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Research Article

SYNTHESIS AND ANTIMICROBIAL EVALUATION OF 2-(5-(SUBSTITUTED PHENYL-1H-TETRAZOL-1-YL) PYRIDINES

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ABSTRCT

A new series of 2-(5-substituted phenyl-1H-tetrazol-1-yl) pyridine has been synthesized by the [3+2] cycloaddition of N-pyridyl-2-yl imidoformylchloride-benzene and sodium azide. The chemical structure of the synthesized compounds was confirmed by means of IR, ^{1}H NMR and Mass spectral analysis. All the synthesized compounds were screened for their antibacterial (*Staphylococcus aureus, Bacillus subtilis, Escherichia coli* and *Pseudomonas aeruginosa*) and antifungal activities (*Aspergillus fumigatus* and *Candida albicans*) by cup plate method. All the synthesized compounds have exhibited significant activity against the bacteria and fungi tested. Compounds 2-(5-(4-chlorophenyl)-1H-tetrazol-1-yl) pyridine and 2-(5-(2,3-dichlorophenyl)-1H-tetrazol-1-yl)pyridine were having a very good antibacterial activity against *Pseudomonas aeruginosa* and *E.coli*. Compound 2-(5-(3-bromophenyl)-1H-tetrazol-1-yl)pyridine having a very good antibacterial activity against *Bacillus subtilis*.

Keywords: Pyridine, Tetrazole, Antibacterial, Antifungal.

INTRODUCTION

The emergence and spread of antimicrobial resistance has become one of the most serious public health concerns across the world. Tetrazoles are medicinally important heterocycles incorporated in a large number of drugs. Tetrazole, an aromatic azapyrrole group, is metabolically stable and has acidic characteristics closely similar to that of the carboxylic group[1]. Tetrazole and its derivatives possessing a wide spectrum of biological activities including antibacterial[2,3], antifungal and anticonvulsant[4], analgesic[5], anti-inflammatory[6], antitubercular activity[7], anticancer activity[8] and anti-hypertensive[9] activities. 1, 5 disubstituted tetrazoles have long been known for their pharmaceutical activity as stimulants or depressants on the central nervous system and are reported to show oral antidiabetic and antithrombotic and antimicrobial properties[10]. In addition pyridines are associated with diverse biological activities. Inspired by the biological profile of tetrazole and pyridine on biologically active heterocycles it was thought worthwhile to synthesize new chemical entities with two active pharmacophores in a single molecular framework in order to prepare molecules having potentially enhanced biological activities. In the present study synthesis of a new series of 2-(5-phenyl-1H-tetrazol-1-yl)pyridine and their antibacterial (*Staphylococcus aureus, Bacillus subtilis, Escherichia coli* and *Pseudomonas aeruginosa*) and antifungal activities (*Aspergillus fumigatus* and *Candida albicans*) by cup plate method.

MATERIALS AND METHOD

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. Microanalysis for C, H and N were performed in a Heraeus CHN Rapid Analyser. The physical data of the title compounds are given in Table 1.

General procedure:

Synthesis of 2- aroylaminopyridines (3a-j)

To a solution of 2-aminopyridine (**1)** was added an equimolar amount of aroyl chloride **(2a-j)** with constant shaking. After the addition was complete the reaction mixture was allowed to stand at room temperature for 2 hrs. 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 hr. When the evolution of fumes of HCl was ceased excess of $POCl_3$ was removed under reduced pressure.

Synthesis of 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.

2-(5-phenyl-1H-tetrazol-1-yl)pyridine (5a): IR (KBr): 1590 (C=N), 1156 (Tetrazole) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 7.22-7.48 (m, 5H, Ar-H), 7.4-8.6 (d, 4H, pyridine); ^{13}C-NMR (DMSO-d_6): δ 122.2, 123.9, 127.5, 128.8, 129.3, 130.7, 136.0, 140.4, 149.1; MS (relative intensity): m/z value 223.09 (M+1); Anal. Calcd. for C_{12} H_9N_5 % C 64.56, H 4.06, N 31.37; found C 64.54, H 4.05, N 31.35

2-(5-(4-nitrophenyl-1H-tetrazol-1-yl)pyridine (5b): IR (KBr): 1598 (C=N), 1525 (NO_2), 1159 (Tetrazole) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 7.74-7.82 (m, 4H, Ar-H), 7.41-8.62 (d, 4H, pyridine); ^{13}C-NMR (DMSO-d_6): δ 121.6, 123.9, 128.4, 136.0, 136.8, 140.4, 148.4, 149.1; MS (relative intensity): m/z value 268.07 (M+1)); Anal. Calcd. for $C_{12}H_8N_6O_2$ % C 53.73, H 3.01, N 31.33; found C 53.71, H 3.01, N 31.30

2-(5-(2-chlorophenyl)-1H-tetrazol-1-yl)pyridine (5c): IR (KBr): 1620 (C=N), 1153 (Tetrazole), 756 (C-Cl) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 7.16-7.42 (m, 4H, Ar-H), 7.41-8.62 (d, 4H, pyridine); ^{13}C-NMR (DMSO-d_6): δ 122.2, 123.9, 127.4, 128.9, 129.4, 130.2, 132.3, 136.0, 138.5, 140.4, 149.1; 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 55.90, H 3.13, N 27.16

2-(5-(4-chlorophenyl)-1H-tetrazol-1-yl)pyridine (5d): IR (KBr): 1608 (C=N), 1151 (Tetrazole), 750 (C-Cl) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 7.33-7.42 (m, 4H, Ar-H), 7.41-8.63 (d, 4H, pyridine); ^{13}C-NMR (DMSO-d_6): δ 122.2, 123.9, 128.8, 128.9, 129.4, 134.3, 136.0, 140.4, 149.1; 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 3.10, N 27.17

2-(5-(4-methoxyphenyl)-1H-tetrazol-1-yl)pyridine (5e): IR (KBr): 1603 (C=N), 1165 (OCH_3), 1154 (Tetrazole) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 6.83-7.37 (m, 4H, Ar-H), 7.41-8.62 (d, 4H, pyridine), 3.73 (s, 3H, OCH_3); ^{13}C-NMR (DMSO-d_6): δ 55.9, 114.8, 122.2, 123.0,

123.9, 128.5, 136.0, 140.4, 149.1, 160.7; MS (relative intensity): m/z value 253.10 (M+1); Anal. Calcd. for $C_{13}H_{11}N_5O$ % C 61.65, H 4.38, N 27.65; found C 61.63, H 4.37, N 27.63

2-(5-p-tolyl-1H-tetrazol-1-yl)pyridine (5f): IR (KBr): 1593 (C=N), 1155 (Tetrazole) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 7.12-7.36 (m, 4H, Ar-H), 7.42-8.63 (d, 4H, pyridine), 2.35 (s, 3H, CH_3); ^{13}C-NMR (DMSO-d_6): δ 24.3, 122.2, 123.9, 127.4, 127.7, 129.6, 136.0, 138.4, 140.4, 149.1; MS (relative intensity): m/z value 237.1 (M+1); Anal. Calcd. for $C_{13}H_{11}N_5$ % C 65.81, H 4.67, N 29.52; found C 65.79, H 4.65, N 29.51

2-(5-(3-bromophenyl)-1H-tetrazol-1-yl)pyridine (5g): IR (KBr): 1609 (C=N), 1157 (Tetrazole), 570 (C-Br) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 7.21-7.65 (m, 4H, Ar-H), 7.42-8.63 (d, 4H, pyridine); ^{13}C-NMR (DMSO-d_6): δ 122.2, 123.6, 123.9, 126.5, 131.5, 131.7, 132.9, 133.1, 136.0, 140.4, 149.1; MS (relative intensity): m/z value 301.00 (M+1); Anal. Calcd. for $C_{12}H_8BrN_5$ % C 47.70, H 2.67, N 23.18; found C 47.69, H 2.65, N 23.17

2-(5-(2,3-dichlorophenyl)-1H-tetrazol-1-yl)pyridine (5h): IR (KBr): 1619 (C=N), 1158 (Tetrazole), 770 (C-Cl) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 7.14-7.30 (m, 3H, Ar-H), 7.42-8.60 (d, 4H, pyridine); ^{13}C-NMR (DMSO-d_6): δ 122.2, 123.9, 127.0, 128.8, 130.3, 131.2, 133.9, 136.0, 139.9, 140.4, 149.1; MS (relative intensity): m/z value 291.01 (M+1); Anal. Calcd. for $C_{12}H_7Cl_2N_5$ % C 49.34, H 2.42, N 23.97; found C 49.33, H 2.41, N 23.95

2-(5-(3,5-dinitrophenyl)-1H-tetrazol-1-yl)pyridine (5i): IR (KBr): 1599 (C=N), 1528 (NO_2), 1154 (Tetrazole) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 8.80-9.08 (d, 3H, Ar-H), 7.41-8.64 (d, 4H, pyridine); ^{13}C-NMR (DMSO-d_6): δ 118.7, 122.2, 123.9, 128.2, 132.5, 136.0, 140.4, 149.1, 149.8; MS (relative intensity): m/z value 313.06 (M+1); Anal. Calcd. for $C_{12}H_7N_7O_4$ % C 46.01, H 2.25, N 31.30; found C 46.02, H 2.23, N 31.32

2-(5-benzyl-1H-tetrazol-1-yl)pyridine (**5j**): IR (KBr): 1593 (C=N), 1155 (Tetrazole) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 3.81 (s, 2H, CH_2), 7.06-7.14 (d, 5H, Ar-H), 7.41-8.61 (d, 4H, pyridine); ^{13}C-NMR (DMSO-d_6): δ 29.4, 122.2, 123.9, 125.8, 128.7, 129.1, 136.0, 136.3, 140.4, 149.1, 154.5; MS (relative intensity): m/z value 237.1 (M+1); Anal. Calcd. for $C_{13}H_{11}N_5$ % C 65.81, H 4.67, N 29.52; found C 65.80, H 4.65, N 29.50

Antibacterial activity

The tetrazole derivatives (**5a-j**) were investigated for their inhibition of growth against *Staphylococcus aureus* (ATCC-25923), *Escherichia coli* (ATCC-25922), *Pseudomonas aeruginosa* (ATCC-27853) and *Bacillus subtilis* bacterial strains by the cup plate method[11, 12]. The test organisms were adjusted to 0.5 McFarland standard. Then by using sterile cotton swab 0.1 ml of suspension is spreaded on to the agar plate and then the plates were allowed to dry for 30 min at room temperature. Then by using sterile agar borer of 6mm diameter the cavities were made on the agar plates and the test drug (50µg/mL) and standard Amoxycillin (30µg/mL) were incorporated into the well and then the plates were kept in a refrigerator for one hour for a period of pre incubation diffusion. The plates were made in triplicate. Solvent and growth controls were made separately. Then the plates were incubated at 37ºC for 24 hours. After incubation zone of inhibition were recorded and tabulate in Table.2.

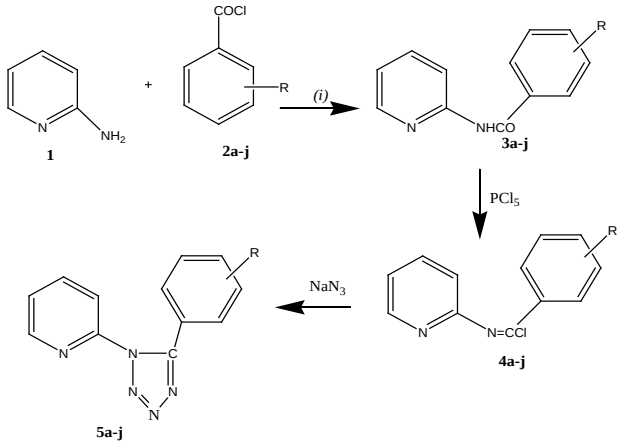
Antifungal activity

The newly synthesized compounds were also investigated for their antifungal activity[13] against two fungal strains, namely *Aspergillus fumigatus* (NCIM No.902) and *Candida albicans* (NCCS 3471). Sabouraud agar media was prepared by dissolving peptone (1 g), D-glucose (4 g) and agar (2 g) in sterile water (100 mL) and the pH was adjusted to 5.7. Normal saline was used to make a suspension of the spores of the fungal strain for seeding. The fungal spores were adjusted to a turbidity of 0.5 McFarland standard. Agar media (20 mL) was poured into each petri dish. Then by using sterile cotton swab 0.1 ml of suspension is spreaded on to the agar plate and then the plates were allowed to dry for 30 min at room temperature. Then by using sterile agar borer of 6mm diameter the cavities were made on the agar plates and the test drug (50µg/mL) and standard Ketoconazole (10µg/mL) were incorporated into the well and then the plates were kept in a refrigerator for one hour for a period of pre incubation diffusion .Controls plates were prepared using DMSO at the same concentration as used with the test compounds. The petri dishes were prepared in triplicate and maintained at 28 °C for 3 to 4 days. The antifungal activity was determined by measuring the diameter of the inhibition zone. The results of antifungal studies are given in Table 2.

RESULTS AND DISCUSSION

The synthetic strategy developed to obtain the target compound 2-(5-substituted phenyl-1H-tetrazol-1-yl)pyridine was prepared by the reaction between aminopyridine and aroylchloride with shaking which undergoes nucleophilic addition to give an intermediate N-pyridyl-2-yl-formamide-benzene **(3a-3j)** in 80% yield. This on reaction with phosphorous pentachloride under reflux for 1h which undergoes halogenation to give N-pyridyl-2-yl imidoformylchloride benzene (**4a-j)** in 70% yield. Further reaction with sodium azide results in title compounds (**5a-j)** in 52-79% yield. The structures of all the newly synthesized compounds were confirmed by their elemental analysis, IR, ^{1}H and ^{13}C NMR and Mass spectral studies. All the newly synthesized compounds exhibited satisfactory spectral data consistent with their molecular structures.

The synthesized compounds were evaluated for their antimicrobial activity. The antibacterial activity of title compounds revealed that 2-(5-(4-chlorophenyl)-1H-tetrazol-1-yl)pyridine **(5d)**, 2-(5-(2,3-dichlorophenyl)-1H-tetrazol-1-yl)pyridine **(5h)** and 2-(5-(3-bromophenyl)-1H-tetrazol-1-yl)pyridine **(5g)** exhibited highest activity against *B.subtilis, S. aureus, E. coli* and *P. aeruginosa.* Compound 2-(5-(2-chlorophenyl)-1H-tetrazol-1-yl)pyridine (5c) having good antifungal effect against *A. fumigatus*



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

Scheme-1

Table 1: Physical Data of Synthesized Compounds

S. No.	Cpd code	Mol. Formula	m.pºC	Yield in %	R_f value
1	5a	$C_{12}H_9N_5$	108	61	0.66
2	5b	$C_{12}H_8N_6O_2$	156	52	0.50
3	5c	$C_{12}H_8N_5Cl$	140	75	0.67
4	5d	$C_{12}H_8N_5Cl$	145	67	0.60
5	5e	$C_{13}H_{11}N_5O$	118	64	0.49
6	5f	$C_{13}H_{11}N_5$	127	79	0.59
7	5g	$C_{12}H_8N_5Br$	110	68	0.75
8	5h	$C_{12}H_7N_5Cl_2$	160	59	0.63
9	5i	$C_{12}H_7N_7O_4$	172	64	0.65
10	5j	$C_{13}H_{11}N_5$	128	76	0.73

Table: 2 Antimicrobial Activities of the Compounds 5a-J

S. No.	Compound code	Zone of inhibition (in mm)					
		Antibacterial activity				Antifungal activity	
		P. aeruginosa	*E. coli*	*B. subtilis*	*S. aureus*	*A. fumigatus*	*C. albicans*
1	5a	14	16	10	16	12	15
2	5b	16	14	18	18	17	14
3	5c	19	20	15	13	21	16
4	5d	24	21	19	16	13	11
5	5e	20	12	17	14	16	17
6	5f	17	14	18	17	15	12
7	5g	13	12	20	12	18	15
8	5h	23	19	12	14	20	13
9	5i	16	15	14	17	14	14
10	5j	20	17	16	15	19	22
Amoxycillin (30 µg/ml)		28	25	27	26	-	-
Ketoconazole (10µg/ml)		-	-	-	-	23	24

CONCLUSION

In conclusion a new series of substituted aryl tetrazole derivatives 5a-j has been synthesized and evaluated for their anti microbial activities. Most of the new compounds showed appreciable activity. Among them the compounds **5d, 5h, 5g** and **5c** were having a very good antimicrobial activity.

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