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Synthesis, Characterization and Evaluation of the Antimicrobial Activity of a New [1,2,4] Triazolo [3,4-B] [1,3,4] Thiadiazoles Derivatives

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Abstract

In this study, synthesis of [D-H] via reaction of compound [C] with p-alkoxybenzaldehyde. The derivative [C] that produces from reaction the 4-amino-5-(4-aminophenyl)-4H-1,2,4-triazole-3-thiol with phosphoryl chloride. The synthesized derivatives are characterized by ^{1}HNMR and FTIR spectroscopies. The antibacterial activity of the obtained thiadiazol derivatives [D-H] was screened against Gram-positive bacteria viz. *(Staphylococcus aureus, Bacillus subtilis)* and Gram-negative bacteria viz. *(Escherichia coli)* by disc diffusion method, the results showed that compound [H] has the highest activity toward *Bacillus subtilis* and *Staphylococcus aureus*, while compounds [D] showed the highest activity toward *E. coli*.

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1. Introduction

The five-membered aromatic azole ring of triazole compounds, which includes three nitrogen atoms, is easily able to interact with a wide range of receptors and enzymes in biological processes by a variety of non-covalent interactions and, as a result, exhibits a wide range of physical activities [1]. Triazole is the most basic group of the triazole family. Triazole is a white-to-pale-yellow crystalline solid with a faint, distinctive odor. It is soluble in water and alcohol, and dissolves at 120 degrees Celsius [2]. Hydrogen bonds may form with triazoles. This property refers to the molecules' higher solubility as well as their ability to bind biomolecular targets. Triazoles have the potential to become enticing linker molecules that join two pharmacophores to generate a novel bifunctional medication. As a result, these molecules are becoming increasingly valuable and essential in the development of bioactive and functional molecules [3]. They are also considered important materials in catalysis, polymer and medicinal chemistry. The 1,3,5-thiadiazol- containing compounds have found many applications. Thiadiazol derivatives that are synthesized can be simply derived from inexpensive start materials [4-6]. Hugo Schiff became the first scientist who identified Schiff bases in 1864 [7], A Schiff's base is a chemical compound also called imine or azomethine, is a functional group that includes a carbon-nitrogen double bond. Schiff bases are used in versatile applications such as inorganic and organic synthesis processes, pigments and catalysts. They are also used as intermediates in the synthesis of organic materials and as polymer stabilizers [8]. Schiff bases exhibit biological activities making them useful as antifungal and antibacterial agents. They found applications against some diseases such as malaria and some viruses due to their antiviral properties. Schiff bases are also used as antipyretic [9-10]. In this study, the new derivatives of thiadiazol derivatives that were synthesized were characterized by FTIR and ^{1}HNMR gives good biological activity against different bacteria by inhibition of the growth zone.

2. Materials and Methods

All the chemicals used in the present work are procured from different sources such as Merck and Fluka. They were carefully weighed to maintain the stoichiometric requirements of the chemical reactions.

2.1. Synthesis of 4-aminobenzohydrazide [A], [11].

A mixture of 0.01 mol (or 1.65 g) of ethyl-4-aminobenzoate and 10 ml of hydrazine hydrate was

added to 30 ml of ethanol and refluxed for 6 hours. The precipitate was infiltrated and washed thoroughly with distilled water. The precipitate was white powder whose melting point is 210–213 °C. The synthesis reaction yielded 83% of the reactants used.

2.2. Synthesis of [B], [12-13]

In 200 ml of absolute ethanol, dissolve 0.15 M of KOH and add it to 3 g of 0.01 M 4-aminobenzohydrazide [A]. In this solution, 15 ml of 0.3 M hydrazine hydrate and 40 mL of 0.15 M carbon disulfide were added in small portions with constant stirring and refluxed for seven hours. The precipitate underwent filtration, a cautious cold-water wash, and ethanol recrystallization. The precipitate has a yellow color 72% yield of 4 g of 0.01 M and a melting point of 162-165 °C.

2.3. Synthesis of [C], [11]

A mixture of 1.5 g of 0.01 mol of p-methoxy benzoic acid, 0.01 mol of derivative [B] and 5 ml of phosphoryl chloride was refluxed for 7 hours. The mixture was left to cool down before distal water was added. The mixture was then infiltrated and weighed. The precipitate has been melting point of 150-153 °C and is yellow.

2.4. Synthesis of [D-H], [14-15]

In a mixture of ethanol and two drops of concentrated glacial acetic acid with 18 mL of 0.01 mol of p-alkoxy benzaldehyde, 0.01 mol of the derivative [C] was refluxed for 5 hours. The solution was left to cool down to room temperature before being precipitated. The precipitate was filtered under a vacuum. Then it was washed with cold ethanol. The product was left to recrystallize in hot ethanol. The reaction yield was 78% which resulted in 5 g of 0.01 M of the product.

3. Results and Discussion

The desired compounds were synthesized using the following scheme: 4-amino-5-(4-aminophenyl)-4H-1,2,4-triazole-3-thiol was produced by cyclization with carbon disulfide in basic media after 4-aminobenzohydrazide was prepared from the reaction of 4-aminoethylbenzoate and hydrazine hydrate. The last compound reacts with different 4-alkoxybenzoic acid to give the titled compounds [D-H]. The synthesized compounds were characterized via FT-IR and ^{1}HNMR spectroscopy.

4-aminobenzohydrazide [A]: Color: white precipitate. Yield: 83%. Melting point: 210 - 213 °C. FT-IR: 3419 and 3338 υ(NH_2), 3028 υ(C-H) Aroma, 1756 υ(C=O), 1594 υ(C=C) Aroma [16] Figure 1. [B]: Color: yellow. Yield: 78%. Melting point: 162 - 165 °C. FT-IR: 3422 and 3338 υ(NH_2) [17], 3128 υ(C-H) Aroma, 1609 υ(C=N), 1580 υ(C=C) Aroma, 1005 υ(C-S), 830 υ para substituted [18] Figure 2. ^{1}H NMR (400 MHz, d6-DMSO, ppm) δ: 12.4 (2H, s, 1H) [19], 7.32-6.70 (4H, m, Ar-H), 7.8 (4H, d, NH_2) [17].

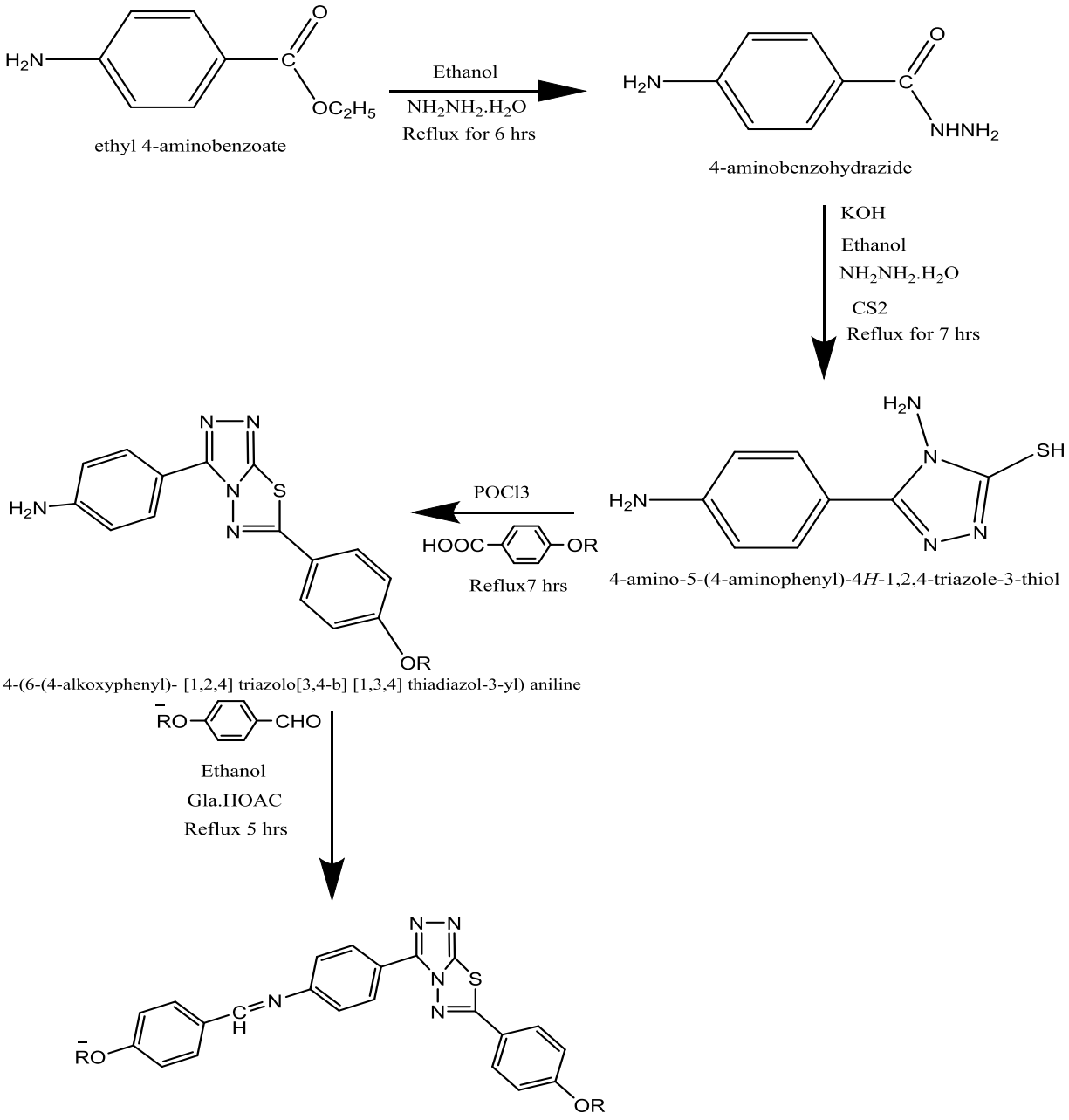
compound [C]: Color: yellow. Yield: 81%. Melting point: 150 - 153 °C. FT-IR: 3420 and 3335 υ(NH_2), 3100 υ(C-H) Arom, 2936 and 2897 υ(C-H) aliph, 1668 υ(C=N), 1600 υ(C=C) Aroma, 1253 υ(C-O), 831 υ para substituted [16] Figure 3. ^{1}H NMR (400 MHz, d6-DMSO, ppm) δ: 7.8 (2H, s, NH_2), 7.22-6.70 (8H, m, Ar-H), 3.8 (3H, OCH_3).

[D]: Color: yellow. Yield: 83%. Melting point: 140 - 143 °C. FT-IR: 3000 υ(C-H) Aroma, 2924 and 2834 υ(C-H) aliph, 1670 υ(HC=N) imine group, 1606 υ(C=C) $_{Aroma}$, 1247 and 1172 υ(C-O), 828 υ para substituted [16] Figure 4. ^{1}H NMR (400 MHz, d6-DMSO, ppm): azomethane proton singlet at 8.7. The protons in the phenyl rings may be responsible for signals at δ 6.9 – 8.1 (12 H). In addition, the 1HNMR showed multiplet signals at 1.6 – 1.9 (6 H), triplet signals at 0.9 (3 H), and a triplet signal at the value of 3.9 (2 H, OCH2). These signals might be attributed to the methoxy group.
The terminal methyoxy group appears as a singlet at (3.8) ppm for (3H) Figure 5.

[E]: Color: yellow. Yield: 74%. Melting point: 160-163°C. FT-IR: 3000 υ(C-H) Aroma, 2932 and 2836 υ(C-H) aliph, 1675 υ(HC=N) imine group, 1598 υ(C=C) Aroma, 1250 and 1173 υ(C-O), 830 υ para substituted [16] Figure 6. ^{1}H NMR (400 MHz, d6-DMSO, ppm): azomethane proton singlet at ≀ 8.5. Protons in the phenyl rings may be responsible for signals at δ 6.5–8.1 (12 H). Additionally, the methoxy group could be identified by the triplet signal at 3.9-4.0 (2 H, OCH2), multiplet signal at 1.7 – 2.0 (6 H), and triplet signal at 1.04-1.06 (3 H) detected by the 1HNMR.
The terminal methoxy group appears as a singlet at 3.8 ppm for (3H) (see Figure 7).

[F]: Color: yellow. Yield: 82%. Melting point: 110-113°C. FT-IR: 3006 υ(C-H) Aroma, 2937 and 2835 υ(C-H) aliph, 1677 υ(HC=N) imine group, 1601 υ(C=C) Aroma, 1248 and 1171 υ(C-O), 829 para-substituted [16] Figure 8. ^{1}H NMR (400 MHz, d6-DMSO, ppm): azomethane proton singlet at 8.3. Protons in the phenyl rings may be responsible for signals at δ 6.9–7.4 (12 H). Figure 9. The ^{1}HNMR also showed a triplet signal at δ 3.9-4.1 (2 H, OCH_2)

and a multiplet signal at δ 1.6 – 1.8 (4 H). additionally, it showed a triplet signal at 0.8 – 1.2 (3 H), which the methoxy group might be associated with. The terminal methoxy group appears as a singlet at 3.8 ppm for (3H).



OR : OCH_3 or $OCH_2CH_2CH_3$

$O\bar{R}$: $OCH_2(CH_2)_6CH_3$, $OCH_2(CH_2)_3CH_3$, $OCH_2CH_2CH_2CH_3$, $OCH_2CH_2CH_3$, OCH_2CH_3

Scheme 1: Synthetic route for the synthesized compounds.

[G]: Color: brown. Yield: 76%. Melting point: 130-133°C. FT-IR: 3055 υ(C-H) Aroma, 2944 and 2890 υ(C-H) aliph, 1684 υ(HC=N) imine group, 1600 υ(C=C) Aroma, 1247 and 1156 υ(C-O), 825 υ para substituted Figure 10 ^{1}H NMR (400 MHz, d6-DMSO, ppm): singlet at 8.5 for azomethane proton; signals at 7.3 – 8.1 (12 H) could be ascribed to the protons of the phenyl rings Figure 11. Additionally, the 1HNMR displayed a triplet signal at 4.7 (2 H, OCH2) and multiple signals at 1.9 – 2.1 (2 H) as well as triplet signal at 0.9 – 1.1 (3 H) that could be ascribed to the methoxy group. The terminal methyoxy group appears as a singlet at (3.8) ppm for (3H).

[H]: Color: brown. Yield: 79%. Melting point: 170-173°C. FT-IR: 3055 υ(C-H) Aroma, 2925 and 2856 υ(C-H) aliph, 1670 υ(HC=N) imine group, 1602 υ(C=C) Aroma, 1250 and 1168 υ(C-O), 831 υ para substituted Figure 12. ^{1}H NMR (400 MHz, d6-DMSO, ppm) Figure 13: singlet at δ (8.7) ppm due to (CH = N,1H), multiplet signs between 6.5 and 8.1 ppm for (Ar. H, 12H). The ethoxyoxy groups appear as a quartet at (3.3) ppm due to (OCH_2, 2H), triplet at (1.9) ppm due to (- CH_3, 3H), the terminal methyoxy group appears as a singlet at (3.8) ppm for 3H.

3.1 Antibacterial Activity

Using the disc diffusion technique, compounds [D–H] were tested for their antibacterial activity against four typical Gram-positive (Staphylococcus aureus, Bacillus subtilis) and Gram-negative (Escherichia coli) bacteria. The produced compounds' antibacterial efficacy was assessed in vitro. Using an agar plate seeded with the test organism, a standard 5mm diameter sterilized filter paper impregnated with the substance (1 mg per 1 ml DMSO and 1 mg per 10 ml DMSO for varying concentrations) was inserted in this way. The plates were incubated at 37 0C for a whole day. Depending on the diameter, the zone of inhibition of bacterial growth was determined in millimeters. Generally speaking, the antibacterial activity of the compounds against *Staphylococcus aureus, Bacillus subtilis* (Gram-positive) was superior to that observed against E. coli (Gram-negative), which is likely attributed to the presence of lipopolysaccharide in E. coli's outer membrane. These components function as a defense mechanism against antibacterial agents, thereby explaining the lesser effectiveness of the compounds against E. coli. Table 1 indicated that compound H was the most effective antibacterial agent against *Staphylococcus aureus and Bacillus subtilis*, with an inhibition zone measuring 22 and 20 mm, respectively, while compound D showed an effect against *Escherichia coli*) with an inhibition zone of 18 mm as shown in Table 1 and Figure 14.

Table 1. Antibacterial activity of the synthesized compounds [D-H].

Comp. No.	Zone of inhibition (mm)				
	D	**E**	**F**	**G**	**H**
Staphylococcus aureus	14	16	18	17	22
Bacillus subtilis	11	12	14	13	20
Escherichia coli	18	11	10	11	16

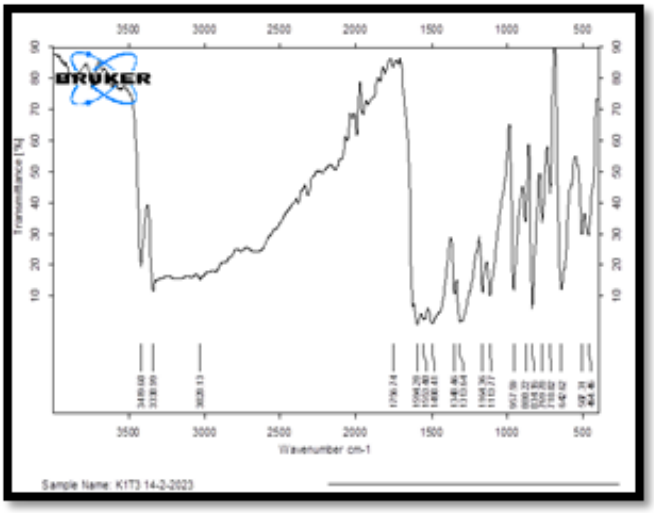


Figure 1. FTIR A.

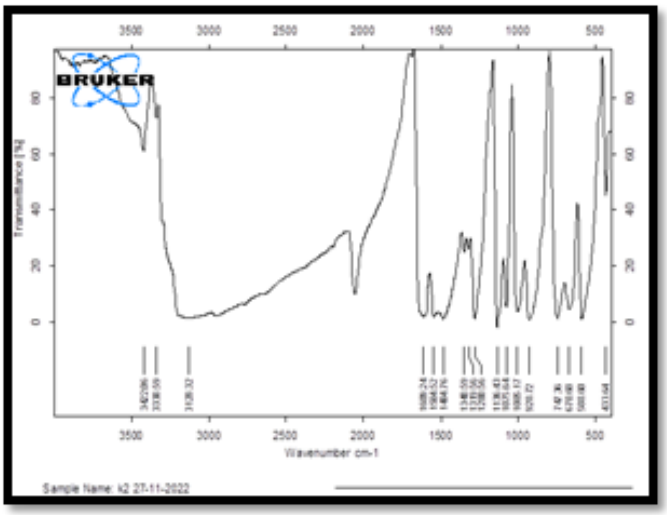


Figure 2: FTIR B.

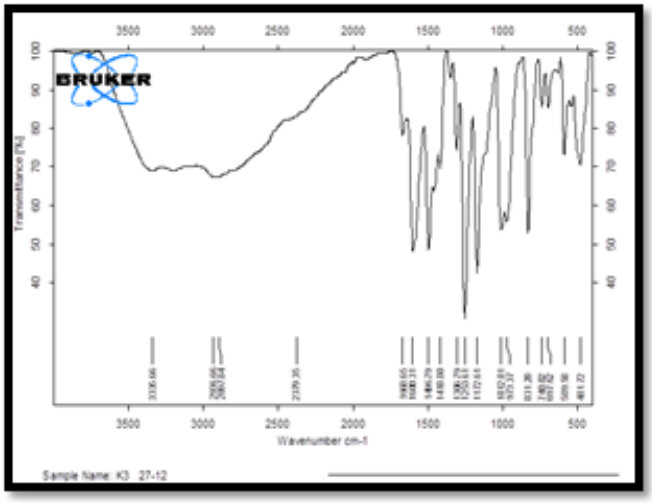


Figure 3: FTIR C.

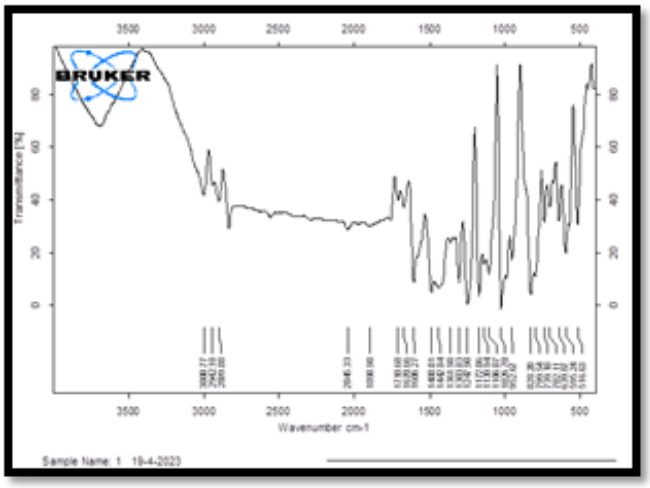


Figure 4: FTIR D.

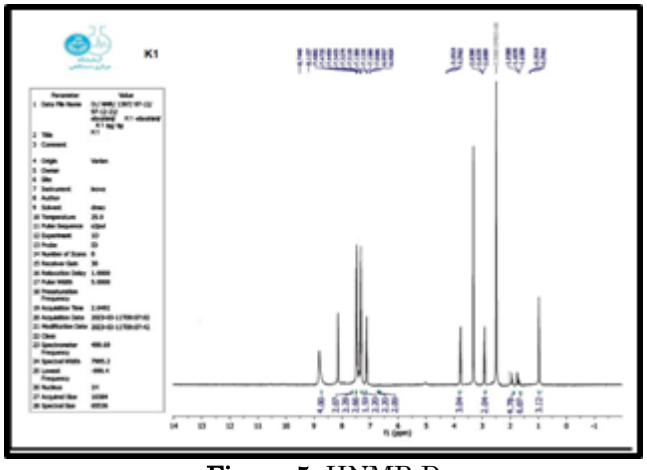


Figure 5. HNMR D.

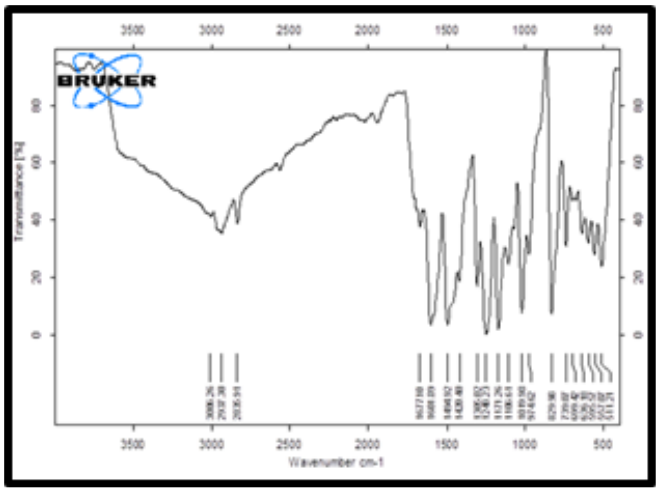


Figure 6. FTIR E.

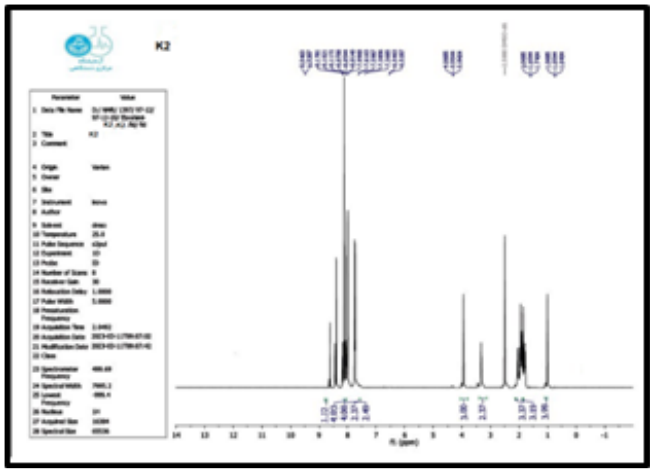


Figure 7. HNMR E.

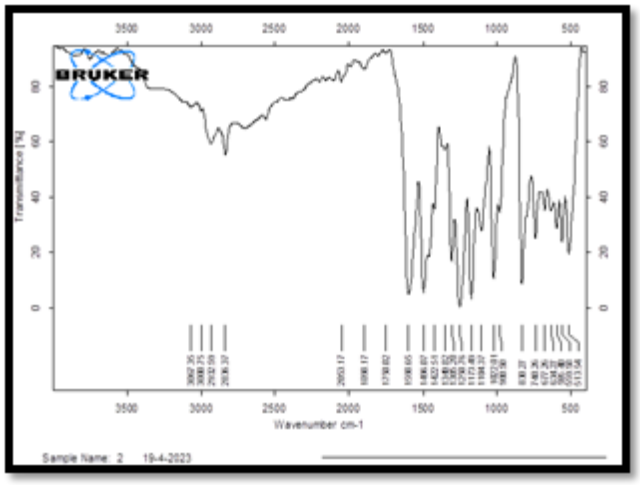


Figure 8. FTIR F.

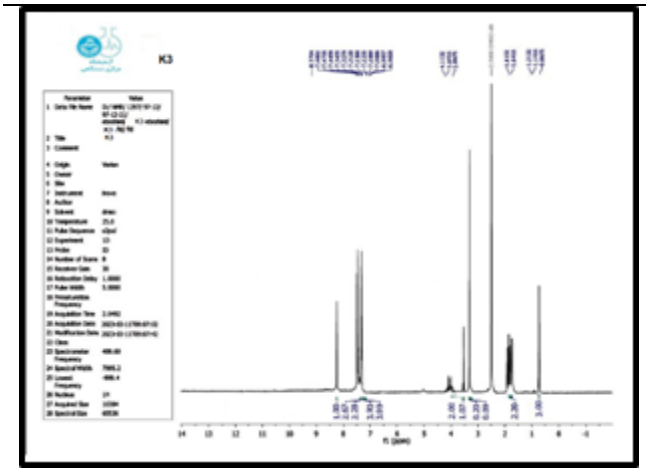


Figure 9. HNMR F.

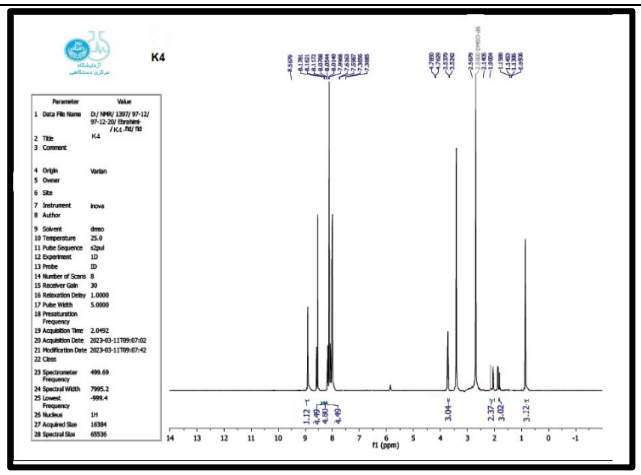


Figure 11. HNMR G.

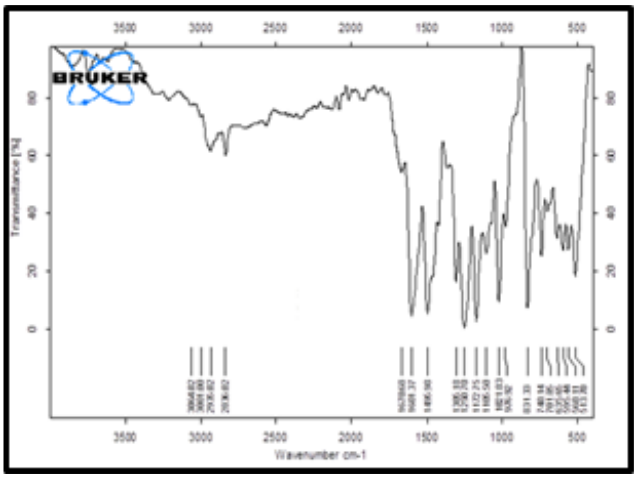


Figure 10. FTIR G.

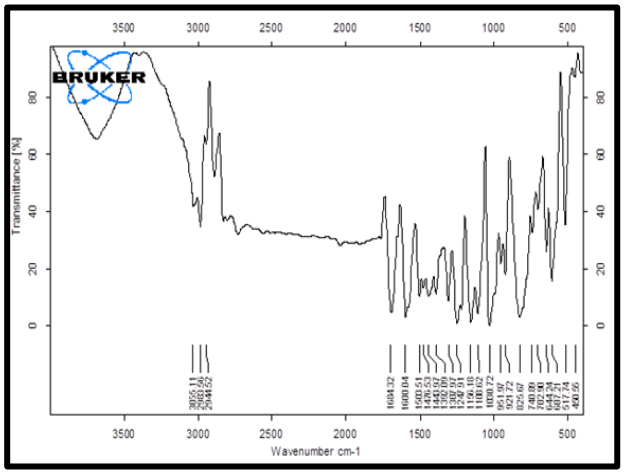


Figure 12. FTIR H.

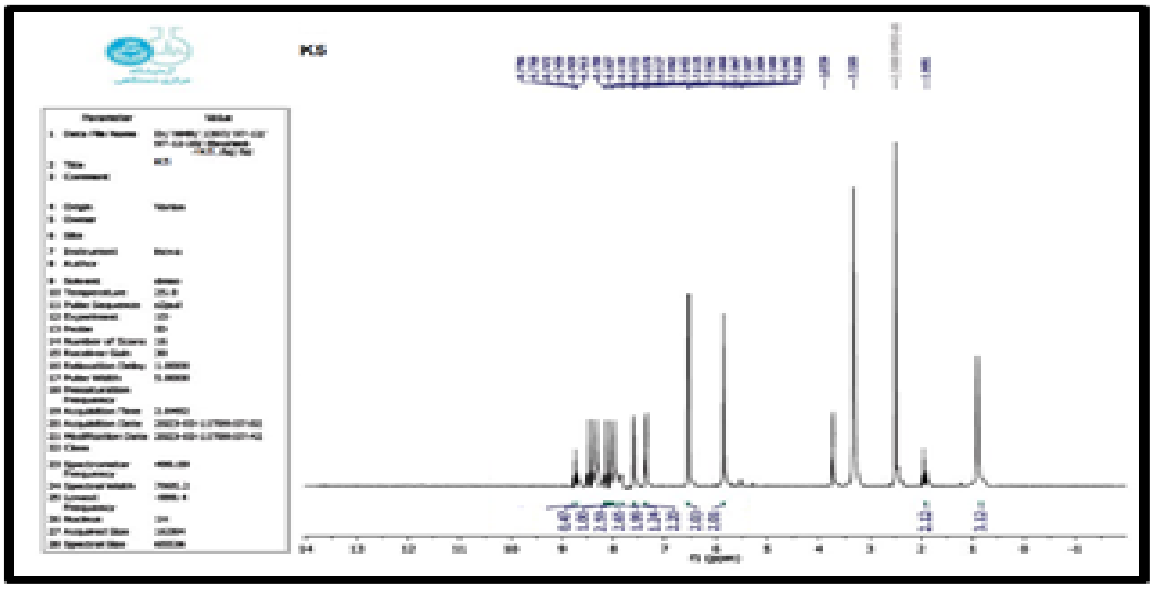


Figure 13. HNMR H.

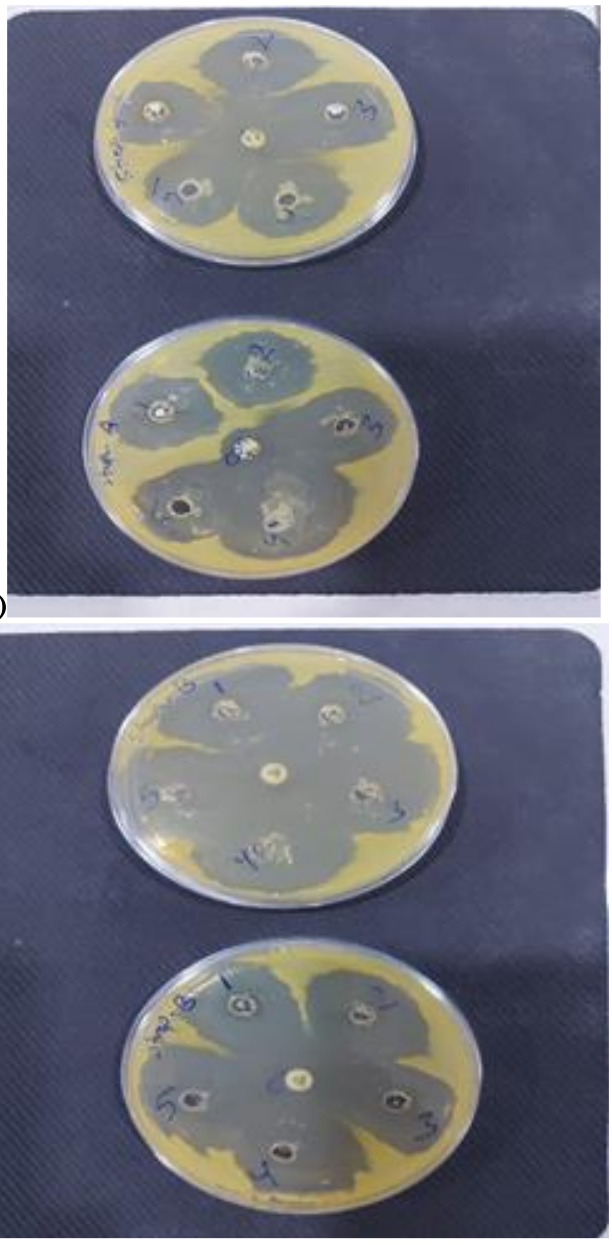


Figure 14. Inhibition zone of synthesized compounds on *Staphylococcus aureus, Bacillus subtilis*) and (*Escherichia coli*).

4. Conclusions

We synthesized many thiazole derivatives (4-8) and investigated their properties using two spectroscopic methods (FT-IR and 1H-NMR). The antibacterial activity of the synthesized compounds was tested against different types of bacteria. The antibacterial properties are believed to be unique for the compounds synthesized in this work thus making these as potential candidates for the respective biological applications.

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