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

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ABSTRACT

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, 1H 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 *Staphylococcus* aureus 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 single molecular framework in order to prepare molecules having two active pharmacophores in a single molecular framework in order to prepare molecules having two active pharmacophores in order to prepare molecules having two active pharmacophores in order to prepare molecules having two active pharmacophores in order to prepare molecules having two active pharmacophores. In addition pyridines are associated with diverse biological activities. Inspired by the biological profile of tetrazole and pyridine on biological 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. 1H and 13C NMR spectra was recorded on a JEOL GXS 400 spectrometer using TMS as an internal standard (chemical shifts in δ, ppm). IR spectra on a Perkin Elmer 1600 FT spectrometer (νmax, cm⁻¹) and Mass spectra on a JEOL MS MAT 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 aryl 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 PCl₅ (0.004 mol) was heated at 100ºC for 1 hr. When the evolution of fumes of HCl was ceased excess of POCI₃ 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 (C–Cl) cm⁻¹; 1H-NMR (DMSO-d₆): δ 7.24-7.82 (m, 4H, Ar-H), 7.41-8.62 (d, 4H, pyridine); 13C-NMR (DMSO-d₆): δ 122.2, 123.9, 127.5, 128.9, 129.4, 130.2, 132.3, 136.0, 136.8, 140.4, 149.1; MS (relative intensity): m/z value 268.07 (M+1); Anal. Calcd. for C₁₂H₈ClN₅: % C 55.93, H 3.13, N 27.18; found  C 55.92, H 3.10, N 27.16.

2-(5-(4-chlorophenyl)-1H-tetrazol-1-yl)pyridine (5d): IR (KBr): 1590 (C=N), 1159 (C–Cl) cm⁻¹; 1H-NMR (DMSO-d₆): δ 7.29-7.82 (m, 4H, Ar-H), 7.41-8.62 (d, 4H, pyridine); 13C-NMR (DMSO-d₆): δ 121.6, 123.9, 128.4, 130.7, 136.0, 136.8, 140.4, 149.1; MS (relative intensity): m/z value 257.05 (M+1); Anal. Calcd. for C₁₂H₈ClN₅: % C 55.93, H 3.13, N 27.16; found C 55.90, H 3.13, N 30.30.

2-(5-(4-nitrophenyl-1H-tetrazol-1-yl)pyridine (5b): IR (KBr): 1590 (C=N), 1525 (NO₂) cm⁻¹; 1H-NMR (Tetrazole, 7.4-8.6 (d, 4H, pyridine); 13C-NMR (DMSO-d₆): δ 122.2, 123.9, 128.4, 130.7, 136.0, 136.8, 140.4, 149.1; MS (relative intensity): m/z value 268.07 (M+1); Anal. Calcd. for C₁₂H₈NO₃: % 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 (C–Cl) cm⁻¹; 1H-NMR (DMSO-d₆): δ 7.16-7.42 (m, 4H, Ar-H), 7.41-8.62 (d, 4H, pyridine); 13C-NMR (DMSO-d₆): 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 C₁₂H₈ClN₅: % 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≡C) cm⁻¹; 1H-NMR (DMSO-d₆): δ 7.33-7.42 (m, 4H, Ar-H), 7.41-8.63 (d, 4H, pyridine); 13C-NMR (DMSO-d₆): δ 122.2, 123.9, 128.8, 129.4, 134.3, 136.0, 140.4, 149.1; MS (relative intensity): m/z value 257.05 (M+1); Anal. Calcd. for C₁₂H₈ClN₅: % C 55.93, H 3.13, N 27.18; found C 55.92, H 3.10, N 30.17.

2-(5-(4-methoxyphenyl)-1H-tetrazol-1-yl)pyridine (5e): IR (KBr): 1603 (C≡N), 1165 (OCHO), 1154 (Tetrazole) cm⁻¹; 1H-NMR (DMSO-d₆): δ 6.83-7.37 (m, 4H, Ar-H), 7.41-8.62 (d, 4H, pyridine), 3.73 (3H, OCHO); 13C-NMR (DMSO-d₆): 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 C12H8BrN5 % C 47.70, H 2.67, N 23.18; found C 47.83, H 2.66, N 23.21

IR (KBr): 1619 (C=N), 1158 (Tetrazole), 770 (C-Cl) cm-1; 1H-NMR (DMSO-d6): δ 118.7, 122.2, 123.9, 127.4, 128.2, 132.5, 136.0, 140.4, 149.8; MS (relative intensity): m/z value 301.00 (M+1);

The newly synthesized compounds were also investigated for their antifungal activity 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 amionopyridine and aroyl chloride with shaking which undergoes nucleophilic addition to give an intermediate N-pyrindyl-2-yl-formamidine benzene (3a-3j) in 80% yield. This 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, 1H and 13C 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*. The plates were made in triplicate. Solvent and growth controls were made separately. Then the plates were incubated at 37°C for 24 hours. After incubation zone of inhibition were recorded and tabulate in Table 2.

**Antifungal activity**

The tetrazole derivatives (5a-j) were investigated for their inhibition of growth against *Staphylococcus aureus* (ATCC-25923), *Escherichia coli* (ATCC-25922), *B.subtilis*, *S. aureus*, *E. coli* and *P. aeruginosa*. Activity against *Staphylococcus aureus* and *Escherichia coli* was 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.

**Scheme-1**
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 5e were having a very good antimicrobial activity.

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REFERENCES