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Thiazolidine-2,4-dione Derivatives Bearing Indole Moiety: Design, Synthesis, Hypoglycaemic activity and Molecular Docking Studies

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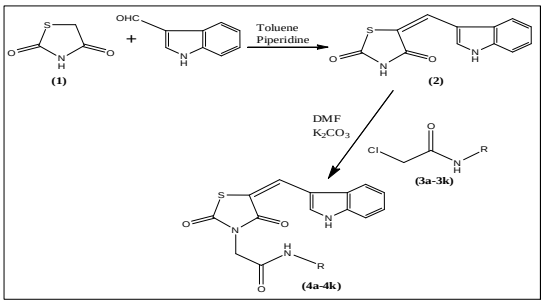
ABSTRACT

A series of novel thiazolidine-2,4-dione derivatives having N-aryl acetamide appendage at 3rd position and indolyl methylene appendage at 5th position was synthesized by using appropriate procedures. The synthesized compounds were characterized physically, FT-IR, ^{1}H-NMR, ^{13}C-NMR and mass spectral analysis. The newly synthesized compounds were evaluated for their hypoglycemic activity by means of tail tipping method in Alloxan induced Wister albino rats of both sexes. Compounds 4a and 4b showed promising hypoglycaemic activity in both acute studies as well as in chronic study when compared with the standard drug Rosiglitazone. Molecular docking studies were carried out using AutoDock software and revealed that compounds 4a and 4b exhibit significant binding interaction with PPARγ receptor compared with the standard ligand Rosiglitazone.

Graphical Abstract



Keywords: Thiazolidine-2,4-dione derivatives, Conventional and microwave methods, *In vivo* hypoglycemic activity, Molecular docking studies.

INTRODUCTION

Diabetes Mellitus (DM) is a group of syndromes characterized by hyperglycemia, altered metabolism of lipids, carbohydrates, proteins and increased risk of complications from vascular disease.

Especially type 2 diabetes mellitus (T2DM) is a chronic metabolic disorder, characterized by hyperglycemia. Characteristically diabetes is a long-term disease with variable clinical manifestations and progression, chronic hyperglycemia from whatever cause, leads to a number of complications including cardiovascular such as hypertension, renal, neurological such as anxiety, stress, ocular and other such inter-current infections.

The thiazolidine-2,4-diones (TZDs) class of molecules normalizes elevated blood glucose level and is of great use in the treatment of Type 2 diabetes. Although the exact mechanism of action remains unclear, a number of reports recommend that TZDs are high affinity towards peroxysomal proliferator activated receptor-γ (PPARγ). At these receptors they act as insulin sensitizers. TZDs are PPARγ agonists improve glycemic control and dyslipidemia in type 2 diabetic patients by down regulating cytokines in adipose tissue. Therefore, PPARγ receptors are justifiable molecular targets for the development of antidiabetic agents. Unlike sulfonylurea drugs glipizide, glyburide (which enhance insulin resistance) and metformin (which reduces hepatic glucose output), TZDs-Rosiglitazone, Pioglitazone improve insulin sensitivity in liver, muscle and fat tissues and thus counteract insulin resistance. However these drugs have been associated with cardiovascular, liver and haematological toxicity and body weight gain [1].

TZDs normalize elevated glucose levels in blood and are of great use in controlling of type 2 diabetes. TZDs had high affinity towards PPARγ receptors and acts as insulin sensitizers. TZDs improve insulin sensitivity in liver, muscle and fat tissues and thus counteract insulin resistance. Many drugs have been developed and approved under the thiazolidinediones class for the treatment of diabetes such as Rosiglitazone, Pioglitazone, Ciglitazone, Troglitazone, Englitazone, Netoglitazone, etc (Figure 1). Ciglitazone is the first synthesized TZD derivative, having hypoglycemic 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 is another TZD derivative, failed to survive due to liver toxicity. Rosiglitazone and Pioglitazone are found to possess good oral hypoglycemic activity, principally they act by increasing tissue sensitivity particularly adipose tissue to the insulin [2]. Pioglitazone and Rosiglitazone are currently in clinical use under this category. These are also having drawbacks like producing hepatotoxicity [3], oedema, haematological toxicity and body weight gain problems [4].

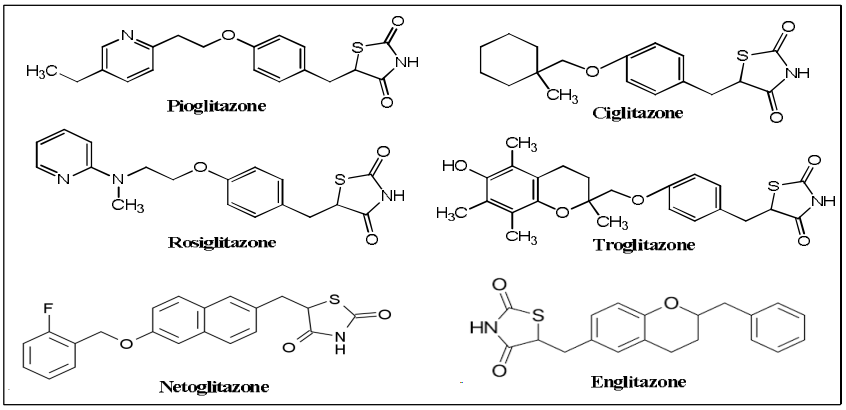


Figure 1. Structures of Pioglitazone, Rosiglitazone, Netogliazone, Ciglitazone, Troglitazone and Englitazone

Recent studies state that TZDs are known to exhibit antidiabetic activity [5] and wide variety of biological activities other than antidiabetic activity such as activators of PPARγ receptors [6], *in vitro* aldose reductase inhibition [7], hypolipidemic [8],anti-inflammatory [9,10], anticonvulsant [11], antitubercular [12], anti-HIV [13], anticancer [14], antifungal [15], Ca^{+2} channel blocking [16] and antioxidant [17], etc.

In the present work a series of novel TZDs having N-arylacetamide attachment at N-3 position with different substituents on aryl moiety and indolylmethylene attachment at C-5 position were developed by both conventional heating and microwave irradiated methods which may increase the production of insulin from the β-cells of pancreas. All the synthesized compounds were evaluated for their glucose lowering capability in Alloxan induced diabetic rat. Molecular docking studies were carried out on designed ligands to observe better efficacy property and binding interaction at the PPARγ target site.

MATERIALS AND METHODS

Chemicals and reagents required for the synthesis of various novel thiazolidine-2,4-diones were obtained from commercial suppliers Merck grade and further those were used without purification. Progress of the reaction as well as completion of the reaction was monitored by TLC (thin layer chromatography) with the help of E.Merck grade silica gel 60GF-254 precoated TLC plates. TLC Spots were observed under iodine chamber and in UV-light. Melting points were determined by using electrical melting point apparatus and those were uncorrected. IR spectra of the compounds were recorded in Bruker FT-IR analyzer spectrophotometer using KBr pressed pellet technique. Chemical shifts (in ppm)of ^{1}H-NMR and ^{13}C-NMR spectra were recorded on Bruker-AMX-400 MHz spectrophotometer using deuteriated dimethyl sulfoxide (DMSO) solvent and tetramethylsilane (TMS) as internal standard. Mass spectra of the compounds were recorded on Agilent-LC-MSD:1200 mass spectrophotometer. The designed compounds were synthesized conventionally as well as microwave assisted method on RGSSIRR modeled Raga's microwave system having different power levels (140W to 700W). Hypoglycemic activity was studied on Alloxan induced Wister albino rats of both sex by means of tail tipping method. Molecular docking studies were carried out using AutoDock software to observe better efficacy property and binding interaction at the PPARγ protein active site.

Synthesis of thiazolidine-2,4-dione (1)Conventional method: Dissolve chloroacetic acid (0.02 mol) and thiourea (0.02 mol) separately in each 5 mL of water. The contents of the vessels were transferred into a three necked round bottom flask and stir the contents until white precipitate obtained. Cool down the contents of the flask and slowly add conc. HCl (6 mL) to it from the dropping funnel. Reflux at a temperature of 100-110°C by applying gentle heat for about 10-12 h. Cool the contents of the flask to solidify and filter to obtain the product. Recrystallization was done using ethanol.

Microwave irradiation method: A mixture of chloroacetic acid (0.01mol) and thiourea (0.01mol) dissolved in 5 mL of water was placed into the microwave synthesizer reaction vessel. The reaction vessel was connected to the condenser, stirred about 30-60 min in cold condition. Add conc. HCl (3 mL) to the reaction mixture and irradiated for 6min at 120°C using 280W power level. Cool down the reaction mixture to room temperature, filtered the obtained solid, dry and recrystallized from ethanol. 78.42% (yield from conventional method), 90.25% (yield from MWI method), white crystalline solid, mp124-126°C, R_f value 0.62 from using chloroform and methanol (9:1 v/v). IR [KBr ν cm^{-1}]: 3321.46 (-NH-), 1689.94 (C=O), 2968.89 (C-H), 1303.29 (C-N), 626.69 (C-S). ^{1}H-NMR [400 MHz, δ, ppm, DMSO-d_6]: 12.015 (s, 1H, NH), 4.132 (s, 2H, CH_2).^{13}C-NMR [400 MHz, δ, ppm, DMSO-d_6]: 35.8, 173.0, 173.8. ESI-MS: (M^+) m/z 117.

Synthesis of 5-[(indol-3-yl)methylene]-thiazolidine-2,4-dione (2) Conventional method: Thiazolidine-2,4-dione (0.01 mol) was added to a solution of indole-3-carboxaldehyde (0.01 mol) in 8 mL of toluene. Catalytic amount of piperidine was added to the reaction mixture and the resulting solution was refluxed in an oil bath for about 5-6 h at 110-120°C. Completion of the reaction was monitored by TLC, the reaction mixture was allowed to cool to the room temperature. Cold water and 1M HCl was added to reaction mixture. The obtained solid product was filtered, washed with cold water and dry toluene. Crude product was purified by recrystallization from ethanol.

Microwave irradiation method: 0.4 mL of piperidine was added to a solution of thiazolidine-2,4-dione (0.01 mol) and indole-3-carboxaldehyde (0.01 mol) in 8mL of toluene. The content was placed in Raga's scientific microwave synthesis reaction vessel and connected to a water condenser, irradiated the reaction mixture at 350W for about 8 min at 120°C. After completion of the reaction, it was cooled and diluted with 15 mL ice-cold water, filtered, washed with cold water and dry toluene. Obtained crude product was purified by recrystallization from ethanol. 70.48%(yield from conventional method), 84.94%(yield from MWI method), yellow crystalline solid, mp186-188°C, Rf value is 0.58 from using benzene and ethyl acetate (8:2 v/v).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]: 12.310 (1H, s, TZD-NH-), 12.140 (1H, s, indole-NH-), 8.067 (1H, s, =CH- methylene), 7.184-7.906 (5H, d and 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.

General procedure for synthesis of substituted 2-chloro-N-aryl acetamide derivatives (3a-3k) (Conventional method): Substituted aryl amine (0.01 mol) was added to the chloroacetyl chloride (0.01 mol) under cold conditions along with a base (triethyl amine) in dichlromethane. Reaction mixture was stirred overnight at room temperature. After completion of the reaction, water was added to the reaction mixture and separated the organic layer. Then it was passed through the anhy.Na_2SO_4 and distilled off the organic layer to get crude product. Further it was purified by using ethanol.

General Procedure for synthesis of thiazolidine-2,4-dione derivatives(4a-4k) Conventional method: A mixture of 5-[(indol-3-yl)methylene]-thiazolidine-2,4-dione (0.01mol) 2 and substituted 2-chloro-N-aryl acetamide 3a-3k (0.01 mol) in DMF along with K_2CO_3 were placed in RBF and refluxed for about 4-6 h. Progress of the reaction was monitored by TLC using mobile phase n-hexane and ethylacetate (9:1 v/v). Allowed to cool the reaction mixture and the content was poured into cold water. The obtained solid product was filtered, dried and recrystallized with absolute ethanol.

Microwave irradiation method: A mixture of 5-[(indol-3-yl)methylene]-thiazolidine-2,4-dione (0.01 mol) 2 and substituted 2-chloro-N-aryl acetamide 3a-3k (0.01 mol) in DMF along with K_2CO_3 were stirred for 20 min at RT. Transfer the contents into Raga's scientific microwave synthesizer vessel and irradiated the reaction mixture at 420W power level for about 6-8 min at 120°C. Cool down the contents of the flask to room temperature and poured into cold water. Collect the obtained solid product by filtration and dry it recrystallized the crude product with absolute ethanol to get desired compound.

3-[N-(4-chlorophenyl)acetamido]-5-[(indol-3-yl)methylene]-thiazolidine-2,4-dione (4a): IR [KBr ν cm^{-1}]: 3331.12 (-NH-), 3220.31 (-NH-), 1720.30 (C=O), 1748.21 (C=O), 1775.25 (C=O), 1348.91 (C-N), 2985.20 (C-H), 3041.21 (=C-H), 611.45 (C-S), 1672.31 (C=C), 870.66 (C-Cl). ^{1}H-NMR [400 MHz, δ, ppm, DMSO-d_6]: 5.310 (1H, s, -CO-N<u>H</u>-), 4.425 (2H, s, -C<u>H</u>$_2$-CO-), 6.982-8.654 (9H, d and t, phenyl and indole), 8.792 (1H, s, =C<u>H</u>- methylene), 12.042 (1H, s, indole-N<u>H</u>-).^{13}C-NMR [400 MHz, δ, ppm, DMSO-d_6]: 174.4, 170.2, 145.3, 137.6, 135.5, 131.8, 129.9, 127.3, 125.1, 123.2, 122.4, 121.8, 119.7, 117.8, 112.6, 110.3, 46.3. ESI-MS: m/z (M^+) 411.

3-[N-(2,4-dichlorophenyl)acetamido]-5-[(indol-3-yl)methylene]-thiazolidine-2,4-dione (4b): IR [KBr ν cm^{-1}]: 3321.40 (-NH-), 3245.35 (-NH-), 1724.51 (C=O), 1742.32 (C=O), 1765.22 (C=O), 1355.62 (C-N), 2975.21 (C-H), 3065.43 (=C-H), 621.15 (C-S), 1680.61 (C=C), 865.11 (C-Cl). ^{1}H-NMR [400 MHz, δ, ppm, DMSO-d_6]: 5.524 (1H, s, -CO-N<u>H</u>-), 4.521 (2H, s, -C<u>H</u>$_2$-CO-), 6.854-8.561 (8H, d and t, phenyl and indole), 8.821 (1H, s, =C<u>H</u>- methylene), 12.149 (1H, s, indole-N<u>H</u>-).^{13}C-NMR [400 MHz, δ, ppm, DMSO-d_6]: 175.5, 169.6, 166.2, 138.6, 13.5, 144.4, 134.1, 132.5, 131.6, 130.5, 127.4, 126.8, 124.1, 123.5, 121.5, 120.8, 118.5, 113.8, 110.6, 48.5. ESI-MS: m/z (M^+) 445.

3-[N-(4-fluorophenyl)acetamido]-5-[(indol-3-yl)methylene]-thiazolidine-2,4-dione (4c): IR [KBr ν cm^{-1}]: 3335.52 (-NH-), 3243.61 (-NH-), 1759.45 (C=O), 1740.57 (C=O), 1721.18 (C=O), 1342.47

(C-N), 2980.42 (C-H), 3064.55 (=C-H), 632.52 (C-S), 1656.31 (C=C), 1232.54 (C-F). ^{1}H-NMR [400 MHz, δ, ppm, DMSO-d_6]: 5.672 (1H, s, -CO-NH-), 4.548 (2H, s, -CH$_2$-CO-), 7.054-8.598 (9H, d and t, phenyl and indole), 8.842 (1H, s, =CH- methylene), 11.954 (1H, s, indole-NH-). ^{13}C-NMR [400 MHz, δ, ppm, DMSO-d_6]: 173.5, 169.2, 165.8, 159.4, 144.7, 136.4, 134.8, 130.9, 127.8, 125.4, 123.7, 121.8, 120.4, 119.8, 118.5, 112.3, 110.8, 47.9. ESI-MS: m/z (M^+) 395.

3-[N-(4-nitrophenyl)acetamido]-5-[(indol-3-yl)methylene]-thiazolidine-2,4-dione (4d): IR [KBr ν cm^{-1}]: 3372.54 (-NH-), 3256.50 (-NH-), 1766.56 (C=O), 1753.45 (C=O), 1725.62 (C=O), 1330.52 (C-N), 2986.44 (C-H), 3078.62 (=C-H), 639.74 (C-S), 1661.84 (C=C), 1535.40 and 1342.51 (-NO$_2$). ^{1}H-NMR [400 MHz, δ, ppm, DMSO-d_6]: 5.585 (1H, s, -CO-NH-), 4.642 (2H, s, -CH$_2$-CO-), 6.978-8.354 (9H, d and t, phenyl and indole), 8.624 (1H, s, =CH- methylene), 12.142 (1H, s, indole-NH-). ^{13}C-NMR [400 MHz, δ, ppm, DMSO-d_6]: 175.8, 169.6, 166.3, 147.5, 145.3, 142.8, 136.6, 133.7, 128.1, 125.2, 123.4, 122.2, 121.6, 119.4, 117.5, 113.4, 110.3, 45.8. ESI-MS: m/z (M^+) 422.

3-[N-(4-hydroxyphenyl)acetamido]-5-[(indol-3-yl)methylene]-thiazolidine-2,4-dione(4e): IR [KBr ν cm^{-1}]: 3573.80 (Ph-OH), 3365.62 (-NH-), 3254.56 (-NH-), 1746.14 (C=O), 1753.13 (C=O), 1730.51 (C=O), 1365.21 (C-N), 2981.32 (C-H), 3067.72 (=C-H), 627.46 (C-S), 1662.50 (C=C). ^{1}H-NMR [400 MHz, δ, ppm, DMSO-d_6]: 10.631 (1H, s, phenyl-OH), 5.462 (1H, s, -CO-NH-), 4.485 (2H, s, -CH$_2$-CO-), 6.775-8.109 (9H, d and t, phenyl and indole), 8.376 (1H, s, =CH- methylene), 12.059 (1H, s, indole-NH-). ^{13}C-NMR [400 MHz, δ, ppm, DMSO-d_6]: 178.6, 167.6, 165.2, 157.9, 144.0, 136.5, 133.8, 130.4, 128.9, 125.5, 123.4, 121.4, 120.2, 119.8, 115.2, 113.4, 110.6, 49.7. ESI-MS: m/z (M^+) 393.

3-[N-(p-tolyl)acetamido]-5-[(indol-3-yl)methylene]-thiazolidine-2,4-dione (4f): IR [KBr ν cm^{-1}]: 3363.62 (-NH-), 3246.53 (-NH-), 1756.18 (C=O), 1740.42 (C=O), 1723.24 (C=O), 1359.51 (C-N), 2973.26 (C-H), 3081.33 (=C-H), 618.40 (C-S), 1671.41 (C=C). ^{1}H-NMR [400 MHz, δ, ppm, DMSO-d_6]: 2.352 (3H, s, phenyl-CH$_3$), 5.664 (1H, s, -CO-NH-), 4.686 (2H, s, -CH$_2$-CO-), 6.775-7.992 (9H, d and t, phenyl and indole), 8.286 (1H, s, =CH- methylene), 12.118 (1H, s, indole-NH-). ^{13}C-NMR [400 MHz, δ, ppm, DMSO-d_6]: 179.1, 169.4, 165.2, 145.6, 137.4, 135.8, 133.4, 130.6, 128.9, 127.6, 124.4, 122.8, 120.6, 119.8, 117.9, 112.6, 110.7, 49.8, 25.8. ESI-MS: m/z (M^+) 391.

3-[N-(4-methoxyphenyl)acetamido]-5-[(indol-3-yl)methylene]-thiazolidine-2,4-dione(4g): IR [KBr ν cm^{-1}]: 3352.42 (-NH-), 3249.23 (-NH-), 1719.44 (C=O), 1726.42 (C=O), 1753.54 (C=O), 1351.60 (C-N), 2974.82 (C-H), 3051.24 (=C-H), 631.35 (C-S), 1125.31 (C-O-C). ^{1}H-NMR [400 MHz, δ, ppm, DMSO-d_6]: 3.754 (3H, s, -OCH$_3$), 5.491 (1H, s, -CO-NH-), 4.562 (2H, s, -CH$_2$-CO-), 6.849-8.162 (9H, d and t, phenyl and indole), 8.442 (1H, s, =CH- methylene), 12.007 (1H, s, indole-NH-).^{13}C-NMR [400 MHz, δ, ppm, DMSO-d_6]: 178.4, 169.3, 166.4, 159.5, 144.9, 137.5, 133.6, 131.8, 127.9, 125.7, 123.4, 122.8, 121.2, 118.3, 115.4, 112.5, 110.6, 58.6, 49.7. ESI-MS: m/z (M^+) 407.

3-[N-(3,4-dimethoxyphenyl)acetamido]-5-[(indol-3-yl)methylene]-thiazolidine-2,4-dione (4h): IR [KBr ν cm^{-1}]: 3345.40 (-NH-), 3234.21 (-NH-), 1698.42 (C=O), 1719.12 (C=O), 1742.26 (C=O), 1306.62 (C-N), 2981.80 (C-H), 3062.56 (=C-H), 622.31 (C-S), 1115.36 (C-O-C). ^{1}H-NMR [400 MHz, δ, ppm, DMSO-d_6]: 3.856 (6H, s, 3'-OCH$_3$ and 4'-OCH$_3$), 5.524 (1H, s, -CO-NH-), 4.518 (2H, s, -CH$_2$-CO-), 6.954-8.274 (8H, d and t, phenyl and indole), 8.562 (1H, s, =CH- methylene), 11.981 (1H, s, indole-NH-).^{13}C-NMR [400 MHz, δ, ppm, DMSO-d_6]: 179.8, 169.5, 165.7, 155.5, 146.4, 143.5, 136.5, 134.2, 131.3, 128.7, 126.2, 123.6, 121.7, 119.2, 117.5, 115.1, 113.6, 110.8, 108.4, 59.8, 48.5. ESI-MS: m/z (M^+) 437.

3-[N-(4-hydroxy-3-methoxyphenyl)acetamido]-5-[(indol-3-yl)methylene]-thiazolidine-2,4-dione(4i): IR [KBr ν cm^{-1}]: 3519.62 (Ph-OH), 3325.32 (-NH-), 3221.42 (-NH-), 1708.40 (C=O), 1722.18 (C=O), 1739.55 (C=O), 1310.65 (C-N), 2967.87 (C-H), 3084.52 (=C-H), 628.41 (C-S), 1119.48 (C-O-C). ^{1}H-NMR [400 MHz, δ, ppm, DMSO-d_6]: 3.915 (3H, s, -OCH$_3$), 5.614 (1H, s, -CO-NH-), 4.624 (2H, s, -CH$_2$-CO-), 7.054-8.312 (8H, d and t, phenyl and indole), 8.442 (1H, s, =CH-

methylene), 9.865 (1H, s, phenyl-OH), 12.181 (1H, s, indole-NH-).^{13}C-NMR [400 MHz, δ, ppm, DMSO-d_6]: 180.5, 167.6, 165.2, 153.7, 145.9, 142.6, 135.4, 133.7, 131.6, 129.6, 127.5, 125.6, 122.3, 119.4, 116.3, 114.1, 112.4, 110.1, 107.5, 57.5, 49.2. ESI-MS: m/z (M^+) 423.

3-[N-(4-bromophenyl)acetamido]-5-[(indol-3-yl)methylene]-thiazolidine-2,4-dione (4j): IR [KBr ν cm^{-1}]: 3373.72 (-NH-), 3240.81 (-NH-), 1754.51 (C=O), 1738.21 (C=O), 1705.25 (C=O), 1354.64 (C-N), 2971.60 (C-H), 3048.40 (=C-H), 629.31 (C-S), 1659.34 (C=C), 524.61 (C-Br). ^{1}H-NMR [400 MHz, δ, ppm, DMSO-d_6]: 5.486 (1H, s, -CO-NH-), 4.681 (2H, s, -CH_2-CO-), 6.952-8.459 (9H, d and t, phenyl and indole), 8.735 (1H, s, =CH- methylene), 12.058 (1H, s, indole-NH-).^{13}C-NMR [400 MHz, δ, ppm, DMSO-d_6]: 178.6, 169.5, 164.3, 143.6, 139.6, 137.4, 134.5, 131.5, 128.2, 126.3, 123.7, 121.9, 120.3, 118.6, 116.7, 114.2, 112.5, 48.6. ESI-MS: m/z (M^+) 456.

3-[N-(4-isopropylphenyl)acetamido]-5-[(indol-3-yl)methylene]-thiazolidine-2,4-dione (4k): IR [KBr ν cm^{-1}]: 3386.53 (-NH-), 3238.85 (-NH-), 1749.55 (C=O), 1733.33 (C=O), 1706.84 (C=O), 1362.62 (C-N), 2986.64 (C-H), 3078.46 (=C-H), 631.37 (C-S), 1653.31 (C=C). ^{1}H-NMR [400 MHz, δ, ppm, DMSO-d_6]: 2.156-2.209 (6H, dd, H_3C-CH-CH_3), 3.277-3.476 (1H, m, H_3C-CH-CH_3), 5.364 (1H, s, -CO-NH-), 4.784 (2H, s, -CH_2-CO-), 7.056-8.557 (9H, d and t, phenyl and indole), 8.642 (1H, s, =CH- methylene), 11.954 (1H, s, indole-NH-). ^{13}C-NMR [400 MHz, δ, ppm, DMSO-d_6]: 175.4, 168.3, 165.3, 146.8, 143.4, 138.6, 136.9, 133.5, 129.2, 127.5, 125.7, 123.9, 121.6, 120.8, 118.2, 116.1, 113.2, 49.2, 35.8, 27.6. ESI-MS: m/z (M^+) 419.

In vivo hypoglycemic activity screening: Wister albino rats (160-200 gm weight) of both sexes have been taken for acute and chronic studies were purchased from Sainadh Agencies- Laboratory animal suppliers, Hyderabad. Acute and chronic studies were carried out on Alloxan induced wister albino rats by tail tipping method [**18**]. Rats were acclimatized for a week to normal laboratory conditions prior to commencing the experiments, fed with tap water and pellet *ad libitum*. Rats were housed in cages for 12 h 12 h^{-1} dark and light cycle at room temperature. Intraperitoneally Alloxan monohydrate 120 mg kg^{-1} in normal saline was administered to the acclimatized animals, kept fasting for 24 h with water *ad libitum*. To overcome the early hypoglycemic phase, 5% dextrose solution was given for a day. After 72 h, by tail tipping method a drop of blood from tail vein was collected and blood glucose levels as well as biochemical parameters were measured using digital Accu-Chek active digital glucometer and Robonik biochemical analyzer respectively. Rats having blood glucose levels beyond 150 mg dL^{-1} were selected for the study and divided into six groups. For acute study, 36 mg kg^{-1} body weight dose was calculated by considering thiazolidinedione derivatives equivalent to average human intake 200 mg kg^{-1}. The test compounds were given orally by mixing with CMC-0.25% solution. 30 mg kg^{-1} body weight dose of Rosiglitazone was used as standard. At 0 h, 1 h, 2 h, 4 h, 6 h and 8 h the blood samples were withdrawn and analyzed for blood glucose level. Based on acute study results, limited compounds were selected for chronic study. A dose of 35 and 70 mg kg^{-1} body weight was taken into consideration in chronic study. On day 7 and day 15, decrease in blood glucose levels and body weight was measured.

Molecular Docking Studies: Docking studies were performed to identify novel insulin sensitizers by observing the molecular interactions of designed ligands with PPARγ receptor protein. The picking of target protein for docking is based on several factors such as it must possess resolution between 2.0 to 3.0Aº, structure should be determined by X-ray diffraction, consists of co-crystallized ligand and does not have any protein breaks in selected protein 3D structure. PPARγ receptor protein is the most promising target for the identification of hypoglycaemic agents possessing thiazolidinedione nucleus [**19, 20**]. The crystal structure of the PPARγ target receptor protein was obtained from the protein data bank PDB ID: 2PRG having a resolution of 2.3Aº. AutoDock 4.2.6 software was utilized to know the type of interactions of designed 3D-structured thiazolidine2,4-diones with the 2PRG active site region. ChemDraw Ultra 8.0 software was used to draw the designed structures and those were converted into suitable 3D models. By applying molecular mechanics they were subjected to energy minimizations which are required for molecular docking and for the preparation of corresponding PDB files. Docking studies were preformed to find out the possible locations for the ligand in active

site region of the receptor. Grid based docking studies was carried out using default parameters and docking was performed by considering Rosiglitazone as standard ligands at PPARγ receptor protein active site.

RESULTS AND DISCUSSION

According to the literature procedures [21, 22], initially thiazolidine-2,4-dione (1) was synthesized conventionally. 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 [23]. Finally the titled compounds (4a-4k) were prepared [24] by the reaction of 5-[(indol-3-yl) methylene]-thiazolidine-2,4-dione (2) with various substituted 2-chloro-N-aryl acetamide derivatives (3a-3k) which were prepared by the chloro acetylation of aryl amines [25]. The titled compounds were also prepared by microwave-assisted irradiation techniques according to the literature procedures [26-28] with different power levels. The scheme of synthesis of titled thiazolidine-2,4-dione derivatives (4a-4k) was depicted in figure 2. The physical characterization data, the comparative studies of conventional synthesis and microwave irradiation synthesis with respect to percentage yields and reaction time intervals were shown in table 1.

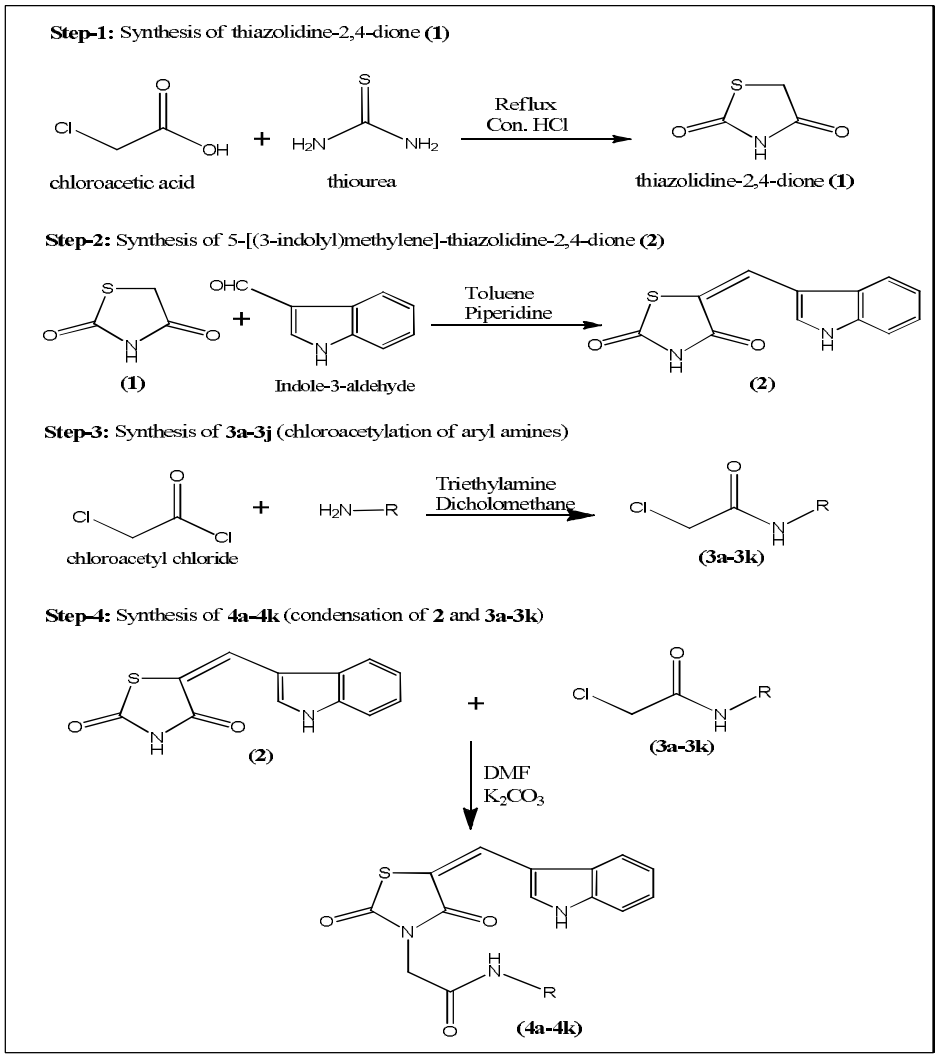


Figure 2. Scheme of synthesis of thiazolidine-2,4-dione derivatives (4a-4k).

Table 1. Physical characterization data of synthesized compounds 4a-4k

Compound	R	m.p. (°C)	Molecular formula	M.W	Conventional synthesis		Microwave synthesis	
					% yield	Reaction time (h)	% yield	Reaction time (min)
4a	4-chlorophenyl	192-194	$C_{20}H_{14}N_3ClO_3S$	411.86	66.65	5	75.91	6
4b	2,4-dichlorophenyl	214-216	$C_{20}H_{13}N_3Cl_2O_3S$	445.32	64.56	6	75.12	8
4c	4-fluorophenyl	198-200	$C_{20}H_{14}N_3FO_3S$	395.41	70.28	5	74.86	6
4d	4-nitrophenyl	212-214	$C_{20}H_{14}N_4O_5S$	422.41	68.55	4.5	75.48	7
4e	4-hydroxyphenyl	188-190	$C_{20}H_{15}N_3O_4S$	393.42	67.62	5	76.45	8
4f	*p*-tolyl	204-206	$C_{21}H_{17}N_3O_3S$	391.44	66.85	6	73.52	8
4g	4-methoxyphenyl	222-224	$C_{21}H_{17}N_3O_4S$	407.44	70.46	5	81.75	7
4h	3,4-dimethoxyphenyl	228-230	$C_{22}H_{19}N_3O_5S$	437.47	68.70	6	75.50	6
4i	4-hydroxy-3-methoxy	236-238	$C_{21}H_{17}N_3O_5S$	423.44	70.23	6	79.45	6
4j	4-bromophenyl	210-212	$C_{20}H_{14}N_3BrO_3S$	456.31	70.56	6	78.28	7
4k	4-isopropylphenyl	232-234	$C_{23}H_{21}N_3O_3S$	419.50	66.24	5	79.54	6

In vivo hypoglycaemic activity efficacy: *In vivo* hypoglycaemic activity study protocols were approved by the Institutional Animal Ethics Committee (IAEC) under the supervision of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi bearing registration number: 1847/PO/Re/S/16/CPCSEA. Blood glucose levels and body weights were expressed as mean ± standard error of mean (SEM). Statistically the values were analyzed by one-way ANOVA (analysis of variance) followed by Dunnet's't' test. Acute and chronic study results were given in table 2 and table 3 respectively. From data of acute study results, compounds 4a and 4b shown significant hypoglycaemic activity and those were tested for chronic study at 35 and 70 mg kg^{-1} body weights. Chronic study results indicate that compounds 4a and 4b showed significant activity at a dose of 70 mg kg^{-1} body weight.

Table 2. Acute study effect of compounds 4a-4k on blood glucose levels

Compound	Mean ± SEM of Blood glucose level, mg dL^{-1}					
	0 h	1 h	2 h	4 h	6 h	8 h
Normal	124.31±3.24	121.55±3.15	121.81±4.28	122.64±4.24	122.19±5.01	121.49±4.12
Standard	397.81±3.45**	248.41±4.35*	195.24±3.21	150.54±4.31*	112.61±3.42	102.45±4.71**
4a	323.3±5.46	280.3±4.41*	200.3±7.23	156.33±4.33**	118.7±6.57	105.12±4.73
4b	335.3±4.42*	275.31±4.1	195.11±4.3	148.15±3.21	116.2±5.24**	103.2±2.34
4c	339.3±4.06**	315±2.89	298.7±3.53	275±5.78**	235±2.89	201.7±6.02
4d	316.5±6.51	297.3±6.37**	285.3±6.02	242.1±8.67	225.3±6.02*	199.8±2.89
4e	345.2±2.63	305.2±1.25	263.4±5.12*	235.6±2.1	255±3.22**	282.1±2.45**
4f	324.25±3.18*	300.51±5.21*	273.2±4.41*	263.41±4.24	275.15±6.12**	300±4.33*
4g	338.5±6.41*	295.31±4.38**	262.31±3.16	241.41±4.61*	270.14±5.37*	284.33±6.42
4h	378.1±7.47	293.51±5.22	258.28±4.51*	237.14±5.32**	200.41±4.63	176.3±5.18
4i	356.42±4.68*	289.61±5.65	245.6±5.22	228.23±5.46**	212.65±6.11*	200.4±6.24**
4j	359.64±5.44	301.14±6.28**	266.61±7.12	232.42±6.15*	206.9±5.61	190.61±2.68
4k	75.41±2.68**	299.44±3.33	271.35±3.48*	254.67±3.22	268.24±4.81**	279.5±5.42*

Standard Drug-Rosiglitazone; Statistical analysis is done by One-Way ANOVA followed by Dunnet's 't' test; ** P<0.01 (considered as significant when compared to normal group), * P<0.001.All the values were expressed as mean±SEM, n=6.

Table 3. Chronic study effect of synthesized compounds 4a and 4b on fasting blood glucose level and body weight

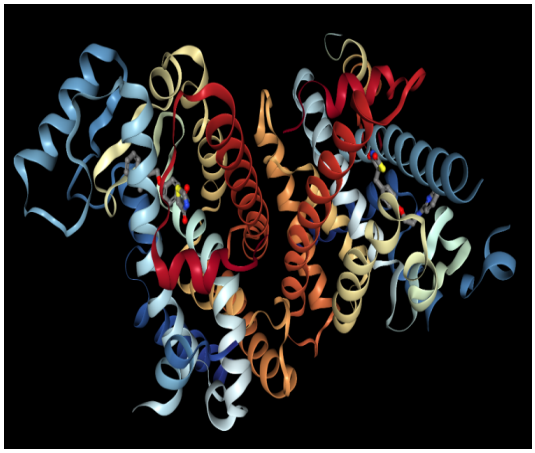
Compound	Blood glucose in mg dL^{-1}			Body Weight in gm		
	Day 0	Day 7	Day 15	Day 0	Day 7	Day 15
Standard	355.31±5.34	210.31±4.18**	162.61±2.35	198.11±4.18	195.72±2.25	195.32±5.24**
4a (35 mg kg^{-1} bw)	363.28±3.65*	226.31 ± 4.51*	180.33±3.24	194.27±3.44	195.41± 1.35**	194.43±3.21**
4a (70 mg kg^{-1} bw)	348.36±4.21	215.67±2.63**	165.45±4.22	196.45±1.59**	194.48±2.28*	195.71±2.64
4b (35 mg kg^{-1} bw)	358.71±3.55*	236.51 ± 4.26	186.43±5.34**	198.32±2.24	196.51 ± 2.67**	196.42±3.45
4b (70 mg kg^{-1} bw)	336.15±2.48	220.28± 1.69**	166.47±2.84**	195.41±3.67**	196.46± 4.54*	195.51±2.38

Standard Drug-Rosiglitazone; Statistical analysis is done by One-Way ANOVA followed by Dunnet's 't' test; ** P<0.01 (considered as significant when compared to normal group), * P<0.001.All the values were expressed as mean±SEM, n=6.

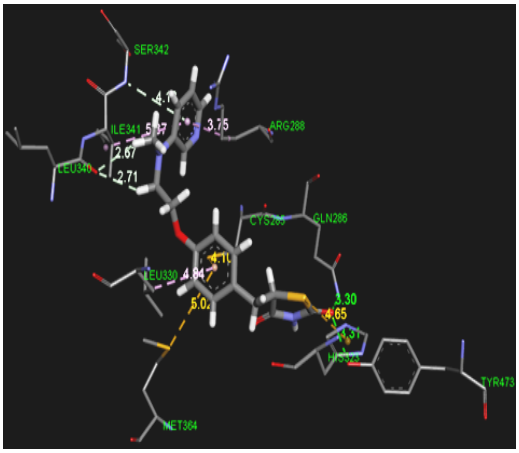
Molecular docking results: Molecular docking studies at PPARγ receptor protein active site regions give the data of binding energy (kcal mol^{-1}), number of hydrogen bonds, hydrogen bond length and interacted amino acid residues were placed in table 4. In comparison with standard ligand Rosiglitazone (binding energy -8.32 kcal mol^{-1}, compound 4a and 4b shown higher binding energy (-9.17 kcal mol^{-1} and -9.25 kcal mol^{-1}) at the active site region of PPARγ receptor protein. Figure 3 depicts the 3D structure of PPARγ receptor protein, binding mode of Rosiglitazone, compound 4a and 4b binding mode at active site region of PPARγ protein PDB ID: 2PRG.

Table 4. Binding energy and amino acid residues interacted with the PPARγ protein target PDB ID - 2PRG

Compound	Binding energy (kcal mol^{-1})	No. of Hydrogen bonds	Hydrogen bond length	Interacted amino acid residues
Rosiglitazone	-8.32	5	3.29, 3.30, 2.70, 2.66, 4.12	Gln286, Tyr473, His323, Arg288, Ile341, Met364, Cys285, Leu330
4a	-9.17	4	3.20, 2.55, 2.91, 3.31	Gln286, Tyr473, Leu453, His449, Cys285, Met364, Ile341, Leu340, Arg288
4b	-9.25	4	3.89, 4.97, 4.88, 4.14	Gln283, Cys285, Leu333, Gly284, Arg288, Ile341, Leu270
4c	-8.10	3	3.72, 2.76, 3.04	Gly284, Leu270, Cys285, Met364, Leu330, Arg288
4d	-8.74	4	2.65, 3.51, 2.48, 3.31	Cys285, Arg288, Ile341, Ser342, Gly284, Gln283, Leu270
4e	-7.59	3	2.95, 2.67, 3.58	Leu340, Cys285, Tyr473, Arg288, Gly284, Met364
4f	-7.19	4	3.14, 4.05, 2.64, 3.68	Cys285, Met364, Ile341, Leu340, Arg288, Leu333
4g	-8.25	3	3.56, 3.45, 2.97	Tyr473, Leu453, Leu270, Cys285, Met364, Ile341
4h	-7.45	5	3.64, 2.91, 3.72, 3.15, 2.17	Leu270, Ile341, Cys285, Tyr473, His323
4i	-8.07	4	2.90, 3.51, 2.85, 3.48	Arg288, Leu270, Ile341, Gln283, Met364, Cys285
4j	-8.01	3	3.57, 3.94, 3.26	Arg288, Cys285, Leu330, Ile341, Met364, Gln286
4k	-7.48	4	3.88, 2.57, 3.81, 2.46	Cys285, Leu453, His449, Tyr473, Ile341, Gly284

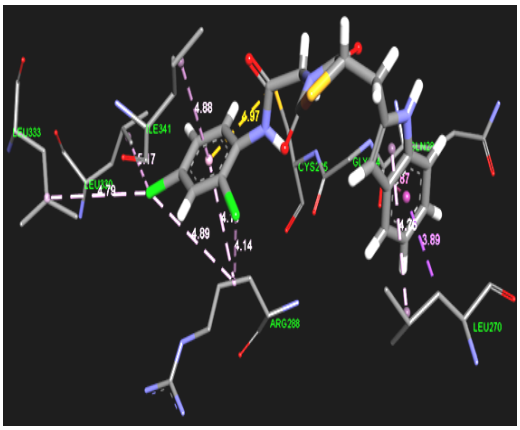


PPARγ protein 3D-structure from PDB ID- 2PRG



Binding mode of Rosiglitazone at active site region of PPARγ protein PDB ID- 2PRG

Binding mode of compound 4a at active site region of PPARγ protein PDB ID- 2PRG



Binding mode of compound 4b at active site region of PPARγ protein PDB ID- 2PRG

Figure 3. Molecular docking studies at active site region of PPARγ protein receptor PBD ID – 2PRG.

APPLICATION

Microwave irradiation technique for compounds 4a-4k produced high yield at less reaction time in comparison with traditional conventional method. *In vivo* hypoglycaemic activity evaluation results reveals that compound 4a and 4b shown significant hypoglycaemic activity in both acute and chronic toxic studies.

CONCLUSION

In this investigation various novel thiazolidinedione derivatives possessing N-aryl acetamide at 3rd position and indolyl methylene at 5th position was developed. Titled compounds 4a-4k were designed and synthesized by conventional and microwave irradiation methods. Microwave irradiation technique produced high yield at less reaction time in comparison with traditional conventional method. Characterization of the compounds was done by physically and spectrally. All the compounds were evaluated for *in vivo* hypoglycemic activity. *In vivo* hypoglycemic activity evaluation reveals that compound 4a and 4b shown significant hypoglycaemic activity in both acute and chronic toxic studies. Molecular docking studies at PPARγ receptor protein (PDB ID-2PRG), the compounds 4a and 4b exhibit significant binding affinity when compared with standard ligand Rosiglitazone.

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Conflict of interest: The authors declare that they do not have any conflict of interest related to the matter or content discussed in this original research article.

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