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Article

Synthesis, Anti-Diabetic and Renoprotective Activity of Some New Benzazole, Thiazolidin-4-one and Azetidin-2-one Derivatives

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A new series of benzazole, thiazolidinone and azetidin-2-one derivatives incorporating to pyrazole moiety were synthesized by condensation of 1,3-diphenyl-1*H*-pyrazol-4-carboxaldehyde with different nucleophiles. The structures of the newly synthesized compounds were confirmed by analytical and spectral methods. Some of these compounds were evaluated for their anti-hyperglycemic and renoprotective activities. Three compounds showed remarkable anti-diabetic potency, whereas other five compounds showed significant renoprotective activity.

Keywords: anti-diabetic, renoprotective, pyrazole, thiazolidine-4-one, azetidine-2-one

Introduction

Diabetes mellitus is increasing in prevalence worldwide, and its complication has grown into a public health problem. It is a condition characterized by both insulin resistance (poor tissue insulin sensitivity) and impaired insulin secretion from pancreatic β-cells.[1] Thiazolidine-4-one ring (Figure 1A) system has appeared as a significant heterocyclic system due to its extensive range of pharmacological activities, and synthetic applications for anti-hyperglycemic agents.[2-4] Thiazolidinediones[5-9] or glitazones, ligands of the peroxisome-proliferator-activated receptor-γ (PPAR-γ), are kind of anti-hyperglycemic agents that reduce insulin resistance and improve insulin action, thereby keeping normoglycemia and potentially preserving β-cell function.[10] Recent data suggest that they might also have therapeutic potential in the treatment of inflammatory diseases and certain cancers.[11] There is increasing evidence that inflammation is closely involved in the pathogenesis of diabetes and associated complications.[12,13] Therefore, drugs with anti-inflammatory properties such as thiazolidinediones can possibly decrease the risk of developing diabetes and diabetes-induced problems.[14-16]

Moreover, inflammation and proinflammatory cytokines have been found to play a critical role in the development of microvascular diabetic complications, including nephropathy, the main cause of diabetes-induced renal failure.[17] This pathogenic perception necessitates new therapeutic considerations and new therapeutic goals for the treatment of diabetes and its complications. Azetidin-2-ones (Figure 1B) have been reported as having a potential anti-inflammatory effect, as well as analgesic and anti-tuberculosis activities.[18,19]

On the other hand, pyrazole derivatives are an important class of heterocyclic compounds. Much attention has been paid to compounds containing pyrazole ring (Figure 1C) due to their biological activities, including anti-inflammatory, anti-depressant, anti-influenza and anti-cancer activities.[20-24]

A pharmacophore hybridization method for the synthesis of novel bioactive molecules is an efficient strategy, and it is being used in current medicinal chemistry (Figure 1).

Hybridization of two diverse bioactive compounds with complementary pharmacophoric purposes or with different mechanisms of action frequently gives synergistic effects.[25,26] In sight of the above mentioned facts and in extension of our interest in the synthesis of heterocycles bearing pyrazole and/or thiazolidine moieties,[27-30] to discover new candidates that may be of importance in designing novel, active, selective and less toxic anti-diabetic and renoprotective drugs, we present herein the synthesis and pharmacological evaluation of some novel structural hybrids containing both the pyrazole moiety with thiazolidin-4-one and azetidin-2-one ring systems during various linkages. This pattern was suggested in an attempt to investigate the influence of such hybridization and structural variation on the anti-hyperglycemic and

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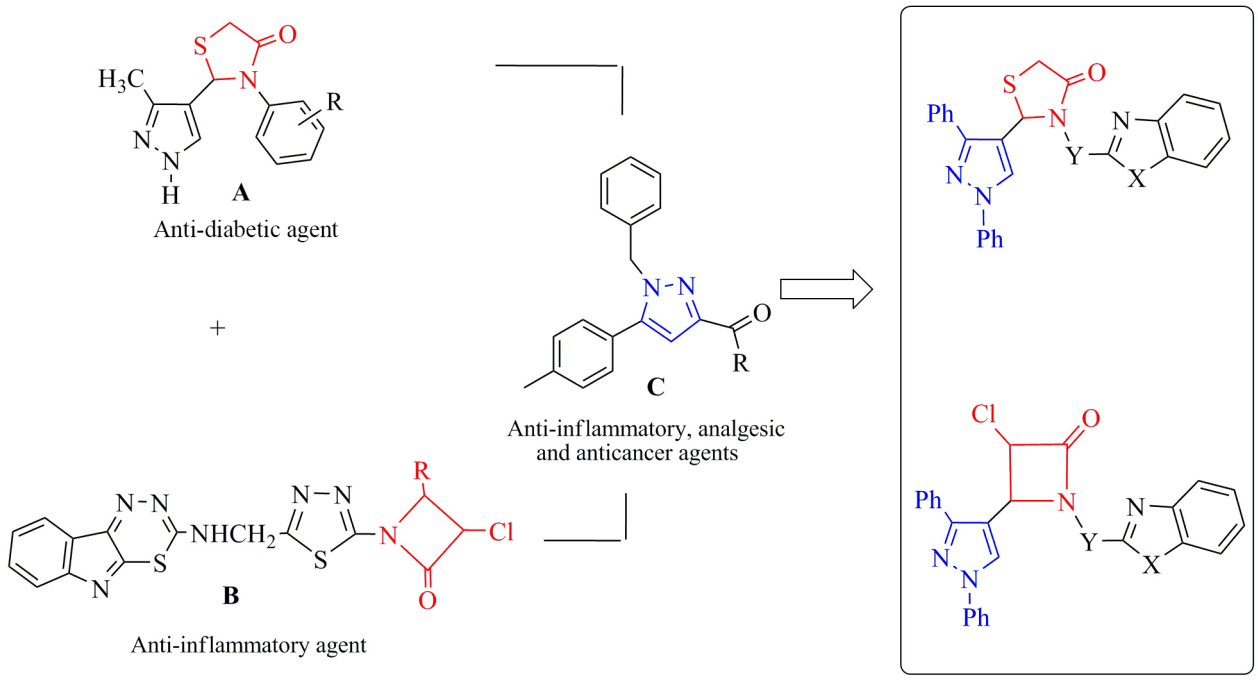


Figure 1. The pharmacophore hybridization process. (A) Thiazolidine-4-one ring; (B) azetidin-2-one; (C) pyrazole ring.

renoprotective activities, hoping to add some synergistic biological significance to the target molecules.

Experimental

All chemicals and reagents used in current study were of analytical grade. Melting points were determined using APP. Digital ST 15 melting point apparatus and are uncorrected. Fourier transfrom infrared (FTIR) spectra were recorded on a Pye-Unicam SP3-100 spectrophotometer in KBr pellet. ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded on AVANCE-III (400 MHz) High Performance FT-NMR Spectrometer (Bruker, Switzerland), JEOL JNM-LA 400 and some ^{1}H NMR spectra were recorded on Varian EM-360L NMR Spectrophotometer (90 MHz) (USA) in $CDCl_3$, DMSO-d_6 as solvent. Chemical shifts (δ) are reported in ppm and the coupling constants (J) are reported in hertz. C, H, N and S analyses were performed with a Vario EL C, H, N, S Analyzer.

General procedure for the synthesis of hydrazide derivatives **3a**,**b**, **7a**,**b**, and **11a**,**b**

A mixture of 1,3-diphenyl-1*H*-pyrazol-4-carboxaldehyde (**1**) (0.74 g, 3 mmol), the corresponding hydrazine (**2a**,**b**, **6a**,**b** and **10a**,**b**) (3 mmol), and 2-3 drops glacial acetic acid in ethanol (20 mL) was refluxed on a water bath for about 5 h. The solvent was removed and the residue was recrystallized from the suitable solvent.

2-(2-((1,3-Diphenyl-1*H*-pyrazol-4-yl)methylene)hydrazinyl)-1*H*-benzimidazole (**3a**)

Yield: 70% (ethanol, brown powder); mp 196-197 °C; IR (KBr) ν / cm^{-1} 3414, 3118, 3017, 1637; ^{1}H NMR (90 MHz, DMSO-d_6) δ 6.40 (s, 1H, CH=N), 7.00-8.00 (m, 14H, Ar-H), 8.20 (s, 1H, NH, D_2O exchanged), 8.35 (s, 1H, pyrazole-H), 9.50 (s, 1H, benzimidazole-NH, D_2O exchanged). Anal. calcd. for $C_{23}H_{18}N_6$ (378.43): C, 73.00; H, 4.79; N, 22.21. Found: C, 72.91; H, 4.70; N, 22.13.

2-(2-((1,3-Diphenyl-1*H*-pyrazol-4-yl)methylene)hydrazinyl) benzoxazole (**3b**)

Yield: 71% (ethanol, pale orange crystals); mp 189-190 °C; IR (KBr) ν / cm^{-1} 3409, 3052, 1638; ^{1}H NMR (90 MHz, DMSO-d_6) δ 6.38 (s, 1H, CH=N), 7.20-8.12 (m, 14H, Ar-H), 8.23 (s, 1H, NH, D_2O exchanged), 8.30 (s, 1H, pyrazole-H). Anal. calcd. for $C_{23}H_{17}N_5O$ (379.41): C, 72.81; H, 4.52; N, 18.46. Found: C, 72.73; H, 4.44; N, 18.38.

2-((1,3-Diphenyl-1'*H*-pyrazol-4-yl)methylidene-hydrazino-acetyl)mercaptobenzimidazole (**7a**)

Yield: 72% (toluene, orange crystals); mp 179-180 °C; IR (KBr) ν / cm^{-1} 3379, 3157, 3049, 1677, 1637; ^{1}H NMR (90 MHz, DMSO-d_6) δ 4.35 (s, 2H, CH_2), 6.35 (s, 1H, CH=N), 7.15-8.10 (m, 14H, Ar-H), 8.25 (s, 1H, NH, D_2O exchanged), 8.35 (s, 1H, pyrazole-H), 9.48 (s, 1H, benzimidazole-NH, D_2O exchanged). Anal. calcd. for

$C_{25}H_{20}N_6OS$ (452.53): C, 66.35; H, 4.45; N, 18.57; S, 7.09. Found: C, 66.28; H, 4.38; N, 18.50; S, 6.99.

2-((1,3-Diphenyl-1'*H*-pyrazol-4-yl)methylidene-hydrazino-acetyl) mercaptobenzoxazole (**7b**)

Yield: 69% (ethanol, pale brown crystals); mp 194-195 °C; IR (KBr) ν / cm^{-1} 3397, 3051, 1671, 1636; ^1H NMR (90 MHz, DMSO-d_6) δ 4.37 (s, 2H, CH_2), 6.38 (s, 1H, CH=N), 7.10-7.90 (m, 14H, Ar-H), 8.20 (s, 1H, NH, D_2O exchanged), 8.35 (s, 1H, pyrazole-H). Anal. calcd. for $C_{25}H_{19}N_5O_2S$ (453.52): C, 66.21; H, 4.22; N, 15.44; S, 7.07. Found: C, 66.13; H, 4.14; N, 15.38; S, 7.00.

(Benzimidazol-2-ylsulfanyl) acetic acid ((1,3-diphenyl-1'*H*-pyrazol-4-yl) methylidene) hydrazide (**11a**)

Yield 78% (toluene, white powder); mp 202-203 °C; IR (KBr) ν / cm^{-1} 3396, 3157, 3096, 1687, 1639; ^1H NMR (90 MHz, DMSO-d_6) δ 4.55 (s, 2H, CH_2), 6.31 (s, 1H, CH=N), 7.10-7.99 (m, 14H, Ar-H), 8.15 (s, 1H, NH, D_2O exchanged), 8.33 (s, 1H, pyrazole-H), 9.50 (s, 1H, benzimidazole-NH, D_2O exchanged). Anal. calcd. for $C_{25}H_{20}N_6OS$ (452.53): C, 66.35; H, 4.45; N, 18.57; S, 7.09. Found: C, 66.26; H, 4.36; N, 18.50; S, 7.01.

(Benzoxazol-2-ylsulfanyl) acetic acid ((1,3-diphenyl-1'*H*-pyrazol-4-yl) methylidene) hydrazide (**11b**)

Yield 79% (toluene, white powder); mp 190-191 °C; IR (KBr) ν / cm^{-1} 3395, 3050, 1675, 1635; ^1H NMR (90 MHz, DMSO-d_6) δ 4.53 (s, 2H, CH_2), 6.38 (s, 1H, CH=N), 7.13-8.00 (m, 14H, Ar-H), 8.11 (s, 1H, NH, D_2O exchanged), 8.36 (s, 1H, pyrazole-H). Anal. calcd. for $C_{25}H_{19}N_5O_2S$ (453.52): C, 66.21; H, 4.22; N, 15.44; S, 7.07. Found: C, 66.13; H, 4.13; N, 15.36; S, 6.96.

General procedure for the synthesis of thiazolidin-5-ones **4a**,**b**, **8a**,**b**, and **12a**,**b**

To a solution of the hydrazide (**3a**,**b**, **7a**,**b**, or **11a**,**b**) (3 mmol) in DMF (20 mL) were added thioglycolic acid (0.27 mL, 3 mmol) and $ZnCl_2$ (0.3 g) and the reaction mixture was refluxed for 8 h, cooled and poured into crushed ice, the solid precipitate was filtered and washed with sodium bicarbonate (10%). The product was dried and recrystallized from the proper solvent.

2-[2'-(1,3-Diphenyl-1*H*-pyrazol-4-yl)thiazolidine-5'-one] hydrazinobenzimidazole (**4a**)

Yield: 68% (ethanol, reddish brown powder); mp 230-231 °C; IR (KBr) ν / cm^{-1} 3427, 3237, 3037, 1676; ^1H NMR (400 MHz, DMSO-d_6) δ 3.74 (s, 2H, thiazolidine-CH_2), 5.14 (s, 1H, CH), 7.19-7.99 (m, 14H, Ar-H), 8.29 (s, 1H, pyrazole-H), 8.58 (s, 1H, NH, D_2O exchanged), 9.50 (s, 1H, benzimidazole-NH, D_2O exchanged). Anal. calcd. for $C_{25}H_{20}N_6OS$ (452.53): C, 66.35; H, 4.45; N, 18.57; S, 7.09. Found: C, 66.27; H, 4.37; N, 18.50; S, 7.00.

2-[2'-(1,3-Diphenyl-1*H*-pyrazol-4-yl)thiazolidine-5'-one] hydrazinobenzoxazole (**4b**)

Yield 66% (ethanol, brown powder); mp 241-242 °C; IR (KBr) ν / cm^{-1} 3415, 3025, 1680; ^1H NMR (90 MHz, DMSO-d_6) δ 3.65 (s, 2H, thiazolidine-CH_2), 5.10 (s, 1H, CH), 7.05-8.10 (m, 14H, Ar-H), 8.30 (s, 1H, pyrazole-H), 8.50 (s, 1H, NH, D_2O exchanged). Anal. calcd. for $C_{25}H_{19}N_5O_2S$ (453.52): C, 66.21; H, 4.22; N, 15.44; S, 7.07. Found: C, 66.14; H, 4.12; N, 15.36; S, 7.00.

2-[2'-(2-(1,3-Diphenyl-1*H*-pyrazol-4-yl)thiazolidine-5-one) acetylaminomercapto] benzimidazole (**8a**)

Yield 70% (dioxane, brown powder); mp 235-236 °C; IR (KBr) ν / cm^{-1} 3414, 3181, 1678, 1648; ^1H NMR (400 MHz, DMSO-d_6) δ 3.77 (s, 2H, thiazolidine-CH_2), 4.13 (s, 2H, $COCH_2$), 5.01 (s, 1H, thiazolidine-CH), 7.15-7.99 (m, 14H, Ar-H), 8.30 (s, 1H, pyrazole-H), 8.45 (s, 1H, NH, D_2O exchanged), 9.55 (s, 1H, benzimidazole-NH, D_2O exchanged). Anal. calcd. for $C_{27}H_{22}N_6O_2S_2$ (526.63): C, 61.58; H, 4.21; N, 15.96; S, 12.18. Found: C, 61.50; H, 4.12; N, 15.90; S, 12.10.

2-[2'-(2-(1,3-Diphenyl-1*H*-pyrazol-4-yl) thiazolidine-5-one) acetylaminomercapto] benzoxazole (**8b**)

Yield: 67% (dioxane, brown powder); mp 205-206 °C; IR (KBr) ν / cm^{-1} 3410, 3020, 1670, 1643; ^1H NMR (400 MHz, $CDCl_3$) δ 3.71 (s, 2H, thiazolidine-CH_2), 4.08 (s, 2H, $COCH_2$), 5.00 (s, 1H, thiazolidine-CH), 7.25-8.12 (m, 14H, Ar-H), 8.33 (s, 1H, pyrazole-H), 8.43 (s, 1H, NH, D_2O exchanged); ^{13}C NMR (100 MHz, $CDCl_3$) δ 37.4, 45.3, 51.3, 119.0, 121.8, 122.1, 125.3, 128.6, 131.3, 132.4, 135.3, 136.7, 139.8, 140.1, 141.5, 151.6, 152.9, 163.6, 174.3. Anal. calcd. for $C_{27}H_{21}N_5O_3S_2$ (527.62): C, 61.46; H, 4.01; N, 13.27; S, 12.15. Found: C, 61.40; H, 3.93; N, 13.20; S, 12.09.

1-[(Benzimidazol-2"-yl)thioacetamidyl]-2-(1',3'-diphenyl-1'*H*-pyrazol-4'-yl) thiazolidin-5-one (**12a**)

Yield: 68% (toluene, white crystals); mp 230-231 °C; IR (KBr) ν / cm^{-1} 3415, 3215, 3019, 1670, 1650; ^1H NMR (90 MHz, DMSO-d_6) δ 3.99 (s, 2H, thiazolidine-CH_2), 4.30 (s, 2H, CH_2), 5.12 (s, 1H, thiazolidine-CH), 7.05-7.99 (m, 15H, Ar-H and pyrazole-H), 8.92 (s, 1H, NH, D_2O exchanged), 9.50 (s, 1H, benzimidazole-NH, D_2O exchanged). Anal. calcd. for $C_{27}H_{22}N_6O_2S_2$ (526.63): C, 61.58; H, 4.21; N, 15.96; S, 12.18. Found: C, 61.49; H, 4.14; N, 15.90; S, 12.10.

1-[(Benzoxazol-2''-yl)thioacetamidyl]-2-(1',3'-diphenyl-1'*H*-pyrazol-4'-yl) thiazolidin-5-one (**12b**)

Yield: 68% (ethanol, white crystals); mp 211-212 °C; IR (KBr) ν / cm^{-1} 3410, 3022, 1672, 16435; ^{1}H NMR (90 MHz, DMSO-d_6) δ 3.95 (s, 2H, thiazolidine-CH_2), 4.18 (s, 2H, CH_2), 5.12 (s, 1H, thiazolidine-CH), 7.02-7.98 (m, 14H, Ar-H), 8.30 (s, 1H, pyrazole-H), 8.41 (s, 1H, NH, D_2O exchanged). Anal. calcd. for $C_{27}H_{21}N_5O_3S_2$ (527.62): C, 61.46; H, 4.01; N, 13.27; S, 12.15. Found: C, 61.38; H, 3.92; N, 13.17; S, 12.01.

General procedure for the synthesis of azetidin-2-ones **5a**,**b**, **9a**,**b**, and **13a**,**b**

A solution of hydrazides (**3a**,**b**, **7a**,**b**, or **11a**,**b**) (3 mmol) in dioxane (20 mL) was added to a well stirred mixture of chloroacetylchloride (0.24 mL, 3 mmol) and triethylamine (3 mmol) in dioxane (10 mL) at 0-5 °C. The reaction mixture was stirred for 3 h, and then refluxed for 8 h. The reaction mixture was cooled, poured into ice-cold and the solid thus obtained was filtered, washed with water and recrystallized from dioxane.

2-[4'-(1,3-Diphenyl-1*H*-pyrazol-4-yl)-3'-chloro-2'-oxoazetidine]hydrazinobenzimidazole (**5a**)

Yield: 61% (pale yellow powder); mp 225-226 °C; IR (KBr) ν / cm^{-1} 3440, 3257, 3024, 1755; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.18 (d, 1H, CH-N), 5.73 (d, 1H, CH-Cl), 7.18-7.94 (m, 14H, Ar-H), 8.37 (s, 1H, pyrazole-H), 8.48 (s, 1H, NH, D_2O exchanged), 9.53 (s, 1H, benzimidazole-NH, D_2O exchanged); ^{13}C NMR (100 MHz, $CDCl_3$) δ 43.4, 58.9, 119.4, 122.3, 122.4, 126.3, 129.7, 133.3, 136.3, 136.7, 139.7, 141.0, 141.3, 151.5, 152.5, 172.2. Anal. calcd. for $C_{25}H_{19}N_6OCl$ (454.91): C, 66.01; H, 4.21; N, 18.47; Cl, 7.79. Found: C, 65.90; H, 4.11; N, 18.40; Cl, 7.65.

2-[4'-(1,3-Diphenyl-1*H*-pyrazol-4-yl)-3'-chloro-2'-oxoazetidine]hydrazinobenzoxazole (**5b**)

Yield: 60% (pale orange crystals); mp 241-242 °C; IR (KBr) ν / cm^{-1} 3440, 3010, 1758; ^{1}H NMR (90 MHz, DMSO-d_6) δ 5.28 (d, 1H, CH-N), 5.50 (d, 1H, CH-Cl), 7.05-8.01 (m, 15H, Ar-H and pyrazole-H), 8.52 (s, 1H, NH, D_2O exchanged). Anal. calcd. for $C_{25}H_{18}N_5O_2Cl$ (455.90): C, 65.86; H, 3.98; N, 15.36; Cl, 7.78. Found: C, 65.75; H, 3.87; N, 15.26; Cl, 7.64.

2-[2'-(4-(1,3-Diphenyl-1*H*-pyrazol-4-yl)-3-chloro-2-oxoazetidine)acetylaminomercapto]benzimidazole (**9a**)

Yield: 70% (orange powder); mp 230-231 °C; IR (KBr) ν / cm^{-1} 3416, 3209, 3080, 1688, 1645; ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.11 (s, 2H, $COCH_2$), 5.30 (d, 1H, CH-N), 5.51 (d, 1H, CH-Cl), 7.18-7.80 (m, 14H, Ar-H), 8.31 (s, 1H, pyrazole-H), 8.51 (s, 1H, NH, D_2O exchanged), 9.56 (s, 1H, benzimidazole-NH, D_2O exchanged). Anal. calcd. for $C_{27}H_{21}N_6O_2SCl$ (529.01): C, 61.30; H, 4.00; N, 15.89; S, 6.06; Cl, 6.70. Found: C, 61.19; H, 3.93; N, 15.79; S, 5.94; Cl, 6.62.

2-[2'-(4-(1,3-Diphenyl-1*H*-pyrazol-4-yl)-3-chloro-2-oxoazetidine)acetylaminomercapto] benzoxazole (**9b**)

Yield: 65% (pale yellow crystals); mp 250-251 °C; IR (KBr) ν / cm^{-1} 3410, 3028, 1674, 1640; ^{1}H NMR (90 MHz, DMSO-d_6) δ 4.10 (s, 2H, $COCH_2$), 5.28 (d, 1H, CH-N), 5.49 (d, 1H, CH-Cl), 7.10-7.98 (m, 15H, Ar-H and pyrazole-H), 8.52 (s, 1H, NH, D_2O exchanged). Anal. calcd. for $C_{27}H_{20}N_5O_3SCl$ (530.00): C, 61.19; H, 3.80; N, 13.21; S, 6.05, Cl, 6.69. Found: C, 61.12; H, 3.72; N, 13.10; S, 5.96, Cl, 6.61.

1-[(Benzimidazol-2''-yl)thioacetamidyl]-3-chloro-4-(1',3'-diphenyl-1'*H*-pyrazol-4'-yl)azetidin-2-one (**13a**)

Yield: 60% (white crystals); mp 258-259 °C; IR (KBr) ν / cm^{-1} 3424, 3228, 3007, 1677, 1633; ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.18 (s, 2H, $COCH_2$), 5.33 (d, 1H, CH-N), 5.53 (d, 1H, CH-Cl), 7.03-7.99 (m, 14H, Ar-H), 8.34 (s, 1H, pyrazole-H), 8.97 (s, 1H, NH, D_2O exchanged), 9.54 (s, 1H, benzimidazole-NH, D_2O exchanged); ^{13}C NMR (100 MHz, $CDCl_3$) δ 33.5, 49.1, 59.1, 118.6, 121.9, 126.4, 129.1, 132.5, 136.5, 139.1, 143.6, 145.6, 149.7, 152.6, 154.6, 169.0, 173.2. Anal. calcd. for $C_{27}H_{21}N_6O_2SCl$ (529.01): C, 61.30; H, 4.00; N, 15.89; S, 6.06, Cl, 6.70. Found: C, 61.22; H, 3.92; N, 15.80; S, 5.95, Cl, 6.60.

1-[(Benzoxazol-2''-yl)thioacetamidyl]-3-chloro-4-(1',3'-diphenyl-1'*H*-pyrazol-4'-yl)azetidin-2-one (**13b**)

Yield: 64% (pale brown powder); mp 250-251 °C; IR (KBr) ν / cm^{-1} 3422, 3020, 1675, 1640; ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.22 (s, 2H, $COCH_2$), 5.30 (d, 1H, CH-N), 5.46 (d, 1H, CH-Cl), 7.05-8.00 (m, 14H, Ar-H), 8.25 (s, 1H, pyrazole-H), 8.92 (s, 1H, NH, D_2O exchanged). Anal. calcd. for $C_{27}H_{20}N_5O_3SCl$ (530.00): C, 61.19; H, 3.80; Cl, 6.69; N, 13.21; S, 6.05. Found: C, 61.09; H, 3.71; Cl, 6.57; N, 13.15; S, 5.96.

Biological evaluation

Animals

Adult male albino Wistar rats, weighing 200-250 g, were obtained from the animal house colony of National Research Centre (NRC). The animals were maintained at a normal temperature of 24 ± 1 °C with a 12-12 h light-dark cycle (light cycle, 7:00 am-7:00 pm). They were

allowed free access to water and standard show *ad libitum*. The animals were treated according to the national and international ethics guidelines stated by the ethics committee of NRC and all procedures and experiments were performed according to its approved protocol.

Evaluation of the anti-diabetic effect of the tested compounds against streptozotocin-induced hyperglycemia in rats

Hyperglycemia was induced in rats using a single i.p. (intraperitoneal) injection of streptozotocin (STZ, 50 mg kg^{-1}) dissolved in a citrate buffer (0.1 M, pH 4.5).[31] After 48 hours, 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^{-1} were selected and considered as hyperglycemic animals to be subjected to further experimentation**.** Hyperglycemic rats were randomly divided into five groups (6 rats each). One group served as a hyperglycemic normal, while the other four groups were treated with gliclazide (Glic, 5 mg kg^{-1} day^{-1}, p.o.), as a reference drug, or one of the tested compounds (**7b**, **12b**, and **13b**), (50 mg kg^{-1} day^{-1}, p.o.) for 10 consecutive days, respectively. Drug treatment was started 48 h after STZ injection (time at which hyperglycemia was confirmed). In addition, a universal normal group which received only the citrate buffer (6 rats) was used. Twenty-four hours after the last dose of either drug treatment, the serum levels of glucose, triglyceride (TG), and cholesterol are determined using specific diagnostic kits (Stanbio, USA).

Evaluation of the renoprotective effects of the tested compounds against renal ischemia/reperfusion-induced acute renal failure in rats

Eight groups of animals were used in this experiment; each group consisted of six rats. The first group was subjected to a sham operation and served as a normal group. In the other seven groups, ischemia/reperfusion acute renal failure (I/R ARF) was induced in anesthetized (ketamine/xylazine) rats by clamping both renal pedicles for 45 min. Reflow was visually confirmed, and to reduce abdominal air, 4 mL warm normal saline was given intraperitoneally before abdominal closure.[32] For ten days prior to I/R, groups 1 and 2 were treated with daily oral dose of saline, while the other six groups were treated with daily oral dose of 50 mg kg^{-1} day^{-1} of the tested compounds (**3a**, **3b**, **5a**, **7a**, **8a**, and **8b**). Twenty-four hours following I/R, blood samples were withdrawn from rats of all groups via retro-orbital vein under light ether anesthesia.[33] Serum was used for estimation of serum urea, creatinine and total protein levels, using specific diagnostic kits (Biodiagnostic, Egypt). Immediately after blood sampling, animals were sacrificed by cervical dislocation under ether anesthesia. The two kidneys from each rat were immediately dissected out, and rinsed with PBS to remove excess blood. Weighed parts from both kidneys were homogenized (MPW-120 homogenizer, MED instruments, Poland) in PBS to obtain 20% homogenate that stored overnight at ≤ –20 °C. After two freeze-thaw cycles performed to break the cell membranes, the homogenates were centrifuged for 5 min at 5000 × g using a cooling centrifuges (Sigma and Laborzentrifugen, 2K15, Germany). The supernatant was removed immediately and assayed for reduced glutathione (GSH), lipid peroxides, measured as malondialdehyde (MDA), and NOx (nitrite and nitrate, stable metabolites of nitric oxide) contents using specific commercial reagent kits (Biodiagnostic, Egypt).

Statistical analysis

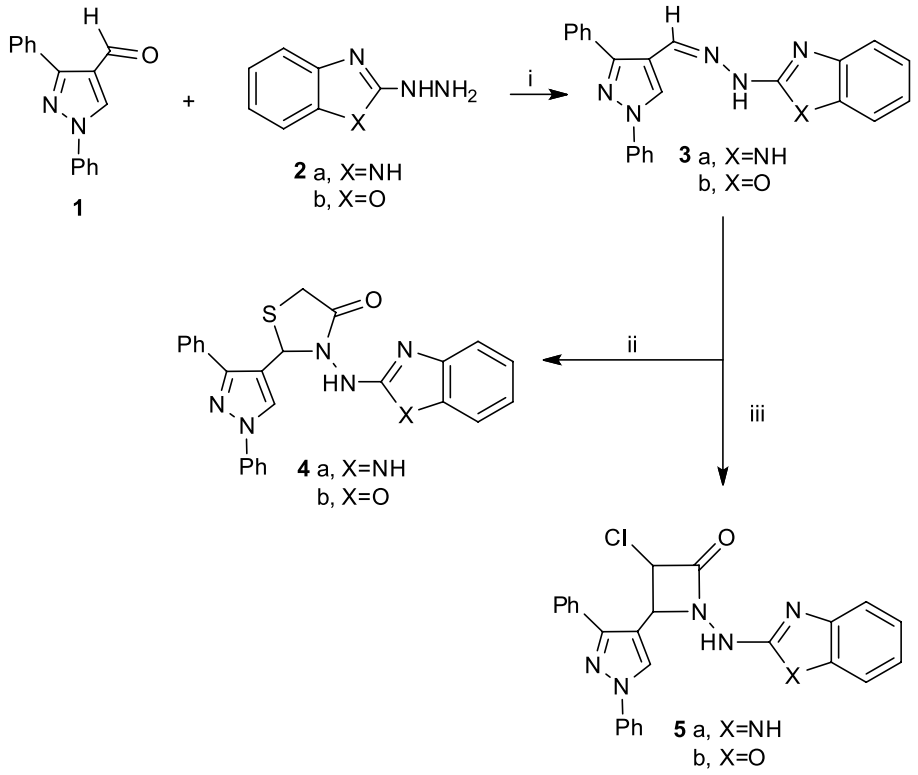
All the values are presented as means ± standard error of the means (SE). Comparisons between different groups were carried out using one-way analysis of variance (ANOVA) followed by Tukey HSD (honest significant difference) test for multiple comparisons.[34] Graphpad Prism software, version 5 (Inc., San Diego, USA) was used to carry out these statistical tests. The difference was considered significant when $p < 0.05$.

Results and Discussion

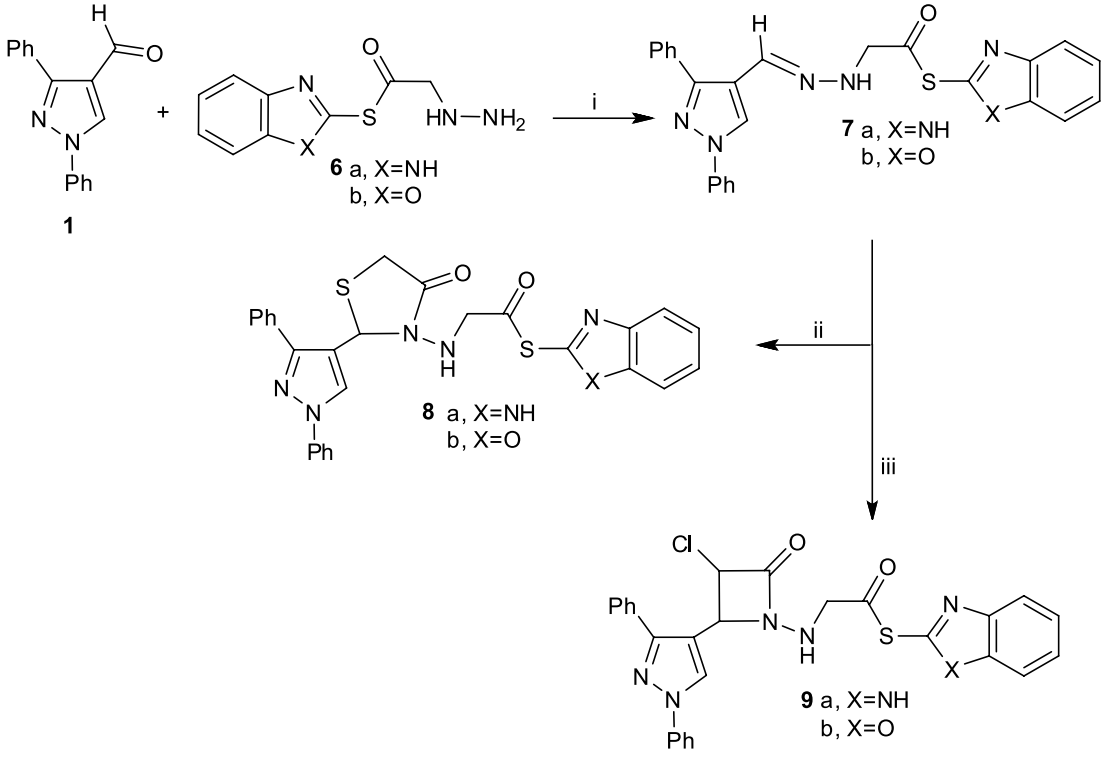
Synthesis

Our synthetic strategy for a novel series of benzazole, thiazolidin-4-one and azetidin-2-one derivatives bearing 1,3-diphenyl-1*H*-pyrazole moiety is illustrated in Schemes 1-3. The required hydrazides **3a**,**b**, **7a**,**b**, and **11a**,**b** were synthesized by condensation of the key aldehyde intermediate[35] **1** with the corresponding hydrazine **2a**,**b**, **6a**,**b** and **10a**,**b** on refluxing with ethanol containing a few drops of acetic acid. The structure of the compounds **3a**,**b**, **7a**,**b**, and **11a**,**b** was assigned by spectral data and elemental analysis (see Experimental section).

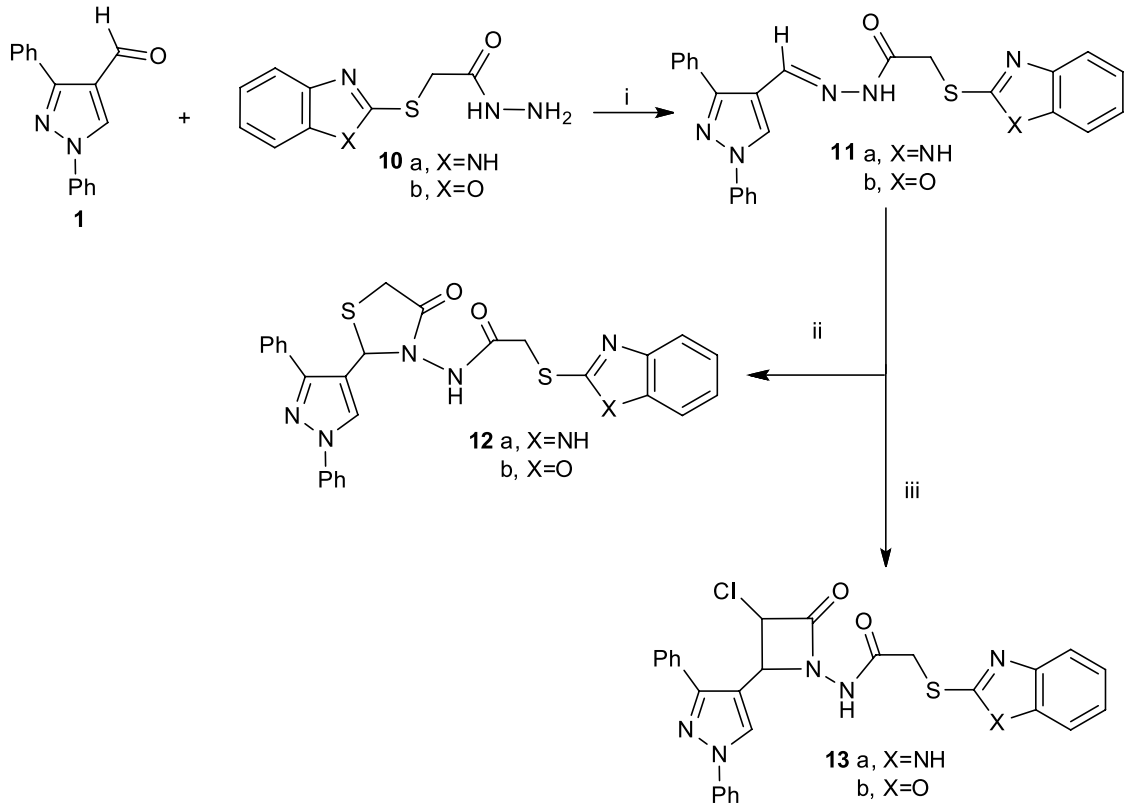
The hydrazide derivatives **3a**,**b**, **7a**,**b**, and **11a**,**b**, when treated with thioglycolic acid in the presence of anhydrous $ZnCl_2$, gave thiazolidine-4-one analogues **4a**,**b**, **8a**,**b** and **12a**,**b**, respectively (Schemes 1-3). The structures of latter compounds was assigned by IR, NMR spectra and elemental analyses which are reliable with the proposed thiazolidine-4-one structures. For example, the ^{1}H NMR of these compounds showed signals at δ 3.65-3.99 ppm and 5.00-5.14 ppm due to CH_2 and CH



Scheme 1. Reagents and conditions: (*i*) ethanol, AcOH, 5 h; (*ii*) thioglycolic acid, DMF, $ZnCl_2$, reflux 8 h; (*iii*) chloroacetyl chloride, dioxane, TEA, stirring then reflux 8 h.



Scheme 2. Reagents and conditions: (*i*) ethanol, AcOH, 5 h; (*ii*) thioglycolic acid, DMF, $ZnCl_2$, reflux 8 h; (*iii*) chloroacetyl chloride, dioxane, TEA, stirring then reflux 8 h.



Scheme 3. Reagents and conditions: (*i*) ethanol, AcOH, 5 h; (*ii*) thioglycolic acid, DMF, $ZnCl_2$, reflux 8 h; (*iii*) chloroacetyl chloride, dioxane, TEA, stirring then reflux 8 h.

of thiazolidine ring. In the ^{1}H NMR spectra of starting hydrazides **3a**,**b**, **7a**,**b**, and **11a**,**b**, the proton of –CH=N appears at about δ 6.31-6.40 ppm. But, in the spectra of thiazolidin-4-ones **4a**,**b**, **8a**,**b** and **12a**,**b**, this proton signal (CH thiazolidine) is shifted slightly downfield in contrast with hydrazides. The reason was due to the deshielding affected by heterocycles.

On the other hand, the cycloaddition reaction of hydrazides **3a**,**b**, **7a**,**b**, and **11a**,**b** with chloroacetyl chloride in dioxane containing triethyl amine as a reaction mediator gave azetidin-2-ones **5a**,**b**, **9a**,**b**, and **13a**,**b**, respectively.

The ^{1}H NMR spectra of these compounds showed the signals at δ 5.46-5.73 ppm due to the CH–Cl in the β-lactam ring. Whereas the ^{13}C NMR spectra of compounds **5a** and **13a** showed signals at δ 58.9 and 59.1 ppm, respectively, due to –CH–Cl. Moreover, signals at δ 172.2 and 173.2 ppm are due to C=O cyclic in the β-lactam moiety.

Biological evaluation

Streptozotocin-induced hyperglycemia experiment

Serum glucose level

A single injection of streptozotocin (STZ) (50 mg kg^{-1}, i.p.) resulted in an elevation of the serum glucose level that was demonstrated 48 h after injection and persisted until the end of the experiment. Oral treatment of hyperglycemic rats with each of the tested compounds (**7b**, **12b**, and **13b**) for 10 days restored the normal serum glucose levels, a similar effect to that produced by the treatment with the reference anti-diabetic drug gliclazide (Glic) (Figure 2).

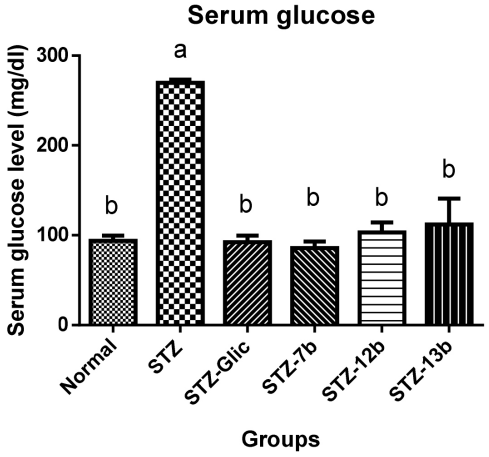


Figure 2. Serum glucose levels of compounds **7b**, **12b** and **13b**. Normal: rats treated with the vehicle; STZ: rats treated with STZ; STZ-Glic: rats treated with STZ and Glic; STZ-T(x), rats treated with STZ and the tested agent of the corresponding number (x). a: Significantly different from Normal group at $p < 0.05$; b: significantly different from STZ group at $p < 0.05$.

Serum cholesterol and triglyceride levels

STZ-induced hyperglycemia in the present study was related with an increase in the serum cholesterol and triglyceride (TG) levels. Gliclazide (Glic) administration restored the normal cholesterol and TG levels. Also, normal serum cholesterol and TG levels were observed in groups treated with **12b** and **13b**. The anti-cholesterolemic effect of **7b** was significantly less than that observed by **12b** and **13b**; however, it restored the normal serum TG level (Figure 3).

Renal ischemia/reperfusion experiment

Renal function markers

A significant elevation in serum urea, creatinine and total protein levels was observed in the rats subjected to I/R as compared with those of normal group. Administration of **3a**, **3b**, **5a**, **7a**, and **8a** significantly reduced the I/R-induced elevation of serum urea and creatinine levels.

On the other hand, compound **8b** reduced these levels, but more than the normal. The normal level of serum total protein was maintained in the rats pretreated with each of the tested agents (Figure 4).

Renal tissue biochemical analysis

Renal GSH content was significantly reduced following I/R in rats. Restoration of normal renal GSH content was observed in the group treated with compound **8a**, while **3a** failed to improve the I/R-induced reduction of renal GSH content. Treatment of rats with the other tested compounds

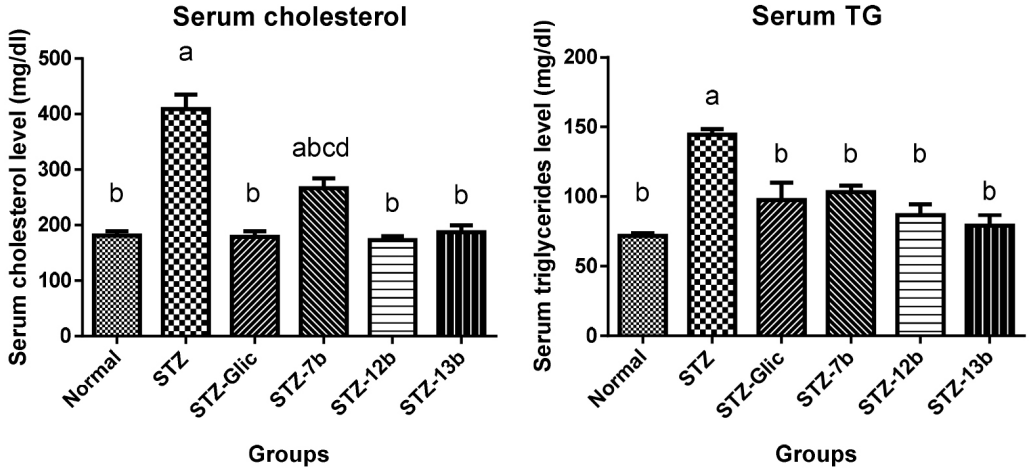


Figure 3. Serum cholesterol and triglyceride (TG) levels of compounds **7b**, **12b** and **13b**. Normal: rats treated with the vehicle; STZ: rats treated with streptozotocin; STZ-Glic: rats treated with streptozotocin and Glic; STZ-T(x): rats treated with STZ and the tested agent of the corresponding number (x). a: Significantly different from normal group at $p < 0.05$; b: significantly different from STZ group at $p < 0.05$; c: significantly different from STZ-Glic group at $p < 0.05$; d: significantly different from the other STZ-T(x) groups at $p < 0.05$.

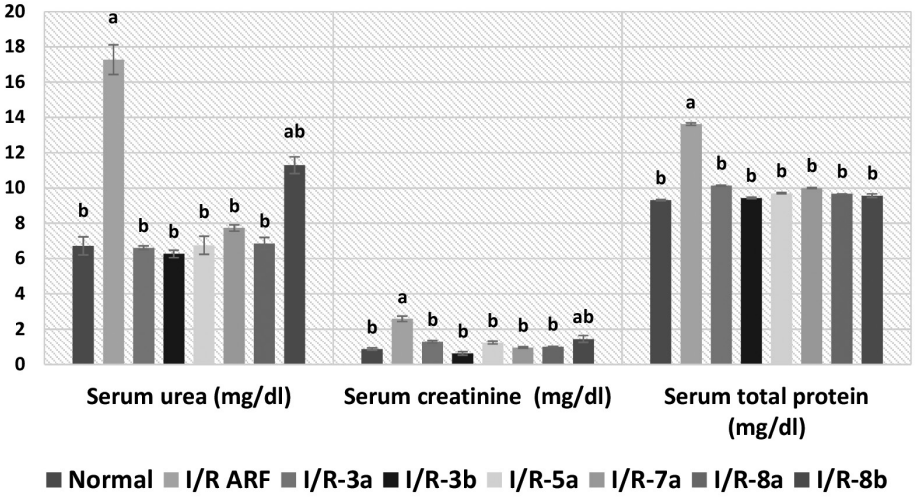


Figure 4. Serum levels of urea, creatinine and total protein of compounds **3a**, **3b**, **5a**, **7a**, **8a**, and **8b**. Normal: rats with sham operation; I/R ARF: rats with ischemia/reperfusion-induced acute renal failure; I/R-T(x): rats with ischemia/reperfusion-induced acute renal failure treated with the tested compounds of the corresponding number (x). a: Significantly different from normal group at $p < 0.05$; b: significantly different from I/R ARF group at $p < 0.05$.

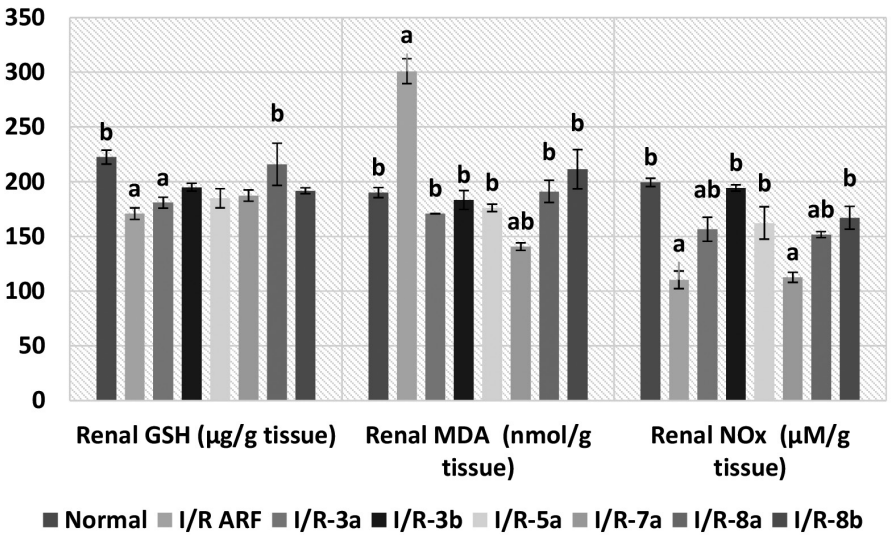


Figure 5. Renal contents of GSH, MDA, and NOx of compounds **3a**, **3b**, **5a**, **7a**, **8a**, and **8b**. Normal: rats with sham operation; I/R ARF: rats with ischemia/reperfusion-induced acute renal failure; I/R-T(x): rats with ischemia/reperfusion-induced acute renal failure treated with the tested agent of the corresponding number (x). a: Significantly different from normal group at $p < 0.05$; b: significantly different from I/R ARF group at $p < 0.05$.

resulted in renal GSH contents that are non-significantly different from normal and I/R groups.

On the other hand, a significant elevation of renal MDA content was detected following I/R. Treatment of rats with all tested compounds significantly retrieved the altered level of MDA. Interestingly, the renal content of MDA in rats treated with **7a** was remarkably lower than those observed in the normal I/R **8b** and I/R **8a** groups. Moreover, a significant decrease in renal NOx content was observed in rats with I/R ARF. Treatment of rats with **5a**, **8b**, and **3b** has been found to restore the normal renal level of NOx. Compounds **3a** and **8a** significantly improved the I/R-induced reduction of NOx level, while **7a** failed (Figure 5).

Conclusions

In conclusion, the objective of the present study was to synthesize and evaluate the anti-diabetic and renoprotective activity of some new benzazole, thiazolidinone and azetidin-2-one derivatives incorporating 1,3-diphenylpyrazole moiety with the hope of discovering new structures leads serving as potent pharmacological agents. Our aim was verified by the synthesis of different groups of structure hybrids comprising basically the pyrazole moiety attached to benzazole, thiazolidinone, azetidin-2-one counter parts through various linkages of synthergistic purpose.

Supplementary Information

Supplementary information is available free of charge at http://jbcs.sbq.org.br.

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