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Original article

Synthesis and evaluation of *N*-acetyl-L-tyrosine based compounds as PPARα selective activators

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Abstract

The development of type 2 diabetes in obese individuals is linked to lipid accumulation in non-adipose tissues. A series of *N*-acetyl-L-tyrosine derivatives were synthesized and evaluated for PPAR transactivation. Compounds **4d** and **4f** were found to show better PPARα transactivation as compared to PPARγ. Molecular docking analysis was carried out to study their important interactions with the active site of PPARα.

Keywords: PPAR-α; Anti-diabetic; Molecular modeling; Synthesis

1. Introduction

The detailed pathophysiology of type 2 diabetes (T2DM) is not fully understood but abnormalities in carbohydrate and fat metabolism play pivotal roles in the development of this disease. Lipid accumulation in non-adipose tissues is increasingly linked to the development of T2DM in obese individuals [1,2]. Peroxisome proliferators activated receptor (PPAR) superfamily, constituted by three different receptor subtypes (PPARα, PPARγ and PPARδ) has been a subject of intensive research for mechanistic importance in glucose and lipid homeostasis [3–6]. PPARα is expressed in organs with high rate of fatty acid catabolism like kidney, liver, skeletal muscle, adrenal glands and cardiac muscles [7,8], PPARγ is expressed in adipose tissue, macrophages and vascular smooth muscles while PPARδ is ubiquitously present in the body [9]. PPARα regulates the expression of genes encoding proteins involved in lipid and lipoprotein metabolism, such as acyl-CoA oxidase, bifunctional enzyme, liver fatty acid binding protein, apo A, apo C-III etc. [10]. PPARα deficient transgenic mouse exhibited massive hepatic and cardiac lipid accumulation owing to the inhibition of cellular fatty acid flux. These results indicate the role of PPARα in lipid homeostasis [11]. Initial interest on PPARγ agonists has waned as they became associated with side effects like weight gain and water retention; instead, the research focus has shifted towards PPARα and PPARδ.

Many new conceptually designed molecules like the PPARα agonists, PPARα/γ dual agonists, triple activators of PPARα/γ/δ and the PPAR modulators have been reported in the literature [12–16]. Selective PPARα agonists have shown

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improvement in insulin action on glucose utilization in two insulin resistant rodent models, perhaps due to increase fatty acid metabolism. Amongst PPARα activators (Fig. 1), fibrate compounds, such as clofibrate, bezafibrate and fenofibrate are antihyperlipidemic drugs. Molecular pharmacological studies have demonstrated that fibrates activate PPARα at high micromolar concentrations [17]. So more potent and selective activators of PPARα are expected to have superior therapeutic utility for the treatment of altered lipid homeostasis in target organs. Recently, (*S*)-isomers of propanoic acid derivatives have been reported to have PPARα selective agonistic activity [18]. KRP-101 and LY-518674 are currently undergoing PPARα selective phase 1 and phase 2 clinical studies, respectively [19].

As a part of the ongoing research to find an effective PPAR-target based drug candidate, which not only would improve the insulin sensitivity but also effectively decrease hyperlipidemia, we have designed low molecular weight L-tyrosine derivatives. Herein, we describe the synthesis and evaluation of a new class of *N*-acetyl-L-tyrosine derivatives as PPARα activators that show potential in the treatment of metabolic disorders like diabetes, obesity and hyperlipidemia. The molecular docking studies performed on this series of molecules also depict good binding affinity of these compounds for PPARα receptor.

2. Chemistry

The synthesis of *N*-acetyl-L-tyrosine derivatives **4a–g** is depicted in Scheme 1. *N*-Acetyl-L-tyrosine (**1**) was esterified by refluxing in ethanol in presence of $SOCl_2$. The resulting ester **2** was condensed with haloalkyl-heterocyclic units to afford **3a–f**. Compounds **3a–f** were further saponified with $LiOH \cdot H_2O$ in THF/MeOH to afford corresponding acids **4a–f**. Compound **2** was also condensed with 2-(*N*-ethyl-*m*-toluidino)ethanol under Mitsunobu reaction conditions to get **3g**, which was further saponified with $LiOH \cdot H_2O$ in THF/MeOH to give the corresponding acid **4g**.

3. Biological evaluation

3.1. PPAR transactivation studies

In vitro screening of compounds was done 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 [20]. Transactivation studies were done for all the compounds at 50 μM concentration for PPARα and 30 μM concentration for PPARγ using WY 14,643 and rosiglitazone as references, respectively. The comparative potencies were determined in terms of fold activation at the same concentrations.

4. Molecular docking

Molecular docking studies were performed on the protein PPARα [21], employing the FlexX docking procedure using Sybyl 6.9 program installed on silicon graphics Octane2 workstation [22]. The 3D coordinates of the active sites were taken from the X-ray crystal structure of PPARα protein reported as complex with GW409544 deposited in Brookhaven Protein Databank with PDB code 1K7L. WY 14,643, the reference compound used for PPARα transactivation study was also docked into the active site of this receptor along with all the synthesized molecules. The active site was assigned at a radius of 8 Å around the reference ligand, GW409544. FlexX run was submitted and the docking scores were obtained and analyzed.

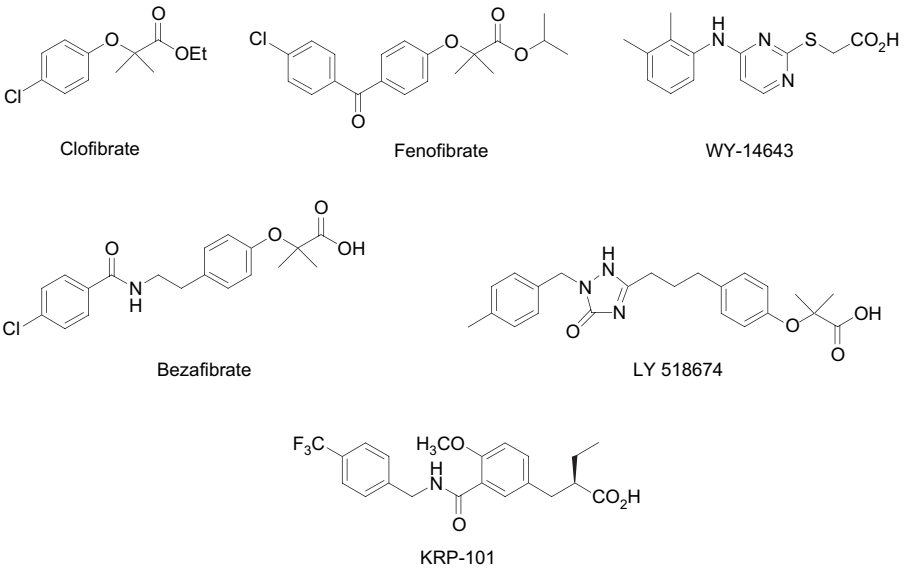
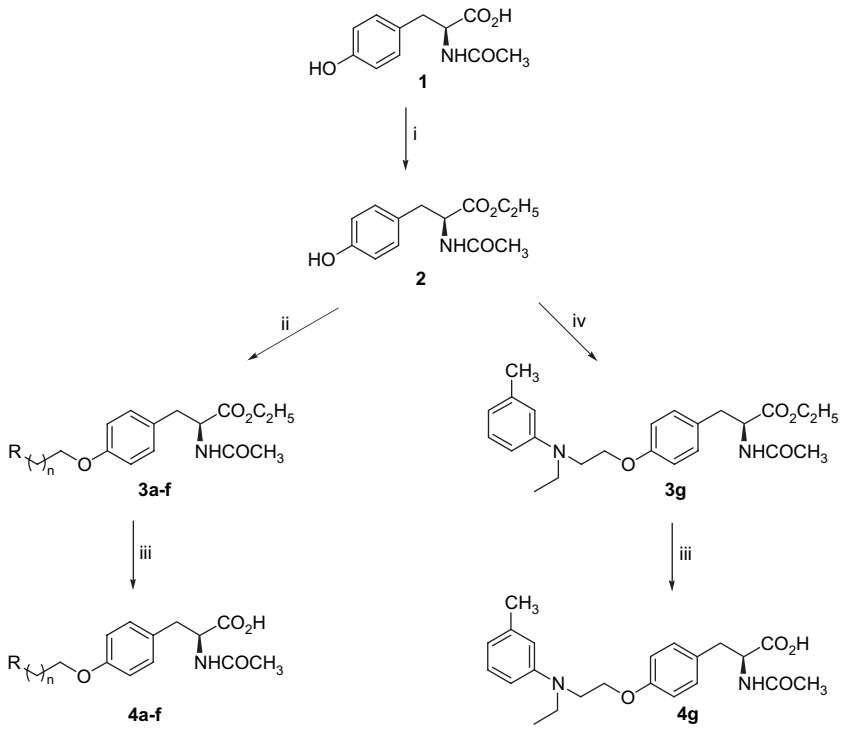


Fig. 1. PPAR activators.



Scheme 1. (i) $SOCl_2$, EtOH, 80 °C, 1 h; (ii) R-$(CH_2)_n$-CH_2X, K_2CO_3, acetone, 70 °C, 14 h; (iii, v) $LiOH \cdot H_2O$, H_2O, THF/CH_3OH (3:1), rt, 12 h; (iv) 2-(*N*-ethyl-2-toluidino)ethanol, DEAD, Ph_3P, THF, 0 °C to rt, 12 h.

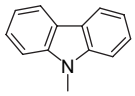
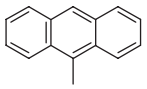
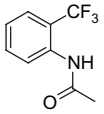
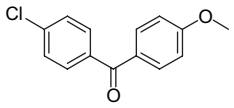
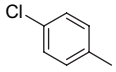
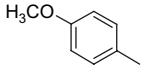
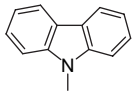
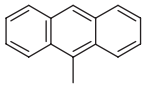
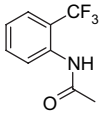
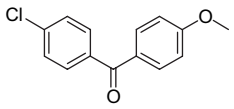
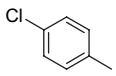
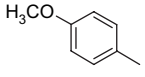
5. Results and discussion

All the synthesized compounds were evaluated by *in vitro* PPAR transactivation studies (Table 1). Evaluation of PPARα/γ activity by the transactivation assay is a widely used functional assay. Rosiglitazone (which showed 27.2-fold activity at 30 μM concentration) was used as the reference standard for PPARγ and WY 14,643 (which showed 4.2-fold activity at 50 μM concentration) was used as standard for PPARα transactivation assay.

In this series, all the molecules show better binding affinity for PPARα in comparison to PPARγ. The acid derivatives have been found to be more active as compared to their ester analogs. It is a well-known fact that the activation of PPARs requires some amount of acidic character and these results are indicative of the same. This series is mainly constituted by the tyrosine moiety as the acidic head group and the changes have been introduced in the hydrophobic side chain. The compounds **3a**, **4a** and **4f** have shown submaximal PPARγ activity while the remaining compounds show very mild transactivation for PPARγ. This indicates that the carbazole moiety is a better hydrophobic substituent for PPARγ. Replacement of carbazole unit of compounds **3a** and **4a** by anthracene unit (**3b** and **4b**) caused a marginal increase in the PPARα activity and a considerable decrease in PPARγ activity. Replacement with 4-(chlorophenyl)phenylmethanone unit (as in fenofibrate) as the hydrophobic side chain in compounds **3d** and **4d** significantly improved the PPARα activity and the compound **4d** showed better activity than the reference compound, WY 14,643. Further substitution of the hydrophobic unit with 4-methoxybenzyl group in compound **4f** also furnished a molecule with better PPARα transactivation than reference compound WY14,643. *N*-(2-Trifluoromethylphenyl)acetamide derivatives (**3c** and **4c**), 4-chlorobenzyl derivatives (**3e** and **4e**) and rosiglitazone mimetics, (**3g** and **4g**) having 2-(*N*-ethyl-*m*-toluidino)ethyl unit, have shown insignificant changes in the PPARα and PPARγ activities. Therefore most of these molecules show better transactivation of PPARα as compared to PPARγ.

Molecular docking studies have been carried out to study the interactions of these molecules in the active site of PPARα (Table 1). All the acid derivatives have been found to dock well in the active site of PPARα and score better than the standard reference compound WY 14,643 (19.92 kcal/mol). They show the H-bonds with the active site residues like Ser280, Tyr314, His440 and Tyr464. The ester derivatives could not bind at the active site due to their inability to form the essential H-bonds of the acidic group, with an exception of compound **3b** and some conformations of compound **3a**. The compounds **4b**, **4c**, **4d** and **4f** occur amongst the best scoring molecules in terms of the flexX docking scores. These results are quite co-relatable to the experimental data. Compound **4b** has the maximum score of −25.59 kcal/mol. The phenanthrene group seems to have good affinity for the active residues of PPARα and the compounds **3b** and **4b** show exceptional binding than the rest of the molecules in this series (Fig. 2). It is able to fit exactly at the same place as the complexed ligand with all the prime interactions and there is another H-bond observed between the side chain ethereal oxygen of the molecule and Thr279. Compound **4d** having the methanone unit and the compound **4c** with its

Table 1
The synthesized molecules with their *in vitro* biological activities for PPARα and PPARγ at 50 μM and their FlexX docking scores at the PPARα receptor

Compound	R	n	1K7L scores	PPARα fold activation	PPARγ fold activation
3a		1	−20.84	1.6	5.4
3b		0	−20.49	2.3	0.8
3c		1	ND	1.0	1.8
3d		1	ND	3.3	0.9
3e		0	ND	2.0	1.0
3f		0	ND	2.4	1.3
3g	**3g**		ND	2.9	1.1
4a		1	−19.57	3.4	4.2
4b		0	−25.59	3.1	1.3
4c		1	−23.02	2.5	1.2
4d		1	−23.22	4.6	2.4
4e		0	ND	3.0	1.9
4f		0	−22.69	4.4	5.4
4g		1	−21.07	3.2	1.4
WY 14,643			−19.92	4.2	–
Rosiglitazone			ND	–	27.2

ND, not determined.

acetamide group also show this additional side chain interaction with Thr279 and another H-bond with a water molecule, besides the acidic group interactions. However, in compound **4f**, the methoxy oxygen forms two-side chain H-bonds with two of the water molecules lying in close vicinity rather than with Thr279 residue.

Thus, from these modeling studies we find that the non-esterified tyrosine derivatives have an edge over the esterified compounds due to the presence of an acidic center and some of the hydrophobic moieties like phenanthrene and 4-(chlorophenyl)phenylmethanone show additional side chain H-bonding interactions which impart better binding capabilities to

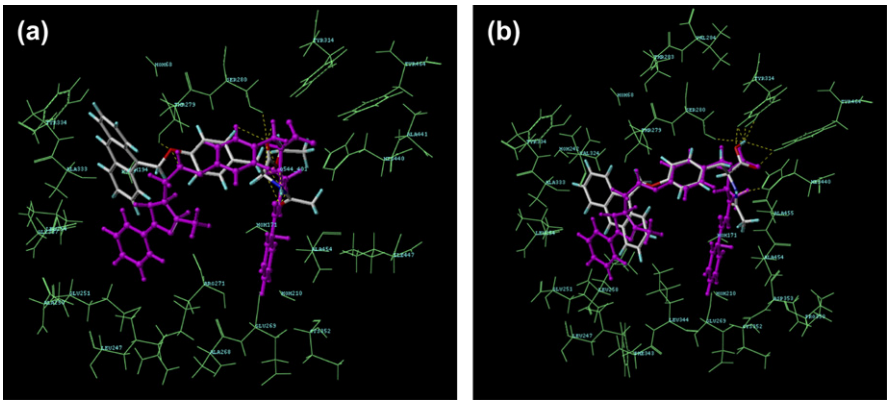


Fig. 2. (a) Compound **3b** in the active site of PPARα. (b) Compound **4b** in the active site of PPARα. The molecule in pink is the reference ligand GW409544 found as a complex with the crystal structure 1K7L. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

them with the PPARα active site residues. These could be used as pointers to design selective PPARα activators.

From this study, compounds **4d** and **4f** have been identified as potent PPARα activators that may be further employed to improve upon the potency of these systems in order to obtain better PPARα activators.

6. Experimental

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. (S)-2-Acetylamino-3-(4-hydroxyphenyl)propionic acid ethyl ester (**2**)*

N-Acetyl-L-tyrosine (10 g, 0.045 mol) was esterified by refluxing in ethanol in presence of thionyl chloride to afford (*S*)-2-acetylamino-3-(4-hydroxyphenyl)-propionic acid ethyl ester (**2**) in 95% yield (1.06 g). IR (cm^{-1}) 3266, 2983, 1734, 1655, 1614, 1516, 1224. ^{1}H NMR (CD_3OD) δ 1.15 (t, $J = 6.9$ Hz, 3H), 1.91 (s, 3H), 2.82–2.98 (m, 2H), 4.11 (q, $J = 6.6$ Hz, 2H), 4.50–4.57 (m, 1H), 6.70 (d, $J = 7.8$ Hz, 2H), 6.98 (d, $J = 7.8$ Hz, 2H), 7.90 (m, 1H). ^{13}C NMR (CD_3OD) δ 14.20, 22.63, 36.77, 54.05, 60.79, 115.34, 127.11, 130.14, 156.25, 170.14, 171.93. MS (APCI): 251 (*m/z* M+1).

*6.2. (S)-2-Acetylamino-3-[4-(2-carbazol-9-yl-ethoxy)phenyl]propionic acid ethyl ester (**3a**)*

(*S*)-2-Acetylamino-3-(4-hydroxyphenyl)propionic acid ethyl ester (**2**) (1 g, 0.004 mol), carbazole-9-ethyl mesylate (1.15 g, 0.004 mol) and potassium carbonate (0.276 g, 0.002 mol) in acetone (10 ml) were refluxed for 14 h. The reaction mixture was concentrated and extracted with DCM. The organic layer was washed with water, brine and dried over sodium sulphate. Purification with column chromatography afforded **3a** in 61% yield (1.06 g). IR (cm^{-1}) 3451, 2922, 1732, 1651, 1513, 1209. ^{1}H NMR ($CDCl_3$) δ 1.25 (t, $J = 6.0$ Hz, 3H), 1.95 (s, 3H), 3.01–3.06 (m, 2H), 4.14 (q, $J = 6.6$ Hz, 2H), 4.31 (t, $J = 6.0$ Hz, 2H), 4.71 (t, $J = 6$ Hz, 2H), 4.77–4.84 (m, 1H), 5.99 (d, $J = 6.0$ Hz, 1H), 6.70–6.74 (m, 3H), 6.95 (m, 3H), 7.23 (m, 2H), 7.45–7.52 (m, 3H), 8.09 (d, $J = 7.8$ Hz, 1H). MS (MALDI): 445, 467 (*m/z* M^+, M+22). Anal. Calcd for ($C_{27}H_{28}N_2O_4$) C, 72.95; H, 6.35; N, 6.30. Found: C, 72.57; H, 6.06; N, 6.08.

*6.3. (S)-2-Acetylamino-3-[4-(2-carbazol-9-yl-ethoxy)phenyl]propionic acid (**4a**)*

(*S*)-2-Acetylamino-3-[4-(2-carbazol-9-yl-ethoxy)phenyl]-propionic acid ethyl ester (**3a**) (1 g, 0.0022 mol) was saponified with 1.5 equiv aqueous $LiOH \cdot H_2O$ in THF:CH_3OH (3:1) to gave (*S*)-2-acetylamino-3-[4-(2-carbazol-9-yl-ethoxy)phenyl]propionic acid in 82% yield (0.77 g). IR (cm^{-1}) 3408, 2897, 1701, 1628, 1507, 1254. ^{1}H NMR (CD_3OD) δ 1.89 (s, 3H), 2.76–2.83 (m, 1H), 3.01–3.08 (m, 1H), 3.30 (s, 1H), 4.27 (t, $J = 6$ Hz, 2H), 4.51–4.56 (m, 1H), 4.67 (t, $J = 6$ Hz, 2H), 6.65 (d, $J = 8.4$ Hz, 2H), 7.00 (d, $J = 8.4$ Hz, 2H), 7.17 (t, $J = 7.5$ Hz, 2H), 7.41 (d, $J = 7.8$ Hz, 2H), 7.52 (d, $J = 8.1$ Hz, 2H), 8.04 (d, $J = 7.5$ Hz, 2H). ^{13}C NMR (CD_3OD) δ 14.49, 22.33, 37.65, 43.51, 55.31, 61.58, 67.71, 110.25, 115.47, 120.10, 121.03, 126.70, 130.74, 131.20, 142.07, 158.88, 173.08, 174.86. MS (APCI): 417 (*m/z* M+1). Anal. Calcd for ($C_{25}H_{24}N_2O_4$) C, 72.10; H, 5.81; N, 6.73. Found: C, 72.05; H, 5.47; N, 6.53.

6.4. (*S*)-2-Acetylamino-3-[4-(anthracen-9-yl-methoxy)phenyl]propionic acid ethyl ester (**3b**)

Compound **3b** was synthesized according to the procedure as mentioned in Section 6.2 from (*S*)-2-acetylamino-3-(4-hydroxyphenyl)propionic acid ethyl ester (**2**) (1 mmol) and 9-chloromethylanthracene (1 mmol). IR (cm^{-1}) 3341, 2925, 1735, 1655, 1515, 1216. ^1H NMR ($CDCl_3$) δ 1.71 (t, $J = 6$ Hz, 3H), 1.99 (s, 3H), 2.05 (s, 2H), 3.01–3.11 (dd, $J = 6$ Hz 6 Hz, 2H), 4.13 (q, $J = 6.6$ Hz, 2H), 4.85 (m, 1H), 6.07 (m, 1H), 6.75 (d, $J = 7.8$ Hz, 2H), 6.95 (d, $J = 7.8$ Hz, 2H), 7.02 (m, 1H), 7.34 (d, $J = 7.8$ Hz, 1H), 7.43 (m, 2H), 8.02 (m, 2H), 7.90 (s, 1H), 8.11 (d, $J = 7.8$ Hz, 1H), 8.43 (m, 1H). MS (MALDI): 442 (*m/z* M^+). Anal. Calcd for ($C_{28}H_{27}NO_4$) C, 76.17; H, 6.16; N, 3.17. Found: C, 76.01; H, 5.89; N, 3.09.

6.5. (*S*)-2-Acetylamino-3-[4-(anthracen-9-yl-methoxy)phenyl]propionic acid (**4b**)

Compound **4b** was synthesized according to the procedure as mentioned in Section 6.3 from (*S*)-2-acetylamino-3-[4-(anthracen-9-yl-methoxy)phenyl]propionic acid ethyl ester. IR (cm^{-1}) 3412, 2921, 1695, 1638, 1512, 1211. ^1H NMR (CD_3OD) δ 2.03 (s, 3H), 2.93–3.10 (m, 2H), 3.56 (s, 2H), 4.30 (m, 1H), 6.11 (m, 1H), 6.77 (d, $J = 7.8$ Hz, 2H), 6.96 (d, $J = 7.8$ Hz, 2H), 7.04 (m, 1H), 7.35 (d, $J = 7.8$ Hz, 1H), 7.45 (m, 2H), 8.02 (m, 2H), 7.90 (s, 1H), 8.10 (d, $J = 7.8$ Hz, 1H), 8.42 (m, 1H). MS (MALDI): 413, 435 (*m/z* M+1, M+23). Anal. Calcd for ($C_{26}H_{23}NO_4$) C, 75.53; H, 5.61; N, 3.39. Found: C, 75.50; H, 5.37; N, 3.17.

6.6. (*S*)-2-Acetylamino-3-{4-[(2-trifluoromethylphenyl carbamoyl)methoxy]-phenyl}propionic acid ethyl ester (**3c**)

Step A: 2-chloro-*N*-(2-trifluoromethylphenyl)acetamide was synthesized by condensing 2-trifluoromethylphenylamine (2 g, 0.012 mol) with chloroacetylchloride (1.37 g, 0.012 mol). ^1H NMR ($CDCl_3$) δ 4.22 (s, 2H), 7.28 (t, $J = 7.5$ Hz, 1H), 7.56–7.66 (m, 2H), 8.22 (d, $J = 8.4$ Hz, 1H), 8.74 (s, 1H). ^{13}C NMR ($CDCl_3$) δ 42.82, 123.96, 125.21, 126.17, 132.94, 164.25. MS (MALDI): 275 (*m/z* M+38).

Step B: compound **3c** was synthesized according to the procedure as mentioned in Section 6.2 from (*S*)-2-acetylamino-3-(4-hydroxyphenyl)propionic acid ethyl ester (**2**) (0.5 g, 0.019 mol) and 2-chloro-*N*-(2-trifluoromethylphenyl)acetamide (0.54 g, 0.019 mol). ^1H NMR ($CDCl_3$) δ 1.25 (t, $J = 6.9$ Hz, 3H), 1.93 (s, 3H), 2.92 (m, 1H), 3.11 (m, 1H), 4.10 (q, $J = 6.9$ Hz, 2H), 4.47 (m, 1H), 4.73 (s, 2H), 6.70 (d, $J = 7.8$ Hz, 2H), 6.98 (d, $J = 7.8$ Hz, 2H), 7.22 (d, $J = 7.2$ Hz, 1H), 7.53–7.63 (m, 3H), 8.26 (d, $J = 8.4$ Hz, 1H), 8.99 (s, 1H). MS (MALDI): 452 (*m/z* M^+). Anal. Calcd for ($C_{22}H_{23}F_3N_2O_5$) C, 58.40; H, 5.12; N, 6.12. Found: C, 58.23; H, 5.03; N, 5.98.

6.7. (*S*)-2-Acetylamino-3-{4-[(2-trifluoromethyl-phenyl carbamoyl)methoxy]-phenyl}propionic acid (**4c**)

Compound **4c** was synthesized according to the procedure as mentioned in Section 6.3 from (*S*)-2-acetylamino-3-{4-[(2-trifluoromethylphenylcarbamoyl) methoxy]phenyl}propionic acid ethyl ester (**3c**). IR (cm^{-1}) 3414, 2925, 1659, 1514, 1233. ^1H NMR (CD_3OD) δ 1.92 (s, 3H), 2.90–2.97 (m, 2H), 4.48 (m, 1H), 4.74 (s, 2H), 6.72 (d, $J = 7.8$ Hz, 2H), 6.97 (d, $J = 7.8$ Hz, 2H), 7.28 (d, $J = 7.2$ Hz, 1H), 7.56–7.66 (m, 3H), 8.21 (d, $J = 8.4$ Hz, 1H), 8.74 (s, 1H). MS (MALDI): 425 (*m/z* M+1). Anal. Calcd for ($C_{20}H_{19}F_3N_2O_5$) C, 56.60; H, 4.51; N, 6.60. Found: C, 56.36; H, 4.37; N, 6.28.

6.8. (*S*)-2-Acetylamino-3-(4-{2-[4-(4-chlorobenzoyl) phenoxy]ethoxy}phenyl)-propionic acid ethyl ester (**3d**)

Step A: [4-(2-bromoethoxy)phenyl]-(4-chlorophenyl) methanone was synthesized by refluxing (4-chlorophenyl)-(4-hydroxyphenyl)methanone (2 g, 0.0086 mol) with 1,2-dibromoethane (1.6 g, 0.0086 mol) in acetone in presence of K_2CO_3 (0.58 g, 0.009 mol). The reaction mixture was concentrated and extracted with ethyl acetate. The organic layer was treated with water, brine and dried over sodium sulphate. The column chromatography afforded the pure title compound in 75% yield (2.19 g). ^1H NMR ($CDCl_3$) δ 3.67 (t, $J = 6.0$ Hz, 2H), 4.38 (t, $J = 6.0$ Hz, 2H), 6.97 (d, $J = 9.0$ Hz, 2H), 7.45 (d, $J = 8.4$ Hz, 2H), 7.73 (d, $J = 9$ Hz, 2H), 7.80 (d, $J = 8.7$ Hz, 2H). MS (MALDI): 339, 341 (*m/z* M^+, M+2).

Step B: [4-(2-bromoethoxy)phenyl]-(4-chlorophenyl) methanone (1 g, 0.003 mol) and (*S*)-2-acetylamino-3-(4-hydroxyphenyl)propionic acid ethyl ester (0.74 g, 0.015 mol) were refluxed to afford 2-acetylamino-3-(4-{2-[4-(4-chlorobenzoyl)-phenoxy]ethoxy}phenyl)propionic acid ethyl ester (**3d**) according to the procedure mentioned in Section 6.2. IR (cm^{-1}) 3426, 2935, 1725, 1644, 1523, 1231. ^1H NMR ($CDCl_3$) δ 1.24 (t, $J = 6.9$ Hz, 3H), 1.98 (s, 3H), 2.93–3.09 (m, 2H), 4.15 (q, $J = 7.2$ Hz, 2H), 4.39 (t, $J = 6$ Hz, 2H), 4.44 (m, 1H), 4.81 (t, $J = 6$ Hz, 2H), 6.28 (d, $J = 7.8$ Hz, 2H), 6.75 (d, $J = 8.1$ Hz, 2H), 6.93 (d, $J = 7.8$ Hz, 2H), 7.06 (m, 2H), 7.44 (d, $J = 8.4$ Hz, 2H), 7.71 (d, $J = 5.1$ Hz, 1H), 7.79 (d, $J = 8.4$ Hz, 2H). MS (MALDI): 510, 532 (*m/z* M^+, M+22). Anal. Calcd for ($C_{28}H_{28}ClNO_6$) C, 65.94; H, 5.53; N, 2.75. Found: C, 65.59; H, 5.34; N, 2.57.

6.9. (*S*)-2-Acetylamino-3-(4-{2-[4-(4-chlorobenzoyl) phenoxy]ethoxy}phenyl)-propionic acid (**4d**)

Compound **4d** was synthesized according to the procedure as mentioned in Section 6.3 from 2-acetylamino-3-(4-{2-[4-(4-chlorobenzoyl)phenoxy]-ethoxy}phenyl)propionic acid ethyl ester (**3d**). IR (cm^{-1}) 3428, 2935, 1646, 1521, 1230. ^1H NMR (DMSO-d_6) δ 1.78 (s, 3H), 2.73–2.81 (m, 1H), 2.93–3.09 (m, 2H), 4.32–4.42 (m, 5H), 6.89 (d, $J = 8.4$ Hz, 2H), 7.16 (m, 3H), 7.61 (d, $J = 9$ Hz, 2H), 7.70–7.79 (m,

4H), 8.14 (d, $J = 7.8$ Hz, 1H). ^{13}C NMR (DMSO-d_6) δ 22.32, 35.97, 53.70, 66.02, 66.83, 114.19, 114.48, 128.57, 129.21 129.91, 131.14, 132.19, 136.39, 136.97, 156.87, 162.20, 169.17, 173.19, 193.27. MS (APCI): 482 (*m/z* M+1). Anal. Calcd for ($C_{26}H_{24}ClNO_6$) C, 64.80; H, 5.02; N, 2.91. Found: C, 64.61; H, 4.84; N, 2.77.

*6.10. (S)-2-Acetylamino-3-[4-(4-chlorobenzyloxy) phenyl]propionic acid ethyl ester (**3e**)*

Compound **3e** was synthesized according to the procedure as mentioned in Section 6.2 from (*S*)-2-acetylamino-3-(4-hydroxyphenyl)propionic acid ethyl ester (**2**) (0.5 g, 0.0019 mol) and 1-chloro-4-chloromethylbenzene (0.31 g, 0.0019 mol) in 62% yield (0.46 g). IR (cm^{-1}) 3348, 2923, 1716, 1612, 1511, 1243. ^{1}H NMR ($CDCl_3$) δ 1.26 (t, $J = 6$ Hz, 3H), 1.99 (s, 3H), 3.00–3.07 (m, 2H), 4.16 (q, $J = 6$ Hz, 2H), 4.81 (m, 1H), 4.98 (s, 2H), 5.98 (d, $J = 7.8$ Hz, 1H), 6.84 (d, $J = 7.5$ Hz, 2H), 6.95 (d, $J = 7.8$ Hz, 2H), 7.01 (d, $J = 7.8$ Hz, 2H), 7.24 (d, $J = 7.8$ Hz, 2H). MS (MALDI): 376 (*m/z* M+1). Anal. Calcd for ($C_{20}H_{22}ClNO_4$) C, 63.91; H, 5.90; N, 3.73. Found: C, 63.54; H, 5.44; N, 3.52.

*6.11. (S)-2-Acetylamino-3-[4-(4-chlorobenzyloxy) phenyl]propionic acid (**4e**)*

Compound **4e** was synthesized according to the procedure as mentioned in Section 6.3 from (*S*)-2-acetylamino-3-[4-(4-chlorobenzyloxy)phenyl]propionic acid ethyl ester (**3e**). IR (cm^{-1}) 3354, 2925, 1702, 1615, 1513, 1247. ^{1}H NMR (CD_3OD) δ 1.90 (s, 3H), 2.84–2.89 (m, 1H), 3.10–3.16 (m, 1H), 4.60 (m, 1H), 6.90 (d, $J = 8.4$ Hz, 2H), 7.15 (d, $J = 8.7$ Hz, 2H), 7.33–7.42 (m, 4H). ^{13}C NMR (CD_3OD) δ 22.36, 37.69, 55.38, 70.16, 115.91, 129.60, 130.11, 130.95, 131.32, 134.58, 137.68, 158.96, 173.12, 174.95. MS (MALDI): 348, 370 (*m/z* M^+, M+22). Anal. Calcd for ($C_{18}H_{18}ClNO_4$) C, 62.16; H, 5.22; N, 4.03. Found: C, 62.07; H, 5.12; N, 3.82.

*6.12. (S)-2-Acetylamino-3-[4-(4-methoxybenzyloxy) phenyl]propionic acid ethyl ester (**3f**)*

Compound **3f** was synthesized according to the procedure as mentioned in Section 6.2 from (*S*)-2-acetylamino-3-(4-hydroxyphenyl)propionic acid ethyl ester (**2**) (0.5 g, 0.0019 mol) and 1-bromomethyl-4-methoxybenzene (0.4 g, 0.0019 mol) in 65% yield (0.48 g). IR (cm^{-1}) 3325, 2925, 1745, 1654, 1612, 1509, 1261. ^{1}H NMR ($CDCl_3$) δ 1.26 (t, $J = 5.4$ Hz, 3H), 1.98 (s, 3H), 2.96–3.05 (m, 2H), 3.81 (s, 3H), 4.18 (q, $J = 6.9$ Hz, 2H), 4.82 (m, 1H), 5.01 (s, 2H), 6.12 (m, 2H), 6.73 (d, $J = 7.8$ Hz, 2H), 6.87–7.00 (m, 2H), 7.28 (m, 1H). MS (MALDI): 372, 394 (*m/z* M^+, M+22). Anal. Calcd for ($C_{21}H_{25}NO_5$) C, 67.91; H, 6.78; N, 3.77. Found: C, 67.68; H, 6.42; N, 3.53.

*6.13. (S)-2-Acetylamino-3-[4-(4-methoxybenzyloxy) phenyl]propionic acid (**4f**)*

Compound **4f** was synthesized according to the procedure as mentioned in Section 6.3 from (*S*)-2-acetylamino-3-[4-(4-methoxybenzyloxy)phenyl]propionic acid ethyl ester (**3f**). IR (cm^{-1}) 3313, 2934, 1659, 1610, 1511, 1267. ^{1}H NMR (CD_3OD) δ 1.98 (s, 3H), 3.05 (m, 2H), 3.81 (s, 3H), 4.67 (m, 1H), 5.00 (s, 1H), 6.86 (d, $J = 9.3$ Hz, 2H), 6.91 (d, $J = 8.1$ Hz, 2H), 7.06 (d, $J = 7.8$ Hz, 2H), 7.28 (d, $J = 7.2$ Hz, 2H). ^{13}H NMR (CD_3OD) δ 14.07, 50.42, 55.18, 60.46, 69.82, 112.22, 113.06, 115.43, 119.61, 129.58, 130.59, 170.68, 173.58. MS (MALDI): 345, 367 (*m/z* M+1, M+23). Anal. Calcd for ($C_{19}H_{21}NO_5$) C, 66.46; H, 6.16; N, 4.08. Found: C, 66.38; H, 6.02; N, 3.73.

*6.14. (S)-2-Acetylamino-3-{4-[2-(ethyl-2-tolylamino) ethoxy]phenyl}propionic acid ethyl ester (**3g**)*

(*S*)-2-Acetylamino-3-(4-hydroxyphenyl)-propionic acid ethyl ester (0.5 g, 0.0019 mol) was condensed with 2-(ethyl-2-tolyl-amino)ethanol (0.35 g, 0.0019 mol) under Mitsunobu reaction condition [i.e. DEAD (0.5 g, 0.0028 mol), triphenylphosphine (0.57 g, 0.0028 mol) in 10 ml THF at 0 °C to rt for 12 h]. The reaction mixture was concentrated and purified by column chromatography to furnish (*S*)-2-acetylamino-3-{4-[2-(ethyl-2-tolyl-amino)-ethoxy]phenyl}propionic acid ethyl ester (**3g**) in 30% yield (0.25 g). IR (cm^{-1}) 3465, 2928, 1726, 1648, 1612, 1507, 1252. ^{1}H NMR (CD_3OD) δ 1.15 (t, $J = 6$ Hz, 3H), 1.24 (t, $J = 6$ Hz, 3H), 2.21 (s, 3H), 2.42 (s, 3H), 2.76–2.87 (m, 2H), 3.37 (q, $J = 6$ Hz, 2H), 3.75 (t, $J = 6$ Hz, 2H), 4.10 (q, $J = 6$ Hz, 2H), 4.26 (t, $J = 6$ Hz, 2H), 4.92 (m, 1H), 6.72 (d, $J = 7.8$ Hz, 2H), 7.12 (d, $J = 7.8$ Hz, 2H), 7.48 (m, 2H), 7.67 (m, 2H), 9.92 (s, 1H). MS (MALDI): 413 (*m/z* M+1). Anal. Calcd for ($C_{24}H_{32}N_2O_4$) C, 69.88; H, 7.82; N, 6.79. Found: C, 69.54; H, 7.67; N, 6.53.

*6.15. (S)-2-Acetylamino-3-{4-[2-(ethyl-2-tolylamino) ethoxy]phenyl}propionic acid (**4g**)*

The compound **3g** was saponified with 1.5 equiv $LiOH \cdot H_2O$ in THF:MeOH (3:1) to give (*S*)-2-acetylamino-3-{4-[2-(ethyl-2-tolyl-amino)-ethoxy]-phenyl}-propionic acid (**4g**). IR (cm^{-1}) 3456, 2918, 1656, 1606, 1513, 1257. ^{1}H NMR ($CDCl_3$) δ 1.22 (t, $J = 6.0$ Hz, 3H), 2.02 (s, 3H), 2.34 (s, 3H), 2.90–2.97 (m, 2H), 3.38 (q, $J = 6$ Hz, 2H), 3.72 (t, $J = 6$ Hz, 2H), 4.16 (t, $J = 6$ Hz, 2H), 4.91 (m, 1H), 6.69 (d, $J = 7.8$ Hz, 2H), 7.07 (d, $J = 7.8$ Hz, 2H), 7.45–7.52 (m, 2H), 7.64–7.70 (m, 2H), 9.89 (s, 1H). MS (MALDI): 384 (*m/z* M^+). Anal. Calcd for ($C_{22}H_{28}N_2O_4$) C, 68.73; H, 7.34; N, 7.29. Found: C, 68.58; H, 7.12; N, 7.03.

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