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Computer aided molecular modeling and docking studies on active compounds of *Maytenus emarginata* (willd.) Ding Hou

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ABSTRACT

Maytenus emarginata is a Ethno medicinal plant and the leaves of this plant it is extensively used by the tribal's of south Indian Nallamala forest region, the aim of our extension study of Project work is to evaluate the Molecular modeling & docking studies on active compounds of Maytenus emarginata. As we already evaluated the Total Phenolic Content, Total Flavanoid Content, Determination of Total Antioxidant Activity, Determination of Reducing Antioxidant Power (FRAP), Nitric Oxide Radical Scavenging Activity, Hydroxyl Radical Scavenging Activity, Superoxide Radical Scavenging Activity, and Evaluation of α-glucosidase inhibition Activity. In our previous study, we also evaluate the gastro protective activity and its effect on antioxidant enzymes of Maytenus emarginata.

Key words: *Maytenus emarginata*, Autodock 4.0, Molecular Modeling, hHH2R, Gastro protective.

INTRODUCTION

In recent years, life sciences have undergone an immense transformation, where technological advancements in genomics, proteomics, and other high-throughput techniques produce floods of data that need to be stored, analyzed, and interpreted in various ways. Biology and medicine have thereby turned into information sciences and new areas of comparative biology have emerged. Bioinformatics is a relatively new discipline integrating life sciences with computational sciences by attracting scientists from a wide range of areas, including physics, chemistry, biology, medicine, mathematics, statistics, and computational sciences. Bioinformatics is crucial by providing tools to enable utilization of these gold mines of data in order to better understand the roles of proteins and genes and to obtain ideas for new experiments. This has made bioinformatics increasingly important in analysis of large-scale data in life sciences.

Bioinformatics and genome science (BGS) are relatively new disciplines, gaining importance across the biomedical research, healthcare and agriculture sectors due to their importance in helping to improve the timeliness and accuracy of disease diagnosis, prognosis and treatment, as well as enhancing crop yield [1,2,3]

Evaluating a Protein structure for SBDD:
Once a target has been identified, it is necessary to obtain accurate structural information. There are three primary methods for structure determination that are useful for drug design: X-ray crystallography, NMR, and homology modeling. The evaluation of structures from each method will be discussed. Crystal structures are the most common source of structural information for drug design, since structures determined to high resolution may be available, and the method is useful for proteins that range in size from a few amino acids to 998 KD [4]

A distinct approach in drug design comprises the use of bioactive small-molecule libraries. The unique chemical diversity available in these libraries represents the space occupied by ligands known to interact with a specific target. This type of information is used in ligand-based drug design (LBDD) methods [5] SBDD and LBDD

approaches have been applied as valuable drug discovery tools both in academia and industry [6] owing to their versatility and synergistic character. The integration of these approaches has been successfully employed in a number of investigations of structural, chemical and biological data [7].

Covalent Bonds in Molecular Docking:
Covalent drugs have demonstrated to be opportune alternatives in several therapeutic areas such as cancer, diabetes, and infectious, cardio-vascular, gastro-intestinal and neurologic diseases. Recent reports have claimed that approximately one-third of the currently marketed enzyme modulators are covalent inhibitors [8]

Despite the recent resurgence of covalent drugs, molecular modeling methods devised to address the problem of covalent docking are not as developed as those dedicated to noncovalent docking [9].

Modeling covalent bonds in molecular docking has been targeted by widely used molecular docking programs such as DOCK, AutoDock and Gold. Each of these programs employs a particular approach to manage covalent docking. Another program—DOCKovalent—is an adaptation of DOCK 3.6 aimed to perform large-scale, covalent virtual screening [10].

Computer Aided Drug design:
Computer-aided drug design makes use of the structural knowledge of either the target (structure-based) or known ligands with bioactivity (ligand-based) to facilitate the determination of promising candidate drugs. Various virtual screening techniques are now being used by both pharmaceutical companies and academic research groups to reduce the cost and time required for the discovery of a potent drug [11]

Proteins as drug targets
At the molecular level, the main targets for drugs are proteins (mainly enzymes, receptors and transport proteins) and nucleic acids (DNA and RNA). In recent years, novel drug identification research has been widely conducted. For example, Hughes *et al* [12] summarized the key preclinical stages of the drug discovery process and Enzymes are the macromolecule responsible for the catalysis of biochemical reactions is an manifestly target when a disease state is associated with production of a biologically active species. Enzymes are a classic target for therapeutic intervention and number of well-studied examples exists. Traditional medicinal chemistry enzyme targets include kinases, phospho diesterases, proteases and phosphatases. Addition to this, Histone methyl transferases, de methylases, acetyl transferases and de acetylases have been recently ascribed an important role as new classes of biological targets for drug discovery [13] An enzyme as Targets for Drug Design is a collection of scientific discussions related to enzyme inhibitors that show the many facets of the drug discovery process from the basic sciences through clinical applications.

Computational Methodology:
Hardware Components:
In present work all the calculations were carried out with high frequency computational analysis such as molecular modeling, energy minimizations, design and optimization of lead molecules, protein ligand interaction studies by molecular docking etc., a Hi-end server (Pentium IV 3.4 MHzs, AMD Athlon 64 bit, Dual processor with 1 GB RAM) manufactured by HCL Corporation, Pondicherry, India was used.

Software Components:
Most of the software's used were either Windows or Linux plat form based which were well accepted and referred in various publications at high rated research journals. Academic license was obtained for the commercial software used in the present study by requesting the concerned suppliers. The software used in the present study was briefly detailed below.

PyMOL:
It is an open-source, user-sponsored, molecular visualization system, and widely known as visualization software, which is well suited to produce high quality 3D images of small molecules and biological macromolecules such as proteins. According to the author, almost a quarter of all published images of 3D protein structures in the scientific literature were made using PyMOL.

AUTODOCK-Tool:
AutoDock is a suite of automated docking tools designed to predict how small molecules, such as substrates or drug candidates, bind to a receptor of known 3D structure. AutoDock actually consists of two main programs: AutoDock performs the docking of the ligand to a set of grids describing the target protein; AutoGrid pre-calculates these grids. In addition to using them for docking, the atomic affinity grids can be visualised. This can help, for example, to

guide organic synthetic chemists design better binders. AutoTors in the Autodock tool kit (ADT) software program (http://autodock.scripps.edu/resources/adt) was used to define the torsion degrees of freedom during the docking process.

Design and selection of Ligand molecules:
From the available drug molecule which is showing an interaction with that of target protein, the scaffold or skeleton molecular structure of ligand molecule was first can drawn by using Hyperchem 7.5. Over thus designed lead molecule (parent) different modifications were performed by taking into the consideration of the database of substituent's and spacers (linkers) containing a collection of current drugs at Molinspiraiton server. Then a series of lead molecules were thus designed as per molecular substitutions and designated the name and number as per instruction manual of Chem Office Ultra 7.0.v [14] These designed lead molecules were then analyzed for following Lipinski's Rule of five. The Lipinski's Rule of five states that in general orally active drug should has

- Not more than 5 hydrogen bond donors (OH and NH groups)
- Not more than 10 hydrogen bond acceptors (notably N and O)
- A molecular weight under 500g/mol
- A partition coefficient log P less than 5.

Among all the designed leads, the molecules of high ranking which follow Lipinski's rule were selected and further analysed for binding with the protein model using docking tools.

Lipinski rule of parameters:
Lipinski's rule of five also known as the Pfizer's rule of five or simply the Rule of five (RO5) is a rule of thumb to evaluate drug likeness or determine if a chemical compound with a certain pharmacological or biological has properties that would make it a orally active drug in humans. The rule was formulated by Christopher A. Lipinski in 1997, based on the observation that most orally administered drugs are relatively small and moderately lipophilic molecules [15].

a) LogP (octanol/water partition coefficient)
LogP is calculated by the methodology developed by Molinspiraiton as a sum of fragment-based contributions and correction factors. This Method is very robust and is able to process practically all organic and most organometallic molecules.

b) Octanol-water partition coefficient logP
LogP is used in QSAR studies and rational drug design as a measure of molecular hydrophobicity. Hydrophobicity affects drug absorption, bioavailability, hydrophobic drug-receptor interactions, metabolism of molecules, as well as their toxicity. LogP has become also a key parameter in studies of the environmental fate of chemicals.

Method for logP prediction developed at Molinspiraiton [16] is based on group contributions. These have been obtained by fitting calculated logP with experimental logP for a training set more than twelve thousand, mostly drug-like molecules. In this way, hydrophobicity values for 35 small simple "basic" fragments have been obtained, as well as values for 185 larger fragments, characterizing intramolecular hydrogen bonding contribution to logP and charge interactions. Molinspiraiton methodology for logP calculation is very robust and is able to process practically all organic and most organo-metallic molecules (Fig.1)

For 50.5% of molecules logP is predicted with error < 0.25, for 80.2% with error < 0.5 and for 96.5% with error < 1.0. Only for 3.5% of structures logP is predicted with error > 1.0. The statistical parameters listed above rank Molinspiraiton miLogP as one of the best methods available for logP prediction. MiLogP is used due to its robustness and good prediction quality in the popular ZINC database for virtual screen.

The designed compounds have given LogP within limits for the drug like compound characterization. With this parameter we have screened the about 50 molecules, among only 13 molecules has shown within range of Log p in the means of miLogP values.

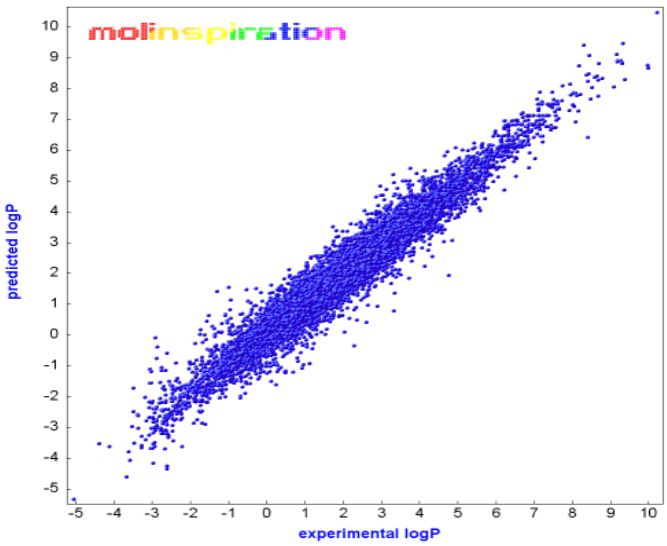

Fig 1 LogP graph has been constructed between predicted logP and experimental logP for standardization of Molinspiration algorithms for calculated drug molecules

c) Molecular Polar Surface Area TPSA:

It is calculated based on the methodology published by Ertl *et al.* as a sum of fragment contributions. O and N atoms centered polar fragments are considered. PSA has been shown to be a very good descriptor characterizing drug absorption, including intestinal absorption and bioavailability. It is a very useful parameter for prediction of drug transport properties. Polar surface area is defined as a sum of surfaces of polar atoms (usually oxygen, nitrogen and attached hydrogen atoms) in a molecule. This parameter has been shown to correlate very well with the human intestinal absorption, Caco-2 monolayer permeability, and blood-brain barrier penetration.

The calculation of PSA in a classical way, however, is rather time consuming, because of the necessity to generate a reasonable 3D molecular geometry and determine the surface itself. Additionally, calculations require specialized software to generate the 3D molecular structures and to determine the surface. In today's era of drug development shaped by high-throughput screening and combinatorial chemistry, fast bioavailability screening of virtual libraries consisting of hundreds of thousands, even millions of molecules is required. That is the reason why in our molecular property prediction toolkit so called topological polar surface area - TPSA is implemented. Briefly, the procedure is based on the summation of tabulated surface contributions of polar fragments (atoms regarding also their environment). These fragment contributions were determined by least squares fitting to the single conformer 3D PSA for 34,810 drugs from the World Drug Index. Topological polar surface area provides results of practically the same quality as the classical 3D PSA, the calculations, however, are two to three orders of magnitude faster.

d) Molecular Volume

Method for calculation of molecule volume developed at Molinspiration is based on group contributions. These have been obtained by fitting sum of fragment contributions to "real" 3D volume for a training set of about twelve thousand, mostly drug-like molecules. 3D molecular geometries for a training set were fully optimized by the semi empirical AM1 method. Molecular volume determines transport characteristics of molecules, such as intestinal absorption or blood-brain barrier penetration. Volume is therefore often used in QSAR studies to model molecular properties and biological activity. Various methods may be used to calculate molecular volume, including methods requiring generation of 3D molecular geometries, or fragment contribution methods such as Mc Gowan volume approximation. Method for calculation of molecule volume developed at Molinspiration is based on group contributions. These have been obtained by fitting sum of fragment contributions to "real" 3D volume for a training set of about twelve thousand, mostly drug-like molecules. 3D molecular geometries for a training set were fully optimized by the semi empirical AM1 method. Calculated volume is expressed in cubic Angstroms (A^3). Molinspiration methodology for calculation of molecular volume is very robust and is able to process practically all organic and most organo-metallic molecules.

ADME Boxes:

As we all know that, *ADME* is an abbreviation in pharmacokinetics and pharmacology for "absorption, distribution, metabolism, and excretion or elimination. Here, the new functionality builds on Pharma Algorithms' molecular property prediction technology based on dynamically defined molecular fragmentation, adding mechanistic modeling of absorption processes such as different routes of permeability and different rates for multiple ionic forms

of a compound. The predictive models have been implemented as automated software applications with a straightforward graphical user interface designed to meet the needs of medicinal chemists who require interactive functionality as well as computational chemists who need to perform virtual screening and high-throughput property filtering of virtual libraries. ADME Works can be used for predicting chemical and biological properties of compounds based on molecular structures (physicochemical, topological, geometrical, and electronic properties) and data on the property of interest [17].

Absorption module contains a mechanistic predictive model of human intestinal permeability. The predictions take into account the transcellular and paracellular routes of permeability and different rates for different ionized forms of a compound. The module can be used to predict the following absorption-related properties.

Protein Binding module predicts plasma protein bound fraction and the equilibrium binding constant to blood serum albumin of a compound in blood. The protein binding properties are predicted from automatically calculated physicochemical properties such as lipophilicity, ionization constants, and hydrogen bonding capacity.

Volume of Distribution module contains a predictive model which generates a quantitative estimate of the apparent volume of distribution of a compound. Physicochemical parameters, charge state, lipophilicity and hydrogen bonding capacity are automatically calculated and used as inputs to the predictive model of the volume of distribution.
The **Absolv module** algorithm has been updated to increase the accuracy of predictions. The user interface has also been enhanced: contributions of each atom to the currently selected Abraham parameter are color-mapped onto the structure, with intensity of the color indicating the degree of contribution of each atom or substructure to the selected parameter.

Homology modeling
Homology modeling, also known as comparative modeling of protein, refers to constructing an atomic-resolution model of the "target" protein from its amino acid sequence and an experimental three-dimensional structure of a homologous protein (the "template"). The 3D homology model of the target protein sequence was predicted using crystal structural coordinates of templates on the basis of sequence alignment. All steps of homology modeling and refinement were carried out through MODELLER 9v1 using base line commands specified by software supplier [18] The method described below is used in the present study to predict the 3D models of proteins.

Preparation of input files for MODELLER:
There are three kinds of input files required to perform homology modeling using MODELLER. They are PDB atom files with co-ordinates for the templates, the alignment file with alignment of the template; structures with the target sequence, and finally .PY file (a MODELLER command file that instructs MODELLER what to do).

Atom file:
Each atom file is named as "**code.Pdb**" where code is a short protein code, preferably the PDB code. The atom file contains the only protein co-ordinates without hetero atoms while modelin g target protein.

Alignment file
One of the formats for the alignment file is related to the PIR data base format which is the preferred format for homology modeling by MODELLER.

Script file (Steering file)
The script file contains commands for MODELLER. Sample scripts file to produce one model of sequence. A number of intermediary files were created as the program proceeds. After 5 minutes on an SGI indigo, the final protein model is written to file protein.B999901. A log file was also created with information about the run.

Flowchart of homology modeling by Modeller
This section describes a flow chart of homology modeling by MODELLER, as implemented in the 'model'. Script file also be used for variety of modeling tasks not only for comparative modeling. Input: script file (steering file; alignment file, PDB file(s) for template(s). Out put::

.log long file
.ini initial conformation for optimization
.rsr restraints file
.sch VTEM schedule file

.B999???? PDB atom file(s) for the model(s) of the target sequence .V9999???? Violation profiles for the model(s) The main MODELLER routines used in each step are given in parentheses.

Read and check the alignment between the target sequence and the template structures

(READ_ALIGNMENT and CHECK_ALIGNMENT).

Calculate restraints on the target from its alignment with the templates

Generate molecular topology for the target sequence GENERATE_TOPOLOGY). Disulfides in the target are assigned here rom equivalent disulfides in the templates PATCH_DISULFIDES). Any user defined patches are also done here (as defined in Top routine 'special patches').

a. Homology modelling by the Modeller Top routine 'model'.

```
Include                                   # include the predefined top routines
set OUTPUT_control = 1 1 1 1 1            # uncomment to produce a large log file
set alnfile ■ 'xxx.ali'                   # alignment filename
set knowns - \x x x x'                    # codes of the templates
set sequence = ' xxxxxxxxxxxx'            # code of the target
set at0m_files_directory = '. / : . . /atom_fi1es'   # directories for input atom files
set starting_model" 1                     # index of the first model
set Ending_model = 2                      # index of the last model
                                          (determines how many models to calculate)
call routine - 'model'                    # do homology modeling
```

b. Calculate coordinates for atom that have equivalent atoms in the templates as an average over all templates (TRANSFER_XYZ) (alternatively, read the initial coordinates from a file).
c. Build the remaining unknown coordinates using internal coordinates from the charm topology library (BUILD_MODEL).
d. Write the initial model to a file with extension. ini (WRITE_MODEL).
e. Generate sterochemical, homology derived, and special restraints (MAKE_RESTRAINTS) (alternatively, skip this and assume the restraints file already exists)
f. Write all restraints to file with extension. rst (WRITE_RESTRAINTS).

Stereochemical RESTRA1NT_TYPE = 'bond angle dihedral Improper'

mainchain dihedrals	RESTRA1NT-TYPE = 'phi-psi binormal'
mainchain dihedral	RESTRAINT-TYPE = "omega dihedral'
sidechain dihedral 01	RESTRAINT-TVPE ='chi!-dihedral'
sidechain dihedral 02	RESTRAINT-TYPE =’chi2_dihedral'
sidechain dihedral 03	RESTRAINT-TYPE = ' chi3_dihedral
sidechain dihedral 04	RESTRAINT-TYPE = 'chi4_dihedral'
main chain CA-CA distance	RESTRAINT-TYPE = 'distance'
mainchain N-O distance	RESTRAINT-TYPE = 'distance'
Sidechain-main chain	RESTRAIN-TYPE = 'distance'
sidechain-side chain	RESTRAINT TYPE = 'distance'
block distance restraints	RESTRAINT_TYPE = 'distance'
user defined	CALL ROUTINE = 'special_ restraints'

Non-bonded pairs distance RESTRAINT-TYPE = 'sphere’; calculated on the fly
1. Calculate model(s) that satisfy the restraints as well as possible, for each model:
a. Generate the optimization schedule for the variable target function method (VTFM)
b. Read the initial model (usually from the .ini file from 2.d) (READ_MODEL).
c. Randomize the initial structure by adding a random number between DEVIATION angstroms to all atomic positions (RANDOMIZE_XYZ).
d. optimize the model

Partially optimize the model by VTFM; Repeat the following steps as many times specified by optimization schedule:

Read all restraints by rd-restraints (READ-RESTRAINTS)

Select only the restraints that operate on the atoms that are close enough in sequence, as specified by the current step of VTFM (PICK_RESTRAINTS).

Optimize the model by conjugate gradients, using only currently selected restraints (OPTIMIZE). Refine the model by simulated annealing with molecular dynamics, if so selected:

- Do a short conjugate gradient optimization (OPTIMIZE).
- Increase temperature in several steps and do molecular dynamics optimization at each temperature (OPTIMIZE).
- Decrease temperature in several steps and do molecular dynamics optimization at each temperature (OPTIMIZE)
- Do a short conjugate gradient optimization (OPTIMIZE)
- Calculate the remaining restraints violations and write them out (ENERGY).
- Write out the final model to a file with extension .B99999???? Where???? Indicates the model number (WRITE_MODELS). Also write out the violation profile. Also write superposed templates and model, if so selected by FINAL_MALIGN3D = 1.

Evaluation of the built 3-D protein model:
A protein 3D model derived from homology modeling technique may have some sources of errors. It is important, therefore, to have an assessment of structure's quality and to be able to identify regions that may need modifications especially at protein folding and turns. The aim of model evaluation is to determine whether the built model is acceptable and suitable to use for molecular analysis such as docking and dynamics.

PROCHECK
The PROCHECK suite of programs provides a detailed check on the stereochemistry of a protein structure. The PROCHECK, a well-known protein structure checking program was carried out to check stereochemical quality of homology modeled structures [19]. The stereo chemical parameter checks implemented in PROCHECK are derived from high-resolution protein structures, against which the structure is compared on a residue-by-residue basis. The criteria are Ramachandran plot, peptide bond planarity, C-alpha tetrahedral distortion, non bonded interactions, hydrogen bond energies, and closeness off side chain dihedral angles to ideal values. PROCHECK can also be able to validate the energy minimized structures.

Preparation of files for AUTODOCK:
The advanced molecular docking program AutoDock 4.2 [20] which uses a powerful Lamarckian genetic algorithm (LGA) (Morris, *et al., 1999)* method for conformational search and docking, was applied for the automated molecular docking simulations. Briefly, The atomic solvation parameters were assigned using the ADDSOL utility of Autodock 4.2. The grid calculation was performed using Autogrid4 program, in which a box dimension of 22.5 Å and grid spacing of 0.375 Å parameters were set. The generated box size allows each member of the tested compound to rotate freely in order to find the conformation with the best binding free energy. LGA is used as a global optimizer and energy minimization for docking simulation. The LGA described the relationship between the antagonists and receptors by the translation, orientation, and conformation of the antagonists. These so-called 'state variables' were the ligands' genotype, and the intramolecular energies were the antagonists' phenotype. The environmental adaptation of the phenotype was reverse transcribed into its genotype and became heritable traits. Each docking cycle or generation, consisted of regimen of fitness evaluation, crossover, mutation, and selection. A Solis and Wets local search [21] was carried out to the energy minimization on a user-specified proportion of the population. The docked structures of the ligands were generated after a reasonable number of evaluations. The whole docking scheme could be stated as follows.

First, the receptor molecules were checked for polar hydrogen and assigned for partial atomic charges, the PDBQS file was created, and the atomic salvation parameters were also assigned for the macromolecules. Meanwhile, all of the torsion angles of the antagonists that would be explored during molecular docking stage were defied. Therefore, it allowed the conformation search for ligands during molecular docking process.

Second, the 3D grid was created by Auto Grid algorithm [21] to evaluate the binding energies between the antagonists and receptors. In this stage, the hHH2R antagonist's receptor was embedded in the 3D grid and probe atom was placed at each grid point. The affinity and electrostatic potential grid were calculated for varies type of atoms in the ligands. The energetic configuration of a particular ligand was found by trilinear interpolation of affinity values and electrostatic interaction of the eight grid points around each atom of the ligand.

Third, a series of the docking parameters were set on. The atom types, generations and run numbers for LGA algorithm were properly assigned according to the requirement of the Amber force field. The number of generations, energy evolutions, and docking runs were set to 370,000, 1,500,000, and 20, respectively. The kind of atomic charges were assigned as Kollman-all-atom for hHH2R receptor and Gasteiger-Marsili for ligands [22].

Protein – Lead molecules binding studies using AutoDock Tool

AutoDock 4.2 has been developed to provide a procedure for predicting the interaction of small molecules with macromolecular targets which can easily separate compounds with micromolar and nanomolar binding constants from those with millimolar binding constants and can often rank molecules with finer differences in affinity. AutoDock can be used to screen a variety of possible compounds, searching for new compounds with specific binding properties or testing a range of modifications of an existing compound [23].

RESULTS AND DISCUSSION

Selection of template to model hHH2R

For building model of hHH2R requires the best templates, this was obtained through PSI-BLAST search of the PDB database revealed 3D crystal structures of the bovine rhodopsin molecules [24] with BLOSUM 62 matrix application. This matrix represents sequences of amino acid substitutions observed in a large number of related receptors, including some quite similar and some quite different protein sequences. The observed substitutions were all lumped together to provide average frequencies of substitutions without regard to the degree of divergence between sequences.

The X-ray structures of bovine rhodopsin [25, 26, 27] were the only crystal structures on any GPCR that include the 7 transmembrane domain. The obtained BLAST(Basic Local Alignment Search Tool) results have shown 1HZX, 1JFP and 1F88 as best templates with same bits score of 65.1 but 2BGK-26.0 and 2IMS 26.6 with less bits score (Fig.7.8). Crystal structures of rhodopsin 1HZX, 1JEP and 1F88 were taken as best templates for homology modeling of hHH2R as reported by Kristiansen *et al*., 2004. The sequence analysis of templates and hHH2R was carried out with clustalW principles [28], critical analysis was done throughout the length of receptor sequence. All the templates have similar amino acids with 20% identity. The hypothesis that most family A receptors share, a similar folding of their 7TM domain was observed in the crystal structures of rhodopsin has been reported by sequence comparison of receptors revealing conserved fingerprint residues and by molecular modeling of receptor micro domains.

Details of the molecular mechanism of structural changes will possibly be revealed by solving the structure of the photo-reaction intermediates of bovine rhodopsin in 3-D crystal or by solving structures of GPCRs complex with hetero-trimeric G-proteins.

It is known fact that most of GPCR proteins cannot be crystallized under specific conditions. Furthermore, many pharmacologically important targets are membrane-bound proteins, such as G-protein-coupled receptors (GPCRs), ion channels, or transporter proteins, for which the experimental determination of the 3D structure is due to technical difficulties, either still impossible or can only be realized with immense experimental effort and complexity.

Homology model for hHH2R was developed through MODELLER 9v1 software with suitable crystallographic structures 1F88, 1HZX, 1JFP as best templates. The modeller was run with default parameters with the algorithms for optimization. Generated model was visualized by Pymol. Molecular visualization investigations were done for the generated model. The homology model has 7 TM regions and one small β sheet determined. hHH2R model is energy minimized with GROMOS 96 force field for further analysis as per Kiss *et al* procedures [29].The homology model is shown in Fig 2 with cartoon representation, top view, electrostatic molecular surface with charge distribution on receptor and position of receptor in lipid bilayer. All seven transmembranes and small beta sheets also represented with cylinders and coils. This indicates positions of helices in three dimensional space co-ordinate system to determine position of small sub units in helices regions within receptor.

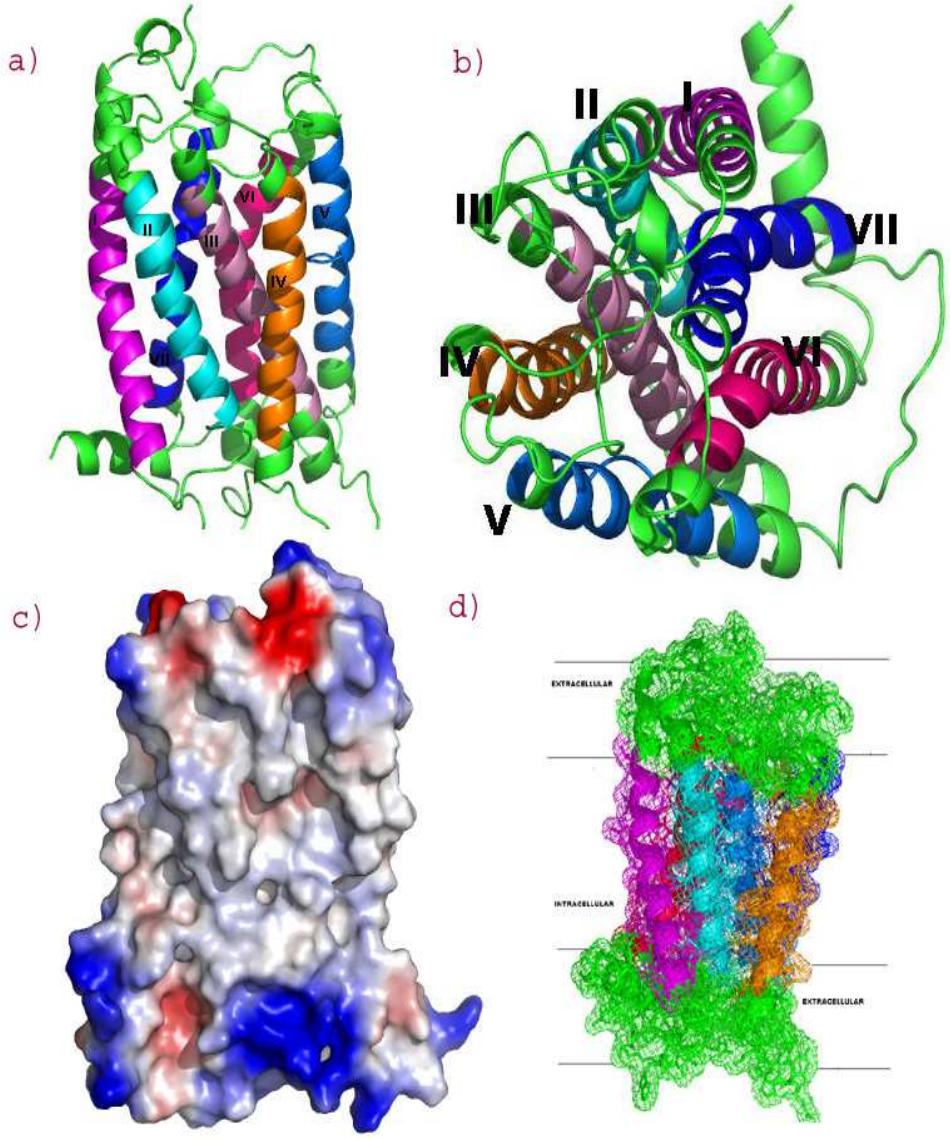

Fig2. Homology model of hHH2R is showing seven transmembranes and each transmembrane shown in different color

5.15 Structural validation

5.15.1 Validation by PROCHECK

The modeled structure as verified by PROCHECK, has given 92.4% in favorable regions, 6.2% in allowed regions, 0.7% generously allowed regions and 0.7% disallowed regions Plot statistics were shown in table 1 According to all these statistics homology model hHH2R is a very good model, which can be used for docking process with newly designed lead molecules based on famotidine drug molecules scaffolds.

Table 1 Ramachandran plot statistics for best model of hHH2r protein structure (PROCHECK)

Ramachandran plot statistics		
Residues in most favoured regions	254	92.40%
Residues in additional allowed regions	17	6.20%
Residues in generously allowed regions	2	0.70%
Residues in disallowed regions	2	0.70%
Number of non-glycine and non proline residues	**275**	**100.00%**
Number of end-residues (excl. Gly and Pro)	2	
Number of Glycine residues (shown as traingle)	14	
Number of proline residues	8	
Total number of residues	**299**	

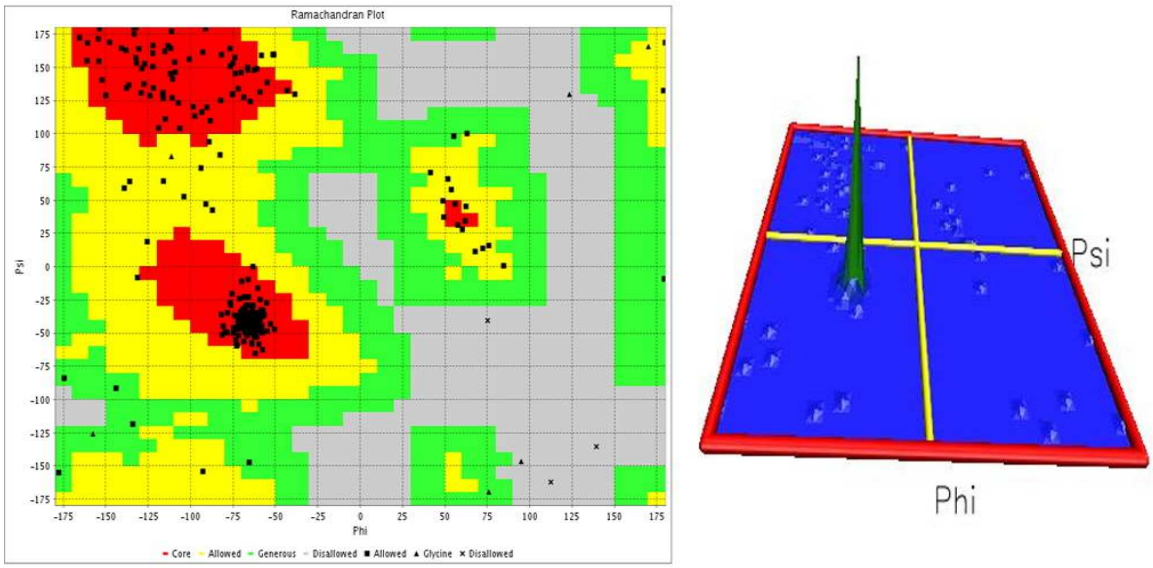

Fig3. Ramachandran plot for hHH2R showing most of the residues falling in coreregion. 3D graphical representation of Ramachandran plot by VMD, green peek indicates the presence of helices in receptor

Refinement of 3D model of H2 Receptor

The modeled Human H2 Receptor energy minimized using Gromacs force fields. Later the energy minimization The Human H2 Receptor model structure was submitted to molecular dynamics simulations using the GROMOS 96 force field. This procedure had the goal of putting both enzymes in the same physiologic conditions for further superposition and also of providing a way for additional validation and refinement of the structure of Human H2 Receptor and after refinement it showing a energy of -1.4571 K.cal.

Docking Analysis on H2 Receptor with phytochemicals

The interactions of Human H2 Receptor with different inhibitors, determined from their crystal structures, have served as a starting point for tests of various docking techniques. The first of such docking studies of Human H2 Receptor was performed by the Kuntz laboratory. who discovered that haloperidol could be an inhibitor of this enzyme. However, subsequent crystal structure determination revealed a different orientation for haloperidol than the one predicted. Docking methods and algorithms were tested using the known structural data and experimental characteristics by Monte Carlo docking or by comparison with de novo constructed inhibitors by a fragment-based method in which the inhibitors were constructed entirely from individual functional groups chosen from a predefined library. A method of continual energy minimization implemented in the program SCULP was a new paradigm for modeling proteins in interactive computer graphic systems. This physically realistic attempt made possible the modeling of very large changes and aided the understanding of how different energy terms interact to stabilize a given conformation. Other recent studies examined empirical free energy as a target function in docking and design, showing the advantages of this approach over studies using the calculation of interaction energy.

The energy minimized H2 receptor docked with *Maytenus emarginata* pytochemicals using Autodock 4.0. The Phytochemical of *Maytenus emarginata* Emarginatine A, B, E, F, G, alpha amyrin, Cynocolchicines and Quercetin has more are less forms hydrogen bond interactions with Asp98, Asp186 and Thr190 residue has made strong hydrogen bonds with all the inhibitors with less than 1.180A^{o} bond length. Asp98 also interacting in all cases with distance of 1.75 A^{0}. Carboxyl group of Asp98 is interacting with protonated all the inhibitors. It is confirmed that protonated amino group plays a vital role in the formation of hydrogen bond at a distance of 1.75 A^{0}. In these interactions, Emarginatine are bigger in their size to dock with hHH2R. But, Cynocolchicines forms a strong H-bond with Asp98 residues of H2 receptor and its release a binding free enrgy of **-5.6 K.cal/mol** at RMSD 0.02 and Ki 40 µM. Whereas, the alpha amyrin forms strong H-bond with Asp98 and asp186 amino acid residues and they are showing a lowest docked energy of **-7.7K.cal/Mol**. But the beauty of Quercetin molecular conformation in the cavity is exactly matching with molecular surface and it showing Lowest Docking energy of **-10.30 K.cal/mol,** RMSD 0.04 and Ki 0.023µM and no other inhibitors showing as much as best interaction as Quercetin and it has been tightly packed with 3 important residues are Asp98, Asp186 and Thr190 of Human H2 Receptor

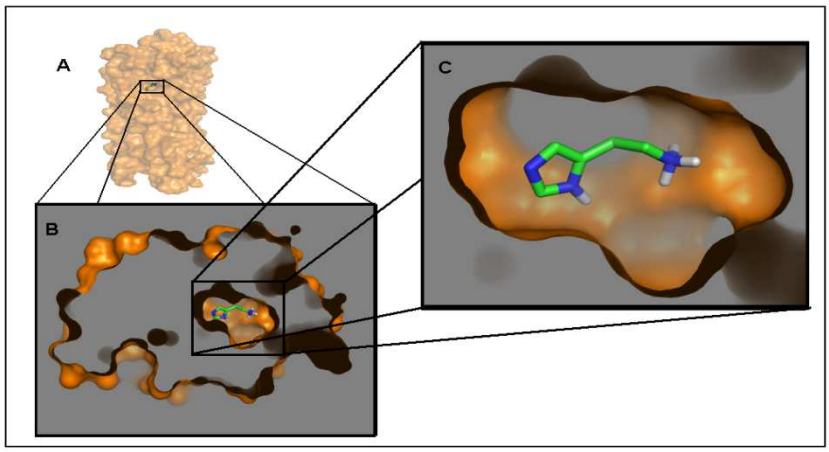

Fig4. Modeled protein of hHH2R protein with molecular surface representation colored in brown. B. Protein surface slab at 35 frame visualization with histamine cavity in protein. C. Histamine stabilization in cavity of modeled protein of hHH2R

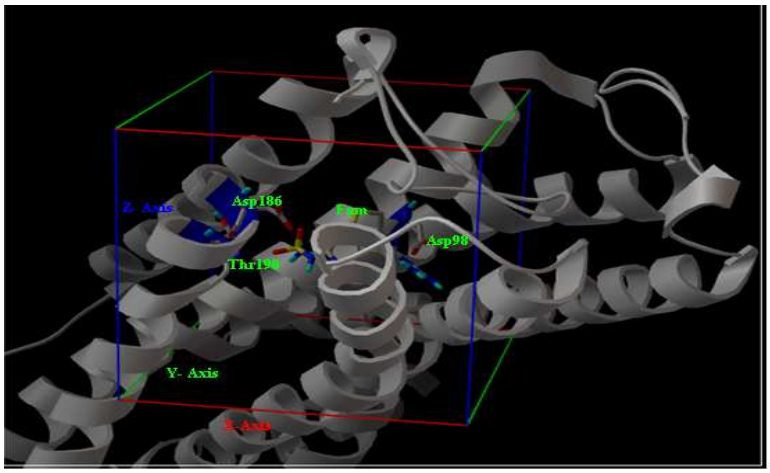

Fig5. Gridbox generated by the mean of X, Y and Z co-ordinates from Asp186, Thr190 and Asp98. Residues and famotidine is shown in stick model and protein as in cartoon representation

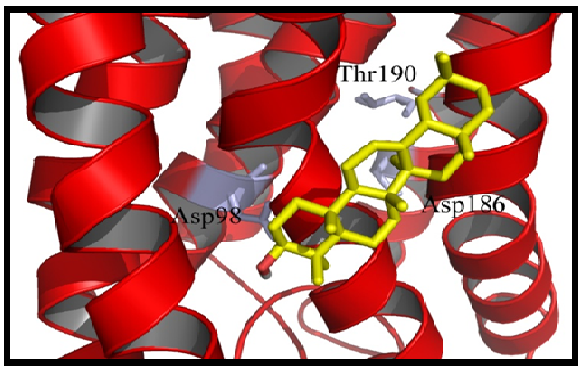

Fig6. Docking study of alpha amyrin

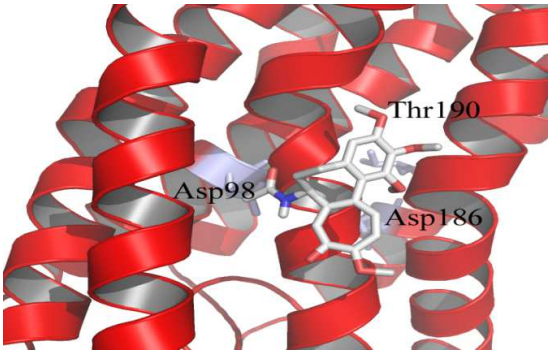

Fig7. Docking study of Cynocolchicines

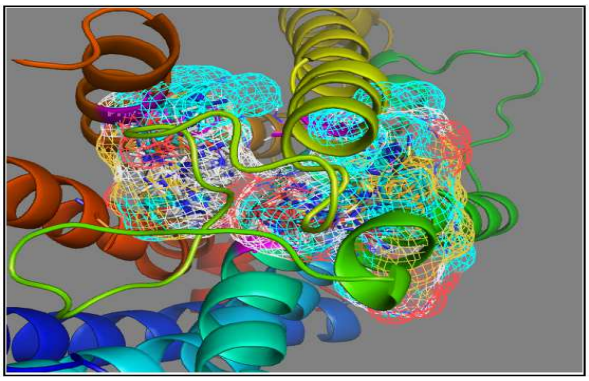

Fig8.Docking space from top view represented

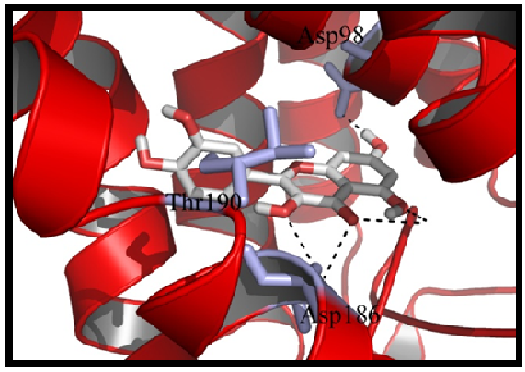

Fig9. Docking study of Quercetin with hH2R by mesh and hHH2R protein in cartoon

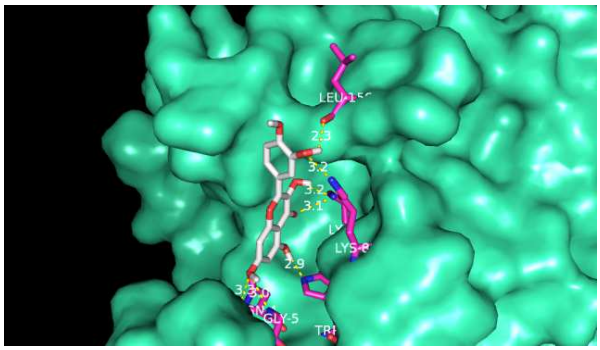

Fig10. Docking study of Quercetin with hH2R

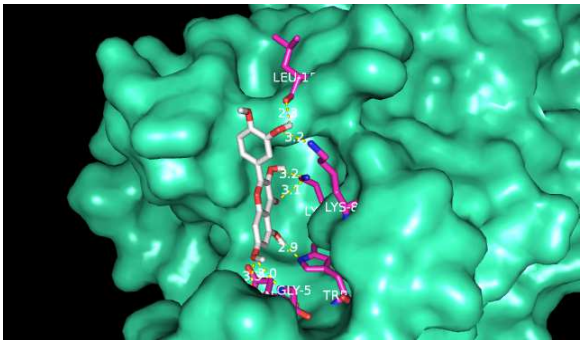

Fig11. Docking study of Quercetin with hH2R

Table2: Docking energies of *Maytenus emarginata* phytochemical constituents with hHH2R (Human Histamine Receptor)

S.No.	Protein	Phyto chemicals	Cluster[a]	RMSD[b]	Lowest Binding Energy[c] (Kcal/mol)	Inhibition Constant[d] (*Ki*)	Amino acids involved in H bond formation
1.	H2 Receptor	Cyno colchicine	05	0.02	-5.6	40µM	Asp98
2.		alpha amyrin	45	0.45	-7.7	9.45 µM	Asp98 and Asp186
3.		Quercetin	32	0.04	-10.3	0.023 µM	Asp98, Asp186 and Thr190

[a]Indicative of the total number of binding modes produced
bHeavy atoms root-mean-square deviation with respect to the experimental structure.
[c]The change in binding free energy is related to the inhibition constant using the equation: ΔG = RT in Ki, where R is the gas constant 1.987 cal K-1 mol-1, and T is the absolute temperature assumed to be 298.15 K.
[d]Estimated inhibition constant at 298.15 K.

CONCLUSION

In our in silico analysis, we have modeled the human histamine receptor 2 protein structure by using the modeler 9v1. Molecular Docking studies were done by using the Auto dock 4.0, The interactions of Human H2 Receptor with different inhibitors, determined from their crystal structures, The docking energy minimization studies were conducted on hHH2R with the phyto chemicals of Maytenus emarginata in which we have found the beauty of Quercetin molecular conformation in the cavity is exactly matching with molecular surface and it showing Lowest Docking energy of -10.30 K.cal/mol, RMSD 0.04 and Ki 0.023µM and no other inhibitors showing as much as best interaction as Quercetin.

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