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SYNTHESES, ANTIFUNGAL AND ANTIBACTERIAL ACTIVITIES OF SUBSTITUTED 1, 2, 4-TRIAZOLES

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Abstract

The reaction of readily available 1-methyl-4-nitropyrrole-2- carboxylic acid (**1**) with thionyl chloride afforded the corresponding acyl chloride **2**. The reaction of compound **2** with thiosemicarbazides yielded 1-(1-methyl-4-nitropyrrole-2-carbonyl)-thiosemicarbazides (**3**) which cyclized in basic medium to 5-(1-methyl-4-nitropyrrole-2-yl)-2,4-dihydro-3H-1,2,4-triazole-3- thione **4**. Alkylation and subsequent oxidation of **4** gave **6**. The antibacterial and antifungal activities of **6a** to **6f** against a number of microorganisms were determined. Only compounds **6b** and **6c** had moderate activity against *Aspergilus niger*.

Introduction

The considerable biological importance of 1, 2, 4-triazoles has stimulated much work on this heterocycle [2-5]. It was also shown that 5-nitropyrrole-2-sulfonamides and 3-chloro-4- (3-chloro-2-nitrophenyl) pyrrole have antifungal and antibacterial activities [6, 7]. We would like to report the syntheses of the title compounds as possible effective drugs against tropical diseases [8].

Results and Discussion

The syntheses of the title compounds were accomplished as shown in Scheme 1.

The reaction of 1-methyl-4-nitropyrrole-2-carboxylic acid (**1**) [9] with thionyl chloride in dry benzene afforded 1-methyl-4- nitropyrrole-2-carbonyl chloride (**2**). Addition of 4-alkyl (or phenyl) thiosemicarbazide in pyridine to compound 2 gave 1-(1-methyl-4-nitropyrrole-2-carbonyl)-4-alkyl- (or phenyl) thiosemicarbazide (**3**). However, the reaction of 1-methyl-4-nitropyrrole-2-carboxylic acid hydrazide (**7**) [9] with alkyl (or phenyl) isothiocyanate in ethanol did not give compound **3**. Refluxing compound **3** with aqueous sodium bicarbonate afforded 4-alkyl-(or phenyl)-5- (1-methyl-4-nitropyrrole-2-yl)-2, 4-dihydro-3H-1,2, 4-triazole-3-thione (**4**). Reaction of compound **4** with alkyl iodides under basic condition afforded 3-(1-methyl-4-nitropyrrole-2-yl)-4-alkyl-(or phenyl) -5-alkylthio-4H-1, 2, 4-triazole (**5**). Oxidation of compound 5 with *m*-chloroperbenzoic acid gave 3-(1-methyl-4-nitropyrrole-2-yl)-4-alkyl-(or phenyl)-5-alkylsulfonyl-4H-1, 2, 4-triazole (**6**). The substituents, melting points and yields of compounds **5** and **6** are given in Table 1.

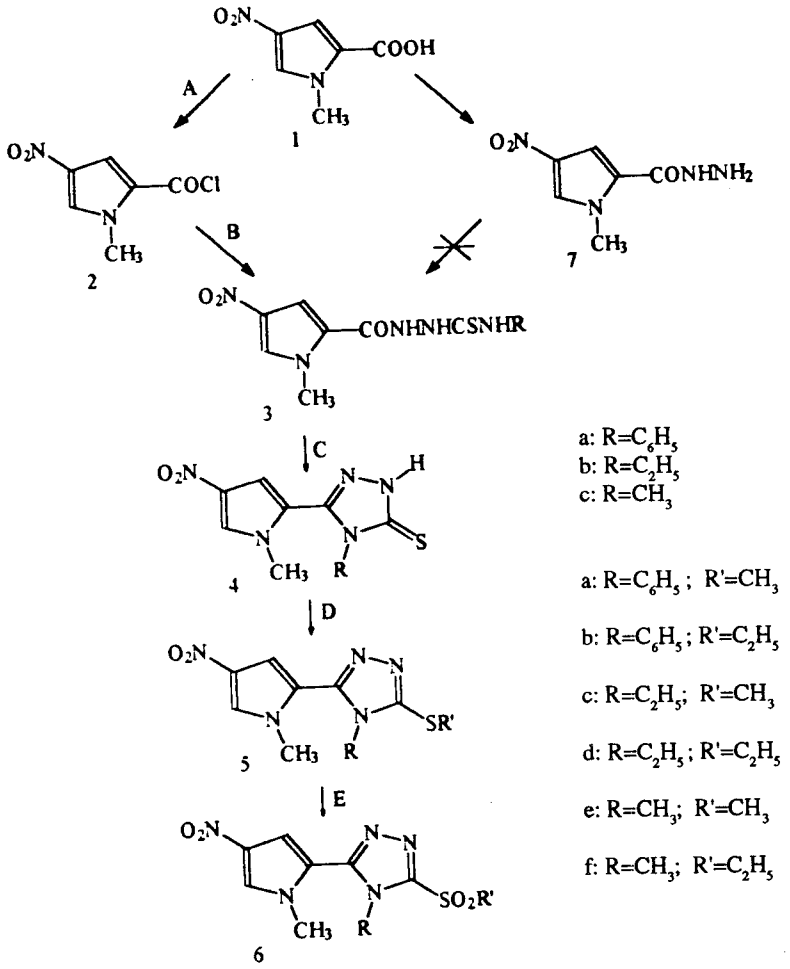
Compounds **6b** and **6c** had moderate activity against *Aspergilus niger*. The antibacterial and antifungal activities of other compounds were not significant (See Table 2).

From this work, we can conclude that a combination of nitropyrrole and substituted triazole does not provide an efficient antibacterial and antifungal compound.

Experimental section

Melting points were taken on a Kofler hot stage apparatus and are uncorrected. The IR spectra were obtained using a Perkin-Elmer Model 781 spectrograph (potassium bromide disks). The ^{1}H NMR spectra were recorded on a Bruker FT-80 spectrometer and chemical shifts (δ) are in ppm relative to internal tetramethylsilane. The mass spectra were run on a Varian model MAT MS-311 spectrometer at 70 eV. All compounds gave satisfactory C, H, N analyses

Keywords: Antibacterial activity; Nitropyrrole; 1,2,4-Triazoles



A: $SOCl_2$, C_6H_6; B: $RNHCSNHNH_2$, C_5H_5N; C: $NaHCO_3$, H_2O
D:IM NaOH, R'I; E: m-$ClC_6H_4CO_3H$, CH_2Cl_2

Scheme 1

Table 1. Melting points and yields for compounds **5a-5f** and **6a-6f**

Compound[a]	R	R	M.P.°C[b]	Yield (%)[c]
5a	C_6H_5	CH_3	154-156	46
5b	C_6H_5	C_2H_5	146-148	50
5c	C_2H_5	CH_3	139-141	62
5d	C_2H_5	C_2H_5	124-126	55
5e	CH_3	CH_3	220-222	80
5f	CH_3	C_2H_5	154-156	67
6a	C_6H_5	CH_3	216-217	34
6b	C_6H_5	C_2H_5	210-211	20
6c	C_2H_5	CH_3	174-175	30
6d	C_2H_5	C_2H_5	130-131	25
6e	CH_3	CH_3	200-201	50
6f	CH_3	C_2H_5	160-161	60

a) All compounds gave satisfactory C, H, N analyses within ±0.4% of theoretical values.
b) All compounds were crystallized from ethanol.
c) The yield is based on the final step.

within ± 0.4% of theoretical values.

1-Methyl-4-nitropyrrole-2-carbonyl chloride (2)

To a solution of 1-methyl-4-nitropyrrole-2-carboxylic acid 1 (1 g, 1.5 mmoles) in dry benzene (10 ml), thionyl chloride (3 ml) was added. The mixture was refluxed for 4 hours (until a clear solution was obtained). The solvent and additional thionyl chloride were evaporated and the residue was crystallized from ether to give 0.88 g (88%) of **2**, m.p. 80-82°C; IR (potassium bromide): ν 3110 (pyrrole), 1740 (C=0), 1500, 1315 cm^{-1} (NO_2); NMR ($CDCl_3$): 3.96 (s, 3H, NCH_3) and 7.75 ppm (s, 2H, aromatic); MS: m/z (%) 188 (M^+, 50) 153, (100), 107 (55) and 79 (15).

1- (1-Methyl-4-nitropyrrole-2-carbonyl)-4-phyenyl-thiosemicarbazide (3a)

To a stirred solution of 4-phenylthiosemicarbazide (1.2 g, 7.2 mmoles) and pyridine (12 ml) at room temperature was added 1-methyl-4-nitropyrrole-2-carbonyl

Table 2. Antifungal and antibacterial activities of compounds **6a** to **6f**

Compound No.	mg/disk	Inhibition Zone (mm)					
		E.Coli endemic	*B. Subtilis* PTCC 1023	*S. aureus* PTCC 1337	*K. pneumoniae* PTCC 1053	*C. albicans* PTCC 5027	*A. niger* PTCC 5013
6a	60	-a	8	-	7	-	7
	90	-	9	-	8	-	8
6b	60	8	-	-	-	-	10
	90	9	-	-	-	7	12
6c	60	7	-	-	-	-	10
	90	8	-	7	-	-	13
6d	60	-	-	7	-	-	-
	90	7	-	8	7	7	-
6e	60	-	-	-	7	-	7
	90	-	8	-	8	-	8
6f	60	7	-	7	-	-	-
	90	8	-	8	-	7	-
gentamycin	10	18	24	8	7	-	-
ketoconazole	10	-	-	-	-	30	18

a) Inhibition diameter was insignificant (0-3 mm).

chloride (**2**, 1.3 g, 6.9 mmoles). After the solution was stirred for 5 hours the excess pyridine was azeotroped with H_2O and the precipitate was collected by filtration. Recrystallization from EtOAc afforded 1.9 g (86%) of **3a**; m.p. >300°C; IR (potassium bromide): ν 1695 (C=O), 1315; 1520 cm^{-1} (NO_2); NMR ($CDCl_3$): 3.99 (s, 3H, NCH_3), 7.44 (m, 6H, H_3-pyrrole, phyenyl) and 7.71 ppm (s, 1H, H_5-pyrrole); MS: m/z (%) 319 (M^+, 10), 184 (43), 153 (70) and 77 (60).

1- (1- Methyl-4-nitropyrrole-2-carbonyl)-4-ethyl-thiosemicarbazide (3b)

This compound was prepared in the same manner as **3a** in 98% yield, m.p. >300°C (EtOAc).

1- (1-Methyl-4-nitropyrrole-2-carbonyl)-4-methyl-thiosemicarbazide (3c)

This compound was prepared in the same manner as **3a** in 56% yield, m.p. >300°C (EtOAc).

5 - (1 - Methyl -4 - nitropyrrole - 2 - yl) - 4 - phenyl - 2, 4 -dihydro-3H-1, 2,4-triazole-3-thione (4a)

Compound **3a** (2.21 g, 6.9 mmoles) and 8% aqueous sodium bicarbonate (300 ml) were stirred and refluxed for 120 hours. The reaction mixture was filtered. When the filtrate had cooled to room temperature it was acidified by the careful addition of concentrated aqueous HCl. The resulting mixture was extracted three times with EtOAc. The EtOAc extracts were combined, washed with saturated aqueous NaCl, and dried over anhydrous Na_2SO_4. The filtrate was evaporated under reduced pressure leaving a solid which was recrystallized from EtOAc to give 1.23 g (56%) of **4a** , m.p.>300°C; IR (potassium bromide): ν 1730 (C=0), 1510, 1315 cm^{-1} (NO_2); NMR ($CDCl_3$): 4.00 (s.3H NCH_3) and 7.61 ppm (m, 7H, pyrrole, phenyl); M.S: m/z (%) 301 (M^+, 15), 167 (25), 149 (100), 91 (54), 77 (65) and 57 (70).

4-Ethyl-5-(1-methyl-4-nitropyrrole-2-yl)-2,4-dihydro-3H-1, 2, 4-triazole-3-thione (4b)

This compound was prepared in the same manner as **4a** in 90% yield, m.p. >300°C (EtOAc).

4-Methyl -5 - (1-methyl-4-nitropyrrole-2-yl) -2, 4-dihydro-3H-1, 2, 4-triazole-3-thione (4c)

This compound was prepared in the same manner as **4a** in 70% yield, m.p. >300°C (EtOAc).

3- (1-Methyl-4-nitropyrrole-2-yl)-5- (methylthio)-4-phenyl-4H-1, 2, 4- triazole (5a)

Compound **4a** (0.7 g, 2 mmoles) was dissolved in 1M aqueous NaOH (7.7 ml). The solution was stirred and a solution of methyl iodide (0.24 ml, 3.8 mmoles) in ethanol (8 ml) was added. After being stirred overnight at room temperature, the reaction mixture was diluted with H_2O and the precipitate was collected by filtration. Recrystallization from ethanol afforded 0.32 g (46%) of **5a**; m.p. 154-156°C; IR (potassium bromide): ν 3120 (pyrrole), 1510, 1315 cm^{-1} (NO_2); NMR ($CDCl_3$): 2.79 (s, 3H, SCH_3), 3.62 (s, 3H, NCH_3 triazole), 3.95 (s, 3H, NCH_3 pyrrole), 6.96 (d, 1H, $J_{3,5}$=1.8 Hz, H_3-pyrrole); MS: m/z (%) 253 (M^+, 15), 225 (15), 167 (35), 149 (100), 113 (18),

94 (24), 71 (60) and 57 (80).

Compounds **5b** to **5f** were prepared similarly (Table 1).

3-(Methylsulfonyl)-5-(1-methyl-4-nitropyrrole-2-yl)-4-phenyl-4H-1,2, 4-triazole (6a)

To a stirred solution of compound **5a** (0.3g, 0.95 mmole) in dichloromethane (9 ml) at 0°C was added *m*-chloroperbenzoic acid (0.5 g, 2.9 mmoles). After being stirred for 48 hours, the reaction mixture was washed twice with saturated aqueous sodium bicarbonate and once with saturated aqueous NaCl. After the solution was dried over anhydrous Na_2SO_4, the dichloromethane was evaporated at reduced pressure leaving a solid. Purification by TLC (silica gel, chloroform/ethanol; 95:5) afforded 200 mg (34%) of **6a**. ,m.p. 216-217°C; IR (Potassium bromide): ν 1350, 1580 (NO_2), 1152, 1340 cm^{-1} (SO_2); NMR ($CDCl_3$): δ 3.59 (s, 3H, CH_3SO_2), 3.97 (s, 3H, NCH_3 triazole), 4.02 (s, 3H, NCH_3 pyrrole), 7.01 (d, 1H, $J_{3,5}$=1.8 Hz, H_3-pyrrole) and 7.73 ppm (d, 1H, $J_{3,5}$=1.8 Hz, H_5-pyrrole); MS: m/z (%) 285 (M^+, 55), 268 (25), 238 (100), 105 (22) and 79 (15). Compounds **6b** to **6f** were prepared similarly (Table 1).

Antibacterial and Antifungal Assay

Compounds **6a** to **6f** were tested against *Escherichia coli* (endemic), *Klebseilla-pneumoniae* (PTCC 1053), *Bacillus subtilis* (PTCC 1023), *Staphylococcus aureus* (PTCC 1337), *Candida albicans* (PTCC 5027) and *Aspergilus niger* (PTCC 5013).

All compounds were dissolved in acetone. They were diluted to 1 mg/ml concentration. To a standard paper disk 6 mm in diameter the latter solution was added until the desired amount of compound was absorbed on inoculated assay medium surface. Ketoconazole and gentamycin were used for comparison (see Table 2).

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