

Synthesis and Characterization of New Diazo Derivatives, and Study of their Biological Activity

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ABSTRACT

This work included the synthesis di azo compound in many steps. The first react is 4-methoxy-2-nitroaniline with 4-hydroxybenzoic acid to get azo derivative. The second step involves react with thiosemicarbazide to get thiadiazol. The last step is reaction with imidazole, 8-hydroxy quinoline, p-hydroxybenzoic acid, 4,5-dichloro imidazole, and 4,5-diphenyl imidazole. All these compounds are characterized by Fourier transform infrared spectroscopy, proton nuclear magnetic resonance (FTIR, ¹H-NMR) spectroscopy and evaluated against two kinds of bacteria and two types of antifungals.

Keyword: 4-hydroxybenzoic acid, 4-methoxy-2-nitroaniline, Biological activate, Diazo.

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Conflict of interest: None

INTRODUCTION

Heterocyclic compounds that are organic compounds containing at least one carbon atom linked to a different atom, and the most common heteroatoms are oxygen, nitrogen, and sulfur, which have a great ability to form heterocyclic compounds,^{1,2} and also heterocyclic compounds may be saturated or aromatic in nature depending on their chemical composition.³ These compounds are distinguished by their pharmacological and biological efficacy; Examples include beta-lactam, thiazolidine, amidazolidine, tetrazole, oxazepine, thiazinone, and hydroquinazoline.^{4,5} Sulfur-containing organic molecules have gained a special interest in the field of medicinal chemistry. Heterocyclic compounds with nitrogen and sulfur in their structures are often used in the design of medicinal chemistry drugs.⁶

Thiadiazole is a heterocyclic and unsaturated organic compound, having the molecular formula C₂H₂N₂S. It's structurally composed of a pentagonal ring containing three heterocyclic atoms, two nitrogen atoms, one sulfur atom, and two double elements.⁷ As it has a wide importance in the pharmaceutical and industrial fields, and this is what encouraged researchers to study these compounds, as a large number of 4,3,1-thiadiazole derivatives were synthesized, as it was observed that these derivatives possess many pharmacological activities⁸ such as antimicrobial, analgesic, and anti-inflammatory, Anti-cancer, central nervous system (CNS) depressants, and antioxidant.^{9,10}

This work also prepare azo compound, it contains the functional group R-N = N-R 'where R and R' may be the alkyl group or aryl and the -N = N functional group is called the azo group.¹¹ These two atoms function as a bridging group linked to many different groups, whether they are aliphatic or aromatic, and azo-aliphatic dyes are of little prevalence due to their rapid dissociation into nitrogen and hydrocarbons.¹² The biological importance of Azo compounds is well known due to their use as inflammatory, anti-diabetic, antibacterial, and antifungal.^{13,14}

MATERIAL

The chemicals used in this research were of a high degree of purity, include: 4-Methoxy-2-nitroaniline, 4,5-diphenylimidazole, Sodium Hydroxide, Potassium Hydroxide (Sigma Aldrich, Germany), 4,5-dichloroimidazole (TGI, China), 4-hydroxybenzoic acid (Riedel de Haen, Germany), thiosemicarbazide, ethanol absolute (Scharlau, Spain), hydrochloric acid (Himedia, India), sodium nitrite, imidazole (B.D.H, England), phosphorous oxychloride (CDH, India) and 8-hydroxyquinoline (SCR, China).

INSTRUMENTS

Melting points were measured using digital (Stuart, UK). FT-IR Spectra (4000–400 cm⁻¹) were recorded on Shimadzu FT-IR 8400S Fourier Transform infrared spectrophotometer as KBr disc. ¹HNMR was recorded on Fourier transformation Bruker spectrometer operating at (500MHz) with (DMSO) measurements were made at Tehran University, Iran.

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EXPERIMENTAL
Synthesis of Azo Compounds (1) 4-hydroxy-3-((4-methoxy-2-nitrophenyl)diazenyl)benzoic Acid¹⁵

In the first line: the azo compound was prepared by dissolving (0.0059 mol, 1 g) of 4-Methoxy-2-nitroaniline in a solution consisting of 4mL of hydrochloric acid and (30ml) of distilled water, and the solution was cooled to 0-5°C, then the prepared sodium nitrite solution was added by dissolved (0.0059 mol, 0.4071 g) of sodium nitrite in (10ml) of distilled water, and then the Diazonium salts solution was added drop by drop with stirring noting that the temperature did not rise above 0-5°C, after which the solution was kept with cooled and stirring for 20 minutes, during addition, the temperature of each the reaction mixture was maintained up to 0-5°C. Then this solution was added drop by drop to the solution consisting of (0.0059 mol, 0.8412 g) of 4-hydroxybenzoic acid and (1 g) of sodium hydroxide dissolved in (30 mL) of distilled water cooled to 0-5°C and (5 mL) of absolute ethyl alcohol, pH = 6 was made for the mixture and stirring for (1-hour) at the same temperature, and the solution was kept for (24 hours), then the precipitate was filtered off and washing by distilled water and recrystallized with absolute ethyl alcohol. The physical properties are shown in Table 1. The general reaction for synthesis of Azo compounds 1 in Scheme 1.

Synthesis of Thiadiazol Derivative Compound (2) 4-(5-amino-1,3,4-thiadiazol-2-yl)-2-((4-methoxy-2-nitrophenyl)diazenyl)phenol.¹⁶

In the second line: the thiadiazol derivative compound was prepared by mix equal moles of azo compound 1 (0.0031mol, 1 g) with thiosemicarbazide (0.0031 mol, 0.2872 g) in 13 mL phosphorous oxychloride (POCl₃), the reaction mixture was 3 hours), after that, the solution cooled and added (40 mL) distill water and refluxed for 4 hours, Then the mixture was filtered,

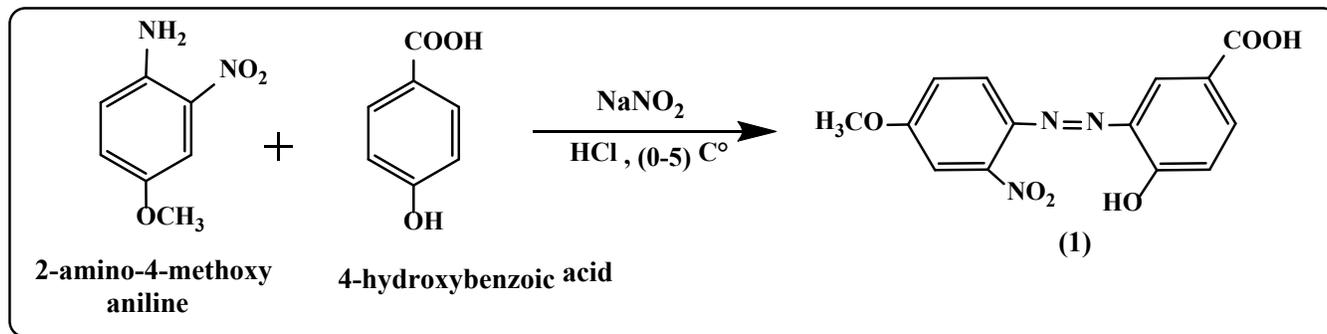
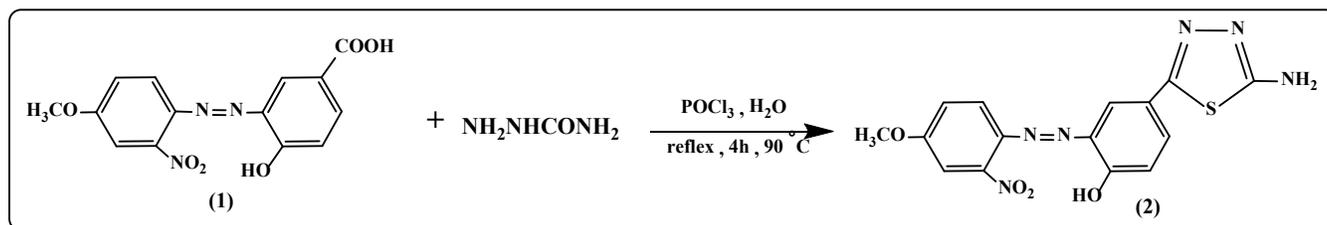
and the filtrate was treated with Potassium Hydroxide solution until the solution became neutral, then the sediment was collected after filtering and washed with distilled water, dried and recrystallized with absolute ethanol, and the reaction was followed by thin-layer chromatography (TLC) using the mobile phase (methanol: benzene) at a ratio of (1: 4). On a dark brown precipitate, they were knowing that R_f = 0.34. The physical properties are shown in Table 1—the general reaction for the synthesis of thiadiazol derivative compound (2) in Scheme 2.

Synthesis of Diazo Compounds (3-7)¹⁷

In the third line: the diazo compounds was prepared by dissolving (0.0013 mol, 0.5 g) of thiadiazol derivative (2) in a solution consisting of 4 mL of hydrochloric acid and (30 mL) of distilled water, and the solution was cooled to 0-5°C, then the prepared sodium nitrite solution was added by dissolved (0.0013 mol) of sodium nitrite in (10 mL) of distilled water, and then the Diazonium salts solution was added drop by drop with stirring noting that the temperature did not rise above 0-5°C, after which the solution was kept with cooled and stirring for (20 min), during addition, the temperature of each the reaction mixture was maintained up to 0-5°C. Then this solution was added drop by drop to the solution consisting of (0.0013 mol) of each of (imidazole, 8-Hydroxyquinoline, 4-hydroxybenzoic

Table 1: Physical properties of the compounds prepared.

Compounds	Colour	M. P (°C)	M, Wt (g/mol)
1	Orange	145	317.26
2	Brown	126	372.36
3	Dark Brown	157	415.42
4	Dark Brown	154	528.50
5	Dark Brown	146	521.46
6	Dark Brown	155	520.31
7	Dark Orange	160	603.62


Scheme 1: Synthesis of 4-hydroxy-3-((4-methoxy-2-nitrophenyl) diazenyl) benzoic acid (1)

Scheme 2: Synthesis of 4-(5-amino-1,3,4-thiadiazol-2-yl)-2-((4-methoxy-2-nitrophenyl) diazenyl) phenol (2)

acid, 4,5-dichloroimidazole and 4,5-diphenylimidazole and (1 g) of sodium hydroxide dissolved in (30 mL) of distilled water cooled to 0–5°C and (5 mL) of absolute ethyl alcohol, pH = 6 was made for the mixture and stirring for (1-hour) at the same temperature, and the solution was kept for (24 hours), then the precipitate was filtered off and washing by distilled water and recrystallized with absolute ethyl alcohol. The physical properties are shown in Table 1. The general reaction for the synthesis of Diazo compounds (3-7) in Scheme 3.

RESULTS AND DISCUSSION

Compound (1)

FT-IR spectrum data showed band at 3415 cm^{-1} for (OH) phenol, 3406 cm^{-1} for (OH) carboxylic acid, 3091 cm^{-1} for (C-H) Aromatic, 2947 cm^{-1} for (C-H) Aliphatic, 1558, 1330 cm^{-1} for (NO_2), 1456 cm^{-1} for (N=N), 1600, 1521 cm^{-1} for (C=C) Aromatic, 1683 cm^{-1} for (C=O), 1244 cm^{-1} for (C-O). The $^1\text{H-NMR}$ spectrum data of compound (1) showed δ : 10.4 (S, 1H, OH) phenol, 11.3 (S, 1H, OH) carboxylic acid, 6.9-8.6 (M, 6H, Ar-H), 3.9 (S, 3H, OCH_3), as in Figures 1 and 2.

Compound (2)

FT-IR spectrum data showed band at 3371, 3487 cm^{-1} for (NH_2), 3425 cm^{-1} for (OH), 3124 cm^{-1} for (C-H) Aromatic, 2954 cm^{-1} for (C-H) Aliphatic, 1643 cm^{-1} for (C=N), 1419 cm^{-1} for (N=N), 1596, 1473 cm^{-1} for (C=C) Aromatic, 1249 cm^{-1} for (C-O).

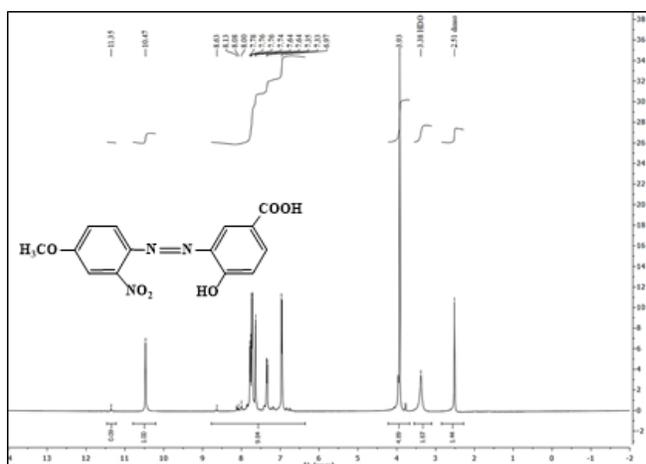


Figure 2: $^1\text{H-NMR}$ Spectrum of the Compound 1

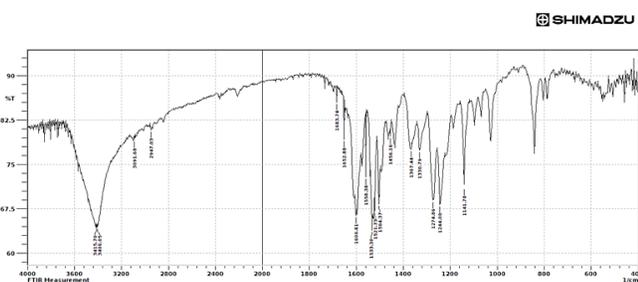


Figure 1: FTIR Spectrum of the Compound 1

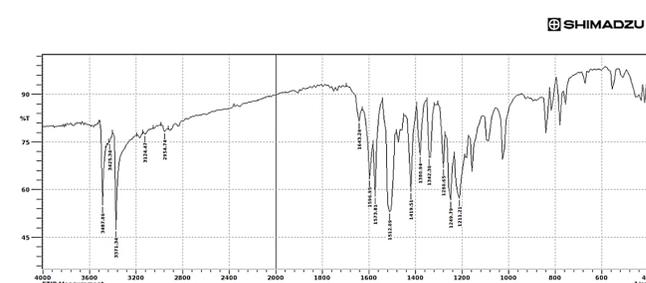
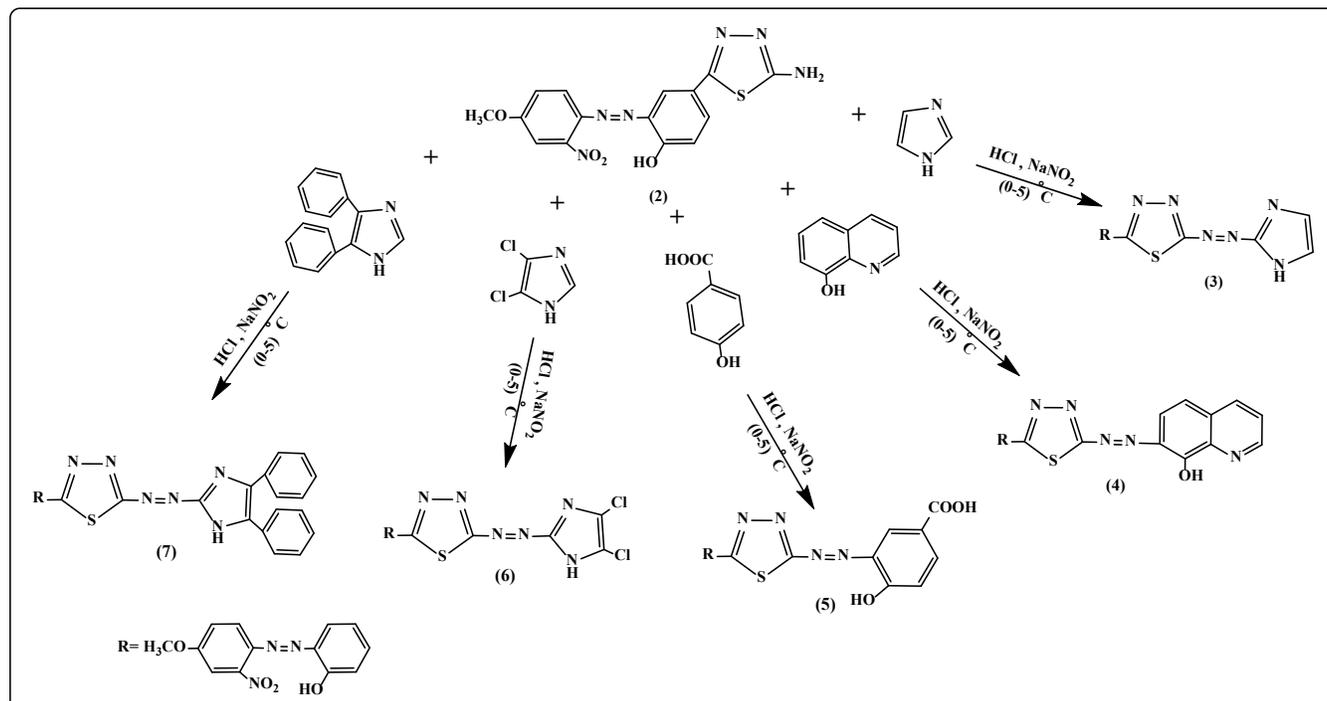


Figure 3: FT-IR Spectrum of the Compound 2



Scheme 3: Synthesis of diazo compounds (3-7)

1512, 1342 cm^{-1} for (NO_2). The $^1\text{H-NMR}$ spectrum data of compound (2) showed δ : 10.1 (S,1H,OH), 6.7-8 (M,6H,Ar-H), 4.7 (S,2H, NH_2), 3.36 (S, 3H, OCH_3), as in Figures 3 and 4.

Compound (3)

FT-IR spectrum data showed band at 3409 cm^{-1} for (OH), 3030 cm^{-1} for (C-H) Aromatic, 2923, 2854 cm^{-1} for (C-H) Aliphatic, 1643 cm^{-1} for (C=N), 1450 cm^{-1} for (N=N), 1604,1535 cm^{-1} for (C=C) Aromatic, 1504, 1350 cm^{-1} for (NO_2). The $^1\text{H-NMR}$ spectrum data of compound (3) showed δ : 10.5 (S,1H,OH), 8.5 (S,1H,NH), 6.6-8.1 (M,8H,Ar-H), 3.8 (S, 3H, OCH_3), as in Figures 5 and 6.

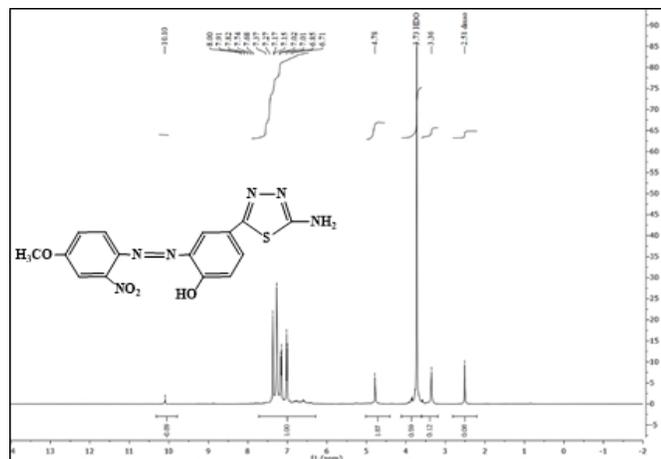


Figure 4: $^1\text{H-NMR}$ Spectrum of the Compound 2

Compound (4)

FT-IR spectrum data showed band at 3417 cm^{-1} for (OH), 3101 cm^{-1} for (C-H) Aromatic, 2923,2854 cm^{-1} for (C-H) Aliphatic, 1650 cm^{-1} for (C=N), 1465 cm^{-1} for (N=N), 1604,1535 cm^{-1} for (C=C) Aromatic, 1504, 1319 cm^{-1} for (NO_2), 1234 cm^{-1} for (C-O). The $^1\text{H-NMR}$ spectrum data of compound (4) showed δ : 9.02 (S,1H,OH), 10.02 (S,1H,OH), 7.02-8.1 (M,11H,Ar-H), 3.9 (S, 3H, OCH_3), as in Figures 7 and 8.

Compound (5)

FT-IR spectrum data showed band at 3425 cm^{-1} for (OH), 3101 cm^{-1} for (C-H) Aromatic, 2923,2846 cm^{-1} for (C-H) Aliphatic, 1643 cm^{-1} for (C=N), 1689 cm^{-1} for (C=O), 1604,1527 cm^{-1} for (C=C) Aromatic, 1458 cm^{-1} for (N=N), 1504, 1357 cm^{-1} for (NO_2). The $^1\text{H-NMR}$ spectrum data of compound (5) showed δ : 11.3 (S,1H,OH) carboxylic acid, 10.4,10.2 (S,2H,OH), 6.9-8.1 (M,9H,Ar-H), 3.9 (S, 3H, OCH_3), as in Figures 9 and 10.

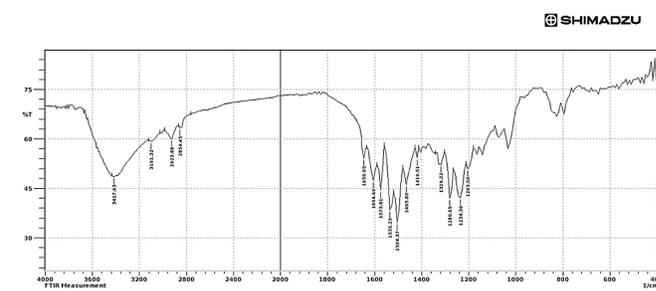


Figure 7: FT-IR Spectrum of the Compound 4

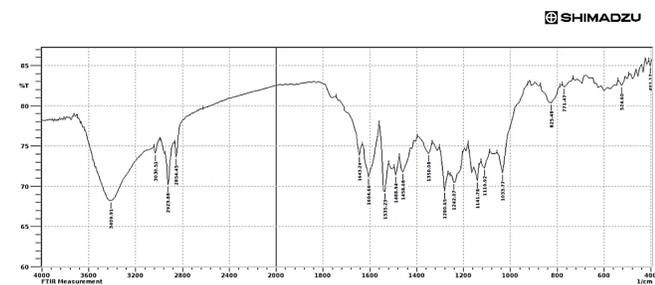


Figure 5: FTIR Spectrum of the Compound 3

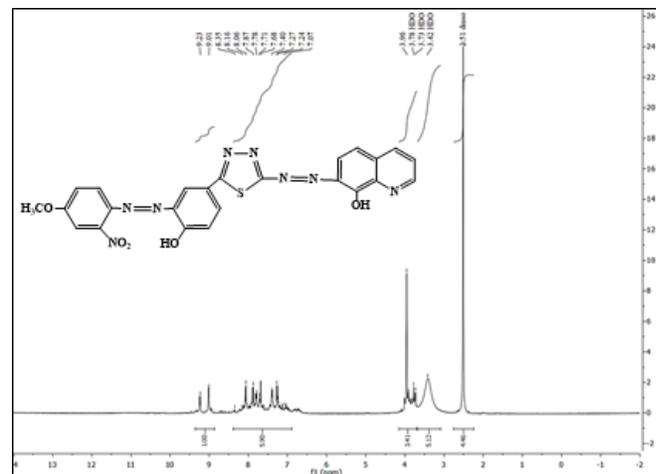


Figure 8: $^1\text{H-NMR}$ Spectrum of the Compound (4)

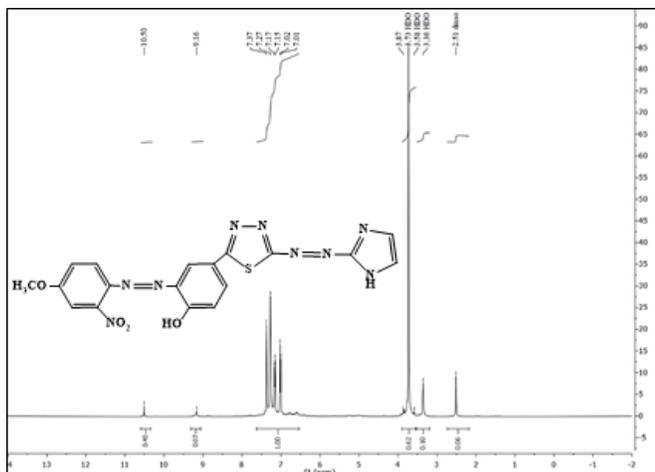


Figure 6: $^1\text{H-NMR}$ Spectrum of the Compound (3)

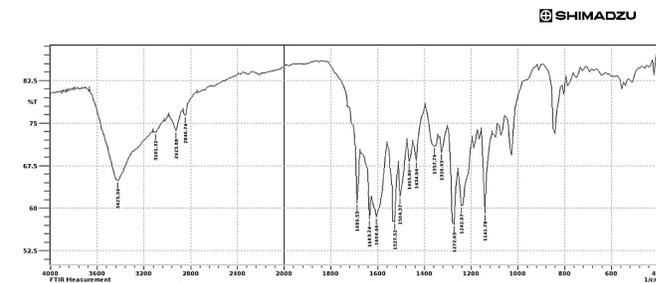


Figure 9: FTIR Spectrum of the Compound 5

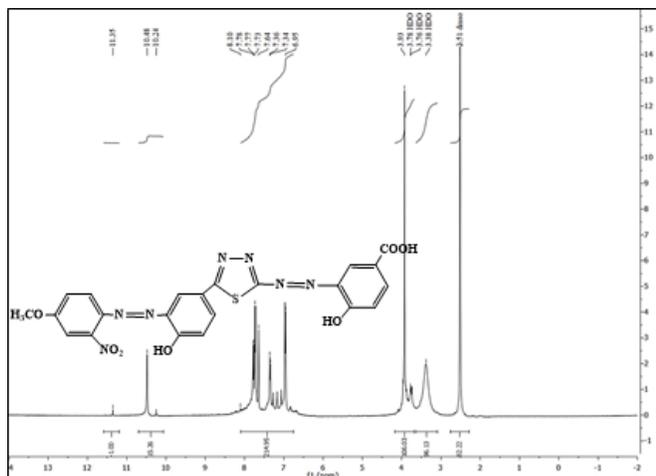
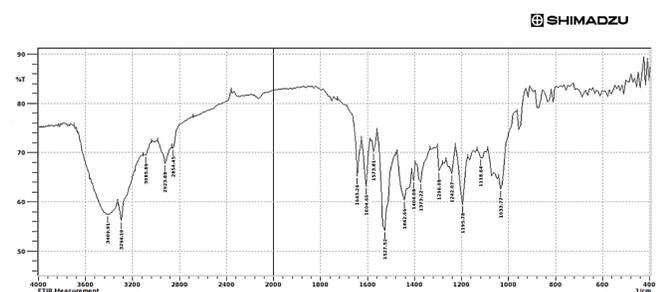
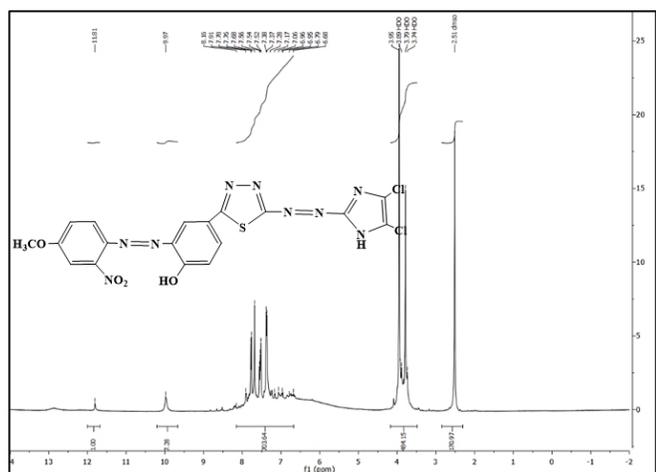

 Figure 10: $^1\text{H-NMR}$ spectrum of the Compound 5


Figure 11: FTIR spectrum of the Compound 6


 Figure 12: $^1\text{H-NMR}$ spectrum of the Compound (6)

Compound (6)

FT-IR spectrum data showed band at 3409 cm^{-1} for (OH), 3294 cm^{-1} for (NH), 3085 cm^{-1} for (C-H) Aromatic, $2923, 2854\text{ cm}^{-1}$ for (C-H) Aliphatic, $1604, 1527\text{ cm}^{-1}$ for (C=C) Aromatic, 1643 cm^{-1} for (C=N), 1442 cm^{-1} for (N=N), $1573, 1319\text{ cm}^{-1}$ for (NO_2). The $^1\text{H-NMR}$ spectrum data of compound (6) showed δ : 11.8 (S,1H,OH), 10 (S,1H,NH), 6.6-7.9 (M, 6H,Ar-H), 3.9 (S, 3H, OCH_3), as in Figures 11 and 12 .

Compound (7)

FT-IR spectrum data showed band at 3440 cm^{-1} for (OH), 3394 cm^{-1} for (NH), 3101 cm^{-1} for (C-H) Aromatic, $2923, 2854$

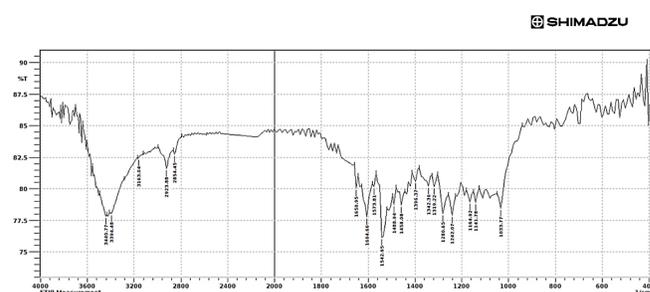


Figure 13: FTIR spectrum of the Compound 7

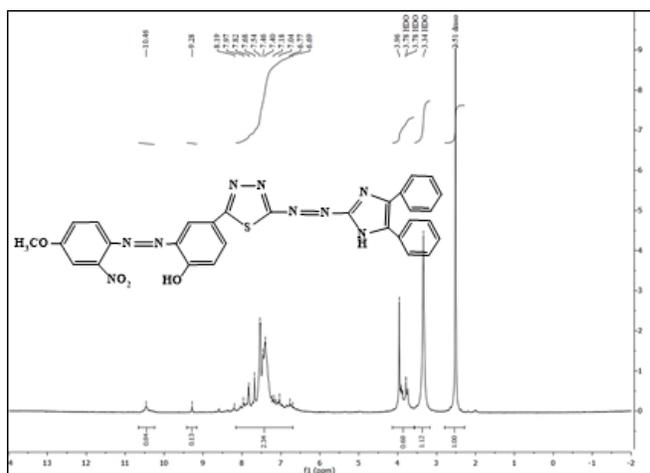

 Figure 14: $^1\text{H-NMR}$ spectrum of the Compound 7

Table 2: The antibacterial and antifungal activities of compounds (3-7)

Compounds	Conc. (M)	Antibacterial activity		Antifungal activity
		<i>Escherichia coli</i> (cm)	<i>Staphylococcus aureus</i> (cm)	<i>Aspergillus niger</i> (cm)
3	0.003	2.5	3	7
4	0.003	Zero	Zero	5.5
5	0.003	0.5	Zero	6
6	0.003	Zero	Zero	7
7	0.003	Zero	1.5	7

cm^{-1} for (C-H) Aliphatic, $1604, 4215\text{ cm}^{-1}$ for (C=C) Aromatic, 1650 cm^{-1} for (C=N), 1458 cm^{-1} for (N=N), $1512, 1342\text{ cm}^{-1}$ for (NO_2). The $^1\text{H-NMR}$ spectrum data of compound (7) showed δ : 10.5 (S,1H,NH), 9.2 (S,1H,OH), 6.9-8.1 (M,7H,Ar-H), 3.7 (S,3H, OCH_3), 3.9 (S,3H, N-CH_3), as in Figures 13 and 14.

BIOLOGICAL STUDY

The biological study of synthesized compounds (3-7) was studied and performed according to the agar plate method.¹⁸ The synthesized compounds were evaluated against representative *E. coli* (Gram-negative), *S. aureus* (Gram-positive), *A. niger* fungicide. Each compound was dissolved in DMSO to give a concentration of 0.003 M. Table 2 showed the zone of inhibition of the compounds (3-7); we noted that compounds (3, 5) have Antibacterial activity against (*E. coli*) and that compounds (3, 7) have Antibacterial activity against (*S. aureus*), while were that compounds (3-7) have higher Antifungal activity against (*A. niger*).

CONCLUSION

This research relied on the preparation of the d-azo compound, which mainly led to the diagnosis of the compounds prepared on several techniques thereof (FT-IR, ¹H-NMR) and their evaluation against two types of bacteria and two types of antifungals.

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