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Simple and efficient method for the preparation of novel tetrazole derivatives spectral characterization and its antibacterial activities

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ABSTRACT

Novel tetrazole analogues with bio-active cores have been synthesized by a simple synthetic methodology. The synthesized compounds are characterized using FT-IR, MS, ^{1}H, ^{13}C and HSQC NMR spectroscopic techniques. The synthesized compounds also screened for their antibacterial activities against Staphylococcus aureus, Escherichia coli, Vibreo cholerae, Pseudomonas aeruginosa and Klebsiella pneumonia. The synthesized compounds are showing very good activities against all the tested bacterial strains.

Key words: Tetrazole, HSQC analysis, Acetic acid, Antibacterial activity.

INTRODUCTION

5-Substituted-1,2,3,4-tetrazoles are reported to possess antibacterial[1], antifungal[2], antiviral[3-5], analgesic[6,7], antiinflammatory[8-10], antiulcer[11] and antihypertensive[12] activities. The tetrazole function is metabolically stable and 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. A series of novel 5-Phenyl-1-acyl-1,2,3,4-tetrazoles have been synthesized[13] via condensation of 5-Phenyl-1,2,3,4-tetrazoles with various acylating reagents.

The tetrazole moiety exhibits a wide and growing number of applications. This nitrogen-rich ring system is used in propellants[14], explosives[15], and pharmaceuticals.[16] Although syntheses of tetrazoles have been reported since the mid-century, there is still a dearth of efficient processes. The [3+2] cycloaddition between hydrazoic acid and cyanide derivatives is a well known synthetic route.[17] Unfortunately, hydrazoic acid is highly explosive. Practically, the use of sodium azide as substrate in place of the hydrazoic acid would be convenient; however, the [3+2] cycloaddition energy barrier is significantly lower with hydrazoic acid than with azide ion. To overcome this energy limitation, syntheses have been designed either to control the hydrazoic acid formation[18] or to use a large excess of azide ions in the presence of metal catalysts[19] or strong Lewis Acids.[20] Overall, these procedures are less than desirable due to the long reactions times, high temperatures, low yields, or non-recoverable catalysts. Also, the use of sensitive catalysts and excess amounts of sodium azide enhance the difficulty and inconvenience of these processes. It has been reported that tetrazoles cover a wider spectrum of bioactive heterocycles with azapyrrole system.[21-23] These compounds have been used for both biological and non-biological applications.[24-26] Among several types of tetrazoles, 5-Amino-1-substituted derivatives have been reported as antibacterial, antiviral, anti-inflammatory and antiallergic agents.[27-28]

The present invention provides a simple synthetic method for the synthesis of tetrazoles by the reaction of amines with sodium azide and triethyl orthoformate in acidic medium.

MATERIALS AND METHODS

The products are analyzed by infra-red spectra, which are recorded on a Thermo Nicolet-Avatar-330 FT-IR spectrophotometer using KBr pellets and noteworthy absorption values (cm^{-1}) are obtained. Mass spectrum was recorded on APPLIED BIO-SYSTEM Mass Spectrometer using Electron Spray Ionization technique. The sample was prepared by dissolving about 2mg of compound in 5mL of HPLC grade methanol. The ^{1}H and ^{13}C NMR spectra are recorded at 293K on BRUKER AMX-400 Spectrometer operating with the frequencies of 400 MHz and 100 MHz respectively using DMSO-d_6 as solvent. Samples are prepared by dissolving about 5 mg of sample in 0.5mL of DMSO-d_6. All the chemical shift values are referenced to TMS.

Preparation of 1-{4-[(Pyrrolidin-1-ylsulfonyl)methyl]phenyl}-1*H*-tetrazole (1)
0.01mole of 4-[(Pyrrolidin-1-ylsulfonyl)methyl]aniline was dissolved in 25 ml of glacial acetic acid or formic acid and to that 0.01mole of sodium azide and 0.012mole of triethyl orthoformate were added and the resultant reaction mixture was heated to 80°C for 5-6 hrs. The flow of the reaction was monitored by TLC. After the completion of the reaction, the reaction mixture was quenched with crushed ice and the solid thrown out was filtered, washed with water and dried under vacuum.

Preparation of N-Acetyl-4-(1*H*-tetrazol-1-yl)benzamide (2a)
0.01mole of 4-Aminobenzamide was dissolved in 25 ml of glacial acetic acid and to that 0.01mole of sodium azide and 0.012mole of triethyl orthoformate were added and the resultant reaction mixture was heated to 80°C for 5-6 hrs. The flow of the reaction was monitored by TLC. After the completion of the reaction, the reaction mixture was quenched with crushed ice and the solid thrown out was filtered, washed with water and dried under vacuum.

In the case of formic acid as solvent the reaction is not at all proceeded to give neither the tetrazole nor its acetyl derivative even after several trials with various reaction conditions.

***In vitro* antibacterial activity:**
Nutrient broth was used to cultivate bacteria. Agar media was prepared by adding 24% w/v agar in the nutrient broth for making agar slants. Bacteria were sub-cultured on the nutrient agar slants. The inoculum was prepared by transferring loop full of the corresponding organism from the stock culture into the sterile broth and incubated at 37°C for bacterial strains. 20mL of sterile nutrient agar media was added to each petri dish and 2mL of 24h broth culture of bacteria was then added to the respective plates and mixed thoroughly by rotatory motion of the plates. The respective test compounds were dissolved in water : DMSO (8:2) in the concentration of 10 mg/mL. This solution was maintained as a stock solution. The different concentrations (100, 200 and 500 ppm) were prepared from the stock solution. Sterile paper disc of 5mm diameter was saturated with these three different concentrations and such discs were placed in each seeded agar plates. The petri plates were incubated at 37°C and zone of inhibitions were measured excluding the diameter of the paper disc (5 mm). Control discs were performed with sterile water. The inhibitory activities were compared with the standard antibacterial drug Ciprofloxacin.

RESULTS AND DISCUSSION

General
The tetrazoles (**1, 2a)** are synthesized in excellent yields by the reaction of sodium azide and triethyl orthoformate with corresponding amines, viz., 1-{4-[(Pyrrolidin-1-ylsulfonyl)methyl]phenyl}-1*H*-tetrazole or N-Acetyl-4-(1H-tetrazol-1-yl)benzamide in acetic acid or formic acid **(Scheme-1)**. The solvent, reaction conditions, melting points and the yields are given in **Table-1**. The structures of the synthesized compounds **(1, 2a)** are confirmed by FT-IR, ^{1}H NMR, ^{13}C NMR, HSQC, Mass spectral studies.

FT-IR Analysis
In the IR spectrum of compound **1** 1-{4-[(Pyrrolidin-1-ylsulfonyl)methyl]phenyl}-1*H*-tetrazole, a strong absorption is observed at 3142cm^{-1} is due to the tetrazole ring C-H stretching frequency. The absorptions observed in the range of 2890-2978cm^{-1} are due to aliphatic C-H stretching frequency. A strong absorption observed at 1693cm^{-1} is due to C=N stretching frequency. The N-H stretching frequency observed at 3369cm^{-1}. The absence of NH_2 stretching frequency and the presence of C=N stretching frequency indicates the formation of the tetrazole ring and hence the product is 1-{4-[(Pyrrolidin-1-ylsulfonyl)methyl]phenyl}-1*H*-tetrazole.

In the IR spectrum of compound **2a** N-Acetyl-4-(1H-tetrazol-1-yl)benzamide, a strong absorption observed at 3179cm^{-1} is due to the tetrazole ring C-H stretching frequency. The absorptions observed at 2909cm^{-1} is due to aliphatic C-H stretching frequency. A strong absorption observed at 1622cm^{-1} is due to C=N stretching frequency. The N-H stretching frequency observed at 3368cm^{-1}. A strong absorption observed at 1659cm^{-1} is due to C=O

stretching frequency. The absence of NH_2 stretching frequency and the presence of C=N stretching frequency indicates the formation of the tetrazole ring and hence the product is N-Acetyl-4-(1H-tetrazol-1-yl)benzamide.

^{1}H NMR Analysis

In the ^{1}H-NMR spectrum of compound **1** 1-{4-[(Pyrrolidin-1-ylsulfonyl)methyl]phenyl}-1*H*-tetrazole, a singlet appeared at 4.30ppm is due to the methylene protons attached between SO_2 and phenyl ring of the C4 carbon. The H2 and H5 protons of the pyrrolidine ring appeared at 3.27ppm and H3 and H4 protons of the same ring are appeared at 1.90ppm. The other aromatic protons appeared in the range of 7.64-7.77ppm. A singlet at 9.04ppm is due to the H5 proton of the tetrazole ring.

In the ^{1}H-NMR spectrum of compound **2a** N-Acetyl-4-(1H-tetrazol-1-yl)benzamide, The tetrazole ring H5 proton appeared as a singlet at 10.19ppm and the signal appeared at 1.86ppm is assigned to the protons of the methyl group attached to the NH of the amide and the amide NH proton appeared at 7.61ppm, The other aromatic protons are appeared in the range of 8.02-8.20ppm.

^{13}C NMR Analysis

In the ^{13}C-NMR spectrum of compound **1** 1-{4-[(Pyrrolidin-1-ylsulfonyl)methyl]phenyl}-1*H*-tetrazole, the methylene carbon attached between the SO_2 and C4 carbon of the phenyl ring appeared at 55.41ppm. The carbon resonance at 48.28ppm and 25.99ppm are due to C2, C5 and C3, C4 carbons of the pyrorolidine ring. Other aromatic carbons are appeared in the range of 121.33-133.90ppm. The tetrazole ring C5 carbon appeared at 140.43ppm.

In the ^{13}C-NMR spectrum of compound **2a** 4 N-Acetyl-4-(1H-tetrazol-1-yl)benzamide, the carbonyl carbon attached between C4 carbon of the phenyl ring and NH appeared at 166.52ppm and the another carbonyl carbon attached between NH and methyl group appeared at 172.36ppm. The carbon resonance observed at21.63ppm is due to methyl carbon attached to the amide NH. The aromatic carbons are appeared in the range of 120.65-135.58ppm and the tetrazole C5 carbon appeared at 142.34ppm.

HSQC NMR Analysis

In the HSQC spectrum of compound **1** 1-{4-[(Pyrrolidin-1-ylsulfonyl)methyl]phenyl}-1*H*-tetrazole, the proton signal at 3.27ppm have correlation with the carbon signal at 48.28ppm which reveals that, the carbon signal at 48.28ppm is due to the C2 and C5 carbons of the pyrrolidine ring and the proton signals at 3.27ppm is due to the H2 and H5 protons of the same ring. The carbon resonance at 25.99ppm correlates with the proton signal at 1.90ppm and this correlation confirms that the carbon signal at 25.99ppm is due to the C3 and C4 carbons of the pyrrolidine ring and the proton signal at 1.90ppm is due to the H3 and H4 protons of the same ring. The proton signal at 4.30ppm correlates with the carbon signal at 55.41ppm and it reveals that the carbon resonance at 55.41ppm is due to the methylene carbon attached in between the aryl ring and the SO_2 group and the proton signal at 4.30ppm is due to its corresponding protons. The ^{13}C resonance at 140.43ppm correlates with the proton singlet signal at 9.04ppm, from this correlation the carbon signal at 140.43ppm is due to the C5 carbon of the tetrazole ring and proton singlet at 9.04ppm is due to the H5 proton of the tetrazole ring. All these entire correlations are shown in Table-2.

In the HSQC spectrum of compound **2a** N-Acetyl-4-(1H-tetrazol-1-yl)benzamide, it is seen that the carbon signal at 166.52 and 172.36ppm are have no correlation with any of the proton signal and hence it is due to the carbonyl carbons of the amide NH and the proton signal at 7.61ppm have no correlation with any carbon signal hence it is due to the amide NH proton. The ^{13}C resonance at 142.34ppm correlates with the proton singlet signal at 10.19ppm, from this correlation the carbon signal at 142.34ppm is due to the C5 carbon of the tetrazole ring and proton singlet at 10.19ppm is due to the H5 proton of the tetrazole ring. A singlet proton signal appeared at 1.86ppm is correlated with the carbon signal at 21.63ppm this correlation confirms that the proton signal at 1.86ppm is due to the methyl proton and the carbon signal at21.63 is due the methyl carbon. All these entire correlations are shown in **Table-3**.

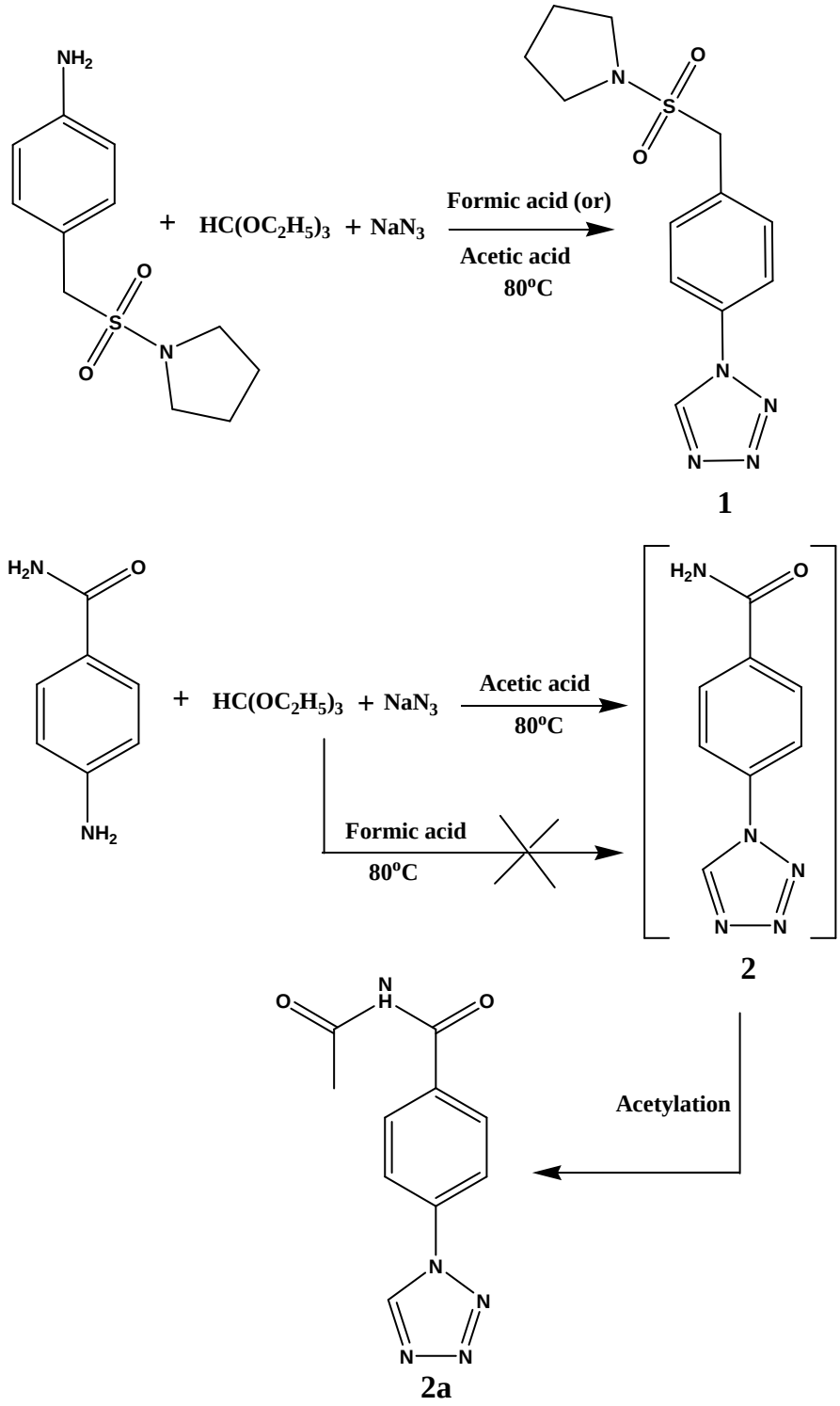
Mass Spectral Analysis

Mass spectrum of compound **1** shows molecular ion peak at *m/z* 294 ($M+H^{+\cdot}$) by the addition of one proton which is consistent with the proposed molecular mass (293) of compound. Mass spectrum of compound **2a** shows molecular ion peak at *m/z* 188 by loss of amide acetyl group which is consistent with the proposed molecular mass (231) of compound.

Anti Bacterial Activity

The synthesized compounds are screened for their antibacterial activities in three different concentrations namely 100ppm, 200ppm and 500ppm against the clinically isolated bacterial strains, viz., *Staphylococcus aureus, Escherichia coli, Vibreo cholerae, Pseudomonas aeruginosa* and *Klebsiella pneumonia*. The compound **1** shows higher activity against *Escherichia coli* and it is shows strong active against the other bacterial strains. Similarly the

compound **2a** shows a good activity against *Pseudomonas aeruginosa Staphylococcus aureus* and *Klebsiella pneumonia* and it is *moderately* active against *Escherichia coli*, and *Vibreo cholerae*. Both the synthesized compounds show a very good activity profile against all the tested bacterial strains which are shown in **Table-4**.



Scheme-1: Synthetic route for the synthesis of novel Tetrazole derivatives

Table-1: Physical properties of compounds 1 & 2a

Compound	Solvent	Reaction conditions		M.p. (^{0}C)	Yield (%)
		Temperature (°C)	Time (hrs)		
1	Acetic acid	80	6	155-157	74
	Formic acid	80	5	154-156	71
2a	Acetic acid	80	6	137-139	81
	Formic acid	80	>24	-	-

Table-2: HSQC Correlations of compound 1

HSQC	**Carbon**	**Aromatic Carbons**	**Tetrazole C5 carbon**	**Methylene carbon attached between SO_2 and phenyl ring**	**C2 and C5 carbons of the pyrrolidine ring**	**C3 and C4 carbons of the pyrrolidine ring**
Proton	**ppm**	121.33	140.43	55.41	48.28	25.99
Aromatic Protons	7.64-7.77	**Bonded**				
Tetrazole H5 proton	9.04		**Bonded**			
Methylene protons attached between SO_2 and phenyl ring	4.03			**Bonded**		
H2 and H5 protons of the pyrrolidine Ring	3.23				**Bonded**	
H3 and H4 protons of the pyrrolidine Ring	1.90					**Bonded**

Table-3: HSQC Correlations of compound 2a

HSQC	**Carbon**	**Aromatic Carbons**	**Tetrazole C5 carbon**	**Methyl carbon**	**Carbonyl carbon between phenyl ring and NH**	**Carbonyl carbon between methyl group and NH**
Proton	**ppm**	120.65-135.58	142.34	21.63	166.52	172.36
Aromatic Protons	8.02-8.20	**Bonded**				
Tetrazole H5 proton	10.19		**Bonded**			
Methyl Protons	1.86			**Bonded**		
Amide NH proton	7.61					

Table-4: *In vitro* antibacterial activities of compounds 1 and 2a by disc diffusion method

Microorganisms	**Compound 1**			**Compound 2a**		
	100 ppm	**200 ppm**	**500 ppm**	**100 ppm**	**200 ppm**	**500 ppm**
Staphylococcus aureus	++	+++	+++	+	++	+++
Escherichia coli	+	+++	++++	+	++	++
Vibreo cholerae	+	++	+++	+	++	++
Pseudomonas aeruginosa	+	+	+++	++	+++	+++
Klebsiella pneumonia	++	++	+++	++	++	+++

(-) = inactive, (+) = weakly active(12-16mm), (+)(+) = moderately active(17-21 mm), (+)(+)(+) = strong active(22-29mm), (+)(+)(+)(+) = highly active(30-33mm).

The commercial antibacterial drug, ciprofloxacin is used as a reference standard.

CONCLUSION

The novel bioactive tetrazole analogues have been synthesized by a simple and less hazardous synthetic method. The synthesized compounds are characterized using FT-IR, MS, ^{1}H, ^{13}C and HSQC NMR spectroscopic techniques. The synthesized compounds are also screened for their antibacterial activities against the clinically isolated bacterial strains, viz., *Staphylococcus aureus*, *Escherichia coli*, *Vibreo cholerae*, *Pseudomonas aeruginosa* and *Klebsiella pneumonia*. These synthesized compounds are showing very good activities against all the tested bacterial strains.

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