

## Synthesis and Microbiological Investigation of Progesterone Derivatives

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### ABSTRACT

New derivatives of progesterone were synthesized by the reaction of progesterone with hydrazine and thiosemicarbazide then cyclized by using of ethylacetoacetate and ethylallylmalonate. The new compounds were characterized by using of Melting point and FT-IR spectroscopy. The new compounds were tested against selected types of gram positive and gram negative bacteria, and show encarge activity.

### تحضير و دراسة الفعالية الحيوية لمشتقات جديدة من هرمون البروجستيرون

#### الخلاصة

تم تحضير مشتقات جديدة لهرمون البروجستيرون و ذلك باستخدام الهيدرازين و الثايوسيميكاربازايد و من ثم الغلق الحلقي بواسطة اثيل مالونيت و اثيل اسيتو اسنتيت. تم تشخيص التركيب الكيميائي للمركبات المحضرة بواسطة قياس درجة الانصهار و مطيافية الاشعة تحت الحمراء. تم اختبار الفعالية البيولوجية لكل من المركبات المحضرة على انواع مختارة من البكتريا الموجبة لصبغة غرام و البكتريا السالبة لصبغة غرام.

### INTRODUCTION

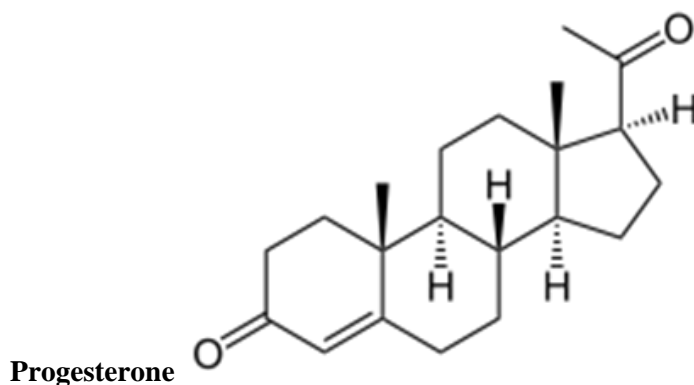
Progesterone is a steroid hormone in mammals that is involved in the female menstrual cycle, pregnancy (supporting gestation), and embryogenesis. The term progesterone also is used for natural or synthetic steroids that have a progesterone-like action on the body[1].

Progesterone belongs to a class of sex hormones called progestogens. Progesterone is the main progestogen produced in ovaries, particularly in the second half of the menstrual cycle after the occurrence of ovulation and the formation of a corpus luteum by the empty follicle[1]. It is synthesized in the initial steps in the biosynthetic pathway involving the conversion of cholesterol to the sex

steroids testosterone (an androgen) and estradiol (an estrogen), among others. It also is a precursor in the conversion to cortisol and aldosterone[2].

In addition to impacts on the reproductive system, progesterone also has impacts on the nervous system, immune system, skeletal system, thyroid function, and numerous other body functions. Human creativity has utilized natural progesterone and many synthetic analogues in many medical applications [3, 4].

Progesterone is a steroid hormone. A steroid is any of a group of natural or synthetic, fat-soluble, organic compounds belonging to the class of lipids and characterized by a molecular core of four fused rings totaling 17 carbon atoms: Three six-carbon rings and one five-carbon ring fused together. The type of steroid is determined by the three-dimensional configuration and the type of additional side chains and rings [5].



progestogens (or progestagens) are a class of hormones that produce effects similar to progesterone. Progestogens that are synthetic are often referred to as progestins[5]. Like other steroids, progesterone consists of four interconnected cyclic hydrocarbons. Progesterone contains ketone and oxygenated functional groups, as well as two methyl branches. Like all steroid hormones, it is hydrophobic. This is mostly due to its lack of very polar functional groups. Progesterone has the chemical formula  $C_{21}H_{30}O_2$  [2,5].

**Experimental:** All chemicals used were of analar grade (supplied by either Merck or Fluka) and used as supplied. FT-IR spectra were recorded using shimadzu-8300 spectrophotometer using KBr.

**Synthesis of compounds [1] and [2]:** A mixture of 0.01mol of progesterone and (0.01mol and 0.02mol ) of hydrazine in 50ml ethanol were refluxed in a water bath for 60min then left to cool in anIce-water. The solid was filtered, and recrystallized from acetonitrile. Melting points and yield are listed in Table (1). Physical and Spectral data are listed in Table (2).

**Synthesis of compounds [3] and [4]:** Hot ethanolic solution of thiosemicarbazide (0.01mol and 0.02 mol) and ethanolic solution of progesterone (0.01mol) were mixed in the presence of few drops of concentrated hydrochloric acid with constant stirring. This mixture was refluxed for 3 hours. The completion of the reaction was confirmed by the TLC. The reaction mass was evaporated on a rotatory evaporator.

Thiosemicarbazones, [3] and [4] filtered, washed with cold ethanol, and dried under vacuum over P<sub>4</sub>O<sub>10</sub>. Melting points and yield are listed in Table (1). Physical and Spectral data are listed in Table (2).

**Synthesis of compounds [5], [6] and [7]:** Dioxocompound (Ethyl acetoacetate) (0.02, 0.01 and 0.02) mol were added to 0.01 mole of [2, 3 and 4] in 30ml absolute ethanol and refluxed for 6-8hrs, and then left to cool; the precipitate thus formed was filtered, and recrystallized from acetonitrile where compounds [5, 6 and 7] are formed. Melting points and yield are listed in Table (1). Physical and spectral data are listed in Table (3).

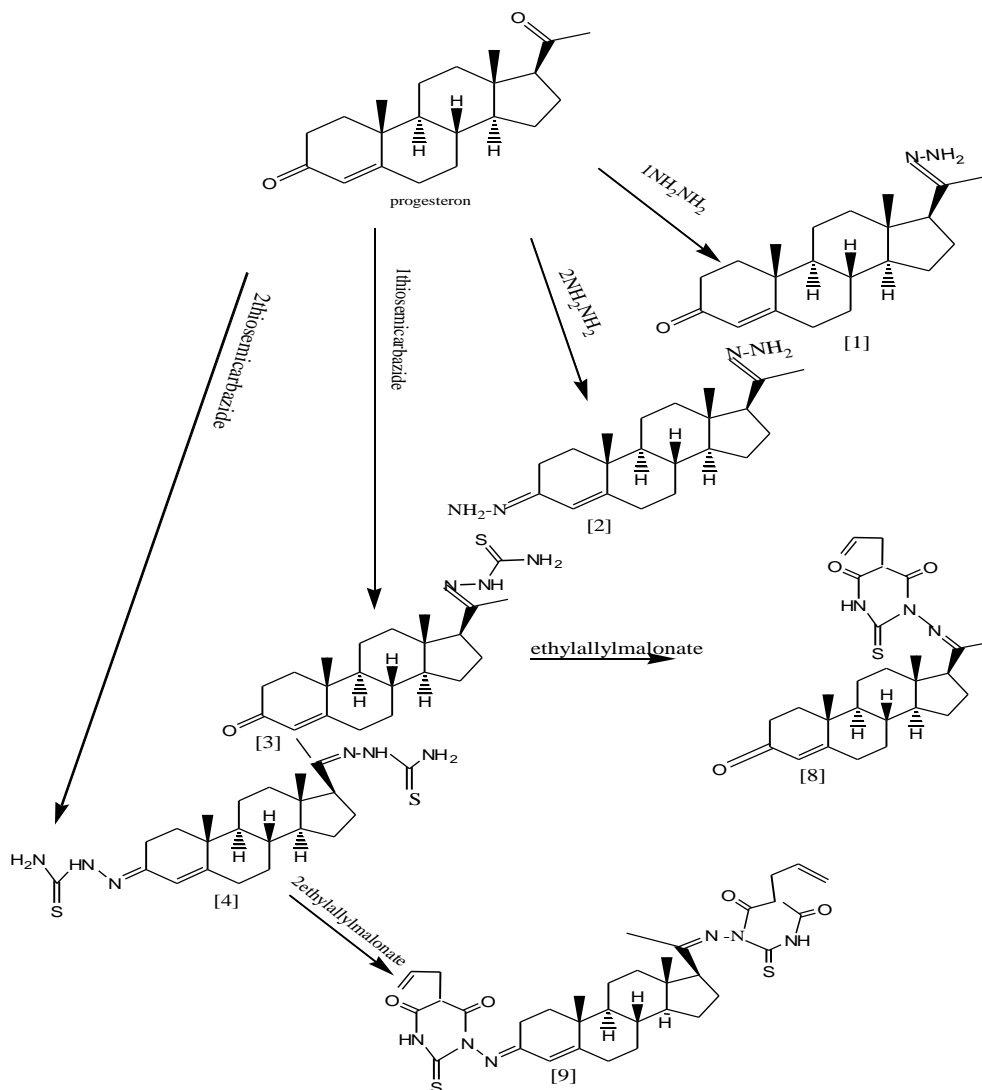
**Synthesis of compounds [8] and [9]:** Dioxocompound (Ethyl allylmalonate) (0.01 and 0.02) mol were added to 0.01 mol [3 and 4] in 30ml ethanol and refluxed for (7-8hrs), then left to cool. The precipitate was filtered, and then recrystallized from acetonitrile where compounds [8] and [9] are formed. Melting points, and yield are listed in Table (1), while physical and spectral data are listed in Table (4).

**Antibacterial activity:** The biological activity of the prepared new progesterone derivatives were studied against selected types of bacteria which included Staphylococcus aureus and Strptococcus pneumoniae as gram positive and Pseudomonas aeruginosa and E. Coli as gram negative, in brain heart broth agar media, which is used DMF as a solvent and as a control for the disc sensitivity test [6-8]. This method involves the exposure of the zone of inhibition toward the diffusion of micro-organism on agar plate. The plates were incubated for 24 hours 37 °C. The antimicrobial activity was recorded as any area of microbial growth inhibition that occurred in the diffusion area. The quantitative antimicrobial activity assay was performed by the nutrient broth for bacterial.

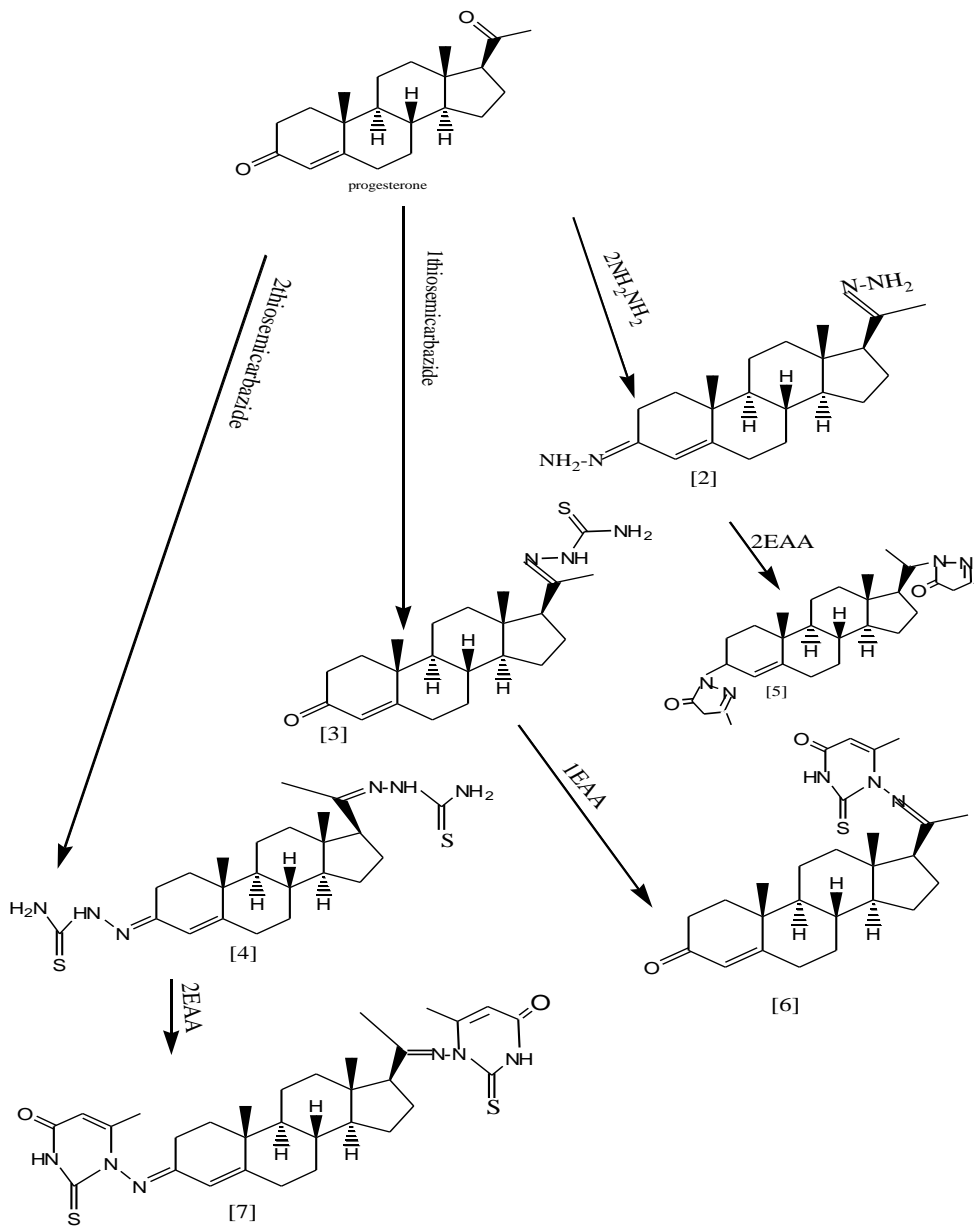
**Table (1) Physical analytical data for the Synthesized of compounds.**

No.	Names	M.P.°C	Yield %	Color
[1]	progesterylhiazide	259-261	80	Yellow
[2]	progesteryldihiazide	Over 300	82	Yellow
[3]	progesteronethaiosemicarbazone	200-202	75	Yellow
[4]	progesteronedithaiosemicarbazone	220-222	77	Milky
[5]	10,13-dimethyl-6,7,8,9,10,11,12,13,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-3-yl)-1,17-di(5-methyl-1,2-dihydropyrazol-3-one)-17-one	oily	60	Brown
[6]	1-((8S,9S,10R,13S,14S,17S,E)-3-hydrazono-10,13-dimethyl-2,3,6,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-17-yl)ethanone-1-pyridine-2,6(1H)-one(3H)-thion	oily	59	Brown
[7]	1-((8S,9S,10R,13S,14S,17S,E)-3-hydrazono-10,13-dimethyl-	oily	61	Brown

	2,3,6,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-17-yl)ethanone-di-(1- pyridine-2,6(1H,3H)-dione)			
[8]	1-((8S,9S,10R,13S,14S,17S,E)-3-hydrazono-10,13-dimethyl-2,3,6,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-17-yl)ethanone-3-allylpiperidine-2,4-dione-6-thion	210-212	45	Rose
[9]	1-((8S,9S,10R,13S,14S,17S,E)-3-hydrazono-10,13-dimethyl-2,3,6,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-17-yl)ethanone-di-(3-allylpiperidine-2,4-dione-6-thion)	188-190	43	Dark rose



Scheme (1) Reaction for synthesis of compounds [1, 2,3,4,8 and 9].

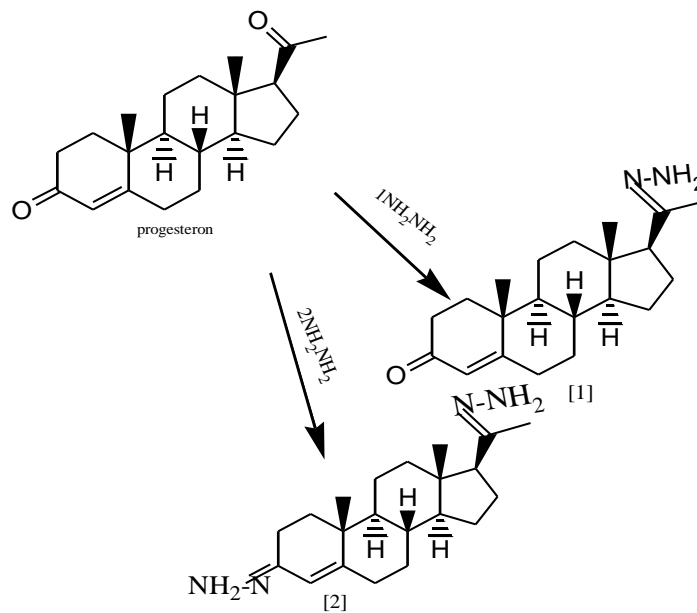


Scheme (2) Reaction for synthesis of compounds [5, 6 and 7].

### Results & Discussion

Reaction of progesterone with (1mole and 2mole) hydrazine in boiling ethanol led to the formation of compounds [1] and [2] (Scheme 1).

**Reaction:**



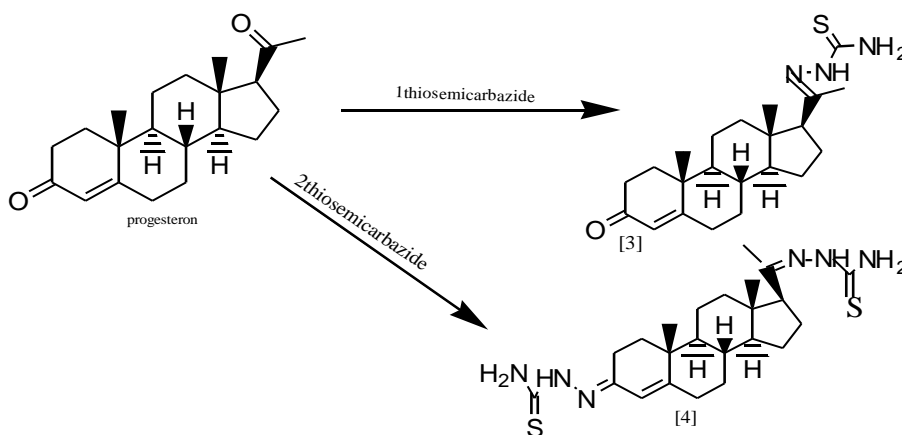
**Scheme (3)**

The FT-IR spectroscopy showed that the peak of carbonyl groups  $\nu$  C=O are disappeared and appearance of  $\text{NH}_2$  peak and the C=N peak. New absorption bands at for  $(3346.61-3205) \text{ cm}^{-1}$  for  $\nu\text{NH}_2$  of the hormone [1] and  $(3346.61-3211) \text{ cm}^{-1}$  for  $\nu\text{NH}_2$  of the hormone [2] .

**Reaction of progesterone with thiosemicarbazide to form [3-4].**

The new hormones were synthesized by the reaction of the progesterone with (1mole and 2mole) thiosemicarbazide in ethanol medium.

**Reaction:**



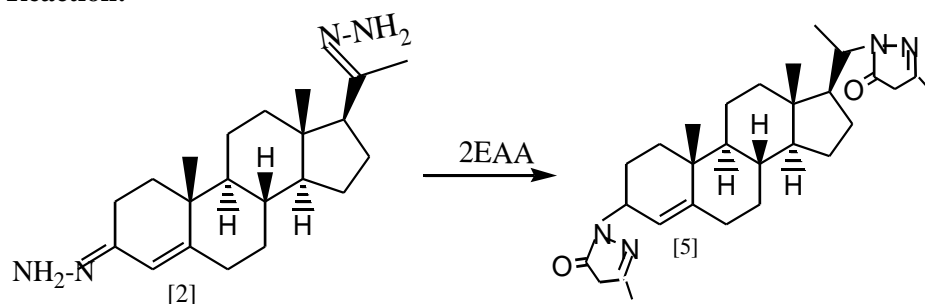
**Scheme (4)**

The IR spectra provide valuable information regarding the nature of functional groups attached to the progesterone. The new hormones [3-4] were characterized mainly using the azomethine and primary amine ( $-\text{NH}_2$ ) bands. The main infrared bands and their assignments are listed in Table (2). The appearance of a broad strong band in the IR spectra of the hormone [3] at  $(3429.55\text{-}3250.16)\text{ cm}^{-1}$  for  $\nu\text{NH}_2$  stretching vibrations of the primary amine group and  $(3431.13\text{-}3245.97)\text{ cm}^{-1}$  for  $\nu\text{NH}_2$  of the hormone [4].

#### Reaction of [2, 3 and 4] with EAA to form [5,6 and 7] respectively

Heating the reaction mixture of ethyl acetoacetate with amines [2, 3 and 4] were yielded cyclized compounds [5, 6 and 7], and the FT-IR spectroscopy showed that the absorption bands of  $\nu\text{C}=\text{O}$  are appeared and the disappearance of the  $\text{NH}_2$  peak (Scheme 5).

#### Reaction:

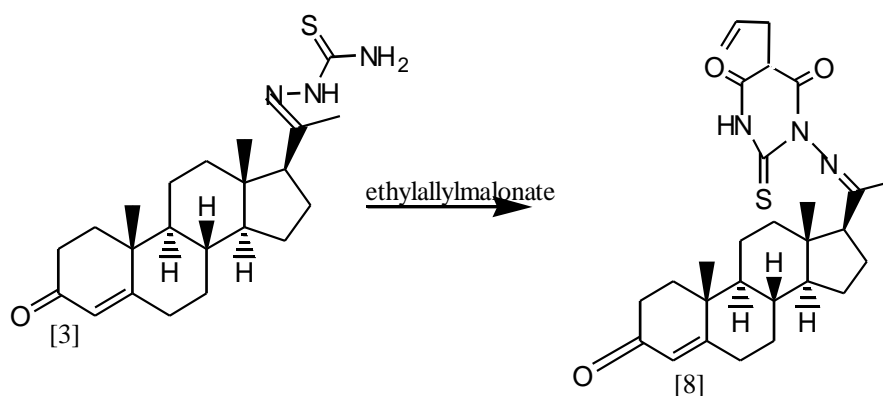


Scheme (5)

#### Reaction of [3 and 4] with ethylallylmalonate to form [8 and 9] respectively

The reaction of [3] or [4] with ethylallylmalonate leads to cyclization of the amino group with the carbonyls. The FT-IR spectroscopy showed that the peak of the  $\text{NH}_2$  group has been disappeared (Scheme 6).

#### Reaction:



Scheme (6)



**Table (2) Selected FT-IR bands in  $\text{cm}^{-1}$  for the compounds.**

Symbol	[1]	[2]	[3]	[4]
-NH <sub>2</sub>	3346.61-3205	3346.61-3211	3429.55-3250.16	3431.13-3245.97
-NH	-	-	3146.00	3176.54
-CH <sub>3</sub>	2929.97	2929.97	2937.66	2962.46
C=O	1569	-	1565.65	-
C=S	-	-	1292.35	1296.08
C=N	1637.61	1633.76	1647.26	1676.02

**Table(3) Selected FT-IR bands in  $\text{cm}^{-1}$  for the compounds.**

Symbol	[5]	[6]	[7]
-NH <sub>2</sub>	-	-	-
C=O	1688.70	1678.61	1675.20
C=N	1633.12	1633.50	1634.00
C=C(adjacent of carbonyl group)	-	1355.11	1356.76
C=C(inside)	1375	1375	1375

**Table(4) Selected FT-IR bands in  $\text{cm}^{-1}$  for the compounds.**

Symbol	[8]	[9]
-NH <sub>2</sub>	-	-
-NH	3136.00	3139.12
C=C(terminal)	1630.11	1637.37
C=C(inside)	1576.00	1576.10
C=O(terminal)	1575.00	-
C=O(inside)	1625.22	1616.00

**Biological Activity:** The antimicrobial screening data show that the compounds exhibit antimicrobial properties and it is important to note that the new derivatives exhibit more inhibitory effects than the original molecule (I). From Table (5) it is clear that the zone of inhibition against the gram-negative bacteria and gram-positive bacteria. The increased activity of the new derivatives can be explained that act as more powerful and potent bactericidal agents, thus killing more of the bacteria than the original molecule (I). The  $\pi$ -electron delocalization over the new derivatives increases the lipophilic character and favors its permeation through the lipid layer of the bacterial membranes. It was reported that progesterone derivatives have interesting antimicrobial activity against different species of Gram positive bacteria, Gram negative bacteria. Heterocyclic compounds have been widely reported to be biologically versatile compounds having antifungal, fungicidal, herbicidal and plant growth regulating properties. The presence of amino linkage (-N=C-) in these compounds has been regarded as being essential for the enhancement of antibacterial and antimicrobial activities [9-16].

Table (5) the effect of Test organism toward synthesized compounds.

Compound	<i>E. Coli</i>	<i>Staphylococcus aureus</i>	<i>Pseudomonas aeruginosa</i>	<i>Streptococcus pneumoniae</i>
[1]	+	-	+	+++
[2]	+	-	++	+++
[3]	++	-	+++	+++
[4]	++	-	+++	+++
[5]	+	+	+++	+++
[6]	+	-	++	+++
[7]	+	-	++	+++
[8]	+	+	+++	+++
[9]	+	-	+++	+++

Results were interpreted in terms of the diameter of the inhibition zone:  
 (-) :< 0.5mm, (+):0.5mm, (++) :1mm, (+++) : => 1.5mm.

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