Tropical Journal of Pharmaceutical Research September 2014; 13 (9): 1445-1454
ISSN: 1596-5996 (print); 1596-9827 (electronic)

Available online at http://www.tjpr.org
http://dx.doi.org/10.4314/tjpr.v13i9.10

Original Research Article

Synthesized 2-Substituted-3-Phenylthiazolidine-4-ones as Potent Antioxidants and Antidiabetic Agents

Sandip Sen[1]*, Biplab De[2] and Trichy Siva Easwari[1]
[1]IIMT College of Medical Sciences, O.Pocket, GangaNagar, Meerut, U.P-250001, [2]Regional Institute of Pharmaceutical Science and Technology, Abhoynagar, Agartala-799006, Tripura, India

For correspondence:* ***Email:* *sandipsen2010@gmail.com;* ***Tel:*** *+91-9997094873, 3812341229*

Received: 11 October 2013 *Revised accepted: 19 July 2014*

Abstract

Purpose: *To employ intramolecular cyclization of Schiff bases of pyrazole-4-carboxaldehydes to form thiazolidine-4-ones and determine the antioxidant and antidiabetic activity of the synthesized compounds.*
Methods: *The Schiff bases were obtained upon reaction between the electrophillic carbon atom of pyrazole-4-carboxaldehyde and the nucleophillic nitrogen atom of the amine. Preparation of thiazolidine-4-one was preceded by attack of sulphur nucleophile of thioglycollic acid on imine carbon followed by intramolecular cyclization using acid catalyst. In vitro antioxidant activity was determined by 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay method using ascorbic acid as standard. Evaluation of antidiabetic activity was carried out in streptazotocine-induced diabetes in Wister rats using rosiglitazone as reference drug. Blood glucose levels were estimated by GOD-POD kit. Serum biochemical parameters like total cholesterol, triglyceride, urea, creatinine and total protein level were also measured.*
Results: *Compounds 7a, 7b, 7c, 7e, 7j showed higher IC_{50} [Half maximal inhibitory concentration] values than the reference antioxidant, ascorbic acid. On the 21st day of treatment, there was significant fall and rise blood glucose level and body weight, respectively, compared to the anti-diabetic standard. There was decrease in serum cholesterol, triglyceride, creatinine and urea levels while high density lipoprotein (HDL) level and total protein levels increased after 21 days of treatment. Compared to rosiglitazone, compounds 7a, 7b, 7c, 7h, 7j showed stronger significant antidiabetic effect in hyperglycemic rats due, probably, to the presence of thiazolidine-4-one nucleus as well as para-substitution on phenyl ring.*
Conclusion: *The 2-(3-Methyl-1H-pyrazol-4-yl)-3-phenylthiazolidin-4-ones possess potent antioxidant and antidiabetic activity but further studies are required to develop them for clinical use.*

Keywords: *Pyrazole-4-carboxaldehydes, Thiazolidine-4-one, Antioxidant, Anti diabetic*

Tropical Journal of Pharmaceutical Research is indexed by Science Citation Index (SciSearch), Scopus, International Pharmaceutical Abstract, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Journal Citation Reports/Science Edition, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

INTRODUCTION

Among the wide variety of heterocyclic compounds, pyrazoles fused with different heterocycles are known to contribute to various pharmacological effects. Due to wide range of pharmacological activities, significant amount of research activity has been directed towards this class. In particular, they are used as antitumor, antibacterial and antifungal, antiviral, antiparasitic, anti-tubercular and insecticidal agents. Some of these compounds have also shown anti-inflammatory, anti-diabetic, anesthetic and analgesic properties [1]. Another hetrocyclic compound, thiazolidine, is a 5 membered saturated ring with a thio ether group

at 1 position and an amine group in the 3 position. It has fungicidal, local anesthetic, anti-seizure, antitubercular, anti-bacterial, anti-amoebic, anti-diabetic and anti-inflammatory activities [2,3].

Thiazolidinones are derivatives of thiazolidine, which is a main pharmacophoric group responsible for antidiabetic activity. It acts by enhancing insulin sensitivity in both muscle and adipose tissue and to a lesser extent by inhibiting hepatic glucose production. These agents have a notable effect on improving insulin resistance, particularly when used in combination with other antidiabetic drugs, but have no effect on insulin secretion. As a class, the thiazolidinediones have also been shown to alter lipid profiles in patients with type 2 diabetes. They also have effected a decrease in triglyceride levels, increase in total and Low density lipoprotein LDL cholesterol levels [4,5]. In patients receiving insulin therapy, the addition of a thiazolidinedione has resulted in significant reductions in daily insulin requirements [6,7]. The thiazolidinediones are dependent on the presence of insulin for activity; however, they do not affect insulin secretion. Patients with chronic pancreatitis are at high risk of antioxidant deficiencies. Furthermore, this disease can lead to diabetes mellitus that could exacerbate the severity of oxidative stress. Oxidative stress and the resulting LDL oxidation are a major cause of atherosclerosis [8].

EXPERIMENTAL

All the chemicals used for the synthesis were reagent grade and obtained from Sigma Aldrich and Mark Lasoraton. The solvents were purified by standard laboratory procedure and free from atmospheric oxygen. The melting points were determined by open capillary method and are not corrected. The IR spectra were recorded in KBr pellets on a Shimadzu 8201 PC FTIR spectrophotometer. Both 1H and 13C NMR were recorded in DMSO-d_6 using Bruker 500 MHz-NMR spectrophotometers using TMS as internal standard. The masses of the compounds were analyzed by ESI method using Thermo Fennigan mass spectrophotometer. Elemental analyses were recorded using Thermo Finnigan FLASH EA 1112 CHN analyzer. TLC was performed in precoated plastic sheet of silica gel g/UV-254 of 0.2 mm thickness.

Synthesis of 3-methyl-1, 2-dihydropyrazol-5-one (3)

One mole of hydrazine hydrate and phenyl hydrazine was weighed into a 500 mL beaker. One mole equivalent of ethyl acetoacetate was added and the mixture allowed to warm up to 100 °C on water bath for 3 h. Cooling of the mixture to room temperature resulted in solidification of crystal, which was washed with ether and recrystallized from hot ethanol [9].

Synthesis of 3-methyl-1H-pyrazole-4-carbaldehyde (4)

3 mmoles of DMF and 1 mmole of $POCl_3$ was added to 1mole 3-methyl-1,2-dihydropyrazol-5-one (3) in round bottom flask. During addition of $POCl_3$ the temperature of reaction mixture was maintained 0-5 °C. After completion of addition, the mixture was refluxed for 6 h and 10% sodium hydroxide was added till neutralization and cooled overnight. After the addition of crushed ice, the resulting precipitate was recrystallized from hot ethanol [10].

General procedure for synthesis of N-((3-methyl-1H-pyrazol-4-yl) methylene) benzenimine (6a-j)

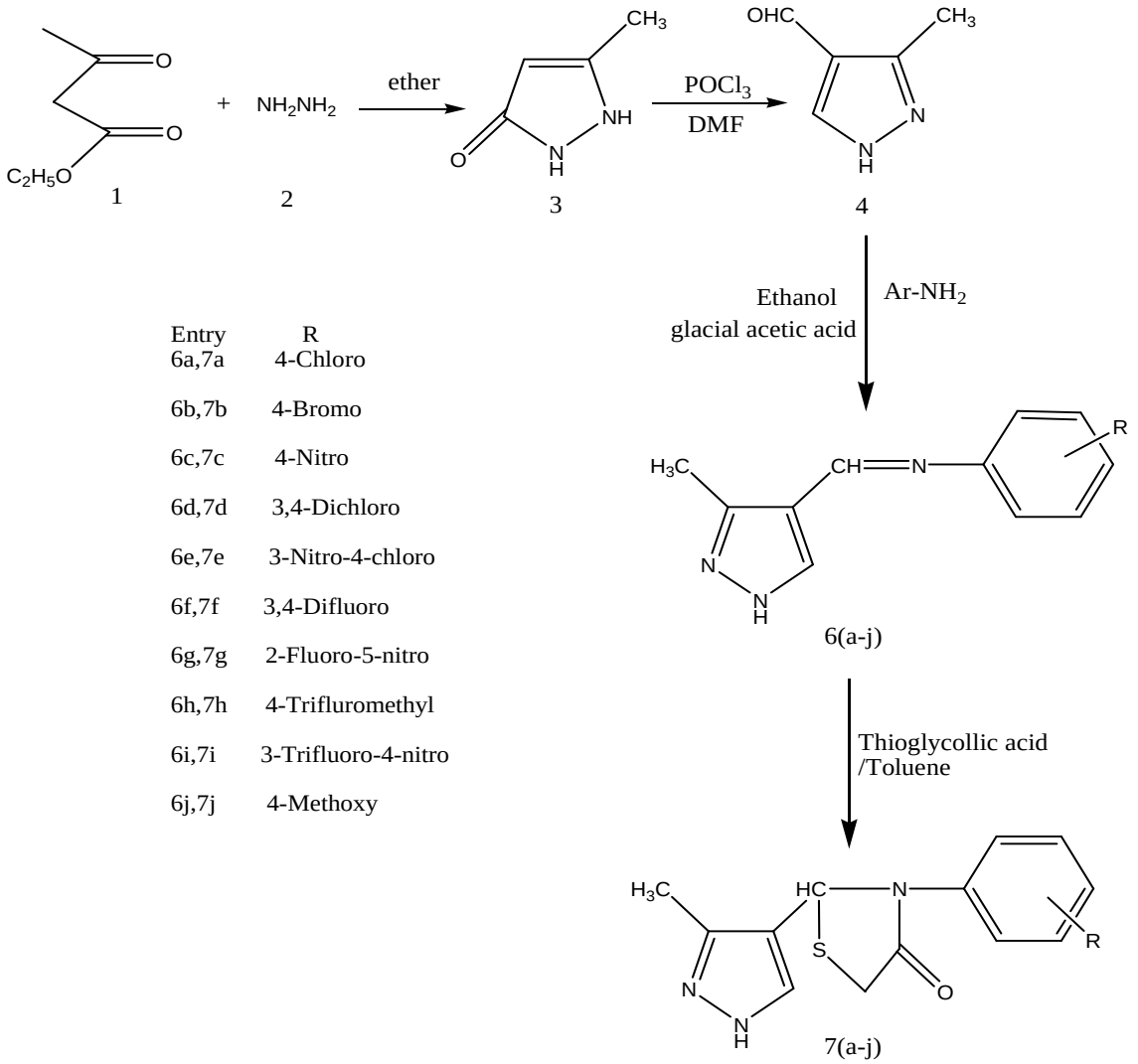
One mole of 3-methyl-1H-pyrazole-4-carboxaldehyde (4) in 50 ml of ethanol was stirred for 30 min under nitrogen atmospherefollowed by 1 ml of glacial acetic acid and 1 mole of substituent aromatic amines, and then refluxed for 1 h. Following the addition of crushed ice, the resulting precipitate was recrystallized from hot ethanol [11].

Synthesis of 2-(3-methyl-1H-pyrazol-4-yl)-3-phenylthiazolidin-4-one (7a-7j)

The substituted imines (0.02 mole) were dissolved in toluene with $SnCl_2$ (0.05 mmole) was used as catalyst. After addition of thioglycollic acid (0.03 mole), reaction mixtures were stirred for an hour. The mixtures were then refluxed in water bath for 12 h and cooled to room temperature. Excess solvents were removed by distillation and compounds were dissolved in dichloromethane. The organic layer were washed with 10 % sodium bicarbonate and finally with brine solutions, dried over anhydrous sodium sulphate and evaporated to dryness. Purification of the compounds were done with petroleum ether and chloroform (1:4 v/v) by chromatographic technique.

Evaluation of antioxidant activity

In vitro antioxidant activity was determined by 1, 1-diphenyl-2-picrylhydrazyl radical method, which was used to evaluate the free radical scavenging capacity of different antioxidants [12,13].



Entry	R
6a,7a	4-Chloro
6b,7b	4-Bromo
6c,7c	4-Nitro
6d,7d	3,4-Dichloro
6e,7e	3-Nitro-4-chloro
6f,7f	3,4-Difluoro
6g,7g	2-Fluoro-5-nitro
6h,7h	4-Trifluromethyl
6i,7i	3-Trifluoro-4-nitro
6j,7j	4-Methoxy

Scheme 1

Animals

Wister rat of either sex weighing between 180-200 g were taken for anti diabetic activity. Animals were maintained under standard environmental condition at temperature of 22 ± 2 ºC and 45-50 % relative humidity for 24 h each of dark and light cycle with proper diet. All the studies were done according to protocol approved by Institutional Animal Ethical Committee (IAEC) of Bansal College of Pharmacy (Reg. no-1252/ac/10/CPCSEA, Ref. no-BCP/IAEC/12/02).

Acute toxicity study

The acute oral toxicity study was carried out according to OECD guideline no 423 [14] in Wister rats. The doses were fixed 2 mg/kg (p.o) to 10 mg/kg (p.o) for rats and contain 5 in each group. The mortality and general behaviours were under observation for 14 days. The test compounds were non-toxic in the dose of 5 mg/kg body weight.

Oral glucose tolerance test on rat (OGTT)

Twelve groups of animals were administered normal saline at the dose of 5 mg/kg for test compounds followed by administration of glucose solution in the dose of 2 g/kg. After 30 mins, blood sample were withdrawn from dorsal vein at interval of 60, 120 and 180 mins. Blood glucose level were estimated using blood glucose test strip with elegance glucometer (Frankenbeng Germany) & GOD-POD kit (Acuurex,India) [15].

Evaluation of antidiabetic activity

Induction of diabetes

Streptozotocine (STZ) was used in the dose of 60 mg/kg to induce insulin dependent diabetes. STZ was injected into rats intraperitoneally. After 48 h of administration of STZ, the blood samples

were collected from the dorsal vein for determination of blood glucose level. The rat with fasting glucose level in range of 275-300 mg/100 ml is considered as diabetic and considered for study [21].

Experimental protocol and dose schedule

This study was carried out over a period of 21 days. The rats were divided into 13 groups consisting of 06 animals in each group.

Group-1: Normal rats treated with normal saline 10 ml/kg p.o.

Group-2: Diabetic control treated with STZ (60 mg/kg) dissolved in citrate buffer.

Group-3: Diabetic rat treated with Rosiglitazone 8 mg/kg.

Group-4-8: Diabetic rat treated with test compounds at 5 mg/kg body weight

On 1, 7, 14 and 21 days of study after 2 h of oral administration of test compounds, blood glucose levels and body weight were measured. Blood samples were withdrawn through dorsal vein. After 21 days, whole blood was collected by cardiac puncture. Blood sample collected was then centrifuged at 3000 rpm for 10 min to obtain serum. Blood glucose levels were estimated by GOD-POD kit (Accurex, India). All biochemical parameters were determined including total cholesterol [16], triglyceride by Hantzsch condensation method [17], Serum urea and creatinine by method of Thomas [18], total protein [19], and HDL cholesterol [20] were also measured.

Statistical analysis

The results were shown as Mean ± SEM and comparison between standard and test compounds were made by one way ANOVA followed by Dunnett's test. Values of $p \leq 0.001$ were considered as potent.

RESULTS

Chemistry

Physical and spectral data

6a: 4-Chloro-N-((3-methyl-1H-pyrazol-4-yl) methylene) benzenamine

Yield - 98 %; Melting Point: 91-94 ^{o}C; IR (KBr, Cm^{-1}): 2927.34 (-CH_3, str); 1435.14 (-CH_3, def); 1681.67 (C=N, str); 764.21 (C-Cl, str). ^{I}H NMR (DMSO-d_6, δppm): 13.7 (s, 1H, -NH); 7.52 (s, 1H,-N=CH-); 7.2-7.18 (d, 2H, J=10 Hz, Ar-H); 7.15 (s, 1H, Ar-H); 6.8-6.78 (d, 2H, J=10Hz, Ar-H); 2.79(s,3H,-CH_3). ^{I3}CNMR (DMSO-d_6 , δppm): 160.1; 147.1; 145.5; 141.4; 132.8; 130.2; 130.2 ; 123.7; 123.7; 105; 11. ESI-MS (m/e): 219.03 (M+). Anal. Calcd (found) for $C_{11}H_{10}C_lN_3$: C-60.14 (60); H- 4.59 (4.49): N-19.13(18.82).

6b: 4-Bromo-N-((3-methyl-1H-pyrazol-4-yl) methylene)benzenamine

Yield – 96 %; Melting Point: 81-83 ^{o}C; IR (KBr, Cm^{-1}): 2924.52 (-CH_3,str); 1459.35 (-CH_3, def); 1624.73 (C=N, str); 621.93 (C-Br, str). ^{I}H NMR (DMSO-d_6, δppm): 12.7 (s,1H,-NH); 7.49 (s, 1H,-N=CH-); 7.21-7.19 (d, 2H, J=10Hz, Ar-H); 7.15 (s, 1H, Ar-H); 6.81-6.78(d, 2H, J=15Hz, Ar-H); 2.69(s,3H,-CH_3). ^{I3}CNMR (DMSO-d_6 , δppm): 161.3; 148.1; 147.5; 141.4; 134.8; 131.2; 129.2 ; 119.7; 119.7; 105; 10.9. MS (m/e): 263.13 (M+). Anal. Calcd (found) for $C_{11}H_{10}BrN_3$: C-50.02 (49.99); H- 3.82 (3.79): N-15.91 (15.87).

6c: 4-Nitro-N-((3-methyl-1H-pyrazol-4-yl) methylene) benzenamine

Yield – 87 %; Melting Point: 85-87 ^{o}C; IR (KBr, Cm^{-1}): 2927.52 (-CH_3, str); 1507.02 (C-NO_2, str); 1457.35 (-CH_3, def); 1624.73 (C=N, str). ^{I}H NMR (DMSO-d_6, δppm): 13.2 (s, 1H, -NH); 7.39 (s, 1H, -N=CH-); 7.19-7.17 (d, 2H, J=10 Hz, Ar-H); 7.2 (s, 1H, Ar-H); 6.39-6.37(d, 2H, J=6Hz, Ar-H); 2.77 (s, 3H, -CH_3). ^{I3}CNMR ($CDCl_3$, δppm)-163.1; 147.1; 146.5; 141.4; 133.8; 130.2; 130.2 ; 126.7; 126.7; 105; 11.2. MS (m/e): 230.03 (M+). Anal. Calcd (found) for $C_{11}H_{10}N_4O_2$: C-57.39 (57.19); H- 4.38 (4.41): N-24.34 (24.12).

6d: 3,4-Dichloro-N-((3-methyl-1H-pyrazol-4-yl)methylene)benzenamine

Yield – 88 %; Melting Point: 88-87 ^{o}C; IR (KBr, Cm^{-1}): 2925.14 (-CH_3, str); 1435.24 (-CH_3, def); 1679.67 (C=N, str); 764.21 (C-Cl, str). ^{I}H NMR (DMSO-d_6, δppm): 12.7 (s, 1H,-NH); 7.53 (s, 1H,-N=CH-); 7.35 (s, 1H, Ar-H); 7.2-7.18 (d, 2H, J=10 Hz, Ar-H); 7.11-7.09 (d, 1H, J=10Hz, Ar-H); 2.69(s, 3H,-CH_3). ^{I3}CNMR (DMSO-d_6, δppm): 162.1; 147.2; 143.5; 141.2; 132.7; 131.2; 131.2 ; 124.7; 124.7; 105; 11. ESI-MS (m/e): 253.02 (M+). Anal. Calcd (found) for $C_{11}H_9Cl_2N_3$: C-51.99 (51.90); H- 3.57 (3.52): N-16.54 (16.51).

6e: 4-Chloro-N-((3-methyl-1H-pyrazol-4-yl) methylene)-3-nitrobenzenamine

Yield – 88 %; Melting Point: 88-87 ^{o}C; IR (KBr,Cm^{-1}): 2927.14 (-CH_3, str); 1513.02 (C-NO_2, str); 1436.24 (-CH_3, def); 1680.67 (C=N, str); 764.21 (C-Cl, str). ^{I}H NMR (DMSO-d_6, δppm): 12.7 (s, 1H,-NH); 7.49 (s, 1H,-N=CH-); 7.25 (s, 1H, Ar-H); 7.11-7.09 (d, 2H, J=10 Hz, Ar-H); 7.09-7.07 (d, 1H, J=10Hz, Ar-H); 2.39 (s,3H,-CH_3). ^{I3}CNMR (DMSO-d_6, δppm): 165.1; 146.2; 143.5; 142.2; 135.7; 133.2; 133.2 ; 121.7; 121.7; 105; 11.5. ESI-MS (m/e): 264.04 (M+). Anal. Calcd (found) for $C_{11}H_9ClN_4O_2$: C-49.92 (49.89); H- 3.43 (3.39): N-21.17 (21.1).

6f: 3, 4-Difluoro-N-((3-methyl-1H-pyrazol-4-yl)methylene) benzenamine

Yield – 89 %; Melting Point: 82-83 ^{o}C; IR (KBr, Cm^{-1}): 2924.14 (-CH_3, str); 1433.24 (-CH_3, def); 1680.67 (C=N, str); 1111.21 (C-F, str). ^{I}H NMR (DMSO-d_6, δppm): 13.7 (s, 1H,-NH); 7.65 (s, 1H,-N=CH-); 7.45 (s, 1H, Ar-H); 7.21-7.19 (d, 2H, J=10 Hz, Ar-H); 7.11-7.09 (d, 1H, J=10Hz, Ar-H); 2.49 (s, 3H,-CH_3). ^{I3}CNMR (DMSO-d_6, δppm): 164.1; 148.2; 143.5; 140.2; 136.7; 133.2; 133.2 ; 126.7; 126.7; 105; 11.5. ESI-MS (m/e): 566.01 (M+). Anal. Calcd (found) for $C_{11}H_9F_2N_3$: C-59.22 (59.49); H- 3.90 (3.86): N-14.80 (21.1).

6g: 2-Fluoro-N-((3-methyl-1H-pyrazol-4-yl)methylene)-5-nitrobenzenamine

Yield – 69 %; Melting Point: 80-81 ^{o}C; IR (KBr, Cm^{-1}): 2927.14 (-CH_3, str); 1534.34 (C-NO_2, str) 1433.24 (-CH_3, def); 1680.67 (C=N, str); 1160.21 (C-F, str). ^{I}H NMR (DMSO-d_6, δppm): 13.5 (s, 1H,-NH); 8.2-8.18 (d, 2H, J=10 Hz, Ar-H); 7.75 (s, 1H,-N=CH-); 7.45 (s, 1H, Ar-H); 7.21-7.19 (d, 2H, J=10 Hz, Ar-H); 2.49 (s, 3H,-CH3). ^{I3}CNMR (DMSO-d_6, δppm): 160.1; 145.5; 143.3; 141.4; 136.2;123.2; 118.7; 117.2 ; 105; 11.5. ESI-MS (m/e): 248.07 (M+). Anal. Calcd (found) for $C_{11}H_9FN_4O_2$: C-53.23 (53.11); H- 3.65 (3.56): N-22.57 (22.41).

6h: N-((3-methyl-1H-pyrazol-4-yl) methylene)-4-(trifluoromethyl)benzenamine

Yield – 85 %; Melting Point: 91-92 ^{o}C; IR (KBr, Cm^{-1}: 2924.14 (-CH_3, str); 1432.24 (-CH_3, def); 1680.67 (C=N, str); 1336.21 (C-F, str). ^{I}H NMR (DMSO-d_6, δppm): 13.7(s, 1H,-NH); 7.49 (s, 1H,-N=CH-); 7.21-7.18 (d, 2H, J=15 Hz, Ar-H); 7.15 (s, 1H, Ar-H); 6.8-6.78 (d, 2H, J=10Hz, Ar-H); 2.69 (s, 3H,-CH_3). ^{I3}CNMR (DMSO-d_6, δppm): 160.1; 147.1; 145.5; 141.4; 132.8; 130.2; 130.2 ; 124.2; 123.7; 123.7; 105; 11.2. ESI-MS (m/e): 253.07 (M+). Anal. Calcd (found) for $C_{12}H_{10}F_3N_3$: C-56.92 (56.52); H- 3.98 (4.2): N-16.59 (15.89).

6i: N-((3-methyl-1H-pyrazol-4-yl) methylene)-4-nitro-3-(trifluoromethyl)benzenamine

Yield – 88 %; Melting Point: 88-87 ^{o}C; IR (KBr, Cm^{-1}): 2925.24 (-CH_3, str); 1513.02 (C-NO_2, str); 1436.24 (-CH_3, def); 1680.67 (C=N, str); 1330.21 (C-F, str). ^{I}H NMR (DMSO-d_6, δppm): 13.1 (s, 1H,-NH); 8.12-8.10 (d,1H,J=10Hz, Ar-H); 7.7(s,1H,Ar-H); 7.62-7.60 (d,1H,J=10Hz,Ar-H); 7.49 (s, 1H,-N=CH-); 7.25 (s, 1H, Ar-H); 2.39 (s,3H,-CH_3). ^{I3}CNMR (DMSO-d_6, δppm): 163.1; 155.2; 145.5; 143.7; 141.7; 127.4;126.5; 122.7; 120.7; 115.6; 105.1; 11.2. ESI-MS (m/e): 298.07 (M+). Anal. Calcd (found) for $C_{12}H_9F_3N_4O_2$: C-48.33 (48.00); H- 3.04 (2.69): N-18.79 (18.59).

6j: 4-Methoxy-N-((3-methyl-1H-pyrazol-4-yl)methylene)benzenamine

Yield – 88 %; Melting Point: 93-94 ^{o}C; IR (KBr, Cm^{-1}): 2924.14 (-CH_3, str); 2835.22 (-OCH_3); 1432.24 (-CH_3, def); 1680.67 (C=N, str). ^{I}H NMR (DMSO-d_6, δppm): 13.7 (s,1H,-NH); 7.49 (s, 1H,-N=CH-); 7.21-7.18 (d, 2H, J=15 Hz, Ar-H); 7.15 (s, 1H, Ar-H); 6.8-6.78 (d, 2H, J=10Hz, Ar-H); 3.73 (s,3H);2.69 (s, 3H, -CH_3). ^{I3}CNMR (DMSO-d_6, δppm): 160.3; 142.1; 140.5; 139.4; 132.8; 130.2; 130.2 ; 123.7; 123.7; 105; ;55.9;11. ESI-MS (m/e): 215.07 (M+). Anal. Calcd (found) for $C_{12}H_{13}N_3O$: C-66.96 (66.56); H- 6.09 (5.92): N-19.52 (19.48).

7a: 3-(4-Chlorophenyl)-2-(3-methyl-1H-pyrazol-4-yl) thiazolidin-4-one

Yield – 68 %; Melting Point: 114-114 ^{o}C; IR (KBr, Cm^{-1}): 2953.14 (-CH_3, str); 1695.67 (C=O, str); 1460.14 (-CH_3, def); 1210.33 (C-N, str); 764.21 (C-Cl, str). ^{I}H NMR (DMSO-d_6, δppm): 13.7 (s, 1H,-NH); 7.2-7.18 (d, 2H, J=10 Hz, Ar-H); 7.15 (s, 1H, Ar-H); 6.8-6.78 (d, 2H, J=10Hz, Ar-H); 4.85 (s,1H,CH-N); 3.49 (s,1H,CH_2-C=O); 3.24 (s,1H,CH_2-C=O); 2.79 (s,3H,-CH_3). ^{I3}CNMR (DMSO-d_6, δppm): 170.9;147.1; 145.5; 141.4; 132.8; 130.2; 130.2 ; 123.7; 123.7; 105; 45; 45; 11. ESI-MS (m/e): 293.07 (M+). Anal. Calcd (found) for $C_{13}H_{12}C_1N_3OS$: C-53.16 (52.86); H-4.12 (4.92): N-14.3 (14.12).

7b: 3-(4-Bromophenyl)-2-(3-methyl-1H-pyrazol-4-yl) thiazolidin-4-one

Yield – 66 %; Melting Point: 113-114 ^{o}C; IR (KBr, Cm^{-1}): 2951.24 (-CH_3, str); 1690.61 (C=O, str); 1460.14 (-CH_3, def); 1300.33 (C-N, str); 664.21 (C-Br, str). ^{I}H NMR (DMSO-d_6, δppm): 12.5 (s, 1H,-NH); 7.21-7.19 (d, 2H, J=10 Hz, Ar-H); 7.17 (s, 1H, Ar-H); 6.81-6.79 (d, 2H, J=10Hz, Ar-H);

4.81 (s,1H,CH-N); 3.47 (s,1H,CH_2-C=O); 3.22(s,1H,CH_2-C=O); 2.70 (s,3H,-CH_3). ^{13}CNMR (DMSO-d_6, δppm): 170.7;147.2; 145.1; 141.3; 132.8; 129.2; 129.2 ; 123.7; 123.7; 105.7; 45.2; 45.2; 11.3. ESI-MS (m/e): 336.92 (M+). Anal. Calcd (found) for $C_{13}H_{12}BrN_3OS$: C-46.16 (45.86); H- 3.58 (3.19): N-12.42 (11.12).

7c: 2-(3-Methyl-1H-pyrazol-4-yl)-3-(4-nitrophenyl) thiazolidin-4-one

Yield – 76 %; Melting Point: 118-119 ^{o}C; IR (KBr, Cm^{-1}): 2949.24 (-CH_3, str); 1700.61 (C=O, str); 1515.02 (C-NO_2, str); 1461.14 (-CH_3, def); 1311.33 (C-N, str). IH NMR (DMSO-d_6, δppm): 13.5 (s, 1H,-NH); 7.23-7.21 (d, 2H, J=10 Hz, Ar-H); 7.19 (s, 1H, Ar-H); 6.80-6.78 (d, 2H, J=10Hz, Ar-H); 4.83 (s,1H,CH-N); 3.41 (s, 1H, CH_2-C=O); 3.22 (s,1H,CH_2-C=O); 2.70 (s,3H,-CH_3). ^{13}CNMR (DMSO-d_6, δppm): 170.5; 147.3; 144.1; 139.3; 132.7; 126.2; 126.2; 121.7; 121.7; 107.7; 44.2; 44.2; 12.3. ESI-MS (m/e): 304.09 (M+). Anal. Calcd (found) for $C_{13}H_{12}N_4O_3S$: C-51.31 (51.00); H- 3.97 (3.79): N-18.41 (18.32).

7d: 3-(3, 4-Dichlorophenyl)-2-(3-methyl-1H-pyrazol-4-yl) thiazolidin-4-one

Yield – 68 %; Melting Point: 115-116 ^{o}C; IR (KBr, Cm^{-1}): 2948.24 (-CH_3, str); 1720.61 (C=O, str); 1471.14 (-CH_3, def); 1321.33 (C-N, str); 764.21 (C-Cl, str). ^{I}H NMR (DMSO-d_6, δppm): 12.7 (s, 1H,-NH); 7.35 (s, 1H, Ar-H); 7.2-7.18 (d, 2H, J=10 Hz, Ar-H); 7.11-7.09 (d, 1H, J=10Hz, Ar-H); 4.83 (s,1H,CH-N); 3.41 (s,1H,CH_2-C=O); 3.22 (s,1H,CH_2-C=O); 2.70 (s,3H,-CH_3). ^{13}CNMR (DMSO-d_6, δppm): 170.3; 147.2; 140.1; 137.3; 132.7; 123.2; 123.2 ; 121.7; 121.7; 105.7; 47.2; 47.2; 11.3. ESI-MS (m/e): 327.15(M+). Anal. Calcd (found) for $C_{13}H_{11}Cl_2N_3OS$: C-47.57 (47.32); H- 3.38 (3.28): N-12.8 (12.21).

7e: 3-(4-Chloro-3-nitrophenyl)-2-(3-methyl-1H-pyrazol-4-yl) thiazolidin-4-one

Yield – 63 %; Melting Point: 119-120 ^{o}C; IR (KBr, Cm^{-1}): 2948.24 (-CH_3, str); 1720.61 (C=O, str); 1525.02 (C-NO_2, str); 1471.14 (-CH_3, def); 1321.33 (C-N, str); 764.21 (C-Cl, str). ^{I}H NMR (DMSO-d_6, δppm): 12.3 (s, 1H,-NH); 7.35 (s, 1H, Ar-H); 7.2-7.18 (d, 2H, J=10 Hz, Ar-H); 7.11-7.09 (d, 1H, J=10Hz, Ar-H); 4.85 (s,1H,CH-N); 3.49 (s, 1H, CH_2-C=O); 3.25 (s,1H,CH_2-C=O); 2.69 (s,3H,-CH_3). ^{13}CNMR (DMSO-d_6, δppm): 170.3; 147.5; 140.3; 137.8; 132.8; 125.2; 125.2 ; 121.7; 121.7; 105.7; 47.2; 47.2; 11.3. ESI-MS (m/e): 338.05 (M+). Anal. Calcd (found) for $C_{13}H_{11}ClN_4O_3S$: C-48.09 (47.90); H- 3.27 (3.42): N-16.54 (16.11).

7f: 3-(3,4-Difluorophenyl)-2-(3-methyl-1H-pyrazol-4-yl)thiazolidin-4-one

Yield – 63 %; Melting Point: 123-125 ^{o}C; IR (KBr, Cm^{-1}): 2948.24 (-CH_3, str); 1720.61 (C=O, str); 1471.14 (-CH_3, def); 1321.33 (C-N, str); 1201 (C-F, str). ^{I}H NMR (DMSO-d_6, δppm): 12.37 (s, 1H,-NH); 7.32 (s, 1H, Ar-H); 7.23-7.21 (d, 2H, J=10 Hz, Ar-H); 7.11-7.09 (d, 1H, J=10Hz, Ar-H); 4.83 (s,1H,CH-N); 3.48 (s,1H,CH_2-C=O); 3.15 (s,1H,CH_2-C=O); 2.89 (s,3H,-CH_3). ^{13}CNMR (DMSO-d_6, δppm): 170.5; 146.5; 141.3; 138.8; 132.8; 127.2; 123.2 ; 121.7; 121.7; 105.7; 47.2; 47.2; 11.3. ESI-MS (m/e): 295.15(M+). Anal. Calcd (found) for $C_{13}H_{11}F_2N_3OS$: C-52.87 (52.47); H- 3.75 (3.52): N-14.23 (14.11).

7g: 3-(2-Fluoro-5-nitrophenyl)-2-(3-methyl-1H-pyrazol-4-yl) thiazolidin-4-one

Yield – 69 %; Melting Point: 130-131 ^{o}C; IR (KBr, Cm^{-1}): 2927.14 (-CH_3, str); 1720.61 (C=O, str); 1534.34 (C-NO_2, str); 1321.33 (C-N, str); 1433.24 (-CH_3, def); 1160.21 (C-F, str). IH NMR (DMSO-d_6, δppm): 13.5 (s, 1H,-NH); 8.2-8.18 (d, 2H, J=10 Hz, Ar-H); 7.45 (s, 1H, Ar-H); 7.21-7.19 (d, 2H, J=10 Hz, Ar-H); 4.81 (s,1H,CH-N); 3.43 (s,1H,CH_2-C=O); 3.15 (s,1H,CH_2-C=O); 2.49 (s, 3H,-CH_3). ^{13}CNMR (DMSO-d_6, δppm): 170.2; 149.5; 143.3; 136.8; 132.7; 123.2; 121.2 ; 119.7; 119.7; 107.7; 47.2; 47.2; 11.3.ESI-MS (m/e): 322.08 (M+). Anal. Calcd (found) for $C_{13}H_{11}FN_4O_3S$: C-48.44 (48.11); H- 3.44 (3.31): N-17.38 (17.10).

7h: 2-(3-Methyl-1H-pyrazol-4-yl)-3-(4-(trifluoromethyl) phenyl) thiazolidin-4-one

Yield – 75 %; Melting Point: 123-124 ^{o}C; IR (KBr, Cm^{-1}): 2927.14 (-CH_3, str); 1720.61 (C=O, str); 1321.33 (C-N, str); 1433.24 (-CH_3, def); 1160.21 (C-F, str). ^{I}H NMR (DMSO-d_6, δppm): 13.7 (s, 1H,-NH); 7.21-7.18 (d, 2H, J=15 Hz, Ar-H); 7.15 (s, 1H, Ar-H); 6.8-6.78 (d, 2H, J=10Hz, Ar-H); 4.81 (s,1H,CH-N); 3.43 (s,1H,CH_2-C=O); 3.15 (s,1H,CH_2-C=O); 2.69 (s,3H,-CH_3). ^{13}CNMR (DMSO-d_6, δppm): 170.3; 149.6; 143.1; 136.7; 132.7;125.3; 123.2; 121.2 ; 120.7; 120.7; 107.7; 47.2; 47.2; 11.3. ESI-MS (m/e): 327.09 (M+). Anal. Calcd (found) for $C_{14}H_{12}F_3N_3OS$: C-51.37 (51.92); H- 3.7 (3.51): N-12.84 (12.79).

7i: 2-(3-Methyl-1H-pyrazol-4-yl)-3-(4-nitro-3-(trifluoromethyl) phenyl) thiazolidin-4-one

Yield – 88 %; Melting Point: 188-189 ^{o}C; IR (KBr, Cm^{-1}): 2925.24 (-CH_3, str); 1720.61 (C=O, str);

1513.02 (C-NO_2, str); 1436.24 (-CH_3, def); 1330.21 (C-F, str); 1321.33 (C-N,str). IH NMR (DMSO-d_6, δppm): 13.1 (s, 1H,-NH); 8.12-8.10 (d,1H,J=10Hz, Ar-H); 7.7 (s, 1H, Ar-H); 7.62-7.60 (d, 1H, J=10Hz, Ar-H); 7.25 (s, 1H, Ar-H); 2.39 (s, 3H, -CH3); 4.81 (s, 1H, CH-N); 3.43 (s, 1H, CH_2-C=O); 3.15 (s, 1H, CH_2-C=O); 2.69 (s, 3H, -CH_3). ^{l3}CNMR (DMSO-d_6, δppm 179.3; 149.6; 147.1; 139.7; 133.7; 124.3; 123.7; 121.5; 120.3; 120.3; 107.7; 47.2; 47.2; 11.3. ESI-MS (m/e): 372.07 (M+). Anal. Calcd (found) for $C_{14}H_{11}F_3N_4O_3S$: C-45.16 (45.10); H- 2.98 (2.56): N-15.07 (14.87).

7j: 3-(4-Methoxyphenyl)-2-(3-methyl-1H-pyrazol-4-yl) thiazolidin-4-one

Yield – 88 %; Melting Point: 133-134 ^oC; IR (KBr, Cm^{-1}): 2924.14 (-CH_3, str); 2855.22 (-OCH_3); 1730.21 (C=O, str); 1432.24 (-CH_3, def); 1321.33 (C-N, str). ^{l}H NMR (DMSO-d_6, δppm): 13.7 (s, 1H, -NH); 7.49 (s, 1H, -N=CH-); 7.21-7.18 (d, 2H, J=15 Hz, Ar-H); 7.15 (s, 1H, Ar-H); 6.8-6.78 (d, 2H, J=10Hz, Ar-H); 4.81(s,1H,CH-N); 3.43(s,1H,CH_2-C=O); 3.73 (s,3H); 3.15 (s,1H, CH_2-C=O); 2.69 (s, 3H, -CH_3). ^{l3}CNMR (DMSO-d_6, δppm): δppm179.3; 149.6; 147.1; 139.7; 133.7; 124.3; 123.7; 121.5 ; 120.3; 120.3; 107.7; 55.1; 47.2; 47.2; 11.3. ESI-MS (m/e): 289.09 (M+). Anal. Calcd (found) for $C_{14}H_{15}N_3O_2S$: C-58.11 (57.86); H- 5.23 (5.12): N-14.52 (14.38).

Antioxidant activity

As Table 1 shows, compounds 7a, 7b, 7c, 7e and 7j showed higher antioxidant activity (IC_{50}) than the standard, ascorbic acid, due to the presence of strong electron donating group.

Antidiabetic activity

The acute toxicity study was performed and revealed that at 5 mg/kg body weight of the compounds was nontoxic. Treatment of STZ animals treated with glucose solution (5 %) for 12 – 24 h, resulted in increase in glucose level in comparison to control animals due to insulin deficiency.

As table 2 shows, blood glucose levels in rats administered with 2 g/kg glucose were significantly decreased by the test compounds within 1 h compared to the standard group. Treatment with compounds 7a - 7j showed that there was significant decrease in blood glucose level compared to the standard, rosiglitazone, on 21 day of study. However, on the 21st day of treatment, it was found that there was significant gain of body weight.

Serum biochemical profile

There was significant decrease in serum cholesterol, triglyceride, creatinine and urea levels whereas the HDL level and total protein levels increased after 21 days of treatment. Compounds 7a,7b,7c,7h,7j produced more significant antiglycemic effect in hyperglycemic rats but had no significant effect in normoglycemic rats (Table 3).

Table 1: Antioxidant activity of synthesized compounds (DPPH method)

Compound code	% Radical scavenging activity (mean ± SEM)					IC_{50} µg/ml
	25 µg/mL	50 µg/mL	75 µg/mL	100 µg/mL	125 µg/mL	
7a	15.61 ±06	21.31±0.026	23.41±0.098	36.97±0.15	47. 32±0.026	137.67
7b	18.06±0.01	28.56±0.06	31.91±0.05	52.75±0.12	60.2 0±0.14	96.38
7c	21.21±0.12	31.37±0.09	38.22±0.24	43.44±0.15	56.5 3±0.16	107
7d	12.39±0.05	25.60±0.025	37.24±0.16	56.52±0.052	63 .70±0.01	88.41
7e	8.83±0.098	14.95±0.026	45.00±0.023	56.24±0.05	65 .01±0.11	86.70
7f	35.00±0.12	58.23±0.01	65.31±0.18	75.00±.0.08	82. 13±0.14	44.31
7g	28.44±0.03	37.09±0.12	48.89±0.14	55.60±0.21	72.4 8±0.12	83.41
7i	25.00±0.17	50.11±0.05	62.03±0.2	75.80±0.03	85.00 ±0.08	49.75
7j	14.10±0.13	32.12±0.25	41.51±0.12	52.97±0.01	62.2 9±0.11	98.30
Ascorbic acid	22.28±0.12	41.03±0.19	52.06±0.2	75.02± 0.09	96.10±0.18	14.66

Table 2: Chronic effect of standard and synthesized compounds on blood glucose level of rat

Group (OGTT, mg/dl)	0 min	60 min	120 min	180 min
Control	91.5±2.23	106.2±3.02	103±7	93.67±1.00
Rosiglitazone (8)	91.5±2.23	110.0±3.94	108±1.89	97.83±0.47*
Compound 7a (5)	95±2.23	125.3±1.22	110.±1.28	99±0.96*
Compound 7b (5)	95±2.23	128 ±0.93	114±2.6	97.8±0.27*
Compound 7c (5)	89.25±2.23	138±0.94	121±2.81	98.17±0.70*
Compound 7h (5)	91.17±1.30	141.3±0.91	130.5±2.43	96±0.85*
Compound 7j (5)	92.83±0.87	136.6±0.87	125.5±1.83	93.5±1.4*
Group (FBG, mg/dL)	0 days	7 days	14 days	21 days
Control	91±1.00	115.7±3.9	114±6.8	100±2.47
Diabetic control	244.7±2.96	281.3±2.51	311±3.99	336±2.12
Rosiglitazone (8)	311.3±3.99	247.8±7.4	126.7±5.8	106.5±1.9
Compound 7a (5)	285.3±5.28	195±6.58	152±7.66	108.17±1.35*
Compound 7b (5)	293.5±4.35	191±6.79	150.5±7.55	115.5±1.76*
Compound 7c (5)	283.2±1.19	190±3.73	157±6.09	110.81±2.8*
Compound 7h (5)	293.5±6.99	158.2±3.20	127.3±2.01	117.33±0.5*
Compound 7j (5)	285.2±4.79	149.8±2.89	129±1.88	98.33±0.76*
Body weight (g)				
Control	160±0.6	169±0.5	166±0.8	163±0.2
Diabetic control	178.5±0.99	190±0.69	215±0.56	253±0.12
Rosiglitazone(8)	171±0.39	144.5±0.67	141±0.60	169±0.6
Compound 7a (5)	163.3±0.61	215.2±0.69	187±0.89	178±0.9*
Compound 7b (5)	174±0.49	133±0.57	127.8±0.27	167±0.61*
Compound 7c (5)	167.7±0.49	143±0.57	129.8±0.55	169.2±0.46*
Compound 7h (5)	166±0.36	142±0.36	125±0.83	160±0.69*
Compound 7j (5)	167±0.41	131±0.5	127±0.56	163±0.26*

**No significant change in level (p < 0.001); OGTT,* Oral Glucose Tolerance Test blood glucose level; FBG, Fasting blood glucose level.

Table 3: Biochemical parameters of rat following treatment with the synthesized compounds

Group (mg/kg body wt)	Cholesterol (mg/dL)	Triglycerides (mg/dL)	Creatinine (mg/dL)	Urea (mg/dL)	HDL Cholesterol (mg/dL)	Total Protein (g/dL)
Control	148±1.5	83.3±4.6	0.6±0.4	22±0.4	34.1±1.8	8.3±1.7
Diabetic control	290±1.9	258±9.8	2±1.9	80±3.2	28±1.8	4±3.2
Rosiglitazone (8)	119±2.8	101±5.2	0.41±1	32.2±3.1	63±2.9	8.4±0.4
Compound 7a (5)	150±2.49*	120.5±3.6*	0.63±0.09*	31±0.44*	46±1.41*	7.6±1.1*
Compound 7b (5)	164.7±2.71*	136.5±1.4*	0.58±0.01*	34.83±0.87*	57±0.85*	8.7±0.9*
Compound 7c (5)	179.8±2.18*	135.7±1.54*	0.53±0.02*	34.5±0.99*	42.5±0.42*	5.4±1.7*
Compound 7h (5)	156.8±0.6*	143±2.09*	0.47±0*	35.5±0.42*	45±2.46*	5±2*
Compound 7j (5)	167.2±0.6*	134.3±1.33*	0.6±0.01*	32.0±0.73*	41.67±3.8*	4.9±1.6*

*Data represent mean ± SEM, (n = 6); *no significant change in level (p < 0.001)*

DISCUSSION

The present work involves cyclization of schiff bases to thiazolidine-4-ones derivatives. The schiff bases (6a-6j) were obtained by the reaction between electrophillic carbon atom of substituted pyrazole-4-aldehyde and nucleophillic nitrogen atom of substituted amines. The preparation of thiazolidine-4-ones (7a - 7j) proceeds by an attack of sulphur nucleophile of thioglycollic acid on imine carbon followed by intramolecular cyclization.

During the reaction, one mole of water was eliminated. Tin chloride dihydrate acted as acid catalyst which counters balance between nucleophilicity and acidity for completion of reaction. The substitution with electron donating group at para- and meta- position of ring increases percentage yield where as it decreases in case of ortho substitution due to steric effect. Purification of compounds was done using hexane: chloroform (1:4 v/v) for thiazolidine-4-ones by column chromatography.

The formations of compounds were again confirmed by elemental analysis (C, H, N analysis), IR, ^{1}HNMR, ^{13}CNMR and MS. The IR peaks at 1775.51-1701.61, 1685-1624, 1321.33-1260.33 and 1290-1200 cm^{-1} indicate presence of C=O, C=N, C-N groups in synthesized compounds .The characteristic ^{1}HNMR peaks at

δ values 4.85-4.81, 3.49-3.43 and 3.25-3.15 ppm indicate presence of thiazolidine-4-ones. The aromaticity of the compounds was also confirmed by ^{1}HNMR. The ^{13}CNMR spectral data also describe characteristic peak according to proposed structure. From the mass spectra, it was found that m/e peaks according to calculated molecular mass of the synthesized compounds.

In vitro antioxidant activity was determined by DPPH method. The DPPH assay method is based on the reduction of DPPH, a stable free radical. The free radical DPPH with an odd electron gives a maximum absorption at 517 nm. When a solution of DPPH is mixed with that of a substance that can donate a hydrogen atom, then this gives rise to the reduced form with the loss of this violet colour [13]. The compounds with strong electron donating groups like 7a, 7b, 7c, 7h, 7j showed more IC_{50} values compared to standard Ascorbic acid The antidiabetic activity was performed by using streptozotocin (STZ) induced model in Wister rats. STZ enters the pancreatic cell via a glucose transporter-GLUT2 and causes alkylation of deoxyribonucleic acid. STZ induces activation of poly adenosine ribosylation and nitric oxide release. Due to destruction of pancreatic cells by STZ a huge release of insulin which makes animals more susceptible to severe hypoglycemia that may be lethal. Thus, following treatment with STZ animals are fed with glucose solution (5 %) for 12 – 24 h, afterwards, an increase of glucose levels is observed in comparison to control animals due to insulin deficiency [21].

The thiazolidinediones are dependent on the presence of insulin for activity; however, they do not affect insulin secretion. They are highly selective and potent agonists for the peroxisome proliferator activated receptor (PPAR) gamma that regulates the transcription of a number of insulin responsive genes. Activation of PPAR-gamma receptors regulates the transcription of insulin-responsive genes involved in the control of glucose production, transport, and utilization. Additionally, PPAR-gamma responsive genes also play a role in the regulation of fatty acid metabolism. Unlike oral sulfonylureas, rosiglitazone enhances tissue sensitivity to insulin rather than stimulates insulin secretion [22]. From the structural activity relationship study it was found that compounds 7a, 7b, 7c, 7h and 7j are biologically active. The presences of thiazolidine-4-one nucleus as well as para substitution of phenyl ring are significant for potentiation of activity.

CONCLUSION

The structures of the synthesized 2-(3-methyl-1H-pyrazol-4-yl)-3-phenylthiazolidin-4-ones have confirmed by spectral and elemental analysis. Compounds 7a, 7b, 7c, 7h and 7j are more potent antioxidant and antidiabetic than the reference standards, ascorbic acid and rosiglitazone, respectively. These activities are probably due to thiazolidine-4-one nucleus present along with pyrazole ring on their structures.

ACKNOWLEDGEMENT

The authors thank the IIMT College of Medical Sciences; Regional Institute of Pharmaceutical sciences and Technology, Govt of Tripura; AIRF, JNU, New Delhi and Arbro Lab, New Delhi. The authors also profoundly thank Arunabaha Mallik, Assistant Professor, Bansal College of Pharmacy, Bhopal for providing the facility for the animal studies.

REFERENCES

1. *Sharshira EM, Hamada NMM. Synthesis and antimicrobial evaluation of some pyrazole derivatives. Molecules 2012; 17: 4962-4971.*
2. *Chaurasia MR. Some 5-Acetamido-3-Aryl-2-Arylamino4-ThiazolidoneAnalogues and their Local Anaesthetic Activity. Indian J Pharm 1971; 33: 17-19.*
3. *Zimenkovsky B, Lesyk R, Vladzimirska O, Nektegayev I, Golota S, Chorniy I. The structure-anti-inflammatory activity relationship among thiazolidines. J Pharm Pharmacol 1999; 51: 264-270.*
4. *Saltiel AR, Olefsky JM. Thiazolidinediones in the treatment of insulin resistance and type II diabetes. Diabetes 1996; 45: 1661–1669.*
5. *Ghazzi MN, Perez JE, Antonucci TK, Driscoll JH, Huang SM, Faja BW. Cardiac and Vglycemic benefits of troglitazone treatment in NIDDM The Troglitazone Study Group. Diabetes 1997; 46: 433–439.*
6. *Schwartz S, Raskin P, Fonseca V, Graveline JF. Effect of troglitazone in insulin-treated patients with type II diabetes mellitus. N Engl J Med 1998; 338: 861–866.*
7. *Buse J, Gumbiner B, Mathias NP, Nelson DM, Faja BW, Whitcomb RW. Troglitazone use in insulin-treated type 2 diabetic patients. Diabetes Care 1998; 21: 1455–1461.*
8. *Quilliot D, Walters E, Bonte JP, Fruchart JC, Duriez P, Ziegler O. Diabetes mellitus worsens antioxidantstatus in patients with chronic pancreatitis. Am J Clin Nutr 2005; 81(5): 1117-1125.*
9. *Furniss BS, Hannaford AJ, Smith PWG, Tetchell AR, Addition Wesley. Vogels text book of practical organic*

chemistry. Fifth edition. England. Long Man Ltd. Edinburgh Gate Harlow; 1998; pp 243-244, 1150

10. *Srivastava A, Singh RM. Vilsmeier-Haack reagent: A facile synthesis of 2-chloro-3-formylquinolines from N-arylacetamides and transformation into different functionalities. Ind. J. Chem. 2005; 44B: 1868–1875.*
11. *Li Y, Yang ZS, Zhang H, Cao BJ, Wang FD. Artemisinin Derivatives Bearing Mannich Base Group: Synthesis and Antimalarial Activity. Biol Org Med Chem 2003; 11: 4363-4368.*
12. *Brand-Willams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity. Leben-Wissen and Techno 1995; 28: 25-30.*
13. *Espin JC, Soler-Rivas C, Wichers HJ. Characterization of total free radical scavenger capacity of vegetable oils and oil fraction using 2,2- diphenyl-1picrylhydrazyl radical. J Agric Food Chem 2000; 48: 648-656.*
14. *OECD. Guidelines No. 423 for the testing of chemicals revised draft guideline 423 (acute oral toxicity). Paris: OECD; 2000.*
15. *Kadnur SV, Goyal RK. Comparitive antidiabetic activity of methanolic extract and ethyl acetate extract of Zingiber officinale Roscoe. Indian J Pharm Sci 2005; 67: 453-457.*
16. *Rifaci N, Bachorik PS, Albers JJ. Lipid, lipoprotein and apolipoprotein, ln Burtis CA and Ashwood ER (Eds). Textbook of Clinical Chemistry. 3rd edn,. Philadolphia: WB Saunder Company 1999; pp 809-861*
17. *Mac Donald RP. Standard methods of clinical chemistry. New York: Academic Press; 1953; pp 215-222.*
18. *Thomas L. Clinical Laboratory Dragnostics.TH. Verlasgesell Schaft ; Frankfurt: 1998; pp 208-214, 366-374*
19. *Tretz NW. Textbook of Clinical Chemistry. 3rd edn, Philadelphia: WB Saumder Company 1986; p 579.*
20. *Burstein M, Scholnick HR, Morfin R. Rapid method for the isolation of lipoprotein from human serum by precipitation with polyamines. J Lipid Res 1970; 11: 583-595.*
21. *Frode TS, Medeiros YS. Animal models to test drugs with potential antidiabetic activity. J Ethnopharmacol 2008; 115: 173–183.*
22. *Imran M, Ilyas B, Deepanjali, Khan SA. Recent thiazolidinediones as antidiabetics. J Sci Ind Res 2007; 66(2): 99-109.*