Synthesis and Biological Activity of Some Triazolothiadiazoles

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ABSTRACT

The synthesis of a series of novel 3-pyridyl-6-aryl-s-triazolo[3,4-b]-[1,3,4]-thiadiazoles is described. Fourteen new compounds were synthesized and characterized by spectral and elemental analyses. Some compounds were screened for antibacterial activity against *S. aureus, E. coli, B. subtilis* and *P. aeruginosa.* Compounds containing aryl substituents at position 6 and the 1,2,4-triazole moiety at position 1 or 2 showed reasonable antibacterial activity.

KEYWORDS

1,2,4-Triazole, thiadiazole, potassium dithiocarbazinate, hydrazine hydrate, antibacterial activity.

1. Introduction

The chemistry of heterocyclic compounds continues to be an active field in organic chemistry. Triazole derivatives have occupied a unique position in heterocyclic chemistry due to their antimicrobial activities.[1,2]

1,2,4-Triazoles exhibit a wide range of therapeutical properties.[3] 1,2,4-Triazoles as antibacterial agents can be grouped according to the mode of action, i.e. the ability to inhibit the synthesis of the cell wall, cell membrane, proteins and nucleic acids of bacteria. The synthesis of 1,2,4-triazoles has also attracted widespread attention due to the diverse agricultural, industrial and biological activities, including anti-inflammatory, analgesic, antitumoral, anticonvulsant and tranquillizing activities shown by these compounds. Triazoles target both prokaryotes as well as eukaryotes but we are interested in their activity against prokaryotes. In view of these observations and in continuation of our earlier work[4-6] on the synthesis of some 1,2,4- and 1,2,3-triazole derivatives, we now report the synthesis of more novel triazole derivatives derived from 4-amino-5-mercapto-3-pyridyl-1,2,4-triazole.

2. Results and Discussion

4-Amino-5-mercapto-3-pyridyl-1,2,4-triazole **(2)**, which is required as starting material, was obtained in a one-pot reaction by heating potassium dithiocarbazinate, hydrazine hydrate and water under reflux conditions for 8 h. H_2S formed and a clear solution was obtained. The 1-NH group is converted to the corresponding potassium dithiocarbazinate, which on cyclization with hydrazine hydrate affords 4-amino-5-mercapto-3-pyridyl-1,2,4-triazole (**2**). 3-Pyridyl-6-aryl-s-triazolo[3,4-b]-[1,3,4]-thiadiazoles (**4a–m**), which are novel compounds, were prepared by the reaction of **2** with aromatic acid in the presence of phosphorus oxychloride. Similarly 4-N-pyridyl carboxamido-5-mercapto-3-pyridyl-1,2,4-triazole (**3**) was obtained by condensation of equimolar quantities of potassium dithiocarbazinate and INH (isonicotinic acid hydrazide or isoniazide) in an oil bath at 175 °C for 6 h; profuse evolution of H_2S was observed. The reaction mixture was cooled, diluted with water and the product (**3**) recrystallized from aqueous ethanol (80 %) (see Scheme 1).

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Elemental analysis of compound **2** corresponds to the molecular formula $C_7H_7N_5S$. The IR spectrum of compound **2** shows characteristic absorption bands at 3275 cm^{-1} for NH_2, 3080 cm^{-1} for ArCH stretching and 2625 cm^{-1} for SH stretching. The absence of absorption bands due to C=O for CONH at 1680, 1620 (C=N), 1600 (C=C), 1515 (CN) and 700 cm^{-1} (CS) stretching vibrations of starting triazoles clearly indicates the formation of the cyclized product.

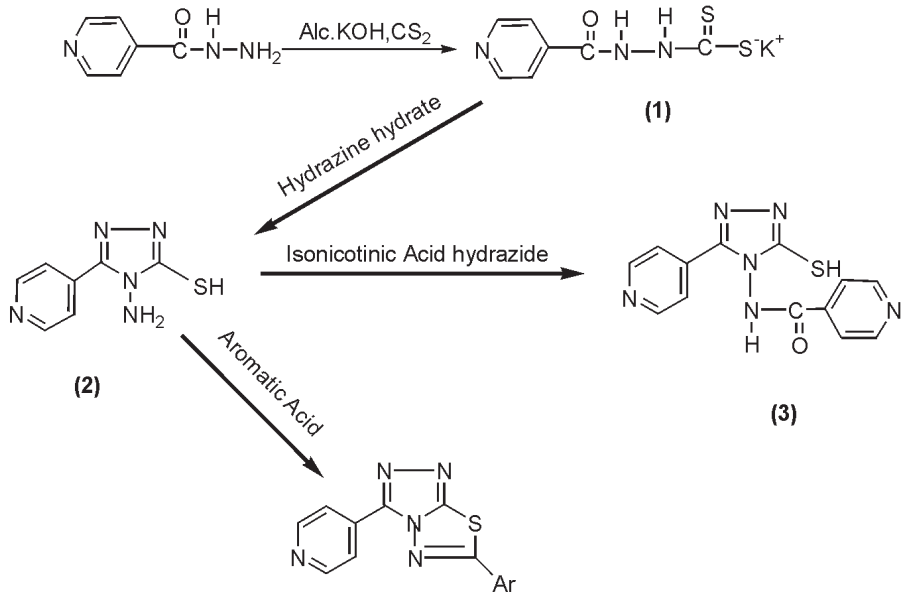
Elemental analysis of compound **3** corresponds to the molecular formula $C_{13}H_{10}N_6SO$. The IR spectrum of compound **3** shows characteristic absorption bands at 3070 cm^{-1} for Ar CH stretching, 2600 cm^{-1} for SH stretching, 1680 cm^{-1} for C=O of CONH, 1620 cm^{-1} for C=N, 1600 cm^{-1} for C=C, and 1510 and 1400 cm^{-1} for CH bending. The proton magnetic resonance (PMR) spectrum of **3** exhibits a singlet at δ 14.6 ppm for the CONH proton and the mass spectrum of **3** shows molecular ion peaks at m/z 298 (M^+) in conformity with the assigned molecular formula.

Elemental analysis of compound **4a** corresponds to the molecular formula $C_{14}H_9N_5S$. The IR spectrum of **4a** shows characteristic absorption bands at 3050 cm^{-1} for Ar CH stretching, 760 cm^{-1} for a monosubtituted benzene and 690 cm^{-1} for CS stretching. The PMR spectrum of **4a** exhibits a multiplet (SH) at δ 7.7–8.1 ppm for the aromatic protons and the mass spectrum of **4a** shows a molecular ion at m/z 297 (M^+) in conformity with the assigned molecular formula.

These compounds were tested against various bacterial strains and the details are provided in the experimental section.

3. Experimental

Melting and boiling points were determined on a Gallenkamp apparatus (Southborough, Leics., UK) in open capillaries and are uncorrected. IR spectra (in KBr) were recorded on a Jasco FT-IR 5300 spectrophotometer (Tokyo, Japan) and proton magnetic resonance (PMR) spectra (DMSO-d6) on a Varian EM-390 spectrometer (Palo Alto, CA, USA) using TMS as an internal standard (chemical shift, δ ppm). Mass spectra were recorded on a Jeol JMS-D 300 mass spectrometer (Amsterdam, the Netherlands) operating at 70 eV. The purity of the compounds was confirmed by TLC using silica gel and they were



Compound **4**

a	Phenyl	b	Chlorophenyl	c	2,4-Dichlorophenyl	d	4-Chloro-3-nitrophenyl
e	4-Nitrophenyl	f	2-Hydroxyphenyl	g	4-Nitro-2-aminophenyl	h	Phenoxymethyl
i	4-Chlorophenoxymethyl	j	4-Nitrophenoxymethyl	k	2-Methyl phenoxymethyl	l	1- Naphthyloxymethyl
m	Benzyl						

Scheme 1

purified by column chromatography. For TLC, Merck silica gel 60G plate (Darmstadt, Germany) was used. For column chromatography, Merck silica gel 60 (0.063–0.200 mm) was used. The necessary chemicals were obtained from Merck and Fluka (Milwaukee, WI, USA). All compounds showed satisfactory elemental analyses.

3.1. Potassium Dithiocarbazinate (1)[7]

Acid hydrazide (0.01 mol) was reacted with carbon disulphide (0.01 mol) in the presence of alcoholic potassium hydroxide (0.08 g KOH in 20 mL aqueous ethanol, 1.5 mol) for a period of 4.5 h. After cooling the separated potassium dithiocarbazinate was taken out, dried and used directly for the next step without further purification (yield 80 %).

3.2. 4-Amino-5-mercapto-3-pyridyl-1,2,4-triazole (2)

A suspension of potassium dithiocarbazinate (0.1 mol), hydrazine hydrate (80 %, 15 mL, 0.3 mol) and water (5 mL) was heated under reflux for 8 h. H_2S evolved after which a clear solution resulted. Dilution of the reaction mixture with cold water (5 mL) and subsequent acidification with dilute HCl gave a white precipitate. The precipitate was filtered, washed with water and crystallized from aqueous ethanol (80 %) to give **2** (yield 80 %), m.p. 208 °C. Anal. calc. for $C_7H_7N_5S$: C, 43.52; H, 3.65; N, 26.27 %; found C, 43.47; H, 3.50; N, 26.19 %; IR (KBr): 3275 (NH_2), 3080 (aromatic CH stretching), 2625 (SH), absence of band for C=O of CONH at 1680 cm^{-1}, 1620 (C=N), 1600 (C=C), 1515 (CN) and 700 cm^{-1} (CS); PMR: δ 5.9 (2H, s, NH_2), 8.0–8.7 (8H, dd, pyridyl) and 14.1 ppm (1H, s, H of SH); MS: m/z 193 (M^+) other peaks were observed at 178, 161, 122, 105, 78, 60 and 44 m/z.

3.3. 4-(N-Pyridyl carboxamido)-5-mercapto-3-pyridyl-1,2,4-triazole (3)

Potassium dithiocarbazinate and INH (isonicotinic acid hydrazide or isoniazide) were heated in equimolar proportions in an oil bath at 175 °C for 6 h; profuse evolution of hydrogen sulphide was observed. The reaction mixture was cooled, diluted with water and the product was recrystallized from aqueous ethanol (80 %) to yield **3** (yield 90 %), m.p. 294 °C. Anal. calc. for $C_{13}H_{10}N_6SO$: C, 52.35; H, 3.36; N, 28.19 %; found C, 52.32; H, 3.32; N, 27.97 %; IR (KBr): 3070 (aromatic CH stretching), 2600 (SH), 1680 (C=O of CONH), 1620 (C=N), 1600 (C=C), 1570, 1490 (CN) and 700 cm^{-1} (SH); PMR: δ 7.8–8.88 (8H, dd, pyridyl), 12.5 (1H, s, 3H) and 14.6 ppm (1H, s, CONH); MS: m/z 298 (M^+) other peaks observed at 282, 265, 220, 193, 170, 162, 149, 120, 106 and 78 m/z.

3.4. 3-Pyridyl-6-aryl-s-triazolo (3,4-b) (1,3,4)-thiadiazoles (4a–m)

General Procedure. A mixture of compound **2** (0.1 mol), aromatic acid (0.1 mol) and phosphorus oxychloride (10 mL) was refluxed for 1.5 h, cooled and poured onto crushed ice. The novel solids were separated and filtered, treated with dilute NaOH, washed with water and crystallized from DMF.

4a (yield 85–90 %), m.p. 236 °C. Anal. calc. for $C_{14}H_9N_5S$, C, 60.22; H, 2.23; N, 25.09 %; found C, 60.19; H, 3.20; N, 24.48 %; IR (KBr): 3050 (aromatic CH stretching), 760 (monosubstituted benzene) and 690 cm^{-1} (CS); PMR: δ 7.7–8.1 (3H, m, aromatic) and 8.3–8.9 ppm (4H, dd, pyridyl); MS: m/z 297 (M^+); other peaks observed at 149, 139, 121, 104, 89, 77, 63, 51 and 44 m/z.

4b (yield 80–90 %), m.p. 232 °C. Anal. calc. for $C_{14}H_8N_5SCl$, C, 60.05; H, 2.25; N, 25 %; found C, 60.11; H, 2.28; N, 24.98 %; IR (KBr): 3039 (aromatic CH stretching), 771 (monosubstituted benzene) and 712 cm^{-1} (CS); PMR: δ 8.3 (3H, m, aromatic) and 8.7 ppm (4H, dd, pyridyl); MS: m/z 283 (M^+); other peaks observed at 168, 137, 108, 103, 69, 49 and 39 m/z.

4c (yield 85 %), m.p. 238 °C. Anal. calc. for $C_{14}H_7N_5SCl_2$, C, 58.62; H, 3.19; N, 25.31 %; found C, 61.3; H, 3.2; N, 23.92 %; IR (KBr): 2989 (aromatic CH stretching), 698 (monosubstituted benzene) and 691 cm^{-1} (CS); PMR: δ 7.1–7.4 (3H, m, aromatic) and 7.5–7.9 ppm (4H, dd, pyridyl); MS: m/z 286 (M^+); other peaks

Table 1 Evaluation of antibacterial activity of the compounds.

Compound	Average zone of inhibition/mm [a]							
	S. aureus		*E. coli*		*B. subtilis*		*P. aeruginosa*	
	100 μg mL^{-1}	10 μg mL^{-1}	100 μg mL^{-1}	10 μg mL^{-1}	100 μg mL^{-1}	10 μg mL^{-1}	100 μg mL^{-1}	10 μg mL^{-1}
3	16	14	14	13	15	13	16	15
4a	15	14	14	13	14	12	19	17
4b	14	13	11	9	11	11	15	14
4c	12	12	10	9	15	13	14	14
4d	–	–	11	10	14	12	14	13
4e	11	11	12	11	13	11	13	11
4f	–	–	12	11	12	10	15	13
4g	12	11	10	9	11	11	–	–
4h	12	10	11	10	–	–	–	–
4i	14	12	15	14	10	10	11	11
4j	–	–	10	9	–	–	–	–
4k	17	15	14	14	13	12	14	13
4l	20	18	15	13	13	12	13	13
4m	16	14	15	13	14	13	14	12
Standard ampicillin	28	22	26	20	24	20	24	20
Control	00	00	00	00	00	00	00	00

[a] – No inhibition zone.

observed at 198, 171, 169, 128, 106, 79, 56 and 41 m/z.

4d (yield 80–85 %), m.p. 235 °C. Anal. calc. for $C_{14}H_7N_6SO_2Cl$, C, 61.02; H, 2.45; N, 26.20 %; found C, 60.93; H, 2.21; N, 25.10 %; IR (KBr): 3053 cm^{-1} (aromatic CH stretching), the signal for SH at 2625 cm^{-1} disappeared, 2361 (OCH_2), 1660 (C=N), 1620 (C=C), 1518 (CN), 1260 (COC), 810 (monosubstituted benzene) and 668 cm^{-1} (CS); PMR: δ 5.4–5.7 (3H, m, aromatic), 5.9 (2H,S,OCH_2) and 8.9–9.2 ppm (4H, dd, pyridyl); MS: m/z 306 (M^+); other peaks observed at 233, 231, 189, 167, 136, 96, 78, 52 and 38 m/z.

4e (yield 80–85 %), m.p. 237 °C. Anal. calc. for $C_{14}H_8N_6SO_2$, C, 60.06; H, 2.55; N, 26.8 %; found C, 58.82; H, 2.91; N, 24.91 %; IR (KBr): 3060 cm^{-1} (aromatic CH stretching), the signal for SH at 2625 cm^{-1} disappeared, 2361 (OCH_2), 1760 (C=N), 1622 (C=C), 1509 (CN), 1339 (COC), 770 (monosubstituted benzene) and 650 cm^{-1} (CS); PMR: δ 5.8 (3H, m, aromatic) and 8.9 ppm (4H, dd, pyridyl); MS: m/z 292 (M^+); other peaks observed at 267, 263, 185, 179, 145, 135, 81, 77 and 44 m/z.

4f (yield 81 %), m.p. 240 °C. Anal. calc. for $C_{14}H_9N_5SO$, C, 59.22; H, 2.82; N, 25.33 %; found C, 62.16; H, 2.52; N, 24.37 %; IR (KBr): 3021 cm^{-1} (aromatic CH stretching), the signal for SH at 2625 cm^{-1} disappeared, 2269 (OCH_2), 1653 (C=N), 1625 (C=C), 1510 (CN), 1331 (COC), 819 (monosubstituted benzene) and 691 cm^{-1} (CS); PMR: δ 4.9–5.2 (3H, m, aromatic) and 8.4 ppm (4H, dd, pyridyl); MS: m/z 287 (M^+); other peaks observed at 269, 265, 187, 175, 174, 139, 81 and 53 m/z.

4g (yield 70–80 %), m.p. 241 °C. Anal. calc. for $C_{14}H_9N_7SO_2$, C, 61.24; H, 3.14; N, 23.95 %; found C, 59.61; H, 2.98; N, 24.72 %; IR (KBr): 2970 cm^{-1} (aromatic CH stretching), the signal for SH at 2625 cm^{-1} disappeared, 2269 (OCH_2), 1631 (C=N), 1611 (C=C), 1556 (CN), 1340 (COC), 801 (monosubstituted benzene) and 667 cm^{-1} (CS); PMR: δ 7.2–7.8 (3H, m, aromatic), 8.1–8.5 (5H, m, aromatic) and 8.9 ppm (4H, dd, pyridyl); MS: m/z 316 (M^+); other peaks observed at 282, 266, 261, 248, 194, 107, 104, 77, 63 and 47 m/z.

4h (yield 87 %), m.p. 242 °C. Anal. calc. for $C_{15}H_{11}N_5SO$, C, 58.6; H, 3.61; N, 27.76 %; found C, 56.78; H, 2.71; N, 28.10 %; IR (KBr): 3079 cm^{-1} (aromatic CH stretching), the signal for SH at 2625 cm^{-1} disappeared, 2258 (OCH_2), 1620 (C=N), 1601 (C=C), 1505 (CN), 1069 (COC), 759 (monosubstituted benzene) and 710 cm^{-1} (CS); PMR: δ 5.5 (2H,s,OCH_2), 7.1–7.5 (5H, m, aromatic), and 8.1–8.7 ppm (4H, dd, pyridyl); MS: m/z 308(M^+); other peaks observed at 268, 263, 241, 215, 197, 177, 165, 147, 132, 121, 105, 84, 77 and 65 m/z.

4i (yield 80–90 %), m.p. 235 °C. Anal. calc. for $C_{15}H_{10}N_5SOCl$, C, 61.24; H, 2.31; N, 25.91 %; found C, 61.17; H, 2.67; N, 24.95 %; IR (KBr.): 3052 cm^{-1} (aromatic CH stretching), the signal for SH at 2625 cm^{-1} disappeared, 2209 (OCH_2), 1690 (C=N), 1598 (C=C), 1509 (CN), 1107 (COC), 790 (monosubstituted benzene) and 710 cm^{-1} (CS); PMR: δ 5.9 (2H,s,OCH_2), 6.8–7.8 (3H, m, aromatic) and 7.9–8.7 ppm (4H, dd, pyridyl); MS: m/z 294 (M^+); other peaks observed at 197, 144, 142, 139, 117, 87, 72, 61 and 49 m/z.

4j (yield 85 %), m.p. 245 °C. Anal. calc. for $C_{15}H_{10}N_6SO_2$, C, 57.8; H, 3.66; N, 26.99 %; found C, 59.2; H, 3.16; N, 25.70 %; IR (KBr): 2985 cm^{-1} (aromatic CH stretching), the signal for SH at 2625 cm^{-1} disappeared, 2200 (OCH_2), 1701 (C=N), 1603 (C=C), 1508 (CN), 1206 (COC), 709 (monosubstituted benzene) and 665 cm^{-1} (CS); PMR: δ 5.7 (2H,s,OCH_2), 7.4–8.2 (3H, m, aromatic) and 8.8 ppm (4H, dd, pyridyl); MS: m/z 284 (M^+); other peaks observed at 274, 257, 232, 179, 159, 104, 86, 64 and 43 m/z.

4k (yield 85–87 %), m.p. 235 °C. Anal. calc. for $C_{16}H_{13}N_5SO$, C, 60.02; H, 2.10; N, 24.96 %; found C, 61.09; H, 2.10; N, 24.91 %; IR (KBr): 3011, 2985 cm^{-1} (aromatic CH stretching), the signal for SH at 2625 cm^{-1} disappeared, 2302 (OCH_2), 1691 (C=N), 1590 (C=C), 1520 (CN), 1199 (COC), 799 (monosubstituted benzene) and 697 cm^{-1} (CS); PMR: δ 5.6–5.9 (2H,s,OCH_2), 7.6–7.9 (3H, m, aromatic) and 7.92 ppm (4H, dd, pyridyl); MS: m/z 296 (M^+); other peaks observed at 197, 159, 137, 115, 89, 77, 68 and 54 m/z.

4l (yield 87 %), m.p. 238 °C. Anal. calc. for $C_{19}H_{13}N_5SO$, C, 60.05; H, 2.15; N, 25.56 %; found C, 59.61; H, 2.63; N, 24.06 %; IR (KBr): 3036 cm^{-1} (aromatic CH stretching), the signal for SH at 2625 cm^{-1} disappeared, 2280 (OCH_2), 1621 (C=N), 1603 (C=C), 1506 (CN), 1139 (COC), 748 (monosubstituted benzene) and 740 cm^{-1} (CS); PMR: δ 5.8 (2H,s,OCH_2), 7.3–7.5 (3H, m, aromatic) and 8.3 ppm (4H, dd, pyridyl); MS: m/z 290 (M^+); other peaks observed at 189, 177, 147, 138, 112, 85, 76, 62 and 57 m/z.

4m (yield 75–80 %), m.p. 230 °C. Anal. calc. for $C_{15}H_{11}N_5S$, C, 58.55; H, 3.12; N, 25.71 %; found C, 57.65; H, 3.15; N, 24.99 %; IR (KBr): 3060 cm^{-1} (aromatic CH stretching), the signal for SH at 2625 cm^{-1} disappeared, 1620 (C=N), 1605 (C=C), 1510 (CN), 750

(monosubstituted benzene) and 700 cm^{-1} (CS); PMR: δ 7.3–7.5 (3H, m, aromatic) and 8.1–8.8 ppm (4H, dd, pyridyl); MS: m/z 293 (M^+); other peaks observed at 259, 235, 179, 148, 135, 121, 103, 91, 77, 70, 65 and 51 m/z.

3.5. Biological Activity

The antibacterial activity of fourteen compounds (**3, 4a–m**), was investigated by employing the filter paper disc method.[8–11] Representative organisms selected for evaluation of antibacterial activity were *S. aureus, E. coli, B. subtilis* and *P. aeruginosa.* The antibacterial activity of each of the compounds was evaluated in triplicate at 100 μg mL^{-1} and 10 μg mL^{-1} concentrations. The compounds were tested as solutions or suspensions in DMF (99.80 % anhydrous). An important and useful control drug, ampicillin, was also tested under similar conditions, with a view to comparing the results.

Ampicillin is a beta-lactam antibiotic[12] that has been used extensively to treat bacterial infections since 1961. Ampicillin is able to penetrate Gram-positive and some Gram-negative bacteria.[13] Ampicillin acts as a competitive inhibitor of transpeptidase enzymes. As a powder ampicillin is white with a slight yellow cast and is soluble in water.

The results indicate that all of the synthesized compounds showed moderate to strong activity against these bacterial strains (see Table 1). Compounds **3, 4a, 4b, 4c, 4i, 4k, 4l** and **4m** showed good activity against *S. aureus, E. coli, B. subtilis* and *P. aeruginosa.* Similarly, compounds **4d** and **4f** showed moderate to mild activity against *E. coli, B. subtilis* and *P. aeruginosa,* **4e** against *S. aureus, E. coli, B. subtilis* and *P. aeruginosa,* **4g** against *S. aureus, E. coli* and *B. subtilis,* **4h** against *S. aureus* and *E. coli* and **4j** against *E. coli* only. From the above observations it is clear that the triazole derivatives of triazolothiadiazoles are more active and also the substituents phenyl, 2-chlorophenyl, 2-methylphenoxymethyl, 1-naphthyloxymethyl and benzyl in triazolothiadiazoles play a prominent role in the biological activity. However, ampicillin is more effective in its antibacterial activity compared with all of the synthesized compounds.

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