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Synthesis and Evaluation of Antimicrobial Activity of Some Heterocyclic Moieties of Pyrazole Derivatives

Mohamad F. Ali[1], Khalid M.[2], Basma S. Baaiu[3], Hussniya al Difar[*,4], Nawal Buzgya[5], Tahani M awin[6], Haneen AlDifar[7]

Abstract

In a successful trial to synthesize pyrazole derivatives, acetophenone was, first, reacted with phenylhydrazine producing the Schiffs's base condensation product (E)-1-phenyl-2-(2-phenylethylidene) hydrazine 1. The application of Vilsmeier Haack's reaction on this product afforded the cyclization product (1,3-diphenyl-1H-pyrazole-4-carbaldehyde 2 as a pyrazole derivative in good yield percentage. The treatment of (1,3-diphenyl-1H-pyrazole-4-carbaldehyde (2) with acetophenone results in the formation of another pyrazole derivative (3), having a chalcone moiety.The chemical structure of newly formed pyrazole derivatives was confirmed based on spectral data, and these compounds were screened for their anti-bacterial activity. Further exploration of the synthetic potential of compound (2) involved its reaction with acetophenone, resulting in the formation of another pyrazole derivative (3) incorporating a chalcone moiety. This newly formed compound exhibited a distinct structural characteristic, distinguished by the presence of a chalcone unit. Comprehensive spectral analysis was employed to elucidate the chemical structures of these newly synthesized pyrazole derivatives. Furthermore, these compounds were evaluated for their potential antibacterial activities. The results obtained from this screening process shed light on the antibacterial efficacy of these pyrazole derivatives, offering valuable insights into their potential applications in the field of medicinal chemistry. To assess their potential applications, these pyrazole derivatives were evaluated for their antibacterial activities. The screening results provided valuable insights into their efficacy against various bacterial strains, highlighting their potential as promising candidates for further investigation in the field of medicinal chemistry.

Keywords: Acetophenone, chalcone, Vilsmeier Haack reagent, bipyrazole, condensation

***Author for Correspondence**
Hussniya al Difar
E-mail: HA-difar@yahoo.com

[1-6]Associated Professor of Organic Chemistry, Chemistry Department, Science Faculty, University of Benghazi, Libya.
[7]Student, Chemistry, Faculty of Science, Benghazi, University Research Scholar, Lingayas's Vidyapeeth, Faridabad, Haryana, India.

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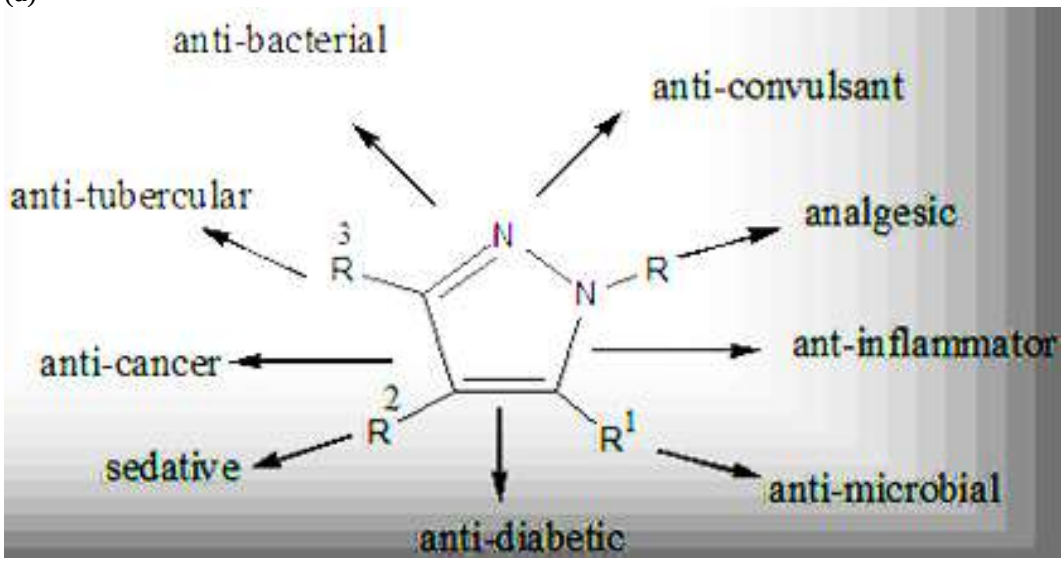
INTRODUCTION

A large and distinct collection of heterocyclic molecules with a wide range of biological activity is the five-membered pyrazole ring, which has two neighboring nitrogen atoms [1–3]. It is a moiety found in many molecules that possess many applications, depending on the substituent on either of the four positions of on pyrazole nucleus (Figure 1). Furthermore, pyrazoles, both naturally occurring and synthesized, are known to possess a wide range of biological features. The chemically named 1,2-diazole, also known as the so-called pyrazole, has many applications and has gained popularity because of its derivatives' wide range of biological activities, including those that are anti-bacterial, anti-convulsant, analgesic, anti-inflammatory, anti-microbial, anti-diabetic, sedative, anticancer and

anti-tubercular [4–11]. Modifications of the pyrazole nucleus made it possible to synthesize new derivatives that have the basic role for the treatment of different diseases like cancer, tuberculosis, inflammation, pain and diseases caused by microorganisms like bacteria [12].

(a)



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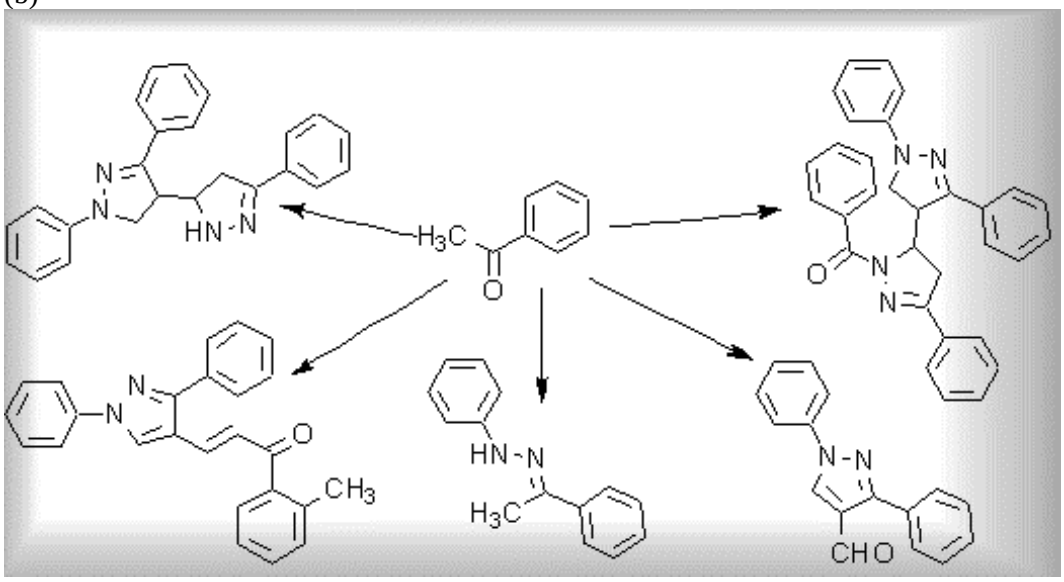


Figure 1. Substitution positions on the pyrazole nucleus which determine its biological activity.

In the present study, we report the synthesis of the pyrazole derivatives. First, we prepared the hydrazones by the reaction of acetophenone with phenylhydrazine and then they were formulated via Vilsmeier reagent (Figure 1), using the so-called Vilsmeier reagent in the Vilsmeier-Haack reaction. This involves creating the Vilsmeier reagent by mixing dimethyl formamide (DMF) with phosphorus oxychloride. This reagent then targets a nucleophilic substrate and finally hydrolyzes to produce the formyl (-CHO) group. Second, based on the literature [13], this product was utilized to synthesize several pyrazole derivatives (see Figure 1).

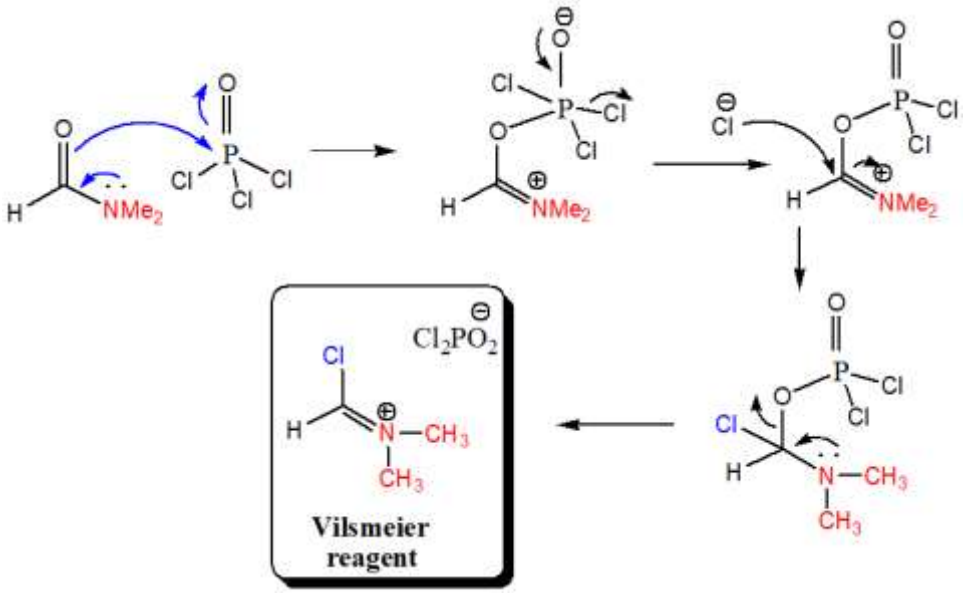


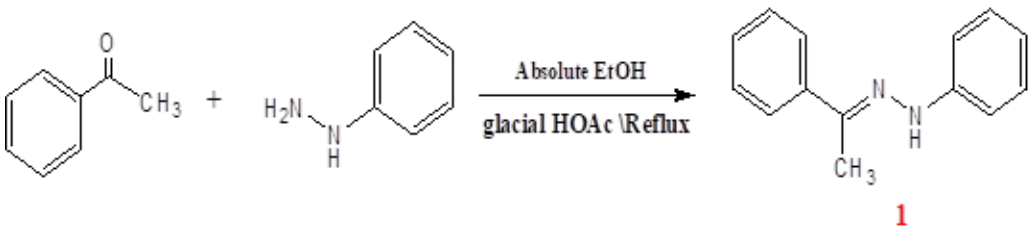
Figure 2. Vilsmeier Reagent.

MATERIALS AND METHODS

An analytical grade of chemicals and solvents was acquired and employed for this experiment. The melting point is determined without calibration using the Griffin apparatus (Stuart 3). Using 1H-NMR, a Varian Gemini 300 MHz, and a JNM-LA 400 FT-NMR system spectrometers, the structure of the obtained compounds was verified. Chemical shifts were represented in ppm units with TMS serving as an internal reference. The solvent employed was DMSO-d6. Mass spectrometry (MS) data were recorded using a Shimadzu GC-MS QP1000 EX.

(2E)-1-phenyl-2-(1-phenyl ethylidene) Hydrazine Synthesis (1)

A mixture of 1.08 g, 0.01M of phenylhydrazine, and 1.2 g, 0.01M of acetophenone in absolute ethanol was refluxed using a water bath for one hour in the presence of one milliliter of glacial acetic acid, as per the literature procedure [14.]. Alcohol affording white crystals of 1, yield 85%, m.p. 105-106 oC (Figure 2)



Scheme 1. Synthetic procedure of compound 1

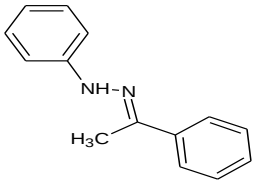
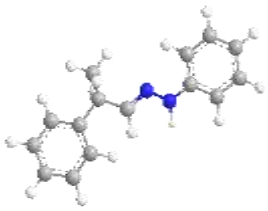
Figure 3. Synthetic procedure of compound 1.

The physical properties of compound 1 are given in Table 1. The molecular formula and structure of compound 1 are given in Table 2.

Table 1. Physical properties of compound 1.

Compound No	Molecular Weight (g/mol)	Molecular Formula	Yield %	Melting Point	Reaction Time	Color of Precipitate
1	210.27	$C_{14}H_{14}N_2$	85%	105-106 C°	1 h	white crystal

Table 2. Molecular formula and molecular structure of compound 1.

IUPAC Name	Structure	3D
(*E*)-1-phenyl-2-(2-phenylpropylidene)hydrazine		

Synthesis Of (1,3-Diphenyl-1h-Pyrazole-4-Carbaldehyde (2)

One (2.1gram, 0.01M) was added to a mixture of Vilsmeier Haack reagent (made by adding 3 ml POCl3 drop-wise to 25 ml DMF that had been cooled on ice) and refluxed for six hours [15]. Sodium bicarbonate was used to neutralize the reaction mixture after it had been placed on ice. After being separated and re-crystallized from ethanol, the product produced two Figure 3.

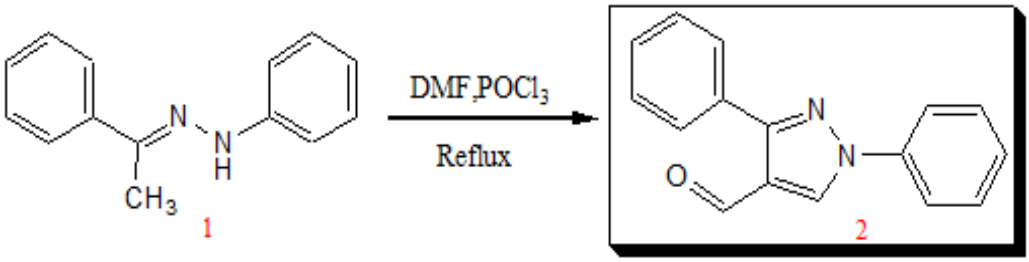


Figure 4. Synthesis of 1,3-diphenyl-1H-pyrazole-4-carbaldehyde (2).

1,3-diphenyl-1H-pyrazole-4-carbaldehyde 2

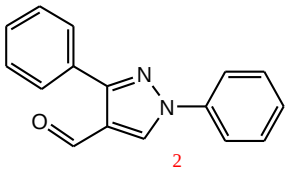
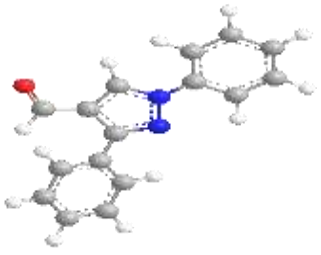
White crystals from ethanol; m.p. 160-162 ^{o}C; yield 80%; Anal. For $C_{16}H_{12}N_2O$ (m.w. 248.28): Found: C, 77.30; H, 4.85; N, 11.11, O, 6.45; Calc: C, 77.33; H, 4.83; N, 11.28, O, 6.44; MS: *m/z*: 248.05 (100 %), 247.06 (79.9 %); ^{1}H-NMR (DMSO-d_6): δ 7.45-7.93 (m, 5 H; Pyrazole-N-Ar-*H*), 7.51-7.65 (m, 5 H; Ar-*H*), 9.67(s, CHO).

The physical properties of compound 2 are shown in Table 3. The molecular formula and structure of compound 2 are shown in Table 4.

Table 3. Physical properties of compound 2.

Compound No	Molecular Weight (g/mol)	Molecular Formula	Yield %	Melting Point	Reaction Time	Color of Precipitate
2	248.28	$C_{16}H_{12}N_2O$	80%	160-162 ^{o}C	6 h	Yellow

Table 4. Molecular formula and molecular structure of compound 2.

IUPAC Name	Structure	3D
1,3-diphenyl-1H-pyrazole-4-carbaldehyde		

Synthesis Of ((E)-3-(1,3-Diphenyl-1h-Pyrazol-4-Yl)-1-Phenylprop-2-En-1-One 3

To an amount of compound 2 (2.48 g, 0.01 M), acetophenone (1.2 g, 0.01 M) in ethanol (25 ml) and 40% NaOH was added until the solution became basic. After being agitated for twenty-four hours, the reaction mixture was transferred to ice, acidified, filtered, and then re-crystallized to produce three Figure 4.

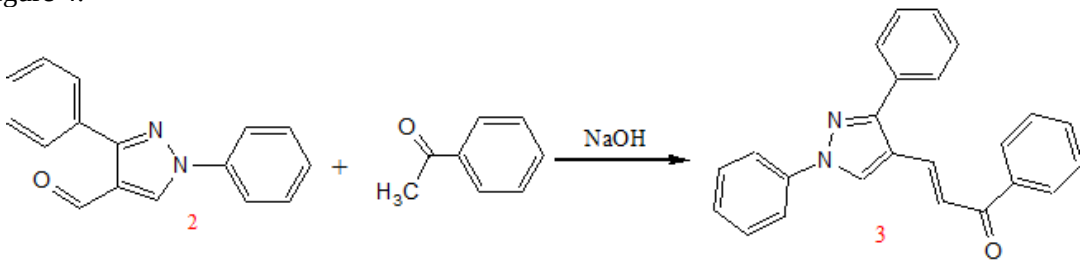


Figure 5. Synthesis of (E)-3-(1,3-diphenyl-1H-pyrazol-4-yl)-1-phenyl prop-2-en-1-one 3.

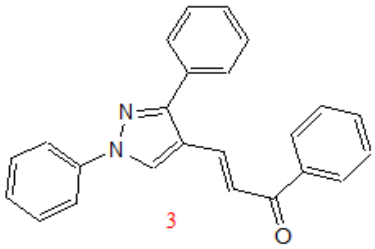
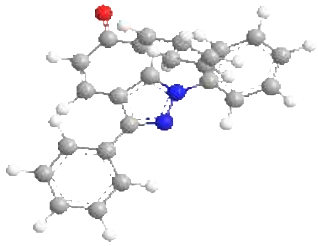
(E)-3-(1,3-diphenyl-1H-pyrazol-4-yl)-1-phenyl prop-2-en-1-one 3: Dark yellow crystals from ethanol; m.p. 163-165 ºC; yield 92%; Anal. For $C_{24}H_{18}N_2O$ (m.w. 350.4): Found: C, 82.22; H, 5.13; N, 8.01, O, 4.57; Calc: C, 82.19; H, 5.14; N, 7.99, O, 4.57; MS: *m/z*: 350.4 (100 %), 349 (11.9 %); ^{1}H-NMR (DMSO-d6): δ 6.72 (d, 1 *H*; -COC*H*), 7.81 (d, 1 *H*; Ar-C*H*), 7.49-7.66 (m, 5 H; Bz-*H*), 7.45-7.93 (m, 5 *H*; Ph-N), 7.51-7.70 (m, 5 *H*; Ph).

The physical properties of compound 3 are shown in Table 5. The molecular formula and structure of compound 3 are shown in Table 6.

Table 5. Physical properties of compound 3.

Compound No	Molecular Weight (g/mol)	Molecular Formula	Yield %	Melting Point	Reaction Time	Color of Precipitate
3	350.4	$C_{24}H_{18}N_2O$	92%	163–165 C°	24 h	Dark yellow

Table 6. Molecular formula and molecular structure of compound 3.

IUPAC Name	Structure	3D
(Z)-3-(1,3-diphenyl-1H-pyrazol-4-yl)-1-phenyl prop-2-en-1-one		

2.4. Synthesis of (1',3',5-triphenyl-3,4-dihydro-1'H,2H-3,4'-bipyrazole (4)

Chalcone **3** (3.5 g, 0.01 M) with hydrazine Hydrate (0.5 g, 0.01 M) in 20 ml ethanol was refluxed in water bath for10 hr. The solution was poured into ice, stirred, filtered, washed with water, dried and re-crystallized from ethanol affording product 4 (Figure 5).

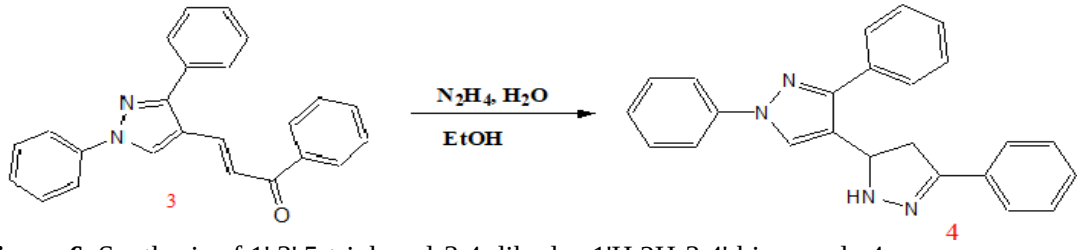


Figure 6. Synthesis of 1',3',5-triphenyl-3,4-dihydro-1'H,2H-3,4'-bipyrazole 4.

(1',3',5-triphenyl-3,4-dihydro-1'H,2H-3,4'-bipyrazole 4

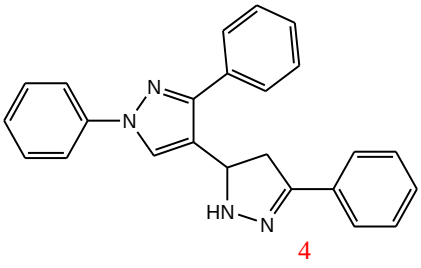
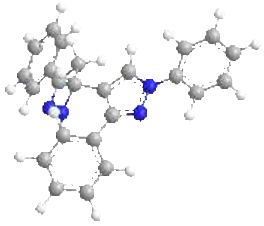
White crystals from ethanol; m.p. 150-152 ºC; yield 70%; Anal. For $C_{24}H_{20}N_4$ (m.w. 364.44): Found: C, 80.01; H, 5.47; N, 15.03; Calc: C, 79.02; H, 5.49; N, 15.37; MS: *m/z*: 364.4 (100 %), 365.4 (8.9 %); ^{1}H-NMR (DMSO-d6): δ 3.17, 3.22 (dd, 2 H; -C*H*$_2$- of dihydropyrazole), 5.3 (t, 1 H; -C*H*- of dihydro-pyraz.), 8.29 (s, 1 H; pyrazole), 7.16-8.02 (m, 15 H; all three Ph-ring-*H*).

The physical properties of compound 4 are shown in Table 7. The molecular formula and structure of compound 4 are shown in Table 8.

Table 7. The physical characteristics of compound 4.

Compound No	Molecular Weight (g/mol)	Molecular Formula	Yield %	Melting Point	Reaction Time	Color of Precipitate
4	364.44	$C_{24}H_{20}N_4$	%70	150 – 152 C°	10 hr	Pale brown

Table 8. Molecular formula and molecular structure of compound 4.

IUPAC Name	Structure	3D
1',3',5-triphenyl-3,4-dihydro-1'H,2H-3,4'-bipyrazole		

Synthesis of Phenyl (1',3',5-Triphenyl-3,4-Dihydro-1'h,2h-[3,4'-Bipyrazol]-2-Yl) Methanone (5)

Compound 4 (3.6 g, 0.01 M) in 25 ml of pyridine and benzoyl chloride (1.4 g, 0.01 M) was stirred at room temperature for an hour and was then treated with cold diluted hydrochloride acid. The solid that was separated was filtered, washed with cold NaOH (2%) then water and re-crystallized from ethanol affording 5 (Figure 6).

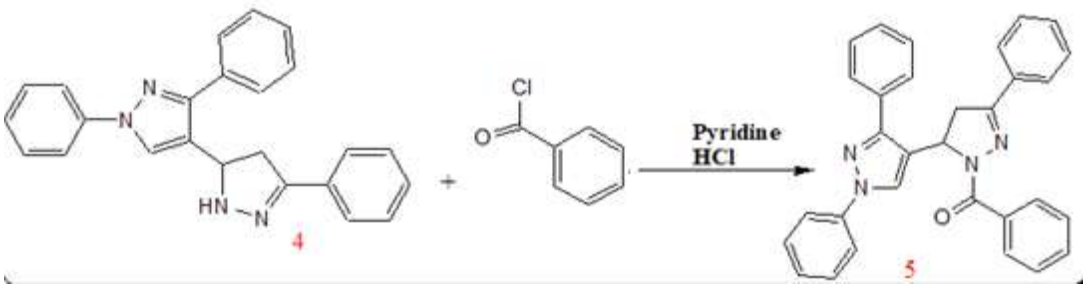


Figure 7. Synthesis of phenyl(1',3',5-triphenyl-3,4-dihydro-1'H,2H-[3,4'-bipyrazol]-2-yl) methanone 5

Phenyl-(1',3',5-triphenyl-3,4-dihydro-1'H, 2H-[3,4'-bipyrazol]-2-yl) Methanone 5

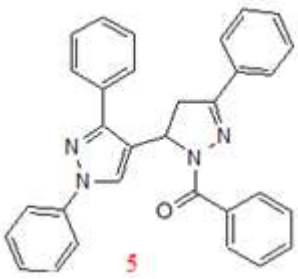
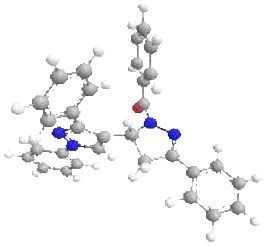
Dark brown crystals from ethanol; m.p. 260-263 °C; yield 80%; Anal. For $C_{31}H_{24}N_4O$ (m.w. 468.55): Found: C, 79.41; H, 5.11; N, 11.94, O, 3.43; Calc: C, 79.39; H, 5.12; N, 11.95, O, 3.41; MS: *m/z*: 468.56 (100 %), 469.1 (13.4 %); ^{1}H-NMR (DMSO-d6): δ3.12, 3.20 (dd, 2 H; -C*H*$_2$- of dihydropyrazole), 5.48 (t, 1 H; -C*H*- of dihydro-pyrazole), 8.02 (s, 1 H; pyrazole), 7.28-8.36 (m, 15 H; all three Ph-ring-*H*), 7.48-7.59 (m, 5 H; Bz ring).

The physical properties of compound 5 are shown in Table 9. The molecular formula and structure of compound 5 are shown in Table 10.

Table 9. The physical characteristics of compound 5.

Compound No	Molecular Weight (g/mol)	Molecular Formula	Yield %	Melting Point	Reaction Time	Color of Precipitate
5	468.55	$C_{31}H_{24}N_4O$	80 %	260-263 C°	2hr	Dark brown

Table 10. Molecular formula and molecular structure of compound 5.

IUPAC name	Structure	3D
phenyl(1',3',5-triphenyl-3,4-dihydro-1'H,2H-[3,4'-bipyrazol]-2-yl) methanone		

RESULTS AND DISCUSSION

The synthesized phenylhydrazone 1 was reacted with Vilsmeier Haack reagent to obtain the pyrazole derivative (2), the ^{1}H NMR spectrum showed the absence of resonance peak of the -CH_3 group of compounds (1), and exhibited characteristic Signals at δ 9.6 due to (-CHO), indicating the formylation of (1) occurred. Compound 2 possesses a reactive formyl group accordingly, and using this aldehydic group, pyrazole derivative 3 was obtained with a comparatively higher yield (92%), and chalcone derivative (3) was refluxed with 99% hydrazine for 10hrs.in absolute ethanol, the phenyl(1',3',5-triphenyl-3,4-dihydro-1'*H*,2*H*-[3,4'-bipyrazol]-2-yl) methanone (4), was obtained in good yields, (Scheme 4), with an active proton on pyrazole nitrogen. Accordingly, this product was in turn reacted with benzoyl chloride to give the final bipyrazole product, 2-benzoyl-1',3',5-triphenyl-3',4,4',5'-tetrahydro-1'*H*,2*H*-3,4'-bipyrazole 5 in high yield. Its mass spectrum showed an M^++1 ion peak at 468 m/z, matching the molecular mass of 5. Based on the data (presented in the experimental part., the structure was assigned for compound 5.

The obtained values for the compounds' structures were congruent with the spectral information, particularly the elemental analysis (calculated values). Tables 1, 3, 5, 7 and 9. The physical characterization for compounds (1;2;3;4;5), including 1H-NMR, MS spectra have been measured and are presented in the experimental part. On the other hand, Tables 2, 4, 6, 8 and 10 each present the IUPAC name, the structural formula, and the 3D structure for the preferred compound.

METHOD USED FOR SCREENING

In the in-vitro evaluation of the antimicrobial activity, the 'agar holes well' method was used for the antimicrobial susceptibility testing. The diameter of inhibitory zones served as an expression for antimicrobial potentialities. Chemical extracts were examined as antimicrobial agents against all microbial isolates.

ANTIBACTERIAL ACTIVITY

The ntibacterial activity was investigated using an agar well diffusion procedure and were studied against *Staphylococus aureus* and *Megaterium* as gram-positive bacteria while *Pseudomonas aeruginosa* and *Escherichia coli* as gram-negative bacteria. The tested materials (10 µg/mL and 100 µg/mL) were placed into the plate wells, and the diameter of the inhibitory zone was measured in millimeters (mm) to assess the activity. As a loading control, all compounds (1–5) were synthesized in dimethyl sulfoxide (DMSO). After 24 hours of incubation at 37° C, the plates were checked to see if zone inhibition had formed. To obtain an average value, each zone inhibition was measured using a caliper three times. For every bacterium culture, the test was run three times. The two main antibacterial medications utilized were streptomycin and penicillin. The following graphical charts show the zone inhibition as they recede. The inhibition zone for each type of bacteria is shown in Table 11, and the inhibition zones of gram-positive and gram-negative bacteria are shown in Figures 2 and 3, respectively. When compared to standards, compounds 1 and 3 demonstrated the strongest inhibition against P. aeruginosa and Escherichia, compound 5 demonstrated moderate inhibition, and compounds 2 and 4 demonstrated comparatively lower inhibition. However, when compared to standards, Figure 4 demonstrated that most of the synthesized compounds exhibited high inhibition against Megaterium but comparatively higher inhibition against *Staphylococus aureus* (Figures 7–10).

Table 11. Inhibition zone in (mm) for both kinds of bacteria for all synthesized compounds.

Compound Code (in µg/mL)	**Gram-Negative**		**Gram-Positive**	
	E. Coli	***P. aerug***	***S. aureus***	***Megaterium***
1	19	17	13	18
2	15	12	14	19
3	17	20	14	16
4	13	14	14	17
5	15	15	15	15
ST	16	14	16	13
ST	17	16	12	14

Solvent Dimethylsulfoxide (DMSO)

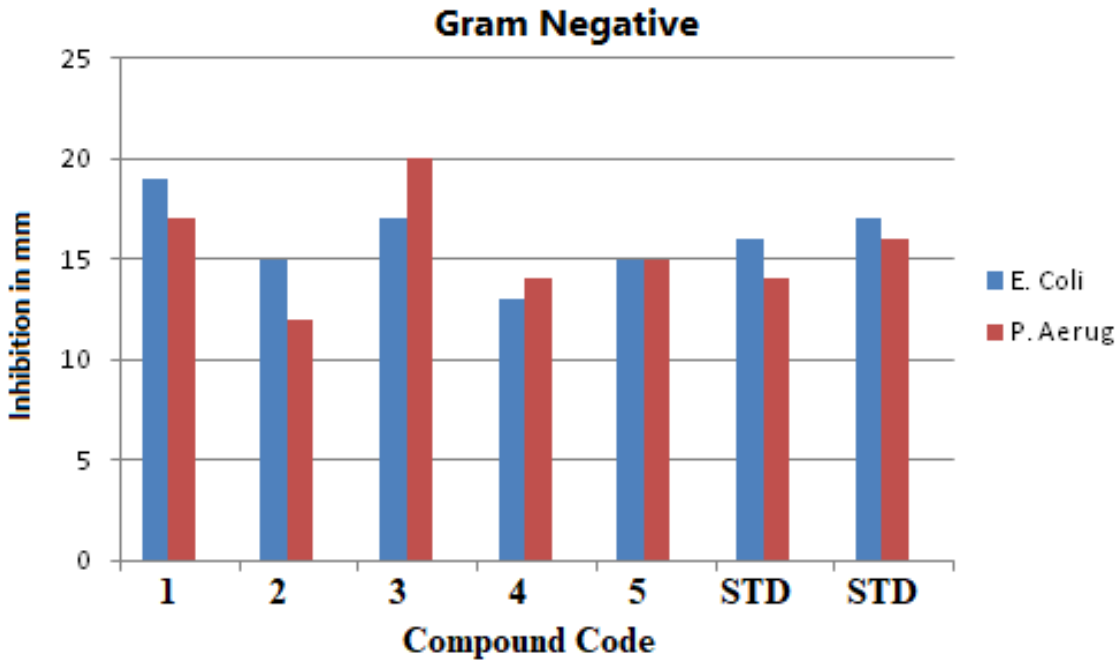


Figure 8. Inhibition zone of compounds against gram-negative microorganisms (in mm).

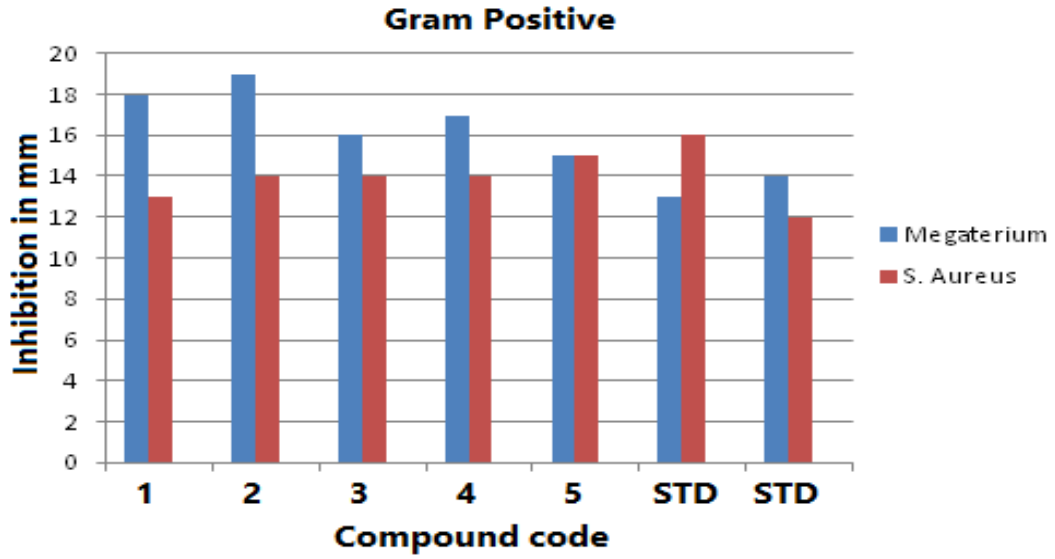


Figure 9. Inhibition zone of synthetic compounds against gram-positive microorganisms (in mm).

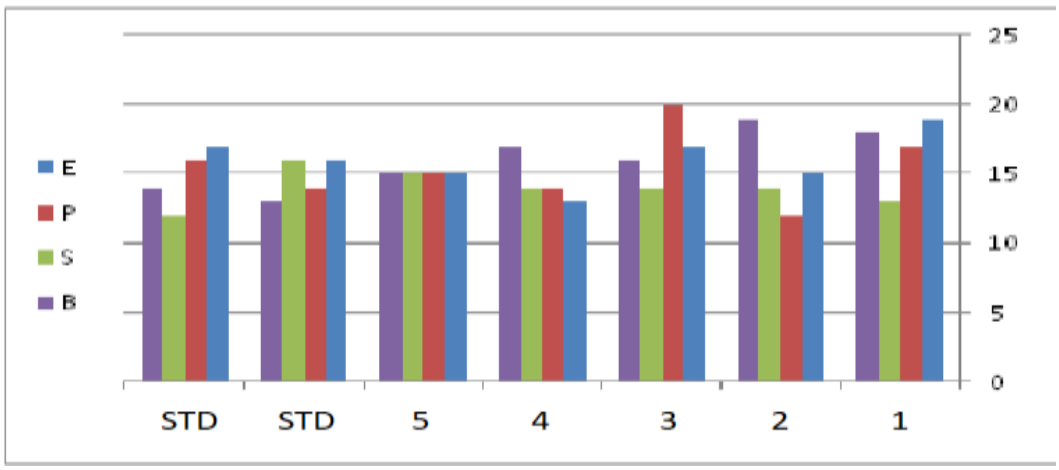


Figure 10. Inhibition zone of compounds against gram-positive and negative microorganisms (mm).

CONCLUSION

In conclusion, it is reported that new bioactive heterocyclic compounds, like pyrazoles, can be easily synthesized. The biological activity of the substances was assessed. As a result, compound 1 exhibited excellent inhibition against all bacteria except *S.Aureus*, and 2 exhibited excellent antibacterial only against *E. coli* and *B. meg.* Compound 3 exhibited excellent inhibition only against *E. coli* and *P.aerug*. but weak inhibition against other species.

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Disclosure Statement

No potential conflict of interest was reported by the authors.

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Authors' Contributions

All authors contributed to data analysis, drafting, and revising of the paper and agreed to be responsible for all aspects of this work.

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