

Synthesis and Antibacterial Activity of 3-(5-Methylisoxazol-3-yl)-1,2,4-triazolo[3,4-*b*]-1,3,4-thiadiazine Derivatives

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The condensed products 2—10 of 4-amino-5-mercapto-3-(5-methylisoxazol-3-yl)-1,2,4-triazole (**1**) with chloroacetaldehyde, 2-bromocyclohexanone, chloranil, ω -bromo- ω -(1*H*-1,2,4-triazol-1-yl)acetophenone, 2-bromo-4'-substituted acetophenones and 2-bromo-6'-methoxy-2'-acetophenone were described. The antibacterial activities were also evaluated.

Keywords 1,2,4-Triazolo[3,4-*b*]-1,3,4-thiadiazine, 5-methylisoxazole, antibacterial activity

Introduction

Substituted 1,2,4-triazoles and the *N*-bridged heterocycles especially *s*-triazolo[3,4-*b*]-1,3,4-thiadiazine derivatives have received considerable attention during the last two decades as their diverse biological activities, including antiparasitic, analgesic, antibacterial and anti-inflammatory activities.¹⁻⁴ Our previous work revealed the antibacterial and plant growth regulative activities of 5-methylisoxazole derivatives.^{5,6} It was worthwhile to synthesize new polyheterocyclic compounds which possess 5-methylisoxazole ring and *s*-triazolo[3,4-*b*]-1,3,4-thiadiazine moiety because the biological activities of structurally hybridized compounds may be improved than those of their parents compounds. In view of the above mentioned facts, we synthesized *s*-triazolo[3,4-*b*]-1,3,4-thiadiazine derivatives (**2**—**10**) by condensation of 4-amino-5-mercapto-3-(5-methylisoxazol-3-yl)-1,2,4-triazole (**1**) with chloroacetaldehyde, 2-bromocyclohex-

anone, chloranil, ω -bromo- ω -(1*H*-1,2,4-triazol-1-yl)-acetophenone, 2-bromo-4'-substituted acetophenone and 2-bromo-6'-methoxy-2'-acetophenone, respectively. The antibacterial activities were also evaluated.

Results and discussion

The starting compound **1** was prepared by the method of Reid and Heindel⁸ in a moderate yield. The condensation of **1** with one equivalent of chloroacetaldehyde in refluxing ethanol catalyzed by concentrated HCl produced 3-(5-methylisoxazol-3-yl)-7*H*-1,2,4-triazolo[3,4-*b*]-1,3,4-thiadiazine (**2**). The ¹H NMR spectrum (DMSO-*d*₆) of **2** showed a doublet at δ 3.87 (d, 2H) and a triplet at 7.93 (t, *J* = 4.0 Hz, 1H), which were assigned to the protons of SCH₂ and N = CH, respectively.

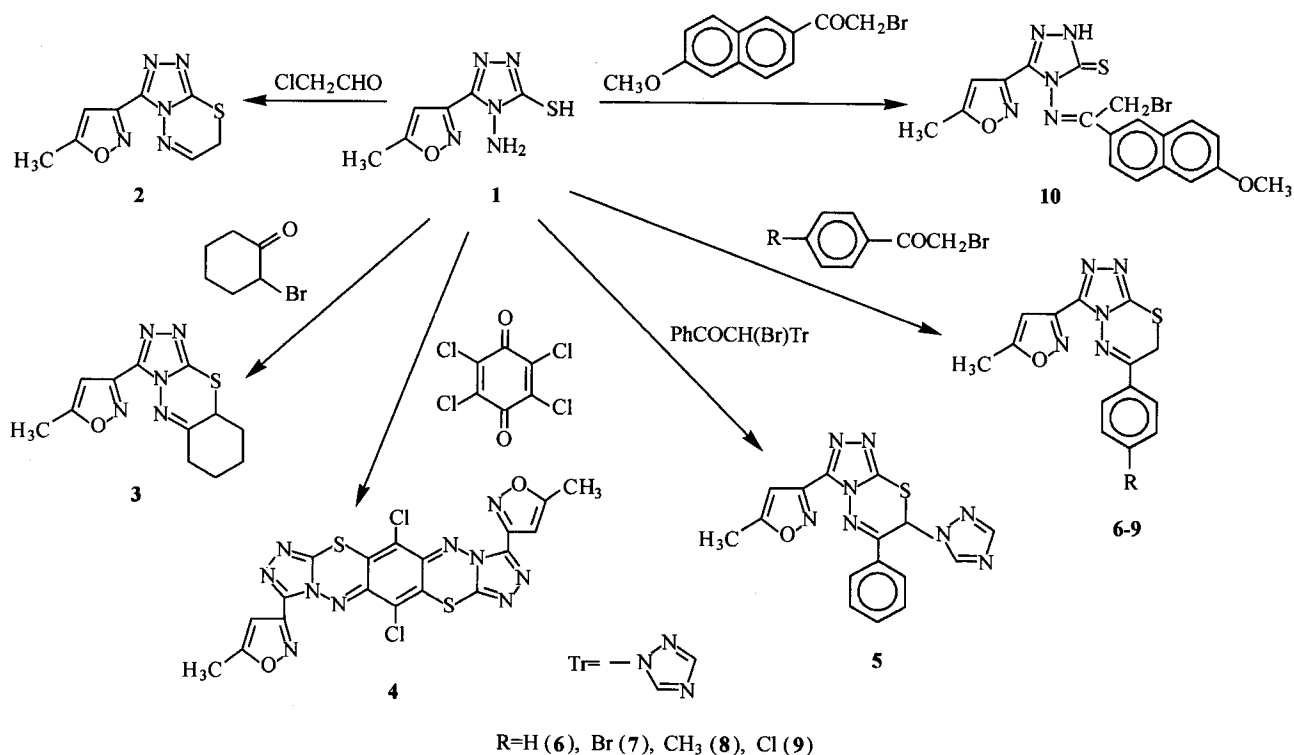
Treatment of **1** with 2-bromocyclohexanone or ω -bromo- ω -(1*H*-1,2,4-triazol-1-yl)acetophenone in absolute ethanol followed by neutralization with potassium carbonate afforded 3-(5-methylisoxazol-3-yl)-6,7,8,9-tetrahydro-1,2,4-triazolo[4,3-*b*][4,1,2]-benzothiadiazine (**3**) and 3-(5-methylisoxazol-3-yl)-6-phenyl-7-(1*H*-1,2,4-triazol-1-yl)-1,2,4-triazolo[3,4-*b*]-1,3,4-thiadiazine (**5**). The ¹H NMR spectrum (DMSO-*d*₆) of **5** exhibited a singlet for one 1,2,4-triazole proton at δ 8.91. The other 1,2,4-triazole proton, SCH proton and aromatic protons resonated as a multiplet at δ 7.42—8.17.

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Scheme 1



When an ethanolic solution of **1** was treated with chloranil in the presence of anhydrous sodium acetate, 3,10-bis(5-methylisoxazol-3-yl)-6,13-dichloro-bis(1,2,4-triazolo[3,4-*b*]-1,3,4-thiadiazino[5',6'-*b*:5',6'-*e*]-cyclohexyl)-1,4-diene (**4**) was obtained. The ¹H NMR spectrum of **4** displayed only the signal of 3-position substituent of 5-methylisoxazole. The IR spectrum showed absorption bands at 1620, 1280 and 723 cm⁻¹ corresponding to C=N, N—N and C—S—C, respectively.

In literature,⁸ the most useful method for the formation of *s*-triazolo[3,4-*b*]-1,3,4-thiadiazine ring system was the condensation of 4-amino-5-mercapto-3-substituted-1,2,4-triazole with 2-bromoacetophenone compounds. This cyclization has afforded different products under various reaction conditions. As anticipated, the reaction of **1** with 2-bromo-4'-substituted acetophenone in the presence of potassium carbonate in absolute ethanol resulted in cyclocondensation to give the corresponding 3-(5-methylisoxazol-3-yl)-6-aryl-7*H*-1,2,4-triazolo[3,4-*b*]-1,3,4-thiadiazines (**6–9**). The IR spectra of **6–9** displayed three characteristic absorption bands; 1584–1619 cm⁻¹ for ν_{C=N}, 1278–1294 cm⁻¹ for ν_{N-N} and 721–723 cm⁻¹ for ν_{C-S-C}, respectively. The

¹H NMR spectra (DMSO-*d*₆) exhibited a singlet for SCH₂ proton at around δ 4.42.

When triazole **1** was treated with 2-bromo-6'-methoxy-2'-acetonaphthone in absolute ethanol, 3-(5-methylisoxazol-3-yl)-4-(2-bromo-6'-methoxy-2'-acetonaphthideneamino)-5-mercapto-1,2,4-triazole (**10**) was yielded instead of the expected cyclic compound due to the steric hindrance. The molecule is of *E*-configuration according to its molecular model. The ¹H NMR spectrum (DMSO-*d*₆) of **10** exhibited signals at δ 4.04 (s, 3H), 4.55 (s, 2H) and 13.14 (s, 1H), which were assigned to the protons of CH₃O, CH₂ and NH, respectively. In addition, the structure was also supported by IR spectrum which revealed the presence of C=S at 1256 cm⁻¹ and NH at 3198 cm⁻¹, respectively. The result indicated the presence of the thione form.

All the compounds synthesized were tested for their antibacterial activities. The antibacterial screening was carried out employing the cup-plate method at a 100 μg/mL concentration against *E. coli*, *S. aureus*, *B. subtilis* and *S. carlsbergensis* in the nutrient agar media (Table 1). The zone of the growth inhibition of bacteria, produced by diffusion of the compounds from the cup

into the surrounding medium, was measured after 24 h. The preliminary results indicated that most of the compounds were active against these bacteria. It is interest-

ing to note that all the compounds were sensitive to *B. subtilis* comparable to chloramphenicol taken as standard drug at the concentration of 100 $\mu\text{g}/\text{mL}$.

Table 1 Antibacterial activities for compounds 2–10

Compd.	<i>E. coli</i>	<i>B. subtilis</i>	<i>S. aureus</i>	<i>S. carlsbergensis</i>
2	-	++	++	+++
3	+++	++	++	++
4	+++	+	++	++
5	+++	+	+++	++
6	+	++	++	++
7	+	+++	++	++
8	-	++	+++	+++
9	++	+++	+	+
10	+	+++	-	+++
DMSO	-	-	-	-
Chloramphenicol	+	+	++	+++

Zone diameter of growth inhibition; < 10 mm (-), 10–12 mm (+), 13–15 mm (++) and 16–20 mm (+++). Diameter of the cup = 8 mm.

Experimental

The melting points of the compounds were determined on an X-4 microscopic melting point apparatus and uncorrected. Elementary analyses were carried out on an Elemental vario EL analyzer. IR spectra were obtained in KBr disc on a 5-DX spectrometer. ^1H NMR spectra (DMSO- d_6) were recorded on a Bruker AC-80 instrument with TMS as an internal standard. MS were performed on a VG-7070E spectro meter (EI at 70 eV) and an HP-5988A spectrometer (FAB).

ω -Bromo- ω -(1*H*-1,2,4-triazol-1-yl) acetophenone and 2-bromocyclohexanone were prepared as described in the literature,^{9,10} respectively. They were used in next steps without further purification.

4-Amino-5-mercapto-3-(5-methylisoxazol-3-yl)-1,2,4-triazole (1)

To a solution of KOH (0.015 mol), absolute ethanol (100 mL) and 5-methylisoxazol-3-formyl hydrazide (0.01 mol), carbon disulfide (0.015 mol) was added and the content was agitated at room temperature for 16 h. It was then diluted with dry ether (50 mL). The precipitated solid was filtered and washed with dry ether. The potassium salt was obtained in a nearly quan-

titative yield (99 %).

A suspension of potassium salt (0.005 mol), 85% hydrazine hydrate (5 mL) and water (1 mL) was refluxed with stirring for 6 h. The mixture was then diluted with ice cold water (20 mL) and neutralized with HCl to pH = 5. The precipitate was filtered, washed with water and recrystallized from ethanol to give **1** in 46% yield, m.p. 189–190°C. ^1H NMR (DMSO- d_6 , 80 MHz) δ : 2.28 (s, 3H, CH₃), 5.91 (s, 2H, NH₂), 6.65 (s, 1H, =CH), 13.77 (s, 1H, NH); IR (KBr) ν : 3368 (w, NH), 2909 (w, C—H), 1643, 1570, 1514, 1476 (m, C=N, C=C) cm^{-1} ; MS (70 eV) m/z (%): 197 (M⁺, 23), 196 (100), 181 (1), 165 (2), 108 (66), 83 (15), 60 (62), 43 (20); Anal. calcd for C₆H₇N₅OS: C 36.54, H 3.58, N 35.51; found C 36.88, H 3.57, N 35.62.

3-(5-Methylisoxazol-3-yl)-7*H*-1,2,4-triazolo[3,4-*b*]-1,3,4-thiadiazine (2)

A mixture of **1** (0.001 mol), chloroacetaldehyde (0.002 mol), concentrated HCl (1 drop) and ethanol (50 mL) was refluxed over oil-bath for 6 h. After removal of the excess of ethanol under reduced pressure, the resulting solid was filtered and washed with water. The crude product was recrystallized from ethanol to give

2 in 72% yield, m.p. 188—190°C. ¹H NMR (DMSO-*d*₆, 80 MHz) δ: 2.28 (s, 3H, CH₃), 3.87 (d, *J* = 4.0 Hz, 2H, CH₂), 6.53 (s, 1H, =CH), 7.93 (t, *J* = 4.0 Hz, 1H, =CHCH₂); IR (KBr) ν: 2961 (w, CH₃), 1591 (m, C=N), 1284 (m, N—N), 727 (m, C—S—C) cm⁻¹; MS (70 eV) *m/z* (%): 221 (M⁺, 13), 220 (M⁺ - 1, 100), 192 (15), 164 (5), 135 (8), 108 (17), 43 (6); Anal. calcd for C₈H₇N₅OS: C 43.43, H 3.19, N 31.66; found C 43.59, H 3.46, N 31.88.

3-(5-Methylisoxazol-3-yl)-6,7,8,9-tetrahydro-1,2,4-triazolo[4,3-*b*][4,1,2]-benzothiadiazine (**3**)

A mixture of **1** (0.001 mol), 2-bromocyclohexanone (0.001 mol) and fused potassium carbonate (0.004 mmol) in absolute ethanol (80 mL) was refluxed with oil-bath for 10 h. After removal of ethanol under reduced pressure, the resulting solid was filtered and washed with water. The crude product was recrystallized from ethanol to give **3** in 47% yield, m.p. 203—205°C. ¹H NMR (DMSO-*d*₆, 80 MHz) δ: 2.28 (s, 3H, CH₃), 1.68—1.96 (m, 6H, (CH₂)₃), 2.57 (t, *J* = 5.6 Hz, 2H, =C(CH₂)-), 4.38 (q, *J* = 5.6 Hz, 1H, SCH), 6.59 (s, 1H, =CH); IR (KBr) ν: 2954 (w, CH₃), 1593 (m, C=N), 1288 (m, N—N), 724 (m, C—S—C) cm⁻¹; MS (70 eV) *m/z* (%): 275 (M⁺, 16), 274 (100), 234 (7), 206 (21), 151 (7), 108 (29), 82 (5), 43 (6); Anal. calcd for C₁₂H₁₃N₅OS: C 52.35, H 4.76, N 25.44; found C 52.07, H 4.80, N 25.71.

3,10-Bis(5-methylisoxazol-3-yl)-6,13-dichloro-bis(1,2,4-triazolo[3,4-*b*]-1,3,4-thiadiazino[5',6'-*b*:5',6'-*e*]-cyclohexyl-1,4-diene (**4**)

A mixture of **1** (0.002 mol), chloranil (0.001 mol) and anhydrous sodium acetate (0.001 mol) in absolute ethanol (100 mL) was refluxed for 8 h and a red solid was obtained. The solid was filtered, washed with water and recrystallized from DMF in 51% yield, m.p. > 300°C. ¹H NMR (DMSO-*d*₆, 80 MHz) δ: 2.32 (s, 6H, 2CH₃), 6.61 (s, 2H, 2 × =CH); IR (KBr) ν: 2928 (w, CH₃), 1620 (m, C=N), 1280 (m, N—N), 723 (m, C—S—C); Anal. calcd for C₁₈H₈N₁₀O₂S₂Cl₂: C 40.69, H 1.52, N 26.36; found C 40.74, H 1.73, N 26.27.

3-(5-Methylisoxazol-3-yl)-6-phenyl-7-(1*H*-1,2,4-triazol-1-yl)-1,2,4-triazolo[3,4-*b*]-1,3,4-thiadiazine (**5**)

A mixture of **1** (0.001 mol) and ω-bromo-ω-(1*H*-1,2,4-triazol-1-yl)acetophenone (0.001 mol) in absolute ethanol (80 mL) was refluxed with oil-bath for 8 h. The reaction mixture was concentrated and cooled. The resulting solid was collected by filtration, treated with aqueous potassium carbonate solution, washed with water and recrystallized from ethanol to give **5** in 56% yield, m.p. 168—169°C. ¹H NMR (DMSO-*d*₆, 80 MHz) δ: 2.30 (s, 3H, CH₃), 6.51 (s, 1H, =CH), 7.42—8.17 (m, 7H, ArH, SCH, TrH), 8.91 (s, 1H, TrH); IR (KBr) ν: 2927 (w, CH₃), 1618 (m, C=N), 1276 (m, N—N), 711 (m, C—S—C); MS (70 eV) *m/z* (%): 364 (M⁺, 6), 297 (13), 287 (3), 261 (41), 191 (9), 108 (13), 77 (40); Anal. calcd for C₁₆H₁₂N₈OS: C 52.74, H 3.32, N 30.75; found C 52.61, H 3.13, N 30.48.

3-(5-Methylisoxazol-3-yl)-6-aryl-7*H*-1,2,4-triazolo[3,4-*b*]-1,3,4-thiadiazines (**6—9**)

The compounds **6—9** were synthesized from **1** (0.001 mol) and 2-bromo-4'-substituted acetophenones (0.001 mol) in a manner similar to that described for the preparation of **3**. They were recrystallized from ethanol to give pure **6—9**.

3-(5-Methylisoxazol-3-yl)-6-phenyl-7*H*-1,2,4-triazolo[3,4-*b*]-1,3,4-thiadiazines (**6**) Yield 81%, m.p. 186—187°C; ¹H NMR (DMSO-*d*₆, 80 MHz) δ: 2.35 (s, 3H, CH₃), 4.43 (s, 2H, CH₂), 6.65 (s, 1H, =CH), 7.75—8.10 (m, 5H, ArH); IR (KBr) ν: 2945 (w, CH₃), 1584 (m, C=N), 1278 (m, N—N), 722 (m, C—S—C) cm⁻¹; MS (70 eV) *m/z* (%): 297 (M⁺, 23), 195 (32), 180 (18), 108 (22), 103 (13), 77 (38), 43 (36); Anal. calcd for C₁₄H₁₁N₅OS: C 56.55, H 3.73, N 23.55; found C 56.63, H 4.03, N 23.78.

3-(5-Methylisoxazol-3-yl)-6-(4-bromophenyl)-7*H*-1,2,4-triazolo[3,4-*b*]-1,3,4-thiadiazine (**7**)

Yield 88%, m.p. 145—146°C. ¹H NMR (DMSO-*d*₆, 80 MHz) δ: 2.30 (s, 3H, CH₃), 4.41 (s, 2H, CH₂), 6.60 (s, 1H, =CH), 7.78 (d, *J* = 8.8 Hz,

2H, ArH), 7.97 (d, $J = 8.8$ Hz, 2H, ArH); IR (KBr) ν : 2969 (w, CH₃), 1589 (m, C=N), 1285 (m, N—N), 723 (m, C—S—C) cm⁻¹; MS (70 eV) m/z (%): 376 (M⁺, 59), 378 (48), 295 (1), 195 (26), 182 (7), 107 (53), 102 (100), 77 (12), 43 (30); Anal. calcd for C₁₄H₁₀N₅OSBr: C 44.69, H 2.68, N 18.61; found C 44.78, H 2.84, N 18.81.

3-(5-Methylisoxazol-3-yl)-6-(4-methylphenyl)-7H-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazine (8)

Yield 78%, m. p. 196—198°C. ¹H NMR (DMSO-*d*₆, 80 MHz) δ : 2.31 (s, 3H, CH₃), 2.40 (s, 3H, CH₃), 4.40 (s, 2H, CH₂), 6.61 (s, 1H, =CH), 7.39 (d, $J = 8.0$ Hz, 2H, ArH), 7.93 (d, $J = 8.0$ Hz, 2H, ArH); IR (KBr) ν : 2920 (w, CH₃), 1608 (m, C=N), 1294 (m, N—N), 721 (m, C—S—C) cm⁻¹; MS (70 eV) m/z (%): 311 (M⁺, 18), 310 (100), 220 (2), 193 (21), 131 (45), 117 (27), 108 (14), 91 (43), 43 (5); Anal. calcd for C₁₅H₁₃N₅OS: C 57.86, H 4.21, N 22.49; found C 57.63, H 4.10, N 22.43.

3-(5-Methylisoxazol-3-yl)-6-(4-chlorophenyl)-7H-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazine (9)

Yield 76%, m. p. 145—146°C. ¹H NMR (DMSO-*d*₆, 80 MHz) δ : 2.31 (s, 3H, CH₃), 4.42 (s, 2H, CH₂), 6.61 (s, 1H, =H), 7.65 (d, $J = 8.8$ Hz, 2H, ArH), 8.04 (d, $J = 8.8$ Hz, 2H, ArH); IR (KBr) ν : 2920 (w, CH₃), 1618 (m, C=N), 1285 (m, N—N), 722 (m, C—S—C) cm⁻¹; MS (70eV) m/z (%): 331 (M⁺, 19), 333 (7), 330 (100), 332 (33), 303 (1), 267 (1), 192 (27), 151 (33), 137 (40), 139 (13), 108 (15), 76 (10), 43 (14); Anal. calcd for C₁₄H₁₀N₅OSCl: C 50.68, H 3.04, N 21.11; found C 50.77, H 3.32, N 21.02.

3-(5-Methylisoxazol-3-yl)-4-(2-bromo-6'-methoxy-2'-acetonephthideneamino)-5-mercapto-1,2,4-triazole (10)

Compound 10 was synthesized from 1 (0.001 mol) and 2-bromo-6'-methoxy-2'-acetonephthone (0.001 mol) in a manner similar to that described for the preparation of 6—9. It was recrystallized from EtOH-DMF in 58% yield, m. p. 246—248°C. ¹H NMR (DMSO-*d*₆, 80 MHz) δ : 2.34 (s, 3H, CH₃), 4.04 (s, 3H, CH₃O), 4.55 (s, 2H, CH₂), 6.68 (s, 1H, =CH), 7.59—8.65 (m, 6H, ArH), 13.14 (s, 1H, NH); IR (KBr) ν : 3198 (w, NH), 2982 (w, CH₃), 1624 (m, C=N), 1276 (m, N—N), 1256 (m, C=S) cm⁻¹; MS (FAB) m/z (%): 457 (M⁺, 22), 459 (19); Anal. calcd for C₁₉H₁₆N₅O₂SBr: C 49.79, H 3.52, N 15.28; found C 49.59, H 3.44, N 15.37.

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