MOLECULAR DOCKING STUDIES OF C-REACTIVE PROTEIN INHIBITORS

BY

RISWANA.M.T.K

(11PBF03)

A Thesis Submitted to Avinashilingam Institute for Home Science and Higher Education for Women,

Coimbatore – 641 043

IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE DEGREE OF MASTER OF SCIENCE IN BIOINFORMATICS

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CERTTIFIED AS BONAFIDE RESEARCH WORK

for

Signature of the Head of the Department

Dr.R.PRVATHAM

07/05/2013

Signature of the Guide

A. Shobana

Mrs.A.SHOBANA

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1. INTRODUCTION

Cardiovascular disease (CVD) contributes disproportionately to premature morbidity and mortality in the developed and developing worlds (Lovallo, 2005).

CVD includes various illnesses such as coronary artery disease, peripheral arterial disease, cerebrovascular disease such as stroke, and congestive heart failure (Zittermann and Gummert, 2010).

According to the Inter Heart study from 2004, nine factors are responsible for 90 % of all cases of CVD. These factors are dyslipidaemia, hypertension, smoking, stress, obesity (especially abdominal fat distribution), physical inactivity, poor diet with insufficient fruit and vegetable intake and excessive alcohol consumption (Schenck-Gustafsson, 2009).

The risk factors of CVD including increased age, gender heredity and race, cigarette smoking, high blood cholesterol, high blood pressure, physical inactivity, obesity and overweight, diabetes mellitus, low bone mineral density and individual response to stress (Perez-Lopez, 2009).

Garlic (*Allium sativum* L.) is one of the most important bulb vegetables grown and used as spice and flavoring agent for foods (Baghalian *et al*., 2005).

Garlic belongs to the botanical family of Liliaceae. It contains water (62-68%), carbohydrate (26-30%), protein (1.5-2.1%), amino acids (1-1.5%), organosulfur compounds (1.1-3.5%) and fiber (1.5%) (Chandrashekar *et al*., 2011).

The health properties of garlic depend on its bioactive compounds. When crushed, garlic yields allicin, a powerful anti-biotic and anti-fungal compound (phytoncide). It also contains the sulfur compounds alliin, ajoene, diallylsulfide,

dithiin, and S-allylcysteine, as well as enzymes, vitamin B, proteins, minerals, saponins, flavonoids and Maillard reaction products, which are non-sulfur containing compounds (Kim *et al*., 2012).

The pharmacological properties of garlic include lowering of blood lipid levels and blood pressure; inhibition of blood clotting; antiviral, antifungal, and antimicrobial activities and even cancerostatic effects (Ota *et al*., 2012).

C-reactive protein (CRP) is a prototypical acute phase protein in humans. Tillet and Francis discovered CRP over 70 years ago in the blood of patients with *Streptococcus pneumoniae* infection, as a substance that precipitated the "C" polysaccharide of the cell wall of the pneumococcus and they called it C-reactive substance, which later changed to C-reactive protein (Marnell *et al*., 2005).

Together with serum amyloid P and pentraxin-3, CRP belongs to the phylogenetically ancient and highly conserved family of pentraxins, which share a common structure with five identical subunits linked by weak non-covalent bonds are arranged in cyclic symmetry (Sjowall *et al*., 2007).

CRP is a pattern recognition molecule, binding to specific molecular configuration that are typically exposed during cell death, or found on the surfaces of pathogens. Its rapid increase in synthesis within hours after tissue injury or infection suggests that it contributes to host defense that is part of the innate immune response (Black *et al*., 2004).

CRP is not only a marker of inflammation, but it has been shown to possess biological function including direct opsonization and regulation of complement activation. CRP is deposited in atherosclerotic lesions, where it may actively participate in various atherosclerotic processes (Taskinen *et al*., 2005).

CRP is synthesized and secreted primarily in human hepatocytes and is regulated mainly by interleukin-6 (IL-6) and inerleukin-1 (IL-1) (Zhang *et al*., 2009). Due to its dramatic rise within the initial 24-72 hours in response to pro-inflammatory stimuli or tissue damage, CRP is an important marker of systemic inflammation (Sjowall *et al*., 2007).

The concentration of CRP increases rapidly and dramatically in response to infection, trauma, tissue infarction and other types of inflammation. Hence, measurement of CRP is widely used to monitor various clinical states, including infectious, autoimmune, and coronary artery diseases (Park *et al*., 2005).

CRP induces an increase in the expression of intercellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1) and E-selectin and increases local infiltration by monocytes and lymphocytes (de Ferranti and Rifai, 2002).

It binds to a wide variety of substances, such as microbial polysaccharide, phosphatidylcholine and damaged cell membrane (Haidari *et al*., 2001).

Expression of CRP is regualted mainly at the transcriptional level, but post-transcriptional mechanisms also play a significant role. The CRP gene has been mapped to human chromosome 1, between 1q21 and 1q23. It contains 2263 nucleotides and has a single intron (Volanakis, 2001).

Following the traumatic event, interleukin-1b (IL-b) and IL6 are released from the activated microphage or monocytes at the sites of injury and these stimulate CRP synthesis in the liver and subsequent secretion from the hepatocytes (Quan *et al*., 2000).

OBJECTIVES

- To study about the molecular interactions between constituents of garlic and CRP through molecular docking.
- To find whether garlic can be used to reduce the level of C-reactive protein.
- To find the details of drug able compounds of garlic.

2. REVIEW OF LITERATURE

Herbal remedies are very useful for treating many of the diseases. Garlic is a good herbal remedy for reducing the level of cardiovascular disease. C-reactive protein is a risk predictor of cardiovascular disease and the level of C-reactive protein increases during the period cardiovascular disease.

The Review of Literature on thesis entitled "**Molecular Docking Studies of C-Reactive Protein Inhibitors**" is discussed under the following headings:

2.1 C-Reactive Protein

2.2 Cardiovascular Disease

2.3 Garlic

2.4 Role of C-reactive Protein and Garlic in Cardiovascular Disease

2.5 Molecular Docking

2.1 C-REACTIVE PROTEIN

C-reactive protein (CRP) was discovered in 1930 by Tillet and Francis during their studies of patients with acute pneumonia. They found that when serum from febrile patients was mixed with a cell-wall component of pneumococci that they called "Fraction C," a precipitate formed (Fage and Szalai, 2007).

CRP is a member of phylogenetically ancient and highly conserved 'pentraxin' family of proteins, which also includes serum amyloid P component, a constituent of all amyloid deposits (Volanakis, 2001).

CRP is an acute phase protein synthesized mainly by hepatocytes in response to severe tissue injury, microbial infections, systemic autoimmune disease, and malignant tumors (Malek *et al*., 2006).

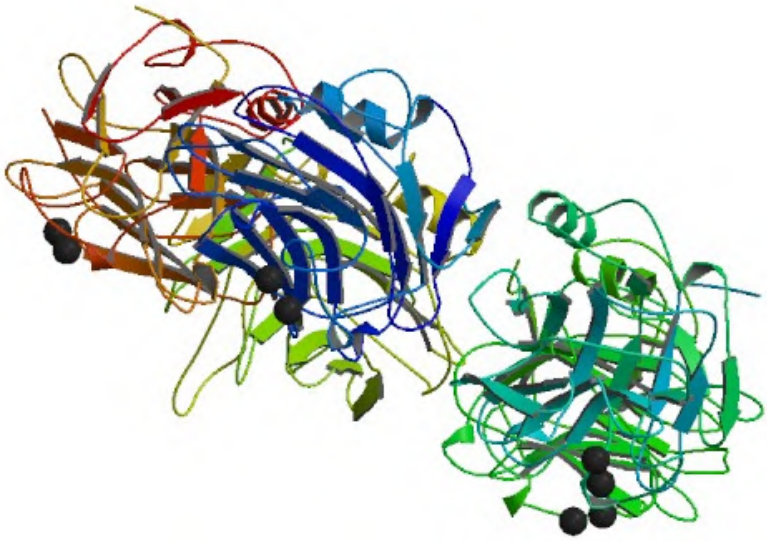


FIGURE - 1

STRUCTURE OF C-REACTIVE PROTEIN

CRP is an important risk predictor for atherosclerosis and coronary heart disease (CHD). Accumulating recent data have indicated that CRP may also modulate or involve in the process of atherosclerosis and CHD (Hu *et al*., 2007).

Prospective studies and primary prevention trials have demonstrated that high-sensitivity CRP, an acute-phase protein and marker of inflammation, predicts overall cardiovascular disease (CVD) risk in those without prior disease and predicts multiple types of CVD including myocardial infarction, stroke, and peripheral arterial disease (Block *et al*., 2009).

CRP starts functioning *in vivo* probably after binding to ligands like phosphocholine-containing substance, such as modified low-density lipoproteins and extra cellular matrix proteins, such as fibronectin (Voleti and Agrawal, 2006).

CRP also enhances the activity of phagocytic cells and activates the classical complement pathway. CRP has also been extracted from human atherosclerotic lesion (Haidari *et al*., 2001).

CRP has a half life of ~19 hours. It is synthesized primarily in hepatocytes and regulated by interleukin 6 (IL-6) and interleukin 1 (IL-1), tumor necrosis factor alpha and other cytokines. CRP has a normal range of <2 mg/l in populations without evidence of acute illness; with illness such as rheumatoid arthritis or sepsis, concentrations can increase to 300 mg/l (de Ferranti and Rifai, 2002).

According to the classic definition of CRP, proclaimed in 1930, it binds specifically to phosphocholine (PC) moiety of pneumococcal C polysaccharide in a characteristic calcium dependent manner (Das *et al*., 2004).

The target structures for CRP in the arterial intima include lipoproteins, and apoptotic and necrotic cells. Studies on the binding of CRP to lipoproteins have been controversial. CRP has been reported to bind to immobilized low density lipoprotein (LDL) and very low density lipoprotein particles, but more recent studies have indicated that CRP does not interact with lipoproteins in their native conformation, but rather binds to LDL modified enzymatically or by oxidation (Taskinen *et al*., 2005).

The first recognized function of CRP was its ability to bind to and precipitate the wall C-polysaccharide of *Streptococcus pneumoniae* (PnC). It was later determined that this interaction depends on the protein's calcium dependent binding specificity for PC, a major constituent of PnC (Szalai, 2002).

CRP is moderate or acute phase protein in most species with the notable exception of the cow and mouse where it is not reacting or weakly reacting during the acute phase response (Heegaard *et al*., 2009).

The structure of CRP consists of five protomers, each with a phosphocholine binding site, arranged as a cyclic pentamer. The five phosphocholine-binding site form a recognition face on the pentamer, and the opposite face binds to the complement C1q (Tanio *et al*., 2009).

CRP mediates monocyte chemo tactic protein -1 induction in the endothelial cells and cause expression of intercellular adhesion molecule-1 by endothelial cells (Blake and Ridker, 2003).

CRP induces the production of inflammatory cytokines, promotes monocyte chemotaxis, reactive oxygen species and tissue factor expression. In endothelial cells, CRP increases the expression of cell adhesion molecules, monocyte-chemotactic protein-1 and endothelin-1, plasminogen activator inhibitor-1, decreases eNOS expression and activity and prostacyclin release. In smooth muscle cells, CRP has been shown to activate angiotensin-1 type receptors. It co-crystalizes with activated fragment of the complement system (Devaraj *et al*., 2004).

The level of CRP may arise as high as 1000 folds with the presence of inflammation. When the CRP level is elevated to be over 3μg mL^{-1}, risk for cardiovascular diseases in considered being high (Choi *et al*., 2012).

CRP levels are associated with age, sex, race (African-American), body mass index, smoking, serum lipids, blood pressure, presence of diabetes mellitus, 2-h post-challenge glucose, frequency of exercise, and cardio-respiratory fitness (Kasayamma *et al.,* 2009).

CRP is a non-glycosylated protein consisting of identical 21 kDa non-covalently bound subunits and is predominantly produced and secreted by hepatocytes in response to the release of inflammatory cytokines. The reference limit of serum CRP concentrations in healthy individuals is less than 10 mg/l, whereas during septic episodes, CRP levels can rise above 100 mg/l. CRP is widely applied in the laboratory detection of severe bacteria-induced inflammation and CRP can be used as a diagnostic parameter for the differentiation between viral and bacterial infection, as well as to identify patients

with prospective complications. Serial determinations of CRP levels are helpful in monitoring the clinical course of sepsis and response to treatment (Tsokos *et al.,* 2001).

CRP has been shown to inhibit angiogenesis through attenuating endothelial cell migration and tube formation, endothelial progenitor cell survival, differentiation, and function, as well as potently attenuating nitric oxide release or activating NF-κB which modulates the transcription or expression of numerous proatherosclerotic genes (Yang *et al*., 2005).

2.2 CARDIOVASCULAR DISEASES

Cardiovascular diseases and their thrombotic complications are the leading cause of morbidity and morality in developed countries (Gopaul and Crook, 2006).

Worldwide in 2005, cardiovascular disease (CVD) was estimated to have caused approximately 17.5 million deaths, of which more than 80% occurred in low-middle-income countries. By 2030, the cardiovascular deaths are projected to increase to 23 million (Joshi *et al*., 2008).

Across the globe, far more people die of cardiovascular disease than any disease in spite of the decline in cardiovascular related deaths since the early 1970s (Thornburg *et al*., 2010).

CVD is the number one killer of patients with diabetes. Men with diabetes are two to three times more likely to die from coronary heart disease than those without diabetes, and the risk for women is even higher (Tuttle *et al*., 2004).

The INTERHEART study recently showed that an unhealthy diet increases the risk of acute myocardial infarction. Globally, the study estimated that an unhealthy diet accounts for 30% of cases (Hoekstra *et al*., 2009).

CVD risk factors such as diabetes, hypertension, high cholesterol, smoking, and obesity tend to occur together in the general and diseased populations. The proportional of adults with two or more of these CVD risk factors increased from 23.6% in 1991 to 27.5% in 1999 in the United States. Increasing prevalence of multiple CVD risk factors has been related to increased risk of all-cause, coronary heart disease, and stroke morality (Li *et al*., 2008).

Inflammation is now recognized as a risk factor for the development and exacerbation of atherosclerotic cardiovascular disease (Phillips *et al*., 2009).

Atherosclerosis is the main etiologic factor for cardiovascular disease and increasingly recognized as a complex phenomenon involving the interaction of several mechanism. Inflammation and thrombosis play an important role in the pathophysiology of atherosclerosis (Gol *et al*., 2006).

Cardiovascular pathophysiology significantly increases the likelihood of developing depressive disorders; and conversely depressive disorders are risk factors for cardiac morbidity and morality. Cardiovascular risk factors (including coronary artery calcification, increased blood glucose levels, hypertension, and diabetes), and increased cardiovascular mortality (Peuler *et al*., 2012).

CVD includes enhanced atherogenesis, with coronary artery, abdominal and peripheral vessel classification in addition to left ventricular dysfunction, with subsequent heart failure and arrhythmias. The pathology of coronay artery disease in the general population is most commonly ischemic, caused by thrombotic occlusion of the artery (Langman and Brooks, 2006).

The relationship between chronic health conditions, such as heart disease, asthma, diabetes, and arthritis, has been highlighted for its public health

significance because depression often develops earlier in the lifespan as compared with most progressive health conditions (Dietz and Matthews, 2011).

Risk algorithms such as the Framingham Risk Score have traditionally incorporated classical clinical and environmental risk factors such as age, gender, blood lipid concentrations, blood pressure, body mass index, family history and smoking habit for primary prevention strategies purposes (Ndiaye *et al*., 2011).

Overweight and especially obese individuals are at greater risk for CVD. Excess visceral fat is associated with these negative consequences possibly because of increased risk factors, for example, circulating triacylglycerols, and reduced high density lipoprotein cholesterol. One important risk factor to modify disease risk long chain n-3 polyunsaturated fatty acids (LC n-3 PUFA), that is, eicosapentaenoic acid and docosahexaenoic acid (Ramel *et al*., 2009).

The increase in life expectancy, and subsequently in the ageing population, is staggering posing tremendous challenges in disease burden, particulary, chronic diseases such as diabetes, hypertension, hypercholesterolemia, cancer and CVD. The prevalence of the majority of the risk factors increases with age, especially in women than men. The risk factors that predispose to CVD in the elderly are the same as those that operate in the middle-aged (Tryrovolas and Panagiotakos, 2010).

Atherosclerosis is the leading cause of CVD related mortality, accounting for nearly three-fourths of all deaths from heart disease (Kinlay, 2007).

Epidemiologic studies present convincing evidence that hyper homocysteinemia is associated with increased risk of various diseases, such as those of a cardiovascular nature (Malinowska and Chmurzynska, 2009).

Lifestyle factors such as westernized diet and physical inactivity in concert with an increasing life expectancy are thought to play a major role in the development of CVD (Zittermann and Gummert, 2010).

CRP is established as a predictor of cardiovascular events in patients with known CVD as well as in asymptomatic individuals with risk factors for CVD (Wethal *et al*., 2010).

Multiple studies show that individuals formally qualifying for metabolic syndrome by current criteria who have high sensitive CRP (hsCRP) µ3 mg/l are at higher risk for future cardiovascular events and diabetes compared with those with metabolic syndrome who have hsCRP <1 mg/l, a risk relationship almost identical to that for high sensitive CRP among individuals without metabolic syndrome (Ridker, 2007).

Nitric oxide is an important vascular regulator involved in vasodilatation, smooth muscle proliferation and antithrombotic processes. Impaired NO production or activity leads to events that promote atherosclerosis (Hov *et al*., 2011).

Many studies have shown that mild increase in CRP is an independent predictor of future cardiovascular events (Nishida *et al*., 2011).

Current dietary advice to reduce risk of CVD includes substituting saturated fat with carbohydrate without changing the protein content. In the USA, the DASH-diet (Dietary Approaches to Stop Hypertension), which is rich in fruits, vegetables and low fat diary products, is recommended to meet this objective. The DASH-diet is based on a reduced intake of saturated fat that is replaced by carbohydrate. Such high carbohydrate, low-protein diets are known to reduce blood pressure and LDL cholesterol, bur also to reduce HDL cholesterol levels and to raise fasting triglycerides (Erdmann *et al*., 2008).

The development of atherosclerosis and related cardiovascular diseases in the majority of sufferers are tightly correlated with diet and the effect this has on the body. Western diets consisting of high fats are correlated with the development of insulin resistance, type II diabetes, and obesity. These disorders result in a number of metabolic perturbations including dyslipidaemia in key organs and blood plasma, alters the proportion of LDL to HDL, and induces hypertension (Waterman *et al*., 2010).

2.3 GARLIC

Garlic's wild progenitor is thought to have originated in the planes of west Central Asia. De Candolle places its origin in the Kiegiz desert of Western Russia. The word 'garlic' originated from the Anglo-Saxon 'gar-leac' or spear plant (Shukla and Kalra, 2007).

Garlic (bulb of *Allium sativum*) has been utilized as a spice and herbal remedy for >4000 years (Ota *et al*., 2012).

Garlic has wide spectrum of actions, including not only its antibacterial, antiviral, antifungal and antiprotozoal functions, but also it beneficial effects on the cardiovascular and immune system (He *et al*., 2008).

Garlic has historically been used to treat earaches, leprosy, deafness, severe diarrhea, constipation and parasitic infections, and to lower fever, fight infections and relieve stomach aches. Garlic and its extracts have been used to treat infections for thousands of years (Omar and Al-Wabel, 2010).

The constituent of garlic are divided into two groups: sulfur-containing compounds. Most of the medicinal effects of garlic are referable to a sulfur compound known as allicin. The intact garlic clove does not contain allicin, but rather its precursor, the non-protein amino acid alliin. Alliin is converted into

allicin, pyruvate and ammonia by the enzyme allinase, when the bulb is cut or crushed (Baghalian *et al*., 2005).

Allicin is a very labile compound that decays spontaneously, especially at high temperature (del Valle *et al*., 2012).

Thermal decomposition of allicin then originates a variety of volatile sulfides, namely 2-propenyl (allyl) derivatives (Avato *et al*., 2000).

Allicin is very unstable compound, soon rearranged and transformed into numerous lipid-soluble sulfur-containing byproducts, mostly DADS, DAS, DATS, allylmethyl trisulfide, and diallyl tetrasulfide. These compounds emit strong odors (Tsai *et al*., 2012).

Alliums contain mainly cysteine sulfoxides, and when tissues are chopped, the enzyme allinase is released, converting the cysteine sulfoxides into the thiosulfinates. These compounds are reactive, volatile, odor producing and lachrymatory (Benkeblia, 2004).

There are many proteins are present in garlic such as high molecular weight glycoprotein agglutinin, the antifungal protein, allivin, from round-cloved garlic and the antimicrobial protein, alliumin (Clement *et al*., 2010).

The characteristic flavors of fresh garlic are associated with thiosulfinates and the volatile substances are formed by the action of the enzyme allinase on hydrolyzing S-alkyl-substituted cysteine sulfoxide derivatives with the corresponding alkyl alkane thiosulfinates, ammonia, and pyruvic acid. This enzyme is separated from its natural substrates until the garlic tissue is disrupted (Yu and Shi-ying, 2007).

The thiosulfinates are present in all the Allium species. They are very unstable compounds and give rise to further rearrangement leading to a wide variety of sulfur compounds, which take part in further transformation and still exhibit biological activity. Among them, thiosulfinates, di- and tri-sulfur compounds, 2-vinyl-2,4-dihydro-1,3-dithiin, 3-vinyl-2,4-dihydro-1,3-dithiin, and ajoene (Lanzotti, 2006).

Thrombus formation plays an important role in the pathogenesis and progression of atherosclerosis, CVDs and diabetic complications. Whole garlic and garlic aqueous extract were able to inhibit platelet aggregation through multiple mechanisms, and is considered as an antithrombotic material (Chan *et al*., 2007).

2.4 ROLE OF C-REACTIVE PROTEIN AND GARLIC IN CARDIOVASCULAR DISEASE

Elevated plasma levels of CRP are predictive future cardiovascular disease events, and there is indeed evidence that CRP plays a predict role in the process of atherogenesis by activation of macrophages via Fcγ-receptors (Kolkhorf *et al*., 2010).

Garlic normalizes serum lipids in both acquired and familial hyperlipidemia and has both anti oxidant and also normalizes blood pressure in some hypertensive. The vascular endothelial cell is an important source of interleukin 6 with hypoxia being a potent stimulant for increased production (Anim-Nyame *et al*., 2004).

The presence of garlic extract modulates the expression of intercellular adhesion molecule 1 and vascular cell adhesion molecule 1 (Rassoul *et al*., 2006).

It is well known that adhesion molecules are expressed at the initial stage of atherosclerosis and play an important role in progression. Patients who developed symptomatic stroke had higher levels of ICAM-1 and hs-CRP at baseline (Kanai *et al*., 2008).

2.5 MOLECULAR DOCKING

In structure-based drug design the structures of known target proteins are used to discover new compounds of therapeutical relevance. The approach can be classified into two categories: de novo design and docking (Clauben *et al*., 2001).

Molecular docking is a process that predicts the conformation of a ligand within the active site of a receptor and finds the low-energy binding modes between them. It has become a useful tool in drug discovery (Kang *et al*., 2012).

Molecular docking method has been extensively used to analyze and elucidate the intermolecular interaction in chemistry (Wang *et al*., 2011).

Molecular docking techniques play an important role in the drug design as well as in the mechanistic study by placing a molecule into the binding site of the target macromolecule into the binding site of the target macromolecule in a non-covalent fashion (Parveen *et al*., 2011).

Docking procedure aims to identify the correct binding poses within the binding site of the protein while the scoring functions aim to predict binding affinity of ligand for the protein binding region (Mascarenhas and Ghoshal, 2008)

Based on the good performance of QSAR and docking studies, the developed models could be helpful to understand the structure-activity relationships of the two series of inhibitors and subsequently for the design of novel potent inhibitors (Cao, 2012).

Absorption, distribution, metabolism, and excretion (ADME) properties of compounds are important in pharmaceutical research. New drug discovery and development are time consuming, expensive and have a high attrition rate. An evaluation of the reasons for attrition showed that poor pharmacokinetics properties accounted for nearly 40% of drug development failure (Chen and Chen, 2012).

Molecular docking is used to predict the binding orientation of small molecule drug candidates to their protein targets in order to predict the affinity and activity of the small molecule (Vijesh *et al*., 2013).

Docking algorithm differ in the transformation search method used to generate the candidate solutions and the scoring functions applied to evaluate them. Some methods have a refinement stage that involves search of side-chain conformations in the interface area. The transformation method can be classified into brute-face search methods, local shape feature-matching algorithms and non-deterministic methods (Inbar *et al*., 2005).

Docking programs were designed to screen multi-compound databases for molecules that fit a binding site on the receptor. For each molecule, many orientations and conformations are sampled; based on these configurations, each molecule is scored for complementarities to the receptor and ranked relative to the other members of the database. This scoring are evaluated for their ability to reproduce known ligand-binding patterns for well-studied receptors (Wei *et al*., 2002).

Several approaches have been employed in attempts to solve the docking problem. Most of the current docking programs perform flexible ligand docking, but treat the receptor as rigid (half-flexible docking). The methods which these programs are based upon include incremental construction approaches, such as

FlexX, shape-based algorithms (DOCK and FLOG), genetic algorithms (GOLD and AutoDock 3.04), systematic search techniques (Glide, and Monte Carlo simulations and simulated annealing (AutoDock 2.4, BoxSearch and Ligand-Fit) (Chen *et al*., 2006).

The quality of a docking application depends on the scoring function for evaluating possible ligand conformations and on an efficient search method for finding a state with minimal binding energy (Janson *et al*., 2008).

3. MATERIALS AND METHODS

C-reactive protein is one of the major causes of cardiovascular diseases and Garlic is a good herbal remedy for heart diseases. The work was done to find how garlic constituents interact with C-reactive protein (ASN 61, GLU 81 and GLU 150 are the active sites, details of hydrogen bonds in the interaction).

Molecular docking studies of garlic and its similar compounds with C-reactive protein using maestro software were done. The complex structure of the C-reactive protein was taken from the PDB and the structures of ligands were taken from the PubChem compound Database. Ligands with best ADME properties can be used as lead compounds for computer aided drug discovery of cardiovascular diseases.

The Methodology on "**Molecular Docking Studies of C-Reactive Protein Inhibitors**" is discussed under the following headings:

3.1 PubChem
3.2 Protein Data Bank
3.3 Target
3.4 Ligand
3.5 Running and Monitoring Jobs
3.6 Preparation of Protein Target Structure
3.7 Docking Analysis Using Maestro
- **3.7.1 Maestro**
- **3.7.2 LigPrep**
- **3.7.3 Qikprop**
- **3.7.4 GLIDE**
- **3.7.5 Receptor Grid Generation**
- **3.7.6 Docking**

3.8 ADME-Toxicity Test

3.8.1 Absorption/Administration

3.8.2 Distribution

3.8.3 Metabolism

3.8.4 Excretion

3.1 PUBCHEM

The PubChem Compound Database contains validated chemical depicted information provided to describe substances in PubChem Substance. Structures stored within PubChem Compounds are pre-clustered and cross-referenced by identity and similarity groups.

PubChem, released in 2004, provides information on the biological activities of small molecules. PubChem also provides a fast chemical structure similarity search tool.

3.2 PROTEIN DATA BANK

The Protein Data Bank (PDB) archive is the single worldwide repository of information about the 3D structures of large biological molecules, including proteins and nucleic acids. This knowledge can be used to help deduce a structure's role in human health and disease and also in drug development. The structures in the archive range from tiny proteins and bits of DNA to complex molecular machines like the ribosome.

3.3 TARGET

The protein molecule chosen for the docking studies was C-reactive protein and also used as target structure in the current study. It was obtained from RCSB Protein Data Bank with the PDB ID **1GNH**.

The structure details of the protein are as follows:

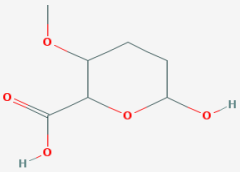
Crystal structure resolution	: 3.00 Å
Space group	: P_{212121}
Ligand chemical component	: Ca
Chains present	: 10 - (A, B, C, D, E, F, G, H, I, J)
Total residues	: 206 amino acids
Crystallography method	: X-ray crystallography
R-value	: 0.239

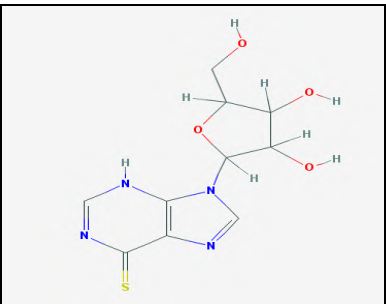
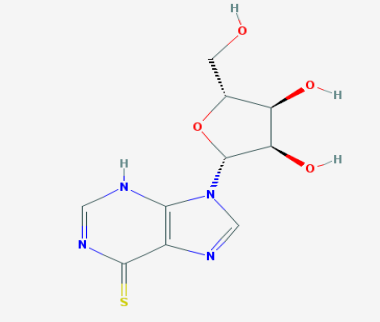
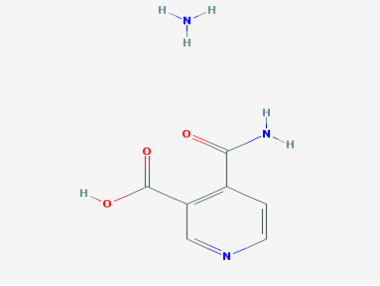
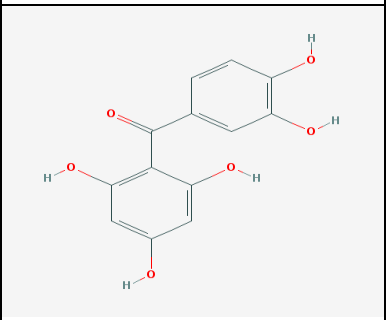
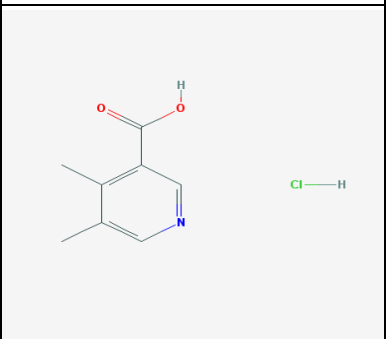
3.4 LIGAND

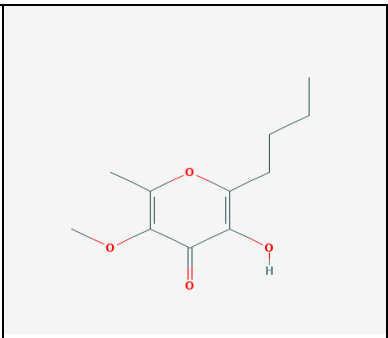
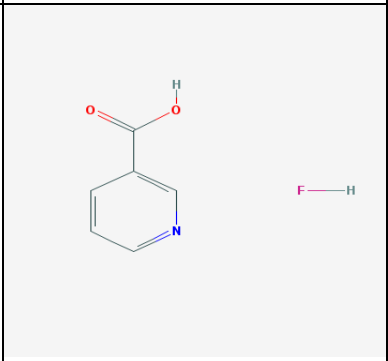
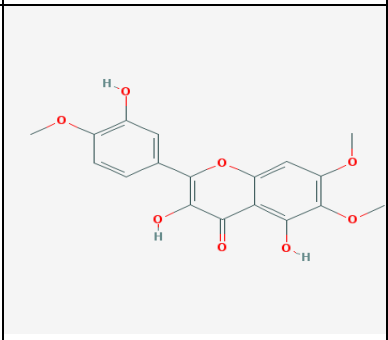
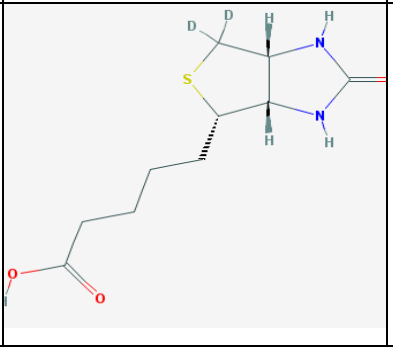
About 70 compounds were selected for docking studies, which were selected using literature studies. The canonical structure of PDB files of the compounds were used for docking. The list of selected compounds is presented in table 1.

TABLE - 1

LIST OF SELECTED COMPOUNDS, ITS STRUCTURE AND IUPAC NAME

S. No	COMPOUNDS (PUBCHEM ID)	STRUCTURE	IUPAC NAME
1	70672104		6-hydroxy-3-methoxyoxane-2-carboxylic acid

2	2861016		9-[3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-3H-purine-6-thione
3	676166		9-[(2R,3R,4R,4S,5R}-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-3H-purine-6-thione
4	70582259		4-carbamoylpyridine-3-carboxylic acid
5	68213		(3,4-dihydroxyphenyl)-(2,4,6-trihydroxyphenyl)methanone
6	70633739		4,5-dimethylpyridine-3-carboxylic acid

7	10536505		2-butyl-3-hydroxy-5-methoxy-6-methyl-pyran-4-one
8	70594729		pyridine-3-carboxylic acid;hydrofluoride
9	5317287		3,5-dihydroxy-2-(3-hydroxy-4-methoxylphenyl)-6,7-dimethoxychromen-4-one
10	60145986		5-[(3aR,6S,6aS)-4,4-dideuterio-2-oxo-3,3a,6,6a-tetrahydro-1H-thiedno[3,4-d]imidazol-6-yl]pentanoic acid

3.5 RUNNING AND MONITORING JOBS

The Job Control facility provides a uniform mechanism for launching, monitoring and controlling calculations. When a Schrödinger job is run on a particular host, Job Control determines where to find the hosts file, which version of the software to use, which environment variables need to be passed to the host, where the scratch directory is located, how to make the input files available to the job, how to retrieve the output files, and how to incorporate them into a Maestro project's.

3.6 PREPARATION OF PROTEIN TARGET STRUCTURE

The Protein Preparation Wizard panel allows you to take a protein from its raw state to a state in which it is properly prepared for use by Schrödinger. The Protein Preparation Wizard panel has three tabs, which contain tools for the stages of protein preparation. In the Import and Process tab, you can import a protein and perform the basic tasks for fixing the structures. The Review and Modify tab allows you to delete unwanted chains and waters, and fix or delete the groups. In the Refine tab you can optimize orientations of hydrogen-bonded groups and minimize the structure.

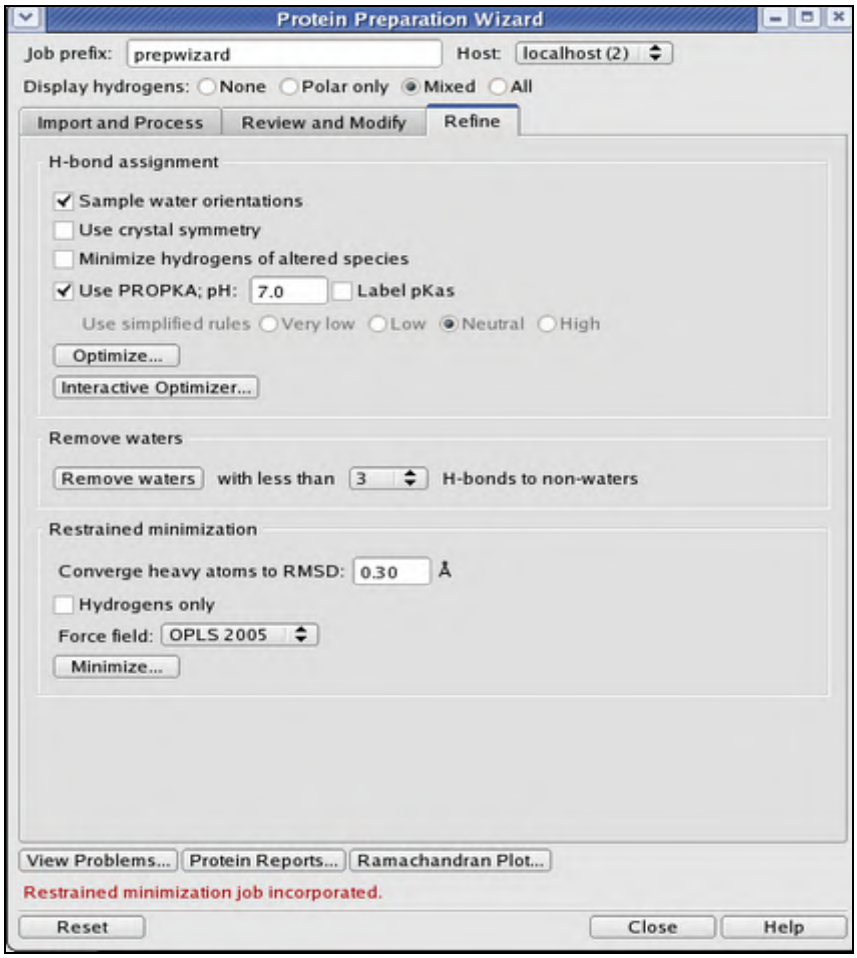


FIGURE - 2

PROTEIN PREPARATION WIZARD

3.7 DOCKING ANALYSIS USING MAESTRO

Maestro is the graphical user interface for all of Schrodinger's products as CombiGlide ™, Epik ™, Glide ™, Impact ™, Liaison ™, LigPrep ™, Phase ™, Maestro Model ™, Prime ™, QikProp ™, QSite ™, and Strike ™. It contains tools for building, displaying and manipulating chemical structures for organizing, loading and storing these structures and associated data and for setting up, monitoring, and visualizing the results of calculations on these structures.

3.7.1 MAESTRO

Maestro is the unified interface for all Schrödinger software. Impressive rendering capabilities, a powerful selection of analysis tools, and an easy-to-use design combine to make Maestro a versatile modeling environment for all researchers. Far more than just a visualization program, Maestro also helps researchers organize and analyze data. Computed results are automatically returned and incorporated into projects for further study. Maestro is a powerful and versatile molecular modeling environment, and the portal to the most advanced science in computational chemistry.

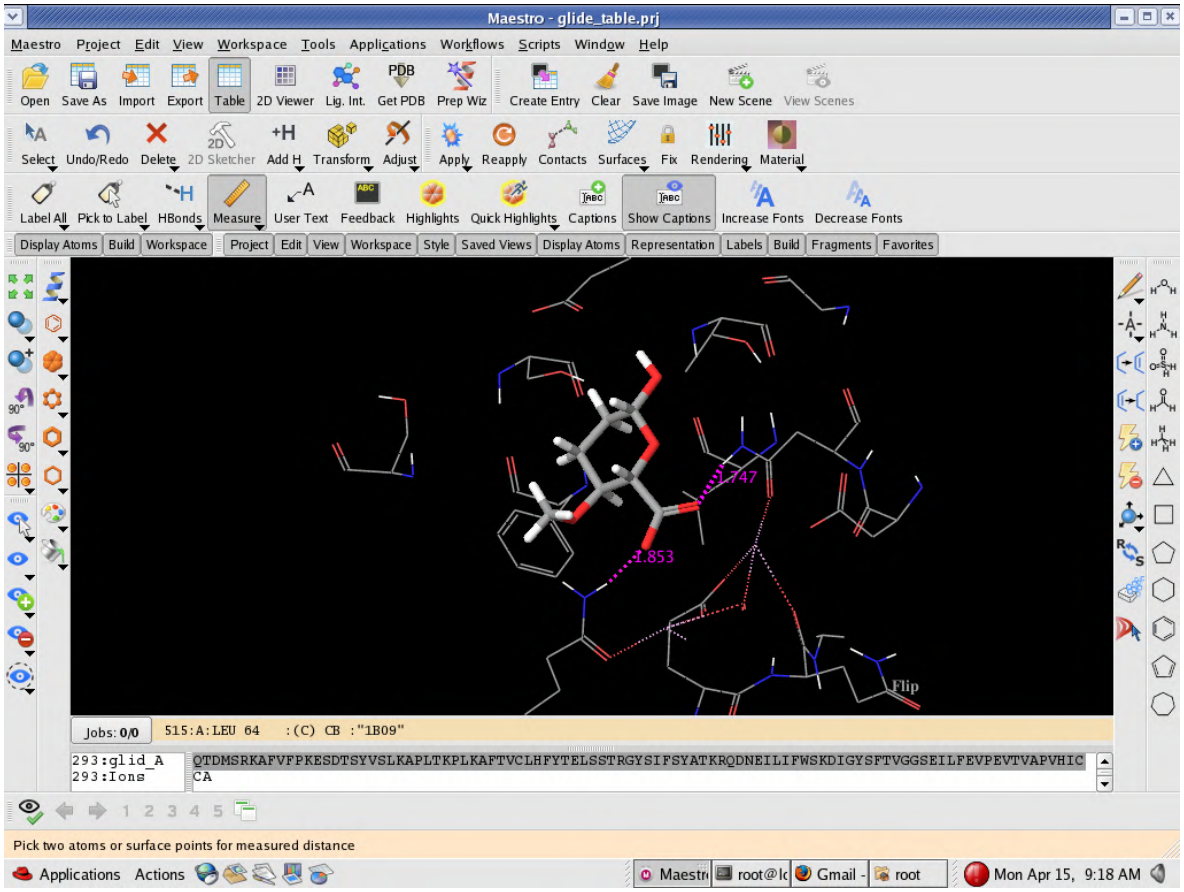


FIGURE – 3

MAESTRO MAIN WINDOW

3.7.2 LIGPREP

LigPrep is a robust collection of tools designed to prepare high quality, all-atom 3D structures for large numbers of drug-like molecules, starting with 2D or 3D structures in SD or Maestro format. The resulting structures can be saved in either SD or Maestro format. The simplest use of LigPrep produces a single, low-energy, 3D structure with correct chiralities for each successfully processed input structure. LigPrep can also produce a number of structures from each input structure with various ionization states, tautomers, stereochemistries, and ring conformations, and eliminate molecules using various criteria including molecular weight or specified numbers and types of functional groups present.

3.7.3 QIKPROP

QikProp is a quick, accurate, easy-to-use absorption, distribution, metabolism, and excretion (ADME) prediction program. It predicts physically significant descriptors and pharmaceutically relevant properties of organic molecules, either individually or in batches. In addition to predicting molecular properties, QikProp provides ranges for comparing a particular molecule's properties with those of 95% of known drugs. QikProp also flags 30 types of reactive functional groups that may cause false positives in high-throughput screening (HTS) assays. QikProp has two modes: normal mode, and fast mode. In fast mode, certain time-consuming calculations are omitted, some properties are not predicted and some have different values.

3.7.4 GLIDE (GRID-BASED LIGAND DOCKING WITH ENERGETICS)

Glide searches for favorable interactions between one or more ligand molecules and a receptor molecule, usually a protein. Glide can be run in rigid or flexible docking modes. The ligand poses that Glide generates pass through a series of hierarchical filters that evaluate the ligand's interaction with the receptor. The initial filters test the spatial fit of the ligand to the defined active site, and

examine the complementarity of ligand-receptor interactions using a grid-based method patterned after the empirical ChemScore function.

Poses that pass these initial screens enter the final stage of the algorithm, which involves evaluation and minimization of a grid approximation to the OPLS-AA (Optimized Potentials for Liquid Simulations- all atom) non bonded ligand-receptor interaction energy.

Final scoring is then carried out on the energy-minimized poses. By default, Schrödinger's proprietary GlideScore multi-ligand scoring function is used to score the poses. If GlideScore was selected as the scoring function, a composite Emodel score is then used to rank the poses of each ligand and to select the poses to be reported to the user. Emodel combines GlideScore, the nonbonded interaction energy, and, for flexible docking, the excess internal energy of the generated ligand conformation.

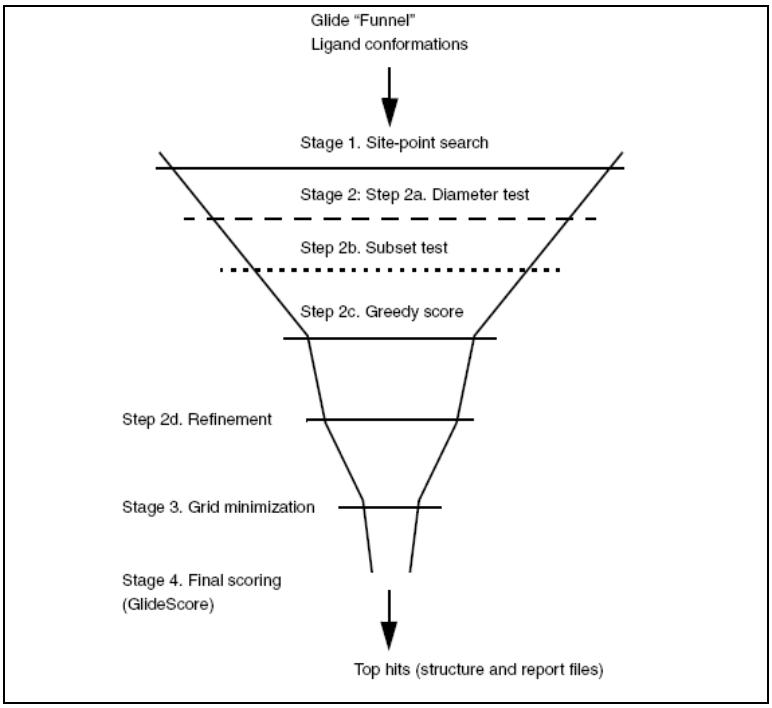


FIGURE – 4

GLIDE DOCKING HIERARCHY

3.7.5 RECEPTOR GRID GENERATION

The shape and properties of the receptor are represented on a grid by several different sets of fields that provide progressively more accurate scoring of the ligand poses.

The Receptor Grid Generation panel has five tabs:

- Receptor
- Site
- Constraints
- Rotatable Groups
- Excluded Volumes

3.7.6 DOCKING

Molecular docking is widely used to discover new ligands for biological targets with a known 3D structure (Ruth *et al*., 2006).

Glide ligand docking jobs require a set of previously calculated receptor grids and one or more ligand structures. The Ligand Docking panel has seven tabs:

- Settings
- Ligands
- Core
- Constraints
- Torsional Constraints
- Similarity
- Output

To specify the receptor grid for the docking job, click Browse in the Receptor grid section of the Settings tab to open a file selector and choose a grid file (.grd) or a compressed grid archive (.zip).

There are three choices of docking precision, given on the Precision option menu in the Docking section.

• HTVS (High-Throughput Virtual Screening) - High-throughput virtual screening (HTVS) docking is intended for the rapid screening of very large number of ligands. HTVS has much more restricted conformational sampling than SP docking, and cannot be used with score-in-place. Advanced settings are not available for HTVS, but are fixed at predetermined values.

• SP (standard precision) - Standard-precision (SP) docking is appropriate for screening ligands of unknown quality in large numbers. Standard precision is the default.

• XP (extra precision) - Extra-precision (XP) docking and scoring is a more powerful and discriminating procedure, which takes longer to run than SP. XP is designed to be used on ligand poses that have a high score using SP docking.

3.8 ADME-TOXICITY TEST

ADME is an acronym in pharmacokinetics and pharmacology for absorption, distribution, metabolism and excretion. It describes the disposition of a pharmaceutical compound within an organism. In order to improve the deliverability of a drug substance in the lead optimization process, it is essential to relate changes in molecular properties to structural modifications. All the four criteria influence the drug levels and kinetics of drug exposure to the tissues and hence influence the performance and pharmacological activity of the compound as a drug.

Criteria:

3.8.1 ABSORPTION / ADMINISTRATION

Before a compound can exert a pharmacological effect in tissues, it has to be taken into the bloodstream usually via mucous surface like the digestive tract. Uptake into the target organs or cells needs to be ensured too. This can be a serious problem at some natural barriers like the blood-barrier brain. Factors such

as poor compound solubility, chemical instability in the stomach, and inability to permeate the intestinal wall can all reduce the extent to which a drug is absorbed after the oral administration. Absorption critically determines the compound's bioavailability. Drugs that absorb poorly must be administrated in some less desirable way, like intravenously or by inhalation.

3.8.2 DISTRIBUTION

The compounds need to be carried out to its effectors site, most often via the bloodstream. From there, the compound may distribute into tissues and organ usually to different extents.

3.8.3 METABOLISM

Compounds need to be broken down as soon as they enter the body. The majority of small molecule drug metabolism is carried out in the liver by redox enzymes. As metabolism occurs, the initial (parent) compound is converted to new compounds called metabolites. When metabolites are pharmacologically inert, metabolism deactivates the administered dose of parent drug and this usually reduces the effects on the body. Metabolites may also be pharmacologically active, sometimes more so than the parent drug.

3.8.4 EXCRETION / ELIMINATION

Compounds and their metabolites to be removed from the body via excretion are usually through the kidneys (urine) or in the feces. Unless excretion is complete, accumulation of foreign substances can adversely affect normal metabolism.

There are three sites where drug excretion occurs. The kidney is the most important site and it is where products are excreted through urine. Biliary excretion is the process that intiates in the liver and passes through the gut until

the products are finally excreted along with waste products or faeces. The last method of excretion is through the lungs e.g. anesthetic gases.

4. RESULTS AND DISCUSSION

C-reactive protein, a member of pentraxin family, has been included in cardiovascular risk stratification and treatment. Its concentration in the circulation can increase up to 1000 fold during acute responses to serious infection or major tissue damage. The oxidative modification of LDL by reactive oxygen species is an important mechanism in the development of atherosclerosis. The beneficial effects of consumption in treating a wide variety of human diseases and disorders have been known for centuries. Garlic has the ability to inhibit the pathogenesis of cardiovascular disease.

The results of the present study entitled "**Molecular docking studies of C-reactive protein Inhibitors**" were presented in this chapter.

DOCKING RESULTS

Docking of 65 ligand molecules with the target C-reactive protein (PDB ID: **1GNH**) was carried out by maestro. Compounds with highest GScore were selected and their structure, interaction with the active site, bond distance of the interaction was discussed.

RESULT OF COMPOUND 70672104

The GScore for the compound 6-hydroxy-3-methoxyoxane-2-carboxylic acid was -0.6247. The interaction of the ligand with receptor molecule was viewed by maestro. From the output, the resulting interaction was analyzed. The ligand has shown good interaction with the residue (GLN150) C-O…H by hydrogen bond length 1.853Å.

RESULT OF COMPOUND 2861016

The GScore for the compound 9-[3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-3H-purine-6-thione was -6.362. The interaction of the ligand and receptor

molecule was viewed by maestro. From the output, the resulting interaction was analyzed. The ligand has shown good interaction with the residue (GLU81) N-H…O by hydrogen bond length 2.125Å.

RESULT OF COMPOUND 676166

The GScore for the compound 9-[(2R,3R,4R,4S,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-3H-purine-6-thione was -6.062. The interaction of the ligand and receptor molecule was viewed by maestro. From the output, the resulting interaction was analyzed. The ligand has shown good interaction with residue (GLU81) N-H…O by hydrogen bond length 1.971Å.

RESULT OF COMPOUND 70582259

The GScore for the compound 4-carbamoylpyridine-3-carboxylic acid was -5.410. The interaction of the ligand and receptor molecule was viewed by maestro. From the output, the resulting interaction was analyzed. The ligand has shown good interaction with residue (ASN61) C-O…H by hydrogen bond length 1.745Å.

RESULT OF COMPOUND 68213

The GScore for the compound (3,4-dihydroxyphenyl)-(2,4,6-trihydroxyphenyl)methanone was -5.223. The interaction of the ligand and receptor molecule was viewed by maestro. From the output, the resulting interaction was analyzed. The ligand has shown good interaction with residue (GLU81) O-H…O by hydrogen bond length 1.861Å.

RESULT OF COMPOUND 70633739

The GScore for the compound 4,5-dimethylpyridine-3-carboxylic acid was -5.244. The interaction of the ligand and receptor molecule was viewed by maestro. From the output, the resulting interaction was analyzed. The ligand has

shown good interaction with residue (ASN61) C-O…H by hydrogen bond length 1.745Å.

RESULT OF COMPOUND 10536505

The GScore for the compound 2-butyl-3-hydroxy-5-methoxy-6-methylpyran-4-one was -5.104. The interaction of the ligand and receptor molecule was viewed by maestro. From the output, the resulting interaction was analyzed. The ligand has shown good interaction with residue (ASN61) C-O…H by hydrogen bond length 1.745Å.

RESULT OF COMPOUND 70594729

The GScore for the compound pyridine-3-carboxylic acid was -5.110. The interaction of the ligand and receptor molecule was viewed by maestro. From the output, resulting interaction was analyzed. The ligand has shown good interaction with residue (GLN150) N-H…O by hydrogen bond length 1.998Å.

RESULT OF COMPUND 5317287

The GScore for the compound 3,5-dihydroxy-2-(3-hydroxy-4-methoxylphenyl)-6,7-dimethoxychromen-4-one was -5.094. The interaction of the ligand and receptor molecule was viewed. From the output, resulting interaction was analyzed. The ligand has shown good interaction with residue (GLU81) C-H…O by hydrogen bond length 1.788Å.

RESULT OF COMPOUND 60145986

The GScore for the compound 5-[(3aR,6S,6aS)-4,4-dideuterio-2-oxo-3,3a,6,6a-tetrahydro-1H-thieno[3,4-d]imidazol-6-yl]pentanoic acid was -5.014. The interaction of the ligand and receptor molecule was viewed. From the output resulting interaction was analyzed. The ligand has shown good interaction with residue (GLU81) C-O…H by hydrogen bond length 1.971Å.

TABLE - 2
BEST COMPOUND RESULTS

S. No	COMPOUND	INTERACTIONS (D-H…A)	BOND DISTANCE BETWEEN DONOR AND ACCEPTOR (Å)	GSCORE	GLIDE ENERGY
1	70652104	(GLN150) C-O…H (ASN61) C-O…H	1.853 1.747	-6.247	-29.479
2	2861016	(GLU81) N-H…O	2.194	-6.362	-35.351
3	676166	(GLU81) N-H…O (ASN61) C-H…O	1.971 2.125	-6.062	-32.958
4	70582259	(ASN61) C-O…H (GLN150) C-O…H	1.745 2.206	-5.410	-24.712
5	68213	(GLU147) O-H…O (GLNN150) O-H…O (ASN61) H-O…H (GLU81) O-H…O (GLU81) O-H…O	2.083 2.137 2.217 2.864 1.861	-5.223	-35.578
6	70633739	(GLN150) C-O…H (ASN61) C-O…H	2.074 1.745	-5.244	-23.005
7	10536505	(ASN61) C-O…H	1.745	-5.104	-23.072
8	70594729	(GLN150) N-H…O (ASN61) C-H…O	1.998 1.792	-5.110	-21.932
9	5317287	(GLU81) C-H…O (GLU81) C-H…O	2.334 1.788	-5.094	-33.579
10	60145986	(GLN150) C-O…H (ASN61) C-O…H	2.111 1.857	-5.014	-33.446

THE BEST COMPOUNDS INTERACTION VIEWS

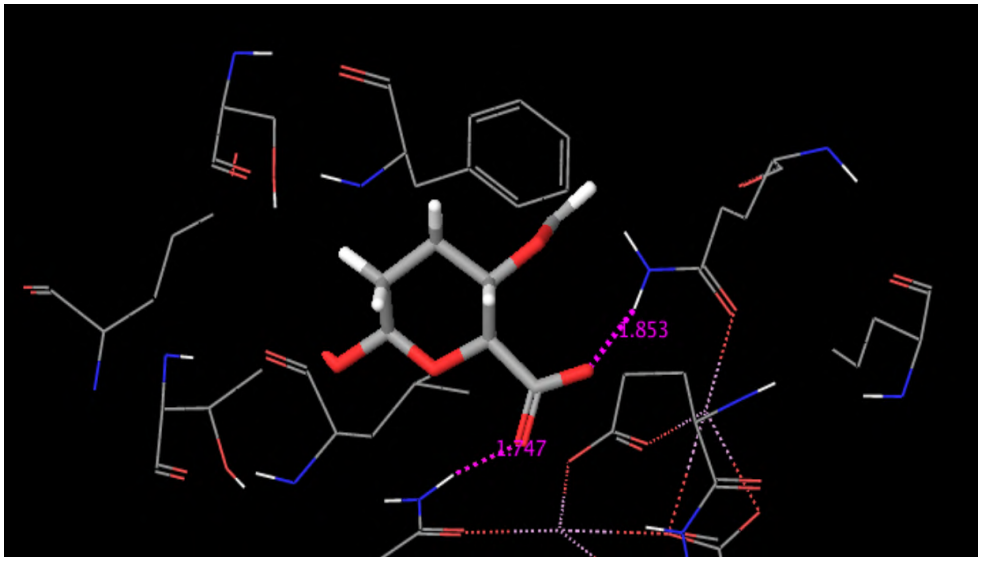


FIGURE - 5

INTERACTION OF COMPOUND 70672104 WITH THE ACTIVE RESIDUES OF TARGET PROTEIN CRP

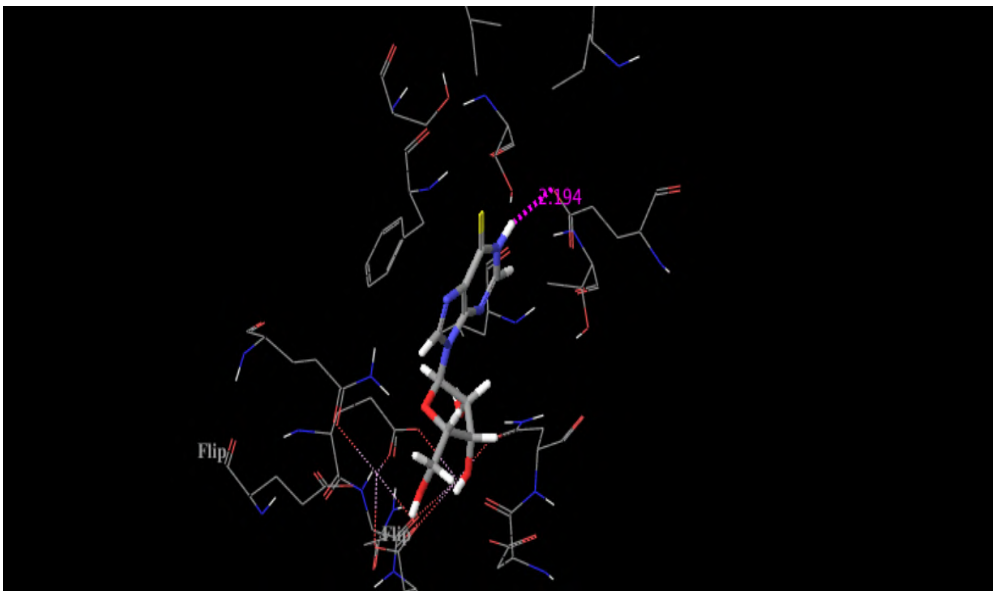


FIGURE - 6

INTERACTION OF COMPOUND 2861016 WITH THE ACTIVE RESIDUES OF THE TARGET PROTEIN CRP

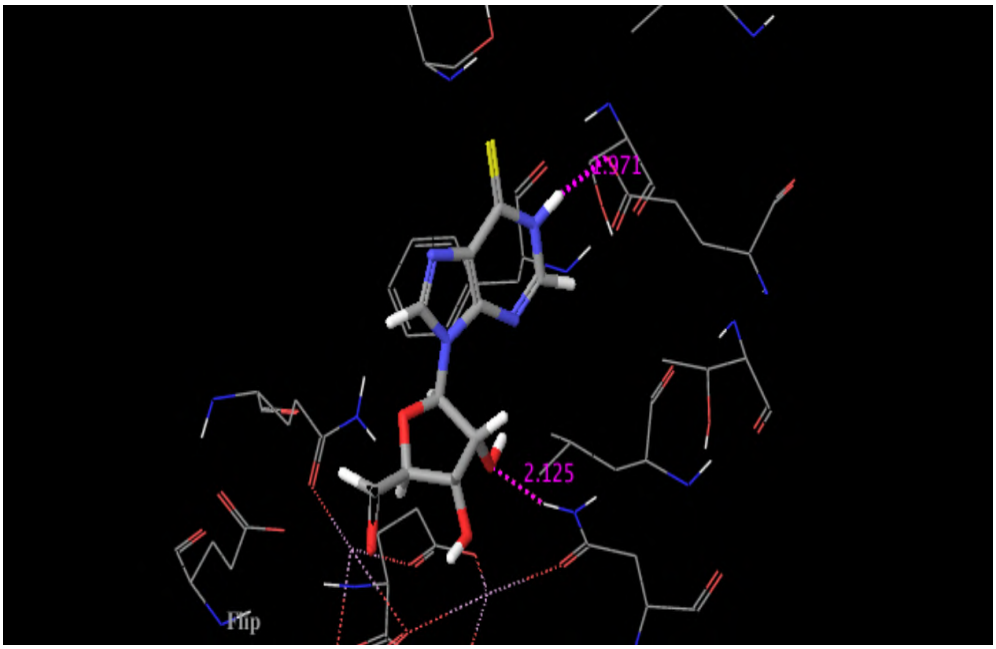


FIGURE - 7

INTERACTION OF COMPOUND 676166 WITH THE ACTIVE RESIDUES OF TARGET PROTEIN CRP

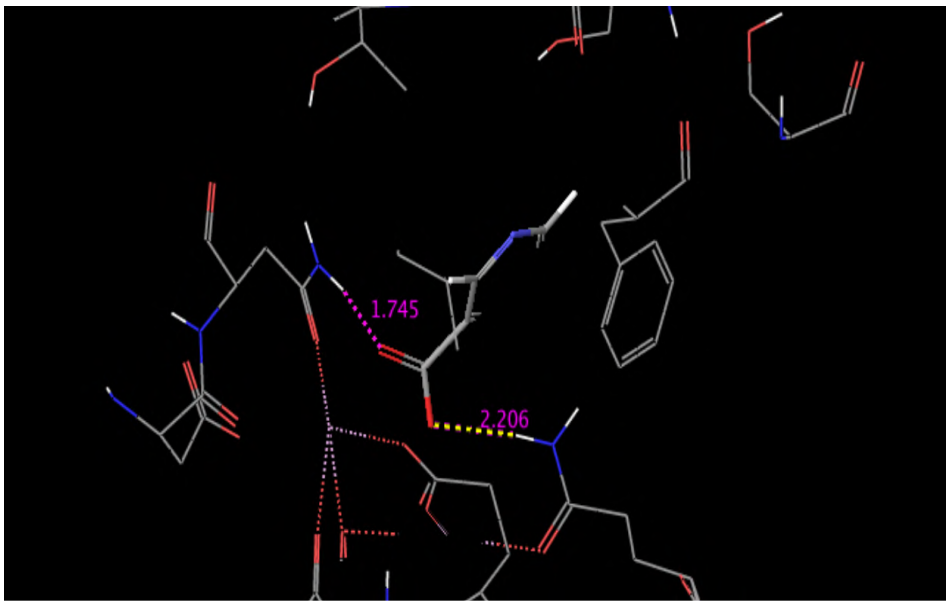


FIGURE - 8

INTERACTION OF COMPOUND 70582259 WITH THE ACTIVE RESIDUES OF TARGET PROTEIN CRP

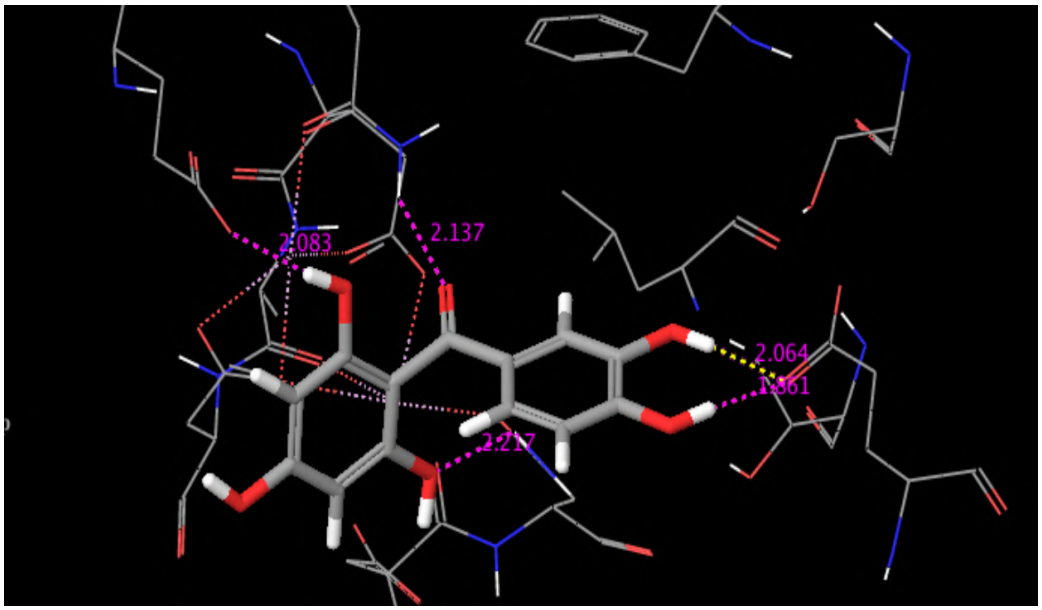


FIGURE - 9

INTERACTION OF COMPOUND 68213 WITH THE ACTIVE RESIDUES OF TARGET PROTEIN CRP

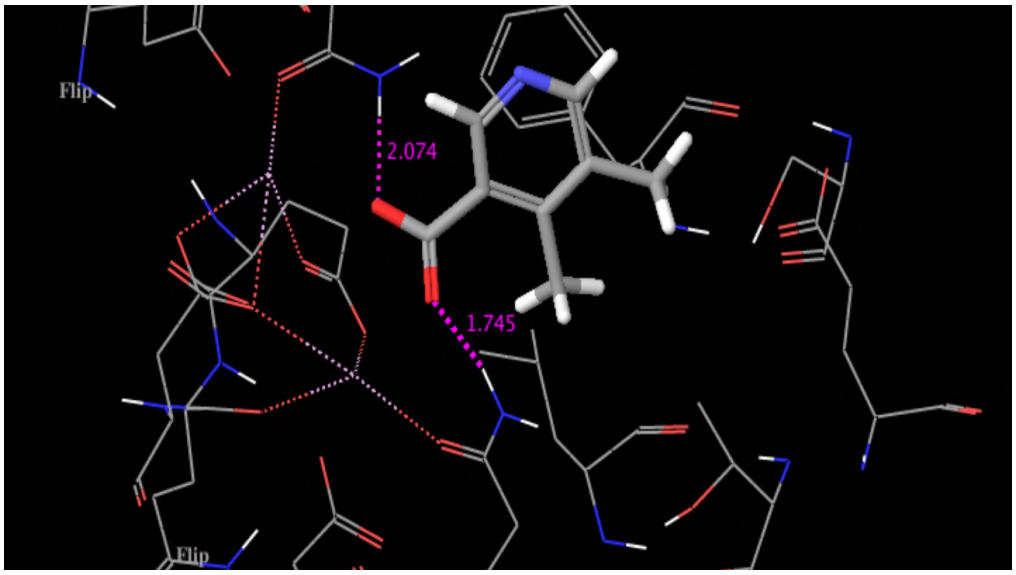


FIGURE - 10

INTERACTION OF COMPOUND 70633739 WITH THE ACTIVE RESIDUES OF TARGET PROTEIN CRP

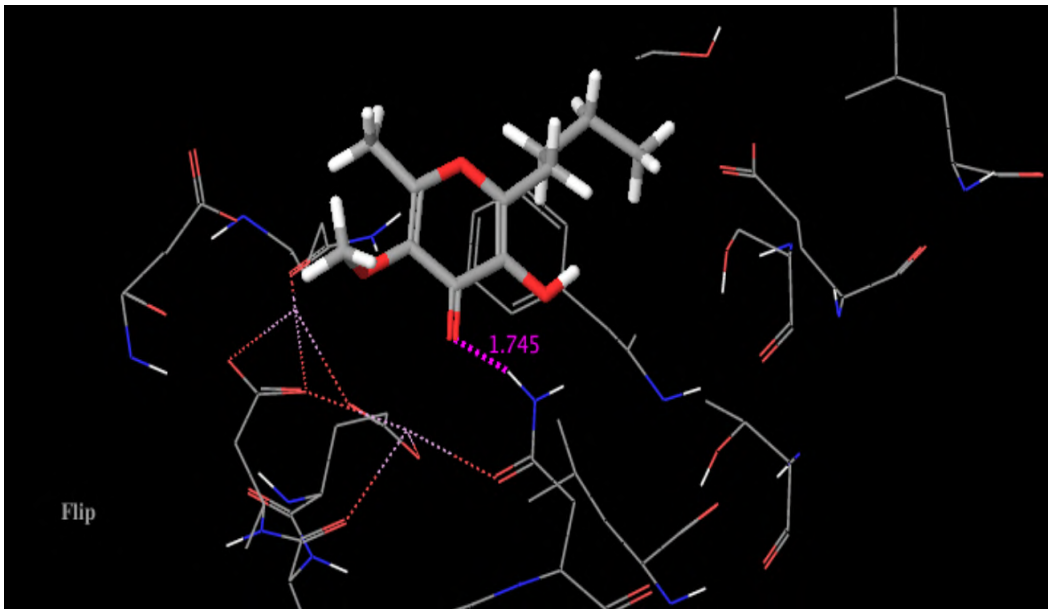


FIGURE - 11

INTERACTION OF COMPOUND 10536505 WITH ACTIVE RESIDUES OF TARGET PROTEIN CRP

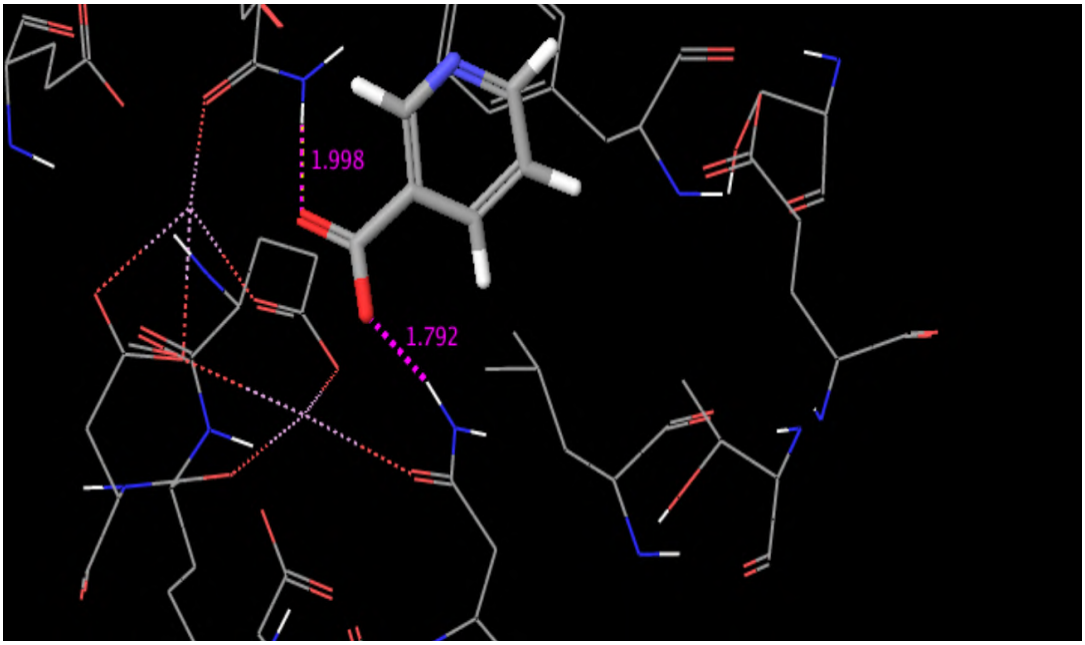


FIGURE - 12

INTERACTION OF COMPOUND 70594729 WITH ACTIVE RESIDUES OF TARGET PROTEIN CRP

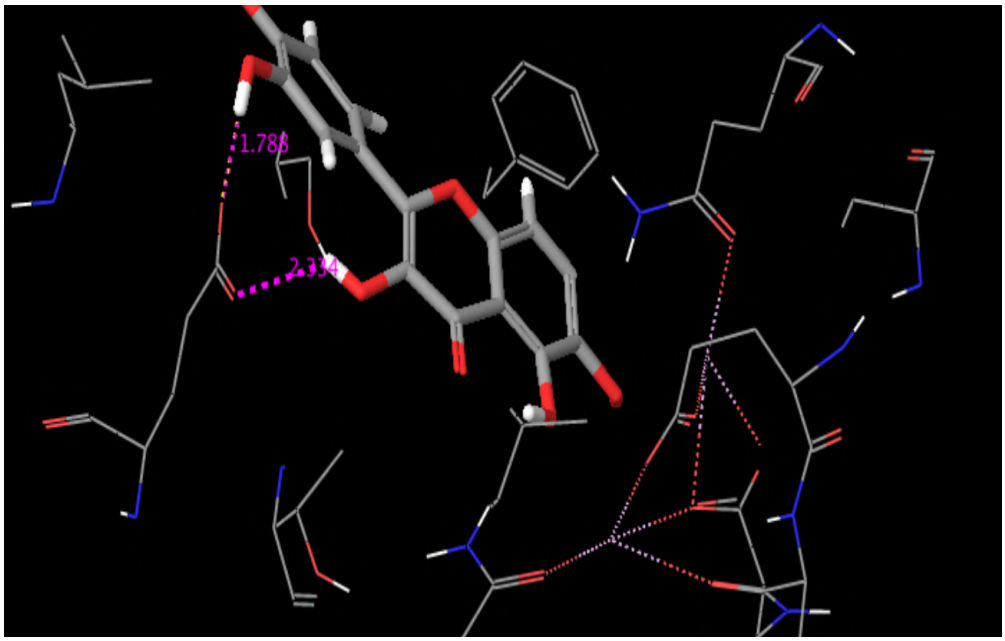


FIGURE - 13

INTERACTION OF COMPOUND 5317287 WITH ACTIVE RESIDUES OF TARGET PROTEIN CRP

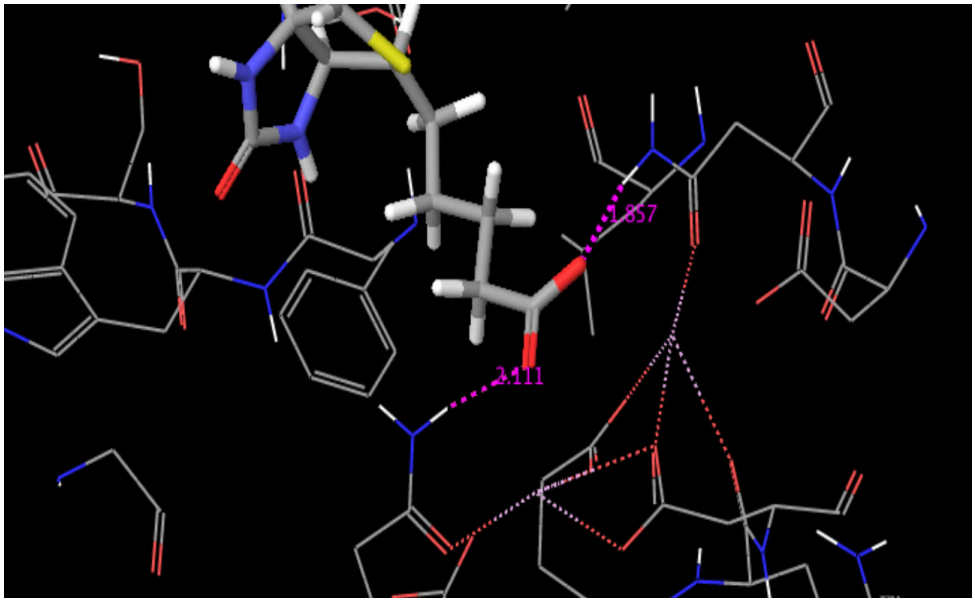


FIGURE - 14

INTERACTION OF COMPOUND 60145986 WITH ACTIVE RESIDUES OF TARGET PROTEIN CRP

TABLE - 3

ADME RESULTS OF THE BEST COMPOUNDS

	COMPOUND 70672104	**COMPOUND 2861016**	**COMPOUND 676166**
Mol wt (130.0 to 725.0)	176.169	284.289	284.289
Volume (500.0 to 2000.0	572.564	805.286	804.389
DonorHB (0.0 to 6.0)	2.0000	4.0000	4.0000
Hb AcceptHB (2.0 to 20.0)	7.1000	11.3000	11.3000
Qlog	-0.226	-1.076	-1.149
QPlogS (-6.5 to -0.5)	-0.770	-2.118	-2.120
Percent Human Oral Absorption (>80% is high & <25% is poor)	62.776	57.168	55.108
Rule of Five (maximum 4)	0	0	0
SASA (Solvent Accessible Surface Area in square angstroms) (300.0-1000.0)	355.669	473.627	473.903
IC50	-0.735	-3.816	-3.839
Ionization Penalty	0.0001	0.1555	0.1555

	COMPOUND 70582259	COMPOUND 68213	COMPOUND 70633739
Mol wt (130.0 to 725.0)	166.136	262.218	151.165
Volume (500.0 to 2000.0	530.590	784.755	549.807
DonorHB (0.0 to 6.0)	3.000	3.000	1.000
Hb AcceptHB (2.0 to 20.0)	6.000	3.750	3.500
Qlog	-0.549	0.625	1.136
QPlogS (-6.5 to -0.5)	-1.056	-2.321	-1.440
Percent Human Oral Absorption (>80% is high & <25% is poor)	47.857	55.406	74.002
Rule of Five (maximum 4)	0	0	0
SASA (Solvent Accessible Surface Area in square angstroms) (300.0-1000.0)	341.222	473.117	349.343
IC50	-1.471	-4.585	-1.295
Ionization Penalty	0.0006	0.0815	0.1001

	COMPOUND 10536505	COMPOUND 70594729	COMPOUND 5317287
Mol wt (130.0 to 725.0)	212.245	123.111	360.320
Volume (500.0 to 2000.0	766.503	448.099	1052.967
DonorHB (0.0 to 6.0)	1.000	1.000	2.000
Hb AcceptHB (2.0 to 20.0)	4.000	3.500	6.000
Qlog	1.836	0.674	2.243
QPlogS (-6.5 to -0.5)	-2.551	-0.715	-4.101
Percent Human Oral Absorption (>80% is high & <25% is poor)	93.576	69.111	81.840
Rule of Five (maximum 4)	0	0	0
SASA (Solvent Accessible Surface Area in square angstroms) (300.0-1000.0)	465.260	301.144	601.314
IC50	-3.722	-1.489	-5.010
Ionization Penalty	0.0026	0.0126	0.0145

	COMPOUND 60145986
Mol wt (130.0 to 725.0)	166.136
Volume (500.0 to 2000.0	530.590
DonorHB (0.0 to 6.0)	3.000
Hb AcceptHB (2.0 to 20.0)	4.500
Qlog	-0.549
QPlogS (-6.5 to -0.5)	-1.056
Percent Human Oral Absorption (>80% is high & <25% is poor)	47.857
Rule of Five (maximum 4)	0
SASA (Solvent Accessible Surface Area in square angstroms) (300.0-1000.0)	485.838
IC50	-2.084
Ionization Penalty	0.0041

DISCUSSION

C-reactive protein is an acute phase reactant that is regulated by microbial infection. CRP is a risk marker for cardiovascular disease. CRP plays direct role in inflammation and pathogenesis of atherosclerosis, including vascular smooth muscle cells migration and proliferation, foam cell formation and inflammatory cell recruitment. Cardiovascular disease is a major and health problem. Garlic possesses beneficial effects for prevention of cardiovascular disease.

In the present work, I proposed and evaluated the interaction of C-reaction protein and compounds of garlic to reduce the level of cardiovascular disease by using maestro software.

To study the molecular interaction of C-reactive protein and compounds of garlic, these compounds were docked into active site of C-reactive protein using GLIDE. The best ten compounds were taken from the result based on the glide score.

Based on ADME results compound 70652104, compound 2861016 and compound 676166 were found to satisfy all the necessary parameters to act as a drug.

Based on overall studies, we can conclude that compound 70652104, compound 2861016 and compound 676166 were found to be more potent inhibitors based on glide score, glide energy and interaction with residues in the active site of C-reactive protein. In future these compounds can be taken as an effective drug candidate in the structure based drug discovery process.

SUMMARY AND CONCLUSION

The Summary and Conclusion of the present study entitled "***Molecular Docking Studies of C - Reactive Protein Inhibitors"*** is presented under this chapter.

C-reactive protein plays an important role in cardiovascular disease. CRP becomes deposited in the infracted area and contributes to myocardial damage by promoting complement activation. Garlic has been shown good effect to reduce cardiovascular disease by lowering blood pressure and low density cholesterol level.

Natural products are the most consistently successful source of drug leads. The 3D structure and its information can be used for developing small-molecule dugs that interact with target protein. The structure of the target will provide a template for the discovery of novel ligands.

Maestro was used to find which compounds of garlic can be used as good inhibitors for C-reactive protein. And we can use that inhibitor as the lead compound for the structure based drug discovery of cardiovascular diseases.

From 70 ligands, three have been selected, Compound 70672104, 2861016 and 676166 based on the ADME properties GScore and Glide Energy and it can be used as good inhibitors for C-reactive protein. Active residue sites of the C-reactive protein (ASN 61, GLU 81 and GLU 150) interact with the ligand molecules. The three compounds have shown drug able properties. These can be used as lead compounds for the structure based drug discovery.

From these results, we can conclude that garlic can be used to reduce the level of CRP level.

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