We synthesized twenty thiazolidin-4-one derivatives, which were then characterized by standard chromatographic and spectroscopic methods. From the in vitro glucose uptake assay, two compounds behaved as insulin sensitizers, where they enhanced glucose uptake in isolated rat diaphragm. In high-carbohydrate diet-induced insulin resistant mice, these two thiazolidin-4-ones attenuated hyperglycemia, hyperinsulinemia, hypertriglyceridemia, hypercholesterolemia, and glucose intolerance. They raised the plasma leptin but did not reverse the diabetes-induced hypoadiponectinemia. Additionally, compound 3a reduced adiposity. The test compounds were also able to reverse the disturbed liver antioxidant milieu. To conclude, these two novel thiazolidin-4-ones modulated multiple mechanisms involved in metabolic disorders, reversing insulin resistance and thus preventing the development of type-2 diabetes.

1. Introduction

Type-2 diabetes is a major health problem worldwide. This multifactorial disease and its complications like diabetic retinopathy, neuropathy, and nephropathy result in considerable morbidity and mortality. Diabetics are also at a greater risk to develop atherosclerosis and coronary heart disease (CHD). Metabolic syndrome characterized by dyslipidemia, hyperglycemia, insulin resistance, hypertension, and obesity also is a predisposing condition for atherosclerosis and CHD [1]. A number of drugs are routinely used to treat the various conditions of metabolic syndrome. This often leads to patient non-compliance besides raising the overall cost of treatment [2, 3]. Further, the drugs employed in treating dyslipidemia and hyperglycemia have their limitations, mainly adverse effects like weight gain and increased cardiac toxicity. Therefore, there is a need for the discovery and development of drugs with multiple actions and lower toxicity profile. This will improve the treatment outcome, reduce the number of drugs required, reduce treatment cost, and consequently, lead to greater patient compliance.

Further, recent understanding of the pathomechanism of metabolic disorder has revealed the central role of inflammation [4, 5]. Downregulation of inflammatory milieu by a hypolipidemic agent such as clofibrate through modulation of PPARα provides insights into adopting the approach for
synthesizing the molecules having an impact on inflammatory and metabolic machinery [6].

Thiazolidin-4-one derivatives have been extensively studied for their varied biological activities. Panetta et al. reported the anti-inflammatory effect of several 4-thiazolidinones [7]. A number of thiazolidinones have been shown to possess antioxidant and calcium overload inhibiting activities [8]. Suitably substituted thiazolidinones have been reported to reduce serum cholesterol, triglyceride, and glucose levels in our laboratory [9,10]. Three thiazolidin-4-ones were reported to exhibit hypolipidemic activity in mice given high-fat diet and fructose [11]. Another study from our laboratory reported the antidiabetic effect of two thiazolidin-4-ones through islet cell protection in streptozotocin-induced diabetes [12]. These molecules carried a substituted benzene ring at C2 of the thiazolidine ring and nicotinoylamino moiety at the 3rd position (N) of the ring.

p-Chlorophenoxyacetic acid is structurally related to the hypolipidemic drug, clofibrate. The latter molecule is a ligand for the peroxisome proliferator activated receptor (PPARα). The present work was carried out on the presumption that a clofibrate or structurally related p-chlorophenoxyacetic acid linked through an amide to the N of the thiazolidine ring would enhance the hypolipidemic and antidiabetic effect of the molecule.

In our earlier studies, a few p-chlorophenoxyacetic acid derivatives were found to possess remediative effect on cellular signalling of inflammation [13]. These molecules suppressed the inflammatory signalling via inhibition of proinflammatory mediators such as interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), and prostaglandin-E2 (PGE2). In this aspect, the proposed study was aimed to synthesize and evaluate a series of thiazolidin-4-ones from the same scaffold and to study their effects on multiple etiological factors of metabolic disorder like glucose intolerance, insulin resistance, hyperglycemia, hypertriglyceridemia, and hypercholesterolemia.

2. Materials and Methods

2.1. Chemical and Instruments. All the chemicals used in the synthesis of compounds were of analytical grade. Compounds were characterized by using various analytical instruments such as melting point apparatus, thin layer chromatography, UV/VIS and IR spectrometer, LC-MS, and RP-HPLC with the specification as reported previously [13].

2.2. General Synthetic Procedure for p-Chlorophenoxyacetic Acid Derivatives. p-Chlorophenoxyacetic acid derivatives (Table I) were synthesized as described previously [13], which is schematically described in Figure I.

2.2.1. 2-(4-Chlorophenoxy)-N-(2-(furan-2-yl)-4-oxothiazolidin-3-yl)acetamide (Compound 2a). 1H NMR (CDCl3) δ: 3.80 (s, 2H, CH2), 4.55 (s, 2H, CO-CH2-O), 5.97 (s, 1H, CH), 6.85 (m, 3H, Furan), 7.42 (m, 4H, Ar), 8.13 (br s, 1H, NH). IR (KBr) cm⁻¹: 3429, 3053, 2978, 1708, 1668, 1589, 1236, 1068, 933, 827, 744, 669. UV λmax (MeOH) 279 nm. LC-MS (ESI) m/z: 353 (Calcd for C15H13 Cl N2O3S: 352.79).

2.2.2. 2-(4-Chlorophenoxy)-N-2-(4-(4-chlorophenyl)-4-oxothiazolidin-3-yl)acetamide (Compound 2b). 1H NMR (CDCl3) δ: 3.70 (s, 2H, CH2), 4.47 (s, 2H, CO-CH2-O), 5.90 (s, 1H, CH), 6.90 (m, 4H, Ar), 7.36 (m, 4H, Ar), 8.0 (br s, 1H, NH). IR (KBr) cm⁻¹: 3450, 3059, 2989, 1710, 1678, 1591, 1236, 1172, 1060, 962, 833, 763, 671. UV λmax (MeOH) 280 nm. LC-MS (ESI) m/z: 397 (Calcd for C17 H14 Cl N2O3S: 397.28).

2.2.3. 2-(4-Chlorophenoxy)-N-2-(3,5-di-tert-butyl-4-hydroxyphenyl)-4-oxothiazolidin-3-yl)acetamide (Compound 2c). 1H NMR (CDCl3) δ: 3.77 (s, 2H, CH2), 4.53 (s, 2H, CO-CH2-O), 6.4 (s, 1H, CH), 7.19 (m, 4H, Ar), 8.0 (m, 4H, Ar), 8.18 (br s, 1H, NH). IR (KBr) cm⁻¹: 3427, 3072, 2914, 1722, 1680, 1597, 1521, 1240, 1170, 1063, 825, 790, 667. UV λmax (MeOH) 258 nm. LC-MS (ESI) m/z: 408 (Calcd for C17 H14 Cl N2O3S: 407.83).

2.2.4. 2-(4-Chlorophenoxy)-N-(2-(nitrophenyl)-4-oxothiazolidin-3-yl)acetamide (Compound 2d). 1H NMR (CDCl3) δ: 3.85 (t, 3H, CH3), 3.88 (s, 2H, CH2), 4.65 (s, 2H, CO-CH2-O), 5.90 (s, 1H, CH), 7.0 (m, 4H, Ar), 7.50 (m, 4H, Ar), 8.6 (br s, 1H, NH). IR (KBr) cm⁻¹: 3446, 3030, 2916, 1703, 1662, 1595, 1208, 1238, 1170, 1074, 950, 823, 763, 669. UV λmax (MeOH) 280 nm. LC-MS (ESI) m/z: 397 (Calcd for C18 H16 Cl N2O3S: 392.86).

2.2.5. 2-(4-Chlorophenoxy)-N-(2-(4-methoxyphenyl)-4-oxothiazolidin-3-yl)acetamide (Compound 2e). 1H NMR (CDCl3) δ: 3.74 (d, 3H, CH3), 4.60 (s, 2H, CO-CH2-O), 4.60 (s, 1H, CH), 6.90 (m, 4H, Ar), 7.47 (m, 4H, Ar), 8.03 (br s, 1H, NH). IR (KBr) cm⁻¹: 3244, 3064, 2987, 1719, 1684, 1589, 1240, 1095, 1055, 958, 825, 758, 669. UV λmax (MeOH) 279 nm. LC-MS (ESI) m/z: 397 (Calcd for C18 H16 Cl N2O3S: 392.86).

2.2.6. 2-(4-Chlorophenoxy)-N-(2-(2-chlorophenyl)-4-oxothiazolidin-3-yl)acetamide (Compound 2f). 1H NMR (CDCl3) δ: 3.72 (s, 2H, CH2), 4.50 (s, 2H, CO-CH2-O), 6.40 (s, 1H, CH), 6.90 (m, 4H, Ar), 7.47 (m, 4H, Ar), 8.03 (br s, 1H, NH). IR (KBr) cm⁻¹: 3427, 3009, 2908, 1716, 1678, 1604, 1236, 1182, 1047, 945, 823, 765, 665. UV λmax (MeOH) 327 nm. LC-MS (ESI) m/z: 397 (Calcd for C15 H13 Cl N2O3S: 386.86).

2.2.7. 2-(4-chlorophenoxy)-N-(4-oxo-2-(thiophen-2-yl)thiazolidin-3-yl)acetamide (Compound 2g). 1H NMR (CDCl3) δ: 3.30 (d, 2H, CH2), 4.65 (s, 2H, COCH2-O), 6.90 (s, 1H, CH), 7.16 (m, 3H, Thiophen), 7.46 (m, 4H, Ar), 8.75 (br s, 1H, NH). IR (KBr) cm⁻¹: 3437, 3030, 2908, 1716, 1678, 1604, 1236, 1182, 1047, 945, 823, 765, 665. UV λmax (MeOH) 327 nm. LC-MS (ESI) m/z: 369 (Calcd for C15 H13 Cl N2O3S: 368.86).

2.2.8. 2-(4-Chlorophenoxy)-N-(2-(4-fluorophenyl)-4-oxothiazolidin-3-yl)acetamide (Compound 2h). 1H NMR (CDCl3) δ: 3.70 (s, 2H, CH2), 4.60 (s, 2H, CO-CH2-O), 5.90 (s, 1H, CH), 6.95 (m, 8H, Ar), 7.95 (br s, 1H, NH). IR (KBr) cm⁻¹: 3209,
**Table 1: List of the twenty synthesized thiazolidin-4-ones.**

| S. no. | Compound code | R                      |
|--------|---------------|------------------------|
| 1      | 2a            | 2-Furyl                |
| 2      | 2b            | 4-Chlorophenyl         |
| 3      | 2c            | 3,5-di-t-Butyl-4-hydroxyphenyl |
| 4      | 2d            | 2-Nitrophenyl          |
| 5      | 2e            | 4-Methoxyphenyl        |
| 6      | 2f            | 2-Chlorophenyl         |
| 7      | 2g            | 2-Thiophenyl           |
| 8      | 2h            | 4-Fluorophenyl         |
| 9      | 2i            | 4-Trifluoromethylphenyl |
| 10     | 2j            | 4-Methylphenyl         |
| 11     | 2k            | 3,4-Dimethoxyphenyl    |
| 12     | 2l            | 4-Bromophenyl          |
| 13     | 2m            | 3,4-Dichlorophenyl     |
| 14     | 3a            | 4-Methoxyphenyl        |
| 15     | 3b            | 4-Fluorophenyl         |
2.2.9. 2-(4-Chlorophenoxy)-N-(4-oxo-2-(4-(trifluoromethyl)phenyl)thiazolidin-3-yl)acetamide (Compound 2i). \(^\text{1}\) HNMR (CDCl\(_3\)) \(\delta\): 3.89 (s, 2H, CH\(_3\)), 4.65 (s, 2H, CO-CH\(_2\)-O), 5.95 (s, 1H, CH), 7.86 (m, 8H, Ar), 8.00 (br s, 1H, NH). IR (KBr) cm\(^{-1}\): 3275, 3090, 2973, 1735, 1716, 1595, 1228, 1163, 1055, 960, 850, 666. UV \(\lambda_{\text{max}}\) (MeOH) 283 nm. LC-MS (ESI) \(m/z\): 431 (Calcd for C\(_{18}\)H\(_{17}\)ClF\(_3\)N\(_2\)O\(_3\): 430.83).

2.2.10. 2-(4-Chlorophenoxy)-N-(2-(3,4-dichlorophenyl)-5-thiazolidin-3-yl)acetamide (Compound 2j). \(^\text{1}\) HNMR (CDCl\(_3\)) \(\delta\): 2.90 (s, 3H, CH\(_3\)), 3.70 (s, 2H, CH\(_2\)), 4.42 (s, 2H, CO-CH\(_2\)-O), 5.90 (s, 1H, CH), 6.95 (m, 7H, Ar), 8.05 (br s, 1H, NH). IR (KBr) cm\(^{-1}\): 3217, 2989, 1722, 1676, 1589, 1292, 1238, 1220, 1170, 1060, 962, 821, 740, 667. UV \(\lambda_{\text{max}}\) (MeOH) 279 nm. LC-MS (ESI) \(m/z\): 377 (Calcd for C\(_{18}\)H\(_{17}\)Cl\(_2\)N\(_2\)O\(_3\): 376.86).

2.2.11. 2-(4-Chlorophenoxy)-N-(2-(3,4-dimethoxyphenyl)-4-thiazolidin-3-yl)acetamide (Compound 2k). \(^\text{1}\) HNMR (CDCl\(_3\)) \(\delta\): 3.85 (s, 2H, CH\(_2\)), 3.88 t, 6H, [(OCH\(_3\))\(_2\)], 4.20 (s, 2H, CO-CH\(_2\)-O), 4.59 (s, 2H, CH\(_2\)), 5.89 (s, 1H, CH), 7.20 (m, 7H, Ar), 8.00 (br s, 1H, NH). IR (KBr) cm\(^{-1}\): 3448, 3026, 2974, 1703, 1662, 1593, 1236, 1072, 950, 821, 796, 669. UV \(\lambda_{\text{max}}\) (MeOH) 279 nm. LC-MS (ESI) \(m/z\): 423 (Calcd for C\(_{19}\)H\(_{19}\)Cl\(_2\)N\(_2\)O\(_4\): 422.88).

2.2.12. N-(2-(4-Bromophenyl)-4-thiazolidin-3-yl)-2-(4-chlorophenoxy)acetamide (Compound 2l). \(^\text{1}\) HNMR (CDCl\(_3\)) \(\delta\): 3.05 (s, 2H, CH\(_2\)), 3.78 (s, 2H, CO-CH\(_2\)-O), 5.64 (s, 1H, CH), 6.72 (m, 4H, Ar), 7.15 (m, 4H, Ar), 7.84 (br s, 1H, NH). IR (KBr) cm\(^{-1}\): 3448, 3051, 2982, 1714, 1674, 1587, 1230, 1176, 1058, 964, 829 & 673. UV \(\lambda_{\text{max}}\) (MeOH) 278 nm. LC-MS (ESI) \(m/z\): 442 (Calcd for C\(_{17}\)H\(_{14}\)BrCl\(_2\)N\(_2\)O\(_3\): 441.73).

2.2.13. 2-(4-Chlorophenoxy)-N-(2-(3,4-dichlorophenyl)-4-oxothiazolidin-3-yl)acetamide (Compound 2m). \(^\text{1}\) HNMR (CDCl\(_3\)) \(\delta\): 3.29 (s, 2H, -CH\(_2\)), 3.88 (s, 2H, -CH\(_{-}\)CO), 5.64 (s, 1H, CH), 6.82 (m, 3H, Ar), 7.33 (m, 4H, Ar), 8.52 (br s, 1H, NH). IR (KBr) cm\(^{-1}\): 3228, 3022, 2974, 1730, 1687, 1585, 1481, 1369, 1219, 1130, 1060, 960, 823, 758, 667. UV \(\lambda_{\text{max}}\) (MeOH) 279 nm. LC-MS (ESI) \(m/z\): 432 (Calcd for C\(_{18}\)H\(_{14}\)Cl\(_3\)N\(_2\)O\(_3\): 431.72).

2.2.14. 2-(4-Chlorophenoxy)-N-(2-(3,4-dichlorophenyl)-5-methyl-4-oxothiazolidin-3-yl)acetamide (Compound 4a). \(^\text{1}\) HNMR (CDCl\(_3\)) \(\delta\): 1.45 (s, 3H, -CH\(_3\)), 3.67 (1H, -CH-CH\(_3\)), 3.90 (s, 2H, -CH\(_{-}\)), 5.68 (s, 1H, -CH), 6.95 (m, 3H, Ar), 7.25 (m, 4H, Ar\(^{2\prime}\)), 8.36 (br s, 1H, -NH). IR (KBr) cm\(^{-1}\): 3277, 3050, 2980, 1726, 1695, 1585, 1490, 1378, 1217, 1126, 1060, 966, 821, 738, 638. UV \(\lambda_{\text{max}}\) (MeOH) 278 nm. LC-MS (ESI) \(m/z\): 448 (Calcd for C\(_{18}\)H\(_{15}\)Cl\(_3\)N\(_2\)O\(_3\): 445.75).

2.2.15. 2-(4-Chlorophenoxy)-N-(2-(4-chlorophenyl)-5-methyl-4-oxothiazolidin-3-yl)acetamide (Compound 4b). \(^\text{1}\) HNMR (CDCl\(_3\)) \(\delta\): 1.55 (s, 3H, -CH\(_3\)), 3.40 (1H, -CH-CH\(_3\)), 3.92 (s, 2H, -CH\(_{-}\)), 5.69 (s, 1H, -CH), 6.77 (m, 4H, Ar), 7.03 (m, 4H, Ar\(^{2\prime}\)), 7.66 (br s, 1H, -NH). IR (KBr) cm\(^{-1}\): 3279, 3057, 2980, 1726, 1693, 1585, 1383, 1205, 1168, 1070, 962, 773, 636. UV \(\lambda_{\text{max}}\) (MeOH) 279 nm. LC-MS (ESI) \(m/z\): 411 (Calcd for C\(_{18}\)H\(_{16}\)Cl\(_2\)N\(_2\)O\(_3\): 411.30).

2.2.16. 2-(4-Chlorophenoxy)-N-(5-methyl-4-oxo-2-(pyridin-2-yl)thiazolidin-3-yl)acetamide (Compound 4c). \(^\text{1}\) HNMR (CDCl\(_3\)) \(\delta\): 1.45 (s, 3H, -CH\(_3\)), 3.55 (1H, -CH-CH\(_3\)), 3.89 (s, 2H, -CH\(_{-}\)), 6.10 (s, 1H, -CH), 6.52 (m, 4H, Pyridine), 6.90
Figure 1: Scheme for the synthesis of thiazolidin-4-ones. Reagents and conditions: (a) aromatic aldehydes, (b) methanol, (c) glacial acetic acid, reflux for 15–45 minutes, (d) dry benzene/toluene, (e) thioglycolic acid, reflux for 24–48 hours or microwave irradiation at power setting of 80% with 3 minutes/cycle for 16 minutes, and (f) thiolactic acid.

Figure 1: Scheme for the synthesis of thiazolidin-4-ones. Reagents and conditions: (a) aromatic aldehydes, (b) methanol, (c) glacial acetic acid, reflux for 15–45 minutes, (d) dry benzene/toluene, (e) thioglycolic acid, reflux for 24–48 hours or microwave irradiation at power setting of 80% with 3 minutes/cycle for 16 minutes, and (f) thiolactic acid.

2.2.17.2-(4-Chlorophenoxy)-N-(2-(4-methoxyphenyl)-5-methyl-4-oxothiazolidin-3-yl)acetamide (Compound 4d). 

$\text{IR (KBr) cm}^{-1}: 3159, 2966, 1732, 1691, 1587, 1496, 1342, 1238, 1180, 1060, 997, 829, 754, 661$. UV $\lambda_{\text{max}}$ (MeOH) 263 nm. LC-MS (ESI) $m/z$: 378 (Calcd for $C_{17}H_{16}ClN_3O_3$: 377.85).

2.3. General Synthetic Procedure for 2-(4-Chlorophenoxy)-2-Methylpropanoic Acid Derivatives [14]. Ethyl-2-(4-chlorophenoxy)-2-methylpropanoate was synthesized by esterifying 2-(4-chlorophenoxy)-2-methylpropanoic acid (clofibric acid, 0.06 mol) with ethanol. The ester was converted into hydrazide by reacting ethyl 2-(4-chlorophenoxy)-2-methylpropanoate (0.1 mol) with hydrazine hydrate (0.1 mol). Schiff’s bases were prepared by reacting 2-(4-chlorophenoxy)-2-methylpropanoic acid with Ar aldehydes (0.1 mol) like anisaldehyde, p-fluorobenzaldehyde, and 3,5-di-tert-butyl-4-hydroxybenzaldehyde in presence of 4-5 drops of glacial acetic acid.

Schiff’s base (0.01 mol) and thioglycolic acid (0.02 mol) were refluxed in dry benzene. The water formed during cyclization was removed azeotropically. The completion of the reaction was checked chromatographically. The period of reflux varied from compound to compound. After the completion of the reaction, the solvent was distilled off under reduced pressure. The reaction mixture was poured into water containing sodium bicarbonate solution. The solid separated was filtered, dried, and recrystallized from methanol, as described schematically in Figure 1.
2.3.1. 2-(4-Chlorophenoxy)-N-(2-(4-methoxyphenyl)-4-oxothiazolidin-3-yl)-2-methylpropanamide (Compound 3a).

\[ \text{HNMR (CDCl}_3; \delta = 1.37 (t, 3H, -CH}_3), 1.47 (t, 3H, -CH}_3), 3.85 (s, 2H, CH2), 5.90 (s, 1H, CH), 7.43 (m, 8H, Ar), 8.17 (br s, 1H, -NH) ppm; IR (KBr); 3281 (-NH), 3080 (Ar, C-H, Str), 2892, 1710 & 1680 (-C=O of -CH}_2, -CONH), 1612 (C=C, Str), 1251 (C-O, Str), 1143, 1095 (C-Cl), 848, 817, 709 & 663 (C-S-C) cm\(^{-1}\), respectively; MS (ESI): \( m/z = 421 \).
the increase in absorbance after addition of epinephrine was measured at 480 nm using a UV-visible spectrophotometer (model UV-1650PC, Shimadzu Co., Kyoto, Japan). The enzyme activity was expressed as U/mg protein.

(iii) Catalase Assay [22]. The catalase activity was determined spectrophotometrically according to the protocol of Claiborne [22]. The reaction was started by adding 0.05 mL supernatant to the reaction mixture (1.95 mL 10 mM H2O2 in 60 mM phosphate buffer, pH 7.0). Absorbance was recorded for 3 min at 240 nm. Phosphate buffer (60 mM, pH 7.0) was kept as a reference. To determine the specific activity of catalase, extinction coefficient of 0.04 mM⁻¹ cm⁻¹ was used.

(iv) Glutathione-S-Transferase (GST) Assay [23]. 0.1 mL 1-chloro-2,4-dinitrobenzene (CDNB) was added to 0.6 mL supernatant of liver homogenate and 2.2 mL phosphate buffer pH 6.5, incubated at 37 °C for 5 min, and added 0.1 mL 30 mM GSH. Absorbance was recorded at 340 nm at intervals of 1,2,3,4,5 min. Blank was carried out in the same manner without homogenate.

(v) Malondialdehyde Assay [24]. One mL of liver homogenate was combined with 2 mL of reaction mixture [15% w/v trichloroacetic acid (TCA) and 0.375% w/v thiobarbituric acid (TBA) in 0.25 N hydrochloric acid (HCl)] and mixed thoroughly. The solution was heated for 20 min on boiling water bath. Samples were cooled and centrifuged at 1000 rpm for 10 min to remove the flocculent precipitate. Supernatant was collected and the absorbance of read at 532 nm against a blank, which contained all the reagents except the liver homogenate. The extinction coefficient of 1.56 × 105 M⁻¹ cm⁻¹ was used to calculate the malondialdehyde concentration.

2.7.3. Histopathological Investigation. For histological examinations, liver, pancreas and white adipose tissue (WAT) from three animals per group were isolated on day 32 after treatment of compounds. The tissue samples were fixed in formalin solution (10%) for one week at room temperature, dehydrated by graded ethanol, cleared using graded xylene, and embedded in paraffin wax. 5 μm thick sections were cut using rotary microtome (RM2245, Leica Microsystems GmbH, Wetzlar, Germany), fixed on glass slides, stained with eosin and hematoxylin, and observed using a microscope (Model BX41, Olympus Corporation, Tokyo, Japan). For Langerhans cells, the average areas of six islets per specimen were measured using ImageJ software (version 1.48, National Institute of Health, MD, USA). Area was expressed as μm². The quantification of adipocytes were performed as reported [25], using adipocyte quantification tool, where area was measured in μm².

2.8. Statistical Analysis. Statistical analysis was performed by comparing the responses of the treatment groups to respective saline control and vehicle treated groups for all experiments and the significance was determined by one-way ANOVA followed by post hoc Dunnett’s test. Values...
Table 3: Effect of thiazolidin-4-ones on in vitro glucose uptake in absence and presence of insulin.

| S. no. | Groups          | Glucose uptake (mg/g/45 min) | In absence of insulin | In presence of insulin |
|--------|-----------------|------------------------------|-----------------------|------------------------|
|        |                 |                              | Mean ± S.E.M. (n = 3)  | Mean ± S.E.M. (n = 3)  |
| 1      | Glucose control | 8.09 ± 0.51                  | 12.11 ± 1.34          |
| 2      | Compound 2a     | 1.89 ± 1.45                  | 10.33 ± 1.21          |
| 3      | Compound 2b     | 6.19 ± 0.31                  | 7.36 ± 0.25           |
| 4      | Compound 2c     | 10.12 ± 0.52                 | 14.88 ± 0.86          |
| 5      | Compound 2d     | 9.84 ± 0.61                  | 8.16 ± 0.11           |
| 6      | Compound 2e     | 19.00 ± 1.27a                | 23.42 ± 0.32b         |
| 7      | Compound 2f     | 6.03 ± 0.33                  | 8.33 ± 0.15           |
| 8      | Compound 2g     | 6.45 ± 0.13                  | 7.08 ± 0.19           |
| 9      | Compound 2h     | 7.61 ± 0.18                  | 7.28 ± 0.38           |
| 10     | Compound 2i     | 5.55 ± 0.14                  | 6.44 ± 1.19           |
| 11     | Compound 2j     | 9.54 ± 0.21                  | 3.68 ± 1.79           |
| 12     | Compound 2k     | 5.82 ± 0.18                  | 7.21 ± 0.15           |
| 13     | Compound 2l     | 6.43 ± 0.10                  | 9.99 ± 0.10           |
| 14     | Compound 2m     | 8.12 ± 0.82                  | 12.87 ± 0.15          |
| 15     | Compound 3a     | 20.44 ± 0.17a                | 25.01 ± 0.29b         |
| 16     | Compound 3b     | 7.77 ± 0.29                  | 11.51 ± 0.02          |
| 17     | Compound 3c     | 12.24 ± 0.10                 | 10.50 ± 0.81          |
| 18     | Compound 4a     | 7.53 ± 1.10                  | 7.98 ± 0.83           |
| 19     | Compound 4b     | 6.10 ± 0.83                  | 7.72 ± 0.21           |
| 20     | Compound 4c     | 0.97 ± 0.59                  | 4.82 ± 0.55           |
| 21     | Compound 4d     | 3.77 ± 0.47                  | 5.54 ± 0.49           |
| 22     | Pioglitazone    | 13.37 ± 0.26a                | 25.23 ± 0.34b         |

a and b represent P < 0.05 as compared to glucose control in absence/presence of insulin, respectively.

Table 4: Effect of thiazolidin-4-ones on body weight and food efficiency ratio in mice.

|                | NPD     | HCD     | Pioglitazone | Compound 2e | Compound 3a |
|----------------|---------|---------|--------------|-------------|-------------|
| Final body weight (g) | 38.0 ± 2.1 | 41.2 ± 2.2 | 38.2 ± 1.4   | 37.6 ± 1.2   | 38.2 ± 2.1   |
| Food efficiency ratio   | 0.0025 ± 0.005 | 0.0083 ± 0.006 | 0.0020 ± 0.005 | 0.0043 ± 0.011 | 0.071 ± 0.010a,b |

a Represents P < 0.05 as compared to NPD group (normal pellet diet).
b Represents P < 0.05 as compared to HCD group (high carbohydrate diet).

were expressed as mean ± S.E.M. P < 0.05 was considered significant.

3. Results

3.1. In Vitro Biological Study

3.1.1. Glucose Uptake Assay. Of the twenty thiazolidin-4-ones tested, compounds 2e and 3a stimulated glucose uptake (P < 0.05) when compared with control. Uptake was stimulated both in the absence and presence of external insulin. The standard drug, pioglitazone, also increased the glucose uptake (Table 3).

3.2. In Vivo Biological Studies

3.2.1. Acute Oral Toxicity Study. Acute toxicity studies were carried out on Swiss albino mice as per the OECD guidelines for test compounds 2e and 3a. Both were found to be safe up to 2000 mg/kg which was the maximum dose tested.

3.2.2. High Carbohydrate Diet- (HCD-) Induced Metabolic Disorder in Mice. Animals put on HCD for 6 months had significant hyperglycemia, hypertriglyceridemia, and hypercholesterolemia. They were randomized to different treatment groups based on plasma glucose level and then treatment was given for 30 days. Firstly, treatment did not have any significant effect on body weight of animals (Table 4). Secondly, energy expenditure was assessed indirectly using FER. None of the treated groups except compound 3a-treated group had any significant effect on FER. The calculated FER was found to be significantly higher in animal treated with compound 3a compared to normal control and HCD group. High FER value indicates the increased energy expenditure in animals treated with compound 3a.
Figure 2: Effect of thiazolidin-4-ones on plasma: (a) glucose; (b) triglyceride (TG); (c) cholesterol in HCD fed mice. Data presented as mean ± S.E.M. (n = 6). * represents P < 0.05 as compared to NPD group and ** represents P < 0.05 as compared to HCD group.

(i) Effect of Thiazolidin-4-Ones on Plasma Glucose, Triglyceride, and Cholesterol Level. Biochemical estimation of metabolic markers such as plasma glucose (PG), triglycerides (TG), and total cholesterol (TC) was performed on day 14 and day 30 after the drug treatment and it was observed that HCD control group consistently had significant increase in PG and TC levels on days 14 and 30 compared to respective day’s normal control group. However, TG levels were significantly increased only on day 30 compared to respective day’s normal control group. Pioglitazone and test compounds (2e and 3a) significantly reversed hyperglycemia and elevated plasma cholesterol compared to HCD control group on both day 14 and day 30 of drug treatment (Figures 2(a) and 2(c)) while elevated TG levels were reduced on day 30 by the test compounds and pioglitazone (Figure 2(b)).

(ii) Effect of Thiazolidin-4-Ones on Plasma Insulin, Leptin, and Adiponectin Level. Development of insulin resistance in mouse was confirmed by estimating the plasma insulin levels on day 30 after drug treatment. HCD control group, in contrast to NPD group, showed two times increase in plasma insulin levels (P < 0.05). Hyperinsulinemia associated with hyperglycaemia and hypertriglyceridemia is considered to be the sign of development of insulin resistance. Thus, high sucrose feeding for 6 months led to the development of insulin resistance in mice. Treatment with pioglitazone and test drugs attenuated the insulin resistance (Figure 3(a)).

HCD feeding to animals did not elicit any significant effect on plasma leptin levels as there was no significant difference between HCD control and NPD group. However, treatment with pioglitazone and test compounds significantly (P < 0.05) raised the circulating leptin level in mice compared with HCD control group (Figure 3(b)). Compound 3a caused a fourfold elevation in leptin levels compared with HCD group. HCD feeding to animals resulted in significant
(P < 0.05) hypoadiponectinemia compared with NPD. Treatment with pioglitazone significantly (P < 0.05) corrected the hypoadiponectinemia. However, both test compounds failed to correct hypoadiponectinemia (Figure 3(c)).

(iii) Effect of Thiazolidin-4-Ones on Oral Glucose Tolerance Test. In oral glucose tolerance test, HCD group animal showed significant (P < 0.05) glucose intolerance (Figure 4). Pioglitazone and test compounds, compound 2e and compound 3a, corrected the glucose intolerance, shown as significant (P < 0.05) percent reductions in glucose excursion (AUC 0-120 min) by 13.00 ± 3.3, 15.46 ± 5.54, and 15.60 ± 3.49, respectively, compared with HCD group (Figures 4(a) and 4(b)).

(iv) Effect of Thiazolidin-4-Ones on Liver Enzymes. Oxidative stress is the hallmark of metabolic disorder, where disturbed homeostasis between oxidative and antioxidative mechanism occurs. HCD feeding to mice resulted in an oxidative stress observed as reduction in liver antioxidant enzymes such as glutathione (GSH), catalase (CAT), superoxide dismutase (SOD), and glutathione-S-transferase (GST). Treatment with pioglitazone and test compounds ameliorated the oxidative stress. They also reversed the elevation in liver malondialdehyde (MDA) levels in HCD-fed mice (Table 5).

(v) Histopathological Examination. Histology of liver showed normal lobular architecture with normal hepatocytes in all groups (Figure 5(a)). Pancreas showed mild to moderate hyperplasia of islets of Langerhans (Figure 5(a)) in pioglitazone and compound 3a groups. Pioglitazone and 3a treatment significantly raised the area of Langerhans islets compared with HCD group (Figure 5(b)). Exocrine portion of the pancreas and vascularity appeared normal in all the groups. Histopathological investigation of white adipose tissue (WAT) from epidydimal showed an increase in the size of adipocytes (Figure 5(c)) in HCD group which was reversed by pioglitazone and compound 3a treatment.
Table 5: Effect of thiazolidin-4-ones on liver antioxidant enzyme and malondialdehyde level.

| Liver biomarkers | NPD          | HCD          | Pioglitazone | Compound 2e | Compound 3a |
|-----------------|--------------|--------------|--------------|-------------|-------------|
| GSH (nmole/mg of protein) | 55.8 ± 2.1  | 46.4 ± 2.1\textsuperscript{a} | 63.2 ± 1.5\textsuperscript{b} | 68.3 ± 2.0\textsuperscript{b} | 67.8 ± 4.3\textsuperscript{b} |
| CAT (U/mg of protein) | 89.2 ± 5.7  | 55.4 ± 3.1\textsuperscript{a} | 93.8 ± 1.0\textsuperscript{b} | 81.9 ± 2.4\textsuperscript{b} | 87.5 ± 7.4\textsuperscript{b} |
| SOD (U/mg of protein) | 186.8 ± 23.2 | 108.1 ± 11.2\textsuperscript{a} | 174.8 ± 14.4\textsuperscript{b} | 202.2 ± 4.7\textsuperscript{b} | 208.0 ± 15.4\textsuperscript{b} |
| GST (U/mg of protein) | 0.43 ± 0.05 | 0.37 ± 0.01\textsuperscript{a} | 0.73 ± 0.02\textsuperscript{b} | 0.70 ± 0.01\textsuperscript{b} | 0.72 ± 0.04\textsuperscript{b} |
| MDA (nmole/mg of protein) | 0.57 ± 0.06 | 1.0 ± 0.33\textsuperscript{a} | 0.46 ± 0.01\textsuperscript{b} | 0.40 ± 0.01\textsuperscript{b} | 0.41 ± 0.02\textsuperscript{b} |

\textsuperscript{a}Represents \( P < 0.05 \) as compared to NPD group (normal pellet diet).
\textsuperscript{b}Represents \( P < 0.05 \) as compared to HCD group (high carbohydrate diet).

Figure 4: Effect of thiazolidin-4-ones on (a) plasma glucose (mg/dL) and (b) area under the curve (AUC\(_{0-120} \) min) against oral glucose tolerance test (OGTT) in HCD fed mice. Data presented as mean ± S.E.M. (\( n = 6 \)). *represents \( P < 0.05 \) as compared to NPD group and **represents \( P < 0.05 \) as compared to HCD group.

However, compound 2e did not show any significant effect on increased adipocyte size.

4. Discussion and Conclusion

The present work was planned as a sequel to earlier studies in our laboratory using thiazolidin-4-ones as antidiabetic, hypolipidemic, and antiinflammatory molecules. The substitutions in the thiazolidine ring were made at C2 and N3. Attached to the latter position was a nicotinamide moiety and the substitution at C2 was either p-methoxyphenyl or 2, 5-di-tert-butyl-4-hydroxyphenyl group. The compounds showed significant antidiabetic and hypolipidemic activities [9–11,26].

In the present study, the substitution at N3 was changed to p-chloro-phenoxyacetylamino, a group that is similar to clofibrate with a methylene bridge instead of gem-dimethyl substitution.

Four compounds out of 20 (i.e., compounds 4a, 4b, 4c, and 4d) had a methyl group attached at the C5 of thiazolidine ring, with cyclisation being made with thiolic acid instead of thioglycolic acid. In three other compounds (i.e., compounds 3a, 3b, and 3c), clofibrate was used to make the moiety for substitution at N3. This was done with a view to examine the effect of the gem-dimethyl on the overall activity of the resulting molecule.

All the 20 synthesised thiazolidin-4-ones were evaluated for glucose uptake in an in vitro system using the isolated rat diaphragm. In this experiment, compounds 2e and 3a significantly raised the amount of glucose uptake by the tissue both in the absence and presence of external insulin. This indicated the potential of the theses compounds in sensitizing the tissues for the external insulin. Hence these two compounds were chosen to study their effect on a diet-induced model of insulin resistance. Both compounds have the same p-methoxyphenyl moiety attached to the C2 of the thiazoline ring. Compound 3a has a gem-dimethyl group instead of methylene in the substituent at N3.

Apart from multiple risk factors, diet-induced metabolic abnormalities contribute to the development of insulin resistance and \( \beta \)-cell failure in type-2 diabetes [16]. Early detection and appropriate treatment are considered beneficial for correcting the abnormality. Among the various animal models, induction of diabetes through diet provides more resemblance to human type-2 diabetes. Chronic intake of diet with high sucrose content has been reported to favour the development of insulin resistance [16]. Similarly in our
study, mice fed a high-sucrose diet for six months developed metabolic abnormalities like hyperglycemia, hyperinsulinemia, hypertriglyceridemia, hypercholesterolemia, and hypoadiponectinemia. Hyperglycemia and hyperinsulinemia suggest the inability of insulin to sensitize the tissue for glucose uptake, allowing glucose to be diverted toward lipogenesis. This led to hyperlipidemia and finally to insulin resistance. In addition, adiponectin and leptin, the adipokines secreted from white adipose tissue (WAT), are reported to be involved in the metabolism of glucose and lipid [27]. In our study, we observed that HCD caused a reduction in plasma adiponectin level and an increase in the size of adipocytes. However, it did not cause any change in plasma leptin levels. Thus, we found a correlation between the adiponectin level and size of adipocytes in HCD model. Further, OGTT results from HCD-fed mice correlated well with glucose intolerance, hyperinsulinemia, and hypoadiponectinemia. The authenticity of the model was validated by the effect of pioglitazone, which was able to correct the metabolic abnormalities. Neither test compounds (2e or 3a) attenuated hypoadiponectinemia. However, they corrected the impaired glucose tolerance and insulin resistance in mice. Compounds 2e and 3a, by their ability to enhance glucose uptake and to sensitize the tissue for available insulin, reduced hyperinsulinemia and raised the leptin levels. This would have resulted in better glucose utilization by peripheral tissue. Thus, these compounds reduced the metabolic abnormalities like hyperglycemia, hypertriglyceridermia, and
hypercholesterolemia. Prospective studies involving direct assessment of insulin sensitivity in OGTT are required to analyze glucose stimulated insulin secretion (GSIS) in presence of compounds to establish the mechanistic role of the test compounds on insulin signalling.

Histological investigation showed no change in liver architecture in the various treatment groups. In HCD control mice, there was an increase in the size of adipocytes in white adipose tissue (WAT), which was reversed by compound 3a and pioglitazone treatment. However, only pioglitazone-treated animals showed positive correlation between reduced size of the cells and correction of hypoadiponectinemia. Apart from correction of hypoadiponectinemia, pioglitazone raised the peripheral leptin levels, which was also true in case of compounds 2e and 3a treatment. Among the tested compounds, 3a raised endogenous leptin levels four times more than HCD group. This finding points to the link between raised leptin levels and reduced adipocyte size caused by compound 3a. The pancreatic islets showed hyperplasia in pioglitazone and other treatment groups. Pioglitazone is a PPAR-γ agonist. This could have been responsible for the proliferation of beta cells of pancreas. The test compounds are thiazolidin-4-ones with some similarity to thiazolidinediones. It is possible that hyperplasia observed in compound 3a-treated animals might have been due to some agonistic activity on PPAR-γ receptors. This needs to be investigated through relevant assay.

Oxidative stress has been implicated in the occurrence of diabetes and compounds reducing the oxidative stress have beneficial role in correcting glucose intolerance and insulin resistance in diabetes [18, 28]. The test compounds and pioglitazone reversed the depletion of endogenous antioxidant enzymes such as GSH, CAT, SOD, and GST. Further, they reduced malondialdehyde levels. This suggests the inhibitory effect of these compounds on oxidative stress.

Leptin serves as an insulin-sensitizing factor in the whole body [29]. However, hyperleptinemia in the obese mouse and human is a sign of leptin resistance, where increased leptin levels are caused by disturbed homeostasis, arising from leptin receptor mutation, ageing or obesity [29]. Thus, hyperleptinemia further worsens impaired insulin action in pathological condition. In these conditions, exogenously administered leptin does not improve glucose tolerance and insulin sensitivity. In our study, diseased animal (HCD fed mouse) per se did not develop hyperleptinemia, which might be the sign of early stage of metabolic abnormality associated with insulin resistance. At this state, test compounds as well as pioglitazone reversed the elevated glucose, TG, TC, and insulin levels while facilitating leptin profile along with insulin sensitivity. Subsequently, compound 3a treatment resulted in increased energy expenditure demonstrated by elevated food efficiency ratio (FER). However, reduction in body weight among the treated groups was not found proportionate to the circulating leptin levels, which suggests that increased leptin level in peripheral blood is not sufficient to induce a proportionate reduction on body weight. A few questions remain unanswered such as (i) whether the compounds directly raised the leptin levels or are the results due to indirect impact on metabolic signaling? (ii) In metabolic disorder combined with hyperleptinemia, how do these compounds affect leptin signaling? Future studies are needed to address these issues.

No single mechanism would suffice to explain the beneficial effects of the test compounds. They do not seem to act through insulinotropic activity unlike the sulfonylureas. They have no significant effect on adiponectin levels, ruling out any involvement of this mechanism. The increase in the level of serum leptin might point to the involvement of leptin in the antihyperlipidemic and antidiabetic potentials of these molecules.

In conclusion, thiazolidin-4-one derivatives act through multiple mechanisms to correct the metabolic abnormalities in type-2 diabetes. In the present work, compounds 2e and 3a were found to be the most effective test compounds to ameliorate insulin resistance and development of type-2 diabetes.

Conflict of Interests

The authors declare that they do not have any conflict of interests.

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References

[1] S. M. Grundy, I. J. Benjamin, G. L. Burke et al., “Diabetes and cardiovascular disease: a statement for healthcare professionals from the american heart association,” Circulation, vol. 100, no. 10, pp. 1134–1146, 1999.
[2] K. E. Thorpe and D. H. Howard, “The rise in spending among Medicare beneficiaries: the role of chronic disease prevalence and changes in treatment intensity. Increasing numbers of beneficiaries being treated for five or more conditions a year are driving Medicare spending upward,” Health Affairs, vol. 25, no. 5, pp. w378–w388, 2006.
[3] S. M. Grundy, “Drug therapy of the metabolic syndrome: minimizing the emerging crisis in polypharmacy,” Nature Reviews Drug Discovery, vol. 5, no. 4, pp. 295–309, 2006.
[4] M. Navab, N. Gharavi, and A. D. Watson, “Inflammation and metabolic disorders,” Current Opinion in Clinical Nutrition and Metabolic Care, vol. 11, no. 4, pp. 459–464, 2008.
[5] G. S. Hotamisligil, “Inflammation and metabolic disorders,” Nature, vol. 444, no. 7121, pp. 860–867, 2006.
[6] M. Schmuth, V. Moosbrugger-Martinez, S. Blunder, and S. Dubrac, “Role of PPAR, LXR, and PXR in epidermal homeostasis and inflammation,” Biochimica et Biophysica Acta, vol. 1841, no. 3, pp. 463–473, 2014.
[7] J. A. Panetta, J. K. Shadle, M. L. Phillips, D. N. Benslay, and P. P. K. Ho, “4-thiazolidinones, potent antioxidants, as antiinflammatory agents,” Annals of the New York Academy of Sciences, vol. 696, pp. 415–416, 1993.
[8] T. Kato, T. Ozaki, K. Tamura, Y. Suzuki, M. Akima, and N. Ohi, “Novel calcium antagonists with both calcium overload inhibition and antioxidant activity. 1. 2-(3,5-Di-tert-butyl-4-hydroxyphenyl)-3-[(aminopropyl)thiazolidinones],” *Journal of Medicinal Chemistry*, vol. 41, no. 22, pp. 4309–4316, 1998.

[9] J. M. Joy, N. Jacob, and G. N. Kutty, “Evaluation of hypoglycemic effects of 4-thiazolidinones,” *Indian Drugs*, vol. 42, no. 1, pp. 17–21, 2005.

[10] N. Jacob and G. N. Kutty, “Synthesis and hypolipidemic activity of a thiazolidinone derivative,” *Indian Drugs*, vol. 41, no. 2, pp. 76–79, 2004.

[11] G. K. Nampurath, S. P. Mathew, V. Khanna, R. T. Zachariah, S. Kanji, and M. R. Chammallamudi, “Assessment of hypolipidaemic activity of three thiazolidin-4-ones in mice given high-fat diet and fructose,” *Chemico-Biological Interactions*, vol. 171, no. 3, pp. 363–368, 2008.

[12] A. Kishore, G. K. Nampurath, S. P. Mathew et al., “Antidiabetic effect through islet cell protection in streptozotocin diabetes: a preliminary assessment of two thiazolidin-4-ones in Swiss albino mice,” *Chemico-Biological Interactions*, vol. 177, no. 3, pp. 242–246, 2009.

[13] J. Mudgal, V. S. Gowdra, G. Mathew et al., “Remedial effects of novel 2, 3-disubstituted thiazolidin-4-ones in chemical mediated inflammation,” *Chemico-Biological Interactions*, vol. 210, pp. 34–42, 2014.

[14] A. R. Surrey, “4-Thiazolidones. IV. the preparation of some 3-alkylaminoalkyl-2-aryl derivatives,” *Journal of the American Chemical Society*, vol. 71, no. 10, pp. 3354–3356, 1949.

[15] B. R. P. Kumar and M. J. Nanjan, “Novel glitazones: design, synthesis, glucose uptake and structure-activity relationships,” *Bioorganic and Medicinal Chemistry Letters*, vol. 20, no. 6, pp. 1953–1956, 2010.

[16] M. Sumiyoshi, M. Sakanaka, and Y. Kimura, “Chronic intake of high-fat and high-sucrose diets differentially affects glucose intolerance in mice,” *Journal of Nutrition*, vol. 136, no. 3, pp. 582–587, 2006.

[17] S. H. Kim, S. H. Hyun, and S. Y. Choung, “Anti-diabetic effect of cinnamon extract on blood glucose in db/db mice,” *Journal of Ethnopharmacology*, vol. 104, no. 1-2, pp. 119–123, 2006.

[18] S. A. Manohara Reddy, J. Mudgal, P. Bansal et al., “Antioxidant, anti-inflammatory and anti-hyperglycemic activities of the flavonoid rich fraction of Pilea microphylla (L.) in high fat diet/streptozotocin-induced diabetes in mice,” *Experimental and Toxicologic Pathology*, vol. 64, no. 6, pp. 651–658, 2012.

[19] C. Pérez, C. Fernández-Galaz, T. Fernández-Agulló et al., “Leptin impairs insulin signaling in rat adipocytes,” *Diabetes*, vol. 53, no. 2, pp. 347–353, 2004.