International Journal of PharmTech Research
CODEN (USA): IJPRIF ISSN : 0974-4304
Vol.3, No.3, pp 1576-1581, July-Sept 2011

Molecular docking Studies of 2α-Hydroxyursolic acid derivatives for hypercholesterolemia

Meena Chandran*[1], Shiny George[2], Kumaran Santhalingam[1], Pallavi Gangwar[1], K Krishnakumar[3]

[1]Gensilico Biosolutions, Zamin Pallavarm, Chennai, Tamil Nadu, India-600043

[2]Department of Pharmaceutical Chemistry, School of Pharmaceutical Sciences, Vels University, Chennai, Tamil Nadu, India-600117

[3]St.James college of Pharmaceutical Sciences, St. James Medical Academy, River bank, Chalakudy-680307, Kerala, India.

Corres. author: meenampha@gmail.com, +91-9043504039

Abstract: Hypercholesterolemia, or high cholesterol, occurs when there is too much cholesterol in the body. Cholesterol is a soft, waxy, fat-like substance that is a natural component of all the cells of the body. Molecular docking is used to predict the binding orientation of small molecule drug candidates to their protein targets in order to in turn predict the affinity and activity of the small molecule. Hence docking plays an important role in the rational design of drugs. The present study is deals with the molecular docking of derivatives of 2α-hydroxyursolic acid which is the active component of the plant Banaba leaves (*Lagerstroemia speciosa* L. Pers, Lythraceae) against HMG CoA reducatse involved in cholesterol biosynthesis using AutoDock software. The protein file of HMG CoA reductase [PDB ID: 1DQ8] was taken from the protein data bank. The lead moiety 2α-hydroxyursolic acid has shown best ligand binding energy -5.32kcal/mol. All the derivatives have shown best ligand binding energy between–2.82 kcal/mol to -12.52kcal/mol. Out of the nine derivatives 1I show best ligand binding energy as -12.52 kcal/mol.
Keywords: Hypercholesterolemia, *Lagerstroemia speciosa,* 2α-hydroxyursolic acid, HMG CoA reducatse, AutoDock.

INTRODUCTION:

Cholesterol is a lipid particle found circulating in the body[1]. Cholesterol, contrary to its popular image as a potent enemy of health and longevity, is actually a crucial substance that performs innumerable vital functions in the body. Cholesterol is needed for the synthesis of bile acids, which are essential for the absorption of fats, and of many hormones such as testosterone, estrogen, dihydroepiandrosterone, progesterone, and cortisol. Together with sun exposure, cholesterol is required to produce vitamin D. Cholesterol is an essential element of cell membranes, where it provides structural support and may even serve as a protective antioxidant. It is essential for conducting nervous impulses, especially at the level of the synapse[2]. Cholesterol is a versatile compound that is vital (in small amounts) to the functioning of the human body. Only animals produce it; no plant product contains cholesterol unless an animal- based product, such as lard, has been added to it in processing. Cholesterol synthesis occurs in the cytoplasm[3]. Normal healthy adults synthesize cholesterol at a rate of approximately 1 g/day and consume approximately 0.3 g/day. A relatively constant level of cholesterol in the body (150–200 mg/dL) is maintained primarily by controlling the level of de novo synthesis. The concentration of cholesterol

in the plasma can serve as a potential source of adipocyte cholesterol and thus influence the extent of adipose tissue cholesterol storage. Hypercholesterolemia produced either by cholesterol feeding or by cholesterol-free, purified diets ("endogenous" hypercholesterolemia) results in the accumulation of cholesterol in adipose tissue. Hypercholesterolemia is primarily due to elevated LDL (Low-density lipoprotein) concentrations in blood. Elevated LDL cholesterol has several causes. One reason is high dietary intake of cholesterol and/or saturated fat or genetic disorders like familial Hypercholesterolemia. Hypercholesterolemia is a well characterized cardiovascular risk factor that is known to initiate inflammatory and thrombogenic responses in the micro vascular[4].

Cholesterol enters the intestinal tract from two major sources, the diet and bile. A third possible source is secretion by intestinal mucosa. Dietary cholesterol is comprised of free and esterified cholesterol, the ratio depending upon dietary source. Cholesterol biosynthesis is a tightly regulated pathway that employs multiple feedback mechanisms to maintain homeostasis. The first committed step in sterol synthesis, the NADPH-dependent reduction of HMG-CoA to mevalonate, is catalyzed by HMG-CoA reductase (HMGR, 3-Hydroxy-3-methylglutaryl coenzyme A reductase) at the endoplasmic reticulum (ER) membrane[5]. Normally in mammalian cells this enzyme is suppressed by cholesterol derived from the internalization and degradation of low density lipoprotein (LDL) via the LDL receptor as well as oxidized species of cholesterol. Competitive inhibitors of the reductase induce the expression of LDL receptors in the liver, which in turn increases the catabolism of plasma LDL and lowers the plasma concentration of cholesterol, an important determinant of atherosclerosis. This enzyme is thus the target of the widely available cholesterol-lowering drugs known collectively as the statins[6-8].HMGR, an eight-span integral membrane protein, is regulated by feedback mechanisms operating at multiple levels. HMGR is generally considered to catalyze the rate-limiting reaction in cholesterol biosynthesis. The enzyme catalyzes the formation of mevalonic acid. The hepatic enzyme is depressed by high-cholesterol diet. Cholesterol feeding lowers HMG-CoA reductase activity by rapid inactivation of preformed enzyme and longer-term reduction in enzyme synthesis.

Plants have been used worldwide in traditional medicines for the treatment of various diseases and it is estimated that even today approximately 65-75% of the world's population rely only on medicinal plants as their primary source of medicines. Plants have been used worldwide in traditional medicines for the treatment of various diseases and it is estimated that even today
approximately 65-75% of the world's population rely only on medicinal plants as their primary source of medicines.Herbal Medicine is derived from medicinal plants are used for the prevention and treatment of diseases. Banaba leaves (*Lagerstroemia speciosa* L. Pers,Lythraceae) have been used in traditional medicine to treat diabetes mellitus in Southeast Asia for a many years. Banaba extracts are also known to have antiobesity, anti-oxidant and anti-gout effects. Corosolic acid (2α-hydroxyursolic acid), an active ingredient in these extracts, displays a otential anti-diabetic activity as well as anti-oxidant, anti-inflammation, and antihypertension properties. Corosolic acid is also known as 2alpha-hydroxyursolic acid. Its structure is a pentacyclic titerpene, which means it contains five rings [10-19].

In the present study we attempt a theoretical study of 2α-Hydroxyursolic acid derivatives (1A-1I) by docking, to inhibit HMG CoA reductase, to identify the inhibitory effect of 2α-Hydroxyursolic acid derivatives.

MATERIALS AND METHODS:

The Structure of the Protein HMG CoA Reductase with the PDB ID (1DQ8) was retrieved from the Protein Data Bank. It is a repository for the 3-D structural data of large biological molecules, such as proteins and nucleicacids.

Active site prediction:

After obtaining the PDB ID (1DQ8), the possible binding sites of HMG CoA Reductase were searched using Computed Atlas of Surface Topography of Proteins (CASTp). These include pockets located on protein surfaces and voids buried in the interior of proteins. CASTp includes a graphical user interface, flexible interactive visualization, as well as on-the-fly calculation for user uploaded structures[20].

Preparing the derivatives of 2α-hydroxyursolic acid:

Medicinal chemistry or Pharmaceutical Chemistry is a discipline at the intersection of chemistry and pharmacology involved with designing, synthesizing and developing pharmaceutical drugs[21]. The 2α-hydroxyursolic acid was synthesized from the lead compound ursolic acid by hydroxylation method and various derivatives in (**Table: 1**) of that were prepared according to the scheme of Yanqiu Meng etal[22].

Docking the inhibitors against the active site of the HMG CoA Reductase:

Docking is a computational technique that samples conformations of small molecules in protein binding sites; scoring functions are used to assess which of these conformations best complements the protein binding site[23].The inhibitor and target protein was geomentrically optimized and docked using docking engine AutoDock.

TABLE: 1 various substitutents of 2α-hydroxyursolic acid derivatives

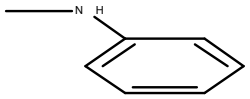
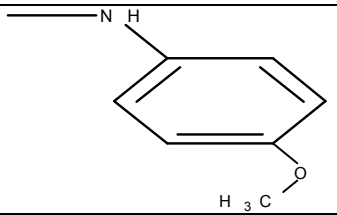
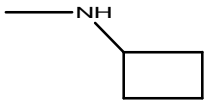
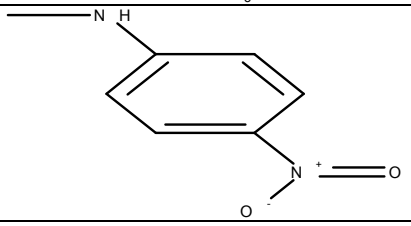
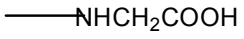
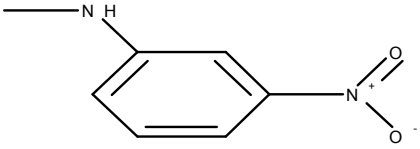
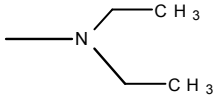
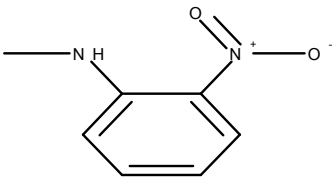
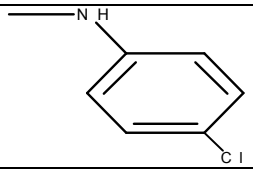
Compound	R	Compound	R
1A		1F	
1B		1G	
1C		1H	
1D		1I	
1E			

TABLE: 2 Binding energy of derivatives of 2-α-hydroxyursolic acid

S.No	Name of the drugs	No of conformation	Binding Energy	Hydrogen bonds
1	1A	10	-4.94	1
2	1B	10	-11.17	1
3	1C	10	-4.81	1
4	1D	10	-5.52	1
5	1E	10	-4.77	1
6	1F	10	-4.42	4
7	1G	10	-3.66	2
8	1H	10	-2.88	1
9	1I	10	-12.52	1
10	2α-hydroxyursolic acid	10	-5.32	1

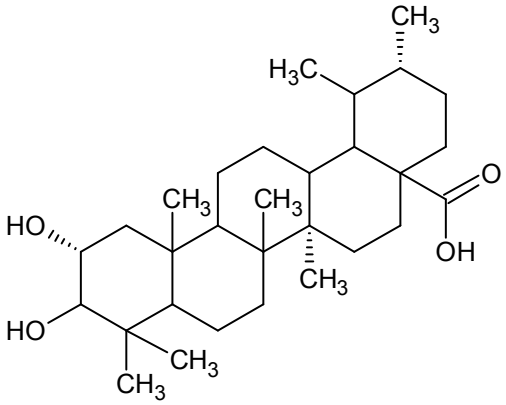


Fig:1 2*α*-hydroxyursolic acid structure

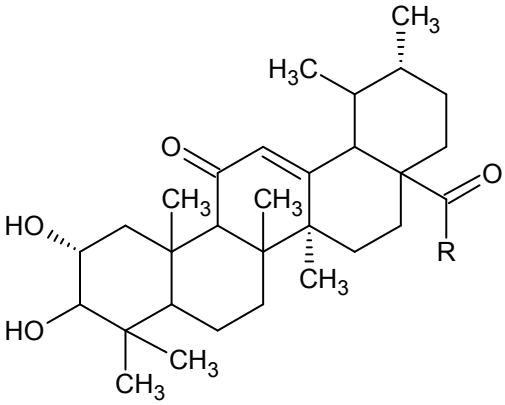


Fig:2 2*α*-hydroxyursolic acid derivatives

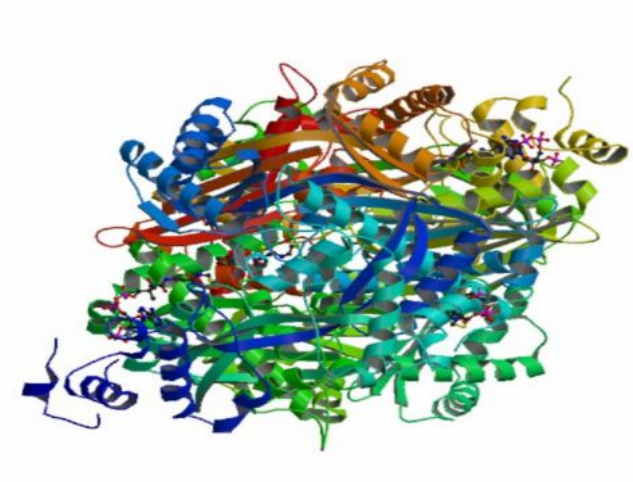


Fig: 3 Structure of HMG CoA reductase protein (PDB ID: 1DQ8)

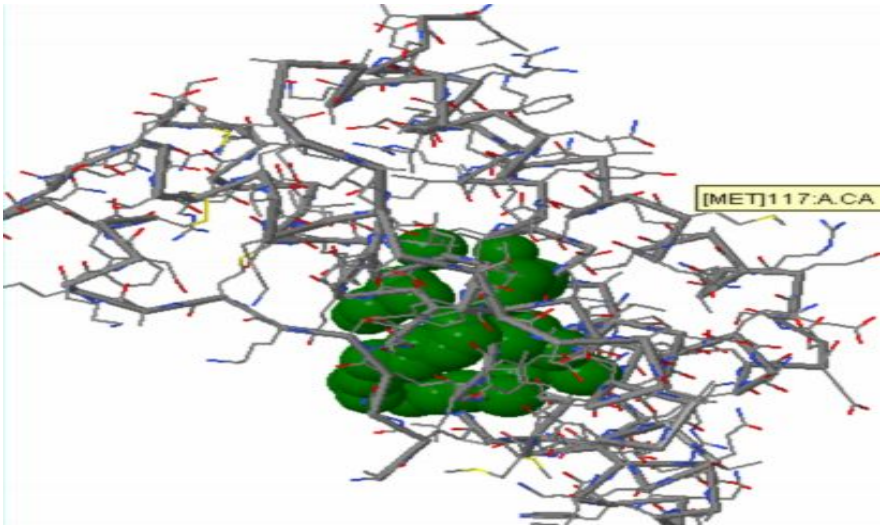


Fig: 4 Binding sites of PDB ID: 1DQ8 from CASTp

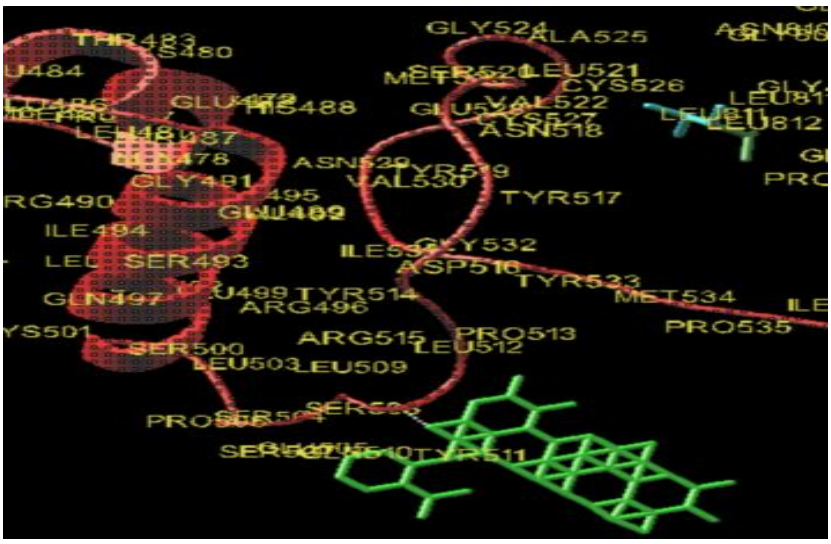


Fig: 5 docking complex of HMG CoA Reductase protein (PDB ID: 1DQ8) with 1I

RESULT AND DISCUSSION:

Molecular modeling (docking) study was carried out for series of 2α-hydroxyursolic acid (1A-1I) fig 2 for HMG CoA reductase. The potential active site amino acids of HMG CoA reductase were predicted using CASTp (Fig.1). Thus, the protein was targeted against pocket 1 .Given the three-dimensional structure of a target receptor molecule usually a protein; chemical compounds having potential affinity toward sit are designed rationally, with the aid of computational methods . Detailed bioinformatics analysis offers a convenient methodology for efficient *in silico* preliminary analysis of possible function of new drug. The target protein and inhibitors were geometrically optimized. All the nine 2α-hydroxyursolic acid inhibitors were docked against active site of the target protein using Auto Dock which gives an insight into the binding modes for the various inhibitors. Out of 10 inhibitors analyzed (i.e. 1A, 1B, 1C, 1D, 1E, 1F, 1G, 1H, 1I) 1I and 1B has showed best binding energy of -12.52 Kcal/mol and -11.17Kcal/mol with 1 hydrogen bond each against the target protein. The binding energy of all the inhibitors was shown in Table: 2. **Fig: 5** represents the docked complex (1I) of the inhibitors to that of target protein.

CONCLUSION:

In conclusion, 2α-hydroxyursolic acid obtained from Banaba leaves (*Lagerstroemia speciosa* L. Pers, Lythraceae) are used in various diseases like diabetic, obesity, hypertension, inflammation and cancer. In this study is mainly done to find out the inhibitory activity of 2α-hydroxyursolic acid and their derivatives against HMG CoA reductase which is the key enzyme involved in the biosynthesis of cholesterol. For this the 2α-hydroxyursolic acid and their derivatives were docked with HMG CoA reductase protein by using AutoDock software to get the best hit. The best drug was selected, depending upon the binding energy. Of which 2-nitroaniline susbstituted derivatie gives better results when compared to 2α-hydroxyursolic acid. So these 1I may act as a better and efficient drug to treat hypercholesterolemia than 2α-hydroxyursolic acid.

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