DESIGN, SYNTHESIS AND BIOLOGICAL EVALUATION OF

PPARα/γ DUAL AGONISTS

2

2 Design, synthesis and biological evaluation of PPARα/γ dual agonists

As described in chapter 1 PPARs have an important role in managing metabolic disorders. During last two decades lot of work has been done for developing ideal PPAR agonists. There has been clear understanding of structural requirement for PPAR agonistic activity. Several PPARα, PPARγ and PPARα/γ dual agonists have been identifier and evaluated. However ideal PPAR agonist that can manage all the manifestation of metabolic disorder and have desirable safety profile is yet to be discovered. In the fore said context, attempt has been made in this work to develop a new class of dual agonists with distinct biological and safety profile consisting of a novel pharmacophore.

2.1 Common structural features of PPAR agonists

As reported by Agnes et al [1] typical PPAR agonists have following skeleton.

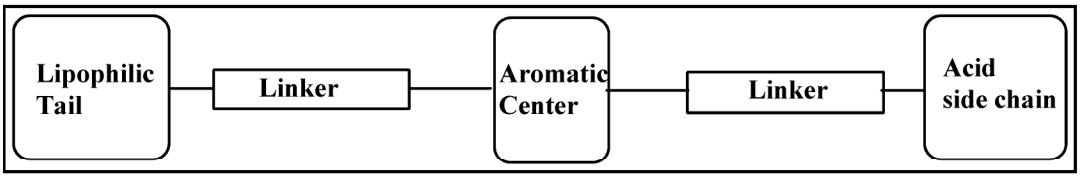


Figure 1 : Common structural features of PPAR agonists

As described in **Figure 1** typical PPAR agonists have lipophilic heterocyclic tail and acidic side chain with aromatic center in-between. The acidic side chain is connected with aromatic center with an alkyl spacer. In most cases aromatic center is phenyl ring. On one side aromatic center is connected with lipophilic heterocyclic tail and on the other side it is connected with acidic side chain. All these three are connected with linker chain having chain length of 1-4

atoms. Acidic side chain binds with receptor via H-bonds which ultimately is responsible for potency. Oxo-acetic acid, fibric acid, thiazolidinedione, α-alkoxyacids, oxybenzylglycine etc acts as acidic side chain in several PPAR agonist.

2.2 Novel PPAR dual agonists

We wished to design compounds with a novel pharmacophore by adopting above structural features mentioned in **Figure 1**. Modifications were done at lipophilic tail and acidic side chain position in newly designed molecules. Our goal was to discover the compounds with very high *in vitro* potency that translates to *in vivo* efficacy. We hypothesized that administration of very low dose of potent compounds would probably be a practical approach to minimize at least some of the adverse effects as these adverse effects exerted by this class of compounds are at high dose.

2.2.1 Modification at acidic side chain

2.2.1.1 Rationale for designing

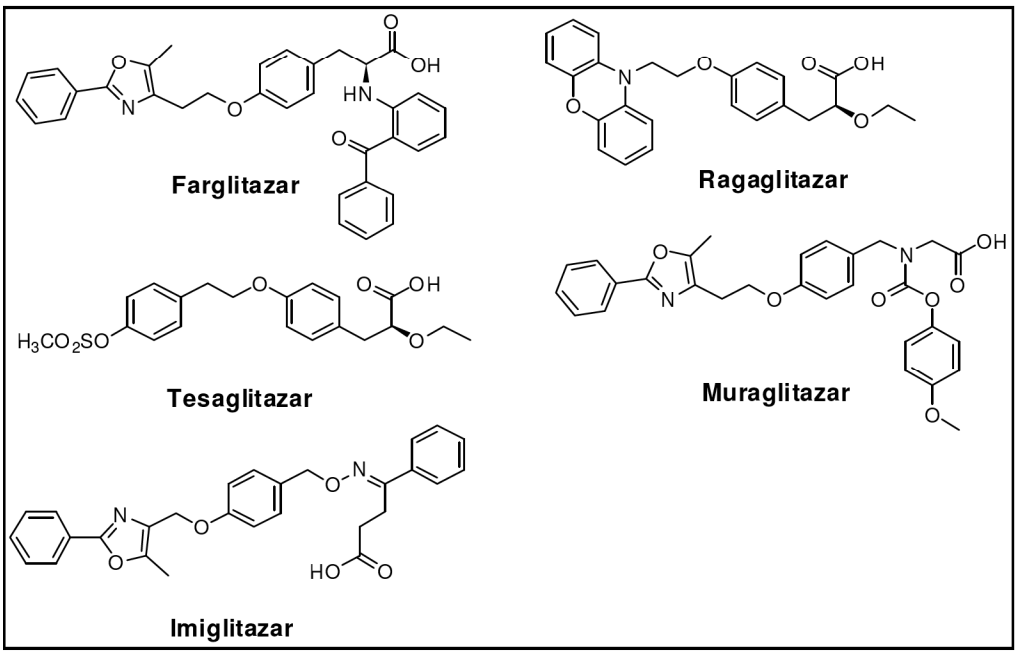


Figure 2 : Structures of PPARα/γ dual agonists having various acidic side chains.

Till date, a number of PPARα/γ dual agonists with structural diversity as well as diversity in ratio of PPARα/γ have been reported. Many of them had advanced up to clinical trials, unfortunately majority of these dual agonists were dropped. For example development of Farglitazar, Ragaglitazar, Tesaglitazar, Muraglitazar, Imiglitazar (**Figure 2**) was terminated due to undesirable adverse events and toxicity concerns [2-6].

For acquiring potency best possible approach was to modify acidic side chain keeping lipophilic tail constant. Acidic side chain is responsible for potency of the molecule because it can form hydrogen bonds with Serine, Tyrosine and Histidine of the protein [1]. α-Alkoxy-substituted propanoic acid is a potent binding motif for the PPAR receptors, which is present in clinically advanced

PPARα/γ dual agonists such as Ragaglitazar, Tesaglitazar, Naveglitazar [7]. These compounds have potency in nM range [8,9].

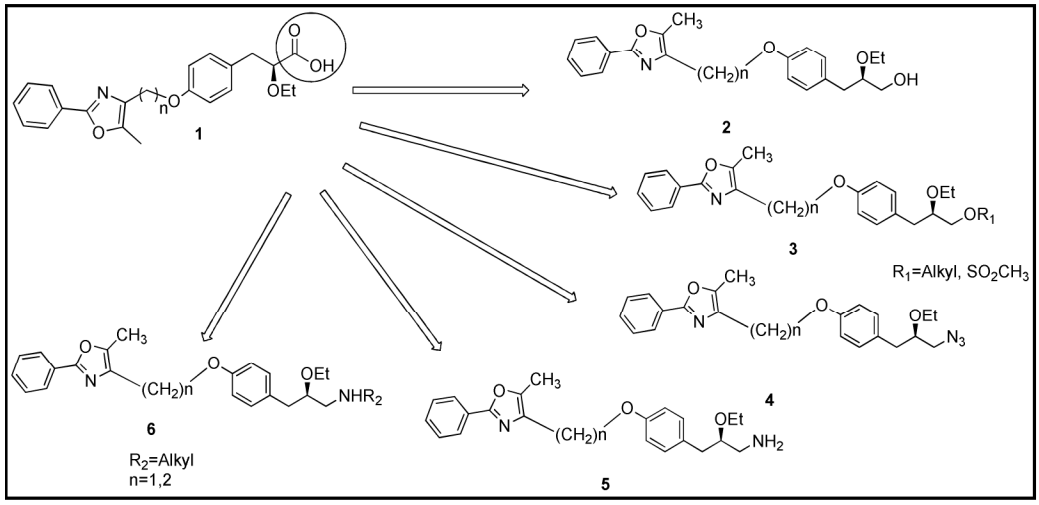


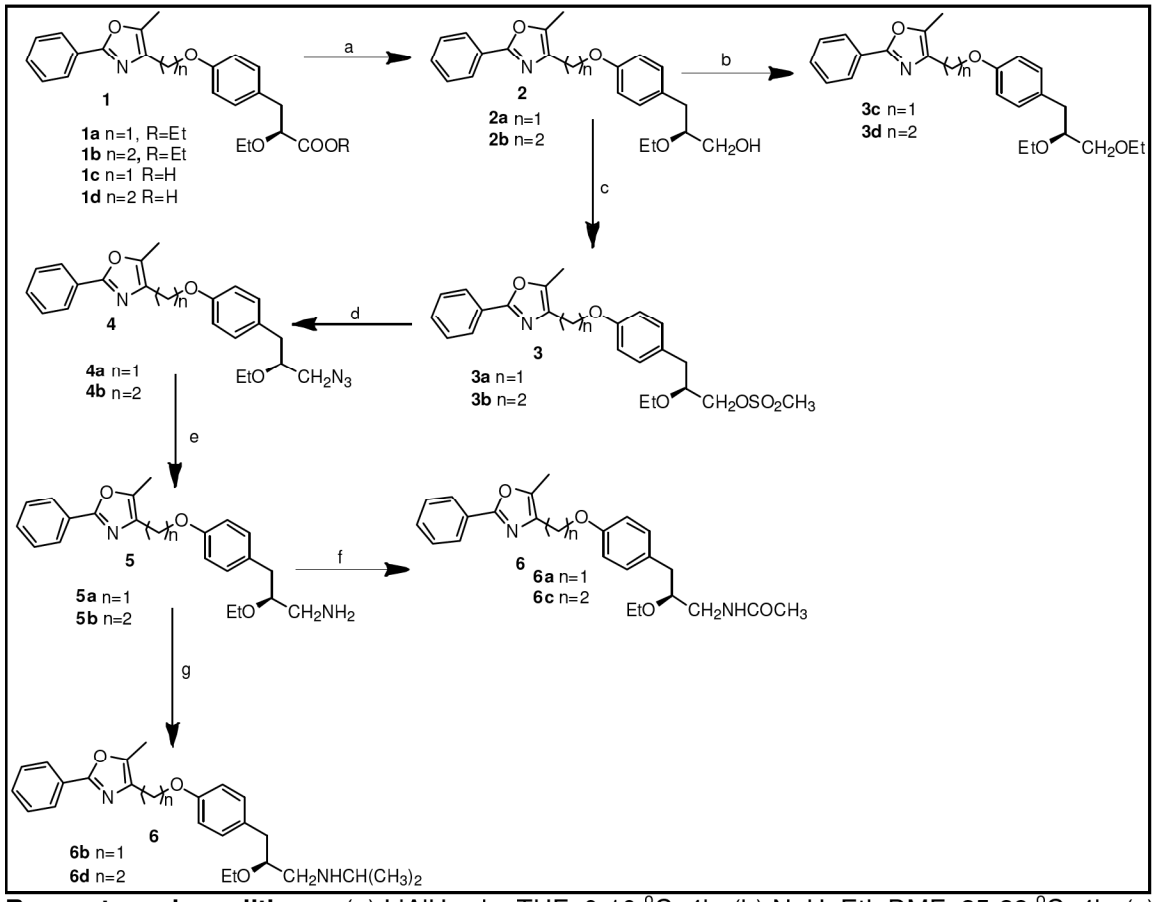
Figure 3 : Modifications at Acidic side chain

Oxazole containing α-ethoxy propanoic acid derivatives was chosen and oxazole at lipophilic tail was fixed. Different carbon chain linkers are explored in many PPAR agonists [10,11]. The planned modification at acidic side chain with different carbon chain linkers are depicted in **Figure 3.**

Few (α)-*(S)*-ethoxyphenyl propane derivatives [12] containing 2-phenyl-5-methyloxazole-4-ylalkoxy moiety were synthesized as per **scheme 1** and evaluated in PPARα and PPARγ transactivation assay in conjugation with *in vivo* studies in male Swiss albino mice (SAM) model.

Synthesis of phenylpropane derivatives

Scheme 1



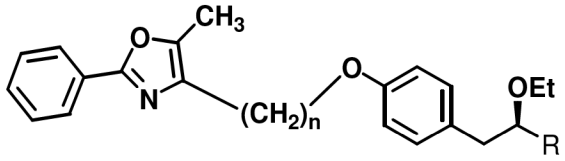
Reagents and conditions : (a) $LiAlH_4$, dry THF, 0-10 ^oC, 4h; (b) NaH, EtI, DMF, 25-28 ^oC, 4h; (c) Et_3N, CH_3SO_2Cl, CH_2Cl_2, 10 ^oC, 2h; (d) NaN_3, DMF, 100 ^oC, 6h; (e) Ph_3P, THF, 25–28 ^oC, 10 h, H_2O, 10h; (f) Et_3N, CH_3COCl, CH_2Cl_2, 25-28 ^oC, 3h; (g) NaH, $ICH(CH_3)_2$, DMF, 25-28 ^oC, 8h.

Ethyl-(2*S*)-ethoxy-3-[4-[2-(5-methyl-2-phenyl-oxazol-4-yl)alkoxy]-phenyl] propionates **1a-b** were synthesized by reacting 5-Methyl-2-phenyl-oxazol-4-yl alkyl methane sulfonate [13] with Ethyl-(2*S*)-ethoxy-3-(4-hydroxy-phenyl)-propanoate [14] in the presence of potassium carbonate in dry DMF. These esters were hydrolysed under aqueous conditions to yield the carboxylic acids **1c** and **1d**. The same esters **1a-b** were treated with lithium aluminum hydride in dry THF at 10 ^oC to yield corresponding alcohols **2a-b** which were either alkylated

with ethyl iodide in the presence of sodium hydride in DMF at 25-28 ^{o}C to obtain ether derivatives **3c-d** or treated with triethyl amine and methane sulfonyl chloride in dichloromethane at 10 ^{o}C to yield the compounds **3a-b**. The azide derivatives **4a-b** were obtained by treating the compounds **3a**-**b** with sodium azide in DMF at 100 ^{o}C which were reduced to amines **5a-b** by the treatment with Ph_3P in THF at 25-28 ^{o}C. The amine derivatives **5a-b** were converted either to amides **6a** and **6c** by reacting with suitable acyl chloride in the presence of triethyl amine in dichloromethane or substituted amines **6b** and **6d** by reacting with alkyl halide in the presence of sodium hydride in DMF (**scheme 1**).

2.2.1.2 Results and discussion

The newly synthesized compounds were screened for activity at human PPARα and PPARγsubtypes by using an established cell-based transactivation assay. Rosiglitazone (which showed 6.2 fold activation at 0.2 μM concentration) and Fenofibrate (which showed 4.4 fold activation at 10 μM concentration) were used as the reference standards for PPARγ and PPARα respectively in our studies. The Swiss albino mice with moderate hypertriglyceridemia were used for the assessment of plasma triglyceride (TG) lowering activity. The results are summarized in the **Table 1**.



(I)

Table 1 : *in vitro* hPPAR transactivation and TG lowering activity of compounds **1-6.**

Compound No.	n	R	hPPAR transactivation[a]		*in vivo* efficacy
			PPARα (10 μM)	PPARγ (0.2 μM)	% Reduction in TG[b]
2a	1	CH_2OH	IA	5.6	71
3a	1	$CH_2OSO_2CH_3$	IA	1.8	24
3c	1	CH_2OEt	5.6	2.8	78
4a	1	CH_2N_3	1.9	6.2	51
5a	1	CH_2NH_2	3.8	3.1	61
6a	1	$CH_2NHCOCH_3$	IA	4.2	27
6b	1	$CH_2NHCH(CH_3)_2$	IA	3.5	44
2b	2	CH_2OH	IA	5.0	57
3b	2	$CH_2OSO_2CH_3$	IA	1.0	10
3d	2	CH_2OEt	5.5	2.7	71
4b	2	CH_2N_3	1.5	6.0	67
5b	2	CH_2NH_2	2.1	3.8	54
6c	2	$CH_2NHCOCH_3$	IA	4.8	60
6d	2	$CH_2NHCH(CH_3)_2$	IA	3.9	24
1c	1	COOH	4.8	11.0	78
1d	2	COOH	5.2	10.6	74
Rosiglitazone			ND	6.2	IA
Fenofibrate (30 mg/Kg)			4.4	ND	28
Bezafibrate (300 mg/kg)					40

a.HepG2 cells were transfected with pSG5 expression vector containing the cDNA of PPARα or PPARγ and cotransfected with PPRE3-TK-luc. The Luciferase activity was determined using commercial firefly luciferase assay and α-galactosidase activity was determined in ELISA reader. Activities are presented by fold induction of PPARα and PPARγ activation. IA indicates inactive.

b. The test compounds were administered orally at a dose of 3 mg/kg/day (fenofibrate at 30 mg/kg/day) to male Swiss albino mice[10] for 6 days. Values (mean±SE) are the % change in plasma triglyceride (TG) concentration of the drug-treated mice relative to vehicle controls. All values are the mean of n=6.

Compounds **1-6** were evaluated for *in vitro* PPAR transactivation potential and subsequently administered orally to male Swiss albino mice at a dose of 3 mg/kg/day for six days and the reduction in plasma triglycerides (TG) was measured at the end.

The carboxylic acid derivatives **1c** and **1d** showed PPARα and PPARγ activity and were two fold more potent towards PPARγ than PPARα and showed excellent TG reduction (78% and 74% respectively). The hydroxy derivative **2a** and its homologue **2b** showed equipotent induction (5.6 and 5.0 respectively) in the PPARγ transactivation assay, whereas they were found inactive towards PPARα and exhibited 71% and 57% TG reduction respectively. When the hydroxy group in compounds **2a** and **2b** were sulfonylated with the bulky methane sulfonyl group the resulting respective compounds **3a** and **3b** lost both *in vitro* and *in vivo* activities. Interestingly the conversion of hydroxy compound **2a** and **2b** to their respective ethyl ether derivatives **3c** and **3d** made them potent PPARα compounds with poor PPARγ activity. Both these compounds were found two fold more potent towards PPARα than PPARγ and both compounds showed excellent triglyceride lowering (78% and 71% respectively) activity comparable to the lead compounds **1c** and **1d**. The azides **4a** and **4b** were found PPARγ selective compounds with moderate reduction in TG. The amines **5a** and **5b** found moderate and equipotent towards PPARα and PPARγ with 61% and 54% TG reduction respectively. The amides **6a** and **6c,** and the isopropyl amines **6b** and **6d** were found inactive towards PPARα and exhibited only PPARγ activity of the similar order. But surprisingly **6a** showed only 27% reduction in TG whereas

its homologue **6c** reduced TG to an extent of 60%. The isopropyl amines **6b** and **6d** exhibited moderate reduction in TG. The standard Fenofibrate showed 28% TG reduction at 30 mg/kg/day whereas Bezafibrate showed 40% TG reduction at a dose of 300 mg/kg/day when administered for 6 days in male Swiss albino mice. Rosiglitazone did not show any significant TG reduction in this model. These results showed that derivatives of (2*S*)-ethoxy-3-[4-[2-(5-methyl-2-phenyl-oxazol-4-yl)-alkoxy]-phenyl]-propanoic acid such as alcohols, ethers, amines, azides, amides etc were useful to modulate the activity towards PPARα and PPARγ subtype and were found to be excellent hypolipidemic agents, which lowered plasma triglycerides to a very significant extent. A most interesting finding is that hydroxy compounds (**2a** and **2b**) were found active towards PPARγ with no activity towards PPARα, whereas the ethyl ether derivatives **3c** and **3d** were two fold more potent towards PPARα than PPARγ. On contrary to these results the parent acids (**1c** and **1d**) had two fold potent activity towards PPARγ than PPARα. These results clearly demonstrate that minor chemical modifications in the functional region of the compounds lead to significant changes in the *in vitro* activity even though they exhibit a similar *in vivo* profile. Compounds **3c** and **3d** which showed the desired predominant PPARα activity were selected as initial lead compounds for further development as hypolipidemic agents.

2.2.1.3 Conclusions

My objective was to increase potency of newly designed series. Acidic side chain can form hydrogen bonds with Serine, Tyrosine and Histidine of the

protein and plays crucial role for the potency. Hence, My first attempt was to alter at acidic side chain. α-Alkoxy acid is one of the common acidic side chain found in many potent PPAR agonists. Therefore, decided to modify at α-alkoxy acid at acidic side chain to newly designed series. Modifications like alcohol, ether, amine, alkyl amine, azide and mesylate were done and compared with the parent acids. Modifications like azide, amines were not comparable to alcohols or ethers. These results indicate that compounds were not potent than parent acids **1c** and **1d**. Although we were not successful to make potent compounds than **1c** to **1d**, learning was that **3c** and **3d** were selective towards PPARα compare to **1c** and **1d**. A minor modification to functional region could change the profile of a molecule.

2.2.2 Modified oxybenzylglycine derivatives

2.2.2.1 Rationale for designing

In another modification, Muraglitazar [N-[(4-methoxy phenoxy) carbonyl]-N-[4-[2-(5-methyl-2-phenyl-1,3-oxazol-4-yl)ethoxy]benzyl]glycine] BMS-298585 which is an oxybenzylglycine derivative (**Figure 4**) was taken as starting point. Structurally it is non-thiazolidinedione or non α-alkoxy phenylpropanoic acid dual PPARα/γ activator. It was in clinical development for the treatment of type 2 diabetes [15]. Unfortunately, because of higher incidence of edema, heart failure and cardiovascular deaths amongst the patients taking Muraglitazar compared with those receiving placebo or treated with Pioglitazone, the molecule was dropped from development [6]. Our intention was to make novel and potent compounds while minimizing at least some of the adverse effects.

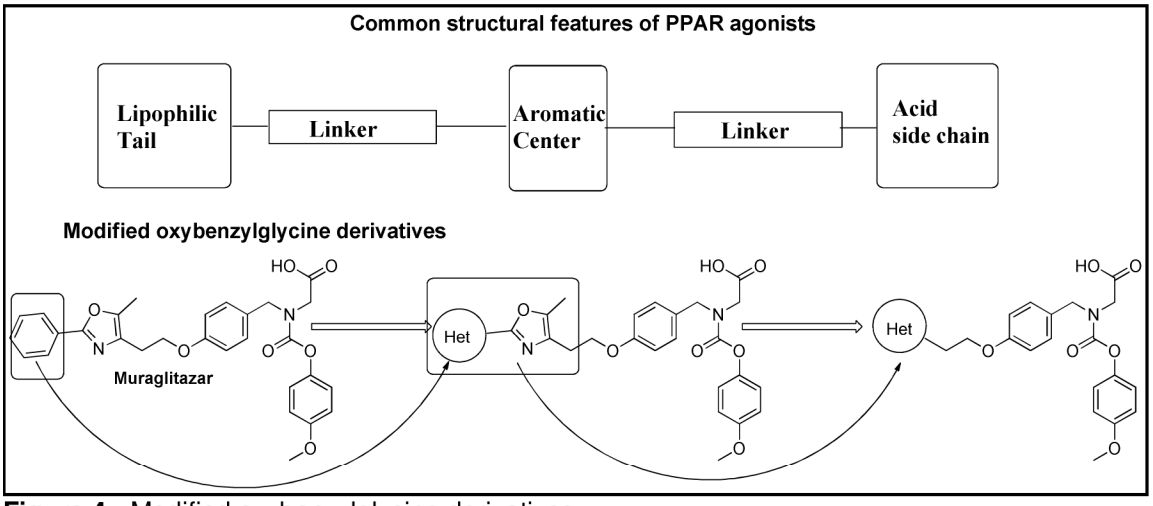
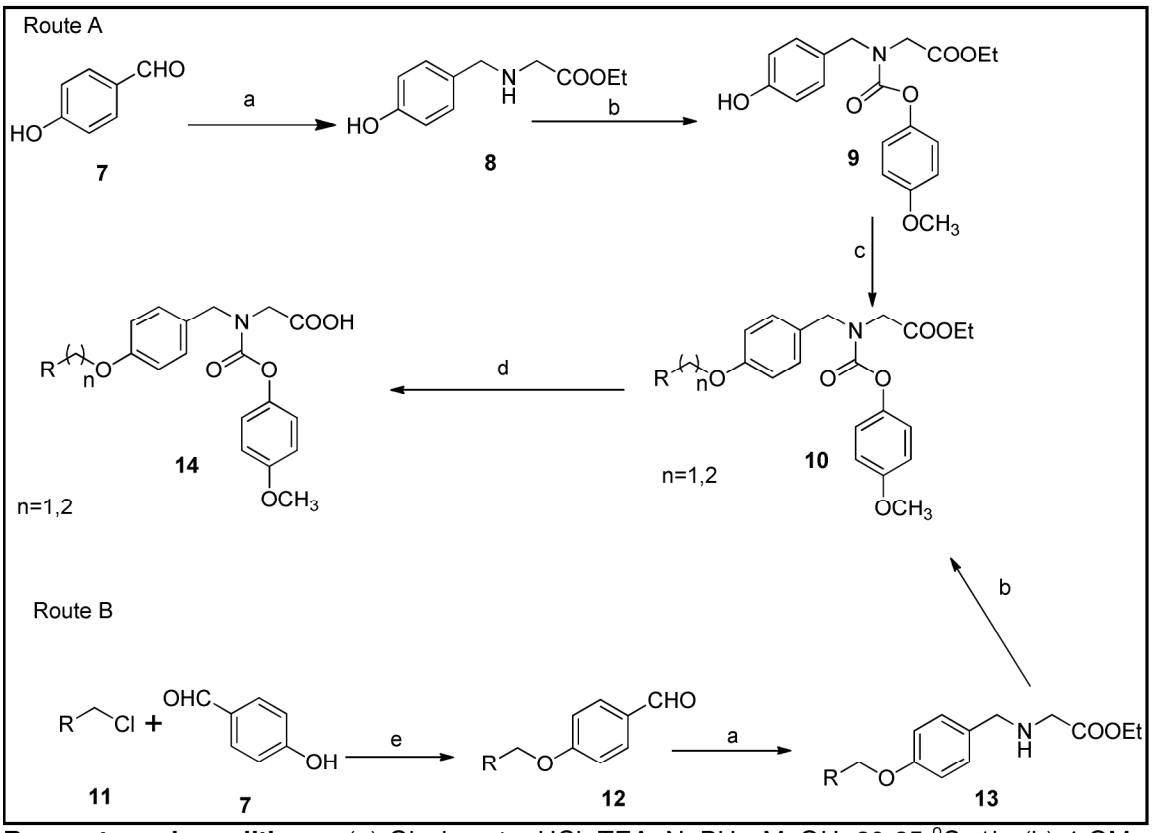


Figure 4 : Modified oxybenzylglycine derivatives

As described earlier, my first approach was to modify at acidic side chain of α-alkoxy phenylpropanoic acid derivatives keeping the oxazole at lipophilic tail constant. In this IInd approach oxybenzylglycine at acidic side chain and oxazole at lipophilic tail were retained. Following classical bioisosterism [16], based on the structures of Muraglitazar, modifications were made on oxazole. Phenyl ring on oxazole was replaced with its bioisoster thiophene and subsequently with the other heterocycles aswell [17]. I anticipated that newly design template will possess the features of both acidic side chain and lipophilic tail.

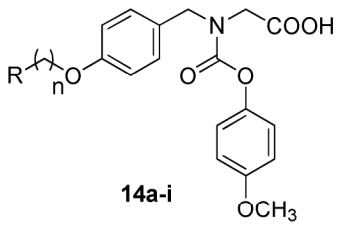
Synthesis of oxybenzylglycine derivatives :

Scheme 2



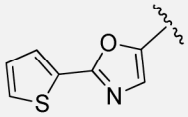
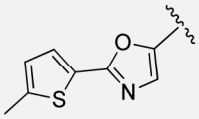
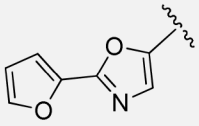
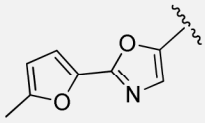
Reagents and conditions : (a) Glycin ester.HCl, TEA, $NaBH_4$, MeOH, 20-25 ^oC, 1h; (b) 4-OMe-PhOCOCl, Pyridine, DCM, 0-5 ^oC, 2h ; (c) Het-$(CH_2)_n$-X(n=2, X=OMs, Cl), K_2CO_3, DMF, 60 ^oC, 5h ; (d) NaOH, H_2O, MeOH, 20-25 ^oC, 4h. (e) K_2CO_3, DMF, 60 ^oC, 5h.

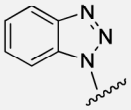
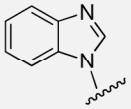
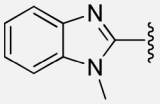
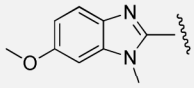
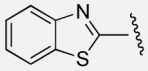
Compounds **14a-i** were synthesized as per the **scheme 2** following two routes (**Route A** and **Route B)**. **Route A** was followed for two carbon linker and **Route B** was used to make compounds having one carbon linker. List of the compounds prepared is given in **Table 2**.



14a-i

Table 2 :

Compound	R	n	Route
14a		2	**A**
14b		2	**A**
14c		2	**A**
14d		2	**A**

Compound	R	n	Route
14e		2	**A**
14f		2	**A**
14g		1	**B**
14h		1	**B**
14i		1	**B**

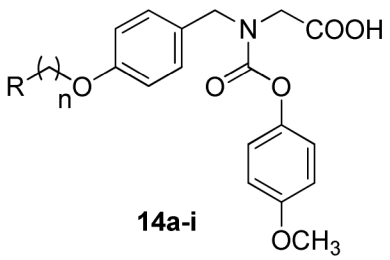
Route A : Starting from 4-hydroxy benzaldehyde **7** in two steps ethyl 2-((4-hydroxy benzyl) ((4-methoxy phenoxy) carbonyl)amino) acetate **9** was prepared. First step was reductive amination of benzaldehyde **7** with glycine ethylester.HCl to form aminoacetate intermediate **8**. It was then reacted with 4-methoxy phenyl chloroformate to form carbamate **9**. Coupling of **9** with Het-$(CH_2)_n$-X (where n=2, X=OMs) [As per scheme 4 intermediate **25**] in presence of K_2CO_3 as base and DMF as solvent afforded precursor **10**, hydrolysis of **10** gave product **14a-f**.

Route B : In the first step, hetrocycle **11** [18] was coupled with 4-hydroxy benzaldehyde **7** to form coupled product **12**. Formyl group of coupled product reacted with glycine ethlester.HCl formed imine which was insitu reduced to form benzyl aminoacetate derivative **13**. This was further reacted with 4-methoxy phenylchloroformate to afford ester compound **10**, which on hydrolysis gave final compounds **14g-i.**

2.2.2.2 Results and discussion

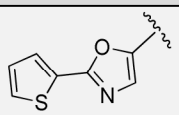
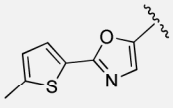
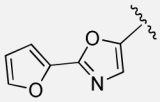
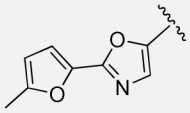
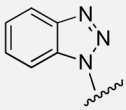
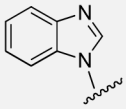
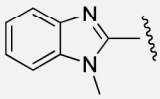
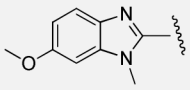
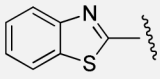
Compounds **14a-i** were screened for hPPARα and hPPARγ agonistic activities on full length PPAR receptor transfected in HepG2 cells. WY-14643 and Rosiglitazone were used as controls for PPARα and PPARγ respectively where the activities are shown as fold activation Vs DMSO at 10 μM concentration towards PPARα and 0.2 μM for PPARγ. Triglyceride lowering activity was measured by administering the compounds orally at a dose of 10 mg/kg/day for 6 days to male Swiss albino mice (SAM) which are moderately hyperlipidemic. Values reported are the % change in serum triglyceride (TG) concentration of the compound treated mice relative to vehicle controls are summarized in **Table 3**.

The first heterocycle selected was thiophene as substitution on oxazole. Thiophene substituted **14a** and 2-methyl thiophene substituted analogue **14b** showed around 5 and 2 fold activation above basal level (activation shown by DMSO) towards PPARα respectively. This was somewhat higher than of WY-14643 for thiophene **14a** and was inferior for 2-methyl thiophene **14b**. Similar trend was observed for PPARγ as fold activation was comparable with standard Rosiglitazone for **14a** and less for **14b**.



14a-i

Table 3 : *in vitro* hPPAR transactivation and TG lowering activity of **14a-i.**

Compound	R	n	hPPARα [a] (10μM) (WY-14643)	hPPARγ [a] (0.2μM) (Rosiglitazone)	% Change in TG in SAM [b] @10 mg/kg
14a		2	5.1 (2.7)	9.5 (6.0)	-19
14b		2	2.2 (3.0)	4.1 (5.3)	-35
14c		2	3.4 (2.2)	10.5 (10.2)	-29
14d		2	3.17 (2.17)	9.4 (10.2)	-29.8
14e		2	3 (2.7)	2.3(6)	-16.6
14f		2	2.2 (2.7)	1.45 (6)	-10.3
14g		1	3.1 (2.7)	1.5 (6.0)	-19.5
14h		1	4.9 (2.7)	2.7 (6.0)	+5.6
14i		1	0.76 (3)	3.7 (5.3)	-9.3
Muraglitazar			2.1(3.7)	10.56(11.75)	IA

[a] hPPAR denotes human PPAR. Activities are presented as fold induction of PPARα, PPARγ activation over the basal level (DMSO). [b]Values indicated are the mean of n=6 animals.

Moving from thiophene to furan similar two analogues of furan **14c** and 2-methyl furan **14d** were prepared. Unlike thiophene here both **14c** and **14d** were better than WY-14643 towards PPARα and equipotent to Rosiglitazone in PPARγ activation. All these four compounds were subjected to *in vivo* efficacy at 10 mg/kg in SAM and serum triglyceride reduction was determined. Thiophene substituted oxazoles **14a** and **14b** showed 19% and 35% reduction in serum TG. Both the furan substituted compounds **14c** and **14d** showed around 29% reduction in serum TG. Unfortunately they were found equipotent for both PPARα and PPARγ so, did not explore oxazoles further. Replacing oxazoles with other heterocycles like benzotriazole **14e** and benzimidazole **14f** were tested for *in vitro* PPARα and PPARγ activity. They showed fold activation comparable to WY-14643 for PPARα and less potent than Rosiglitazone for PPARγ. However, their PPAR agonistic activity was not reflected *in vivo* as there was only 10-15% reduction in serum TG. All these tested compounds were having two carbon spacer linked with N. Compound **14g-i** where spacer was one carbon and linked at carbon of the benzimidazole and benzthiazole. Compounds **14g-i** were equipotent or less potent for PPAR and this was not reflected in serum TG reduction.

2.2.2.3 Conclusions

After altering at acidic side chain to acquire potency my next approach was to modify at lipophilic tail position. To work on this approach Muraglitazar (oxazole analogue) was chosen as starting point. The oxybenzylglycine acidic side chain was fixed and series of substituted oxazole was evaluated. The

substitutions on oxazole was replacement of phenyl ring with other heterocycles like thiophene, furan, benzimidazole, benzthiazole etc. Chain length of linker was kept two carbon and one carbon. Compounds belong to this series demonstrated moderate PPAR *in vitro* activity which could not be translated to *in vivo*. One of the reason for not getting potent compound could be the selection of in appropriate *in vivo* model.

2.2.3 Thiophene substituted oxazoles

2.2.3.1 Rationale for designing

Earlier modification was carried out at acidic side chain of α-alkoxy acid and lipophilic tail of Muraglitazar. In next approach α-alkoxy acid at acidic side chain and oxazole at lipophilic tail were frozen. As mentioned earlier, α-alkoxy acids at acidic side chain can form hydrogen bonds with Serine, Tyrosine and Histidine of the protein and a strong hydrogen acceptor is essential for PPAR affinity [1].

Oxazole group as lipophilic tail and α-alkoxy phenylpropanoic acid as acidic pharmacophore are extensively studied during the development of PPAR agonists. Few PPARα/γ dual agonists containing oxazole group as lipophilic tail and non α-alkoxy phenylpropanoic acid group as acidic head had entered clinical trial stage, for example, Imiglitazar, Muraglitazar, Farglitazar (**Figure 5**) and several other compounds having oxazoles as cyclic tail have also been reported to be potent dual PPAR agonists [19]. Further non oxazole α-alkoxy phenylpropanoic acid derivatives like Tesaglitazar and Naveglitazar (**Figure 5**) were found to be efficacious in animal models and in humans [20]. In addition to

these α-alkoxy phenylpropanoic acids containing 2-phenyloxazole-4ylalkyl moiety are also reported to be potent dual agonists [21].

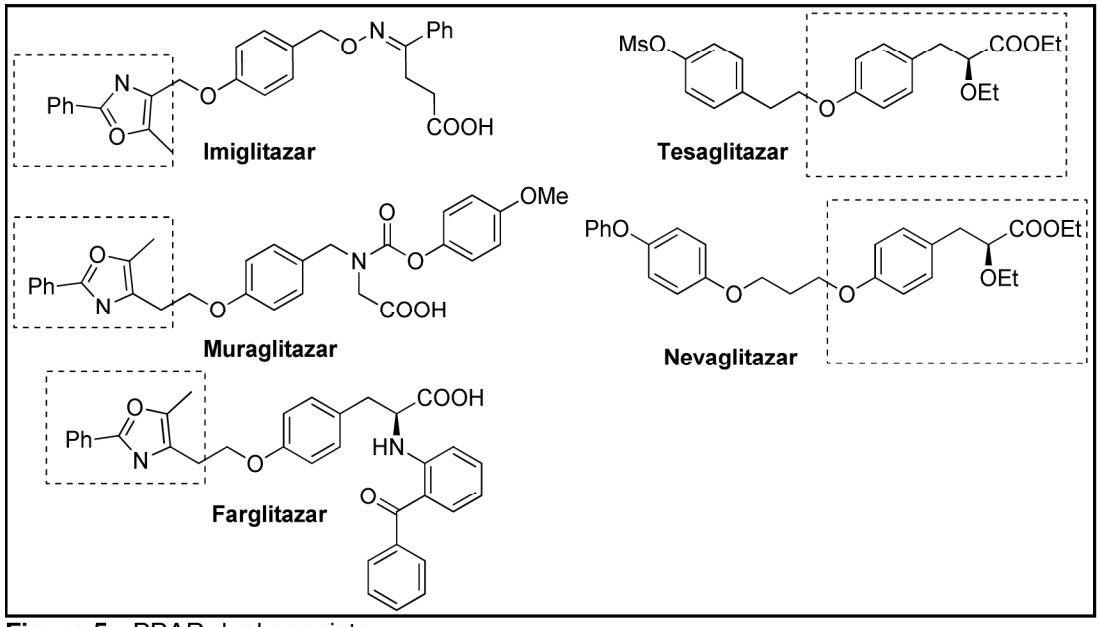


Figure 5 : PPAR dual agonists

My goal obviously was to discover the compounds with very high *in vitro* potency that translates to *in vivo* efficacy. In order to achieve this 2-phenyloxazole containing α-alkoxy phenylpropanoic acid derivative (**Figure 6**) was chosen as chemical lead.

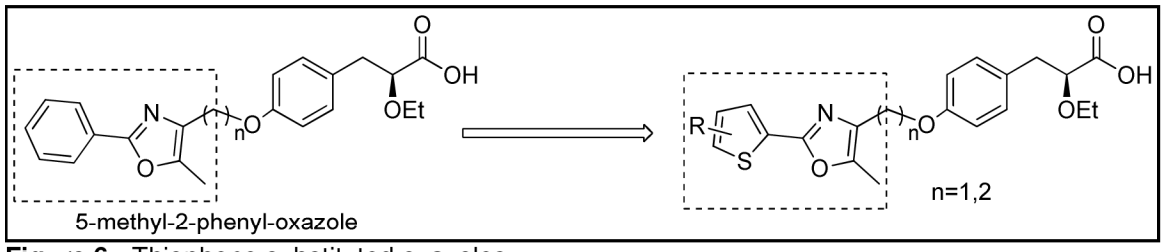


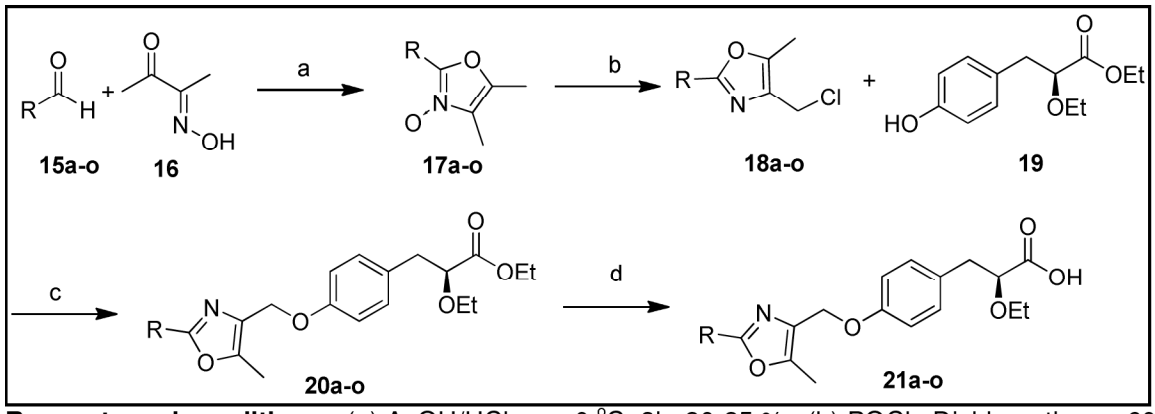
Figure 6 : Thiophene substituted oxazoles

5-methyl-2-phenyl-oxazole at lipophilic tail is known to be a potent fragment for PPAR binding [21]. Ring bioisosterism is the most frequent strategy followed by drugs discovery process [17]. Phenyl ring on 5-methyl-2-phenyl-oxazole was replaced with substituted thiophenes (**Figure 6**). Before I

synthesized these compounds, I did some molecular docking studies which are discussed in the later part of this chapter. Several analogues were designed and synthesized [22] and elaborated in the subsequent **scheme 3** and **scheme 4.**

Synthesis

Scheme : 3



Reagents and conditions : (a) AcOH/HCl gas, 0 ^{o}C, 3h, 20-25 %; (b) $POCl_3$, Dichloroethane, 60 ^{o}C, 3h, 50-70 %; (c) K_2CO_3, DMF, 80-90 ^{o}C, 2h, 40-50 %; (d) NaOH/MeOH/H_2O, 20-25 ^{o}C, 4h, 60-80%.

List of the compounds prepared is given in **Table 4**.

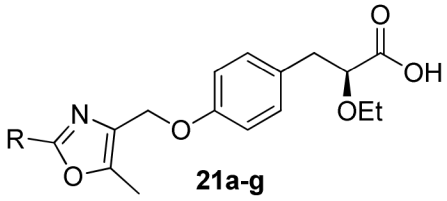
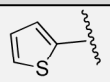
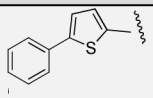
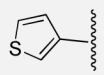
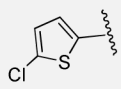
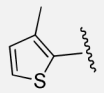
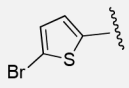
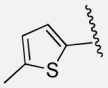


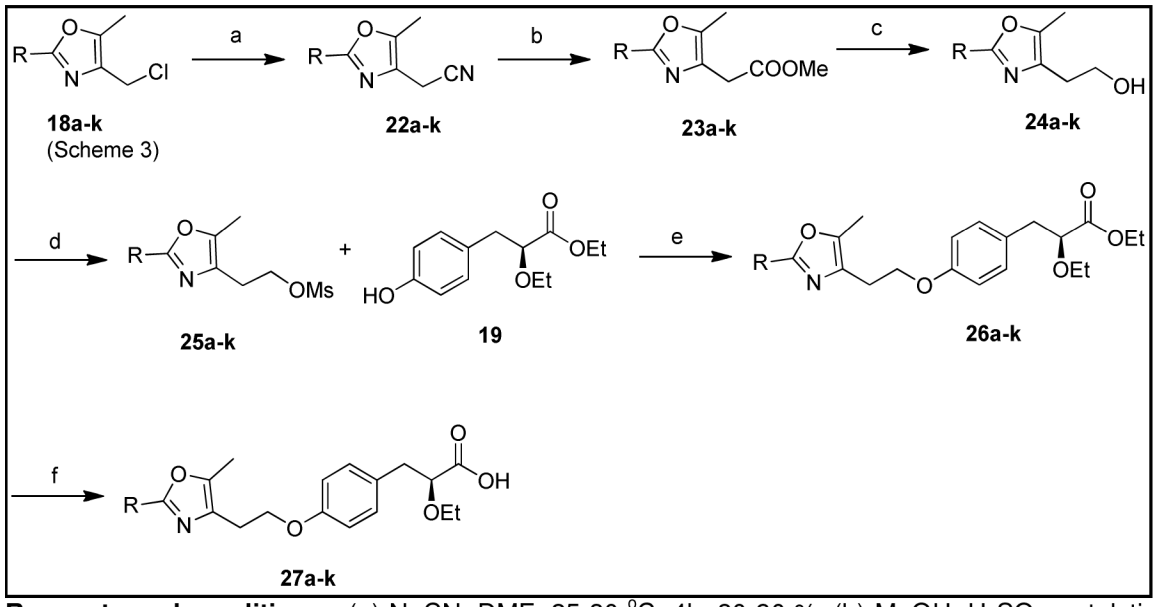
Table 4 :

Compound	R	Compound	R
21a		21e	
21b		21f	
21c		21g	
21d			

Synthetic route of compounds **21a-o** is outlined in **Scheme 3**. Synthesis of intermediate **18** was accomplished in two steps following the reported method [13]. Benzaldehydes **15** were reacted with diacetyl mono-oxime **16** in acetic acid in presence of dry HCl (gas) to afford N-Oxides **17**. Treatment of **17** with $POCl_3$ gave the corresponding chloromethyl oxazole **18**. Nucleophilic substitution of **18** with *(S)*-ethyl 2-ethoxy-3-(4-hydroxyphenyl)propanoate **19** [14] using K_2CO_3 as base in DMF afforded precursor esters **20**. Hydrolysis of these ester compounds **20** under aqueous basic conditions yielded final acid derivatives **21a-o.**

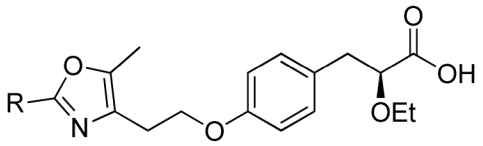
Synthesis

Scheme : 4



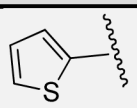
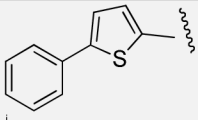
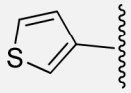
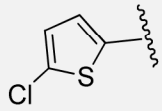
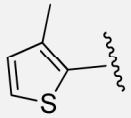
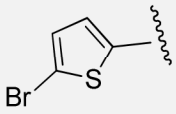
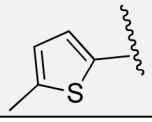
Reagents and conditions : (a) NaCN, DMF, 25-30 ^oC, 4h, 80-90 %, (b) MeOH, H_2SO_4, catalytic H_2O, reflux, 16h, 60-70 %, (c) $LiAlH_4$, THF, 0-10 ^oC, 1h, 70-80 %, (d) $MeSO_2Cl$, NEt_3, CH_2Cl_2, 25 ^oC, 4h, 85-90%, (e) K_2CO_3, DMF, 80-90 ^oC, 2h, 40-50%, (f) NaOH/MeOH/H_2O, 20-25 ^oC, 4h, 60-70 %.

List of the compounds prepared is given in **Table 5**.



27a-g

Table 5 :

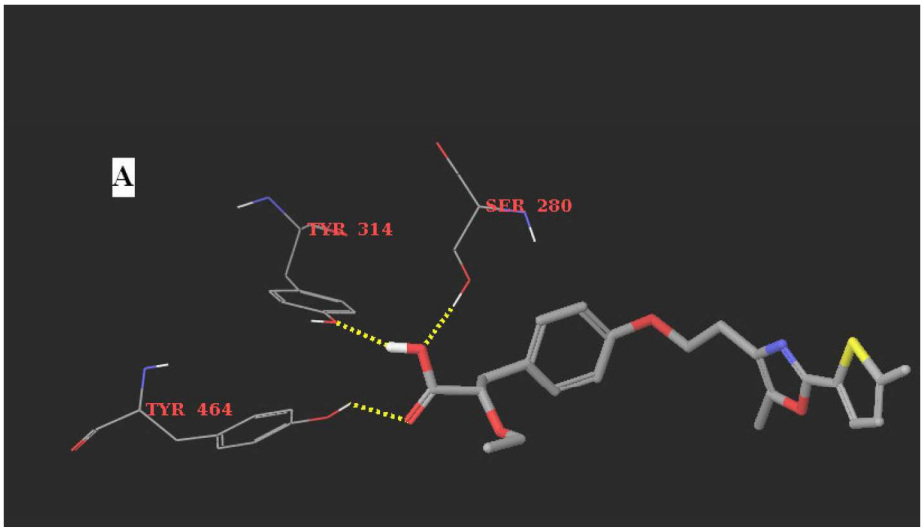
Compound	R	Compound	R
27a		**27e**	
27b		**27f**	
27c		**27g**	
27d			

The synthesis of compounds **27a-k** is outlined in **Scheme 4**. Intermediate mesylate derivatives **25a-k** were prepared from their corresponding lower homologues **18a-k** in four steps. Chloromethyl oxazoles **18a-k** were converted to corresponding cyano derivatives **22a-k** using NaCN in DMF at ambient temperature in good yields. Cyano derivatives **22a-k** were refluxed in a mixture of methanol, sulfuric acid and water (catalytic amount) to yield corresponding esters **23a-k** which were then reduced to alcohols **24a-k** using $LiAlH_4$. Alcohols **24a-k** were converted to corresponding mesylates **25a-k**. Coupling of **25a-k** with *(S)*-ethyl 2-ethoxy-3-(4-hydroxyphenyl)propanoate **19** [14] as shown in **Scheme 3** afforded ester compounds **26a-k**. Aqueous basic hydrolysis of **26a-k** gave the

corresponding acids **27a-k**. The structure of all the final compounds and intermediates are confirmed by their spectral analysis.

Molecular docking study

Docking was carried out using Discovery Studio software version 1.6. The geometry of compound docked was subsequently optimized using the CHARMM force field. The energy minimization was carried out using smart minimize option in the software until the gradient value was smaller than 0.001 kcal/ mol A°.



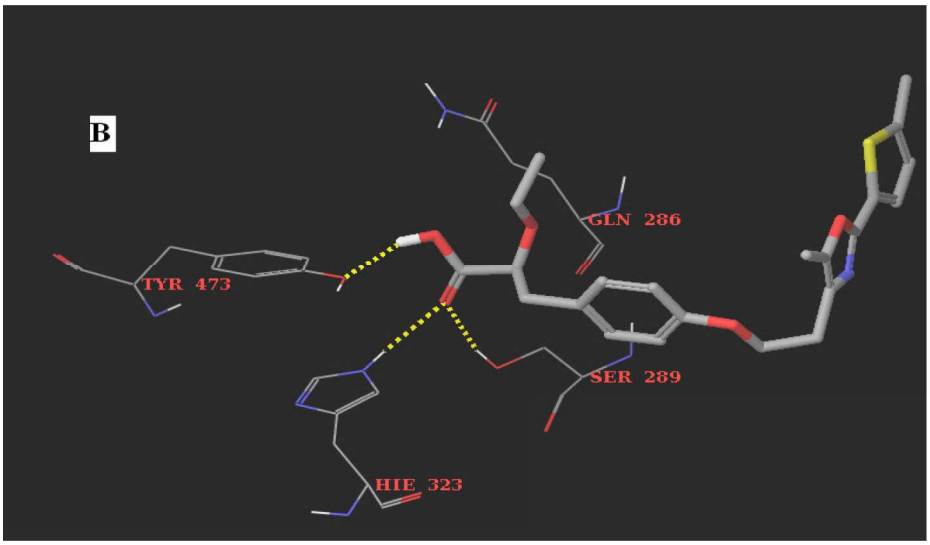


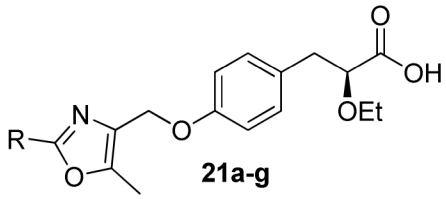
Figure 7 : Molecular docking of **27b** into PPARα (A) and PPARγ (B) binding pockets: H-bond interactions with amino acids are shown in dashed lines.

The complexed X-ray crystal structure of the ligand binding domain (LBD) of PPARα with GW-409544 (1k7l.pdb) [23] and PPARγ with Rosiglitazone (2PRG.pdb) were obtained from RCSB Protein Data Bank. When docked into PPARα binding pocket the most stable docking model of thiophene substituted derivatives adopts a confirmation that allows the carboxylic group to form hydrogen bond with Tyr464, Tyr314 and Ser280 (**Figure 7A**).

Whereas in PPARγ binding pocket thiophene substituted derivatives adopts a confirmation that allows the carboxylic group to form hydrogen bonds with His323, Tyr473 and Ser289, and are reported H-bond interaction for a PPARγagonist (**Figure 7B**). We eventually found by molecular modeling analysis that compound **27d** which is methyl substituted thiophene analogue shows good fit.

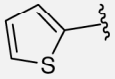
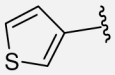
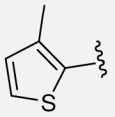
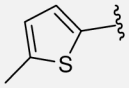
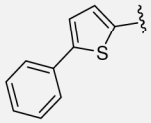
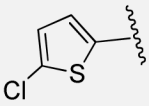
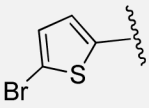
2.2.3.2 Results and discussion

All the compounds synthesized were screened for human PPARα and PPARγ transactivation assay using hPPAR receptor trasfected HEPG2 cells described in literature [24], WY14643, Rosiglitazone and GW501516 were used as control for PPARα, PPARγ and PPARδ respectively (**Table 6**).



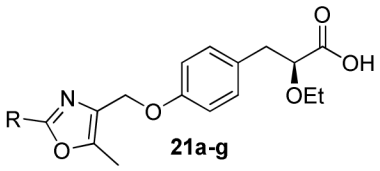
21a-g

Table 6 : *in vitro* hPPAR transactivation of compounds **21a–g.**

Compd. No	R	hPPAR transactivation[a] hPPARα EC_{50} (nM)	h*PPAR*γ EC_{50} (nM)	hPPARα %max[b]	hPPARγ %max[b]
21a		0.026	0.0015	134	107
21b		3	2.7	147	99
21c		0.00006	0.0003	106	88
21d		0.00005	0.0002	151	120
21e		20	3	93	104
21f		0.1	0.45	95	105
21g		0.04	0.3	102	115
WY-14643		4800	ND	100	ND
Rosiglitazone		ND	50	ND	100

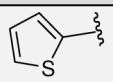
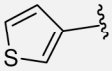
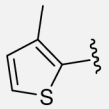
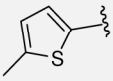
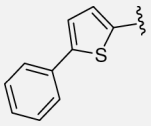
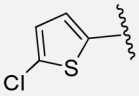
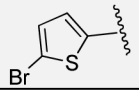
[a]HepG2 cells transfected with pSG5 expression vector containing the cDNA of hPPARα or hPPARγ or hPPARδ and cotransfected with PPRE3-TK-luc. The Luciferase activity determined using commercial fire-fly luciferase assay and β-galactosidase activity determined in ELISA reader. None of the compound showed activation above basal level against PPARδ.

[b]Percent of maximal efficacy (% max) of all compounds compared to reference compounds (WY-14643 for PPARα and Rosiglitazone for PPARγ) normalized to 100%.



21a-g

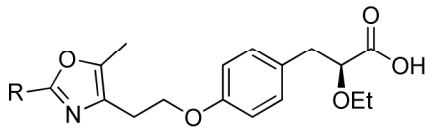
Table 7: TG lowering activity of **21a-g**

Compd. No	R	% Change TG[a]
21a		-72
21b		-70
21c		-72
21d		-69
21e		-57
21f		-70
21g		-81

[a]The test compounds were administered orally at a dose of 10 mg/kg/day to male *Swiss albino* mice (SAM) of 6-8 weeks of age for 6 days. Mean values (n=6) are the % change in serum triglyceride (TG) concentration of the compound-treated mice versus vehicle controls. All values are the mean of n= 6. ND denotes not determined.

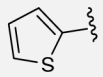
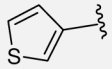
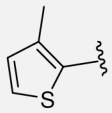
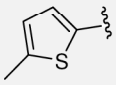
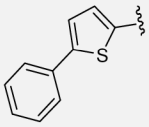
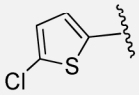
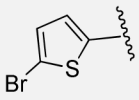
It appears from the **Table 6** and **Table 7** that *in vitro* potency of all the test compounds are in the range of low nM to pM in general. Methyl substitution on thiophene **21c** and **21d** are more potent than of compounds possessing unsubstituted thiophene **21a** and **21b** towards both PPARα and PPARγ. When the substitution on of thiophene was Cl and Br at 5th position of thiophene **21f** and **21g** were less potent than methyl substituted and unsubstituted analogues. Phenyl substitution on thiophene **21e** is lowest potent among all analogues

having one carbon chain length. All compounds demonstrated significant TG lowering effect.



27a-g

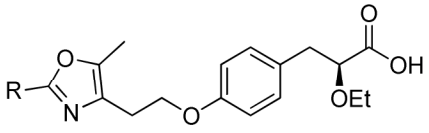
Table 8 : *in vitro* hPPAR transactivation of compounds **27a-g**.

Compd. No	R	hPPAR transactivation[a]			
		hPPARα EC_{50} (nM)	hPPARγ EC_{50} (nM)	hPPARα %max[b]	hPPARγ %max[b]
27a		0.1	0.05	126	104
27b		1.9	0.6	114	87
27c		0.00007	0.0001	96	192
27d		0.00031	0.018	116	165
27e		22	4.5	90	103
27f		1.2	3.9	110	102
27g		0.7	2.1	115	90
WY-14643		100	ND	100	ND
Rosiglitazone		ND	50	ND	100

[a]HepG2 cells transfected with pSG5 expression vector containing the cDNA of hPPARα or hPPARγ or hPPARδ and cotransfected with PPRE3-TK-luc. The Luciferase activity determined using commercial fire-fly luciferase assay and β-galactosidase activity determined in ELISA reader. None of the compound showed activation above basal level against PPARδ.

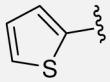
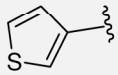
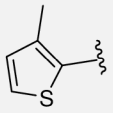
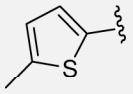
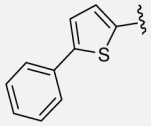
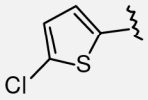
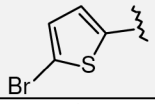
[b]Percent of maximal efficacy (%max) of all compounds compared to reference compounds (WY-14643 for α and Rosiglitazone for γ) normalized to 100%.

The *in vitro* SAR of spacer having chain length of two carbon was similar to one carbon (**Table 8**). All the analogues having chain length of two carbon showed potency in nM range and methyl substitution on thiophene **27c** and **27d** are more potent than unsubstituted thiophene **27a** and **27b** towards both PPARα and PPARγ.



27a-g

Table 9 : TG lowering activity of **27a-g**

Compd. No	R	% Change TG[a]
27a		-78
27b		-62
27c		-67
27d		-88
27e		-62
27f		-62
27g		-67

[a]The test compounds were administered orally at a dose of 10 mg/kg/day to male *Swiss albino* mice (SAM) of 6-8 weeks of age for 6 days. Mean values (n=6) are the % change in serum triglyceride (TG) concentration of the compound-treated mice versus vehicle controls. All values are the mean of n= 6. ND denotes not determined.

Compounds bearing Cl and Br at 5th position of thiophene **27f** and **27g** were less potent than methyl substituted or unsubstituted thiophene analogues. Here also phenyl substitution on thiophene **27e** was least potent. TG lowering effects of all analogues were more or less similar (**Table 9**).

In general *in vitro* potency of all the test compounds **21** and **27** are in the range of low nM to pM and subtype selectivity ranges between 1-15 folds. The initial compound **21a** possessing unsubstituted thiophene linked through 2nd position to oxazole showed 26 pM and 1.5 pM potency towards PPARα and PPARγ respectively, whereas its higher homologue **27a** is found detrimental in terms of *in vitro* potency as compared to **21a**. Both the compounds demonstrated significant TG lowering effect. When the linkage on thiophene was changed to 3rd position, resulting compounds **21b** and **27b** showed relatively poor *in vitro* potency but still exhibited comparable TG reduction to their 2^{nd} position analogues **21a** and **27a**. These results indicate that linkage on thiophene through its 2nd position is favorable over 3^{rd} position. Then we intended to substitute 3^{rd} & 5^{th} position of thiophene and synthesized compounds **21c-e** and **27c-e**. Compound **21c** bearing methyl group at 3rd position of thiophene showed remarkable increase in potency (0.06 pM for PPARα and 0.3 pM for PPARγ) and also reduced TG by 72%. When the methyl group was introduced at 5th position of thiophene, the resulting compound **21d** demonstrated similar *in vitro* potency as well as TG lowering effect. When chain length of the spacer of **21c** was increased to ethylene group the resulting compound **27c** showed similar profile as exhibited by **21c**. But compound **27d** which is higher homologue of **21d**

though showed marginally inferior PPAR potency as compared to **21d**, demonstrated 88% of TG reduction and emerged as a lead compound in the series. To know the effect of bulkier substituent, we have introduced phenyl group at 5th position of thiophene and synthesized compounds **21e** and **27e**. Both this compounds are found detrimental in terms of *in vitro* potency with respect to other compounds of the series. So as we increase bulkier group on thiophene ring compounds (**21e-g** and **27e-g**) are weak in terms of *in vitro* potency. Based on *in vitro* activity and TG lowering results, compound **27d** and its methylene analogue **21d** were selected for further *in vivo* evaluation.

21d and **27d** were subjected for glucose lowering effect in db/db mice and the results are summarized in **Table 10**.

Table 10 : *in vivo* glucose lowering effect of **21d** and **27d** in db/db mice[a]

Compd. No	Dose (mg/kg/day)	% Change in Glucose[a]
21d	3	-67
27d	3	-72
Tesaglitazar	3	-55

[a]Male db/db mice of 6–8 weeks old were dosed with test compounds daily for 6 days and Plasma glucose, triglycerides were measured. Values reported are % change of compound-treated mice versus vehicle controls.

Compounds **21d** and **27d** produced excellent glucose reduction of 67% and 72% respectively when dosed orally at 3 mg/kg/day dose. Finally **27d** is selected as the lead compound of the series and its pharmacokinetic parameters were determined and presented in **Table 11.** 27d exhibited excellent profile with

a Cmax of 91.7 μg/mL and 455.8 h.μg/mL of AUC. This compound exhibited extended $T_{1/2}$ of 7.4h.

Subsequently **27d** was then evaluated for its dose dependent hypolipidemic and antihyperglycemic activity in Swiss albino mice (SAM) and db/db mice and the data is presented in **Table 12**. In SAM model **27d** reduced TG by 41.5% at a dose of 0.003 mg/kg and the ED_{50} was found to be 0.01 mg/kg. In db/db mice the ED_{50} values for the reduction of PG and TG were 0.1657 and 0.0188 mg/kg respectively.

Table 11 : Mean pharmacokinetic parameters[a] of **27d** in fasted male Wistar rat

Compd	Route	dose (mg/kg)	T_{max} (h)	C_{max} (μg/mL)	$T_{1/2}$ (h)	AUC(0-∞) (h.μg/mL)
27d	Oral	30	0.667	91.7	7.4	455.8

[a] Values indicate mean ± SD for n=3

Table 12 : Dose dependent effect of **27d** on TG and PG in SAM and db/db mice.

Dose (mg/kg)	SAM		*db/db*			
	% change in TG	ED_{50} (mg/kg)	% change in PG	ED_{50} (mg/kg)	% change in TG	ED_{50} (mg/kg)
0.003	-41.5		-47.1		-7.2	
0.01	-61.9		-52.7		-6.6	
0.03	-65.6		-52.1		-25.9	
0.1	-71.8	0.01	-51.7	0.1657	-49.4	0.0188
0.3	-80.8		-58.2		-51.8	
1	-82.0		-59.7		-64.2	
3	-83.1		-62.6		-55.1	

Having achieved the primary goal of identifying potent and efficacious PPARα/γ dual agonist, our next end point of this endeavor was to study the toxicity profile of the lead compound **27d**. Oral acute toxicity of this molecule was studied in male and female wistar rats at 3, 30 mg/kg for 28 days. 15x and 150x doses were selected based on the ED_{50} (considering as ~0.2 mg) values in SAM and db/db mice. There were no significant treatment related clinical manifestations noted in any of the treated group animals and there was no treatment related mortality. Food consumption was comparable to that of control groups throughout the study period in both the treated groups of male and female animals. Animals were sacrificed on day 29 and data analysis of blood biochemical parameters, organ weight ratios and histopathological findings.

Body weights were recorded weekly and the data is presented in **Figure 8** (male) and **Figure 9** (female). No increase in body weight was observed due to compound treatment in male animals while in female animals an increase in body weight was observed till week 3 and thereafter marginal decrease was observed by the end of the treatment. These results clearly indicate that **27d** does not cause significant weight gain, the main side effect of PPAR class of compounds even at a dose of 15x and 150x of ED_{50} values. Analysis of organ to body weight ratios (**Table 13)** did not show evidence of toxicity attributed to compound treatment at least at 3 mg/kg dose, which is 15x of ED_{50}, except the liver weights. The results clearly indicate the hepatomegaly (liver enlargement) in both male and female animals treated with **27d** at 3 and 30 mg/kg. However it is well established by now that this phenomenon is specific to rodents and the literature

precedence clearly established that such an effect does not occur in non-rodents [25]. There was marginal increase (7%) in heart weight at 3 mg dose and significant increase (25%) at 30 mg dose, which is 150 times of ED_{50} was observed. Similarly no significant alterations were observed in biochemical parameters (**Table 14**) except the increase in liver enzymes (ALP, SGOT, SGPT) which are in correlation with rodent specific hepatomegaly. No significant changes were observed in hemoglobin, albumin urea and creatinine at 3 mg/kg while at 30 mg/kg dose an increase in urea levels was observed. More interestingly a marginal decrease in creatinine was observed at both the doses in male and female animals, while elevation of creatinine is common side effect of PPAR agonists. These results clearly indicate that treatment with **27d** in rodents did not exert any significant side effects even at 150 times higher dose than ED_{50} value. The evaluation of the toxicity of this molecule in non-rodent species will be carried out and the results will be published subsequently.

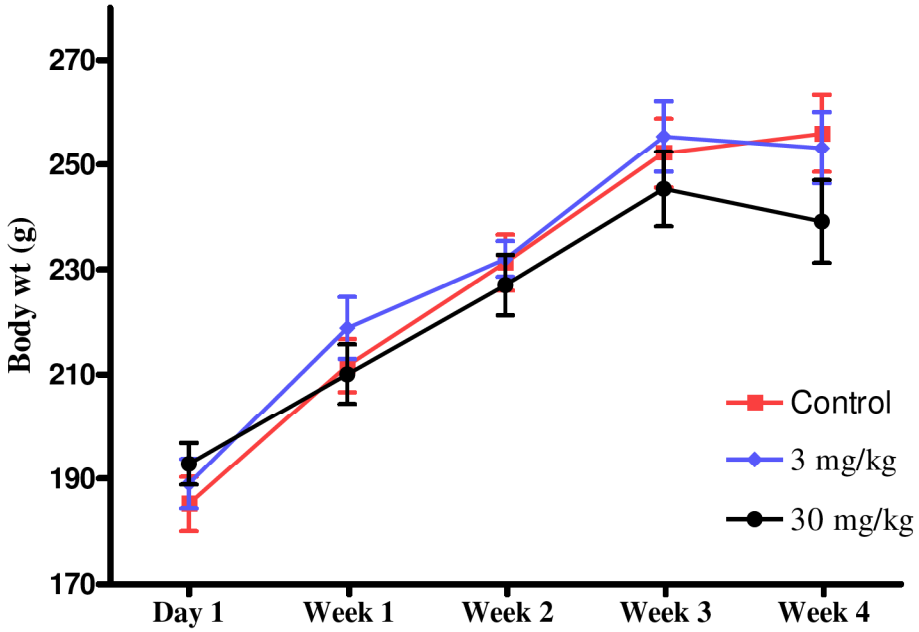


Figure 8 : Body weight values of male Wistar rats treated with **27d** for 28 days

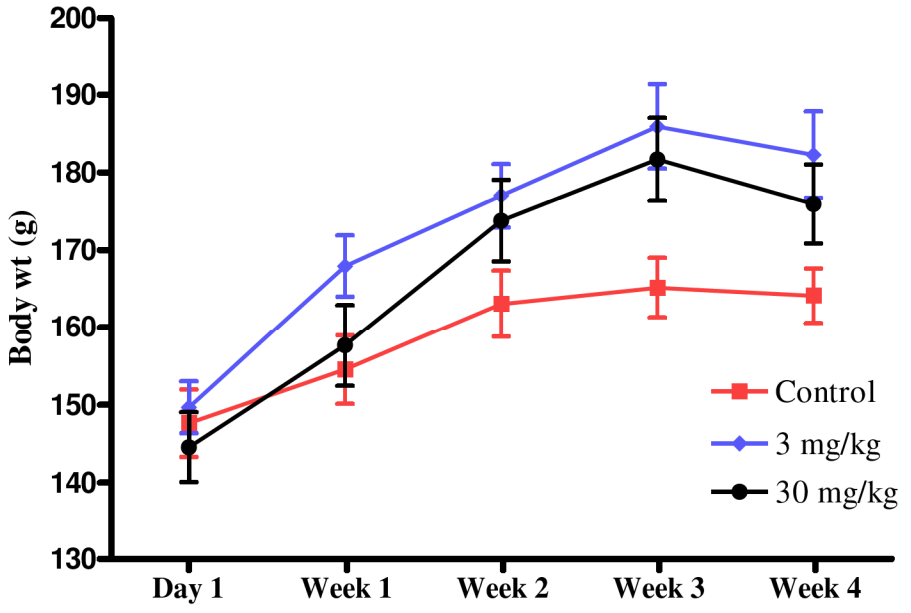


Figure 9 : Body weight values of female Wistar rats treated with **27d** for 28 days

Table 13 : Relative Organ Weights[a] of wistar rats administered orally with **27d** for 28 days

Dose (mg/kg)	Heart	Liver	Kidney	Spleen	Adrenal	Brain	Testes	Epididymine	Thymus	% change in BodyWt.
Males										
Control	0.383 ±0.012	3.257 ±0.069	0.745 ±0.012	0.215 ±0.007	0.018 ±0.001	0.658 ±0.015	1.075 ±0.106	0.391 ±0.017	0.081 ±0.013	38.25
3	0.412 ±0.006	7.505 ±0.151	0.807 ±0.007	0.223 ±0.004	0.015 ±0.001	0.720 ±0.024	1.152 ±0.033	0.360 ±0.018	0.089 ±0.013	33.99
30	0.479 ±0.007	8.099 ±0.124	0.955 ±0.024	0.252 ±0.005	0.018 ±0.001	0.757 ±0.021	1.356 ±0.043	0.432 ±0.016	0.075 ±0.008	24.01
Females							**Ovary**	**Uterus**		
Control	0.403 ±0.010	3.091 ±0.087	0.733 ±0.007	0.237 ±0.012	0.028 ±0.001	0.970 ±0.023	0.039 ±0.005	0.233 ±0.041	0.094 ±0.01	11.13
3	0.460 ±0.010	6.221 ±0.165	0.804 ±0.024	0.257 ±0.024	0.020 ±0.001	0.947 ±0.034	0.020 ±0.002	0.176 ±0.011	0.101 ±0.013	21.77
30	0.501 ±0.009	7.075 ±0.314	0.915 ±0.037	0.260 ±0.011	0.022 ±0.001	0.939 ±0.050	0.024 ±0.002	0.189 ±0.029	0.066 ±0.005	21.71

a Presented as organ-to-body weight percent ratio.

Table 14 : Biochemical Parameters of wistar rats administered orally with **27d** for 28 days

Dose (mg/kg)	HGB (g/dl)	Glu (mg/dl)	CREA (mg/dl)	ALP (U/L)	SGOT (U/L)	SGPT (U/L)	ALB (g.dl)	Urea (mg/dl)
Males								
Control	13.66 ±0.23	70 ±3.49	0.47 ±0.03	243.88 ±20.34	135.14 ±6.89	34.24 ±2.13	3.66 ±0.10	30.52 ±0.79
3	12.20 ±0.18	75.74 ±2.65	0.36 ±0.04	470.50 ±23.93	193.51 ±9.76	28.54 ± 0.89	3.91 ±0.15	31.24 ±1.19
30	12.46 ±0.21	83.28 ±2.90	0.37 ±0.02	679.13 ±59.03	245.24 ±26.13	33.03 ±3.19	4.12 ±0.10	34.58 ±1.14
Females								
Control	11.90 ±0.19	69.94 ±2.30	0.42 ±0.04	172.38 ±7.18	155.70 ±6.56	30.44 ±1.59	3.92 ±0.18	33.05 ±2.02
3	11.84 ±0.18	91.88 ±5.71	0.36 ±.03	248.13 ±18.00	157.09 ±10.32	22.46 ±0.77	4.03 ±0.10	29.19 ±1.48
30	11.83 ±0.19	91.79 ±9.14	0.41 ±0.03	248.38 ±32.75	224 ±41.94	35.21 ±4.41	4.12 ±0.12	37.39 ±7.93

Above results suggest that thiophene substituted oxazole containing α-ethoxy phenylpropanoic acid derivative are highly potent and efficacious dual PPARα/γ agonists.

2.2.3.3 Conclusions

I have synthesized a series of thiophene substituted oxazole containing α-alkoxy phenylpropanoic acid derivatives. Thiophene substituted oxazoles and α-alkoxy phenylpropanoic acid were linked through one carbon and two carbon chain. Increasing from the unsubstituted, methyl, chloro, bromo and phenyl substitution on thiophene was evaluated. Interestingly methyl substituted analogues were the most potent. By increasing bulkier group on thiophene chloro and bromo substitution gave moderate activity, in contrast, phenyl substitution resulted least potent compound. The possible reason could be binding site of the

PPAR receptor might not accommodate large groups, such as phenyl. Compounds **21d** and **27d** were the most potent compounds identified. Both compounds demonstrated significant TG reduction in SAM model, further they were subjected for glucose lowering effect in db/db mice. Finally **27d** was selected as lead compound and toxicity of this molecule was studied in male and female wistar rats at 3, 30 mg/kg for 28 days. The outcome of toxicity study of compound **27d** in rodents clearly indicated that there was no significant side effects even at 150 times higher dose than ED_{50} value.

Development of two glitazar drugs Tesaglitazar (α-alkoxy phenylpropanoic acid derivative) and Muraglitazar (5-methyl-2-phenyl-oxazole derivative) were discontinued after completion of phase III clinical trials. Muraglitazar increased incidence of heart failure and toxicity associated with Tesaglitazar was decreased glomerular filtration [6,26]. Combining the structural features of both the compounds thiophene substituted oxazole containing α-ethoxy phenylpropanoic acid derivatives were designed. The compounds of this series emerge as of highly potent and efficacious PPARα/γ dual agonists and showed clean toxicity profile in rodents. The lead candidate **27d** showed excellent anti-hyperglycemic, hypolipidemic effects than Muraglitazar and Tesaglitazar. The evaluation of the lead compound for its toxicity profile in rodents was encouraging the further development of this compound for the treatment of metabolic disorder is under progress.

2.2.4 Replacement of thiophene with other heterocycles

As thiophene substituted oxazoles were subjected for toxicology evaluation we further explore this template in search of backup candidate.

2.2.4.1 Rationale for designing

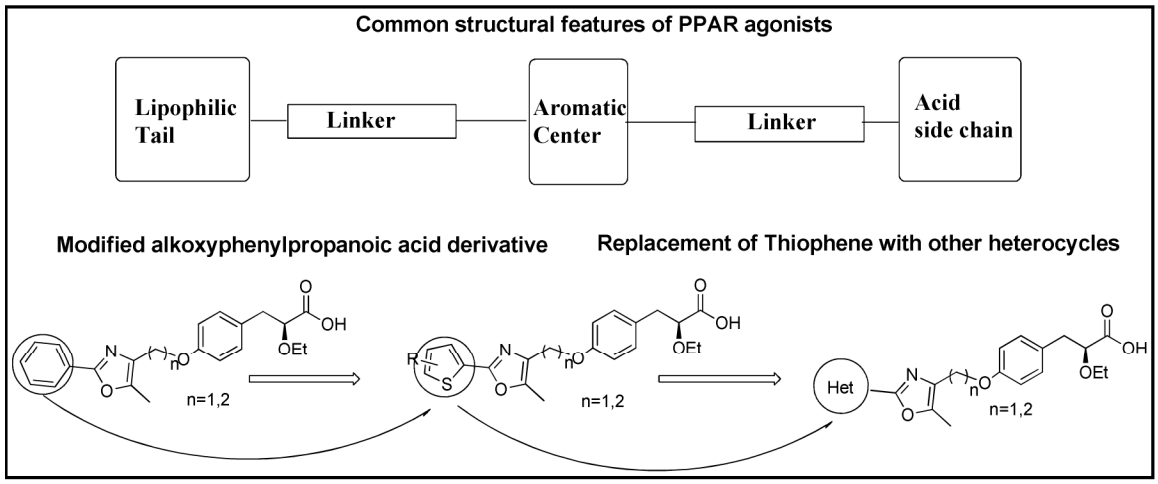
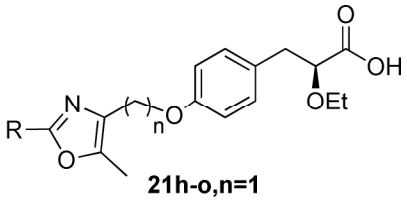


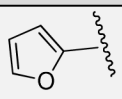
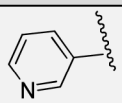
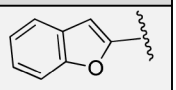
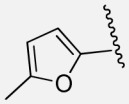
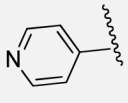
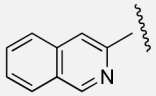
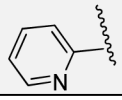
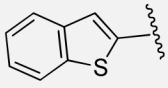
Figure 10 : Replacement of thiophene with other heterocycles

Further, thiophene was replaced with other ringisosters like pyridine, furan, bezofuran, benzoxazole etc (**Figure 10**). Following compounds were synthesized (**Table 15**) and subjected for *in vitro* as well as *in vivo* study.



21h-o,n=1

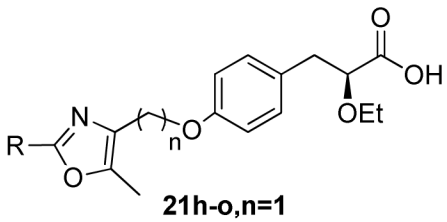
Table 15 :

Compound	R	Compound	R	Compound	R
21h		21k		21n	
21i		21l		21o	
21j		21m			

Synthesis of the above mentioned molecules remained same as per **Scheme 3**.

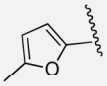
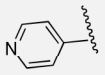
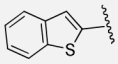
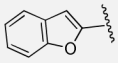
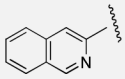
2.2.4.2 Result and discussion

in vitro results of the modified oxazoles are given in **Table 16**.

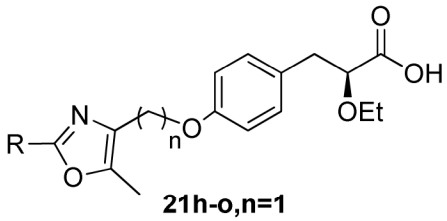


21h-o,n=1

Table 16 : *in vitro* hPPAR transactivation of compounds **21h-o.**

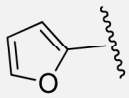
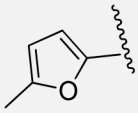
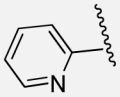
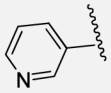
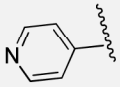
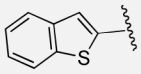
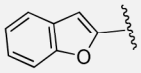
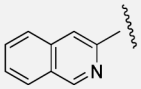
Compd. No	R	hPPAR transactivation[a]	
		PPARα EC_{50} (nM)	PPARγ EC_{50} (nM)
21h		6.1	2.3
21i		40	100
21j		62	12
21k		89	39
21l		115	82
21m		60	10,1
21n		73	44
21o		79	25
WY-14643		100	ND
Rosiglitazone		ND	50

[a]HepG2 cells transfected with pSG5 expression vector containing the cDNA of hPPARα or hPPARγ or hPPARδ and cotransfected with PPRE3-TK-luc. The Luciferase activity determined using commercial fire-fly luciferase assay and β-galactosidase activity determined in ELISA reader. None of the compound showed activation above basal level against PPARδ.



21h-o,n=1

Table 17 : TG lowering activity of **21h-o**

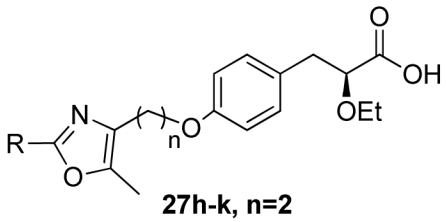
Compd No	R	% Change TG[a]
21h		-59
21i		-65
21j		-40
21k		-30
21l		-14
21m		-71
21n		-76
21o		-68

[a] The test compounds were administered orally at a dose of 10 mg/kg/day to male *swissalbino* mice (SAM) of 6-8 weeks of age for 6 days. Mean values (n=6) are the % change in serum triglyceride (TG) concentration of the compound-treated mice versus vehicle controls. All values are the mean of n= 6. ND denotes not determined.

The *in vitro* and *in vivo* data of compounds with one carbon chain spacer are presented in **Table 16** and **Table 17**. Furan substitution on oxazole **21h** showed potency in nM range for both PPARα and PPARγ. Unlike thiophene, methyl

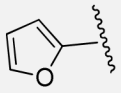
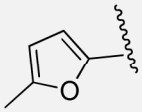
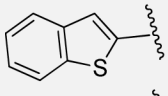
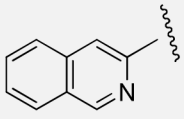
substitution at 5th position **21i** did not emerge as very potent compound. But both the compounds produce similar TG lowering effect. Replacing furan with 2, 3 and 4-pyridyl rendered the compounds **21j-l** respectively. Relatively 2-pyridyl substituted oxazole **21j** was best among pyridine substituted analogues. Compounds with bulky tail groups like benzothiophene, bezofuran, and isoquinoline substituted oxazoles **21m-o** were synthesized and screened. They were found to be having similar *in vitro* potency to pyridine substituted analogues. Surprisingly these compounds were comparable to potent analogues in reducing TG levels *in vivo*.

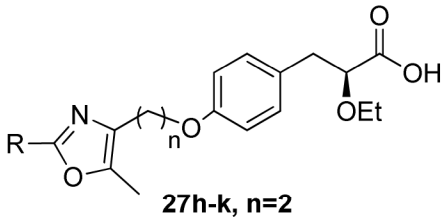
Few compounds were synthesized as per **Scheme 4** with ethylene spacer (**Table 18**).



27h-k, n=2

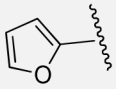
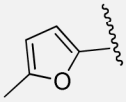
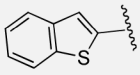
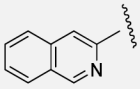
Table 18 :

Compound	R
27h	
27i	
27j	
27k	



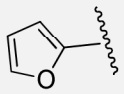
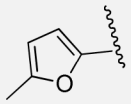
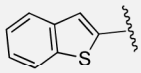
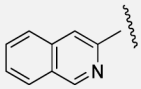
27h-k, n=2

Table 19 : *in vitro* hPPAR transactivation of compounds **27h-k.**

Compd. No	R	hPPAR transactivation[a]	
		PPARα EC_{50} (nM)	PPARγ EC_{50} (nM)
27h		0.0004	1.8
27i		0.3	0.06
27j		100	0.1
27k		85	5.5
WY-14643		100	ND
Rosiglitazone		ND	50

[a]HepG2 cells transfected with pSG5 expression vector containing the cDNA of hPPARα or hPPARγ or hPPARδ and cotransfected with PPRE3-TK-luc. The Luciferase activity determined using commercial fire-fly luciferase assay and β-galactosidase activity determined in ELISA reader. None of the compound showed activation above basal level against PPARδ

Table 20 : TG lowering activity of **27h-k**

Compd No	R	% Change TG[a]
27h		-65
27i		-14
27j		-72
27k		-52

[a]The test compounds were administered orally at a dose of 10 mg/kg/day to male *swissalbino* mice (SAM) of 6-8 weeks of age for 6 days. Mean values (n=6) are the % change in serum triglyceride (TG) concentration of the compound-treated mice versus vehicle controls. All values are the mean of n= 6. ND denotes not determined.

Both the furan substituted analogues **27h** and **27i** showed potency less than 1 nM for both PPARα and PPARγ. Compounds bearing bulky tail groups benzothiophene and isoquinoline **27j** and **27k** were less potent for PPARα. All the compounds produce moderate TG lowering efficacy *in vivo* (**Table 19, 20**).

2.2.4.3 Conclusions

To continue, newly designed oxazole containing α-alkoxy phenylpropanoic acid derivatives thiophene was replaced with other ringisosters like pyridine, furan, bezofuran, benzoxazole etc. Like thiophene substitution on oxazole, any other heterocycle did not turn out fruitful. All the compounds were moderately active in both *in vitro* and *in vivo*. Considering above results it clearly indicate that thiophene substitution on oxazole is most favorable and produced high *in vitro* potent compounds having picomolar potency.

2.3 Summary of chapter 2

The objective of this work was to increase potency of newly designed series to avoid the toxicity. It was envisioned that by altering at various structural component of PPAR agonists (**Figure 11**) may significantly enhance potency.

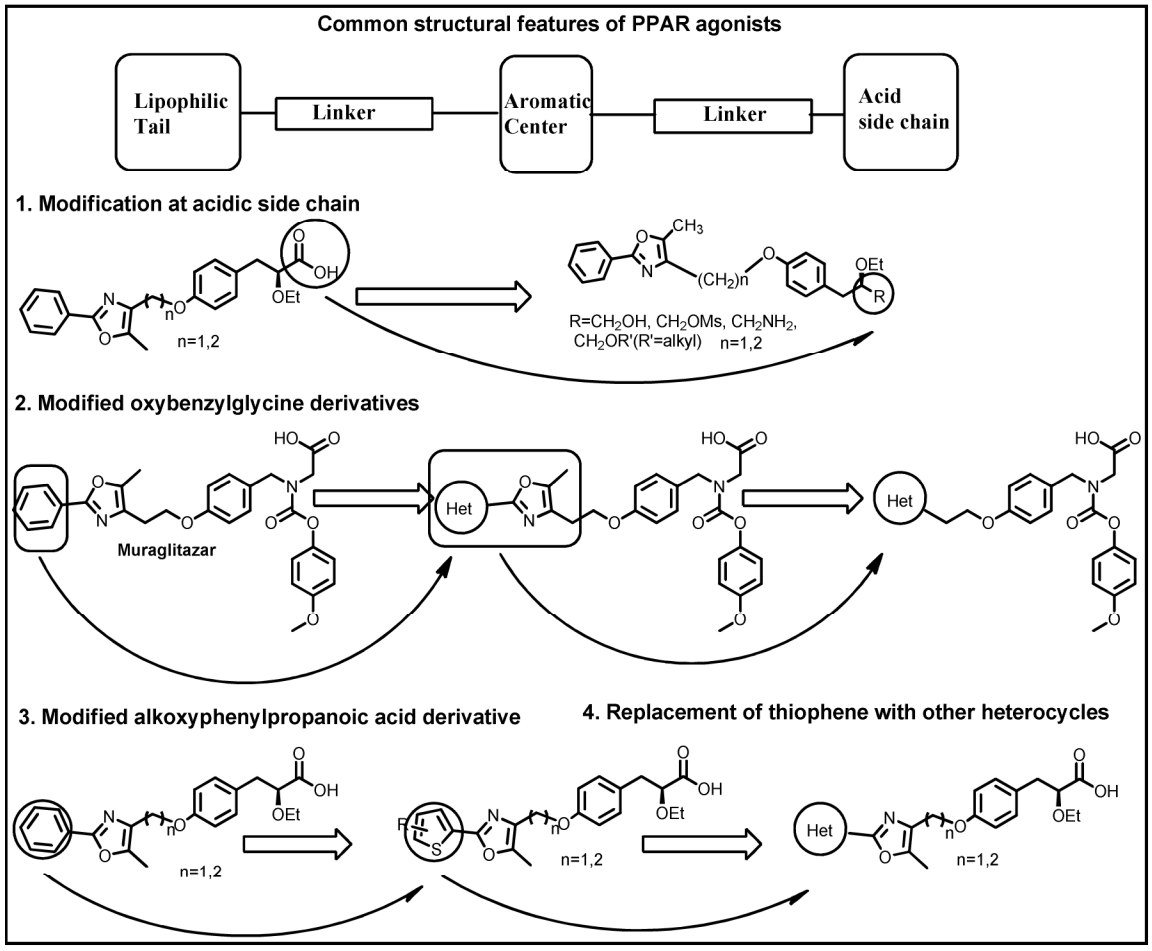


Figure 11 : Summary of novel PPARα/γ dual agonists

- Initial attempt to improve the potency of molecule was focused on modification at acidic side chain. Many potent dual PPARα/γ agonists are known with α-alkoxy acid at acidic side chain, therefore it was selected to modify further. Modifications like alcohol, ether, amine, alkyl amine, azide and mesylate were carried out. Few compounds of this series exhibited TG lowering activity but desired potency was not achieved.

- The next attempt was to modify at lipophilic tail of oxybenzylglycin derivatives. Muraglitazar which is oxybenzylglycin derivative with 5-methyl-2-phenyl-oxazole at lipophilic tail was modified. Phenyl substitution on oxazole was replaced with other heterocycles like thiophene, furan, pyridine, benzthiazole, benzoxazole, benzimidazole. Hypothesis was heterocyclic replacement of phenyl ring may increase the potency of compound. These compounds were evaluated for *in vitro* PPAR agonistic activity and *in vivo* hypolipidemic efficacy in animal models. Compounds of this series exhibited moderate PPAR *in vitro* activity. However, the *in vitro* activity was not translated to *in vivo*.
- In first attempt, modification at acidic side chain of α-alkoxy acid which is present in many of the potent PPARα/γ dual agonists was carried out. 5-methyl-2-phenyl-oxazole at lipophilic tail is known to be a potent fragment for PPAR binding. Combining these structural features, series of oxazole containing α-alkoxy acid was designed. It is hypothesized that replacement of phenyl ring on oxazole with heterocycles might increase the potency. Closely related thiophene substituted compounds turned out as potent PPARα/γ dual agonists. Thus establishing the evidence for hypothesis of combining the structural features of α-alkoxy acid and 5-methyl-2-phenyl-oxazole in single chemotype, the thiophene ring served as a good replacement of the phenyl ring. Compound **27d** exhibited potent hypoglycemic, hypolipidemic effects in SAM and db/db mice. Furthermore, there was no toxicity observed in rodents.

Thus discovered a series of novel thiophene substituted oxazole derivatives to aid in the characterization of PPARα/γ dual agonists.

- Having hypothesis validated, further modified the same scaffold with other 5-membered heterocycle furan, six membered pyridine, fused heterocycles like benzofuran, bezothiophene etc were evaluated for their PPARα/γ agonistic activity. Furan and other heterocyclic replacement shows moderate activity but not comparable to thiophene substituted analogues.
- The use of the classical bioisosterism approach by replacing phenyl ring with other heterocycles like thiophene, furan, pyridine, benzthiazole, benzoxazole, benzimidazole etc resulted retention of biological activity within different series of newly designed PPARα/γ dual agonists. Thiophene substitution gave most potent analogues. It is expected that the strategy to develop potent PPARα/γ dual agonist will provide a comprehensive treatment for type II diabetes and dyslipidemia.

2.4 References

1. Benardeau A, Benz J, Binggeli A, Blum D, Boehringer M, Grether U, Hilpert H, Kuhn B, Marki HP, Meyer M, Puntener K *et al*. **Aleglitazar, a new, potent, and balanced dual PPARalpha/gamma agonist for the treatment of type II diabetes.** *Bioorganic & Medicinal Chemistry Letters* (**2009**) 19(9):2468-2473.
2. Fagerberg B, Edwards S, Halmos T, Lopatynski J, Schuster H, Stender S, Stoa-Birketvedt G, Tonstad S, Halldorsdottir S, Gause-Nilsson I. **Tesaglitazar, a novel dual peroxisome proliferator-activated receptor alpha/gamma agonist, dose-dependently improves the metabolic abnormalities associated with insulin resistance in a non-diabetic population.** *Diabetologia* (**2005**) 48(9):1716-1725.
3. Fievet C, Fruchart JC, Staels B. **PPARalpha and PPARgamma dual agonists for the treatment of type 2 diabetes and the metabolic syndrome.** *Current Opinion in Pharmacology* (**2006**) 6(6):606-614.
4. Imoto H, Matsumoto M, Odaka H, Sakamoto J, Kimura H, Nonaka M, Kiyota Y, Momose Y. **Studies on non-thiazolidinedione antidiabetic agents. 3. Preparation and biological activity of the metabolites of tak-559.** *Chemical & Pharmaceutical Bulletin* (**2004**) 52(1):120-124.
5. Lohray BB, Lohray VB, Bajji AC, Kalchar S, Poondra RR, Padakanti S, Chakrabarti R, Vikramadithyan RK, Misra P, Juluri S, Mamidi NV *et al.* **(-)3-[4-[2-(phenoxazin-10-yl)ethoxy]phenyl]-2-ethoxypropanoic acid [(-)drf 2725]: A dual PPAR agonist with potent antihyperglycemic and lipid modulating activity.** *Journal of Medicinal Chemistry* (**2001**) 44(16):2675-2678.
6. Nissen SE, Wolski K, Topol EJ. **Effect of muraglitazar on death and major adverse cardiovascular events in patients with type 2 diabetes mellitus.** *JAMA : The Journal of the American Medical Association* (**2005**) 294(20):2581-2586.
7. Martin JA, Brooks DA, Prieto L, Gonzalez R, Torrado A, Rojo I, Lopez de Uralde B, Lamas C, Ferritto R, Dolores Martin-Ortega M, Agejas J *et al.* **2-alkoxydihydrocinnamates as PPAR agonists. Activity modulation by the incorporation of phenoxy substituents.** *Bioorganic & Medicinal Chemistry Letters* (**2005**) 15(1):51-55.

8. Ebdrup S, Pettersson I, Rasmussen HB, Deussen HJ, Frost Jensen A, Mortensen SB, Fleckner J, Pridal L, Nygaard L, Sauerberg P. **Synthesis and biological and structural characterization of the dual-acting peroxisome proliferator-activated receptor alpha/gamma agonist Ragaglitazar.** *Journal of Medicinal Chemistry* (**2003**) 46(8):1306-1317.

9. Ljung B, Bamberg K, Dahllof B, Kjellstedt A, Oakes ND, Ostling J, Svensson L, Camejo G. **AZ 242, a novel PPARalpha/gamma agonist with beneficial effects on insulin resistance and carbohydrate and lipid metabolism in ob/ob mice and obese zucker rats.** *Journal of Llipid Research* (**2002**) 43(11):1855-1863.

10. Kim E, Park CS, Han T, Bae MH, Chong W, Lee CH, Shin YA, Ahn BN, Kim MK, Shin CY, Son MH *et al*. **Design, synthesis, and evaluation of novel aryl-tetrahydropyridine PPARalpha/gamma dual agonists.** *Bioorganic & Medicinal Chemistry Letters* (**2008**) 18(18):4993-4996.

11. Kuhn B, Hilpert H, Benz J, Binggeli A, Grether U, Humm R, Marki HP, Meyer M, Mohr P. **Structure-based design of indole propionic acids as novel PPARalpha/gamma co-agonists.** *Bioorganic & Medicinal Chemistry Letters* (**2006**) 16(15):4016-4020.

12. Pingali H, Raval S, Raval P, Makadia P, Zaware P, Goel A, Suthar D, Jain M, Patel P. **Novel oxazole containing phenylpropane derivatives as peroxisome proliferator activated receptor agonists with hypolipidemic activity.** *Die Pharmazie* (**2008**) 63(7):497-502.

13. Goto S, Tsuzuki O, Iguchi S. **Effect of combination of pharmaceuticals on gastrointestinal absorption. 3. Combination of aminopyrine and barbital.** *Chemical & Pharmaceutical Bulletin* (**1971**) 19(5):944-953.

14. Brooks DA, Etgen GJ, Rito CJ, Shuker AJ, Dominianni SJ, Warshawsky AM, Ardecky R, Paterniti JR, Tyhonas J, Karanewsky DS, Kauffman RF *et al*. **Design and synthesis of 2-methyl-2-[4-(2-[5-methyl-2-aryloxazol-4-yl]ethoxy)phenoxy]propionic acids: A new class of dual PPARalpha/gamma agonists.** *Journal of Medicinal Chemistry* (**2001**) 44(13):2061-2064.

15. Devasthale PV, Chen S, Jeon Y, Qu F, Shao C, Wang W, Zhang H, Cap M, Farrelly D, Golla R, Grover G *et al*. **Design and synthesis of n-[(4-**

methoxyphenoxy)carbonyl]-n-[[4-[2-(5-methyl-2-phenyl-4-oxazolyl)ethoxy] phenyl]methyl]glycine[Muraglitazar/BMS-298585], a novel peroxisome proliferator-activated receptor alpha/gamma dual agonist with efficacious glucose and lipid-lowering activities. *Journal of Medicinal Chemistry* (**2005**) 48(6):2248-2250.

16. Patani GA, LaVoie EJ. **Bioisosterism: A rational approach in drug design.** *Chemical Reviews* (**1996**) 96(8):3147-3176.
17. Burger A. **Isosterism and bioisosterism in drug design.** *Progress in Drug Research Fortschritte der Arzneimittelforschung Progres Des Recherches Pharmaceutiques* (**1991**) 37(287-371.
18. Flosi WJ, DeGoey DA, Grampovnik DJ, Chen HJ, Klein LL, Dekhtyar T, Masse S, Marsh KC, Mo HM, Kempf D. **Discovery of imidazolidine-2,4-dione-linked hiv protease inhibitors with activity against lopinavir-resistant mutant HIV.** *Bioorganic & Medicinal Chemistry* (**2006**) 14(19):6695-6712.
19. Balakumar P, Rose M, Ganti SS, Krishan P, Singh M. **PPAR dual agonists: Are they opening pandora's box?** *Pharmacological Research :The Official Journal of the Italian Pharmacological Society* (**2007**) 56(2):91-98.
20. Cho N, Momose Y. **Peroxisome proliferator-activated receptor gamma agonists as insulin sensitizers: From the discovery to recent progress.** *Current Topics in Medicinal Chemistry* (**2008**) 8(17):1483-1507.
21. Hulin B, Newton LS, Lewis DM, Genereux PE, Gibbs EM, Clark DA. **Hypoglycemic activity of a series of alpha-alkylthio and alpha-alkoxy carboxylic acids related to Ciglitazone.** *Journal of Medicinal Chemistry* (**1996**) 39(20):3897-3907.
22. Raval P, Jain M, Goswami A, Basu S, Gite A, Godha A, Pingali H, Raval S, Giri S, Suthar D, Shah M *et al.* **Revisiting glitazars: Thiophene substituted oxazole containing alpha-ethoxy phenylpropanoic acid derivatives as highly potent PPARalpha/gamma dual agonists devoid of adverse effects in rodents.** *Bioorganic & Medicinal Chemistry Letters* (**2011**) 21(10):3103-3109.
23. Xu HE, Lambert MH, Montana VG, Parks DJ, Blanchard SG, Brown PJ, Sternbach DD, Lehmann JM, Wisely GB, Willson TM, Kliewer SA *et al.*

Molecular recognition of fatty acids by peroxisome proliferator-activated receptors. *Molecular Cell* (**1999**) 3(3):397-403.

24. Pingali H, Jain M, Shah S, Makadia P, Zaware P, Goel A, Patel M, Giri S, Patel H, Patel P. **Design and synthesis of novel oxazole containing 1,3-dioxane-2-carboxylic acid derivatives as PPAR alpha/gamma dual agonists.** *Bioorganic & Medicinal Chemistry* (**2008**) 16(15):7117-7127.

25. Palmer CN, Hsu MH, Griffin KJ, Raucy JL, Johnson EF. **Peroxisome proliferator activated receptor-alpha expression in human liver.** *Molecular Pharmacology* (**1998**) 53(1):14-22.

26. Hellmold H, Zhang H, Andersson U, Blomgren B, Holland T, Berg AL, Elebring M, Sjogren N, Bamberg K, Dahl B, Westerberg R *et al*. **Tesaglitazar, a PPARalpha/gamma agonist, induces interstitial mesenchymal cell DNA synthesis and fibrosarcomas in subcutaneous tissues in rats.** *Toxicological Sciences : An Official Journal of the Society of Toxicology* (**2007**) 98(1):63-74.