

SYNTHESIS OF NOVEL 2-AMINO-5-ARYLAZOTHIAZOL DERIVATIVES AND THEIR BIOLOGICAL IMPACTS: ASSESSMENT OF TOXICITY AND ANTIOXIDANT ENZYME ACTIVITIES

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A reactivity study of both the amino group and the aryl substituent of a newly synthesized 2-amino-5-(4-acetylphenylazo)-thiazole compound and its derivatives *via* various electrophilic reagents was performed to obtain new bioactive chalcone, imine, and pyrazole-thiazolidine derivatives. The synthesized compounds were chemically elucidated by analytical and spectral methods, and biologically evaluated *in vitro* and *in vivo* for their toxicity and antioxidant activity based on liver function enzymes.

Keywords: 2-aminothiazole; chalcone; imine; pyrazole; azo coupling; biological activities

СИНТЕЗА НА НОВИ ДЕРИВАТИ НА 2-АМИНО-5-АРИЛТИАЗОЛ И НИВНА БИОЛОШКА АКТИВНОСТ: ПРОЦЕНА НА НИВНАТА ТОКСИЧНОСТ И АНТИОКСИДАТИВНА ЕНЗИМСКА АКТИВНОСТ

Извршено е испитување како на аминокрупата така и на арилсупституентот на ново синтетизирано соединение на 2-амино-5-(4-ацетилфенилазо)-тиазол и неговите деривати преку разни електрофилни реагенси за да се добијат нови биоактивни халконски, имински и пиразолтиазолидински деривати. Синтетизираните соединенија беа идентификувани преку аналитички и спектрални методи, а потоа беа и биолошки евалуирани *in vitro* и *in vivo* за нивната токсичност и антиоксидативна активност преку ензими од црниот дроб.

Клучни зборови: 2-аминотиазол; халкон; имин; пиразол; азоспајување; биолошка активност

1. INTRODUCTION

Azo compounds are of great importance due to their versatile application in various fields, such as the dyeing of textile fibers, coloring of different materials, biomedical studies, and advanced applications in organic synthesis [1–4]. In continuation of our previous work [5–8] on the synthesis of S/N heterocyclic azo compounds with different applications, such as dyeing polyester fabrics and bioactive function, the present work describes the synthesis of several new S/N heterocyclic mono azo

compounds based on the 2-aminothiazole moiety, and their applications as biologically active materials. Diverse biological activities have been found to be associated with thiazolidine derivatives, including anesthetic [3], antiviral [4], antimicrobial [9], bactericidal [10], anti-inflammatory [11], fungicidal [12, 13], and anticonvulsant [14–16] properties. It is well known that 2-aminothiazole can exhibit amino-imino tautomeric configurations in nature, and the amino configuration is the predominant tautomer [17]. Considering the reactivity of 2-aminothiazoles and their enormous biological

importance, the chemical and biological study of selected 5-arylazo-2-aminothiazole and its various derivatives is a hot and interesting topic and is focus of our research.

2. EXPERIMENTAL

2.1. Materials and methods

All chemicals and reagents used were of analytical grade or were chemically pure, and were supplied by Sigma Aldrich Co. (Germany). All kits for biochemical parameters were supplied by Diagnostic Company (UK). Elemental analyses (C, H, N) were conducted using the Perkin-Elmer 2400 Analyzer, series II (Perkin Elmer Co., Shelton, UK). All of the melting points (uncorrected) are in °C and were measured using a Stuart SMP 20 melting point apparatus (Bibby Scientific Limited, Staffordshire, UK). The infrared spectra were recorded on a Perkin Elmer Alpha platinum-ATR spectrometer, and the ^1H NMR and ^{13}C NMR spectra were measured on a Bruker WP 300 (Bruker, MA, USA) using trimethylsilane (TMS) as an internal standard. All of the microanalyses and spectral analyses were performed by the Micro Analytical Centers of Taif (IR, CHN) and King Abdel-Aziz Universities (^1H NMR, ^{13}C NMR), Kingdom of Saudi Arabia. Mass spectra were recorded on a Finnigan MAT 212 instrument (Micro Analytical Center, Faculty of Science, Mansoura University, Egypt). Male albino rats (180 ± 10 g) were provided by the Animal Science Department (Faculty of Agriculture, Cairo University, Egypt) and the biological tests were performed by the Biotechnology Unit (Faculty of Agriculture, Cairo University, Egypt). Methods of biological assays are described in detail in the attached supplementary file S1.

2.2. Synthesis

2.2.1. Synthesis of 2-amino-5-(4-acetylphenylazo)-thiazole **3**

The corresponding aryl diazonium chloride was prepared by adding cold sodium nitrite solution (10 mmol, 0.69 g) in H_2O (15 ml) to a cold suspension of 4'-aminoacetophenone **2** (10 mmol, 1.35 g) in concentrated HCl (4 ml) with stirring. To a cold solution of 2-aminothiazole **1** (10 mmol, 1 g) in ethanol (20 ml) and sodium acetate (20 mmol, 1.6 g), a cold aqueous solution from the corresponding aryl diazonium chloride was added dropwise with stirring at -5 °C for 2 hours. The

solid products obtained were filtered, washed with water followed by cold ethanol, and then dried. The obtained product was recrystallized from ethanol to give the 2-amino-5-(4-acetylphenylazo)-thiazole **3**.

Brown solid, yield: 73%; m.p. 153–154°C. IR ($\bar{\nu}/\text{cm}^{-1}$): 3358, 3244 (NH_2), 1668 ($\text{C}=\text{O}$). ^1H NMR ($\text{DMSO}-d_6$) (δ/ppm): 2.45 (s, 3H, COCH_3), 6.90 (s, 1H, thiazole $\text{C}_4\text{-H}$), 7.50 (d, 2H, Ar-H), 7.85 (d, 2H, Ar-H), 8.15 (s, 2H, NH_2). ^{13}C NMR (300 MHz, DMSO , TMS): δ 199.67 (COCH_3), 169.35 (C-NH_2), 162.17, 147.23, 108.21 (thiazole C) and 136.74, 133.12, 128.72, 128.80, 128.72. MS (M^+ ; EI): m/z = 246 (34.7%), 238 (7.1%), 203 (7.8%), 152 (7.1%), 147 (12.9%), 135 (18.5%), 129 (31.4%), 120 (33.3%), 100 (25.5%), 71 (23.2%), 57 (36.5%), 44 (100.0%). Anal. calcd. for $\text{C}_{11}\text{H}_{10}\text{N}_4\text{OS}$ (mol. wt.: 246.29): C, 53.64; H, 4.09; N, 22.75. Found: C, 53.49; H, 4.17; N, 22.81.

2.2.2. Synthesis of 2-acetylamino-5-(4-acetylphenylazo)-thiazole **4**

A mixture of 2-amino-5-(4-acetylphenylazo)-thiazole **3** (20 mmol, 4.9 g) and acetic anhydride (10 ml) was heated in a water bath at 100 °C for 2 hours. The reaction mixture was allowed to cool at room temperature, and was then recrystallized from ethanol to obtain the corresponding 2-(*N*-acetylamino)-5-(4-acetylphenylazo)-thiazole **4**.

Brown solid, yield: 52%; m.p. 178–179 °C. IR ($\bar{\nu}/\text{cm}^{-1}$): 3164 (NH), 1682 ($\text{C}=\text{O}$), 1665 ($\text{C}=\text{O}$). ^1H NMR ($\text{DMSO}-d_6$) (δ/ppm): 2.25 (s, 3H, CH_3), 2.45 (s, 3H, COCH_3), 6.90 (s, 1H, $\text{C}_4\text{-thiazole -H}$), 7.45 (d, 2H, Ar-H), 7.85 (d, 2H, Ar-H), 11.15 (s, 1H, NH). ^{13}C NMR (300 MHz, DMSO , TMS): δ 199.77 (COCH_3), 168.91 (C-NH), 162.74, 147.11, 108.01 (thiazole C) and 136.81, 133.11, 128.68, 128.70, 128.68. MS (M^+ ; EI): m/z = 288 (22.6%), 245 (12.2%), 223 (15.7%), 120 (44.8%), 100 (25.5%), 77 (11.6%), 44 (100.0%). Anal. calcd. for $\text{C}_{13}\text{H}_{12}\text{N}_4\text{O}_2\text{S}$ (mol. wt.: 288.32): C, 54.15; H, 4.20; N, 19.43. Found: C, 54.02; H, 4.28; N, 19.40.

2.2.3. Synthesis of 2-benzoylamino-5-(4-acetylphenylazo)-thiazole **5**

A mixture of 2-amino-5-(4-acetylphenylazo)-thiazole **3** (3 mmol, 0.74 g) and benzoyl chloride (3 mmol, 0.35 ml) was stirred in 15 ml pyridine at room temperature. The reaction mixture was diluted with a solution of sodium acetate and filtered off. The 2-benzoylamino-5-(4-acetylphenylazo)-thiazole **5** obtained was dried and recrystallized from an ethanol/dimethyl formamide (DMF) mixture.

Brown solid; yield: 53%; m.p.: 210–212 °C. IR ($\bar{\nu}/\text{cm}^{-1}$): 3147 (NH), 1675 (COCH₃) and 1662 (COPh) cm^{-1} . ¹H NMR (CF₃COOD): δ/ppm = 2.57 (s, 3H, CH₃), 7.26–7.90 (m, 9H, Ar-H), 8.16 (s, 1H, C₄-thiazole-H). ¹³C NMR (300 MHz, DMSO, TMS): δ 199.81 (COCH₃), 164.83 (C-NH), 162.80, 147.11, 108.08 (thiazole C) and 136.78, 133.13, 127.47, 127.48, 128.88, 128.80, 128.88, 128.90, 128.89. MS (M⁺; EI): m/z = 350 (61.3%), 245 (19.7%), 203 (27.7%), 120 (73.6%), 77 (100.0%). Anal. calcd. for C₁₈H₁₄N₄O₂S (mol. wt.: 350.39): C, 61.70; H, 4.03; N, 15.99. Found: C, 61.48; H, 4.17; N, 15.84.

2.2.4. Synthesis of 2-chloroacetyl-amino-5-(4-acetylphenylazo)-thiazole **6**

A mixture of 2-amino-5-(4-acetylphenylazo)-thiazole **3** (3 mmol, 0.74 g) in 10 ml DMF and chloroacetyl chloride (3 mmol, 0.34 ml) was stirred in the presence of 3 ml triethylamine (TEA) for 4 hours. The reaction mixture was poured onto ice-cold water and filtered off to produce the chloroacetyl amino derivative **6**. The collected product was dried and recrystallized from an ethanol/DMF mixture.

Brown solid; yield: 76%; m.p.: 132–134 °C. IR ($\bar{\nu}/\text{cm}^{-1}$): 3172 (NH), 1684 (C=O) and 1670 (C=O, acetyl) cm^{-1} . ¹H NMR (CF₃COOD): δ/ppm = 2.62 (s, 3H, CH₃), 4.11 (s, 2H, CH₂), 7.26 (d, 2H, Ar-H), 7.78 (d, 2H, Ar-H), 8.07 (s, 1H, C₄-thiazole-H). ¹³C NMR (300 MHz, DMSO, TMS): δ 199.78 (COCH₃), 165.43 (C-CONH), 162.79, 147.01, 108.10 (C-thiazole ring), and 136.80, 128.69, 128.70, 128.80, 128.82. MS (M⁺; EI): m/z = 322 (3.3%), 238 (5.9%), 223 (7.3%), 196 (11.85%), 120 (13.0%), 77 (9.5%), 44 (100.0%). Anal. calcd. for C₁₃H₁₁N₄O₂SCl (mol. wt.: 322.77): C, 48.37; H, 3.44; N, 17.36. Found: C, 48.49; H, 3.48; N, 17.27.

2.2.5. General procedure for the synthesis of the chalcones (**8a–c**) and the chalcone - imine derivatives **9a–c**

A mixture of the appropriate aromatic aldehydes **7a–c** (5 mmol) and compound **3** or **4** (1 mmol, 2.46 g, and/or 5 mmol, 1.44 g, respectively) dissolved in ethanol (50 ml) was added slowly to an aqueous solution of sodium hydroxide (12 mmol, 0.48 g, and/or 6 mmol, 0.24 g, respectively) in water (10 ml). The reaction mixture was stirred at 20–25 °C for 4 hrs. The solid product obtained was washed with cold water and recrystallized from ethanol to give **8a–c** and **9a–c** derivatives, respectively.

N-{5-[[4-[3-Phenylprop-2-enoyl]phenyl]diazonyl]-1,3-thiazol-2-yl}acetamide **8a.** Brown solid; yield: 43%; m.p.: >300 °C. IR ($\bar{\nu}/\text{cm}^{-1}$): 3153 (NH), 1672 (NHCOCH₃) and 1659 (COCH=CH) cm^{-1} . ¹H NMR (CDCl₃/CF₃COOD): δ/ppm = 2.25 (s, 3H, CH₃), 7.21–7.90 (m, 11H, Ar-H and two olefinic protons CH=CH), 8.05 (s, 1H, C₄-thiazole-H). ¹³C NMR (300 MHz, DMSO, TMS): δ 189.71 (CO), 168.83 (C-CONH), 162.80, 147.03, 108.08 (C-thiazole ring), 145.18, 121.33 (C=C), 137.80, 134.52, 135.22, 129.87, 129.88, 129.27, 129.28, 128.66, 128.67, 128.04, 126.39, 126.40. MS (M⁺ +H; CI *iso*-butane): m/z = 377 (100.0%). Anal. calcd. for C₂₀H₁₆N₄O₂S (mol. wt.: 376.43): C, 63.81; H, 4.28; N, 14.88. Found: C, 63.64; H, 4.13; N, 14.71.

N-{5-[[4-[(3-(1H-Indol-3-yl)prop-2-enoyl]phenyl]diazonyl]-1,3-thiazol-2-yl]acetamide **8b.** Brown solid; yield: 63%; m.p.: 220 °C. IR ($\bar{\nu}/\text{cm}^{-1}$): 3214 (NH), 1678 (NHCOCH₃) and 1655 (COCH=CH) cm^{-1} . ¹H NMR (CDCl₃/CF₃COOD): δ/ppm = 2.25 (s, 3H, CH₃), 7.15–7.80 (m, 10H, Ar-H and two olefinic protons CH=CH), 8.05 (s, 1H, C₄-thiazole-H), 9.15 (s, 1H, C₂-pyrrol-H). ¹³C NMR (300 MHz, DMSO, TMS): δ 189.73 (CO), 168.91 (C-CONH), 162.78, 147.01, 108.04 (C-thiazole ring), 145.20, 121.38 (C=C), 135.48, 130.77, 126.08, 122.17, 120.10, 111.13, 110.58 (C-indole), 137.85, 134.44, 129.88, 129.86, 129.31, 129.30. MS (M⁺ +H; CI *iso*-butane): m/z = 416 (82.4%). Anal. calcd. for C₂₂H₁₇N₅O₂S (mol. wt.: 415.47): C, 63.60; H, 4.12; N, 16.86. Found: C, 63.42; H, 3.98; N, 16.77.

N-{5-[[4-[3-(3-Methylthiophen-2-yl)prop-2-enoyl]phenyl]diazonyl]-1,3-thiazol-2-yl]acetamide **8c.** Brown solid; yield: 69%; m.p.: 140–141 °C. IR ($\bar{\nu}/\text{cm}^{-1}$): 3133 (NH), 1670 (NHCOCH₃) and 1662 (COCH=CH) cm^{-1} . ¹H NMR (CDCl₃/CF₃COOD): δ/ppm = 2.25 (s, 3H, CH₃), 2.40 (s, 3H, CH₃), 7.10–7.80 (m, 8H, Ar-H and two olefinic protons CH=CH), 8.10 (s, 1H, C₄-thiazole-H). ¹³C NMR (300 MHz, DMSO, TMS): δ 189.68 (CO), 168.90 (C-CONH), 162.80, 147.03, 107.97 (C-thiazole ring), 134.21, 127.39 (C=C), 139.18, 131.07, 130.38, 126.11 (C-thiophene), 137.90, 134.47, 129.90, 129.89, 129.30, 129.28. MS (M⁺; EI): m/z = 396 (8.1%), 144 (95.9%), 116 (34.2%), 97 (20.3%), 89 (20.3%), 73 (100.0%), 57 (33.3%). Anal. calcd. for C₁₉H₁₆N₄O₂S₂ (mol. wt.: 396.49): C, 57.56; H, 4.07; N, 14.13. Found: C, 57.34; H, 3.98; N, 14.22.

3-Phenyl-1-{4-[2-[[phenylmethylidene]amino]-1,3-thiazol-5-yl]diazonyl]-phenyl}prop-2-en-1-one **9a.** Brown solid; yield: 41%; m.p.: >300 °C. IR

($\bar{\nu}/\text{cm}^{-1}$): 1662 (COCH=CH), 1644 (C=N) cm^{-1} . ^1H NMR ($\text{CDCl}_3/\text{CF}_3\text{COOD}$): δ/ppm = 7.15–7.90 (m, 16H, Ar-H and two olefinic protons CH=CH), 8.05 (s, 1H, C₄-thiazole-H), 8.25 (s, 1H, N=CH). ^{13}C NMR (300 MHz, DMSO, TMS): δ 189.71 (CO), 160.12 (C=N), 171.80, 151.48, 118.96 (C-thiazole ring), 144.21, 121.39 (C=C), 137.92, 135.16, 134.51, 129.90, 129.89, 129.30, 129.28, 128.71, 128.70, 128.00, 126.34, 126.33. MS (M^+ +H; CI *iso*-butane): m/z = 423 (67.1%). Anal. calcd. for $\text{C}_{25}\text{H}_{18}\text{N}_4\text{OS}$ (mol. wt.: 422.5): C, 71.07; H, 4.29; N, 13.26. Found: C, 70.88; H, 4.13; N, 13.08.

1-{4-[(2-{[1H-Indol-3-ylmethylidene]amino}-1,3-thiazol-5-yl)diazanyl]-phenyl}-3-(1H-indol-3-yl)prop-2-en-1-one 9b. Brown solid; yield: 76%; m.p.: 198–200 °C. IR ($\bar{\nu}/\text{cm}^{-1}$): 3207 (NH), 1655 (COCH=CH), 1638 (C=N) cm^{-1} . ^1H NMR ($\text{CDCl}_3/\text{CF}_3\text{COOD}$): δ/ppm = 7.15–7.80 (m, 15H, Ar-H and two olefinic protons CH=CH), 8.05 (s, 1H, C₄-thiazole-H), 8.20 (s, 1H, N=CH), 9.20 (s, 1H, C₂-pyrrol-H). ^{13}C NMR (300 MHz, DMSO, TMS): δ 189.70 (CO), 171.65, 151.43, 118.64 (C-thiazole ring), 145.19, 126.38 (C=C), 160.08 (C=N), 135.51, 130.74, 126.13, 122.14, 120.11, 110.93, 102.28 (C-indole), 137.90, 134.43, 129.83, 129.81, 129.29, 129.28. MS (M^+ +H; CI *iso*-butane): m/z = 501 (85.6%). Anal. calcd. for $\text{C}_{29}\text{H}_{20}\text{N}_6\text{OS}$ (mol. wt.: 500.57): C, 69.58; H, 4.03; N, 16.79. Found: C, 69.41; H, 3.87; N, 16.88.

3-(3-Methylthiophen-2-yl)-1-{4-[(2-{[3-methylthiophen-2-yl)methylidene]amino}-1,3-thiazol-5-yl)diazanyl]phenyl}prop-2-en-1-one 9c. Brown solid; yield: 39%; m.p.: 186–187 °C. IR ($\bar{\nu}/\text{cm}^{-1}$): 1661 (COCH=CH), 1641 (CH=N) cm^{-1} . ^1H NMR ($\text{CDCl}_3/\text{CF}_3\text{COOD}$): δ/ppm = 2.40 (s, 3H, CH₃), 2.45 (s, 3H, CH₃), 7.10–7.80 (m, 10H, Ar-H and two olefinic protons CH=CH), 8.05 (s, 1H, C₄-thiazole-H), 8.30 (s, 1H, N=CH). ^{13}C NMR (300 MHz, DMSO, TMS): δ 189.70 (CO), 172.01, 150.98, 117.98 (C-thiazole ring), 134.17, 127.34 (C=C), 139.22, 131.08, 130.39, 126.01 (C-thiophene), 137.97, 134.51, 129.90, 129.89, 129.27, 129.26. MS (M^+ ; EI): m/z = 462 (5.7%), 253 (10.3%), 238 (20.4%), 222 (12.4%), 192 (42.3%), 177 (33.9%), 162 (52.2%), 135 (61.4%), 120 (100.0%), 97 (23.9%), 92 (39.3%). Anal. calcd. for $\text{C}_{23}\text{H}_{18}\text{N}_4\text{OS}_3$ (mol. wt.: 462.61): C, 59.71; H, 3.92; N, 12.11. Found: C, 59.56; H, 3.84; N, 11.98.

2.2.6. General procedure for the preparation of pyrazole derivatives 10a–c

To a solution of appropriate chalcones **8a–c** (2mmol) in ethanol (30 ml), 80%hydrazine hydrate

(5 mmol, 0.3 ml) was added. The reaction mixture was refluxed for 6 hours, then left to cool to room temperature, and the formed solid product was filtered and washed with ethanol.

N-(5-[[4-(5-Phenyl-4,5-dihydro-1H-pyrazol-3-yl)phenyl]diazanyl]-1,3-thiazol-2-yl)acetamide 10a. Brown solid; yield: 77%; m.p.: >300 °C. IR ($\bar{\nu}/\text{cm}^{-1}$): 3205, 3164 (NH), 1675 (C=O) and 1635 (C=N) cm^{-1} . ^1H NMR ($\text{CDCl}_3/\text{CF}_3\text{COOD}$): δ/ppm = 2.20 (s, 3H, CH₃), 2.85 (dd, 1H, CH), 3.20 (dd, 1H, CH), 4.60 (t, 1H, CH), 7.10–7.80 (m, 9H, Ar-H), 8.10 (s, 1H, C₄-thiazole-H). ^{13}C NMR (300 MHz, DMSO, TMS): δ 168.90 (C-CONH), 162.80, 146.93, 108.01 (C-thiazole ring), 151.11, 49.13, 42.49 (C-pyrazole), 184.06, 143.45, 131.47, 129.91, 129.89, 129.20, 129.21, 128.55, 127.03, 126.76. MS (M^+ +H; CI *iso*-butane): m/z = 391 (100.0%). Anal. calcd. for $\text{C}_{20}\text{H}_{18}\text{N}_6\text{OS}$ (mol. wt.: 390.46): C, 61.52; H, 4.65; N, 21.52. Found: C, 61.35; H, 4.57; N, 21.64.

N-{5-[[4-[5-(1H-Indol-3-yl)-4,5-dihydro-1H-pyrazol-3-yl]phenyl]diazanyl]-1,3-thiazol-2-yl}acetamide 10b. Brown solid; yield: 38%; m.p.: 282–283 °C. IR ($\bar{\nu}/\text{cm}^{-1}$): 3258, 3214 (NH), 1675 (C=O) and 1645 (C=N) cm^{-1} . ^1H NMR ($\text{CDCl}_3/\text{CF}_3\text{COOD}$): δ/ppm = 2.20 (s, 3H, CH₃), 2.80 (dd, 1H, CH), 3.35 (dd, 1H, CH), 4.70 (t, 1H, CH), 7.15–7.80 (m, 8H, Ar-H), 8.10 (s, 1H, C₄-thiazole-H), 9.10 (s, 1H, C₂-pyrrol-H). ^{13}C NMR (300 MHz, DMSO, TMS): δ 168.90 (C-CONH), 162.80, 146.93, 108.01 (C-thiazole ring), 151.11, 49.13, 42.49 (C-pyrazole), 184.06, 143.45, 131.47, 129.91, 129.89, 129.20, 129.21, 128.55, 127.03, 126.76. MS (M^+ +H; CI *iso*-butane): m/z = 430 (100.0%). Anal. calcd. for $\text{C}_{22}\text{H}_{19}\text{N}_7\text{OS}$ (mol. wt.: 429.5): C, 61.52; H, 4.46; N, 22.83. Found: C, 61.42; H, 4.36; N, 22.68.

N-{5-[[4-[5-(3-Methylthiophen-2-yl)-4,5-dihydro-1H-pyrazol-3-yl]phenyl]diazanyl]-1,3-thiazol-2-yl}acetamide 10c. Brown solid; yield: 60%; m.p.: 215–218 °C. IR ($\bar{\nu}/\text{cm}^{-1}$): 3211 (NH), 1668 (C=O) and 1642 (C=N) cm^{-1} . ^1H NMR ($\text{CDCl}_3/\text{CF}_3\text{COOD}$): δ/ppm = 2.20 (s, 3H, CH₃), 2.45 (s, 3H, CH₃), 2.75 (dd, 1H, CH), 3.30 (dd, 1H, CH), 4.75 (t, 1H, CH), 7.10–7.70 (m, 6H, Ar-H), 8.10 (s, 1H, C₄-thiazole-H). ^{13}C NMR (300 MHz, DMSO, TMS): δ 168.88 (C-CONH), 162.75, 147.04, 107.91 (C-thiazole ring), 151.09, 43.53, 41.74 (C-pyrazole), 133.91, 124.60, 124.09 (C-thiophene), 184.04, 131.11, 129.88, 129.86, 129.20, 129.21. MS (M^+ ; EI): m/z = 410 (7.4%), 236 (8.1%), 213 (15.3%), 185 (18.2%), 149 (40.0%), 129 (50.8%), 111 (40.4%), 97 (59.5%), 83 (61.6%), 77 (20.1%), 57 (100.0%). Anal. calcd. for $\text{C}_{19}\text{H}_{18}\text{N}_6\text{OS}_2$ (mol. wt.: 410.52): C, 55.59; H, 4.42; N, 20.47. Found: C, 55.41; H, 4.37; N, 20.29.

2.3. Biological assays

The potential antioxidant activities of the synthesized compounds were carried out using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging activity assay [13], and the percentage inhibition of the DPPH radical by the samples was calculated according to the following equation:

$$\text{Inhibition \%} = \frac{A_b - A_a}{A_b} \cdot 100 \quad (1)$$

A_b is the absorption of the blank sample ($t = 0$ min) and A_a is the absorption of the tested compounds or standard substance solution ($t = 30$ min).

The acute toxicity of the selected compounds **3**, **5**, **9b** and **10c** based on *in vitro* antioxidant potential results was monitored in an animal model system by the LD₅₀ value and biochemical analysis, including glutathione-S-transferase (GST) and lactic dehydrogenase (LDH) activities according to OECD guideline [14–16]. The toxicological effects were observed in terms of mortality, and are expressed as LD₅₀ and the number of animals dying during the period was noted. Antioxidant activity, based on the *in vitro* antioxidant potential results, of the selected compounds **3**, **5**, **9b** and **10c** was determined *in vivo*. Male albino rats were quarantined and handled according to the institutional ethical guidelines and under the standard laboratory conditions as detailed in S1. For the enzyme activity measurements, glutathione-S-transferase (GST) is expressed in μmol of glutathione conjugated with 1-chloro-2,4-dinitrobenzene (CDNB) produced/mg protein/min [17], Super Oxide Dismutase (SOD) is expressed in units/mg protein [18], and glutathione reduced (GSH-Rd) levels in the liver and kidney tissues were determined according to the Ellman method [19] and the results are expressed in mg/g protein. The protein levels were determined at 595 nm using Coomassie Brilliant Blue G-250 as a protein-binding dye, and bovine serum albumin (BSA) was used as a protein standard [20].

Each of the measurements described was carried out in three replicate experiments, and the results are recorded as mean \pm standard deviation. Significant differences were calculated at the level of $p \leq 0.05$.

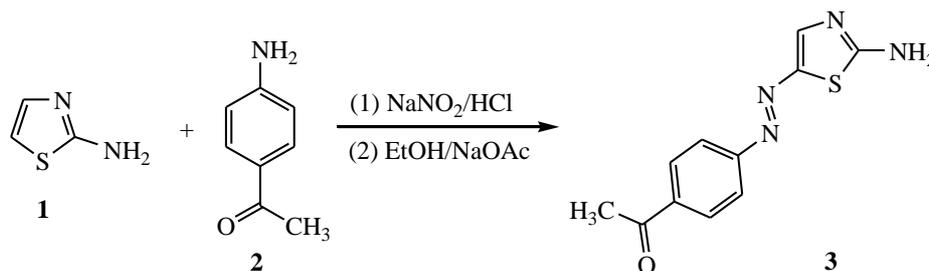
3. RESULTS AND DISCUSSION

3.1. Chemistry

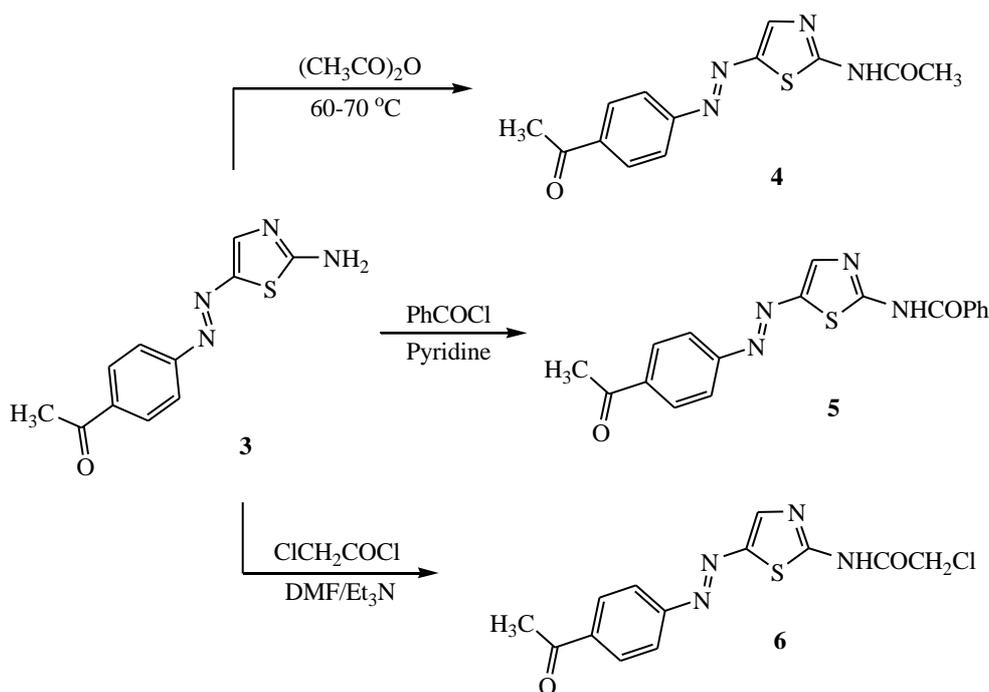
The biological properties associated with the thiazolidine derivatives prompted us to synthesize new thiazolidine derivatives **4–10** using standard functional group transformation, and to study their *in vitro* and *in vivo* toxicity and antioxidant function.

Firstly, 2-aminothiazole **1** was allowed to couple with 4-aminoacetophenone **2** to form a new 2-amino-5-(4-acetylphenylazo)-thiazole **3** under mild basic conditions (Scheme 1). The chemical structure of **3** was confirmed by its analytical and spectral data. The IR spectrum of **3** revealed intense bands at 3358 and 3244 cm^{-1} (NH_2), and 1668 cm^{-1} (CO, acetyl). The ¹H NMR spectrum of **3** displayed a singlet signal at δ 2.45 ppm due to the methyl protons (COCH_3), a singlet at δ 6.90 ppm due to the C₄-thiazole proton, two doublet signals at δ 7.50 ppm and 7.85 ppm due to the aromatic protons, and a broad singlet at δ 8.15 ppm due to NH_2 protons. The molecular structure of the synthesized 2-amino-5-(4-acetylphenylazo)-thiazole **3** was confirmed by mass spectroscopy. The mass spectrum of **3** showed a molecular ion peak at $m/z = 246$ (intensity 34.7%) corresponding to the molecular weight of the molecular formula $\text{C}_{11}\text{H}_{10}\text{N}_4\text{OS}$.

The new 5-arylo-2-aminothiazolidine **3** was investigated as the precursor compound of our study to explore its reactivity, where a series of chemical reactions were performed at the terminal amino and acetyl groups as shown in schemes 2–5. The reactivity of the thiazolyl amino group was detected *via* a series of custom reactions with various active carbonyl reagents (Scheme 2).



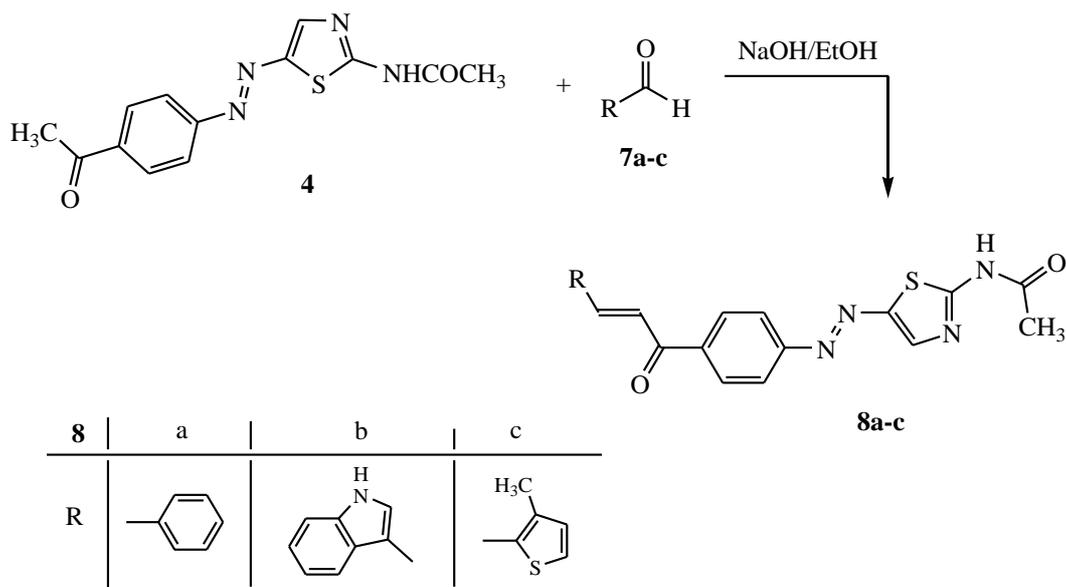
Scheme 1. Synthesis of 2-amino-5-(4-acetylphenylazo)-thiazole compound **3**



Scheme 2. Reactivity of amino groups towards various electrophilic reagents

As shown in scheme 2, a convenient acetylation reaction of 2-amino-5-(4-acetylphenylazo)-thiazole **3** by solvent-free acetylation with acetic anhydride at 60–70 °C afforded the *N*-acetylated product **4**. The chemical structure of 2-acetylamino-5-(4-acetylphenylazo)-thiazole **4** was confirmed by its analytical and spectral data. The IR spectrum revealed intense bands at 3164 cm^{-1} (NH stretching), and 1682 and 1665 cm^{-1} (two C=O stretching bands). The ^1H NMR spectrum of **4** displayed two singlet signals at δ 2.25 and 2.45 ppm for two methyl protons, a singlet at δ 6.90 for the C₄-thiazole proton, two doublet signals at δ 7.45 ppm and 7.85 ppm due to the aromatic protons, and a singlet signal at δ 11.15 ppm for the NH proton. The electrophilic attack of the benzoyl cation towards the 2-amino-5-(4-acetylphenylazo)-thiazole **3** in presence of pyridine and at room temperature yielded the benzoyl amino derivative **5**. The synthesized 2-benzoylamino-5-(4-acetylphenylazo)-thiazole **5** was confirmed by its analytical and spectral data. The IR spectrum of **5** was characterized by the presence of strong absorption bands at 3147 cm^{-1} corresponding to NH (NHCOMe), at 1675 cm^{-1} corresponding to the C=O stretching (COCH₃), and at 1662 cm^{-1} corresponding to the carbonyl group of the benzoyl function (COPh). The ^1H NMR spectrum of **5** in CF_3COOD was characterized by the presence of a singlet peak at δ 2.57 ppm corresponding to methyl protons, as well as multiplet peaks in the region δ 7.26–7.90 ppm corresponding

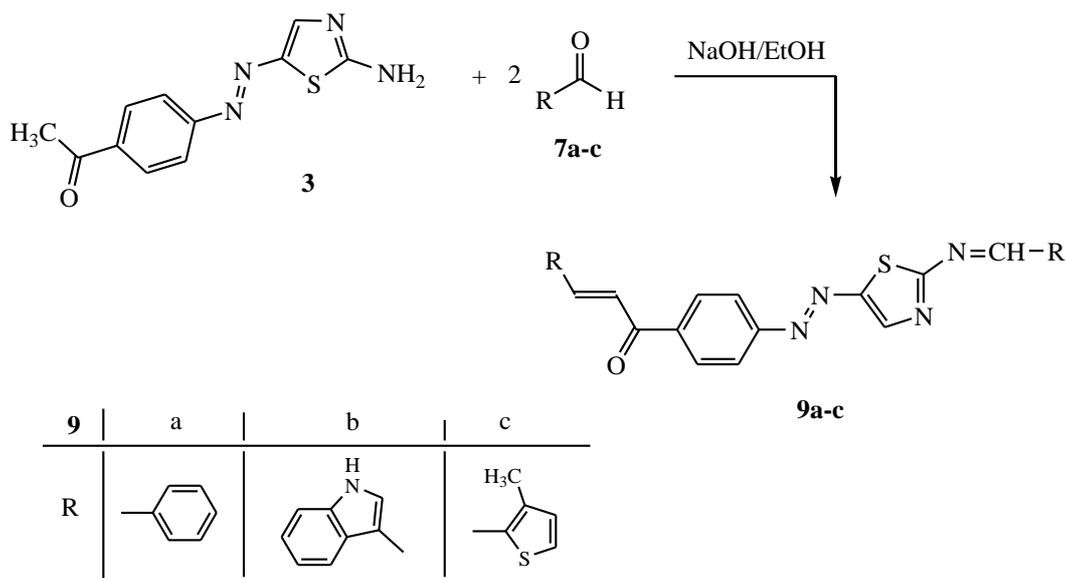
to the aromatic protons, and a singlet signal at δ 8.16 ppm corresponding to the C₄-thiazole proton. A further reaction of the highly activated chloroacetyl chloride reagent with 2-amino-5-(4-acetylphenylazo)-thiazole **3** under basic conditions using triethylamine (TEA) in the presence of dimethylformamide (DMF) was performed to produce the chloroacetyl amino derivative **6**. The chemical structure of 2-chloroacetyl amino-5-(4-acetylphenylazo)-thiazole **6** was elucidated by its analytical and spectral data. The IR spectrum of **6** revealed intense bands at 3172 cm^{-1} corresponding to NH (CONH), at 1684 cm^{-1} corresponding to the carbonyl of the NHCOCH_2Cl function, and at 1670 cm^{-1} due to the carbonyl group of the acetyl function. The ^1H NMR spectrum of **6** displayed the singlet signal of methylene protons (NHCOCH_2Cl) at δ 4.11 ppm in addition to other signals at δ 2.62 ppm (singlet for three protons, CH₃), δ 7.26 and 7.78 ppm (two doublets for the aromatic protons), and a singlet signal at δ 8.07 due to the C₄-thiazole proton. On the other hand, the reactivity of the functionalized terminal acetyl groups was studied *via* Claisen-Schmidt condensation using aromatic and/or heterolytic aldehydes in alkaline medium [18–23]. Therefore, condensation of 2-acetylamino-5-(4-acetylphenylazo)-thiazole **4** with an equimolar ratio of aromatic and/or heterocyclic aldehydes **7a–c** in sodium hydroxide and water/ethanol medium led to the exclusive formation of chalcones **8a–c** (Scheme 3).



Scheme 3. Condensation reaction of 2-acetyl-5-(4-acetylphenylazo)-thiazole **4** with an equimolar ratio of aromatic and/or heterocyclic aldehydes **7a-c** to form chalcones **8a-c**

The chemical structures of *N*-{5-[4-(3-aryl(heteryl)prop-2-enoyl)phenyl]diazonyl]-1,3-thiazol-2-yl}acetamide derivatives **8a-c** were confirmed by their elemental analyses and spectral data. The IR spectrum of **8a-c** generally exhibited an intense band in the range 1655–1662 cm^{-1} for the acetyl carbonyl group, whereas another band in the range 1670–1678 cm^{-1} was seen due to the amidic carbonyl group. The ^1H NMR spectrum displayed a shift for the thiazole proton at δ 8.05–8.10 and a multiplet signal in the region δ 7.10–7.90 due to the aromatic and the two olefinic pro-

tons CH=CH. The reaction of aldehydes **7a-c** with the appropriate 5-arylo-2-aminothiazole starting compound **3** (2:1) under the same reaction conditions led to the formation of the chalcone-imine derivatives **9a-c** (Scheme 4), where the electrophilic carbon atoms of aldehydes **7a-c** can be targeted by nucleophilic attack of the amines. As a result, the compound in which the C=O double bond is replaced by a C=N double bond was formed. This type of compound is known as an imine, or Schiff base.



Scheme 4. Reaction of 5-arylo-2-aminothiazole **3** with aldehydes **7a-c** (1:2) to form the chalcone-imine derivatives **9a-c**

Mechanistically, the formation of an imine is analogous to hemiacetal and hemiketal formation, and involves two steps. First, the amine's nitrogen acts as a nucleophile, attacking the carbonyl carbon [24]. The next step might be expected that the carbonyl carbon was attacked by a second amine to

form a compound with a carbon bound to two amine groups (the nitrogen version of a ketal). Instead, the nitrogen is deprotonated, and the electrons from this N-H bond 'push' the oxygen off of the carbon, leaving a C=N double bond (an imine) and a displaced water molecule, as shown in Figure 1.

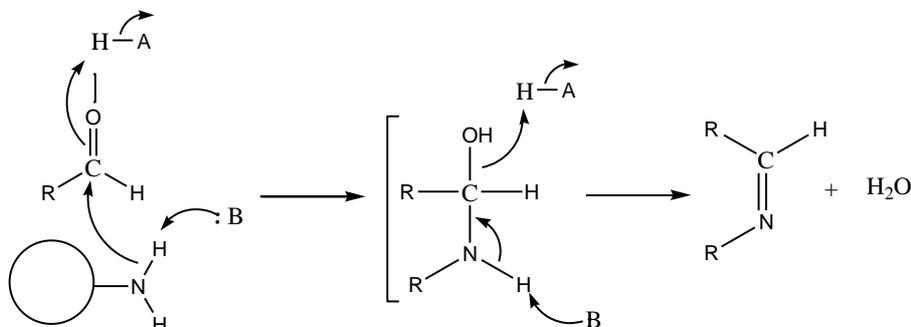
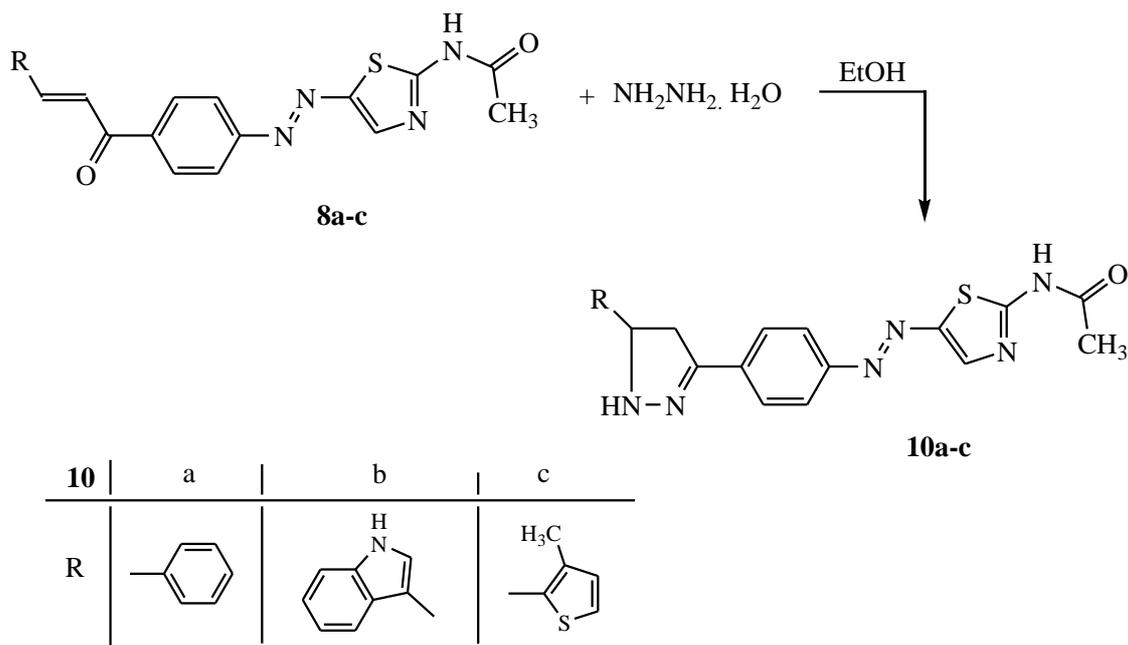


Fig. 1. Mechanism of imine formation

The chemical structures of 3-aryl(heteroaryl)-1-{4-[(2-{[aryl(heteryl)-methylidene]amino}-1,3-thiazol-5-yl)diazenyl]phenyl}-prop-2-en-1-one derivatives **9a-c** were confirmed by their analytical and spectral data. A band in the range of 1655–1661 cm^{-1} due to the acetyl group was investigated, while the ¹H NMR spectrum displayed a singlet signal at δ 8.05 due to the thiazole proton, and a multiplet signal in the region δ 7.10–7.90 due to the aromatic protons and the two olefinic protons CH=CH.

Further reaction of α,β -unsaturated carbonyl derivatives **8a-c** with hydrazine hydrate, proceed-

ing throughout the hydrazone intermediate and subsequent dehydrogenation, yielded the pyrazole derivatives **10a-c** (Scheme 5) [22]. The chemical structures of *N*-(5-{(E)-[4-(5-phenyl-4,5-dihydro-1H-pyrazol-3-yl)phenyl]diazenyl}-1,3-thiazol-2-yl)acetamide **10a-c** were confirmed by analytical and spectral data. The IR spectrum revealed sharp bands in the range 1675–1668 cm^{-1} due to the amidic carbonyl group, and 1635–1645 cm^{-1} due to the pyrazole CH=N, whereas the ¹H NMR spectrum exhibited a singlet signal at δ 8.10 due to the thiazole proton and a multiplet signal in the region δ 7.10–7.80 due to the aromatic protons.



Scheme 5. Reaction of α,β -unsaturated carbonyl derivatives **8a-c** with hydrazine hydrate to form the pyrazole derivatives **10a-c**

3.2. Biological assessments

In the present study, several monoazo S/N heterocyclic compounds (**3-6**, **8a-c** – **10a-c**) were evaluated for toxicity and antioxidant activity. Firstly, antioxidant activities of the synthesized compounds were screened *in vitro* using the DPPH assay. Toxicity of the selected compounds based on *in vitro* antioxidant results was monitored in an animal model system, including the LD₅₀ value, and GPT and LDH enzyme activities. *In vivo* antioxidant activities of the selected compounds **3**, **5**, **9b**, and **10c**, based on their *in vitro* antioxidant and toxicity results, were performed and also biochemical analyses, including SOD and GST enzymes and reduced glutathione (GSH-Rd) activities were evaluated.

3.2.1. *In vitro* antioxidant activity of synthesized compounds

Antioxidant capacities for the synthesized compounds were screened using the DPPH radical-scavenging assay incorporating a metastable free radical that is capable of accepting hydrogen radicals from antioxidants in solution. The reaction between DPPH and antioxidant can be monitored by the decrease in absorbance of the colored free radical. Results of the inhibition of the DPPH radical by tested compounds are listed in Table 1 and plotted in Figure 2. The inhibition affects against DPPH were not noticeably changed by doubling the concentration, and were in the order **10c** > **9b** > **5** > **3**. This may be due to the presence of thiazole, pyrazole, and thiophene rings in the same compound **10c** and fewer rings in the others.

Table 1

Antioxidant activity (%) of the synthesized compounds 3-6 and 9a-c -10a-c at different concentrations using the DPPH free radical method

Compound #	% Antioxidant activity	
	50 mg	100 mg
Trolox (Control)	91.21	90.81
3	45	46.9
4	32.8	54.6
5	66.8	71.3
6	42.6	39.43
9a	37.9	33.9
9b	40.1	42
9c	31.6	34.8
10a	27.9	30.2
10b	72.87	78.4
10c	38.9	40.5

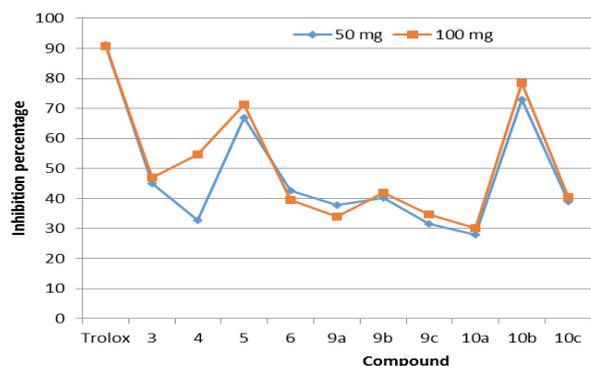


Fig. 2. Inhibition percentage of DPPH free radical by the synthesized compounds **3-6** and **9a-c -10a-c** at different concentrations

3.2.2. Acute toxicity studies

Acute toxicity of selected compounds **3**, **5**, **9b**, and **10c** were monitored in an animal model system. Biochemical parameters, including LD₅₀, and GPT (an enzyme that allows determination of the liver function as an indicator of liver cell damage) and LDH (an enzyme that is used as a marker of tissue breakdown) activities, were recorded during treatment with graded doses of 50–500 mg/kg body weight of each selected synthesized compound. Toxicity results showed that no mortality was observed during administration with the selected synthesized compounds up to 500 mg/kg body weight. The GPT and LDH enzyme activities are listed in Table 2 and plotted in Figure 3. There was no significant effect after administration with the selected synthesized compounds up to 500 mg/kg body weight, which indicates that there was no toxicity of the synthesized compounds up to high concentrations.

Table 2

Effect of the selected synthesized compounds 3, 5, 9b and 10c on LDH and GPT enzymes at different concentrations

Compound #	Enzymes activity units/liter of blood serum				
	50 mg	100 mg	200 mg	300 mg	500 mg
3-LDH	25	32	34	29	36
3-GPT	45	43	35	40	41
5-LDH	27	30	35	37	28
5-GPT	42	41	46	44	41
9b-LDH	22	21	24	26	25
9b-GPT	40	35	38	32	36
10c-LDH	20	21	24	19	18
10c-GPT	39	41	42	43	40

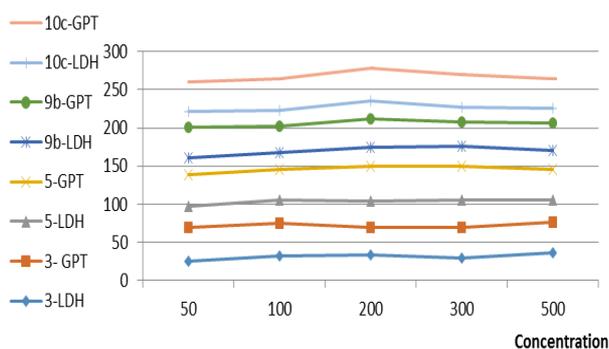


Fig. 3. Effect of different compounds on LDH and GPT enzymes

Table 3

Effect of compounds 3, 5, 9b and 10c on SOD, GST, and GSH-Rd in control, vitamin E and treated rat groups

Treatment compound #	SOD (Units/mg protein)	GST $\mu\text{mol/mg protein}$	GSH-Rd (mg/g protein)
Normal control (Group 1)	12.15 \pm 0.14 ^b	3.32 \pm 0.09 ^b	5.11 \pm 0.61 ^b
Vitamin E (Group 2)	15.35 \pm 0.43 ^{a,b}	5.12 \pm 0.25 ^{a,b}	7.41 \pm 0.43 ^{a,b}
3 (50 mg/kg; Group 3)	11.21 \pm 0.11 ^{a,b}	3.90 \pm 0.19 ^{a,b}	6.34 \pm 0.22 ^{a,b}
3 (100 mg/kg; Group 4)	10.61 \pm 0.73 ^{a,b}	3.15 \pm 0.11 ^{a,b}	5.81 \pm 0.61 ^{a,b}
5 (50 mg/kg; Group 5)	11.02 \pm 0.52 ^{a,b}	2.89 \pm 0.15 ^{a,b}	5.04 \pm 0.31 ^{a,b}
5 (100 mg/kg; Group 6)	10.76 \pm 0.13 ^{a,b}	2.91 \pm 0.07 ^{a,b}	4.74 \pm 0.21 ^{a,b}
9b (50mg/kg; Group 7)	11.02 \pm 0.17 ^{a,b}	1.87 \pm 0.09 ^{a,b}	5.34 \pm 0.41 ^{a,b}
9b (100mg/kg; Group 8)	11.21 \pm 0.19 ^{a,b}	1.95 \pm 0.15 ^{a,b}	5.67 \pm 0.54 ^{a,b}
10c (50mg/kg; Group 9)	13.48 \pm 0.42 ^a	4.91 \pm 0.70 ^a	5.82 \pm 0.32 ^a
10c (100 mg/kg; Group 10)	13.97 \pm 0.29 ^a	4.25 \pm 0.15 ^a	5.70 \pm 0.63 ^a

Values are mean \pm SD, $n = 6$.

a: $p < 0.05$ compared with vehicle control group.

b: $p < 0.05$ compared with vitamin E group.

n : number of rats in each group.

There were significant increases ($p < 0.05$) in SOD and GST activities in the 10c treated groups at doses of 50 and 100 mg/kg compared to the control (untreated) group ($p < 0.05$) and nearly matched the treated group with vitamin E as standard antioxidant. No significant differences were observed between the doses (50 and 100 mg/kg) with any tested compound.

4. CONCLUSIONS

A new 2-amino-5-(4-acetylphenylazo)-thiazole was synthesized, and the reactivity of both the amino group and the aryl substituent *via* various active carbonyl reagents and custom chemical reactions yielded new bioactive chalcone, imine, and pyrazole derivatives. The synthesized derivatives were structurally confirmed and biologically screened *in vitro* and *in vivo* for their toxicity and

3.2.3. In vivo antioxidant activity

SOD, GST, and GSH-Rd levels were measured *in vivo* to monitor the antioxidant activities, and are listed in Table 3. SOD and GST are antioxidant enzymes that protect cells from oxidative stress of highly reactive free radicals formed in normal conditions (food metabolites) or abnormal conditions (presence of environment pollutants) [25]. These enzymes are induced by the generation of free radicals in cells.

antioxidant activity. The synthesized compounds showed a noticeable inhibition effect against DPPH and significant increases in antioxidant enzyme activities in the treated rats groups at doses of 50 and 100 mg/kg compared to the control group ($p < 0.05$). In addition, the synthesized compounds showed no significant toxicity at high concentrations based on liver function enzyme evaluation.

Acknowledgments. The authors would like to extend their thanks to the staff of the Chemistry Department, Faculty of Science, Taif University, KSA, for their technical support.

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