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Hyperglycedemia 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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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, acetoacetanilide 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 **b** were prepared via reaction of hydrogen peroxide with arylidene spirothiazole derivatives **1a** and **d**. 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.*

Keywords: Spiro(cyclohexane-1,2'-thiazolidine), Spiro(cyclohexane-1,2'-thiazolo-pyridine), anti hyperglycedemia and anti hypertriglyceridemia.

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- inflamatory [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 moitey 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 hyperglyeamic 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 (δ 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 Microanalysis Unit, National Research Centre, and the results were within the accepted range (± 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, υ, cm^{-1}): 3135, 3143 (2 NH); ^{1}H NMR (DMSO-d$_6$): δ (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$_2$Oexchangeable); MS *m/z* (%): 381.17 (M$^+$, 29), 383.16 (M$^+$+2, 9). Anal. calcd. for $C_{22}H_{24}FN_3S$ (381.51): C, 69.26; H, 6.34; N, 11.01; S,8.40. Found, C, 69.30; H, 6.20; N, 10.89; S, 8.37.

6'-(4-Fluorophenyl)-1'-phenyl-3'-*p*-tolyl-1',2',3',6'-tetrahydrospiro[cyclohexane-1,5'-pyrazolo [3,4-*d*]thiazole] (2b).

Yellow crystals, mp 206-208 ˚C (dioxane), yield 70%, IR (KBr, υ, cm^{-1}): 3133 (NH); ^{1}H NMR (DMSO-d$_6$): δ (ppm) 1.50- 2.12 (m, 10H, 5CH$_2$), 2.3 (s, 3H, CH$_3$), 2.7 (s, 1H, CH pyrazole) 7.4-7.8 (m, 13H, Ar-H), 9.2 (s, 1H, NH, D$_2$O exchangeable); MS *m/z* (%): 456.21 (M$^+$, 12), 458.21 (M$^+$+2, 5). Anal. calcd. for $C_{28}H_{28}FN_3S$ (457.61): C, 73.49; H, 6.17; N, 9.18; S, 7.01. Found, C, 73.52; H, 6.09; N, 9.00; S, 6.96.

General procedure for the synthesis of spiro[cyclohexane-1,2'-thiazolo[5,4-*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^{o}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$_6$): δ (ppm) 1.5 -1.98 (m, 10H, 5CH$_2$), 2.6 (s, 3H, CH$_3$), 7.0-7.5 (m, 9H, Ar-H+ SH, D$_2$O exchangeable); MS *m/z* (%): 423 (M$^+$, 14.57), 424 (M$^+$+1, 6.8). Anal. calcd. for $C_{23}H_{22}FN_3S_2$ (423.57): C, 65.22; H, 5.24; N, 9.92; S, 15.14, found, C, 65.20; H, 5.18; N, 9.76; S, 15.10.

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$_6$): δ (ppm) 1.45-2.0 (m, 10H, 5CH$_2$), 7.2-7.7 (m, 10H, Ar-H+ SH, D$_2$O exchangeable); MS *m/z* (%): 409 (M$^+$, 18.5), 410 (M$^+$+1, 6.0). Anal. calcd. for $C_{22}H_{20}FN_3S_2$ (409.54): C, 64.52; H, 4.92; N, 10.19; S, 15.66, found, C, 64.48; H, 4.87; N, 10.26; S, 15.61.

2-(2'-(6'-(4-Fluorophenyl)-3'-*p*-tolyl-3,3a'-dihydrospiro[cyclohexane-1,5'-pyrazolo[3,4-*d*]thia-zole]-2'(6*H*)-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, υ, cm^{-1}): 3341 (OH); ^{1}H NMR (DMSO-d_6): δ (ppm) 1.25-1.95 (m, 10H, $5CH_2$), 2.2 (m, 4H, $2CH_2$-O), 2.37(s, 3H, CH_3), 2.56 (s, 1H, CH pyrazole), 3.6 (s, 1H, CH thiazole, bridged hydrogen), 3.75 (m, 4H, $2CH_2$-O), 5.44 (s, 1H, OH, D_2O exchangeable), 7.1-7.51 (m, 8H, Ar-H). Anal. calcd. For $C_{26}H_{32}FN_3O_2S$ (469.22): C, 66.50; H, 6.87; N, 8.95; S, 6.83, found, C, 66.56; H, 6.78; N, 8.83; S, 6.90.

General procedure for the synthesis of compounds 5a,b
A mixture of compound **1b** or **1d** (0.01 mole) and acetoacetanilide (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. The formed solid was filtered off, dried and recrystallized from dioxane to give compounds **5a**,**b**.

6'-Acetyl-3'-(4-fluorophenyl)-4'-phenyl-7'-*p*-tolyl-3'*H*-spiro[cyclohexane-1,2'-thiazolo[4,5-*b*]pyridin]-5'(4'*H*)-one (5a).
Yellow powder, mp 226-228˚C (dioxane),yield 60%, IR (KBr, υ, cm^{-1}): 1675.7, 1710 (2C=O); ^{1}H NMR (DMSO-d_6): δ (ppm) 1.45 -1.97 (m, 10H, $5CH_2$), 2.4 (s, 3H, CH_3), 3.6 (s, 3H, $COCH_3$), 7.0-7.7 (m, 13H, Ar); ^{13}C NMR: 21.6 (CH_3), 24.2-38 (5 CH_2+CH_3, acetyl), 67.12 (C-spiro), 114.5-139.6 (aromatic carbons), 159.5 (C=O), 179 (O=C-CH_3); MS m/z (%): 524.19 (M^+, 7.4), 526.20 (M^++2, 6.5). Anal. calcd. for $C_{32}H_{29}FN_2O_2S$ (524.65): C, 73.26; H, 5.57; N, 5.34; S, 6.11. Found, C, 73.24; H, 5.48; N, 5.27; S, 6.00.

6'-Acetyl-3'-(4-bromophenyl)-7'-(4-chlorophenyl)-4'-phenyl-3'*H*-spiro[cyclohexane-1,2'-thia-zolo[4,5-*b*]pyridin]-5'(4'*H*)-one (5b).
Pale yellow powder, mp 255-257˚C (dioxane/methanol), yield 65%, IR (KBr, υ, cm^{-1}): 1675.3, 1714 (2C=O); ^{1}H NMR (DMSO-d_6): δ (ppm) 1.49-2.0 (m,10H, $5CH_2$), 3.6 (s, 3H, $COCH_3$), 7.07-7.6 (m, 13H, Ar); MS m/z (%): 604 Anal. calcd. for $C_{31}H_{26}BrClN_2O_2S$ (605.97): C, 61.44; H, 4.32; N, 4.62; $^+$(M^+), 606. (M^++2, 46.5), 607.9 (M^++4, 16). S, 5.29. Found, C, 61.48; H, 4.23; N, 4.53; S, 5.27.

3'-(4-Bromophenyl)-7'-(4-chlorophenyl)-6'-(3-(4-chlorophenyl)acryloyl)-4'-phenyl-3'*H*-spiro-[cyclohexane-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 benzaldehyde (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 coold 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, υ, cm^{-1}): 1675.84, 1701 (2C=O); ^{1}H NMR (DMSO-d_6): δ(ppm) 1.24-1.96 (m, 10H, $5CH_2$), 6.5 (s, 1H, CH), 7.3-7.62 (m, 17H, Ar), 7.95 (s,1H, CH-Ar); MS m/z (%):726 (M^+,18.62), 728 (M^+ +2, 21.78). Anal. calcd. for $C_{38}H_{29}BrCl_2N_2O_2S$ (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-halophenyl)-1-thia-4-azaspiro[4.5]decan-3-one 7a,b.
To a cold mixture of arylidene spiro thiazolidinone **1a** or **1d** (0.01 mole) in acetone: methanol (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 compound **7a** and **b**

2-[3-(4-Chlorophenyl)oxiran-2-yl]-4-fluorophenyl)-1-thia-4-azaspiro[4.5]decan-3-one (7a).
White powder, mp 220-222 ˚C (EtOH), Yield 65 %, IR (KBr, υ, cm^{-1}): 1677 (CO) ; ^{1}H NMR (DMSO-d_6): δ (ppm) 1.43 -2.09 (m, 10H, $5CH_2$), 3.6 (s,1H, CH-Oxirane), 7.1-7.5 (m, 8H, Ar-H); ^{13}CNMR: 24.2-38.9 ($5CH_2$), 67.3 (C-spiro, thiazolidine ne), 71.6 (HC-O, oxirane), 73.1 (C, oxirane), 115.6-134.7 (sp^2 carbons),162.4 (CF), 173.2 (C=O), Anal. calcd. for $C_{21}H_{19}ClFNO_2S$ (403,90): C, 62.45; H, 4.74; N, 3.47; S, 7.94. Found, C, 62.49; H, 4.67; N, 3.39; S, 7.91.

2-[3-(4-Chlorophenyl)oxiran-2-yl]-4-bromophenyl)-1-thia-4-azaspiro[4.5]decan-3-one (7b).
White powder, mp 212-214˚C (EtOH), yield 72%, IR (KBr, υ, cm^{-1}): 1678.8 (CO); ^{1}H NMR (DMSO-d_6): δ (ppm) 1.48-1.99 (m, 10H, $5CH_2$), 3.66 (s,1H, CH-oxirane), 7.0-7.6 (m, 8H, Ar-H); ^{13}CNMR: 24.1-38.5 ($5CH_2$), 67.9 (C-spiro, thiazolone), 72.1 (HC-O, oxirane), 73.15 (C, oxirane), 119.1-135.3 (sp^2 carbons), 164 (CF), 171.6 (C=O), Anal. calcd. for $C_{21}H_{19}BrClNO_2S$ (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, υ, cm^{-1}): 1676.8 (CO), 3461.6 (OH), 3133.48 (NH); ^{1}H NMR (DMSO-d_6): δ (ppm) 1.6-1.97 (m, 10H, $5CH_2$), 3.66 (s,1H, OH, D_2O exchangeable), 3.73 (s, 1H, CH-N), 7.2 -7.72 (m, 13H, Ar-H+ NH, D_2O exchangeable); MS m/z (%):634 (M^+, 10), 636 (M^+ +2 , 20.04), 638 (M^+ +4 , 14), 640 (M^+ +6, 2). Anal. calcd. for $C_{27}H_{25}Br_2ClN_2O_2S$ (636.83) : C, 50.92; H, 3.96; N, 4.40; S, 5.04; 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%, ^{1}H NMR (DMSO-d_6): δ (ppm) 1.45 - 1.9 (m, 10H, $5CH_2$), 3.4 (s, 1H, OH, D_2O exchangeable), 3.6 (s, 6H, 2 CH_3), 4.3 (s, 1H, CH-N), 7.3-7.8 (m, 8H, Ar-H); MS *m/z* (%): 508 (M^+, 12.75), 510 (M^++2, 10.59). Anal. calcd. for $C_{23}H_{26}BrClN_2O_2S$ (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 9a,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 compound **9a** and **b** respectively.

4-(4-Bromophenyl)-2-((4-chlorophenyl)(hydrazinyl)methyl)-2-hydroxy-1-thia-4-azaspiro[4.5]-decan-3-one (9a).
White powder, mp 176-178 ˚C (methanol), yield 62% , IR (KBr, υ, cm^{-1}): 1675.84 (CO), 3212.83 (NH), 3340.11, 3443 (NH_2), 3426.89 (OH); ^{1}H NMR (DMSO-d_6): δ(ppm) 1.58-1.98 (m, 10H, $5CH_2$), 2.7 (s, 2H, NH_2, D_2O exchangeable), 3.66 (s, 1H, OH, D_2O exchangeable), 3.70 (s, 1H, CH-N), 7.38-7.62 (m, 9H, Ar-H + NH, D_2O exchangeable); MS *m/z* (%): 496 (M^++1, 4.78), 497 (M^+ +2, 5.39). Anal. calcd. for $C_{21}H_{23}BrClN_3O_2S$ (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-aza-spiro[4.5]decan-3-one (9b).
Beige fine needle crystals, mp 237-239˚C (dioxane/ methanol), yield 65%, IR (KBr, υ, cm^{-1}): 1675.84 (CO), 3133.48, 3162 (2 NH), 3339.14 (OH); ^{1}H NMR (DMSO-d_6): δ(ppm) 1.55-1.95 (m, 10H, $5CH_2$), 3.4 (s, 1H, NH, D_2O exchangeable), 3.60 (s, 1H, OH, D_2O exchangeable), 3.9 (s, 1H, CH-N) ,7.38-7.62 (m, 14H, Ar-H + NH, D_2O exchangeable); MS *m/z* (%): 571 (M^+ -1, 64.67), Anal. calcd. for $C_{27}H_{27}BrClN_3O_2S$ (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 a**nd **b** respectively.

4-(4-Bromophenyl)-2-((4-chlorophenyl)(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, υ, cm^{-1}): 1675.84 (CO), 3431.71(OH); ^{1}H NMR (DMSO-d_6): δ (ppm) 1.55-2.2 (m, 20H,$10CH_2$), 3.66 (S,1H, OH), 3.70 (s,1H, CH), 7.3 -7.62 (m, 8H, Ar); MS *m/z* (%): 549 (M^+ +1, 11.0). Anal. calcd. for $C_{26}H_{30}BrClN_2O_2S$ (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-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%, ^{1}H NMR (DMSO-d_6): δ (ppm) 1.56-2.1 (m, 18H, 9 CH_2), 3.62 (s, 2H, CH+OH, D_2O exchangeable), 7.0 -7.62 (m, 8H, Ar-H); MS *m/z* (%): 551 (M^+ , 10.6), 553 (M^+ +2, 9.1) Anal. calcd. for $C_{25}H_{28}BrClN_2O_3S$ (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, υ, cm^{-1}):1674.87 (CO), 3136.37 (NH), 3432.67, 3398 (2 OH); ^{1}H NMR($CDCl_3$): δ (ppm) 1.62-1.98 (m, 10H, $5CH_2$), 3.66 (s, 1H, OH, D_2O exchangeable), 3.70 (s, 1H, CH-N), 3.73 (s, 1H, OH, D_2O exchangeable), 7.38-7.62 (m, 9H, Ar-H + NH, D_2O exchangeable); MS *m/z* (%): 496 (M^+, 11.30), 498 (M^++2, 11.59). Anal. calcd. for $C_{21}H_{22}BrClN_2O_3S$ (497.83); C, 50.66 ; H, 4.45; N, 5.63; S, 6.44 Found, C, 50.61; H, 4.31; N, 5.58; S, 6.40.

7'-(4-Bromophenyl)-4'-(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, υ, cm^{-1}): 3200.17 (NH), 3430.80 (OH); ^{1}H NMR ($CDCl_3$): δ (ppm) 1.68-2.08 (m, 10H, $5CH_2$), 3.7 (s, 1H, OH, D_2O exchangeable), 3.8 (s, 1H, CH, oxadiazine), 7.1-7.66 (m, 9H, Ar-H + NH, D_2O exchangeable); MS *m/z* (%): 493 (M^+, 37.39), 495 (M^++2, 23.48). Anal. calcd. for $C_{21}H_{21}BrClN_3O_2S$ (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 hrs , 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, υ, cm^{-1}): 1678.7(CO), 3155.94 (NH), 3278.39, 3312 (NH_2), 3440.39 (OH); ^{1}H NMR (DMSO-d_6): δ (ppm) 1.5-1.95 (m, 10H, $5CH_2$), 3.65 (s, 2H, NH + OH, D_2O exchangeable), 3.80 (s, 1H, CH-N), 7.1 (br, 2H, NH_2, D_2O exchangeable), 7.3-7.65 (m, 8H, Ar-H); MS *m/z* (%): 540 (M^+, 25.48), 542 (M^++2, 9). Anal. calcd. for $C_{22}H_{23}BrClN_3O_2S_2$ (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.
Methode 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 gm 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 **14 a-c**.

Methode B: A solution of compound **13** (5.4 g, 0.01 mole) in ethanol containing 1gm 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, υ, cm^{-1}): 1221 (C=S), 3145.89 (NH), 3435.56 (OH); ^{1}H NMR (DMSO-d_6): δ (ppm) 1.5-1.9 (m, 10H, $5CH_2$), 3.12 (s, 1H, CH thiazole), 3.6 (s, 1H, OH, D_2O exchangeable), 6.95-7.6 (m, 8H, Ar-H), 8.6 (s, 1H, NH, D_2O exchangeable); MS *m/z* (%): 522 (M^+, 21.65), Anal. calcd. for $C_{22}H_{21}BrClN_3OS_2$ (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*]pyrimi-dine]-5'(7a'*H*)-thione (14b).
White fine needle crystals, mp 237-239 ˚C (dioxane), yield 66%, ^{1}H NMR (DMSO-d_6): δ (ppm) 1.6-1.97 (m, 10H,$5CH_2$), 3.3(s, 1H, CH thiazole), 3.7 (s,1H, OH, D_2O exchangeable), 7.2-7.8 (m, 8H, Ar-H), 8.8(s,1H, NH, D_2O exchangeable); MS *m/z* (%): 462 (M^+, 8) Anal. calcd. for $C_{22}H_{21}ClFN_3OS_2$ (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*]pyrimi-dine]-5'(7a'*H*)-thione (14c).
White rectangle crystals, mp 222-224˚C (dioxane), yield 60%, ^{1}H NMR (DMSO-d_6): δ(ppm) 1.51-2.0 (m, 10H, $5CH_2$), 2.6 (s, 3H, CH_3), 3.2 (s, 1H, CH thiazole), 3.6 (s, 1H, OH, D_2O exchangeable), 7.3-7.8 (m, 8H, Ar-H), 8.7(s, 1H, NH, D_2O exchangeable); MS *m/z* (%): 441.5 (M^+, 10.68) Anal. calcd. for $C_{23}H_{24}FN_3OS_2$ (441.58): C, 62.56; H, 5.48; N, 9.52; S, 14.52. Found C, 62.44; H, 5.28; N, 9.44; 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-chloro benzylidene)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,υ,cm^{-1}): 1676.8 (CO), 3440.06 (OH), 3141.07 (NH); ^{1}H NMR (DMSO-d_6): δ(ppm) 1.55-1.98 (m, 10H, $5CH_2$), 3.6 (s,1H, OH, D_2O exchangeable), 3.7 (s,1H, CH-Ar) ,7.3-7.62 (m, 13H, Ar-H+NH, D_2O exchangeable); 7.9 (s,1H, CH, Schiff base); MS m/z (%): 661 (M^+, 17.88), 663 (M^++2, 27.15). Anal. calcd. for $C_{29}H_{26}BrCl_2N_3O_2S_2$ (663.48): C, 52.50; H, 3.95; N,6.33; S,9.67. Found, C, 52.38; H, 3.88; N, 6.20; S, 9.59.

4-(4-Bromophenyl)-2-(1-(4-chlorophenyl)-3-(2-hydroxyethoxy)propyl)-2-hydroxy-1-thia-4-azaspiro[4.5]decan-3-one (16).
To a solution of DMF containing Na_2CO_3, 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, υ, cm^{-1}): 1676.8 (CO), 3418, 3339 (2OH); ^{1}H NMR (DMSO-d_6): δ (ppm) 1.5- 1.95 (m, $10CH_2$, $5CH_2$), 2.1(t, 4H, *J*=2.8 Hz, $2CH_2$),3.2 (t, 1H, *J*=2.6 Hz, CH-Ar), 3.4 (s,1H, OH, D_2O exchangeable), 3.8 (t, 4H, *J*=2.8 Hz, $2CH_2$), 7.1 -7.8 (m, 9H, Ar-H+ OH, D_2O exchangeable); ^{13}CNMR; 24.0-39 ($5CH_2$), 73.5 (C-spiro), 76 ($4CH_2$), 123-133 (aromatic-carbons), 135 (C-N), 137(C-F), 167 (C=O). Anal. calcd. for $C_{25}H_{29}BrClNO_4S$ (554.92): C, 54.11; H, 5.27; N, 2.52; S, 5.78; 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'-hydroxytetrahy-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, υ, cm-1): 1229 (C=S), 3150, 3213 (2 NH), 3435.56 (OH); ^{1}H NMR (DMSO-d_6): δ (ppm) 1.52-1.96 (m, 10H, $5CH_2$), 3.64 (s, 1H, OH, D_2O exchangeable), 4.3 (s,1H, CH, thiazole), 4.6 (s, 1H, CH, primidine), 6.9 (s, 1H, NH, D_2O exchangeable), 7.1-7.6 (m, 9H, Ar-H + NH, D_2O exchangeable); MS *m/z* (%): 522 (M^+, 21.65), Anal. calcd. for $C_{22}H_{23}BrClN_3OS_2$ (524.92): C, 50.34; H, 4.42; N, 8.00; S, 12.22, Found, C, 50.19; H, 4.26; N, 7.89; S, 12.21.

7'-(4-Chlorophenyl)-3'-(4-fluorophenyl)-7a'hydroxytetrahydro-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%, ^{1}H NMR (DMSO-d_6): δ (ppm) 1.55-1.98 (m, 10H, $5CH_2$), 3.5 (s, 1H, OH, D_2O exchangeable), 4.2 (s, 1H, CH, thiazole), 4.7 (s, 1H, CH, primidine), 6.8 (s, 1H, NH, D_2O exchangeable), 6.9-7.6 (m, 9H, Ar-H + NH, D_2O exchangeable); MS *m/z* (%): 463 (M^+, 19), 465 (M^++2, 10.1). Anal. calcd. for $C_{22}H_{23}ClFN_3OS_2$ (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*-tolyltetrahydro-3'*H*-spiro[cyclohexane-1,2'-thiazolo[4,5-*d*]pyrimidine-]-5'(6'*H*)-thione (17c).
White powder, mp 224-226˚C (dioxane/methanole), yield 60%,^{1}H NMR (DMSO-d_6): δ (ppm) 1.49-1.95 (m, 10H, $5CH_2$), 2.6 (s, 3H, CH_3), 3.4 (s,1H, OH, D_2O exchangeable), 4.3 (s,1H, CH, thiazole), 4.5 (s,1H, CH, primidine), 6.9 (s,1H, NH, D_2O exchangeable), 7. 1-7.6 (m, 9H, Ar-H + NH, D_2O exchangeable); MS *m/z* (%): 522 (M^+, 21.65), Anal. calcd. for $C_{23}H_{26}FN_3OS_2$ (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 1g of NaOH a mixture of compound **17a** or **17b** (0.01 mole) and CH_3I (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',7',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,υ,cm^{-1}): 3340.1(OH), 3190 (NH); ^{1}HNMR (DMSO-d_6): δ (ppm) 1.62-1.99 (m,10H, $5CH_2$), 2.1(s, 3H, CH_3), 3.7(s,1H, OH, D_2O exchangeable), 7.38-7.6 (m, 9H, Ar-H+NH, D_2O exchangeable); MS m/z (%): 537 (M^+, 15.92). Anal. calcd. for $C_{23}H_{25}BrClN_3OS_2$ (538.95): C, 51.26; H, 4.68; N, 7.80; S, 11.90, Found, C, 51.14; H, 4.55; N, 7.66; S, 11.87.

7'-(4-Chlorophenyl)-1'-(4-fluorophenyl)-5'-(methylthio)-3a',4',7',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,υ,cm^{-1}): 3453.8 (OH), 3110.6 (NH); ^{1}HNMR (DMSO-d_6): δ (ppm) 1.54-1.9 (m, 10H, $5CH_2$), 2.6 (s, 3H, CH_3), 3.7 (s,1H,OH, D_2O exchangeable), 7.18-7.51 (m, 9H, Ar-H+NH, D_2O exchangeable); MS m/z (%): 477 (M^+, 10.0), Anal. calcd. for $C_{23}H_{25}ClFN_3OS_2$ (478.05) : C, 57.79; H, 5.27; N, 8.79; S, 13.42, Found, C, 57.77; H, 5.09; N, 8.66; S, 13.50

1'-(4-Bromophenyl)-6'-(4-chlorobenzylidene)-9'-(4-chlorophenyl)-6',9'-dihydrospiro[cyclohexane-1,2'-dithiazolo[3,2-a:5',4'-*d*]pyrimidin]-7'(1'*H*)-one (19).

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, υ, cm^{-1}): 1676.8 (CO), ^{1}H NMR (DMSO-d_6): δ (ppm) 1.25-1.95 (m, 10H, $5CH_2$), 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^++2, 9.76); Anal. calcd. for $C_{31}H_{24}BrCl_2N_3OS_2$ (669.48): C, 55.61; H, 3.61; N, 6.28; S, 9.58 Found, C, 55.69; H, 3.44; N, 6.11; S, 9.60.

6'-(4-Bromophenyl)-3',9'-bis(4-chlorophenyl)-3',3'a-dihydro-2'H,9'H-spiro{cyclohexane-1,7'-thiazolo [5',4':4,5]}pyrazolo[3',4':4,5]thiazolo [3,2-a]pyrimidine (20).

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 , 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, υ, cm^{-1}): 3140.39 (NH); ^{1}H NMR (DMSO-d_6): δ (ppm) 1.25-1.8 (m, 10H, $5CH_2$), 3.70 (s, 1H, CH thiazole), 4.95 (s, 1H, CH pyrimidine), 6.9 (s,1H, CH pyrazole) 7.07 (s, 1H, NH, D_2O exchangeable,), 7.38-7.62 (m, 12H, Ar-H); MS *m/z* (%): 682.0 (M^+, 39.39), Anal.calcd. for $C_{31}H_{26}BrCl_2N_5S_2$ (683.51): C, 54.47; H, 3.83; N, 10.25; S, 9.38, Found C, 54.56; H, 3.71; N, 10.07; S, 9.31.

6'-(4-Bromophenyl)-3',9'-bis(4-chlorophenyl)-2',3'-dihydro-9'H-spiro{cyclohexane-1,7'-thiazolo[5',4':4,5]} isoxazolo [5',4':4,5] thiazolo [3,2-a]pyrimidine] (21).

A mixture of compound **19** (6.6 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 was poured into water and neutralized with diluted HCl then the solid was filtered off and recrystallized from dioxane to give compound **21.** White powder, mp 285-287˚C (dioxane), yield 70%, IR (KBr, υ, cm^{-1}): 3133.48 (NH); ^{1}H NMR (DMSO-d_6): δ (ppm) 1.25-1.8 (m, 10H, $5CH_2$), 4.6 (s, 1H, CH pyrimidine), 6.2 (s, 1H, CH oxazole), 7.0 (s, 1H, NH, D_2O exchangeable), 7.09-7.60 (m, 12H, Ar-H); MS *m/z* (%): 682.1 (M^+, 71.31), 684.1(M^++2, 57.38); Anal. calcd. for $C_{31}H_{25}BrCl_2N_4OS_2$ (684.50): C, 54.39; H, 3.68; N, 8.19; S, 9.37, Found, C, 54.44; H, 3.53; N, 8.00; S, 9.45.

Serum glucose and trigyceride 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.

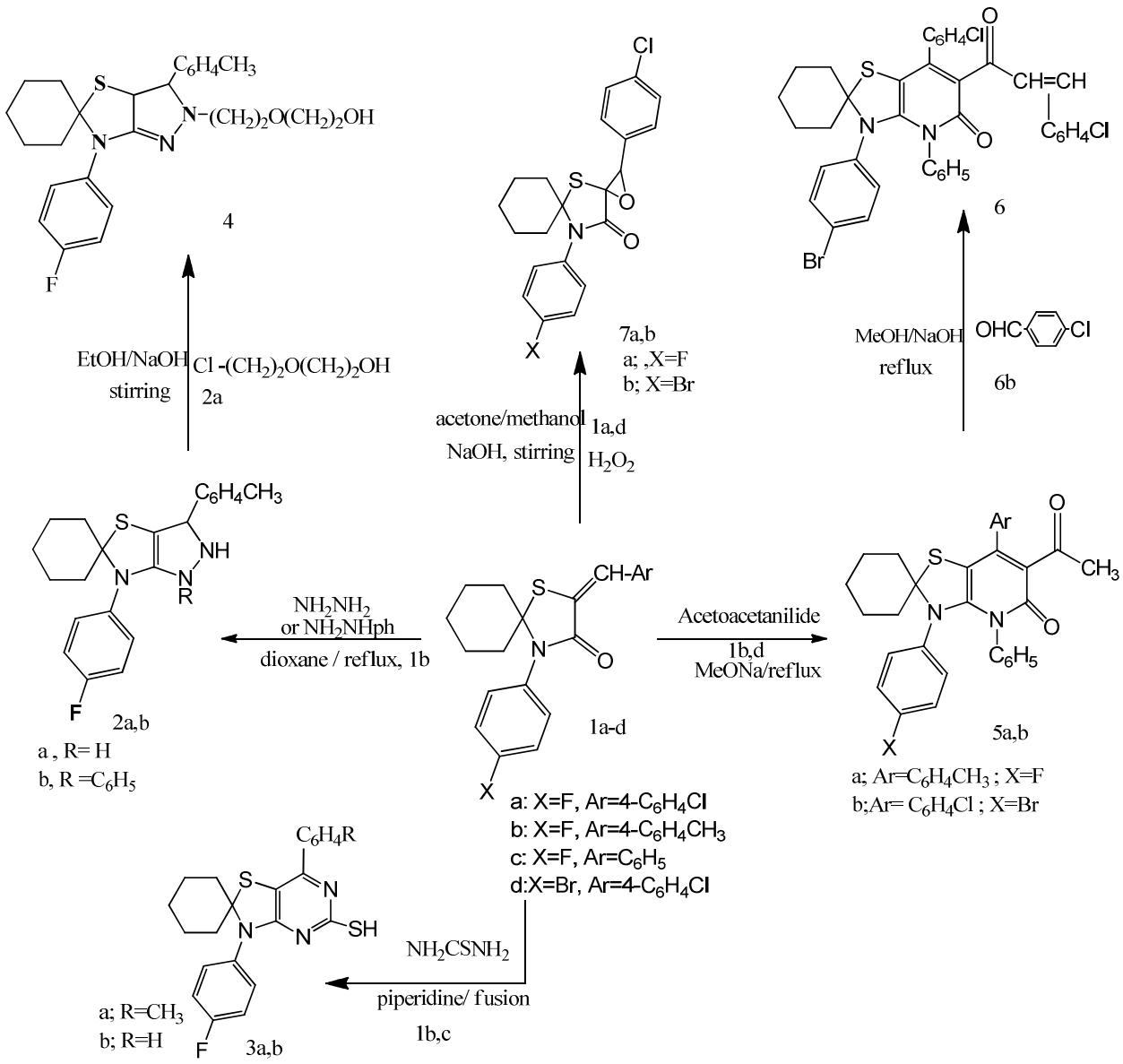
Determination of serum triglyceride level

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

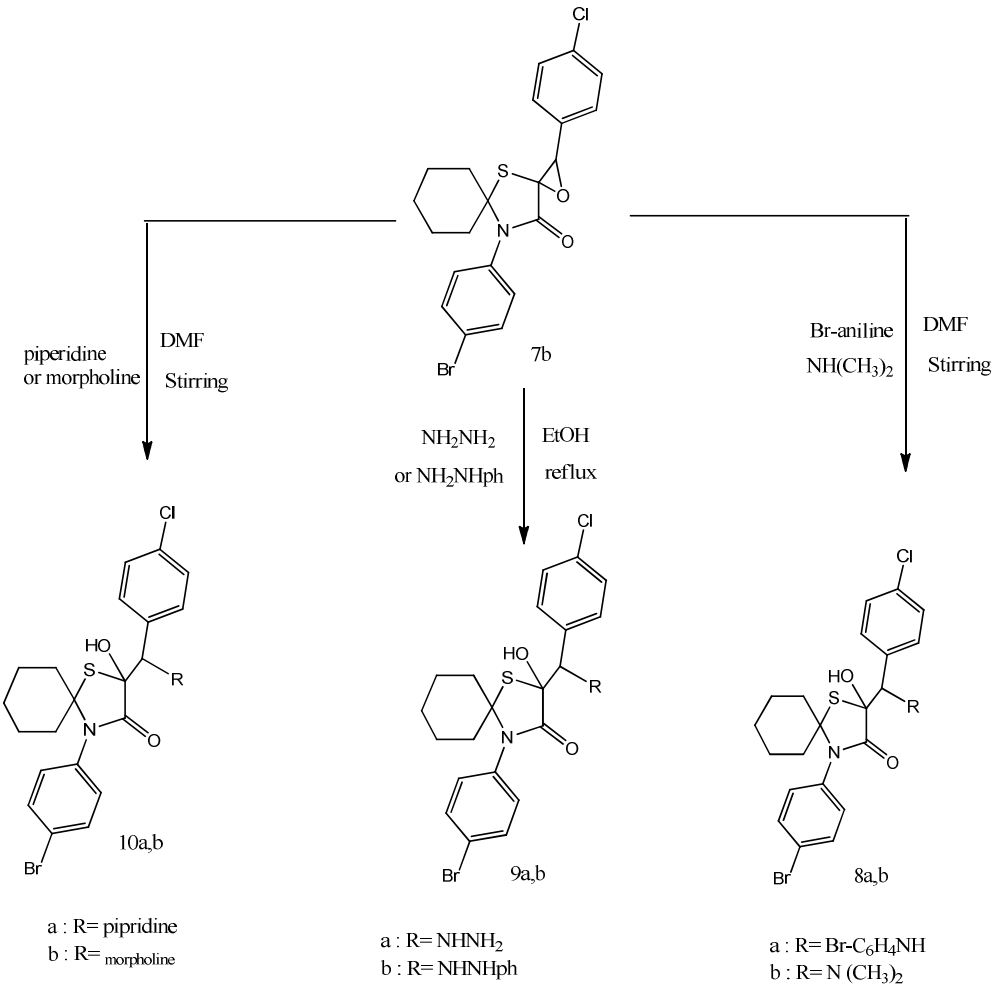
The starting compounds **1a-d** were prepared by condensation of 3-fluoro- or 3-bromophenyl)-spiro-(cyclohexane-1,2'-thiazolidin)-5-one with some aromatic aldehydes namely, 4-chlorobenzal-dehyde, 4-methylbenzaldehyde and benzaldehyde in ethanolic NaOH under reflux to give the corresponding arylidene 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. ^{1}H NMR showed absorption band at δ 2.6 ppm CH pyrazole and its mass spectrum showed Ms 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}. ^{1}H NMR spectrum of **4** showed signals at δ 2.2 (4H, 2CH_2), 2.37 (s, 3H, CH_3), 2.56 (s, 1H, CH pyrazole), 3.6 (s, 1H, CH thiazole, bridged hydrogen), 3.75 (4H, 2CH_2), 5.44 (s, 1H, OH, D_2O 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.$^{-1}$ ^{1}H NMR spectrum of **6** showed signals at δ 6.5, 7.9 (2H, methylene). The mass spectrum showed M$^+$ *m/z* at 726 (18.62). In addition, α,β-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).



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_N2 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 (nucleophilis) using different conditions to produce compounds **8-10** *via* ring opening. ^{1}H NMR for compound **8b** as an example showed at ᵟ ppm 3.4 (s, 1H, OH, D_2O 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).

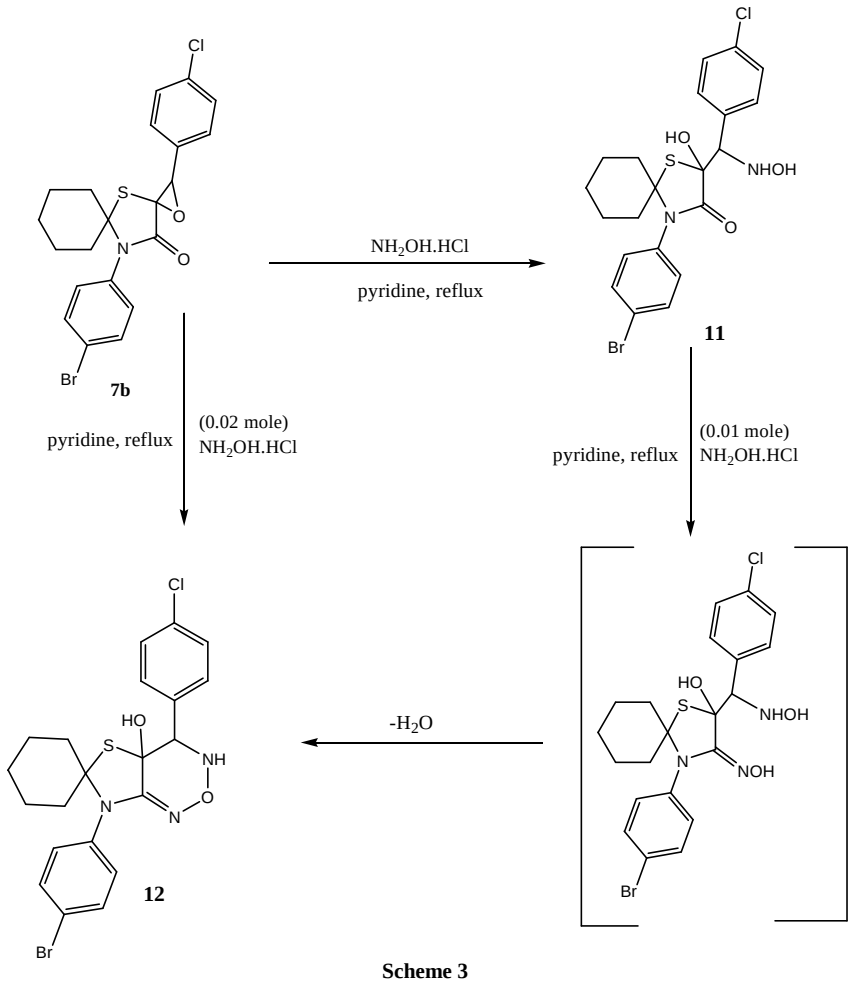


Scheme 2

Oxirane derivative **7b** and hydroxylamine hydrochloride were refluxed in molar ratio 1:1 to afford 4-(4-bromophenyl)-2((4chlorophenyl)-(hydroxyamino)methyl)-2-hydroxy-1-thia-4-azaspiro-[4.5]decan-3-one (**11**). Its IR spectrum showed C=O at 1674.87cm^{-1}, NH at 3136.37 cm^{-1} and OH at 3432.67, 3398 cm^{-1}, its ^{1}H NMR spectrum showed signals at δ 1.62-1.98 (m, 10H, 5CH_2), 3.66 (s, 1H, OH, D_2O exchangeable), 3.70 (s, 1H, CH-N), 3.73 (s, 1H, OH, D_2O exchangeable),7.38-7.62 (m, 9H, Ar-H + NH, D_2O exchangeable). Addition of another mole of hydroxylamine hydrochloride to compound **11** afforded the oxadiazine product: 7'-(4-bromophenyl)-4'-(4-chlorophenyl)-3',4',4a',7'-tetrahydrospiro[cyclohexane-1,6'-thiazolo [4,5-*c*][1,2,6]oxadiazin]-4a'-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 ^{1}H 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, NH_2 at 3278.39, 3312 and OH at 3440.39 cm^{-1}, its ^{1}H NMR spectrum showed signals at δ 3.6 (s,1H, NH D_2O exchangeable) and 7.1 (br, 2H, NH_2, D_2O exchangeable). Its mass spectrum showed the molecular ion peak at *m/z* 540 (25.48%). While The IR spectrum of compound **14a** as example showed the absence of the (CO) group and the presense of C=S at 1221 cm^{-1}, NH at 3145.89 cm^{-1} and OH at 3435.56 cm^{-1}. ^{1}H NMR spectrum of **14a** showed signals at δ 3.6 (s, 1H, OH, D_2O exchangeable) and 8.6 (br, 1H, NH, D_2O exchangeable). Compound **14a** however,

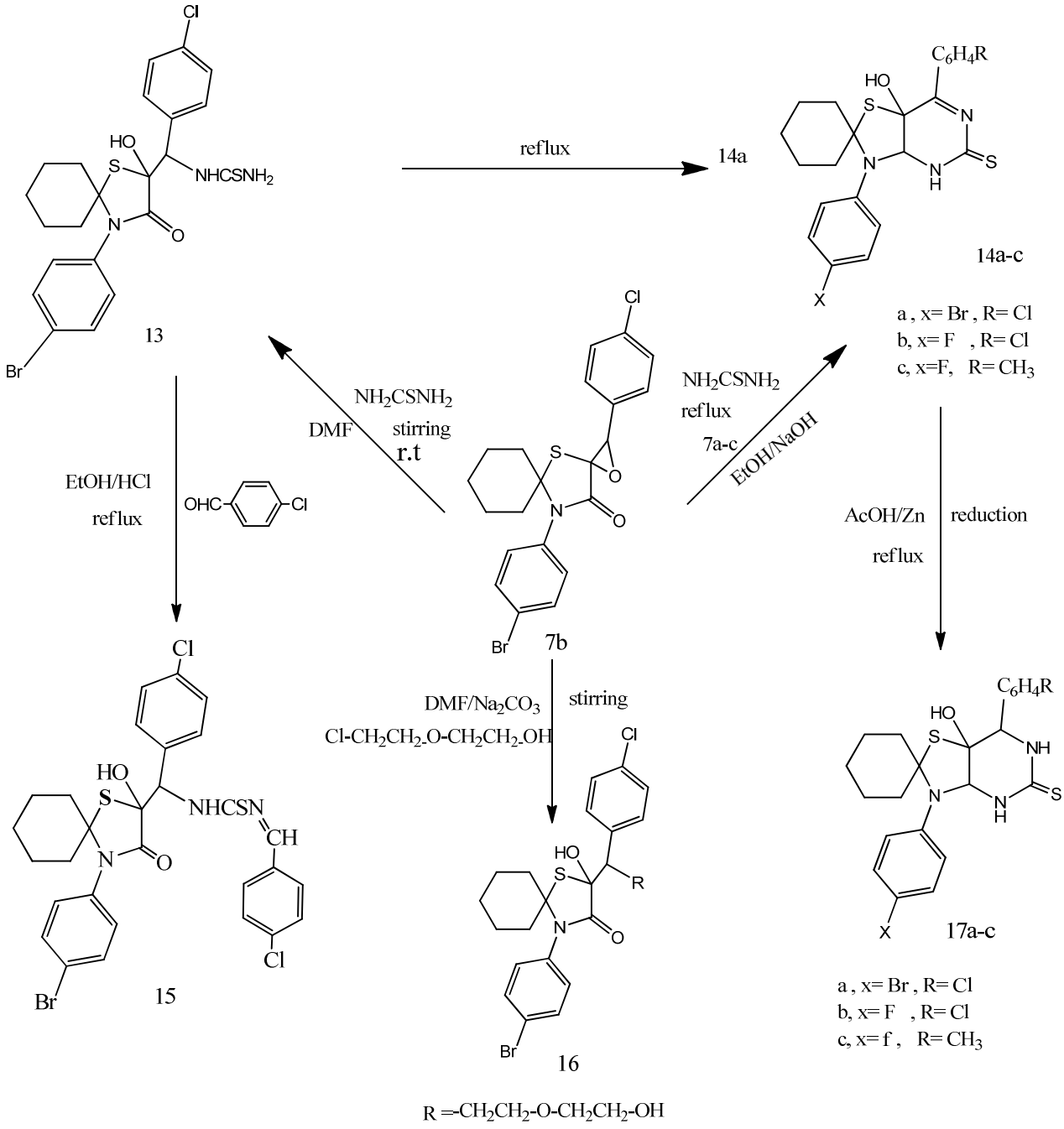
was prepared from compound **7b** by refluxing it with thiourea in alkaline medium, or by refluxing compound **13** in alkaline medium. The T.L.C. and m.p. for the product **14a** which was prepared by two methods are the same, with no depression in the mixed m.p.



Scheme 3

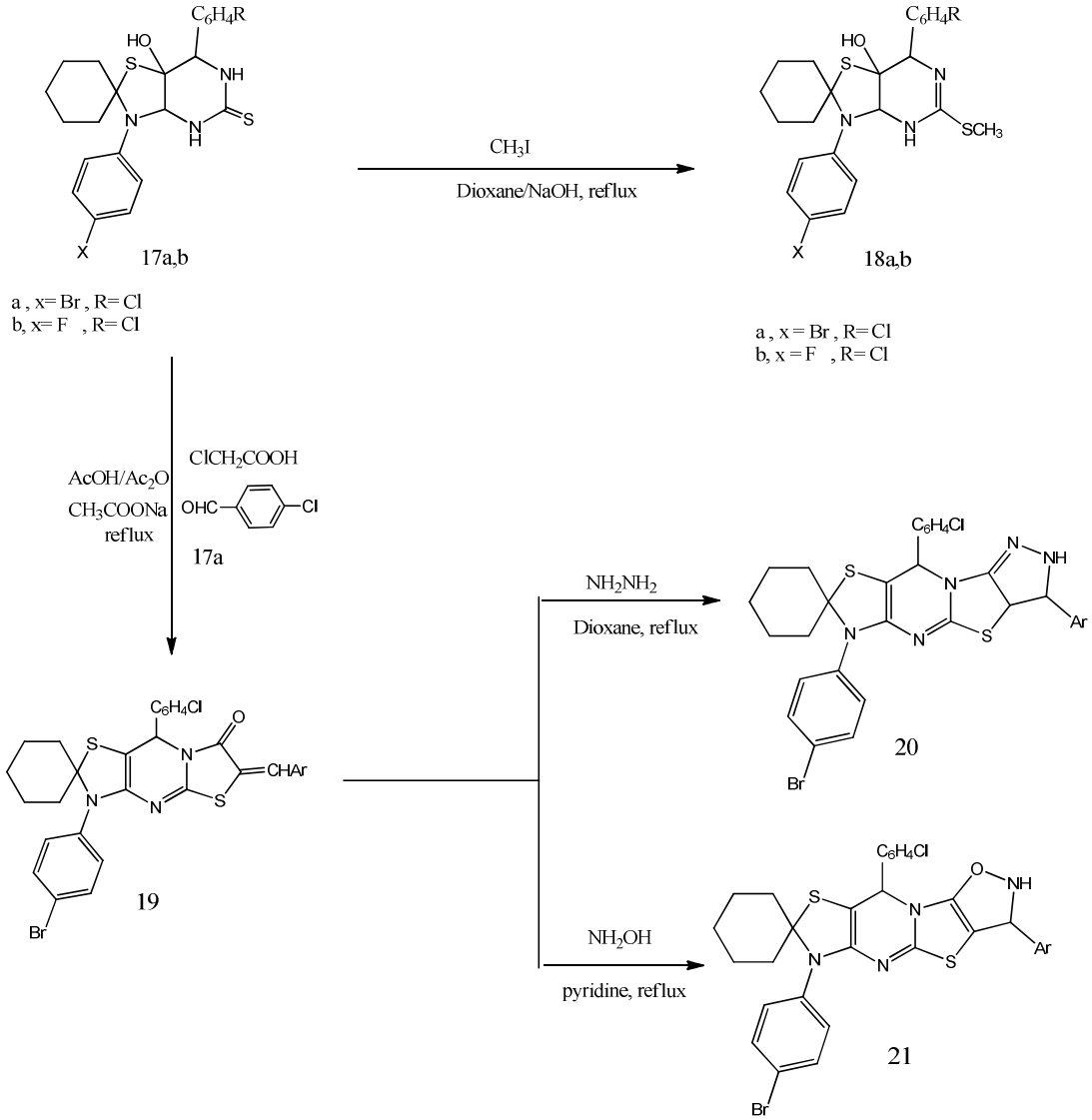
The structure of compound **13** was further confirmed *via* condensation with *p*-chloro-benzaldehyde to afford 1-((4-(4-bromophenyl)-2-hydroxy-3-oxo-1-thia-4-azaspiro[4.5]decan-2-yl)(4-chlorophenyl)methyl)-3-(4-chloro benzylidene)thiourea (**15**) (Scheme 4); its IR spectrum showed the absence of NH_2 group, while its ^{1}H NMR showed signals at δ 7.3-7.62 ppm (13H, aromatic + NH, D_2O 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_2CO_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**).



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.



Scheme 5

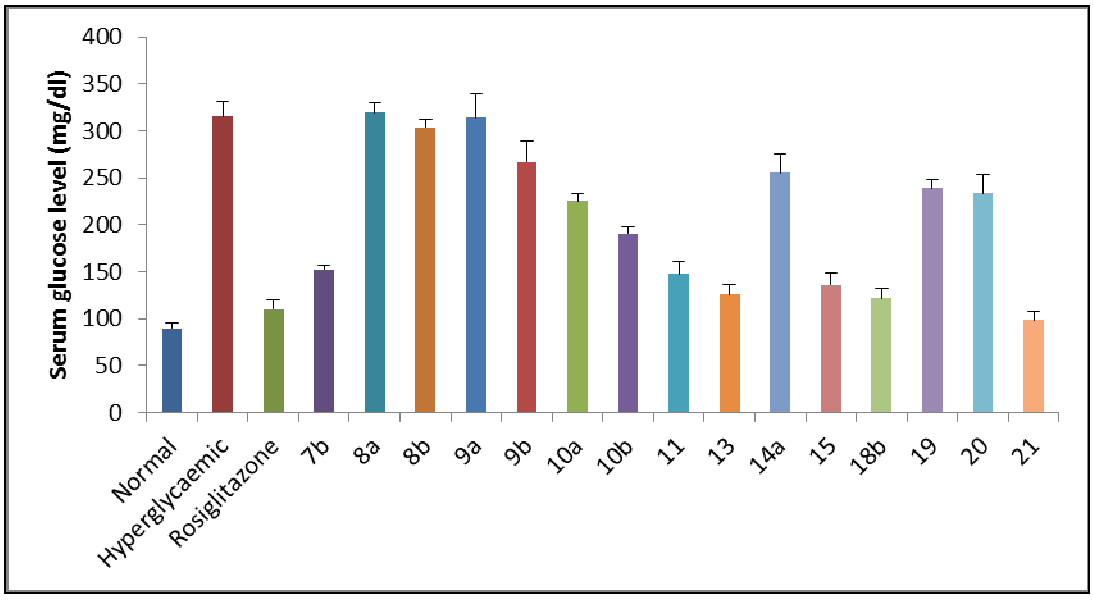
RESULTS

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.

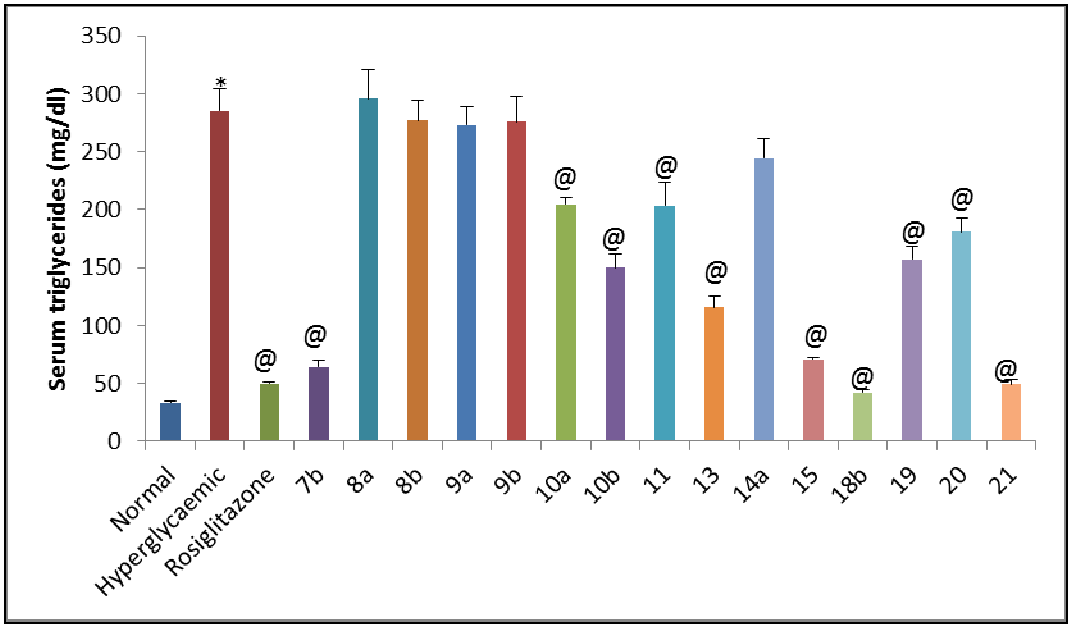
Table 1: Effect of 3'-(4-halophenyl)-5'-arylidene spiro(cyclohexane-(1,2')-thiazolidin)-4'-one derivatives on the serum levels of glucose and triglycerides in in alloxan-treated rats

	Triglycerides	Glucose
Control	**89.93±5.79**	**33.54±1.10**
Hyperglycaemic	**315.18±15.86***	**285.24±18.98***
Rosiglitazone	**110.04±10.56@**	**49.92±1.84@**
Compounds		
7b	**152.09±5.17@**	**64.00±6.12@**
8a	**319.14±10.40**	**296**.00±**24.89**
8b	**303.58±9.04**	**277.14±16.12**
9a	**313.91±25.12**	272.85±**15.59**
9b	**266.85±21.64**	**276.00±21.33**
10a	**225.08±8.57@**	**204.57±5.67@**
10b	**190.29±7.04@**	**150.00±12.01@**
11	147.46±**13.17@**	**203.42±20.10@**
13	**125.87±10.94@**	**115.42±10.23@**
14a	**255.37±20.12**	**244.57±16.70**
15	**136.04±13.07@**	**70.85±0.93@**
18b	121.78±**10.46@**	**42.28±2.14@**
19	**238.71±10.08@**	**157.02±10.82@**
20	233.60±**20.42@**	180.57±**12.66@**
21	**98.792±8.74@**	**49.14±3.82@**



Effect of 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 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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