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Facile synthesis and *in vivo* hypoglycemic activity of novel 2, 4-hiazolidinedione derivatives

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ABSTRACT

A series of novel 2, 4-thiazolidinedione derivatives 2-{4-[(E)-(2,4-dioxo-1,3-thiazolidin-5-ylidene)methyl] phenoxy}-N-(substituted phenyl) acetamides ***d (1-12)*** *have been accomplished in good yields by stirring 2,4-thiazolidinedione* ***a*** *and 2-(4-formyl phenoxy) N-substituted acetamide* ***c (1-12)*** *at room temperature. Some of the reaction sequences were carried under microwave irradiation which resulted in increased reaction rate and yield enhancement. The synthesized compounds were evaluated for their hypoglycemic activity in Wister albino mice animal model and histopathological studies for kidney and Liver were performed. Some of the derivatives have exhibited promising hypoglycemic activity.*

Key words: Thiazolidinedione, acetamide, Anti-hyperglycemic, Alloxan-induced diabetes.

INTRODUCTION

Diabetes Mellitus (DM) consists of a group of syndromes characterized by hyperglycemia, altered metabolism of lipids, carbohydrates, proteins and increased risk of complications from vascular disease. Type 2 diabetes mellitus (T2DM) is a chronic metabolic disorder, characterized by hyperglycemia [1].

The thiazolidinediones (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 unknown, a number of reports suggest that TZDs are high affinity ligands of peroxysome proliferator activated receptor-γ. TZDs at these receptors act as insulin sensitizers. PPARα and PPARγ receptor subtypes show different tissue and ligand specificities, PPARγ agonists improve glycemic control and dyslipidemia in type 2 diabetic patients by down regulating cytokines in adipose tissue, while agonists of the PPAR subtype improve the atherogenic lipoprotein profile of insulin resistance [2–5]. Therefore, PPARs are legitimate molecular targets for the development of antidiabetic agents. 2,4-Thiazolidinedione moiety is the generic feature of the glitazone antidiabetic agents[6]. Unlike sulfonylureas (e.g., glipizide , glyburide), which enhance insulin resistance and metformin, which reduces hepatic glucose output, TZDs (e.g. rosiglitazone, pioglitazone) improve insulin sensitivity in liver, muscle and fat tissues and thus counteract insulin resistance [7]. These drugs, however, have been associated with liver, cardiovascular and hematological

toxicity and body weight gain. This situation emphasizes the need to develop new antidiabetic agents[, , ,] that could retain the insulin sensitizing properties of TZD, but be safer and have better efficacy.

The use of microwave (MW) heating in last years has revolutionized the organic synthesis and is indispensible tool [8]. Advantages of microwave include reduced pollution, reduced reaction times, increased reaction rates; yield enhancements, cleaner and greener eco friendly synthetic protocols. All these facts prompted us to investigate novel TZD as selective PPARγ agonist as candidate for the development of antidiabetic agents. The structural feature of compounds synthesized in the present work was based on rosiglitazone as the lead compound. The possible diagrammatic representation of synthesized derivative with PPAR γ receptor is shown in Fig.1. We modified the hydrophobic ring tail with various substituents like aryl/heteryl / aliphatic- alicyclic groups; also the phenoxy ethyl group was replaced by phenoxy methyl group, in order to investigate the effect of substitution. Herein, we report the synthesis of novel 2,4-thiazolidinedione derivatives as hypoglycemic agents. Like rosiglitazone and pioglitazone, the newly synthesized derivatives 2-{4-[-(2, 4-dioxo-1, 3-thiazolidin-5-ylidene) methyl] phenoxy}-*N*-(substituted phenyl) acetamide **d (1-12)** are potent hypoglycemic agents.

Chemistry :We here in report the Claisen- Schmidt reaction of 2, 4- thiazolidinedione **a** with the 2-(4-formyl phenoxy) N-substituted acetamide **c (1-12)** to give novel derivatives 2-{4-[(*E*)-(2,4-dioxo-1,3-thiazolidin-5-ylidene)methyl]phenoxy}-*N*-(substituted phenyl) acetamide **d (1-12).** Multi component 1, 3-dipolar cycloaddition reactions are considered to be one of the most useful processes for the construction of five member heterocyclic ring systems [9-11]. 2, 4-Thiazolidinedione was synthesized according to the procedure from mono chloro acetic acid and thiourea [12]. The 4-(formyl phenoxy) acetic acid **b** [13] was synthesized by refluxing 4-hydroxy benzaldehyde, monochloro acetic acid and NaOH. In the next step 4-(formyl phenoxy acetic acid) **b** was condensed with different substituted aromatic, heterocyclic, alicyclic amines to get 2-(4-formylphenoxy)-*N*- substituted phenylacetamide) **c (1-12)**; these when condensed with **a** give the final derivatives **d (1-12)**. All these compounds were synthesized in accordance with the literature procedure [14]. The protocol for the synthesis of the presented TZD derivatives is as shown in Scheme 1.

IR, ^{1}H NMR, mass spectral studies and elemental analysis confirmed the ring structures of products **a, b** and **c.** The IR spectrum of **d1** revealed the presence of a thiocarbonyl stretching vibration band at 1750 cm^{-1} showing an increase of 40 cm^{-1}from the normal value of 1710 cm^{-1}.The ^{1}H NMR spectrum of **d2** revealed one sharp singlet at 2 ppm due to the methyl protons. A doublet of doublets was observed at δ 1.97 and 3.42 for the protons of pyrolidine ring in the ^{1}H NMR spectrum of **d8**. The O-CH_2 proton appeared as a singlet at δ 4.8 in the ^{1}H NMR spectrum of **d2**. The aromatic protons appeared as a multiplet in the region δ 6.80–7.80. Two broad singlets at δ 8.261 and δ 10.1 ppm, confirms the NH proton of amide and thiazolidinedione ring, respectively. Similarly, the olefinic proton appears as a singlet at δ 7.15 ppm. The structure of the products **d1** and **d2** were confirmed by its ^{1}H NMR spectrum. The mass spectrum of **d 1** showed a molecular ion peak at m/z 356.14 (M^+). The mass spectrum of **c (1-4)** showed the molecular ion peak at m/z 256.14 (M^+), 270.24 (M^+), 301.35 (M^+), 290.41 (M^+) and confirmed the formation of products. The structures were further confirmed by the elemental analysis.

MATERIALS AND METHODS

Synthesis

Melting points were determined in an open capillary melting point apparatus and are uncorrected. All the chemicals and solvents used in this study were purchased from E. Merk and Qualigens .Synthetic microwave Micro Synth Microwave Lab station-Ethosy Milestone was used. IR spectra were recorded on *JASCO FT-IR 5300* spectrophotometer using KBr powder. ^{1}HNMR spectra were recorded on Varian Mercury YH 300 FT-NMR Spectrometer at 300 MHz Frequency in deuteriated DMSO and $CDCl_3$ using TMS as internal standard and chemical shifts (δ) are given in ppm relative to TMS. Mass spectra were recorded on Shimadzu's GCMS-QP5050A System bench top quadrupole mass spectrometer. Elemental analyses were performed at Shimadzu Analytical Centre, Chemistry Department, Pune on FLASH EA 1112 CHN analyzer and found within ± 0.4% of theoretical values(C, H, N) were obtained. All the reactions were monitored by thin layer chromatography, performed on silica gel plates with iodine vapors as indicator and the R_f values were calculated. The structures of synthesized compounds were confirmed by spectral and elemental analysis. The physical characterization of synthesized compounds **c (1-12)** and **d (1-12)** is summarized in Table 1.

Synthesis of 2, 4-thiazolidinedione
General Procedure
Solution of chloro acetic acid (5.64 g, 0.06mol) in 6 ml of water was mixed with solution of thiourea (4.56g, 0.06 mol) in 6 ml of water in a 100 ml RBF. The mixture was stirred for 15 minutes, the white solid separated out. To this solid, 6 ml of concentrated HCl was added slowly from dropping funnel. The flask was connected to reflux condenser, and heated gently to effect complete solution after which the reaction mixture is stirred and refluxed for 8-10 hours at 100-110 ^{0}C. On cooling the contents of the flask, the cluster of colorless crystalline product was separated out. The product was filtered, washed with water to remove traces of HCl and dried. It was purified and recrystallized by hot water. Molecular Wt. 117 gm; Yield: 83%, mp 125-127^{0}C; Rf - 0.43 (Benzene: Methanol 9:1).

Synthesis of (4-Formyl phenoxy) acetic acid (b)
General Procedure
To 4-hydroxy benzaldehyde (1g) and sodium hydroxide solution (3.5ml,33%), mono chloracetic acid(2.5ml,50%) was added and gently heated on water bath for one hour. It was then acidified with dil. HCl, extracted with ether and 5% sodium carbonate solution. Sodium carbonate extract was acidified with dil. HCl. The product thus obtained was filtered and recrystallized from ethanol. Molecular Wt. 180 g ; Yield:75% ; mp 130-132^{0}C; Rf ;-0.48 (Benzene: Methanol 4.5:0.5).

Synthesis of 2-(4-Formyl phenoxy) N-substituted acetamide c (1-12)
(a) Conventional procedure for synthesis of c
4-Formylphenoxy acetic acid (1gm) and aniline (2gm), or other aromatic, alicyclic, heterocyclic amine was taken in a dry RBF fitted with a short air condenser and the mixture was heated in oil bath at 140-160 ^{0}C, for 2-5 hours. The reaction mass was allowed to cool to room temperature and then acidified with 20-30 ml of 10% hydrochloric acid. The solid thus obtained was filtered and recrystallized from aqueous ethanol (50:50).

(b) Microwave Assisted Synthesis for c8 and c12:
Some of the alicyclic secondary amines like morpholine, pyrolidine required too long reaction times to react with (4-formylphenoxy) acetic acid **b** and gave poor yield by conventional method, hence the same reaction was carried out by microwave irradiation at 700 w for 3-5 minutes (amine and acid in 2:1 proportion) in solvent free condition at temperature of 182^{0}C. The cooled reaction mass was acidified with 20-30 ml of 10% hydrochloric acid. The solid thus obtained was filtered and recrystallized from aqueous ethanol (50:50).

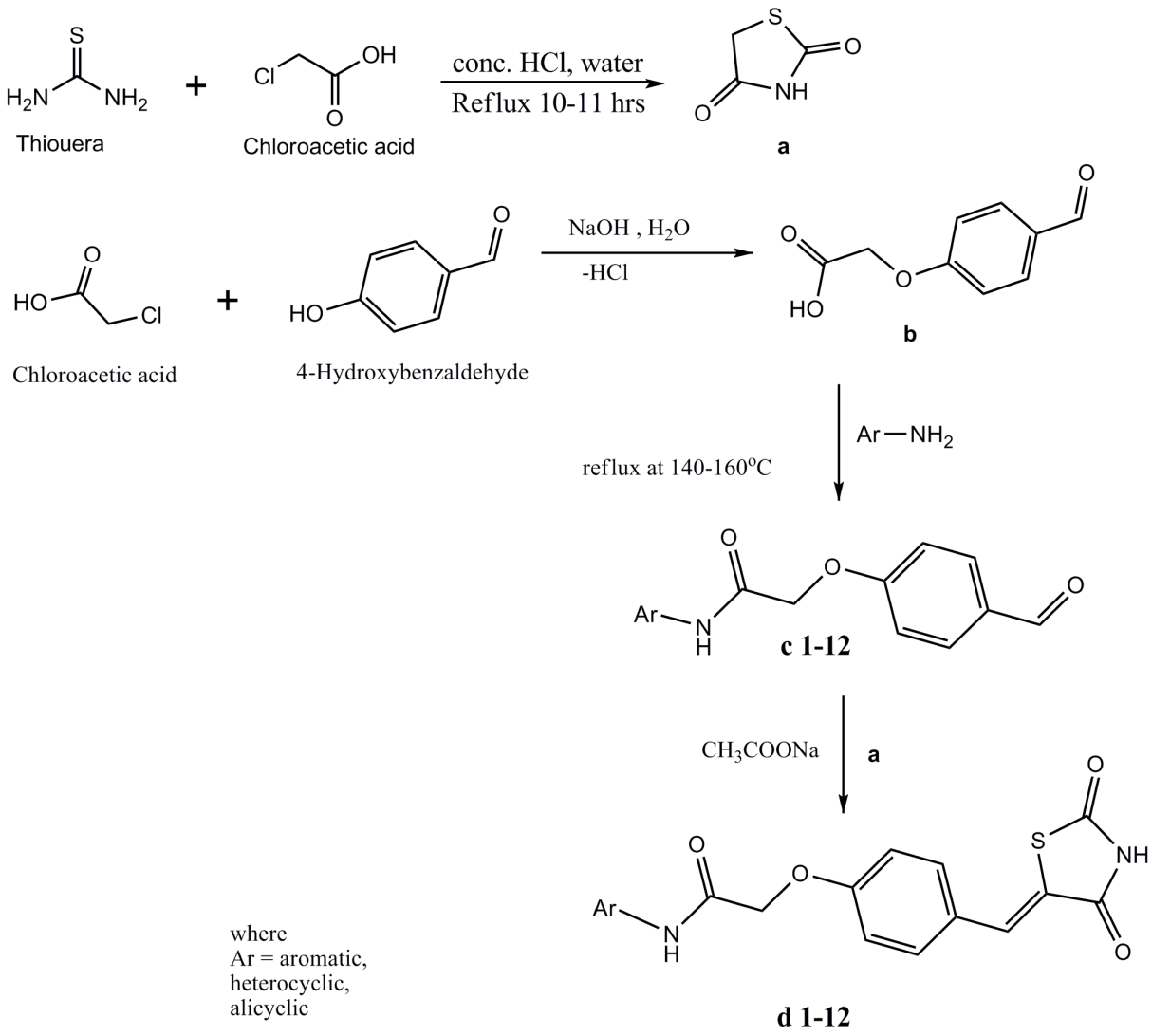
Preparation of 2-{4-(2, 4-thiazolidinedione -5- ylidine) N- substituted phenoxy} phenyl acetamide d (1-12)
General procedure
In Claisen- Schmidt reaction, time requirement is 48 hours which is too long , therefore, in the present work synthesis of 2-{4-(2,4-thiazolidinedione -5- ylidine) N- substituted phenoxy}phenyl acetamide was carried out as per the following procedure[15] .

In 100 ml of conical flask, 2,4-thiazolidinedione (2.5 gm, 0.0236 mole) was dissolved in glacial acetic acid (2.13ml) and to the resulting solution sodium acetate (0.51 gm, 0.006 mole) was added and the mixture was stirred. To this mixture 2-(4-formyl phenoxy) N-substituted acetamide (0.021mole) was added and heated for few minutes with stirring, without boiling glacial acetic acid. Upon cooling, the product separated out. The product was filtered, dried and recrystallized from DMF and ethanol. The structures of the synthesized compounds were confirmed by spectral data.

Spectral characterization of 2-(4-formyl phenoxy) N-substituted acetamide derivatives c (1-12)
Synthesis of (4-formylphenoxy)-*N*- phenylacetamide) (c 1) IR(KBr, cm-1): 3490 (-NH), 3061(Ar-CH),2845 (-CH), 1753 (-CO), 1714 (C=O), 1649 (C=C), 1378-1393(C-O-C),1321(C-O); ^{1}HNMR($CDCl_3$) 4.8(s, H, -CH_2), 6.9-8.0 (m, 9H, Ar), 8.2(s, 1H, -NH), 10(s,H, -CHO) ; m/z 256.14 (M^+). Analysis calculated for $C_{15}H_{13}N\ O_{3:}$ C, 70.58; H, 5.13; N, 5.49 Found: C, 69.93; H,5.64; N, 5.81.

Synthesis of 2-(4-formylphenoxy)-*N*-p-tolylacetamide (c 2) IR(KBr, cm-1): 3490 (-NH), 3061(Ar-CH),2845 (-CH), 1753 (-CO), 1714 (C=O), 1649 (C=C), 1378-1393(C-O-C),1321(C-O); ^{1}HNMR($CDCl_3$) 2.33 (s,-3H, CH_3), 4.8 (s, 2H, -CH_2), 6.9,7.78 (m, 8H, Ar), 10 (s, H, -CHO); m/z 269.14 (M^+). Analysis calculated for $C_{16}H_{15}NO_{3:}$ C, 71.36; H, 5.61; N,5.20 Found: C,71.56; H,5.72; N,5.62.

Scheme 1 Synthesis of 2-{4-(2,4-thiazolidinedione -5- ylidine) N- phenoxy} phenyl acetamide



Synthesis of 2-(4-formylphenoxy)-*N*-(4-nitrophenyl) acetamide (c 3) IR(KBr, cm-1): 3490 (-NH), 3061(Ar-CH),2845 (-CH), 1753 (-CO), 1714 (C=O), 1649 (C=C), 1378-1393(C-O- C), 1421(C-O); ^{1}HNMR($CDCl_3$): 6.63, 6.9,7.78 (m, 8H, Ar), 4.8(s, 2H, -CH_2), 8 (s, 1H , –NH), 10(s ,H, -CHO); m/z 301.32 (M^+). Analysis calculated for $C_{15}H_{12}N_2O_5$: C,60; H, 4.03; N, 9.33. Found: C, 59.49; H, 4.01;N, 8.43.

Synthesis of 2-(4-formylphenoxy)-*N*-(4-cholrophenyl) acetamide) (c 4) IR(KBr, cm-1): 3490 (-NH), 3061(Ar-CH),2845 (-CH), 1753 (-CO), 1714 (C=O), 1649 (C=C), 1378-1393(C-O- C), 1421(C-O); ^{1}HNMR($CDCl_3$) 4.8(s,2H, -CH_2), 6.8-7.80 (m, 8H, Ar), 8.0 (s, 1H ,–NH), 10(s,1H, -CHO); m/z 290.54 (M^+). Analysis calculated for $C_{15}H_{12}ClNO_3$: C, 62.19; H, 4.17; N, 4.83. Found: C, 59.49; H,4.01;N, 4.43

Synthesis of 2-(4-formylphenoxy)-*N*-(3-nitrophenyl) acetamide (c 5) IR(KBr, cm-1): IR(KBr, cm-1): 3490 (-NH), 3061(Ar-CH),2845 (-CH), 1753 (-CO), 1714 (C=O), 1649 (C=C), 1378-1393 (C-O- C), 1421(C-O) ^{1}HNMR($CDCl_3$) 4.8 (H,s, -CH_2), 6.63, 6.9,7.78 (m, 8H), 8 (,s –NH), 10 (s, 1H, -CHO) m/z 301.22 (M^+). Analysis calculated for $C_{15}H_{12}N_2O_5$ C,60; H, 4.03; N, 9.33. Found: C, 59.49; H,4.01;N, 8.43

Synthesis of 2-(4-formylphenoxy)-*N*-(4-methoxyphenyl) acetamide (c 6) IR(KBr, cm-1): 3490 (-NH), 3061(Ar-CH),2845 (-CH), 1753 (-CO), 1714 (C=O), 1649 (C=C), 1378-1393(C-O-C),1321(C-O); ^{1}HNMR($CDCl_3$) 2.33 (s,-3H, -OCH_3), 4.8(s,2H, -CH_2), 6.9-7.78 (m, 8H, Ar), 10(s, 1H, -CHO); m/z 280.48 (M^+). Analysis calculated for $C_{16}H_{15}NO_3$: C,71.36; H,5.61; N,5.20. Found: C, 71.56; H,5.72; N,5.62.

Synthesis of 2-(4-formylphenoxy)-*N*-(pyridin-2yl) acetamide (c 7) IR (KBr, cm-1): 3490 (-NH), 3061 (Ar-CH), 2845 (-CH), 1753 (-CO), 1714 (C=O), 1649 (C=C), 1378-1393(C-O- C), 1421(C-O); ^{1}HNMR ($CDCl_3$) 3.4 (s,3H,) , 6.9-7.78 (m, 7H, Ar), 4.8 (s, 2H,CH_2), 8 (s, 1H, –NH), 10 (s,1H, -CHO); m/z 276.14 (M^+). Analysis calculated for $C_{13}H_{11}N_3O_2$: C,60.70; H, 4.31; N, 16.33. Found: C,59.49; H,4.01;N, 16.43.

Synthesis of 4-(2-oxo-2-(pyrolidine-1-yl)ethoxy)-benzaldehyde (c 8) IR(KBr, cm-1): 3061(Ar-CH),2845 (-CH), 1753 (-CO), 1714 (C=O), 1649 (C=C), 1378-1393(C-O- C), 1421(C-O); ^{1}HNMR($CDCl_3$) 1.97 (d,2H, -CH_2) ,3.46 (d,2H, -CH_2) , 4.83(s, 2H,-OCH_2) ; 6.9-7.1 (m, 4H, Ar), 4.8(s, -CH_2), 10 (s,1H, -CHO)) ; m/z 233.42 (M^+). Analysis calculated for $C_{13}H_{15}NO_3$: C,66.94; H,6.48; N, 6.00. Found: C,65.94; H,6.31;N, 6.43.

Synthesis of 2-(4-formylphenoxy)-*N*-(4-hydroxyphenyl) acetamide (c 9) IR(KBr, cm-1): 3490 (-NH), 3061(Ar-CH),2845 (-CH), 1753 (-CO), 1714 (C=O), 1649 (C=C), 1378-1393(C-O- C), 1421(C-O); ^{1}HNMR ($CDCl_3$) 4.8(s, 2H,-CH_2), 5.0 (s,1H, -OH) ,6.9-7.1-7.78(m, 8H, Ar), 8 (s, 1H,–NH), 10 (s, 1H, -CHO) ; m/z 271.14 (M^+). Analysis calculated for $C_{15}H_{13}NO_4$: C, 66.41; H,4.83; N, 5.61 Found: C,66.94; H,4..81;N, 5.43.

Synthesis of 2-(4-formylphenoxy)-*N*-(3-hydroxyphenyl) acetamide (c 10) IR(KBr, cm-1): 3500(- OH), 3490 (-NH), 3061(Ar-CH),2845 (-CH), 1753 (-CO), 1714 (C=O), 1649 (C=C), 1378-1393(C-O- C), 1421(C-O); ^{1}HNMR ($CDCl_3$) 4.8(s, 1H, -CH_2), 5.0 (s,1H, -OH) ,6.9,7.1, 7.78(m, 8H, Ar), 8 (s,1H, –NH), 10(s,1H, -CHO); m/z 271.14 (M^+). Analysis calculated for $C_{15}H_{13}NO_4$ C,66.41; H,4.83; N, 5.61. Found: C,66.94; H,4..81;N, 5.43.

Synthesis of 2-(4-(-2-(formyl phenoxy)acetamide)phenyl)acetic acid (c 11) IR(KBr, cm-1): 3500(-OH), 3490 (-NH), 3061(Ar-CH), 2845 (-CH), 1753 (-CO), 1714 (C=O), 1649 (C=C), 1378-1393(C-O- C), 1421(C-O); ^{1}HNMR($CDCl_3$) 3.4(s,2H, CH_2) , 6.9,7.1,7.7, 8.8 (m, 7H, Ar), 4.8(s, 1H, -CH_2), 8 (s, 1H,–NH), 10(s, 1H,-CHO) , 11 (s,1H, OH); m/z 399.75 (M^+). Analysis calculated for $C_{16}H_{13}NO_5$ C, 65.17; H, 4.83; N,4.47. Found: C, 65.94; H, 4.55;N, 4.43.

Synthesis of 4-(2-(morpholino-2-oxoethoxy)-benzaldehyde (c 12) IR(KBr, cm-1): 3061(Ar-CH), 2845 (-CH_2), 1753 (-CO), 1714 (C=O), 1649 (C=C), 1378-1393(C-O- C), 1421(C-O); ^{1}HNMR($CDCl_3$) 3.4 (dd,2H, -CH_2) , 3.6 (dd,2H, -CH_2) , 4.8(s,1H, -CH_2), 6.9,7.1,7.78, 8.81 (m, 7H, Ar), 10(s,1H,-CHO); m/z 249.4 (M^+). Analysis Calculated for $C_{13}H_{15}NO_4$ C, 62.64 ; H,6.07; N, 5.62. Found: C,62.94; H,6.23;N, 5.43.

Spectral characterization of 2-{4-(2,4-thiazolidinedione -5- ylidine) N- substituted phenoxy}phenyl acetamide derivatives d (1-12)

Synthesis of 2-{4-[(*E*)-(2,4-dioxo-1,3-thiazolidin-5-ylidene)methyl] phenoxy}-*N*-(phenyl) acetamide (d1) IR (KBr, cm-1) 3490 (-NH), 3366 (-NH amide) ,3030 (Ar-CH),2914, 2862 (-CH), 1683 (-CO), 1575,1585 (C=O), 1649 (C=C), 1435(C-H bend alkane), 1378-1390 (C-O-C), 1244 (C-S); ^{1}HNMR($CDCl_3$) 4.8 (s, 1H, -OCH_2), 7.15(s,1H, =CH), 6.8-7.6 (m, 8H, Ar), 8.0(s,1H, NH amide), 10.1 (s, 1H, -NH thiazolidinedione); m/z 356.26 (M^+). Analysis calculated for C_{18} H_{14} N_2 O_4S: C, 61.01; H, 3.98; N,7.90. Found: C, 61.21; H, 3.48; N,8.10.

Synthesis of 2-{4-[(*E*)-(2,4-dioxo-1,3-thiazolidin-5-ylidene)methyl]phenoxy}-*N*-(4-methyl phenyl)acetamide (d2) IR(KBr, cm-1): 3490 (-NH), 3366(-NH amide) 3030(Ar-CH),2914, 2862 (-CH), 1683 (-CO), 1575,1585 (C=O), 1649 (C=C), 1435(C-H bend alkane), 1378-1390 (C-O-C),1244 (C-S); ^{1}HNMR ($CDCl_3$) 2.4 (s,3H, CH_3) , 4.8(s, 2H, -OCH_2), 7.15(s,1 H, =CH), 6.8-8.2 (m, 8H,Ar), 8.0 (s,1H, NH amide), 10 (s,1H,-NH thiazolidinedione); m/z 369.64 (M^+).Analysis calculated for C_{19} H_{16} N_2 0_4S C, 61.94; H,4.38; N, 7.60. Found: C,61.84; H,4.31;N, 7.43.

Synthesis of 2-{4-[(*E*)-(2,4-dioxo-1,3-thiazolidin-5-ylidene)methyl]phenoxy}-*N*-(4-nitro phenyl) acetamide (d3)IR (KBr, cm-1) 3564 (-NH), 3476 (-NH amide) 3030(Ar-CH),2916, (-CH), 1682 (-C=O), 1575,1575 (C=C), 1409 (C=C), 1378-1393(C-O-C),1245(C-S); ^{1}HNMR ($CDCl_3$) 4.8(s, 2H, -OCH_2), 7.15(s, 1H, =CH), 6.8-8.2 (m,

8H, Ar), 8.0 (s,1H, NH amide), 10 (s, 1H, -NH thiazolidinedione) $C_{18}H_{13}N_3O_6S$ m/z 399.61 (M^+). Analysis calculated for C,54.67; N,4.59; N,10.07 . Found : C,54.70; N,4.75; N,10.11.

Synthesis of 2-{4-[(*E*)-(2,4-dioxo-1,3-thiazolidin-5-ylidene)methyl]phenoxy}-*N*-(4-chloro phenyl) acetamide (d4) IR(KBr, cm-1): 3564 (-NH), 3476 (-NH amide) 3030(Ar-CH),2916, (-CH), 1682 (-C=O), 1575,1575 (C=C), 1409 (C=C), 1378-1393(C-O-C),1245(C-S); ^{1}HNMR ($CDCl_3$) 4.8(s, 2H, -OCH_2), 6.9-7.8 (m, 7H, Ar), 7.15(s, 1H, =CH), 8 (s,1H ,–NH), 10(s,1H, NH thiazolinedione) ; m/z 389.14 (M^+). Analysis calculated for $C_{18}H_{13}ClN_2O_4S$ C,55.60; H,3.37; N, 7.20. Found: C,55.94; H,3.31;N, 7.40.

Synthesis of 2-{4-[(*E*)-(2,4-dioxo-1,3-thiazolidin-5-ylidene)methyl]phenoxy}-*N*-(3-nitro phenyl)acetamide (d5) IR (KBr, cm-1) 3564 (-NH), 3476 (-NH amide) 3030(Ar-CH),2916, (-CH), 1682 (-C=O), 1575,1575 (C=C), 1409 (C=C), 1378-1393(C-O-C),1245(C-S); ^{1}HNMR ($CDCl_3$) 4.8(s, 2H, -OCH_2), 6.9-7.8 (m, 7H, Ar), 7.15(s, 1H, =CH), 8 (s,1H ,–NH amide), 10.1 (H,s, -NH thiazolidinedione). $C_{18}H_{13}N_3O_6S$ m/z 399.14 (M^+). Analysis calculated for C, 54.67; N, 4.59; N, 10.07. Found: C, 54.70; N, 4.75; N, 10.11.

Synthesis of 2-{4-[(*E*)-(2,4-dioxo-1,3-thiazolidin-5-ylidene)methyl]phenoxy}-*N*-(4-methoxy phenyl)acetamide (d6) IR(KBr, cm-1): 3490 (-NH), 3366(-NH amide) 3030(Ar-CH),2914, 2862 (-CH), 1683 (-CO), 1575,1585 (C=O), 1649 (C=C), 1435(C-H bend alkane), 1378-1390 (C-O-C),1244 (C-S); ^{1}HNMR ($CDCl_3$) 3.7 (s,3H, OCH_3) , 4.8(S, 2H,-CH_2), 7.15(s 1H, =CH), 6.8-8.2 (m, 8H,Ar), 8.0 (s,1H, NH amide), 10 (s, 1H, -NH thiazolidinedione) ;m/z 385.21 (M^+). Analysis calculated for $C_{19}H_{16}N_2O_5S$: C,61.84; H,4.31;N, 7.43. Found: C, 61.84; H,4.31;N, 7.43.

Synthesis of *N*-(5-aminopyrimidin-2-yl)-2-{4-[(*E*)-(2,4-dioxo-1,3-thiazolidin-5- ylidene) methyl]phenoxy}acetamide (d7) IR(KBr, cm-1): 3490 (-NH), 3366(-NH amide) 3030(Ar-CH),2914, 2862 (-CH), 1683 (-CO), 1575,1585 (C=O), 1649 (C=C), 1435(C-H bend alkane), 1378-1390 (C-O-C),1244 (C-S); ^{1}HNMR ($CDCl_3$) 4.0(s,1H,NH_2), 4.8(s, 2H,-CH_2), 6.8,7.2(m, 4 H), 7.15(s,1H,=CH), 8.5 (d, 2H,Ar), 8.0 (s,1H,NH amide), 10 (s,1H, -NH thiazolidinedione); m/z 356.14 (M^+). Analysis calculated for C_{16} H_{13} N_5 0_4S C, 51.75; H,3.53; N, 18.86. Found: C,51.84; H,3.51;N, 17.39.

Synthesis of (5*E*)-5-{4-[2-oxo-2-(pyrrolidin-1-yl)ethoxy]benzylidene}-1,3-thiazolidine-2,4-dione (d8) IR(KBr, cm-1): 3490 (-NH), 3366(-NH amide) 3030(Ar-CH),2914, 2862 (-CH), 1683 (-CO), 1575,1585 (C=O), 1649 (C=C), 1435(C-H bend alkane), 1378-1390 (C-O-C),1244 (C-S); ^{1}HNMR($CDCl_3$) 1.97 (d,2H, -CH_2) ,3.4(d,2H, -CH_2) , 4.8(s,2H, -CH_2), 6.8-7.1, (m, 4H, Ar), 7.15(s,1H,=CH), 8 (s,1H,–NH amide), 10(s,1H,-NH thiazolidinedione); m/z 333.44 (M^+). Analysis calculated for $C_{16}H_{16}N_2O_4S$ C,57.82; H,4.85; N, 8.43. Found: C, 57.94; H, 4.31;N, 8.53.

Synthesis of 2-{4-[(*E*)-(2,4-dioxo-1,3-thiazolidin-5-ylidene)methyl]phenoxy}-*N*-(4-hydroxy phenyl) acetamide(d9) IR(KBr, cm-1): 3490 (-NH), 3366(-NH amide) 3030(Ar-CH),2914, 2862 (-CH), 1683 (-CO), 1575,1585 (C=O), 1649 (C=C), 1435(C-H bend alkane), 1378-1390 (C-O-C),1244 (C-S); ^{1}HNMR($CDCl_3$) 4.8(s, 2H, -CH_2), 5.0 (s,1H,-OH) , 6.9,7.1,7.5 (m, 7H,Ar), 7.15(s,1H, =CH), 8 (s,1H,–NH amide), 10(s,1H, -NH thiazolidinedione); m/z 371.34 (M^+). Analysis calculated for $C_{18}H_{14}N_2O_5S$ C,58.37 H,3.81; N, 7.56. Found: C, 58.47; H, 3.71;N, 7.43.

Synthesis of 2-{4-[(*E*)-(2,4-dioxo-1,3-thiazolidin-5-ylidene)methyl]phenoxy}-*N*-(3-hydroxy phenyl) acetamide (d10) IR (KBr, cm-1): 3490 (-NH), 3366 (-NH amide) 3030 (Ar-CH),2914, 2862 (-CH), 1683 (-CO), 1575,1585 (C=O), 1649 (C=C), 1435(C-H bend alkane), 1378-1390 (C-O-C),1244 (C-S); ^{1}HNMR($CDCl_3$) 4.8(s, 2H, -CH_2), 5.0 (s,1H,- OH) , 6.9,7.1,7.5 (m, 7H,Ar), 7.15(s,1H, =CH), 8 (s,1H, –NH amide), 10(s, 1H,-NH thiazolidinedione) ; m/z 371.34 (M^+). Analysis calculated for $C_{18}H_{14}N_2O_5S$ C, 58.37 H,3.81; N, 7.56. Found: C, 58.47; H, 3.71;N, 7.43

Synthesis of {4-[({4-[(*E*)-(2,4-dioxo-1,3-thiazolidin-5- ylidene)methyl] phenoxy} acetyl) amino]phenyl}acetic acid (d11) IR (KBr, cm-1): 3490 (-NH), 3366 (-NH amide) 3030 (Ar-CH), 2914, 2862 (-CH), 1683 (-CO), 1575,1585 (C=O), 1649 (C=C), 1435 (C-H bend alkane), 1378-1390 (C-O-C),1244 (C-S); ^{1}HNMR ($CDCl_3$), 3.5 (s,2H, -CH_2) , 4.8(s,2H, -CH_2), 6.9,7.5 (m, 8H), 7.15(s,1H,=CH), 8 (s,1H,–NH amide), 10(s,1H, -NH thiazolidinedione), 11.0 (s,1H, -COOH); m/z 412.34 (M^+). Analysis calculated for $C_{20}H_{16}N_2O_6S$ C,58.25 H,3.91; N, 6.79 .Found: C,58.75; H,3.31;N, 6.84.

Synthesis of (5*E*)-5-{4-[2-(morpholin-4-yl)-2-oxoethoxy]benzylidene}-1,3-thiazolidine-2,4-dione (d12) IR (KBr, cm-1): 3490(-NH), 3061 (Ar-CH), 2845 (-CH), 1753 (-CO), 1714 (C=O), 1649 (C=C), 1378-1393 (C-O- C), 1421(C-O) ; ^{1}HNMR ($CDCl_3$), 3.4,-3.6 (m,4H) , 4.8 (s,2H, -CH_2),6.8,7.1 (m, 4H), 7.15 (s,1H,=CH), 8 (s,1H ,–NH amide), 10 (s,1H, -NH thiazolidinedione); m/z 348.46 (M^+). Analysis calculated for $C_{16}H_{16}N_2O_5S$: C, 55.16; H, 4.63; N, 8.04. Found: C, 55.14; H, 4.31;N, 7.93.

Pharmacology

Animals and Treatment

Wister Albino mice of either sex weighing between 25-30 gm were selected for the study. Animals were housed in polypropylene cages and under standard condition of temperature (25 ± 5^{o}C), 12h/12h, light/dark cycles and were fed with standard mice pelleted diet and water was given *ad libitum*. All the study protocols related to hypoglycemic activity testing were approved from the Institutional Animal Ethics Committee constituted under the supervision of Committee for the Purpose of Control and Supervision of Experiments on Animals, New Delhi. (CPCSEA/IAEC/Pharm. Chem. 05/2009-10/12).

Mice were kept on overnight fasting befor the drug treatment and free access to water was allowed. Blood samples were collected through the tail vein and the basal fasting blood glucose (BG) was estimated by digital Blood Glucometer. The injected with alloxan (120 mg/kg) made i. p. Dextrose 5% solution was administered via feeding bottle, to recover from early hypoglycemic phases [16]. The blood glucose level was again moniter after 48 hrs. The animals showing elevated blood glucose (BG) levels above 250 mg/dl were selected for study.

Acute Hypoglycemic Study [17]

The synthesized compounds were screened for hypoglycemic activity in vivo by alloxan induced hypoglycemic mice model. For acute study animals were fasted overnight and the fasting blood glucose (BG); 0hr level was calculated. The quantity of thiazolidinedione derivatives equivalent to average human intake of 200 mg/kg at a time was calculated for a single dose of 30 mg/kg body weight and the homogenized suspension in 0.5% carboxy methyl cellulose injected intra peritoneally to mice. The vehicle treated group were given an equal amount of 0.5% CMC i.p. Blood samples were removed from animals at 2, 4, 6 and 24 hrs after drug treatment. The results were expressed as mean ± standard error of mean (SEM) and are shown in Table 2 and percent decrease in blood glucose was calculated. The data obtained was analyzed by one-way ANOVA followed by Dunnett test, $p<0.01$ was considered as statistically significant.

Chronic 21 days study [17]

Study animals were fasted overnight and the fasting Blood glucose (BG) levels were calculated on Day 1. The compounds were homogenized and suspended in 0.5% CMC, administered at a fixed dose of 30 mg/kg i.p. for 21 days at a fixed time. Blood glucose level was measured after 30 minutes of the administration of the dose and decrease in Blood glucose (BG) was calculated on 7th, 14th and 21st day. The results were expressed as mean ± standard error of mean (SEM) and are shown in Table 3 and percent decrease in blood glucose was calculated. The data obtained was analyzed by one-way ANOVA followed by Dunnett test, $p<0.01$ was considered as statistically significant. Evaluation of hypoglycemic activity.

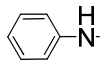
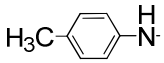
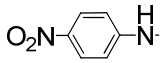
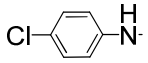
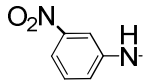
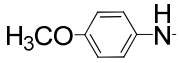
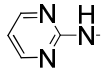
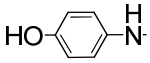
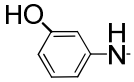
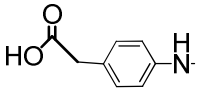
Histopathology [18-22]: Mice having pretreatment for 21 days of synthesized derivative which has shown promising hypoglycemic activity was selected for this study. The animals were selected randomly from each of the following groups; normal control, diabetic treated with pioglitazone and treated with **d1**. Histopathological studies for kidney and Liver were performed. Effect of compounds on liver histopathology & kidney histopathology is shown in Fig. 2.

RESULTS AND DISCUSSION

In the present work twelve novel 2, 4- thiazolidinedione derivatives 2-{4-[(*E*)-(2,4-dioxo-1,3-thiazolidin-5-ylidene)methyl]phenoxy}-*N*-(substituted phenyl) acetamide **d (1-12)** were synthesized by conventional as well as microwave –assisted method; which required very short duration, 3-5 minutes for completion of reaction and gave better yield as compared to conventional synthesis which requires 2-5 hrs refluxing.

The in vivo hypoglycemic study the activity of derivatives was evaluated by alloxan induced hyperglycemia in Wistar albino mice model [23-30]. Biological activity was expressed in terms of percent decrease in blood glucose level and shown in Table 2 and Table 3. Most of the synthesized compounds showed moderate hypoglycemic activity and compound no. **d1**, **d2** and **d9** have shown significant decrease in blood glucose level; compound no. **d1** has shown highest hypoglycemic activity amongst the synthesized derivatives and comparatively better activity than the standard drug, Pioglitazone. Pretreatment with the compounds for 21 days showed the normal hepatic as well as renal histopathology and enzymology when compared with diabetic control group among diabetic animals. In general thiazolidinediones exhibit hemotoxicity and hepatotoxicity in the animals [31]. In the present investigation, it was observed that the serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT) and serum alkaline phosphatase (ALP) were elevated in alloxan induced diabetes indicating that alloxan administration produces hepatic and renal damage. This obviously specifies that the elevation of liver serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT) and serum alkaline phosphatase (ALP) values in alloxan-induced diabetes was reduced when treated with synthesized compounds; this shows that the newly synthesized thiazolidinedione derivatives 2-{4-[(*E*)-(2,4-dioxo-1,3-thiazolidin-5-ylidene)methyl] phenoxy}-*N*-(substituted phenyl) acetamide **d (1-12)** are nontoxic.

Table 1: Physical Characterization of intermediates (2-(4-formylphenoxy)-*N*- substituted phenyl acetamide), c (1-12) and 2-{4-[(2, 4-thiazolidindione-5- ylidene) methyl] Phenoxy}-*N*-phenylacetamide, final Derivatives d (1-12).

Sr. No.	R	% Yield		m. p.^{0}C *		R_f value**	
		c	d	c	d	c	d
1		65%	75%	145-147	167-168	0.49	0.38
2		70%	78%	140-142	145-147	0.41	0.49
3		68%	78%	152-155	140-142	0.53	0.41
4		70%	80%	170-172	152-155	0.45	0.43
5		68%	78%	140-142	170-172	0.42	0.41
6		66%	68%	182-185	140-142	0.38	0.37
7		73%	73%	214-216	182-185	0.46	0.38
8		[a]75%	72%	165-170	214-216	0.43	0.48
9		67%	75%	168-170	165-170	0.45	0.44
10		68%	79%	220-222	168-170	0.42	0.38
11		74%	85%	255-257	220-222	0.48	0.44
12		[a]73%	83%	176-179	255-257	0.49	0.38

*a Yield by microwave-assisted reaction; * Melting points were uncorrected; ** Solvent system chosen for Rf value determination was Benzene: Methanol (9.6: 0.4)*

Structure Activity Relationship (SAR)

The following general remarks can be stated regarding SAR of the synthesized compounds: Thiazolidinedione ring, the polar head, is important for activity. Unsubstituted N-H at 3rd position of thiazolidinedione ring was important for activity; may be for hydrogen bonding at the receptor site. Attachment of phenyl ring (either with or without substitution) at 5th position has resulted in moderate hypoglycemic activity. Electron donating groups like methyl

(d2) and polar group like hydroxyl **(d9)** attached to the phenyl ring increases hypoglycemic activity; especially when attached at para position of phenyl ring than when group is in ortho position (**d10**). Electron withdrawing groups like $-NO_2$ when present in para position (**d3)** gives better activity than when present in meta position (**d5)**. When substituents are heterocyclic; like 2-amino pyrimidine **(d10**), gives activity less significant than aromatic substituent. The presence of alicyclic substituent i.e. morpholin and pyrolidine in the final derivatives have contributed towards non significant activity. This might be due to non aromatic nature of the ring and unavailability of free primary nitrogen required for hydrogen bonding at the receptor binding sites. The literature reveals that the presence of phenoxy ethyl chain (an ether linkage) increases the potency of the parent compound; which was replaced here in the present work by phenoxy methyl chain (an ether linkage) and gives promising activity. There should be proper balance between hydrophilicity and lipophilicity of the molecule so as to show anti-diabetic / hypoglycemic activity. To summarize, the designed **2-{4-[(*E*)-(2, 4-dioxo-1,3-thiazolidin-5-ylidene)methyl]phenoxy}-*N*-(substituted phenyl) acetamide derivatives d (1-12)** have shown remarkable hypoglycemic activity in alloxan induced diabetic mice model.

Table 2: Evaluation of hypoglycemic activity: Effect of d (1-12) on % decrease in blood glucose in diabetic mice (acute study).

Groups	0hr mg/dl	2hr	4hr % decrease	6hr	24hr
Diabetic Control	252.53±4.254.	4.74±0.68	7.9±4.32	13.43±2.68	3.18±4.35
Piogiltazone	250.75±5.21.	31.07±6.74**	37.48±5.37**	45.41±3.67**	10.3±6.53**
d1	251.52±3.45	30.03±5.43**	40.4±7.74**	46.81±5.21**	9.42±3.62**
d2	254.79±4.65	24.40±5.8*	31.88±8.64**	39.37±6.71**	7.32±5.74**
d3	252.84±2.85	29.34±4.53**	36.52±5.43**	46.64±4.52**	6.70±6.51**
d4	252.19± 4.35	24.7±3.97**	34.76±6.51*	37.89±5.43**	5.19±7.74**
d5	254.38± 4.53	26.64±5.28**	34.26±5.67**	37.05±4.62**	4.19±5.43**
d6	253.60±5.64	22.9±4.72*	35.6±5.53*	40.41±5.97**	3.87±6.53**
d7	250.17± 5.43	22.13±5.74**	30.83±7.74**	39.13±6.51**	7.0±4.43**
d8	254.91± 2.75	26.8±4.75**	34.8±6.53**	39.2±3.84**	5.8±6.51**
d9	256.56± 6.13	28.96±6.87**	38.49±6.51**	43.65±5.62**	4.77±7.63**
d10	252.73± 5.23	29.01±6.54**	36.47±4.65**	39.21±5.74**	3.0±3.75**
d11	254.88± 4.17	20.55±4.67*	24.9±7.74*	30.46±6.41*	2.58±6.75*
d12	258.20± 5.13	19.52±6.54*	27.73 ±3.98*	30.03±5.48*	1.82±6.54*

0 hr the basal blood glucose level in mg/dl considered as 100% respectively for calculation of % decrease.
*n=6, The results expressed as Mean ±SEM and the data analyzed using One-way ANOVA followed by Dunnett test; ***P < 0.001, ** P < 0.01, *P < 0.05*

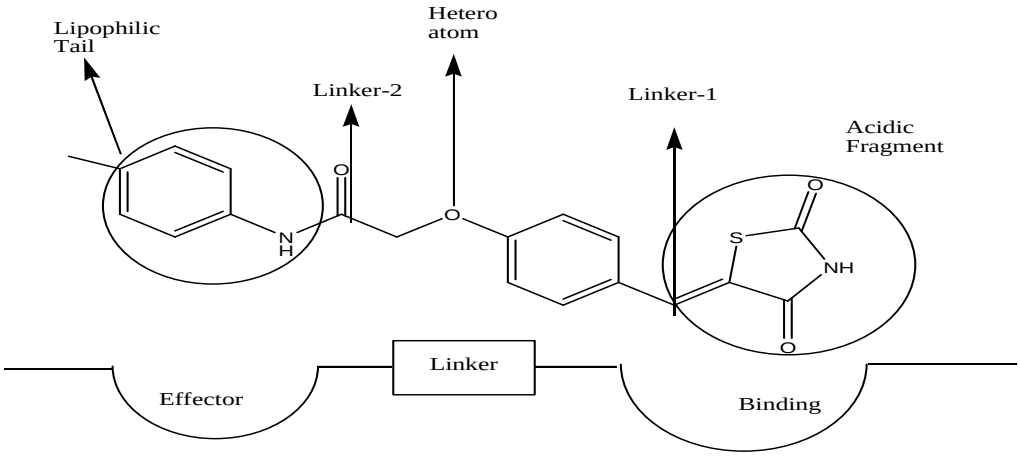


Fig. 1 Possible diagrammatic representation of synthesized derivative with PPAR γ receptor.

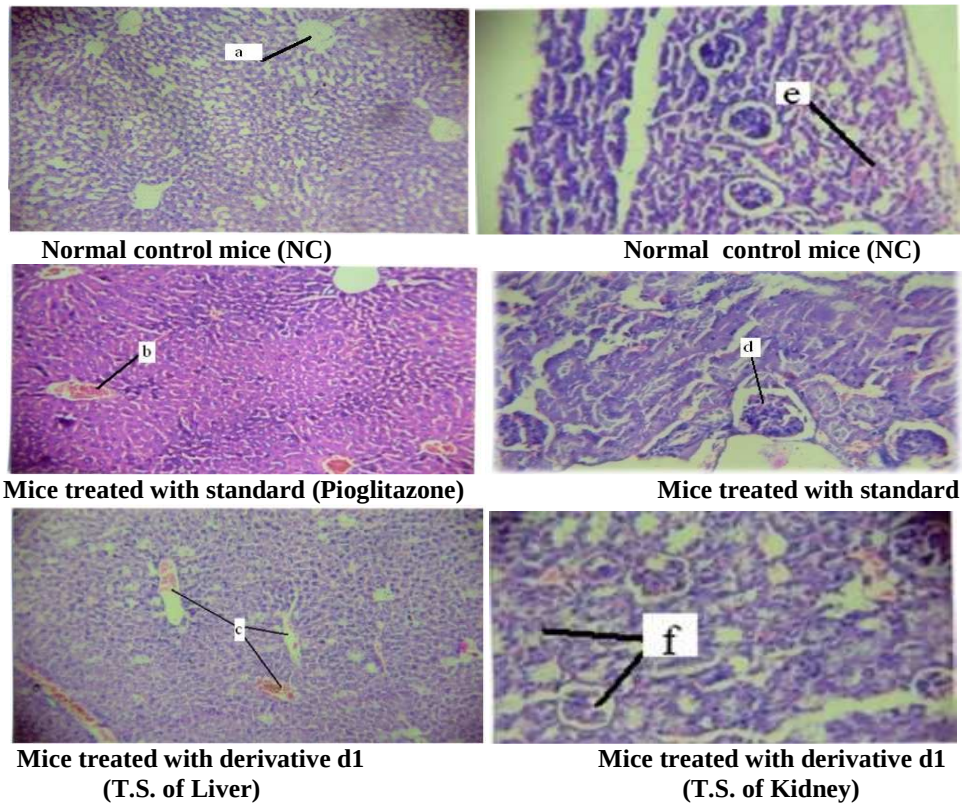


Fig.2. Effect on Liver Histopathology & Kidney Histopathology

Table 3: Evaluation of hypoglycemic activity: Effect of d (1-12) on % decrease in blood glucose in diabetic mice (chronic 21 days study)

Groups	7 Day	15 Day % decrease	21 Day
Diabetic Control	0.39±4.25	1.17±2.27	5.54±3.53
Piogiltazone	12.59±5.21**	23.22±4.13**	51.55±4.73***
d1	12.74±3.45**	23.90±3.15**	52.33±5.64***
d2	08.30±4.65*	18.97±4.63**	51.51±2.27***
d3	8.76±2.85**	19.52±3.76**	48.73 ±4.65**
d4	7.81±4.35*	18.35±4.65**	46.49±2.85**
d5	9.72±4.53**	18.28±5.42**	46.89±4.53**
d6	23-6.34±5.64*	15.07±4.76*	43.81±4.13**
d7	12.30±5.43**	21.03±4.36**	49.59±5.42**
d8	7.03±2.75*	19.92±5.13**	45.82±4.17*
d9	12.40±6.13**	21.31±3.87**	52.39±4.13**
d10	8.20±5.23*	18.35±3.21**	45.57±3.23*
d11	7.87±4.17*	14.17± 2.52*	39.43±2.75**
d12	4.32±5.13 ns	13.04±2.49*	37.84±4.36**

the basal blood glucose level considered as 100% respectively for calculation of % decrease

*n=6, The results expressed as Mean ±SEM and the data analyzed using One-way ANOVA followed by Dunnett test; ***P < 0.001, ** P < 0.01, *P < 0.05*

CONCLUSION

This communication illustrates a facile and efficient synthesis of 2-{4-[-(2, 4-dioxo-1,3-thiazolidin-5-ylidene)methyl] phenoxy}-*N*-(substituted phenyl) acetamide derivatives **d (1-12)** which are found to exhibit significant hypoglycemic activity. One of the synthesized compound **d1** (R = -NHC_6H_5) is more effective than

pioglitazone at the dose of 30mg/kg body weight. Overall, it was observed that derivatives having R = aromatic amines have given excellent hypoglycemic activity.

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