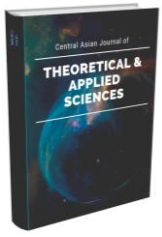

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Article

Synthesis and Characterization of Ethyl 4-(Trifluoromethyl) Benzoate Derivatives of Formazan and Evaluation of Biological Activity

Asmaa Badr Saber*[1]

1. Department of chemistry/college of education for pure science/ Tikrit university/ Iraq

* Correspondence: asmaa.b.sabr@tu.edu.iq

Abstract: The study included the use of the prepared hydrazide as a nucleophile in the formation of several organic compounds. This hydrazide was reacted with substituted benzaldehydes in the presence of ethanol as a solvent to produce hydrazones, which are nucleophiles used in the preparation of many organic compounds, where they react with 2,4-dichloroaniline to produce pharmaceutical preparations, and in the presence of pyrimidine as a solvent. The reaction was monitored by thin-layer chromatography, and the formed compounds exhibited physical changes such as color, yield, and melting point. The biological activity of some of the generated compounds was evaluated using two antibiotic-resistant bacterial isolates, Gram-positive (Staphylococcus aureus) and Gram-negative (Klebsiella pneumoniae). Fourier transform infrared spectroscopy (FTIR), hydrogen and carbon (1H&13C-NMR) spectroscopy, mass spectrometry, and quantitative C.H.N. analysis were used to confirm the accuracy of the prepared results.

Keywords: Formazan, Hydrazones, biological activity.

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Introduction

Formazan The functional groups of formazan compounds are arranged in an azohydrazon configuration, giving them a general structure. Bamberger and Von Pechman synthesized the first formazan independently, and many more were subsequently made and studied. Because of their $\pi \rightarrow \pi^*$ transitions of π-electrons, formazans are colored compounds that range from cherry-red to a deep purple-black and include atoms of (-N=N-C=NNH-)[1,2]. Formazans are typically solids with enormous structural dimensions and a comparatively low melting point[3]. Because of their accessibility, diverse chemical reactivity, and biological activity, formazans have been extensively researched [4]. Analgesic and anti-inflammatory [5], antitubercular [6], anticonvulsant [7], Antioxidant [8], and anticancer [9]. It also shows antibacterial activity[10]. The condensation reaction between hydrazine and different aldehydes or ketones [11] results in the formation of hydrazones, which are compounds made up of two nitrogen atoms (one hydrogen and the other double bonded to a third atom in the system, a carbon atom). This reaction happens through an unstable intermediate state [12] also known as hydrazone. az=(N)(C=O) hydra=(NH)[13].The most active part of the hydrazone system is the imine (azomethyne) group (C=N). Compounds derived from the condensation of ketones with primary amine groups are called ketimines, while compounds condensed with aldehydes are called aldehyde amines [14]. The study aims to prepare hydrazine derived from hydrazide and

use it as a nucleotide to prepare pharmazan derivatives. Then, the bacterial sensitivity against two types of Gram-positive and Gram-negative bacteria will be tested.

Materials and Methods

Chemicals used: Fluka, Merck, BDH Thomas, and Aldrich roduced the chemicals.

Preparation of hydrazone (SH_1-SH_5):

Equal moles (0.001 moles) of 4-(trifluoromethyl)benzo hydrazide and benzaldehyde equivalents were mixed in 20 mL of absolute ethanol. A drop of ice acetic acid was added, and the mixture was stirred for 4-6 hours. After that, it was concentrated, filtered, and recrystallized from ethanol, and the reaction's progress was monitored using TLC [15].

Preparation of formazan (SH_6-SH_{10}):

Drop by drop, while stirring, an aqueous solution of sodium nitrate (0.0069 g) was added to the crushed ice after the amine derivative solution (0.001 mol) had been dissolved in 10 mL of aqueous hydrochloric acid.A transparent solution of the amine's diazonium salt was the result of this procedure. The temperature was kept between (0-5) °C. This combination was then added to a 5 mL solution of Htdrazone (0.001 mol) dissolved in pyridine. For two hours, the reaction mixture was stirred while the temperature was kept between (0-5) °C. The mixture was then added to water while being constantly stirred. Following that, it underwent cleaning, filtering, and ethanol recrystallization [16].

(Z)-1-(2,4-dichlorophenyl)-3-(4-(dimethylamino)phenyl)-5-(4-(trifluoromethyl)benzoyl)formazan SH6

Dusty *(52%); mp* = 201 °*C; Elemental analysis* $C_{23}H_{18}Cl_2F_3N_5O$*; C.H.N Calcd: C, 54.35; H, 3.57; N, 13.78; Found: C, 54.9; H, 3.86; N, 14.06; IR v(cm⁻¹)= 3179 (NH),3076 (Ar-CH), 1658 (-C=O), 1598 (-C=N-), 1548,1523 (-C=C-), 1440 (N=N), 1230 (-C-N-), 1124(-N-N-), 915 (-C-F), 767 (-C-Cl) cm⁻¹;* ^{1}*H-NMR (400 MHz): δ(ppm) = 8.22 (s, 1H, -NH), 7.94-7.14 (11H, m, Ar–H), 2.38 (s, 6H,* $-CH_3$*);* ^{13}*C-NMR (101 MHz): δ(ppm) = 163.14 (-C=O), 149.04 (-C=N-), 138.63-125.23 (Ar-C=C-), 122.99 (-C-F), 27.93 (*$-CH_3$*);* The EI-MS m/z [M]+: 508 ($C_{23}H_{18}Cl_2F_3N_5O$), 155 ($C_8H_{16}N_3^+$).

(Z,E)-1-(2,4-dichlorophenyl)-3-(4-nitrophenyl)-5-(4-(trifluoromethyl)benzoyl)formazan **SH7**

Pink *(52%); mp* = 195 °*C; Elemental analysis* $C_{21}H_{12}Cl_2F_3N_5O_3$*; C.H.N Calcd: C, 49.43; H, 2.37; N, 13.73; Found: C, 50.17; H, 2.65; N, 13.31; IR v(cm⁻¹)= 3217 (NH),3070 (Ar-CH), 1666 (-C=O), 1604 (-C=N-), 1571,1508 (-C=C-), 1450 (-N=N-), 1249 (-C-N-), 1124(-N-N-), 966 (-C-F), 769 (-C-Cl) cm⁻¹;* ^{1}*H-NMR (400 MHz): δ(ppm) = 8.87 (s, 1H, NH), 7.85-7.13 (11H, m, Ar–H);* ^{13}*C-NMR (101 MHz): δ(ppm) = 162.77 (-C=O), 148.45 (-C=N-), 146.65-126.03 (Ar-C=C), 124.60 (C-F);* The EI-MS m/z [M]+: 510 ($C_{21}H_{12}Cl_2F_3N_5O_3$), 57 ($C_4H_9^+$)

(Z,E)-3-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-5-(4-(trifluoromethyl)benzoyl)formazan **SH8**

Light Brown *(66%); mp* = 194-195 °*C; Elemental analysis* $C_{21}H_{12}Cl_3F_3N_4O$*; C.H.N Calcd: C, 50.48; H, 2.42; N, 11.21; Found: C, 50.79; H, 2.83; N, 11.53; IR v(cm⁻¹)= 3166 (NH),3024 (Ar-CH), 1664 (-C=O), 1595 (-C=N-), 1541,1492 (-C=C-), 1463 (-N=N-), 1282 (C-N), 1128(-N-N-), 943 (-C-F), 771 (C-Cl) cm⁻¹;* ^{1}*H-NMR (, 400 MHz): δ(ppm) = 8.75 (s, 1H, NH), 7.92-7.09 (11H, m, Ar–H);* ^{13}*C-NMR (101 MHz): δ(ppm) = 164.94 (-C=O), 151.36 (-C=N-), 138.60-116.56 (Ar-C=C), 116.34 (C-F);* The EI-MS m/z [M]+: 499 ($C_{21}H_{12}Cl_3F_3N_4O$); 223 ($C_8H_{11}F_3N_3O^+$)

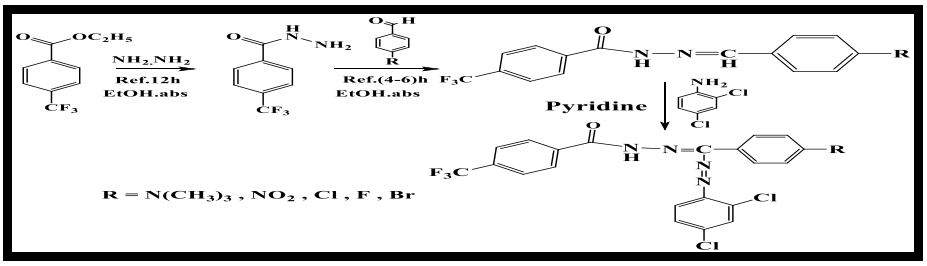
(Z,E)-1-(2,4-dichlorophenyl)-3-(4-fluorophenyl)-5-(4-(trifluoromethyl)benzoyl)formazan SH8

Light Brown *(66%); mp* = 194-195 °*C; Elemental analysis* $C_{21}H_{12}Cl_2F_4N_4O$*; C.H.N Calcd: C, 52.19; H, 2.50; N, 11.59; Found: C, 51.94; H, 2.82; N, 11.09; IR v(cm⁻¹)= 3172 (NH),3024 (Ar-CH), 1654 (-C=O), 1620 (-C=N-), 1558,1512 (-C=C-), 1465 (N=N), 1236 (-C-N-), 1132 (-N-N-), 960 (-C-F), 754 (C-Cl) cm⁻¹;* ^{1}*H-NMR (400 MHz): δ(ppm) = 8.94 (s, 1H, NH), 7.84-7.21 (11H,*

m, Ar–H); ^{13}C-NMR (101 MHz): δ(ppm) = 161.21 (-C=O), 147.28 (-C=N-), 137.37-125.69 (Ar-C=C), 124.20 (C-F);. The EI-MS m/z [M]+: 483 ($C_{21}H_{12}Cl_2F_4N_4O$), 58 ($C_3H_8N^+$)

(Z,E)-3-(4-bromophenyl)-1-(2,4-dichlorophenyl)-5-(4-(trifluoromethyl)benzoyl)formazan SH10

Off-Whit *(58%); mp* = 202-204 °*C; Elemental analysis $C_{21}H_{12}BrCl_2F_3N_4O$; C.H.N Calcd: C, 46.35; H, 2.33; N, 10.30; Found: C, 45.95; H, 2.03; N, 10.69; IR ν(cm^{-1})= 3218 (NH),3049 (Ar-CH), 1658 (-C=O), 1600 (-C=N-), 1544,1517 (-C=C-), 1488 (-N=N-), 12244 (-C-N-), 1091 (-N-N-), 916 (-C-F), 771 (C-Cl), 680 (C-Br) cm^{-1}; ^{1}H-NMR (, 400 MHz): δ(ppm) = 8.88 (s, 1H, NH), 7.96-7.13 (11H, m, Ar–H); ^{13}C-NMR (101 MHz): δ(ppm) = 164.85 (-C=O), 158.10 (-C=N-), 151.15-124.60 (Ar-C=C), 122.98 (C-F);* The EI-MS m/z [M]+: 544 ($C_{21}H_{12}BrCl_2F_3N_4O$); 136 ($C_5H_5Cl_2^+$)



Scheme 1: Prepared compounds (SH-SH10)

Biological activity study: Two types of antibiotic-resistant bacteria were obtained from the pathology laboratory of Tikrit University. Two types of bacteria were Gram-negative (*Klebsiella pneumoniae*) and Gram-positive (*Staphylococcus aureus*) [17-19]. Culture media were prepared according to the instructions provided by the supplier. Agar well diffusion technique The biological activity of the compounds developed in this study against the growth of some pathogenic bacteria used in the laboratory was measured using microplates. The culture medium was placed on a Petri dish, sterilized in a pressure vessel, and then solidified [20-22]. The surface was covered with a bacterial solution. Three holes were then drilled in each Petri dish using a cork drill, and each well was filled with different concentrations of the previously synthesized compounds [23-25]. The plates were then incubated at 37 °C for 24 h or approximately one day. The next day, the diameter of the inhibitor was increased to take the readings. Each studied substance was diluted in three (0.01, 0.001, 0.0001) mg/ml of each component [26,27].

Results and Discussions

Characterization of prepared compounds (SH1-SH10)

In the first step, hydrazone derivatives (SH1-SH5) were prepared from the reaction of hydrazide prepared in one step with benzaldehyde substitutes, where the prepared compounds were confirmed by spectroscopic measurements such as the infrared spectrum, which showed the presence of the azomethine bond that appears at (1622-1598) cm^{-1}, which is evidence for the formation of these compounds. This was also supported by the ^{1}H-NMR spectrum, which showed a signal returning (N=CH) in the range (8.38-8.72) ppm, another explanation for this distinctive group in hydrazones. A signal at (143.79-153.04) ppm was discovered after examining the ^{13}C-NMR spectra, indicating the existence of the azomethine group in the produced molecules [28].

In the second step, Formazan compounds (SH6-SH10) were prepared by the reaction of hydrazones prepared in step 2 from 2,4-dichloroaniline. The infrared spectrum confirmed the reaction, which showed the presence of a band in the range (1440-1488) cm^{-1} attributed to the azo group (N=N). The spectrum also showed the disappearance of the NH_2 group, which appears as a double band from the compound 2,4-dichloroaniline, which is evidence of forming formazan compounds. As for the ^{1}H-NMR spectrum, the disappearance of the proton belonging to the azomethine group was observed due to its

association with the azo. The production of novel compounds with a shift in values was indicated by an increase in the number of carbon signals in the ^{13}C NMR spectra [29].

The mass spectra of all studied compounds showed molecular ion peaks $[M]^+$ that are completely consistent with the proposed structures. The spectrum gave peaks at (508), (510), (499), (483) and (544) respectively, which match the molecular weights of the prepared compounds, indicating the accuracy and correctness of the synthesis products. The spectrum showed peaks at (155), (57), (223), (58), and (136) respectively, which reflects the peak intensity of the fragments' stability [30].

When studying the quantitative analysis of the element, C.H.N. it was found that the calculated values were close to the values found in the quantitative analysis, which confirms the validity and accuracy of the results [31].

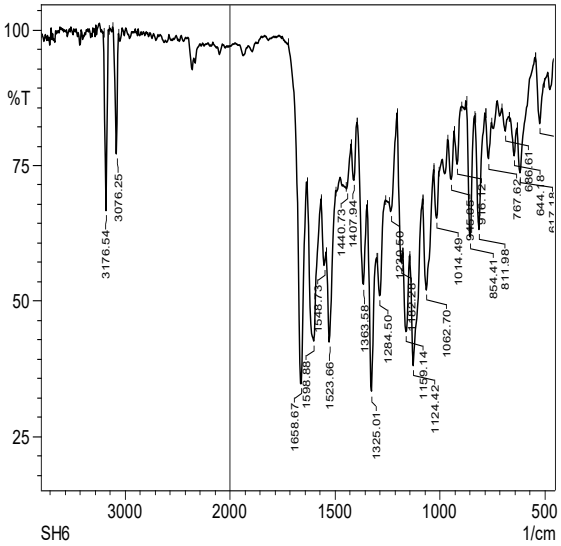


Figure (1): (SH6) of FT-IR

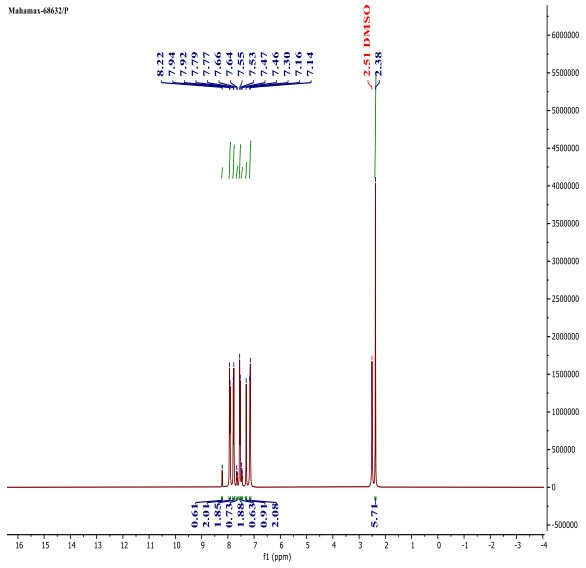


Figure (2): (SH6) of ^{1}H-NMR.

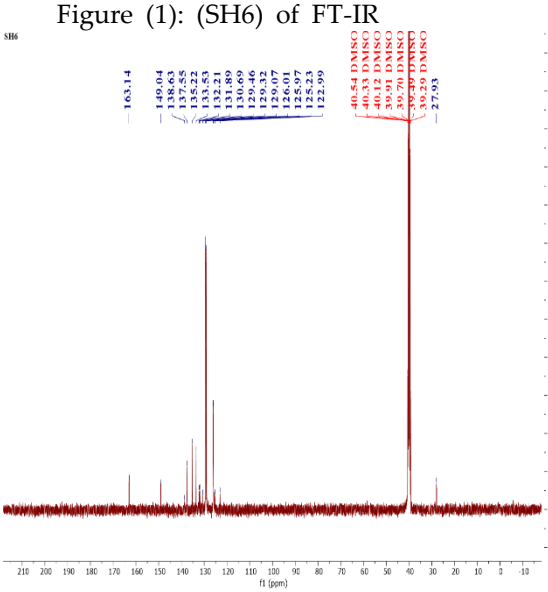
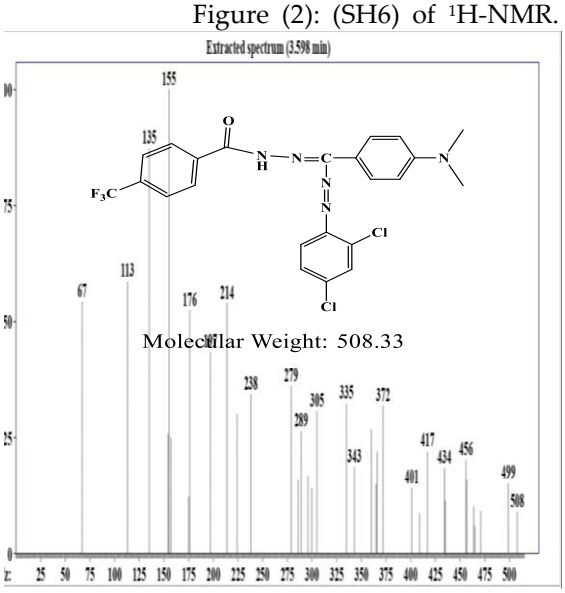


Figure (3): (SH6) of ^{13}C-NMR



. Figure (4): (SH6) of Mass spectrum.

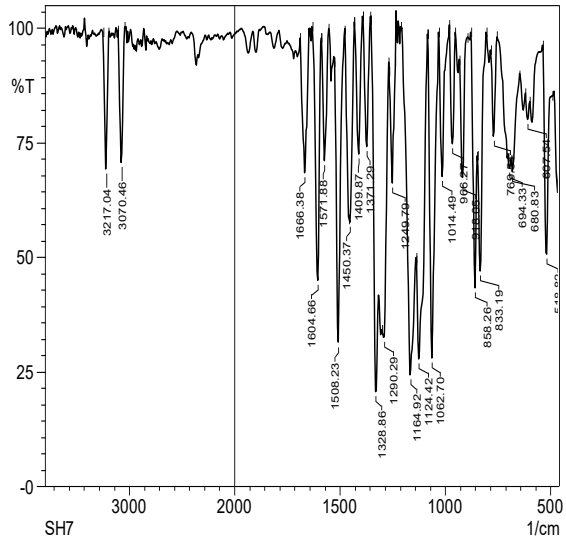


Figure (5): (SH7) of FT-IR

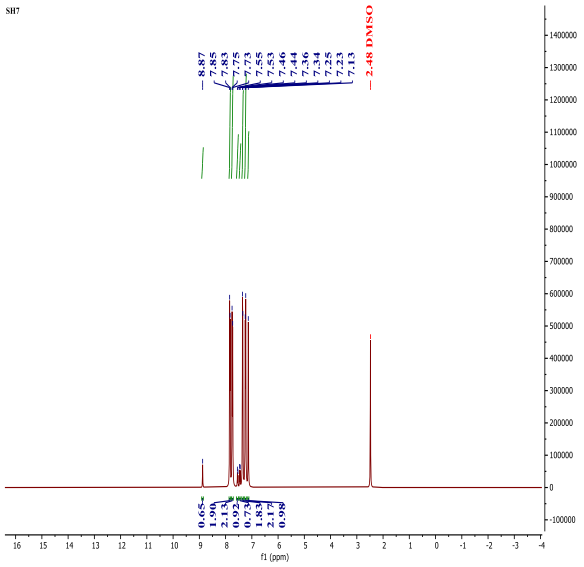


Figure (6): (SH7) of ^{1}H-NMR.

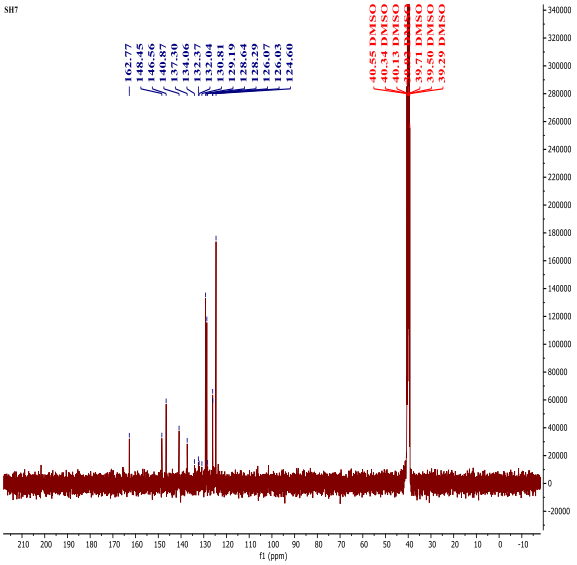


Figure (7): (SH7) of ^{13}C-NMR

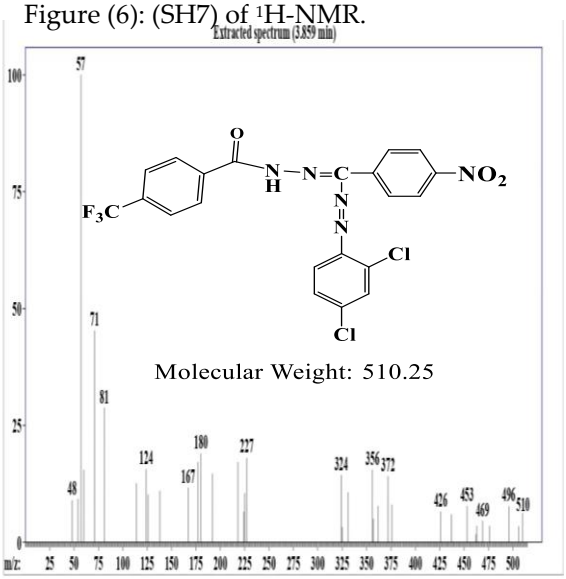


Figure (8): (SH7) of Mass spectrum.

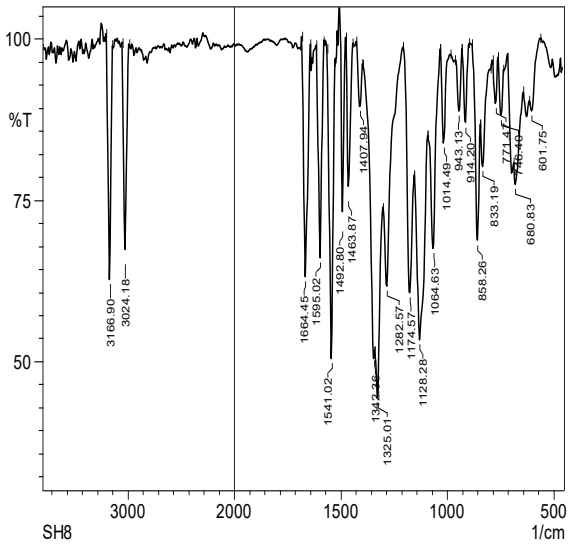


Figure (9): (SH8) of FT-IR

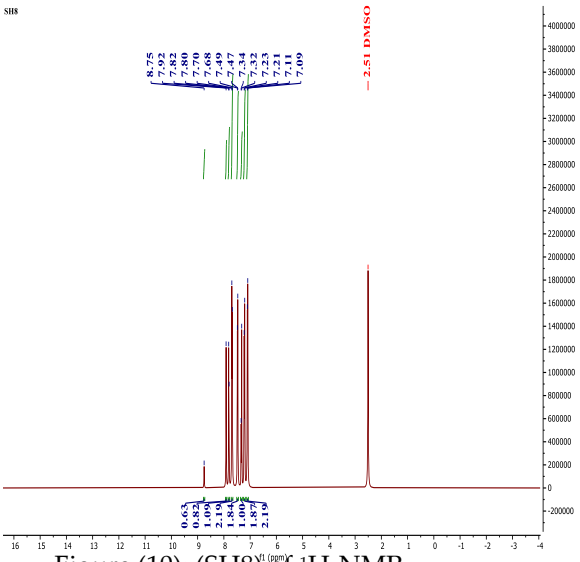


Figure (10): (SH8) of ^{1}H-NMR.

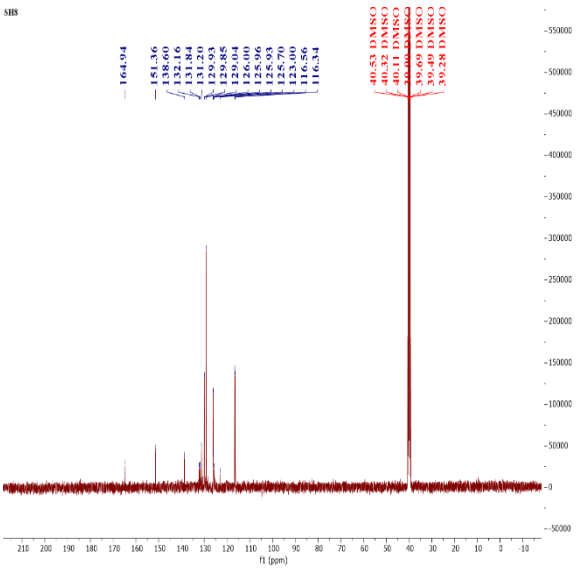


Figure (11): (SH8) of ^{13}C-NMR

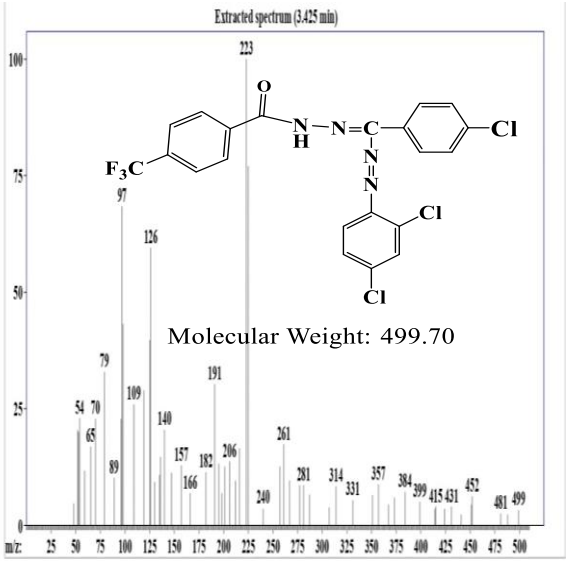


Figure (12): (SH8) of Mass spectrum.

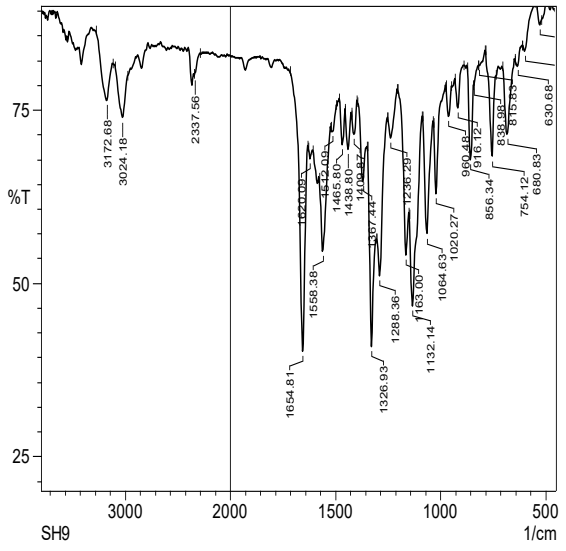


Figure (13):(SH9) of FT-IR

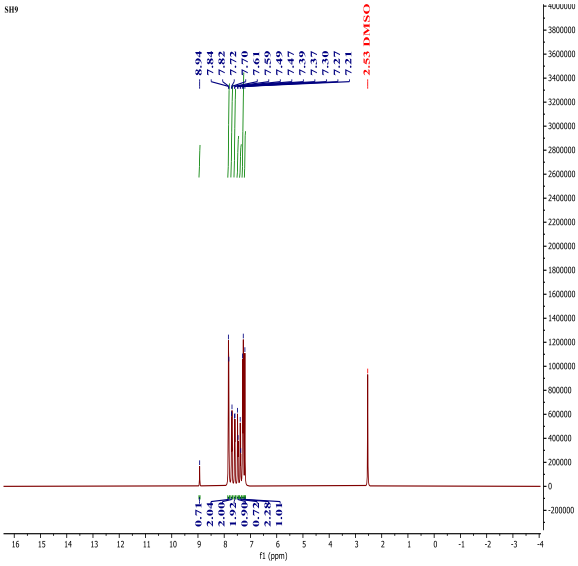


Figure (14): (SH9) of ^{1}H-NMR.

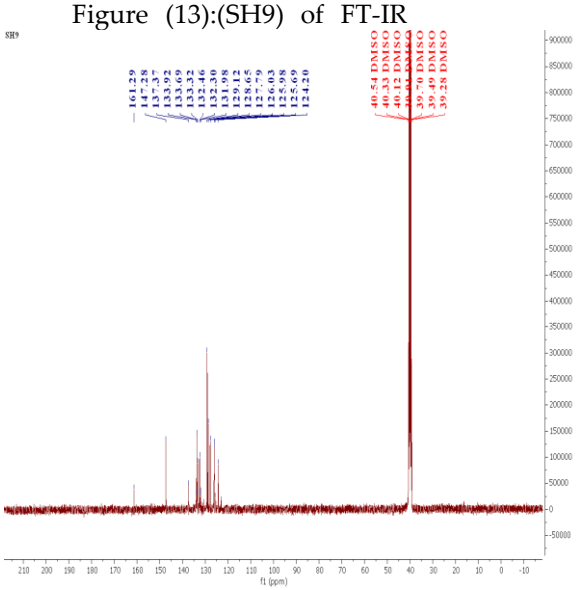


Figure (15): (SH9) of ^{13}C-NMR

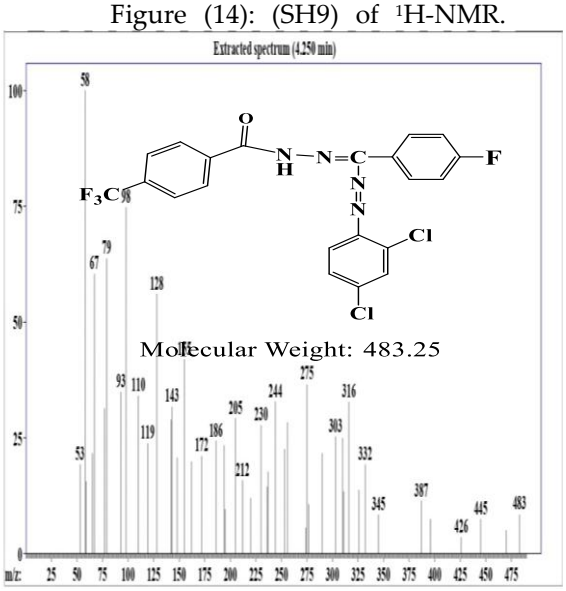


Figure (16): (SH9) of Mass spectrum.

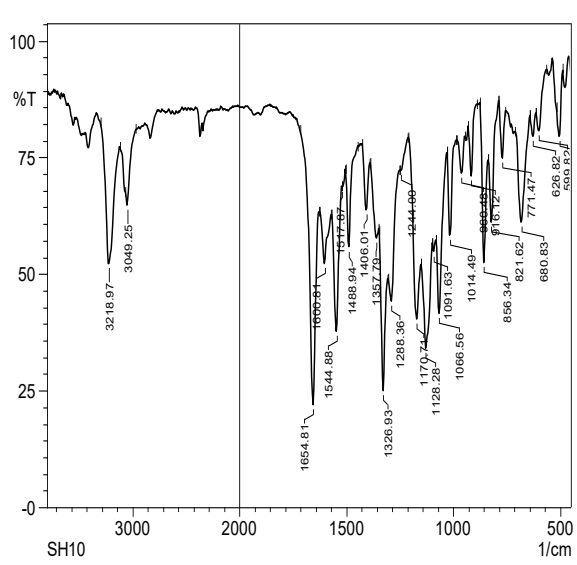


Figure (17): (SH10) of FT-IR

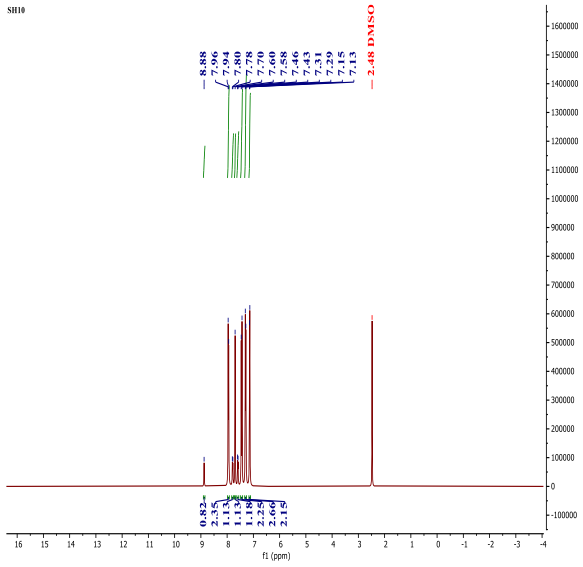


Figure (18): (SH10) of ^{1}H-NMR.

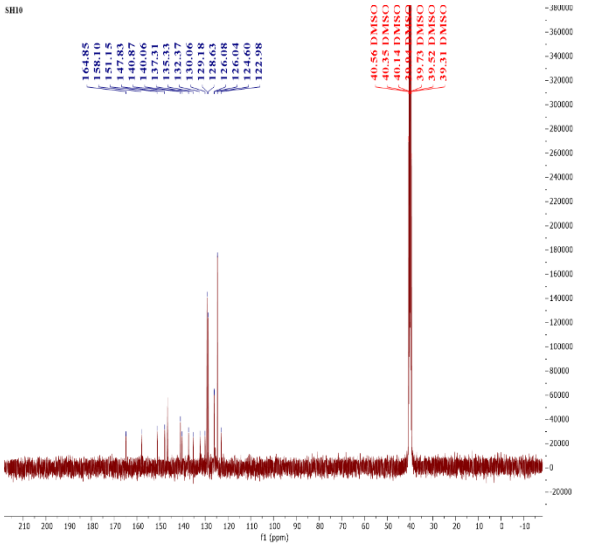


Figure (19): (SH10) of ^{13}C-NMR .

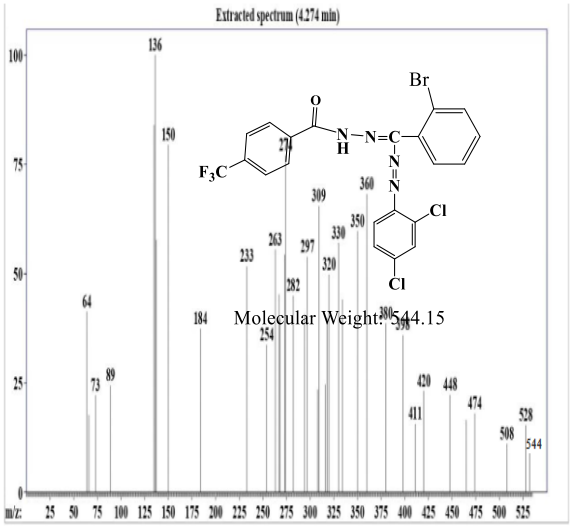


Figure (20): (SH10) of Mass spectrum.

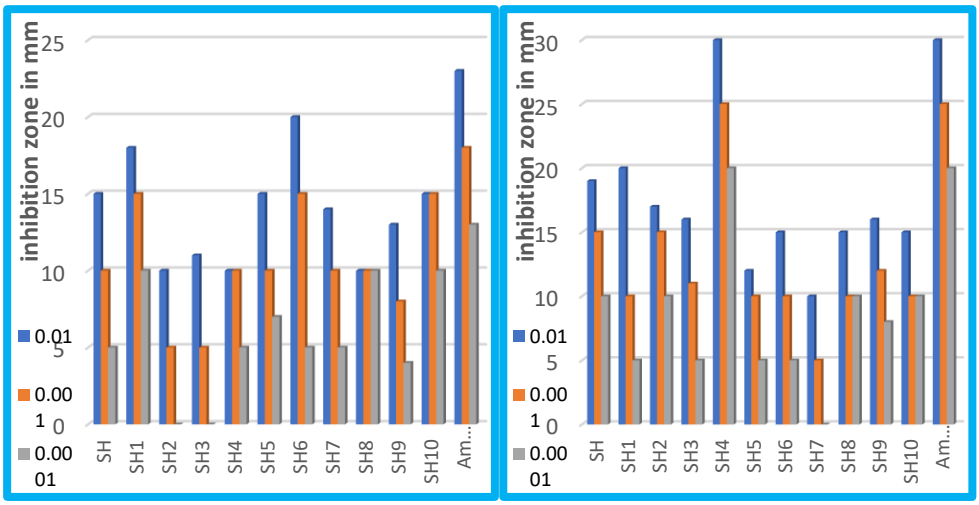
Assessing the Biological Activity of a Few Prepared Substances:

Gram-positive Gram-negative bacteria and Staphylococcus aureus The two bacterial species that were tested against a few of the compounds created in this investigation were K. pneumoniae. The test was performed on Petri plates using the diffusion technique [32, 33]. The inhibitory zone width of a few of the generated compounds was measured at doses of 0.01, 0.001, and 0.0001 mg/ml using the Mueller-Hinton medium. The outcomes were contrasted with those of conventional antibiotics [34, 35]. When compared to the first kind of bacteria, some of the chemicals that were produced were demonstrated to have a definite influence on the first type, while others had a clear effect on the second type [36,37]. With diameters of 30 and 20 mm, respectively, compounds of the various types (SH1, SH4) showed the highest rate of inhibition against Staphylococcus aureus. At a pace of (20,15) mm, compound (SH5, SH6) showed the highest inhibition of K. pneumoniae bacteria. At a concentration of 0.01 mg/ml, the compounds showed the greatest inhibition, suggesting that the inhibition increases with concentration [38, 39].

Table (1): The produced compounds' antibacterial activity (inhibition zone in millimeters).

	***K. pneumoniae* mg/ml**	***Staph. epidermidis* mg/ml**

Comp. No.	0.01	0.001	0.0001	0.01	0.001	0.0001
SH	15	10	5	19	15	10
SH1	18	15	10	20	10	5
SH2	10	5	0	17	15	10
SH3	11	5	0	16	11	5
SH4	10	10	5	30	25	20
SH5	15	10	7	12	10	5
SH6	20	15	5	15	10	5
SH7	14	10	5	10	5	0
SH8	10	10	10	15	10	10
SH9	13	8	4	16	12	8
SH10	15	15	10	15	10	10
Ampicillin.	23	18	13	30	25	20



Scheme (1): Inhibitory activity of (SH-SH10) for K.pneumoniae and *Staphylococcus aureus*

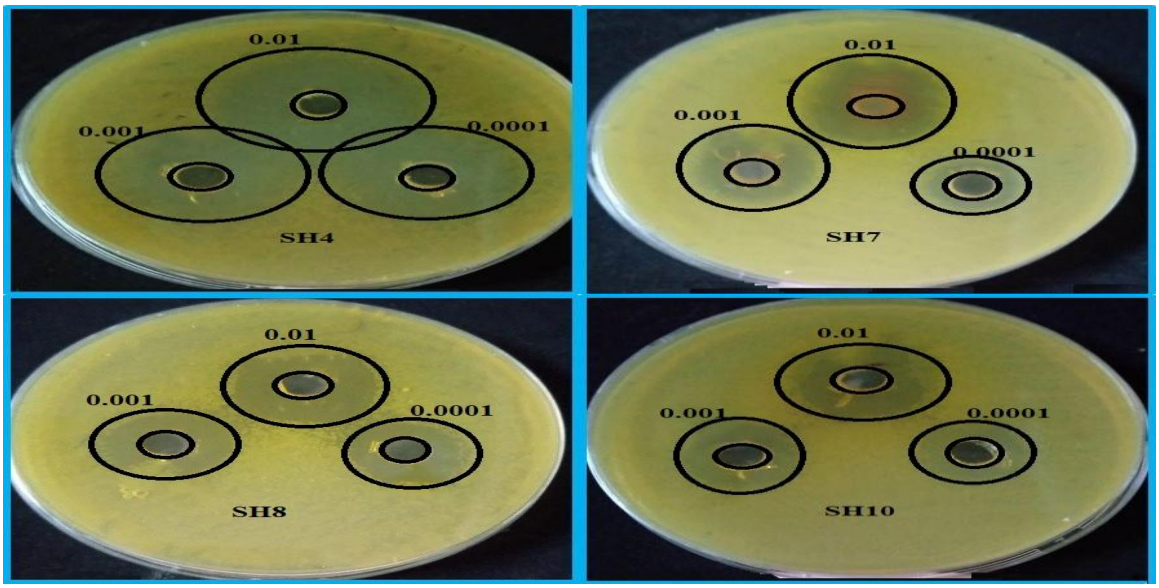


Figure 21: Activity of the compounds (SH4,7,8,10) against Staph aureus and

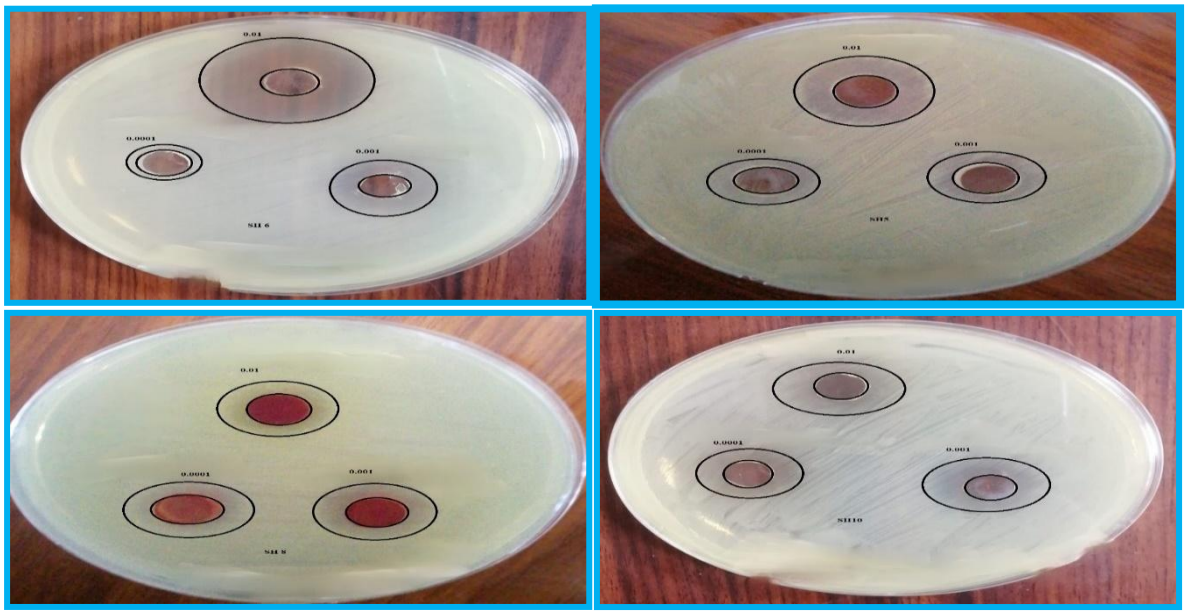


Figure 22: Activity of the compounds (SH5,6,8,10) against K. pneumoniae.

Conclusion

In this study, a series of novel hydrazone and formazan derivatives were successfully synthesized from ethyl 4-(trifluoromethyl) benzoate through multi-step reactions involving hydrazide intermediates. produced using hydrazide intermediates in a series of steps from ethyl 4-(trifluoromethyl) benzoate. Fourier transform infrared spectroscopy was used to confirm the structures of the resultant compounds, ^{1}H-NMR, ^{13}C-NMR, and quantitative C,H,N elemental analysis, which revealed characteristic signals consistent with the functional groups of hydrazone and formazan, confirming the success of the synthesis route. Biological evaluation against two antibiotic-resistant bacterial strains—*Staphylococcus aureus* (Gram-positive) and *Klebsiella pneumoniae* (Gram-negative)—showed promising antibacterial activity for many of the derivatives. Notably, compounds SH1 and SH4 exhibited significant inhibitory effects against Staphylococcus aureus, while compounds SH5 and SH6 demonstrated significant activity against pneumococcus, with inhibition zones similar to those of the reference antibiotic (*ampicillin*) at the highest concentration tested. The results indicate that structural modifications of the benzaldehyde molecule, as well as the addition of various substituents, play a pivotal role in enhancing biological activity. These findings support the potential use of the synthesized compounds as candidates for further development in antimicrobial drug research, particularly against resistant bacterial strains. Future work may focus on mechanistic studies, structure-activity relationship (SAR) analysis, and cytotoxicity testing to improve the pharmacological properties of these formazan-based structures.

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