

# PREPARATION AND DIAGNOSIS OF NEW DERIVATIVES OF THE TETRAZOLE RING DERIVED FROM 2-BROMOISOPHTHALALDEHYDE AND EVALUATION OF THEIR BIOLOGICAL EFFECTIVENESS

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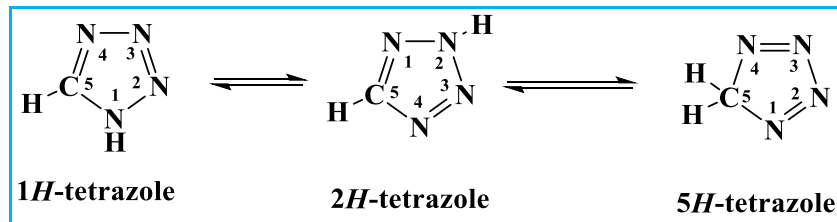
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**Abstract:** This study included the preparation of Schiff base derivatives (Z1-Z5) derived from 2-bromoisophthalaldehyde with aniline substitutes. By the reaction of prepared compounds with sodium azide using dioxane as a solvent a pentacyclic rings derived from tetrazole (Z6-Z10) were obtained. The prepared compounds (Z6-Z10) were characterized through measurements of fluorescence infrared (FTIR) spectra and proton ( $^1\text{H-NMR}$ ) and carbon nuclear magnetic resonance spectroscopy ( $^{13}\text{C-NMR}$ ). The melting points were measured, and the biological efficacy of several compounds prepared for two types of Gram-positive and Gram-negative bacteria was evaluated.

**Keywords:** tetrazole, Schiff bases, biological activity.

## 1. Introduction

**Tetrazole** is a heterocyclic pentacyclic compound containing one carbon atom and four nitrogen atoms. The molecular formula  $\text{CH}_2\text{N}_4$  [1] contains the following three isomers (**Scheme 1**):



**Scheme 1.** Izomers of tetrazole

These compounds are considered among the most vital cyclic compounds. Because it contains four pairs of free electrons, equivalent to four nitrogen atoms, it is one of the electron-pushing compounds [2]. Previous studies have shown that tetrazole compounds and their derivatives are essential in medicine, especially in the biological field and show antibacterial [3] and antifungal [4] activity. In addition, they are an antidote against viral immunodeficiency [5], and the following compounds show good anticancer activity [6].

**Schiff bases** are compounds containing an azomethine group ( $-\text{HC}=\text{N}-$ ) and are usually yellow. They are named after the chemist Hugo Schiff [7]. Hydrazone compounds and their derivatives have received extensive attention recently, especially after discovering their biological effects. They are widely used in the medical and pharmaceutical fields as antifungal [8], antibacterial [9], and antitumor [10] agents. They are also effective against viruses [11].

## 2. Experimental part

**2.1. Materials:** All compounds used in this research, including 2-bromoisophthalaldehyde, which was obtained from Sigma pure, has a molecular weight of 213.03 g/mol, its melting point is 139-144 degrees Celsius, and its boiling point is 298 + 25 degrees Celsius, while the rest of the materials are from BDH, Fluka, and Aldrich without further purification.

**2.2. Devices used:** A thermoelectric melter 9300 was used to determine melting points. Using KBr disk at a scale of (400–4000) cm<sup>-1</sup>, Shimadzu FT-IR 8400S spectrophotometer; <sup>1</sup>H-NMR and <sup>13</sup>C-NMR spectra using Bruker apparatus operating at 400 MHz. Thickness at 0.2 mm, Fluka silica gel plates were employed in thin-layer chromatography (TLC). The plates were activated with fluorescent silica gel G, and visibility was achieved by UV light.

### 2.3. Preparation of Schiff bases (Z1-Z5) [12]

Schiff bases were prepared by dissolving an amount of the compound 2-bromozophthaldehyde (0.0004 moles and 0.8 g) in 10 ml of ethanol solvent, adding it to the reaction flask, then adding a few drops (3-5) of acid glacial acetic as a catalyst with stirring. Then one of the substituted aromatic amine derivatives was dissolved in an amount of (0.0008 mol) in 10 ml of ethanol solvent and then added to the aldehyde with stirring and sublimation for (4-6) hours, and residues of different proportions and colors were obtained. The mixture was cooled, filtered, dried, and recrystallised with ethanol, and the melting points were measured. The completion of the reaction was confirmed using the TLC technique as shown in Table 1.

### 2.4. Preparation of Tetrazole (Z6-Z10) [13]

These compounds are prepared as follows: (0.0004 moles) of previously prepared Schiff bases are dissolved in (10 ml) of dioxane solvent and placed in a circular flask, and gradually added (3 drops of triethylamine) (Et<sub>3</sub>N) and progressively added to a mixture of (0.05 g, 0.0008 mol sodium azide (NaN<sub>3</sub>), dissolved in (10 ml) dioxane, heated in a water bath (8 hours), added to crushed ice after cooling, the sediment was filtered, dried and repeated with ethanol. The physical properties are shown in **Table 1**

**Table 1.** The produced chemicals' physical attributes (Z1-Z10)

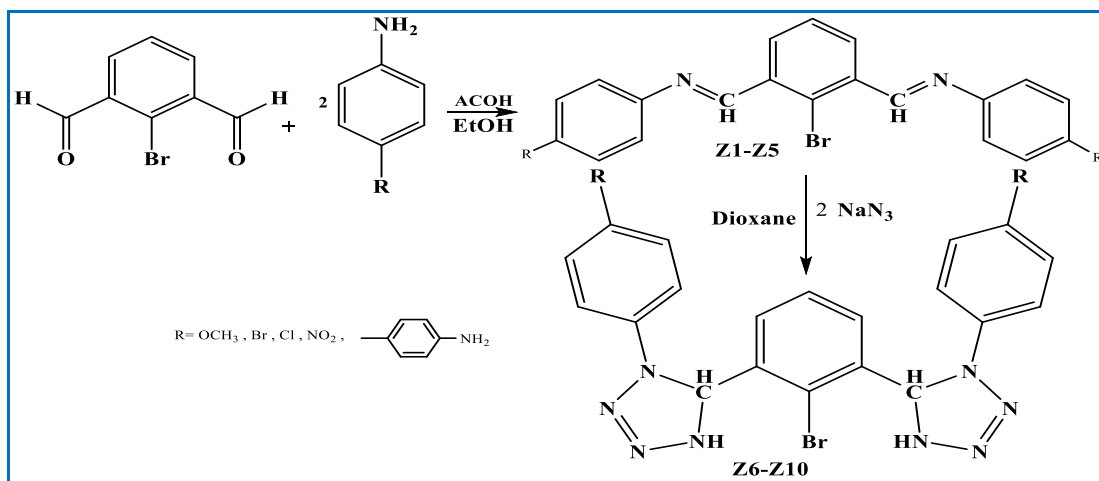
| Comp. No. | R                               | Molecular formula   | m.p. °C | Yield% | Color       |
|-----------|---------------------------------|---|---------|--------|-------------|
| Z1        | OCH <sub>3</sub>                | C <sub>22</sub> H <sub>19</sub> N <sub>2</sub> O <sub>2</sub> Br  | 172-174 | 85%    | Gray light  |
| Z2        | Br                              | C <sub>20</sub> H <sub>13</sub> N <sub>2</sub> Br <sub>3</sub>    | 186-188 | 86%    | White       |
| Z3        | Cl                              | C <sub>20</sub> H <sub>13</sub> Cl <sub>2</sub> N <sub>2</sub> Br | 175-178 | 75%    | White       |
| Z4        | NO <sub>2</sub>                 | C <sub>20</sub> H <sub>13</sub> N <sub>4</sub> O <sub>4</sub> Br  | 155-157 | 74%    | Yellow      |
| Z5        | C <sub>6</sub> H <sub>6</sub> N | C <sub>32</sub> H <sub>25</sub> N <sub>4</sub> Br                 | 168-170 | 77%    | Green       |
| Z6        | OCH <sub>3</sub>                | C <sub>22</sub> H <sub>21</sub> N <sub>8</sub> O <sub>2</sub> Br  | 192-194 | 59%    | White       |
| Z7        | Br                              | C <sub>20</sub> H <sub>15</sub> N <sub>8</sub> Br <sub>3</sub>    | 212-214 | 60%    | White       |
| Z8        | Cl                              | C <sub>20</sub> H <sub>15</sub> Cl <sub>2</sub> N <sub>8</sub> Br | 220-222 | 64%    | White       |
| Z9        | NO <sub>2</sub>                 | C <sub>20</sub> H <sub>15</sub> N <sub>10</sub> O <sub>4</sub> Br | 174-177 | 61%    | Yellow      |
| Z10       | C <sub>6</sub> H <sub>6</sub> N | C <sub>32</sub> H <sub>27</sub> N <sub>10</sub> Br                | 230-232 | 62%    | Green Light |

### 2.5. Evaluation of the biological activity of (Z1, Z4, Z6, Z7, Z8)

The propagation approach of the Kirby Bauer movement, which involves spreading 0.1 ml of bacterial solution on agar Muller Hinton plates and letting them absorb the fluid for five minutes, has been used to measure biological activity [14]. A cork plunger and a five mm diameter cork were used to create holes in each dish. Then, 0.1 ml of the produced solutions for the fourth hole, which employed Ciprofloxacin as a reference sample, were incubated at 37 °C for twenty-four hours. Using Prescott's approach [15], the inhibitory zone widths surrounding each hole have been determined in millimeters.

## 3. Results and discussion

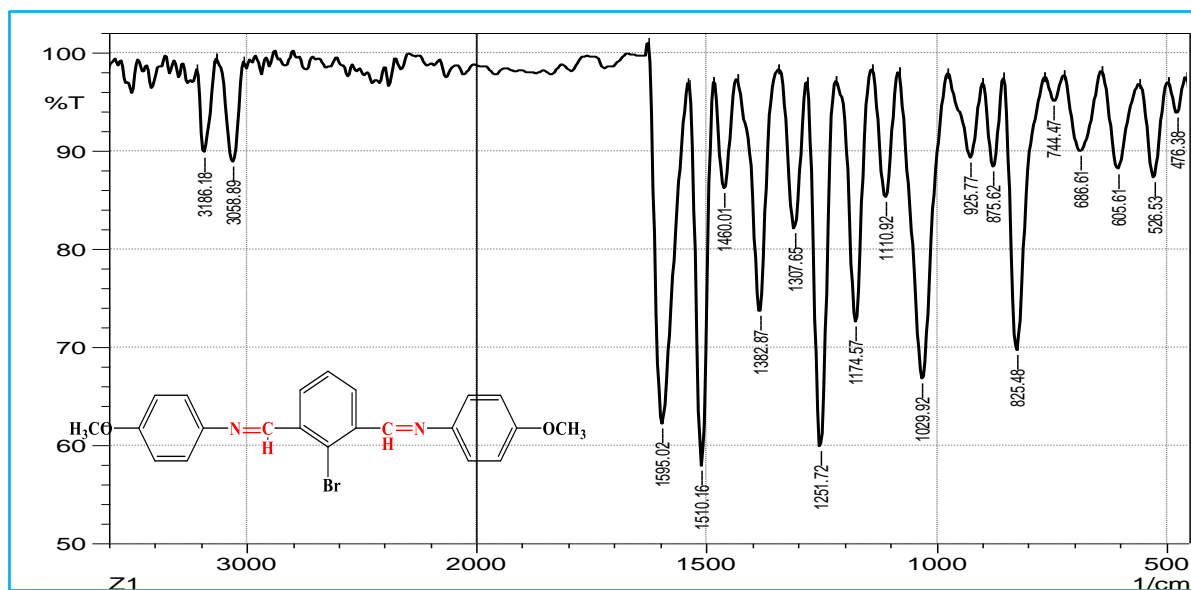
This study included using 2-bromoisophthalaldehyde as a nucleus in preparing Schiff bases (Z1-Z5) and then reacting them with sodium azide to form pentagonal heterocyclic rings called tetrazoles (Z6-Z10) as in **Scheme 2**.



**Scheme 2.** Path of the preparation of compounds (Z1-10)

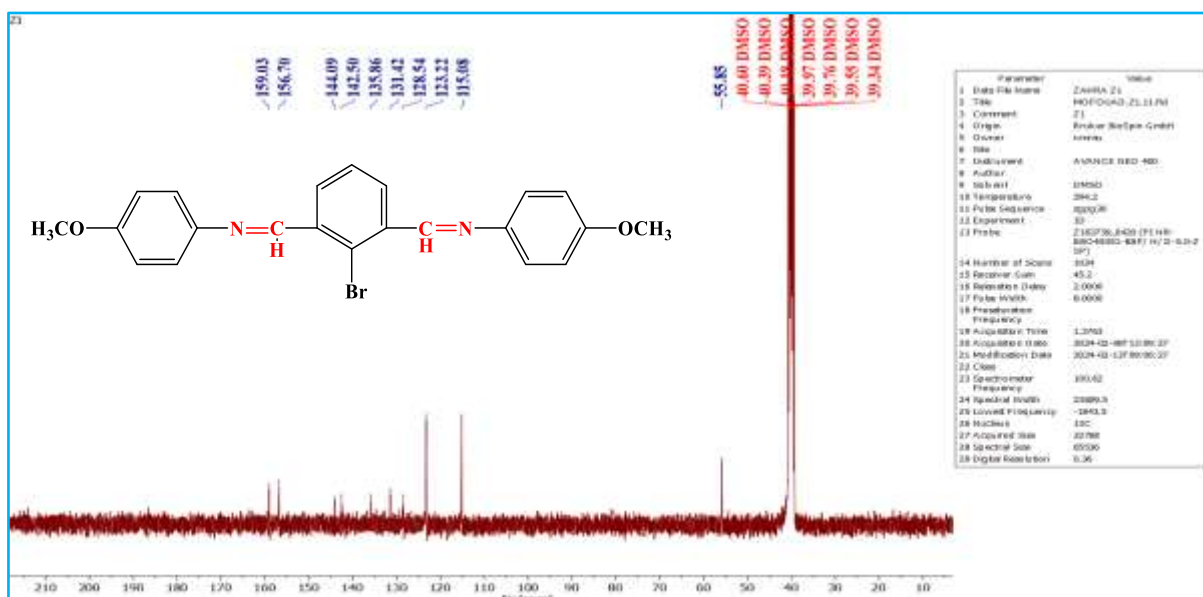
### 3.1. Characterization of Schiff bases

Examining the produced Schiff base compounds' (Z1-Z5) infrared spectra (FTIR), the appearance of an elastic band in the range (1623-1595) cm<sup>-1</sup> belongs to the something group (C=N), which is the distinctive band of labial compounds and is evidence of the formation of the product. In addition to these bands, absorption bands related to the stretching of the aromatic bond (C-H) began to develop in the region (3065-3025) cm<sup>-1</sup>. And the emergence of two absorption bands indicative of the bond's (C=C) aromaticity in the ranges of (1565-1510) cm<sup>-1</sup> and (1489-1456) cm<sup>-1</sup> [16], as shown in **Table 2 and Figure 1**.



**Fig.1.** Infrared spectrum of compound (Z1)

When studying the proton NMR spectrum of the compound [Z1], A solitary signal was detected at the ppm location (8.97), which was identified as the proton of the azomethine group (HC=N). The sole signal that appeared at the ppm spot (3.80) was recognised as the group proton (CH<sub>3</sub>). Likewise, multiple signals appear in the ppm site (7.02-8.25) attributed to the aromatic ring's protons (C=C), and the protons of the solvent (DMSO-d<sub>6</sub>) are responsible for a signal in the ppm spot (2.51), as in **Figure 2**.

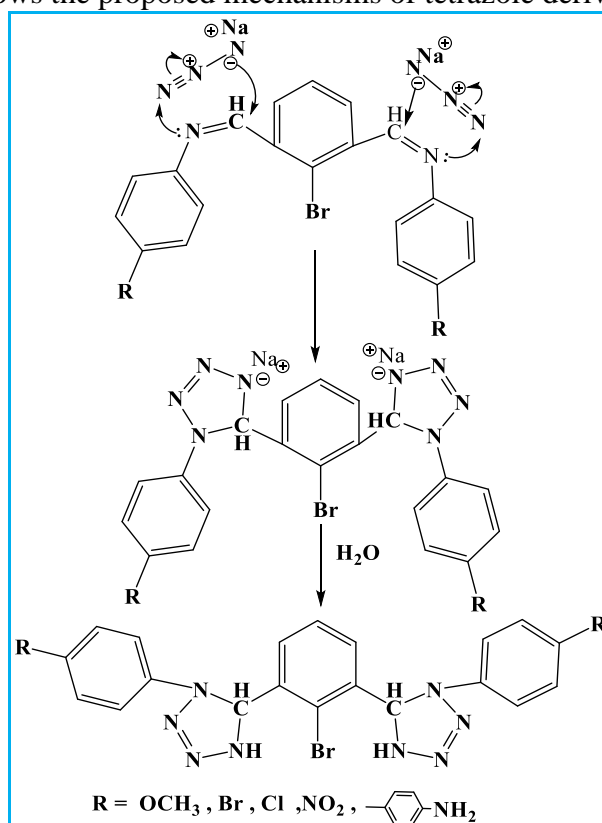


**Fig. 2.** The <sup>13</sup>C-NMR spectrum of the compound (Z1)

Examining the chemical [Z1's] carbon NMR spectra. A signal was shown to emerge at the ppm locations (159.03), which was linked to the azomethine group's carbon (C=N). A signal was detected at the ppm locations (55.85 ppm) related to the group's carbon (CH<sub>3</sub>). Signals linked to the aromatic benzene ring's carbons may be seen at the location (115.08–156.70) ppm; a signal is detected at the ppm location (40.18) that is linked to the solvent's carbon (DMSO-d<sub>6</sub>). as in **Figure 2**.

### 3.2.Characterization of Tetrazole derivatives [17]:

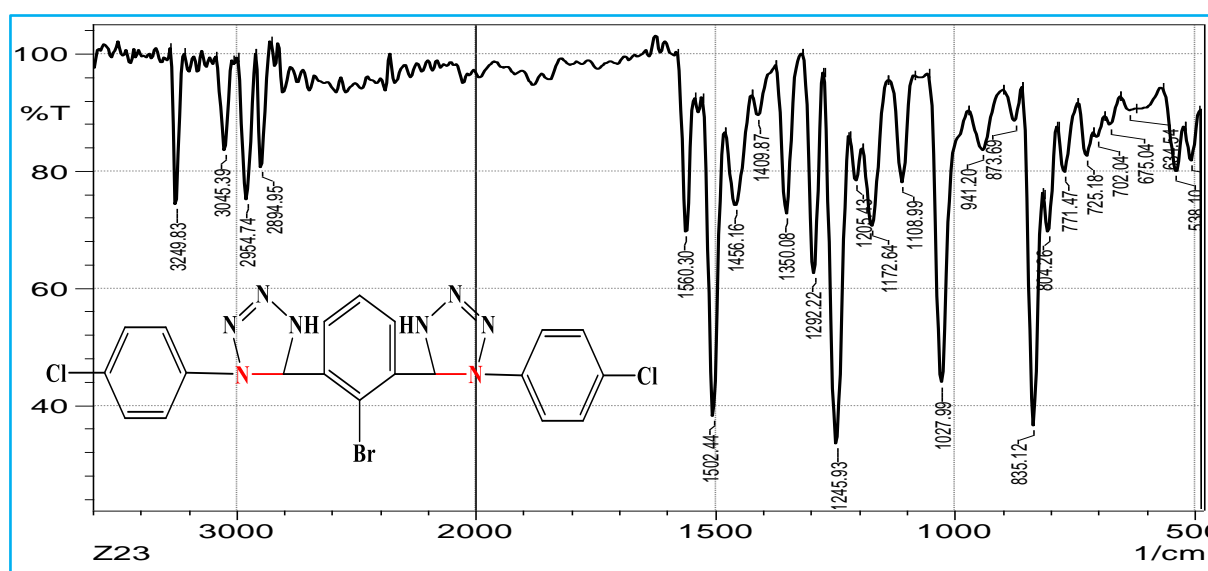
Scheme 3 below shows the proposed mechanisms of tetrazole derivatives.



**Scheme 3.** The proposed mechanisms of tetrazole derivatives

By examining the produced compounds' infrared spectra [Z6-Z10], the stretching of the (N-H) group on the tetrazole ring is the cause of the formation of an intermediate band in the range (3264-3195)  $\text{cm}^{-1}$ . The stretching of the aromatic bond (C-H) is what causes an absorption band to form in the region of (3085-3038)  $\text{cm}^{-1}$ . The stretching of the aliphatic (CH) of the tetrazole ring is responsible for the formation of two symmetrical and asymmetric absorption bands, respectively, in the range of (2963-2931)  $\text{cm}^{-1}$  and (2894-2850)  $\text{cm}^{-1}$ .

Besides the emergence of two bands in the intervals of (1533-1487) and (1579-1553)  $\text{cm}^{-1}$  is caused by the aromatic bond's vibration (C=C). The center bands that emerge in the region of (1449-1488)  $\text{cm}^{-1}$  are part of the group (N=N) that is linked to the formation of the tetrazole ring., and the appearance of other bands in the range (1230-1271)  $\text{cm}^{-1}$  due to synergistic stretching (C-N), in addition to the appearance of absorption bands in the range (1071-1012)  $\text{cm}^{-1}$  due to synergistic stretching (N-N) [18]. IR data is in **Table 3 in Figure 3**.



**Fig. 3.** The compound's infrared spectrum (Z8)

When examining the compound's  $^1\text{H-NMR}$  spectra [Z7] using a solvent (DMSO- $d_6$ ), it was observed that multiple signals appeared in the ppm range (6.98-8.20) attributed to the protons of the aromatic rings, and a single signal after the chemical shift, ppm (5.56). It is attributed to the protonation of two (CH) groups on the tetrazole ring, and the appearance of a single signal after the ppm chemical shift (3.54) is attributed to the protonation of two. Groups (NH) and the appearance of a signal unique to the chemical shift ppm (3.33) are attributed to the protons of water ( $\text{H}_2\text{O}$ ). When a signal first appears, the solvent's protons (DMSO- $d_6$ ) are the cause of the chemical shift ppm (2.48), as shown in **Figure 4**.

When examining the compound's  $^{13}\text{C-NMR}$  spectra [Z7] using a solvent (DMSO- $d_6$ ), multiple signals were observed in the ppm chemical shift (115.05-153.73) attributed to the carbons of the aromatic ring, and the appearance of a signal at the ppm chemical shift (87.50) is attributed to the carbon group (CH) on the tetrazole ring, and the solvent's carbon (DMSO- $d_6$ ) is responsible for the signal that appears at the ppm chemical shift (39.12-40.79). The spectrum is shown in **Figure 5**.

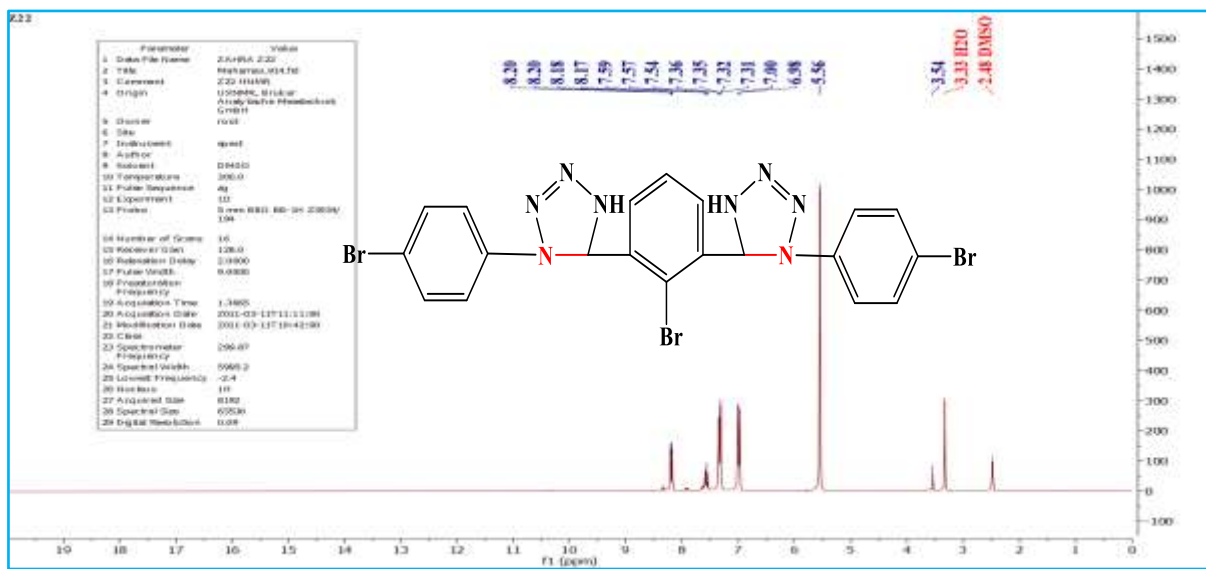


Fig. 4. The  $^1\text{H-NMR}$  spectrum of the compound (Z7)

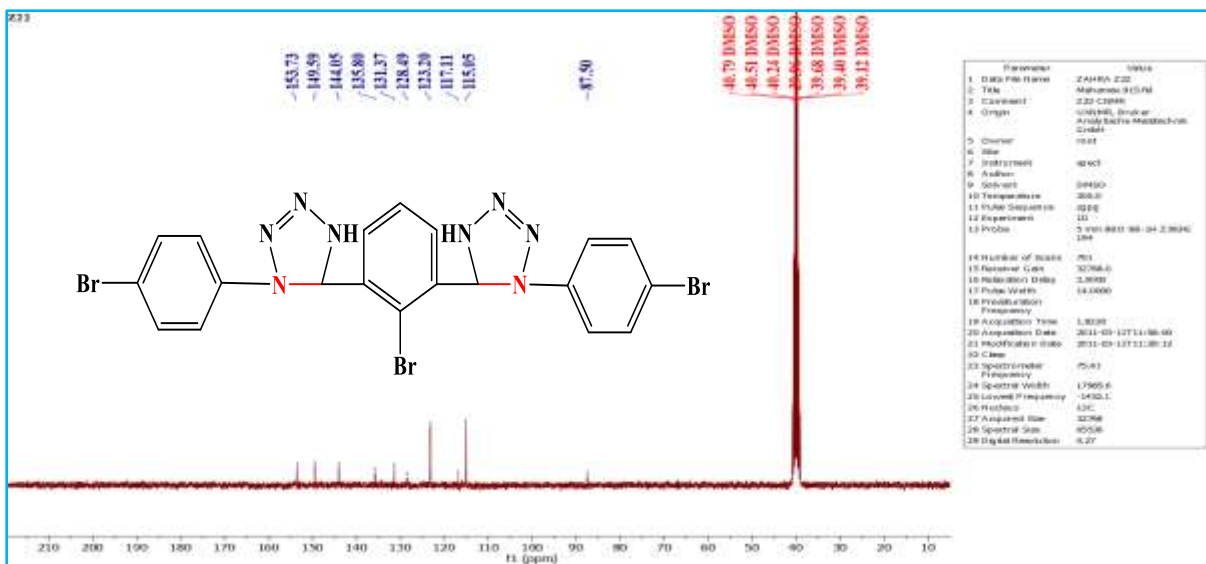


Fig. 5. The  $^{13}\text{C-NMR}$  spectrum of the compound (Z7)

Table 2. The synthesised compounds' FT-IR data (Z1-Z5)  $\text{cm}^{-1}$

| Compound No | IR (KBr) $\text{cm}^{-1}$         |                            |                   |                            |   |
|-------------|-----------------------------------|----------------------------|-------------------|----------------------------|---|
|             | R                                 | $\nu(\text{C-H})$<br>Arom. | $\nu(\text{C=N})$ | $\nu(\text{C=C})$<br>Arom. | Others  |
| Z1          | 4-OCH <sub>3</sub>                | 3058                       | 1595              | 1510<br>1460               | $\nu(\text{C-O})$ 1251                                      |
| Z2          | 4-Br                              | 3039                       | 1600              | 1527<br>1467               | $\nu(\text{C-Br})$ 597                                      |
| Z3          | 4-Cl                              | 3065                       | 1612              | 1516<br>1478               | $\nu(\text{C-Cl})$ 614                                      |
| Z4          | 4-NO <sub>2</sub>                 | 3025                       | 1623              | 1565<br>1489               | $\nu(\text{NO}_2)$ <i>asy.</i> (1524)<br><i>sym.</i> (1318) |
| Z5          | 4-C <sub>6</sub> H <sub>6</sub> N | 3051                       | 1598              | 1534<br>1456               | $\nu(\text{NH}_2)$ <i>asy.</i> 3271<br><i>sym.</i> 3230     |

**Table 3.** The synthesised compounds' FT-IR data (Z6-Z10)  $\text{cm}^{-1}$ 

| Comp. No. | R                                 | IR (KBr) $\text{cm}^{-1}$ |                            |                |                            |  |                             | Others  |
|-----------|-----------------------------------|---------------------------|----------------------------|----------------|----------------------------|--|-----------------------------|---|
|           |                                   | $\nu(\text{NH})$          | $\nu(\text{C-H})$<br>Arom. | $\nu$<br>(N=N) | $\nu(\text{C=C})$<br>Arom. | $\nu(\text{C-N})$<br>$\nu(\text{N-N})$ | $\nu(\text{C-H})$<br>Aliph. |   |
| Z6        | 4-OCH <sub>3</sub>                | 3195                      | 3081                       | 1468           | 1553<br>1492               | 1223<br>1045                           | 2954<br>2871                | $\nu$ (C-O) 1306                              |
| Z7        | 4-Br                              | 3252                      | 3085                       | 1449           | 1579<br>1487               | 1238<br>1071                           | 2963<br>2886                | $\nu$ (C-Br) 567                              |
| Z8        | 4-Cl                              | 3249                      | 3045                       | 1456           | 1560<br>1502               | 1245<br>1027                           | 2945<br>2894                | $\nu$ (C-Cl) 675                              |
| Z9        | 4-NO <sub>2</sub>                 | 3264                      | 3038                       | 1473           | 1575<br>1495               | 1230<br>1034                           | 2931<br>2850                | $\nu(\text{NO}_2)$ .asy(1541)<br>sym.(1330)   |
| Z10       | 4-C <sub>6</sub> H <sub>6</sub> N | 3211                      | 3074                       | 1488           | 1564<br>1533               | 1271<br>1012                           | 2935<br>2864                | $\nu$ (NH <sub>2</sub> ) asy.3442<br>sym.3361 |

### 3.4. Evaluation of Biological activity of (Z1, Z4, Z6, Z7, Z8)

At first, the agar diffusion method carried out the biological activity against two types of pathogenic bacteria, namely the gram-positive bacteria *Staphylococcus aureus* and the second bacteria, the gram-negative coliform bacteria *E.coli* [19]. The compounds (Z4, Z8, and Z6) were biologically effective, while compound Z4 showed activity only against positive bacteria and showed no activity against harmful bacteria [20], while both compounds Z8 and were 100% efficient against the two varieties of the chosen bacterial species, as shown in the following Table 4.

**Table 4.** Antibiotics and other generated chemicals can prevent the growth

| Comp. | Inhibition diameter of the compounds (potency) measured in millimeters |     |      |                       |     |      |
|-------|--|-----|------|-----------------------|-----|------|
|       | Escherichia coli   |     |      | Staphylococcus aureus |     |      |
| Conc. | 25%  | 50% | 100% | 25%                   | 50% | 100% |
| Z4    | 0  | 0   | 0    | 0                     | 0   | 12   |
| Z6    | 0  | 0   | 13   | 0                     | 0   | 13   |
| Z8    | 0  | 0   | 20   | 0                     | 0   | 31   |
|       | N.A  |     |      | N.A                   |     |      |

### Conclusion

The authenticity and effectiveness of the prepared compounds were verified through spectroscopic and physical tests. The effectiveness of the obtained composition was verified by carbon nuclear magnetic resonance, proton and infrared spectroscopy, and some of the compounds produced had an inhibitory effect on Gram-positive and Gram-negative bacteria, where compound Z8 showed an activity comparable to the antibiotic. Ciprofloxacin was used as a control sample, while the others were inactive. The results were compared with antibiotic control samples.

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## 2-BROMIZOFTALALDEHİDDƏN ALINAN TETRAZOL HALQASININ YENİ TÖRƏMƏLƏRİNİN ALINMASI, DİAQNOSTİKASI VƏ ONLARIN BİOLOJİ EFFEKTİVLİYİNİN QIYMƏTLƏNDİRİLMƏSİ

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**Xülasə:** İşdə anilin əvəzediciləri ilə 2-bromizoftalaldehiddən Şiff əsaslı törəmələr (Z1-Z5) alınmışdır. Dioksandan həlledici kimi istifadə etməklə tetrazolun pentatsiklik halqaları (Z6-Z10) alınmış və sintez edilmiş birləşmələrin natrium azidlə reaksiyası öyrənilmişdir. Alınmış birləşmələr (Z6-Z10) infraqırmızı spektroskopiyaya (FTIR), proton ( $^1\text{H-NMR}$ ) və karbon nüvə maqnit rezonans spektroskopiyaları ( $^{13}\text{C-NMR}$ ) ilə xarakterizə edilmişdir. Birləşmələrin ərimə temperaturları təyin olunmuşdur. Alınmış bir sıra birləşmələrin iki növ qram-müsbət və qram-mənfi bakteriyalara qarşı bioloji effektivliyi qiymətləndirilmişdir.

**Açar sözlər:** tetrazol, Şiff əsasları, bioloji aktivlik.

## ПОЛУЧЕНИЕ И ДИАГНОСТИКА НОВЫХ ПРОИЗВОДНЫХ ТЕТРАЗОЛЬНОГО КОЛЬЦА ИЗ 2-БРОМИЗОФТАЛАЛДЕГИДА И ОЦЕНКА ИХ БИОЛОГИЧЕСКОЙ ЭФФЕКТИВНОСТИ

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**Абстракт:** В данном исследовании были получены производные Шиффовых оснований (Z1-Z5) на основе 2-бромизофталаaldeгида с анилиновыми заместителями. Взаимодействием полученных соединений с азидом натрия с использованием диоксана в качестве растворителя получены пентациклические кольца тетразола (Z6-Z10). Полученные соединения (Z10-Z6) были охарактеризованы измерениями инфракрасной спектральной флуоресценции и спектроскопии протонного ( $^1\text{H-NMR}$ ) и углеродного ядерного магнитного резонанса ( $^{13}\text{C-NMR}$ ). Измерены температуры плавления и оценена биологическая эффективность ряда синтезированных соединений для двух типов грамположительных и грамотрицательных бактерий.

**Ключевые слова:** тетразол, основания Шиффа, биологическая активность.