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RESEARCH ARTICLE

Benign by Design Synthesis of Novel 1*H*-Tetrazoles: Spectral Characterization and Antibacterial Activities

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Abstract: New tetrazole analogues with bio-active cores have been synthesized by a novel synthetic methodology. The synthesized compounds were characterized using FT-IR, MS, elemental analysis, ^{1}H, ^{13}C and HSQC NMR spectroscopic techniques. The synthesized compounds were 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.

Keywords: ^{1}*H*-tetrazole, HSQC analysis, Antibacterial activity

Introduction

Sumatriptan is an anti-migraine drug that is chemically related to the endogenous neurotransmitter, serotonin (5-hydroxytryptamine, 5-HT), which is believed to be involved in the pathogenesis of migraine. In preclinical studies, sumatriptan selectively constricted large cranial (cerebral and dural) blood vessels, through activation of vascular 5-HT, receptors[1]. This action may be the basis of its therapeutic efficacy in migraine. However, there is also evidence that sumatriptan may also work by acting on presynaptic 5-HT, receptors on trigeminal sensory nerve terminals in the cranial vasculature, blocking neuropeptide release and inhibiting neurogenic inflammation[2]. The precursor for the preparation of sumatriptan is 1-[3-(2-aminoethyl)-1*H*-indol-5-yl]-*N*-methylmethanesulfonamide. zolmitriptan, an active pharmaceutical ingredient is also a selective serotonin receptor agonist of the 1B and 1D subtype. The precursor for the preparation of zolmitriptan is 4-(4-aminobenzyl)-1,3-oxazolidin-2-one.

5-Substituted 1,2,3,4-tetrazoles are reported to possess antibacterial[3], antifungal[4], antiviral[5-7], analgesic[8,9], antiinflammatory[10-12], antiulcer[13], andantihypertensive[14], activities. The tetrazole function is metabolically stable this feature 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[15] via condensation of 5-phenyl-1,2,3,4-tetrazoles with various acylating reagents. 5-Phenyl-1,2,3,4-tetrazoles was synthesized by the cycloaddition of benzonitrile with sodium azide and ammonium chloride in presence of dimethylformamide as solvent.

The tetrazole moiety exhibits a wide and growing number of applications. This nitrogen-rich ring system is used in propellants[16], explosives[17] and pharmaceuticals[18]. 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 and one of the most efficient routes[19]. 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[20] or to use a large excess of azide ions in the presence of metal catalysts[21] or strong Lewis Acids[22]. 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. Hence we developed a new synthetic method for the synthesis of tetrazoles by the reaction of amines with sodium azide and triethyl orthoformate.

It has been reported that tetrazoles cover a wider spectrum of bioactive heterocycles with azapyrrole system[16,23,24]. These compounds have been used for both biological and non-biological applications[25-27]. Among several types of tetrazoles, 5-amino -1-substituted derivatives have been reported as antibacterial, antiviral, anti-inflammatory and antiallergic agents[28-30].

Experimental

The products were analyzed by infra-red spectra, which were recorded on a thermo nicolet-avatar-330 FT-IR spectrophotometer using KBr pellets and noteworthy absorption values (cm^{-1}) were obtained. Mass spectrum was recorded on applied bio-system mass spectrometer using electron spray ionization technique. The sample was prepared by dissolving about 2 mg of compound in 5 mL of HPLC grade methanol. The ^{1}H and ^{13}C NMR spectra were recorded at 293 K on BRUKER AMX-400 spectrometer operating with the frequencies of 400 MHz and 100 MHz respectively using DMSO-d_6 as solvent. Samples were prepared by dissolving about 5 mg of sample in 0.5 mL of DMSO-d_6. All the chemical shift values are referenced to TMS.

*Synthesis of 1H-Tetrazole (**1** & **2**)*

0.01 Mole of appropriate amine was dissolved in 25 mL of glacial acetic acid or formic acid and to that 0.01 mole of sodium azide and 0.012 mole of triethyl orthoformate were added and the resultant reaction mixture was heated to 80 ^{o}C for 5-6 h. 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.

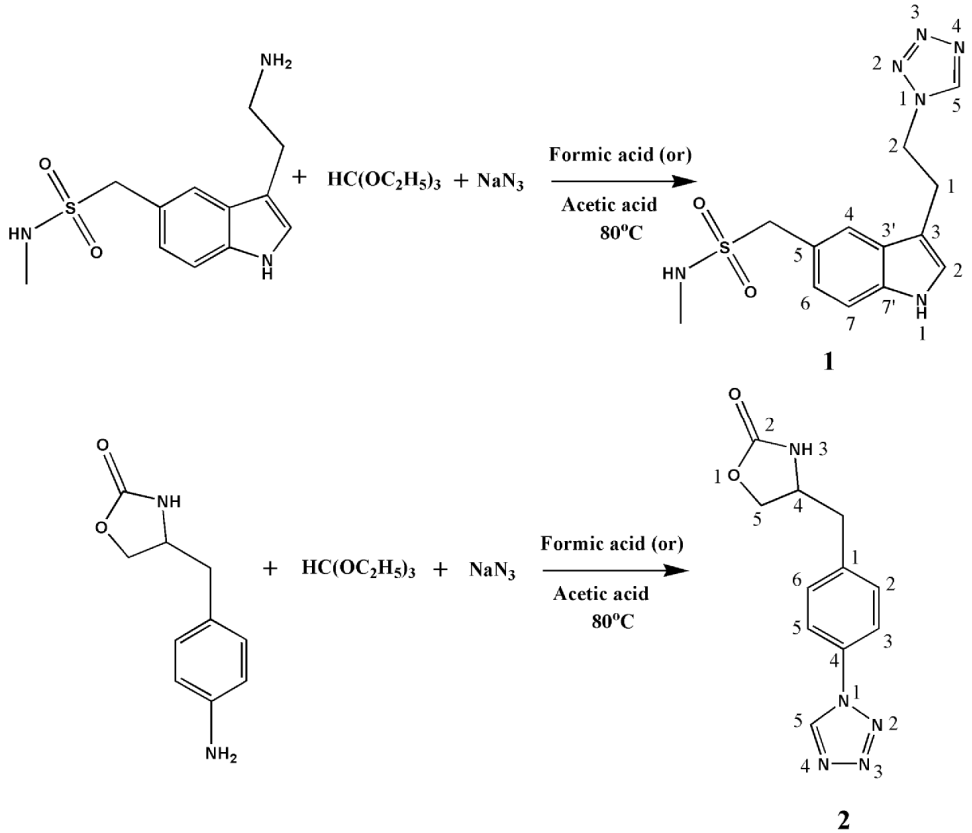
In vitro antibacterial activity

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. 20 mL of sterile nutrient agar media was added to each petri dish and 2 mL of 24 h 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 5 mm 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

The tetrazoles (**1, 2)** were synthesized in excellent yields by the reaction of sodium azide and triethyl orthoformate with corresponding amines, *viz*., 1-[3-(2-aminoethyl)-1*H*-indol-5-yl]-*N*-methylmethanesulfonamide or 4-(4-aminobenzyl)-1,3-oxazolidin-2-one 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, 2)** were confirmed by FT-IR, ^{1}H NMR, ^{13}C NMR, HSQC, Mass spectral studies and elemental analysis.



Scheme 1. Synthetic route for the synthesis of novel 1*H*-tetrazoles

Table 1. Physical properties of compounds **1** & **2**

Compd.	Solvent	Reaction conditions		M.P. ^{0}C	Yield %	Calculated				Experimental			
		Temperature, °C	Time, h			C_{cal}	H_{cal}	N_{cal}	S_{cal}	C_{exp}	H_{exp}	N_{exp}	S_{exp}
1	Acetic acid	80	6	170-172	74	48.74	5.03	26.23	10.01	48.70	5.00	26.18	9.98
	Formic acid	80	5	169-171	71					48.71	4.97	26.16	9.95
2	Acetic acid	80	6	143-145	81	53.87	4.52	28.56	-	53.81	4.45	28.53	-
	Formic acid	80	5	144-146	78					53.83	4.46	28.49	-

Table 2. HSQC correlations of compound **2**

HSQC	Carbon	Aromatic Carbons	C4 carbon of Oxazolidinone ring	C5 carbon of Oxazolidinone ring	C=O carbon of Oxazolidinone ring	-CH_2 carbon attached to Oxazolidinone ring	C5 carbon of Tetrazole ring
Proton	ppm	121.04-138.47	52.22	67.88	158.60	40.1	142.15
Aromatic protons	7.52-7.89	Bonded					
H4 proton of Oxazolidinone ring	4.13		Bonded				
H5 proton of Oxazolidinone ring	4.0 & 4.30			Bonded			
CH_2 protons attached to Oxazolidinone ring	2.89					Bonded	
H5 proton Of tetrazole ring	10.07						Bonded

FT-IR analysis

In the IR spectrum of compound **1** *N*-methyl-1-{3-[2-(1*H*-tetrazol-1-yl)ethyl]-1*H*-indol-5-yl}methanesulfonamide, a strong absorption is observed at 3142 cm^{-1} is due to the tetrazole ring C-H stretching frequency. The absorptions observed in the range of 2854-2924 cm^{-1} are due to aliphatic C-H stretching frequency. A strong absorption observed at 1648 cm^{-1} is due to C=N stretching frequency. The N-H stretching frequency observed at 3384 cm^{-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 *N*-methyl-1-{3-[2-(1*H*-tetrazol-1-yl)ethyl]-1*H*-indol-5-yl}methanesulfonamide.

In the IR spectrum of compound **2** 4-[4-(1*H*-tetrazol-1-yl)benzyl]-1,3-oxazolidin-2-one, a strong absorption observed at 3125 cm^{-1} is due to the tetrazole ring C-H stretching frequency. The absorptions observed in the range of 2849-1978 cm^{-1} are due to aliphatic C-H stretching frequencies. A strong absorption observed at 1682cm^{-1} is due to C=N stretching frequency. The N-H stretching frequency observed at 3302 cm^{-1}. A strong absorption observed at 1785 cm^{-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 4-[4-(1*H*-tetrazol-1-yl)benzyl]-1,3-oxazolidin-2-one.

^{1}H NMR analysis

In the ^{1}H NMR spectrum of compound **1** *N*-methyl-1-{3-[2-(1*H*-tetrazol-1-yl)ethyl]-1*H*-indol-5-yl}methanesulfonamide, a singlet appeared at 2.55 ppm is due to the methyl proton attached at the nitrogen of the sulfonamide. A singlet appeared at 4.34 ppm is due to the methylene protons attached to the SO_2 of the sulfonamide group. The H2 proton of the indole ring appeared at 7.53 ppm and the other aromatic protons appeared in the range of 7.00-7.34 ppm. The H1 proton of the ethyl chain appeared as a triplet centered at 3.30 ppm and a triplet centered at 4.75 ppm is due to the H2 proton of the ethyl chain. A singlet at 9.30 ppm is due to the H5 proton of the tetrazole ring.

In the ^{1}H-NMR spectrum of compound **2** 4-[4-(1*H*-tetrazol-1-yl)benzyl]-1,3-oxazolidin-2-one, the H5 axial and equatorial protons of the oxazolidinone ring appeared at 4.00 and 4.30 ppm. A multiplet centered at 4.13 is due to the H4 proton of the oxazolidinone ring. The tetrazole ring H5 proton appeared as a singlet at 10.07 ppm and the signal appeared at 2.89 ppm is assigned to the protons of the methylene group, which is attached between the aryl ring and the C4 carbon of the oxazolidinone ring. The other aromatic protons are appeared in the range of 7.52-7.89 ppm.

^{13}C NMR analysis

In the ^{13}C-NMR spectrum of compound **1** *N*-methyl-1-{3-[2-(1*H*-tetrazol-1-yl)ethyl]-1*H*-indol-5-yl}methanesulfonamide, the methyl carbon attached to the NH group of the sulfonamide appeared at 25.27 ppm and the methylene carbon attached to the SO_2 of the sulfonamide appeared at 56.37 ppm. The carbon resonance at 111.32 ppm and 109.36 ppm are due to C2 and C3 carbons of the indole ring. Other aromatic carbons are appeared in the range of 120.06-135.82 ppm. The C1 and C2 carbons of the ethyl group appeared at 28.93 ppm and 47.94 ppm respectively. The tetrazole ring C5 carbon appeared at 143.82 ppm. In the ^{13}C NMR spectrum of compound **2** 4-[4-(1*H*-tetrazol-1-yl)benzyl]-1,3-oxazolidin-2-one, the carbonyl carbon of the oxazolidinone ring appeared at 158.60 ppm. The carbon resonance observed at 52.22 ppm and 67.88 ppm are due to C4 and C5 carbons of the oxazolidinone ring. A carbon signal at 40.01 ppm is due to the methylene carbon attached in

between the aryl ring and the C4 carbon of the oxazolidinone ring. The aromatic carbons are appeared in the range of 121.04-138.47 ppm and the tetrazole C5 carbon appeared at 142.15 ppm.

HSQC NMR analysis

Compound **2** is further analyzed by HSQC analysis for the further confirmation of its structure. In the HSQC NMR spectrum of compound **2** it is seen that the carbon signal at 158.60 ppm have no correlation with any of the proton signal and hence it is due to the carbonyl carbon of the oxazolidinone ring. The proton signals at 4.00 ppm and 4.30 ppm have correlation with the carbon signal at 67.88 ppm which reveals that, the carbon signal at 67.88 ppm is due to the C5 carbon of the oxazolidinone ring and the proton signals at 4.00 ppm and 4.30 ppm are due to the H5 protons of the same ring. The carbon resonance at 52.22 ppm correlates with the proton signal centered at 4.13 ppm and this correlation confirms that the carbon signal at 52.22 ppm is due to the C4 carbon of the oxazolidionone ring and the proton signal at 4.13 ppm is due to the H4 proton of the same ring. The proton signal at 2.89 ppm correlates with the carbon signal at 40.01 ppm and it reveals that the carbon resonance at 40.01 ppm is due to the methylene carbon attached in between the aryl ring and the oxazolidinone ring and the proton signal at 2.89 ppm is due to its corresponding protons. The ^{13}C resonance at 142.15 ppm correlates with the proton singlet signal at 10.07 ppm, from this correlation the carbon signal at 142.15 ppm is due to the C5 carbon of the tetrazole ring and proton singlet at 10.07 ppm is due to the H5 proton of the tetrazole ring. All these entire correlations are shown in Table 2.

Mass spectral analysis

Mass spectrum of compound **1** shows molecular ion peak at *m/z* 321 ($M+H^{+\cdot}$) by the addition of one proton which is consistent with the proposed molecular mass (320) of compound. Mass spectrum of compound **2** shows molecular ion peak at *m/z* 246 ($M+H^{+\cdot}$) by the addition of one proton which is consistent with the proposed molecular mass (245) of compound.

Antibacterial activity

The synthesized compounds are screened for their antibacterial activities in three different concentrations namely 100 ppm, 200 ppm and 500 ppm against the clinically isolated bacterial strains, *viz*., *Staphylococcus aureus, Escherichia coli, Vibreo cholerae, Pseudomonas aeruginosa* and *Klebsiella pneumonia*. The compound **1** at 100 ppm concentration shows moderate activity only against *Staphylococcus aureus* and it is weakly active against the other bacterial strains. At 200 ppm concentration it shows strong inhibitory activity against *Staphylococcus aureus,* but it shows only a moderate activity against the other bacterial strains. At 500 ppm concentration the compound **1** is highly active against *Staphylococcus aureus* and *Escherichia coli* and it shows a strong inhibitory activity against *Vibreo cholerae, Pseudomonas aeruginosa* and *Klebsiella pneumonia.* Similarly the compound **2** at 100 ppm concentration shows a moderate activity against *Pseudomonas aeruginosa* and *Klebsiella pneumonia* but it is weakly active against *Staphylococcus aureus, Escherichia coli* and *Vibreo cholerae*. At 200 ppm concentration it shows a strong activity only against *Klebsiella pneumonia* and it shows only a moderate activity against the other bacterial strains. At 500ppm concentration the compound **2** is highly active against *Staphylococcus aureus* and *Klebsiella pneumonia* and it shows a strong inhibitory activity against *Escherichia coli, Vibreo cholerae* and *Pseudomonas aeruginosa*. Both the synthesized compounds show a very good activity profile against all the tested bacterial strains which are shown in Table 3.

Table 3. *In vitro* antibacterial activities of compounds 1 and 2 by disc diffusion method

Microorganisms	Compound 1			Compound 2		
	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 (12-16 mm), (+)(+) = moderately active (17-21 mm), (+)(+)(+) = strong active (22-29 mm), (+)(+)(+)(+) = highly active (30-33 mm). The commercial antibacterial drug, ciprofloxacin is used as a reference standard

Conclusion

The novel bioactive tetrazole analogues have been synthesized by a novel synthetic method. The synthesized compounds are characterized using FT-IR, MS, elemental analysis, ^{1}H, ^{13}C and HSQC NMR spectroscopic techniques. The synthesized compounds are 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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