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ORIGINAL ARTICLE

Synthesis and antibacterial activity of some new 4-anilino-5-phenyl-4*H*-1,2,4-triazole-3-thiol derivatives

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KEYWORDS

4-Anilino-5-phenyl-4*H*-1,2,4-triazole-3-thiol;
Mannich bases;
Triazolotriazoles;
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Triazinotriazoles;
Antibacterial activity

Abstract 4-Anilino-5-phenyl-4*H*-1,2,4-triazole-3-thiol (**1**) reacted with formaldehyde and different amines to give Mannich bases **2a**–**i**. Treatment of compound **1** with formaldehyde afforded the corresponding 2-hydroxymethyl derivative **3**, which upon its reaction with thionyl chloride yielded the corresponding chloromethyl derivative **4**. Treatment of compound **4** with some thiols gave the corresponding sulfides **5a**–**f**. The ring closure reaction of chloromethyl derivative **4** with hydrazine hydrate, phenyl hydrazine, hydroxylamine, urea and thiourea afforded triazolo-, oxadiazolo- and triazinotriazoles **6**–**10**, respectively.

1. Introduction

Various 1,2,4-triazoles found extensive investigations due to their useful application in different areas of biological activity and as industrial intermediates such as antiasthmatic (Naito et al., 1996), antiviral (ribavirin) (De Clercq, 2004), antifungal (fluconazole) (Collin et al., 2003), antimicrobial (Kidwai et al., 2001), antibacterial (Papakonstantinou-Garoufalias et al., 2002), insecticidal (Ghorab et al., 1999), amoebicidal (Andotra and Sharma, 1988), hypnotic (Hester et al., 1971), cytotoxic (Milton, 2001) and hypotensive (Burell et al., 1994; Ghorab et al., 1996) activities. This moiety was also found in potent agonist and antagonist receptor ligands, (Wadsworth et al., 1992; Chen et al., 2001) in HIV-1 protease inhibitors (Thompson et al., 1994) and in thrombin inhibitors (Duncia et al., 1998). Along with these significant pharmaceutical uses, 1,2,4-triazole derivatives are effectively used in polymers, dyestuff, photographic chemicals and agricultural chemicals (Potts, 1961). In view of these findings and in continuation of our work in the same field (Ghattas and El-saraf, 1989; Ghattas et al., 1989, 2001, 2003; El-wassaimy et al., 1992; Abdel-Rahman et al., 1993; Moustafa, 2001), we report herein the synthesis of some new s-triazole derivatives and tested the latter for antibacterial activity.

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2. Experimental

Melting points are uncorrected and were determined by Kofeler melting point apparatus. IR (cm^{-1}) spectra were recorded (KBr disk) on a Nicolet 710 FT-IR spectrophotometer. ^1H NMR (DMSO-d_6) spectra were recorded at 300 MHz on a Varian Gremini NMR spectrometer at the Cairo University, the chemical shift is expressed in δ value (ppm) using TMS as an internal reference. Element analyses carried out on an elemental analyzer 240 °C. Mass spectra were performed on Micromass 7070 E spectrometer operating at 70 eV, using direct inlet.

*2.1. Synthesis of Mannich bases **2a–i** (general procedure)*

Formaldehyde (1.5 mL, 40% solution) was added to a solution of compound **1** (0.5 g, 1.8 mmol) in ethanol (15 mL) and the reaction mixture was refluxed for 1 h. The appropriate amine (0.001 mol) was added and the reaction mixture was refluxed for 4 h. After cooling, the formed precipitate was filtered and recrystallized from the appropriate solvent to give the corresponding Mannich bases **2a–i**.

*2.1.1. 4-Anilino-5-phenyl-2-[(1,3-thiazol-2-yl-amino)methyl]-2,4-dihydro-3H-1,2,4-triazole-3-thione (**2a**)*

White crystals from ethanol, yield 0.39 g (55%), m.p. 155 °C; IR (KBr): V_{max}/cm^{-1}: 3431 (NH), 3195 (NH), 3081 (Ar, CH), 2918 (Aliph., CH), 1592 (C═N); MS, *m/z* (I_{rel},%): 380 $[M]^+$ (10.6), 312 (21.3), 239 (27.7), 178 (51), 135 (34), 103 (100), 77 (81), 51 (72.3). Anal. for $C_{18}H_{16}N_6S_2$ (380.48) Calcd. %: C 56.77; H 4.20; N 22.07; S 8.41. Found %: C 56.51; H 4.35; N 22.30; S 8.61.

*2.1.2. 4-Anilino-5-phenyl-2-[(1,3,4-thiadiazol-2-yl-amino)methyl]-2,4-dihydro-3H-1,2,4-triazole-3-thione (**2b**)*

White crystals from ethanol, yield 0.7 g (98%), m.p. 113 °C; IR (KBr): V_{max}/cm^{-1}: 3415 (NH), 3203 (NH), 3072 (Ar, CH), 2940 (Aliph., CH), 1568 (C═N); MS, *m/z* (I_{rel},%): 381 $[M]^+$ (0.04), 312 (21.3), 103 (60.6), 60 (100). Anal. for $C_{17}H_{15}N_7S_2$ (381.47) Calcd. %: C 53.47; H 3.93; N 25.69; S 8.38. Found %: C 53.33; H 3.67; N 25.53; S 8.41.

*2.1.3. 4-Anilino-5-phenyl-2-[(pyridin-2-yl-amino)methyl]-2,4-dihydro-3H-1,2,4-triazole-3-thione (**2c**)*

White-yellow crystals from ethanol, yield 0.65 g (94%), m.p. 175 °C; IR (KBr): V_{max}/cm^{-1}: 3428 (NH), 3240 (NH), 3015 (Ar, CH), 2954 (Aliph., CH), 1600 (C═N); MS, *m/z* (I_{rel},%): 374 $[M]^+$ (0.07), 312 (37.7), 279 (19), 181(13.2), 149 (22.6), 103 (100), 55 (96.2). Anal. for $C_{20}H_{18}N_6S$ (374.46) Calcd. %: C 64.06; H 4.80; N 22.43; S 8.54. Found %: C 64.15; H 4.66; N 22.52; S 8.37.

*2.1.4. 1-[4-(4-Anilino-5-phenyl-2,4-dihydro-3H-1,2,4-triazole-3-thione-2-yl-methylamino)phenyl]-1-ethanone (**2d**)*

Yellow crystals from ethanol, yield 0.75 g (97%), m.p. 168 °C; IR (KBr): V_{max}/cm^{-1}: 3328 (NH), 3185 (NH), 3048 (Ar, CH), 2930 (Aliph., CH), 1657 (C═O), 1589 (C═N); ^1H NMR (DMSO-d_6), δ, ppm: 7.95–7.58 (m, 15H, Ar-H), 6.95 (s, 2H, 2NH), 5.54 (s, 2H, N–CH_2–N), 2.39 (s, 3H, CH_3). Anal. for $C_{23}H_{21}N_5OS$ (415.51) Calcd. %: C 66.42; H 5.05; N 16.84; S 7.70. Found %: C 66.31; H 5.22; N 16.75; S 7.53.

*2.1.5. 4-Anilino-2-[(diethylamino)methyl]-5-phenyl-2,4-dihydro-3H-1,2,4-triazole-3-thione (**2e**)*

Brown crystals from ethanol, yield 0.37 g (56%), m.p. 92 °C; IR (KBr): V_{max}/cm^{-1}: 3369 (NH), 3055 (Ar, CH), 2966 (Aliph., CH), 1617 (C═N); ^1H NMR (DMSO-d_6), δ, ppm: 7.92–7.33 (m, 11H, Ar-H + NH), 3.07–3.04 (q, 4H, CH_2, J = 7.8 Hz), 2.68 (s, 2H, CH_2), 1.15–1.20 (t, 6H, CH_3, J = 14.7 Hz). Anal. for C_{19} $H_{23}N_5S$ (353.48) Calcd. %: C 64.50; H 6.50; N 19.80; S 9.00. Found %: C 64.31; H 6.72; N 19.63; S 9.12.

*2.1.6. 4-Anilino-2-{[ethyl(phenyl)amino]methyl}-5-phenyl-2,4-dihydro-3H-1,2,4-triazole-3-thione (**2f**)*

White crystals from ethanol, yield 0.68 g (90%), m.p. 107 °C; IR (KBr): V_{max}/cm^{-1}: 3424 (NH), 3048 (Ar, CH), 2964 (Aliph., CH), 1606 (C═N); ^1H NMR (DMSO-d_6), δ, ppm: 7.91–6.92 (m, 10H, Ar-H), 5.68 (s, NH), 5.20 (s, 2H, N–CH_2–N), 3.69–3.63 (q, 2H, N–CH_2, J = 18.0 Hz), 1.21–1.19 (t, 3H, CH_3, J = 6 Hz). Anal. for $C_{23}H_{23}N_5S$ (401.52) Calcd. %: C 68.73; H 5.72; N 17.43; S 7.97. Found %: C 68.61; H 5.83; N 17.35; S 7.81.

*2.1.7. 4-Anilino-2-(morpholin-4-yl-methyl)-5-phenyl-2,4-dihydro-3H-1,2,4-triazole-3-thione (**2g**)*

White crystals from ethanol, yield 0.48 g (71%), m.p. 118 °C; IR (KBr): V_{max}/cm^{-1}: 3427.9 (NH), 3051.8 (Ar, CH), 2937, 2841 (Aliph., CH,), 1615 (C═N); ^1H NMR (DMSO-d_6), δ, ppm: 7.91–7.33 (m, 10H, Ar-H), 5.16 (s, NH), 5.01 (s, 2H, N–CH_2–N), 3.58–3.36 (m, 4H, 2N–CH_2), 2.79–2.49 (m, 4H, 2O–CH_2). Anal. for $C_{19}H_{21}N_5OS$ (367.46) Calcd. %: C 62.04; H 5.71; N 19.05; S 8.70. Found %: C 62.23; H 5.82; N 19.31; S 8.65.

*2.1.8. 4-Anilino-5-phenyl-2-(piperidin-1-ylmethyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione (**2h**)*

White crystals from ethanol, yield 0.63 g (92%), m.p. 93 °C; IR (KBr): V_{max}/cm^{-1}: 3422 (NH), 3058 (Ar, CH), 2925, 2845 (Aliph., CH), 1608 (C═N); ^1H NMR (DMSO-d_6), δ, ppm: 7.91–7.55 (m, 10H, Ar-H), 4.99 (s, 2H, N–CH_2), 3.36 (s, 1H, NH), 2.72–2.50 (m, 4H, 2N–CH_2), 1.48–1.44 (m, 4H, CH_2CH_2N), 1.33–1.32 (m, 2H, $CH_2\underline{CH_2}CH_2$). Anal. for $C_{20}H_{23}N_5S$ (365.49) Calcd. %: C 65.66; H 6.29; N 19.15; S 8.75. Found %: C 65.42; H 6.33; N 19.31; S 8.61.

*2.1.9. 4-Anilino-1-[4-(4-anilino-3-phenyl-5-thioxo-4,5-dihydro-1H-1,2,4-triazole-1-yl-methyl) piperazino methyl-3-phenyl-4,5-dihydro-1H-1,2,4-triazole-5-thione (**2i**)*

White crystals from ethanol, yield 0.98 g (81%), m.p. 240 °C; IR (KBr): V_{max}/cm^{-1}: 3430 (NH), 3059 (Ar, CH), 2938, 2841 (Aliph., CH), 1615 (C═N); ^1H NMR (DMSO-d_6), δ, ppm: 7.89–7.56 (m, 20H, Ar-H), 5.0 (s, 4H, 2N–CH_2–N), 3.21 (s, 2H, NH), 2.80 (m, 8H, 4N–CH_2). Anal. for $C_{34}H_{34}N_{10}S_2$ (646.83): Calcd. %: C 63.07; H 5.25; N 21.64; S 9.89. Found %: C 63.19; H 5.11; N 21.55; S 9.97.

*2.2. Synthesis of 4-anilino-2-hydroxymethyl-5-phenyl-2,4-dihydro-3H-1,2,4-triazole-3-thione (**3**)*

Formaldehyde (1.5 mL, 40% solution) was added to a solution of compound **1** (1.34 g, 5 mmol) in ethanol (15 mL) and the reaction mixture was refluxed for 1 h. The solvent was evaporated to dryness, the formed solid was collected and

recrystallized from ethanol to give compound **3**. White crystals from ethanol, yield 1.4 g (94%), m.p. 132 °C; IR (KBr): V_{max}/cm^{-1}: 3418 (OH), 3107 (NH), 2944 (Ar, CH), 2768 (Aliph., CH), 1610 (C═N); ^{1}H NMR (DMSO-d_6), δ, ppm: 7.92–7.58 (m, 10H, Ar-H), 6.06 (s, 2H, CH_2), 5.4 (s, 1H, NH), 4.99 (s, 1H, OH). Anal. for $C_{15}H_{14}N_4OS$ (298.36) Calcd. %: C 60.32; H 4.69; N 18.76; S 10.72. Found %: C 60.41; H 4.59; N 18.65; S 10.81.

*2.3. Synthesis of 4-anilino-2-chloromethyl-5-phenyl-2,4-dihydro-3H-1,2,4-triazole-3-thione (**4**)*

Thionyl chloride (10 mL) was added dropwise to compound **3** (2.98 g, 10 mol) and the reaction mixture was warmed on a water bath for 1 h. After cooling, the reaction mixture was poured into petroleum ether (100 mL, 80:100 °C). The formed precipitate was filtered and recrystallized from ethanol to give compound **4**. White crystals from ethanol, yield 2.76 g (87%), m.p. 106 °C; IR (KBr): V_{max}/cm^{-1}: 3439 (NH), 3037 (Ar, CH), 2929 (Aliph., CH), 1616 (C═N), 767 (C–Cl); ^{1}H NMR (DMSO-d_6), δ, ppm: 7.91–7.28 (m, 10H, Ar-H), 5.41 (s, 1H, NH), 5.39 (s, 2H, CH_2). Anal. for $C_{15}H_{13}ClN_4S$ (316.8) Calcd. %: C 56.81; H 4.10; N 17.67; S 10.10. Found %: C 56.57; H 4.13; N 17.54; S 10.21.

*2.4. Synthesis of compounds **5a–f** (general procedure)*

To a solution of compound **4** (0.5 g 1.58 mmol) in dimethylformamide (30 mL) and triethylamine (1.58 mmol), the appropriate thiol (1.58 mmol) was added. The reaction mixture was refluxed for 3 h, the solvent was evaporated under reduced pressure. The formed precipitate was collected and recrystallized from the appropriate solvent to give the corresponding sulfides **5a**–**f**.

*2.4.1. 4-Anilino-5-phenyl-2-[(propylthio) methyl]-2,4-dihydro-3H-1,2,4-triazole-3-thione (**5a**)*

Brown crystals from dioxane, yield 0.44 g (79%), m.p. 89 °C. IR (KBr): V_{max}/cm^{-1}: 3438 (NH), 3046 (Ar, CH), 2961, 2931 (Aliph., CH), 1615 (C═N); ^{1}H NMR (DMSO-d_6), δ, ppm: 7.87–7.54 (m, 10H, Ar-H), 6.15 (br, 3H, CH_2–N + NH), 3.206–3.157 (t, 2H, S–CH_2, J = 14.7 Hz), 1.70–1.60 (m, 2H, –CH_2–), 0.99–0.97 (t, 3H, CH_3–, J = 8.4 Hz). Anal. for $C_{18}H_{20}N_4S_2$ (356.5) Calcd. %: C 60.58; H 5.61; N 15.70; S 17.95. Found %: C 60.48; H 5.55; N 15.61; S 17.99.

*2.4.2. 4-Anilino-2-[(cyclohexylthio)methyl]-5-phenyl-2,4-dihydro-3H-1,2,4-triazole-3-thione (**5b**)*

White crystals from dimethylformamide, yield 0.39 g (62%), m.p. 222 °C; IR (KBr): V_{max}/cm^{-1}: 3439 (NH), 3055 (Ar, CH), 2929 (Aliph., CH), 1608 (C═N); ^{1}H NMR (DMSO-d_6), δ, ppm: 7.89–7.57 (m, 11H, Ar-H + NH), 6.36 (s, 2H, CH_2), 3.59–3.27 (m, 11H, cyclohexyl). Anal. for $C_{21}H_{24}N_4S_2$ (396.56) Calcd. %: C 63.54; H 6.05; N 14.12; S 16.13. Found %: C 63.48; H 6.22; N 14.11; S 16.25.

*2.4.3. 4-Anilino-5-phenyl-2-[(phenylthio)methyl]-2,4-dihydro-3H-1,2,4-triazole-3-thione (**5c**)*

White crystals from dimethylformamide, yield 0.41 g (67%), m.p. 98 °C; IR (KBr): V_{max}/cm^{-1}: 3433 (NH), 3054 (Ar, CH), 2928 (Aliph., CH), 1581 (C═N); ^{1}H NMR (DMSO-d_6), δ, ppm: 7.82–7.31 (m, 15H, Ar-H), 5.58 (br, 3H, NH + CH_2). Anal. for $C_{21}H_{18}N_4S_2$ (390.52) Calcd. %: C 64.52; H 4.61; N 14.33; S 16.38. Found %: C 64.64; H 4.55; N 14.47; S 16.35.

*2.4.4. 4-Anilino-2-{[(4-chlorophenyl)thio]methyl}-5-phenyl-2,4-dihydro-3H-1,2,4-triazole-3-thione (**5d**)*

White crystals from dimethylformamide, yield 0.56 g (82.5%), m.p. 138 °C; IR (KBr): V_{max}/cm^{-1}: 3439 (NH), 3055 (Ar, CH), 2929 (Aliph., CH), 1608 (C═N); ^{1}H NMR (DMSO-d_6), δ, ppm: 7.89–7.57 (m, 11H, Ar-H + NH), 6.36 (s, 2H, CH_2). Anal. for $C_{21}H_{17}ClN_4S_2$ (424.96) Calcd. %: C 59.29; H 4.00; N 13.17; S 15.06. Found %: C 59.41; H 4.12; N 13.32; S 15.12.

*2.4.5. 4-Anilino-2-{[(4-anilino-5-phenyl-4H-1,2,4-triazol-3-yl)thio]methyl}-5-phenyl-2,4-dihydro-3H-1,2,4-triazole-3-thione (**5e**)*

Pale-pink crystals from dimethylformamide, yield 0.65 g (75.5%), m.p. 148 °C; IR (KBr): V_{max}/cm^{-1}: 3440 (2NH), 3028 (Ar, CH), 2932 (Aliph., CH), 1610 (C═N); ^{1}H NMR (DMSO-d_6), δ, ppm: 7.99–7.54 (m, 20H, Ar-H), 5.9 (br, 4H, N–CH_2 + 2NH). Anal. for $C_{29}H_{24}N_8S_2$ (548.68) Calcd. %: C 63.42; H 4.37; N 20.41; S 11.66. Found %: C 63.35; H 4.43; N 20.37; S 11.81.

*2.4.6. 4-Anilino-5-phenyl-2-{[(5-phenyl-1,3,4-oxadiazol-2-yl)thio]methyl}-2,4-dihydro-3H-1,2,4-triazole-3-thione (**5f**)*

Pale-white crystals from dimethylformamide, yield 0.54 g (75%), m.p. 217 °C; IR (KBr): V_{max}/cm^{-1}: 3445 (NH), 3065 (Ar, CH), 2931 (Aliph., CH), 1612 (C═N); ^{1}H NMR (DMSO-d_6), δ, ppm: 7.95–7.11 (m, 16H, Ar-H + NH), 5.43 (s, 2H, N–CH_2). Anal. for $C_{23}H_{18}N_6OS_2$ (458.55) Calcd. %: C 60.18; H 3.92; N 18.31; S 13.95. Found %: C 60.22; H 3.87; N 18.42; S 13.76.

*2.5. Synthesis of compounds **6–10:** (general procedure)*

To a solution of compound **4** (2.2 mmol) and triethylamine (2.2 mmol) in xylene (40 mL) were added hydrazine hydrate, phenyl hydrazine, hydroxylamine hydrochloride, urea and/or thiourea (2.2 mmol), respectively. The reaction mixture was refluxed for 10 h. The solvent was evaporated under reduced pressure, the formed precipitate was collected and recrystallized from ethanol to give the corresponding compound **6–10**.

*2.5.1. 7-Anilino-6-phenyl-2,7-dihydro-3H-1,2,4-triazolo[4,3-b]-1,2,4-triazole (**6**)*

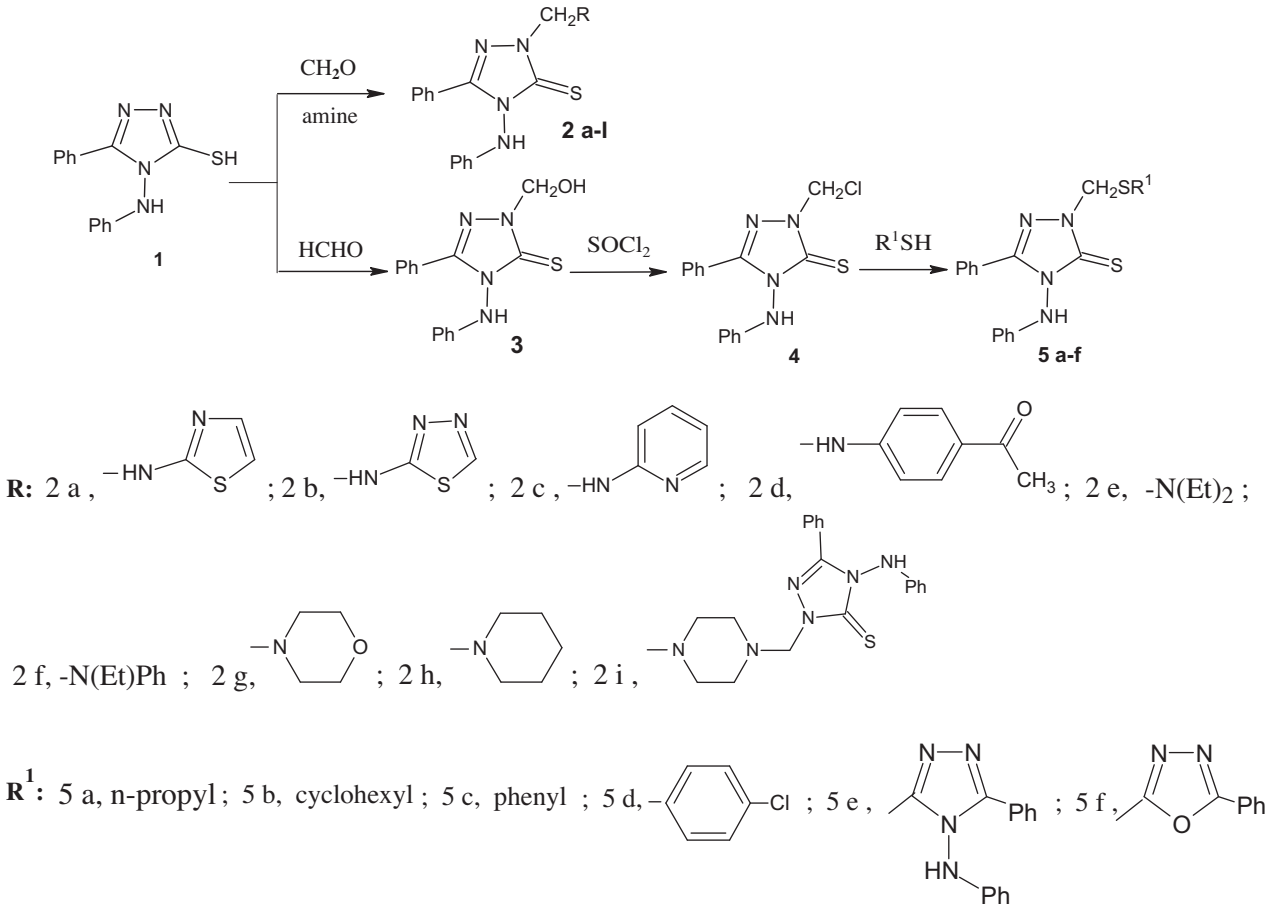
White crystals from ethanol, yield 0.68 g (97%), m.p. 200 °C; IR (KBr): V_{max}/cm^{-1}: 3106 (NH), 3015 (Ar, CH), 2932 (Aliph., CH), 1622 (C═N); MS, m/z (I_{rel},%): 276 [M]$^+$ (7.7), 252 (58.5), 192 (69.2), 161 (100), 133 (32.3), 104 (66.2),77 (67.7), 51 (72.3). Anal. for $C_{15}H_{12}N_6$ (276.3) Calcd. %: C 67.34; H 4.48; N 31.42. Found %: C 67.21; H 4.35; N 31.27.

2.5.2. 7-Anilino-2,6-diphenyl-2,7-dihydro-3H-1,2,4-triazolo[4,3-b]-1,2,4-triazole (7)

Dark brown crystals from ethanol, yield 0.56 g (72%), m.p. 115 °C; IR (KBr): V_{max}/cm^{-1}: 3414 (NH), 3045 (Ar, CH), 2928 (Aliph., CH), 1598 (C═N); ^{1}H NMR (DMSO-d_6), δ, ppm: 7.98–7.04 (m, 16H, Ar-H + NH), 5.54 (s, 2H, N–CH_2–N). Anal. for $C_{21}H_{18}N_6$ (354.41) Calcd. %: C 71.10; H 5.07; N 23.7. Found %: C 71.24; H 5.11; N 23.67.

Table 1 Results of anti-bacterial evaluation of compounds **2b**, **2c**, **2e**, **2g**, **2h**, **2i**, **4**, **5a**, **5b**, **5d**, **5e** and **6**.

Types of bacteria	*Bacillus cereus*			*Pseudomonas aeruginosa*			*Escherichia coli*		
	Concentrations			Concentrations			Concentrations		
Compound	10,000 ppm	30,000 ppm	50,000 ppm	10,000 ppm	30,000 ppm	50,000 ppm	10,000 ppm	30,000 ppm	50,000 ppm
2b	0.9 cm	2.3 cm	2.6 cm	0.7 cm	1.2 cm	1.4 cm	0.4 cm	0.8 cm	1.4 cm
2c	1.0 cm	1.7 cm	1.7 cm	–	0.5 cm	–	0.2 cm	0.4 cm	0.7 cm
2e	0.7 cm	1.3 cm	1.8 cm	0.7 cm	1.3 cm	1.8 cm	0.5 cm	1.1 cm	1.2 cm
2g	1.4 cm	3.1 cm	3.4 cm	0.8 cm	1.4 cm	1.9 cm	0.9 cm	1.8 cm	1.9 cm
2h	1.7 cm	2.6 cm	3.5 cm	1.1 cm	1.4 cm	2.3 cm	1.0 cm	1.2 cm	1.7 cm
2i	0.8 cm	0.9 cm	1.0 cm	0.8 cm	0.9 cm	1.3 cm	0.5 cm	0.8 cm	0.9 cm
4	1.0 cm	1.1 cm	2.0 cm	0.9 cm	1.2 cm	1.5 cm	1.1 cm	1.3 cm	2.3 cm
5a	0.5 cm	0.6 cm	0.7 cm	0.5 cm	0.7 cm	1.0 cm	0.5 cm	0.6 cm	0.8 cm
5b	0.6 cm	0.9 cm	1.1 cm	–	–	0.5 cm	0.5 cm	0.8 cm	0.4 cm
5d	0.7 cm	0.9 cm	0.9 cm	0.5 cm	0.7 cm	0.9 cm	0.6 cm	0.8 cm	1.0 cm
5e	0.3 cm	0.4 cm	0.8 cm	–	–	–	0.7 cm	0.8 cm	0.6 cm
6	0.4 cm	0.5 cm	0.7 cm	0.9 cm	1.3 cm	2.1 cm	0.5 cm	0.8 cm	0.9 cm



Scheme 1 Synthesis of Mannich bases and sulfides.

*2.5.3. 7-Anilino-6-phenyl-7H-1,2,4-triazolo[5,1-c]-1,2,4-oxadiazole (**8**)*

Brown crystals from ethanol, yield 0.55 g (90%), m.p. 138 °C; IR (KBr): V_{max}/cm^{-1}: 3422 (NH), 3022 (Ar, CH), 2932 (Aliph., CH), 1607 (C═N); ^{1}H NMR (DMSO-d_6), δ, ppm: 8.0–7.54 (m, 11H, Ar-H + NH), 5.9 (s, 2H, CH_2). Anal. for $C_{15}H_{13}N_5O$ (279.3) Calcd. %: C 64.44; H 4.65; N 25.06. Found %: C 64.36; H 4.58; N 25.31.

*2.5.4. 1-Anilino-2-phenyl-1,5,6,7-tetrahydro-1,2,4-triazolo[1,5-a]-1,3,5-triazin-7-one (**9**)*

Pale-brown crystals from ethanol, yield 0.41 g (61%), m.p. 140 °C; IR (KBr): V_{max}/cm^{-1}: 3398 (NH), 3038 (Ar, CH), 2923 (Aliph., CH), 1720 (C═O), 1612 (C═N); ^{1}H NMR (DMSO-d_6), δ, ppm: 8.0–7.55 (m, 12H, Ar-H + 2NH), 5.9 (s, 2H, CH_2). Anal. for $C_{16}H_{14}N_6O$ (306.32) Calcd. %: C 62.67; H 4.57; N 27.42. Found %: C 62.51; H 4.43; N 27.56.

*2.5.5. 1-Anilino-2-phenyl-1,5,6,7-tetrahydro-1,2,4-triazolo[1,5-a]-1,3,5-triazine-7-thione (**10**)*

Brown crystals from ethanol, yield 0.45 g (90%), m.p. 176 °C; IR (KBr): V_{max}/cm^{-1}: 3430 (2NH), 3091 (Ar, CH), 2924 (Aliph., CH), 1607 (C═N), 1175 (C═s); MS, *m/z* (I_{rel},%): 322 $[M]^+$ (2.77), 253 (100), 194 (10.22), 149 (10.09), 126 (41.39), 118 (19.53), 103 (12.7), 91 (18.3), 77 (26.62), 51(6.5). Anal. for $C_{16}H_{14}N_6S$ (322.38) Calcd. %: C 59.55; H 4.34; N 26.05. Found %: C 59.64; H 4.27; N 26.22.

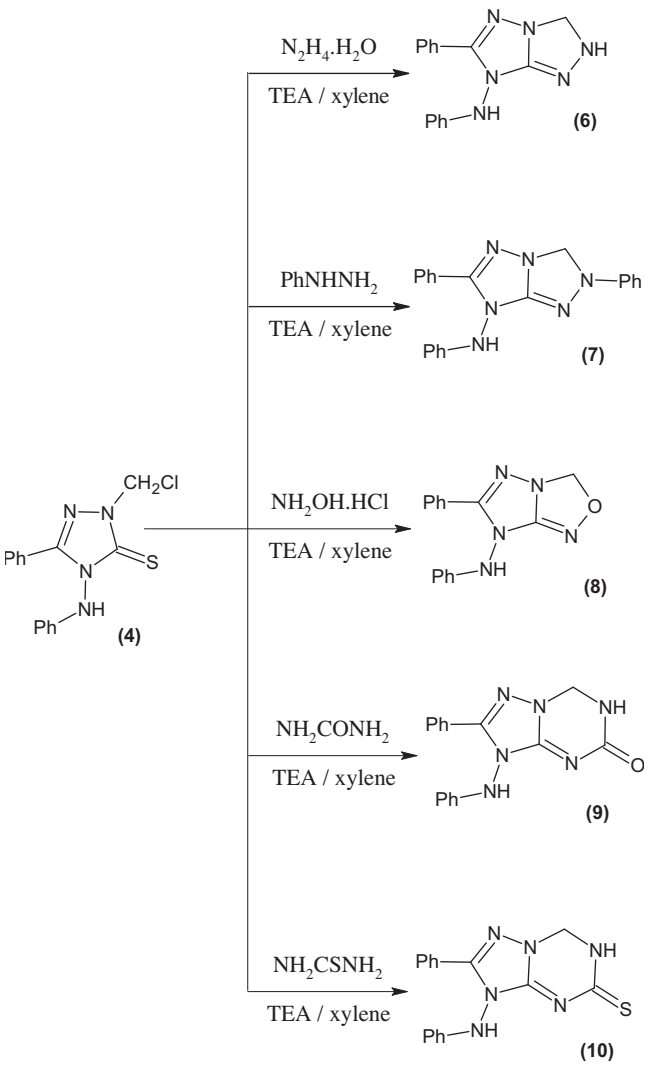
2.6. Antibacterial activity

The compounds were dissolved in DMSO. In order to ensure that the solvent per se had no effect on bacterial growth or enzymatic activity, negative control tests were performed using DMSO at the same concentrations. The inhibitory effect of compounds **2b**, **2c**, **2e**, **2g**, **2h**, **2i**, **4**, **5a**, **5b**, **5d**, **5e** and **6** on the *in vitro* growth of broad spectrum of bacteria representing one gram positive bacterium, namely *Bacillus cereus* and two gram negative bacteria, namely; *Pseudomonas aeruginosa* and *Escherichia coli* was evaluated using agar diffusion method (cup and plate method) (Barry, 1976) by measuring the zone of inhibition on agar plates at three different concentrations 10,000, 30,000 and 50,000 ppm. DMSO was used as solvent control. All plates were incubated at 37 ± 0.5 °C for 24 h. The zone of inhibition of compounds was measured using cm scale. The results indicated in Table 1.

3. Results and discussion

3.1. Chemistry

Mannich reaction on 4-anilino-5-phenyl-4*H*-1,2,4-triazole-3-thiol (**1**) (Chande et al., 1993) using formaldehyde and different amines namely; 2-aminothiazole, 2-amino-1,3,4-thiadiazole, 2-aminopyridine, *p*-aminoacetophenone, diethylamine, *N*-ethylaniline, morpholine, piperidine and piperazine afforded the corresponding Mannich bases **2a**–**i**. (*cf.* Scheme 1). The structure of these compounds was established on the basis of their elemental and spectral analyses. MS of compounds **2a**, **2b** and **2c** showed the molecular ion peaks at *m/z* = 380 (M^+, 10.6%), 381(M^+, 0.04%) and 374(M^+, 0.07%), respectively, (*cf.* experimental). 4-Anilino-2-hydroxymethyl-5-phenyl-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (**3**) was obtained *via* the reaction of compound **1** with formaldehyde, which in turn reacted with thionyl chloride to give 4-anilino-2-chloromethyl-5-phenyl-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (**4**). IR spectrum (KBr, cm^{-1}) of compound **3** showed new absorption band corresponding to OH at 3418.3 cm^{-1}. Treatment of compound **4** with the respective aliphatic, aromatic or heterocyclic thiols namely; propanethiol, cyclohexanethiol, thiophenol, 4-chlorothiophenol, 4-anilino-5-phenyl-4*H*-1,2,4-triazole-3-thiol and 5-phenyl-1,3,4-oxadiazole-2-thiol, in boiling dimethylformamide in the presence of triethylamine, yielded the corresponding sulfides **5a**–**f** in good yields (*cf.* Scheme 1). The IR spectra (KBr, cm^{-1}) of compounds **5a**–**f** showed disappearance of absorption band corresponding to C–Cl at 767 cm^{-1}. Condensation of chloromethyl derivative **4** with hydrazine hydrate, phenyl hydrazine, hydroxylamine, urea and thiourea in refluxing *p*-xylene and triethylamine gave; 7-anilino-6-phenyl-2,7-dihydro-3*H*-1,2,4-triazolo[4,3-*b*]-1,2,4-triazole (**6**), 7-anilino-2,6-diphenyl-2,7-dihydro-3*H*-1,2,4-triazolo[4,3-*b*]-1,2,4-triazole (**7**), 7-anilino-6-phenyl-7*H*-1,2,4-triazolo[5,1-*c*]-1,2,4-oxadiazole (**8**), 1-anilino-2-phenyl-1,5,6,7-tetrahydro-1,2,4-triazolo[1,5-*a*]-1,3,5-triazin-7-one (**9**) and 1-anilino-2-phenyl-1,5,6,7-tetrahydro-1,2,4-triazolo[1,5-a]-1,3,5-triazine-7-thione (**10**) respectively (cf. Scheme 2). The structure of these compounds was proved by elemental and spectral analyses (*cf.* experimental). MS of compounds **6** and **10** showed the molecular ion peak at *m/z* 276 (M^+, 7.7%) and 322 (M^+, 2.77%), respectively.



Scheme 2 Synthesis of triazolo-, oxadiazolo- and triazinotriazoles.

3.2. Antibacterial activity

The results of antibacterial activity are shown in Table 1. Mannich bases derivatives exhibited good activity, specially compounds **2g** and **2h**, showed the highest inhibitory effect against all types of bacteria excepting *E. coli* which effected highly by chloromethyl compound **4** (2.3 cm). Sulfides display good activity against Bacillus cereus and *E. coli* but possess moderate to poor activities against *P. aeruginosa*. Triazolo-triazole **6** showed good activities against all types of bacteria.

The zone of inhibition of all compounds was increased by increasing the concentrations excepting compound **2c** that has activity against *P. aeruginosa* at 30,000 ppm only.

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