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SYNTHESIS AND IN VITRO EVALUATION OF ANTIBACTERIAL ACTIVITY OF 1, 5-DISUBSTITUTED TETRAZOLES CATALYZED BY ZIRCONIUM OXYCHLORIDE

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ABSTRACT

Synthesis and Antibacterial Activity of 1, 5-disubstituted tetrazoles (1-(2-Fluorphenyl)-5-phenyl-1H-tetrazole, 1-(2-chlorophenyl)-5-phenyl-1H-tetrazole) in 87-88% yields using Zirconium Oxychloride in the presence of sodium azide and acetonitrile as solvent. With Zirconium Oxychloride and Sodium Azide was used as azide transfer reagent as it transformed the amide to imidoylazide intermediate and, then, by ring closing to tetrazole. The formation of hindered 1, 5-disubstituted tetrazoles was confirmed by1H-, 13C- and 19F-NMR, HRMS and FT-IR. A possible mechanism is described to clarify the effect of electron-withdrawing groups on anilines ring in the conversion to tetrazoles. In fact, substituent effect on nitrogen of amide group has key role in ring closing imidoylazide intermediate to tetrazoles.

Keywords: 1, 5-Disubstituted tetrazoles, Zirconium Oxychloride and Sodium Azide, Acetonitrile, N-benzoyl amide

INTRODUCTION:

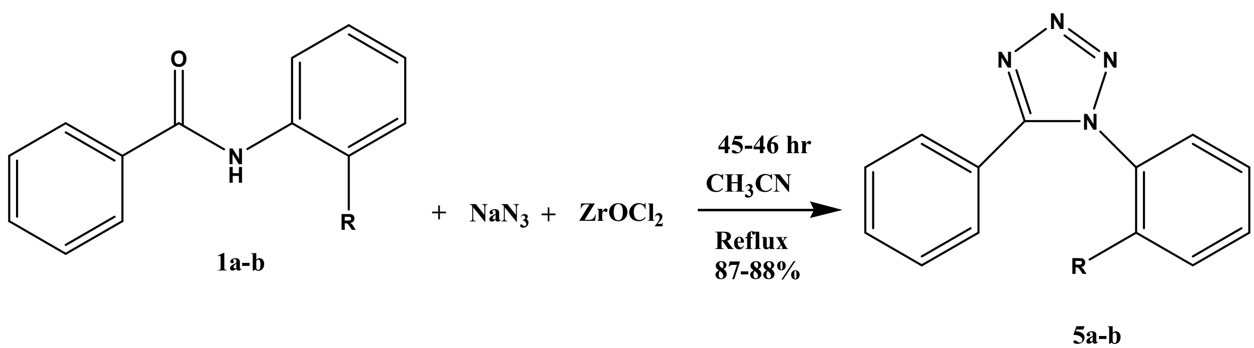
The tetrazoles important in medicinal, pharmaceutical research and pharmacological effectives. The class of tetrazole compounds has been recently used both as anticancer and antimicrobial agents **[1, 2]**. They have received increased attention due to their potential biological activity and industrial applications **[3, 4]**. Esikov and coworkers have synthesized the 1,5-disubstituted tetrazoles **[5, 6]**. They reported some intrinsic limitations to the azidating amides. Unlike N-acetyl derivatives of amino acids, N-benzoyl derivatives almost do not react with tetrachlorosilane sodium azide which has been attributed to steric effect of the benzoyl substituent. Duncia and coworkers reported synthesis of a sterically hindered ortho-tetrazole group by three different routes. However, their methods were only applied to prepare 5-substituted-1H-tetrazoles **[7]**. Katritzky and coworkers prepared 1,5-disubstituted tetrazoles with diverse substituents (aliphatic, aromatic or heteroaromatic) on imidoylbenzotriazoles precursor **[8]**. Imidoylbenzotriazoles were prepared from corresponding secondary carboxamides and benzotriazoles by two methods: oxalyl chloride and pyridine or thionyl chloride under microwave (80W/80ºC). However, these methods suffer from two disadvantages. Firstly, there is an added step in the preparation of imidoylbenzotriazoles using non-eco-friendly materials. Secondly, only para-substituted aromatic tetrazoles are obtained. Schroeder and coworkers reported improved conditions for converting sterically hindered amides into their corresponding 1,5-disubstituted tetrazoles in good yield **[9]**. The optimum reaction conditions were applied by using diisopropyl azodicarboxylate (DIAD), diphenylphosphoryl azide (DPPA), and diphenyl-2- pyridyl phosphine in THF at 45°C. However, it should be mentioned that expensive, toxic and non-eco-friendly materials were utilized in this method as well as applying just aliphatic amines. Here, we wish to report synthesis of sterically hindered 1, 5-disubstituted tetrazoles from the bulky secondary N-benzoyl amides in one step using silicon tetrachloride in the presence of sodium azide (triazidochlorosilane) according to procedure reported by Esikov and co-workers. This method has been overcome the above mentioned disadvantages with high yield as well. In fact, anilines containing electron-withdrawing groups in ortho position gave satisfactory results and anilines containing electronreleasing groups such as 2-methyl, 2-ethyl, 2-sec-butyl and 2-methoxy anilines were unsuccessful. Furthermore, we propose a reasonable mechanism of how substituent effects on ring closing of imidoylazide intermediates and converting them to tetrazole.

MATERIALS AND METHODS:

2-fluoro aniline, 2-chloro aniline and benzoyl chloride were used for amides preparation and purchased from Merck. Sodium Azide, Zirconium Oxychloride and acetonitrile were used for tetrazole preparation and purchased from Merck **[9]**. Ethyl acetate, acetonitrle and n-hexane were purchased from Merck and used as the organic solvents. Amides 2-fluoro aniline, 2-chloro aniline, as shown in Scheme 1, I prepared according to reported procedure by Ghosh and coworkers of benzoyl chloride and the corresponding aniline in solid-state **[10]**.

Table: The resulted products from reaction of bulky secondary amide With Zirconium Oxychloride and Sodium azide

Entry	Amide	Time (hr.)	Tetrazole	m.p. (°C)	Yield (%)
1	1a	43	1	105-106	87
2	1b	44	1	103-104	88



R=F
R=Cl

INSTRUMENTATION:

The obtained tetrazoles were characterized by ^{1}H-, ^{13}C- and ^{19}F-NMR spectra recorded on a Bruker Avanace DRX 500 (500 MHz) using the solvent signal as reference ($CDCl_3$). The FT-IR spectra were obtained on a Shimadzu-470 (potassium bromide tablet). The progress of the reaction and purity of the products were monitored by TLC on Kieselgel 60 F_{254} plates (Merck). The eluent user petroleum etherethyl acetate 95:5, spots were visualized by UV irradiation. Melting points were recorded by an Electro Thermal 9100 and were uncorrected. HRMS spectra were obtained on Q-TOF Micromass (Wakes Inc. UK).

PREPARATION OF 1,5-DISUBSTITUTED TETRAZOLE:

Tetrazoles were synthesized according to reported procedure by Esikov and co-workers. As typical procedure for 1-(2-Fluorphenyl)-5-phenyl-1H-tetrazole (1a) from amide a mixture of (4 mmol), sodium azide (8 mmol) and Zirconium Oxychloride (8 mmol) in dry acetonitrile (16 ml) were refluxed and stirred with exclusion of moisture **(Scheme 1)**. In order to determine the end of the reaction, TLC test was used to check the reaction every 6 hours. After each TLC test, 1 mmol sodium azide and 2 mmol Zirconium Oxychloride were added to the mixture of the reaction. The last TLC test showed the pure hindered 1, 5-disubstituted tetrazole clearly. After the completion of reaction, the mixture was poured into the saturated solution of Na_2CO_3 (pH ~ 7). Then the precipitate of silica was filtered. The pure products were obtained by extracting the mixture with ethyl acetate. The organic solvents (ethyl acetate and acetonitrile) were evaporated under the vacuum **[8]**. The final products were kept at room temperature for more characterization.

1-(2-Fluorphenyl)-5-phenyl-1H-tetrazole (5a):

1H-NMR spectrum of 5b (500 MHz, CDCl3), δ (ppm): 7.25 (t, J= 9.1 Hz, 1H), 7.35-7.37 (m, 3H), 7.45 (t, J= 7.5 Hz, 1H), 7.51 (td, J= 8.0 Hz, J= 1.5 Hz, 1H), 7.55 (d, J= 7.5 Hz, 2H), 7.56-7.61 (m, 2H). 13C-NMR spectrum of 5b (125 MHz, CDCl3), δc (ppm): 157.11, 155.09, 154.73, 132.94, 132.88, 131.47, 129.02, 128.31, 128.13, 125.47, 125.44, 123.33, 122.78, 122.67, 117.40 and 117.26, (77.34, 77.08 and 76.83 for solvent). 19F-NMR spectrum of 5b (470 MHz, CDCl3), δF (ppm): -120.48 (m). FT-IR (KBr) spectrum of 5b [11]: 1097 and 1268 (-CN4 tetrazole ring), 1116 and 1142 (tetrazole ring), 1235 (Ar-F), 1285 (N-N=N), 1320 (C=N tetrazole ring), 1448 (C-H), 1506 (N=N tetrazole ring), 1579 (-N=N-), 1609 (C=N), 3062 (Ar-CH)cm-1. Mass spectrum (HRMS) of 5b (ESI) m/z: 241.0909 (M+ + 1).

1-(2-chlorophenyl)-5-phenyl-1H-tetrazole (5b):

1-(2-chlorophenyl)-5-phenyl-1H-tetrazole (1b) from amide a mixture of (4 mmol), sodium azide (8 mmol) and Zirconium Oxychloride (8 mmol) in dry acetonitrile (16 ml) were refluxed and stirred with exclusion of moisture (Scheme 1). In order to determine the end of the reaction, TLC test was used to check the reaction every 6 hours. After each TLC test, 1 mmol sodium azide and 2 mmol Zirconium Oxychloride were added to the mixture of the reaction. The last TLC test showed the pure hindered 1, 5-disubstituted tetrazole clearly. After the completion of reaction, the mixture was poured into the saturated solution of Na_2CO_3 (pH ~ 7). Then the precipitate of silica was filtered. The pure products were obtained by extracting the mixture with ethyl acetate. The organic solvents (ethyl acetate and acetonitrile) were evaporated under the vacuum **[8]**. The final products were kept at room temperature for more characterization.

1H-NMR spectrum of 5c (500 MHz, CDCl3), δ (ppm): 7.36 (t, J= 7.7 Hz, 2H), 7.46 (t, J= 7.5 Hz, 1H), 7.46- 7.51 (m, 2H), 7.53 (d, J= 7.8 Hz, 2H), 7.57-7.59 (m, 2H). 13C-NMR spectrum of 5c (125 MHz, CDCl3), δc (ppm): 154.57, 132.57, 132.42, 131.62, 131.43, 131.03, 129.02, 128.95, 128.34, 128.11 and 123.35, (77.33, 77.07 and 76.82 for solvent). FT-IR (KBr) spectrum of 5c [11]: 776 (C-Cl), 1086 and 1270 (-CN4 tetrazole ring), 1100 and 1143 (tetrazole ring), 1284 (N-N=N), 1318 (C=N tetrazole ring), 1442 (C-H), 1488 (N=N tetrazole ring), 1579 (-N=N-), 1606 (C=N), 3071 (Ar-CH) cm-1. Mass spectrum (HRMS) of 5c (ESI) m/z: 257.0593 (M+ + 1)

ANTIBACTERIAL ACTIVITY:

Tetrazole Compounds 5(a-b) were screened for their antibacterial activity against Gram-positive bacteria viz. Bacillus subtilis (MTCC 441), Bacillus sphaericus (MTCC 11) and Staphylococcus aureus (MTCC 96), and Gram-negative bacteria viz. Pseudomonas aeruginosa (MTCC 741), Klobsinella aerogenes (MTCC 39) and Chromobacterium violaceum (MTCC 2656) by disc diffusion method (NCCLS, 1982). For the antibacterial assay standard inoculums (1-2 × 107 c.f.u/mL 0.5 Mc Farland standards) were introduced on to the surface of sterile agar plates, and a sterile glass spreader was used for even distribution of the inoculums. The discs measuring 6.26 mm in diameter were prepared from Whatman no.1 filter paper and sterilized by dry heat at 140 o C for 1 h. The sterile discs previously soaked in a known concentration of the test compounds were placed in nutrient agar medium. The plates were inverted and incubated for 24 h at 37^{O}C.

Table 1: Antibacterial activity of compounds 5(a-b)

Compound	zone inhibition at 50 µg/mL (mm)					
	B.subtilis	B.sphaericus	S.aureus	P.aeruginosa	K.aerogenes	C.violaceum
5a	13	23	16	16	18	22
5b	10	17	18	15	22	20
Ciprofloxacin	25	30	30	30	25	30

RESULTS AND DISCUSSION:

To confirm the formation of 1, 5-disubstituted tetrazoles and antibiotic activity, characterizations such as ^{1}H-, ^{13}C- and ^{19}F-NMR, HRMS and FT-IR were used. TLC was utilizes to monitor the progress of the reaction and purity of the products **[11]**. Melting point was used to verify the purity of the products. The reaction of secondary amide 1a, 1b with Zirconium Oxychloride and Sodium Azide is shown in Table. With Zirconium Oxychloride and Sodium Azide was used as azide transfer reagent. It transforms amides to nitriles or acid azides (imidoyazides) however ketones are transformed with rearrangement into their corresponding tetrazoles **[12]** and the spread of general synthetic achieves for chemo selective formation of tetrazole derivatives.

CONCLUSIONS:

Synthesis and Antibacterial Activity of 1,5-disubstituted tetrazoles from bulky secondary N-benzoyl amides in 87-88% yield by Esikov and co-workers method using Zirconium Oxychloride in the presence of sodium azide and acetonitrile as a solvent.

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