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Article in International Journal of Pharmaceutical Quality Assurance · March 2020

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# Synthesis and Characterization of some new Formazan Derivatives from 2-Amino-4-Hydroxy-6-Methyl Pyrimidine and Study the Biological Activity (Anti-Bacteria and Anti-Cancer)

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*Received: 22th Dec, 2019; Revised: 24th Jan, 2020; Accepted: 17th Feb, 2020; Available Online: 25th Mar, 2020*

## ABSTRACT

This study involves a synthesis of some formazan derivatives starting from react chloro acetyl chlorid with 2-amino-4-hydroxy-6-methyl pyrimidine to gate compound (a), (a) react with hydrazine hydrate to give compound (b) also (b) react with 3-4-dimethoxy benzaldehyd to product Schiff base derivative (c) then (c) react with deferent amin derivatives to get formazan derivatives. All these compounds characterized by  $^{13}\text{C-NMR}$ , fourier transform infrared spectroscopy (FTIR),  $^1\text{HMNR}$ . After that, we study the biological activity for all formazan derivatives toward two different kinds of bacteria and anti-cancer.

**Keywords:** Azo, Formazan, Schiff bases.

International Journal of Pharmaceutical Quality Assurance (2020); DOI: 10.25258/ijpqa.11.1.8

**How to cite this article:** Shakir A, Adnan S. Synthesis and Characterization of Some New Formazan Derivatives From 2-Amino-4-Hydroxy-6-Methyl Pyrimidine and Study the Biological Activity (Anti-Bacteria and Anti-Cancer). International Journal of Pharmaceutical Quality Assurance. 2020;11(1):53-59.

**Source of support:** Nil

**Conflict of interest:** None

## INTRODUCTION

Formazan derivatives are organic compounds containing four nitrogen atoms, General formula (-N=N-C=N-N-),<sup>1</sup> Formazan compounds are studied extensively because of applications.<sup>2</sup> It has report to possess wide effected of biological active, such as antiviral, antimicrobial, anti HIV,<sup>3</sup> anti-filamentary,<sup>4</sup> Anticancer,<sup>5</sup> Cyclic formazan derivatives are lithium-specific good indicators of lithium-ion in biological fluids such as blood,<sup>6</sup> Formazan derivatives are used widely to cytotoxicity and evaluate cell viability,<sup>7</sup> Formazan derivatives are used widely as reagents for analysis of trace metal,<sup>8</sup> it's are colored compounds because their  $\pi - n^*$  and  $\pi - \pi^*$  transition, formazan compounds have been generally solids of relatively low melting point although of large the size of the molecules.<sup>9</sup>

## MATERIALS

FTIR Spectra (400 -4000  $\text{cm}^{-1}$ ) in KBr disk were recorded on SHIMADZU FTIR-8400S Fourier transform.  $^{13}\text{C-NMR}$  and  $^1\text{HNMR}$  were recorded on Fourier transformation Bruker spectrometer operating at (500MHz) with (DMSO- $d_6$ ) measurements were made at Department of Chemistry,kashan University, Iran.

## METHODS

### Preparation of Compound (a)<sup>10, 11</sup>

Mix equal moles of chloro acetyl chloride compounds (0.0079 mol, 0.6 ml) with the amino compound (2-amino-4-hydroxy-

6-methyl bromide) (0.0079 mol, 1 g) in 30 mL of dry benzene at room temperature with stirring for 6 hours The reaction was followed by TLC technique. The precipitation was then filtered and reconstituted with absolute ethyl alcohol. to resulted whit precipitation and molecular weight (202.598) and a percentage (87%) were obtained. The melting point was within range (228–230) $^{\circ}\text{C}$  ( $R_f = 0.27$ ).

### Preparation Compound (b)<sup>12,13</sup>

Mix equal moles of hydrazine hydrate (0.004 mol, 0.29 mL) with compound (1) at room temperature for 8 hours where the reaction process was followed using TLC technique. The precipitate was then filtered and reconstituted using  $\text{CHCl}_3$  . to resulted whit precipitation and and its melting point was within the range 193–195 $^{\circ}\text{C}$  and ( $R_f$  0.43) (78.1%).

### Preparation of Schiff Base Compound (c)<sup>14,15</sup>

Mix equal moles of compound (2) (0.005 m, 1 g) with 3, 4-dimethoxy benzaldehyde (0.005 m, 0.83 g) and reflex at a temperature of(74-78)  $^{\circ}\text{C}$  for 8 hours. where the reaction process was followed using TLC technique,The precipitation was then filtered and reconstituted using methanol . to resulted yellow precipitation and molecular weight of 345.359 and a percentage of 90% were obtained. The melting point was within range (208-212) $^{\circ}\text{C}$  and( $R_f = 0.35$ ).

### Preparation of Dizonum salt (d)<sup>16,17</sup>

Dissolve (0.003mol) of the aromatic Amin is dissolved in 5 mL HCl + 8 mL distilled water). The solution is cooled to a

temperature of (0-5 °C). After that added to 0.003 mol sodium nitrate dissolved in 5 mL distilled water drop by drop to the mixture and leave for 20 minutes inside the refrigerator

### Preparation of Formazan Derivatives<sup>18,19</sup>

Dissolve (0.003mol) of the Schiff base derivative (c) to the preparation of derivatives from (c1-c8) in (10 mL dioxane + 20 mL ethano).

The dizonium salt produced from the above step is added to the solution of the Schiff base drop by drop in snow bath (0-5)°C. It is left with stirring for a period of 2- 3.5 hours, the reaction process was followed using TLC technique. Obtain color derivatives to be weighed and calculate the percentage and physical properties as shown in Table 1.

## RESULTS AND DISCUSSION

### Compound (a) :- 2-chloro-N-(4-hydroxy-6-methylpyrimidin-2-yl)acetamide

FT-IR spectrum data for derivative (a) show band at 3386 cm<sup>-1</sup>

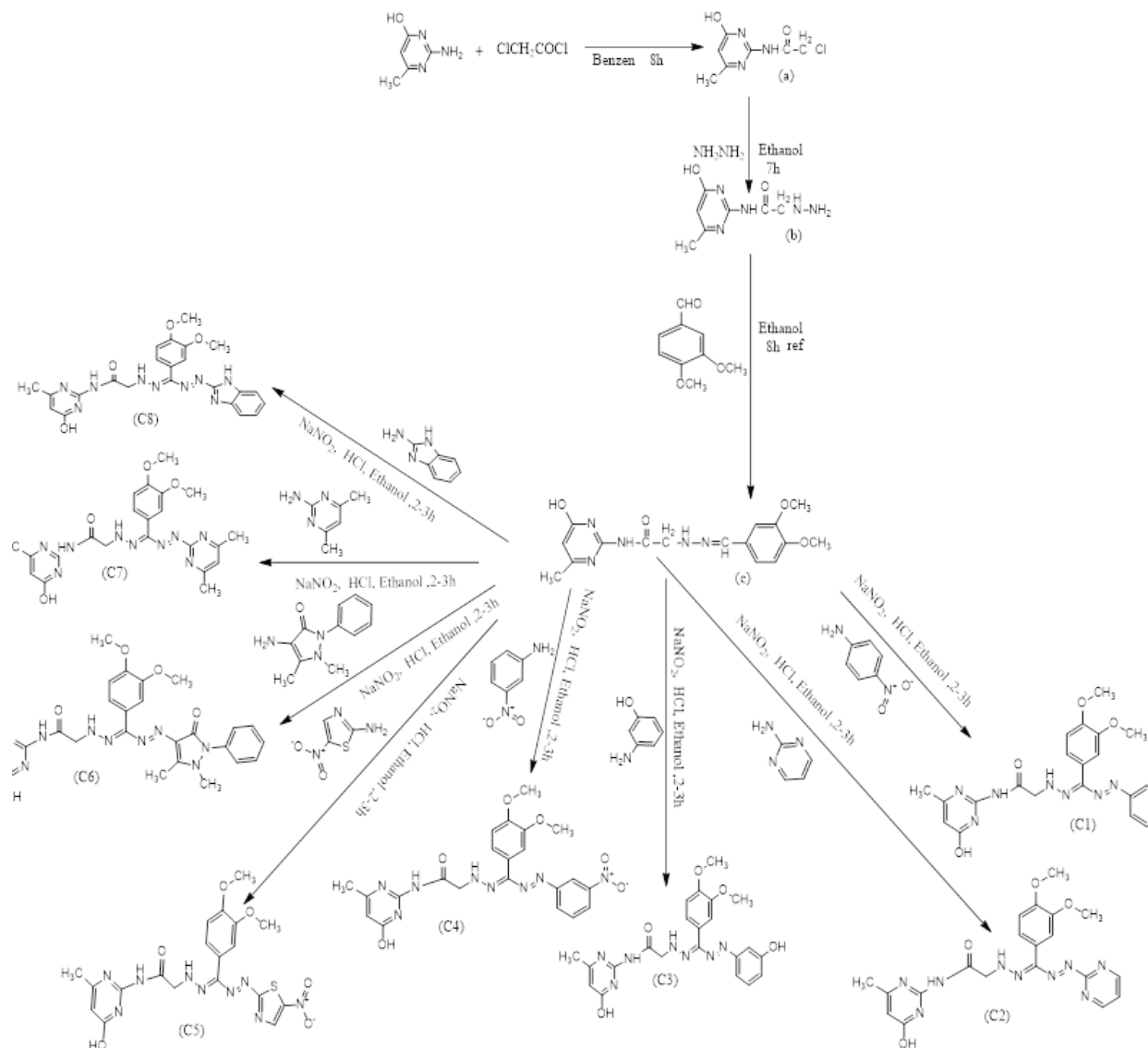
for (O – H), 3278 cm<sup>-1</sup> for (N – H), 3070 cm<sup>-1</sup> for (Ar – H), 2839 cm<sup>-1</sup> for (C – H) in (CH<sub>3</sub>), 1712 cm<sup>-1</sup> for (C=O), 1642 cm<sup>-1</sup> for (C=N), 1582 cm<sup>-1</sup> for (C=C) aromatic .

### Compound (b) :- 2-hydrazinyl-N-(4-hydroxy-6-methylpyrimidin-2-yl)acetamide

FT-IR spectrum data for derivative (b) show band at (3332-3178) cm<sup>-1</sup> for (O – H) and (N – H), 3070 for (Ar – H), 2926 cm<sup>-1</sup> for (C – H) in (CH<sub>3</sub>), 1700 cm<sup>-1</sup> for (C=O), 1666 cm<sup>-1</sup> for (C=N), 1604 cm<sup>-1</sup> for (C=C) aromatic

### Compound (c):- 2-(2-(3,5-dimethoxybenzylidene)hydrazinyl)-N-(4-hydroxy-6-methylpyrimidin-2-yl)acetamide

FT-IR spectrum data for derivative (c) show band at 3394 cm<sup>-1</sup> for (O – H), (3278-3178) cm<sup>-1</sup> for two kind of (N – H), 3078 for (Ar – H), 2968 cm<sup>-1</sup> for (C – H) in CH<sub>3</sub>, 1750 cm<sup>-1</sup> for (C=O), 1620 cm<sup>-1</sup> for (C=N), 1600 cm<sup>-1</sup> for (C=C) . <sup>1</sup>HMR spectrum data of compound (c) show 2.50ppm (DMSO), 10ppm (s, 1H, OH), 2ppm (s,3H, CH<sub>2</sub>) Figure 1, 2.4ppm (S,6H, OCH<sub>3</sub>), 3.9ppm (s,



Scheme 1: Synthesis of some formazan derivatives

2H, CH<sub>2</sub>), 7.4-7.9 ppm (m, 4H, Ar-H), 8.2 ppm (s, 1H, N=CH). The C<sub>13</sub>-NMR spectrum data (DMSO) compound (c) show : 23.76 ppm (C<sub>13</sub>), 26.24 ppm (C<sub>14</sub>, C<sub>15</sub>), 55 ppm (C<sub>6</sub>), 160 ppm (C<sub>5</sub>), 129 ppm (C<sub>7</sub>), 145 ppm (C<sub>4</sub>), 143 (C<sub>1</sub>), 136 (C<sub>14</sub>, C<sub>15</sub>), 117-134 ppm (C<sub>Arom</sub>), 39 ppm DMSO .

**Compound (C<sub>1</sub>):- (E,E)-3-(3,4-dimethoxyphenyl)-5-(2-((4-hydroxy-6-methylpyrimidin-2-yl)amino)-2-oxoethyl)-1-(4-nitrophenyl)formazan**

FT-IR spectrum data for derivative (C<sub>1</sub>) show band at 3340 cm<sup>-1</sup> for (O – H), 3224 cm<sup>-1</sup> for (N – H), 3078 for (Ar – H), 2931 cm<sup>-1</sup> for (C – H) in (CH<sub>3</sub>), 1728 cm<sup>-1</sup> for (C=O), 1600 cm<sup>-1</sup> for (C=N), 1596 cm<sup>-1</sup> for (C=C) aromatic . <sup>1</sup>H-NMR spectrum data of derivative (C<sub>1</sub>) show 2.43 ppm (DMSO) 10.5 ppm (s, 1H, OH), 1.22 ppm (s, 3H, CH<sub>3</sub>), 3.2 ppm (s, 6H, OCH<sub>3</sub>), 3.9 ppm (s, 2H, CH<sub>2</sub>), 6.90, 6.92 ppm (s, 2H, NH), 7.1, 7.7 ppm (m, 8H, Ar-H) . The C<sub>13</sub>-NMR spectrum data (DMSO) derivative (C<sub>1</sub>) show : 26.7 ppm (C<sub>18</sub>), 28.6 ppm (C<sub>19</sub>, C<sub>20</sub>), 29.7 ppm (C<sub>6</sub>), 161 ppm (C<sub>5</sub>), 154 ppm (C<sub>4</sub>), 148 ppm (C<sub>3</sub>), 139 ppm (C<sub>17</sub>), 129 ppm (C<sub>7</sub>), 137 ppm (C<sub>14</sub>), 100-130 ppm (C<sub>Arom</sub>), 39 ppm (DMSO) Figure 2.

**Compound (C<sub>2</sub>):- (E,E)-3-(3,4-dimethoxyphenyl)-5-(2-((4-hydroxy-6-methylpyrimidin-2-yl)amino)-2-oxoethyl)-1-(pyrimidin-2-yl)formazan**

FT-IR spectrum data for derivative (C<sub>2</sub>) show band at 3425 cm<sup>-1</sup> for (O – H), 3224 cm<sup>-1</sup> for (N – H), 3332 cm<sup>-1</sup> for (Ar – H), 2931 cm<sup>-1</sup> for (C – H) in (CH<sub>3</sub>), 1735 cm<sup>-1</sup> for (C=O), 1697 cm<sup>-1</sup> for (C=N), 1581 cm<sup>-1</sup> for (C=C) aromatic . <sup>1</sup>H-NMR spectrum data of derivative (C<sub>2</sub>) show 2.50 ppm (DMSO) 10.3 ppm (s, H, OH), 2.4 ppm (s, 3H, CH<sub>3</sub>), 3.3 ppm (s, 6H, OCH), 3.9 ppm (s, 2H, CH<sub>2</sub>), 6.6, 6.8 ppm (s, 2H, NH), 7.1- 7.8 ppm (m, 7H, Ar-H) . The C<sub>13</sub>-NMR spectrum data (DMSO) derivative (C<sub>2</sub>) show : 22.1 ppm (C<sub>17</sub>), 26 ppm (C<sub>18</sub>), 28 ppm (C<sub>6</sub>), 164 ppm (C<sub>5</sub>), 159 ppm (C<sub>4</sub>), 129 ppm (C<sub>7</sub>), 155 ppm (C<sub>14</sub>), 151 ppm (C<sub>3</sub>), 147 ppm (C<sub>10</sub>, C<sub>11</sub>), 100 ppm -138 ppm (C<sub>Arm</sub>) See Figure 3 and 4.

**Compound (C<sub>3</sub>):- (E,E)-3-(3,4-dimethoxyphenyl)-5-(2-((4-hydroxy-6-methylpyrimidin-2-yl)amino)-2-oxoethyl)-1-(3-hydroxyphenyl)formazan**

FT-IR spectrum data for derivative (C<sub>3</sub>) show band at 3425 cm<sup>-1</sup> for (O – H), 3276 cm<sup>-1</sup> for (N – H), 3132 cm<sup>-1</sup> for (Ar – H), 2958 cm<sup>-1</sup> for (C – H) in (CH<sub>3</sub>), 1797 cm<sup>-1</sup> for (C=O), 1604 cm<sup>-1</sup>

for (C=N), 1419 cm<sup>-1</sup> for (C=C) aromatic . <sup>1</sup>H-NMR spectrum data of derivative (C<sub>3</sub>) show 10 ppm (s, 2H, OH), 1.2 ppm (s, 3H, CH<sub>3</sub>), 2.0 ppm (s, 6H, OCH), 3 ppm (s, 2H, CH<sub>2</sub>), 6.4 ppm, 6.8 ppm (s, 2H, NH), 6.9-8.3 ppm (m, 8H, Ar-H) . The C<sub>13</sub>-NMR spectrum data (DMSO) derivative (C<sub>3</sub>) show : 26 ppm (C<sub>20</sub>), 28 ppm (C<sub>22</sub>, C<sub>23</sub>), 37 ppm (C<sub>6</sub>), 163 ppm (C<sub>5</sub>), 158 ppm (C<sub>4</sub>), 129 ppm (C<sub>7</sub>), 147 ppm (C<sub>9</sub>, C<sub>10</sub>), 151 ppm (C<sub>3</sub>, C<sub>16</sub>), 101 ppm -137 ppm (C<sub>Arm</sub>) .

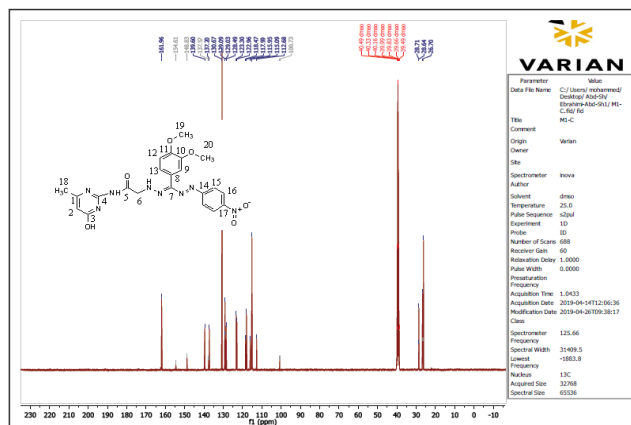


Figure 2: <sup>13</sup>C-NMR spectrum of compound (c<sub>1</sub>)

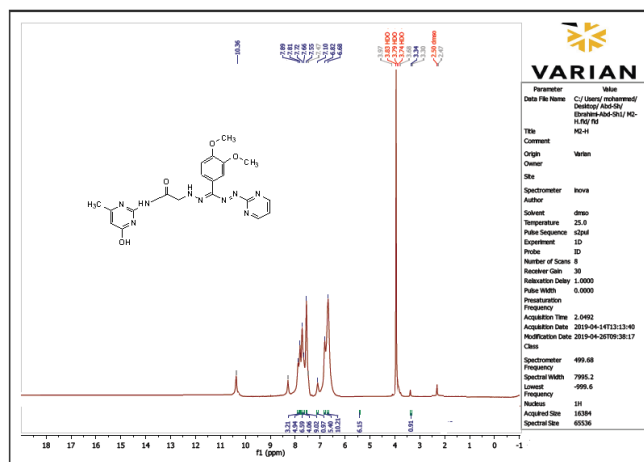


Figure 3: <sup>1</sup>H-NMR spectrum of compound (c<sub>2</sub>)

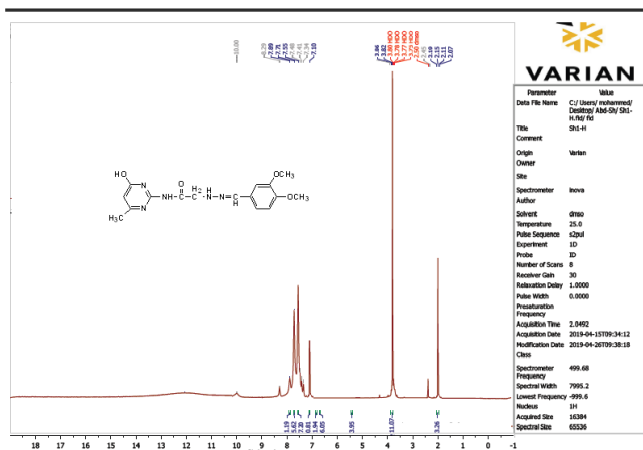


Figure 1: <sup>1</sup>H-NMR spectrum of compound (c)

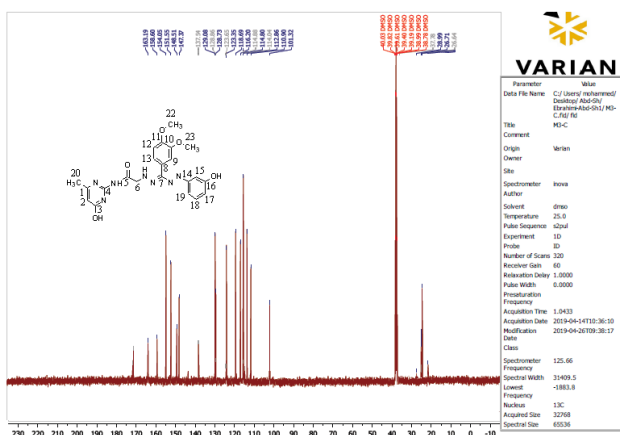


Figure 4: <sup>13</sup>C-NMR spectrum of compound (c<sub>3</sub>)

**Compound<sub>4</sub> :- (E,E)-3-(3,4-dimethoxyphenyl)-5-(2-((4-hydroxy-6-methylpyrimidin-2-yl)amino)-2-oxoethyl)-1-(3-nitrophenyl)formazan**

FT-IR spectrum data for derivative (c<sub>4</sub>) show band at 3440 cm<sup>-1</sup> for (O – H), 3261 cm<sup>-1</sup> for (N – H), 3078 cm<sup>-1</sup> for (Ar – H), 2931 cm<sup>-1</sup> for (C – H for CH<sub>3</sub>), 1735 cm<sup>-1</sup> for (C=O), 1581 cm<sup>-1</sup> for (C=N), 1512 cm<sup>-1</sup> for (C=C) aromatic. <sup>1</sup>HMNR spectrum data of derivative (c<sub>4</sub>) show 10ppm (s, H, OH), 2ppm (s,3H, CH<sub>3</sub>), 3.3ppm (s,6H, OCH), 3.9ppm (s, 2H, CH<sub>2</sub>), 5.3ppm, 6.5 ppm (s, 2H, NH), 6.8ppm – 8.1 ppm (m,8H, Ar-H). The C13-NMR spectrum data (DMSO) derivative (c<sub>4</sub>) show : 20ppm (C<sub>19</sub>), 26ppm, 29ppm (C<sub>20</sub>, C<sub>21</sub>), 33ppm (C<sub>6</sub>), 162ppm (C<sub>5</sub>),159ppm(C<sub>4</sub>), 129ppm (C<sub>7</sub>), 155ppm (C<sub>14</sub>), 130ppm (C<sub>16</sub>), 138ppm (C<sub>10</sub>, C<sub>11</sub>),100 ppm-129ppm (C<sub>Arm</sub>), 39ppm (DMSO).

**Compound<sub>5</sub>:- (E,E)-3-(3,4-dimethoxyphenyl)-5-(2-((4-hydroxy-6-methylpyrimidin-2-yl)amino)-2-oxoethyl)-1-(5-nitrothiazol-2-yl)formazan**

FT-IR spectrum data for derivative (c<sub>5</sub>) show band at 3409 cm<sup>-1</sup> for (O – H), 3224 cm<sup>-1</sup> for (N – H), 3032 cm<sup>-1</sup> for (Ar – H), 2839 cm<sup>-1</sup> for (C – H for CH<sub>3</sub>), 1560 cm<sup>-1</sup> for (C=O), 1581 cm<sup>-1</sup> for (C=N), 1481 cm<sup>-1</sup> for (C=C) aromatic. <sup>1</sup>HMNR spectrum data of derivative (c<sub>5</sub>) show 10.7ppm (s, 1H, OH), 1.2ppm (s,3H, CH<sub>3</sub>), 2ppm (s,6H, OCH), 3.8ppm (s, 2H, CH<sub>2</sub>), 8.1ppm (s, 1H, thiazol), 5.3, 6.7 ppm (s, 2H, NH), 6.9ppm – 7.4 ppm (m,4H, Ar-H). The C13-NMR spectrum data (DMSO) derivative (c<sub>5</sub>) show : 21ppm (C<sub>17</sub>), 26.1ppm (C<sub>18</sub>, C<sub>19</sub>), 26.6 ppm (C<sub>6</sub>), 160ppm (C<sub>5</sub>),145ppm(C<sub>4</sub>), 129ppm (C<sub>7</sub>), 143ppm (C<sub>14</sub>), 147ppm (C<sub>10</sub>, C<sub>11</sub>),136.3ppm(C<sub>3</sub>), 117 ppm-129ppm (C<sub>Arm</sub>), 39ppm (DMSO) See Figure 5 and 6.

**Compound<sub>6</sub>:- (E,E)-3-(3,4-dimethoxyphenyl)-1-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)-5-(2-((4-hydroxy-6-methylpyrimidin-2-yl)amino)-2-oxoethyl)formazan**

FT-IR spectrum data for derivativ (c<sub>6</sub>) show band at 3425 cm<sup>-1</sup> for (O – H), 3217 cm<sup>-1</sup> for (N – H), 3124 cm<sup>-1</sup> for (Ar – H), 2931 cm<sup>-1</sup> for (C – H for CH<sub>3</sub>), 1720 cm<sup>-1</sup> for (C=O), 1581 cm<sup>-1</sup> for (C=N), 1419 cm<sup>-1</sup> for (C=C) aromatic. <sup>1</sup>HMNR spectrum data of derivative (c<sub>6</sub>) show 10ppm (s, H, OH), 2.1ppm (s,3H,

CH<sub>3</sub> in pyrazoline derivative, 3.6ppm, 3.7ppm (s,6H, OCH), 3.97ppm (s, 2H, CH<sub>2</sub>), 6.77ppm, 6.79 ppm (s, 2H, NH), 2.5 ppm (s,3H, in pyrazoline derivative). The C13-NMR spectrum data (DMSO) derivative (c<sub>6</sub>) show : 20ppm (C<sub>23</sub>), 26.21ppm, 26.62ppm, 29ppm (C<sub>24</sub>, C<sub>25</sub>), 29.32ppm (C<sub>26</sub>), 29.72ppm (C<sub>27</sub>) 33.62ppm (C<sub>6</sub>), 162ppm (C<sub>5</sub>),149ppm(C<sub>4</sub>), 139ppm (C<sub>14</sub>), 155ppm (C<sub>15</sub>), 137ppm (C<sub>22</sub>),100 ppm-130ppm (C<sub>Arm</sub>), 39ppm (DMSO) See Figure 7 and 8.

**Compound<sub>7</sub> :- (E,E)-3-(3,4-dimethoxyphenyl)-1-(4,6-dimethylpyrimidin-2-yl)-5-(2-((4-hydroxy-6-methylpyrimidin-2-yl)amino)-2-oxoethyl)formazan**

FT-IR spectrum data for derivative (c<sub>7</sub>) show band at 3440 cm<sup>-1</sup> for (O – H), 3240 cm<sup>-1</sup> for (N – H), 3132 cm<sup>-1</sup> for (Ar – H), 2839 cm<sup>-1</sup> for (C – H for CH<sub>3</sub>), 1720 cm<sup>-1</sup> for (C=O), 1581 cm<sup>-1</sup> for (C=N), 1419 cm<sup>-1</sup> for (C=C) aromatic. <sup>1</sup>HMNR spectrum data of derivative (c<sub>7</sub>) show 10.9ppm (s, 1H, OH), 1.91ppm,1.97ppm, 2.1ppm (s,9H, CH<sub>3</sub>), 3.6ppm (s,6H, OCH), 3.7ppm (s, 2H, CH<sub>2</sub>), 6.5ppm, 6.7 ppm (s, 2H, NH), 6.8ppm – 7.9 ppm (m,4H, Ar-H). The C13-NMR spectrum data (DMSO) derivative (c<sub>7</sub>) show : 22ppm (C<sub>18</sub>), 26.65ppm, 26.23ppm (C<sub>21</sub>), 28.6 ppm, 28.7 ppm (C<sub>19</sub>, C<sub>20</sub>), 30.9 ppm (C<sub>6</sub>), 167ppm (C<sub>5</sub>),153ppm(C<sub>4</sub>), 129ppm (C<sub>7</sub>), 152ppm (C<sub>14</sub>), 141ppm (C<sub>10</sub>, C<sub>11</sub>),121 ppm -135ppm (C<sub>Arm</sub>), 39ppm (DMSO) See Figure 9.

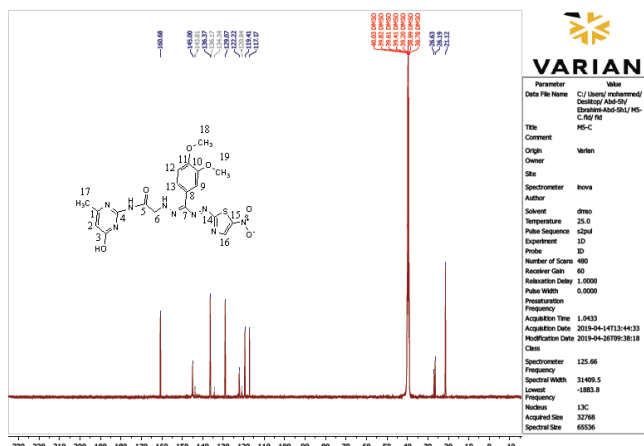


Figure 6: <sup>13</sup>C-NMR spectrum of compound (c<sub>5</sub>)

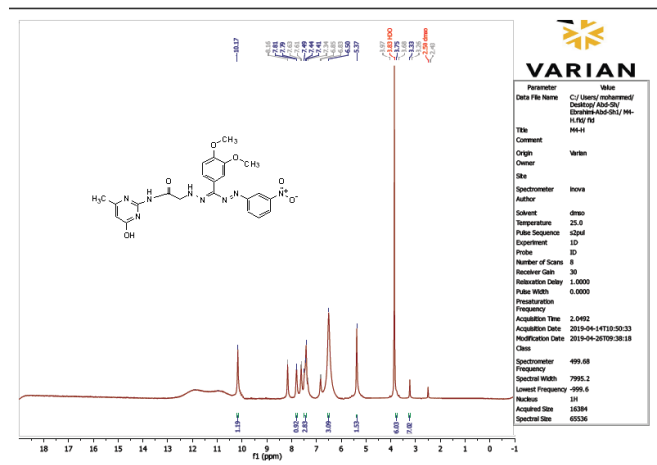


Figure 5: <sup>1</sup>H-NMR spectrum of compound (c<sub>4</sub>)

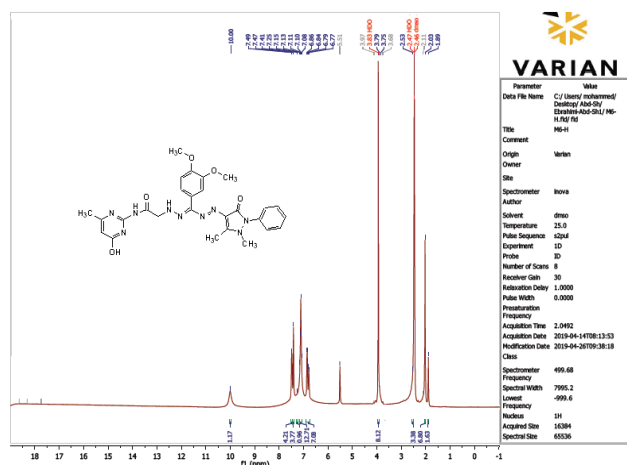


Figure 7: <sup>1</sup>H-NMR spectrum of compound (c<sub>6</sub>)

**Compound c<sub>8</sub>:- (E,E)-1-(1H-benzo[d]imidazol-2-yl)-3-(3,4-dimethoxyphenyl)-5-(2-((4-hydroxy-6-methylpyrimidin-2-yl)amino)-2-oxoethyl)formazan**

FT-IR spectrum data for derivative (c<sub>8</sub>) show band at 3487 cm<sup>-1</sup> for (O – H), 3217 cm<sup>-1</sup> for (N – H), 3116 cm<sup>-1</sup> for (Ar – H), 2908 cm<sup>-1</sup> for (C– H in CH<sub>3</sub>), 1735 cm<sup>-1</sup> for (C=O), 1620 cm<sup>-1</sup> for (C=N), 1504 cm<sup>-1</sup> for (C=C) aromatic. <sup>1</sup>H-NMR spectrum data of compound (c<sub>8</sub>) show 10ppm (s, 1H, OH), 2ppm, 3.3, 3.6 (s,3H, CH<sub>3</sub>), 3.7ppm (s,6H, OCH), 3.7ppm (s, 2H, CH<sub>2</sub>), 5.3ppm,6.6ppm (s, 3H, NH), 6.8ppm – 8.33 ppm (m,9H, Ar-H). The C13-NMR spectrum data (DMSO) derivative (c<sub>5</sub>) show : 23ppm (C<sub>21</sub>), 26ppm, 29ppm (C<sub>22</sub>, C<sub>23</sub>), 37 ppm (C<sub>6</sub>), 164ppm (C<sub>5</sub>),163ppm(C<sub>4</sub>), 161ppm (C<sub>14</sub>), 155ppm (C<sub>15</sub>,C<sub>20</sub>), 129ppm (C<sub>7</sub>), 149ppm (C<sub>3</sub>), 137ppm (C<sub>10</sub>,C<sub>11</sub>), 100 ppm-130ppm (C<sub>Ar</sub>), 39ppm (DMSO).

### BIOLOGICAL ACTIVITY

in the above studies it can be conclusion that the prepare compounds reduce significant antibacterial effectiveness against bacteria staphylococcus aureus and Escherichia coli. The compounds that show good activity are (2,3,6) against (staphylococcus aureus), and compound that show good activity are (1,2,6) against (Escherichia coli), the results of the antibacterial activity are shown in the Figure 10.

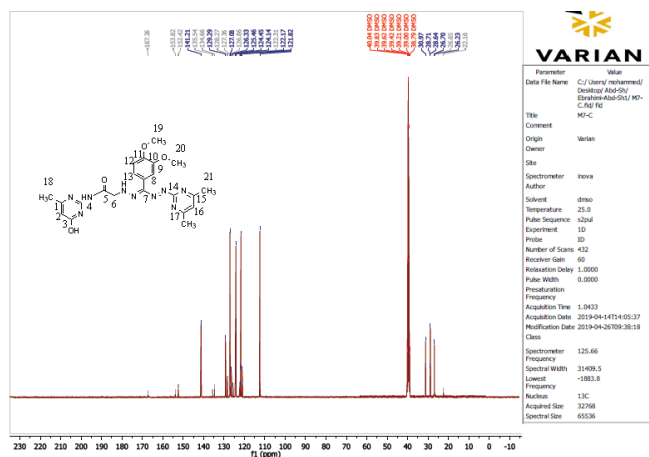


Figure 8: <sup>13</sup>C-NMR spectrum of compound (c<sub>7</sub>)

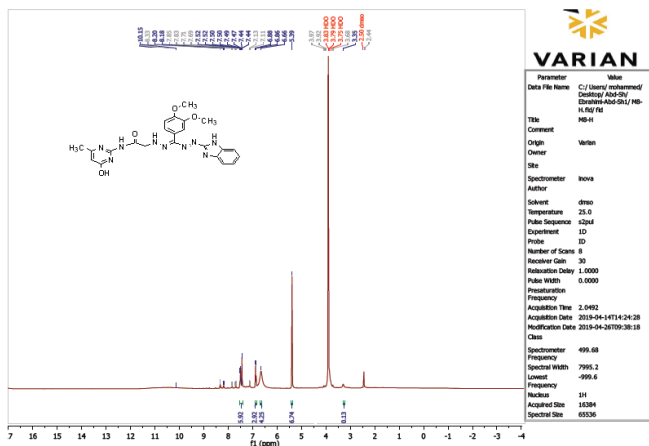


Figure 9: <sup>1</sup>H-NMR spectrum of compound (c<sub>8</sub>)

The biological activity of the prepared compounds was investigated and the most effective compound (2) The drug was studied on two types of cells: MCF-7 and WRL The study showed that this type of compounds was effective with good pharmacologic effect (Figure 11). At concentration 400, we found a response rate of 61% in MCF-7 cells versus 21% in WRL cells and table (1-2) showing the effect of the compound on cells versus concentrations Which was developed

The IC<sub>50</sub> values for the cells were also calculated and there was a significant difference between the values of the IC<sub>50</sub> in the WRL and MCF-7 cells as shown in the Figure 11.

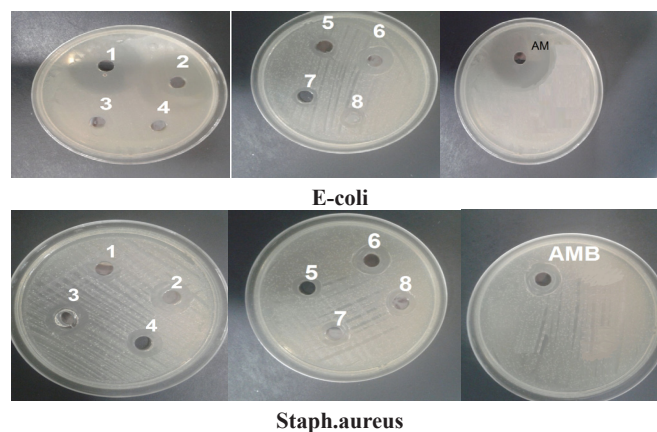


Figure 10: Activity of staphylococcus aureus against inhibition

Table 1: Show Biological activity for compounds (1-8)

Comp No	<i>E.Coli</i>	<i>Mm</i>	<i>Staph aureus</i>	<i>Mm</i>
1	+++	28	+	7
2	+++	31	+++	25.5
3	-	0	++	17
4	-	0	+	9
5	+	5.2	+	6.5
6	++	13	++	12
7	+	7.5	+	3
8	+	9	+	5
Ampicillin	+++	29	+++	23

=-No inhibition active = in active, += (5-10)mm =slightly active, ++= (11-20)mm moderately +++ = More than 20, good active

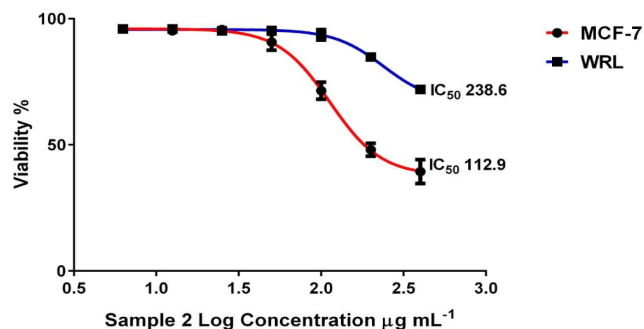


Figure 11: biological activity of the prepared compounds on two types of cells: MCF-7 and WRL

**Table 2:** Showing the effect of the compound on cells versus concentrations Which was developed

WRL		A	B	C	D	E	F	G
		400	200	100	50	25	12.5	6.25
		Y	Y	Y	Y	Y	Y	Y
1	Number of values	3	3	3	3	3	3	3
2								
3								
4	Mean	71.95	84.80	93.60	95.33	95.22	95.95	95.95
5	Std. Deviation	0.8128	1.205	2.100	1.183	0.8209	1.028	0.2003
6	Std. Error of Mean	0.4693	0.6955	1.212	0.6828	0.4739	0.5937	0.1157

MCF-7		A	B	C	D	E	F	G
		400	200	100	50	25	12.5	6.25
		Y	Y	Y	Y	Y	Y	Y
1	Number of values	3	3	3	3	3	3	3
2								
3								
4	Mean	39.35	48.03	71.49	90.70	95.72	95.18	95.95
5	Std. Deviation	4.783	2.554	3.399	3.183	0.8099	1.280	0.5306
6	Std. Error of Mean	2.762	1.475	1.962	1.838	0.4676	0.7393	0.3063

**Table 3:** Physical and analytical data of compounds (1-8)

Comp	Nam of compound	M.F M.wat	m.p	Rf	Colour	%
1	((E,E)-3-(3,4-dimethoxyphenyl)-5-(2-((4-hydroxy-6-methylpyrimidin-2-yl)amino)-2-oxoethyl)-1-(4-nitrophenyl)formazan	C <sub>22</sub> H <sub>22</sub> N <sub>8</sub> O <sub>6</sub> 494.47	169	0.31	Yellow	62
2	(E,E)-3-(3,4-dimethoxyphenyl)-5-(2-((4-hydroxy-6-methylpyrimidin-2-yl)amino)-2-oxoethyl)-1-(pyrimidin-2-yl)formazan	C <sub>20</sub> H <sub>21</sub> N <sub>9</sub> O <sub>4</sub> 451.45	204	0.16	Yellow	64
3	(E,E)-3-(3,4-dimethoxyphenyl)-5-(2-((4-hydroxy-6-methylpyrimidin-2-yl)amino)-2-oxoethyl)-1-(3-hydroxyphenyl)formazan	C <sub>22</sub> H <sub>23</sub> N <sub>7</sub> O <sub>5</sub> 465.47	201	0.27	Pink	72
4	((E,E)-3-(3,4-dimethoxyphenyl)-5-(2-((4-hydroxy-6-methylpyrimidin-2-yl)amino)-2-oxoethyl)-1-(3-nitrophenyl)formazan	C <sub>22</sub> H <sub>22</sub> N <sub>8</sub> O <sub>6</sub> 494.47	192	0.43	Yellow	62
5	((E,E)-3-(3,4-dimethoxyphenyl)-5-(2-((4-hydroxy-6-methylpyrimidin-2-yl)amino)-2-oxoethyl)-1-(5-nitrothiazol-2-yl)formazan	C <sub>19</sub> H <sub>19</sub> N <sub>9</sub> O <sub>6</sub> S 501.48	215	0.25	Brown red	69
6	((E,E)-3-(3,4-dimethoxyphenyl)-1-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)-5-(2-((4-hydroxy-6-methylpyrimidin-2-yl)amino)-2-oxoethyl)formazan	C <sub>27</sub> H <sub>29</sub> N <sub>9</sub> O <sub>5</sub> 559.59	183	0.28	Yellow	63
7	((E,E)-3-(3,4-dimethoxyphenyl)-1-(4,6-dimethylpyrimidin-2-yl)-5-(2-((4-hydroxy-6-methylpyrimidin-2-yl)amino)-2-oxoethyl)formazan	C <sub>22</sub> H <sub>25</sub> N <sub>9</sub> O <sub>4</sub> 479.50	220	0.12	Yellow	61
8	((E,E)-1-(1H-benzof[d]imidazol-2-yl)-3-(3,4-dimethoxyphenyl)-5-(2-((4-hydroxy-6-methylpyrimidin-2-yl)amino)-2-oxoethyl)formazan	C <sub>23</sub> H <sub>23</sub> N <sub>9</sub> O <sub>4</sub> 489.50	176	0.22	Brown	67

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