DESIGN AND SYNTHESIS OF NOVEL OXADIAZOLE, HYDANTOIN AND BENZOXAZOLE DERIVATIVES AS POTENTIAL ANTIDIABETIC AGENT

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DECLARATION

I declare that the thesis entitled "Design and Synthesis of Novel Oxadiazole, Hydantoin and Benzoxazole Derivatives as Potential Antidiabetic Agent" has been prepared by me under the guidance of Dr.Gopal Lal Khatik, Associate Professor, School of Pharmaceutical Sciences, Lovely Professional University, Phagwara. No part of this thesis has formed the basis for the award of any degree or fellowship previously.

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PARANJEET KAUR

Abstract:

Diabetes is ubiquitously life threatening subject, which introduces need for patient's life patterns to the disease and number of advanced therapeutic treatments. Diabetes mellitus (type 2 diabetes) is commonly diagnosed disease that poses greater challenges to the physicians. Diabetes Mellitus is metabolic syndrome carrying the cluster of risk factors mainly arises due to abnormal carbohydrate; lipid and protein metabolism concomitant with insulin resistance and hyperglycemia due to deficiency of insulin in diabetic patients.

Worldwide, around 422 million adults are surviving with type 2 DM which is projected to rise to 592 million by 2035, every year, 1.6 million deaths occurred due to diabetes. In India, approximately 95% of the diabetic patients are of type 2 DM. Maximum numbers of the deaths in the age group of 30-69 years, which represents the economically productive segment of the nation. Despite of health burden issues, it creates an economic burden as well, such as: Cost of care, disability and productivity loss leads to the costs associated with diabetes. Thereby, these antidiabetic drugs makeup the second largest market by sales in the pharmaceuticals industry after cancer.

Several novel therapeutic targets have been identified for the type 2 Diabetes intervention. Most of the known oral hypoglycemic are peroxisome-proliferator activated receptor (PPAR) agonist. PPARs are receptors that bind to ligand and undergo transcription that plays significant roles in the glucose and lipid metabolism regulation and agonists of PPAR α/γ are being used therapeutically. TZD agonists mainly acts on PPAR γ, which therapeutically used to attenuate insulin resistance whereas PPAR α ligands such as fibrates, lower serum triglycerides levels.

With an advancement of the pleiotropic effects, Potential indications are being exposed including roles in the cardiovascular disease intervention and metabolic syndrome. A perusal of literature shows that many marketed products do not address relevant aspects of the disease. Currently available drugs are associated with various side effects like severe cardiac toxicity, weight gain, and hepatotoxicity. PPAR α/γ dual agonists tend to collaborate the functions of both thiazolidinediones and fibrates and they hold substantial promise for ameliorating the type 2 diabetic treatments and providing potential therapeutic diabetic interventions. Hence, we proposed three potential heterocyclic series, oxadiazole, hydantoin and benzoxazole scaffolds as potential PPAR α/γ dual activators on the basis of rational that compounds possessing polymethylene linker in between

pharmacophore head and lipophilic tail showed PPARα agonism, while those with an aryl phenylene linker showed dual PPARα/γ agonism. So, on the basis of these studies three classes were generated from the proposed pharmacophore as: oxadiazole, haydantoin, benzoxazole derivatives. Among various derivatives docked by AutoDock Vina (ADT) 1.5.6 software, total 8 compounds coded as P152, P155, P158, P163, P170, P171, P172, P173 were selected comparative to standard drug (Pioglitazone) on the basis of their affinity scores and further their *in-silico* toxicity and *in-silico* ADME properties were predicted which showed these compounds having non-carcinogenecity and non-mutagenecity effect and moderate solubility in water and suppose to have high GI absorption with no permeation to BBB. Calculated liphophilicity (iLogP) was found to be 0.92 to 3.19. Further, on the basis of feasibility, compounds were synthesized through various proposed schemes in good yield. Later, compounds were subjected to both PPAR-gamma and PPAR-alpha assay which revealed that compound P158 and P155 were found to be most potent on both receptor with EC_{50} of 0.07µM, 0.06µM and 0.781µM, 3.29µM respectively as compared to pioglitazone having EC_{50} of 32.38 and 38.03 for both receptors. Further *in-vivo* evaluation revealed that both compounds P158 and P155 were found to reduce the plasma glucose level and total cholesterol level significantly in diabetic rats compared to standard drug pioglitazone at 5mg/kg/day dose for 7 days treatment. Various biochemical parameters in liver and kidney like TBARS, GSH and CAT were also observed to be normal as compared to diabetic group after treatment with both compounds. Further, histology of kidney and pancreas revealed that compounds are effective in the tissue regeneration after treatment with P155 and P158. Hence, compounds P155 and P158 could be used as a novel therapeutic agent for the diabetic interventions which are potentially expected to prevent morbidity and improve lifestyle in fair economical ways.

Abbreviations

S.No	Abbreviations	Full form
1	ACN	Acetonitrile
2	AF	Activation factor
3	ADME	Adsorption, distribution, metabolism and excretion
4	ANOVA	Analysis of variance
5	BBB	Blood brain barrier
6	CAT	Catalase
7	CTFA	Cayman's transcription factor
8	CBP	CREB pinding protein
9	DM	Diabetes mellitus
10	DCM	Dichloromethane
11	DBD	DNA binding domain
12	EC	Effective concentration
13	ELISA	Enzyme-linked immunosorbent assay
14	ET	Exocrine tissue
15	FDA	Food and drug approval
16	GI	Gastric intestinal
17	GL	Glomeruli
18	GSH	Glutathione
19	HB	Hemoglobin
20	HDL	High density lipoprotein
21	HFD	High fat density
22	HOBT	Hydroxybenzotriazole
23	HRE	Human response element
24	IL	Islets of Langerhans
25	LSB	Low salt buffer
26	LBD	Ligand binding domain
27	MAP	Mitogen-activated protein
28	MD	Molecular dynamic

29	MM	Molecular mechanics
30	NPD	Normal pallet diet
31	NTD	N-terminal domain
32	NR	Nuclear receptor
33	PBP	PPAR binding protein
34	PGC-1	PPAR co-activator-1
35	PDB	Protein data bank
36	PRIP	PPAR interacting protein
37	PPAR-α	Peroxisome proliferator activated receptor-alpha
38	PPAR-γ	Peroxisome proliferator activated receptor-gamma
39	PPRE	Peroxisome proliferative response element
40	PBS	Phosphate buffer saline
41	PGL	Plasma glucose
42	RXR	Retinoid X receptor
43	ROS	Reactive oxygen species
44	SMRT	Silencing mediator for retinoid and thyroid hormone receptor
45	SD	Standard deviation
46	SEM	Standard error of mean
47	STZ	Streptozotocin
48	SAR	Structure activity relationship
49	TBAR	Thiobarbituric acid reactive substance
50	TF	Transcription factor
51	VC	Vacuolations

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Abstract:

Diabetes is ubiquitously life threatening subject,which introduces need for patient's life patterns to the disease and number of advanced therapeutic treatments. Diabetes mellitus (type 2 diabetes) is commonly diagnosed disease that poses greater challenges to the physicians. Diabetes Mellitus is metabolic syndrome carrying the cluster of risk factors mainly arises due to abnormal carbohydrate; lipid and protein metabolism concomitant with insulin resistance and hyperglycemia due to deficiency of insulin in diabetic patients.

Worldwide, around 422 million adults are surviving with type 2 DM which is projected to rise to 592 million by 2035, every year, 1.6 million deaths occurred due to diabetes. In India, approximately 95% of the diabetic patients are of type 2 DM. Maximum numbers of the deaths in the age group of 30-69 years, which represents the economically productive segment of the nation. Despite of health burden issues, it creates an economic burden as well, such as: Cost of care, disability and productivity loss leads to the costs associated with diabetes. Thereby, these antidiabetic drugs makeup the second largest market by sales in the pharmaceuticals industry after cancer.

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polymethylene linker in between pharmacophore head and lipophilic tail showed PPAR*α* agonism, while those with an aryl phenylene linker showed dual PPAR*α/γ* agonism. So, on the basis of these studies three classes were generated from the proposed pharmacophore as: oxadiazole, haydantoin, benzoxazole derivatives. Among various derivatives docked by AutoDockVina (ADT) 1.5.6 software, total 8 compounds coded as P152, P155, P158, P163, P170, P171, P172, P173 were selected comparative to standard drug (Pioglitazone) on the basis of their affinity scores and further their *in-silico* toxicity and *in-silico* ADME properties were predicted which showed these compounds having non-carcinogenecity and non-mutagenecity effect and moderate solubility in water and suppose to have high GI absorption with no permeation to BBB. Calculated liphophilicity (iLogP) was found to be 0.92 to 3.19. Further, on the basis of feasibility, compounds were synthesized through various proposed schemes in good yield. Later, compounds were subjected to both PPAR-*γ* and PPAR-*α* assay which revealed that compound P158 and P155 were found to be most potent on both receptor with EC_{50} of 0.07µM, 0.06µM and 0.781µM, 3.29µM respectively as compared to pioglitazone having EC_{50} of 32.38 and 38.03 for both receptors. Further *in-vivo* evaluation revealed that both compounds P158 and P155 were found to reduce the plasma glucose level and total cholesterol level significantly in diabetic rats compared to standard drug pioglitazone at 5mg/kg/day dose for 7 days treatment.Various biochemical parameters in liver and kidney like TBARS, GSH and CAT were also observed to be normal as compared to diabetic group after treatment with both compounds. Further,histology of kidney and pancreas revealed that compounds are effective in the tissue regeneration after treatment with P155 and P158. Hence, compounds P155 and P158 could be used as a novel therapeutic agent for the diabetic interventions which are potentially expected to prevent morbidity and improve lifestyle in fair economical ways.

Chapter 1: Introduction

1. Introduction

Among the life style diseases obesity and diabetes are increasing at a tremendous rate in India. Worldwide, around 422 million adults are surviving with type 2 diabetes mellitus (DM), according to the latest data from the World Health Organization (WHO).[1] An estimate by International Diabetes Federation says that among other countries in the world, India had more diabetics comparative to other country in the world until recently and According to the World Health Organization (WHO), India had 69.2 million people living with diabetes in 2015 and are believed to increase approximately up to 98 million (approximately) by 2025.[2] As per the study by the American Diabetes Association states that India would face highest number of diabetic people being diagnosed by 2030.[3] This is probably the highest number of diabetics in any country. Diabetes Mellitus is very expensive disease and has profound long-term complications and their associated cost.[4,5] World Health Organization also estimated that mortality related to heart disease and diabetes costs nearly $210 billion each year and is expected to rise to $335 billion in the coming 10 years.[4,6] This economic burden results in declining life expectancy and quality of life.[7,8]

Diabetes mellitus (DM) is characterized by hyperglycemia which is a consequence of changed metabolism of carbohydrates, lipids, proteins. Diabetes can be subdivided as Type 1 or Type 2 DM. Type 1 DM (insulin dependent), is caused by lack of insulin, which must be injected or inhaled to sustain the patient. Type 2 DM (non-insulin dependent) is a metabolic disorder characterized by increased blood glucose level. In India, approximately 95% of the diabetic patients are of Type 2 DM (**Table 1**). [4,6,9]

Table 1: Diabetes in India- 2015[10]

Population comprising of adults aged between 20 -79 yrs.	798,988	Adults deaths in because of diabetes	1,027,911
Adults suffering by diabetes. (20-79 yrs) (%)	8.7	Economical rate of diabetes per person (cost/person)	94.9
Total diabetic cases of adults aged between 29-79yrs	69,188.6	Undiagnosed cases of diabetes in adults	36,061.1

1.1 Diabetes mellitus (DM)

"Diabetes" is derived from Greek and it means "to siphon out". Its symptoms are excessive loss of water through polyuria, and urination. **"Mellitus"**, originated from Latin, means "sweet".[11] Diabetes mellitus is considered as a chronic biochemical disease causes deficiency of insulin (the pancreatic hormone) and an irregular release of glucagon (a polypeptide hormone). It may promote many other symptoms or diseases such as arteriosclerosis, blindness etc. The normal blood sugar in human is between 80 to 120 mg/dl (empty stomach), and 120 to 160 mg/dl after meal. But during bedtime, it varies from 100 to 140 mg/dl. Any kind of variation from the above mentioned value range is indicative of diabetes.

1.2 Complications caused by DM:[12]

(i) Diabetic nephropathy: Causes swelling in the legs and feet, which progresses further to other body parts.

(ii) Diabetic retinopathy: Pain in the eyes, which may lead to loss of vision.

(iii) Diabetic neuropathy: Tingling, burning, tightness, numbness, shooting pain in the feet, hands and other body parts.

1.3 Treatment of DM:[13,7]

Insulin therapy

Insulin used currently is in seven forms classified as per their duration of action, i.e. fast, intermediate, or long-acting. A majority of the insulin is being prepared from beef or beef/pork pancreas. This insulin contains pancreatic enzymatic impurities such as somatostatin, glucagon, pancreatic proinsulin and polypeptide. This impure insulin was reported to have adverse effects on many patients. Normally the body of an organism refuses foreign protein or abnormal protein injected into it, recognizing it as a poison which is quickly excreted by the body.[14]

Oral hypoglycemic agents [15,16,17]

Janbon et al. reported that sulfonamides are found to be hypoglycemic. Thereafter, 1-butyl-3-sulfonylurea (carbutamide) was clinically used first as an effective treatment for diabetes. Although later it was withdrawn due to its side effect like bone marrow depression. This compound led to the discovery and development of sulfonylureas class of drugs (**Table 2**). Tolbutamide was the first

widely used drug in patients having Type 2 DM. Repaglinid, a new compound related to insulin secretagogues was developed and was called as meglitinides derivatives. This was further approved as a cure for diabetes. Thereafter in 1920s, a new class of antidiabetic agent was developed and categorized into biguanides, but was surpassed due to the discovery of insulin. The hypoglycemic effect of antimalarial agent chloroguanide, promoted for the development of this class of compounds. Phenformin from his class was withdrawn due to lactic acidosis associated with its use. Further developed biguanides such as metformin is being used broadly in Europe and the United States without any noteworthy undesirable effects.[18,19]

Table 2: Oral hypoglycemic agents.

Class	Drug	Class	Drug
(I)Insulin secretagogues: Sulfonylureas(These bind at SUR1 subunits and block the ATP-sensitive K^+ Channel)	*1st generation* Chlorpropamide Acetohexamide Tolazamide Tolbutamide *2nd generation* Glipizide Glyburide Glimepiride	Non-sulfonylureas Meglitinides (Block ATP sensitive K^+ Channel)	Repaglinide (Prandin) Nateglinide (Starlix)
(II) Insulin sensitizers Biguanides (activation AMP kinase)	Metformin	PPARα agonist	Fibrates/Rexinoids
Thiazolidinediones (PPARγ agonists)	Pioglitazone Rosiglitazone	Protein tyrosine kinase inhibitors	CLX 0300/0301/ 0900/0901
Alpha-glucosidase Inhibitors	Acarbose, Miglitol		

Thiazolidinediones, considered as the second major class of "insulin sensitizers", were introduced in 1997. They interact with peroxisome proliferator-activated receptors (PPARγ), which results in increased glucose uptake in muscles as well as reduced glucose production. One of the drugs of this class, troglitazonewas withdrawn due to hepatic toxicity but other agents of this class, like

pioglitazone and rosiglitazone did not show liver toxicity so these agents continued to be used widely.[20, 21]

1.4 PPARs as a promising target for DM [22]

PPARs are considered as promising target for treatment of diabetes since evidences support that stimulus to PPAR*γ* increases insulin resistance. Insulin-sensitizing drugs such as thiazolidinediones (TZDs) were found with high affinity toward PPAR*γ*. In addition, these agents' binding affinity appears to intimately relate to their potency. There are some Non-TZD-related PPAR*γ* agonists, i.e. oxyiminoacetic acid derivatives which also show strong anti-diabetic activity.

The PPARs are of 3 types: *α*, *β/δ*, and *γ*.[23]

α (alpha) NR1C1- It expresses mainly in kidney, liver, muscle, heart, and adipose tissue.

β/δ (beta/delta) NR1C2- It expresses mainly in adipose tissue, brain, and skin.

γ (gamma) NR1C3- This PPAR*γ* expresses in three different subtypes:

γ1 –It usually expresses in colon, heart, kidney, muscle, spleen and pancreas.

γ2 – It expresses in adipose tissue.

γ3 – It expresses in white adipose tissue, macrophages and large intestine.

These receptors, recognized in Xenopus frogs, provoke the proliferation of peroxisomes.[24] The first PPAR was discovered and classified as PPAR*α* and is referred to as *peroxisome proliferators*, because it accelerates the amount of peroxisomes in the liver tissue, apart from ameliorating insulin sensitivity.[25] The fibrates agents discovered in the early 1980s turned out to be ligands of PPAR. Conformational changes of PPARs are brought in by the ligand or various co-activator or co-repressor proteins at LBD (Ligand Binding Domain) which stimulate or inhibit the functions of the receptor.[26]

1.5 Structure of PPARs

PPARs, classified as nuclear receptors, possess three main functional domains (**Figure 1**) with the following segments:[27,18]

I. NTD (N-terminal domain) -A/B
II. DBD (DNA binding domain) - C
III. flexible hinge region -D
IV. LBD (ligand binding domain) -E
V. C-terminal region –F

Figure 1. Structure of PPAR[27,18]

When the receptor is activated, the DBD which has 2 zinc finger motifs binds to DNA by hormone response elements (HRE). The LBD consists of one β-sheet and 13 α-helices.[29]Various natural or synthetic ligands bind at the LBD, and stimulate or inhibit the receptor.

1.6Mechanisms of PPAR activation[30]

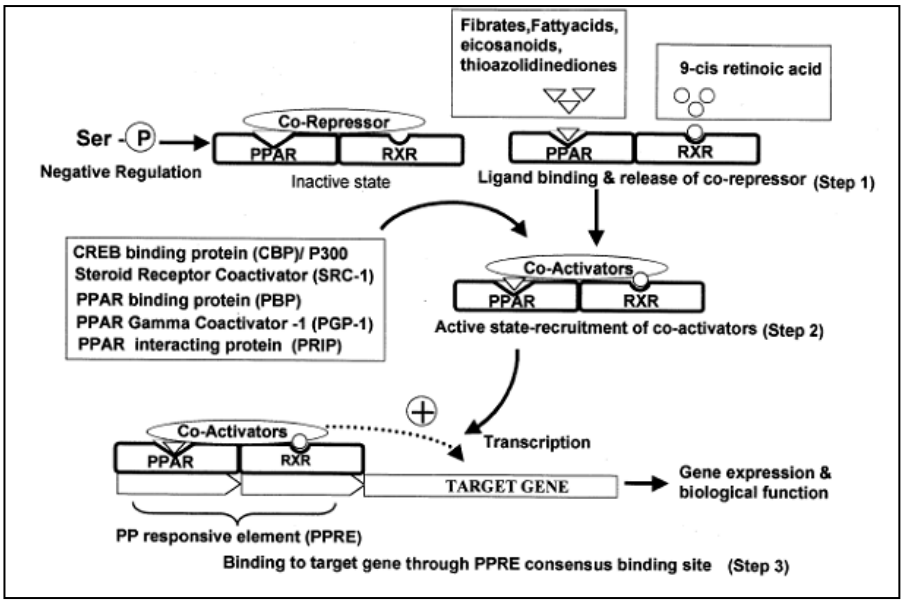


Figure 2.PPAR activation and its transcriptional mechanism.[32]

PPARs activated by various ligands regulate the transcriptional activity as shown in **Figure 2**. Natural ligands or molecules like thiazolidinone bind to PPARγ, leading to activation and complexation with retinoid X receptor (RXR) on DNA. Further, it forms heterodimers and binds to DNA via peroxisome proliferative response elements (PPREs).[31]One of the co-repressors such as SMRT (Silencing Mediator for Retinoid and Thyroid hormone receptor) usually involved in PPARγ-mediated gene transcription leads to down-regulation (**Figure 2**).[32]

Several proteins [33,34]are identified and characterized as coactivators of PPARγi.e. P300, CREB binding protein (CBP), PPARγ co-activator-1(PGC-1), and PPAR binding protein (PBP). Other co-activator recently reported by Zhu *et al*[35], is PPAR interacting protein (PRIP). Normally, nuclear

receptors remain inactive with co-repressors, and their activation led by conformational changes, further facilitates required gene transcription.[36] Other than the cofactor, PPARγcan be downed by Mitogen-Activated Protein (MAP) kinase.[37] The mechanism involved is phosphorylation, particularly at Ser114, diminishes the PPARγ activity as well as adipocyte differentiation.[38-42]

1.7 PPARs interactions with ligands

Ligand Binding Domain (LBD) of PPARγ has a binding site in Y-shape pocket and is twice in volume in comparison to other nuclear receptors. Agonists, such as rosiglitazone, bind in the form of "U-shaped" conformation and occupy about just 40% of the total volume of the pocket. Rosiglitazone comprising of thiazolidinedione head group directly interacts with thePPARγ in AF2 helix and lock it in such a manner where coactivators can bind.[43]Nolte *et al*. identified the space by virtual atoms fitting and reported total unoccupied volume at approximately 1,300 A^{o} LBD. The binding mode of BRL49653 is depicted in **Figure 3**.

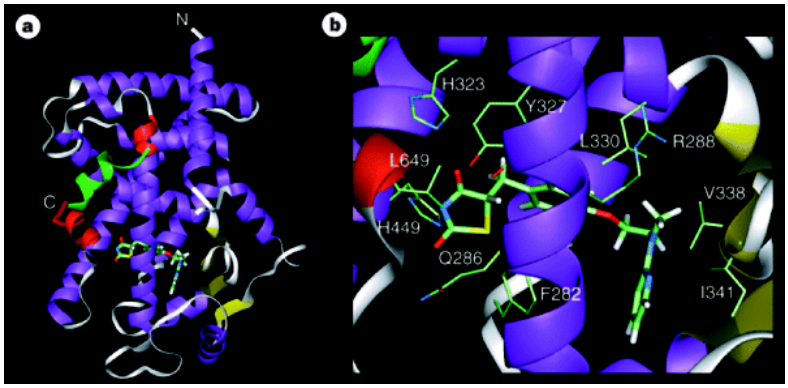


Figure 3. Ternary complex of PPARγ LBD, and binding of BRL49653 [44]

TZDs are known to have specificity toward PPARγ, whereas other subtypes of receptors are not conserved; amino acid residue e.g. H323, Q286 are not found in PPARα or PPARδ respectively.[44]

1.8 Dual activator of PPARα, γ receptor

Mortality and morbidity associated with diabetes mellitus is due to its complications such as cardiovascular disorders.[45] It is observed that PPARα activation involves in reduction of triglycerides and regulates energy homeostasis,[46] and also reduces the diabetic complications particularly cardiovascular disorders (**Figure 4**).[47,48]

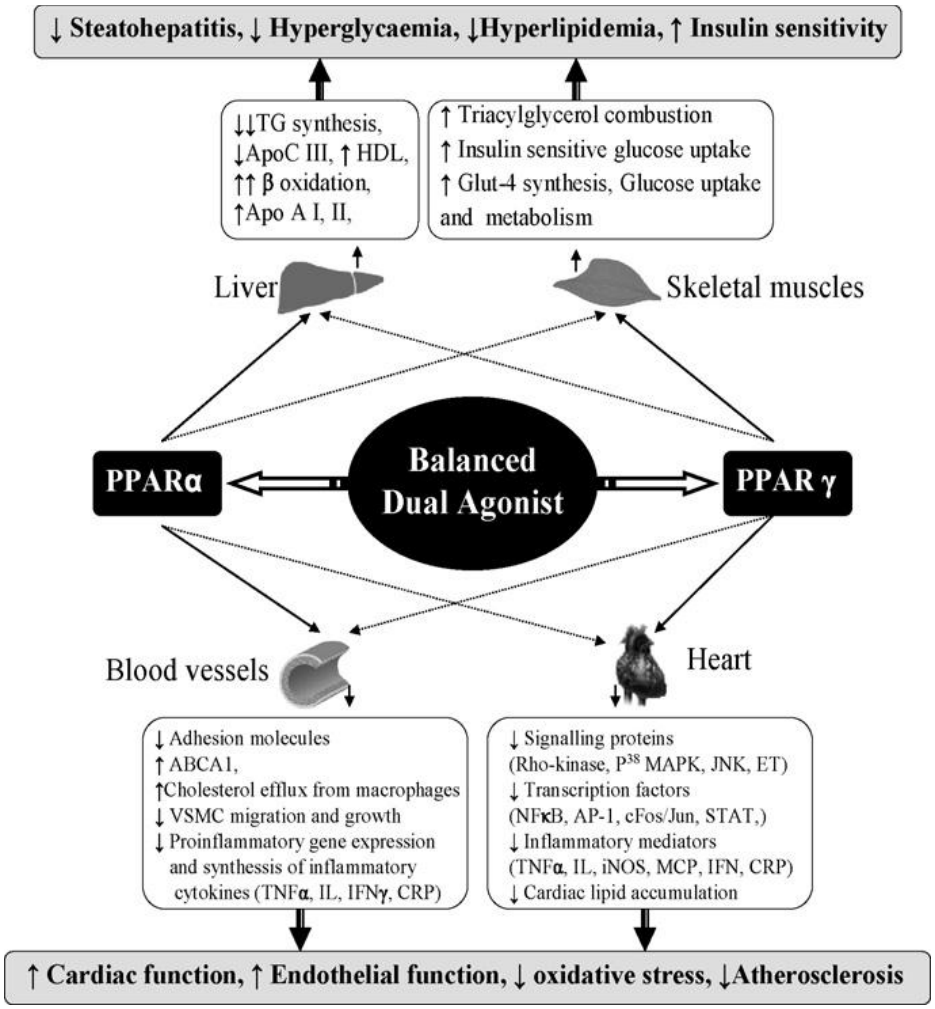


Figure 4. PPAR*α/γ* dual agonists association to regulate metabolic activity [49-53]

α and *γ*-PPAR agonists

1. Thiazolidinediones ("glitazones") are PPAR-*γ* agonists
2. Fibric acid analogs are PPAR-*α* agonists.
3. Reduce HbA1c (PPAR-*γ* mediated)
4. Increase HDL (PPAR-*α* mediated)
5. Reduce triglycerides (PPAR-*α* mediated)

PPAR dual agonist such as muraglitazar and tesaglitazar were found to improve lipid metabolism and hyperglycemia respectively but were discontinued due to their adverse effects like heart failure, oedema which were associated with muraglitazar and increased serum creatinine level due to tesaglitazar, which have raised several questions about safety concerns in the use of PPAR *α/γ* dual agonist. [54]

Chapter 2: Literature Review

2.1 Development of PPAR dual agonists:

As discussed earlier balanced affinity is required to increase effectiveness and minimize side effects so the supra activation of PPAR *α/γ* is the main cause of adverse effects.[55,56] Muraglitazar has high affinity for PPAR*γ*, whereas tesaglitazar is more specific for PPAR*α*, consequently these agonists are associated with toxic or adverse effects [57] (**Figure 5**).

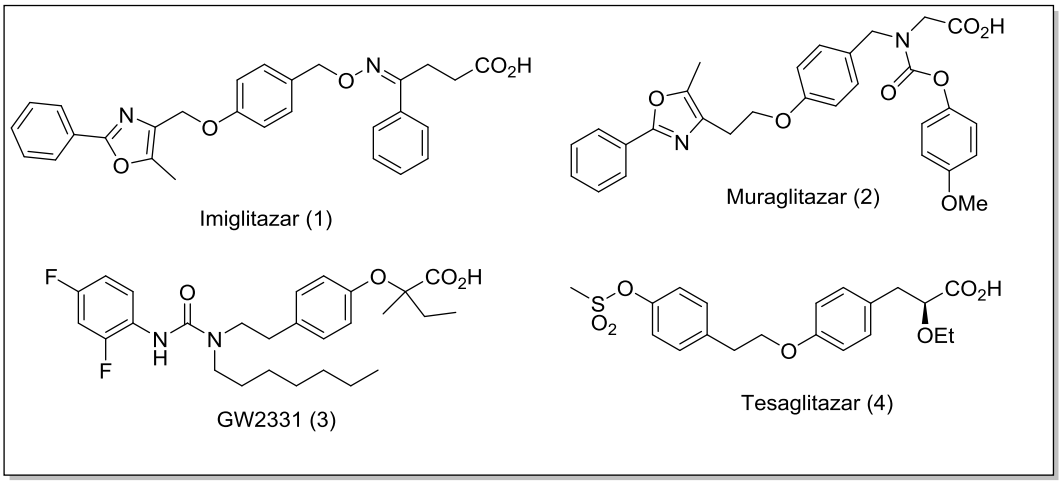


Figure 5. PPAR dual agonist

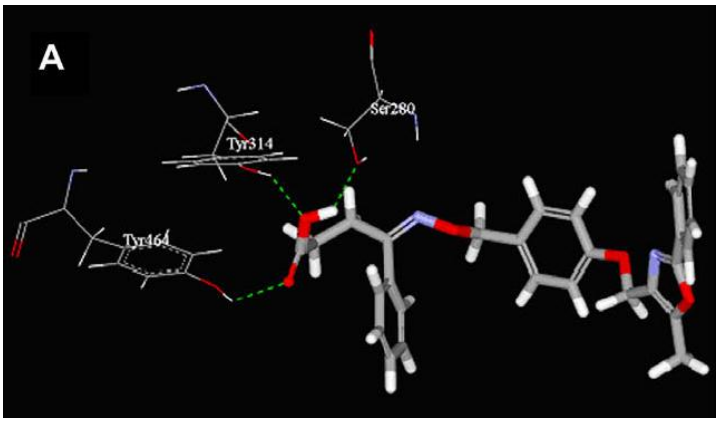


Figure 6. Molecular docking of Imiglitazar (A) into PPAR*α* LBD[58]

A PPAR agonist[59] in its design normally contains a lipophilic heterocyclic tail and an acidic head connected with a spacer. A recently reported PPAR*α* agonist imiglitazar has been found to have hydrogen bonding with amino acid residues Tyr314, Tyr 464, and Ser280 as essential characteristics for PPAR*α* agonist activity. Besides this, imiglitazar has moderate PPAR*γ* activity

through interaction with Cys295 amino acid residue (**Figure 6**). The influence of chiral centre, demonstrated in butanoic acid, a conformationally constrained analog of the well-known PPAR*α*/*γ* agonist GW2331, was found stereocontrolled (**Figure 7**).[60] Introduction of the benzooxazole group as a lipophilic part, where the *R*-enantiomer, acting dual on both PPAR*α* and PPAR*γ*, shows higher potency on PPAR*α*. Whereas, *S*-enantiomer is appear as a partial agonist by exhibiting less efficacy toward PPAR*γ*.

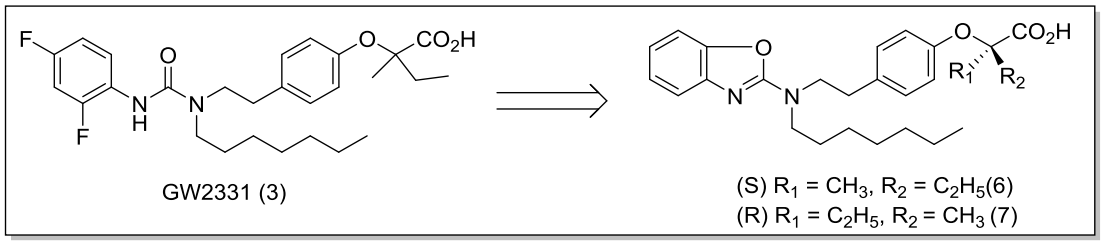


Figure 7. Chiral centre effect

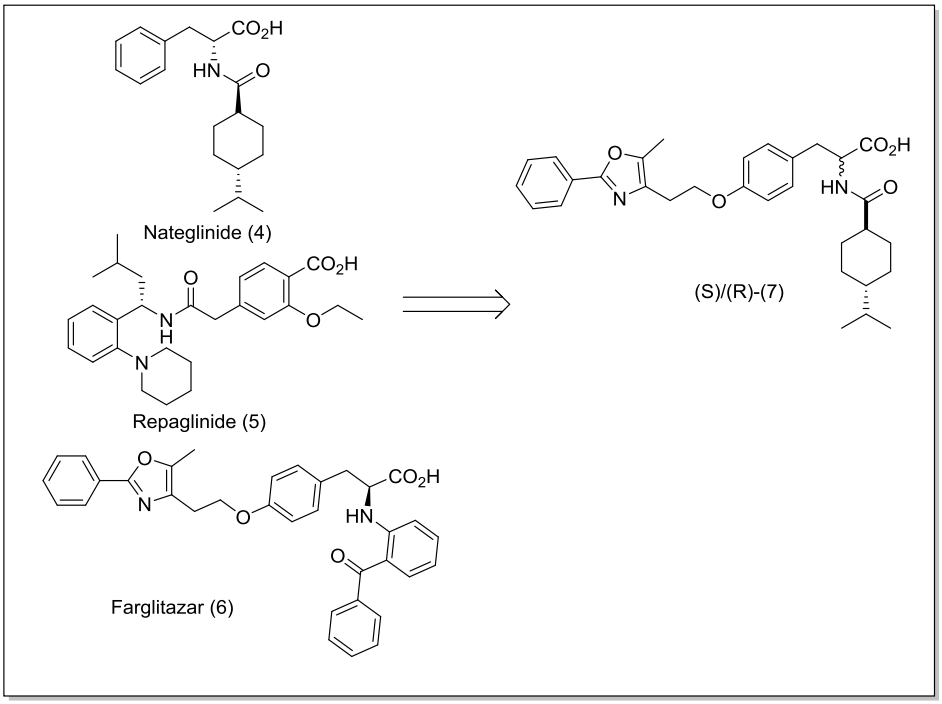


Figure 8. Stereoelectronic effect on binding affinity of dual PPAR agonist

The Nateglinide's D-phenylalanine derivatives showed influence of stereochemistry on antihyperglycemic activity.[61] This demonstrated that *R*-enantiomer was essential for activity along with carboxylic acid moiety,[62] similarly Repaglinide's *S*-enantiomer was found potent and exhibited its stereoselective effect (**Figure 8**).[63]

Isohumulones, called iso- α acids , which is obtained from the hop plant (*Humulus lupulus* L.), imparts it's flavor in beer and used as preservative as well. Previously, humulone used to possess anti-bacterial activity. Recently, among the three major isohumulone homologs, isohumulone and isocohumulone are found to activate PPAR α and γ. In an *in-vivo* study, With respect to standard drug pioglitazone these compounds showed reduced plasma glucose level, triglycerides and free fatty acid in 65.3, 62.6, and 73.1%, respectively, for isohumulone. However, unlike pioglitazone, this iso- α acids therapy did not cause any undesired body weight gain. Hence, isohumulones tend to possess the potential of being novel therapeutic option for diabetic interventions. [64]

Oxazole derivatives containing polymethylene spacer instead of phenylene group showed PPAR-α agonist. Further tetramethylene spacer of NS-220**(8)** modified to afford 1,3-dioxane-2-carboxylic acid derivative **(9)** was reported to be selective PPAR α as well as moderate dual agonist toward PPARα/γ (**Figure 9**).[65]

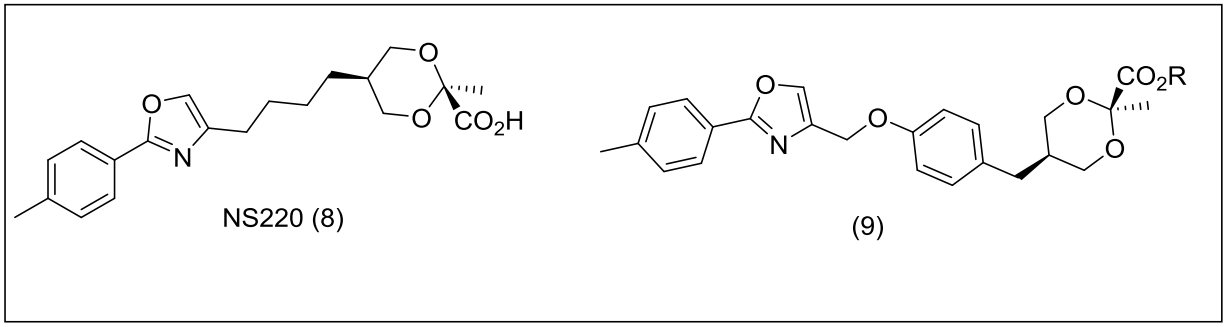
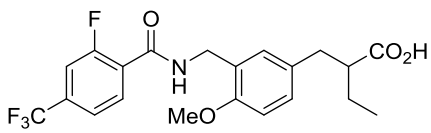


Figure 9. Chiral dual agonist

Based on the novel selective PPAR alpha and gamma scaffolds, various propionic acid derivatives were designed and synthesized which exhibited potent PPAR α/γ dual agonist activities. Futher *in-vitro* and *in-vivo* studies demonstrated their anti-diabetic activity which proved to be effective moiety for further exploration for the intervention of diabetes.[66]Further, a compound named (*S*)-2-methyl-3-{4-[2-(5-methyl-2-thiopen-2-yl-oxazol-4-yl)ethoxy]-phenyl}-2-phenoxypropionic acid was designed effectively to potentially act against type 2 diabetes by acting on dual PPAR α/γ receptor. There preclinical studies revealed that compound significantly ameliorates insulin sensitivity and actively shoot back diabetic hyperglycemia while substantially improving overall lipid homeostasis.[67]Alkoxy phenyl propanoic acid (selective PPAR-α agonist) based on their finding, they developed a potent PPAR α/γ dual agonist such as TIPP-401 **(10)** (**Figure 10**).[51] Likewise, novel scaffold 3,4-disubstituted pyrrolidine acid analog was explored and series of such

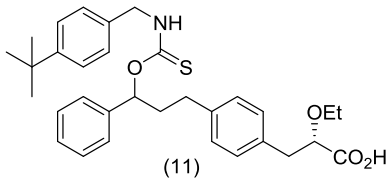
analogs were generated by carrying out it's structure activity relationship among various analogs N-4-trifluoromethyl-pyrimidinyl pyrrolidine acid was found to be more efficacious in lowering fasting glucose and triglyceride levels in diabetic db/db mice.[68]Further, compound (*RS*)-2-ethoxy-3-{4-[2-(4-trifluoro-8-methanesulfonyloxy-phenyl)-ethoxy]-phenyl}-propionic acid was designed and synthesized by Cai *et al*., which substantially activated both PPAR*α* and PPAR*γ* *in-vitro*. *In vivo*, compound (*RS*)-2-ethoxy-3-{4-[2-(4-trifluoro-8-methanesulfonyloxy-phenyl)-ethoxy]-phenyl}-propionic acidimpoves the insulin resistance and glucose tolerance and effectively alleviates plasma triglyceride levels after 14d of treatment as compared to the standard drug.[69]



TIPP-401 (10)

Figure 10. TIPP-401 as PPAR *α*/*γ* dual agonist

Later, a new class of *α*-aryloxyphenylacetic acids have been explored as agonists on both PPAR *α*/*γ* receptors and led to the emergence of subseries comprising of fused heterocyclic ring systems with modified ketone group. Their in-vitro and *in-vivo* study revealed that these compounds showedgreater potential anti-diabetic activity as compared to commercially available anti-diabetic drugs without stimulating any undesired effects associated with already available drugs in the market.[70]



(11)

Figure 11. PPAR*α*/*γ* dual agonist

Thiamide derivative (*R*)-**(11)** also showed PPAR*α*/*γ* dual agonist with good EC_{50} = 0.136 lM in PPAR*γ* and 0.377M in PPAR*α*. The SAR of these derivatives captivatingly exhibited that their stereochemistry governs the PPAR*α*/*γ* dual agonism (**Figure 11**).[71]

In heterocyclic compounds carrying aryl-pyridyl and aryl-pyrimidinyl tail, lipophilic group was changed which led to the generation of indanylacetic acid derivative **(12)**, a new class of PPAR *α*/*γ* and PPAR *α*/*γ*/*δ* agonist **(13)** (**Figure 12**).[72]

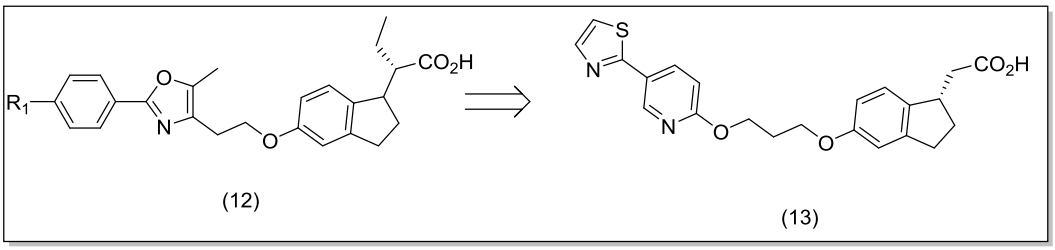


Figure 12. Indanylacetic acid anologs

Novel oximes and amides with thiazolidinone moiety have shown antihyperglycemic activity. In these series (*S*)-**(14)** compound was found more potent PPAR*α*/*γ* dual agonist than rosiglitazone (**Figure 13**).[73]

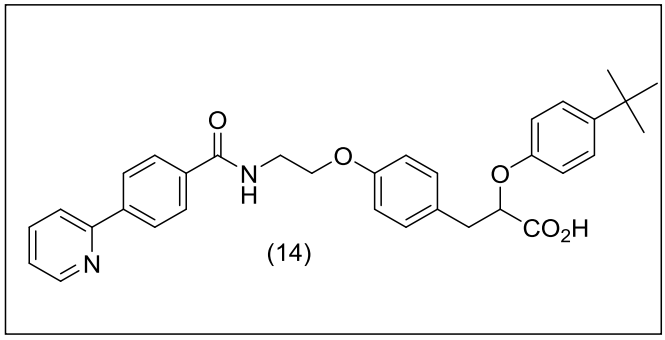


Figure 13. PPAR a/g dual agonist

In a recent study Das *et al*. developed a new thieno-oxazine analog **(15)** which was found as antihyperglycemic and lipid modulating agent (**Figure 14**).[73] This dual agonist of PPAR*α* /*γ* showed better activity than standard drugs like pioglitazone and fenofibrate.

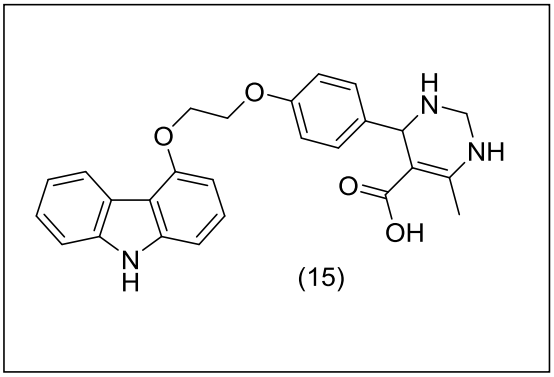


Figure 14. Thieno-oxazine analog

Barbituric acid derivatives using virtual screening and molecular docking approach were designed and having good affinity on PPAR γ with IC_{50} of 0.1µM **(16)** (**Figure 15**).[74]

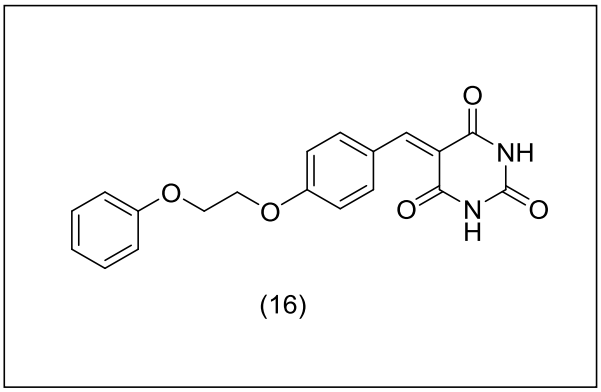


Figure 15. Barbituric acid derivatives

PPAR$\alpha/\gamma/\delta$ activators of oxadiazole series when subjected to Comparative Molecular Field Analysis (CoMFA) by Bharatam *et al.* showed the potential of oxadizoles **(17)** as PPAR$\alpha/\gamma/\delta$ activators (**Figure 16**).[75]

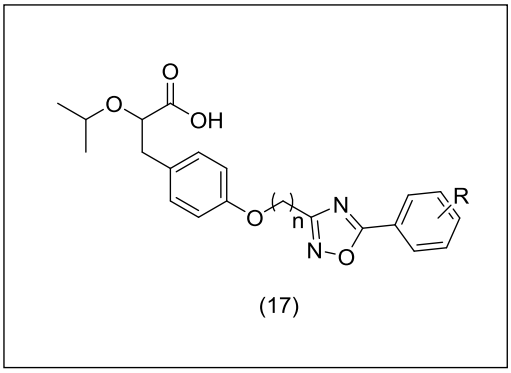


Figure 16. Oxadiazole derivative

Recently, Amorphastilbol (APH-1),is a natural trans-stilbene compound possesses dual agonistic activity for PPAR *α*/*γ*. Their biological studies revealed that constituent of Amorphastilbol were found to be effective PPAR*α*/*γ* transcriptional activators and thus, poses effective novel scaffold as dual PPAR*α*/*γ* agonists for diabetes intervention and undesired effects associated with it.[76]

Furthermore, a series of novel tetrahydrocarboline derivatives were explored by designing and synthesizing and characterizing potent PPAR*α*/*γ* dual regulators. Their *in-vitro* studies showedtheir greater PPAR-regulating activity for both receptor as compared to WY14643 and rosiglitazone.[77]

In non-thiazolidinedione class of compounds, novel benzylpyrazole acylsulfonamides were discovered as non carboxylicacid PPAR-*γ* agonists **(18,19).**[78] Their further optimization led to development of potent compound that was an antidiabetic agent having good metabolic stability (**Figure 17**).

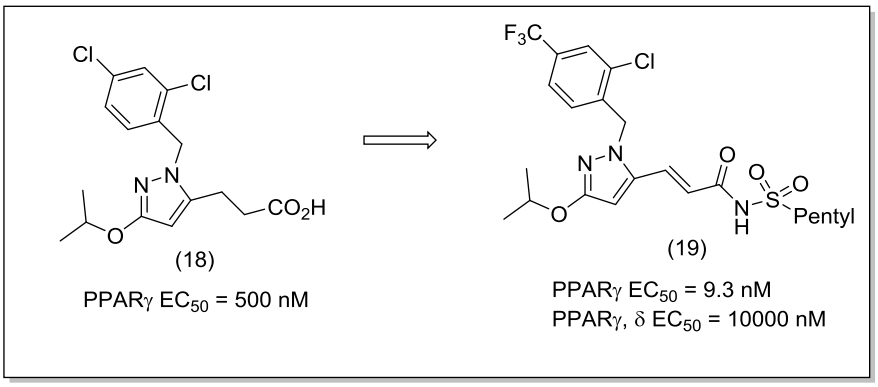


Figure 17. Benzylpyrrole analogs

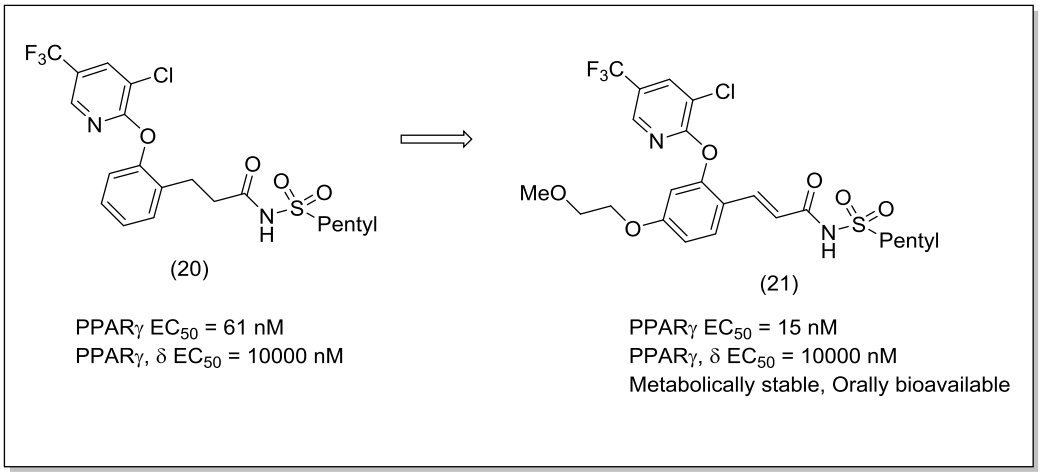


Figure 18. Sulfonyl analogs

Studies on benzylpyrazole and pyridyloxybenzyl acylsufonamide **(20,21)** and sufonyl anlogs derivatives from fibrates by Rikimaru *et al.* showed that their sufonamide analog were more potent on PPAR gamma receptor (**Figure 18**) [79,80]

New class of classical fibrate analogs which were synthesized containing *N*-(methyl sulfonyl) amides scaffold **(23)** showed good agonistic potency on PPAR-α (**Figure 19**).

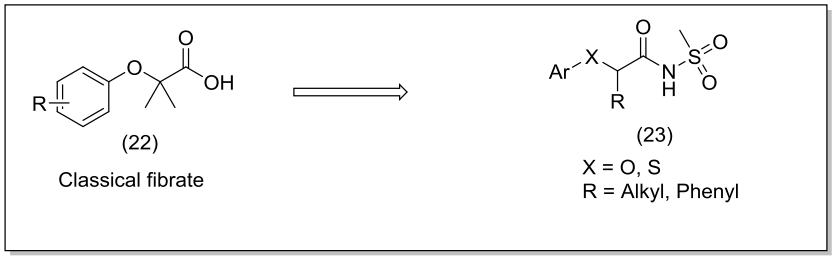


Figure 19. Sulfonyl analogs of classical fibrate compound

Various series of synthesized carbazole or phenoxazine having alkoxyindole acetic acid **(24)**[81] (**Figure 20**) and aryl peptoid **(25)**[82] were reported for transactivation of PPAR$\alpha/\gamma/\delta$ (**Figure 21**).

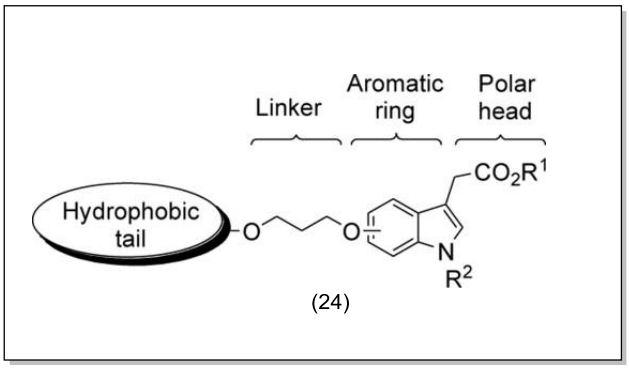


Figure 20.Alkoxyindole-3-acetic acid analogs

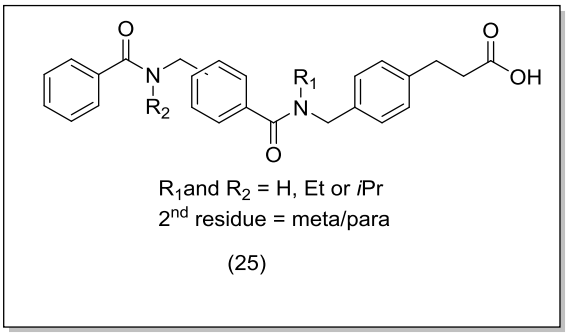


Figure 21. Arylpeptoid derivatives

Novel benzimidazolone derivatives were also evaluated for PPAR-δ modulator as a central part of molecules, with increased selectivity for PPAR-γ (**Figure 22**).[83]

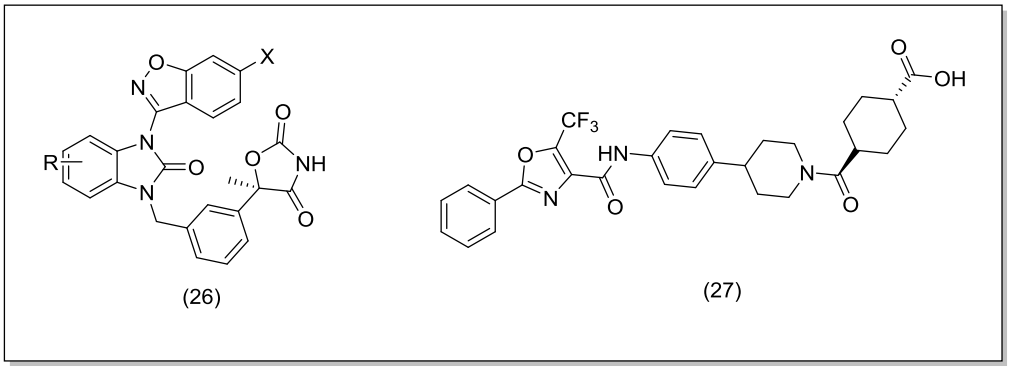


Figure 22. Heterocyclic scaffolds

2.2 Recently developed PPAR *α*/*γ* dual agonists:

Recently in the year 2015, Gao *et al.* identified 10 potential dual PPAR *α*/*γ* dual agonist lead compounds on the basis of combination of ligand based and structure based virtual screening of natural product libraries and studied their effect on lipid metabolism.[84] One of the potent lead compound as shown in **Figure 23**, exhibited no statistically significant cell toxicity, and has comparable biological activity in respect to fatty acid and cholesterol metabolism. Further Piemontese *et al.* investigated new ligand from the slight modification of previously reported ligand LT175 as PPAR *α*/*γ* dual agonist.[85,86] By surrogating oxime moiety in place of diphenyl aromatic ring of LT175, showed greater potency for PPAR *α* and partial agonistic activity for PPAR *γ*. therefore, MOIMM oximes (**28**) considered as an therapeutic novel tool for the emergence of further novel PPAR ligands.

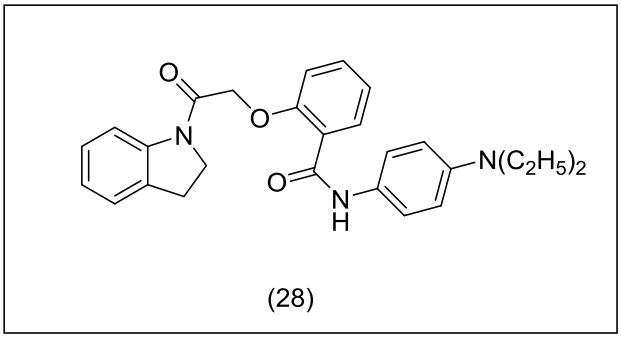


Figure 23. Modified PPAR dual agonist

Pharmacological assays and molecular modeling studies of novel PPAR ligands comprising an oxadiazolidinone ring reveal that oxadiazolidinone pharmacophore is promising scaffold for PPAR agonist activity. So, recentlybased on oxazolidinone motif, 14 new PPAR chiral ligands have been designed following 3 module structures comprising of polar head, linker region, hydrophobic tail described by Pirat *et al.*[87] With the help of docking studies, SAR was generated by changing or modifying hydrophobic tail and polar head. Among all the designed ligands, two of them showed dual well balanced PPAR *α/γ* agonist activity (**29,30**) and their significant *in-vivo* properties, made them promising therapeutic tool for further research in the management of type 2 diabetes and associated metabolic syndrome (**Figure 24**).

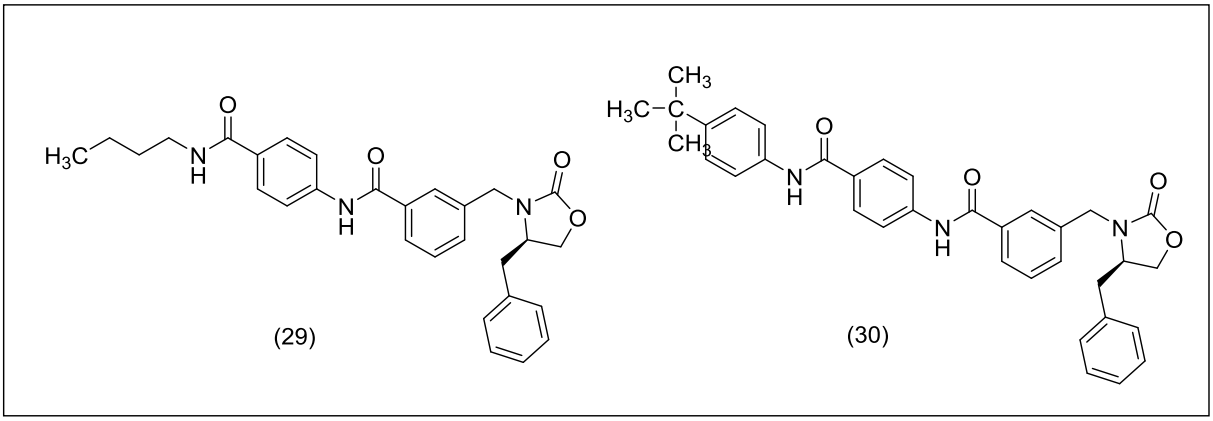


Figure 24. Dual PPAR *α/γ* agonist

17-oxodocosahexaenoic acid (17-oxoDHA), possesses anti-inflammatory activity due to oxofatty acid group (**Figure 25**). Recently in the study it has proven to exhibited PPAR*α/γ* agonist activity in transcriptional assays manner and thus comes under the category of PPAR*α/γ* dual agonists.[88]Also it has been shown to bind covalently through Cys285 for PPAR*γ* and Cys275 for PPAR*α* by ESI mass spectroscopy and X-ray crystallographic analysis, hence, 17-oxoDHA proved to be first novel class of PPAR agonist,

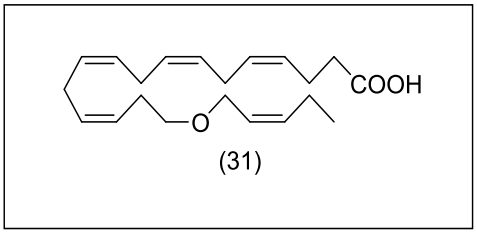
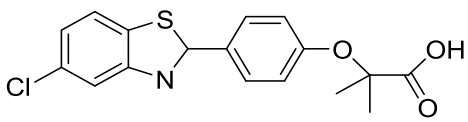


Figure 25. 17-oxodocosahexaenoic acid

With ongoing research a large number of PPAR *α/γ* dual agonists have been investigated for their functional properties to diminish type 2 diabetes and insulin resistance. Active compound as PPAR *α/γ* dual agonist named MHY908 "2-[4-(5-chlorobenzothiazothiazol-2-yl)phenoxy]-2-methyl-propionic acid" **(32a)** was found to be potential novel agent by diminishing ER stress resulting insulin resistanceand increasing adipogenesis in the liver and adipose tissue respectively, concomitant with attenuating age- related inflammation. Thus MHY908 employed as active compound used for aging intervention (**Figure 26**).[89]



(32a)

Figure 26. MHY908 a potent novel PPAR *α/γ* dual agonist

Abelmoschus manihot (L) medic (AM) is another natural compound, which is a natural medicinal plant used for the chronic kidney disease treatment in China.[90]HKC (human kidney cell) was found to activate PPAR *α/γ* target genes by CHIP and Reporter gene assays and further in vivo studies revealed that HKC potentially act as an anti diabetic nephropathy (DN) agent for treatment of diabetic nephropathy in humans amendedadipogenic disorders by activating PPAR *α/γ* and attenuated ER stress as well as ameliorated renal inflammation in DN ratsdose dependently.[91]

Similarly in the year 2017, PPAR alpha/gamma dual agonist, being an effective treatment against diabetic metabolic syndrome, more research has been focused on this regimen and by the help of virtual screening, ADMET techniques, a molecule ASN15761007 is identified with good binding affinity and low toxicity. This showed more favourable conformation for binding to both the receptors and acts as more promising lead compound as novel PPAR *α/γ* duals against insulin resistance and hyperlipidemia.[92] Later, potential of Chlorogenic acid (CGA) (**32b**) has been explored which indicated it's multiple physicochemical properties resultant in the intervention of metabolic diseases such as type 2 diabetes. CGA is being natural moiety isolated from plant *Cecropia Obtusifolia*. Biological evaluation studies revealed that, in the cellular functionality test, antihyperglycemic properties and lipid lowering properties were evaluated through increase in $[Ca^{2+}]$I levels and second, by activation of mRNA of PPAR*γ* and PPAR*α*. Therefore, compound

represents its significant effect on different organ through multiple mechanism of action **(Figure 27)**.[93]

Based on the pharmacophoric scaffold of PPAR *γ* and GPR40 agonists, some new compounds were found with improved biological activity. Similarly two novel compounds were synthesized showed promising antidiabetic activity through *in-vitro* and *in-vivo* studies which potentially act as therapeutic targets PPAR *γ* and GPR40 by increasing mRNA expression. Additionaly *in-silico* studies revealed it's polar interaction carboxylic group with residues in binding pocket site showing great affinity for both targets through molecular docking studies. The proposed scaffold (**32 c**) not only reducing insulin resistance but also effective in improvingtheinternalization of blood glucose by increasing insulin secretion **(Figure 27)**.[94]

Likewise, Yujung *et al*. predicted and validated the anti-diabetic potential of SN158**(32d)** through PPAR *α/γ* dual activation. It has been found that SN158 tend to increase their transcriptional activation resulting in boosting fatty acid oxidation in liver cells and fat cells differentiation of 3T3-L1 preadipocytes as well as glucose uptake in myotubes despite of severe weight gain and hepatomegaly **(Figure 27)**.[95]

However, several commercially available PPAR-selective drugs possess some adverse side effects. In an attempt to nullify such drawbacks, amarine organism has been explored to develop potential molecular scaffold as novel and safer PPAR-targetting drugs. Its molecular identification led several hits, among which two isomeric oxo-fatty acids from the microalgae *Chaetoceros Karianus* showed dual PPAR*α/γ* specificity. The biological evaluation of**32e1**and **32e2** showed greater antidiabetic activity resulting in treating metabolic syndrome as compared to other conventional PPAR *α/γ* dual acting antidiabetic drugs**(Figure 27)**.[96]

Likewise, **32f** has been emerged as novel PPAR *α/γ* agonists acting against insulin resistance and dislipidemia *in-vivo* and *in-vitro*. *In-vivo* and *in-vitro* study revealed it as highly efficacious and safe on insulin resistance regulation in high fat diet (HFD) and streptozotocin (STZ), palmitic acid (PA) induced diabetic mice and HepG2 cells. Thus, it's benefitial effects on glucose and lipid metabolism without increasing any obesity and hepatocytoxicity in mice adds key feature to this compound and proves it as interesting motif for treating type 2 diabetes and related metabolic disorders **(Figure 27).**[97]

However, various PPAR *α/γ* dual agonists tend to show several undesired effects, including heart failure, edema and body weight gain. To overcome such drawback, more and more advanced research is focused on developing novel PPAR *α/γ* dual agonist and similarly an antimalarial agent amodiaquine was investigated as PPAR *α/γ* dual agonist with lesser undesired effects by screening a Prestwick library (Prestwick chemical, IIIkierch, France). Further, *in-vitro* and *in-vivo* studies revealed that amadiaquine (4-[(7-chloroquinolin-4-yl)amino]-2-[(diethylamino)methyl]phenol) selectively activated the transcriptional activities of PPAR *α/γ* and showed that amodiaquine not only significantly improved hyperlipidemia, fatty liver and insulin resistancebut diminished body weight gain as well. [98]

With past diverse studies, it has been evidenced that dual PPAR *α/γ* agonist have come up as an interesting alternative to selective PPAR agonists to treat metabolic syndrome. Similarly, recently study was carried out on phytocannabinoids (**32g1, 32g2, 32g3**) which were known to activate PPAR *γ*, with no reported data on its PPAR *α* activity. *In-silico* studies assessed by molecular docking and molecular dynamics showed PPAR *α/γ* dual agonisms of phytocannabinoids followed by Luciferase assays validating these computational predictions through its effects on downstream gene transcription in adipocytes and hepatocytes resulting in their functional dual agonism **(Figure 27)**.[99]

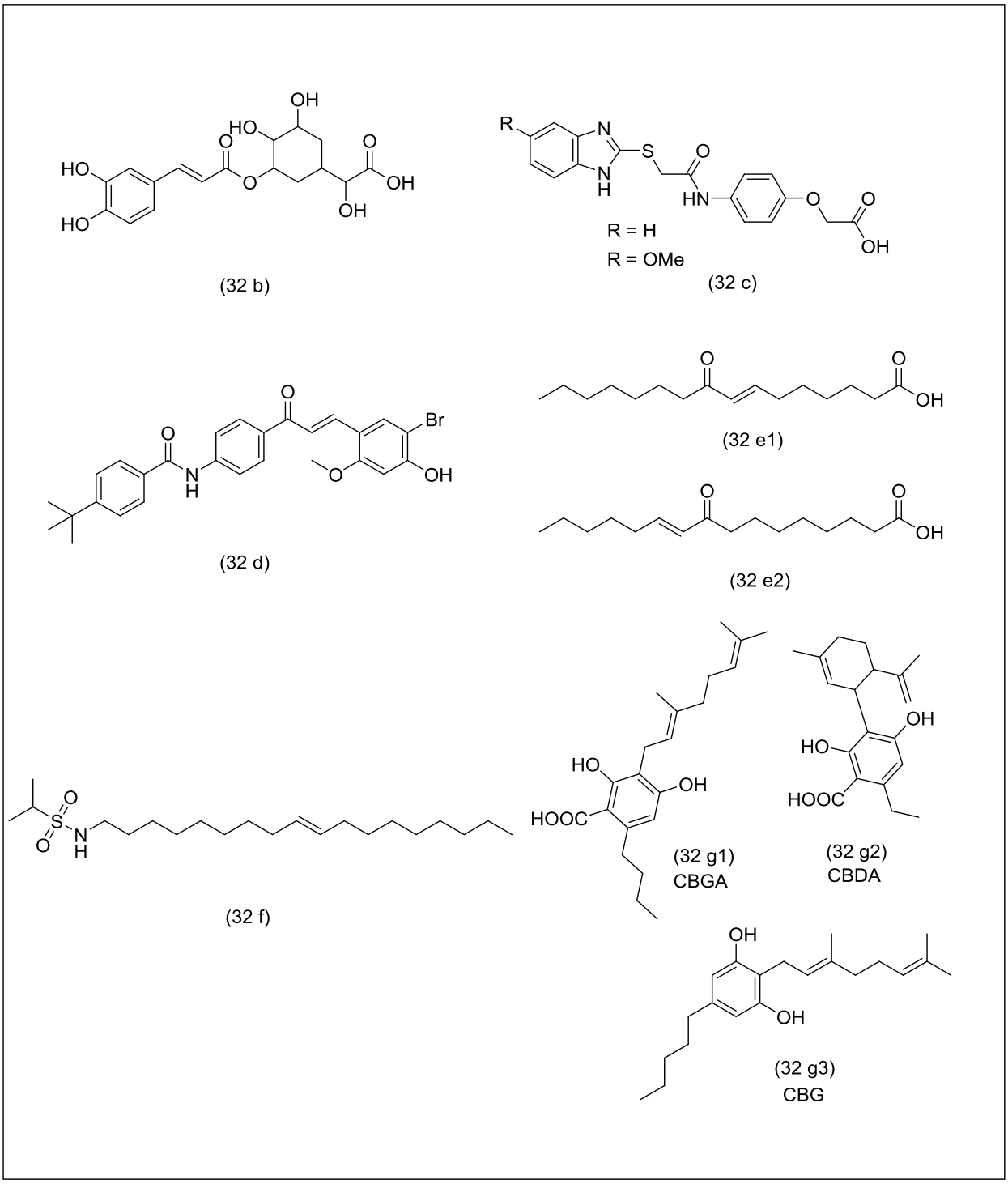


Figure 27. Recent developed ppar *α/γ* dual agonists

Compounds **33(a-e)** were found to be potent antihypoglycemic agents with PPAR*γ* as a molecular target and on their further biological evaluations, revealed that the (*S*)-enantiomers **(33)** evidently showed better potency in comparison to (*R*)-enantiomer, *in vitro* [100,101] as well as *in vivo* antihyperglycaemic activity (**Figure 28**).[102-104] Various substituted derivatives of phenylpropanoic acid (**35** and **36**), synthesized based on compound **34** by Kasuga*et al*.[105] were found effective human PPAR*α* selective agonist (**Figure 29**).

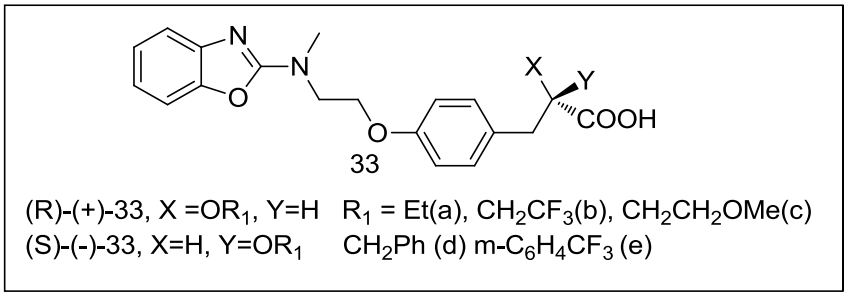


Figure 28.Structural modification of proposed scaffold

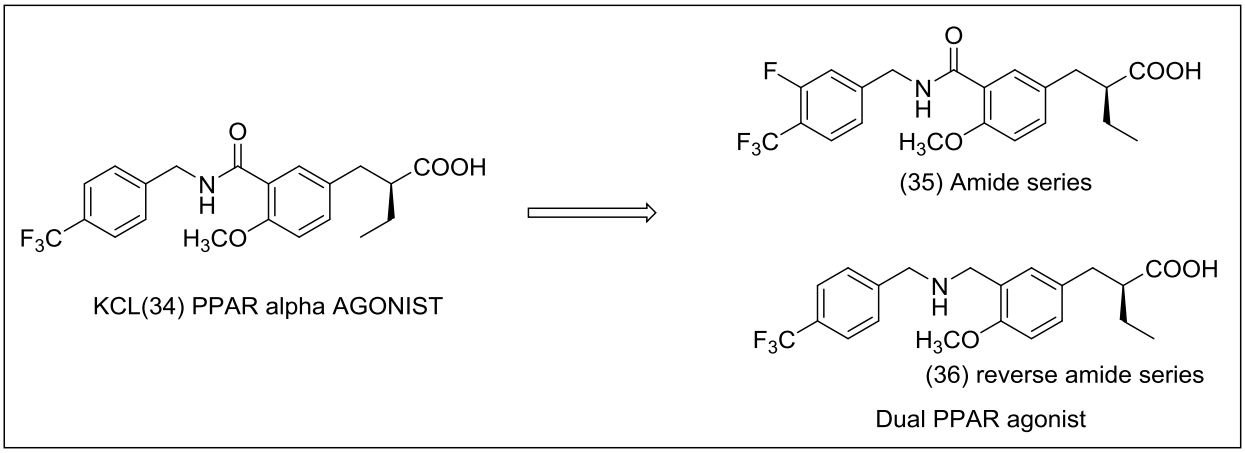


Figure 29. Substituted phenylpropanoic acid derivative

From a detailed survey of the literature, it can be concluded that to reduce toxicity and improve efficacy, a good ligand should possess a balanced affinity towards the PPAR*α/γ* receptor. So, a pharmacophore is proposed in which the acidic head group is linked with lipophilic aryl moeity through a linker which contains aryl polymethylene group, since compounds possessing polymethylene linker in between pharmacophore head and lipophilic tail showed PPAR*α* agonism,

while those with an aryl phenylene linker showed dual PPAR*α/γ* agonism (**Figure 30**).

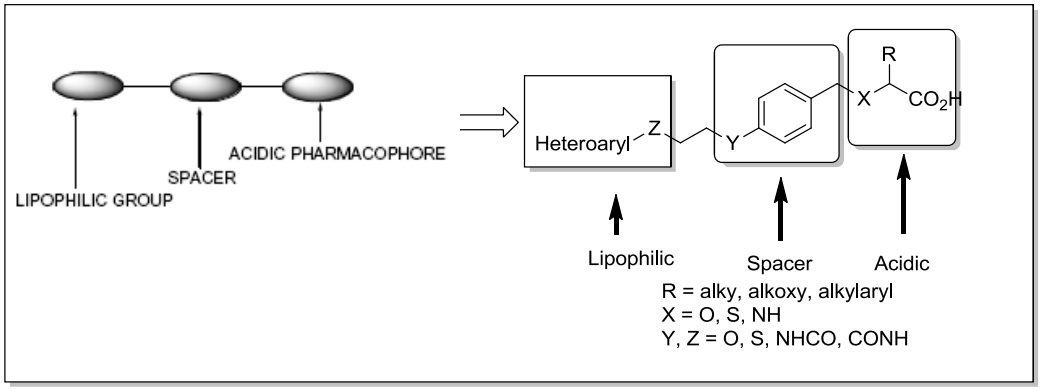


Figure 30.Designed pharmacophore for PPAR*α/γ* agonism.

Objectives:

1. Design of novel 1,2,4-oxadiazole, hydantoin, benzoxazole derivatives.
2. Study the binding interactions of the aforesaid derivatives by molecular docking and identify the most potent compounds.
3. Synthesis of most potent compound as identified through molecular docking.
4. Characterization of synthesized compound through spectroscopy (IR, NMR, Mass analysis)
5. *In-vitro* and *in-vivo* antidiabetic activity of synthesized target molecules by using Pioglitazone as standard.

Chapter 4: Work Plan

To develop novel heterocyclic antidiabetic scaffolds, it is necessary to have the knowledge of the binding interactions of existing drugs with the PPARα and γ receptors. By taking into consideration of various aspects of these ligand-receptor interactions, new scaffolds will be designed to develop new PPARα and γ dual agonist as given below:

Molecular docking study:

1. Ligand preparation: Database of various ligands will be prepared and MM2 method will be employed for minizing energy as well as geometry through ChemDraw.[106]All the optimized ligands will be saved in pdb format.
2. Protein preparation: Molecular docking performed by Autodock Vina software.[107] Protein structures will be downloaded from protein data bank [108] and prior to docking, protein is prepared by adding hydrogen atoms, optimizing hydrogen bonds, removing atomic clashes, and performing other operations by selecting the protein chain, heteroatoms, ligands and waters present in pdb file.
3. Docking study: Select the protein and ligand from liberary, setup the docking parameters and start docking calculations by analyzing the interactions between protein and ligand.
4. Analysis of docking result: Analysis of results will be carried out by comparison of docking results and ranking them with their docking score as calculated by binding affinity toward the peroxisome proliferator activated receptor alpha and gamma (with RMSD 0-1).

Chemical synthesis:

Synthesis of synthetically feasible most potent compounds: Synthesis of most potent and synthetically feasible compound will be carried out as shown in scheme 1,6, and 12 for series 1,2, and 3 respectively (refer section 6.2)

***In-vitro* evaluation by transcription factor (TF) binding assay:**

For *in-vitro* transcription factor assay, Cayman's PPARα, γ CTFA Kit is used because of its non-radioactivity as well as good sensitivity. It detects the specific transcription factor by DNA binding activity in nuclear extracts as well as whole cell lysates. It contains a specific dsDNA (double

stranded DNA) sequence with PPAR response element which immobilizes onto the bottom of wells of a 96 well plate [ELISA (Enzyme-linked immunosorbent assay)]. The PPARα, γ are sensed by adding specific antibodies bound for individual PPARs, while secondary antibody is added for increasing sensitivity for colorimetric assay at 450 nm.[109,110]

In-vivo evaluation of streptozotocin induced diabetes [111-113]

A high fat diet along with single low dose of streptozotocin induces type 2 diabetes which is causes pancreatic insulitis as well as resistance to insulin producing diabetes in rats. It is believed that the mechanism behind this is pathogenic involvement pancreas leading to low insulin production. This method is very much useful in case of IIDDM type of diabetes or type 2 diabetes.

Chapter 5: Material, Method and Experimental

5.1 Molecular docking

To identify potentially active ligands, analysis of different designed molecules wereconducted by Auto dock Vina 1.5.6 software. Trott *et al*, 2010 "Both PPAR *α,γ* proteins were downloaded from protein data bank for the preparation and extraction of ligands. Preparation of protein was carried by reloading it and fixing any problems associated with it such as removing extraneous structures like water molecules, missing bonds or atoms. While adding Kollman charges, Polar hydrogens were also added this is next step of the protein preperation. After saving the protein, the 2p54.pdbqt and 3SZ1.pdbqt was opened and both were set as map type by choosing ligand and generated the grid box. By extracting the ligand and docking it in the same manner as actual ligand, the protein was validated. Then with the help of ChemDraw Ultra, designed molecules were drawn and converted to 3D structures. By the semi-empirical MM2 method, the geometry of all compounds were optimized and on these optimized structures, Molecular docking was performed. To develop new molecules it is necessary to know affinity of the proposed molecules towards our target molecule which will be obtained by docking studies. Select the protein and ligand from liberary, setup the parameters and start calculations by analyzing the interactions between protein and ligand".

5.2 Chemical synthesis

BrukerAvance 400 (400MHz) spectrometer was employed for conducting ^{1}H-NMR spectra using TMS as an internal standard and solvent as $CDCl_3$. The chemical shifts (δ) were observed for 1H(Hz). To indicate the multiplicity, the following abbreviations are used: s, singlet; d, doublet; m, multiplet. TLC (Merck) was used to monitor the reactions and rotator evaporator was utilized to carry out evaporation of solvents under reduced pressure. Commercial grade reagents, chemicals and solvents were used without further purification.

5.2.1 General procedure for the synthesis of *trans*-Cinnamic acid (39):

Method 1: To a stirred solution of benzaldehyde (**37a**, 1.7 ml, 16.04 mmol, 1 equiv) and malonic acid (**38**, 2 g, 24.06 mmol, 1.5 equiv) in pyridine (5 mL) was added $LiClO_4$ (0.34 g, 3.2 mmol, 0.2 equiv) and refluxed for 5 hr. With the help of TLC, reaction progress was monitored using eluent EtOAc: n-hexane (4:6). After completion of the reaction the solution acidified with concentrated HCl to afford a white precipitate. The precipitate was filtered and washed with water (10 ml) twice,

further dried to afford 2.2 g (93%) of **39a** as the final product. All synthesized compounds were characterized by IR, NMR, and mass with satisfactory spectral data.

Method 2: This method was used in case of 4-hyroxy benzaldehyde as a starting material to prepare *trans*-4-Hydroxy cinnamic acid (**39f**) where method 1 afford low yield. In a typical experiment, 2g of malonic acid (**38)** (24.06 mmol, 1.5 equiv) was taken and dissolved in 5 mL pyridine in RBF fitted with reflux condenser. 1 g of 4-hydroxy benzaldehyde (**37f**) (16.04 mmol, 1 equiv) and 0.036 ml piperidine (3.2 mmol, 0.2 equiv) was added to the reaction mixture. The mixture is heated at 100 °C under reflux for 1 hour (CO_2 produced). It was then cooled down by ice bath and 4 mL conc. HCl was added slowly into the solution. Vacuum filtrate the solution and crude product was obtained. The crude *trans*-4-hydroxy cinnamic acid (**39f**) was recrystallized from ethanol/water (3:1).

***trans*-Cinnamic acid** (**39a**): "Physical and spectroscopic data were identical to previous literature reports for this compound. Yield: 93%; white solid; mp: 135-139 °C; *Rf*: 0.5 (ethyl acetate / n-hexane 4/6); IR(cm^{-1}): 1627 (C=C), 1681 (C=O), 2833 (C-H); ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.46 (d, 1H, *J* = 16Hz, *t*-CH=CH); 7.39-7.43 (m, 3H, Ar-H), 7.55-7.57 (m, 2H, Ar-H); 7.80 (d, 1H, *J* = 16Hz, *t*-CH=CH); ^{13}C NMR (100 MHz, $CDCl_3$): δ 117.19, 128.39, 128.99, 129.92, 134.04, 147.14, 171.91; MS-ESI (m/z): 149.142 (M+1); Anal. Calcd for $C_9H_8O_2$: C, 72.96; H, 5.44; Found: C, 72.91; H, 5.42".

***trans*-4-Chlorocinnamic acid** (**39b**): "Physical and spectroscopic data were identical to previous literature reports for this compound. Yield: 82%; white solid; mp: 245-250 °C; *Rf*: 0.4 (ethyl acetate / n-hexane 4/6);, IR (cm^{-1}): 1620 (C=C), 1685 (C=O), 2920 (C-H); ^{1}H NMR (400 MHz, $CDCl_3$): 6.42 (d, 1H, *J* = 16 Hz, *t*-CH=CH); 7.37 (d, 2H, *J* =4Hz, Ar-H), 7.55 (d, 2H, *J* = 4Hz, Ar-H), 7.58 (d, 1H, *J* = 16Hz, *t*-CH=CH); ^{13}C NMR (100 MHz, $(CD_3)_2SO$): 119.59, 128.64, 129.12, 132.81, 134.94, 142.24, 167.39; MS-ESI (m/z): 183.12 (M+1); Anal. Calcd for $C_9H_7ClO_2$: C, 59.20; H, 3.86; Found: C, 59.22; H, 3.87".

***trans*-2-Chlorocinnamic acid** (**39c**): "Physical and spectroscopic data were identical to previous literature reports for this compound. Yield: 80%; white solid; mp: 170-180 °C; *Rf*: 0.4 (ethyl acetate / n-hexane 4/6);, IR (cm^{-1}): 1627 (C=C), 1687 (C=O), 2880 (C-H). ^{1}H NMR (400 MHz, $CDCl_3$): 6.44 (d, 1H, *J* = 16Hz, *t*-CH=CH), 7.34 (d, 2H, *J* = 8Hz, Ar-H), 7.43 (d,1H, *J* = 8Hz, Ar-H); 7.70 (d, 1H, *J*= 8Hz, Ar-H); 7.98 (d, 1H, *J*=16Hz, *t*-CH=CH); ^{13}C NMR (100 MHz, $(CD_3)_2SO$): 122.01,

127.39, 127.89, 129.74, 131.20, 131.94, 133.66, 138.75, 167.07; MS-ESI (m/z): 183.14 (M+1); Anal. Calcd for $C_9H_7ClO_2$: C, 59.20; H, 3.86; Found: C, 59.24; H, 3.89."

***trans*-4-Fluorocinnamic acid** (**39d**): "Physical and spectroscopic data were identical to previous literature reports for this compound. Yield: 94%; white solid, mp. 205-211 °C; *Rf*: 0.4 (ethyl acetate / n-hexane 4/6);, IR (cm^{-1}): 1622 (C=C), 1681 (C=O), 2910 (C-H). ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ: 6.45 (d, 1H, *J* = 16 Hz, *t*-CH=CH), 7.14-7.21 (m, 2H, ArH), 7.57 (d, 1H, J = 16.2, *t*-CH=CH), 7.62-7.67 (m, 2H); ^{13}C NMR (100 MHz, $(CD_3)_2SO$) δ: 115.82, 120.11, 129.54, 130.10, 141.81, 162.23, 168.71; MS-ESI (m/z): 167.11 (M+1); Anal. Calcd for $C_9H_7FO_2$: C, 65.06; H, 4.25; Found: C, 65.12; H, 4.31."

***trans*-4-Methoxycinnamic acid** (**39e**): "Physical and spectroscopic data were identical to previous literature reports for this compound. Yield = 81%; pale yellow solid; mp: 181-183 °C, *Rf* : 0.4 (ethyl acetate / n-hexane 4/6); IR (cm^{-1}): 1622 (C=C), 1680 (C=O), 2921 (C-H). ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ: 3.81 (s, 3H, OCH_3); 6.31 (d, 1H, *J* = 16Hz, *t*-CH=CH); 6.94 (d, 2H, 8Hz, Ar-H), 7.51-7.56 (d, 2H, *J* = 8Hz, Ar-H), 7.56 (d, 1H, *J* = 16Hz , *t*-CH=CH); ^{13}C NMR (100 MHz, $CDCl_3$): 55.07, 114.12, 116.28, 126.68, 129.55, 143.53, 160.81, 167.79; MS-ESI (m/z): 179.58 (M+1); Anal. Calcd for $C_{10}H_{10}O_3$: C, 67.41; H, 5.66; Found: C, 67.38; H, 5.62."

***trans*-4-Hydroxycinnamic acid** (**39f**): "Physical and spectroscopic data were identical to previous literature reports for this compound. Yield 61%: white solid, mp 201-208°C, *Rf* : 0.3 (ethyl acetate / n-hexane 4/6); IR (cm^{-1}): 1620 (C=C), 1690 (C=O), 2910 (C-H), 3510 (O-H); ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ: 6.45 (d, 1H, *J* = 16 Hz, *t*-CH=CH), 7.14-7.21 (m, 2H, ArH), 7.57 (d, 1H, *J* = 16.2, *t*-CH=CH), 7.62-7.67 (m, 2H); ^{13}C NMR (100 MHz, $(CD_3)_2SO$) δ: 115.82, 117.12, 128.12, 129.44, 144.12, 159.21, 169.32; MS-ESI (m/z): 165.56 (M+1); Anal. Calcd for $C_9H_8O_3$: C, 65.85; H, 4.91; Found: C, 65.91; H, 4.95."

***trans*-4-Hydroxy-3-Methoxycinnamic acid** (**39g**): "Yield: 78%, white solid; mp: 160-161 °C, *Rf* : 0.3 (ethyl acetate / n-hexane 4/6); IR (cm^{-1}): 1514 (C=C), 1689 (C=O), 2964 (C-H), 3437 (O-H); ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ: 3.86 (s, 3H, OCH_3); 6.26 (d, 1H, *J* = 16Hz, *t*-CH=CH); 6.81 (d, 1H, *J* = 8Hz, Ar-H), 7.01 (dd, 1H, *J* = 1.8 Hz (1,2), 8 Hz(1,3), Ar-H), 7.14 (d, 1H, *J* = 8Hz, Ar-H); 7.48 (d, 1H, *J* = 16Hz, *t*-CH=CH); 9.36 (s, 1H, OH); 12.00 (bs, 1H, COOH); ^{13}C NMR (100 MHz, $(CD_3)_2SO$) δ: 55.49, 110.51, 115.36, 122.40, 125.67, 144.24, 147.68, 148.90, 167.97; MS-ESI (m/z): 195.57 (M+1); Anal. Calcd for $C_{10}H_{10}O_4$: C, 61.85; H, 5.19; Found: C, 61.92; H, 5.21."

***trans*-4-*N,N*-Dimethylcinnamic acid** (**39h**): "Yield: 70%, yellow solid; mp: 221-222 °C;*Rf* : 0.4 (ethyl acetate / n-hexane 4/6); IR (cm^{-1}): 1514 (C=C), 1689 (C=O), 2964 (C-H), 3437 (O-H); ^1H NMR (400 MHz, $(CD_3)_2SO$) δ: 3.03 (s, 6H, N-CH_3), 6.21 (d, 1H, *J* = 16Hz, *t*-CH=CH); 6.83 (d, 2H, *J* = 8Hz, Ar-H), 7.48 (d, 1H, *t*-CH=CH); 8.10-8.14 (d, 2H, *J* = 8Hz, Ar-H); ^{13}C NMR (100 MHz, $(CD_3)_2SO$) δ: 40.14, 110.75, 112.72, 127.18, 129.32, 141.50, 150.41, 168.13; MS-ESI (m/z): 192.63 (M+1); Anal. Calcd for $C_{11}H_{13}NO_2$: C, 69.09; H, 6.85; N, 7.32; Found: C, 69.22; H, 6.91; N, 8.01."

***trans*-Pent-2-enoic acid** (**39i**): "Yield: 66%; colorless liquid; *Rf* : 0.6 (ethyl acetate / n-hexane 4/6); IR (cm^{-1}): 1654 (C=C), 1698 (C=O), 2970 (C-H).). ^1H NMR (400 MHz, $(CD_3)_2SO$) δ:1.08 (t, 3H, *J* = 6 Hz, CH_3), 2.22-2.27 (m, 2H, CH_2), 5.80-5.84 (dd, 1H, *J* =16Hz, CH=CH), 7.10-7.17 (m, 1H, CH); 10.19 (s, 1H, COOH); ^{13}C NMR (100 MHz, ($CDCl_3$) δ: 11.84, 25.32, 119.73, 154.51, 172.00; MS-ESI (m/z): 101.50 (M+1); Anal. Calcd for $C_5H_8O_2$: C, 59.98; H, 8.05; Found: C, 60.45; H, 8.21."

5.2.2 Procedure for the synthesis of (*E*)-methyl 3-(4-hydroxyphenyl)acrylate (40): "A 5 g of*trans* -4-hydroxy cinnamic acid (**39f**) (30.49 mmol, 1 equiv) was dissolved in 20 mL of methanol. A 0.33 mL of conc. H_2SO_4 (6.10 mmol, 0.2 equiv) was added. This reaction mixture was refluxed for 2 hr. Methanol was evaporated and the reaction mixture was washed with aq. $NaHCO_3$ solution. Further it was extracted in ethyl acetate and concentrated to get desired compound. Light pale yellow liquid, Yield: 75%, Rf: 0.5 (2:8 EtOAc: hexane), IR (cm^{-1}): 1595 (C=C), 1680 (C=O), 2357(C-H), 3281(O-H). ^1H NMR (400 MHz, $CDCl_3$) : δ 3.71(s, 3H, CH_3); 6.31 (d, 1H, *J* = 16Hz, CH=CH); 6.79 (m, 2H); 7.44-7.47(m, 2H); 7.55 (d, 1H, *J* = 16Hz, CH=CH); 9.85 (s, 1H, OH); ^{13}C NMR ($CDCl_3$) : δ 50.00, 113.69, 115.66, 124.91, 129.85, 144.53, 159.76, 166.87; MS- ESI (m/z):179.59 (M+1)."

5.2.3 Procedure for the synthesis of (*E*)-2-(4-(3-methoxy-3-oxoprop-1-en-1-yl)phenoxy)acetic acid (41):"3 g of (*E*)-methyl 3-(4-hydroxyphenyl)acrylate (**40**) (16.84 mmol, 1 equiv) was dissolved in 50 mL of acetone. To this solution, 7 g of K_2CO_3 (50.52 mmol, 3 equiv) was mixed properly and thereafter a solution of 3.18g of chloroacetic acid (33.68 mmol, 2 equiv) was addeddropwise in 10 mL of acetone. Further, the stirring of the mixture was carried at room temperature for 4hr and reaction progress was monitored by TLC. The mixture was acidified with dil. HCl and organic phase was extracted with ethyl acetate. The organic phase was dried with anhydrous Na_2SO_4 and filtered. The filtrate was dried to afford (*E*)-2-(4-(3-methoxy-3-oxoprop-1-en-1-yl)phenoxy)acetic acid (**41**). White solid mass; Yield: 80%, Rf: 0.5 (4:6 EtOAc: hexane),

mp90-110 °C; IR (cm^{-1}): 1510 (C=C), 1600 (C=N), 1728 (C=O), 3421 (O-H); ^{1}H NMR (400 MHz, $CDCl_3$) : δ 3.72(s, 3H, CH_3); 5.01 (s, 2H), 6.30 (d, 1H, *J* = 16Hz, CH=CH); 6.79 (d, 2H, *J*= 8Hz); 7.45 (d, 2H, *J* = 8Hz); 7.55 (d, 1H, *J* = 16Hz, CH=CH); 9.83 (s, 1H, OH);^{13}C NMR (100 MHz, $CDCl_3$) δ 50.95, 65.21, 78.94, 113.67, 115.66, 124.90, 129.80, 144.51, 159.74, 166.86. MS (m/z): 236.58 (M+)."

5.2.4 General procedure for the synthesis of *N*-hydroxybenzimidamide (amidoxime) (43): "A 1.3 g of solution of 4-chorobenzonitrile (**42a**,9.69mmol, 1 equivalent) was dissolved in 50 mL of methanol. To this 2 g of K_2CO_3 (14.53 mmol, 1.5 equiv) was added and mixed properly. Reaction mixture was cooled at 0 °C, and 1.35 g of hydroxylamine hydrochloride (19.38 mmol, 2 equiv) was added in portion wise. After completion of the addition it was refluxed for 6hr. Then evaporated the solvent and excess of water was added to afford the precipitate of the desired product which was filtered and dried."

4-Chloro-*N*-hydroxybenzimidamide (43a): "Yield 66%; white solid;mp 132–136 °C; IR (cm^{-1}): 1494 (C=N), 1614 (C=C), 3379 (O-H), 3470 (N-H); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.50 (s, 2H, NH), 6.55-6.58 (m, 2H), 6.72 (s, 2H), 7.46 (s, 1H, OH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 127.18, 128.93, 130.85, 136.01, 151.87; MS-APCI (m/z): 193.63 (M+23)."

4-Fluoro-*N*-hydroxybenzimidamide (43b): "Yield 68%; Light brown solid; mp 125–129 °C; IR (cm^{-1}): 1589 (C=N), 1708 (C=C), 2980 (O-H), 3373 (N-H);^{1}H NMR (400 MHz, $CDCl_3$) δ5.81(s, 2H, NH), 6.98-7.10 (m, 2H), 7.95 -7.97(m, 2H), 9.87(s, 1H, OH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 115.04, 115.17, 127.49, 130.08, 161.26; MS-APCI (m/z): 177.59 (M+23)."

***N*-Hydroxybenzimidamide (43c): "**Yield 78%; white solid;mp 74–78 °C; IR (cm^{-1}): 1589 (C=N), 1659 (C=C), 3400 (O-H), 3514 (N-H);^{1}H NMR (400 MHz, $CDCl_3$) δ 5.76 (s, 2H, NH), 7.16-7.18 (m, 3H), 7.71 (d, 2H, *J* = 8Hz), 9.58 (s, 1H, OH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 128.09, 128.19, 128.38, 131.24, 168.01; MS-APCI (m/z): 137.07 $(M+1)^{+}$."

4-Methyl-*N*-hydroxybenzimidamide (43d): "Yield = 77%; Off white solid; mp 80-84^{0}C; Rf: 0.55 (2:8 EtOAC: Hexane),IR (cm^{-1}): 1602 (C=N), 1678 (C=C), 2879 (O-H), 3184 (N-H); ^{1}H NMR (400 MHz, $CDCl_3$); δ 3.11 (s, 3H, CH_3), 5.76 (s, 2H, NH), 7.17 (d, 2H, *J*=8Hz), 7.71 (d, 2H, *J*=8Hz), 9.58 (s, 1H, OH).^{13}C NMR (100 MHz, $CDCl_3$) δ 21.27, 125.24, 128.71, 130.3, 139.46, 138.26, 167.87; MS-APCI (m/z): 151.09 $(M+1)^{+}$."

2-Methoxy-*N*-hydroxybenzimidamide (43e): "Yield 63%; Reddish brown solid; mp 128–133^{0}C; Rf: 0.66 (2:8 EtOAC : Hexane), IR (cm^{-1}): 1610 (C=N), 1666 (C=C), 3200 (O-H), 3500 (N-H);^{1}H NMR (400 MHz, $CDCl_3$) δ 4.01 (s, 3H, OCH_3), 6.19 (d, 2H, NH), 6.88-7.14 (m, 2H), 7.26-7.34 (m, 2H), 8.13 (s, 1H, OH). MS-APCI (m/z): 166.18 $(M)^+$."

3-Methoxy-*N*-hydroxybenzimidamide (43f): "Yield 78%; Light brown solid; mp 90–94^{0}C; Rf: 0.68 (2:8 EtOAC : Hexane), IR (cm^{-1}): 1598 (C=N), 1667 (C=C), 3000 (O-H), 3650 (N-H); ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.77 (m, 3H, OCH_3), 6.39 (d, 2H, NH), 7.11 -7.15 (m, 2H), 7.53 – 7.63 (m, 2H), 8.18 (s, 1H, OH); MS-APCI (m/z): 168.09 $(M+1)^+$."

5.2.5 General procedure for the synthesis of substituted (*E*)-3-Phenyl-5-styryl-1,2,4-oxadiazole (44):

"(*E*)-2-(4-(3-Methoxy-3-oxoprop-1-en-1-yl)phenoxy)acetic acid (**41**) (2 g, 8.47 mmol, 1 equiv) and hydroxybenzotriazole (HOBt) (1.44 g, 8.47 mmol, 1 equiv) were slurried in dry acetonitrile (20 mL). Addition of *N*-(3-Dimethylaminopropyl)-*N′*-ethylcarbodiimide hydrochloride (EDC.HCl) (1.6 g, 8.47 mmol) to this mixture was done at room temperature. To this solution, 4-Chloro-*N*-hydroxybenzimidamide (1.45 g, 8.47 mmol, 1 equiv) was added and stirred for 1 h. The solvent evaporated to afford the intermediate product and to this toluene was added and refluxed for 3 h. The reaction mixture was partitioned with ethyl acetate and aqueous bicarbonate solution. Then dried the organic layer and concentrated to afford the crude product which was purified by recrystallization in DCM to afford the desired product."

Methyl (*E*)-3-(4-((3-(4-chlorophenyl)-1,2,4-oxadiazol-5-yl)methoxy)phenyl)acrylate (44a): "off white solid; Yield: 72%, mp: 200-215°C, Rf: 0.76 (2:8 EtOAc: hexane), IR (cm^{-1}): 1602 (C=C), 1678 (C=O), 3184(O-H). ^{1}H NMR (400 MHz, DMSO): δ 3.72 (s, 3H, CH_3), 5.04 (s, 2H, CH_2), 5.59 (d, 1H, J = 16Hz, CH=CH), 6.45 (d, 1H, J = 16Hz, CH=CH), 7.00-7.02 (m, 4H), 7.48(d, 2H, J= 8Hz), 7.76 (d, 2H, J = 8Hz); ^{13}C NMR (100 MHz, DMSO) δ 47.83, 78.27, 112.12, 114.51, 116.33, 118.91, 120.22, 126.60, 127.93, 128.82, 129.18, 129.70, 134.21, 143.44, 161.99. MS-APCI (m/z): 409.76 $(M+39)^+$".

Methyl(*E*)-3-(4-((3-(4-Florophenyl)-1,2,4-oxadiazol-5-yl)methoxy)phenyl)acrylate (44b):"white solid; Yield: 50%; mp: 180-220°C, Rf: 0.68, (2:8 EtOAc: hexane), IR (cm^{-1}): 1604 (C=C), 1710 (C=O. ^{1}H NMR (400 MHz, DMSO): δ 3.71 (s, 3H, CH_3), 5.01 (s, 2H), 6.41(d, 1H, J=16Hz,

CH=CH), 6.91(d, 2H, *J* = 16Hz, CH=CH), 7.34-7.36 (m, 2H), 7.51-7.61 (m, 3H), 7.66 (d, 2H, *J* = 8Hz); MS-APCI (m/z): 355.13 $(M+1)^+$".

Methyl (*E*)-3-(4-((3-phenyl-1,2,4-oxadiazol-5-yl)methoxy)phenyl)acrylate (44c): "white solid, Yield: 78%; mp: 188 -200 °C, Rf: 0.77 (2:8 EtOAc: hexane); IR (cm^{-1}): 1627 (C=N), 1712 (C=C), 1754 (C=O). ^1H NMR (400 MHz,DMSO) : δ 3.72 (s, 3H, CH_3), 5.03(s, 2H, CH_2), 5.55 (d, 1H, *J*=8Hz, CH=CH), 6.43(d, 1H, *J* = 16Hz, CH=CH), 7.01(d, 2H, *J* = 8Hz), 7.43-7.45 (m, 2H), 7.62-7.63 (m, 3H), 7.73 (d, 2H, *J*=8Hz). ^{13}C NMR (100 MHz, DMSO) δ 51.32, 64.01, 114.08, 115.15, 115.47, 126.80, 127.41, 128.41, 130.11, 131.20, 130.22, 144.15, 157.62, 159.49, 166.89; MS-APCI (m/z): 353.32 $(M+1)^+$".

Methyl (*E*)-3-(4-((3-(p-tolyl)-1,2,4-oxadiazol-5-yl)methoxy)phenyl)acrylate (44d): "Reddish brown; Yield: 88%; mp: 260-268 °C, Rf: 0.81 (2:8 EtOAc: hexane); IR (cm^{-1}): 1609 (C=C), 1712 (C=O), 3389 (O-H); ^1H NMR (400 MHz, DMSO) : δ 2.51 (s, 3H, CH_3), 3.69 (s, 3H, CH_3), 5.43 (s, 2H, CH_2), 6.19 (d,1H, *J*=16Hz, CH=CH), 6.30 (d, 2H, *J* =16Hz), 6.75 (d, 2H, *J*= 8Hz), 6.86 (d, 2H, *J*= 8Hz), 7.21 (d, 2H, *J*= 8Hz), 7.33 (d, 1H, *J* = 8Hz, CH=CH); ^{13}C NMR (100 MHz, DMSO) δ 21.05, 60.84, 64.03, 114.08, 115.46, 126.65, 127.85, 129.38, 130.09, 130.20, 141.79, 144.14, 158.96, 159.50, 166.88, 175.35;MS-APCI (m/z): 367.35 $(M+17)^+$".

(*E*)-Methyl 3-(4-((3-(2-methoxyphenyl)-1,2,4-oxadiazol-5-yl)methoxy)phenyl)acrylate (44e): "Reddish brown; Yield: 79%; mp 210-223°C, Rf : 0.80 (2:8 EtOAc: hexane); IR (cm^{-1}) 1494 (C=C), 1679 (C=O), 3099 (C-H), 3321 (O-H);^1H NMR (400MHz, $CDCl_3$) : δ 3.02 (s, 3H, CH_3), 3.30 (s, 3H, CH_3), 6.05 – 6.25 (m, 2H, CH_2), 6.71 (d, 1H, *J* = 16Hz, CH=CH), 6.83 (d, 1H, *J*=16Hz, CH=CH), 7.09-7.12 (m, 4H); 7.27 – 7.59 (m, 4H);MS-APCI (m/z): 383.35 $(M+23)^+$".

(*E*)-Methyl 3-(4-((3-(3-methoxyphenyl)-1,2,4-oxadiazol-5-yl)methoxy)phenyl)acrylate (44f): "Light brown; Yield: 86%; mp 208-215 °C, Rf: 0.77 (2:8 EtOAc: hexane); IR (cm^{-1}): 1494 (C=C), 1679 (C=O), 3099 (C-H), 3321 (O-H); ^1H NMR (400MHz, $CDCl_3$) : δ 3.24 (s, 3H, CH_3); 3.84 (s, 3H, CH_3); 5.95 (s, 2H, CH_2); 6.42 (d, 1H, *J*= 16Hz, CH=CH); 6.63 (d, 1H, CH=CH), 7.51-7.67 (m, 4H); 7.90-7.94 (m, 1H); 8.02-8.27 (m, 3H). MS-APCI (m/z): 405.35$(M+39)^+$".

5.2.6 General procedure for the synthesis of (*E*)-3-(4-((3-Phenyl-1,2,4-oxadiazol-5-yl)methoxy)phenyl)acrylic acid (46):

"Methyl (*E*)-3-(4-((3-(4-chlorophenyl)-1,2,4-oxadiazol-5-yl)methoxy)phenyl)acrylate (**44a**) (1g,

2.7 mmol, 1equiv) was directly hydrolyzed by dissolving in 20 mL of THF and a suspension of $LiOH.H_2O$ (0.12 g, 2.97 mmol, 1.1 equiv) in THF : water (6 mL, 1:1, v/v) was added and then stirred for 2hr. TLC was analyzed , then the lithium salt of carboxylic acid was converted to the corresponding acid by neutralizing with dil. HCl (1N)".

(*E*)-3-(4-((3-(4-Chlorophenyl)-1,2,4-oxadiazol-5-yl)methoxy)phenyl)acrylic acid (46a, P152): "Dark brown solid;Yield 71%; mp: 200-210 °C ; Rf 0.5 (4:6, EtOAc : hexane); IR (cm^{-1}): 1597 (C=C), 1638 (C=O), 3327(O-H); ^{1}H NMR (400 MHz, DMSO) : δ 4.69 (s, 2H, CH_2), 6.31-6.38 (m, 1H, CH=CH), 6.94 (d, 2H, *J* = 8Hz), 7.11 (d, 1H, *J* = 12Hz), 7.20 (d, 1H, *J*=16Hz, CH=CH), 7.52-7.61 (m, 4H), 8.03 (d, 1H, *J*= 8Hz); ^{13}CNMR (100MHz, DMSO): δ64.42, 114.64, 116.55, 124.46, 127.22, 128.03, 129.46, 134.47, 136.67, 143.19, 158.59, 167.79, 169.75, 175.14; MS-APCI (m/z): 373 $(M+NH_3)^+$; Anal. Calcd for $C_{18}H_{13}ClN_2O_4$: C, 60.60; H, 3.67; N, 7.85; Found: C, 60.55; H, 3.71; N, 7.81".

(*E*)-3-(4-((3-(4-Fluorophenyl)-1,2,4-oxadiazol-5-yl)methoxy)phenyl)acrylic acid (46b, P158): "Yellow solid, Yield**:** 80%; mp: 205-282°C ; Rf: 0.45 (4:6, EtOAc : hexane); IR (cm^{-1}) 1601 (C=C), 1690 (C=O), 2932(O-H); ^{1}H NMR (400 MHz, DMSO) : δ 4.67 (s, 2H, CH_2), 6.31(d, 1H, *J*=16Hz, CH=CH), 6.91-6.94(m, 2H), 7.11 (d, 1H, *J*=16Hz, CH=CH), 7.68-7.70 (m, 2H), 7.51-7.56 (m, 4H); ^{13}CNMR (100MHz , DMSO) : δ 67.61, 114.62, 116.01, 116.65, 127.26, 127.51, 129.11, 130.21, 143.30, 159.96, 165.60, 167.74, 169.70, 171.50; MS-APCI (m/z): 378.21 $(M+K)^+$; Anal. Calcd for $C_{18}H_{13}FN_2O_4$: C, 63.53; H, 3.85; N, 8.23; Found: C, 63.59; H, 3.91; N, 8.27".

(*E*)-3-(4-((3-Phenyl-1,2,4-oxadiazol-5-yl)methoxy)phenyl)acrylic acid (46c, P155): "brown color,Yield: 88%; mp: 238-265°C ; Rf: 0.44 (4:6, EtOAc : hexane); IR (cm^{-1}): 1521 (C=C), 1778 (C=O), 3436(O-H);^{1}H NMR (400 MHz, DMSO) : δ 4.69 (s, 2H, CH_2), 6.33 (d, 1H, *J* =16 Hz, CH=CH); 6.92 -6.95 (m, 2H), 6.98 (d, 1H, CH=CH), 7.10 – 7.13 (m, 1H), 7.51 – 7.64 (m, 4H); 8.02- 8.04 (m, 2H). MS-APCI (m/z): 345.06 (M+Na); Anal. Calcd for $C_{18}H_{14}N_2O_4$: C, 67.07; H, 4.38; N, 8.69; Found: C, 67.10; H, 4.41; N, 8.71".

(*E*)-3-(4-((3-(p-Tolyl)-1,2,4-oxadiazol-5-yl)methoxy)phenyl)acrylic acid (46d, P167): "white solid, Yield: 90%; mp: 188-200°C ; Rf = 0.39 (4:6, EtOAc : hexane); IR (cm^{-1}): 1498 (C=C), 1771 (C=O), 3229(O-H). ^{1}H NMR (400 MHz,DMSO) δ 2.38 (s, 3H, CH_3), 4.73 (s, 2H), 6.38 (d, 1H, *J*= 16Hz, CH=CH); 6.93 – 6.95 (m, 2H), 7.12 – 7.14 (m, 1H); 7.37 – 7.39(m, 1H), 7.53 (d, 1H, *J*=16Hz, CH=CH); 7.61 – 7.63 (m, 2H); 7.71 – 7.73 (m, 2H); ^{13}CNMR (100MHz, DMSO): δ 21.06, 64.46, 114.82, 115.94, 116.79, 127.00, 127.27, 129.81, 130.20, 143.54, 143.93, 159.35,

167.74, 169.87, 175.35; MS-APCI (m/z): 376.02 (M+K); Anal. Calcd for $C_{19}H_{16}N_2O_4$: C, 67.85; H, 4.79; N, 8.33; Found: C, 67.90; H, 4.81; N, 8.38".

(*E*)-3-(4-((3-(3-Methoxyphenyl)-1,2,4-oxadiazol-5-yl)methoxy)phenyl)acrylic acid (46e, P170): **"**Dark brown;Yield: 92%, mp: 210-250°C ; Rf: 0.44 (4:6, EtOAc : hexane); IR (cm^{-1}): 1508 (C=N), 1687 (C=O), 2980(O-H), 3414(N-H); ^{1}H NMR (400MHz, DMSO) δ 3.79 (s, 3H, OCH_3), 5.45 (s, 2H, CH_2), 6.29 (d, 1H, *J*=16Hz, CH=CH); 6.81(d, 1H, *J*= 16Hz, CH=CH); 6.86-6.88 (m, 2H), 7.27 -7.30 (m, 2H), 7.39 – 7.42 (m, 2H), 7.47 – 7.52 (m, 2H), 7.68 (m, 2H); 12.11 (brs, 1H, OH). ^{13}CNMR (100MHz, DMSO): δ 21.05, 55.60, 112.77, 115.24, 115.71, 116.77, 118.65, 119.86, 124.41, 125.19, 130.03, 130.78, 144.15, 159.35, 167.90, 171.99; MS-APCI (m/z): 375.11 $(M+Na)^+$; Anal. Calcd for $C_{19}H_{16}N_2O_5$: C, 64.77; H, 4.58; N, 7.95; Found: C, 64.81; H, 4.55; N, 7.98".

(*E*)-3-(4-((3-(2-Methoxyphenyl)-1,2,4-oxadiazol-5-yl)methoxy)phenyl)acrylic acid (46f, P171): **"**dark brown solid; Yield: 88%; mp: 210-215°C; Rf: 0.49 (4:6, EtOAc : hexane), IR (cm^{-1}): 1510 (C=N), 1766 (C=O), 2974(O-H), 3363 (NH);^{1}H NMR (400MHz, DMSO): δ 3.77 (s, 3H, OCH_3), 5.54 (s, 2H, CH_2), 6.29 (d, 1H, *J*=16Hz, CH=CH), 6.88 (m, 2H), 7.27 -7.30 (m, 1H), 7.38 – 7.41 (m, 1H), 7.44(d, 1H, *J*= 16Hz, CH=CH); 7.47 – 7.52 (m, 1H), 7.68 (d, 2H, *J* = 12Hz), 7.96 (d, 1H, *J*= 16Hz), 12.11 (1H, OH); Anal. Calcd for $C_{19}H_{16}N_2O_5$: C, 64.77; H, 4.58; N, 7.95; Found: C, 64.76; H, 4.53; N, 7.99".

5.2.7 General procedure for the synthesis of hydantoin (49)

"An aq.solution of sodium hydroxide (30 mL, 10%w/v) was added to a mixture of glycine (5g, 66.67 mmol, 1equiv) and urea (4g, 66.67 mmol, 1 equiv) in RBF. The mixture was thoroughly mixed and heated at 110-115°C for 1-2hr. Reaction mixture was cooled to 60°C and acidified with conc. HCl to congo red. The separated intermediate hydantoic acid was filtered and mixed in 50 mL of dist. water,.It was then heated at 110-115°C for an hour and then cooled and filtered and the separated product was then recrystallized from dist. water".

Imidazolidine-2,4-dione (49a): **"**Off white color semi solid; Yield: 92%; Rf: 0.5 (4:6, EtOAc : hexane); IR (cm^{-1}): 1458 (C=N), 1627 (C=C), 1720 (C=O), 3450 (N-H); ^{1}H NMR (400MHz, $CDCl_3$): δ 3.84(s, 2H, CH_2); 8.22 (s, 1H, NH); 10.62 (s, 1H, NH). MS-APCI (m/z): 101.50 $(M+1)^+$".

1-Phenylimidazolidine-2,4-dione (49b): **"**Off white color semi solid; Yield: 78%; Rf: 0.5(4:6,

EtOAc : hexane); IR (cm^{-1}): 1626 (C=C), 1689 (C=O), 3024 (N-H); ^{1}H NMR (400MHz, $CDCl_3$): δ 5.58(s, 2H, CH_2), 7.51-7.58(m, 2H), 7.63-7.69 (m, 3H), 7.78(d, 1H, NH). MS-APCI (m/z): 177.55 $(M+1)^+$".

5.2.8 Procedure for the synthesis of (*E*)-Methyl 3-(4-(2-(2,5-dioxoimidazolidin-1-yl)-2-oxoethoxy)phenyl)acrylate (50):

"Method B was used which includes addition of (*E*)-2-(4-(3-Methoxy-3-oxoprop-1-en-1-yl)phenoxy)acetic acid (**41**) (2 g, 8.47 mmol, 1 equiv) and triethylamine (0.9 mL, 8.47 mmol, 1 equiv) were slurried in dry acetonitrile (20 mL). Further added *N*,*N*'-dicyclohexylcarbodiimide (DCC) (1.7 g, 8.47 mmol) and 4-dimethylaminopyridine (DMAP) (0.1 g, 0.847 mmol, 0.1 equiv) to this mixture and stirred at RT for 1h. To this solution, imidazolidine-2,4-dione (**49a**) (0.86 g, 8.47 mmol, 1 equiv) was added and stirred for 4 h. Then, desired product was extracting from the solvent by separating it with ethyl acetate and aqueous bicarbonate solution followed with drying of the organic layer and concentrated to afford the crude product which was purified by recrystallization in DCM to afford the desired product".

(*E*)-Methyl 3-(4-(2-(2,5-dioxoimidazolidin-1-yl)-2-oxoethoxy)phenyl)acrylate (50): "Reddish brown;mp: 235-239 °C yield**:** 80%; Rf: 0.48 (4:6, EtOAc : hexane); IR (cm^{-1}): 1597 (C-N), 1612 (C=C), 1880 (C=O), 2333 (C-H), 3029 (C-H), 3471 (N-H); ^{1}H NMR (400 MHz, DMSO): δ 3.72 (s, 3H), 3.88 (s, 2H), 4.75 (s, 2H), 5.54 (d, 1H, *J*= 8Hz,CH=CH), 6.40 (d, 2H, *J*=16Hz), 6.88 (d, 1H, *J*= 8Hz, CH=CH), 7.55-7.61(m, 2H). MS-APCI (m/z): 335.09 $(M+NH_3)^+$."

5.2.9 Procedure for the synthesis of (*E*)-3-(4-(2-(2,5-Dioxoimidazolidin-1-yl)-2-oxoethoxy)phenyl)acrylic acid (51):

"(*E*)-methyl 3-(4-(2-(2,5-dioxoimidazolidin-1-yl)-2-oxoethoxy)phenyl)acrylate (**50**) (1 g, 3.14 mmol, 1 equiv) was dissolved in 20 mL of THF and a suspension of $LiOH.H_2O$ (0.15 g, 3.45 mmol, 1.1 equiv) in THF : water (6 mL, 1:1, v/v) was added and then stirred for 2hr. TLC was analyzed, then the lithium salt of carboxylic acid was converted to the corresponding acid by neutralizing with dil. HCl (1N)".

(*E*)-3-(4-(2-(2,5-Dioxoimidazolidin-1-yl)-2-oxoethoxy)phenyl)acrylic acid (51, P172): "Reddish brown;mp: 240-249 °C Yield**:** 82% ; Rf: 0.5 (4:6, EtOAc : hexane); ^{1}H NMR (400 MHz, DMSO): δ 4.22 (s, 2H, CH_2), 5.12 (s, 2H, CH_2), 6.36 (d, 1H, *J* = 16Hz, CH=CH), 6.91-6.96 (m, 2H), 7.51 (d,

1H, *J* = 16Hz, CH=CH), 7.60(d, 2H, *J* = 12Hz), 8.01 (s, 1H, NH). ^{13}CNMR (100MHz , DMSO): δ 39.77, 63.3, 115.03, 116.5, 129.80, 130.2, 144.2, 156.92, 158.2, 167.82, 168.31, 171.5; MS-APCI (m/z): 336 $(M+NH_3)^+$ Anal. Calcd for $C_{14}H_{12}N_2O_6$: C, 55.27; H, 3.98; N, 9.21; Found: C, 55.31; H, 3.95; N, 9.27".

5.2.10 General procedure for synthesis of Bezoxazole-2-amine derivatives (53): "At room temperature, cyanogen bromide (5.3 g, 50.40 mol, 1.1 equiv) was stirred in MeOH/H_2O (20 mL, 7:3, v/v), thereafter a solution of 2-aminophenol (5 g, 45.81 mol, 1 equiv) in methanol (5 mL) was added to the mixture. The resulting mixture was further stirred for 1 h. The solution was subsequently neutralized with conc. aq. NaOH and the residue was obtained by evaporating methanol. The residue was treated with water and filtered to collect the crude. Further the crude was purified by hot water mixed with charcoal to obtain the pure product".

Benzo[d]oxazol-2-amine (53a): "Reddish brown; mp: 135-139 °C; Yield: 90%; Rf: 0.5(4:6, EtOAc : hexane); IR (cm^{-1}): 1504 (CN), 1603 (C=O), 3486 (N-H); ^{1}H NMR (400MHz, $CDCl_3$): δ 6.06(s, 2H, NH_2), 7.03-7.08(m, 1H), 7.14-7.18(m, 1H), 7.25 (d, 1H, *J*= 8Hz), 7.32 (d, 1H, *J*= 8Hz). MS-APCI (m/z): 135.05 $(M+1)^+$".

5-Nitrobenzo[d]oxazol-2-amine (53b): "Reddish brown;mp: 139-145°C; Yield: 90%; Rf: 0.5(4:6, EtOAc : hexane); IR (cm-^{1}):1588 (C-N), 1683 (C=O), 3317 (N-H); ^{1}H NMR (400MHz, $CDCl_3$): δ 6.39(s, 1H), 7.12 (d, 2H, *J* = 8Hz), 7.41 (d, 1H, *J* = 8Hz), 7.90 (d, 2H). MS-APCI (m/z): 180.13 $(M+1)^+$".

5.2.11 Procedure for coupling with Benzoxazole-2-amine derivatives (54):

"(*E*)-2-(4-(3-Methoxy-3-oxoprop-1-en-1-yl)phenoxy)acetic acid (**41**) (2 g, 8.47 mmol, 1 equiv) and triethylamine (0.9 mL, 8.47 mmol, 1 equiv) were slurried in dry acetonitrile (20 mL). Further added *N*,*N*'-Dicyclohexylcarbodiimide (DCC) (1.7 g, 8.47 mmol) and 4-Dimethylaminopyridine (DMAP) (0.1 g, 0.847 mmol, 0.1 equiv) to this mixture and mixture was stirred for 1hr at RT. To this solution benzo[d]oxazol-2-amine (**53a**) (1.1 g, 8.47 mmol, 1 equiv) was added and stirred for 4 h.Then, desired product was extracting from the solvent by separating it with ethyl acetate and aqueous bicarbonate solution followed with drying of the organic layer and concentrated to afford the crude product which was recrystallized in DCM to afford the desired product".

Methyl (*E*)-3-(4-(2-(benzo[d]oxazol-2-ylamino)-2-oxoethoxy)phenyl)acrylate (54a): "Light brown semi-solid; Yield: 82%; Rf: 0.77(2:8, EtOAc : hexane); IR (cm^{-1}): 1536 (C-N), 1632 (C=C),

1730 (C=O), 3326 (N-H); ^{1}H NMR (400MHz, $CDCl_3$): δ 3.74(s, 3H), 4.97(s, 2H), 6..83(d, 2H, *J* = 8Hz), 7.01 -7.14 (m, 4H), 7.20- 7.28 (m, 4H), 7.40 (d, 1H, *J* = 8Hz), 7.46 (d, NH). MS-APCI (m/z): 180.13 $(M+1)^{+}$".

Methyl (E)-3-(4-(2-((5-nitrobenzo[d]oxazol-2-yl)amino)-2-oxoethoxy)phenyl)acrylate (54b): **"**Reddish brown semi-solid; Yield: 80%; Rf: 0.78 (2:8, EtOAc : hexane); IR (cm^{-1}): 1514 (C-N), 1600 (C=C), 1687 (C=O), 3377 (N-H); ^{1}H NMR (400MHz, $CDCl_3$): δ 3.70 – 3.80 (m, 3H, CH_3), 5.80 (S, 2H, *J* = 16Hz, CH=CH), 6.41(d, 2H, *J* = 16Hz, CH), 6.69 (d, 2H, *J* = 16Hz, CH), 7.35-7.47 (m, 4H, CH), 7.55-7.63 (m, 3H, CH), 8.14 (d, 3H, J = 16Hz, NH). MS-APCI (m/z): 412.55 $(M+CH_3)^{+}$".

5.2.12 Procedure for synthesis (*E*)-3-(4-((Benzo[d]oxazole-2-carboxamido)methoxy)phenyl)acrylic acid (55, P173):

"Methyl (*E*)-3-(4-(2-(Benzo[d]oxazol-2-ylamino)-2-oxoethoxy)phenyl)acrylate (**54a**) (1g, 2.8 mmol, 1equiv) was directly hydrolyzed by dissolving in 20 mL of THF and a suspension of $LiOH.H_2O$ (0.13 g, 3.1 mmol, 1.1 equiv) in THF : water (6 mL, 1:1, v/v) was added and then stirred for 2hr. TLC was analyzed, then the lithium salt of carboxylic acid was converted to the corresponding acid by neutralizing with dil. HCl (1N). Dark brown;mp: 239-245°C; Yield: 83%; Rf: 0.2 (EtOAc); IR (cm^{-1}): 1512 (C-N), 1602 (C=C), 1674 (C=O), 2916 (O-H), 3346 (N-H) ^{1}H NMR (400MHz, $CDCl_3$): δ 3.72-3.76 (m, 2H), 6.26 (d, 1H, *J*= 16Hz, CH=CH), 6.83 (d, 1H, *J*= 8Hz, CH=CH), 7.41-7.45(m, 2H), 7.38-7.41 (m, 2H), 7.56-7.58 (m, 2H), 10.57 (s, 1H, NH); ^{13}CNMR (100MHz , DMSO): *δ*39.99, 108.38, 114.20, 115.21, 119.95, 123.46, 127.70, 130.10, 135.00, 143.48, 147.85, 150.00, 143.48, 147.85, 150.00, 162.66, 166.27, 171.01.MS-APCI (m/z): 339.75 $(M+1)^{+}$Anal. Calcd for $C_{18}H_{14}N_2O_5$: C, 63.90; H, 4.17; N, 8.28; Found: C, 63.94; H, 4.21; N, 8.31".

5.3 *In-silico* toxicity study:

The compounds which were synthesized in the laboratory were analyzed online to predict the toxicity. For this the SMILES of compounds based on their structure obtained from ChemBioDraw software were given as input using Lazar toxicity predictor available on-line at https://lazar.in-silico.ch/predict.[117,118]

ADME (Adsorption, distribution, metabolism,and excretion) profile of the compounds were predicted the *in-silico* ADME for correlation of its lipophilicity and water solubility with its toxicity. The *in-silico* ADME properties of the compounds were obtained as a calculated value for physicochemical properties, water solubility, lipophilicity, pharmacokinetics (http://www.swissadme.ch/index.php).[119,120]

5.4 *In-vitro* PPAR alpha/gamma agonist activity

5.4.1 Cell lysate preparation: L6 Cells were cultured in 25mM glucose containing DMEM supplemented with 10% fetal bovine serum and antibiotics (Penicillin 100 IU/ml and Streptomycin 100 mg/ml) in 5% CO_2 incubator at 37 °C. Till 80–90% confluence, cells were kept in serum-free DMEM for 5 Hours. Cells will be then treated with the 0.1-100 µM concentration of pioglitazone, test compounds, and positive control for 1 hour. Cells were then washed twice with ice-cold phosphate buffer saline (PBS) and lysed in a low salt buffer (LSB) (10 mMTris–HCl, pH 7.4, 150 mM EDTA, 1 mM sodium orthovanadate, 10 mM sodium fluoride, 10mM sodium pyrophosphate, 1 mMphenylmethylsulfonyl fluoride, 10 µg/ml aprotinin, 1 mM sodium butyrate and 0.05% NP-40).

5.4.2 Transcription factor binding assay method: For *in-vitro* transcription factor assay, Cayman's PPAR*α, γ* CTFA Kit was used, it is a non-radioactivity method with high sensitivity. It detects the specific transcription factor by DNA binding activity in nuclear extracts as well as whole cell lysates. It contains a specific dsDNA (double-stranded DNA) sequence with PPAR response element which immobilizes onto the bottom of wells of a 96 well plate [ELISA (Enzyme-linked immunosorbent assay)]. The PPAR*α, γ* is sensed by adding specific antibodies bound for individual PPARs, while secondary antibody is added for increasing sensitivity for colorimetric assay at 450 nm.

5.4.3 Pre-assay reagents preparation (Table 3):

Transcription factor antibody binding buffer (10X): "One vial with item no. 10006882 containing 3mL of a 10X stock of transcription factor antibody binding buffer (ABB) was used for dilution of the primary and secondary antibodies. A 1X ABB solution was prepared to dilute 1:10 by adding 27mL of UltraPure water. It was stored at 4 °C till use".

Wash buffer concentrate (400X): "One vial (item no. 400062) contains 5mL of 400X wash buffer. By using ultra pure water, the content of the vial was diluted up to 2 liters and added 1mL of polysorbate 20. Wash buffer concentrate in small volume was prepared by adding polysorbate 20 (0.5 ml/liter of wash buffer) and diluting it (1:400) and. stored at 4 °C till use".

Transcription factor binding assay buffer (4X): "A complete TFB assay buffer (CTFB) solution was prepared immediately prior to use in 1.5mL centrifuge tubes".

Table 3: Preparation of complete transcription factor binding assay buffer

Component	Volume/well	Volume/strip	Volume/96-well plate
UltraPure water	73µL	584 µL	7008 µL
4X Transcription factor binding assay buffer	25 µL	200 µL	2400 µL
Reagent A	1 µL	8 µL	96 µL
300 mM DTT	1 µL	8 µL	96 µL
Total required	100 µL	800 µL	9600 µL

Transcription factor PPARα/γ positive control: "One vial (item no. 10006881/10007441) contains 150µL of clarified cell lysate. A serial 2-fold dilution of this positive control was used for monitoring the dynamic range of the assay. This positive control was aliquoted at 25µL per vial and stored at -80 °C to avoid loss due to repeated freeze-thaw cycles".

5.4.4 Assay procedure: The reagents were added to wells as indicated on the plate and incubated one hour at 37 °C. Each well was washed for five times with 200 µL of 1X wash buffer. A100 µL of diluted PPAR *γ* primary antibody was added to pre well (except blk wells) and again incubated for one hour at room temperature. Each well was washed with 200 µL of 1X wash buffer for five times. A100 µL of diluted secondary antibody was added to each well (except blk wells). The plate was again incubated for one hour at 37 °C. Each well was washed for five times with 200 µL of 1X wash buffer. Thereafter a 100 µL of developing solution was added in wells and incubated for 30 minutes with gentle agitation. At the end, a100 µL of stop solution was added in each well and the absorbance was measured at 450nm using ELISA plate reader. All samples were performed in triplicates.

% agonist activity = (absorbance of test- absorbance of blank/ absorbance of standard-absorbance of blank)*100

5.5 *In-vivo*antidiabetic activity:

5.5.1 Development of type 2 diabetic rat model: For the development of diabetic rat model, nine treatment groups were used as specified in Table 24. All the groups were treated as per the treatment protocol is given below.

Female Sprague-Dawley rats were induced with insulin resistance, type 2 diabetes by feeding high-fat diet (HFD) and administering a low dose of streptozotocin (STZ) as per Srinivasan*et al*. 2005,[111] and Gupta et al., 2010[112]. The rats were divided into two dietary regimens, either normal pellet diet (NPD) or HFD (58% fat, 25% protein, and 17% carbohydrate, as a percentage of total kcal) *ad libitum*, respectively, for the initial period of 2 weeks (day 1 to 14). The composition and preparation of HFD are according to Gaikwad*et al.* (2010).[113] After the 2 weeks of dietary manipulation (on day 15), the rats from HFD-fed group was injected with a low dose of STZ (35 mg/kg; i.p.) and NPD-fed group was injected with 0.5% CMC (vehicle). One week after STZ injection i.e. on 22nd day, blood glucose was estimated and rats having non-fasting value above 250 mg/dl were identified and further divided into different treatment groups to receive varying as HFD + STZ (negative control group), HFD + STZ + pioglitazone; 10 mg/kg (positive control group), HFD + STZ + test compound P155 at 5 and 10 mg/kg (test compound 1 treatment groups) and HFD + STZ + test compound P158 at 5 and 10 mg/kg (test compound 2 treatment groups) for 1 week (day 22 to 28). Parallel to this treatment the NPD rats were further divided into different groups to receive varying treatment as NPD (vehicle control group), NPD + test compound-P155 at 10 mg/kg (test compound 1 *per se* group), NPD + test compound P158 at 10 mg/kg (test compound 2 *per se* group) for 1 week (day 22 to 28) Plasma glucose levels and body weight were evaluated on 1st day (before dietary modification), on 15th day, on 22nd day (before STZ or vehicle injection) and on 28th day. The respective treatments (pioglitazone, test compound-P155, and test compound-P158) to different groups were given orally every day from day 22 to 28. The rats were allowed to fed on their respective diets until the end of the study (28th day). Histopathology of the pancreas and biochemical estimations will be carried out after sacrificing the animals at the end of the protocol.

5.5.2 Parameters of evaluation: Following parameters were evaluated at the end of the study to confer the effect of the test compounds in type 2 diabetic rat model.

5.5.2.1 Body weight: Prior to the *in-vivo* study, the body weights of animals will be assessed and thereafter every week before behavioral assessment.All samples were performed in triplicates.

5.5.2.2 Biochemical parameters:

a) Plasma glucose (PGL): It estimates the glucose after oxidation to gluconic acid and hydrogen peroxide (H_2O_2) by glucose oxidase. The intensity of the pinkcolor is directly proportional to glucose concentration

Glucose evaluated weekly using kits based on GOD/POD method. Briefly, 10µL of plasma will be mixed with Reagent 1 (containing Glucose oxidase enzyme) of the kit (**Table 4**). The blank was aspirated followed by standard and tests for glucose estimation. All mixture was incubated for 15 minutes at 37°C and then, at 510nm, the absorbances of the test and standard samples were observed in comparision to the reagent blank. All samples were performed in triplicates.

Table 4: Preparation of sample for GOD/POD method of glucose estimation.

Pipette into tubes marked	Blank	Standard	Test
Working reagent	1000 µL	1000 µL	1000 µL
Distilled water	10µL	--	--
Standard	--	10 µL	--
Test	--	--	10 µL

The formula used to calculate glucose concentration:

Glucose (mg/dL) = (Absorbance of test/Absorbance of Standard) x 200 mg/dL (Standard Conc.)

b) Total cholesterol: It involves the estimation of cholesterol after the conversion of cholesterol ester to cholesterol and fatty acid in the presence of cholesterol esterase (CE). The intensity of the redcolor is directly proportional to cholesterol concentration.

To estimate cholesterol Eraba cholesterol kit was used. The 20µL plasma of fasted animal was used and mixed with working reagent. Reagents got appropriately mixed and incubated at 37°C for 10 minutes. Blank was aspirated followed by standard and tests (**Table 5**), further at 505 nm,absorbances of the test and standard samples were noted in comparision to the reagent blank. All values were carried in triplicates.

Table 5: Preparation of sample for cholesterol estimation.

Pipette into tubes marked	Blank	Standard	Test
Working reagent	1000 µL	1000 µL	1000 µL
Distilled water	20µL	--	--
Standard	--	20 µL	--
Test	--	--	20 µL

The formula used to calculate cholesterol:

Cholesterol (mg/dL) = (Absorbance of test/Absorbance of standard) x 200 mg/dL (Standard Conc.)

c) Oxidative biomarkers: Once the study is completed, on the 28th day of treatment, marked animals for biochemical studies were sacrificed by cervical dislocation. The tissues were taken off the organ and triturated to make a 10% (w/v) tissue homogenate in 0.1 M phosphate buffer (ph 7.4). After centrifugation at 3000 rpm for 15 minutes,The clear supernatant was collected and used further for various biochemical studies to estimate the levels of CAT, GST, and LPO.

i) Estimation of thiobarbituric acid reactive substances (TBARS): [127]

it involve the estimation of TBARS which is biomarker for lipid peroxidation in the brain , method; Ohkawa*et al*., (1979).

Assay procedure: **"**0.2mL of Supernatant homogenate was taken out in a test tube, followed by addition of 8.1%; 0.2 mL sodium dodecyl sulphate, 30%; 1.5 mL; pH 3.5acetic acid, 1.5 mL thiobarbituric acid and up the volume upto 4 mL with distill water. For 1 h at 95 °C,The test tubes were incubated, cooled and distilled water (1 mL) was added followed by addition of n-butanol-pyridine mixture (15:1 v/v; 5 mL). The tubes were centrifuged at 4000 g for 10 min. The pink color was developed for which the absorbance was measured spectrophotometrically (Shimadzu UV spectrophotometer 1240) at 532 nm. A calibration curve was prepared using 1-10 nM 1, 1, 3, 3-tetra methoxy propane. By using linear regression equation the TBARS concentration was estimated with respect of absorbance measured at 532 nm. The TBARS value was expressed as nanomoles per mg of protein. All samples were performed in triplicates".

ii)Estimation of reduced glutathione (GSH):[128] Beutler*et al.*, 1963 method was employed in estimating the GSH content in tissue.

Assay procedure: **"**In brief, the superficial floating layer of homogenate was mixed with trichloroacetic acid (10% w/v) in 1:1 ratio in test tubes. For 10min these tubes were centrifuged at 1000 g at 4 ^{o}C. The supernatant (0.5 mL) obtained was mixed with 2 mL disodium hydrogen phosphate (0.3 M). Then freshly prepared DTNB [5,5-dithiobis (2-nitrobenzoic acid)dissolved in 1% w/v sodium citrate; 0.001 M; 0.25 mL] was added andat 412 nm, absorbance was noted spectrophotometrically. By using 10-100 μM of the reduced form of glutathione, a standard curve was plotted and results were expressed in micromoles of reduced glutathione per mg of protein. All samples were performed in triplicates".

iii) Estimation of catalase (CAT) activity:[129]Aebi, 1974 method was employed to determine it's activity.

Assay procedure: "To a 3.0 mL cuvette that contained phosphate buffer (50 mM; 1.95 mL; pH 7.0), the supernatant (50 µL) was added, to which Hydrogen peroxide (30 mM; 1.0 mL) was added and variations in absorbance were observed at 15-sec intervals for 30 sec at240 nm. By using the millimolar extinction coefficient of H_2O_2 (0.071 mmol cm^{-1}),the catalase activity was calculated (Bisswanger, 2004).[130] All samples were performed in triplicates".

$$\text{CAT activity} = \frac{\delta \text{O. D.}}{\varepsilon \text{ X Vol. of sample (mL) X mg of protein}}$$

Where 'δ O.D.' changes in absorbance/minute; 'ε' is the extinction coefficient of hydrogen peroxide (0.071 m mol cm^{-1}).

Histopathological evaluation: The kidneys and pancreases were obtained after sacrifices of SD rats from each group and preserved in 10 % formalin immediately after dissection to avoid drying. Tissue slides were prepared and stained with hematoxylin and eosin. Histopathological examination was performed by a pathologist at Gargi Diagnostic Laboratory, Jalandhar.

5.6 Statistical analysis: All the results were articulated as mean SEM. The statistics for behavioral studies and Biochemical data was analyzed using one-way ANOVA followed by Tukey test (Sigma Stat Software, 4.0). The difference was considered to be significant at 5% level ($p < 0.05$).

Chapter 6: Results and discussion

6.1 Molecular docking

Series 1 having oxadiazole derivatives, Series 2 having hydantoin/imidazolidinone derivatives and Series 3 having benzoxazole derivatives were designed as shown in **Figure 31** below by varying the substituents for molecular docking purpose so that we can identify the most probable PPAR dual agonist.

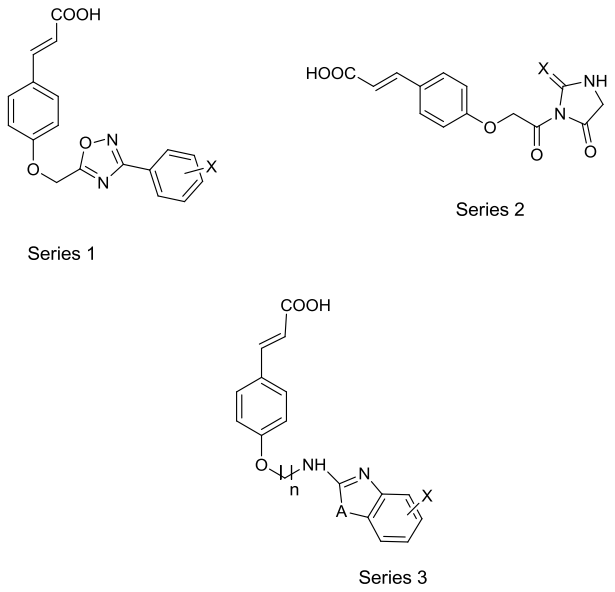


Figure 31. Designed series 1, 2 and 3 as PPAR dual agonist

To determine most potentially active ligands towards PPAR by using Autodock-vina.[107] For molecular docking the PPAR receptors were downloaded from PDB (https://www.rcsb.org/).[108] We used 3SZ1 for PPAR*γ* and 2P54 for PPAR*α* to evaluate the all designed compound with piogliazone as a standard drug known to dual agonist. All designed molecule structures were drawn using ChemBioDraw software.[106]A step wise discussion on molecular docking is given below:

6.1.1 Preparation of protein (3SZ1 for PPAR*γ* or 2P54 for PPAR*α*):

For the preparation of protein go to File menu, click on Read Molecule, select the protein PDB file and select Open or, right click on Python Molecular Viewer (PMV) Molecules at the bottom of the window and choose the protein pdb file followed with fixing the problems associated with the PDB files like missing atoms and water molecules. Further, keeping only the protein and naturally bound

cofactors to it and save it as pdbqt file (**Figure 32 and 33**).

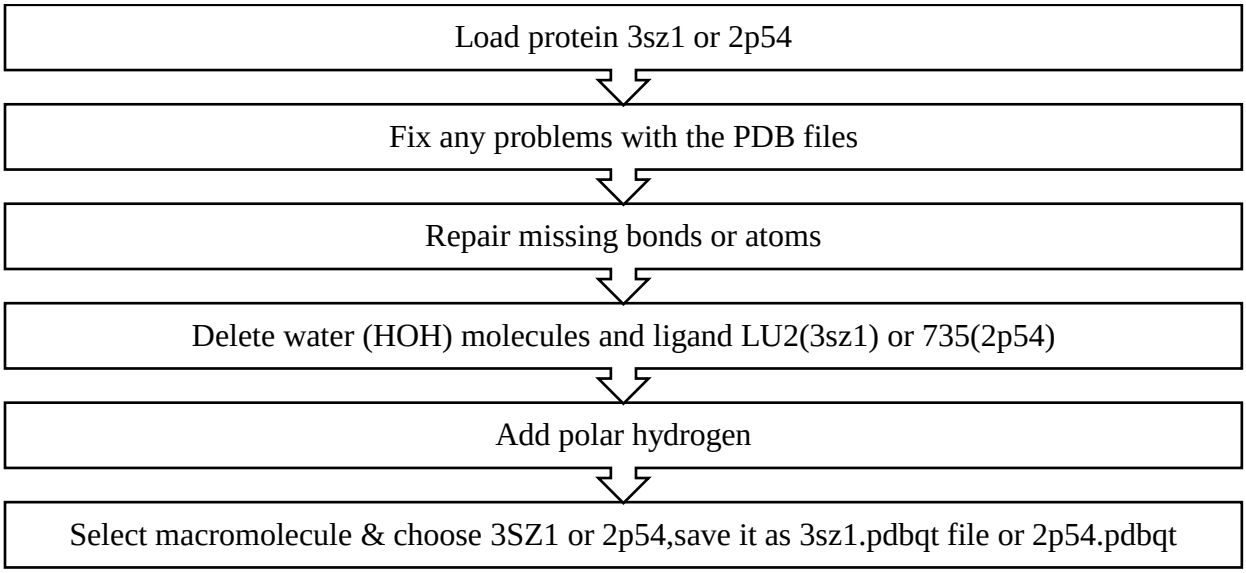


Figure 32. Flow chart of protein preparation procedure in Autodock-vina

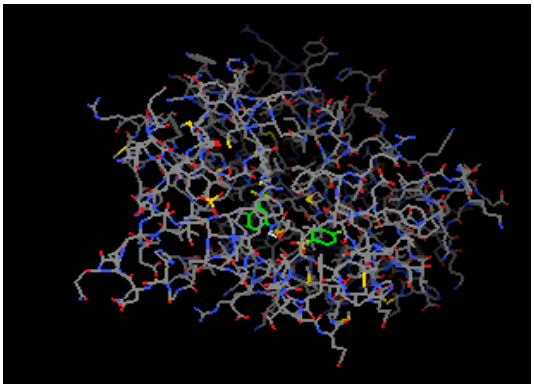
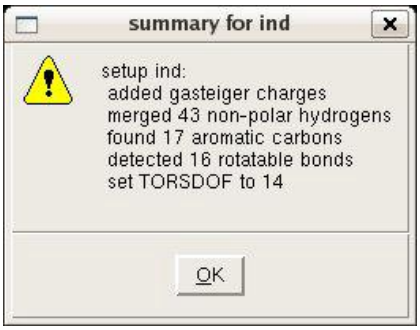


Figure 33. Protein view in Autodock-vina

6.1.2 Preparation of ligand:

Go to ligand on menu bar, then click on input molecule and then open the ligand and select pdb files. Then select the file comprising the ligand, and click Open. Then message will be pop up on screen as shown below and Ligand was selected and prepared by choosing Torsion Tree, later Detect Root and saving as pdbqt file (**Figure 34**).



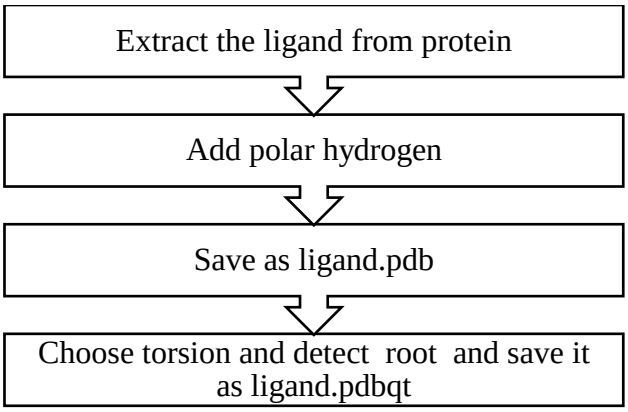


Figure 34. Flow chart of ligand preparation procedure in Autodock-vina

6.1.3 Docking and validation of protein:

Load ligand.pdbqt file and set it as map type by choosing ligand. After this centralize ligand by setting grid box and then save it by close saving current. Then save the protein as pdbqt file and then prepare configuration file and save it as conf.txt. Then analyze the docking results in command prompt as shown below (**Figure 35**).

Configuration file "conf.txt" was prepared and for AutodockVina molecular docking, command prompt was used by giving command "program files\the scripps research institute\vina\vina.exe --config conf.txt --log log.txt" and thus, generated the output file including the docking score or binding affinity (Kcal/mol), similarly, all the designed molecules were studied and their binding affinities were showed in Table 3, 4 and 5 for series 1, 2 and 3 respectively.

Load ligand.pdbqt and set it as map type by choosing ligand

Set the grid box by selecting “center on ligand” and save it by close saving current

Search space volume should be less then 27000A³

Save protein as 3sz1.pdbqt or 2p54.pdbqt ; grid --- macromolecule ---choose ---3sz1 or 2p54 --- replace

Prepare configuration file as parameter in grid output txt file– and –save as –- conf.txt

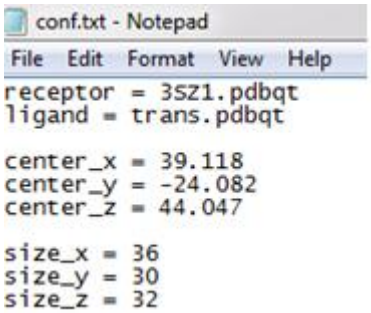


Figure 35. Flow charts of configuration file preparation procedure in Autodock-vina.

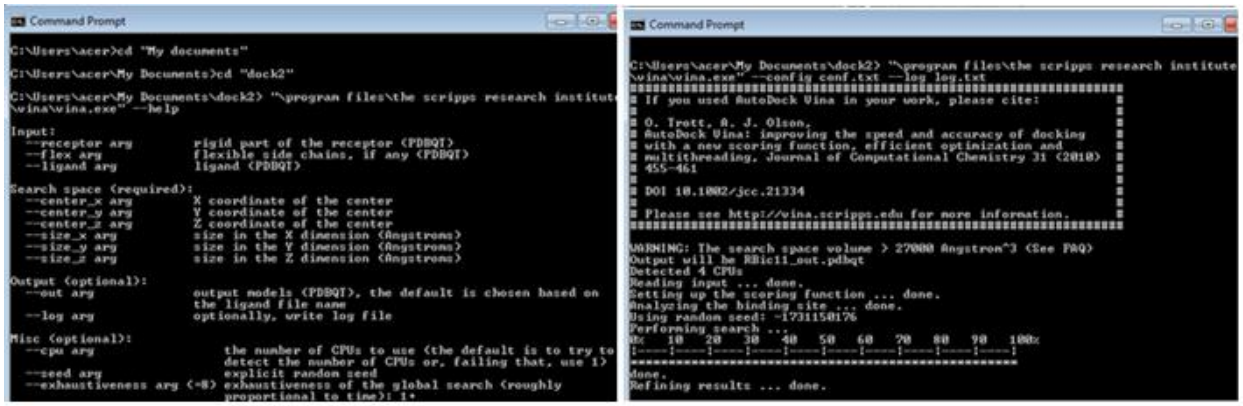
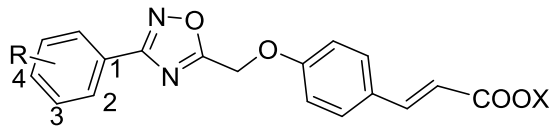


Figure 36. A command prompt used in Autodock-vina

Table 6: Binding energy of series 1 designed molecules with PPARα (2p54) and PPARγ (3sz1) protein. (NA = not available)

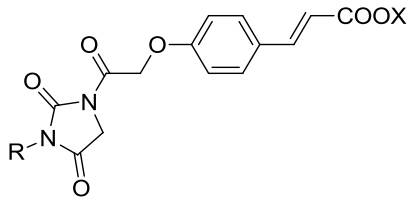


S.No.	Code	R	X	Binding affinity (kcal/mol)	
				PPARα (2p54)	PPARγ (3sz1)
1	Standard Ligand (pioglitazone)			-11.3	-10.3
2	PA2Cl	2-Cl	H	-9.1	-9.7
3	PA2F	2-F	H	-9.0	-10.1
4	PA2I	2-I	H	-9.6	-9.8
5	PA3Br	3-Br	H	NA	NA
6	PA3Cl	3-Cl	H	-9.7	-9.4
7	PA3F	3-F	H	-8.9	-10.0
8	PA3I	3-I	H	-9.3	-10.0
9	PA3Me	3- CH_3	H	-9.4	-10.0
10	PA4Br	4-Br	H	NA	NA
11	PA4I	4-I	H	-9.0	-9.0
12	PA4MeO	4- CH_3O	H	-9.0	-9.2
13	P4Cl	4-Cl	H	-8.2	-9.2
14	P4H	H	H	-9.3	-9.6
15	P4F	4-F	H	-9.6	-9.3
16	P4CH_3	4-CH_3	H	-9.0	-9.0
17	P2OCH_3	2-OCH_3	H	-8.9	-9.4
18	P3OCH_3	3-OCH_3	H	-9.4	-9.1
19	PE2Br	2-Br	CH_3	NA	NA
20	PE2Cl	2-Cl	CH_3	-9.5	-10.0
21	PE2F	2-F	CH_3	-9.0	-9.2
22	PE2I	2-I	CH_3	-9.3	-9.3

Cont. **Table 6**: Binding energy of series 1 designed molecules with PPAR*α* (2p54) and PPAR*γ* (3sz1) protein. (NA = not available)

23	PE3Br	3-Br	CH_3	NA	NA
24	PE3Cl	3-Cl	CH_3	-9.3	-9.8
25	PE3I	3-I	CH_3	-9.5	-8.9
26	PE3Me	3- CH_3	CH_3	-9.4	-9.4
27	PE4Br	4-Br	CH_3	NA	NA
28	PE4I	4-I	CH_3	-8.8	-9.0
29	PE4Me	4- CH_3	CH_3	-9.2	-9.7

Table 7: Binding energy of series 2 designed molecules with PPAR*α* (2P54) and PPAR*γ* (3SZ1) protein. (NA = not available)

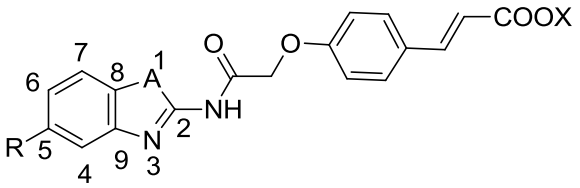


S.No.	Code	R	X	Binding affinity (kcal/mol)	
				PPAR*α* (2P54)	PPAR*γ* (3SZ1)
1	Standard ligand (pioglitazone)			-11.3	-10.3
2	PHABr	Br	H	NA	NA
3	PHACl	Cl	H	-7.4	-8.4
4	PHAEt	CH_2CH_3	H	-8.1	-8.6
5	PHAF	F	H	-8.0	-8.7
6	PHAI	I	H	-8.0	-8.0
7	PHAMe	CH_3	H	-8.3	-8.6
8	PHAMeO	CH_3O	H	-8.1	-7.9
9	PHAPh	Ph	H	-9.8	-9.4
10	PHAH	H	H	-8.9	-8.3

Cont. **Table 7**: Binding energy of series 2 designed molecules with PPAR*α* (2P54) and PPAR*γ* (3SZ1) protein. (NA = not available)

11	PHEBr	Br	CH_3	NA	NA
12	PHECl	Cl	CH_3	-7.8	-9.2
13	PHEEt	CH_2CH_3	CH_3	-8.1	-8.9
14	PHEF	F	CH_3	-7.9	-9.4
15	PHEI	I	CH_3	-7.9	-9.1
16	PHEMe	CH_3	CH_3	-7.6	-8.4
17	PHEMeO	CH_3O	CH_3	-7.8	-8.8
18	PHEPh	Ph	CH_3	-9.6	-9.2
19	PHEH	H	CH_3	-8.3	-7.9

Table 8: Binding energy of series 3 designed molecules with PPAR*α* (2P54) and PPAR*γ* (3SZ1) protein. (NA = not available)



S.No	Code	R	A	X	Binding afinity (kcal/mol)	
					PPAR*α* (2P54)	PPAR*γ* (3SZ1)
1	Standard ligand (pioglitazone)				-11.3	-10.3
2	PBABr	Br	O	H	NA	NA
3	PBACl	Cl	O	H	-9.1	-9.5
4	PBAH	H	O	H	-8.9	-9.0
5	PBAMe	CH_3	O	H	-9.5	-9.3
6	PBEBr	Br	O	CH_3	NA	NA
7	PBECl	Cl	O	CH_3	-9.1	-8.8

Cont. **Table 8**: Binding energy of series 3 designed molecules with PPARα (2P54) and PPARγ (3SZ1) protein. (NA = not available)

8	PBEF	F	O	CH_3	-9.8	-9.2
9	PBEH	H	O	CH_3	-8.9	-9.1
10	PBEMe	CH_3	O	CH_3	-9.1	-9.2
11	PBEMeO	OCH_3	O	CH_3	-9.2	-8.9
12	$PBENO_2$	NO_2	O	CH_3	-9.9	-8.9

6.1.4 Potent compounds observed by molecular docking:

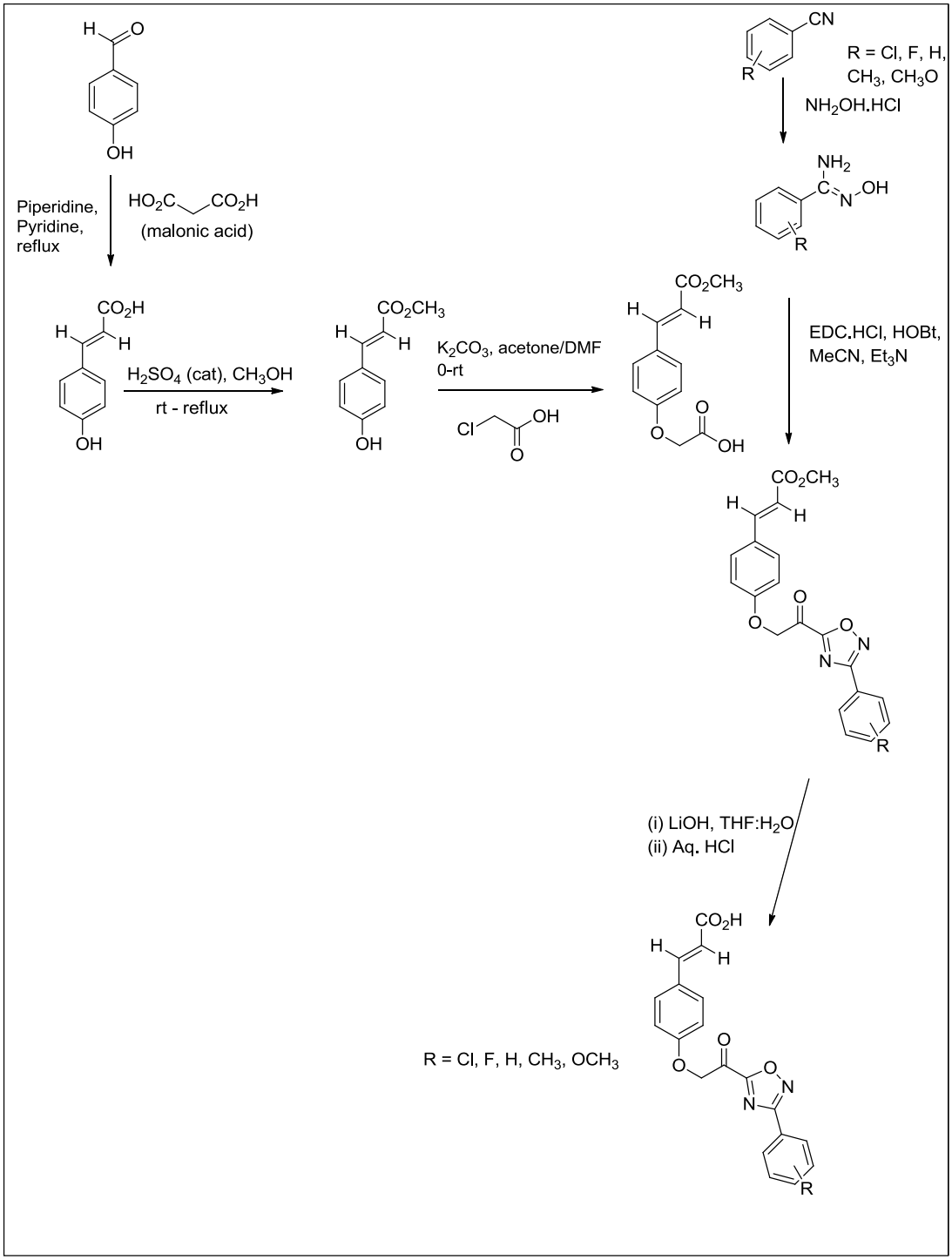
Most potentially active compounds on the basis of their binding affinity and feasibility in chemical synthesis were identified and these were proposed for the synthesis (**Table 9**).

Table 9: Proposed compounds from series 1,2 and 3 based on molecular docking.

Series	Code for synthesized compound	Code for docked compound	Binding affinity (kcal/mol)	
			PPARα (2P54)	PPARγ (3SZ1)
	Standard ligand (pioglitazone)		-11.3	-10.3
1	P152	P4Cl	-8.2	-9.2
2	P155	P4H	-9.3	-9.6
3	P158	P4F	-9.6	-9.3
4	P167	$P4CH_3$	-9.0	-9.0
5	P170	$P2OCH_3$	-8.9	-9.4
6	P171	$P3OCH_3$	-9.4	-9.1
7	P172	PHAH	-8.9	-8.3
8	P173	PBAH	-8.9	-9.0

6.2 Synthesis of most potent compounds

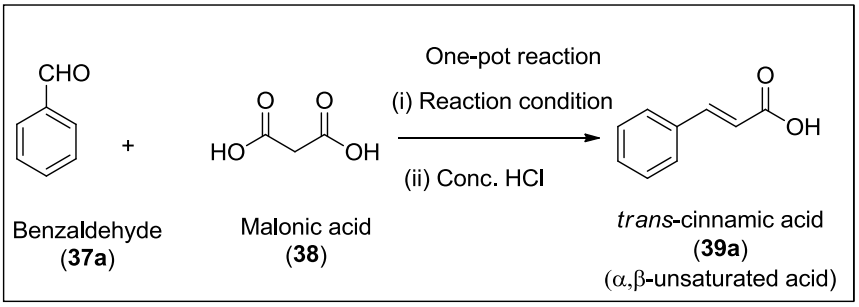
6.2.1 Series 1: Oxadiazole derivatives



Scheme 1: Proposed scheme for the synthesis of oxadiazole derivatives (Series 1).

So, based on the molecular docking results we synthesized selected compounds mostly from series 1 (**Scheme 1**) and each compounds for series 2 and 3. The designed compounds to further study the effects of different aromatic heterocyclic as a linker to head and tail part of pharmacophore. Oxadiazole derivatives will be prepared by coupling oximes with carboxylic acid after condensation (**Scheme 1**).

trans -Cinnamic acid was prepared in series 1 which is based on the Knoevenagel condensation reaction.[114] For the development of method, a model reaction was performed using benzaldehyde (**37a**) and malonic acid (**38**) to afford *trans* -cinnamic acid or *trans-α*,β-unsaturated phenyl carboxylic acid (**39a**) (**Scheme 2**). When benzaldyhyde and malonic acid were refluxed for 10 hr in pyridine only afforded moderate yield (Entry 1) and with the help of catalytic piperidine afforded good yield (Entry 2; **Table 10**) which is known as Doebner modification which undergo through imine intermediate.



Scheme 2: A prototype reaction for synthesis of *trans* -cinnamic acid or *trans-α,β*-unsaturated phenyl carboxylic acid (**39a**).

Table 10: A model reaction of one-pot synthesis of *trans* -cinnamic acid or *trans-α*,β-unsaturated phenyl carboxylic acid (**39a**).[a]

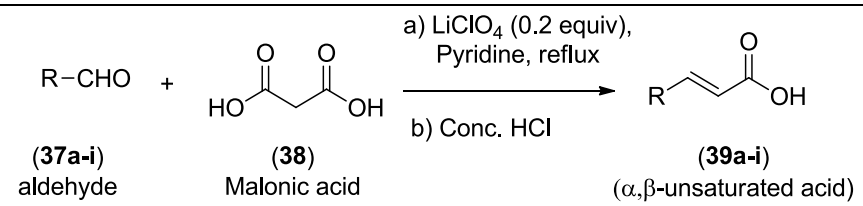
Entry	Reaction conditions	**39a**, isolated yield (%)
1	Pyridine, reflux, 10 hr[b]	30
2	Pyridine, piperidine (0.2 equiv), 10 hr	85
3	Pyridine, $LiClO_4$ (0.2 equiv), 10 hr	94
4	Pyridine, $LiClO_4$ (0.2 equiv), 5 hr	93
5	Pyridine, $NaClO_4$ (0.2 equiv), 5 hr	55
6	Pyridine, $KClO_4$ (0.2 equiv), 5 hr	42
7	Pyridine, $MgClO_4$ (0.2 equiv), 5 hr	20
8	Et_3N, $LiClO_4$ (0.2 equiv), 5 hr	20
9	DIPEA, $LiClO_4$ (0.2 equiv), 5 hr	32
10	DCM, $LiClO_4$ (0.2 equiv), 5 hr	NA[c]

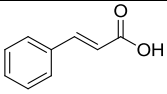
[a]All the reactions were performed one-pot and the product wherever isolated by acidification with conc. HCl. [b]The yield did not improve even after 24 hr. [c]NA = reactants recovered as such.

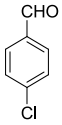
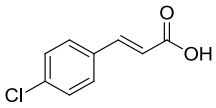
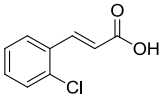
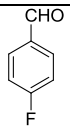
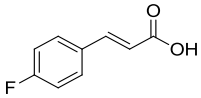
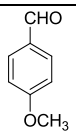
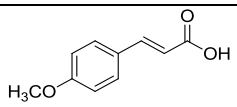
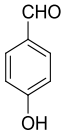
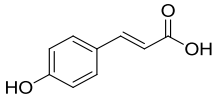
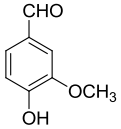
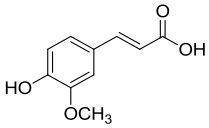
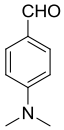
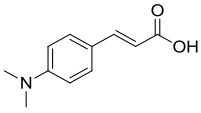
We have been working in the area of synthesis of bioactive molecules which led us to use the catalysis in this reaction and the notion to try $LiClO_4$ is pedestal from our previous work.[115,116] Hence to improve further we substituted the piperidine with lithium perchlorate ($LiClO_4$) in catalytic amount (0.2 equiv) which afforded excellent yield (Entry 3; **Table 10**) even after decreasing the reaction time from 10 hr to 5 hr (Entry 4, **Table 10**). Thus $LiClO_4$ catalyzed reaction lead to development of one-pot synthesis of *trans-α*,β-unsaturated phenyl carboxylic acid via full conversion of intermediate aldol adduct. To understand the role of lithium perchlorate we tried other perchlorate catalytsi.e sodium perchlorate ($NaClO_4$), potassium perchlorate ($KClO_4$) and magnesium perchlorate ($MgClO_4$) (Entries 5, 6, and 7; **Table 10**) but afforded low yield compare to $LiClO_4$. It can be due to better coordination power of Li through high charge to size ratio than other metals (Compare entry 4 with 5-7; **Table 10**). Thus lithium has better interaction to the electron rich atoms like carbonyl oxygen and lead to increase the electrophilicity of such carbonyl compounds. Therefore the lithium catalyst coordinated carbonyl compound would be more reactive in comparison to other studied metal catalysts. Various other solvents were tried but only basic

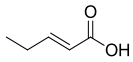
solvents showed good results. The basic solvents such as pyridine, triethyl amine (Et_3N), diisopropylethylamine (DIPEA) and ammonia found reliable base with $LiClO_4$ (Entries 4, 8, 9, 10; **Table 10**). Among the basic solvents, pyridine found to be best to afford final product 3a in excellent yield (Entry 4; **Table 10**). Pyridine along with $LiClO_4$ serves as the solvent as well as base to paved aldol adduct and further to the concerted *trans* elimination and decarboxylation to yield the final product. The generalized protocol was envisaged and explored to synthesize the various substituted *trans-α,β*-unsaturated carboxylic acids (**Table 11**). The effect of electron withdrawing group (entry 2-4 and 6; **Table 11**) and electron donating group (Entry 5 and 8; **Table 11**) was assessed on aromatic aldehydes, along with vaniline containing both electron withdrawing and electron donating functional group (Entry 7; **Table 11**) as well as aliphatic aldheydes (Entry 9; **Table 11**) were also studied. Results showed that in all cases good to excellent yields were obtained of desired products. The aromatic aldehydes were afforded in good to excellent yields than aliphatic aldehyde due to resonance effect (Compare entries 1-8 and 9-10; **Table 11**). The effect was very much prominent of electron withdrawing groups on aromatic aldehyde which increases the electrophilicity of aldehyde and gave excellent yield than electron donating groups containing aromatic aldehydes (Compare entries 1,2, and 3 with 5 and 8; **Table 11**), but in comparison to *para* substituents, an *ortho*substituents yielded low yield of product which may be accounted due to steric hindrance offered by *ortho* substituent (Entry 3; **Table 11**).

Table 11: Synthesis of *trans*-cinnamic acid (*α*,β-unsaturated carboxylic acid) derivatives (**39a-j**).[a] 117



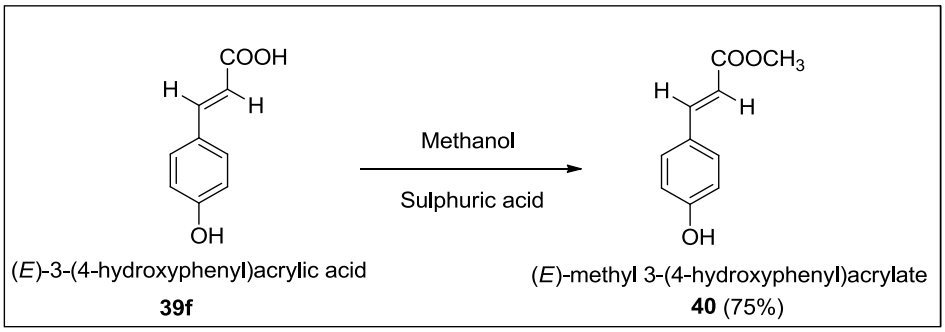
Entry	R-CHO (Aldehyde)	Time	*trans-α*,β-unsaturated carboxylic acid	Isolated yield (%)[a]
1	(**37a**)	5 hr	 (**39a**)	93

2	 (**37b**)	5 hr	 (**39b**)	82
3	(**37c**)	5 hr	 (**39c**)	80
4	 (**37d**)	5 hr	 (**39d**)	94
5	 (**37e**)	5 hr	 (**39e**)	81
6	 (**37f**)	5 hr	 (**39f**)	61
7	 (**37g**)	5 hr	 (**39g**)	78
8	 (**37h**)	5 hr	 (**39h**)	70

9	**(37i)**	7 hr	 **(39i)**	66

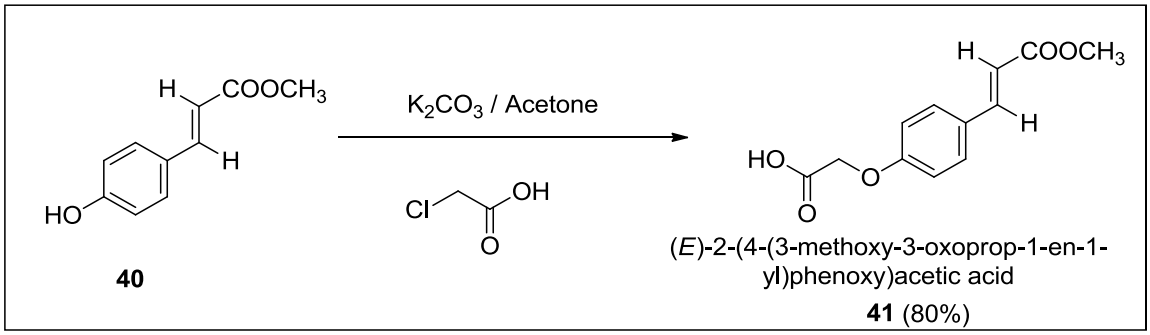
[a]All the reactions were performed one-pot and the products were isolated after acidification with conc. HCl.

Further the *trans*cinnamic acid was converted to the corresponding methyl ester by adding methanol and few drops of conc. H_2SO_4 to the solution of cinnamic acid and refluxed for 1-2 hour. Reaction mixture was then concentrated and checked for TLC (**Scheme 3**).



Scheme 3: Preparation of methyl ester of *trans*cinnamic acid.

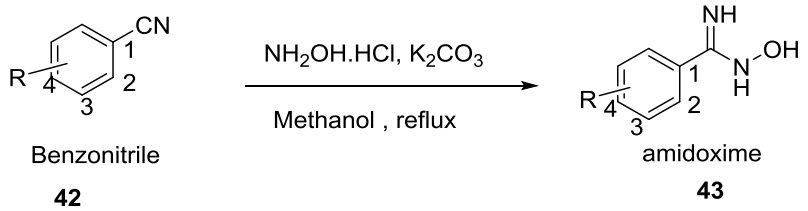
The methyl ester was converted to the (*E*)-2-(4-(3-methoxy-3-oxoprop-1-en-1-yl)phenoxy)acetic acid by reacting, chloroacetic acid in presence of K_2CO_3 in acetone. The addition of chloroacetic acid was dropwise and further stirred at room temperature for 2hr. The TLC was checked to confirm the completion of the reaction, if still reaction is incomplete then further continue the reaction on refluxing (**Scheme 4**)



Scheme 4: Preparation of (*E*)-2-(4-(3-methoxy-3-oxoprop-1-en-1-yl)phenoxy)acetic acid.

For coupling we used amidoxime and its derivative which were synthesizedby adding benzonitrile (**42**) and potassium carbonate in methanol. Reaction mixture was cooled and hydroxylamine hydrochloride was added portion wise and later, refluxed for 6hr after completion of the addition. The solvent evaporated and desired product was precipitated out by adding excess water which was filtered and dried. All amidoximes (**43**) were obtained in good yield (**Table 12**).

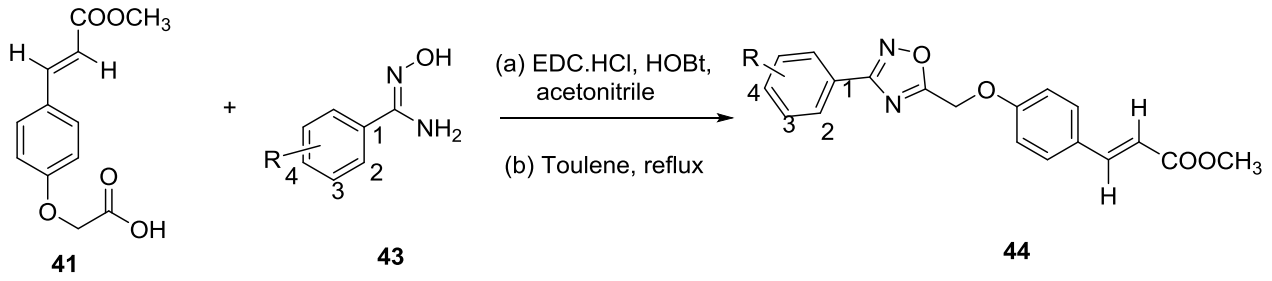
Table 12: Preparation of *N*-hydroxybenzimidamide (amidoxime)



Entry	R	Compound	Isolated yield (%)
1	4-Cl	**43a**	66
2	4-F	**43b**	68
3	H	**43c**	78
4	4-CH_3	**43d**	77
5	2-OCH_3	**43e**	63
6	3-OCH_3	**43f**	78

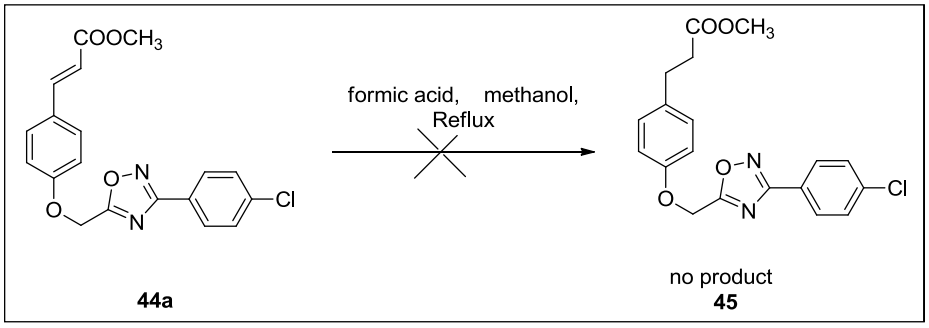
After synthesis of (*E*)-2-(4-(3-methoxy-3-oxoprop-1-en-1-yl)phenoxy)acetic acid and amidoxime derivatives, we used both to prepare 1,2,4,-oxadiazole derivatives by coupling. 1,2,4,-oxadiazole were prepared by mixing α-β unsaturated acid and amidoxime with cyclizing agents *N*-(3-dimethylaminopropyl)-*N′*-ethylcarbodiimide hydrochloride (EDC.HCl) or dicyclohexylcarbodiimde (DCC) and hydroxybenzotriazole (HOBt) as activating reagent in acetonitrile. Therefore crude intermediate was refluxed in toluene for 2hr which afforded crude oxadiazole. The crude was purified by recrystallizing it in DCM and filtered it and concentrated the filtrate to afford the desired product (**Table 13**).

Table 13: Preparation of 1,2,4-oxadiazole derivative



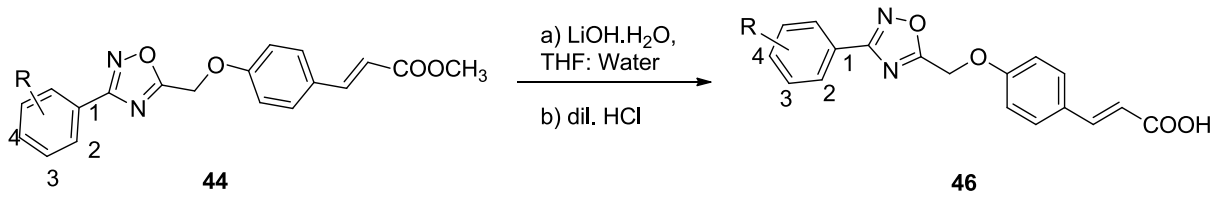
Entry	R	Compound	Yield (%)
1	4-Cl	**44a**	72
2	4-F	**44b**	50
3	H	**44c**	78
4	4-CH_3	**44d**	88
5	2-OCH_3	**44e**	79
6	3-OCH_3	**44f**	86

Further we tried to reduce the unsaturated bond via formic acid mediated reduction (**Scheme 5**) but we did not get the desired product.Due to failure of this reaction, later we directly hydrolyzed the unsaturated 1,2,4-oxadiazole. (*E*)-methyl 3-(4-((3-phenyl-1,2,4-oxadiazol-5-yl)methoxy)phenyl)acrylate was dissolved in THF and a suspension of LiOH.H_2O in THF : water was added and then stirred for 2hr. TLC was analyzed, then the lithium salt of carboxylic acid was converted to the corresponding acid by neutralizing with dil. HCl (1N) (**Table 14**).



Scheme 5: Trial for reduction of (*E*)-3-(4-((3-phenyl-1,2,4-oxadiazol-5-yl)methoxy)phenyl)acrylic ester.

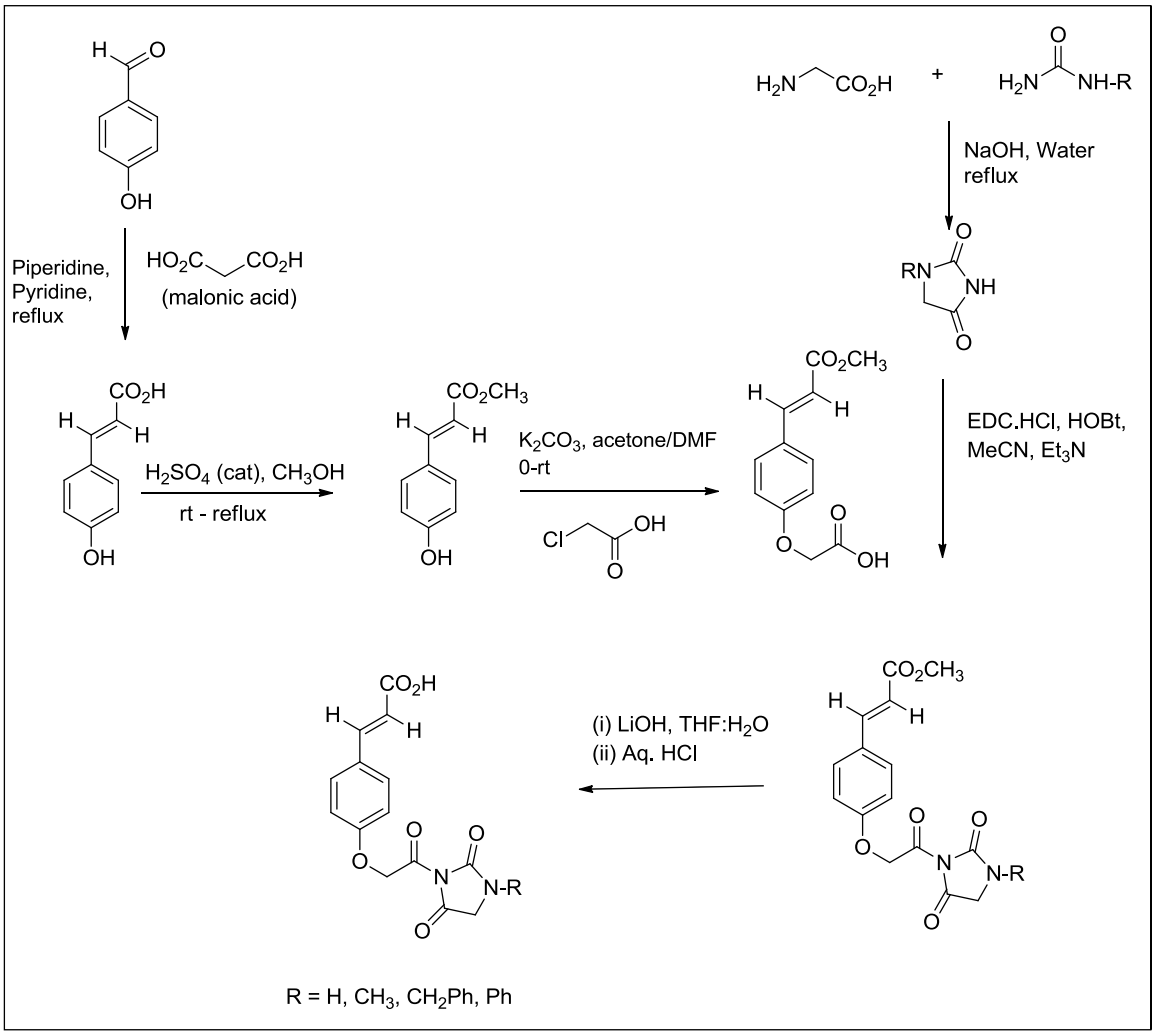
Table 14: Preparation of (*E*)-3-(4-((3-phenyl-1,2,4-oxadiazol-5-yl)methoxy)phenyl)acrylic acid.



Entry	Code (docked compounds)	Code (synthesized compounds)	R	Compound, Yield
1	P4Cl	P152	4-Cl	**46a**,71%
2	P4F	P158	4-F	**46b**,80%
3	P4H	P155	H	**46c**, 88%
4	P4CH_3	P167	4-CH_3	**46d**,90%
5	P2OCH_3	P170	2-OCH_3	**46e,** 92%
6	P3OCH_3	P171	3-OCH_3	**46f,** 88%

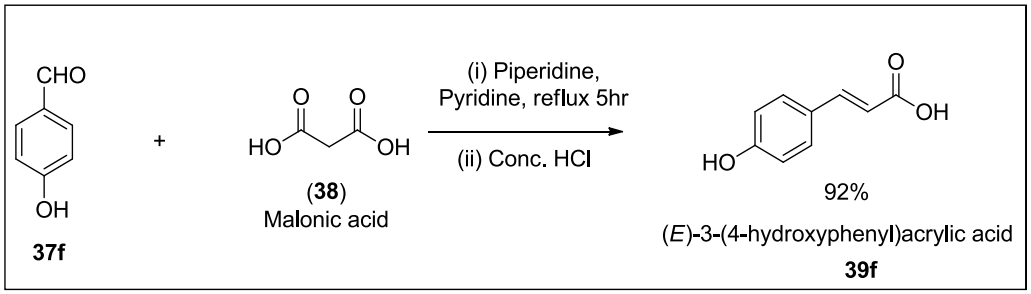
6.2.2 Series 2: Hydantoin derivative

Hydantoin derivatives will be prepared by coupling hydantoin with carboxylic acid after condensation (**Scheme 6**).



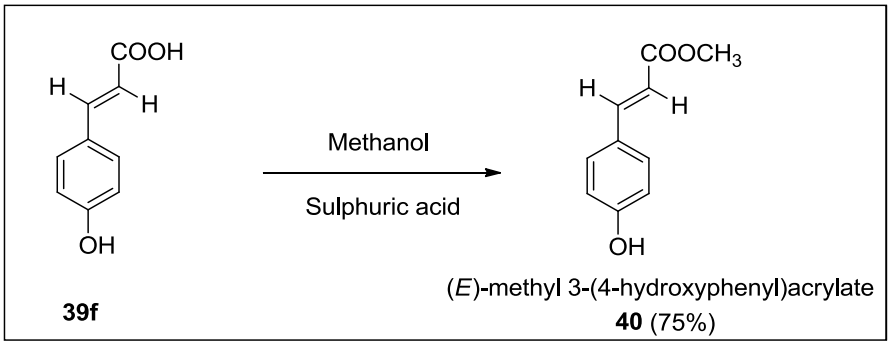
Scheme 6: Proposed scheme for hydantoin derivatives (Series 2).

In first step for series 2, we prepared *(E)*-3-(4-hydroxyphenyl)acrylic acid (**39f**) as previously discussed and obtained in good yield (**Scheme 7**). The reaction was performed one-pot and isolated the product after acidification with conc. HCl.



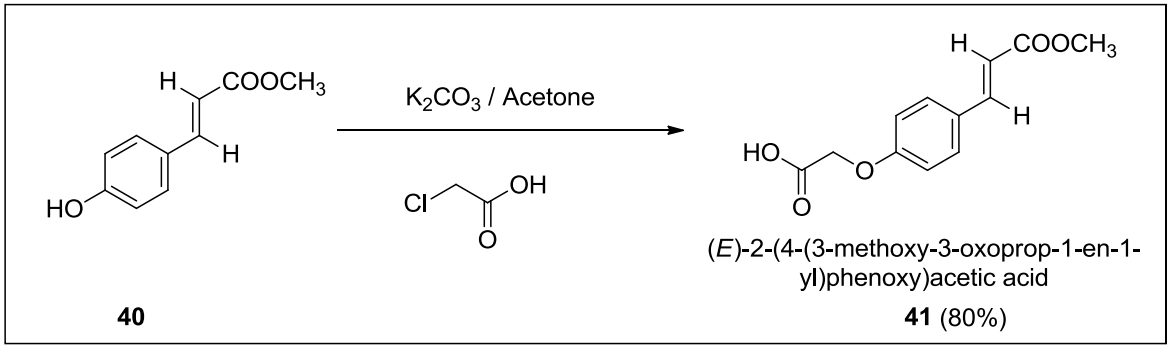
Scheme 7: Preparation of *(E)*-3-(4-hydroxyphenyl)acrylic acid.

Further *(E)*-3-(4-hydroxyphenyl)acrylic acid was esterified using methanol and few drops of conc. H_2SO_4 at refluxing for 2 hour and then concentrated the reaction mixture(**Scheme 8**).



Scheme 8: Preparation of (*E*)-methyl 3-(4-hydroxyphenyl)acrylate.

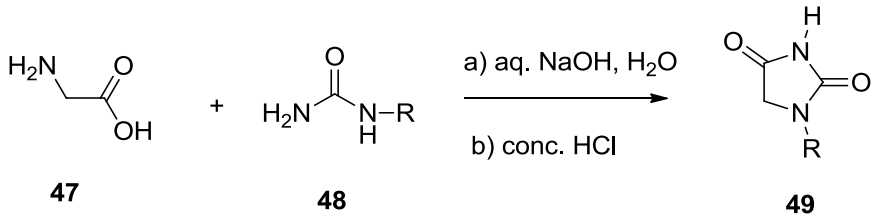
The (*E*)-methyl 3-(4-hydroxyphenyl)acrylate ester was dissolved in acetone and added K_2CO_3 and chloroacetic acid was added dropwise to this solutionand stirred for 2 hr at room temperature. The reaction progress was checked and completion was analyzed by TLC. If still, reaction was incomplete, then further continued for 5hr on refluxing (**Scheme 9**).



Scheme 9: Preparation of (*E*)-2-(4-(3-methoxy-3-oxoprop-1-en-1-yl)phenoxy)acetic acid.

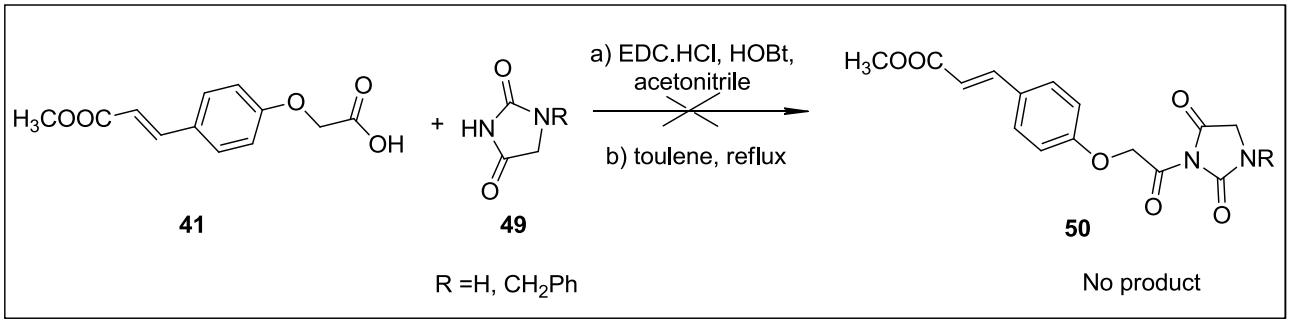
For the preparation of hydantoin derivatives, we used glycine and urea derivative. Sodium hydroxide solution was added to a mixture of glycine and urea in RBF. The mixture was thoroughly mixed and heated at 110-115°C. Reaction mixture was cooled and acidified with conc. HCl to congo red. The separated product (hydantoic acid) was filtered and recrystallized from water. Then the mixture of hydantoic acid and water was heated at 110-115°C. The reaction mixture was cooled and filtered and the separated product was then recrystallized from water (**Table 15**).

Table 15: Preparation of hydantoin derivatives.



Entry	R	Compound, Yield
1	H	**49a**, 92%
2	Ph	**49b**, 78%

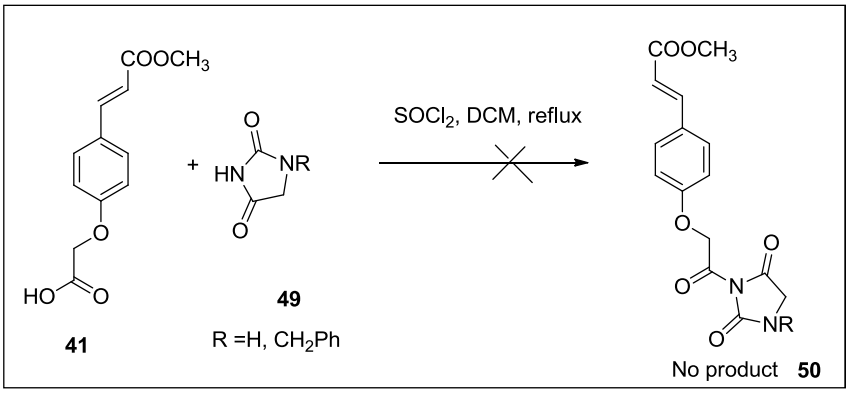
Preparation of (*E*)-methyl 3-(4-(2-(2,5-dioxoimidazolidin-1-yl)-2-oxoethoxy)phenyl)acrylate derivatives was done by mixing *α*-β unsaturated acid and hydantoin with cyclizing agents EDC.HCl and HOBt in acetonitrile. Further a crude intermediate was refluxed in toluene for 2hr but did not afford the desired product (**Scheme 10**).



Scheme 10: Trial to prepare of (*E*)-methyl 3-(4-(2-(2,5-dioxoimidazolidin-1-yl)-2-oxoethoxy)phenyl)acrylate derivatives:

Because of the above reaction did not afford the product thus other methods were tried as following:

Method A: A solution of carboxylic acid in $SOCl_2$was heated to reflux for 1hr. After evaporation of$SOCl_2$, DCM (5mL) was added. In DCM and TEA,a solution of amine was added dropwise at 0^oC. Overnight, the mixture was stirred, then added ethyl acetate and washed the organic layer with saturated aq. Na_2CO_3. Finally, evaporated the organic layer via vacuum, the crude product was separated (**Scheme 11**).

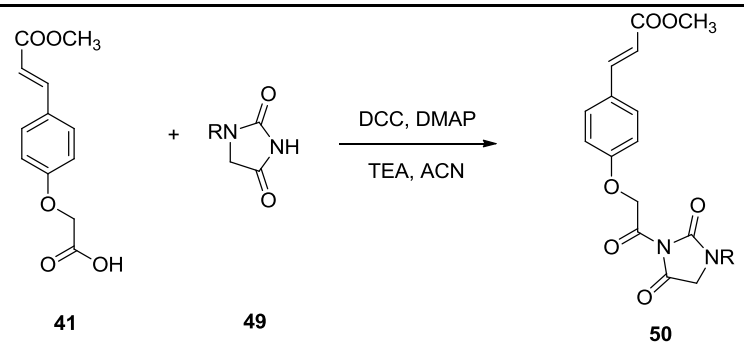


Scheme 11: Method A to prepare of (*E*)-methyl 3-(4-(2-(2,5-dioxoimidazolidin-1-yl)-2-oxoethoxy)phenyl)acrylatederivatives.

Since method A, also failed in synthesis of desired product. Further the reaction conditions have been modified slightly to get the final product of series 2.

Method B: Allderivativeswere prepared by mixing α-β unsaturated acid andhydantoinwith cyclizingagents DCC, DMAP, TEA in acetonitrile. Further crude intermediate was refluxed in toluene for 2hr which afforded crude product. It was then purified by recrystallizing it in DCM and filtered it and concentrated the filtrate to afford the desired product (**Table 16**).

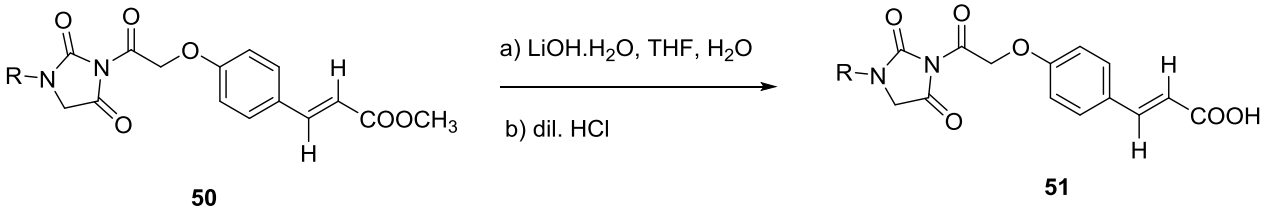
Table 16: Preparation of (*E*)-methyl 3-(4-(2-(2,5-dioxoimidazolidin-1-yl)-2-oxoethoxy)phenyl)acrylate.NA = no product



Entry	R	Compound	Yield
1	H	**50a**	80%
2	Ph	**50b**	NA

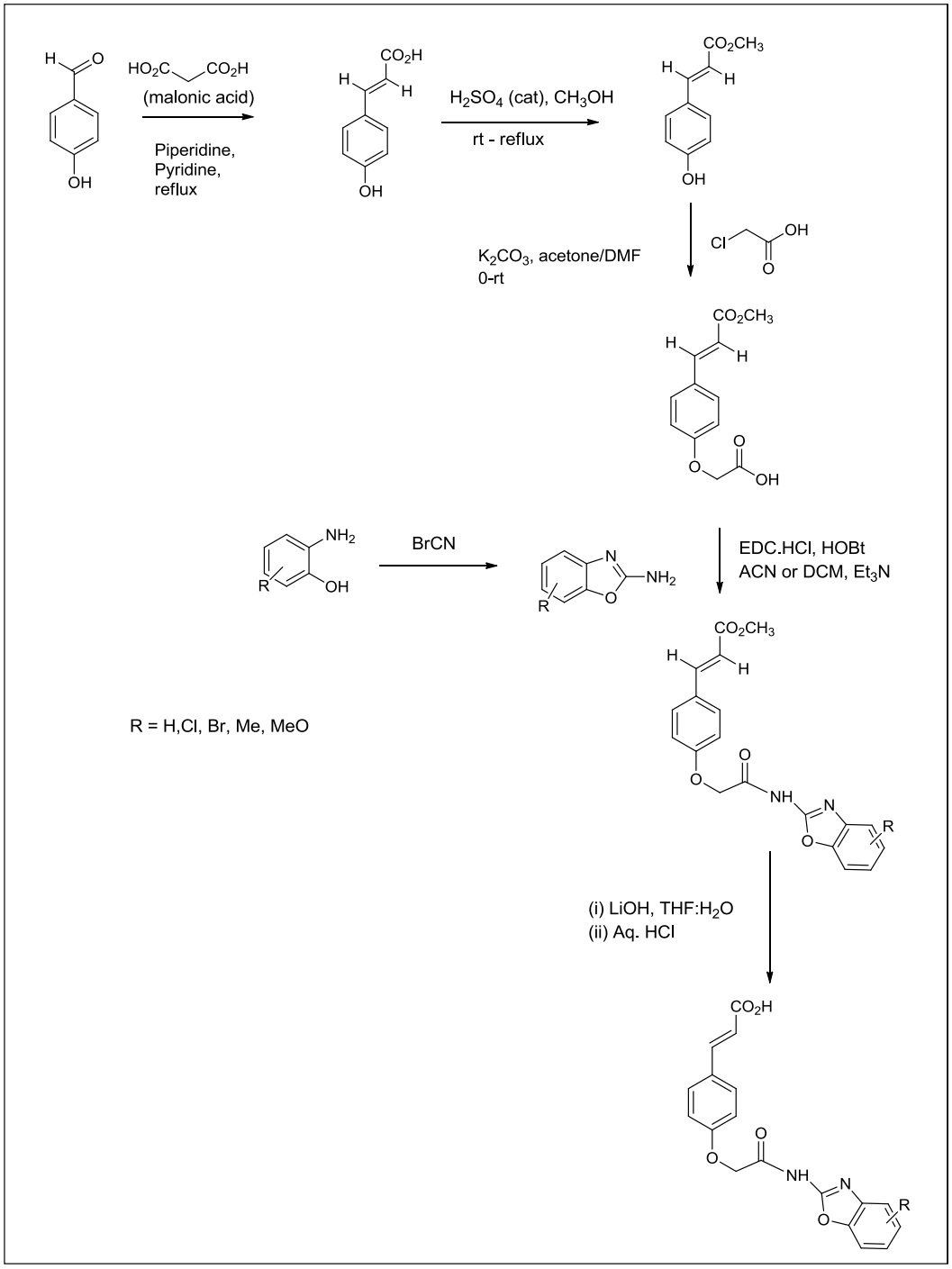
Further a suspension of ester and LiOH.H_2O in THF : water was stirred for 2h, TLC was analyzed, then to convert lithium salt of acid, it was neutralized by dil. HCl (1N) to get corresponding carboxylic acid (**Table 17**). This reaction only worked for hydantoin whereas in case of phenylhydantoin failed to give the desired product.

Table 17: Synthesis of (*E*)- 3-(4-(2-(2,5-dioxoimidazolidin-1-yl)-2-oxoethoxy)phenyl)acrylic acid.



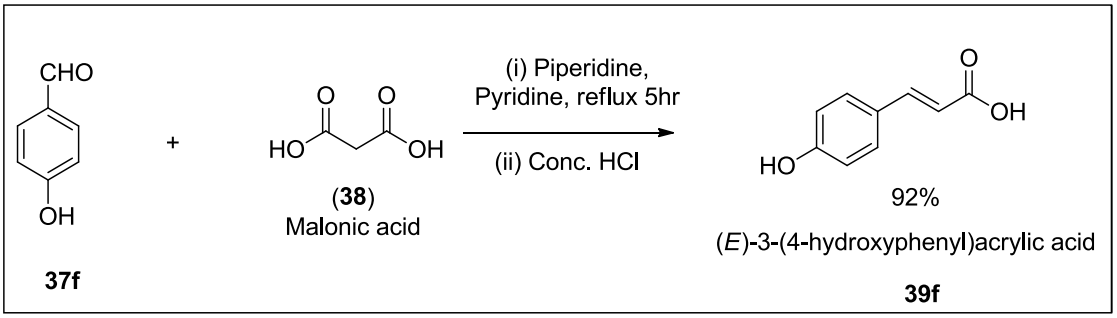
Entry	CODE	R	Compound	Yield
1	172	H	**51**	82%

6.2.3 Series 3: Benzoxazole derivatives: Benzoxazole derivatives were prepared by alkylation of benzo[d]oxazol -2-amine (**Scheme 12**).



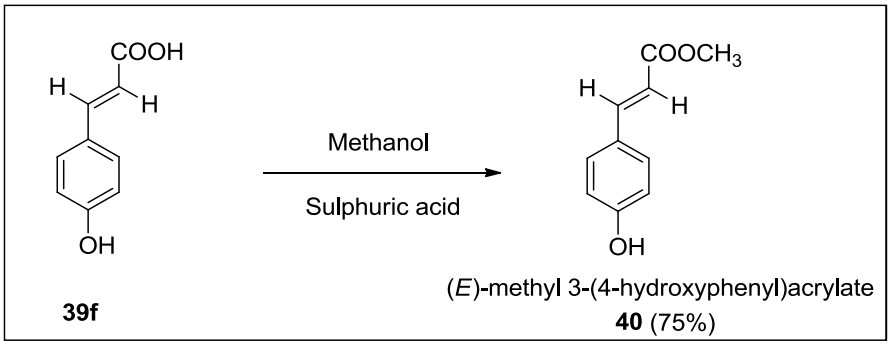
Scheme 12: Proposed scheme for synthesis of benzoxazoles (series 3).

In first step for series 2 compounds we prepared *(E)*-3-(4-hydroxyphenyl)acrylic acid as previously discussed and obtained in good yield (**Scheme 13**). The reaction was performed one-pot and the product was isolated after acidification with conc. HCl.



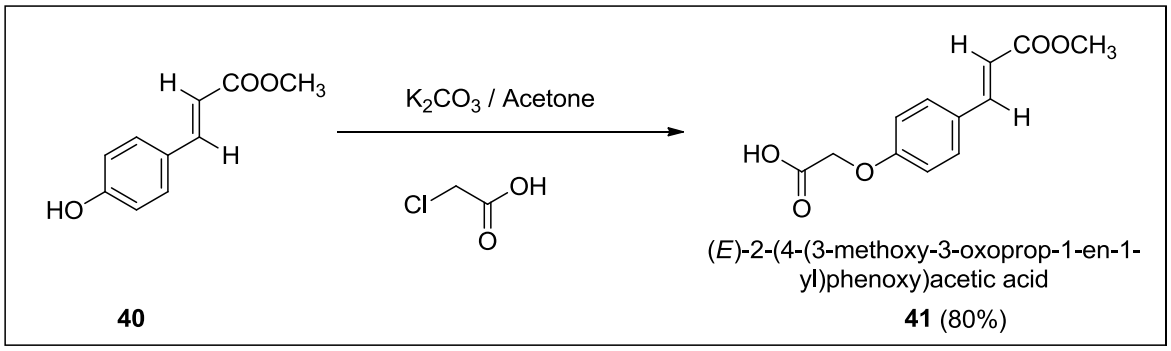
Scheme 13: Preparation of *(E)*-3-(4-hydroxyphenyl)acrylic acid.

Further Further*(E)*-3-(4-hydroxyphenyl)acrylic acid was esterified using methanol and few drops of conc. H_2SO_4 at refluxing for 2 hour. Reaction mixture was then concentrated (**Scheme 14**).



Scheme 14: Preparation of (*E*)-methyl 3-(4-hydroxyphenyl)acrylate.

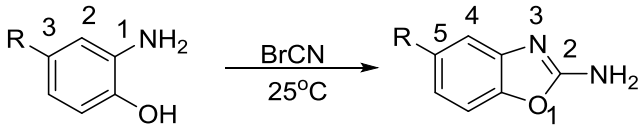
Further (*E*)-methyl 3-(4-hydroxyphenyl)acrylate ester was dissolved in acetone and added K_2CO_3 , to this solution, dropwise added chloroacetic acid and stirred for 2hr at room temperature. The reaction progress was monitored by TLC. If still reaction was incomplete, then further it was continued for 5hr on refluxing (**Scheme 15**).



Scheme 15: Preparation of (*E*)-2-(4-(3-methoxy-3-oxoprop-1-en-1-yl)phenoxy)acetic acid.

For coupling with acrylic ester we prepared benzoxazoles using cyanogen bromide mediated cyclization of ortho-aminophenol. At room temperature, BrCN was stirred in MeOH/H_2O (7:3). Then a solution of 2-aminophenol (1 mol) was mixed with minimum amount of methanol which was then added to the mixture, further stirred for 40min. The solution was subsequently neutralized with conc. aq. NaOH and distillation was carried out to evaporate the methanol. The residue was treated with water followed by the filteration to collect the crude material. Later the crude was purified by hot water mixed with charcoal led to pure products (**Table 18**).

Table 18: Preparation of benzoxazole-2-amine derivatives.

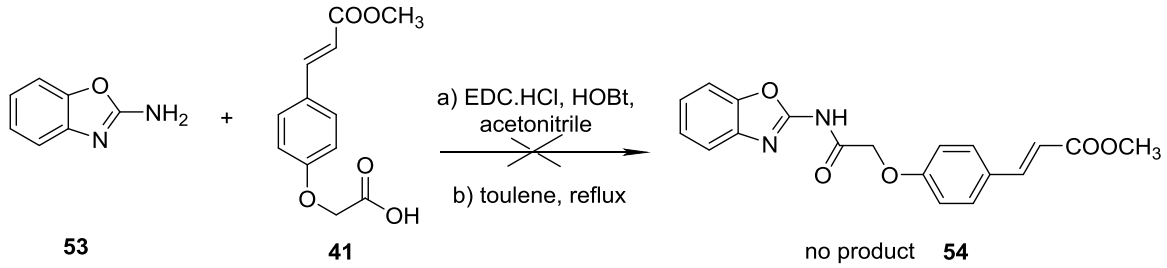


52 **53**

Entry	R	X	Compound	Yield (%)
1	H	O	**53a**	90
2	3-NO_2	O	**53b**	90

(E)-3-(4-((benzo[d]oxazol-2-ylamino)methoxy)phenyl)acrylate derivatives were prepared by mixing 3-(4-(chloromethoxy)phenyl)propanoic acid and benzoxazole-2-amine with cyclizing agents EDC.HCl and HOBt in acetonitrile. Therefore crude intermediate was refluxed in toluene for 2hr

which afforded crude product. The crude was purified by recrystallizing it in DCM and filtered it and concentrated the filtrate to afford the desired product (**Scheme 16**).

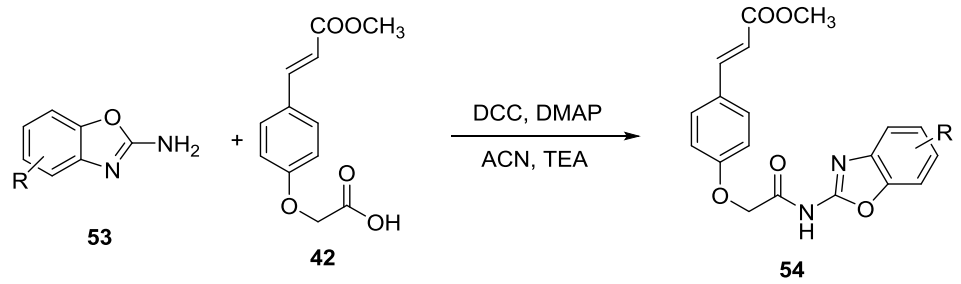


Scheme 16: Trial to prepare methyl (*E*)-3-(4-((benzo[d]oxazol-2-ylamino)methoxy)phenyl)acrylate derivatives.

The Above method didn't work well for the above synthesis. So another method has beentried with slight modification in reaction condition as follow:

All derivatives were prepared by mixing α-β unsaturated acid and substituted amine with coupling agents DCC, DMAP, TEA in acetonitrile. Thereafter crude intermediate was refluxed in toluene for 2h which afforded crude product. Finally, the recrystallization was done to purify desired product from crude(**Table 19**).

Table 19: Preparation of methyl (*E*)-3-(4-(2-(benzo[d]oxazol-2-ylamino)-2-oxoethoxy)phenyl)acrylate.

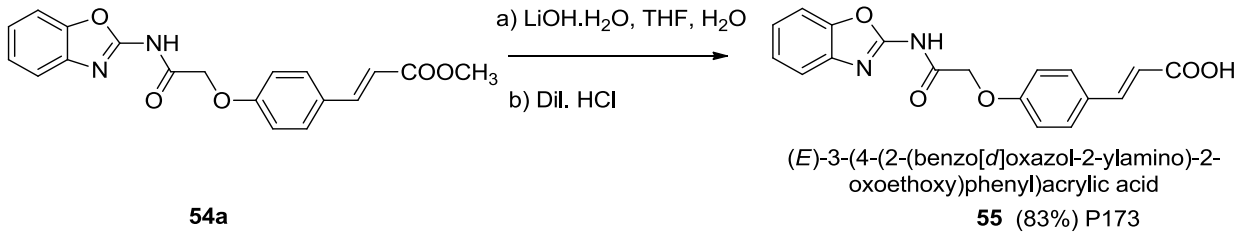


Entry	code	R	X	Compound, Yield
1	P161	H	O	**54a**, 82%
2	P174	3-NO_2	O	NA

NA = no product

For preparation of methyl (*E*)-3-(4-(2-(benzo[d]oxazol-2-ylamino)-2-oxoethoxy)phenyl)acrylic acidderivatives. A suspension of ester and $LiOH.H_2O$ was dissolved in THF : water which was then

stirred for2h, the progress of reaction was then detected by TLC. To convert lithium salt of acid, it was neutralized by dil. HCl (1N) to get corresponding (*E*)-3-(4-(2-(benzo[d]oxazol-2-ylamino)-2-oxoethoxy)phenyl)acrylic acid (**Scheme 17**).



Scheme 17: Preparation of methyl (*E*)-3-(4-(2-(benzo[d]oxazol-2-ylamino)-2-oxoethoxy)phenyl)acrylic acid derivatives

6.3 Characterization of representative compound (46a or P152):

All the compounds synthesized in each steps were analyzed and characterized by Melting point, TLC, IR, NMR, Mass. Completion of reaction was checked using TLC (precoated) was used in 3 X 6 cm dimension and spotted the solution of starting material, co-spot and reaction mixture. The TLC was eluted in optimized eluent i.eethyleacetate : hexane(4:6) with rf 0.54 for **46a** or **P152** (**figure 37**). IR spectrum obtained by making KBr pellet.

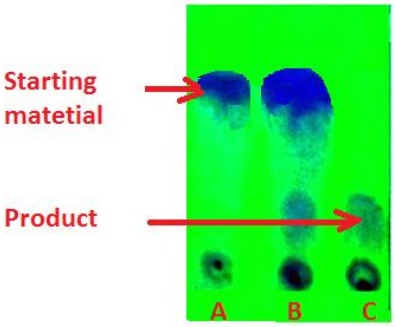


Figure 37: TLC of representative compound 46a or P152 [Rf 0.5 (4:6, EtOAc : hexane); (A) starting material 44a, (B) mixture of starting and product, (C) product].

IR specteum useful in determination of functional groups, the characterization of compound was done by preparing the KBr pellet and sprtium taken which showed peaks like 1597 cm^{-1} (C=C), 1638 cm^{-1} (C=O), 2929cm^{-1} (C-H), and 3327cm^{-1} (O-H) (**Figure 38**). Mass characterstic peak for

compound coded as **46a or P152**. MS (m/z): 373 $(M+NH_3)+$ (**Figure 39**). The ^1H NMR characterstic peak represented as:^1H NMR(400MHz, *d*-DMSO): δ 4.69 (s, 2H, CH_2), 6.31-6.38 (m, 1H, CH=CH), 6.94 (d, 2H, J = 8Hz), 7.11 (d, 1H, J = 12Hz), 7.20 (d, 1H, J=16Hz, CH=CH), 7.52-7.61 (m, 4H), 8.03 (d, 1H, J= 8Hz) (**Figure 40**). ^{13}C NMR spectra for compound coded as **46a or P152** represented as^{13}CNMR (100MHz, *d*-DMSO): δ 64.42 (CH_2, alkenyl), 114.64 (CH_2, aromatic), 116.55 (CH, alkenyl), 124.46 (CH, aromatic), 127.22 (C, aromatic), 128.03 (C, aromatic), 129.46 (CH, aromatic), 134.47 (CH, aromatic), 136.67 (CH, aromatic), 143.19 (CH, alkenyl), 158.59 (C, aromatic), 167.79 (CN), 169.75 (CN), 175.14 (COOH) (**Figure 41**).

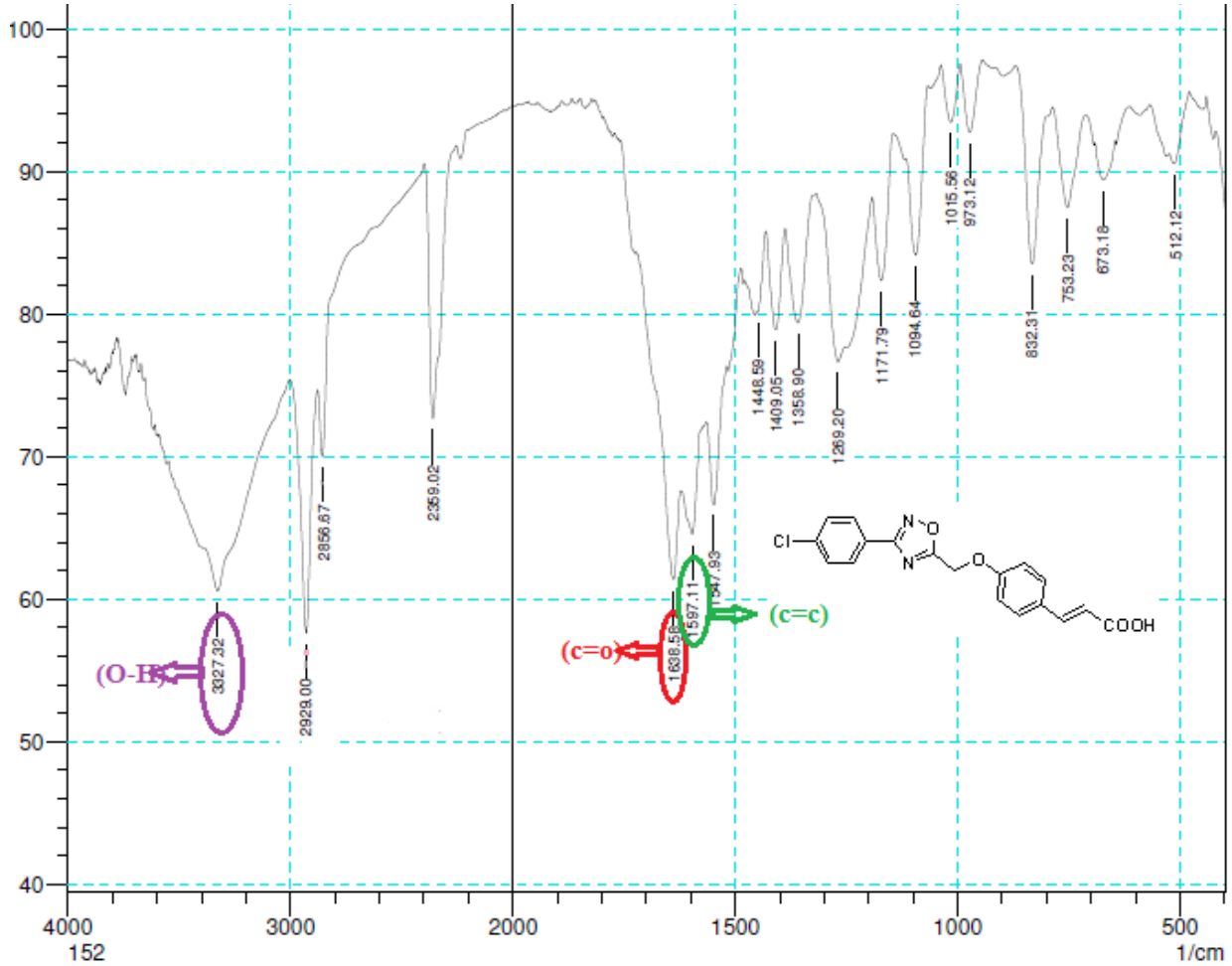


Figure 38: IR spectra for compound coded as **46a** or**P152**. IR(cm^{-1}): 1597 (C=C), 1638 (C=O), 3327(O-H).

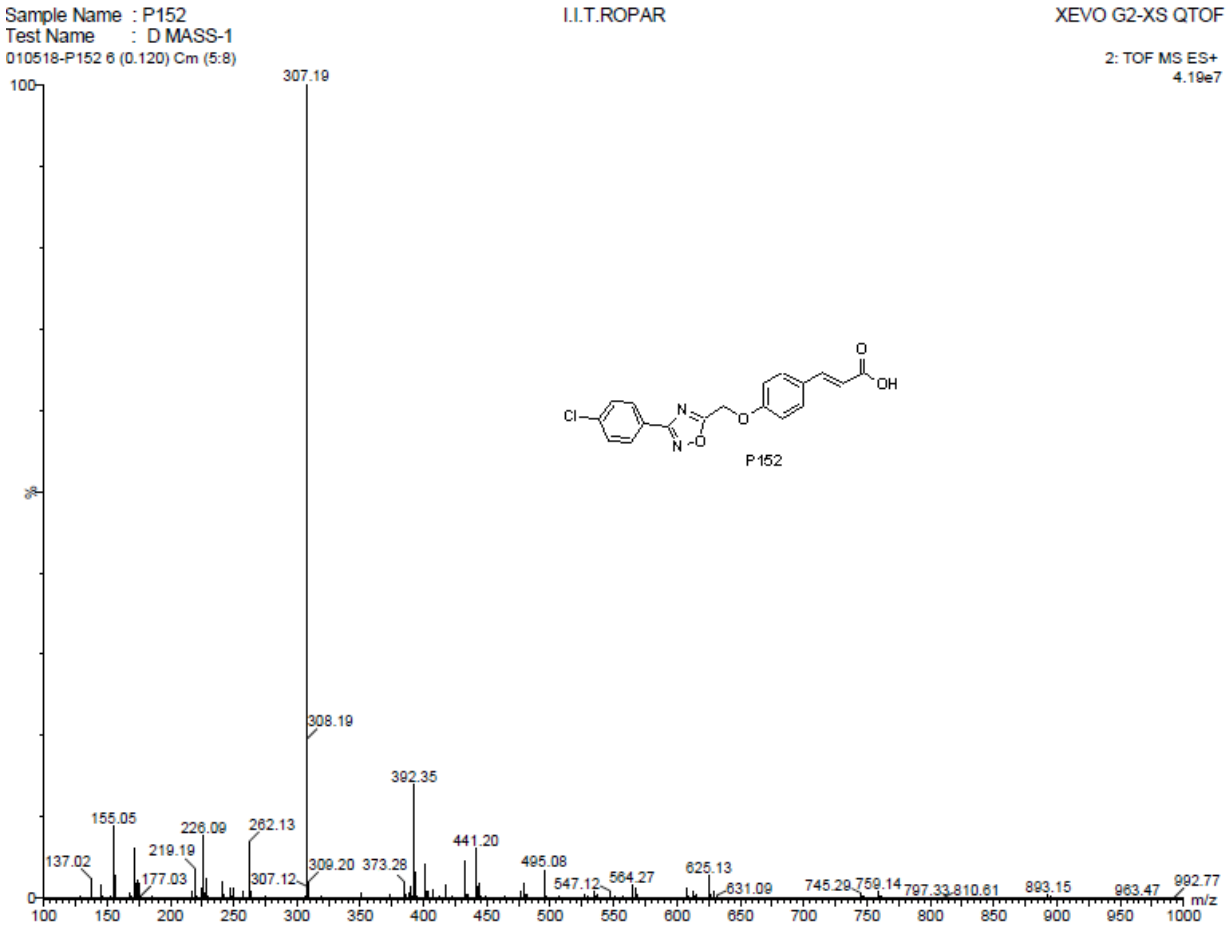


Figure 39: Mass spectra for compound coded as **46a**. MS(m/z): 373 $(M+NH_3)+$.

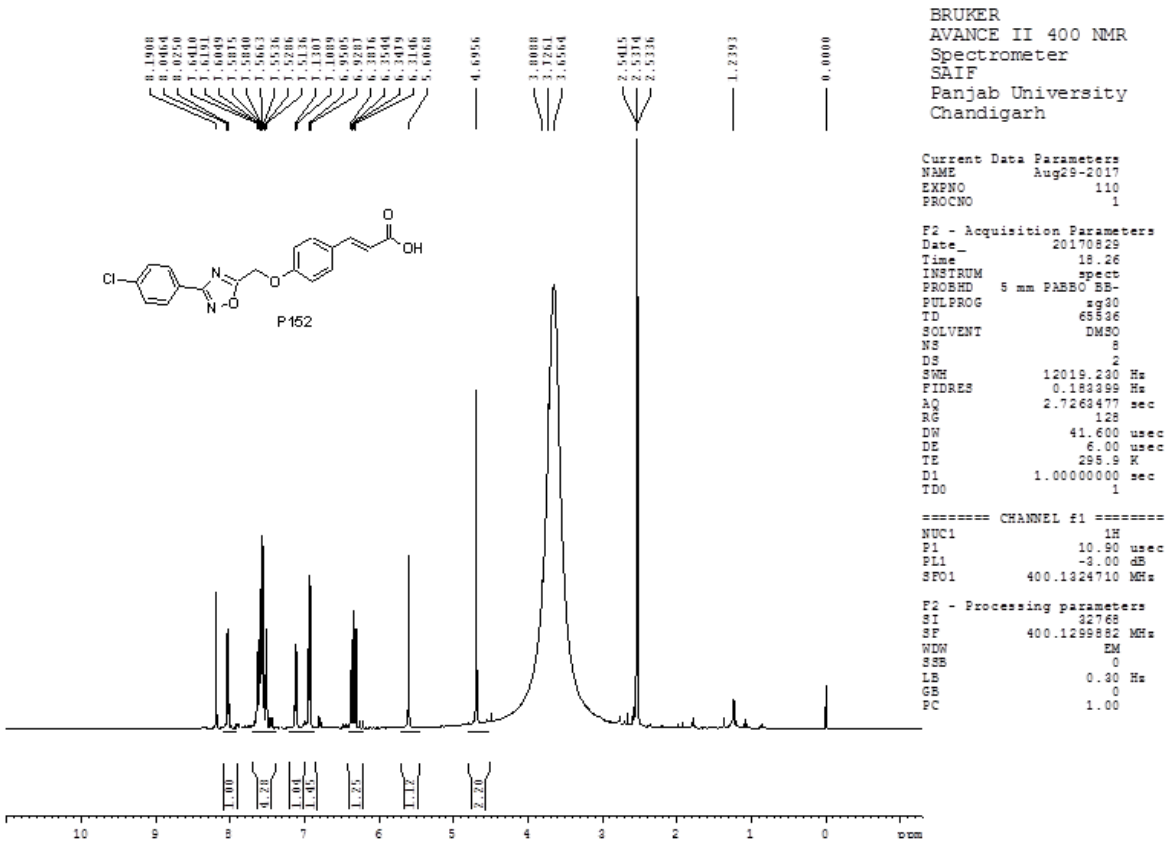


Figure 40: ^{1}H NMR spectra for compound coded as **46a or P152**. ^{1}H NMR(400MHz,*d*-DMSO): δ 4.69 (s, 2H, CH2), 6.31-6.38 (m, 1H, CH=CH), 6.94 (d, 2H, J = 8Hz), 7.11 (d, 1H, J = 12Hz), 7.20 (d, 1H, J=16Hz, CH=CH), 7.52-7.61 (m, 4H), 8.03 (d, 1H, J= 8Hz).

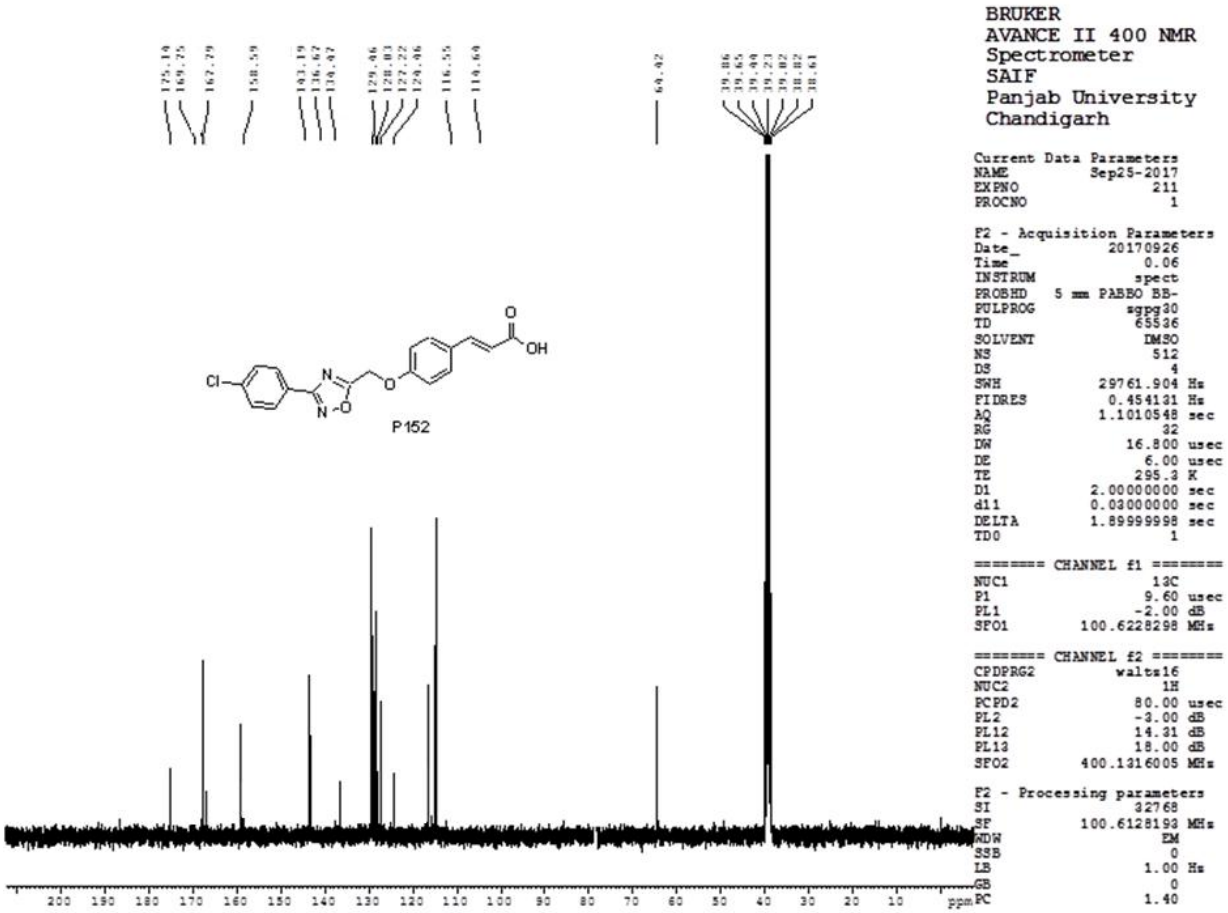


Figure 41: ^{13}C NMR spectra forcompound coded as **46a or P152**. ^{13}CNMR (100MHz, *d*-DMSO): δ 64.42, 114.64, 116.55, 124.46, 127.22, 128.03, 129.46, 134.47, 136.67, 143.19, 158.59, 167.79, 169.75, 175.14

6.4 *In-silico* toxicity prediction:

Currently toxicity is a major concern of newly developed and existing drug molecules therefore we looked into to find out toxicity issue in our designed compounds to be considering them for further development. We studied the eight compounds from series 1, and one from each series 2 and 3 which were synthesized in the laboratory (**Figure 42**).

The toxicity of these synthesized compounds was predicted by using Lazar toxicity predictor available on-line at https://lazar.in-silico.ch/predict.[118,119]

Following are the steps to predict the toxicity:

- Open or draw a 2D structure of designed compound in ChemBiodraw software.

- Copy the smileys for each structure from ChemBiodraw
- Paste the smileys into the webpage.
- Thereafter predict all type of toxicity.

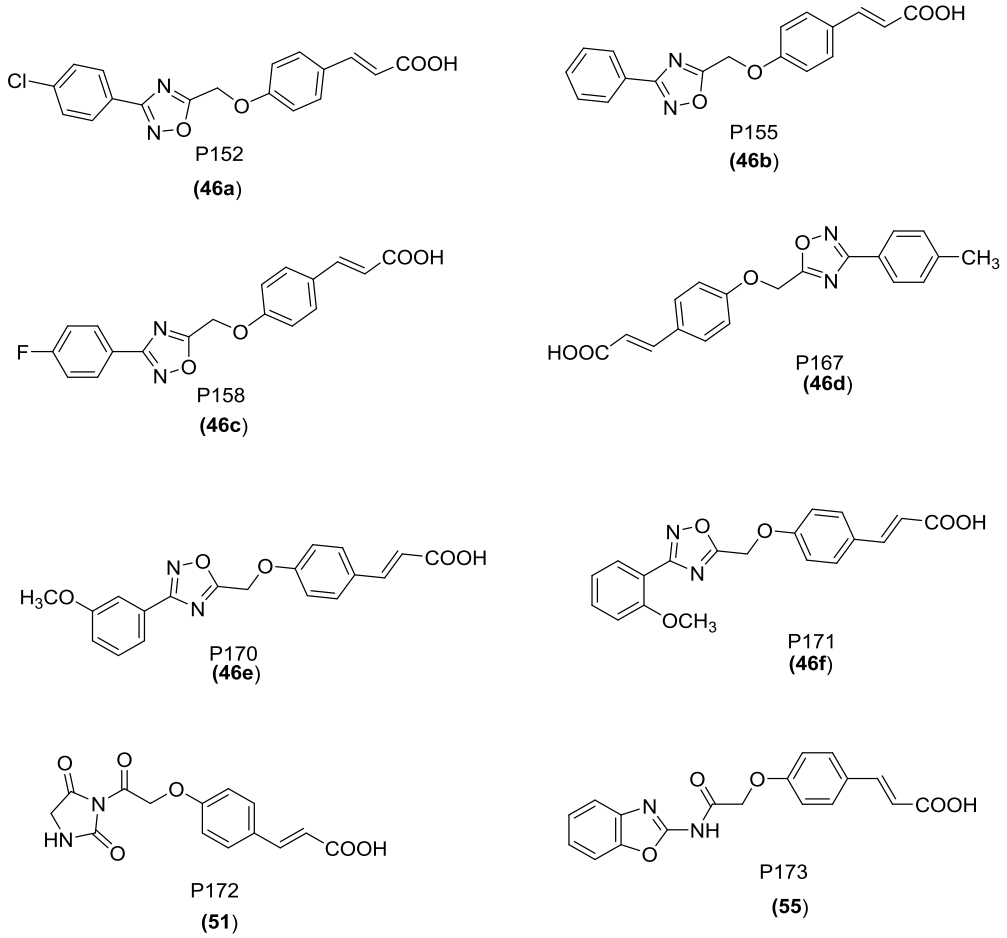


Figure 42: Designed and synthesized compounds

Table 20. Prediction of *in-silico* toxicity by Lazar toxicity predictions (https://lazar.in-silico.de/predict). NA = not available

S.No	Compound	Carcinogenicity (rodent multiple species/sites)	Carcinogenicity (mouse)	Maximum recommended daily dose (human)	Mutagenicity (Salmonella typhimurium)
1	P152	non-carcinogenic (carcinogenic: 0.11 vs non-carcinogenic: 0.14)	non-carcinogenic(carcinogenic: 0.112 vs non-carcinogenic: 0.138)	NA	non-mutagenic (mutagenic: 0.174 vs non-mutagenic: 0.276)
2	P155	Non-carcinogenic (carcinogenic: 0.162	Non-carcinogenic (carcinogenic 0.134	NA	Mutagenic (mutagenic :

		vs non-carcinogenic 0.172)	vsnon-carcinogenic 0.199)		0.214 vs non-mutagenic 0.175)
3	P158	Non-carcinogenic (carcinogenic 0.153 vs non-carcinogenic 0.193)	Non-carcinogenic (carcinogenic 0.112 vs non-carcinogenic 0.193)	NA	Non-mutagenic (mutagenic 0.128 vs non-mutagenic 0.176)
4	P167	Non-carcinogenic (carcinogenic 0.149 vs non-carcinogenic 0.155)	Non-carcinogenic (carcinogenic 0.132 vs non-carcinogenic 0.173)	NA	Mutagenic (mutagenic 0.175 vs non-mutagenic 0.129)
5	P170	Carcinogenic (carcinogenic 0.167 vs non-carcinogenic 0.125)	Non-carcinogenic (carcinogenic 0.124 vs non-carcinogenic 0.168)	0.016 (mmol/kg-bw/day) 5.62 (mg/kg_bw/day) 95% Prediction interval: 0.0099 - 0.0257 (mmol/kg-bw/day) 3.49 - 9.06 (mg/kg_bw/day)	Mutagenic (mutagenic 0.217 vs non-mutagenic 0.143)
6	P171	Carcinogenic (carcinogenic 0.169 vs non-carcinogenic 0.122)	Non-carcinogenic (carcinogenic 0.126 vs non-carcinogenic 0.166)	0.016 (mmol/kg-bw/day) 5.65 (mg/kg_bw/day) 95% Prediction interval: 0.00962 - 0.0267 (mmol/kg-bw/day) 3.39 - 9.41 (mg/kg_bw/day)	Mutagenic (mutagenic 0.214 vs non-mutagenic 0.146)
7	P172	Non-carcinogenic (carcinogenic 0.163 vs non-carcinogenic 0.171)	Non-carcinogenic (carcinogenic 0.136 vs non-carcinogenic 0.198)	NA	Non-mutagenic (mutagenic 0.096 vs non-mutagenic 0.237)
8	P173	Carcinogenic (carcinogenic, 0.148 vs non-carcinogenic 0.144)	Non-carcinogenic (carcinogenic 0.121 vs non-carcinogenic 0.171)	NA	Non-mutagenic (mutagenic 0.143 vs non-mutagenic 0.19)

The **Table 20** above showed *in-silico* toxicity profile, the results showed that compounds P152, P155, P158, P172, and P173 are proposed to be non-carcinogenic, non-mutagenic whereas, compound 170 found to be carcinogenic.

Moreover toxicity is associated with the ADME (Adsorption, distribution, metabolism and excretion) profile of the compounds thus we predicted the *in-silico* ADME for correlation of its lipophilicity and water solubility with its toxicity (**Table 21**). The *in-silico* ADME properties of the

compounds were obtained as a calculated value for physicochemical properties, water solubility, lipophilicity, pharmacokinetics(http://www.swissadme.ch/index.php).[120-122]

All compounds showed moderate solubility in water and suppose to have high GI absorption with no permeation to BBB. Calculated liphophilicity (iLogP) was found to be 0.92 to 3.19.

Table 21. Prediction of *in-silico*ADME properties.

Compound	Physico-chemical properties		Water solubility		Lipophilicity Log $P_{o/w}$ (iLOGP)	Pharmacokinetics	
P152	Formula	$C_{18}H_{13}ClN_2O_4$	Log *S* (Ali)	-5.37	3.19	GI absorption	High
	Molecular weight	356.76 g/mol	Solubility	1.52e-^{03} mg/ml ; 4.25e-^{06}mol/l		BBB permeate	No
	Molar Refractivity	92.39	Class	Moderately soluble		P-gp substrate	No
						CYP1A2 inhibitor	Yes
						CYP2C19 inhibitor	Yes
						CYP2C9 inhibitor	Yes
						CYP2D6 inhibitor	No
						CYP3A4 inhibitor	No
						Log K_p (skin permeation)	-5.72 cm/s
						Druglikeness	Yes
P155	Formula	$C_{18}H_{14}N_2O_4$	Log *S* (Ali)	-4.72	2.87	GI absorption	High
	Molecular weight	322.31 g/mol	Solubility	6.17e-^{03} mg/ml ; 1.91e-^{05}mol/l		BBB permeate	No
	Molar Refractivity	87.38	Class	Moderately soluble		P-gp substrate	No
						CYP1A2 inhibitor	Yes
						CYP2C19 inhibitor	Yes
						CYP2C9 inhibitor	Yes
						CYP2D6 inhibitor	No
						CYP3A4 inhibitor	No
						Log K_p (skin permeation)	-5.96 cm/s
						Druglikeness	Yes
P158	Formula	$C_{18}H_{13}FN_2O_4$	Log *S* (Ali)	-4.82	3.00	GI absorption	High
	Molecular weight	340.31 g/mol	Solubility	5.13e-^{03} mg/ml ; 1.51e-^{05}mol/l		BBB permeant	No
	Molar Refractivity	87.34	Class	Moderately soluble		P-gp substrate	No
						CYP1A2 inhibitor	Yes
						CYP2C19 inhibitor	Yes
						CYP2C9 inhibitor	Yes
						CYP2D6 inhibitor	No
						CYP3A4 inhibitor	No
						Log K_p (skin permeation)	-6.00 cm/s
						Druglikeness	Yes
P163	Formula	$C_{19}H_{16}N_2O_4$	Log *S* (Ali)	-5.10	3.12	GI absorption	High
	Molecular weight	336.34 g/mol	Solubility	2.66e-^{03} mg/ml ; 7.90e-^{06}mol/l		BBB permeant	No
	Molar Refractivity	92.35	Class	Moderately soluble		P-gp substrate	No
						CYP1A2 inhibitor	Yes
						CYP2C19 inhibitor	Yes
						CYP2C9 inhibitor	Yes

				CYP2D6 inhibitor: No CYP3A4 inhibitor: No Log K_p (skin permeation): -5.78 cm/s Druglikeness: Yes
P170	Formula: $C_{19}H_{16}N_2O_5$ Molecular weight: 352.34 g/mol Molar Refractivity: 93.87	Log *S* (Ali): -5.10 Solubility: 2.66e-^{03} mg/ml ; 7.90e-^{06}mol/l Class: Moderately soluble	2.97	GI absorption: High BBB permeant: No P-gp substrate: No CYP1A2 inhibitor: Yes CYP2C19 inhibitor: Yes CYP2C9 inhibitor: Yes CYP2D6 inhibitor: No CYP3A4 inhibitor: No Log K_p (skin permeation): -5.78 cm/s Druglikeness: Yes
P171	Formula: $C_{19}H_{16}N_2O_5$ Molecular weight: 352.34 g/mol Molar Refractivity: 93.87	Log *S* (Ali): -4.89 Solubility: 4.53e-^{03} mg/ml ; 1.28e-^{05}mol/l Class: Moderately soluble	3.12	GI absorption: High BBB permeant: No P-gp substrate: No CYP1A2 inhibitor: Yes CYP2C19 inhibitor: Yes CYP2C9 inhibitor: Yes CYP2D6 inhibitor: No CYP3A4 inhibitor: No Log K_p (skin permeation): -6.16 cm/s Druglikeness: Yes
P172	Formula: $C_{13}H_{10}N_2O_6$ Molecular weight: 290.23 g/mol Molar Refractivity: 76.82	Log *S* (Ali): -2.79 Solubility: 4.75e-^{01} mg/ml ; 1.64e-^{03} mol/l Class: Soluble	0.92	GI absorption: High BBB permeant: No P-gp substrate: No CYP1A2 inhibitor: No CYP2C19 inhibitor: No CYP2C9 inhibitor: No CYP2D6 inhibitor: No CYP3A4 inhibitor: No Log K_p (skin permeation): -7.48 cm/s Druglikeness: Yes
P173	Formula: $C_{18}H_{14}N_2O_5$ Molecular weight: 338.31 g/mol Molar Refractivity: 91.00	Log *S* (Ali): -4.72 Solubility: 6.50e-^{03} mg/ml ; 1.92e-^{05}mol/l Class: Moderately soluble	1.82	GI absorption: High BBB permeant: No P-gp substrate: No CYP1A2 inhibitor: Yes CYP2C19 inhibitor: No CYP2C9 inhibitor: Yes CYP2D6 inhibitor: No CYP3A4 inhibitor: No Log K_p (skin permeation): -6.29 cm/s Druglikeness: Yes

6.5 *In-vitro* evaluation

In-vitro PPARα/γ dual agonist activity study was done by PPAR alpha and gamma transcriptional assay method using Abcam transcription factor assay kit.The reagents were added to wells as indicated on the plate and incubated one hour at 37°C (**Figure 43**).

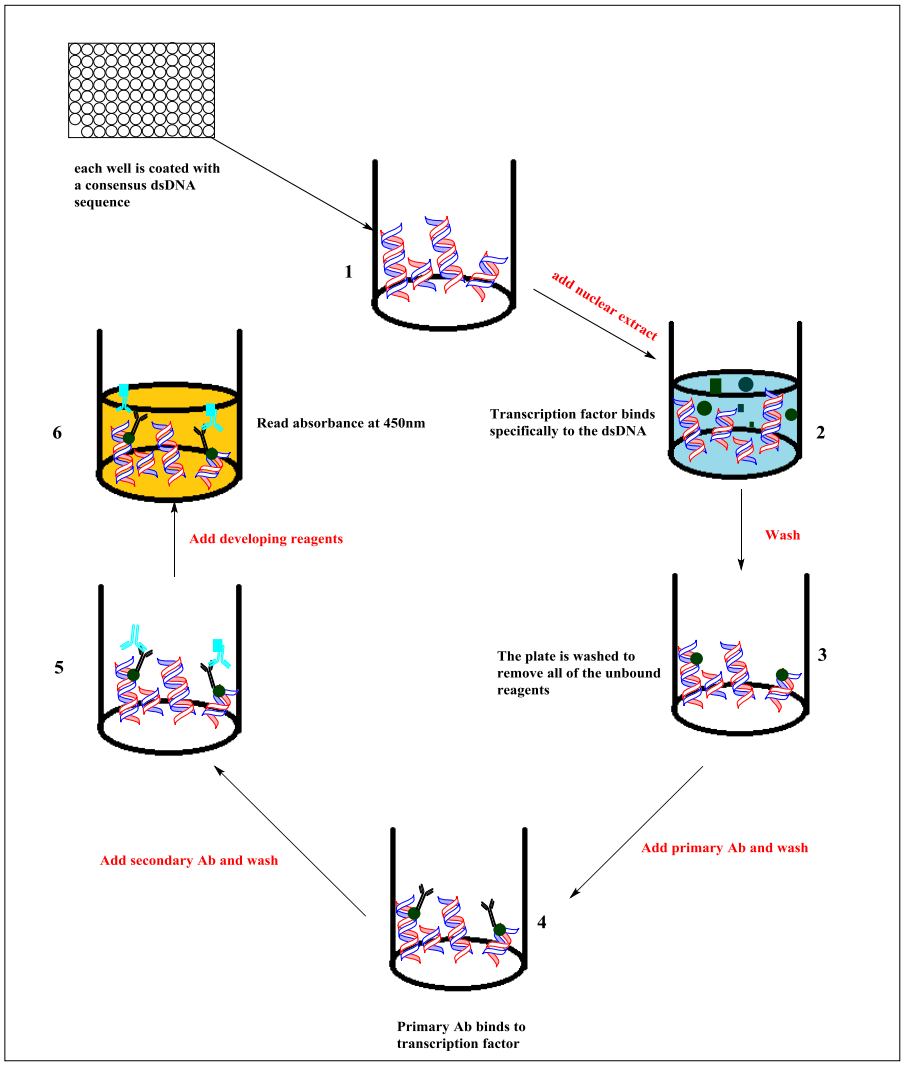


Figure 43. Schematic of the transcription factor binding assay

Each well was washed 5 times with required amount of wash buffer. To each pre well, added a given amount of diluted PPARα/γ primary antibody (except blank wells) and for one hour again incubated at room temperature. Again washing was repeated for five times for each well with required amount of wash buffer. A given amount of diluted secondary antibody was added to each well (except blank wells). The plate was again incubated for one hour at 37 °C. Again washing to

each well was done five times with wash buffer. Thereafter, added a developing solution to each well and for 30 minutes incubated with gentle agitation. At the end a stop solution was added in each well and with the help of ELISA plate reader the absorbance was measured at 450nm.[123]

There was dose-dependent non-linear increase in % agonist activity of test compounds with 0.1-100 µM concentrations to PPAR- γ receptor. The EC_{50} were calculated from EC_{50} calculator (https://www.aatbio.com/tools/ec50-calculator).[110] Results showed that compounds found to be potent agonist to PPAR-gamma receptor and having lower EC_{50} compared to pioglitazone (**Figure 44**, **Table 22**). Compounds P171, P172 and P173 found to be inactive. Compounds P152, P155, P158, P167 and P170 found to have better efficacy with EC_{50} of 0.988µM, 0.781µM, 0.07µM, and 2.68µM respectively. Whereas EC_{50} value of standard drug pioglitazone was found to be 32.38µM.

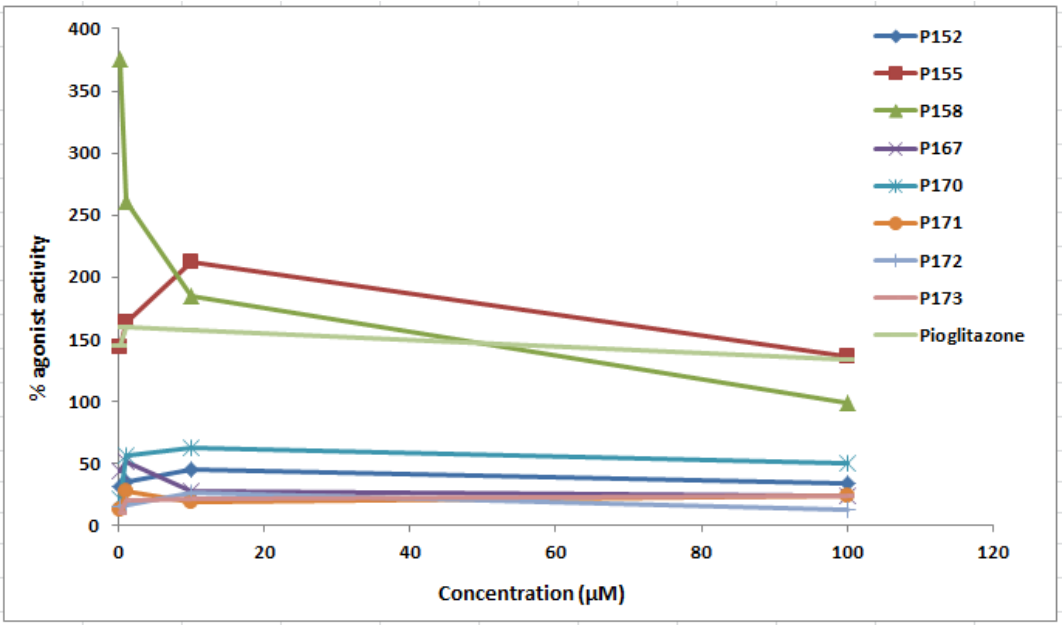


Figure 44. PPAR gamma agonist activity (at 0.1-100 µM concentrations of test compounds and pioglitazone).

Similar of PPAR-gamma assay, there was dose-dependent non-linear increase in % agonist activity of test compounds with 0.1-100 µM concentrations to PPAR- α receptor. The EC_{50} were calculated from EC_{50} calculator (https://www.aatbio.com/tools/ec50-calculator).[110] Results showed that compounds found to be highly selective to PPAR-α receptor than PPAR- γ and having lower EC_{50} compared to pioglitazaone (**Figure 45, Table 23**). Compound P173 found to be inactive. Compounds P155, P158, P167 and P170 were found to have better efficacy with the EC_{50} of 3.29

µM, 0.06 µM, 3.16, and 0.13 µM respectively. Whereas EC_{50} value of standard drug pioglitazone was found to be 38.03 µM.

Table 22: PPAR gamma agonist activity of test compounds

S.No.	Test compound	% agonist activity (at Concentration)*				EC_{50}
		0.1µM	1 µM	10 µM	100 µM	(µM)
1	P152	30.82±0.02	35.27±0.04	44.58±0.05	34.40±0.02	0.988
2	P155	143.61±1.11	163.13±1.45	211.63±2.22	136.48±1.35	0.781
3	P158	375.69±3.44	260.35±2.38	185.17±1.61	98.24±0.88	0.07
4	P167	44.29±0.08	51.24±0.10	27.96±0.04	23.95±0.02	7.39
5	P170	60.18±0.03	56.39±0.21	98.36±0.13	49.84±0.11	2.68
6	P171	12.99±0.01	27.77±0.02	19.32±0.01	24.29±0.04	NA
7	P172	15.33±0.01	16.61±0.01	26.72±0.02	12.87±0.01	NA
8	P173	10.29±0.02	20.25±0.03	21.61±0.03	23.51±0.02	NA
9	Pioglitazone	145.24±1.06	160.21±1.12	157.68±1.01	134.12±0.98	32.38

*All values are expressed as the mean±SEM and determinations were carried out in triplicate manner. NA = not active

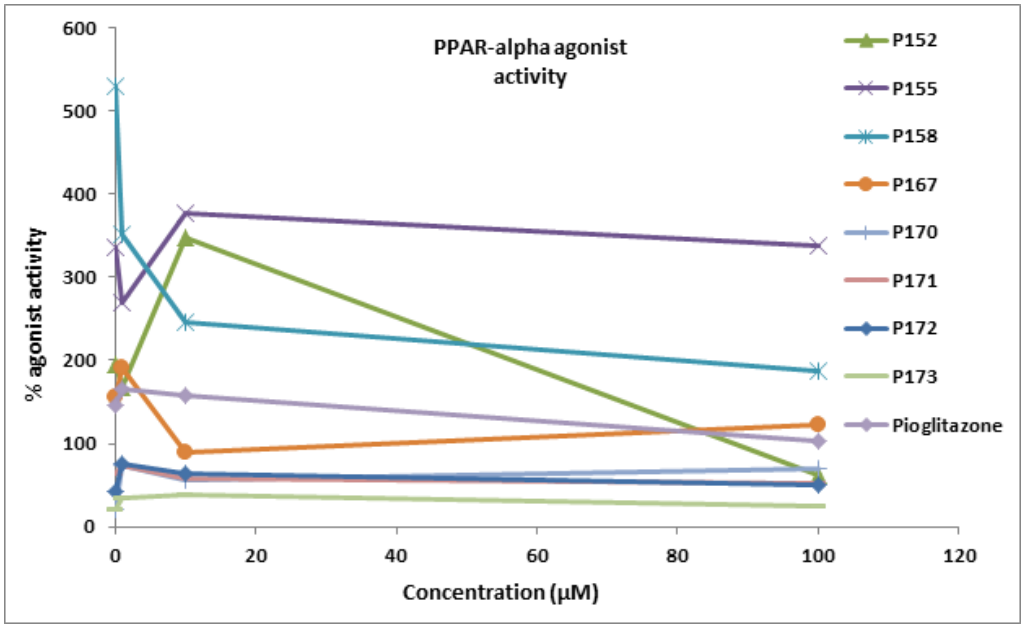


Figure 45: PPAR alpha agonist (activity at 0.1-100 µM concentrations of test compounds and pioglitazone).

Table 23: PPAR alpha agonist EC_{50} value of test compounds

S.No.	Test compound	% agonist activity (at Concentration)*				EC_{50}(µM)
		0.1µM	1 µM	10 µM	100 µM	
1	P152	195.76±3.12	167.74±1.55	346.48±3.33	61.73±0.44	34.57
2	P155	335.65±3.44	269.35±2.24	376.31±4.51	337.48±3.41	3.29
3	P158	529.67±4.45	350.45±2.81	245.08±2.11	186.47±2.01	0.06
4	P167	155.43±1.31	191.72±1.55	89.66±0.33	121.77±0.43	3.16
5	P170	20.99±0.01	73.29±0.11	56.07±0.31	69.36±0.21	0.13
6	P171	63.96±1.72	73.29±2.81	58.26±0.84	53.11±0.41	8.60
7	P172	42.94±0.81	75.71±0.27	63.28±0.29	49.63±0.11	47.13
8	P173	20.35±0.06	35.23±0.05	39.22±0.03	25.29±0.01	NA
9	Pioglitazone	145.23±0.78	166.45±0.76	157.68±0.53	102.23±0.34	38.03

*All values are expressed as the mean±SEM and determinations were carried out in triplicate manner. NA = not active

The analysis of both PPAR-gamma and PPAR-alpha assay reveals that compound P155 and 158 was found to be most potent on both receptors. Therefore for further *in-vivo* evaluation, we selected P158 and P155 whereas eliminating P170 which is highly selective on PPAR-alpha rather than PPAR-gamma receptor.

For rational and detail molecular interaction study, these two compounds were studied in detail using AutodockVina molecular docking software. The close contacts have shown for the selected potent compounds or ligands P155 at PPAR-alpha in **Figure 46, 47** whereas for PPAR-gamma in **Figure 48 and 49**. In **figure 50, 51** depicted of P158 at PPAR alpha receptor whereas **Figure 52, 53 and 54** depicted the interaction of P158 with PPAR gamma receptor. In **Figure 55, 56** shown the interactions of pioglitazone (standard drug) with both the PPAR receptors.

Binding interaction of PPAR- α with active isomer P155: Ring A (Phenoxy), NH of MET 220 forms H-bond with OH of carboxyl group, NN of THR279 forms H-bond with 'O'ofphenoxy ring and COOH of ring A forms hydrophilic interaction with TYR334, ASN219, ALA333. Whereas ring B (oxadiazole) situated in hydrophobic region (**Figure 46, 47**).

Binding interaction of PPAR-γ with active isomer P155: Ring A (Phenoxy): COOH of ring A

undergoes hydrophilic interaction with ARG542, LYS265 and Ring B (Oxadiazole) situated in hydrophobic region and shows interaction with ILE595, LEU584 (**Figure 48, and 49**).

Binding interaction of PPAR- *α* with active isomer P158: Ring A (Phenoxy): NH of TYR729 forms H-bond with 'O' of ring and COOH of ring A forms hydrophilic interaction with TYR334, MET220, ALA333. Whereas ring B (Oxadiazole) situated in hydrophobic region (**Figure 50, 51, and 52**).

Binding interaction of PPAR-*γ* with active isomer P158: Ring A (Phenoxy): NH of TYR725 forms H-bond with COOH of ring A , GLN540 form 2 H-bonds with "O" of phenoxy ring. COOH of ring A undergoes hydrophilic interaction with HIS266, LEU721 and Ring B (Oxadiazole) is situated in hydrophobic region and shows interaction with LEU707, LYS711 **(Figure 53, 54**).

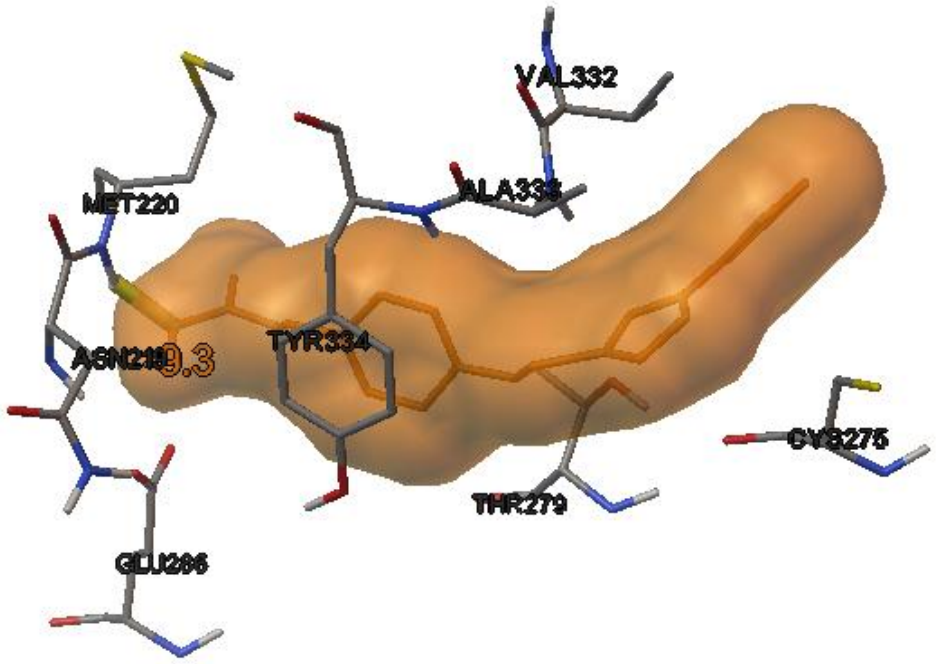


Figure 46: Overlay of hydrogen bonding between isomer: P155 and neighboring amino acid residues of PPAR-*α*.

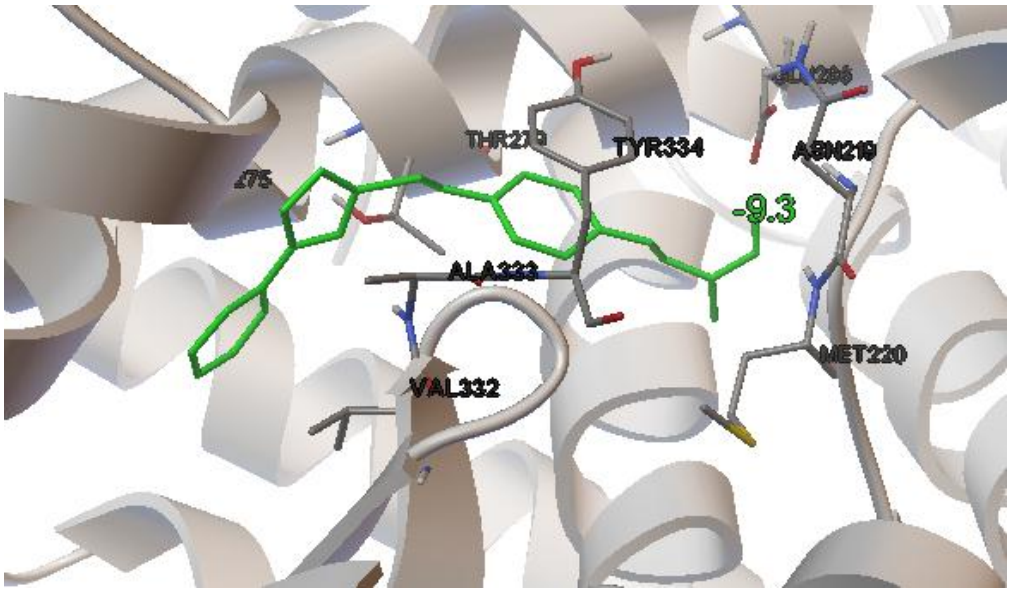


Figure 47: Overlay of close contacts of isomer: P155 with neighboring amino acid residues of PPAR-*α* protein

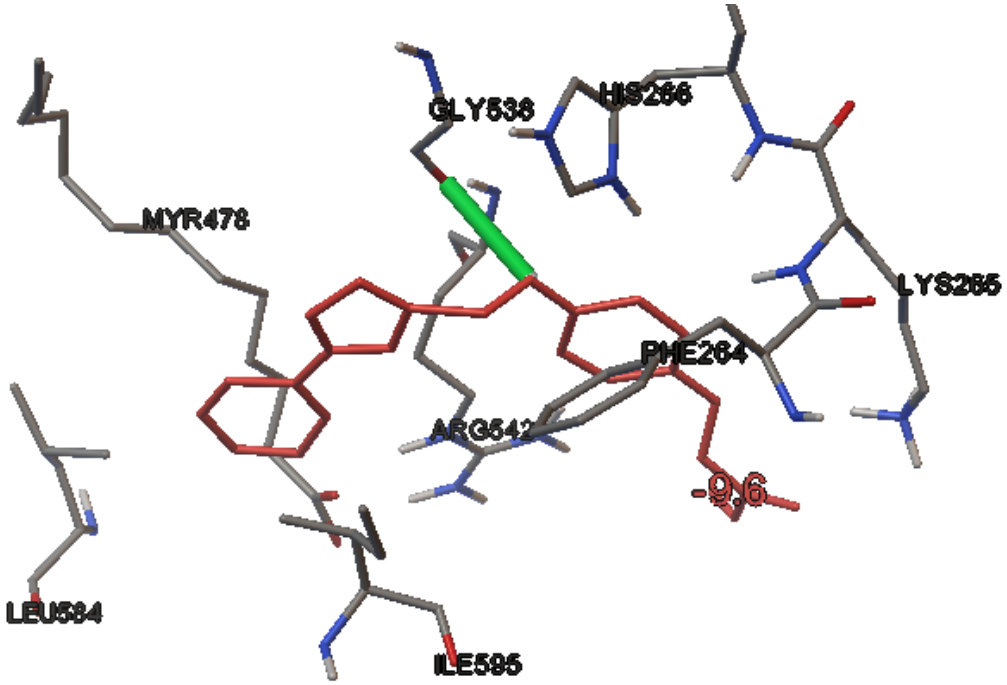


Figure 48: Overlay of close contacts and hydrogen bonding of isomer: P155 with neighboring amino acid residues of PPAR-*γ* protein

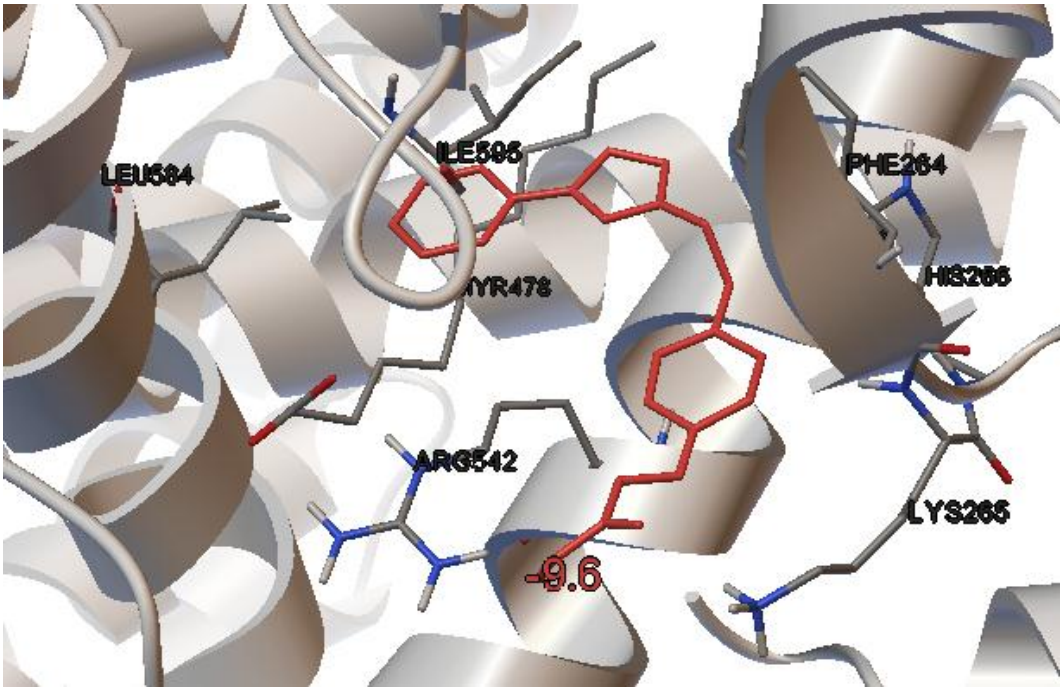


Figure 49: Visualization of active binding sites of protein (PPAR-γ) with bound ligand P155.

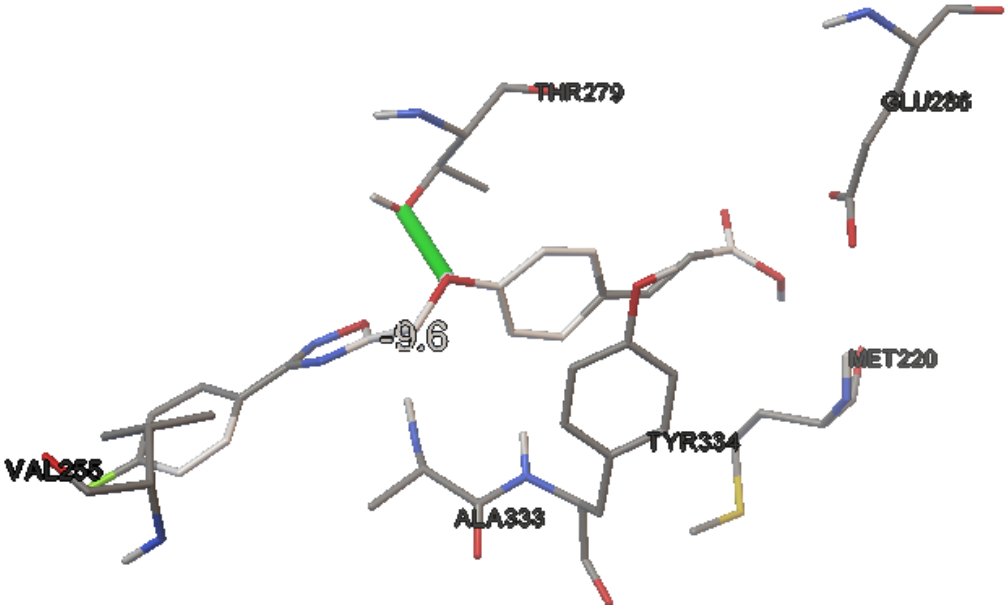


Figure 50: Overlay of hydrogen bonding between of isomer: P158 and neighboring amino acid residues of PPAR-α protein

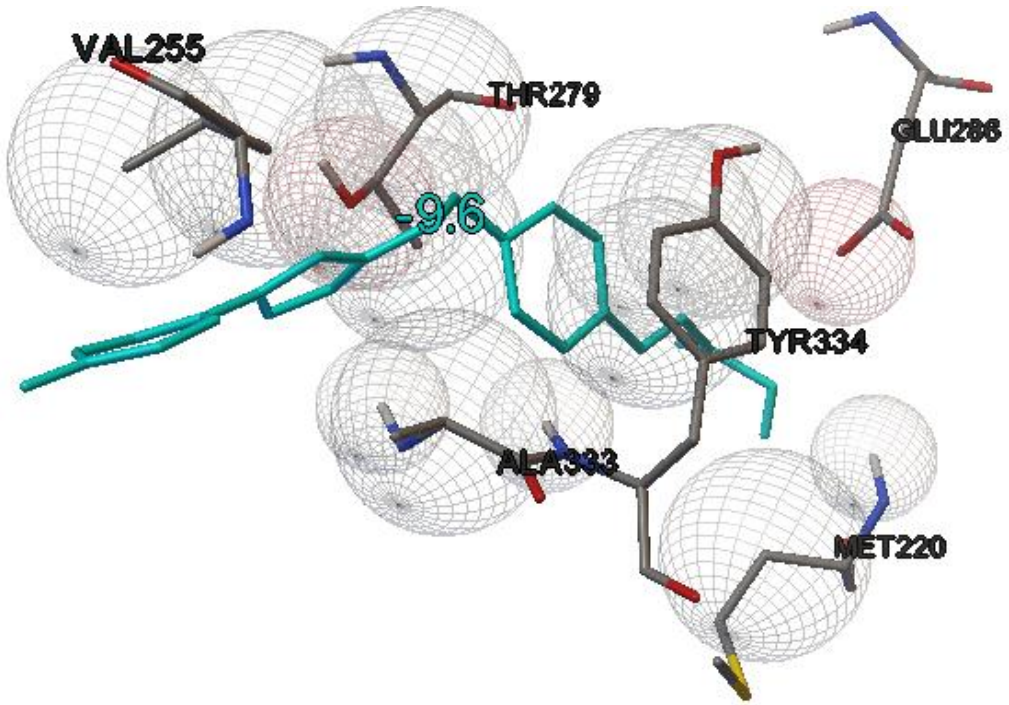


Figure 51: Overlay of close contacts of isomer: P158 with neighboring amino acid residues of PPAR-α protein

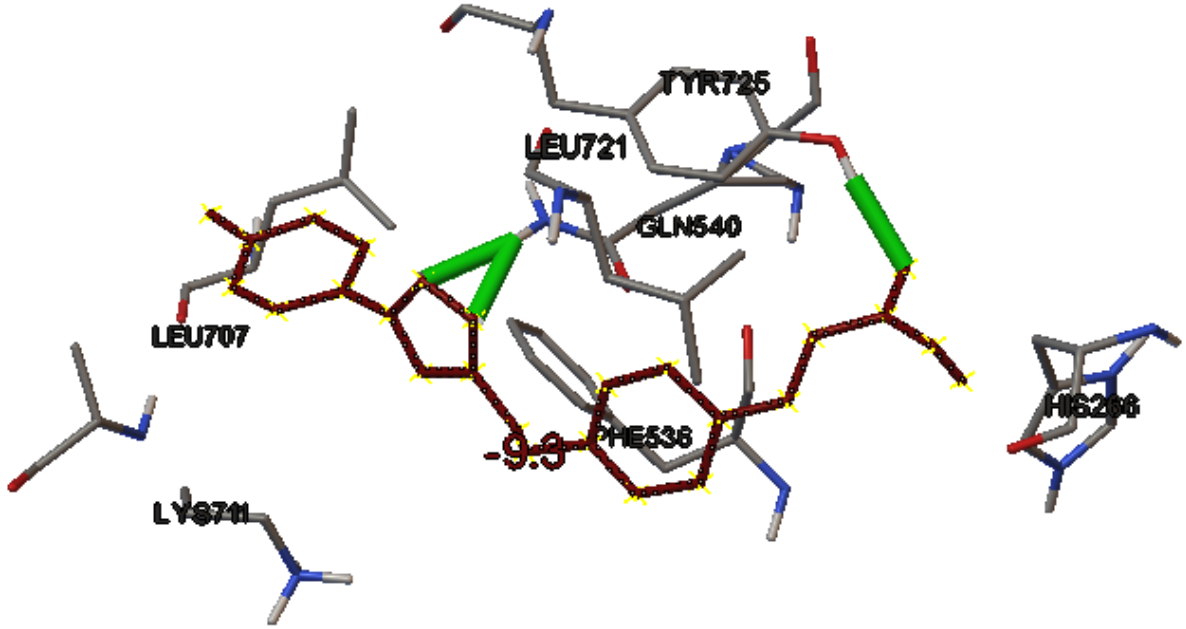


Figure 52: Overlay of hydrogen bonding between isomer: P158 and neighboring amino acid residues of PPAR-γ protein

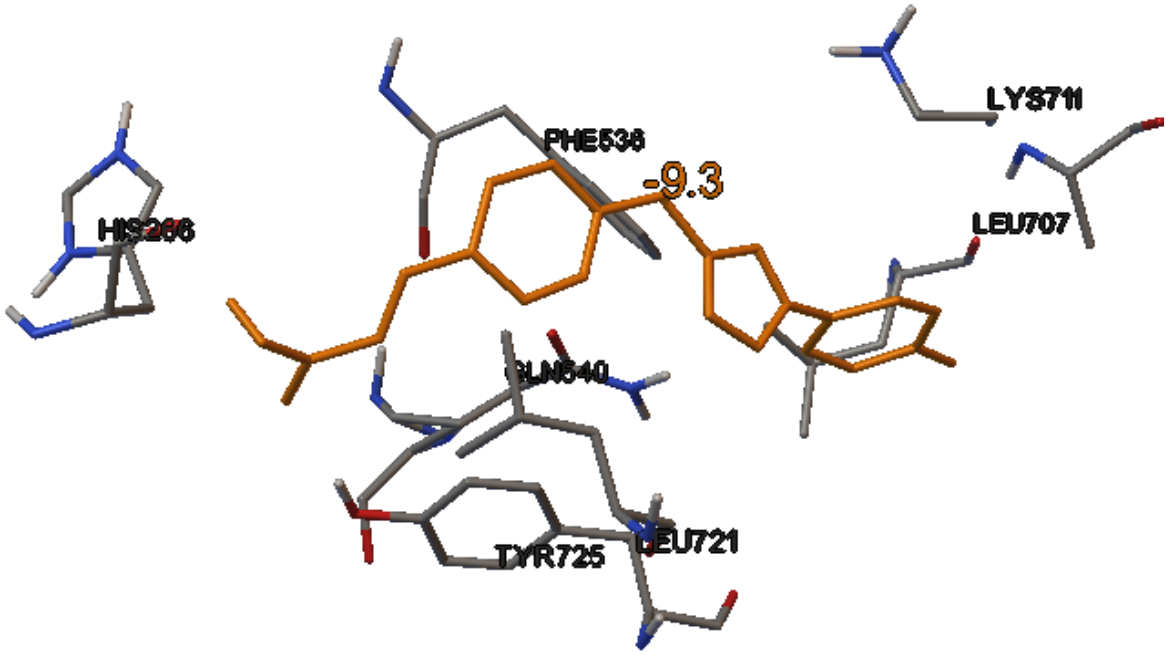


Figure 53: Overlay of close contact between isomer: P158 and neighboring amino acid residues of PPAR-γ protein

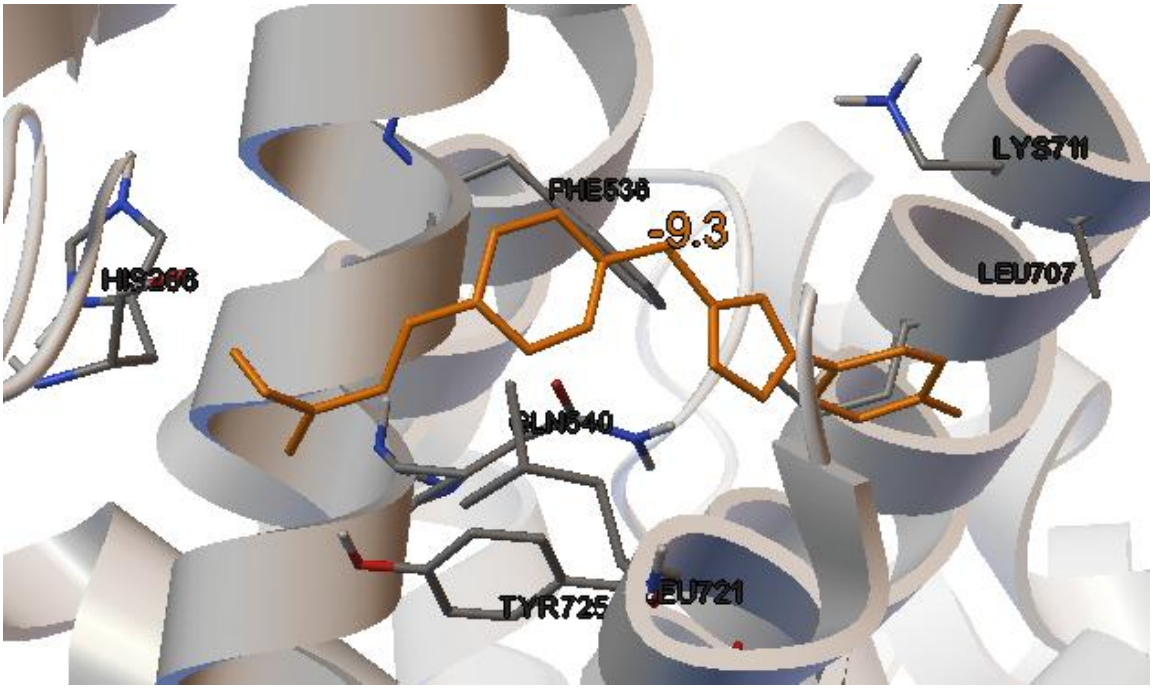


Figure 54: Visualization of active binding sites of protein (PPAR-γ) with bound ligand P158.

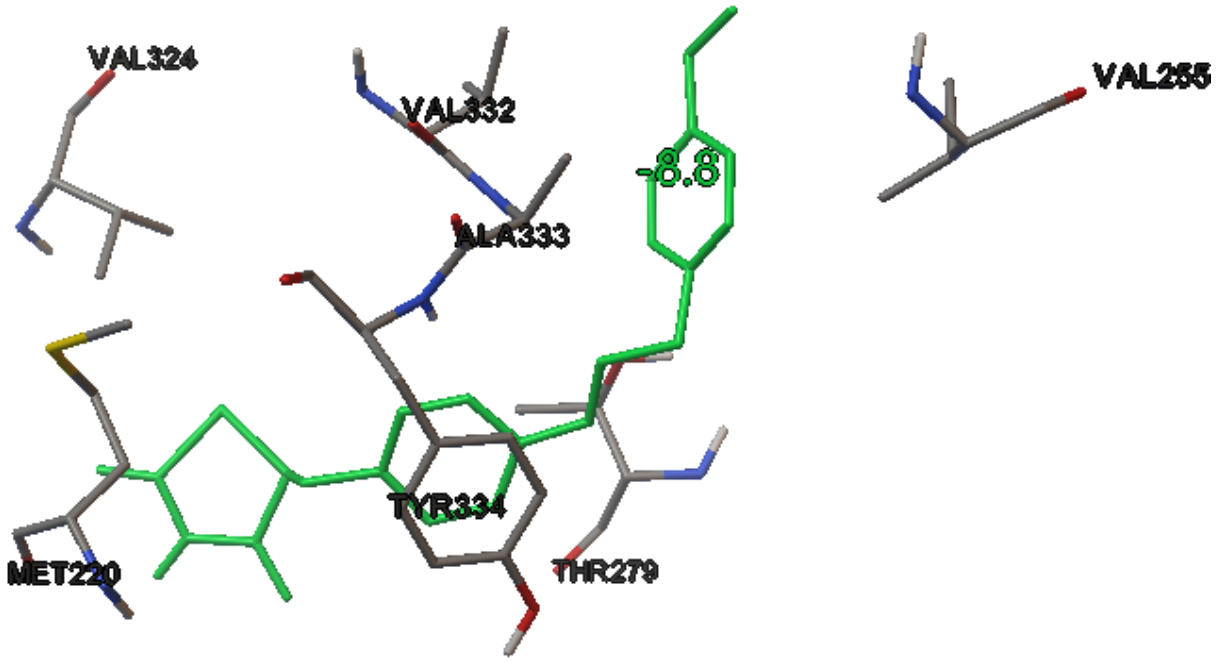


Figure 55: Overlay of close contacts of pioglitazone with neighboring amino acid residues of PPAR-*α* protein

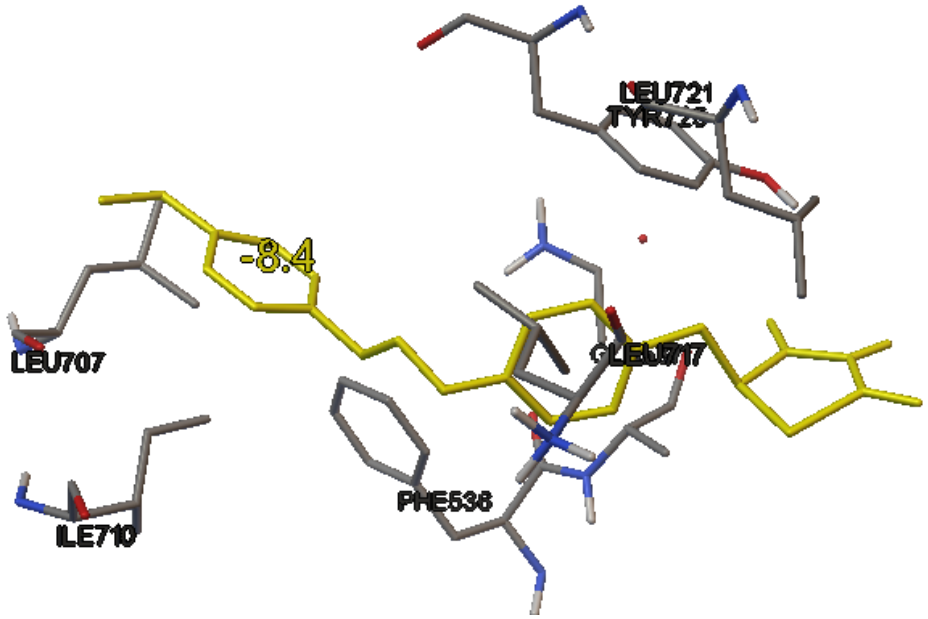


Figure 56: Overlay of close contacts of pioglitazone with neighboring amino acid residues of PPAR-*γ* protein.

6.6 *In-vivo* evaluation:

Sprague Dawley female rats (160-200g) were acquired from "NIPER, Mohali, India,"prior to the animal study, animals were permitted to acclimatize for 1 week under standard animal lab conditions, which include "polypropylene cages for accommodation , free rodent chow eating regimen and water ad libitum." Animals were kept up at 25±20°C and relative humidity of the room was 65 under 12 hour's light/dark cycle. The examination convention endorsed by "IAEC (LPU/IAEC/2018/ 30)," dated 14-07-2018**.** SD rats were divided into nine groups as per **Table 24** and treated as per approved protocol is given below (**Table 24**).

Table 24: Treatment protocol of diabetes-induced model

Group	**Group Name**	**Diet + Dose and route of drug treatment**	**No. of animals**
Group 1	Vehicle control	NPD + 0.5% CMC (p.o.) (Vehicle)	6
Group 2	Test compound P155 *per se*	NPD + 10 mg/kg of test compound P155 (p.o.)	6
Group 3	Test compound P158 *per se*	NPD + 10 mg/kg of test compound P158 (p.o.)	6
Group 4	Negative control	HFD + 35 mg/kg of STZ (i.p.)	8
Group 5	Positive control	HFD + 35 mg/kg of STZ (i.p.) +10 mg/kg of Pioglitazone (p.o.)	8
Group 6	Test compound P155 low dose treated	HFD + 35 mg/kg of STZ (i.p.) + 5 mg/kg of test compound P155 (p.o.)	8
Group 7	Test compound P155 high dose treated	HFD + 35 mg/kg of STZ (i.p.) + 10 mg/kg of test compound P155 (p.o.)	8
Group 8	Test compound P158 low dose treated	HFD + 35 mg/kg of STZ (i.p.) + 5 mg/kg of test compound P158 (p.o.)	8
Group 9	Test compound P158 high dose treated	HFD + 35 mg/kg of STZ (i.p.) + 10 mg/kg of test compound P158 (p.o.)	8

Female Sprague-Dawley rats were induced with Insulin resistance, type 2 diabetes by feeding high-fat diet (HFD) and administering a low dose of Streptozotocin (STZ) as per Srinivasan *et al*. 2005,[111] and Gupta *et al*., 2010[112]. "The rats were divided into two dietary regimens, either normal

pellet diet (NPD) or HFD (58% fat, 25% protein, and 17% carbohydrate, as a percentage of total kcal) *ad libitum*, respectively, for the initial period of 2 weeks (day 1 to 14). The composition and preparation of HFD are according to Gaikwad*et al.* (2010).[113] After the 2 weeks of dietary manipulation (on day 15), the rats from HFD-fed group was injected with a low dose of STZ (35 mg/kg; i.p.) and NPD-fed group was injected with 0.5% CMC (vehicle). One week after STZ injection i.e. on 22nd day, blood glucose was estimated and rats having non-fasting value above 250 mg/dl were identified and further divided into different treatment groups to receive varying as HFD + STZ (negative control group), HFD + STZ + pioglitazone; 10 mg/kg (positive control group), HFD + STZ + test compound P155 at 5 and 10 mg/kg (test compound 1 treatment groups) and HFD + STZ + test compound P158 at 5 and 10 mg/kg (test compound 2 treatment groups) for 1 week (day 22 to 28). Parallel to this treatment the NPD rats were further divided into different groups to receive varying treatment as NPD (vehicle control group), NPD + test compound-P155 at 10 mg/kg (test compound 1 *per se* group), NPD + test compound P158 at 10 mg/kg (test compound 2 *per se* group) for 1 week (day 22 to 28) Plasma glucose levels and body weight were evaluated on 1st day (before dietary modification), on 15th day, on 22nd day (after STZ or vehicle injection) and on 28th day. The respective treatments (pioglitazone, test compound-P155, and test compound-P158) to different groups were given orally every day from day 22 to 28. The rats were permitted to fed on their respective diets until the last day of study (28th day). Histopathology along with biochemical estimations of the pancreaswere carried out after sacrificing the animals on the 28th day."

6.6.1 Body weight estimation:

The body weights in grams of animals were recorded on the 1st day, 15th day, on 22nd day and on the 28th day (**Table 25**). No significant difference in body weight of rats among different groups was observed on day 1. After 2 weeks of dietary modifications, a substantial increase in body weight was noticed in rats on HFD (group 4 to 9), as compared to rats on NPD (vehicle control; group 1). Similarly, after 1 week of STZ/vehicle injection i.e. on day 22, a substantial rise in body weight was noticed in rats of group 4 to 9, as compared to rats of a vehicle control group of the respective day. On day 28 i.e. 1 week after respective drug treatments, a substantial rise in the body weight of rats from group 5 to 9 was observed, as compared to group 4 (negative control). The effect of different treatments on the weight of rats was shown in **Table 25** and **Figure 57**.

Table 25: Effect of different treatments on body weight (g±SD) of rats

Group	Group Name	Day 1	Day 15	Day 22	Day 28
Group 1	Vehicle control	204.66±15.77	208.66±10.34	211.66±5.56	212.66±5.34
Group 2	Test compound P155 *per se*	203.33±10.77	209.33±12.17	207±10	212±6.43
Group 3	Test compound P158 *per se*	196.66±14.77	200.66±10.54	205.45±10	208.44±5.45
Group 4	Negative control	180.12±13.66	230.45±7.09**	226.25±4.16*	225.25±3.91***
Group 5	Positive control	182.12±13.27	232.5±12.8***	234±6.25***	235±4.25$^{***, ##}$
Group 6	Test compound P155 low dose treated	182.37±14.31	229.37±5.22**	231.87±8.4**	236±6.18$^{***, ##}$
Group 7	Test compound P155 high dose treated	181.87±14.86	227±8.34*	230.75±7.6**	237.5±5.07$^{***, ###}$
Group 8	Test compound P158 low dose treated	198.75±15.5	233±10.69***	234.37±6.99***	240.5±4$^{***, ###}$
Group 9	Test compound P158 high dose treated	183.12±14.32	226.5±9.45*	231.12±5.24***	238.5±4.88$^{***, ###}$

Data represented as mean±SD. $^{*, **, ***}$ represents $p < 0.05$, $p < 0.01$, $p < 0.001$, respectively, when compared with vehicle control group; $^{\#, \#\#, \#\#\#}$ represents $p < 0.05$, $p < 0.01$, $p < 0.001$, respectively, when compared with negative control group.

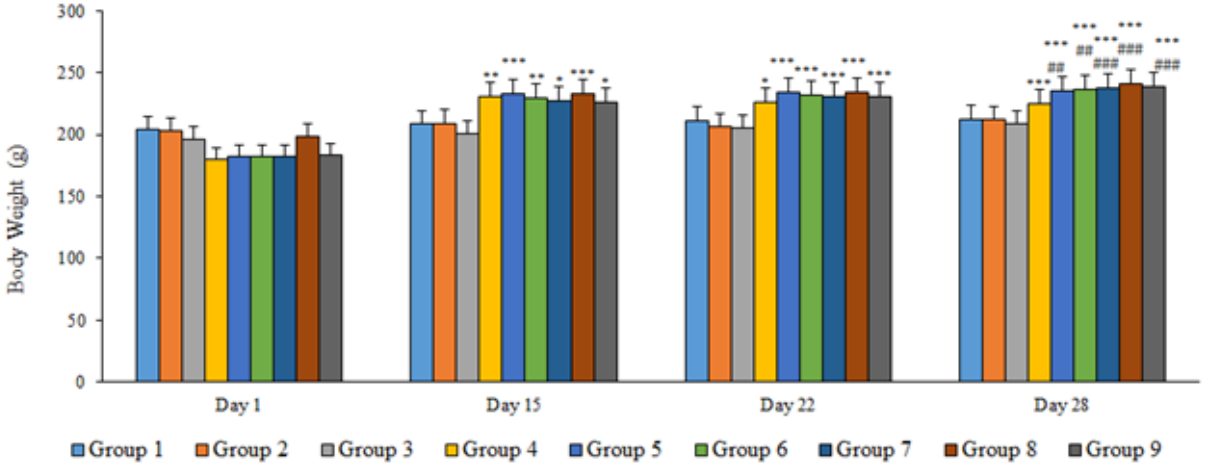


Figure 57: Effect of different treatments on body weight (g) of rats. Data represented as mean±SD. *, **, *** represents $p < 0.05$, $p < 0.01$, $p < 0.001$, respectively, when compared with vehicle control group; #, ##, ### represents $p < 0.05$, $p < 0.01$, $p < 0.001$, respectively, when compared with negative control group.

6.6.2 Biochemical parameters

6.6.2.1 Plasma glucose (PGL): It was evaluated on day 22 and 28 using Erba Glucose kit and method therein.[124]The plasma glucose levels (mg/dL) were compared on the 21st day and 28th day of study. The results revealed that the plasma glucose levels of group 4 to 9 (HFD+STZ treated groups) were significantly increased when compared to group 1 (vehicle control). After one week of respective treatments in group 5 to 9, plasma glucose levels were significantly reduced when compared to group 4 (negative control group). The effect of different treatments on plasma glucose levels of rats was represented in **Table 26** and **Figure 58**.

Table 26: Effect of different treatments on plasma glucose level (mg/dL±SD)

Group	Group Name	Day 22	Day 28
Group 1	Vehicle control	119.68±10.92	112.76±7.12
Group 2	Test compound P155 *per se*	132.15±8.76	124.84±9.46
Group 3	Test compound P158 *per se*	112.23±9.11	105.49±8.69
Group 4	Negative control	328.29±14.25***	355.6±15.43***
Group 5	Positive control	341.21±15.47***	183.68±12.23###
Group 6	Test compound P155 low dose treated	325.18±12.55***	171.52±9.36###
Group 7	Test compound P155 high dose treated	336.25±17.4***	169.39±14.85###
Group 8	Test compound P158 low dose treated	328.93±13.93***	180.97±14.63###
Group 9	Test compound P158 high dose treated	339.76±14.65***	173.43±10.69###

Data represented as mean±SD. *** represents p < 0.001, when compared with vehicle control group; ### represents p < 0.001, when compared with negative control group.

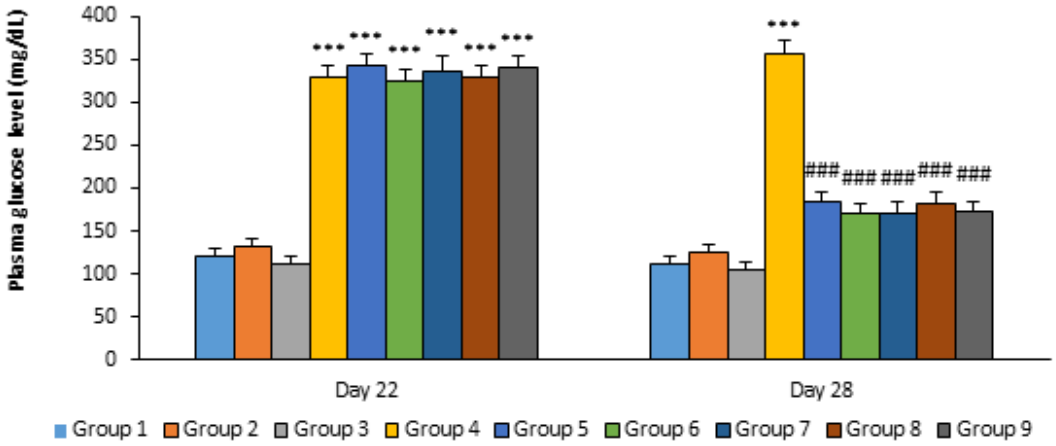


Figure 58: Effect of different treatments on plasma glucose (g/dL) level. Data represented as mean±SD. *** represents p < 0.001, when compared with vehicle control group; ### represents p < 0.001, when compared with negative control group.

6.6.2.2 Total cholesterol: It was evaluated by a commercially available kit from Eraba and method therein.[125] using 10µL plasma from the fasted animal. The total cholesterol levels were compared on the 21st day and 28th day of study. The results revealed that the total cholesterol levels of group 4 to 9 (HFD+STZ treated groups) were significantly increased when compared to group 1 (vehicle control). After one week of respective treatments in group 5 to 9, plasma cholesterol levels were significantly reduced when compared to group 4 (negative control group). The effect of different treatments on plasma cholesterol levels of rats was represented in **Table 27** and **Figure 59**.

Table 27: Effect of different treatments on plasma cholesterol levels

Group	Group Name	Day 22	Day 28
Group 1	Vehicle control	99.07±7.12	96.75±5.61
Group 2	Test compound P155 *per se*	112.5±8.44	100.92±7.85
Group 3	Test compound P158 *per se*	102.31±6.85	95.64±9.24
Group 4	Negative control	159.25±9.74***	190.5±8.94***
Group 5	Positive control	150.5±8.11***	161.34±9.93###
Group 6	Test compound P155 low dose treated	161.32±7.38***	163.31±8.66###
Group 7	Test compound P155 high dose treated	148.55±8.26***	155.5±8.23###
Group 8	Test compound P158 low dose treated	149.67±9.62***	157.23±9.2###
Group 9	Test compound P158 high dose treated	158.45±8.24***	166.45±7.38###

Data represented as mean±SD. *** represents $p < 0.001$, when compared with vehicle control group; ### represents $p < 0.001$, when compared with negative control group.

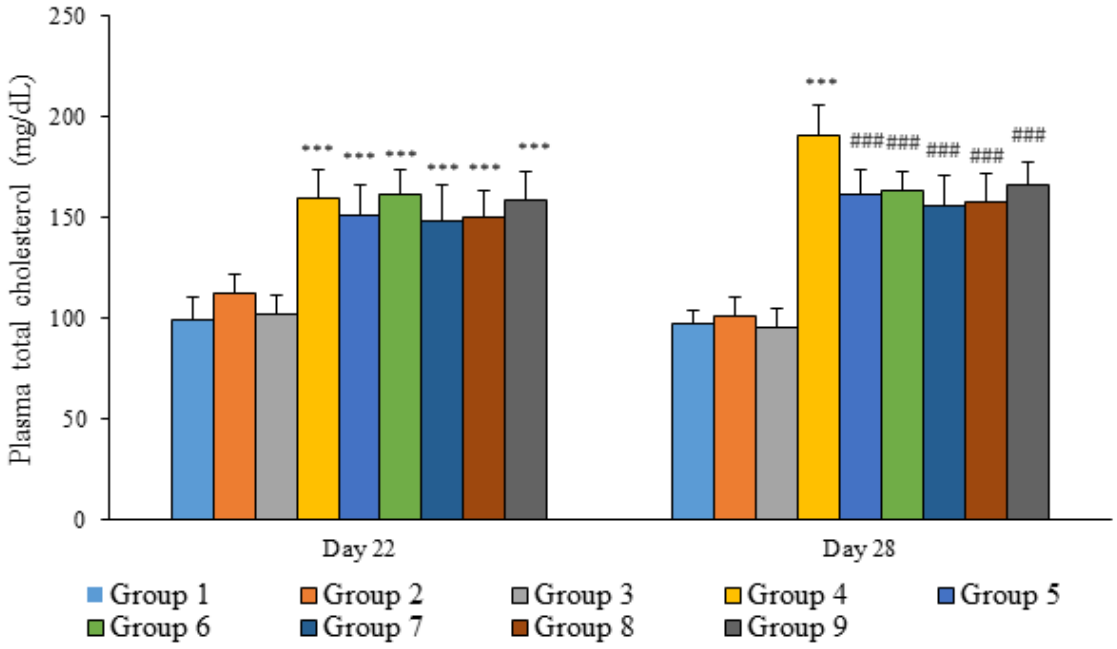


Figure 59: Effect of different treatments on plasma total cholesterol level. Data represented as mean±SD. *** represents $p < 0.001$, when compared with vehicle control group; ### represents $p < 0.001$, when compared with negative control group.

6.6.2.3 Oxidative biomarkers: at the end ofstudy on the 28th day of treatment, animals marked for biochemical studies were sacrificed by dislocating their cervical. Tissue homogenate was prepared by removing tissues and triturated them to make 10% (w/v) in 0.1 M phosphate buffer (pH 7.4).

After centrifugating them at 3000 rpm for 15 minutes, the clear supernatant was obtained, which was used to estimate the levels of TBARS, GSH, and CAT.

6.6.2.3.1 Estimation of thiobarbituric acid reactive substances (TBARS)[126]

TBARSis biomarker for the estimation of degradation of fats due to lipid peroxidation (Ohkawa*et al*., 1979).[127]"0.2 mL superficial floating liquid was taken intotest tube, followed by adding sodium dodecyl sulphate, acetic acid (pH 3.5), thiobarbituric acid and made up the volume with distill water and incubated for 1 h at 95 °C, cooled and distilled water (1 mL) was added followed by addition of *n*-butanol-pyridine mixture (15:1 v/v) and then, for 10min again centrifugation at 4000 gwas done and the pink color was developed for which the absorbance was measured (Shimadzu UV spectrophotometer 1240) at 532 nm. By using 1, 1, 3, 3-tetra methoxypropane,A calibration curve was prepared in the concentration range of 1-10 Nm" (**Figure 60**).

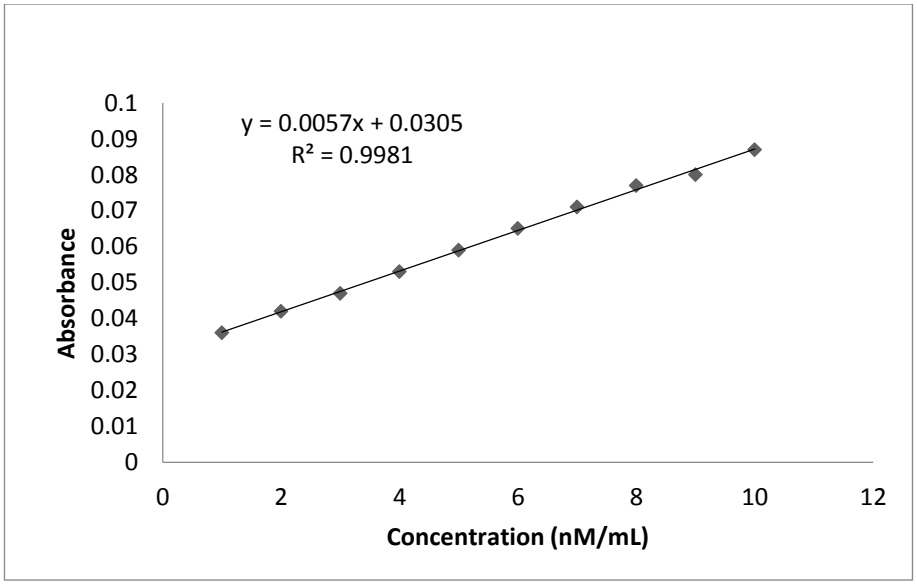


Figure 60: Calibration curve of 1, 1, 3, 3-tetramethoxy propane

The overall comparison for different groups by considering at the end of the study was also carried out. In a comparison of data by considering different days as a subject factor, no significant change in TBARS was observed in control group and placebo control group, whereas a significant increase is observed in the 4th group which is diabetic rats in the pancreas (**Table 28, Figure 61**).

Table 28: Effect of different treatments on TBARS levels in pancreas

Group	Group Name	TBARS (nM/mg protein)
Group 1	Vehicle control	14.59±2.6
Group 2	Test compound P155 *per se*	15.52±4.73
Group 3	Test compound P158 *per se*	11.72±0.53
Group 4	Negative control	36.75±3.5***
Group 5	Positive control	14.41±1.12###
Group 6	Test compound P155 low dose treated	18.04±1.33###
Group 7	Test compound P155 high dose treated	16.16±1.68###
Group 8	Test compound P158 low dose treated	18.04±2.67###
Group 9	Test compound P158 high dose treated	14.53±0.44###

Data represented as mean±SD. *** represents $p < 0.001$, when compared with vehicle control group; ### represents $p < 0.001$, when compared with negative control group.

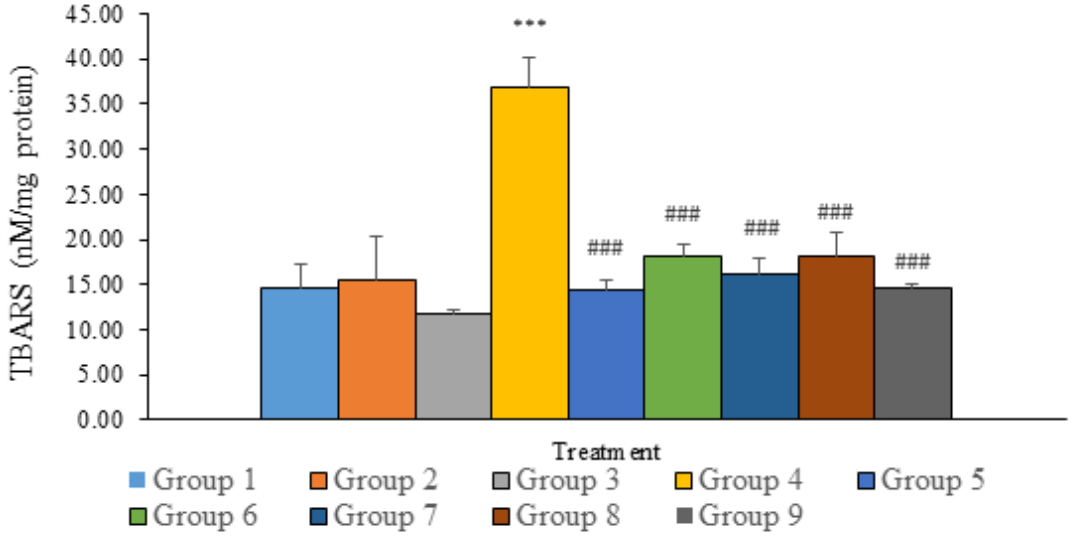


Figure 61: Effect of different treatments on TBARS levels in pancreas. Data represented as mean±SD. *** represents $p < 0.001$, when compared with vehicle control group; ### represents $p < 0.001$, when compared with negative control group.

6.6.2.3.2 Estimation of reduced glutathione (GSH)

Reduced glutathione is an essential tool for the antioxident system and lower value is an index of an increased susceptibility to oxidative stress(Beutler*et al*., 1963).[128] As described "In brief, the superficial floating layer of homogenate was mixed with trichloroacetic acid in 1:1 ratio in test tubes and centrifugation was carried at 1000 g at 4 ^{o}C for 10min. Then, Disodium hydrogen phosphate (0.3 M) was added to superficial floating layer. Then DTNB [5,5-dithiobis (2-nitrobenzoic acid)dissolved in 1% w/v sodium citrate] prepared freshly was added andat 412 nm,noted the absorbance spectrophotometrically. By using reduced glutathione,A standard curve was plotted (**Figure 62**)".

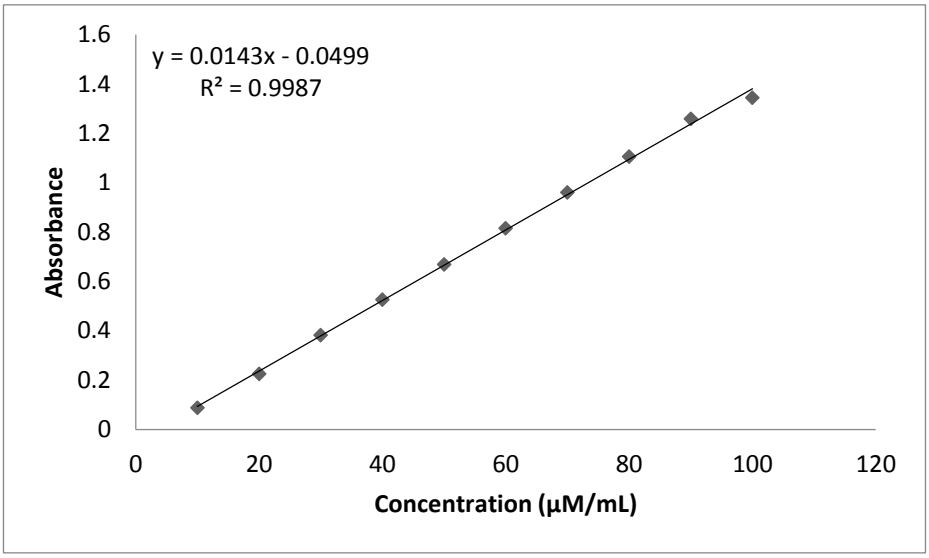


Figure 62: Calibration curve of reduced glutathione

The GSH levels were compared on the 28th day of study. The results revealed that the GSH levels of group 4 (HFD+STZ treated negative control groups) was significantly decreased as compared to group 1 (vehicle control). This decreased GSH level was significantly reversed in group 5 to 9 by respective treatment with standard and test drugs when compared to group 4 (HFD+STZ treated negative control groups). The effect of different treatments on GSH levels of rats was represented in **Table 29** and **Figure 63**.

Table 29: Effect of different treatments on GSH levels in pancreas

Group	Group Name	GSH (mM/mg protein)
Group 1	Vehicle control	0.507±0.019
Group 2	Test compound P155 *per se*	0.474±0.031
Group 3	Test compound P158 *per se*	0.498±0.033
Group 4	Negative control	0.213±0.027***
Group 5	Positive control	0.405±0.029###
Group 6	Test compound P155 low dose treated	0.542±0.031###
Group 7	Test compound P155 high dose treated	0.412±0.043###
Group 8	Test compound P158 low dose treated	0.531±0.04###
Group 9	Test compound P158 high dose treated	0.468±0.032###

Data represented as mean±SD. *** represents $p < 0.001$, when compared with vehicle control group; ### represents $p < 0.001$, when compared with negative control group.

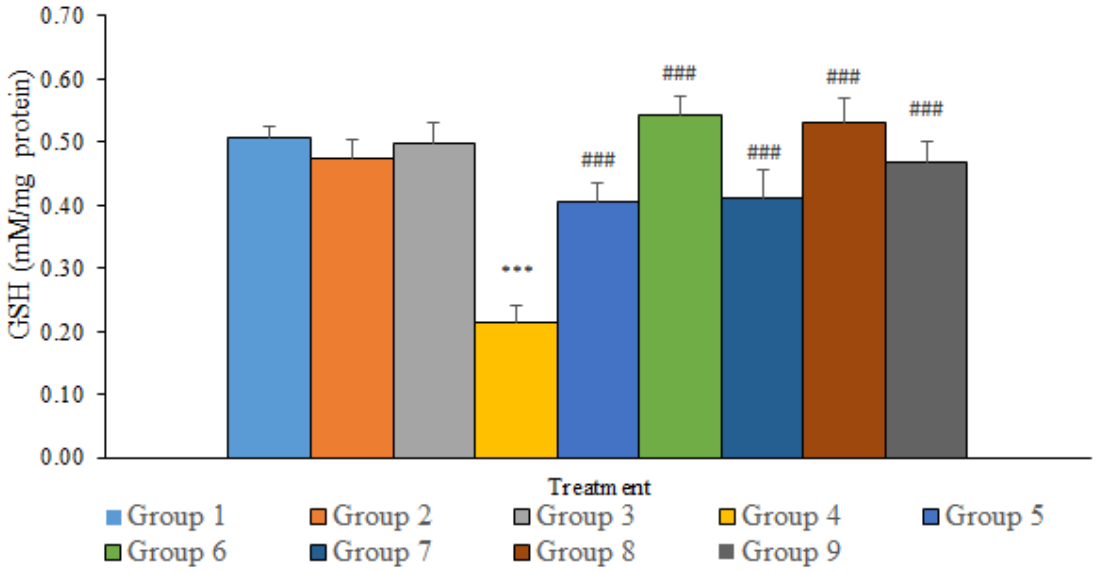


Figure 63: Effect of different treatments on GSH levels in pancreas. Data represented as mean±SD. *** represents $p < 0.001$, when compared with vehicle control group; ### represents $p < 0.001$, when compared with negative control group.

6.6.2.3.3 Estimation of catalase (CAT) activity

Catalase (CAT) is an enzyme mainly exists in living organisms and it functions by catalyzing the decomposition of oxidative product as hydrogen peroxide to water and oxygen leading to neutralization of reactive oxygen species (ROS). The CAT activity was estimated using the

methodof Aebi, 1974.[129] As described "In brief, to a 3.0 mL cuvette that contained phosphate buffer, the supernatant was added, to whichHydrogen peroxide was added and variations in absorbance were noted at 15-sec intervals for 30 sec at240 nm. By using the millimolar extinction coefficient of H_2O_2 (0.071 mmol cm^{-1}),the catalase activity was calculated.(Bisswanger, 2004)".[130]

Table 30: Effect of different treatments on CAT levels in pancreas

Group	Group Name	CAT (mM/mg protein)
Group 1	Vehicle control	0.81±0.0567
Group 2	Test compound P155 *per se*	0.85±0.0765
Group 3	Test compound P158 *per se*	0.83±0.0415
Group 4	Negative control	0.47±0.0329***
Group 5	Positive control	0.64±0.0256###
Group 6	Test compound P155 low dose treated	0.73±0.0438###
Group 7	Test compound P155 high dose treated	0.78±0.039###
Group 8	Test compound P158 low dose treated	0.76±0.0532###
Group 9	Test compound P158 high dose treated	0.82±0.0656###

Data represented as mean±SD. *** represents $p < 0.001$, when compared with vehicle control group; ### represents $p < 0.001$, when compared with negative control group.

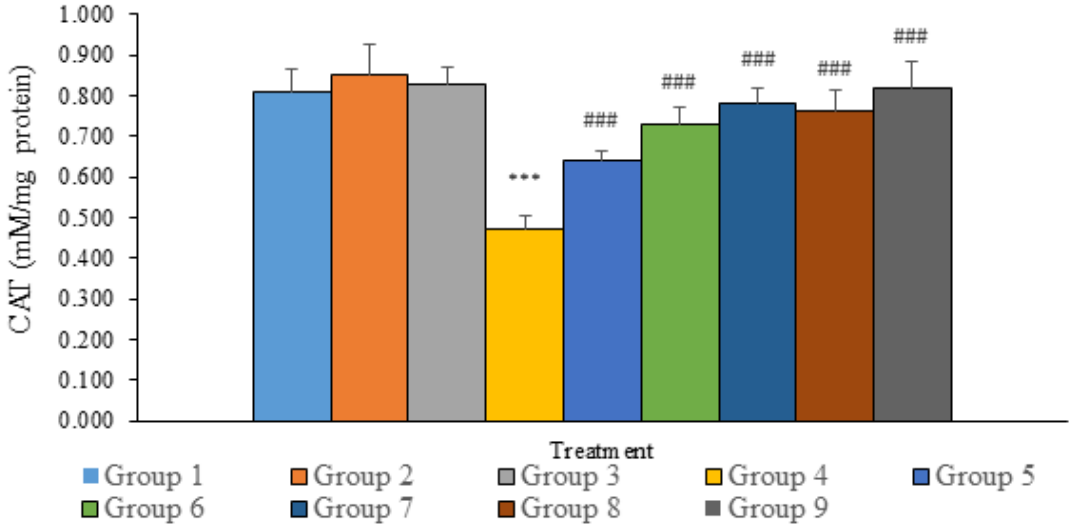


Figure 64: Effect of different treatments on CAT levels in pancreas. Data represented as mean±SD. *** represents $p < 0.001$, when compared with vehicle control group; ### represents $p < 0.001$, when compared with negative control group.

The CAT levels were compared on the 28th day of study. The results revealed that the CAT levels of group 4 (HFD+STZ treated negative control groups) was significantly decreased as compared to group 1 (vehicle control). This decreased CAT level was significantly reversed in group 5 to 9 by respective treatment with standard and test drugs when compared to group 4 (HFD+STZ treated negative control groups). The effect of different treatments on CAT levels of rat pancreases was represented in **Table 30** and **Figure 64**.

6.6.3 Histopathological evaluation:

Histopathologyofvarious tissue sections i.e. kidney, and pancrease were done at Gargi diagnostic Lab Jalandhar. All panels were stained with hematoxylin (H) &eosin (E) and magnified at 40X.

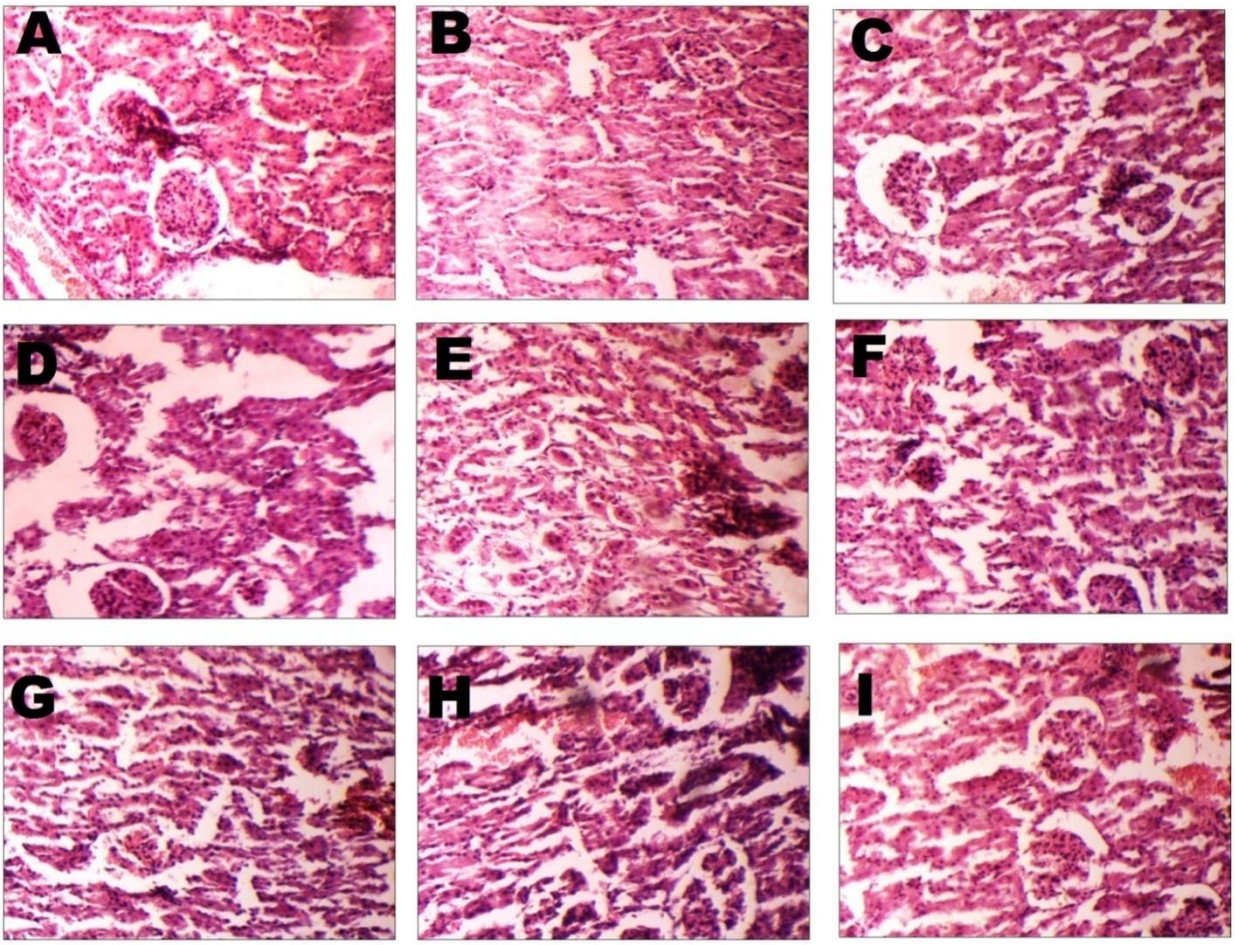


Figure 65: Histology of kidney on 28th day (A) NPD+vehicle, (B) NPD+P155, (C) NPD+P158, (D) HFD+STZ, (E) HFD+STZ+Pio, (F) HFD+STZ+P155(5 mg/kg), (G) HFD+STZ+P155(10 mg/kg), (H) HFD+STZ+P158(5 mg/kg). (I) HFD+STZ+P158(10 mg/kg).

Histology of the kidney showed in **Figure 65** which represent the of control rat with vehicle group 1 (A), Test compound P155 (10mg) *per se* group 2 (B), Test compound P158 (10mg) *per se* group 3 (C), were showing show normal appearance of the glomeruli (GL). Image (D) is the kidney of STZ-HFD-induced diabetic rat group 4, showing marked degeneration of the glomeruli (GL) with glomerular atrophies and severe vacuolations (VC). E, F, G, H, I were the kidneys of pioglitazone-induced diabetic rat group 5, Test compound P155 (5mg) group 6, Test compound P155 (10mg) group 7, Test compound P158 (5mg) group 8, and Test compound P158 (10mg) group 9 respectively, showing normal appearance of the glomeruli (**Figure 65**).

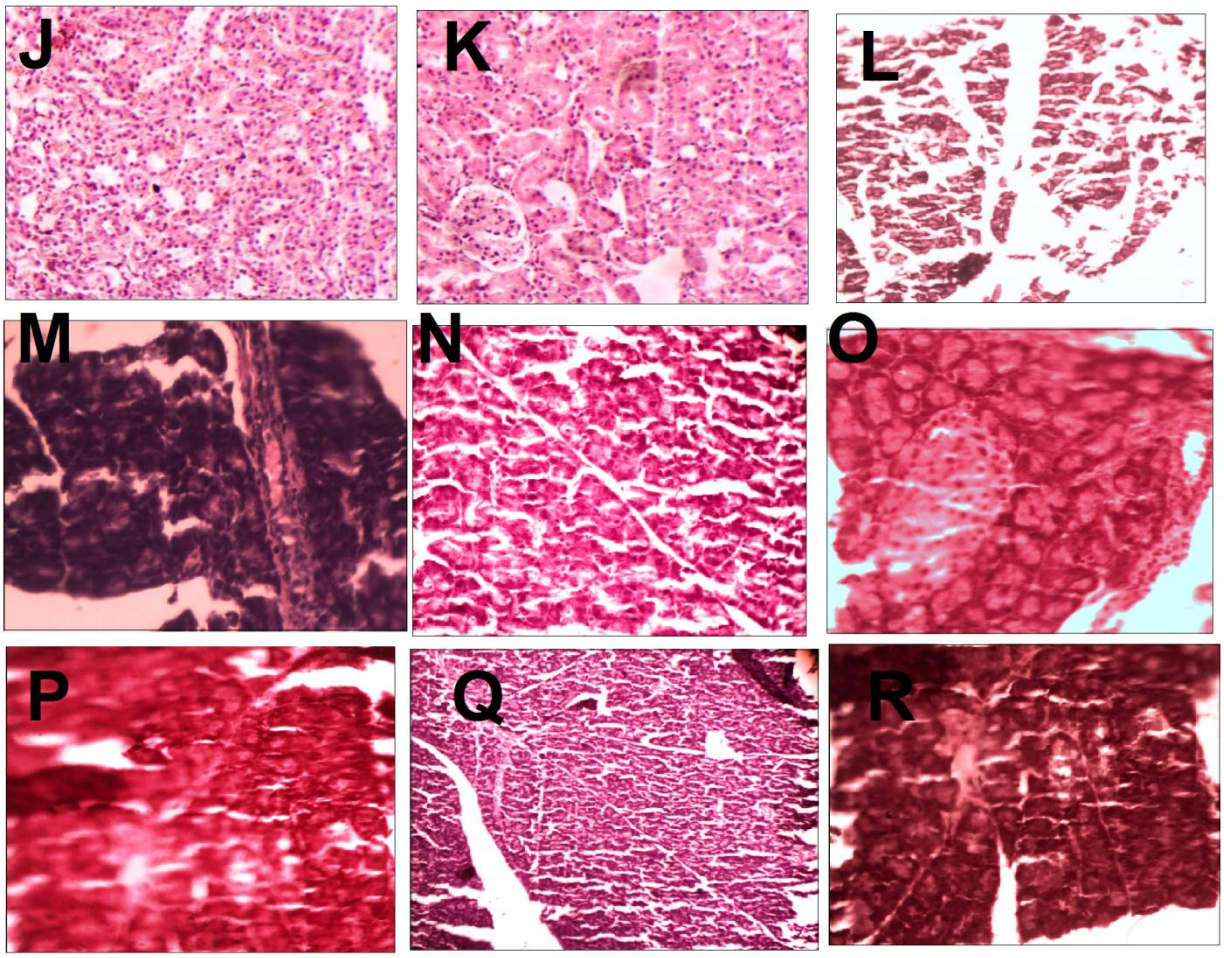


Figure 66: Histology of pancrease on 28th day (J) NPD+vehicle, (K) NPD+P155, (L) NPD+P158, (M) HFD+STZ, (N) HFD+STZ+Pio, (O) HFD+STZ+P155(5 mg/kg), (P) HFD+STZ+P155(10 mg/kg), (Q) HFD+STZ+P158(5 mg/kg), (R) HFD+STZ+P158(10 mg/kg).

Histology of pancreas was showed in **Figure 66**, it represent the control rat with vehicle group 1 (J), Test compound P155 (10mg) *per se* group 2 (K), Test compound P158 (10mg) *per se* group 3 (L), were showing normal, appearance of the islet of Langerhans (IL) located in the exocrine tissue (ET). Image (D) is the pancreas of STZ-HFD-induced diabetic rat group 4, showing marked degeneration of the Islets of Langerhans (IL). M, N, O, P, Q were the pancreas of pioglitazone-induced diabetic rat group 5, Test compound P155 (5mg) group 6, Test compound P155 (10mg) group 7, Test compound P158 (5mg) group 8, and Test compound P158 (10mg) group 9 respectively, showing normal appearance of the islets of Langerhans (IL).

Chapter 7 : Conclusions

Diabetes is one of the major concerns worldwide having various metabolic disorders. Drugs currently available in the market are associated with various side effects. From a detailed survey of the literature, it was concluded that to reduce toxicity and improve efficacy, a good ligand should possess a balanced affinity towards the PPAR *α/γ* receptor. So, a pharmacophore is proposed in which the acidic head group is linked with lipophilic aryl moeity through a linker which contains aryl polymethylene group, since compounds possessing polymethylene linker in between pharmacophore head and lipophilic tail showed PPAR*α* agonism, while those with an aryl phenylene linker showed dual PPAR*α/γ* agonism.

So, on the basis of these studies three classes are generated from the proposed pharmacophore as: oxadiazole, haydantoin, benzoxazole derivatives. Further, various derivatives were screened by preliminary molecular docking procedures with the help of Autodock vina software. So, on the basis of affinity score comparative to standard pioglitazone, eight actively docked compounds were selected i.e. P152, P155, P158, P163, P170, P171, P172, P173. Also *in-silico* toxicity profiles were performed on these selected compounds. This showed most of the compounds showed non-carcinogenecity and non-mutagenecity effect. Further, the *in-silico* ADME properties of the compounds were obtained as a calculated value for physicochemical properties, water solubility, lipophilicity, and pharmacokinetics. All compounds showed moderate solubility in water and suppose to have high GI absorption with no permeation to BBB. Calculated liphophilicity (iLogP) was found to be 0.92 to 3.19. On the basis of their binding affinity and toxicity profile, most feasible and potent compounds were synthesized because of their cost effectiveness and availability of starting material. Oxadiazole derivatives were prepared by coupling oximes with carboxylic acid after condensation, Hydantoin derivatives were prepared by coupling hydantoin with carboxylic acid after condensation and Benz-oxazole derivatives were prepared by alkylation of benzo[d]oxazol -2-amine. All the compounds synthesized in each series were analyzed and characterized by Melting point, TLC, IR, NMR, Mass.

Further, the *in-vitro* analysis of both PPAR-*γ* and PPAR- *α* assay revealed that there was dose-dependent non-linear increase in % agonist activity of test compounds with 0.1-100 µM concentrations to PPAR-*α* & *γ* receptor. The EC_{50} were calculated from EC_{50} calculator (https://www.aatbio.com/tools/ec50-calculator), which showed that compounds found to be potent

agonist to PPAR- γ receptor and having lower EC_{50} compared to pioglitazone. Compounds P171, P172 and P173 found to be inactive. Compounds P152, P155, P158, P167 and P170 found to have better efficacy with EC_{50} of 0.988µM, 0.781µM, 0.07µM, and 2.68µM respectively. Whereas EC_{50} value of standard drug pioglitazone was found to be 32.38µM. Results showed that compounds found to be highly selective to PPAR-α receptor than PPAR- γ and having lower EC_{50} compared to pioglitazaone. Compound P173 found to be inactive. Compounds P155, P158, P167 and P170 were found to have better efficacy with the EC_{50} of 3.29 µM, 0.06 µM, 3.16, and 0.13 µM respectively. Whereas EC_{50} value of standard drug pioglitazone was found to be 38.03 µM. Therefore, analysis of both PPAR- γ and PPAR- α assay revealed that compound P155 and 158 were found to be most potent on both receptors.

For rational and detail molecular interaction study, these two compounds were studied in detail using Autodock Vina molecular docking software. The close contacts were studied for potent compounds or ligands P155 and P158 at PPAR- α and PPAR- γ espectively and compared with the interactions of pioglitazone (standard drug) with both the receptors. Proposed compounds P155, P158 undergoes similar kind of hydrophobic and hydrophilic interactions as that of pioglitazone (standard drug) with common amino acid residues on both receptors (MET, ALA, GLU, VAL, TYR, THR).

For further *in-vivo* evaluation, we selected P158 and P155 to carry out their antidiabetic effect by using diabetic rat model. Both compounds were found to reduce the plasma glucose level significantly in diabetic rats compared to standard drug pioglitazone at 5mg/kg/day dose for 7days treatment despite of any body weight changes. These are also found to be effective in reduction in the total cholesterol level treatment. Various biochemical parameters in liver and kidney like TBARS, GSH and CAT were also observed to be normal after treatment with both compounds P155 and P158 as compared to negative control group (diabetic group). Histology of kidney and pancreas proved these compounds effective and reverse the tissue regeneration after treatment with P155 and P158 as compared to negative control group (diabetic group). Thus it can be concluded that P155 and P158 are the dual PPAR agonist found to be effective at 5 mg/kg/day in the significant reduction of plasma glucose level compared to pioglitazone which offers to be an alternative in better management of type 2 diabetes mellitus.

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Chapter 9: Annexures:

LOVELY PROFESSIONAL UNIVERSITY

Transforming Education Transforming India

School of Research Degree Programmes

LPU/SRDP/EC/170913/065

Dated: 13th Sep 2017

Paranjeet Kaur
Registration Number: 11512770
Program Name: Ph.D. (Pharmaceutical Chemistry) [Full Time]

Subject: Letter of Candidacy for Ph.D.

Dear Candidate,

We are very pleased to inform you that the Department Doctoral Board has approved your candidacy for the Ph.D Programme on 4th March 2017 by accepting your research proposal entitled: "DESIGN AND SYNTHESIS OF NOVEL OXADIAZOLE, HYDANTOIN AND BENZOXAZOLE DERIVATIVES AS POTENTIAL ANTIDIABETIC AGENT" under the supervision of Dr. Gopal L Khatik.

As a Ph.D. candidate you are required to abide by the conditions, rules and regulations laid down for Ph.D. Programme of the University, and amendments, if any, made from time to time.

We wish you the very best!!

In case you have any query related to your program, please contact School of Research Degree Programme.

HOS
School of Research Degree Programme

Jalandhar-Delhi G.T.Road, Phagwara, Punjab (India) - 144411
Ph : +91-1824-444594 Fax : +91-1824-240830 E-mail : drp@lpu.co.in website : www.lpu.in

LOVELY PROFESSIONAL UNIVERSITY

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Provisional Academic Transcript

Student Name	: Paranjeet Kaur	Programme	: PSD0-NNJ :: Doctorate of Philosophy in Pharmaceutical Chemistry
Registration no.	: 11512770		
Father's Name	: Mr. Baljeet Singh		
Mother's Name	: Ms. Bhajan Kaur	Mode : Regular	Date of initial registration : August,2016

TERM-I TGPA : 9.50 Equivalent percentage : 85.50 %

S.No.	Course	Credits	Grade
1	GEN703 RESEARCH METHODOLOGY	4	A
2	GEN709 WORKSHOP ON PROJECT WRITING	2	A+
3	GEN730 DATA ANALYSIS AND REPORTING	2	A+

This is a provisional transcript and is valid till a regular transcript is issued

Student yet not completed the programme.

CGPA : 9.50

Equivalent percentage : 85.50 %

Dy. Dean Examination
Lovely Professional University
Phagwara (Punjab)

Print Date: 15-02-2017

Place : Phagwara (Punjab)

REF0057/61

(CGPA Incharge)
Checked & Verified by

CGPA & Grades
Verified

Gargi Diagnostics Laboratory
An Associate of
MANN DIAGNOSTIC & RESEARCH CENTRE PVT. LTD.
S.C.O. 202/5, GUJRAL NAGAR, NEAR T.V. CENTRE, JALANDHAR CITY
Phones : 0181-2258034, 4618034 Mobile : 98158-09352
E-mail : gargidiagnosticslaboratory@gmail.com

For: Dr. Gopal Lal Khatik, Lovely Professional Univeristy, Phagwara (Punjab)-144411

Report on histology

Group	Kidney	Pancreas
1	Showing normal appearance of the glomeruli	Normal appearance of the islet of Langerhans located in the exocrine tissue
2	Showing normal appearance of the glomeruli	Normal appearance of the islet of Langerhans located in the exocrine tissue
3	Showing normal appearance of the glomeruli	Normal appearance of the islet of Langerhans located in the exocrine tissue
4	Showing marked degeneration of the glomeruli with glomerular atrophies	Showing marked degeneration of the Islets of Langerhans
5	Showing normal appearance of the glomeruli with low glomerular atrophies	Showing marked recovery to normal appearance of the islets of Langerhans
6	Showing normal appearance of the glomeruli with low glomerular atrophies	Showing moderate recovery to normal appearance of the islets of Langerhans
7	Showing normal appearance of the glomeruli with low glomerular atrophies	Showing moderate recovery to normal appearance of the islets of Langerhans
8	Showing normal appearance of the glomeruli with low glomerular atrophies	Showing moderate recovery to normal appearance of the islets of Langerhans
9	Showing normal appearance of the glomeruli with low glomerular atrophies	Showing moderate recovery to normal appearance of the islets of Langerhans

Facilities Available :
Fully Automated Immunoassay System (Chemiluminescence) for Thyroid, Fertility, TORCH, Cancer Markers, Hepatitis Markers & Drug Assays.
Fully Automated – Hematology Cell Counter, Fully Automated – Biochemistry & Electrolyte Analyser (also for Lithium), Elisa system for Elisa tests
* Quantitative Serology (CRP, ASO, RA) * Histo Pathology * Micro Biology

Dr. (Mrs.) Gargi Sharma
M.D.
Consultant Pathologist

CENTRAL ANIMAL HOUSE FACILITY (CAHF)

Lovely School of Pharmaceutical Sciences, Lovely Professional University

Ludhiana- Jalandhar G.T. Road, Phagwara (Punjab), 144402

Registration Number -954/ac/06/CPCSEA

CERTIFICATE

This is to certify that the project titled ***"Design, Synthesis and Evaluation of Novel Heterocyclic Scaffolds as Potential Anti-Diabetic Agents Targeted to PPAR α/γ Dual Agonist"*** has been approved by the IAEC.

Name of Principal Investigator: Dr Gopal Lal Khatik

IAEC approval number: LPU/IAEC/2018/ 30

Date of Approval: 14.7.2018

Animals approved: 66 rats

Remarks if any: -

Dr. Monica Gulati	**Dr. Navneet Khurana**	**Dr. Bimlesh Kumar**
Biological Scientist, Chairperson IAEC	Scientist, COD Pharmacology	Scientist In-Charge of Animal House, Member Secretary IAEC

Publications from current research work:

1. Kaur P. Khatik G.L. Lithium perchlorate catalyzed electrophilic activation: A convenient one-pot synthesis of trans-cinnamic acids. Letters in Organic Chemistry, 2018; 15(8): 688-692.
2. Khatik, G.L., Datusalia, A.D., Ahsan, W., Kaur, P., Vyas, V., Mittal, A., Nayak, S.K.. A Retrospect study on thiazole derivatives as the potential antidiabetic agents in drug discovery and developments. Current Drug Discovery Technologies, 2018;15(3):163-177.
3. Kaur, P., Mittal, A., Nayak, S.K, Vyas, M., Mishra, V., Khatik, G.L. Current strategies and future perspectives in the management of type 2 diabetes mellitus. Current Drug Targets, 2018;19(15):1738-1766.

Publications from allied research work:

1. Kaur, P., & Khatik, G. L. (2016). Advancements in non-steroidal antiandrogens as potential therapeutic agents for the treatment of prostate cancer. *Mini Reviews in Medicinal Chemistry2016;16(7):531-546.*
2. Kaur, P., Khatik, G.L. Identification of novel 5-styryl- 1,2,4-oxadiazole/Triazole derivatives as the potential antiandrogens. through molecular docking study. *Int. J. Pharm. Sci.,* 2016; 8(10):72-77.
3. Kaur, P., Khatik, G.L & Nayak, S.K. A Review on Advances in Organoborane-Chemistry: Versatile Tool in Asymmetric Synthesis. Current Organic Synthesis 2017; 14(5): 665 – 682.
4. Chaurasiya,S.; Kaur, P.; Nayak, S.K.; Khatik, G. L. (2016) Virtual screening for identification of novel potent EGFR inhibitors through Autodock Vina molecular modeling software. *J. Chem. Pharm. Res.,* 8(4):353-360.
5. Bhardwaj, S., Khatik, G.L., Kaur, P., Nayak, S.K. (2017) Computer aided drug design through molecular docking: identification of selective COX-2 inhibitors as potential NSAIDs. *J. Pharm. Res.,* 11(6): 604-608.
6. Kaur, K., Kaur, P., Mittal, A., Nayak, S.K., Khatik, G.L. (2017). Design and molecular docking studies of novel antimicrobial peptides using autodock molecular docking software. *Asian J. Pharm. Clin. Res.,* 2455-3891.

Abstract publication :

1. Kaur, P., Khatik, G. L. (2017) Computer aided drug design of pharmaceuticals : A n valuable tool in the drug discovery and development. *Med. Chem.(los angeles)*. DOI: 10.4172/2161-0444-C1-037.

Certifications/workshop/seminars:

1. Presented POSTER at 18th international conference on Targeted Drug delivery and Drug designing during December 06-08, 2017 at Dallas, Texas, USA. (Conference series and Editors of Journal of Medicinal Chemistry, Journal of Drug Designing)
2. Presented POSTER at international conference of pharmacy (ICP-2017). entitled as Lithium perchlorate catalyzed stereoselective synthesis of trans-α,β-unsaturated carboxylic acid. (LPU, Punjab).
3. Participated in "National Seminar Cum Workshop on pharmacovigilance" held on 26th March, 2016.
4. Participated in the PHYTOCON 2018. International Conference on "commercialization of Medicinal Plant Products: Lab Techniques to Trade"held on April 14, 2018.
5. Presented poster at 29th annual conference of Indian Pharmacy Graduates Association at LPU, Punjab. (*In-silico* molecular docking analysis of novel heterocyclic scaffolds as a potential androgen receptor modulator, Nov. 22-23, 2014)
6. Attended workshop on role of HPLC and Analytical Method Development in Dosage Form Design. (Feb 2-3, 2018). At LPU, Punjab.
7. Attended workshop on Design of experiments. At LPU, Punjab.

Certificates for honors/award or conference attended

Certificate No. 53411

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UNIVERSITY HONOR ROLL

As an appreciation for excellent academic performance

Paranjeet Kaur

D/o Mr. Baljeet Singh Student of Lovely Faculty of Applied Medical Sciences

Registration No. 11512770 of Programme Ph.D. (Pharmaceutical Chemistry) [FullTime] Batch of 2015

has been placed on University Honor Roll for Academic Session 2015-16

Date of Issue: 25-04-2017
Place : Phagwara (Punjab)

Prepared by (Admin. Officer-Records) Registrar Vice - Chancellor

International Conference of Pharmacy [ICP - 2017]

Society of Pharmaceutical Education & Research (SPER)

LOVELY PROFESSIONAL UNIVERSITY

Theme : "Pharmacists: Catalysts for Change"

April 7-8, 2017

Venue :- School of Pharmaceutical Sciences, Lovely Professional University, Phagwara [Punjab] India

Certificate

This is to certify that Prof./Dr./Mr./Mrs./Ms. PARANJEET KAUR

has successfully participated in the International Conference of Pharmacy [ICP - 2017]

as delegate and presented a poster on

LITHIUM PERCHLORATE CATALYSED STEREOSELECTIVE SYNTHESIS OF TRANS -A, B-UNSATURATED CARBOXYLIC ACIDS

Prof. (Dr.) S. H. Ansari, President, Society of Pharmaceutical Education & Research, Gwalior [M.P.] India

Dr. Upendra Nagaich, Secretary, Society of Pharmaceutical Education & Research, Gwalior [M.P.] India

Prof. (Dr.) Monica Gulati, Chairperson-LOC, Sr. Dean, LFAMS, Lovely Professional University, Phagwara [Punjab] India

Mr. Bimlesh Kumar, Organizing Secretary, Associate Professor, SPS, Lovely Professional University, Phagwara [Punjab] India

SPER Official Publication: Journal of Advanced Pharmaceutical Technology & Research [JAPTR]

Publication Partner: SPER Publications and Solutions Pvt. Ltd.

International Conference of Pharmacy [ICP - 2017]

Society of Pharmaceutical Education & Research [SPER]

LOVELY PROFESSIONAL UNIVERSITY

Theme : "Pharmacists: Catalysts for Change"

April 7-8, 2017

Venue :- School of Pharmaceutical Sciences, Lovely Professional University, Phagwara [Punjab] India

Certificate

This is to certify that Prof./Dr./Mr./Mrs./Ms. Paranjeet Kaur

has successfully participated in the International Conference of Pharmacy [ICP - 2017]

as delegate and presented a poster on

Lithium Perchlorate catalyzed stereoselective synthesis of trans-α,β-unsaturated carbonyl fatty acids

and won "Best Poster Presentation Award"

Prof. (Dr.) S. H. Ansari
President,
Society of Pharmaceutical Education & Research, Gwalior [M.P.] India

Dr. Upendra Nagaich
Secretary,
Society of Pharmaceutical Education & Research, Gwalior [M.P.] India

Prof. (Dr.) Monica Gulati
Chairperson-LOC
Sr. Dean, LFAMS,
Lovely Professional University, Phagwara [Punjab] India

Mr. Bimlesh Kumar
Organizing Secretary,
Associate Professor, SPS
Lovely Professional University, Phagwara [Punjab] India

SPER Official Publication
Journal of Advanced Pharmaceutical Technology & Research [JAPTR]

Publication Partner
SPER Publications and Solutions Pvt. Ltd.

Certificate of Recognition

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Conference Series and the Editors of Journal of Medicinal Chemistry, Journal of Drug Designing: Open Access and Journal of Pharmaceutical Sciences & Emerging Drugs wish to thank

Prof/Dr/Mr/Ms. **Paranjeet Kaur**

Lovely Professional University, India

for her Poster Presentation on

Computer Aided Drug Design of Pharmaceuticals: An valuable tool in the drug discovery& development

at the "18th International Conference on Medicinal Chemistry & Targeted Drug Delivery" held during December 06-08, 2017 in Dallas, USA

T. Takagi
Tatsuya Takagi
Osaka University, Japan

Certificate of Participation

This is to certify that Dr./Mr./Ms. Paranjeet Kaur has participated in **"National Seminar Cum Workshop on Pharmacovigilance"** held on 26th March, 2016 organized by IPGA Phagwara Chapter, School of Pharmaceutical Sciences, Lovely Professional University, Punjab.

Mr. Atul Nasa
President, IPGA

Dr. Monica Gulati
Chairperson, IPGA Phagwara Chapter

Mr. Bimlesh Kumar
Organizing Secretary

Certificate No. 91281

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PHYTOCON 2018

Certificate of Participation

This is to certify that Prof./Dr./Mr./Ms. Paranjeet Kaur has participated as **Delegate / Volunteer** in the **PHYTOCON 2018** - International Conference on **"Commercialization of Medicinal Plant Products : Lab Techniques to Trade"** held on April 14, 2018 organized by School of Pharmaceutical Sciences, Lovely Professional University, Punjab.

Date of Issue : 14-04-2018
Place of Issue: Phagwara (India)

Prepared by
(Administrative Officer-Records)

Dr. Meenu
Chairman, Registrations

Dr. Saurabh Satija
Organizing Secretary

Prof. Monica Gulati
Chairman - LOC

HUMAN RESOURCE DEVELOPMENT CENTER

[Under the Aegis of Lovely Professional University, Jalandhar-Delhi G.T. Road, Phagwara (Punjab)]

Certificate No. 73580

Certificate of Participation

This is to certify that **Ms. Paranjeet Kaur** D/o S. Baljeet Singh Randhawa participated in **Workshop on Role of HPLC and Analytical Method Development in Dosage Form Design** organized by Human Resource Development Center, Lovely Professional University from **February 02, 2018** to **February 03, 2018** and obtained **'B'** Grade.

Prepared by
(Administrative Officer –Records)

Head
Division of Human Resource

Head
Human Resource Development Center

Date of Issue: 03-02-2018
Place: Phagwara (Punjab)

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CERTIFICATE OF PARTICIPATION

This is to certify that Prof. / Dr. / Mr. / Ms. **Paranjeet Kaur**
has participated as a **Delegate** / **Resource person** in Conference on **"Design of Experiments"** jointly organized by **Qsutra** & **School of Pharmaceutical Sciences, Lovely Professional University**, Punjab on 13th - 14th July 2018.

Prof. Monika Gulati
Sr. Dean - LFAMS, LPU

Mr. Madhu Madhavan
Founder & CEO - Qsutra

Dr. Saurabh Satija
Organizing Secretary