

# Synthesis and Characterization of Formazan Derivatives from Schiff's Base and Studying their Biological Activity

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## ABSTRACT

A group of substituted formazine derivatives was prepared through several steps where the first step was the preparation of compound (1) by reacting chloroacetyl chloride with 2-aminopyrimidine, and the second step was the reaction of compound (1) with hydrazine hydrate to obtain the derivative (2) and in the third step was prepared Schiff's base (3) by reaction of derivative (2) with para-dimethyl benzaldehyde and then a number of formazine derivatives were synthesized by reaction of derivative (3) with a number of substituted aromatic amines. The newly prepared derivatives are distinguished by their melting points (1H-nuclear magnetic resonance (1H-NMR), fourier transform infrared spectroscopy (FTIR)) spectra. The biological activity of the prepared derivatives was studied on two types of bacteria that cause urinary tract and measuring the diameter of the inhibition zone against the growth of these bacteria.

**Keywords:** Biological activity, Formazan, Schiff bases.

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**Conflict of interest:** None

## INTRODUCTION

Formazan has one of the following organic chemical formulas  $N=N-C=N-$  or  $N=N-C=N-N$  depending on the type and structure of the reactive aromatic amines.<sup>1</sup> Formazan compounds have attracted the attention of many researchers because of their wide biological and industrial applications in analytical chemistry and the synthesis of heterocyclic compounds.<sup>2</sup> The backbone of formazan compounds is rich in nitrogen, and in addition to its intense color, it has been widely used in dyes.<sup>3</sup> Formazans are characterized by  $\pi-\pi^*$  transitions, and these transitions are sensitive to the nature of the substituents in the phenyl rings, the nature of organic solvents in solution, and the acidity and basicity of the medium.<sup>4</sup> Formazan derivatives have different pharmacological activities; therefore, a number of these compounds were synthesized through coupling reactions.<sup>5</sup> Formazan has a high biological activity, especially on cancer cells, due to the possession of the tetrazolium-formazan system produced by redox reactions.<sup>6</sup> Formazan compounds were first described at the end of the 19th century.<sup>7</sup> Biogenic formazans are used as antiviral, antimicrobial, antifungal, and also anti-human immunodeficiency virus (HIV).<sup>8</sup> The melting point of formazan is generally relatively low despite its large molecular size.<sup>9</sup>

## THE METHOD OF WORK

FT-IR spectra, the instrument was of the Shimadzu type (8400S). The heat of melting was modified using (Stewart, UK).

<sup>1</sup>HNMR (the device was a Borchert type, running at 400MHz with dimethylsulfoxide (DMSO)-d<sub>6</sub>).

### Prepare Derivative 1.<sup>10</sup>

Chloroacetyl chloride 0.04 mol was slowly added to the 2-aminopyrimidine solution 0.02 mol dissolved in dry benzene at a temperature of 0-5°C. The reaction mixture was refluxed for 4 hours, and then the precipitate was filtered and recrystallized by using absolute ethanol.

### Prepare of Derivative 2<sup>11</sup>

A mixture of compound (2) (0.006 mol) with hydrazine hydrate (0.012 mol) in ethanol 20 mL was refluxed for 4 hours in water bath. After cooling the reaction mixture, the precipitate formed is collected by filtration, dried, and recrystallized with ethanol.

### Prepare Derivative 3<sup>12</sup>

Equimolar quantities of compound (2) (0.04 mol), para-dimethyl benzaldehyde (0.04 mol) and drops of acetic acid were refluxed for 3 hours with 20 mL of absolute ethanol. The resultant mixture was cooled at room temperature. Then the obtained precipitate was filtered and washed with water.

### Prepare of Derivative (4-8)<sup>13</sup>

A cold stirred solution of different aromatic amine (0.04 mol) previously dissolved in aqueous HCl (5 + 8 mL water) was diazotized over crushed ice by dropwise addition of cold aqueous solution of NaNO<sub>2</sub> (0.04 mol) with stirring at 0-5°C

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and Leave the mixture for 20 minutes in the refrigerator. This mixture was then poured into a cold solution of compound (3) (0.04 mol) dissolved in dry pyridine (10 mL). The reaction mixture was further stirred for 3 hours, maintaining temperature 0-5 °C. The mixture was then poured into water with continuous stirring. The resultant dark colored solids were

filtered and washed with water till free from pyridine, dried, and recrystallized from ethanol. The physical properties of the synthesized compounds 1 to 8 are given in Table 1.

### Biological Activity

The culture medium was used (Muller-Hinton Agar) for the purpose of measuring the biological activity of two types of pathogenic bacteria (*Escherichia Coli* and *Staphylo coccus aureus*) isolated from patients with urinary tract infection in the maternity and children's hospital in AL-Diwaniyah city.

Bacteria were cultured in the culture medium for about (0.1 μmol) and left to settle for half an hour, then holes were made in the culture medium, and a concentration (0.05mol) of the prepared material was added after dissolving it in DMSO. Another culture dish was used as a control to see the effect of the solvent on bacterial growth. After that, all dishes were left for 15 minutes until the medium was dry, and then placed in the Container for 24 hours. Then, the diameter of the inhibition area was measured in millimeters by using a ruler.<sup>14</sup>

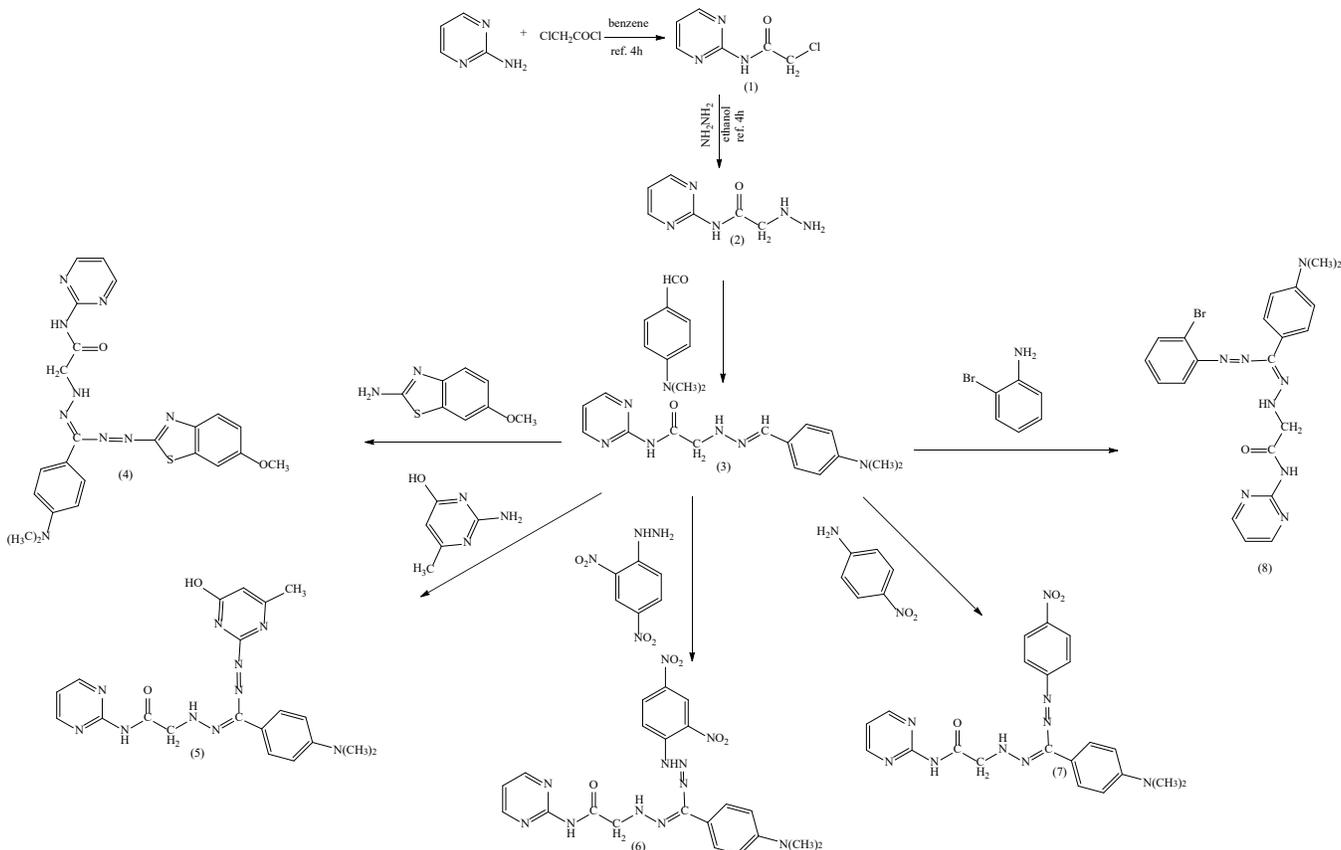
### RESULTS AND DISCUSSION

#### The Derivative (1) (2-chloro-N-(pyrimidin-2-yl)acetamide)

The FTIR data are shown in the range (1641.31, 3344.34, 2879.52, 3087.82, 1672.17, 1618.17)  $\text{cm}^{-1}$  to (C = N, NH, Aliphatic C-H, C-H pyrimidine, C = O, C = C) respectively (Figure 1).

**Table 1:** Physical data of derivatives (1-8)

NO.	M F MW	Color	MP°C	RF	yield%
1	C <sub>6</sub> H <sub>6</sub> ClN <sub>3</sub> O 171.58	Dark gray	180	0.38	80
2	C <sub>6</sub> H <sub>9</sub> N <sub>5</sub> O 167.17	Dark yellow	170	0.29	78
3	C <sub>15</sub> H <sub>18</sub> N <sub>6</sub> O 298.35	Orange	215	0.31	88
4	C <sub>23</sub> H <sub>23</sub> N <sub>9</sub> O <sub>2</sub> S 489.56	Dark orange	225	0.35	75
5	C <sub>20</sub> H <sub>22</sub> N <sub>10</sub> O <sub>2</sub> 434.46	Dark orange	217	0.25	80
6	C <sub>21</sub> H <sub>21</sub> N <sub>11</sub> O <sub>5</sub> 507.47	Dark orange	228	0.14	78
7	C <sub>21</sub> H <sub>21</sub> N <sub>9</sub> O <sub>3</sub> 447.46	Dark orange	214	0.52	75
8	C <sub>21</sub> H <sub>21</sub> BrN <sub>8</sub> O 481.36	Dark orange	219	0.15	77



**Scheme 1:** Preparation of compounds (1-8)

The  $^1\text{H-NMR}$  ( $\text{DMSO-d}_6$ ) data are shown in the range  $\delta$ : 10.99 (S, 1H, NH), 4.5 (S, 2H,  $\text{CH}_2$ ), 6.97 – 8.69 (3H, CH, pyrimidine ring), 2.5 (DMSO).

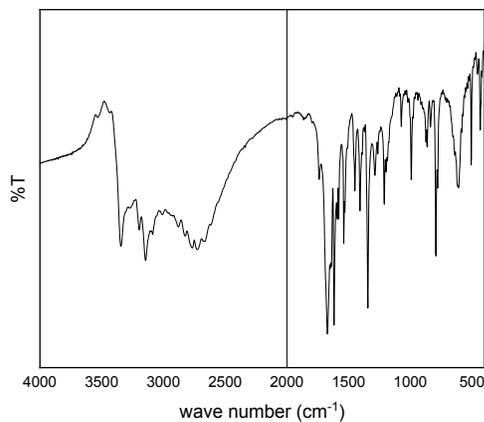


Figure 1: FTIR for derivative (1)

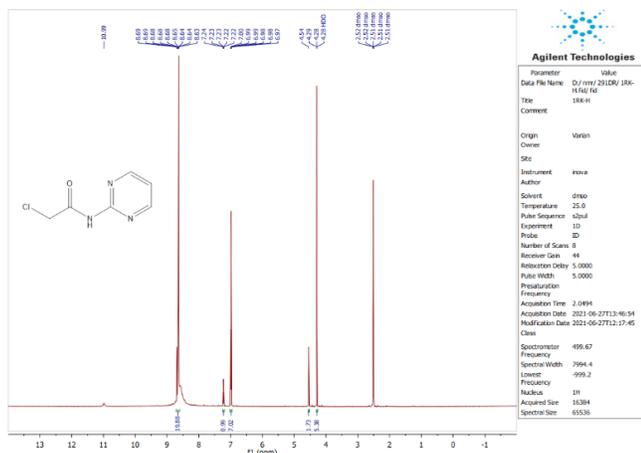


Figure 2:  $^1\text{H-NMR}$  of derivative (1)

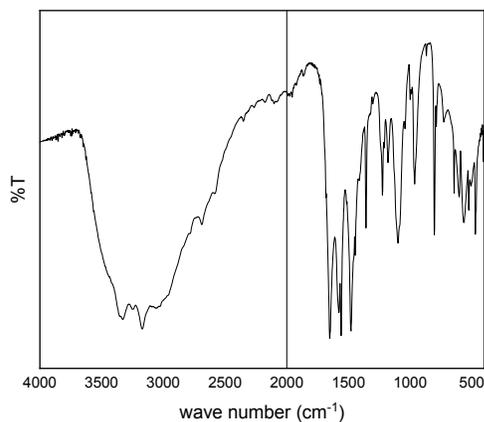


Figure 3: FT. IR for derivative (2)

### The Derivative (2) 2-hydrazineyl-N-(pyrimidin-2-yl) acetamide

The FTIR data are shown in the range (1577.66, 3323.12-3244.05, 2985.60, 3002.96, 1652.88, 1558.38)  $\text{cm}^{-1}$  to (C=N, NH, C-H, C-H pyrimidine, C=O, C=C) respectively (Figure 2).

The  $^1\text{H-NMR}$  ( $\text{DMSO-d}_6$ ) data are shown in the range  $\delta$ : 10.18 (S, 1H, NH),  $\delta$ : 4.4 (d, 2H,  $\text{NH}_2$ ), 3.5 (d, 2H,  $\text{CH}_2$ ), 6.05 – 8.2 (3H, CH, pyrimidine ring), 2.5 (DMSO).

### The derivative (3) ((E)-2-(2-(4-(dimethyl amino) benzylidene) hydrazonyl)-N-(pyrimidin-2-yl) acetamide)

The FTIR data are shown in the range (1519.80, 3325.05-3232.47, 2885.31-2970.17, 3002.96, 1604.66)  $\text{cm}^{-1}$  to (C=N, NH, C-H, C-H aromatic, C=O) respectively (Figure 3).

The  $^1\text{H-NMR}$  ( $\text{DMSO-d}_6$ ) data are shown in the range  $\delta$ : 9.1 (S, 1H, NH),  $\delta$ : 4.4 (T, 1H, NH-N), 3.1 (S, 1H, CH), 3.4 (d, 2H,  $\text{CH}_2$ ), 7.7 – 8.32 (m, 4H, Ar-H), 2.5 (DMSO).

### The Derivative (4) ((Z)-3-(4-(diethylamino) phenyl)-1-(6-methoxybenzo[d]thiazol-2-yl)-5-(2-oxo-2-(pyrimidin-2-ylamino) ethyl) formazan

The FTIR data are shown in the range (1604.66, 3425.34, 2808.16-2908.45, 3002.96, 1733.12, 1434.94)  $\text{cm}^{-1}$  to (C=N, NH, C-H aliphatic, C-H aromatic, C=O, N=N) respectively (Figure 4).

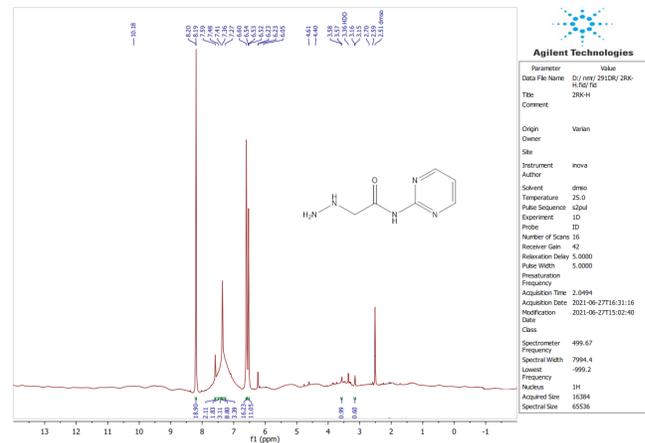


Figure 4:  $^1\text{H-NMR}$  of derivative (2)

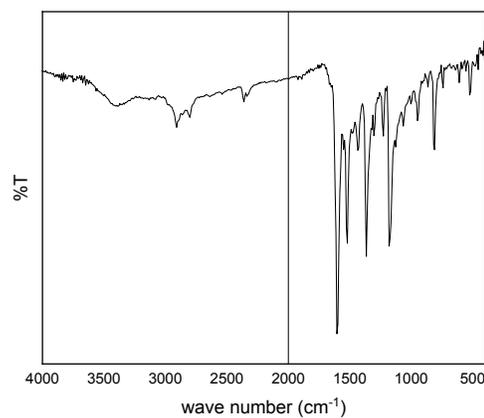


Figure 5: FT. IR for derivative (3)

The  $^1\text{H-NMR}$  ( $\text{DMSO-d}_6$ ) data are shown in the range  $\delta$ : 9.43 (S, 1H, NH),  $\delta$ : 3.72 (T, 1H, NH-N), 3.08 (S, 1H,  $\text{CH}_3$ ), 3.19 (S, 1H,  $\text{OCH}_3$ ), 3.72 (d, 2H,  $\text{CH}_2$ ), 7.76 – 8.72 (m, 7H, Ar-H), 2.5 (DMSO).

**The derivative (5) ((Z)-3-(4-(dimethyl amino) phenyl)-1-(4-hydroxy-6-methylpyrimidin 2-yl)-5-(2-oxo-2-(pyrimidin-2-ylamino) ethyl) formazan)**

(FT-IR) data are shown in the range (1604.66, 3263.33, 2862.17-2908.45, 3139.90, 1735.81, 1519.80, 3417.63)  $\text{cm}^{-1}$  to (C=N, NH, C-H aliphatic, C-H aromatic, C=O, N=N, OH) respectively.

The  $^1\text{H-NMR}$  ( $\text{DMSO-d}_6$ ) data are shown in the range  $\delta$ : 9.1 (S, 1H, NH),  $\delta$ : 8.51 (T, 1H, NH-N), 1.9 (S, 1H,  $\text{CH}_3$ ), 3.01 (S, 6H,  $\text{N}(\text{CH}_3)_2$ ), 4.78 (d, 2H,  $\text{CH}_2$ ), 9.68 (S, 1H, OH), 7.77 – 8.69 (m, 4H, Ar-H), 2.5 (DMSO) (Figure 5).

**The derivative (6) (2-(2-((Z)-4-(dimethyl amino) phenyl) ((E)-3-(2,4-dinitrophenyl) triaz-1-en-1-yl) methylene) hydrazonoyl)-N-(pyrimidin-2-yl) acetamide)**

(FT-IR) data are shown in the range (1604.66, 3278.76, 2862.17-2908.45, 3085.89, 1712.67, 1527.52)  $\text{cm}^{-1}$  to (C=N, NH, C-H) aliphatic, C-H aromatic, C=O, N=N) respectively (Figure 6).

The  $^1\text{H-NMR}$  ( $\text{DMSO-d}_6$ ) data are shown in the range  $\delta$ : 10.02 (S, 1H, NH),  $\delta$ : 6.78 (T, 1H, NH-N), 3.01 (S, 6H,  $\text{N}(\text{CH}_3)_2$ ), 3.18 (d, 2H,  $\text{CH}_2$ ), 11.55 (S, 1H, N=N-NH), 6.79 – 8.86 (m, 7H, Ar-H), 2.5 (DMSO).

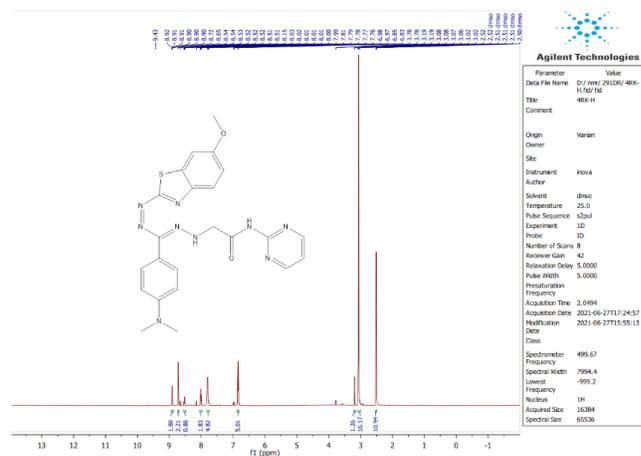


Figure 8:  $^1\text{H-NMR}$  of derivative (4)

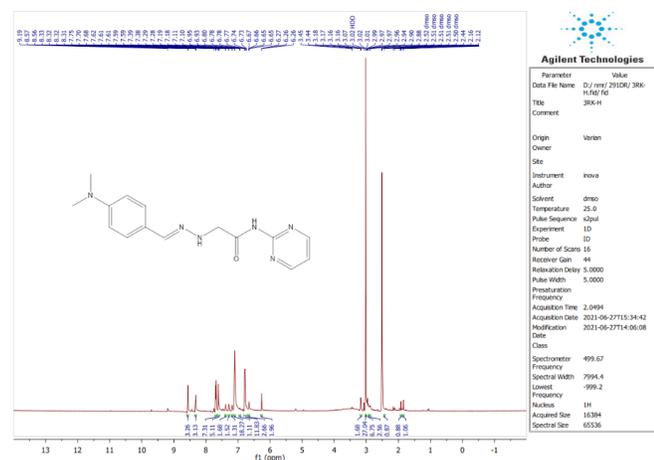


Figure 6:  $^1\text{H-NMR}$  of derivative (3)

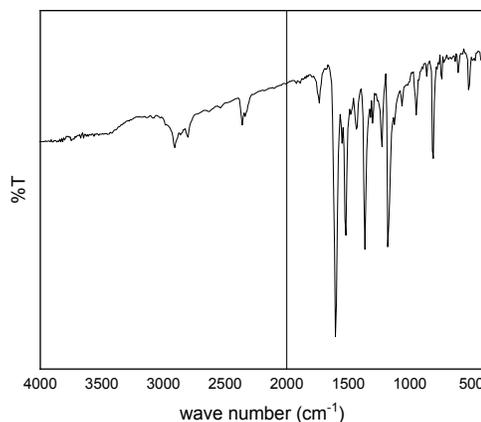


Figure 9: FTIR for derivative (5)

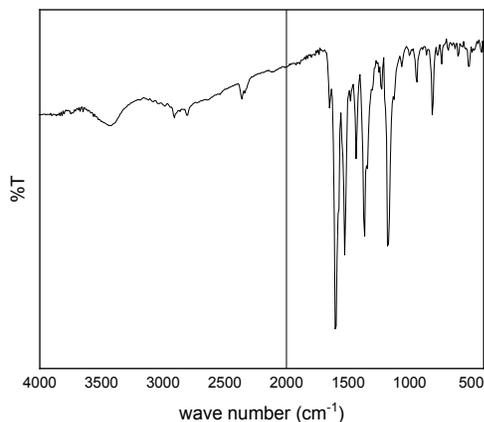


Figure 7: FT. IR for derivative (4)

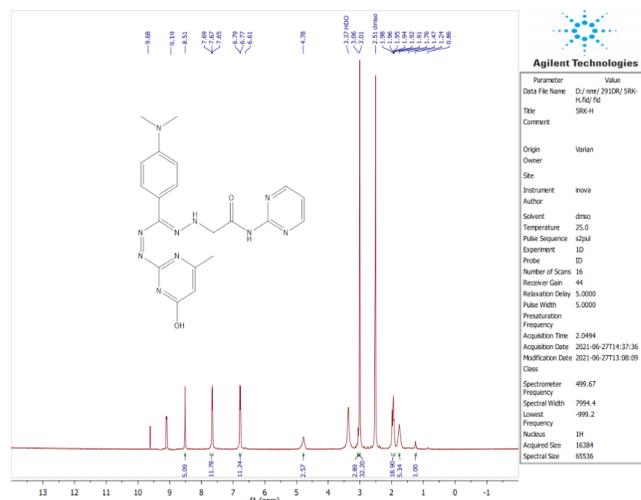


Figure 10:  $^1\text{H-NMR}$  of derivative (5)

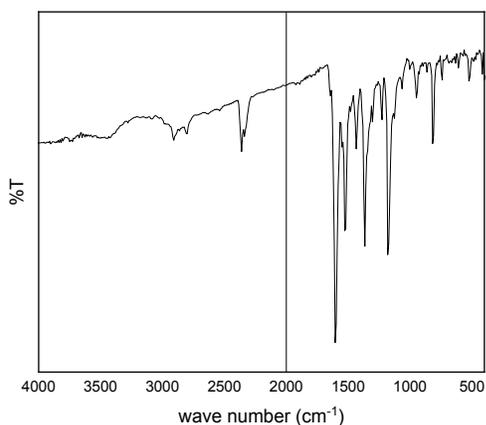


Figure 11: FTIR for derivative (6)

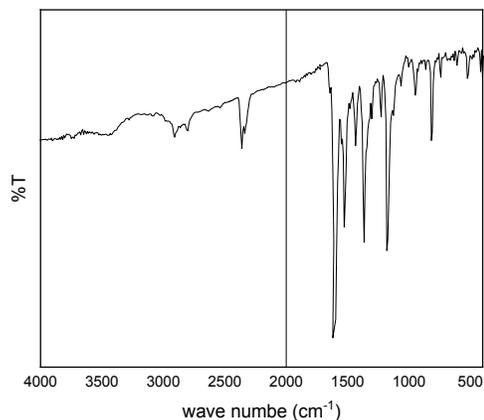
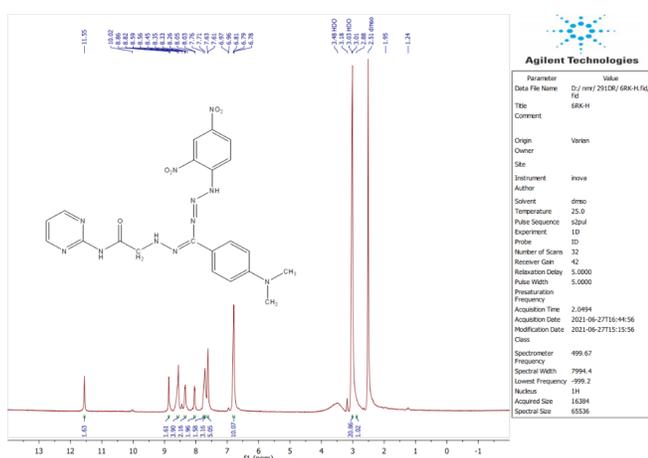
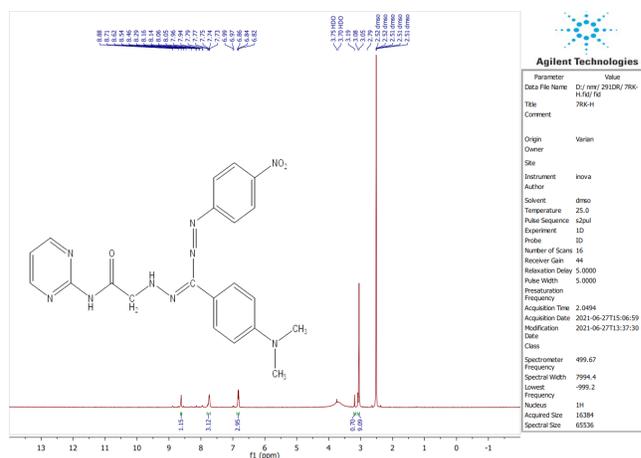


Figure 13: FTIR for derivative (7)


 Figure 12: <sup>1</sup>H-NMR of derivative (6)

 Figure 14: <sup>1</sup>H-NMR of derivative (7)

**The derivative (7) ((Z)-3-(4-(dimethyl amino) phenyl)-1-(4-nitrophenyl)-5-(2-oxo-2-(pyrimidin-2-ylamino) ethyl) formazan (**

The FTIR data are shown in the range (1620.55, 3278.76, 2862.17-2908.45, 3085.89, 1720.23, 1525.90)  $\text{cm}^{-1}$  to (C=N, NH, C-H aliphatic, C-H aromatic C=O, N=N) respectively (Figure 7).

The <sup>1</sup>H-NMR (DMSO- $d_6$ ) data are shown in the range  $\delta$ : 8.8 (s, 1H, NH),  $\delta$ : 6.82 (t, 1H, NH-N), 3.08 (s, 6H, N(CH<sub>3</sub>)<sub>2</sub>), 3.19 (d, 2H, CH<sub>2</sub>), 6.84 – 8.71 (m, 8H, Ar-H), 2.5 DMSO.

**The derivative (8) ((Z)-1-(2-bromophenyl)-3-(4-(dimethyl amino) phenyl)-5-(2-oxo-2-(pyrimidin-2-ylamino) ethyl) formazan)**

The FTIR data are shown in the range (1615.66, 3278.76, 2862.17-2908.45, 3085.89, 1718.97, 1530.77)  $\text{cm}^{-1}$  to (C=N, NH, C-H aliphatic-H aromatic=C=O, N=N) respectively (Figure 8).

The <sup>1</sup>H-NMR (DMSO- $d_6$ ) data are shown in the range  $\delta$ : 8.68 (s, 1H, NH),  $\delta$ : 6.7 (t, 1H, NH-N), 3.06 (s, 6H, N(CH<sub>3</sub>)<sub>2</sub>), 3.14 (d, 2H, CH<sub>2</sub>), 6.79 – 8.52 (m, 8H, Ar-H), 2.5 (DMSO).

**Biological Activity Results**

Table 2 and images indicate the results of measuring the

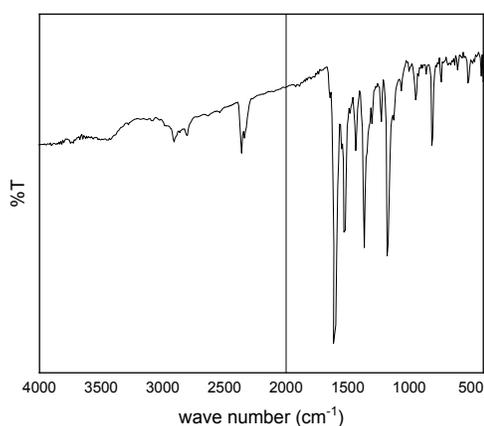


Figure 15: FT. IR for derivative (8)

area inhibition of bacterial growth using solutions of organic substances prepared at a concentration (0.05 mol) (Figure 9-16).

It is clear from Table 2 and images that there is a clear growth inhibition zone against all types of bacteria.



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