Synthesis, Characterization and Antibacterial Studies of Some 1,2,4-Triazole Derivatives Containing a 6-Chloropyridin-3-yl methyl Moiety

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5-(6-Chloropyridin-3-yl methyl)-4-phenyl-1,2,4-triazole-3-thiol (**2**) and 5-substituted-4-phenyl-1,2,4-triazole-3-thiols (**3**) were synthesized. Alkylation and aminomethylation reactions of these triazoles were also carried out. Some of the newly synthesized compounds were screened for their antibacterial activities.

Key words: 6-Chloropyridin-3-yl methyl Moiety, Triazoles, Aminomethylation, Antibacterial Activity, Serial Dilution Method

Introduction

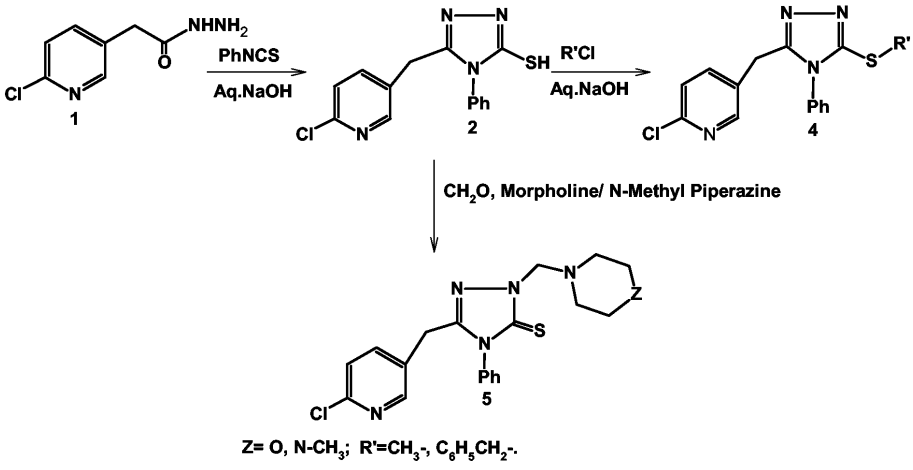
Many pyridine derivatives find important applications in the field of agriculture, medicine and industry [1 – 3]. Pyridine derivatives are also used as effective insecticides, fungicides and herbicides [4 – 6]. Triazoles gained increasing attention because of their immense biological activities [7 – 9]. Further, the presence of 6-chloro-pyridin-3-yl methyl group is known to enhance the biological activities of the molecules [10]. Prompted by these findings and in continuation of our studies on 3,6-disubstituted pyridine derivatives [11, 12], it was contemplated to synthesize a series of new 1,2,4-triazoles containing 6-chloropyridin-3-yl methyl moiety and screen them for their antibacterial activity.

Results and Discussion

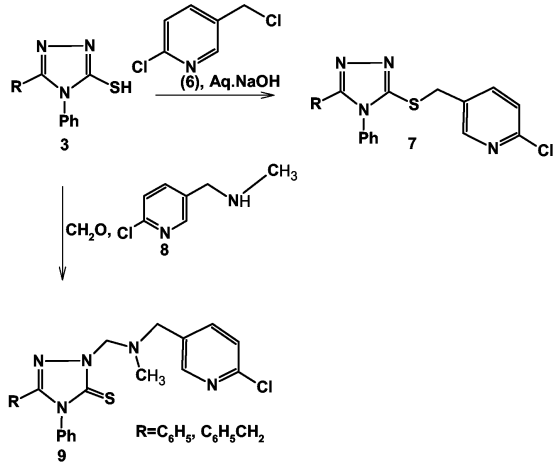
6-Chloropyridin-3-yl acetic acid hydrazide (**1**) was prepared from 6-chloro-3-chloromethylpyridine (**6**) by the method reported by our group [11]. 6-Chloro-3-chloromethyl pyridine (**6**) was also converted into (6-chloropyridin-3-yl methyl)methyl amine (**8**) [13]. 5-Phenyl/benzyl-4-phenyl-1,2,4-triazole-3-thiols (**3**) were synthesized from the respective acid hydrazides [14]. 6-Chloropyridin-3-yl acetic acid hydrazide (**1**) was reacted with phenyl isothiocyanate followed by aqueous sodium hydroxide to obtain 5-(6-chloropyridin-3-yl methyl)-4-phenyl-1,2,4-triazole-3-thiol (**2**). The triazole **(2)** was then transformed into 5-(6-chloropyridin-3-yl methyl)-4-phenyl-3-alkylsulfanyl-1,2,4-triazoles (**4**) by reacting it with alkyl/aryl halides using aqueous sodium hydroxide. Aminomethylation of triazole (**2**) was carried out with formaldehyde and a secondary amine to yield 5-(6-chloropyridin-3-yl methyl)-4-phenyl-2-morpholino/*N*-methylpiperazino-4-yl methyl-3*H*-1,2,4-triazole-3-thiones (**5**) (Scheme 1).

On the other hand, 5-phenyl/benzyl-4-phenyl-3-(6-chloropyridin-3-yl methyl)sulfanyl-1,2,4-triazoles (**7**) and 2-{[(6-chloropyridin-3-yl methyl)methylamino]-methyl}-5-phenyl/benzyl-4-phenyl-3*H*-1,2,4-triazole-3-thiones (**9**) were prepared from 5-phenyl/benzyl-4-phenyl-1,2,4-triazole-3-thiols (**3**) using 6-chloro-3-chloromethyl pyridine (**6**) and (6-chloropyridin-3-yl methyl) methylamine (**8**), respectively (Scheme 2).

All newly synthesized compounds gave satisfactory analysis for their nitrogen content. They also exhibited characteristic spectral patterns pertaining to 6-chloropyridin-3-yl moiety. In the IR spectra, the absorption bands corresponding to C-Cl vibrations for these molecules were observed in the region 725 – 737 cm^{-1}. In the ^{1}H NMR spectra, sharp singlets corresponding to -CH_2 group present in all compounds were seen at δ ranging from 3.85 to 4.43. The characteristic signals due to three protons of 3,6-disubstituted pyridine ring appeared as a quartet and two doublets. The proton (H_a) in position-2 of pyridine ring was coupled with the proton (H_m) in position-4 to give rise to a doublet in the region $\delta = 7.88 - 8.43$ ($J_{am} = 2.1 - 2.5$ Hz). Sim-



Scheme 1.



Scheme 2.

ilarly, proton (H_x) in the position-5 was coupled with proton (H_m) in the position-4 to give rise to another doublet in the region $\delta = 7.22-7.48$ ($J_{mx} = 8.2$ Hz), while proton (H_m) in the position-4 was coupled with other two protons to give rise to a doublet of doublet in the region $\delta = 7.36-7.89$ ($J_{am} = 2.1-2.5$ Hz, $J_{mx} = 8.2$ Hz) in all compounds. These data support the presence of a 6-chloropyridin-3-yl methyl moiety. In the mass spectra, fragment corresponding to the 6-chloropyridin-3-yl methyl cation was seen at m/z 126 which again supported the presence of a 6-chloropyridin-3-yl methyl moiety in all newly synthesized compounds.

The IR spectrum of triazole (**2**) showed an absorption band at 3114 cm^{-1} indicating the presence of NH/SH group. It could be seen that the characteristic absorption bands of hydrazide group of parent compound (**1**) were absent in the spectrum. In the IR spectra of the alkylated products (**4**) as well as Mannich bases (**5**), the NH/SH band was absent. The IR spectra of compounds (**7**) and (**9**) also showed the absence of NH/SH absorption bands which were found in the parent triazoles. In the ^{1}H NMR spectrum of triazole (**2**), two singlets corresponding to the protons of $NHNH_2$ group of parent hydrazide (**1**) were absent. The aromatic protons of the phenyl ring were seen as a multiplet in the region of $\delta = 7.16-7.54$ and the signal of the SH proton was observed as a broad singlet at $\delta = 13.75$. The downfield shift of this signal indicates the thiol-thione tautomerism in the triazole. This downfield signal was absent in the ^{1}H NMR spectra of the alkylated product (**4**) and the Mannich base (**5**) thus confirming their formation.

The mass spectra of triazole (**2**), its alkylated derivative (**4a**) and Mannich base (**5a**) were found to be consistent with the assigned structures. Molecular ion peaks for these compounds were observed at m/z 302, 316 and 401 corresponding to their molecular formulae $C_{14}H_{11}ClN_4S$, $C_{15}H_{13}ClN_4S$ and $C_{19}H_{20}ClN_5OS$, respectively. The molecular ion of compound (**2**) underwent fragmentation to produce an ion at m/z 152 which corresponds to 6-chloropyridin-3-yl acetonitrile cation ($ClC_5H_3NCH_2CN^+$). A peak at m/z 301 seen in the mass spectrum of the compound (**4a**) was due to the elimination of the methyl group from parent ion during fragmentation. The molecular ion of Mannich base (**5a**) underwent characteristic fragmentation to give the base peak at m/z 100, which corresponds to the molecular ion of N-methyl morpholine cation. Compounds (**7a**) and (**9a**) also exhibited similar mass spectral fragmentation pattern. The molecular ion

Table 1. Antibacterial activity data of the newly synthesized compounds.

Compound	Minimum inhibition concentration* (μg/ml)			
	E. coli	*P. aeruginosa*	*S. aureus*	*B. subtilis*
2	10	20	10	10
4a	100	200	100	100
4b	–	–	–	–
5a	–	–	–	–
5b	–	50	100	200
7a	10	20	10	10
7b	–	–	–	–
9a	100	200	100	100
9b	–	–	–	–
Furacin	6	12.5	12.5	12.5

* Index for antibacterial activity; diameter of the disc: 5 mm; amount of the sample: 100 μg/ml; control(solvent): dimethyl formamide.

peaks (M^+) were found at m/z 378 and 421, respectively. Compound (**9a**) being a Mannich base, showed a base peak at m/z 169 corresponding to its characteristic fragment [N-methyl-N-(6-chloropyridin-3-yl-methyl)amino]methyl cation.

Antibacterial Activity

All the newly synthesized compounds were initially screened for their *in vitro* antibacterial activities against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus subtilis* according to serial dilution method [15]. The compounds were tested at a concentration of 200 μg/ml in dimethylformamide against all organisms. Furacin was used as standard drug for comparison of antibacterial activity and solvent control was kept. The minimum inhibitory concentrations (MIC values) of the above compounds were determined. The results of antibacterial screening studies are reported in Table 1.

A stock solution of concentration 100 μg/ml was prepared by dissolving 5 mg of the test compound in 50 ml of dimethyl formamide. One loopful of an 18 h broth culture was inoculated into 5 ml of nutrient broth and this was incubated at 37 °C for 4 h. An assay was prepared by diluting 4 h sub-culture in 1/1000 in nutrient broth.

Nutrient broth (0.5 ml) was taken in tubes with labeled numbers 1 – 11 and 0.5 ml of the solution of the test compound (100 μg/ml) was added to the first tube no. 2. This process was repeated serially to obtain the quantities indicated in each of the test tubes. The eleventh tube was taken as control. A drop of diluted broth culture of the test organization (approximately 0.05 ml) was added to all the tubes using a sterilized Pasteur pipette. The solutions were mixed gently and the incubation was carried out at 37 °C for 16 – 18 h. Furacin was dissolved in dimethylformamide and was used as a standard drug for comparison. The minimum concentration at which there was no turbidity was taken as the minimum inhibitory concentration (MIC value).

The antibacterial screening data indicate that among the compounds tested, only triazole (**2**) and S-alkylated derivative (**7a**) showed good antibacterial activities against *S. aureus* and *B. subtilis* compared to Furacin.

Experimental Section

The melting points were determined by open capillary method and are uncorrected. IR spectra were recorded in KBr pellets on a Perkin-Elmer 882 IR spectrophotometer. ^{1}H NMR spectra were recorded in (DMSO-d_6/$CDCl_3$ or a mixture of them) on a Bruker AC-300F / amx 400 (300/400 MHz) NMR spectrometer using TMS as an internal standard. The mass spectra were recorded on a VG-Micromass or Hewlett-Packard 6890-GC mass spectrometer operating at 70 eV. Progresses of all the reactions and purity of the products were monitored by TLC using ethyl acetate as eluent and UV/iodine as visualizer.

*Preparation of 4-phenyl-5-(6-chloropyridin-3-yl methyl)-4H-1,2,4-triazole-3-thiol (**2**)*

To a suspension of hydrazide (1.85 g, 0.01 mol) in dry benzene (30 ml), phenyl isothiocyanate (1.35 g, 0.01 mol) was added and refluxed for 5 h. The reaction mixture was then cooled to room temperature. The solid obtained was filtered and washed with light petrol. The resulting solid was then dissolved in 2N sodium hydroxide solution (20 ml) and heated on water bath for 2 h, cooled to room temperature and filtered. The filtrate was neutralized with dilute hydrochloric acid. The precipitate obtained was filtered, dried and recrystallized.

White crystals; m. p. 222 – 224 °C; yield 69%. – IR (KBr): $\tilde{\nu}$ = 3114 (N-H str.), 3043 – 2930 (C-H str.), 1581 – 1459 (C=N, C=C str.), 1423 (CH_2 bend), 841 (Py-H bend), 723 (C-Cl str.), 699 (Ar-H bend) cm^{-1}. -^{1}H NMR (300 MHz, DMSO-d_6): δ = 3.85 (s, 2H, -CH_2-), 7.16 – 7.54 (m, 5H, Ar-H), 7.22 (d, 1H, Hx, J = 8.2 Hz), 7.36 (dd, 1H, Hm, J = 2.5 Hz, 8.2 Hz), 7.88 (d, 1H, Ha, J = 2.5 Hz), 13.75 (s, 1H, NH/SH). -MS (VG, 70 eV): m/z (%) = 302 (100) [M^+], 304 (36) [M^++2], 152 (12) [$ClC_5H_3NCH_2CN^+$], 126 (26) [$ClC_5H_3NCH_2$], 77 (15) [$C_6H_5^+$].

*General method of alkylation of 4-phenyl-5-substituted-1,2,4-triazole-3-thiols (**4**, **7**)*

To a mixture of 4-phenyl-5-substituted-1,2,4-triazole-3-thiol (0.01 mol) and sodium hydroxide (0.01 mol) in water

(25 ml), alkylating agent (0.01 mol) in methanol (10 ml) was added in drops. The contents were stirred for 8 h at room temperature. The solid separated was filtered, washed with water and recrystallized from appropriate solvent to yield the title compound.

4a: White powder; m. p. 86 – 88 °C; yield 91%. – IR (KBr): $\tilde{\nu} = 3049 - 2931$ (C-H str.), 1592 – 1455 (C=N, C=C str.), 1422 (CH_2 bend), 827 (Py-H bend), 735 (C-Cl str.), 700 (Ar-H) cm^{-1}. – ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 2.56$ (s, 3H, -CH_3), 4.03 (s, 2H, -CH_2-), 7.36 – 7.57 (m, 7H, Ar-H, Hx & Hm), 8.02 (d, 1H, H_A, $J = 2.0$ Hz). – MS(VG, 70 eV): m/z (%) = 316 (100) [M^+], 318 (38) [M^++2], 301 (45) [M^+-CH_3], 281 (17) [M^+-Cl], 269 (9) [M^+-SCH_3], 239 (8) [M^+-$C_6H_5{}^+$], 126 (66) [$ClC_5H_3NCH_2{}^+$], 91 (42) [126-Cl].

4b: Yellow powder; m. p. 97 – 99 °C; yield 82.5%. – IR (KBr): $\tilde{\nu} = 3054 - 2946$ (C-H str.), 1591 – 1453 (C=N, C=C str.), 1409 (CH_2 bend), 838 (Py-H bend), 737 (C-Cl str.), 703 (Ar-H bend) cm^{-1}.

7a: Yellow powder; m. p. 113 – 116 °C; yield 73.9%. – IR (KBr): $\tilde{\nu} = 3057 - 2977$ (C-H str.), 1591 – 1462 (C=N, C=C str.), 1441 (CH_2 bend), 835 (Py-H bend), 735 (C-Cl str.), 704 (Ar-H bend) cm^{-1}. – ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 4.43$ (s, 2H, -CH_2-), 7.19 – 7.55 (m, 10H, Ar-H), 7.48 (d, 1H, Hx, $J = 8.2$ Hz), 7.89 (dd, 1H, Hm, $J = 2.4$ Hz, 8.2 Hz), 8.43 (d, 1H, Ha, $J = 2.1$ Hz). – MS(VG, 70 eV): m/z (%) = 378 (100) [M^+], 395 (32) [M^++2], 126 (26) [$ClC_5H_3NCH_2{}^+$], 77 (23) [$C_6H_5{}^+$].

7b: White powder; m. p. 170 – 171 °C; yield 72%. – IR (KBr): $\tilde{\nu} = 3055$ (C-H str.), 1597 – 1452 (C=N, C=C str.), 1421 (CH_2 bend), 836 (Py-H bend), 725 (C-Cl str.), 702 (Ar-H bend) cm^{-1}.

*General method of aminomethylation of 4-phenyl-5-substituted-1,2,4-triazole-3-thiols (***5**, **9***)*

4-Phenyl-5-substituted-1,2,4-triazole-3-thiol (0.01 mol) was dissolved in ethanol (25 ml). Then formaldehyde (40%, 1.5 ml) and secondary amine (0.01 mol) were added to this mass. The mixture was stirred and heated to reflux for 30 min and left overnight at r. t. The resulting solid was collected by filtration, washed with ethanol and recrystallized from appropriate solvent to yield the title compound.

5a: Yellow powder; m. p. 143 – 144 °C; yield 76.7%. – IR (KBr): $\tilde{\nu} = 3088 - 2807$ (C-H str.), 1596 – 1467 (C=N, C=C str.), 1430 (CH_2 bend), 1288 (C=S str.), 852 (Py-H bend), 734 (C-Cl str.), 707 (Ar-H str.) cm^{-1}. – ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 2.72$ (t, 4H, -CH_2-N-CH_2-, $J = 4.3$ Hz), 3.58 (t, 4H, -CH_2-O-CH_2-, $J = 4.3$ Hz), 3.90 (s, 2H, -CH_2-), 5.05 (s, 2H, N-CH_2-N), 7.37 – 7.54 (m, 5H, Ar-H), 7.41 (d, 1H, Hx, $J = 8.2$ Hz), 7.57 (dd, 1H, Hm, $J = 2.3$ Hz, 8.2 Hz), 8.05 (d, 1H, Ha, $J = 2.3$ Hz). – MS(VG, 70 eV): m/z (%) = 401 (46) [M^+], 403 (16) [M^++2], 302 (76) [$ClC_5H_3NCH_2C_7H_6N_3S^+$], 126 (24) [$ClC_5H_3NCH_2{}^+$], 100 (100) [$C_4H_8O^+$].

5b: Yellow powder; m. p. 119 – 121 °C; yield 63.8%. – IR (KBr): $\tilde{\nu} = 3072 - 2827$ (C-H str.), 1594 – 1463 (C=N, C=C str.), 1444 (CH_2 bend), 1299 (C=S str.), 825 (Py-H bend), 727 (C-Cl str.), 702 (Ar-H str) cm^{-1}.

9a: White powder; m. p. 154 – 155 °C; yield 72.4%. – IR (KBr): $\tilde{\nu} = 3068 - 2831$ (C-H str.), 1592 – 1454 (C=N, C=C str.), 1420 (CH_2 bend), 1288 (C=S str.), 849 (Py-H bend), 732 (C-Cl str.), 708 (Ar-H bend) cm^{-1}. – ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 2.47$ (s, 3H, CH_3), 3.98 (s, 2H, -CH_2-), 5.27 (s, 2H, N-CH_2-N), 7.34 – 7.53 (m, 11H, Ar-H & Hx), 7.88 (dd, 1H, Hm, $J = 2.3$ Hz, 8.2 Hz), 8.41 (d, 1H, Ha, $J = 2.3$ Hz). – MS(VG, 70 eV): m/z (%) = 421 (18) [M^+], 423 (7) [M^++2], 295 (7) [M^+-$ClC_5H_3NCH_2{}^+$], 253 (11) [$C_6H_5C_8H_6N_3S^+$], 169 (100) [$ClC_5H_3NCH_2N(CH_3)CH_2{}^+$], 126 (82) [$ClC_5H_3NCH_2{}^+$], 91 (11) [126-Cl], 77 (17) [$C_6H_5{}^+$].

9b: Paste, yield 64.3%. – IR (KBr): $\tilde{\nu} = 3059 - 2829$ (C-H str.), 1596 – 1463 (C=N, C=C str.), 1422 (CH_2 bend), 1291 (C=S str.), 855 (Py-H bend), 731 (C-Cl str.), 701 (Ar-H str) cm^{-1}.

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