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Conventional and MW induced synthesis of some bridgehead nitrogen containing triazolo-dithiadiazines by sulphur-sulphur bond formation through cyclocondensation and antimicrobial study

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Abstract : Conventional and MW induced synthesis of 3-aryl/alkylimino-6-pyridin-4-yl-[1,2,4]-triazolo-(3,4-*c*)-[1,2,4,5]-dithiadiazines have been carried out by reacting 4-amino-3-mercapto-5-pyridin-4-yl-4*H*-[1,2,4]-triazole with *N*-aryl/alkyl-*S*-chloro isothiocarbamoyl chlorides followed by the basification with dilute ammonium hydroxide solution. 4-Amino-3-mercapto-5-pyridin-4-yl-4*H*-[1,2,4]-triazole was synthesized by the interaction of isoniazide with carbondisulphide and potassium hydroxide followed by the addition of hydrazine hydrate. 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 : Conventional, MW, synthesis, triazolo-dithiadiazines, antimicrobial study.

Introduction

Synthesis of organic compounds by microwave technique provides a number of advantages over the standard heating[1]. High density microwave irradiation technology has emerged as a reliable and useful methodology for accelerating time consuming reactions[2] and can be adapted for high speed parallel synthesis of a library of bioactive compounds[3,4]. Heterocyclic compounds especially those containing sulphur and nitrogen atoms possess a wide variety of biological activities[5,6]. Synthesis, structural properties and antimicrobial activities of substituted [1,2,4,5]-dithiadiazines have been reported earlier in some communications[7–9]. 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[10,11]. Fused [1,2,4]-triazoles are found to possess diverse applications in the field of medicine[12,13]. Synthetic applications of *N*-aryl/alkyl-*S*-chloro isothiocarbamoyl chlorides have been investigated earlier and shown to have enough potentiality in the synthesis of nitrogen and sulphur containing 5 and 6 membered heterocyclic compounds[14,15]. On perusal of literature, it has been observed that there is scanty work on the synthesis of [1,2,4]-triazolo-[1,2,4,5]-dithiadiazines. In view of these findings and as a part of wider programme to provide alternative routes of synthesis[16,17], we report herein the synthesis of substituted [1,2,4]-triazolo-(3,4-*c*)-[1,2,4,5]-dithiadiazines by conventional heating as well as MW irradiation.

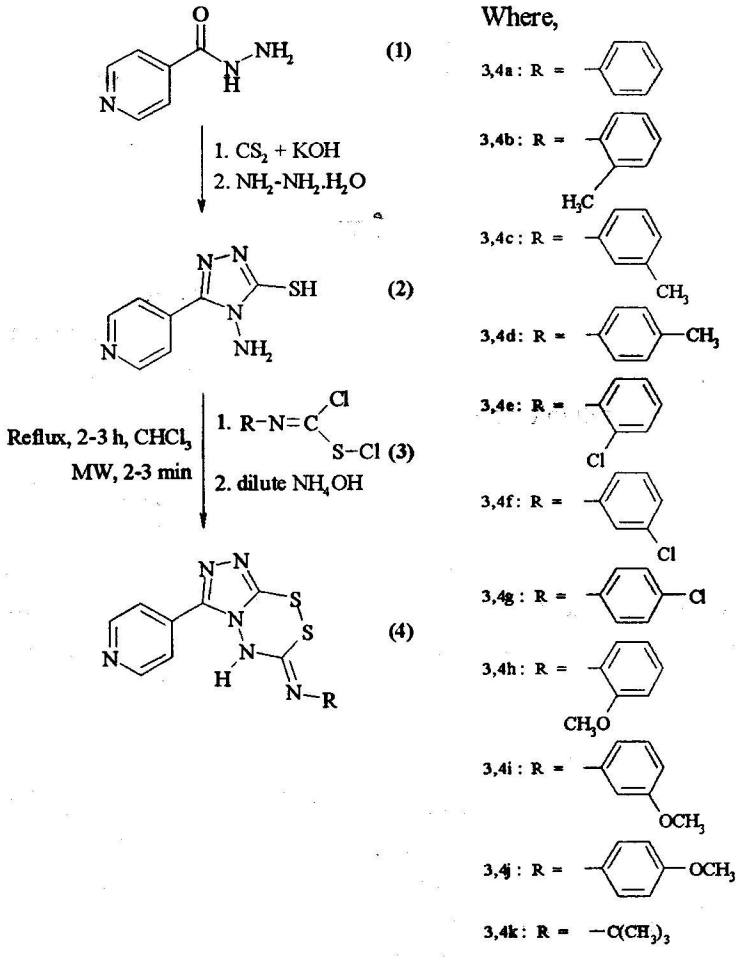
Results and discussion

Isoniazide was transformed into 4-amino-3-mercapto-5-pyridin-4-yl-4*H*-[1,2,4]-triazole (**2**) by interacting with carbondisulphide (0.01 mol) and potassium hydroxide (1 *M*, 10 ml) followed by the addition of hydrazine hydrate (0.01 mol). Compound (**2**) was then reacted with *N*-aryl/alkyl-*S*-chloro

isothiocarbamoyl chlorides (**3a-k**) (0.01 mol) in boiling chloroform for 2–30 h. 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 by titrating these (0.1 g) with sodium hydroxide solution (0.01 *N*) found to be the hydrochlorides. These on basification with dilute ammonium hydroxide solution afforded free bases, 3-aryl/alkylimino-6-pyridin-4-yl-[1,2,4]-triazolo-(3,4-*c*)-[1,2,4,5]-dithiadiazines (**4a-k**). Simultaneously compounds (**4a-k**) were also synthesized by MW irradiation under solvent free conditions (Scheme 1).

Antimicrobial activity :

The synthesized compounds (**4a-k**) were screened for their antibacterial activity using cup plate diffusion method[18,19]. 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 1×10^6 CIU ml^{-1} and each well (diameter 10 mm) was loaded with 0.1 ml of test compound solution (1000 μg ml^{-1}) in DMF, so that concentration of each test compound was 100 μg ml^{-1}. The zones of inhibition were recorded after incubation for 24 h at 37 °C, using vernier caliper. Inhibition zone record of the compounds clearly indicated that (**4c**), (**4d**) and (**4i**) were highly active against



Scheme 1

E. coli and moderately active against *S. typhi*. Compounds (**4h**) and (**4j**) were also moderately active against *S. aureus*. 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[20] was followed using nutrient broth medium. The MIC values of compounds (**4c**), (**4d**) and (**4i**), which were determined against *E. coli*, found to be 60, 65 and 55 μg ml^{-1} respectively.

Screening of these compounds (**4a-k**) having the concentration 1% and 2%, for antifungal activity using paper disc method[21,22] showed that (**4c**) and (**4i**) were 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 °C (Table 1).

Table 1. Antibacterial and antifungal activity of compounds (**4a-k**)

Compds.	Antibacterial activity					Antifungal activity	
	E. coli	*S. aureus*	*S. typhi*	*B. subtilis*	*P. vulgaris*	*A. niger*	
						(Conc. 1%)	(Conc. 2%)
4a	–	–	+	+	–	+	+
4b	+	+	+	+	+	++	++
4c	+++	+	++	–	+	+++	+++
4d	+++	–	++	+	–	+	–
4e	++	–	–	–	–	–	+
4f	+	+	+	+	–	++	++
4g	+	+	+	–	+	+	+
4h	+	++	+	–	+	+	++
4i	+++	+	++	+	–	+++	+++
4j	+	++	+	+	–	++	++
4k	+	–	+	–	+	–	+

(–) : Inactive (10 mm and less) (+) : Weakly active (11–15 mm)
(++) : Moderately active (16–20 mm) (+++) : Highly active (21 mm and above)

Experimental

The MW induced reactions were carried out using commercially available microwave oven (800 W). Melting points of all synthesized compounds were recorded using the Veego, VMP-D digital melting point apparatus and are uncorrected. Chemicals used were of A.R. 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. Mass spectral measurements were carried out by EI method on Jeol-JMC 300 spectrometer at 70 eV. Purity of the compounds was checked on silica gel-G plates by TLC.

The reagents *N*-aryl/alkyl-*S*-chloro isothiocarbamoyl chlorides were prepared by passing chlorine gas (0.01 mol) through the solution of aryl/alkyl isothiocyanates (0.01 mol) in chloroform (10 ml), maintaining the temperature at 10 °C. The reaction mixture was then diluted with petroleum ether (60–80 °C) and the solvent was distilled off under vacuum to get pale yellow coloured oily liquids (**3a-k**); ν_{max} 1652, 1703 (C=N), 840 cm^{-1} (C-S).

Synthesis of 4-amino-3-mercapto-5-pyridin-4-yl-4H-[1,2,4]-triazole (**2**) :

The compound 4-amino-3-mercapto-5-pyridin-4-yl-4*H*-[1,2,4]-triazole (**2**) was prepared by the interaction of isoniazide (**1**) (0.01 mol) with carbondisulphide

(0.01 mol) and potassium hydroxide solution (1 *M*, 10 ml) followed by the dropwise addition of hydrazine hydrate (0.01 mol) with constant stirring. The stirring was continued for 30 min at room temperature. The reaction mixture was cooled and poured in distilled water, a white precipitate was obtained. It was crystallized from ethanol, (**2**) (85%), m.p. 145 °C (Found : C, 43.11; H, 3.31; N, 35.82; S, 16.08. Calcd. for $C_7H_7N_5S$: C, 43.52; H, 3.62; N, 36.26; S, 16.58%).

Conventional synthesis of 3-phenylimino-6-pyridin-4-yl-[1,2,4]-triazolo-(3,4-c)-[1,2,4,5]-dithiadiazine (**4a**) :

The compound 4-amino-3-mercapto-5-pyridin-4-yl-4*H*-[1,2,4]-triazole (**2**) (0.01 mol) was reacted with *N*-phenyl-*S*-chloro isothiocarbamoyl chloride (**3a**) (0.01 mol) in boiling chloroform medium over a water bath for 2 h. 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 °C) gave a granular solid. It was acidic to litmus and on determination of equivalent weight (362.46) by titrating it (0.1 g) with sodium hydroxide solution (0.01 *N*) found to be the hydrochloride. It on basification with dilute ammonium hydroxide solution afforded a free base, 3-phenylimino-6-pyridin-4-yl-[1,2,4]-triazolo-(3,4-*c*)-[1,2,4,5]-dithiadiazine (**4a**). It was crystallized from ethanol, (75%), m.p. 160–162 °C (Found : C, 48.77; H, 3.01; N, 25.04; S, 19.66. Calcd. for $C_{14}H_{10}N_6S_2$: C, 51.53; H, 3.06; N, 25.76; S, 19.63%). This reaction was extended to synthesize other hydrochlorides whose equivalent weights were found to be 376.11, 375.91, 374.81, 396.22, 397.12, 395.97, 392.02, 390.68, 391.77 and 341.22. These hydrochlorides were then basified to compounds (**4b-k**) : **4b** (70%), m.p. 192 °C (Found : C, 51.11; H, 3.44; N, 24.63; S, 18.52. Calcd. for $C_{15}H_{12}N_6S_2$: C, 52.94; H, 3.52; N, 24.70; S, 18.82%); **c** (80%), m.p. 243 °C (Found : C, 50.92; H, 3.16; N, 24.32; S, 18.79. Calcd. for $C_{15}H_{12}N_6S_2$: C, 52.94; H, 3.52; N, 24.70; S, 18.82%); **d** (60%), m.p. 130 °C (Found : C, 51.83; H, 3.47; N, 24.03; S, 18.41. Calcd. for $C_{15}H_{12}N_6S_2$: C, 52.94; H, 3.52; N, 24.70; S, 18.82%); **e** (75%), m.p. 205 °C (Found : C, 44.98; H, 2.30; N, 23.29; S, 17.01. Calcd. for $C_{14}H_9N_6S_2Cl$: C, 46.60; H, 2.49; N, 23.30; S, 17.75%); **f** (65%), m.p. 122 °C (Found : C, 45.13; H, 2.28; N, 23.05; S, 16.96. Calcd. for $C_{14}H_9N_6S_2Cl$: C, 46.60; H, 2.49; N, 23.30; S, 17.75%); **g** (80%), m.p. 178 °C (Found : C, 45.88; H, 2.40; N, 22.94; S, 16.87. Calcd. for $C_{14}H_9N_6S_2Cl$: C, 46.60; H, 2.49; N, 23.30; S, 17.75%); **h** (75%), m.p. 211 °C (Found : C, 50.31; H, 3.33; N, 23.61; S, 17.64. Calcd. for $C_{15}H_{12}N_6OS_2$: C, 50.56; H, 3.37; N, 23.59; S, 17.97%); **i** (60%), m.p. 168 °C (Found : C, 49.78; H, 3.18; N, 23.12; S, 17.90. Calcd. for $C_{15}H_{12}N_6OS_2$: C, 50.56; H, 3.37; N, 23.59; S, 17.97%); **j** (65%), m.p. 263 °C (Found : C, 50.12; H, 3.21; N, 22.98; S, 17.81. Calcd. for $C_{15}H_{12}N_6OS_2$: C, 50.56; H, 3.37; N, 23.59; S, 17.97%); **k** (70%), m.p. 170 °C (Found : C, 46.83; H, 4.39; N, 27.41; S, 20.67. Calcd. for $C_{12}H_{14}N_6S_2$: C, 47.05; H, 4.57; N, 27.45; S, 20.91%).

MW induced synthesis of 3-phenylimino-6-pyridin-4-yl-[1,2,4]-triazolo-(3,4-c)-[1,2,4,5]-dithiadiazine (**4a**) :

A mixture of 4-amino-3-mercapto-5-pyridin-4-yl-4*H*-[1,2,4]-triazole (**2**) (0.01 mol) and *N*-phenyl-*S*-chloro isothiocarbamoyl chloride (**3a**) (0.01 mol) was irradiated under microwave conditions for 2 min (100 to 115 °C). An acidic solid mass was obtained, it was basified with dilute ammonium hydroxide solution and crystallized from ethanol to give 3-phenylimino-6-pyridin-4-yl-[1,2,4]-triazolo-(3,4-*c*)-[1,2,4,5]-dithiadiazine (**4a**), (92%), m.p. 162 °C. The complete reaction was monitored by TLC and extended to synthesize the other compounds (**4b-k**), (90–96%).

Spectral data of compounds (**2**) *and* (**4a,d**) :

2, ν_{max} 3423, 3370 (NH), 1682 (C=N), 1298 (C-N), 1210 (N-N), 758 cm^{-1} (C-S)[23]; **4a**, ν_{max} 3416 (NH), 1667, 1608 (C=N), 1351, 1308, 1289 (C-N),

1231 (N-N), 742, 699 (C-S), 498 cm^{-1} (S-S); δ ($CDCl_3$+DMSO-d_6) 9.66 (1H, s, NH), 7.01–8.63 (9H, m, Ar-H and Py-H); MS : *m/z* 326 (M^+), 325 (M^+-H), 248 (M^+-C_5H_4N), 235 (M^+-C_6H_4-CH_3), 171 ($C_3HN_4S_2$-N^+), 157 ($C_3HN_4S_2^+$)[23,24]; **d**, ν_{max} 3437 (NH), 1668, 1613, 1534 (C=N), 1349, 1310, 1299 (C-N), 1222 (N-N), 767, 715 (C-S), 494 cm^{-1} (S-S); δ ($CDCl_3$+DMSO-d_6) 9.78 (1H, s, NH), 7.09–8.72 (8H, m, Ar-H and Py-H), 2.36 (3H, s, Ar-CH_3); MS : *m/z* 339 (M^+-H), 325 (M^+-CH_3), 262 (M^+-C_5H_4N), 249 (M^+-C_6H_4-CH_3), 235 (M^+-N-C_6H_4-CH_3), 171 ($C_3HN_4S_2$-N^+), 157 ($C_3HN_4S_2^+$).

Conclusion

In present work, conventional and MW induced synthesis of 3-aryl/alkylimino-6-pyridin-4-yl-[1,2,4]-triazolo-(3,4-*c*)-[1,2,4,5]-dithiadiazines (**4a-k**) have been reported. The MW induced synthesis is quite simple, efficient and completed within a very short period of time with good yield as compared to conventional method. Antimicrobial study of synthesized compounds revealed that, compounds (**4c**), (**4d**) and (**4i**) have very good antibacterial and antifungal activities.

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