New Heterocyclic Condensed Systems with Bridgehead Nitrogen. Ring Formation of Thiadiazine Cycle Fused to 1,2,4-Triazole by Reactions with Alpha-Halocarbonyl Compounds

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Synthesis of a series of 3-[4-(4-X-phenylsulfonyl)phenyl]-6-(substituted-phenyl)-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazines ***2-7a,b*** *(X=Cl, Br) by reaction of different substituted phenacyl bromide with 4-amino-5-[4-(4-X-phenylsulfonyl)phenyl]-4H-1,2,4-triazole-3-thiols* ***1*** *(X=Cl, Br) is described. The newly synthesized compounds were characterized by elemental analysis IR, ^{1}H-NMR and ^{13}C-NMR data. These compounds were tested for their antimicrobial activities against Gram-negative bacteria: Escherichia coli ATCC 11775; Pseudomonas aeruginosa ATCC 9027 and Gram-positive bacteria: Staphylococcus aureus ATCC 12600; Bacillus subtilis ATCC 6633, by using disc-diffusion assay. The preliminary results of antimicrobial activities indicated that the tested compounds exhibited a moderate to low activity against tested strains.*

Keywords: α-halocarbonyl compound structure; triazolothiadiazines; antibacterial activity

In the last few decades, the chemistry of 1,2,4-triazoles and their fused heterocyclic derivatives has received considerable attention owing to their synthetic and effective biological importance. Moreover, sulphur containing heterocycles represents an important group of sulphur compounds that are promising for use in practical applications. Among these heterocycles, the mercapto-substituted 1,2,4-triazole ring systems have been well studied and so far a variety of biological activities have been reported for a large number of their derivatives, such as antimicrobial, antifungal, anti-tubercular, anti-mycobacterial, anticancer, diuretic and hypoglycemic properties [1-8].

In addition to these important biological applications, 4-amino-3-mercapto-1,2,4-triazoles are also of great utility in preparative organic chemistry, for example, in the present of various reagents, undergo different types of reactions to yield other heterocyclic compounds, *e.g.*, thiazolotriazoles, triazolothiadiazoles, triazolothiazines, triazolothiazepines and triazolothiadiazines. Thus, by reaction with α-halocarbonyl compounds results in the formation of triazolothiadiazines [9], which may exhibit antimicrobial, analgesic, antihelmintic, antitubercular, plant growth regulating, antiviral, diuretic and anticancer properties [10-15].

On the other hand, many of the halogen containing heterocycles has attracted attention due to the ability of halogen to act as polar hydrogen or hydroxy mimic. Substitution of hydrogen by halogen has been a strategy in designing molecules for biological studies [16]. Therefore it was envisaged that chemical entities with 1,2,4-triazolo[3,4-b][1,3,4]thiadiazines containing halogenoaryl moiety would result in compounds with enhanced biological activities [17-20].

Prompted by these investigations and in continuation of our research work on the synthesis of novel condensed heterocyclic compounds with potential biological activity [21,22], we selected 4-amino-5-[4-(4-X-phenylsulfonyl) phenyl]-4*H*-1,2,4-triazole-3-thiols **1** (X=Cl, Br), a versatile substrate for mono and difunctional electrophiles by virtue of its vicinal amino and thiol groups, as key intermediate to obtain new [1,2,4]triazolo[3,4-b] [1,3,4]thiadiazines which may exhibit antibacterial activity.

Experimental part

The melting points were determined with Boetius apparatus and are uncorrected. The IR spectra were recorded on a FTS-135 BIO-RAD or Vertex 70 Brucker apparatus in KBr pellets (4000-400 cm^{-1} range). The NMR spectra were registered on a VARIAN GEMINI 300 BB apparatus working at 300 MHz for ^{1}H and at 75 MHz for ^{13}C and using TMS as internal standard.

Chemistry

The starting material, 4-amino-5-[4-(4-X-phenylsulfonyl)phenyl]-4*H*-1,2,4-triazole-3-thiol **1** (X = Cl, Br), was prepared in good yield earlier, by the reaction of the corresponding oxadiazole with hydrazine hydrate [23].

General procedure for the preparation of 3-[4-(4-X-phenylsulfonyl)phenyl]-6-(4-fluoro/3,4-dichlorophenyl)-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazines **2a,b; 3a,b**

A mixture of 4-amino-5-[4-(4-X-phenylsulfonyl)phenyl]-4*H*-1,2,4-triazole-3-thiol **1** (X =Cl, Br) (0.001 mol), 4-fluoro/3,4-diclorophenacyl bromide (0.001 mol) and a solution of ethanolic potassium hydroxide (10%, 1.5 mL) in ethanol (15 mL) was kept under reflux on a water bath for about 4 h. The reaction mixture was cooled and the precipitated solid was filtered, washed with water, dried and recrystallised from ethyl acetate/petroleum ether (1:1, v/v).

***3-[4-(4-chloro-phenylsulfonyl)phenyl]-6-(4-fluorophenyl)-7H-[1,2,4]triazolo[3,4-b] [1,3,4] thiadiazine* 2a**

60% yield; m.p.: 181-183°C; Anal. Calc. (%) for $C_{22}H_{14}ClFN_4O_2S_2$ (484.95 g/mol): C-54.49; H-2.91; N-11.55; Found: C-54.38; H-2.85; N-11.59

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IR (KBr, cm^{-1}): 3085m (CH$_{aryl}$), 2950m, 2899w (CH$_2$), 1600s, 1573s (C=C, C=N), 1326s, 1287s, 1160vs (SO$_2$), 1103s (C-F), 1009m (N-N), 690w (C-S-C), 763vs (C-Cl)

^{1}H-NMR (DMSO - d$_6$, δ, ppm): 8.28 (d; *J*=8.9 Hz; 2H; Ar-H); 8.17 (d; *J*=8.9 Hz; 2H; Ar-H); 8.10 (dd; J_{H-H}=8.7 Hz; J_{H-F}=7.2 Hz; 2H; 4-fluorophenyl protons); 7.94 (d; *J*=8.9 Hz; 2H; Ar-H); 7.86 (d; *J*=8.9 Hz; 2H; Ar-H); 7.42 (t; $J_{H-F\ ortho}$=8.8 Hz; 2H; 4-fluorophenyl protons); 4.45 (s; 2H; SCH$_2$)

^{13}C-NMR (DMSO-d$_6$, δ, ppm): 163.16 (d, *J*=274.3 Hz, C-F); 161.86 (C-3 triazolic ring), 156.55 (C-5 triazolic ring); 150.32 (C=N thiadiazine ring); 130.50 (d, *J*=8.9 Hz, 2C, C-F$_{meta}$); 141.54, 139.88, 133.01, 130.70, 129.54, 128.88, 128.47, 128.04, 127.41 (C-aromatic rings), 116.28 (d, *J*=22.0 Hz, 2C, C-F$_{ortho}$); 22.83 (SCH$_2$)

***3-[4-(4-bromo-phenylsulfonyl)phenyl]-6-(4-fluorophenyl)-7H-[1,2,4]triazolo[3,4-b] [1,3,4] thiadiazine* 2b**

65% yield; m.p.: 192-193°C; Anal. Calc. (%) for $C_{22}H_{14}BrFN_4O_2S_2$ (529.40 g/mol): C-49.91; H-2.67; N-10.58; Found: C-49.86; H-2.61; N-10.63

IR (KBr, cm^{-1}): 3089m (CH$_{aryl}$), 2947m, 2912w (CH$_2$), 1571s, 1504m (C=C, C=N), 1325vs, 1290m, 1158vs (SO$_2$), 1101s (C-F), 1008m (N-N), 696w (C-S-C), 574s (C-Br)

^{1}H-NMR (DMSO-d$_6$, δ, ppm): 8.14 (d; *J*=8.1 Hz; 2H; Ar-H); 8.05 (d; *J*=8.1 Hz; 2H; Ar-H); 7.92 (dd; J_{H-H} =8.3 Hz; $J_{H-F\ meta}$=6.9 Hz; 2H; 4-fluorophenyl protons); 7.75 (d; *J*=8.4 Hz; 2H; Ar-H); 7.66 (d; *J*=8.4 Hz; 2H; Ar-H); 7.26 (t; $J_{H-F\ ortho}$=8.3 Hz; 2H; 4-fluorophenyl protons); 4.49 (s; 2H; SCH$_2$)

^{13}C-NMR (DMSO-d$_6$, δ, ppm): 163.34 (d, *J*=265.4 Hz, C-F); 162.72 (C-3-triazolic ring), 160.68 (C-5 triazolic ring); 150.71 (C=N thiadiazine ring); 131.65 (d, *J*=8.7 Hz, 2C, C-F $_{meta}$); 142.01, 140.30, 133.57, 132.81, 130.19, 130.17, 129.40, 129.20, 128.54 (C-aromatic rings), 115.96 (d, *J*=22.8 Hz, 2C, C-F $_{ortho}$); 22.83 (SCH$_2$)

***3-[4-(4-chloro-phenylsulfonyl)phenyl]-6-(3,4-dichlorophenyl)-7H-[1,2,4]triazolo [3,4-b][1,3,4] thiadiazine* 3a**

73% yield; m.p.: 186-187°C; Anal. Calc. (%) for $C_{22}H_{13}Cl_3N_4O_2S_2$ (535.85 g/mol): C-49.31; H-2.45; N-10.46; Found: C-49.27; H-2.39; N-10.52

IR (KBr, cm^{-1}): 3088m (CH$_{aryl}$), 2953w, 2915m (CH$_2$), 1573s, 1549m, 1464m (C=C, C=N), 1319s, 1288m, 1159vs (SO$_2$), 1009m (N-N), 692w (C-S-C), 761vs (C-Cl)

^{1}H-NMR (DMSO-d$_6$, δ, ppm): 8.04 (d; *J*=8.1 Hz; 2H; Ar-H); 8.01 (d; *J*=8.1 Hz; 2H; Ar-H); 7.77 (d; *J*=8.5 Hz; 1H; Ar-H); 7.62 (d; *J*=8.5 Hz; 1H; Ar-H); 7.56 (s, 1H, Ar-H); 7.51 (d; *J*=8.3 Hz; 2H; Ar-H); 4.59 (s; 2H; SCH$_2$)

^{13}C-NMR (DMSO-d$_6$, δ, ppm): 162.70 (C-3-triazolic ring), 158.10 (C-5 triazolic ring); 150.70 (C=N thiadiazine ring); 141.65, 139.60, 138.64, 134.85, 133.03, 130.60, 129.60, 129.44, 129.00, 128.90, 128.80, 128.40, 127.72, 126.21 (C-aromatic rings), 22.78 (SCH$_2$)

***3-[4-(4-bromo-phenylsulfonyl)phenyl]-6-(3,4-dichlorophenyl)-7H-[1,2,4]triazolo [3,4-b][1,3,4] thiadiazine* 3b**

71% yield; m.p.: 202-204°C; Anal. Calc. (%) for $C_{22}H_{13}BrCl_2N_4O_2S_2$ (580.30 g/mol): C-45.53; H-2.26; N-9.65; Found: C-45.49; H-2.17; N-9.70

IR (KBr, cm^{-1}): 3083m (CH$_{aryl}$), 2948m, 2917w (CH$_2$), 1587s, 1553m, 1510m (C=C, C=N), 1322s, 1291m, 1160vs (SO$_2$), 1010m (N-N), 689w (C-S-C), 759vs (C-Cl), 570s (C-Br)

^{1}H-NMR (DMSO-d$_6$, δ, ppm): 8.02 (d; *J*=8.00 Hz; 2H; Ar-H); 7.98 (d; *J*=8.00 Hz; 2H; Ar-H); 7.90 (d; *J*=8.2 Hz; 2H; Ar-H); 7.72 (d; *J*=8.3 Hz; 1H; Ar-H); 7.68 (d; *J*=8.3 Hz; 1H; Ar-H); 7.54 (s, 1H, Ar-H); 7.50 (d; *J*=8.3 Hz; 2H; Ar-H); 4.52 (s; 2H; SCH$_2$)

^{13}C-NMR (DMSO-d$_6$, δ, ppm): 164.30 (C-3-triazolic ring), 158.60 (C-5 triazolic ring); 150.30 (C=N thiadiazine ring); 143.15, 141.12, 139.86, 134.60, 133.12, 131.20, 130.54, 130.31, 130.20, 129.44, 129.10, 128.64, 128.40, 128.02, 127.46 (C-aromatic rings), 22.80 (SCH$_2$)

General procedure for the preparation of 4-amino-5-[4-(4-X-phenylsulfonyl)phenyl]-3-(4-chloro/4-bromo-phenacylthio)-4H-1,2,4-triazole **4a,b; 5a,b**

A mixture of 4-amino-5-[4-(4-X-phenylsulfonyl)phenyl]-4*H*-1,2,4-triazole-3-thiol **1** (X=Cl, Br) (0.002 mol), 4-chloro/4-bromophenacyl bromide (0.002 mol), and triethylamine (0.2 mL, 0.002 mol) in ethanol (30 mL) was heated under reflux for 2 h, then cooled. The formed precipitate was filtered off, washed with ethanol, dried and finally recrystallized from absolute ethanol to give the corresponding derivatives **4,5a,b** in good yield.

***4-amino-5-[4-(4-chloro-phenylsulfonyl)phenyl]-3-(4-chloro-phenacylthio)-4H-1,2,4-triazole* 4a**

81% yield; m.p.: 202-204°C; Anal. Calc. (%) for $C_{22}H_{16}Cl_2N_4O_3S_2$ (519.42 g/mol): C-50.87; H-3.10; N-10.79; Found: C-50.80; H-3.16; N-10.71

IR (KBr, cm^{-1}): 3385m (NH$_2$), 3092m (CH$_{aryl}$), 2956w, 2916m (CH$_2$), 1677s (C=O), 1584s, 1472vs (C=C, C=N), 1324s, 1287m, 1157vs (SO$_2$), 1010m (N-N), 766vs (C-Cl), 632m (C-S-C)

^{1}H-NMR (DMSO-d$_6$, δ, ppm): 8.16 (d; *J*=8.5 Hz; 2H; Ar-H); 8.07 (d; *J*=8.5 Hz; 2H; Ar-H); 8.02 (d; *J*=8.5 Hz; 2H; Ar-H); 7.72 (d; *J*=8.5 Hz; 4H; Ar-H); 7.66 (d; *J*=8.5 Hz; 2H; Ar-H); 5.18 (s; 2H; SCH$_2$)

^{13}C-NMR (DMSO-d$_6$, δ, ppm): 191.74 (C=O), 164.50 (C-3-triazolic ring), 163.90 (C-5 triazolic ring); 143.12, 139.60, 139.04, 133.74, 133.10, 130.47, 129.65, 129.31, 129.10, 128.60, 127.77, 127.60 (C-aromatic rings), 37.16 (SCH$_2$)

***4-amino-5-[4-(4-bromo-phenylsulfonyl)phenyl]-3-(4-chloro-phenacylthio)-4H-1,2,4-triazole* 4b**

80% yield; m.p.: 222-223°C; Anal. Calc. (%) for $C_{22}H_{16}BrClN_4O_3S_2$ (563.87 g/mol): C-46.86; H-2.86; N-9.94; Found: C-46.79; H-2.90; N-9.87

IR (KBr, cm^{-1}): 3376m (NH$_2$), 3095m (CH$_{aryl}$), 2958m, 2920w (CH$_2$), 1680s (C=O), 1590vs, 1485s (C=C, C=N), 1328s, 1288m, 1160vs (SO$_2$), 1011m (N-N), 764vs (C-Cl), 638m (C-S-C), 576s (C-Br)

^{1}H-NMR (DMSO-d$_6$, δ, ppm): 8.50 (d; *J*=8.5 Hz; 2H; Ar-H); 8.21 (d; *J*=8.1 Hz; 2H; Ar-H); 8.00 (d; *J*=8.7 Hz; 2H; Ar-H); 7.90 (d; *J*=8.7 Hz; 2H; Ar-H); 7.70 (d; *J*=8.5 Hz; 2H; Ar-H); 7.63 (d; *J*=8.1 Hz; 2H; Ar-H); 5.24 (s; 2H; SCH$_2$)

^{13}C-NMR (DMSO-d$_6$, δ, ppm): 191.65 (C=O), 163.66 (C-3-triazolic ring), 161.90 (C-5 triazolic ring); 142.00, 140.84, 133.92, 133.63, 131.13, 131.61, 130.20, 129.40, 129.10, 128.51, 128.04 (C-aromatic rings), 37.66 (SCH$_2$)

***4-amino-5-[4-(4-chloro-phenylsulfonyl)phenyl]-3-(4-bromo-phenacylthio)-4H-1,2,4-triazole* 5a**

75% yield; m.p.: 208-210°C; Anal. Calc. (%) for $C_{22}H_{16}BrClN_4O_3S_2$ (563.87 g/mol): C-46.86; H-2.86; N-9.94; Found: C-46.80; H-2.94; N-9.88

IR (KBr, cm^{-1}): 3375m (NH$_2$), 3097m (CH$_{aryl}$), 2957m, 2917w (CH$_2$), 1678s (C=O), 1588m, 1573s, 1471vs (C=C, C=N), 1328s, 1292w, 1156vs (SO$_2$), 1010m (N-N), 764vs (C-Cl), 636m (C-S-C), 572s (C-Br)

^{1}H-NMR (DMSO-d$_6$, δ, ppm): 8.27 (d; *J*=8.5 Hz; 2H; Ar-H); 8.17 (d; *J*=8.8 Hz; 2H; Ar-H); 8.02 (d; *J*=8.5 Hz; 2H; Ar-H); 7.94 (d; *J*=8.8 Hz; 2H; Ar-H); 7.78 (d; *J*=8.5 Hz; 2H; Ar-H); 7.72 (d; *J*=8.5 Hz; 2H; Ar-H); 5.20 (s; 2H; SCH$_2$)

^{13}C-NMR (DMSO-d$_6$, δ, ppm): 191.90 (C=O), 161.72 (C-3-triazolic ring), 155.70 (C-5 triazolic ring); 143.11, 141.60, 139.14, 134.02, 132.20, 132.20, 130.50, 130.10, 129.70, 129.60, 128.03, 127.70 (C-aromatic rings), 37.03 (SCH$_2$)

***4-amino-5-[4-(4-bromo-phenylsulfonyl)phenyl]-3-(4-bromo-phenacylthio)-4H-1,2,4-triazole* 5b**

78% yield; m.p.: 228-230°C; Anal. Calc. (%) for $C_{22}H_{16}Br_2N_4O_3S_2$ (608.32 g/mol): C-43.44; H-2.65; N-9.21; Found: C-43.37; H-2.71; N-9.27

IR (KBr, cm^{-1}): 3326m (NH$_2$), 3094m (CH$_{aryl}$), 2955m, 2910m (CH$_2$), 1675s (C=O), 1586m, 1570s, 1478vs (C=C, C=N), 1326s, 1294m, 1158vs (SO$_2$), 1009m (N-N), 641m (C-S-C), 574vs (C-Br)

^{1}H-NMR (DMSO-d$_6$, δ, ppm): 8.21 (d; *J*=8.3 Hz; 2H; Ar-H); 8.02 (d; *J*=8.5 Hz; 2H; Ar-H); 7.95 (d; *J*=8.3 Hz; 2H; Ar-H); 7.82 (d; *J*=8.5 Hz; 2H; Ar-H); 7.72 (d; *J*=8.3 Hz; 2H; Ar-H); 7.61 (d; *J*=8.3 Hz; 2H; Ar-H); 5.18 (s; 2H; SCH$_2$)

^{13}C-NMR (DMSO-d$_6$, δ, ppm): 192.53 (C=O), 162.87 (C-3-triazolic ring), 154.90 (C-5 triazolic ring); 143.11, 141.58, 140.30, 134.12, 133.47, 132.17, 130.80, 130.27, 129.40, 129.34, 128.97, 128.20, 126.98 (C-aromatic rings), 37.75 (SCH$_2$)

General procedure for the preparation of 3-[4-(4-X-phenylsulfonyl)phenyl]-6-(4-chloro/4-bromo-phenyl)-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazines **6a,b; 7a,b**

Method 1

A mixture of 0.001 mol of 4-amino-5-[4-(4-X-phenylsulfonyl)phenyl]-3-(4-chloro/4-bromo-phenacylthio)-4*H*-1,2,4-triazole **4a,b** or **5a,b** and 20-30 mL of POCl$_3$ was heated for 2-3 h under reflux. Excess POCl$_3$ was evaporated under reduced pressure, and the oily residue was ground with 30 mL of diethyl ether. After crystallization, the precipitate was filtered off and recrystallized from appropriate solvent.

Method 2

A mixture of 4-amino-5-[4-(4-X-phenylsulfonyl)phenyl]-2,4-dihydro-3*H*-1,2,4-triazole-3-thione **1** (X=Cl, Br) (0.001 mol), 4-chloro/bromophenacyl bromide (0.0012 mol), in 10 mL of absolute ethanol was refluxed for 7 h. The reaction mixture was slowly quenched onto crushed ice with stirring and it was neutralized with solid sodium bicarbonate. The solid which separated after standing overnight was filtered, washed with cold water, dried and recrystallized from appropriate solvent to afford the title compounds **6,7a,b**.

***3-[4-(4-chloro-phenylsulfonyl)phenyl]-6-(4-chlorophenyl)-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazine* 6a**

70% yield; m.p.: 175-177°C (absolute ethanol); Anal. Calc. (%) for $C_{22}H_{14}Cl_2N_4O_2S_2$ (501.41 g/mol): C-52.70; H-2.81; N-11.17; Found: C-52.63; H-2.75; N-11.22

IR (KBr, cm^{-1}): 3090m (CH$_{aryl}$), 2953m, 2913m (CH$_2$), 1588s, 1554m, 1471vs (C=C, C=N), 1324s, 1292m, 1156vs (SO$_2$), 998s (N-N), 689w (C-S-C), 768vs (C-Cl)

^{1}H-NMR (DMSO-d$_6$, δ, ppm): 8.04 (d; *J*=8.5 Hz; 2H; Ar-H); 7.93 (d; *J*=9.0 Hz; 2H; Ar-H); 7.86 (d; *J*=9.0 Hz; 2H; Ar-H); 7.83 (d; *J*=8.5 Hz; 2H; Ar-H); 7.65 (d; *J*=8.8 Hz; 2H; Ar-H); 7.61 (d; *J*=8.8 Hz; 2H; Ar-H); 4.55 (s; 2H; SCH$_2$)

^{13}C-NMR (DMSO-d$_6$, δ, ppm): 162.74 (C-3-triazolic ring), 161.00 (C-5 triazolic ring); 157.53 (C=N thiadiazine ring); 141.12, 139.33, 139.14, 135.00, 133.72, 130.43, 130.12, 129.60, 129.10, 128.60, 127.73, 127.60 (C-aromatic rings), 20.60 (SCH$_2$)

***3-[4-(4-bromo-phenylsulfonyl)phenyl]-6-(4-chlorophenyl)-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazine* 6b**

68% yield; m.p.: 190-192°C (ethyl-acetate/petroleum ether 1:1, v/v); Anal. Calc. (%) for $C_{22}H_{14}BrClN_4O_2S_2$ (545.86 g/mol): C-48.41; H-2.59; N-10.26; Found: C-48.37; H-2.53; N-10.32

IR (KBr, cm^{-1}): 3085m (CH$_{aryl}$), 2958m, 2910m (CH$_2$), 1587s, 1573m, 1465s (C=C, C=N), 1322s, 1290m, 1159vs (SO$_2$), 1001m (N-N), 690m (C-S-C), 763vs (C-Cl), 578s (C-Br)

^{1}H-NMR (DMSO-d$_6$, δ, ppm): 7.98 (d; *J*=8.7 Hz; 2H; Ar-H); 7.71 (d; *J*=8.3 Hz; 2H; Ar-H); 7.66 (d; *J*=8.3 Hz; 2H; Ar-H); 7.56 (d; *J*=8.7 Hz; 2H; Ar-H); 7.52 (d; *J*=8.3 Hz; 2H; Ar-H); 7.41 (d; *J*=8.3 Hz; 2H; Ar-H); 4.42 (s; 2H; SCH$_2$)

^{13}C-NMR (DMSO-d$_6$, δ, ppm): 163.68 (C-3-triazolic ring), 159.20 (C-5 triazolic ring); 156.90 (C=N thiadiazine ring); 142.10, 139.90, 139.60, 134.90, 134.33, 131.20, 130.20, 129.62, 129.00, 128.10, 127.60, 126.21 (C-aromatic rings), 20.61 (SCH$_2$)

***3-[4-(4-chloro-phenylsulfonyl)phenyl]-6-(4-bromophenyl)-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazine* 7a**

77% yield; m.p.: 202-204°C (ethyl-acetate/petroleum ether 1:1, v/v); Anal. Calc. (%) for $C_{22}H_{14}BrClN_4O_2S_2$ (545.86 g/mol): C-48.41; H-2.59; N-10.26; Found: C-48.36; H-2.51; N-10.31

IR (KBr, cm^{-1}): 3087m (CH$_{aryl}$), 2955m, 2915m (CH$_2$), 1583s, 1473vs (C=C, C=N), 1325s, 1287m, 1157vs (SO$_2$), 1007m (N-N), 693m (C-S-C), 767vs (C-Cl), 578s (C-Br)

^{1}H-NMR (DMSO-d$_6$, δ, ppm): 8.08 (d; *J*=8.8 Hz; 2H; Ar-H); 8.02 (d; *J*=8.8 Hz; 2H; Ar-H); 7.96 (d; *J*=8.8 Hz; 2H; Ar-H); 7.80 (d; *J*=8.8 Hz; 2H; Ar-H); 7.61 (d; *J*=8.5 Hz; 2H; Ar-H); 7.44 (d; *J*=8.5 Hz; 2H; Ar-H); 4.42 (s; 2H; SCH$_2$)

^{13}C-NMR (DMSO-d$_6$, δ, ppm): 163.94 (C-3-triazolic ring), 160.10 (C-5 triazolic ring); 158.60 (C=N thiadiazine ring); 142.42, 140.00, 139.60, 133.12, 131.33, 130.50, 128.90, 128.52, 128.01, 127.80, 127.40, 127.32 (C-aromatic rings), 22.70 (SCH$_2$)

***3-[4-(4-bromo-phenylsulfonyl)phenyl]-6-(4-bromophenyl)-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazine* 7b**

75% yield; m.p.: 195-196°C (ethanol/DMF 2:1, v/v); Anal. Calc. (%) for $C_{22}H_{14}Br_2N_4O_2S_2$ (590.31 g/mol): C-44.76; H-2.39; N-9.49; Found: C-44.70; H-2.32; N-9.56

IR (KBr, cm^{-1}): 3090m (CH$_{aryl}$), 2950m, 2909m (CH$_2$), 1584s, 1470vs (C=C, C=N), 1324s, 1288m, 1156vs (SO$_2$), 1003m (N-N), 690m (C-S-C), 570vs (C-Br)

^{1}H-NMR (DMSO-d$_6$, δ, ppm): 8.04 (d; *J*=8.6 Hz; 2H; Ar-H); 8.01 (d; *J*=8.6 Hz; 2H; Ar-H); 7.98 (d; *J*=8.5 Hz; 2H; Ar-H); 7.83 (d; *J*=8.5 Hz; 2H; Ar-H); 7.66 (d; *J*=8.2 Hz; 2H; Ar-H); 7.51 (d; *J*=8.2 Hz; 2H; Ar-H); 4.45 (s; 2H; SCH$_2$)

^{13}C-NMR (DMSO-d$_6$, δ, ppm): 162.72 (C-3-triazolic ring), 160.68 (C-5 triazolic ring); 158.81 (C=N thiadiazine ring); 142.01, 140.30, 139.60, 134.62, 133.60, 131.40, 131.15, 130.20, 129.40, 128.10, 126.22, 124.70 (C-aromatic rings), 22.74 (SCH$_2$)

Antibacterial activity

The newly prepared compounds were screened for their antibacterial activity against Gram-negative bacteria: *Escherichia coli (Ec)* ATCC 11775, *Pseudomonas aeruginosa (Pa)* ATCC 9027 and Gram-positive bacteria: *Staphylococcus aureus (Sa)* ATCC 12600, *Bacillus subtilis (Bs)* ATCC 6633, by using disc-diffusion assay [24].

Suspensions in sterile peptone water from 24 h cultures of microorganisms were adjusted to 0.5 McFarland. Muller-Hinton Petri dishes of 90 mm were inoculated using these suspensions. The synthesized compounds were dissolved in dimethylsulfoxide (DMSO) to a final concentration of 2048 μg/mL. The discs (6 mm in diameter) were impregnated with 10 μL of each compounds and placed on the inoculated agar. DMSO impregnated discs were used as negative controls. Toxicity tests of the solvent, DMSO, showed that the concentrations used in antibacterial activity assays did not interfere in the microorganism's growth [25].

Chloramphenicol was used as a reference substance. The inoculated plates were incubated at 37°C for 24 h. Antibacterial activity in disc diffusion assay was evaluated by measuring the diameters of the inhibition zones generated by the tested substances using a ruler. Each assay in this experiment was repeated three times.

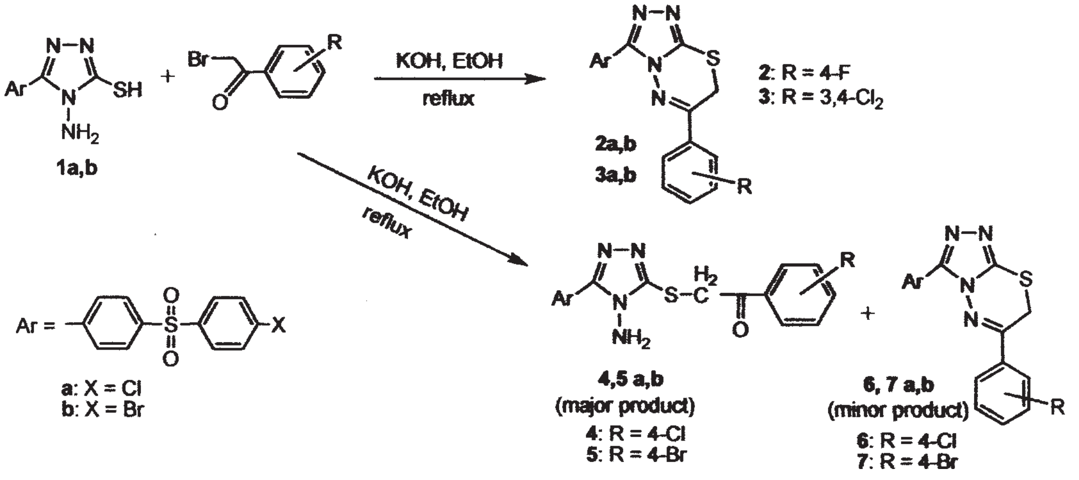
Results and discussions

Chemistry

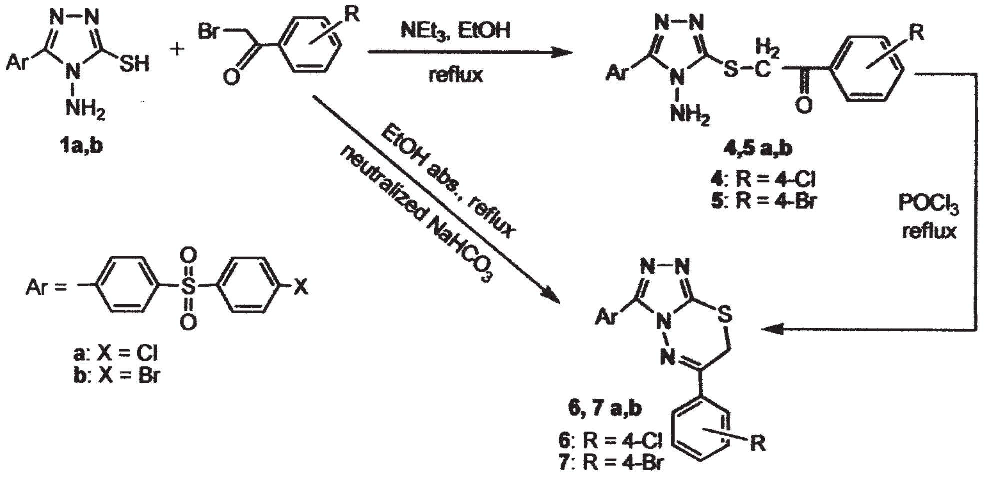
The synthetic utility of α-halocarbonyl compounds is well known for more than a century. They have been widely used as versatile intermediates for the synthesis of variety of heterocycles. So, generally, by reaction of different substituted phenacyl bromide with 4-amino-1,2,4-triazol-3-thiols should obtain triazolothiadiazine heterocycles by one-pot synthesis [26-30]. But, in our case, this reaction, depending on the α-halocarbonyl compound structure, has afforded different products under various reaction conditions.

We carried out a series of experiments on substrate **1a,b** with 4-chloro/4-bromo/4-fluoro/3,4-dichloro-phenacyl bromide and the reactions did not follow similar trends in all these cases. Initially in each experiment, a mixture of **1a,b**, substituted phenacylbromide (mole:mole) and a solution of ethanolic potassium hydroxide (10%) in ethanol was kept under reflux on a water bath for about 4 h. In case of 4-fluoro- and 3,4-dichlorophenacylbromide we got the expected products 3-[4-(4-X-phenylsulfonyl)phenyl)-6-(substituted-aryl)-7*H*-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazine **2a,b** and **3a,b** whereas using 4-chloro- or 4-bromo-phenacyl bromide the obtained product was formulated as phenacylthio derivative **4a,b**/**5a,b** in addition with the corresponding triazolothiadiazines **6a,b**/**7a,b** derivatives (scheme 1).

In the first case, the disappearance of the characteristic primary amino and carbonyl stretching frequencies in the IR spectra of the starting materials indicates that the open chain compound underwent intermolecular cyclization. The detection of new C=N and C-S-C stretching bands at about 1571-1587 and 689-696 cm^{-1} respectively, are evidences for ring closure of 1,3,4-thiadiazines **2,3 a,b**. In addition, the ^{1}H-NMR spectrum of these compounds showed a singlet at 4.45-4.59 ppm, integrating for two protons, which was attributable to the methylene protons of the thiadiazine ring. An additional evidence of



Scheme 1



Scheme 2

triazolothiadiazine ring closure is the appearance of a new peak in the 150.30 - 150.70 ppm resonance region of ^{13}C-NMR spectra, which can be attributed to the new C=N bond in thiadiazine ring.

^{1}H-NMR analysis of the resulting mixture obtained in the second case, indicated the presence of two singlet at α 5.20 and 4.45 ppm, which are attributed to –SCH_2 protons in uncyclized products **4,5 a,b** (76%) and in corresponding cyclized products **6,7 a,b** (24%), respectively. Also, the IR spectra show so characteristic absorption bands due to stretching of the -NH_2 moiety of the triazoles **1a,b** and carbonyl absorption of 4-chloro/4-bromo phenacyl bromide.

In order to establish that the formation of triazolothiadiazines **6a,b 7a,b** from triazole **1a,b** has taken place via the cyclocondensation process, we changed the working conditions. Thus, the reaction can take place either through S-alkylation (by refluxing 2 hrs in ethanol in the presence of triethylamine), following by cyclodehydration by boiling in phosphoryl chloride, or directly, in one step, by refluxing for 7 h a mixture of triazole **1a,b** and corresponding substituted phenacyl bromide in absolute ethanol and neutralized the reaction mass with sodium bicarbonate (scheme 2).

The structures of all obtained compounds were confirmed on the basis of their elemental analysis and spectral data (IR, NMR).

The IR spectrum of compounds **4,5 a,b** showed a broad absorption band at 3326-3385 cm^{-1} indicating the presence of primary amino group of triazole. The carbonyl stretching frequency was observed at 1675-1680 cm^{-1} and the C-S-C stretching bands appear in the 632-641 cm^{-1} region.

All data were consistent with the proposed structures [21, 31]. In the NMR spectra of the title compounds **4,5 a,b** the observation of additional resonances assigned to the SCH_2 (β 5.18-5.24 ppm in ^{1}H-NMR spectra and 37.03-37.75 ppm in ^{13}C-NMR spectra) confirmed the S-alkylation of triazole **1**.

The structure of **6,7 a,b** derivatives was confirmed by the absence of the amino and carbonyl bands in its IR spectrum and the appearance of a characteristic singlet signal at 4.42-4.55 ppm due to methylene protons of SCH_2 group, in its ^{1}H-NMR spectrum. As a result of the overall action of inductive effect and magnetic anisotropic effect of the aromatic rings, the chemical shift of the SCH_2 group in the thiadiazine moiety appears downfield in comparison with the obtained values for **4,5 a,b**. ^{13}C-NMR spectra of triazolothiadiazines **6,7 a,b** exhibit a new peak at 156.90-158.81 ppm, which corresponding to quaternary carbon of the azo-methynic group formed by cyclodehydration of **4,5 a,b** and the peaks with δ values in 20.60-22.74 ppm region are attributed to the SCH_2 in the thiadiazine rings.

The structure of the products **6,7 a,b** obtained by cyclodehydration of **4,5 a,b** was confirmed by the direct comparison (m.p. and mixed m.p.) with those obtained by the direct reaction of the 4-amino-5-[4-(4-X-phenylsulfonyl)phenyl]-2,4-dihydro-3*H*-1,2,4-triazole-3-thione **1** (X=Cl, Br) with 4-chloro- or 4-bromophenacyl bromide.

Antibacterial activity

The preliminary results of antimicrobial activities indicated that the tested compounds exhibited a moderate activity against Gram-negative bacteria: *Escherichia coli* ATCC 11775, *Pseudomonas aeruginosa* ATCC 9027 and Gram-positive bacteria: *Staphylococcus aureus* ATCC 12600, *Bacillus subtilis* ATCC 6633 (table 1).

From the results of these studies it is evident that the triazolothiadiadiazines **2a** and **3a**, which presented a 4-fluoro- or 2,4-dichlorophenyl substituent attached to the thiadiazine ring, were more active than other tested compounds against all the bacterial species taken in the study.

These results can be attributed to the electronic and steric effects of fluoro and chloro atoms. Presence of more bulky bromine atom, exerts a visible decrease and even cancellation of antibacterial action (**7b** was found to have no activity against *B.subtilis, P.aeruginosa, E.coli*).

The structure of the tested compounds seemed to be the principal factor influencing the antibacterial activity. This property is certainly correlated with the ability of a compound to diffuse through the biological membranes to reach its site of action. Thus, for 4-amino-5-[4-(4-X-phenylsulfonyl)phenyl]-3-(4-chloro/4-bromo-phenacylthio)-4*H*-1,2,4-triazole **4a,b/5a,b**, which have a linear and bulky structure (and due to the presence of carbonyl group), the antibacterial activity was diminished as compared to the corresponding derivatives **6,7 a,b**

Table 1

IN VITRO ANTIBACTERIAL SPECTRUM OF **2-7 a,b** USING DISC-DIFFUSION ASSAY

Compd.	**X**	**R**	**Diameter of inhibition zone (mm)***			
			Sa	***Bs***	***Pa***	***Ec***
2a	Cl	4-F	13.33±0.577	14.66±0.707	13.66±0.707	12.33±1.527
2b	Br	4-F	10.66±0.707	12.33±0.577	11.33±0.707	12.66±0.707
3a	Cl	3,4-Cl_2	12.33±0.577	12.66±0.707	14.66±0.707	12.00±1.00
3b	Br	3,4-Cl_2	11.33±0.707	11.66±0.707	12.66±0.577	11.33±0.577
4a	Cl	4-Cl	11.33±0.577	11.66±0.577	14.33±0.577	-
4b	Br	4-Cl	9.66±0.707	10.66±0.707	11.33±0.707	-
5a	Cl	4-Br	8.33±0.577	9.33±0.577	11.33±0.707	-
5b	Br	4-Br	7.33±0.577	10.66±0.577	9.66±0.577	-
6a	Cl	4-Cl	11.33±0.577	13.66±0.707	13.66±0.707	10.33±0.577
6b	Br	4-Cl	10.66±0.707	9.66±0.707	9.66±0.707	9.66±0.707
7a	Cl	4-Br	10.33±1.527	10.33±1.527	10.33±0.577	10.33±0.577
7b	Br	4-Br	9.66±0.707	-	-	-
Chloramphenicol			16.66±0.707	21.66±0.577	24.66±0.577	18.00±0.00

* mean of three replicate ± standard deviation

- no activity

Sa - *Staphylococcus aureus* (ATCC 12600); Bs - *Bacillus subtilis* (ATCC 6633);
Ec - *Escherichia coli* (ATCC 11775); Pa - *Pseudomonas aeruginosa* (ATCC 9027)

possessing the more compact structure. Better than, **4,5 a,b** were found to have no antimicrobial activity against *E. coli*. MIC determination tests are in progress.

Conclusions

The research study reports the successful synthesis and antimicrobial activity of new 6-(substituted-aryl)-7*H*-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazines possessing (phenylsulfonyl) phenyl moiety, by reaction of different substituted phenacyl bromide with 4-amino-5-[4-(4-X-phenylsulfonyl)phenyl]-4*H*-1,2,4-triazole-3-thiols .

The potential antimicrobial effects (diameter of the inhibition zones) of the synthesized compounds were investigated by disc-diffusion method using the *Escherichia coli* ATCC 11775, *Pseudomonas aeruginosa* ATCC 9027, *Staphylococcus aureus* ATCC 12600 and *Bacillus subtilis* ATCC 6633 strains.

The most active compounds were **2a** and **3a** containing fluoro or chloro substituents attached to the thiadiazine rings, which exhibited promising activities against all the tested strains.

The remaining compounds differed in their ability to inhibit the growth of micro-organisms in a close relationship with their chemical structure and all are less effective than chloramphenicol.

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