Turk J Pharm Sci 2019;16(4):380-91
DOI: 10.4274/tjps.galenos.2018.82612

ORIGINAL ARTICLE

Synthesis and Hypoglycemic and Anti-inflammatory Activity Screening of Novel Substituted 5-[Morpholino(Phenyl)Methyl]-Thiazolidine-2,4-Diones and Their Molecular Docking Studies

Yeni Sübstitüe 5-[Morfolino(Fenil)Metil]-Tiazolidin-2,4-Dionların Sentezi ve Hipoglisemik ve Antienflamatuvar Aktivitelerinin Taranması ile Moleküler Doking Çalışmaları

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ABSTRACT

Objectives: The aim was the synthesis of novel substituted 5-[morpholino(phenyl)methyl]-thiazolidine-2,4-diones and screening for their *in vivo* hypoglycemic activity and *in vitro* anti-inflammatory activity, as well as molecular docking studies to find out active potential lead molecules.

Materials and Methods: Substituted aromatic aldehydes, thiazolidine-2,4-dione, and morpholine on Mannich reaction gave the title compounds. They were characterized by physical and spectral methods. In vivo hypoglycemic activity was examined in alloxan induced Wistar albino rats by tail tipping method. In vitro anti-inflammatory activity was tested by human red blood cell (HRBC) membrane stabilization and protein denaturation. Using AutoDock, molecular docking studies were carried out to find out the best fit ligands.

Results: Series of substituted 5-[morpholino(phenyl)methyl]-thiazolidine-2,4-diones were synthesized and chemically they were confirmed by spectral techniques. Acute toxic studies of *in vivo* hypoglycemic activity results revealed that compounds 4c, 4h, and 4n exhibited good activity at 35 mg/kg body weight. Chronic toxic study results indicated that compounds 4h and 4n exhibited good activity at 70 mg/kg body weight. Anti-inflammatory activity results indicated the highest inhibition was shown by compounds 4k and 4f at 500 µg/mL in HRBC membrane stabilization. In protein denaturation, the highest inhibition was shown by compound 4k at 500 µg/mL. In molecular docking studies, compounds 4h and 4n exhibited higher binding affinity at PPARγ receptor protein and compound 4k exhibited higher binding affinity at COX-1 and COX-2 actives sites.

Conclusion: Microwave irradiation produced high yield in short reaction times. The presence of electron releasing groups at the para position of the phenyl ring may give the ability to produce hypoglycemic activity and the presence of electron withdrawing groups at the para position of the phenyl ring causes anti-inflammatory activity. The results showed that some compounds exhibited good hypoglycemic and anti-inflammatory activities. Compounds 4h and 4n exhibited higher binding affinity at PPARγ receptor protein and compound 4k exhibited higher binding affinity at COX isoenzymes' active sites in molecular docking studies.

Key words: Thiazolidinediones bearing morpholine, Mannich reaction, *in vivo* hypoglycemic activity, *in vitro* anti-inflammatory activity, docking studies

ÖZ

Amaç: Bu çalışmanın amacı, yeni sübstitüe 5-[morfolino(fenil)metil]-tiyazolidin-2,4-dionların sentezi ve *in vivo* hipoglisemik ve *in vitro* anti-enflamatuvar aktivitelerinin taranması ile olası aktif moleküller için moleküler doking çalışmalarının yapılmasıdır.

Gereç ve Yöntemler: Bileşikler; sübstitüe aromatik aldehidler, tiyazolidin-2,4-dion ve morfolinin mannich reaksiyonu sonucu elde edilmiş, elde edilen bileşikler fiziksel ve spektral yöntemlerle karakterize edilmiştir. *İn vivo* hipoglisemik aktivite, alloxan ile indüklenen Wistar albino farelerde "tail

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Received: 04.05.2018, Accepted: 20.07.2018

tipping" yöntemi, *in vitro* anti-enflamatuar aktivite ise, insan kırmızı kan hücrelerinde membran stabilizasyonu ve protein denatürasyon yöntemleri ile gerçekleştirilmiştir. AutoDock kullanarak, en uygun ligandları bulmak için moleküler doking çalışmaları yapılmıştır.

Bulgular: Sübstitüe 5-[morfolino(fenil)metil]-tiyazolidin-2,4-dion serileri sentezlenmiş ve spektral tekniklerle kimyasal yapıları teyit edilmiştir. *İn vivo* hipoglisemik aktivite sonuçlarıyla ilgili akut toksik çalışmaları, 4c, 4h ve 4n bileşiklerinin 35 mg/kg'da, kronik toksisite çalışmaları da, bileşik 4h ve 4n'nin 70 mg/kg'da iyi aktivite sergilediklerini göstermektedir. Antienflamatuvar aktivite sonuçları, membran stabilizasyon yönteminde en yüksek inhibisyonunun 500 µg/mL'de 4k ve 4f bileşikleri ile görüldüğünü göstermektedir. Protein denatürasyonunda, 500 µg/mL'de bileşik 4k en yüksek inhibisyonu göstermiştir. Moleküler doking çalışmalarında, 4h ve 4n bileşiklerinin PPARγ reseptör proteinine, 4k bileşiğinin de, COX-1 ve COX-2 aktif bölgelerine daha yüksek bağlanma afinitesi sergilediği görülmüştür.

Sonuç: Mikrodalga tekniği düşük reaksiyon sürelerinde yüksek verim sağlamıştır. Fenil halkasının para pozisyonunda; elektron salıcı grupların hipoglisemik aktivite oluşturabileceği, elektron çekici grupların ise antienflamatuvar aktiviteye neden olabileceği belirtilmiştir. Sonuçla, bazı bileşiklerin iyi hipoglisemik ve anti-enflamatuar aktivite sergilediklerini göstermiştir.

Moleküler doking çalışmalarında; bileşik 4h ve 4n, PPARγ reseptör proteininde, bileşik 4k da COX izoenzimleri aktif bölgelerinde daha yüksek bağlanma afinitesi sergilemiştir.

Anahtar kelimeler: Morfolin taşıyan tiyazolidinonlar, mannich reaksiyonu, *in vivo* hipoglisemik aktivite, *in vitro* antienflamatuvar aktivite, doking çalışmaları

INTRODUCTION

Diabetes mellitus (DM) is a common chronic metabolic syndrome in which high blood sugar levels occur over a prolonged time and symptoms include frequent urination, increased hunger, and increased thirst. DM is associated with severe degenerative complications such as nephropathy, neuropathy, cataract, retinopathy, accelerated atherosclerosis, stroke, and increased risk of myocardial infarction. Onset of these pathologies is a remarkable event in the course of both type 1 and type 2 diabetes. Prevention and control are still serious challenging therapeutic problems as they stand for the foremost causes of morbidity and mortality for diabetic patients.[1] Type 1 diabetes is characterized by deficient insulin production and symptoms include excessive thirst (polydipsia) and excretion of urine (polyuria), weight loss, constant hunger, fatigue, and vision changes requiring daily administration of insulin. Type 2 diabetes results from the body's ineffective impaired insulin action. The majority of cases around the world are type 2 diabetes and it is principally the result of physical inactivity and excess body weight. Symptoms are similar to type 1 diabetes, but are less marked. In the past this type of diabetes was seen only in the adults but at present it is also happening frequently even in children. Normally in humans up to 80% of insulin stimulated glucose disposal takes place in the skeletal muscle, which is the major site of insulin resistance in type 2 diabetes.[2]

Worldwide the frequency of diabetes for all age groups was estimated to be 2.8% in 2000 and 4.4% in 2030. The total number of people with diabetes is expected to increase from 171 million in 2000 to 366 million in 2030. The occurrence of diabetes is higher in men than in women; however, there are more women with this diabetes than men. In developing countries, the urban population is expected to double between 2000 and 2030. Across the world the most important demographic change to diabetes prevalence appears to be an increase in the proportion of people greater than 65 years of age. Therefore, there is a need to develop novel drug candidates in this region.[3]

Heterocyclic compounds and their derivatives are a fascinating field in medicinal chemistry because of their remarkable biological and pharmacological properties. In the family of heterocyclic compounds, heterocycles with O, S, and N atoms have attracted more attention in chemistry research due to their wide range of biological activities. O and N atoms containing morpholine have a unique position in heterocyclic chemistry. Morpholine is a six-member saturated heterocyclic system having O and N as heteroatoms at the 1st and 4th positions, respectively. Clinically the morpholine ring contains drugs used in the market that have considerable importance, such as timolol (β-adrenergic blocker used in ocular hypertension and glaucoma), moclobemide (mono amino oxidase inhibitor used to treat depression and anxiety), reboxetine (norepinephrine reuptake inhibitor used as an antidepressant), pholcodine (opioid cough suppressant), emorfazone (nonsteroidal anti-inflammatory agent), gefitinib (epidermal growth factor receptor inhibitor used for certain breast, lung, and other cancers), and linezolid (antibiotic used to treat infections caused by gram-positive bacteria).[4]

Similarly, the thiazolidine-2,4-dione (TZD) nucleus is also one of the most important heterocyclics that has received much attention especially in the treatment of diabetes. TZD is a five-member heterocyclic compound that consists of S and N as heteroatoms in the ring at the 1st and 3rd positions, respectively, along with ketone functional groups at the 2nd and 4th positions. The TZD class of drugs includes troglitazone, ciglitazone, rosiglitazone, and ciglitazone with an insulin sensitizing property. Troglitazone was withdrawn from the market in 2000 due to its hepatotoxicity producing nature. TZDs are found to produce hypoglycemic activity by lowering the blood glucose levels significantly through the activation of peroxisome proliferator-activated receptor gamma (PPARγ) receptors.[5] TZDs enhance the insulin action and promote glucose utilization in the peripheral tissues. The exact mechanism of TZDs has not been elucidated, but is expected to exhibit pharmacological actions by binding and agonistic properties at the PPARγ nuclear receptor.[6]

Recently TZDs have been found to possess a wide range of biological activities such as antidiabetic,[7] protein tyrosine phosphatise 1B inhibitory,[8] 15-hydroxyprostaglandin

dehydrogenase (15-PGDH) inhibitory,[9] hypolipidemic,[10] aldose reductase inhibitory,[11] anti-inflammatory,[12] antimicrobial,[13] antitubercular,[14] antioxidant,[15] antitumor,[16] and antiproliferative.[17] From the literature observations, we made an attempt to design and develop novel TZD derivatives bearing morpholine in their structures using the Mannich reaction by both conventional heating and microwave irradiated methods. Microwave techniques give high yields, decrease by-product formation, and decrease the decomposition of products as compared to the conventional heating reaction methods.[18,19] The developed compounds were characterized and screened for biological activities. By using AutoDock molecular docking software the binding energies of the designed ligands and their interactions at the target protein were studied.

MATERIALS AND METHODS

All the chemicals required for the synthesis of novel thiazolidinediones such as reagents and solvents were obtained from commercial suppliers in Merck grade and further they were used without purification. Reaction progress and completion were monitored by thin layer chromatography with the help of 0.25 mm E. Merck grade silica gel 60GF-254 precoated TLC plates; spots were observed under ultraviolet (UV)-light and in an iodine chamber. Fourier Transform Infrared Spectrometer (FT-IR) spectra of the compounds were recorded with a Bruker FT-IR analyzer spectrophotometer by compression of compound with anhydrous KBr under vacuum using the KBr pressed pellet technique. Chemical shifts in δ, ppm of proton nuclear magnetic resonance (^{1}H-NMR) and carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectra were recorded on a Bruker AMX 400 MHz spectrometer using deuterated dimethyl sulfoxide (DMSO) solvent and tetramethylsilane as internal standard. Mass spectra of the compounds were recorded on an Agilent LC-MSD 1200 mass spectrometer. For conventional synthesis normal reflux condenser setup and microwave assisted synthesis was performed on an RGSSIRR model Raga's scientific microwave system having different power levels from 140 W to 700 W. Melting points were determined by electrical melting point apparatus and were uncorrected. The Wistar albino rats used in the hypoglycemic study were divided into various groups and each group contained 6 rats (n=6).

Chemistry

*Synthesis of thiazolidine-2,4-dione (**3**)*

A. Conventional synthesis: Chloroacetic acid (20 mmoL) and thiourea (20 mmoL) were separately dissolved in 5 mL of water. The contents of the vessels were transferred into a three-necked round bottom flask and stirred until white precipitate was obtained. The reaction mixture was cooled and conc. Hydrochloric acid [(HCl) 6 mL] was added slowly to it from the dropping funnel. It was refluxed by applying gentle heat for about 10-12 h at 100-110°C. The contents of the flask were cooled to solidify them and they were filtered to obtain the product by washing with water. Recrystallization was done using ethyl alcohol.[20]

B. Microwave irradiation synthesis: A mixture of chloroacetic acid (10 mmoL) and thiourea (10 mmoL) dissolved in 5 mL of water was transferred into the microwave synthesizer reaction vessel. The reaction vessel was closed with the help of lids and condenser and stirred for about 1 h in cold condition. Then 3 mL of conc. HCl was added to the reaction mixture and irradiated for 6 min using 280 W power level at 120°C. The reaction mixture was cooled to room temperature and the obtained solid was filtered, dried, and recrystallized from ethyl alcohol.[21]

78.42% (conventional synthesis yield), 90.25% (MWI synthesis yield), white crystalline powder, melting point 124-126°C, R_f value 0.62 from using 9:1 v/v of chloroform and methanol. 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]: 35.80, 173.06, 173.82. ESI-MS: (M^+) m/z 117.

*General procedure for synthesis of compounds **4a-4n***

A. Conventional synthesis: 0.01 mol of TZD (**3**) was dissolved in 5 mL of ethanol and 0.01 moL of substituted aromatic benzaldehyde was added to this solution. The mixture was stirred for about 30 min at room temperature. To the reaction mixture 0.01 moL of morpholine and a catalytic amount of conc. HCl (3-5 drops) were added, followed by refluxing for 5-6 h. Completion of the reaction was monitored by TLC using mobile phase n-hexane and ethylacetate (9:1). The reaction mixture was allowed to cool for about 2-4 h and then it was poured into ice cold water. The product was collected by filtration and washed with cold water followed by dry toluene. It was dried and recrystallized with absolute ethanol.[22]

B. Microwave irradiation synthesis: 0.01 moL of TZD (**3**) was dissolved in 5 mL of ethanol and 0.01 moL of substituted aromatic benzaldehyde was added to it. The reaction mixture was stirred for 30 min at RT. To the above solution 0.01 moL of morpholine and 3-5 drops of conc. HCl were added. It was mixed well and placed in a Raga's scientific microwave synthesizer vessel and the reaction mixture was irradiated at 420 W power level for about 6-10 min at 120°C. The reaction mixture was cooled to room temperature and treated with ice cold water for the work up process. The obtained precipitate was filtered, washed, and dried to get the desired compound. Completion of the reaction was monitored by TLC using the same mobile phase mentioned in the above conventional synthesis.[23]

5-[(4-chlorophenyl)-morpholin-4-yl-methyl]-thiazolidine-2,4-dione **(4a)**

IR [KBr ν cm^{-1}]: 3331.29 (-NH-), 1660.56 (C=O), 1263.44 (C-N), 2984.21 (C-H), 3045.20 (=C-H), 615.50 (C-S), 1104.13 (C-O-C), 785.56 (C-Cl). ^{1}H-NMR [400 MHz, δ, ppm, DMSO-d_6]: 12.105 (s, 1H, NH), 4.305-4.317 (d, 1H, TZD CH), 4.012-4.041 (d, 1H, N-CH), 7.355-7.421 (d, 2H, 3'-H&5'-H), 6.945-7.014 (d, 2H, 2'-H&6'-H), 3.642-3.685 (t, 4H, CH_2-O-CH_2), 2.475-2.635 (t, 4H, CH_2-N-CH_2). ^{13}C-NMR [400 MHz, δ, ppm, DMSO-d_6]: 173.15, 166.56, 138.45, 133.74, 130.32, 126.46, 68.44, 59.20, 56.48, 51.52. ESI-MS: (M^+) m/z 326.

5-[(2,4-dichlorophenyl)-morpholin-4-yl-methyl]-thiazolidine-2,4-dione **(4b)**

IR [KBr ν cm^{-1}]: 3456.54 (-NH-), 1672.55 (C=O), 1284.56 (C-N), 2949.85 (C-H), 3084.78 (=C-H), 632.04 (C-S), 1131.45 (C-O-C), 796.74 (C-Cl). ^{1}H-NMR [400 MHz, δ, ppm, DMSO-d_6]: 12.214 (s, 1H, NH), 4.321-4.348 (d, 1H, TZD CH), 4.124-4.168 (d, 1H, N-CH), 7.409 (s, 1H, 3'-H), 7.358-7.414 (d, 1H, 5'-H), 6.984-7.021 (d, 1H, 6'-H), 3.748-3.894 (t, 4H, CH_2-O-CH_2), 2.546-2.798 (t, 4H, CH_2-N-CH_2). ^{13}C-NMR [400 MHz, δ, ppm, DMSO-d_6]: 176.15, 167.08, 140.84, 136.56, 133.45, 131.63, 128.23, 125.46, 69.52, 60.45, 54.62, 50.45. ESI-MS: (M^+) m/z 361.

5-[(4-fluorophenyl)-morpholin-4-yl-methyl]-thiazolidine-2,4-dione **(4c)**

IR [KBr ν cm^{-1}]: 3425.45 (-NH-), 1673.52 (C=O), 1274.25 (C-N), 2983.44 (C-H), 3063.74 (=C-H), 623.24 (C-S), 1121.47 (C-O-C), 1345.25 (C-F). ^{1}H-NMR [400 MHz, δ, ppm, DMSO-d_6]: 12.046 (s, 1H, NH), 4.215-4.298 (d, 1H, TZD CH), 4.112-4.156 (d, 1H, N-CH), 7.284-7.301 (d, 2H, 3'-H&5'-H), 6.845-7.001 (d, 2H, 2'-H&6'-H), 3.542-3.599 (t, 4H, CH_2-O-CH_2), 2.546-2.741 (t, 4H, CH_2-N-CH_2). ^{13}C-NMR [400 MHz, δ, ppm, DMSO-d_6]: 175.26, 168.54, 139.74, 134.52, 130.66, 124.48, 67.47, 58.45, 54.74, 50.62. ESI-MS: (M^+) m/z 310.

5-[(4-hydroxyphenyl)-morpholin-4-yl-methyl]-thiazolidine-2,4-dione **(4d)**

IR [KBr ν cm^{-1}]: 3415.41 (-NH-), 1681.46 (C=O), 1264.74 (C-N), 2986.21 (C-H), 3085.46 (=C-H), 631.42 (C-S), 1116.22 (C-O-C), 3505.46 (Ph-OH). ^{1}H-NMR [400 MHz, δ, ppm, DMSO-d_6]: 11.965 (s, 1H, NH), 4.317-4.365 (d, 1H, TZD CH), 9.845 (s, 1H, C_6H_4-O<u>H</u>), 4.205-4.246 (d, 1H, N-CH), 6.935-7.459 (m, 4H, C_6H_4-OH), 3.645-3.726 (t, 4H, CH_2-O-CH_2), 2.685-2.784 (t, 4H, CH_2-N-CH_2). ^{13}C-NMR [400 MHz, δ, ppm, DMSO-d_6]: 176.46, 167.89, 159.56, 138.46, 133.47, 115.48, 65.32, 59.64, 53.12, 51.32. ESI-MS: (M^+) m/z 308.

5-[(4-methylphenyl)-morpholin-4-yl-methyl]-thiazolidine-2,4-dione **(4e)**

IR [KBr ν cm^{-1}]: 3335.66 (-NH-), 1666.22 (C=O), 1272.65 (C-N), 2980.67 (C-H), 3095.26 (=C-H), 615.22 (C-S), 1128.27 (C-O-C). ^{1}H-NMR [400 MHz, δ, ppm, DMSO-d_6]: 12.025 (s, 1H, NH), 4.225-4.308 (d, 1H, TZD CH), 2.865 (s, 3H, C_6H_4-C<u>H</u>$_3$), 4.056-4.116 (d, 1H, N-CH), 6.849-7.554 (m, 4H, C_6<u>H</u>$_4$-CH_3), 3.720-3.842 (t, 4H, CH_2-O-CH_2), 2.784-2.951 (t, 4H, CH_2-N-CH_2). ^{13}C-NMR [400 MHz, δ, ppm, DMSO-d_6]: 178.66, 165.42, 158.45, 137.45, 134.62, 118.61, 66.30, 60.52, 54.11, 52.55, 30.28. ESI-MS: (M^+) m/z 306.

5-[(4-nitrophenyl)-morpholin-4-yl-methyl]-thiazolidine-2,4-dione **(4f)**

IR [KBr ν cm^{-1}]: 3364.22 (-NH-), 1693.45 (C=O), 1274.66 (C-N), 2968.41 (C-H), 3095.45 (=C-H), 618.62 (C-S), 1135.84 (C-O-C), 1362.04, 1546.15 (NO_2). ^{1}H-NMR [400 MHz, δ, ppm, DMSO-d_6]: 12.546 (s, 1H, NH), 4.015-4.068 (d, 1H, TZD CH), 4.325-4.366 (d, 1H, N-CH), 6.846-7.387 (m, 4H, C_6H_4-NO_2), 3.862-3.984 (t, 4H, CH_2-O-CH_2), 2.855-2.948 (t, 4H, CH_2-N-CH_2). ^{13}C-NMR [400 MHz, δ, ppm, DMSO-d_6]: 178.79, 168.46, 150.62, 143.52, 130.46, 120.84, 68.32, 59.23, 55.74, 50.85. ESI-MS: (M^+) m/z 337.

5-[(4-methoxyphenyl)-morpholin-4-yl-methyl]-thiazolidine-2,4-dione **(4g)**

IR [KBr ν cm^{-1}]: 3381.47 (-NH-), 1679.22 (C=O), 1269.61 (C-N), 2971.52 (C-H), 3068.94 (=C-H), 629.41 (C-S), 1130.49 (C-O-C). ^{1}H-NMR [400 MHz, δ, ppm, DMSO-d_6]: 11.985 (s, 1H, NH), 4.211-4.285 (d, 1H, TZD CH), 4.389-4.401 (d, 1H, N-CH), 6.956-7.421 (m, 4H, C_6<u>H</u>$_4$-OCH_3), 3.986 (s, 3H, 4'-OCH_3), 3.745-3.889 (t, 4H, CH_2-O-CH_2), 2.651-2.894 (t, 4H, CH_2-N-CH_2). ^{13}C-NMR [400 MHz, δ, ppm, DMSO-d_6]: 180.22, 169.56, 160.55, 133.52, 129.44, 118.22, 68.45, 64.21, 59.45, 54.66, 51.23. ESI-MS: (M^+) m/z 322.

5-[(3,4-dimethoxyphenyl)-morpholin-4-yl-methyl]-thiazolidine-2,4-dione **(4h)**

IR [KBr ν cm^{-1}]: 3421.46 (-NH-), 1685.26 (C=O), 1284.62 (C-N), 2979.85 (C-H), 3089.13 (=C-H), 620.45 (C-S), 1115.65 (C-O-C). ^{1}H-NMR [400 MHz, δ, ppm, DMSO-d_6]: 12.264 (s, 1H, NH), 4.145-4.189 (d, 1H, TZD CH), 4.374-4.485 (d, 1H, N-CH), 6.452 (s, 1H, 2'-H), 6.678-6.894 (d, 2H, 5'-H&6'-H), 3.964 (s, 6H, 3'-OCH_3&4'-OCH_3), 3.745-3.865 (t, 4H, CH_2-O-CH_2), 2.746-2.894 (t, 4H, CH_2-N-CH_2). ^{13}C-NMR [400 MHz, δ, ppm, DMSO-d_6]: 177.88, 169.46, 153.56, 149.25, 132.45, 125.26, 118.24, 113.46, 69.05, 61.85, 58.47, 55.62, 50.43. ESI-MS: (M^+) m/z 352.

5-[(3,4,5-trimethoxyphenyl)-morpholin-4-yl-methyl]-thiazolidine-2,4-dione **(4i)**

IR [KBr ν cm^{-1}]: 3385.41 (-NH-), 1676.74 (C=O), 1268.46 (C-N), 2959.74 (C-H), 3094.25 (=C-H), 628.71 (C-S), 1134.84 (C-O-C). ^{1}H-NMR [400 MHz, δ, ppm, DMSO-d_6]: 12.114 (s, 1H, NH), 4.058-4.194 (d, 1H, TZD CH), 4.405-4.492 (d, 1H, N-CH), 6.248 (s, 2H, 2'-H&6'-H), 3.954 (s, 9H, 3',4',5'-triOCH_3), 3.725-3.849 (t, 4H, CH_2-O-CH_2), 2.652-2.845 (t, 4H, CH_2-N-CH_2). ^{13}C-NMR [400 MHz, δ, ppm, DMSO-d_6]: 179.05, 168.19, 151.42, 140.74, 135.65, 112.27, 68.91, 63.64, 57.41, 53.44, 50.22. ESI-MS: (M^+) m/z 382.

5-[(4-hydroxy-3-methoxyphenyl)-morpholin-4-yl-methyl]-thiazolidine-2,4-dione **(4j)**

IR [KBr ν cm^{-1}]: 3325.41 (-NH-), 1675.64 (C=O), 1291.45 (C-N), 2980.65 (C-H), 3091.47 (=C-H), 622.11 (C-S), 1125.62 (C-O-C), 3512.22 (Ph-OH). ^{1}H-NMR [400 MHz, δ, ppm, DMSO-d_6]: 12.005 (s, 1H, NH), 4.105-4.184 (d, 1H, TZD CH), 4.357-4.462 (d, 1H, N-CH), 6.348 (s, 1H, 2'-H), 6.548-6.754 (d, 1H, 5'-H), 6.874-6.994 (d, 1H, 6'-H), 9.548 (s, 1H, C_6H_4-O<u>H</u>), 3.895 (s, 3H, 3'-OCH_3), 3.765-3.910 (t, 4H, CH_2-O-CH_2), 2.747-2.897 (t, 4H, CH_2-N-CH_2). ^{13}C-NMR [400 MHz, δ, ppm, DMSO-d_6]: 175.44, 167.41, 155.22, 148.45, 131.58, 123.21, 119.55, 112.41, 68.08, 62.44, 59.75, 56.32, 50.85. ESI-MS: (M^+) m/z 338.

5-[(4-bromophenyl)-morpholin-4-yl-methyl]-thiazolidine-2,4-dione **(4k)**

IR [KBr ν cm^{-1}]: 3389.81 (-NH-), 1686.35 (C=O), 1271.64 (C-N), 2992.67 (C-H), 3089.45 (=C-H), 621.12 (C-S), 1131.83 (C-O-C), 578.63 (C-Br). ^{1}H-NMR [400 MHz, δ, ppm, DMSO-d_6]: 12.158 (s, 1H, NH), 4.317-4.397 (d, 1H, TZD CH), 4.135-4.187 (d, 1H, N-CH), 7.278-7.314 (d, 2H, 3'-H&5'-H), 6.942-7.108 (d, 2H, 2'-H&6'-H), 3.618-3.685 (t, 4H, CH_2-O-CH_2), 2.612-2.732 (t, 4H, CH_2-N-CH_2). ^{13}C-NMR [400 MHz, δ, ppm, DMSO-d_6]: 178.54, 166.64, 138.18, 133.79, 130.52, 122.15, 66.35, 57.72, 53.23, 50.48. ESI-MS: (M^+) m/z 371.

5-[(3-bromophenyl)-morpholin-4-yl-methyl]-thiazolidine-2,4-dione **(4l)**

IR [KBr ν cm^{-1}]: 3412.54 (-NH-), 1678.75 (C=O), 1269.18 (C-N), 2987.2 (C-H), 3086.23 (=C-H), 619.84 (C-S), 1128.46 (C-O-C), 584.45 (C-Br). ^{1}H-NMR [400 MHz, δ, ppm, DMSO-d_6]: 11.846 (s, 1H, NH), 4.365-4.381 (d, 1H, TZD CH), 4.208-4.299 (d, 1H, N-CH), 7.875 (s, 1H, 2'-H), 7.457-7.510 (d, 1H, 4'-H), 7.108-7.201 (t, 1H, 5'-H), 6.847-6.912 (d, 1H, 6'-H), 3.748-3.8414 (t, 4H, CH_2-O-CH_2), 2.546-2.698 (t, 4H, CH_2-N-CH_2). ^{13}C-NMR [400 MHz, δ, ppm, DMSO-d_6]: 175.51, 168.25, 139.17, 136.85, 133.79, 130.52, 126.62, 121.87, 68.55, 58.74, 54.76, 50.08. ESI-MS: (M$^+$) m/z 371.

5-[(4-dimethylaminophenyl)-morpholin-4-yl-methyl]-thiazolidine-2,4-dione **(4m)**

IR [KBr ν cm^{-1}]: 3426.45 (-NH-), 1684.24 (C=O), 1289.47 (C-N), 2992.35 (C-H), 3084.47 (=C-H), 627.74 (C-S), 1118.64 (C-O-C). ^{1}H-NMR [400 MHz, δ, ppm, DMSO-d_6]: 12.248 (s, 1H, NH), 4.462-4.510 (d, 1H, TZD CH), 4.256-4.347 (d, 1H, N-CH), 7.415-7.511 (d, 1H, 2'-H&6'-H), 6.917-7.125 (d, 1H, 3'-H&5'-H), 3.148 (s, 6H, H_3C-N-CH_3), 3.839-3.990 (t, 4H, CH_2-O-CH_2), 2.847-2.954 (t, 4H, CH_2-N-CH_2). ^{13}C-NMR [400 MHz, δ, ppm, DMSO-d_6]: 178.77, 169.71, 152.82, 135.54, 129.64, 119.55, 68.74, 65.46, 59.44, 57.62, 43.25. ESI-MS: (M$^+$) m/z 335.

5-[(4-isopropylphenyl)-morpholin-4-yl-methyl]-thiazolidine-2,4-dione **(4n)**

IR [KBr ν cm^{-1}]: 3386.62 (-NH-), 1693.24 (C=O), 1276.48 (C-N), 2989.42 (C-H), 3090.48 (=C-H), 613.75 (C-S), 1125.64 (C-O-C). ^{1}H-NMR [400 MHz, δ, ppm, DMSO-d_6]: 12.156 (s, 1H, NH), 4.324-4.398 (d, 1H, TZD CH), 4.158-4.205 (d, 1H, N-CH), 7.146-7.205 (d, 1H, 2'-H&6'-H), 7.452-7.512 (d, 1H, 3'-H&5'-H), 2.421-2.564 (dd, 6H, $\underline{H}_3C$-CH-C$\underline{H}_3$), 2.875-3.105 (m, 1H, H_3C-C$\underline{H}$-CH_3), 3.754-3.897 (t, 4H, CH_2-O-CH_2), 2.759-2.851 (t, 4H, CH_2-N-CH_2). ^{13}C-NMR [400 MHz, δ, ppm, DMSO-d_6]: 174.32, 168.46, 150.41, 136.52, 129.22, 122.48, 67.71, 64.41, 58.32, 54.40, 35.46, 27.56. ESI-MS: (M$^+$) m/z 334.

Biological evaluation

In vivo hypoglycemic activity screening

Wistar albino rats of both sexes weighing 160-200 g for acute and chronic studies were purchased from Sainadh Agencies Laboratory animal suppliers, Hyderabad. Acute and chronic studies were carried out on alloxan induced Wistar albino rats by tail tipping method.[24,25] The rats were acclimatized for a week to the normal laboratory conditions prior to commencing the experiments, with *ad libitum* access to tap water and pellets. The rats were housed in cages with 12 h/12 h dark and light cycle at room temperature. Intraperitoneally alloxan monohydrate 120 mg/kg 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 the tail vein was collected and blood glucose levels as well as biochemical parameters were measured using a digital Accu-Chek active digital glucometer and Robonik biochemical analyzer, respectively. Rats having blood glucose levels above 150 mg/dL were selected for the study and divided into six groups. For the acute study, 36 mg/kg body weight dose was calculated by considering thiazolidinedione derivatives equivalent to average human intake 200 mg/kg. The test compounds were given orally by mixing with CMC-0.25% solution. Rosiglitazone at 30 mg/kg body weight dose was used as the standard drug. At 0 h, 1 h, 2 h, 4 h, 6 h, and 8 h blood samples were withdrawn and analyzed for blood glucose level. Based on the acute study results limited compounds were selected for the chronic study. Doses of 35 and 70 mg/kg body weight were taken into consideration in the chronic study. On day 7 and day 15, decrease in blood glucose was observed by measuring the blood glucose levels.

In vitro anti-inflammatory activity screening

In vitro anti-inflammatory activity was evaluated by human red blood cell (HRBC) membrane stabilization method and protein denaturation method.[26-28]

HRBC membrane stabilization method

Fresh whole blood was collected from a healthy human volunteer who had not used any nonsteroidal anti-inflammatory drug 2 weeks prior to the experiment and mixed with an equal volume of sterilized Alsever's solution (0.8% sodium citrate, 2% dextrose, 0.42% sodium chloride, and 0.05% citric acid in water). At 3000 rpm the blood was centrifuged for 10 min and the packed cells were washed three times with 0.85% isosaline (pH 7.2). The volume of blood was measured and reconstituted with isosaline as 10% v/v suspension. Different concentrations (50, 100, 300, and 500 µg/mL) of the test solutions were prepared in isosaline. To 1 mL of test solution were added 2 mL of hyposaline (0.25% w/v NaCl), 1 mL of 0.15 M phosphate buffer (pH 7.4), and 0.5 mL of 10% HRBC in isosaline. For the test control, 1 mL of distilled water used instead of hyposaline, while the product control lacked red blood cells. The mixtures were incubated at 37°C for 30 min and centrifuged at 3000 rpm for about 20 min. Diclofenac sodium (100 µg/mL and 200 µg/mL) was used as the reference drug. The hemoglobin content in the suspension was estimated using a UV-spectrophotometer at 560 nm. Percentage inhibition activity was calculated as follows:

$$\% \text{ inhibition} = 100 - \left[\frac{\text{OD of test}}{\text{OD of control}} \times 100\right]$$

where optical density (OD) of test sample is OD or absorbance of the test sample absorbance and OD of control is OD or absorbance of the negative control.

Protein denaturation method

Denaturation of proteins is a well-documented cause of inflammation. Salicylic acid, phenylbutazone, diclofenac, and flufenamic acid are anti-inflammatory drugs that have shown dose dependent ability for thermally induced protein denaturation. Agents that can prevent protein denaturation would be advisable for anti-inflammatory drug development. Each 0.5 mL of test sample solution consists of 0.45 mL of 5% w/v aqueous bovine serum albumin solution and 0.05 mL of test sample of different concentrations (50, 100, 300, and 500

μg/mL), while 0.5 mL of test control solution consists of 0.45 mL of 5% w/v aqueous bovine serum albumin solution and 0.05 mL of distilled water, and 0.5 mL of standard solution consists of 0.45 mL of 5% w/v aqueous bovine serum albumin solution and 0.05 mL of different concentrations (100 and 200 μg/mL) of diclofenac sodium. All the solutions were adjusted to pH 6.3 using 1 N HCl. The samples were incubated at 37°C for 20 min and the temperature was raised to keep the samples at 57°C for 3 min. After cooling, 2.5 mL of phosphate buffer was added to the above solutions. The absorbance was measured using a UV-visible spectrophotometer at 600 nm. The percentage inhibition of protein denaturation was calculated as follows:

$$\% \text{ inhibition} = 100 - \left[\left\{\frac{\text{OD of test} - \text{OD of product control}}{\text{OD of test control}}\right\} \times 100\right]$$

MOLECULAR DOCKING

Docking studies were performed to identify novel insulin sensitizers by observing the molecular interactions of designed ligands with PPARγ receptor protein and to determine the potential anti-inflammatory agents at cyclooxygenase (COX)-1 and COX-2 isoenzymes. The selection of the target protein for docking is based on several factors such as it must possess resolution between 2.0 and 3.0 Å, the structure should be determined by X-ray diffraction, it consists of co-crystallized ligand, and it does not have any protein breaks in the selected protein's 3D structure. PPARγ receptor protein is the most promising target for the identification of antidiabetic drugs possessing a thiazolidinedione nucleus.[29,30] The crystal structure of the PPARγ target receptor protein was obtained from the protein databank PDB ID: 2PRG having resolution of 2.3 Å. COX isoenzymes were the targets for determining the anti-inflammatory activity and the target proteins were downloaded from a protein databank PDB ID: 1EQG (COX-1) and PDB ID: 1CX2 (COX-2) having a resolution of 2.6 Å and 3.0 Å, respectively.[31] AutoDock 4.2.6 software was utilized to know the type of interactions of the designed 3D-structured thiazolidinediones with the 2PRG, 1EQG, and 1CX2 active site regions. ChemDraw Ultra 8.0 software was used to draw the designed structures and they 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 the active site region of the receptor. Grid-based docking studies was carried out using default parameters and docking was performed by considering rosiglitazone, indomethacin, and celecoxib as standard ligands at PPARγ receptor protein, COX-1, and COX-2 active sites, respectively.

RESULTS AND DISCUSSION

Chemistry

Initially TZD (**3**) was synthesized conventionally, which on further reaction with various substituted aromatic aldehydes and morpholine as secondary amines underwent the Mannich reaction to yield the series of designed and title Mannich bases represented in Scheme 1. TZD (**3**) and the title Mannich base compounds **4a-4n** were also prepared by microwave-assisted irradiation with 280 W and 420 W power levels at different steps of the synthesis. The physical characterization data, the comparative studies of conventional synthesis and microwave irradiation synthesis with respect to percentage yields, and their reaction time intervals are shown in Table 1.

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

Compound	R	m.p. (°C)	Molecular formula	m.w.	Conventional synthesis		Microwave synthesis	
					% yield	Reaction time	% yield	Reaction time
4a	4-chloro	186-188	$C_{14}H_{15}N_2ClO_3S$	326.80	63.45	5 h	74.95	8 min
4b	2,4-dichloro	202-204	$C_{14}H_{14}N_2Cl_2O_3S$	361.24	68.42	6 h	74.68	7 min
4c	4-fluoro	192-194	$C_{14}H_{15}N_2FO_3S$	310.34	59.32	5 h	72.35	10 min
4d	4-hydroxy	186-188	$C_{14}H_{16}N_2O_4S$	308.35	70.45	5.5 h	77.46	7 min
4e	4-methyl	204-206	$C_{15}H_{18}N_2O_3S$	306.38	69.15	6.5 h	80.72	6 min
4f	4-nitro	200-202	$C_{14}H_{15}N_3O_5S$	337.35	71.48	6 h	82..94	10 min
4g	4-methoxy	190-192	$C_{15}H_{18}N_2O_4S$	322.38	69.74	6 h	79.48	9 min
4h	3,4-dimethoxy	200-202	$C_{16}H_{20}N_2O_5S$	352.41	64.84	5.5 h	78.22	8 min
4i	3,4,5-trimethoxy	212-214	$C_{17}H_{22}N_2O_6S$	382.43	63.48	5 h	79.18	8 min
4j	4-hydroxy-3-methoxy	234-236	$C_{15}H_{18}N_2O_5S$	338.38	69.23	6 h	80.46	8 min
4k	4-bromo	214-216	$C_{14}H_{15}N_2BrO_3S$	371.25	70.51	6.5 h	79.25	10 min
4l	3-bromo	198-200	$C_{14}H_{15}N_2BrO_3S$	371.25	68.26	5.5 h	80.15	9 min
4m	4-dimethylamino	210-212	$C_{16}H_{21}N_3O_3S$	335.42	69.43	6 h	78.67	8 min
4n	4-isopropyl	216-218	$C_{17}H_{22}N_2O_3S$	334.43	70.24	6 h	81.64	8 min

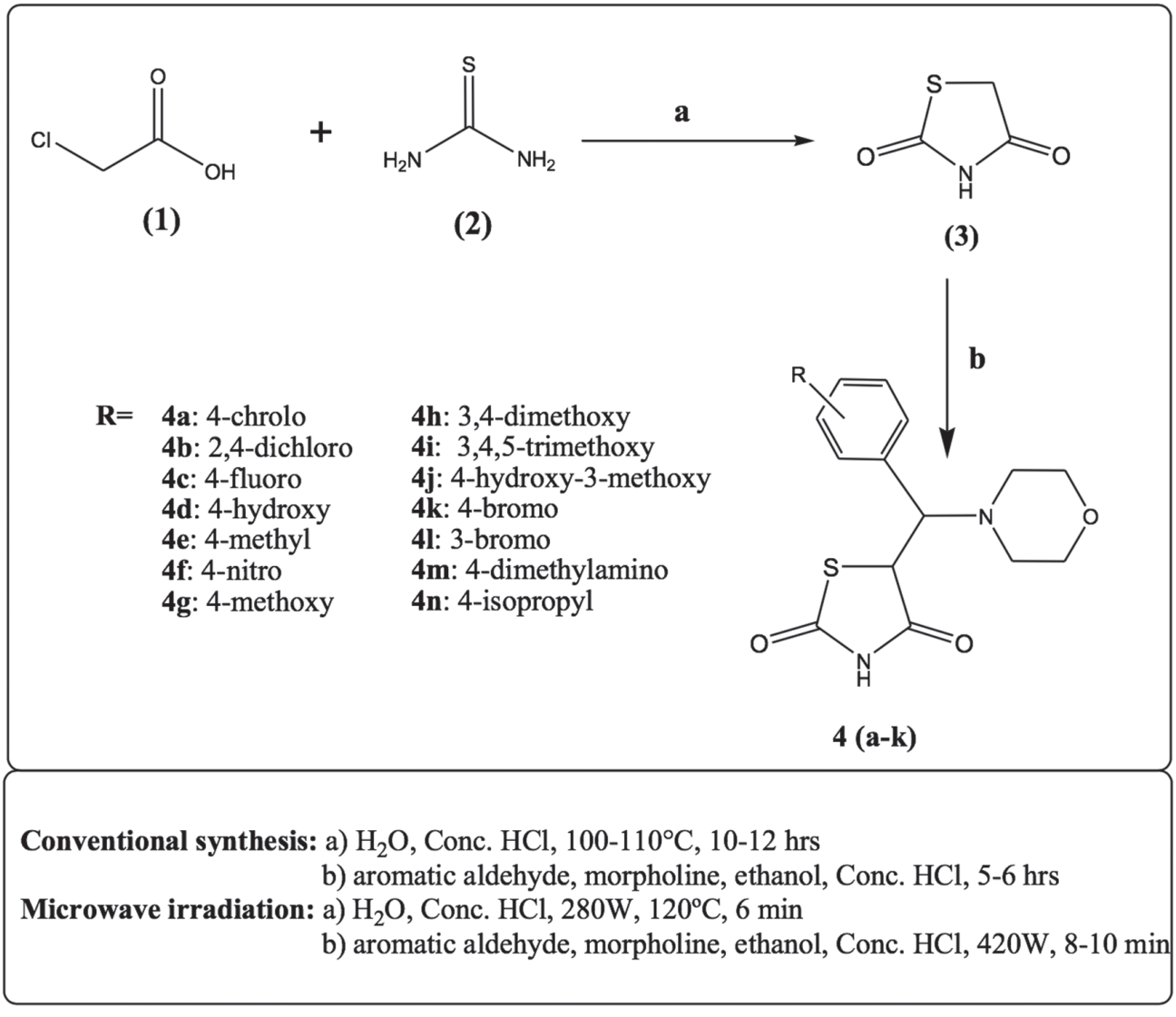
The designed and synthesized Mannich bases **4a-4n** were confirmed by the IR absorption bands at 3300-3500 cm^{-1} characteristic of -NH- of TZD, 1640-1690 cm^{-1} characteristic of –C=O of TZD, 3000-3100 cm^{-1} characteristic of =C-H stretching of the aromatic phenyl ring, and 1050-1300 cm^{-1} characteristic of C-O stretching of morpholine. The ^{1}H-NMR spectra produced a singlet at 11-12.5 ppm that is characteristic of the NH proton of TZD, triplets at 2-3.8 ppm indicating methylene group protons of morpholine, doublets at 4-4.5 ppm indicating CH of TZD, and N-CH protons, aromatic protons, were exhibited at 6.5-8 ppm. In ^{13}C-NMR, the signal appeared at 165-200 ppm indicating C=O of TZD, signals at 120-160 ppm indicate aromatic carbons, 10-50 ppm signals indicate aliphatic carbons, and 30-60 ppm signals indicate $-CH_2$-N-CH_2- carbons.

In vivo hypoglycemic activity

In vivo hypoglycemic activity study protocols were approved by the Institutional Animal Ethics Committee 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 the mean (SEM). The values were analyzed by one-way analysis of variance followed by Dunnet's "t" test and they were considered significant when compared to the normal group. The acute and chronic study results are given in Tables 2 and 3, respectively. The acute study results showed compounds **4c, 4h,** and **4n** exhibited good hypoglycemic activity and they were subjected to the chronic study at 35 and 70 mg/kg body weight. The chronic study results indicate that compounds **4h** and **4n** exhibited good activity at a dose of 70 mg/kg body weight.

In vitro anti-inflammatory activity

As a part of the *in vitro* anti-inflammatory activity investigation, the compounds were tested for HRBC membrane stabilization and protein denaturation by taking diclofenac sodium as



Scheme 1. Reaction conditions and reagents

standard. Percentage inhibition of test compounds at various concentration levels was calculated and the results are given in Tables 4 and 5. HRBC membrane stabilization indicated the standard drug diclofenac sodium showed 90.78±0.87% inhibition at 300 µg/mL concentration. The highest inhibition of 90.64±0.26% and 89.61±0.25% was exhibited by compounds **4k** and **4f**, respectively, at 500 µg/mL concentration. Protein denaturation indicated the standard drug diclofenac sodium showed 95.34±0.92% inhibition at 300 µg/mL concentration. In protein denaturation, the highest inhibition of 93.72±0.61% was exhibited by compound **4k** at 500 µg/mL concentration. All the values were expressed as mean ± SEM, n=3.

Molecular docking

Molecular docking studies at PPARγ receptor protein, COX-1, and COX-2 active site regions give the data of binding energy (kcal/moL), number of hydrogen bonds, hydrogen bond length, and interacted amino acid residues, shown in Tables 6, 7, and 8. In comparison with the standard ligand rosiglitazone (binding energy -8.26 kcal/moL), compounds **4h** and **4n** showed higher

Table 2. Effect of synthesized compounds 3a-3l on blood glucose level in alloxan induced diabetic rats (acute study)

Compound	Mean ± SEM of blood glucose level mg/dL					
	0 h	1 h	2 h	4 h	6 h	8 h
Normal	121.24±2.56	123.64±2.04	122.5±5.11	121.54±3.47	120.5±4.22	120.33±2.3
Standard	398.26±4.22*	242.34±5.48	192.1±4.29*	148.64±3.46*	110.35±4.65	100.55±5.26**
4a	326.45±2.64	294.32±4.41**	265.61±4.68	250.31±8.61	253.12±8.32**	290.5±4.85
4b	315.22±8.15**	254.26±6.78	235.81±6.34*	175.52±3.51	165.45±5.61*	200.61±2.46
4c	325.64±8.55	249.6±5.44**	199.72±7.14*	150.4±6.45	118.12±6.47**	125.47±3.84
4d	322.3±4.22	299.52±6.38*	235.42±4.28**	168.5±2.64	154.8.3±6.55	198.24±5.24**
4e	315.88±5.64	305.11±7.34	296.58±5.42	235.25±2.65**	258.44±6.54	272.05±7.56
4f	305.44±5.26**	297.53±6.48	285±7.12*	252.56±5.29	278.32±5.48*	308±6.24*
4g	316.0±8.54*	291.5±6.44*	252.5±4.62	230.45±4.69	268.2±5.48*	274.68±8.47
4h	335.78±8.95	250.55±4.37**	200.48±5.48**	153.51±4.62**	112.4±5.12	125.42±5.46
4i	334.54±4.18*	309.85±4.68**	295.23±6.18	254±6.47*	289.14±5.61*	306.47±5.28
4j	309.52±2.84	300.15±6.15*	285.3±7.52	265.9±4.28**	273.58±4.35**	298.09±5.51
4k	338.4±6.41*	310.68±3.48	279.5±6.45**	254.30±5.49	281.21±5.41	298.45±6.32**
4l	325.64±2.54	308±5.48	285.46±8.17	265.48±6.48	285.15±5.48**	310.56±4.29*
4m	319.25±5.35*	318.62±4.65	294.32±6.21*	268.48±7.15**	294.54±4.62	300.58±5.42**
4n	320.61±5.16**	260.46±3.16	210.45±4.65*	145.68±5.49	120.64±4.68	130.64±6.48*

All values expressed as mean ± standard error of the mean, n=6. Standard drug: Rosiglitazone; Statistical analysis is done by one-way One-Way Analysis of Variance followed by Dunnet's "t" test; **p<0.01 (considered significant when compared to normal group), *p<0.001.
SEM: Standard error of the mean

Table 3. Effect of synthesized compounds 4c, 4h, and 4n 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 g		
	Day 0	Day 7	Day 15	Day 0	Day 7	Day 15
Standard	368.25±4.24	200.65±3.63	178.91±5.61	196.5±4.27	193.22±3.25	194.2±5.24
4c (35 mg/kg bw)	325.56±2.59*	260.46±3.24*	216.22±2.44	194.20±2.43	196.5±3.34	194.22±5.17**
4c (70 mg/kg bw)	327.18±4.41	228.46±3.54*	209.16±3.28	199.31±2.64*	197.22±2.26*	195.21±2.77
4h (35 mg/kg bw)	336.64±4.22**	250.61±2.64	228.42±5.16	196.31±4.14	198.47±2.67**	195.44±3.12
4h (70 mg/kg bw)	319.61±2.58	205.22±4.51	179.62±3.61**	199.42±3.16	197.61±3.21	198.55±4.67*
4n (35 mg/kg bw)	320.38±4.55	254.35±3.14	211.28±2.45	198.66±2.61*	199.34±2.61	195.24±2.34*
4n (70 mg/kg bw)	320.64±3.27	206.37±2.48**	180.55±3.22**	200.61±3.64	198.46±3.48	197.41±4.54

All values expressed as mean ± standard error of the mean, n=6. Standard drug: rosiglitazone; Statistical analysis is done by one-way One-Way Analysis of Variance followed by Dunnet's "t" test; **p<0.01 (considered significant when compared to normal group), *p<0.001.

Table 4. Effect of compounds 4a-4n on human red blood cell membrane stabilization

Compound	% Inhibition at different concentrations			
	50 µg/mL	100 µg/mL	300 µg/mL	500 µg/mL
Diclofenac sodium	-----	86.79±0.43	90.78±0.87	-----
4a	53.33±0.38	58.67±0.32	61.33±0.46	68.00±0.62
4b	37.33±0.18	48.00±0.46	52.67±0.22	65.33±0.32
4c	42.67±0.32	52.00±0.62	61.33±0.28	68.00±0.32
4d	46.00±0.32	54.67±0.22	65.33±0.46	72.00±0.52
4e	40.00±0.32	46.00±0.52	52.00±0.36	54.00±0.42
4f	64.25±0.45	82.62±0.40	85.46±0.32	89.61±0.25
4g	52.34±0.36	61.48±0.85	72.42±0.60	85.82±0.46
4h	58.64±0.42	70.84±0.23	76.55±0.44	81.12±0.52
4i	51.65±0.75	60.33±0.48	71.64±0.68	78.45±0.72
4j	49.25±0.24	58.64±0.44	65.98±0.52	74.23±0.75
4k	61.42±0.61	80.43±0.64	84.28±0.48	90.64±0.26
4l	54.40±0.44	63.44±0.74	71.68±0.80	78.71±0.64
4m	58.64±0.62	65.49±0.82	70.18±0.42	76.48±0.34
4n	57.82±0.48	62.47±0.62	72.14±0.54	78.91±0.46

All values expressed as mean ± standard error of the mean, n=3

Table 5. Effect of compounds 4a-4n on protein denaturation

Compound	% Inhibition at different concentrations			
	50 µg/mL	100 µg/mL	300 µg/mL	500 µg/mL
Diclofenac sodium	-----	88.89±0.36	95.34±0.92	-----
4a	80.27±0.20	83.47±0.35	86.80±0.15	88.80±0.42
4b	66.53±0.28	84.53±0.42	86.00±0.35	86.80±0.45
4c	54.67±0.18	61.33±0.12	71.93±0.45	84.93±0.13
4d	61.33±0.46	71.80±0.35	78.67±0.38	86.00±0.28
4e	71.47±0.35	78.60±0.42	82.93±0.36	86.80±0.25
4f	83.42±0.35	86.49±0.28	87.42±0.33	94.82±0.44
4g	78.64±0.54	82.66±0.42	88.48±0.40	91.62±0.64
4h	81.80±0.24	86.48±0.22	88.64±0.86	92.75±0.32
4i	74.63±0.26	80.46±0.26	87.42±0.34	90.66±0.28
4j	78.62±0.42	81.66±0.48	86.94±0.55	90.48±0.36
4k	81.26±0.32	85.46±0.46	90.46±0.22	93.72±0.61
4l	81.46±0.62	85.92±0.68	88.33±0.82	92.64±0.46
4m	72.61±0.64	79.45±0.40	84.24±0.28	88.49±0.62
4n	69.46±0.52	72.24±0.64	76.18±0.38	82.81±0.12

All values expressed as mean ± standard error of the mean, n=3

binding energy (-8.32 kcal/moL and -8.29 kcal/moL) at the active site region of PPARγ receptor protein. Figure 1 depicts the 3D structure of PPARγ receptor protein, binding mode of rosiglitazone and compounds **4h** and **4n** at the active site region of PPARγ protein (PDB ID: 2PRG). Compound **4k** gave the highest binding energy (-7.47 kcal/moL and -10.98 kcal/moL) with the two COX targets used in comparison with the binding energy of the standard ligands indomethacin (-7.14 kcal/moL) and celecoxib (-10.89 kcal/moL). Figure 2 depicts the binding mode of indomethacin at the active site region of COX-1 (PDB ID: 1EQG), celecoxib at the active site region of COX-2 (PDB ID: 1CX2), and compound **4k** interaction with the two targets.

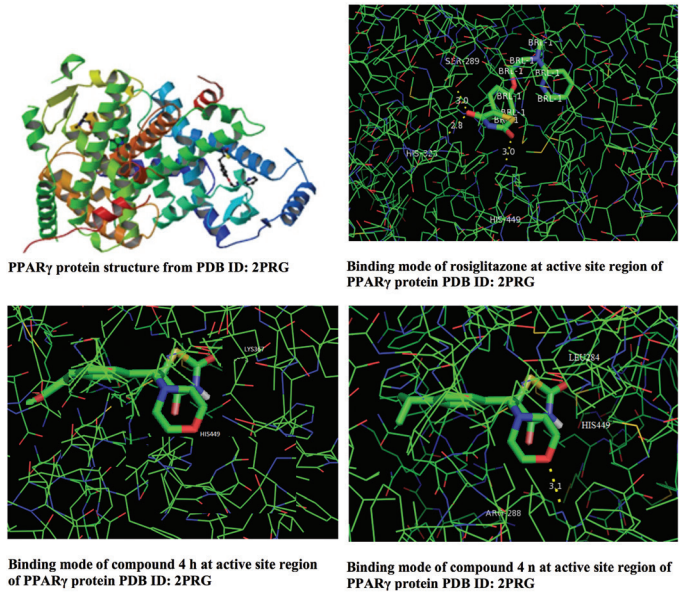


Figure 1. Molecular docking studies at active site region of PPARγ protein receptor (PBD ID: 2PRG)

PPARγ: Proliferator-activated receptor gamma

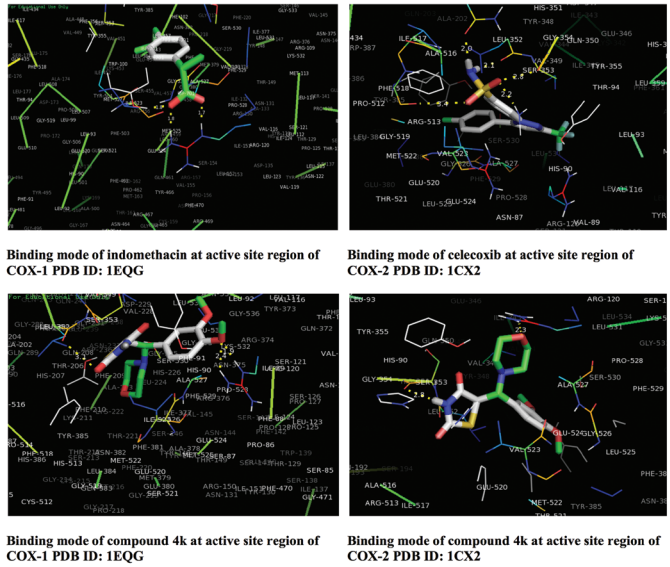


Figure 2. Molecular docking studies at active site region of COX isoenzymes (COX-1 PBD ID: 1EQG & COX-2 PDB ID: 1CX2)

CONCLUSION

In this investigation various thiazolidinedione derivatives possessing morpholine and substituted phenyl appendages that are attached to the common methyl group were developed as Mannich bases by simple Mannich reaction. The title compounds were synthesized by conventional as well as microwave-assisted synthesis. Microwave irradiation produced high yield in a shorter reaction time in comparison with traditional conventional heating synthesis. Characterization of the compounds was done physically and spectrally. They were evaluated for *in vivo* hypoglycemic activity and *in vitro* anti-inflammatory activity because of the existing thiazolidinedione

Table 6. Binding energy and amino acid residues interacted by the compounds 4a-4n with the target PPARγ protein (PDB ID - 2PRG)

Compound	Binding energy (kcal/moL)	Number of hydrogen bonds	Hydrogen bond length	Interacted amino acid residues
Rosiglitazone	-8.26	3	3.11, 3.01, 2.82	Ser289, His449, His323
4a	-7.64	2	3.15, 2.64	Leu284, Ser289
4b	-8.12	2	2.94, 3.22	Ser289, Cys258
4c	-7.64	3	2.84, 3.12, 2.61	Gln284, Tyr388, Met348
4d	-7.86	2	3.26, 2.82	Leu288, His449
4e	-7.85	3	3.24, 2.84, 2.28	Ser289, Met326, Leu284
4f	-7.24	2	2.98, 2.42	Cys258, Tyr471
4g	-7.61	3	1.86, 2.64, 1.91	Thr246, Ser289, His449
4h	-8.32	2	2.93, 3.08	Lys367, His449
4i	-7.12	3	2.48, 2.27, 1.92	Leu284, Ser289, Met298
4j	-7.57	2	3.40, 2.50	Cys255, Gln286
4k	-7.28	2	2.53, 1.94	Ser289, Thr246,
4l	-7.08	3	3.14, 1.92, 2.68	Tyr388, Ser289, His449
4m	-6.89	2	1.96, 2.44	Tyr471, Met348
4n	-8.29	3	3.10, 2.94, 3.22	Arg288, His449, Leu284

PPARγ: Proliferator-activated receptor gamma

Table 7. Binding energy and amino acid residues interacted by the compounds 4a-4n with the target COX-1 isoenzyme (PDB ID: 1EQG)

Compound	Binding energy (kcal/moL)	No. of hydrogen bonds	Hydrogen bond length	Interacted amino acid residues
Indomethacin	-7.14	2	2.62, 3.21	Leu352, Tyr355
4a	-7.01	2	2.32, 2.81	Arg120, Tyr355
4b	-6.58	3	1.87, 2.65, 3.17	Ala527, Ile523, Met522
4c	-6.48	1	2.84	Tyr355
4d	-6.74	3	2.56, 3.11, 1.98	Met522, Leu352, Tyr385
4e	-7.02	2	2.22, 2.95	Tyr385, Ser530
4f	-6.91	4	2.91, 2.05, 3.46, 2.07	Met522, Ile523, Tyr385, Arg120
4g	-6.65	2	1.97, 3.08	Leu352, Tyr355
4h	-6.46	3	1.91, 1.97, 3.12	Tyr385, Met522, Ile523
4i	-7.01	4	2.15, 2.04, 3.26, 1.91	Tyr355, Leu352, Ala527, Ser530
4j	-6.54	1	3.16	Arg120
4k	-7.47	3	1.96, 2.42, 3.17	Arg120, Leu352, Tyr385
4l	-6.22	2	2.64, 1.78	Arg120, Tyr355
4m	-6.81	2	2.74, 3.26	Tyr385, Leu352
4n	-6.74	5	2.08, 2.34, 2.65, 2.84, 2.26	Tyr385, Ser530, Ala527, Ile523, Met522

Table 8. Binding energy and amino acid residues interacted by the compounds 4a-4n with the target COX-2 isoenzyme (PDB ID: 1CX2)

Compound	Binding energy (kcal/moL)	Number of hydrogen bonds	Hydrogen bond length	Interacted amino acid residues
Celecoxib	-10.89	5	2.25, 2.08, 2.16, 2.84, 3.46	His90, Gln192, Leu352, Ser353, Phe518
4a	-8.59	3	1.95, 2.64, 3.66	Ala527, His90, Ala527
4b	-7.99	3	3.22, 1.94, 2.65	Ala527, Ser530, Val523
4c	-9.47	2	3.42, 2.68	Tyr355, VAL349
4d	-8.98	4	2.31, 3.18,1.97, 3.18	Leu352, Arg120, Ser353, Tyr385
4e	-9.19	2	1.88, 2.86	Ser253, Val349
4f	-9.86	3	1.94, 2.09, 2.72	His90, Arg120, Ser353
4g	-8.89	2	2.55, 3.74	Arg120, Val349
4h	-7.49	3	2.19, 2.92, 3.15	His90, Ser353, Val523
4i	-9.05	3	3.22, 2.10, 1.94	Val349, Ser253, Leu352
4j	-9.39	4	1.89, 2.54, 3.30, 2.38	Tyr355, Tyr385, Ala527, Ser530
4k	-10.98	2	2.32, 2.87	Arg120, Ser353
4l	-8.33	2	1.95, 2.40	Tyr355, Tyr385
4m	-9.81	3	3.62, 2.31, 2.48	Arg120, Val349, Ser353
4n	-7.45	2	2.85, 3.23	Arg120, Val349

and morpholine moieties. From the data of *in vivo* hypoglycemic activity screening, compounds **4h** and **4n** exhibited good hypoglycemic activity in both acute and chronic toxic studies. In the *in vitro* anti-inflammatory activity investigation by HRBC membrane stabilization method and protein denaturation method, compound **4k** exhibited good activity among all. From the viewpoint of structure–activity relationship, it was identified that compounds with electron releasing groups at the para position of the phenyl ring may have the ability to produce hypoglycemic activity and the presence of electron withdrawing groups at the para position of the phenyl ring causes anti-inflammatory activity. Molecular docking studies with PPARγ receptor protein (PDB ID-2PRG) and compounds **4h** and **4n** exhibited higher binding affinity. In molecular docking studies with COX isoenzymes (PDB IDs: 1EQG and 1CX2) for potential anti-inflammatory activity, compound **4k** exhibited higher binding affinity.

ACKNOWLEDGEMENTS

The authors are thankful to the Management of V. V. Institute of Pharmaceutical Sciences, Gudlavalleru-521356, Andhra Pradesh, India, for providing necessary facilities to carry out the research work. The authors are grateful to Dr. K. Ravishankar, Principal, Aditya College of Pharmacy, Surampalem, Andhra Pradesh, for helping with the biological evaluation.

Conflicts of interest: No conflict of interest was declared by the authors.

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