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SYNTHESIS AND ANTI-MICROBIAL ACTIVITY OF SOME HETEROCYCLES: *PART-II*

Khandker M M Rahman, A Z M Shaifullah Chowdhury, M M H Bhuiyan*, M K Hossain and M K Uddin

Department of Chemistry, University of Chittagong, Chittagong-4331, Bangladesh

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Thieno[2,3-d]pyrimidines **(2)**, **(3)** and **(4)** have been obtained via the reaction of 2-amino-4, 5-dimethylthiophen-3-carbonitrile (1) with formamide, acetonitrile and benzonitrile respectively. Treatment of **(2)**, **(3)** and **(4)** with chloroacetaldehyde respectively afforded imidazo[1,2-c]thieno[3,2-e]pyrimidines **(5)**, **(6)** and **(7)**. Anti-microbial activity of the above compounds was determined.

Key words: Thieno[2,3-d]pyrimidines, Chloroacetaldehyde, Imidazo[1,2-c]thieno[3,2-e]pyrimidines.

Introduction

The compounds incorporating imidazole ring in their structures possess diverse biological activities (Kihara *et al* 1986; Salaby *et al* 1986). The imidazolo[4,5-e]pyrido [1,2-a] pyrimidines are effective for herbicidal activity (Nizamuddin *et al* 2000). Derivatives of thieno[2,3-d] pyrimidine systems are of great interest because of antibacterial and antimalarial activities (Albert 1986). For the past few years we are interested in the syntheses of heterocycles containing the thieno-pyrimidine systems with the aim of finding compounds with antihypertensive, antifungal and antibacterial activities (Sauter *et al* 1996; Shaifullah Chowdhury *et al* 1997, 2000 a & b and 2001). In continuation of earlier work (Rahman *et al* 1999), the syntheses and anti-microbial activities of some thieno-pyrimidines and imidazo-thienopyrimidines has been reported.

Experimental

All melting points were determined on an electrothermal melting point apparatus and are uncorrected. ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker AC 200 (200 MHz) at the National Institute for Environmental Studies (NIES), Japan. TMS was used as internal standard and chemical shifts are expressed in ppm (δ units). Elemental analyses were also performed at the same Institute. The progress of the reaction was monitored on TLC, which was performed on G. Merck silica Gel 60. All evaporations were conducted under reduced pressure at bath temperature below 50^{0}C.

*2-Amino-4,5-dimethylthiophen-3-carbonitrile **(1)***. The title compound **(1)** was prepared from butanone by reacting with malononitrile and sulfur using the literature procedure (Gewald *et al* 1966), yellowish crystals, m.p. 140-141^{0}C.

^{1}H-NMR ($CDCl_3$): δ_H 4.89 (s, 2H, NH_2), 1.34 (s, 3H, CH_3), 0.97 (s, 3H, CH_3).

*Author for correspondence

*4-Amino-5,6-dimethylthieno[2,3-d]pyrimidine **(2)***. A mixture of **(1)** (1g, 6.58 mmol) and freshly distilled formamide (5 mL) was heated at 180^{0}C for 4 h. After cooling, the mixture was then poured into ice-water and stirred for additional 1 h. The resultant precipitate was collected by filtration and recrystallized from ethanol to give 4-amino-5,6-dimethylthieno [2,3-d] pyrimidine **(2)** as brown crystals, m.p. 175-177^{0}C, yield 0.9g (76.41%). Anal. calcd. for $C_8H_9N_3S$ (179): C, 53.63, H, 5.03; N, 23.46; Found: C, 53.40; H, 5.00; N, 23.89%. ^{1}H-NMR (DMSO-d_6): δ_H 8.82 (s, 1H, 2-H), 7.38 (s, 2H, NH_2), 2.34 (s, 6H, 2 CH_3). ^{13}C-NMR (DMSO-d_6): δ_C 163 (d, C-2), 156.69 (s, C-4), 149.96 (s, C-4a), 129.53 (s, C-7a), 125.30 (s, C-5), 116.11 (s, C-6), 23.40 (q, 5-CH_3), 21.59 (q, 6-CH_3).

*4-Amino-2,5,6-trimethylthieno[2,3-d]pyrimidine **(3)***. A solution of **(1)** (1g, 6.58 mmol), sodium methoxide (0.71g, 13.16 mmol) and acetonitrile (0.27g, 6.58 mmol) was refluxed in 2-propanol (15 ml) for 24 h. After cooling, the precipitate was collected by filtration and recrystallized from ethanol to give 4-amino-2,5,6-trimethylthieno[2,3-d]pyrimidine **(3)**, m.p. 191-192^{0}C, yield 0.8g (63%). Anal. calcd. for $C_9H_{11}N_3S$ (193): C, 55.95; H, 5.70; N, 21.76; Found: C, 55.79; H, 5.66; N, 21.56%. ^{1}H-NMR (DMSO-d_6): δ_H 5.70 (s, 2H, NH_2), 1.20 (s, 6H, 2 CH_3) 1.1.02 (s, 3H, 2-CH_3).

*4-Amino-5,6-dimethyl-2-phenylthieno[2,3-d] pyrimidine **(4)***. A mixture of **(1)** (1g, 6.58 mmol), sodium methoxide (0.71g, 13.16 mmol) and benzonitrile (0.68g, 6.58 mmol) was refluxed in 2-propanol (10 ml) for 24 h. After cooling, the precipitate was collected by filtration and recrystallized from ethanol to afford 4-amino-5,6-dimethyl-2-phenylthieno[2,3d] pyrimidine **(4)** as brown crystals, m.p. 210-211^{0}C, yield 1.1g (65%). Anal. calcd. for $C_{14}H_{13}N_3S$ (255): C 65.88; H, 5.09; N, 16.47; Found: C, 65.63; H, 5.13; N, 16.42%. ^{1}H-NMR ($CDCl_3$):δ_H 8.36 (m, 2H, Ar-H), 7.42 (m, 3H, Ar-H), 5.27 (s, 2H, NH_2), 2.88 (s, 3H, 5-CH_3), 2.79 (s, 3H, 6-CH_3). ^{13}C-NMR ($CDCl_3$): δ_C 167.67 (s, C-2), 159.20 (s, C-4), 157.62 (s, C-7a), 138.08 (s, C-1), 133.65

Table 1
Antibacterial screening for the compounds **(1-7)**

	Diameter of zone of inhibition in mm (100 µg(dw)/disc)							
Name of bacteria	Comp **1**	Comp **2**	Comp **3**	Comp **4**	Comp **5**	Comp **6**	Comp **7**	Ampicillin 25µg/(dw)/disc
B.subtilis (G^+)	8	–	9	–	–	8	10	21
B. cereus (G^+)	10	8	9	6	9	15*	9	20
B megaterium (G^+)	7	9	8	6	7	8	–	19
S.aureus (G^+)	11	7	8	7	–	–	–	20
S.dysenteriae (G^+)	12	–	10	–	7	–	–	30
S.typhi (G)	9	6	7	–	–	–	8	24
E. coli (G)	7	–	7	7	8	9	–	12
Vibrio (G)	7	7	6	13	6	7	16*	17

*, Inhibition against *B.cereus* and *vibrio.*

Table 2
Fungicidal screening for the compounds **(1-7)**

	% Inhibition of mycelial growth (100µg(dw)/ml PDA)							
Name of fungi	Comp **1**	Comp **2**	Comp **3**	Comp **4**	Comp **5**	Comp **6**	Comp **7**	Nystatin
M.Phaseolina	35.00	60.00*	66.67*	53.33	34.47	38.00	13.34	71.78
A.alternata	29.07	52.69*	52.40*	48.20	22.90	61.30*	27.10	51.55
F.equiseti	7.79	80.05*	41.17*	35.50	47.05	58.82*	21.00	44.70
C. corchori	48.78*	32.33	26.68	20.45	22.23	33.30	35.50*	40.51
C.lunata	45.20	36.00	46.00*	42.88	28.50	55.20*	23.00	75.00

(s, C-6′), 129.78 (d, C-2′ and C-6′), 128.27 (d, C-3′ and C-5′), 128.02 (d, C-4′), 125.57 (s, C-4a), 114.43 (s, C-5), 26.12 (q, 5-CH_3), 25.47 (q, 6-CH_3).

*8,9-Dimethylimidazo[1,2-c] thieno[3,2-e] pyrimidine **(5)**.* A mixture of **(2)** (0.5g, 2.79 mmol), sodium acetate (0.35g) and 40% chloroacetaldehyde (0.55 ml, 2.79 mmol) in water (4 ml) was heated on steam bath for 2 h. The mixture was then extracted with chloroform (30 ml x 4), dried over anhydrous sodium sulfate. The solvent was evaporated to dryness under reduced pressure. The resulting residue was recrystallized from aqueous ethanol to give 8, 9-dimethylimidazo [1, 2-c] thieno [3, 2-e] pyrimidine **(5)**, m.p. 195-196^{0}C, yield 0.4g (70.6%). Anal. calcd. for $C_{10}H_9N_3S$ (203): C, 59.11; H, 4.43; N, 20.69; Found: C, 59.58; H, 4.37; N, 20.48%. ^{1}H-NMR ($CDCl_3$): δ_H 8.82 (s, 1H, 5-H), 7.76 (d, 1H, 2-H), 7.67 (d, 1H, 3-H), 1.96 (s, 6H, 2 CH_3). ^{13}C-NMR ($CDCl_3$): δ_C 148.98 (d, C-5), 142.87 (s, C-9), 142.70 (s, C-6a), 134.37 (s, C-9a), 133.10 (s, C-9b), 128.78 (d, C-2), 119.02 (d, C-3), 110.09 (s, C-8), 22.99 (q, 8-CH_3), 22.26 (q, 9-CH_3).

*5,8,9-Trimethylimidazo[1,2-c]thieno[3,2-e] pyrimidine **(6)**.* A mixture of **(3)** (0.77g, 4 mmol), sodium acetate (0.5g) and 40% chloroacetaldehyde (0.8 ml, 4 mmol) in water (6 ml) was heated at 100^{0}C for 1 h with constant stirring. The reaction mixture was then extracted with chloroform (30 ml x 4) and the organic layer was dried over anhydrous sodium sulfate. The solvent was evaporated to dryness *in vacuo* and the residue was recrystallized from diethyl ether to give 5, 8, 9-trimethylimidazo [1,2-c] thieno[3,2-e] pyrimidine **(6)**, m.p. 117-118^{0}C, yield 0.6g (69%). Anal. calcd. for $C_{11}H_{11}N_3S$ (217): C, 60.83; H 5.07; N, 19.35; Found: C, 60.91; H, 5.12; N, 19.21%. ^{1}H-NMR ($CDCl_3$): δ_H 8.81 (d, 1H, 2-H), 8.64 (d, 1H, 3-H), 2.19 (s, 3H, 5-CH_3), 1.63 (s, 6H, 2 x CH_3). ^{13}C-NMR ($CDCl_3$): δ_C 149.25 (s, C-5), 142.69 (s, C-9), 142.36 (s, C-6a), 135.37 (s, C-9a), 132.38 (s, C-9b), 128.67 (d, C-2), 118.81 (d, C-3), 109.11 (s, C-8), 22.82 (q, 8-CH_3), 22.04 (q, 9-CH_3), 20.71 (q, 5-CH_3).

*8,9-Dimethyl-5-phenylimidazo[1,2-c]thieno[3,2-e] pyrimidine **(7)**.* A solution of **(4)** (0.5g, 1.96 mmol), sodium acetate (0.25g) and 40% chloroacetaldehyde (0.4 ml, 1.96 mmol) in water (4 ml) was heated on steam bath for 1.5 h with

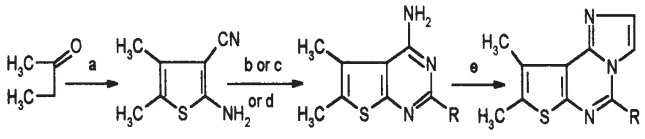
stirring. The solution was then extracted with chloroform (30 ml x 4) and the organic layer was dried over anhydrous sodium sulfate. The solvent was evaporated to dryness *in vacuo* and the resulting residue was recrystallized from ethanol to furnish 8,9-dimethyl-5-phenylimidazo[1,2-c]thieno[3,2-e] pyrimidine **(7)**, m.p. 114-115^{0}C, yield 0.35g (64%). Anal. calcd. for $C_{16}H_{13}N_3S$ (279): C, 68.81; H, 4.65, N, 15.05; Found: C, 68.65; H, 4.61; N, 14.85%. ^{1}H-NMR ($CDCl_3$): δ_H 7.85 (m, 2H, Ar-H), 7.75 (d, 1H, 2-H), 7.66 (d, 1H, 3-H), 7.60 (m, 3H, Ar-H), 3.23 (s, 3H, 5-CH_3), 2.89 (s, 3H, CH_3). ^{13}C-NMR ($CDCl_3$): δ_C 150.75 (s, C-5), 144.40 (s, C-9a), 143.06 (s, C-6a), 137.59 (s, C-9b), 133.32 (s, C-9), 131.78 (s, C-1), 130.91 (d, C-2 and C-6), 129.89 (d, C-4), 128.75 (d, C-2), 128.44 (d, C-3 and C-5), 119.34 (d, C-3), 11.08 (s, C-8), 23.01 (q, 8-CH_3), 22.31 (q, 9-CH_3).

Antibacterial and antifungal screenings. The antibacterial activities of the synthesized compounds **(1-7)** were studied against eight bacteria, *viz., Bacillus subtilis* BTCC 17 (G^+), *Bacillus cereus* BTCC 19 (G^+), *Bacillus megaterium* BTCC 18 (G^+), *Staphylococcus aureus* ATCC 6538 (G^+), *Shigella dysenteriae* AE 14396 (G^-), *Salmonella typhi* AE 14612 (G^-), *Escherichia coli* ATCC 25922 (G^-) and INABA-ET *(Vibrio)* AE 14748 (G^-). Antifungal activities of the same compounds were also studied against five fungi, *viz, Macrophomina phaseolina* (Tassi) Goid, *Alternaria alternata* (Fr.) Kedissler, *Fuserium equiseti* (Corda) Sacc, *Collectotrichum corchori* Ikata (Yoshida), *Curvularia lunata* Wakker Boedijn. For the detection of antibacterial activities, the disc diffusion method (Bauer *et al* 1966) was followed. Poisoned-food technique (Grover and Moore 1962) assessed the antifungal activities. Ampicillin and Nystatin were used as standard antibiotics for the antibacterial and antifungal activities respectively. Nutrient Agar (NA) and Potato Dextrose Agar (PDA) were used as basal medium for test bacteria and fungi respectively. Dimethyl formamide (DMF) was used, as a solvent to prepare desired solution (1%) of the compounds initially. Proper control was maintained with DMF.

Results and Discussion

Reaction of **(1)** (Gewald *et al* 1966) with formamide at 180^{0}C afforded 4-amino-5,6-dimethylthieno[2,3-d]-pyrimidine **(2)** in 76.41% yield as brown crystal, m.p. 175-177^{0}C (Scheme-I). The ^{1}H-NMR spectrum of **(2)** showed a one-proton singlet at δ 8.82 for 2-H, a two-proton singlet at δ 7.38 for NH_2 group and a six proton singlet at δ 2.34 for two methyl groups in the molecule. The structure of **(2)** was also confirmed from its micro-analytical data and ^{13}C-NMR spectrum. The spectrum gave signal at δ 163.04 as doublet indicated for C-2. The rest peaks were accorded to its structure.

The compound **(1)**, on reaction with acetonitrile or benzonitrile in presence of sodium methoxide in 2-propanol under reflux furnished 4-amino-2,5,6-trimethylthieno[2,3-d]pyrimidine **(3)** and 4-amino-5,6-dimethyl-2-phenylthieno[2,3-d]pyrimidine **(4)** respectively. The ^{1}H-NMR spectrum of **(3)** showed a two proton singlet at δ 5.70 for NH_2 group, a six-proton singlet at d 1.20 for two methyl groups (5-CH_3 and 6-CH_3) and a three-proton singlet at δ 1.02 for 2-CH_3 group, which provided the formation of a new pyrimidine ring. The ^{1}H-NMR spectrum of **(4)** exhibited signals confirming the presence of NH_2, 5-CH_3, 6-CH_3 and aromatic protons at δ 5.27, 2.88, 2.79, 8.36 and 7.42 respectively. The ^{13}C-NMR spectrum displayed the presence of fourteen carbons corresponding to its molecular formula $C_{14}H_{13}N_3S$.



Scheme I

Reagents. (a) $CNCH_2CN$, S, EtOH, Et_2NH; (b) $HCONH_2$, reflux, 4 h (to give **2**); (c) CH_3CN, CH_3ONa, 2-propanol, reflux, 24 h (to give **3**); (d) PhCN, CH_3ONa, 2-propanol, reflux, 24 h (to give **4**); (e) $ClCH_2CHO$, CH_3COONa, 100^{0}C, 1-2 h.

The compounds **(2)** or **(3)** or **(4)** on reaction with 40% chloroacetaldehyde in presence of sodium acetate in water at 100^{0}C for 1-2 h yielded novel tricyclic compounds, 8, 9-dimethyl imidazo [1, 2-c] thieno [3, 2-e] pyrimidine **(5)**, 5, 8, 9-trimethylimidazo [1, 2-c] thieno [3, 2-e] pyrimidine **(6)** and 8,9-dimethyl-5-phenylimidazo [1,2-c] thieno [3,2-e] pyrimidine **(7)** respectively. The structures of compounds **(5-7)** were established on the basis of their micro-analytical data and spectral studies. In the ^{1}H-NMR of these compounds indicate the disappearance of NH_2 peaks and appearance of 2- and 3-H protons as doublets confirming the formation of imidazole ring. The other peaks are also in good agreement with the structures. Further confirmation of structures of **(5-7)** was based on their ^{13}C-NMR spectra.

Antibacterial activities. The antibacterial activities of compounds **(1-7)** have been studied against eight human pathogenic bacteria. Among them, four were Gram-positive and the rest were Gram-negative. The results are shown in Table-1.

Compounds **(6)** and **(7)** were found to show marked inhibition against *B. cereus* and *Vibrio* respectively. Most of the tested compounds showed good inhibition, some are unable to show any inhibition and some compounds showed weak to moderate activity against all the organisms tested.

Antifungal activities. Five plant pathogenic fungi were employed to study the antifungal properties of the compounds **(1-7)**. The results of the percentage inhibitions of mycelial growth are presented in Table 2.

Amongst the synthesized compounds screened for the antifungal activity, compounds **(2, 3)** and **(6)**showed excellent results against *A. alternata* and *F. equiseti*, which were also greater than that of the standard antibiotic, Nystatin. The other compounds also exhibited moderate to good activity against the tested organisms.

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