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Article

Synthesis and Characterization of New Tetrazole Ring Substitutes and Testing of Their Antimicrobial Activity

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Abstract: In this manuscript, several compounds containing a tetrazole ring were produced by reacting hydrazone precursors with sodium azide in a conventional manner using DMF as a solvent. The correctness of the synthesis was confirmed by key organic chemistry assays (FT-IR, ^{1}H, and ^{13}C-NMR), which showed a clear disappearance of azomethine, indicating complete consumption of the reactants. The antimicrobial activity was also evaluated against two widespread bacteria: *Staphylococcus aureus* and *Escherichia coli*. The results showed that the inhibition increased with increasing concentration, and preparation (M2) exhibited the highest antimicrobial activity against the bacterial strains used in the study, compared to the antibiotic *amepicillin*, which served as the control for inhibition. Overall, these compounds have potential for future pharmacological applications.

Keywords: heterocyclic, Tetrazoles, bacterial bioactivity

Introduction

Due to their importance in pharmacology, heterocyclic compounds have received a lot of attention recently [1,2]. In the fields of industrial chemistry, synthetic organic chemistry, and medicine, nitrogenous heterocyclic molecules appear to be very intriguing vectors[3]. Chemists are also expected by society to develop more ecologically friendly and sustainable chemical processes. Tetrazoles are important heterocyclic compounds with five members, one carbon atom and four nitrogen atoms. Applied chemistry continues to focus on the different applications of tetrazole heterocycles, notwithstanding the progress made in the study of heterocyclic chemistry. Since Bladin first synthesized tetrazoles in 1855, the number of papers discussing their synthesis and use has increased yearly [4].Because they include more carbon-nitrogen and nitrogen-nitrogen bonds, tetrazoles also have a greater heat of production. Tetrazoles are very resistant to shock, impulse, and electrostatic discharge because of their low ratio of C to H bonds [5]. A number of bioactive substances that fight diseases brought on by bacteria and viruses have been developed thanks in large part to the tetrazole ring [6]. In medicinal chemistry, tetrazoles are used as spacers and alternatives to carboxylic acids [7].Tetrazoles have been used in the development of energy materials. Additionally, tetrazoles offer the ideal starting point for synthetic procedures that produce the required heterocyclic units. Another significant pharmacophore fragment that has been used in the creation of possible

anti-cancer drugs is tetrazole, which is made up of five-membered heterocyclic molecules. Numerous chemicals, including naturally occurring compounds with a tetrazole moiety and coordination compounds, have demonstrated potential[8]. The tetrazole ring possesses strong antibacterial action, according to earlier research [9].The purpose of this study is to prepare several tetrazole compounds using environmentally friendly and alternative methods by reacting hydrazones with sodium azide to form pentacycles. The compounds will then be tested for their sensitivity against two types of Gram-positive and Gram-negative bacteria.

Materials and methods

2.1. Materials: Research materials were obtained from reputable and leading companies in the field such as BDH, Aldrich, and FLUKA, and were used without additional filtering.

2.2. Preparation of Tetrazoles derivatives (M_1-M_5) [10,11]

Neutral moles (0.001 mol) of the previously obtained hydrazones were homogenized with sodium azide in 20 ml of DMF in the bottom of a 100 ml round flask. The flask containing the solution was sublimated for six hours, after which the mixture was concentrated, allowed to cool, filtered, dried, and the product was collected and recrystallized from the ethanol as indicated in Table 1.

Table 1. Data related to the practical part of synthetic compounds (M1-M5)

Comp. No.	R	Molecular formula	m.p. °C	Yield%	Color
M1	Br	$C_{20}H_{14}BrN_3O_4$	246-248	76	Yellow
M2	NO_2	$C_{20}H_{14}N_4O_6$	263-265	72	Brown
M3	Cl	$C_{20}H_{14}ClN_3O_4$	296-298	73	Orange
M4	OH	$C_{20}H_{15}N_3O_5$	277-279	77	Blue
M5	H	$C_{20}H_{15}N_3O_4$	255-257	80	Yellow

2.3. Evaluation of bacterial bioactivity

Because of their high prevalence and the ease of obtaining them through the Biology Laboratories at Tikrit University, we obtained two of the most widespread types of bacteria that are mainly found in soil and water, namely *Staphylococcus aureus*, which is a class of positive bacteria, and *Escherichia coli*, which belongs to the class of negative bacteria. Initially, the medium on which these bacteria grow, Mueller-Hinton agar, was prepared by dissolving 40 grams in only one liter of distilled water [12-15]. This medium was heated to a temperature of 121°C at a pressure of 1.5 bar in an autoclave, then the mixture was cooled and poured into Petri dishes at room temperature. After cooling, these dishes were smeared with the bacteria we obtained in three different directions in order to ensure the distribution of bacteria in all directions, and then the dish was pierced with three holes of a mm diameter [16-19]. The compound solutions were prepared using DMSO solvent at three concentrations (0.01, 0.0001, 0.001) mg/ml by continuous addition. Each concentration was then poured into a well, with three different concentrations being poured into a single dish. The dishes were then incubated in a special incubator at 37°C for a full day. The diameter of the inhibition was measured in mm [20-22].

Results and discussion

3.1. Characterization of Tetrazole derivatives (M1-M5)

When performing the tests, beginning with FT-IR spectroscopy, it became clear that the azomethine bond disappeared, indicating that the substance had reacted well. New bands appeared, indicating the formation of a new compound. The stratigraphic layer showed the presence of a ligand related to the (NH) ligand, which was targeted at (3390-3311) cm^{-1}. New bonds not previously present were also detected, such as an aliphatic ligand, located at (2947, 2858) cm^{-1}. The bond characteristic of

tetrazole, an azo bond, was located at (1447-1433) cm^{-1}. Furthermore, the remaining bonds present in the original compound retained their positions with slight displacements that did not affect their location or disrupt the spectrum [23,24], as shown in Table 2 and Figures 1 and 2.

Table 2. Metadata of the FT-IR spectrum for compounds (M1-M5)

Comp. No.	R	ν(NH) Tetrazole	ν(N-H)	ν(C-H) Arom	ν(C-H) Aliph.	ν(C=C) Arom.	ν(N=N)	ν(C=O)	Others
M1	Br	3390	3313	3053	2924,2860	1512,1492	1437	1647	ν (C-Br)630
M2	NO_2	3367	3257	3078	2947,2867	1548,1488	1445	1656	ν (NO)1332,1521
M3	Cl	3324	3288	3067	2938,2872	1529,1491	1447	1651	ν (C-Cl)734
M4	OH	3311	3271	3055	2922,2858	1516,1483	1433	1654	ν (OH)3390
M5	H	3332	3276	3042	2927,2882	1532,1480	1441	1655	--

However, the ^{1}H-NMR spectroscopy also revealed the disappearance of hydrogen, which is always attributed to the azomethine bond, along with signals indicating the presence of substances formed after confirming that the reactant had been consumed. The spectrum showed a signal at 2.14 ppm, attributed to the presence of NH_2, belonging to the target ring. The data did not stop there; a signal at 6.03 ppm was found, attributed to CH_2 in the same ring. These signals are characteristic of tetrazol. The remaining groups, such as the benzene ring, retained their positions, with their presence at 6.97–8.31 ppm. In addition to these data, signals attributed to NH_2 were found at 8.76 and 9.44 ppm [25,26], as shown in Figure 3

The ^{13}C-NMR spectroscopy confirmed the disappearance of azomethine, while signals indicated the formation of the targeted ring, tetrazole. A signal of 96.35 ppm was attributed to the lone carbon atom in the target ring. The remaining atoms maintained their positions with slight, but unaffected, shifts. The benzene signals were located between 122.78 and 144.54 ppm, while the carbonyl hydrazide was located at 164.97 ppm, as shown in Figure 4.

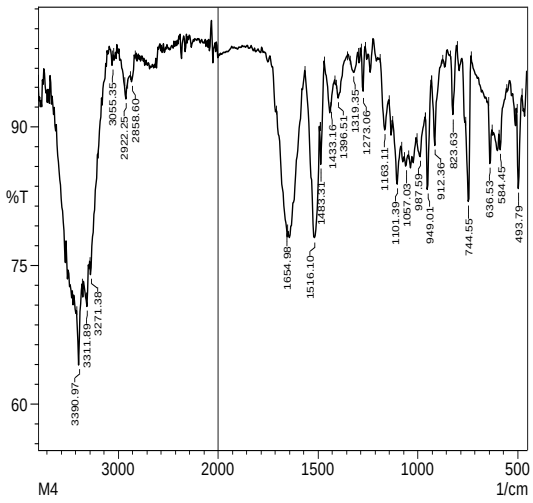

Figure 1. FT-IR OF (M1)

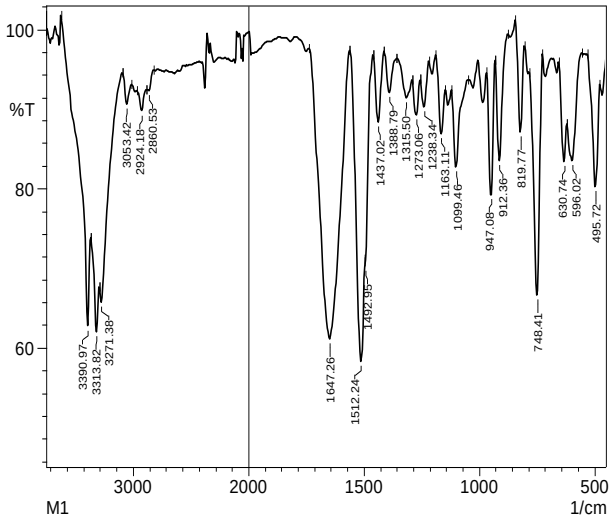

Figure 2. FT-IR OF (M4)

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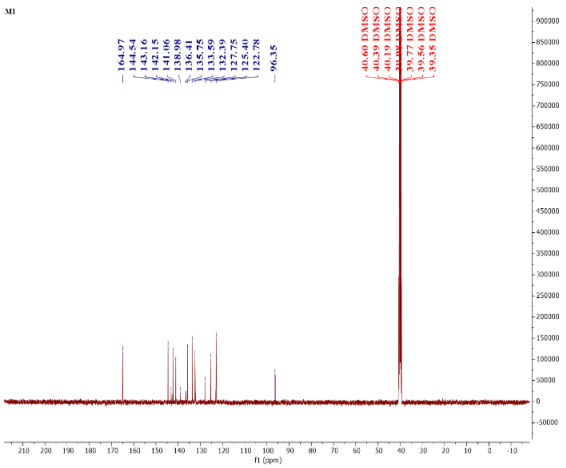


Figure 3. ^{1}H-NMR OF (M1)

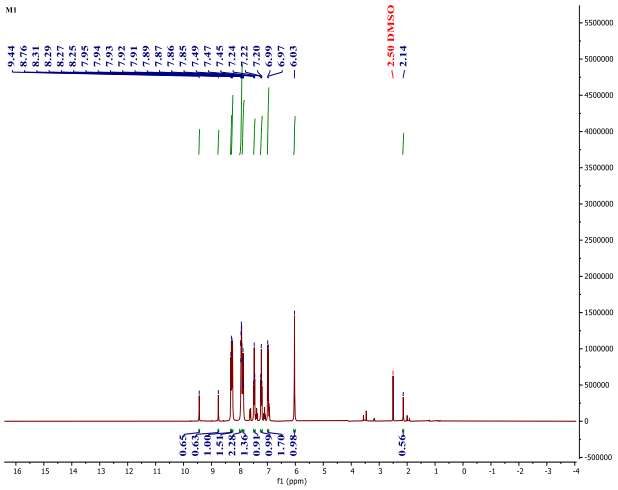


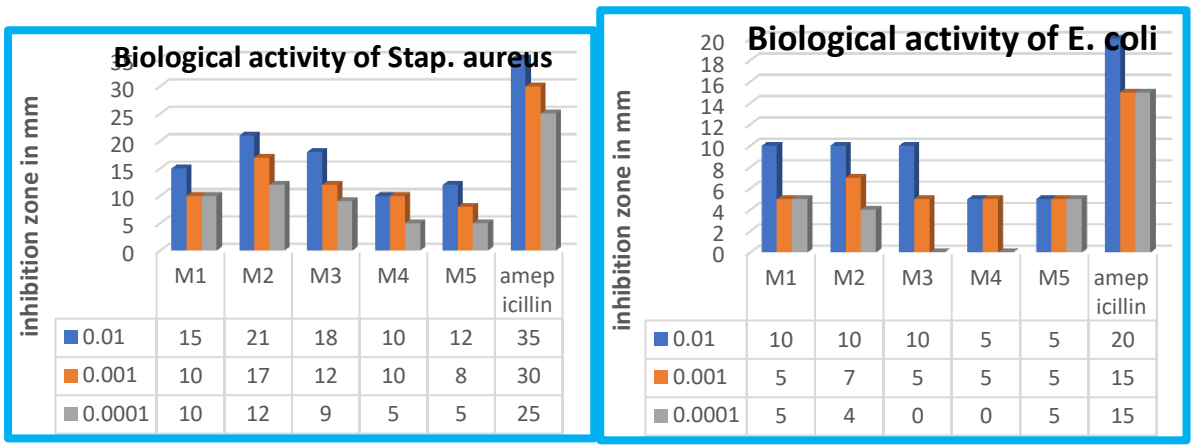
Figure 4. ^{13}C-NMR OF (M1)

3.2. Bacterial susceptibility to prepared compounds

From the data provided in Table (4), it is clear that the synthesized compounds have varying inhibitory activity against the targeted microbes at three different concentrations. It is also clear that the activity increases with increasing concentration, so the higher the concentration, the greater the ability of the synthesized compounds to stop the growth of these microbes on the bacterial plates [27,28]. In general, the microbes were more effective against Gram-positive bacteria than Gram-negative bacteria. This can be attributed to the fact that Gram-negative bacteria possess an outer membrane that limits the diffusion of the compounds into the cells, while Gram-positive bacteria lack this membrane, allowing the compounds to penetrate them easily [29,30]. Regarding the types of bacteria, compound (M2) exhibited the highest activity against Gram-positive bacteria with a diameter of up to 21 mm at the highest concentration. It maintained its high activity at medium and low concentrations. Although its activity did not match that of an antibiotic, it was the most promising compound as a potential future antibiotic if treated under different conditions. Compound (M3) followed, exhibiting activity with a diameter of 18 mm [31,32]. Against Gram-negative bacteria, activity was similar at all concentrations and almost negligible at low concentrations in some microbes. Compounds (M1, M2, and M3) showed equal activity at the highest concentrations. As mentioned earlier, this low activity is attributed to their outer membrane [33,34].

Table 4. Data concerning the effectiveness of compounds (M1-M5) against microbes

Comp	E.coli mg/ml			Staph. aureus mg/ml		
No.	0.01	0.001	0.0001	0.01	0.001	0.0001
M1	10	5	5	15	10	10
M2	10	7	4	21	17	12
M3	10	5	0	18	12	9
M4	5	5	0	10	10	5
M5	5	5	5	12	8	5
amepicillin	20	15	15	35	30	25



Scheme 2. Data concerning the effectiveness of compounds (M1-M5) against microbes

Conclusions

Five-membered rings produced from tetrazole are created when the azomethine molecule in hydrazone combines with sodium azide. High purity was demonstrated by the produced compounds, particularly in nuclear magnetic resonance and infrared spectra, which indicated full reactant absorption and the presence of unique groups indicative of the target ring. Additionally, they demonstrated moderate action against Escherichia coli and high activity against Staphylococcus aureus, with activity rising with concentration. Compound (M2) in particular shown promising activity that may make it an antibacterial in the future.

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