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In-silico docking studies of Plant Derived Natural Products to identify potential drugs for the treatment of Diabetes

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In

Bioinformatics

Submitted by

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CERTIFICATE

This is to certify that the dissertation entitled ***In-silico*** **docking studies of Plant Derived Natural Products to identify potential drugs for the treatment of Diabetes (2k14/bio/12)** in the partial fulfillment of the requirements for the award of the degree of Masters of Engineering, Delhi Technological University (Formerly Delhi College of Engineering, University of Delhi), is an authentic record of the candidate's own work carried out by him under my guidance. The information and data enclosed in this thesis is original and has not been submitted elsewhere for honoring of any other degree.

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I declare that my major project entitled **"*In-silico* docking studies of Plant Derived Natural Products to identify potential drugs for the treatment of Diabetes"**, submitted to Department of Biotechnology, Delhi Technological University as a result of the work carried out by me at "Plant Biotechnology Laboratory", Department of Biotechnology, as Major project.

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ABSTRACT

Natural products are main sources of drug discovery. In this context groups of different set of flavonoids were taken and docked into the different cavities of the GPR40 (PDB ID: 4PHU) of Diabetes Mellitus (DM) and results were discussed. Natural compounds were found to very effective according to its binding energy and ligand efficiency score. Those compounds also have no adverse effect as carcinogenicity and mutagenicity and favorable drug likeness score. Identification and growth of naturally occurring compounds, such as flavonoids, as drugs against EDBC is in demand due to their smaller toxicity when equated to those of synthetic ones. Medicinal plants have been widely used to cure a range of contagious and non-contagious diseases. According to a study, 25% of the commonly used medicines comprise compounds extracted from plants. Several plants could propose a great reserve for drug discovery of infectious diseases, mainly in an era when the modern separation techniques are available on one hand, and the human population is confronted by a number of evolving infectious diseases on the other hand. Currently available therapeutic options for NDDM, such as dietary alteration, oral hypoglycemic, and insulin, have limitations of their own. Many natural products and herbal medicines have been suggested for the cure of diabetes. Many kinds of natural products, such as terpenoids, alkaloids, flavonoids, phenolic, and some others, have shown antidiabetic potential. Among active medicinal herbs, Momordica charantia L. (Cucurbitaceae), Pterocarpus marsupium Roxb. (Leguminoceae), and Trigonella foenum graecum L. (Leguminosae) have been reported as beneficial for treatment of type 2 diabetes. Binding interactions of drugs using docking studies is an important component of computer aided drug design paradigms. Lamarckian genetic algorithm methodology was employed for docking simulations using AutoDock4.2. The three important parameters like binding energy, inhibition constant and intermolecular energy were determined.

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1. INTRODUCTION

Diabetes mellitus is a heterogeneous group of disorders characterized by persistent hyperglycemia. The two most common forms of diabetes are type 1 diabetes (T1D, previously known as insulin-dependent diabetes or IDDM) and type 2 diabetes (T2D, previously known as non-insulin-dependent diabetes or NIDDM). Both are caused by a combination of genetic and environmental risk factors. However, there are other rare forms of diabetes that are directly inherited. These include maturity onset diabetes in the young (MODY), and diabetes due to mutations in mitochondrial DNA.

All forms of diabetes have very serious effects on health. In addition to the consequences of abnormal metabolism of glucose (e.g., hyperlipidemia, glycosylation of proteins, etc.), there are a number of long-term complications associated with the disease. These include cardiovascular, peripheral vascular, ocular, neurologic and renal abnormalities, which are responsible for morbidity, disability and premature death in young adults. Furthermore, the disease is associated with reproductive complications causing problems for both mothers and their children. Although improved glycemic control may decrease the risk of developing these complications, diabetes remains a very significant cause of social, psychological and financial burdens in populations worldwide.

1.1. Type 1 Diabetes

Epidemiology- T1D is caused by the autoimmune destruction of the beta cells of the pancreas, and represents approximately 10% of all cases with diabetes. At present, lifelong insulin therapy is the only treatment for the disease. Without exogenous insulin injections, individuals with T1D will not survive. Although the prevalence of T1D is <1% in most populations, the geographic variation in incidence is enormous, ranging from <1/100,000 per year in China to approximately 40/100,000 per year in Finland (Figure 1) (Karvonen et al., 1993). The only chronic childhood disorder more prevalent than T1D is asthma. It has been estimated that approximately 20 million people worldwide, mostly children and young adults, have T1D (Holt, 2004). The incidence of T1D is increasing worldwide at a rate of about 3% per year (Onkamo et al., 1999). This trend appears to be most dramatic in the youngest age groups, and is completely unrelated to the current increase in T2D in children. More children with beta cell autoantibodies, a hallmark of T1D, are being diagnosed with the T1D around the world each year. Although the peak age at onset is at puberty, T1D can also develop in adults. Epidemiologic studies have revealed no significant gender differences in incidence among individuals diagnosed before age 15 (Kyvik et al., 2004). However, after age 25, the male to female incidence ratio is approximately 1.5. There is also a notable seasonal variation in the incidence of T1D in many countries, with lower rates in the warm summer months, and higher rates during the cold winter (Dorman et al., 2003).

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Environmental Risk Factors- The epidemiological patterns described above suggest that environmental factors contribute to the etiology of the T1D. In particular, the recent temporal increase in T1D incidence points to a changing global environment rather than variation in the gene pool, which require the passage of multiple generations. Twin studies also provide evidence for the importance of environmental risk factors for T1D. T1D concordance rates for monozygous twins are higher than those for dizygous twins (approximately 30% vs. 10%, respectively) (Hirschhorn, 2003). However, most monozygous twin pairs remain discordant. Thus, T1D cannot be completely genetically determined.

Environmental risk factors are thought to act as either 'initiators' or 'accelerators' of beta cell autoimmunity, or 'precipitators' of overt symptoms in individuals who already have evidence of beta cell destruction. They also may function by mechanisms that are directly harmful to the pancreas, or by indirect methods that produce an abnormal immune response to proteins normally present in cells. The T1D environmental risk factors that have received most attention are viruses and infant nutrition. Another hypothesis that has been the subject of considerable interest relates to early exposure to cow's milk protein and the subsequent development of T1D. The first epidemiologic observation of such a relationship was by Borch-Johnsen et al., who found that T1D children were breast-fed for shorter periods of time than their non-diabetic siblings or children from the general population (Borsh-Johnsen et al., 1984). The authors postulated that the lack of immunologic protection from insufficient breast-feeding may increase risk for T1D later during childhood. It was also postulated that shorter duration of breast feeding may indirectly reflect early exposure to dietary proteins that stimulate an abnormal immune response in newborns. Most recently it has been hypothesized that the protective effect of breast-feeding may be due, in part, to its role in gut maturation (Kolb and Pozzilli, 1999; Harrison and Honeyman, 1999; Vaarala, 1999). Breast milk contains growth factors, cytokines, and other substances necessary for the maturation of the intestinal mucosa. Breast-feeding also protects against enteric infections during infancy, and promotes proper colonization of the gut. Interestingly, enteroviral infections can also interfere with gut immunoregulation, which may explain the epidemiologic associations between viral infections and T1D. The role of hygiene in the etiology of T1D is also currently being explored (McKinney et al., 1997; Marshall et al., 2004). It has been hypothesized that delayed exposure to microorganisms due to improvements in standard of living hinders the development of the immune system, such that it is more likely to respond inappropriately when introduced to such agents at older (compared to younger) ages. This explanation is consistent with recent reports indicating that factors such as day care attendance (McKinney et al. 2000), sharing a bedroom with a sibling, and contact with pets are protective against T1D (Marshall et al., 2004). Further studies are needed to determine if improved hygiene can explain the temporal increase in the incidence of T1D worldwide.

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1.2. Type 2 Diabetes

Epidemiology- T2D is the most common form of the disease, accounting for approximately 90% of all affected individuals. A diagnosis of T2D is made if a fasting plasma glucose concentration is ≥ 7.0 mmol/L (≥ 126 mg/dl) or plasma glucose 2 hours after a standard glucose challenge is ≥ 11.1 mmol/L (≥ 200 mg/dl) (WHO, 1999) T2D is caused by relative impaired insulin secretion and peripheral insulin resistance. Typically, T2D is managed with diet, exercise, oral hypoglycemic agents and sometimes exogenous insulin. However, it is associated with the same long-term complications as T1D. In addition to the burden of T2D there are an even larger number of people with raised levels of blood glucose but below the level for diabetes. The World Health Organization defines impaired fasting glucose as a fasting plasma glucose level of ≥ 6.1 $mmoll^{-1}$ and < l7 $mmoll^{-1}$, and impaired glucose tolerance as 2 hour plasma glucose, post glucose challenge, of 7.8 to less than 11.1 $mmoll^{-1}$ (WHO, 1999). The prevalence of T2D increases with age of population (Wild et al., 2004). In developing countries, the largest numbers of people with diabetes are in the age group 45 to 64 years, while in developed the largest number is found in those aged 65 years and over. These differences largely reflected differences in population age structure between developed and developing countries. Worldwide rates are similar in men and women, although they are slightly higher in men < 60 years of age and in women > age 65 years.

Environmental Risk Factors- As early as 1962, Neel hypothesized that T2D represented a 'thrifty genotype', which had a selective advantage (Neel, 1962). He postulated that in primitive times, individuals who were 'metabolically thrifty' and able to store a high proportion of energy as fat when food was plentiful were more likely to survive times of famine. However, in recent years, most populations experience a continuous supply of calorie-dense processed foods, as well as a decrease in physical activity. This likely explains the rise in T2D prevalence worldwide. The major environmental risk factors for T2D are obesity (≥ 120% ideal body weight or a body mass index ≥ 30 k/m^2) and a sedentary lifestyle (van Dam, 2003; Shaw and Chisholm, 2003). Thus, the tremendous increase in the rates of T2D in recent years has been attributed, primarily, to the dramatic rise in obesity worldwide (Zimmet et. al., 2001). It has been estimated that approximately 80% of all new T2D cases are due to obesity (Lean, 2000). This is true for adults and children

1.3. Plant derived natural product in diabetes

The chemical compounds/substances found in living organisms are known as natural compounds. The various sources of these natural compounds include plants, animals, and microorganisms. Natural bioactive compounds are a source of novel pharmaceuticals because of their diversity, which enables the synthesis of drugs that differ from other chemical compounds

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in terms of their complex structures and biological potency. About 50% of the drugs approved by the US Food and Drug Administration are phytogenic compounds or derivatives thereof. Aspirin, metformin, morphine, vinblastine, vincristine, quinine, artemisinin, etoposide, teniposide, paclitaxel, and camptothecin are examples of natural compound-derived pharmaceuticals (Kingston et al., 2010). About 1200 plants have been claimed to contain compounds with antidiabetic properties, and over 400 plants and their bioactive compounds have been scientifically evaluated for type 2 diabetes treatment (Singh et al., 2011). However, very little is known about the mechanism of action of plants traditionally used as antidiabetics, preventing them from being used in diabetes care. Recently, more research is being focused on elucidating the mechanism of action of these plants and their active compounds. The modern oral hypoglycemic agents produce undesirable and side effects. Thus, alternative therapy is required; a need of hour is to shift towards the different indigenous plant and herbal formulations (Satyanarayana et al., 2006).

The main objective of this study is to find out the Plant Derived Natural Products which can further be studied by means of docking so that they can be used as drugs for the treatment of Diabetes.

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2. REVIEW OF LITERATURE

2.1. DIABETES

Diabetes is not an epidemic anymore but has turned into pandemic (Lal et al., 2009). The worldwide survey reported that diabetes is affecting nearly 10% of the population (Doss et al., 2009). According to the WHO projections, the prevalence of diabetes is likely to increase by 35% by the year 2025 (Asaduzzaman et al., 2010). India has a high prevalence of diabetes and the numbers are increasing at an alarming rate. In India alone, diabetes is expected to increase from 40.6 million in 2006 to 79.4 million by 2030 (Mehta et al., 2009).

2.1.1. Diagnostic approaches for Diabetes

Diagnosis of diabetes at an earlier stage is important in preventing diabetes related complications. The tests commonly used to diagnose diabetes are fasting blood glucose, postprandial blood glucose and HbA1c. Recent clinical studies have shown that acute glucose swings in addition to chronic hyperglycemia can trigger oxidative stress mechanisms in type 2 diabetes, demonstrating the importance for therapeutic interventions during acute and sustained hyperglycemic episodes (Monnier et al., 2006).

i. Blood sugar as a biomarker for diabetes

The criteria for the diagnosis of diabetes mellitus in clinical practice is fasting plasma glucose that is equal or greater than 126 mg/dL or two-hours post prandial plasma glucose greater than 200 mg/dL (Nwankwo et al., 2008)

ii. HbA1c as a biomarker for glycemic control

Glycated hemoglobin (HbA1c) is the best measure of long-term glycemic control, since it represents the average blood glucose levels over several months (Thomas and Elliott, 2009). Glycemic control is defined as excellent if the measured HbA1c is < 6.5 %,very good if HbA1c is 6.5 to 7.0 % , good if HbA1c is 7.1 to 7.5 %, acceptable if HbA1c is 7.6 to 8.0 % and poor if HbA1c is > 8.0 % (Al-Shoumer et al., 2008).

iii. C-peptide as a biomarker for differential diagnosis of type 1 and type 2 diabetes

Type 1 diabetes is distinguished from type 2 diabetes on the basis of the need for exogenous insulin for survival (Maghsoudi et al., 2008). C-peptide level may be used to distinguish people with new-onset type 2 diabetes from those with type 1 diabetes in addition to obesity, family history of type 2 diabetes and absence of glutamic acid decarboxylase (GAD)-65 antibodies (Aggarwal et al., 2010). Fasting C-peptide level < 0.6 ng/ml is considered as an indicator of poor insulin reserve.

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2.1.2. Complications of diabetes

Severe long term abnormalities can result such as eye complications, heart disease, kidney and foot problems if blood sugar levels are poorly controlled (Brophy et al., 2007). These complications are of two types- microvascular complications that include retinopathy, nephropathy, neuropathy and peripheral vascular disorders and macrovascular complications that include cardiovascular and cerebrovascular disorders. The complications of diabetes can involve multiple systems throughout the body that are susceptible to the detrimental effects of oxidative stress and apoptotic cell injury (Maiese et al., 2010). The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction and failure of various organs especially the eyes, kidneys, nerves, heart and blood vessels (Chandramohan et al., 2009).

i. Causes of diabetic complications

The complications of diabetes are the result of multiple factors in particular, cellular pathways that lead to diabetes. The complications of diabetes have been tied to oxidant stress (Szabo, 2009). Studies with diabetic animals have shown that oxidative stress leads to DNA damage in renal cortical cells (Simone et al., 2008). Although early effects of elevated glucose may increase the presence of potentially protective pathways, more prolonged exposure of elevated glucose with the rise in insulin levels can lead to reactive oxygen species (ROS) and can be detrimental even if glucose levels are controlled (Barbosa et al., 2008).

ii. Mechanisms leading to complications

Oxidative stress may promote the onset of diabetes by decreasing insulin sensitivity and destroying the insulin-producing cells. ROS can penetrate through cell membranes and cause damage to β-cells of pancreas (Chen et al., 2005). A high fat diet or free fatty acids also has been shown to release ROS and contribute to mitochondrial DNA damage and impaired pancreatic β-cell function (Rachek et al., 2006). Oxidant stress and ROS exposure can result in the opening of the mitochondrial membrane permeability transition pore, reduce mitochondrial NAD+ stores and result in apoptotic cell injury (Chong and Maiese, 2005). Free fatty acids also can lead to ROS release, mitochondrial DNA damage and impaired pancreatic β-cell function (Li et al., 2008). The development of diabetes has been associated with a decrease in the levels of mitochondrial proteins and mitochondrial DNA (Choo et al., 2006). Long standing diabetes mellitus is associated with an increased prevalence of microvascular and macrovascular diseases (Mehta et al., 2009). Cellular pathways in diabetes are closely associated to cellular energy maintenance and intact mitochondrial function (Newsholme et al., 2007).

2.1.3. Interventions for diabetes

Once diabetes is diagnosed, adequate treatment requires a significant amount of resources for patients i.e. access to glucometers, medications, regular access to health care and referral to

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specialists for management of complications. Life style changes/interventions and drugs are the current strategies that exist to prevent or reduce the onset of diabetes (Mehta et al., 2009). These complications of diabetes are the result of multiple factors, but argue for the implementation of novel drug development strategies (Szabo, 2009). Type 1 diabetes is the classical form of diabetes and these subjects cannot survive without insulin treatment. Type 2 diabetes is a group of genetically determined diseases which may be controlled by diet, hypoglycemic agents and /or exogenous insulin (Ahmed et al., 2010).

i. Diet

Diet therapy is the cornerstone of treatment in diabetes, especially for type 2 diabetes patients. It is difficult to maintain dietary control for long periods, but dietary control is important and necessary (Shabbidar et al., 2006). With more sedentary lives and more available food, our waistlines are growing and chronic diseases related to nutrition – like diabetes and cardiovascular disease are on the rise. Our diets, although abundant, are relatively less healthy than in the past (Livesey and Taylor, 2008). Nutrition therapy is an essential component of successful diabetes management and carbohydrate accounts for the largest percentage of energy intake (Wylie-Rosett and Albright, 2007). Different carbohydrate foods have different effects on blood glucose and can be ranked by the overall effect on the blood glucose levels using the glycemic index (Thomas and Elliott, 2009). The glycemic index (GI) is a system for ranking carbohydrates according to their effects on postprandial glucose concentrations. Although low-GI foods are known to produce less postprandial hyperglycemia and hyperinsulinemia than are highGI foods, the role of low-GI foods in the prevention and treatment of diabetes remains unclear (Miles, 2008). Glycemic index may be beneficial in improving weight regulation, postprandial glucose level, insulin action and risk for cardiovascular disease (Miller et al., 2009). Both the amount and the type of carbohydrate induce distinct plasma glucose and insulin responses that are quantified by the glycemic index (Bove et al., 2006). In the past, diabetic patients were advised to avoid carbohydrates, but it is now accepted and recommended by diabetic associations that 60-70% of the calories in a diabetic diet should be provided by carbohydrate and that carbohydrate should be in the form of complex polysaccharides (starch) and nonstarch polysaccharide (dietary fiber). Intake of food high in dietary fiber (such as whole grain, unrefined cereals and legumes) instead of more rapidly digested forms of carbohydrates improve glycemic control because of the slow release of carbohydrate due to the high fiber content (Weickert et al., 2006).

ii. Physical activity

During the past 50 years several studies have underlined the central role of physical exercise in the management of patients with both type 1 and type 2 diabetes mellitus. Children, adolescents and young adults with diabetes must be educated on the metabolic changes occurring during

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physical activity in order to acquire the ability to individually modulate their diet and insulin therapy before and after exercise (Giannini et al., 2007). Regular aerobic exercise reduces visceral fat mass and body weight without decreasing lean body mass, ameliorates insulin sensitivity, glucose and blood pressure control, lipid profile and reduces the cardiovascular risk (Feo et al., 2006).

iii. Oral antidiabetic drugs

Pharmacologic treatment of type 2 diabetes to improve glycemic control, to control hypertension and to reduce blood lipid concentrations reduces the occurrence and progression of diabetes complications (Wolever et al., 2008). With a better understanding of the molecular mechanisms of diabetes, patients with genetic defects encoding the β-cell pathways were found to be more responsive to sulphonylurea therapy than to metformin treatment. Phenotyping and targeted therapy can minimize risk and maximize efficacy (Ko et al., 2009).

α αα α- Glucosidase inhibitors- Alpha- glucosidase inhibitors (AGIs) such as voglibose are known to inhibit disaccharide hydrolysis in intestinal mucosa, thereby reducing the hydrolysis of disaccharides to monosaccharides. This impedes absorption of carbohydrate and therefore reduces glucose levels in type 2 diabetes patients. Voglibose treatment was found to prevent the increase in body weight (Negishi et al., 2008). Alpha- glucosidase inhibitors (acarbose, miglitol, voglibose) are widely used in the treatment of patients with type 2 diabetes that have a lowering effect on postprandial blood glucose and insulin levels (Van de Laar et al., 2005).

Biguanides- Metformin, a biguanide, is one of the most commonly used first-line antihyperglycemic agents in the treatment of type 2 diabetes, which acts primarily by lowering hepatic glucose production and may also improve insulin resistance (Charbonnel et al., 2006). Metformin is the only antidiabetic agent that has been shown to reduce mortality in patients newly diagnosed with type 2 diabetes and the only antidiabetic agent not shown to be associated with increased morbidity and mortality in patients with cardiac disease, including heart failure (Eurich et al., 2009).

Third generation sulphonylurea drugs- The sulfonylureas stimulate insulin release from pancreatic β cells and have been a cornerstone of type 2 diabetes pharmacotherapy for over 50 years. Although sulfonylureas are effective antihyperglycemic agents, interindividual variability exists in drug response namely pharmacodynamics, disposition namely pharmacokinetics and adverse effects (Aquilante, 2010). The third generation of sulphonylurea, glimepiride stimulates nitric oxide production and thereby inhibits cytokine-induced nuclear factor (NF)-κB activation in endothelial cells and confers protective effects on vascular endothelial cells. They are

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preferable sulphonylurea agents in the treatment of type 2 diabetes and vascular diseases (Jojima et al., 2009).

Thiazolidinediones- Pioglitazone, a member of the thiazolidinedione drug family, is widely used for the treatment of type 2 diabetic patients. This antihyperglycemic drug is a selective ligand of the nuclear transcription factor, peroxisome proliferator-activated receptor (PPAR-γ). PPAR-γ receptor activation increases glucose and lipid uptake, increases glucose oxidation, decreases free fatty acid concentration and decreases insulin resistance. PPAR-γ receptor activation also stimulates adipocyte differentiation resulting in more and smaller fat cells (Smith et al., 2005).

Dipeptidyl peptidase-4 (DPP- 4) inhibitors- DPP-4 inhibitors offer a new therapeutic approach for the management of patients with type 2 diabetes (Charbonnel et al., 2006).Sitagliptin is a oncedaily, orally active, competitive and fully reversible inhibitor of dipeptidyl peptidase-4, the enzyme that is responsible for the rapid degradation of the incretin hormone glucagon-like peptide-1 (Deacon, 2007).

iv. Insulin

Insulin is the primary treatment for all patients with type 1 diabetes and for type 2 diabetic patients who cannot adequately control their blood sugar by diet and exercise or by oral hypoglycemic agents (Nathan et al., 2006).

Newer Insulins- Novel long and short acting insulin analogues, the so-called 'designer insulins', developed through genetic engineering in the 1990s. Newer analogues exist as monomers and are absorbed much faster (insulin aspart or lispro) or absorbed very slowly (insulin glargine or detemir). The newer analogues have increased the stability, less variability and selective action which will help in developing individualized treatment suitable to specific patient characteristics and will improve glycemic control (Kaur and Badyal, 2008).

Other newer insulins- Albulin is the newest insulin analogue. Albulin displays characteristics of a potent long acting insulin analogue that can be evaluated for use as a novel insulin therapy for patients with insulin-dependent diabetes (Duttaroy et al., 2005). Inhaled insulin drugs have faster onset of action, even faster than intravenous route and large surface area of lungs causes more systemic absorption. If become the first non-subcutaneous route of insulin administration for widespread clinical use. Exubera, an insulin product for pulmonary delivery in powder form is the first inhalational drug to be approved by food and drug administration (Mandal, 2005).

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2.1.4. Plant compounds as antidiabetic agents

The use of plant by man for the treatment of diseases is an age long practice (Prohp et al., 2008). Diabetes mellitus was known in ancient times and some medicinal plants have been used for its control in traditional medicine (Mukherjee et al., 2006). The oral antihyperglycemic agents currently used in clinical practice have characteristic profiles of serious side effects. This leads to increasing demand for herbal products with antidiabetic activity and less side effects (Doss et al., 2009). The efficacy of plant for the management of diabetes requires confirmation and WHO has recommended the assessment of traditional plant.

Recently, some medicinal plants have been reported to be useful in diabetes worldwide and have been used empirically as antidiabetic and antihyperlipidemic remedies. Despite the presence of known antidiabetic medicine in the pharmaceutical market, diabetes and the related complications continued to be a major medical problem. Antihyperglycemic effects of these plants are attributed to their ability to restore the function of pancreatic tissues by causing an increase in insulin output or inhibit the intestinal absorption of glucose or to the facilitation of metabolites in insulin dependent processes. More than 400 plant species having hypoglycemic activity have been available in literature, however, searching for new antidiabetic drugs from natural plants is still attractive because they contain substances which demonstrate alternative and safe effects on diabetes mellitus. Most of plants contain glycosides, alkaloids, terpenoids, flavonoids, cartenoids, etc., that are frequently implicated as having antidiabetic effect.

Since Phytomedicine has globally been the matter of interest in primary source of healthcare (Farnsworth et al., 1976) that encouraged its utilization as a source of chemical diversity in drug development. Plant-derived molecule (PDM) structures are known to have evolved under evolutionary pressure with diverse properties that make them suitable as lead structures in drug discovery (Evans et al., 1988). PDMs have also been recognized to provide specific substructures or scaffolds that make them comparable to trade drugs and their potential utilization in combinatorial chemistry (Basmadjian et al., 2014). Such exceptional properties exhibited by PDMs make their direct use in drug discovery as well as by using them as scaffolds to synthesize combinatorial repertoire proficient enough to bind against wide range of disease-specific targets. In fact, it could be argued that plants with medicinal values may have co-evolved with humans. Various disease treatments have become dependent now upon natural products importantly diabetes (Hung et al., 2012).

2.1.5. Emerging potential drugs targeting GPR40 for the regulation of insulin secretion

Due to tissue distribution, the pharmacological activation of GPR40 provides a novel target for the treatment of type 2 diabetes. Certain synthetic GPR40 agonists are very promising to become

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the drug for mediating insulin secretion. For example, GW9508, a small molecule agonist, activates GPR40 and stimulates GSIS in MIN6 cells, implicating a potential glucose-sensitive insulin secretagogue (CP Briscoe et al., 2006). A phenylpropanoic acid derivative named 3-{2-fluoro- 4-[({4'- [(4-hydroxy-1,1- dioxidotetrahydro- 2H-thiopyran- 4-yl) methoxy]- 2',6'-dimethylbiphenyl-3-yl}methyl)amino]phenyl} propanoic acid has been shown to exhibit a robust plasma glucose-lowering effect and insulinotropic action during an oral glucose tolerance test in rats with impaired glucose tolerance (Mikami et al., 2012). Recently, Zhou et al. in 2009 discovered a series of thiazolidinediones (TZDs) as potent GPR40 agonists by systematic structure-activity relationship studies of a screening. Among these, compound C demonstrated an acute mechanism-based glucose lowering in an intraperitoneal glucose tolerance test (IPGTT) in lean mice, while no effects were observed in GPR40 knockout mice. However, it is necessary to determine whether compound C has the same adverse effects.

3.2. Docking

Docking is a process by which the best configuration of binding molecules is determined. In this process, a complex structure is obtained having stable structure (Lengauer and Rarey, 1996). Knowledge of favored orientation in turn can be used in predicting strength of association between the two molecules and binding energy can be measured in terms of scoring function.

Docking is often used for predicting the binding of drug candidates to their target protein to predict the activity and affinity of the drug candidates. Thus, docking perform a very significant character in the rational drug design (Kitchen *et al.*, 2004).

Docking approaches- There are 2 approaches popular in molecular docking community. First approach employs a method of matching where protein and ligand molecular surfaces are reported as complementary to each other (Goldman and Wipke, 2000; Elaine C. Meng *et al.*, 2004; Morris *et al.*, 1998). In the next approach, process of docking takes place and the interaction energy of protein-ligand complex is determined (Feig *et al.*, 2004).

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3. METHODOLOGY

3.1. Online Tools and Database

3.1.1. NCBI

NCBI is one of the part of National Institutes of Health branch (United States National Library of Medicine). There are a number of databases available in NCBI which are useful for biomedicine and biotechnology. Major databases are: -GenBank: For DNA sequences, -PubMed: For biomedical literature, -Protein: For protein sequences, etc.

3.1.2. PDB

The Protein Data Bank is a repository for 3D biological molecules (nucleotides and proteins) structural data. The structural data found experimentally by NMR spectroscopy or X-ray crystallography and put in by the biochemists or biologists and are accessible freely on Internet. Users can search in this database by PDB ID, macromolecule, author, sequence or ligands and download the required files in pdb format.

3.1.3. ZINC Database

The ZINC database comprises of commercially available chemical compounds. ZINC database is mainly used for virtual screening. ZINC is used by various scholars in research field as well as by the investigators in pharmaceutical companies or biotech companies. Users can search in this database by IDs, SMILES, etc. The ZINC database finds the compounds based on similarity to the query compound. The output result of query can be downloaded in the mol2, sdf, SMILES, ddb (flexibase) format. Other uses of the ZINC database: -obtaining a compound for purchasing, -obtaining compounds which can be used as a drug molecule, etc.

3.1.4. SCF BIO-IITD

Supercomputing facility at IIT Delhi has various online softwares which can be used for ligand screening and ligand optimization. Active site prediction, Lipinski filter etc are some of the few online resources which are accessible online on this site.

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3.1.5. RASPD

RASPD is used to excluding the ligand molecules in the beginning based on physicochemical properties of ligands and active site of the target protein molecule. This tool searches based on various physicochemical properties like chemical formula, H-bond donors as well as acceptors, number of rings, etc. for every molecules. Four methods are available for users in this tool: -Method A: If protein-ligand complex information is available, -Method B: If protein 3D structure is available but no information related to ligand is available, -Method C: Modified Dataset, -Method D: Modified Molecule.

3.1.6. Toxicity checker

As the name suggests, Toxicity Checker aims to identify whether any toxic substructure of the query compound is available or not and it also calculates the different properties of the compounds. It is available freely to the users. It helps the scholars, companies and research institutes by allowing them to use the available tools online. In this tool, users have two options to check for the toxic substructure in the compound. They can check it either by drawing the molecule or by providing molecule ID, SMILES, InChI, InChIKey.

3.2. Software

3.2.1. AUTODOCK 4.2.5

Auto Dock 4.2.5 is software used for the purpose of molecular docking of ligand to macromolecules like DNA, proteins, etc. There are two main programs in Auto Dock: (a) Auto Grid program for the identification of pre-computing grids, and (b) Auto Dock program for docking ligand molecule to a number of grids of the target protein. Binding energy calculated is the combination of intermolecular and torsional energies.

3.2.2. OSIRIS DATA WARRIOR

OSIRIS Data Warrior is data analysis and visualization software. OSIRIS data warrior is helpful in predicting various physico-chemical properties and toxicity risk indication that must be optimized while designing pharmaceutically active compounds.

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3.2.3. Chemsketch

Chemsketch is a software which is used to draw chemical structure and create a file in desired format. This was used to draw chemical structure for molecules whose mol2 format was not available online.

3.2.4. Pymol

Pymol is a protein molecule visualization software. This software can further be used to edit protein structure by removing unnecessary ligands which are attached to the protein molecule file.

3.3. STEPS

3.3.1. Protein structure

The X-ray crystal structure of the matrix protein GPR40 at 2.33 ˚A resolution (PDB ID: 4PHU), were retrieved from Protein Data Bank. Processing of protein structures were carried out by "Protein preparation wizard". Before protein preparation process, all the water molecules and hetero-molecule attached with the structures were removed from the original crystal structure of GPR40. Hydrogen atoms were added and the geometry of all the hetero groups was corrected. For optimizing the network of H-bonds, hydrogen bonds assignment tool was implemented.

3.3.2. Active site Prediction

The Active site prediction server designed by IIT delhi can be used to determine the active sites. The server computes the cavities in a given protein using the uploaded pdb file. The active sites of GPR40 were identified using SCFBIOIIT Delhi, The most essential property produced by SCFBIO site is an overall Site score, which has demonstrated to be successful at differentiating identified binding sites in co-crystallized centers. Active sites with best site scores were taken as a prerequisite for receptor grid generation. The active sites identified by this program have been in accordance with the literature available for the GPR40.

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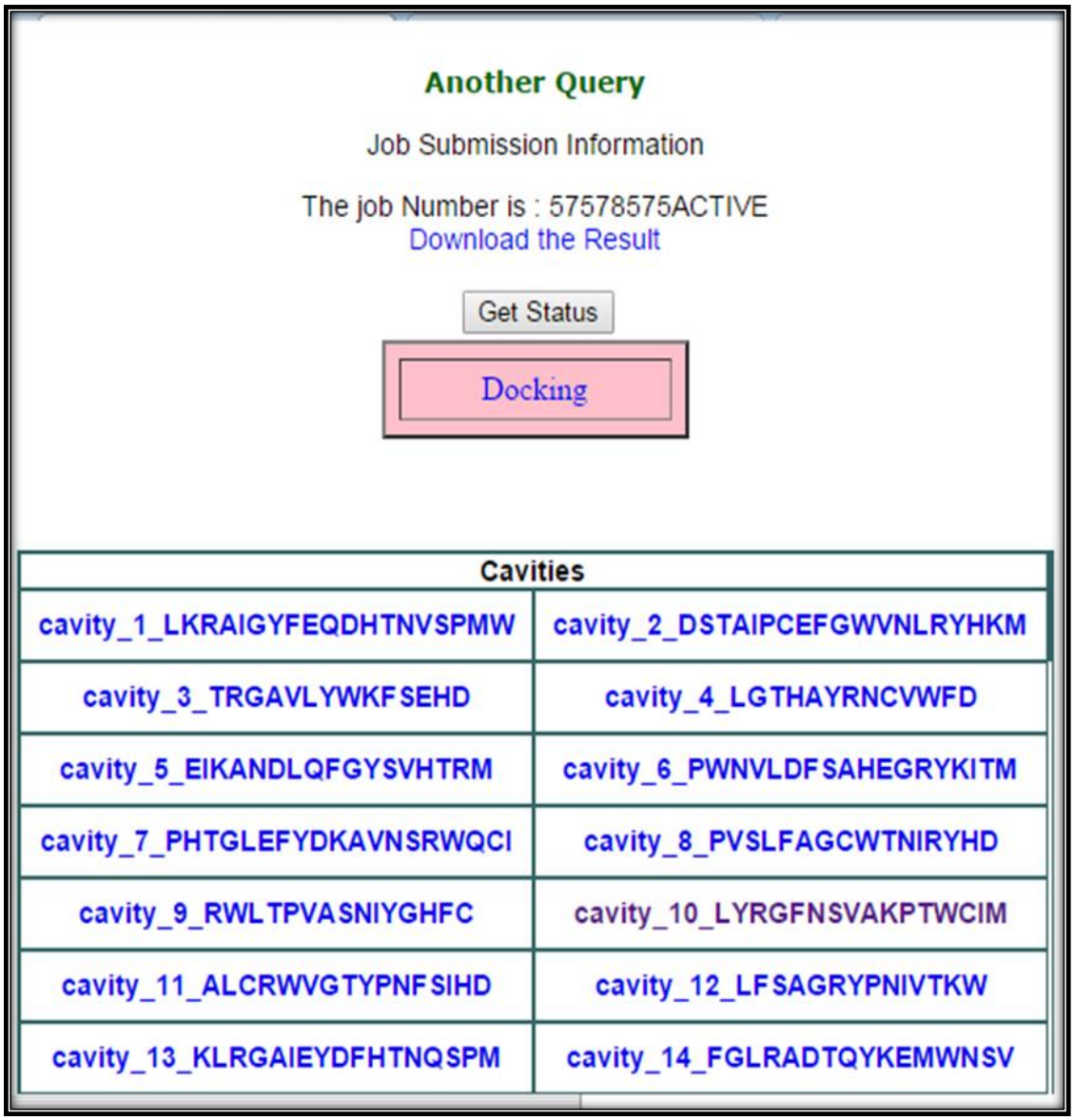

Figure.1. Active site predicted cavities of GPR40

3.3.3. Docking of ligand with the target protein molecule

From Protein Data Bank, the natural ligand of our target protein molecule was found. And the ligand molecule was downloaded in mol2 format from ZINC database. Then, we had used the docking software, AutoDock 4.2.5 for docking the natural ligand with the target protein molecule and binding energy had been recorded. Now we have to identify the ligands having lesser binding energy.

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3.3.4. Finding ligands based on the target protein active sites

Method B of the online available tool RASPD had been used to identify various ligands. This method is useful when only mark protein molecule is available. RASPD-Method B determines ligands depending upon the active grooves present on the target molecule. So, we obtained the library of ligands with their ZINC ID, IUPAC name and the 3D coordinates of the atoms involved. Then, all the ligands were downloaded from the ZINC database and thus we had generated the virtual library of ligands.

3.3.5. Docking of different ligands with the target protein molecule

Each of the ligands as well as drug molecules was docked one by one with the target molecule using AutoDock 4.2.5 software. And obtained binding energies were noted down and the data is shown at Table.

3.3.6. Checking Lipinski's filters

Lipinski's rule of five had been checked for top 10 ligand molecules, according to binding energy. These filters are obtained from one of the drug design tools at scfbio-IIT Delhi. The results are shown in Table 2.

3.3.7. Cheking Toxicity of ligand molecules

a) Using Toxicity checker

Using SMILES sequence of the ligand, molecule structures was drawn and was checked for the toxic substructure. It is done for top 10 ligands and is shown in the Result section.

b) Using OSIRIS data warrior software

Using OSIRIS data warrior software, toxicity as well as physicochemical properties was obtained for top 10 ligand molecules and compared with the available drug molecules and summarized in Table3.

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4. RESULTS

4.1. 3D structure view of the protein with PDB ID 4PHU

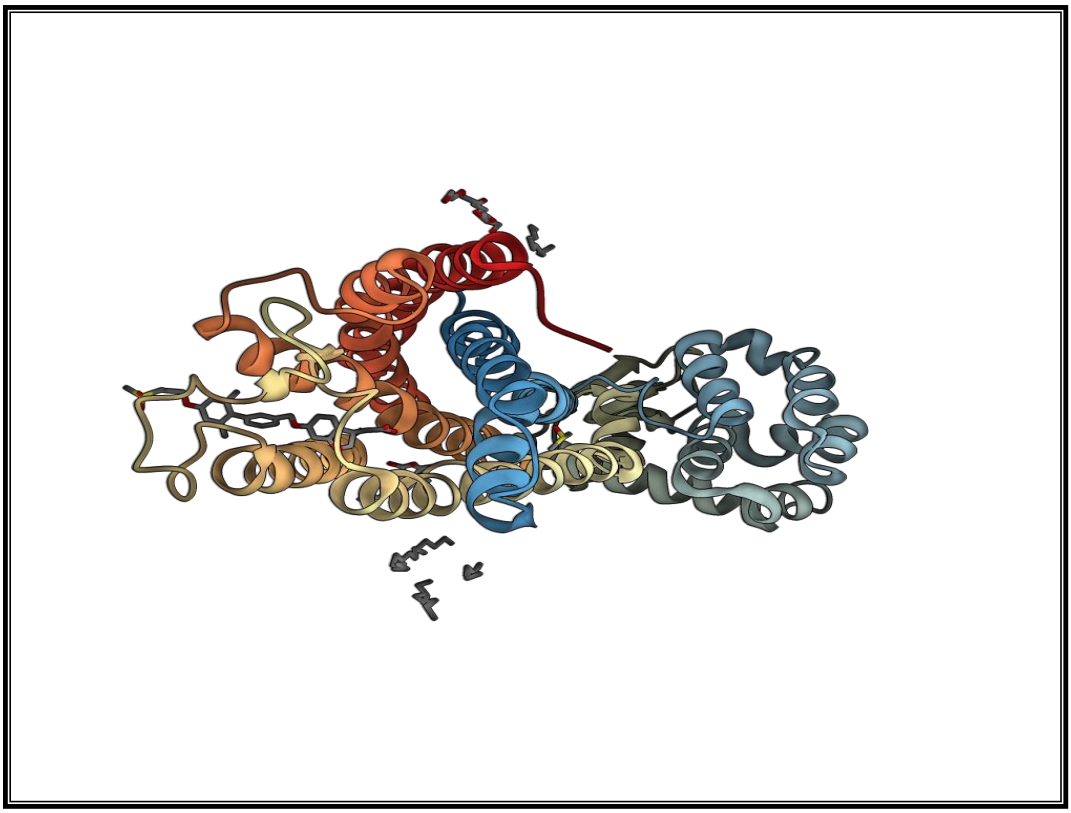

Figure.2. 3D structure of 4PHU

4.2. Docking of ligand with the target protein molecule

The ligand identified for protein with PDB ID 4PHU were 7-hydroxyflavone; 7,8-dihydroxyflavone; 7-methoxyflavone; Flavan-4-ol; Apigenin; Chrysin,3-hydroxyflavone; Flavone,3,4Dimethoxyflavone;Flavanone. After docking of ligand with the target protein; results into the complex with binding energy -5.24 kcal/mol. Now we have to identify the ligands having lesser binding energy.

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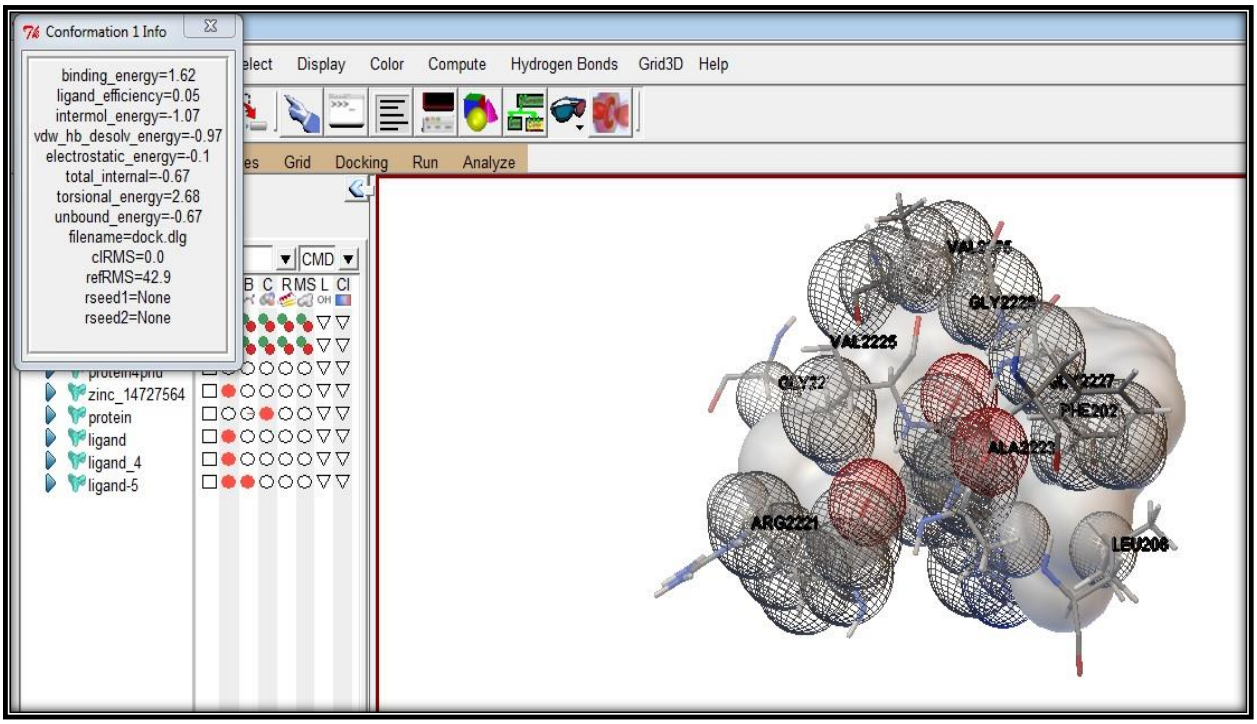

Figure.3. Docking result of 7-hydroxyflavone with the target protein 4PHU

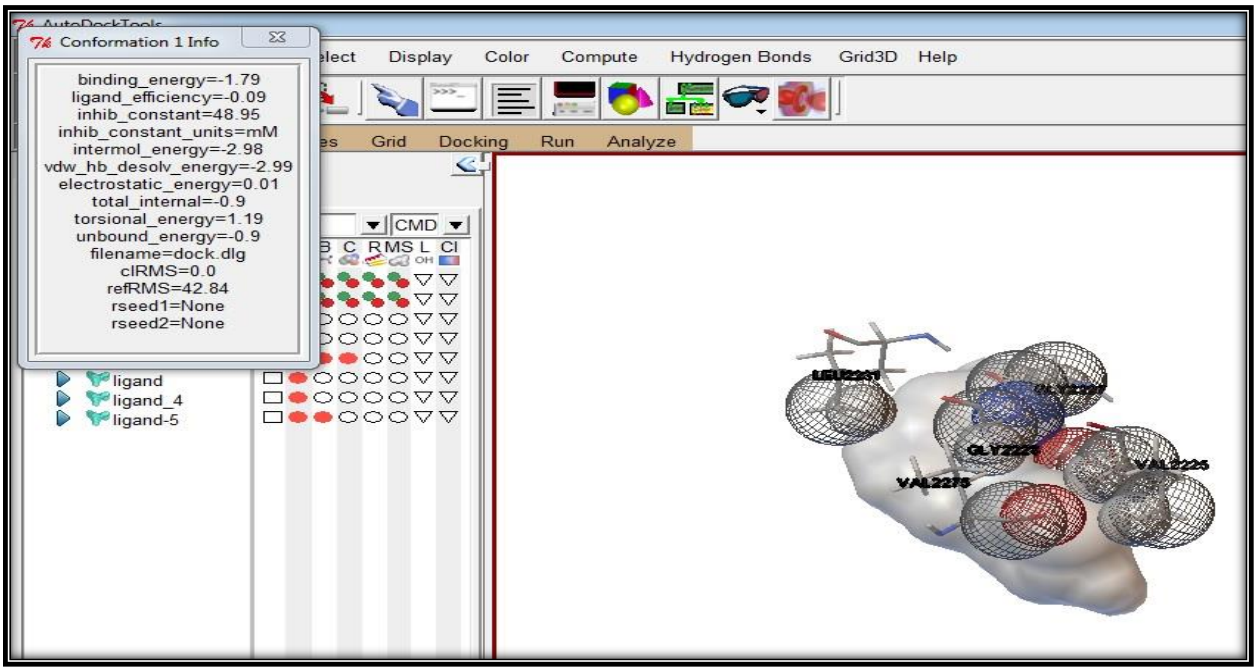

Figure.4. Docking result of 7, 8-dihydroxyflavone with the target protein 4PHU

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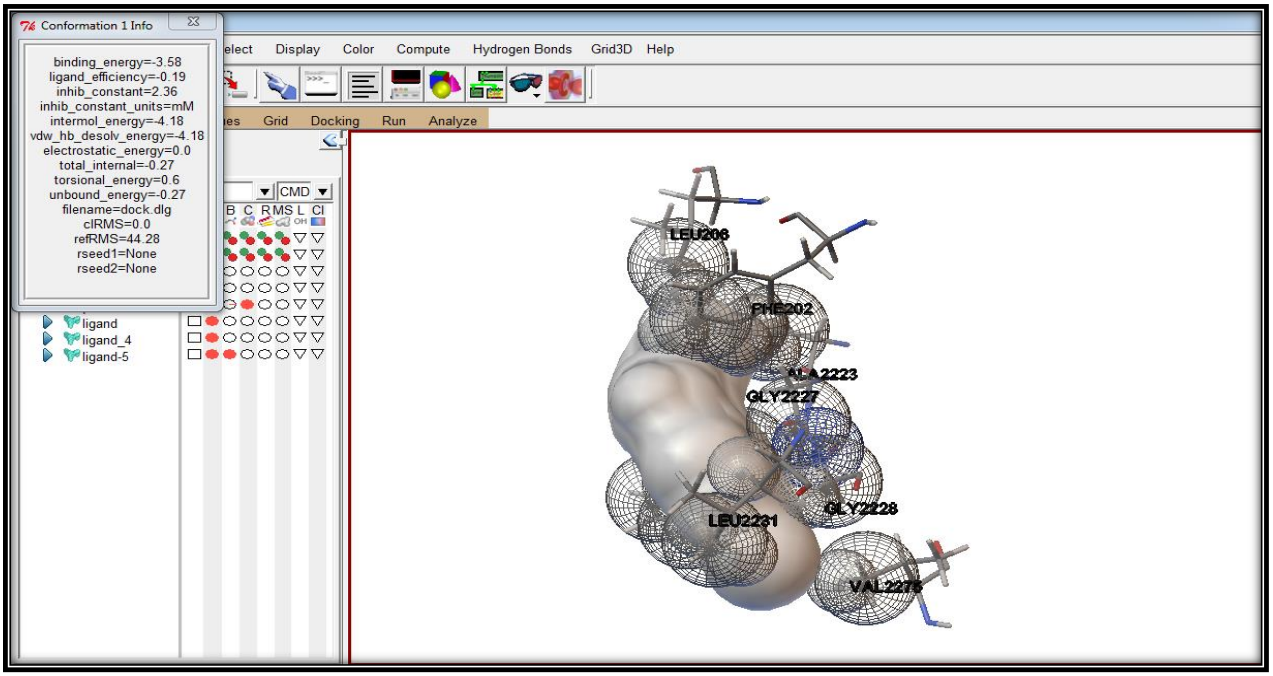

Figure.5. Docking result of 7- methoxyflavone with the target protein 4PHU

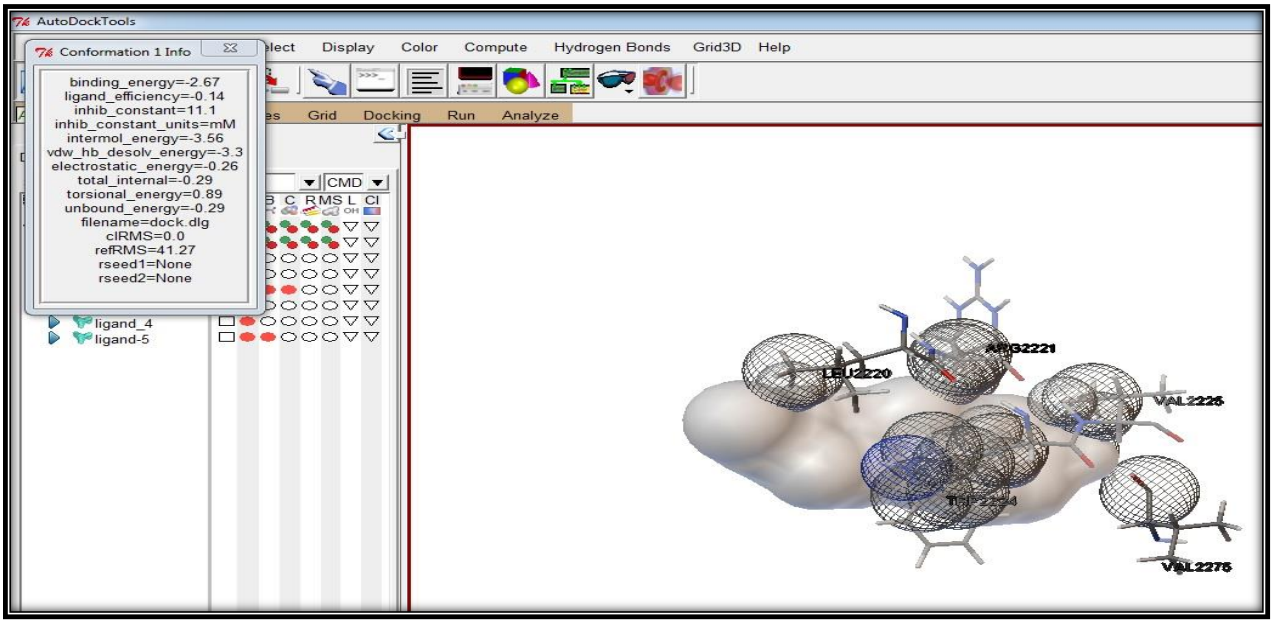

Figure.6. Docking result of flavan-4-ol with the target protein 4PHU

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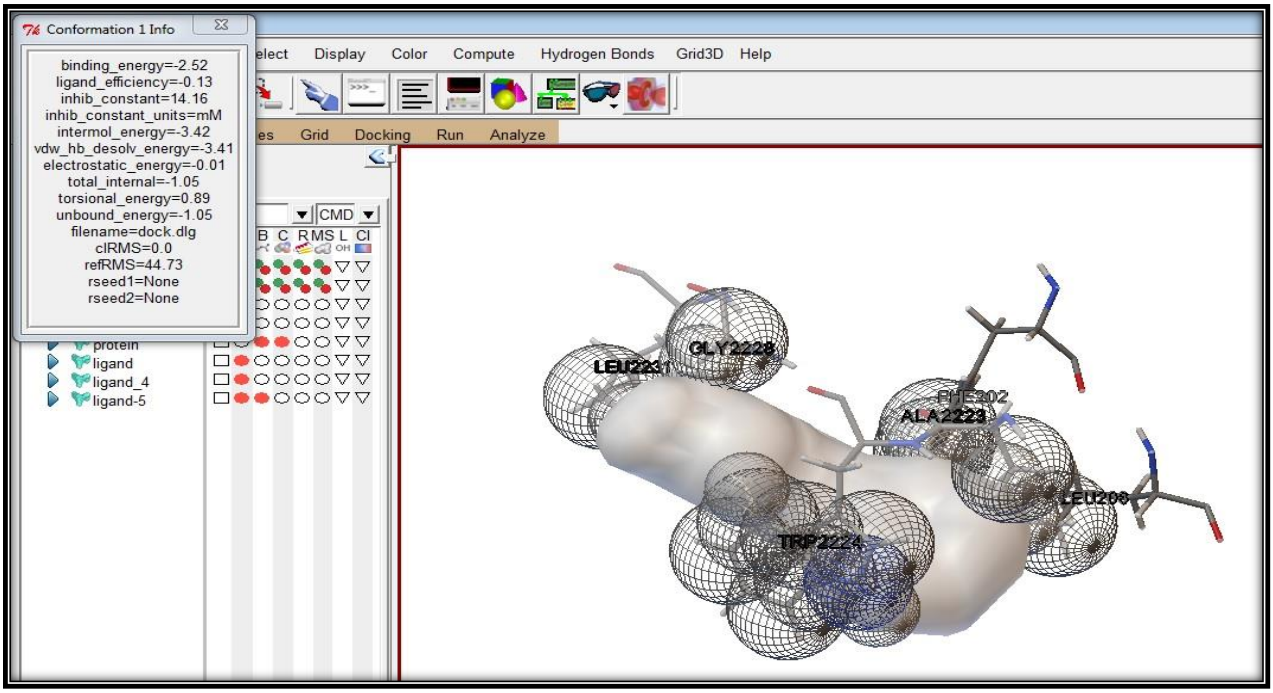

Figure.7. Docking result of apigenin with the target protein 4PHU

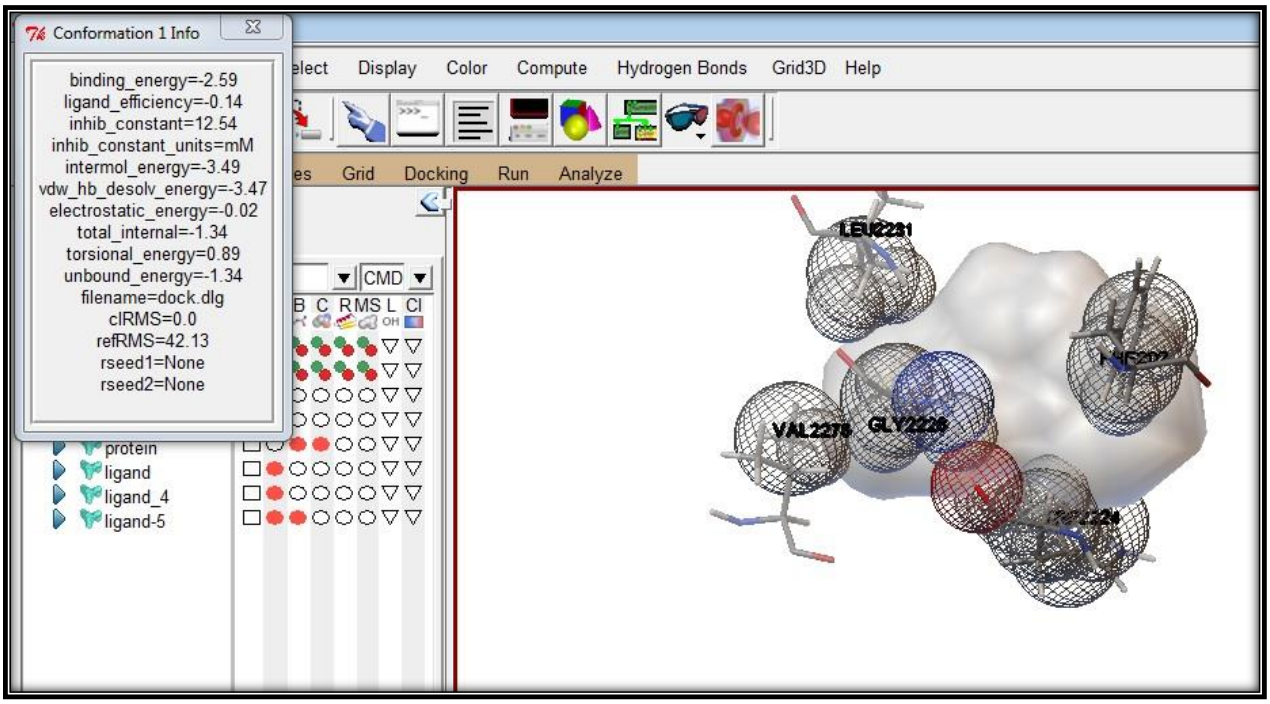

Figure.8. Docking result of chrysin with the target protein 4PHU

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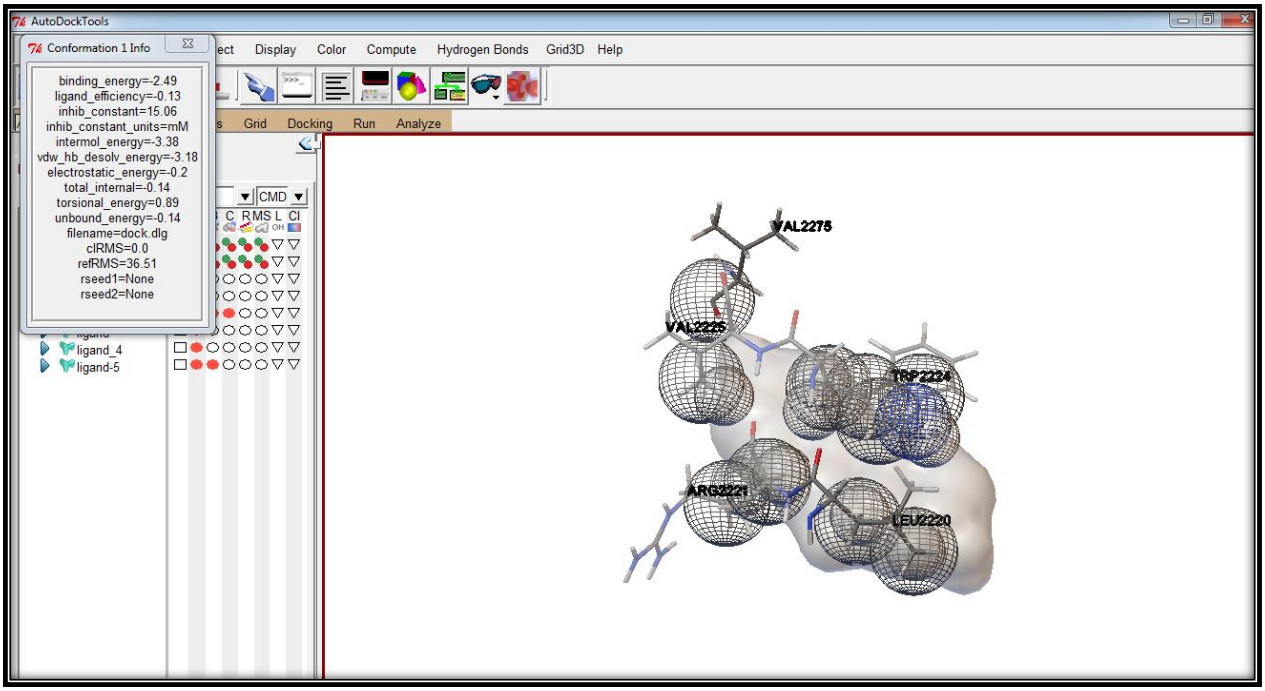

Figure.9. Docking result of 3-hydroxyflavanone with the target protein 4PHU

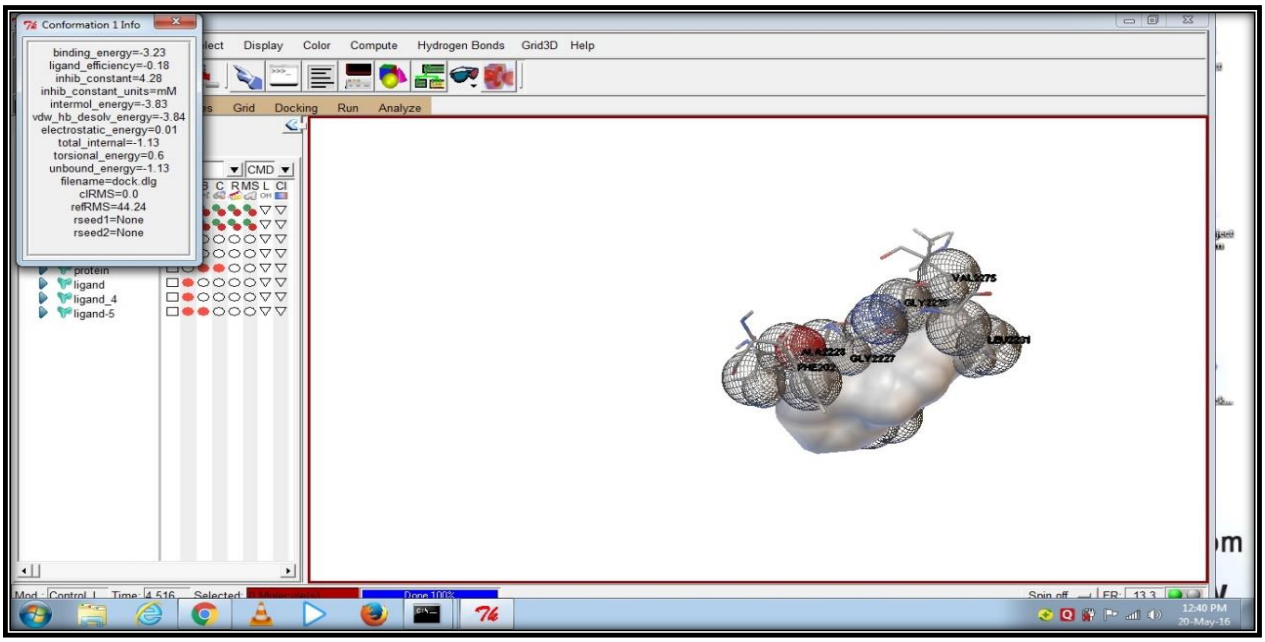

Figure.10. Docking result of flavone with the target protein 4PHU

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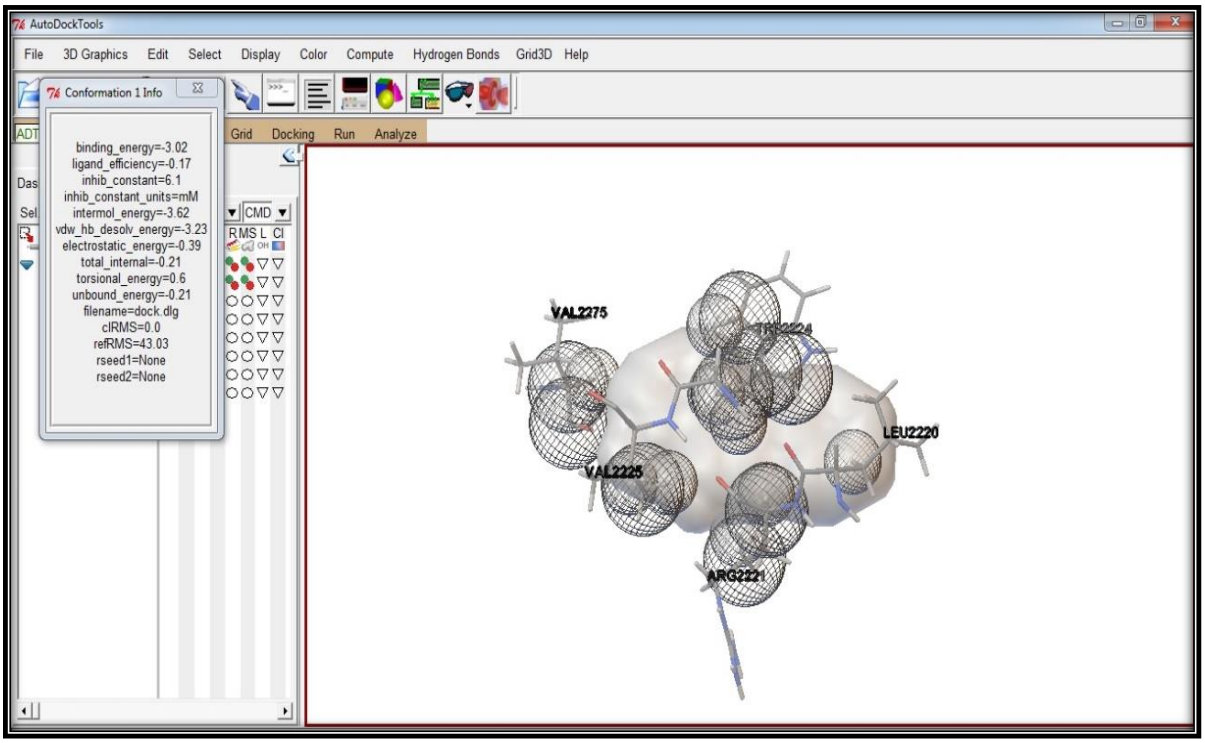

Figure.11. Docking result of 3,4-Dimethoxyflavone with the target protein 4PHU

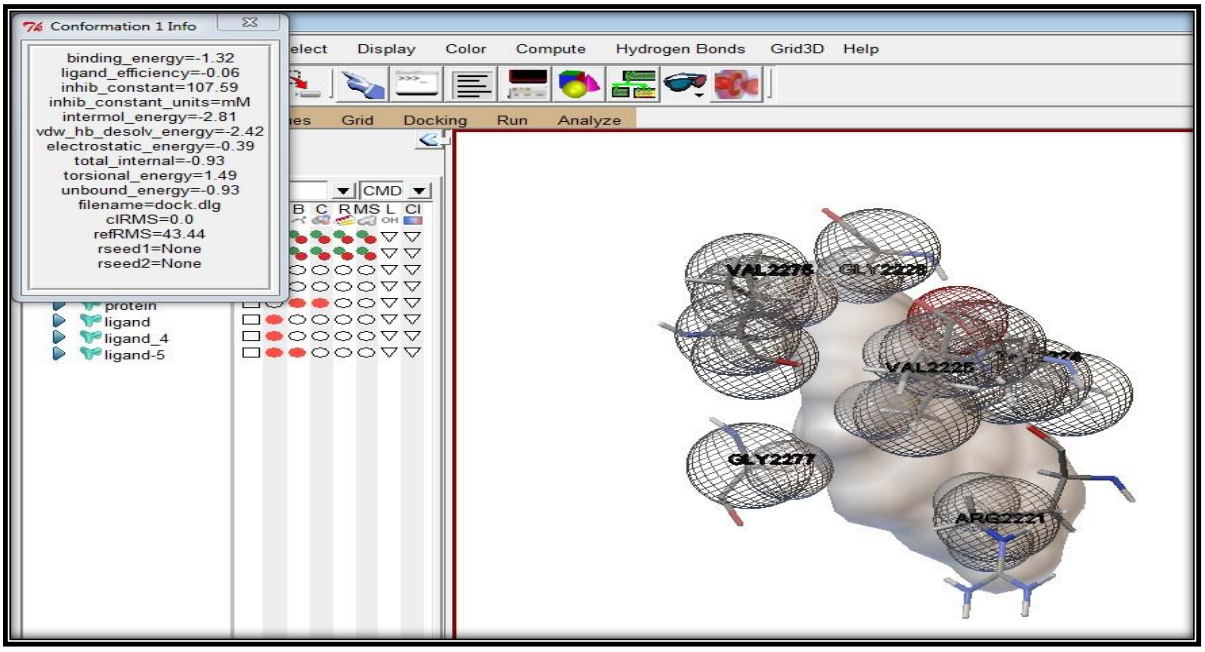

Figure.12. Docking result of Flavanone with the target protein 4PHU

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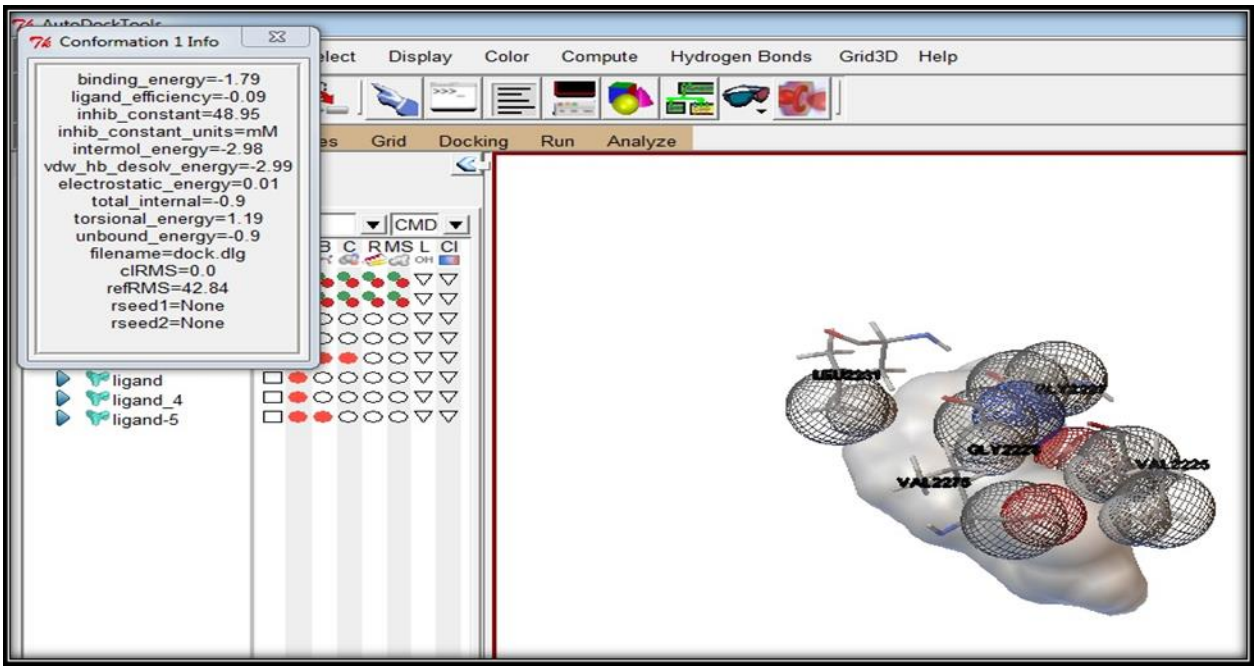

Figure.13. Docking result of naringenin with the target protein 4PHU

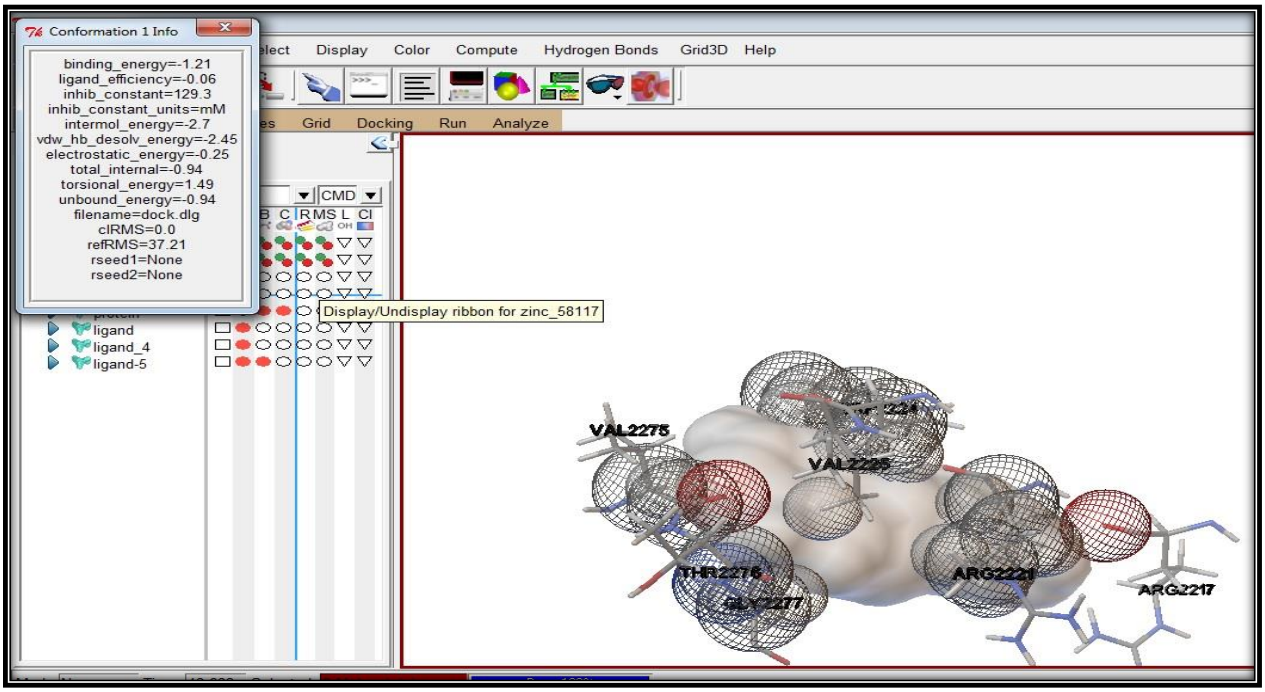

Figure.14. Docking result of 4-hydroxyflavanone with the target protein 4PHU

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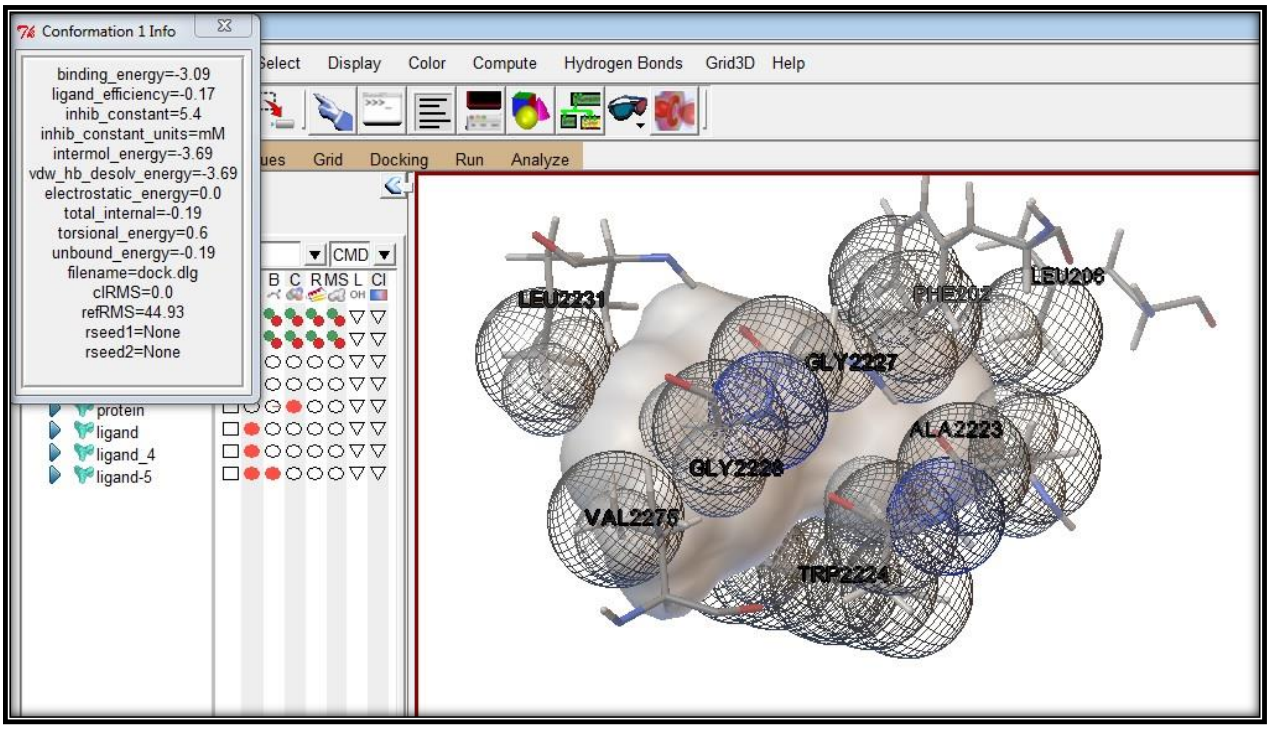

Figure.15. Docking result of 5-Hydroxyflavone with the target protein 4PHU

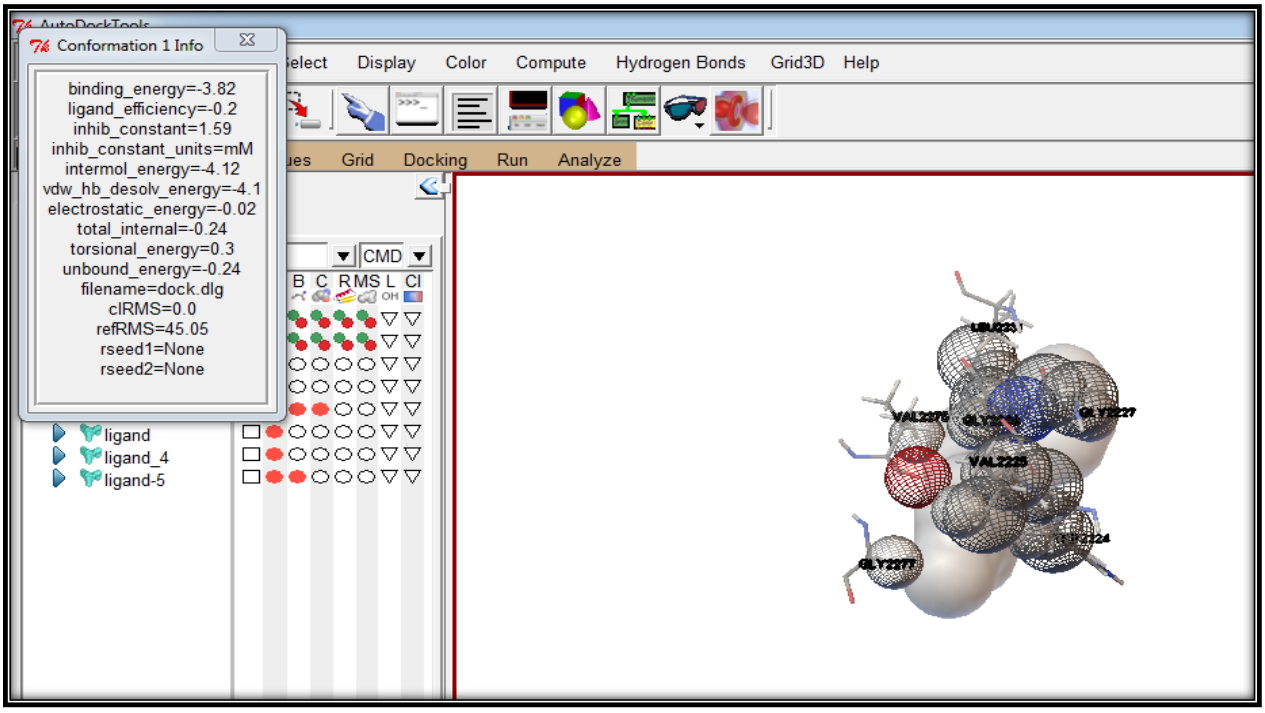

Figure.16. Docking result of Isoflavanone with the target protein 4PHU

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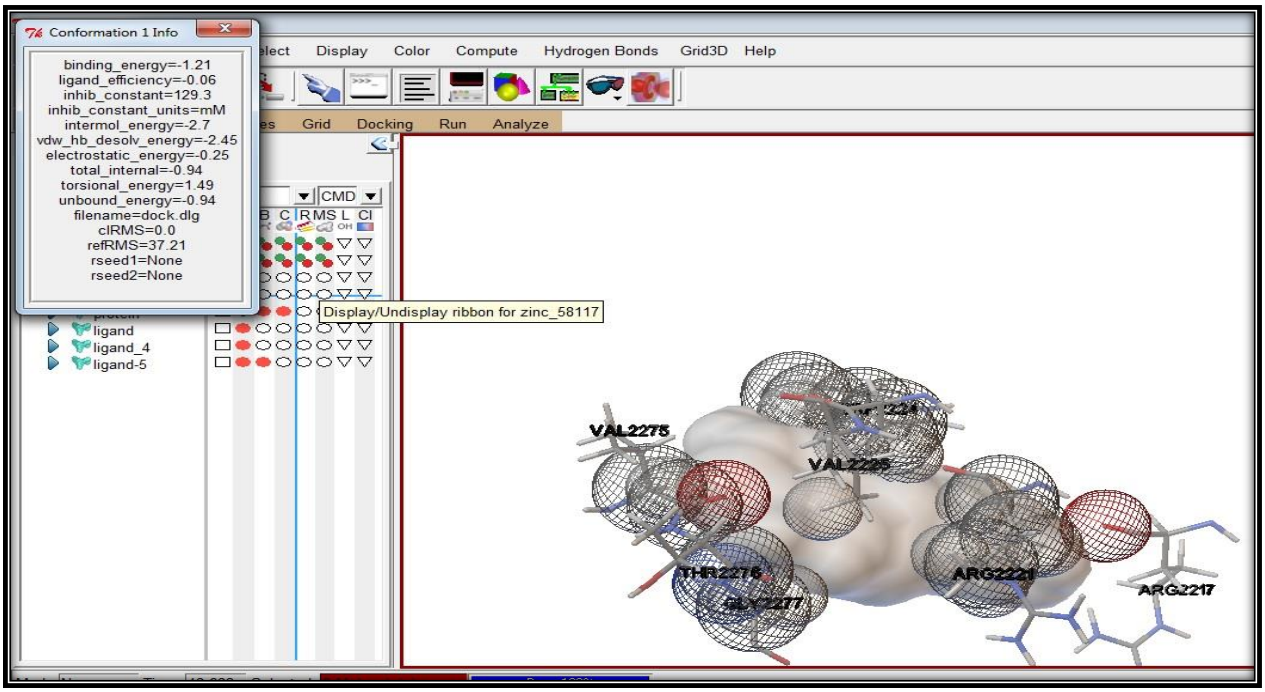

Figure.17. Docking result of 5,7-dihydroxyflavone with the target protein 4PHU

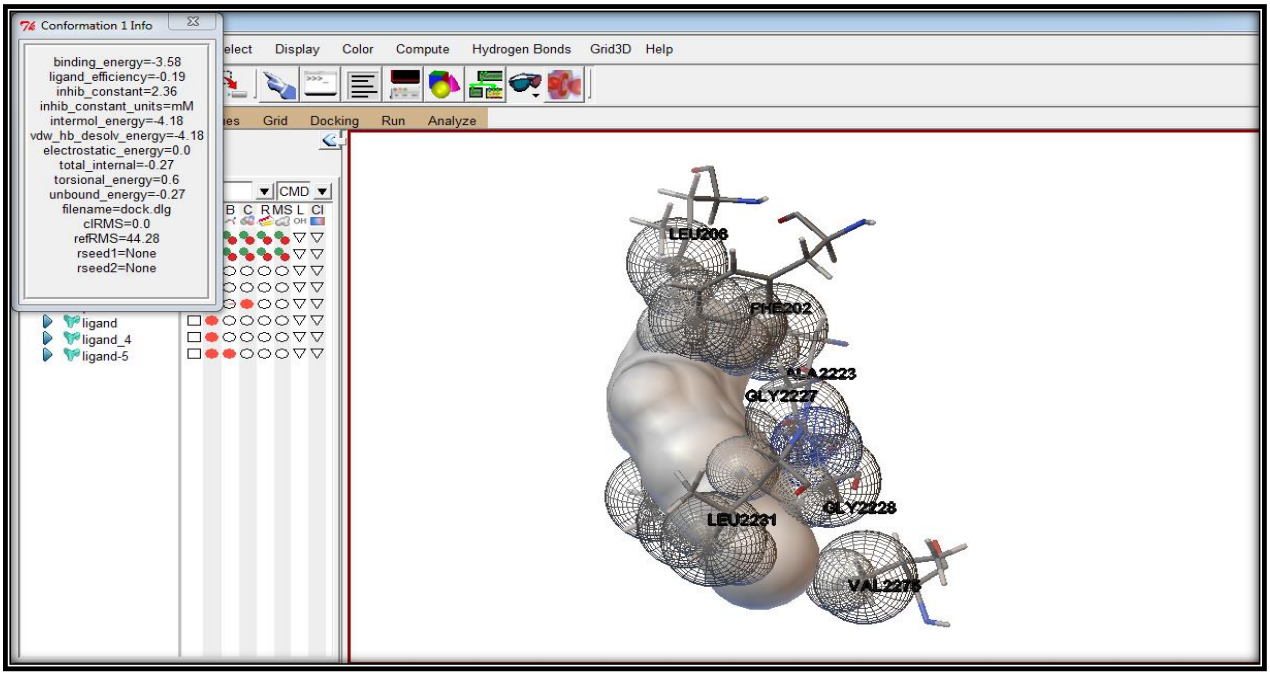

Figure.18. Docking result of 4-o-methylequol with the target protein 4PHU

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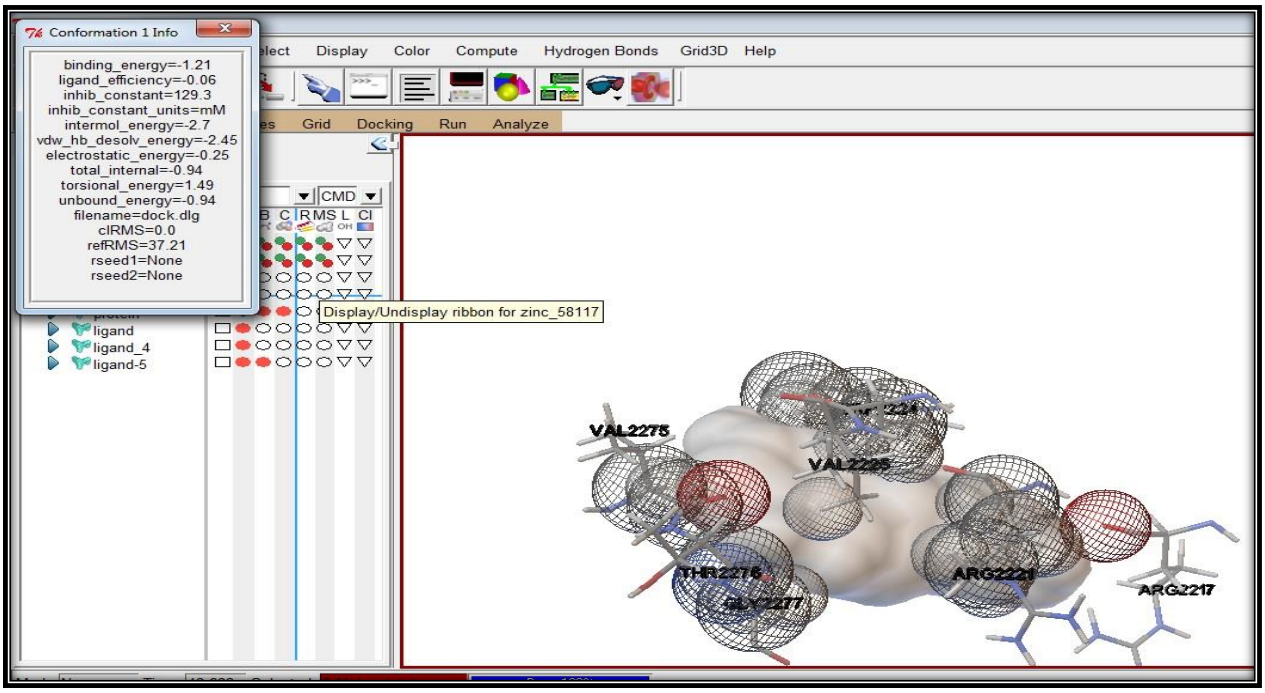

Figure.19. Docking result of Quercetin with the target protein 4PHU

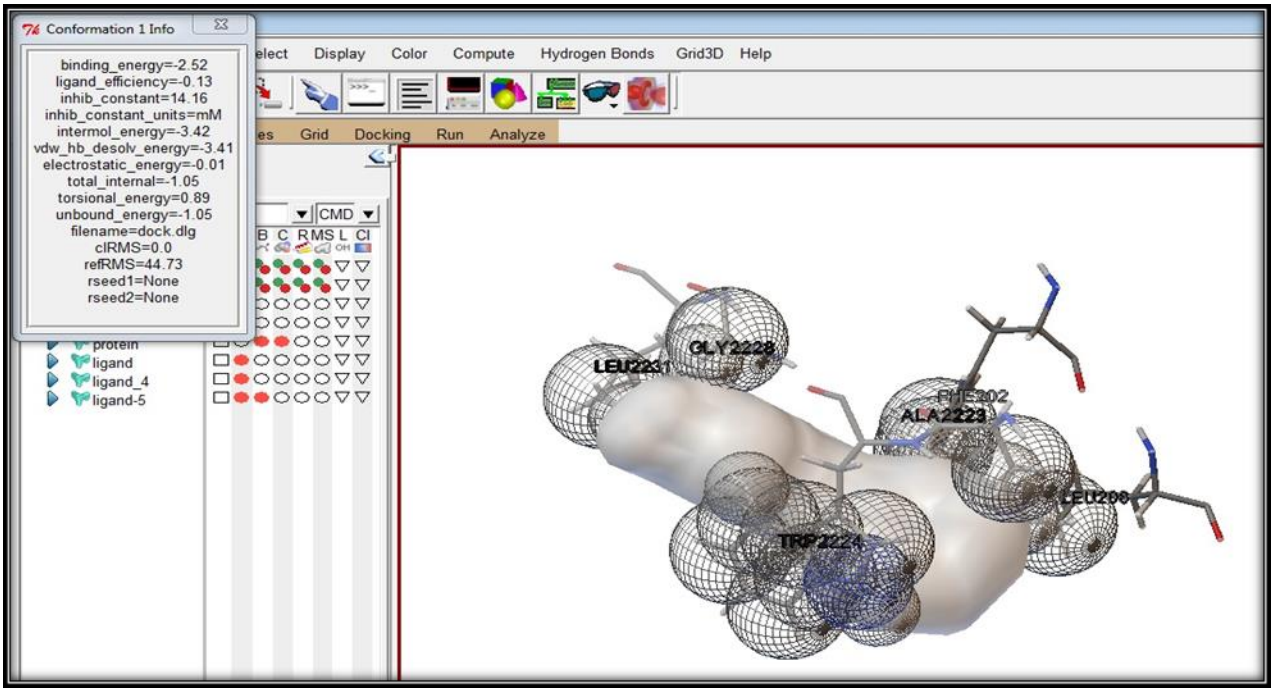

Figure.20. Docking result of Eriodictyol with the target protein 4PHU

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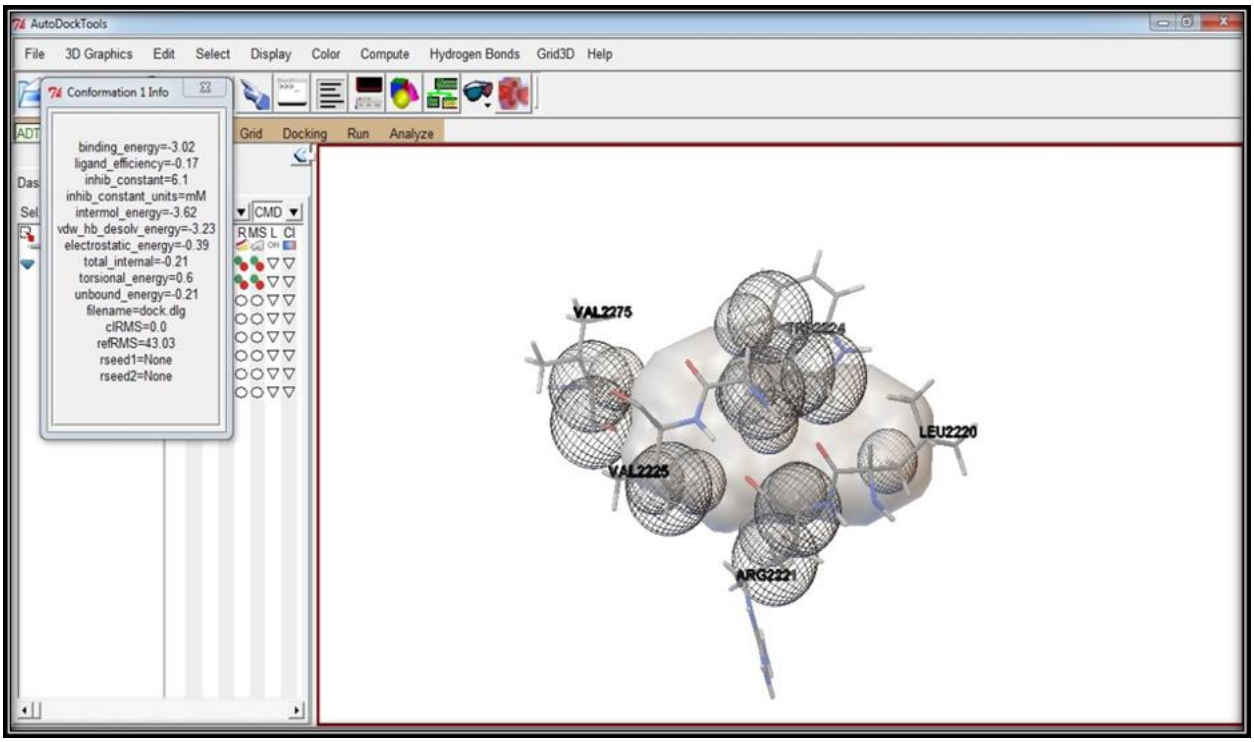

Figure.21. Docking result of Luteolin with the target protein 4PHU

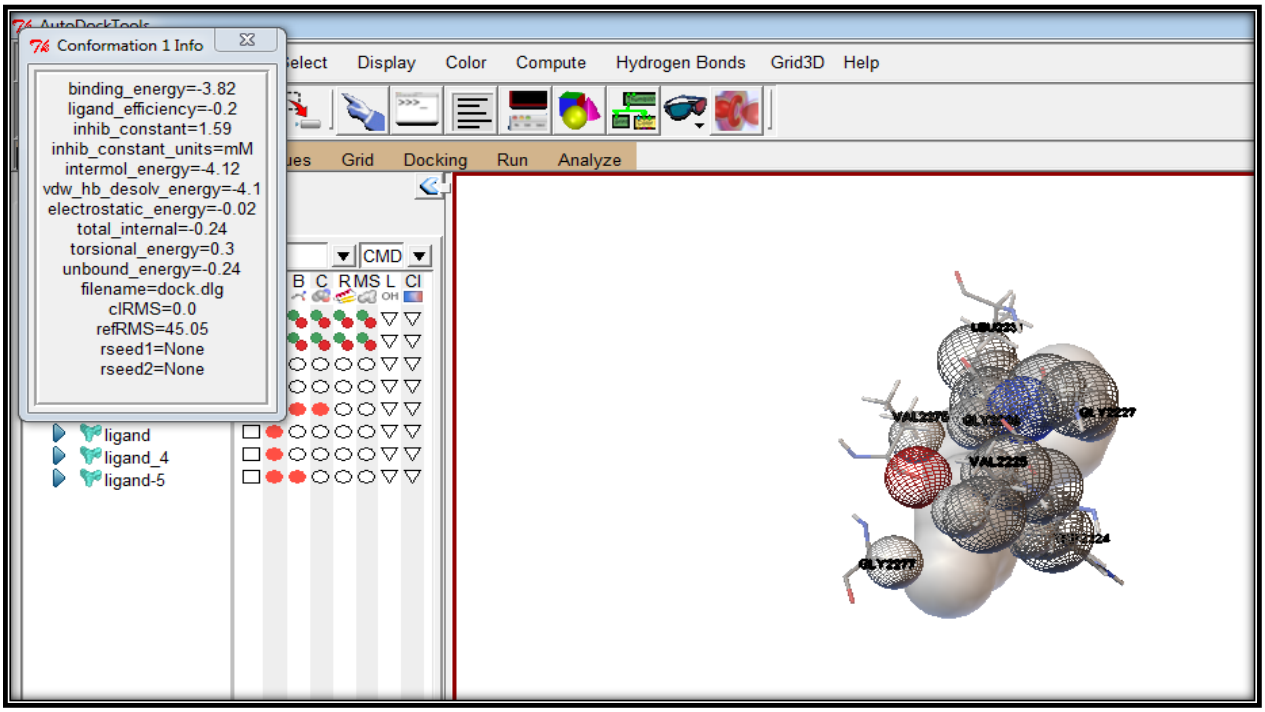

Figure.22. Docking result of Taxifolin with the target protein 4PHU

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S.No.	Name	ZINC ID	Binding energy
1	7-hydroxyflavone	zinc_5934541	-5.24
2	7,8-dihydroxyflavone	zinc_57657	-4.99
3	7- methoxyflavone	zinc_18056	-4.96
4	Flavan-4-ol	zinc_85342	-4.53
5	Apigenin	zinc_3871576	-4.47
6	Chrysin	zinc _3872070	-4.47
7	3-hydroxyflavone	zinc_57678	-4.44
8	Flavone	zinc_57674	-4.32
9	3,4-Dimethoxyflavone	zinc_57672	-4.26
10	Flavanone	zinc_58113	-4.26
11	Naringenin	zinc_156701	-4.23
12	4-hydroxyflavanone	zinc_57922.mol2	-4.21
13	5-Hydroxyflavone	zinc_57676	-4.16
14	Isoflavanone	zinc_134619	-3.92
15	5,7-dihydroxyflavone	zinc_4935	-3.84
16	4-o-methylequol	zinc_2558138	-3.82
17	Quercetin	zinc_3869685	-3.76
18	Eriodictyol	zinc_58117	-3.6
19	Luteolin	zinc_18185774	-3.18
20	Taxifolin	zinc_105086	-3.06

Table.1. Binding energy of different ligands with the target protein

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4.3. Checking Lipinski's filter

Lipinski's rule of five had been checked for top 20 ligand molecules selected based on the binding energy.

S.N	Ligands (ZINC ID)	Molecular Mass	LogP	Hydrogen Bond Donors	Hydrogen Bond Acceptors
1	ZINC _5934541	238	3.00	1	3
2	Zinc_57657	254	2.71	2	4
3	Zinc_18056	252	3.31	0	3
4	Zinc_85342	226	3.24	1	2
5	Zinc_3871576	270	2.41	3	5
6	Zinc _3872070	254	2.71	2	4
7	Zinc_57678	238	3.00	1	3
8	Zinc_57674	222	3.30	0	2
9	Zinc_57672	282	3.31	0	4
10	Zinc_156701	272	2.50	3	5
11	Zinc_57676	238	3.00	1	3
12	Zinc_134619	224	3.04	0	2
13	Zinc_4935	256	2.80	2	4
14	Zinc_2558138	256	3.11	1	3
15	Zinc_3869685	302	2.01	5	7
16	Zinc_58117	288	2.21	4	6
17	zinc_18185774	286	2.12	4	6
18	Zinc_105086	304	1.18	5	7
19	Zinc_58113	224	3.39	0	2
20	zinc_57922.mol2	240	3.09	1	3

Table2. Lipinski's filters for top 20 ligands

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5.4. Cheking Toxicity of ligand molecules

5.4.1. Using Toxicity checker

Presence of any toxic substructure was checked by the online tool Toxicity checker by providing the SMILES sequence of the ligand. It is done for the top 10 ligands and the result is shown below.

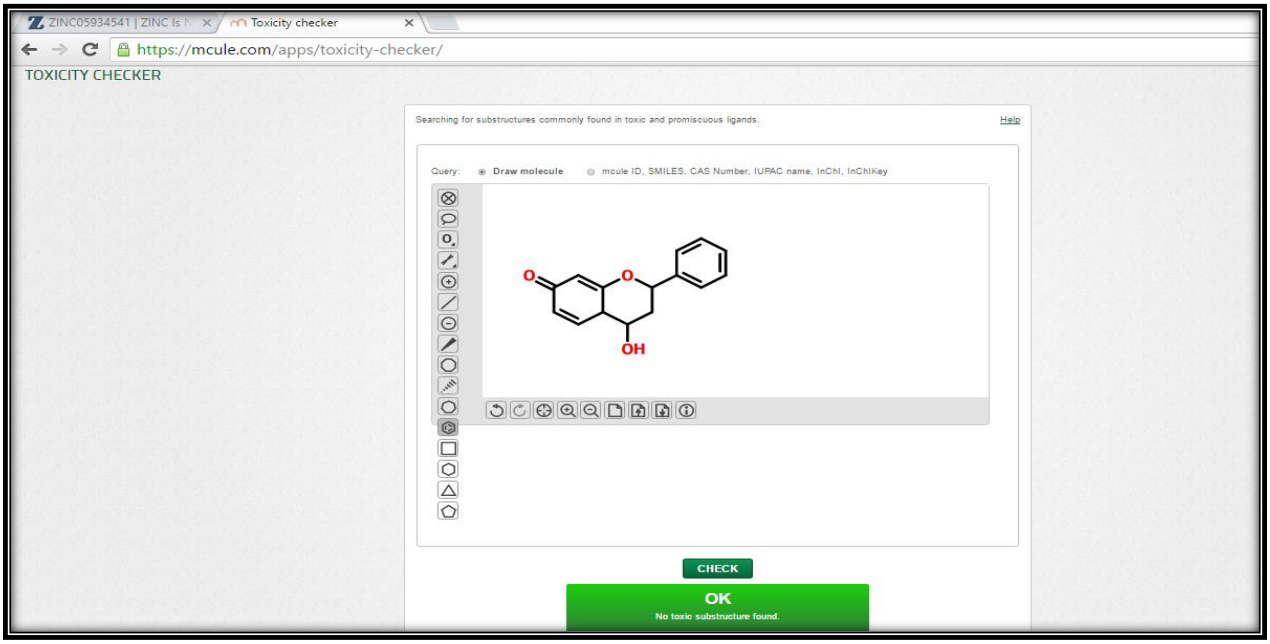

Figure.23. Toxicity checker result of ligand 1

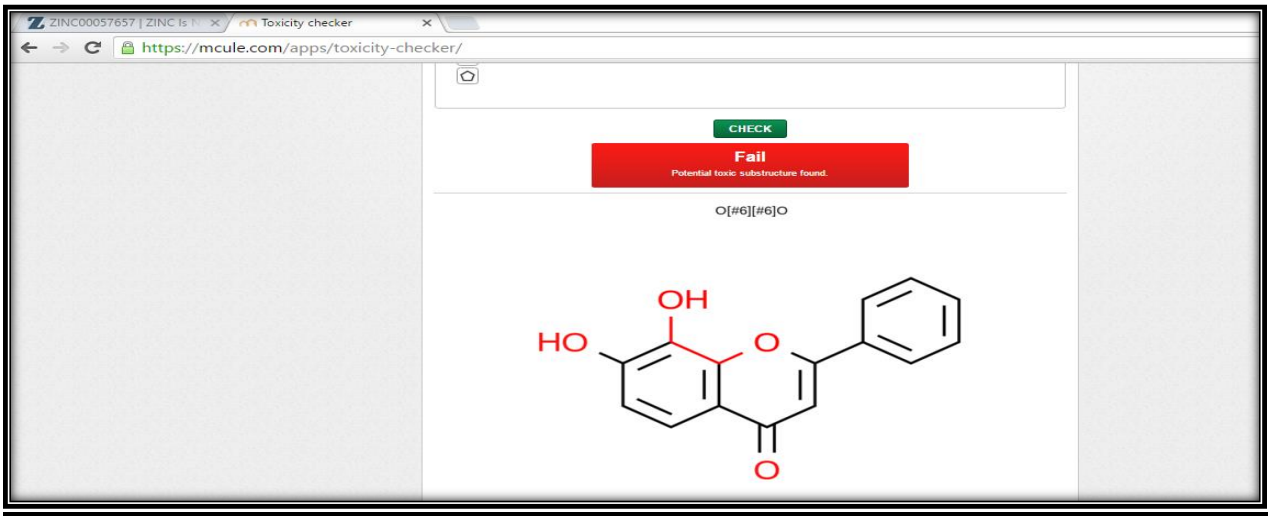

Figure.24. Toxicity checker result of ligand 2

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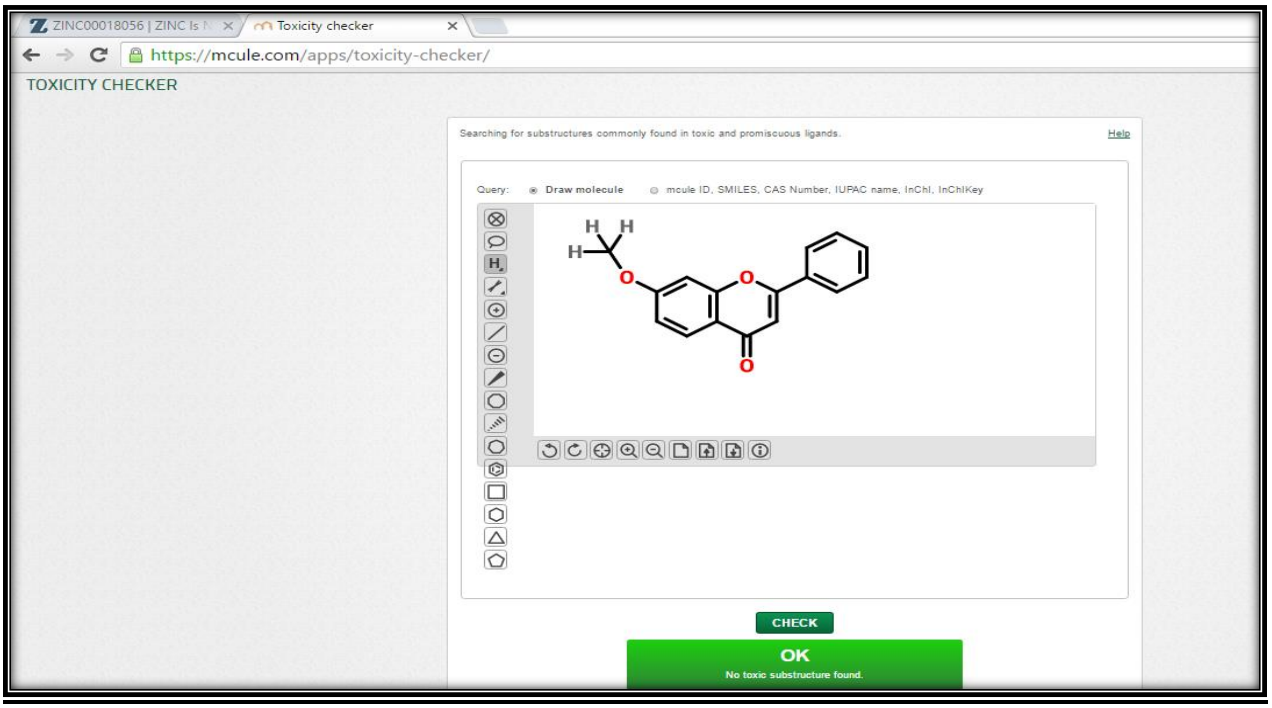

Figure.25.Toxicity checker result of ligand 3

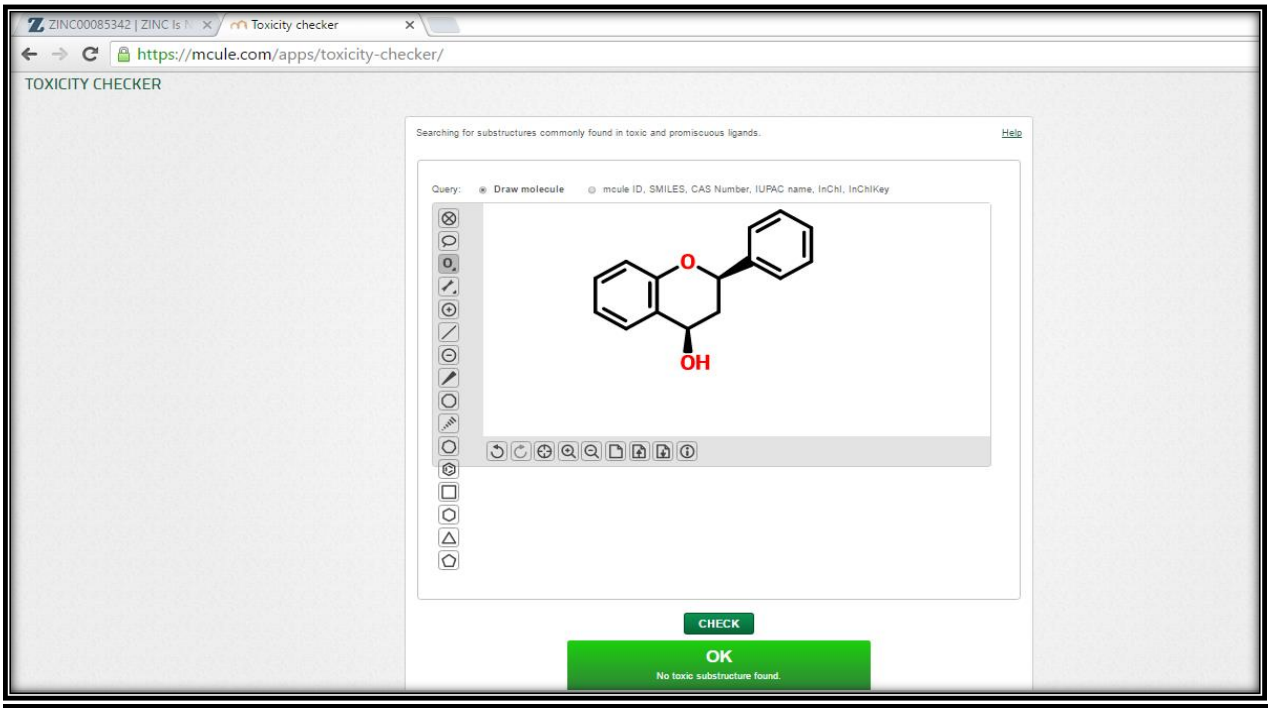

Figure.26.Toxicity checker result of ligand 4

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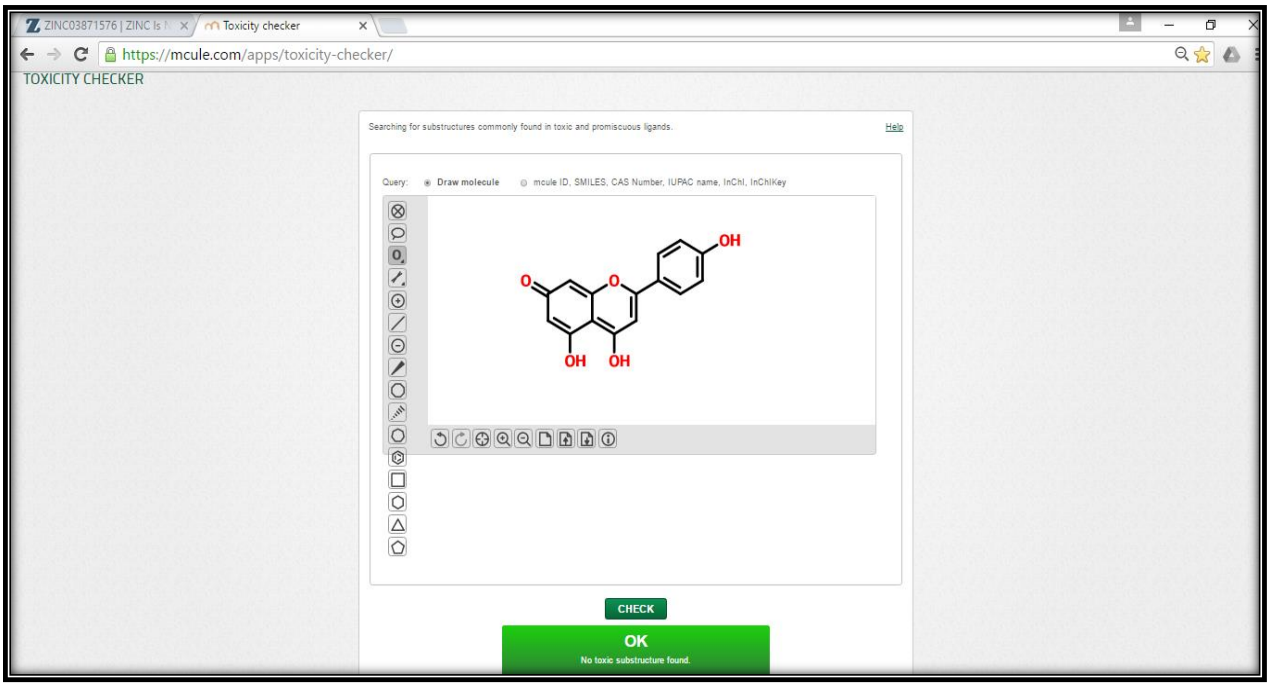

Figure.27.Toxicity checker result of ligand 5

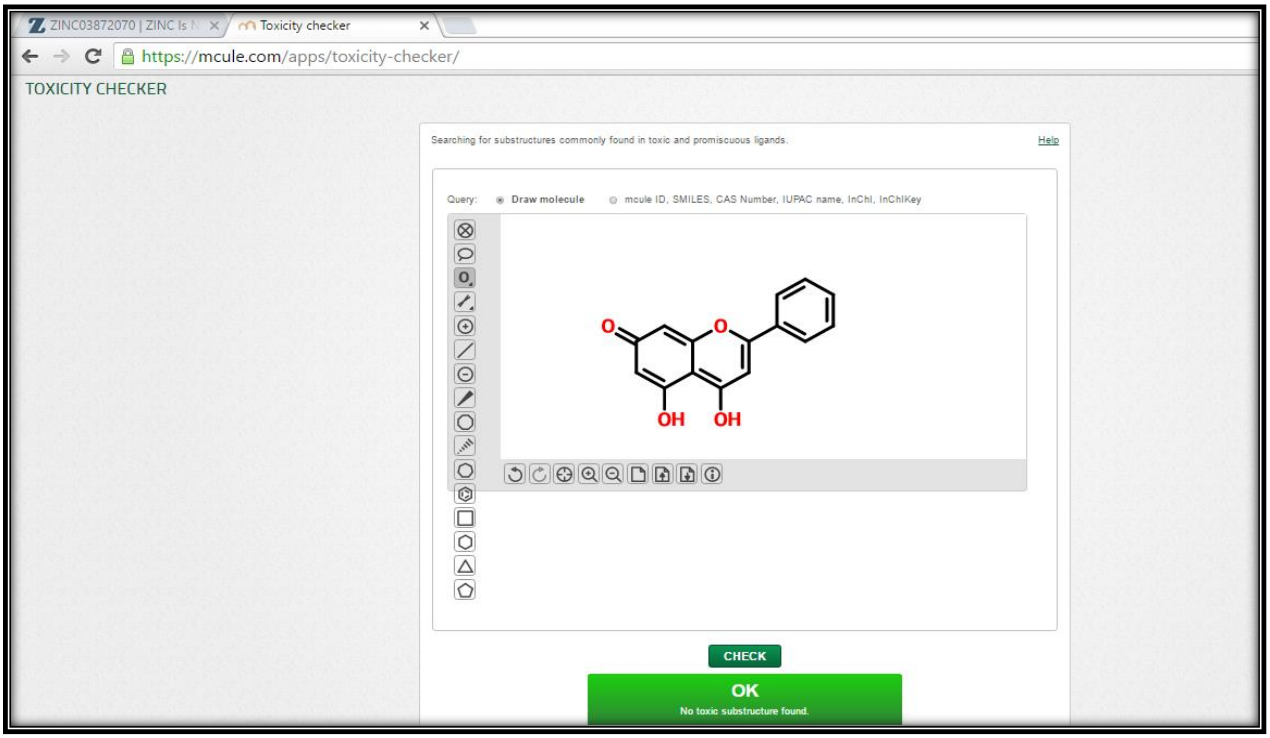

Figure.28.Toxicity checker result of ligand 6

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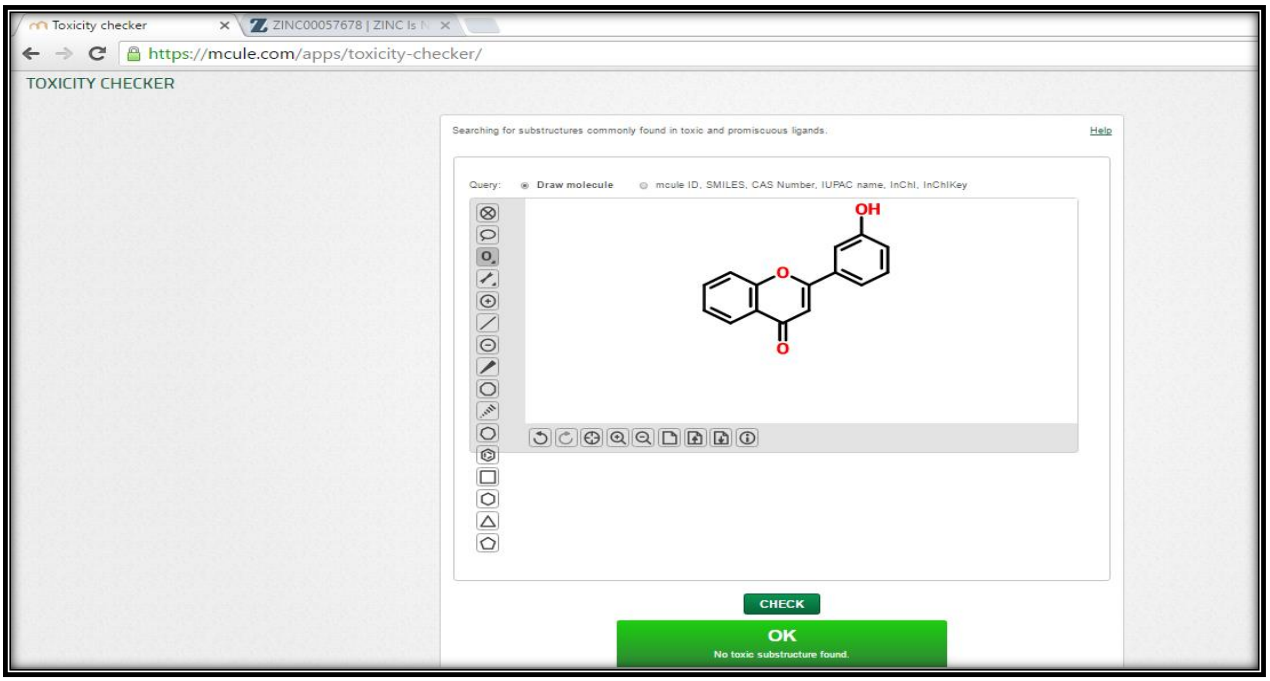

Figure.29.Toxicity checker result of ligand 7

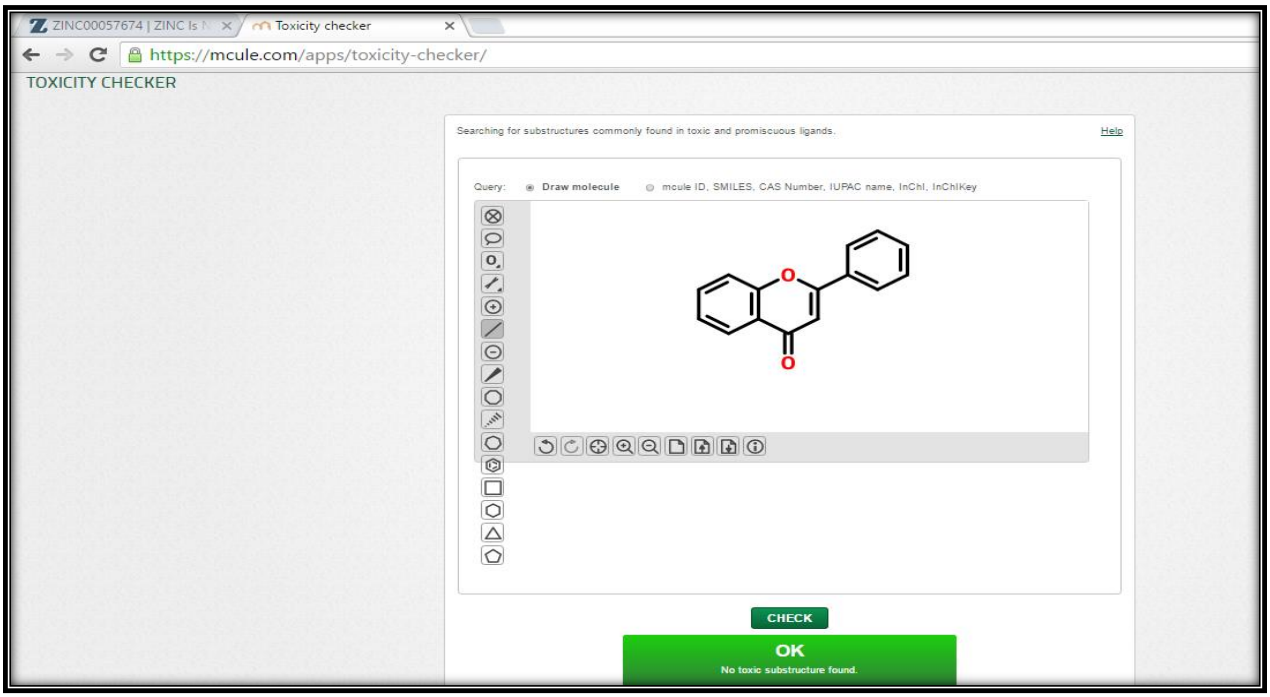

Figure.30.Toxicity checker result of ligand 8

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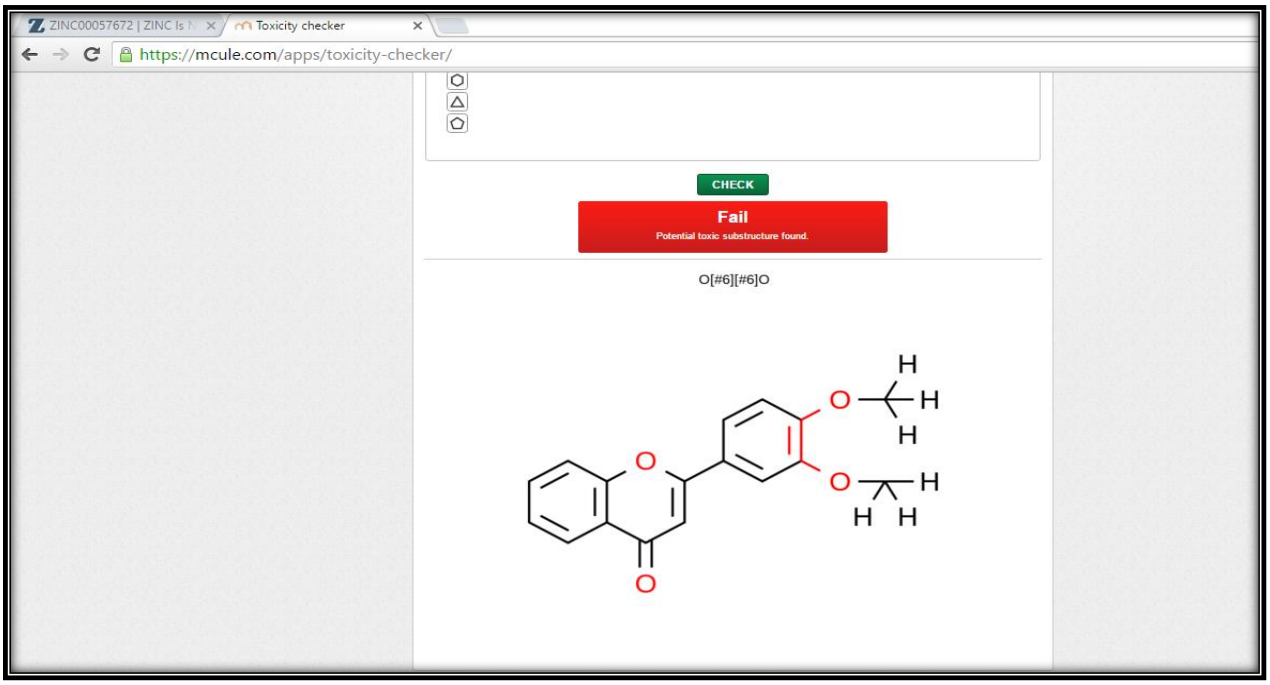

Figure.31.Toxicity checker result of ligand 9

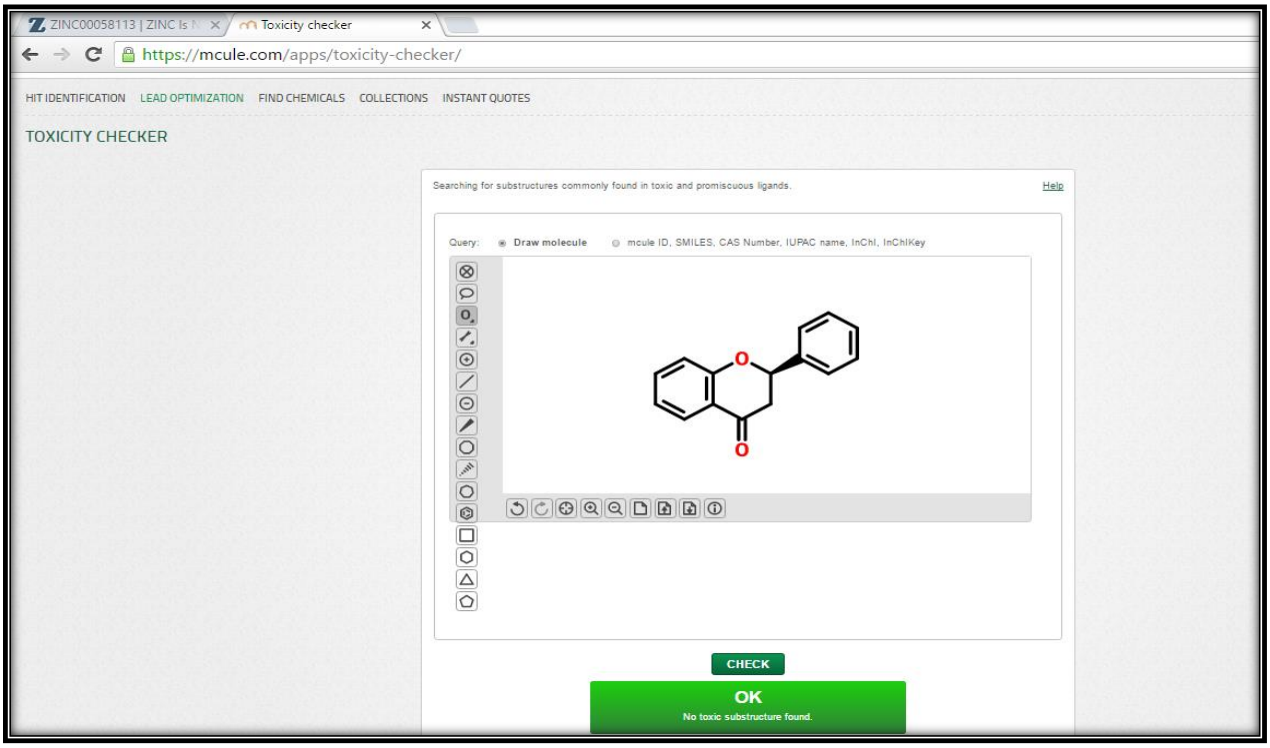

Figure.32.Toxicity checker result of ligand 10

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5.4.2. Using OSIRIS data warrior software

Toxicity as well as various physicochemical properties was again checked by OSIRIS data warrior software for top 10 ligand molecules. These results are summarized in Table 3.

Ligand	zinc_18056	zinc_39289	zinc_57656	zinc_57675	zinc_57676	zinc_57756	zinc_58076	zinc_58117	zinc_156701	zinc_2558138
Total mol. Wt.	252.268	254.240	254.240	238.241	238.241	286.238	270.714	288.254	272.255	256.300
CLogP	3.3028	2.5273	2.6814	2.873	3.0271	1.8359	4.3227	1.81	2.1557	3.5561
CLogS	-3.762	-3.379	-3.152	-3.675	-3.448	-2.787	-4.824	-2.344	-2.64	-3.208
H-Acceptors	3	4	4	3	3	6	2	6	5	3
H- Donars	0	2	2	1	1	4	0	4	3	1
Polar Surface Area	35.53	66.76	66.76	46.53	46.53	107.22	26.3	107.22	86.99	38.69
Drug likeness	0.35997	-0.082832	0.28194	-0.082832	0.28194	-0.082832	0.31301	-0.22006	-0.22006	-1.09
Mutagenic	none	none	none	none	none	none	none	none	none	none
Tumorigenic	none	none	none	none	none	none	none	none	none	none
Reproductive Effect	none	none	none	none	none	none	None	none	none	high
Irritant	none	none	none	none	none	none	none	none	none	none
Non C/H Atoms	3	4	4	3	3	6	3	6	5	3
Stereo centers	0	0	0	0	0	0	0	1	1	1
Rotatable bonds	2	1	1	1	1	1	1	1	1	2
Rings	3	3	3	3	3	3	3	3	3	3
Aromatic Rings	2	2	2	2	2	2	2	2	2	2

Table3. Physicochemical properties and toxicity results for top 10 ligands

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6. CONCLUSION

Diabetes is an alarming disorder of the third world. The prevalence of diabetes is likely to increase by 35% by the year 2025 according to the World Health Organization (WHO) projections. Currently, India is the diabetic capital of the world. Diabetes mellitus, a group of metabolic diseases is characterized by hyperglycemia resulting from defects in insulin secretion, insulin action or both. In conclusion, the results of the current study clearly demonstrated that screened flavonoids i.e., ST001551 are better inhibitors in comparisons to reported lead for protein targets. Also the screened inhibitors are effective against multiple targets of Diabetes therapy. The reliability of the docked result depends on the similarity of its final docked conformation. The groups indicate that all the agonists mainly take one conformation. Besides analysis, AutoDock also uses binding free energy evaluation to find the best binding mode. Energy items calculated by AutoDock are characterized by intermolecular energy (consist of van der Walls energy, hydrogen bonding energy, desolvation energy, and electrostatic energy), internal energy of ligand, and torsional free energy. The first two of these combined give the docking energy while the first and third terms build up the binding energy. During all these interactions, the electrostatic interaction between ligands and receptor is the most important, because in most cases it can decide the binding strength and the location of ligand, while the hydrophobic interaction of some certain groups can affect the agonistic activity to a larger extent. In the present study, the molecular docking of the five diverse agonists was performed, which revealed the main differences of binding modes of these agonists and the critical amino acid residues involved in the recognition of the agonists. Our results suggested that agonists can exactly bind to the active pocket of GPR40 to display agonistic activity. The leads figured out, could potentially restrain the disease. Notwithstanding, these leads ought to experience different preclinical analysis and optimization before going into clinical trials. Therefore, these new molecular entities were suggested as possible versatile agonist for these proteins.

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7. DISCUSSION AND FUTURE PERSPECTIVE

Our aim is to identify the potential drug molecule against our protein target molecule. The ligand is determined from PDB as hydroxy ion and docking of natural ligand with the target protein gives a cut-off value of binding energy for any ligand to be the drug molecule.

Then based on the active sites present on our target protein, 100 ligands were identified as suitable ligands. Thus, virtual library of ligands were generated. And each ligand was separately docked with the target protein to determine top 10 ligands having better binding energy. And the best shows better binding energy than the natural ligand as well as available drugs.

Then, various physicochemical properties and toxicity of the selected ligands were determined. Finally, we come up with ligand, 7-hydroxyflavone (ZINC ID 05934541) as the best ligand molecule and have the potential to be a drug molecule. So, in future one can go for clinical trials with the best ligand and study their different properties like pharmacodynamic, pharmacokinetics, solubility etc.

Diabetes milletus is a chronic disease which leads to various complications on long standing. Allopathic medicines are not effective in treating the disease leading to various adverse effects. Hence medicinal plants are the best alternative for the treatment of diabetes milletus. The plant species have proved their efficacy in reducing blood glucose levels. Discovery of novel compounds can be developed through extensive research work on bioactivity of various constituents. In near future herbal plants will play a crucial role in modern system of medicine. Thousands of herbal medicines are used by people from every culture and various indigenous medicines are gradually being introduced into modern therapeutics. In developing countries about 80% of the people, especially the rural population, rely on the traditional medical remedies for their health care needs. In developed countries, there has been a resurgence of interest in herbal medicines due to a large extent on the preference of many consumers for products of natural origin. It is important however; to distinguish between herbal medicine supplied by a “qualified” medical practitioner as a result of a consultation and those herbal remedies (in the form of “teas”) freely available to the public for selfmedication. The rapidly increasing incidence of diabetes mellitus is a serious threat to human health in all parts of the world. Recently, new bioactive drugs have been isolated from plants and have shown anti-diabetic activity with more efficacy than oral hypoglycaemic agents used in clinical therapy. Therefore in recent years, Attention has been drawn towards identification of plants with anti-diabetic ability that may be useful to man. They may also provide clue for the development of a new and better oral drugs for diabetes mellitus.

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In this study the lead molecule with binding energy of -3.92 i.e ***Isoflavanone*** was previously been shown to control the diabetes (Zhao-min Liu et al., 2009). Anti-inflammatory and anti-oxidative effects of ***luteolin*** were also proved (Yanqing Zang et al., 2016). ***Taxifolin*** prevents diabetic cardiomyopathy in vivo and in vitro by inhibition of oxidative stress and cell apoptosis (Xiao Sunet al., 2013). Treatment with ***eriodictyol*** significantly suppressed diabetes-related lipid peroxidation (Bucolo et al., 2012). ***Apigenin*** causes biochemical modulation, GLUT4 and CD38 alterations to improve diabetes and to protect damages of some vital organs in experimental diabetes (Hossain et al., 2014). ***Naringenin*** has shown to have Anti-inflammatory and Antifibrotic Effects (Shih-Jei Tsai et al., 2012). From these results we could conclude that we can use these lead molecules for further studies as potential drug molecules.

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