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Research Article

SYNTHESIS, ANTIMICROBIAL AND ANTI-INFLAMMATORY ACTIVITIES OF 3-(1-SUBSTITUTED PHENYL-1H-TETRAZOL-5-YL)PYRIDINE DERIVATIVES

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ABSTRACT

A number of 3-(1-substituted phenyl-1H-tetrazol-5-yl) pyridine derivatives were synthesized by the reaction of nicotinic acid with different types of primary amines followed by the treatment with sodium azide. The structure of the newly synthesized compounds has been established on the basis of IR, ^{1}H and ^{13}C NMR, mass spectra and elemental analysis. Investigation of *invitro* anti-bacterial and anti-fungal activity of synthesized compounds was done by cup plate method against *P. aeruginosa, E. coli, B. subtilis, S. aureus, A. fumigatus* and *C. albicans.*. All the compound exhibited moderate to good anti-bacterial and anti-fungal activity. The synthesized compounds were screened for antiinflammatory activity by carragenan induced paw edema method using Diclofenac sodium as standard drug. All the compounds of the series exhibited 22–70% protection against carageenin induced edema in the tested animals.

Keywords: Tetrazole, pyridine, antimicrobial, anti-inflammatory.

INTRODUCTION

The synthesis of heterocyclic rings containing nitrogen atoms became of great importance in medicinal chemistry. Increasing attention has been paid over the past two decades to the chemistry of tetrazoles and tetrazole derivatives. The first tetrazole was prepared in 1885 by the Swedish chemist, J. A. Bladin[1]. Tetrazoles are regarded as a biological equivalent for carboxylic acid group[2]. A close similarity between the acidic character of the tetrazole group and carboxylic acid group have inspired medicinal chemists to synthesize substituted tetrazoles as potential medicinal agents. The major area of interest has been the application of tetrazoles in pharmacological compounds with glycosidase inhibitory[3], antihypertensive[4], antiinflammatory[5], antibacterial[6], antifungal[7], analgesic[8], antinociceptive[9], anticancer[10], anticonvlsant[11], antidiabetic[12], antiulcer1[3] and antitubercular[14] activities. In addition Pyridine derivatives are reported to possess anticonvulsant[15], antiviral[16], antimicrobial[17], antitumor[18] and antiinflammatory[19] activities. After the extensive literature search, it was observed that tetrazole and pyridine are the important pharmacophore and an effort have been made to combine these two moieties as a single molecular scaffold in the present work. So, our object was to synthesize a series of new compounds incorporating these two moieties and explore the possibility of anti inflammatory and antimicrobial activity in the present nucleus.

Table 1: Physical Data Of Synthesized Compounds 5a-5j

Cpd code	Mol. Formula	m.pºC	Yield in %	R_f value
5a	$C_{12}H_9N_5$	136	62	0.64
5b	$C_{12}H_8BrN_5$	134	58	0.69
5c	$C_{12}H_8ClN_5$	142	74	0.61
5d	$C_{12}H_8N_6O_2$	146	69	0.67
5e	$C_{12}H_8N_6O_2$	152	60	0.68
5f	$C_{12}H_8ClN_5$	164	65	0.56
5g	$C_{13}H_{11}N_5$	128	71	0.72
5h	$C_{13}H_{11}N_5O$	170	64	0.65
5i	$C_{13}H_{11}N_5O$	158	67	0.63
5j	$C_{12}H_7Cl_2N_5$	178	63	0.58

MATERIALS AND METHODS

All protocols of animal experiments have been approved by the Institutional Animal Ethics Committee (IAEC). All the reagents were of Analytical grade. The melting points of compounds were determined by open capillary tubes and are uncorrected. Completion of the reaction was monitored by thin layer chromatography on perforated sheets of silica gel-G using iodine vapour for detection. The synthetic pathway is enumerated in the scheme and the physical data is given in Table 1. IR spectra was recorded on a Perkin Elmer 1600 FT spectrometer (ν_{max} cm^{-1}), ^{1}H and ^{13}C NMR spectra on JEOL GSX 400 spectrometer using TMS as an internal standard (chemical shifts in δ, ppm) and Mass spectra on a JEOL MSMATE spectrometer. Elemental analysis for C, H and N were performed and were found to be within 0-4% of the theoretical values.

Synthesis of 3-benzamidopyridine (3a-j)

Equimolar amount of nicotinic acid (1) and aromatic primary amine (2a-j) was mixed with constant shaking and allowed to stand at room temperature for 2 hours. The crude product that separated out on dilution was filtered and recrystallised from ethanol.

Synthesis of 3-imidoylchloride pyridine (4a-j)

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

Synthesis of 3-(1-substituted phenyl-1H-tetrazol-yl)pyridine (5a-j)

The residual imidoyl chloride (4a-j) 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.

3-(1-phenyl-1H-tetrazol-5-yl)pyridine (5a)

IR (KBr): 3075 (Ar C-H str), 1591(Ar C=C str), 1158 (Tetrazole) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 7.30-7.39 (m, 5H, Ar-H), 7.4-8.81 (d, 4H, pyridine); ^{13}C-NMR (DMSO-d_6): δ 124.0, 128.5, 128.8, 129.9, 133.0, 134.1, 148.0, 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 64.53, H 4.04, N 31.36

3-(1-(4-bromophenyl)-1H-tetrazol-5-yl)pyridine (5b)

IR (KBr): 3070 (Ar C-H str), 1595(Ar C=C str), 1152 (Tetrazole), 570 (C-Br) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 7.21-7.43 (m, 4H, Ar-H), 7.45-8.80 (d, 4H, pyridine); ^{13}C-NMR (DMSO-d_6): δ 123.1, 124.0, 127.5, 131.7, 132.1, 133.0, 134.1, 148.0, 149.1; MS (relative intensity): m/z value 301.0 (M+1); Anal. Calcd. for $C_{12}H_8BrN_5$ % C 47.70 H 2.67, N 23.18; found C 47.71 H 2.65, N 23.16

3-(1-(2-chlorophenyl)-1H-tetrazol-5-yl)pyridine (5c)

IR (KBr): 3078 (Ar C-H str), 1597 (Ar C=C str), 1154 (Tetrazole), 770 (C-Cl) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 7.12-7.32 (m, 4H, Ar-H), 7.45-8.82

(d, 4H, pyridine); ^{13}C-NMR (DMSO-d_6): δ 124.0, 126.5, 128.9, 130.2, 131.3, 132.1, 133.0, 134.1, 143.2, 148.0, 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 55.91, H 3.12, N 27.16

3-(1-(2-nitrophenyl)-1H-tetrazol-5-yl)pyridine (5d)

IR (KBr): 3076 (Ar C-H str), 1592 (Ar C=C str), 1525 (NO_2), 1155 (Tetrazole) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 7.52-8.21 (m, 4H, Ar-H), 7.45-8.80 (d, 4H, pyridine); ^{13}C-NMR (DMSO-d_6): δ 121.1, 124.0, 129.7, 130.2, 133.0, 134.3, 134.9, 143.2, 148.0, 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 3.03, N 31.33; found C 53.71, H 3.02, N 31.31

3-(1-(4-nitrophenyl)-1H-tetrazol-5-yl)pyridine (5e)

IR (KBr): 3079 (Ar C-H str), 1590 (Ar C=C str), 1522 (NO_2), 1157 (Tetrazole) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 7.51-8.21 (m, 4H, Ar-H), 7.44-8.81 (d, 4H, pyridine); ^{13}C-NMR (DMSO-d_6): δ 121.1, 124.0, 129.7, 130.8, 133.0, 134.1, 134.6, 148.0, 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 53.70, H 3.02, N 31.32

3-(1-(4-chlorophenyl)-1H-tetrazol-5-yl)pyridine (5f)

IR (KBr): 3071 (Ar C-H str), 1593 (Ar C=C str), 1158 (Tetrazole), 770 (C-Cl) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 7.22-7.315 (m, 4H, Ar-H), 7.43-8.83 (d, 4H, pyridine); ^{13}C-NMR (DMSO-d_6): δ 124.0, 126.6, 128.7, 131.8, 133.0, 134.1, 134.6, 148.0, 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 3.11, N 27.15

3-(1-p-tolyl-1H-tetrazol-5-yl)pyridine (5g)

IR (KBr): 3075 (Ar C-H str), 1594 (Ar C=C str), 1157 (Tetrazole) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 2.35 (s, 3H, CH_3), 7.12-7.2 (m, 4H, Ar-H), 7.43-8.83 (d, 4H, pyridine); ^{13}C-NMR (DMSO-d_6): δ 24.3, 124.0, 125.6, 129.1, 129.8, 133.0, 134.1, 138.6, 148.0, 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 65.80, H 4.65, N 29.50

3-(1-(4-methoxyphenyl)-1H-tetrazol-5-yl)pyridine (5h)

IR (KBr): 3076 (Ar C-H str), 1592 (Ar C=C str), 1164 (OCH_3), 1154 (Tetrazole) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 3.72 (s, 3H, OCH_3), 6.82-7.2 (m, 4H, Ar-H), 7.43-8.81 (d, 4H, pyridine); ^{13}C-NMR (DMSO-d_6): δ 55.9, 114.3, 120.8, 124.0, 130.9, 133.0, 134.1, 138.6, 148.0, 149.1, 160.7; MS (relative intensity): m/z value 253.1 (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 4.36, N 27.62

3-(1-(2-methoxyphenyl)-1H-tetrazol-5-yl)pyridine (5i)

IR (KBr): 3078 (Ar C-H str), 1590 (Ar C=C str), 1165 (OCH_3), 1153 (Tetrazole) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 3.73 (s, 3H, OCH_3), 6.83-7.2 (m, 4H, Ar-H), 7.45-8.81 (d, 4H, pyridine); ^{13}C-NMR (DMSO-d_6): δ 55.9, 113.8, 114.3, 121.8, 124.0, 129.8, 130.9, 133.0, 134.1, 138.6, 148.0, 149.1, 154.2; MS (relative intensity): m/z value 253.1 (M+1); Anal. Calcd. for $C_{13}H_{11}N_5O$ % C 61.65, H 4.38, N 27.65; found C 61.64, H 4.37, N 27.63

3-(1-(2,3-chlorophenyl)-1H-tetrazol-5-yl)pyridine (5j)

IR (KBr): 3073 (Ar C-H str), 1591 (Ar C=C str), 1157 (Tetrazole), 773 (C-Cl) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 7.12-7.25 (m, 3H, Ar-H), 7.42-8.81 (d, 4H, pyridine); ^{13}C-NMR (DMSO-d_6): δ 124.0, 125.6, 128.3, 129.4, 130.3, 133.0, 133.4, 133.6, 134.1, 148.0, 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 49.32, H 2.40, N 23.95

Antibacterial activity

The synthesized compounds (**5a-5j**) 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[20]. 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.

Table 2: Antimicrobial Activities Of The Compounds 5a-5j

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	13	15	11	14	11	13
2	5b	18	16	13	12	17	19
3	5c	17	14	15	16	15	17
4	5d	14	12	15	13	12	11
5	5e	12	15	14	11	14	13
6	5f	15	14	19	18	16	15
7	5g	16	13	12	11	12	14
8	5h	13	10	11	12	11	13
9	5i	11	12	13	15	10	12
10	5j	22	21	16	17	18	16
	Amoxycillin (30 µg/ml)	28	25	27	26	-	-
	Ketoconazole (10µg/ml)	-	-	-	-	23	24

Antifungal activity

The newly synthesized compounds were also investigated for their antifungal activity[21] 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.

Anti-inflammatory activity

All the synthesized compounds were screened for *in vivo* anti-inflammatory activity by carrageenan induced paw edema test in

rats[22]. Diclofenac sodium (20 mg/kg) was administered as standard drug for comparison. Rats were divided in to 12 groups each group containing 6 rats. Group I was treated with tween-80 (1%) suspension which served by vehicle control. Group II to XI were treated with the suspension of the test compounds (**5a-j**) at a dose of 50 mg/kg. Group XII was administered with standard drug Diclofenac sodium. After 30 minutes, the animals were injected with 0.1 mL of carrageenan (1%w/v), in the sub planter region of left hind paw of rats. The paw volume was measured using the mercury displacement technique with the help of a plethysmometer after 2 h and 4 h of carrageenan injection. The formula used for calculating the percentage inhibition of edema is

% Inhibition = [1-(Vt/Vc)]*100

Where, Vt and Vc are the mean relative changes in the volume of paw edema in the test and control respectively. The results are analysed statistically by student "*t*" test and recorded in Table 3.

Table 3:Effect Of 5a-5j On Carrageenan Induced Paw Edema

Treatment	Mean paw edema volume (ml)		Percentage inhibition	
	2h	4h	2h	4h
Control (Tween-80, 1%)	0.78±0.02	0.84±0.01	-	-
5a	0.42±0.03	0.37±0.03	46.15	55.95
5b	0.45±0.02	0.40±0.02	42.31	52.38
5c	0.31±0.01**	0.25±0.01**	60.26	70.24
5d	0.35±0.02**	0.31±0.03**	55.13	63.10
5e	0.32±0.01**	0.28±0.01**	58.97	66.67
5f	0.39±0.02	0.34±0.02	50.0	59.52
5g	0.71±0.01	0.65±0.02	8.97	22.62
5h	0.69±0.03	0.63±0.02	11.54	25.0
5i	0.68±0.03	0.61±0.01	12.82	27.38
5j	0.54±0.02	0.49±0.01	30.77	41.67
Diclofenac sodium (20mg/kg)	0.24±0.03*	0.17±0.01*	69.23	79.76

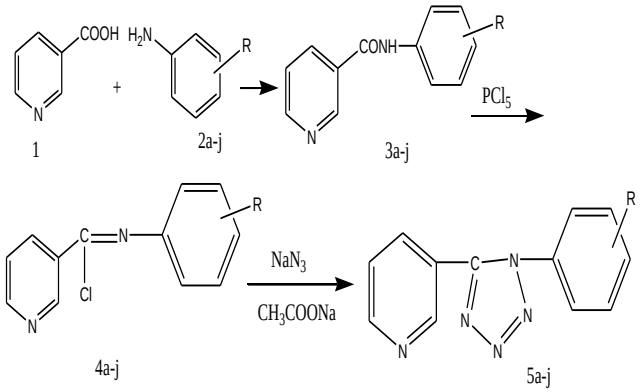
*** *P* < 0.001 and ** *P* < 0.01 represent significant difference when compared with control groups. Values are expressed in mean ± SEM (n = 6)**

Acute toxicity on oral administration in mice

Acute toxicity tests were performed according OECD guideline for testing of chemicals[23]. Male and female albino mice weighing 25-30 g were used for the study. Each group of 3 animals were fasted for 4 h, but allowed to free access to water throughout. The test compounds were administered orally in doses 5mg/kg-2000 mg/kg body weight by suspending in 1% C.M.C solution and were kept under observation for period of 24 hours. Mortality in each group was observed for 14 days.

RESULT AND DISCUSSION

The target compounds were prepared by using the reaction sequence in Scheme 1. Different tetrazole derivatives were synthesized by [3+2] cycloaddition of 3-imidoylchloride pyridine and sodium azide. All the compounds gave satisfactory elemental analysis (±0.4%). The chemical structures of the synthesized compounds were confirmed by means of their IR, ^{1}H, ^{13}C NMR and mass spectral analysis. All the synthesized compounds have exhibited significant activity against the bacteria and fungi tested. Compounds 3-(1-(2,3-chlorophenyl)-1H-tetrazol-5-yl)pyridine and 3-(1-(4-bromophenyl)-1H-tetrazol-5-yl)pyridine were having a very good antibacterial activity against *Pseudomonas aeruginosa* and *E.coli*. 3-(1-(4-bromophenyl)-1H-tetrazol-5-yl)pyridine showed good antifungal activity against *A. fumigates* and *C.albicans.* The synthesized compounds showed significant anti-inflammatory activity. It is apparent from Table 3 that compounds 5a-5j afforded 22–70% protection against carageenin induced edema, whereas the standard drug diclofenac sodium under similar conditions showed 79.76% inhibition. Among the compounds tested, compound 5c and 5e were found to be most potent compounds as they exhibited 70% and 66% inhibition, respectively. It was found that the introduction of CH_3 and OCH_3 groups at C-4 of phenyl ring caused marked decrease in the anti-inflammatory activity.



R= H, 4-Br, 2-Cl, 2-NO_2, 4-NO_2, 4-Cl, 4-CH_3, 4-OCH_3, 2-OCH_3, 2,3-Cl

Scheme I

CONCLUSION

We prepared a series of 3-(1-substituted phenyl-1H-tetrazol-yl)pyridine and demonstrated that these compounds possessed good antibacterial and antifungal acticity in cup plate method and anti-inflammatory activity tested by carrageenin induced paw edema method. The most promising compounds having anti bacterial and antifungal activity were 3-(1-(2,3-chlorophenyl)-1H-tetrazol-5-yl)pyridine and 3-(1-(4-bromophenyl)-1H-tetrazol-5-yl)pyridine and anti-inflammatory activity were 3-(1-(2-chlorophenyl)-1H-tetrazol-5-yl)pyridine and 3-(1-(4-nitrophenyl)-1H-tetrazol-5-yl)pyridine.

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