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Syntheses, biological evaluation of some novel substituted benzoic acid derivatives bearing hydrazone as linker

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Abstract

On the basis of rational drug design fourteen novel compounds having benzoic acid as acidic head, hydrazone as linker and substituted diaryl sulfanyl/aryl-cyclohexylsulfanyl as a hydrophobic tail were synthesized and characterized by physicochemical and spectrophotometric (FTIR, Mass, ^{1}HNMR and ^{13}CNMR) analysis. The spectral data were satisfactory with their structures. The designed compounds were docked against peroxisome proliferated activated receptors (PPARγ) and further evaluated for in vitro PPARγ agonist activity and in vivo hypoglycemic activity in wistar strain of albino rats. Compound **3k** and **3m** exhibited potent anti-diabetic activity without ulcerogenic toxicity and minimum side effects as weight gain. Therefore these compounds would be considered as promising agents for the development of novel antidiabetic agents.

Keywords PPARγ agonists · Benzoic acid derivatives · Hydrazone · Thioether · Anti-diabetic · Ulcerogenic toxicities

Introduction

PPAR(peroxisome proliferator activated receptor) γ is a member of the peroxisome proliferator activated receptor family and has been the subject of extensive research for mechanistic importance in glucose and lipid homeostasis [1]. The receptor is widely distributed in the spleen, colon, adipose tissue and macrophages, and found to a lesser extent in the liver, pancreas and skeletal

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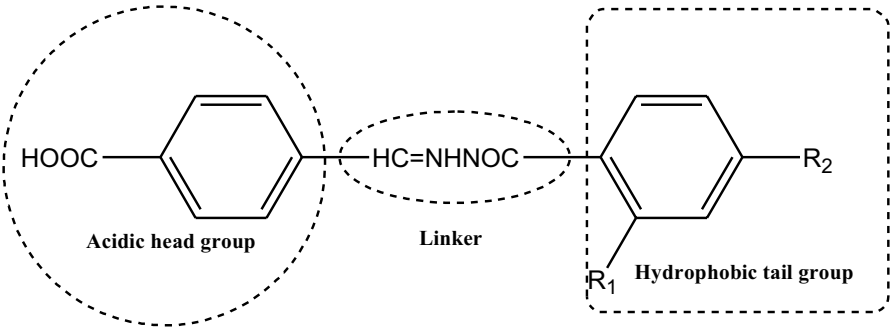
muscle [2]. PPARγ agonist actions are known to improve hyperglycemia and hyperlipidemia, and to reduce cardiovascular risk factors such as atherosclerosis, arterial hypertension and inflammatory mediators. Hyperlipidemia is usually associated with hyperglycemia and frequently leads to the development of type-2 diabetes mellitus (T2DM) and cardiovascular disease [3], thus posing a substantial worldwide economic burden [4].

PPARγ agonists, such as thiazolidinediones (TZDs) have proven to be efficacious as insulin sensitizing agents in the treatment of persistent hyperglycemia [5–9]. Due to unwanted adverse effects of long term administered TZDs, such as weight gain and fluid retention, search of new PPARγ agonists without adverse effects is essential in the development of new antidiabetic agents.

Literature was reported that benzoic acid derivatives stimulated expression and exhibited PPARγ agonistic activity [10–12]. Benzoic acid possesses a simple skeleton that belong to open chain acidic group in place of close chain acidic TZD and that binds comfortably in the active site of PPARγ [13, 14]. These findings prompted us to search novel benzoic acid derivatives containing different hydrophobic tail part as PPARγ agonists.

Design

Compounds having trifunctional unit (acidic head part, hydrophobic tail part and a connecting part as linker) have been shown good PPARγ agonistic activity [15]. Keeping these structural features in mind novel benzoic acid derivatives were designed. The different hydrophobic tail parts are conveniently selected according to physicochemical properties such as hydrophobicity and electronic distribution. The hydrophobic tail part which differs in the compounds is of major interest. In designed compound, the benzoic acid as acidic head part is linked with substituted aryl/cyclohexylsulfanyl via hydrazone moiety as linker (Fig. 1). Earlier hydrazone was effectively evaluated as anti-diabetic agents [16, 17].



$R_1 = R_2$ = Substituted aryl/cyclohexylsulfanyl

Fig. 1 Design of benzoic acid derivatives

Experimental section

Molecular docking

The Program Molegro Virtual Docker (MVD 2012. 5.5, Molegro Bioinformatics, Aarchus C, Denmark) was employed to generate grid, calculate dock score and evaluate conformers [18]. The compounds were docked using PPARγ protein coordinates. Protein coordinates were downloaded from the Protein Data Bank, accession code 2PRG [19]. Chain A was prepared for docking within the MVD by removing chain B and all water molecules. Maximum number of cavities was fixed to 5 for detection of possible binding cavities, grid resolution was 0.60 Å with center at coordinates $x=(-17.45)$, $y=(-16.26)$ and $z=(18.04)$ Å, and the binding site radius was set to 16 Å, while other parameters was set as default. All compounds were stored in a MVD file and an original conformation was generated for each compound. Compounds were docked into the protein coordinates and the highest scoring pose was selected for each of the compounds. The best docking poses are predicted to be the most stable conformation of each compound for binding to the PPARγ receptor. The validation of the docking process was performed and determines whether the molecular docking algorithm is able to recover the crystallographic.

position with root mean square distance value less than 2.0 Å [see Supplementary File 1].

Synthetic materials and methods

All synthetic starting material, reagents, and solvents were procured from Sigma Aldrich and Merck and used without further purification. Thin-layer chromatography (TLC) and column chromatography was used to reach the completion of the reaction and purity of the compounds synthesized respectively. Melting points were recorded using an open capillary tube electrothermal melting point apparatus and are uncorrected. IR spectra were obtained in KBr discs on a Shimadzu 8400S Fourier- transform infrared (FTIR) spectrophotometer. ^{1}H NMR spectra were recorded on a FT NMR (400 MHz) and ^{13}C NMR were recorded at 100 MHz on a BRUKER AVANCE II 400 NMR spectrometer; DMSO-d_6 was used as a solvent. Chemical shifts are reported as δ (ppm). The Mass spectra were recorded on a Waters Q-TOF Micro mass spectrometer. Elemental analysis was performed using EURO Vector EA 3000 analyzer. The spot on sample loaded TLC (E-Merck pre-coated plates) plates were identified by exposing to UV light and iodine vapour. The CsF-Celite reagent was prepared by stirring an aqueous solution of CsF with celite 521 at room temperature for 20 min [20].

Preparation of intermediates

Procedure for preparation of ester

A mixture of substituted benzoic acid (1.0 mmol), methanol (10 mmol) and few drops of Conc. sulfuric acid as catalyst was refluxed at 70 °C for 4–5 h. and reaction

was monitored by TLC. After completion of reaction, the reaction mixture was cooled and filtered. The solid residue (IM1) was washed with distilled water and dried.

Procedure for preparation of thioether

A mixture of aryl/acylthiol (100 mmol) and CsF-Celite as a solid base (150 mmol) in 20 ml of acetonitrile containing substituted ester (200 mmol) was refluxed at 80 °C for 03–18 h and the progress of the reaction was monitored by TLC. After completion of reaction, the reaction mixture was cooled at room temperature and filtered. The CsF-Celite was separated as residue and thioether as filtrate. The filtrate was evaporated to get desired product (IM2).

Procedure for preparation of hydrazide

Hydrazide intermediates were obtained from reaction of the substituted thioether (10 mmol) with excess of hydrazine 90% (10 mmol) under reflux at 110 °C for 45 min. The solid was obtained by cooling the reaction vessels, filtered off and washed with distilled water and dried to get desired products (IM3).

General method for the preparation of compounds (3a–3n)

The compounds were synthesized by reacting equimolar proportion of substituted aryl hydrazides (IM3; 10 mmol) and p-formyl benzoic acid (10 mmol) in absolute ethanol (25 mL) under reflux at 78 °C for 40 min. The precipitate was filtered off and washed with distilled water and finally recrystallized with dimethylformamide to obtain **3a**–**3n**.

(1) 4-{[2-(4-Chloro-phenylsulfanyl)-benzoyl]-hydrazonomethyl}-benzoic acid (3a)

White solid; Yield: 83%; mp: 219–221 °C; IR (KBr, υ cm^{-1}): 3348(NH), 3059(CH), 2988(OH), 1749(C=O), 1680 (C=O), 1530(C=C), 1058(C–Cl), 680(C–S). ^{1}HNMR (DMSO-d_6): δ 12.13(s, 1H, COOH), 11.56(*s*, 1H, NH), 10.10 (*s*, 1H, –N=CH), 7.95–7.92(*d*, J = 7.37 Hz, 2H), 7.73–7.70 (*d*, J = 6.70 Hz, 2H), 7.59–7.54 (*m*, 6H), 7.39–7.35. (*d*, J = 8.62 Hz, 2H). ^{13}C NMR (DMSO-d_6): δ 192.35, 171.53, 164.98, 145.32, 138.15, 137.46, 135.33, 129.92, 125.70, 125.19, 125.30, 125.21, 125.19, 125.09, 124.90, 124.84, 124.53, 124.31, 124.17, 124.11, 124.05. MS: m/z. 412.70 (M^+ + 2). Anal. Calcd for $C_{21}H_{15}ClN_2O_3S$: C, 61.40; H, 3.69; N, 6.83; S, 7.80. Found: C, 61.37; H, 3.53; N, 6.70; S, 7.47.

(2) 4-{[2-(4-Fluoro-phenylsulfanyl)-benzoyl]-hydrazonomethyl}-benzoic acid (3b)

White solid; Yield: 93%; mp: 229–231 °C; IR(KBr, υ cm^{-1}): 3391(NH), 3077(CH), 3019(OH), 1747(C=O), 1698(C=O), 1527(C=C), 1157(C–F), 683(C–S). ^{1}HNMR (DMSO-*d*6): δ 12.14(*s*, 1H, COOH), 11.65(*s*, 1H, NH), 10.13

(*s*, 1H, –N=CH), 7.89–7.86 (*d*, *J* = 8.93 Hz, 2H), 7.74–7.71(*d*, *J* = 7.05 Hz, 2H), 7.59–7.50 (*m*, 6H), 7.46–7.42(*d*, *J* = 7.23 Hz, 2H). ^{13}C NMR (DMSO-*d*6): δ 192.96, 171.34, 166.53, 145.76, 140.34, 138.62, 135.77, 129.88, 125.70, 125.10, 124.47, 124.29, 124.23, 124.21, 124.17, 124.11, 124.07, 124.01, 123.83, 123.76, 123.65. MS: m/z 395.43 (M^+). Anal. Calcd for $C_{21}H_{15}FN_2O_3S$: C, 63.94; H, 3.84; N, 7.11; S, 8.13. Found: C, 63.85; H, 3.64; N, 7.02; S, 8.08.

(3) 4-{[2-(4-Bromo-phenylsulfanyl)-benzoyl]-hydrazonomethyl}-benzoic acid (3c)

White solid; Yield: 81%; mp: 230–232 °C; IR(KBr, υ cm^{-1}): 3388(NH), 3113(CH), 3013(OH),1758(C=O), 1683 (C=O), 1514(C=C), 1041(C–Br), 669(C–S), ^{1}H NMR (DMSO-*d*6): δ 12.13(*s*, 1H, COOH), 11.64(*s*, 1H, NH), 10.09(*s*,1H, –N=CH), 7.84–7.81(*d*, *J* = 7.19 Hz, 2H), 7.73–7.70(*d*, *J* = 7.81 Hz, 2H), 7.61–7.55(*m*, 6H), 7.47–7.43 (*d*, *J* = 8.01 Hz, 2H). ^{13}C NMR (DMSO-*d*6): δ 192.21, 171.11, 165.82, 145.47, 139.46, 135.71, 131.19, 126.47, 125.21, 125.13, 125.01, 124.93, 124.61, 124.18, 124.11, 124.03, 124.95, 124.61, 124.33, 124.13, 124.11.MS:m/z 456.63 ($M^+ + 2$). Anal. Calcd for $C_{21}H_{15}BrN_2O_3S$: C, 55.39; H, 3.32; N, 6.15; S, 7.08. Found: C, 55.35; H, 3.04; N, 6.12; S, 7.03.

(4) 4-[(2-p-Tolylsulfanyl-benzoyl)-hydrazonomethyl]-benzoic acid (3d)

Off-white solid; Yield: 78%; mp: 224–226 °C; IR(KBr,υcm^{-1}):3331(NH),312 1(CH), 3008(OH),1751(C=O), 1689 (C=O), 1521(C=C), 691(C–S). ^{1}H NMR (DMSO-*d*6): δ 2.08(*s*, 1H, COOH), 11.56(*s*, 1H, NH), 10.11(*s*, 1H, –N=CH), 7.88–7.85(*d*, *J* = 7.03 Hz, 2H), 7.65–7.63(*d*, *J* = 7.12 Hz, 2H), 7.59–7.53(*m*, 6H), 7.39–7.35 (*d*, *J* = 7.33 Hz,2H), 2.22 (s, 3H).^{13}C NMR (DMSO-*d*6): δ 191.02, 171.51, 165.21, 145.74, 138.52, 135.43, 129.94, 125.71, 125.58, 125.53, 125.33, 125.21,125.10, 125.03, 124.93, 124.77, 124.71, 124.11,123.33, 123.09, 122.70, 21.42. MS: m/z 391.10 ($M^+ + 1$). Anal. Calcd for $C_{22}H_{18}N_2O_3S$: C, 67.69; H, 4.65; N, 7.17; S, 8.22. Found: C, 67.35; H, 4.64; N, 7.12; S, 8.18.

(5) 4-{[2-(4-Methoxy-phenylsulfanyl)-benzoyl]-hydrazonomethyl}-benzoic acid (3e)

Off white solid; Yield: 89%; mp: 261–263 °C; IR (KBr, υ cm^{-1}); 3359(NH), 3100(CH), 2991(OH), 2831(OCH3), 1733(C=O), 1687(C=O), 1530(C=C), 684(C–S).^{1}H NMR (DMSO-*d*6): δ 12.10(s, 1H, COOH), 11.63(*s*, 1H, NH), 10.11(*s*, 1H, –N=CH), 7.84–7.82(*d*, *J* = 7.83 Hz, 2H), 7.69–7.67(*d*, *J* = 6.71 Hz, 2H), 7.60–7.50(*m*, 4H),7.41–7.37(*d*, *J* = 5.22 Hz, 2H), 3.43(*s*, 3H). ^{13}C NMR (DMSO-*d*6): δ 191.91, 170.40, 167.15, 157.46, 144.97, 138.29, 135.74, 129.52, 125.89, 125.31, 125.28, 125.19, 125.17, 125.14, 125.07, 124.92, 124.71, 124.57, 124.38, 124.17, 124.07, 55.36.MS: m/z 407.08 ($M^+ + 1$). Anal. calcd for $C_{22}H_{18}N_2O_4S$; C, 65.05; H, 4.48; N, 6.89; S,7.89. Found: C, 64.95; H, 4.34; N, 6.42; S, 7.68.

(6) 4-{[2-(2,4-Dimethyl-phenylsulfanyl)-benzoyl]-hydrazonomethyl}-benzoic acid (3f)

White solid;Yield: 81%; mp: 205–207 °C; IR (KBr, υ cm^{-1}); 3369(NH), 3122(CH), 3011(OH), 1724(C=O), 1681(C=O), 1516(C=C), 659(C–S).^{1}H NMR (DMSO-*d*6): *δ* 12.09(*s*, 1H, COOH), 11.61(*s*, 1H, NH), 10.12(*s*, 1H, -N=CH), 7.85–7.83(*d*, *J* = 6.22 Hz, 2H), 7.77–7.73(*d*, *J* = 5.19 Hz, 2H), 7.61–7.56(*m*, 5H), 7.42–7.39(*d*, *J* = 7.57 Hz, 2H), 2.46(*s*, 6H).^{13}C NMR (DMSO-*d*6): *δ* 191.29, 171.23, 165.41, 145.33, 137.43, 135.46, 130.45, 126.91, 125.62, 125.33, 125.30, 125.21, 125.11, 125.01, 124.73, 124.33, 124.24, 124.17, 124.09, 123.82, 123.71, 22.37, 21.13. MS: m/z 405.13 ($M^+ + 1$). Anal. calcd for $C_{23}H_{20}N_2O_3S$: C,68.32; H,4.95; N, 6.93; S,7.94. Found: C, 68.03; H, 4.64; N, 6.62; S, 7.78.

(7) 4-[(2-Cyclohexylsulfanyl-benzoyl)-hydrazonomethyl]-benzoic acid (3 g)

White solid; Yield: 67%; mp: 209–211 °C; IR (KBr, υ cm^{-1}): 3311(NH), 3113(CH), 2941(OH), 1733(C=O), 1684(C=O), 1525(C=C), 621(C–S). ^{1}H NMR (DMSO-*d*6): *δ* 11.98 (*s*, 1H,COOH), 11.41(*s*, 1H, NH), 10.04(*s*, 1H, -N=CH), 7.84–7.82(*d*, *J* = 7.20 Hz, 2H), 7.66–7.64(*d*, *J* = 5.28 Hz), 7.36–7.33(*d*, *J* = 5.43 Hz, 2H), 7.11–7.06(*m*, 2H), 3.33(*m*, 1H), 1.63–1.39(m, 10 H) ^{13}C NMR (DMSO-*d*6): *δ* 190.08, 170.03, 164.93, 144.42, 137.12, 135.37, 129.41, 125.61, 125.05, 124.39, 124.33, 124.23, 124.13, 124.09, 123.08, 43.15, 39.43, 39.42, 26.45, 25.19, 25.18. MS: m/z 383.33($M^+ + 1$). Anal. Calcd for $C_{21}H_{22}N_2O_3S$: C, 65.94; H, 5.81; N, 7.33; S, 8.38. Found: C, 65.83; H, 5.64; N, 7.12; S, 8.08.

(8) 4-{[4-(4-Chloro-phenylsulfanyl)-benzoyl]-hydrazonomethyl}-benzoic acid (3 h)

White solid; Yield: 84%; mp: 278–280 °C; IR (KBr, υ cm^{-1}): 3359(NH), 3101(CH), 2989(OH) 1733(C=O), 1697(C=O), 1518(C=C), 1069(C–Cl), 683(C–S). ^{1}H NMR (DMSO-*d*6): *δ* 12.13(*s*, 1H, COOH), 11.57(*s*, 1H, NH), 10.08(*s*, 1H, –N=CH), 7.93–7.91(*d*, *J* = 7.02 Hz, 2H), 7.72–7.70(*d*, *J* = 8.73 Hz, 2H), 7.58–7.54(*m*, 6H), 7.37–7.33(*d*, *J* = 6.88 Hz,2H).^{13}CNMR(DMSO-*d*6): *δ* 192.32, 171.46, 165.93, 145.72, 139.46, 138.40, 135.33, 129.92, 126.71, 125.60, 125.39, 125.33, 125.21, 125.11, 125.09, 124.48, 124.27, 124.11, 124.03, 123.81, 123.76. MS: m/z 412.94($M^+ + 2$). Anal. Calcd for $C_{21}H_{15}ClN_2O_3S$: C, 61.40; H, 3.69; N, 6.83; S, 7.80. Found: C, 61.31; H, 3.64; N, 6.60; S, 7.71.

(9) 4-{[4-(4-Fluoro-phenylsulfanyl)-benzoyl]-hydrazonomethyl}-benzoic acid (3i)

White solid; Yield: 93%; mp: 270–272 °C; IR(KBr, υ cm^{-1}): 3402(NH), 3049(CH),3023(OH),1741(C=O), 1701(C=O), 1529(C=C), 1148(C–F), 689(C–S).^{1}H NMR (DMSO-*d*6): *δ* 12.14 (*s*, 1H COOH), 11.63. (*s*, 1H, NH), 10.13(*s*, 1H, –N=CH), 7.88–7.85(*d*, *J* = 7.17 Hz, 2H), 7.73–7.71(*d*, *J*=7.06 Hz, 2H),7.59–7.54(*m*, 6H), 7.43–7.39(*d*, *J* = 8.07 Hz, 2H). ^{13}CNMR (DMSO- *d*6): *δ* 192.90, 171.74, 166.51, 145.73, 140.12, 138.40, 135.77, 130.31, 126.70, 125.58, 125.41, 125.30, 125.23, 125.11, 124.59, 124.44, 124.31, 124.25, 124.23, 124.19,

124.10. MS: m/z 395.14 ($M^+ + 1$). Anal. Calcd for $C_{21}H_{15}FN_2O_3S$: C, 63.94; H, 3.84; N, 6.83; S, 8.13. Found: C, 63.83; H, 3.74; N, 6.70; S, 8.01.

(10) 4-{[4-(4-Bromo-phenylsulfanyl)-benzoyl]-hydrazonomethyl}-benzoic acid (3j)

White solid; Yield: 82%; mp: 279–281 °C; IR (KBr, υ cm^{-1}): 3349 (NH), 3101(CH), 2999 (OH), 1719(C=O), 1682(C=O), 1522(C=C), 1051(C–Br), 661(C–S),.^{1}H NMR (DMSO-*d*6): δ 12.12(s,1H COOH), 11.62(*s*, 1H, NH), 10.09(*s*, 1H, –N=CH), 7.85–7.83(*d*, $J = 7.20$ Hz, 2H),7.73–7.70(*d*, $J = 8.03$ Hz, 2H), 7.60–7.54(*m*, 6H),7.47–7.42(*d*, $J = 6.91$ Hz,2H).^{13}CNMR(DMSO-*d*6): δ 192.21, 170.11, 165.41, 145.07, 138.40, 136.72, 135.12, 129.91, 125.91, 125.59, 125.41, 125.30, 125.26, 125.18, 125.15, 124.80, 124.61, 124.55, 124.30, 124.09, 124.01.MS: m/z 456.61 ($M^+ + 2$). Anal. calcd for $C_{21}H_{15}BrN_2O_3S$: C, 55.39, H, 3.32, N, 6.15, S, 7.08. Found: C, 55.27, H, 3.14, N, 6.12, S, 7.06.

(11) 4-[(4-p-Tolylsulfanyl-benzoyl)-hydrazonomethyl]-benzoic acid (3 k)

Off-white solid; Yield: 76%; mp: 256–258 °C; IR(KBr, υ cm^{-1}): 3341(NH), 3103(CH), 2943(OH), 1721. (C=O), 1681(C=O), 1527(C=C), 663(C–S). ^{1}H NMR (DMSO-*d*6): δ 12.10(*s*,1H,COOH), 11.58(*s*, 1H, NH), 10.12(*s*, 1H, –N=CH), 7.88–7.85(*d*, $J = 6.20$ Hz, 2H),7.67–7.65(*d*, $J = 5.01$ Hz, 2H), 7.61–7.56(*m*, 6H), 7.37–7.34(*d*, $J = 4.97$ Hz, 2H), 2.23(*s*, 3H). ^{13}C NMR (DMSO-*d*6): δ 192.19, 171.10, 166.41, 145.77, 138.40, 135.30, 129.94, 126.71, 125.69, 125.41, 125.33, 125.23, 125.11, 125.05, 124.93, 124.84, 124.71, 124.51, 124.33, 123.19, 123.10, 20.42. MS: m/z 391.02 ($M^+ + 1$). Anal. calcd for $C_{22}H_{18}N_2O_3S$: C, 67.68; H, 4.65; N,7.18; S, 8.21. Found: C, 67.51; H, 4.64; N, 7.02; S, 8.05.

(12) 4-[4-(4-Methoxy-phenylsulfanyl)-benzylidene-hydrazinocarbonyl]-benzoic acid (3l)

Off white solid; Yield: 88%; mp: 285–287 °C; IR (KBr, υ cm^{-1}); 3359(NH), 3107(CH), 2979(OH), 2833(OCH3),1733(C=O), 1687(C=O), 1533(C=C),684(C–S).^{1}HNMR(DMSO-*d*6): δ 12.10(*s*, 1H, COOH), 11.61 (*s*, 1H, NH), 10.15(*s*, 1H, –N=CH), 7.86–7.82(*d*, $J = 7.10$ Hz, 2H), 7.70–7.67(d, $J = 7.03$ Hz, 2H), 7.60–7.57(m, 6H), 7.41–7.38(*d*, $J = 6.73$ Hz, 2H), 3.44(*s*, 3H). ^{13}C NMR (DMSO-*d*6): δ 191.66, 170.40, 166.44, 157.34, 144.77, 138.44, 130.44, 125.61, 125.30, 125.22, 125.20, 125.18, 125.11, 125.05, 124.93, 124.80, 124.71, 124.51, 124.23, 124.09, 123.95, 55.48. MS: m/z 407.21 ($M^+ + 1$). Anal.calcd for $C_{22}H_{18}N_2O_4S$: C, 65.05: H, 4.48; N, 6.89; S, 7.90. Found: C, 65.00; H, 4.34; N, 6.42; S, 7.61.

(13) 4-{[4-(2,4-Dimethyl-phenylsulfanyl)-benzoyl]-hydrazonomethyl}-benzoic acid (3m)

White solid; Yield: 79%; mp: 259–261 °C; IR (KBr, υ cm^{-1}); 3311(NH), 3094(CH), 2981(OH), 1715(C=O). 1680(C=O), 1531(C=C), 653(C–S).^{1}H NMR (DMSO-*d*6):

δ 12.09(s, 1H, COOH), 11.61(*s*, 1H, NH), 10.11(*s*, 1H, -N=CH), 7.84–7.82(*d*, *J* = 6.77 Hz, 2H),7.79–7.77(*d*, *J* = 7.03 Hz, 2H), 7.61–7.56(*m*, 5H),7.51–7.47(*d*, *J* = 5.54 Hz, 2H), 2.43(*s*, 6H). ^{13}C NMR (DMSO-*d*6): δ 191.11, 171.16, 165.33, 145.75, 138.40, 130.35, 125.94, 125.71, 125.49, 125.33, 125.21, 125.20, 125.11, 125.05, 124.93, 124.74, 124.61, 124.41, 124.23, 124.09, 123.90, 21.62, 20.50. MS: m/z 405.31($M^+ + 1$). Anal. calcd for $C_{23}H_{20}N_2O_3S$: C,68.30; H, 4.95; N,6.92; S,7.94. Found: C, 68.17; H, 4.64; N, 6.62; S, 7.78.

(14) 4-[(4-Cyclohexylsulfanyl-benzoyl)-hydrazonomethyl]-benzoic acid (3n)

White solid; Yield: 63%; mp:252–254 °C; IR (KBr,υ cm^{-1}):3313(NH),3104(CH), 2953(OH), 1742(C=O),1681 (C=O), 1517(C=C), 648(C–S).^{1}H NMR (DMSO-*d*6): δ 12.01(*s*, 1H COOH), 11.44(*s*, 1H, NH), 10.02(*s*, 1H, –N=CH), 7.86–7.83(*d*, *J* = 4.26 Hz, 2H), 7.68–7.65(*d*, *J* = 5.73 Hz, 2H), 7.39–7.35(*d*, *J* = 5.88 Hz, 2H), 7.15–7.09(*m*, 2H), 3.41(*m*, 1H), 1.76–1.43(*m*, 10H).^{13}C NMR (DMSO-*d*6): δ 190.10, 170.06, 163.53, 145.72, 138.45, 135.33, 129.52, 125.71, 124.28, 124.21, 124.15, 124.01, 123.12, 123.01, 122.77, 44.14, 39.39, 39.40, 26.43, 25.20, 25.21. MS: m/z 383.43 ($M^+ + 1$). Anal. Calcd for $C_{21}H_{22}N_2O_3S$: C, 65.90; H, 5.80; N, 7.31; S, 8.40. Found: C, 65.67; H, 5.64; N, 7.23; S, 8.11.

Biological studies

Wistar strain of albino rats(normal and hyperglycemic; 150–190 g) were maintained under standard conditions, i.e., room temperature (25 ± 2 °C) and photoperiod of 12 h day/night cycles each day for 1 week before and during the experiment. The rats were allowed free access to tap water and pellet diet. Rats were divided into following five groups for evaluation of intraday, interday hypoglycemic activity, biochemical parameters and body weight measurement:

Group	Description
Group I	Normal control rats treated with vehicles (10% Tween 80 suspension)
Group II	Hyperglycemic control rats treated with vehicles (10% Tween 80 suspension)
Group III	Normal control rats treated with compound **3m** (**3m** in 10% Tween 80 suspension at a dose of 28 mg/kg)
Group IV	Hyperglycemic rats treated with rosiglitazone (Rosiglitazone in 10% Tween 80 suspension at a dose of 10 mg/kg)
Group V	Hyperglycemic rats treated with synthesized compounds (Synthesized compounds in 10% Tween 80 suspension at a dose of 28 mg/kg)

Intraday blood glucose level was measured at 0th, 1st, 3rd and 6th hrs duration after the administration of vehicle, standard drug (rosiglitazone) and test compounds (**3a**–**3n**). For evaluation of the effect of compound **3k** and **3m** on interday study of blood glucose, biochemical parameters (TC, TG, HDL, LDL, AP), the same rats were continued with the same dose of test compounds, standard drug and vehicle once daily for 3 sweek (21 days). Standard kit (Beacon Diagnostics, India) were used

to determined TC, TG, HDL, LDL and AP after period of 21 days. All the procedures were performed in accordance with guidelines of institutional animal ethics committee, CPCSEA, Government of India. All values were expressed as mean ± S.E.M. Statistical significance was estimated by analysis of variance (ANOVA).

Induction of hyperglycemia

Hyperglycemia was induced in overnight fasted rats by a single intraperitoneal injection of 65 mg/kg streptozotocin in citrate buffer (pH 4.5), 15 min after the intraperitoneal administration of 110 mg/kg.

of nicotinamide. Blood glucose level was checked after 72 h by one touch Accucheck glucometer. Rats with blood glucose levels greater than 250 mg/dl were considered hyperglycemic and were used for the study [21].

Intraday evaluation of hypoglycemic activity of benzoic acid derivatives in normal and STZ induce rats

After overnight fasted rats, intraday blood glucose level was measured at 0th, 1st, 3rd and 6th hrs duration after the administration of vehicle, standard drug (rosiglitazone) and test compounds (**3a**–**3n**) through gastric intubation using a force feeding needle. Blood samples were collected from the tail vein and blood glucose level was determined with one touch Accucheck glucometer.

Interday evaluation of hypoglycemic activity of benzoic acid derivatives in normal and STZ induce rats

The vehicle, standard drug and test compounds (**3k–3m**) were administered into the rats of the respective groups every day morning for 21 days by gastric intubation using force feeding needle. Blood samples were collected for the measurement of blood glucose from the tail vein at 1st day, 7th,14th and 21st day duration after the administration of vehicle, standard drug and test compounds (**3k** and **3m**) and blood glucose levels were determined with basic one touch Accucheck glucometer.

Effects of compound 3k and 3m on biochemical parameters of normal and STZ induce rats at 21 days

Biochemical parameters such as TC, TG, HDL, LDL and AP were also estimated on overnight fasted rats at the end of 21st day by using Biochemistry Analyzer Chem-7(Erba Mannhelm) [22–25].

Effects of compound 3k and 3m on body weight of normal and STZ induce rats at 21 days

At the beginning (1st day) and at the end of study (21st day), overnight fasted rats were subjected to weight measurement).

Gastro-ulcerogenic toxicity studies

Albino rats of wistar strain (150–200gm) of both sexes were divided into different groups, control, test and standard (containing six animals each).The test group, control group and standard group were received test drug, vehicle and standard drug respectively. The test compounds and standard compound were suspended in 10% tween-20 and administered orally to each animal by using gastric gavage needle. The control group animals, however received the same volume of vehicle. In this study,the animals were administered a 28 mg/kg (body weight) dose of the test drugs and 10 mg/kg (body weight) dose of standard drug (indomethacin) [26].

Results and Discussion

Molecular docking

Figure 2 illustrates the best docking pose of the most active compound **3m** with amino acid residues of PPARγ. The compound **3m** showed hydrogen bond interaction with Tyr473, Ser289, His323, and Cys285. This hydrogen bonding pattern is conserved in most PPARγ agonist complex structures and essential for the activity of the compound [14, 27]. The crystal form of 2PRG [19] consists 2 molecules in the asymmetric unit, denoted A and B. Chain A was chosen for the PPARγ agonist docking study. The redocking results with chain A gave most reliable docked conformation for the synthesized compound- protein interactions. This is reason behind selection of chain A over chain B. The crystal structure of the 2PRG complex was re-docked for validation.

Chemistry of synthesized compounds

A simple and convenient synthetic route has been developed for synthesis of designed compounds [28–31]. In the first step, the starting material was esterified in strongly acidic conditions and obtained intermediates (**IMI**) were subjected to thioetherification (**IM2**). Thioetherification follows an addition–elimination two-step reaction; in the first step, aryl/cyclohexylsulfanyl as nucleophile attacks on aryl ring bearing chloride leaving group. In the second step, elimination of the chloride leads to generation of thioethers. Here, the utility of cesium fluoride-celite (CsF-Celite) was to activation of the aryl halide groups by the lewis acid type effect. In next step **IM2** were subjected to ammonolysis to get hydrazides (**IM3**) which on further reaction with absolute ethyl alcohol containing 4-formylbenzoic acid yielded desired compounds **3a**–**3n**. This reaction condition is essential for nucleophilic addition of the amino group to the carbonyl function of the aldehyde. The synthesized compounds (**3a–3n)** were confirmed by FTIR, ^{1}H NMR, ^{13}C NMR, Mass and Elemental analysis. The FT-IR spectrum of intermediates clearly indicated the desired substitution in the intermediates. The absence of OH group, SH

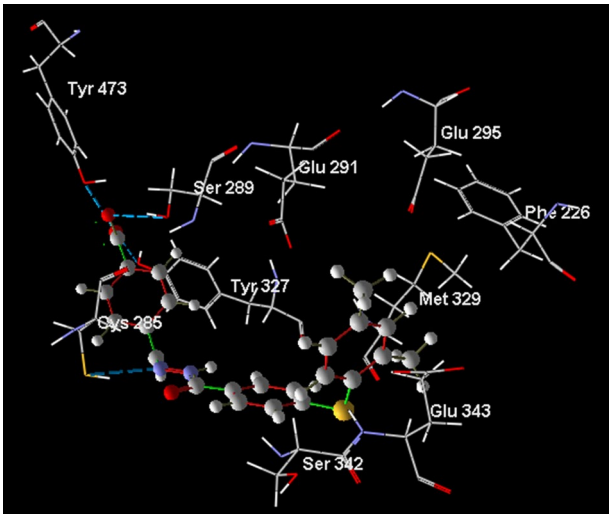
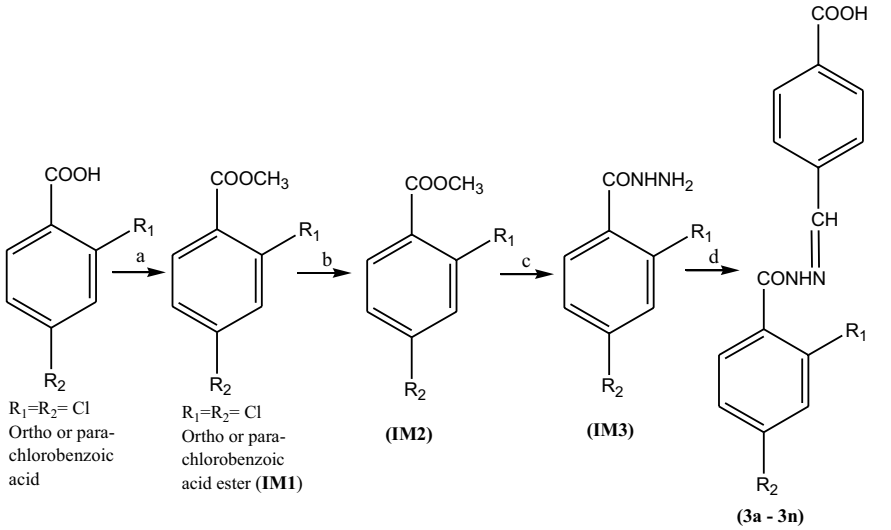


Fig. 2 The most active compound **3m** (ball and stick tube) is interacted with Phe226, Glu291, Glu295, Met329, Ser 342 and Glu343 of amino acid residues in the active binding sites of the receptor. H-bond interactions with key amino acids Tyr473, Ser289, His323 (not appeared) and Cys285 are shown in magenta dashed lines

group absorption and sharp decrease in C=O stretching of hydrazide in the FT-IR spectra of the **IM1**, **IM2** and **IM3** respectively confirmed the reactions of esterification, thioetherification and ammonolysis (Scheme 1).

The FT-IR spectra of synthesized compounds **3a**–**3n** showed absorption bands around 3023–2943 cm^{-1} for OH (COOH), 3402–3311 cm^{-1} for NH hydrazone, while the distinguished absorption peaks C=O for acid was observed in the range 1758–1715 cm^{-1}, C=O for hydrazone was observed in the range 1701–1680 $cm^{-1}$and C–S for the thioether was observed in the range of 691–621 cm^{-1}.^{1}H NMR spectrum revealed the lack of –SH signal and the presence of characteristic singlets around δ 12.14–11.98 for carboxylic acid protons, around δ 11.65–11.41 for the NH protons of hydrazone while condensation of p-formyl benzoic acid with various substituted aryl hydrazides was confirmed by downfield region of δ 10.15–10.02 observed as singlet for=CH group at phenyl ring. In addition, the ^{1}H NMR spectra of compounds **3a**–**3n** showed aromatic group protons as three doublets around δ 7.93–7.82, 7.77–7.63 and 7.51–7.33. On the other hand, the spectrum of **3d, 3f, 3k** and **3m** exhibited singlets for CH_3 groups at δ 2.23, 2.43, 2.22 and 2.46 respectively. The ^{1}H NMR spectroscopic data of compound **3e** and **3 l** showed the presence of singlet at δ 3.44 and 3.43 respectively for three protons in the methoxy group. The compound **3g** and **3n** showed the presence of multiplets around δ 7.15–1.39 for aromatic and aliphatic protons. Moreover, compounds **3a**–**3f** and **3h**–**3 m** showed the presence of a multiplets around δ 7.61–7.50 for aromatic protons. ^{13}C NMR spectra, which were in conformity with the assigned structures, displayed most characteristic signals appearing at around δ 192.96–190.10 ppm for carbonyl carbon peak, δ 171.74–170.03 ppm for carboxylic acid carbon peak, δ



(1) IM2, IM3 and 3a **:** R_1= 4-Cl-C_6H_4S- , R_2 = H

(2) IM2, IM3 and 3b **:** R_1= 4-F-C_6H_4S- , R_2 = H

(3) IM2, IM3 and 3c **:** R_1= 4-Br-C_6H_4S- , R_2 = H

(4) IM2, IM3 and 3d **:** R_1= 4-CH_3-C_6H_4S- , R_2 = H

(5) IM2, IM3 and 3e **:** R_1= 4-OCH_3-C_6H_4S-, R_2 = H

(6) IM2, IM3 and 3f **:** R_1= 2,4-$(CH_3)_2$-C_6H_3S-, R_2 = H

(7) IM2, IM3 and 3g **:** R_1= $C_6H_{11}S$- , R_2 = H

(8) IM2, IM3 and 3h **:** R_1 = H, R_2 = 4-Cl-C_6H_4S-

(9) IM2, IM3 and 3i **:** R_1 = H, R_2 = 4-F-C_6H_4S-

(10) IM2, IM3 and 3j **:** R_1 = H, R_2 = 4-Br-C_6H_4S-

(11) IM2, IM3 and 3k **:** R_1 = H, R_2 = 4-CH_3-C_6H_4S-

(12) IM2, IM3 and 3l **:** R_1 = H, R_2 = 4-OCH_3-C_6H_4S-

(13) IM2, IM3 and 3m **:** R_1 = H, R_2 = 2,4-$(CH_3)_2$-C_6H_3S-

(14) IM2, IM3 and 3n **:** R_1 = H, R_2 = $C_6H_{11}S$- ;

Scheme 1 Reaction pathways, Reagents and Conditions: **a** Esterification; absolute Methanol, conc. H_2SO_4, Reflux, 4–5 h; **b** Thioetherification; substituted aryl/cyclohexylthiols, CsF-Celite, CH_3CN, Reflux, 3–18 h; **c** Ammonolysis; NH_2NH_2(90%), Reflux, 45 min.; **d** Condensation; 4-Formylbenzoic acid, absolute ethanol, Reflux, 40 min.

167.12–163.53 ppm for C=N peak, aromatic carbons around δ 145.77–122.70 ppm and aliphatic carbon around δ 78.67–25.18 ppm. Two carbons of the aromatic ring (**3e** and **3l**) displayed chemical shifts at δ 157.34 and 157.46 due to carbon attached with methoxy group. Mass spectra of all the newly synthesized compounds exhibited a prominent molecular ion peak and isotopic peaks with different intensities. All the newly synthesized compounds were also characterized by elemental chemical analysis and gave satisfactory experimental values and acceptable error range ($\pm$0.4%) compared to calculated values.

PPARγ in vitro activity and structure–activity relationships

The synthesized compounds were screened on full length PPAR receptor transfected in HepG2 cells (Table 1) following the procedure described in our earlier

publication [32]. Two compounds **3 k** (80.21%) and **3 m** (94.60%) showed significant PPARγ transactivation activity as fold activation and rest of the compounds were exhibited moderate to weak activity in the range of 67.26–17.26% (Table 1). The effect of substituents at R_1 and R_2 position of the compound on hPPARγ transactivation activity was determined. Dimethyl substitution (**3m**) provides more hPPARγ transactivation activity as compare to monomethyl substitution (**3k**) at para position of the phenyl ring(Hydrophobic tail part). Potency of the compounds also indicates that para phenylsufanyl substituents are more effective than ortho phenylsufanyl substituents. The potency for PPARγ agonistic activity was significantly increased by the introduction of electron donating groups at the phenyl ring of the tail part of the compounds. The compounds (**3g**, **3n**) having unsubstituted cyclohexyl moiety in place of substituted phenyl ring were least active. These findings may be due to, the bulky moieties containing tail part comprise at least one substituted phenyl group capable of vander waals interaction to the receptor surface and one substituted phenylsulfanyl moiety for hydrophobic bonding interactions. Moreover, it has been hypothesized that two phenyl groups as bulky moieties exhibited more potency than one phenyl plus a cyclohexyl ring. This would imply that two flat- surfaced benzene rings are better than one and their combined vander walls forces and hydrophobic interactions important for increased biological potency.

Table 1 In vitro PPARγ agonistic activity as hPPARγ transactivation activities#

Compound	In vitro activation(Mean ± SEM)*
3a	0.81 ± 0.51 (29.13%)
3b	0.99 ± 0.22 (35.61%)
3c	1.45 ± 0.21 (52.15%)
3d	1.54 ± 0.40 (55.39%)
3e	1.00 ± 0.42 (35.95%)
3f	1.87 ± 0.42 (67.26%)
3g	0.48 ± 0.41 (17.26%)
3h	0.90 ± 0.51 (32.37%)
3i	0.76 ± 0.35 (27.33%)
3j	1.23 ± 0.18 (44.24%)
3k	2.23 ± 0.38 (80.21%)
3l	1.13 ± 0.18 (40.64%)
3m	2.63 ± 0.50 (94.60%)
3n	0.62 ± 0.41 (22.30%)
Rosiglitazone	2.78 ± 0.20 (100.00%)

*A mean from three determinations at concentration 0.02 μM. #Activities are presented as fold induction of hPPARγ activation. EC_{50} of the compounds were not determined. Fold activation relative to maximum activation obtained with rosiglitazone (100%)

In vivo activity

In vivo studies were carried in wistar strain of albino rats. Streptozotocin (STZ) was used for induction of hyperglycemia in rats. STZ is well known for its selective pancreatic β-cell cytotoxicity and has been extensively used to induce diabetes mellitus in animals and allows a consistent production of hyperglycemia as well as hyperlipidemia as diabetic like symptoms within a short period of time [33–35].

The intraday fasting blood glucose level at 6th hour for group I, II, III and IV were found 92.66, 303.50, 79.83 and 110.00 mg/dl respectively. While the synthesized benzoic acid derivatives (**3a**–**3n**) has exhibited intraday fasting blood glucose level at 6th hour in the range of 94.65 mg/dl to 137.50 mg/dl (Table 2). Thus, all the synthesized compounds showed significant reduction in fasting blood glucose level in comparison to diabetic control (group II).

The interday fasting blood glucose level at 21st day of group I, II, III and IV were found 86.66, 337.65, 78.16 and 104.16 mg/dl respectively. The interday fasting blood glucose level at 21st day of **3k** and **3m** were found 84.33 and 106.33 mg/dl respectively. The fasting blood glucose levels of interday study showed consistent blood glucose reduction at 1st, 7th, 14th, 21st day (Fig. 3). Treatment with benzoic acid derivatives in normal rats for 21 days did not produce hypoglycemic conditions. In the diabetic untreated rats (group II) the glucose levels remained higher without much change in the whole experimental period.

After 21 days treatment with compound **3k** and **3m** biochemical parameters such as serum total cholesterol (TC), triglycerides (TG), high density lipoprotein (HDL), low density lipoprotein (LDL) and alkaline phosphatase (AP) were also determined. It was found that the levels of HDL were significantly increased and TC, TG and LDL levels were significantly decreased compared to diabetic untreated rats (Fig. 4). It is interesting to observe in the present study that treatment with benzoic acid derivatives have not only lowered the TG, TC and LDL levels but also enhanced the cardio protective lipid HDL after 21st day treatment. AP which is a hepatic toxicity marker was found higher in diabetic rats treated with standard drug and untreated diabetic control group compared to diabetic rats treated with compound **3k** and **3m**. Thus, benzoic acid derivatives might have protective effect against liver toxicity caused by STZ.

Further, at the end of 21st day treatment, the body weights of the overnight fasted experimental rats were also determined. It was found that, compound treated rats showed less weight gain compare to rosiglitazone treated rats, whereas the body weights of untreated rats (control diabetic) decreased significantly. Untreated rats have shown marked reduction in their body weights compared to normal rats, which could be due to their uncontrolled diabetes (Fig. 5).

At the end of study (21st day), compound **3k** and **3m** treated rat were sacrificed and the stomachs and intestines were removed. A longitudinal incision along the lesser curvature is made. The stomach and intestine of animals was rinsed in running water and the presence and absence of ulcers were determined in control group, test group and standard group and data reported in Table 3.

Table 2 Effects of test compounds on intraday fasting blood glucose levels (mg/dl) of normal and STZ induce rats

Groups/compounds	0 h	1 h	3 h	6 h
Group I	89.50 ± 3.76	89.16 ± 3.49	90.66 ± 3.43	92.66 ± 3.15
Group II	309.30 ± 16.65	307.00 ± 15.10	305.50 ± 16.84	303.50 ± 17.75
Group III	81.66 ± 2.41	76.66 ± 2.96	80.40 ± 3.76	79.83 ± 3.68
Group IV	307.16 ± 16.52	252.16 ± 4.82	130.66 ± 6.19	110.00 ± 1.82
3a	293.17 ± 13.28[b]	189.00 ± 17.76a	121.83 ± 0.83[a]	119.86 ± 1.02[a]
3b	311.52 ± 12.60[c]	164.50 ± 20.31[a]	114.33 ± 4.07[a]	112.66 ± 2.37[a]
3c	287.66 ± 12.43[b]	205.65 ± 14.56[a]	120.83 ± 0.30[a]	120.33 ± 0.33[a]
3d	311.66 ± 18.15[c]	199.83 ± 16.40[a]	118.50 ± 0.93[a]	116.16 ± 0.47[a]
3e	303.34 ± 14.05[c]	163.00 ± 6.44[a]	132.34 ± 2.06[a]	119.46 ± 2.65[a]
3f	295.56 ± 20.55[b]	193.16 ± 7.82[a]	117.16 ± 0.98[a]	115.83 ± 1.06[a]
3g	296.00 ± 15.28[b]	175.85 ± 18.23[a]	130.16 ± 3.62[a]	118.16 ± 2.27[a]
3h	312.00 ± 12.80[c]	248.50 ± 13.05[a]	133.60 ± 3.7[a]	117.03 ± 6.30[a]
3i	286.66 ± 11.60[b]	205.67 ± 22.82[a]	149.66 ± 5.81[a]	137.50 ± 4.93[a]
3j	296.83 ± 9.33[b]	194.43 ± 7.71[a]	102.66 ± 3.79[a]	94.65 ± 5.46[a]
3k	307.06 ± 9.96[c]	206.16 ± 7.03[a]	102.00 ± 5.93[a]	96.83 ± 4.62[a]
3l	309.83 ± 8.03[c]	220.03 ± 25.15[a]	149.66 ± 5.81[a]	127.50 ± 4.23[a]
3m	298.83 ± 12.63[b]	182.66 ± 7.00[a]	112.33 ± 3.20 [a]	109.33 ± 3.01[a]
3n	288.83 ± 19.96[b]	180.66 ± 10.96[a]	150.50 ± 15.96 [a]	132.13 ± 2.16 [a]

[a]$P < 0.001$ indicates statistically more significant when compared with Group II. [b]$P < 0.05$ indicates statistically significant when compared with Group II. [c]$P > 0.05$ not significant compared with Group II. Each value in table is represented as (mean ± SD)

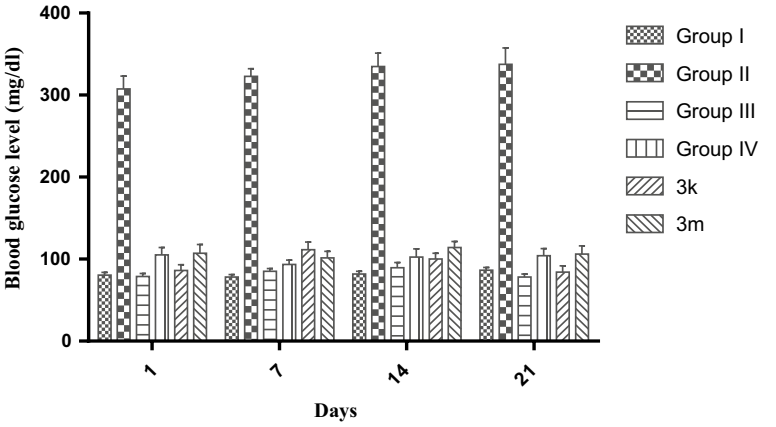


Fig. 3 Effect of the most active compounds on interday fasting blood glucose levels (mg/dl) of normal and STZ induce rats

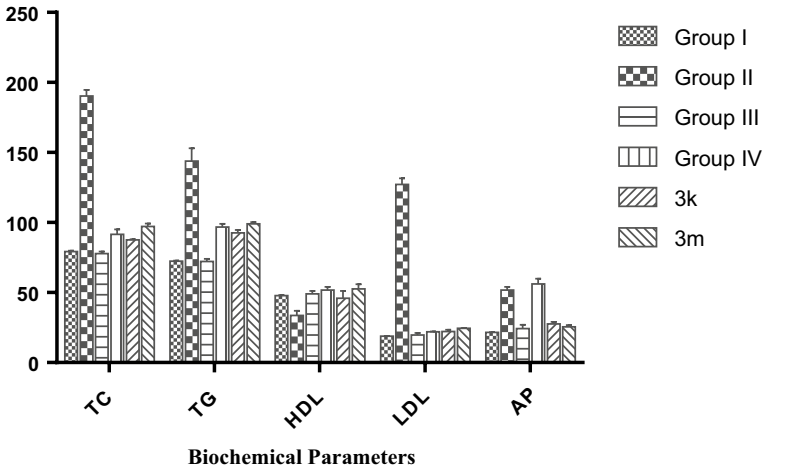


Fig. 4 Effect of the most active compounds on biochemical parameters of normal and STZ induce rats after 21 days of treatmentChange in body weight of normal and STZ induce rats at day 1 and day 21

Conclusion

Here, we described the design, syntheses and evaluation of novel benzoic acid derivatives as PPARγ agonists. In this investigation, the phenyl variants were utilized aiming to enhance the hydrophobicity and the effects of substituents on the phenyl ring at tail group examined successfully. Among these compounds, compound **3k** and **3m** have displayed marked in vitro PPARγ agonist and in vivo hypoglycemic activity in comparison to rosiglitazone. Further the values of change in body weight by compound **3k** and **3m** in comparison to rosiglitazone

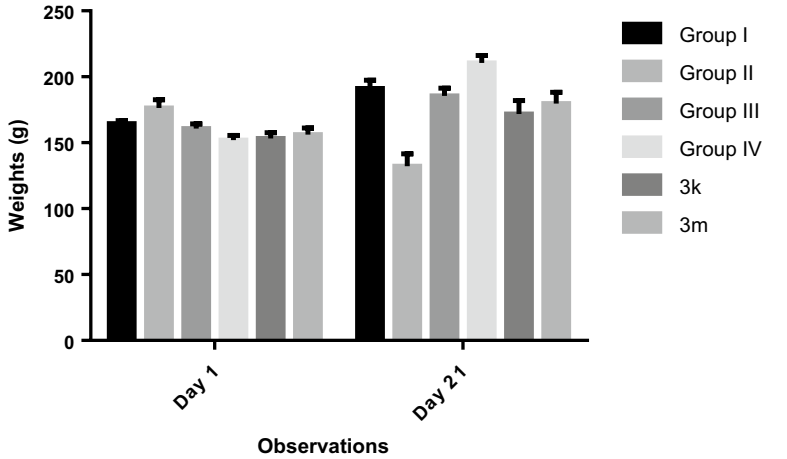


Fig. 5 Change in body weight of normal and STZ induce rats at day 1 and day 21

Table 3 Gastro-ulcerogenic toxicity of the most active compounds in rats

Compounds	Ulcer score	Ulceration (%)
Vehicle	0/6	0.0
3k	0/6	0.0
3m	0/6	0.0
Indomethacin	6/6	100

Bold values provides us information whether synthesized compounds are causing gastric ulcer or not

exhibited that the compounds might be devoid of weight gain, the common adverse effect of TZDs.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11164-021-04555-y.

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Declarations

Conflicts of interest Authors declares no conflict of interest.

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