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Synthesis and Antimicrobial Screening of Novel Bis-Triazolo-Thiadiazoles Containing Bridgehead Nitrogen

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ABSTRACT

A facile synthesis of 1,4-bis-(6-arylimino-[1,2,4]-triazolo-(3,4-b)-[1,3,4]-thiadiazol-3-yl)-benzenes have been carried out by reacting 1,4-bis-(4-amino-3-mercapto-4H-[1,2,4]-triazol-5-yl-benzene with *N*-aryl isocyanodichlorides followed by the basification with dilute ammonium hydroxide solution. 1,4-bis-(4-amino-3-mercapto-4H-[1,2,4]-triazol-5-yl-benzene was synthesized by the interaction of terephthalic acid dihydrazide with carbondisulphide and potassium hydroxide followed by the addition of hydrazine hydrate. The acetylation of bis-triazolo-dithiadiazines afforded di-acetyl derivatives. The structures of synthesized compounds have been established on the basis of chemical transformation, elemental analysis, equivalent weight determination and IR, ^{1}H-NMR, mass spectral studies. The title compounds have been assayed for their antimicrobial activity against gram-positive as well as gram-negative micro-organisms.

Keywords: Synthesis; antimicrobial screening; novel bis-triazolo-thiadiazoles.

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INTRODUCTION

Heterocyclic compounds especially those containing sulphur and nitrogen atoms possess a wide variety of biological activities. Therapeutic effect of [1,2,4]-triazole and [1,2,4]-triazole-3-one containing compounds have been well studied for a number of pathological conditions including inflammation, cancer, pain, tuberculosis and hypertension[1,2]. Fused [1,2,4]-triazoles are found to possess diverse applications in the field of medicine[3,4]. Triazolo-thiadiazoles are reported to show a broad spectrum of pharmacological properties like antifungal, antibacterial, antiviral, anticonvulsant, analgesic, anti-inflammatory and antituberculor activities[5-8]. These two fused systems are reported to possess significant CNS depressant, anthelmentic and other pharmaceutical activities[9,10]. Synthetic applications of *N*-aryl isocyanodichlorides have been investigated earlier and shown to have enough potentiality in the synthesis of nitrogen and sulphur containing 5 and 6 membered heterocyclic compounds[11]. In view of these findings and as a part of wider programme to provide alternative routes of synthesis[12,13], we report herein the synthesis of bis-(substituted)-[1,2,4]-triazolo-(3,4-b)-[1,3,4]-thiadiazoles.

MATERIALS AND METHODS

The melting points of all synthesized compounds were recorded using the Veego, VMP-D digital melting point apparatus and are uncorrected. Chemicals used were of AR grade. ^{1}H-NMR spectra were recorded on a Bruker Avance II 400 NMR spectrometer with TMS as internal standard using $CDCl_3$ and DMSO-d_6 as solvents. IR spectra were recorded on Perkin-Elmer spectrophotometer in the range 4000-400 cm^{-1} in Nujol mull and as KBr pellete. Purity of the compounds was checked on silica gel-G plates by TLC.

The parent compound terephthalic acid dihydrazide (**1**) was prepared by refluxing the mixture of terephthalic acid (0.01 mol) and thionyl chloride (0.02 mol) for 30 minute, followed by the dropwise addition of hydrazine hydrate (0.02 mol) with constant stirring.

Synthesis of 1,4-bis-(4-amino-3-mercapto-4H-[1,2,4]-triazol-5-yl-benzene (2)

The compound 1,4-bis-(4-amino-3-mercapto-4H-[1,2,4]-triazol-5-yl-benzene (**2**) was prepared by the interaction of terephthalic acid dihydrazide (**1**) (0.01 mol) with carbondisulphide (0.02 mol) and potassium hydroxide solution (2M, 10 ml) followed by the dropwise addition of hydrazine hydrate (0.02 mol) with constant stirring. The stirring was continued for 30 minute at room temperature. The reaction mixture was cooled and poured in distilled water, a white precipitate was obtained. It was crystallized from ethanol, (**2**) (80%), m.p. 214°C(d).

Synthesis of 1,4-bis-(6-phenylimino-[1,2,4]-triazolo-(3,4-b)-[1,3,4]-thiadiazol-3-yl)-benzene (4a)

The compound 1,4-bis-(4-amino-3-mercapto-4H-[1,2,4]-triazol-5-yl-benzene (**2**) (0.01 mol) was reacted with *N*-phenyl isocyanodichloride (**3a**) (0.02 mol) in boiling chloroform medium over a water bath for 3 hour. The evolution of hydrogen chloride gas was noticed. Cooling the reaction mixture and distilling off chloroform afforded a sticky mass, which on washing with petroleum ether (60-80^{o}C) gave a granular solid. It was acidic to litmus and on determination of equivalent weight found to be the hydrochloride. It on basification with dilute ammonium hydroxide solution afforded a free base, 1,4-bis-(6-phenylimino-[1,2,4]-triazolo-(3,4-b)-[1,3,4]-thiadiazol-3-yl)-benzene (**4a**). It was crystallized from ethanol, (65%), m.p. 50^{0}C

Spectral and elemental analysis of 1,4-bis-(6-phenylimino-[1,2,4]-triazolo-(3,4-b)-[1,3,4]-thiadiazol-3-yl)-benzene (4a)

(Found: C, 55.12; H, 3.08; N, 26.18; S, 12.61. Calcd. for $C_{24}H_{16}N_{10}S_2$: C, 56.69; H, 3.14; N, 27.55; S, 12.59%); ν_{max} 3439 (NH), 1616, 1538, 1504 (C=N), 1315, 1293 (C-N), 1236 (N-N), 780, 742 cm^{-1} (C-S); δ ($CDCl_3$+DMSO-d_6) 9.50 (2H, s, NH), 7.02-8.21 (14H, m, Ar-H); MS: m/z 431 (M^+-C_6H_5), 417 (M^+-N-C_6H_5), 260 (M^+-$C_3HN_4S_2$-N-C_6H_5), 216 (C_3HN_4S-N-$C_6H_5^+$), 139 (C_3HN_4S-N^+), 125 ($C_3HN_4S^+$)[14,15]. This reaction was extended to synthesize other compounds (4b-g): 4b (70%), m.p. 184^{0}C (Found: C, 58.11; H, 3.68; N, 26.05; S, 11.88. Calcd. for $C_{26}H_{20}N_{10}S_2$: C, 58.20; H, 3.73; N, 26.11; S, 11.94%); c (68%), m.p. 206^{0}C (Found: C, 57.84; H, 3.69; N, 25.85; S, 11.81. Calcd. for $C_{26}H_{20}N_{10}S_2$: C, 58.20; H, 3.73; N, 26.11; S, 11.94%); d (60%), m.p. 241^{0}C (Found: C, 57.96; H, 3.62; N, 25.88; S, 11.50. Calcd. for $C_{26}H_{20}N_{10}S_2$: C, 58.20; H, 3.73; N, 26.11; S, 11.94%); ν_{max} 3438 (NH), 1614, 1537, 1503 (C=N), 1311, 1291 (C-N), 1235 (N-N), 780, 741 cm^{-1} (C-S); δ ($CDCl_3$+DMSO-d_6) 9.58 (2H, s, NH), 7.05-8.16 (12H, m, Ar-H), 2.28 (6H, s, Ar-CH_3); e (70%), m.p. 107^{0}C (Found: C, 49.08; H, 2.18; N, 23.99; S, 11.11. Calcd. for $C_{24}H_{14}N_{10}S_2Cl_2$: C, 49.91; H, 2.42; N, 24.26; S, 11.09%); f (70%), m.p. 164^{0}C (Found: C, 48.79; H, 2.39; N, 24.21; S, 10.97. Calcd. for $C_{24}H_{14}N_{10}S_2Cl_2$: C, 49.91; H, 2.42; N, 24.26; S, 11.09%); g (71%), m.p. 213^{0}C (Found: C, 48.81; H, 2.33; N, 24.16; S, 11.05. Calcd. for $C_{24}H_{14}N_{10}S_2Cl_2$: C, 49.91; H, 2.42; N, 24.26; S, 11.09%).

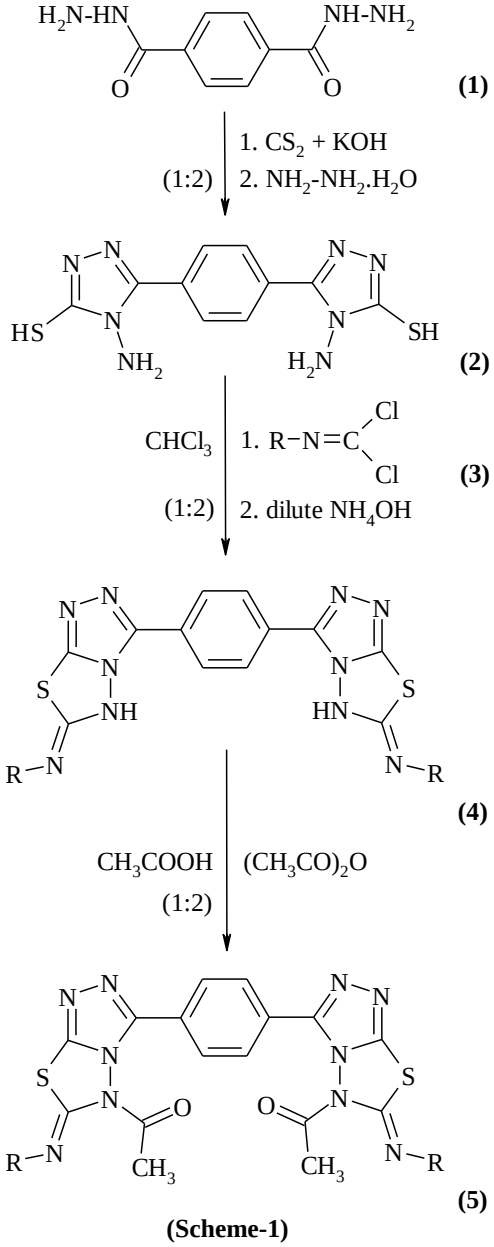
Synthesis of 1,4-bis-(5-acetyl-6-phenylimino-[1,2,4]-triazolo-(3,4-b)-[1,3,4]-thiadiazol-3-yl)-benzene (5a)

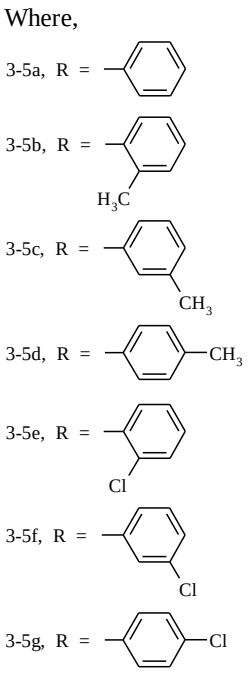
The mixture of 1,4-bis-(6-phenylimino-[1,2,4]-triazolo-(3,4-b)-[1,3,4]-thiadiazol-3-yl)-benzene (**4a**) (0.01 mol) and acetic anhydride (0.02 mol) in glacial acetic acid (10 ml) was refluxed for 2 hour. The reaction mixture was cooled and poured in a little crushed ice with water, a cream

coloured solid precipitated was crystallized from aqueous ethanol to give (**5a**), (78%), m.p. 112^0C

Spectral and elemental analysis of 1,4-bis-(5-acetyl-6-phenylimino-[1,2,4]-triazolo-(3,4-b)-[1,3,4]-thiadiazol-3-yl)-benzene (5a)

(Found: C, 54.71; H, 3.13; N, 23.65; S, 10.77. Calcd. for $C_{28}H_{20}N_{10}O_2S_2$: C, 56.75; H, 3.37; N, 23.64; S, 10.81%); ν_{max} 1696 (C=O), 1618, 1594 (C=N), 1340, 1323 (C-N), 1241 (N-N), 708, 693 cm^{-1} (C-S); δ ($CDCl_3$+DMSO-d_6) 6.96-8.02 (14H, m, Ar-H), 2.16 (6H, s, CH_3). This reaction was extended to synthesize other compounds (5b-g): 5b (65%), m.p. 151^0C; c (71%), m.p. 197^0C; d (60%), m.p. 204^0C; e (75%), m.p. 222^0C; f (72%), m.p. 181^0C; g (70%), m.p. 202^0C.





(Scheme-1)

RESULTS AND DISCUSSION

The parent compound terephthalic acid dihydrazide (1) was prepared by refluxing the mixture of terephthalic acid (0.01 mol) and thionyl chloride (0.02 mole) for 30 minute, followed by the addition of hydrazine hydrate (0.02 mol). It was transformed into 1,4-bis-(4-amino-3-mercapto-4H-[1,2,4]-triazol-5-yl-benzene (2) by interacting with carbondisulphide (0.02 mol) and potassium hydroxide (2M, 10 ml) followed by the addition of hydrazine hydrate (0.02 mol). Compound (2) was then reacted with *N*-aryl isocyanodichlorides (3a-g) (0.02 mol) in boiling chloroform for 3 hour. The evolution of hydrogen chloride gas was clearly noticed as tested with moist blue litmus paper. Cooling the reaction mixture and distilling off chloroform afforded sticky masses, which on washing with petroleum ether gave granular solids. These were acidic to litmus and on determination of equivalent weight found to be the hydrochlorides. These on basification with dilute ammonium hydroxide solution afforded free bases, 1,4-bis-(6-arylimino-[1,2,4]-triazolo-(3,4-b)-[1,3,4]-thiadiazol-3-yl)-benzenes (4a-g). Compounds (4a-g) on acetylation with acetic anhydride in 1:2 ratio afforded di-acetyl derivatives (5a-g) (Scheme 1).

Antimicrobial activity

The synthesized compounds (4a-g) were screened for their antibacterial activity using cup plate diffusion method[16,17]. The bacterial organisms used included both gram-positive as well as gram-negative strains like *E. coli, S. aureus, S. typhi, B. subtilis* and *P. vulgaris*. Sensitivity plates were seeded with a bacterial innoculum of $1x10^6$ CIU/ml and each well (diameter 10 mm) was loaded with 0.1 ml of test compound solution (1000 µg/ml) in DMF, so that concentration of each test compound was 100 µg/ml. The zones of inhibition were recorded after incubation for 24 hour at 37^0C, using vernier caliper. Inhibition zone record of the compounds clearly indicated that (4b), (4c) and (4d) were highly active against *S. aureus* and moderately active against *S. typhi* and *E. coli*. Majority of the compounds were found to be inactive against *B. subtilis and P. vulgaris* (Table 1). To determine minimum inhibitory concentration (MIC), the serial dilution technique[18] was followed using nutrient broth medium. The MIC values of compounds (4b), (4c) and (4d), which were determined against *S. aureus*, found to be 82, 85 and 89 µg/ml respectively.

Screening of these compounds (4b-g) having the concentration 1% and 2%, for antifungal activity using paper disc method[19] showed that (4b) was highly active against *A. niger*, whereas other compounds showed low to moderate activity. The zones of inhibition were recorded after incubation for 48 h at 37^0C (Table 1).

Table 1: Antibacterial and antifungal activity of compounds (4a-h).

Compounds	Antibacterial activity					Antifungal activity	
	E. coli	***S. aureus***	***S. typhi***	***B. subtilis***	***A. aerogenes***	***A. niger*** (Conc. 1%)	(Conc. 2%)
4a	+	+	+	–	–	–	–
4b	++	+++	++	+	+	++	++
4c	++	+++	++	–	+	+	+
4d	++	+++	++	+	–	+++	+++
4e	+	+	+	+	+	+	++
4f	+	+	+	–	–	–	+
4g	+	+	–	–	+	++	++

(–) : Inactive (12 mm and less) (+) : Weakly active (13-16 mm)

(++) : Moderately active (17-20 mm) (+++) : Highly active (21 mm and above)

CONCLUSION

In present work, synthesis of 1,4-bis-(6-arylimino-[1,2,4]-triazolo-(3,4-b)-[1,3,4]-thiadiazol-3-yl)-benzenes (4a-g) and their di-acetyl derivatives (5a-g) have been reported. The method applied for the synthesis is quite simple, efficient and completed within a short period of time with good yield. Antimicrobial study of synthesized compounds revealed that, compounds (4b), (4c) and (4d) have very good antibacterial activities and compound (4b) has very good antifungal activities.

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