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Synthesis and Antibacterial Activity of Some New Functionalized Derivatives of 4-amino-5-benzyl-4*H*-[1,2,4]-triazole-3-thiol

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Abstract: ***The use of 4-amino-5-benzyl-4H-[1,2,4]triazole-3-thiol (1) as a precursor to synthesize some new biologically active heterocycles has been found to be effective. Condensation of 1 with appropriate aldehydes gave the new Schiff bases 2a, b, which either by cyclization with thioglycolic acid gave 3a, b, or by Mannich reaction using morpholine gave 4a, b. Reaction of 1 with different halogen compounds such as, benzenesulphonyl chloride, chloroacetamide, chloroacetone, phenyl acetyl chloride, chloroacetic acid, oxalyl chloride and dichloroacetic acid afforded the newly compounds 5, 6, 7, 8, 9, 10 and 11 respectively. The chemical structures of the prepared compounds were characterized by considering the data of their elemental analyses as well as their spectral data of their FT-IR, ^{1}H NMR, ^{13}C NMR and Mass spectra. Investigation of the antibacterial activity of these compounds was done by the paper disc technique. Some of the tested compounds showed high and favorable antibacterial activity.***

Keyword: Schiff bases, Mannich reaction, halogen compounds, 1,2,4-triazoles, antibacterial activity.

1. Introduction

A huge volume of published literature about 1,2,4-triazoles and their derivatives plays an important role among the class of heterocycles and have received much attention due to their versatile biological and therapeutical activities including antibacterial activity [1]-[4], antifungal activity [5]-[7], antiviral activity [8]-[9], antitubercular activity [10]-[11], anticonvulsant activity [12]-[14], antioxidant activity [15], anti-inflammatory activity [16]-[18], antitumor activity [19], [20], analgesic activity [21]-[23], antidepressant activity [24], and anthelmintic activity [25]. Owing to the above significance and the existing biological activity of 1,2,4-triazoles, it is of interest to synthesize new derivatives of 4-Amino-5-benzyl-4*H*-[1,2,4]triazole-3-thiol (**1**) as well as the investigation of their antibacterial activities.

2. Materials and Methods

Melting points (uncorrected) were recorded on an Electrothermal melting apparatus. The IR spectra were recorded on a Shimadzu FT-IR 8101 PC spectrometer. The ^{1}H and ^{13}C NMR spectra were determined in DMSO-d_6 at 300 MHz on a Varian Mercury VX 300 NMR spectrometer; Chemical shifts are reported in ppm with TMS as an internal standard and are given in δ units. Electron impact mass spectra were obtained at 70 eV using a GCMS-QP 1000 EX spectrometer. Elemental analyses, mass and NMR spectra were carried out at the Microanalytical Center of Cairo University.

2.1 ***Synthesis of 5-Benzyl-4-[(4-benzyloxy-benzylidene)-amino]-2,4-dihydro-[1,2,4]triazole -3-thione (2a) and 5-Benzyl-4-[(2,4-dimethoxy-benzylidene)-amino]-2,4-dihydro -[1,2,4]triazole-3-thione (2b)***: General procedure: A mixture of compound **1** (2 gm, 10 mmol) and 4-benzyloxybenzaldehyde and/or 2,4-dimethoxybenzaldehyde (10 mmol) in 50 ml absolute ethanol in presence of few drops of hydrochloric acid was refluxed for 1 h. After cooling, the solid crystals were filtered off and crystallized from ethanol to give the Schiff bases **2**a and/or **2b** respectively.

5-Benzyl-4-[(4-benzyloxy-benzylidene)-amino]-2,4-dihydro -[1,2,4]triazole-3-thione (2a). This compound was obtained as yellow crystals, 3.3 g (82%); mp 130- 132 °C; IR (KBr): 3329 (NH), 3044 (CH-aromatic), 2886 (CH_2's), 1594 (C=N) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 10.08 (s, 1H, NH), 9.9 (s, 1H, N=C-H), 7.86-7.05 (m, 14H, Ar'H), 5.16 (s, 2H, O-CH_2Ph), 4.16 (s, 2H, CH_2ph); ^{13}C NMR (DMSO-*d6*): δ= 30.64 (CH_2Ph), 69.54 (O-CH_2ph), 115.40, 124.75, 126.82, 127.71, 127. 95, 128. 43, 128. 60, 128. 84, 130.46, 135.11 and 136.40 (Ar-C), 150.21 (N=C-N, triazole), 161.48 (N=CH-), 161.86 (Ar-C-O-benzyl), and 162. 83 (C=S); Ms: m/z 400 (M^{+}), 380, 333, 290, 227, 168, 132; Anal. Calcd. for $C_{23}H_{20}N_4OS$: C, 68.98; H, 5.03; N, 13.99; S, 8.01. Found: C, 68.87; H, 5.11; N, 13.91; S, 8.12.

5-Benzyl-4-[(2,4-dimethoxy-benzylidene)-amino]-2,4-dihydro-[1,2,4]triazole-3-thione (2b). This compound was obtained as yellow crystals, 2.8 g (79%); mp170- 172 °C; IR (KBr): 3212 (NH), 3004 (CH-aromatic), 2825 (CH-aliphatic), 1594 (C=N) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 10.42 (s, 1H, NH), 9.72 (s, 1H, N=C-H), 7.55-6.95 (m, 8H, Ar'H), 4.16 (s, 2H, CH_2ph), 3.87 and 3.84 two (s, 3H, OCH_3); Ms: m/z 354 (M^{+}), 326, 291, 267, 191, 163, 132; Anal. Calcd. for $C_{18}H_{18}N_4O_2S$: C, 61.00; H, 5.12; N, 15.81; S, 9.05. Found: C, 61.07; H, 5.21; N, 15.59; S, 9.10.

2.2 ***Synthesis of 3-(3-Benzyl-5-mercapto-[1,2,4]triazol-4-yl)-2-(4-benzyloxy-phenyl)-thiazolidin-4-one (3a) and 3-(3-Benzyl-5-mercapto-[1,2,4]triazol-4-yl)-2-(2,4-dimethoxy-phenyl)-thiazolidin-4-one (3b)***: Thioglycolic acid (0.9 ml, 10 mmol) was added to a solution of the Schiff base **2**a and/or **2b** (10 mmol) in 30 ml dioxane, then the reaction mixture was refluxed for 5 hrs. After cooling, it was gradually poured onto crushed ice and kept overnight. The solid formed was filtered off and crystallized from ethanol to give compound **3a** and/or **3b** respectively.

3-(3-Benzyl-5-mercapto-[1,2,4]triazol-4-yl)-2-(4-benzyloxy-phenyl)-thiazolidin-4-one (3a). This compound was obtained as white crystals, 3 g (63%); mp 100- 102 °C; IR (KBr): 3301 (NH), 3059 (CH-aromatic), 2858 (CH_2's), 1671 (C=O) cm^{-1}; ^1H NMR (DMSO-*d6*): δ 9.76 (s, 1H, 1NH), 7.85-7.16 (m, 14H, Ar'H), 5.42 (s, 1H, thiazolidin-H), 5.22 (s, 2H, O-CH_2Ph), 4.16 (s, 2H, CH_2ph), 3.3 (s, 2H, CH_2, thiazolidin); Ms: m/z 474 ($M^{\mp}$), 432, 404, 362, 207, 165, 132; Anal. Calcd. for $C_{25}H_{22}N_4O_2S_2$: C, 63.27; H, 4.67; N, 11.81; S, 13.51. Found: C, 63.38; H, 4.73; N, 11.89; S, 13.26.

3-(3-Benzyl-5-mercapto-[1,2,4]triazol-4-yl)-2-(2,4-dimethoxy-phenyl)-thiazolidin-4-one (3b). This compound was obtained as white crystals, 2.6 g (60%); mp 163-165 °C; IR (KBr): 3204 (NH), 3034 (CH-aromatic), 2825 (CH_2's), 1682 (C=O) cm^{-1}; ^1H NMR (DMSO-*d6*): δ 10.35 (s, 1H, 1NH), 7.85-7.16 (m, 8H, Ar'H), 5.47 (s, 1H, thiazolidin-H), 4.16 (s, 2H, CH_2ph), 3.81 and 3.77 two (s, 3H, OCH_3) 3.24 (s, 2H, CH_2, thiazolidin); Ms: m/z 428 ($M^{\mp}$), 398, 326, 263, 206, 147, 132; Anal. Calcd. for $C_{20}H_{20}N_4O_3S_2$: C, 56.06; H, 4.70; N, 13.07; S, 14.97. Found: C, 56.00; H, 4.68; N, 13.39; S, 14.73.

2.3 ***Synthesis of 5-Benzyl-4-[(4-benzyloxy-benzylidene)-amino]-2-morpholin-4-ylmethyl-2,4-dihydro-[1,2,4]triazole -3-thione (4a) and 5-Benzyl-4-[(2,4-dimethoxy-benzylidene)-amino]-2-morpholin-4-ylmethyl-2,4-dihydro-[1,2,4] triazole-3-thione (4b)***: General procedure: The Schiff base **2a** and/or **2b** (10 mmol) was dissolved in 20 ml dioxane at RT. Then, a solution of formaldehyde (37%, 1mL) and morpholine (0.87 ml, 10 mmol) in 20 ml dioxane was added dropwise with stirring. The reaction mixture was stirred at RT for 12 hours and left overnight in a freeze. Then the resulting mixture was poured on to crushed ice and the solid product was filtered off and recrystallized from ethanol to give compound **4a** and/or **4b** respectively.

5-Benzyl-4-[(4-benzyloxy-benzylidene)-amino]-2-morpholin-4-ylmethyl-2,4-dihydro-[1,2,4] triazole-3-thione (4a). This compound was obtained as yellowish white crystals, 3.6 g (72%); mp 118-120 °C; IR (KBr): 3023 (CH-aromatic), 2826 (CH_2's), 1595 (C=N) cm^{-1}; ^1H NMR (DMSO-*d6*): δ 9.71 (s, 1H, N=C-H), 7.88-7.17 (m, 14H, Ar'H), 5.45 (s, 2H, N-CH_2-N), 5.26 (s, 2H, O-CH_2Ph), 4.16 (s, 2H, CH_2ph), 3.33 (t, 4H, CH_2, morpholine), 2.4 (t, 4H, CH_2, morpholine); Ms: m/z 499 ($M^{\mp}$), 478, 320, 242, 209,104, 77; Anal. Calcd. for $C_{28}H_{29}N_5O_2S$: C, 67.31; H, 5.85; N, 14.02; S, 6.42. Found: C, 67.22; H, 5.98; N, 14.16; S, 6.24.

5-Benzyl-4-[(2,4-dimethoxy-benzylidene)-amino]-2-morpholin-4-ylmethyl-2,4-dihydro-[1,2,4]triazole-3-thione (4b). This compound was obtained as yellow crystals, 3 g (66%); mp 124- 126 °C; IR (KBr): 2940 (CH-aromatic), 2856 (CH_2's), 1591 (C=N) cm^{-1}; ^1H NMR (DMSO-*d6*): δ 10.2 (s, 1H, N=C-H), 7.39-7.13 (m, 8H, Ar'H), 5.06 (s, 2H, N-CH_2-N), 4.19 (s, 2H, CH_2ph), 3.81 and 3.77 two (s, 3H, OCH_3), 3.58 (t, 4H, CH_2, morpholine), 2.72 (t, 4H, CH_2, morpholine); ^{13}C NMR (DMSO-*d6*): δ= 30.57 (CH_2Ph), 50.37 (OCH_3), 55.48 (N-CH_2, morpholine), 56.45 (O-CH_2, morpholine), 66.05 (N-CH_2-N), 88,98, 109.37, 113.88, 120.52, 121.00, 126.86, 128.47 and 135.05 (Ar-C), 149.17 (N=C-N, triazole), 153.18 and 153.84 (2 Ar-C-OMe), 157.84 (N=CH-) and 162. 25 (C=S); Ms: m/z 453 ($M^{\mp}$), 382, 323, 262, 220, 147, 132; Anal. Calcd. for $C_{23}H_{27}N_5O_3S$: C, 60.91; H, 6.00; N, 15.44; S, 7.07. Found: C, 60.84; H, 6.07; N, 15.40; S, 7.11.

2.4 ***Synthesis of N-(3-Benzyl-5-mercapto-[1,2,4]triazol-4-yl)benzenesulfonamide (5)***: Benzenesulfonyl chloride (ml, 10 mmol) was added dropwisely with stirring in ice bath to a solution of the triazole **1** (2 gm, 10 mmol) in 10 ml pyridine . The reaction mixture was vigorously stirred for 3hrs. The reaction mixture was poured on to crushed ice and the solid product was filtered off and recrystallized from ethanol/water (1:1) as white crystals. 2.4 g (69%); mp 158-160 °C; IR (KBr): 3287 (NH), 3085 (CH-aromatic), 2935 (CH_2), 2325 (SH), 1624 (C=N) cm^{-1}; ^1H NMR (DMSO-*d6*): δ13.51(s, 1H, SH), 10.15 (s, 1H, NH), 7.34-7.24 (m, 10H, Ar'H), 4.08 (s, 2H, CH_2Ph); Ms: m/z 346 ($M^{\mp}$), 255, 205, 190, 189, 161, 132; Anal. Calcd. for $C_{15}H_{14}N_4O_2S_2$: C, 52.01; H, 4.07; N, 16.17; S, 18.51. Found: C, 52.12; H, 4.01; N, 16.24; S, 18.39.

2.5 ***Synthesis of 2-(4-Amino-5-benzyl-4H-[1,2,4]triazol-3-ylsulfanyl)-acetamide (6)***: To a solution of the triazole **1** (2 gm, 10 mmol) in dil. ethanolic KOH (30 mL, 10%), chloroacetamide (0.93 gm, 10 mmol) was added, and the reaction mixture was stirred at RT for 6 hrs. The reaction mixture was poured on to crushed ice and HCl. The solid formed was filtered off and crystallized from ethanol as white crystals. 2.1 g (79%); mp 162- 164 °C; IR (KBr): 3444, 3317 (NH_2's), 3085 (CH-aromatic), 2926 (CH_2's), 1673 (C=O), 1615 (C=N) cm^{-1}; ^1H NMR (DMSO-*d6*): δ 7.61 (b, 2H, $CONH_2$), 7.33-7.20 (m, 5H, Ar'H), 5. 91 (s, 2H, NH_2), 4.08 (s, 2H, CH_2CO), 3.81 (s, 2H, CH_2Ph); Ms: m/z 263 ($M^{\mp}$), 248, 232, 188, 176, 144, 132; Anal. Calcd. for $C_{11}H_{13}N_5OS$: C, 50.17; H, 4.98; N, 26.60; S, 12.18. Found: C, 50.36; H, 4.88; N, 26.53; S, 12.15.

2.6 ***Synthesis of 3-Benzyl-6-methyl-5H-[1,2,4] triazolo[3,4-b][1,3,4]thiadiazine (7), Phenyl-thioacetic acid S-(4-amino-5-benzyl-4H-[1,2,4] triazol-3-yl) ester (8) and 3-Benzyl-[1,2,4] triazolo[3,4-b][1,3,4]thiadiazin-6-one (9)***: General procedure: A mixture of compound **1** (2 gm, 10 mmol) and chloroacetone, phenyl acetylchloride and/or chloroacetic acid (10 mmol) in 20 ml methanol in presence of sodium acetate (0.82 gm, 10 mmol) was refluxed for 6 hrs. After cooling, the reaction mixture was poured onto crushed ice and kept overnight, the precipitate formed separated by filtration and crystallized from a proper solvent to give compound **7**, **8** and/or **9** respectively.

3-Benzyl-6-methyl-5H-[1,2,4]triazolo[3,4-b][1,3,4] thiadiazine (7). This compound was obtained as brown crystals, 1.7 g (69%); mp 220-222 °C; IR (KBr): 3198 (NH), 3030 (CH-aromatic), 2870 (CH-aliphatic), 1589 (C=N) cm^{-1}; ^1H NMR (DMSO-*d6*): δ 10.07 (s, 1H, NH), 7. 62 (s, 1H, C=CH-S), 7.31-7.21 (m, 5H, Ar'H), 3.45 (s, 2H, CH_2Ph), 1.32 (s, 3H, CH_3); Ms: m/z 244 ($M^{\mp}$); 226, 208, 197, 185, 155, 126; Anal. Calcd. for $C_{12}H_{12}N_4S$: C, 58.99; H, 4.95; N, 22.93; S, 13.12. Found: C, 58.90; H, 4.89; N, 22.88; S, 13.33.

Phenyl-thioacetic acid S-(4-amino-5-benzyl-4H-[1,2,4] triazol-3-yl) ester (8). This compound was obtained as brown crystals, 2.4 g (70%); mp 198- 200 °C; IR (KBr): 3277 (NH_2), 2935 (CH-aromatic), 2876 (CH_2's), 1698 (C=O) cm^{-1}; ^1H NMR (DMSO-*d6*): δ 7.38-7.08 (m, 10H, Ar'H), 3.85 (s, 2H, CH_2CO), 3.68 (s, 2H, CH_2Ph), 3.20 (b, 2H, NH_2); Ms: m/z 324 ($M^{\ddagger}$), 294, 217, 194, 150, 108, 91; Anal. Calcd. for $C_{17}H_{16}N_4OS$: C, 62.94; H, 4.97; N, 17.27; S, 9.88. Found: C, 62.81; H, 4.92; N, 17.55; S, 9.79.

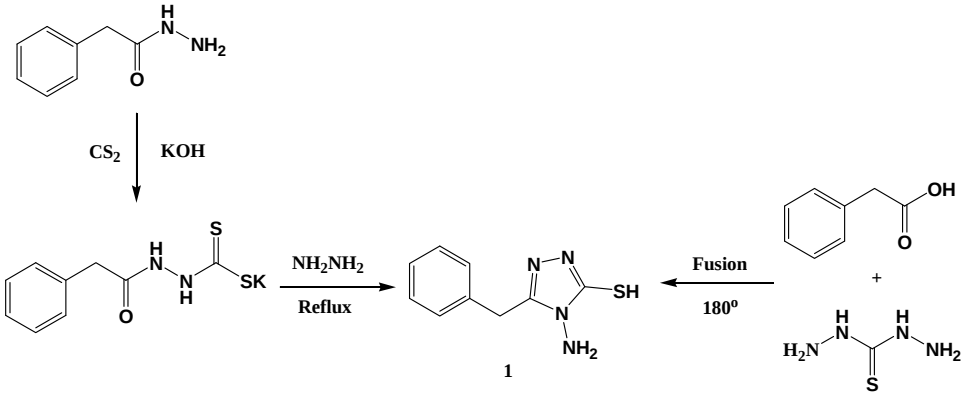
3-Benzyl-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazin-6-one (9). This compound was obtained as white crystals, 2 g (81%); mp 242- 244 °C; IR (KBr): 3197 (NH), 3030 (CH-aromatic), 2886 (CH_2's), 1685 (C=O) cm^{-1}; ^1H NMR (DMSO-*d6*): δ 10.08 (s, 2H, NH), 7.31-7.20 (m, 5H, Ar'H), 3.90 (s, 2H, S-CH_2CO), 3.62 (s, 2H, CH_2Ph),; Ms: m/z 246 ($M^{\ddagger}$), 229, 218, 201, 173, 155, 132; Anal. Calcd. for $C_{11}H_{10}N_4OS$: C, 53.64; H, 4.09; N, 22.75; S, 13.02. Found: C, 53.77; H, 4.01; N, 22.53; S, 13.19.

2.7 ***Synthesis of 3-Benzyl-[1,2,4]triazolo[3,4-b][1,3,4] thiadiazine-6,7-dione (10)***: A mixture of **1** (2 gm, 10 mmol) and oxalyl chloride (1.2 ml, 10 mmol) in 15 ml DMF was refluxed for 4 hrs. After cooling, the reaction mixture was poured on to crushed ice, the solid formed was filtered off and crystallized from acetic acid as white crystals. 1.85 g (71%); mp 216- 218 °C; IR (KBr): 3212 (NH), 3046 (CH-aromatic), 2871 (CH_2), 1701, 1679 (C=O's) cm^{-1}; ^1H NMR (DMSO-*d6*): δ10.22 (b, H, NH), 7.33-7.20 (m, 5H, Ar'H), 4.12 (s, 2H, CH_2Ph); Ms: m/z 260 ($M^{\ddagger}$), 217, 204, 189, 169, 132, 104; Anal. Calcd. for $C_{11}H_8N_4O_2S$: C, 50.76; H, 3.10; N, 21.53; S, 12.32. Found: C, 50.65; H, 3.18; N, 21.47; S, 12.41.

2.8 ***Synthesis of 7-(4-Amino-5-benzyl-4H-[1,2,4] triazol-3-ylsulfanyl)-3-benzyl-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazin-6-one (11)***: Compound **1** (2 gm, 10 mmol) and dichloroacetic acid (1.9 ml, 15 mmol) were added to an ethanolic KOH solution (30 mL, 10%) and the reaction mixture was refluxed for 3 hrs. The reaction mixture was poured onto crushed ice, a brown resin was formed and after decantation, the resin was trituration with petroleum ether until solidification. The solid formed was collected by filtration and crystallized from ethanol/ water (1:2) as brown crystals. 2.9 g (64%); mp 156- 158 °C; IR (KBr): 3436-3249 (NH_2, NH), 2926 (CH-aromatic), 2891 (CH_2's), 1670 (C=O) cm^{-1}; ^1H NMR (DMSO-*d6*): δ10.19 (s, 1H, NH), 7.44-7.06 (m, 10H, Ar'H), 4.34 (s, 1H, S-CH-S), 3.78 and 3.64 two (s, 2H, CH_2Ph), 3.36 (b, 2H, NH_2); Ms: m/z 450 ($M^{\ddagger}$), 407, 333, 268, 245, 202, 147, 56; Anal. Calcd. for $C_{20}H_{18}N_8OS_2$: C, 53.32; H, 4.03; N, 24.87; S, 14.23. Found: C, 53.25; H, 4.12; N, 24.96; S, 14.12.

3. Results and Discussion

In the literature, two methods have been reported for the preparation of 4-amino-5-benzyl-4*H*-[1,2,4]triazole-3-thiol (**1**), either by treatment of phenylacetic acid hydrazide with carbon disulfide in ethanolic potassium hydroxide, followed by refluxing the resulted potassium salt with hydrazine hydrate (Method **A**, 53% yield) [26], [27], or by fusion of phenylacetic acid with thiocarbohydrazide at 180° (Method **B**, 68% yield) [28]. In the present work, the method **B** was used for the preparation of compound **1** due to the higher yield reaction (Scheme 1).

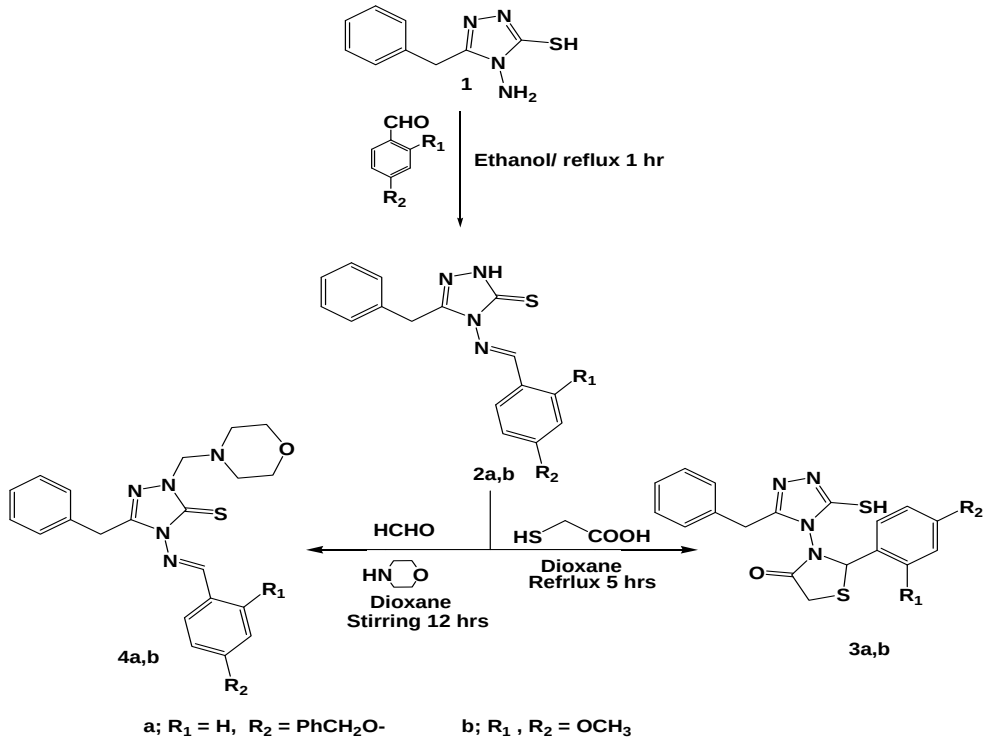


Scheme 1

Scheme 1: Two methods for the preparation of compound **1**

Thus, boiling of compound **1** in ethanolic solution of 4-benzloxy-benzaldehyde and/or 2,4-dimethoxy-benzaldehyde in presence of few drops of HCl afforded the corresponding new Schiff bases **2a** and **2b**, respectively. The ^1H NMR spectra of **2a, b** showed the absence of the amino group of compound **1** and the presence of the proton of azomethine linkage (N=CH) as a singlet downfield at 9.9 and 9.72 ppm, respectively. The ^{13}C NMR spectrum of compound **4a** showed seventeen different signals for seventeen different carbon atoms which gives great evidence for the proposed structure [Experimental part].

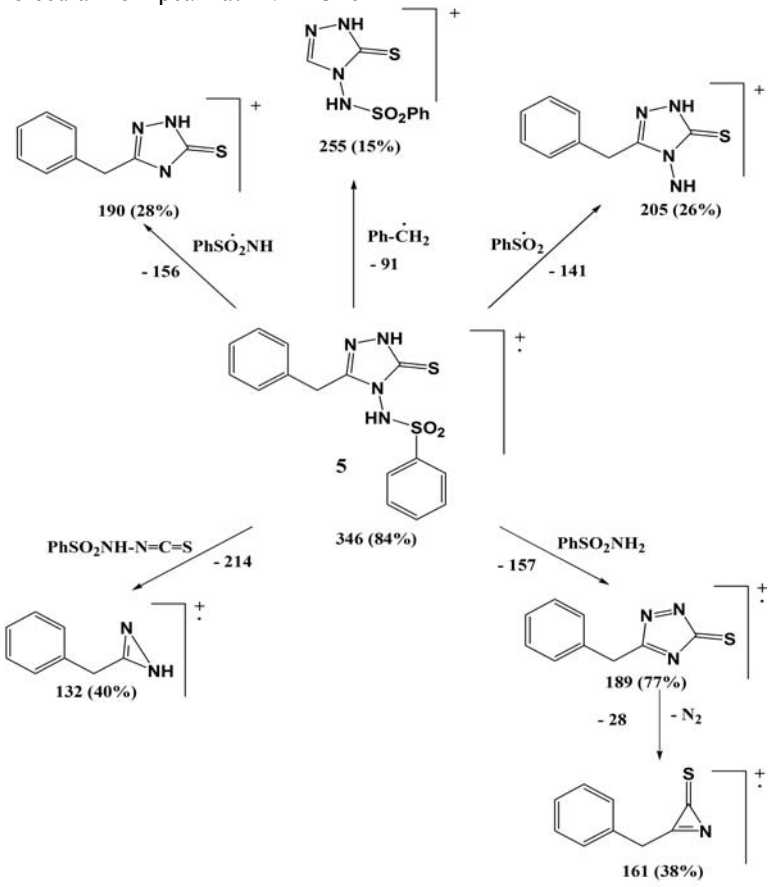
Refluxing of both Schiff bases **2a**, **b** with thioglycolic acid in dioxane gave 1,2,4-triazol-4-yl-thiazolidin-4-one derivatives **3a, b**. Also, the Schiff bases **2a**, **b** were reacted with formaldehyde in the presence of morpholine to obtain the corresponding Mannich bases **4a, b** (Scheme 2). The elemental analyses and spectroscopic data are consistent with the assigned structures of **3a, b** and **4a, b.** The ^{13}C NMR of compound **4b** showed eighteen different signals for eighteen different carbon atoms which adds additional confirmation for the proposed structure [Experimental part].



Scheme 2

Scheme 2: Formation of Schiff bases **2a,b**, thiazolidin-4-one derivatives **3a,b** and Mannich bases **4a,b**.

The reactivity of the triazole **1** towards different halogen compounds has been investigated. Thus, stirring of compound **1** with benzenesulphonyl chloride in pyridine gave compound **5** via HCl elimination. The mass spectrum of compound **5** showed molecular ion peak at m/z= 346 (84%) and various characteristic peaks at 255 (15%), 205 (26%), 190 (28%), 189 (77%), 161 (38%) and 132 (40%). Scheme 3 shows the fragmentation pattern for compound **5**, which confirms its structure.



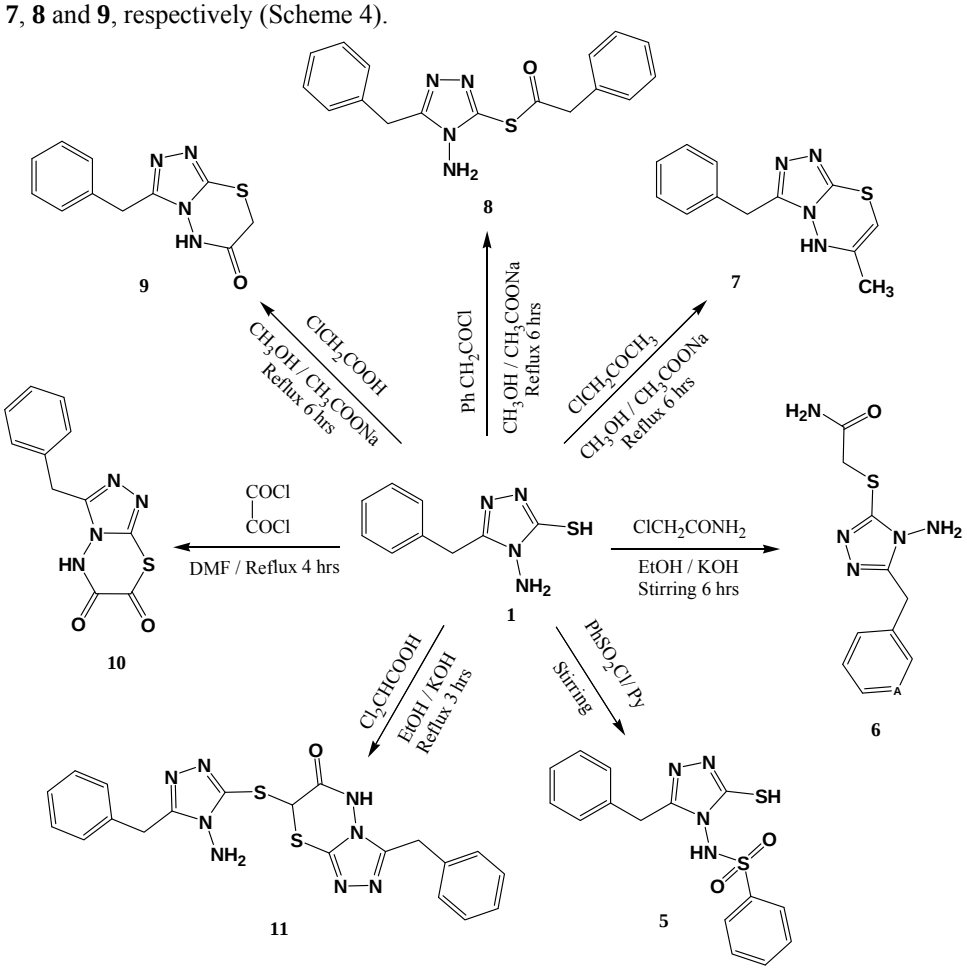
Scheme 3

Scheme 3: Mass fragmentation pattern of compound **5**

When compound **1** was allowed to react with chloroacetamide in ethanolic KOH, compound **6** assigned as 2-(4-Amino-5-benzyl-4H-[1,2,4]triazol-3-ylsulfanyl)-acetamide was obtained (Scheme 4). The IR spectrum of **6** showed absorption bands at 3444, 3317 for (NH_2), 3085 (CH-aromatic), 2926 (CH_2's) and 1673 for (C=O) cm^{-1}.

Hoping to expand the biological activity investigation of these derivatives, compound **1** was next reacted with chloro-compounds such as chloroacetone, phenyl acetylchloride and chloroacetic acid in methanol/ sodium acetate to give the corresponding **7**, **8** and **9**, respectively (Scheme 4).

The structure of **7** was confirmed from its Mass and ^1H NMR data. The Mass spectrum showed molecular ion peak at m/z= 244 and the ^{1} H NMR showed signals at 10.07 corresponding to (NH), singlet at δ 7. 62 for the alkene proton (C=CH-S) and a singlet at 1.32 for the (CH_3) group. The IR of **8** showed bands at 3277 for (NH_2), 2876 for (CH_2's) and 1698 cm^{-1} for (C=O) and ^1H NMR showed signals at δ 3.85 and 3.68 for the two methylene groups of (CH_2CO) and (CH_2Ph) and appearance (NH_2) at δ 3.20. The structure of compound **9** was confirmed from its full analysis [Experimental part].

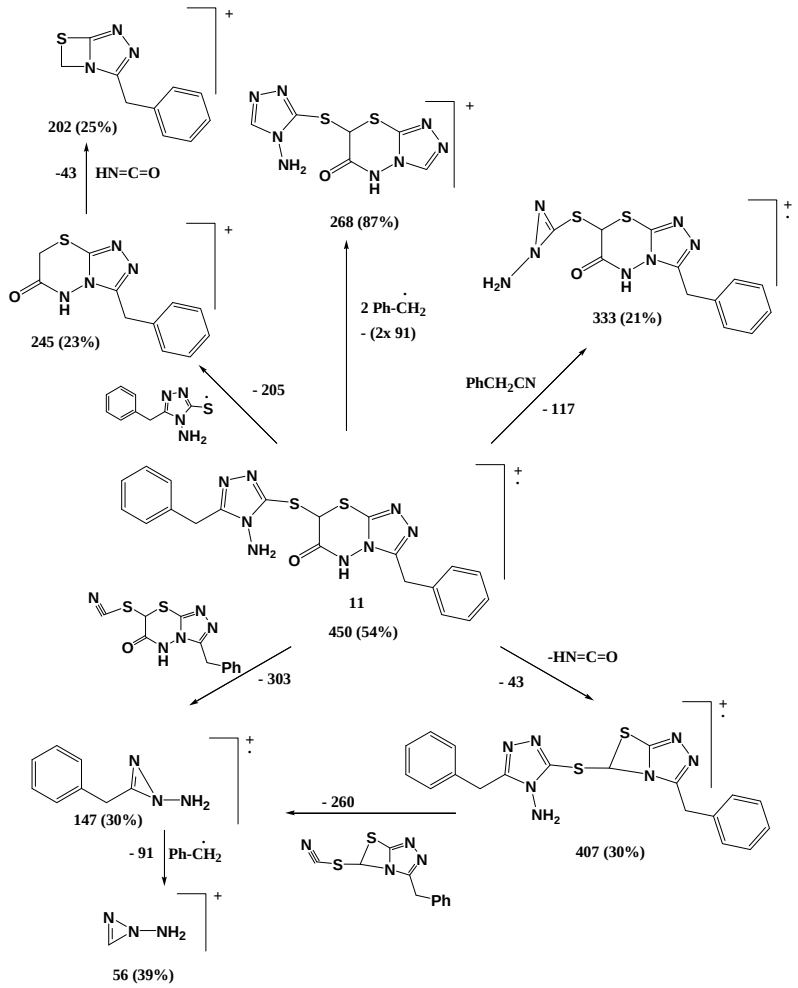


Scheme 4

Scheme 4: Reactions of compound **1** with different halogen reagents

In dimethylformamide, compound **1** was refluxed with oxalyl chloride to give the corresponding triazolo[3,4-b][1,3,4]thiadiazine-6,7-dione derivative **10**. The structure of **10** was confirmed from its IR, ^{1}H-NMR, MS and elemental analysis [Experimental part].

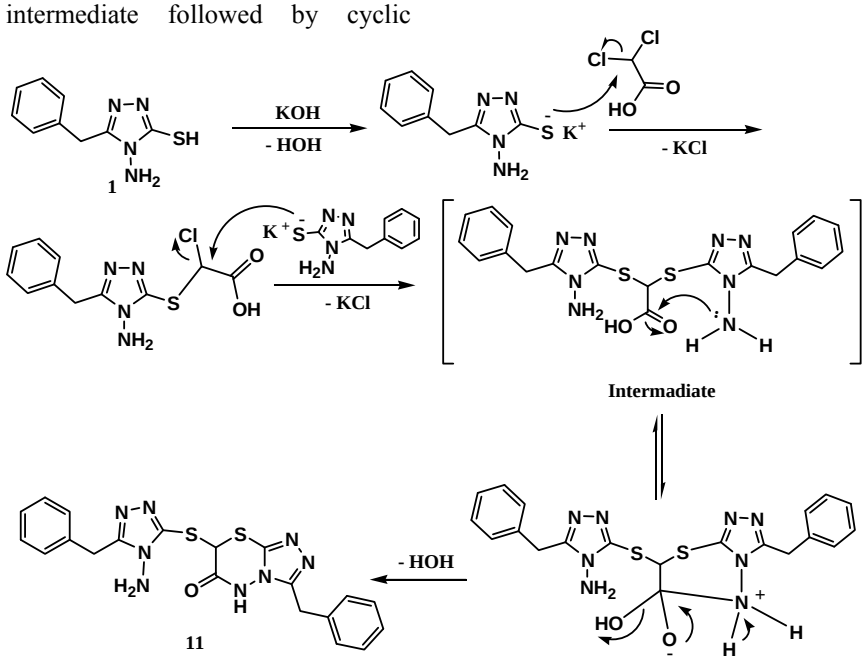
Compound **11** assigned as 7-(4-Amino-5-benzyl-4H-[1,2,4]triazol-3-ylsulfanyl)-3-benzyl-[1,2,4]triazolo[3,4-b] [1,3,4] thiadiazin-6-one was obtained from the reaction of compound **1** with dichloroacetic acid in ethanolic KOH (Scheme 4). The elemental analysis and spectroscopic data are in agreement with the assigned structure. Thus, the IR showed bands at 3436-3249 for (NH_2, NH) and at 1670 cm^{-1} for (C=O), ^{1}H NMR showed two singlets at δ10.19 and 3.36 for NH and NH_2, respectively, presence of singlet proton at δ 4.34 for (S-CH-S) and two peaks at δ 3.78 and 3.64 for the two (CH_2Ph), and the mass spectrum showed molecular ion peak at m/z= 450 (54%) corresponding to the formula ($C_{20}H_{18}N_8OS$), and characteristic peaks at 407 (30%), 333 (28%), 268 (87%), 245 (23%), 202 (25%), 147 (30%) and 56 (39%). Scheme 5 shows the fragmentation pattern for compound **11**, which supports the proposed structure.



Scheme 5

Scheme 5: Mass fragmentation pattern of compound **11**.

Formation of compound **11** may proceed via reaction of two molecules of **1** with one molecule of dichloroacetic acid to give an unstable intermediate followed by cyclic condensation through losing of water according to the proposed mechanism (Scheme 6).



Scheme 6

Scheme 6: The suggested reaction mechanism of **1** to give compound **11**

3.1 Antimicrobial Activity

Bacterial source and culture condition:

The used Bacterial strains were Gram negative bacteria including *E. Coli* (ATCC 25922) and Gram positive bacteria *Enterococcus faecalis* (ATCC 29212). Mueller-Hinton Agar was used as culture media (gl^{-1}) [29], Beef extract, 3.0; Peptone, 17.5; Starch, 1.5; Agar, 17, pH= 7.3 ± 0.1. The plates were incubated at 37ºC for 24 – 48 hrs.

Paper disc technique: Antibacterial activity was determined against the above strains using the paper disc assay method [30]. Whatman number 1 filter paper disc of 6.0 mm diameter was sterilized by autoclaving for 20 min at 121 °C. The sterile discs were impregnated with the spaced apart and plates were incubated at 37°C for 24- 48 hrs [31]. Chloramphenicol 30 µg/disc was used as a positive control. Diameter of the growth inhibition halos caused by the tested compounds were measured and expressed in millimeter. All the assays were carried out in triplicate.

Table 1: Effect of the synthesized compounds (**1- 11**) on bacterial growth (mm).

Sample No.	Bacterial growth inhibition zone diameter (mm)	
	Gram (-ve) Bacteria	Gram (+ve) Bacteria
	E. Coli	*Enterococcus faecalis*
1	**7**	**5**
2a	**6**	-----
2b	**6**	-----
3a	**8**	**7**
3b	**6**	-----
4a	**9**	**7**
4b	-----	-----
5	**9**	-----
6	**9**	**7**
7	**9**	-----
8	**7**	**7**
9	**7**	**7**
10	**7**	**6**
11	-----	-----
***Choramphenicol* 30 µg (Control)**	**18**	**18**

E. coli (Escherichia coli) is the name of a germ, or bacterium that lives in the digestive tracts of humans and animals. Many types of *E. coli* can cause bloody diarrhea and urinary tract infections. Some strains of E. coli bacteria may also cause severe anemia or kidney failure [32]. Also, *Enterococci* are Gram-positive *cocci* that often occur in pairs (diplococci) or short chains. The important clinical infections caused by *Enterococcus* include urinary tract infections, bacteremia, bacterial endocarditis, diverticulitis, and meningitis [33].

The antibacterial activity of the synthesized compounds **1-11** were carried out on the growth of two pathogenic bacteria (*E. Coli* and *Enterococcus faecalis*). The data obtained in Table (1) indicate that 12/14 of these compounds have effects on *E. Coli* bacteria where the great inhibition (9 mm) was observed by the Mannich base **4a**, benzenesulfonamide **5**, acetamide **6** and the triazolothiadiazine **7**, but the low inhibition (6 mm) was appeared by Schiff bases **2a**, **b** and the thiazolidinone **3b**. The triazole **1**, thiazolidinone **3a**, Phenyl-thioacetic acid ester **8**, thiadiazinone **9** and thiadiazinedione **10** showed moderate inhibition (7- 8 mm), while the Mannich base **4b** and the triazolothiadiazinone **11** showed no activity.

The inhibition effect was decreased on *Enterococcus faecalis* and also 7/14 only of the tested compounds showed moderate and low inhibition effect (5- 7 mm).

4. Conclusion

In summary, 4-amino-5-benzyl-4*H*-[1,2,4]triazole-3-thiol (**1**) has been utilized as a key starting material in the synthesis of many novel heterocyclic compounds **2- 11**. The constitution of these compounds assigned on the basis of IR, ^{1}H ^{13}C NMR, mass spectra and elemental analyses were found to be in correlation with the desired structure. The antimicrobial activity screening revealed that the compounds **1- 10** have significant antimicrobial activity. Compounds **4a**, **5**, **6** and **7** have high biological activity against gram (-ve) bacteria, and compounds **1**, **3a**, **8, 9** and **10** showed moderate inhibition effect.

On the other hand, compounds **1**, **3a**, **4a**, **6**, **8**, **9** and **10** showed moderate inhibitions effect against gram (+ve) bacteria, while compounds **2a**, **2b**, **3b**, **4b**, **5**, **7** and **11** showed no activity. The results are promising and show that the fine tuning of the structures **4a**, **5**, **6** and **7** can lead to some new antimicrobial agents in treating microbial infections.

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