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Synthesis, in vitro and in silico evaluation of L-tyrosine containing PPARα/γ dual agonists

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Abstract—A novel series of L-tyrosine derivatives have been reported with potential PPARα/γ dual agonistic activity. In vitro cell based PPARα/γ transactivation studies have shown compound **4a** and compound **4f** to be the most potent PPARγ and PPARα activators, respectively. Molecular docking studies performed on these series of compounds have complemented the experimental results and have led to interesting inferences.

1. Introduction

Diabetes is the root cause of several chronic and progressive diseases that adversely affect a number of organs including nervous and vascular systems.[1] Type 2 diabetes mellitus (T2DM) is a complex metabolic disorder that affects 90% of total diabetic population.[2] This disease is characterized by insulin resistance in liver and peripheral tissues accompanied by a defect in pancreatic β-cells.[3] The drugs that reverse insulin resistance fulfill the major medical need in treating T2DM and hence have the potential to reduce long-term complications of T2DM.[4] Insulin sensitizers bind to peroxisome proliferator-activated receptor (PPARs) belonging to the hormone receptors superfamily of ligand-activated transcription factors that govern glucose and lipid homeostasis.[5] The PPARs have three subtypes, PPARα, PPARγ, and PPARδ each of which is differentially expressed in a tissue specific manner.[6–8] Two pharmaceutically important PPARγ agonists, pioglitazone and rosiglitazone, are blockbuster drugs (Chart 1).[9] Though current research trend has shifted toward non-thiazolidinedione insulin sensitizers, there are many groups working on PPARα/γ dual agonists as they are involved in both insulin sensitization and improving lipidemic profile of the diabetic patients.[10–15] Many compounds belonging to the category of dual activators have advanced to clinical trials.[16]

Based on the crystal structure analysis of PPARα and PPARγ and the molecular modeling studies, a common U-shaped pharmacophore has been derived for PPARγ agonists which is constituted by an acidic moiety and a bulky hydrophobic fragment, on the two terminals of the active compounds connected by a flat aromatic linker. A similar pharmacophoric pattern holds true for the PPARα/γ dual agonists except that there are differences in the size of the acidic head group. The thiazolidinedione (TZD) head group is anchored by four important H-bonds in the active site—Ser289, His323, His449, and Tyr473. However, His323 is replaced by Tyr314 in PPARα. The relatively larger size of Tyr314 leads to steric problems in accommodating bulkier TZD ring. Sterically favorable open chain acid derivatives have been found to bind comfortably in the active sites of both PPARα and PPARγ and therefore show better dual activity.[17,24]

Recently, we reported carbazole derivatives with the acyclic acidic head groups as PPARα/γ dual agonists

Keywords: PPAR; Dual agonists; L-Tyrosine; Transactivation; Molecular docking.
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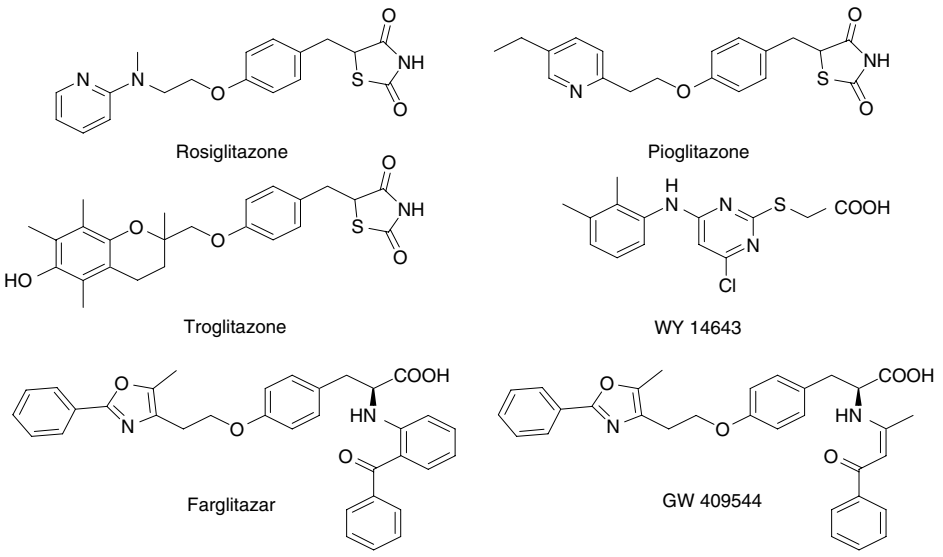
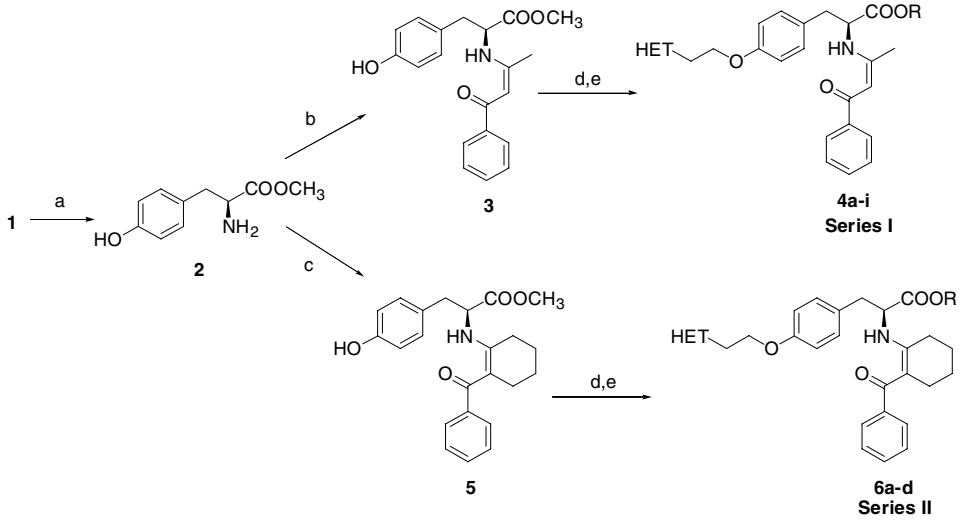


Chart 1. Compounds known to be PPARα, PPARγ and PPARα/γ dual agonists.

with antioxidant property.[18] Antioxidant is an added property of such compounds, as it scavenges free radicals generated and thus offers additional benefits to the diabetic patients.[19] In this work, we have made an attempt to improve the potency of the active PPARα/γ dual agonists identified from the previously published work. It is an N-derivatized L-tyrosine analogue. Since compounds based on tyrosine derivatives have reached up to phase II clinical trials,[20–23] so the compounds with this moiety were explored further. Two types of N-derivatised tyrosine derivatives have been synthesized (Scheme 1). Different substituents have been tried in the hydrophobic side chains of the molecules. This exercise has lead to some potent PPARα/γ dual agonists that have been evaluated by in vitro assays. Molecular docking studies were performed to evaluate and analyze these compounds for important interactions at the active sites of PPARα and PPARγ.

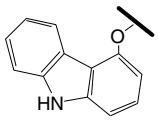
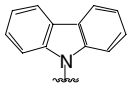
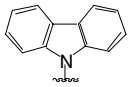
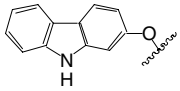
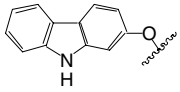
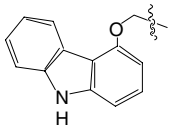
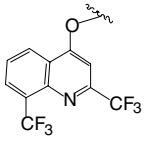
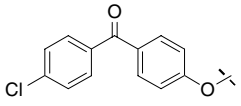
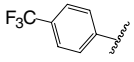
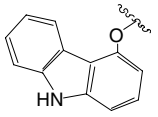
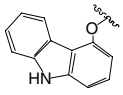
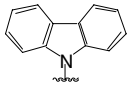
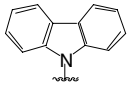
2. Chemistry

Two series of compounds were synthesized as described in Scheme 1 in chiral non-racemic form starting with L-tyrosine methyl ester hydrochloride **1**, which was converted to its free ester form **2** by treatment with sodium bicarbonate in methanol. **2** was refluxed with 2-benzoylacetone (**series I**) or 2-benzoylcyclohexanone (**series II**) in toluene in presence of 4 Å molecular sieves, to adsorb in situ generated water, to afford **3** and **5**, respectively, in excellent yields. The intermediates **3** and **5** were condensed with various haloalkoxyheteroaryl moieties (HET substituents in Table 1) by refluxing in acetone in presence of K_2CO_3 to furnish **4a–i** and **6a–d**, respectively. Saponification with LiOH in THF/MeOH afforded corresponding acids **4a–i** and **6a–d** (Table 1) in enantiomerically pure fashion. The struc-



Scheme 1. Reagents and conditions: (**1**) L-tyrosine methyl ester HCl; (a) $NaHCO_3$, MeOH, 1 h; (b) benzoylacetone, toluene, 4 Å molecular sieves, reflux, 12 h; (c) benzoylcyclohexanone, toluene, 4 Å molecular sieves, reflux, 12 h; (d) haloalkoxyheteroyl groups (HET), K_2CO_3, acetone, reflux, 14 h; (e) 1.5 equiv LiOH in H_2O, THF/CH_3OH (3:1).

Table 1. List of synthesized compounds, their PPAR fold activation values, and their FlexX docking scores in 1K7L (PPARα) and 1FM9 (PPARγ)

Sr. No.	Compound	HET	R	PPARα fold activation	1K7L docking score	PPARγ fold activation	1FM9 docking score
1	**4a**		H	4.6	−33.91	48.2	−41.17
2	**4b**		CH_3	1.2	ND	1.9	ND
3	**4c**		H	1.4	−35.77	1.7	−39.05
4	**4d**		CH_3	1.9	ND	1.8	ND
5	**4e**		H	2.0	−29.69	4.2	−37.90
6	**4f**		H	5.1	−34.55	15.1	−29.5
7	**4g**		H	1.1	−28.18	2.3	−35.03
8	**4h**		H	2.8	−29.16	22.6	−34.49
9	**4i**		H	0.3	−28.86	0.7	−30.97
10	**6a**		CH_3	1.4	ND	4.7	ND
11	**6b**		H	1.2	−29.24	11.2	−34.05
12	**6c**		CH_3	0.5	ND	3.0	ND
13	6d		H	1.0	ND	8.4	−37.25

PPAR fold activation at 50 μM and FlexX docking scores in kcal/mol, ND = not docked.

tures of all the synthesized compounds were confirmed by various spectral analysis techniques and their enantiomeric purity was confirmed by chiral HPLC.

3. Biological evaluation

In vitro screening of compounds was performed to evaluate their PPARα/γ dual agonistic activity. The cells

were transfected with an expression plasmid for PPAR receptors and activation of luciferase gene was measured. Potencies of PPAR gene activation were evaluated in cell based transcription assays using GAL4-PPAR chimeric receptors.[25] Transactivation studies were done for all the compounds at 50 μM concentration both for PPARα and PPARγ using WY14,643 and rosiglitazone as references, respectively. The comparative potencies were determined in terms of fold activation at 50 μM concentration.

4. Molecular docking

Molecular docking studies were performed on two proteins, viz. PPARα and PPARγ, employing the FlexX docking procedure using Sybyl6.9 program installed on silicon graphics Octane2 workstation. FlexX is a fast automated program based on incremental construction procedure. In this method flexibility of the ligands is considered by including several conformations of ligands, while maintaining a rigid structure for the biomolecule. The 3D coordinates of the active sites were taken from the X-ray crystal structures of PPARα and PPARγ proteins reported as complexes with their corresponding agonists, GW409544 and farglitazar deposited in Brookhaven Protein Data Bank with PDB codes 1K7L and 1FM9, respectively. WY14643 and rosiglitazone, the reference compounds used for PPARα and PPARγ transactivation studies, respectively, were docked into the active sites of the two receptors to judge the discriminatory strength of the docking procedures and the choice of the crystal structures. The result of this initial docking exercise was fruitful as WY14643 docked well in the active site of PPARα but failed to dock in the active site of PPARγ. Similarly rosiglitazone was able to dock well in the active site of PPARγ, while it failed to do so in PPARα. Encouraged by these results, all the synthesized molecules were docked into the active sites of the PPARα and PPARγ. The active sites were assigned at a radius of 8 Å around the reference ligand. FlexX run was submitted and the docking scores were obtained and analyzed.

5. Results and discussion

The results obtained from the transactivation studies exhibited that **series I** (**4a**–**i**) showed better PPARα/γ dual agonistic activity as compared to **series II** (**6a**–**d**). In both the series, the acid derivatives have been found to be much more active as compared to their ester analogues. It reinforces the fact that PPARγ agonistic activity requires trivial amount of acidic character. Some changes in the hydrophobic side chain were introduced by substitution of the carbazole ring. The results indicated that the carbazoles substituted at the C4 position are more active than those substituted at other positions. The compounds obtained with 4-hydroxy carbazoles as the starting material and substituted at this hydroxyl group showed better activity than the compounds substituted at the –NH of the carbazole moiety.

The results of the transactivation studies are listed in Table 1. In the first series of molecules, the lead compound **4a** showed maximum agonistic activity for both the receptors followed by compounds **4f** and **4h**. The lead compound **4a** exhibits more than 2-fold activity for PPARγ receptor (48.2) as compared to rosiglitazone (22.5) and also shows very good activity for PPARα. The compound **4f**, with three carbon chain linker, showed higher potency for PPARα receptor (5.1) as compared to WY14643 (4.0) and moderate PPARγ fold activation. Compound **4h** showed good PPARγ fold agonistic activity while showing moderate activity for PPARα. The compounds **4a** and **4f** constitute the carbazole derivatives, whereas the compound **4h** has 4-chlorobenzophenone unit of fenofibrate in place of carbazole as the hydrophobic side chain. Other substitutions were also tried in the hydrophobic region of the compounds. Replacing carbazole ring with trifluoromethyl-substituted ring systems (**4g** and **4i**) did not improve the potency of these compounds. In the second series, the compound **6b** showed the best results.

Molecular docking studies were carried out on the synthesized compounds to get insight into their binding preferences at the active sites of the two receptors. Rosiglitazone when docked in the active site of PPARγ receptor attained a score of −15.96 kcal/mol, however, it could not fit well in the active site of PPARα receptor. It showed the important H-bonding interactions with the residues at the active site of PPARγ. Similarly, WY14643 docked well and achieved the score of −19.92 kcal/mol in the active site of PPARα but could not bind well in the active site of PPARγ. These results are on the expected lines as rosiglitazone is a PPARγ agonist, while WY14643 is a PPARα agonist. These molecules in their docked conformations are shown in the Figure 1a and b indicating the important interactions they make at the active site. Similar FlexX based docking studies were carried out to evaluate the synthesized compounds. The FlexX docking scores of these compounds are listed in Table 1. From the observed results, the following general conclusions can be drawn: the acidic derivatives docked well in both the receptors and maintained all the essential H-bonding interactions required for good anchoring at the active site. However, the ester analogues of these compounds could not fit into active sites of the two receptors, as they were devoid of the acidic factor and could not interact through the H-bonds. All the active compounds docked very well in the active sites of both the receptors. Scores of some of these molecules correlated well with their in vitro activity profiles. The most active compound **4a** fitted the best in the active site of PPARγ and attained the best score of −41.17 kcal/mol amongst all the molecules. It also docked well in the active site of PPARα and scored a FlexX score of −33.91 kcal/mol. It showed all the prime interactions to anchor well in the active sites of both the receptors (Fig. 1d). The other active compounds in the **series I**, **4f** and **4 h**, also achieved very good docking scores and could fit well into the active sites of both the receptors. The compound **4f** has a three-carbon linker between the hydrophobic region and the central aromatic unit. The extended length introduces a strong H-bond with Thr279 which is not observed with

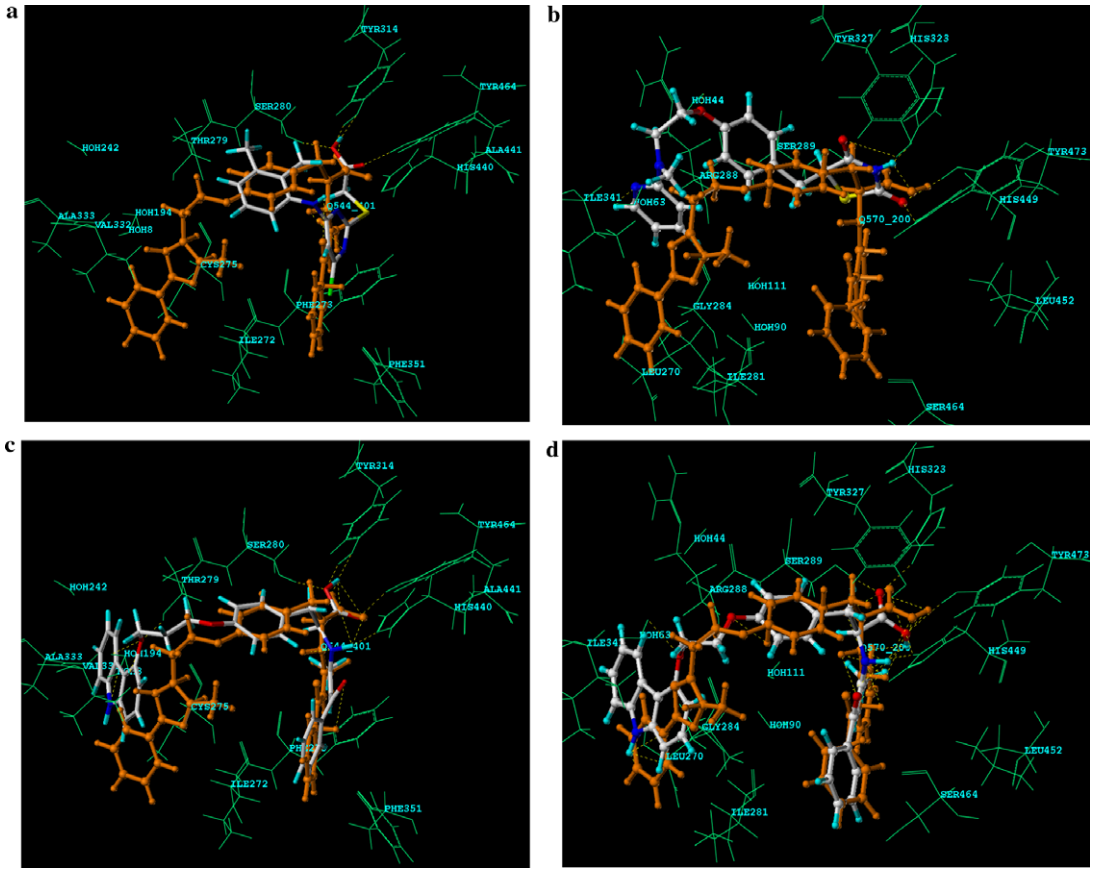


Figure 1. (a) WY14643 with PPARα (1K7L). (b) Rosiglitazone with PPARγ (1FM9). (c) Compound **4f** with PPARα (1K7L). (d) Compound **4a** with PPARγ (1FM9). The molecules in orange are the reference ligands found as complexes with the crystal structures (farglitazar in 1FM9 and GW409544 in 1K7L).

the other molecules (Fig. 1c). The new H-bond provides it a better score over the others. In case of the compound **4h**, it shows a good π-stacking interaction with the Phe287 that makes this particular hydrophobic unit more active than the others. In the second series, compounds **6b** and **6d** docked well and attained good docking scores at the active site of PPARα and PPARγ. But no additional interactions were observed in these molecules. The reason for the better activity of 4-hydroxy-substituted carbazole derivatives over compounds with N-substitution and 2-hydroxy substituents can be attributed to the fact that the 4-hydroxy substituent orients its oxygen such that there is an additional H-bonding interaction that is observed with this oxygen atom and a water molecule lying in close vicinity. Hence the strategic position of this group is important for its activity. Thus, with these studies we could get some insights into the nature of binding of the ligands and these studies can be applied for designing better molecules having dual PPARα/γ agonistic activity.

6. Experimental

6.1. General experimental techniques

Thin-layer chromatography analyses were performed on precoated silica gel plates (GF254, Merck). Chromatography was performed on flash silica gel (230–400 mesh). Melting points were recorded on a Buchi capillary melting point apparatus and are uncorrected. Parr shaker used for hydrogenation is from Perfit-India. Infrared (IR) spectra were recorded on a Nicolet Impact-410 FTIR spectrometer. Proton magnetic resonance (NMR) spectra were recorded on a Bruker 300 MHz spectrometer in $CDCl_3$, CD_3OD, D_2O or DMSO-d_6 solution. The chemical shifts are reported in δ (ppm) relative to internal standard tetramethylsilane (TMS) and coupling constants J are given in Hertz. Mass spectroscopy was conducted using Shimadzu QP5000 mass spectrometer, LCQ Finnigan MAT, and Bruker Daltonics MALDI Tandem TOF mass spectrometer. Elemental analyses were obtained from Elementar Vario®EL.

6.1.1. (*S*)-3-{4-[2-(9H-Carbazol-4-yloxy)-ethoxy]-phenyl}-2-(1-methyl-3-oxo-3-phenyl-propenylamino)-propionic acid (4a). *Step A.* Synthesis of (*S*)-3-(4-hydroxy-phenyl)-2-(methyl-3-oxo-3-phenyl-propenylamino)-propionic acid methyl ester. To a 10 ml dry toluene suspension of L-tyrosine methyl ester (2.0 g, 10.2 mmol), benzoylacetone (1.7 g, 10.2 mmol) was added, in presence of 4°A molecular sieve. The suspension was refluxed for 12 h. Toluene was removed and residue was extracted with dichloromethane. The organic layer was separated and dried over anhydrous sodium sulfate, decanted, concentrated, and chromatographed on silica gel. Elution with a gradient mixture of ethyl acetate/hexane yielded the title compounds (90–95%). IR (cm^{-1}) 3177.8, 1744.1, 1596.1, 1438.5, 1118.2, 745.6 ^1H NMR ($CDCl_3$) δ 1.81

(s, 3H), 2.99–3.16 (m, 4H), 3.72 (s, 3H), 5.65 (m, 1H), 6.74 (d, J = 8.34 Hz, 1H), 7.02 (d, J = 6.57 Hz, 1H), 7.38 (d, J = 6.78 Hz, 1H), 7.84 (d, J = 7.02 Hz, 1H), 11.63 (d, J = 9.00 Hz, 1H). MS (APCI): 339 (*m/z* M+1).

Step B. Synthesis of 4-(2-bromo-ethoxy)-9H-carbazole. To a 10 ml acetone solution of 4-hydroxycarbazole (2.0 g, 10.8 mmol) and 1, 2-dibromoethane (2.0 g, 12.0 mmol) was added K_2CO_3 (2.2 g, 12.0 mmol). The suspension was refluxed overnight. The reaction mixture was concentrated, and residue was extracted with ethyl acetate. The organic layer was separated and dried over anhydrous sodium sulfate, decanted, concentrated and chromatographed on silica gel. Elution with a gradient mixture of ethyl acetate/hexane yielded the title compounds as white powder mp 115 °C (60–70%). ^{1}H NMR ($CDCl_3$) δ 3.84 (t, J = 6 Hz, 2H), 4.55 (t, J = 6 Hz, 2H), 6.64 (d, J = 8.1 Hz, 1H), 7.07 (d, J = 8.1 Hz, 1H), 7.25 (m, 2H), 7.36 (d, J = 9 Hz, 1H), 8.06 (s, 1H), 8.38 (d, J = 6.9 Hz, 1H) MS (EI): 289, 291 (*m/z* M+1, M+2).

Step C. To a 10 ml acetone solution of (*S*)-3-(4-Hydroxy-phenyl)-2-(methyl-3-oxo-3-phenyl-propenylamino)-propionic acid methyl ester (1.0 g, 2.9 mmol) and 4-(2-bromo-ethoxy)-9H-carbazole (0.9 g, 3.19 mmol) was added K_2CO_3 (0.6 g, 4.35 mmol). The suspension was refluxed for 14 h, concentrated, and residue was extracted with ethyl acetate. The organic layer was separated and dried over anhydrous sodium sulfate, decanted, concentrated, and chromatographed on silica gel. Elution with a gradient mixture of ethyl acetate/hexane yielded the (*S*)-3-{4-[2-(9H-carbazol-4-yloxy)-ethoxy]-phenyl}-2-(1-methyl-3-oxo-3-phenyl-propenylamino)-propionic acid methyl ester. ^{1}H NMR ($CDCl_3$) δ 1.94 (s, 3H), 3.16 (m, 1H), 3.20 (m, 1H), 3.54 (t, J = 6 Hz, 1H), 3.76 (s, 3H), 4.49 (t, J = 6 Hz, 2H), 4.57 (t, J = 6 Hz, 2H), 4.74 (m, 1H), 5.6 (s, 1H), 6.74 (d, J = 8.4 Hz, 2H), 6.93 (t, J = 8.4 Hz, 1H), 7.09 (d, J = 8.4 Hz, 2H), 7.20 (m, 5H), 7.33 (d, J = 9 Hz, 2H), 7.87 (m, 3H), 8.01(s, 1H), 8.25 (d, J = 8.4 Hz, 1H) MS (EI): 548 (*m/z* M^+) Anal. Calcd for $C_{34}H_{32}N_2O_5$ (548.23) C, 74.43; H, 5.88; N, 5.11; Found: C, 74.27; H, 5.76; N, 5.08.

Step D. To ethanol solution of compound (*S*)-3-{4-[2-(9H-carbazol-4-yloxy)-ethoxy]-phenyl}-2-(1-methyl-3-oxo-3-phenyl-propenylamino)-propionic acid methyl ester (1.0 g, 1.8 mmol) was added 1.5 equiv aqueous LiOH in THF/CH_3OH and stirred at room temperature overnight. The reaction mixture was concentrated, acidified with acetic acid (pH 4). The acidic solution was extracted with ethyl acetate. The organic layer was separated and dried over anhydrous sodium sulfate, decanted, and concentrated to yield the title compounds (*S*)-3-{4-[2-(9H-carbazol-4-yloxy)-ethoxy]-phenyl}-2-(1-methyl-3-oxo-3-phenyl-propenylamino)-propionic acid (75%). Chiral HPLC (chiral-AGP, 150*4 mm, 5 μm, hexane/isopropanol (4:1), 1 ml/min), t_R = 8.2, 98% ee. ^{1}H NMR ($CDCl_3$) δ 1.94 (s, 3H), 3.16 (m, 1H), 3.25–3.30 (m, 1H), 3.54 (t, J = 6 Hz, 1H), 3.60 (m, 1H), 4.49 (t, J = 6 Hz, 2H), 4.57 (t, J = 6 Hz, 2H), 5.6 (s, 1H), 6.74 (d, J = 8.4 Hz, 2H), 6.93 (t, J = 8.4 Hz, 1H), 7.09 (d, J = 8.4 Hz, 2H), 7.20 (m, 5H), 7.33 (d, J = 9 Hz, 2H), 7.87 (m, 3H), 8.01 (s, 1H), 8.25 (d, J = 8.4 Hz, 1H) ^{13}C NMR δ ($CDCl_3$) 13.24, 20.02, 28.60, 29.08, 60.09, 60.47, 68.79, 100.05, 100.24, 103.41, 103.64, 109.48, 114.26, 114.76, 118.21, 122.35, 124.04, 125.71, 126.41, 127.74, 128.09, 129.17, 130.00, 131.86, 132.37, 138.81, 141.08, 143.94, 154.55, 171.49 MS (MALDI): 535 M^+. Anal. Calcd for $C_{33}H_{30}N_2O_5$; C, 74.14; H, 5.66; N, 5.24; Found: C, 73.93; H, 5.53; N, 5.04.

6.1.2. (*S*)-3-[4-(2-Carbazol-9-yl-ethoxy)-phenyl]-2-(1-methyl-3-oxo-3-phenyl-propenylamino)-propionic acid methyl ester (4b). (*S*)-3-(4-Hydroxy-phenyl)-2-(methyl-3-oxo-3-phenyl-propenylamino)-propionic acid methyl ester (1 equiv), carbazole-9-ethyl mesylate (1 equiv), and potassium carbonate (2 equiv) in ethanol and toluene (1:1) were refluxed for 10 h. The reaction mixture was concentrated and extracted with DCM. The organic layer was washed with water, brine and dried over sodium sulfate. Purification with column chromatography afforded **4b**. IR (cm^{-1}) 2920, 1592, 1458, 1079, 1018, 750 ^{1}H NMR ($CDCl_3$) δ 1.98 (s, 3H), 3.91 (m, 4H), 4.11 (m, 3H), 4.31 (m, 4H), 7.20 (m, 5H), 7.42 (m, 8H), 8.05 (d, J = 7.6 Hz, 4H) MALDI: 535 (*m/z* M^+) Anal. Calcd for ($C_{34}H_{32}N_2O_4$) C, 76.67; H, 6.06; N, 5.26; Found: C, 76.61; H, 5.93; N, 5.04.

6.1.3. (*S*)-3-[4-(2-Carbazol-9-yl-ethoxy)-phenyl]-2-(1-methyl-3-oxo-3-phenyl-propenylamino)-propionic acid (4c). (*S*)-3-[4-(Carbazol-9-yloxy)-phenyl]-2-(1-methyl-3-oxo-3-phenyl-propenylamino) propionic acid methyl ester was saponified with 1.5 equiv aqueous LiOH in THF/CH_3OH to give (*S*)-3-[4-(carbazol-9-yloxy)-phenyl]-2-(1-methyl-3-oxo-3-phenyl-propenylamino) propionic acid ^{1}H NMR ($CDCl_3$) δ 1.97 (s, 3H), 3.90 (m, 1H), 4.13 (m, 3H), 4.33 (m, 4H), 7.21 (m, 5H), 7.42 (m, 8H), 8.03 (d, J = 7.6 Hz, 4H) MALDI: 518 (*m/z* M^+) Anal. Calcd for ($C_{33}H_{30}N_2O_4$) C, 76.43; H, 5.83; N, 5.40; Found: C, 76.35; H, 5.73; N, 5.02.

6.1.4. (*S*)-3-{4-[2-(9H-carbazol-2-yloxy)-ethoxy]-phenyl}-2-(1-methyl-3-oxo-3-phenyl-propenylamino)-propionic acid methyl ester (4d). Compound **4d** was synthesized according to procedure as mentioned in 6.1.2 from 2-(2-bromo-ethoxy)-9H-carbazole (1 equiv) and (*S*)-3-(4-hydroxy-phenyl)-2-(methyl-3-oxo-3-phenyl-propenylamino)-propionic acid methyl ester (1 equiv). ^{1}H NMR ($CDCl_3$) δ 1.98 (s, 3H), 3.04–3.05 (m, 2H), 3.57 (t, J = 6 Hz, 1H), 3.72 (s, 3H), 4.35 (t, J = 6 Hz, 2H), 4.46 (t, J = 6 Hz, 2H), 5.61 (s, 1H), 6.75 (d, J = 8.4 Hz, 2H), 6.93 (t, J = 8.4 Hz, 1H), 7.08 (d, J = 8.4 Hz, 2H), 7.20 (m, 5H), 7.32 (d, J = 9 Hz, 2H), 7.89 (m, 4H), 8.12 (s, 1H). MS (MALDI): 549 (*m/z* M^+) Anal. Calcd for ($C_{34}H_{32}N_2O_5$) C, 74.43; H, 5.88; N, 5.11; Found: C, 74.22; H, 5.68; N, 5.07.

6.1.5. (*S*)-3-{4-[2-(9H-Carbazol-2-yloxy)-ethoxy]-phenyl}-2-(1-methyl-3-oxo-3-phenyl-propenylamino)-propionic acid (4e). Compound **4e** was synthesized according to procedure as mentioned in 6.1.3 from (*S*)-3-{4-[2-(9H-carbazol-2-yloxy)-ethoxy]-phenyl}-2-(1-methyl-3-oxo-3-phenyl-propenylamino)-propionic acid methyl ester. ^{1}H NMR ($CDCl_3$) δ 1.99 (s, 3H), 2.99 (m, 1H), 3.05 (m, 1H), 3.58 (t, J = 6 Hz, 1H), 4.34 (t, J = 6 Hz, 2H), 4.45

(t, J = 6 Hz, 2H), 5.59 (s, 1H), 6.74 (d, J = 8.4 Hz, 2H), 6.93 (t, J = 8.4 Hz, 1H), 7.09 (d, J = 8.4 Hz, 2H), 7.20 (m, 5H), 7.33 (d, J = 9 Hz, 2H), 7.87–7.91 (m, 4H), 8.13 (s, 1H). MS (MALDI): 535 (*m*/*z* M^+) Anal. Calcd for ($C_{33}H_{30}N_2O_5$) C, 74.14; H, 5.66; N, 5.24; Found: C, 74.02; H, 5.32; N, 4.95.

6.1.6. (*S*)-3-{4-[3-(9H-Carbazol-4-yloxy)-propoxy]-phenyl}-2-(1-methyl-3-oxo-3-phenyl-propenylamino)-propionic acid (4f). *Step A.* Synthesis of 4-(3-chloro-propoxy)-9H-carbazole: 4-hydroxycarbazole was refluxed with 3-bromo-1-chloropropane in acetone in presence of K_2CO_3 to afford 4-(3-chloro-propoxy)-9H-carbazole with 55% yield. ^{1}H NMR ($CDCl_3$) δ 2.43 (tt, J = 6.0 Hz, 2H), 3.89 (t, J = 6.0 Hz, 2H), 4.38 (t, J = 6.0 Hz, 2H), 6.67 (d, J = 8.1 Hz, 1H), 7.03 (d, J = 7.8 Hz, 1H), 7.23 (m, 2H), 7.37 (m, 2H), 8.02 (s, 1H), 8.23 (d, J = 7.5 Hz, 1H). MS (MALDI): 259 (*m*/*z* M^+).

Step B. (*S*)-3-{4-[3-(9H-Carbazol-4-yloxy)-propoxy]-phenyl}-2-(1-methyl-3-oxo-3-phenyl-propenylamino)-propionic acid was synthesized according to procedure as mentioned in 6.1.2 and 6.1.3 by condensing (*S*)-3-(4-hydroxy-phenyl)-2-(methyl-3-oxo-3-phenyl-propenylamino)-propionic acid methyl ester with 4-(3-chloro-propoxy)-9H-carbazole. ^{1}H NMR ($CDCl_3$) δ 1.94 (s, 3H), 2.43 (t, J = 6 Hz, 2H), 3.16 (m, 1H), 3.25–3.30 (m, 1H), 3.54 (t, J = 6 Hz, 1H), 4.49 (m, 4H), 5.6 (s, 1H), 6.73 (d, J = 8.4 Hz, 2H), 6.92 (t, J = 8.4 Hz, 1H), 7.09 (d, J = 8.4 Hz, 2H), 7.19–7.24 (m, 5H), 7.31 (d, J = 9 Hz, 2H), 7.85 (m, 3H), 8.22 (d, J = 8.4 Hz, 2H). MS (MALDI): 549 (*m*/*z* M^+) Anal. Calcd for ($C_{34}H_{32}N_2O_5$) C, 74.43; H, 5.88; N, 5.11; Found: C, 74.21; H, 5.63; N, 5.01.

6.1.7. (*S*)-3-{4-[2-(2,8-Bis-trifluoromethyl-quinolin-4-yloxy)-ethoxy]-phenyl}-2-(1-methyl-3-oxo-3-phenyl-propenylamino)-propionic acid (4g). Compound **4g** was synthesized according to procedure as mentioned in 6.1.2 and 6.1.3 by condensing (*S*)-3-(4-hydroxy-phenyl)-2-(methyl-3-oxo-3-phenyl-propenylamino)-propionic acid methyl ester with 4-(2-bromo-ethoxy)-2,8-bis-trifluoromethyl-quinoline. ^{1}H NMR ($CDCl_3$) δ 1.94 (s, 3H), 3.04–3.14 (m, 2H), 4.26 (m, 4H), 4.38 (m, 1H), 5.65 (s, 1H), 6.92 (d, J = 8.4 Hz, 2H), 7.21 (d, J = 8.4 Hz, 2H), 7.44 (m, 3H), 7.64 (m, 3H), 8.13 (m, 2H), 8.47 (m, 1H). MS (MALDI): 632 (*m*/*z* M^+) Anal. Calcd for ($C_{32}H_{26}F_6N_2O_5$) C, 60.76; H, 4.14; N, 4.43; Found: C, 60.26; H, 4.08; N, 4.17.

6.1.8. (*S*)-3-(4-{2-[4-(4-Chloro-benzoyl)-phenoxy]-ethoxy}-phenyl)-2-(1-methyl-3-oxo-3-phenyl-propenylamino)-propionic acid (4h). Compound **4h** was synthesized according to procedure as mentioned in 6.1.2 and 6.1.3 by condensing (*S*)-3-(4-hydroxy-phenyl)-2-(methyl-3-oxo-3-phenyl-propenylamino)-propionic acid methyl ester with [4-(2-bromo-ethoxy)-phenyl]-(4-chloro-phenyl)-methanone. ^{1}H NMR ($CDCl_3$) δ 1.94 (s, 3H), 3.04–3.14 (m, 2H), 4.26 (m, 4H), 4.38 (m, 1H), 5.65 (s, 1H), 6.89 (d, J = 8.4 Hz, 2H), 7.07 (d, J = 9 Hz, 2H), 7.21 (d, J = 8.4 Hz, 2H), 7.5 (d, J = 8.1 Hz, 2H), 7.58–7.77 (m, 7H), 7.93 (d, J = 9 Hz, 2H). MS (MALDI): 584 (*m*/*z* M^+) Anal. Calcd for ($C_{34}H_{30}ClNO_6$) C, 69.92; H, 5.18; N, 2.40; Found: C, 69.67; H, 5.01; N, 2.13.

6.1.9. (*S*)-2-(1-Methyl-3-oxo-3-phenyl-propenylamino)-3-{4-[2-(4-trifluoromethyl-phenyl)-ethoxy]-phenyl}-propionic acid (4i). Compound **4i** was synthesized according to procedure as mentioned in 6.1.2 and 6.1.3 by condensing (*S*)-3-(4-hydroxy-phenyl)-2-(methyl-3-oxo-3-phenyl-propenylamino)-propionic acid methyl ester with methanesulfonic acid 2-(4-trifluoromethyl-phenyl)-ethyl ester. ^{1}H NMR ($CDCl_3$) δ 1.94 (s, 3H), 2.93 (t, J = 6 Hz, 2H), 3.04–3.16 (m, 2H), 4.14 (t, J = 6 Hz, 2H), 4.38 (m, 1H), 5.65 (s, 1H), 6.77 (d, J = 8.4 Hz, 2H), 6.90 (d, J = 8.4 Hz, 2H), 7.39 (m, 3H), 7.53 (d, J = 9 Hz, 2H), 7.72 (d, J = 8.7 Hz, 2H), 7.85 (d, J = 8.7 Hz, 2H). MS (MALDI): 497 (*m*/*z* M^+) Anal. Calcd for ($C_{28}H_{26}F_3NO_4$) C, 67.61; H, 5.27; N, 2.82; Found: C, 67.21; H, 5.18; N, 2.74.

6.1.10. (*S*)-2-(2-Benzoyl-cyclohex-1-enylamino)-3-{4-[2-(9H-carbazol-4-yloxy)-ethoxy]-phenyl}-propionic acid methyl ester (6a). *Step A.* Synthesis of (*S*)-2-(2-benzoyl-cyclohex-1-enylamino)-3-(4-hydroxy-phenyl)-propionic acid methyl ester: L-tyrosine ester was refluxed with 2-benzoyl-cyclohexanone in toluene in presence of 4 Å molecular sieve for 12 h. Reaction mixture was concentrated under vacuum and extracted with methylene chloride, washed with brine, and then dried over sodium sulfate. ^{1}H NMR ($CDCl_3$) δ 1.32 (m, 4H), 2.18 (m, 4H), 3.04 (m, 2H), 3.72 (s, 3H), 4.42 (m, 1H), 6.73 (d, J = 9.0 Hz, 2H), 7.03 (d, J = 9.0 Hz, 2H), 7.32–7.43 (m, 5H), 12.14 (d, J = 9.0 Hz, 1H). MS (MALDI): 379 (*m*/*z* M^+).

Step B. (*S*)-2-(2-Benzoyl-cyclohex-1-enylamino)-3-{4-[2-(9H-carbazol-4-yloxy)-ethoxy]-phenyl}-propionic acid methyl ester was synthesized from (*S*)-2-(2-benzoyl-cyclohex-1-enylamino)-3-(4-hydroxy-phenyl)-propionic acid methyl ester and 4-(2-bromo-ethoxy)-9H-carbazole according to procedure as mentioned in 6.1.1. ^{1}H NMR ($CDCl_3$) δ 1.40–1.45 (m, 2H), 1.55 (m, 2H), 1.75–1.79 (m, 2H), 2.34 (m, 2H), 2.91 (m, 1H), 3.00–3.05 (m, 1H), 3.72 (s, 3H), 4.30–4.45 (m, 5H), 6.56 (m, 2H), 6.81 (m, 1H), 6.95 (m, 2H), 7.15 (m, 2H), 7.32–7.39 (m, 5H), 7.41 (m, 2H), 7.86 (m, 1H), 8.25 (d, J = 7.8 Hz, 2H), 12.22 (d, J = 8.7 Hz, 1H). ^{13}C NMR ($CDCl_3$) δ 21.93, 23.69, 27.74, 29.56, 32.65, 39.16, 52.51, 57.55, 67.56, 100.90 (2), 104.20 (2), 110.01 (2), 115.60 (2), 119.40, 122.76 (2), 124.88, 126.53 (2), 127.53 (2), 128.43 (2), 130.46 (2), 138.76, 142.54, 148.15, 155.57, 157.86, 163.17, 171.91, 195.34 MS (EI): 589 (*m*/*z* M^+) Anal. Calcd for ($C_{37}H_{36}N_2O_5$) C, 75.49; H, 6.16; N, 4.76; Found: C, 75.21; H, 5.98; N, 4.74.

6.1.11. (*S*)-2-(2-Benzoyl-cyclohex-1-enylamino)-3-{4-[2-(9H-carbazol-4-yloxy)-ethoxy]-phenyl}-propionic acid (6b). Compound **6b** was synthesized from (*S*)-2-(2-benzoyl-cyclohex-1-enylamino)-3-{4-[2-(9H-carbazol-4-yloxy)-ethoxy]-phenyl}-propionic acid methyl ester according to procedure as mentioned in 6.1.3. ^{1}H NMR ($CDCl_3$) δ 1.41–1.46 (m, 2H), 1.67–1.77(m, 4H), 2.31 (m, 2H), 2.99 (m, 2H), 4.30–4.45 (m, 5H), 6.64 (m, 2H), 7.00 (m, 1H), 7.09 (m, 2H), 7.18 (m, 2H),

7.41–7.48 (m, 5H), 7.67 (d, $J = 8.7$ Hz, 1H), 7.76 (d, $J = 7.8$ Hz, 1H), 7.86 (m, 1H), 7.93 (d, $J = 8.7$ Hz, 1H), 8.06 (d, $J = 7.8$ Hz, 1H), 12.23 (d, $J = 8.7$ Hz, 1H). MS (MALDI): 575 (*m/z* M^+) Anal. Calcd for ($C_{36}H_{34}N_2O_5$) C, 75.24; H, 5.96; N, 4.87; Found: C, 75.11; H, 5.48; N, 4.72.

6.1.12. (*S*)-2-(2-Benzoyl-cyclohex-1-enylamino)-3-[4-(2-carbazol-9-yl-ethoxy)-phenyl]-propionic acid methyl ester (6c). Compound **6c** was synthesized from (*S*)-2-(2-benzoyl-cyclohex-1-enylamino)-3-(4-hydroxy-phenyl)-propionic acid methyl ester and carbazole-9-ethyl mesylate according to procedure mentioned in 6.1.2. ^{1}H NMR ($CDCl_3$) δ 1.39–1.46 (m, 2H), 1.53–1.61 (m, 2H), 2.16–2.23 (m, 4H), 2.90 (m, 1H), 3.01–3.07 (m, 1H), 3.67 (s, 3H), 4.50 (m, 3H), 4.56 (t, $J = 6$ Hz, 2H), 6.69 (d, 9i = 7.8 Hz, 2H), 6.95 (d, $J = 7.8$ Hz, 2H), 7.23–7.26 (m, 2H), 7.32–7.35 (m, 5H), 7.37–7.44 (m, 4H), 8.06 (d, $J = 7.8$ Hz, 2H), 12.15 (d, $J = 8.4$ Hz, 1H). ^{13}C NMR δ ($CDCl_3$) 14.05, 20.92, 22.91, 27.16, 36.86, 38.94, 41.89, 52.41, 65.81, 101.30, 108.44 (2), 108.71, 115.56, 115.78 (2), 119.03, 119.51 (2), 120.36 (2), 125.60, 126.43 (2), 127.06 (2), 127.96 (2), 128.51, 130.29 (2), 139.99, 142.22, 155.84, 163.35, 171.81, 194.93 MS (EI): 573 (*m/z* M^+) Anal. Calcd for ($C_{37}H_{36}N_2O_4$) C, 77.60; H, 6.34; N, 4.89; Found: C, 77.21; H, 6.18; N, 4.69.

6.1.13. (*S*)-2-(2-Benzoyl-cyclohex-1-enylamino)-3-[4-(2-carbazol-9-yl-ethoxy)-phenyl]-propionic acid (6d). Compound **6d** was synthesized according to procedure as mentioned in 6.1.3 from (*S*)-2-(2-benzoyl-cyclohex-1-enylamino)-3-[4-(2-carbazol-9-yl-ethoxy)-phenyl]-propionic acid methyl ester. ^{1}H NMR δ ($CDCl_3$) 1.43–1.47 (m, 2H), 1.63 (m, 2H), 1.78 (m, 2H), 2.23 (m, 2H), 2.90 (m, 1H), 3.01–3.07 (m, 1H), 4.41 (m, 1H), 4.60 (t, $J = 6$ Hz, 2H), 4.69 (t, $J = 6$ Hz, 2H), 6.67 (d, $J = 7.8$ Hz, 2H), 6.95 (d, $J = 7.8$ Hz, 2H), 7.23–7.26 (m, 2H), 7.32–7.35 (m, 5H), 7.37–7.44 (m, 4H), 8.06 (d, $J = 7.8$ Hz, 2H), 12.15 (d, $J = 8.4$ Hz, 1H). MS (MALDI): 559 (*m/z* M^+) Anal. Calcd for ($C_{36}H_{34}N_2O_4$) C, 77.43; H, 6.13; N, 5.01; Found: C, 77.08; H, 5.93; N, 4.64.

6.2. Biological evaluation

6.2.1. PPAR transactivation assay. Comparative dose–response study was performed with all mentioned compounds and Wyeith (WY)14,643 {2-[4-chloro-6-(2,3-dimethyl phenyl amino) pyrimidin-2-ylsulfanyl] acetic acid} for PPARα and all mentioned compounds with rosiglitazone for PPARγ. Most of compounds show dual activation less or more than the reference. The response element (UASGAL4*5) was cloned upstream of the Pgl2-sv 40-Luc reporter (Promega, Madison, WI, USA), which contains the Simian virus early promoter for luciferase assay. GAL4 fusions were made by fusing human PPARγ1 or PPARα ligand-binding domain (amino acids: 174–475) to the C-terminal end of the yeast GAL4 DNA-binding domain (amino acids: 1–147) of the pM1 vector. PadVantage (Promega, Madison, WI, USA) vector was used to enhance luciferase expression. HEK 293T cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum (DMEM-FBS) at 37 °C in 5% CO_2. At 1 day prior to transfection, cells were plated to 50–60% confluence in DMEM containing 10% delipidated FBS (DMEM-DFBS). Cells were transfected by Superfect as per the manufacturer's protocol. At 3 h after transfection, the reagent was removed and cells were maintained in DMEM-DFBS. At 42 h after transfection, the cells were placed in phenol red-free DMEM-DFBS and treated for 18 h with the test compounds or vehicle alone. The cells were lysed and assayed for luciferase activity. Luciferase activity was determined by using Lucite kit (Packard, CT, USA) in a Packard Top count and expressed as fold activation relative to untreated cells.

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