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Synthesis and Antimicrobial Activity of Some Heterocycles: Part-V

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Abstract. *Ortho*-aminonitrile **(1)** was prepared from ethoxymethylenemalononitrile. 4-Allylamino-1-methyl-6-methylthiopyrazolo[3,4-d]pyrimidine **(4)** was prepared by an initial treatment of compound **(1)** with carbon disulfide in pyridine followed by methylation with methyl iodide and subsequent reaction with allylamine in acetonitrile. *Ortho*-aminoester **(5)** was prepared from ethyl (ethoxymethylene) cyanoacetate. Reaction of compound **(5)** with formamide yielded compound **(6)**, which was then tosylated. All compounds were screened for their antibacterial and antifungal activities.

Keywords: ethoxymethylenemalononitrile, pyrazolo[3,4-d]pyrimidine, antimicrobial activity, synthesis of heterocycles

Introduction

Substituted heterocyclic compounds offer a high degree of structural diversity, which have proven to be broadly useful as therapeutic agents (Thompson and Ellman, 1996). Fused heterocyclic systems containing pyrazole ring are ranked among the most versatile bioactive compounds, possessing diverse biological activities, such as fungicidal (Sasse *et al.,* 1986), herbicidal (Ohyama *et al.,* 1986), virucidal (Zikan *et al.,* 1986),and insecticidal (Hasan *et al.,* 1994). The pyrazolo[3,4-d] pyrimidines have been described as biologically active agents (Elmaati, 2002). The most widely used pyrazolo[3,4-d] pyrimidines, allopurinol and oxyallopurinol, are established inhibitors of xanthine oxidase and thus interfere in the biosynthesis of uric acid, the causative agent of gout. This group of compounds also exhibits antineoplastic activity (Hansch *et al.,* 1990a). Antitumor and antiviral compounds have been synthesized based on the antibiotic formycin, a nucleoside of pyrazolo[3,4-d]pyrimidene (Hansch *et al.,* 1990b). Potent anti-inflammatory and analgesic activities have been reported in a number of 5-arylpyrazolo[3,4-d]pyrimidines (Machon and Witkiewiz 1985; Shishoo *et al.,* 1999). Prompted by these observations, and as a continuation of the ongoing programme on fused heterocycles (Rahman *et al.,* 2003; Chowdhury *et al.,* 2001; 2000; Chowdhury and Bhuiyan, 2000; 1997), the synthesis and antimicrobial activity of some new pyrazolo[3,4-d] pyrimidine derivatives is reported here.

Materials and Methods

Melting points were determined in open capillary tubes and are reported as uncorrected values. ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker AC 200 spectrometer, using DMSO-d_6/$CDCl_3$ as the solvents and TMS as an internal standard (chemical shifts in δ, ppm). TLC was carried out on silica gel-G plates and spots were located by iodine vapour. All evaporations were conducted under reduced pressure at the waterbath temperature below 50 °C. Various steps involved in the synthesis of seven heterocyclic compounds **(1-7)** are shown in Fig. 7 as scheme 1 (compounds **1-4**) and scheme 2 (compounds **5-7**).

Synthesis of 5-amino-3-cyano-1-methylpyraxole (1). To a solution of ethoxymethylenemalononitrile (10.85 g, 88.9 ml) in absolute ethanol (35 ml) was added methylhydraxine (3.6 g, 57.5 mmol) in absolute ethanol (30 ml). Slight heat was produced during the addition. The resulting solution was then refluxed with continuous stirring for 1 h, after which the solvent was evaporated under reduced pressure. The obtained solid was washed with ether and recrystallized from water to give compound **(1)**, as shining white plates; yield: 7.6 g (70%), m. p. = 222-223 °C; ^{1}H-NMR ($CDCl_3$): δ 7.50 (s, 1H, 3-H), 6.51 (s, 2H, NH_2), 3.51 (s, 3H, CH_3); ^{13}C-NMR ($CDCl_3$): δ 151.4, 139.7, 115.2, 72.1, 34.5.

Synthesis of 1-methylpyraxolo[3,4-d]pyrimidine-4, 6(5H, 7H)-dithion (2). A mixture of compound **(1)** (1 g, 8.196 mmol) and carbon disulfide (3.53 ml, 40.69 mmol) in pyridine (5 ml) was refluxed for 10 h. The progress of the reaction was monitored by TLC (*n*-hexane : ethyl acetate, 1:1 v/v). After completion of the reaction, the mixture was cooled to room temperature and ethanol (30 ml) was added to the mixture. The orange coloured solid thus obtained was then collected by filtration, washed with ether (10 ml) and recrystallized from ethanol to yield compound **(2)**; yield: 1.23 g (75.9%), m.p. > 250 °C. Without further analysis, the compound was taken to the next step synthesis.

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Synthesis of 1-methyl-4,6-dimethylthiopyrazolo[3,4-d]pyrimidine (3). To the solution of compound **(2)** (0.25 g, 1.26 mmol) in aqueous sodium hydroxide (10%, 1.2 ml) was dropwise added methyl iodide (0.25 g, 1.26 mmol) and stirred at room temperature for 20 h. The yellowish coloured solid was collected by filtration, washed with distilled water and recrystallized from ethanol to furnish compound **(3)**; yield: 0.20 g (71.4%), m. p. = 103-105 °C; ^{1}H-NMR ($CDCl_3$); δ 7.81 (s, 1H, CH), 4.0 (s, 3H, CH_3), 2.65 (s, 3H, SCH_3); ^{13}C-NMR ($CDCl_3$): δ 168.48, 164.66, 151.76, 131.24, 109.24, 33.61, 14.22, 11.70.

Synthesis of 4-allylamino-1-methyl-6-methylthiopyrazolo [3,4-d]pyrimidine (4). A mixture of compound **(3)** (0.15 g, 0.66 mmol) and allylamine (1.02 ml, 13.5 mmol) in acetonitrile (3 ml) was heated in a stainless steel vessel at 120-130 °C for 30 h. The mixture was concentrated *in vacuo* to 1 ml and was then allowed to set in a refrigerator for 6 h. The obtained solid was collected by filtration and washed with acetonitrile to yield compound **(4)**; yield: 0.082 g (52.9%), m.p. = 86-88 °C; ^{1}H-NMR ($CDCl_3$): δ 7.73 (s, 1H, CH), 6.94-6.82 (m, 1H, CH), 5.30-5.19 (m, 2H, CH_2), 4.23 (s, 2H, CH_2), 3.93 (s, 3H, CH_3), 2.58 (s, 3H, SCH_3), 1.22 (s 1H, NH); ^{13}C-NMR ($CDCl_3$): δ 172.12, 151.10, 117.44, 114.56, 106.22, 98.64, 82.82, 33.69, 29.67, 14.14. Analytical calculated values for $C_{10}H_{13}N_5S$ (235.31): C 51.04, H 5.57, N 29.76; experimentally found values: C 51.25, H 5.53, N 29.70%.

Synthesis of ethyl-5-amino-1-methylpyrazole-4-carboxylate (5). The compound **(5)** was prepared from ethyl (ethoxymethylene)cyanoacetate by reacting with methyl hydrazine using the same method as was used for the preparation of compound **1** as shining white plates; yield: 70% yield, m.p. = 98-100 °C; ^{1}H-NMR ($CDCl_3$): δ 7.60 (s, 1H, 3H), 5.00 (s, 2H, NH_2), 4.15 (q, 2H, OCH_2), 3.60 (s, 3H, NCH_3), 1.35 (t, 3H, CH_3); ^{13}C-NMR ($CDCl_3$): δ 164.30, 149.30, 138.90, 95.80, 59.30, 33.90, 14.30.

Synthesis of 1-methylpyrazolo[3,4-d]pyrimidine-4 (5H)-one (6). A mixture of *ortho*-amino ester compound **(5)** (1 g, 5.92 mmol) and formamide (4 ml) was refluxed for 2 h at 180 °C. After cooling, the mixture was poured onto crushed ice and stirred for 1h. The precipitate was collected by filtration and recystallized from ethanol to give compound **(6)** as white crystals: yield; 0.68 g (68.8%), m.p. > 250 °C; ^{1}H-NMR ($CDCl_3$): δ 8.05 (s, 1H, CH), 8.00 (s, 1H, CH), 4.48 (s, 1H, NH), 3.88 (s, 3H, CH_3); ^{13}C-NMR ($CDCl_3$): δ 157.27, 151.78, 147.78, 134.05, 105.60, 34.01.

Synthesis of 1-methyl-4-oxo-pyrazolo[3,4-d]pyrimidine-5-p-toluene sulfonate ester (7). A solution of compound **(6)** (0.25 g, 1.67 mmol) and *p*-toluene sulfonyl chloride (0.32 g, 1.67 mmol) in ether (6 ml) was stirred at 30 °C for 6 h. The progress of the reaction was monitored by TLC [*n*-hexane : ethyl acetate, 1:3 v/v). After completion of the reaction, the solvent was evaporated to dryness and the resulting solid was recrystallized from ethanol to give compound **(7)** as white crystals; yield: 0.43 g (84.3%), m.p. = 220-222 °C; ^{1}H-NMR ($CDCl_3$): δ 8.11 (d, *J* = 8.0 Hz, 2H, Ar-H), 8.00 (d, *J* = 8.0 Hz, 2H, Ar-H), 7.77 (s, 1H, CH), 7.47 (s, 1H, CH), 3.73 (s, 3H, CH_3), 2.27 (s, 3H, CH_3); ^{13}C-NMR ($CDCl_3$): δ 157.27, 151.79, 147.8, 145.3, 137.8, 134.05, 128.60, 125.50, 105.67, 67.40, 34.02.

Antibacterial and antifungal screenings. All the synthesized compounds (**1-7**) were screened for their antibacterial activity against the gram-positive bacteria *Bacillus cereus*, *B. megaterium*, *Staphylococcus aureus,* and the gram-negative bacteria *Shigella dysenteriae*, *Salmonella typhi, Escherichia coli* (Table 1). The antifungal activity was tested against the fungi *Macrophomina phaseolina*, *Fusarium equiseti*, *Alternaria alternata* and *Colletotricum corchori* (Table 2). For the detection of antibacterial activities, the disc diffusion method was followed (Bauer *et al.,* 1966). Poisoned-food technique was used to assess the antifungal activities (Grover and

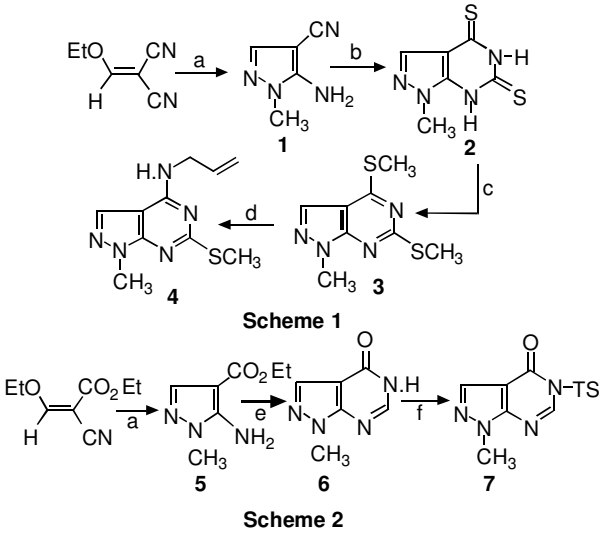

Fig. 1. Schemes 1 and 2 for the synthesis of some heterocyclic compounds; reagents for various steps and conditions: CH_3NHNH_2, EtOH, reflux, 1 h; (b) CS_2, pyridine, reflux, 10 h; (c) CH_3I, 10% NaOH, stirred at room temperature, 20 h; (d) allylamine, CH_3CN, 12-130 °C, 30 h; (e) $HCONH_2$, 180 °C, 2 h; (f) p-TSCl, ether, 30 °C, 6 h; compounds synthesized: **1:** 5-amino-3-cyano-1-methylpyrazole; **2:** 1-methylpyrazole[3,4-d] pyrimidine-4, 6(5H, 7H)-dithione; **3:** 1-methyl-4, 6-dimethylthiopyrazolo[3, 4-d]pyrimidine; **4:** 4-allylamino-1-methyl-6-methylthiopyrazolo[3, 4-d]pyrimidine; **5:** ethyl-5-amino-1-methylpyrazole-4-carboxylate; **6:** 1-methylpyrazolo[3, 4-d]pyrimidine-4(5H)-one; **7:** 1-methyl-4-oxo-pyrazolo[3, 4-d]pyrimidine-5-*p*-toluene sulfonate ester; *p*-TSCl = p-toluene sulfonyl chloride.

Moore,1962). A commercial antibacterial Ampicillin and an antifungal Nystatin were also tested under similar conditions for comparison. Nutrient agar (NA) and potato dextrose agar (PDA) were used as the basal media for the culture of the tested bacteria and fungi, respectively. Dimethyl formamide (DMF) was used, as a solvent, to prepare 1% solution of the compounds. Proper control was maintained with DMF.

Results and Discussion

Ortho-aminonitrile, 5-amino-3-cyano-1-methylpyrazol (**1**), was prepared from ethoxyemthylenemalononitrile as described in scheme 1 (Fig. 1) (Chowdhury and Bhuiyan, 1997). The structure of compound **(1)** was confirmed by ^{1}H-NMR spectrum. It showed a one-proton singlet at δ 7.50 for 3-H, a two-proton singlet at δ 6.51 for NH_2 and a three-proton 3.51 for N-CH_3 group. The cyclized compound **(1)** was further proved by ^{13}C-NMR spectrum. The resonances displayed at δ 151.4 for C-5, 139.7 for C-3, 72.1 for C-4 carbon atoms and down field shift at δ 34.5 for N-CH_3 carbon atom, respectively. Treatment of compound **(1)** with carbon disulfide in pyridine under reflux afforded 1-methylpyrazolo[3,4-d]pyrimidine-4,6(5H, 7H)-dithione **(2)** in good yield. Compound **(2)** was then methylated with methyl iodide in aqueous sodium hydroxide to afford 1-methyl-4,6-dimethylthiopyrazolo[3,4-d]pyrimidine **(3)** with 71.4% yield, m.p. 103-105 °C. The formation of compound **(3)** was established by its ^{1}H-NMR spectrum, which displayed two three-proton singlets at δ 2.65 and 2.59 corresponding to two SCH_3 groups. This structure was also supported by ^{13}C-NMR spectrum, which displayed two peaks at 14.22 and 11.70 for two SCH_2 groups in the molecule.

A substitution reaction of compound **(3)** with allylamine in acetonitrile at 120-130 °C afforded 4-allylamino-1-methyl-6-methylthio-pyrazolo[3,4-d] pyrimidine (**4**). The disappearance of one SCH_3 peak in the ^{1}H-NMR spectrum of compound **(4)** and appearance of allylic two-proton multiplet at δ 5.30-5.90 and one-proton multiplet at δ 6.94-6.82 confirmed the introduction of allylic group in the molecule. The peaks were also in good agreement with ^{13}C-NMR spectrum. The microanalytical data of the compound **(4)** for C, H, N were in accordance with the calculted values.

Ortho-aminoester, ethyl-5-amino-1-methylpyrazole-4-carboxylate **(5)**, was prepared from ethyl (ethoxymethylene) cyanoacetate following the same method as was used for the preparation of compound **(1)** as shining white plates in 70% yield, m. p. 98-100 °C, as shown in scheme 2 (Fig.1). The ^{1}H-NMR spectrum of compound **(5)** showed a one-proton singlet at δ 7.60 for 3-H, a two-proton singlet at δ 5.00 for NH_2, and a two-proton quartet at δ 4.15 for OCH_2 and a three-proton trip-

Table 1. Antibacterial screening of some heterocyclic compounds **(1-7)**

Bactrial species	Dia of the zone of inhibition by 100 µg dry wt of different compounds/disc (mm)							
	comp 1	comp 2	comp 3	cmp 4	comp 5	comp 6	comp 7	Ampicilin 25 µg/disc
Bacillus cereus	9	15	9	21	14	9	–	21
B. megaterium	6	9	5	7	–	–	7	20
Staphylococcus aureus	10	9	11	8	–	–	–	19
Shigella dysenteriae	10	11	12	8	–	–	–	30
Salmonella typhi	9	9	13	10	–	–	10	24
Escherichia coli	6	6	9	20	8	7	–	12

1: 5-amino-3-cyano-1-methylpyrazole; **2:** 1-methylpyrazole[3, 4-d]pyrimidine-4, 6(5H, 7H)-dithione; **3:** 1-methyl-4, 6-dimethylthiopyrazolo[3, 4-d]pyrimidine; **4:** 4-allylamino-1-methyl-6-methylthiopyrazolo[3, 4-d]pyrimidine; **5:** ethyl 5-amino-1-methylpyrazole-4-carboxylate; **6:** 1-methylpyrazolo[3, 4-d]pyrimidine-4-(5H)-one; **7:** 1-methyl-4-oxo-pyrazolo[3, 4-d]pyrimidine-5-*p*-toluene sulfonate ester

Table 2. Fungicidal screening of some heterocyclic compounds **(1-7)**

Fungal species	Inhibition of mycelial growth by 100 µg dry wt of different compounds/ml PDA (%)							
	comp 1	comp 2	comp 3	comp 4	comp 5	comp 6	comp 7	Nystatin
Macrophomina phaseolina	35.00	46.00	23.00	95.10	30.30	22.00	69.00	71.78
Alternaria alternata	13.00	29.07	51.50	32.34	43.43	49.50	34.00	51.55
Fusarium equiseti	40.10	32.00	43.30	90.20	21.00	19.10	29.16	44.50
Colletotrichum corchori	17.00	48.78	32.00	24.44	49.11	22.22	29.00	40.51

let at δ 1.35 for CH_3, which were coming from ethyl ester. The ^{13}C-NMR spectrum of **(5)** was also consistent with the structure.

Compound **(5)** in formamide under reflux furnished 1-methylpyrazolo[3,4-d]-4(5H)- one **(6)** in 68.8% yield. In its ^{1}H-NMR spectrum the disappearance of NH_2 peak at δ 5.00 and appearance of one-proton singlet at δ 8.00 for H-6 confirmed the formation of pyramidine ring. The ^{13}C-NMR spectrum displayed the presence of six carbons corresponding to its molecular formula $C_6H_6N_4O$. The structure of compound **(6)** was futher confirmed by its conversion to tosylate derivative **(7)** with *p*-toluene sulfonyl chloride.

Most of the compounds showed moderate to significant antibacterial and antifungal activities (Tables 1 and 2). Generally, allylamino pyrimidine derivative exhibited higher activities. Compound **(4)** showed the highest antibacterial activity against *B. cereus* and *E. coli*. Compounds **(3, 4, 5, 6)** showed high antifungal activities against *A. alternata*; *M. phaseolina* and *F. equiseti*; *C. corchori*; and *A. alternata*, respectively. However, none of the synthesized compounds showed better antifungal and antibactrial activities than the standards Nystatin and Ampilicilin, respectively, used during the present investigations for the purpose of comparison.

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