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Synthesis and Antitubercular Evaluation of 5-Chloro-2-(5-(Substituted Phenyl)-1H-tetrazol-1-yl) Pyridine

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Abstract: Several new 5-chloro-2-(5-(substituted phenyl)-1H-tetrazol-1-yl) pyridines has been synthesized by reaction of 2- amino pyridine derivative with various aromatic acid chlorides and sodium azide. The chemical structure of the synthesized compounds was confirmed by means of IR, ^{1}H and ^{13}C NMR and Mass spectral analysis. All the synthesized compounds were screened for their antitubercular activity by Microplate Alamar Blue assay (MABA) method. All the synthesized compounds have exhibited significant activity against *Mycobacterium tuberculosis* $H_{37}Rv$. The activities expressed as the minimum inhibitory concentration (MIC) fall into the range of 3.125-25 µg/ml.
Key words: pyridine, tetrazole, antimycbacterial, antitubercular.

Introduction

Mycobacterium tuberculosis, the causative agent of tuberculosis (TB), is a remarkably successful pathogen that has latently infected two million casualities worldwide[1]. TB is contagious and spreads through the air; if not treated properly, each person infects average 10-15 people every year with TB bacilli[2]. It is termed as "a global health emergency" by world health organization (WHO) in 1993 as it affects 1.7 billion people per year, that is equal to one-third of the entire world population. The first line of drugs used in the treatment of tuberculosis (TB) is a combination of isoniazid, rifampicin, pyrazinamide and ethambutol. The narrow choice of antibiotics, lengthy treatment regimens, and patient noncompliance has provided conditions for acquired antibiotic resistance that led to worldwide emergence of strains resistant to virtually all available drugs[3]. Thus the increasing clinical importance of tuberculosis has lent additional urgency for researchers to identify new and effective antimycobacterial compounds.

Tetrazoles are an increasingly popular functionality with wide-ranging applications. They have found use in pharmaceuticals as lipophilic spacers and carboxylic acid surrogates[4]. Tetrazoles are regarded as biologically equivalent to the carboxylic acid group, and extensive work on tetrazoles has been carried out in the field of medicinal chemistry. Literature survey reveals that tetrazole and its derivatives possessing a wide spectrum of biological activities including antibacterial[5,6], antifungal[7], anticonvulsant[8], analgesic[9], anti-inflammatory[10], antitubercular[11], anticancer[12], anti-hypertensive[13] and antidiabetic[14] activities. In addition pyridines are associated with diverse biological activities[15-17]. In view of these facts and in continuation of our studies on the synthesis of biologically active substituted tetrazoles, it was considered of interest to synthesize 1,5

disubstituted tetrazoles. We report here a simple and facile one pot procedure by cycloaddition method for the synthesis of 5-chloro-2-(5-substituted-1H-tetrazol-1-yl) pyridines and their antimycobacterial activity.

Experimental

Melting points were determined in open capillaries and **were uncorrected.** The purity of the synthesized compounds was routinely checked by TLC on silica gel G. ^{1}H and ^{13}C NMR spectra was recorded on JEOL GSX 400 spectrometer using TMS as an internal standard (chemical shifts in δ, ppm), IR spectra on a Perkin Elmer 1600 FT spectrometer (ν_{max} cm^{-1}) and Mass spectra on a JEOL MSMATE spectrometer. The physical data of the title compounds are given in Table 1.

General Procedure

Synthesis of 2- aroylamino-5-chloropyridines (3a-j)

To a solution of 2-amino-5-chloropyridine (**1)** was added an equimolar amount of aroyl chloride **(2a-j)** in pyridine with constant shaking. After the addition was complete the reaction mixture was allowed to stand at room temperature for 2 hours. The crude products that separated out on dilution was filtered and recrystallised from ethanol.

Synthesis of N-Pyridyl-2-yl imidoformylchloride-benzene (4a-j)

A mixture of (**3a-j)** (0.004 mol) and PCl_5 (0.004 mol) was heated at 100^0 C for 1 hour. When the evolution of fumes of HCl was ceased excess of $POCl_3$ was removed under reduced pressure.

Synthesis of 5-chloro-2-(5-(substituted phenyl-1H-tetrazol-1-yl) pyridine (5a-j)

The residual imidoyl chloride was treated with an ice cold solution of sodium azide (0.0075 mol) and excess of sodium acetate in water (25 ml) and acetone (30 ml) with stirring. Stirring was continued overnight, acetone was removed under reduced pressure, remaining aqueous portion was extracted with chloroform and dried.

5-chloro-2-(5-phenyl-1H-tetrazol-1-yl)pyridine (5a): IR (KBr): 1592 (C=N), 1157 (Tetrazole) 754 (C-Cl) cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 7.23-7.48 (m, 5H, Ar-H), 7.61-8.80 (d, 3H, pyridine); ^{13}C-NMR ($CDCl_3$): δ 122.9, 127.5, 128.8, 129.3, 134.1, 134.8, 139.0, 151.5; MS (relative intensity): m/z value 257.05 (M+1); Anal. Calcd. for $C_{12}H_8ClN_5$ % C 55.93, H 3.13, N 27.18; found C 55.92, H 3.11, N 27.16

5-chloro-2-(5-(4-nitrophenyl)-1H-tetrazol-1-yl)pyridine (5b): IR (KBr): 1592 (C=N), 1527 (NO_2), 1158 (Tetrazole), 753 (C-Cl) cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 7.73-8.25 (m, 4H, Ar-H), 7.61-8.88 (d, 3H, pyridine); ^{13}C-NMR ($CDCl_3$): δ 121.6, 122.9, 128.4, 134.1, 134.8, 136.8, 139.0, 148.4, 155.9; MS (relative intensity): m/z value 302.03 (M+1); Anal. Calcd. for $C_{12}H_7ClN_6O_2$ % C 47.62, H 2.33, N 27.77; found C 47.63, H 2.32, N 27.75

5-chloro-2-(5-(2-chlorophenyl)-1H-tetrazol-1-yl)pyridine (5c): IR (KBr): 1628 (C=N), 1152 (Tetrazole), 757 (C-Cl) cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 7.17-7.43 (m, 4H, Ar-H), 7.61-8.81 (d, 3H, pyridine); ^{13}C-NMR ($CDCl_3$): δ 122.9, 127.4, 128.9, 129.4, 130.2, 132.3, 134.1, 134.8, 136.8, 139.0, 151.9; MS (relative intensity): m/z value 291.05 (M+1); Anal. Calcd. for $C_{12}H_7Cl_2N_5$ % C 49.34, H 2.42, N 23.97; found C 49.35, H 2.41, N 23.96

5-chloro-2-(5-(4-chlorophenyl)-1H-tetrazol-1-yl)pyridine (5d): IR (KBr): 1603 (C=N), 1150 (Tetrazole), 752 (C-Cl) cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 7.32-7.43 (m, 4H, Ar-H), 7.62-8.83 (d, 3H, pyridine); ^{13}C-NMR ($CDCl_3$): δ122.9, 128.8, 128.9, 129.4, 134.1, 134.3, 134.8, 139.0, 151.9; MS (relative intensity): m/z value 291.05 (M+1); Anal. Calcd. for $C_{12}H_7Cl_2N_5$ % C 49.34, H 2.42, N 23.97; found C 49.36, H 2.40, N 23.98

5-chloro-2-(5-(4-methoxyphenyl)-1H-tetrazol-1-yl)pyridine (5e): IR (KBr): 1606 (C=N), 1168 (OCH_3), 1155 (Tetrazole), 750 (C-Cl) cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 6.84-7.38 (m, 4H, Ar-H), 7.60-8.82 (d, 3H, pyridine), 3.74 (s, 3H, OCH_3); ^{13}C-NMR ($CDCl_3$): δ 55.8, 113.8, 122.2, 123.0, 128.5, 134.1, 134.8, 139.0, 151.1, 160.7; MS (relative intensity): m/z value 287.06 (M+1); Anal. Calcd. for $C_{13}H_{10}ClN_5O$ % C 54.27, H 3.50, N 24.34; found C 54.28, H 3.52, N 24.35

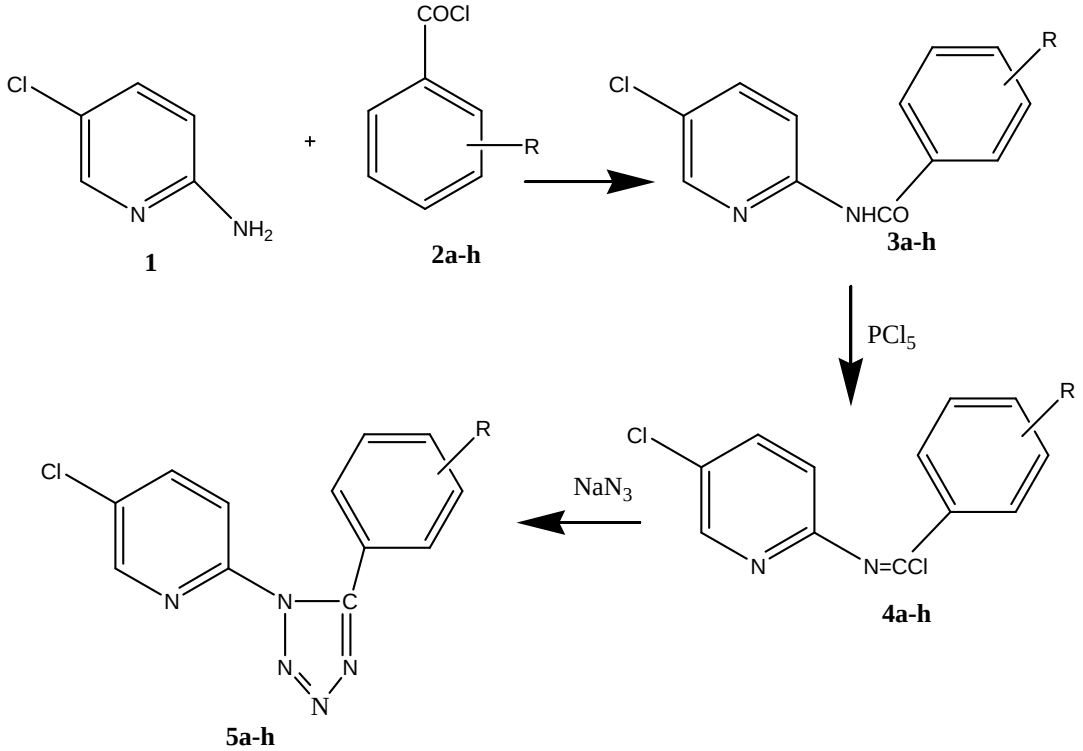
5-chloro-2-(5-p-tolyl-1H-tetrazol-1-yl)pyridine (5f): IR (KBr): 1596 (C=N), 1155 (Tetrazole), 752 (C-Cl) cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 7.13-7.37 (m, 4H, Ar-H), 7.64-8.83 (d, 3H, pyridine), 2.37 (s, 3H, CH_3); ^{13}C-NMR ($CDCl_3$): δ 24.5, 122.2, 127.4, 127.7, 129.6, 134.1, 134.8, 138.4, 139.2, 152.1; MS (relative intensity): m/z value 271.06 (M+1); Anal. Calcd. for $C_{13}H_{10}ClN_5$ % C 57.47, H 3.71, N 25.78; found C 57.48, H 3.70, N 25.79

5-chloro-2-(5-(3-bromophenyl)-1H-tetrazol-1-yl)pyridine (5g): IR (KBr): 1612 (C=N), 1159 (Tetrazole), 751 (C-Cl), 572 (C-Br) cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 7.22-7.66 (m, 4H, Ar-H), 7.64-8.86 (d, 3H, pyridine); ^{13}C-NMR ($CDCl_3$): δ 122.2, 123.6, 126.5, 131.5, 131.7, 132.9, 133.1, 134.1, 134.8, 140.4, 152.1; MS (relative intensity): m/z value 334.96 (M+1); Anal. Calcd. for $C_{12}H_7BrClN_5$ % C 42.82, H 2.10, N 20.81; found C 42.80, H 2.12, N 20.82

5-chloro-2-(5-(2,3-dichlorophenyl)-1H-tetrazol-1-yl)pyridine (5h): IR (KBr): 1629 (C=N), 1159 (Tetrazole), 768 (C-Cl) cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 7.15-7.31 (m, 3H, Ar-H), 7.63-8.80 (d, 3H, pyridine); ^{13}C-NMR ($CDCl_3$): δ 122.2, 127.0, 128.8, 130.3, 133.9, 134.1, 134.9, 139.0, 139.9, 150.1; MS (relative intensity): m/z value 324.97 (M+1); Anal. Calcd. for $C_{12}H_6Cl_3N_5$ % C 44.13, H 1.85, N 21.45; found C 44.10, H 1.88, N 21.44

5-chloro-2-(5-(3,5-dinitrophenyl)-1H-tetrazol-1-yl)pyridine (5i): IR (KBr): 1592 (C=N), 1526 (NO_2), 1153 (Tetrazole), 762 (C-Cl) cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 8.87-9.09 (d, 3H, Ar-H), 7.63-8.85 (d, 3H, pyridine); ^{13}C-NMR ($CDCl_3$): δ 118.7, 122.2, 128.2, 132.5, 134.0, 134.8, 149.1, 151.8; MS (relative intensity): m/z value 347.02 (M+1); Anal. Calcd. for $C_{12}H_6ClN_7O_4$ % C 41.46, H 1.74, N 28.20; found C 41.47, H 1.75, N 28.18

5-chloro-2-(5-benzyl-1H-tetrazol-1-yl)pyridine (**5j**): IR (KBr): 1594 (C=N), 1159 (Tetrazole), 769 (C-Cl) cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 3.82 (s, 2H, CH_2), 7.06-7.14 (d, 5H, Ar-H), 7.61-8.81 (d, 3H, pyridine); ^{13}C-NMR ($CDCl_3$): δ 29.6, 122.2, 125.8, 128.7, 129.1, 134.0, 134.9, 136.3, 139.3, 152.1, 155.5; MS (relative intensity): m/z value 271.06 (M+1); Anal. Calcd. for $C_{13}H_{10}ClN_5$ % C 57.47, H 3.71, N 25.78; found C 57.45, H 3.70, N 25.79.



R= hydrogen; 4-nitro; 2-chloro; 4-chloro; 4-methoxy; 4-methyl; 3-bromo; 2,3-dichloro; 3,5-dinitro, benzyl

Scheme-1

Antitubercular Activity

The antimycobacterial activity of compounds were assessed against *M. tuberculosis* using microplate Alamar Blue assay (MABA)[18]. The 96 well plates received 100 µl of the Middlebrook 7H9 broth containing *Mycobacterium tuberculosis* and serial dilution of compounds were made directly on plate. The final drug concentrations tested were 0.01 to 20.0 µl/ml. Plates were covered and sealed with parafilm and incubated at 37º C for seven days. After this time, 25µl of freshly prepared 1:1 mixture of Alamar Blue reagent and 10% tween 80 was added to the plate and incubated for 24 hours. A blue color in the well was interpreted as of no bacterial growth, and pink color was scored as growth. The MIC was defined as lowest drug concentration which prevented the color change from blue to pink. The MIC data is given in table 2.

Table 1 –Physical Data of Synthesized Compounds

Cpd code	Mol. Formula	m.pº C	Yield in %	R_f value
5a	$C_{12}H_8ClN_5$	124	54	0.62
5b	$C_{12}H_7ClN_6O_2$	144	62	0.55
5c	$C_{12}H_7N_5Cl_2$	138	68	0.64
5d	$C_{12}H_7Cl_2N_5$	150	75	0.71
5e	$C_{13}H_{10}ClN_5O$	180	69	0.54
5f	$C_{13}H_{10}ClN_5$	148	71	0.63
5g	$C_{12}H_7BrClN_5$	106	59	0.59
5h	$C_{12}H_6Cl_3N_5$	166	75	0.57
5i	$C_{12}H_6ClN_7O_4$	174	67	0.76
5j	$C_{13}H_{10}ClN_5$	132	73	0.60

Table: 2 Antitubercular Activity of the Compounds 5a-j

Sample Code	MIC in µg/ml
5a	12.5
5b	6.25
5c	3.125
5d	12.5
5e	25
5f	25
5g	12.5
5h	12.5
5i	12.5
5j	25
Rifampicin	0.2

Results And Discussion

The synthetic strategy developed to obtain the target compound 5-chloro-2-(5-substituted phenyl-1H-tetrazol-1-yl)pyridine was prepared by the reaction between aminopyridine and aroylchloride derivatives. Tetrazoles were synthesized under mild conditions in a short reaction time with good overall yield as outlined in Scheme 1. Cycloaddition of N-Pyridyl-2-yl imidoformylchloride-benzene with sodium azide produced 5-chloro-2-(5-(substituted phenyl-1H-tetrazol-1-yl) pyridine. The synthesized compounds were characterized by IR, ^{1}H and ^{13}C NMR and mass spectral data. The spectral data confirms the successful formation of the newly synthesized compounds.

In vitro activity of the synthesized derivatives against *M. tuberculosis* H_{37}Rv strains was carried out using Microplate Alamar Blue assay (MABA) method. Rifampicin was included as a standard drug for comparison (MIC = 0.2µg/ml). The anti tuberular screening data showed that all the pyridyl tetrazoles showed good anti tubercular activity. Derivatives 5b and 5c, which has respectively the nitro group and chlorine atom at 2 and 3

positions in the aromatic ring presented increased anti-tubercular activity, indicating that chlorine atom at 3 position is important for the biological activity.

Conclusion

A new series of 1,5 disubstituted tetrazoles (5a-j) starting from 2-amino pyridine derivative with aromatic acid chlorides have been synthesized. 1,5 disubstituted tetrazoles are promising anti-TB compounds and the structural optimization of this class may result in analogs with greater potency.

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