Hyperglycemia and hypertriglyceridemia activities of newly synthesized compounds derived from 3’-(4-halophenyl)-5’-arylidene spiro(cyclohexane-(1,2’)-thiazolidin)-4’-one

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\textbf{ABSTRACT}

5-Arylidene spiro[cyclohexane-(1,2’)-thiazolidin-4’-one derivatives 1a-d were treated with some nucleophiles. So, when 1b was treated with hydrazine hydrate or phenylhydrazine it gave the pyrazolothiazole derivatives 2a,b, respectively. Compounds 1b,c were reacted with thiourea to give the corresponding thiazolopyrimidine derivatives 3a,b, respectively. Compound 1b, d were reacted with active methylene compound, namely, acetocetanilide to afford the thiazolopyridine derivatives 5a,b, respectively. The latter compound was confirmed chemically via reaction with p-chlorobenzaldehyde to afford the condensation product 6. The oxirano derivatives 7a and 7b were prepared via reaction of hydrogen peroxide with arylidene spirothiazole derivatives 1a and 1d. Compound 7b was reacted with different amine derivatives using different conditions to afford compounds 8a,b-14a-c through ring opening of the oxirane ring. Compound 7b was reacted with 2-(2-chloroethoxy)ethanol to afford compounds 16. Compounds 14a-c were reduced using zinc dust to afford compound 17a-c. The structure of compound 17a, b were confirmed chemically via alkylation with methyl iodide or treatment with chloroacetic acid in the presence of p-chlorobenzaldehyde to give the corresponding S-methylpyrimidine or thiazolo-pyrimidine derivatives 18a,b and 19, respectively. Compound 19 was reacted with hydrazine hydrate or hydroxylamine to afford the thiazolo[5’,4’:4,5]pyrazolo[3’,4’:4,5]thiazolo [3,2-a]pyrimidine derivative 20 or the thiazolo [5’,4’:4,5] isoxazolo [5’,4’:4,5] thiazolo [3,2-a]pyrimidine 21, respectively. Moreover, some of the newly prepared products were screened for determination of the serum glucose level and triglycerides level. Oral treatment of hyperglycaemic rats with compounds 7b, 13, 15, 18b and 21 (0.01 mM/kg/day) for 10 consecutive days caused a marked decrease in the elevated serum level of glucose reaching about 169\%, 140\%, 151\%, 135\% and 110\% of the normal values, respectively. Similar treatment of hyperglycaemic rats showed a prominent decrease in the serum level of triglycerides reaching about 190\%, 344\%, 211\%, 126\% and 147\%, respectively.

\textbf{Keywords:} Spiro(cyclohexane-1,2’-thiazolidine), Spiro(cyclohexane-1,2’-thiazolo-pyridine), anti hyperglycemia and anti hypertriglyceridemia.

\textbf{INTRODUCTION}

Design of new substances based on privileged scaffolds is one of the successful directions in drug discovery. According to this approach, the use of thiazolidinones gives access to series of compounds with a broad spectrum of biological activity. Thiazolidinone derivatives are of great interest as sources of innovative drug candidates with antimicrobial [1-11], antiviral [12-14], antifungal [15], antitubercular [16,17], anti-inflammatory [18,19], antidiabetic effects [20]. Recently research of thiazolidinone’s pharmacological effects became interesting and promising for anticancer agents design [21-24], antioxidant [25,26], antihyperglycemic [27], nematicidal[28]. Thiazolidine
derivatives represent a family of compounds with great industrial interest, which have found applications in food and flavor chemistry [29,30]. Thiazolidines have also attracted very significant biochemical interest, owing to the presence of the thiazoline moiety in the structures of several compounds with important pharmacological properties such as antibiotics [31]. So the aim of the present study is to synthesize the biologically important scaffold spiro(cyclohexane-thiazolidine) derivatives and study the treatment of hyperglycemic rats with some of the newly prepared compounds and evaluate the level of glucose as well the serum level of the triglycerides.

**MATERIALS AND METHODS**

**Chemistry**

All melting points are uncorrected and measured using Electro-thermal IA 9100 apparatus, (Shimadzu, Tokyo, Japan). IR spectra were recorded as potassium bromide pellets on a Perkin-Elmer 1650 spectrophotometer (Perkin-Elmer, Norwalk, CT, USA). $^1$H NMR and $^{13}$C NMR spectra were determined on a Jeol-Ex-300 NMR spectrometer (JEOL, Tokyo, Japan) and chemical shifts were expressed as part per million; ppm ($\delta$ values) against TMS as internal reference. Mass spectra were recorded on VG 2AM-3F mass spectrometer (Thermo electron corporation, USA) [products containing Br, Cl showed some pattern of their isotope abundance]. Microanalyses were operated using Mario El Mentar apparatus, Organic Microanalyis Unit, National Research Centre, and the results were within the accepted range ($\pm$ 0.20) of the calculated values. Follow up of the reactions and checking the purity of the compounds was made by TLC on silica gel-precoted aluminum sheets (Type 60 F254, Merck, Darmstadt, Germany).

Compounds 1a-d were prepared according to previous reported procedure [25].

**General procedure for the synthesis of spiro[cyclohexane-1,5'-pyrazolo[4,3-d][thiazole] derivatives (2a,b).**

A mixture of compound 1b (3.6 g, 0.01 mole) and hydrazine hydrate (0.02 mole) or phenylhydrazine (1.08 g, 0.01 mole) was refluxed in 30 ml dioxane for 10 h. The reaction mixture was concentrated under reduced pressure, the formed solid was filtered off, dried and crystallized from dioxane to give compound 2a,b.

6'-[4-Fluorophenyl]-3'-p-tolyl-1',2',3',6'-tetrahydrospiro[cyclohexane-1,5'-pyrazolo[3,4-d][thiazole] (2a). Pale yellow powder, mp 222-224 °C (dioxane), yield 68%, IR (KBr, cm$^{-1}$): 3135, 3143 (2 NH); $^1$H NMR (DMSO-d$_6$): $\delta$ (ppm) 1.49-2.0 (m, 10H, 5CH$_2$), 2.4 (s, 3H, CH$_3$), 2.6 (s, 1H, CH pyrazole), 3.4 (s, 1H, CH thiazole (H 3a'), 7.3-7.9 (m, 8H, Ar-H), 8.3 (s, 1H, NH, D$_2$O exchangeable); MS m/z (%): 381.17 (M$^+$, 29), 383.16 (M$^+$+2, 9).

**General procedure for the synthesis of spiro[cyclohexane-1,2'-thiazolo[4,5-d][pyrimidine]-5'-thiol derivatives (3a,b).**

A mixture of compound 1b or 1c (0.01 mole) and thiourea (0.76 g, 0.01 mole) were fused in the presence of 10 drops of piperidine in oil bath for 3 h at 180°C. The product was poured onto ice, the solid was filtered off and recrystallized from acetic acid to give compound 3a,b.

3'-(4-Fluorophenyl)-7'-p-tolyl-3'H-spiro[cyclohexane-1,2'-thiazolo[4,5-d][pyrimidine]-5'-thiol (3a). Brown powder, 218-220°C (AcOH), yield 50%, $^1$H NMR (DMSO-d$_6$): $\delta$ (ppm) 1.5-1.98 (m, 10H, 5CH$_2$), 2.3 (s, 3H, CH$_3$), 7.0-7.5 (m, 9H, Ar-H), 9.2 (s, 1H, NH, D$_2$O exchangeable); MS m/z (%): 429.23 (M$^+$, 14.57), 424 (M$^+$+1, 6.8).

3'-(4-Fluorophenyl)-7'-phenyl-3'H-spiro[cyclohexane-1,2'-thiazolo[4,5-d][pyrimidine]-5'-thiol (3b). Brown powder, 232-234°C (AcOH), yield 55%, $^1$H NMR (DMSO-d$_6$): $\delta$ (ppm) 1.45-2.0 (m, 10H, 5CH$_2$), 7.2-7.7 (m, 10H, Ar-H), 3.4 (s, 3H, CH$_3$), 8.3 (s, 1H, NH, D$_2$O exchangeable); MS m/z (%): 409 (M$^+$, 18.5), 410 (M$^+$+1, 6.0).
2-(2′-(6′-(4-Fluorophenyl)-3′-p-tolyl-3,3a′-dihydrospirocyclohexane-1,5′-pyrazolo[3,4-d]thiazole-2′(6H)-yl)ethoxy)ethanol (4).

A mixture of compound 2a (3.8 g, 0.01 mole) and 2-(2-chloroethoxy)ethanol (0.01 mole) was stirred in ethanol containing (0.2 g NaOH) at room temperature for 10 h. The solid substance was filtered off, dried and recrystallized from dioxane to give compound 4. White powder, mp 214-216 °C (dioxane), yield 45%, IR (KBr, v, cm⁻¹): 3341 (OH); 1H NMR (DMSO-d₆): δ (ppm) 1.25-1.95 (m, 10H, 5CH₂), 2.4 (s, 3H, CH₃), 3.6 (s, 3H, COCH₃), 7.0-7.7 (m, 13H, Ar); 13C NMR: 24.2-38.9 (5CH₂), 67.1 (C-spiro), 114.5-139.6 (aromatic carbons), 159.5 (C=O), 173.2 (C=O), 179.2 (C=O).

6′-Acetyl-3′-(4-fluorophenyl)-4′-phenyl-3′H-spirocyclohexane-1′,2′-thiazolo[4,5-b]pyridin-5′(4′H)-one (5a).

Yellow powder, mp 226-228°C (dioxane), yield 60%, IR (KBr, v, cm⁻¹): 1675.7, 1710 (2C=O); 1H NMR (DMSO-d₆): δ (ppm) 1.45-1.97 (m, 10H, 5CH₂), 2.4 (s, 3H, CH₃), 3.6 (s, 3H, COCH₃), 7.0-7.7 (m, 13H, Ar); 13C NMR: 21.6 (CH₂), 24.2-38.5 (5CH₂), 67.1 (C-spiro), 114.5-139.6 (aromatic carbons), 159.5 (C=O), 179 (C=O), 179.2 (C=O); MS m/z (%): 524.6 (M⁺+2, 4.5), 526.8 (M⁺+2, 6.5). Anal. calcd. for C₂₁H₁₉BrClN₂O₂S (523.5): C, 73.15; H, 5.34; N, 5.29; S, 5.27. Found, C, 73.15; H, 5.34; N, 5.29; S, 5.27.

General procedure for the synthesis of compounds 5a,b

A mixture of compound 1b or 1d (0.01 mole) and acetoacetaamide (1.7 g, 0.01 mole) was refluxed in sodium methoxide (0.5 g in 50 ml methanol) for 10 h. The reaction mixture was cooled and poured into water, neutralized with dilute HCl, then the formed solid was filtered off and recrystallized from dioxane to give compounds 5a,b.

6′-Acetyl-3′-(4-bromophenyl)-4′-phenyl-3′H-spirocyclohexane-1′,2′-thiazolo[4,5-b]pyridin-5′(4′H)-one (5a).

White powder, mp 220-222°C (EtOH), yield 65%, IR (KBr, v, cm⁻¹): 1675.3, 1714 (2C=O); 1H NMR (DMSO-d₆): δ (ppm) 1.24-1.96 (m, 10H, 5CH₂), 2.4 (s, 3H, CH₃), 3.6 (s, 3H, COCH₃), 7.3-7.62 (m, 17H, Ar); MS m/z (%): 607.9 (M⁺+2, 4.5), 609.5 (M⁺+2, 4.5), 610.1 (M⁺+2, 6.5). Anal. calcd. for C₂₁H₂₁BrClN₂O₂S (605.97): C, 73.26; H, 5.34; N, 5.34; S, 5.34. Found, C, 73.24; H, 5.48; N, 5.27; S, 6.00.

3′-(4-Bromophenyl)-7′-(3-(4-chlorophenyl)acryloyl)-4′-phenyl-3′H-spirocyclohexane-1′,2′-thiazolo[4,5-b]pyridin-5′(4′H)-one (6).

A mixture of compound 5b (6.05 g, 0.01 mole) and p-chloro benzoaldehyde (1.4 g, 0.01 mole) in sodium hydroxide (0.2 g in 30 ml methanol) was refluxed for 2 hrs. The reaction mixture was poured into cold water and neutralized with dilute HCl, then the formed solid was filtered off and recrystallized from acetic acid to give compound 6. White fine needle crystals, mp 272-274°C (AcOH), yield 50%, IR (KBr, v, cm⁻¹): 1675.4, 1701 (2C=O); 1H NMR (DMSO-d₆): δ (ppm) 1.24-1.96 (m, 10H, 5CH₂), 2.4 (s, 3H, CH₃), 3.6 (s, 3H, COCH₃), 7.3-7.62 (m, 17H, Ar), 7.95 (s, 1H, CH-Ar); MS m/z (%): 726 (M⁺+2, 18.62), 728 (M⁺+2, 21.78). Anal. calcd. for C₂₁H₁₇BrClN₂O₂S (728.52): C, 62.65; H, 4.01; N, 3.85; S, 4.30. Found, C, 62.62; H, 3.89; N, 3.73; S, 4.35.

2-[3-(4-Chlorophenyl)oxiran-2-yl]-4-halogenophenyl-1-1H-1-azaspiro[4.5]decan-3-one 7a,b.

To a cold mixture of arylidine spiro thiazolidinone 1a or 1d (0.01 mole) in acetonitrile (30:10) containing 1 gm NaOH, hydrogen peroxide (5 mL, 36%) was added portionwise with stirring for 1 h. The solvent was concentrated under reduced pressure. The solid formed was filtered off, washed with water and crystallized from dioxane to give compounds 7a and b.

2-[3-(4-Chlorophenyl)oxiran-2-yl]-4-bromophenyl-1H-1-azaspiro[4.5]decan-3-one 7b.

White powder, mp 212-214°C (EtOH), yield 72%, IR (KBr, v, cm⁻¹): 1678.8 (CO); 1H NMR (DMSO-d₆): δ (ppm) 1.48-1.99 (m, 10H, 5CH₂), 3.66 (s, 1H, CH-Oxirane), 7.0-7.6 (m, 8H, Ar-H); 13C NMR: 24.8-38.5 (5CH₂), 67.3 (C-spiro, thiazolidine), 72.1 (HC-O, oxirane), 73.15 (C, oxirane), 119.1-135.3 (sp² carbons), 164 (CF), 171.3 (C=O), Anal. calcd. for C₂₁H₁₇BrClNO₃S (464.80): C, 54.26; H, 4.12; N, 3.01; S, 6.90. Found, C, 54.22; H, 4.01; N, 2.93; S, 6.89.
General procedure for the synthesis of compounds 8a,b
A mixture of compound 7b (0.01 mole) and p-bromoaniline or dimethylamine in DMF was stirred at room temperature for 8 h. The product poured into water then the solid was filtered off and recrystallized from dioxane to give compounds 8a and b, respectively.

4-(4-Bromophenyl)-2-((4-bromophenylamino)(4-chlorophenyl)methyl)-2-hydroxy-1-thia-4-azaspiro[4.5]decan-3-one (8a).
White powder, mp 238-240 °C (dioxane), yield 60%, IR (KBr, v cm⁻¹): 1676.8 (CO), 3461.6 (OH), 3133.48 (NH); ¹H NMR (DMSO-d₆): δ (ppm) 1.6-1.97 (m, 10H, 5CH₂), 3.66 (s, 1H, OH, D₂O exchangeable), 3.73 (s, 1H, CH-N), 7.2-7.72 (m, 13H, Ar-H+ NH, D₂O exchangeable); MS m/z (%):634 (M⁺, 10), 636 (M⁺+2 , 20.04), 638 (M⁺+4 , 14), 640 (M⁺+6, 2). Anal. calcd. for C₂₇H₂₈BrClN₂O₂S (636.83) : C, 50.92; H, 3.96; N, 4.40; S, 6.29; Found, C, 50.89; H, 3.88; N, 4.30; S, 5.01.

4-(4-Bromophenyl)-2-((4-chlorophenyl)(dimethylamino)methyl)-2-hydroxy-1-thia-4-azaspiro[4.5]decan-3-one (8b).
White powder, mp 226-228 °C (dioxane), yield 55%, ¹H NMR (DMSO-d₆): δ (ppm) 1.45 - 1.9 (m, 10H, 5CH₂), 3.4 (s, 1H, OH, D₂O exchangeable), 3.6 (s, 6H, 2CH₃), 4.3 (s, 1H, CH-N), 7.3-7.78 (m, 8H, Ar-H); MS m/z (%): 508 (M⁺, 12.75), 510 (M⁺+2, 10.59). Anal. calcd. for C₂₅H₂₆BrClN₂O₂S (509.89): C, 54.18; H, 5.14; N, 5.49; S, 6.29; Found, C, 54.22; H, 5.18; N, 5.39; S, 6.33.

General procedure for the synthesis of compounds 9 a,b.
A mixture of compound 7b (4.6 g, 0.01 mole) and hydrazine hydrate or phenylhydrazine (0.01 mole) in ethanol was refluxed for 4 h, the reaction mixture was concentrated, then the solid was filtered off and recrystallized from methanol to give compounds 9a and b respectively.

4-(4-Bromophenyl)-2-((4-chlorophenyl)(3-phenylhydrazinyl)methyl)-2-hydroxy-1-thia-4-azaspiro[4.5]decan-3-one (9a).
White powder, mp 233-237 °C (methanol), yield 62% , IR (KBr, v cm⁻¹): 1675.84 (CO), 3212.83 (NH), 3340.11, 3443 (NH₂), 3426.89 (OH); ¹H NMR (DMSO-d₆): δ (ppm) 1.58-1.98 (m, 10H, 5CH₂), 2.7 (s, 2H, NH₂, D₂O exchangeable), 3.66 (s, 1H, OH, D₂O exchangeable), 3.70 (s, 1H, CH-N), 7.38-7.62 (m, 9H, Ar-H + NH, D₂O exchangeable); MS m/z (%): 496 (M⁺+1, 4.78), 497 (M⁺+2, 5.39). Anal. calcd. for C₂₇H₂₈BrClN₂O₂S (496.85): C, 50.76; H, 4.67; N, 8.46; S, 6.45; Found C, 50.80 ; H, 4.61; N, 8.34, S, 6.41.

4-(4-Bromophenyl)-2-((4-chlorophenyl)(2-phenylhydrazinyl)methyl)-2-hydroxy-1-thia-4-azaspiro[4.5]decan-3-one (9b).
Beige fine needle crystals, mp 237-239°C (dioxane/ methanol), yield 65%. IR (KBr, v cm⁻¹): 1675.84 (CO), 3133.48, 3162 (2 NH), 3339.14 (OH); ¹H NMR (DMSO-d₆): δ (ppm) 1.55-1.95 (m, 10H, 5CH₂), 3.4 (s, 1H, NH, D₂O exchangeable), 3.60 (s, 1H, OH, D₂O exchangeable), 3.9 (s, 1H, CH-N), 7.38-7.62 (m, 14H, Ar-H + NH, D₂O exchangeable); MS m/z (%): 571 (M⁺-1, 64.67). Anal. calcd. for C₂₇H₂₈BrClN₂O₂S (572.94): C, 56.60 ; H, 4.75 ; N, 7.33; S, 5.60; Found C, 56.54 ; H, 4.63 ; N, 7.12; S, 5.69.

General procedure for the synthesis of compounds 10a,b
A mixture of compound 7b (4.6 g, 0.01 mole) and piperidine or morpholine in DMF was stirred at room temperature for 7 h. The product poured into water then the solid was filtered off and recrystallized from dioxane to give compounds 10a and b respectively.

4-(4-Bromophenyl)-2-((4-(piperidin-1-yl)methyl)-2-hydroxy-1-thia-4-azaspiro[4.5]decan-3-one (10a).
White powder, mp 231-233 °C(dioxane/methanol), yield 40%, IR (KBr, v cm⁻¹): 1675.84 ( CO), 3431.71(OH); ¹H NMR (DMSO-d₆): δ (ppm) 1.55-2.2 (m, 20H,10CH₂), 3.66 (S,1H, OH), 3.70 ( s,1H, CH), 7.37 -7.62 (m, 8H, Ar); MS m/z (%): 549 (M⁺+1, 11.0). Anal. calcd. for C₂₉H₂₈BrClN₂O₂S (549.95): C, 56.78; H, 5.50; N, 5.09; S, 5.83. Found, C, 56.76; H, 5.39; N, 4.89; S, 5.80.

4-(4-Bromophenyl)-2-((4-(4-chlorophenyl)morpholino)methyl)-2-hydroxy-1-thia-4-azaspiro[4.5]decan-3-one (10b).
Pale yellow powder, mp 244-246°C (dioxane/methanol), yield 45%, ¹H NMR (DMSO-d₆): δ (ppm) 1.56-2.1 (m, 18H, 9 CH₂), 3.62 ( s, 2H, CH₂OH, D₂O exchangeable), 7.0 -7.62 (m, 8H, Ar-H); MS m/z (%): 551 (M⁺, 10.6), 553 (M⁺+2, 9.1) Anal. calcd. for C₂₉H₂₈BrClN₂O₂S (551.92): C, 54.40; H, 5.11; N, 5.08; S, 5.81. Found, C,54.38; H, 5.01; N, 4.91; S, 5.72.
4-(4-Bromophenyl)-2-((4-chlorophenyl)(hydroxyamino)methyl)-2-hydroxy-1-thia-4-azaspiro[4.5]decan-3-one (11).

A mixture of compound 7b (4.6 g, 0.01 mole) and hydroxylamine (0.33 g, 0.01 mole) in pyridine was refluxed for 3 h, the reaction mixture was concentrated then, the product poured into water and neutralized with dilute HCl, the formed solid was filtered off and recrystallized from dioxane to give compound 11. Beige powder, mp 213-215°C (dioxane), yield 58%, IR (KBr, u, cm⁻¹): 1674.87 (CO), 3136.37 (NH), 3432.67, 3398 (2OH); ¹H NMR(CDCl₃): δ (ppm) 1.62-1.98 (m, 10H, 5CH₃), 3.66 (s, 1H, OH, D₂O exchangeable), 7.38-7.62 (m, 9H, Ar-H + NH, D₂O exchangeable); MS m/z (%): 496 (M⁺, 11.30), 498 (M⁺+2, 11.59). Anal. calcd. for C₁₇H₁₂BrClN₃O₄S (497.83): C, 50.66; H, 4.45; N, 8.63; S, 6.44. Found: C, 50.61; H, 4.31; N, 5.58; S, 6.40.

7'-(4-Bromophenyl)-1-(4-chlorophenyl)-3',4',4a',7'-tetrahydrospiro[cyclohexane-1,6'-thia-zolo-[4,5-c][1,2,6]oxadiazin]-4a'-ol (12).

Method A: A mixture of compound 7b (4.6 g, 0.01 mole) and hydroxylamine (0.66 g, 0.02 mole) in pyridine was refluxed for 3 h, the reaction mixture was concentrated then, the product poured into water/HCl then, the solid was filtered off and recrystallized from dioxane to give compound 12.

Method B: (0.01 mole) of compound 11 and hydroxylamine (0.33 g, 0.01 mole) in pyridine was refluxed for 6 h, the reaction mixture was concentrated then, the product poured into water/HCl then, the solid was filtered off and recrystallized from dioxane to give compound 12.

White powder, mp 262-264°C (dioxane), yield 62%, IR (KBr, u, cm⁻¹): 3278.39, 3312 (NH); ¹H NMR(CDCl₃): δ (ppm) 1.68-2.08 (m, 10H, 5CH₃); 3.70 (s, 1H, CH-N), 3.73 (s, 1H, OH, D₂O exchangeable), 7.1-7.66 (m, 9H, Ar-H + NH, D₂O exchangeable); MS m/z (%): 493 (M⁺, 37.39), 495 (M⁺+2, 23.48). Anal. calcd. for C₁₇H₁₂BrClN₃O₄S (494.83): C, 50.97; H, 4.28; N, 8.49; S, 6.48. Found: C, 50.90; H, 4.09; N, 8.33; S, 6.52.

1-(4-(4-Bromophenyl)-2-hydroxy-3-oxo-1-thia-4-azaspiro[4.5]decan-2-yl-(4-chlorophenyl)-methyl thiourea (13).

A mixture of compound 7b (4.6 g, 0.01 mole) and thiourea (0.76 g, 0.01 mole) in DMF was stirred at room temperature for 6 h, the product poured into water then the solid was filtered off and recrystallized from dioxane to give compound 13. White powder, mp 229-231°C (dioxane), yield 65%, IR (KBr, u, cm⁻¹): 1678.7 (CO), 3155.94 (NH), 3278.39, 3312 (NH₂), 3440.39 (OH); ¹H NMR(DMSO-d₆): δ (ppm) 1.5-1.95 (m, 10H, 5CH₃), 3.65 (s, 2H, NH + OH, D₂O exchangeable), 3.80 (s, 1H, CH-N), 7.1 (br, 2H, NH₂, D₂O exchangeable), 7.3-7.65 (m, 8H, Ar-H); MS m/z (%): 540 (M⁺, 25.48), 542 (M⁺+2, 9). Anal. calcd. for C₁₇H₁₂BrClN₃O₃S (540.92): C, 48.85; H, 4.29; N, 7.77; S, 11.86; Found: C, 48.88; H, 4.14; N, 7.57; S, 11.79.

General procedure for the synthesis of compounds 14a-c.

Method A: A mixture of compound 7a-c (4.6 g, 0.01 mole) and thiourea (0.76 g, 0.01 mole) were added then, the reaction mixture was refluxed in 50 mL ethanol containing 0.5 g NaOH for 4 h. The product was poured into water and neutralized with dilute HCl then, the solid was filtered off and recrystallized from dioxane to give compounds 14a-c.

Method B: A solution of compound 13 (5.4 g, 0.01 mole) in ethanol containing 1 gm of NaOH was refluxed for two h. The product was poured into water and neutralized with dilute HCl then, the solid was filtered off and recrystallized from dioxane to give compound 14a.

7'-(4-Chlorophenyl)-3'-(4-bromophenyl)-7a'-hydroxy-3a',4'-dihydro-3'H-spiro[cyclohexane-1,2'-thiazolo[4,5-d]pyrimidine]-5'-(7a'H)-thione (14a).

White powder, mp 272-274°C (dioxane), yield 70%, IR (KBr, u, cm⁻¹): 1221 (C=S), 3145.89 (NH), 3435.56 (OH); ¹H NMR (DMSO-d₆): δ (ppm) 1.5-1.9 (m, 10H, 5CH₃), 3.12 (s, 1H, OH, D₂O exchangeable), 6.95-7.6 (m, 8H, Ar-H), 8.6 (s, 1H, NH, D₂O exchangeable); MS m/z (%): 522 (M⁺, 21.65). Anal. calcd. for C₁₇H₁₂BrClN₃O₃S (522.91): C, 50.53; H, 4.05; N, 8.04; S, 12.26. Found, C, 50.48; H, 3.91; N, 7.94; S, 12.29.

7'-(4-Chlorophenyl)-3'-(4-fluorophenyl)-7a'-hydroxy-3a',4'-dihydro-3'H-spiro[cyclohexane-1,2'-thiazolo[4,5-d]pyrimidine]-5'-(7a'H)-thione (14b).

White fine needle crystals, mp 237-239°C (dioxane), yield 66%, ¹H NMR (DMSO-d₆): δ (ppm) 1.6-1.97 (m, 10H, 5CH₃), 3.3 (s, 1H, CH-thiazole), 7.3 (s, 1H, OH, D₂O exchangeable), 7.2-7.8 (m, 8H, Ar-H), 8.6(s,1H, NH, D₂O exchangeable); MS m/z (%): 462 (M⁺, 8). Anal. calcd. for C₁₇H₁₂ClFN₃O₃S (462.00): C, 57.19; H, 4.58; N, 9.10; S, 13.88. Found, C, 57.29; H, 4.38; N, 8.98; S, 13.91.
7"-(4-Methylphenyl)-3"-(4-fluorophenyl)-7a'-hydroxy-3a',4'-dihydro-3'H-spiro[cyclohexane-1,2'-thiazolo[4,5-d][pyrimidine]-5'(7a'H)-thione (14c).

White rectangle crystals, mp 222-224°C (dioxane), yield 60%. 1H NMR (DMSO-d6): δ (ppm) 1.51-2.0 (m, 10H, 5CH2), 2.6 (s, 3H, CH3), 3.2 (s, 1H, CH thiazole), 3.6 (s, 1H, OH, D2O exchangeable), 7.3-7.8 (m, 8H, Ar-H), 8.7 (s, 1H, NH, D2O exchangeable); MS m/z (%): 441.5 (M+, 10.68). Anal. calcd. for C23H22FN4O2S2 (441.58): C, 62.56; H, 5.48; N, 9.52; S, 14.39.

1-(4-(4-Bromophenyl)-2-hydroxy-3-oxo-1-thia-4-azaspiro[4,5]decan-2-yl)(4-chlorophenyl)methyl)-3-(4-chlorobenzylieidene)thiourea (15).

A mixture of compound 13 (5.4 g, 0.01 mole) and p-chlorobenzaldehyde (1.4 g, 0.01 mole) in ethanol containing 1 ml of HCl was refluxed for 3 h. The product was poured into water then the solid was filtered off and recrystallized from dioxane to give compound 15. White powder, mp 208-210°C (dioxane), yield 58%. IR (KBr, v, cm⁻¹): 1676.8 (CO), 3440.06 (OH), 3141.07 (NH); 1H NMR (DMSO-d6): δ (ppm) 1.55-1.98 (m, 10H, 5CH2), 3.6 (s, 1H, OH, D2O exchangeable), 3.7 (s, 1H, CH-Ar), 7.3-7.62 (m, 13H, Ar-H + NH, D2O exchangeable); MS m/z (%): 661 (M+, 17.88), 663 (M+ +2, 27.15). Anal. calcd. for C22H25BrClN3O2S4 (663.48): C, 52.50; H, 3.95; N, 6.33; S, 9.67. Found, C, 52.38; H, 3.88; N, 9.44; S, 14.39.

4-(4-Bromophenyl)-2-(4-chlorophenyl)-3-(2-hydroxyethoxy)propyl)-2-hydroxy-1-thia-4-azaspiro[4,5]decan-3-one (16).

To a solution of DMF containing Na2CO3, a mixture of compound 7b (4.6 g, 0.01 mole) and 2-(2-chloroethoxy)ethanol (0.01 mole) were added, then the reaction mixture was stirred at room temperature for 6 h. The product was poured into water then the solid was filtered off and recrystallized from methanol to give compound 16. White needle crystals, mp 235-236°C (MeOH), yield 50%. IR (KBr, v, cm⁻¹): 1673.2 (C=O), 3435.56 (OH); 1H NMR (DMSO-d6): δ (ppm) 1.5-1.95 (m, 10CH2, 5CH2), 2.1 (t, 4H, J=2.8 Hz, 2CH2), 2.25 (t, 1H, J=2.6 Hz, CH-Ar), 3.1 (s, 1H, OH, D2O exchangeable), 3.8 (t, 4H, J=2.8 Hz, 2CH2), 7.1-7.8 (m, 9H, Ar-H), 8.7 (s, 1H, Schiff base); MS m/z (%): 522 (M+, 17.88), 663 (M+ +2, 27.15). Anal. calcd. for C22H22BrClN2O4S4 (522.92): C, 54.34; H, 4.42; N, 8.00; S, 12.29. Found, C, 54.09; H, 5.13; N, 2.39; S, 5.83.

General procedure for the synthesis of Compounds 17a-c.

Compound 14a-c (0.01 mole) was dissolved in 20 ml glacial acetic acid and 1gm of zinc dust was added. The reaction mixture was refluxed for 2h. The inorganic product was removed and the mother liquor was poured into water and neutralized with ammonia, the solid was filtered off and crystallized from dioxane to give compound 17a-c.

3"-(4-Bromophenyl)-7"-(4-chlorophenyl)-7a'-hydroxytetrahydro-dro-3'H-spiro[cyclohexane-1,2'-thiazolo[4,5-d][pyrimidine]-5'(6'H)-thione (17a).

White powder, mp 266-268°C (dioxane/methanol), yield 58%, IR (KBr, v, cm⁻¹): 1229 (C=S), 3150, 3213 (2 NH); 1H NMR (DMSO-d6): δ (ppm) 1.52-1.96 (m, 10H, 5CH2), 2.1 (t, 4H, J=2.8 Hz, 2CH2), 2.15 (t, 1H, J=2.6 Hz, CH-Ar), 3.4 (s, 1H, OH, D2O exchangeable), 3.8 (t, 4H, J=2.8 Hz, 2CH2), 7.1-7.8 (m, 9H, Ar-H + NH, D2O exchangeable); 13CNMR: 24.0-39 (5CH2), 73.5 (C-spiro), 76 (4CH2), 123-133 (aromatic-carbons), 135 (C-N), 137(C-F), 167 (C=O). Anal. calcd. for C23H23BrClN2O4S4 (554.92): C, 54.11; H, 5.27; N, 2.54; S, 5.78; Found, C, 54.09; H, 5.13; N, 2.39; S, 5.83.

7"-(4-Chlorophenyl)-3"-(4-fluorophenyl)-7a'-hydroxytetrahydro-dro-3'H-spiro[cyclohexane-1,2'-thiazolo[4,5-d][pyrimidine]-5'(6'H)-thione (17b).

White powder, mp 237-239°C (dioxane/methanol), yield 66%, 1H NMR (DMSO-d6): δ (ppm) 1.55-1.98 (m, 10H, 5CH2), 3.5 (s, 1H, OH, D2O exchangeable), 4.2 (s, 1H, CH thiazole), 4.7 (s, 1H, CH, primidine), 6.9 (s, 1H, NH, D2O exchangeable), 6.9-7.6 (m, 9H, Ar-H + NH, D2O exchangeable); MS m/z (%): 463 (M+, 19), 465 (M+ +2, 10.1). Anal. calcd. for C22H22ClF2N2O4S4 (464.02): C, 56.94; H, 5.00; N, 9.06; S, 13.82, found, C, 56.98; H, 4.45; N, 8.97; S, 13.90.

3"-(4-Fluorophenyl)-7a'-hydroxy-7"-p-tolytetrahydro-dro-3'H-spiro[cyclohexane-1,2'-thiazolo[4,5-d][pyrimidine]-5'(6'H)-thione (17c).

White powder, mp 224-226°C (dioxane/methanol), yield 60%. 1H NMR (DMSO-d6): δ (ppm) 1.49-1.95 (m, 10H, 5CH2), 2.6 (s, 3H, CH3), 3.4 (s, 1H, OH, D2O exchangeable), 4.3 (s, 1H, CH thiazole), 4.5 (s, 1H, CH, primidine), 6.9 (s, 1H, NH, D2O exchangeable), 7.1-7.6 (m, 9H, Ar-H + NH, D2O exchangeable); MS m/z (%): 522 (M+, 21.65). Anal. calcd. for C22H22F2N2O4S4 (443.60): C, 62.27; H, 5.91; N, 9.47; S, 14.46, found, C, 62.09; H, 5.73; N, 9.27; S, 14.51.
General procedure for the synthesis of compounds 18a,b

To a solution of dioxane containing 1 g of NaOH a mixture of compound 17a or 17b (0.01 mole) and CH₂Cl₂ (0.01 mole) were added. Then the reaction mixture was refluxed in water bath for 10 h. The product was poured into water then the solid was filtered off and recrystallized from methanol to give compound 18a,b.

1’-(4-Bromophenyl)-7-(4-chlorophenyl)-5’-(methylthio)-3a’,4’,7a’-tetrahydro-1’H-spiro[cyclohexane-1,2’-thiazolo[5,4-d][pyrimidin]-3a’-ol (18a).
White fine needle crystals, mp 236-238˚C (MeOH), yield 56%, IR (KBr, cm⁻¹): 3453.8 (OH), 3110.6 (NH); ¹H NMR (DMSO-d₆): δ (ppm) 1.54-1.9 (m, 10H, 5CH₂), 2.6 (s, 3H, CH₃), 3.7 (s,1H,OH, D₂O exchangeable); MS m/z (%): 669 (M⁺, 10.0). Anal. calcd. for C₂3H₁₂BrClN₂O₂ (669.08): C, 51.26; H, 4.68; N, 7.80; Found C, 51.14; H, 4.55; N, 7.66; S, 11.87.

7’-(4-Chlorophenyl)-1’-(4-fluorophenyl)-5’-(methylthio)-3a’,4’,7a’,-tetrahydro-1’H-spiro[cyclohexane-1,2’-thiazolo[5,4-d][pyrimidin]-3a’-ol (18b).
White powder. 254-256˚C (MeOH), yield 52%, IR(KBr, cm⁻¹): 3453.8 (OH), 3110.6 (NH); ¹H NMR (DMSO-d₆): δ (ppm) 1.54-1.9 (m, 10H, 5CH₂), 2.6 (s, 3H, CH₃), 3.7 (s,1H,OH, D₂O exchangeable); MS m/z (%): 669 (M⁺, 10.0). Anal. calcd. for C₂3H₁₂ClN₂O₂ (669.08): C, 51.26; H, 4.68; N, 7.80; Found C, 51.14; H, 4.55; N, 7.66; S, 11.87.

To a solution of (20 ml acetic acid/ 10 mL acetic anhydride) containing 1 g of sodium acetate, a mixture of compound 17a (5.2 g, 0.01 mole) and chloroacetic acid (0.93 g, 0.01 mole) were added. The reaction mixture was refluxed for 1 h, then (1.4 g, 0.01 mole) of p-chlorobenzaldehyde was added and the mixture refluxed for additional 4 h. The product was poured into ice, then the solid was filtered off and recrystallized from dioxane to give compound 19.

White powder, mp 242-246˚C (dioxane), yield 65%, IR (KBr, cm⁻¹): 1676.8 (CO), 1H NMR (DMSO-d₆): δ (ppm) 1.25-1.95 (m, 10H, 5CH₂), 5.8 (s, 1H, CH pyrimidine), 7.07-7.62 (m, 12H, Ar-H), 8.1(s, 1H, CH methylene); MS m/z (%): 669 (M⁺, 9.76). Anal. calcd. for C₂₅H₂₆BrClN₂O₂ (669.48): C, 55.61; H, 3.61; N, 6.28; Found C, 55.69; H, 3.44; N, 6.11; S, 9.60.

A mixture of compound 19 (6.6g, 0.01 mole) and hydrazine hydrate (0.5 ml, 0.01 mole) in dioxane was refluxed for 3 hrs, then the reaction mixture was concentrated then the solid was filtered off and recrystallized from dioxane to give compound 20.

White fine needle crystals, mp 271-273˚C (dioxane), yield 69%. IR (KBr, cm⁻¹): 3140.39 (NH); ¹H NMR (DMSO-d₆): δ (ppm) 1.25-1.8 (m, 10H, 5CH₂), 3.70 (s, 1H, CH thiazole), 4.95 (s, 1H, CH pyrimidine), 6.9 (s,1H, CH pyrazole) 7.07 (s, 1H, NH, D₂O exchangeable), 7.38-7.62 (m, 12H, Ar-H); MS m/z (%): 682.0 (M⁺, 39.39), Anal. calcd. for C₃₃H₂₆BrClN₂S₂ (683.51): C, 54.47; H, 3.83; N, 10.25; Found C, 54.56; H, 3.71; N, 10.07; S, 9.31.

Serum glucose and triglyceride levels evaluation in hyperglycemic rates

Adult male albino rats, weighing 180-250 g, were used in all experiments of this study. They were obtained from the Animal House Colony of the National Research Centre (Dokki, Giza, Egypt), and were housed under conventional laboratory conditions throughout the period of experimentation. The animals were fed a standard rat pellet diet and allowed free access to water.

Induction of hyperglycemia

Rats were weighed and injected intraperitoneally (I.P.) with alloxan (150 mg/kg) [32] which was purchased from Sigma Aldrich (St. Louis, MO, USA) dissolved in distilled water. After 48 h blood samples were withdrawn from
the retro-orbital venous plexus under light ether anesthesia and the serum was separated by centrifugation for the determination of glucose level. Only rats with serum glucose levels more than 250 mg/dl were selected and considered as hyperglycemic animals that have been used for further experimentation.

Thiazolidin-4-ones was started 48h after STZ injection at which time hyperglycemia was confirmed at dose 0.01mM/kg for 10 consecutive days orally for 10 days. Twenty-four hours after the last dose of either drug treatment, blood samples were withdrawn from the retro-orbital venous plexus from 18 h food-deprived rats which was centrifuged at 3000 rpm for 10 min and the serum was obtained for determination of the serum glucose level and triglycerides level.

**Determination of glucose level**
Glucose level was determined as quinineamine using a test reagent kit (Biodiagnostic, Egypt) according to the reported method [33]. The absorbance was measured at 510 nm and the results were expressed as mg/dl.

**Determination of serum triglyceride level**
Triglycerides were estimated by enzymatic methods by using diagnostic kit (Biodiagnostic, Egypt) according to the reported method [34]. The absorbance was measured at 510 nm and the results were expressed as mg/dl.

**RESULTS AND DISCUSSION**

The starting compounds 1a-d were prepared by condensation of 3-fluoro- or 3-bromophenyl)-spiro-(cyclohexane-1,2'-thiazolidin)-5-one with some aromatic aldehydes namely, 4-chlorobenzaldehyde, 4-methylbenzaldehyde and benzaldehyde in ethanolic NaOH under reflux to give the corresponding arylidene derivatives in good yields [25]. The arylidene of spirothiazolidines 1a-d, containing conjugate exocyclic α,β-unsaturated ketonic function (-CH=CH-CO-), have been used as a component of Michael addition with different amines to yield the novel spiro(cyclohexane-thiazole) derivatives. So, compound 1b was reacted with hydrazine hydrate or phenylhydrazine to give 2a or b, while, compound 1b and c were reacted with thiourea to give 3a and b, respectively. The IR spectrum of compound 2a as an example showed the absence of CO and the presence of 2NH groups. 

\[ ^1H \text{NMR} \text{ spectrum of } 2a \text{ showed absorption band at } \delta \text{ 2.6 ppm CH pyrazole and its mass spectrum showed } M^+ \text{ m/z 381.17.} \]

Compound 2a was alkylated with 2-(2-chloroethoxy)ethanol to afford compound 4. The IR spectrum of compound 4 showed absence of the 2NH groups and the presence of OH group at 3341 cm\(^{-1}\). 

\[ ^1H \text{NMR spectrum of } 4 \text{ showed signals at } \delta \text{ 2.2 (4H, 2CH}_2\text{), 2.37 (s, 3H, CH}_3\text{), 2.56 (s, 1H, CH pyrazole), 3.6 (s, 1H, CH thiazole, bridged hydrogen), 3.75 (4H, 2CH}_2\text{), 5.44 (s, 1H, OH, D}_2\text{O exchangeable), 7.1-7.51 (m, 8H, Ar-H).} \]

Also, when compounds 1b and d were treated with acetoacetanilide they afforded 6-acetyl pyridin-5-one derivatives 5a and b, respectively. The structure of the latter compound 5b was confirmed chemically via condensation with p-chlorobenzaldehyde to afford compound 6 via Claisen-Schmidt’s condensation reaction. The IR spectrum of compound 6 showed the presence of two CO groups at δ 1675.84, 1701 cm\(^{-1}\). 

\[ ^1H \text{NMR spectrum of } 6 \text{ showed signals at } \delta \text{ 6.5, 7.9 (2H, methylene). The mass spectrum showed } M^+ \text{ m/z at 726 (18.62). In addition, } \alpha,\beta\text{-unsaturated ketone 1a and d were used for the synthesis of the corresponding oxirano derivatives 7a and b, respectively by treatment with hydrogen peroxide (30%) in the presence of sodium hydroxide (Scheme 1).} \]
Compounds were $7a,b$ used as key molecules for preparation of different heterocyclic compounds. In general, an epoxide has a considerable ring strain and reacts with a nucleophile to open its ring, the reaction proceeds by an $S_N^2$ mechanism. The nucleophile attacks the least hindered side of the ring carbons (the less substituted end), and the ring oxygen serves as the leaving group. The reaction finishes with the protonation of the negatively charged oxygen. So, compound $7b$ reacted with different amines (nucleophiles) using different conditions to produce compounds $8-10$ via ring opening. $^1$H NMR for compound $8b$ as an example showed at $\delta$ ppm 3.4 (s, 1H, OH, D$_2$O exchangeable), 3.6 (s, 6H, 2NCH$_3$), 4.3 (s, 1H, CH-N) and its mass spectrum showed the molecular ion peak at $m/z$ (%) 508.0 (12.75) (Scheme 2).
Oxirane derivative 7b and hydroxylamine hydrochloride were refluxed in molar ratio 1:1 to afford 4-(4-bromophenyl)-2((4-chlorophenyl)-(hydroxymino)methyl)-2-hydroxy-1-thia-4-azaspiro-[4,5]decan-3-one (11). Its IR spectrum showed C=O at 1674.87 cm⁻¹, NH at 3136.37 cm⁻¹ and OH at 3432.67, 3398 cm⁻¹, its ¹H NMR spectrum showed signals at δ 1.62-1.98 (m, 10H, 5CH₂), 3.66 (s, 1H, OH, D₂O exchangeable), 3.70 (s, 1H, CH-N), 3.73 (s, 1H, OH, D₂O exchangeable), 7.38-7.62 (m, 9H, Ar-H + NH, D₂O exchangeable). Addition of another mole of hydroxylamine hydrochloride to compound 11 afforded the oxadiazine product: 7'-(4-bromophenyl)-4'-(4-chlorophenyl)-3',4',4'a,7'-tetrahydropyrimido[cyclohexane-1,6'-thiazolo [4,5-c][1,2,6]oxadiazin-4'a-ol (12). The IR spectrum of compound 12 showed the absence of the C=O group, and presence of NH group at 3200.17 cm⁻¹ and OH at 3430.80 cm⁻¹ and its ¹H NMR spectrum showed the presence of the oxadiazine ring proton at δ 3.8. Compound 12 also was prepared from compound 7b via addition of 2 moles of hydroxylamine hydrochloride. The T.L.C. and m.p. of the product from the two reactions are similar with no depression in the mixed m.p. Therefore, addition of hydroxylamine to the oxirane ring occurred at first, then addition to the C=O ring to form oxime, finally cyclization took place to form the oxadiazine derivative 12 (Scheme 3).

On the other hand, the reaction of thiourea with compound 7b at room temperature with stirring proceeded at the oxiranyl ring with no cyclization to give 1-((4-(4-bromophenyl)-2-hydroxy-3-oxo-1-thia-4-azaspiro[4,5]decan-2-yl)(4-chlorophenyl)methyl)thiourea (13). In the meantime, when compounds 7a-c were treated with thiourea under reflux in ethanol, oxiranyl ring was opened followed by intramolecular cyclization to form 3'-(4-halophenyl)-7'-(4-halo- or 4-methyl-phenyl)-5'-mercapto-3a,7a'-dihydro-3'H-spiro [cy-clohexane-1,2'- thiazolo [4,5-d] pyrimidin]-7a'-ol (14a-c), respectively (Scheme 4). The IR spectrum of compound 13 showed absorption of the C=O group at 1678.7 cm⁻¹, NH at 3155.94, NH₂ at 3278.39, 3312 and OH at 3440.39 cm⁻¹, its ¹H NMR spectrum showed signals at δ 3.6 (s,1H, NH D₂O exchangeable) and 7.1 (br, 2H, NH₂, D₂O exchangeable). Its mass spectrum showed the molecular ion peak at m/z 540 (25.48%). While the IR spectrum of compound 14a-c as example showed the absence of the (CO) group and the presence of C=S at 1221 cm⁻¹, NH at 3145.89 cm⁻¹ and OH at 3435.56 cm⁻¹. ¹H NMR spectrum of 14a showed signals at δ 3.6 (s, 1H, OH, D₂O exchangeable) and 8.6 (br, 1H, NH D₂O exchangeable). Compound 14a however,
was prepared from compound 7b by refluxing it with thiourea in alkaline medium, or by refluxing compound 13 in alkaline medium. The T.L.C. and m.p. for the product 14a which was prepared by two methods are the same, with no depression in the mixed m.p.

The structure of compound 13 was further confirmed via condensation with \textit{p}-chloro-benzaldehyde to afford 1-((4-(4-bromophenyl)-2-hydroxy-3-oxo-1-thia-4-azaspiro[4.5]decan-2-yl)(4-chlorophenyl)methyl)-3-(4-chlorobenzylidene)thiourea (15) (Scheme 4); its IR spectrum showed the absence of NH$_2$ group, while its $^1$H NMR showed signals at $\delta$ 7.3-7.62 ppm (13H, aromatic + NH, D$_2$O exchangeable), 7.9 (1H, N=CH). Its mass spectrum showed the molecular ion peak at m/z (%) 661(17.88).

Also, compound 7b (0.01 mole) was reacted with 2-(2-chloroethoxy)ethanol (0.01 mole) in the presence of Na$_2$CO$_3$ to give compound 16. On the other hand, compounds 14a-c were reduced by zinc dust to form more active pyrimidine-thione derivatives 17a-c, which were used as key components to prepare different poly-condensed heterocyclic compounds 18-21 (Scheme 4).
They reacted with different halo compounds namely, methyl iodide and chloroacetic acid in presence of p-chlorobenzaldehyde to afford S-methylthiopyrimidine and benzylidene-dithiazolo-pyrimidine derivatives 18a, b and 19 respectively. The IR spectrum of compound 19 as an example showed the presence of CO at 1676.8 cm⁻¹, ¹H NMR showed signal at δ 8.1 (s, 1H, CH methylene). Mass spectrum showed the molecular ion peak +2 at m/z (%) 669 (9.7). Compound 19 was reacted with some amines namely hydrazine hydrate or hydroxylamine to afford condensed heterocyclic compounds, pyrazolothiazole and oxazolothiazole derivatives 20 and 21 respectively (Scheme 5). The ¹H NMR of compound 20 as an example showed signals at δ 4.9 (s, 1H, pyrimidine), 6.9 ppm (s, 1H, pyrazole). The mass spectrum of compound 20 showed the molecular ion peak at m/z (%) 682.6 (39.4). The structure of all new compounds were confirmed by elemental analysis and spectral data. The ring systems of the new novel products 12, 19, 20, 21, are hitherto unknown ring system.
A single i.p. injection of alloxan (150 mg/kg) produced an elevation of serum levels of glucose and triglycerides which were evidenced 48 h after administration which reached nearly about 350% and 850% of the normal value, respectively. Oral treatment of hyperglycaemic rats with compounds 7b, 13, 15, 18b or 21 (0.01 mM/kg/day) for 10 consecutive days caused a marked decrease in the elevated serum level of glucose reaching about 169%, 140%, 151%, 135% and 110% of the normal values, respectively. Similar treatment of hyperglycaemic rats showed a prominent decrease in the serum level of triglycerides reaching about 190%, 344%, 211%, 126% and 147%, respectively.

Oral treatment of hyperglycaemic rats with compounds 19, 20, 10a, 10b or 11 (0.01 mM/kg/day) for 10 consecutive days caused a slight decrease in the elevated serum level of glucose reaching about 265%, 259%, 250%, 211% and 163% of the normal values, respectively. Similar treatment of hyperglycaemic rats showed a decrease in the serum level of triglycerides reaching about 468%, 538%, 609%, 447% and 606, respectively. On the other hand, oral treatment of hyperglycaemic rats with compounds 9b, 9a, 8b, 8a or 14a (0.01 mM/kg/day) for 10 consecutive days did not show any change in the serum levels of glucose and triglycerides.
Table 1: Effect of 3’-(4-halophenyl)-5’-arylidene spiro(cyclohexane-(1,2’)-thiazolidin)-4’-one derivatives on the serum levels of glucose and triglycerides in alloxan-treated rats

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Triglycerides (mg/dl)</th>
<th>Glucose (mg/dl)</th>
</tr>
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<tbody>
<tr>
<td>Control</td>
<td>89.93±5.79</td>
<td>33.54±1.10</td>
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<tr>
<td>Hyperglycaemic</td>
<td>315.18±15.86*</td>
<td>285.24±18.98*</td>
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<td>Rosiglitazone</td>
<td>110.04±10.56@</td>
<td>49.92±1.84@</td>
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<td>7b</td>
<td>152.09±5.17@</td>
<td>64.00±6.12@</td>
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<tr>
<td>8a</td>
<td>319.14±10.40</td>
<td>296.00±24.89</td>
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<tr>
<td>9a</td>
<td>313.91±25.12</td>
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<td>18b</td>
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<td>42.30±1.14@</td>
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<td>180.57±12.66@</td>
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<td>21</td>
<td>98.79±8.74@</td>
<td>49.14±3.82@</td>
</tr>
</tbody>
</table>

Effect of 3’-(4-halophenyl)-5’-arylidene spiro(cyclohexane-(1,2’)-thiazolidin)-4’-one derivatives on serum glucose level in alloxan-treated rats

Rats were rendered hyperglycaemic by a single i.p. injection of alloxan (150 mg/kg). Thiazolidine-4-ones (0.01 mM/kg; p.o.) were administered for 10 consecutive days. Treatment started 48 h after alloxan injection. Blood samples from 18 h food-deprived animals were withdrawn using heparinized capillary tubes and serum was used for glucose determination twenty-four hours after the last dose. Results are expressed as means ±SEM (n =6–10). *Significant difference from normal rats P < 0.05. @ Significant difference from hyperglycaemic rats P < 0.05.
Effect of 3’-(4-halophenyl)-5’-arylidene spiro(cyclohexane-(1,2’)-thiazolidin)-4’-one derivatives on serum triglycerides level in alloxan-treated rats

Rats were rendered hyperglycaemic by a single i.p. injection of alloxan (150 mg/kg). Thiazolidine-4-ones (0.01 mM/kg; p.o.) were administered for 10 consecutive days. Treatment started 48 h after alloxan injection. Blood samples from 18 h food-deprived animals were withdrawn using heparinized capillary tubes and serum was used for triglycerides determination twenty-four hours after the last dose. Results are expressed as means ±SEM (n =6–10). *Significant difference from normal rats P < 0.05. @ Significant difference from hyperglycaemic rats P < 0.05.

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