Publications

"Information is a source of learning. But unless it is organized, processed and available to the right people in the format for decision making, it is a burden not a benefit"

William pollard

8. PUBLICATIONS

List of Publications

1. Design and Synthesis of Novel Oxazole Containing 1,3-Dioxane-2-carboxylic acid Derivatives as PPARα/γ Dual Agonists. Harikishore Pingali, Mukul Jain, Shailesh Shah, Pankaj Makadia, Pandurang Zaware, Ashish Goel, Megha Patel, Suresh Giri, Harilal Patel and Pankaj Patel *Bioorganic & Medicinal Chemistry*, **2008**, *16*, 7117-7127.

2. Discovery of a highly orally bioavailable *c*-5-[6-(4-Methanesulfonyloxyphenyl)hexyl]-2-methyl-1,3-dioxane-*r*-2-carboxylic acid as a potent hypoglycemic and hypolipidemic agent. Harikishore Pingali, Mukul Jain, Shailesh Shah, Sujay Basu, Pankaj Makadia, Amitgiri Goswami, Pandurang Zaware, Atul Godha, Suresh Giri, Ashish Goel, Megha Patel, Harilal Patel and Pankaj Patel *Bioorganic & Medicinal Chemistry Letters,* **2008**, *18*, 5586-5590.

3. Modulation of PPAR receptor sub type selectivity of the ligands: Aliphatic chain vs aromatic ring as a spacer between pharmacophore and the lipophilic moiety. Harikishore Pingali, Mukul Jain, Shailesh Shah, Pravin Patil, Pankaj Makadia, Pandurang Zaware, Kalapatapu V. V. M. Sairam, Jeevankumar Jamili, Ashish Goel, Megha Patel, and Pankaj Patel *Bioorganic & Medicinal Chemistry Letters,* **2008**, *18*, 6471-6475.

4. Design and synthesis of novel bisoximinoalkanoic acids as potent PPARα agonists. Harikishore Pingali, Mukul Jain, Shailesh Shah, Pandurang Zaware, Pankaj Makadia, Suresh Pola, Baban Thube, Darshit Patel, Pravin Patil, Priyanka Priyadarshini, Dinesh Suthar, Maanan Shah, Suresh Giri and Pankaj Patel *Bioorganic & Medicinal Chemistry Letters,* **2010**, 20, 1156-1161.

5. Design and Synthesis of Novel 1,3-Dioxane-2-carboxylic acid Derivatives as PPARα/γ Dual Agonists. Harikishore Pingali, Mukul Jain, Shailesh Shah, Pankaj Makadia, Pandurang Zaware, Jeevankumar Jamili, Kalapatapu V. V. M. Sairam, Pravin Patil, Dinesh Suthar, Suresh Giri, Harilal Patel and Pankaj Patel. (Communicated).

Bioorganic & Medicinal Chemistry 16 (2008) 7117–7127

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Design and synthesis of novel oxazole containing 1,3-Dioxane-2-carboxylic acid derivatives as PPAR α/γ dual agonists ☆

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ARTICLE INFO

Article history:
Received 30 May 2008
Revised 24 June 2008
Accepted 26 June 2008
Available online 28 June 2008

Keywords:
PPAR agonist
Type 2 diabetes
Oxazole
1,3-Dioxane carboxylic acid

ABSTRACT

A few novel 1,3-dioxane carboxylic acid derivatives were designed and synthesized to aid in the characterization of PPAR α/γ dual agonists. Structural requirements for PPARα/γ dual agonism of 1,3-dioxane carboxylic acid derivatives included the structural similarity with potent glitazones in fibric acid chemotype. The compounds with this pharmacophore and substituted oxazole as a lipophilic heterocyclic tail were synthesized and evaluated for their in vitro PPAR agonistic potential and in vivo hypoglycemic and hypolipidemic efficacy in animal models. Lead compound 2-methyl-c-5-[4-(5-methyl-2-(4-methylphenyl)-oxazol-4-ylmethoxy)-benzyl]-1,3-dioxane-r-2-carboxylic acid **13b** exhibited potent hypoglycemic, hypolipidemic and insulin sensitizing effects in *db/db* mice and Zucker *fa/fa* rats.

1. Introduction

Type 2 diabetes is a complex metabolic disorder characterized by hyperglycemia, insulin resistance, and defects in insulin secretion and is usually associated with dyslipidemia, hypertension and obesity. Detailed pathophysiology of this disease remains incompletely understood. However, the probable reasons for the development of this disease are metabolic defects in the liver, pancreatic β-cells, adipose tissue and skeletal muscle. Though it was thought to be mainly a disorder of carbohydrate metabolism. Since hyperglycemia being the main symptom of this disease, it is evident from the advanced research that abnormalities in fat metabolism play a central role in the pathogenesis of this disease.[1–3] The peroxisome proliferation-activated receptors (PPARs) are ligand-activated transcription factors belonging to nuclear hormone receptor superfamily.[4,5] Three distinct PPAR subtypes (PPARα, PPARγ and PPARδ) have been identified in most mammalian species and their physiological roles in glucose homeostasis, fatty acid metabolism and cellular differentiation have been reviewed extensively.[6–10] PPARγ is well known for its role in adipogenesis at a cellular level and insulin sensitization.[11,12] PPARγ agonists, such as thiazolidinediones (TZDs or glitazones) have proven to be efficacious as insulin sensitizing agents in the treatment of type 2 diabetes.[13–15] Rosiglitazone and Pioglitazone (Fig. 1) belong to this class and are currently available in the market. PPARα is known to play an important role in fatty acids oxidation and lipoprotein metabolism.[16] Fibrates (**Fenofibrate, Clofibrate, and Bezafibrate**) and similar compounds like **WY-14643** (Fig. 2) show effects such as lowering triglycerides and elevating HDL levels through activation of PPARα.[17–23] The majority of type 2 diabetes patients suffer from atherogenic lipid abnormalities in addition to insulin resistance, termed as metabolic syndrome,[24] and given the importance of controlling both glucose and lipid levels in metabolic syndrome. This gave rise to the concept of identifying dual agonists, which can activate both PPARα and PPARγ. In addition to their hypolipidemic effects, fibrates reduce body weight gain in rodents without affecting food intake[25,26] and led to a hypothesis that probably activation of PPARα may mitigate the weight gain induced by PPARγ activation in humans. This hypothesis that PPARα/γ dual agonism would provide synergistic pharmacological effects has encouraged many research groups to develop these agents (Fig. 3) but none of these dual agonists including Farglitazar,[27] Ragaglitazar,[28] Tesaglitazar,[29] and Muraglitazar[30–32] has been marketed. These facts made the development of PPARα/γ dual agonists with distinct biological and safety profiles a challenge among the drug discovery groups around the world as the medical need for metabolic disorders is largely remain unmet. In continuation of our research in the field of PPARs to develop novel therapeutic agents to treat metabolic disorders[33–35] we herein report an initial SAR of novel 1,3-dioxane carboxylic acid derivatives which are shown to be potent PPARα/γ dual agonist.

☆ ZRC communication # 232.

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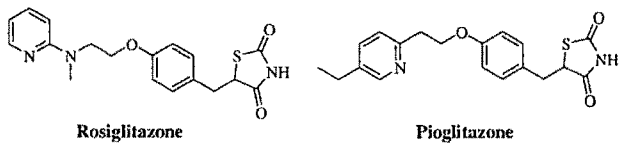
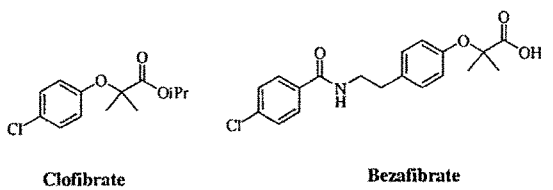
doi:10.1016/j.bmc.2008.06.050

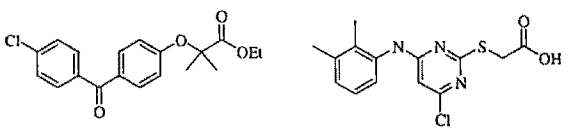


Rosiglitazone Pioglitazone

Figure 1. PPARγ agonists.

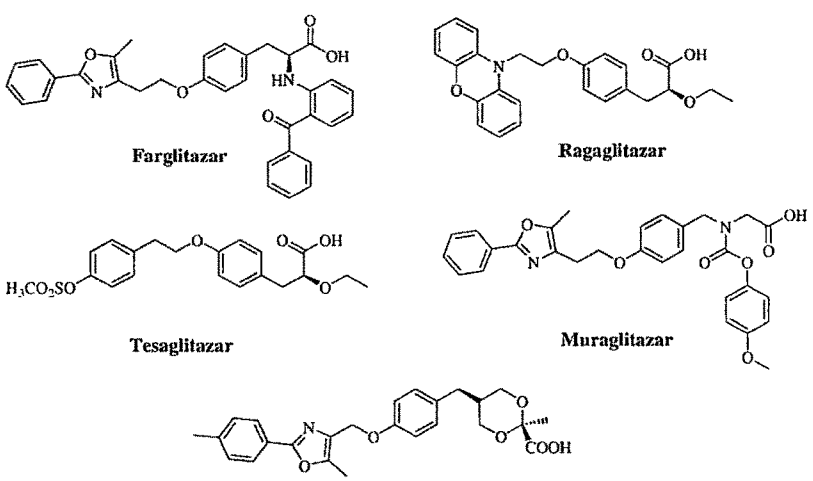


Clofibrate Bezafibrate



Fenofibrate WY-14643

Figure 2. PPARα agonists.



Farglitazar Ragaglitazar

Tesaglitazar Muraglitazar

13b

Figure 3. PPARα/γ dual agonists.

2. Design concept for 1,3-dioxane-2-carboxylic acids as PPAR α/γ dual agonists

Fibric acid is the key pharmacophore of PPARα ligands like **Fenofibrate**, **Clofibrate**, and **Bezafibrate** (Fig. 2). All the glitazone class of compounds possess 2,4-thiazolodinedione as key pharmacophore as in **Rosiglitazone** and **Pioglitazone** (Fig. 1). We intended to design compounds with a novel pharmacophore possessing the features of both fibric acid and glitazone hoping that these compounds can be developed as PPARα/γ dual agonists (Fig. 4). We started the structural design by introducing an oxygen atom on the carbon alpha to carboxylic acid of fibric acid chemotype and cyclizing with the aryl oxygen forming a 1,3-dioxane ring connected to phenyl ring either directly through a bond or with a methylene or ethylene group in-between forming the key pharmacophore of the novel compounds. The remaining part of the structure mimics the typical PPAR agonist comprising of a heterocyclic tail connected to a acidic head with a alkoxy spacer. The newly designed pharmacophore resembles glitazones structurally and possesses free carboxylic function resembling fibric acid pattern and

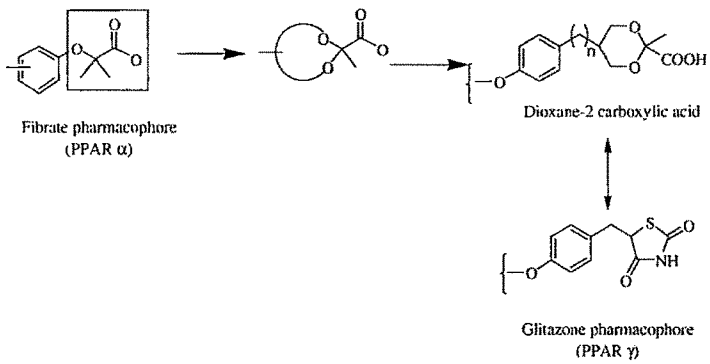
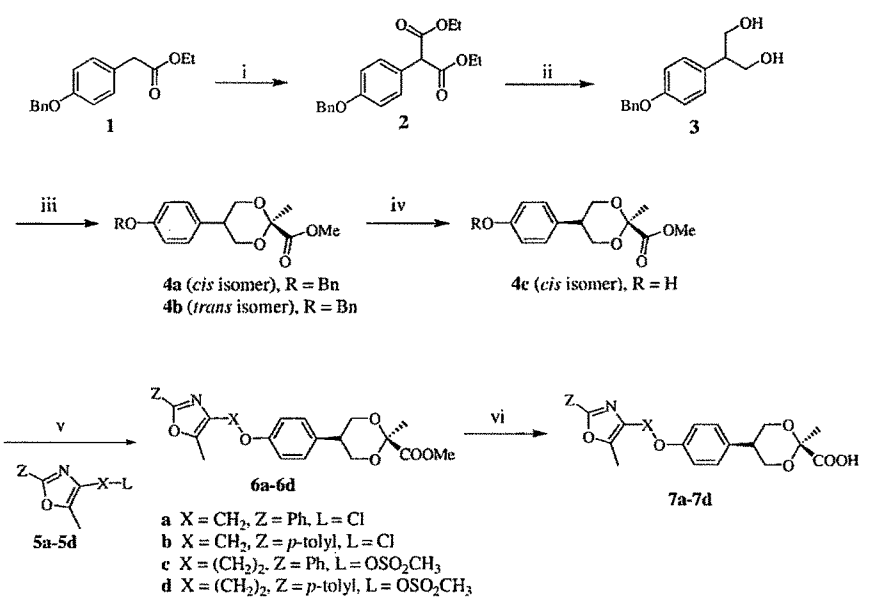


Figure 4. Design of dioxane carboxylic acids.

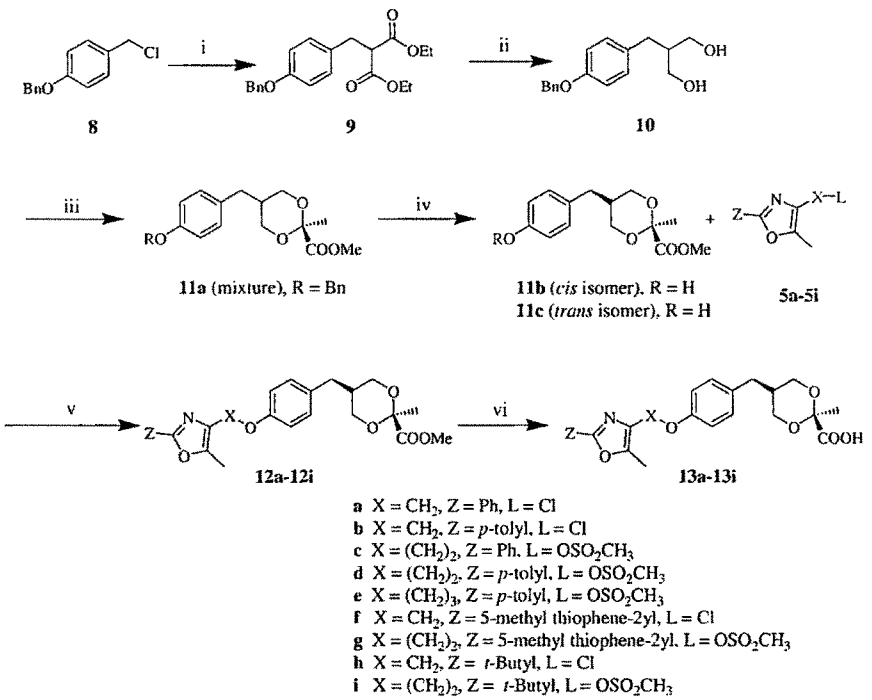
provides rationale to study these compounds as dual PPARα/γ agonists. Few compounds containing 1,3-dioxane carboxylic acid pharmacophore are reported earlier as selective PPARα agonists.[36,37]

3. Chemistry

Compounds **7a–d** were synthesized as described in Scheme 1. Reaction of phenylacetate **1** with diethylcarbonate in presence of NaH gave the diester **2** which was reduced with $LiAlH_4$ to give the diol **3**. Dioxane ring formation was brought about by the Reaction of **3** with methyl pyruvate in presence of borontrifluoride diethylether complex which gave the cyclized compound as a mixture of diastereomeric isomers and the ratio of *cis*- to *trans*-isomers determined by HPLC was found to be 3:1. These isomers were separated by means of column chromatography to give *cis*-isomer **4a** and *trans*-isomer **4b**. Both of these isomers showed ^{1}HNMR chemical shifts identical with other 1,3-dioxane derivatives reorted.[37,38] Pure *cis*-isomer **4a** was subjected to debenzylation under hydrogenation conditions to obtain phenolic intermediate **4c**. Reaction of the intermediate **4c** with **5a–d** (Compounds **5a–i** were synthesized following the procedures reported.[39]) in presence of K_2CO_3 gave the esters **6a–d**, which upon hydrolysis under basic conditions gave the acids **7a–d**. Synthesis of compounds **13a–i** is illustrated in Scheme 2. Diester **9**, synthesized from **8** by reacting with diethylmalonate in presence of NaH was reduced to diol **10** with $LiAlH_4$. Transformation of **10** to dioxane **11a** (mixture of *cis*- and *trans*-isomers) was achieved by the treatment of **10** with methyl-pyruvate under the conditions described above, and the ratio of *cis*- to *trans*-isomer in this case as determined by HPLC was



Scheme 1. Reagents and conditions: (i) 60% NaH, diethyl carbonate, THF, 25 °C, 18 h; (ii) $LiAlH_4$, THF, 25 °C, 6 h; (iii) methyl pyruvate, 98% BF_3 ethyrate complex, CH_3CN, 25 °C, 4 h; (iv) 10% Pd/C, ammonium formate, MeOH, reflux, 2 h; (v) K_2CO_3, DMF, 60 °C, 18 h; (vi) $LiOH{\cdot}H_2O$, THF, H_2O, MeOH, 25 °C, 18 h.



Scheme 2. Reagents and conditions: (i) 60% NaH, diethyl malonate, THF, 25 °C, 14 h; (ii) $LiAlH_4$, THF, 25 °C, 6 h; (iii) methyl pyruvate, 98% BF_3 ethyrate complex, CH_3CN, 25 °C, 4 h; (iv) 10% Pd/C, ammonium formate, MeOH, reflux, 2 h; (v) K_2CO_3, DMF, 60 °C, 18 h; (vi) $LiOH{\cdot}H_2O$, THF, H_2O, MeOH, 25 °C, 18 h.

approximately 4:1. Surprisingly, the attempts to separate the *cis*- and *trans*-isomers of **11a** by column chromatography were unsuccessful. However, separation by recrystallization was successful after debenzylating the mixture of isomers **11a** under hydrogenation conditions using Pd/C and ammoniumformate. Pure *cis*-isomer **11b** was obtained quantitatively as first crop on recrystallization from a mixture of 1:2 ethyl acetate and hexane, whereas *trans*-isomer **11c** was obtained only after repeated crystallizations from a mixture of 1:1 ethyl acetate and hexane in minor quantity. Coupling of *cis* intermediate **11b** with **5a–i** in presence of potassium carbonate in DMF gave the esters **12a–i**, which were hydrolyzed under aqueous alkaline conditions to give the carboxylic acids **13a–i**.

4. Results and discussion

Compounds **7a–d** and **13a–i** were screened for hPPAR α, γ, and δ agonistic activity on full length PPAR receptor transfected in HepG2 cells. **WY-14643** (Fig. 2), **Rosiglitazone** (Fig. 1), and **GW-501516**[40] were used as controls for PPARα, and δ, respectively, and the results are summarized in Table 1 where the activities were reported as fold induction as well as EC_{50}. A typical chemical structural design of PPAR ligands comprises of an acidic head as pharmacophore which is connected to an aromatic ring mostly the phenyl ring which inturn connected to a lipophilic tail group through a teether-like alkoxy group. Initially to start with the synthesis of novel compounds we chose phenyl dioxane carboxylic acid as pharmacophore which contained acidic head connected to phenyl ring mimicking a typical pharmacophore chemotype of PPAR agonist. Then we have selected 5-methyl-2-phenyl-oxazole group as lipophilic tail for the reason that this heterocycle being used extensively in PPAR drug discovery research. With this plan we have synthesized compounds **7a–d**. Both compounds **7a** and **7c** containing phenyl ring at 2-position on oxazole with a teether of methylene and ethylene, respectively, showed moderate PPARα activity. When the phenyl ring at 2-position on oxazole was substituted with a methyl group at metabolically susceptible *para* position compound **7b** with methylene teether showed similar activity as **7a** but the compound **7d** did not show any PPAR activity. But none of the compounds showed superior activity than the control compounds in terms of their fold induction and hence these compounds were not evaluated for their EC_{50} values. Then we intended to synthesize compounds **13a–i**, which resembles glitazone structure more closely. Compound **13a** with phenyl ring at 2-position of oxazole and a methylene teether showed 0.096 μM activity on PPARγ and 1 μM activity on PPARα, whereas the compound **13c** with ethylene spacer showed inferior and contradictory results with 0.27 μM activity on PPARα and 4 μM on PPARγ. When the phenyl ring in compounds **13a** and **13c** was substituted with a methyl group at *para* position the respective resulting compounds **13b** and **13d** exhibited superior activity compared to parent compounds and surprisingly compound **13b** was found equipotent towards PPARα and γ with 0.07 and 0.015 μM EC_{50}, respectively. The further elongation of the teether to propylene group as in compound **13e** found detrimental to PPAR affinities. Having done this we then wanted to replace the phenyl ring on oxazole with 5-methyl thiophene which was selected from a lead compound of our in-house library and the resulting compounds **13f** and **13g** showed interesting PPAR activity. **13f** found to be 30-fold more selective towards PPARγ whereas **13g** was 8-fold more selective

Table 1
In vitro data of compounds **7** and **13**

Compound	X	Z	hPPAR transactivation[a]				
			α (10 μM)[b]	γ (0.2 μM)[b]	δ (10 μM)[b]	$EC_{50}\alpha$ (μM)	$EC_{50}\gamma$ (μM)
7a	Methylene	Phenyl	3.0	1.6	1.4	ND	ND
7b	Methylene	4-methylphenyl	2.7	1.5	1.2	ND	ND
7c	Ethylene	Phenyl	3.0	1.5	1.3	ND	ND
7d	Ethylene	4-methylphenyl	IA	1.6	1.5	ND	ND
13a	Methylene	Phenyl	6.5	11.4	1.7	1.09	0.096
13b	Methylene	4-methylphenyl	8.2	12.7	IA	0.072	0.015
13c	Ethylene	Phenyl	5.3	3.9	IA	0.272	4.096
13d	Ethylene	4-methylphenyl	6.3	3.4	IA	0.089	1.385
13e	Propylene	4-methylphenyl	1.8	1.8	IA	ND	ND
13f	Methylene	5-methyl thiophene-2yl	5.6	11.5	1.0	0.6	0.0198
13g	Ethylene	5-methyl thiophene-2yl	6.8	8.9	1.1	0.03	0.239
13h	Methylene	*tert*-Butyl	2.3	3.2	IA	ND	ND
13i	Ethylene	*tert*-Butyl	5.8	4.2	1.2	4.2	5.01
Vehicle			1.0	1.0	1.0		
WY-14643			4.4	ND	ND	4.8	ND
Rosiglitazone			ND	11.6	ND	ND	0.05
GW-501516@2 nM			ND	ND	4.3	ND	ND

[a] IA denotes inactive where compounds did not shows any fold induction above the basal level shown by vehicle and ND denotes not determined.
[b] Activities are presented as fold induction of PPAR α, γ and δ activation.

towards PPARα. Replacing phenyl ring with *tert*-butyl group made the compounds **13h** and **13i** inactive towards PPARα and γ. In all of the above compounds except **13b** methylene teether made the compound more potent towards PPARγ whereas the ethylene group made the compounds more selective towards PPARα. Compound **13b** which was found equally potent towards PPARα and γ was selected as a lead compound and its pharmacokinetic behaviour was studied in male *Wistar* rats and the results are summarized in Table 2. Based on the cell-based activities and pharmacokinetic behaviour we then wished to evaluate compound**13b** in *db/db* mice and Zucker *fa/fa* rats for its hypolipidemic and hypoglycemic activities. When dosed orally to *db/db* mice at a dose of 3 mg/kg/day for 6 days **13b** reduced plasma glucose (PG) by 57% and triglycerides (TG) by 50% as depicted in Table 3. Subsequently compound **13b** when administered orally to male Zucker *fa/fa* rats at a dose of 3 mg/kg/day for 14 days normalized glucose tolerance and significantly reduced fasted insulin to an extent of 77%. Additionally, **13b** reduced plasma TG by 71% and total cholesterol (TC) by 30% (Table 4). The above results indicate that compound **13b** showed hypoglycemic, hypolipidemic and insulin sensitizing comparable to **Rosiglitazone** and **Tesaglitazar**.

Table 2
Mean pharmacokinetic parameters[a] of **13b** 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)
13b	Oral	30	1.7 (±0.06)	54 (±4.6)	2.7 (±0.2)	351 (±51)

[a] Values indicate mean ± SD for $n = 6$.

Table 3
In vivo efficacy of the compound **13b** in *db/db* mice

Compound	Dose (mg/kg/day)	% Change	
		TG	PG
13b	3	−50	−57
Rosiglitazone	30	−41	−54
Tesaglitazar	3	−60	−54

Table 4
In vivo efficacy of the compound **13b** in Zucker *fa/fa* rats

Compound	Dose (mg/kg/day)	% Change			% Improvement in glucose AUC
		TG	TC	Fasted insulin	
13b	3	−71	−30	−77	51
Rosiglitazone	30	−57	−17	−78	49
Tesaglitazar	3	−67	−16	−91	51

5. Conclusion

We discovered a novel series of oxazole containing 1,3-dioxane-2-carboxylic acid which are PPARα/γ dual agonists as exemplified by the lead compound **13b**. The pharmacophore was designed by incorporating structural features of glitazones in fibric acid chemotype and optimized using oxazole tail. Lead compound **13b** was found to be a potent PPARα/γ dual agonist and reduced plasma glucose and triglycerides significantly in *db/db* mice. The same compound normalized glucose tolerance and reduced fed insulin in Zucker *fa/fa* rats and exhibited favourable pharmacokinetic parameters in rodent model. Further work in the development of SAR of this lead series based on the benzyl dioxane carboxylic acid core will be described in a subsequent publication.

6. Experimental Section

6.1. In vitro PPAR transactivation assay

6.1.1. Cell culture

HepG2 cells (ATCC, USA) were maintained in growth medium composed of MEM (Sigma) supplemented with 10% FBS (Hyclone), 1× MEM non-essential amino acid (Sigma) and 1 mM sodium pyruvate and 1% penicillin/streptomycin (Sigma).

6.1.2. Transient transfection

HepG2 cells were seeded in 24-well plates at a density of 400,000 cells/well in 1 mL of medium per well. Cells were transfected using the transfection reagent Superfect (Qiagen). Cells were transfected with 0.08 μg of the pSG5 expression vector containing the cDNA of PPARα or 0.08 μg of the pSG5 expression vector containing the cDNA of PPARγ was cotransfected with PPRE3-TK-luc. Cells were incubated at 37 °C, 5% CO_2 for 3 h. After this, 1.0 mL of the medium containing the respective ligands to the respective wells were added. The cells were then incubated at 37 °C, 5% CO_2 for 20–22 h. After the incubation period, cells were first washed with PBS, lysed and supernatant collected. Supernatant was then

assayed for luciferase and β-galactosidase activity. The luciferase activity was determined using commercial fire-fly luciferase assay according to the suppliers's instructions [Promega] in white 96-well plate [Nunc]. β-Galactosidase activity was determined in ELISA reader at 415 nm.

6.2. In vivo studies (mice and rats)

All animals were used from inbred colony which are maintained on standard laboratory rodent chow ad libitum, and the study protocols were approved by Institutional Animal Ethics Committee.

6.2.1. *db/db* Mice and Zucker *fa/fa* rats experiments

Male *db/db* mice of 12–14 weeks age and 30–40 g body weight and male Zucker *fa/fa* rats of 13–15 weeks age and body weight of 450–470 g were selected for the study. The animals were weighed and tail-bled prior to the start of study. Plasma was analyzed for glucose (PG), triglyceride (TG) levels in *db/db* mice and PG, TG and cholesterol (TC) levels in Zucker *fa/fa* rats. The animals were arranged into the appropriate number of groups with each group having 6 animals of the same mean PG, TG and TC levels prior to dosing. All animals then were orally dosed once daily with vehicle (0.5% methylcellulose in water) and test compounds for 6 days in *db/db* mice and for 14 days in Zucker *fa/fa* rats. All animals were fed ad libitum throughout the study. Approximately, 1 h after the last dose, the animals were bled and the plasma was analyzed for glucose and triglycerides (also cholesterol in Zucker *fa/fa* rats) to calculate percent change due to drug treatment (This takes into account any changes that may have occurred in the vehicle-treated animals during the study).

6.2.2. Glucose tolerance experiments

On day 15 Zucker *fa/fa* rats were fasted overnight, insulin levels were measured and given a 2 g/kg oral glucose load. Blood glucose was measured just prior to the glucose load and after 30, 60 and 120 min by collecting blood from tail tip. The glucose area-under-the-curve (AUC) was calculated over 0 to 120 min using the trapezoidal method and result was reported as percent improvement in glucose AUC versus vehicle treated control group.

6.2.3. Pharmacokinetics experiment

Pharmacokinetics of the test compound **13b** was studied *via* per-oral route of administration in *wistar* rats of 8 to 10 weeks of age. Animals were fasted for 18 hours and food was supplied after 4 hours of administration of the test compound. There was free access to water throughout the study. A homogenous suspension of the test substance was prepared in 0.5% w/v CMC in normal saline and a per-oral dose of 30 mg/kg was administered. After the administration of the test compounds, blood samples were withdrawn at various time intervals through retro-orbital plexus and collected into heparinized micro centrifuge tubes. Plasma was separated by centrifugation at 4000 rpm for 5 min at ambient temperature and analyzed immediately. Remaining samples were stored at −20 °C until analyzed.

Analysis was carried out by taking an aliquot of 180 μL plasma and 20 μL of internal standard (Atorvastatin), and was extracted with 2.5 mL of extracting solvent (ethyl acetate: acetonitrile 80:20, v/v) in glass test-tube by vortexing with spinix vortex mixture for a minute. This was then centrifuged at 2000 rpm for 2.0 min. The supernatant was transferred to another glass test-tube and the solvent was evaporated under nitrogen using Zymark evaporator at 40 °C. Finally, the tubes were reconstituted with 0.1 mL diluent (acetonitrile:methanol:water 40:40:20, v/v/v). The reconstituted samples were analyzed on Agilent 1100 Series HPLC system with a mobile phase of 0.05% v/v trifluoroacetic acid in water: acetonitrile (32:68, v/v); flowing at a flow rate of 1.0 mL/min through a Kromasil 250 mm × 4.6 mm × 5 μ column maintained at 30 °C. Chromatographic separation was achieved within 15 min. Agilent software version Chemstation Rev.A.09.01. (1206) was used to acquire and process all chromatographic data. Quantification was based on a series of calibrators ranging from 0.031 to 32 μg/mL, prepared by adding test compound to drug free rat plasma. Quality control samples were analyzed in parallel to verify that the system performs in control. Pharmacokinetic parameters namely maximum plasma concentration (C_{max}), time point of maximum plasma concentration (t_{max}), area under the plasma concentration-time curve from 0 h to infinity ($AUC_{0-\infty}$) and half-life of drug elimination during the terminal phase ($t_{1/2}$) were calculated from plasma concentration *versus* time data, by standard non-compartmental methods, using the WinNonLin software version 4.0.1 procured from Pharsight Corporation, USA.

6.3. Synthesis

6.3.1. Synthetic materials and methods

Reagents and solvents were obtained from commercial suppliers and used without further purification. Flash chromatography was performed using commercial silica gel (230-400 mesh). Melting points were determined on a capillary melting point apparatus and are uncorrected. IR spectra were recorded on a Shimadzu FT IR 8300 spectrophotometer (Vmax in cm^{-1}, using KBr pellets or Nujol). The ^1H NMR spectra were recorded on a Brucker Avance-300 spectrometer (300 MHz). The chemical shifts (δ) are reported in parts per million (ppm) relative to TMS, either in $CDCl_3$ or DMSO-d_6 solution. Signal multiplicities are represented by s (singlet), d (doublet), dd (doublet of doublet), t (triplet), q (quartet), br s (broad singlet), and m (multiplet). $^{13}CNMR$ spectra were recorded on Brucker Avance-400 at 100 MHz either in $CDCl_3$ or in DMSO-d_6 solution. Mass spectra (ESI-MS) were obtained on Shimadzu LC–MS 2010-A spectrometer. HPLC analysis was carried out at λ_{max} 220 nm using column ODS C-18, 150 nm * 4.6 nm * 4 μ on AGILENT 1100 series.

6.3.2. Diethyl 2-(4-benzyloxyphenyl)malonate (2)

To an ice-cold suspension of NaH (60%) (4.4 g, 0.111 mol) in THF (30 mL), a solution of **1** (10.0 g, 0.037 mol) in THF (50 ml) was added drop wise over a period of 30 min at 0–10 °C and stirred at the same temperature for 30 min. Diethyl carbonate (18 mL, 0.148 mol) was added to the reaction mixture at 0–10 °C and stirred at 25 °C for 18 h. The reaction mixture was poured into ice-cold water (200 mL) and extracted with ethyl acetate (3× 100 mL). The organic layer was washed with water and brine, dried over Na_2SO_4 and evaporated under reduced pressure to yield 15 g crude product as thick oil. The crude product was purified by column chromatography (10% ethyl acetate in hexane, 230–400 mesh silica gel) to give title compound **2** as off-white solid (6.5 g). Yield: 51%; mp: 58–60 °C; purity by HPLC: 90%; IR (KBr): 1743, 1726, 1247, 1226, 1177, 1010, 750 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.25 (t, J = 7.11 Hz, 6H), 4.15–4.55 (m, 4H), 4.55 (s, 1H), 5.05 (s, 2H), 6.91 (d, J = 8.6 Hz, 2H), 7.28 (d, J = 8.6 Hz, 2H), 7.35–7.45 (m, 5H); ESI-MS *m/z*: 343.2 $(M+H)^+$.

6.3.3. 2-(4-Benzyloxyphenyl)propane-1,3-diol (3)

To a solution of **2** (6.0 g, 0.0175 mol) in THF (100 mL), $LiAlH_4$ (1.33 g, 0.035 mol) was added in small portions at 0 °C over a period of 30 min. and stirred at 25 °C for 6 h. The excess $LiAlH_4$ was quenched by dropwise addition of saturated aqueous Na_2SO_4 solution at 0–10 °C. Solid residue was filtered and washed with ethyl acetate. Filtrate was evaporated under reduced pressure. Crude product was triturated in diisopropyl ether to give title compound **3** as white solid (1.76 g). Yield: 39%; mp: 130–132 °C; purity by HPLC: 93%; IR (KBr): 3278, 2943, 2292, 2868, 1514, 1226, 1026,

and 740 cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.01 (br s, 2H), 3.02–3.11 (m, 1H), 3.93–3.97 (m, 4H), 5.05 (s, 2H), 6.95 (d, J = 8.6 Hz, 2H), 7.16 (d, J = 8.6 Hz, 2H), 7.32–7.44 (m, 5H); ESI-MS m/z: 276.2 $(M+NH_4)^+$.

6.3.4. Methyl *c*-5-(4-benzyloxyphenyl)-2-methyl-1,3-dioxane-*r*-2-carboxylate (4a)

To a solution of **3** and methyl pyruvate (1.14 mL, 15.5 mmol) in acetonitrile (15 mL), BF_3 etherate (98%) (0.98 mL, 7.7 mmol) was added dropwise at 25 °C and stirred at the same temperature for 4 h. The reaction mixture was poured into an ice cold aqueous sodium bicarbonate solution (50 mL) and extracted with ethyl acetate (3× 20 mL). The organic layer was washed with water and brine, dried over Na_2SO_4 and evaporated under reduced pressure to give mixture of *cis*- and *trans*-isomers which were separated by means of flash chromatography on a silicagel column using 10% ethylacetate in hexane as eluent to yield 0.55 g of title compound **4a** and 0.52 g of *trans*-isomer **4b** as white solids. Yield: 41%; mp: 84–86 °C; purity by HPLC: 98%; IR (KBr): 1739, 1514, 1236, 1218, 1195, 1139, 1043, and 748 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.58 (s, 3H), 3.15–3.24 (m, 1H), 3.82 (d, J = 11.8 Hz, 2H), 3.87 (s, 3H), 4.02–4.08 (dd, J = 11.8 and 4.7 Hz, 2H), 5.03 (s, 2H), 6.91 (d, J = 8.6 Hz, 2H), 7.03 (d, J = 8.6 Hz, 2H), 7.32–7.42 (m, 5H); ESI-MS m/z: 360.3 $(M+NH_4)^+$.

6.3.5. Methyl *t*-5-(4-benzyloxyphenyl)-2-methyl-1,3-dioxane-*r*-2-carboxylate (4b)

The latter fractions eluted from the column in previous experiment gave 0.52 g of *trans*-isomer **4b** as white solid. Yield: 39%; mp: 110–112 °C; purity by HPLC: 97%; IR (KBr): 1739, 1514, 1236, 1218, 1195, 1139, 1043, and 748 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 1.60 (s, 3H), 2.69–2.71 (m, 1H), 3.86 (s, 3H), 4.04–4.09 (dd, J = 11.9 and 2.3 Hz, 2H), 4.20–4.25 (dd, J = 12 and 3.6 Hz, 2H), 5.06 (s, 2H), 6.94 (d, J = 8.6 Hz, 2H), 7.29–7.44 (m, 7H); ESI-MS m/z: 360.3 $(M+NH_4)^+$.

6.3.6. Methyl *c*-5-(4-hydroxyphenyl)-2-methyl-1,3-dioxane-*r*-2-carboxylate (4c)

To a suspension of Pd/C (10%) (55 mg) in MeOH (5 mL), a solution of **4a** (550 mg, 1.6 mmol) in MeOH (10 mL) and ammonium formate (405 mg, 6.4 mmol) was added and refluxed for 2 h. The reaction mixture was filtered through Celite, and filtrate was evaporated under reduced pressure. The residue was dissolved in ethyl acetate (50 mL), washed with water and brine, dried over Na_2SO_4 and evaporated under reduced pressure to give title compound **4c** as white solid (400 mg). Yield: 99%; mp: 65–67 °C; purity by HPLC: 99%; IR (KBr): 1724, 1519, 1263, 1028, and 817 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.58 (s, 3H), 3.14–3.23 (m, 1H), 3.81 (d, J = 11.8 Hz, 2H), 3.88 (s, 3H), 4.02–4.13 (dd, J = 11.9 and 4.6 Hz, 2H), 4.98 (br s, 1H), 6.77 (d, J = 8.4 Hz, 2H), 6.99 (d, J = 8.4 Hz, 2H); ^{13}C NMR: (100 MHz, DMSO-d_6): δ 25.72, 38.43, 52.39, 67.48, 97.51, 115.39, 127.44, 128.56, 156.59, 170.43; ESI-MS m/z: 274.8 $(M+Na)^+$.

6.3.7. Methyl 2-methyl-*c*-5-[4-(5-methyl-2-phenyloxazol-4-ylmethoxy)phenyl]- 1,3-dioxane-*r*-2-carboxylate (6a)

To a solution of **4c** (600 mg, 2.38 mmol) and 4-chloromethyl-5-methyl-2-phenyl-oxazole **5a** (494 mg, 2.38 mmol) in dry DMF (10 mL), K_2CO_3 (657 mg, 4.76 mmol) was added and reaction mixture was stirred at 60 °C for 20 h. Reaction mixture was poured into ice-cold water (20 mL) and extracted with ethyl acetate (3× 20 mL). The organic layer was washed with water and brine, dried over Na_2SO_4 and evaporated under reduced pressure. The crude product was purified by column chromatography (8% ethyl acetate in hexane) to give title compound **6a** as white solid (850 mg). Yield: 84%; mp: 82–84 °C; purity by HPLC: 96%; IR (KBr): 1739, 1610, 1585, 1269, 1234, 1218, 1147, and 700 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.58 (s, 3H), 2.42 (s, 3H), 3.16–3.25 (m, 1H), 3.83 (d, J = 11.8 Hz, 2H), 3.88 (s, 3H), 4.03–4.12 (dd, J = 11.8 and 4.6 Hz, 2H), 4.96 (s, 2H), 6.96 (d, J = 8.6 Hz, 2H), 7.05 (d, J = 8.6 Hz, 2H), 7.42–7.44 (m, 3H), 7.99–8.02 (m, 2H); ESI-MS m/z: 424.2 $(M+H)^+$.

6.3.8. Methyl {2-Methyl-*c*-5-[4-(5-methyl-2-(4-methylphenyl) oxazol-4-ylmethoxy)phenyl]}-1,3-dioxane-*r*-2-carboxylate (6b)

This compound was prepared from **4c** and **5b** by means of a procedure similar to that reported for **6a**. White solid; yield: 90%; mp: 145–147 °C; purity by HPLC: 96%; IR (KBr): 1741, 1515, 1267, 1242, 1215, 1141, 1045, 1012, and 732 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.58 (s, 3H), 2.40 (s, 3H), 2.42 (s, 3H), 3.18–3.21 (m, 1H), 3.82 (d, J = 11.6 Hz, 2H), 3.88 (s, 3H, s), 4.03–4.08 (dd, J = 11.8 and 4.6 Hz, 2H), 5.00 (s, 2H), 6.96 (d, J = 8.6 Hz, 2H), 7.05 (d, J = 8.6 Hz, 2H), 7.26 (d, J = 7.65 Hz, 2H), 7.95 (d, J = 8.0 Hz, 2H); ESI-MS m/z: 438.2 $(M+H)^+$.

6.3.9. Methyl {2-Methyl-*c*-5-[4-(2-(5-methyl-2-phenyloxazol-4-yl)ethoxy)phenyl]}-1,3-dioxane-*r*-2-carboxylate (6c)

This compound was prepared from **4c** and **5c** by means of a procedure similar to that reported for **6a**. Thick oil; yield: 57%; purity by HPLC: 91%; IR (Nujol): 1745, 1514, 1218, 1260, 1143, 1049, and 756 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.57 (s, 3H), 2.36 (s, 3H), 2.95 (d, J = 6.6 Hz, 2H), 3.13–3.21 (m, 1H), 3.81 (d, J = 11.8 Hz, 2H), 3.87 (s, 3H, s), 4.01–4.11 (dd, J = 11.8 and 4.7 Hz, 2H), 4.20 (t, J = 6.6 Hz, 2H), 6.83 (d, J = 8.6 Hz, 2H), 7.02 (d, J = 8.6 Hz, 2H), 7.40–7.42 (m, 3H), 7.94–7.98 (m, 2H); ESI-MS m/z: 438.2 $(M+H)^+$.

6.3.10. Methyl 2-Methyl-*c*-5-{4-[2-(5-methyl-2-(4-methylphenyl) oxazol-4-yl)ethoxy]-phenyl}-1,3-dioxane-*r*-2-carboxylate (6d)

This compound was prepared from **4c** and **5d** by means of a procedure similar to that used for **6a**. White solid; yield: 42%; mp: 121–123 °C; purity by HPLC: 98%; IR (KBr): 1718, 1515, 1269, 1244, 1045, and 823 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.58 (s, 3H), 2.35 (s, 3H), 2.38 (s, 3H), 2.95 (t, J = 6.6 Hz, 2H), 3.16–3.21 (m, 1H), 3.80 (t, J = 11.9 Hz, 2H), 3.88 (s, 3H), 4.01–4.07 (dd, J = 12.0 and 4.7 Hz, 2H), 4.20 (t, J = 6.7 Hz, 2H), 6.83 (d, J = 8.6 Hz, 2H), 7.02 (d, J = 8.6 Hz, 2H), 7.22 (d, J = 8.0 Hz, 2H), 7.84 (d, J = 8.16 Hz, 2H); ESI-MS m/z: 452.2 $(M+H)^+$.

6.3.11. 2-Methyl-*c*-5-[4-(5-methyl-2-phenyloxazol-4-ylmethoxy) phenyl]-1,3-dioxane-*r*-2 carboxylic acid (7a)

To a solution of **6a** (850 mg, 2.0 mmol) in THF (9 mL), MeOH (3 mL) and H_2O (3 mL), LiOH·H_2O (168 mg, 4.0 mmol) was added and stirred at 25 °C for 18 h. The reaction mixture was concentrated in vacuo. Twenty millilitres of water were added to the reaction mixture, acidified by HCl and extracted with ethyl acetate (3× 20 mL). The organic layer was washed with water and brine, dried over Na_2SO_4 and evaporated under reduced pressure to give title compound **7a** as white solid. (425 mg) Yield: 51%; mp: 172–174 °C; purity by HPLC: 99%; IR (KBr): 3448, 1710, 1271, 1240, 1215, and 1151 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.61 (s, 3H), 2.46 (s, 3H), 3.02–3.10 (m, 1H), 3.65 (t, J = 11.5 Hz, 2H), 3.71–3.77 (dd, J = 11.7 and 4.8 Hz, 2H), 5.05 (s, 2H, s), 6.88–6.96 (m, 4H), 7.46–7.48 (m, 3H), 8.04–8.07 (m, 2H); ^{13}C NMR ($CDCl_3$): δ 10.46, 26.13, 39.74, 60.75, 68.15, 98.04, 114.83, 126.44, 126.66, 129.11, 129.94, 131.05, 131.26, 147.74, 157.80, 160.89, 172.9; ESI-MS m/z: 410.1 $(M+H)^+$.

6.3.12. {2-Methyl-*c*-5-[4-(5-methyl-2-(4-methylphenyl)oxazol-4-ylmethoxy)phenyl]}-1,3-dioxane-*r*-2-carboxylic acid (7b)

This compound was prepared from **6b** by means of a procedure similar to that used for **7a**. White solid; yield: 90%; mp: 193–195 °C; purity by HPLC: 98%; IR (KBr): 2922, 1735, 1514, 1244,

122, 1145, and 1049 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.61 (s, 3H), 2.40 (s, 3H), 2.45 (s, 3H), 3.03–3.08 (m, 1H), 3.60 (t, J = 11.5 Hz, 2H), 3.69–3.74 (dd, J = 11.6 and 4.0 Hz, 2H), 5.05 (s, 2H), 6.87 (d, J = 8.6 Hz, 2H), 6.94 (d, J = 8.6 Hz, 2H), 7.28 (d, J = 8.0 Hz, 2H), 7.94 (d, J = 8.0 Hz, 2H); ^{13}C NMR ($CDCl_3$): δ 10.40, 21.14, 26.11, 39.74, 60.67, 68.11, 98.02, 114.79, 123.67, 126.63, 129.10, 129.49, 129.80, 130.37, 141.49, 147.36, 157.79, 161.14, 172.89; ESI-MS m/z: 424.2 $(M+H)^+$.

6.3.13. {2-Methyl-*c*-5-[4-(2-(5-methyl-2-phenyloxazol-4-yl) ethoxy)phenyl]}-1,3-dioxane-*r*-2-carboxylic acid (7c)

This compound was prepared from **6c** by means of a procedure similar to that used for **7a**. Off white solid; yield: 81%; mp: 145–147 °C; purity by HPLC: 98%; IR (KBr): 2964, 2927, 1720, 1550, 1269, 144, 1218, 1110, 1024, and 759 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.63 (s, 3H), 2.40 (s, 3H), 3.06 (t, J = 6.5 Hz, 2H), 3.12–3.21 (m, 1H), 3.88 (t, J = 11.5 Hz, 2H), 3.94–4.01 (dd, J = 11.6 and 4.8 Hz, 2H), 4.17 (t, J = 6.6 Hz, 2H), 6.77 (d, J = 8.5 Hz, 2H), 6.95 (d, J = 8.5 Hz, 2H), 7.42–7.44 (m, 3H), 7.97–8.00 (m, 2H); ^{13}C NMR ($CDCl_3$): δ 10.26, 21.58, 26.03, 39.37, 66.72, 68.36, 98.13, 114.85, 124.12, 126.34, 128.64, 129.59, 131.81, 140.92, 145.25, 158.05, 160.33, 173.13; ESI-MS m/z: 424.2 $(M+H)^+$.

6.3.14. 2-Methyl-*c*-5-[4-[2-(5-methyl-2-(4-methylphenyl) oxazol-4-yl)ethoxy]phenyl]-1,3-dioxane-*r*-2-carboxylic acid (7d)

This compound was prepared from **6d** by means of a procedure similar to that used for **7a**. White solid; yield: 94%; mp: 184–186 °C; purity by HPLC: 99%; IR (KBr): 2925, 1724, 1651, 1515, 1269, 1244, 1218, and 759 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.64 (s, 3H), 2.38 (s, 3H), 2.39 (s, 3H), 3.07 (t, J = 6.5 Hz, 2H), 3.15–3.18 (m, 1H), 3.88 (t, J = 11.5 Hz, 2H), 3.96–4.01 (dd, J = 11.7 and 4.8 Hz, 2H), 4.19 (t, J = 6.6 Hz, 2H), 6.77 (d, J = 8.5 Hz, 2H), 6.94 (d, J = 8.5 Hz, 2H), 7.24 (d, J = 9.96 Hz, 2H), 7.87 (d, J = 8.1 Hz, 2H); ^{13}C NMR ($CDCl_3$): δ 10.26, 21.58, 25.87, 26.03, 39.37, 66.72, 68.36, 98.13, 114.85, 124.12, 126.34, 128.64, 129.59, 131.81, 140.92, 145.25, 158.05, 160.33, 173.13; ESI-MS m/z: 437.9 $(M+H)^+$.

6.3.15. Diethyl-2-(4-benzyloxybenzyl)malonate (9)

To an ice-cold suspension of NaH (60%, 178 g, 3.7 mol) in THF (1000 mL), diethyl malonate (704 mL, 4.66 mol) was added dropwise over a period of 30 min at 0–10 °C and stirred at the same temperature for 30 min. A solution of 4-benzyloxybenzyl chloride **8** (434 g, 1.864 mol) in THF (500 mL) was added to the reaction mixture at 0–10 °C and stirred at 25 °C for 14 h. The reaction mixture was poured into ice-cold water (2 L) and extracted with ethyl acetate (3× 1000 mL). The organic layer was washed with water and brine, dried over Na_2SO_4 and evaporated under reduced pressure. Excess diethyl malonate was distilled out under vacuum to give tittle compound **9** as thick oil. (525 g) Yield: 79%; purity by HPLC: 90%; IR (Nujol): 1728, 1512, 1217, and 756 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.20 (t, J = 7.1 Hz, 3H), 1.28 (t, J = 6.9 Hz, 3H), 3.15 (d, J = 7.7 Hz, 2H), 3.59 (t, J = 7.9 Hz, 1H), 4.08–4.24 (m, 4H), 5.03 (s, 2H), 6.88 (d, J = 8.55 Hz, 2H), 7.12 (d, J = 8.49 Hz, 2H), 7.31–7.43 (m, 5H); ESI-MS m/z: 357.2 $(M+H)^+$.

6.3.16. 2-(4-Benzyloxybenzyl)propane-1,3-diol (10)

This compound was prepared from **9** by means of a procedure similar to that reported for **3**. White solid (4.0 g). Yield: 47%; mp: 81–82 °C; purity by HPLC: 98%; IR (KBr): 3064, 2922, 1635, 1514, 1245, and 748 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.99–2.06 (m, 3H), 2.57 (d, J = 7.5 Hz, 2H), 3.64–3.70 (dd, J = 10.4 and 7.0 Hz, 2H), 3.78–3.70 (dd, J = 10.5 and 3.9 Hz, 2H), 5.04 (s, 2H), 6.90 (d, J = 8.5 Hz, 2H), 7.09 (d, J = 8.5 Hz, 2H), 7.29–7.44 (m, 5H); ESI-MS m/z: 273.2 $(M+H)^+$.

6.3.17. Methyl 5-(4-benzyloxybenzyl)-2-methyl-1,3-dioxane-2-carboxylate (11a)

This compound was prepared from **10** by means of a procedure similar to that described in section **6.4.3** and obtained as a thick oil containing mixture of *cis*- and *trans*-isomers. Separation of these isomers by column chromatography was unsuccessful and the mixture was subjected to debenzylation as described in the following experiment. Yield: 76%; ESI-MS m/z: 357.2 $(M+H)^+$.

6.3.18. Methyl *c*-5-(4-hydroxybenzyl)-2-methyl-1,3-dioxane-*r*-2-carboxylate (11b)

The mixture of isomers **11a** obtained in the previous experiment was subjected to debenzylation by method similar to that used for **4c**. The crude product was recrystallized from a mixture of ethylacetate and hexane (1:2). The first crop yielded pure *cis*-isomer as white solid. Yield: 46%; mp: 119–120 °C; purity: 99% by HPLC; IR (KBr): 1724, 1610, 1267, 1184, and 1099 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.50 (s, 3H), 2.27 (s, 3H), 3.46 (t, J = 10.9 Hz, 2H), 3.84 (s, 3H), 3.86–3.91 (dd, J = 11.8 and 3.3 Hz, 2H), 5.02 (s, 1H), 6.74 (d, J = 8.4 Hz, 2H), 6.93 (d, J = 2.3 Hz, 2H); ^{13}C NMR: (100 MHz, $CDCl_3$): δ 25.64, 32.81, 35.27, 52.23, 67.08, 97.40, 115.15, 127.90, 129.17, 155.64, and 170.43; ESI-MS m/z: 288.9 $(M+Na)^+$.

6.3.19. Methyl *t*-5-(4-hydroxybenzyl)-2-methyl-1,3-dioxane-*r*-2-carboxylate (11c)

The filtrate from the previous experiment on subjecting to repeated crystallizations from a mixture of ethylacetate and hexane (1:1) for 2 times gave the *trans*-isomer as white solid. Yield: 30%; mp: 62–64 °C; purity by HPLC: 98%; IR (KBr): 3373, 2972, 1710, 1517, 1442, 1265, 1211, 1145, 1055, and 956 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.51–1.57 (m, 1H), 1.59 (s, 3H), 2.93 (d, J = 8.0 Hz, 2H), 3.76 (d, J = 12.0 Hz, 2H), 3.92–3.95 (dd, J = 10.8 and 1.6 Hz, 2H), 5.02 (s, 1H), 6.77 (d, J = 8.4 Hz, 2H), 7.07 (d, J = 8.4 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 25.64, 33.85, 34.39, 52.23, 65.06, 97.97, 115.15, 129.89, 30.16, 155.56, 170.59; ESI-MS m/z: 288.9 $(M+Na)^+$.

6.3.20. Methyl 2-methyl-*c*-5-[4-(5-methyl-2-phenyloxazol-4-ylmethoxy)benzyl]-1,3-dioxane-*r*-2-carboxylate (12a)

This compound was prepared from **11b** and **5a** by means of a procedure similar to that used for **6a**. Thick oil; yield: 62%; purity by HPLC: 95%; IR (Nujol): 1741, 1508, 1262,116, and 754 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.51 (s, 3H), 2.26–2.28 (m, 3H), 2.42 (s, 3H), 3.46 (t, J = 10.8 Hz, 2H), 3.74–76 (dd, J = 11.8 and 3.6 Hz, 2H), 3.82 (s, 3H), 4.96 (s, 2H), 6.93 (d, J = 8.5 Hz, 2H), 7.00 (d, J = 8.5 Hz, 2H), 7.43–7.45 (m, 3H), 8.00–8.03 (m, 2H); ESI-MS m/z: 438.2 $(M+H)^+$.

6.3.21. Methyl 2-methyl-*c*-5-[4-(5-methyl-2-(4-methylphenyl) oxazol-4-ylmethoxy)benzyl]-1,3-dioxane-*r*-2-carboxylate (12b)

This compound was prepared from **11b** and **5b** by means of a procedure similar to that used for **6a**. Thick oil; yield: 97%; purity by HPLC: 97%; IR (Nujol): 1745, 1500, 1244, 1141, 1116, and 1022 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.58 (s, 3H), 2.20–2.2 (m, 3H), 2.39 (s, 3H), 2.41 (s, 3H), 3.46 (t, J = 10.9 Hz, 2H), 3.84 (s, 3H), 3.86–3.90 (m, 2H), 4.95 (s, 2H), 6.92 (d, J = 8.5 Hz, 2H), 7.02 (d, J = 8.5 Hz, 2H), 7.22 (d, J = 8.3 Hz, 2H), 7.90 (d, J = 8.1 Hz, 2H); ESI-MS m/z: 452.4 $(M+H)^+$.

6.3.22. Methyl 2-methyl-*c*-5-[4-{2-(5-methyl-2-phenyloxazol-4-yl)ethoxy}benzyl]-1,3-dioxane-*r*-2-carboxylate (12c)

This compound was prepared from **11b** and **5c** by means of a procedure similar to that reported for **6a**. Thick oil; yield: 76%; pur-

ity by HPLC: 96%; IR (Nujol): 1732, 1537, 1217, 1188, 1143, and 785 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.49 (s, 3H), 2.27 (s, 3H), 2.32 (s, 3H), 2.96 (t, J = 6.7 Hz, 2H), 3.45 (t, J = 10.4 Hz, 2H), 3.83–3.90 (m, 5H), 4.21 (t, J = 6.7 Hz, 2H), 6.73–6.75 (d, J = 8.5 Hz, 2H), 6.97 (d, J = 8.5 Hz, 2H), 7.39–7.44 (m, 3H), 7.97 (m, 1H); ESI-MS *m/z*: 452.0 $(M+H)^+$.

6.3.23. Methyl 2-methyl-*c*-5-{4-[2-(5-methyl-2-(4-methyl-phenyl)oxazol-4-yl)ethoxy]benzyl}-1,3-dioxane-*r*-2-carboxylate (12d)

This compound was prepared from **11b** and **5d** by means of a procedure similar to that reported for **6a**. Thick oil; yield: 79%; purity by HPLC: 95%; IR (Nujol): 1747, 1514, 1245, 1217, 1190, 1167, and 785 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.49 (s, 3H), 2.04 (s, 3H), 2.35 (s, 3H), 2.38 (s, 3H), 2.95 (t, J = 6.7 Hz, 2H), 3.45 (t, J = 9.0 Hz, 2H), 3.84–3.90 (m, 5H), 4.20 (t, J = 13.5 Hz, 2H), 6.74 (d, J = 8.4 Hz, 2H), 6.80 (d, J = 8.5 Hz, 2H), 6.97 (d, J = 8.4 Hz, 2H), 7.85 (d, J = 8.2 Hz, 2H); ESI-MS *m/z*: 466.1 $(M+H)^+$.

6.3.24. Methyl 2-methyl-*c*-5-{4-[3-(5-methyl-2-(4-methylphenyl)oxazol-4-yl)propoxy]benzyl}-1,3-dioxane-*r*-2-carboxylate (12e)

This compound was prepared from **11b** and **5e** by means of a procedure similar to that reported for **6a**. Thick oil; yield: 83%; purity by HPLC: 92%; IR (Nujol): 1747, 1512, 1245, 1217, 1143, 1116, and 786 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.50 (s, 3H), 2.09–2.18 (m, 2H), 2.25 (s, 3H), 2.27 (s, 3H), 2.38 (s, 3H), 2.68 (t, J = 7.2 Hz, 2H), 3.46 (t, J = 11.3 Hz, 2H), 3.84 (s, 3H), 3.86–3.96 (m, 4H), 6.81 (d, J = 8.5 Hz, 2H), 6.99 (d, J = 8.5 Hz, 2H), 7.22 (d, J = 8.2 Hz, 2H), 7.84 (d, J = 8.2 Hz, 2H); ESI-MS *m/z*: 480.2 $(M+H)^+$.

6.3.25. Methyl 2-methyl-*c*-5-{4-[5-methyl-2-(5-methylthiophen-2-yl)oxazol-4-ylmethoxy]benzyl}-1,3-dioxane-*r*-2-carboxylate (12f)

This compound was prepared from **11b** and **5f** by means of a procedure similar to that reported for **6a**. Thick oil; yield: 80%; purity by HPLC: 95%; IR (Nujol): 1747, 1591, 1234, 1213, and 1087 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.62 (s, 3H), 2.22–2.28 (m, 3H), 2.38 (s, 3H), 2.52 (s, 3H), 3.46 (t, J = 11.0 Hz, 2H), 3.84 (s, 3H), 3.86–3.91 (dd, J = 12.0 and 3.6 Hz, 2H), 4.92 (s, 2H), 6.74 (d, J = 2.8 Hz, 1H), 6.90 (d, J = 8.6 Hz, 2H), 7.01 (d, J = 8.6 Hz, 2H), 7.42 (d, J = 3.5 Hz, 1H); ESI-MS *m/zs*: 458.3 $(M+H)^+$.

6.3.26. Methyl 2-methyl-*c*-5-{4-[2-(5-methyl-2-(5-methylthiophen-2-yl)oxazol-4-yl)ethoxy]benzyl}-1,3-dioxane-*r*-2-carboxylate (12g)

This compound was prepared from **11b** and **5g** by means of a procedure similar to that reported for **6a**. Thick oil; yield: 55%; purity by HPLC: 94%; IR (Nujol): 1747, 1512, 1245, 1218, 1116, and 758 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.49 (3H, s), 2.21–2.26 (3H, m), 2.32 (s, 3H), 2.51 (s, 3H), 2.92 (t, J = 6.5 Hz, 2H), 3.42–3.48 (m, 2H), 3.82–3.90 (m, 5H), 4.17 (t, J = 6.6 Hz, 2H), 6.74 (d, J = 2.8 Hz, 1H), 6.90 (d, J = 8.6 Hz, 2H), 7.01 (d, J = 8.6 Hz, 2H), 7.42 (d, J = 3.5 Hz, 1H); ESI-MS *m/z*: 472.1 $(M+H)^+$.

6.3.27. Methyl *c*-5-[4-(2-*tert*-Butyl-5-methyloxazol-4-ylmethoxy)benzyl]-2-methyl-1,3-dioxane-*r*-2-carboxylate (12h)

This compound was prepared from **11b** and **5h** by means of a procedure similar to that reported for **6a**. Thick oil; yield: 95%; purity by HPLC: 95%; IR (Nujol): 1743, 1612, 1566, 1238, 1217, 1118, and 756 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.38 (s, 9H), 1.51 (s, 3H), 2.12 (d, J = 7.2 Hz, 2H), 2.22–2.26 (m, 1H), 2.33 (s, 3H), 3.43 (t, J = 11.3 Hz, 2H), 3.72–3.80 (dd, J = 11.8 and 4.1 Hz, 2H), 3.84 (s, 3H), 4.90 (s, 2H), 6.82 (d, J = 8.3 Hz, 2H), 6.91 (d, J = 8.7 Hz, 2H); ESI-MS *m/z*: 418.1 $(M+H)^+$.

6.3.28. Methyl *c*-5-[4-{2-(2-*tert*-Butyl-5-methyloxazol-4-yl)ethoxy}benzyl]-2-methyl-1, 3-dioxane-*r*-2-carboxylate (12l)

This compound was prepared from **11b** and **5i** by means of a procedure similar to that reported for **6a**. Thick oil; yield: 97%; purity by HPLC: 94%; IR (Nujol): 1735, 1512, 1251, 1143, 1109, and 756 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.35 (s, 9H), 1.54 (s, 3H), 2.24 (s, 3H), 2.27 (s, 3H), 2.89–2.96 (dd, J = 15.0 and 8.1 Hz, 2H), 3.50 (t, J = 9.5 Hz, 2H), 3.84–3.89 (m, 5H), 4.12 (t, J = 6.7 Hz, 2H), 6.78 (d, J = 8.3 Hz, 2H), 6.92 (d, J = 8.1 Hz, 2H); ESI-MS *m/z*: 432.2 $(M+H)^+$.

6.3.29. 2-Methyl-*c*-5-[4-(5-methyl-2-phenyloxazol-4-ylmethoxy)benzyl]-1,3-dioxane-*r*-2-carboxylic acid (13a)

To a solution of **12a** (1.0 g, 2.2 mmol.) in EtOH (10 mL), a solution of NaOH (176 mg, 4.4 mmol) in H_2O (5 mL) was added and stirred at 25 °C for 18 h. The reaction mixture was concentrated in vacuo. Twenty millilitres water were added to the residue, acidified by HCl and extracted with ethyl acetate (3× 20 mL). The organic layer was washed with water and brine, dried over Na_2SO_4 and evaporated under reduced pressure to give title compound **13a** as off-white solid. (0.78 g) Yield: 85%; mp: 170–171 °C; purity by HPLC: 98%; IR (KBr): 2930, 1720, 1512, 1271, 1236, 1124, and 1056 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.52 (s, 3H), 2.21–2.24 (m, 3H), 2.43 (s, 3H), 3.60 (t, J = 10.8 Hz, 2H), 3.83–86 (dd, J = 11.8 and 3.6 Hz, 2H), 4.96 (s, 2H), 6.93 (d, J = 8.5 Hz, 2H), 7.00 (d, J = 8.5 Hz, 2H), 7.43–7.45 (m, 3H), 8.00–8.03 (m, 2H); ^{13}C NMR (DMSO-d_6): δ 9.96, 25.54, 32.89, 34.38, 61.38, 67.09, 97.78, 114.98, 125.59, 126.87, 129.05, 129.63, 129.99, 130.30, 132.04, 147.36, 156.56, 158.82, 171.32; ESI-MS *m/z*: 424.3 $(M+H)^+$.

6.3.30. 2-Methyl-*c*-5-[4-(5-methyl-2-(4-methylphenyl)oxazol-4-ylmethoxy)benzyl]-1,3-dioxane-*r*-2-carboxylic acid (13b)

This compound was prepared from **12b** by means of a procedure similar to that reported for **13a**. White solid; yield: 95%; mp: 197–198 °C; purity by HPLC: 99%; IR (KBr): 2929, 1759, 1610, 1502,1286, 1226, 1182, and 827 cm^{-1}; ^1H NMR (DMSO-d_6): δ 1.32 (s, 3H), 2.09–2.13 (m, 1H), 2.27 (d, J = 6.9 Hz, 2H), 2.36 (s, 3H), 2.42 (s, 3H), 3.42 (t, J = 11.4 Hz, 2H), 3.69-3.74 (dd, J = 11.6 and 4.1 Hz, 2H), 4.94 (s, 2H), 6.94 (d, J = 8.4 Hz, 2H), 7.11 (d, J = 8.4 Hz, 2H), 7.32 (d, J = 8.1 Hz, 2H), 7.83 (d, J = 8.1 Hz, 2H); ^{13}C NMR (DMSO-d_6): δ 9.93, 20.96, 25.52, 32.87, 34.86, 61.38, 67.07, 97.56, 114.64, 124.24, 125.57, 129.62, 130.58, 131.85, 140.15, 146.95, 156.65, 158.98, 171.31; SI-MS *m/z*: 438.4 $(M+H)^+$.

6.3.31. 2-Methyl-*c*-5-[4-{2-(5-methyl-2-phenyloxazol-4-yl)ethoxy}benzyl]-1,3-dioxane-*r*-2-carboxylic acid (13c)

This compound was prepared from **12c** by means of a procedure similar to that reported for **13a**. Thick oil; yield: 32%; purity by HPLC: 97%; IR (Nujol): 2925, 1732, 1512, 1245, 1217, and 1145 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.54 (s, 3H), 2.29 (s, 3H), 2.37 (s, 3H), 2.97 (t, J = 6.7 Hz, 2H), 3.51–3.55 (m, 2H), 3.89–3.93 (dd, J = 12.6 and 4.2 Hz, 2H), 4.21 (t, J = 6.7 Hz, 2H), 6.82 (d, J = 8.6 Hz, 2H), 6.99 (d, J = 8.5 Hz, 2H), 7.40–7.43 (m, 3H), 7.95–7.99 (m, 2H); ^{13}C NMR (DMSO-d_6): δ 9.78, 25.54, 32.88, 34.88, 66.07, 67.09, 97.79, 114.33, 125.43, 126.92, 128.69, 129.60, 129.96, 130.27, 132.95, 145.01, 156.72, 158.38, 171.49; ESI-MS *m/z*: 438.2 $(M+H)^+$.

6.3.32. 2-Methyl-*c*-5-{4-[2-(5-methyl-2-(4-methylphenyl)oxazol-4-yl)ethoxy]benzyl}-1,3-dioxane-*r*-2-carboxylic acid (13d)

This compound was prepared from **12d** by means of a procedure similar to that reported for **13a**. Off-white solid; yield: 31%; mp: 129–131 °C; purity by HPLC: 99%; IR (KBr): 2923, 1718, 1500, 1247, 1217, and 1143 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.54 (s, 3H), 2.27 (s, 3H), 2.36 (s, 3H), 2.38 (s, 3H), 2.98 (t, J = 6.7 Hz, 2H),

3.52 (t, J = 10.7 Hz, 2H), 3.89–3.93 (dd, J = 12.6 and 4.2 Hz, 2H), 4.20 (t, J = 6.7 Hz, 2H), 6.81 (d, J = 8.5 Hz, 2H), 6.95 (d, J = 8.5 Hz, 2H), 7.23 (d, J = 8.3 Hz, 2H), 7.85 (d, J = 8.1 Hz, 2H); ^{13}C NMR (DMSO-d_6): δ 9.78, 20.94, 25.51, 32.84, 34.88, 66.08, 67.01, 97.56, 114.33, 124.56, 125.41, 129.60, 129.97, 130.26, 132.50, 139.76, 144.59, 156.65, 158.54, 171.32; ESI-MS m/z: 452.2 (M+H)$^+$.

6.3.33. 2-Methyl-*c*-5-{4-[3-(5-methyl-2-(4-methylphenyl) oxazol-4-yl)propoxy]benzyl}-1,3-dioxane-*r*-2-carboxylic acid (13e)

This compound was prepared from **12e** by means of a procedure similar to that reported for **13a**. White solid; yield: 73%; mp: 126–128 °C; purity by HPLC: 97%; IR (KBr): 2922, 1716, 1556, 1469, 1244, 1215, 1120, and 1035 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 1.56 (s, 3H), 2.05–2.15 (m, 2H), 2.27–2.29 (m, 6H), 2.38 (s, 3H), 2.72 (t, J = 7.2 Hz, 2H), 3.55 (t, J = 10.8 Hz, 2H), 3.89–3.97 (m, 4H), 6.80 (d, J = 8.5 Hz, 2H), 6.98 (d, J = 8.5 Hz, 2H), 7.22–7.26 (m, 2H), 7.84 (d, J = 8.2 Hz, 2H); ^{13}C NMR (DMSO-d_6): δ 9.62, 20.93, 21.42, 25.53, 27.98, 32.87, 34.89, 66.33, 67.09, 97.56, 114.29, 124.65, 125.38, 129.52, 130.12, 134.89, 139.66, 143.39, 157.02, 158.50, 171.31; ESI-MS m/z: 466.2 (M+H)$^+$.

6.3.34. 2-Methyl-*c*-5-{4-[5-methyl-2-(5-methylthiophen-2-yl) oxazol-4-ylmethoxy]benzyl}-1,3-dioxane-*r*-2-carboxylic acid (13f)

This compound was prepared from **12f** by means of a procedure similar to that reported for **13a**. Pale yellow solid; yield: 64%; mp: 172–173 °C; purity by HPLC: 99%; IR (KBr): 2923, 1718, 1508, 1259, 1224, and 1122 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 1.54 (s, 3H), 2.21–2.28 (m, 3H), 2.39 (s, 3H), 2.52 (s, 3H), 3.49 (t, J = 11.1 Hz, 2H), 3.89–3.93 (dd, J = 12.6 and 4.2 Hz, 2H), 4.96 (s, 2H), 6.75 (d, J = 2.8 Hz, 1H), 6.91 (d, J = 8.6 Hz, 2H), 7.00 (d, J = 8.5 Hz, 2H), 7.48 (d, J = 3.6 Hz, 1H); ^{13}C NMR (DMSO-d_6): δ 9.86, 14.95, 25.53, 32.89, 34.86, 61.23, 67.08, 97.56, 114.64, 126.74, 127.54, 129.62, 130.61, 131.68, 142.95, 146.49, 155.13, 156.61, 171.31; ESI-MS m/z: 444.2 (M+H)$^+$.

6.3.35. 2-Methyl-*c*-5-{4-[2-(5-methyl-2-(5-methylthiophen-2-yl) oxazol-4- yl)ethoxy]benzyl}-1,3-dioxane-*r*-2-carboxylic acid (13g)

This compound was prepared from **12g** by means of a procedure similar to that reported for **13a**. Off-white solid; yield: 46%; mp: 122–124 °C; purity by HPLC: 96%; IR (KBr): 2922, 1718, 1514, 1249, and 1118 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 1.55 (s, 3H), 2.20–2.26 (m, 3H), 2.34 (s, 3H), 2.51 (s, 3H), 2.95–2.97 (t, J = 6.7 Hz, 2H), 3.5 (t, J = 10.7 Hz, 2H), 3.90 (d, J = 9.9 Hz, 2H), 4.18 (t, J = 6.3 Hz, 2H), 6.73 (d, J = 2.8 Hz, 1H), 6.80 (d, J = 8.2 Hz, 2H), 6.95 (d, J = 8.0 Hz, 2H), 7.42 (d, J = 3.6 Hz, 1H); ^{13}C NMR (DMSO-d_6): δ 9.71, 14.93, 25.55, 32.90, 34.89, 66.01, 67.10, 97.58, 114.33, 126.72, 127.01, 127.20, 129.60, 130.27, 132.40, 142.41, 144.17, 154.73, 156.71, 171.35; ESI-MS m/z: 458.2 (M+H)$^+$.

6.3.36. *c*-5-[4-(2-*tert*-Butyl-5-methyloxazol-4-ylmethoxy) benzyl]-2-methyl-1,3-dioxane-*r*-2-carboxylic acid (13h)

This compound was prepared from **12h** by means of a procedure similar to that reported for **13a**. White solid; yield: 49%; mp: 117–118 °C; purity by HPLC: 99%; IR (KBr): 2968, 1735, 1514, 1217, 1120, and 1029 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 1.38 (s, 9H), 1.51 (s, 3H), 2.12 (d, J = 7.2 Hz, 2H), 2.22–2.26 (m, 1H), 2.33 (s, 3H), 3.43 (t, J = 11.3 Hz, 2H), 3.72–3.80 (dd, J = 11.8 and 4.1 Hz, 2H), 4.90 (s, 2H), 6.82 (d, J = 8.3 Hz, 2H), 6.91 (d, J = 8.7 Hz, 2H); ^{13}C NMR (DMSO-d_6): δ 10.35, 25.84, 28.53, 33.69, 33.86, 34.77, 61.63, 68.10, 98.24, 114.97, 129.39, 129.64, 130.66, 146.63, 157.28, 170.17, 173.09; ESI-MS m/z: 404.1 (M+H)$^+$.

6.3.37. *c*-5-[4-[2-(2-*tert*-Butyl-5-methyloxazol-4-yl)ethoxy] benzyl]-2-methyl-1, 3-dioxane-*r*-2-carboxylic acid (13i)

This compound was prepared from **12i** by means of a procedure similar to that reported for **13a**. Off-white solid; yield: 60%; mp: 140–141 °C; purity by HPLC: 98%; IR (KBr): 2960, 2923, 1718, 1514, 1249, 1120, and 767 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 1.35 (s, 9H), 1.54 (s, 3H), 2.24 (s, 3H), 2.27 (s, 3H), 2.89–2.96 (dd, J = 15.0 and 8.1 Hz, 2H), 3.50 (t, J = 9.5 Hz, 2H), 3.84–3.89 (m, 2H), 4.12 (t, J = 6.7 Hz, 2H), 6.78 (d, J = 8.3 Hz, 2H), 6.92 (d, J = 8.1 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 10.45, 25.84, 28.83, 33.69, 33.86, 34.87, 61.43, 68.10, 98.24, 114.97, 129.39, 129.64, 130.66, 146.63, 157.28, 170.17, 173.09; ESI-MS m/z: 418.1 (M+H)$^+$.

Acknowledgements

Authors are grateful to Dr. B. B. Lohray, Dr. V. B. Lohray for valuable suggestions, management of Zydus Group for encouragement, and the analytical department for support.

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Bioorganic & Medicinal Chemistry Letters 18 (2008) 5586–5590

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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Discovery of a highly orally bioavailable *c*-5-[6-(4-Methanesulfonyloxyphenyl)-hexyl]-2-methyl-1,3-dioxane-*r*-2-carboxylic acid as a potent hypoglycemic and hypolipidemic agent ☆

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ARTICLE INFO

Article history:
Received 27 June 2008
Revised 24 August 2008
Accepted 29 August 2008
Available online 3 September 2008

Keywords:
Type 2 diabetes
PPAR agonist
1,3-Dioxane-2 carboxylic acid
Metabolic disorders

ABSTRACT

A series of novel 1,3-dioxane-2-carboxylic acid derivatives containing alkyl chain tether and substituted phenyl group as a lipophilic tail have been prepared as agonists of PPARα and γ. *c*-5-[6-(4-Methanesulfonyloxyphenyl)hexyl]-2-methyl-1,3-dioxane-*r*-2-carboxylic acid **13c** exhibited potent hypoglycemic and lipid lowering activity with high oral bioavailability in animal models.

Prevalence of Type 2 diabetes and adverse cardiovascular conditions associated with this disease are increasing world wide and are considered as a major threat to human health in the 21st century.[1] The defects in lipid and carbohydrate metabolism are the main causes of this disease and the nuclear hormone receptors PPARα and PPARγ agonists have shown therapeutic benefits in the treatment of diabetes and dyslipidemia.[2] PPARγ has been identified as a key regulator for insulin sensitivity, and two PPARγ agonists, **Rosiglitazone** and **Pioglitazone** have demonstrated clinical success in the treatment of Type 2 diabetes. Unfortunately, these agents are found to cause adverse effects including weight gain, edema and anemia in both animal models and humans. Fibrate class of compounds have been shown to lower plasma lipid, triglyceride (TG), levels and have been in clinical use for the treatment of dyslipidemia. However these are poor activators of PPARα and need high doses to exert therapeutic effect. Treatment with PPARγ and PPARα dual agonists found to be useful in the treatment of hyperglycemia and hyperlipidemia in animal models and a number of PPARα/γ dual agonists have been discovered and evaluated by different research groups.[3] Till date none of these dual agonists are marketed including **Farglitazar**,[4] **Ragaglitazar**,[5] **Tesaglitazar**[6] and **Muraglitazar**[7] which were dropped from late clinical development due to adverse effects like edema, carcinogencity in rodent toxicity models, heart failure or cardiovascular deaths and elevated serum creatinine. The unsuccessful efforts to develop dual agonist and recent research findings that activation of PPARα lower triglycerides, elevate HDL and exert insulin-sensitizing effects[8] led to the discovery of potent and selective activators of PPARα as remedy for disorders mediated by lipid and carbohydrate metabolism. A potent and selective PPARα agonist **NS-220**[9] is reported to exert hypoglycemic and lipid modulating effects in animal models but the further development of this compound is discontinued for unknown reasons. Another compound **K-111**,[10] a relatively weak PPARα agonist is presently undergoing clinical trials for the treatment of Type 2 diabetes. As a part of our research in the field of PPAR,[11] we have recently reported a series of oxazole containing 1,3-dioxane-2-carboxylic acid derivatives as PPARα/γ dual agonist.[12] In this communication we report a series of novel 1,3-dioxane carboxylic acid derivatives designed by the hybridization of the compounds **NS-220** and **K-111** and mimicking **Tesaglitazar** (Fig. 1) in order to optimize the lead candidate.

The synthesis of compounds **7a–e** is outlined in Scheme 1. Starting materials **1a–e**, procured from commercial sources were reduced initially with Zn/Hg to compounds **2a–e** which were further reduced with $LiAlH_4$ to yield the hydroxy compounds

☆ ZRC communication.240.

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doi:10.1016/j.bmcl.2008.08.112

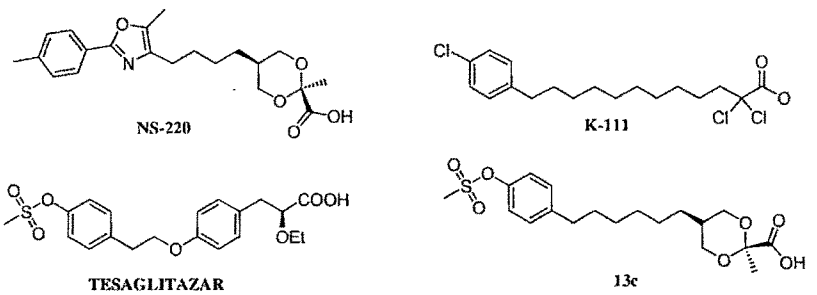
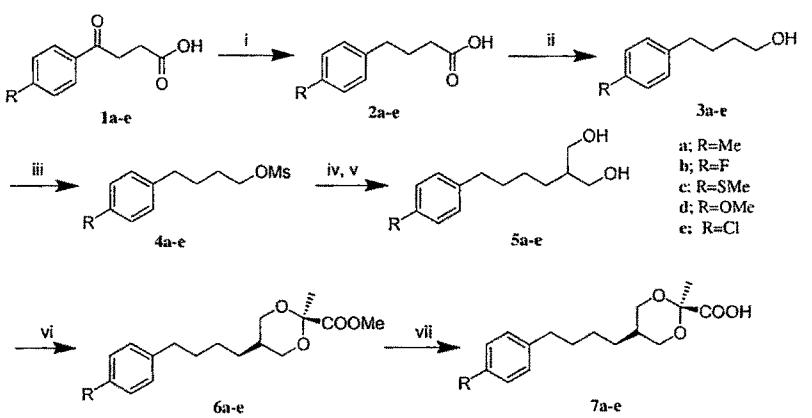
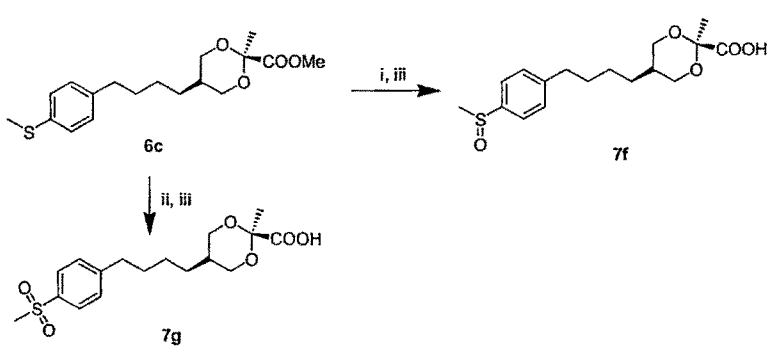


Figure 1. Structures of PPAR agonists.



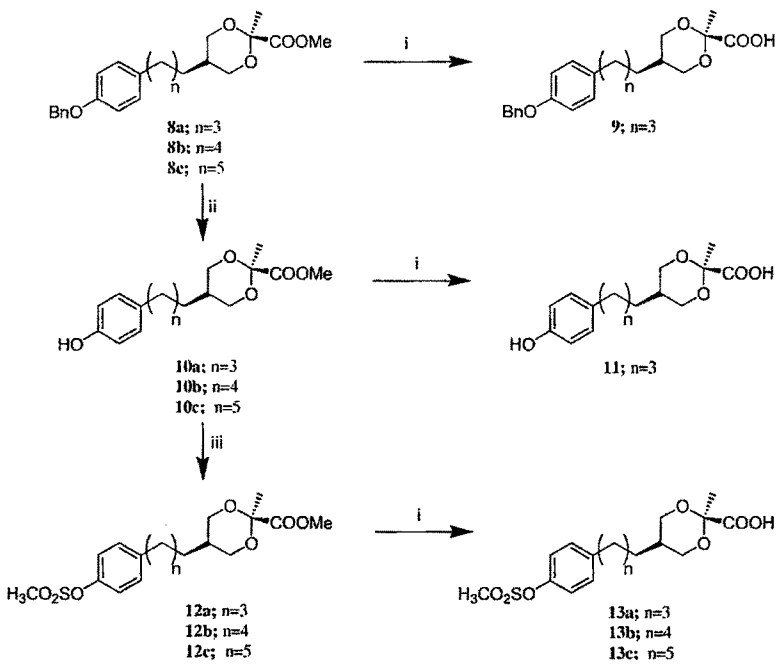
Scheme 1. Reagents and conditions: (i) Zn/Hg, Toluene, AcOH, H_2O, reflux, 4 h; (ii) $LiAlH_4$, THF, 0–10 °C, 1 h; (iii) CH_3SO_2Cl, Et_3N, CH_2Cl_2, 0–10 °C, 30 min; (iv) diethyl malonate, NaH, THF, 60 °C, 48 h; (v) $LiAlH_4$, THF, 0–10 °C, 2 h; (vi) BF_3-etherate, methyl pyruvate, CH_3CN, 25 °C, 2 h; (vii) $LiOH.H_2O$, H_2O, THF, MeOH, 25 °C, 18 h.

3a–e. These compounds (**3a–e**) were converted to their corresponding mesylate derivatives (**4a–e**) and treated with diethylmalonate in the presence of sodium hydride. The diesters so obtained were reduced with $LiAlH_4$ to diols **5a–e**. These diols when reacted with methylpyruvate in the presence of borontrifluoride etherate in acetonitrile yielded the esters as a mixture of diastereomeric isomers. The mixture was separated by chromatography to get the *cis*-isomers **6a–e** which showed a chemical shift pattern in ^1H NMR identical to that of the other *cis* derivatives reported.[13] These esters **6a–e** were hydrolyzed using aqueous NaOH to obtain the acids **7a–e**. Compounds **7f** and **7g** were synthesized as illustrated in Scheme 2. Sulfanyl derivative **6c** when treated with 1.2 molar equivalent of *m*-CPBA gave corresponding sulfinyl derivative which on hydrolysis gave the acid



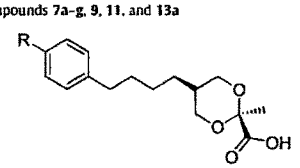
Scheme 2. Reagents and conditions: (i) *m*-CPBA (1.2 equiv), $CHCl_3$, 0–10 °C, 30 min; (ii) *m*-CPBA (3 equiv), $CHCl_3$, 25 °C, 1 h; (iii) LiOH, H_2O, THF, MeOH, 25 °C, 18 h.

7f where as the same compound **6c** on treatment with 3 molar equivalents of *m*-CPBA gave the sulfonyl derivative which upon hydrolysis yielded the acid **7g**. The synthesis of compounds **9**, **11**, and **13a–c** was as illustrated in Scheme 3. Compounds **8a–**



Scheme 3. Reagents and conditions: (i) LiOH, H_2O, THF, MeOH, 25 °C, 18 h; (ii) $HCOONH_4$, Pd/C (10%), MeOH, reflux, 2 h; (iii) CH_3SO_2Cl, Et_3N, CH_2Cl_2, 0–10 °C, 30 min.

Table 1
In vitro hPPAR transactivation and TG reducing activity of compounds **7a–g**, **9**, **11**, and **13a**



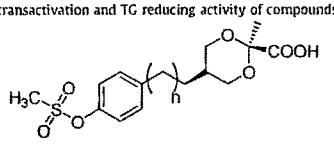
Compound	R	hPPAR transactivation[a,b]			% Change in TG in SAM[c]
		α (10 μM)	γ (0.2 μM)	δ (0.2 μM)	
7a	Me	3.2	1.5	1.1	−22
7b	F	2.3	2.1	1.2	−9
7c	SMe	4	1.9	1.9	−14
7d	OMe	2.5	1.2	1.2	−11
7e	Cl	2.6	2.2	2.2	−10
7f	SOMe	2	1.8	1.8	−12
7g	SO_2Me	1	2.2	2.2	−7
9	OBn	2.3	1.0	1.0	−25
11	OH	1.2	1.4	1.4	−15
13a	OSO_2Me	3.0	1.0	1.0	−32
Vehicle		1.0	1.0	1.0	0.0
WY-14643		3.3	ND	ND	ND
Rosiglitazone		ND	10.2	ND	ND
GW-501516		ND	ND	5.6	

[a] HepG2 cells were transfected with pSG5 expression vector containing the cDNA of hPPARα or hPPARγ or hPPARδ and cotransfected with PPRE3-TK-luc. The Luciferase activity was determined using commercial fire-fly luciferase assay and β-galactosidase activity was determined in ELISA reader. Activities are presented by fold induction of PPARα and γ activation. IA denotes inactive.

[b] In hPPARδ transactivation assay, any of these compounds did not show any activation above basal level.

[c] 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.

Table 2
In vitro hPPAR transactivation and TG reducing activity of compounds **13a–c**



Compound	n	hPPAR transactivation EC_{50} (μM)		% Change in TG in SAM
		α	γ	
13a	3	20	IA	−32
13b	4	2.5	IA	−28
13c	5	2	15	−71
NS-220		0.05	7	−67
Tesaglitazar		0.82	0.013	−79

Table 3
Hypoglycemic and hypolipidemic activities of compound **13c** in *db/db* mice[a]

Compound	Dose (mg/kg/day)	% Change	
		TG	PG
13c	3	−50	−53
NS-220	3	−54	−44
Tesaglitazar	3	−60	−54

[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.

Table 4
Hypolipidemic activity of compound **13c** in HC fed SD rats[a]

Compound	Dose (mg/kg/day)	% Change			
		TG	TC	LDL-C	HDL-C
13c	3	−63	−56	−46	51
NS-220	3	−54	−49	−68	88
Tesaglitazar	3	−51	−59	−31	18

[a] Male Sprague–Dawley (SD) rats were fed with diet containing high cholesterol for 15 days then dosed with vehicle or the indicated doses of test compound daily for 4 days by oral gavage. Serum triglycerides (TG), total cholesterol (TC), LDL-cholesterol (LDL-C) and HDL-cholesterol (HDL-C) were measured. Values reported are % change versus control group.

Table 5
Mean pharmacokinetic parameters[a] of **13c** in fasted male wistar rat

Compound	Route	Dose (mg/kg)	T_{max} (h)	C_{max} (μg/mL)	$T_{1/2}$ (h)	AUC(0–∞) (h μg/mL)
13c	Oral	30	4(±0.0)	127(±4.6)	12(±0.9)	1491(±78)
NS-220	Oral	30	0.67(±0.0)	41(±3.8)	1.5(±0.3)	99(±5.2)

[a] Values indicate mean ± SD for n = 6.

c are prepared following the procedures similar to those described in Scheme 1. Compound **8a** was hydrolyzed to obtain the compound **9** where as the compounds **8a–c** were debenzylated under hydrogen transfer reaction condition to yield the compounds **10a–c**. Compound **10a** was hydrolyzed to compound **11** while **10a–c** were treated with mathanesulfonyl chloride to obtain the compounds **12a–c** which were subsequently hydrolyzed to compounds **13a–c**.

The newly synthesized compounds[14] have been screened for hPPARα, γ and δ agonistic activity by using PPAR receptor transfected HepG2 cells. **WY-14643**, **Rosiglitazone** and **GW501516** were used as PPARα, γ and δ controls, respectively. Initial compounds **7a–g**, **9**, **11**, and **13a** are designed by hybridizing the structures of **NS-220** and **K-111** wherein the phenyl ring is connected with 1,3-dioxane acidic head with an alkyl tether. Phenyl ring has been substituted at para-position as the activity of these compounds is sensitive to substituent at this position. In vitro hPPAR transactivation is reported as fold induction and their ability to reduce plasma triglycerides (TG) in male *swiss albino mice* (SAM) which is a moderately hyperlipidemic model and the results are shown in Table 1. These results reveal that the substitution at para-position on the phenyl ring contributes significantly to the in vitro as well as in vivo triglyceride lowering activity of these compounds. Based on these results **13a** was selected for further modifications. We fixed the tail group as *para*-methanesulfonyloxy phenyl group and opted to optimize the tether length. Compounds **13b** and **13c** were synthesized by elongating the tether length to pentamethylene and hexamethylene, respectively. PPAR in vitro activities of these compounds (**13b–c**) and their TG lowering activity in SAM model are summarized in Table 2. Compound **13b** containing pentamethylene chain as tether found inferior to **13a** but surprisingly compound **13c** containing hexamethylene chain as tether reduced plasma TG significantly (71%). The transient transactivation results of these compounds are reported as EC_{50} (Table 2) which shows that the compound **13c** is a moderate activator of PPAR. Having surprised and encouraged with the initial results of compound **13c**, we evaluated it in *db/db* mice and high cholesterol fed Sprague–Dawley rats (HC fed SD rats). In *db/db* mice model the compound **13c** showed excellent plasma glucose and TG reductions which are comparable to **NS-220** (Table 3). In HC fed SD rat model the same compound exhibited excellent reduction in TG and cholesterol superior to **NS-220** (Table 4). To further understand and to draw correlation between moderate in vitro potency and high in vivo efficacy of compound **13c** we evaluated its pharmacokinetic parameters which are presented in Table 5. Compound **13c** showed C_{max} of 127 μg/mL and an AUC of 1491 h μg/mL when administered orally to male wistar rat at a dose of 30 mg/kg body weight. These results clearly established the compound **13c** as highly efficacious and bioavailable hypoglycemic and hypolipidemic agent with moderate in vitro potency and this compound is currently undergoing pre-clinical toxicity studies in order to evaluate the other effects of this compound in animal models.

In summary c-5-[(phenyl)-alkyl]-2-methyl-1,3-dioxane-r-2-carboxylic acid derivatives have been prepared with substitutions on phenyl ring and are evaluated in vitro for their PPAR agonistic potential followed by in vivo hypoglycemic and hypolipidemic activities. Lead compound *c*-5-[6-(4-Methanesulfonyloxyphenyl)hexyl]-2-methyl-1,3-dioxane-*r*-2-carboxylic acid **13c** showed excellent hypoglycemic and hypolipidemic activities with very modest in vitro potency and has exhibited high oral bioavailability.

Acknowledgments

Authors are grateful to Dr. B.B. Lohray, Dr. V.B. Lohray for valuable suggestions, management of Zydus Group for encouragement, and the analytical department for support.

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14. Spectroscopic analysis of the compounds **7a–7g**, **9**, **11**, **13a–13c**:**7a**: *2-Methyl-c-5-(4-(4-methylphenyl)-butyl)-1,3-dioxane-r-2-carboxylic acid*: White solid; mp: 111–113 °C; Yield: 86%; Purity: 99% by HPLC; IR (KBr): 3003, 2923, 1716, 1284, 1207, 1047 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 1.02–1.09 (m, 2H), 1.23–1.31 (m, 2H), 1.51–1.64 (m, 5H), 1.95–2.05 (m, 1H), 2.31 (s, 3H), 2.54 (t, J = 7.5 Hz, 2H), 3.44 (t, J = 11.6 Hz, 2H), 3.94–3.99 (dd, J = 11.8 and 4.6 Hz, 2H), 7.01–7.09 (m, 4H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 20.63, 25.34, 25.63, 27.38, 31.24, 32.92, 34.54, 67.32, 97.76, 128.11, 128.61, 134.44, 139.04, 171.46; ESI-MS *m/z*: 310.2 $(M+NH_4)^+$.**7b**: *c-5-[4-(4-Fluoro-phenyl)-butyl]-2-methyl-1,3-dioxane-r-2-carboxylic acid*: White solid; mp: 90–92 °C; Yield: 88%; Purity: 98% by HPLC; IR (KBr): 3020, 2923, 2852, 1716, 1284, 1209, 806 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 1.02–1.10 (m, 2H), 1.23–1.31 (m, 2H), 1.51–1.64 (m, 5H), 1.95–2.05 (m, 1H), 2.55 (t, J = 7.5 Hz, 2H), 3.44 (t, J = 11.5 Hz, 2H), 3.94–3.99 (dd, J = 11.8 and 4.6 Hz, 2H), 6.94 (t, J = 8.6 Hz, 2H), 7.06–7.14 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 25.33, 25.65, 27.47, 31.41, 33.05, 33.93, 67.07, 98.07, 115.01, 128.64, 132.14, 155.25, 171.60; ESI-MS *m/z*: 313.6 $(M+NH_4)^+$.**7c**: *2-Methyl-c-5-[4-(4-methylsulfonyl-phenyl)-butyl]-1,3-dioxane-r-2-carboxylic acid*: White solid; mp: 103–105 °C; Yield: 90%; Purity: 98% by HPLC; IR (KBr): 3040, 2931, 1720, 1492, 1284, 1043 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 1.02–1.09 (m, 2H), 1.25–1.33 (m, 2H), 1.51–1.61 (m, 5H), 1.98–2.02 (m, 1H), 2.46 (s, 3H), 2.51 (t, J = 7.6 Hz, 2H), 3.40 (t, J = 11.8 Hz, 2H), 3.94–3.99 (dd, J = 11.8 and 4.4 Hz, 2H), 7.05 (d, J = 8.1 Hz, 2H), 7.20 (d, J = 8.3 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 15.09, 25.14, 25.57, 27.27, 31.03, 32.82, 34.27, 67.26, 97.50, 126.30, 128.87, 134.72, 139.07, 171.35; ESI-MS *m/z*: 342.2 $(M+NH_4)^+$.**7d**: *c-5-[4-(4-Methoxy-phenyl)-butyl]-r-2-methyl-1,3-dioxane-2-carboxylic acid*: White solid; mp: 84–86 °C; Yield: 80%; Purity: 98% by HPLC; IR (KBr): 3020, 2938, 1727, 1556, 1228, 1136, 823 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 1.02–1.09 (m, 2H), 1.23–1.30 (m, 2H), 1.50–1.52 (m, 2H), 1.60 (s, 3H), 1.98–2.0 (m, 1H), 2.52 (t, J = 7.5 Hz, 2H), 3.44 (t, J = 11.5 Hz, 2H), 3.78 (s, 3H), 3.94–3.99 (dd, J = 11.9 and 4.4 Hz, 1H), 6.83 (d, J = 8.4 Hz, 2H), 7.07 (d, J = 8.4 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 25.09, 25.54, 27.26, 31.29, 32.80, 33.93, 54.90, 67.24, 97.48, 113.90, 129.07, 133.98, 157.27, 171.32; ESI-MS *m/z*: 309.3 $(M+H)^+$.**7e**: *c-5-[4-(4-Chloro-phenyl)-butyl]-r-2-methyl-1,3-dioxane-2-carboxylic acid*: White solid; mp: 94–96 °C; Yield: 86%; Purity: 99%; IR (KBr): 3030, 2936, 1723, 1516, 1228, 1136, 813 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 0.92–1.00 (m, 2H), 1.13–1.21 (m, 2H), 1.31 (s, 3H), 1.38–1.49 (m, 2H), 1.75–1.83 (m, 1H), 2.37 (t, J = 7.5 Hz, 2H), 3.22 (t, J = 11.5 Hz, 2H), 3.76–3.82 (dd, J = 11.7 and 4.4 Hz, 2H), 6.61 (d, J = 8.3 Hz, 2H), 6.91 (d, J = 8.2 Hz, 2H), 9.06 (br s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 25.33, 25.66, 27.47, 31.39, 32.99, 34.03, 67.07, 98.07, 114.97, 128.60, 132.10, 155.24, 171.60; ESI-MS *m/z*: 335.1 $(M+Na)^+$.**7f**: *2-Methyl-c-5-[4-(4-methylsulfinyl-phenyl)-butyl]-1,3-dioxane-r-2-carboxylic acid*: White solid; mp: 147–149 °C; Yield: 63%; Purity: 99% by HPLC; IR (KBr): 3040, 2939, 1730, 1265, 1197, 1147, 1001 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 0.98–1.06 (m, 2H), 1.21–1.32 (m, 2H), 1.53 (s, 3H), 1.57–1.65 (m, 2H), 1.98–2.04 (m, 1H), 2.65 (t, J = 7.48 Hz, 2H), 2.72 (s, 3H), 3.39 (t, J = 11.5 Hz, 2H), 3.88–3.93 (dd, J = 11.6 and 4.2 Hz, 2H,), 7.52 (d, J = 8.07 Hz, 2H), 7.6 (d, J = 8.0 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 25.18, 25.55, 27.22, 30.86, 32.77, 34.59, 43.20, 67.24, 97.48, 123.60, 129.10, 143.46, 145.26, 171.33; ESI-MS *m/z*: 341.1 $(M+H)^+$.**7g**: *2-Methyl-c-5-[4-(4-methylsulfonyl-phenyl)-butyl]-1,3-dioxane-r-2-carboxylic acid*: White solid; mp: 152-154 °C; Yield: 88%; Purity: 97% by HPLC; IR (KBr): 3020, 2933, 1743, 1299, 1134, 1118, 763 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 1.04–1.11 (m, 2H), 1.26–1.36 (m, 2H), 1.56 (s, 3H), 1.59–1.67 (m, 2H), 2.01–2.06 (m, 1H), 2.68 (t, J = 7.6 Hz, 2H), 3.05 (s, 3H), 3.45 (t, J = 11.5 Hz, 2H), 3.93–3.99 (dd, J = 11.9 and 4.4 Hz, 2H), 7.34 (d, J = 8.2 Hz, 2H), 7.84 (d, J = 8.3 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 25.22, 25.60, 27.25, 30.72, 32.81, 34.72, 67.28, 97.58, 127.01, 129.21, 138.31, 148.59, 171.43; ESI-MS *m/z*: 379.2 $(M+Na)^+$.**9**: *c-5-[4-(4-Benzyloxy-phenyl)-butyl]-r-2-methyl-1,3-dioxane-r-2-carboxylic acid*: White solid; mp: 102–104 °C: Yield: 84%; Purity: 99% by HPLC; IR (KBr): 3020, 2929, 1728, 1510, 1236, 1047, 746 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 1.04-1.09 (m, 2H), 1.23–1.33 (m, 2H), 1.50–1.62 (m, 5H), 1.97–2.03 (m, 1H), 2.50 (t, J = 7.5 Hz, 2H), 3.40 (t, J = 11.5 Hz, 2 H), 3.94-3.99 (dd, J = 11.8 and 4.4 Hz, 2H), 5.03 (s, 2H), 6.87 (d, J = 8.4 Hz, 2H), 7.04 (d, J = 8.4 Hz, 2H), 7.29–7.44 (m, 5H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 25.17, 25.61, 27.32, 31.36, 32.85, 34.02, 67.31, 69.13, 97.56, 114.56, 127.66, 128.42, 129.18, 134.34, 137.30, 156.43, 171.42, 185.15; ESI-MS *m/z*: 402.3 $(M+NH_4)^+$.**11**: *c-5-[4-(4-Hydroxy-phenyl)-butyl]-2-methyl-1,3-dioxane-r-2-carboxylic acid*: White solid; mp: 157–159 °C; Yield: 86%; Purity: 99% by HPLC; IR (KBr): 3073, 2933, 1720, 1514, 1228, 1136, 813 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 0.92–1.00 (m, 2H), 1.13–1.22 (m, 2H), 1.31 (s, 3H), 1.38–1.52 (m, 2H), 1.75–1.83 (m, 1H), 2.37 (t, J = 7.5 Hz, 2H), 3.22 (t, J = 11.5 Hz, 2H), 3.76–3.82 (dd, J = 11.7 and 4.4 Hz, 2H), 6.61 (d, J = 8.3 Hz, 2H), 6.91 (d, J = 8.2 Hz, 2H), 9.06 (br s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 25.32, 25.63, 27.42, 31.39, 32.99, 34.03, 67.07, 98.07, 114.97, 128.60, 132.10, 155.24, 171.60; ESI-MS *m/z*: 317.1 $(M+Na)^+$.**13a**: *c-5-[5-(4-Methanesulfonyloxy-phenyl)-butyl]-2-methyl-1,3-dioxane-r-2-carboxylic acid*: White solid; mp: 140–142 °C; Yield: 84%; Purity: 98% by HPLC; IR (KBr): 3020, 2927, 1753, 1506, 1346, 1261, 1170, 987 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$), δ 1.03–1.10 (q, J = 7.5 Hz, 2H), 1.28–1.34 (m, 2H), 1.53 (s, 3H), 1.98–2.04 (m, 1H), 2.59 (t, J = 7.5 Hz, 2H), 3.13 (s, 3 H), 3.44 (t, J = 11.6 Hz, 2H), 3.93–3.99 (dd, J = 4.7 and 11.9 Hz, 2H), 7.18 (s, 4H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 25.20, 25.56, 27.25, 30.94, 32.80, 34.19, 37.23, 67.26, 97.50, 121.91, 129.72, 141.51, 147.15, 171.34; ESI-MS *m/z*: 395.0 $(M+Na)^+$.**13b**: *c-5-[5-(4-Methanesulfonyloxy-phenyl)-pentyl]-2-methyl-1,3-dioxane-r-2-carboxylic acid*: White solid; mp: 102–104 °C; Yield: 94%; Purity: 98% by HPLC; IR (KBr): 3036, 2930, 1720, 1498, 1363, 1290, 1211, 1168, 1145, 974, 867, 775, 538, 526 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 1.0 (m, 2H), 1.2 (m, 4H), 1.5 (m, 5H), 2.0 (m, 1H), 2.6 (t, J = 7.4 Hz, 2H), 3.1 (s, 3H), 3.4 (t, J = 11.6 Hz, 2H), 3.9 (dd, J = 12.0 and 4.5 Hz, 2H), 7.2 (m, 4H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 25.34, 25.54, 27.41, 28.71, 30.54, 32.86, 34.33, 37.22, 67.27, 97.49, 121.86, 129.70, 141.54, 147.12, 171.32; ESI-MS *m/z*: 409.0 $(M+Na)^+$.**13c**: *c-5-[6-(4-Methanesulfonyloxy-phenyl)-hexyl]-2-methyl-1,3-dioxane-r-2-carboxylic acid*: White solid; mp: 104–106 °C; Yield: 93%; Purity: 97.5% by HPLC; IR (KBr): 3026, 2922, 1755, 1467, 1259, 1147 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.02–1.05 (m, 2H), 1.27 (bm, 6H), 1.57 (bm, 5H), 1.98–2.06 (m, 1H), 2.59 (t, J = 7.6 Hz, 2H), 3.12 (s, 3H), 3.46 (t, J = 11.6 Hz, 2H), 3.96–4.00 (dd, J = 12.0 and 4.4 Hz, 2H), 7.16–7.28 (m, 4H); ^{13}C NMR: (100 MHz, DMSO-d_6): δ 25.60, 26.72, 28.46, 29.02, 32.47, 33.06, 34.46, 37.23, 67.35, 97.13, 121.64, 129.47, 141.35, 147.50, 171.40; ESI-MS *m/z*: 423.0 $(M+Na)^+$.

Bioorganic & Medicinal Chemistry Letters 18 (2008) 6471–6475

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Modulation of PPAR receptor subtype selectivity of the ligands: Aliphatic chain vs aromatic ring as a spacer between pharmacophore and the lipophilic moiety ☆

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ARTICLE INFO

Article history:
Received 27 June 2008
Revised 11 September 2008
Accepted 14 October 2008
Available online 17 October 2008

Keywords:
PPARα agonist
Oxazole
Imiglitazar
Muraglitazar

ABSTRACT

Oxazole containing glycine and oximinobutyric acid derivatives were synthesized as PPARα agonists by incorporating polymethylene spacer as a replacement of commonly used phenylene group that connects the acidic head with lipophilic tail. Compound **13a** was found to be a selective and potent PPARα agonist. Further 1,3-dioxane-2-carboxylic acid derivative **20** was synthesized by replacing the tetramethylene spacer of NS-220, a selective PPARα agonist with phenylene group and found to exhibit PPARα/γ dual agonism. These results suggest that compounds possessing polymethylene spacer between pharmacophore and lipophilic tail exhibit predominantly PPARα agonism whereas those with an aromatic phenylene spacer shows PPARα/γ dual agonism.

The peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors in the nuclear hormone receptor superfamily.[1] Three distinct PPAR subtypes (PPARα, PPARγ, and PPARδ) have been identified in most mammalian species. The multiple roles of the PPARs in physiological regulation of glucose homeostasis, fatty acid metabolism, inflammation, and cellular differentiation have been reviewed extensively in recent years.[2] PPARγ is well known at a cellular level for its role in adipogenesis and has been implicated as the primary receptor modulating the antidiabetic activity through insulin sensitization.[3] PPARγ agonists, such as TZDs, have proven to be efficacious as insulin-sensitizing agents in the treatment of type 2 diabetes. Two of these TZDs namely Rosiglitazone and Pioglitazone are currently available in the market. Unfortunately, they are also known to cause undesirable side effects including weight gain, edema, and anemia in both animal models and humans. PPARα is known to play a pivotal role in the uptake and oxidation of fatty acids and also in lipoprotein metabolism.[4] Fibrate compounds such as Fenofibrate and Bezafibrate used to treat hyperlipidemia are effective in reducing triglycerides, increasing HDL cholesterol and lowering LDL cholesterol are poor activators of PPARα and need high doses to show significant efficacy. The hypothesis that PPARα/γ dual agonism provides an additive, and possibly synergistic, pharmacology has resulted in an intensive effort within the pharmaceutical industry to develop and evaluate these agents.[5] The first dual agonist Farglitazar[6] which is a potent PPARγ agonist with a moderate PPARα activation was dropped in an advanced stage due to the emergence of edema. Two more dual agonists with substantial PPARα/γ dual activity Ragaglitazar[7] and Tesaglitazar[8] were also dropped from late clinical development due to carcinogenicity in rodent toxicity models and elevated serum creatinine and associated decrease in glomular filtration rate, respectively. The only dual agonist that has been advanced to NDA filing, Muraglitazar[9] was dropped very recently due to the incidence of edema, heart failure, and cardiovascular complications. Activation of PPARα is known to lower triglycerides, elevate HDL, and exert insulin-sensitizing effects.[10] These findings suggest that even chronic administration of selective PPARα agonist will serve as a better remedy for the treatment of metabolic disorder and led us to develop useful chemical tools to aid discovery of novel PPARα agonists. A typical structural design of a PPAR agonist as shown in Figure 1 comprises of a lipophilic heterocyclic tail and an acidic pharmacophore with a spacer in-between. As a part of our research in the field of PPARs to develop novel therapeutic agents to treat metabolic disorders[11] we have recently reported a series of 1,3-dioxane-2-carboxylic acid derivatives as PPARα/γ dual agonist[12] and the lead compound of this series **20** differs structurally from a recently reported PPARα agonist

☆ ZRC communication # 229.

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doi:10.1016/j.bmcl.2008.10.062

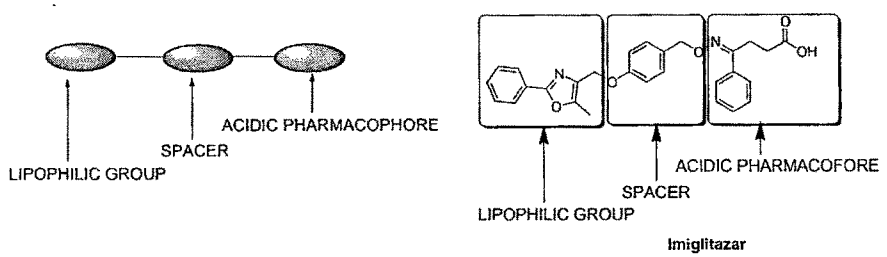


Figure 1. Structural design of PPAR ligand.

NS-220[13] in spacer region as compound **20** and other dual agonists like Imiglitazar[14] and Muraglitazar possess aromatic phenylene spacer whereas NS-220 possesses an alkyl chain as a spacer between acidic head and lipophilic tail (Fig. 2). Based on these observations we envisioned that PPAR subtype selectivity of ligands may be sensitive to chemical variations in the spacer region of the structure and intended to study the effect of changing the phenylene spacer of Imiglitazar and Muraglitazar to a polymethylene spacer in order to develop selective PPARα agonist. In this context we herein report few oxazole containing glycine and oximinobutyric acid derivatives as PPARα agonists designed by incorporating polymethylene spacer as a replacement of phenylene group of Imiglitazar and Muraglitazar. We also report a previously discovered dual agonist **20** synthesized by replacing the polymethylene spacer of NS-220, a selective PPARα agonist with phenylene group in order to show that the replacement of phenylene spacer of dual agonist with a polymethylene spacer makes the compounds PPARα selective agonists.

Synthesis of intermediate hydroxy compound **4a–b** and **6a–b** was illustrated in Scheme 1. Carboxylic acids **3a–b** were synthesized by reacting **1** or **2** with diethylmalonate in presence of sodium hydride followed by hydrolysis of the diester followed by decarboxylation under thermal condition. Esterification of monoacids **3a–b** followed by reduction of the resulted esters **3c–d** with lithium aluminum hydride yielded the hydroxy compounds **4a–b**. The mesylate derivatives **4c–d** were subjected to a similar series of reactions described for the synthesis of **4** in order to elongate the methylene chain to yield compounds **6a–b**. Compounds **9a–c** were synthesized according to Scheme 2. The hydroxy compounds **4b** and **6a–b** were oxidized to corresponding aldehydes **7a–c** with pyridiniumchlorochromate in dichloromethane which were reacted with glycine ethyl ester under reductive amination conditions followed by acylation of the intermediate with 4-methoxyphenylchloroformate to yield the ester **8a–c**. Hydrolysis of **8a–c** in aqueous basic medium resulted to yield acid compounds **9a–c**. Synthesis of compounds **13a–c** were described in Scheme 3. The hydroxy compounds **4b** and **6a–b** were converted to their mesylate derivatives **4d** and **10a–b**, respectively, and coupled with compound **11** in presence of base and the corresponding esters **12a–c** obtained were hydrolyzed with aqueous base to afford acids **13a–c**. Compound **20**[12] was synthesized as illustrated in Scheme 4 by coupling the intermediate **18** with **1** and hydrolyzing the ester **19** in presence of aqueous base. Intermediate **18** was prepared by reducing the diester **15** to diol **16** which was then cyclized to 1,3-dioxane by treating with methylpyruvate in the presence of lewis acid to afford compound **17** and debenzylated under hydrogen transfer reaction conditions. Imiglitazar,[14] Muraglitazar,[9] NS-220[13], Intermediates **1**, **2**,[15] and **11**[14] were synthesized following the methods reported in the literature.

Newly synthesized compounds[16] **9a–c**, **13a–c**, and **20** along with Imiglitazar, Muraglitazar, and NS-220 were screened for hPPARα, γ, and δ agonistic activity on full length PPAR receptor transfected in HepG2 cells following the procedure described in our earlier publication.[12] WY-14643, Rosiglitazone, and GW-

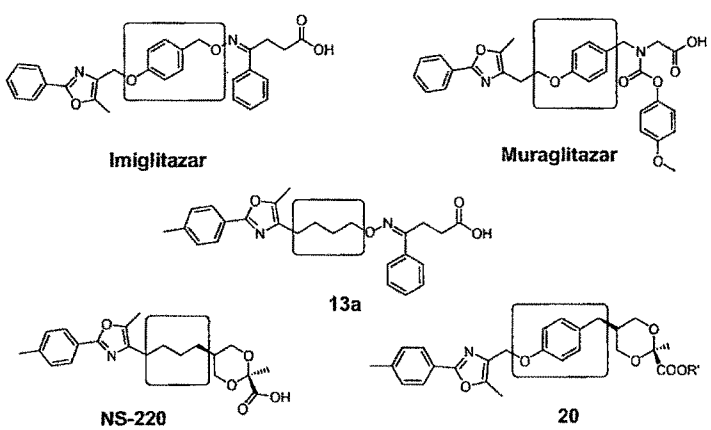
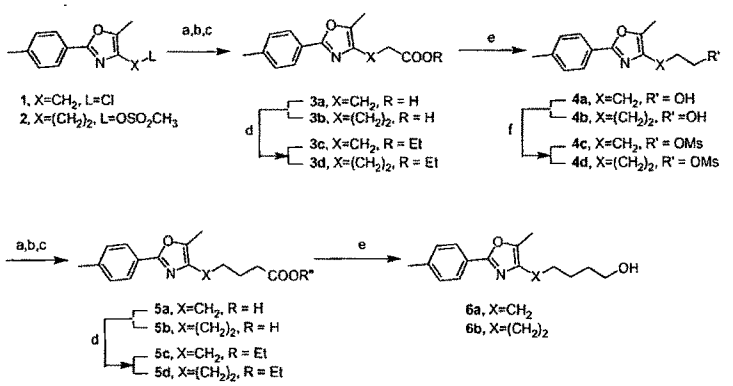
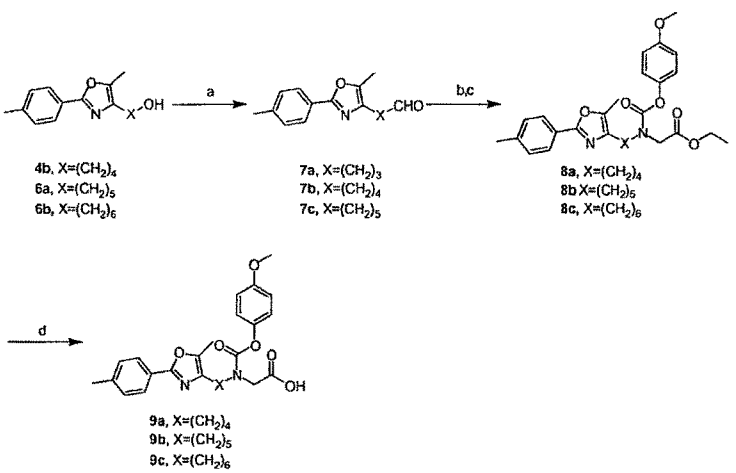


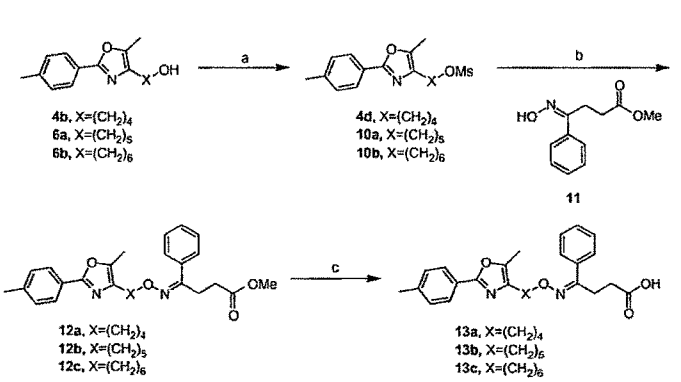
Figure 2. Chemical structures of PPAR ligands. Imiglitazar, Muraglitazar and **20** possess aromatic phenylene spacer between acidic head and lipophilic tail and are PPAR α/γ dual agonists whereas NS-220 and **13a** contain polymethylene spacer and are PPARα selective agonists.



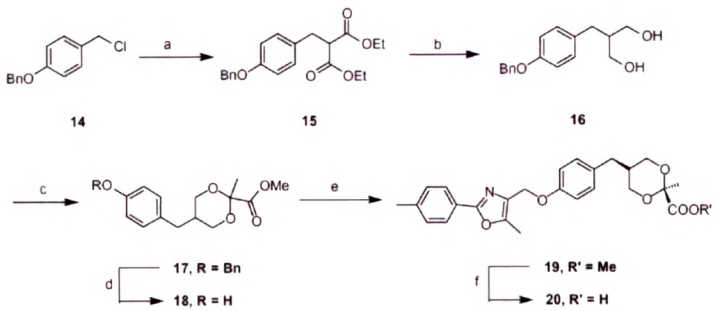
Scheme 1. Reagents and conditions: (a) diethyl malonate, NaH, DMF, 25 °C, 18 h; (b) aq NaOH, MeOH, 25 °C, 0.25 h; (c) xylene, reflux, 5 h; (d) EtOH, H_2SO_4, reflux, 24 h; (e) $LiAlH_4$, THF, 25 °C, 0.5 h; (f) CH_3SO_2Cl, Et_3N, CH_2Cl_2, 10 °C, 0.25 h.



Scheme 2. Reagents and condition: (a) PCC, celite, CH_2Cl_2, 25 °C, 2 h; (b) glycine ethyl ester hydrochloride, MeOH, $NaBH(OAc)_3$, 25 °C, 1.5 h; (c) 4-methoxyphenylchloroformate, CH_2Cl_2, 25 °C, 2 h; (d) LiOH·H_2O, THF, H_2O, MeOH, 25 °C, 3 h.



Scheme 3. Reagents and conditions: (a) CH_3SO_2Cl, Et_3N, CH_2Cl_2, 10 °C, 0.25 h; (b) NaH (50%), DMF, 60 °C, 24 h; (c) LiOH·H_2O, THF, H_2O, MeOH, 25 °C, 5 h.



Scheme 4. Reagents and conditions: (a) diethyl malonate, NaH (50%), THF, 20 °C, 14 h; (b) $LiAlH_4$, THF, 20 °C, 6 h; (c) methyl pyruvate, BF_3 etherate, CH_3CN, 25 °C, 4 h; (d) $HCOONH_4$, Pd/C (10%), MeOH, reflux, 2 h; (e) **1**, K_2CO_3, DMF, 60 °C, 20 h; (f) NaOH, MeOH, H_2O, 25 °C, 18 h.

Table 1
hPPAR transactivation data.

Compound	hPPAR transactivation[a,b] EC_{50} (μM)			
	α	γ	Ratio γ/α	δ
9a	IA	IA	–	IA
9b	0.044	0.56	12	IA
9c	0.034	0.45	13	IA
13a	0.0000038	0.43	113157	IA
13b	0.002	0.031	15	IA
13c	0.0001	0.1	1000	IA
20	0.072	0.015	0.2	IA
Imiglitazar	0.008	0.004	0.5	IA
Muraglitazar	0.15	0.09	0.6	IA
NS-220	0.052	6.85	131	IA

[a] IA denotes inactive where compounds did not show any fold induction above the basal level (shown by vehicle) up to 1 μM concentration.
[b] EC_{50} is the concentration of the test compound that affords half-maximum transactivation activity.

501516 were used as controls for PPARα, γ, and δ, respectively, and the results were summarized in Table 1. As described earlier we intended to replace the central phenylene spacer of Muraglitazar with an alkyl chain and synthesized compounds **9a–c** and evaluated their PPAR transactivation potentials. Among these three compounds, **9a** with tetramethylene spacer was found inactive towards both PPARα and γ whereas **9b** and **9c** were found less potent towards PPARγ compared to PPARα increasing the ratio of EC_{50} γ/α to 12 and 13, respectively, against 0.6 as in case of Muraglitazar. Having felt encouraged with these results and in urge of potent and highly selective PPARα agonist we then synthesized the compounds **13a–c** taking this time Imiglitazar as initial lead. The transactivation results of the compounds **13a–c** were found very interesting and were in line with our hypothesis. Compounds **13a** and **13c** found very potent and selective PPARα agonists with picomolar and nanomolar range EC_{50}, respectively, and multi-fold selectivity towards PPARα over PPARγ whereas Compounds **13b** showed inferior results in terms of selectivity towards PPARα transactivation. To support our hypothesis further and cross validate the same we synthesized the compound **20** by replacing the tetramethylene group of the compound NS-220 with phenylene group. This compound **20** turned out to be a dual PPARα/γ agonist with equipotent activation towards both PPARα and γ whereas its parent compound NS-220 is a selective PPARα agonist.

To better understand the activity of **13a** at molecular level, docking simulations were carried out for this compound and Imiglitrazar using Discovery Studio software version 1.6. The geometry of compounds docked was subsequently optimized using the CHARMM force field. The energy minimization was carried out using smart minimizer option in the software until the gradient value was smaller than 0.001 kcal/mol Å. The complexed X-ray crystal structure of the ligand binding domain (LBD) of PPARα with GW409544[17] (1k7l.pdb) and PPARγ with Rosiglitazone (2PRG.pdb) were obtained from RCSB Protein Data Bank. When docked into PPARα binding pocket the most stable docking models both **13a** and Imiglitazar adopted a confirmation that allows the carboxylic group to form hydrogen bonds with Tyr 314, Tyr 464, and Ser280 (Fig. 3) which are reported to be essential interaction for a PPARα agonist. However, in the docking model of **13a** an additional H-bond between the nitrogen atom of oxazole ring and Cys276 was observed. This additional H-bond may actually be responsible for improved activity of this compound over Imiglitazar. Compound **13a** when docked into PPARγ binding pocket none of the conformations showed H-bond interactions with any of the amino acids though the molecular orientation was similar to that of Imiglitazar which correlates with moderate PPARγ activity of this compound

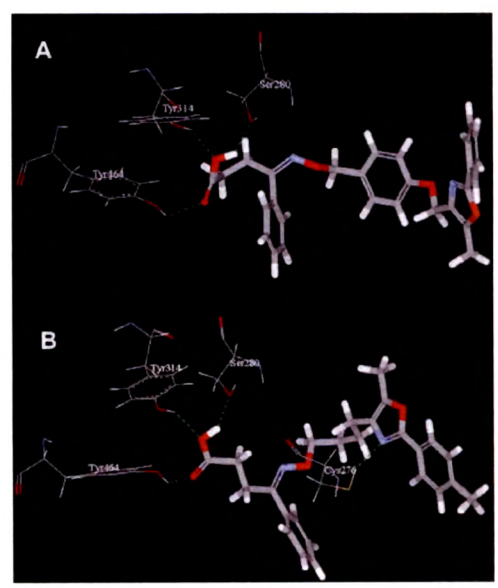


Figure 3. Molecular docking of **13a** (B) and Imiglitazar (A) into PPARα binding site. H-bond interactions with amino acids are shown in dashed lines.

whereas Imiglitazar showed H-bond interactions with Cys295 while Ser289 and Tyr473 are lying in close proximity of the ligand.

In summary we discovered novel oxazole containing glycine and oximinobutyric acid derivatives as PPARα agonists by incorporating polymethylene spacer as a replacement of commonly used phenylene group that connects the acidic head with lipophilic tail as exemplified by **13a**. On the other hand we also designed compound **20** as PPARα/γ dual agonist by replacing tetramethylene spacer of a known compound NS-220 with phenylene group. The above results clearly established that the spacer of polymethylene chain of varying length between the pharmacophore and the lipophilic heterocycle improves selectivity of the compound towards PPARα receptor compared to the compounds having phenylene group as a spacer. Further work in the development of SAR of this lead series based on **13a** will be described in a subsequent publication.

Acknowledgments

Authors are thankful to management of Zydus Cadila for the encouragement and to analytical department for the support.

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16. *Spectroscopic analysis of the compounds* **9a–c**, **13a–c**, *and* **20**: **9a**: [(4-methoxyphenoxycarbonyl)-[4-(5-methyl-2-(4-methylphenyl)oxazol-4-yl)-butyl]-amino]acetic acid: thick liquid; yield: 96%; purity by HPLC: 99%; IR (Nujol): 3018, 2939, 1716, 1458, 1180, 761 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.69–1.72 (m, 4H), 2.29 (s, 3H), 2.36 (s, 3H), 2.52–2.57 (m, 2H), 3.52–3.56 (m, 2H), 3.77 (s, 3H), 4.17 (d, *J* = 8.0 Hz, 2H), 6.83 (d, *J* = 9.0 Hz, 2H), 7.01–7.05 (m, 2H), 7.21 (d, *J* = 6.4 Hz, 2H), 7.82 (d, *J* = 7.8 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 9.70, 20.94, 23.68, 24.24, 24.65, 25.80, 26.66, 28.23, 47.80, 53.40, 55.31, 62.96, 113.96, 122.5, 124.69, 125.39, 129.53, 135.52, 139.61, 143.17, 145.14, 155.07, 156.16, 157.76, 158.37. ESI-S *m/z*: 453.1 [M+H]$^+$; **9b**: [(4-methoxyphenoxycarbonyl)-[5-(5-methyl-2-(4-methylphenyl)oxazol-4-yl)-pentyl]-amino]acetic acid: white solid; mp: 140–141 °C; yield: 32%; purity by HPLC: 96%; IR (KBr): 2925, 1720, 1450, 1182, 827 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.40–1.44 (m, 2H), 1.68–1.72 (m, 4H), 2.22 (s, 3H), 2.31 (s, 3H), 2.52 (t, *J* = 7.4 Hz, 2H), 3.39–3.47 (m, 2H), 3.81 (s, 3H), 4.12 (d, *J* = 8.0 Hz, 2H), 6.81 (d, *J* = 8.8 Hz, 2H), 6.97–7.04 (m, 2H), 7.21 (d, *J* = 8.1 Hz, 2H), 7.82 (d, *J* = 8.2 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 9.71, 20.97, 24.90, 25.64, 27.15, 28.23, 48.48, 59.78, 114.16, 122.56, 124.71, 125.39, 129.56, 135.63, 139.64, 143.13, 144.62, 154.57, 156.48, 158.37.; ESI-S *m/z*: 467.3 [M+H]$^+$; **9c**: [(4-Methoxy-phenoxycarbonyl)-[6-(5-methyl-2-(4-methylphenyl)oxazol-4-yl)-hexyl]amino]acetic acid: thick liquid; yield: 82%; purity by HPLC: 98%; IR (Nujol): 3016, 2935, 1720, 1508, 1199, 1180, 756 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.41–1.45 (m, 2H), 1.59–1.63 (m, 6H), 2.32 (s, 3H), 2.36 (s, 3H), 2.52 (t, *J* = 7.3 Hz, 2H), 3.48–3.52 (m, 2H), 3.72 (s, 3H), 4.12 (d, *J* = 12.5 Hz, 2H), 6.81 (d, *J* = 8.1 Hz, 2H), 7.01–7.06 (m, 2H), 7.22 (d, *J* = 7.9 Hz, 2H), 7.91 (d, *J* = 8.0 Hz, 2H); ^{13}C NMR: (100 MHz, DMSO-d_6): δ 9.69, 20.93, 24.93, 26.10, 27.25, 27.69, 28.42, 48.16, 50.22, 55.30, 113.90, 114.08, 122.45, 122.84, 124.70, 125.35, 129.49, 135.69, 139.57, 142.95, 145.03, 154.44, 154.82, 156.23, 158.32.; ESI-S *m/z*: 481.3 [M+H]$^+$; **13a**: 4-[4-(5-methyl-2-(4-methylphenyl)oxazol-4-yl)-butoxyimino]-4-phenylbutyric acid: white solid; mp: 102–104 °C; yield: 85%; purity by HPLC: 99%; IR (KBr): 3429, 2922, 1649, 1596, 1577, 1307, 1265, 1147 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.76–1.82 (bm, 4H), 2.31 (s, 3H), 2.36 (s, 3H), 2.47–2.54 (m, 4H), 3.17 (t, *J* = 6.8 Hz,2H), 4.26 (bt, 2H), 7.21 (d, *J* = 8.0 Hz, 2H), 7.32–7.38 (m, 3H), 7.60–7.66 (m, 2H), 7.84 (d, *J* = 8.0 Hz, 2H),; ^{13}C NMR (100 MHz, DMSO-d_6): δ 9.94, 20.93, 21.93, 24.76, 24.97, 28.49, 30.46, 73.41, 124.75, 125.39, 126.08, 128.44, 129.08, 129.46, 135.00, 135.57, 139.81, 143.09, 154.93, 158.40, 173.51; ESI-MS *m/z*: 421.2 [M+H]$^+$: **13b**: 4-[5-(5-methyl-2-(4-methylphenyl)oxazol-4-yl)-pentyloxyimino]-4-phenylbutyric acid: off white solid; mp: 91–93 °C; yield: 86%; purity by HPLC: 97%; IR (KBr): 3413, 2931, 1718, 1500, 1269, 1163, 732 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.47–1.54 (m, 2H), 1.64–1.69 (m, 2H), 1.72–1.79 (m, 2H), 2.30 (s, 3H), 2.36 (s, 3H), 2.52 (t, *J* = 7.5 Hz, 2H), 2.58 (t, *J* = 7.5 Hz, 2H), 3.11 (t, *J* = 7.5 Hz, 2H), 4.20 (t, *J* = 6.0 Hz, 2H), 7.22 (d, *J* = 8.1 Hz, 2H), 7.34–7.36 (m, 3H), 7.60–7.63 (m, 2H), 7.85 (d, *J* = 8.1 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 9.67, 20.92, 21.90, 24.90, 28.25, 28.56, 30.40, 73.55, 124.72, 125.36, 126.05, 128.40, 129.08, 129.47, 133.88, 134.97, 135.61, 139.54, 143.02, 156.33, 158.36, 173.33; ESI-MS *m/z*: 435.1 [M+H]$^+$: **13c**: 4-[6-(5-methyl-2-(4-methylphenyl)oxazol-4-yl)-hexyloxyimino]-4-phenyl-butyric acid: white solid; mp: 79–81 °C; yield: 63%; purity by HPLC: 99%; IR (KBr): 3020, 1714, 1625, 1402, 1217, 771 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.43–1.45 (m, 2H), 1.59–1.64 (m, 4H), 1.71–1.74 (m, 2H), 2.31 (s, 3H), 2.38 (s, 3H), 2.53 (t, *J* = 7.5 Hz, 2H), 2.64 (t, *J* = 7.5 Hz, 2H), 3.09 (t, *J* = 7.4 Hz, 2H), 4.22 (t, *J* = 5.7 Hz, 2H), 7.21–7.25 (m, 2H), 7.35–7.37 (m, 3H), 7.61–7.63 (m, 2H), 7.85 (d, *J* = 8.1 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 9.65, 20.89, 21.86, 24.84, 25.18, 28.26, 28.65, 73.56, 124.70, 125.32, 126.02, 128.40, 129.04, 129.44, 134.95, 135.67, 139.51, 142.94, 156.29, 158.29, 173.27; ESI-MS *m/z*: 449.1 [M+H]$^+$; **20**: 2-methyl-c-5-[4-(5-methyl-2-(4-methylphenyl)oxazol-4-ylmethoxy)benzyl]-1,3 dioxane-r-2-carboxylic acid: white solid; mp: 197–198 °C; yield: 95%; purity by HPLC: 99%; IR (KBr): 2929, 1759, 1610, 1502,1286, 1226, 1182, 827 cm^{-1}; ^1H NMR (DMSO-d_6): δ 1.32 (s, 3H), 2.09–2.13 (m, 1H), 2.27 (d, *J* = 6.9 Hz, 2H), 2.36 (s, 3H), 2.42 (s, 3H), 3.42 (t, *J* = 11.4 Hz, 2H), 3.69–3.74 (dd, *J* = 11.6 and 4.1 Hz, 2H), 4.94 (s, 2H), 6.94 (d, *J* = 8.4 Hz, 2H), 7.11 (d, *J* = 8.4 Hz, 2H), 7.32 (d, *J* = 8.1 Hz, 2H), 7.83 (d, *J* = 8.1 Hz, 2H); ^{13}C NMR (DMSO-d_6): δ 9.93, 20.96, 25.52, 32.87, 34.86, 61.38, 67.07, 97.56, 114.64, 124.24, 125.57, 129.62, 130.58, 131.85, 140.15, 146.95, 156.65, 158.98, 171.31; ESI-MS *m/z*: 438.4 [M+H]$^+$.
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Bioorganic & Medicinal Chemistry Letters 20 (2010) 1156–1161

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Design and synthesis of novel bis-oximinoalkanoic acids as potent PPARα agonists ☆

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ARTICLE INFO

Article history:
Received 9 September 2009
Revised 16 November 2009
Accepted 3 December 2009
Available online 6 December 2009

Keywords:
Type 2 diabetes
PPARα agonist
Oximinoalkanoic acids
PPARα/γ dual agonist

ABSTRACT

Bis-oximinoalkanoic acid derivatives were designed and synthesized to aid in the characterization of selective PPARα agonists by replacing the oxazole ring with flexible oximino group in the lipophilic tail part of a previously reported compound **3**. Selected compounds **9d** and **9m** showed excellent potency and high selectivity towards PPARα in vitro. These compounds found effective in reducing serum triglycerides (TG) in vivo.

The peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors in the nuclear hormone receptor superfamily[1] and are activated by endogenous saturated and unsaturated fatty acids and their metabolites, as well as synthetic ligands.[2] Three distinct PPAR subtypes (PPARα, PPARγ and PPARδ) have been identified in most mammalian species. Each PPAR subtype is differentially expressed in a tissue-specific manner. PPARα is mostly expressed in the tissues involved in lipid oxidation, such as liver, kidney, skeletal, cardiac muscle, and adrenal glands.[3] PPARγ is expressed in adipose tissue, macrophages, and vascular smooth muscles.[4] PPARδ is ubiquitously expressed, though it is mainly found in skeletal muscle and adipose tissues.[5] PPARs form heterodimers with another nuclear receptor partner, retinoid X receptor (RXR), and become functional to regulate gene expression by binding to a specific DNA sequence, termed PPRE (peroxisome proliferator responsive element), located in the promoter region of target genes[6] The multiple roles of the PPARs in physiological regulation of glucose homeostasis, fatty acid metabolism, inflammation and cellular differentiation have been reviewed extensively in recent years.[7] PPARγ is known to play a vital role at a cellular level in adipogenesis and identified as the primary receptor modulating insulin sensitization and thereby exerting antidiabetic activity.[8] Two of these PPARγ agonists, namely Rosiglitazone and Pioglitazone are currently available in the market. Unfortunately, they are also known to cause undesirable side effects including weight gain, edema and anemia in both animal models and humans. PPARα is known to play a pivotal role in the uptake and oxidation of fatty acids and also in lipoprotein metabolism.[9] Fibrate compounds such as Fenofibrate and Bezafibrate were originally developed without knowing their molecular target and are in use to treat hyperlipidemia as they are effective in reducing triglycerides, increasing HDL cholesterol and lowering LDL cholesterol. Subsequent research proved these fibrates to be poor activators of PPARα and need high doses to show significant efficacy. In 1990s the hypothesis that PPARα/γ dual agonism provides an additive, and possibly synergistic, pharmacology has resulted in an intensive effort within the pharmaceutical industry to develop and evaluate these agents.[10] but none of these dual agonists including Farglitazar,[11] Ragaglitazar,[12] Tesaglitazar[13] and Muraglitazar,[14] has been marketed. The unsuccessful efforts to develop dual agonist and recent research findings that activation of PPARα lower triglycerides, elevate HDL and exert insulin-sensitizing effects[15] led to the discovery of potent and selective activators of PPARα as remedy for disorders mediated by lipid and carbohydrate metabolism. Disclosure of LY518674[16] and GW590735[17] as potent and selective PPARα agonists hightened the interest among several research groups to develop such agents. Recently a potent and selective PPARα agonist NS-220[18] (Fig. 1) is reported to exert hypoglycemic and lipid modulating effects in animal models but

☆ ZRC communication # 249.

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doi:10.1016/j.bmcl.2009.12.022

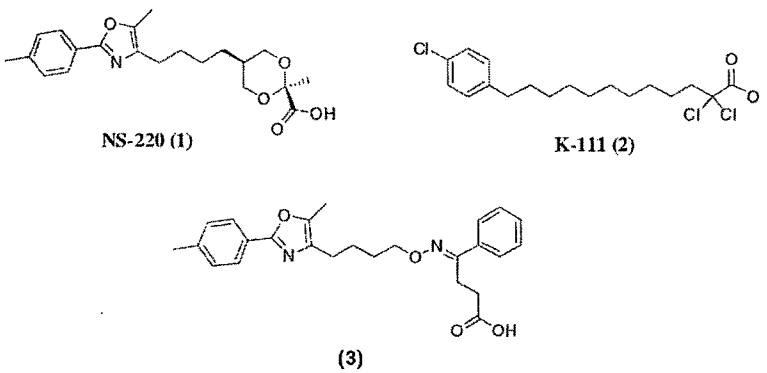


Figure 1. Selective PPARα agonists.

the further development of this compound is discontinued for unknown reasons. Another compound K-111[19] (Fig. 1) a relatively weak PPARα agonist is presently undergoing clinical trails for the treatment of type 2 diabetes. In continuation of our research in the field of PPARs to develop novel therapeutic agents to treat metabolic disorders[20] we have recently reported selective PPARα agonist (3)[21] (Fig. 1) derived from the known α/γ dual agonist Imiglitazar employing the chemical modifications in the central spacer region of the typical chemotype of PPAR agonist. Although this compound exhibited high degree of selectivity towards PPARα over γ, its picomolar potency raised concerns of possible toxicity for further development of this compound. Inorder to identify PPARα selective agonists further based on compound **3**, molecular modeling experiments were under taken and based on the results (data not shown) we envisioned that replacement of rigid oxazole heterocycle with a flexible bioisostere would be a good strategy and designed a novel series of bis-oximinoalkanoic acid derivatives centering the modifications in the lipophilic tail as depicted in Figure 2.

Synthesis of intermediate **6** was depicted in Scheme 1. Intermediate **4** was synthesized by Friedel–Crafts acylation of benzene with succinic anhydride. Treatment of **4** with hydroxylamine gave

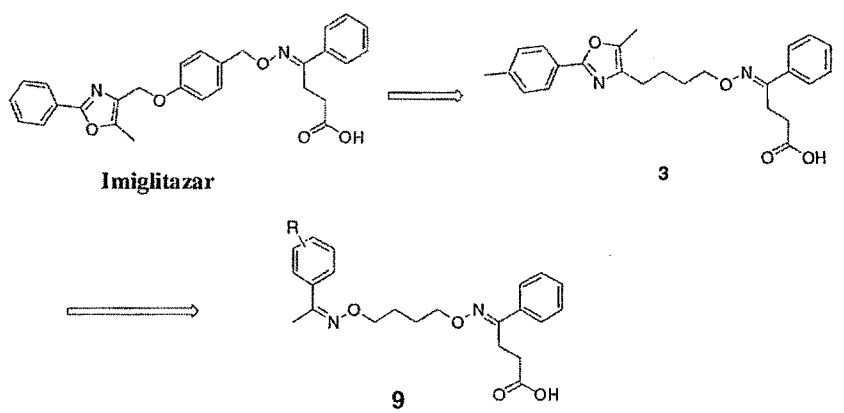
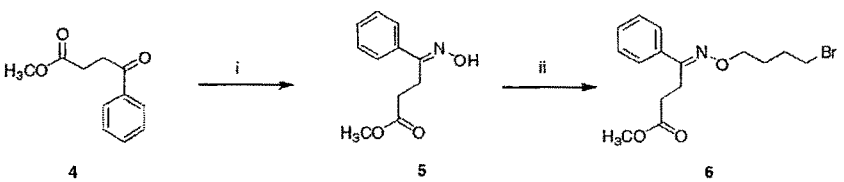
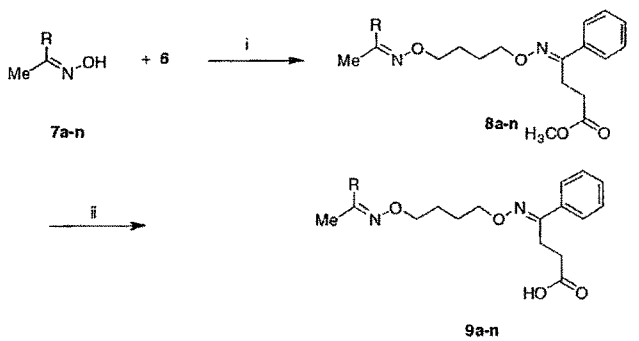


Figure 2. Designing selective PPARα agonist.



Scheme 1. Reagents and conditions: (i) hydroxylammonium chloride, NaOAc, EtOH, reflux, 2 h; (ii) 1,4-dibromobutane, K_2CO_3, DMF, 60 °C, 24 h.

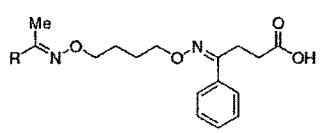


Scheme 2. Reagents and conditions: (i) K_2CO_3, DMF, 60 °C, 8 h; (ii) NaOH, H_2O, MeOH, 25 °C, 18 h.

the oxime derivative as a mixture of *E* and *Z*-isomers and the *E*-isomer **5** was isolated by column chromatography as a major product which showed ^{1}H NMR chemical shifts identical with reported values.[22] Alkylation of **5** with 1,4-dibromobutane gave the intermediate **6** in good yields. Synthesis of compounds **9a–n** were described in Scheme 2. Oximes **7a–n** were synthesized by reacting the corresponding acetophenone with hydroxylamine and are considered to be *E*-isomers as these are reported to be thermodynamically more stable than *Z*-isomers.[23] Coupling reaction between intermediate **6** and oximes **7a–n** under basic conditions gave the ester derivatives **8a–n** which upon hydrolysis under aqueous basic conditions gave the acids **9a–n**.

Compounds **9a–n**[24] were screened for hPPARα, γ and δ agonistic activity on full length PPAR receptors transfected in HepG2 cells. WY-14643, Rosiglitazone and GW-501516 were used as controls for PPARα, γ and δ, respectively, and the results are summarized in Table 1 where the activities were reported as EC_{50} values and percent maximal activity of each compound compared to

Table 1
In vitro hPPAR transactivation and TG reducing activity of compounds **9a–n**



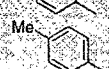
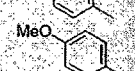
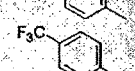
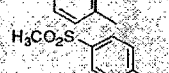
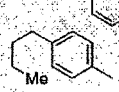
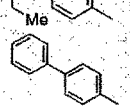
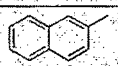
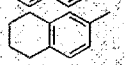
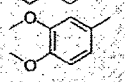
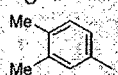
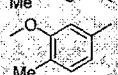
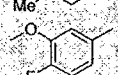
Compd	R	PPAR transactivation: EC_{50} (% activation)[b]		% reduction in TG in SAM[c]
		α	γ	
9a		2.3 (46)	IA	ND
9b		0.05 (101)	1.4 (69)	18
9c		0.05 (78)	2.1 (69)	13
9d		0.01 (102)	1.1 (13)	35
9e		0.4 (79)	1.4 (37)	0.6
9f		2.4 (41)	1.9 (49)	14
9g		0.5 (85)	0.2 (64)	31
9h		0.02 (83)	0.1 (52)	23

Table 1 (*continued*)

Compd.	R	PPAR transactivation[a] EC_{50} (% activation)[b]		% reduction in TG in SAM[c]
		α	γ	
9i		0.03 (61)	0.2 (50)	25
9j		0.003 (86)	0.2 (61)	36
9k		0.1 (136)	0.8 (45)	30
9l		0.002 (89)	1.0 (48)	12
9m		0.005 (257)	1.6 (60)	36
9n		0.4 (122)	0.7 (76)	19
Imiglitazar		0.005 (116)	0.004 (89)	37

[a] HepG2 cells were transfected with pSG5 expression vector containing the cDNA of hPPARα or hPPARγ or hPPARδ and cotransfected with PPRE3-TK-luc. The Luciferase activity was determined using commercial fire-fly luciferase assay and β-galactosidase activity was determined in ELISA reader.

[b] Percent of maximal activation of all compounds was compared to reference compounds (WY-14643 for α and Rsiglitazone for γ) normalized to 100%. IA denotes inactive.

[c] 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.

reference compound normalized to 100%. None of the compounds show any fold induction above the basal level (shown by vehicle) up to 1 μM concentration towards PPARδ. Triglyceride lowering activity was measured by administering the compounds orally at a dose of 10 mg/kg/day for 6 days to male *swissalbino* mice (SAM) which are moderately hyperlipidemic. Values reported are the % change in plasma triglyceride (TG) concentration of the compound-treated mice relative to vehicle controls and are given in Table 1. Our goal was to develop potent and selective PPARα agonist that did not contain phenyloxazole group starting from compound **3** reported previously by our group. We decided to design the compounds **9** by replacing the oxazole ring with an oximino group expecting it to behave as a bioisostere of oxazole and synthesised the compounds **9a–n**. As the initial compound **9a** was found to be inactive, we envisioned based on our experience that substitution at metabolically susceptible *para* position of phenyl ring of tail part may play an important role in the modulation of potency and selectivity of the compounds which became evident from the in vitro activity of **9b**. Compounds **9c** and **9d** with electron-donating methyl and methoxy groups respectively were found to be potent and selective towards PPARα. **9d** exhibited 110-fold selectivity towards PPARα over γ and reduced plasma triglycerides by 35% in the SAM model whereas **9c** though exhibited potency similar to **9d** in vitro did not show significant TG reduction in vivo. Substitution on this position with electron-withdrawing groups exhibited detrimental effects both in vitro and in vivo, which is evident from the activity of **9e** and **9f** possessing trifluoromethyl and methanesulfonyl groups, respectively. We then intended to study the effect of bulky substituents on the phenyl ring. **9g**, with an *n*-butyl group, was found to be a weak activator of PPARα and γ with an EC_{50} of 0.5 and 0.2 μM, respectively. To our surprise this compound reduced TG by 31%. Further increase of the bulk at this position by introducing a phenyl ring made the compound **9h** a potent activator of PPARα but found to be only fivefold selective over PPARγ. Replacing the flexible *n*-butyl chain with a rigid group by fusing the 3- and 4-positions into a naphthyl or tetrahydronaphthyl group gave the respective compounds **9i** and **9j** showed surprising and interesting results. Compounds **9i** and **9j** found to be equipotent towards PPARγ. **9i** exhibited sixfold selectivity towards PPARα over γ and reduced TG by 25% in vivo whereas **9j** is found to be 10-fold more potent than **9i** towards PPARα and showed 36% reduction in TG. These results suggests that increasing the bulk of the lipophilic tail increases the affinity of the compounds towards PPARγ and guided us to study the effect of substituents on both 3- and 4-positions of the phenyl ring. Since electron-donating groups appeared to be favorable, we chose methoxy and methyl as well as electron-withdrawing fluoro groups and synthesised the compounds **9k**, **9l**, **9m** and **9n**. Among these compounds **9k** and **9n** showed weak and equal affinity towards PPARα and γ but **9k** reduced TG by 30% in vivo whereas **9n** did not show significant reduction in TG. Compound **9l** with two methyl groups found to be the most potent and highly selective towards PPARα with an EC_{50} of 0.002 μM and 500-fold selectivity over γ. But this compound is not efficacious in reducing TG in vivo. Compound **9m** with methoxy group at *meta* position and methyl group at *para* position exhibited similar potency and selectivity as **9l** in vitro with an EC_{50} of 0.002 μM towards PPARα and 320-fold selectivity over γ. This compound reduced TG by 36% in vivo. Compounds **9d** and **9m** were identified as lead compounds for further evaluation in lipid lowering animal models and pharmacokinetic parameters.

In summary bis-oximinoalkanoic acids were designed as potent and selective PPARα agonists based on Imiglitazar chemotype and evaluated for their in vitro PPAR agonism and two compounds were found to be potent and selective PPARα agonists. Further evaluation of the lead compounds for their in vivo efficacy in relevant animal models and pharmacokinetic properties is currently being undertaken.

Acknowledgments

Authors are grateful to management of Zydus Group for encouragement, and the analytical department for support.

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24. *Spectroscopic analysis of the compounds* **9a–n**: Compound **9a**: *(E,E)-4-Phenyl-4-[4-(1-phenylethylideneaminooxy)-butoxyimino]-butyric acid*; yellow oil. Purity by HPLC: 97.4%; Yield: 60%; IR (neat): 3412, 3020, 1712, 1600, 1444, 1219, 759 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.84–1.88 (m, 4H), 2.23 (s, 3H), 2.58–2.62 (m, 2H), 3.03–3.07 (m, 2H), 4.19–4.26 (m, 4H), 7.32–7.43 (m, 6H), 7.61–7.65 (m, 4H); ^{13}C NMR: (100 MHz, DMSO-d_6): δ 12.90, 21.17, 25.98, 30.78, 73.90, 74.23, 125.14, 125.31, 127.89, 128.28, 129.35, 128.82, 135.31, 136.88, 154.65, 156.26, 178.57; ESI/MS *m/z*: 404.9 $(M+Na)^+$; compound **9b**: *(E,E)-4-[4-[1-(4-Fluorophenyl)-ethylideneaminooxy]-butoxyimino]-4-phenyl-butyric acid*; white solid; mp: 51 °C; purity by HPLC: 95.4%; yield: 90%; IR (KBr): 3411, 3018, 1712, 1662, 1510, 1215, 758 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.83–1.85 (m, 4H), 2.21 (s, 3H), 2.58–2.62 (m, 2H), 3.03–3.07 (m, 2H), 4.22–4.26 (m, 4H), 7.05 (t, J = 8.8 Hz, 2H), 7.35–7.37 (m, 3H), 7.59–7.63 (m, 4H); ^{13}C NMR: (100 MHz, DMSO-d_6): δ 12.24, 22.06, 25.42, 30.46, 73.23, 73.37, 115.10, 115.32, 126.32, 128.15, 128.43, 128.57, 132.55, 134.94, 152.83, 156.43, 161.41, 163.85, 173.47; ESI/MS *m/z*: 422.9 $(M+Na)^+$; compound **9c**: *(E,E)-4-[4-[1-(4-Methylphenyl)-ethylideneaminooxy]-butoxyimino)-4-phenyl-butyric acid*; purity by HPLC: 95.6%; Yield: 83%; IR (KBr): 3408, 3018, 1710, 1560, 1384, 1215, 758 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.83–1.85 (m, 4H), 2.21 (s, 3H), 2.34 (s, 3H), 2.57–2.61 (m, 2H), 3.04–3.08 (m, 2H), 4.19–4.24 (m, 4H), 7.15 (d, J = 7.6 Hz, 2H), 7.33–7.43 (m, 3H), 7.52 (d, J = 8.0 Hz, 2H), 7.60–7.63 (m, 2H); ^{13}C NMR: (100 MHz, DMSO-d_6): δ 12.22, 21.02, 21.92, 25.49, 25.58, 30.19, 72.98, 73.14, 125.72, 126.11, 128.49, 128.96, 129.25, 133.06, 134.69, 138.56, 153.54, 156.30, 173.40; ESI/MS *m/z*: 419.0 $(M+Na)^+$; compound **9d**: *(E,E)-4-[4-[1-(4-Methoxyphenyl)-ethylideneaminooxy]-butoxyimino)-4-phenyl-butyric acid*; White solid; mp: 56 °C; Purity by HPLC: 97.5%; Yield: 81%; IR (KBr): 3100, 2941, 1699, 1608, 1512, 1228, 1051 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 1.81–1.84 (m, 4H), 2.20 (s, 3H), 2.57–2.61 (m, 2H), 3.03–3.07 (m, 2H), 3.81 (s, 3H), 4.22–4.24 (m, 4H), 6.85 (d, J = 8.8 Hz, 2H), 7.34–7.36 (m, 3H), 7.56–7.60 (m, 4H); ^{13}C NMR: (100 MHz, DMSO-d_6): δ 12.16, 21.91, 25.56, 30.96, 55.12, 73.02, 73.42, 113.73, 126.09, 127.16, 128.46, 129.10, 134.97, 153.22, 156.45, 160.00, 173.34; ESI/MS *m/z*: 435.1 $(M+Na)^+$; compound **9e**: *(E,E)-4-[4-[1-(4-Trifluoromethylphenyl)-ethyli deneaminooxy]-butoxyimino]-4-phenyl-butyric acid*; white solid; mp: 52 °C; purity by HPLC: 97.2%; yield: 87%; IR (KBr): 3412, 3018, 1710, 1408, 1327, 1215, 758 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.82–1.87 (m, 4H), 2.24 (s, 3H), 2.58–2.62 (m, 2H), 3.03–3.07 (m, 2H), 4.21–4.28 (m, 4H), 7.34–7.38 (m, 3H), 7.58–7.64 (m, 4H), 7.73 (d, J = 8.4 Hz, 2H); ^{13}C NMR: (100 MHz, DMSO-d_6): δ 12.67, 22.20, 25.99, 30.86, 74.28, 125.55, 126.36, 128.71, 129.03, 131.26, 140.23, 153.23, 156.23, 178.80; ESI/MS *m/z*: 472.8 $(M+Na)^+$; compound **9f**: *(E,E)-4-[4-[1-(4-Methanesulfonylphenyl)-ethylideneaminooxy]-butoxyimino]-4-phenyl-butyric acid*; white solid; mp: 88 °C; purity by HPLC: 99.0%; yield: 49%; IR (KBr): 3412, 2929, 1708, 1454, 1408, 1315, 1151, 1051, 970 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.77–1.87 (m, 4H), 2.25 (s, 3H), 2.59–2.63 (m, 2H), 3.03–3.07 (m, 5H), 4.22–4.30 (m, 4H), 7.34–7.39 (m, 3H), 7.59–7.63 (m, 2H), 7.82 (dd, J = 6.8 and 1.8 Hz, 2H), 7.91 (dd, J = 6.8 and 1.6 Hz, 2H); ^{13}C NMR: (100 MHz, $CDCl_3$): δ 12.61, 22.16, 25.94, 30.60, 44.58, 74.10, 74.45, 126.24, 126.86, 127.53, 128.68, 129.37, 133.71, 140.38, 142.05, 152.75, 156.21, 178.28; ESI/MS *m/z*: 460.9 $(M+H)^+$; compound **9g**: *(E,E)-4-[4-[1-(4-Butylphenyl)-ethylideneaminooxy]-butoxyimino]-4-phenyl-butyric acid*; oil; purity by HPLC: 98.1%; yield: 71%; IR (neat): 3398, 3018, 2931, 1712, 1614, 1500, 1382, 1215, 758 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 0.91 (t, J = 7.2 Hz, 3H), 1.29–1.38 (m, 2H), 1.56–1.62 (m, 2H), 1.83–1.85 (m, 4H), 2.21 (s, 3H), 2.58–2.66 (m, 4H), 3.03–3.07 (m, 2H), 4.21–4.25 (m, 4H), 7.15 (d, J = 8.4 Hz, 2H), 7.32–7.36 (m, 3H), 7.54 (d, J = 8.4 Hz, 2H), 7.60–7.63 (m, 2H); ^{13}C NMR: (100 MHz, DMSO-d_6): δ 12.59, 14.28, 21.95, 22.13, 25.73, 25.89, 29.44, 33.18, 34.98, 73.53, 125.80, 126.12, 128.19, 128.41, 129.30, 132.55, 133.99, 139.06, 143.79, 153.94, 153.98, 173.73; ESI/MS *m/z*: 461.0 $(M+Na)^+$; compound **9h**: *(E,E)-4-[4-(1-Biphenyl-4-yl-ethylideneaminooxy)-butoxyimino]-4-phenyl-butyric acid*; white solid; mp: 79 °C; purity by HPLC: 95.2%; yield: 64%; IR (KBr): 3411, 3018, 1710, 1691, 1564, 1384, 1215, 758 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.86–1.87 (m, 4H), 2.26 (s, 3H), 2.58–2.62 (m, 2H), 3.04–3.08 (m, 2H), 4.24–4.27 (m, 4H), 7.35–7.37 (m, 4H), 7.44 (t, J = 7.6 Hz, 2H), 7.57–7.63 (m, 6H), 7.71 (d, J = 8.0 Hz, 2H); ^{13}C NMR: (100 MHz, DMSO-d_6): δ 12.38, 21.93, 25.50, 31.53, 73.31, 73.44, 126.00, 126.37, 126.61, 127.70, 128.50, 129.09, 129.15, 134.97, 135.09, 138.71, 140.66, 153.36, 156.30, 173.41; ESI/MS *m/z*: 481.0 $(M+Na)^+$; compound **9i**: *(E,E)-4-[4-(1-Naphthalen-2-yl-ethylideneaminooxy)-butoxyimino]-4-phenyl-butyric acid*; white solid; mp: 72 °C; purity by HPLC: 97.8%; yield: 71%; IR (KBr): 3409, 2929, 1733, 1629, 1498, 1438, 1215, 758 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.86–1.90 (m, 4H), 2.34 (s, 3H), 2.58–2.62 (m, 2H), 3.02–3.06 (m, 2H), 4.24–4.31 (m, 4H), 7.34–7.36 (m, 3H), 7.46–7.49 (m, 2H), 7.61–7.64 (m, 2H), 7.78–7.86 (m, 3H), 7.90–7.93 (dd, J = 8.7 and 1.8 Hz, 1H), 7.98 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 11.97, 21.94, 25.43, 30.55, 73.33, 123.07, 125.53, 126.03, 126.33, 126.56, 127.39, 127.67, 128.33, 128.37, 129.01, 132.72, 133.08, 133.42, 134.94, 153.53, 156.47, 173.35; ESI/MS *m/z*: 455.0 $(M+Na)^+$; compound **9j**: *(E,E)-4-Phenyl-4-[4-[1-(5,6,7,8-tetrahydro-naphthalen-2-yl)-ethylideneaminooxy]-butoxyimino)-butyric acid*; oil; purity by HPLC: 95.4%; yield: 90%; IR (KBr): 3411, 3018, 1710, 15001384, 1215, 758 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.76–1.80 (m, 4H), 1.86–1.89 (m, 4H), 2.20 (s, 3H), 2.58–2.62 (m, 2H), 2.75–2.77 (m, 4H), 3.04–3.08 (m, 2H), 4.21–4.25 (m, 4H), 7.02 (d, J = 8.0 Hz, 1H), 7.32–7.43 (m, 5H), 7.61–7.63 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 12.19, 21.08, 22.64, 25.52, 28.56, 28.77, 30.44, 72.91, 73.02, 122.89, 126.04, 126.23, 128.41, 128.83, 129.00, 129.06, 133.83, 134.92, 136.08, 136.48, 153.64, 156.40, 173.42; ESI/MS *m/z*: 459.1 $(M+Na)^+$; compound **9k**: *(E,E)-4-[4-[1-(3,4-Dimethoxyphenyl)-ethylideneaminooxy]-butoxyimino)-4-phenyl-butyric acid*; oil;

purity by HPLC: 95.3%; yield: 72%; IR (KBr): 3411, 3020, 1712, 1579, 1512, 1384, 1215, 758 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.84–1.87 (m, 4H), 2.21 (s, 3H), 2.57–2.61 (m, 2H), 3.03–3.07 (m, 2H), 3.88 (s, 3H), 3.90 (s, 3H), 4.22–4.26 (m, 4H), 6.82 (d, J = 8.4 Hz, 1H), 7.11–7.14 (dd, J = 8.4 and 2.0 Hz, 1H), 7.28 (d, J = 2.0 Hz, 1H), 7.34–7.36 (m, 3H), 7.60–7.63 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 12.27, 21.95, 25.51, 25.62, 30.21, 55.41, 72.99, 73.09, 108.80, 111.49, 118.98, 126.01, 128.51, 128.73, 133.92, 147.83, 148.81, 153.41, 156.50, 173.45; ESI/MS *m/z*: 465.1 (M+Na)$^+$: compound **9l**: *(E,E)-4-{4-[1-(3,4-Dimethylphenyl)-ethylideneaminooxy]-butoxyimino}-4-phenyl-butyric acid*; oil; purity by HPLC: 95.5%; yield: 76%; IR (Nujol): 3124, 3018, 1735, 1610, 1498, 1404, 1215, 758 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.83–1.85 (m, 4H), 2.21 (s, 3H), 2.25 (s, 3H), 2.26 (s, 3H), 2.57–2.62 (m, 2H), 3.03–3.07 (m, 2H), 4.20–4.23 (m, 4H), 7.09 (d, J = 7.7 Hz, 1H), 7.33–7.35 (m, 4H), 7.42 (s, 1H), 7.60–7.63 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 12.24, 19.18, 19.41, 21.93, 25.36, 30.45, 73.11, 73.46, 123.33, 126.10, 126.75, 128.48, 129.12, 129.44, 133.70, 134.99, 136.11, 137.30, 153.63, 156.43, 173.41; ESI/MS *m/z*: 411.1 (M+H)$^+$; compound **9m**: *(E,E)-4-{4-[1-(3-Methoxy-4-methylphenyl)-ethylideneaminooxy]-butoxyimino}-4-phenyl-butyric acid*; oil; purity by HPLC: 96.5%; yield: 50%; IR (neat): 3411, 3018, 1710, 1610, 1508, 1384, 1215, 758 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.83–1.86 (m, 4H), 2.20 (s, 3H), 2.21 (s, 3H), 2.58–2.62 (m, 2H), 3.04–3.08 (m, 2H), 3.83 (s, 3H), 4.21–4.26 (m, 4H), 6.77 (d, J = 8.4 Hz, 1H), 7.34–7.36 (m, 3H), 7.39–7.42 (dd, J = 8.4 and 2.0 Hz, 1H), 7.44 (s, 1H), 7.61–7.63 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 12.18, 16.06, 21.90, 25.32, 25.45, 30.47, 55.28, 72.96, 73.41, 109.87, 124.90, 125.77, 126.09, 127.63, 128.04, 128.48, 129.13, 134.95, 153.31, 156.29, 158.14, 173.37; ESI/MS *m/z*: 449.1 (M+Na)$^+$; compound **9n**: *(E,E)-4-{4-[1-(4-Fluoro-3-methoxyphenyl)-ethylideneaminooxy]-butoxyimino}-4-phenyl-butyric acid*; oil; purity by HPLC: 95.5%; yield: 82%; IR (neat): 3411, 3018, 1710, 1562, 1502, 1384, 1215, 758 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.83–1.86 (m, 4H), 2.18 (s, 3H), 2.58–2.62 (m, 2H), 3.04–3.08 (m, 2H), 3.89 (s, 3H), 4.21–4.26 (m, 4H), 6.91 (t, J = 8.8 Hz, 1H), 7.32–7.37 (m, 4H), 7.42–7.46 (dd, J = 12.8 and 2.0 Hz, 1H), 7.60–7.63 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 12.46, 21.26, 25.83, 25.93, 30.90, 56.45, 73.63, 73.78, 113.48, 113.86, 113.87, 122.85, 126.39, 128.45, 129.54, 134.29, 148.25, 150.46, 152.90, 156.93, 173.85; ESI/MS *m/z*: 453.0 (M+Na)$^+$.