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Full Length Original Research Paper

Synthesis of hydrazine based novel HMG coA inhibitor and its docking studies

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Abstract:
Heart attack is the recent attention to the medical sciences and cardiac low density cholesterol (LDL) has a significant role in inhibiting the arrest. Various pathways having the keypoints which can interrupt the synthesis of bad cholesterol. In this study Hydrazine based compound is choosen due to its wide applications, the 1,2-Bis (2-hydroxy-5-methylbenzylidene) - hydrazine crystals were synthesized. It was taken as a ligand for molecular docking. The target receptor used for docking is HMG-CoA reductase whose crystal structure is available on the PDB database as PDB ID: 1DQ8. For docking analysis, Autodock tool v4. 2 program used. The synthesized crystals are subjected to single crystal X-ray studies in order to investigate their molecular structure.

Keywords: HMG-CoA, BMET, Docking, Autodock, X-ray diffraction, B3LYP

Introduction:

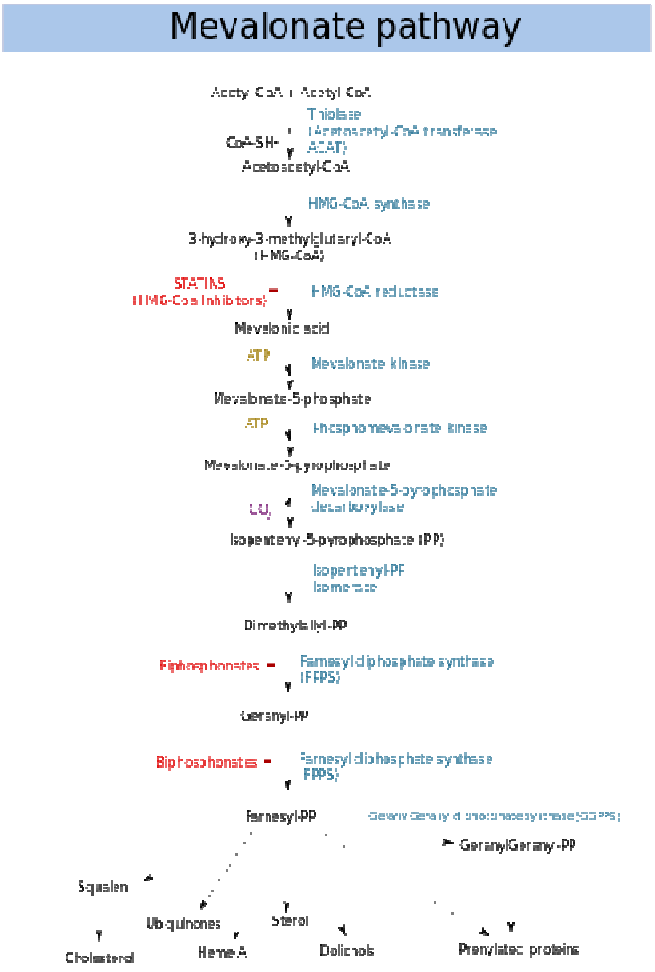
Current scenario of the young death is tremendous and shocking. Recent study estimated 78% men under the age of 38 having risk of heart arrest due to LDL. In India the research in cardiology shows valuable results in youngsters. Large number of study demonstrated that traditional cardiovascular disease risk factors play crucial role in the prior estimation of myocardial infarction in populations around the world, including India. Hence the factor responsible for cause need to be focoused.

Hydroxymethylglutaryl-coenzyme A (HMG-CoA) is the precursor for cholesterol synthesis HMG-CoA is also an intermediate on the pathway for synthesis of ketone bodies from acetyl-CoA. Virtual screening has become a widely used approach to lead discovery in the pharmaceutical industry. Molecular docking programs are commonly used to position small molecules within a three-dimensional representation of the protein structure. As the number of protein X-ray structures has increased dramatically in recent years [1], these programs have become standard computational tools used in structure-based optimization of lead compounds [2, 3]. Now a days virtual screening is the simplest and targeted way to find inhibitors.

Cholesterol is a remarkably versatile molecule [4]. It determines the biophysical properties of cellular membranes [5], serves as a precursor for steroid hormones and regulates the function of signaling molecules like a hedgehog [6]. Given this multitude of functions, it is not surprising that acquired or genetic defects in cholesterol metabolism cause severe diseases [7–11] including arteriosclerosis [12,13], Smith–Lemli–

Opitz syndrome [14] and Niemann–Pick type C disease [15].

Hydrazine is mainly used as a foaming agent in preparing polymer foams, but significant applications also include its uses as a precursor to polymerization catalysts and pharmaceuticals.



Cholesterol synthesis pathway

1. Cholesterol synthesis:

The mitochondrial matrix having the enzymes for ketone body production. HMG-CoA destined for cholesterol synthesis is made by equivalent, but different, enzymes in the cytosol.HMG-CoA is formed by condensation of acetyl-CoA and acetoacetyl-CoA, catalyzed by HMG-CoA Synthase. The HMG-CoA Reductase reaction is rate-limiting for cholesterol synthesis. This enzyme is highly regulated and the targeted. Feedback inhibition of the HMG reductase is responsible for the cholesterol inhibition.[22] There are many rate limiting factors available, it suggested that phosporylation of the reductase can be regulate the synthesis and stop the activity of the enzymes[23]

Hence to understand and focus the newly synthesized hydrazine based compound have been incorporated for its biological activity testing insilico aganist the HMG CoA reductase.

2. Experimental

2.1. Synthesis

The title compound was synthesized by mixing a solution (1:2 molar ratio) of hydrazine hydrate (0.20 ml, 4 mmol) and 2- hydroxy-5-methylbenzaldehyde (1.08 g, 8 mmol) in ethanol (30 ml). The resulting solution was refluxed for 4 h, yielding (65%) the pale yellow crystalline solid. The resultant solid was filtered off and washed with methanol. Pale Yellow single crystals of the title compound suitable for X-ray structure determination were recrystalized from dimethylformamide by slow evaporation at room temperature over several days.

3. Computational Details

3.1. X-ray Crystallography

A single crystal of the title complex suitable for X−ray structural analysis was selected from the crystals obtained above. All measurements were made on a Bruker MART APEX-II CCD area detector with graphite monochromated Mo-Ka radiation (0.71073 Å). The structure was solved by direct methods and refined by full−matrix least squares on F2. All non-hydrogen atoms were refined anisotropically. The H atoms were introduced in calculated positions and refined

with fixed geometry with respect to their carrier atoms.

3.2. Molecular docking

The structure of the target receptor, Human reductase with HMG and CoA (PDB id: 1DQ8) were obtained from the RCSB protein databank [http://www.rcsb.org/pdb]. Protein retrieved from the database was analyzed for pockets before docking studies, to ensure the possible number of binding sites of protein and ligand [16]. The binding sites for the target receptor were searched from the Q - site finder [www.bioinformatics.leeds.ac.uk/qsitefinder]. The small molecule or ligand is optimized by Gaussian 03W [17] package in the basis set B3LYP/6-31G(d,p). The ligand-protein docking simulations are carried out by Autodock tools [18] V1.5.4. and AutodockV4.2. Programs. Autodock tools are part of MGL tools of the Molecular Graphics Laboratory at The Scripps Research Institute, is built on the python molecule viewer (PMV).

The non bonded atoms in the target receptor like an oxygen atom of H_2O molecules that were present in the crystal structure of Human reductase with HMG and CoA, is cleaned up by removing H_2O molecules and hydrogen atoms also added. AutoDock docks a flexible ligand to a rigid receptor. Affinity maps for all the atom types present as well as electrostatic map, were computed with grid spacing of 0.375 E. Evaluation of the results was done by sorting the different complexes with respect to predicted binding energy.

4. Result and Discussion

4.1. X-ray structure determination

The details of the single crystal X-ray diffraction data collection, experimental conditions and structure refinements are given in Table 1. Some selected bond length and bond angles are shown in table 2. The Fig 1. Shows the schematic diagram of BMET. The ORTEP3 [19] diagram of BMET shown in Fig 2 with atom labels and 30% probability displacement ellipsoids for non-H atoms. The structure was solved by direct methods using SHELX 97 program and final R-factor was 0.050 [20].

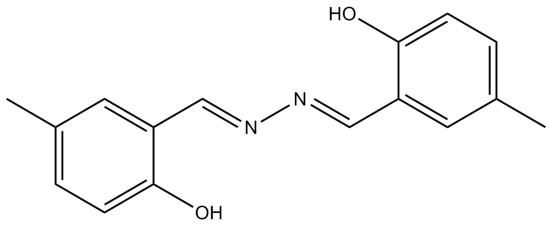


Fig. 1: Schematic diagram of BMET

Table 1: Details of the experimental diffraction data collections and refinements.

Empirical Formula	$C_{16}H_{16}N_2O_2$
Formula weight	268.31
Colour, shape	Yellow, Block
Temperature (K)	295
Crystal size (mm)	0.22 0.18 0.16
Crystal system	Orthorhombic
Space group	$P2_12_12_1$
Lattice constants	
a (Å)	6.0108(5)
b (Å)	7.3394(5)
c (Å)	31.674(2)
Volume (Å^3)	1397.32(17)
Z	4
λ(Å)	0.71073
Calculated density, ρ (Mg m^{-3})	1.275
Reflections collected	5699
Independent reflections	2952 [R(int)=0.023]
Goodness-of-fit	1.02
R1 [I > 2σ(I)]	0.050
wR2 (all data)	0.154

Table 3: Experimental Bond length (Å) in angstrom and Bond angles (0) in degrees of BMET

Bond lengths (Å)	Value	Bond angles (0)	Value
C1—C2	1.393 (3)	C2—C1—C6	117.9 (2)
C1—C6	1.415 (3)	C2—C1—C8	119.9 (2)
C1—C8	1.445 (3)	C6—C1—C8	122.3 (2)
C6—O1	1.354 (3)	C11—C10—C5	118.2 (2)
C8—N1	1.281 (3)	C11—C10—C9	119.3 (3)
N2—N1	1.404 (3)	C15—C10—C9	122.5 (2)
C10—C11	1.400 (3)	O1—C6—C5	118.5 (2)
N2—C9	1.277 (3)	O2—C15—C14	118.6 (3)
C10—C15	1.404 (3)	O2—C15—C10	121.7 (2)
C11—C12	1.381 (3)	C8—N1—N2	113.7 (2)
C15—O2	1.357 (3)	C9—N2—N1	113.9 (2)

In the molecule, two aromatic rings are almost co-planar, with a dihedral angle of 1.82 (12) ˚, the hydroxyl groups form intramolecular O-H… N hydrogen bonds like O1-H1… N1 and O2-H2A… N2 are listed in table 3. The yellow colour crystals of BMET crystallizes in the orthorhombic lattice with $P2_12_12_1$ symmetry with the unit cell dimensions a=6. 0108 (5) Å, b=7. 3394 (5) Å and c=31. 674 (2).

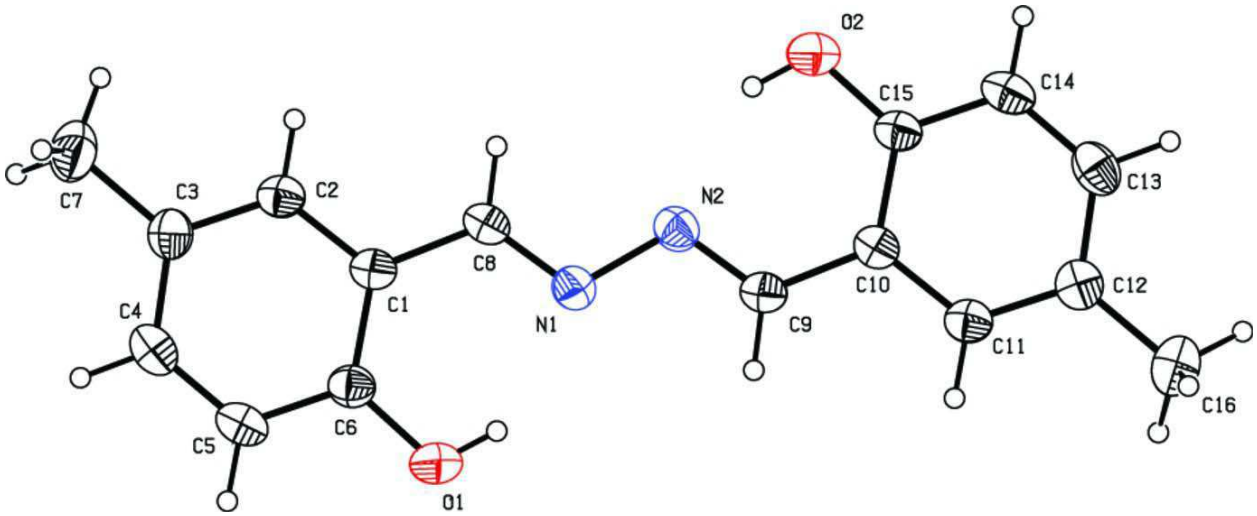


Fig. 2: Molecular structure of the BMET, with atom labels and 30% probability displacement ellipsoids for non-H atoms.

Table 3: Hydrogen-bond geometry (Å, °)

D—H…A	D—H (Å)	H…A (Å)	D…A (Å)	D—H…A (°)
O(2)—H(2A)…N(2)	0.82	1.91	2.635(3)	146
O(1)—H(1)…N(1)	0.82	1.93	2.646(3)	145
C(5)—H(5)…Cg(1)[(i)]	0.93	2.84	3.519(3)	130
C(14)—H(14)…Cg(2)[(ii)]	0.93	2.85	3.519(3)	130
Symmetry Codes (i) −x−1, y−1/2, −z+1/2; (ii) x+1/2, −y+5/2, −z+1.				

4.2. Molecular Docking

The Autodock 4.2 software is used to simulate the binding mode of the target receptor and ligand. Fig. 3 shows the protein structure of HMG-CoA reductase (PDB ID: 1DQ8). Fig. 4 shows the binding of BMET with HMG-CoA receptor. HMG-CoA reductase is the rate controlling enzyme of the mevalonate pathway, the metabolic pathway that produces cholesterol and isosprenoids. The 50 docking candidates were ranked by energy and the one with the lowest energy was regarded as the best mimic structure. There are five hydrogen bonds joining BMET and HMG-CoA reductase.

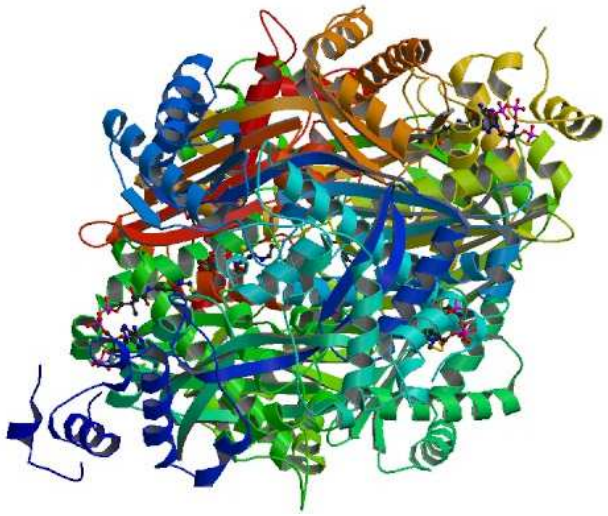


Fig. 3: shows the protein structure of HMG-CoA receptor

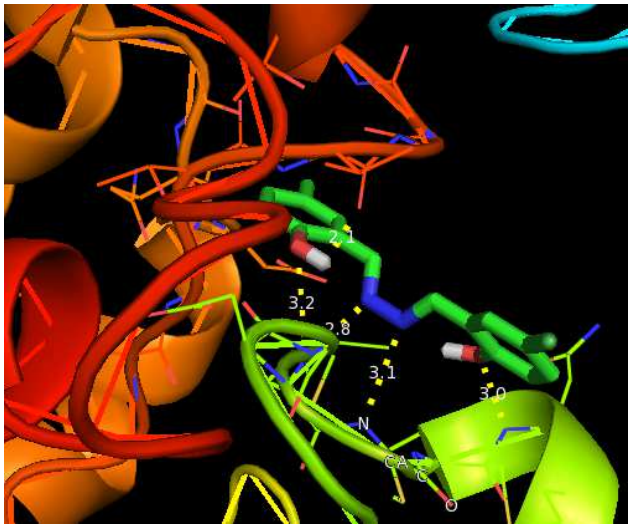


Fig. 4: shows the binding of BMET with HMG-CoA receptor, yellow dotted lines shows hydrogen bonds

According to the determination of the counterparts of experiment. The hydrogen bonds between HMG-CoA and BMET also shown in Fig. 3. Hydrogen bonding in docking plays a significant role in interaction studies [21]. The hydrogen bonds formed between O1 and N1 with MET655, the distance are 3.2 and 2.8Å. The bonds O2, N2 and H2A of BMET with ASN658, GLY656 and VAL805 respectively with the distance 3.0, 3.1 and 2.1Å. The energy value between binding sites of HMG-CoA and BMET are -7.23 kcal/Mol.

5. Conclusion

Heart disease has a sifnificant importance in the world, cholesterol is the limiting factor for the mortality rate due to coronary blockage. It has long been known that high blood cholesterol is a key risk factor for developing atherosclerosis - sometimes called hardening of the arteries. The condition causes the arteries of the heart and other tissues to become damaged and narrowed, preventing blood from pumping through as it should and increasing the risk of heart attack and stroke.

During the last few years, a variety of experimental methods developed by physicists and

Biologists allowed direct monitoring of ligand-receptor interaction at the single molecule level. The continual progress within the field of crystallography and bioinformatics made available the structure of many ligand – receptor complexes with angstrom resolution. In this paper, the structure of the synthesized BMET crystals was determined by single crystal x-ray structure determination. The molecular docking studies focused on the interactions of HMG-CoA receptor and BMET. The Hydrogen bonding between receptor and the small molecule is very important. The binding between small molecule and target receptor is also important to pharmacology and biochemistry the Ultimate aim of the research in this area is to inhibit cholesterol synthesis in excess. The said docking score explains the possibility of the compound to be desgnated as drug in future ofcourse after more illutrated testings. Feedback inhibition of pathway now carried out using the diffrenet drugs in martket eg. Stanin, lovastanin, cerastanin, atrovastanin. Which are modified

depending on generations. Hence this hydrazine based new compound has also tested to estimate its potential against cholestrol biosynthesis. This research may lead to open new generation drug discovery in future.

Conflict of Interest:

Authours has no conflict of interest.

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