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Synthesis, characterization and evaluation of the bacterial, antioxidant and anticancer activity of pyrimidine derivatives

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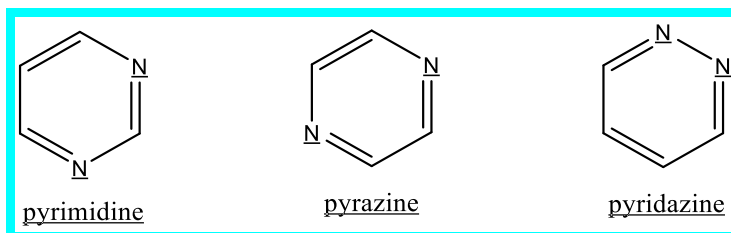
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Abstract---In this paper, heterocyclic hexagonal rings were prepared for pyrimidine derivatives [I9-I16] by reacting a mole of gluconate derivatives with a mole of cyanoguanidine and using ethanol as a solvent. The accuracy of the compositions of the prepared compounds was confirmed by measuring the physical properties, including melting point, color, and molecular weight, as well as through spectroscopic measurements, including the infrared spectrum, the proton nuclear magnetic resonance spectrum, and the carbon-13 nuclear resonance spectrum. The biological activity of some prepared compounds and on two types of pathogenic bacteria, one of which is Gram-positive, which is *Staphylococcus aureus*, and one of which is Gram-negative, which is *Escherichia coli*, was studied. And the culture medium of the Muller Hinton Agar type was used, and chemical solutions were prepared for the two compounds (I9, I16) with concentrations (0,01, 0.001, 0.0001) mg /ml using a solvent dimethyl sulfoxide (DMSO). The sensitivity test of bacterial isolates used in the study was carried out by diffusion method, and the antibiotic Ciprofloxacin was used as a control sample. The effect of compound I15 on the removal of free radicals was also studied using DPPH root and at different concentrations. The effectiveness of some prepared compounds against breast cancer cells was studied, cancer cells were obtained from the Medical City – Baghdad. Holes were made to place the prepared compounds, and 200 µl of the prepared concentrations of the compounds prepared for the study were added (25, 50, 100, 200) µg/ml to the pits at three pits for each concentration, cytotoxicity tests were performed in three replicates and (IC₅₀) values were calculated. In this test, the cytotoxicity effect of the compounds was determined and prepared on breast cancer cells and regular cell line WRL68 for comparison, as examined using MTT and solute solutions.

Keywords---Heterocyclic Sextants, Pyrimidines, Staphylococcus Aureus, Escherichia Coli, Antioxidant Activity, Breast Cancer.

1. Introduction

The pyrimidines are heterocyclic compounds identical to the pyridine ring [1]. The pyrimidine ring consists of four carbon atoms and two nitrogen atoms in the form of 1,3-pyrimidines heterocyclic compounds, which determines the type of the compound Its name in the ring is the position of the two nitrogen atoms [2], and according to their work in the atoms.



Pyrimidine derivatives are used in biological and pharmaceutical activities such as anti-cancer, anxiolytic, antioxidant, antiviral, And antifungal [3]. Pyrimidines have many properties in common with pyridine, such as the number of nitrogen atoms. The PKA value of the protons of pyrimidines is 1.23 compared to 5.30 for pyridine [4]. And his group [5] prepared pyrimidine derivatives from the reaction of chalcones with various reagents. Pyrimidines and their products have wide applications [6], including thymine, nucleotides (V B), and (Alloxan), which have significant biological activity. Pyrimidines [7] are prepared from the reaction of substituted chalcones with urea and thiourea and ethanol as a solvent. And the developments in medicinal chemistry showed that pyrimidine has a vital efficacy in the development of anti-cancer, anti-inflammatory, and anti-bacterial [8], and it succeeded in its use as antifungals [9] and also proved its efficiency as an anti-tuberculosis and antioxidant [10]. Bacteria are micro-organisms that can only be seen with a microscope. Bacteria are found everywhere, in the air, in the water, in the human body, and inside the digestive canal and respiratory system [11]. Bacteria can live for long years, tolerating all unsuitable conditions such as high or low temperature or other harsh environmental conditions. When environmental conditions improve, the bacteria get rid of the thick membrane and return to their previous activity and vitality [12]. Staphylococcus aureus is spherical germ cells with a diameter of about (3 μm), positive for Gram-positive, very thick, immobile, non-sporulating and fermenting sugars [13], all types of which produce catalase. This enzyme breaks down oxygen water. Its colonies join in the form of groupings resembling grape clusters, they increase on regular media aerobically or anaerobically (Facultative), and they are heat-resistant spores (70-60) 0 C and drought-resistant [14]. Escherichia coli is a Gram-negative bacillus that proliferates on standard culture media without growth stimulants. These bacteria are destroyed at a temperature of 60°C for 30 min. This type of bacteria can grow on MacConkey agar culture, and some strains of this bacteria can form capsules [15]. This type of Bacteria is one of the well-known types of intestinal bacteria in humans and animals, and it is responsible for inflammation of the bladder,

urinary tract, meninges, and bile sacs [16]. Cancer is a group of diseases characterized by the unlimited growth and division of cells in the tissues of the body and the ability of these cells to invade and destroy neighboring tissues or to move to distant tissues through the blood or lymphatic system by the process of metastasis, and these capabilities are the characteristics of a malignant tumor. Unlike Benign's adenoma [17], they are characterized by a specific growth and inability to invade and cannot metastasize or metastasize. A benign tumor can sometimes develop into cancer. Cancer may affect people of all ages, but the risk tends to increase with age, and it is one of the leading causes of death in developed and developing countries [18]. Breast cancer is a form of cancerous tumor that affects the breast tissue and appears in the ducts of the tubes that carry milk to the nipple and milk glands [19]. It affects both men and women, but the incidence in males is rare, as, for every 200 Injuries to women, there is only one injury to men [20]. Breast cancer can be classified into invasive and non-invasive, located in either the duct or the lobes (inside the lobes) [21]. Risk factors can be divided into two categories: modifiable risk factors (such as consumption of alcoholic beverages) and fixed risk factors (such as age and biological sex) [22]. The main factor for breast cancer is gender, as women are more likely than men and have an increased risk of developing with increasing age [23].

2. Experimental

2.1. Material: All chemicals used in this work were purchased from Fluka, Aldrich, and BDH and used without further purification.

2.2. Devices used: The melting points were measured using Electrothermal Melting Apparatus 9300. The FT-IR spectra were captured using a Shimadzu FT-IR 8400S spectrophotometer with a scale of (400-4000) cm^{-1} by KBr disc. DMSO- d_6 as solvents were used to capture ^1H -NMR and ^{13}C -NMR spectra on Bruker instruments running at 400 MHz.

2.3. Preparation of pyrimidine derivatives (I9-I16) [24]:

In a round flask of 100 ml volume, 0.01 mole of the prepared chalcone derivatives [I1-I8] is dissolved in 10 ml of ethanol. A solution of 0.01 mole of cyanoguanidine dissolved in 10 ml of ethanol is added, then 10 ml of a 10% solution is added. Sodium hydroxide and the mixture were raised for 6 hours with continuous stirring. The answer was cooled and added to crushed ice. The solution was neutralized by adding concentrated hydrochloric acid (HCl) drops. The precipitate was separated by filtering, washed with cold water, and recrystallized from methanol; table (1) shows Some physical properties of pyrimidine derivatives (I9-I16).

Table (1): Physical properties of pyrimidine derivatives (I9-I16)

Comp. No.	X	R	Molecular Formula/ M.Wt g/mol	Color	M.P. ($^{\circ}\text{C}$)	Yield (%)
I ₉	F	Cl	$\text{C}_{17}\text{H}_{12}\text{N}_4\text{FCl}$ 326.76	Light brown	121-122	60
I ₁₀	F	Br	$\text{C}_{17}\text{H}_{12}\text{N}_4\text{FBr}$	White	150-	71

			371.21		151	
I ₁₁	F	CH ₃	C ₁₈ H ₁₅ N ₄ F	Light	109-	66
			306.34	yellow	110	
I ₁₂	F	OCH ₃	C ₁₈ H ₁₅ N ₄ OF	Light	137-	59
			322.34	yellow	139	
I ₁₃	Cl	Cl	C ₁₇ H ₁₂ N ₄ Cl ₂	Brown	188-	54
			343.21		190	
I ₁₄	Cl	Br	C ₁₇ H ₁₂ N ₄ ClBr	Brown	169-	67
			387.67		170	
I ₁₅	Cl	CH ₃	C ₁₈ H ₁₅ N ₄ Cl	Light	145-	78
			322.80	brown	147	
I ₁₆	Cl	OCH ₃	C ₁₈ H ₁₅ N ₄ OCl	Brown	104-	72
			338.80		106	

2.4. Biological activity study

Two types of pathogenic bacteria were used in this study, one of which is Gram-positive, which is *Staphylococcus aureus*, and one of which is Gram-negative, which is *Escherichia coli*; and these types of bacteria. It is essential in the medical field because of its resistance to antibiotics. These bacteria were taken from the laboratories of the College of Education for Pure Sciences, Department of Life Sciences, and the culture medium was used, a type of Muller Hinton Agar, which is used to measure the biological activity of antibiotics and substances. Chemicals with medicinal uses. It is used to measure and determine the minimum inhibitory concentration (MIC), and chemical solutions of (I9, I16) were prepared in concentrations (0.01, 0.001, 0.0001) mg/ml and using a solvent Dimethyl sulfoxide (DMSO). The sensitivity test for the bacteria isolates used in the study was carried out by diffusion method in the nutrient medium Mueller-Hinton agar, which is a transparent food medium with a dark yellow color that is useful in testing the sensitivity of microorganisms to antibiotics because it contains on an animal infusion, casein and starch are extracted. It supports the growth of most microbes and microorganisms. The medium was prepared and sterilized by autoclave, then distributed in dishes and left to harden, then small pits were made at a rate of four holes in each plate. Then it was incubated at (37 °C) for a period of (24 hours). The results were read on the next day to show the derivatives sensitivity derivatives used, which depends on the diameter of the inhibition evident in the dishes around the holes used, as the increase in diameter Inhibition means the increase in the biological activity of the prepared compounds and compare that with the diameter of inhibition for antibiotics [25, 26].

2.5. Measuring the antioxidant activity of some compounds prepared ex vivo (DPPH inhibition activity test)

DPPH root (2,2-Diphenyl-2-Picryl Hydrazyl) was used to evaluate natural oxidation's free radical scavenging activity. At a concentration of 0.1 mM by dissolving 4 mg in 100 mL of methanol, then 3 mL of DPPH prepared solution was added to 1 mL of the prepared compounds of several concentrations ranging from (25, 50, 100, 200) µg/mL, as well as Ascorbic acid prepared from dilute concentrations of (25, 50, 100, 200) µg/mL, left the mixture in the dark for 30 minutes, then read the absorbance at a wavelength of 516 nm, and calculated the

percentage of scavenging ability of the prepared compounds DPPH for free radicals for each compound as well as for Ascorbic acid AA, which represents the positive witness for comparison through the following equation:

$$I\% = (Abc_0 - Abc_1) / Abc_0 \times 100$$

The standard solution was made by adding DPPH solution without ascorbic acid or extracts.

2.6. Testing the cytotoxicity of I15 on breast cancer cells (MCF-7)

Cancer cells were obtained from the Medical City - Baghdad, and the cancer cells were preserved in liquid nitrogen; they were perpetuated, grown, and tested at the Biotechnology Research Center at Al-Nahrain University; and work began with steps:

First: The natural cell line WRL 68 Cell Line: The human liver cell line represents a thank similar to hepatocytes and primary liver transplants. It has been proven that the cells secrete albumin and alpha-fetoprotein and express enzymes specific to the liver. Like alanine aminotransferase

Second: Solutions Used in Tissue Culture Technique: Several solutions used for cell culture were prepared, namely: Antibiotic Solution (Streptomycin (1g/vial), Benzyl Penicillin, Sodium Bicarbonate Solution, Phosphate Buffer Saline (PBS), Trypsin Solution, EDTA, Trypsin - EDTA

Third: Media: several media attended, namely: Roswell Park Memorial Institute - 1640 Medium (RPMI), Serum-Free Medium, and Freezing Medium.

2.6.1. Breast Cancer Cell Growth (MCF-7): The freshener method was used to grow cancer cells as follows

The tumor cells were thawed using a water bath at 37 °C. Then, the tumor cells were placed in a 25 cm² animal cell culture vessel (Falcon) containing culture medium (RPMI-1640) and 10% calf serum. Then the culture vessels containing the cell suspension and medium were incubated in a 5% CO₂ incubator at 37 °C for 24 hours. After incubation, and when it was confirmed that there was growth on the farm and that it was free from pollution, secondary farms were conducted for it. The cells were examined using an inverted microscope to ensure their viability, freedom from contamination, and growth to approximately 500000-800000 cells/ml. Then the cells were transferred to the growth booth, and the used culture medium was discarded. Cells were washed using PBS solution and discarded, and the process was repeated twice for 10 minutes each time. A sufficient amount of trypsin/EDTA enzyme solution was added to the cells and incubated for (30-60) seconds at a temperature of 37 °C and monitored until they changed from a monolayer of cells to single cells. Then, the enzyme was stopped by adding a new development medium containing serum. Then the cells were collected in centrifugal tubes and placed in the apparatus at a speed of 2000 rpm for 10 minutes at room temperature to precipitate the cells and get rid of the trypsin and the used medium. The filtrate was discarded, and the cells were suspended in a fresh medium containing 10% serum [27]. The cell number was examined by taking a specific volume of the cell suspension and adding to it the same volume of Trypan Blue dye to find out the number of cells and their vitality percentage using a Haemocytometer chip, according to the equation:

$$C = N \times 10^4 \times F/ml$$

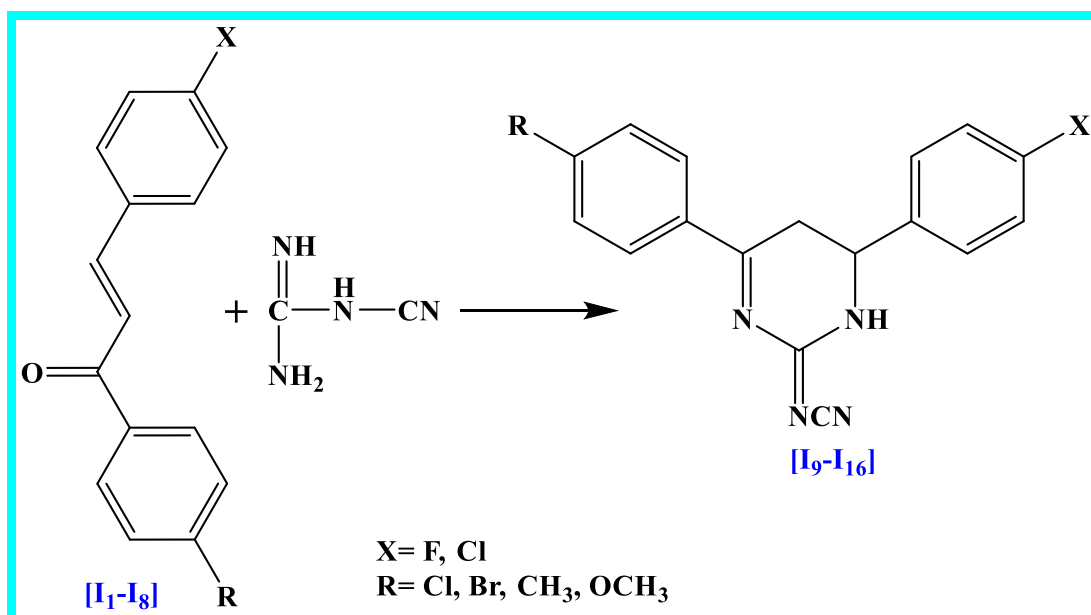
The cell suspension was distributed in new containers and then incubated in a 5% CO₂ incubator at 37 °C for 24 hours: Total Cell Count/ ml = Cell Count x dilution factor (Sample Volume) x 10⁴.

2.6.2. MTT Cytotoxicity Assay

Cytotoxicity tests were performed in three replicates, and the (IC₅₀) values were calculated. 3-(4,5 dimethylthiazol - 2-yl) 2,5-Diphenyl tetrazolium bromide (MW = 414), and solute solution [28]. The manufacturer's instructions were followed as they prepared breast cancer cells as previously mentioned, then placed the cell suspension at a concentration of (1 x 10⁴ to 1 x 10⁶) cells/ml in a 96-hole newspaper to a final volume of 200 µl of complete culture medium for each hole and the plates were covered with sterile parafilm, gently stirred and incubated. After incubation, the medium was removed, and 200 µl of the prepared concentrations of the studied compounds (25, 50, 100, 200) µg/ml were added to the pits at three pits for each engagement with the control sample and incubated in a 5% incubator. CO₂ at 37 °C for 24 hours. After exposure to the compounds under study, ten µl of MTT solution was added to each hole; then, the plate was incubated in a 5% CO₂ incubator at 37 °C for 4 hours. 100 µl of DMSO was added to each pit and set for 5 minutes. Then, the absorbance was read using an ELISA device at a wavelength of 570 nm [29]. Statistical analysis was performed on the optical density readings to calculate the IC₅₀.

3. Results and Discussion

The pyrimidine derivatives (I9-I16) were prepared by reacting a mole of chalcone derivatives with a mole of cyanoguanidine and using ethanol as a solvent, as in the following equation:



Scheme (1): Route of prepared compounds (I9-I16)

3.1. Diagnosis of pyrimidine derivatives (I9-I16)

The reaction to the pyrimidine derivatives (I9-I16) was confirmed by observing the changes in the physical properties of the melting point and the significant color difference. During measurements of infrared (IR), proton nuclear magnetic resonance (^1H -NMR), and carbon (^{13}C -NMR) spectra. When studying the infrared (IR) spectrum of pyrimidine derivatives [I9-I16], it was observed that absorption bands appeared at a frequency (3190-3231) cm^{-1} due to the stretching of the NH bond and the appearance of an absorption band at the frequency (3032-3097) cm^{-1} it returns to the extension of the (CH) aromatic band, as well as the appearance of two absorption bands at the frequency (2904-3005) cm^{-1} and (2825-2896) cm^{-1} due to the stretching of the (CH) aliphatic band. In addition to the appearance of an absorption band at frequency (2255-2262) cm^{-1} due to the stretching of the (CN) bond and an absorption band at frequency (1650-1667) cm^{-1} due to the extension of the azomethine group (C=N), it was also observed that two absorption bands appeared at the frequency (1577-1599) cm^{-1} and (1479-1498) cm^{-1} due to the stretching of the aromatic bond (C=C) aromatic, and an absorption band appeared at the frequency (1201-1237) cm^{-1} belongs to the extension of the (C-N) group, as shown in table (2), and these bundles were close to what is found in the literature [30, 31].

Table (2): Infrared absorption results (cm^{-1}) pyrimidine (I9-I16)

Comp. No.	X	R	$\nu(\text{NH})$	$\nu(\text{C-H})$ Arom. Aliph.	IR (KBr) cm^{-1}				Others
					$\nu(\text{CN})$	$\nu(\text{C=N})$	$\nu(\text{C=C})$ Arom.	$\nu(\text{C-N})$ Arom.	
I ₉	F	Cl	3192	3037 2972 2835	2262	1654	1577 1491	1201	$\nu(\text{C-F})$ 1022 $\nu(\text{C-Cl})$ 785
I ₁₀	F	Br	3203	3045 2904 2876	2259	1650	1590 1488	1214	$\nu(\text{C-F})$ 1018 $\nu(\text{C-Br})$ 610
I ₁₁	F	CH ₃	3215	3032 2920 2889	2257	1661	1596 1487	1206	$\nu(\text{C-F})$ 1027
I ₁₂	F	OCH ₃	3190	3097 2908 2896	2255	1655	1593 1498	1237	$\nu(\text{C-F})$ 998
I ₁₃	Cl	Cl	3200	3068 2913 2881	2259	1667	1580 1495	1224	$\nu(\text{C-Cl})$ 756
I ₁₄	Cl	Br	3207	3061 2972 2825	2256	1656	1599 1489	1217	$\nu(\text{C-Cl})$ 775
I ₁₅	Cl	CH ₃	3231	3092 2922 2840	2261	1658	1582 1479	1217	$\nu(\text{C-Cl})$ 781
I ₁₆	Cl	OCH ₃	3213	3064 3005	1260	1660	1589 1485	1228	$\nu(\text{C-Cl})$ 763

When studying the nuclear magnetic resonance spectrum of the proton for the compound (I9), it was observed that multiple signals appeared in the range (7.093-8.182) ppm attributed to the aromatic ring protons, and a single sign appeared in the position (4.510) ppm attributed to the proton of (NH), and the appearance of a quadrupole signal in the range (4.481-4.492) ppm attributed to the proton of (CH) aliphatic, and the appearance of a binary signal at sites (3.968, 3.939) ppm attributed to the protonation of (CH₂), and the formation of a signal at the position (2.505, 2.509) ppm attributed to the protons of the solvent (DMSO-d⁶) [32], and as in figure (4).

When studying the nuclear magnetic resonance spectrum of the proton for the compound (I16), it was observed that multiple signals appeared in the range (6.936-7.578) ppm that was attributed to the aromatic ring protons, and a single signal appeared in the position (4.481) ppm that was attributed to the proton of (NH), and the appearance of a tetragonal signal in the range (3.381-3.519) ppm attributed to the proton of (CH) aliphatic, as well as the appearance of a binary signal at sites (2.608, 2.606) ppm attributed to the protonation of the (CH₂) group, as well as the appearance of a signal at the site (2.407) ppm attributed to the protons of the methoxy group (OCH₃), and the appearance of a signal at the site (2.509) ppm attributed to the protons of the solvent (DMSO-d⁶) [33, 34], and as in figure (5).

When studying the nuclear magnetic resonance spectrum of carbon for the compound (I16), it was observed that a signal appeared at the site (163.22) ppm attributed to the carbon of the group (C=N) adjacent to the group (CN), and the appearance of a signal at the site (158.87) ppm attributed to the carbon of the second (C=N) group, and the appearance of a signal at the site (152.68) ppm attributed to the carbon of the (CN) group, as well as the appearance of signals at the site (110.43-149.52).) ppm belonging to the aromatic benzene ring carbons, and a signal appeared at the site (106.24) ppm attributed to the (OCH₃) group carbon, as well as the appearance of a signal at the site (70.24) ppm attributed to the (OCH₃) group carbon. CH) aliphatic, the appearance of a signal at position (62.68) ppm attributed to the carbon group (CH₂), and the formation of signals at the range (38.27-39.98) ppm attributed to the solvent carbonate (DMSO-d⁶) [35]. and as in figure (6).

3.2. Biological activity of some prepared compounds

The study of the biological activity of the compounds prepared at certain concentrations showed that most of these compounds contain antagonistic activity against the types of bacteria studied compared with the antibiotic (Ciprofloxacin), which is a broad-based antibiotic, especially these two types of bacteria studied in addition to many types. It also has an inhibitory diameter. It is great as it gives a high selectivity when studying the sensitivity of bacteria to the prepared compounds since this antibiotic is used to treat many infections and diseases such as infections of the urinary tract, especially those that occur as a result of infection with colon bacteria and *Staphylococcus aureus* bacteria. It also treats simple cystitis in females caused by bacteria Colon. It treats chronic

bacterial prostatitis caused by colon bacteria and *Staphylococcus aureus* and infections of the lower respiratory tract, sinusitis, arthritis, and bones. It is also used to treat diarrhea caused by colon bacteria and effectively treat typhoid. Therefore, two compounds of the compounds prepared in this research (I9, I16) were studied on different types of chromium-positive and negative bacteria, which recorded a global antagonistic activity against the bacteria studied and compared with the mentioned antibiotic, it is possible to use this Compounds as a treatment for the same infections and pathological conditions above after investigating the biological pathway of these compounds, their side effects, and the amount of their accumulation in animal tissues (0.01, 0.001, 0.0001) mg/ml where the inhibition diameter ranges between (10 mm the lowest inhibition diameter, to 35 mm, the highest measured inhibition diameter) and the table below shows the inhibitory activity of some of the prepared compounds [36, 37], and as in table (3).

Table (3): The inhibitory activity of the two compounds (I9, I16) in the growth of several positive and negative bacteria (the diameter of inhibition is measured in mm)

Comp. No	Conc. mg/m	<i>E. coil</i>	<i>Staph. aur</i>
I9	0.01	20	10
	0.001	25	15
	0.0001	22	20
I16	0.01	15	10
	0.001	31	15
	0.0001	10	20
Ciprofloxacin	0.01	25	10
	0.001	21	10
	0.0001	25	10

3.3. Antioxidant Activity

The qualitative evaluation of the anti-oxidant activity of free radical scavenging is done by utilizing the TLC method to determine the prepared compound (I15) ability to reduce the oxidative stress, besides studying the quantitative free radical scavenging activity. Any compound's action is shown when it changes the purple color of DPPH to yellow color, and the intensity of the spot color stated the positive marker. The results indicate that the prepared compound (I15) has an excellent scavenging ability. There is a change in color for yellow, and that is detected that (I15) was a good source of anti-oxidant, but with the least activity than ascorbic acid (as standard). It is clear from the collected data that the free radical scavenging activity of the compound under search increased with increasing concentration exhibiting; for that, the highest free radical scavenging percentage is given with 200 µg/mL of with 80 %, and had the lowest percentage of 40% when 25 µg/mL. Also, this search displays that pyrimidine derivatives were a leading source of anti-oxidant, besides it has an excellent free radical scavenging activity and functions as an anti-bacterial [38,39]. As shown in figure (9).

3.4. Results of the breast cancer cell cytotoxicity (MCF-7) test

The test results for compound (I15) showed good inhibitory activity against (MCF-7) breast cancer cells and normal cell line (HdFn). When calculating the IC_{50} of the compound (I15) against breast cancer cells was 183.4. Its value was 561.5 against normal cells. When calculating IC_{50} , the results showed significant differences, $P \leq 0.0001$, when treated with the compound (I15) for breast cancer cells and normal cells [40], as shown in figures (10-12).

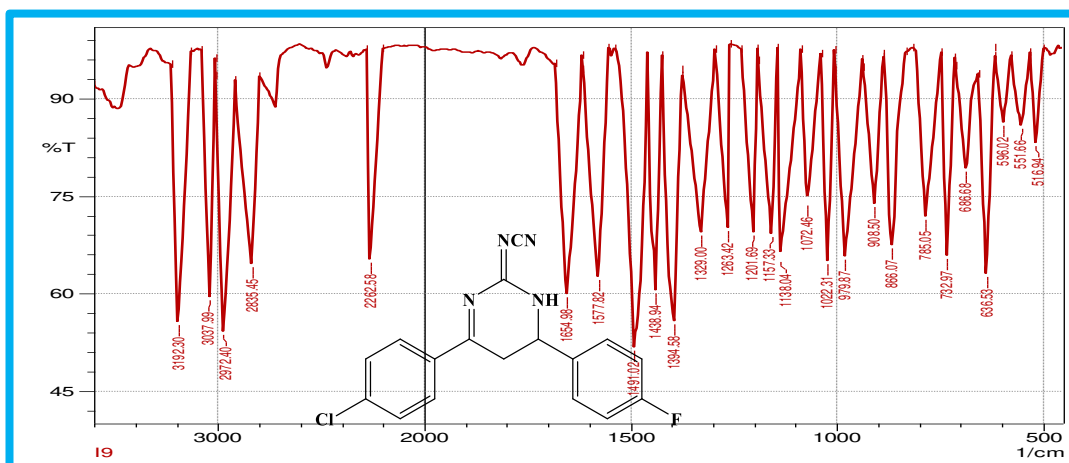


Figure (1): Infrared spectrum of the compound (I9)

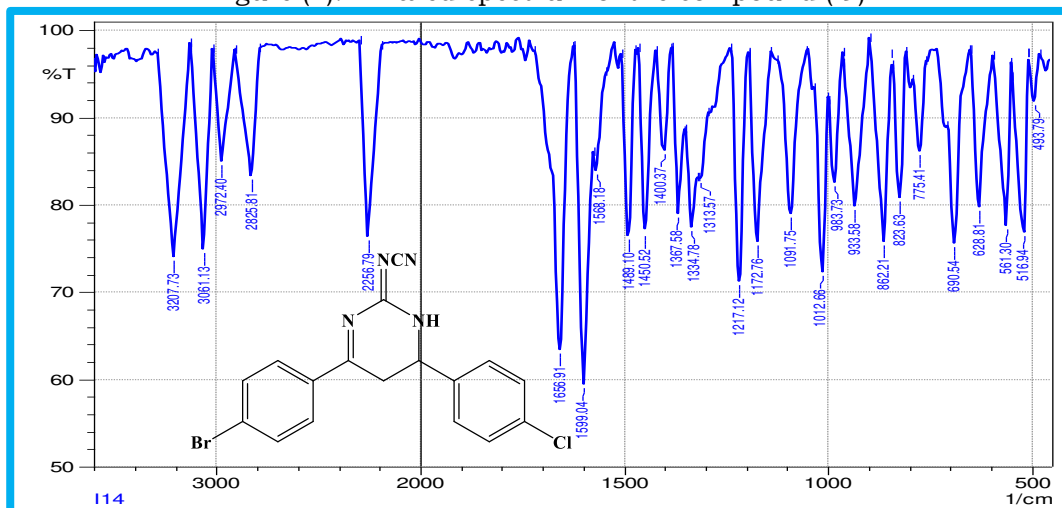


Figure (2): Infrared spectrum of the compound (I14)

Chemical Structure: N#CC1=NC(C2=CC=C(C=C2)c3cc(F)ccc31)c4ccc(Cl)cc4

¹H NMR Spectrum (DMSO-d₆):

Chemical Shift (ppm)	Integration
8.182 (s)	1.00
8.161 (s)	1.00
8.112 (s)	1.00
7.961 (s)	1.00
7.939 (s)	1.00
7.923 (s)	1.00
7.902 (s)	1.00
7.887 (s)	1.00
7.884 (s)	1.00
7.753 (s)	1.00
7.739 (s)	1.00
7.722 (s)	1.00
7.718 (s)	1.00
7.709 (s)	1.00
7.687 (s)	1.00
7.624 (s)	1.00
7.588 (s)	1.00
7.584 (s)	1.00
7.567 (s)	1.00
7.558 (s)	1.00
7.545 (s)	1.00
7.493 (s)	1.00
7.322 (s)	1.00
7.317 (s)	1.00
7.300 (s)	1.00
7.095 (s)	1.00
7.093 (s)	1.00
4.510 (s)	1.00
4.492 (s)	1.00
4.491 (s)	1.00
4.484 (s)	1.00
4.481 (s)	1.00
3.968 (s)	1.00
3.939 (s)	1.00
2.509 (s)	1.00
2.505 (s)	1.00

Peak Labels: 38.48, 5.08, 0.92, 35.98, 7.84

Figure (4): The ^1H -NMR spectrum of compound (19)

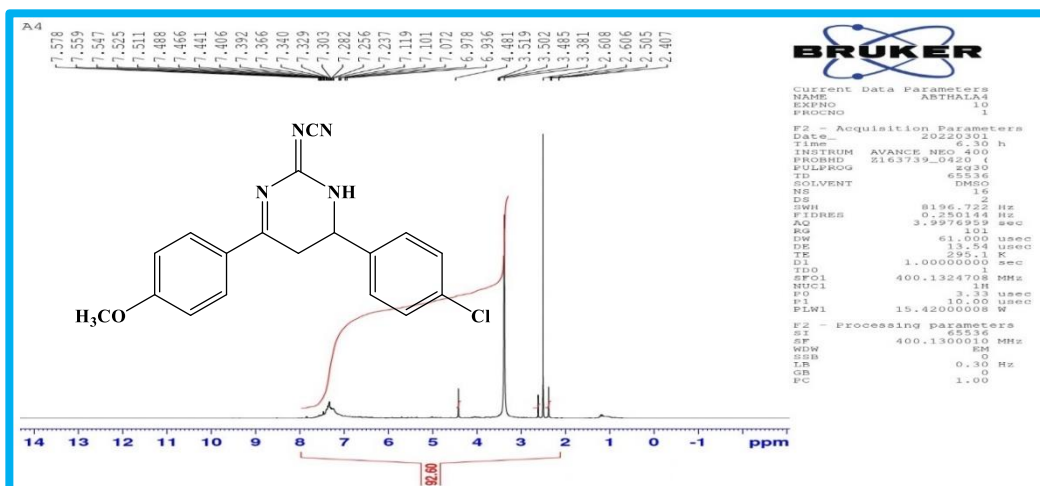


Figure (5): The ^1H - NMR spectrum of compound (I16)

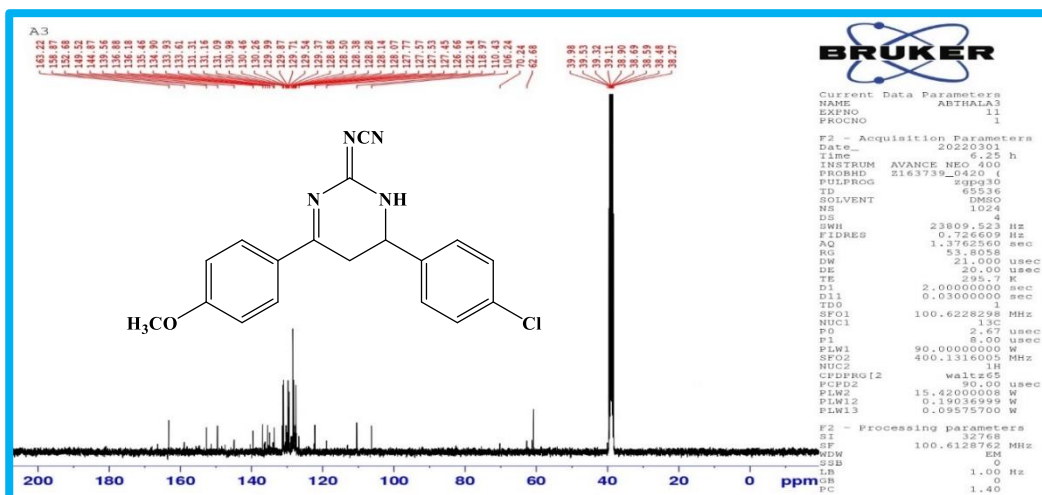


Figure (6): The ^{13}C - NMR spectrum of compound (I16)

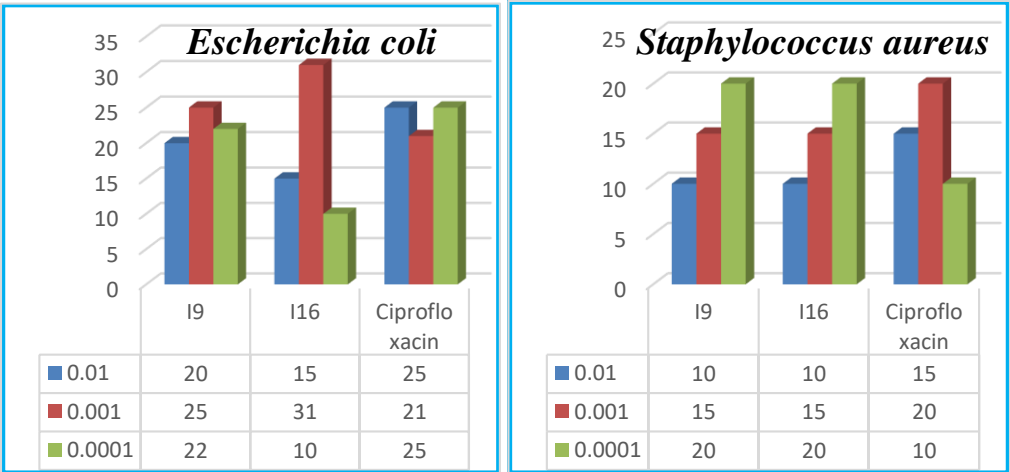


Figure (7): Complex inhibition (I9, I16) against *Escherichia coli* and *Staphylococcus aureus*

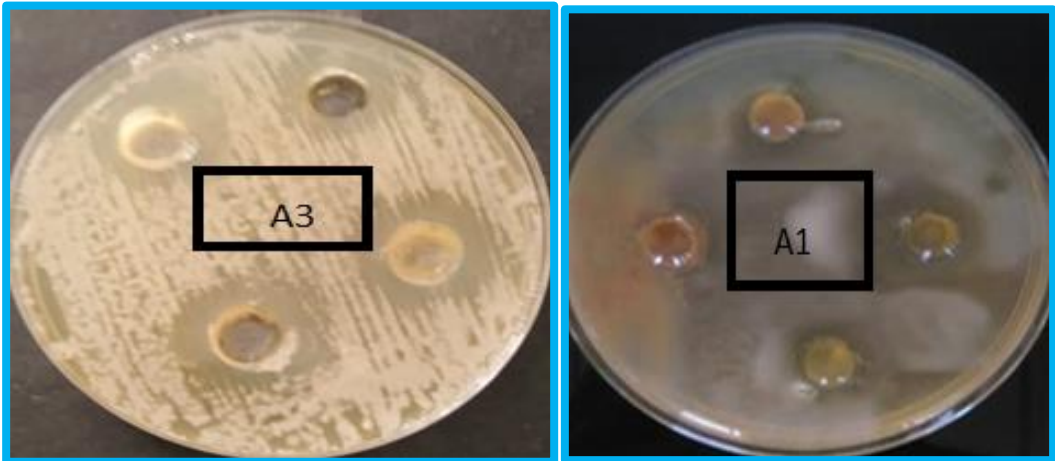


Figure (8): Inhibition of compound (I9) against *Escherichia coli* and inhibition of compound (I16) against *Staphylococcus aureus*

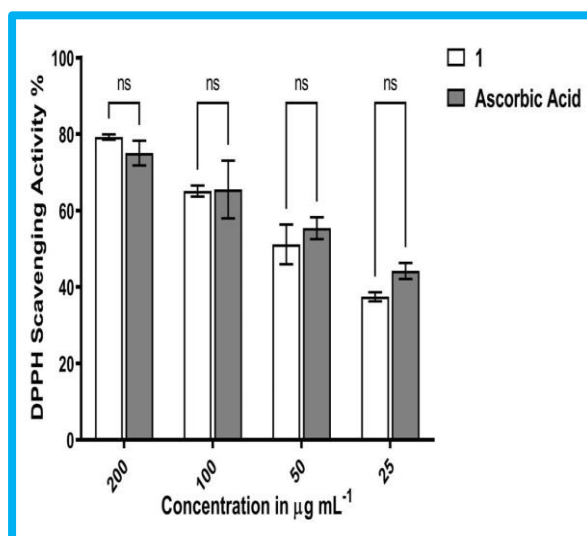


Figure (9): Antioxidant activity for compound (I15)

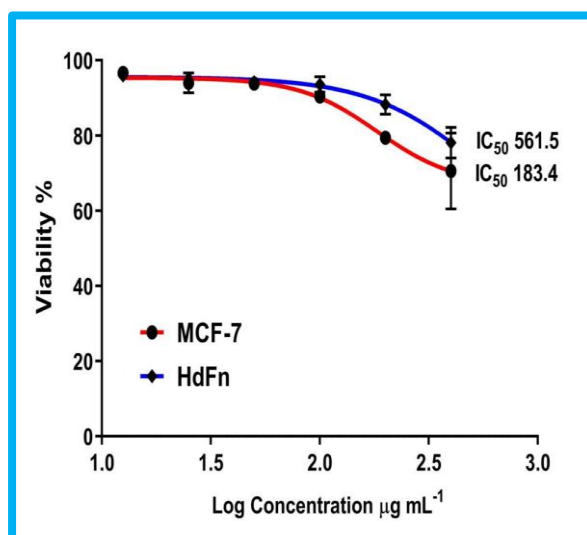


Figure (10): Anticancer efficacy of MCF-7 and HdFn for compound (I15)

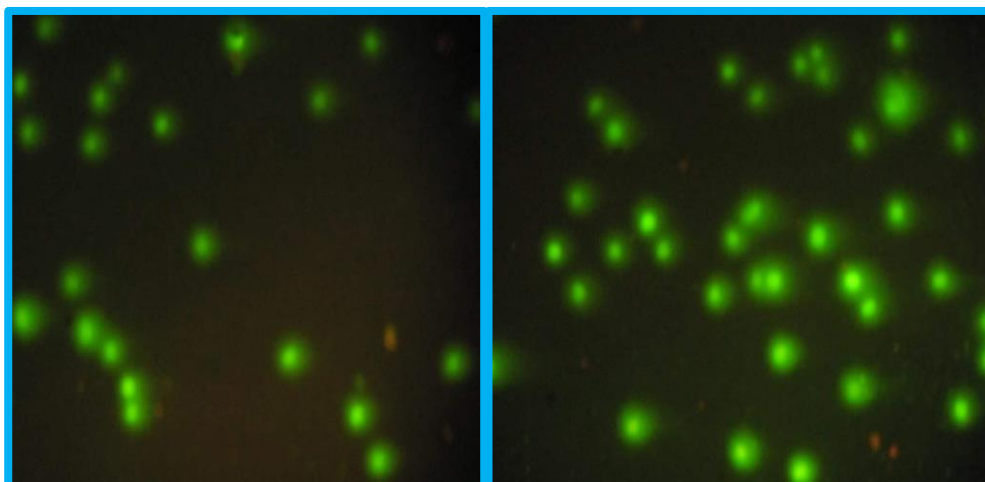


Figure (11): Anticancer efficacy of control sample

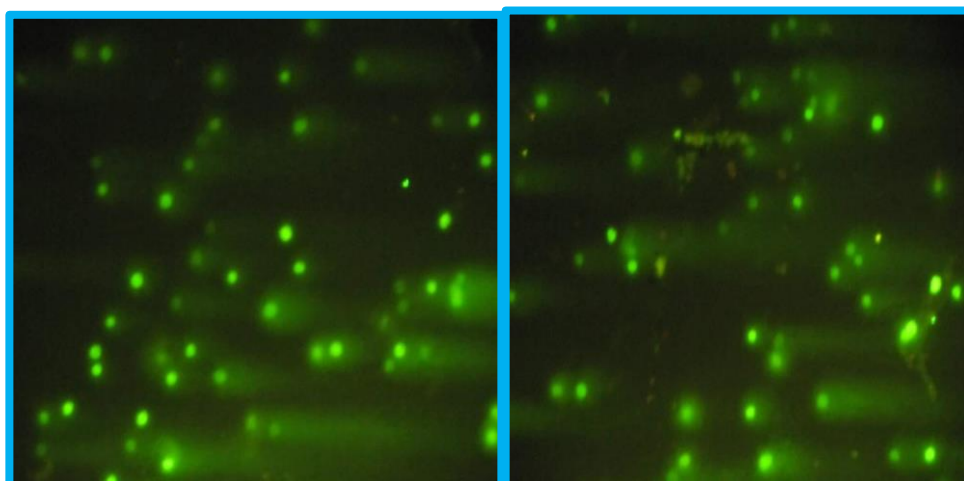


Figure (12): Anticancer efficacy of compound (I15)

4. Conclusions

Through spectroscopic and physical measurements, it was found that the accuracy and validity of the prepared compounds. The biological activity study showed that the compounds had the effect of inhibiting *Gedo* compared to the control sample. I16 compound gave the highest percentage of 31 mm against *E. coli* bacteria, and the two compounds gave the same percentage of inhibition of 20 mm against *Staphylococcus aureus* bacteria. The results indicate that the prepared compound (I15) has an excellent scavenging ability. That the free radical scavenging activity of the compound under search increased with increasing concentration exhibiting; for that, the highest free radical scavenging percentage is given with 200 $\mu\text{g/mL}$ of with 80 %, and had the lowest percentage of 40% when 25 $\mu\text{g/mL}$. The test results for compound (I15) showed good inhibitory activity against (MCF-7) breast cancer cells and normal cell line (HdFn). When calculating the IC_{50} of the compound (I15) against breast cancer cells was 183.4.

Its value was 561.5 against normal cells. When calculating IC₅₀, the results showed significant differences, $P \leq 0.0001$, when treated with the compound (II5) for breast cancer cells and normal cells.

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