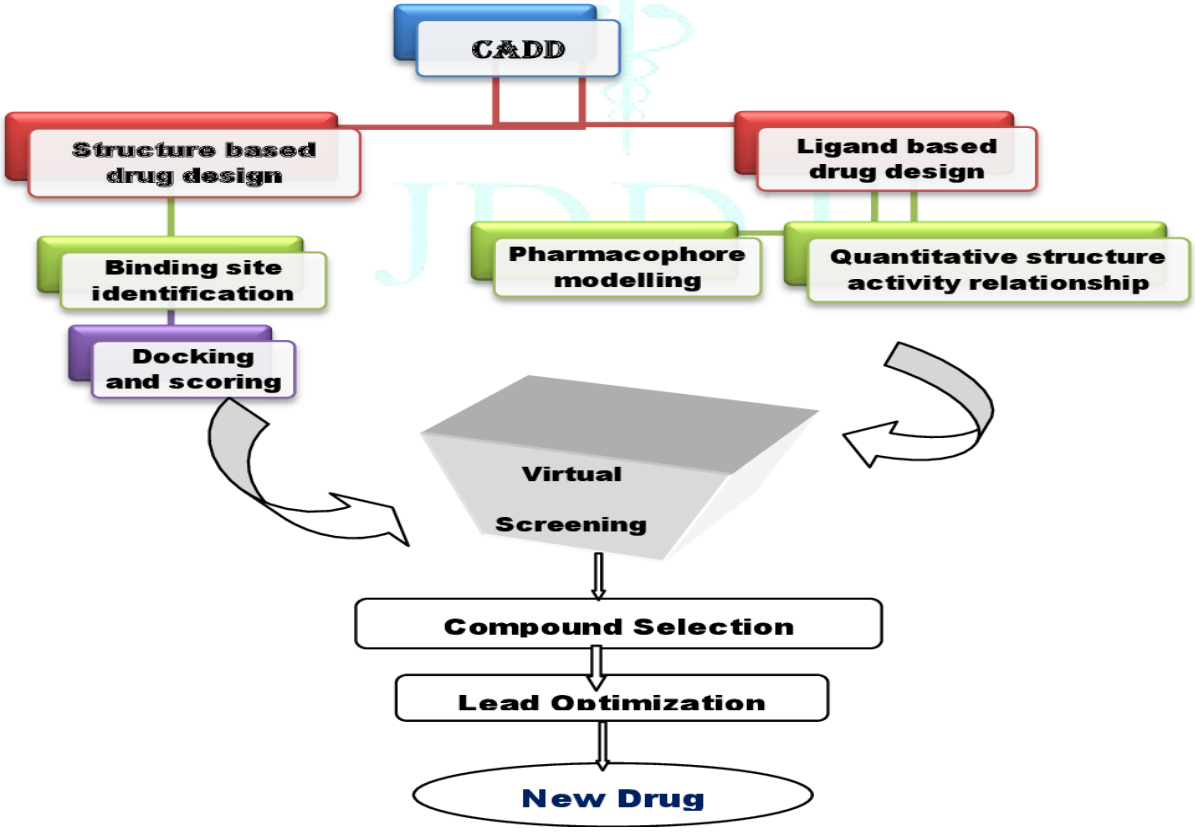


Figure no.4:- General representation of workflow for CADD

I. Structure-based drug design In SBDD, structure of the target protein is known and interaction or bio-affinity for all tested compounds calculate after the process of docking; to design a new drug molecule, which shows better interaction with target protein [48] The layout of SBDD is represented in figure 5 and the steps of SBDD is represented in figure 6

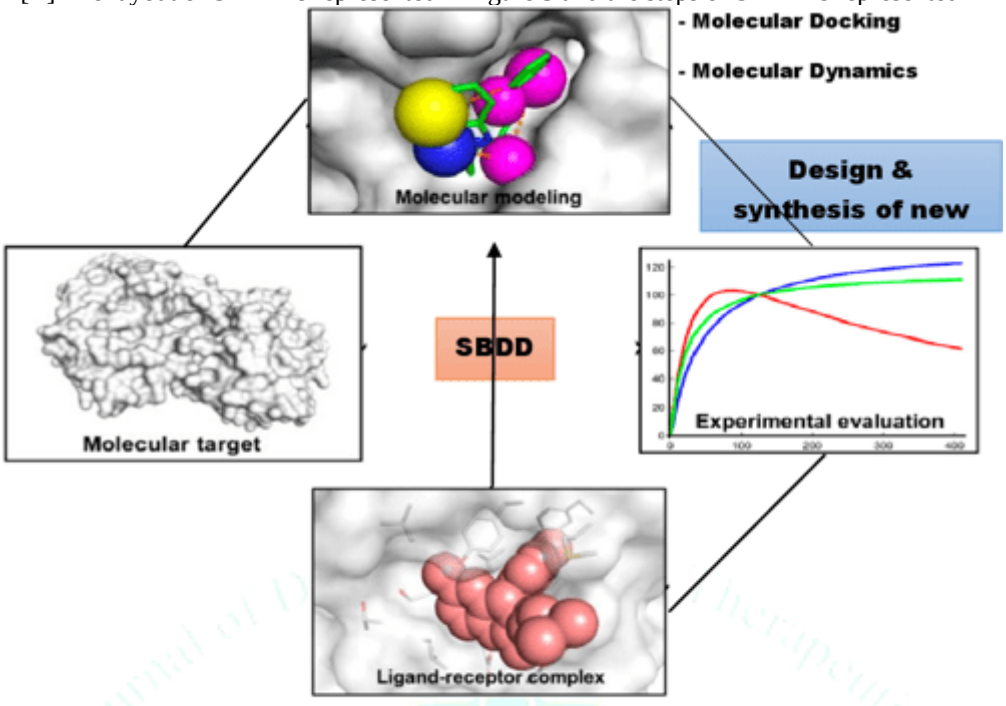


Figure 4: Layout of SBDD [5].

Figure no. 5 :- Layouts of SBDD

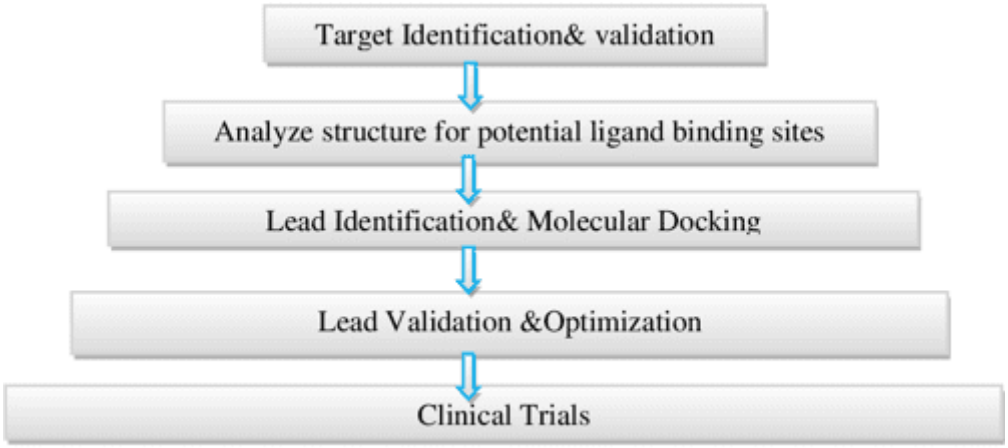


Figure 5: Steps involved in SBDD.

Figure no.6:- Steps involved in SBDD

Overview of the process involved in SBDD runs through multiple cycles before the optimized lead reached into clinical trials. The first cycle comprises isolation, purification and structure determination of the target protein by one of three key methods: like X-ray crystallography, homology modelling or NMR. Using compounds comes through virtual screening of different databases are placed into a selected region (active site) of the protein. These compounds are scored and ranked on the bases of steric, hydrophobic, electrostatic interaction of these molecules with the active site of target protein. Top ranked compounds are tested with biochemical assays. Second cycle comprises structure determination of the protein in complex with the most optimistic lead of the first cycle, the one with minimum micro-molar inhibition in-vitro, and shows sites of the compound which can be optimized for further increment in the potency. After several additional cycles like synthesis of lead, further optimization of lead through complex structure of protein with lead compound, the optimized compounds generally show marked increment in the target specificity and binding affinity[7].

II. Ligand-Based drug design

In LBDD, 3D structure of the target protein is not known but the knowledge of ligands which binds to the desired target site is known. These ligands can be used to develop a pharmacophore model or molecule which possesses all necessary structural features for bind to a target active site.

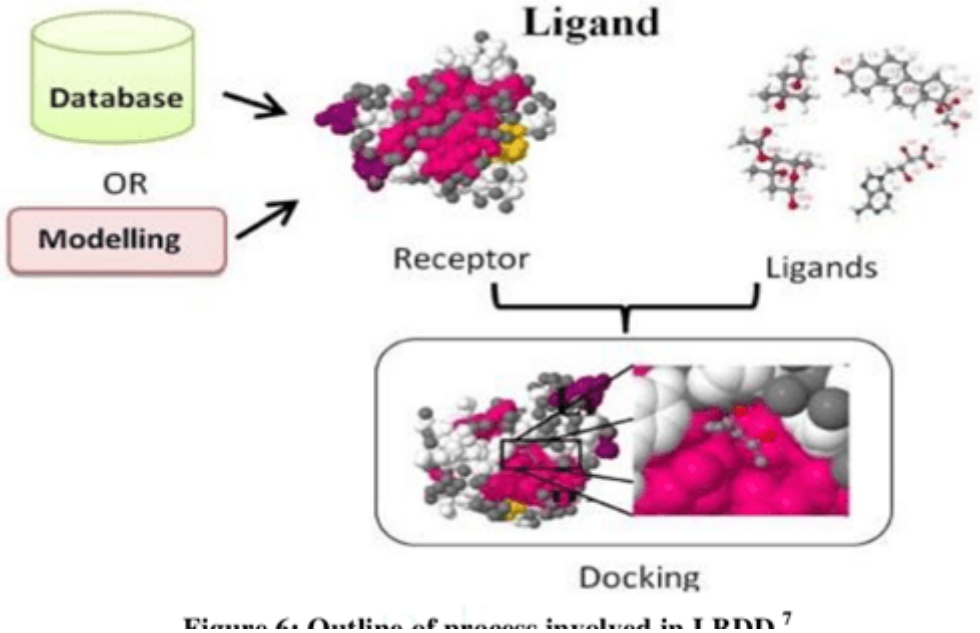


Figure no. 7 :- Outline of Process involved in LBDD

Generally ligand-based techniques are pharmacophore based approach and quantitativestructure activity relationships (QSARs). In LBDD it is assumed that compounds which having similarity in their structure also having the same biological action and interaction with the target protein[8].

1.3 Virtual screening

Virtual screening has been worked as a most convenient tool now a day to find out the most favorable bioactive compounds with the help of information about the protein target or known active ligands. In the recent time virtual screening is known as a mind blowing alternative of high-throughput screening mainly in terms of cost effectiveness and probability of finding most appropriate novel hit through filter the large of libraries of compounds.

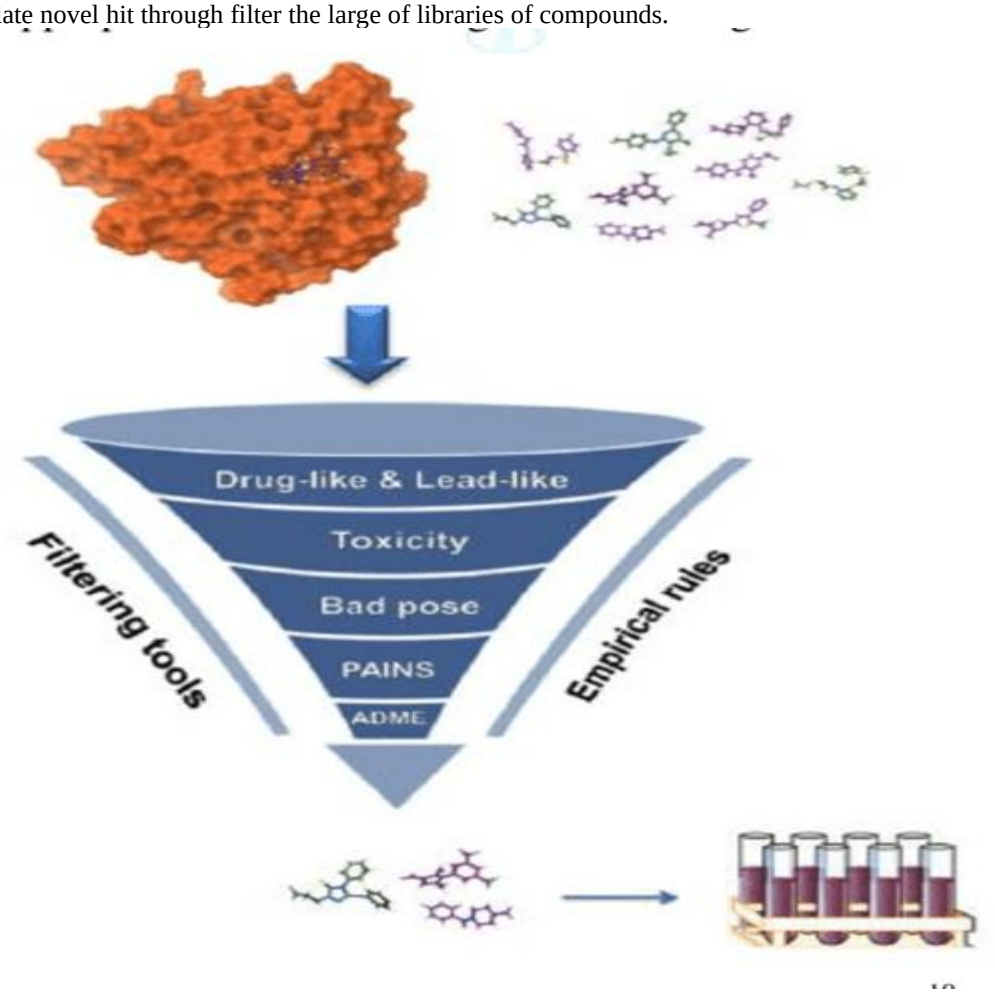


Figure no. 8 :- Overview of Virtual Screening Process

There are generally two types of virtual screening approaches like structure-based virtual screening (SBVS) and ligand-based virtual screening (LBVS), SBVS method rely on the structure of target protein active site and LBVS method is based on estimation of calculated similarity between the known active and compound come from databases[9]..

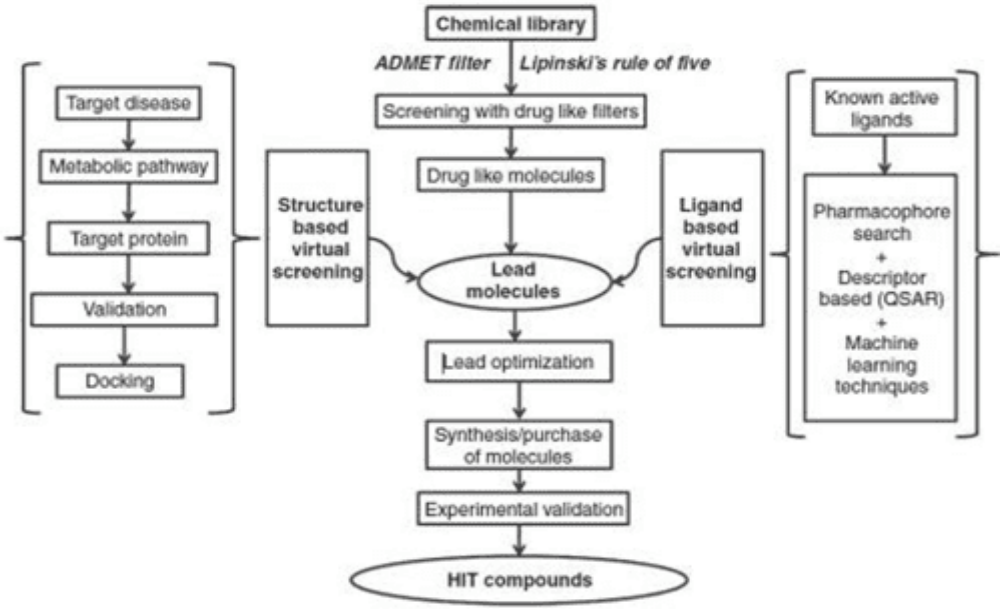


Figure no.9:- Schematic Diagram of VS Process for SBDD & LBDD

1.4 ADVANTAGES OF CADD [52] [53] [54]

- It can reduce the synthetic and biological testing efforts.
- It gives the most promising drug candidate by eliminate the compounds with undesirable properties (poor efficacy, poor ADMET etc.) through in silico filters.
- It is a cost-effective, time saving, rapid and automatic process.
- It know about the drug-receptor interaction pattern.
- It gives compounds with high hit rates through searching huge libraries of compounds in silico in comparison to traditional high throughput screening.
- These approaches minimize chances of failures in the final phase

1.6 ADVANTAGES OF INSILICO APPROACHES

The term in-silico stems from the computer component silicium; in silico methods, therefore, refer to methods or prediction using computational approaches. In silico methods have the advantage that they can make fast predictions for a large set of compounds in a high-throughput mode[10].

1.7 5-HYDROXYTRYPTAMINE-2a[5ht2a] INHIBITORS

5ht2a receptor inhibitor are the drug which inhibit the all the actions of 5ht2a receptors. 5ht2a receptors is the most widely expressed postjunctional 5HT receptors located on vascular and visceral smooth muscle,platelates and cerebral neurones especially prefrontal cortex. It mediates most of the direct actions of 5-HT like vasoconstriction,intestinal,uterine and bronchial contraction platelate aggregation and activation of cerebral neurones.the drugs which inhibit 5-HT2a receptors give vasodilation which can be used in hypertension,bronchodilation which can given in ashthama and also give for uterine relaxation and prevent aggregation of platelates. **5-HT2A are Gq protein** which activate **phospholipase C** and function through generation **of IP3/DAG pathway**. 5-HT2A receptors also **inhibit K channel** resulting is **slow depolarization of neurones**[11]**.**

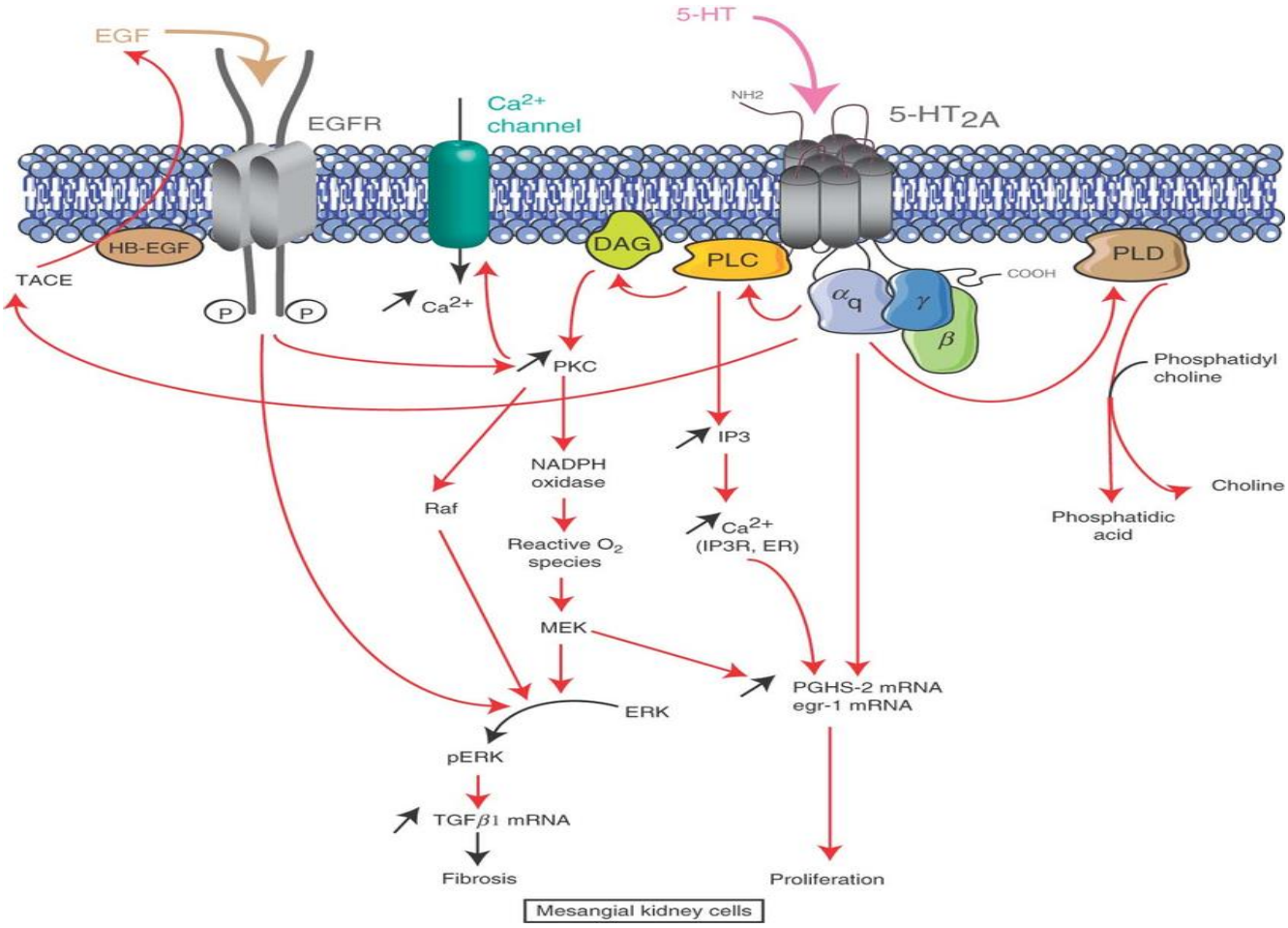


Figure no.10:- Diagram of 5HT2A Rceptors Mechanism

2.AIM:

I. To Screening of Biologically Active Ligands as **5HT2A receptors inhibitor**

2.1 OBJECTIVE:

II.To find out best ligand showing good receptor binding.

3. PLAN OF WORK :

a) Downloading of Software Program
b) Preparation of ligand
c) Preparation of receptor
d) Virtual screening

4. EXPERIMENTAL WORK:

(a) Softwares and programs

Chemsketch open source software, a chemical molecule drawing tool was used to draw the ligand compounds. Avogadro software was used to convert the .mol file to .pdb format. Pyrx software was used for virtual screening of library of derivatives. Discovery studio 3.5 was used for molecular interaction and visualization

(b) Preparation of ligand

Ligand structure was drawn using Chemsketch software and the structure was cleaned using the clean structure tool. The structure was saved in the working folder as .mol file. The .mol file was then accessed in Avogadro software and structure was optimized using optimization tool. The optimized structure was saved in the working directory as .pdb file. A set of 3 ligands were planned ligand and the binding affinity was calculated

After that in which one ligand was showing better binding affinity than other two ligand and fig.no.11 showning that ligand in pdb format[12].

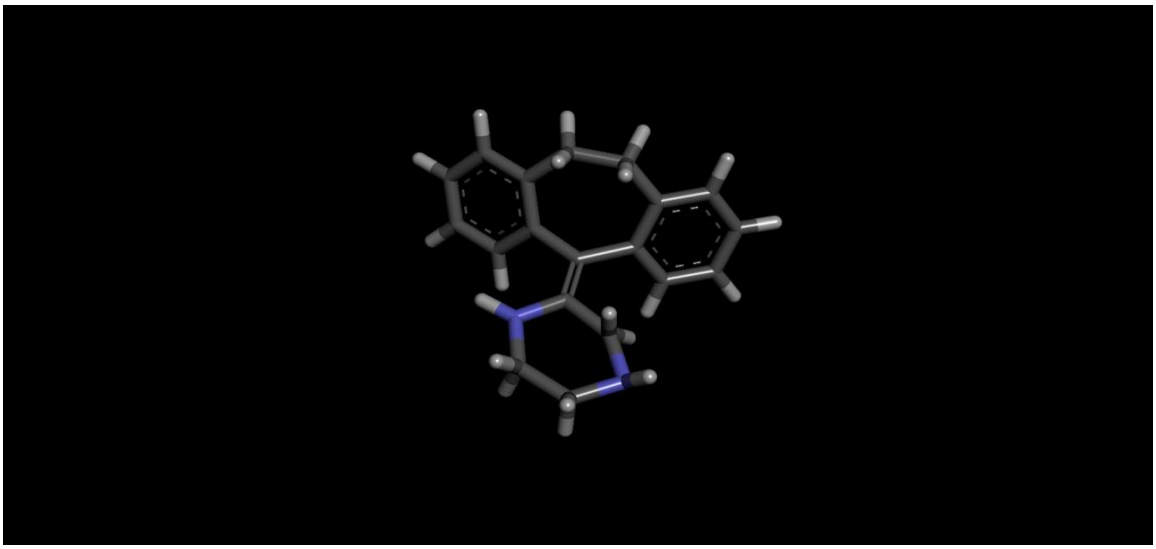


Figure no.11:- 3D Diagram of ligand (4-(5H-Dibenzo[a,d]cycloheptene-5-ylidene)-1,4-pyrazine)in .pdb format

(c) Preparation of receptor

The structure downloaded in .pdb(4oaj5HT2a.pdb) format from the online database and was rectified using Autodock v4.0 software. The energy was minimized by spreading the charges all over the receptor. The pre-associated ligands to the receptors were removed and the active site were identified[13].

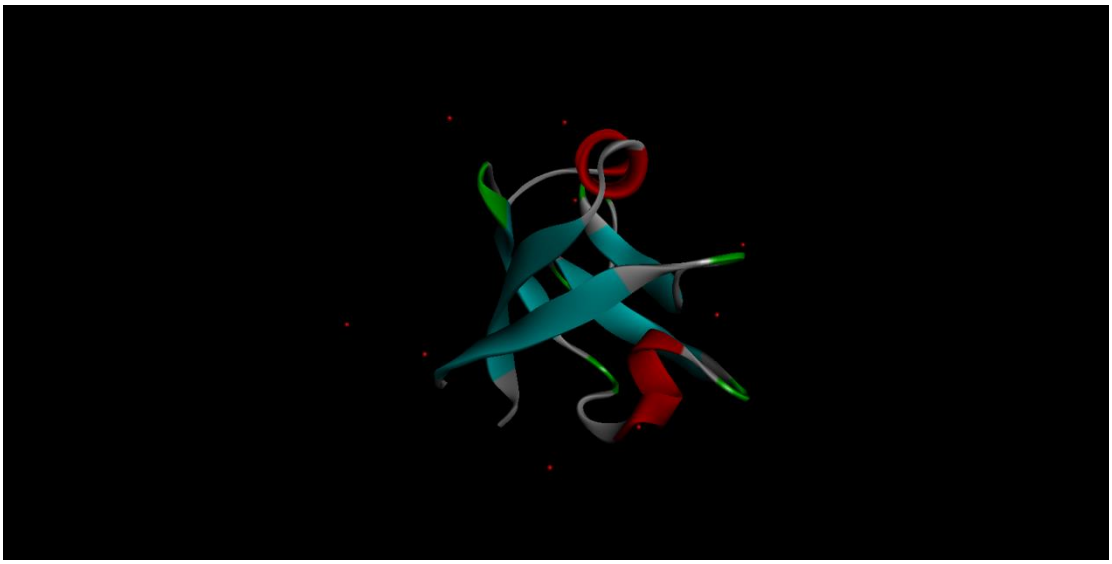


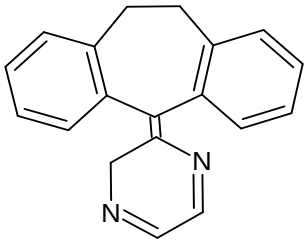
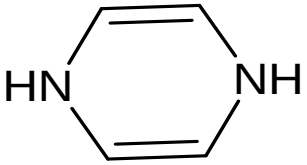
Figure no.11:- 3D Diagram of receptors 4oaj5HT2a.pdb format

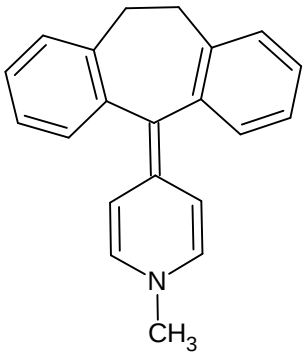
(d) Virtual screening

The Pyrx software was used for virtual screening protocols. The vina wizard module was selected for our study. The ligands and receptors were selected and converted into .pdbqt formats. The grid were selected and screening was carried out.

5. RESULTS & DISCUSSION :

- **Table of total screening molecule with their structure,IUPAC,and their binding affinity.**

Sr.no	NAME OF MOLECULE	structure	Binding affinity (kcal/mol)
1.	4-(5H-Dibenzo[a,d]cycloheptene-5-ylidene)-1,4-pyrazine		**-8.6**
2.	Pyrazine		**-3.2**

3.	Cyproheptadine- {4-(5H-Dibenzo[a,d]cycloheptene-5-ylidene)-1-methyl-pyridine		**-8.1**

- After performing virtual screening of different molecules with active ligand as 5-HT2A receptors inhibitors The **Dibenzcycloheptenes** and **Pyrazine** derivative showed better binding affinity than the other molecules. **4-(5H-Dibenzo[a,d]cycloheptene-5-ylidene)-1,4-pyrazine, Pyrazine,** and **Cyproheptadine- {4-(5H-Dibenzo[a,d]cycloheptene-5-ylidene)-1-methyl-pyridine** one showed good binding affinities. **4-(5H-Dibenzo[a,d]cycloheptene-5-ylidene)-1,4-pyrazine(novel 5HT2a antagonist)** – this one had the best binding affinity of **-8.6 Kcal/mol**

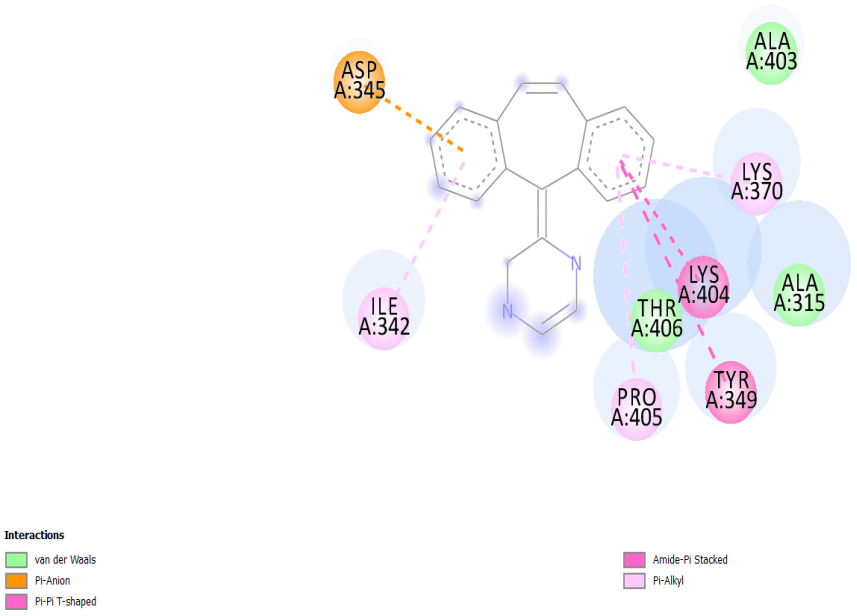


Figure no.12:- 2D Diagram of receptors ligand interactions

- The ligand i.e. 4-(5H-Dibenzo[a,d]cycloheptene-5-ylidene)-1,4-pyrazine are bind to the maximum amino acids of 5HT2A receptors and 2D diagram of these recptors ligand interactions are shown in above figure no.11. this interaction shows binding affinity is -8.6

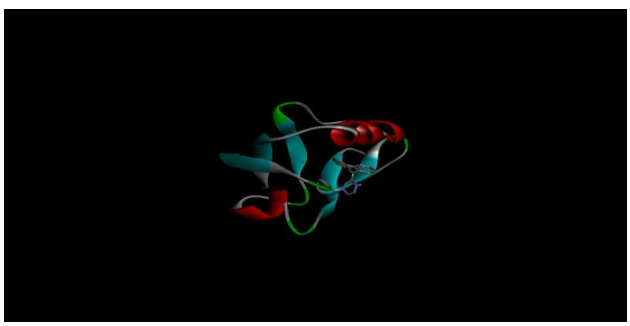


Figure no.13:- receptors ligand interactions by DS visualizer

6. CONCLUSION:

- The study showed **4-(5H-Dibenzo[a,d]cycloheptene-5-ylidene)-1,4-pyrazine (novel 5HT2a antagonist)** one molecule having potent **Antihypertensive,antiasthmatic,antiplatelate aggregation**. The results revealed binding affinity as -8.6 kcal/mol and interactions with active sites. These helped us to confirm the potency of the molecule towards receptor responsible for treatment of **Antihypertension.**

Conflict of Interest

All authors declare no conflict of interest.

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