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SYNTHESIS AND ANTIMICROBIAL EVALUATION OF 5-SUBSTITUTED 1(H)-TETRAZOLES

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ABSTRACT

An efficient and economical protocol for the synthesis of 5-substituted 1H-tetrazoles from various nitriles and sodium azide is described using ZnO as catalyst. A wide variety of aryl nitriles underwent [2+3] cycloaddition under mild reaction conditions to afford tetrazoles in moderate to excellent yields. All the synthesized compounds were screened for their antibacterial activities were tested against Bacillus subtilis (Gram-positive), Pseudomonas aeruginosa (Gramnegative) and Streptomyces species (Actinomycetes).

Keywords: Aryl nitriles, 5-substituted 1H tetrazoles.

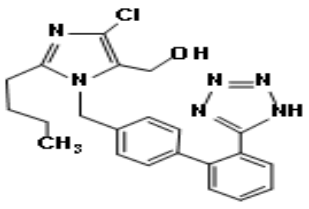
INTRODUCTION

Tetrazoles have been studied extensively since they were first described in 1885[1] and have been used in a variety of synthetic and medicinal chemistry applications.[2-4] Although many 5-substituted 1*H*-1,2,3,4-tetrazoles are known, only a few 1-substituted 1*H*-1,2,3,4-tetrazoles have been described. In 1947, Benson reported a review on tetrazole chemistry listing only seven examples of 1-substituted 1*H*-1, 2, 3, 4- tetrazoles including the questionable 1-hydroxy-1*H*-1, 2, 3, 4- tetrazole.

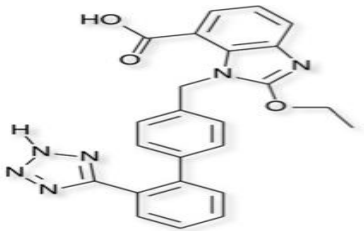
After that time, several methods for the preparation of 1-substituted 1*H*-1,2,3,4-tetrazoles were reported,[5] because of their wide utility.[6] These include the cyclization reaction of amines, or its hydrochloride salt, with an orthocarboxylic acid ester and a hydrazoic acid metal salt in the presence of acetic acid or trifluoroacetic acid. Unfortunately, all of these known methods suffered from some limitations, such as drastic reaction conditions, tedious work up procedures, the use of excessive amounts of glacial acetic acid or trifluoroacetic acid as the solvent, or even the need for excess amounts of dangerous and harmful hydrazoic acid. Therefore, it is desirable to develop a more efficient and convenient method for the synthesis of 1-substituted 1*H*-1, 2, 3, 4-tetrazoles.

Tetrazoles are a class of synthetic organic heterocyclic compound, consisting of a 5-member ring of four nitrogen and one carbon atom (plus hydrogens). The simplest is tetrazole itself, CN_4H_2. They are unknown in nature. There are several pharmaceutical agents which are tetrazoles, but they are generally undesirable due to safety concerns for process-scale synthesis; tetrazoles are usually explosive.

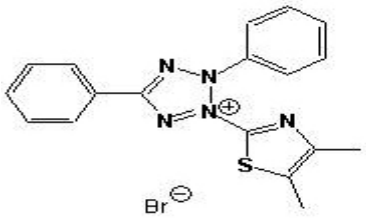
However, tetrazoles can act as a bioisostere for the carboxylate group, increasing their utility. Angiotensin II receptor blockers, in particular, often contain tetrazoles, such as Losartan[3], candesartan. A well-known tetrazole is MTT, which is a dimethyl thiazolyl diphenyl tetrazolium salt. This tetrazole is used in the MTT assay to quantify the respiratory activity of live cells in cell culture, although it generally kills the cells in the process.



LOSARTAN



CANDESARTAN



MTT

Other tetrazoles are used for their explosive or combustive properties, such as tetrazole itself and 5-aminotetrazole, which are sometimes used as a component of gas generators in automobile airbags[4] They produce high-temperature, non-toxic reaction products, and have a high burn rate and (relative) stability, all of which are desirable properties. Tetrazole was first prepared by the reaction of anhydrous hydrazoic acid and hydrogen cyanide under pressure.

MATRRIALS AND METHODS

All chemicals were purchased from Sigma-Aldrich and S.D Fine Chemicals, Pvt. Ltd. India and used as received. ACME silica gel (100–200 mesh) was used for column chromatography and thin-layer chromatography was performed on Merck-precoated silica gel 60-F_{254} plates. All the other chemicals and solvents were obtained from commercial sources and purified using standard methods.

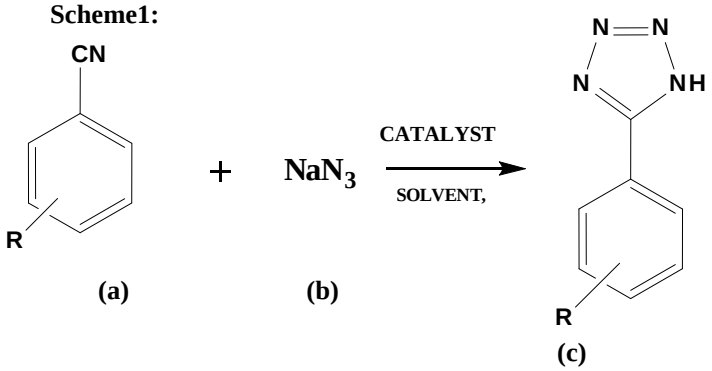
The IR spectra of all compounds were recorded on a Perkin-Elmer, Spectrum GX FTIR spectrometer. The IR values are reported in reciprocal centimeters (cm^{-1}). The ^{1}H, ^{13}C NMR spectra were recorded on a Varian- 400 MHz, Bruker-Avance 300 MHz Spectrometer. Chemical shifts (δ) are reported in ppm, using TMS (δ =0) as an internal standard in $CDCl_3$. ESI mass spectra were recorded on a Finnigan LCQ Advantagemax spectrometer. EI mass spectra were recorded on a GC-MS QP2010 Plus (Shimadzu).

Typical experimental procedure for the direct synthesis of 5-substituted 1(H) tetrazoles: The solution of (1 mmol) nitrile, Sodium azide (1.5 mmol) and Cu_2O (15 mol %) in dimethyl formamide (3ml) was stirred at 100 ^{o}C and monitored by TLC. After completion of the reaction, the reaction mixture was treated with HCl (5 n, 10 mL) and ethyl acetate with stirring. The organic layer was separated and the aqueous solution left behind extracted further with ethyl acetate (3 x 20 mL). The combined organic extracts were washed with water and concentrated to furnish the desired tetrazole. And the combined organics were dried over anhydrous Na_2SO_4, concentrated in vacuo and recrystallised from ethanol. All products were characterized by IR, ^{1}H NMR, ^{13}C NMR and mass spectroscopic techniques. Pure products will be examined for anti- microbial activity.

RESULTS AND DISCUSSION

Initially, we optimize an effective catalytic system for the synthesis of 5-substituted-1(H) tetrazoles from the reaction between nitriles and sodium azide. A range of reaction conditions were tested and some of the results are listed in table 1.

Scheme1:



(a) **(b)** **(c)**

(a): Nitriles
(b): Sodium Azide
(c): 5-substituted-1(H)-tetrazoles

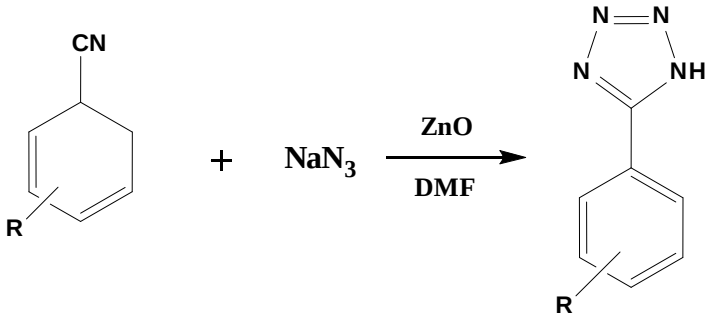
Initially we tried to find suitable reaction medium for the synthesis of of 5-substituted-1(H) tetrazoles from the reaction between nitriles and sodium azide. Different catalysts under varied reaction conditions were studied and the results are presented in table 1.

Among the several catalysts screened Cu_2O gave the product in low yield using H_2O as the solvent, where as with DMSO gave the product in high yield. $ZnBr_2$ gave the product in moderate yield using DMF as solvent. ZnO gave the product in low yield using EtOH and THF as solvents. Whereas ZnO gave the product in very high yield using DMF as solvent.

Having determined the optimum reaction conditions, we investigated the generality of this process. As can

be seen from table 2, a variety of aryl nitriles give corresponding products in moderate to excellent yields.

Scheme2:



(a) (b) (c)

(a): Nitriles
(b): Sodium Azide
(c): 5-substituted-1(H)-tetrazoles

Unsubstituted as well as benzonitriles with electron-donating substituents at both Para and Meta positions reacted well and gave the corresponding products in good to excellent yields. Different halogen substituted benzonitriles, such as 4-chlorobenzonitrile, 4-chlorobenzonitrile, 4-bromobenzonitrile and 4-bromobenzonitrile reacted smoothly and gave the desired products in decent yields Benzonitriles with electron-donating substituents at both Para and Meta positions reacted well and gave the corresponding products in moderate yield.

ANTIBACTERIAL ACTIVITY

The synthesized compounds were tested for their antimicrobial activity against three microorganisms, and the minimal inhibitory concentrations (MICs) of the tested compounds were determined by the dilution method.

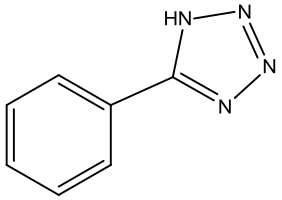
Bacterial strains were supplied, namely *Bacillus subtilis* (ATCC 6633) (Gram-positive), *Pseudomonas aeruginosa* (ATCC 27853) (Gram-negative) and *Streptomyces species* (Actinomycetes). The bacterial strains were maintained on MHA (Mueller - Hinton agar) medium (Oxoid, Chemical Co.) for 24 h at 37°C. The medium was molten on a water bath, inoculated with 0.5 mL of the culture of the specific microorganism, and poured into sterile Petri dishes to form a layer of about 3-4 mm. The layer was allowed to cool and harden. With the aid of cork-borer, cups of about 10 mm diameter were produced.

AGAR DIFFUSION TECHNIQUE

Antibacterial activities were tested against Bacillus subtilis (Gram-positive), Pseudomonas aeruginosa (Gramnegative) and Streptomyces species (Actinomycetes) using MH medium (17.5 g casein hydrolysate, 1.5 g soluble starch, 1000 mL beef extract) are listed in table 3. A stock solution of each synthesized compound (500 µg/mL) in DMSO was prepared and incorporated in sterilized liquid MH medium. Different concentrations of the test compounds in DMF were placed separately in cups in the agar medium. All plates were incubated at 37oC overnight. The inhibition zones were measured after 24 h. The minimum inhibitory concentration (MIC) was defined as the intercept of the grave of logarithm concentrations versus diameter of the inhibition zones .

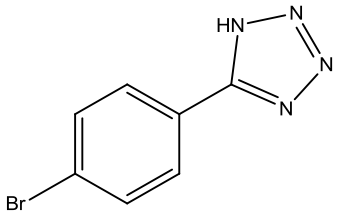
SPECTROSCOPIC DATA

5-phenyl tetrazole:



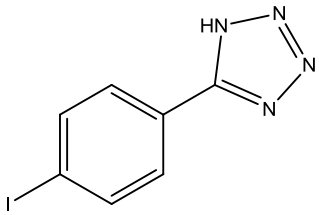
white solid, m.p. 215–216 °C. ^{1}H NMR (500 MHz, DMSO): δ = 7.57–7.63 (m, 3 H), 8.01–8.03 (m, 2 H) ppm.

5-(4-Bromophenyl)tetrazole:



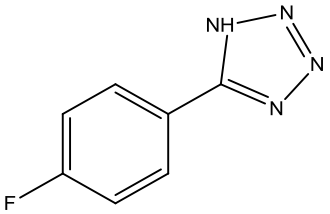
Off-white solid. ^{1}H NMR (500 MHz, DMSO): δ = 7.46 (t, J= 9.0 Hz, 2 H), 8.08–8.10 (m, 2 H) ppm.

5-(4-Iodophenyl)tetrazole:



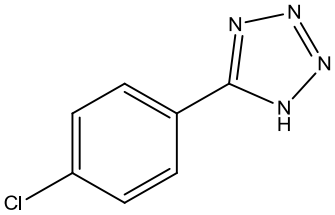
White solid.^{1}H NMR (500 MHz, DMSO): δ = 7.46 (t, J= 9.0 Hz, 2 H), 8.08–8.10 (m, 2 H) ppm.

5-(4-Fluorophenyl)tetrazole:



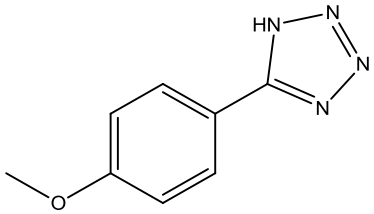
Off-white solid. ^{1}H NMR (500 MHz, DMSO): δ = 7.46 (t, J= 9.0 Hz, 2 H), 8.08–8.10 (m, 2 H) ppm.

5-(4-Chlororophenyl)tetrazole



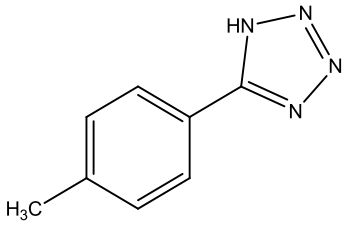
Off-white solid ^{1}H NMR (500 MHz, DMSO): δ = 7.46 (t, J= 9.0 Hz, 2 H), 8.08–8.10 (m, 2 H) ppm.

5-(4-Methoxyphenyl)tetrazole :



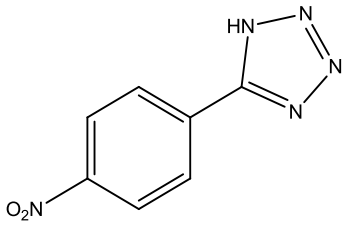
Off-white solid,. ^{1}H NMR (500 MHz, DMSO): *δ* = 3.82 (s, 3 H); 7.14 (d, *J* = 9.0 Hz, 2 H), 7.96 (d, *J* = 9.0 Hz, 2 H) ppm

5-(4-Methylphenyl)tetrazole :



Off-white solid,. ^{1}H NMR (500 MHz, DMSO): *δ* = 2.30 (s, 3 H); 7.14 (d, *J* = 9.0 Hz, 2 H), 7.96 (d, *J* = 9.0 Hz, 2 H) ppm

5-(4-Nitrophenyl)tetrazole:



Yellow solid, ^{1}H NMR (500 MHz, DMSO): δ = 8.28 (d, J =8.8 Hz, 2 H), 8.42 (d, J = 8.8 Hz, 2 H) ppm.

CONCLUSION

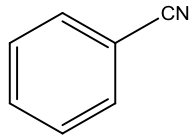
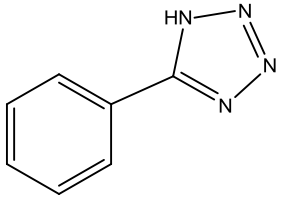
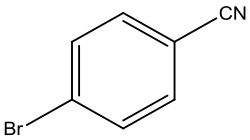
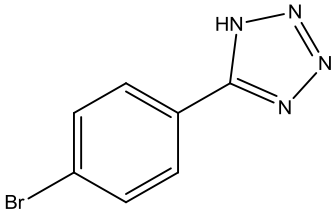
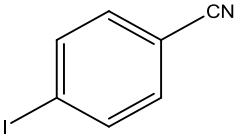
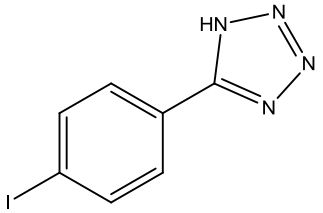
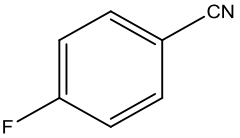
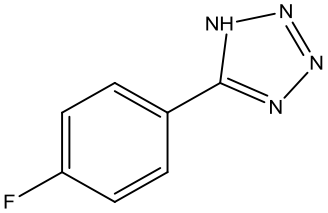
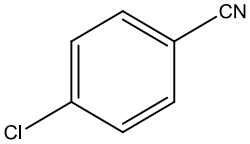
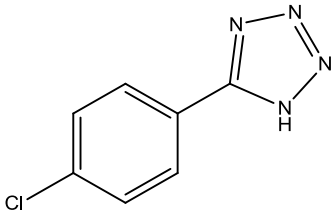
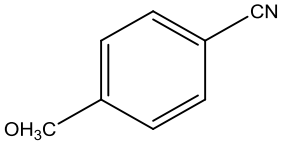
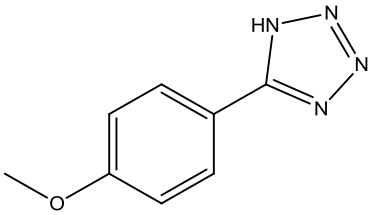
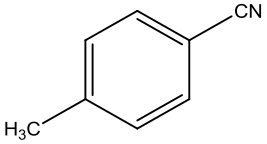
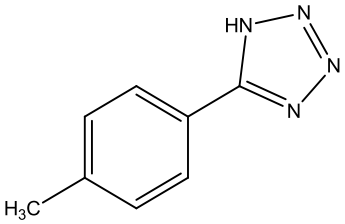
In conclusion we have developed a simple and efficient method for the synthesis of 5-substituted 1H-tetrazoles using ZnO as the catalyst under mild reaction conditions. This catalyst has been used to generate a diverse range of 5-substituted 1H-tetrazoles of using different nitriles in good to excellent yields. The antimicrobial screening suggests that all the synthesized compounds showed moderate to good activity against the tested organisms.

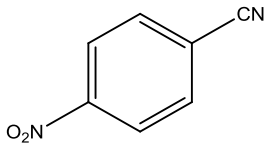
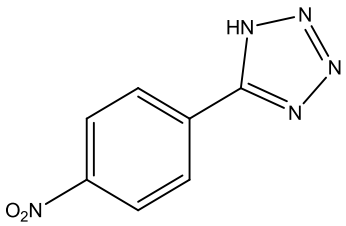
Table 1. Optimization of the reaction conditions for the synthesis of 5-substituted-1(H) tetrazoles from the reaction between nitriles and sodium azide

ENTRY	CATALYST	SOLVENT	TIME(hr)	TEMP(°C)	YIELD (%)
1	ZnO	DMF	12	100	92
2	Cu_2O	DMSO	12	100	85
3	$ZnBr_2$	DMF	12	100	78
4	ZnO	EtOH	12	100	56
5	ZnO	THF	12	100	45
6	Cu_2O	H_2O	12	100	35

Conditions: Catalyst (20 mol %), nitrile (1 mmol), sodium azide (1.5 mmol), Solvent (3ml). [b] Isolated yield.

TABLE 2: Preparation of 5-substituted 1H-tetrazoles from various nitriles and sodium azide is using ZnO as catalyst

S.NO	SUBSTRATE	PRODUCT	TIME	YIELD (%)
1.			12 hr	90
2.			12 hr	85
3.			12 hr	88
4.			12 hr	65
s.			12 hr	70
6.			12 hr	75
7.			12 hr	72

8.			12 hr	70

Conditions: (1 mmol) nitrile, Sodium azide (1.5 mmol) and ZnO (15 mol%) in dimethyl formamide (3 ml) was stirred at 100 ^{o}C [b] Isolated yield.

Table 3:Minimum inhibitory concentrations (MIC-µg/mol) of the title compounds:

Compound	Gram positive *Bacillus subtilis*	Gram negative *Pseudomonas aeruginosa*	Actinomycetes *Streptomyces Species*
1	125	75	100
2	75	100	75
3	75	100	75
4	100	100	125
5	125	125	100
6	75	100	75
7	75	100	75
8	75	100	75
Penicillin	31	45	34

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