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Preparation, Characterization, and Study of Antibacterial some new Pyrazole Derivatives

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الخلاصة

تضمن البحث تحضير اربع مشتقات للبايرازول من خلال تفاعل الجالكونات مع الهيدرازينات المعوضه. شخصت هذه المركبات بطرق التشخيص الطيفية (الاشعة تحت الحمراء، طيف الكتلة والرنين النووي المغناطيسي للبروتون). مركبات البايرازول المحضرة (Py1-Py4) درست كمثبطات للبكتريا السالبة والموجبة لصبغة كرام حيث اعطت هذه المركبات نسب تثبيط عالية نسبيا اعتمادا على نوع المجموعة المعوضة. البايرازول المعوض بمجموعة COOH (Py1) اعطى اعلى نسبة تثبيط حيث كانت قطر التثبيط (28) ملم، بينما البايرازول المعوض بمجموعة OH في الموقع ميتا كان اقل قدرة على التثبيط.

Abstract

The papear include synthesis of four of pyrazole derivatives from the reaction of chalcones with substituted hydrazine. FT-IR, Mass, and H^1-NMR spectra identified the compounds. The new pyrazoles (Py1-Py4) studies as anti-bacterial against negative and positive gram stain. The prepared compounds gave the high and different abilities to inhibit these bacteria according to the type of the substituted group. The inhibition ratio of pyrazole substituted by the COOH group (Py1) was 28 mm, while pyrazole substituted by OH in the meta position was less able to inhibit.

Key words:chalcone, pyrazole,hydrazine,cyclization,microbial,gram stain.

Introduction

Heterocyclic compounds are included in the composition of many compounds. They have a wide range of biological activities as antioxidants, agricultural chemicals, and veterinary products. They also have clinical applications described as anti-fungal, anti-viral, anti-inflammatory, and anti-tumor drugs. That is what made researchers focus on preparing these compounds [(1)]. The most important heterocyclic compounds have five- and six rings. Pyrazole compounds have

biological activity and are included in the synthesis of many pharmaceutical compounds.

Pyrazole is considered a heterocyclic organic compound with a five-membered ring consisting of three carbon atoms and two adjacent nitrogen atoms and has two double bonds[2]. pyrazoline is a pyrazole added to two hydrogen atoms and contains only a double bond [3]. In comparison, pyrazolidine is a pyrazole involved four hydrogen atoms and thus does not have a double bond [4].Figure(1).

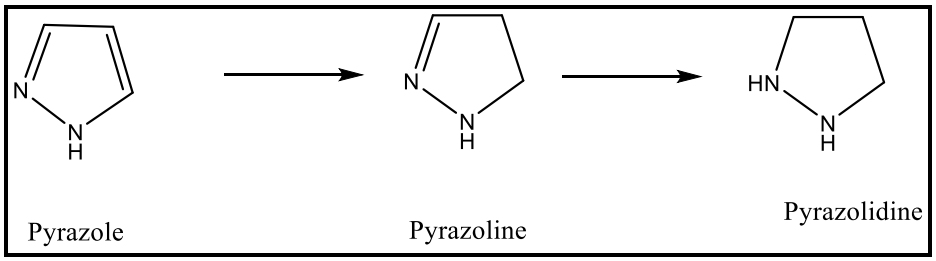
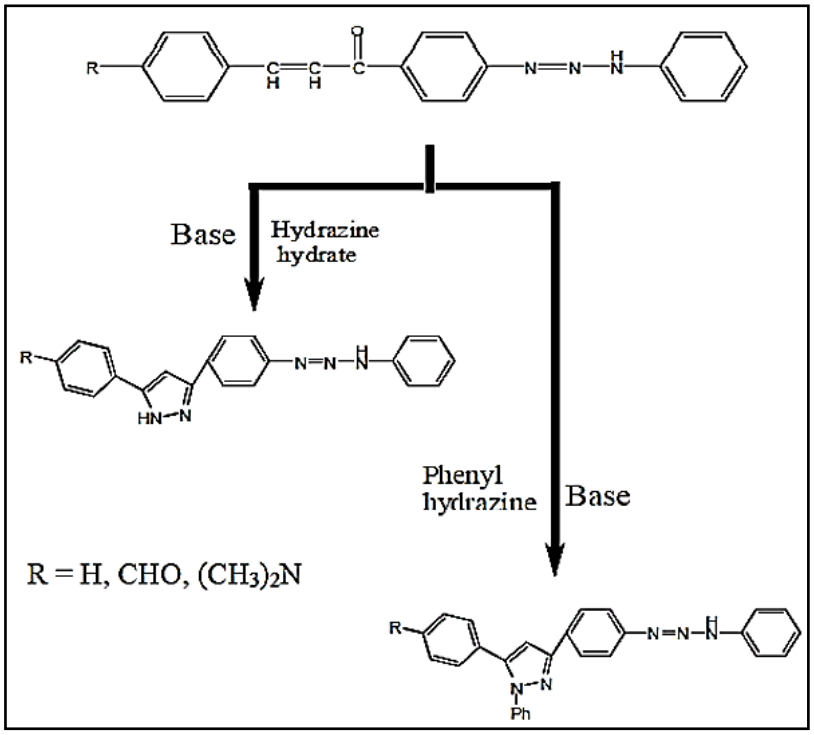


Figure (1) structure of pyrazole, pyrazolin and pyrazolidin

Pyrazole derivatives have biological and pharmacological activity. For example, they are used as antidepressants [5], for epilepsy, convulsions, infections, microbes, fungi, bacteria, cancer, and antioxidants [6]. It is also used as apain relief [7], antipyretics [8], and blood pressure and cholesterol suppressants [9].

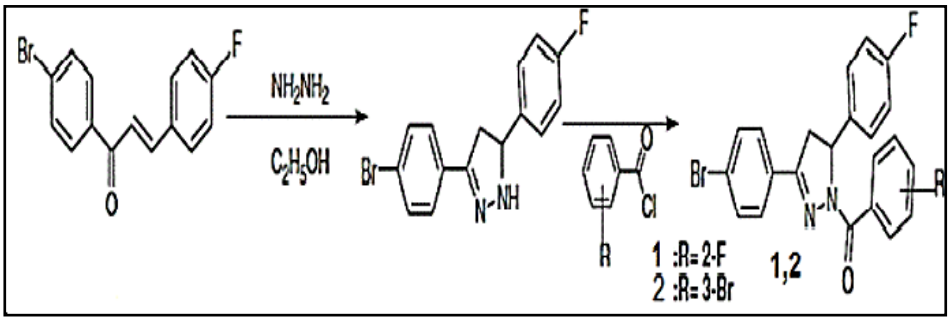
In the last of nineteenth century, Fischer and Knoevenagell described the reaction of acrolein with phenylhydrazine to prepare a compound of the type (2-pyrazoline). The scientist (Auwers) [10] and his colleagues confirmed that the product of this reaction is (1-phenyl- 2-pyrazoline). After these pioneering studies in the last century, many (2-pyrazoline) were prepared by reacting (β,α-enone) with hydrazines. This convenient and straightforward preparation method is one of the most common methods of preparing (2-pyrazoline) [11].

Due to the multiplicity of use of pyrazole compounds and its derivatives,the researchers were interested in their preparing them. The researchers (Khudhair & Shehab) prepared pyrazole derivatives through the interaction of Chalcone compounds with hydrazine and phenylhydrazine compounds, and they were effective as corrosion inhibitors[12]. schemes(1).



schemes (1) prepared cycles by researcher (Khudhair & Shehab)

Also, the researchers (Khalil & Refaat) [13] prepared a series of pyrazoline derivatives and studied their effectiveness as an anti-inflammatory on mice. the equation (1).



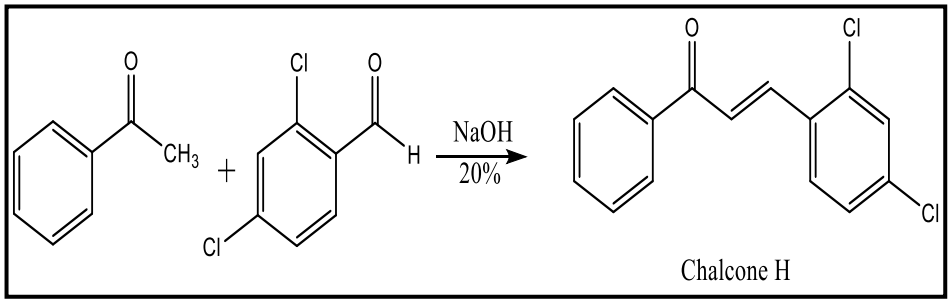
…. (1)

Materials and Methods:

The incorrect melting point was measured with the Electrothermal melting point apparatus. I.R. spectra were recorded using KBr disk on Shimadzu FT-IR-8300 spectrophotometer in Basrah University, Science college, Chemistry department. H^1-NMR spectra were taken at Tehran University (Iran) on Avance DRX 500 MHz (from Bruker), using dimethylsulphoxide (DMSO) as the internal standard. The antibacterial experience was done on Biotech Technology in Basrah(Iraq) .

Preparation of Chalcones [14]

An equivalent mixture (0.01mmole) of 2,4-dichlorobenzaldehyde (1.72g) and acetophenones was dissolved in 45 ml of Ethanol (96%). 25 ml of aqueous NaOH 20 % was added gradually to the mixture. The reaction mixture was stirred at 0◦C overnight. 100 ml of cold water was added to the mixture and acidified with acetic acid. The precipitate was filtrated and recrystallized in absolute Ethanol. ethyl acetate: n-hexane (3:1) was used as eluent solvents. Equation 2



…. (2)

Preparation of Pyrazole Compounds

Equivalent moles (0.01 mol) of chalcone H with hydrazine derivatives, Table (1) according to the weights shown in Table (2) were dissolved in around bottom flask with (35) ml of absolute Ethanol with continuous mechanical stirring. A few drops of glacial acetic acid were added to the mixture with stirring. Reflux was carried out for 3-6 hours, where the reaction was followed up by TLC (thin layer chromatography) technique with ethyl acetate: n-hexane (3:1), and the R_f value was calculated. The mixture was left to cool, then filtered, dried, and recrystallized

using absolute Ethanol, and the resultant yield was calculated, and its melting point was measured.

Table (1) Structure of the used hydrazine compounds

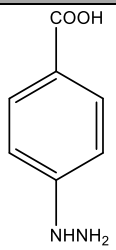
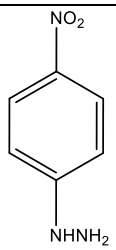
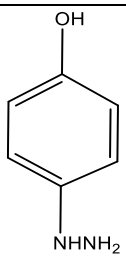
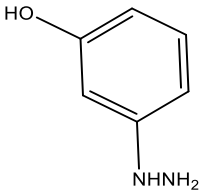
Structure	Formula	Name	symbole	No
	$C_7H_8N_2O_2$	4-Hydrazinobenzoicacid	Hy1	1
	$C_6H_7N_3O_2$	4-Nitrophenylhydrazine	Hy2	2
	$C_6H_8N_2O$	4-Hydrazinylphenol	Hy3	3
	$C_6H_8N_2O$	3-Hydrazinylphenol	Hy4	4

Table (2) weights of starting materials used in the preparation of pyrazole compounds

Hydrazine	Chalcone H	No.
Hy1 (0.152 gm)	(0.276 gm)	1

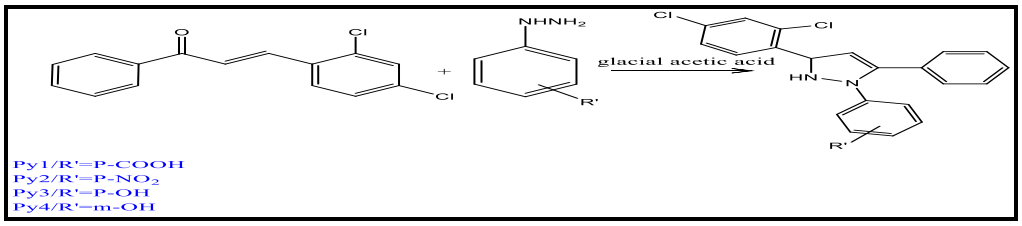
Hydrazine	Chalcone H	No.
Hy2 (0.153 gm)	(0.276 gm)	2
Hy3 (0.124 gm)	(0.276 gm)	3
Hy4 (0.124 gm)	(0.276 gm)	4

Anti-bacterial Activity

The effectiveness of the prepared compounds on the most common types of bacteria that develop from diseases, namely, Escherichia coli and Staphylococcus aureus, with two concentrations (500-1000 µg/ml). DMSO is used as a solvent and control for the hole sensitivity test.

Results and Discussion:

Pyrazole derivatives were prepared using cyclization of chalcone H with hydrazine derivatives to give the products. (Equation 3).



……..(3)

According to the proposed mechanism in the diagram (1)

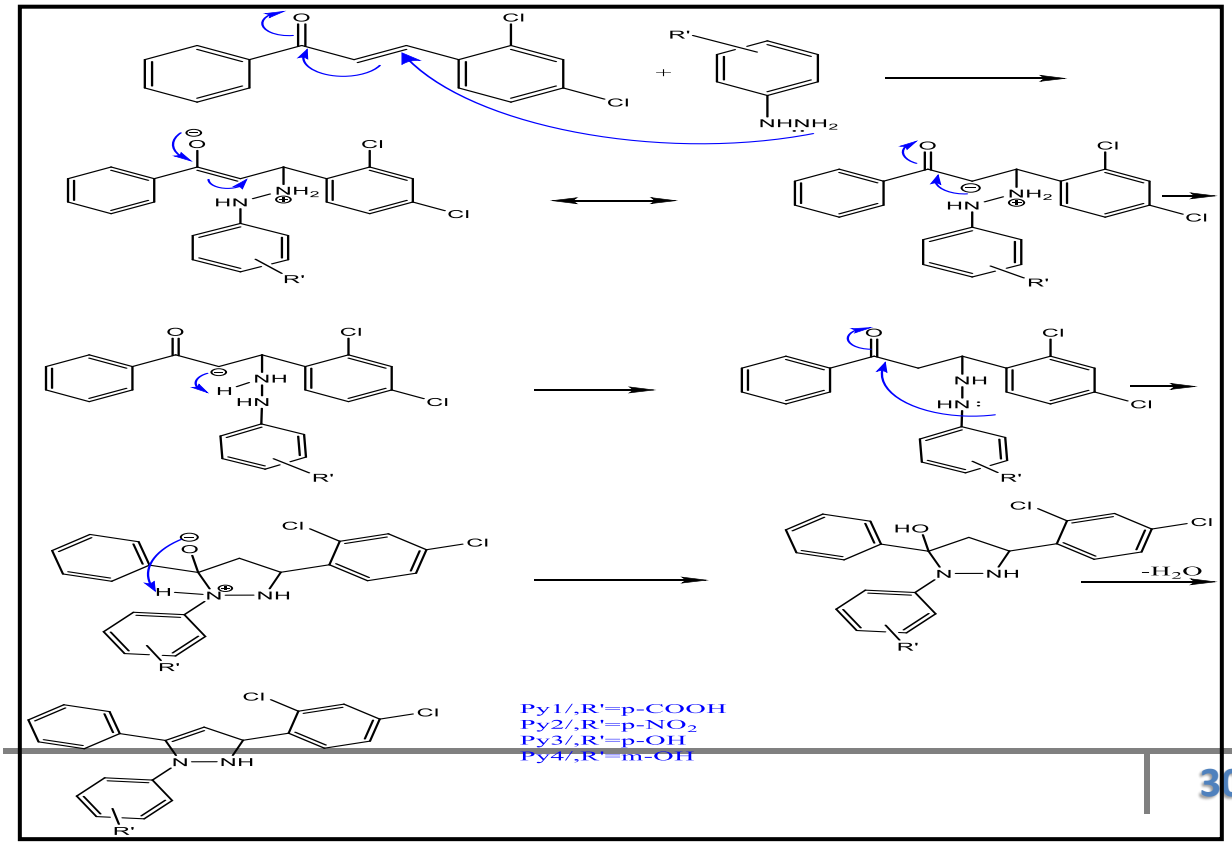


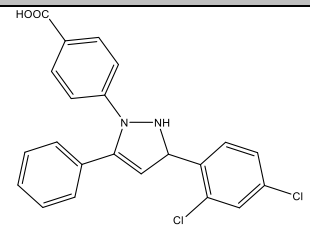
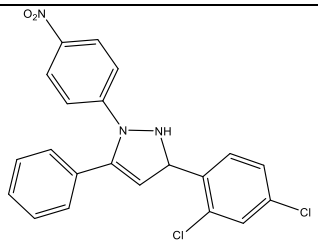
Diagram (1) the proposed mechanisms of prepared pyrazole compounds

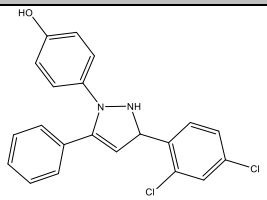
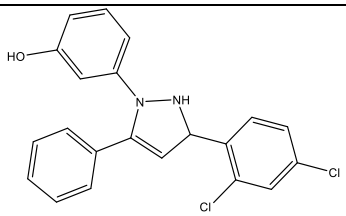
The physical properties of prepared compounds are included in Table (3). Table (4) shows the structure of the prepared pyrazole compounds.

Table (3) Physical properties of the prepared pyrazole compounds

R_f	Color	Yield %	M.P. ^0C	M.Wt.	Symbole	No.
0.6o	light yellow	55	63-65	411	Py1	1
0.53	Yellow	54	56-58	412	py2	2
0.55	White	77	58-60	383	Py3	3
0.58	Ashen	75	60-62	383	Py4	4

Table (4) Structure of the prepared Pyrazoles compounds

Structure	Formula	Name	symbole	No.
	$C_{23}H_{18}Cl_2N_2O_3$	4-(3-(2,4-dichlorphenyl)-5-phenyl-2,3-dihydro-1*H*-pyrazol-1-yl)benzoic acid	Py1	1
	$C_{22}H_{17}Cl_2N_3O_3$	3-(2,4-dichlorphenyl)-1-(4-nitropheny)-5-phenyl-2,3-dihydro-1*H*- pyrazol	Py2	2

Structure	Formula	Name	symbole	No.
	$C_{22}H_{18}Cl_2N_2O_2$	4-(3-(2,4-dichlorphenyl)-5-phenyl-2,3-dihydro-1*H*-pyrazol-1-yl)phenol	Py3	3
	$C_{22}H_{18}Cl_2N_2O_2$	3-(3-(2,4-dichlorphenyl)-5-phenyl-2,3-dihydro-1*H*-pyrazole-1-yl)phenol	Py4	4

The products were characterized by infra-red (Table5), ^{1}H-NMR (Table 6 and Figures 1-4), and Mass spectra (Table7).

Table (5): FT-IR Spectra data of prepared compounds

Compounds	IR data(cm^{-1})
Py1	N-$H_{sec.}$(3421),C- C-H(2854), C=C-H(2924), Ar-H(3063), C=C_{Ar} (1585, 1469),C=C_{olf}.(1662), C-N(1138),C-Cl(1099),OH(3421).
Py2	N-$H_{sec.}$(3421),C-C-H(2850), C=C-H(2920),Ar-H(3063), C=C_{Ar} (1585, 1469),C=C_{olf}.(1662), C-N(1145),C-Cl(1103),NO_2(Asym.)1550,NO_2(Sym.)1334.
Py3	N-$H_{sec.}$(3421),C- C-H(2854), C=C-H(2924),Ar-H(3063),C=C_{Ar} (1585, 1469),C=C_{olf}.(1662), C-N(1138),C-Cl(1103),OH(321).
Py4	N-$H_{sec.}$(3448),C- C-H(2854), C=C-H(2924),Ar-H(3063),C= C_{Ar} (1585, 1469),C=C_{olf}.(1662), C-N(1141),C-Cl(1103),OH(3448).

Table(6): Chemical Shifts of ^{1}H.NMR Spectra

Compounds	N.M.R. data(ppm)
Py1	2.09 (1H,NH), 3.51 (dd,1H, -C-H), 7.47 (d,1H, =C-H), 7.52-8.29 (12H,aromatic rings).
Py2	2.12 (1H,NH), 7.31 (dd,1H, -C-H), 7.195 (d,1H, =C-H), 7.53-8.32 (12H,aromatic rings).
Py3	2.12 (1H,NH), 7.32 (dd,1H, -C-H), 7.27 (d,1H, =C-H), 7.51-8.32 (12H,aromatic rings).
Py4	2.08 (1H,NH), 7.30 (dd,1H, -C-H), 7.25 (d,1H, =C-H), 7.51-8.32 (12H,aromatic rings).

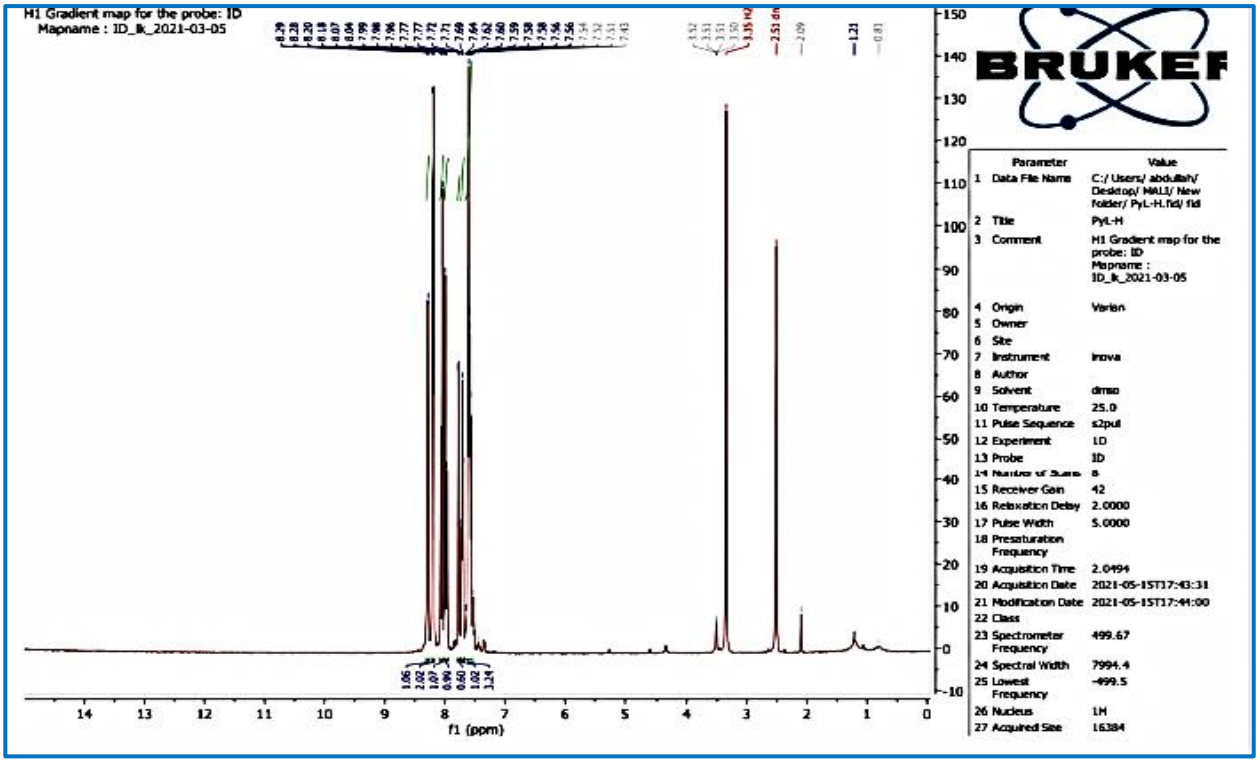


Figure (1) ^{1}H-NMR spectrum of compound Py1

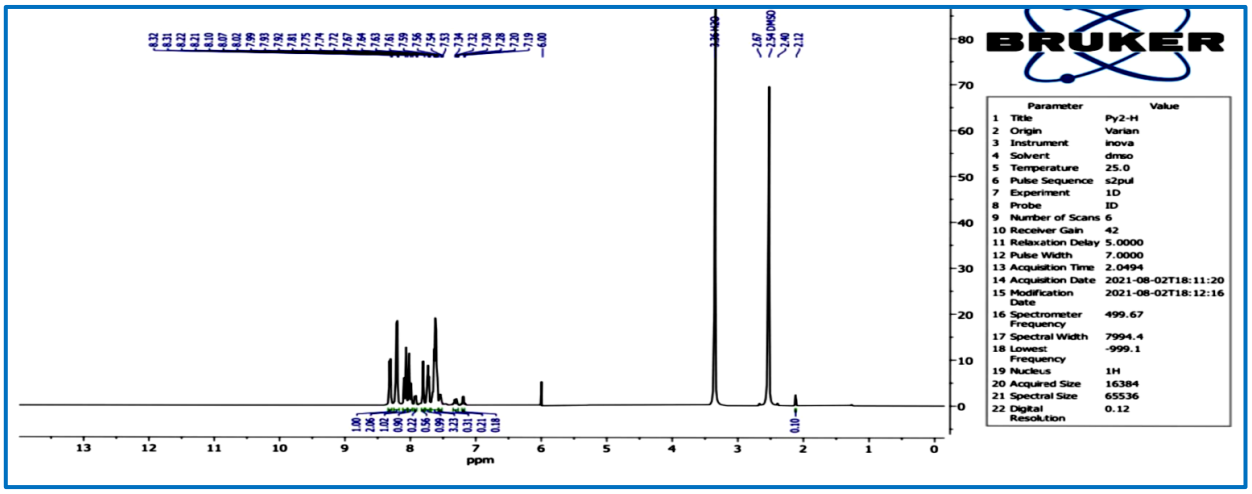


Figure (2) ^{1}H-NMR spectrum of compound Py2

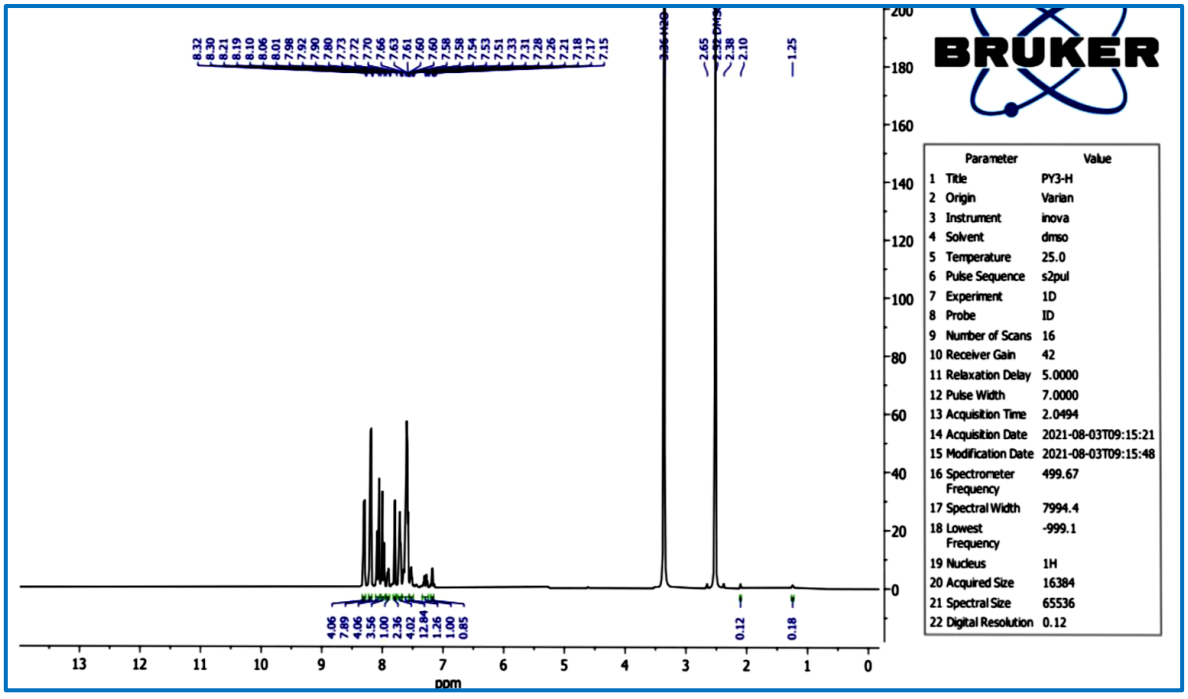


Figure (3) ^{1}H-NMR spectrum of compound Py3

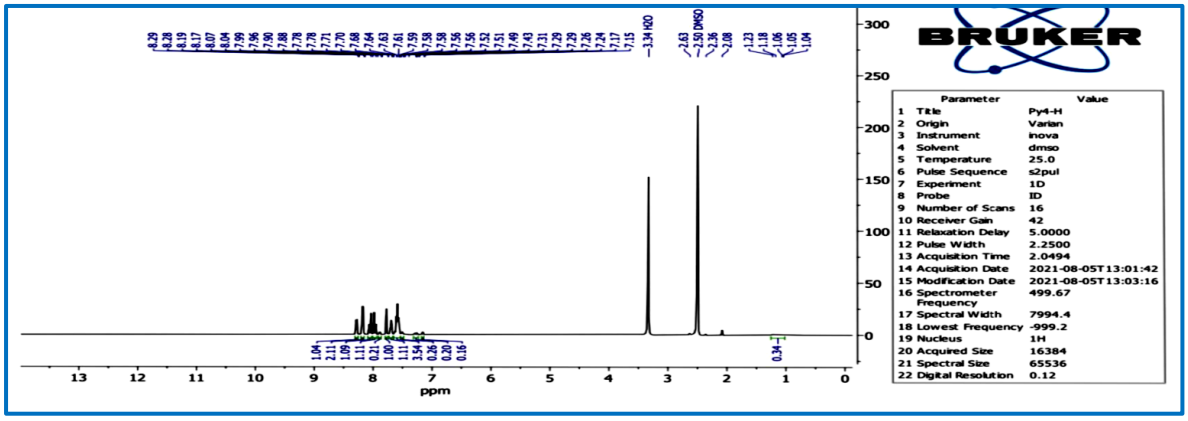


Figure (4) ^{1}H-NMR spectrum of compound Py4

Table(7): Mass spectra of prepared compound

Fragment \ Comp.	Py1	Py2	Py3	Py4
$C_6H_5^+$	77.1	77.1	77.1	77.1
$C_{15}H_{11}Cl_2N^+$	276.1	276.1	276.1	276.1
$C_{16}H_{13}Cl_2NO^+$	199.0	199.0	199.0	199.0
$C_5H_4Cl_2^{\bullet+}$	135.2	135.2	135.2	135.2
$C_{15}H_{12}ClN^{\bullet+}$	241.1	241.1	241.1	241.2
$C_{12}H_{18}NH_2^{\bullet+}$	178.1	178.1	178.2	178.2
M+	410. 4	412.9	383.6	383.6
M.Wt	411	412	383	383

Anti-Bacterial Activity

Staphylococcus aureus bacteria

The activity of pyrazole compounds was tested on Gram-positive *Staphylococcus aureus*, where all compounds showed high inhibition activity as shown in Table (8), and Figure

Table (8) Inhibition Zoon of the prepared pyrazoles against *S-aureus* bacteria

Zoon (mm)Inhibition		Compounds	No.
1000(µg/ml)	500(µg/ml)		
26	23	Py1	1
26	22	Py2	2
27	24	Py3	3
27	23	Py4	4

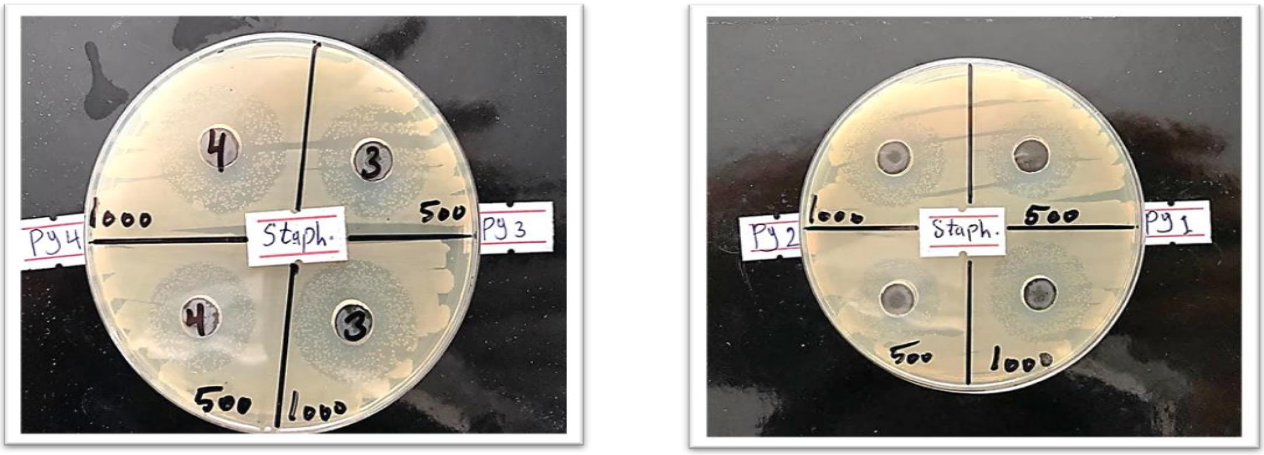


Figure (5) Activity of the prepared pyrazole compounds against *Staphylococcus aureus* bacteria.

We found compounds that have withdrawing groups (COOH and NO_2), the Py1compound is more effective than the Py2 compound. It was also noted that the compounds containing the donor group (OH) in the para-site are more effective than the compounds containing the withdrawing groups (COOH and NO2), which indicates that the activity depends on the charge of the compound, meaning that the attachment is electrostatic between the compound and the target (the receptor inside the bacteria) so that the higher the charge, the more effective the inhibition, because the NO2 group was more withdrawn, so the charge decreased and the

effectiveness decreased, while the COOH group was less cloudy, the charge increased and the efficiency increased. Table (9) [15]

Table (9) values of the electronic effect of the donor and withdrawing groups

Group	σ_p	σ_m
-OH	-0.38	0.13
-H	0	0
-COOH	0.44	0.35
$-NO_2$	0.81	0.71

In the case of the donor groups (compounds containing the OH group), the activity of the substituted compound in the para-site Py3 was more effective than the substituted compound in the meta-site Py4 (Figure 6), which indicates that the steric shape of the compound affected the way it binds to the target [16].

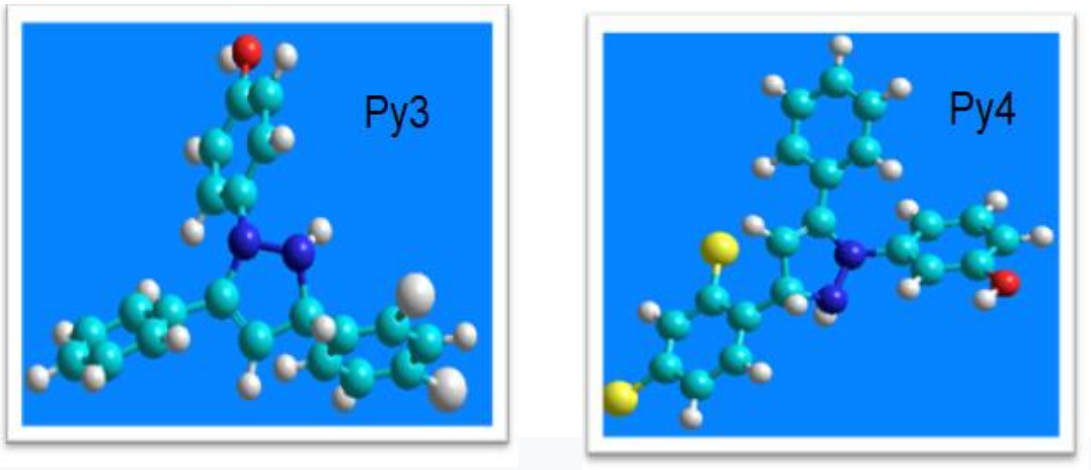


Figure (6) Geometry of the Py3 and Py4 compounds

Escherichia coli bacteria

The activity of the prepared pyrazole compounds was tested on Gram-negative *Escherichia coli* bacteria, where all compounds showed high inhibition activity as shown in Table (9), and Figure (7).

Table (10) Inhibition Zoon of the prepared pyrazoles against *E.Coli* bacteria

Inhibition Zoon (mm)	Compound	No.

Inhibition Zoon (mm)		Compound	No.
1000(µg/ml)	500(µg/ml)		
30	26	Py1	1
29	24	Py2	2
32	28	Py3	3
34	26	Py4	4

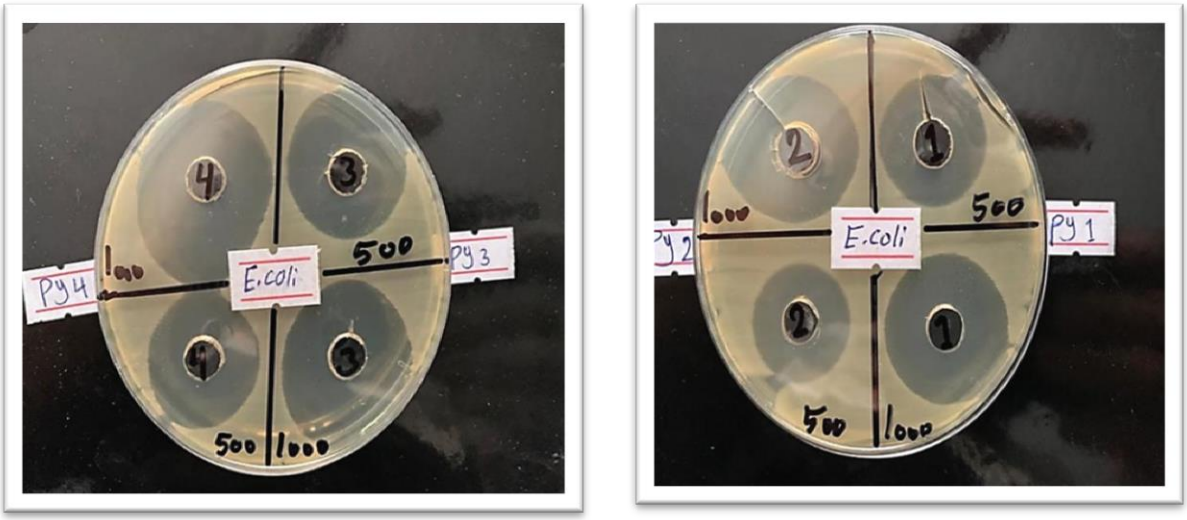


Figure (7) Activity of the prepared pyrazole compounds against *E.Coli* bacteria.

When comparing the compounds (Py1) containing the COOH group (the least drawn) was more effective than the compound (Py2) containing the NO2 group (the most withdrawn) Table (9). The compound (Py3) containing the donor group OH in the para-site was more effective than the substituted compound (Py4) in the meta-site Figure(6). We also note that the effectiveness of the compounds containing the donor groups is more than the compounds containing the withdrawing groups.

Finally, from the observation of Figures (5) and (7), we find that the compounds have an inhibitory effect on bacteria gram-positive *S.aureus* (because there are some colonies of bacteria within the measurement area) and a "bactericidal effect on gram-negative *E.Coli* bacteria (the absence of any bacteria colony inside measuring area)

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