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RESEARCH ARTICLE

Synthesis and antidiabetic activity of morpholinothiazolyl-2,4-thiazolidindione derivatives

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Abstract

We report the synthesis and the *in vitro* insulin releasing and glucose uptake activity of the morpholino thiazolyl-2,4-thiazolidinediones (**1-15**). Compounds **5, 11–15** (at lower concentration; 0.001 mg/ml) were able to increase insulin release in the presence of 5.6 mmol/l glucose. The compounds, except derivative **3** show an increase of glucose uptake. Various compounds are interesting potential antidiabetic leads showing pancreatic and extrapancreatic effects.

Keywords: Antidiabetic drugs, 2;4-thiazolidinediones, thiazolyl-2;4-thiazolidinediones, insulinotropic activity, glucose uptake

Introduction

One of the most serious metabolic diseases worldwide is diabetes mellitus. Insulin binds to its receptor and increases the content of the GLUT 4 leading to enhanced glucose uptake[1]. One of the problems in diabetes is that the glucose uptake in peripheral tissues in response to insulin is not sufficient, elevated blood levels of glucose are the consequence[2]. A typically feature of type 2 diabetes is the insulin resistance; many organs such as liver or muscle may become resistant to the action of the hormone. Another consequence is an increased not inhibited glucose output from the liver[3].

It is known that peroxisome proliferator-activated receptor γ (PPAR-γ) plays an important role in the regulation of genes involved in glucose metabolism, insulin signal transduction and lipid storage[4]. PPAR γ is the target for the treatment of insulin resistance using thiazolidinediones (TZDs) (insulin sensitizer)[5].

Despite the clear clinical benefit of TZDs as a treatment for type 2 diabetes, the use of the current generation of thiazolidinedione is associated with side effects of clinical importance, such as fluid retention and possibly heart failure[6].

There is a greater need to develop a safe and effective insulin sensitizier for type 2 diabetes. For these reasons, significant efforts are ongoing to develop the novel TZDs, which retain their insulin-sensitizing activity and are devoid of activities that cause adverse effects. The structural characteristic common to all TZDs is a thiazolidinedione ring, to which divergent molecular moieties are attached.

In the last few years, we reported the synthesis and insulin releasing activity of flavonyl-TZDs[7–11], chromonyl-TZDs[12–14] and thiazolyl-TZDs[15,16]. A significant insulinotropic effect was seen with those TZD compounds.

Herein, in our screening program to search for antidiabetic compounds, the 2,4-TZD N-acetic acid, acetic acid ethyl ester, benzyl and phenacyl derivatives containing morpholinothiazole ring were synthesized and their insulin releasing activities in INS-1 cells and glucose uptake activities were evaluated. It can be derived from structure-activity data that, instead of imidic hydrogen on the TZD ring at N-3 position carboxylic acid, carboxylic acid ester groups and benzyl or phenacyl groups are important for increasing the insulin releasing activity; moreover it can be evaluated whether the compounds

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possessing insulin releasing activity show glucose uptake activity or not.

Experimental

Chemistry

Melting points were measured on an electrothermal 9100 type apparatus (Electrothermal Engineering, Essex, UK) and uncorrected. All instrumental analyses were performed in Central Lab. of Pharmacy Faculty of Ankara University. ^{1}H NMR spectra were measured with a VARIAN Mercury 400 FT-NMR spectrometer (Palo Alto, CA) in $CDCl_3$ and DMSO-d_6. All chemical shifts were reported as δ (ppm) values. Elementary analyses were determined on a Leco CHNS 932 analyzer (Leco, St. Joseph, MI) and satisfactory results ±0.4% of calculated values (C, H, N) were obtained. For the chromatographic analysis Merck Silica Gel 60 (230–400 mesh ASTM = American Society for Testing and Materials) was used. The chemical reagents used in synthesis were purchased from E. Merck (Darmstadt, Germany) and Aldrich (Milwaukee, MI). 2,4-TZD (**I**[17]), 2,4-dichlorothiazole-5-carbaldehyde (**II**[18]), 4-chloro-2-(morpholin-4-yl)-thiazole-5-carbaldehyde (**III**[19]), ethyl 2,4-dioxothiazolidine-3-ylacetate **VI**[20], substituted benzyl-2,4-TZDs (**IVa, c, e, f**[21]**, IVb**[17]**, IVd**[22]) and substituted phenacyl-2,4-TZDs (**Va, c, f**[23]**, Vb**[24]**, Vd**[25]**, Ve**[21]) were synthesized according to the literature.

Crystals suitable for X-ray analysis were obtained by recrystalization of compound **2** from ethylacetate: n-hexane (4:1) The data collection was performed on a CAD-4 diffractometer employing graphite-monochromated CuK_α radiation (λ = 1.54184Å). Three standard reflections were measured every two hours. The structure was solved by direct methods. The refinement was made with anisotropic temperature factors for all non-hydrogen atoms. The hydrogen atoms were generated geometrically. An empirical Ψ scan absorption correction was applied. Crystallographic data (excluding structure factors) for compound **2** have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no CCDC 804860 Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44–1223–336033; e-mail: depositcdc.cam.ac.uk)

Synthesis of 4-chloro-2-(morpholin-4-yl)-thiazole-5-carbaldehyde (III)

To a stirred suspension of 2,4-dichlorothiazole-5-carbaldehyde **(II)** (1.0 g, 5.5 mmol) and sodium carbonate (0.583 g, 5.5 mmol) in acetonitrile (25 ml) was added morpholine (0,5 ml, 5.5 mmol), followed by stirring for 12 h at room temperature. The crude product was purified by column chromatography using silica gel 60 (230–400 mesh ASTM) as adsorbent and chloroform: ethyl acetate (5:1) as eluant. Yield: 1.3 g, 93.0%, m. p.: 200°C (Ref. 19, 200°C).

Synthesis of compounds 1,2,4-15

A mixture of 4-chloro-2-(morpholin-4-yl)-thiazole-5-carbaldehyde (**III**) (0.001 mol) and **I/IVa-f/Va-f/VI** (0.001 mol) was heated at 100–110°C in the presence of 0.5 ml acetic acid glacial and sodium acetate (0.001 mol) for 5 h. The reaction mixture was extracted with $CHCl_3$ (3 × 50 ml) and the organic layer was washed with water, dried over anhydrous Na_2SO_4 and evaporated to dryness. The residue was purified by column chromatography silica gel 60 (230–400 mesh ASTM) using hexane:dichloromethane (1:2) as eluant.

5-((4-chloro-2-(morpholin-4-yl)-1,3-thiazol-5-yl)methylidene) thiazolidine-2,4-dione (1). Yield: 43.0%, m.p.: 304–306°C, IR (KBr): C=O (cm^{-1}): 1759, 1687; ^{1}H NMR, δ, ppm (DMSO-d_6): 3.56 (t, 4H, NCH_2), 3.72 (t, 4H, OCH_2), 7.65 (s, 1H, =CH), 12.59 (s, 1H, TZD-NH); Anal. for $C_{11}H_{10}ClN_3O_3S_2$: Calc. C: 39.82, H: 3.04, N: 12.66, S: 19.33. Found C: 40.16, H: 2.93, N: 12.49, S: 19.34.

(Z)-Ethyl 2-(5-((4-chloro-2-(morpholin-4-yl)-1,3-thiazol-5-yl) methylidene)-2,4-dioxothiazolidin-3-yl)acetate (2). Yield: 54.0%, m.p.: 187°C, IR (KBr): C=O (cm^{-1}): 1729, 1676; ^{1}H NMR, δ, ppm (DMSO-d_6): 1.21 (t, 3H, CH_3), 3.59 (t, 4H, NCH_2), 3.73 (t, 4H, OCH_2), 4.17 (q, 2H, CH_2CH_3), 4.46 (s, 2H, CH_2COOEt), 7.82 (s, 1H, =CH); Anal. for $C_{15}H_{16}ClN_3O_5S_2$: Calc. C: 43.16, H: 3.87, N: 10.06, S: 15.33. Found C: 43.00, H: 3.94, N: 9.96, S: 15.05.

3-Benzyl-5-((4-chloro-2-(morpholin-4-yl)-1,3-thiazol-5-yl) methylidene) thiazolidine-2,4-dione (4). Yield: 33.0%, m.p.: 205°C, IR (KBr): C=O (cm^{-1}): 1729, 1682; ^{1}H NMR, δ, ppm (DMSO-d_6): 3.57 (t, 4H, NCH_2), 3.72 (t, 4H, OCH_2), 4.81 (s, 2H, TZD-NCH_2), 7.29–7.31 (m, 3H, Ar-o,p-H), 7.33–7.35 (m, 2H, Ar-m-H), 7.80 (s, 1H, =CH); Anal. for $C_{18}H_{16}ClN_3O_3S_2$. 0.5 H_2O: Calc. C: 50.22, H: 3.98, N: 9.76, S: 14.86. Found C: 50.19, H: 3.59, N: 9.72, S: 14.59.

5-((4-chloro-2-(morpholin-4-yl)-1,3-thiazol-5-yl) methylidene)-3-(4-fluorobenzyl)-thiazolidine-2,4-dione (5). Yield: 64.0%, m.p.: 197.6°C, IR (KBr): C=O (cm^{-1}): 1734, 1684; ^{1}H NMR, δ, ppm (DMSO-d_6): 3.57 (t, 4H, NCH_2), 3.72 (t, 4H, OCH_2), 4.79 (s, 2H, TZD-NCH_2), 7.15–7.20 (m, 2H, Ar-o-H), 7.34–7.37 (m, 2H, Ar-m-H), 7.79 (s, 1H, =CH); Anal. for $C_{18}H_{15}ClFN_3O_3S_2$: Calc. C: 49.14, H: 3.44, N: 9.55, S: 14.58. Found C: 48.94, H: 3.33, N: 9.55, S: 14.69.

5-((4-chloro-2-(morpholin-4-yl)-1,3-thiazol-5-yl)methylidene)-3-(4-chlorobenzyl)-thiazolidine-2,4-dione(6). Yield: 83.0%, m.p.: 179.4°C, IR (KBr): C=O (cm^{-1}): 1738, 1682; ^{1}H NMR, δ, ppm (DMSO-d_6): 3.54 (t, 4H, OCH_2), 3.69 (t, 4H, NCH_2), 4.77 (s, 2H, TZD-NCH_2), 7.30 (d, 2H, Ar-o-H), 7.39 (d, 2H, Ar-m-H), 7.76 (s, 1H, =CH); Anal. for $C_{18}H_{15}C1_2N_3O_3S_2$: Calc. C: 47.37, H: 3.31, N: 9.21, S: 14.05. Found C: 47.42, H: 3.09, N: 9.13, S: 13.76.

5-((4-chloro-2-(morpholin-4-yl)-1,3-thiazol-5-yl) methylidene)-3-(4-bromobenzyl)-thiazolidine-2,4-dione (7). Yield: 86.0%, m.p.: 192.6°C, IR (KBr): C=O (cm^{-1}): 1738, 1680; ^1H NMR, δ, ppm (DMSO-d_6): 3.57 (t, 4H, NCH_2), 3.72 (t, 4H, OCH_2), 4.78 (s, 2H, TZD-NCH_2), 7.26 (d, 2H, Ar-o-H), 7.54 (d, 2H, Ar-m-H), 7.79 (s, 1H, = CH); Anal. for $C_{18}H_{15}BrClN_3O_3S_2$: Calc. C: 43.17, H: 3.02, N: 8.39, S: 12.81. Found C: 43.02, H: 3.07, N: 8.40, S: 12.47.

5-((4-chloro-2-(morpholin-4-yl)-1,3-thiazol-5-yl)methylidene)-3-(2,4-dichlorobenzyl)-thiazolidine-2,4-dione (8). Yield: 76.0%, m.p.: 238.4°C, IR (KBr): C=O (cm^{-1}): 1717, 1699; ^1H NMR, δ, ppm (DMSO-d_6): 3.58 (t, 4H, NCH_2), 3.73 (t, 4H, OCH_2), 4.86 (s, 2H, TZD-NCH_2), 7.31 (d, 1H, Jo = 8.40Hz, Ar-6'-H), 7.40 (dd, 1H, Jo = 8.40Hz, Jm = 2.00Hz, Ar-5'-H), 7.67 (d, 1H, Jm = 2.00Hz, Ar-3'-H), 7.81 (s, 1H, = CH); Anal. for $C_{18}H_{14}Cl_3N_3O_3S_2$: Calc. C: 44.05, H: 2.88, N: 8.56, S: 13.07. Found C: 43.71, H: 2.69, N: 8.57, S: 12.72.

5-((4-chloro-2-(morpholin-4-yl)-1,3-thiazol-5-yl)methylidene)-3-(4-nitrobenzyl)-thiazolidine-2,4-dione (9). Yield: 81.0%, m.p.: 253.1°C, IR (KBr): C=O (cm^{-1}): 1725, 1666; ^1H NMR, δ, ppm ($CDCl_3$): 3.62 (t, 4H, NCH_2), 3.83 (t, 4H, OCH_2), 4.96 (s, 2H, TZD-NCH_2), 7.59 (d, 2H, Ar-o-H), 8.04 (s, 1H, = CH), 8.19 (d, 2H, Ar-m-H); Anal. for $C_{18}H_{15}ClN_4O_5S_2$: Calc. C: 46.30, H: 3.24, N: 12.00, S: 13.73. Found C: 46.67, H: 3.26, N: 11.76, S: 13.35.

5-((4-chloro-2-(morpholin-4-yl)-1,3-thiazol-5-yl)methylidene)-3-(2-oxo-2-phenylethyl)-thiazolidine-2,4-dione (10). Yield: 40.0%, m.p.: 283.6°C, IR (KBr): C=O (cm^{-1}): 1738, 1684; ^1H NMR, δ, ppm (DMSO-d_6): 3.51 (t, 4H, NCH_2), 3.68 (t, 4H, OCH_2), 5.30 (s, 2H, TZD-NCH_2), 7.59–7.63 (m, 2H, Ar-m-H), 7.68–7.73 (m, 2H, Ar-o-H), 8.07–8.09 (m, 1H, Ar-p-H), 8.18 (s, 1H, = CH); Anal. for $C_{19}H_{16}ClN_3O_4S_2$: Calc. C: 50.72, H: 3.58, N: 9.34, S: 14.25. Found C: 50.72, H: 3.74, N: 9.76, S: 14.62.

5-((4-chloro-2-(morpholin-4-yl)-1,3-thiazol-5-yl)methylidene)-3-(2-(4-fluoro-phenyl)-2-oxoethyl)-thiazolidine-2,4-dione (11). Yield: 74.0%, m.p.: 210.3°C, IR (KBr): C=O (cm^{-1}): 1729, 1700, 1681; ^1H NMR, δ, ppm (DMSO-d_6): 3.60 (t, 4H, NCH_2), 3.74 (t, 4H, OCH_2), 5.30 (s, 2H, TZD-NCH_2), 7.42-7.46 (m, 2H, Ar-m-H), 7.83 (s, 1H, = CH), 8.16–8.19 (m, 2H, Ar-o-H); Anal. for $C_{19}H_{15}ClFN_3O_4S_2$. 0.1 H_2O: Calc. C: 48.63, H: 3.26, N: 8.96, S: 13.63. Found C: 48.28, H: 3.20, N: 8.92, S: 13.46.

5-((4-chloro-2-(morpholin-4-yl)-1,3-thiazol-5-yl)methylidene)-3-(2-(4-chlorophenyl)-2-oxoethyl)-thiazolidine-2,4-dione (12). Yield: 73.0%, m.p.: 236.5°C, IR (KBr): C=O (cm^{-1}): 1733, 1695, 1683; ^1H NMR, δ, ppm (DMSO-d_6): 3.60 (t, 4H, NCH_2), 3.74 (t, 4H, OCH_2), 5.31 (s, 2H, TZD-NCH_2), 7.68 (d, 2H, Ar-m-H), 7.83 (s, 1H, = CH), 8.10 (d, 2H, Ar-o-H); Anal. for $C_{19}H_{15}Cl_2N_3O_4S_2$. 0.5 H_2O: Calc. C: 46.34, H: 3.27, N: 8.54, S: 12.99. Found C: 46.15, H: 3.18, N: 8.72, S: 12.71.

5-((4-chloro-2-(morpholin-4-yl)-1,3-thiazol-5-yl)methylidene)-3-(2-(4-bromophenyl)-2-oxoethyl)-thiazolidine-2,4-dione (13). Yield: 54.0%, m.p.: 285–286°C, IR (KBr): C=O (cm^{-1}): 1734, 1688, 1680; ^1H NMR, δ, ppm ($CDCl_3$): 3.63 (t, 4H, NCH_2), 3.84 (t, 4H, OCH_2), 5.11 (s, 2H, TZD-NCH_2), 7.67 (d, 2H, Ar-m-H), 7.85 (d, 2H, Ar-o-H), 8.05 (s, 1H, = CH); Anal. for $C_{19}H_{15}BrClN_3O_4S_2$: Calc. C: 43.15, H: 2.86, N: 7.95, S: 12.13. Found C: 43.14, H: 2.72, N: 8.14, S: 12.30.

5-((4-chloro-2-(morpholin-4-yl)-1,3-thiazol-5-yl)methylidene)-3-(2-(2,4-dichlorophenyl)-2-oxoethyl)-thiazolidine-2,4-dione (14). Yield: 55.0%, m.p.: 216°C, IR (KBr): C=O (cm^{-1}): 1733, 1683; ^1H NMR, δ, ppm ($CDCl_3$): 3.63 (t, 4H, NCH_2), 3.84 (t, 4H, OCH_2), 5.08 (s, 2H, TZD-NCH_2), 7.38 (dd, 1H, Jo = 8.80Hz, Jm = 2.00Hz, Ar-5'-H), 7.51 (d, 1H, Jm = 2.00Hz, Ar-3′-H), 7.71 (d, 1H, Jo = 8.40Hz, Ar-6′-H), 8.05 (s, 1H, = CH); Anal. for $C_{19}H_{14}Cl_3N_3O_4S_2$: Calc. C: 43.98, H: 2.72, N: 8.10, S: 12.36. Found C: 43.80, H: 2.65, N: 8.22, S: 12.44.

5-((4-chloro-2-(morpholin-4-yl)-1,3-thiazol-5-yl)methylidene)-3-(2-(4-nitrophenyl)-2-oxoethyl)-thiazolidine-2,4-dione (15). Yield: 70.0%, m.p.: 243°C, IR (KBr): C=O (cm^{-1}): 1728, 1700, 1683; ^1H NMR, δ, ppm (DMSO-d_6): 3.60 (t, 4H, NCH_2), 3.74 (t, 4H, OCH_2), 5.40 (s, 2H, TZD-NCH_2), 7.84 (s, 1H, = CH), 8.32 (d, 2H, Ar-H), 8.40 (d, 2H, Ar-H); Anal. for $C_{19}H_{15}ClN_4O_6S_2$: Calc. C: 46.15, H: 3.06, N: 11.34, S: 12.94. Found C: 46.03, H: 2.89, N: 11.32, S: 12.91.

Synthesisof(Z)-2-(5-((4-chloro-2-(morpholin-4-yl)-1,3-thiazol-5-yl) methylidene)-2,4-dioxothiazolidin-3-yl)acetic acid (3). A mixture of acetic acid ester compound **2** (0.075 g, 0.18 mmol), glacial acetic acid (4 mL) and HCl 12 N (1 mL) was refluxed for 2 h. After evaporation in vacuo, the residue was refluxed again with glacial acetic acid (4 mL) and HCl 12 N (1 mL) for 2 h. After evaporation to dryness in vacuo, the crude solid was crystallized from ethanol providing pure carboxylic acid **3**.

Yield: 52 mg, 74.0%, m.p.: 269°C, IR (KBr): C=O (cm^{-1}): 1729, 1676; ^1H NMR, δ, ppm (DMSO-d_6): 3.59 (t, 4H, NCH_2), 3.73 (t, 4H, OCH_2), 4.35(s, 2H, CH_2COOH), 7.81 (s, 1H, = CH), 13.45 (broad s, 1H, COOH); Anal. for $C_{13}H_{12}ClN_3O_5S_2$: Calc. C: 40.05, H: 3.10, N: 10.78, S: 16.45. Found C: 39.66, H: 3.03, N: 10.79, S: 16.45.

Biological activity studies

Insulin releasing activity

Cell culture of INS-1 cells. INS-1 cells, generously provided by Dr. C. Wollheim, Geneva, Switzerland[26], were grown in plastic culture bottles or micro-wells for 4–6 days (half confluence: $1–2 \times 10^6$ cells per ml) in RPMI medium supplemented with 10% (v/v) fetal calf serum, 100 U of penicillin per ml and 0.1 mg of streptomycin per ml. Cells were seeded at a density of 5×10^5 cells/ml. The medium was changed every 5 days, and the cells were detached from the culture flask with trypsin 1 week after seeding, centrifuged and reseeded as described above.

Prior to the experiment cells were washed two times and then incubated in Krebs-Ringer buffer containing 10 mM HEPES and 0.5% bovine serum albumin (KRBH).

Insulin release. To measure insulin secretion, half-confluent cells in micro-wells were incubated for 90 min. at 37°C in the aforementioned KRBH buffer. Insulin released into the medium was assayed with a radioimmunoassay using rat insulin (Novo Nordisk, Bagsvaerd, Denmark) as a standard, (mono 125I-Tyr A14)-porcine insulin as the labelled compound (Sanofi-Aventis, Germany) and anti-insulin antibodies from Linco (St. Louis, MO). Each compound had been checked for non-interference with the insulin radioimmunoassay. The data were corrected for the effects of solubilizing compounds (ethanol, DMSO).

Glucose uptake activity

Reagents. Dulbecco modified Eagle medium high glucose (DMEM) and fetal calf serum was purchased from PAA (COlbe, Germany). Rosiglitazone was isolated from Avandia®. 2-NBDG (2-[*N*-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino]-2-deoxyglucose), as the fluorescence probe, was obtained from Invitrogen (Darmstadt, Germany). TNF-α and bovine Insulin was purchased from Sigma-Aldrich (Steinheim, Germany).

Cell culture and treatment. HepG2 cells were obtained from Boehringer Ingelheim (Biberach, Germany). The human hepatocellular liver carcinoma cells were grown at 37°C in a 5% CO_2 humidified atmosphere in Dulbecco' modified Eagle's medium (DMEM) supplemented with 10% heat-inactivated fetal calf serum (FCS), 100 U of penicillin per ml and 0.1 mg of streptomycin per ml, 4 mM Glutamine. Medium was changed every third day and was split after reaching 80% of confluence.

Glucose uptake measurement by fluorescence microplate reader. The method used was first described by Zou et al[2]. It uses the fluorescent probe 2-NBDG for the direct measurement of glucose uptake followed by the detection of the fluorescence within the cells. 2-NBDG is a fluorescent derivate of glucose modified with a 2-[N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl) amino group at the C-2 position. This indicator was excited at 467 nm and showed fluorescence at 542 nm[27].

HepG2 cells were seeded at 1.5×10^4 cells /well into 96-well plates, and after the cells adhere on the bottom of the well the supernatant was collected. Then the cells were treated with or without 100 μM Rosiglitazone as a positive control, with or without 1 nM TNF-α to induce insulin resistance and the compounds were added to reverse the insulin resistance. Also their direct effect was measured. All substances except insulin and TNF-α were diluted in DMSO (final concentration in the well: 1% DMSO). To show if the compounds or Rosiglitazone reverse the TNF-α effect we combined Rosiglitazone and the compounds with TNF-α and insulin.

After 4 d of preincubation the supernatant was collected and the cells were treated with or without 50 nM insulin for 30 min. Then the cells were incubated with 1 mM 2-NBDG for 5 min. The 2-NBDG uptake reaction was stopped by removing the incubation medium and washing the cells three times with cold phosphate buffered saline (PBS). All substances were checked for adhering to the plastic wells and can easily be drained away.

Fluorescence intensity of 2-NBDG was recorded using FLUOstar Galaxy, a multifunctional microplate reader.

Results and discussion

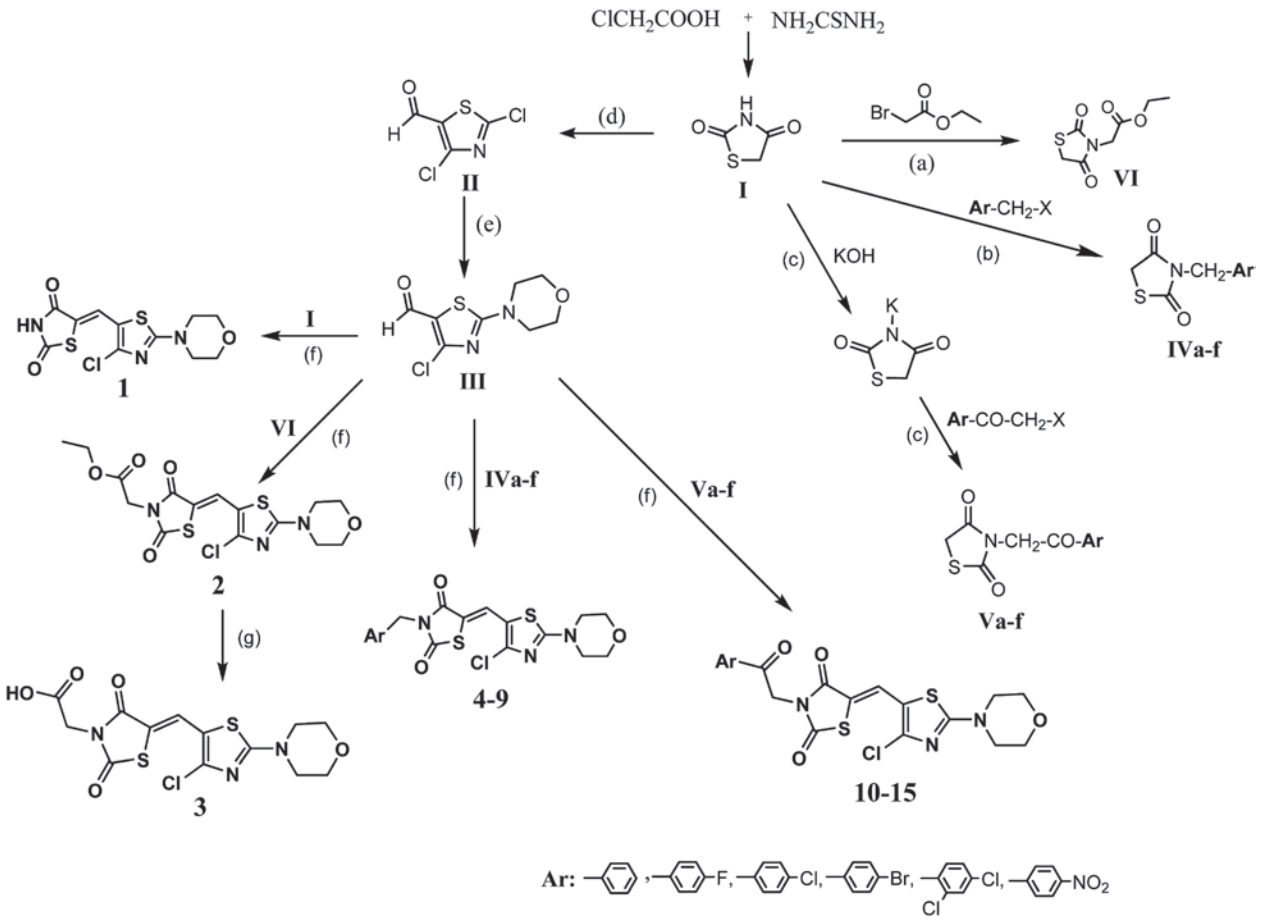
Chemistry

Thiazolyl-2,4-thiazolidinedione compounds **1-15** were synthesized according to the synthetic pathway described in Scheme 1. 2,4-dichlorothiazole-5-carbaldehyde (**II**) was obtained with 2,4-TZD[17] and N,N-dimethylformamide in phosphoryl chloride[18]. 4-chloro-2-(morpholin-4-yl)-thiazole-5-carbaldehyde (**III**) was synthesized with 2,4-dichlorothiazole-5-carbaldehyde (**II**) and morpholine in sodium carbonate/acetonitrile[19]. Ethyl 2,4-dioxothiazolidine-3-ylacetate (**VI**) was prepared by N-alkylation of 2,4-TZD with ethyl bromoacetate in THF/NaH[20].

Substituted benzyl-2,4-thiazolidinediones **IVa-f** were obtained with 2,4-TZD and appropriate benzyl halide derivatives in NaOH/ethanol. Substituted phenacyl-2-,4-thiazolidinediones **Va-f** were synthesized by reacting potassium 2,4-thiazolidinedione with appropriate phenacylbromide derivatives in hot methanol.

The condensation of 4-chloro-2-(morpholin-4-yl)-thiazole-5-carbaldehyde (**III**) with 2,4-thiazolidinedione **I**, ethyl 2,4-dioxothiazolidine-3-ylacetate **VI**, substituted benzyl-2,4-thiazolidinediones **IVa-f** and phenacyl-2,4-thiazolidinediones **Va-f** in the presence of sodium acetate/acetic acid glacial by Knoevenagel reaction, led to morpholino thiazolyl-2,4-thiazolidinediones **1**, 2,4-thiazolidinedione acetic acid ethyl ester **2**, morpholino thiazolyl-substituted benzyl-2,4-thiazolidinediones **4-9** and morpholino thiazolyl-substituted phenacyl-2,4-thiazolidinediones **10–15**, respectively. The acidic hydrolysis of **2** provided corresponding carboxylic acid **3**.

In our previous paper, the Z configuration of the methyne proton was confirmed via X-ray diffractometric analysis and it was resonated at lower field than that of the E configuration in ^{1}H NMR. Additionally, the calculated values of the methyne protons of Z and E isomeric form of the compound were seen as a singlet at 8.05 ppm and 7.34 ppm, respectively[28]. In E isomers, due to the lesser deshielding effect of 1-S of the TZD ring, such a proton should resonate at lower chemical shift values[29]. In this study, only one isomer of the synthesized compounds was obtained. Furthermore, the X-ray diffractometric analysis of compound **2** unambiguously confirmed the Z configuration at the chiral axis (Figure 1). Methyne proton of **2** was observed as a singlet at 7.82 ppm. As for methyne proton of the compound **3** which was obtained



Scheme 1. (a) NaH/THF; (b) Ethanol/NaOH; (c) Methanol; (d) $POCl_3$/DMF; (e) morpholin; (f) CH_3COOH/CH_3COONa; (g) CH_3COOH/HCl.

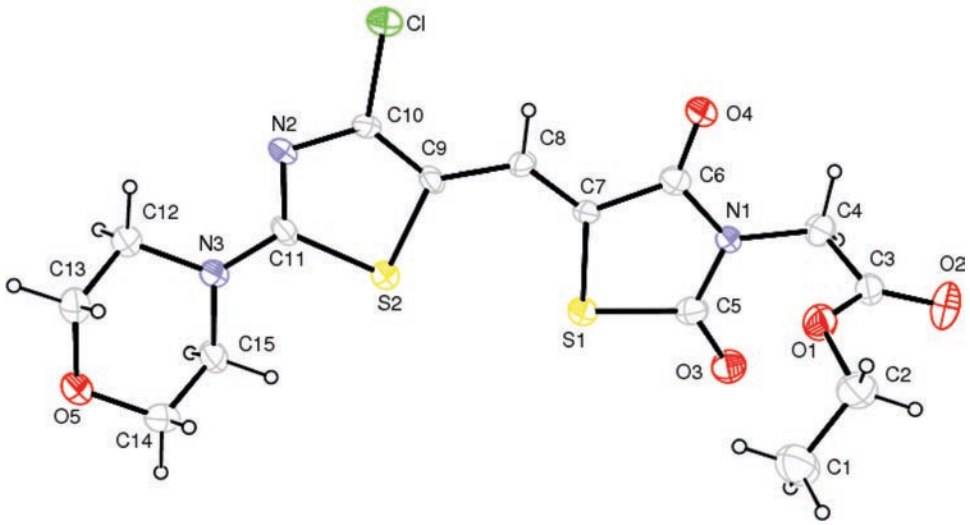


Figure 1. The molecular structure and atomic labeling scheme of compound **2**.

by acidic hydrolysis of ester compound **2**, was seen as a singlet at 7.81 ppm. Methyne protons of the compounds **1–15** were seen at 7.65–8.18 ppm as a singlet.

Biological activity

Derivatives of morpholino thiazolyl TZD compounds **1-15** were tested comparing with glibenclamide for

their insulinotropic activities in INS-1 cells at two different concentrations (Table 1). Compounds **5, 11–15** (at lower concentration; 0.001 mg/ml) were able to increase insulin release in the presence of 5.6 mmol/l glucose. Compounds **3-6, 8-10, 12–15** (at higher concentration; 0.01 mg/ml) were able to increase insulin release. In this series, the most potent compounds are **12** and **14** which are having phenacyl chloride and dichloride at N-3 position of TZD ring, respectively.

Insulin and rosiglitazone show an increase of glucose uptake by the HepG2 cells. TNF-α is able to reverse the stimulatory effect of insulin. Rosiglitazone is able to reverse the inhibitory effect of TNF-α and increases the glucose uptake (Figure 2).

Different concentrations of Compound **4** were tested to find out the optimal concentration for glucose uptake (Figure 3). We choose the highest tested concentration of 0.1 mg/ml for further experiments.

Table 1. Effects of various compounds on glucose-mediated insulin release from INS-1 cells*.

Compound [concentration]	Insulin release (%)	Concentration	Insulin release (%)
Glucose [3.0 mM]	74.10 ± 4.78		
Glucose [5.6 mM]	100		
Plus **1** [1 μg/ml]	37.83 ± 12.33	Plus **1** [10 μg/ml]	47.03 ± 7.88
Plus **2** [1 μg/ml]	76.97 ± 10.24	Plus **2** [10 μg/ml]	91.19 ± 6.18
Plus **3** [1 μg/ml]	79.23 ± 9.76	Plus **3** [10 μg/ml]	122.0 ± 25.01
Plus **4** [1 μg/ml]	84.50 ± 4.97	Plus **4** [10 μg/ml]	145.5 ± 32.58
Plus **5** [1 μg/ml]	101.4 ± 9.41	Plus **5** [10 μg/ml]	143.3 ± 20.16
Plus **6** [1 μg/ml]	92.85 ± 7.56	Plus **6** [10 μg/ml]	143.3 ± 33.42
Plus **7** [1 μg/ml]	87.31 ± 10.33	Plus **7** [10 μg/ml]	97.51 ± 10.81
Plus **8** [1 μg/ml]	70.87 ± 12.93	Plus **8** [10 μg/ml]	106.8 ± 18.89
Plus **9** [1 μg/ml]	91.53 ± 4.83	Plus **9** [10 μg/ml]	146.1 ± 26.69
Plus **10** [1 μg/ml]	96.95 ± 18.91	Plus **10** [10 μg/ml]	139.0 ± 39.47
Plus **11** [1 μg/ml]	104.9 ± 12.00	Plus **11** [10 μg/ml]	94.46 ± 6.51
Plus **12** [1 μg/ml]	121.0 ± 21.80	Plus **12** [10 μg/ml]	107.7 ± 24.86
Plus **13** [1 μg/ml]	102.8 ± 3.12	Plus **13** [10 μg/ml]	116.3 ± 7.89
Plus **14** [1 μg/ml]	122.3 ± 11.34	Plus **14** [10 μg/ml]	129.4 ± 26.18
Plus **15** [1 μg/ml]	109.8 ± 22.65	Plus **15** [10 μg/ml]	127.5 ± 3.25
Plus glibenclamide (1 μg/ml)	160.4 ± 18.04		
DMSO 0.01%	93.88 ± 9.68	DMSO 0.1%	104.5 ± 13.86

*INS-1 cells in multi-wells were washed three times and incubated in KRBH buffer for 90 min at 5.6 mM glucose. The results are expressed as percent insulin release at 5.6 mM glucose alone. Values obtained in the presence of 3.0 mM glucose (substimulatory concentration) and glibenclamide (1 μg/ml) served as negative and positive controls. The final concentration of the solvent DMSO was either 0.01 or 0.1%; a DMSO control (even at 1%) had no effect as shown in the table data. Each value represents the mean ± SEM of six independent experiments.

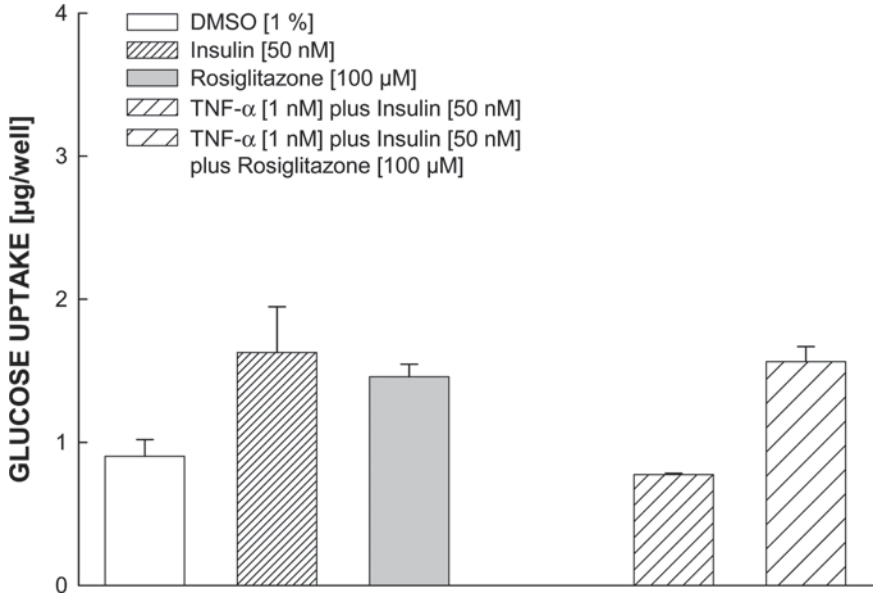


Figure 2. Effect of insulin, rosiglitazone and TNF-α on glucose uptake by HEP G2 cells. Rosiglitazone and TNF-α were added for four days, insulin for the final 30 minutes and the labeled glucose 2-NBDG for 5 min. Mean ± SEM three experiments.

The compounds except compound number **3** show an increase of glucose uptake (Figure 4).

Not in all experiments TNF-α was able to inhibit the insulin stimulatory effect, but when it was possible the compounds were able to reverse this effect and increase the glucose uptake in the cells (Table 2).

Only compounds **3,4,5,6,9** and **10** have been tested which had positive effects with respect to insulin release. Compounds **4,5,6,9** and **10** also increased glucose uptake by themselves. To simulate the pathophysiological situation HepG2 cells were rendered resistant by TNF-α.

The compounds were able to reverse this effect. Various compounds are interesting antidiabetic drugs in that they possess a dual effect.

In our previous studies[7–16], we showed that 2,4-TZD N-acetic acid, acetic acid ethyl ester, benzyl and phenacyl derivatives were more potent than unsubstituted TZDs with regard to their insulin releasing activities. As seen in this study, 2,4-TZD-N-substituted derivatives

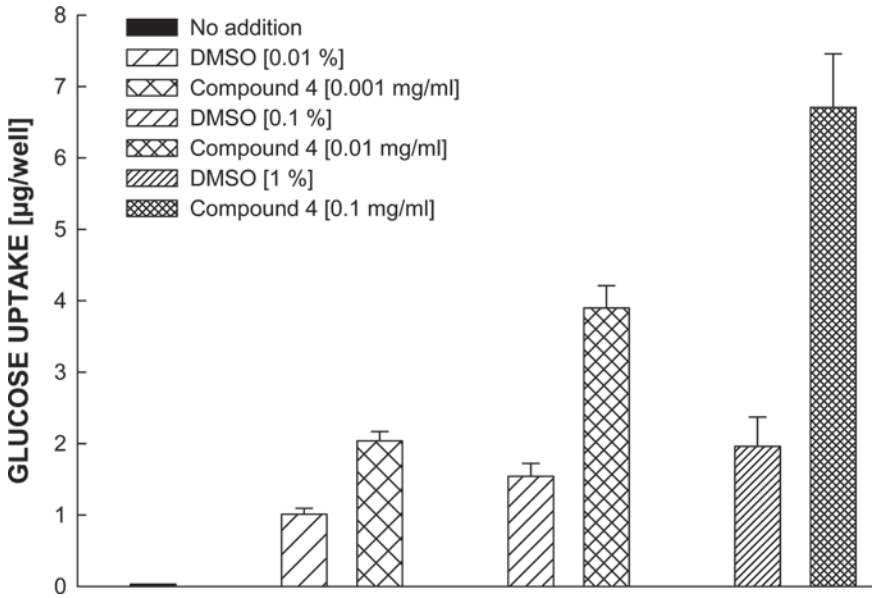


Figure 3. Concentration-response curve of compound 4. Mean ± SEM three experiments.

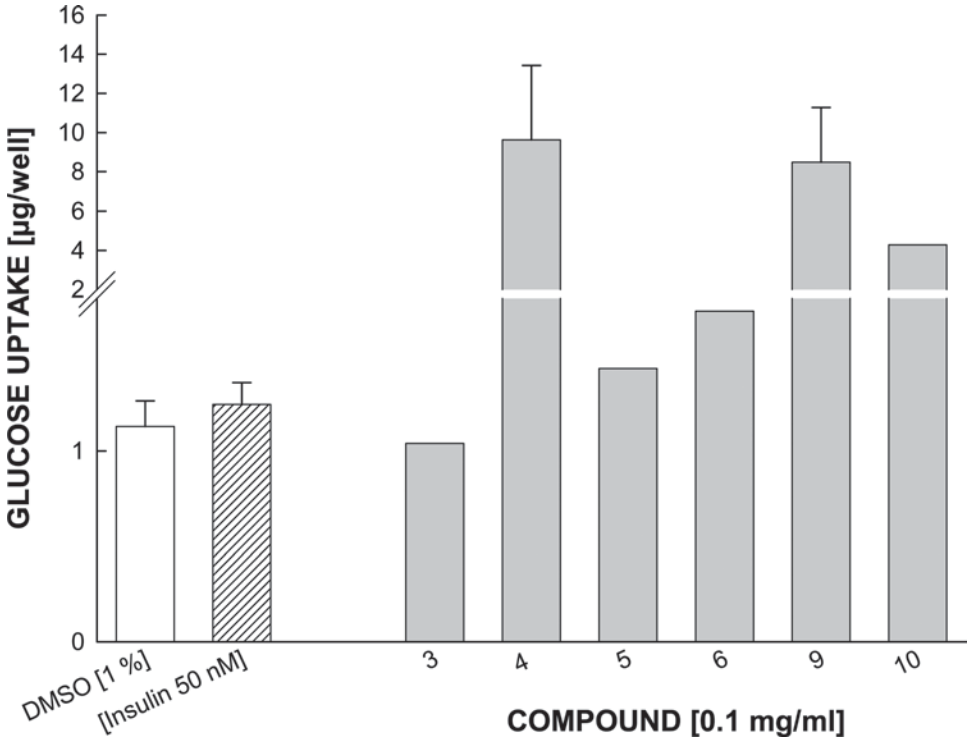


Figure 4. Effect of insulin and tested compounds on glucose uptake. Mean ± SEM two to four experiments.

Table 2. Effect of insulin, a combination of TNF-α plus insulin and the addition of various compounds. Mean ± SEM three experiments.

	Glucose uptake		
Compound	Insulin	TNF-α plus Insulin	TNF-α plus Insulin plus Compound
3	1.2094 μg Glucose/well	0.5776 μg Glucose/well	0.8664 μg Glucose/well
4	1.4488 μg Glucose/well	1.0902 μg Glucose/well	8.2828 μg Glucose/well
5	0.9747 μg Glucose/well	0.5292 μg Glucose/well	1.6584 μg Glucose/well
6	0.9747 μg Glucose/well	0.5299 μg Glucose/well	1.4202 μg Glucose/well
9	1.4488 μg Glucose/well	1.0902 μg Glucose/well	7.1834 μg Glucose/well
10	1.2094 μg Glucose/well	0.5776 μg Glucose/well	3.5378 μg Glucose/well

had also effect on insulin releasing activity in INS-1 cells. According to these results, it should be pointed out that compared with imidic hydrogen carboxylic acid, carboxylic acid ester, benzyl or phenacyl groups on the TZD ring at N-3 position played a noticeable role for increasing the insulin releasing activity in INS-1 cells. On the other hand, 2,4-TZD-N-benzylsubstituted compounds are the most potent compounds in terms of glucose uptake activity. As a result, we can say that instead of imidic hydrogen on the TZD ring at N-3 position benzylic groups are important for increasing the glucose uptake and the insulin releasing activity.

Conclusion

We report the synthesis and the *in vitro* insulin releasing and glucose uptake activity of the morpholino thiazolyl-2,4-thiazolidinediones **1-15.** Only compounds **3,4,5,6,9** and **10** have been tested for glucose uptake activity which had positive effects with respect to insulin release. Compounds **4,5,6,9** and **10** also increased glucose uptake by themselves. To simulate the pathophysiological situation HepG2 cells were rendered resistant by TNF-α. The compounds were able to reverse this effect. In conclusion, we can say that compounds **4,5,6,9** and **10** are interesting antidiabetic drugs in that they possess pancreatic and extrapancreatic effects.

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Declaration of interest

The authors report no conflicts of interest.

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