Docking of Phytochemicals of Medicinal Plant Ficus religiosa with Human Histamine H2 Receptor

A DISSERTATION

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE AWARD OF THE DEGREE
OF

MASTER OF TECHNOLOGY
IN
BIOINFORMATICS

Submitted by:

Varsha kumari
2K16/BIO/08

Under the Supervision of

Dr. ASMITA DAS

DEPARTMENT OF BIOTECHNOLOGY
DELHI TECHNOLOGICAL UNIVERSITY
(Formerly Delhi College of Engineering)
Bawana Road, Delhi-110042

JUNE, 2018

DELHI TECHNOLOGICAL UNIVERSITY
(Formerly Delhi College of Engineering)
Bawana Road, Delhi-110042

CANDIDATE'S DECLARATION

I, Varsha Kumari, Roll No. 2K16/BIO/08 student of M.Tech (Bioinformatics), hereby declare that the project Dissertation titled "Docking of Phytochemicals of Medicinal Plant Ficus religiosa with Histamine H2 Receptor" which is submitted by me to the Department of Biotechnology, Delhi Technological University, Delhi in partial fulfillment of the requirement for the award of the degree of Master in Technology, is original and not copied from any source without proper citation. This work has not previously formed the basis for the award of any Degree, Diploma Associateship, Fellowship or other similar title or recognition.

Place: Delhi **VARSHA KUMARI**

Date:

DEPARTMENT OF BIOTECHNOLOGY
DELHI TECHNOLOGICAL UNIVERSITY
(Formerly Delhi College of Engineering)
Bawana Road, Delhi-110042

CERTIFICATE

I hereby certify that the project dissertation titled "Docking of Phytochemicals of Medicinal Plant Ficus with Human Histamine H2 Receptor" which is submitted by Varsha kumari, Roll No. 2K16/BIO/08, Department of Biotechnology, Delhi Technological University, Delhi in partial fulfillment of the requirement for the award of the degree of Master in Technology (Bioinformatics), is a record of a project work carried out by the student under my supervision. To the best of my knowledge this work has not been submitted in part or full for ant Degree or Diploma to this University or elsewhere.

Place: Delhi **(Dr. ASMITA DAS)**

Date: **SUPERVISOR**

Assistant Professor

Department of Biotechnology

Delhi Technological University

ABSTRACT

Medicinal plants have been used as a source of medicine to treat health disorders and to prevent diseases including epidemics. Plants produces active compounds during secondary metabolism, and these active compounds are usually responsible for the biological properties of plants. Medicinal plants are used for various purposes including treatment of infectious diseases and allergy. The biological name of Peepal is Ficus religiosa. It belongs to the family of Moraceae. The tree has various therapeutic values as it is a source of several useful chemical compounds. Histamine has been recognized as a major mediator in allergic diseases plays a much wider and critical role as a pro-inflammatory mediator in allergic disease**.** This study is to focus on the range of phytochemicals present in F.religiosa and perform docking of these phytochemicals with the human Histamine H2. Docking was performed to access binding affinities between phytochemical and modeled receptor. The analysis suggested that lanosterol, α-amyrin acetate, Ergost-5-en-3beta-ol, germacrene, bergaptol, bergapten, gamma-cadinene form the most stable complex with human histamine H2 receptor. These compounds may be a suitable as therapeutic agent against inflammatory and allergic diseases

ACKNOWLEDGEMENT

I am most thankful to my family for constantly encouraging me and giving me time and unconditional support while pursuing this research.

I am extremely grateful to Dr. Asmita Das, Asst. Professor, Delhi Technological University, Delhi, India for providing invaluable guidance and being a constant source of inspiration throughout my research. I will always be indebted to her for her extensive support and encouragement.

I also convey my heartfelt gratitude to all the research scholars of the bioinformatics department for their valuable suggestions and helpful discussions throughout the course of this research work.

VARSHA KUMARI

(2K16/BIO/08)

Table of Contents

LIST OF TABLES

LIST OF FIGURES

LIST OF ACRONYM

ACRONYM NAMES	DESCRIPTION
NK	Natural killer cells
GPCRs	

Chapter 1 INTRODUCTION

Herbs have been the important source of medicine. Medicinal plants have therapeutic properties because of the presence of various chemical substances of different compositions, which are formed as secondary plant metabolites. They have therapeutic properties against diabetes, cardiac diseases, tuberculosis, liver diseases, asthma, and several other diseases.

Histamine plays an important role in a variety of pathophysiological conditions. Histamine binds with four different separate rhodopsin-like G protein-coupled receptors-histamine H1, H2, H3, and H4 and exerts its biological effects by activating them. Every one of the histamine receptors has a functional response, but their action mechanism is different from each other.*Ficus religiosa* is an Indian tree and generally known as Peepal which belongs to the family *Moraceae.* The phytochemical of various parts of *F. religiosa* plant shows anti-inflammatory and anti-allergic effects against several diseases.

Plants have been the basis of many traditional medicine systems throughout the World for thousands of years and still remain as the main new source of structurally important chemical substances that lead to the development of innovative drugs. The use of medicinal plants for the treatment of many diseases is associated with folk medicine from different parts of the World. Nowadays, the search for new anti-inflammatory and anti-allergic agents from the huge array of medicinal plant resources is intensifying. In fact, a variety of bioactive components have been shown to modulate inflammatory responses. The inflammatory response is a critical protective reaction to irritation, injury, or infection, characterized by redness, heat, swelling, loss of function and pain. Redness and heat result from an increase in blood flow, swelling is associated with increased vascular permeability, and pain is the consequence of activation and sensitization of primary afferent nerve fibers. The understanding of the cellular and molecular mechanisms involved in the inflammatory process has increased considerably in recent decades and this has permitted the discovery of many promising targets for the development of new drugs to treat chronic inflammatory diseases. A great number of inflammatory mediators including, prostaglandins, leukotriene, amines, purines, cytokines, chemokines and adhesion molecules, has been found to act on specific targets, leading to the local release of other mediators from

leukocytes and the further attraction of leukocytes, such as neutrophils, to the site of inflammation. The constant advent of new findings from Immunohistochemical, biochemical, molecular and functional animal models, together with clinical trials, has greatly increased the interest in the study of the mechanisms that underlie the inflammatory process.

Recently, roles have been identified for several inflammatory cells and for a large number of inflammatory mediators in important pathologies not previously known to be linked to inflammation, such as Alzheimer's disease and cardiovascular disorders including atherosclerosis, as well as cancer. Natural products have long been, over the years, contributed to the development of modern therapeutic drugs. Evidence exists that drugs derived from natural products can modulate various inflammatory mediators and action of second .In parallel, the allergic process has an important inflammatory component in which mast cell activation and degranulation are the first phenomena observed. During this process, mast cells release several inflammatory mediators including histamine, platelet aggregating factor, leukotrienes, and a variety of cytokines. Hypersensitivity type I, an allergic reaction, is an IgE mediated immune response, resulting in histamine secretion from mast cells and blood basophils. The early phase reaction of allergy occurs within minutes after allergen exposure, whereas the late phase reaction occurs hours later and involves in cytokines secretion.The discovery of drugs that can be used for the treatment of inflammatory and allergic diseases is important in human health. Drug discovery from plants involves a multidisciplinary approach combining botanical, ethnobotanical, phytochemical and biological techniques. Medicinal plants for medical care have increased significantly during the last few years. Currently, there is a renewed interest in the search for new phytochemicals that could be developed as useful anti-inflammatory and anti-allergic agents to reduce the risk of many diseases. The activation of nuclear transcription factor-kappa B has now been linked to a variety of inflammatory diseases, while data from numerous studies underline the importance of phytochemicals in inhibiting the pathway that activates this transcription factor. Moreover, the incidence of type I allergic disorders has been increasing worldwide, particularly, the hypersensitivity to food. Thus, a good number of plant products with anti-inflammatory and anti-allergic activity have been documented, but very few of these compounds have reached clinical use and there is scant scientific evidence that could explain their mode of action.

Chapter2 REVIEW OF LITERATURE

2.1 PHYTOCHEMICALS

Plants have an important role in maintaining human health and up the quality of human life for thousands of years. Phytochemicals are the chemicals compounds that are naturally presents in the plants. Phytochemicals play a crucial role against varied diseases like respiratory disorder, arthritis, cancer, peptic ulcer etc. These phytochemicals don't have any side effects, not like pharmaceutical chemicals. Phytochemicals may be thought of as "man friendly medicines" as a result of it cures diseases while not cause any damage to people. Phytochemicals are generally divided into 2 categories- primary and secondary constituents. Proteins, sugar and amino acids are primary constituents. Secondary constituents contain terpenoids and alkaloids. Plants have antifungal, bactericide and anti-inflammation activities.

2.2 PHYTOCHEMICALS ACTION MECHANISM

Phytochemicals show both anti-inflammatory and anti-allergic activities in vitro and in vivo. Several cellular action mechanisms are proposed to explain their mode of action. Any single mechanism could not explain all of their in vivo activities. They probably have multiple cellular mechanisms acting on multiple sites of cellular machinery. The continual efforts will provide new insight into the anti-inflammatory and anti-allergic activities of phytochemicals, and eventually lead to development of a new class of anti-inflammatory and anti-allergic agents. However, the concern and difficulties related to the investigation of herbal medicines have precluded the financial incentives that could be provided to pharmaceutical industries. As a function of such difficulties, few herbal drugs have been studied adequately and well-controlled double-blind clinical trials to prove their safety and efficacy have been lacking.

The trend today, especially in an industrial setting, is to seek bioactive compounds from plants that will serve as lead compounds for synthetic or semi synthetic development, and knowledge of the main pharmacologically active plant compounds is an essential requirement to standardize

procedures for obtaining herbal remedies in order to replace crude products with modern pharmacological formulations.

The ranges of phytoconstituents were responsible for anti-inflammatory activity including phenolics, alkaloids, and terpenoids. However, efforts have focused on a class of compounds to elucidate the mechanisms of action of herbs, characterize and establish their potential utility as therapeutic agents in the treatment of inflammatory diseases.

Several mechanisms of action have been proposed to explain the anti-inflammatory actions of phytoconstituents, it consist broadly in:

(1) Antioxidative and radical scavenging activities;

(2) Modulation of cellular activities of inflammation-related cells (mast cells, macrophages, lymphocytes, and neutrophils);

(3) Modulation of proinflammatory enzyme activities such as phospholipase A2 (PLA2), cyclooxygenase (COX), and lipoxygenase (LOX) and the nitric oxide (NO) producing enzyme, nitric oxide synthase (NOS);

(4) Modulation of the production of other proinflammatory molecules;

(5) Modulation of proinflammatory gene expression.

The inflammatory process can be initiated by various inflammatory stimuli including viruses, chemicals, and reactive oxygen/nitrogen species, which subsequently increases the synthesis and secretion of proinflammatory cytokines.

Moreover, the unchecked activation of NF-κB/AP-1 and the production of TNF-α signaling have provided compelling evidence about the critical role for these factors in coupling inflammation and many chronic diseases.

Phytochemicals have been shown to modulate various points in these inflammatory processes. These modulations serve as controlling points where the amplification of the inflammatory processes can be disconnected and thereby reduce subsequent diseases risk.

Table 1: List of phytochemicals in Ficus religiosa

Parts of plants	Phytochemicals
Bark	Lanosterol, Bergapten, Stigmasterol, Bergaptol, beta-sitosterol, lupen-3-one ,beta-sitosterol-d-glucoside, vitamin K1, wax
Leave	Stigmasterol, campestrol, isofucosterol, lupeol, tannic acid, serine, isolucine, aspartic acid, glycine, threonine, alanine, proline, trytophan, tyrosine, methionin, valinine, leucine, n-hetricontanen, hexacosanol, n-octacosan
Fruit	Asgragine, undecan, tetradecan, (e)-beta-ocimen, alloaromadendrene, alpha-thujene ,beta-pinene, alpha-pinene, limonene, alpha-terpinene, dendrolasin, aromadendrene, dendrolasine, alpha-yalangene, alpha-copaene, beta-bocerbonene, beta-caryophyllene, alpha-trans bergamoten, alpha-humulene, germacrene, bicyclogermacrene, gamma-cadinene, delta-cadinene
Seed	Alanine, tyrosine, threonine

2.3 HISTAMINE RECEPTOR

Histamine is one of the aminergic neurotransmitters, playing an important role in the regulation of several pathophysiological processes. In peripheral tissues histamine is stored in mast cells, basophils, and probably also in some specific neurons. Mast cell histamine plays an important role in the pathogenesis of various allergic conditions. After mast cell degranulation, release of histamine leads to various well-known symptoms of allergic conditions in the skin and the airway system. Based on these observations histamine is considered as one of the most important mediators of allergy and inflammation.The clinical expression of allergic diseases, which is mediated in part by IgE, actually depends on the actions of multiple mediators, many of which are derived from mast cells; histamine is prominent among them.

2.3.1 HISTAMINE EFFECTS

Most of the important histamine effects in allergic diseases are mediated through the H2 receptor. These include smooth muscle contraction, increased vascular permeability, pruritus, prostaglandin generation, decreased atrioventficular node conduction time, with resultant tachycardia, activation of vagal reflexes, and increased cyclic guanosine monophosphate. The H2-mediated effects include gastric acid secretion, increased lower airway mucus secretion, increased cyclic adenosine monophosphate, esophageal contraction, inhibition of basophil, but not mast cell, histamine release, inhibition of neutrophil activation, and induction of suppressor T cells.

Histamine's ability to induce smooth muscle contraction forms the basis for the histamine bioassay. Diseases that feature smooth muscle contraction include asthma and food allergy. Histamine is also the only proved mediator of pruritus; it is prominent in urticaria, eczema, and allergic rhinitis. The mechanism by which histamine mediates pruritus is indirect and involves stimulation of sensory nerve endings. Neurohormones probably partially mediate histamine-induced cutaneous vasodilation and flushing because capsaicin treatment inhibits the histamine-induced flare but not the wheal. Vasodilation is a prominent component of urticaria and anaphylaxis. Increased mucus production from both vascular and glandular sources is prominent in asthma, allergic rhinitis, and food allergy. In the nose the glandular component is indirectly

mediated by histamine through a vagal reflex; unilateral nasal histamine challenge resuits in contralateral glandular secretion that is inhibited by atropine.

Finally, histamine induces prostaglandin formation, possibly contributing to both asthma and allergic rhinitis. Although histamine is a primary mediator in allergic disease, effects known to be mediated by histamine can also be caused by other mediators derived from mast cells.

A systematic review of the pathophysiology of individual allergic diseases, including potential contributing mediators, will facilitate an assessment of the importance of histamine in the pathophysiology of allergic diseases.

2.3.2 H2 RECEPTOR

H_2 receptors are positively coupled to adenylate cyclase via G_s. It is a potent stimulant of cAMP production, which leads to activation of protein kinase A. PKA functions to phosphorylate certain proteins, affecting their activity.

FUNCTION

Histamine is a ubiquitous messenger molecule released from mast cells , and neurons. Its various actions are mediated by histamine receptors H_1, H_2, H_3 and H_4.

The histamine receptor H_2 belongs to the rhodopsin-like family of G protein-coupled receptors. It is an integral membrane protein and stimulates gastric acid secretion.

It also regulates gastrointestinal motility and intestinal secretion and is thought to be involved in regulating cell growth and differentiation.

TISSUE DISTRIBUTION

Histamine H_2 receptors are expressed in the following tissues:

Peripheral tissues –

Gastric parietal cells ,Vascular smooth muscle, Neutrophils, Mast cells, Heart, Uterus

Central nervous system tissues-
Caudate–putamen, Cerebral cortex, Hippocampal formation, Dentate nucleus of the cerebellum

2.4 ROLE OF HISTAMINE IN ALLERGIC REACTION

Histamine has been recognized as a major mediator in allergic diseases. Histamine is a primary amine synthesized from histidine in the Golgi apparatus, from where it is transported to the granule for storage in ionic association with the acidic residues of the glycosaminoglycans side chains of heparin and with proteinases. Following mast cell activation, histamine is rapidly dissociated from the granule matrix by exchange with sodium ions in the extracellular environment. Proteoglycans comprise the major supporting matrix of the mast cell granule with the sulfate groups binding to histamine, proteinases and acid hydrolases.

The allergic process is believed to consist of two phases: early and late. The early phase reaction is mainly induced by histamine released from mast cells. Histamine is a potent vasoactive agent, bronchial smooth muscle constrictor, and stimulant of nociceptive itch nerves. In addition to its known effects on glands, vessels and sensory nerves, recent data have provided further evidence of histamine's proinflammatory actions. Histamine binding specific cell receptors produces clinical allergic symptoms

Histamine, which is stored mainly in mast cells and basophils, is a prominent contributor to allergic disease. Elevations in plasma or tissue histamine levels have been noted during anaphylaxis and experimental allergic responses of the skin, nose, and airways. Of the four cardinal signs of asthma (bronchospasm, edema, inflammation, and mucus secretion), histamine is capable of mediating the first two through its H1 receptor and mucus secretion through its H2 receptor. Of the five cardinal signs of allergic rhinitis (pruritus, mucosal edema, sneezing, mucus secretion, and late-phase inflammatory reactions), histamine is capable of mediating the first three through its H1 receptor. In the nose, mucus secretion can be reflexively mediated by H1 and possibly also by H2 receptors. In the skin the cardinal features of urticaria (vasodilation, vascular permeability, and pruritus) can be mediated by stimulation of the H1 receptor. In anaphylaxis histamine H1-receptor stimulation can mediate vascular permeability, smooth muscle contraction, and tachycardia, whereas H2-receptor stimulation can mediate mucus secretion.

Stimulation of both receptors can mediate vasodilation and reduce peripheral vascular resistance. Thus although histamine is only one of many mediators of allergic disease, it plays a primary role in allergic rhinitis, urticaria, anaphylaxis, and to a lesser degree, asthma.

2.5 Pro-inflammatory effects of histamine

Although the classical effects of histamine, expressed at the organ level, are generally well documented and much emphasized in allergic disease, this is not the case for the increasing amount of evidence which suggests that histamine also directly or indirectly influences the activity of several inflammatory or immunologic cell types involved in the pathogenesis of allergic disease. Consequently, it is likely that histamine plays a much wider and critical role as a pro-inflammatory mediator in allergic disease that is considered presently. Indeed, several studies have demonstrated that histamine receptors are expressed on basophils, mast cells, neutrophils, eosinophils, lymphocytes, macrophages, epithelial cells and endothelial cells, and therefore are likely to modulate the function of these cells.

Basophils and mast cells

Basophils express predominantly H2 receptors and these are involved in the regulation of IgE-induced histamine release, as indicated by enhanced histamine release in the presence of anti-IgE and cimetidine, but not in the presence of anti-IgE and thioperamide.

Studies of partially purified human conjunctival mast cells, which are thought to be similar to human connective tissue mast cells in their response to a variety of secretagogues, have indicated the expression of H1, H2 and H3 receptors, of which the H1 -receptors appear to be most active with respect to mediator release from these cells.

Neutrophils

Neutrophils have been shown to express H1 and H2 receptors .Functional studies have suggested that the effects of histamine on neutrophils are also mostly inhibitory and mediated via H2 receptors.

Monocytes and macrophages

Some recent studies have demonstrated the presence of H1 and H2 histamine receptors on human monocytes and macrophages, suggesting that histamine may also modulate the activity of these cells in allergic disease. Furthermore, histamine induced H1 receptor signaling induces the release of pro-inflammatory compounds such as TNF-a, prostaglandin D2, and b-glucuronidase.

2.6 ROLE OF MAST CELL IN ALLERGIC REACTION

Mast cell has a long history of being recognized as an important mediator-secreting cell in allergic diseases. It has a high capacity to release an array of both preformed and newly generated mediators in response to environmental stimuli, especially allergen exposure. Cross linkage of IgE bound to high affinity receptors on mast cells not only results in the rapid release of autacoids mediators, but also the sustained synthesis and release of cytokines, chemokines and growth factors.

Mature mast cells are ubiquitous in human tissues and can thus participate in the processes of inflammation at different sites. Systemic anaphylaxis, a life-threatening disease, involves mast cell activation in multiple organs. In bronchial asthma, a disease characterized by widespread but potentially reversible bronchial obstruction, there are increased numbers of mast cells and a greater degree of continuous mast cell degranulation in bronchoalveolar lavage fluid from asthmatics compared with normal controls. Increased mast cell numbers and evidence for continuous degranulation of mast cells have been observed also in nasal lavage fluid and the nasal epithelium of the patients with allergic rhinitis.

Intestinal mast cells, as well as eosinophils, have been shown to be involved in the pathogenesis of food-allergy-related enteropathy. Although allergic drug reactions are just one type of adverse reactions to medications, they are clinically very important because of the morbidity and mortality they cause. Allergic drug reactions may result in anaphylaxis, urticaria, bronchospasm and angioedema. During these reactions, allergic drugs cause direct histamine release from mast cells.

The histochemical characteristics of human basophils and tissue mast cells were described over a century ago by Paul Ehrlich. When mast cells are activated by an allergen that binds to serum IgE attached to their FcεRI receptors, they release cytokines, eicosanoids and their secretory granules.

Mast cells are now thought to exert critical proinflammatory functions, as well as potential immunoregulatory roles, in various immune disorders through the release of mediators such as

histamine, leukotrienes, cytokines chemokines, and neutral. Mast cells interact directly with bacteria and appear to play a vital role in host defense against pathogens.

2.7 ROLE OF NATURAL KILLER (NK) CELL IN ALLERGIC REACTION

Despite high numbers of NK cells in the lung and their ability to generate a variety of immunomodulatory mediators, the potential of NK cells as therapeutic targets in allergic airway disease has been largely overlooked. The fact that IgE, acting through FcγRIII, can activate NK cells resulting in cytokine/chemokine production implies that NK cells may contribute to IgE-mediated allergic responses.

NK cells are the key to the natural defenses of the body and have been detected in virtually all species from invertebrates to mammals. As components of the innate immune response, NK cells do not require previous sensitization to instigate their action.

NK cells mediate cellular toxicity and release chemokines and cytokines involved in combating tumors. The function of NK cells is regulated by a large repertoire of inhibitory and activating receptors. It is also important that NK cells remain tolerant of healthy tissue, and as such they express receptors that can prevent cell activation. NK cells develop in the bone marrow and migrate to the blood as circulating cells, or become resident cells in tissues such as the liver, spleen, lymph nodes, uterus, and lungs. Innate immune cells in the lung have the job of defending the organ's vital function against the constant barrage of exposures to environmental antigens, commensal organisms, and potential pathogens. The role of NK cells in protecting against respiratory infection by fungi, bacteria, IFN signaling, and cytokine and chemokine secretion which bring about cytolysis of the virally infected cells. The contribution of NK cells to the defense against viral infection may also afford the cells a pivotal role in regulating the pathogenesis of asthma. Mouse models indicate NK cells may also have an important role in the allergic airway response beyond a relationship with viral infection. NK cells have been demonstrated to contribute to the initiation and development of T-cell mediated allergic airway inflammation.NK cell recruitment and accumulation at the site of immunization and generation of a panel of cytokines which are involved in the pathogenesis of allergic inflammation suggest that these cells play a critical role at several steps in the development of the acquired immune response to allergens.

Chapter 3 METHODOLOGY

3.1 H2 receptor from PDB

Histamine H2 receptor isoform X1 (Homo sapiens) sequence was taken from database of NCBI

Histamine H2 receptor sequence database in NCBI contains protein sequence and their encoding regions derived from their nucleotide sequences.

The sequences of Histamine H2 receptor with GI: 1034644802 were selected contains 422 amino acid residues.

BLAST (Basic local alignment search tool) is used for suitable template search against PDB (Protein data bank).

Histamine receptor 2VT4 from PDB was considered the basis of similarity search.

3.2 Ligand preparation

Ligand was prepared by using Ligprep.

Ligand was obtained from Pubchem database and PDB chem in structure format.

Ligprep uses OPLS (Optimized potential liquid simulation).

All the structures which were included have low 3D conformers with satisfactory bond length and angles.

For the respective ligand, all the possible protonation states and ionization states were computed by using Ionizer at PH 7.4.

Only the lowest energy conformers were kept for all ligand.

3.3 Molecular docking of modeled protein with phytochemicals using Chimera tools

Chimera is a program for visualization and analysis of molecular structures and related data including sequence alignments, density maps, trajectories, conformational ensembles, docking results. It can create high quality images and movies.

General structure analysis-

automatic identification of atom types, hydrogen addition and partial charge assignment, measurements: distances, angles, surface area, Ramachandran plot, protein contact map,structure building and bond rotation, molecular dynamics trajectory playback, ViewDock tool to facilitate interactive screening of docking results, AutoDock Vina single-ligand docking.

Presentation of images and movies-

high-resolution images, standard molecular representations (sticks, spheres, ribbons, molecular surfaces) ,pipes-and-planks for helices and strands, ellipsoids to show anisotropic B-factors, renderings of density maps and other volume data,different structures can be clipped differently and at any angle,optional raytracing with bundled POV-Ray, simple graphical interface for creating movies interactively, MD trajectory playback.

Volume data tools-

many formats of volume data maps (electron density, electrostatic potential, others) read, fitting of atomic coordinates to maps and maps to maps,density maps can be created from atomic coordinates,display of individual data planes or multiple orthogonal planes,Gaussian smoothing, Fourier transform, other filtering and normalization, measurements: surface area, surface-enclosed volume, map symmetry.

Sequence structure tools-

many sequence alignment formats read, protein BLAST search via Web service, multiple sequence alignment via Clustal Omega and MUSCLE Web services, interfaces to MODELLER for homology modeling and loop building, structure superposition with or without pre-existing sequence alignment, generation of structure-based sequence alignments from multiple superpositions, several methods for calculating conservation and displaying values on associated structures, UniProt and CDD feature annotations shown as colored boxes on sequences

Chapter 4 RESULTS AND DISCUSSION

4.1 Human Histamine H2 receptor

The human histamine H2 receptor 2VT4 from PDB (Protein data bank) was considered for docking with selected phytochemicals of *Ficus religiosa medicinal plant.*

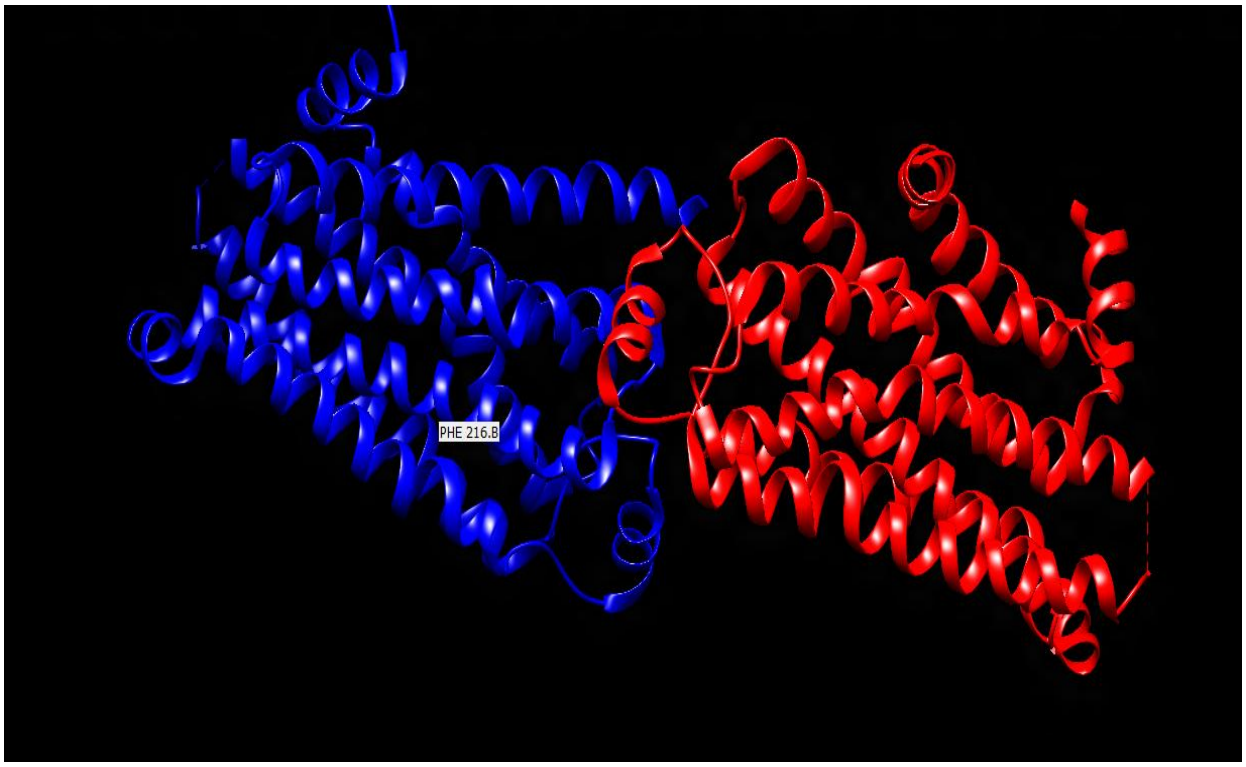


Figure 1: Three-dimensional representation of histamine H2 receptor 2VT4

4.2 Docking of phytochemicals with histamine H2 receptor

Ligands were docked at the active site of the histamine H2 receptor.

The top seven ligands germacrene, bergaptol, lanosterol, Ergost-5-en-3beta-ol, α-amyrin acetate, bergapten, and γ-cadinene in the case of docking with histamine H2 receptor were found to be −5.838, −5.472, −5.423, −5.387, −5.255, −5.109, and −5.029, respectively. These seven phytochemical shows good binding affinity with active sites residues of human histamine H2 receptor. Others parameters such as Glide energy were also used for the evaluation of the docking result.

Docking steps of phytochemical Germacrene is shown in figure-2 and the docking steps for others phytochemicals are shown in the appendix.

1. Phytochemical (Germacrene) from Pubchem

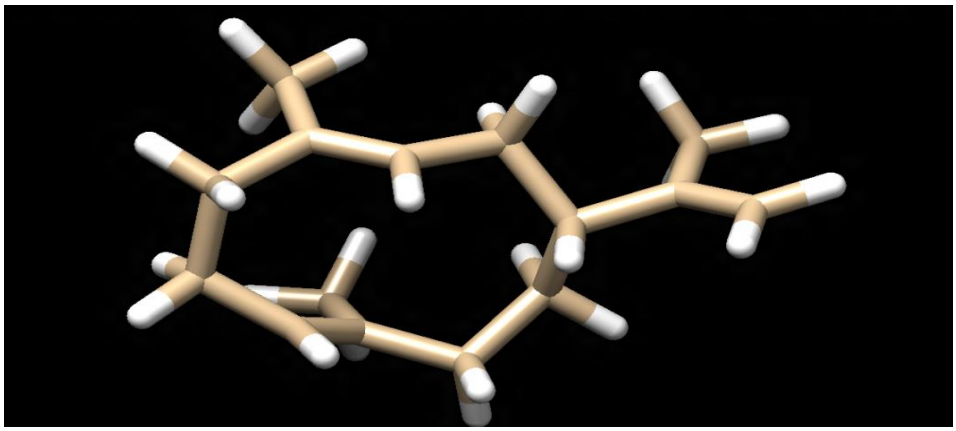


Figure 2: Phytochemical (Germacrene) from Pubchem

2. Minimize structure (addition of charges and addition of hydrogens)

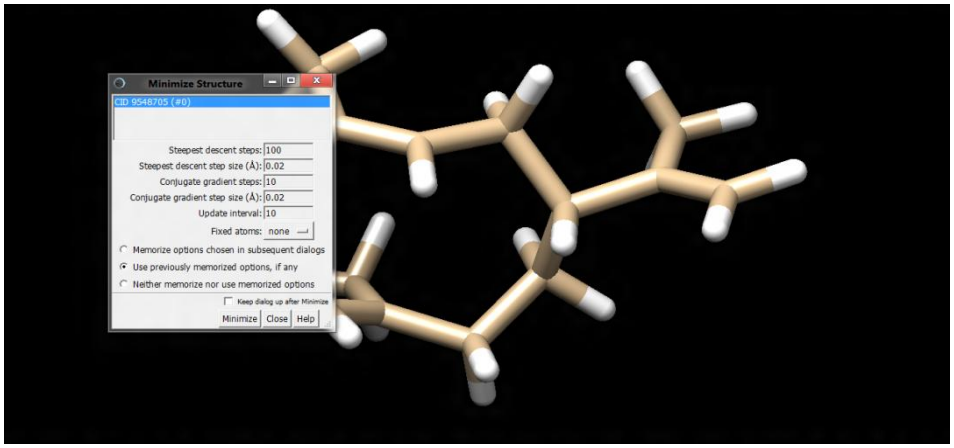


Figure 3: Minimize structure (addition of charges and addition of hydrogens)

3. Histamine H2 receptor from PDB (2VT4)

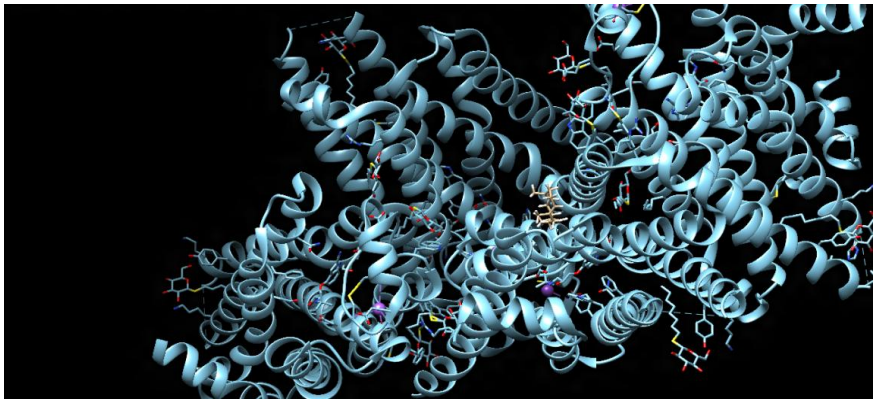


Figure 4: Histamine H2 receptor from PDB (2VT4)

4. DOCK prep.

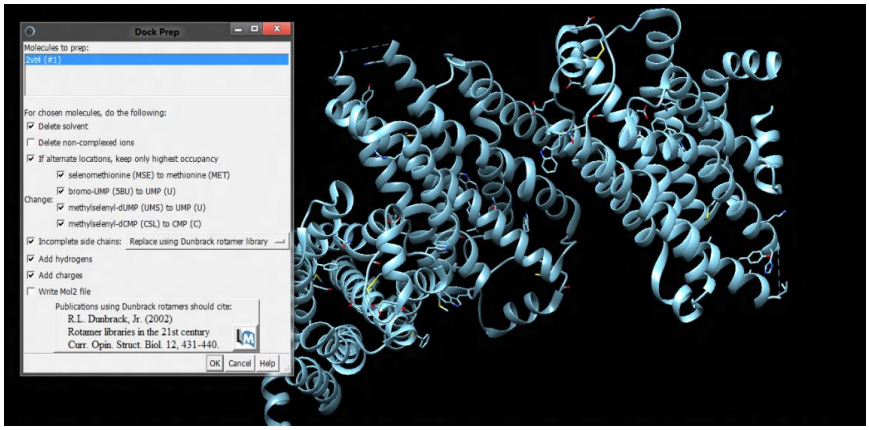


Figure 5: DOCK prep

5. Auto dock vina (Box shows interaction)

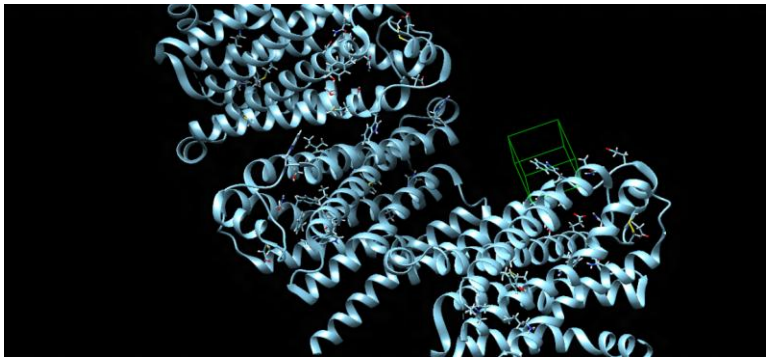


Figure 6: Auto dock vina (Box shows interaction)

Figure: 2-6 Shows docking steps of phytochemical Germacrene with H2 receptor

Table 2: Phytochemicals with docking score

Phytochemicals	Entry ID	Docking score	Glide energy
Germacrene	9548705	-5.836	-23.946
Bergaptol	5280371	-5.469	-25.177
Lanosterol	2469831	-5.419	-28.663
Ergost-5-en-3-beta-ol	5283637	-5.385	-33.347
Alpha-amyrin acetate	92842	-5.251	-26.889
Bergapten	2355	-5.107	-30.988
Gamma-cadinene	6432404	-5.025	-28.946
Delta-cadinene	441005	-4.989	-21.842
Beta-sitosterol	441005	-4.954	-19.788
Lupeol	222284	-4.886	-23.963
Aromadendrene	259846	-4.864	-17.561
Alpha-humulene	11095734	-4.863	-19.455
Dendrolasin	5281520	-4.863	-20.514
Alpha-amyrin	5316534	-4.837	-16.371
Bicyclogermacrene	73145	-4.773	-23.133
Alloramadendrene	5315347	-4.773	-14.183
Beta-bourbonene	95315347	-4.750	-22.216
Alpha-thujene	10899740	-4.713	-18.327
Beta-caryophyllene	62566	-4.669	-21.162
Isofucosterol	17868	-4.616	-31.706
Bergamotene	5281515	-4.558	-23.571
Limonene	5281326	-4.537	-21.115
Beta-pinene	6429302	-4.366	-19.698
Alpha-pinene	440917	-4.322	-17.659
Lupeol acetate	14896	-4.247	-15.468
Stigmasterol	82227	-4.081	-15.328
Beta-ocimen	92157	-2.796	-22.352
n-octacosan	5280794	-2.693	-12.587
n-hentricontanen	5281533	-1.956	-14.96
Tetradecane	12408	-1.956	-20.714
Tridecane	12410	-1.232	-10.166
Undecane	76737	-1.214	-13.923

Table 3: Phytochemicals which shows most stable complex with human histamine H2 receptor

Phytochemicals	Entry ID	Docking score	Glide energy
Germacrene	9548705	-5.836	-23.946
Bergaptol	5280371	-5.469	-25.177
Lanosterol	2469831	-5.419	-28.663
Ergost-5-en-3-beta-ol	5283637	-5.385	-33.347
Alpha-amyrin acetate	92842	-5.251	-26.889
Bergapten	2355	-5.107	-30.988
Gamma-cadinene	6432404	-5.025	-28.946

Table 4: Role of phytochemicals which shows maximum binding with Human histamine H2 receptor

Phytochemicals	Effects
Germacrene	-Anti-oxidant and anti-inflammatory activities by acting on the following target molecules : nitrite, TNF-α, IL-1β
Bergaptol	-Inhibition of chemical mediator release and - cytokine production by mast, basophil, Inhibited the release of histamine
Lanosterol	-Anti-inflammatory effects in experimental allergic asthma, -Inhibited itching in atopic dermatitis by preventing the skin from drying
Ergost-5-en-3-beta-ol	-Inhibition of transport ATPase in histamine secretion -Decreased the histamine release (mast cell degranulation and superoxide dismutase activity, and increased catalase activity in tissues
Alpha-amyrin acetate	-Effective on patients with rheumatoid arthritis
Bergapten	-Inhibited the release of histamine, leukotrienes and prostaglandin D2 Inhibited IgE-mediated TNF-α and IL-6 production -Psoriasis, skin cancer
Gamma-cadinene	-Anti-microbial and anti-bacterial activity

Chapter 5 CONCLUSION

Molecular docking was performed with selected phytochemicals from *F. religiosa*. Docking was performed with thirty phytochemicals of *F. religiosa.* The analysis suggests that lanosterol, α-amyrin acetate, Ergost-5-en-3beta-ol, germacrene, bergaptol, bergapten, gamma-cadinene form the most stable complex with human histamine H2 receptor.

The binding scores of germacrene, bergaptol, lanosterol, Ergost-5-en-3beta-ol, α-amyrin acetate, bergapten, gamma-cadinene are-5.836,-5.469,-5.419,-5.385,-5.251,-5.107,-5.025 respectively shows good binding affinity with the active site residues of human histamine H2 receptor.

Germacrene shows anti-oxidant and anti-inflammatory activities by acting on the following target molecules-nitrite, TNF-α, IL-1β.

Bergaptol shows Inhibition of chemical mediator release and cytokine production by mast, basophil.

Lanosterol shows anti-inflammatory effects in experimental allergic asthma, Inhibited itching in atopic dermatitis by preventing the skin from drying, Inhibited the release of histamine.

Ergost-5-en-3beta-ol shoes Inhibition of transport ATPase in histamine secretion, Decreased the histamine release (mast cell degranulation and superoxide dismutase activity, and increased catalase activity in tissues.

Alpha (α)-amyrin acetate is effective on patients with rheumatoid arthritis.

Bergapten inhibits the release of histamine, leukotrienes and prostaglandin D2 Inhibited IgE-mediated TNF-α and IL-6 production, Psoriasis, skin cancer.

Gamma-cadinene have anti-microbial and anti-bacterial activity. These compounds may be a suitable therapeutic agent against histamine H2 receptor as these compounds shows better binding with human histamine H2 receptor and have anti-allergic and anti-inflammatory effect. This study facilitates initiation of the herbal drug discovery process for inflammatory and allergic diseases.

Chapter 6 REFERENCES

[1]. Brahmachari HD, Augusti KT. Orally effective hypoglycemic agents from plants. J Pharm Pharmacol. 1962; 14:254–5. [PubMed]

[2]. Kirtikar KR, Basu BD. Indian Medicinal Plants. 2nd ed. III. New Delhi, India: Periodical Experts Book Agency; 1993. pp. 2317–9.

[3]. Khanom F, Kayahara H, Tadasa K. Superoxide-scavenging and prolyl endopeptidase inhibitory activities of Bangladeshi indigenous medicinal plants. Biosci Biotechnol Biochem. 2000;64:837–40. [PubMed]

[4]. Kotoky J, Das PN. Medicinal plants used for liver diseases in some parts of Kamrup district of Assam, a North Eastern State of India. Fitoterapia. 2008;79:384–7. [PubMed]

[5]. Mahishi P, Srinivasa BH, Shivanna MB. Medicinal plant wealth of local communities in some villages in Shimoga District of Karnataka, India. J Ethnopharmacol. 2005;98:307–12. [PubMed]

[6]. Rout SD, Panda T, Mishra N. Ethno-medicinal plants used to cure different diseases by tribals of Mayurbhanj district of North Orissa. Stud Ethno Med. 2009;3:27–2.

[7]. Williamson EM, Hooper PM. Major Herbs of Ayurveda. London: Churchill Livingstone; 2002. pp. 145–9.

[8]. Poonam K, Singh GS. Ethnobotanical study of medicinal plants used by the Taungya community in Terai Arc Landscape, India. J Ethnopharmacol. 2009;123:167–76. [PubMed]

[9]. Lansky EP, Paavilainen HM, Pawlus AD, Newman RA. *Ficus* spp. (fig): Ethnobotany and potential as anticancer and anti-inflammatory agents. J Ethnopharmacol. 2008;119:195–213. [PubMed]

[10]. Kirana H, Agrawal SS, Srinivasan BP. Aqueous extract of *Ficus religiosa* linn. reduces oxidative stress in experimentally induced type 2 diabetic rats. Indian J Exp Biol. 2009;47:822–6. [PubMed]

[11]. Balachandran P, Govindarajan R. Cancer – an ayurvedic perspective. Pharmacol Res. 2005;51:19–30.[PubMed]

[12]. Warrier PK, Nambiar VP, Ramankutty C. Indian Medicinal Plants: A Compendium of 500 Species. III. Chennai: Orient Longman Pvt. Ltd; 1995. pp. 38–42.

[13]. Jain A, Katewa SS, Chaudhary BL, Galav P. Folk herbal medicines used in birth control and sexual diseases by tribals of southern Rajasthan, India. J Ethnopharmacol. 2004;90:171–7. [PubMed]

[14]. Dale MM, Rang HP, Dale MM. Rang and Dale's Pharmacology. Edinburgh: Churchill Livingstone; 2007.

[15]. Tripathi KD. Essentials of Medical Pharmacology. New Delhi: Jaypee Brothers, JP Medical Ltd; 2013.

[16]. Hill SJ, Ganellin CR, Timmerman H, Schwartz JC, Shankley NP, Young JM, et al. International union of pharmacology. XIII. Classification of histamine receptors. Pharmacol Rev. 1997;49:253–78. [PubMed]

[17]. Code CF. Ciba Foundation Symposium-Histamine. Chichester, UK: John Wiley and Sons, Ltd; 1956. Histamine and gastric secretion; pp. 189–9.

[18]. Cooper DG, Young RC, Durant GJ, Ganellin CR. Histamine receptors. Compr Med Chem. 1990;3:323–1.

[19]. Chandrasekar SB, Bhanumathy M, Pawar AT, Somasundaram T. Phytopharmacology of *Ficus religiosa*. Pharmacogn Rev. 2010;4:195–9. [PMC free article] [PubMed]

[20]. Rajiv P, Sivaraj R. Screening for phytochemicals and antimicrobial activit of aqueous extract of *Ficus religiosa* Linn. Int J Pharm Pharm Sci. 2012;4:207–9.

[21]. Ambika SH, Rao MR. Studies on a phytosteroin from the bark of *Ficus religiosa*. Indian J Pharm. 1967;29:91–4.

[22]. Swami KD, Bisht NP. Constituents of *Ficus religiosa* and *Ficus infectoria* and their biological activity. J Indian Chem Soc. 1996;73:631.

[23]. Behari M, Rani KU, Matsumoto T, Shimizu N. Isolation of active-principles from the leaves of *Ficus religiosa*. Curr Agric. 1984;8:73–6.

[24]. Verma RS, Bhatia KS. Chromatographic study of amino acids of the leaf protein concentrates of *Ficus religiosa* Linn and *Mimusops elengi* Linn. Indian J Hosp Pharm. 1986;23:231–2.

[25]. Ali M, Qadry JS. Amino acid composition of fruits and seeds of medicinal plants. J Indian Chem Soc. 1987;64:230–1.

[26]. Mali S, Borges RM. Phenolics, fibre, alkaloids, saponins, and cyanogenic glycosides in a seasonal cloud forest in India. Biochem Syst Ecol. 2003;31:1221–6.

[27]. Saha S, Goswami G. Study of antiulcer activity of *Ficus religiosa* L. on experimentally induced gastric ulcers in rats. Asian Pac J Trop Med. 2010;3:791–3.

[28]. Bansal VK, Goyal SK, Goswami DS, Singla S, Rahar S, Kumar S. Herbal approach to peptic ulcer disease: Review. J Biosci Tech. 2009;1:52–8.

[29]. Feldman M, Burton ME. Histamin. E2-receptor antagonists. Standard therapy for acid-peptic diseases 1. N Engl J Med. 1990;323:1672–80. [PubMed]

[30]. Parsons ME, Ganellin CR. Histamine and its receptors. Br J Pharmacol. 2006;147(Suppl 1):S127–35.[PMC free article] [PubMed]

[31]. Hirschowitz BI. H-2 histamine receptors. Annu Rev Pharmacol Toxicol. 1979;19:203–4. [PubMed]

[32]. Webb B, Sali A. Comparative Protein Structure Modeling Using MODELLER. Curr Protoc Bioinformatics. 2014;47(5.6):5.6.1–5.6.32. [PubMed]

[33]. Schmutz J, Martin J, Terry A, Couronne O, Grimwood J, Lowry S, et al. The DNA sequence and comparative analysis of human chromosome 5. Nature. 2004;431:268–274. [PubMed]

[34]. Berman HM, Westbrook J, Feng Z, Gilliland G, Bhat TN, Weissig H, et al. The Protein Data Bank. Nucleic Acids Research. 2000;28:235–42. [PMC free article] [PubMed]

[35]. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool. J Mol Biol. 1990;215:403–10. [PubMed]

[36]. Laskowski RA, MacArthur MW, Moss DS, Thornton JM. PROCHECK: A program to check the stereochemical quality of protein structures. J Appl Crystallogr. 1993;26:283–1.

[37]. LigPrep. Ver. 2.3. New York: Schrödinger, LLC; 2009.

[38]. Bolton EE, Wang Y, Thiessen PA, Bryant SH. PubChem: Integrated platform of small molecules and biological activities. Ann Rep Comput Chem. 2008;4:217–1.

[39]. Sastry GM, Adzhigirey M, Day T, Annabhimoju R, Sherman W. Protein and ligand preparation: Parameters, protocols, and influence on virtual screening enrichments. J Comput Aided Mol Des. 2013;27:221–34. [PubMed]

[40]. Dinesh KB, Vignesh KP, Bhuvaneshwaran SP, Mitra A. Advanced drug designing softwares and their applications in medical research. Int J Pharm Pharm Sci. 2010;2:16–8.

[41]. Halgren T. New method for fast and accurate binding-site identification and analysis. Chem Biol Drug Des. 2007;69:146–8. [PubMed]

[42]. Friesner RA, Banks JL, Murphy RB, Halgren TA, Klicic JJ, Mainz DT, et al. Glide: A new approach for rapid, accurate docking and scoring 1. Method and assessment of docking accuracy. J Med Chem. 2004;47:1739–49. [PubMed]

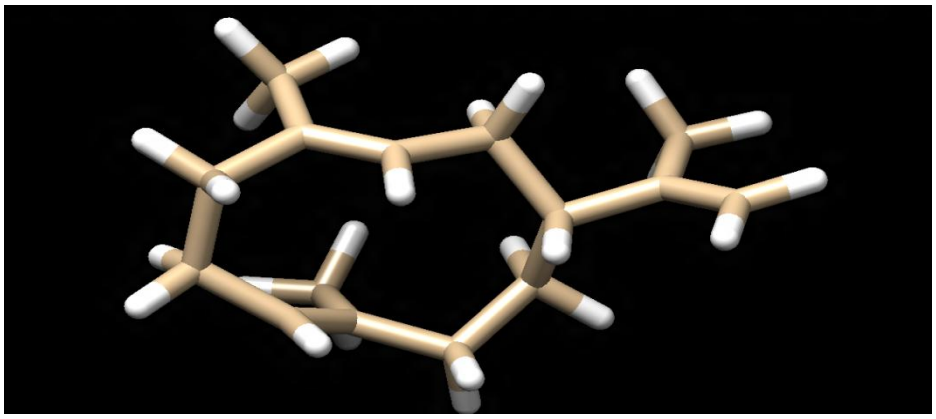
[43]. Schrödinger Release. 2015-2: LigPrep version 2.3. New York, NY: Schrödinger, LLC; 2015.

Chapter 7 APPENDIX

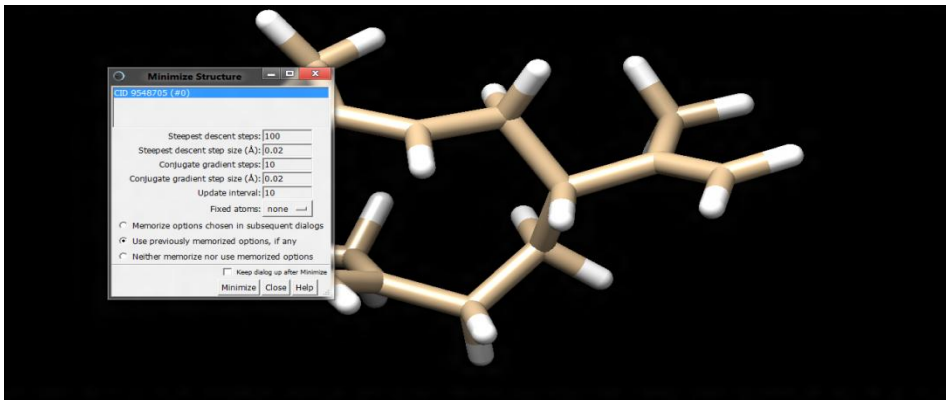
Figures (shows docking steps of selected phytochemicals with H2 receptor)

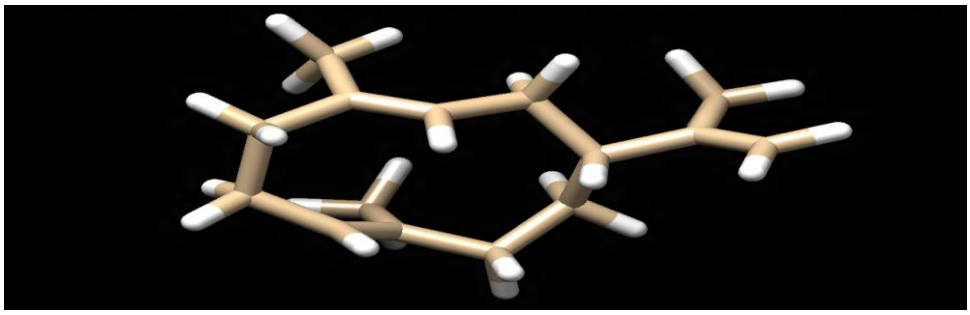
1. Germacrene

1. Phytochemical from Pubchem

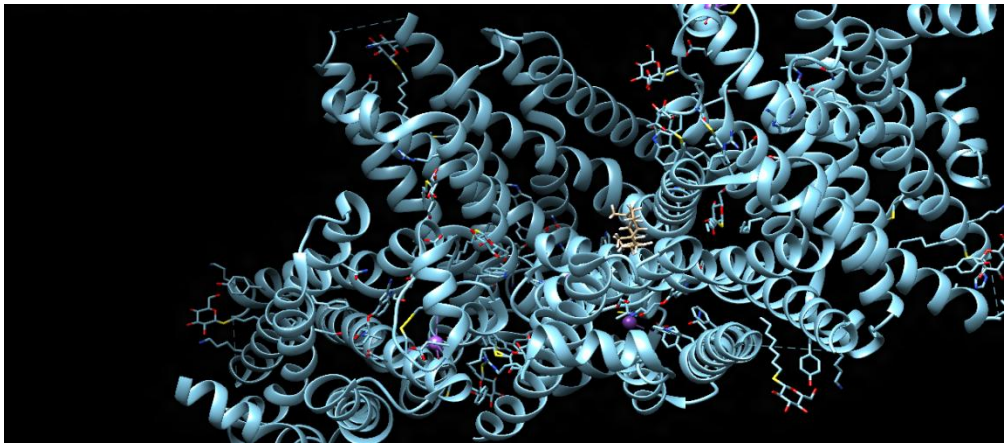


2. Minimize structure

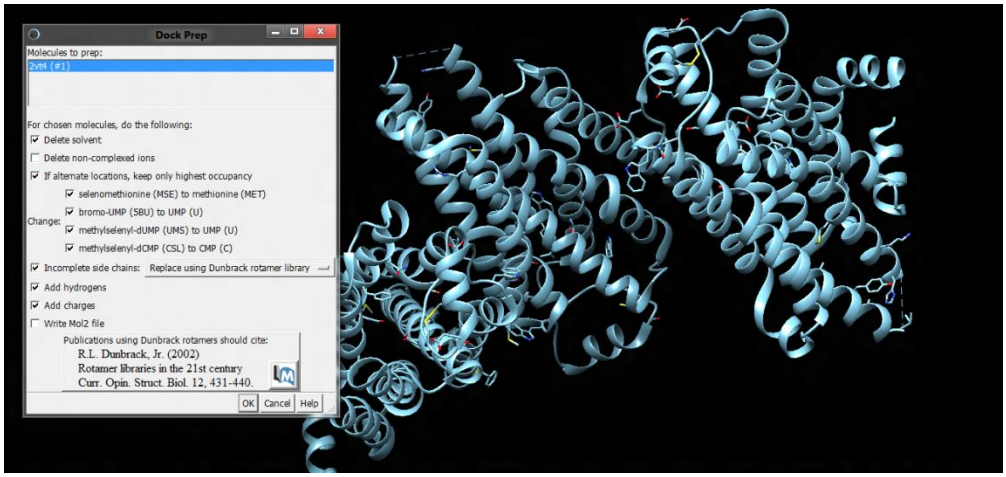




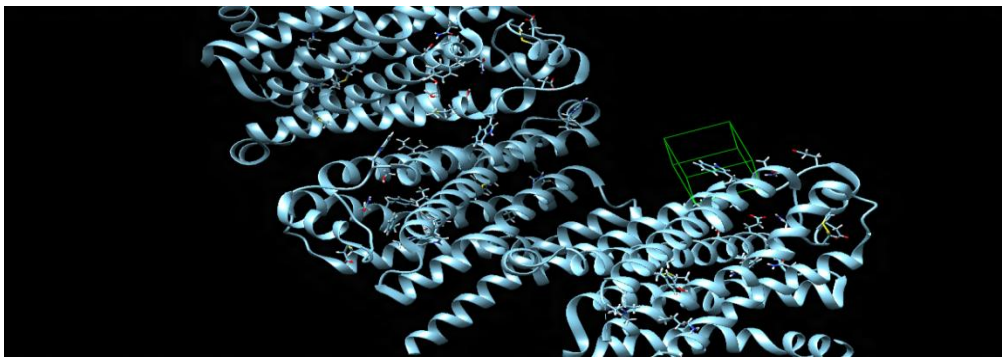
3. Histamine H2 receptor from PDB



4. DOCK prep.

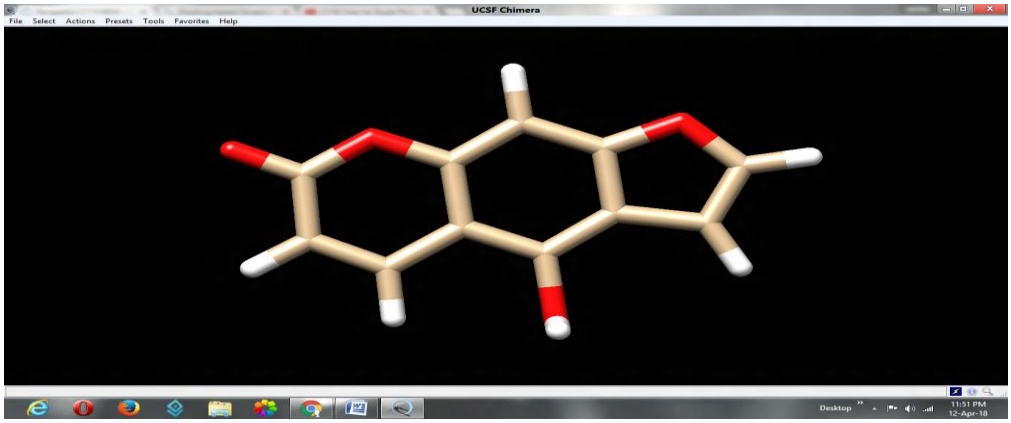


5. Auto dock vina (Box shows interaction)

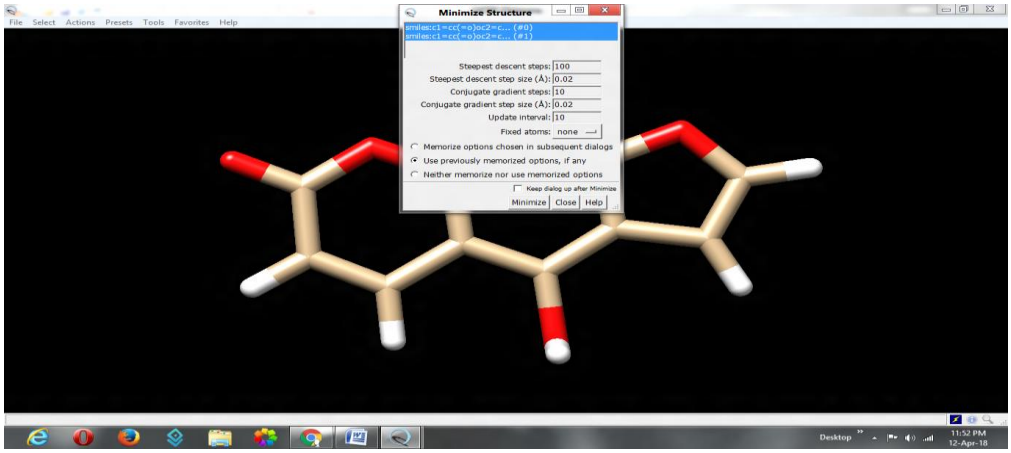


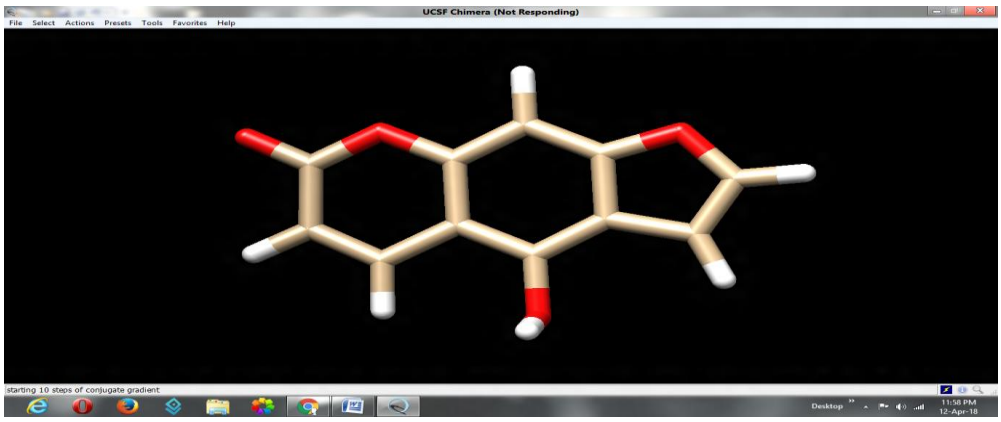
2. BERGAPTOL

1. Phytochemical from Pubchem



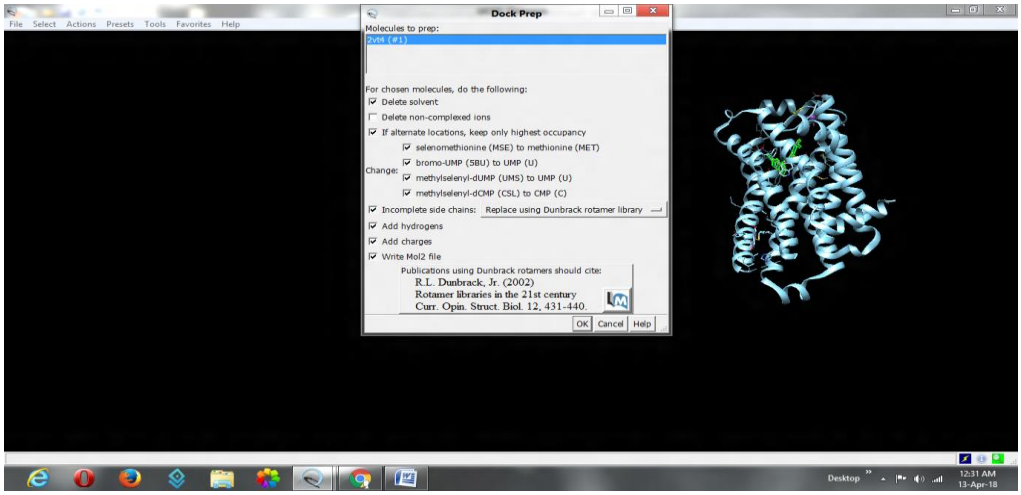
2. Minimize structure



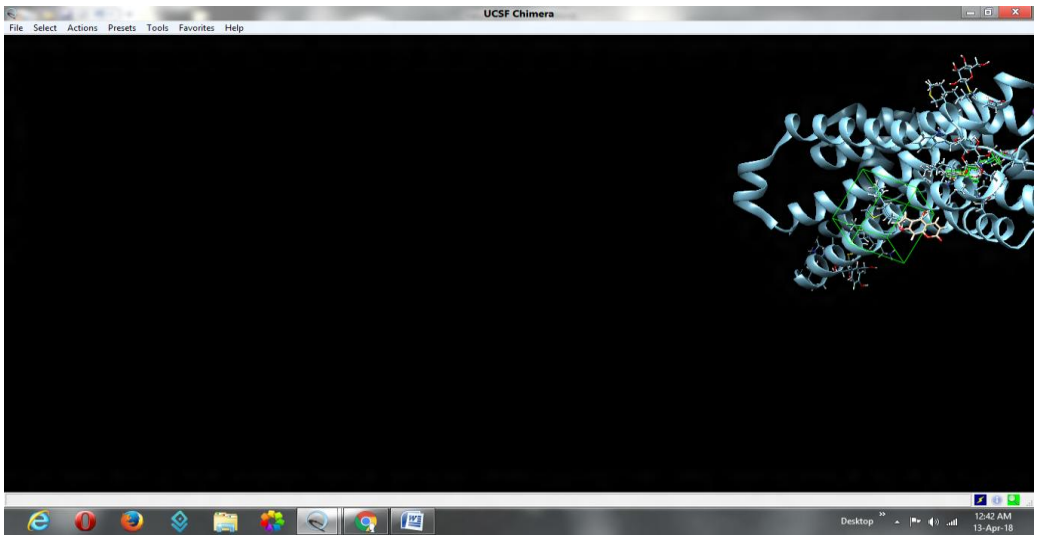


4. Open human histamine H2 receptor from PDB.

5. Dock prep

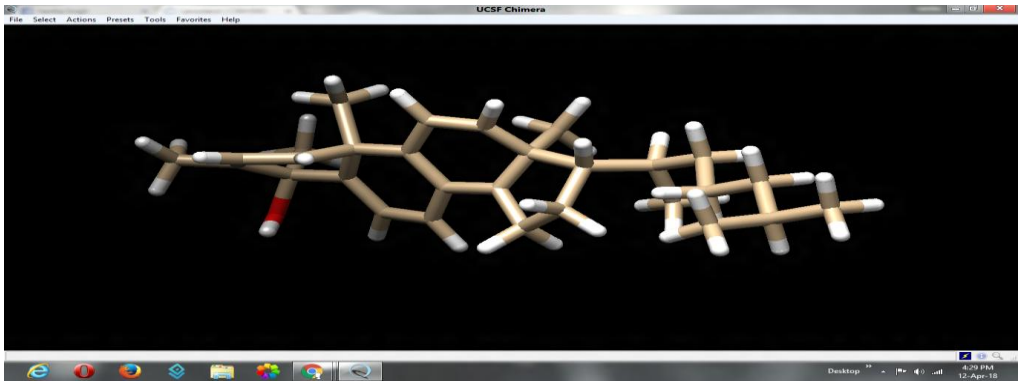


6. Auto dock vina

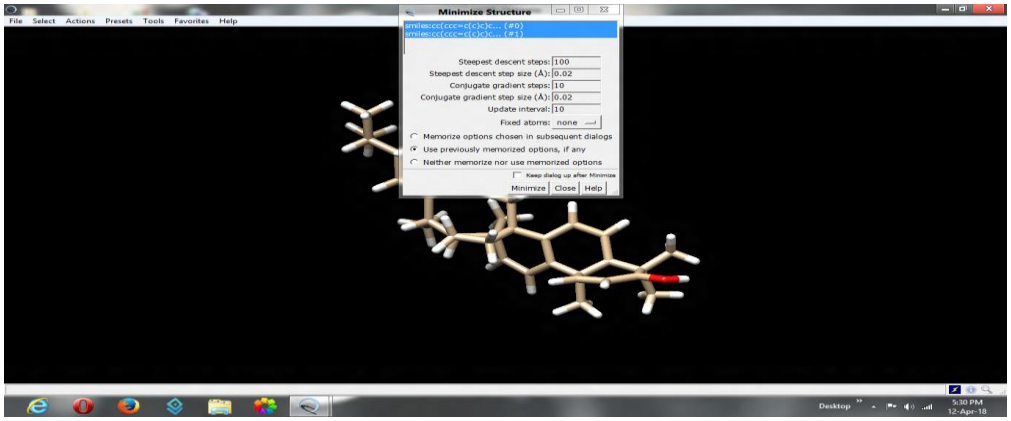


3. LANOSTEROL

1. Phytochemical from Pubchem.

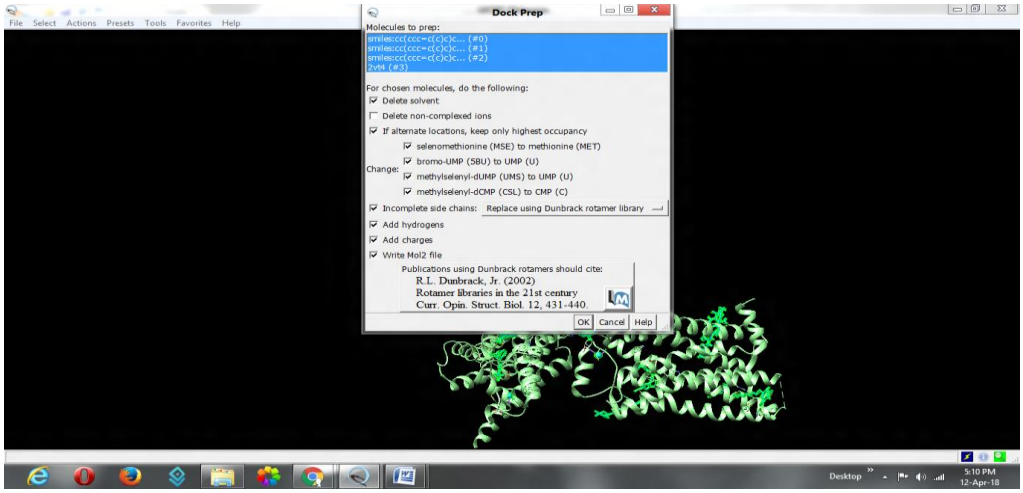


2. Minimize structure.

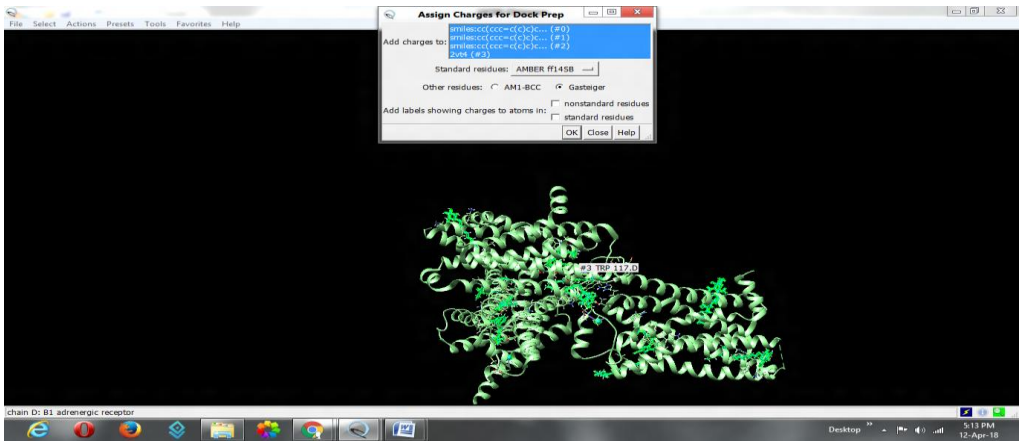


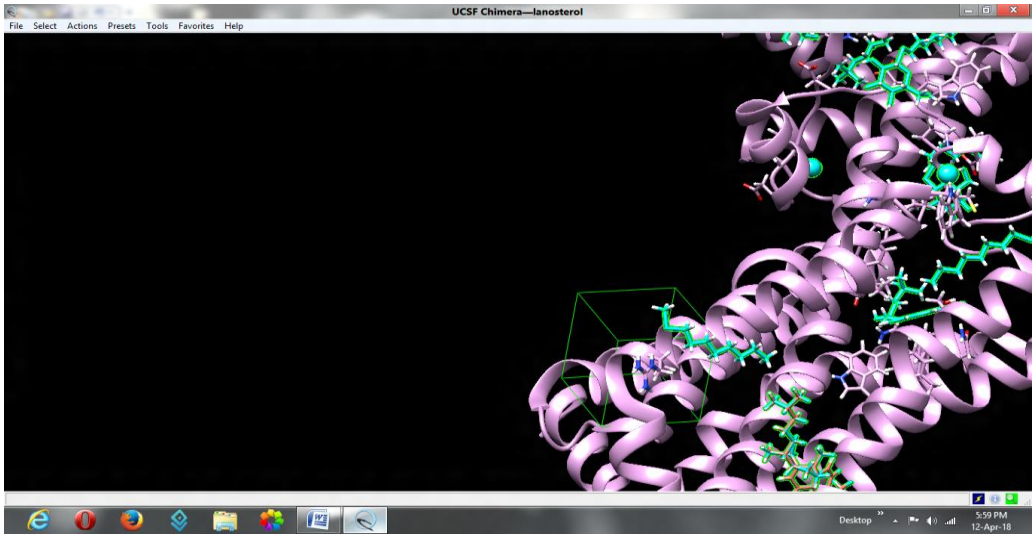
3. Open H2 receptor from PDB.

4. Dock prep



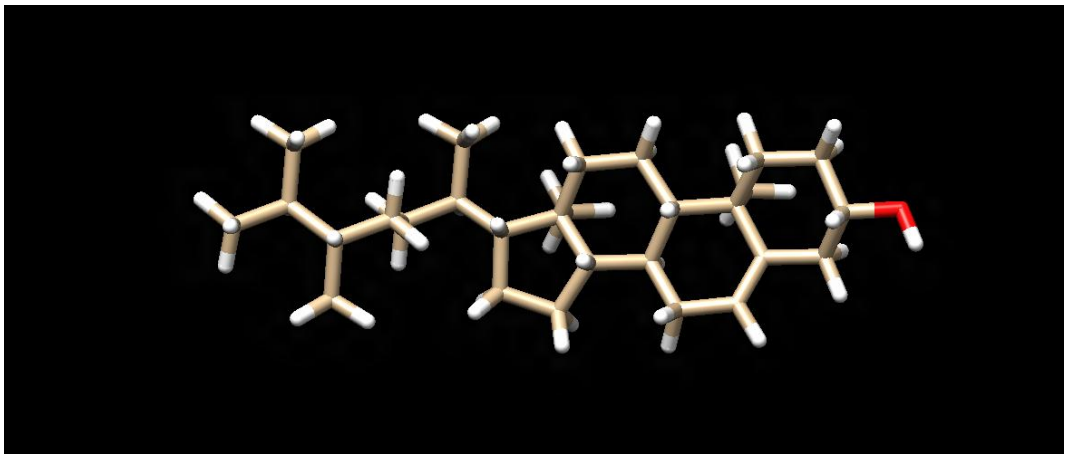
5. Autodock vina



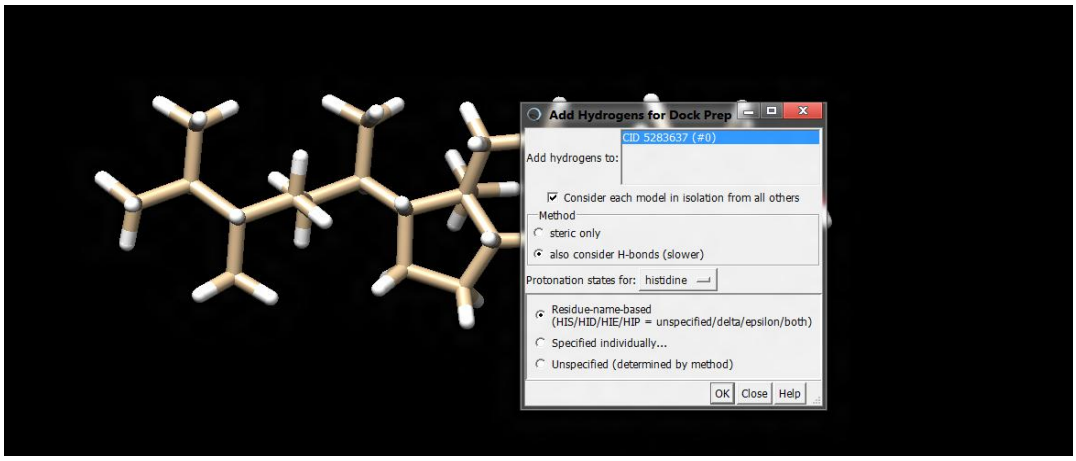


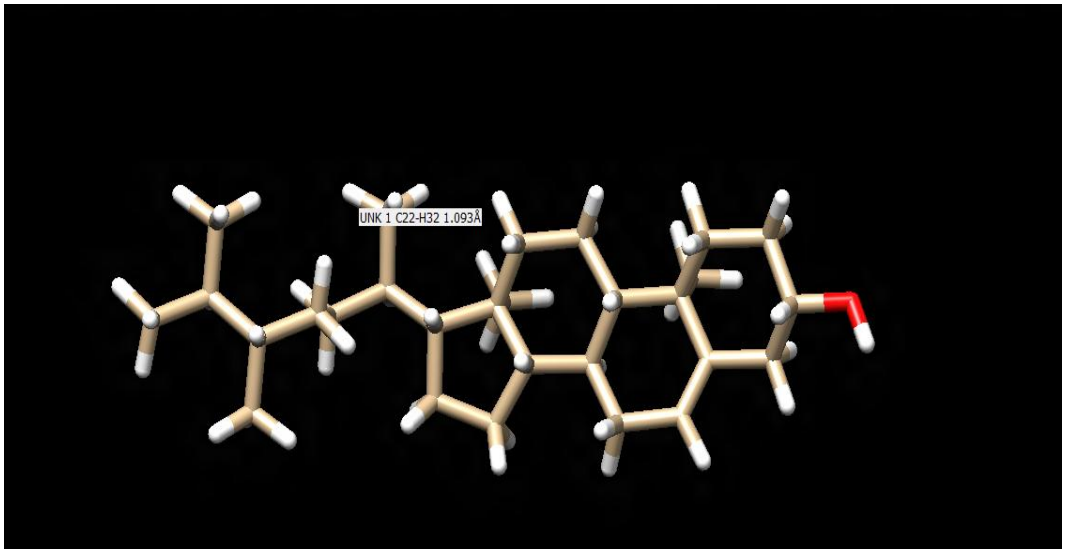
4. ERGOST-5-EN-3-BETA-OL

1. phytochemical from Pubchem.

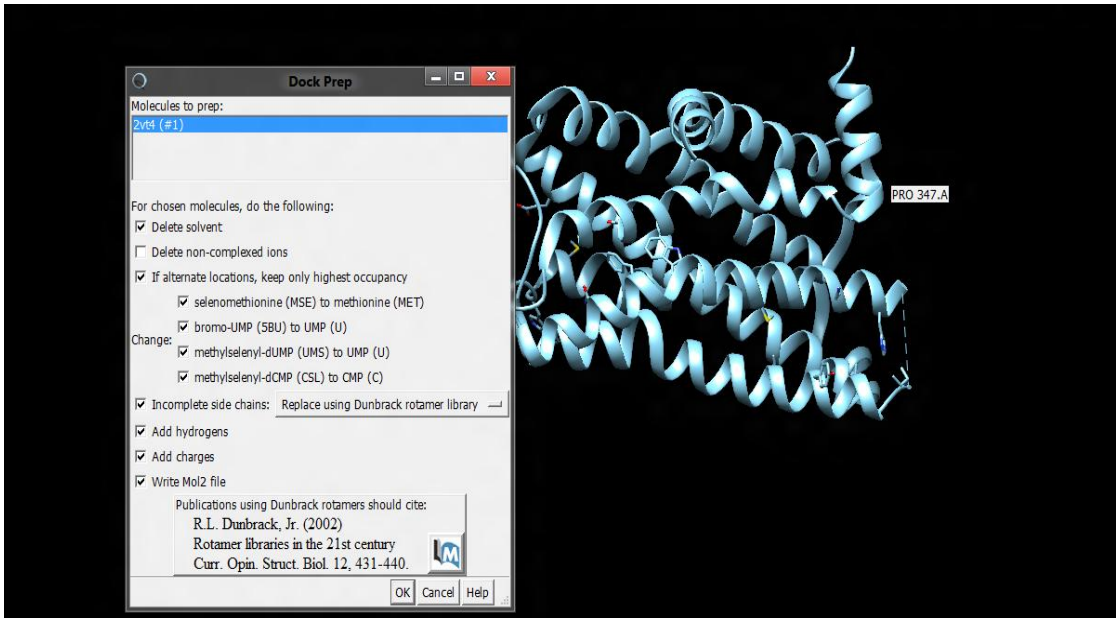


2. Minimize structure

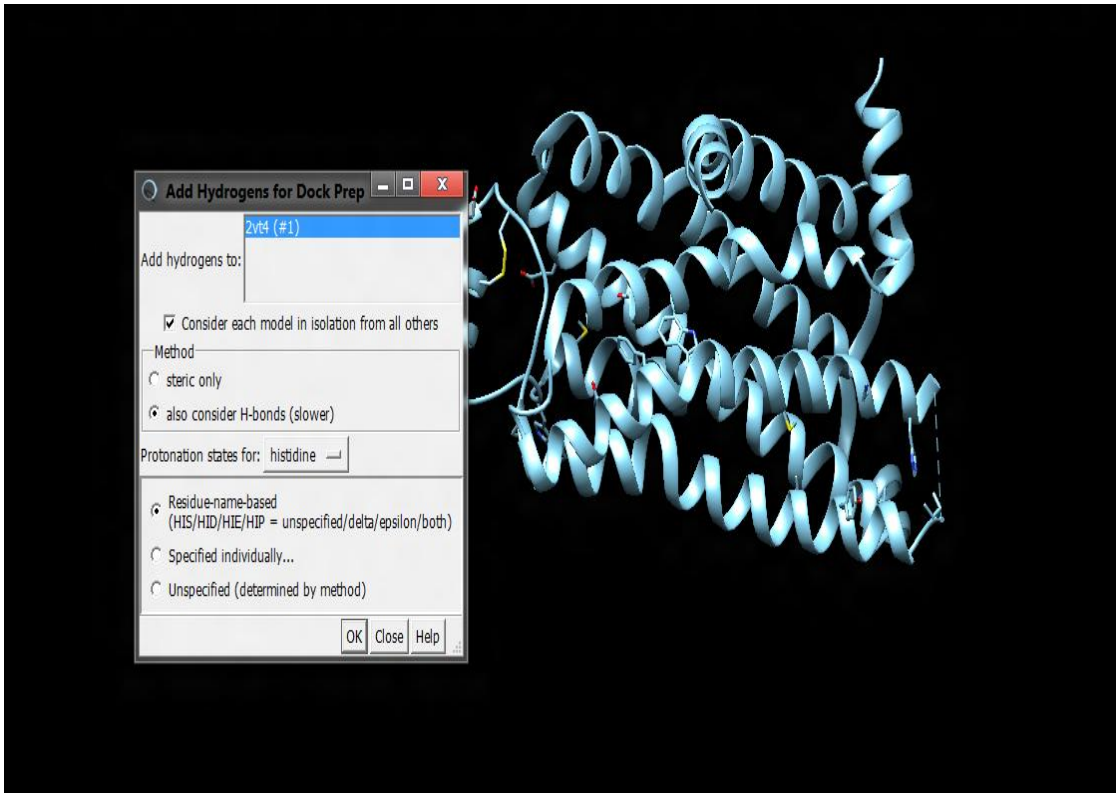




3. DOCK PREP

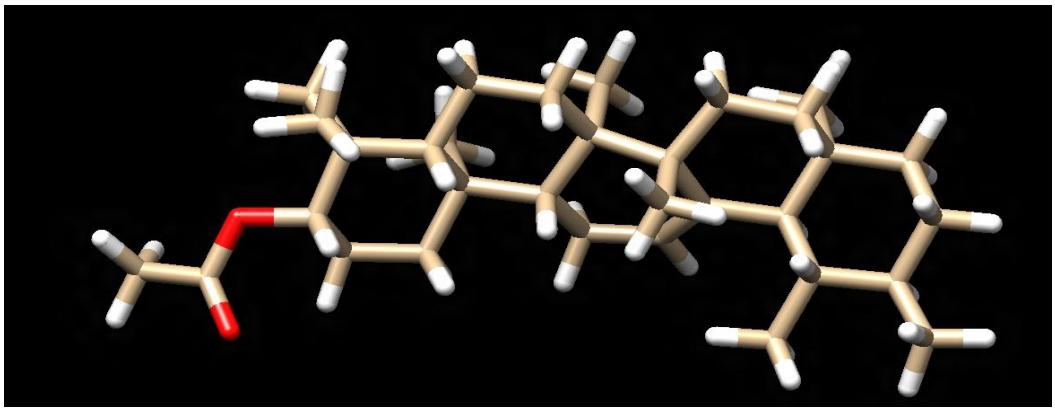


4. Autodock vina

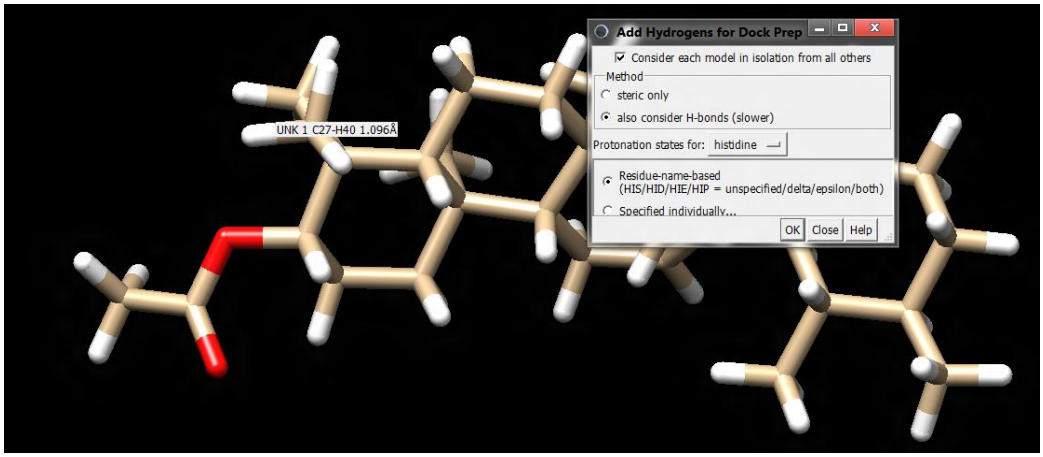


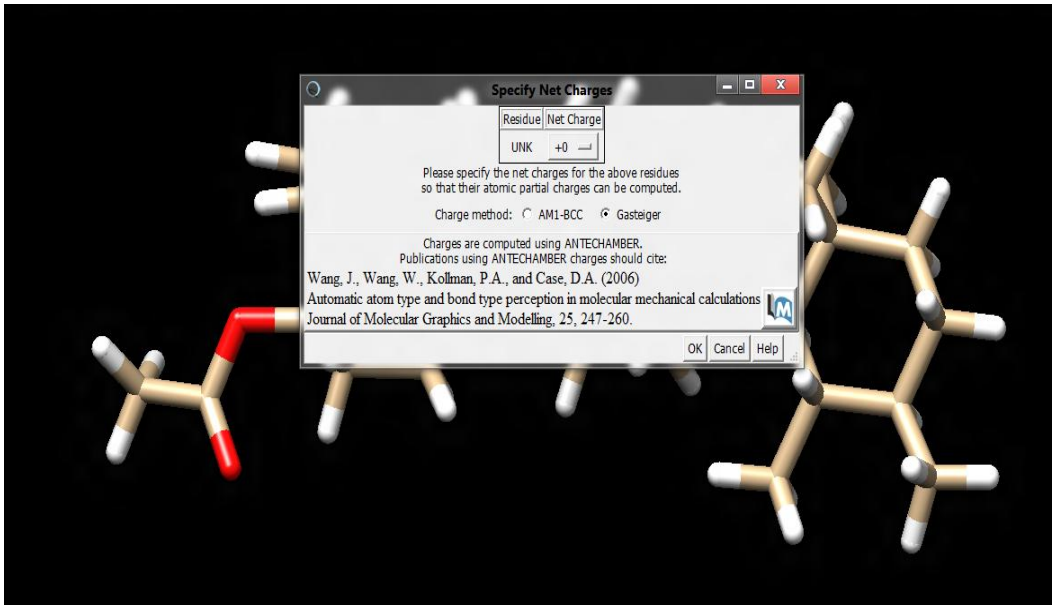
5. ALPHA-AMYRIN ACETATE

1. Phytochemical from Pubchem

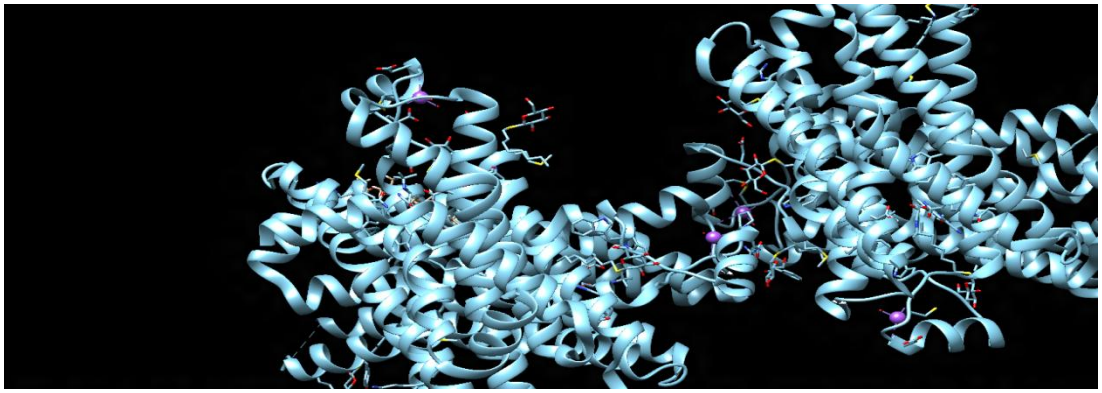


2. Minimize structure

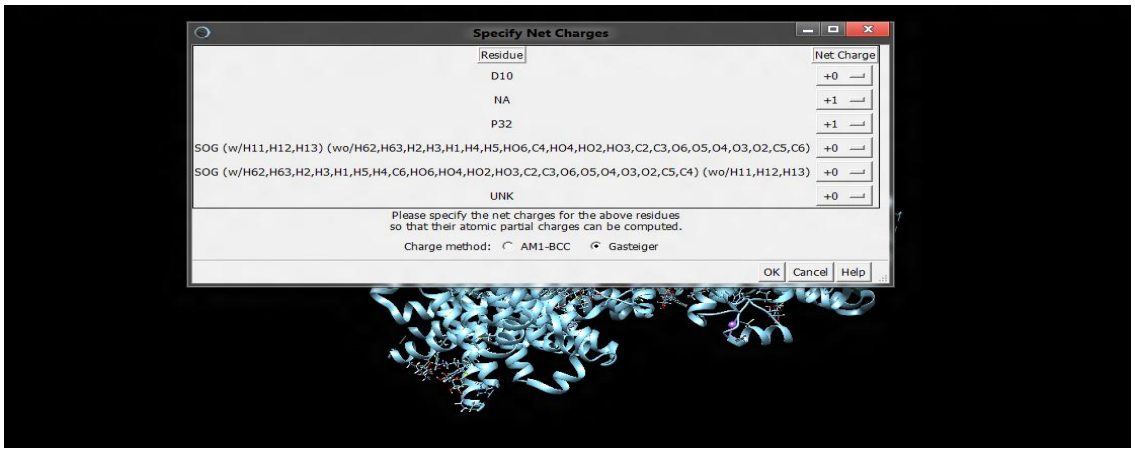




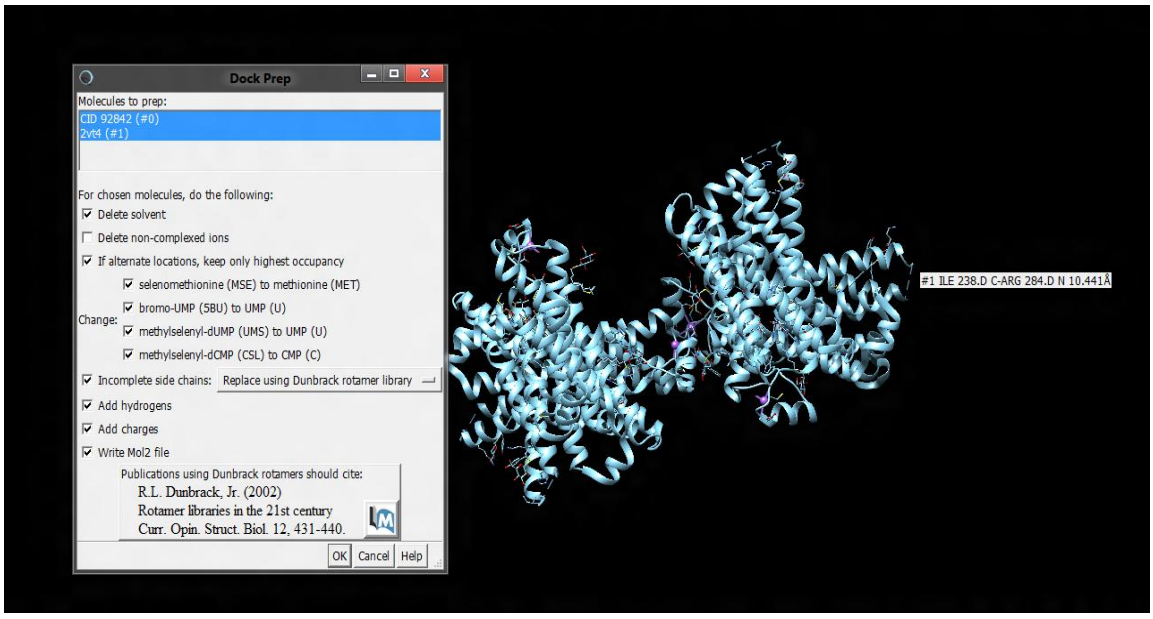
3. Open histamine H2 receptor



4. DOCK prep

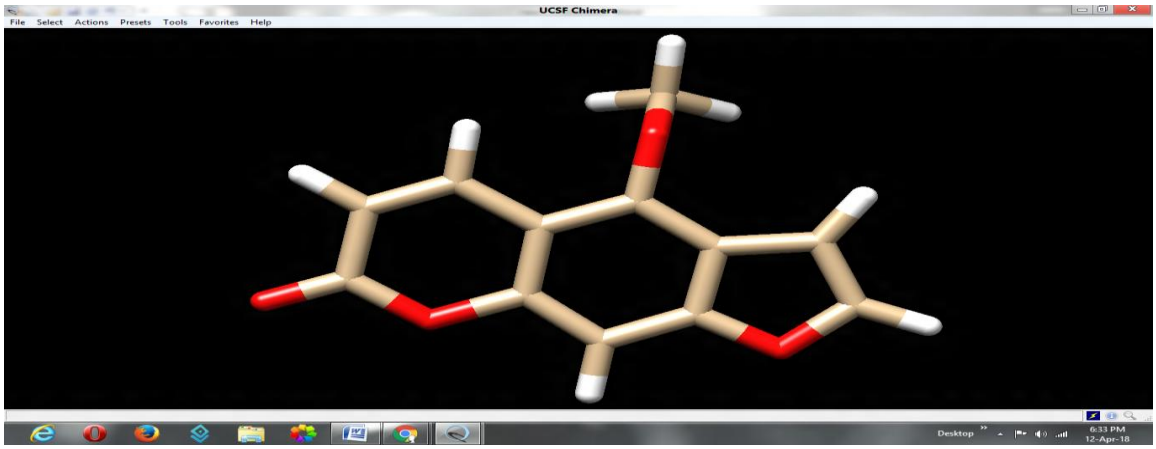


5. Auto dock

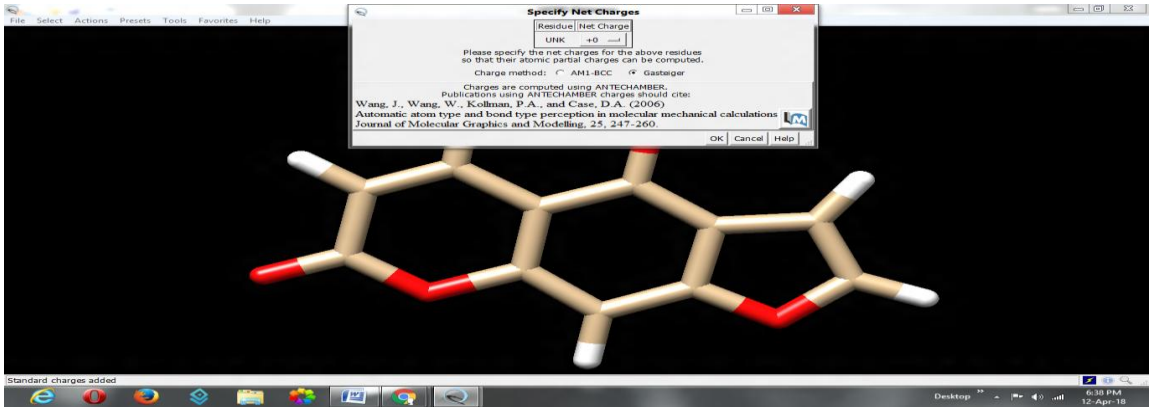


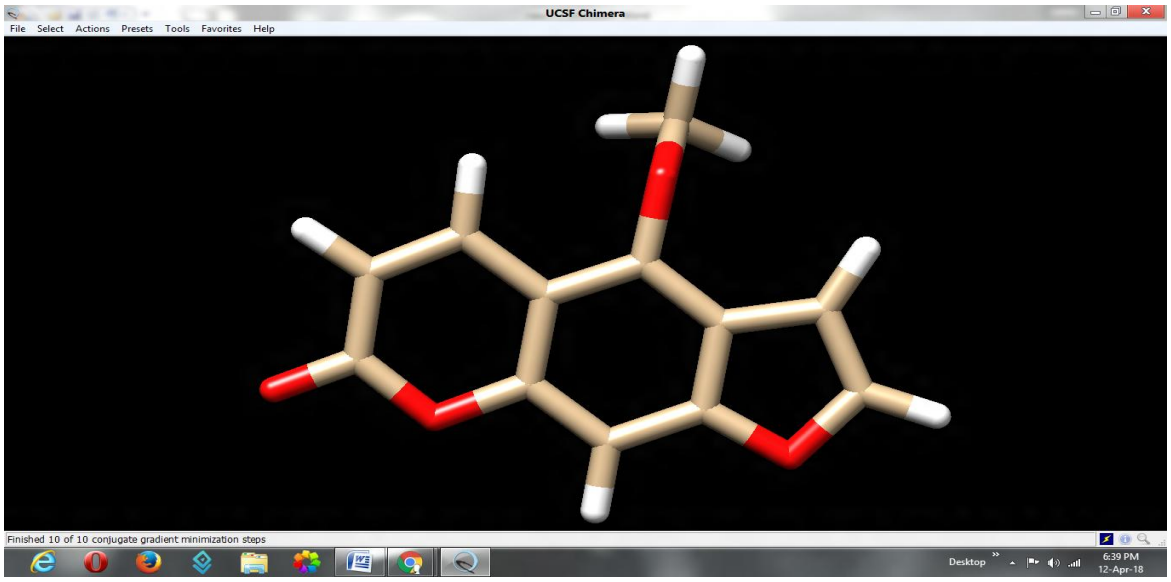
6. BERGAPTEN

1. Phytochemical from Pubchem

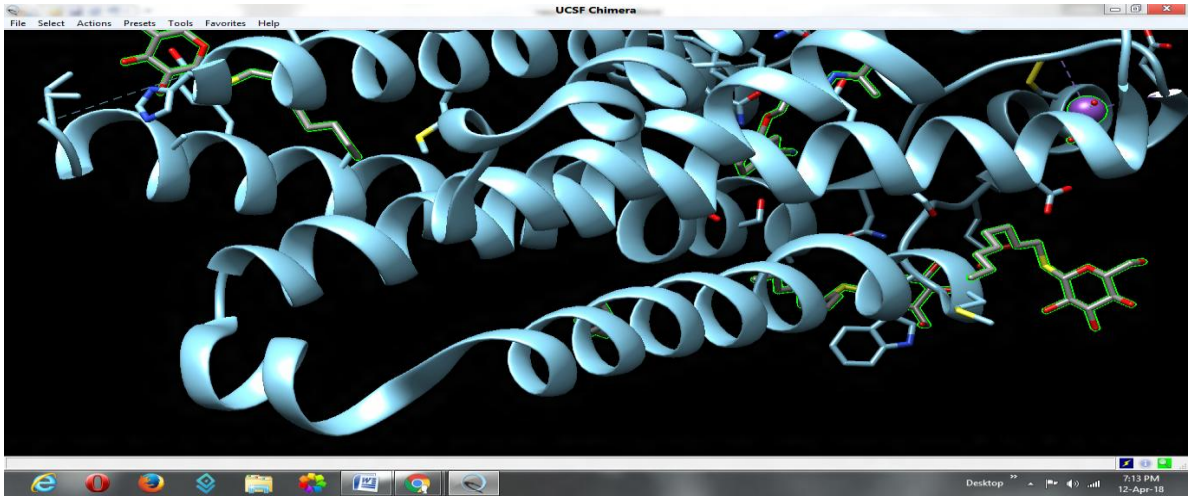


2. Minimize structure

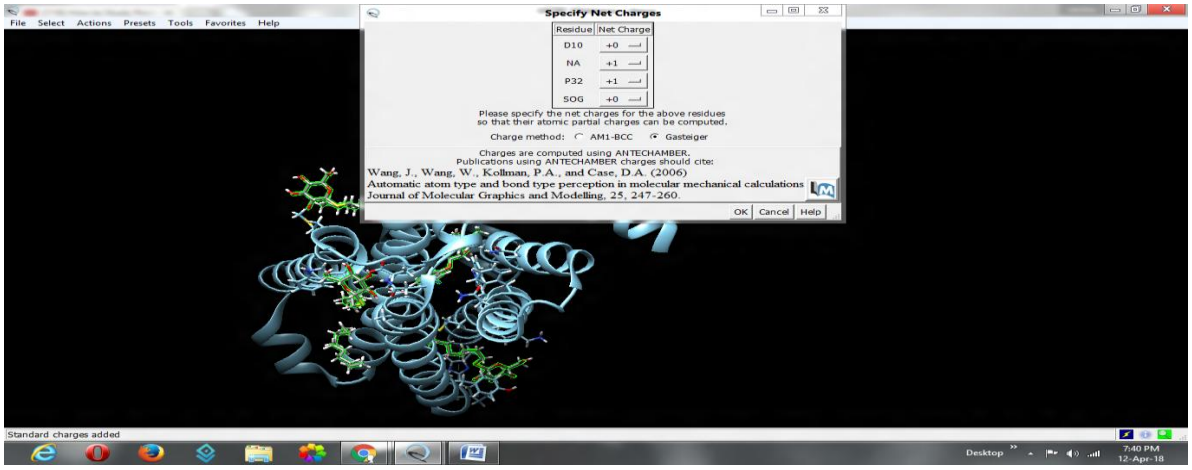




3. Open H2 receptor



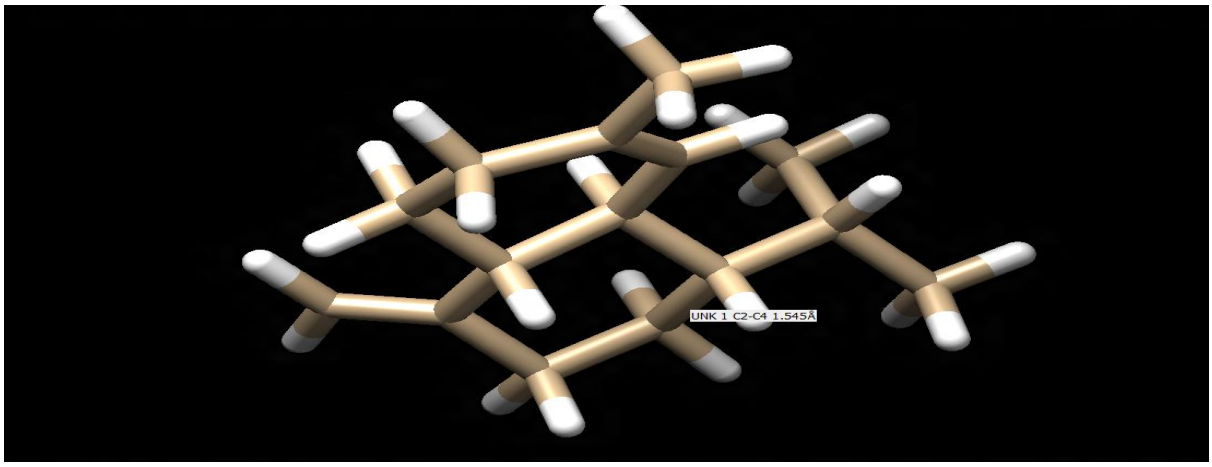
4. Dock prep



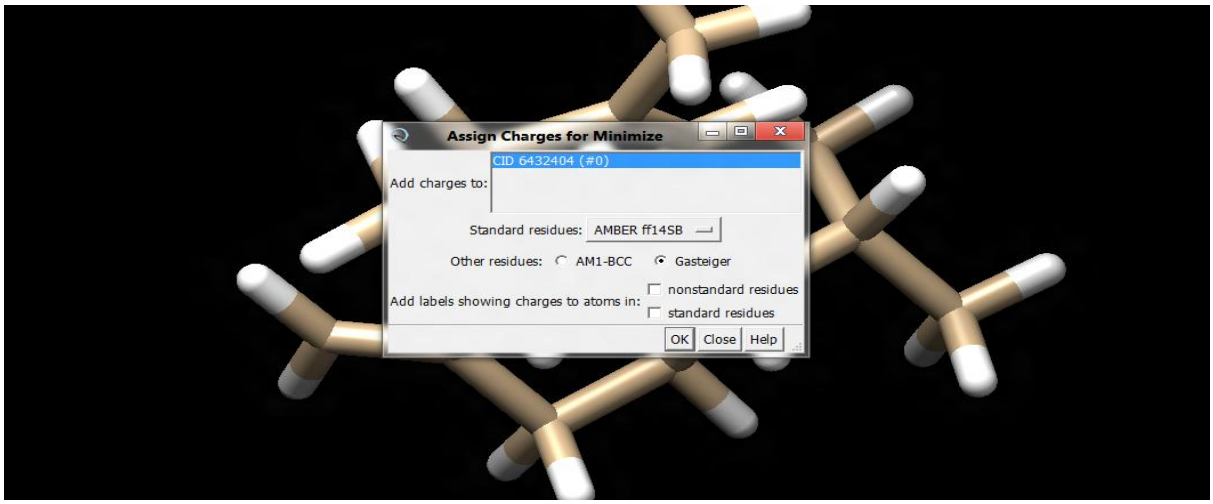
5. Autodock vina

7. GAMMA-CADINENE

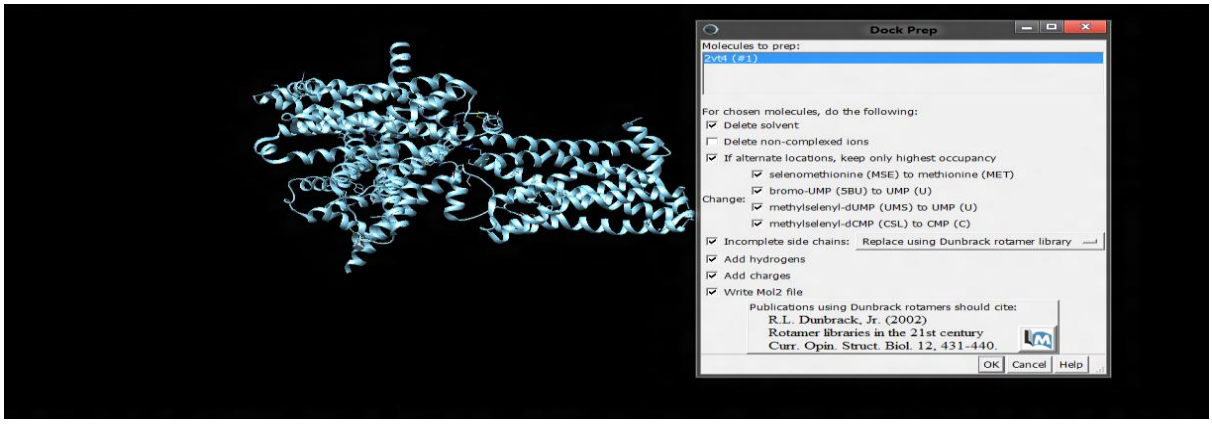
1. Phytochemical from Pubchem.



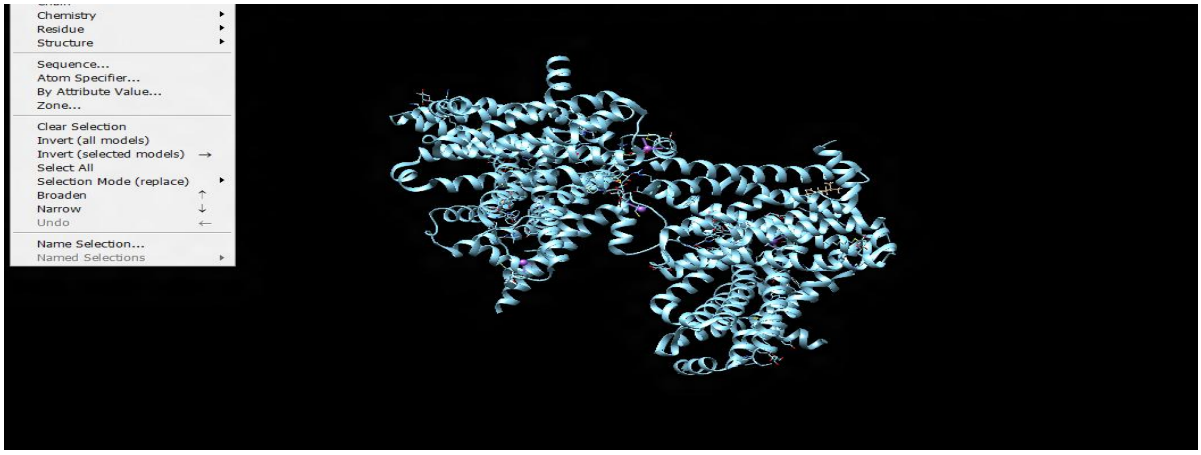
2. Minimize structure

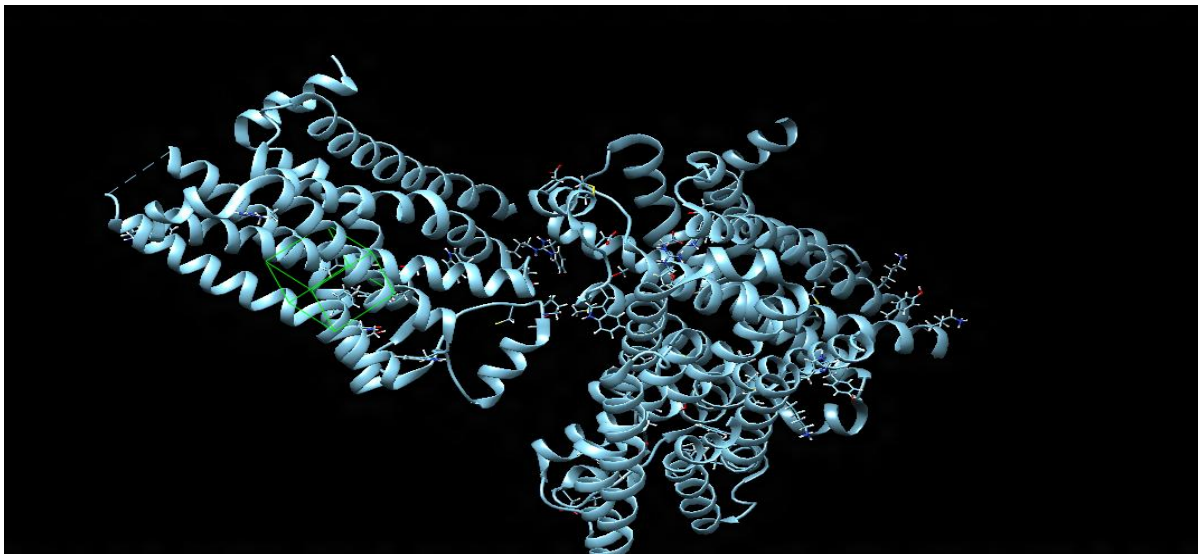


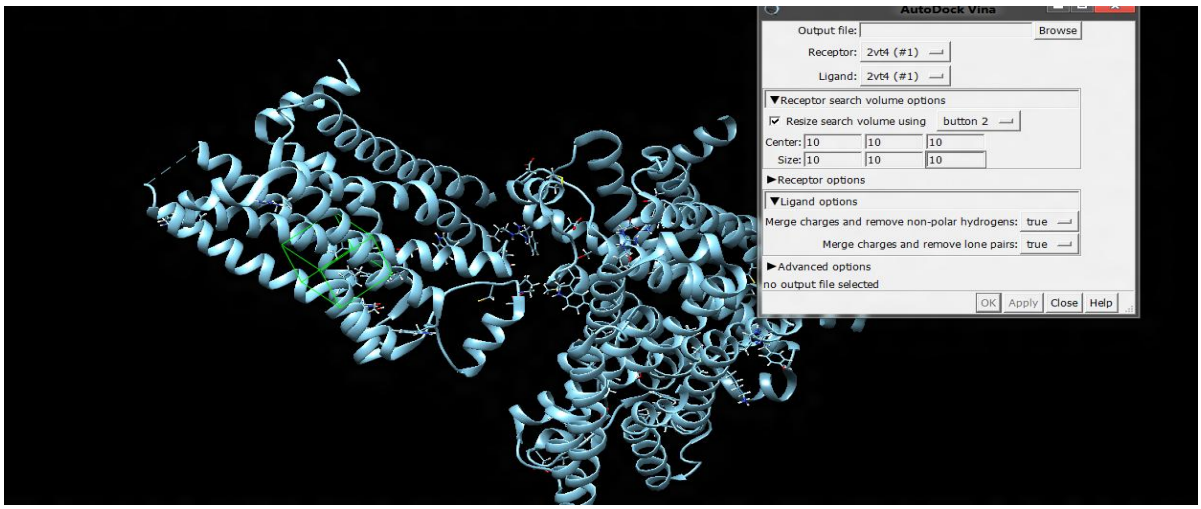
3. Dock prep



4. Autodock vina

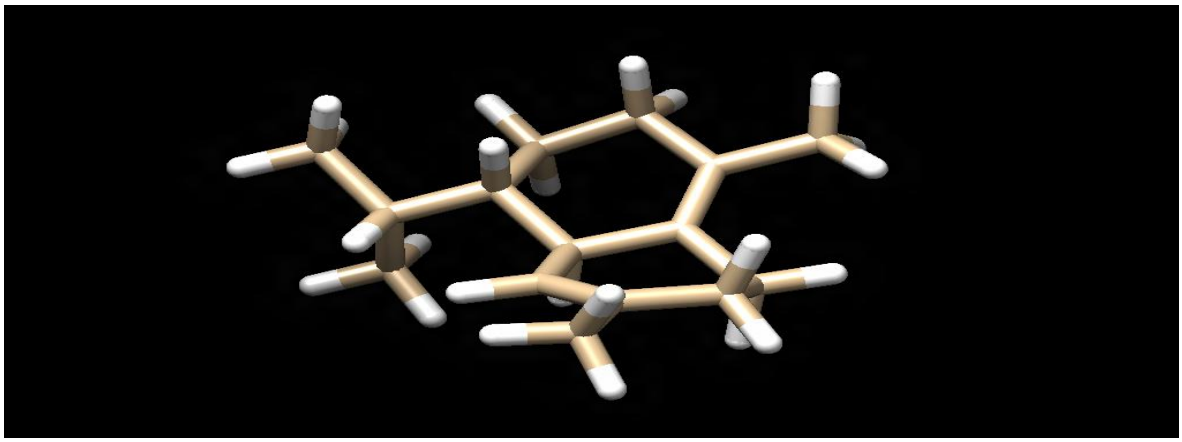




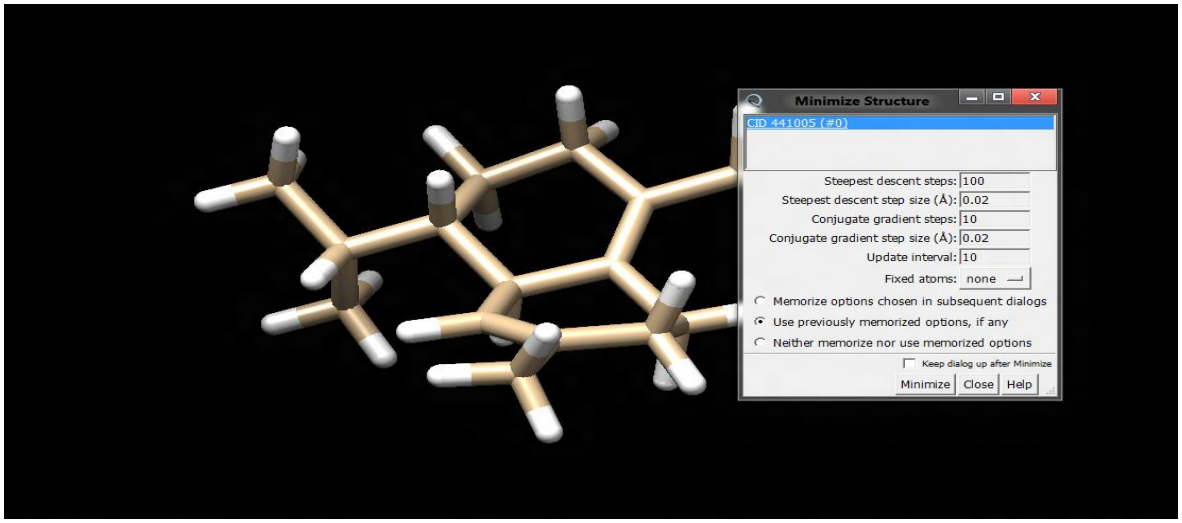


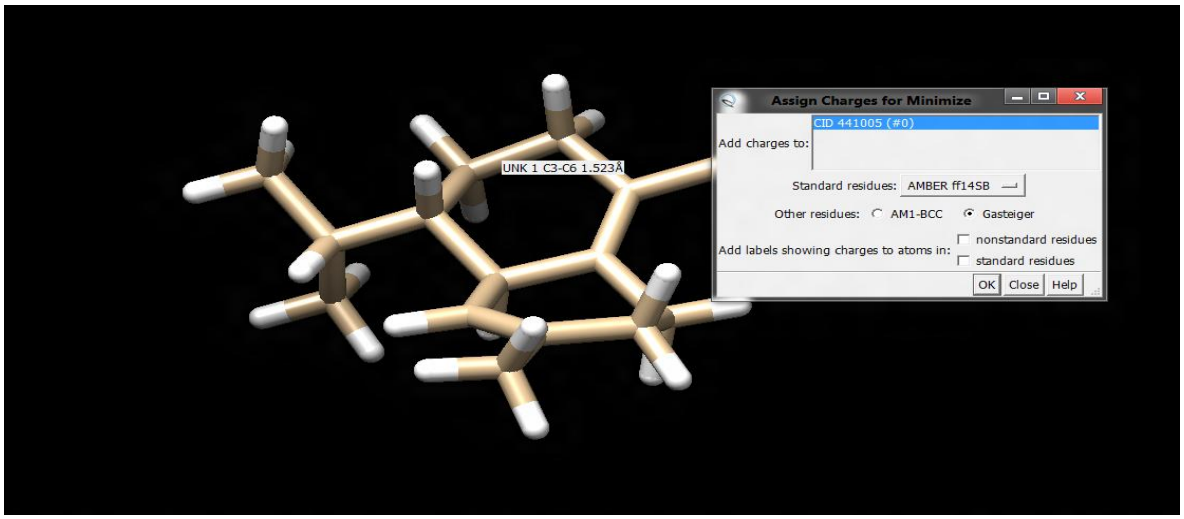
8. Delta-cadinen

1. Phytochemical from Pubchem

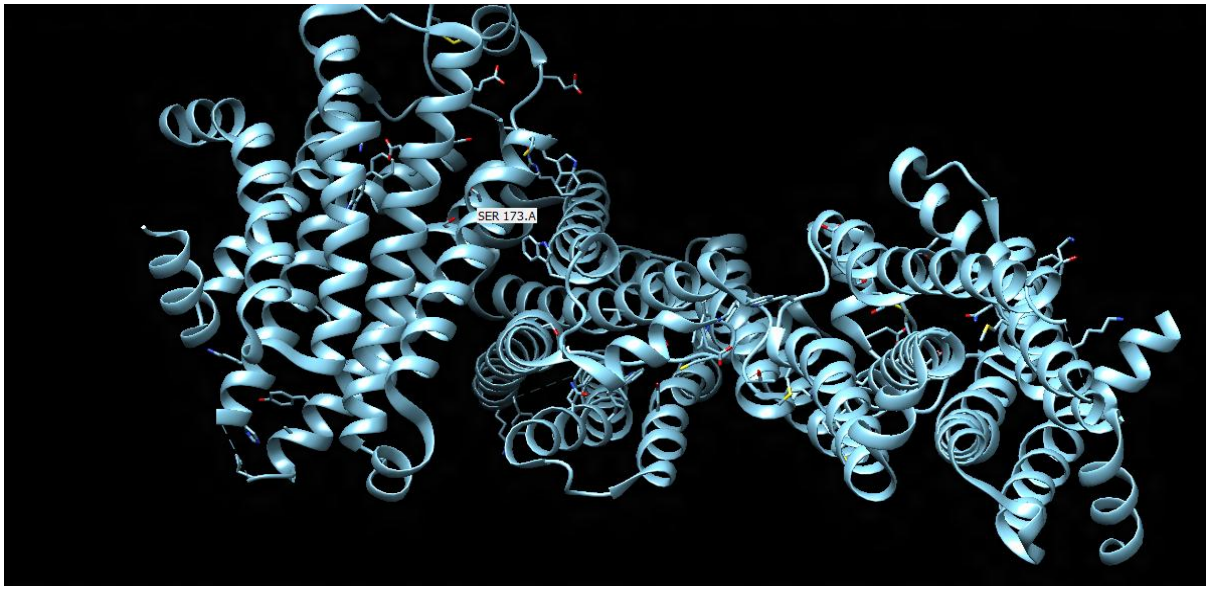


2. Minimize structure

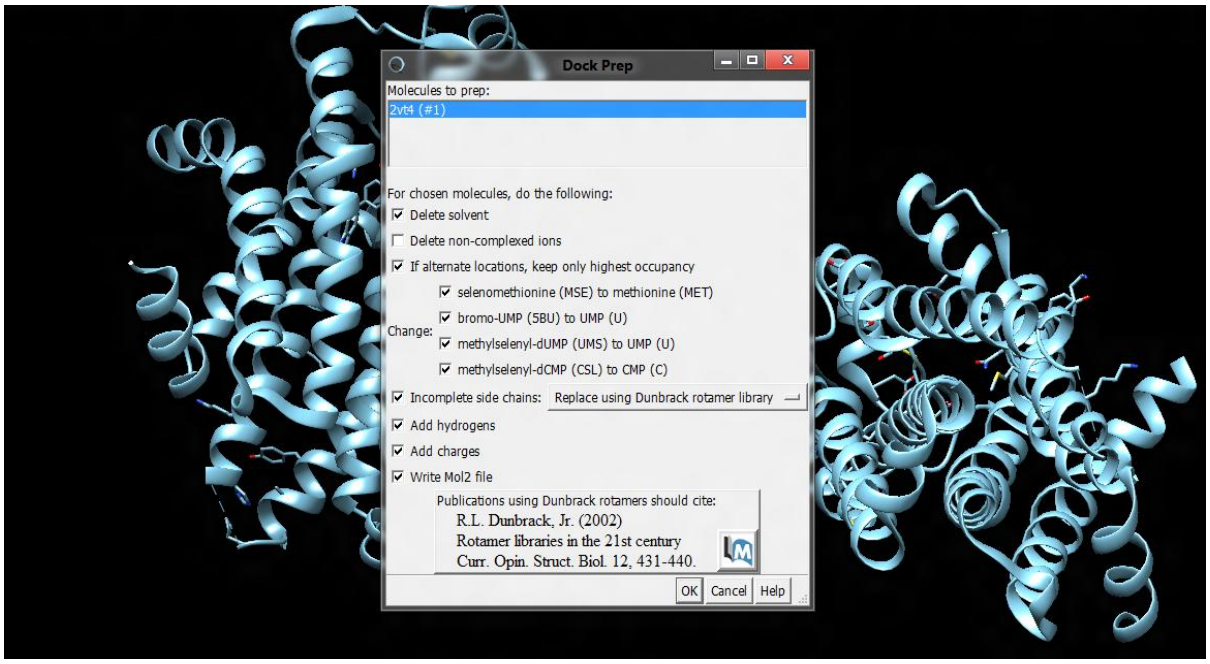




3. Open histamine receptor H2

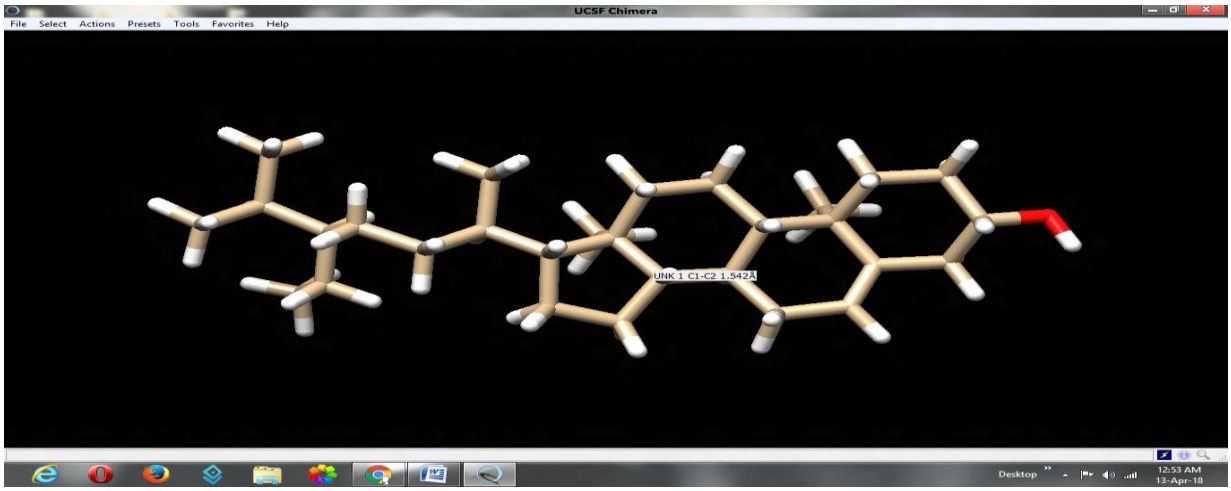


4. Dock prep

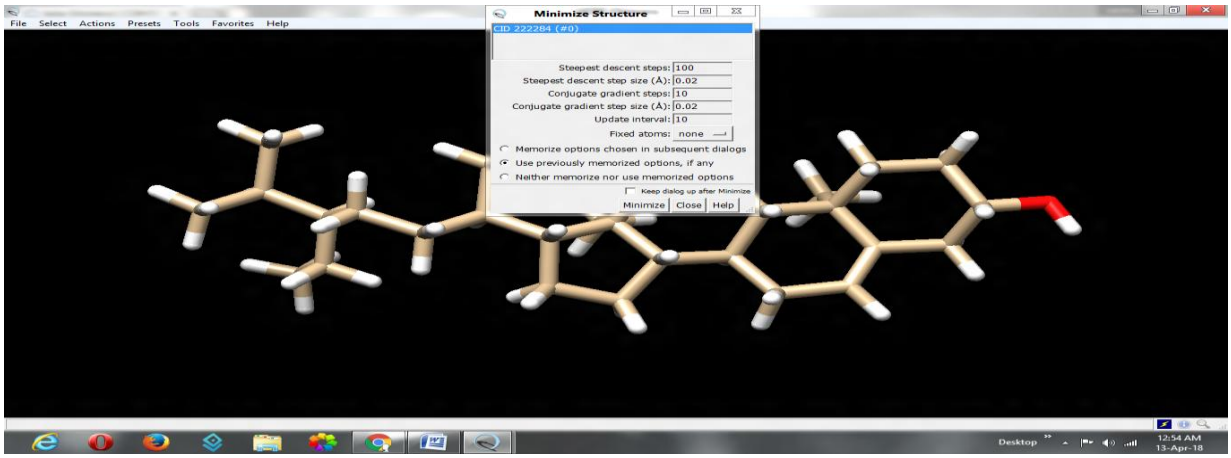


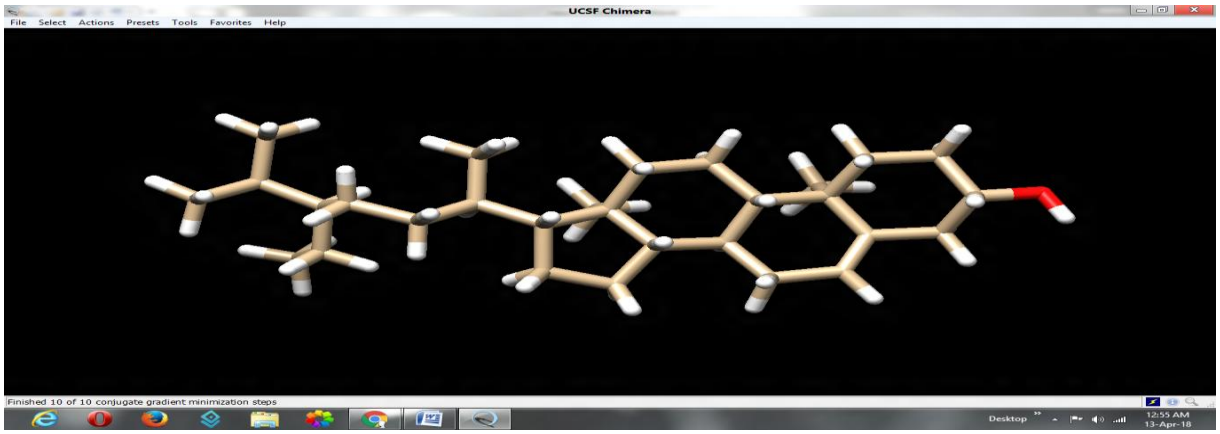
9. BETA-SITOSTEROL

1. Phytochemical from Pubchem

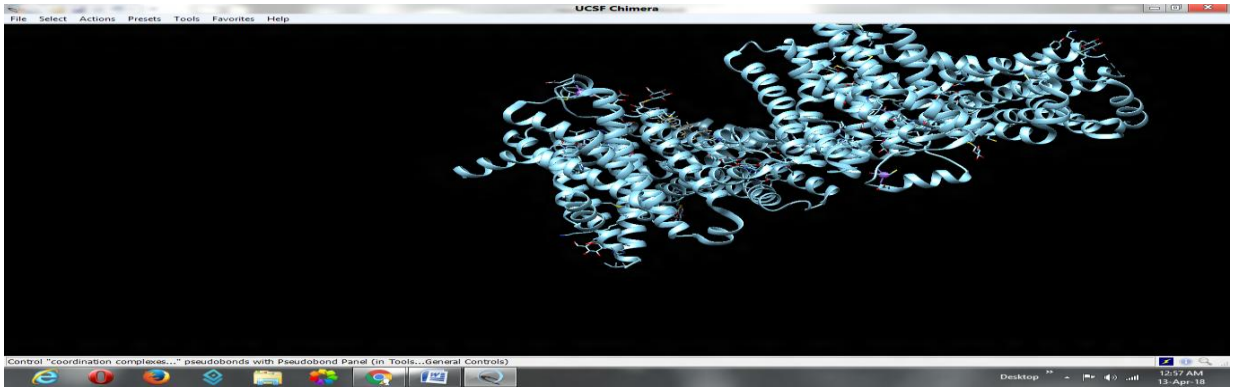


2. Minimize structure

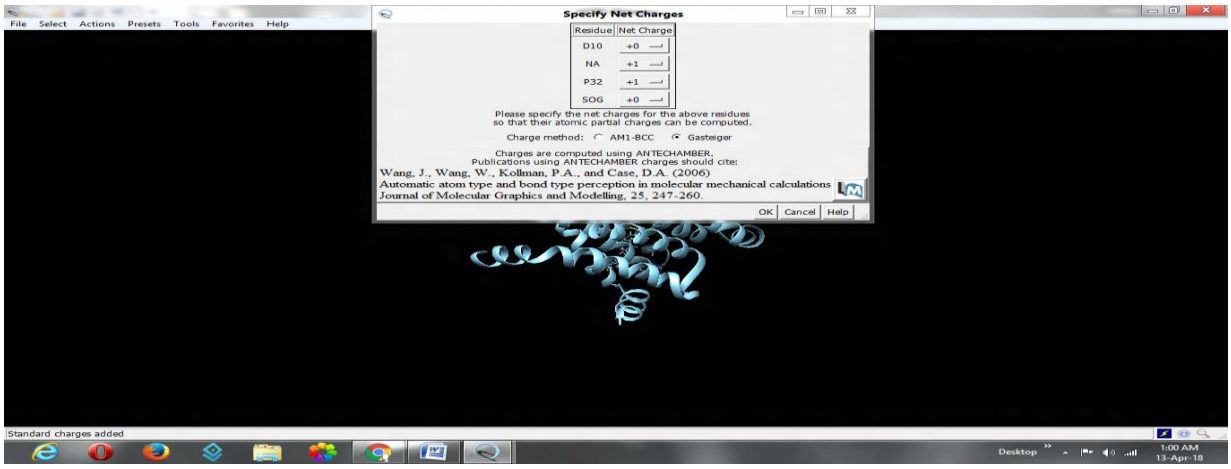




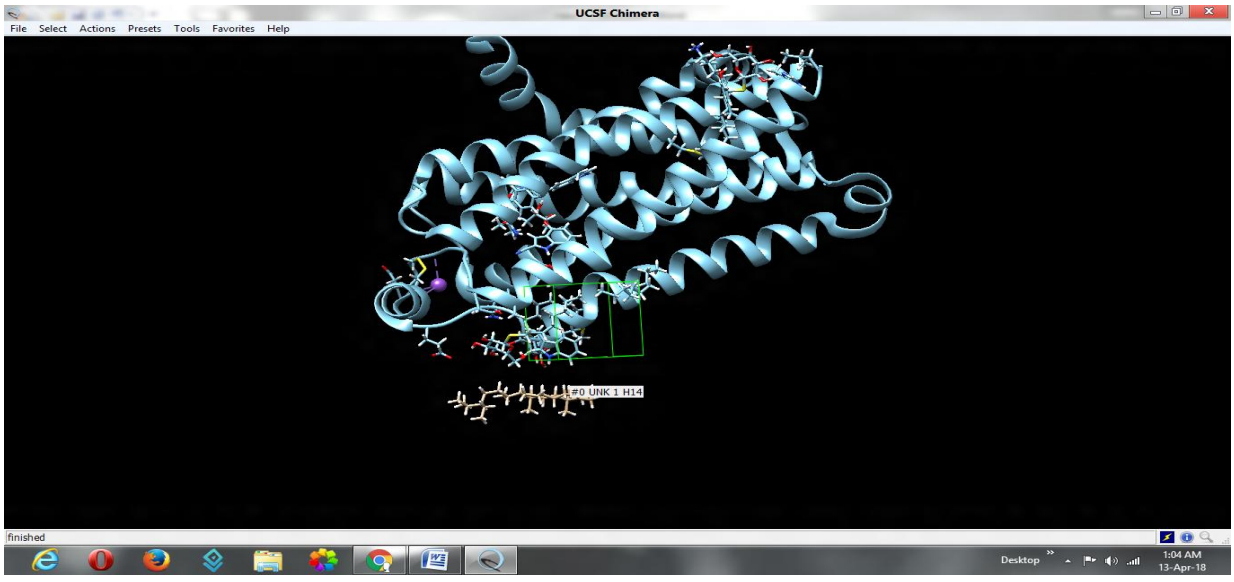
3. Open histamine H2



4. Dock prep

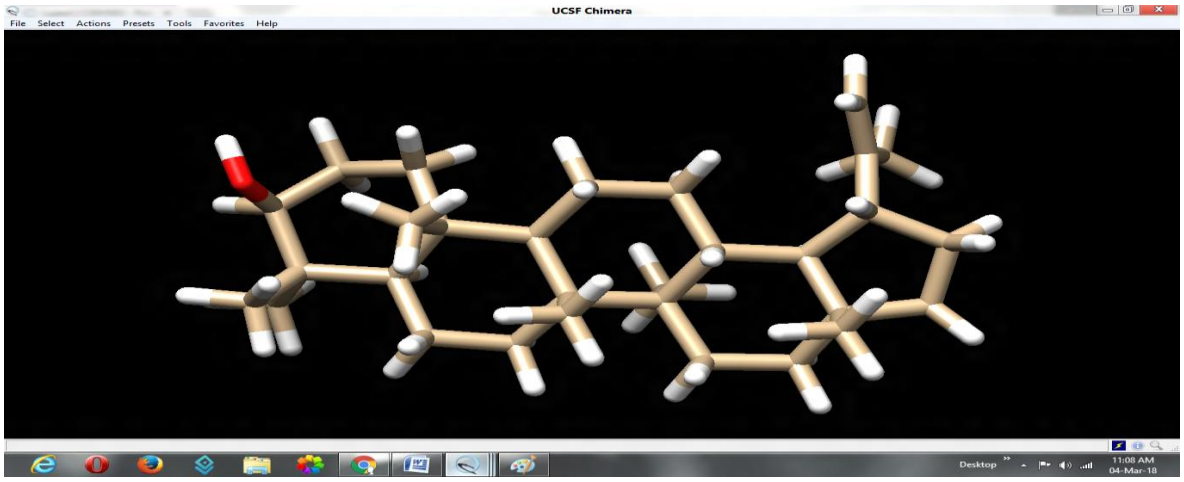


5. Autodock vina

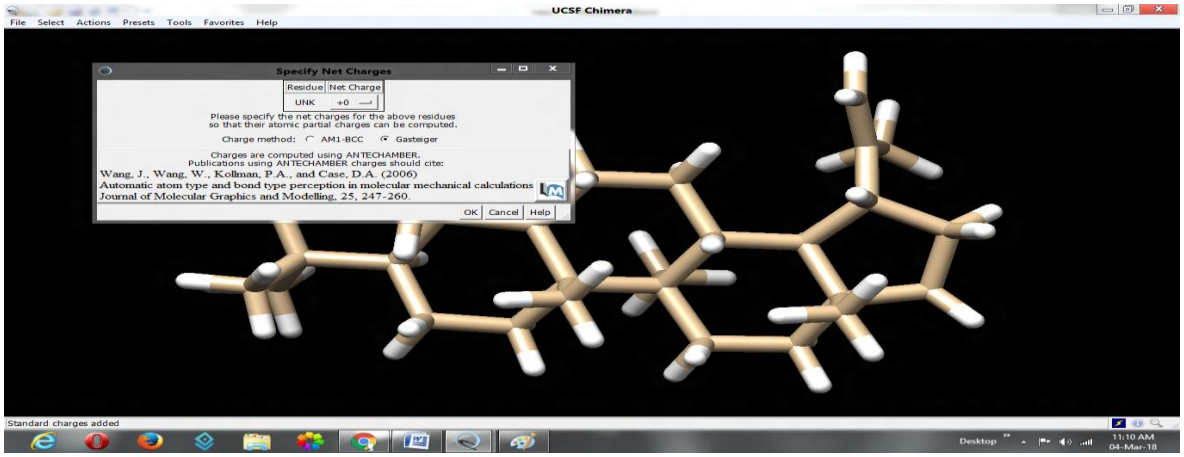


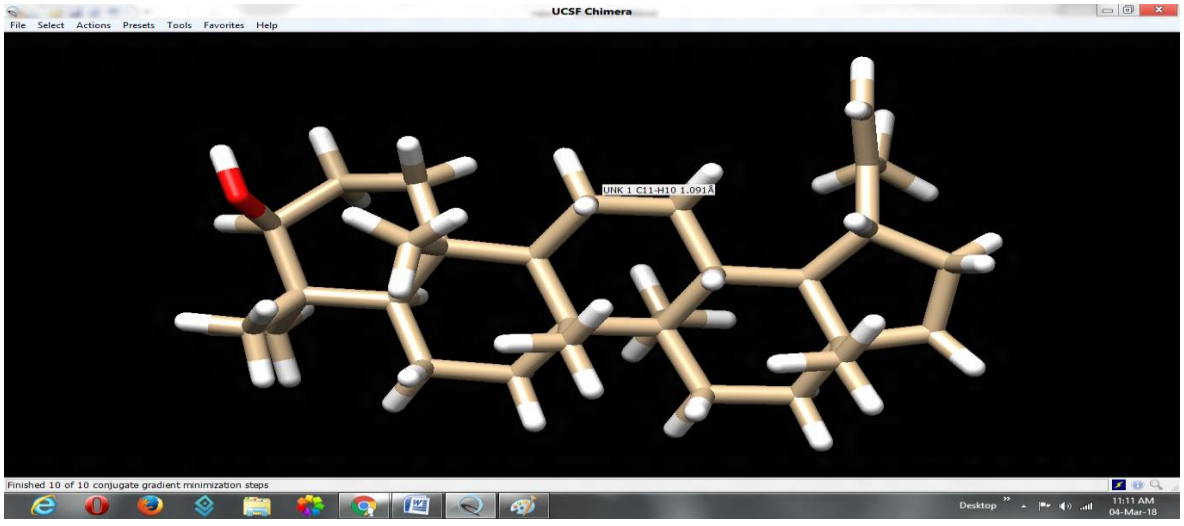
10. LUPEOL

1. Phytochemical from Pubchem

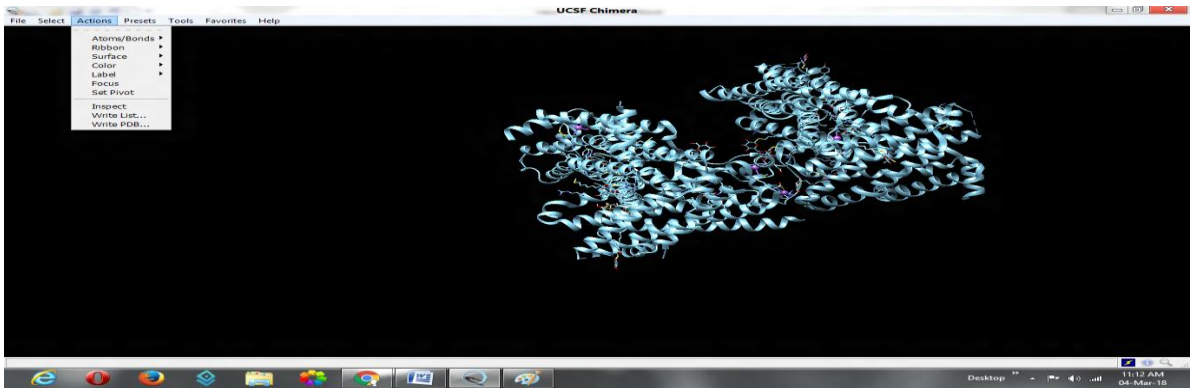


2. Minimize structure

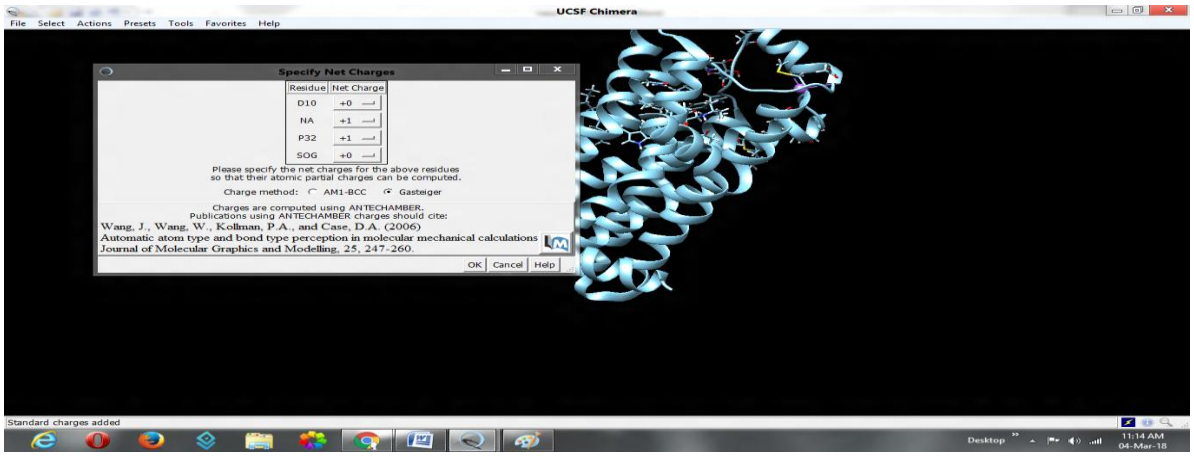




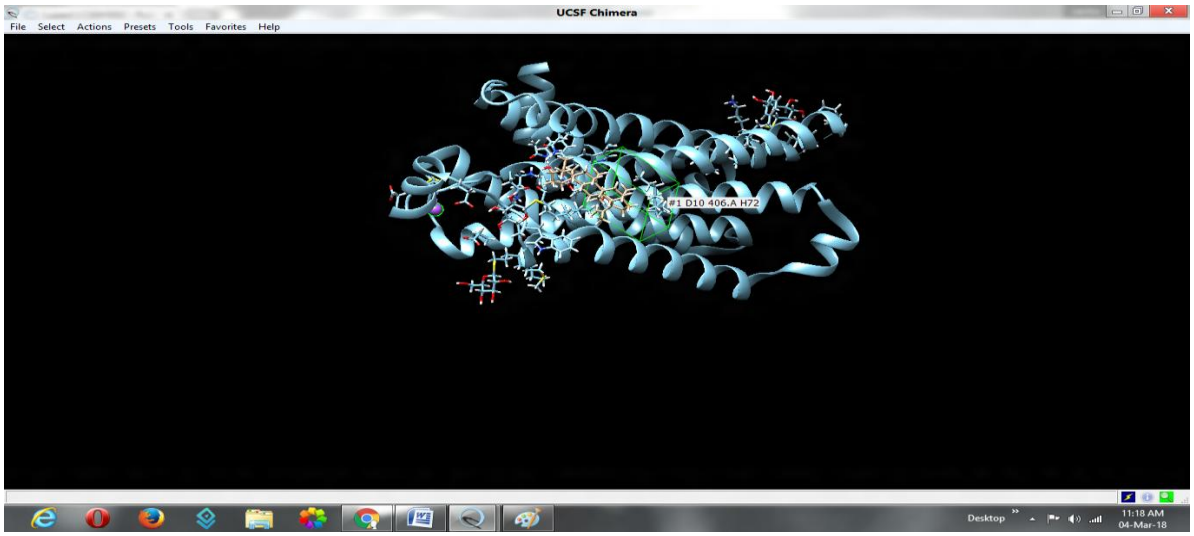
3. OpenH2 from PDB



4. Dock prep

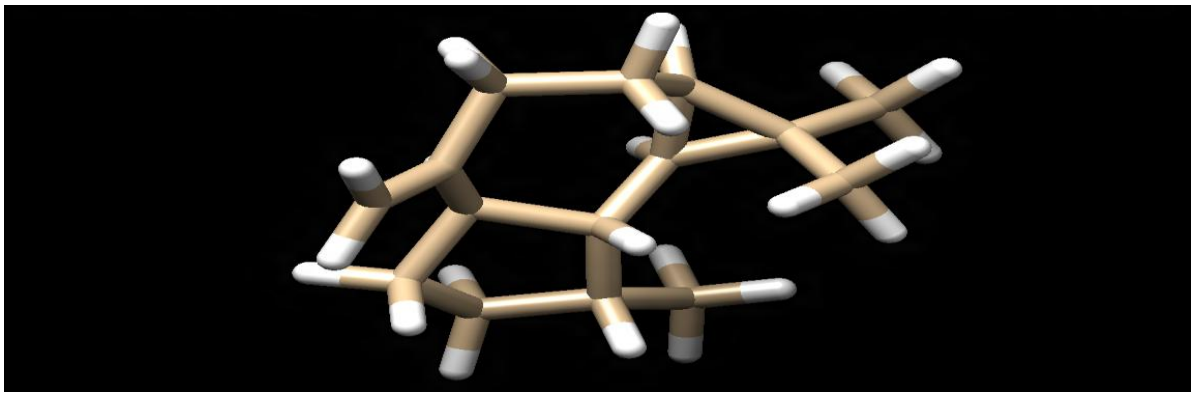


5. Autodock vina

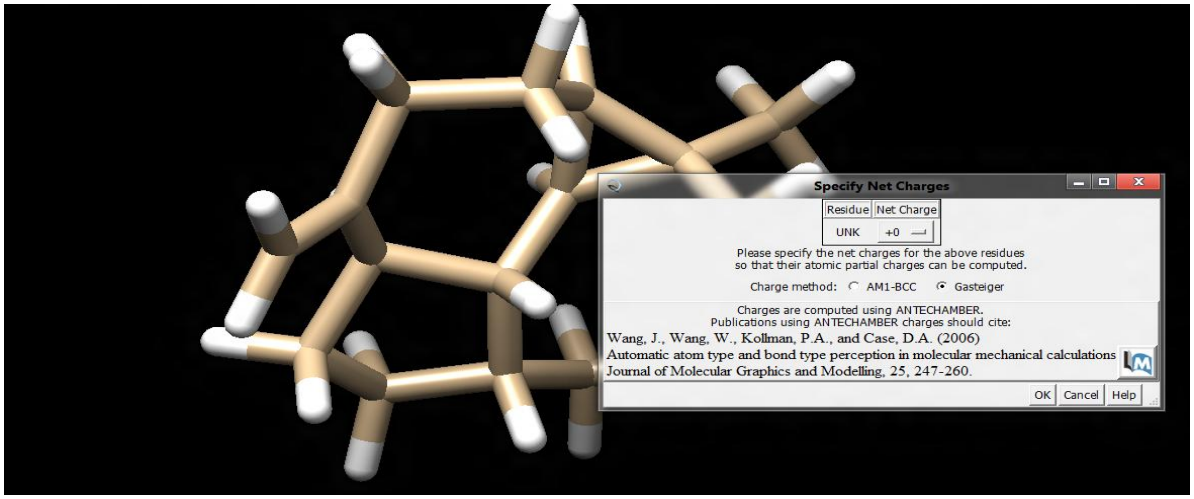


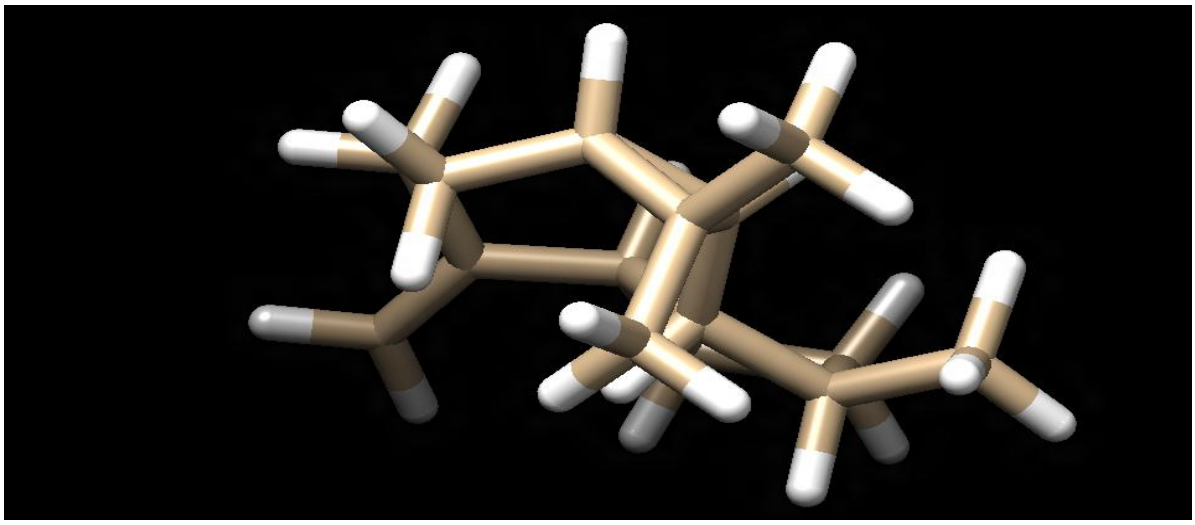
11. AROMADENDRENE

1. Phytochemical from Pubchem

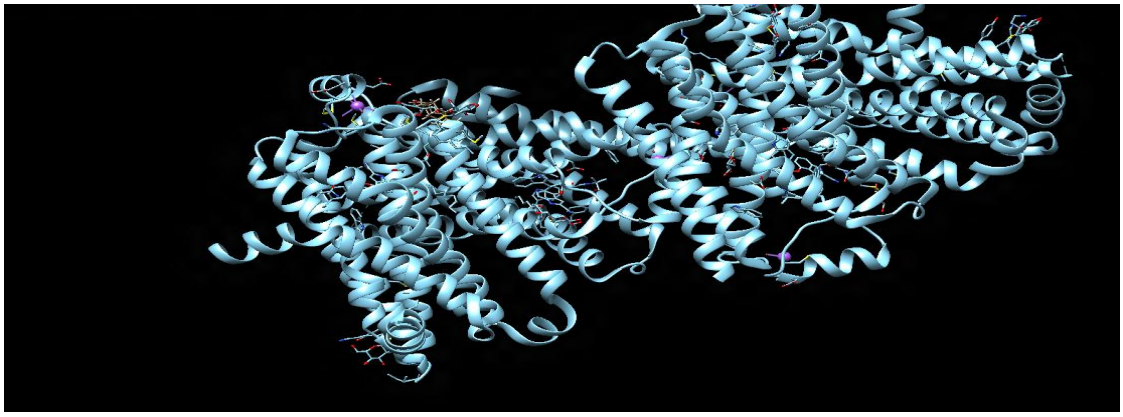


2. Minimize structure

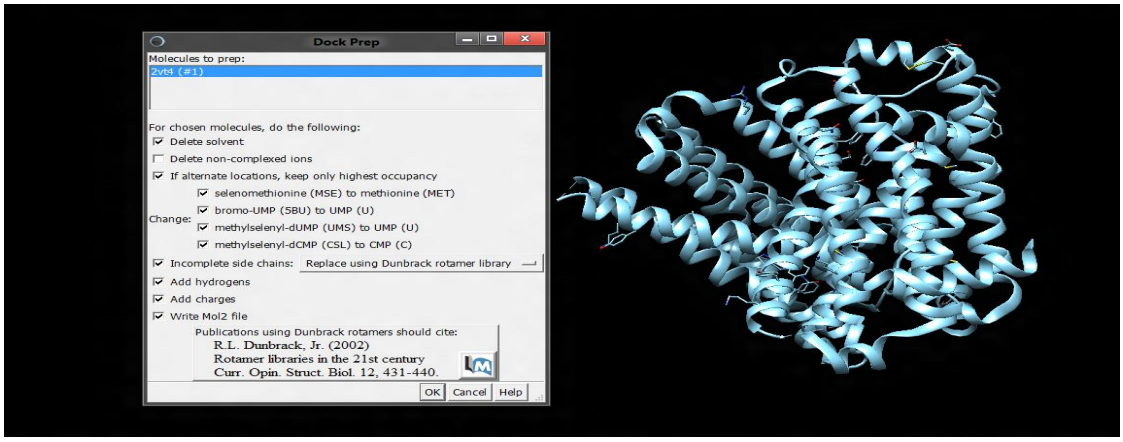




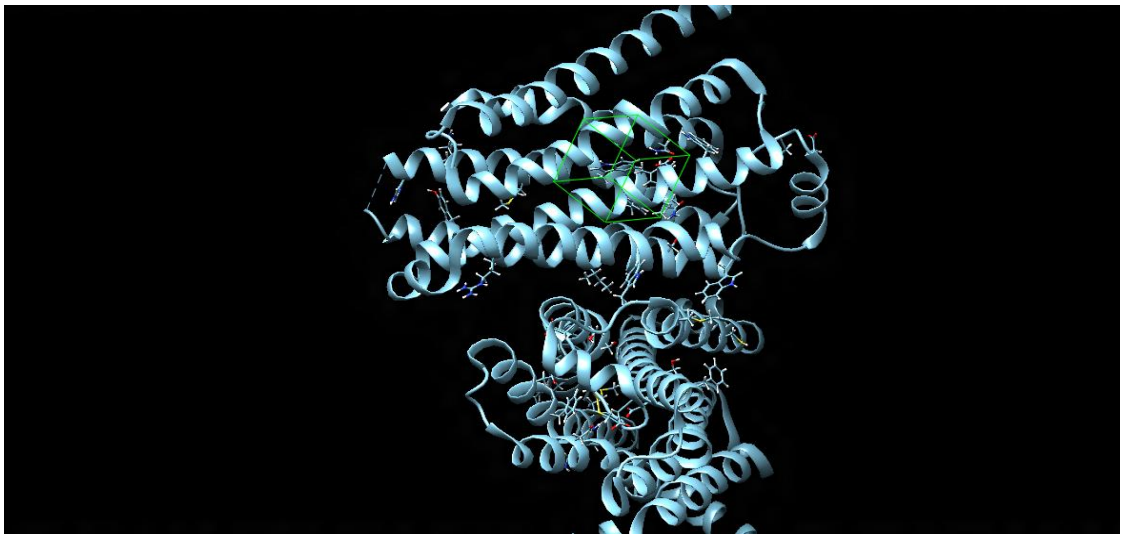
3. Open H2 from PDB



4. Dock prep

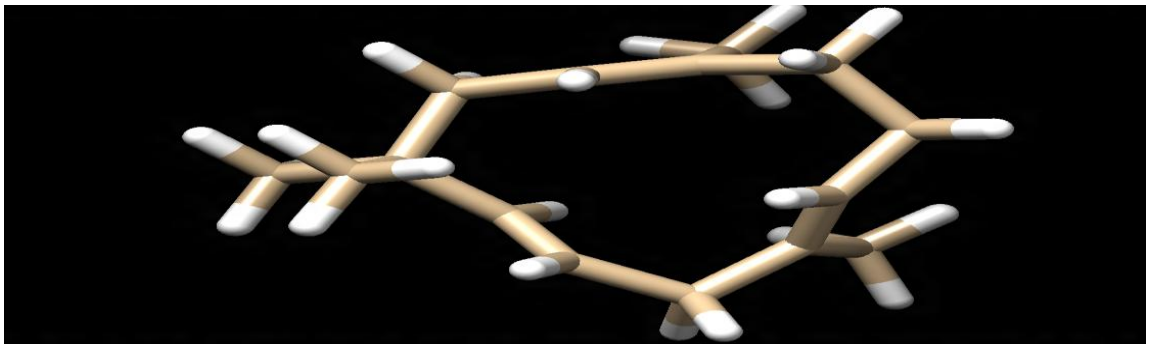


5. Autodock vina

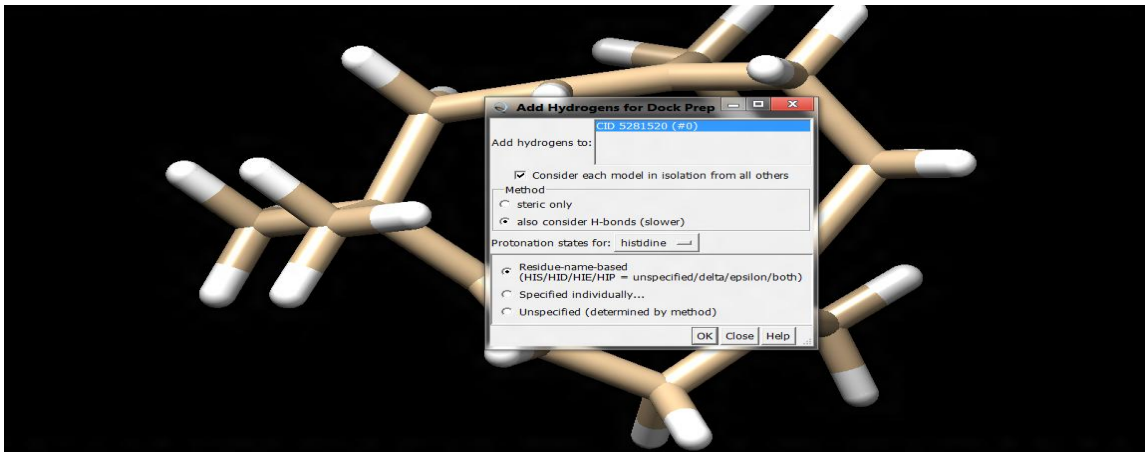


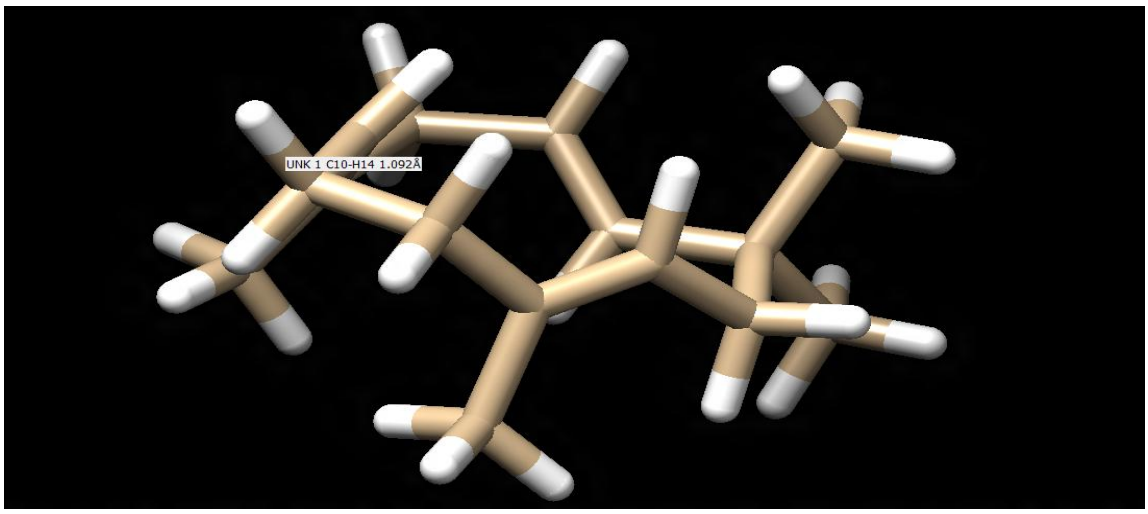
12. ALPHA-HUMULEN

1. Phytochemical from Pubchem

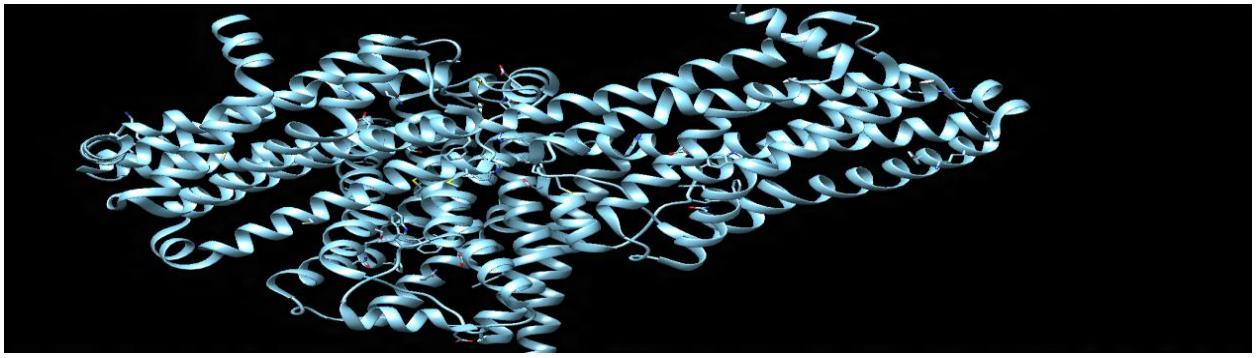


2. Minimize structure

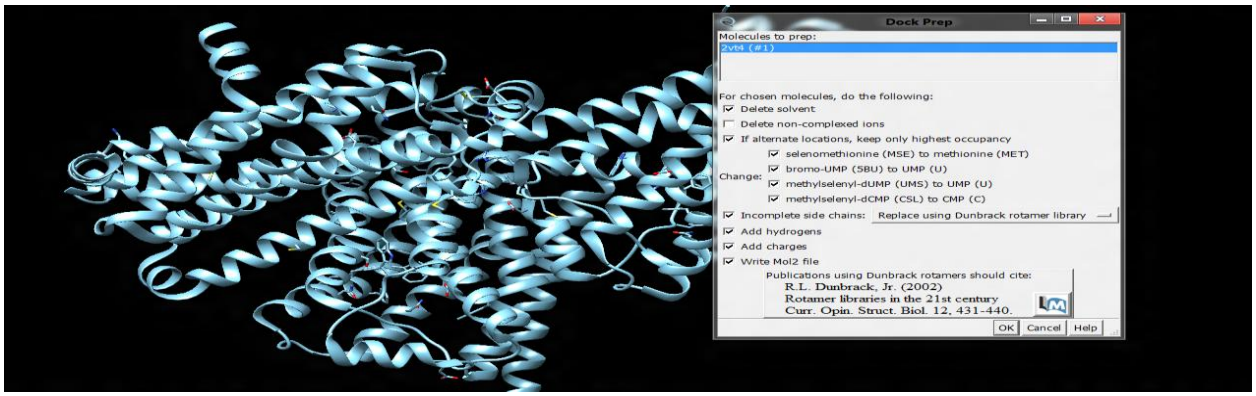




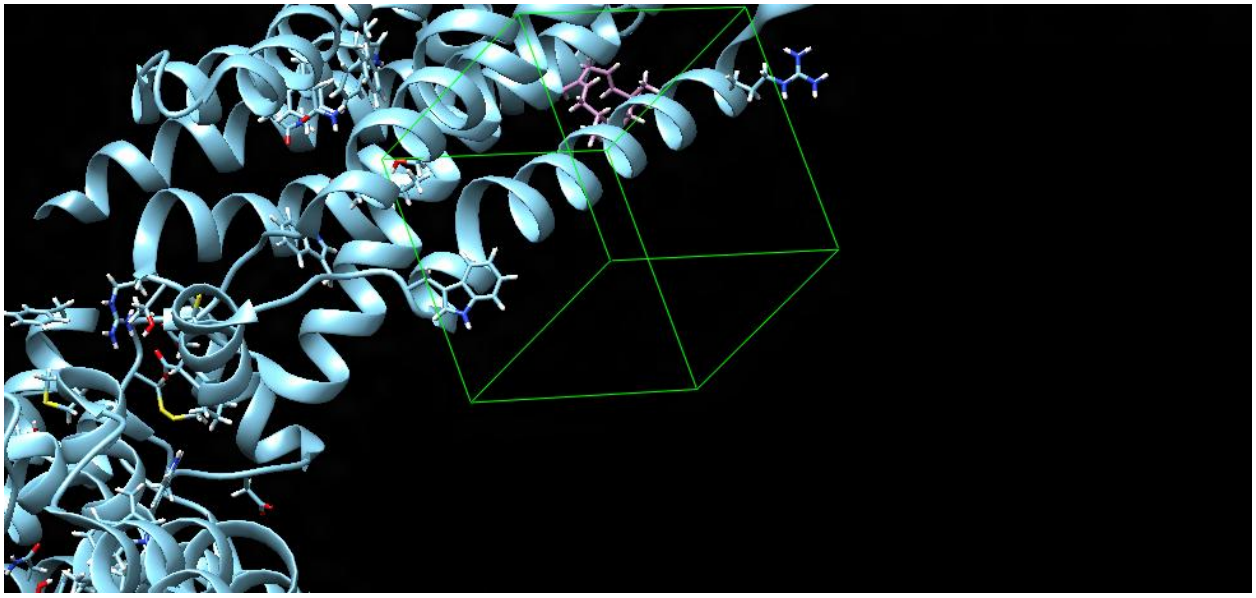
3. Open H2 from PDB



4. Dock prep

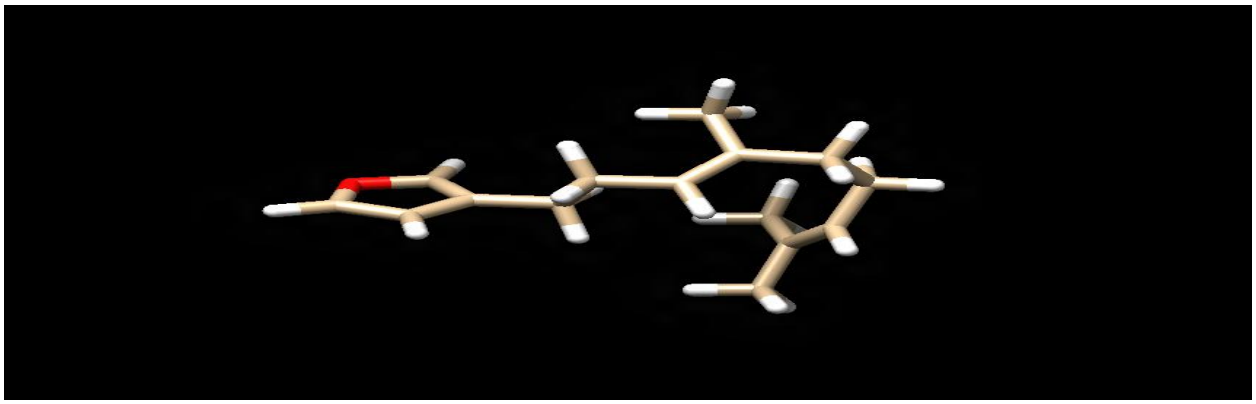


5. Autodock vina

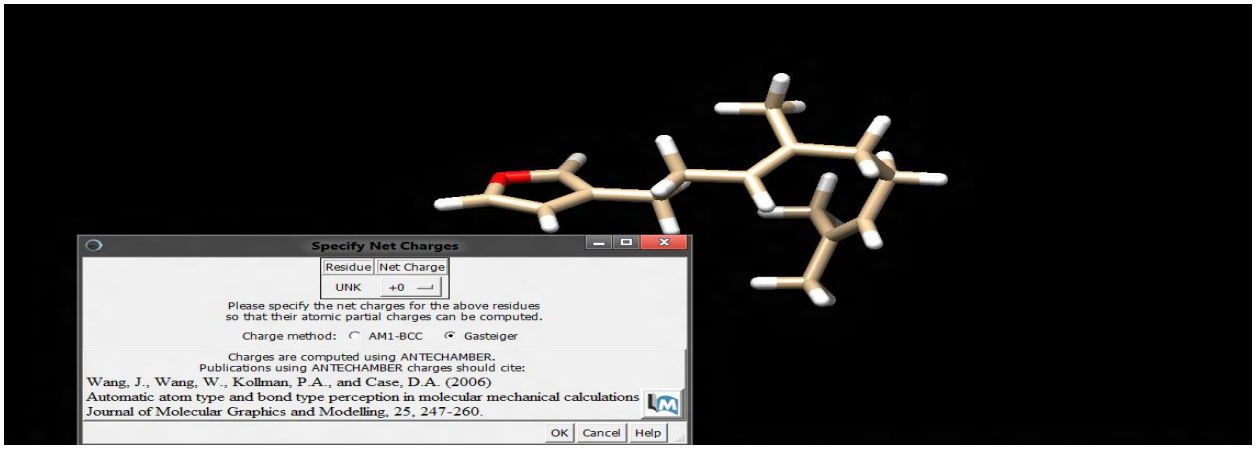


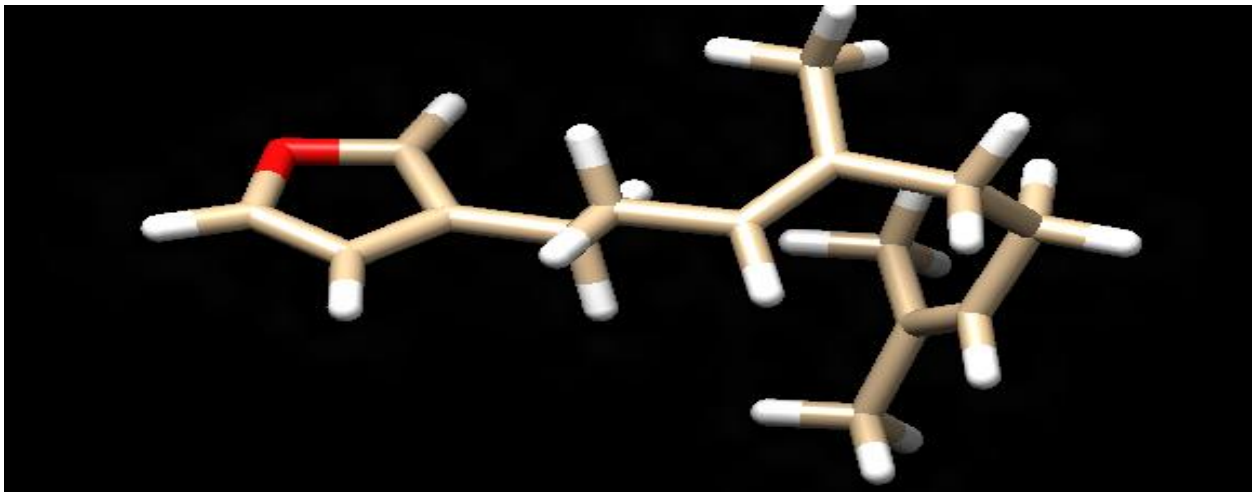
13. DENDROLASIN

1. Phytochemical from Pubchem

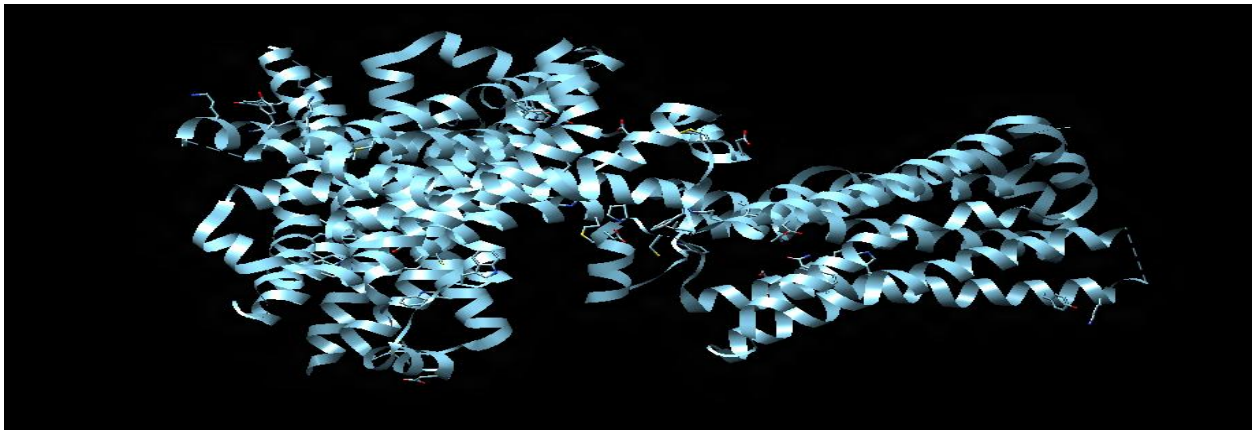


2. Minimize structure

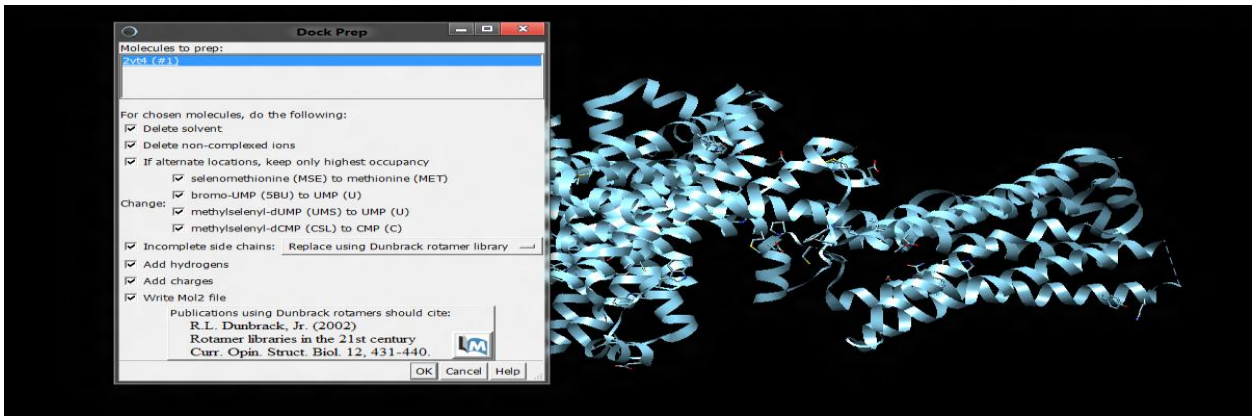




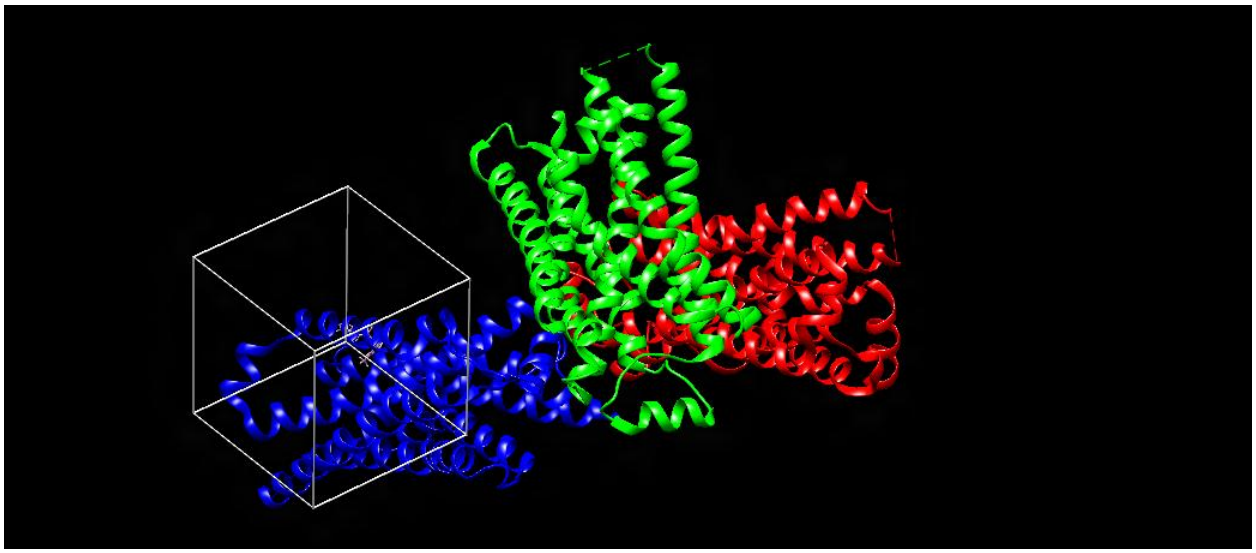
3. Open H2 from PDB



4. DOCK PREP

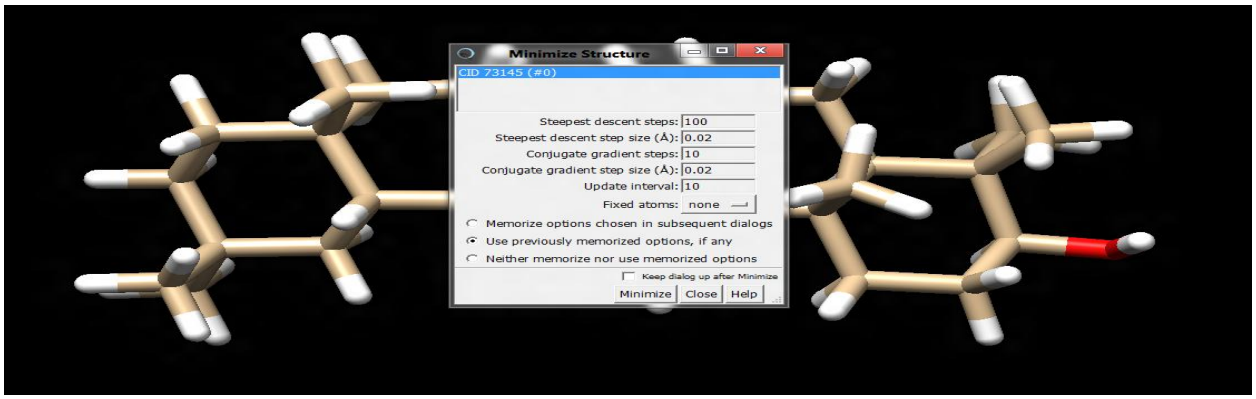


5. Autodock vina

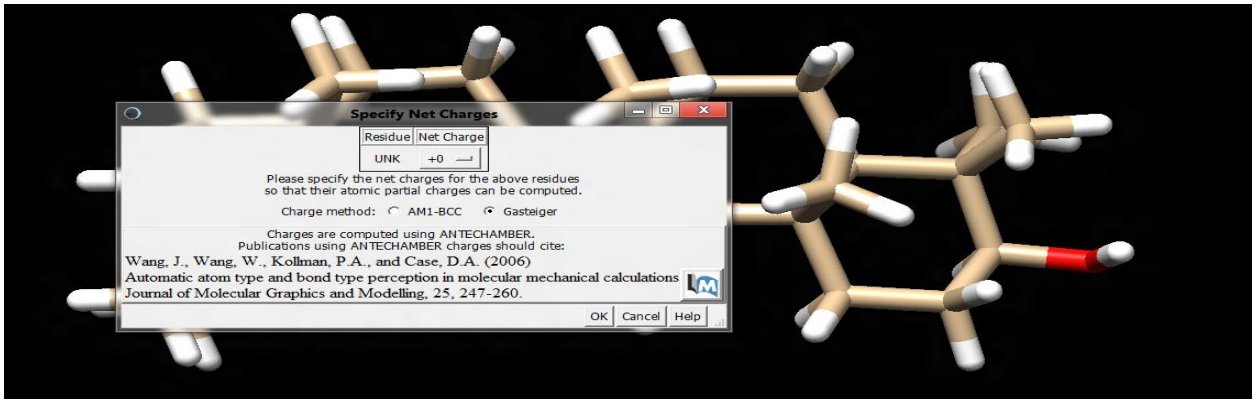


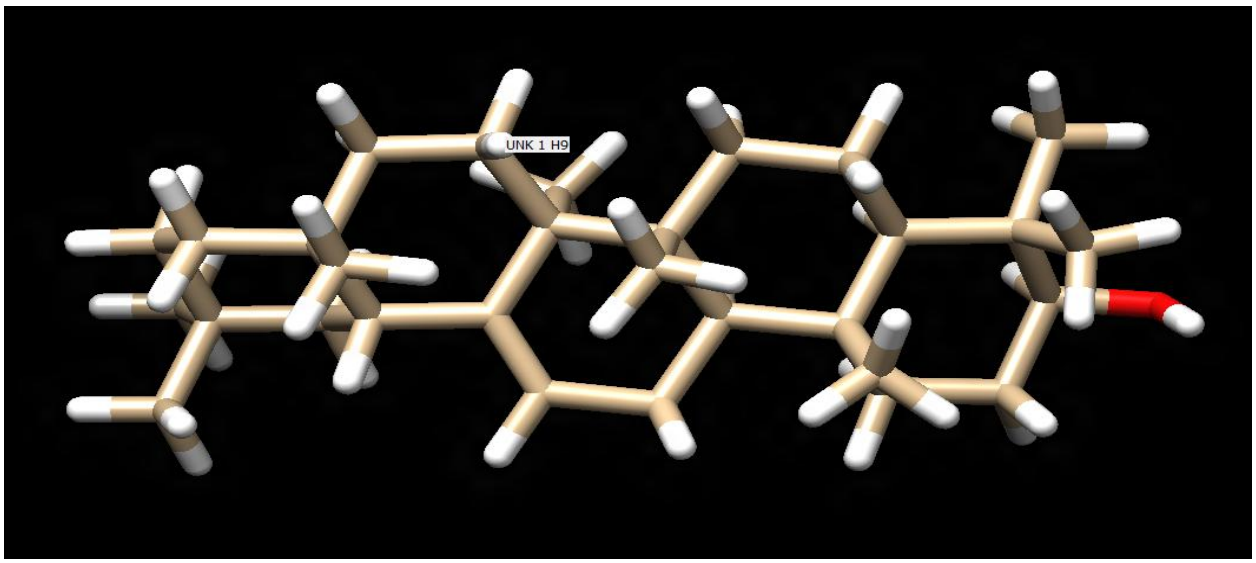
14. ALPHA-AMYRIN

1. Phytochemical from Pubchem

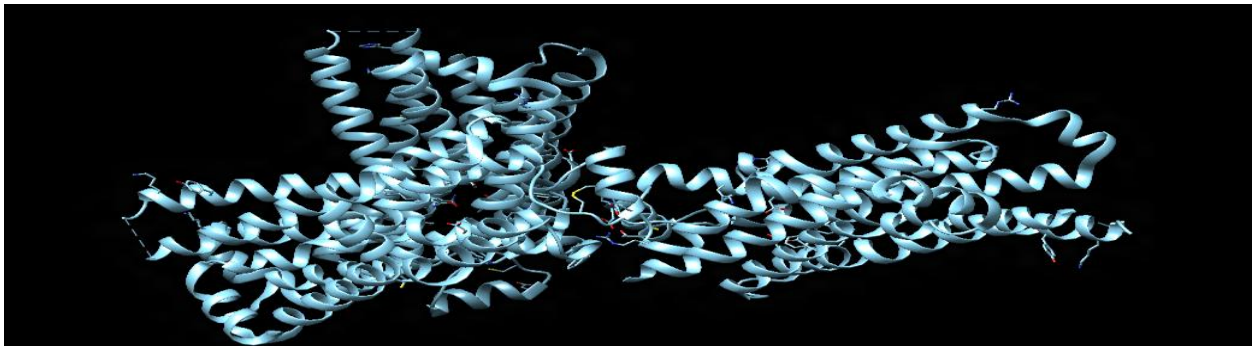


2. Minimize structure

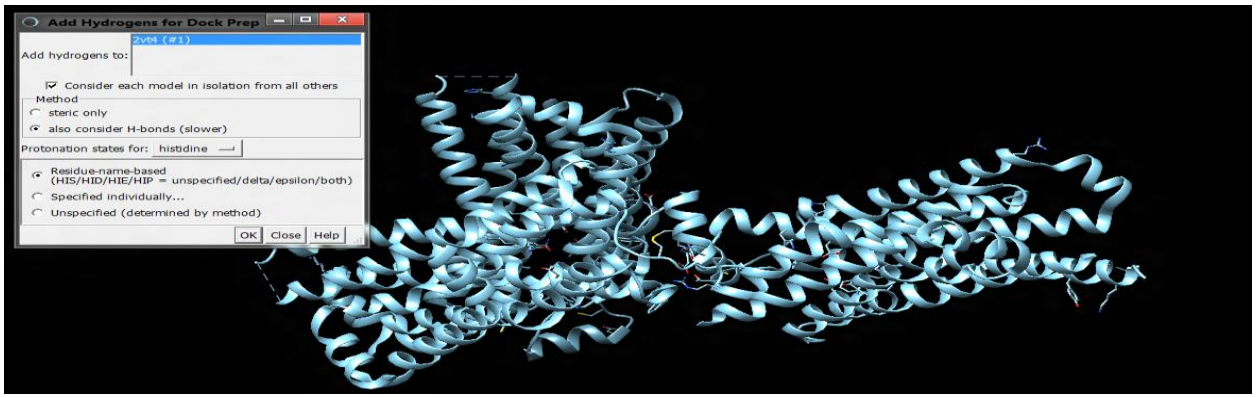




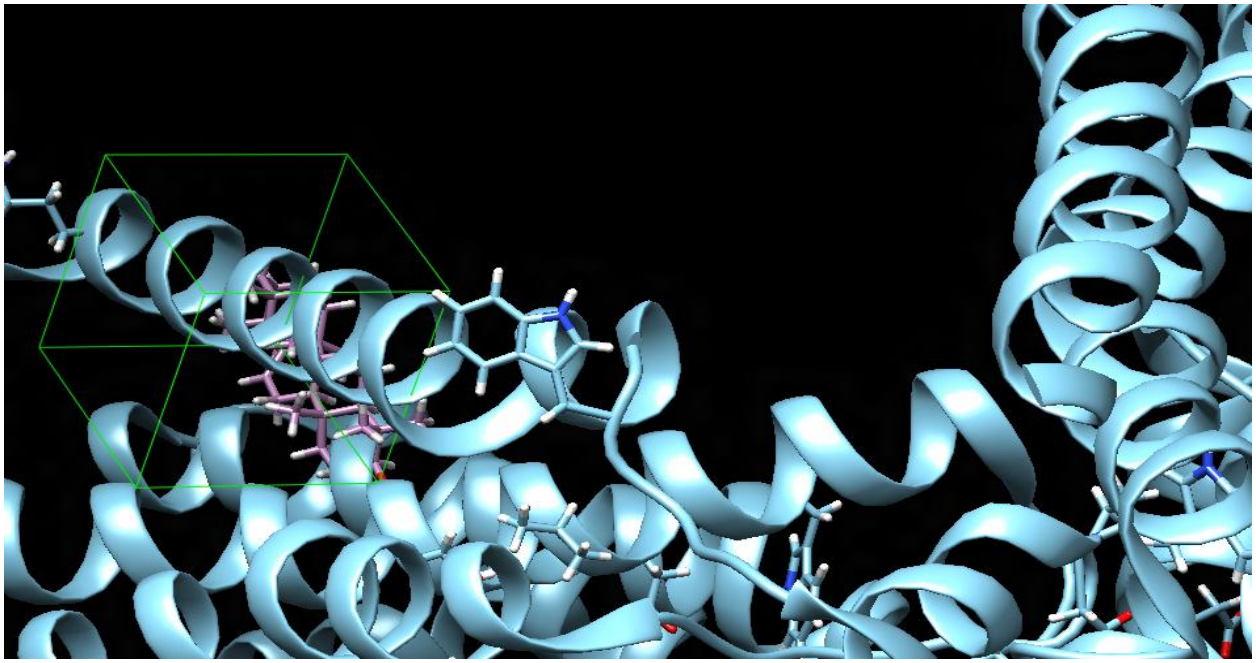
3. Open H2 from PDB



4. Dock prep

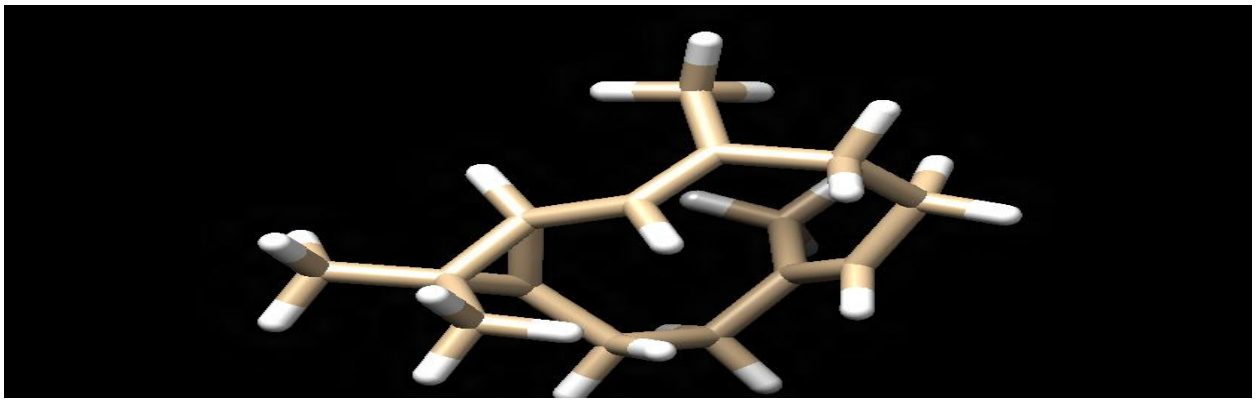


5. Autodock vina

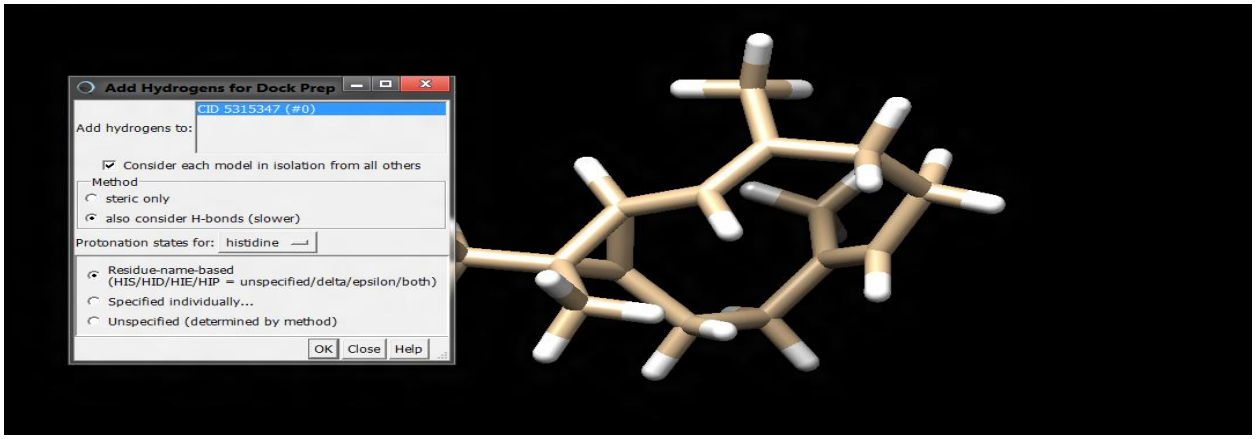


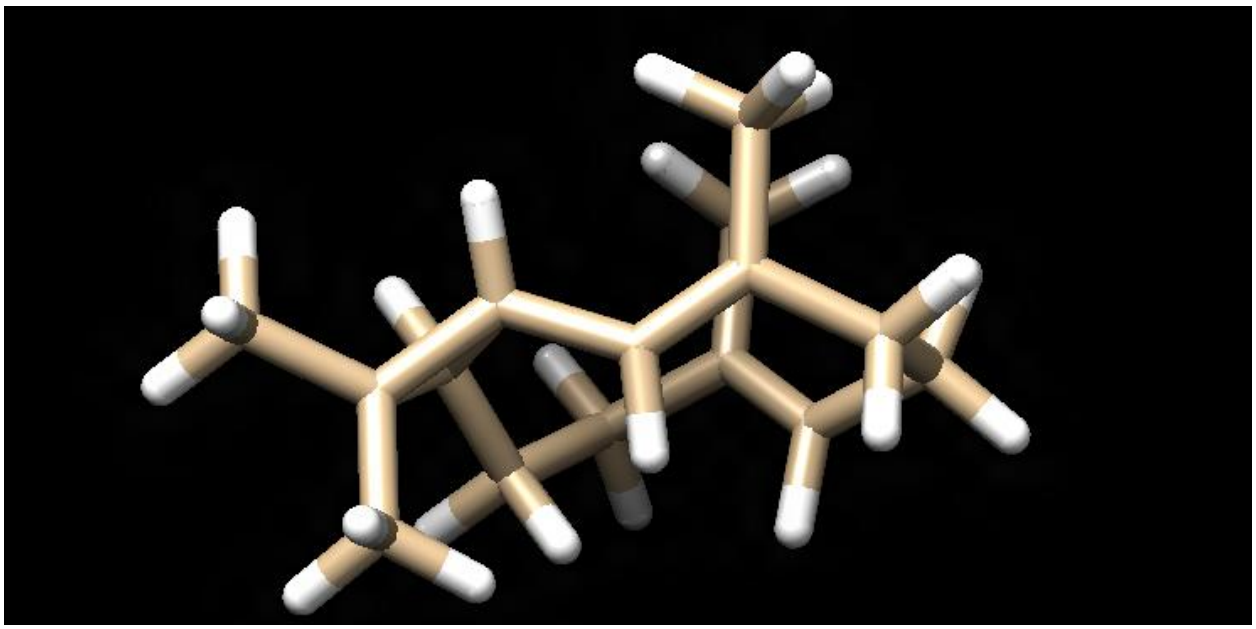
15. BICYCLOGERMACRENE

1. Phytochemical from Pubchem

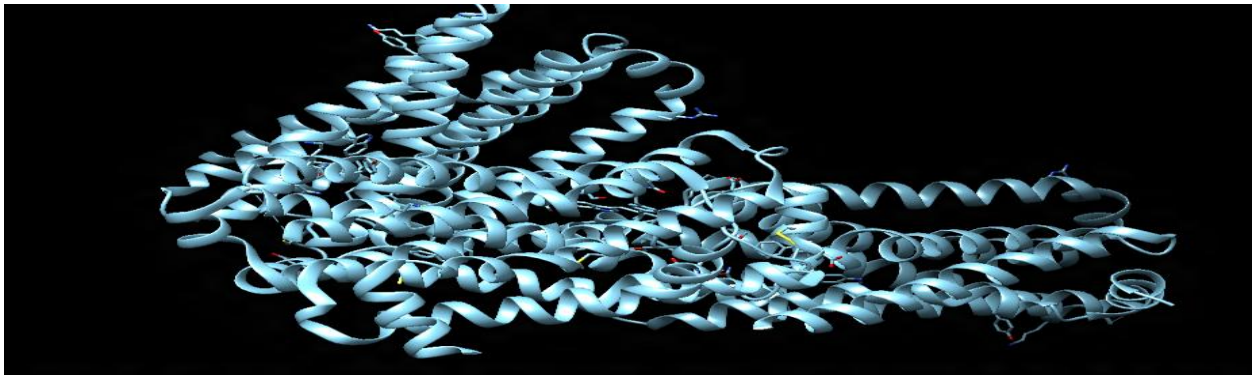


2. Minimize structure

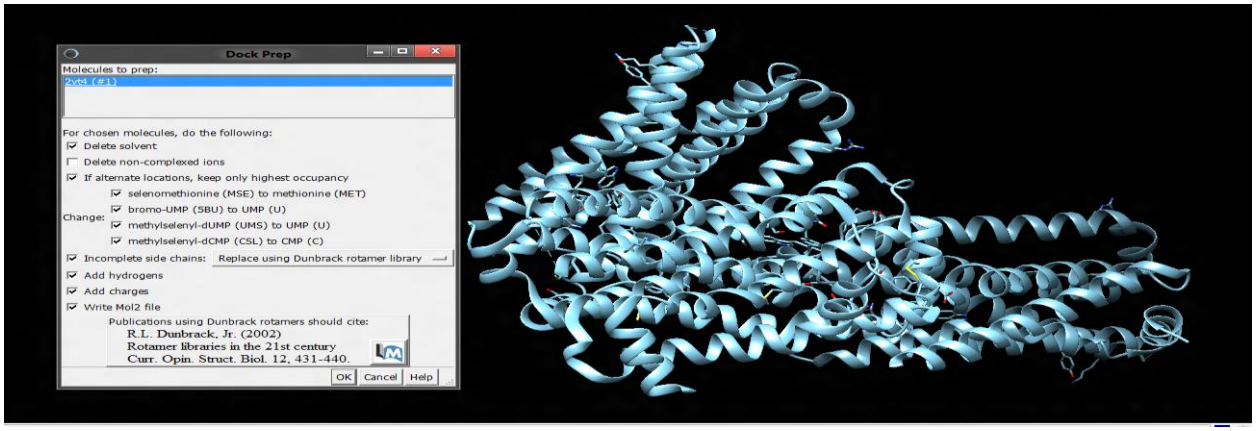




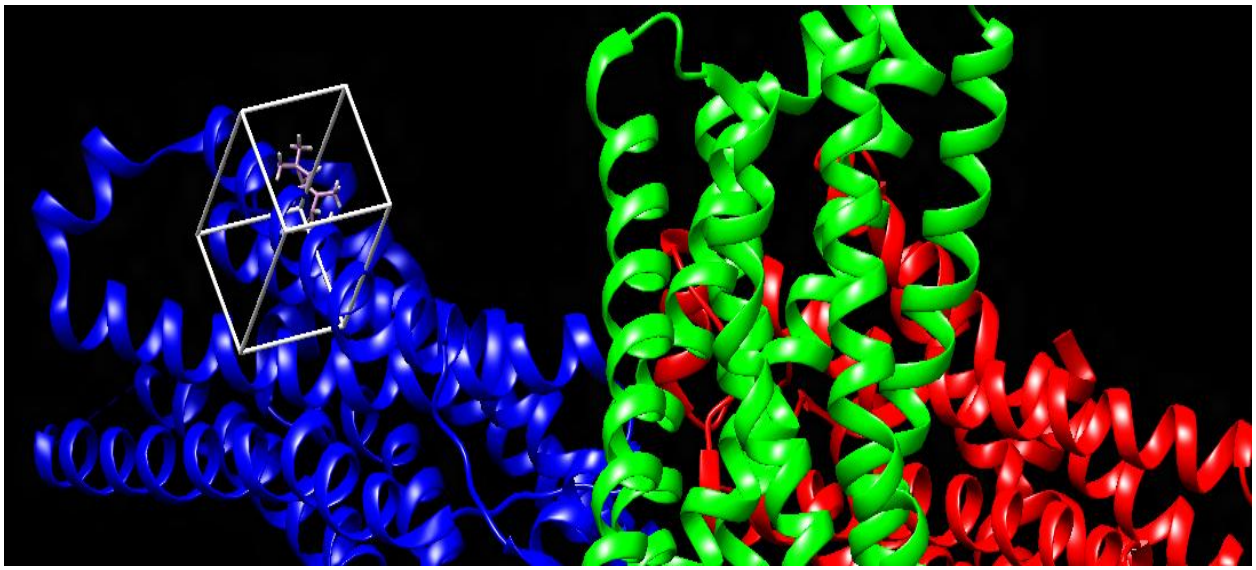
3. Open H2 from PDB



4. Dock prep

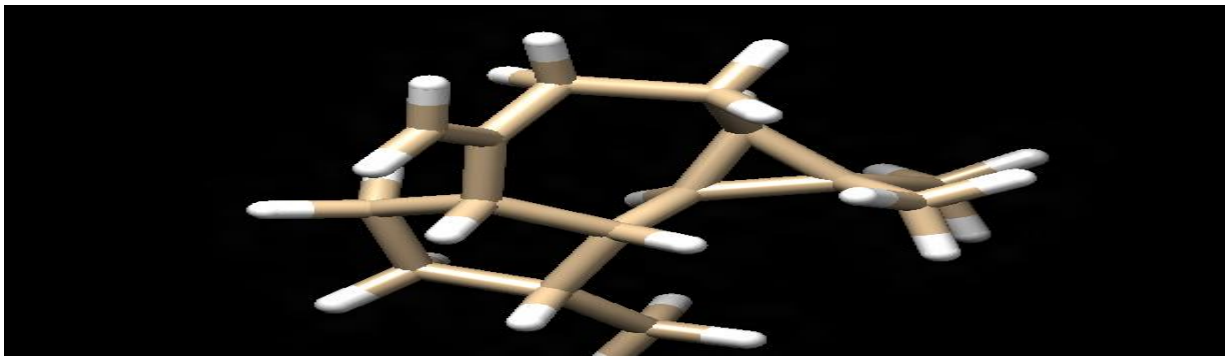


5. Autodock vina

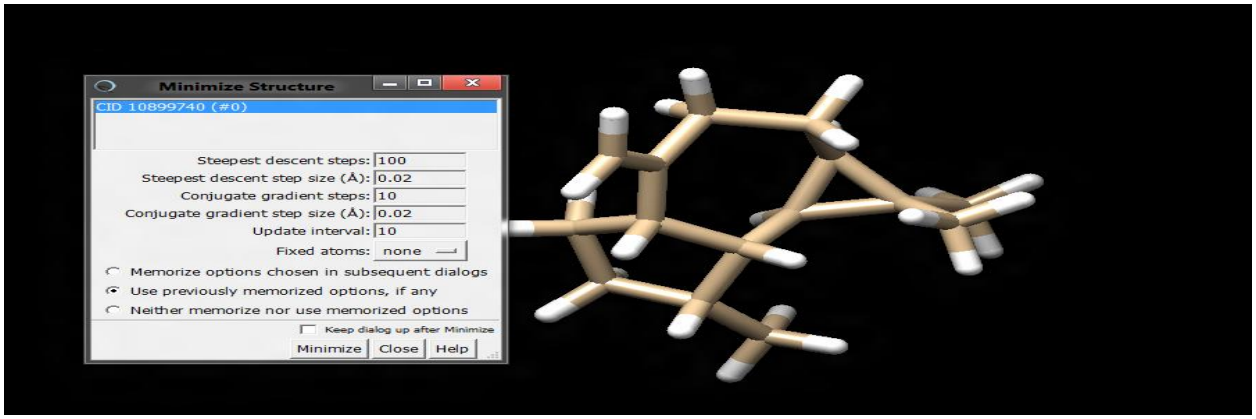


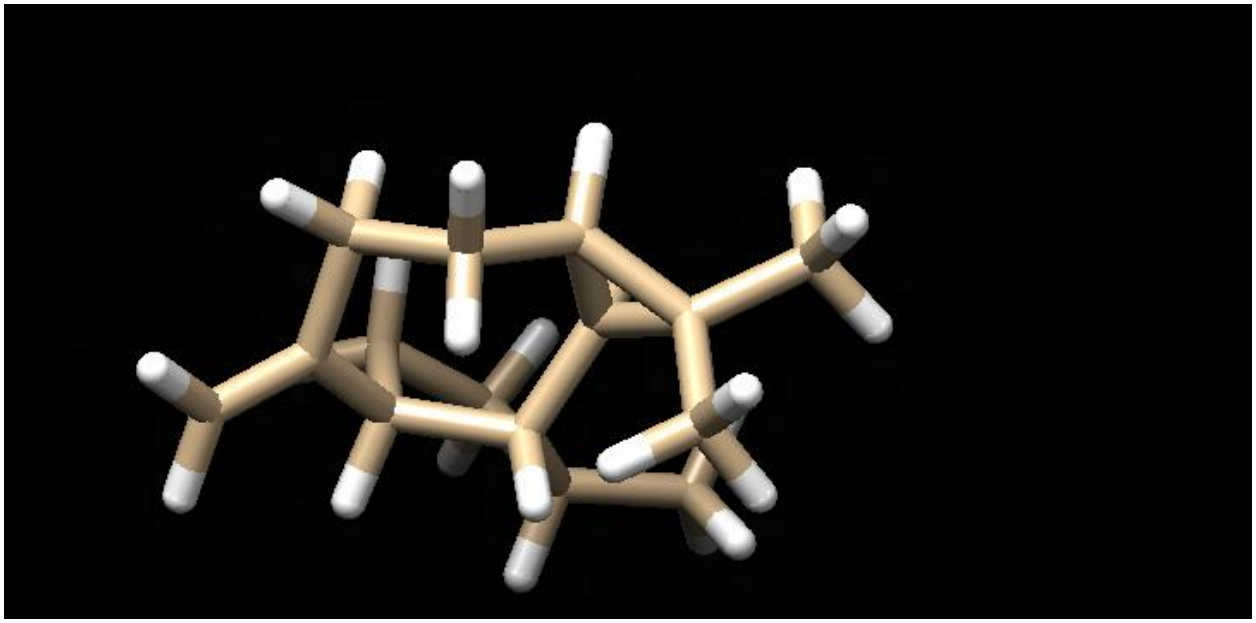
17. ALLORAMADENDRENE

1. Phytochemical from Pubchem

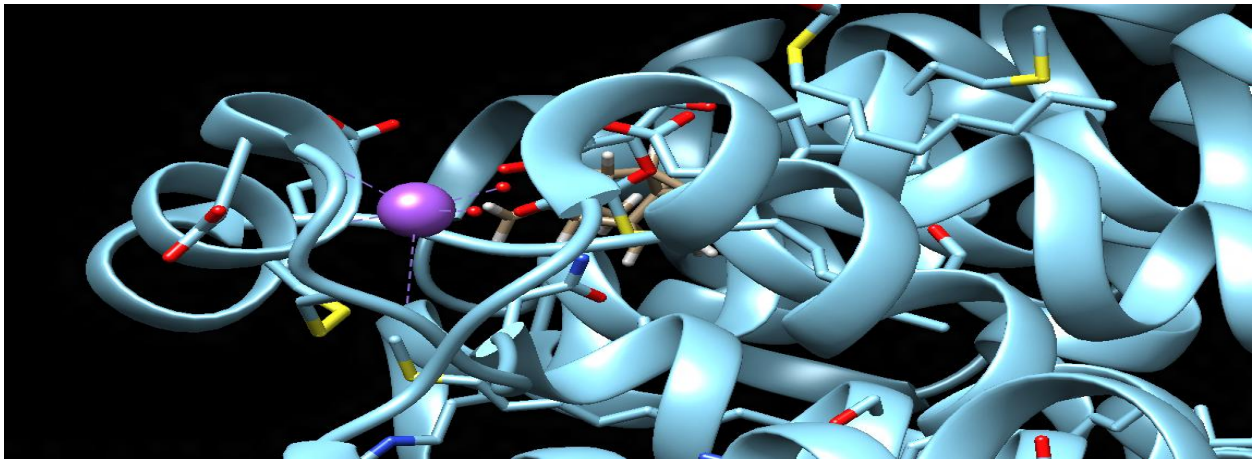


2. Minimize structures

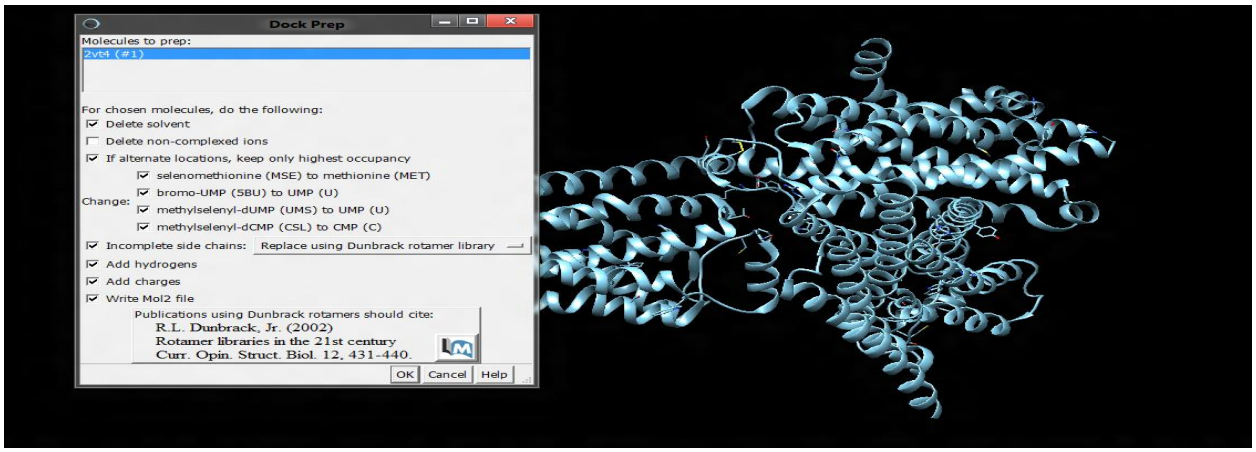




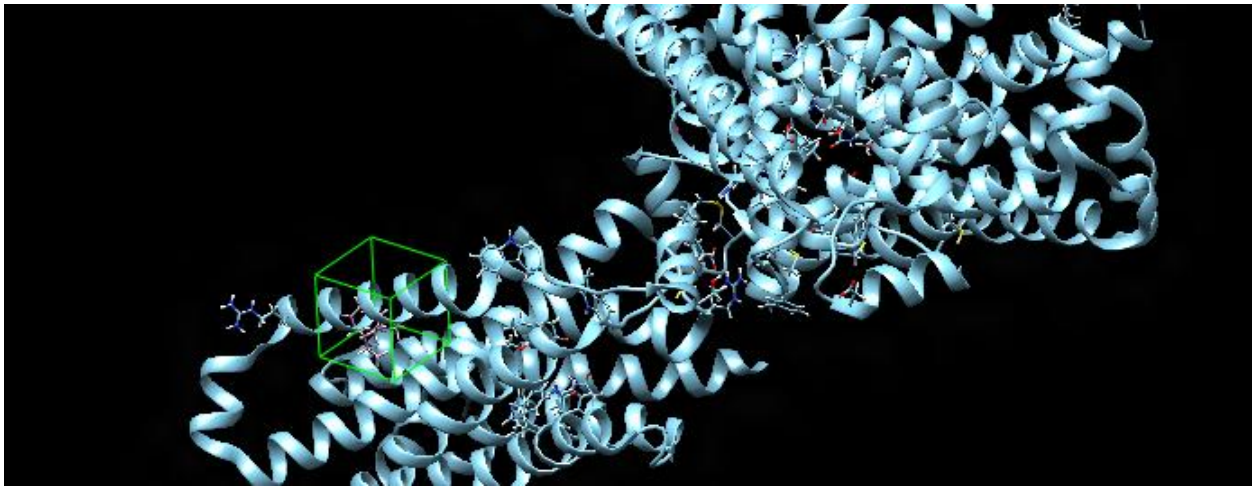
3. Open H2 from PDB



4. Dock prep

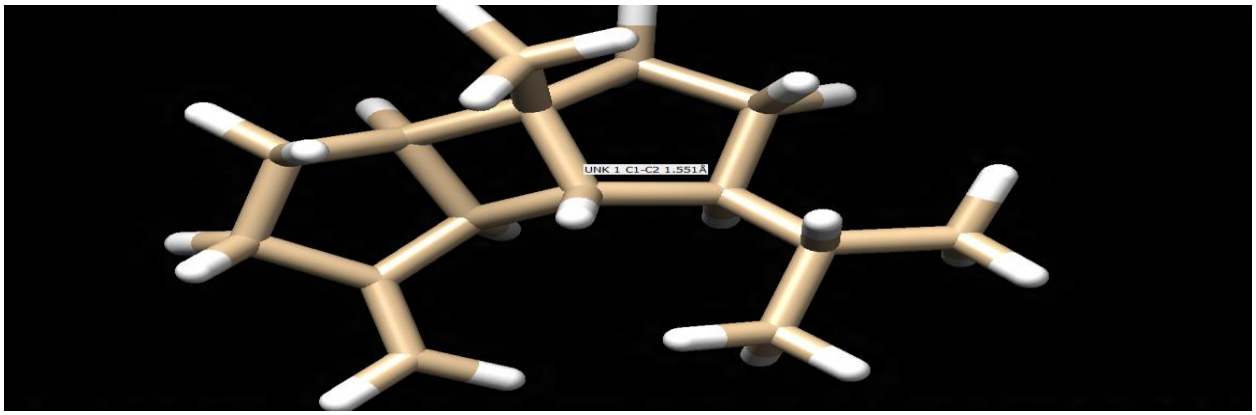


5. Autodock vina

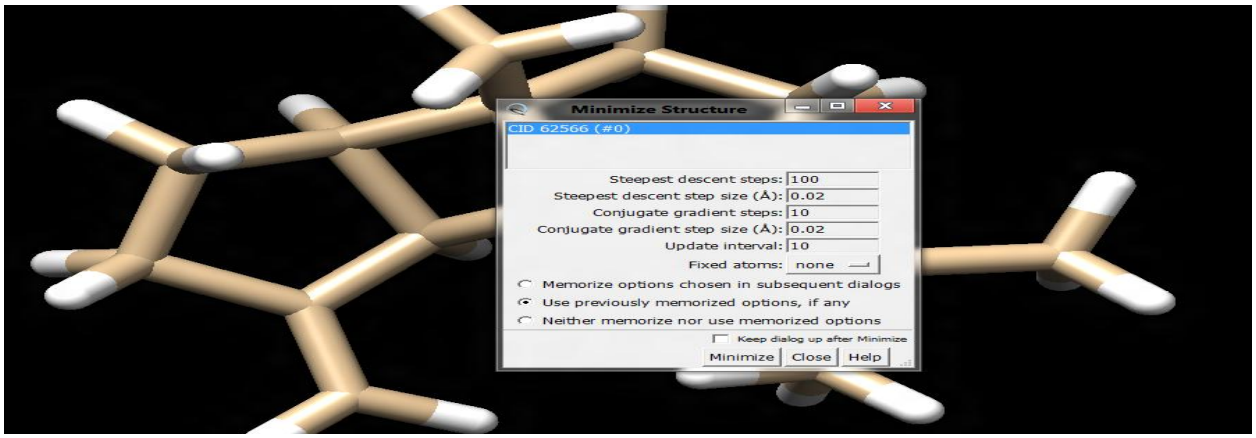


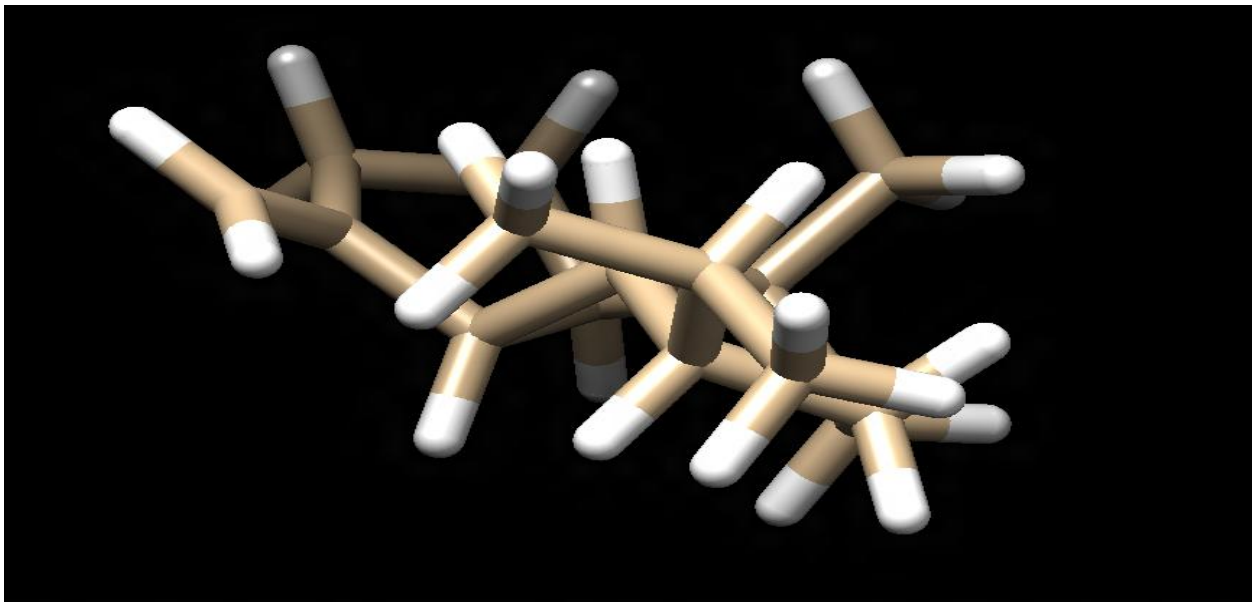
18. BETA-BOURBONENE

1. Phytochemical from Pubchem

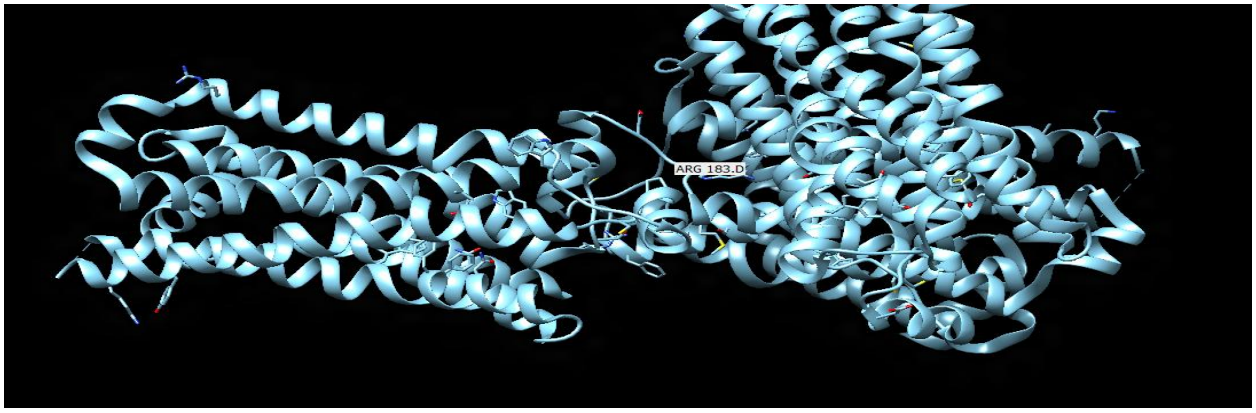


2. Minimize structure

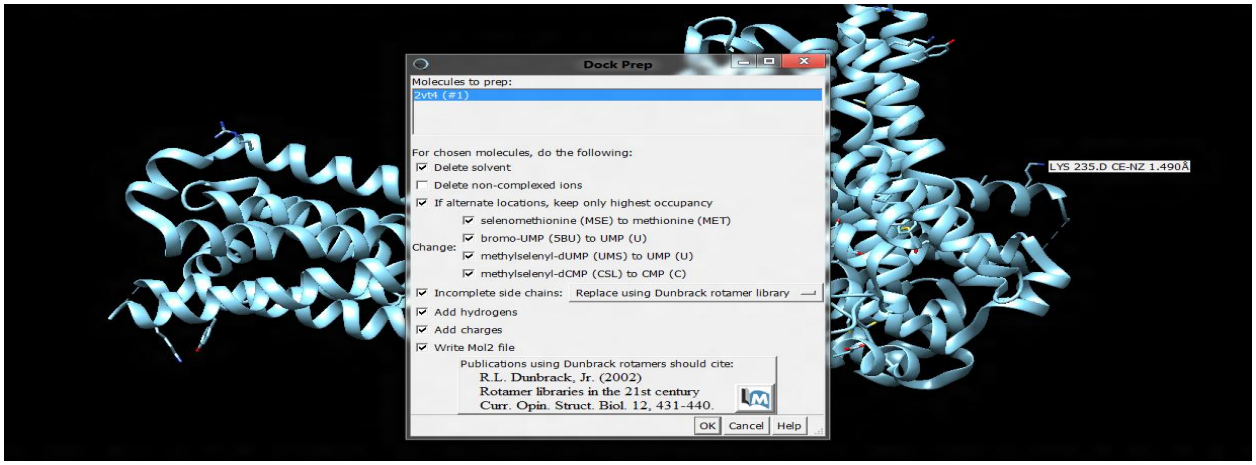




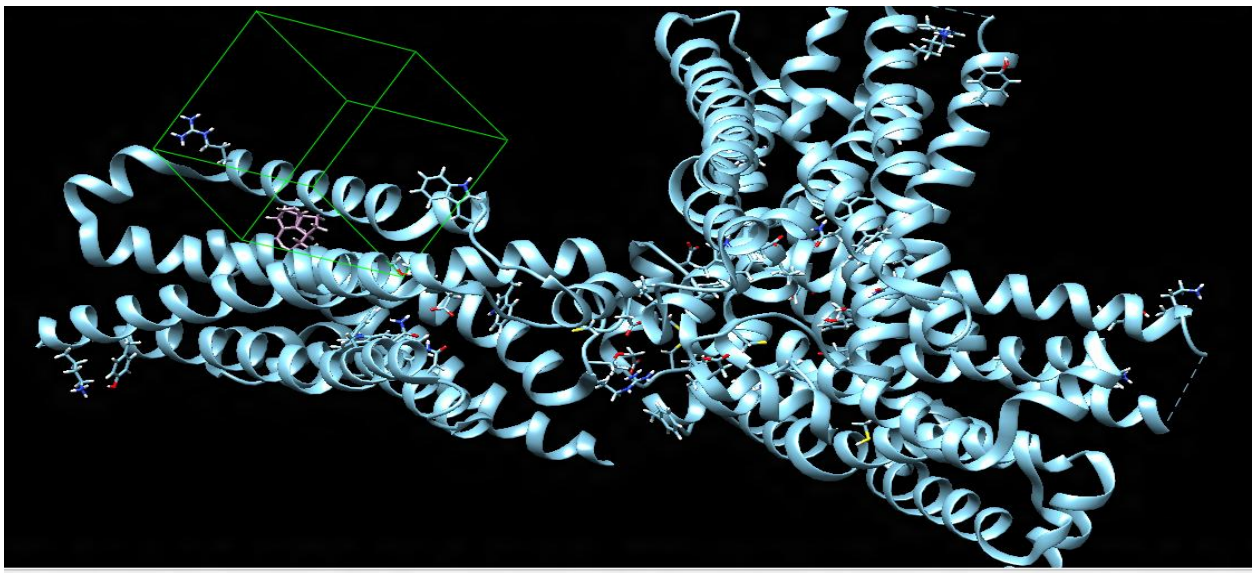
3. Open H2 from PDB



4. Dock prep

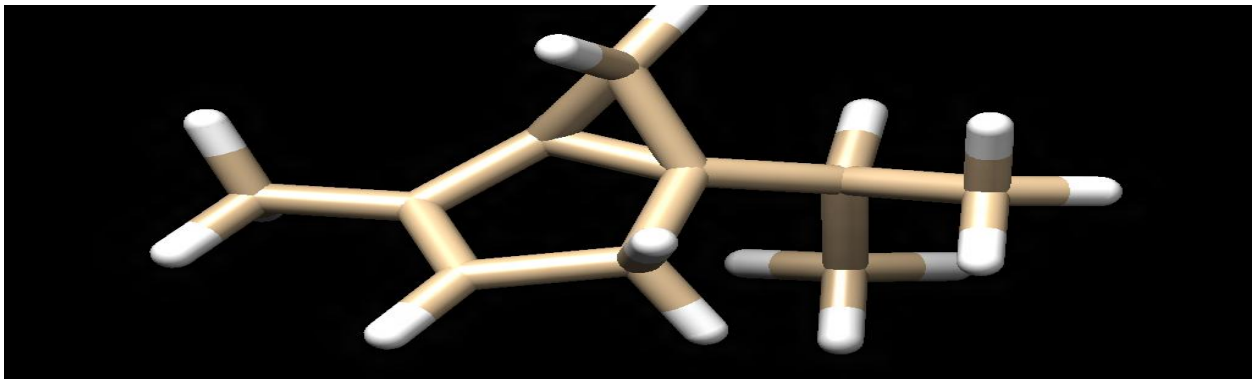


5. Autodock vina

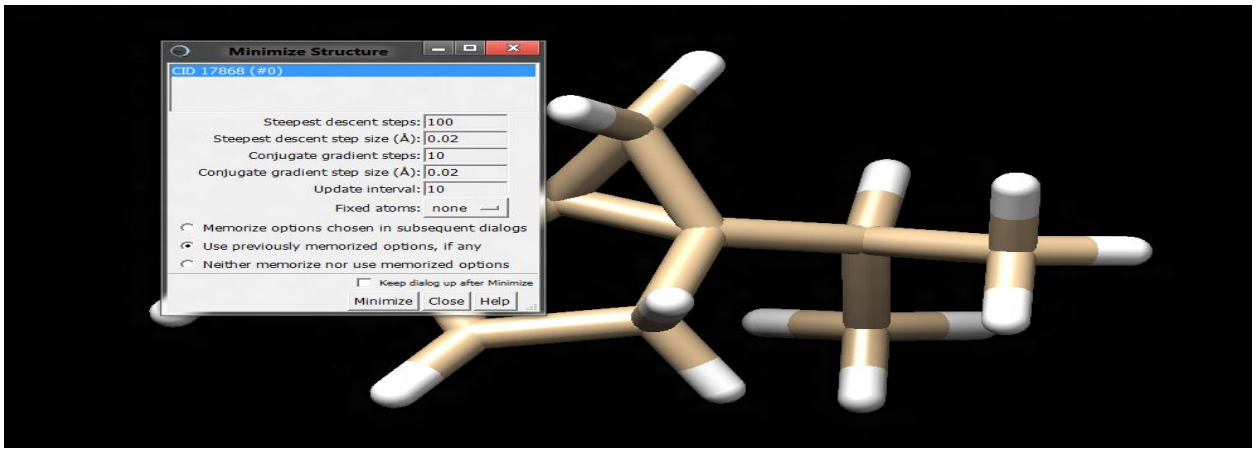


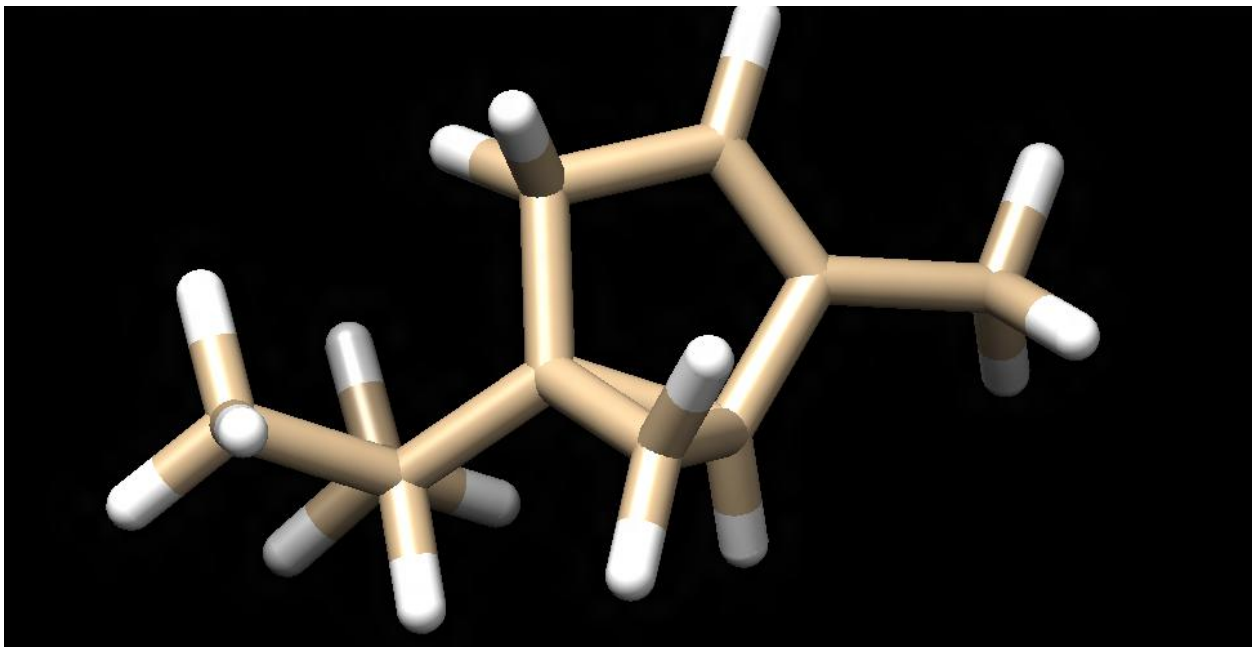
19. ALPHA-THUJENE

1. Phytochemical from Pubchem

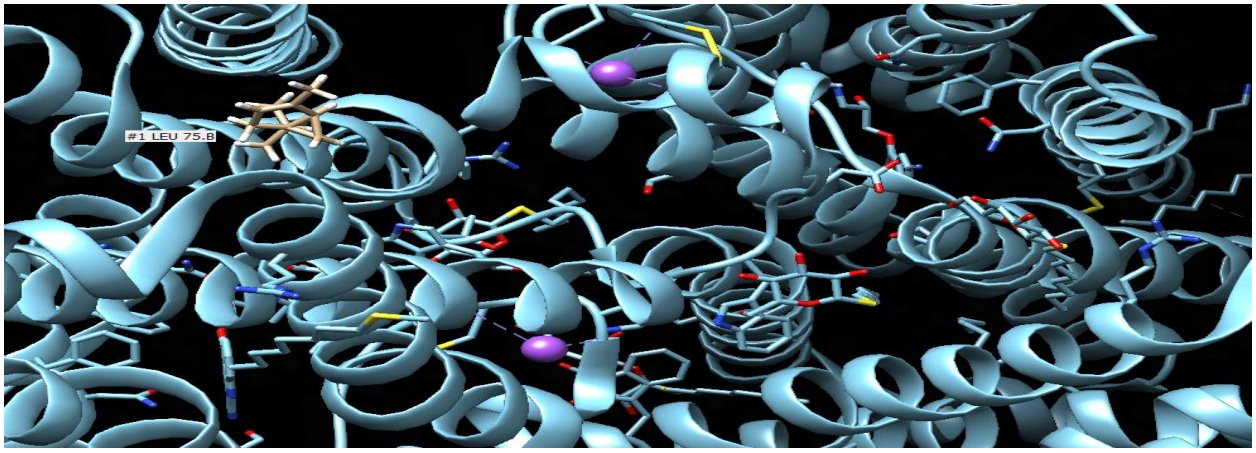


2. Minimize structures

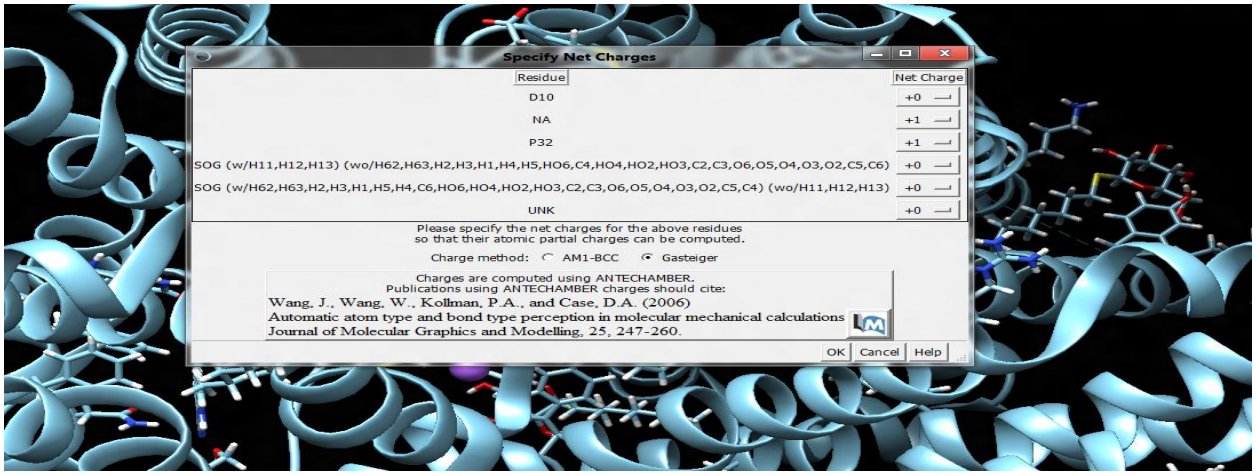




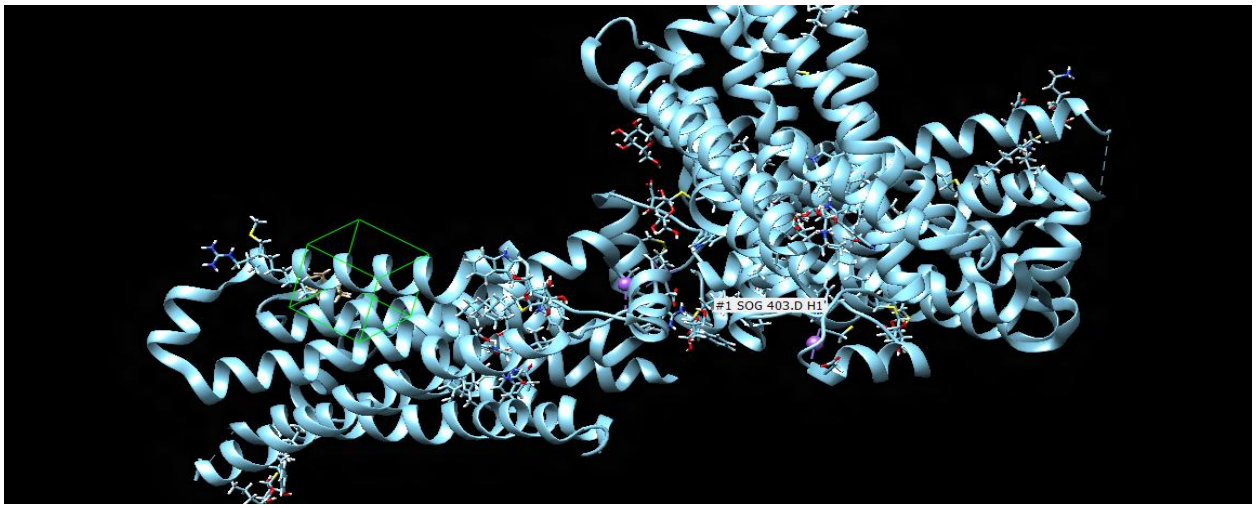
3. Open H2 from PDB



4. Dock prep

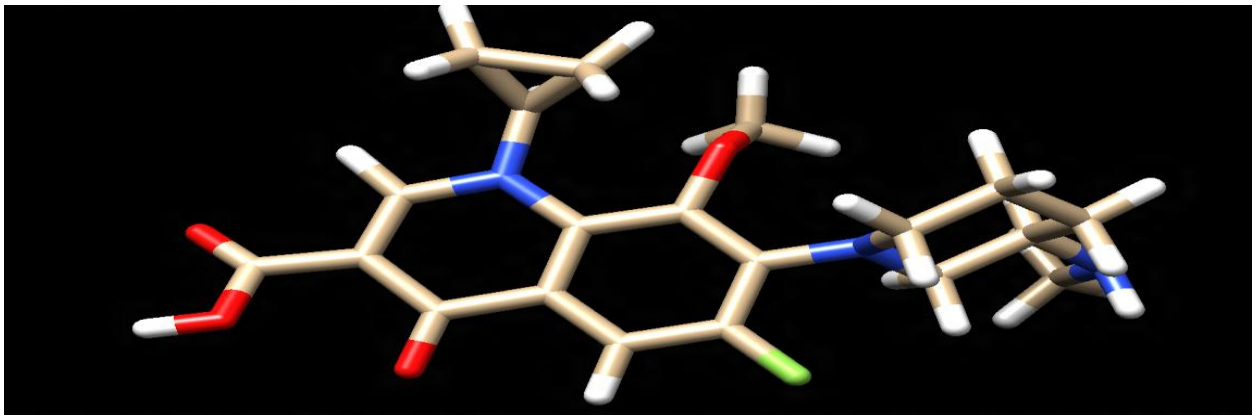


5. Autodock vina

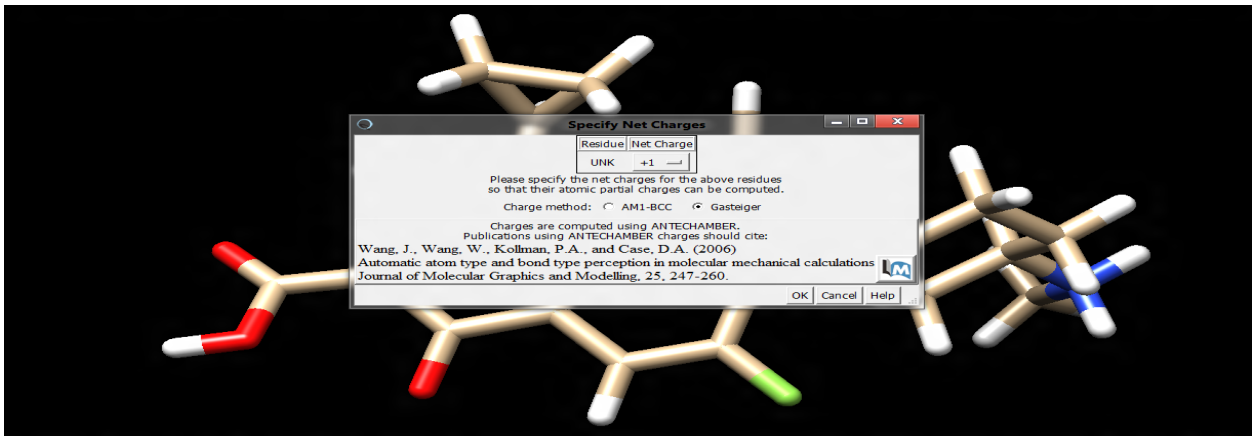


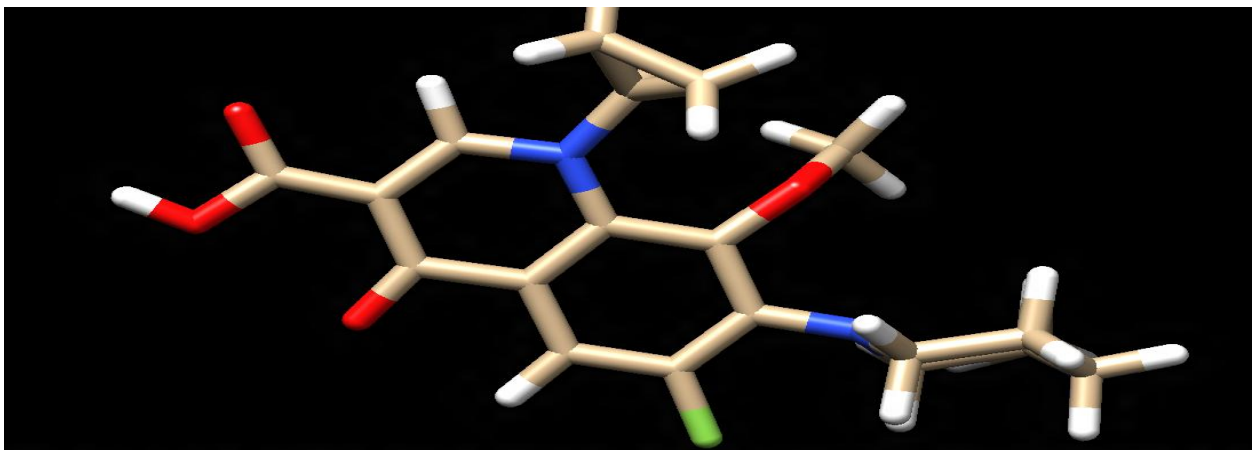
20. BETA-CARYOPHYLLENE

1. Phytochemical from Pubchem

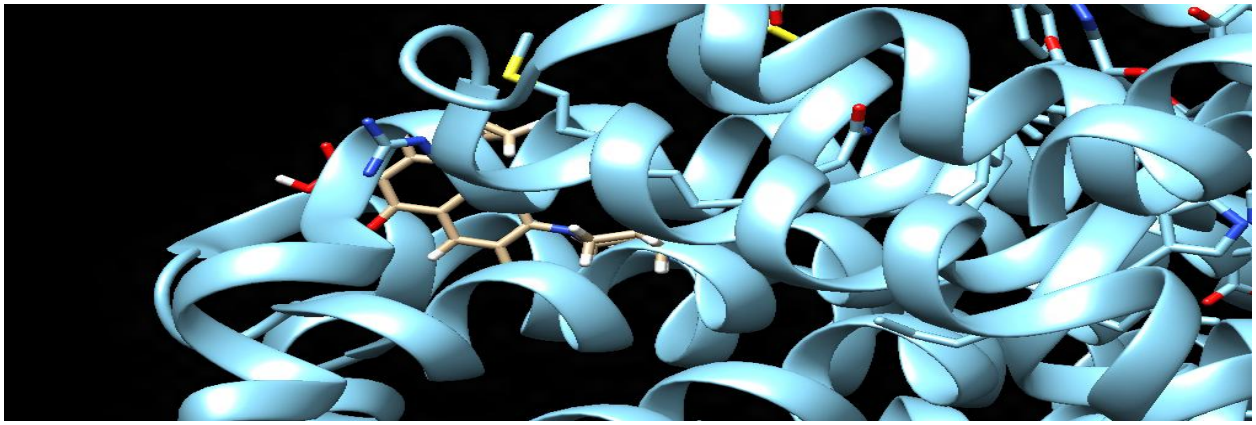


2. Minimize structures

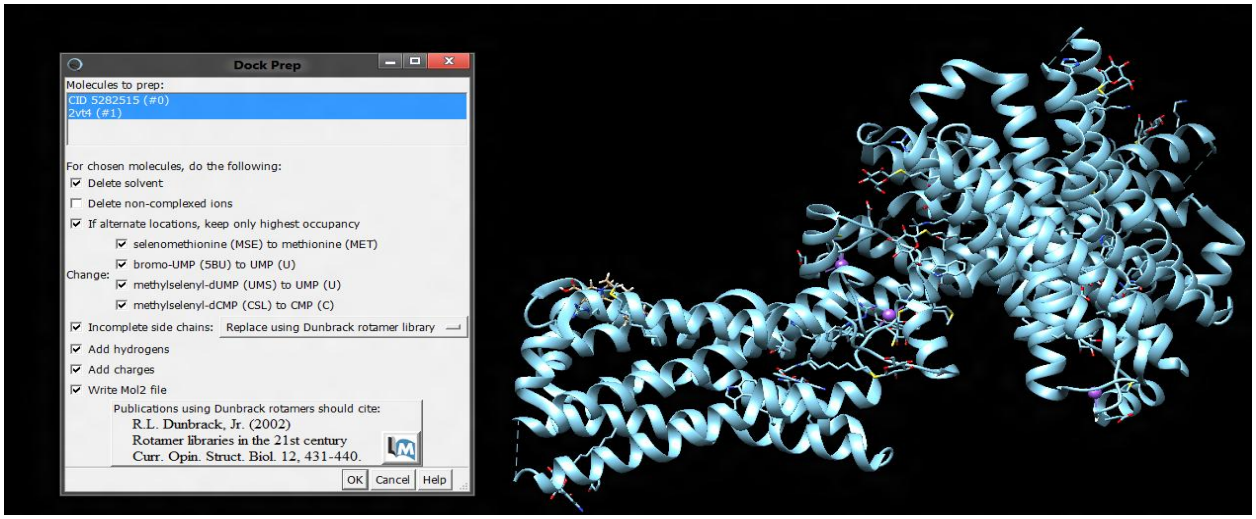




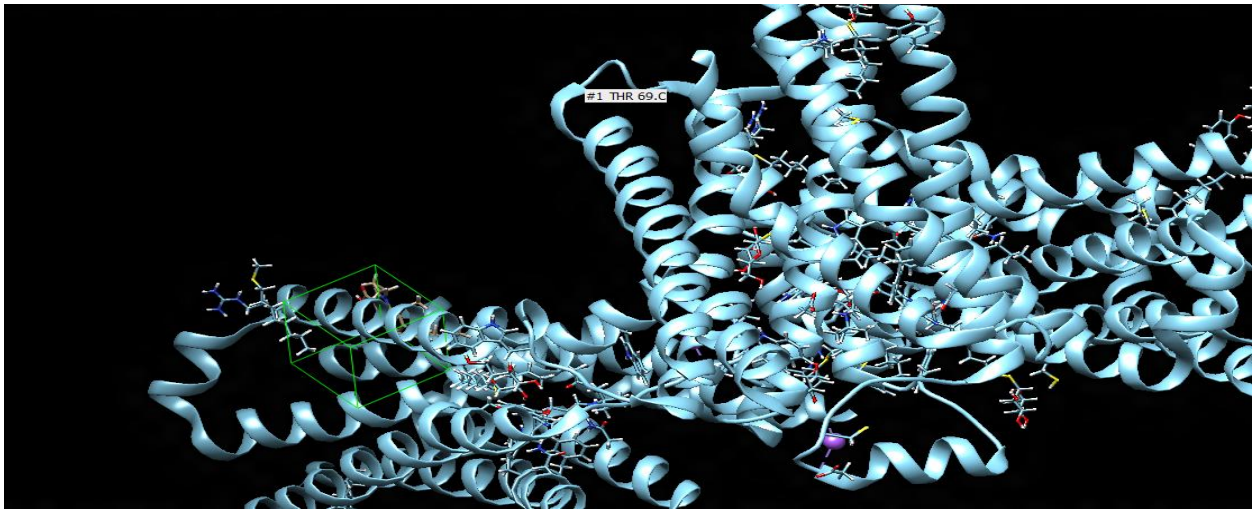
3. Open H2 from PDB



4. Dock prep

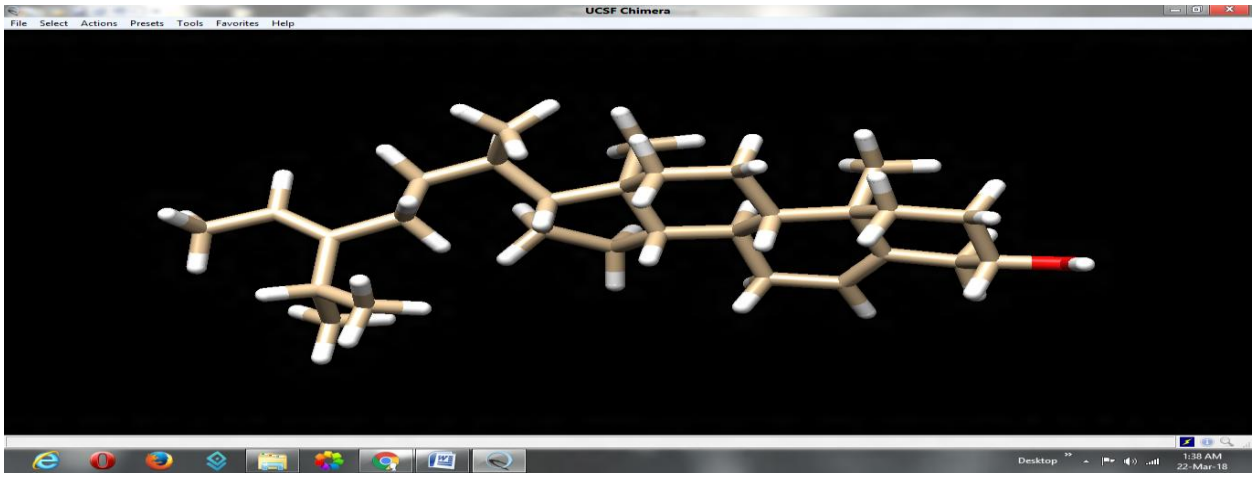


5. Autodock vina

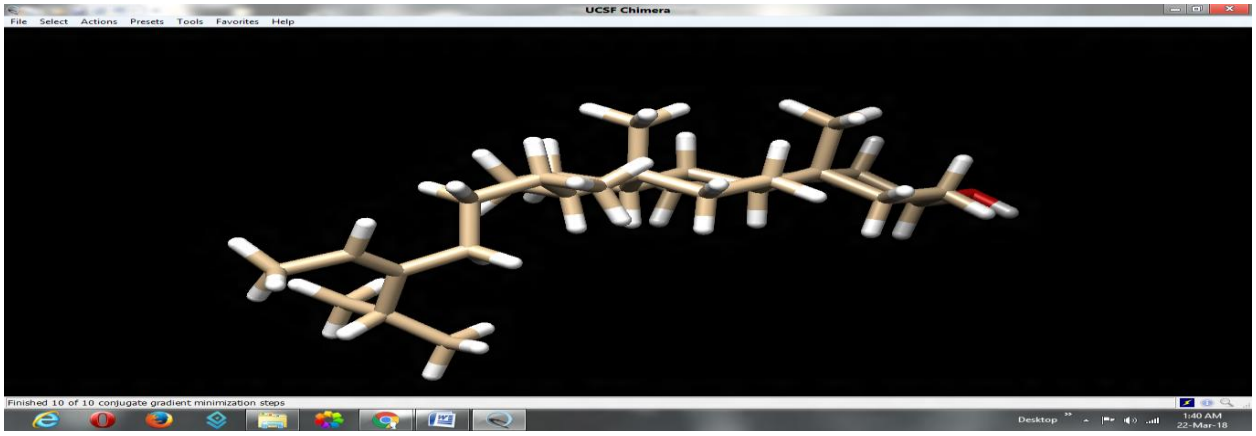


21. ISOFUCOSTEROL

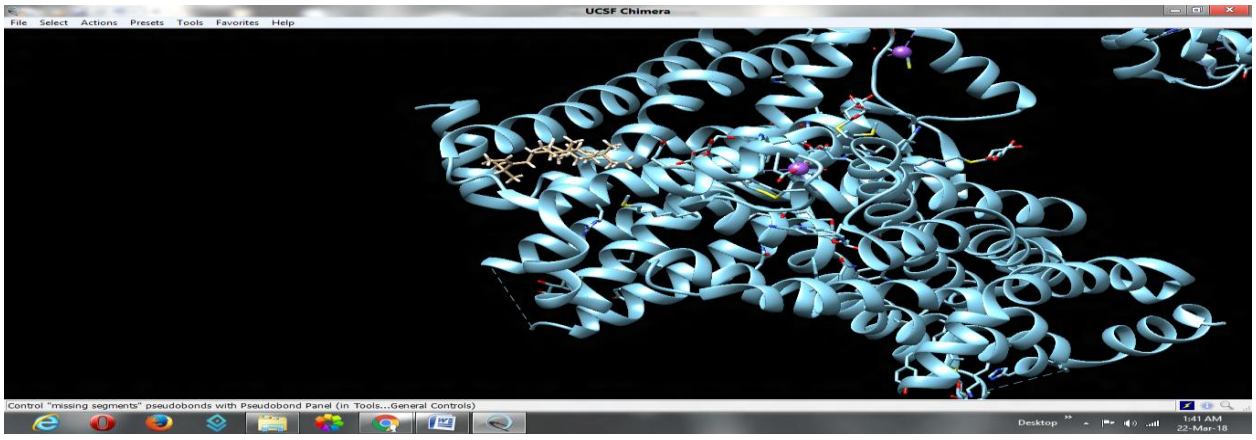
1. Phytochemical from Pubchem



2. Minimize structures

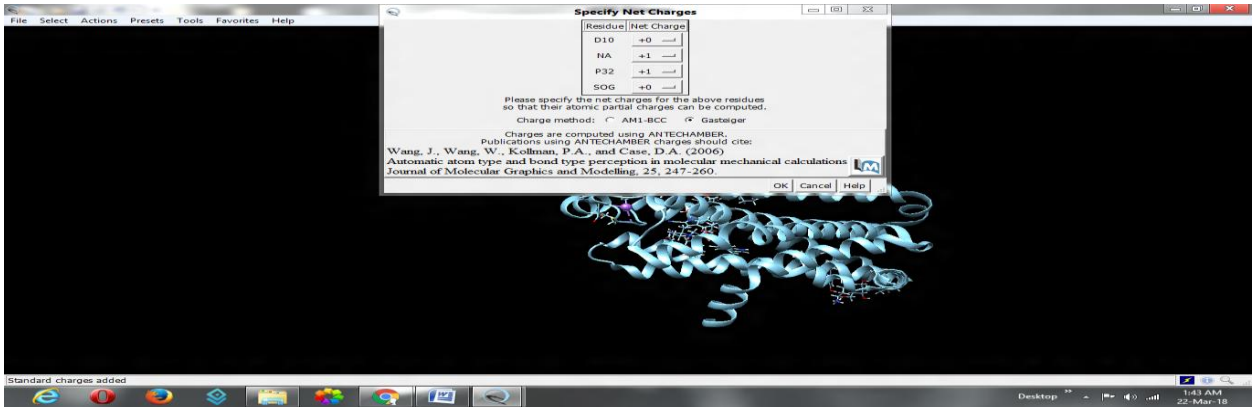


3. Open H2 from PDB



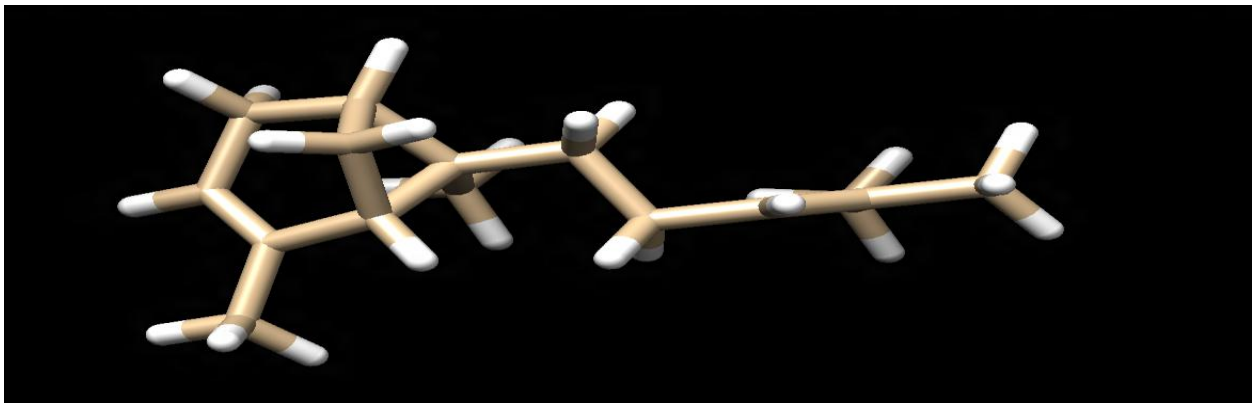
4. Dock prep

5. Autodock vina

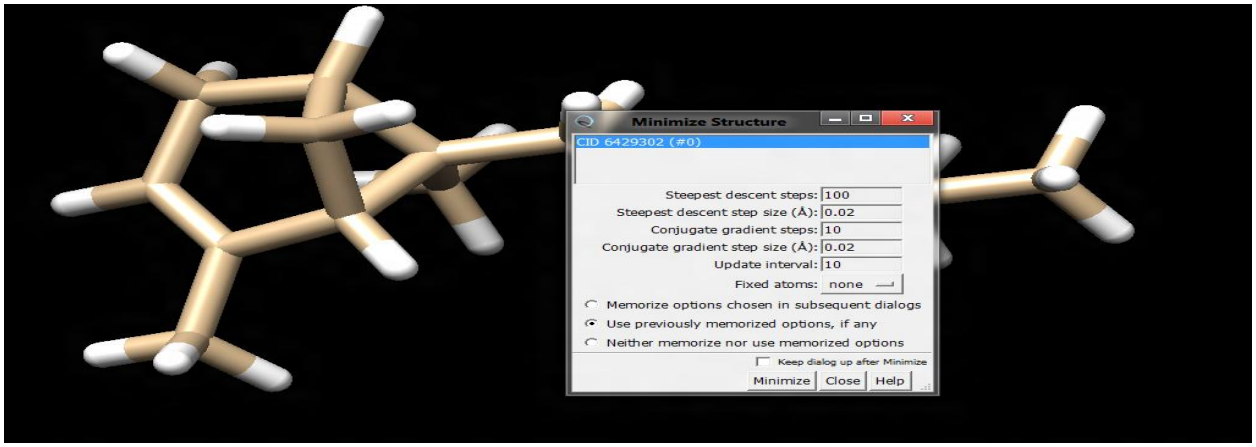


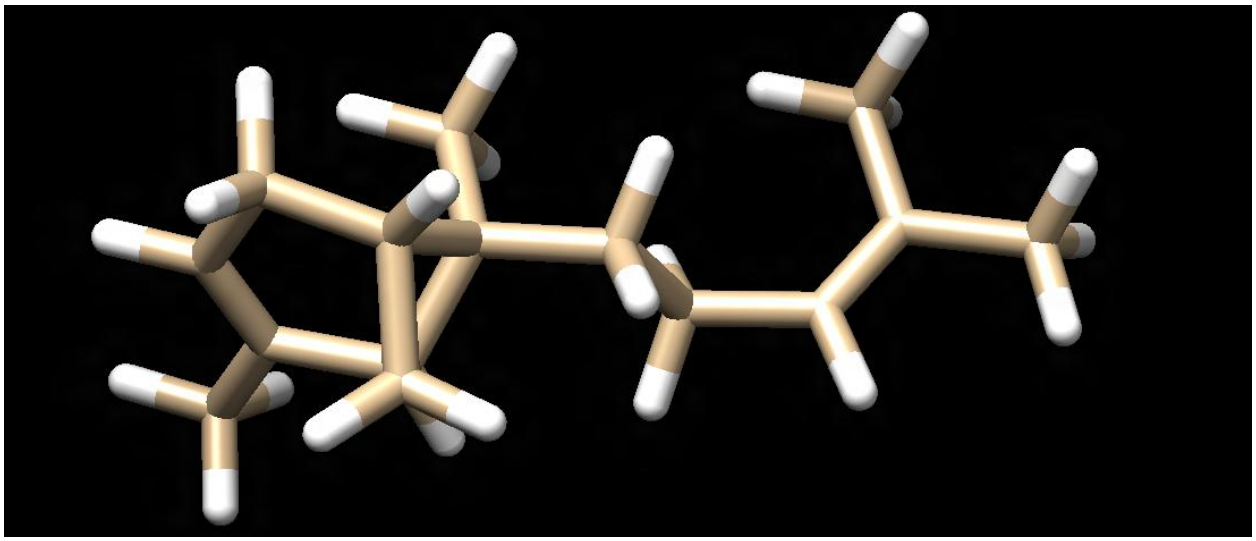
22. BERGAMOTENE

1. Phytochemicals from Pubchem

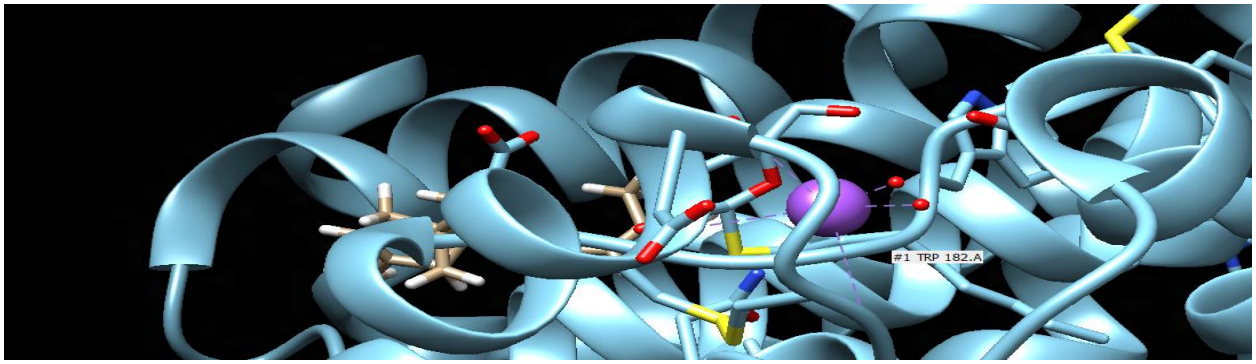


2. Minimize structures

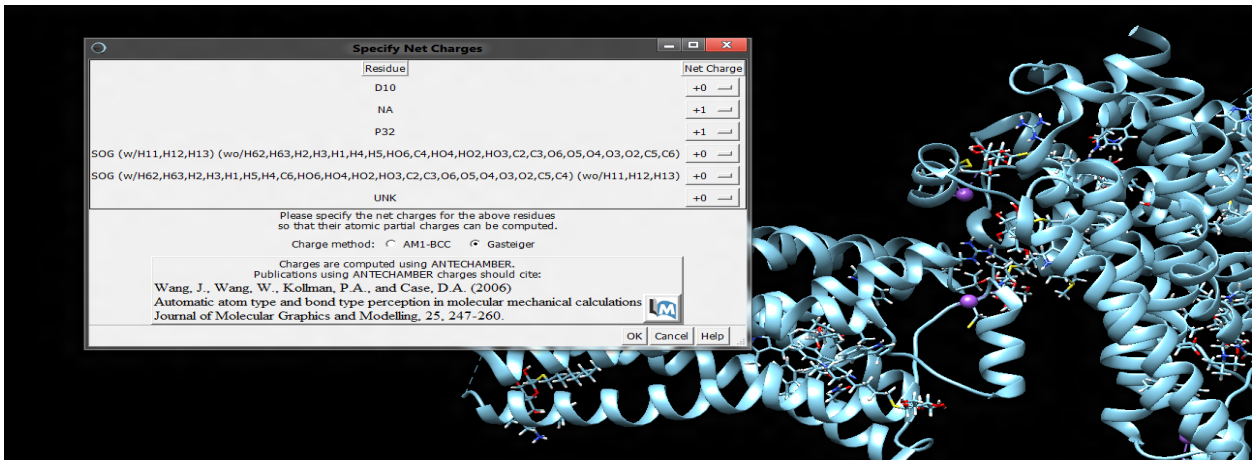




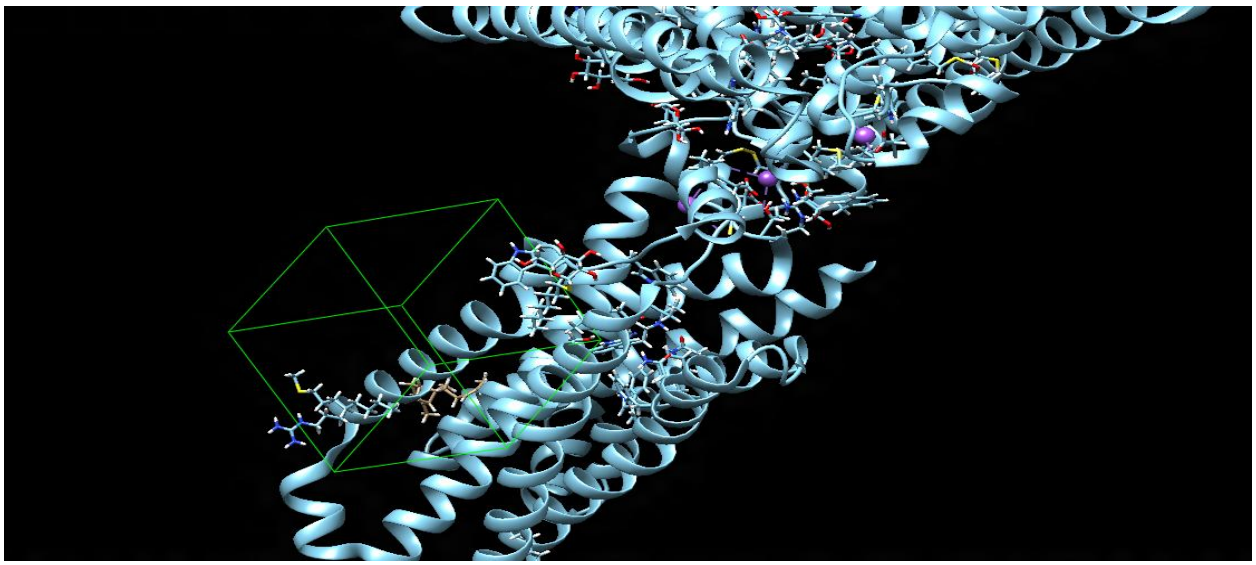
3. Open H2 from PDB



4. Dock prep

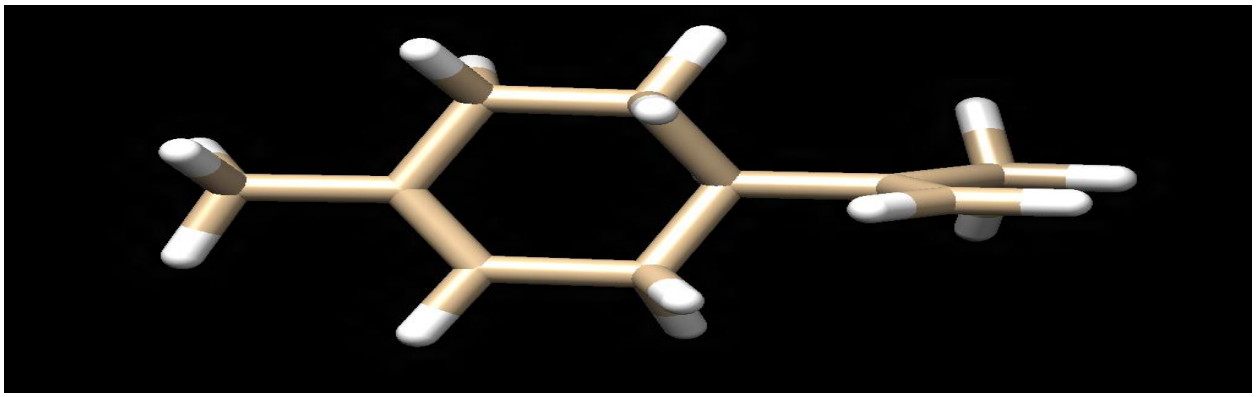


5. Autodock vina

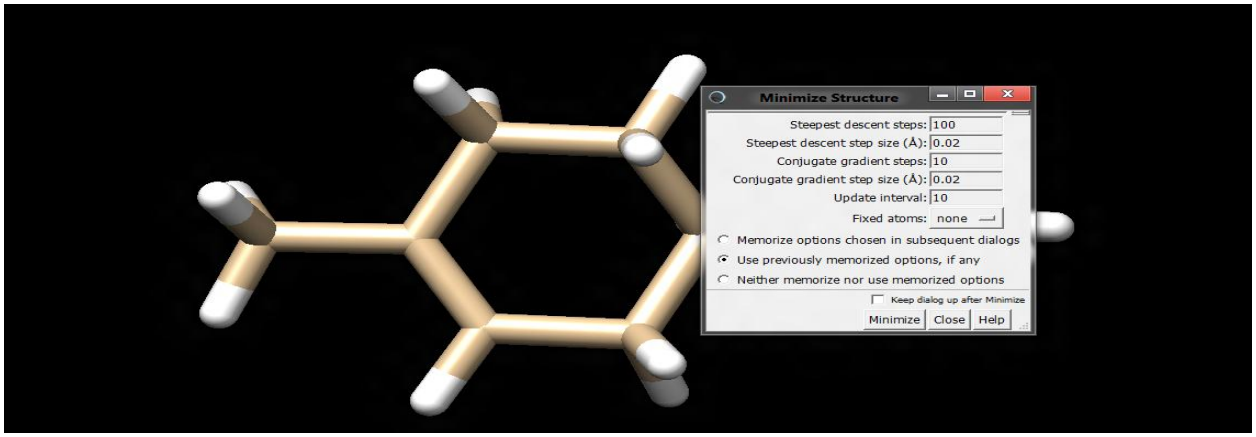


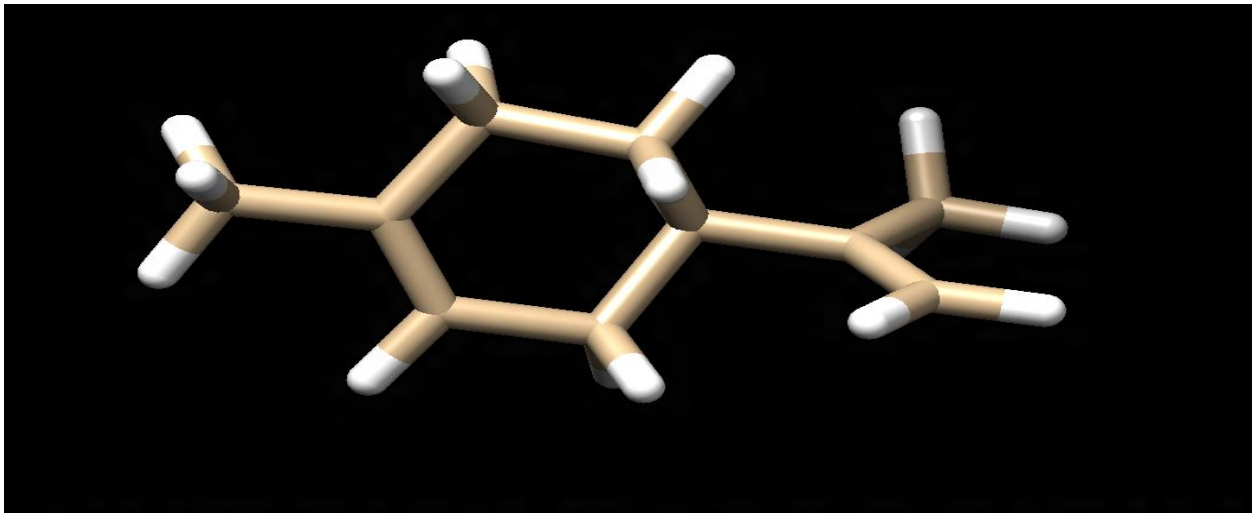
23. LIMONENE

1. Phytochemical from Pubchem

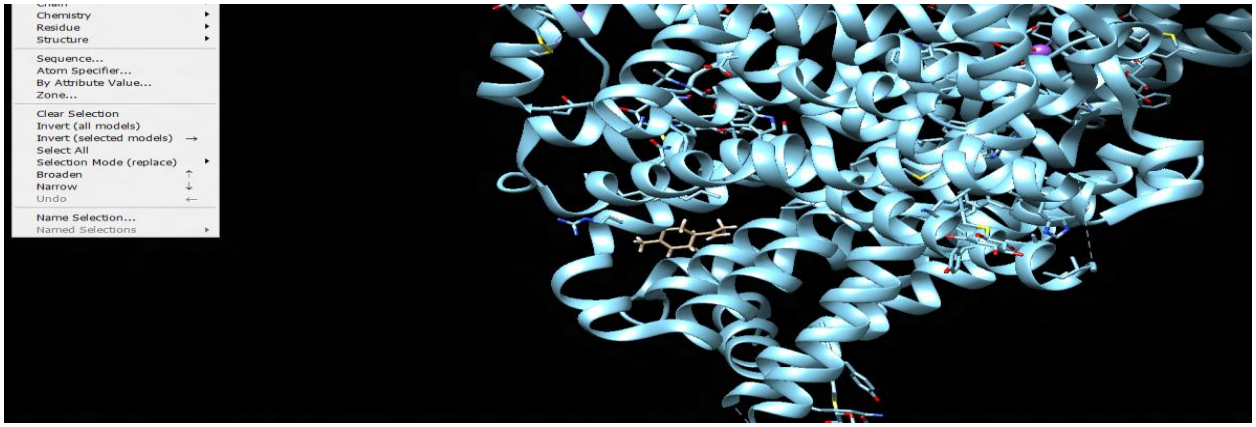


2. Minimize structures

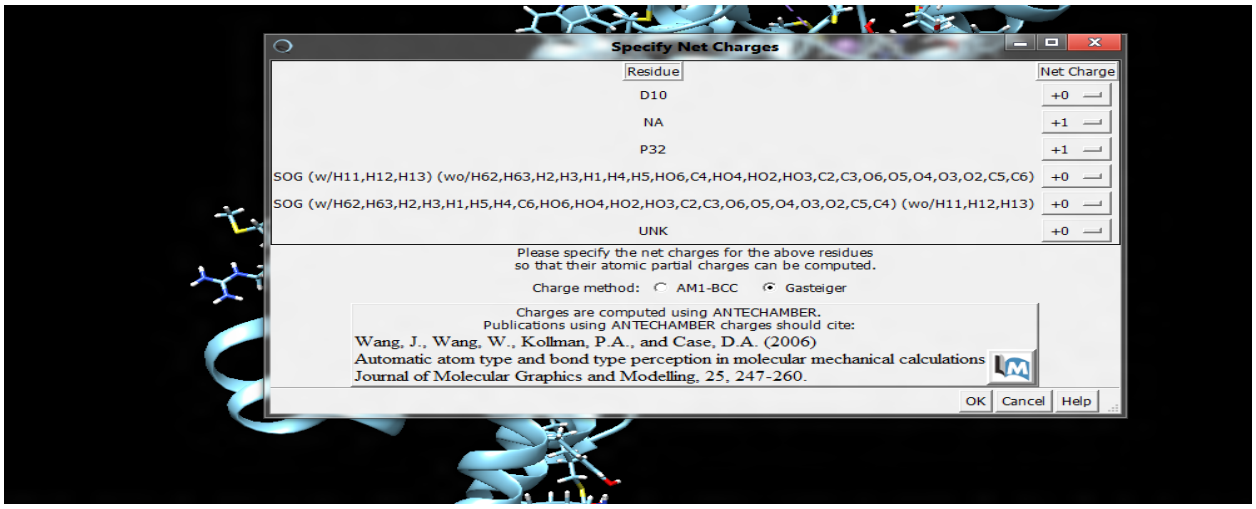




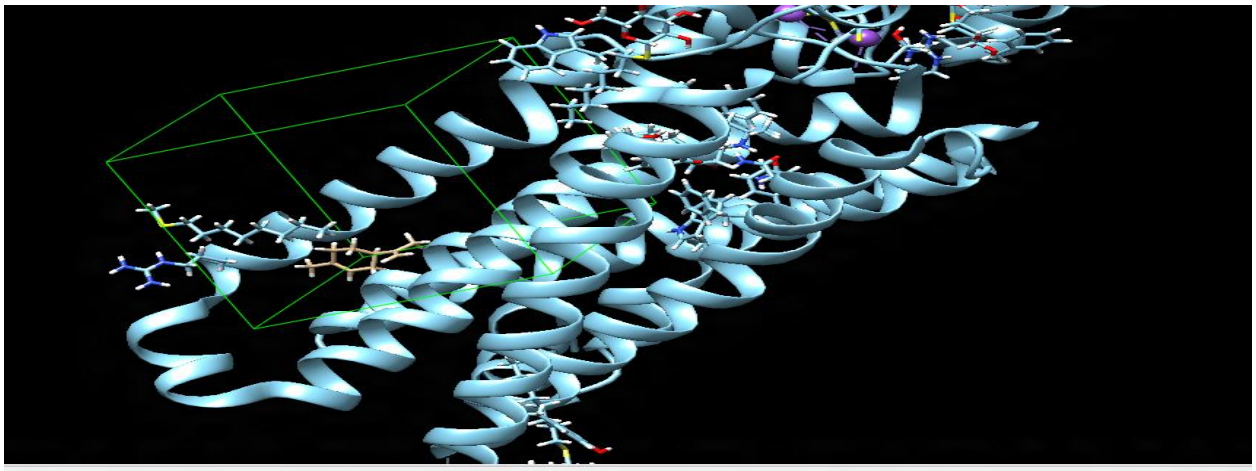
3. Open H2 from PDB



4. Dock prep

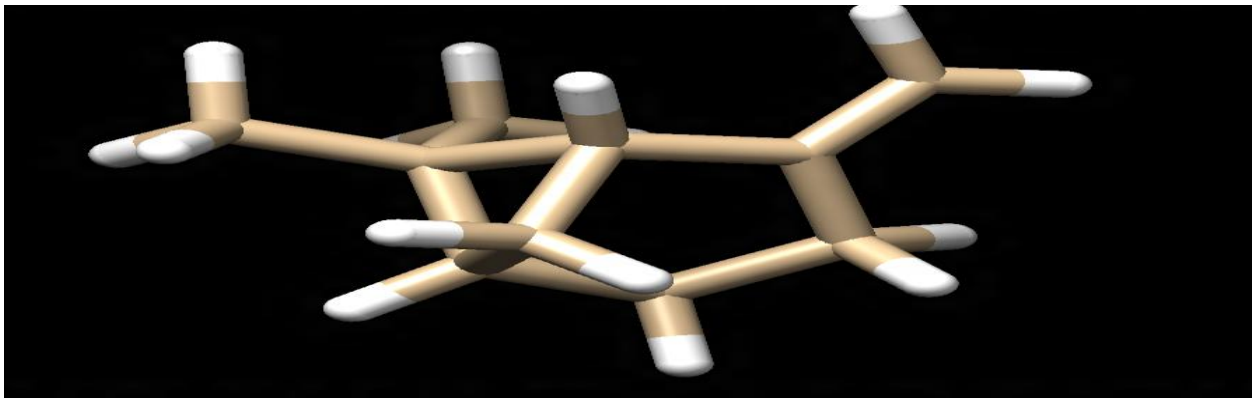


5. Autodock vina

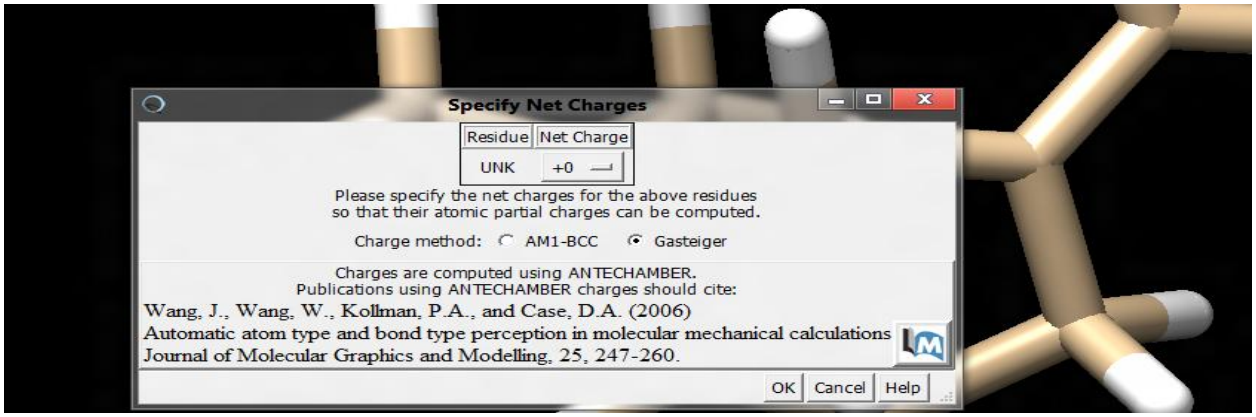


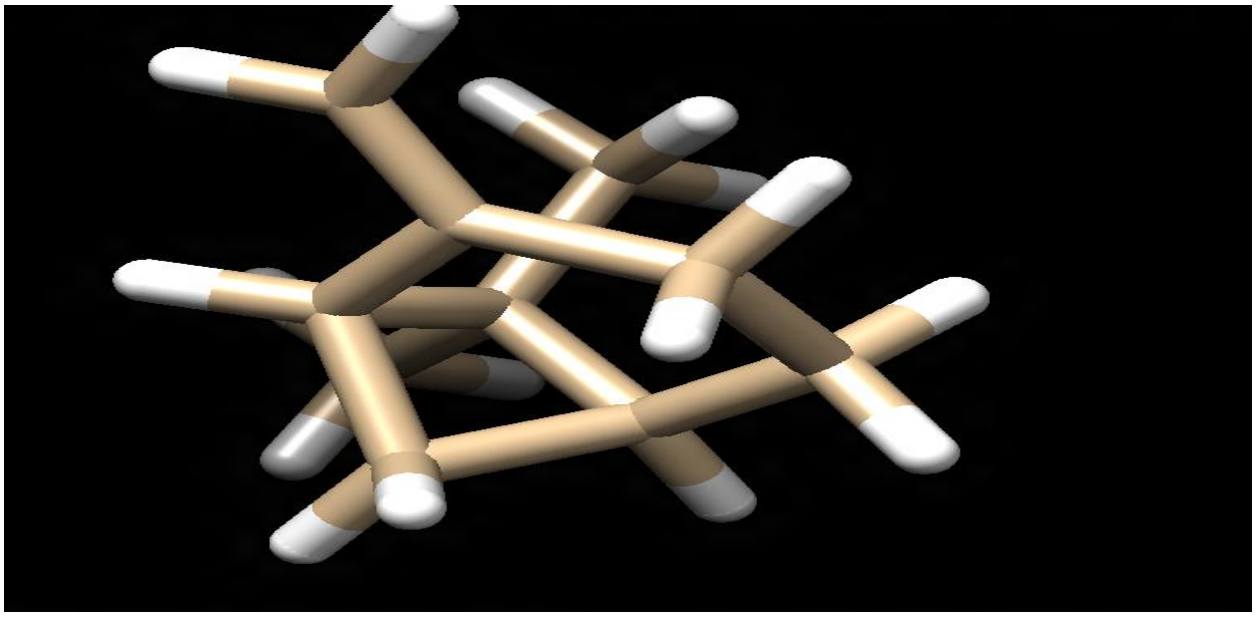
24. BETA-PINENE

1. Phytochemical from Pubchem

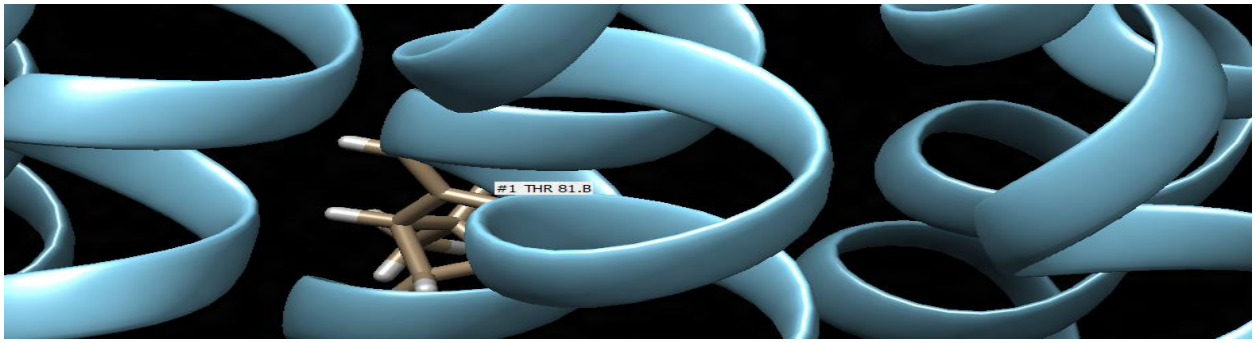


2. Minimize structures

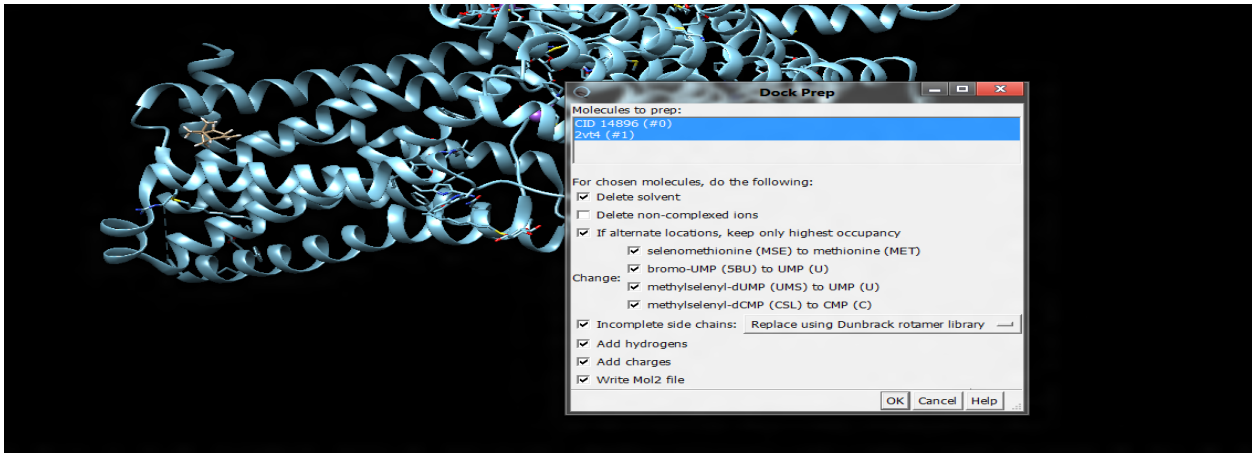




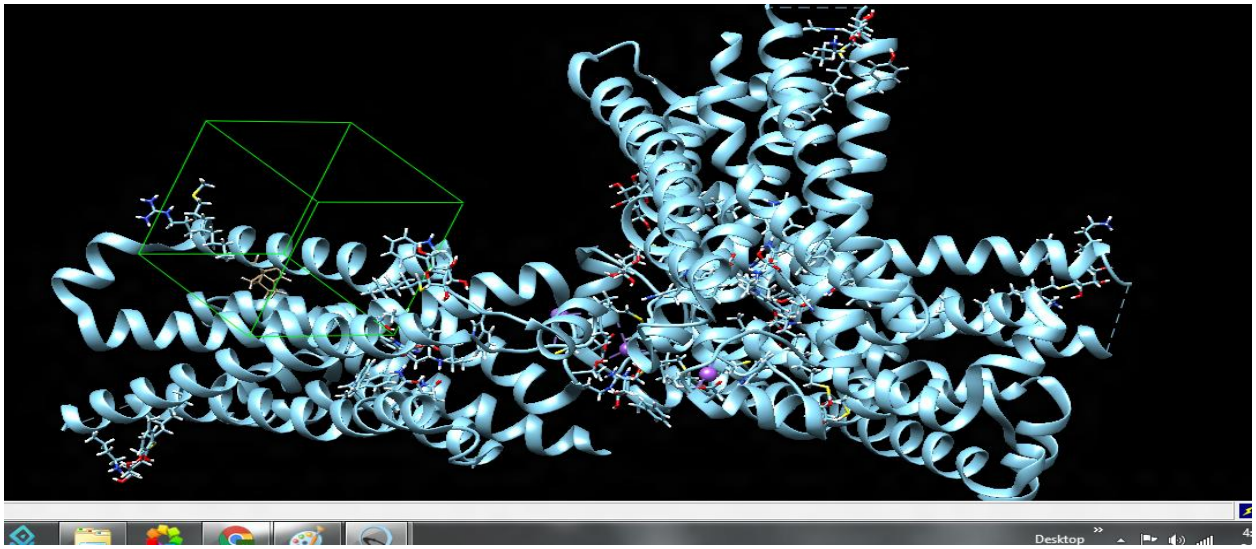
3. Open H2 from PDB



4. Dock prep

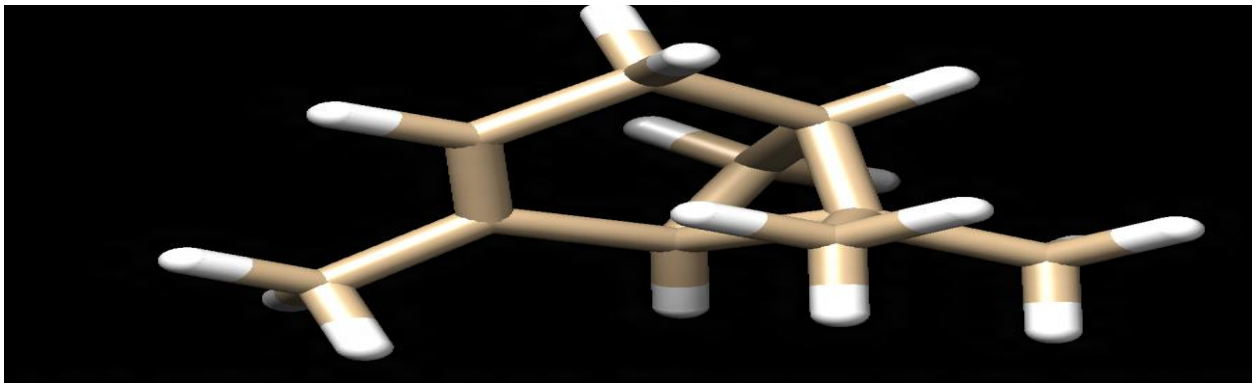


5. Autodock vina

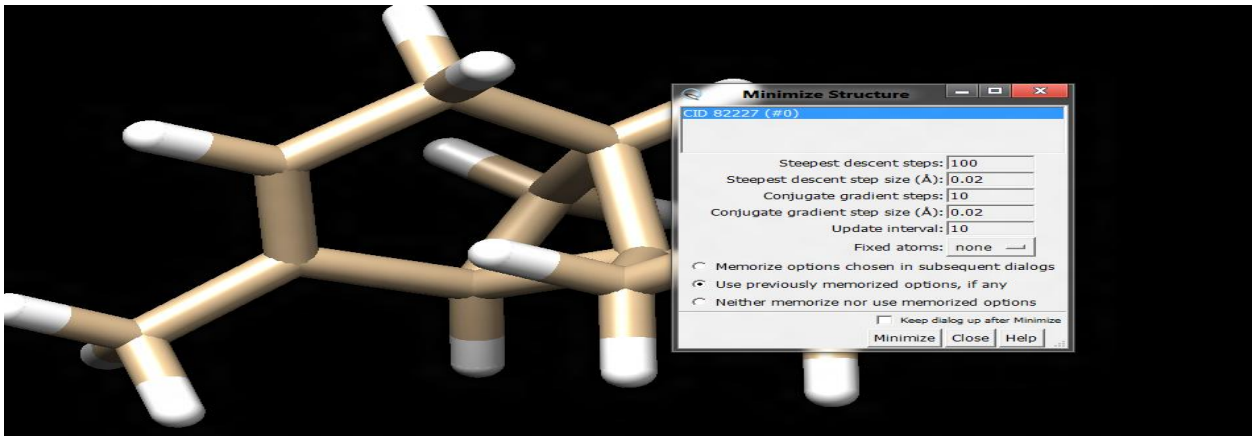


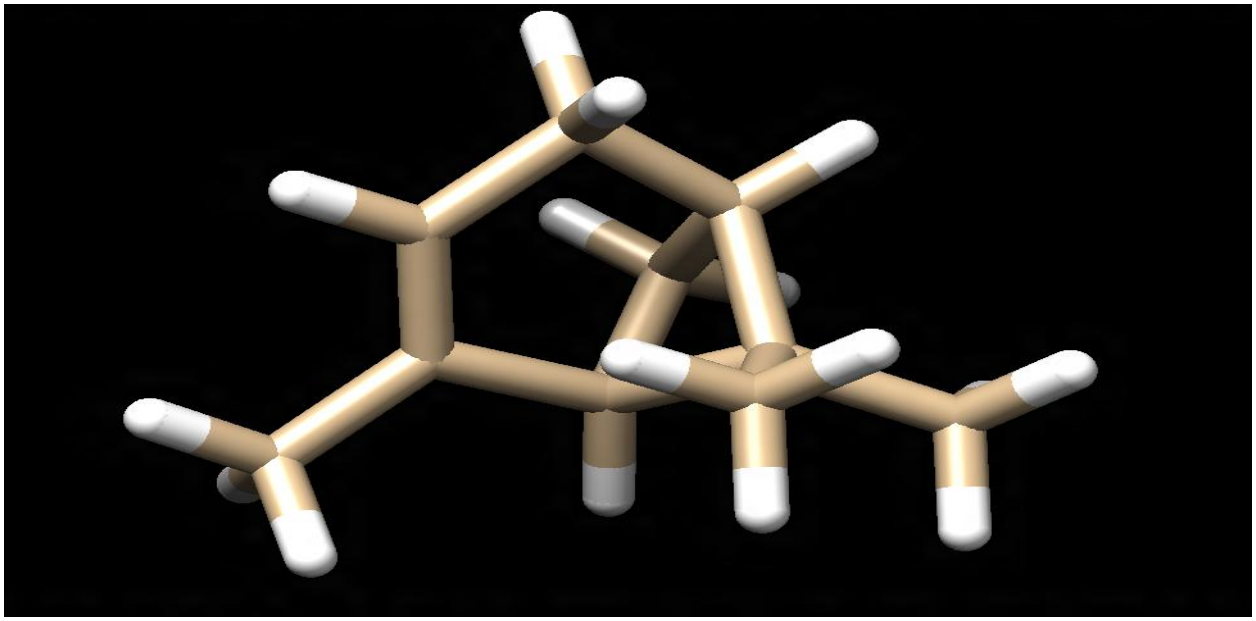
25. ALPHA PINENE

1. Phytochemicals from Pubchem

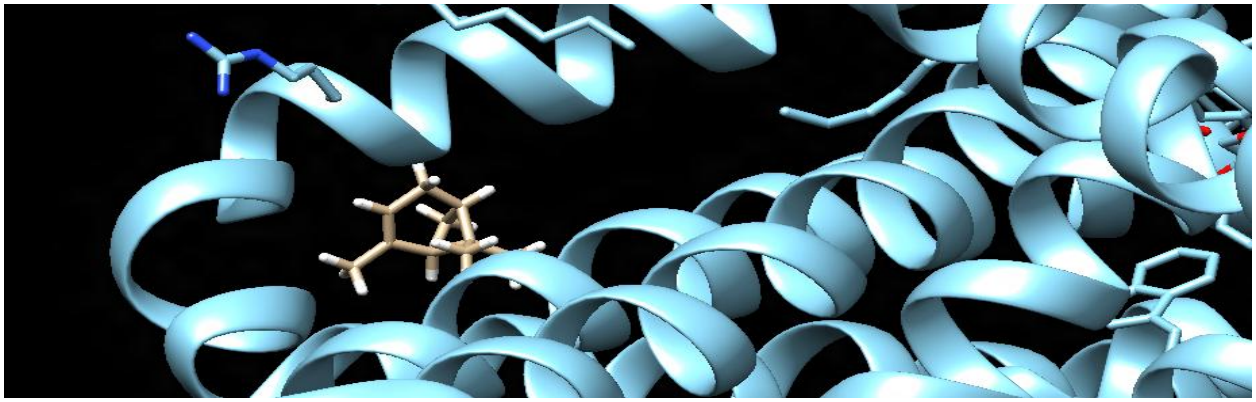


2. Minimize structures

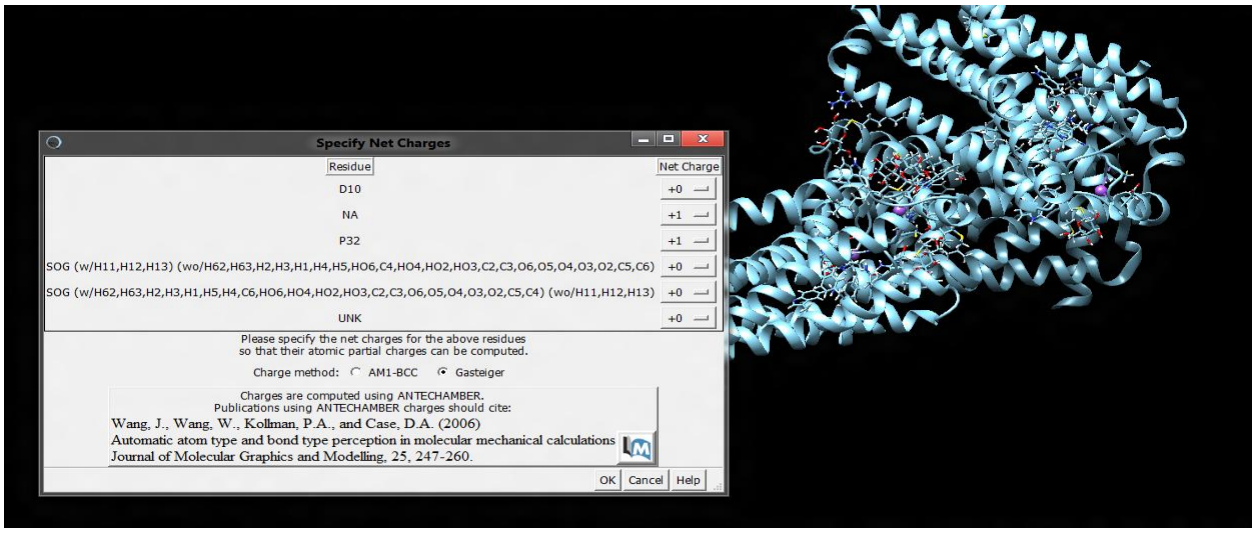




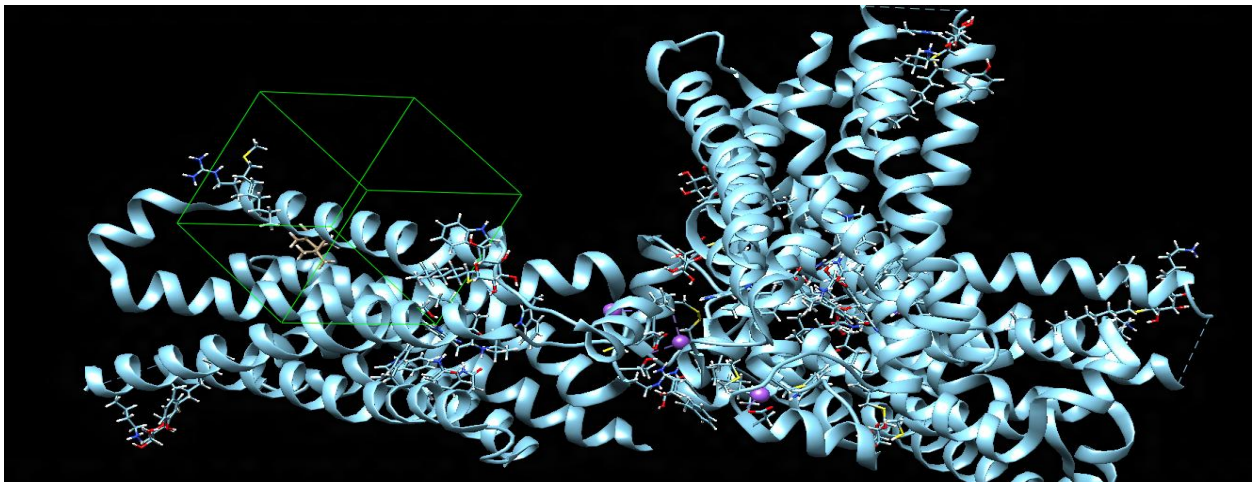
3. Open H2 from PDB



4. Dock prep

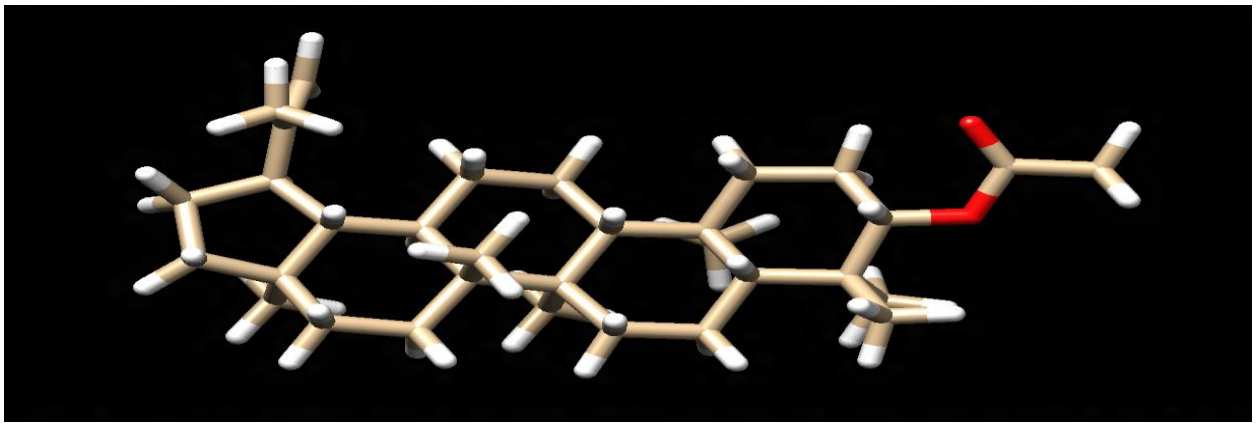


5. Autodock vina

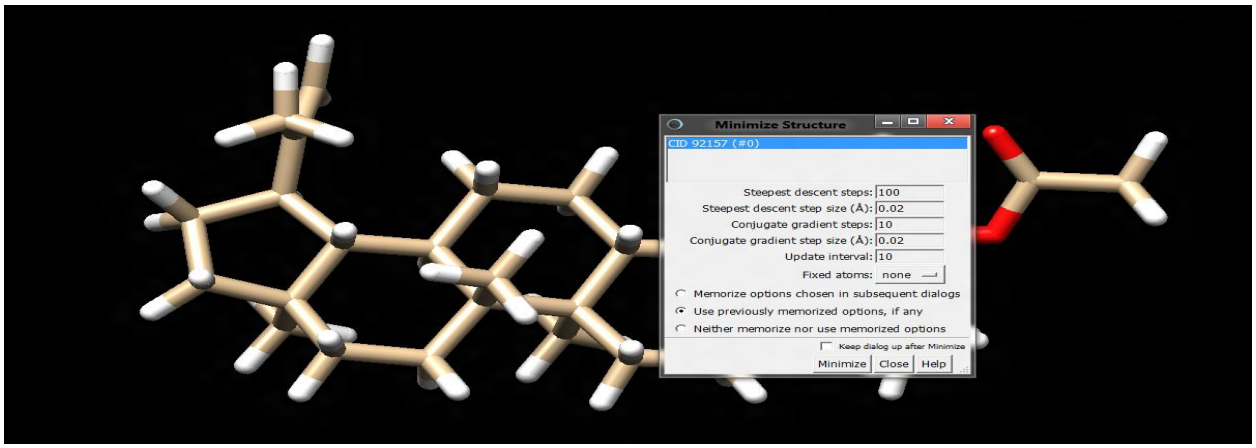


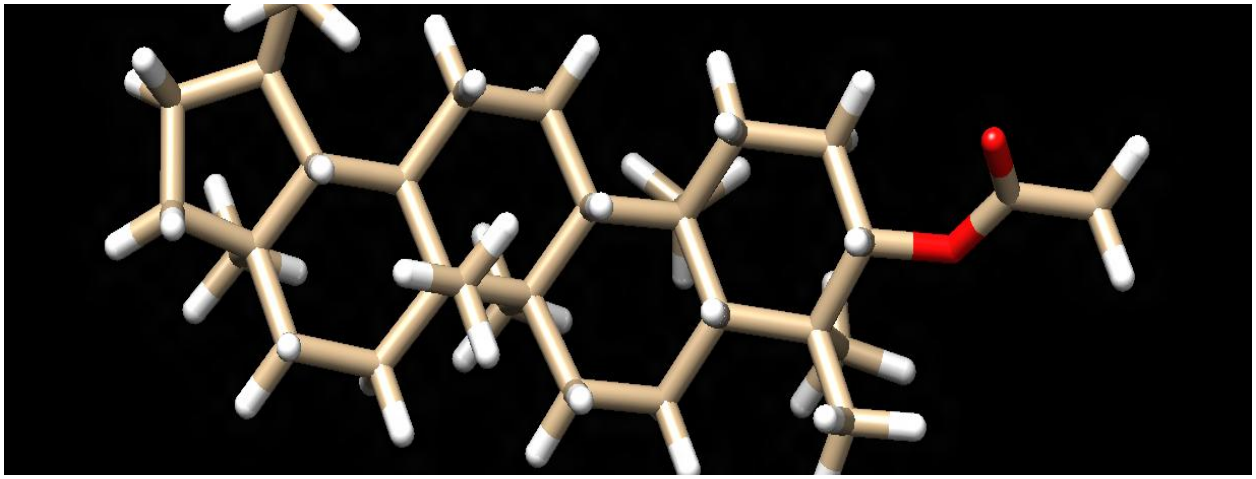
26. LUPEOL ACETATE

1. Phytochemical from Pubchem

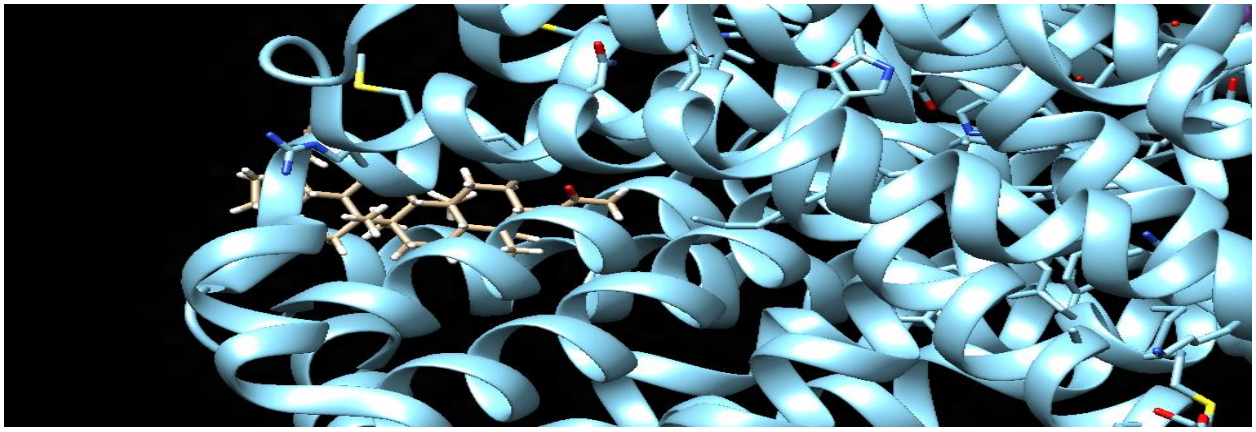


2. Minimize structures

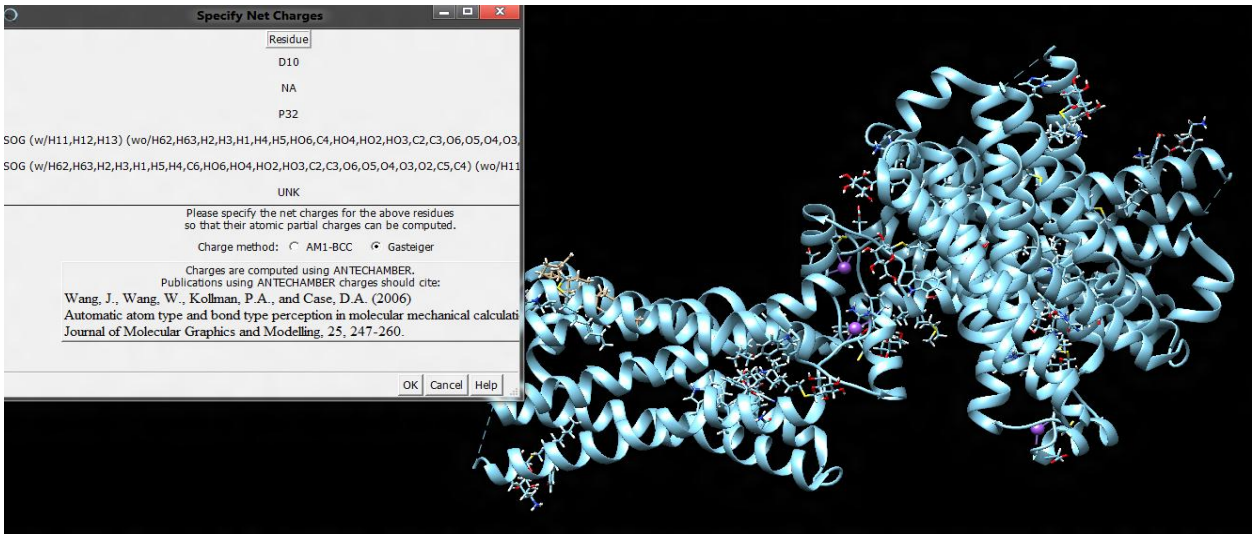




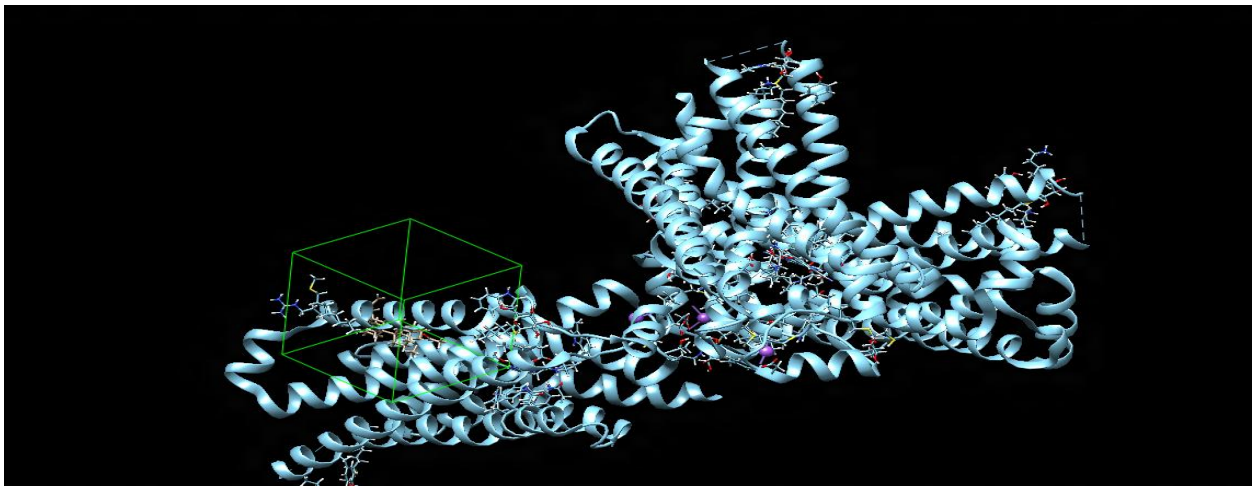
3. Open H2 from PDB



4. Dock prep

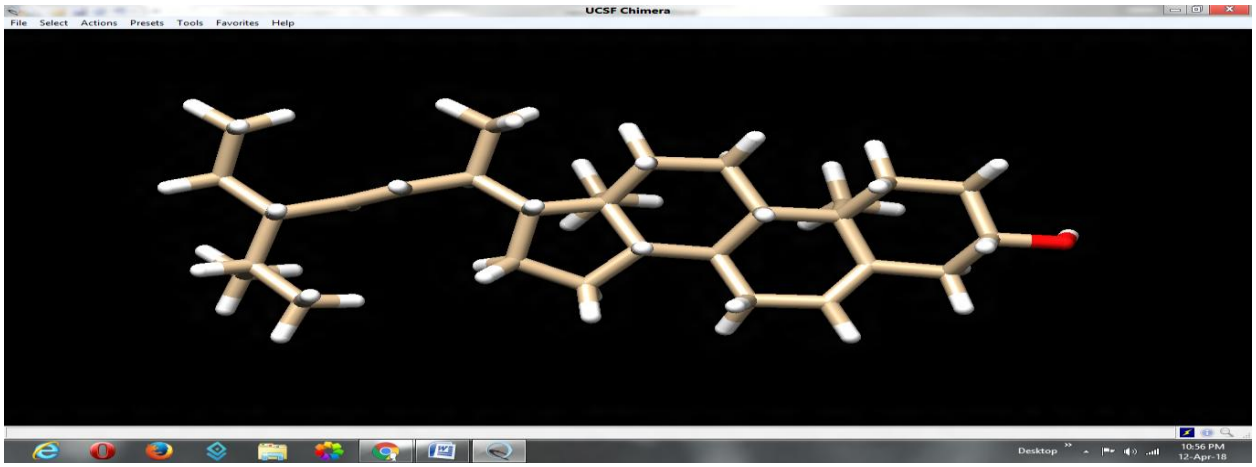


5. Autodock vina

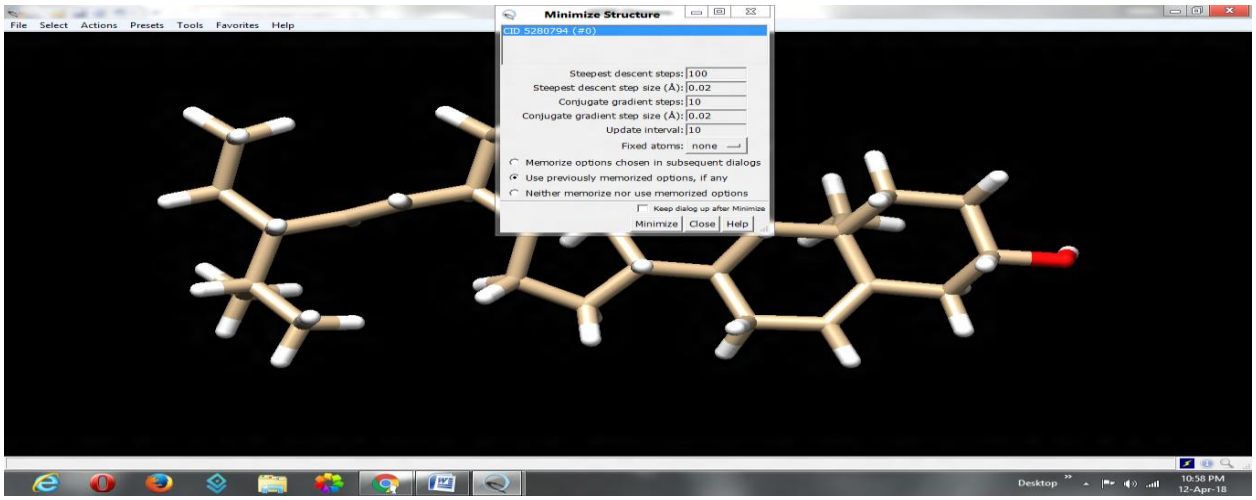


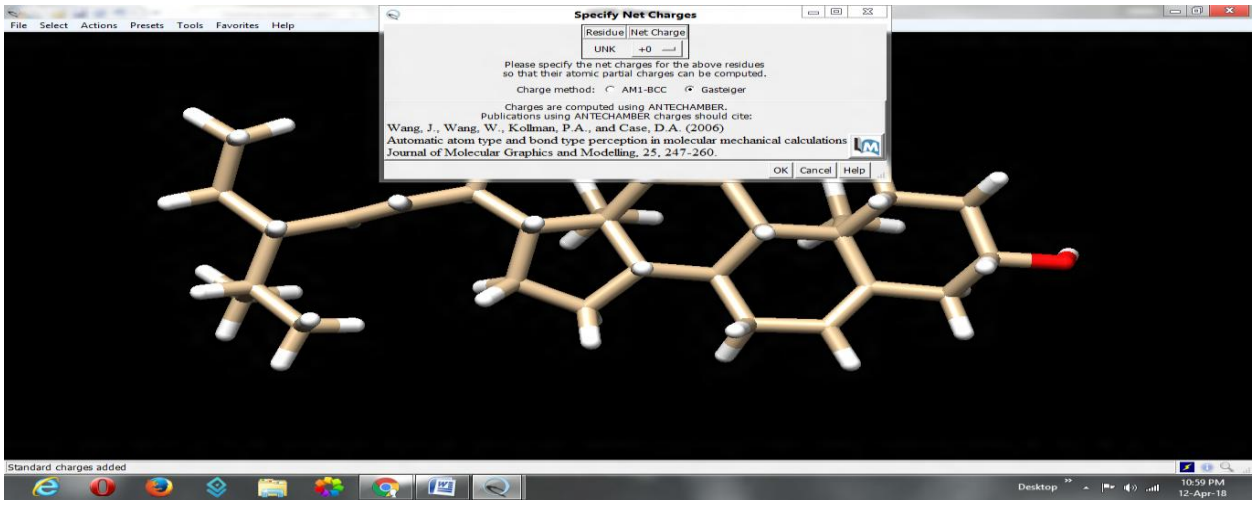
27. STIGMASTEROL

1. Phytochemicals from Pubchem



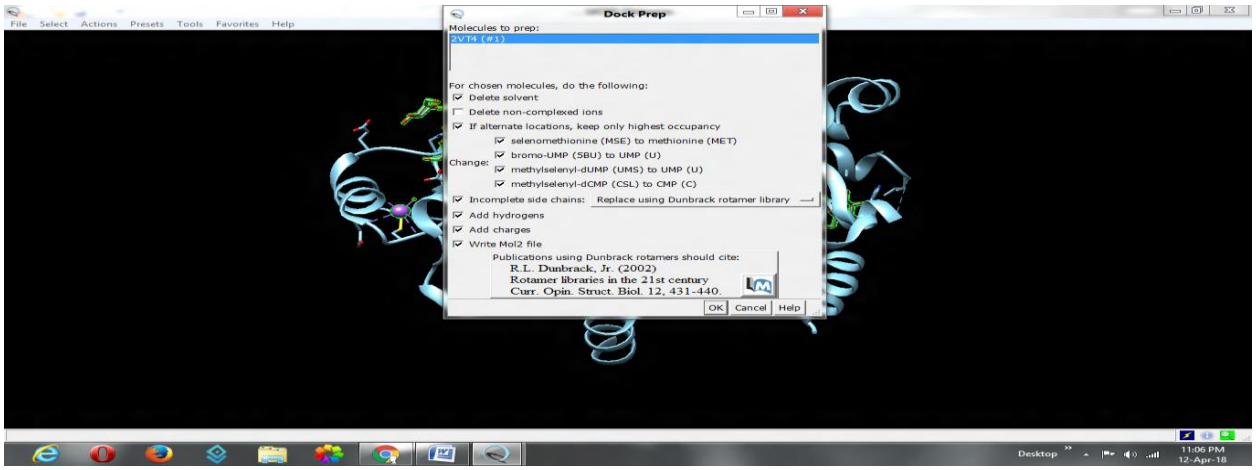
2, Minimize structures



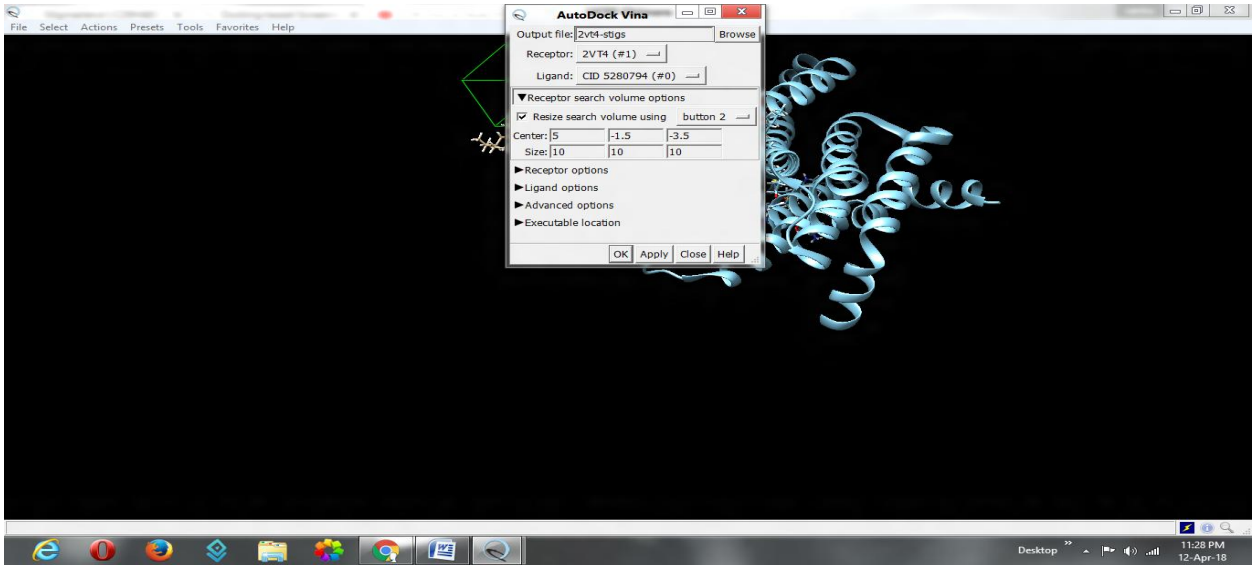


3. Open H2 receptor from PDB

4, Dock prep

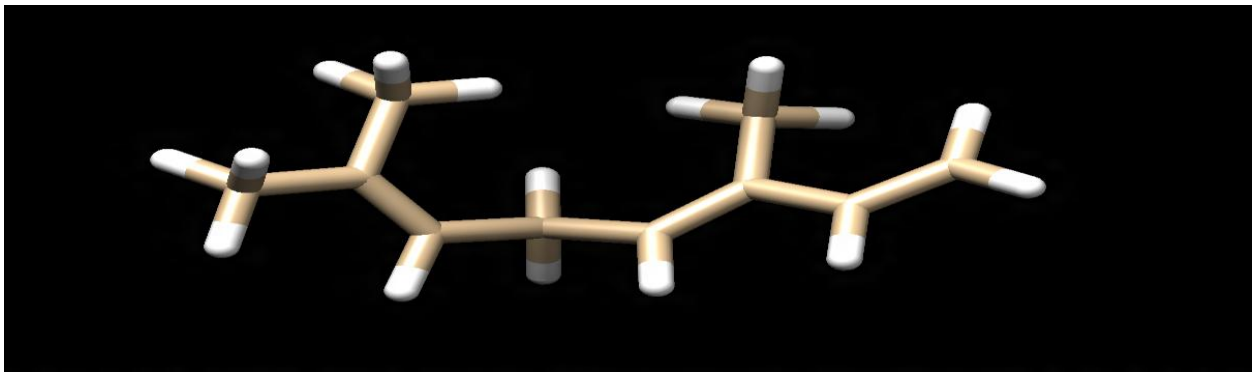


5. Autodock vina

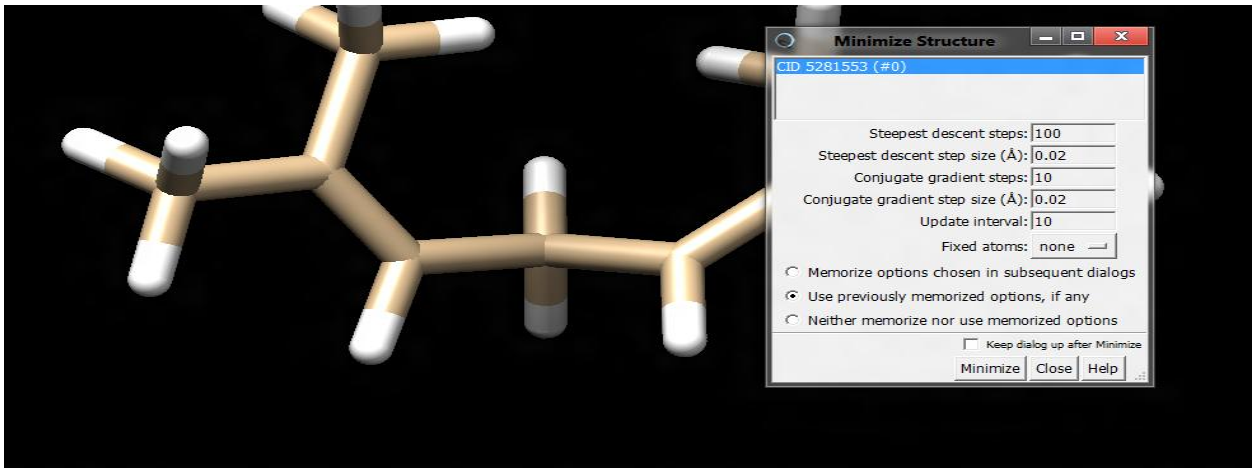


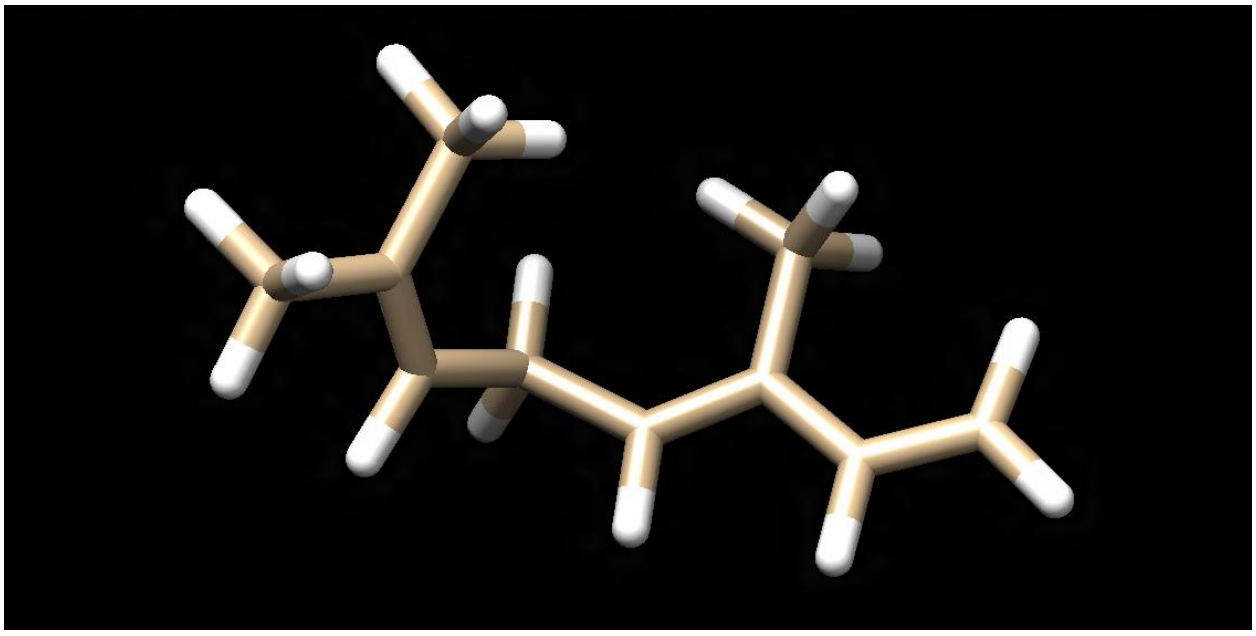
28. BETA-OCIMENE

1. Phytochemical from Pubchem

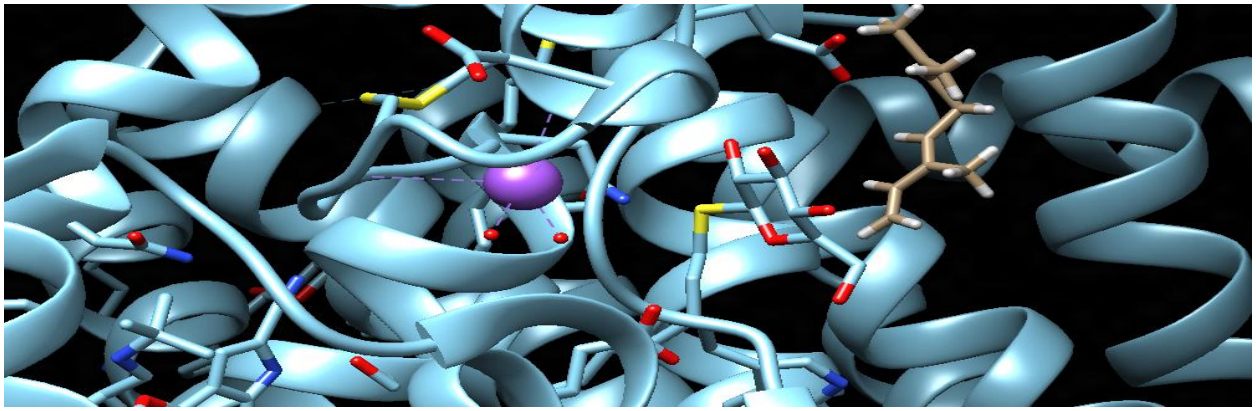


2. Minimize structures

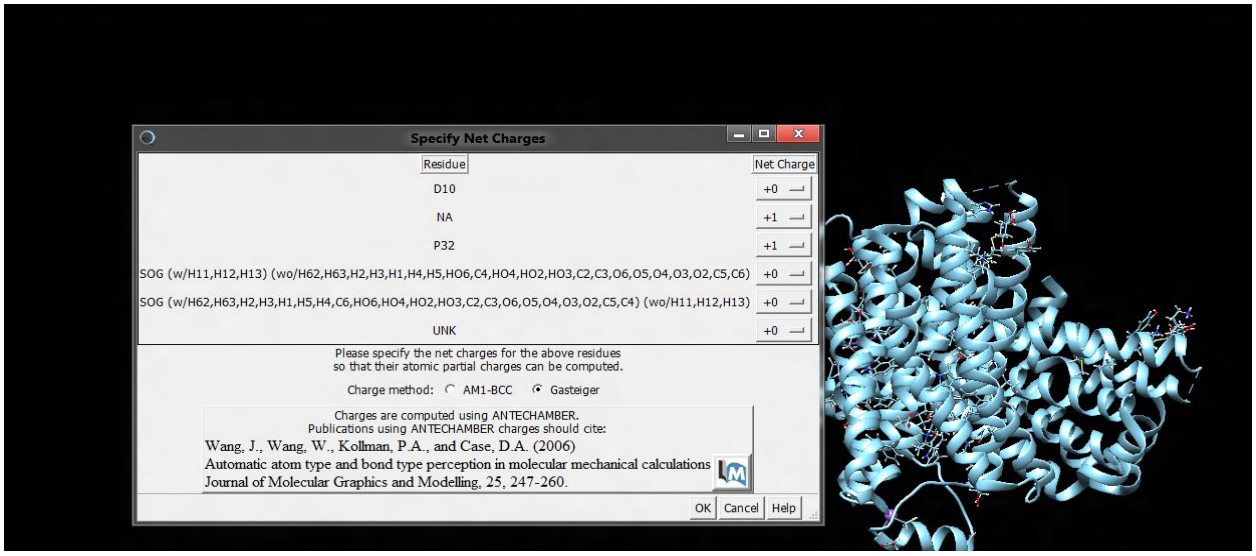




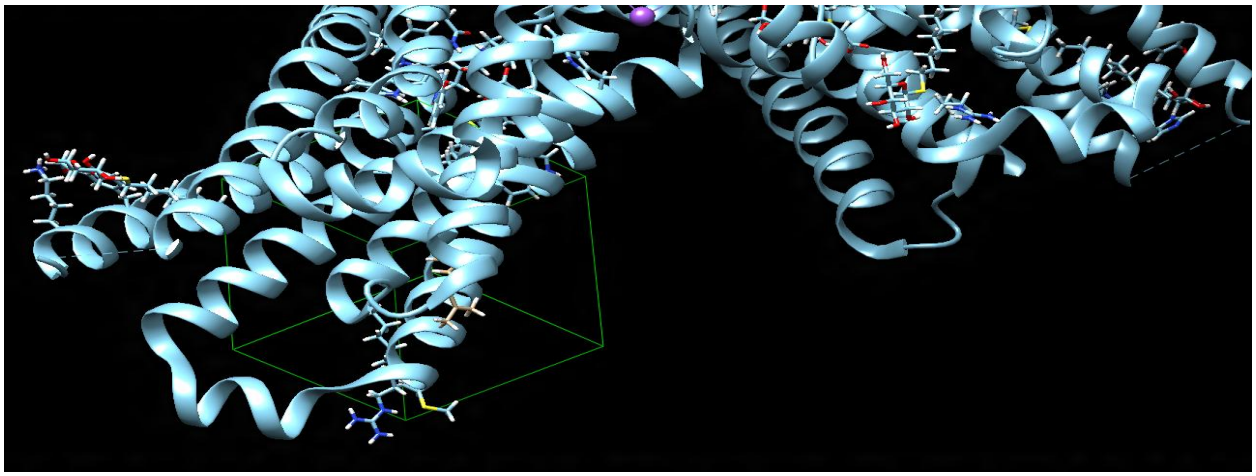
3, Open H2 from PDB



4. Dock prep

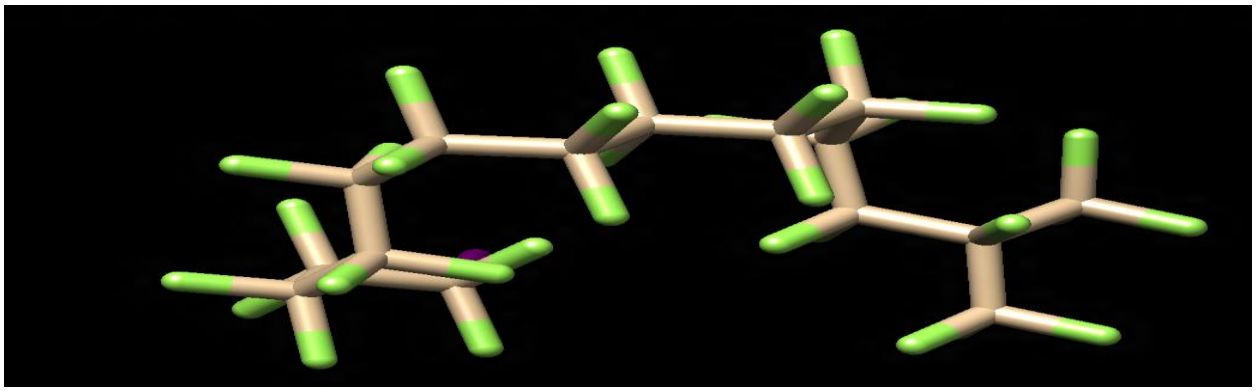


5. Autodock vina

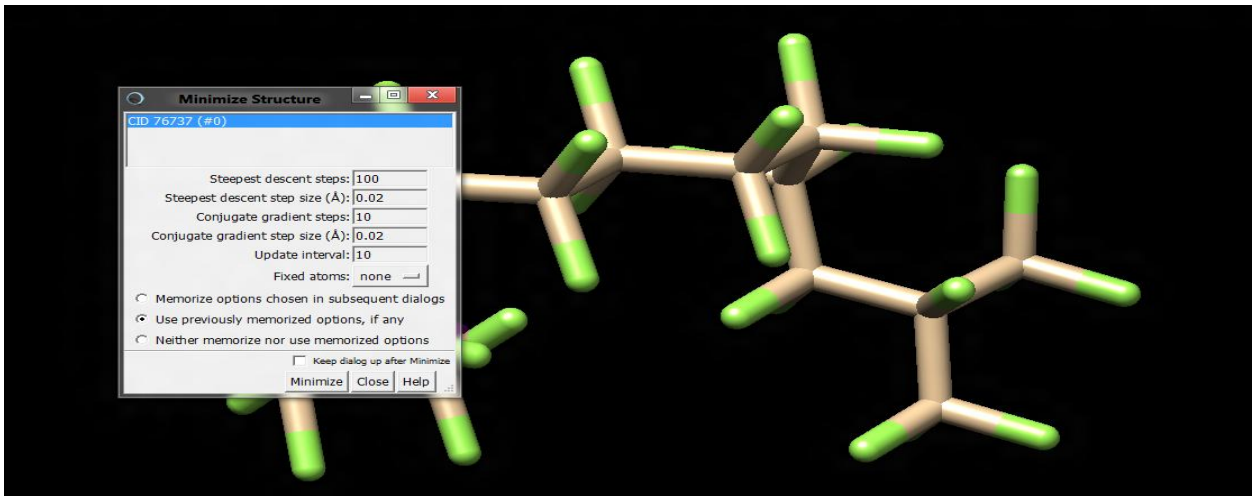


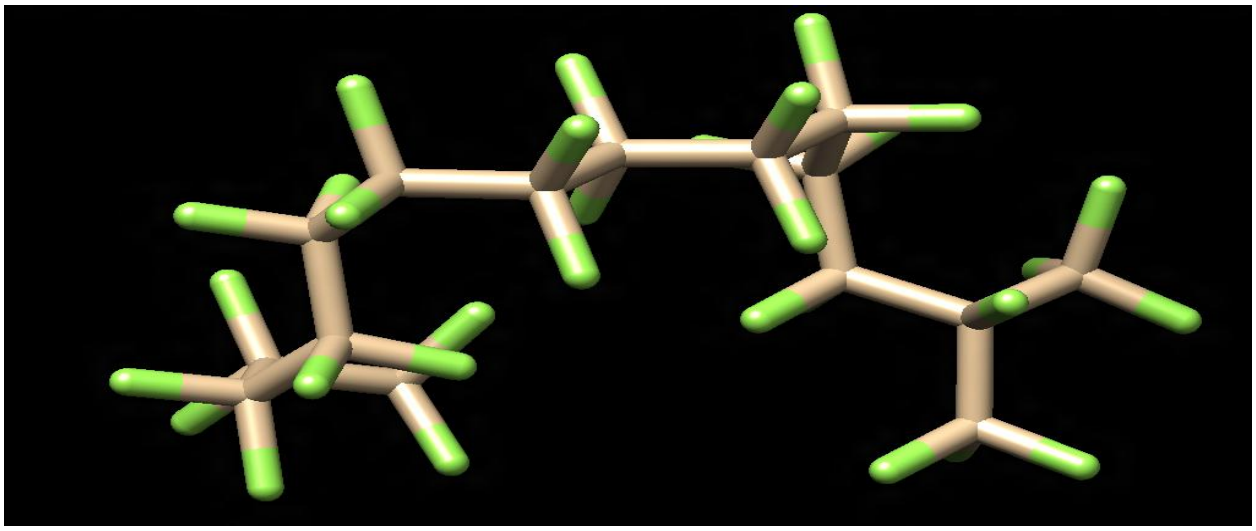
29. TETRADECANE

1. Phytochemical from Pubchem

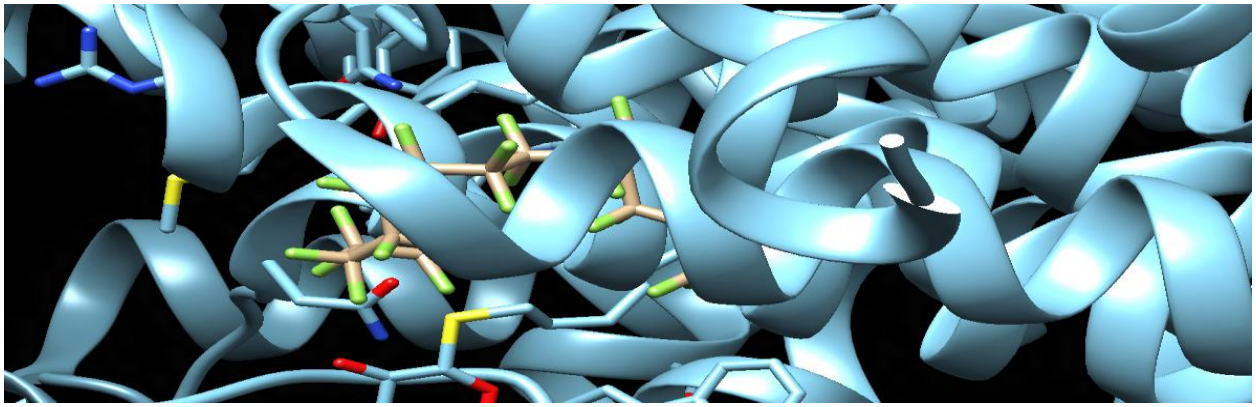


2. Minimize structures

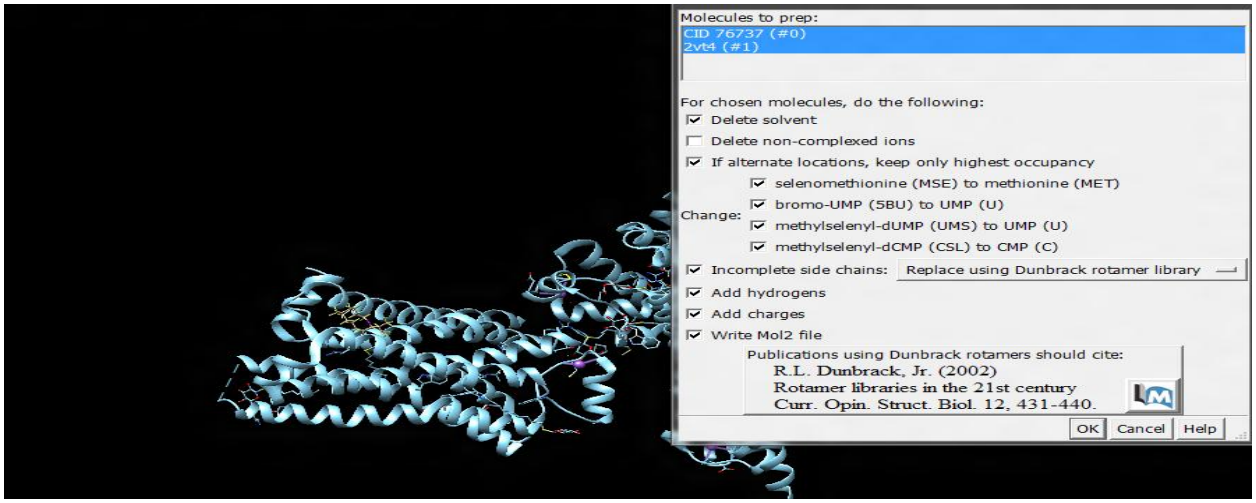




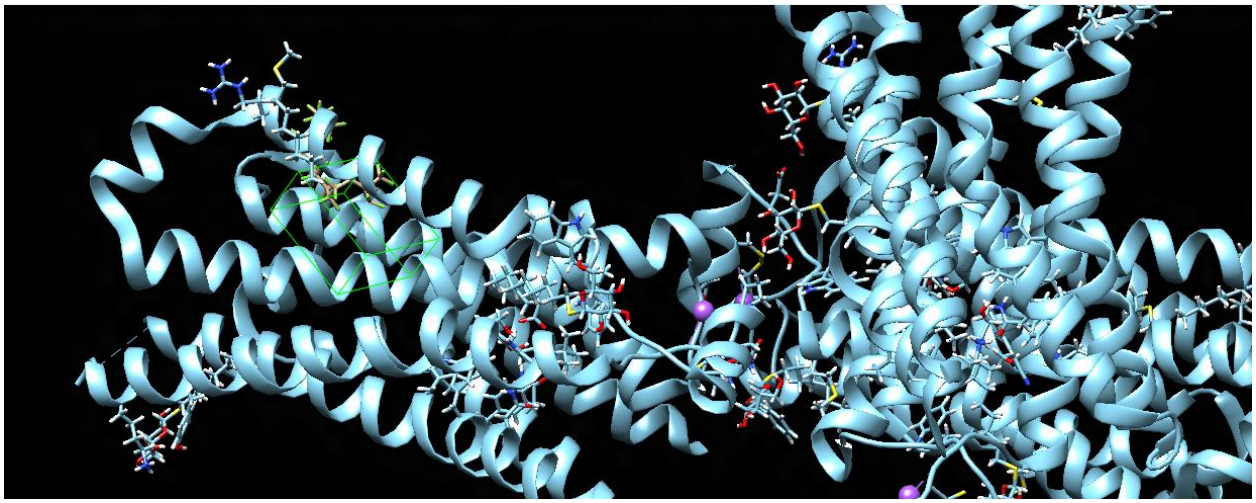
3. Open H2 from PDB



4, Dock prep

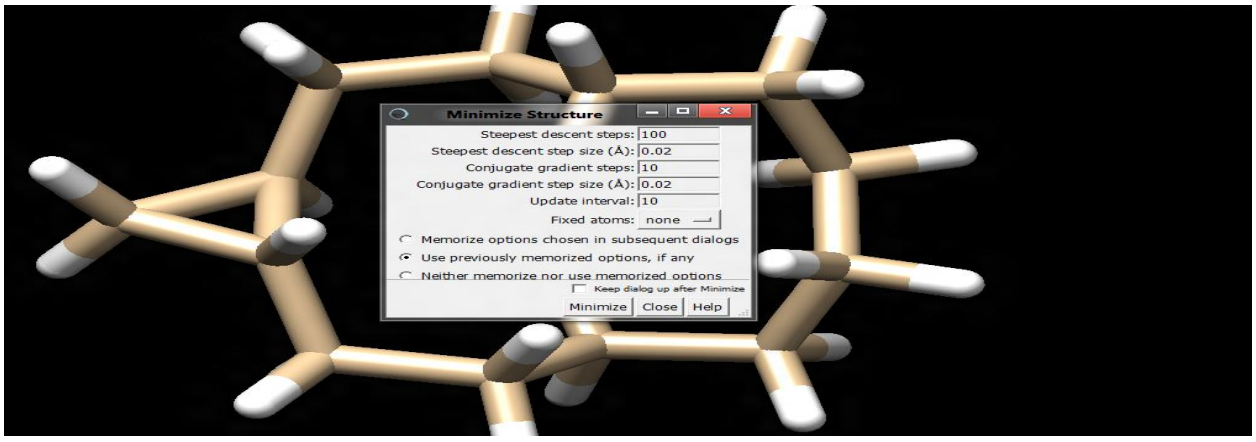


5. Autodock vina

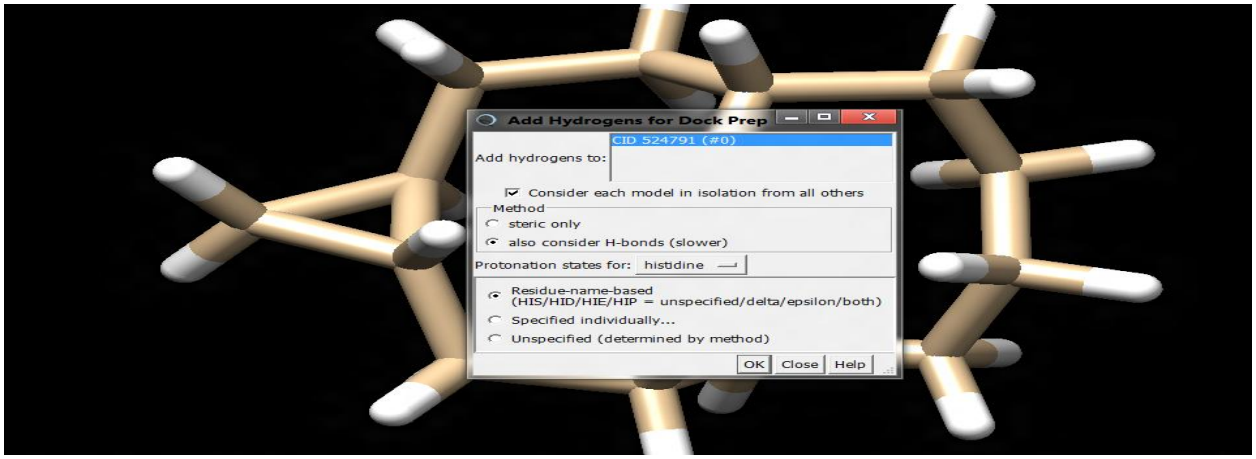


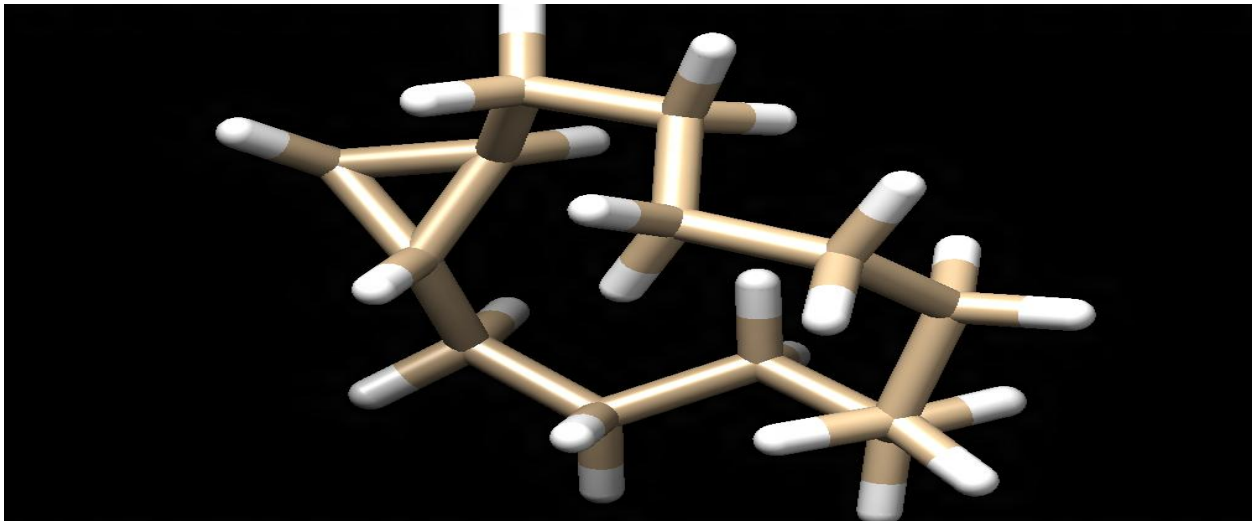
30. TRIDECANE

1. Phytochemical from Pubchem

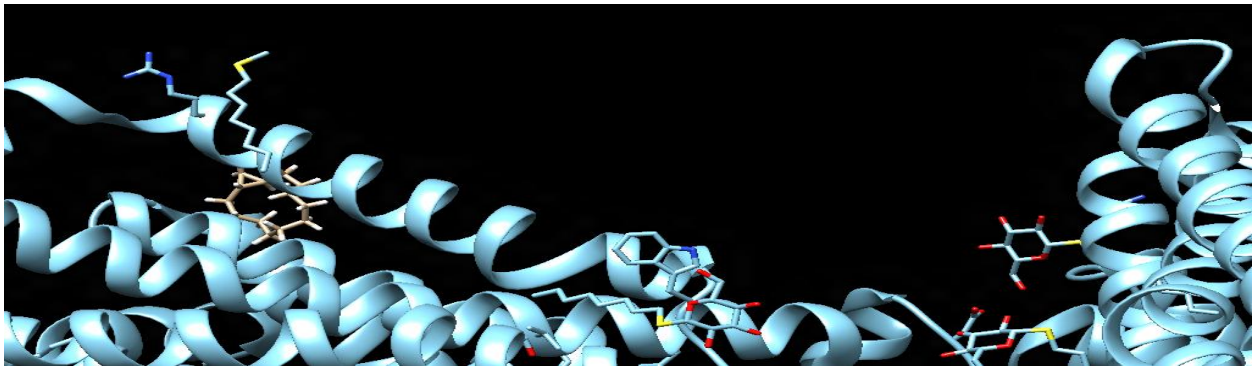


2. Minimize structures

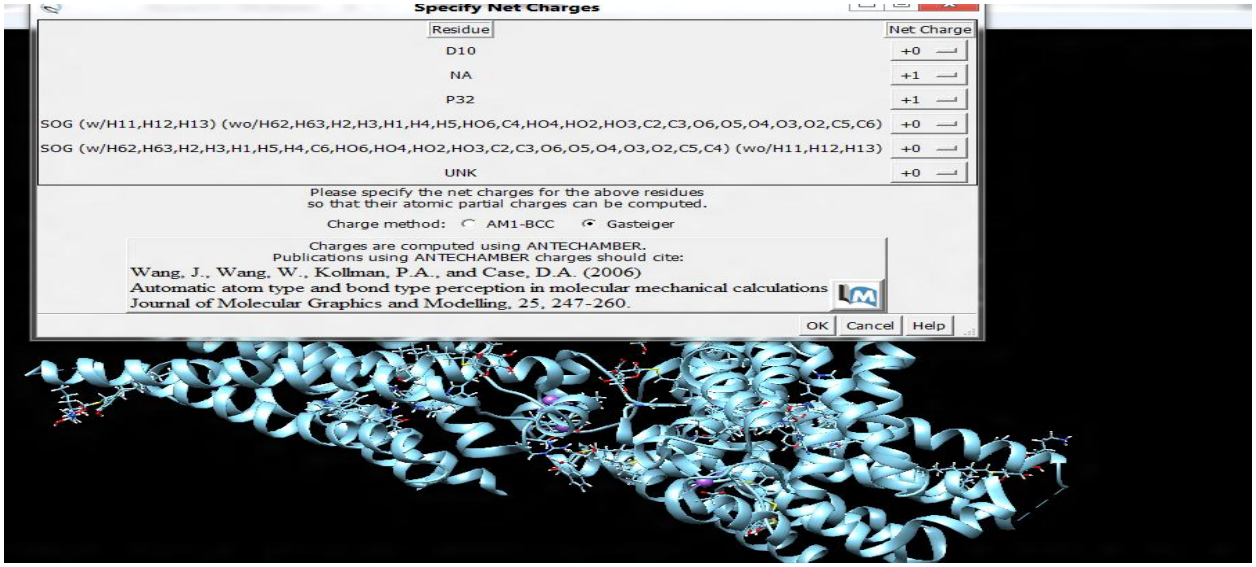




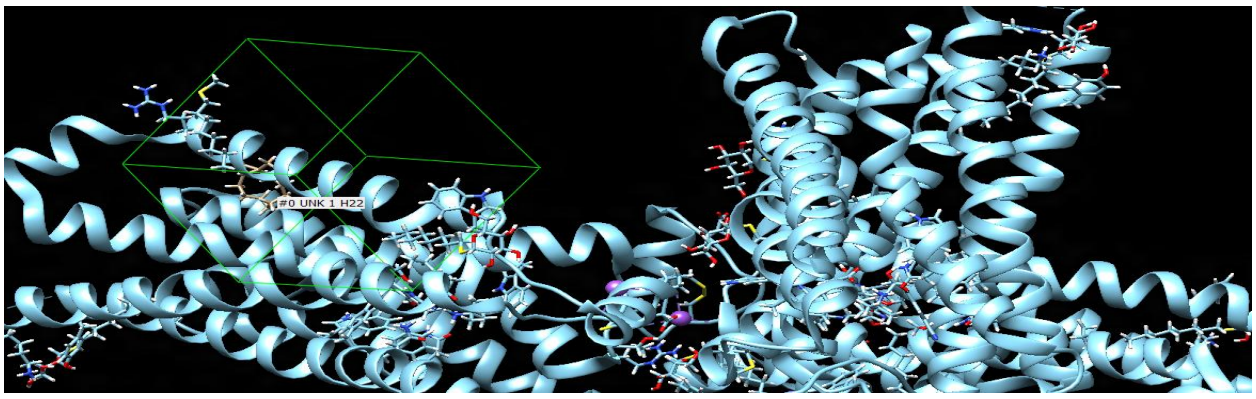
3. Open H2 from PDB



4. Dock prep

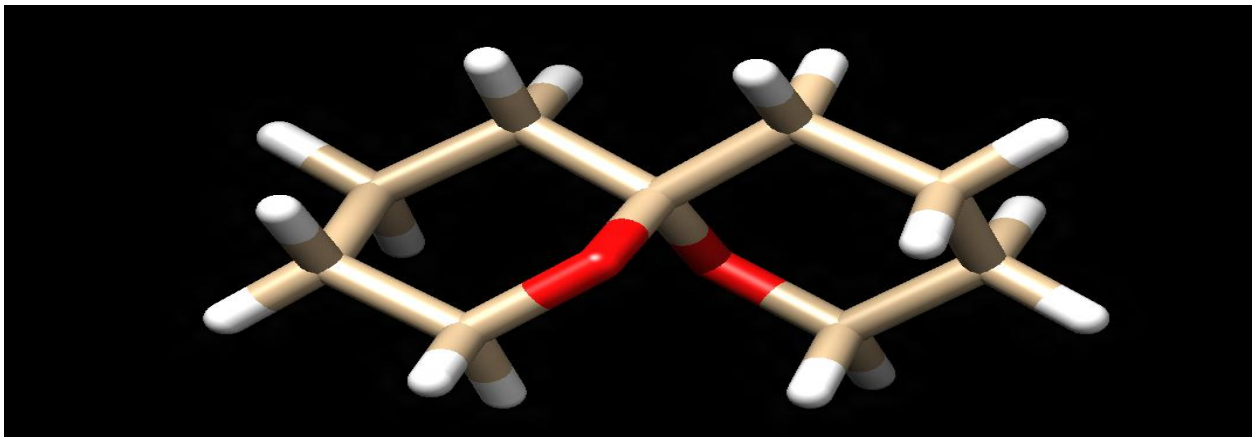


5. Autodock vina

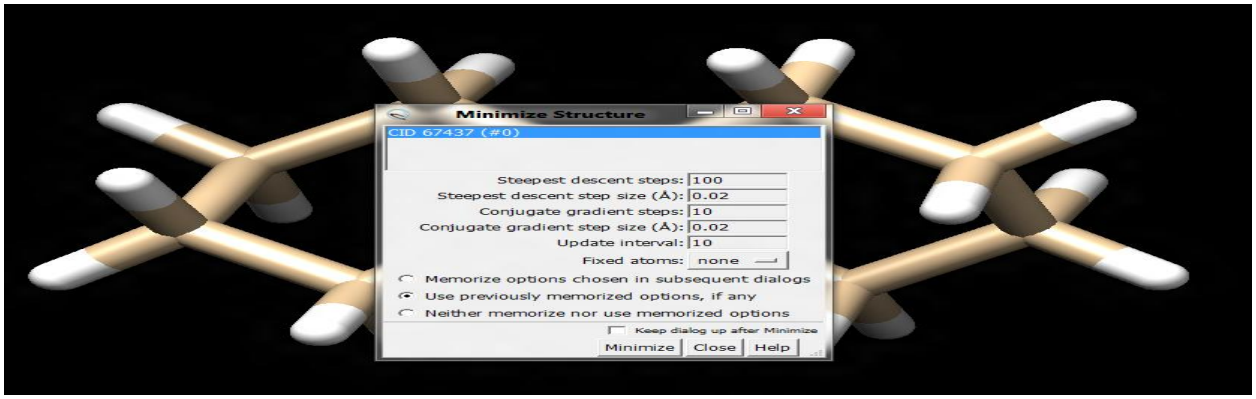


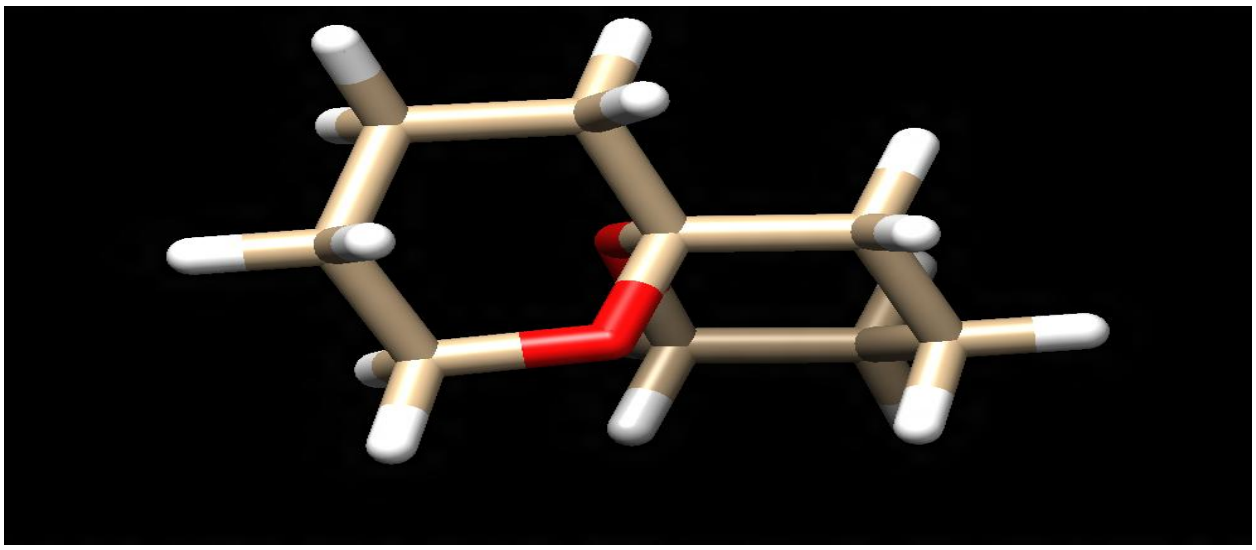
31.UNDECANE

1. Phytochemicals from Pubchem

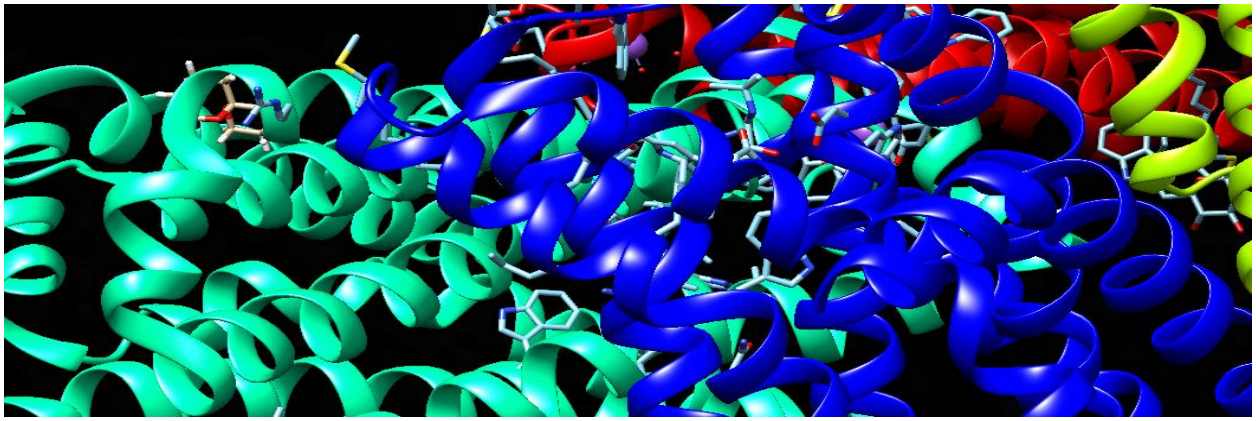


2. Minimize structures

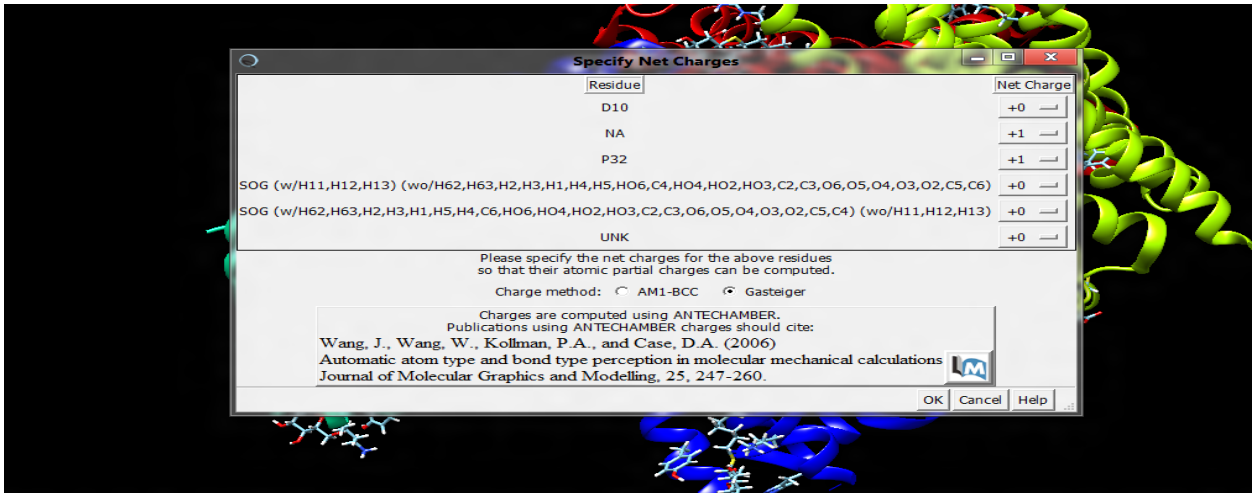




3. Open H2 from PDB



4. Dock prep



5. Autodock vina