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Design, synthesis, biological evaluation and molecular docking studies of novel 3-substituted-5-[(indol-3-yl) methylene]-thiazolidine-2,4-dione derivatives

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Abstract

Various thiazolidine-2,4-dione derivatives **3a-l** possessing indole moiety were designed, synthesized using appropriate conventional heating as well as microwave irradiation methods. All the synthesized compounds were characterized physically and spectrally. The compounds were evaluated for *in vitro* antibacterial activity, *in vitro* antioxidant activity and *in vivo* hypoglycemic activity in relation to the standard drugs. Most of the new compounds exhibited moderate activity and some showed considerable activity. Molecular docking studies were carried out using AutoDock software and revealed that compound **3b** has significant binding interaction with PPARγ receptor compared with the standard ligand Rosiglitazone.

Keyword: Pharmaceutical chemistry

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1. Introduction

Diabetes mellitus is one of the life threatening causes found in the majority of the countries in the world due to impaired carbohydrate, protein and lipid metabolism. Thiazolidinediones (TZDs) are a novel class of hypoglycemic agents for the management of NIDDM (Non-insulin dependent diabetes mellitus); initially they were identified as antidiabetic drugs known to sensitize tissues to insulin. A deficient insulin discharge which translates into impaired glucose use is a characteristic feature of diabetes mellitus and results in hyperglycemia [1].

TZDs normalize elevated glucose levels in blood and therefore are used in the controlling type 2 diabetes. TZDs have high affinity towards Peroxisomal Proliferator Activated Receptor gamma type (PPARγ) receptors and act as insulin sensitizers at PPARγ receptors. Moreover, they stimulate peripheral adiposity increasing the free fatty acids uptake, which leads to decline the fat stored in muscles, liver and deposits of visceral fat. TZDs improve insulin sensitivity in liver, muscle and fat tissues and thus counteract insulin resistance. Ciglitazone is the first synthesized thiazolidinedione derivative having antihyperglycemic activity in the insulin resistant animal models, but it was withdrawn because of low potency and appearance of cataracts, anemia and oedema in animals. Troglitazone was failed to survive due to liver toxicity. Pioglitazone and Rosiglitazone are currently in clinical use (Fig. 1). These are also having drawbacks like hepatotoxicity [2], oedema, haematological toxicity and body weight gain problems [3].

Recent studies with various thiazolidinedione derivatives were developed as they possess a wide variety of biological activities such as antimicrobial activity [4, 5], antihyperglycemic activity [6, 7], anti-inflammatory [8, 9], hypolipepidemic [10, 11], *in vitro* aldose reductase inhibitory activity [12], protein tyrosine phosphatase 1B inhibitory activity [13], 15-hydroxy prostaglandin dehydrogenase inhibitors [14], activators of PPARγ receptors [15], cytotoxic activity on different cell lines [16], antitubercular activity [17], antioxidant activity [18].

Nowadays microwave irradiated reaction techniques are broadly used in the development of organic compounds with or without presence of solvents because of the

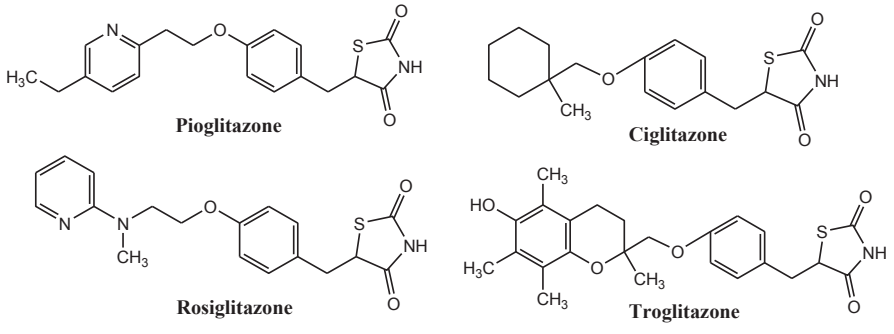

Fig. 1. Structures of Pioglitazone, Rosiglitazone, Ciglitazone and Troglitazone.

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simplicity in reaction handling, the eco-friendly nature and high yields [19]. Microwave irradiation method can quickly increase the temperature, uniform heat transfer into the reaction, improve the yield and reduce the formation of by-products or the decomposition of products, in comparison to the conventional synthetic reactions [20, 21]. These considerations led us to develop novel bioactive TZDs substituted at 3rd position and 5th position using both conventional heating and microwave irradiated methods. Synthesized compounds were screened for antibacterial, antioxidant, antidiabetic activities and molecular docking studies were carried out on designed ligands to observe better efficacy property and binding interaction at the target site.

2. Materials and methods

2.1. Chemicals and instruments

All the chemicals (reagents and solvents) were purchased from commercial suppliers (Merck grade) and they were used further without purification. Raga's scientific microwave synthesis system (RGSSIRR model) with different power levels from 140 W to 700 W was used for microwave irradiation. Melting points were determined by using electrical melting point apparatus and were uncorrected. Progress and completion of the reaction was monitored by using commercially available pre-coated TLC plates (E. Merck 0.25 mm silica gel 60GF-254), spots were visualized by exposing the dry plates under UV-light and in iodine vapours. IR spectra were recorded (λ_{max} in cm^{-1}) on Bruker analyzer FT-IR spectrophotometer using KBr pressed pellet technique. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on Bruker AMX-400 MHz spectrometer (chemical shifts in δ, ppm) in DMSO-d_6 solvent using internal standard TMS. The mass spectra of the compounds were recorded on Agilent LC-MSD. Elemental analysis for C, H and N was carried out using elemental analyzer.

2.2. Chemistry

2.2.1. Synthesis of 1,3-thiazolidine-2,4-dione (1)

Conventional heating method: A solution of chloroacetic acid (1.89 g, 20 mmol) in water (5 mL) was added into a stirring solution of thiourea (1.52 g, 20 mmol) in a three-necked round bottom flask. The reaction mixture was stirred until white precipitate was formed. Concentrated solution of HCl (6 mL) was added dropwise slowly into the reaction mixture by a fitted dropping funnel. A reflux condenser is connected in the middle of the flask. The reaction mixture was heated at 100−110 °C for 10−12 hrs and then cooled down to room temperature. The resulting suspension was filtered off and the precipitate was well washed with water to remove the traces of HCl. The product was further purified by recrystallization from ethanol.

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Microwave irradiation method: A mixture of chloroacetic acid (0.95 g, 10 mmol) and thiourea (0.76 g, 10 mmol) in water (3 mL) was added into Raga's scientific microwave synthesis reaction vessel. The vessel was sealed and the reaction mixture was stirred for about 1 hr at room temperature under ventilation. Conc. HCl (3 mL) was added into the reaction mixture, which was, then, irradiated by 280W power at 120 °C for 6 min. The reaction mixture was cooled to room temperature and the resulting solid was filtered off, well washed with water, dried and recrystallized from ethanol.

Yield 78.42% (conventional synthesis), 90.25% (MWI synthesis), white crystalline powder, mp 124−126 °C, R_f value is 0.61 from TLC of chloroform and methanol (9:1). IR [KBr ν cm^{-1}]: 3321.46 (−NH−), 1718.95 (C═O), 1776.94 (C═O), 1303.29 (C−N), 2968.89 (C−H), 626.69 (C−S). ^{1}H-NMR [400 MHz, δ, ppm, DMSO-d_6]: 12.01 (1H, s, NH), 4.13 (2H, s, CH_2). ^{13}C-NMR [400 MHz, δ, ppm, DMSO-d_6]: 173.8, 173.0, 35.8. ESI-MS: m/z (M^+) 117.

*2.2.2. Synthesis of 5-[(indol-3-yl)methylene]-thiazolidine-2,4-dione (**2**)*

Conventional heating method: Thiazolidine-2,4-dione (1.17 g, 0.01 mol) ***1*** was added in a solution of indole-3-carboxaldehyde (1.45 g, 0.01 mol) in toluene (8 mL). Catalytic amount of piperidine (0.4 mL) was added to reaction mixture and the resulting mixture was refluxed for about 5−6 hrs at 110−120 °C using an oil bath. Upon completion of the reaction, monitored by TLC, the reaction mixture was allowed to cool to room temperature. 1M HCl and cold water was added to reaction mixture. The resulting solid was filtered and washed with cold water and dry toluene, then dried and further purified by recrystallization from ethanol.

Microwave irradiation method: Piperidine (0.4 mL) was added to a solution of thiazolidine-2,4-dione (1.17 g, 0.01 mol) and indole-3-carboxaldehyde (1.45 g, 0.01 mol) in toluene (8 mL) The reaction mixture was placed in Raga's scientific microwave synthesis reaction vessel, which was connected with a water condenser. The reaction mixture was irradiated at 350W for about 8 min at 120 °C. After completion of the reaction, the mixture was cooled and diluted with ice-water (15 mL), filtered, washed with cold water and dry toluene. The product was further purified by recrystallization from ethanol.

Yield 70.48% (conventional synthesis), 84.94% (MWI synthesis), yellow powder, mp186-188 °C, R_f value is 0.58 from TLC of benzene and ethyl acetate (8:2), IR [KBr ν cm^{-1}]: 3320.08 (−NH−), 3288.49 (−NH−), 1687.44 (C═O), 1718.98 (C═O), 1352.89 (C−N), 3018.25 (C−H), 624.81 (C−S), 1687.44 (C═C). ^{1}H-NMR [400 MHz, δ, ppm, DMSO-d_6]: 12.31 (1H, s, TZD-N<u>H</u>-), 12.14 (1H, s, indole-N<u>H</u>-), 8.06 (1H, s, ═C<u>H</u>− methylene), 7.18−7.90 (5H, d & t, indole-H). ^{13}C-NMR [400 MHz, δ, ppm, DMSO-d_6]: 167.6, 167.2, 136.2, 128.5, 126.7, 124.4, 123.0, 121.0, 118.2, 116.2, 112.3, 110.4. ESI-MS: m/z (M^+) 244.

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2.2.3. General procedure for synthesis of 3-[(substituted phenylamino)methyl]-5-[(indol-3-yl)methylene]-thiazolidine-2,4-dione (3a-3l)

Conventional heating method: Formaldehyde (0.01 mol) was added in a solution of 5-[(indol-3-yl)methylene]-thiazolidine-2,4-dione **(2)** (0.005 mol) in DMF. The reaction mixture was stirred at room temperature for about 30 min. The solution of aryl amine (0.005 mol) in DMF was added to the above reaction mixture and then catalytic amount of conc. HCl (3−5 drops) was added. The reaction mixture was refluxed for 10−14 h at 120 °C and then, cooled at 2−8 °C for about 24 hrs. The reaction mixture was poured into crushed ice and the resulting solid was filtered, washed with cold water and dry toluene. The product was dried and recrystallized from ethanol. Completion of the reaction was monitored by TLC using an eluent, a mixture of solvents, *n*-hexane:ethylacetate (9:1).

Microwave irradiation method: To a solution of 5-[(indol-3-yl)methylene]-thiazolidine-2,4-dione **(2)** (0.005 mol) in of DMF (3 mL), formaldehyde (0.01 mol) was added and the reaction mixture stirred for about 20−30 min at room temperature. The solution of aryl amine (0.005 mol) in DMF and catalytic amount of conc. HCl (3−5 drops) were added to the above reaction mixture. The resulting mixture was placed in Raga's scientific microwave synthesis reaction vessel and was irradiated at 420W for about 8−12 min at 120 °C. The reaction mixture was cooled and diluted with ice cold water and the resulting solid was filtered, washed with cold water and dry toluene. The product was further purified by recrystallization from boiling ethanol.

2.2.3.1. 3-(phenylaminomethyl)-5-[(indol-3-yl)methylene]-thiazolidine-2,4-dione (3a)

IR [KBr ν cm^{-1}]: 3394.73 (−NH−), 3222.36 (−NH−), 1720.82 (C═O), 1757.43 (C═O), 1353.93 (C−N), 2921.84 (C−H), 3115.36 (═C-H), 616.05 (C−S), 1672.14 (C═C). ^{1}H-NMR [400 MHz, δ, ppm, DMSO-d_6]: 12.10 (1H, s, indole-N<u>H</u>-), 7.91 (1H, s, ═C<u>H</u>− methylene), 6.68−7.66 (10H, d & t, phenyl-H and indole-H), 4.88−4.91 (2H, d, −C<u>H</u>$_2$−NH−), 3.99−4.10 (1H, t, −CH$_2$−N<u>H</u>−). ESI-MS: m/z (M^+) 349.

2.2.3.2. 3-[(4-chlorophenyl)aminomethyl]-5-[(indol-3-yl)methylene]-thiazolidine-2,4-dione (3b)

IR [KBr ν cm^{-1}]: 3320.09 (−NH−), 3223.39 (−NH−), 1719.32 (C═O), 1777.35 (C═O), 1351.97 (C−N), 2971.21 (C−H), 3021.23 (═C−H), 612.51 (C−S), 1675.42 (C═C), 872.88 (C−Cl). ^{1}H-NMR [400 MHz, δ, ppm, DMSO-d_6]: 12.04 (1H, s, indole-N<u>H</u>-), 8.29 (1H, s, ═C<u>H</u>− methylene), 6.78−8.04 (9H, d & t, phenyl-H and indole-H), 4.81−4.83 (2H, d, −C<u>H</u>$_2$−NH−), 4.01−4.10 (1H, t,

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−CH_2−N<u>H</u>−). ^{13}C-NMR [400 MHz, δ, ppm, DMSO-d_6]: 167.5, 167.2, 149.7, 146.1, 138.2, 129.5, 128.5, 126.7, 1246.4, 123.0, 121.0, 118.2, 116.1, 112.3, 111.4, 110.4, 65.6. ESI-MS: m/z (M^+) 383.

*2.2.3.3. 3-[(2-chlorophenyl)aminomethyl]-5-[(indol-3-yl)methylene]-thiazolidine-2,4-dione (**3c**)*

IR [KBr ν cm^{-1}]: 3323.39 (−NH−), 3221.32 (−NH−), 1719.69 (C═O), 1764.35 (C═O), 1357.91 (C−N), 2911.25 (C−H), 3070.50 (═C−H), 623.11 (C−S), 1690.32 (C═C), 879.19 (C−Cl). ^{1}H-NMR [400 MHz, δ, ppm, DMSO-d_6]: 12.30 (1H, s, indole-N<u>H</u>-), 8.06 (1H, s, ═C<u>H</u>− methylene), 7.18−7.90 (9H, d & t, phenyl-H and indole-H), 4.29−4.33 (1H, t, −CH_2−N<u>H</u>−), 3.96−3.98 (2H, d, −C<u>H</u>$_2$−NH−).

*2.2.3.4. 3-[(4-fluorophenyl)aminomethyl]-5-[(indol-3-yl)methylene]-thiazolidine-2,4-dione (**3d**)*

IR [KBr ν cm^{-1}]: 3338.07 (−NH−), 3215.60 (−NH−), 1742.56 (C═O), 1723.14 (C═O), 1330.43 (C−N), 2974.02 (C−H), 3038.51 (═C−H), 635.12 (C−S), 1629.31 (C═C), 1237.07 (C−F). ^{1}H-NMR [400 MHz, δ, ppm, DMSO-d_6]: 12.31 (1H, s, indole-N<u>H</u>-), 8.06 (1H, s, ═C<u>H</u>− methylene), 7.18−7.90 (9H, d & t, phenyl-H and indole-H), 4.54−4.56 (2H, d, −C<u>H</u>$_2$−NH−), 4.06−4.10 (1H, t, −CH_2−N<u>H</u>−). ^{13}C-NMR [400 MHz, δ, ppm, DMSO-d_6]: 167.6, 167.2, 156.7, 144.2, 143.2, 136.2, 128.6, 126.7, 124.4, 123.0, 121.0, 118.3, 116.1, 112.3, 111.3, 110.4, 65.9. ESI-MS: m/z (M^+) 367.

*2.2.3.5. 3-[(4-bromophenyl)aminomethyl]-5-[(indol-3-yl)methylene]-thiazolidine-2,4-dione (**3e**)*

IR [KBr ν cm^{-1}]: 3375.76 (−NH−), 3238.86 (−NH−), 1754.58 (C═O), 1763.85 (C═O), 1354.35 (C−N), 2965.65 (C−H), 3075.45 (═C−H), 628.33 (C−S), 1659.35 (C═C), 526.56 (C−Br). ^{1}H-NMR [400 MHz, δ, ppm, DMSO-d_6]: 12.30 (1H, s, indole-N<u>H</u>-), 8.06 (1H, s, ═C<u>H</u>− methylene), 7.18−7.89 (9H, d & t, phenyl-H and indole-H), 5.14 (1H, s, −CH_2−N<u>H</u>−), 4.32−4.34 (2H, d, −C<u>H</u>$_2$−NH−).

*2.2.3.6. 3-[(3-nitrophenyl)aminomethyl]-5-[(indol-3-yl)methylene]-thiazolidine-2,4-dione (**3f**)*

IR [KBr ν cm^{-1}]: 3382.25 (−NH−), 3265.74 (−NH−), 1785.52 (C═O), 1747.45 (C═O), 1348.65 (C−N), 2987.64 (C−H), 3065.65 (═C−H), 632.21 (C−S), 1663.33 (C═C), 1533.26 & 1363.89 (−NO_2). ^{1}H-NMR [400 MHz, δ, ppm, DMSO-d_6]: 12.30 (1H, s, indole-N<u>H</u>-), 8.06 (1H, s, ═C<u>H</u>− methylene),

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7.18−7.90 (9H, s, d & t, phenyl-H and indole-H), 5.28 (1H, s, −CH_2−N<u>H</u>−), 4.10−4.12 (2H, d, −<u>CH_2</u>−NH−). ^{13}C-NMR [400 MHz, δ, ppm, DMSO-d_6]: 169.1, 167.2, 149.5, 144.3, 143.3, 135.2, 127.2, 126.0, 125.4, 123.9, 121.1, 117.3, 116.6, 114.3, 112.3, 111.0, 110.4, 108.5, 66.9. ESI-MS: m/z (M^+) 394.

*2.2.3.7. 3-[(4-nitrophenyl)aminomethyl]-5-[(indol-3-yl)methylene]-thiazolidine-2,4-dione (**3g**)*

IR [KBr ν cm^{-1}]: 3376.82 (−NH−), 3275.52 (−NH−), 1766.56 (C═O), 1753.45 (C═O), 1339.41 (C−N), 2967.56 (C−H), 3085.12 (═C−H), 628.52 (C−S), 1669.78 (C═C), 1539.45 & 1348.23 (−NO_2). ^{1}H-NMR [400 MHz, δ, ppm, DMSO-d_6]: 12.50 (1H, s, indole-N<u>H</u>-), 8.09 (1H, s, ═C<u>H</u>− methylene), 7.10−7.90 (9H, d & t, phenyl-H and indole-H), 5.32 (1H, s, −CH_2−N<u>H</u>−), 4.32−4.34 (2H, d, −<u>CH_2</u>−NH−).

*2.2.3.8. 3-[(2,4-dinitrophenyl)aminomethyl]-5-[(indol-3-yl)methylene]-thiazolidine-2,4-dione (**3h**)*

IR [KBr ν cm^{-1}]: 3365.46 (−NH−), 3256.42 (−NH−), 1774.22 (C═O), 1765.52 (C═O), 1328.78 (C−N), 2964.54 (C−H), 3104.28 (═C−H), 618.45 (C−S), 1668.65 (C═C), 1545.25 & 1339.56 (−NO_2). ^{1}H-NMR [400 MHz, δ, ppm, DMSO-d_6]: 11.78 (1H, s, indole-N<u>H</u>-), 8.29 (1H, s, ═C<u>H</u>− methylene), 6.78−8.04 (8H, s, d & t, phenyl-H and indole-H), 4.81−4.83 (2H, d, −<u>CH_2</u>−NH−), 4.01−4.10 (1H, t, −CH_2−N<u>H</u>−). ^{13}C-NMR [400 MHz, δ, ppm, DMSO-d_6]: 175.6, 166.2, 151.2, 144.8, 137.5, 136.8, 134.6, 130.7, 128.9, 127.6, 123.4, 121.9, 121.5, 119.9, 118.6, 115.6, 111.5, 110.5, 63.8. ESI-MS: m/z (M^+) 439.

*2.2.3.9. 3-[(3-methylphenyl)aminomethyl]-5-[(indol-3-yl)methylene]-thiazolidine-2,4-dione (**3i**)*

IR [KBr ν cm^{-1}]: 3368.85 (−NH−), 3248.45 (−ENH−), 1726.09 (C═O), 1765.60 (C═O), 1352.44 (C−N), 2965.50 (C−H), 3075.90 (═C−H), 622.46 (C−S), 1673.20 (C═C). ^{1}H-NMR [400 MHz, δ, ppm, DMSO-d_6]: 11.25 (1H, s, indole-N<u>H</u>-), 8.12 (1H, s, ═C<u>H</u>− methylene), 6.59−7.88 (9H, s, d & t, phenyl-H and indole-H), 4.26 (1H, s, −CH_2−N<u>H</u>−), 4.12−4.13 (2H, d, −<u>CH_2</u>−NH−), 2.21 (3H, s, phenyl-CH_3).

*2.2.3.10. 3-[(4-methylphenyl)aminomethyl]-5-[(indol-3-yl)methylene]-thiazolidine-2,4-dione (**3j**)*

IR [KBr ν cm^{-1}]: 3376.65 (−NH−), 3250.60 (−NH−), 1758.11 (C═O), 1743.55 (C═O), 1364.43 (C−N), 2959.05 (C−H), 3082.44 (═C−H), 620.42 (C−S), 1669.08 (C═C). ^{1}H-NMR [400 MHz, δ, ppm, DMSO-d_6]: 12.00 (1H, s, indole-

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NH-), 8.14 (1H, s, =CH− methylene), 6.89−7.99 (9H, d & t, phenyl-H and indole-H), 4.52 (1H, s, $-CH_2-NH-$), 4.10−4.11 (2H, d, $-CH_2-NH-$), 2.33 (3H, s, phenyl-CH_3). ^{13}C-NMR [400 MHz, δ, ppm, DMSO-d_6]: 175.1, 167.4, 145.2, 142.6, 137.4, 129.8, 129.4, 128.9, 127.6, 122.4, 121.8, 119.8, 118.9, 115.6, 111.2, 110.7, 65.8, 25.8. ESI-MS: m/z (M^+) 363.

*2.2.3.11. 3-[(4-methoxyphenyl)aminomethyl]-5-[(indol-3-yl)methylene]-thiazolidine-2,4-dione (**3k**)*

IR [KBr ν cm^{-1}]: 3356.46 (−NH−), 3255.21 (−NH−), 1732.45 (C=O), 1754.22 (C=O), 1348.64 (C−N), 2956.85 (C−H), 3063.74 (=C−H), 634.32 (C−S), 1126.38 (C−O−C). ^{1}H-NMR [400 MHz, δ, ppm, DMSO-d_6]: 12.03 (1H, s, indole-NH-), 8.15 (1H, s, =CH− methylene), 6.87−7.84 (9H, d & t, phenyl-H and indole-H), 4.42 (1H, s, $-CH_2-NH-$), 3.90−4.01 (2H, d, $-CH_2-NH$), 3.65 (3H, s, $-OCH_3$). ESI-MS: m/z (M^+) 379.

*2.2.3.12. 3-[(4-hydroxyphenyl)aminomethyl]-5-[(indol-3-yl)methylene]-thiazolidine-2,4-dione (**3l**)*

IR [KBr ν cm^{-1}]: 3582.84 (−OH), 3376.65 (−NH−), 3263.44 (−NH−), 1746.14 (C=O), 1753.13 (C=O), 1374.28 (C−N), 2965.08 (C−H), 3078.78 (=C−H), 624.33 (C−S), 1658.54 (C=C). ^{1}H-NMR [400 MHz, δ, ppm, DMSO-d_6]: 11.93 (1H, s, indole-NH-), 10.54 (1H, s, phenyl-OH), 8.02 (1H, s, =CH− methylene), 6.95−7.98 (9H, d & t, phenyl-H and indole-H), 4.35 (1H, s, $-CH_2-NH-$), 4.00−4.11 (2H, d, $-CH_2-NH-$). ^{13}C-NMR [400 MHz, δ, ppm, DMSO-d_6]: 173.5, 165.2, 147.9, 144.0, 141.7, 135.5, 130.8, 127.4, 122.8, 121.5, 120.4, 119.8, 117.4, 113.4, 112.4, 110.8, 64.3. ESI-MS: m/z (M^+) 365.

2.3. Biological evaluation

2.3.1. In vitro antibacterial activity

Minimum inhibitory concentrations (MIC) of the compounds were measured by two-fold serial dilution method [22, 23, 24] for screening the *in vitro* antibacterial activity against gram positive bacteria (*Staphylococcus aureus*: MTCC-1134, *Bacillus subtilis*: MTCC-1144) and gram negative bacteria (*Escherichia coli*: MTCC-1089, *Pseudomonas aeruginosa*: MTCC-424). Test compounds and reference standard Ampicillin were dissolved in DMSO at a concentration of 1280 μg/mL. Further dilutions were made using DMSO only, tested at a concentration of 640, 320, 160, 80, 40, 20 μg/mL and DMSO as a control. Drug solution was added to the each tube containing 5 mL sterilized nutrient broth medium. MIC tests were carried out in nutrient broth with inoculums of (1−2) × 10^6 Colony Forming Unit/ml (CFU/mL) bacterial strains. The test compounds and standard of nutrient broth serial

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tube dilutions inoculated with each bacterial strain were incubated at 37 ± 2 °C for 18−24 hrs.

2.3.2. In vitro antioxidant activity evaluation

DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay and hydrogen peroxide scavenging assay methods were carried out to evaluate the *in vitro* antioxidant activity.

2.3.2.1. DPPH free radical scavenging assay method

The use of DPPH free radical scavenging assay [25, 26] provides a simple and speedy way to estimate antioxidant property by spectrophotometer and it is useful to evaluate different compounds at a time. DPPH (2,2-diphenyl-1-picrylhydrazyl) is stable free radical. Methanolic solution of DPPH is used to estimate the antioxidant activity of numerous synthetic compounds. Interaction of antioxidant compound with DPPH, both transfer electron or hydrogen atom to DPPH, neutralizing its free radical nature and converted to 2,2-diphenyl-1-picrylhydrazine. The scavenging activity of the compound was indicated by degree of discoloration. At 517 nm, the change in absorbance was used to measure antioxidant activity. DPPH solution in methanol (0.002%) was prepared and 1.0 ml of this solution was added to 3.0 ml of the test solutions in DMSO at different concentrations (50, 100, 300 and 500 μg/mL). The mixture was shaken well and was incubated at 37 °C for 30 minutes; the absorbance was measured at 517 nm. A blank was prepared without adding test solution. Ascorbic acid in methanol at various concentrations (50, 100, 300 and 500 μg/mL) was used as standard. The experiment was repeated triplicate. The percentage inhibition capability of scavenging the DPPH radical was calculated using the following equation:

$$\%\ \text{DPPH Scavenged} = [(A_0 - A_1)/A_0] \times 100$$

Where A_0 is the absorbance of the control reaction (containing all reagents except the test solution) and A_1 is the absorbance of the test solution. Ascorbic acid was used as positive controls.

2.3.2.2. Hydrogen peroxide scavenging assay method

The ability of synthesized compounds to scavenge hydrogen peroxide was determined according to the literature procedures [27, 28, 29]. 40 mM concentration of hydrogen peroxide was prepared in phosphate buffer (pH 7.4). At 230 nm, the concentration of hydrogen peroxide was determined using a spectrophotometer. Different concentrations of the test compounds (50, 100, 300, and 500 μg/mL) in 3.4 ml of phosphate buffer were added to 0.6 ml of 40 mM hydrogen peroxide solution. The absorbance of reaction mixture was measured at 230 nm against a blank

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solution consisting of phosphate buffer without hydrogen peroxide. The experiment was repeated triplicate and the percentage scavenging of hydrogen peroxide by the test samples and standard compound was calculated as follows:

$$\% \text{ Scavenged } [H_2O_2] = [(A_0 - A_1)/A_0] \times 100$$

Where A_0 is the absorbance of the control reaction (containing all reagents except the test solution) and A_1 is the absorbance of the test solution. Ascorbic acid was used as positive control.

2.3.3. *In vivo* hypoglycemic activity evaluation

All the synthesized compounds were screened for *in vivo* hypoglycemic activity using Alloxan induced wister albino rats by tail tipping method [30, 31]. Wister albino rats of either sex having 160−200 g weight were taken for this study. Rats were purchased from Sainadh Agencies- Laboratory animal suppliers, Hyderabad. All the rats were acclimatized for one week to the laboratory conditions before commencing the experiments and fed with pellet and tap water *ad libitum*. At room temperature the animals were housed in the polypropylene cages for 12 hrs/12 hrs dark and light cycle. Acclimatized animals were kept fasting for 24 hrs with water *ad libitum* and Alloxan monohydrate was administered at120 mg/kg i.p. in normal saline. The animals were given *ad libitum* after one hour of Alloxan administration. To overcome the early hypoglycemic phase 5% dextrose solution was given in feeding bottle for a day. The blood glucose levels were monitored after alloxination by withdrawing a drop of blood from tail vein by Tail tipping method. The blood glucose levels as well as biochemical parameters were measured using digital Accu-Chek active digital glucose monitoring system and Robonik biochemical analyzer respectively.

After 72 hrs, the rats having blood glucose levels beyond 150 mg/dL were selected for the study and divided into six groups. The quantity of thiazolidine-2,4-dione derivatives equivalent to average human intake 200 mg/kg was calculated for single dose 36 mg/kg for acute study. The test compounds were administered orally by mixing with CMC-0.25% solution. Glibenclamide was administered as standard drug at 500 μg/kg body weight. All samples were administered at a dose of 35 mg/kg body weight for acute study. The blood samples were withdrawn and analyzed for blood glucose level at different time intervals 0 hr, 1 hr, 2 hr, 4 hr, 6 hr and 8 hr respectively. Based on the results of acute study few samples were selected for chronic study and they were administered at a dose of 35 and 70 mg/kg body weight. After 30 minutes of the administration of the dose the blood glucose level was measured and decrease in blood glucose was calculated on 7^{th} day and 15^{th} day. The effect of samples **3b, 3d, 3g** and **3h** on insulin, urea, creatinine, lipid profiles, HDL, LDL and VLDL levels in control and in Alloxan induced diabetic rats in serum or plasma were studied on Day 15.

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2.4. Molecular docking studies

The choice of protein for docking studies is based upon numerous factors such as structure should be determined by X-ray diffraction, it should have a resolution between 2.0 to 2.5A°, contain a co-crystallized ligand and the selected protein 3D structure should not have any protein breaks [32]. The co-crystal structure of the target receptor was obtained from the protein data bank (http://www.rcsb.org/pdb) PDB ID: 2PRG having resolution of 2.3A°. Then it was prepared for docking by removing all the heteroatoms, nonreceptor atoms, water and other ions, etc. Molecular docking was performed on the designed compounds **3a-l** as potential PPARγ agonists [33]. PPARγ receptor is the major target for some of the antidiabetic drugs consisting of thiazolidine-2,4-dione nucleus [34]. The docking procedure was applied on a set of designed ligands within the region of 2PRG active site using AutoDock 4.2.6 software. Based on the validations and hydrogen bond interactions of various substituents, they were considered for the evaluation. It was done to understand the kind of interactions that occurred between various substituted thiazolidine-2,4-diones with 2PRG binding site region. The active site was considered as a rigid molecule, while the ligands were treated as being flexible. Series of compounds **3a-l** were modeled by using ChemDraw Ultra 8. 0 software and converted into suitable 3D model, subjected to energy minimization using molecular mechanics. The energy minimized structures are required for molecular docking and for the preparation of corresponding pdb files. Docking studies were preformed on prepared ligands to predict the binding energy at the region of 2PRG active site to find out the possible locations for the ligand in active site region of the receptor. Using default parameters Grid based docking studies were carried out and docking was performed on all the designed compounds using standard ligand Rosiglitazone.

3. Results and discussion

3.1. Chemistry

Initially, thiazolidine-2,4-dione (**1**) was synthesized conventionally according to the literature procedure [35, 36]. Thiazolidine-2,4-dione **(1)** was condensed with indole-3-aldehyde to form 5-[(indol-3-yl)methylene]-thiazolidine-2,4-dione (**2**), under Knoevenagel reaction conditions [37]. Compound **2** was then coupled with formaldehyde and substituted aromatic amines under Mannich reaction conditions [38, 39] to afford the desired final derivatives **3a-l**, depicted in Fig. 2. The titled compounds were also prepared by microwave-assisted irradiation techniques according to the literature procedures [40, 41, 42, 43] with different power levels. All the compounds were characterized physically and most of the compounds were characterized spectrally. The physical characterization data, the comparative study of conventional and

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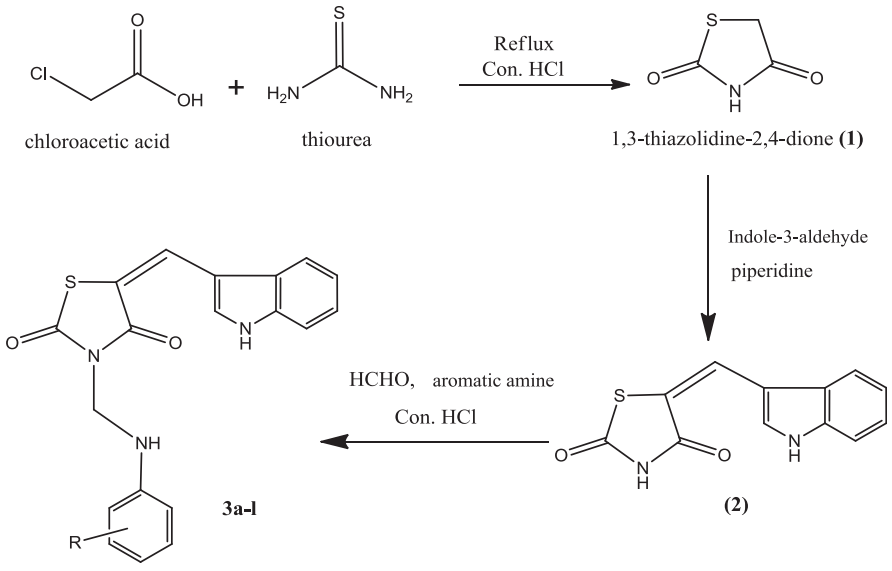

Fig. 2. Scheme of synthesis.

microwave irradiation methods with respect to their percentage yield and reaction time were given in Table 1.

3.2. Biological evaluation efficacy

3.2.1. In vitro antibacterial efficacy

All the synthesized compounds were evaluated for their *in vitro* antibacterial activity against the both gram positive and gram negative bacteria. The MIC of each test compound was recorded as the lowest concentration in the tubes with no growth i.e. no turbidity of inoculated bacterial strains. The MIC values were determined by using serial dilution technique in nutrient broth medium by observing the presence or absence of turbidity. The lowest concentration that completely inhibits macroscopic growth was determined and MICs were reported. Antibacterial activity results of synthesized test compounds and Ampicillin as reference standard were depicted in Table 2. The comparative antimicrobial activity of the synthesized compounds was given in Fig. 3. All the tested compounds showed MIC values between 320-40 μg/mL. *In vitro* antibacterial evaluation states that compounds **3c, 3f, 3g** shown good activity against *B. subtilis* at 40 μg/mL while compound **3d, 3g, 3l** shown good activity against *S. aureus* at 40 μg/mL. Compounds **3d, 3i** shown good activity against *E. coli* and *P. aeruginosa* at 40 μg/mL.

3.2.2. In vitro antioxidant efficacy

Antioxidant activity of the synthesized compounds **3a-l** was performed by using DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging free radical activity assay

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Table 1. Physical characterization data of synthesized compounds **3a-l**.

Compd.	R	M.p. (°C)	Molecular formula	M.w.	% yield (Reaction tine)		Elemental analysis (%)
					Conventional	Microwave	C, H, N-Calculated (found)
3a	H	210–212	$C_{19}H_{15}N_3O_2S$	349.41	72.45 (10 hrs)	81.56 (8 min)	65.31(65.27), 4.33(4.25), 12.03(11.93)
3b	4-chloro	224–226	$C_{19}H_{14}ClN_3O_2S$	383.05	60.30 (12 hrs)	75.46 (10 min)	59.45(59.38), 3.68(3.56), 10.95(10.89)
3c	2-chloro	218–220	$C_{19}H_{14}ClN_3O_2S$	383.05	74.05 (11 hrs)	85.36 (9 min)	59.45(59.36), 3.68(3.51), 10.95(10.85)
3d	4-fluoro	228–230	$C_{19}H_{14}FN_3O_2S$	367.40	68.50 (12 hrs)	79.25 (10 min)	62.11(62.02), 3.84(3.76), 11.44(11.35)
3e	4-bromo	198–200	$C_{19}H_{14}BrN_3O_2S$	428.30	77.30 (12 hrs)	86.94 (12 min)	53.28(53.15), 3.29(3.18), 9.81(9.74)
3f	3-nitro	240–242	$C_{19}H_{14}N_4O_4S$	394.40	65.88 (14 hrs)	80.24 (9 min)	57.86(57.68), 3.58(3.39), 14.21(14.10)
3g	4-nitro	236–238	$C_{19}H_{14}N_4O_4S$	394.40	80.50 (12 hrs)	89.64 (10 min)	57.86(57.70), 3.58(3.42), 14.21(14.15)
3h	2,4-dinitro	200–202	$C_{19}H_{13}N_5O_6S$	439.40	72.30 (13 hrs)	88.46 (10 min)	51.94(51.85), 2.98(2.85), 15.94(15.85)
3i	3-methyl	256–258	$C_{20}H_{17}N_3O_2S$	363.43	70.85 (12 hrs)	87.48 (8 min)	66.10(66.01), 4.71(4.62), 11.56(11.42)
3j	4-methyl	220–222	$C_{20}H_{17}N_3O_2S$	363.43	69.75 (10 hrs)	76.28 (9 min)	66.10(66.03), 4.71(4.59), 11.56(11.45)
3k	4-methoxy	234–236	$C_{20}H_{17}N_3O_3S$	379.43	68.60 (12 hrs)	82.65 (12 min)	63.31(63.20), 4.52(4.49), 11.07(10.99)
3l	4-hydroxy	204–206	$C_{19}H_{15}N_3O_3S$	365.41	70.42 (11 hrs)	89.46 (10 min)	62.45(62.28), 4.14(4.05), 11.50(11.42)

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Table 2. *In vitro* antibacterial activity of compounds **3a-l**.

Compounds	MIC values of tested compounds (μg/mL) against			
	Gram positive bacteria		Gram negative bacteria	
	B. subtilis MTCC 1134	*S. aureus* MTCC 1144	*E. coli* MTCC 1089	*P. aeruginosa* MTCC 424
3a	160	320	80	160
3b	160	80	80	160
3c	40	80	320	40
3d	160	40	40	40
3e	160	320	160	40
3f	40	80	320	80
3g	40	40	160	80
3h	320	80	80	40
3i	160	160	40	40
3j	320	40	160	80
3k	160	80	320	160
3l	80	40	320	160
Ampicillin	20	20	20	20

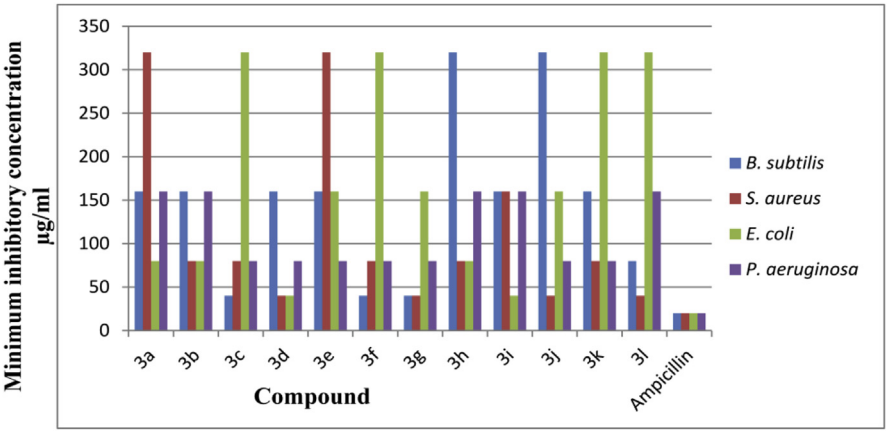

Fig. 3. Comparative antibacterial activity of the synthesized compounds.

method and hydrogen peroxide method in comparison with ascorbic acid as reference standard. The % inhibition of DPPH scavenging activity and the % inhibition of H_2O_2 scavenging activity along with their IC_{50} values was calculated and the results were given in Tables 3 and 4.

3.2.2.1. DPPH scavenging efficacy

DPPH assay results states that, the compounds **3b**, **3d** and **3g** were found to be shown significant antioxidant activity with IC_{50} values 52.36 ± 0.12, 56.36 ±

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Table 3. *In vitro* antioxidant activity evaluation of samples against DPPH radicals.

Compound	% inhibition (DPPH scavenging) at different concentrations				IC_{50} μg/mL
	50 μg/mL	100 μg/mL	300 μg/mL	500 μg/mL	
3a	33.75 ± 0.42	36.04 ± 0.18	55.83 ± 0.26	74.79 ± 0.32	145.21 ± 0.09
3b	52.26 ± 1.25	58.63 ± 0.92	69.33 ± 0.45	78.84 ± 0.58	52.36 ± 0.12
3c	35.28 ± 0.44	48.22 ± 1.01	51.06 ± 0.63	60.71 ± 0.52	187.49 ± 0.11
3d	51.33 ± 0.36	59.83 ± 0.42	67.33 ± 0.36	76.29 ± 0.38	56.36 ± 0.07
3e	48.26 ± 0.11	55.23 ± 0.68	65.85 ± 1.05	73.75 ± 0.84	67.60 ± 0.15
3f	28.33 ± 0.24	42.08 ± 0.46	44.38 ± 0.58	59.58 ± 0.24	331.13 ± 0.21
3g	53.78 ± 0.66	59.28 ± 0.32	69.86 ± 0.86	78.52 ± 0.23	50.11 ± 0.14
3h	45.46 ± 0.24	56.92 ± 0.36	62.83 ± 0.56	71.00 ± 0.42	76.91 ± 0.32
3i	34.26 ± 0.44	40.23 ± 0.65	52.65 ± 0.46	63.71 ± 1.03	200.91 ± 0.25
3j	36.04 ± 0.36	41.46 ± 0.26	45.63 ± 0.26	47.08 ± 0.42	438.53 ± 0.45
3k	38.26 ± 0.15	45.26 ± 0.32	56.52 ± 0.13	68.25 ± 0.52	128.82 ± 0.62
3l	43.82 ± 0.42	51.25 ± 0.41	60.41 ± 0.33	67.65 ± 0.84	97.72 ± 0.22
Ascorbic acid	55.36 ± 0.18	60.32 ± 0.24	70.85 ± 0.42	80.32 ± 0.12	46.99 ± 0.15

All the values are expressed as Mean ± SEM, n = 3.

Table 4. *In vitro* antioxidant activity evaluation of samples against H_2O_2 radicals.

Compound	% inhibition (H_2O_2 scavenging) at different concentrations				IC_{50} μg/mL
	50 μg/mL	100 μg/mL	300 μg/mL	500 μg/mL	
3a	40.87 ± 0.35	43.62 ± 0.42	49.8 ± 0.28	59.25 ± 0.36	203.23 ± 0.33
3b	67.98 ± 0.58	80.91 ± 0.12	81.85 ± 0.44	85.54 ± 1.01	26.79 ± 0.14
3c	64.27 ± 0.56	69.78 ± 0.91	74.28 ± 0.75	83.46 ± 0.72	34.83 ± 0.41
3d	70.93 ± 0.42	80.31 ± 0.23	82.87 ± 0.36	86.31 ± 0.22	26.48 ± 0.22
3e	74.98 ± 0.84	78.26 ± 0.12	85.45 ± 0.45	88.24 ± 1.02	24.15 ± 0.13
3f	41.25 ± 0.24	58.31 ± 0.22	74.25 ± 0.42	88.93 ± 0.22	45.91 ± 0.25
3g	49.26 ± 0.35	54.28 ± 0.47	60.45 ± 0.32	74.61 ± 0.65	73.96 ± 0.16
3h	65.40 ± 0.42	76.25 ± 0.24	79.50 ± 0.26	82.51 ± 0.38	30.62 ± 0.09
3i	60.60 ± 1.10	65.46 ± 0.43	72.28 ± 0.75	78.44 ± 0.56	42.26 ± 0.25
3j	60.25 ± 0.36	75.56 ± 0.22	77.25 ± 0.24	80.12 ± 0.18	34.35 ± 0.16
3k	55.24 ± 0.56	61.43 ± 0.42	72.25 ± 1.04	79.28 ± 0.66	46.23 ± 0.17
3l	60.45 ± 0.44	66.24 ± 0.25	75.26 ± 0.33	82.14 ± 0.48	37.84 ± 0.45
Ascorbic acid	69.47 ± 0.32	82.55 ± 0.26	83.46 ± 0.12	87.65 ± 0.16	24.94 ± 0.16

All the values are expressed as Mean ± SEM, n = 3.

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0.07 and 50.11 ± 0.14 μg/mL respectively when compared with standard Ascorbic acid IC_{50} value 46.99 ± 0.15 μg/mL. The compounds **3e** and **3h** exhibited moderate activity with the IC_{50} values 67.60 ± 0.15 and 76.91 ± 0.32 μg/mL respectively.

3.2.2.2. Hydrogen peroxide efficacy

Hydrogen peroxide assay results revealed the compounds **3b**, **3d** and **3e** were found to exhibit significant antioxidant activity with the IC_{50} values 26.79 ± 0.14, 26.48 ± 0.22 and 24.15 ± 0.13 μg/mL respectively when compared with standard Ascorbic acid IC_{50} value 24.94 ± 0.16. The compounds **3c**, **3h** and **3j** exhibited moderate activity with the IC_{50} values 34.83 ± 0.41, 30.62 ± 0.09 and 34.35 ± 0.16 μg/mL respectively.

3.2.3. In vivo hypoglycaemic efficacy

Study protocols related to *in vivo* hypoglycaemic activities were approved by the Institutional Animal Ethics Committee under the supervision of Committee for the Purpose of Control and Supervision of Experiments on Animals, New Delhi bearing registration number 1847/PO/Re/S/16/CPCSEA. Blood glucose levels, body weight and serum biochemical parameters were expressed as mean ± standard error of mean (SEM). The values were analyzed by one-way analysis of variance (ANOVA) followed by Dunnet's 't' test. The acute study data of all the synthesized compounds were depicted in Table 5 in relation to the standard drug Glibenclamide. The compounds **3b, 3d, 3g** and **3h** have shown significant hypoglycaemic activity. Chronic study analysis results were depicted in Table 6 revealed that compound **3b** and **3h** at 70 mg/kg body weight possess significant activity. On day 15, effect of compounds **3b, 3d, 3g** and **3h** on insulin, urea, creatinine, lipid profiles, HDL, LDL and VLDL levels in control and Alloxan induced diabetic rats in serum or plasma were placed in Table 7, revealed that the compounds shows significant to moderate reduction.

3.3. Molecular docking results

In this study, all the designed compounds were subjected to docking to explore their binding mode at PPARγ receptor. Biological target PPARγ receptor was downloaded from the protein data bank PDB ID- 2PRG. AutoDock molecular docking technique was employed to dock the designed compounds against PPARγ receptor (PDB ID-2PRG) to trace the interaction between various compounds and PPARγ receptor. Non-polar hydrogen atoms were removed from the receptor and their partial charges were added to the corresponding carbon atoms. PPARγ receptor agonist Rosiglitazone was used as a reference ligand. The docking study has been conducted to predict the binding mode and to rationalize the observed biological activity. Molecular docking was performed using recently updated version AutoDock docking

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Table 5. Effect of synthesized compounds **3a-l** on blood glucose level in Alloxan induced diabetic rats (Acute Study).

Compound	Mean ± SEM of blood glucose level mg/dL					
	0 hr	1 hr	2 hr	4 hr	6 hr	8 hr
Normal	122.22 ± 2.4	124.12 ± 1.46	123.5 ± 5.11	120.54 ± 3.22	122.5 ± 4.22	120.33 ± 2.3
Standard	383.8 ± 14.28	222.8 ± 8.05**	180.3 ± 6.92	120.42 ± 9.86*	93.6 ± 4.95	85.42 ± 2.53*
3a	313.3 ± 5.46	288.3 ± 4.41	259.3 ± 7.23	242.33 ± 4.33**	250.7 ± 6.57*	282.7 ± 2.34
3b	305.3 ± 5.46*	290.3 ± 7.32	200.3 ± 9.29**	145.33 ± 1.76	102 ± 5.78*	90.58 ± 4.73
3c	339.3 ± 4.06	315 ± 2.89	298.7 ± 3.53*	275 ± 5.78	285 ± 2.89	301.7 ± 6.02**
3d	316 ± 6.51**	297.3 ± 6.37*	195.3 ± 6.02	142 ± 8.67	105.3 ± 6.02**	95 ± 2.89
3e	317.3 ± 6.18	300.7 ± 5.21**	276.7 ± 4.41	249.3 ± 8.70*	263.3 ± 6.02	285.0 ± 2.89
3f	320.0 ± 2.00*	303.3 ± 6.02	276.7 ± 3.53**	250.0 ± 2.89	281.7 ± 6.02	300.0 ± 5.30*
3g	309.0 ± 5.51*	282.3 ± 4.37**	200.3 ± 4.22	168.01 ± 7.65*	128.7 ± 6.02**	100.02 ± 2.89**
3h	306.0 ± 2.08	280.3 ± 3.85**	208.3 ± 3.39	155.6 ± 3.48**	110.3 ± 6.02	94.7 ± 4.41
3i	333.0 ± 5.87**	311.3 ± 5.21	292.7 ± 6.37	264.0 ± 5.87**	285.0 ± 2.89*	301.7 ± 6.02
3j	316.7 ± 2.41	301.3 ± 5.24*	273.3 ± 6.02	243.00 ± 3.22**	266.7 ± 6.02	288.0 ± 2.65
3k	311.4 ± 5.42*	302.61 ± 2.16	289.45 ± 4.11*	265.32 ± 8.12	279.65 ± 2.35	295.44 ± 3.51**
3l	319.12 ± 4.15	310.52 ± 3.05	283.64 ± 4.22**	272.62 ± 6.42	284.61 ± 3.15	301.82 ± 4.56*

Standard Drug: Glibenclamide; Statistical analysis is done by One-way ANOVA followed by Dunnet's 't' test; **P < 0.01 (considered as significant), *P < 0.001.

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Table 6. Effect of compounds **3a, 3d, 3f** and **3j** on fasting blood glucose level and body weight in Alloxan induced diabetic rats (Chronic Study 15 days).

Compound	Blood glucose in mg/dL			Body weight in gm		
	Day 0	Day 7	Day 15	Day 0	Day 7	Day 15
Standard	308.3 ± 6.51	214.3 ± 1.15	147.0 ± 4.36	193 ± 7.00	189.3 ± 3.79	192.0 ± 5.29
3b (35 mg/kg bw)	316.7 ± 1.70	252.3 ± 3.06**	199.3 ± 5.03	195.3 ± 3.06	200.7 ± 1.53	192.3 ± 3.06**
3b (70 mg/kg bw)	313 ± 4.58	221.3 ± 5.03*	157 ± 6.24	201 ± 3.61*	197 ± 1.00*	195.3 ± 2.52
3d (35 mg/kg bw)	311.67 ± 2.19**	262.33 ± 1.77	212.00 ± 4.17	194.33 ± 1.45	200.67 ± 0.88**	192.33 ± 1.77
3d (70 mg/kg bw)	314.33 ± 2.24	261.33 ± 2.91	197 ± 3.61**	205.00 ± 3.22	196.67 ± 0.67	195.33 ± 2.03
3g (35 mg/kg bw)	311.67 ± 2.19	260.33 ± 6.65	207.00 ± 3.61	204.57 ± 2.50*	196.63 ± 0.32	190.33 ± 1.02*
3g (70 mg/kg bw)	314.33 ± 2.61	255.67 ± 2.03**	192.67 ± 3.18	204.67 ± 3.53	196.67 ± 1.30	195.33 ± 2.11
3h (35 mg/kg bw)	312.33 ± 2.03*	271.00 ± 2.00	218.00 ± 1.16**	195.67 ± 1.33**	200.67 ± 0.60*	192.33 ± 1.77
3h (70 mg/kg bw)	314.33 ± 2.61	227.67 ± 2.85	165.00 ± 3.52**	194.33 ± 2.19	204.33 ± 1.20	195.33 ± 2.03**

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Table 7. Effect of compounds **3b, 3d, 3g** and **3h** on insulin, urea, creatinine, lipid profiles, HDL, LDL and VLDL levels in control and Alloxan induced diabetic rats in serum or plasma on Day 15.

Compound	Insulin (μIU/mL)	Urea (mg/dL)	Creatinine (mg/dL)	Total cholesterol (mg/dL)	Triglyceride (mg/dL)	Free fatty acids (mg/dL)	HDL-cholesterol (mg/dL)	LDL-cholesterol (mg/dL)	VLDL-cholesterol (mg/dL)
Control	16.3 ± 0.68	17.7 ± 0.2	0.71 ± 0.12	87.16 ± 6.12	13.29 ± 1.08	65.21 ± 4.12	45.16 ± 3.61	23.67 ± 1.67	19.72 ± 1.21
Diabetic control	6.9 ± 0.26	37.2 ± 1.6	1.21 ± 0.16	258.13 ± 19.98	45.17 ± 3.11	132.22 ± 9.92	22.68 ± 1.81	79.66 ± 4.95	47.51 ± 3.79
Diabetic + Glibenclamide (500 μg/kg)	11.3 ± 0.12	20.1 ± 0.98	0.78 ± 0.32	80 ± 0.26	28.60 ± 1.35	68.21 ± 4.12	22.30 ± 1.52	25.67 ± 1.67	20.50 ± 0.25
Diabetic + sample 3b (35 mg/kg bw)	10.3 ± 0.51	29.4 ± 1.6	0.96 ± 0.27	91.32 ± 7.12	16.72 ± 1.62	59.65 ± 5.16	41.67 ± 3.05	41.56 ± 4.12	28.91 ± 2.07
Diabetic + sample 3b (70 mg/kg bw)	14.3 ± 0.26	21.1 ± 2.5	0.85 ± 0.19	85.65 ± 7.73	18.94 ± 1.92	64.12 ± 7.07	40.12 ± 3.01	32.14 ± 2.71	25.71 ± 1.86
Diabetic + sample 3d (35 mg/kg bw)	12.3 ± 0.21	27.4 ± 0.25	0.86 ± 0.32	95.26 ± 0.50	36.60 ± 1.85	70.65 ± 2.56	35.67 ± 2.12	40.21 ± 1.56	19.38 ± 0.25
Diabetic + sample 3d (70 mg/kg bw)	16.3 ± 0.46	19.1 ± 1.06	0.65 ± 0.26	90.23 ± 1.63	58.40 ± 2.05	85.12 ± 1.76	40.12 ± 1.52	35.14 ± 1.27	14.97 ± 0.72
Diabetic + sample 3g (35 mg/kg bw)	10.5 ± 0.12	20.1 ± 0.98	0.68 ± 0.32	95.26 ± 0.50	46.60 ± 1.55	69.65 ± 1.56	38.67 ± 2.32	38.21 ± 1.32	20.50 ± 0.25
Diabetic + sample 3g (70 mg/kg bw)	15.3 ± 0.36	25.4 ± 0.32	0.89 ± 0.23	90.23 ± 1.63	58.40 ± 2.05	83.23 ± 1.26	42.12 ± 2.01	35.14 ± 1.25	16.23 ± 0.34
Diabetic + sample 3h (35 mg/kg bw)	10.3 ± 1.32	29.4 ± 1.23	0.96 ± 0.32	91.26 ± 0.50	45.60 ± 1.25	62.65 ± 1.56	35.67 ± 1.32	40.21 ± 0.26	22.30 ± 0.42
Diabetic + sample 3h (70 mg/kg bw)	12.3 ± 2.26	22.1 ± 2.47	0.78 ± 0.23	102.23 ± 1.63	50.40 ± 1.85	72.23 ± 1.26	45.12 ± 0.26	32.14 ± 1.32	18.52 ± 0.26

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Table 8. Binding energy and amino acid residues interacted by the compounds **3a-l** with the target PPARγ protein PDB ID − 2PRG.

Compound	Binding energy (kcal/mol)	No. of H bonds	H-bond length	Amino acid residues interacted
Rosiglitazone	−8.26	3	3.01, 2.82, 3.11	His449, His323, Ser289
3a	−7.85	2	3.25, 2.56	Arg288, Ser289
3b	−9.65	2	3.10, 3.28	Ser289, Gln286
3c	−7.99	3	2.72, 2.42, 2.41	Lys296, Leu268, Met348
3d	−8.76	2	3.10, 2.32	Thr246, His449
3e	−8.85	2	2.80, 2.51	Met329, Leu268
3f	−9.05	2	3.11, 2.16	Met348, Tyr473
3g	−8.45	2	2.91, 1.95	Tyr396, His449
3h	−9.04	2	3.16, 2.91	HIS449, LYS367
3i	−7.22	3	2.15, 3.25, 1.98	Leu298, Ser289, Met329
3j	−9.42	2	2.54, 3.20	Gln286, Cys255
3k	−7.68	2	2.53, 1.94	Ser289, Leu292
3l	−6.48	2	1.97, 2.24	Ser289, His449

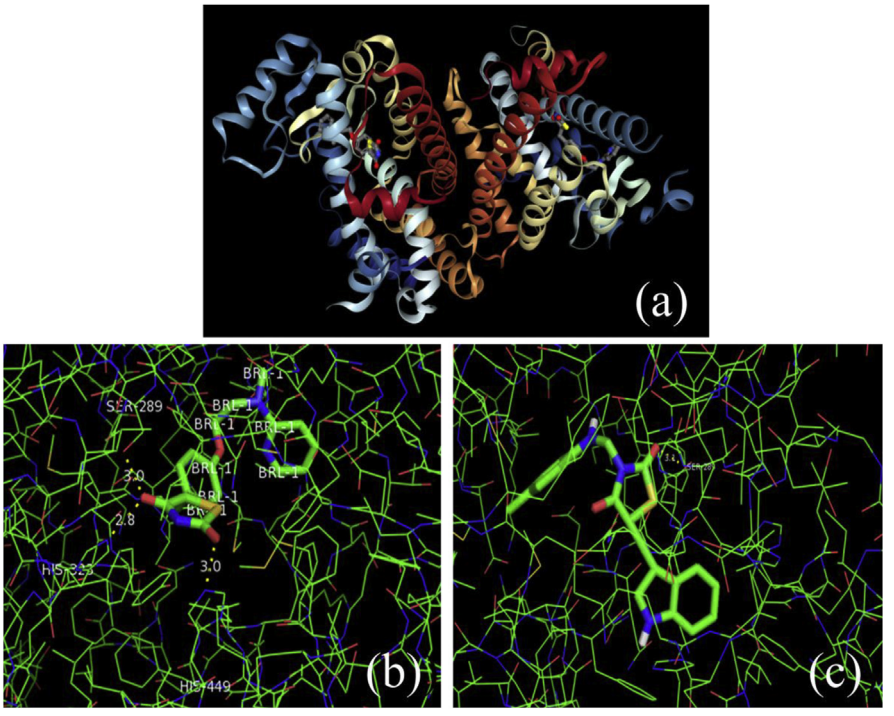

Fig. 4. Molecular docking studies at PPARγ protein. **(a)** Structure of PPARγ protein from PDB ID-2PRG. **(b)** Docking complex of PPARγ protein (PDB ID- 2PRG) with Rosiglitazone. **(c)** Docking complex of PPARγ protein (PDB ID- 2PRG) with compound 3b.

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engine 4.2.6 software. Default settings were used for all the calculations. The interactions between the receptor protein and ligands were studied in Pymol 1.7.4.5. The binding energy (kcal/mol) with hydrogen bonds, number of hydrogen bonds, hydrogen bond length and amino acid residues interacted were identified. The binding energy values revealed that most of the compounds had good binding affinity toward the PPARγ receptor and the computed values were depicted in Table 8. The interaction of Rosiglitazone at the active site of the receptor has showed binding energy of −8.26 kcal/mol and forms three H-bonds with His449, His323 and Ser289. Fig. 4 shown the 3D structure of PPARγ receptor, docking complex of PPARγ protein-2PRG against Rosiglitazone and compound **3b**. The compound **3b** shown promising binding affinity i.e. −9.65 kcal/mol and forms two H-bonds with Ser289 and Gln286.

4. Conclusion

A Series of 3-substituted-5-[(indol-3-yl)methylene]-thiazolidine-2,4-dione derivatives were developed by incorporating different aromatic amines, using conventional and microwave irradiation methods and compared. The results of microwave irradiation technique indicated drastic fall of reaction time and improvement in percentage yield in comparison with traditional conventional synthesis. All the compounds were characterized physically and most of the compounds were characterized spectrally by FT-IR, ^{1}H-NMR, ^{13}C-NMR and mass spectroscopy. *In vitro* antibacterial evaluation indicated that, compound **3g** has shown good activity against gram positive bacteria (*B. subtilis and S. aureus*) at 40 μg/mL while compound **3i** has shown good activity against gram negative bacteria (*E. coli* and *P. aeruginosa*) at the same concentration. *In vitro* antioxidant results stated that, compound **3b** and **3d** were found to exhibit significant antioxidant activity in both DPPH assay and hydrogen peroxide assay methods. *In vivo* hypoglycemic activity evaluation revealed that, the compounds **3b** and **3h** have shown promising hypoglycaemic activity in acute study as well as in chronic study. Molecular docking studies revealed that, compound **3b** shown highest binding affinity at PPARγ receptor protein. All these results indicate that the novel synthesized TZDs may be beneficial compounds.

Declarations

Author contribution statement

K. Srikanth Kumar: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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A. Lakshmana Rao: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

M.V. Basaveswara Rao: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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