DESIGN, SYNTHESIS AND PHARMACOLOGICAL EVALUATION OF NOVEL HETEROCYCLIC *PCSK9* INHIBITORS AS ANTIHYPERLIPIDEMIC AGENTS

A Dissertation submitted to

THE TAMIL NADU Dr. M.G.R. MEDICAL UNIVERSITY
CHENNAI-600 032

In partial fulfilment of the requirements for the award of the Degree of

MASTER OF PHARMACY
IN
PHARMACEUTICAL CHEMISTRY

Submitted by
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COLLEGE OF PHARMACY
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CERTIFICATE

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EXAMINERS

1.

2.

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LIST OF ABBREVIATIONS

AHA	America Heart Association
AI	Atherogenic index
AMA	America Medical Association
ANGPTL3	Angiopoietin-like 3
ANOVA	Analysis of Variance
CMC	Carboxy methyl cellulose
CVD	Cardiovascular disease
CPCSEA	Committee for the Purpose of Control and Supervision of Experiments on Animals
DMSO	Dimethyl sulfoxide
HDL-c	High Density Lipoprotein cholesterol
HFD	High Fat Diet
HMG CoA	3-Hydroxy-3-methylglutaryl-Coenzyme A
LDL-c	Low Density Lipoprotein cholesterol
LDLR	Low Density Lipoprotein Receptor
mg/dl	Milligram per decilitre
OECD	Organization for Economic Co-operation and Development
PPAR α	Peroxisome Proliferator Activated Receptor α
SEM	Standard Error of the Mean

SREBP	Sterol Regulatory Element Binding Protein
TC	Total cholesterol
TG	Triglycerides
TLC	Thin layer chromatography
VDLC-c	Very Low Density Lipoprotein cholesterol
WHO	World Health Organization

I.INTRODUCTION

I.A.HYPERLIPIDEMIA

Hyperlipidemia is a term that encompasses various genetic and acquired disorders that describe elevated lipid levels within the human body. It is a systemic disease, which is characterized by elevated lipid levels in blood including total cholesterol (TC), total glyceride (TG), and low density lipoprotein cholesterol (LDL-c) and so on. Hyperlipidemia represents the subset of dyslipidaemia and a superset of hypercholesterolemia. It is considered as one of the five leading cause of death[1].

Hyperlipidemia is one of the most important risk factors responsible for many cardiovascular and cerebrovascular diseases such as atherosclerosis (increased plasma level of low density lipoprotein), hypertension, stroke, fatty liver and becomes the first killer of human health[2,3]. It has been reported that nearly 23.6 million people will die from cardiovascular diseases originating from hyperlipidemia by 2030[4,]. Many clinical studies revealed that reduction of LDL and TC level significantly reduced the risk of the first stroke.

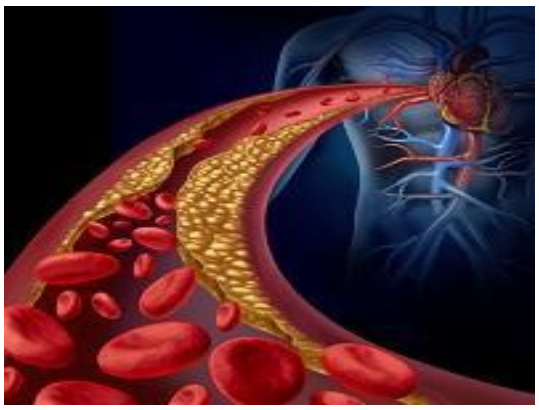


Figure.no.1: Hyperlipidemia

CLASSIFICATION

Hyperlipidemia subdivides into two broad classifications[5].

- **Primary (familial) hyperlipidemia**
- **Secondary (acquired) hyperlipidemia**

Familial hyperlipidemia

Familial hyperlipidemia derives from plethora of genetic disorders that a patient may inherent through birth. The primary hyperlipidemia may be treated by antihyperlipidemic

drugs. Primary hyperlipidemia was classified according to the Fredrickson classification, which is based on the pattern of lipoproteins on electrophoresis or ultracentrifugation[6]. It was later adopted by the World Health Organization (WHO).

Table.no.1: Fredrickson Classification of Primary hyperlipidemia[7].

Type	Disorder	Cause	Occurrence	Elevated plasma lipoprotein
I	Familial hyperchylomicronemia or Primary hyperlipoproteinemia	Lipoprotein lipase deficiency or Altered ApoC2	Very rare	Chylomicrons
IIb	Familial combined hyperlipidemia	Decreased LDL receptor and increased Apo B	Commonest	LDL and VLDL
III	Familial dysbetalipoprotenemia	Defect in Apo E- 2 synthesis	Rare	IDL
IV	Familial hypertriglyceridemia	Increased VLDL production and decreased excretion	Common	LDL
V	Endogenous hypertriglyceridemia	Increased VLDL production and decreased LPL	Less common	VLDL and chylomicrons

Acquired hyperlipidemia

Acquired hyperlipidemia (also called secondary dyslipoproteinemias) often mimic primary forms of hyperlipidemia and can have similar consequences. It typically originates from alternate underlying etiologic factors.

ETIOLOGY

- Genetic factors
- Unhealthy diet
- Excessive alcohol consumption
- Obesity
- Use of medications such as thiazide diuretics, beta blockers, corticosteroids, estrogen-progestin contraceptives[8].
- Diabetes Mellitus
- Hypopituitarism
- Chronic renal failure
- Nephritic syndrome
- Hypothyroidism
- Age
- Sedentary lifestyle
- Smoking[9]

SYMPTOMS

A person with hyperlipidemia usually has no signs or symptoms but they are usually discovered during routine examination or until it reaches the danger stage of a stroke or heart attack.

- Excessive fat in the blood forming plaques on the walls of the arteries and blood vessels which narrows the openings.
- If hyperlipidemia results in CHD or atherosclerosis at other sites, symptoms may include chest pain (angina), heart attack or stroke, acute pancreatitis[10].
- In familial, or inherited, hyperlipidemia, there may be yellowish fatty growths around the eyes or the joints.
- Very high triglyceride levels may result in the formation of nodules on the elbows or knees, or the appearance of multiple, pimple-sized, yellowish skin eruptions.
- Swelling of organs such as the liver, spleen, or pancreas (pancreatitis).
- Blockage of blood vessels in brain and heart.

COMPLICATIONS OF HYPERLIPIDEMIA

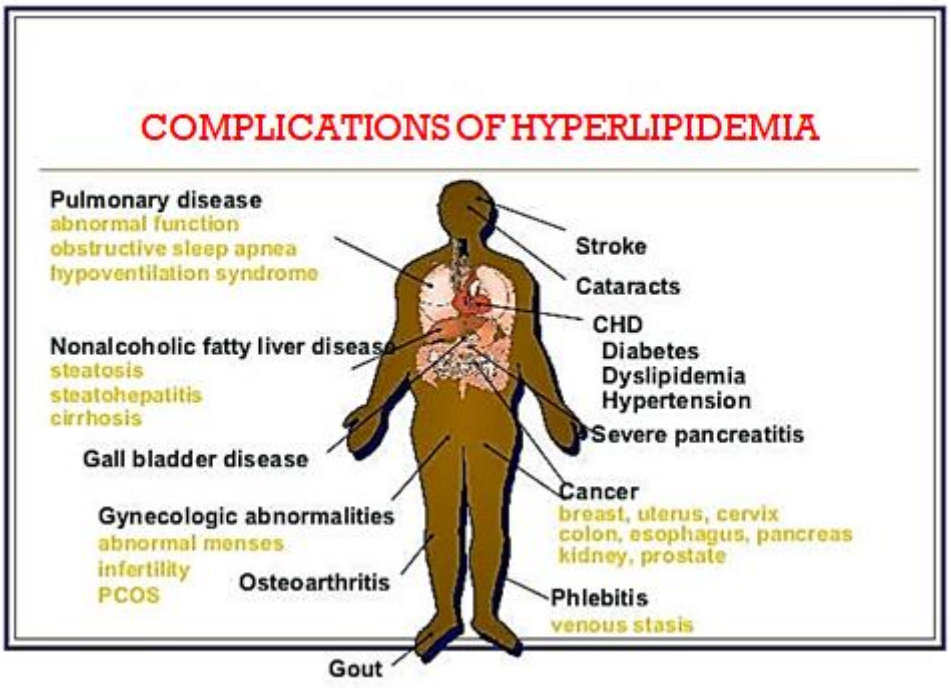


Figure.no.2: Complications of hyperlipidemia[11]

DIAGNOSIS

To determine if and when medications are needed, a physician will look at the patient's lipid profile and their risk factors. If the person has high cholesterol levels, monitoring and treatment are likely to be necessary.

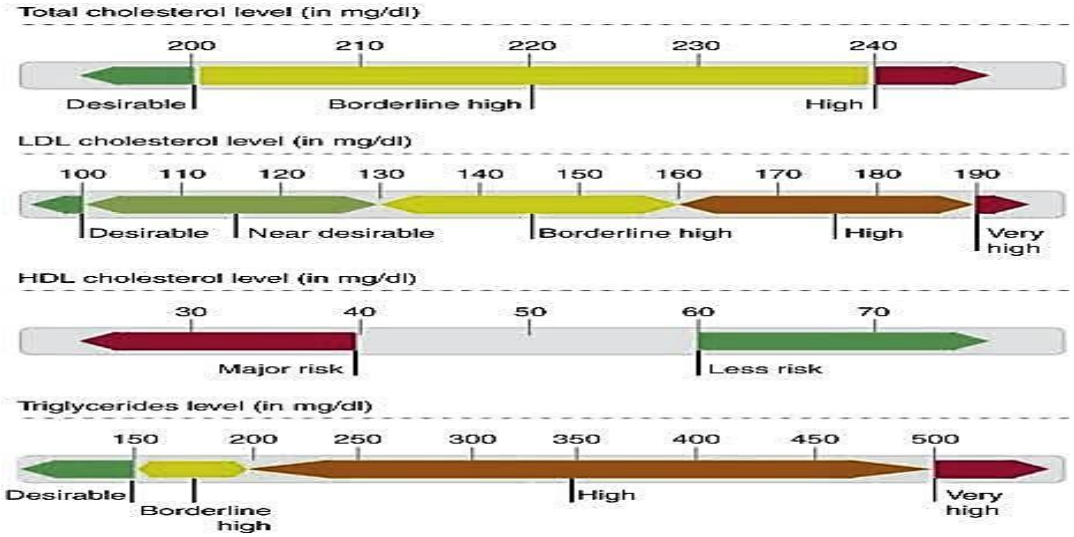


Figure.no.3: Lipid profile

PREVENTION

- Lifestyle options are the best way to prevent and treat hyperlipidemia. This involves a "heart-healthy" diet, regular exercise, avoiding or quitting tobacco use, and maintaining a healthy weight.
- A low-fat diet include a variety of whole fruits and vegetables, plenty of fibre, whole grains, fish, nuts, legumes, olive oil or oil rich in monounsaturated fats are recommended
- Regular exercise can help reduce LDL, total cholesterol, and triglyceride levels. It can also boost HDL, which helps to remove the bad cholesterol out of the blood encourage weight loss.
- Smoking promotes plaque build-up on the walls of the arteries, increases LDL levels, and it encourages the formation of blood clots and inflammation. Quitting smoking will result in higher HDL reduces the risk of cardiovascular disease[12].

TREATMENT

The most commonly prescribed medicines for hyperlipidemia treatment are

- HMG CoA Reductase Inhibitors – Atorvastatin, Simvastatin
- Fibrates – Fenofibrate, Clofibrate
- Bile acid sequestrants – Colesevelam, Colestipol, Cholestyramine
- Nicotinic acid – Niacin
- Cholesterol Absorption Inhibitors – Ezetimibe
- Other drugs – Vitamin E[13].

There are also new medications called PCSK9 inhibitors being studied for people with cardiovascular disease that need additional lowering of their LDL-c.

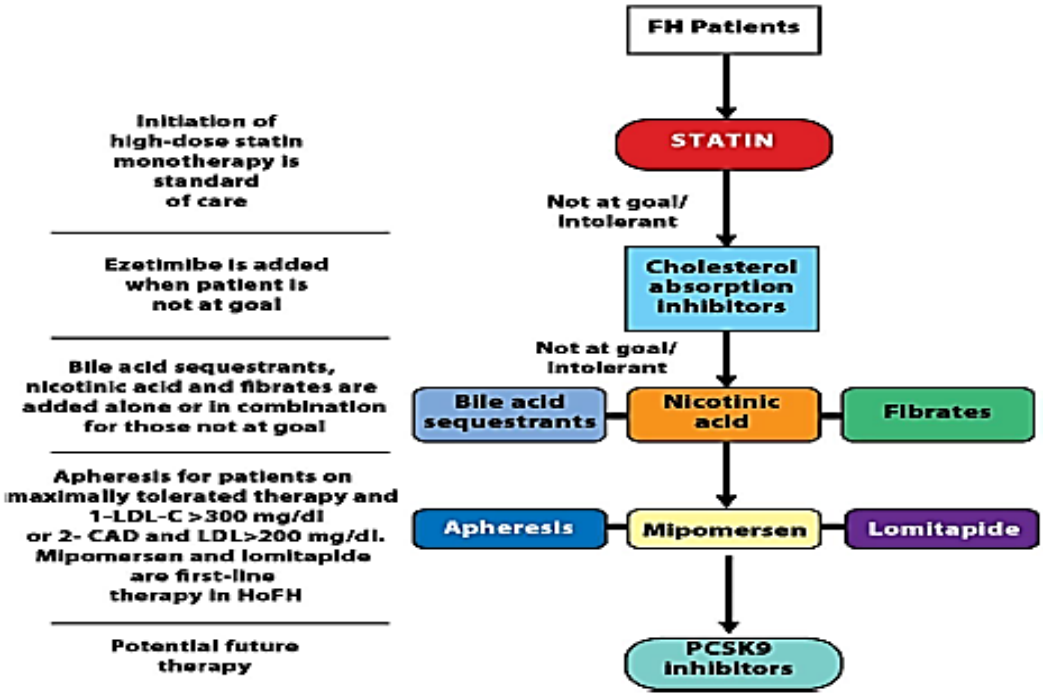


Figure.no.4: Agents used in the treatment of Hyperlipidemia

NEED FOR NEW ANTIHYPERLIPIDEMIC DRUGS

Some of the reasons necessitating the emergence of newer antihyperlipidemic drugs are

- To improve the current course of treatment by shortening its total duration.
- To facilitate drug compliance by providing with less intensive supervision.
- Drug with lesser frequency of administration which permits widely spaced intermittent treatment.
- To reduce the side effects such as stomach irritation, heart problems, and liver and kidney damage.
- To design a drug with higher efficacy and more specific in action by inhibiting the enzyme target which is responsible for causing hyperlipidemia.

I.B. DRUG DESIGN

In the field of new discovery and development, Computational techniques are rapidly gaining popularity, implementation and appreciation. Drug design is an inventive process of finding a new medication based on the knowledge of the biological target also known as rational drug design[14]. Drug design is frequently based on computer modelling techniques which are often referred to as Computer Aided Drug Design (CADD).

Different terms are being applied to this area, including computer aided drug design (CADD), computational drug design, Computer aided molecular drug design (CAMD), computer aided molecular modelling (CAMM), rational drug design, *In silico* drug design and computer aided rational drug design. Both computational and experimental techniques have complementary roles in drug discovery and development[15].

CADD ENTITIES

- Drug discovery and development process is streamlined by using the computing power.
- Chemical and biological information about ligands, targets in process of identification and optimization of new analogs.
- Design of *In silico* filters to eliminate compounds with undesirable properties (ADMET) and select the most promising candidates.

Drug Design

- Ligand based Drug Design
- Structure based Drug Design

Ligand Based drug design

- ✓ Ligand based drug design (Indirect drug design) depends on knowledge of all other molecules which binds to the biological target.

Structure based drug design

- ✓ Structure based drug design (Direct drug design) mainly depends on knowledge of the three dimensional structure of the biological target[16].

PHARMACOPHORE MODELING

Pharmacophore modeling studies have become one of the major tools in the field of drug discovery. In 1909, Paul Ehrlich introduced the concept of Pharmacophore, who defined the pharmacophore as "a molecular framework that carries (phoros) the essential features responsible for a drug's (Pharmacon) biological activity". The IUPAC defines "A pharmacophore is ensemble of stearic and electronic features that is necessary to ensure the optimal supra-molecular interaction with a specific biological target and to trigger or block its biological response"[17].

Pharmacophore features

- Hydrogen bond acceptor
- Hydrogen bond donor
- Hydrophobic
- Hydrophobic aliphatic
- Hydrophobic aromatic
- Positive ionizable
- Negative ionizable
- Ring aromatic

In order to identify novel ligands, the pharmacophoric features should match different chemical moieties with similar properties. A well-defined pharmacophore model includes both hydrophobic volumes and Hydrogen bond vectors.

Various ligands based and structure based methods involving pharmacophore modeling have been developed and extensively applied in the field of virtual screening, De novo design and lead optimization.

DOCKING STUDIES

Virtual screening techniques range from simple one up to sophisticated virtual docking methods aimed at fitting putative ligand molecules into the target receptor site[18]. In the field of drug design, docking predicts the preferred orientation of one molecule to the other when they bound to each other to form a stable complex[19]. Docking plays an important role in the molecular modelling as it is used to predict the binding orientation (affinity) of drug candidate to their protein targets[20].

Docking process

The docking process mainly involves the prediction of ligand conformation and orientation (posing) with active binding site of the target. Molecular docking process is compared to "lock-and-key" model. Here, the protein is considered as the "lock" and the ligand as a "key". The ligand and the protein adjust their conformation to achieve an overall "best-fit" which is referred as "induced-fit"[21].

Docking methodology

Docking is an interactive procedure which generates the random ligand conformations for specified number of times, number of maximum trials[22].

Steps involved in docking

- ✓ Protein preparation.
- ✓ Ligand Preparation.
- ✓ Docking Procedure.
- ✓ Visualization / Interpretation of Docking.

Protein Preparation

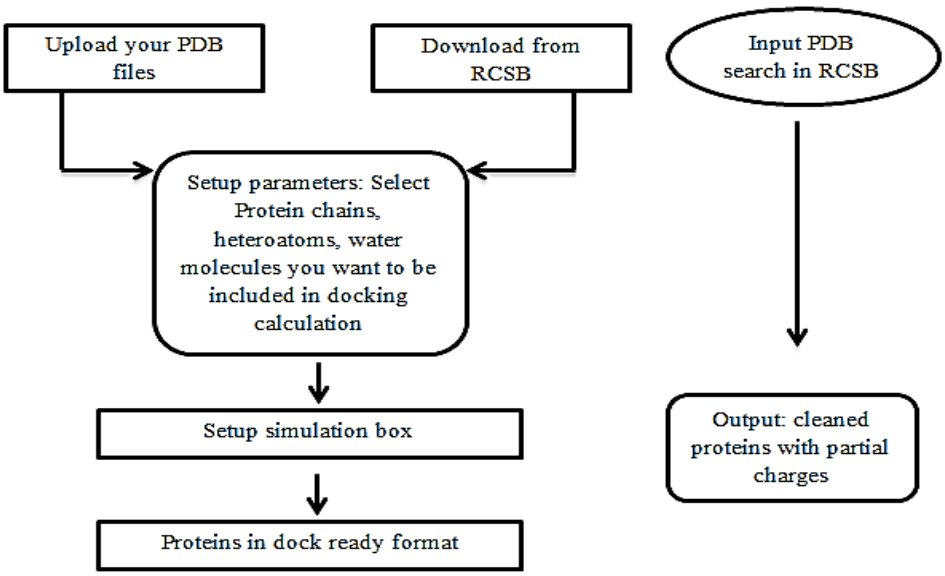


Figure.no.5: Preparation of Protein

Ligand Preparation

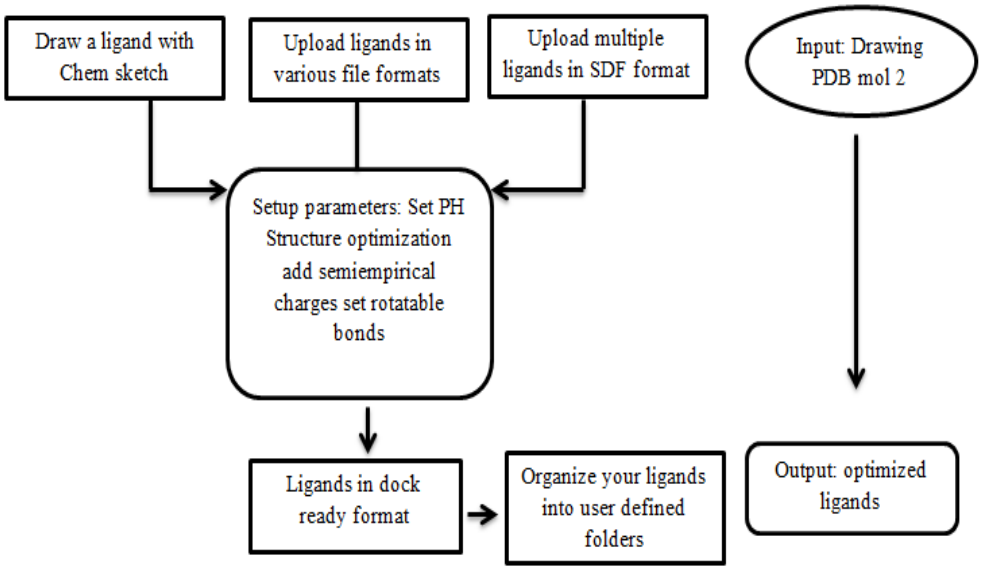


Figure.no.6: Preparation of ligands

Docking procedure

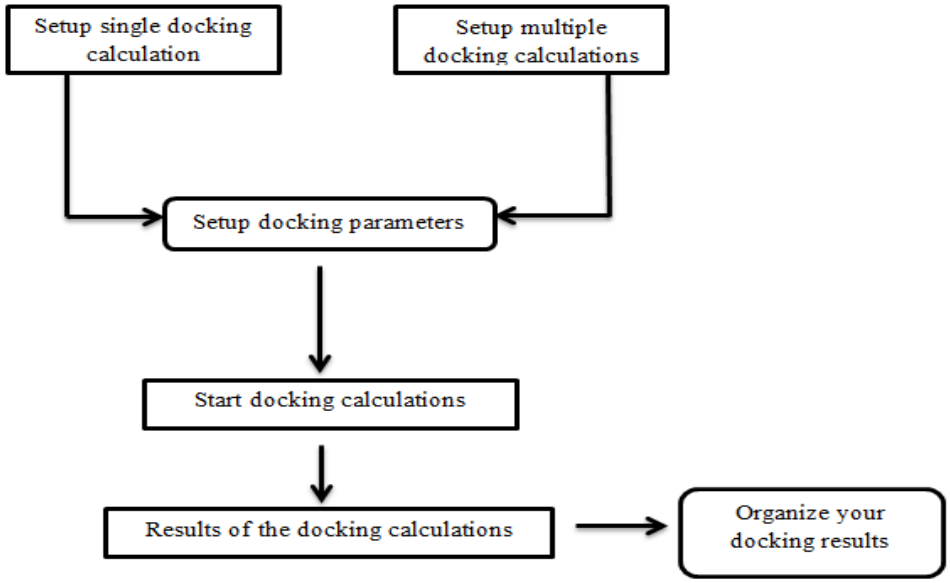


Figure.no.7: Docking calculations

I.C. TARGET PROFILE

PROPROTEIN CONVERTASES

Proprotein convertases are a family of enzymes involved in converting precursors of secretory proteins such as hormones, enzymes and receptors into bioactive molecules at their intended target tissue. These enzymes are part of regulatory pathways that help the body to maintain homeostasis.

PCSK9- PROPROTEIN CONVERTASE SUBTILISIN/KEXIN TYPE 9

PCSK9 (Proprotein convertase subtilisin/kexin type 9) was first described in 2003 in its active form, *PCSK9* regulates cell surface receptors, in particular the LDL receptor. The enzyme encoded by the *PCSK9* gene is primarily expressed in the liver.

3D structure of *PCSK9*

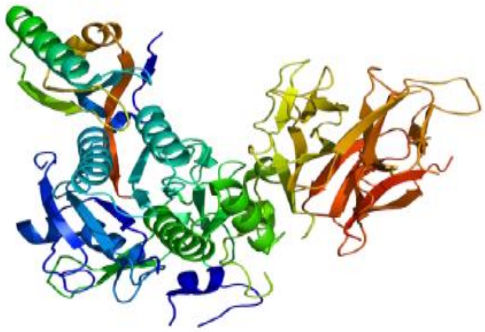


Figure.no.8:3D Structure of *PCSK9* protein

Biogenesis and structure of *PCSK9*

The human *PCSK9* gene, initially called ***'neural apoptosis-regulated convertase 1'*** (*NARC1*), was discovered by Seidah *et al*., who reported the identification of the ninth member of the mammalian *proprotein convertase* family located on chromosome 1p32.3. The *PCSK9* gene on the short arm of chromosome 1 is 25 kb long and contains 12 exons and 11 introns. *PCSK9* encodes an inactive glycoprotein (pre-*PCSK9*) with 692 amino acids comprising four major components: a signal sequence (1-30) and *N*-terminal prodomain (31-152), followed by a subtilisin-like catalytic domain (153-425) and a *C*-terminal domain (426-692, also called the V domain).

Once the signal peptide is cleaved from pre-*PCSK9* in the endoplasmic reticulum, pro-*PCSK9* (31-692) is formed and then converted to mature secretory *PCSK9* through autocatalytic cleavage of the prodomain between Gln152 and Ser153 in the Golgi apparatus. However, unlike the other *proprotein convertases*, the cleaved prodomain of *PCSK9* remains tightly associated with the catalytic domain, where it inhibits further catalytic activity.

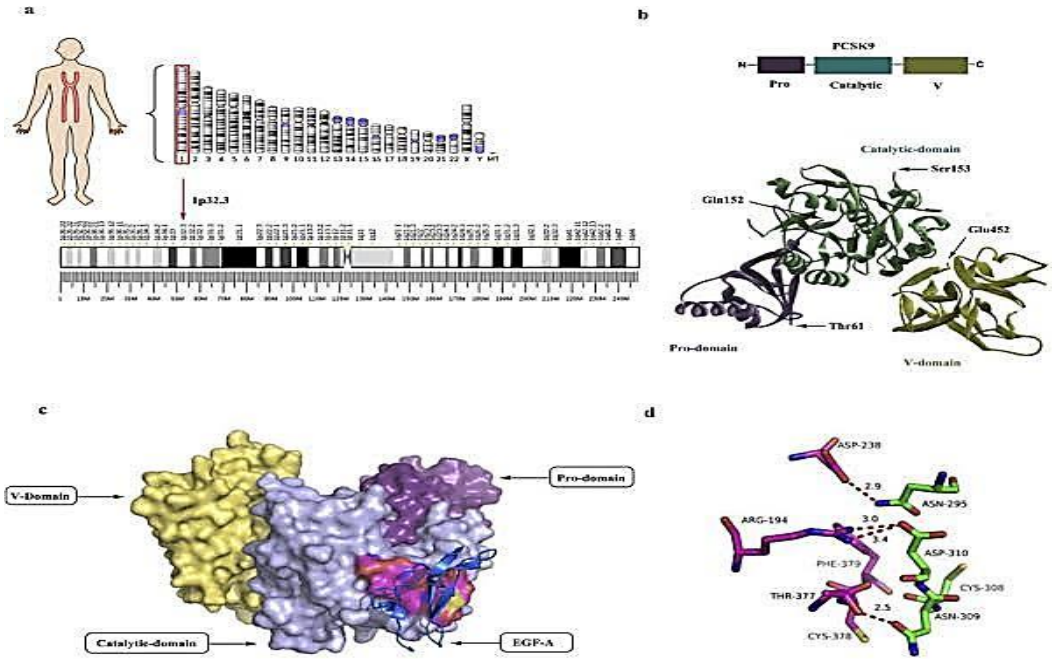


Figure.no .9: Gene location (human) and structure of *PCSK9*

PCSK9 binds to LDLRs on the cell surface, leading to their degradation. The binding site of *PCSK9* localizes to the EGF(A) (epidermal growth factor A) domain of the LDLR. The binding surface of *PCSK9* is formed primarily by residues 367-381, constructing an exposed, slightly convex ~500 Å 2 region. The key interactions of *PCSK9* with EGF(A) are made by an antiparallel β-sheet formed between residues 377-379 of *PCSK9* and 308-310 of EGF(A).

Physiological Role of *PCSK9*

One of the most important *PCSK9* functions is participation in LDLR expression regulation, leading to regulation of the LDL cholesterol level in the blood. Additionally, *PCSK9* carries out two quasi-independent functions: on the one hand, it decreases the LDLR density on hepatocytes surface, and on the other hand, it prevents the reverse capture of the newly synthesized VLDL, letting them reach the peripheral tissues. However, it should be

kept in mind that *PCSK9* is able to interact with other receptors and thus may possess a far broader spectrum of activity which has not been completely investigated to date.

Mechanism of Regulation of PCSK9-Mediated LDLR Expression in Hepatocytes

The clearance and catabolism of serum LDL occurs in the liver. These are the hepatocytes which are major regulators of the LDL level, expressing LDLRs which capture the LDLs and remove them from the blood plasma. The LDL/LDLR complex enters a hepatocyte within clathrin vesicles, gradually merging with the endosomes. The endosomal acidic medium activates a complex dissociation and the released LDLRs return to the hepatocyte surface, continuing their job of removing LDL from the serum.

It is known that the calcium-mediated interaction of the *PCSK9* catalytic domain with the A-EGFP repeat of LDL takes place on the hepatocytes surface. After the LDL binds to the LDLR, the LDL/LDLR/*PCSK9* complex relocates into the cell within the clathrin vesicle. The low-pH medium in the endosomes promotes the separation of the LDL from the complex and increases the *PCSK9*/LDLR binding due to additional ion–ionic interactions between the *PCSK9* prodomain and the LDLR.

As a result, the *PCSK9* holds the LDLR in the open conformation like a pillar, without acting proteolytically, and prevents the transition in the closed conformation that is necessary for the return to the cell surface, which leads to a decrease in receptor expression on the hepatocyte surface and, accordingly, a decrease in LDL clearance from the plasma. Consequently, *PCSK9* plays a very important role in the serum LDL cholesterol level regulation.

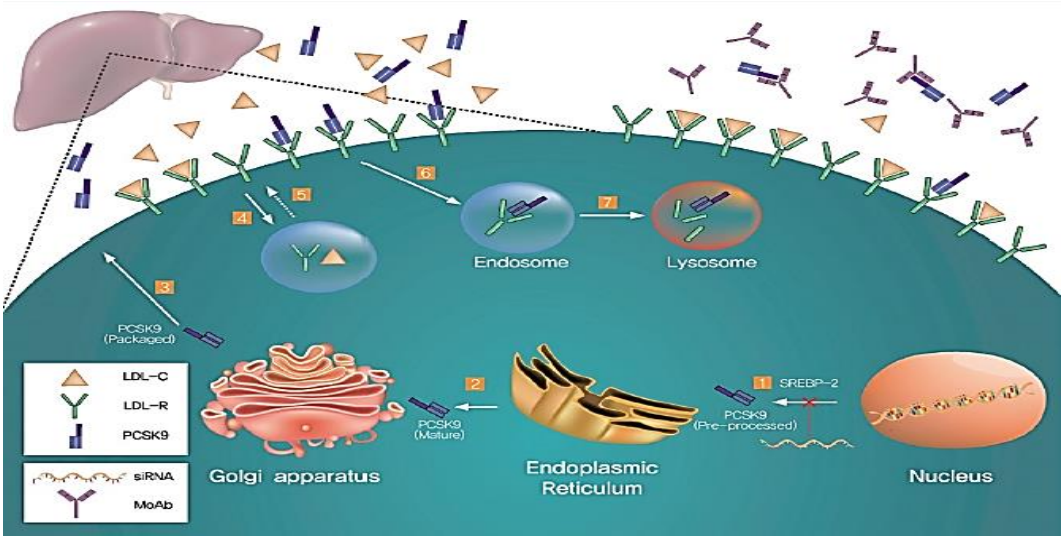


Figure.no.10: *PCSK9*-mediated regulation of LDLRs

TIMELINE OF DEVELOPMENTS IN THE HISTORY OF *PCSK9* INHIBITORS

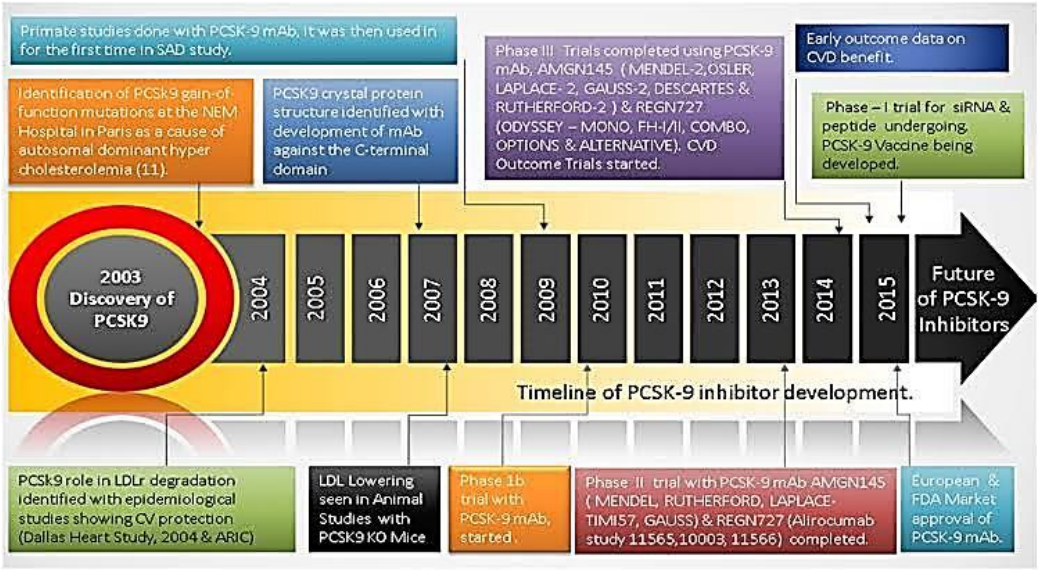
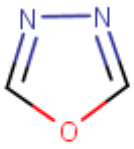


Figure.no.11: Timeline of *PCSK9* inhibitors development.

II. SYNTHETIC CHEMISTRY

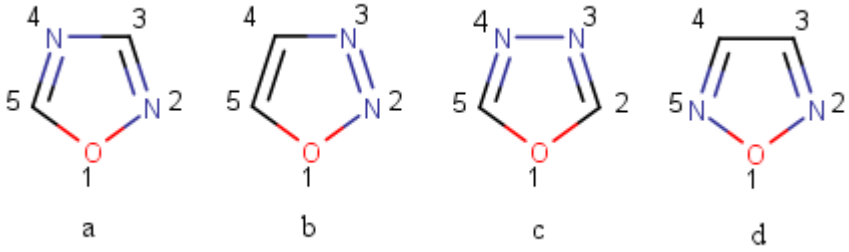
OXADIAZOLE

Oxadiazole is a five membered heterocycle which contain two carbons, two nitrogen, one oxygen and two double bonds having general formula $C_2H_2ON_2$. Oxadiazole is considered to be derived from furan by replacement of two methylene (-CH=) group by two pyridine type nitrogen (-N=)[24].



Oxadiazole

It undergoes number of reactions including electrophilic substitution, nucleophilic substitution, thermal and photochemical reaction. There are four possible isomers of oxadiazole (a,b,c,d) depending on the position of nitrogen atom in the ring and are numbered as shown below.

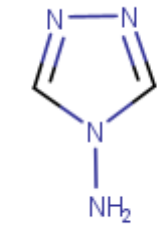


Isomers of Oxadiazole

Out of its four isomer 1,3,4- Oxadiazole is widely exploited for various applications. A wide variety of substituted 1,3, 4-oxadiazole have attracted attention in the field of drug discovery because of their wide range of pharmacological activities such as antibacterial, antifungal, analgesic, anti-inflammatory, antiviral, anticancer, hypolipidemic, anticonvulsant, and CNS stimulants properties[25-27].

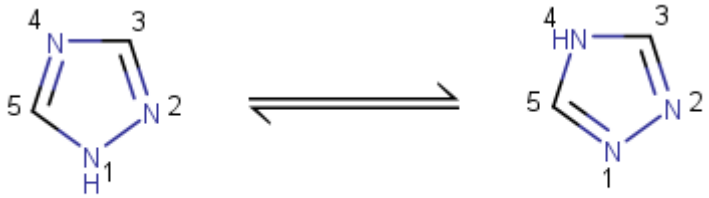
AMINO TRIAZOLE

Triazoles are five-membered rings, which contain two carbon and three nitrogen atoms, with a molecular formula of $C_2H_3N_3$.



Amino triazole

According to the position of nitrogen atoms, triazoles exist in two isomeric forms – 1,2,3-triazole and 1,2,4-triazole[28]. 1,2,4-triazoles and their fused heterocyclic derivatives are the primary heterocycles in the field of medicine. 1,2,4-triazole exist in two tautomeric forms.

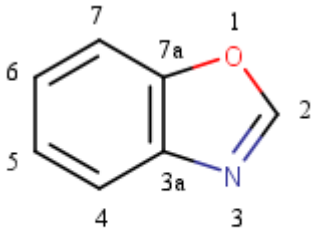


1-H tautomer **4-H tautomer**

It possesses various pharmacological activities such as anti-inflammatory, antifungal, antidiabetic, anticancer, antioxidant, antihyperlipidemic and anticonvulsant activities[29].

BENZOXAZOLE

Benzoxazole (1-oxa-3aza-1H indene) is an aromatic organic compound with a with a molecular formula C_7H_5NO, benzene fused oxazole ring structure[30].



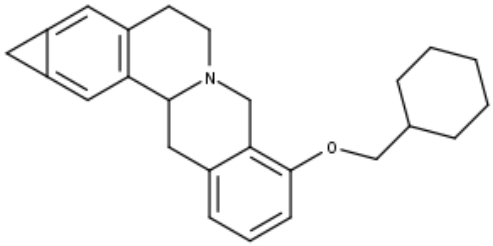
Benzoxazole

A slight change in the substitution pattern of benzoxazole nucleus causes distinguishable difference in their pharmacological activities. The substitution at second position in benzoxazole skeleton is influential for the biological activity of the molecule[31]. Benzoxazole derivatives possess diverse variety of pharmacological activities such as anticancer, anticonvulsant, antimicrobial, antiviral, antihyperglycemic, hypolipidemic, herbicidal, antiparasitic and anti-inflammatory activity[32].

III. LITERATURE REVIEW

LITERATURE REVIEWS RELATED TO HYPERLIPIDEMIA

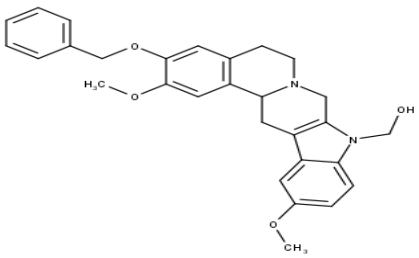
Haixia Ge *et al.*, (2021)[33] designed and performed biological evaluation of novel tetrahydroprotoberberine derivatives to reduce *SREBPs* expression for the treatment of hyperlipidemia. Results showed that two compounds significantly reduced both intracellular TC and TG content. Compound 49 displayed superior bioavailability and hence it was a promising candidate for the treatment of hyperlipidemia.



Compound 49

Hill M *et al.*, (2021)[34] briefly outlined the etiology, pathophysiology and treatment of hyperlipidemia condition.

Chenglin Wu *et al.*, (2019)[35] designed and synthesized series of novel tetrahydroprotoberberine derivatives as *Proprotein convertase subtilisin/kexin type 9(PCSK9)* modulators for the treatment of hyperlipidemia. Results showed that 8 compounds exhibited excellent activities in downregulating hepatic *PCSK9* expression than berberine. Among this compound 22 promoted hepatic LDLR expression in a dose dependent manner in HepG2 cells. Hence, compound 22 found to be a promising lead compound for the development of *PCSK9* modulator for the treatment of hyperlipidemia.



Compound 22

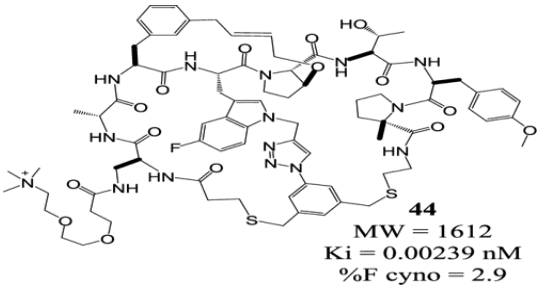
LITERATURE REVIEWS RELATED TO BIOLOGICAL TARGET *PCSK9*

Nikolay Kuzmich *et al.*, (2022)[36] reviewed *PCSK9* as a target for development of a new generation of hypolipidemic drugs. The prevention of its interaction with LDL receptors leads to an increase in the uptake of cholesterol rich atherogenic LDL from the blood stream. Hence low-molecular weight *PCSK9* inhibitors could be a worthy alternative to produce drug of minimal side effects.

Nabil A Elshourbagy *et al.*, (2022)[37] discovered and developed oral small molecule *PCSK9/LDLR* antagonist. *PCSK9* is a well validated target for the treatment of hypercholesterolemia. Virtual screening and SAR study was performed to identify the potent antagonist. Further *in vivo* studies carried out in the most potent nano formulation P-21 at 1,3,10 and 30mg/kg in C57BL/6 mice fed a high fat diet shows a 20, 40, 60 and 90% LDL-C lowering respectively.

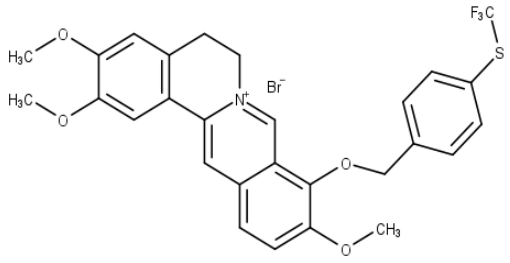
Munchalika Jaitrong *et al.*, (2021)[38] designed novel series of Berberine (BBB) derivatives based on molecular docking studies to serve as MTDLs for *PCSK9* and *HMGCR*. Results showed that all the designed compounds were identified as the potent multitarget inhibitors because the increase of hydrogen bond, hydrophobic and electrostatic interactions were observed. The introduction of nitro-group plays a vital role in the binding pose of the BBB derivative. Finally, *in silico* study confirmed that most of the compounds pass the drug likeliness properties.

***Thomas* J Tucker *et al.*, (2021)**[39] developed a series of novel, highly potent and orally bioavailable next generation Tricyclic peptide *PCSK9* inhibitors. Optimized molecules such as 44 demonstrated sufficient oral bioavailability to maintain therapeutic levels in rats and cynomolgus monkeys after dosing with an enabled formulation.



44
MW = 1612
Ki = 0.00239 nM
%F cyno = 2.9

Tian-Yun Fan *et al.*, (2021)[40] synthesized series of berberine derivatives and evaluated for their activities on down regulating the transcription of biological *PCSK9* in HepG2 cells, taking BBR as the lead. SAR analysis revealed that 2,3-dimethoxy moiety might be beneficial for activity. Among them, compound 9k showed increased LDLR expression and LDL-C clearance via down-regulating *PCSK9* protein. Therefore, 9k might have the potential to be a novel *PCSK9* transcription inhibitor for the treatment of Atherosclerosis, worthy for further investigation.



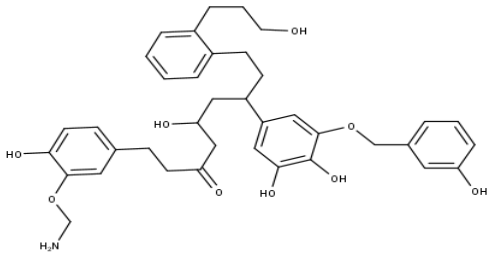
Compound 9k

Shengato Xu *et* al., (2019)[41] summarised the overview recent development of small molecules as inhibitors of *PCSK9*. Two *PCSK9* blocking monoclonal antibodies alirocumab and evolocumab were approved in 2015. However the high cost of *PCSK9* antibody drugs impede their prior authorization practices and reduced their long term adherence. Hence, different approaches have been pursued to modulate the functional activity of small molecules as inhibitors of *PCSK9.*

Johan Frostegard *et al.*, (2018)[42] identified that *Proprotein convertase subtilisin/kexin type 9(PCSK9)* inhibitors have unexpected anti-inflammatory effects. Researchers at karolinska instituted have examined how immune cell from human atherosclerotic plaques are effected by *Proprotein convertase subtilisin/kexin type 9 (PCSK9).*These dendritic cell then mediated the activation of T cell in to pro-inflammatory phenotype *Proprotein convertase subtilisin/kexin type 9(PCSK9)* inhibition reversed the effect of oxidized LDL on immune activity.

Niki Katsikii *et al.*, (2017)[43] identified that the *Proprotein convertase subtilisin/kexin type 9* that shaping the future for further cardiovascular outcomes research with *Proprotien convertase subtilisin/kexin type 9* inhibition in subjects with elevated risk by (FOURIER TRIAL). They concludes that *Proprotein convertase subtilisin/kexin type 9* inhibitors are now an evidence based option for patients who do not tolerate statins.

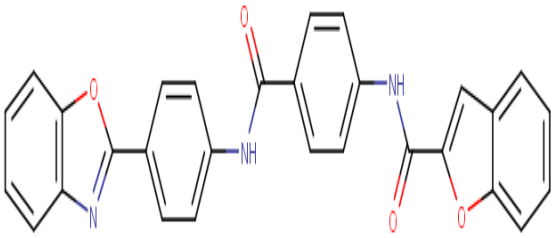
Vikas reddy *et al.*, (2016)[44] identified the potential inhibitors for lower cholesterol level by inhibiting *Proprotein convertase subtilisin/kexin type 9 (PCSK 9).* In these docking studies, they conclude that two compounds such as ZINC85625485 and ZINC85625406 may act as the potential inhibitors of *Proprotein convertase subtilisin/kexin type 9* receptor.



ZINC85625485

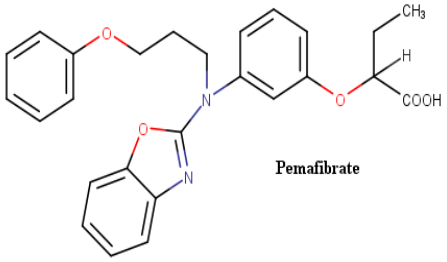
LITERATURE REVIEWS RELATED TO BASIC NUCLEUS

Xi Khai Wong *et al.*, (2020)[45] summarized the current developments of benzoxazole in drug discovery. Shifa biomedical corp. explored the possibility of benzoxazole based compounds in inhibiting *PCSK9*. Among all the compounds synthesized, the benzoxazole 73 is most potent with an IC_{50} value of 0.6µM. when the benzoxazole moiety is replaced with other functional groups, the potency was compromised. Hence it is inferred that the benzoxazole moiety play a vital role in providing good *PCSK9* inhibitory activity for an excellent LDL-c lowering effect.



Benzoxazole 73

Jean Charles Fruchart (2017)[46] outlined modulation of the unique receptor cofactor binding profile to identify the most potent molecule induce *PPAR-γ* mediated beneficial effects. A new therapeutic approach for development of pemafibrate, a novel selective *PPAR-γ* modulator. A major outcome study, PROMINENT will provide definitive evaluation of the role of pemfibroate for management of atherogenic dislipidemia.

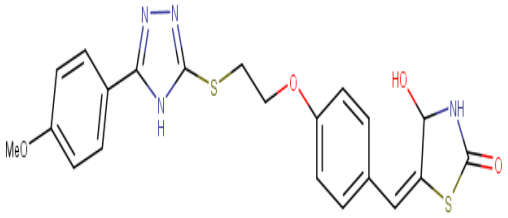


Pemafibrate

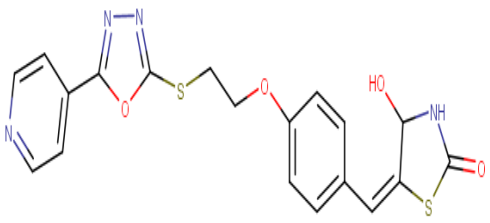
Wenbin Wang *et al.*, (2015)[47] designed a series of isoflavone amide with isoflavone in place of scaffold of 2- aryl benzoxazole of *CETP* inhibitors. Twelve new compounds were synthesized and their inhibitory activities were assayed. The results indicate that HY-2C exhibited favourable antihyperlipidemic activity.

Mayura Kale *et al.*, (2013)[48] summarized the biological potential of various heterocyclic scaffolds with antihyperlipidemic potential.

Ashraf Y. Khan *et al.*, (2012)[49] designed and synthesized series thiazolidinedione derivatives by incorporating pharmacological significant heterocycles like substituted thiazole, triazole and oxadiazole moieties. These heterocycles are linked to central phenyl ring via heteroatom linkage with one or two carbon spacer as the structure analogue of pioglitazone by employing multistep synthetic protocol. The synthesized compounds were screened for their in vivo hypolipidemic and hypoglycemic activity. Results showed that compound 10h, 11c and 11d significantly decreased serum TC level.



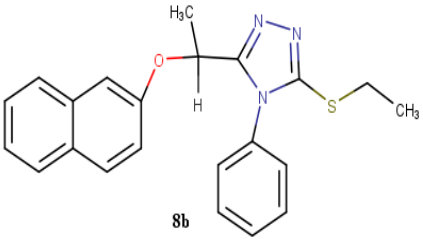
Compound 10h



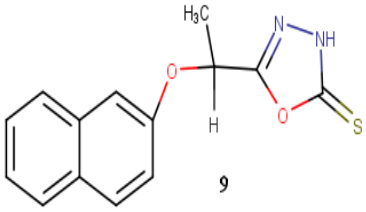
Compound 11c

Camerson J Smith *et al.*, (2010)[50] synthesized series of 2-arylbenzoxazole as inhibitor of *CETP*. SAR studies focused on variation of the benzoxazole moiety were found to be beneficial for *CETP* Inhibition. Substitution at 5th and 7th position of benzoxazole moiety was found to be beneficial for *CETP* inhibition. Compound 47 was found to be the most potent inhibitor in this series and inhibited *CETP* with an IC_{50} of 28 nm.

Gamal A. Idrees *et al.*, (2009)[51] synthesised series of 2-(naphthalene-2-yloxy)propionic acid derivatives and the hypolipidemic activity of the new compounds as well as the intermediate acid 2 was evaluated in the high cholesterol diet fed hyperlipidemic rat model. The results showed that the S-alkylated mercaptotriazole 8b and the 1,3,4-oxadiazole 9 produced striking reduction of serum levels of total cholesterol (TC) ,triglycerides (TGs), low density lipoproteins (LDLs) and elevation of serum high- density lipoprotein being more active than the reference gemfibrozil.



8b



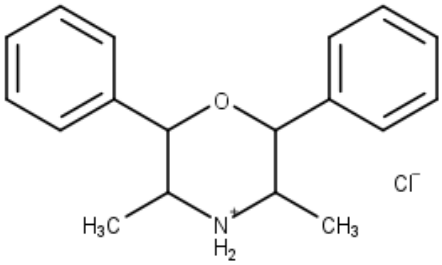
9

LITERATURE REVIEWS RELATED TO PHARMACOLOGICAL ACTIVITY

Anusha Govindula *et al.*, (2019)[52] investigated *in vivo* antihyperlipidemic activity of ethanolic extract of *Bauchinia accuminata* in Atherogenic diet induced rats. Preliminary photochemical screening showed the presence of flavonoids, alkaloids, steroids, terpenoids. Results showed that a significant reduction in the TC, VLDL, TG and increase in HDL level with ethanolic extract at a dose of 400 mg/kg then 200 mg/kg and the standard Atorvastain.

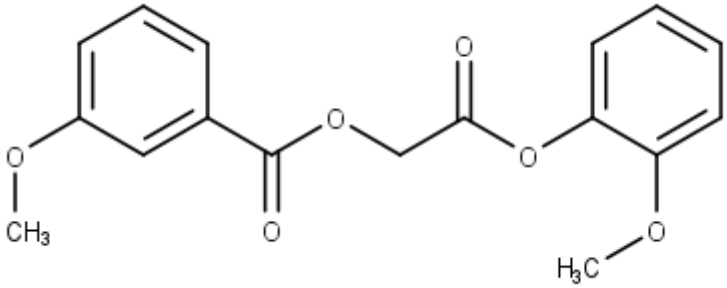
Prabhu Mahendra *et al.*, (2019)[53] synthesized some substituted morpholine derivatives and evaluated it's *in vivo* antihyperlipidemic activity. *In silico* molecular docking studies for the synthesised Morpholine derivatives with *PPAR-α* protein showed the energy level ranging from -6.74kcal/mol to -8.83kcal/mol. the compound **1C** which shows least binding energy taken for further *in vivo* studies. Morpholine derivatives shows significant reduction in TC

and TG in blood compared to the standard drug. Hence the morpholine compounds exhibit significance towards the anorectic activity.



1C

Muhammad Tahir Aeel *et al.*, (2018)[54] synthesized series of phenolic derivatives 4a-e and 6a-e with the aim of developing antihyperlipidemic agents. *In silico* docking studies revealed that compound 4a and 6a exhibited maximum binding affinity. *In vivo* studies showed that compound 4a as more hypolipidemic than the Atorvastatin against hyperlipidimic model.



4a

Safia A K *et al.*, (2013)[55] studied the antioxidant, hyperlipidemic and anti-obesity effect of *Mommordica diocia Roxb* fruit extracts. The results suggest that the MDR extract shows hypolipidemic and anti-obesity activity. Methanolic extract showed more potential than FMD because of high phenolic and flavonoid content.

LITERATURE REVIEWS RELATED TO COMPUTER AIDED DRUG DESIGN

Rida Zainab *et al.*, (2021)[56] described finding inhibitors for *PCSK9* using computational methods. The protein ligand interaction helps to understand the actual mechanism for the pharmacological action. The result showed that (S) - candine may act as a potential inhibitor against atherosclerosis for the development of new *PCSK9* inhibitory drugs in future *in vivo* research.

Fernando, D.Prielo Martinez (2019)[57] outlined the importance of the computational drug design methods in the Drug Discovery process.

Praveen K Guttula *et al*., (2017)[58] performed molecular docking studies on selected phytocompounds against *PCSK9*-LDL receptors (homosapiens) for coronary artery disease. The docking studies performed for Resveratrol, Ellagic acid, quercetin with target protein using *Autodock* docking software and the results showed that Ellagic acid compound have greater inhibition of enzyme activity compare to Resveratrol and Quercetin.

IV. AIM AND OBJECTIVE

AIM

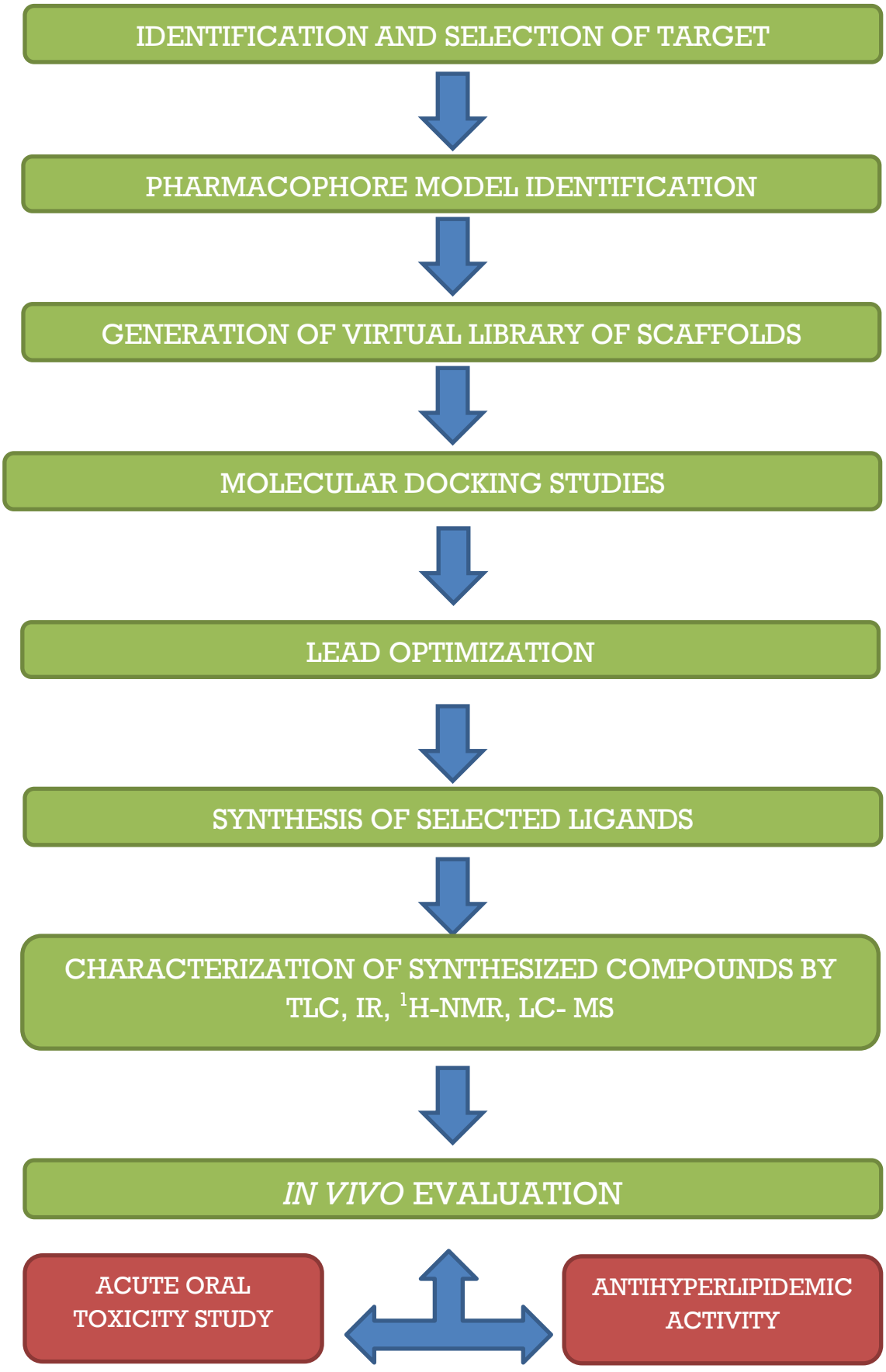
The main aim of the study is to identify, design, and synthesize certain newer heterocycles as potent antihyperlipidemic agents with good predicted capability to inhibit the *PCSK9* involving Computational drug designing methods.

OBJECTIVE OF WORK

The plan of work includes the following steps

- Selection of target responsible for producing hyperlipidemia from the literature review which is carried out as part of the current research.
- Identification of common pharmacophoric features responsible for inhibiting *Proprotein Convertase Substilsin/Kexin type 9.*
- Designing series of leads that selectively modulate the activities of *Proprotein Convertase Substilsin/Kexin type 9* for exhibiting antihyperlipidemic activity.
- The binding mechanism of *PCSK9* receptor and newly designed leads has to be studied using molecular docking with *Autodock4.2.6* (PDB ID: 2P4E).
- Optimization of designed leads based on the Drug likeliness, ADMET and Toxicity prediction using *Molinspiration, Osiris software.*
- Synthesis of certain optimized leads based on the synthetic feasibility and high dock score.
- Characterization of chemical nature of the synthesized compounds by IR, ^{1}H-NMR, and LC-MS.
- *In vivo* Pharmacological screening studies of the synthesized compounds.
    - Evaluation of acute oral toxicity studies.
    - Evaluation of Antihyperlipidemic activity.

PLAN OF WORK


IDENTIFICATION AND SELECTION OF TARGET
PHARMACOPHORE MODEL IDENTIFICATION
GENERATION OF VIRTUAL LIBRARY OF SCAFFOLDS
MOLECULAR DOCKING STUDIES
LEAD OPTIMIZATION
SYNTHESIS OF SELECTED LIGANDS
CHARACTERIZATION OF SYNTHESIZED COMPOUNDS BY TLC, IR, 1H-NMR, LC- MS
IN VIVO EVALUATION
ACUTE ORAL TOXICITY STUDY
ANTIHYPERLIPIDEMIC ACTIVITY

V.A. DRUG DESIGN

V.A.I. MATERIALS AND METHODS

1. SELECTION OF TARGET

Protein Data Bank (PDB) is a crystallographic database for three-dimensional structural data of large biological molecules, such as Proteins, Nucleic acid and Complex assemblies. The targets creating the greatest enthusiasm at this time for the treatment of Hyperlipidemia includes *HMG Co-A reductase, ATP Citrate lyase, Apolipoprotein B, PCSK9, Angiopoietin-like 3(ANGPTL3), Sterol regulatory element binding protein(SSEBP)* and *PPAR*-α activators[36]. Ultimately human trials will help to understand the potential risks and benefits of these novel approaches across a number of diseases.

An elaborate literature survey was done to understand the concepts and facts behind the hyperlipidemia diseases. Based on the gained knowledge ***Proprotein convertase substilsin/kexin 9(PCSK9)*** was chosen as the target for hyperlipidemia treatment. *PCSK9* has medical importance because it acts in lipoprotein homeostasis. Agents that block *PCSK9* can lower the LDL-C particle concentration. Hence, *PCSK9* is considered as an attractive and most effective target in the field of discovering newer antihyperlipidemic drugs.

Some of the efficient PDB enzyme targets were selected with lower resolution and the results are illustrated in Table.no:2, from which the highlighted best PDB target **(2P4E)** was employed in this study.

Table.no.2: List of PDB for *PCSK9* target for Hyperlipidemia

S.NO	CODE	RESOLUTION (A^O)
1	4NMX	1.85
2	2QTW	1.90
3	***2P4E***	**1.98**
4	4LKC	2.20
5	2PMW	2.30
6	3H42	2.30
7	2W2N	2.30
8	5OCA	2.30
9	2W2Q	2.33
10	2W2M	2.40
11	3BPS	2.41
12	4NE9	2.60

13	2W2P	2.62
14	2W2O	2.62
15	4OV6	2.60

2. PHARMACOPHORE MODELING[59]

a. Pharmacophore identification

A Pharmacophore is defined as "a set of structural features in a molecule that is recognized at a receptor site and is responsible for that molecule's biological activity". Pharmacophore modeling correlates the biological activity with the spatial arrangement of various features in set of active analogues.

When reviewing the efficient journals and research articles, pharmacophore model consisting of one hydrogen bond acceptor (HBA), one hydrogen bond donor (HBD), aromatic ring features was identified as the best model for designing as *PCSK9* inhibitor[44]. Hence the above best pharmacophoric model was used as 3D structural query to screen the chemical databases for retrieving new potent *PCSK9* inhibition.

b. **Database screening**

Scaffold hopping, or chemo type switching, is a technology that modifies the chemical scaffold of a bioactive compound retaining the activity and key interaction points, or the interacting molecular fragments of the parent compound.

Based on the above quoted literature facts in designing potent *PCSK9* inhibitors, the target screening library was designed by using molecular fragments from a relatively narrow and low molecular weight, selected diversity at both the putative "scaffold" core. The analogue library was generated by modifying the respective functional groups with sterically and conformationally allowed substituents using the reagent database and a combinatorial design model.

Construction of a Large Virtual Scaffold Library

A library consisting of nearly new150 lead molecules as potent *PCSK9* inhibitors was generated based on the knowledge of binding interaction of ligand with the protein and also the common features necessary for the biological activity of molecule. The chemical features like one hydrogen bond acceptor (HBA), one hydrogen bond donor (HBD), one aromatic ring

features were used to screen knowledge database (Table.no:3). Virtual scaffold library consisting of newly designed 150 molecules each as *Proprotein convertase subtilisin/kexin type 9 (PCSK9)* inhibitor was constructed as depicted in the Figure.no:12

Table.no:3 Molecular fragments used in construction of library of *PCSK9* inhibitor

HBD	HBA	AROMATIC RING
Imidazole, Thiadiazole, Benzimidazole, Aminothiazole, Phenolic-OH, Aniline, Alkyl amines, Oxazole.	C-O-C of Oxadiazole, C=O of aliphatic and aromatic amides, C=O of aromatic ketones, C=O of diamide.	Phenol, Pyrrole, Pyridine, Indole,Quinoline, Benzimidazole, Benzthiazole, Thiadiazole, Pyrazole.

2. MOLECULAR DOCKING STUDY[38]

In the current molecular simulation study, *Autodock 4.2.6* software was used for the prediction of binding energy of the ligands with *PCSK9.*

a. Preparation of target protein

The three-dimensional structure of *Proprotein convertase substilsin/kexin type9* (*PCSK9)* was acquired from the Protein Data Bank as PDB format *(*PDB ID:2P4E-Homosapien, Resolution 1.8 Å) (www.rcsb.org/pdb).Co-crystalized ligands, cofactors and water molecules were removed from the crystal structure using *Molegro Molecular viewer*. The two chains (P, A) of the target enzyme were exported from the *Molegro Molecular viewer* in .pdb format and saved as protein.pdb in a repository work folder – destination folder.

b. Ligands preparation

The two-dimensional (2D) chemical structures of the ligand molecules were sketched by using *ChemDraw Ultra 12.*0 and saved in MDL Mol format. The energy minimizations of the ligands were carried out with *Chem3D Pro 12.0.* Energy minimized ligand molecules were saved as ligand.pdb in the same repository workfolder.

c. Docking studies

Steps involved in docking

i.Preparation of protein:

- File > Read molecule > Select Protein file in .pdb format
- Edit > Charges > Compute Gasteiger (for arbitrary molecules)
- Edit >Hydrogens> Add > Polar only
- Edit >Hydrogens> Merge Non-polar
- Edit >Misc> Repair Missing Atoms
- File > Save > Write PDB > Sort Nodes(Check)

ii. Preparation of Ligand

- Ligand > Input > Open > Select Ligand file in pdb format
- Edit > Charges > Add Kollman charges
- Ligand > Torsion > Choose torsion [Rotatable bonds are shown in green, and non-rotatable bonds are shown in red. Bonds that are potentially rotatable but treated as rigid, such as amide bonds and bonds that are made rigid by the user, are shown in magenta].
- Ligand > Torsion > Set number of torsions
- Ligand > Output > Save as .pdbqt

iii. Grid generation:

- Grid > Macromolecule > choose Protein > Select molecule > Save as .pdbqt
- Grid > Set map types > choose Ligand > Select ligand
- Grid > Grid Box > Set the Grid Box dimensions (i.e., XYZ co-ordinates100 x 100 x 100) > File > Close Saving current
- Grid > Output > Save as grid.gpf (Grid Parameter file)

iv. Preparation of Docking Parameters:

- Docking > Macromolecule > Set Rigid Filename > Select protein.pdbqt> Open
- Docking > Ligand > Select Ligand > Accept
- Docking > Search Parameters > Genetic Algorithm
- Docking > Output > Lamarckian GA > Save as dock.dpf (Dock Parameter File)

  - ✓ **autogrid4.exe –p grid.gpf–l grid.glg**(wait for the response) and
  - ✓ **autodock4.exe –p dock.dpf–l dock.dlg.**

v. Visualization / Interpretation of Docking

- Analyze> Docking >Select dock.dlg
- Analyze> Macromolecule > Select Protein
- Analyze> Conformations > Play ranked by energy
- Set Play options > Build H-Bonds > Show info > Build Current > Write complex > Save as result.pdb.
- Analysis > Docking > Show interaction (Specialized visualization which highlights the interactions between the docked conformation of the ligand with the receptor)

The Binding energy and the inhibitor constant values were predicted by performing the docking studies. The binding scores of ligands were tabulated in Table.no.4. then the result.pdb file was viewed in the *Molegro Molecular viewer*. Electrostatic, hydrogen bond and steric interactions between the various amino acids in the target protein and ligands were observed. These interactions are depicted in Figure.no.13.

4. *IN SILICO* DRUG LIKELINESS SCREENING

Drug likeness is a qualitative concept indicated by the molecular properties that affect absorption, distribution, metabolism, excretion and toxicity (ADMET) of a compound. Drug likeliness is described as a complex balance of various molecular properties and structural feature which determine whether particular molecule is similar to the known drugs[60]. These properties are mainly hydrophobicity, electronic distribution, hydrogen bonding characteristics, molecule size and flexibility and also presence of various pharmacophoric features that influence the behaviour of molecule in a living organism including bioavailability, affinity to proteins and many others[61].

Drug likeliness properties of the newly designed *PCSK9* inhibitors was determined by employing different Online softwares like *Molinspiration and Osiris property explorer.*

a. *Lipinski's* Rule of Five

Lipinski's rule of five is a rule of thumb to evaluate drug likeliness, or to determine if a chemical compound with a certain biological activity. The rule was formulated by Christopher A. Lipinski in 1997, based on the observation that most medication drugs are relatively small and lipophilic molecules.

Lipinski's rule says that, in general, an orally active drug has no more than one violation of the following criteria,

- Log P value should be less than 5
- Hydrogen bond donor less than 5
- Hydrogen bond acceptor less than 10
- Molecular weight under 500 Daltons and
- Not more than 10 rotatable bonds.

Molinspiration online software tool

Molinspiration is an online tool used to evaluate the *in silico* pharmacokinetic properties of ligands based on the Lipinski rule of five. It was employed to predict the molecular properties like Molecular weight, Log P, Total Polar Surface Area, number of Hydrogen Bond Donors & Acceptors, number of atoms, number of rotatable bonds etc. and also predict bioactivity score of ligands

The molecular structure of the designed ligands was drawn using online *Molinspiration software* (www.molinspiration.com)for calculation of molecular properties. The calculated value of the drug likeliness score and various parameters of all designed ligands were given in Table.no.6 and the bioactivity scores are shown in Figure.no.14.

b. ADMET Properties

Osiris Property explorer

Osiris Property explorer is an online cheminformatics tool employed to determine toxicity potential of designed molecular compounds. The virtual toxicity results are colour coded either in green or red.

- Toxicity properties shown in green indicates the molecule are safe and non-toxic
- Toxicity properties shown in red indicate the molecule are toxic and have undesired effects (Tumorogenicity, Mutagenicity, Irritant effect and Reproductive effect).

The *in silico* toxicity of the designed compounds were determined by sketching the structures in online tool and the results are illustrated in Figure.no.15.

http://www.cheminfo.org/Chemistry/Cheminformatics/Property_explorer/index.html#

V.A. II. RESULTS AND DISCUSSION

2. Virtual Scaffold Library of newly designed ligands as *PCSK9* Inhibitors

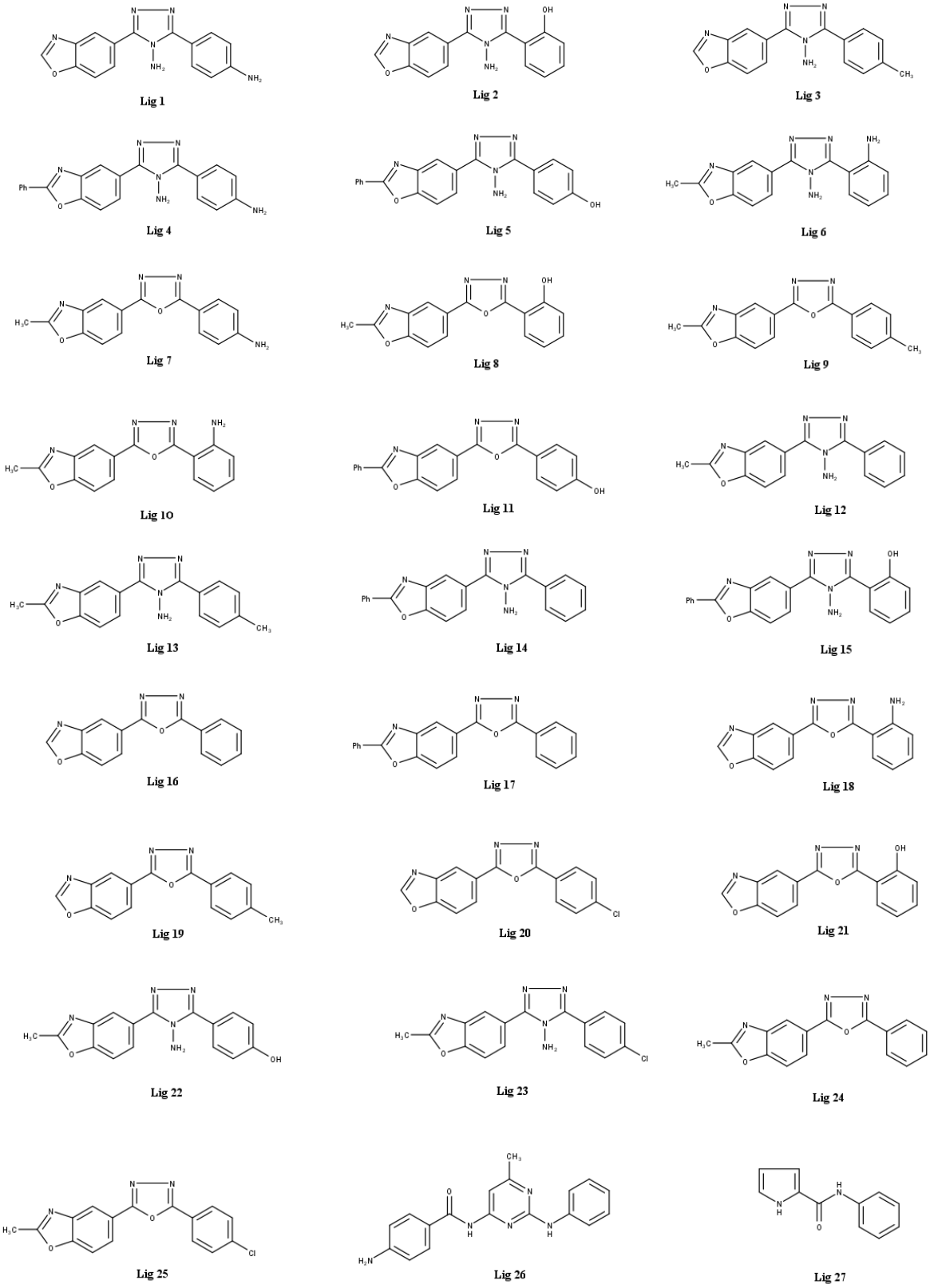


Figure.no.12:2D structure of newly designed ligands

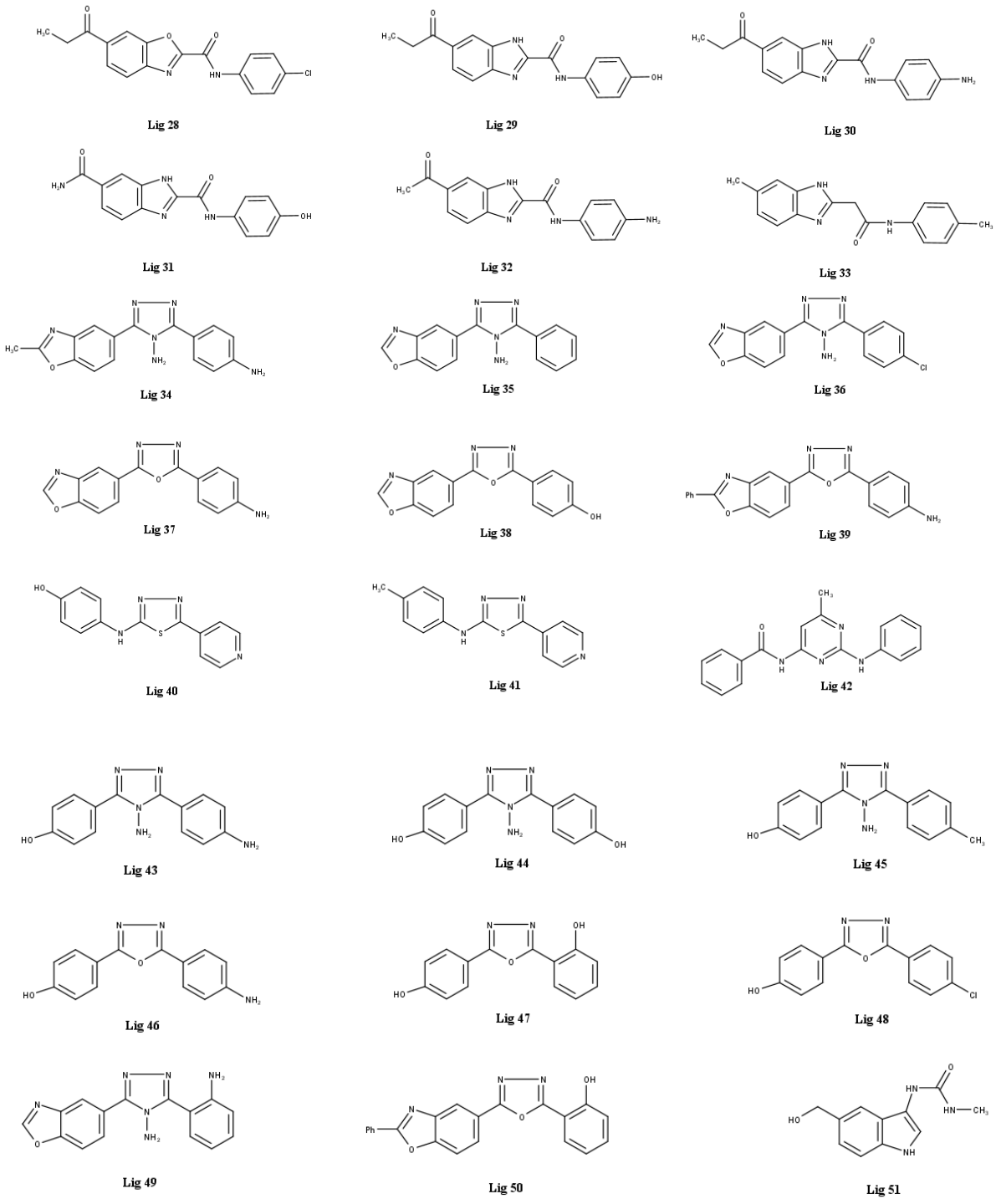


Figure.no.12:2D structure of newly designed ligands

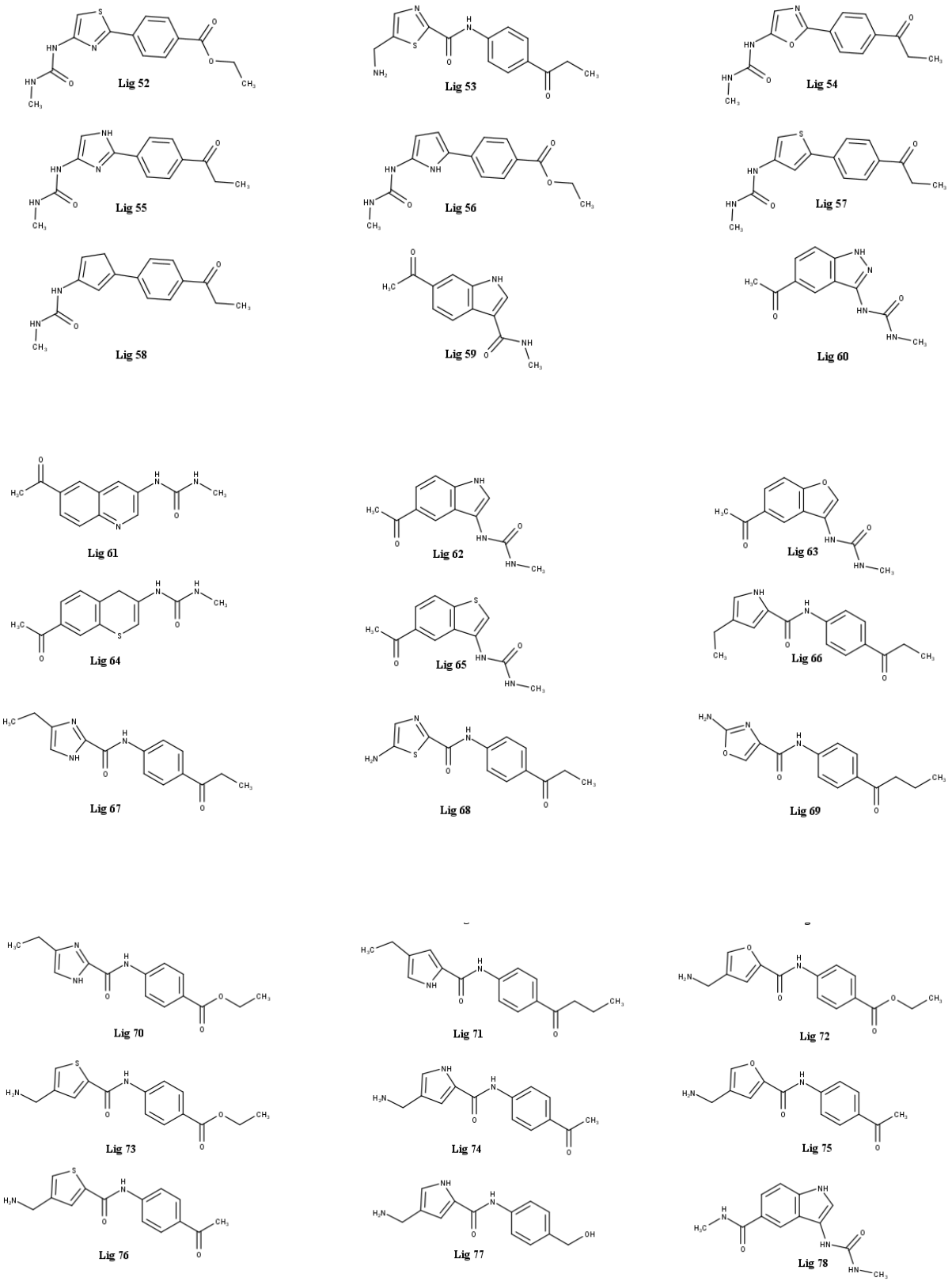


Figure.no.12:2D structure of newly designed ligands

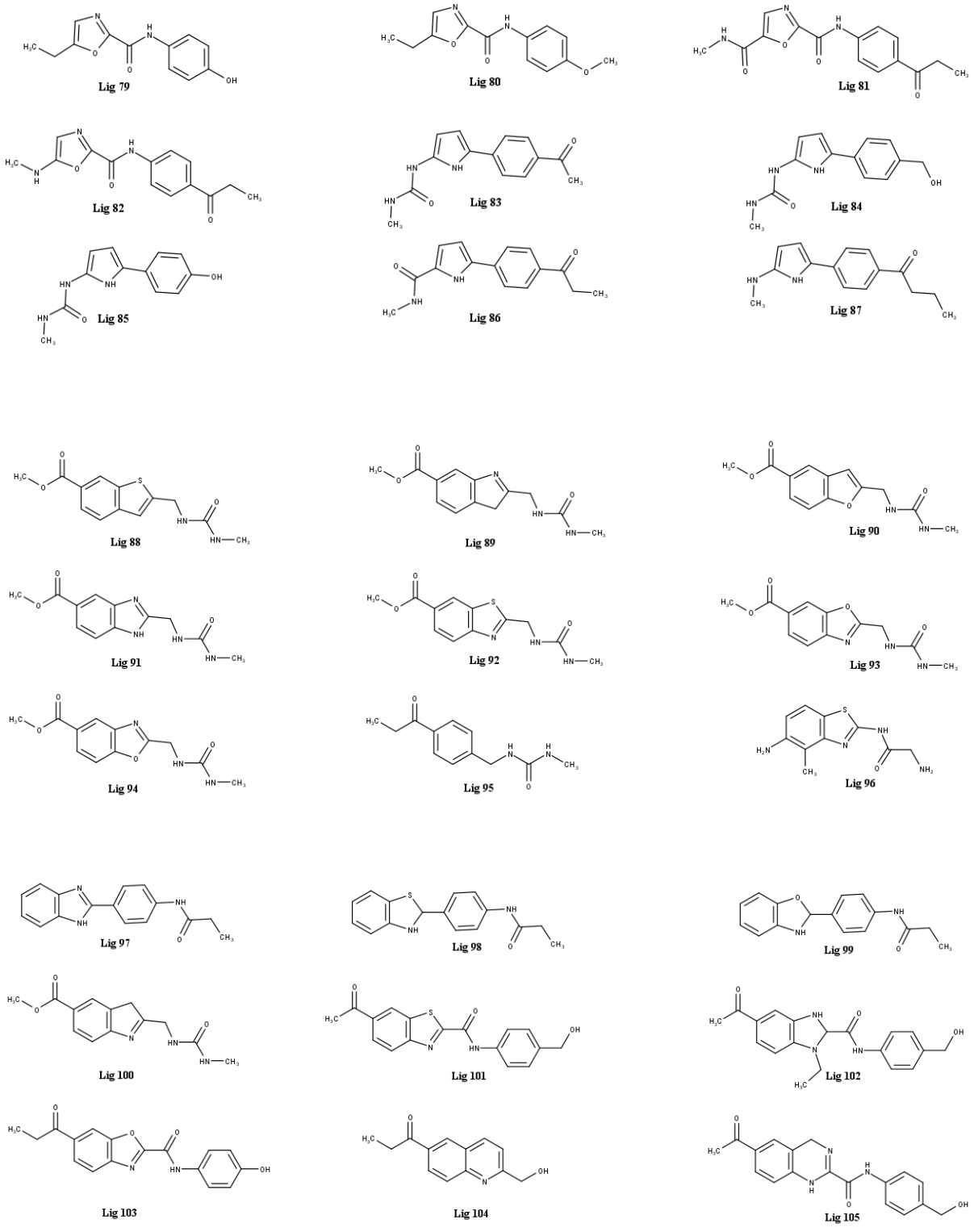


Figure.no.12:2D structure of newly designed ligands

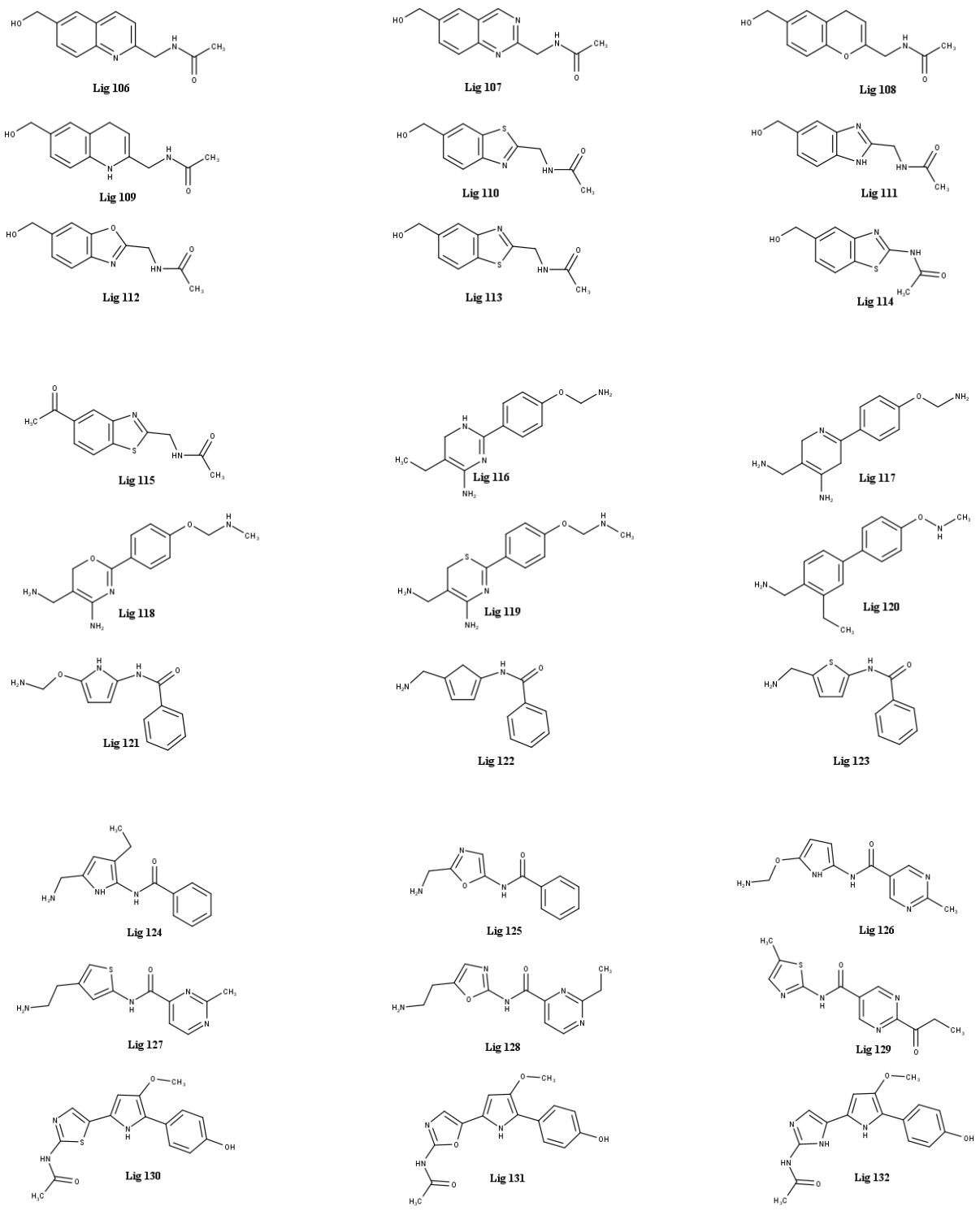


Figure.no.12: 2D structure of newly designed ligands

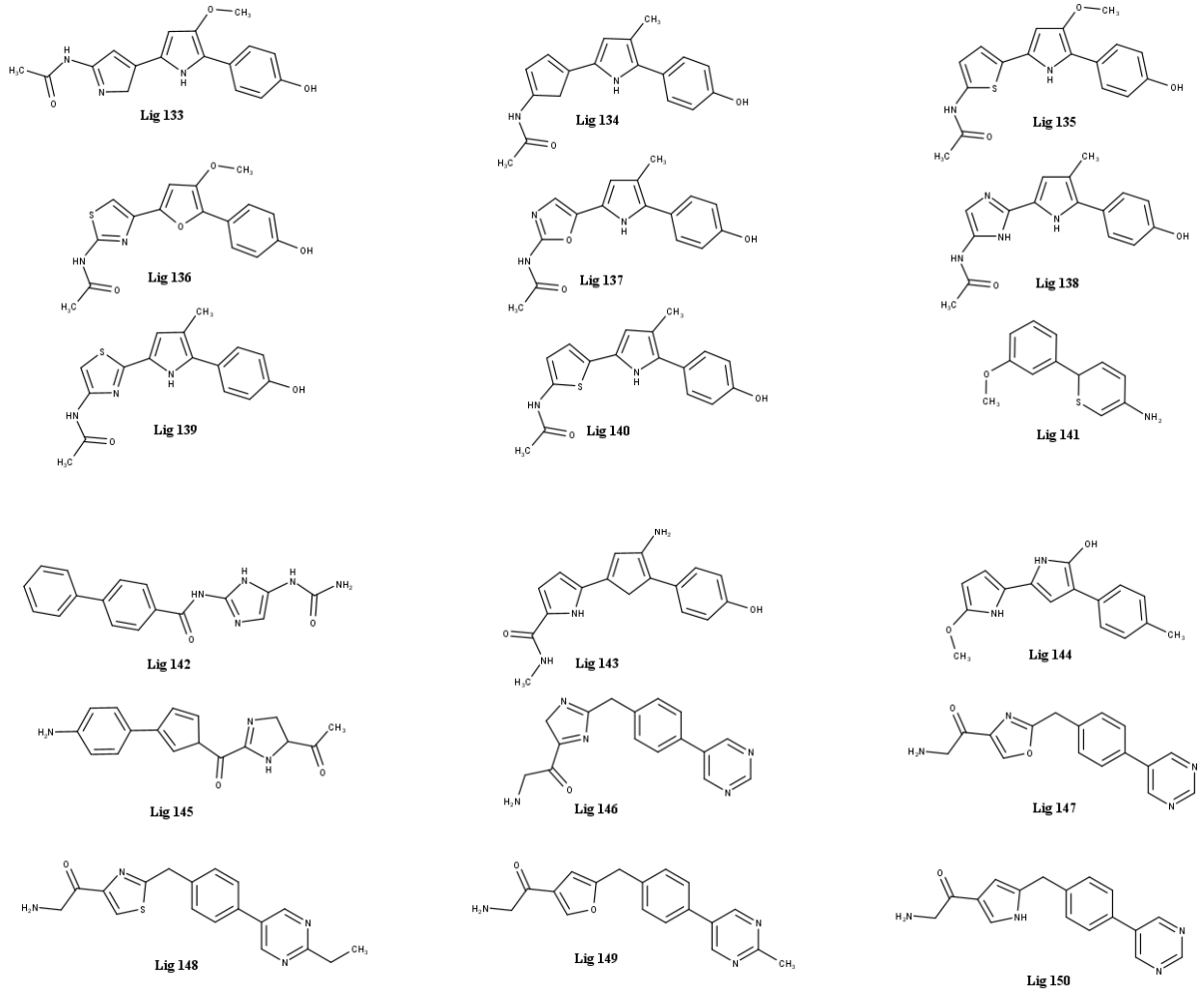


Figure.no.12: 2D structure of newly designed leads

3. DOCKING STUDIES

Docking studies was performed on all the newly designed 150 *PCSK9* inhibitors retrieved from virtual screening using *Autodock4.2.6* to identify which ligand interacts better with the target protein. The bonding interactions of the selected molecules at the active sites of *PCSK9* inhibitors was also determined. Results of predicted activity of all the designed ligands with fitness score are given below in Table.no.4.

Table.no.4: Docking score for designed ligands

S.NO	LIGAND	DOCKING SCORE (kcal/mol)
1	Lig 1	-8.48.
2	Lig 2	-8.73
3	Lig 3	-9.12
4	Lig 4	-8.05
5	Lig 5	-8.86
6	Lig6	-8.26
7	Lig 7	-10.24
8	Lig 8	-8.53
9	Lig 9	-8.43
10	Lig 10	-8.98
11	Lig 11	-7.98
12	Lig 12	-8.78
13	Lig 13	-8.01

14	Lig 14	-8.23
15	Lig 15	-10.96
16	Lig 16	-9.04
17	Lig 17	-9.58
18	Lig 18	-9.52
19	Lig 19	-8.61
20	Lig 20	-8.54
21	Lig 21	-9.64
22	Lig 22	-8.74
23	Lig 23	-8.61
24	Lig 24	-8.28
25	Lig 25	-9.34
26	Lig 26	-7.78
27	Lig 27	-6.75
28	Lig 28	-8.8
29	Lig 29	-7.49
30	Lig 30	-9.15
31	Lig 31	-8.88
32	Lig32	-8.96

33	Lig 33	-8.53
34	Lig 34	-10.24
35	Lig 35	-9.37
36	Lig 36	-9.06
37	Lig 37	-8.49
38	Lig 38	-8.36
39	Lig 39	-9.06
40	Lig 40	-7.25
41	Lig41	-8.18
42	Lig42	-7.26
43	Lig43	-6,68
44	Lig44	-8.77
45	Lig45	-8,42
46	Lig 46	-8.34
47	Lig 47	-7.66
48	Lig 48	-8.18
49	Lig 49	-10.05
50	Lig 50	-10.19
51	Lig 51	-8.25

52	Lig 52	-8.59
53	Lig 53	-8.14
54	Lig 54	-8.80
55	Lig 55	-8.88
56	Lig 56	-9.16
57	Lig 57	-9.16
58	Lig 59	-5.8
59	Lig 59	-8.25
60	Lig 60	-8.29
61	Lig 61	-7.95
62	Lig 62	-8.83
63	Lig 63	-6.17
64	Lig 64	-8.68
65	Lig 65	-9.30
66	Lig 66	-8.04
67	Lig 67	-5.39
68	Lig 68	-8.23
69	Lig 69	-8.67
70	Lig 70	-7.74

71	Lig 71	-8.18
72	Lig 72	-8.58
73	Lig 73	-6.80
74	Lig 74	-7.90
75	Lig 75	-7.73
76	Lig 76	-9.05
77	Lig 77	-8.23
78	Lig 78	-8.72
79	Lig 79	-7.87
80	Lig 80	-7.42
81	Lig 81	-8.94
82	Lig 82	-7.75
83	Lig 83	-8.37
84	Lig 84	-7.01
85	Lig 85	-7.44
86	Lig 86	-8.72
87	Lig 87	-7.68
88	Lig 88	-9.49
89	Lig 89	-8.16

90	Lig 90	-8.35
91	Lig 91	-9.79
92	Lig 92	-8.23
93	Lig 93	-9.42
94	Lig 94	-8.93
95	Lig 95	-7.99
96	Lig 96	-8.79
97	Lig 97	-9.17
98	Lig 98	-8.87
99	Lig 99	-8.20
100	Lig 100	-8.59
101	Lig 101	-9.54
102	Lig 102	-8.79
103	Lig 103	-10.37
104	Lig 104	-7.26
105	Lig105	-9.18
106	Lig 106	-7.54
107	Lig 107	-7.82
108	Lig 108	-8.11

109	Lig 109	-8.14
110	Lig 110	-7.73
111	Lig 111	-8.43
112	Lig 112	-7.90
113	Lig 113	-7.07
114	Lig 114	-7.59
115	Lig 115	-7.60
116	Lig 116	-8.04
117	Lig 117	-7.21
118	Lig 118	-8.19
119	Lig 119	-8.74
120	Lig 120	-8.15
121	Lig 121	-6.90
122	Lig 122	-7.52
123	Lig 123	-7.41
124	Lig 124	-8.42
125	Lig 125	-8.19
126	Lig 126	-8.03
127	Lig 127	-8.30

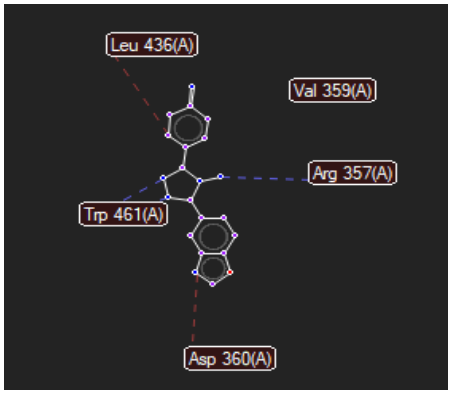
128	Lig 128	-7.95
129	Lig 129	-8.03
130	Lig 130	-9.15
131	Lig 131	-9.67
132	Lig 132	-6.10
133	Lig 133	-6.69
134	Lig 134	-8.06
135	Lig 135	-7.44
136	Lig 136	-7.01
137	Lig 137	-9.76
138	Lig 138	-6.80
139	Lig 139	-8.44
140	Lig 140	-6.96
141	Lig 141	-6.32
142	Lig 142	-6.88
143	Lig 143	-9.04
144	Lig 144	-8.33
145	Lig 145	-8.87
146	Lig 146	-7.80

147	Lig 147	-8.15
148	Lig 148	-8.19
149	Lig 149	-9.67
150	Lig 150	-9.36

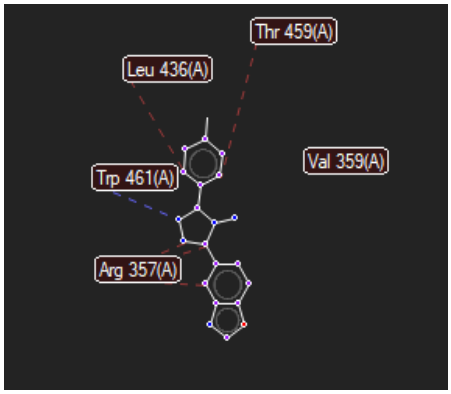
Based on the docking results, all the 150 newly designed ligands were categorized as highly active, moderately active and low active hits.

Table 5: Docking results of *PCSK9* inhibitors using *Autodock4.2.6*

Receptor	**Highly active (>10)**	**Moderately active (8-10)**	**Low active (<8)**
PCSK9 Inhibitors	Lig7, Lig15,Lig34, Lig49,Lig73	Lig1-6, Lig8, Lig10 Lig12, Lig13, Lig14,Lig16-25,Lig28,Lig30-33 Lig35-39,Lig41,Lig44-46,Lig48,Lig50,Lig59,Lig60, Lig62,Lig64-66,Lig68, Lig71,Lig72, Lig76-78, Lig81, Lig83, Lig84, Lig86, Lig88-94, Lig96-102, Lig105, Lig108, Lig109, Lig111, Lig116, Lig118-120, Lig124-127, Lig129, Lig131, Lig134-135, Lig139, Lig143-145, Lig147-150.	Lig26-27,Lig29,Lig40,Lig42-43,Lig47,Lig58, Lig61, Lig63, Lig67, Lig70, Lig73-75, Lig79, Lig80, Lig82, Lig85, Lig87, Lig95, Lig104, Lig106, Lig107, Lig110, Lig112-115, Lig117, Lig121-123, Lig128, Lig132, Lig133, Lig135-137, Lig140, Lig141, Lig142, Lig146



Lig 1



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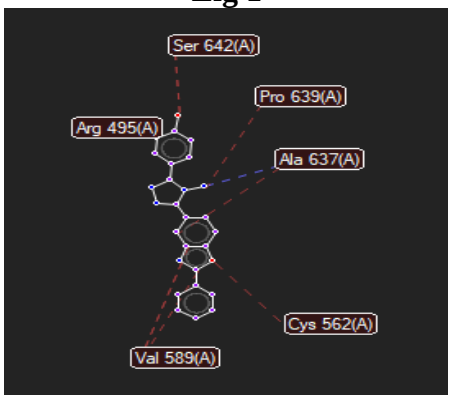

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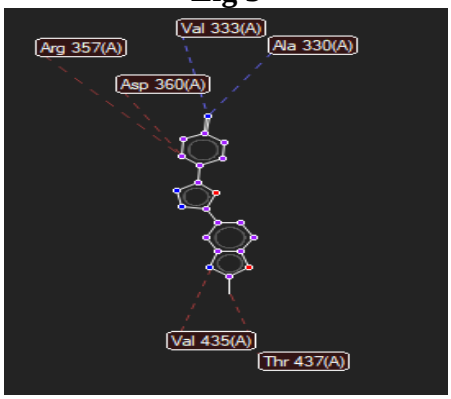

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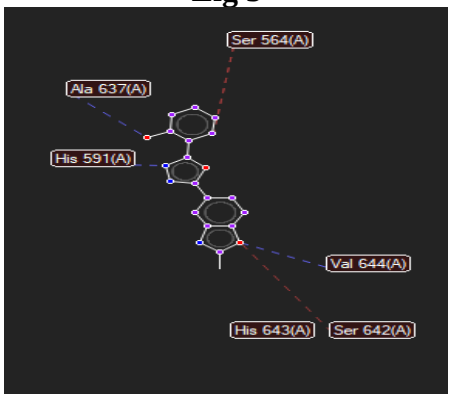

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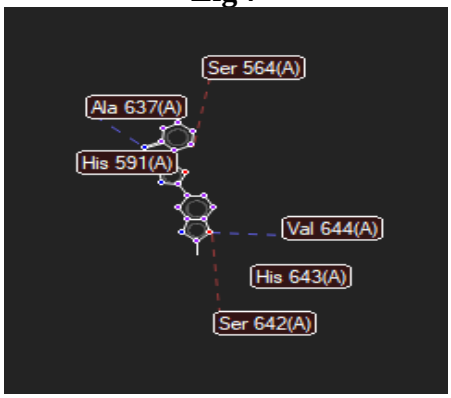

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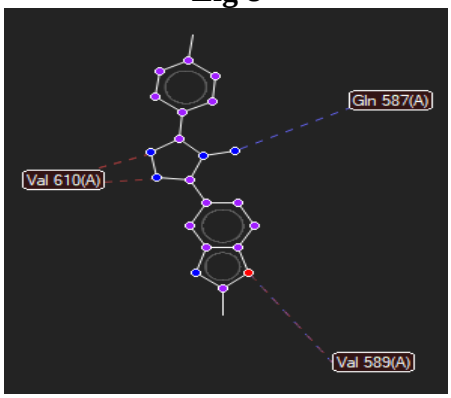

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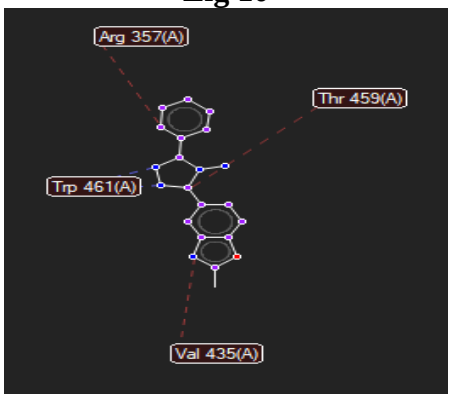
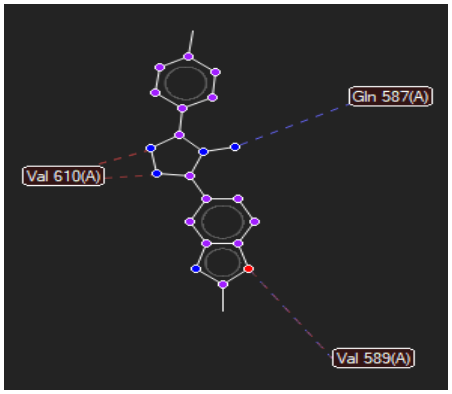


Lig 12

Figure.no.13: Docking interactions of ligands



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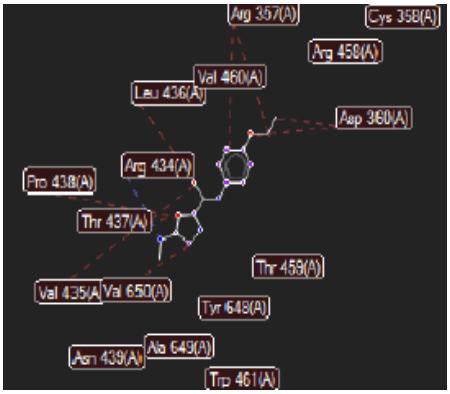

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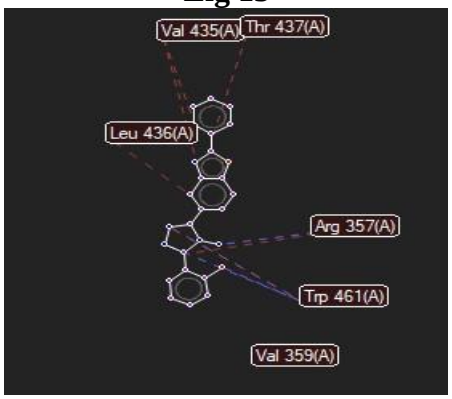

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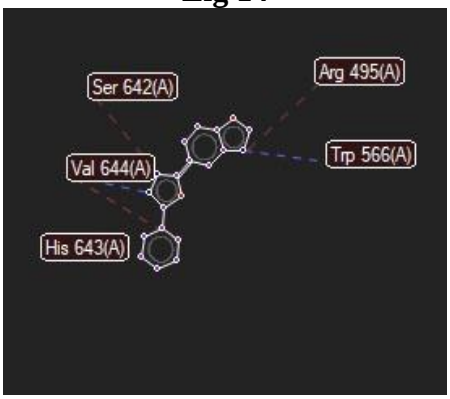

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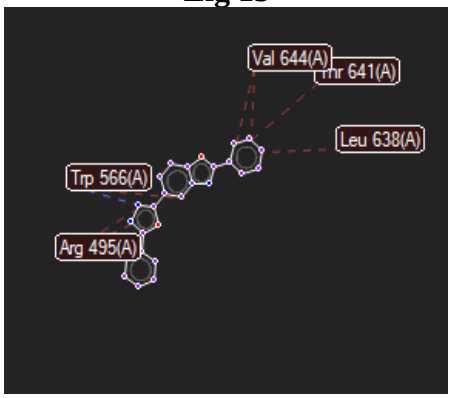

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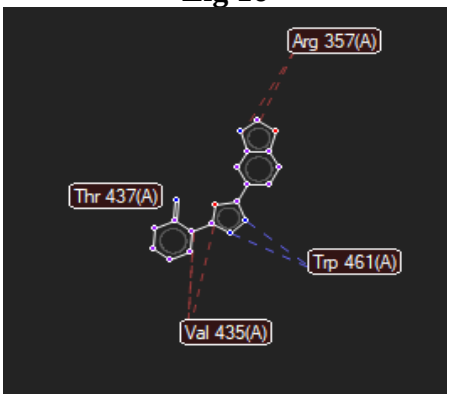

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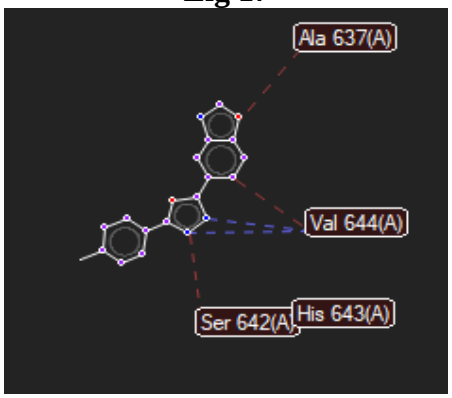

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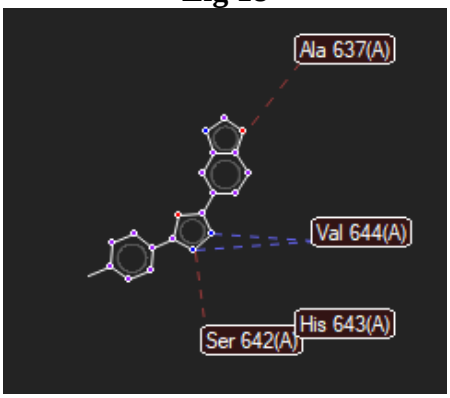
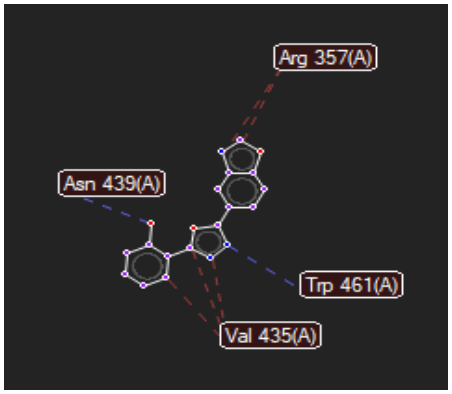
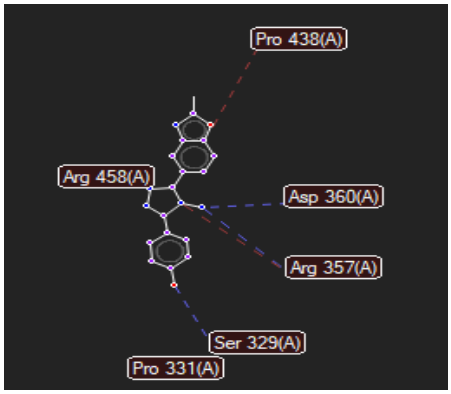


Lig 20

Figure.no.13: Docking interactions of ligands



Lig 21



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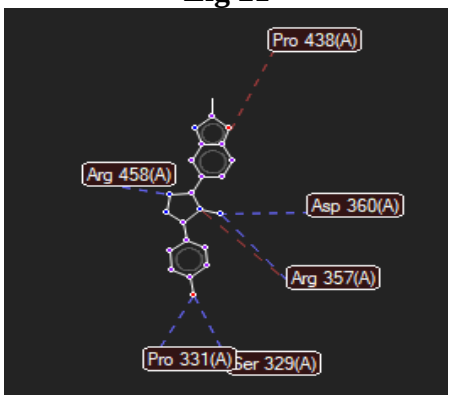

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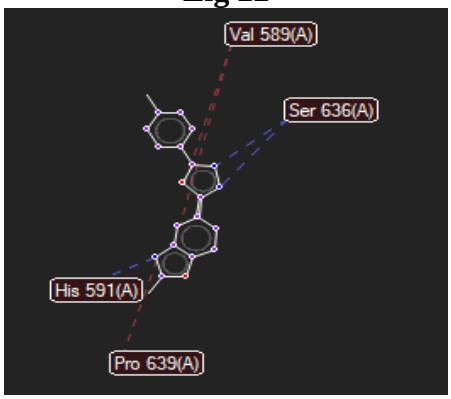
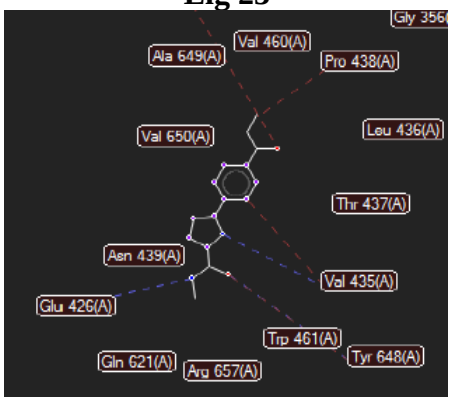
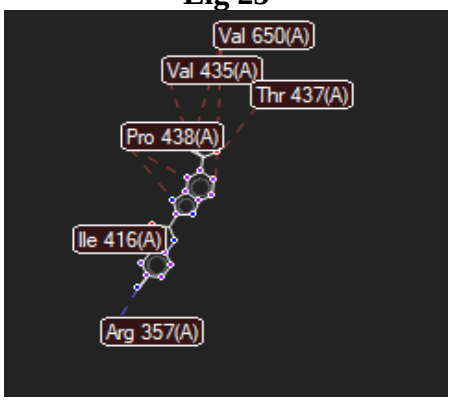


Lig 25



Lig 28



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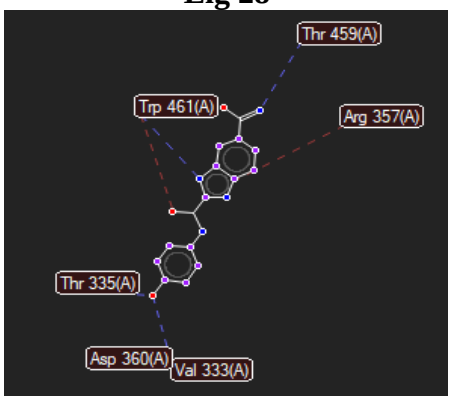

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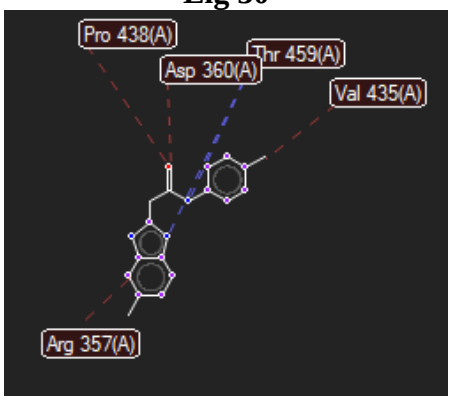
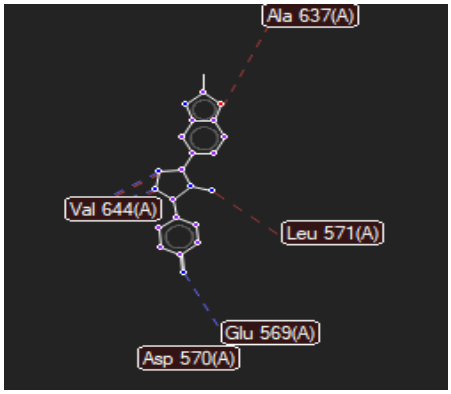
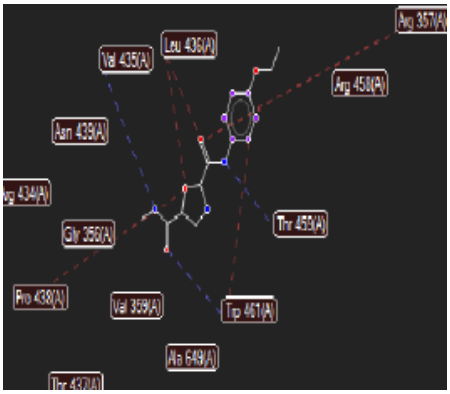


Lig 33

Figure.no.13: Docking interactions of ligands



Lig 34



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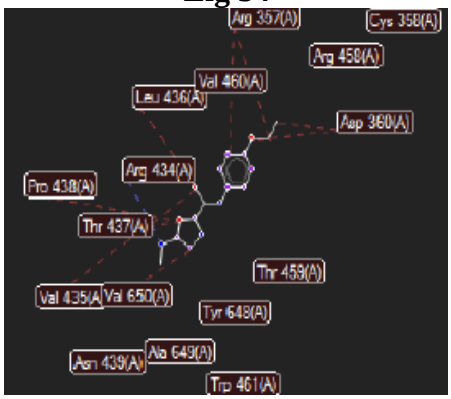

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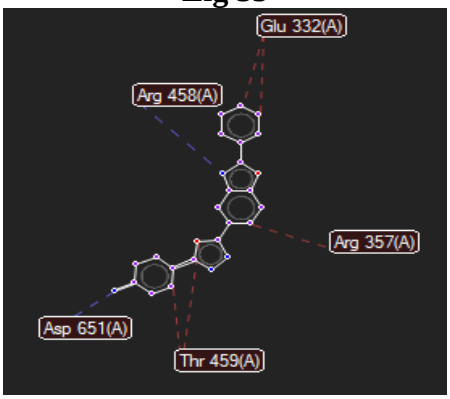

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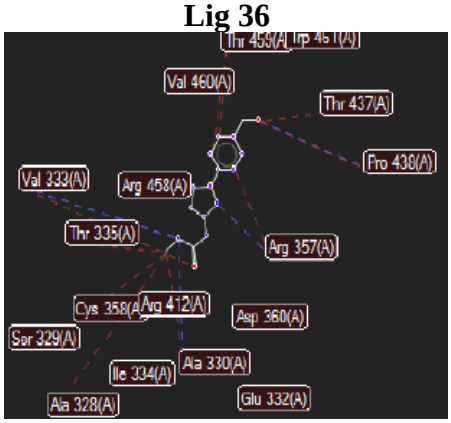
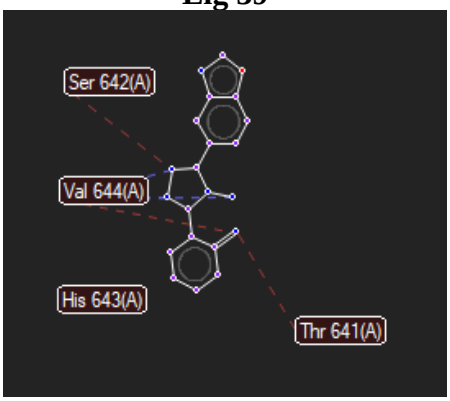


Lig 44



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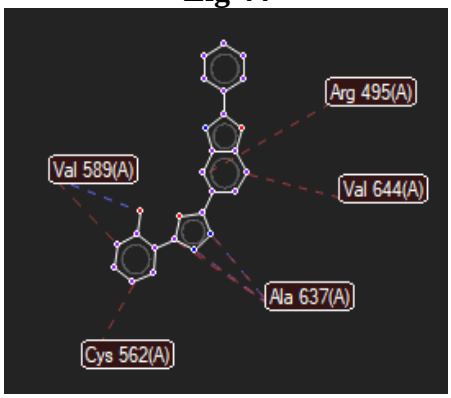
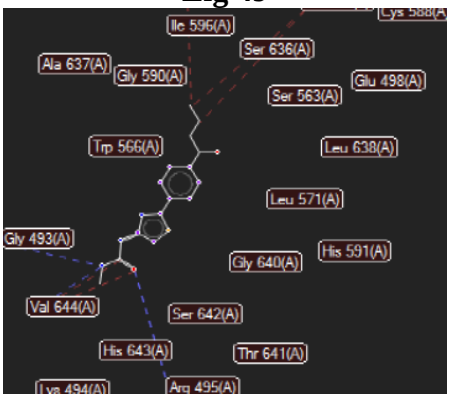
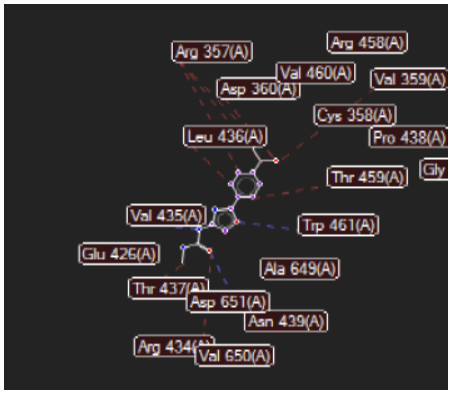


Lig 50

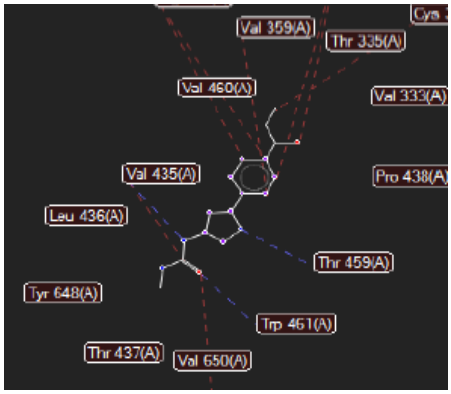


Lig 52

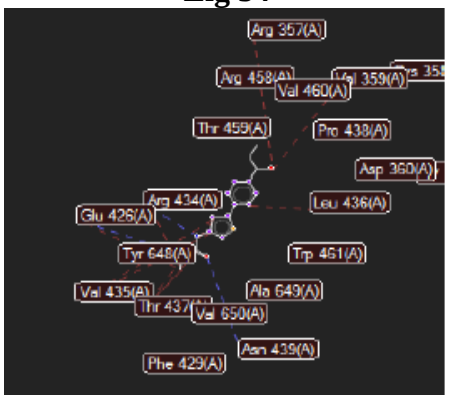
Figure.no.13: Docking interactions of ligands



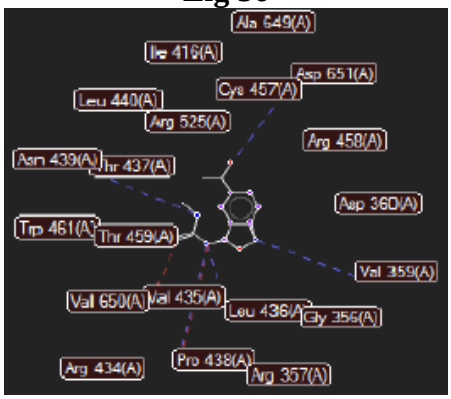
Lig 54



Lig 56



Lig 57



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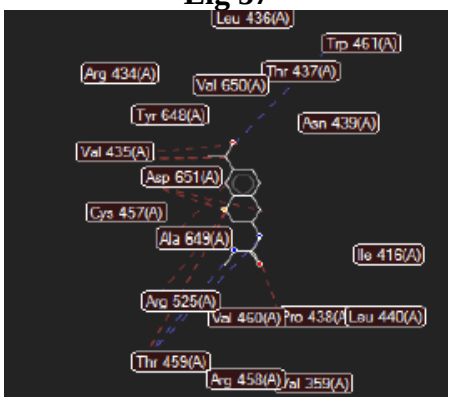

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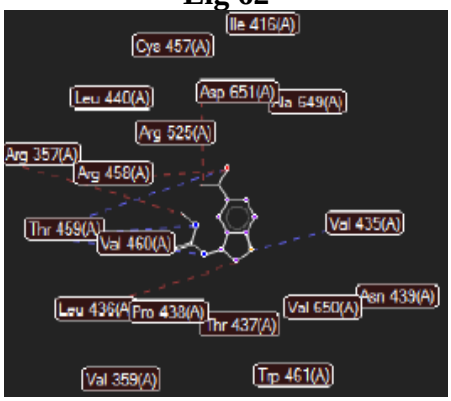

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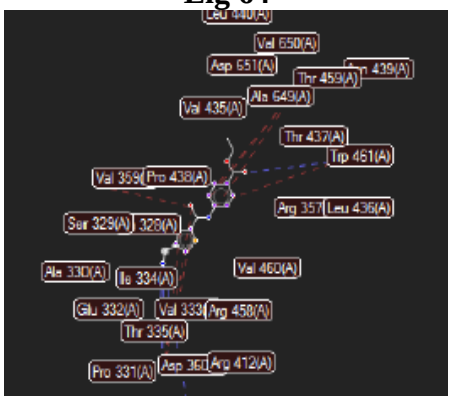

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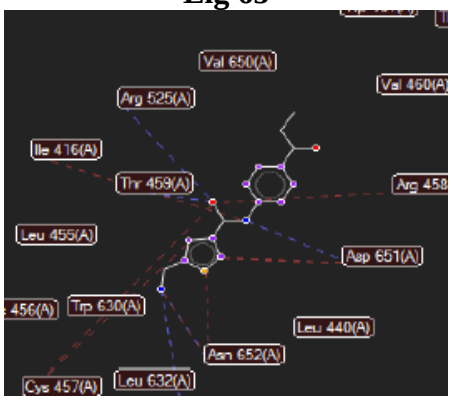
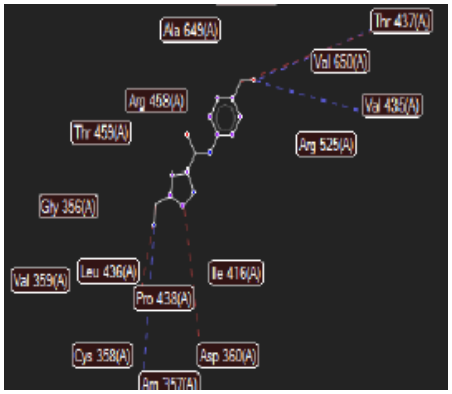


Lig 76

Figure.no.13: Docking interactions of ligands



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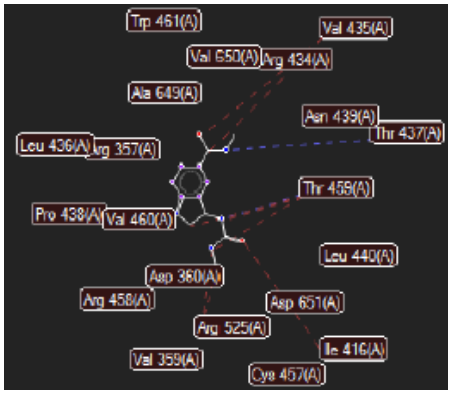

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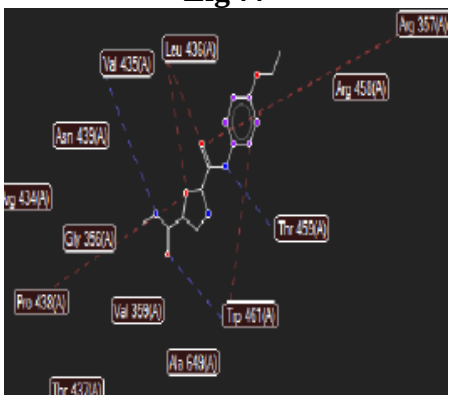

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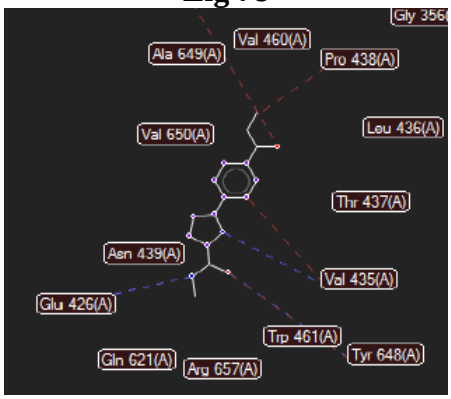

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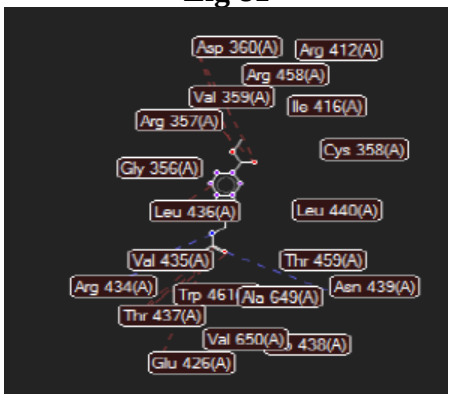

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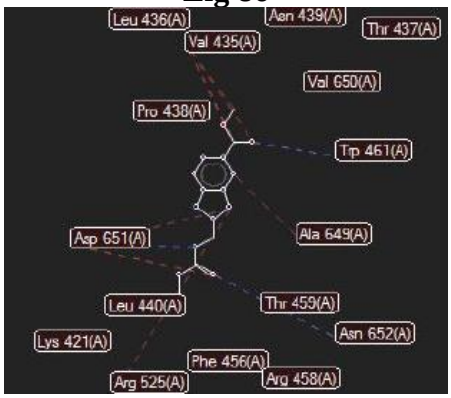

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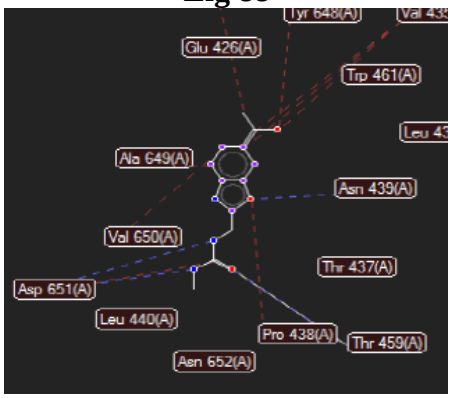

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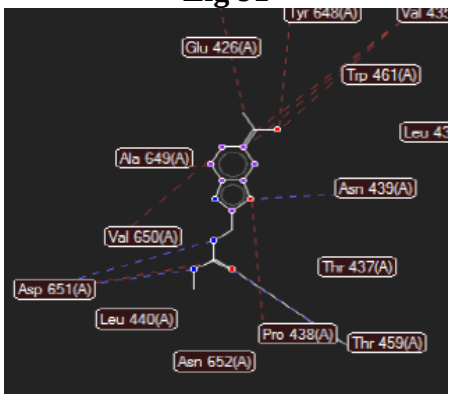
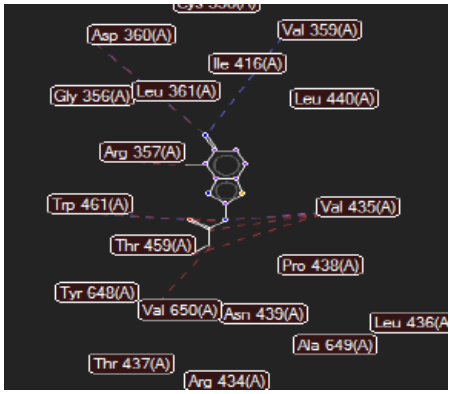


Lig 94

Figure.no.13: Docking interactions of ligands



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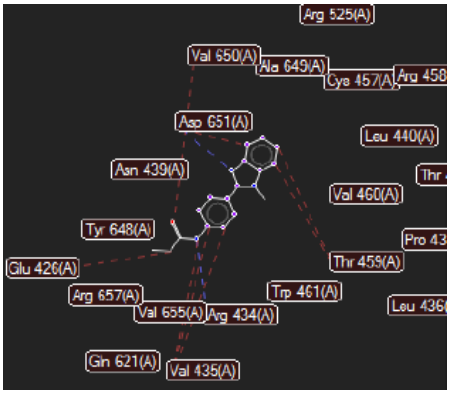

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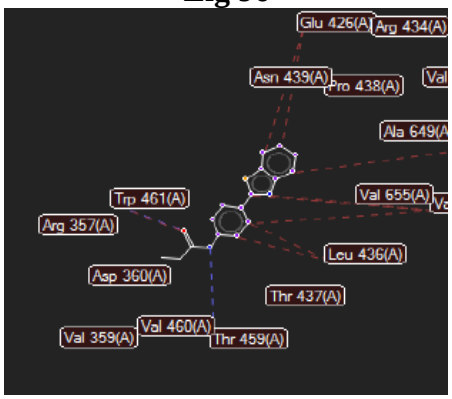

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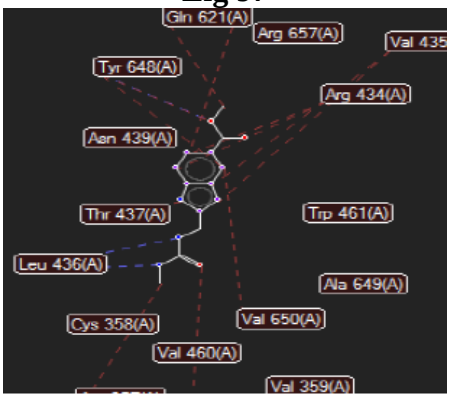

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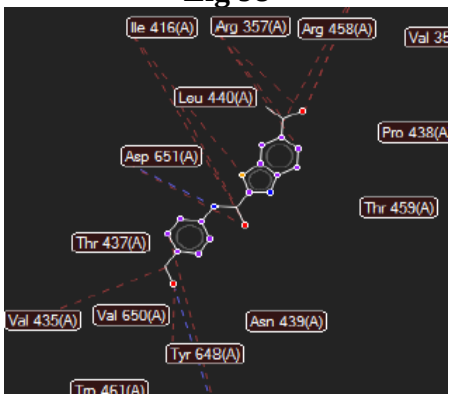

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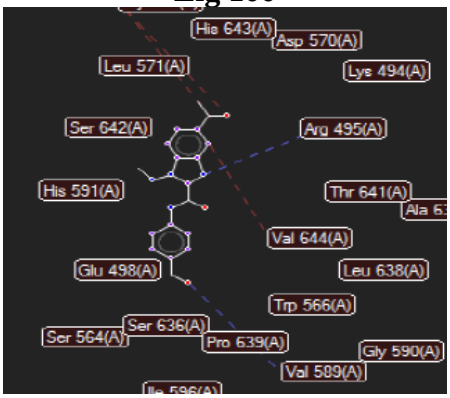

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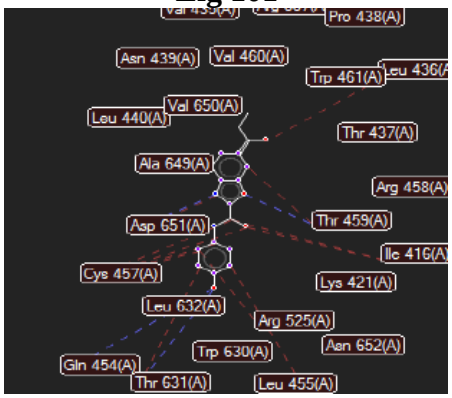

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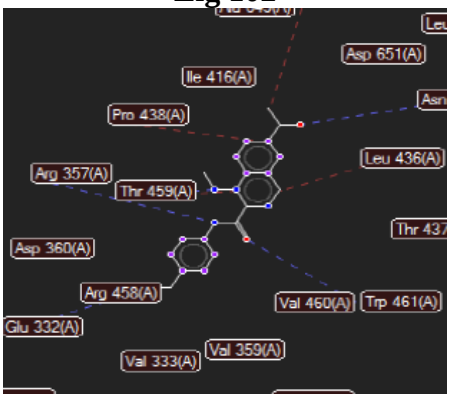
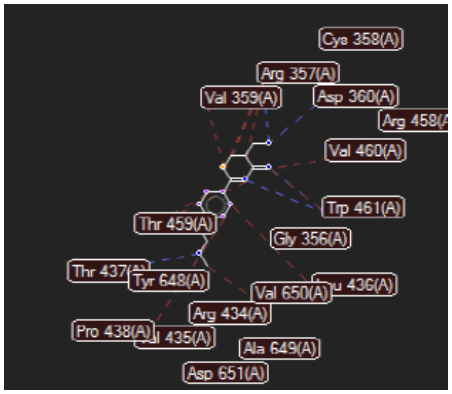


Lig 105

Figure.no.13: Docking interactions of ligands



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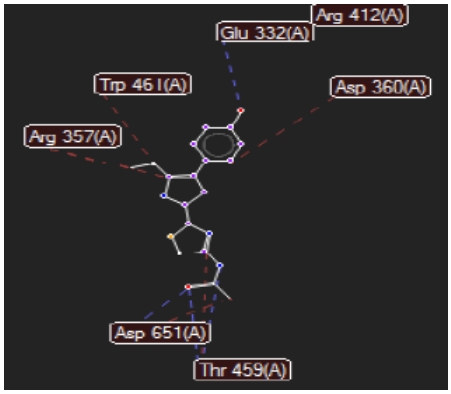

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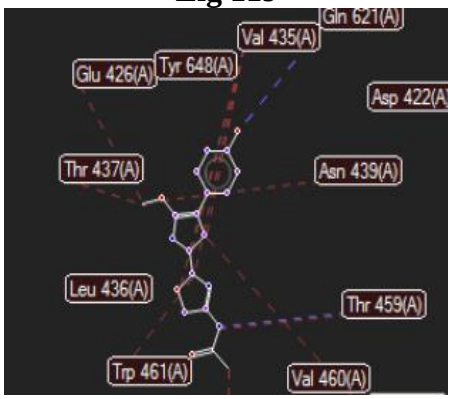

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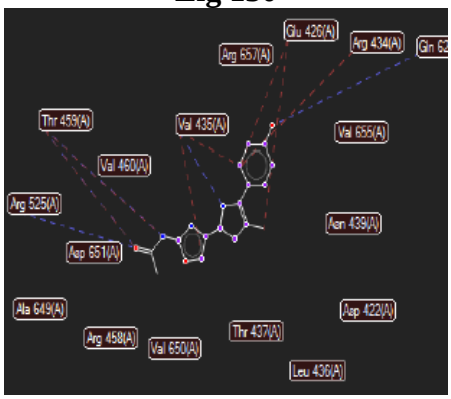

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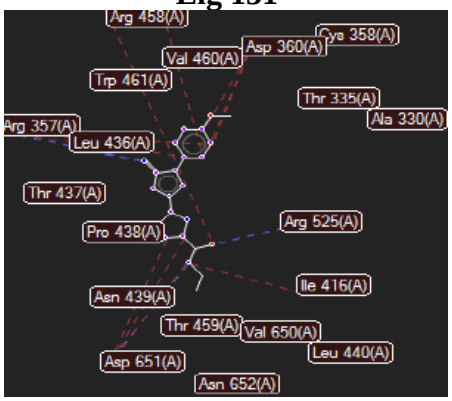

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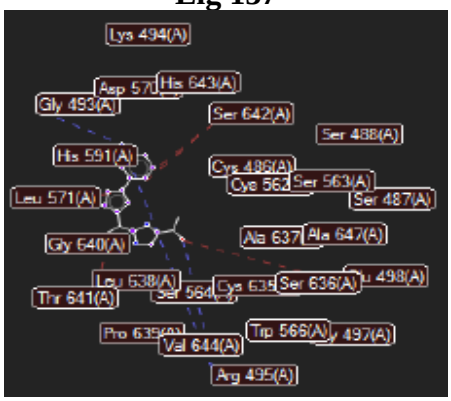

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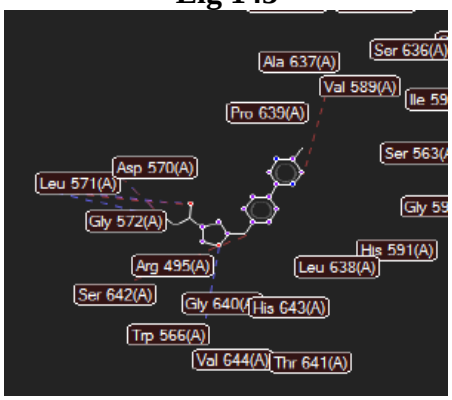

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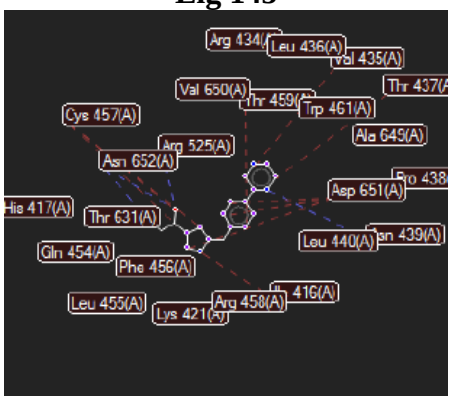


Lig 150

Figure.no.13: Docking interactions of ligands

4. Drug likeliness properties

When all the newly designed 150 ligands of *PCSK9* inhibitors were subjected to drug likeliness prediction studies Lipinski rule of five, ADMET like all were found to exhibit the drug likeliness properties

Lipinski rule of five

The Lipinski rule of five was performed using *Molinspiration Online tool*. All the newly designed *PCSK9* ligands were found to pass Lipinski's rule of five and the results were tabulated below.

Table.no.6: Drug likeliness reports for *PCSK9* inhibitors using *Molinspiration Online tool*

Ligands	Mol.Wt	Log P	nOHNH	nON	TPSA	nrotb	n-Violations
Lig 1	292.30	1.92	4	7	108.80	2	0
Lig 2	293.29	2.58	3	7	103.00	2	0
Lig 3	291.31	3.29	2	6	82.77	2	0
Lig 4	368.40	3.84	4	7	108.80	3	0
Lig 5	369.38	4.28	3	7	103.00	3	0
Lig 6	306.33	2.50	4	7	108.80	2	0

Lig 7	292.30	2.79	2	6	90.98	2	0
Lig 8	293.28	3.45	1	6	85.18	2	0
Lig 9	291.31	4.16	0	5	64.96	2	0
Lig 10	292.30	3.15	3	6	90.98	2	0
Lig 11	355.35	4.93	1	6	85.18	3	0
Lig 12	291.31	3.06	2	6	82.77	2	0
Lig 13	305.34	3.51	2	6	82.77	2	0
Lig 14	353.38	4.76	2	6	82.77	3	0
Lig 15	369.38	4.50	3	7	103.00	3	0
Lig 16	263.26	3.49	0	5	64.96	2	0
Lig 17	296.29	0.74	5	7	121.10	3	0
Lig 18	278.27	2.93	2	6	90.98	2	0
Lig 19	277.28	3.94	0	5	64.96	2	0

Lig 20	297.70	4.17	0	5	64.96	2	0
Lig 21	279.25	3.23	1	6	85.18	2	0
Lig 22	307.31	2.59	3	7	103.00	2	0
Lig 23	325.76	3.74	2	6	82.77	2	0
Lig 24	277.28	3.71	0	5	64.96	2	0
Lig 25	311.73	4.39	0	5	64.96	2	0
Lig 26	318.38	4.23	2	5	66.91	4	0
Lig 27	186.21	1.98	2	3	44.89	2	0
Lig 28	327.77	3.48	2	5	74.85	4	0
Lig 29	309.32	2.32	3	6	95.08	4	0
Lig 30	308.34	1.88	4	6	100.88	4	0
Lig 31	296.29	0.74	5	7	121.10	3	0
Lig32	326.38	2.21	2	5	79.29	4	0

Lig 33	337.38	2.08	2	6	84.22	5	0
Lig 34	310.31	2.76	2	6	92.43	4	0
Lig 35	215.25	1.73	1	3	50.19	3	0
Lig 36	352.39	1.33	3	7	94.03	5	0
Lig 37	230.27	0.25	2	4	62.22	3	0
Lig 38	231.25	-0.12	2	5	75.11	3	0
Lig 39	233.27	1.26	2	4	58.56	3	0
Lig 40	232.28	0.88	3	4	61.35	3	0
Lig 41	250.32	0.70	2	4	61.69	3	0
Lig 42	338.44	1.89	2	5	81.77	6	0
Lig 43	307.35	1.60	2	5	82.02	5	0
Lig 44	292.34	1.27	3	5	84.67	5	0
Lig 45	261.28	1.75	3	6	83.22	4	0

Lig 46	262.26	1.86	2	6	80.57	4	0
Lig 47	262.27	0.89	3	7	96.11	4	0
Lig 48	279.32	1.46	2	6	80.32	4	0
Lig 49	247.25	1.05	2	6	84.23	3	0
Lig 50	305.34	3.51	2	6	82.77	2	0
Lig 51	219.24	1.10	4	5	77.14	2	0
Lig 52	303.39	3.38	2	5	71.09	5	0
Lig 53	279.37	0.65	3	5	76.72	5	0
Lig 54	273.29	2.18	2	6	84.23	4	0
Lig 55	272.31	2.08	3	6	86.88	4	0
Lig 56	287.32	2.51	3	6	83.22	5	0
Lig 57	288.37	3.55	2	4	58.20	4	0

Lig 59	270.33	2.37	2	4	58.20	4	0
Lig 59	216.24	1.16	2	4	61.96	2	0
Lig 60	232.24	1.13	3	6	86.88	2	0
Lig 61	243.27	1.47	2	5	71.09	2	0
Lig 62	231.25	1.47	3	5	73.99	2	0
Lig 63	232.24	1.57	2	5	71.34	2	0
Lig 64	262.33	1.96	2	4	58.20	2	0
Lig 65	248.31	2.21	2	4	58.20	2	0
Lig 66	270.33	3.23	2	4	61.96	5	0
Lig 67	260.30	0.78	4	6	93.04	5	0
Lig 68	275.33	1.50	3	5	85.08	4	0
Lig 69	273.29	1.92	3	6	98.22	5	0
Lig 70	288.31	0.89	4	7	110.11	6	0

Lig 71	285.35	2.06	4	5	87.98	6	0
Lig 72	302.33	2.25	3	6	94.57	7	0
Lig 73	304.37	2.39	3	5	81.43	6	0
Lig 74	257.29	0.99	4	5	87.98	4	0
Lig 75	258.28	1.10	3	5	85.33	4	0
Lig 76	288.37	2.24	3	4	72.19	5	0
Lig 77	245.28	0.43	5	5	91.14	4	0
Lig 78	246.27	0.77	4	6	86.01	2	0
Lig 79	232.24	1.67	2	5	75.36	3	0
Lig 80	246.27	2.21	1	5	64.36	4	0
Lig 81	301.30	1.19	2	7	101.30	5	0
Lig 82	273.29	1.74	2	6	84.23	5	0
Lig 83	257.29	2.06	3	5	73.99	3	0

Lig 84	245.28	1.50	4	5	77.14	3	0
Lig 85	231.25	1.68	4	5	77.14	2	0
Lig 86	256.31	2.36	2	4	61.96	4	0
Lig 87	258.32	3.56	2	4	54.12	6	0
Lig 88	278.33	2.50	2	5	67.43	4	0
Lig 89	261.28	1.75	3	6	83.22	4	0
Lig 90	262.26	1.86	2	6	80.57	4	0
Lig 91	262.27	0.89	3	7	96.11	4	0
Lig 92	279.32	1.46	2	6	80.32	4	0
Lig 93	247.25	1.05	2	6	84.23	3	0
Lig 94	265.27	1.22	3	7	88.69	4	0
Lig 95	220.27	1.55	2	4	58.20	4	0
Lig 96	235.31	2.39	3	4	68.01	2	0

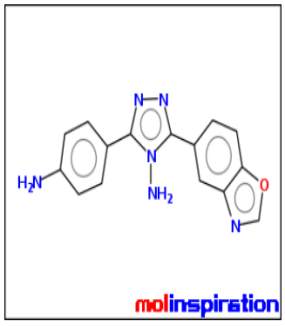
Lig 97	279.34	3.67	1	4	46.92	3	0
Lig 98	282.37	4.35	1	3	41.99	3	0
Lig 99	266.30	3.71	1	4	55.13	3	0
Lig 100	261.28	1.52	2	6	79.79	4	0
Lig 101	326.38	2.21	2	5	79.29	4	0
Lig 102	337.38	2.08	2	6	84.22	5	0
Lig 103	310.31	2.76	2	6	92.43	4	0
Lig 104	215.25	1.73	1	3	50.19	3	0
Lig 105	352.39	1.33	3	7	94.03	5	0
Lig 106	230.27	0.25	2	4	62.22	3	0
Lig 107	231.25	-0.12	2	5	75.11	3	0
Lig 108	233.27	1.26	2	4	58.56	3	0
Lig 109	232.28	0.88	3	4	61.35	3	0

Lig 110	250.32	0.70	2	4	61.69	3	0
Lig 111	219.24	-0.23	3	5	78.01	3	0
Lig 112	220.23	0.20	2	5	75.36	3	0
Lig 113	236.30	0.34	2	4	62.22	3	0
Lig 114	222.27	1.03	2	4	62.22	2	0
Lig 115	248.31	0.90	1	4	59.06	3	0
Lig 116	246.31	0.07	5	5	85.67	4	0
Lig 117	245.33	2.29	4	4	73.64	4	0
Lig 118	262.31	-0.75	5	6	94.91	5	0
Lig 119	277.39	1.79	3	4	59.65	5	0
Lig 120	256.35	3.58	3	3	47.28	5	0
Lig 121	231.25	1.45	4	5	80.15	4	0
Lig 122	214.27	1.15	3	3	55.12	3	0

Lig 123	232.31	2.04	3	3	55.12	3	0
Lig 124	243.31	2.13	4	4	70.91	4	0
Lig 125	217.23	0.66	3	5	81.15	3	0
Lig 126	247.26	-0.77	4	7	105.93	4	0
Lig 127	262.34	-0.50	3	5	80.91	4	0
Lig 128	261.29	-0.99	3	7	106.94	5	0
Lig 129	276.32	0.50	1	6	84.84	4	0
Lig 130	329.38	2.10	3	6	87.24	4	0
Lig 131	313.31	1.46	3	7	100.38	4	0
Lig 132	312.33	1.11	4	7	103.03	4	0
Lig 133	311.34	1.89	4	6	90.14	4	0
Lig 134	294.35	2.80	3	4	65.12	3	0
Lig 135	328.39	2.63	3	5	74.35	4	0

Lig 136	330.37	2.70	2	6	84.59	4	0
Lig 137	297.31	1.60	3	6	91.15	3	0
Lig 138	296.33	1.75	4	6	93.80	3	0
Lig 139	313.38	2.49	3	5	78.01	3	0
Lig 140	312.39	3.03	3	4	65.12	3	0
Lig 141	219.31	2.55	2	2	35.26	2	0
Lig 142	322.33	0.50	3	8	112.66	4	0
Lig 143	293.37	3.10	4	4	70.91	4	0
Lig 144	268.32	3.58	3	4	61.04	3	0
Lig 145	293.33	1.21	3	5	88.85	4	0
Lig 146	293.33	0.53	3	6	97.56	5	0
Lig 147	294.31	0.96	2	6	94.91	5	0
Lig 148	338.44	1.89	2	5	81.77	6	0

Lig 149	307.35	1.60	2	5	82.02	5	0
Lig 150	292.34	1.27	3	5	84.67	5	0



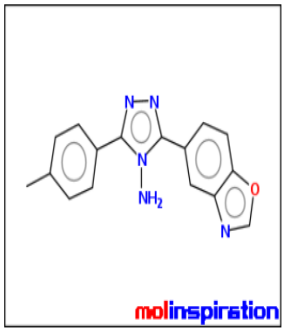
Molinspiration bioactivity score v2021

GPCR ligand	-0.03
Ion channel modulator	-0.01
Kinase inhibitor	0.09
Nuclear receptor ligand	-0.34
Protease inhibitor	-0.29
Enzyme inhibitor	0.18

Get data as text (for copy / paste).

Get 3D geometry BETA

Lig1



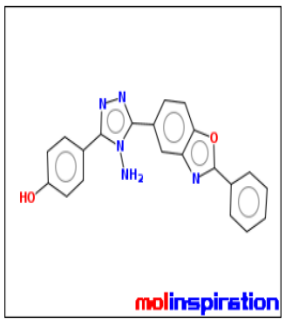
Molinspiration bioactivity score v20

GPCR ligand	-0.11
Ion channel modulator	-0.15
Kinase inhibitor	-0.06
Nuclear receptor ligand	-0.31
Protease inhibitor	-0.41
Enzyme inhibitor	0.04

Get data as text (for copy / paste).

Get 3D geometry BETA

Lig 3



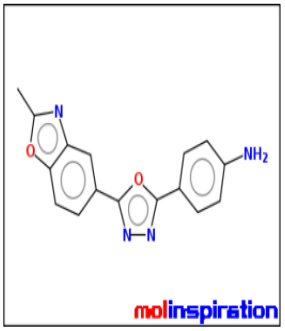
Molinspiration bioactivity score v2021

GPCR ligand	-0.29
Ion channel modulator	-0.36
Kinase inhibitor	-0.10
Nuclear receptor ligand	-0.13
Protease inhibitor	-0.55
Enzyme inhibitor	-0.06

Get data as text (for copy / paste).

Get 3D geometry BETA

Lig 5



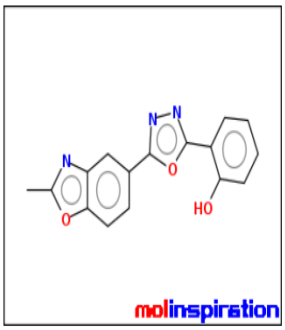
Molinspiration bioactivity score v20

GPCR ligand	-0.06
Ion channel modulator	-0.18
Kinase inhibitor	0.11
Nuclear receptor ligand	-0.33
Protease inhibitor	0.00
Enzyme inhibitor	0.16

Get data as text (for copy / paste).

Get 3D geometry BETA

Lig 7



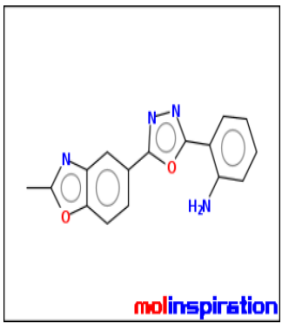
Molinspiration bioactivity score v2021

GPCR ligand	-0.11
Ion channel modulator	-0.35
Kinase inhibitor	-0.03
Nuclear receptor ligand	-0.19
Protease inhibitor	-0.14
Enzyme inhibitor	0.06

Get data as text (for copy / paste).

Get 3D geometry BETA

Lig 8



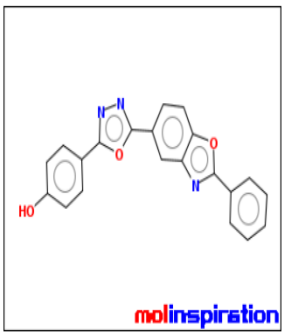
Molinspiration bioactivity score v202

GPCR ligand	-0.07
Ion channel modulator	-0.25
Kinase inhibitor	-0.04
Nuclear receptor ligand	-0.47
Protease inhibitor	0.07
Enzyme inhibitor	0.10

Get data as text (for copy / paste).

Get 3D geometry BETA

Lig 10



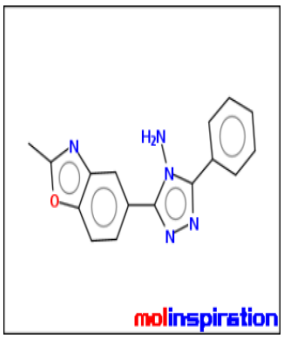
Molinspiration bioactivity score v2021

GPCR ligand	-0.10
Ion channel modulator	-0.31
Kinase inhibitor	-0.01
Nuclear receptor ligand	0.10
Protease inhibitor	-0.31
Enzyme inhibitor	0.04

Get data as text (for copy / paste).

Get 3D geometry BETA

Lig 11



Molinspiration bioactivity score v20

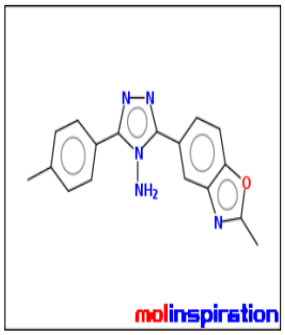
GPCR ligand	-0.30
Ion channel modulator	-0.25
Kinase inhibitor	-0.08
Nuclear receptor ligand	-0.60
Protease inhibitor	-0.36
Enzyme inhibitor	-0.03

Get data as text (for copy / paste).

Get 3D geometry BETA

Lig 12

Figure.no.14: Biological properties of selected *PCSK9* inhibitors



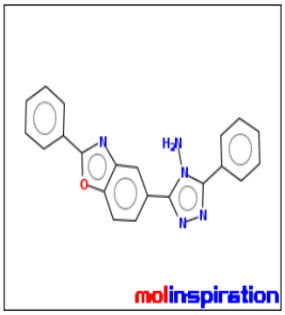
Molinspiration bioactivity score v2021

GPCR ligand	-0.31
Ion channel modulator	-0.29
Kinase inhibitor	-0.11
Nuclear receptor ligand	-0.58
Protease inhibitor	-0.36
Enzyme inhibitor	-0.08

Get data as text (for copy / paste).

Get 3D geometry BETA

Lig13



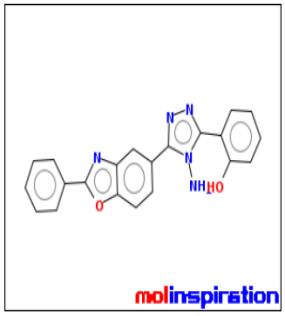
Molinspiration bioactivity score v202

GPCR ligand	-0.34
Ion channel modulator	-0.41
Kinase inhibitor	-0.14
Nuclear receptor ligand	-0.26
Protease inhibitor	-0.57
Enzyme inhibitor	-0.11

Get data as text (for copy / paste).

Get 3D geometry BETA

Lig 14



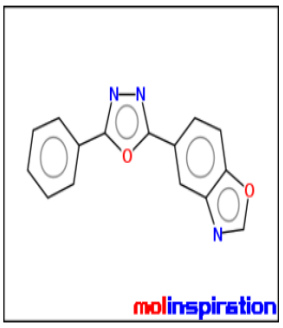
Molinspiration bioactivity score v2021

GPCR ligand	-0.33
Ion channel modulator	-0.43
Kinase inhibitor	-0.14
Nuclear receptor ligand	-0.21
Protease inhibitor	-0.58
Enzyme inhibitor	-0.08

Get data as text (for copy / paste).

Get 3D geometry BETA

Lig 15



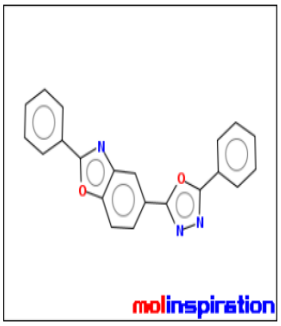
Molinspiration bioactivity score v202

GPCR ligand	0.09
Ion channel modulator	-0.06
Kinase inhibitor	0.02
Nuclear receptor ligand	-0.01
Protease inhibitor	-0.16
Enzyme inhibitor	0.22

Get data as text (for copy / paste).

Get 3D geometry BETA

Lig 16



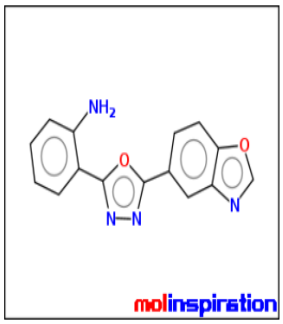
Molinspiration bioactivity score v2021.

GPCR ligand	-0.15
Ion channel modulator	-0.36
Kinase inhibitor	-0.05
Nuclear receptor ligand	-0.02
Protease inhibitor	-0.34
Enzyme inhibitor	-0.01

Get data as text (for copy / paste).

Get 3D geometry BETA

Lig 17



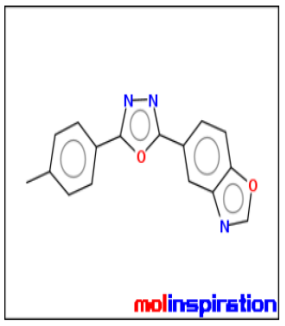
Molinspiration bioactivity score v202

GPCR ligand	0.16
Ion channel modulator	-0.07
Kinase inhibitor	0.01
Nuclear receptor ligand	-0.18
Protease inhibitor	0.05
Enzyme inhibitor	0.24

Get data as text (for copy / paste).

Get 3D geometry BETA

Lig 18



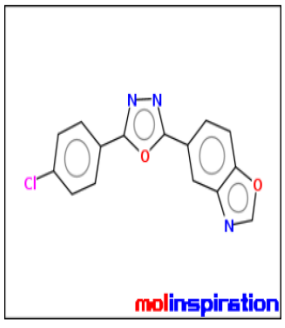
Molinspiration bioactivity score v2021.

GPCR ligand	0.07
Ion channel modulator	-0.15
Kinase inhibitor	-0.01
Nuclear receptor ligand	0.00
Protease inhibitor	-0.18
Enzyme inhibitor	0.14

Get data as text (for copy / paste).

Get 3D geometry BETA

Lig 19



Molinspiration bioactivity score v20

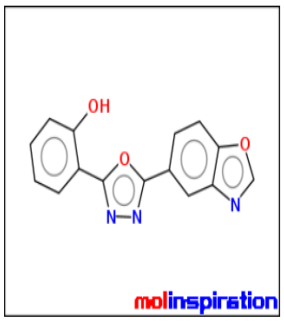
GPCR ligand	0.12
Ion channel modulator	-0.07
Kinase inhibitor	0.03
Nuclear receptor ligand	0.01
Protease inhibitor	-0.16
Enzyme inhibitor	0.17

Get data as text (for copy / paste).

Get 3D geometry BETA

Lig 20

Figure.no.14: Biological properties of selected *PCSK9* inhibitors

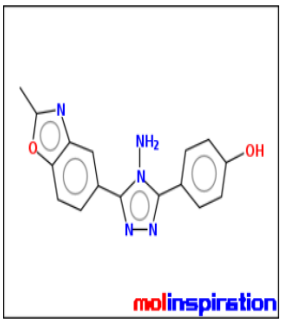


Molinspiration bioactivity score	v2021
GPCR ligand	0.11
Ion channel modulator	-0.17
Kinase inhibitor	0.03
Nuclear receptor ligand	0.11
Protease inhibitor	-0.17
Enzyme inhibitor	0.20

Get data as text (for copy / paste).

Get 3D geometry BETA

Lig 21

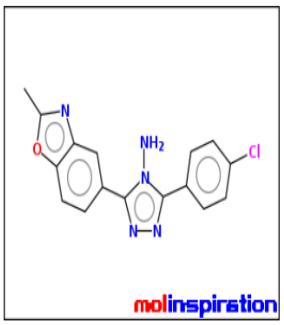


Molinspiration bioactivity score	v20
GPCR ligand	-0.23
Ion channel modulator	-0.19
Kinase inhibitor	-0.02
Nuclear receptor ligand	-0.41
Protease inhibitor	-0.31
Enzyme inhibitor	0.03

Get data as text (for copy / paste).

Get 3D geometry BETA

Lig 22

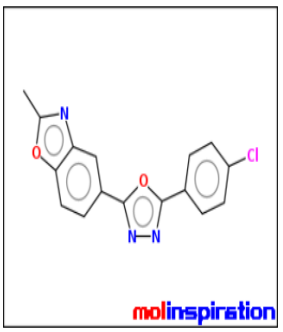


Molinspiration bioactivity score	v2021
GPCR ligand	-0.28
Ion channel modulator	-0.24
Kinase inhibitor	-0.09
Nuclear receptor ligand	-0.58
Protease inhibitor	-0.37
Enzyme inhibitor	-0.07

Get data as text (for copy / paste).

Get 3D geometry BETA

Lig 23

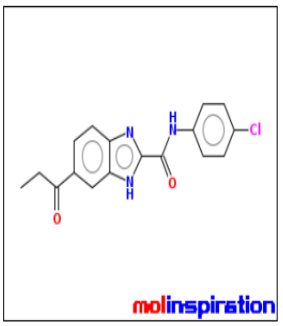


Molinspiration bioactivity score	v202
GPCR ligand	-0.10
Ion channel modulator	-0.25
Kinase inhibitor	-0.03
Nuclear receptor ligand	-0.28
Protease inhibitor	-0.13
Enzyme inhibitor	0.03

Get data as text (for copy / paste).

Get 3D geometry BETA

Lig 25

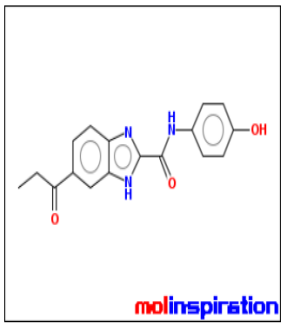


Molinspiration bioactivity score	v2021.
GPCR ligand	0.02
Ion channel modulator	-0.02
Kinase inhibitor	0.10
Nuclear receptor ligand	-0.29
Protease inhibitor	-0.14
Enzyme inhibitor	-0.10

Get data as text (for copy / paste).

Get 3D geometry BETA

Lig 28

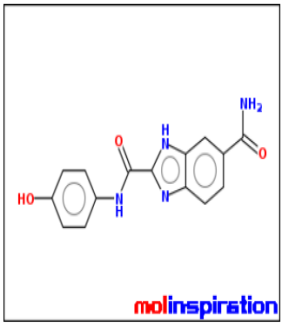


Molinspiration bioactivity score	v20
GPCR ligand	0.07
Ion channel modulator	0.03
Kinase inhibitor	0.16
Nuclear receptor ligand	-0.12
Protease inhibitor	-0.08
Enzyme inhibitor	-0.01

Get data as text (for copy / paste).

Get 3D geometry BETA

Lig 30

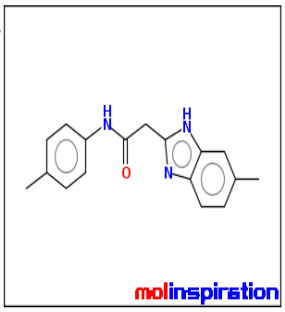


Molinspiration bioactivity score	v2021.
GPCR ligand	0.12
Ion channel modulator	0.04
Kinase inhibitor	0.43
Nuclear receptor ligand	-0.23
Protease inhibitor	0.03
Enzyme inhibitor	0.08

Get data as text (for copy / paste).

Get 3D geometry BETA

Lig 31



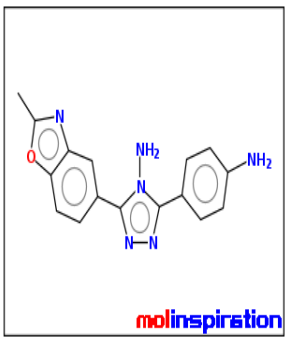
Molinspiration bioactivity score	v20
GPCR ligand	-0.07
Ion channel modulator	-0.27
Kinase inhibitor	-0.14
Nuclear receptor ligand	-0.57
Protease inhibitor	-0.27
Enzyme inhibitor	-0.15

Get data as text (for copy / paste).

Get 3D geometry BETA

Lig 33

Figure.no.14: Biological properties of selected *PCSK9* inhibitors



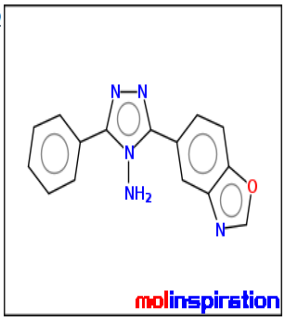
Molinspiration bioactivity score v202

GPCR ligand	-0.24
Ion channel modulator	-0.19
Kinase inhibitor	0.02
Nuclear receptor ligand	-0.61
Protease inhibitor	-0.27
Enzyme inhibitor	0.04

Get data as text (for copy / paste).

Get 3D geometry BETA

Lig 34



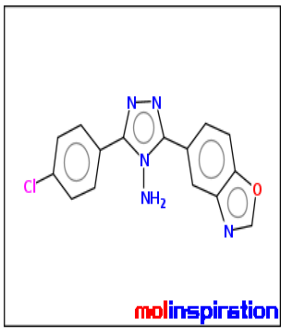
Molinspiration bioactivity score v20

GPCR ligand	-0.09
Ion channel modulator	-0.06
Kinase inhibitor	-0.03
Nuclear receptor ligand	-0.33
Protease inhibitor	-0.40
Enzyme inhibitor	0.11

Get data as text (for copy / paste).

Get 3D geometry BETA

Lig 35



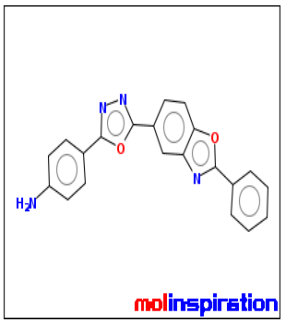
Molinspiration bioactivity score v2021

GPCR ligand	-0.07
Ion channel modulator	-0.07
Kinase inhibitor	-0.03
Nuclear receptor ligand	-0.31
Protease inhibitor	-0.40
Enzyme inhibitor	0.07

Get data as text (for copy / paste).

Get 3D geometry BETA

Lig 36



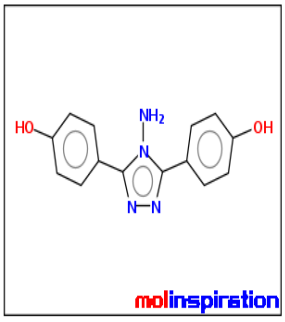
Molinspiration bioactivity score v202

GPCR ligand	-0.11
Ion channel modulator	-0.30
Kinase inhibitor	0.04
Nuclear receptor ligand	-0.09
Protease inhibitor	-0.26
Enzyme inhibitor	0.06

Get data as text (for copy / paste).

Get 3D geometry BETA

Lig 39



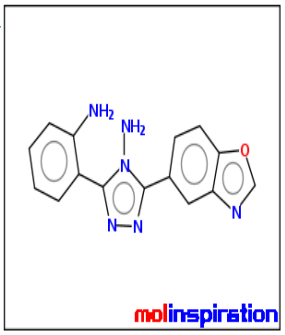
Molinspiration bioactivity score v2021

GPCR ligand	-0.36
Ion channel modulator	-0.24
Kinase inhibitor	-0.19
Nuclear receptor ligand	-0.51
Protease inhibitor	-0.57
Enzyme inhibitor	-0.10

Get data as text (for copy / paste).

Get 3D geometry BETA

Lig 44



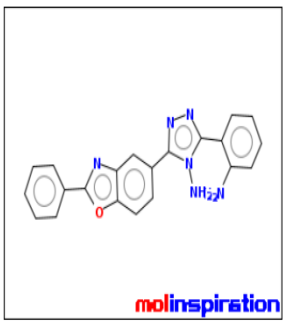
Molinspiration bioactivity score v202

GPCR ligand	-0.03
Ion channel modulator	0.00
Kinase inhibitor	0.01
Nuclear receptor ligand	-0.48
Protease inhibitor	-0.35
Enzyme inhibitor	0.10

Get data as text (for copy / paste).

Get 3D geometry BETA

Lig 49



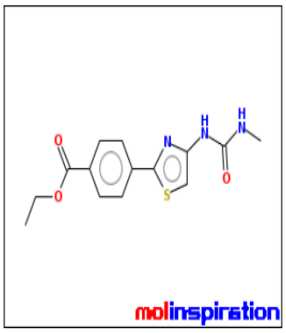
Molinspiration bioactivity score v2021

GPCR ligand	-0.30
Ion channel modulator	-0.35
Kinase inhibitor	-0.12
Nuclear receptor ligand	-0.41
Protease inhibitor	-0.56
Enzyme inhibitor	-0.11

Get data as text (for copy / paste).

Get 3D geometry BETA

Lig 50



Molinspiration bioactivity score v20

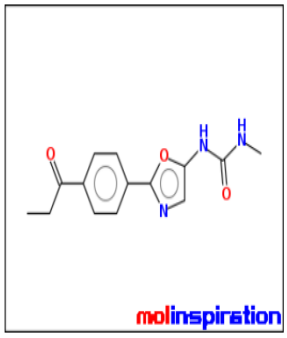
GPCR ligand	-0.08
Ion channel modulator	-0.13
Kinase inhibitor	0.37
Nuclear receptor ligand	-0.39
Protease inhibitor	-0.37
Enzyme inhibitor	-0.14

Get data as text (for copy / paste).

Get 3D geometry BETA

Lig 52

Figure.no.14: Biological properties of selected *PCSK9* inhibitors



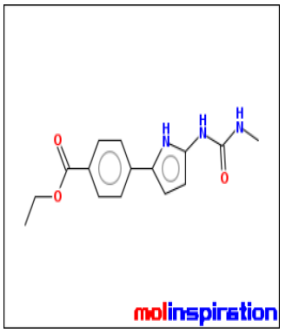
Molinspiration bioactivity score v2021

GPCR ligand	-0.04
Ion channel modulator	-0.36
Kinase inhibitor	0.04
Nuclear receptor ligand	-0.40
Protease inhibitor	-0.36
Enzyme inhibitor	-0.02

Get data as text (for copy / paste).

Get 3D geometry BETA

Lig 54



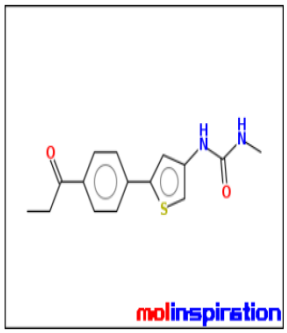
Molinspiration bioactivity score v202

GPCR ligand	0.06
Ion channel modulator	0.10
Kinase inhibitor	0.22
Nuclear receptor ligand	0.04
Protease inhibitor	-0.16
Enzyme inhibitor	0.05

Get data as text (for copy / paste).

Get 3D geometry BETA

Lig 56



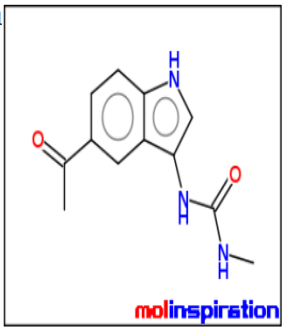
Molinspiration bioactivity score v2021

GPCR ligand	0.01
Ion channel modulator	0.01
Kinase inhibitor	0.16
Nuclear receptor ligand	-0.46
Protease inhibitor	-0.03
Enzyme inhibitor	0.06

Get data as text (for copy / paste).

Get 3D geometry BETA

Lig 57



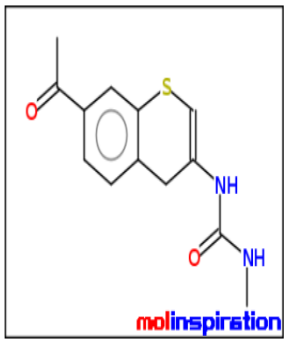
Molinspiration bioactivity score v202

GPCR ligand	-0.20
Ion channel modulator	-0.09
Kinase inhibitor	-0.04
Nuclear receptor ligand	-1.02
Protease inhibitor	-0.58
Enzyme inhibitor	-0.29

Get data as text (for copy / paste).

Get 3D geometry BETA

Lig 62



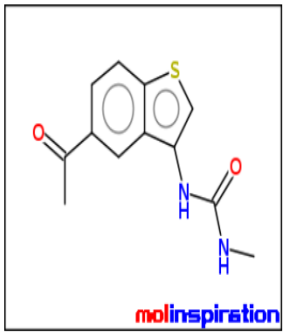
Molinspiration bioactivity score v2021

GPCR ligand	-0.50
Ion channel modulator	-0.51
Kinase inhibitor	-1.04
Nuclear receptor ligand	-0.62
Protease inhibitor	-0.40
Enzyme inhibitor	-0.28

Get data as text (for copy / paste).

Get 3D geometry BETA

Lig 64



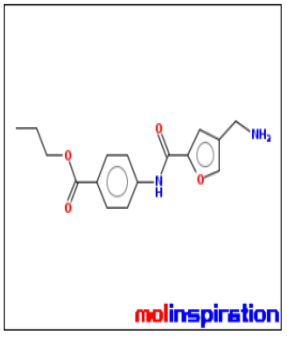
Molinspiration bioactivity score v20

GPCR ligand	-0.16
Ion channel modulator	-0.09
Kinase inhibitor	-0.15
Nuclear receptor ligand	-0.93
Protease inhibitor	-0.47
Enzyme inhibitor	-0.10

Get data as text (for copy / paste).

Get 3D geometry BETA

Lig 65



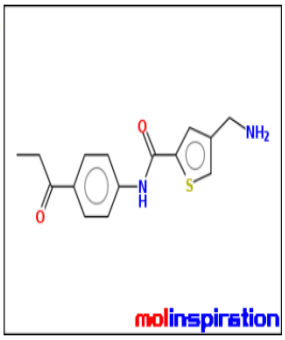
Molinspiration bioactivity score v2021

GPCR ligand	-0.19
Ion channel modulator	-0.37
Kinase inhibitor	-0.20
Nuclear receptor ligand	-0.62
Protease inhibitor	-0.13
Enzyme inhibitor	-0.21

Get data as text (for copy / paste).

Get 3D geometry BETA

Lig 73



Molinspiration bioactivity score v20

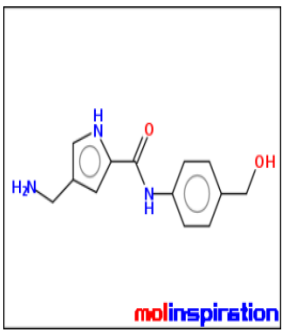
GPCR ligand	-0.19
Ion channel modulator	-0.37
Kinase inhibitor	-0.14
Nuclear receptor ligand	-0.73
Protease inhibitor	-0.06
Enzyme inhibitor	-0.14

Get data as text (for copy / paste).

Get 3D geometry BETA

Lig 76

Figure.no.14: Biological properties of selected *PCSK9* inhibitors

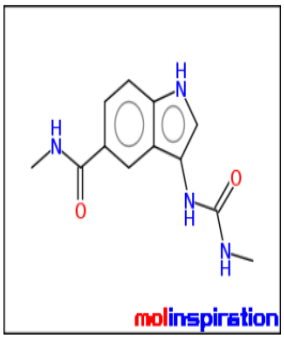


Molinspiration bioactivity score	v2021
GPCR ligand	0.18
Ion channel modulator	0.25
Kinase inhibitor	0.38
Nuclear receptor ligand	-0.58
Protease inhibitor	0.17
Enzyme inhibitor	0.24

Get data as text (for copy / paste).

Get 3D geometry BETA

Lig 77

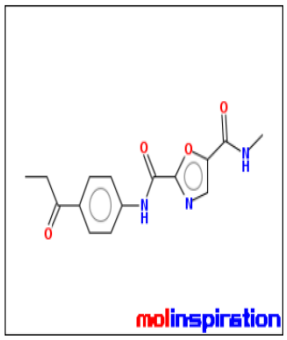


Molinspiration bioactivity score	v20
GPCR ligand	-0.03
Ion channel modulator	-0.06
Kinase inhibitor	0.29
Nuclear receptor ligand	-1.02
Protease inhibitor	-0.34
Enzyme inhibitor	-0.17

Get data as text (for copy / paste).

Get 3D geometry BETA

Lig 78

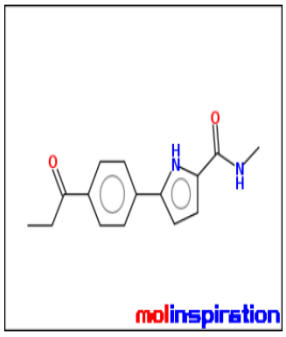


Molinspiration bioactivity score	v2021
GPCR ligand	0.15
Ion channel modulator	-0.10
Kinase inhibitor	0.22
Nuclear receptor ligand	-0.47
Protease inhibitor	0.12
Enzyme inhibitor	0.12

Get data as text (for copy / paste).

Get 3D geometry BETA

Lig 81

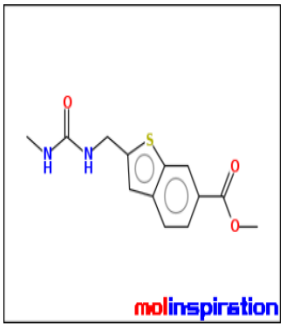


Molinspiration bioactivity score	v20
GPCR ligand	0.13
Ion channel modulator	-0.09
Kinase inhibitor	0.23
Nuclear receptor ligand	-0.06
Protease inhibitor	-0.17
Enzyme inhibitor	0.10

Get data as text (for copy / paste).

Get 3D geometry BETA

Lig 86

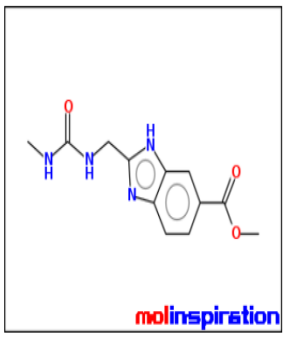


Molinspiration bioactivity score	v2021
GPCR ligand	-0.19
Ion channel modulator	-0.17
Kinase inhibitor	-0.10
Nuclear receptor ligand	-0.49
Protease inhibitor	-0.18
Enzyme inhibitor	0.00

Get data as text (for copy / paste).

Get 3D geometry BETA

Lig 88

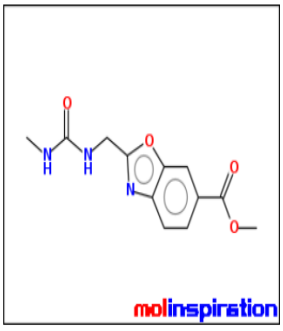


Molinspiration bioactivity score	v20
GPCR ligand	-0.06
Ion channel modulator	0.09
Kinase inhibitor	-0.07
Nuclear receptor ligand	-0.80
Protease inhibitor	-0.23
Enzyme inhibitor	-0.02

Get data as text (for copy / paste).

Get 3D geometry BETA

Lig 91

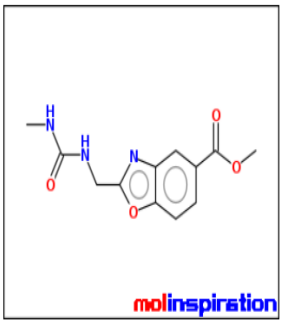


Molinspiration bioactivity score	v2021
GPCR ligand	-0.33
Ion channel modulator	-0.37
Kinase inhibitor	-0.53
Nuclear receptor ligand	-0.74
Protease inhibitor	-0.21
Enzyme inhibitor	-0.19

Get data as text (for copy / paste).

Get 3D geometry BETA

Lig 93



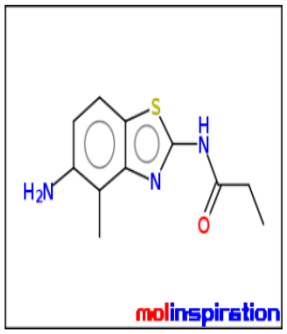
Molinspiration bioactivity score	v202
GPCR ligand	-0.33
Ion channel modulator	-0.35
Kinase inhibitor	-0.48
Nuclear receptor ligand	-0.70
Protease inhibitor	-0.17
Enzyme inhibitor	-0.16

Get data as text (for copy / paste).

Get 3D geometry BETA

Lig 94

Figure.no.14: Biological properties of selected *PCSK9* inhibitors



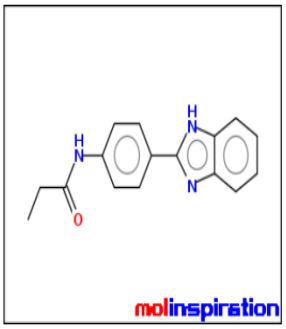
Molinspiration bioactivity score v2021

GPCR ligand	-0.57
Ion channel modulator	-0.55
Kinase inhibitor	-0.36
Nuclear receptor ligand	-1.14
Protease inhibitor	-0.88
Enzyme inhibitor	-0.25

Get data as text (for copy / paste).

Get 3D geometry BETA

Lig 96



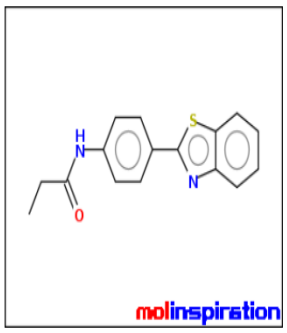
Molinspiration bioactivity score v20

GPCR ligand	0.01
Ion channel modulator	-0.11
Kinase inhibitor	0.16
Nuclear receptor ligand	-0.29
Protease inhibitor	-0.23
Enzyme inhibitor	0.03

Get data as text (for copy / paste).

Get 3D geometry BETA

Lig 97



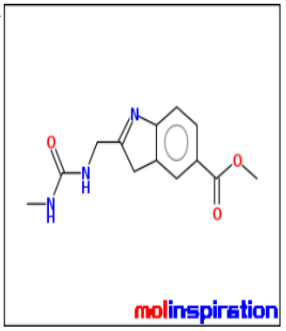
Molinspiration bioactivity score v2021

GPCR ligand	-0.40
Ion channel modulator	-0.63
Kinase inhibitor	-0.07
Nuclear receptor ligand	-0.47
Protease inhibitor	-0.42
Enzyme inhibitor	-0.22

Get data as text (for copy / paste).

Get 3D geometry BETA

Lig 98



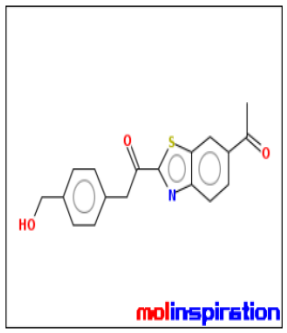
Molinspiration bioactivity score v202

GPCR ligand	-0.22
Ion channel modulator	-0.32
Kinase inhibitor	-0.40
Nuclear receptor ligand	-0.53
Protease inhibitor	-0.41
Enzyme inhibitor	-0.19

Get data as text (for copy / paste).

Get 3D geometry BETA

Lig 100



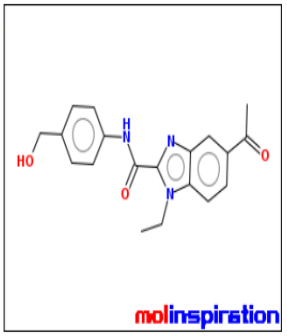
Molinspiration bioactivity score v2021

GPCR ligand	-0.09
Ion channel modulator	-0.17
Kinase inhibitor	-0.04
Nuclear receptor ligand	-0.36
Protease inhibitor	0.16
Enzyme inhibitor	0.13

Get data as text (for copy / paste).

Get 3D geometry BETA

Lig 101



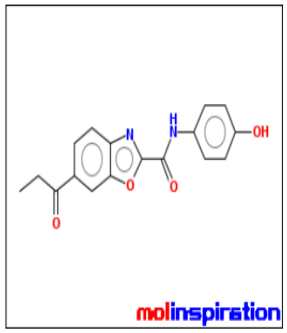
Molinspiration bioactivity score v20

GPCR ligand	0.01
Ion channel modulator	-0.11
Kinase inhibitor	-0.01
Nuclear receptor ligand	-0.23
Protease inhibitor	-0.09
Enzyme inhibitor	-0.04

Get data as text (for copy / paste).

Get 3D geometry BETA

Lig 102



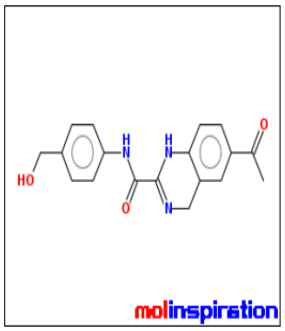
Molinspiration bioactivity score v2021

GPCR ligand	-0.08
Ion channel modulator	-0.16
Kinase inhibitor	-0.14
Nuclear receptor ligand	-0.08
Protease inhibitor	-0.04
Enzyme inhibitor	-0.06

Get data as text (for copy / paste).

Get 3D geometry BETA

Lig 103



Molinspiration bioactivity score v20

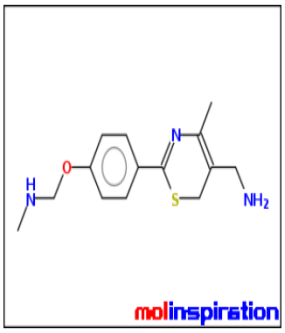
GPCR ligand	-0.00
Ion channel modulator	0.05
Kinase inhibitor	-0.15
Nuclear receptor ligand	-0.36
Protease inhibitor	0.01
Enzyme inhibitor	0.04

Get data as text (for copy / paste).

Get 3D geometry BETA

Lig 105

Figure.no.14: Biological properties of selected *PCSK9* inhibitors



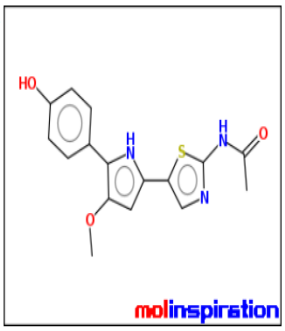
Molinspiration bioactivity score v2021

GPCR ligand	-0.46
Ion channel modulator	-0.52
Kinase inhibitor	-0.81
Nuclear receptor ligand	-0.68
Protease inhibitor	-0.34
Enzyme inhibitor	-0.29

Get data as text (for copy / paste).

Get 3D geometry BETA

Lig119



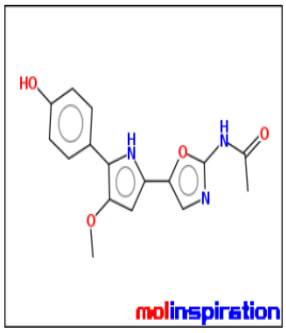
Molinspiration bioactivity score v20

GPCR ligand	0.19
Ion channel modulator	-0.16
Kinase inhibitor	0.63
Nuclear receptor ligand	0.10
Protease inhibitor	-0.27
Enzyme inhibitor	0.23

Get data as text (for copy / paste).

Get 3D geometry BETA

Lig 130



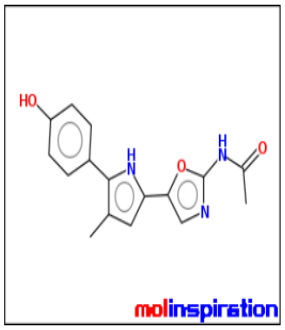
Molinspiration bioactivity score v2021

GPCR ligand	0.32
Ion channel modulator	0.02
Kinase inhibitor	0.50
Nuclear receptor ligand	0.09
Protease inhibitor	-0.22
Enzyme inhibitor	0.39

Get data as text (for copy / paste).

Get 3D geometry BETA

Lig 131



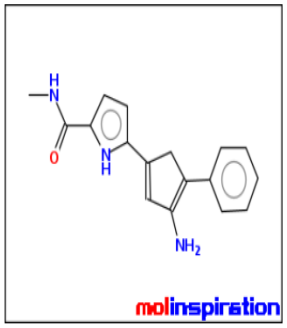
Molinspiration bioactivity score v20

GPCR ligand	0.36
Ion channel modulator	0.05
Kinase inhibitor	0.62
Nuclear receptor ligand	0.17
Protease inhibitor	-0.32
Enzyme inhibitor	0.47

Get data as text (for copy / paste).

Get 3D geometry BETA

Lig 137



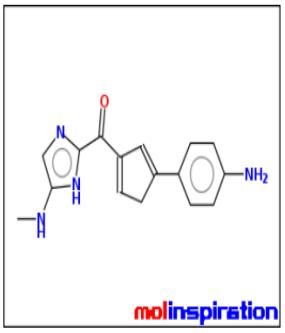
Molinspiration bioactivity score v2021

GPCR ligand	0.09
Ion channel modulator	-0.09
Kinase inhibitor	0.13
Nuclear receptor ligand	-0.11
Protease inhibitor	-0.07
Enzyme inhibitor	0.06

Get data as text (for copy / paste).

Get 3D geometry BETA

Lig 143



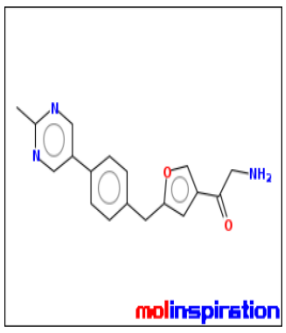
Molinspiration bioactivity score v20

GPCR ligand	0.35
Ion channel modulator	-0.04
Kinase inhibitor	0.41
Nuclear receptor ligand	-0.40
Protease inhibitor	-0.09
Enzyme inhibitor	0.32

Get data as text (for copy / paste).

Get 3D geometry BETA

Lig 145



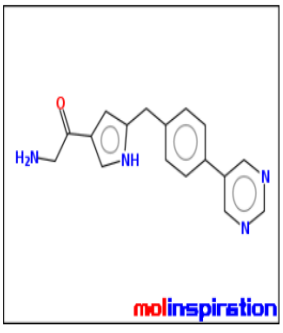
Molinspiration bioactivity score v2021

GPCR ligand	0.36
Ion channel modulator	-0.02
Kinase inhibitor	0.20
Nuclear receptor ligand	-0.18
Protease inhibitor	0.15
Enzyme inhibitor	0.39

Get data as text (for copy / paste).

Get 3D geometry BETA

Lig 149



Molinspiration bioactivity score v20

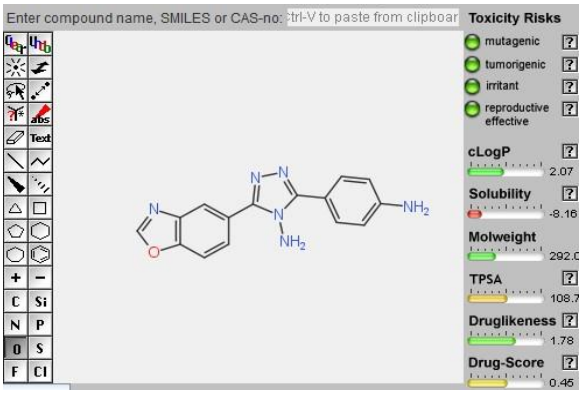
GPCR ligand	0.30
Ion channel modulator	0.17
Kinase inhibitor	0.56
Nuclear receptor ligand	-0.11
Protease inhibitor	0.21
Enzyme inhibitor	0.53

Get data as text (for copy / paste).

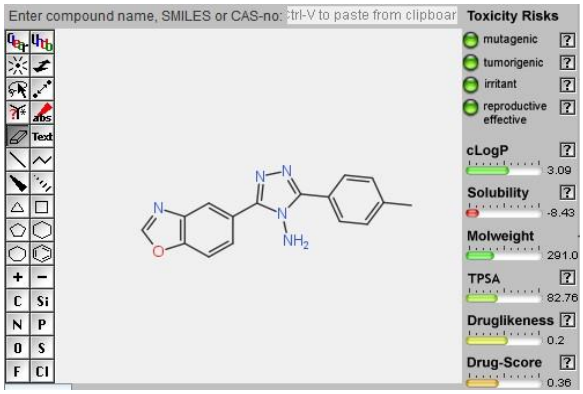
Get 3D geometry BETA

Lig 150

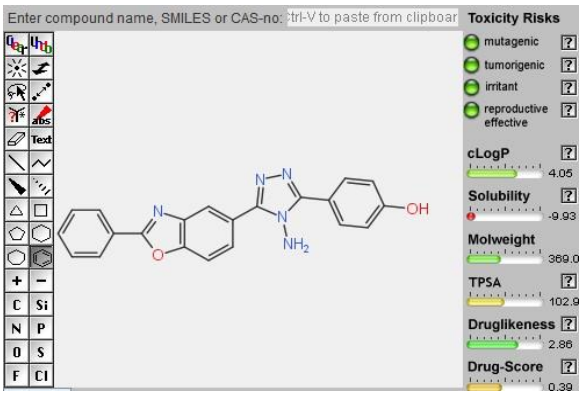
Figure.no.14: Biological properties of selected *PCSK9* inhibitors



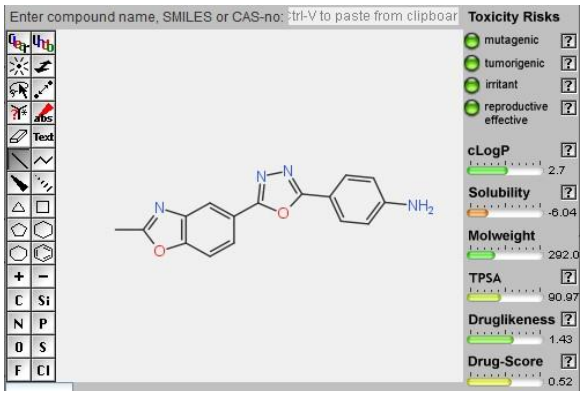
Lig 1



Lig 3



Lig 5



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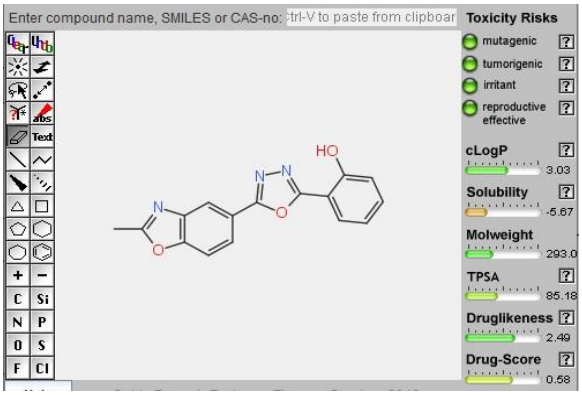
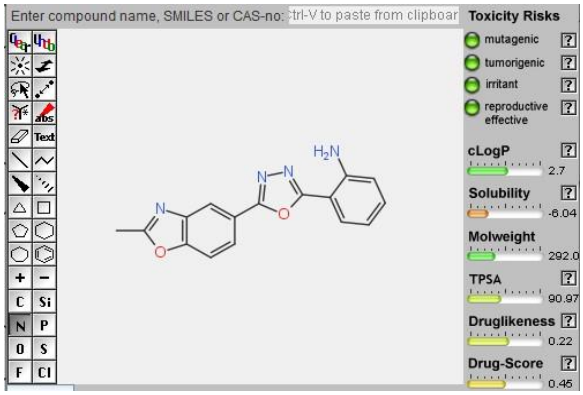
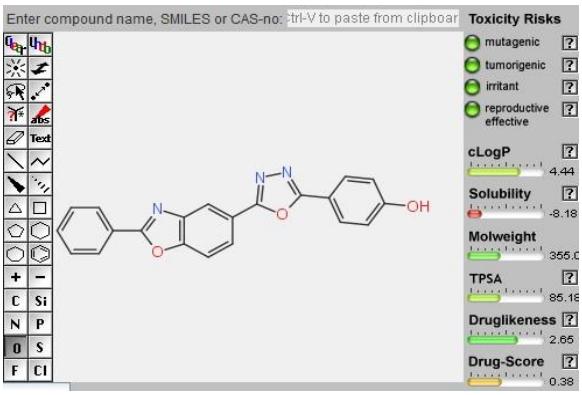


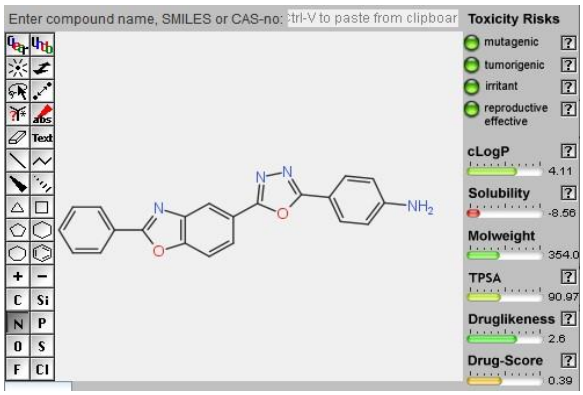
Lig 8



Lig 10

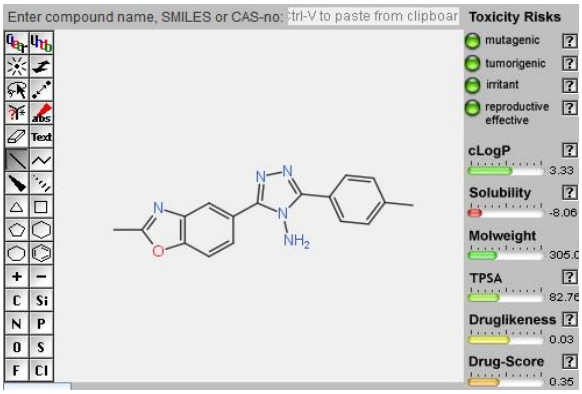


Lig 11

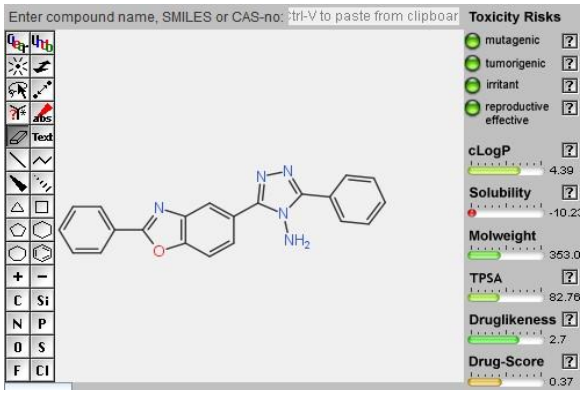


Lig 12

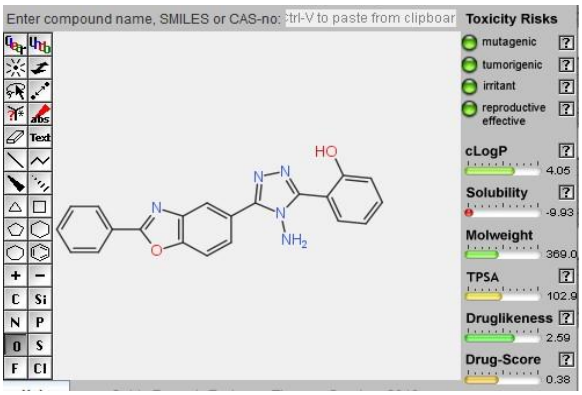
Figure.no.15: Toxicity profile of selected *PCSK9* inhibitors



Lig 13



Lig 14



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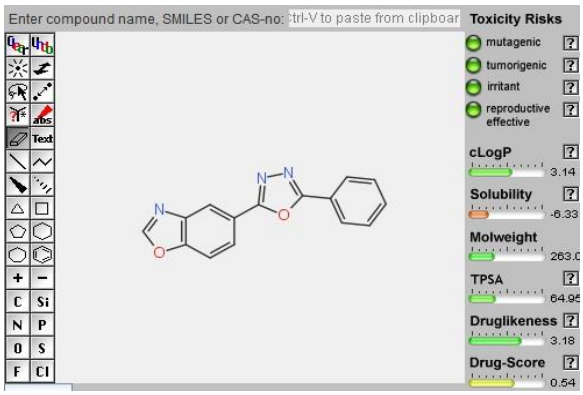

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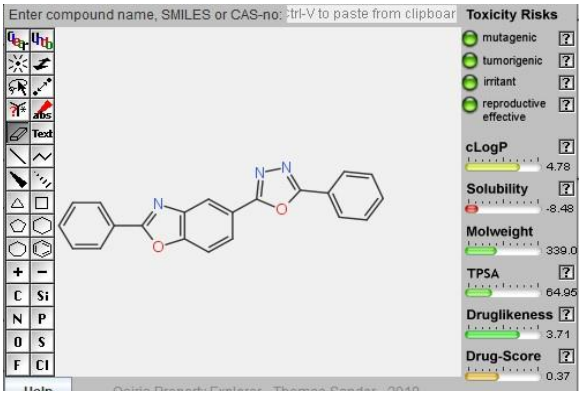

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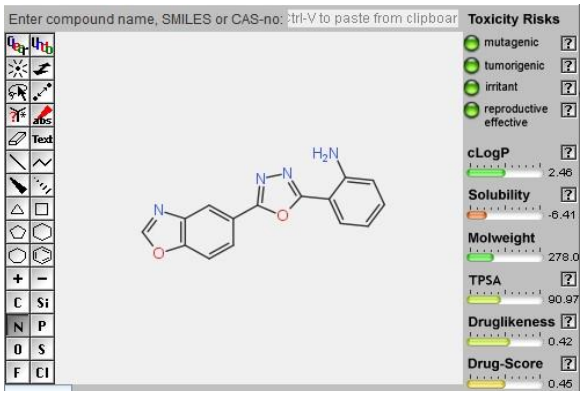

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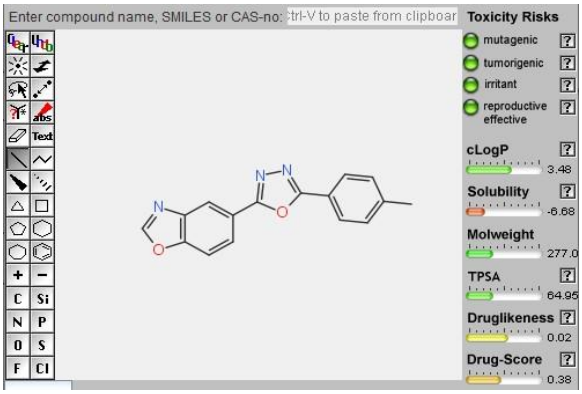

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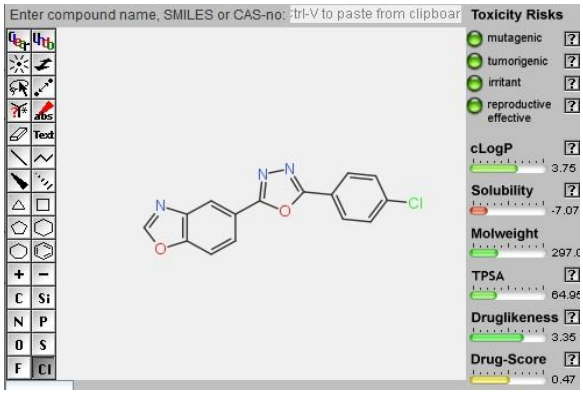
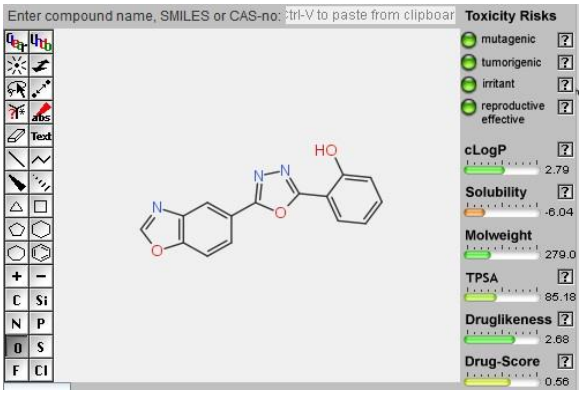


Lig 20

Figure.no.15: Toxicity profile of selected *PCSK9* inhibitors



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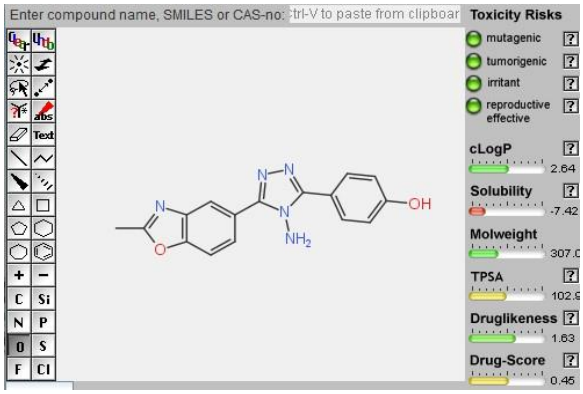

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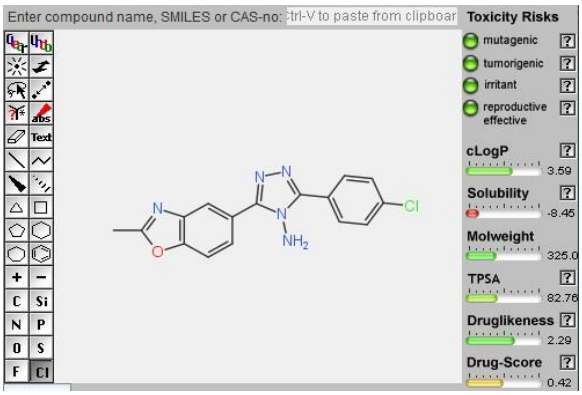

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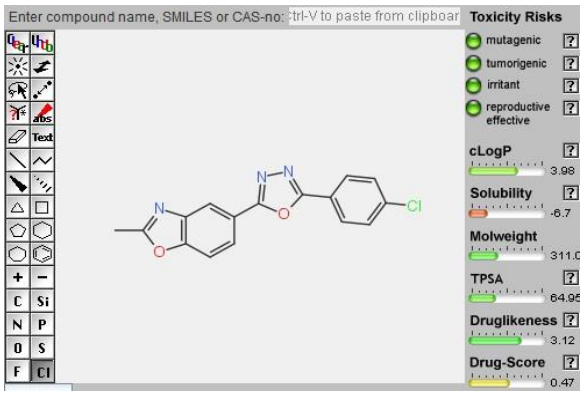

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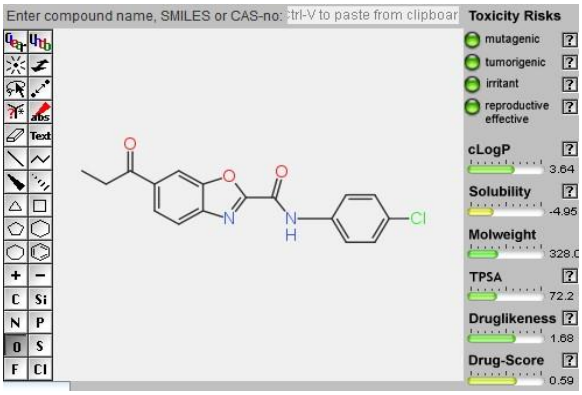

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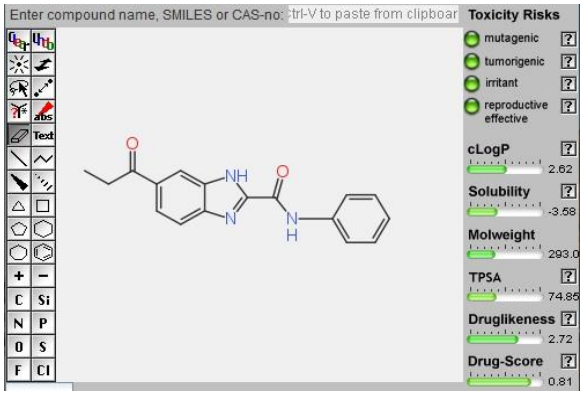

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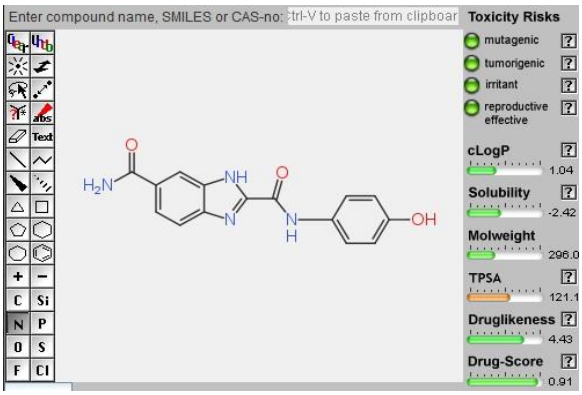

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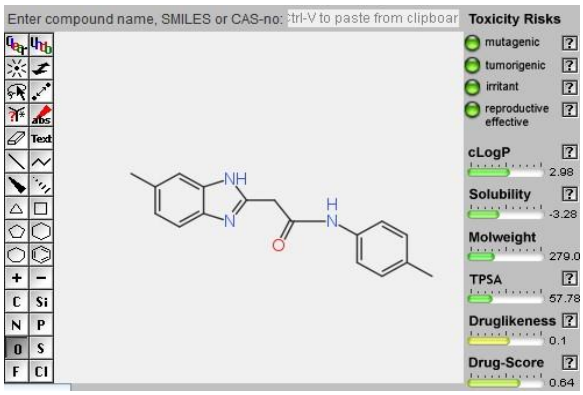

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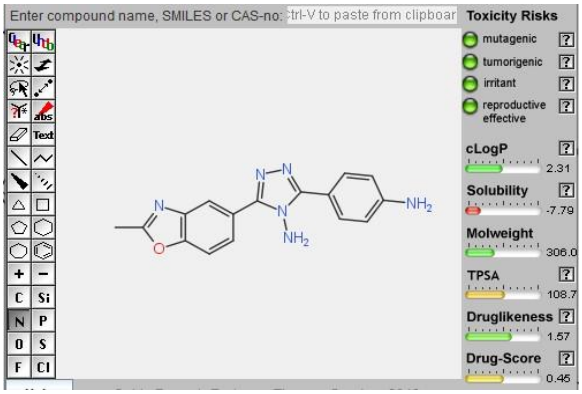
Figure.no.15: Toxicity profile of selected *PCSK9* inhibitors



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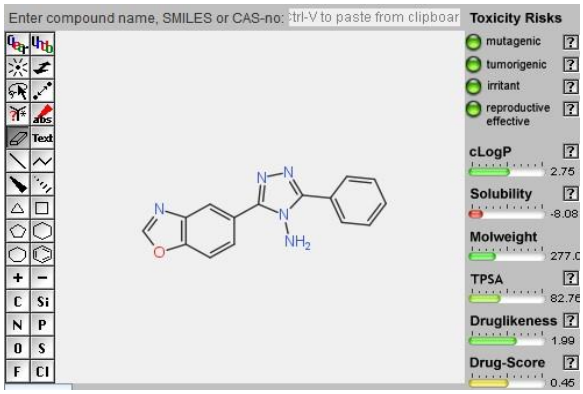

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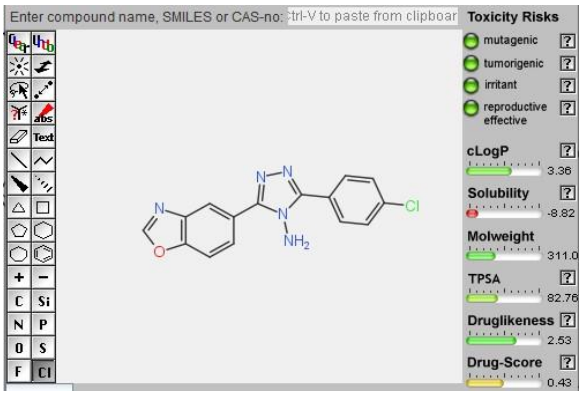

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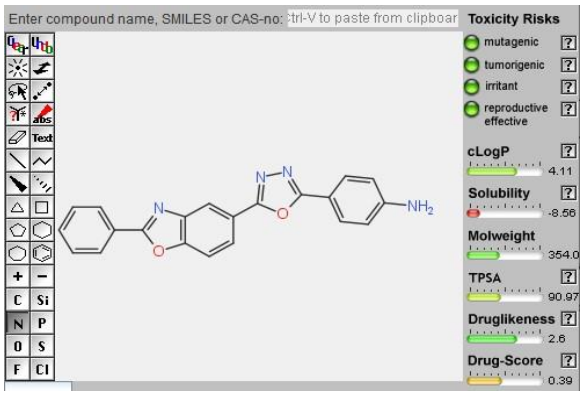

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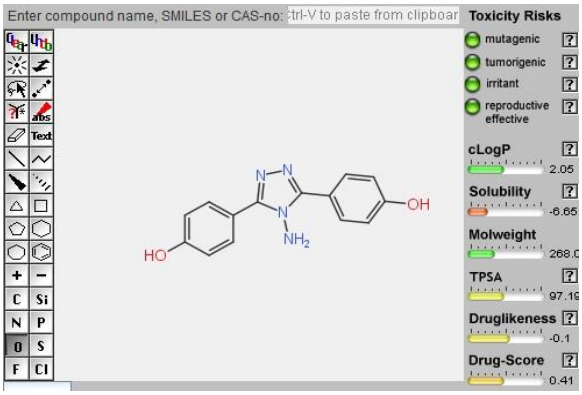

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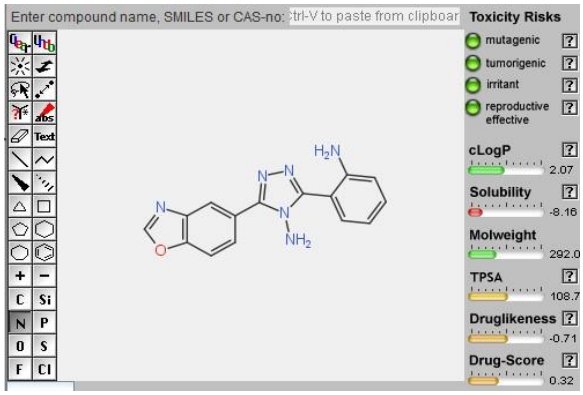

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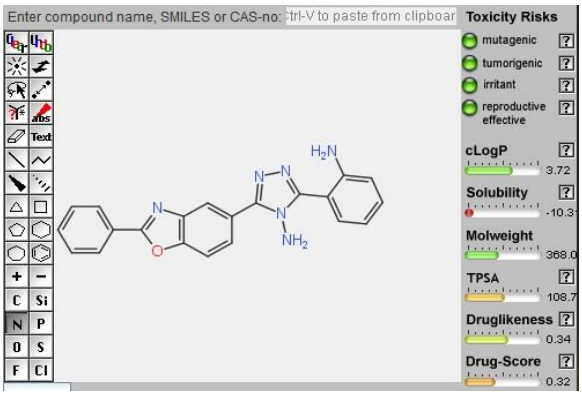

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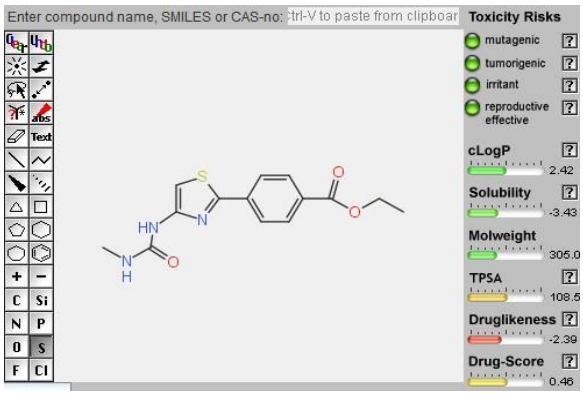
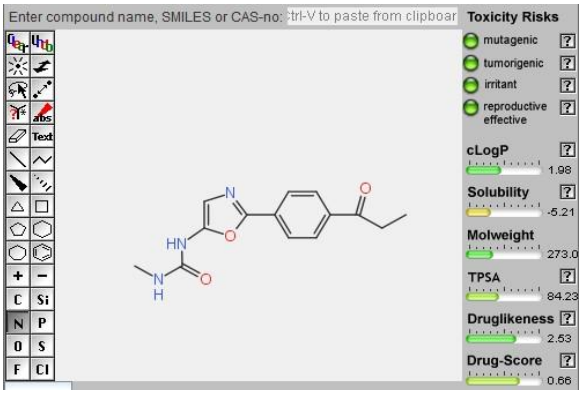


Lig 52

Figure.no.15: Toxicity profile of selected *PCSK9* inhibitors



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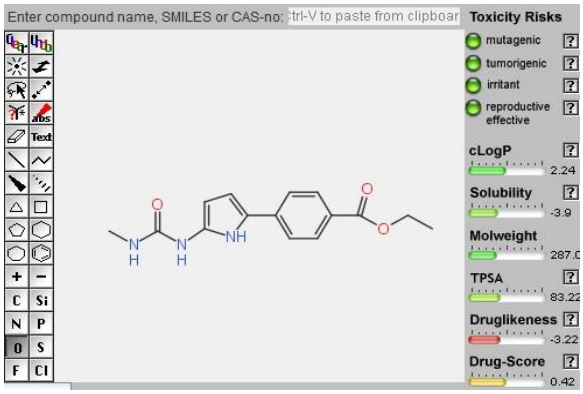

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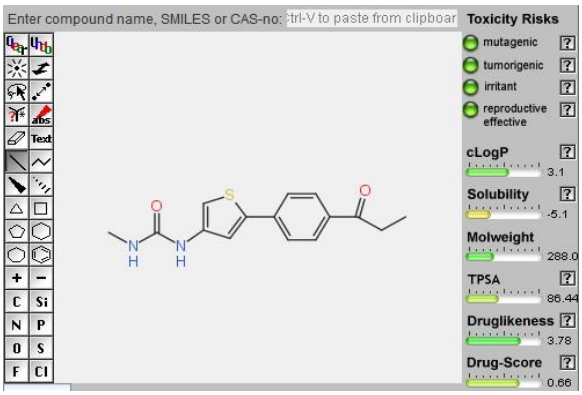

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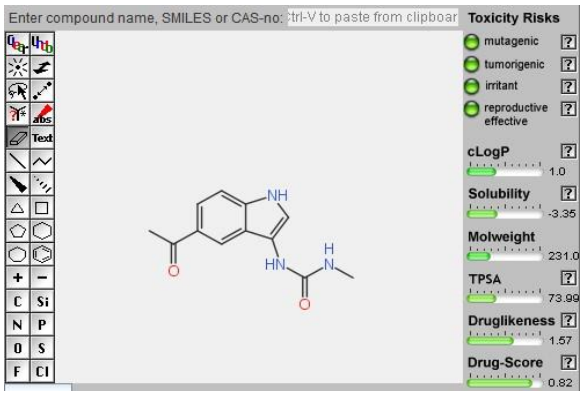

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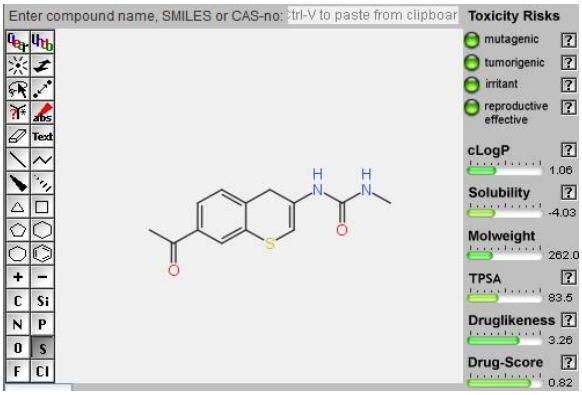

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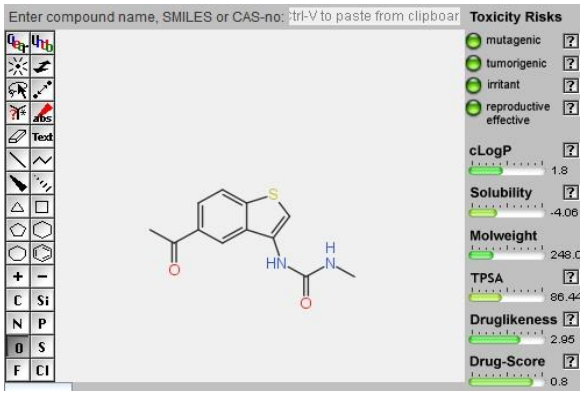

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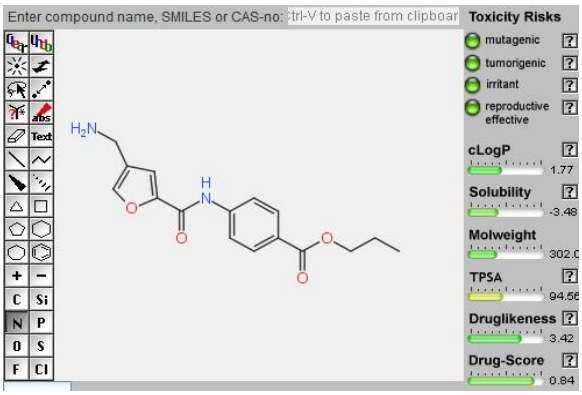

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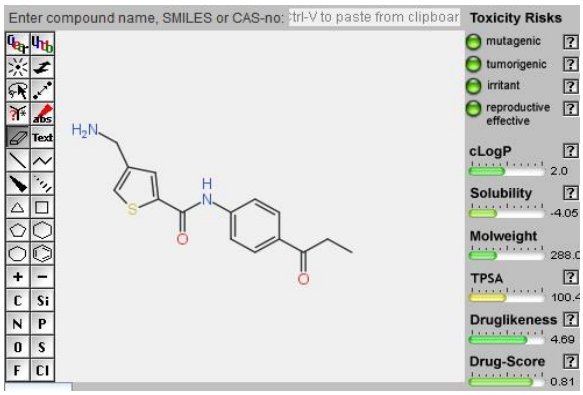
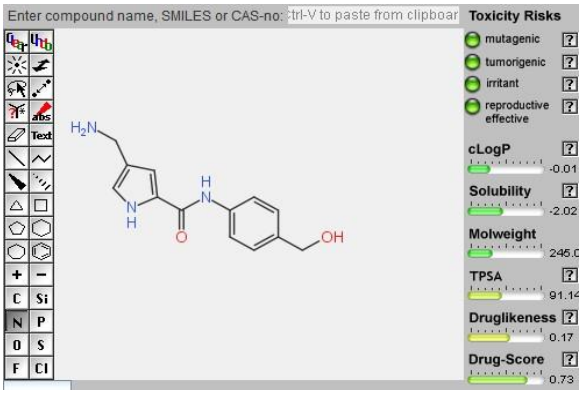


Lig 76

Figure.no.15: Toxicity profile of selected *PCSK9* inhibitors



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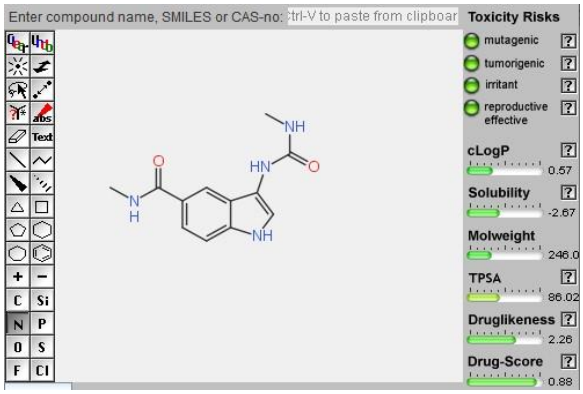

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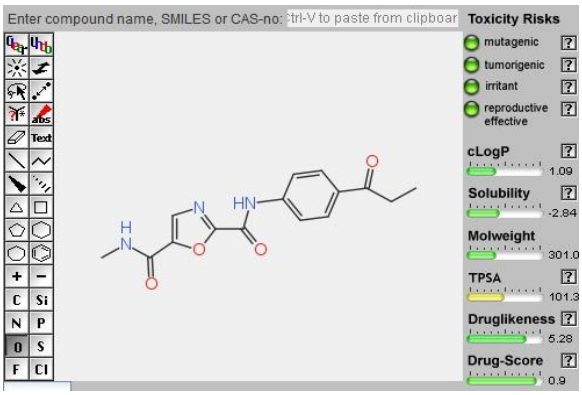

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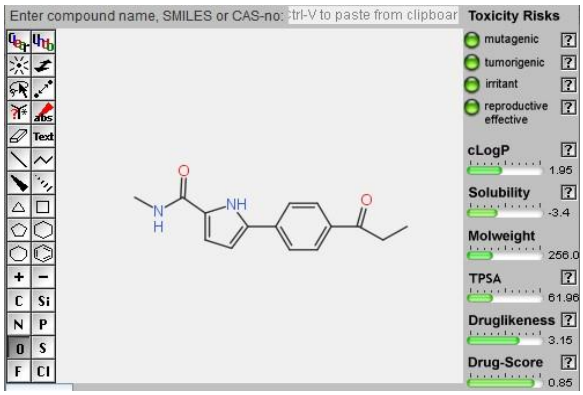

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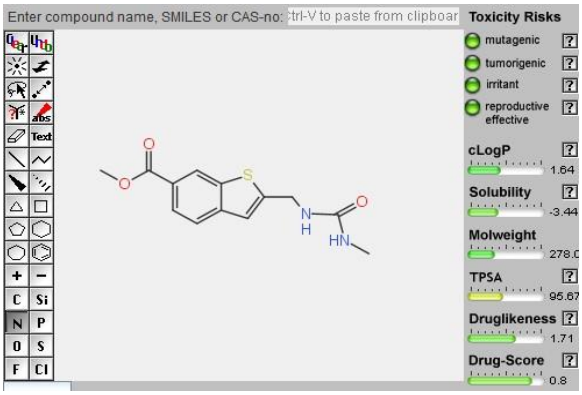

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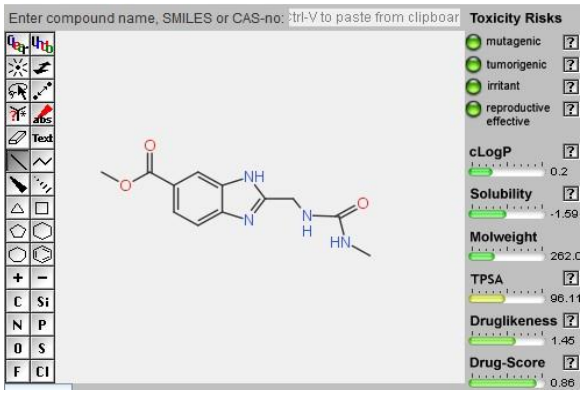

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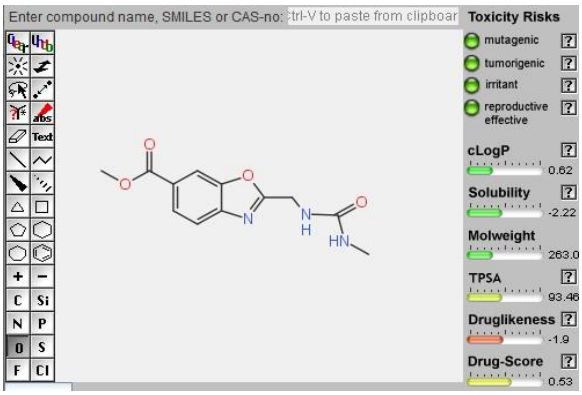

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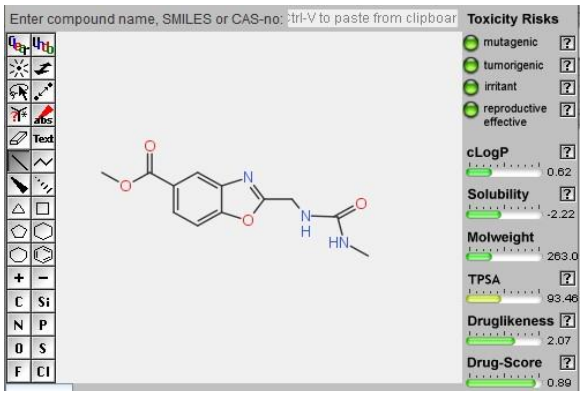
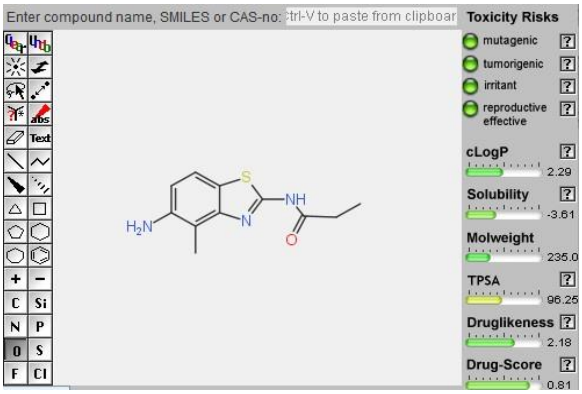


Lig 94

Figure.no.15: Toxicity profile of selected *PCSK9* inhibitors



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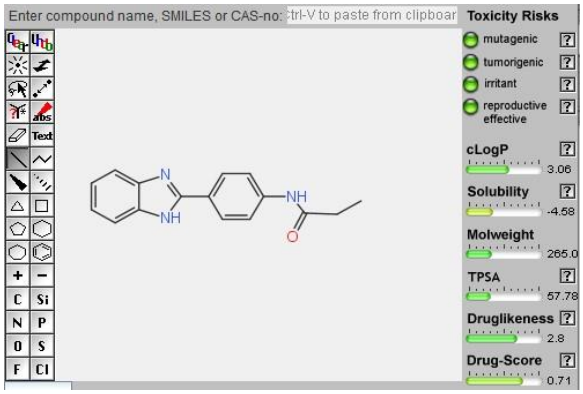

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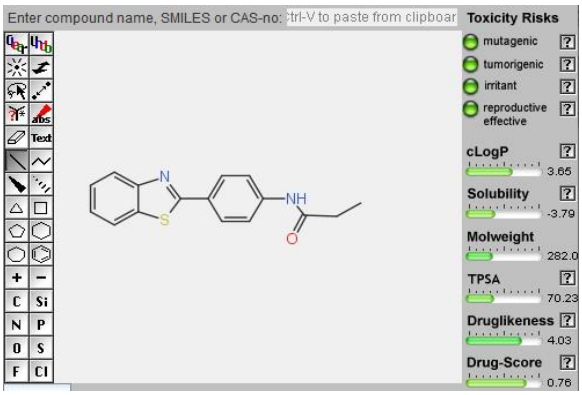

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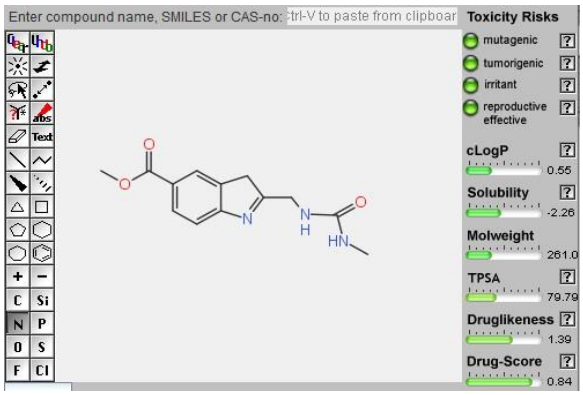

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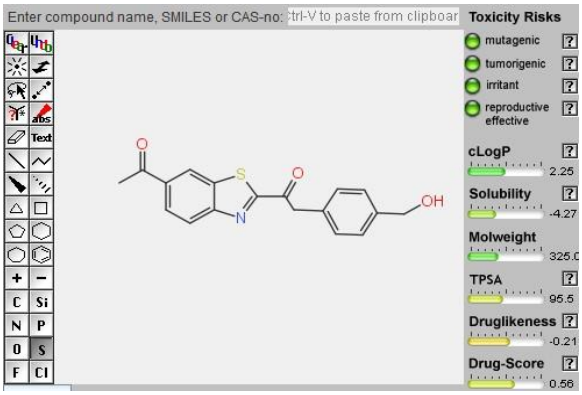

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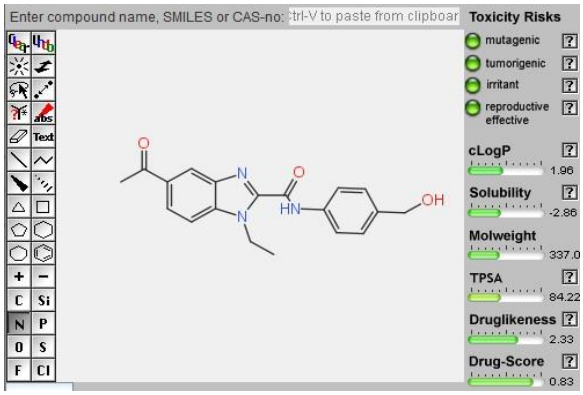

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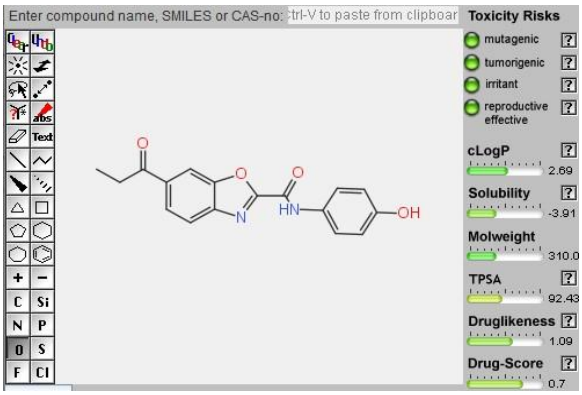

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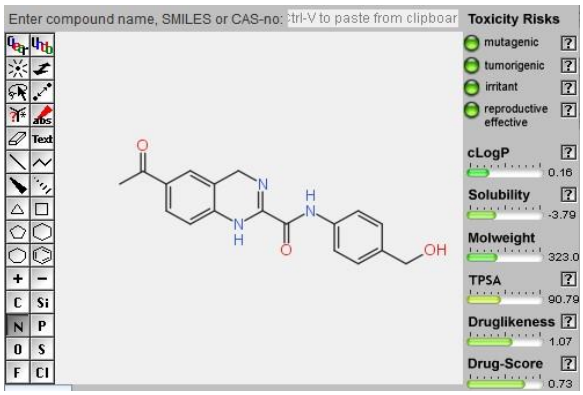
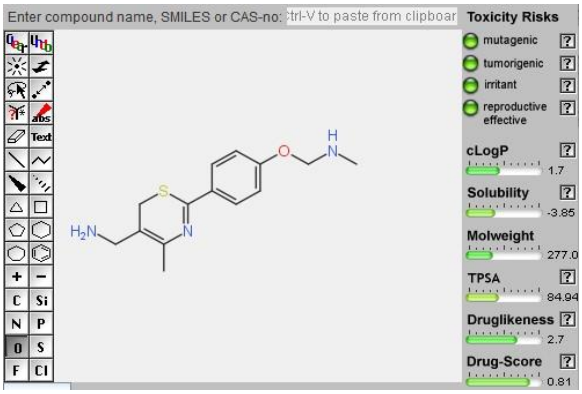


Lig 105

Figure.no.15: Toxicity profile of selected *PCSK9* inhibitors



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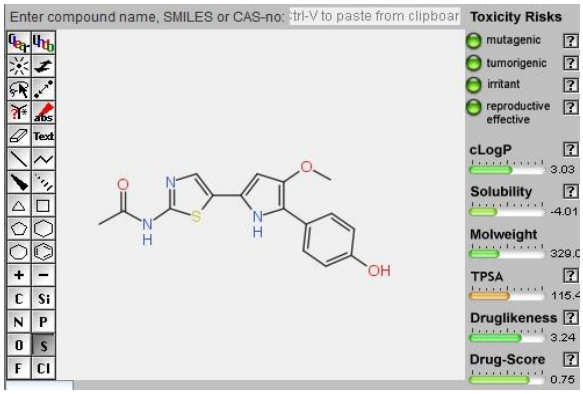

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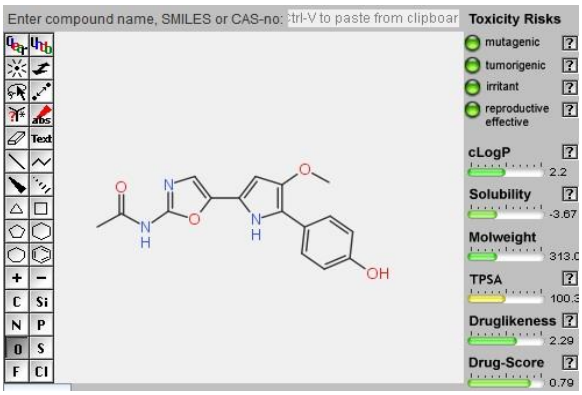

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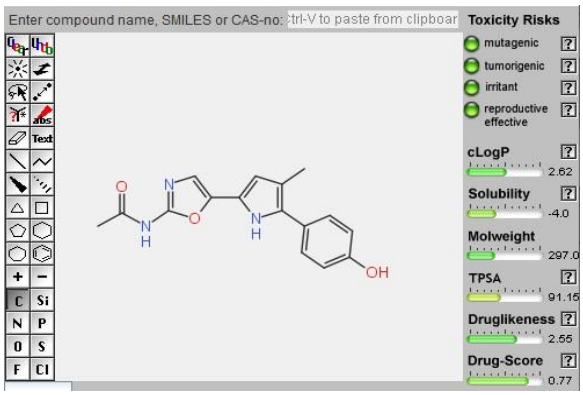

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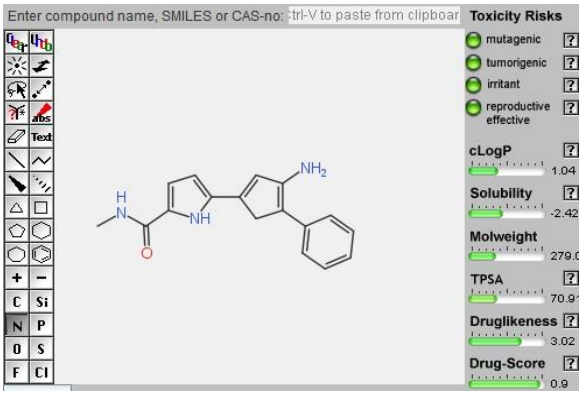

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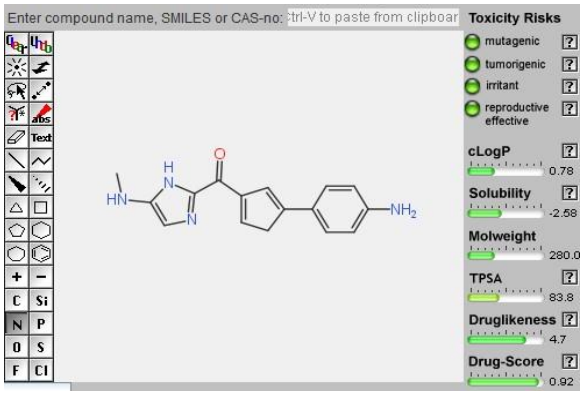

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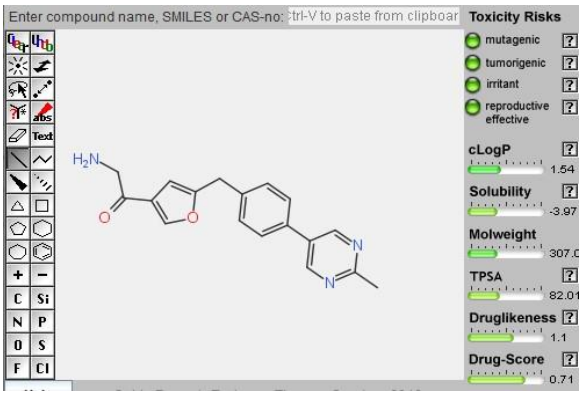

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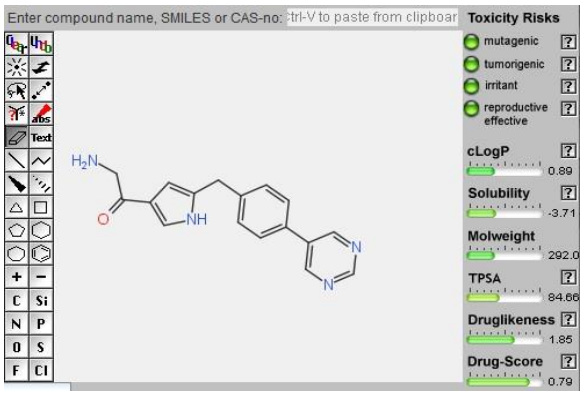


Lig 150

Figure.no.15: Toxicity profile of selected *PCSK9* inhibitors

Thus the proposed ligands like **ligand 7, ligand 8, ligand 10, ligand 12, and ligand 13** for synthesis have satisfied all the above filtering method of good predictive activity with good docking scores and also drug likeliness properties confirming that these molecules are accepted to be orally bioavailable.

V. B. SYHTHESIS AND CHARECTERIZATION

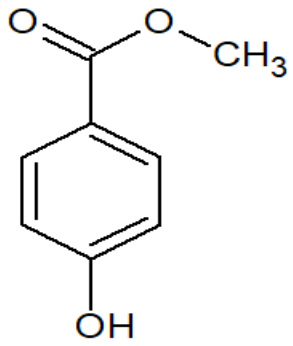
MATERIALS AND METHODS

V.B.1. SYNTHESIS

The ligands with top docking score and synthetic feasibility were selected for synthesis. All reagents and solvents used were of analytical grade.

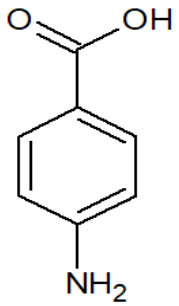
Chemicals used for synthesis

PARA HYDROXY METHYL BENZOATE



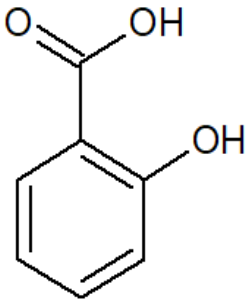
Molecular formula	:	$C_7H_7NO_2$
Molecular weight	:	152.15g/mol
Description	:	white crystalline powder
Melting point	:	125-128^{0}C
Boiling point	:	275^{0}C

PARA AMINO BENZOIC ACID



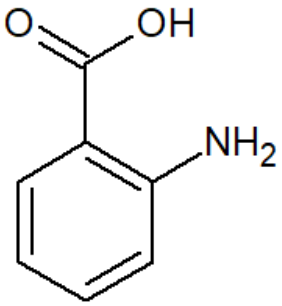
Molecular formula	:	$C_7H_7NO_2$
Molecular weight	:	137.14g/mol
Description	:	white crystalline powder
Melting point	:	187^0C
Boiling point	:	340^0C

SALICYLIC ACID



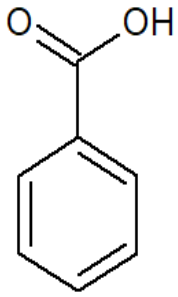
Molecular formula	:	$C_7H_6O_3$
Molecular weight	:	138.12g/mol
Description	:	white to off-white crystalline poeder
Melting point	:	159^0C
Boiling point	:	211^0C

ANTHRANILIC ACID



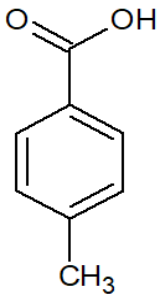
Molecular formula	:	$C_7H_7NO_2$
Molecular weight	:	137.14g/mol
Description	:	white solid
Melting point	:	144-148^{0}C
Boiling point	:	200^{0}C

BENZOIC ACID



Molecular formula	:	$C_7H_6O_2$
Molecular weight	:	122.123g/mol
Description	:	white solid
Melting point	:	122^{0}C
Boiling point	:	250^{0}C

TOLUIC ACID



Molecular formula	:	$C_8H_8O_2$
Molecular weight	:	136.15g/mol
Description	:	white solid
Melting point	:	180-181^{0}C
Boiling point	:	274-275^{0}C

SYNTHETIC METHODOLOGY

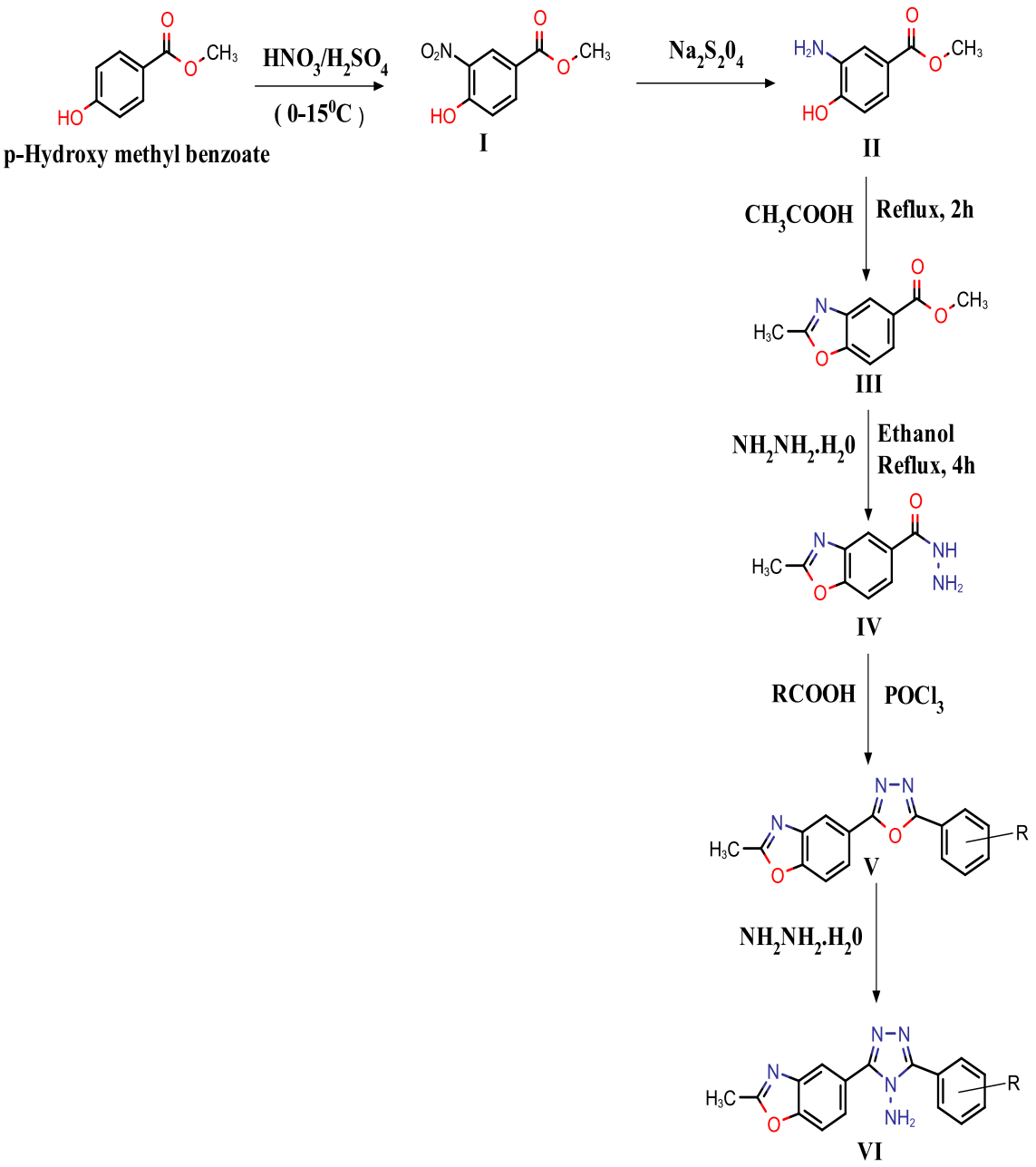


Figure.no.20: Scheme

Procedure

STEP1: Synthesis of 4-Hydroxy-3-nitro-benzoic acid methyl ester (I):

4- hydroxyl methyl benzoate (10g, 0.74 mol) was placed in a round bottomed flask equipped with reflux condenser and thermometer. A mixture of concentrated sulphuric acid (6.2 ml) and nitric acid (6.2 ml) was added drop wise in p- hydroxyl methyl benzoate with stirring. Cool the flask in ice bath to 0-10^{0}C. Then pour the reaction mixture into crushed ice. Filtered off the crude product m- nitro-p-hydroxy-methyl benzoate and washed with cold water. Transfer the solids into 500 ml flask and stirred it with ice cold methanol in order to remove a small amount of ortho isomer and other impurities. The mixture was filtered and recrystallized using ethanol as solvent. The purity of product was established by single spot-on TLC. The percentage yield was found to be 85%.

STEP2: Synthesis of 3- Amino-4-hydroxy-benzoic acid methyl ester (II):

In a 500 ml three necked flask bottom flask equipped with reflux condenser with guard tube, compound I (10g) was dissolved in boiling alcohol (50%, 100 ml) and sodium dithionite was added to this boiling alcohol solution until it becomes almost colourless. Then the alcohol was reduced to one third of its volume by distillation and the residual liquid was triturated with ice cold water. The resulting product was filtered, washed with cold water, dried and recrystallized using ethanol as solvent. The purity of product was established by single spot-on TLC. The percentage yield was found to be 83%

STEP3: Synthesis of 2- substituted benzoxazole-5-carboxylic acid methyl ester (III)

Compound II (0.01 mol) was heated with an appropriate aliphatic acid in excess under reflux for 2 hours. The reaction mixture was poured in crushed ice with stirring. The product thus separated was filtered and washed with cold water. The products were recrystallized by using ethanol as solvent. The purity of product was established by single spot-on TLC. The percentage yield was found to be 86%

STEP4: Synthesis of 2- substituted benzoxazole-5-carboxylic acid hydrazide (IV)

A mixture of an appropriate 2- substitutedbenzoxazole-5-carboxylic acid methyl ester III (0.001 mol) in alcohol (25 ml) and hydrazine hydrate (99%, 0.015 mol) was heated under reflux on water bath for 4 hours. The alcohol was reduced to half of its volume and cooled.

The product separated was filtered and washed with small portions of cold alcohol and then with cold water repeatedly and dried. The resultant product was recrystallized using ethanol as solvent. The purity of product was established by single spot-on TLC. The percentage yield was found to be 74%.

STEP5: Synthesis of 5-(5-phenyl-1,3,4 oxadiazol-2-yl)-benzo(d)oxazole derivatives (V)

Compound IV (0.01 mol), aromatic acids (0.01 mol), Phosphorus oxychloride was refluxed for about 5-6 hrs. Then the mixture was made alkaline with sodium bicarbonate. The product was dried and recrystallized using ethanol. The purity of product was established by single spot-on TLC.

STEP6: Synthesis of 3-(benzo(d)oxazol-5-yl-)-5-phenyl-4H-1,2,4 -triazole-4-amine derivatives (VI)

Compound (V) (0.01 mol) was treated with hydrazine hydrate (0.015 mol) and refluxed for about 2 hrs in dry pyridine (15ml). Then neutralized with hydrochloric acid. The obtained corresponding amino triazoles were filtered, dried and recrystallized with ethanol. The purity of this product was established by single spot on the TLC. The purity of product was established by single spot-on TLC.

V.B.2. CHARECTERIZATION

The purity of the synthesized compounds was checked by TLC method and observance of the sharp melting point. Progress of the reactions was monitored by thin layer chromatographic technique. TLC was performed using Aluminum plates pre-coated with Silica gel 60F 254 (E-Merck); the mobile phase used for all synthesized compound was Methanol: $CHCl_3$ in the ratio of 9:1. Spots were detected using UV light chamber and Iodine chamber (Solvent system- methanol,). Melting points of all the synthesized compounds were determined by open-capillary tubes and values were uncorrected. All the synthesized compounds were characterized by the following instrumental methods.

- IR spectroscopy by ABB MB3000-PH FT-IR Spectrometer
- ^{1}H-NMR spectroscopy by BRUKER Topspin Advance 400MHZ NMR Spectra using Deuterated DMSO
- LC-MS spectrometry by Waters - Xevo G2- XS - QToF High Resolution Mass spectrometer

IR SPECTROSCOPY

IR spectroscopy (which is short for infrared spectroscopy) deals with the infrared region of the electromagnetic spectrum, i.e. light having a longer wavelength and a lower frequency than visible light. Infrared Spectroscopy generally refers to the analysis of the interaction of a molecule with infrared light. The major use of infrared spectroscopy is to determine the functional groups of molecules, relevant to both organic and inorganic chemistry.

Regions of the Infrared spectrum

Most of the bands that indicate what functional group is present are found in the region from 4000 $cm^{-1}$to 1300 cm^{-1}. Their bands can be identified and used to determine the functional group of an unknown compound.

Bands that are unique to each molecule, similar to a fingerprint, are found in the fingerprint region, from 1300 cm^{-1} to 400 cm^{-1}. These bands are only used to compare the spectra of one compound with another.

MASS SPECTROMETRY

Mass spectrometry is an analytical technique used to establish the molecular structure and the molecular weight of the analyte under investigation. In this technique, the compound under investigation is bombarded with a beam of electrons producing ionic fragments of the original species. The relative abundance of the fragment ion formed depends on the stability of the ion and of the lost radical. The resulting charged particles are then separated according to their masses. Mass spectrum is a record of information regarding various masses produced and their relative abundances.

HYPHENATED TECHNIQUES

A technique where a separation technique is coupled with an online spectroscopic detection technology is known as hyphenated technique. Chromatography produces pure or nearly pure fractions of chemical components in a mixture. Spectroscopy produces selective information for identification using standards or library spectra. These hyphenated techniques offer shorter analysis time, higher degree of automation, higher sample throughput, better reproducibility, reduction of contamination because it is a closed system, enhanced combined selectivity and therefore higher degree of information.

Various hyphenated techniques:

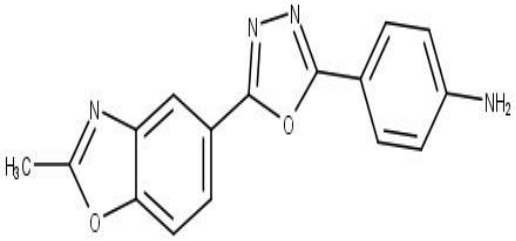
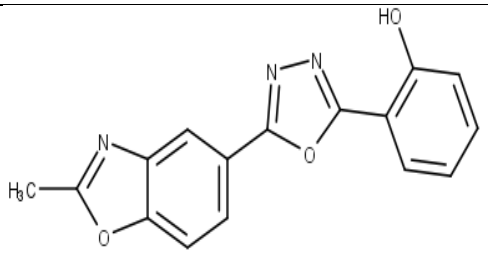
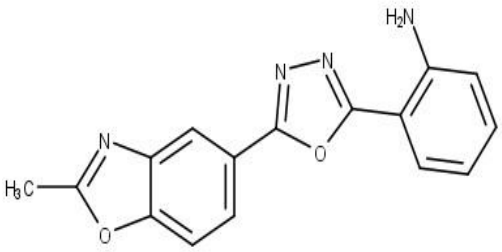
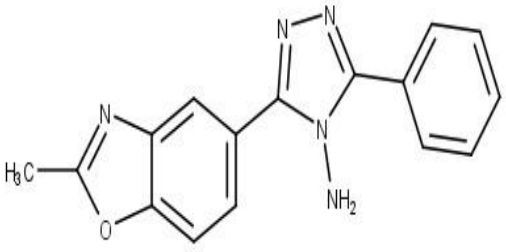
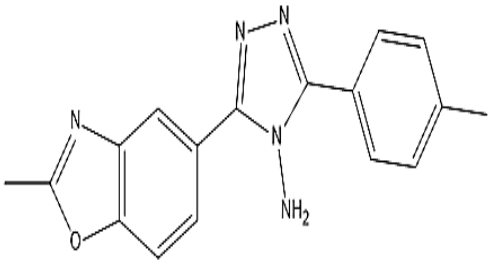
- GC-MS
- LC-MS
- LC-FTIR
- LC-NMR
- CE-MS

Liquid Chromatography–Mass Spectrometry (LC-MS)

LC-MS is an analytical chemistry technique that combines the physical separation capabilities of liquid chromatography (or HPLC) with the mass analysis capabilities of mass spectrometry (MS). Coupled chromatography - MS systems are popular in chemical analysis because the individual capabilities of each technique are enhanced synergistically. While liquid chromatography separates mixtures with multiple components, mass spectrometry provides structural identity of the individual components with high molecular specificity and detection sensitivity.

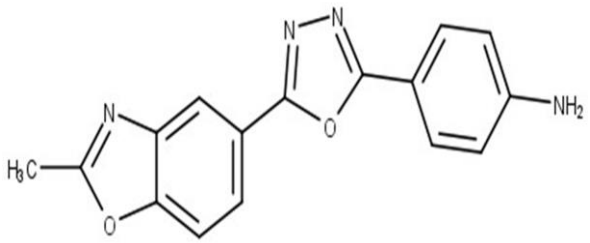
RESULTS AND DISCUSSION

Table.no.7: List of compounds synthesized

Compound Code	IUPAC Name	Structure
VD1	4- [5- (2- methyl- 1,3- benzoxazol- 5- yl)- 1,3,4- oxadiazol- 2- yl]aniline	
VD2	2- [5- (2- methyl- 1,3- benzoxazol- 5- yl)- 1,3,4- oxadiazol- 2- yl]phenol	
VD3	2- [5- (2- methyl- 1,3- benzoxazol- 5- yl)- 1,3,4- oxadiazol- 2- yl]aniline	
VD4	3- (2- methyl- 1,3- benzoxazol- 5- yl)- 5- phenyl- 4H- 1,2,4- triazol- 4- amine	
VD5	3- (2- methyl- 1,3- benzoxazol- 5- yl)- 5- (4- methylphenyl)- 4H- 1,2,4- triazol- 4- amine	

PRODUCT PROFILE

VD1



4- [5- (2- methyl- 1,3- benzoxazol- 5- yl)- 1,3,4- oxadiazol- 2- yl]aniline

Molecular Formula	:	$C_{16}H_{12}N_4O_2$
Formula Weight	:	292.29208
Appearance	:	Yellow
Melting Point	:	172^0C
Rf Value	:	0.73
Yield	:	75.36%
Composition	:	C(65.75%) H(4.14%) N(19.17%) O(10.95%)
Molar refractivity	:	81.21 ± 0.3 cm^3
Molar volume	:	217.9 ± 3.0 cm^3
Parachor	:	611.7 ± 4.0 cm^3
Index of Refractivity	:	1.667 ± 0.02
Surface Tension	:	62.0 ± 3.0 dyne/cm
Polarizability	:	32.19 ± 0.5 $10^{-24}cm^3$

INFRARED SPECTRUM OF VD1:

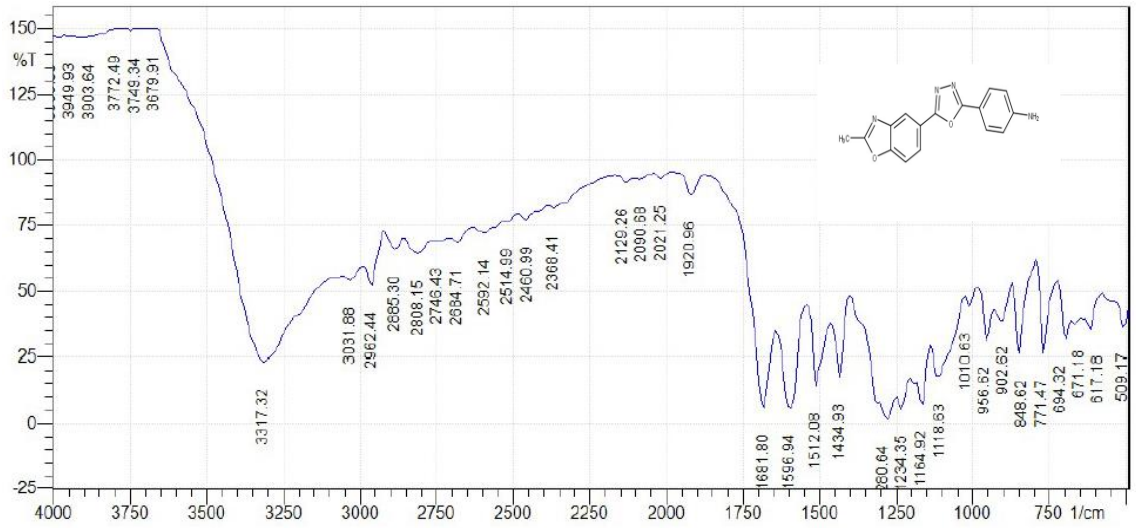


Figure.no.17: IR Spectrum of Compound VD1

Table.no.8: IR Interpretation of Compound VD1

S.NO	WAVE NUMBER (cm^{-1})	FUNCTIONAL GROUPS
1	1681	C=N Stretching
2	1596	-C=C-
3	3317	NH2 Stretching
4	2885	SP_2-C-H stretching
5	1234	C-O-C Stretching

LC-MS SPECTRUM OF VD1:

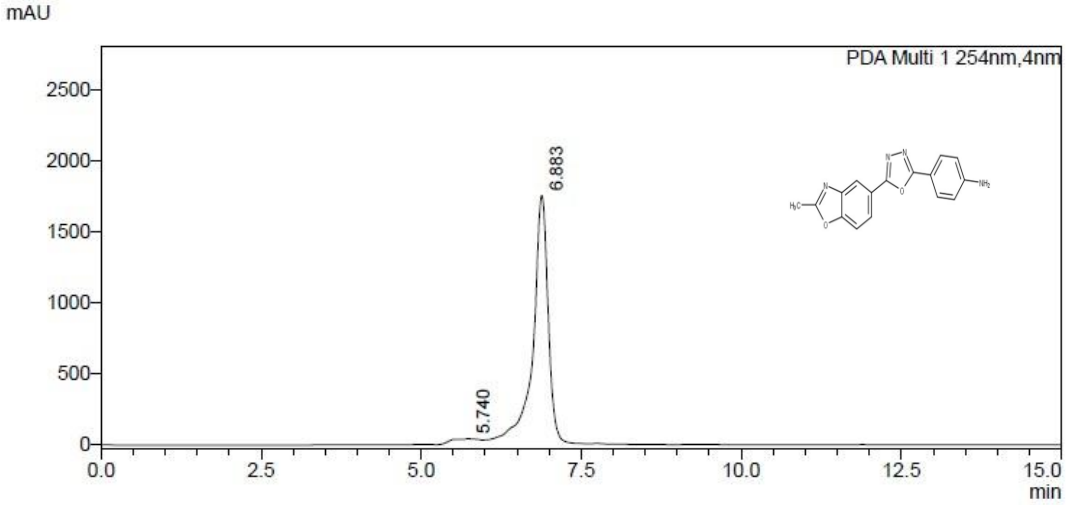


Figure.no.18: Chromatogram of Compound VD1

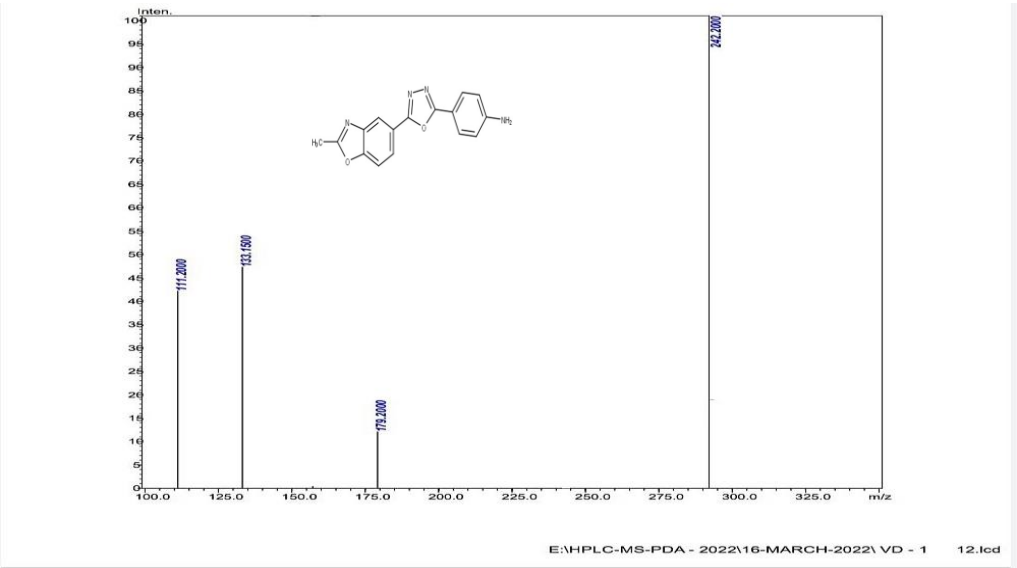


Figure.no.19: Mass Spectrum of Compound VD1

PROTON NMR SPECTRUM OF VD1:

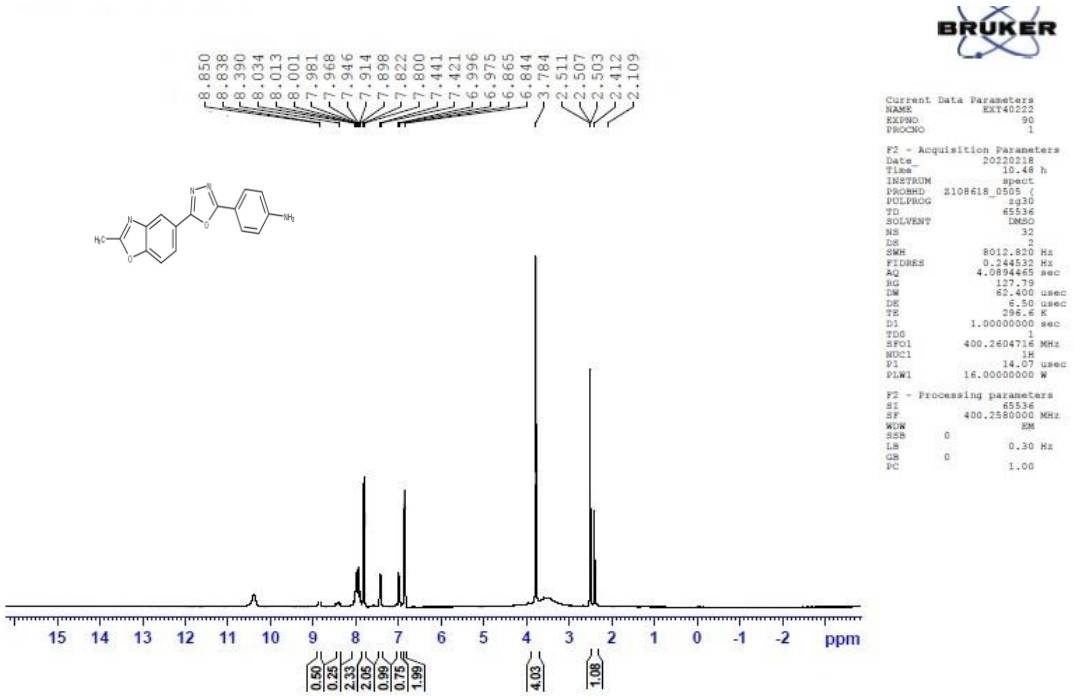
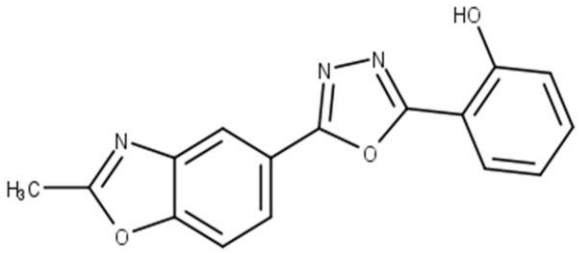


Figure.no.20: ^{1}H NMR Spectrum of Compound VD1

Table.no.9: ^{1}H NMR Interpretation of Compound VD1

S.NO	DELTA VALUE (PPM)	NATURE OF PROTON	NATURE OF PEAK	NUMBER OF PROTONS
1	2.4	Methyl C-H	Singlet	3
2	7.4-8.5	Aromatic C-H	Multiplet	7
3	6.8	NH_2	Singlet	2

VD2



4- 2- [5- (2- methyl- 1,3- benzoxazol- 5- yl)- 1,3,4- oxadiazol- 2- yl]phenol

Molecular Formula	:	$C_{16}H_{11}N_3O_3$
Formula Weight	:	293.27684
Appearance	:	Yellow
Melting Point	:	177^0C
Rf Value	:	0.77
Yield	:	78.36%
Composition	:	C(65.53%) H(3.78%) N(14.33%) O(16.37%)
Molar refractivity	:	78.85 ± 0.3 cm^3
Molar volume	:	214.0 ± 3.0 cm^3
Parachor	:	600.8 ± 4.0 cm^3
Index of Refractivity	:	1.658 ± 0.02
Surface Tension	:	62.0 ± 3.0 dyne/cm
Polarizability	:	31.26 ± 0.5 $10^{-24}cm^3$

INFRARED SPECTRUM OF VD2:

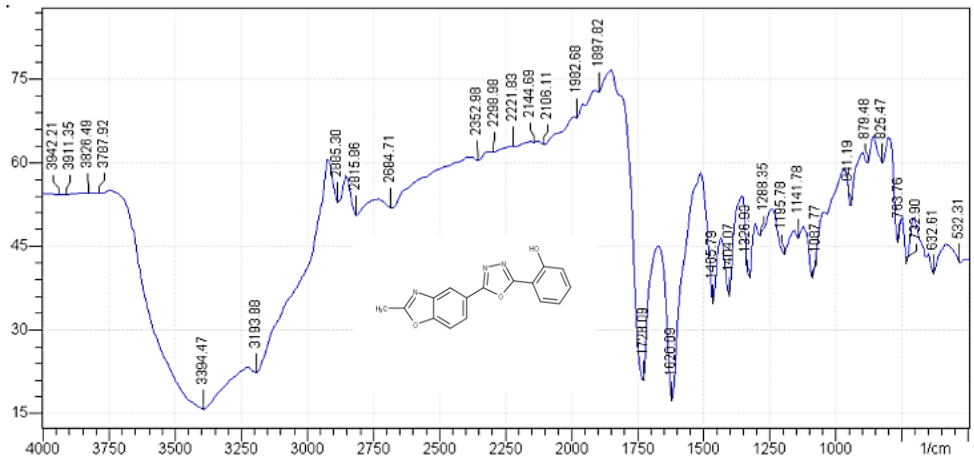


Figure.no.20: IR Spectrum of Compound VD2

Table.no.10: IR Interpretation of Compound VD2

S.NO	WAVE NUMBER (cm^{-1})	FUNCTIONAL GROUPS
1	1620	C=N Stretching
2	1589	-C=C-
3	3394	OH Stretching
4	2815	SP_2.C-H Stretching
5	1288	C-O-C Stretching

LC-MS SPECTRUM OF VD2:

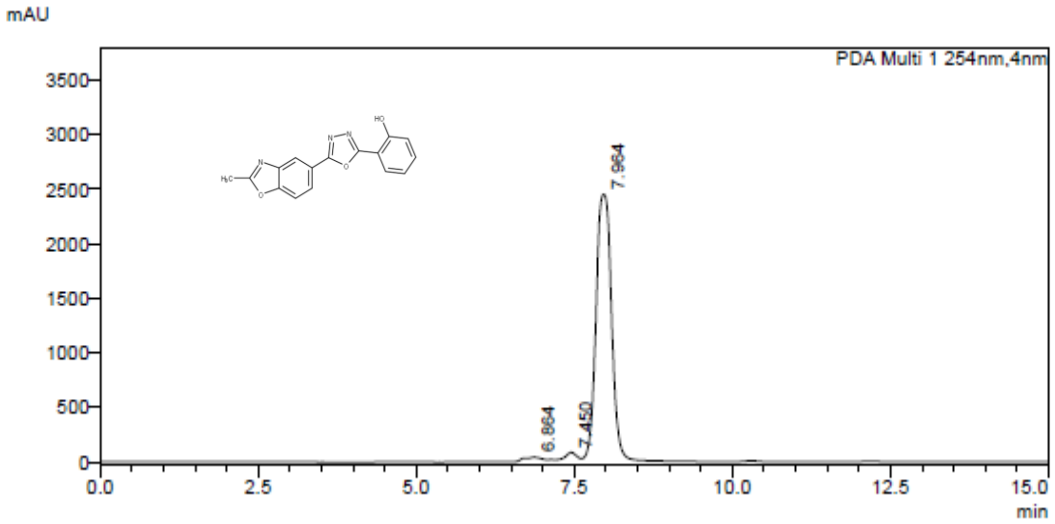


Figure.no.22: Chromatogram of Compound VD2

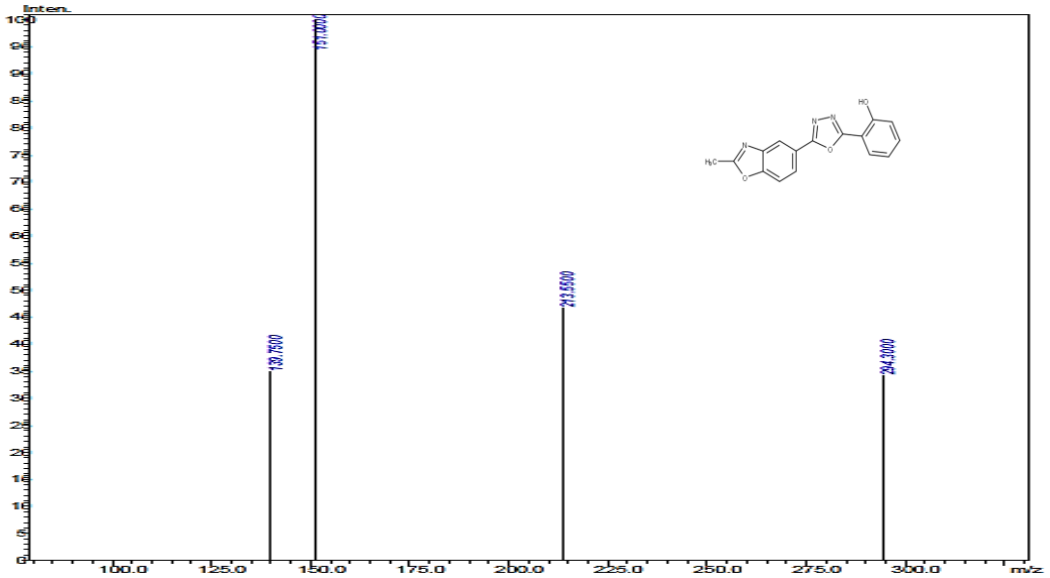


Figure.no.23: Mass Spectrum of Compound VD2

PROTON NMR SPECTRUM OF VD2:

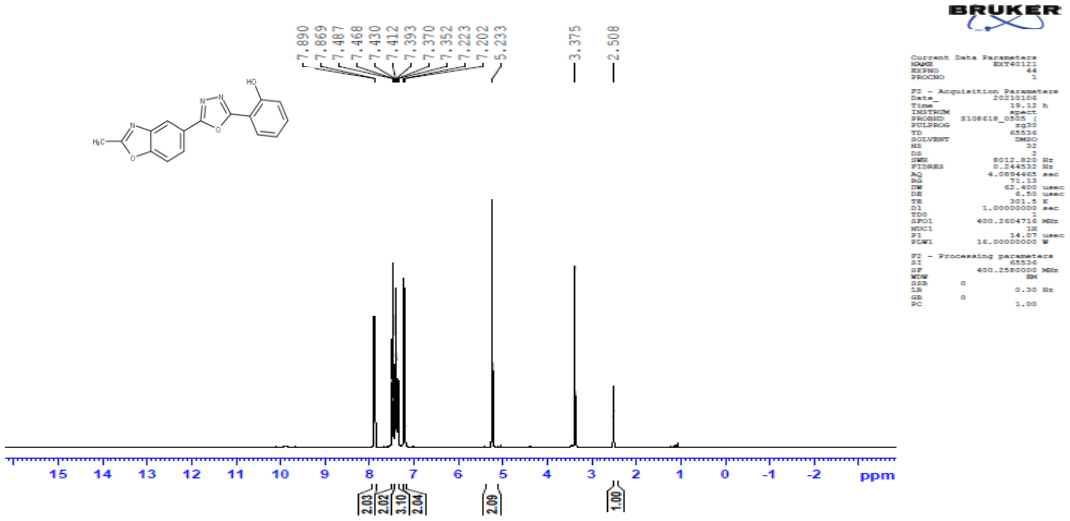
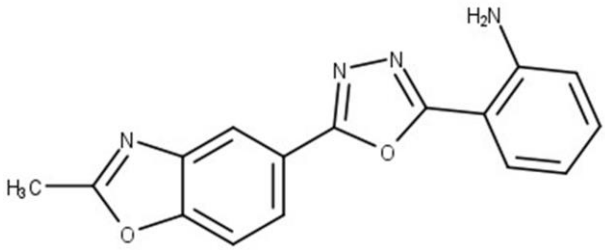


Figure .no.24: ^{1}H NMR Spectrum of Compound VD2

Table.no.11: ^{1}H NMR Interpretation of Compound VD2

S.NO	DELTA VALUE (PPM)	NATURE OF PROTON	NATURE OF PEAK	NUMBER OF PROTONS
1	2.508	Methyl C-H	Singlet	3
2	7.2-7.8	Aromatic C-H	Multiplet	7
3	5.23	Hydroxy-OH	Singlet	1

VD3



2- [5- (2- methyl- 1,3- benzoxazol- 5- yl)- 1,3,4- oxadiazol- 2- yl]aniline

Molecular Formula	:	$C_{16}H_{12}N_4O_2$
Formula Weight	:	292.29208
Appearance	:	Yellow
Melting Point	:	172^0C
Rf Value	:	0.83
Yield	:	73.36%
Composition	:	C(65.75%) H(4.14%) N(19.17%) O(10.95%)
Molar refractivity	:	81.21 ± 0.3 cm^3
Molar volume	:	217.9 ± 3.0 cm^3
Parachor	:	611.7 ± 4.0 cm^3
Index of Refractivity	:	1.667 ± 0.02
Surface Tension	:	62.0 ± 3.0 dyne/cm
Polarizability	:	32.19 ± 0.5 $10^{-24}cm^3$

INFRARED SPECTRUM OF VD3:

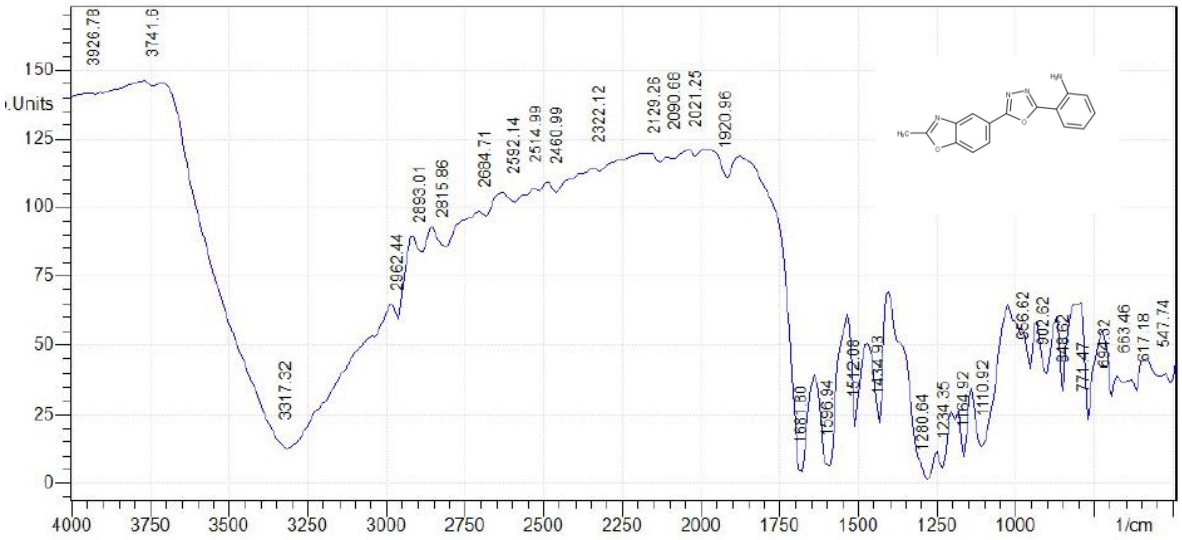


Figure.no.25: IR Spectrum of Compound VD3

Table.no.12: IR Interpretation of compound VD3

S.NO	WAVE NUMBER (cm^{-1})	FUNCTIONAL GROUPS
1	1681	C=N Stretching
2	1589	- C=C-
3	3317	NH2
4	2962	SP_2-C-H stretching
5	1280	C-O-C Stretching

LC-MS SPECTRUM OF VD3:

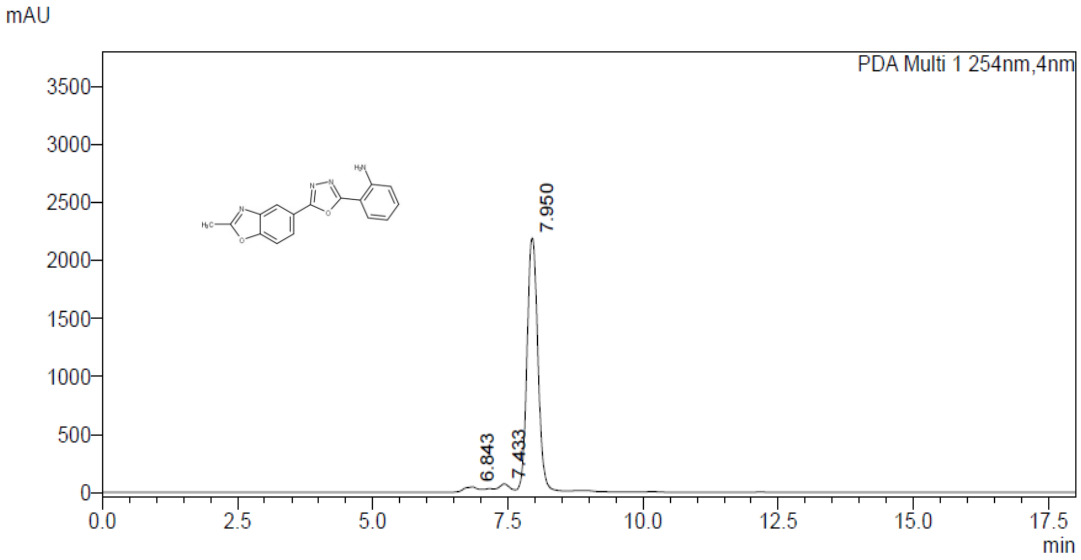


Figure.no.26: Chromatogram of Compound VD3

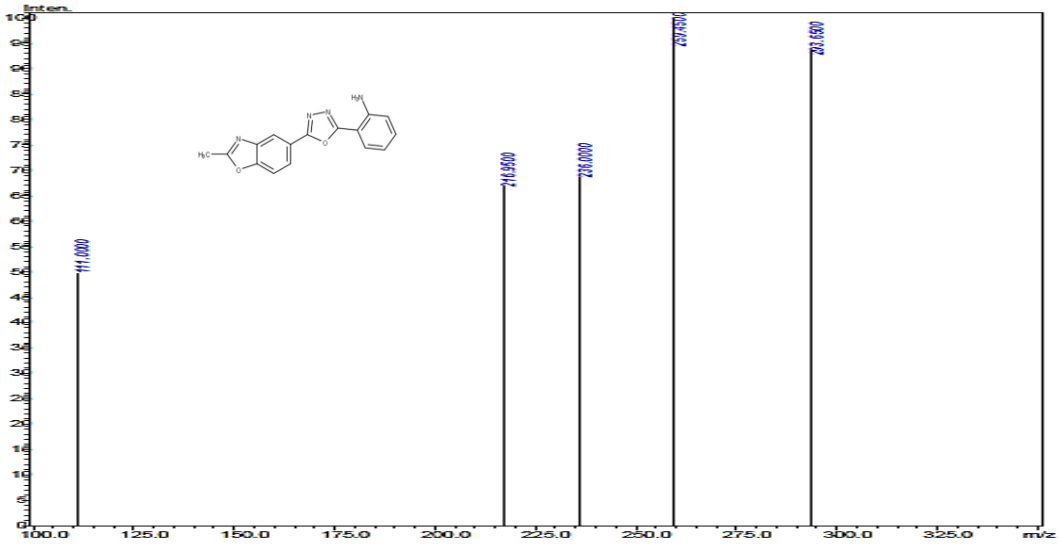


Figure.no.27: Mass Spectrum of Compound VD3

PROTON NMR SPECTRUM OF VD3:

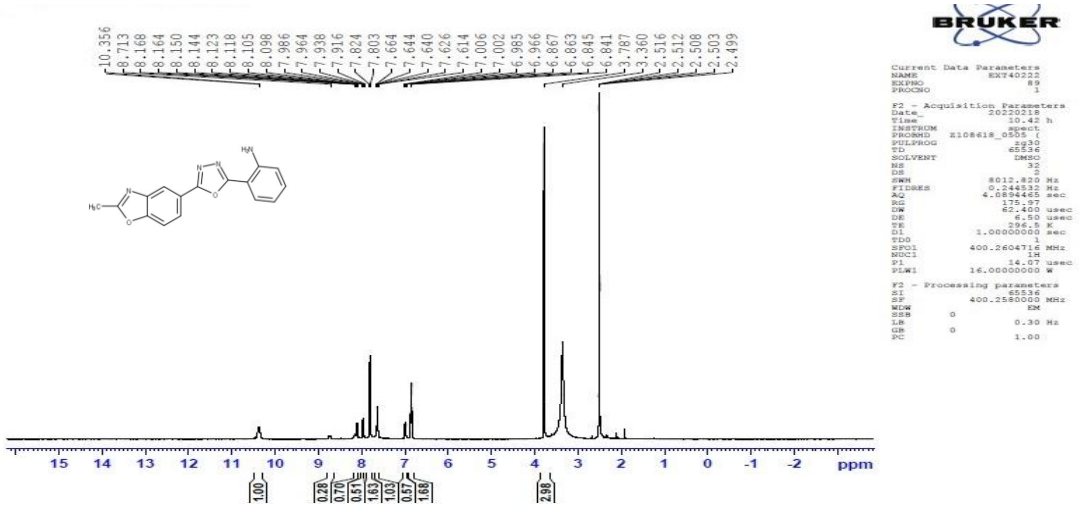
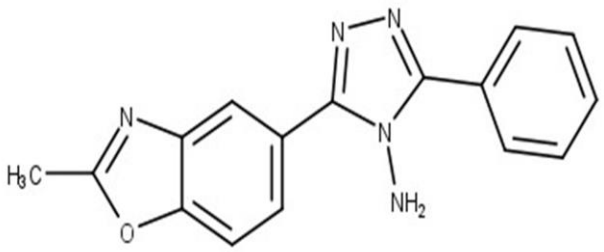


Figure .no.28: ^{1}H NMR Spectrum of Compound VD3

Table.no.13: ^{1}H NMR Interpretation of Compound VD3

S.NO	DELTA VALUE (PPM)	NATURE OF PROTON	NATURE OF PEAK	NUMBER OF PROTONS
1	2.51	Methyl C-H	singlet	3
2	7.4-8.7	Aromatic C-H	Multiplet	7
3	6.8	NH_2	Singlet	2

VD4



3- (2- methyl- 1,3- benzoxazol- 5- yl)- 5- phenyl- 4H- 1,2,4- triazol- 4- amine

Molecular Formula	:	$C_{16}H_{13}N_5O$
Formula Weight	:	291.30732
Appearance	:	Pink
Melting Point	:	180^0C
Rf Value	:	0.54
Yield	:	75.36%
Composition	:	C(65.97%) H(4.50%) N(24.04%) O(5.49%)
Molar refractivity	:	$82.32 \pm 0.3\ cm^3$
Molar volume	:	$203.4 \pm 3.0\ cm^3$
Parachor	:	$565.1 \pm 8.0\ cm^3$
Index of Refractivity	:	1.743 ± 0.05
Surface Tension	:	59.5 ± 7.0 dyne/cm
Polarizability	:	$32.63 \pm 0.5\ 10^{-24} cm^3$

INFRARED SPECTRUM OF VD4:

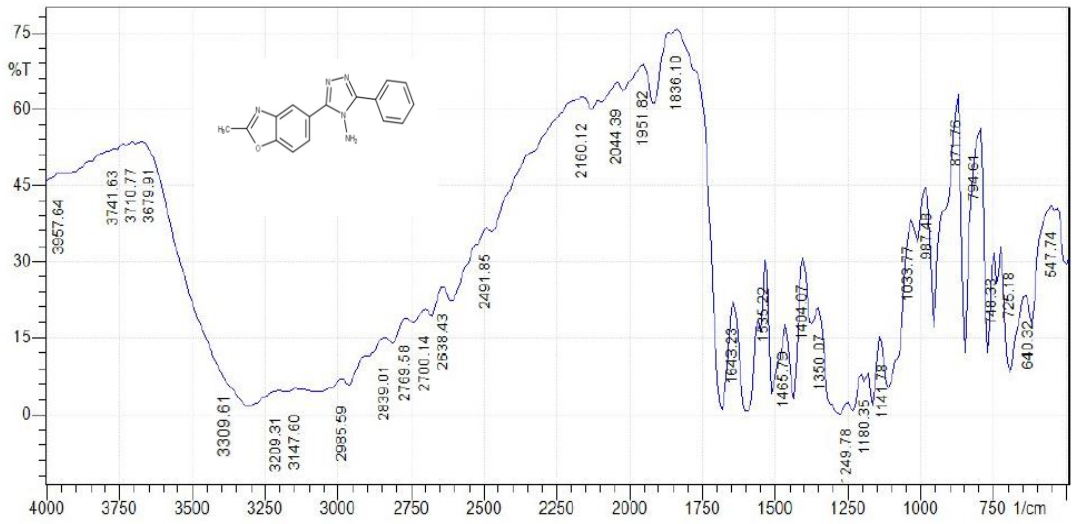


Figure.no.29: IR Spectrum of Compound VD4

Table.no.14: IR Interpretation of compound VD4

S.NO	WAVE NUMBER (cm^{-1})	FUNCTIONAL GROUPS
1	1643	C=N Stretching
2	1535	-C=C-
3	3309	NH2
4	3147	SP-C-H Stretching
5	2839	$-SP_2$-C-H stretching

LC-MS SPECTRUM OF VD4:

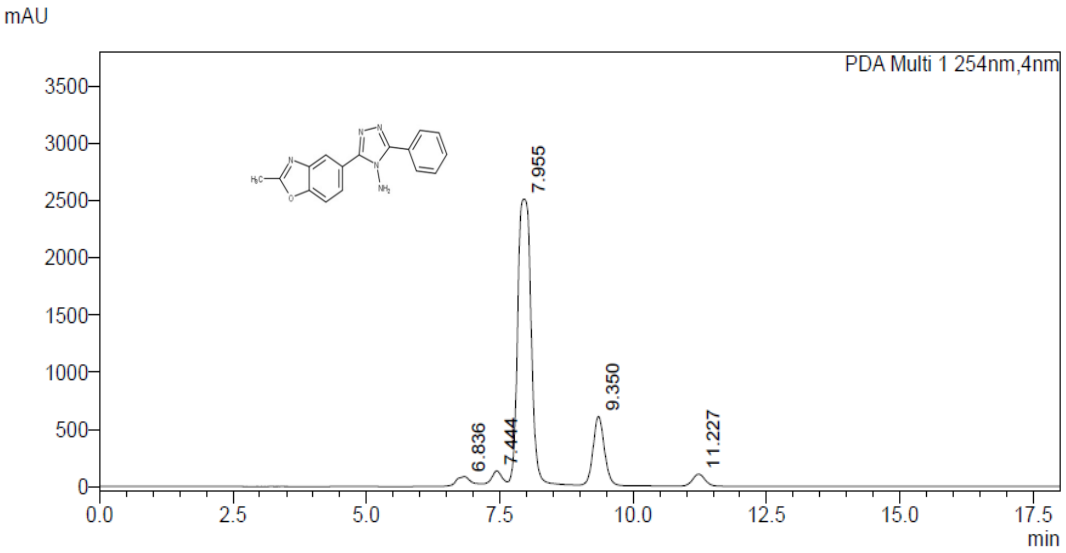


Figure.no.30: LC-MS Spectrum of Compound VD4

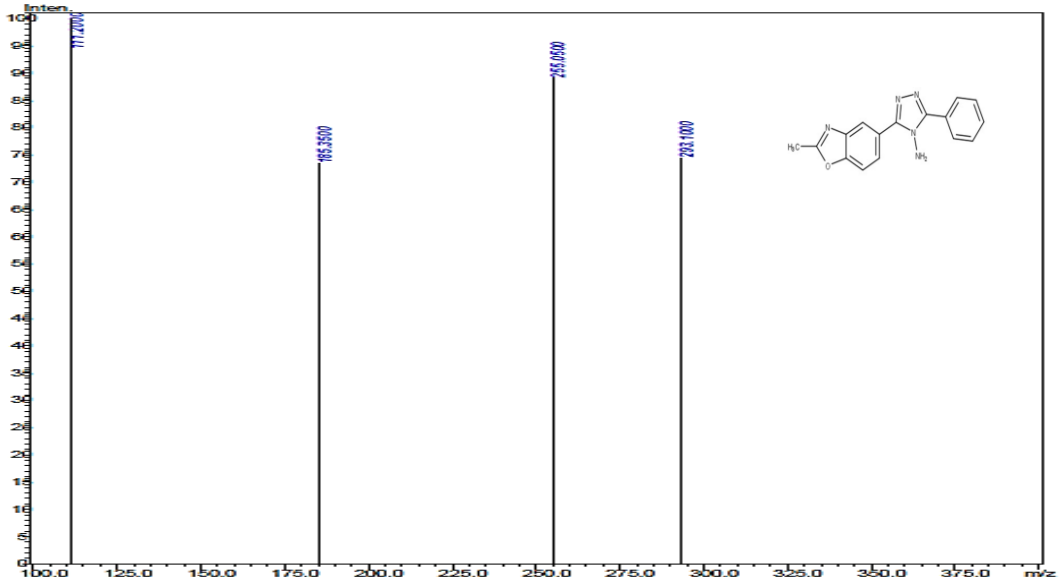


Figure.no.31: Mass Spectrum of Compound VD4

PROTON NMR SPECTRUM OF VD4:

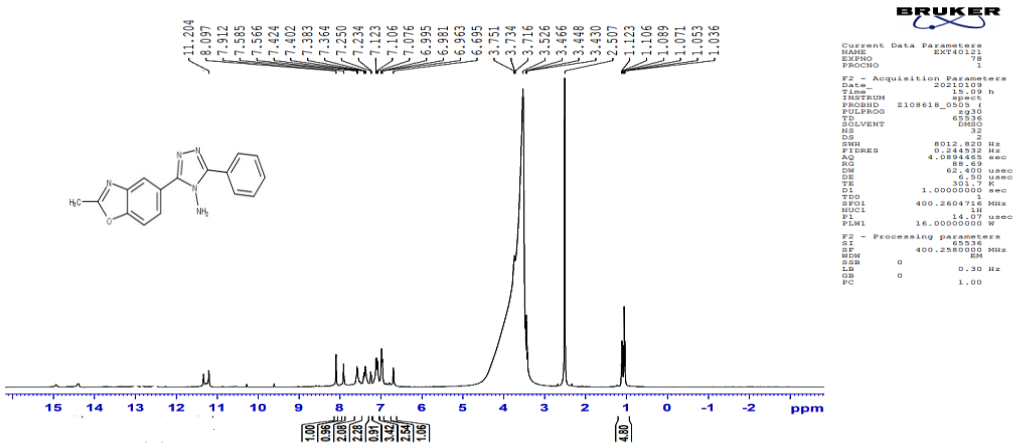
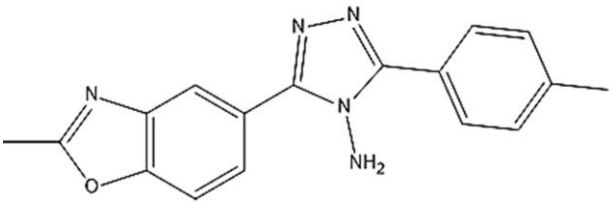


Figure.no.32: ^{1}H NMR Spectrum of Compound VD4

Table.no.15: ^{1}H NMR Interpretation of Compound VD4

S.NO	DELTA VALUE (PPM)	NATURE OF PROTON	NATURE OF PEAK	NUMBER OF PROTONS
1	2.5	Methyl C-H	singlet	3
2	7.7-8.0	Aromatic C-H	Multiplet	8
3	6.6	NH_2	Singlet	2

VD5



3- (2- methyl- 1,3- benzoxazol- 5- yl)- 5- (4-methylphenyl)- 4H- 1,2,4-triazol- 4- amine

Molecular Formula	:	$C_{17}H_{15}N_5O$
Formula Weight	:	305.3339
Appearance	:	Pink
Melting Point	:	190^0C
Rf Value	:	0.71
Yield	:	74.36%
Composition	:	C(66.87%) H(4.95%) N(22.94%) O(5.24%)
Molar refractivity	:	86.74 ± 0.5 cm^3
Molar volume	:	218.6 ±7.0 cm^3
Parachor	:	592.2 ± 8.0 cm^3
Index of Refractivity	:	1.724 ± 0.05
Surface Tension	:	55.2 ± 7.0 dyne/cm
Polarizability	:	34.38 ± 0.5 $10^{-24}cm^3$

INFRARED SPECTRUM OF VD5:

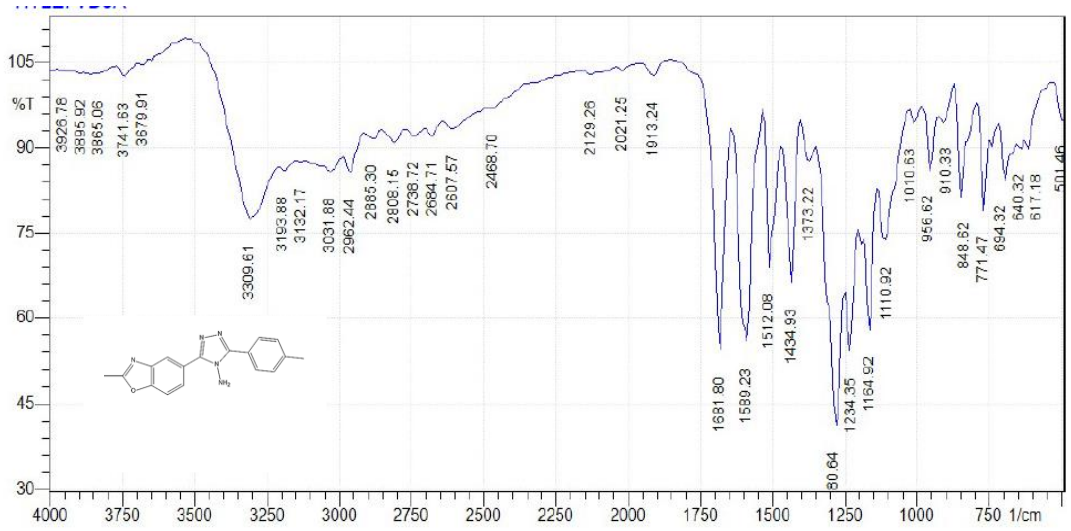


Figure.no.33: IR Spectrum of Compound VD5

Table.no.16: IR interpretation of compound VD5

S.NO	WAVE NUMBER (cm^{-1})	FUNCTIONAL GROUPS
1	1681	C=N Stretching
2	1589	- C=C-
3	3309	NH2 Stretching
4	3031	SP-C-H Stretching
5	2962	SP_2-C-H Stretching

LC-MS SPECTRUM OF VD5:

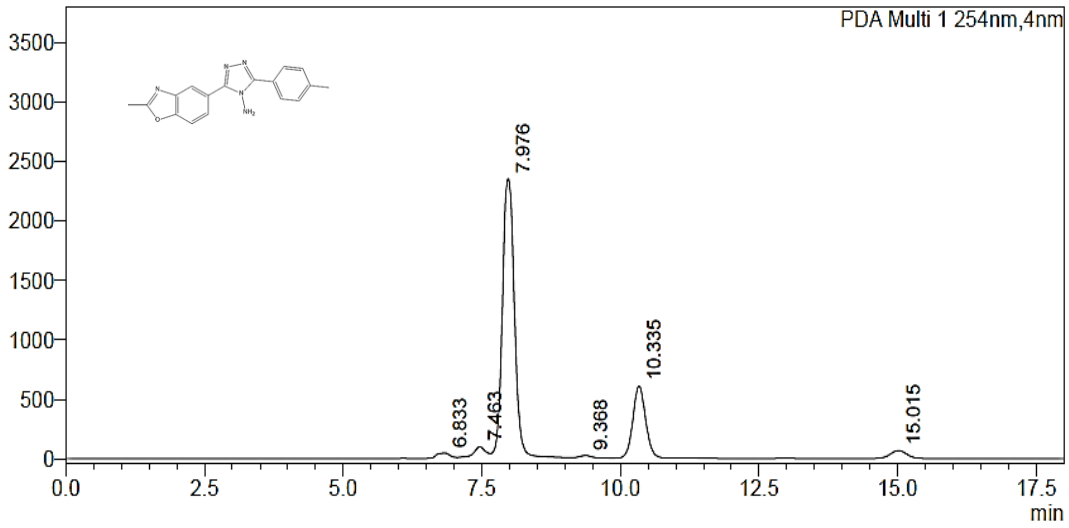


Figure.no.34: Chromatogram of Compound VD5

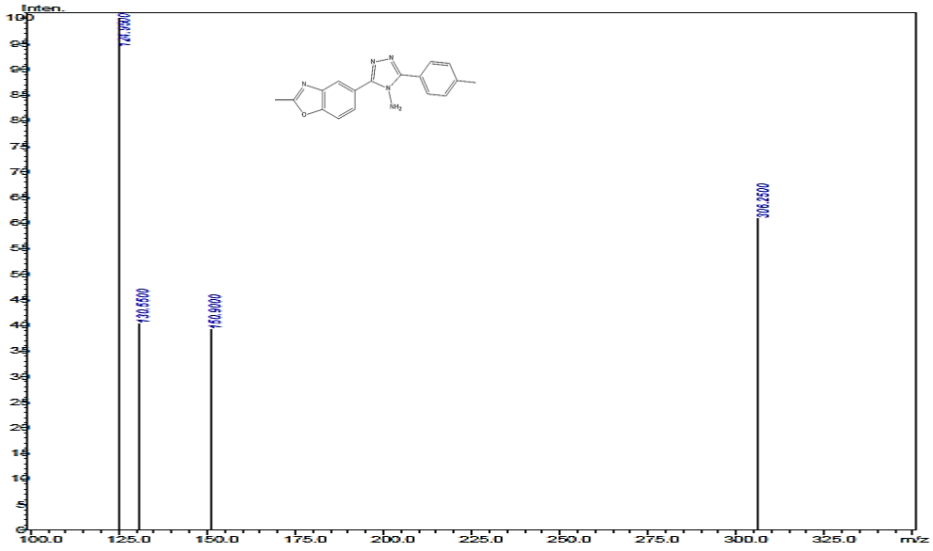


Figure.no.35: Mass Spectrum of Compound VD5

PROTON NMR SPECTRUM OF VD5:

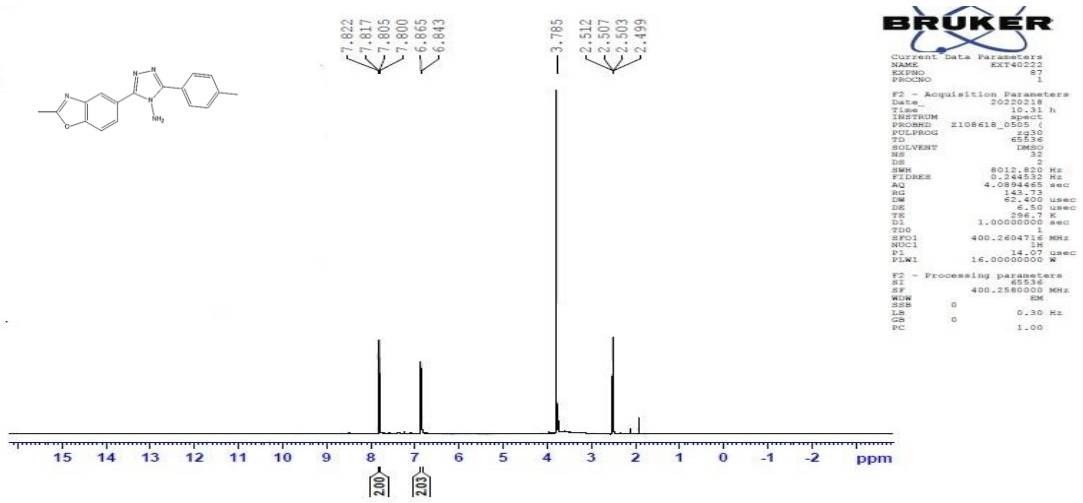


Figure.no.36: ^{1}HNMR Spectrum of Compound VD5

Table.no.17: ^{1}H NMR interpretation of Compound VD5

S.NO	DELTA VALUE (PPM)	NATURE OF PROTON	NATURE OF PEAK	NUMBER OF PROTONS
1	2.4	Methyl C-H	Singlet	3
2	7.80-7.82	Aromatic C-H	Multiplet	7
3	6.8	NH_2	Singlet	2

MOLECULAR MASS OF THE SYNTHESIZED COMPOUNDS

Table.no.18: Molecular mass of synthesized compounds

SAMPLE CODE	CALCULATED MASS	ACTUAL MASS
VD1	292.30	292.05
VD2	293.28	294.300
VD3	292.30	293.65
VD4	291.31	293.100
VD5	305.34	306.25

V.C. PHARMACOLOGICAL EVAUATION

V.C.I. MATERIALSAND METHODS

IN VIVO ANTIHYPERLIPIDEMIC ACTIVITY

Experimental animals

Male Wistar Albino rats of (150-180g) were procured from the Animal Experimental Laboratory, Madras Medical College, Chennai-03. The study was approved by the Institutional Animal Ethical Committee (IAEC) of Madras Medical College, Chennai which was certified by the Committee for the Purpose of Control and Supervision of Experiments of Animals (CPCSEA), India. (CPCSEA Registration number:1917/GO/ReBi/2016/CPCSEA). **Approval Number: 13/2021-2022**

Maintenance of animals

Animals were kept in clean and dry polypropylene cages with 12:12 hours light and dark cycle at 25±5^{0}C and 55-58% relative humidity in the animal house. Animals were allowed freely to access standard pellet diet and purified water *ad libitum*.

i. ACUTE ORAL TOXICITY STUDY(ACUTE TOXIC CLASS METHOD)

Acute oral toxicity defines to the adverse effects occurring following oral administration of single dose of substances or multiple doses given within 24hrs.

The different methods used to evaluate the acute oral toxicity studies are as follows,

i. Fixed dose procedure(OECD Guidelines- 420)
ii. Acute toxic class method (OECD Guidelines- 423)
iii. Ups and down procedure(OECD Guidelines- 425)

Our study was done following acute toxic class method (OECD Guidelines- 423)

OECD Guidelines – 423

OECD Guidelines for the testing of chemicals are periodically reviewed in the light of scientific progress or changing assessment practices. The original guidelines 423 was adopted in March 1996 as the second alternative to the conventional acute toxicity test, described in the guideline 401. Based on the recommendation of several expert meetings, revision was considered timely because international agreement has been reached on harmonized LD_{50}.

Cut-off value for the classification of the chemical substances, which differ from the cut-off recommended in the 1996 version of the guidelines and testing in one sex (usually female) is now considered sufficient.

A. Acute toxic class method

In the present study, the oral toxicity of the synthesized compounds were performed by acute toxic class method. In these methods, the toxicity of the synthesized compounds was tested using a stepwise procedure, each step using 3 rats of a single sex. The various concentration of test drug as per OECD Guidelines are as follows, wistar rats were fasted overnight prior to dosing (food but not water should be withheld). Following the period of fasting the animal should be weighed and the synthesized compounds administered orally at the dose of 2000mg/kg body weight. Animals were observed individually after dosing at least during the first four hours and daily thereafter, for a total of 14 days[63]. The test procedure with starting doses of 2000mg/kg body weight as per OECD-423 Guidelines was shown as follows.

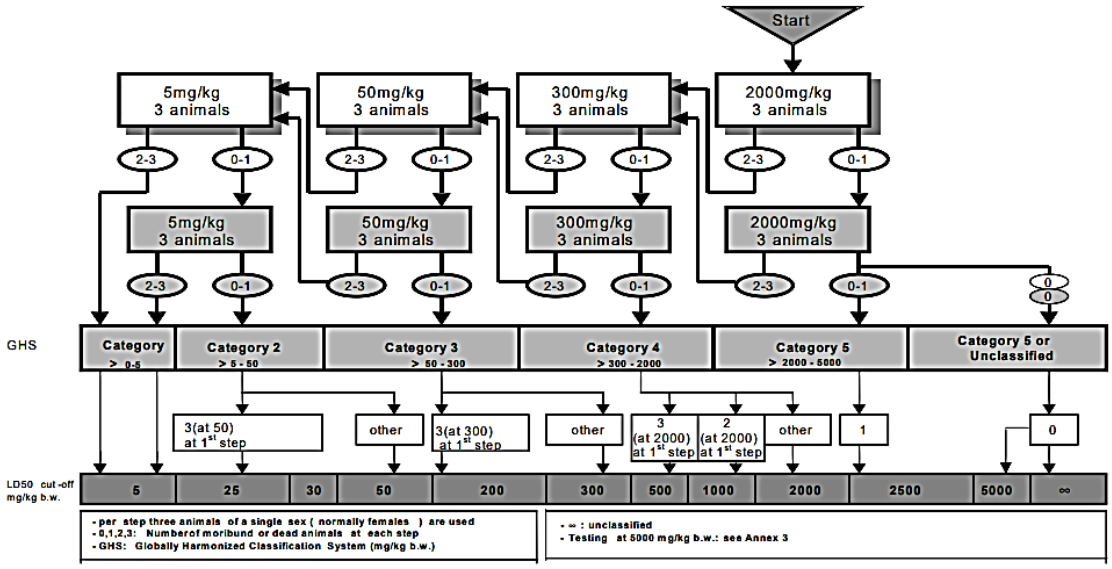


Figure.no.37: Treatment protocol for the acute toxicity study (OECD-423)

ii. *IN VIVO* ANTIHYPERLIPIDEMIC ACTIVITY

The model used to evaluate the antihyperlipidemic activity was High Fat Diet induced hyperlipidemia in rats. Hyperlipidemia in rats was induced by administration of high fat diet (43% carbohydrate, 17%protein and 40% fat, each nutrient per 100g) for 60 days with standard rat chow diet[65].

Experimental Design

30 Male Wistar rats were used in this study. The animals were divided into 5 groups of 6 animals each.

Table.no.19: Experimental design

GROUP (n=6)	NAME OF THE GROUP	TREATMENT SCHEDULE
A	Normal Control	Standard Rat chow diet for 60 days
B	Disease Control	High fat diet for 60 days
C	Low dose	High fat diet for 60 days +Low dose of VD1 p.o from 31st to 60th day
D	High dose	High fat diet for 60 days +High dose of VD1 p.o from 31st to 60th day
E	Standard control	High fat diet for 60 days + Atorvastatin, (1.2mg/kg) p.o from 31st to 60th day

EVALUATION PARAMETERS

A. Body weight

During study period, the rats were weighed periodically using electronic balance and their body weight was recorded.

B. **Biochemical estimation**

At the end of experiment, the rats were fasted overnight. The rats were weighed and euthanized using light anaesthesia of Isoflurane. The blood was collected by cardiac puncture in non-heparinised tubes for serum separation. The clear serum was separated by centrifugation at 2500 rpm for 10 minutes and used for the determination of biochemical parameters such as total cholesterol, LDL-cholesterol, HDL-cholesterol, VLDL and triglycerides. Atherogenic index were calculated using the formula

AI = (TC-HDL) /HDL

C. Histopathological analysis

The liver and heart was isolated from each rat in all groups and then fixed in 10% neutral buffered formalin. The specimen were cleared in xylene and embedded in wax with paraffin. Then cut into 4-6 microns thickness using a rotary microtome and stained with haematoxylin and eosin. This section was observed under microscope.

STATISTICAL ANALYSIS

Data are presented as Mean ± SEM and the values of $P< 0.01$ were considered statistically significant. Statistical analysis between the control and experimental groups was analyzed using one-way ANOVA followed by Dunnetts's multiple comparison test using *Graph Pad Prism 8.0.2.*

V.C.II.RESULTS AND DISCUSSION

i. Acute toxicity study

Oral acute toxicity of newly synthesized heterocyclic compounds such as VD1, VD2, VD3, VD4 and VD5 were performed and the results were illustrated in Table.no.20.

Table.no.20: Acute toxicity study

OBSERVATIONS	30 mins	4 hrs	24 hrs	14thday
Alertness	Present	Present	Present	Present
Aggressiveness	Absent	Absent	Absent	Absent
Touch response	Present	Present	Present	Present
Gripping	Present	Present	Present	Present
Motor co-ordination	Present	Present	Present	Present
Catatonia	Absent	Absent	Absent	Absent
Righting reflux	Present	Present	Present	Present
Corneal reflux	Present	Present	Present	Present
Lacrimation	Normal	Normal	Normal	Normal
Salivation	Normal	Normal	Normal	Normal
Writhing effect	Absent	Absent	Absent	Absent
Diarrhoea	Absent	Absent	Absent	Absent
Tremors	Absent	Absent	Absent	Absent
Convulsions	Absent	Absent	Absent	Absent
Death	Absent	Absent	Absent	Absent

In acute toxicity studies it was found that the animals were safe up to a maximum dose of 2000mg/kg of body weight. There were no changes in normal behaviour pattern, no signs and symptoms of toxicity and mortality were observed up to the dose of 2000mg/kg of body weight. As no mortality was observed with the above doses a series of doses 100 & 200mg/kg body weight were selected for the further pharmacological evaluation

ii. *IN VIVO* ANTIHYPERLIPIDEMIC ACTIVITY

A. Effect of compound VD1 on body weight of animal

Rats treated with HFD (Hyperlipidemic rats) showed a considerable increase in body weight whereas control rats remained the same. Atrovastatin and test compound treated group showed significant reduction in the body weight of animals. Results are illustrated in Table.no.21.

Table.no.21: Body weight variation in control and experimental wistar rats

Table 1

GROUP	INITIAL WEIGHT (g)	FINAL WEIGHT (g)	WEIGHT GAIN (g)
A	132.91±1.74	174.06±2.38	35.26±4.39
B	136.84±2.53	254.14±8.58	117.44±6.05
C	150.66±1.26	220.90±7.51	70.33±5.87
D	153.58±2.32	195.78±3.10	42.26±3.34
E	177.83±2.29	218.47±3.78	40.76±3.37

All the values are expressed as Mean ± SEM (n=6). $^{###}P < 0.001$ compared to normal control group, $^{***}P < 0.001$ compared to disease control group

B. Biochemical Estimation

1. Effect of compound VD1 on TC in hyperlipidemic rats

Table.no.22: Effect of compound VD1 on TC in hyperlipidemic rats

GROUP	TREATMENT	TC(mg/dl)
A	Normal Control	111.83±1.137
B	Disease control	180.83±1.013
C	HFD + Test compound (100mg/kg)	145.51±1.335
D	HFD + Test compound (200mg/kg)	120.16±1.137
E	HFD + Atorvastatin (1.2mg/kg)	113.5±1.408

All the values are expressed as Mean ± SEM (n=6). $^{###}P < 0.001$ compared to normal control group, $^{***}P < 0.001$ compared to disease control group

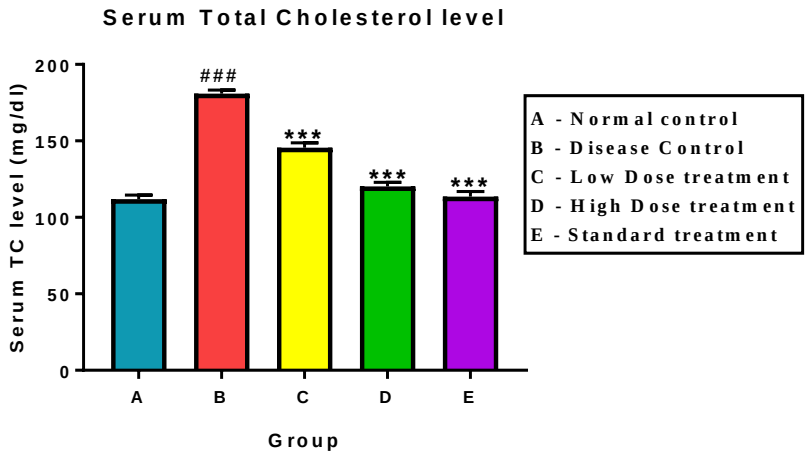


Figure.no.38: Effect of compound VD1 on TC in hyperlipidemic rat

As illustrated in Table.no.22 and figure.no.38, there was a significant rise (P<0.001) in TC levels in HFD induced hyperlipidemic rats when compared to normal group. The administration of Atorvastatin has significant (P<0.001) lowering of TC level compared to group B. The administration of compound VD1at 200mg/kg has showed a significant lowering (P<0.001) of TC level when compared to Group B (HFD induced hyperlipidemic rats).

2. Effect of compound VD1 on TG in hyperlipidemic rats

Table.no.23: Effect of compound VD1 on TG in hyperlipidemic rats

GROUP	TREATMENT	TG (mg/dl)
A	Normal Control	84.66±1.382
B	Disease control	159.83±1.301
C	HFD + Test compound (100mg/kg)	115.16±1.424
D	HFD + Test compound (200mg/kg)	96.83±1.249
E	HFD + Atorvastatin (1.2mg/kg)	92.66±1.406

All the values are expressed as Mean ± SEM (n=6). $^{###}P < 0.001$ compared to normal control group, $^{***}P < 0.001$ compared to disease control group

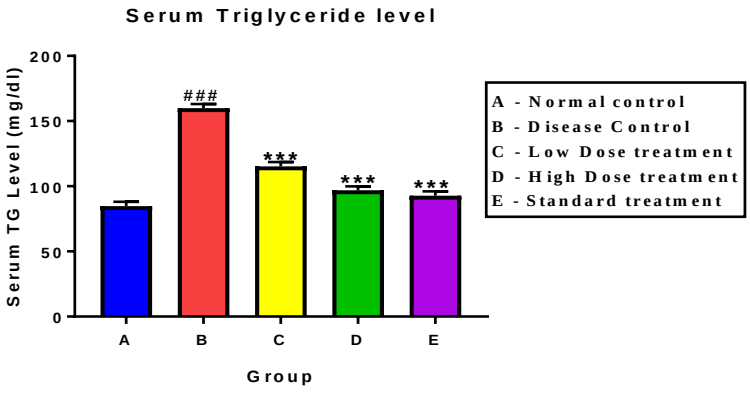


Figure.no.39: Effect of compound VD1 on TG in hyperlipidemic rat

As illustrated in Table.no.23 and Figure.no.39, there was a significant rise (P<0.001) in TG levels in HFD induced hyperlipidemic rats when compared to normal group. The administration of Atorvastatin has significant (P<0.001) lowering of TG level compared to group B. The administration of compound VD1at 200mg/kg has showed a significant lowering (P<0.001) of TG level when compared to Group B (HFD induced hyperlipidemic rats).

3. Effect of compound VD1 on HDL in hyperlipidemic rats

Table.no.24: Effect of compound VD1 on HDL in hyperlipidemic rats

GROUP	TREATMENT	HDL (mg/dl)
A	Normal Control	58.66±1.282
B	Disease control	25.66±0.954
C	HFD + Test compound (100mg/kg)	39.66±1.115
D	HFD + Test compound (200mg/kg)	51.5±1.586
E	HFD + Atorvastatin (1.2mg/kg)	54.5±1.335

All the values are expressed as Mean ± SEM (n=6). $^{###}P < 0.001$ compared to normal control group, $^{***}P < 0.001$ compared to disease control group.

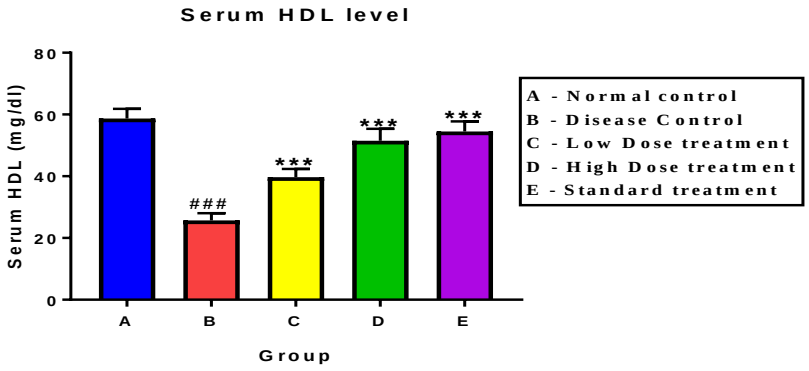


Figure.no.40: Effect of compound VD1 on HDL in hyperlipidemic rat

As illustrated in Table.no.24 and Figure.no.40, there was a significant decline (P<0.001) in HDL levels in HFD induced hyperlipidemic rats when compared to normal group. The administration of Atorvastatin has significant (P<0.001) increase of HDL level compared to group B. The administration of compound VD1at 200mg/kg has showed a significant increase (P<0.001) of HDL level when compared to Group B (HFD induced hyperlipidemic rats).

4. Effect of compound VD1 on LDL in hyperlipidemic rats

Table.no.25: Effect of compound VD1 on LDL in hyperlipidemic rats

GROUP	TREATMENT	LDL (mg/dl)
A	Normal Control	37.83±1.740
B	Disease control	113.5±1.231
C	HFD + Test compound (100mg/kg)	66.33±1.145
D	HFD + Test compound (200mg/kg)	46.83±1.249
E	HFD + Atorvastatin (1.2mg/kg)	42.16±1.833

All the values are expressed as Mean ± SEM (n=6). $^{###}P < 0.001$ compared to normal control group, $^{***}P < 0.001$ compared to disease control group.

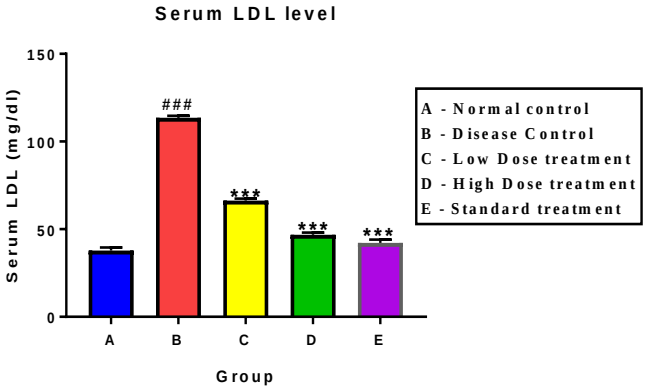


Figure.no.41: Effect of compound VD1 on LDL in hyperlipidemic rat

As illustrated in Table.no.25, there was a significant rise (P<0.001) in LDL levels in HFD induced hyperlipidemic rats when compared to normal group. The administration of Atorvastatin has significant (P<0.001) decrease of LDL level compared to group B. The administration of compound VD1at 200mg/kg has showed a significant decrease (P<0.001) of LDL level when compared to Group B (HFD induced hyperlipidemic rats).

5. Effect of compound VD1 on VLDL in hyperlipidemic rats

Table.no.26: Effect of compound VD1 on VLDL in hyperlipidemic rats

GROUP	TREATMENT	VLDL (mg/dl)
A	Normal Control	19.66±1.333
B	Disease control	35.16±1.337
C	HFD + Test compound (100mg/kg)	29.16±1.301
D	HFD + Test compound (200mg/kg)	23.83±1.424
E	HFD + Atorvastatin (1.2mg/kg)	21.83±1.137

All the values are expressed as Mean ± SEM (n=6). $^{###}P < 0.001$ compared to normal control group, $^{***}P < 0.001$ compared to disease control group.

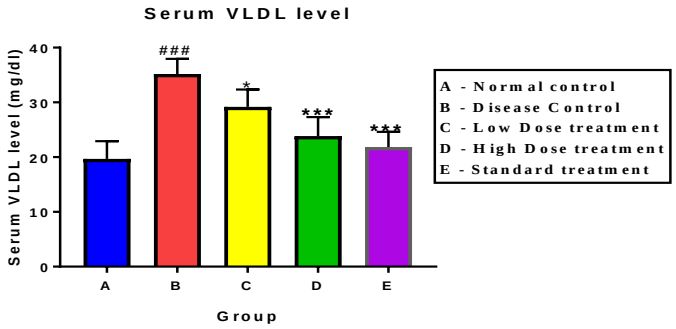


Figure.no.42: Effect of compound VD1 on VLDL in hyperlipidemic rat

As illustrated in Table**.**no.26, there was a significant rise (P<0.001) in VLDL levels in HFD induced hyperlipidemic rats when compared to normal group. The administration of Atorvastatin has significant (P<0.001) decrease of VLDL level compared to group B. The administration of compound VD1at 200mg/kg has showed a significant decrease (P<0.001) of VLDL level when compared to Group B (HFD induced hyperlipidemic rats).

6. Effect of compound VD1 on AI in hyperlipidemic rats

Table.no. 27: Effect of compound VD1 on AI in hyperlipidemic rats

GROUP	TREATMENT	AI
A	Normal Control	0.91±0.046
B	Disease control	6.09±0.278
C	HFD + Test compound (100mg/kg)	2.68±0.123
D	HFD + Test compound (200mg/kg)	1.34±0.076
E	HFD + Atorvastatin (1.2mg/kg)	1.08±0.054

All the values are expressed as Mean ± SEM (n=6). $^{###}P < 0.001$ compared to normal control group, $^{***}P < 0.001$ compared to disease control group.

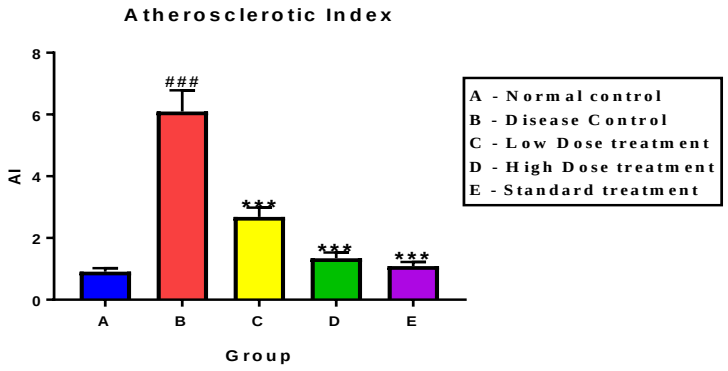


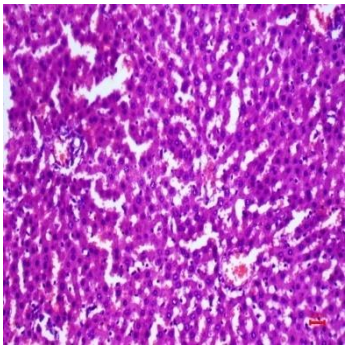
Figure.no.43: Effect of compound VD1 on AI in hyperlipidemic rat

As illustrated in Table.no.27, there was a significant rise (P<0.001) in AI levels in HFD induced hyperlipidemic rats when compared to normal group. The administration of Atorvastatin has significant (P<0.001) decrease of AI level compared to group B. The administration of compound VD1at 200mg/kg has showed a significant decrease (P<0.001) of AI level when compared to Group B (HFD induced hyperlipidemic rats).

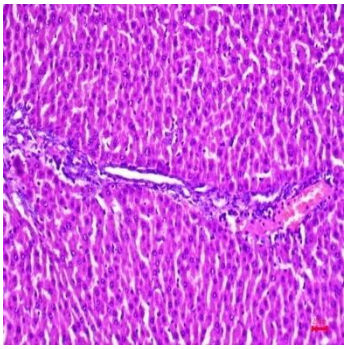
C. Histopathological studies

Effect on liver

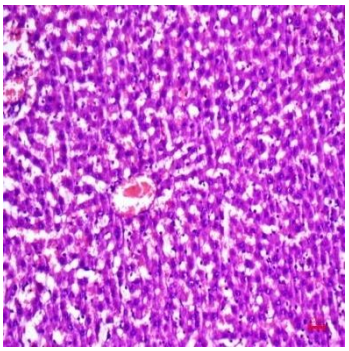
The liver section was examined for Histopathological changes and the results were illustrated in the Figure.no.44



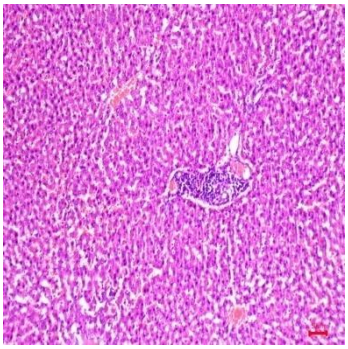
Normal Control



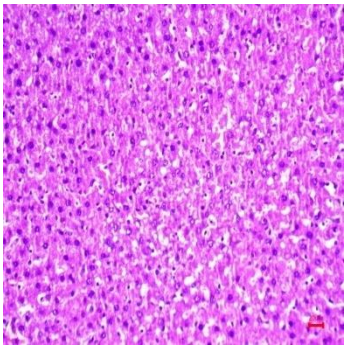
Disease Control



Test Low dose



Test High dose

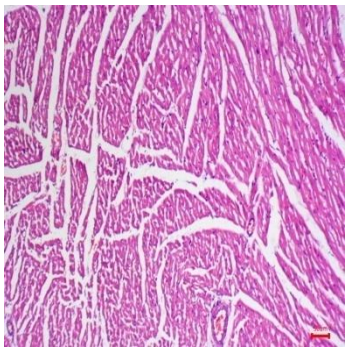


Standard control

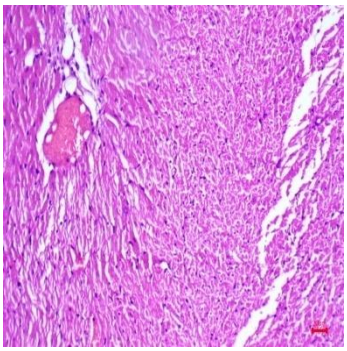
Figure 44: Histopathology of Rat Liver

Effect on Heart

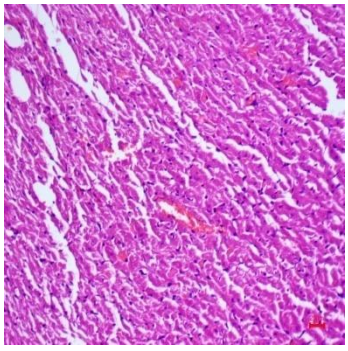
The heart section was examined for Histopathological changes and the results were illustrated in the Figure.no.45



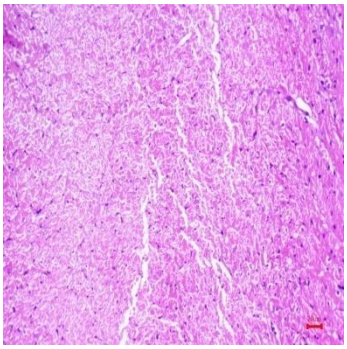
Normal Control



Disease Control



Test Low dose



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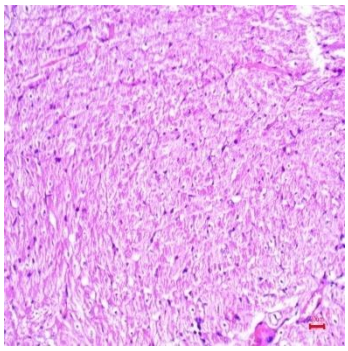

Standard control

Figure.no.45: Histopathology of Rat Heart

Histopathology of liver:

The HFD induced hyperlipidemia and abnormal lipid metabolism all collectively associated with inflammation, congestion, minimal to mild periportal mononuclear cell infiltration. Histopathological studies showed that the liver section was normal in normal control group. The HFD treated group showed marked ballooning, cellular degeneration. These changes were absent in atorvastatin treated standard group. Test compound VD1(100mg/kg) treated group showed decrease in cellular degeneration and congestion when compared to normal group. Test compound VD1(200mg/kg) treated group showed maximum suppression of cellular degeneration and inflammation.

Histopathology of Heart:

Histopathological section of heart of normal control showed normal cardiac muscle fiber. Section of heart of HFD induced hyperlipidemic group showed excessive fatty infiltration of the myocardium. Heart section of standard group showed cardiac muscle with minimal infiltration. Cross-section of test group (200 mg/kg) showed cardiac muscle with minimal fatty infiltration.

VI. SUMMARY AND CONCLUSION

SUMMARY

Medicinal chemistry primarily aims to discover novel chemical molecule which have the potential to prevent or treat a disease / infection. According to the WHO reports, Hyperlipidemia is one of the leading causes of death globally. Therefore, the current work aimed to design and synthesize some novel antihyperlipidemic compounds.

Based on the literature review, ***PCSK9*** has been considered as potential therapeutic target for selective **LDLR regulation** in the treatment of hyperlipidemia. Based on the literature review, pharmacophoric features such as **1 HBD, 1 HBA and 1 Aromatic ring** were identified. Hence a scaffold library has been generated with 150 newly designed ligands which were screened with high docking score against *PCSK9* using *Autodock4.2.6. software* and further optimized by drug likeliness properties such as Lipinski rule of five and ADMET properties. All the designed ligands were found to obey lipinski's rule of five and possess drug likeliness property. Results of the *in silico* toxicity studies showed that all the designed compounds found to be non-toxic.

Based on high docking scores and synthetic feasibility **ligand 7, ligand 8, ligand 10, ligand 12 and ligand 13** was selected for synthesis. All the selected ligands were chemically synthesized with different aromatic carboxylic acids. Completion of the reaction was determined by TLC. The synthesized compounds are labelled as **VD1, VD2, VD3, VD4 and VD5.** The purity of the synthesized compounds was checked by determining Melting point. The chemical nature of synthesized compounds was characterized by different spectral studies such as IR, ^{1}HNMR and LC- MS spectroscopy.

All the synthesized compounds were subjected to acute oral toxicity studies as per the OECD Guidelines 423 to access the toxicity and also to fix the dose. The LD50 value of the test compound VD1, VD2, VD3, VD4 and VD5 does not found to 2000mg/kg and also no mortality was observed. Based on high docking score CompoundVD1 was selected for further pharmacological evaluation. The dose 100mg/kg and 200mg/kg were selected..

In vivo antihyperlipidemic activity was evaluated for compound VD1 against HFD induce Hyperlipidemia in wistar rats. The rats were divided into five groups of 6 animals each.

From *In vivo* activity study, it was found that the body weight of Group B rats (rats treated with HFD) were significantly increased (P<0.001) in comparison with normal control rats. The increment in the body weight was reduced considerably (P<0.001) by the administration of Atorvastatin and test compound VD1 (200mg/kg and 100mg/kg). However, Group D (200mg/kg) showed decrease in body weight to normal as that of Group E.

The level of serum **TC, TG, LDL, VLDL** were significantly increased **(P<0.001)** in disease Control group in comparison with normal control group. Administration of test compound VD1 at the dose of 100mg/kg and 200mg/kg showed considerable reduction in serum TC, TG, LDL, VLDL in comparison with disease control. In comparison of the two doses of test group, the test compound at the dose of 200mg/kg was revealed considerable reduction of LDL and VLDL as that of Atorvastatin treated group.

The level of serum **HDL** were significantly decreased **(P<0.001)** in disease Control group in comparison with normal control group. Administration of test compound VD1 at the dose of 100mg/kg and 200mg/kg showed considerable raised serum HDL in comparison with disease control. In comparison of the two doses of test group, the test compound at the dose of 200mg/kg was revealed considerable raise in HDL as that of Atorvastatin treated group.

It has been shown that the Atherogenic index is strong marker to predict the risk of atherosclerosis and CVD. Test compound showed significant reduction of **AI** in compared to disease control group.

From the histopathological studies, the compound VD1 at 200mg/kg normalize the tissue of both liver and heart compared to disease control group.

CONCLUSION

Drug design approach as well as clinical studies has revealed that the *PCSK9* have a crucial role in LDLR regulation for the treatment of Hyperlipidemia. The present study also provides important structural insight of benzoxazole, amino triazole and oxadiazole in designing better *PCSK9* inhibitor as potent antihyperlipdimic agents. The designed compounds were docked against *PCSK9* using *Autodock4.2.6.* The synthesized compounds VD1, VD2, VD3, VD4 and VD5 found to obey Lipinski's rule of five and also possess drug likeliness property. Based on high docking score Compound VD1 has been selected for evaluating in vivo antihyperlipidemic activity and it found to reduce LDL level effectively with significance of P value 0.001 when compared to the standard drug Atorvastatin.

Further evaluation studies including *in vitro, in vivo* antihyperlipidemic screening and *PCSK9* enzyme Inhibition assay method using ELISA kit. will be performed for all the remaining synthesized compound **(VD2, VD3, VD4, VD5)** in future.

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MADRAS MEDICAL COLLEGE, CHENNAI – 600003

INSTITUTIONAL ANIMAL ETHICS COMMITTEE

PROCEEDINGS

PRESENT: Dr. A. JERAD SURESH, M.Pharm., Ph.D., MBA

Roc. No: 5/AEL/IAEC/MMC/2022 Dated: 01-11-2021

Sub: IAEC, MMC, Ch-3 – Approval of Laboratory Animals – Regarding

Ref: IAEC Meeting held on 21-10-2021

This order is issued based on the approval by the Institutional Animal Ethics Committee Meeting held on 21-10-2021, Thursday.

Project Proposal ID Number	13/2021-2022
CPCSEA Registration Number	1917/GO/ReBi/2016/CPCSEA Valid till 19-9-2026
Name of the Researcher with ID Number	V. DINESH KUMAR 261915703
Name of the Guide	Dr. R. Priyadarsini, M.Pharm., Ph.D
Project Title	Design, Synthesis and Pharmacological Evaluation of Novel Heterocyclic *PCSK9* Inhibitors as Antihyperlipidemic Agents
Date of submission of proposal to IAEC	07-10-2021
Date of IAEC meeting	21-10-2021
Date of submission of modified proposal to IAEC	22-10-2021
Date of Approval	21-10-2021
Validity of the Approved Proposal	One Year
Number & Species of Laboratory Animals Approved	**36 Wistar Rats Approved. Disease control group can be removed.**

Chairperson 5/11/21
Institutional Animal Ethics Committee
Madras Medical College
Chennai-600003

PRINCIPAL
COLLEGE OF PHARMACY
MADRAS MEDICAL COLLEGE
CHENNAI-600 003

To
Dr. R. Priyadarsini, M.Pharm., Ph.D.,
Assistant Professor, Dept. of Pharmaceutical Chemistry,
College of Pharmacy,
MMC, Ch-3.

Copy to:
Special Veterinary Officer, Animal Experimental Laboratory,
Madras Medical College, Ch-3.

71st Indian Pharmaceutical Congress

Theme: Healthcare System - Role of Regulators

Certificate

This is to certify that Prof./Dr./Mr./Ms.....

of ..... has participated in the 71st Indian Pharmaceutical Congress held at Sri Ramachandra Institute of Higher Education and Research (DU), from 20th to 22nd December 2019, Chennai, Tamil Nadu.

Shri. Ravi Uday Bhaskar	**Dr. P.V.Vijayaraghavan**	**Dr. M. Dhilip Kumar**	**Dr. T.V. Narayana**
President - 71st IPC 2019	Chairman - LOC	Secretary - LOC	General Secretary - IPCA

AIDCOC

Organised by
Indian Pharmaceutical Congress Association

Hosted by
All India Drugs Control Officers' Confederation

CERTIFICATE

of Participation

51 Rank - 2019

This Certificate is awarded to

V. Dinesh Kumar

For attending the Webinar on " 2D & 3D QSAR Tools" organized by Department of Pharmaceutical Analysis, Chalapathi Institute of Pharmaceutical Sciences (AUTONOMOUS), Guntur on 5th June 2020.

Sri.Y.V.Anjaneyulu
President,
Chalapathi Educational Society
Guntur

Prof.K.N.Rajini Kanth
HOD,
Department of Pharmaceutical Analysis
Chalapathi Institute of Pharmaceutical Sciences

Prof.N.Rama Rao
Principal
Chalapathi Institute of Pharmaceutical Sciences
Guntur

JSS ACADEMY OF HIGHER EDUCATION & RESEARCH
(DEEMED TO BE UNIVERSITY) MYSURU

SCHRÖDINGER

JSS COLLEGE OF PHARMACY, OOTY

(A constituent of JSS Academy of Higher Education & Research, Mysuru)

Webinar on

Recent Advances in Drug Design: State of the Art Tools for Drug Design and Drug Discovery

This is to certify that

DINESH KUMAR V

has participated in the above mentioned webinar
Organized by Pharmacy Education Unit &
Department of Pharmaceutical Chemistry, JSS College of Pharmacy, Ooty
in association with Schrödinger LLC, Bengaluru on **23rd June 2020**.

Dr S.P. DHANABAL
Principal, JSSCP Ooty

Dr PRITESH BHAT
Senior Scientist, Schrödinger LLC, Bengaluru

Dr R. RAGHU
Vice President, Schrödinger LLC, Bengaluru

VINAYAKA MISSION'S COLLEGE OF PHARMACY,

Vinayaka Mission's Research Foundation,

Deemed to be University as per Section 3 of UGC Act 1956

Salem, Tamilnadu, India

Accredited by NAAC

INTERNATIONAL WEBINAR

"Dyslipidemia- Pharmacotherapy and Clinical Management"

Certificate of Appreciation

This Certificate is presented to

Mr. DINESH KUMAR.V

In appreciation for participating in the International webinar delivered by Dr. Christapher Parayil Varghese AIMST University, Malaysia, which was organized by Vinayaka Mission's College of Pharmacy, VMRF-DU, Salem, Tamilnadu, India on 27th of May 2020.

Dr.B.Jayakar
Registrar

Dr.B.S.Venkateswarlu
Principal

VINAYAKA MISSION'S COLLEGE OF PHARMACY

Vinayaka Mission's Research Foundation, Salem.

Deemed to be University under Section 3 of UGC Act 1956

Accredited by NAAC

In association with

Indian Pharmaceutical Association, Salem Local Branch, *Organised*

WEBINAR

Certificate of Participation

Awarded to

DINESH KUMAR.V

In appreciation for participating in the webinar on "Drug design strategies and molecular docking studies" delivered by DR.Bijo Mathew, International Research Consultant, Drug Design Group, China, which was organised by Vinayaka Mission's College of Pharmacy, VMRF-DU, Salem & IPA Salem Local Branch, on 05.06.20 Friday 2020

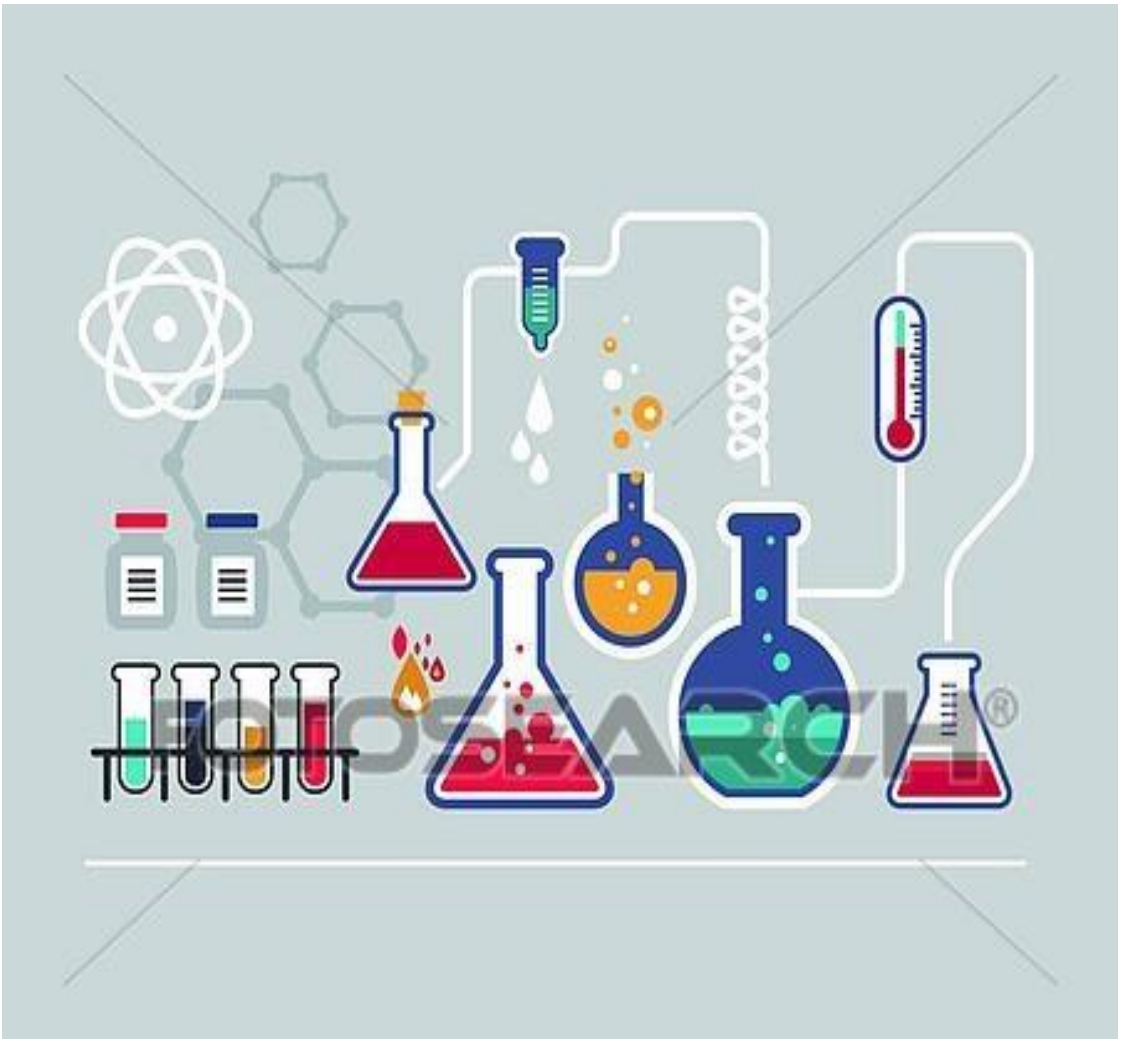
Dr.B.Jayakar
Registrar

Dr.B.S.Venkateswarlu
Principal


INTRODUCTION

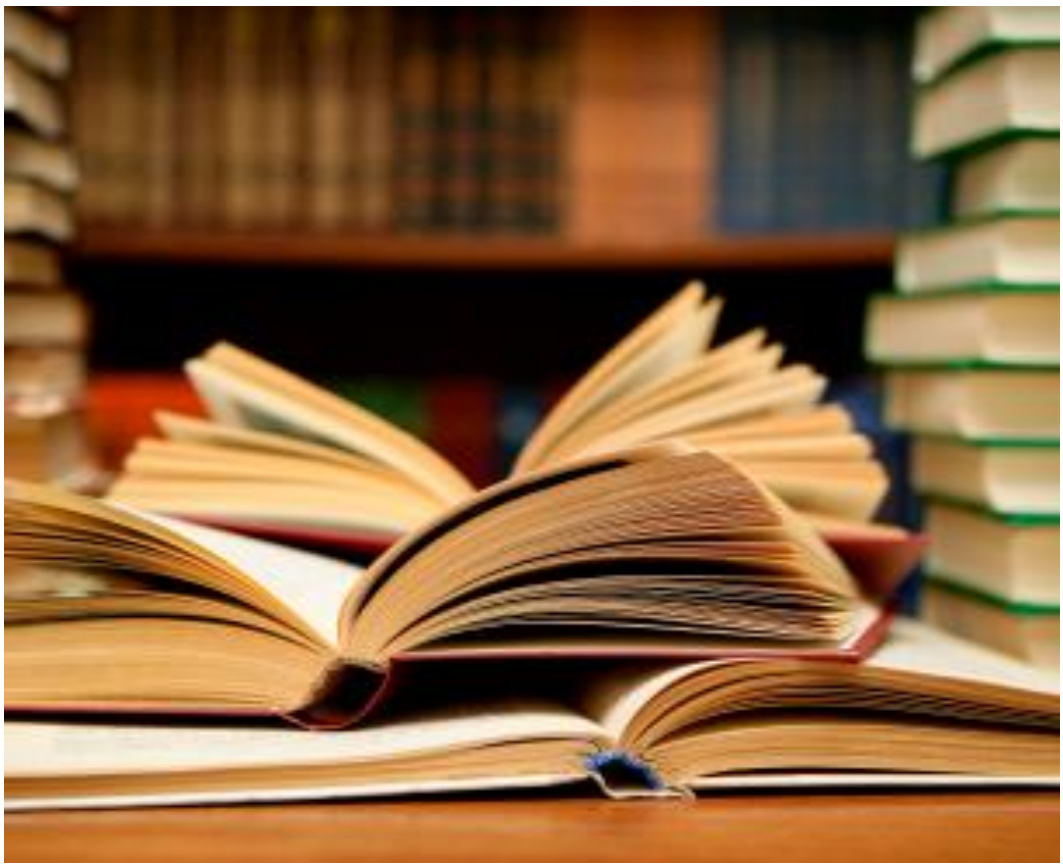
Review of literature

Aim and objectives



Experimental work

Summary and Conclusion



References

annexure