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# Synthesis and Antibiotic Activity of 1-(2-Fluorphenyl)-5-phenyl-1H-tetrazole and 1-(2-chlorophenyl)-5-phenyl-1H-tetrazole(1,5-disubstituted Tetrazoles) catalyzed by EuFOD

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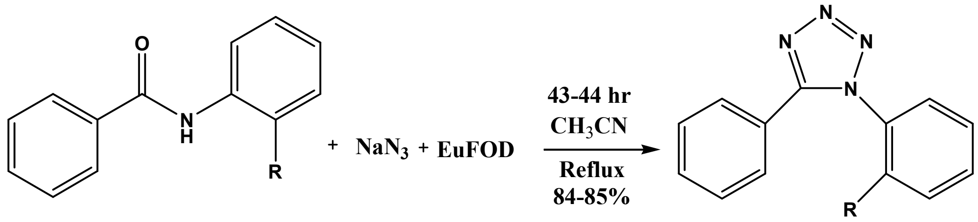
## ABSTRACT

Synthesis and Antibiotic Activity of 1, 5-disubstituted Tetrazoles in 84–85% yields using EuFOD in the presence of sodium azide and acetonitrile as solvent with EuFOD (Europium(3+)tris [(3Z)-6,6,7,7,8,8,8-heptafluoro-2,2-dimethyl-5-oxo-3-octen-3-olate]), Sodium Azide was used as an azide transfer reagent as it transformed the amide to an imidoyl azide intermediate and, then, by ring closing, to tetrazole. The formation of hindered 1, 5-disubstituted tetrazoles was confirmed by $^{1}$H-, $^{13}$C-, and $^{19}$F-NMR, HRMS, and FT-IR.



**Keywords: 1, 5-Disubstituted tetrazoles, EuFOD and Sodium Azide, Acetonitrile, N-benzoyl amide**

## INTRODUCTION:

A chemical molecule known as EuFOD (Europium(3+)tris [(3Z)-6,6,7,7,8,8,8-heptafluoro-2,2,5-octen-3-olate]) exists. Is the substance with the formula Eu(OCC(CH3)3CHCOC3F7)3, sometimes referred to as Eu(fod)3. This coordination molecule is commonly used as a shift reagent in NMR spectroscopy. It is the best lanthanide shift reagent on the market. Tetrazoles is essential for research in medicine and pharmacology. Recently, compounds in the tetrazole class have been used as antibacterial and anticancer agents **[1]**. Their possible biological activities and industrial uses have attracted increased interest **[2]**. Furthermore, the metabolically stable tetrazole fragment can be used to substitute the carboxy group and amide link in peptidomimetics molecules **[3]**. The first description of amino acid derivatives containing a 5-tetrazolyl substituent was given by McManus and Herbst **[4]**. Later, Zabrocki *et al*. proposed making peptidomimetics with cis-block peptide links by utilizing the 1,5-diyl fragment of tetrazole **[5]**. The increasing need for the synthesis of tetrazoles containing peptides and peptidomimetics has prompted a number of investigations aimed at developing effective protocols for the synthesis of amide derivatives possessing a tetrazoles moiety.

Esikov and associates are the ones who produced the 1,5-disubstituted tetrazoles **[6]**. They stated that there were some intrinsic restrictions on the azidating amides. Unlike N-acetyl derivatives of amino acids, N-benzoyl derivatives of amino acids hardly ever react with Eu(fod)3,sodium azide due to the steric influence of the benzoyl group. Duncia and colleagues reported the synthesis of an ortho-tetrazole group that was sterically inhibited using three different approaches. However, they only made 5-substituted-1H-tetrazoles **[7]** using their methods.

Katritzky and colleagues produced 1,5-disubstituted tetrazoles with different substituents (aliphatic, aromatic, or heteroaromatic) on the precursor of imidoylbenzotriazoles **[8]**.

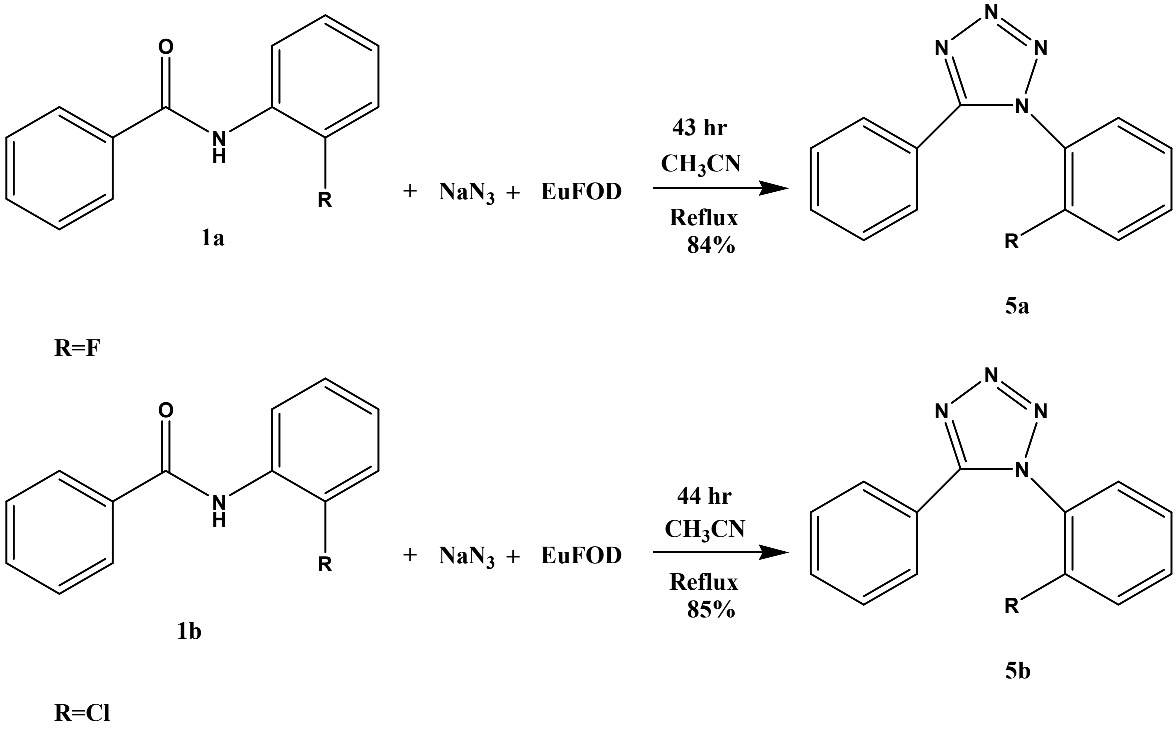
Imidoylbenzotriazoles were synthesized from corresponding secondary carboxamides and benzotriazoles by two distinct methods: oxalyl chloride and pyridine or thionyl chloride in a microwave (80 W/80oC). There are two problems with these methods, though. To begin with, there is an additional step involved in the manufacture of imidoylbenzotriazoles using non-sustainable resources. Only para-substituted aromatic tetrazoles are generated. Schroeder and colleagues reported improved conditions for the production of sterically hindered amides into equivalent 1,5-disubstituted tetrazoles **[9]**. The ideal reaction conditions were obtained by using diisopropyl azodicarboxylate (DIAD), diphenylphosphoryl azide (DPPA), and diphenyl-2-pyridyl phosphine in THF at 45°C. It should be mentioned, nonetheless, that this procedure also included the employment of pricy, dangerous, and unfavorable substances in addition to aliphatic amines. In this paper, we report on the synthesis of sterically hindered 1, 5-disubstituted tetrazoles by Esikov and coauthors using a one-step, EUFOD-based method in the presence of sodium azide. The aforementioned constraints have been effectively surmounted by this method.

**Table: The resulted products from reaction of bulky secondary amide With EuFOD and Sodium azide**

| Entry | Amide | Time (hr.) | Tetrazole | M.P. (°C) | Yield (%) |
|---|---|---|---|---|---|
| 1 | 1a | 43 | 5a | 104-105 | 84 |
| 2 | 1b | 44 | 5b | 103-104 | 85 |

## MATERIALS AND METHODS:

Benzoyl chloride, 2-fluoro aniline, and 2-chloro aniline were all purchased from Merck and used in the preparation of the amides. Acetonitrile, sodium azide, and EUFOD were obtained from Merck and used to produce tetrazoles. The organic solvents, n-hexane, acetonitrile, and ethyl acetate, were purchased from Merck. Using benzoyl chloride and the corresponding solid-state anilines, Amides 1a and 1b, as shown in Scheme 1, were prepared using the procedure outlined by Ghosh and coauthors **[10]**.



scheme 1

## PREPARATION OF 1,5-DISUBSTITUTED TETRAZOLE:

Tetrazoles were created in accordance with the method that Esikov and associates had previously published. A combination of (4 mmol), sodium azide (8 mmol), and EuFOD (8 mmol) in dry acetonitrile (16 ml) were refluxed and agitated with the exclusion of moisture as a standard process for 1-(2-Fluorphenyl)-5-phenyl-1H-tetrazole (1a) from amide (Scheme 1). Every six hours, the reaction was checked using the TLC test to identify when the reaction had ended. Following every TLC test, the reaction mixture was supplemented with 1 mmol of sodium azide and 2 mmol of EuFOD. The pure hindered 1, 5-disubstituted tetrazole was clearly visible in the most recent TLC test. Once the reaction was finished, the mixture was added to the $Na_2CO_3$ saturated solution (pH ~ 7). After that, the silica precipitate was filtered. Ethyl acetate was used to extract the mixture and yield the pure components. Under vacuum, the organic solvents (acetonitrile and ethyl acetate) evaporated. For further characterization, the finished compounds were stored at room temperature.

**1-(2-Fluorphenyl)-5-phenyl-1H-tetrazole (5a):** $^1$H-NMR spectrum of 5a (500 MHz, $CDCl_3$), δ (ppm): 7.26 (t, J= 9.1 Hz, 1H), 7.36-7.36 (m, 3H), 7.44 (t, J= 7.5 Hz, 1H), 7.52 (td, J= 8.0 Hz, J= 1.5 Hz, 1H), 7.56 (d, J= 7.5 Hz, 2H), 7.55-7.62 (m, 2H). $^{13}$C-NMR spectrum of 5a (125 MHz, $CDCl_3$), δc (ppm): 157.12, 155.10, 154.72, 132.93, 132.89, 131.48, 129.01, 128.32, 128.12, 125.48, 125.43, 123.32, 122.76, 122.68, 117.42 and 117.27, (77.34, 77.08 and 76.83 for solvent). $^{19}$F-NMR spectrum of 5a (470 MHz, $CDCl_3$), δF (ppm): -120.48 (m). FT-IR (KBr) spectrum of 5a [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) was prepared by refluxing and stirring a combination of 4 mmol, 8 mmol sodium azide, and 8 mmol EuFOD in 16 ml of dry acetonitrile while keeping moisture out (Scheme 1). Every six hours, the reaction was checked using the TLC test to identify when the reaction had ended. Following every TLC test, the reaction mixture was supplemented with 1 mmol of sodium azide and 2 mmol of EuFOD. The pure hindered 1, 5-disubstituted tetrazole was clearly visible in the most recent TLC test. Once the reaction was finished, the mixture was added to the Na2CO3 saturated solution (pH ~ 7). After that, the silica precipitate was filtered. Ethyl acetate was used to extract the mixture and yield the pure components. Under vacuum, the organic solvents (acetonitrile and ethyl acetate) evaporated. For further characterization, the finished compounds were stored at room temperature.

$^{1}$H-NMR spectrum of 5b (500 MHz, CDCl$_3$), δ (ppm): 7.37 (t, J= 7.7 Hz, 2H), 7.45 (t, J= 7.5 Hz, 1H), 7.45- 7.52 (m, 2H), 7.52 (d, J= 7.8 Hz, 2H), 7.56-7.58 (m, 2H). $^{13}$C-NMR spectrum of 5b (125 MHz, CDCl$_3$), δc (ppm): 154.58, 132.58, 132.43, 131.63, 131.42, 131.03, 129.03, 128.94, 128.35, 128.12 and 123.34, (77.33, 77.07 and 76.82 for solvent). FT-IR (KBr) spectrum of 5b [11]: 775 (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 5b (ESI) m/z: 257.0593 (M+ + 1).

## ANTIBIOTIC ACTIVITY:

By using the disc diffusion method (NCCLS, 1982), Tetrazole Compounds 5(a-b) were tested for their antibacterial activity against Gram-positive bacteria such as Bacillus subtilis (MTCC 441), Bacillus sphaericus (MTCC 11), and Staphylococcus aureus (MTCC 96), as well as Gram-negative bacteria such as Pseudomonas aeruginosa (MTCC 741), Klobsinella aero genes (MTCC 39), and Chromobacterium violaceum (MTCC 2656). Standard inoculums (1-2 × 107 c.f.u/mL 0.5 Mc Farland standards) were applied to the surface of sterile agar plates for the antibacterial assay, and a sterile glass spreader was utilized to ensure uniform dispersion of the inoculums. The 6.26 mm-diameter discs were made using Whatman No. 1 filter paper and dried at 140 degrees Celsius for one hour. Nutrient agar medium was added to the sterile discs that had previously been soaked in a known concentration of the test chemicals. After being inverted, the plates were incubated at 37°C for 24 hours.

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

| **Zone of inhibition at 50 µg/mL (mm)** | | | | | | |
|---|---|---|---|---|---|---|
| **Compound** | ***B.subtilis*** | ***B.sphaericus*** | ***S.aureus*** | ***P.aeruginosa*** | ***K.aerogenes*** | ***C.violaceum*** |
| **5a** | **13** | **24** | **16** | **16** | **18** | **23** |
| **5b** | **10** | **17** | **18** | **15** | **23** | **20** |
| **Streptomycin** | **25** | **30** | **30** | **30** | **24** | **30** |

## RESULTS AND DISCUSSION:

1H-, 13C-, and 19F-NMR, HRMS, and FT-IR characterizations were used to show the synthesis of 1, 5-disubstituted tetrazoles and antibiotic activity. The purity of the product and the progress of the reaction were monitored using TLC **[11]**. We used the melting point to verify the materials' purity. The table shows the reaction between sodium azode and EuFOD in secondary amides 1a and 1b. Sodium azide and EuFOD were combined with an azide transfer reagent. While ketones are converted into the corresponding tetrazoles by rearrangement and the expansion of general synthetic techniques for the chemo selective synthesis of tetrazole derivatives, amidates are turned into nitriles or acid azides (imidoyl azides) **[12-28]**.

## CONCLUSIONS:

The method developed by Esikov and coauthors provides 84–85% of 1,5-disubstituted tetrazoles from bulky secondary N-benzoyl amides with antibiotic activity. It uses EuFOD in combination with sodium azide and acetonitrile as a solvent.

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