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# SYNTHESIS, MOLECULAR DOCKING STUDY AND ANTIBACTERIAL ACTIVITY OF NEW PYRAZOLINE DERIVATIVES

Kale Mohammed Qadir[1], Hawzhin Yassen Hussein[2], Marlin Yousef Aziz[2], Mzgin Mohammed Ayoob[2*], Mohammed Kareem Samad[2], Awaz Jameel Hussien[2], Nabard Ghafur Ahmed[2] and Farouq Emam Hawaiz[2]

[1]Biology Department, College of Education, Akre University for Applied Science, Duhok, Kurdistan, Iraq
[2]Department of Chemistry, College of Education Salahaddin-University-Erbil, Erbil, Kurdistan, Iraq

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**ABSTRACT**. In this study, a new series of pyrazoline compounds with five members was synthesized by cyclization of chalcone derivatives in presence of phenyl hydrazine (**3a-i**). The development began with 4-hydroxy acetophenone, which was benzylated via Williamson ether synthesis to give 1-(4-(benzyloxy) phenyl)-3-phenylprop-2-en-1-one (**1**), many derivatives of chalcones (**2a-i**) were synthesized by Claisen-Schmidt condensation of the compound (**1**) with substituted benzaldehyde, chalcones undergo cyclization with phenyl hydrazine to give target pyrazoline derivatives. The structure of all newly obtained compounds was supported by spectral data (FT-IR, $^{1}$H-NMR, $^{13}$C-NMR and $^{13}$C DEPT-135). Some pyrazoline derivatives were estimated for their antibacterial activity against *Escherichia coli* as Gram-negative and *Staphylococcus aureus* as Gram-positive. The results showed the high sensitivity of the synthesized compounds to both types of bacteria. Furthermore, this study examines a molecular docking analysis of synthesized compounds, the results revealed a favorable binding contact with the target bacterial DNA gyrase (PDB ID: 1KZN) of *E. coli*. Finally, combined findings indicate that these compounds exhibit significant antibacterial activity, with computer models suggesting their ability to target bacterial enzymes or receptors, thereby inhibiting their function and suppressing bacterial growth.

**KEY WORDS**: Chalcone, Pyrazoline, Antibacterial activity, Docking study

## INTRODUCTION

Given the significant prevalence of diseases in society, driven by environmental pollution and the increasing resistance of microorganisms and bacteria to conventional medicines, it becomes essential to explore the development of new and effective antibiotics. These innovations aim to address or minimize the impact of diseases that current antibiotics can no longer combat. Consequently, prioritizing the design, enhancement, and synthesis of novel compounds with improved antibacterial efficacy and reduced risks is highly recommended [1], particularly a heterocyclic compounds containing nitrogen and sulphur atoms [2]. The Claisen–Schmidt (CS) condensation between acetophenone and benzaldehyde derivatives is a valuable C-C bond-forming reaction which allows α,β-unsaturated ketones called chalcones to be obtained [3]. Chalcones are flavonoid natural products that act as an intermediary in the biosynthesis of flavonoids and are found in a wide range of plants [4]. Chalcone is an α,β-unsaturated ketones linked between two aromatic rings[5]. Chalcone is a class of compounds, which has many applications in different fields. α,β unsaturated carbonyls used as a key intermediates for the preparation of number of heterocyclic compounds such as thiazine, oxazines, isoxazoles and pyrazoles, chalcone can be used as an initial compound for synthesis of a lots of compounds [6]. Chalcones have been prepared by the Claisen-Schmit condensation reaction [7], which is

*Corresponding authors. E-mail: Mzgin.ayoob@su.edu.krd

classified within the aldol condensation, where the aromatic aldehydes which do not contain alpha hydrogen interact with the aromatic ketones containing alpha hydrogen presence of a strong base medium. Chalcones are versatile and convenient precursors for the synthesis of a wide variety of organic compounds such as pyrazoline [8], pyrimidines [9] and thiazepines [10]. Pyrazolines are well-known as an important type of heterocyclic compounds containing two N atoms in the five-membered ring. In medicine, pyrazolines derivatives have been shown some remarkable properties, especially as antimicrobial [11, 12], antitumor [13], anti-HIV [14], antioxidant [15, 16], antidepressant [17], anti-bacterial [18, 19], anti-fungal [20], antiinflammatory [20, 21], anti-malarial [22], anti-depresent [23] and anti-pyretic [24-26]. Pyrazolines can be made in a variety of ways, but one of the most used is Michael reaction strategy, instead of making a direct nucleophilic addition to the carbonyl, some nucleophiles make a conjugate addition to the alkene of an α,β-unsaturated carbonyl molecule [27]. In the present research, a new compound known as 3-(4-(benzyloxy) phenyl)-4,5-dihydro-1,5-diphenyl-1H-pyrazole was synthesized and analysed its molecular structure utilizing FT-IR, $^{1}$H-NMR ,$^{13}$C-NMR and $^{13}$C-DEPT-135 spectroscopy with evaluation their biological studies.

## RESULTS AND DISCUSSION

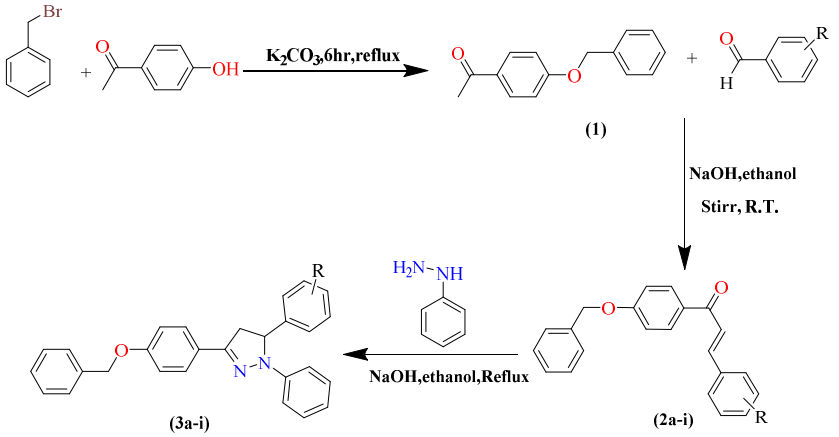
### *Chemistry section*

Novel pyrazoline derivative were synthesized by cyclization of substituted chalcone derivatives (**2a**–**i**) in presence of phenyl hydrazine (Scheme 1). Chalcones were synthesized via Claisen--Schmidt condensation, which involves condensing substituted aromatic acetophenone with substituted benzaldehydes in ethanol with sodium hydroxide as a catalyst, was the basis for the generic synthesis method used to create exceptional yields of chalcones. Chalcones serve as a useful starting material for the synthesis of a number of heterocyclic substances, including flavonoids, pyridines, thiazepines, isoxazoles, pyrazolines, and pyrimidines [28]. The structure of newly synthesized chalcones (**2a-h**) and pyrazolines (**3a-h**) were confirmed on the basis of their spectral data FT-IR, $^{1}$H-NMR, $^{13}$C-NMR and $^{13}$C-DEPT-135. The FT-IR spectrum of intermediate benzyloxy compound (**1**) shows a strong carbonyl group absorption band appearing at 1676 cm$^{-1}$, 1240 cm$^{-1}$ (C-O), and in the FT-IR spectrum of chalcones (**2a-i**) shifting carbonyl group to lower value (1653-1660 cm$^{-1}$), this is a strong evidence for the formation of chalcones [29]. In the FT-IR spectrum of pyrazolines (**3a-h**) disappearance of carbonyl group band at 1660-1653 cm$^{-1}$ for enone system is a good evidence for the formation of imine and the occurrence of cyclization reaction to give 2-pyrazolines [30]. The $^{1}$H-NMR spectra of chalcones, showed that the Cα-H and Cβ-H protons are considerably shifted downfield to the extent that they appear in aromatic region δ 7.47-8.06, and disappearance of aldehydic protons of the reactants at 9-10 ppm confims the formation of chalcones [31]. In the $^{1}$H-NMR spectra of the pyrazolines the protons attached to the C-4′ and C-5′ carbon atoms in the 2-pyrazoline ring gave an (ABX) spin system, which appeared three doublet to doublets (dd) signals at δ 3.0 , 3.8 , 5.6 ppm for two geminal and one vicinal protons unequivocally prove a 2-pyrazoline structure. From the $^{13}$C-NMR spectrum of **3e** the chemical shift values of carbon atoms C-4′ δ (55) and C-5′ δ (77) corroborate the 2-pyrazoline structure and the disappearance of two singlets for C-α and C-β of intermediate chalcones is considered further evidence for 2-pyrazoline structure [32].

### *Antibacterial activity*

Antibacterial activities were also performed as *in-vitro* screening against bacterial strain respectively. The inhibition zone (mm) values for all active compounds were determined. The synthesized chalcones and pyrazolines showed the different biological activities against two types of bacteria Gram positive and Gram negative bacteria including *S-aureus* and *E-coli*. The findings

of this study revealed that tested compounds (**3a** and **3d**) had highest biological activity at 400 µg/mL against both *S. aureus* but with *E. coli* only compound **3d** had a highest activity with inhibition zone 23 mm at 400 µg/mL. Compound **3i** has lowest activity against *S. aureus* but with *E. coli* compound **3f** has lowest activity, the results was shown in Tables 1 and 2. According to the results obtained from this study most of synthesized compounds has moderate to good antibacterial activity in comparison to the standard ciprofloxacin as control.




**(a) R=4-$CH_3$, (b) R=4-F, (c) R=4-Cl, (d) R=4-Br, (e) R=3,4-$OCH_3$, (f) R=4-$NO_2$, (g) R=3-Cl, (h) R=3-$NO_2$, (i) R=4-$OCH_3$**

Scheme 1. The protocol for the synthesis of pyrazoline derivatives.

Table 1. The antibacterial activity data for synthesized pyrazolines (**3a-i**), with different concentration against *S. aureus* as gram-positive.

| Conc. (µg/mL) | Zone of inhibition in (mm) | | | | | |
|---|---|---|---|---|---|---|
| | **3a** | **3b** | **3d** | **3f** | **3i** | Ciprofloxacin |
| 200 | 17 | 19 | 21 | 20 | 12 | 22 |
| 400 | 22 | 19 | 22 | 14 | 16 | 34 |
| 600 | 21 | 17 | 17 | 13 | 19 | 25 |
| 800 | 19 | 0 | 15 | 16 | 11 | 26 |

Table 2. The antibacterial activity data for synthesized pyrazolines (**3a-i**), with deferent concentration against *E. coli* gram-negative.

| Concentration (µg/mL) | Zone of inhibition in (mm) | | | | | |
|---|---|---|---|---|---|---|
| | **3a** | **3b** | **3d** | **3f** | **3i** | Ciprofloxacin |
| 200 | 19 | 17 | 18 | 14 | 19 | 33 |
| 400 | 18 | 17 | 23 | 13 | 18 | 34 |
| 600 | 18 | 16 | 17 | 13 | 18 | 37 |
| 800 | 20 | 17 | 0 | 0 | 19 | 41 |

*Docking study*

Bacterial DNA gyrase is essential for bacterial growth, and recent research has focused on developing synthetic inhibitors that target this enzyme as potential antibacterial agents [33]. Consequently, we conducted a molecular docking analysis on the synthesized compounds to

evaluate their binding interactions with DNA gyrase and compared these interactions to those of the clinical pharmacological inhibitor ciprofloxacin.

Table 3. The docking study output of the synthesized compounds in which docked against *E. coli* DNA gyrase B (PDB ID: 1KZN).

| Entry | ΔG binding (kcal/mol) | Hydrogen bond contacts | Pi-cation | pi-H contacts |
|---|---|---|---|---|
| 3a | -7.8054 | Asn46 | -- | Asn46,Ile78 |
| 3b | -6.8084 | --- | -- | Glu50, Thr 165 |
| 3c | -7.5419 | -- | -- | Gly117 |
| 3d | -7.4105 | -- | Arg136, Arg136 | Pro79 |
| 3e | -8.3222 | -- | Arg136, Arg136 | Pro79 |
| 3f | -7.3847 | Ala96, Ser 121 | -- | -- |
| 3g | -7.2530 | -- | Arg136 | Pro79 |
| 3h | -7.5409 | Arg76 | Ile78 | -- |
| 3i | -7.7897 | -- | Arg136, Arg136 | Pro79 |
| Ciprofloxacin | -6.7012 | -- | -- | Asn 46 |

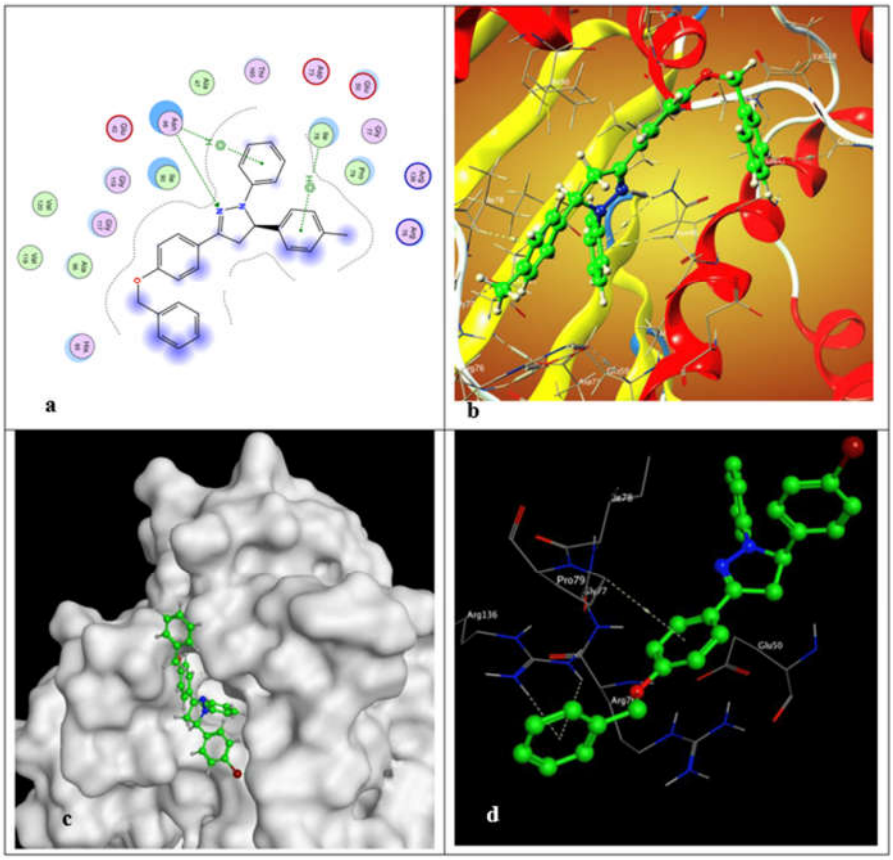



Figure 1. 3D structure of **3a** and **3d**: a 2D ligand interaction, b 3a docked into active site of (1KZN) c- active site of site of (1KZN) with 3d, and d-ligand active site interaction.

The binding energies of the synthesized compounds (**3a-i**) ranged from -6.7012 to -8.3222 kcal/mol, with compound 3e showing the most favourable binding energy of -8.32 kcal/mol. The data in Table 3 also provides information on binding affinity, hydrogen bonding, and residual interactions of the synthesized compounds compared to ciprofloxacin. Most of the compounds, as detailed in Table 3, exhibited predicted binding energies lower than that of ciprofloxacin.

Hydrogen bonding interactions were predominantly observed between the pyrazoline ring compounds and residues such as Asn 46, Ala 96, Ser 121, and Arg 76 in DNA Gyrase Subunit B (PDB ID: 1KZN). For example, compound **3a** formed one hydrogen bond with Asn 46 and three π-H interactions with Asn 46 and Ile 78 at the DNA Gyrase active site. The 3D binding mode of compound **3a** within the DNA Gyrase Subunit B (PDB ID: 1KZN) is shown in Figure 1.

## EXPERIMENTAL

*Chemicals and instruments*

All chemical that used are analytical grade purchased from different brand (Scharlau, Fluka, Riedel-de-Haen). Infrared affinity-1 (Shimadzu) spectrometers with KBr pellets were used to record IR spectrum. $^{1}$H NMR and $^{13}$C-NMR and $^{13}$C-DEPT-135 with $CDCl_3$ and DMSO as solvents were used to record spectra of nuclear magnetic resonance (Bruker spectrometer 400 MHz). Electro-thermal melting point devise 9100 were used to record the melting point of compounds (uncorrected).

*Synthesis of 1-(4-(benzyloxy) phenyl)ethanone*

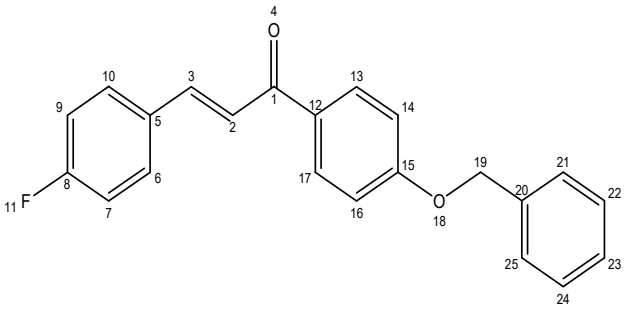
A mixture of 1-(4-hydroxyphenyl)ethanone (50 mmol), [1-(bromomethyl)benzene (60 mmol)] and anhydrous $K_2CO_3$ (100 mmol), in ethanol (100 mL-99.9%) was refluxed for 6 h. with stirring. When the reaction was completed by the changing of the colour, the cooled solution poured into water, solid materials immediately was obtained. The desired product was filtered off, washed several times with water and cold ethanol, dried and recrystallized from ethanol to obtain white crystal [34]**.**

*Synthesis of chalcone derivatives (**2a-i**)*

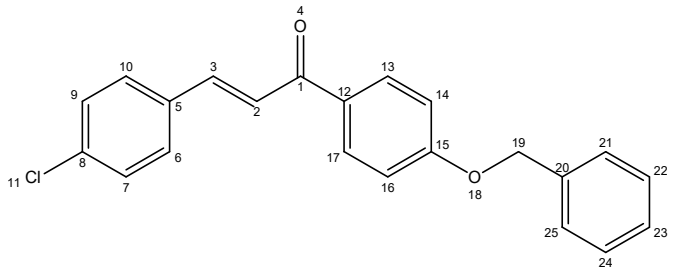
*Synthesis of (E)-1-(4-(benzyloxy)phenyl)-3-phenylprop-2-en-1-one*

To the solution of 4-(4'-benzyloxy)acetophenone (0.0025 mol) in 20 mL ethanol, in equivalent of benzaldehyde (0.5 mL) 30% aqueous sodium hydroxide were added and stirred at room temperature for about (30-45 min) until the formation of pale yellow crystals then the solution was kept under stirring overnight at room temperature. The solid crystals were separated by suction filtration, washed with ethanol and water to neutralize, dried and recrystallized from ethanol [9, 35]**.**

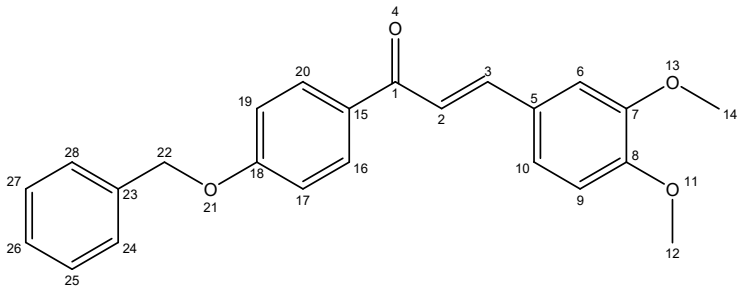
*1-(4-(Benzyloxy)phenyl)-3-(4-fluorophenyl)prop-2-en-1-one (**2b**)*. $C_{22}H_{17}FO_2$ ; Mol. Wt.: 332 ( 95%), m.p. 161-163 °C. $^{1}$H NMR (400 MHz, $CDCl_3$) δ 7.81 (d, *J* = 15.8 Hz, 1H, CH=CHβ), 7.66 – 7.58 (m, 2H Ar-H), 7.47 (d, *J* = 15.8 Hz, 1H, CHα=CH), 7.39 (dd, *J* = 7.7, 1.6 Hz, 1H, ph-H), 7.26 – 7.17 (m, 5H, Ar-H), 6.93 – 6.75 (m, 5H, Ar-H), 4.91 (s, 2H, $OCH_2$-) . $^{13}$C-NMR (101 MHz, $CDCl_3$) δ 189.18: C=O, 166.77, 164.24: 158.00, 140.92, 134.88, 134.65, 134.24, 131.71, 131.29, 131.08, 130.99, 129.43, 129.03, 123.97, 122.94, 121.37, 115.71, 115.50, 112.33, 69.80: O-$CH_2$-. $^{13}$C-DEP-135 (101 MHz, $CDCl_3$) δ 140.92, 131.71, 131.30, 131.08, 130.99, 129.43, 129.03, 122.94, 121.37, 115.72, 115.50, 112.33.



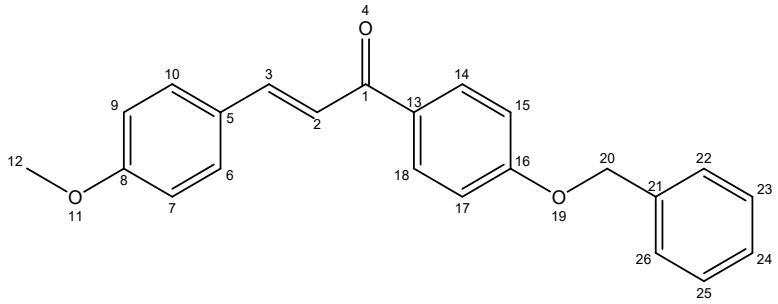
*1-(4-(Benzyloxy)phenyl)-3-(4-chlorophenyl)prop-2-en-1-one (**2c**)*. $C_{22}H_{17}FO_2$; Mol. Wt.: 349 (95%), m.p. 158-160 °C. $^1$H NMR (400 MHz, chloroform-*d*) δ 8.06 (d, *J* = 15.8 Hz, 1H, CH=CH ß), 7.78 (d, *J* = 8.6 Hz, 2H, Ar-H), 7.73 – 7.60 (m, 3H Ar-H), 7.50 – 7.41 (m, ,6H, Ar-H), 7.39 (d, *J* = 1.7 Hz, ,1H CHα=CH), 7.11 – 7.00 (m, 2H, Ar-H), 5.16 (s, 2H, $OCH_2$). $^{13}$C-NMR (101 MHz, $CDCl_3$) δ 189.61: C=O, 158.01, 141.20:C3, 139.03, 136.61, 134.85, 131.82, 131.19, 129.87, 129.39, 129.04, 128.83, 123.92, 122.87, 121.38, 112.35, 69.81: O-$CH_2$-. $^{13}$C-DEP-135 (101 MHz, $CDCl_3$) δ 141.20 131.82, 131.20, 129.87, 129.39, 129.04, 128.83, 122.87, 121.38, 112.35.



*1-(4-(Benzyloxy)phenyl)-3-(3,4-dimethoxyphenyl)prop-2-en-1-one (**2e**)*. $C_{24}H_{22}O_4$; Mol. Wt.: 374 (92.8%), m.p. 137-139 °C. $^1$H-NMR (400 MHz, Chloroform-*d*) δ 8.01 (d, *J* = 15.7 Hz,CH=CHβ, 1H), 7.79 (d, *J* = 15.7 Hz, CHα=CH, 1H), 7.66 – 7.60 (m, 2H,Ar-H), 7.49 (d, *J* = 8.6 Hz, 2H, Ar-H), 7.48 – 7.42 (m, 3H, Ar-H), 7.42 – 7.35 (m, 1H, Ar-H), 7.24 (dd, 1H Ar-H), 7.04 (d, *J* = 8.3 Hz, 2H, Ar-H), 6.82 (d, *J* = 8.4 Hz, 1H, Ar-H), 5.16 (s, $OCH_2$,2H,), 3.98 (s, 2($OCH_3$), 6H). $^{13}$C -NMR (101 MHz, $CDCl_3$) δ 189.02: C=O, 157.98, 153.08, 149.16, 139.90, 135.00, 134.14, 131.54, 131.43, 131.34, 129.64, 129.05, 124.24, 123.28, 123.01, 121.34, 112.22, 110.56, 109.71, 69.79: O-$CH_2$, 56.14, 56.00, 2O-$CH_3$.$^{13}$C-DEP-135 (101 MHz, $CDCl_3$) δ 139.91:C3, 131.54, 131.34, 129.64, 129.05, 123.28, 123.01, 121.34:C2, 112.21, 110.56, 109.71, 77.25, 56.14:C12, 56: C14.



*1-(4-(Benzyloxy)phenyl)-3-(4-methoxyphenyl)prop-2-en-1-one* ***(2i)***. $C_{23}H_{20}O_3$; Mol. Wt. 344: (95.2%), m.p.132-134 °C. $^1$H NMR (400 MHz, chloroform-*d*) δ 8.02 (d, *J* = 15.7 Hz, 1H CH=CHβ), 7.88 – 7.78 (m, 3H, Ar-H), 7.75 (s, 1H, Ar-H), 7.63 (dd, *J* = 7.7, 1.7 Hz, 1H, -CHα=CH), 7.55 – 7.45 (m, 3H, Ar-H), 7.45 – 7.39 (m, 1H, Ar-H), 7.39 – 7.32 (m,1H, Ar-H), 7.14 – 6.98 (m,2H, Ar-H), 6.97 – 6.87 (m, 2H, Ar-H), 5.21 – 5.01 (m, 2H, $OCH_2$), 3.92 (s, 3H, $OCH_3$). $^{13}$C- DEP-135 (101 MHz, $CDCl_3$) δ 139.91: C3, 131.45, 131.34, 130.77, 130.53, 129.58, 129.04, 123.41, 121.32 , 113.70, 112.22, 55.53: C12.

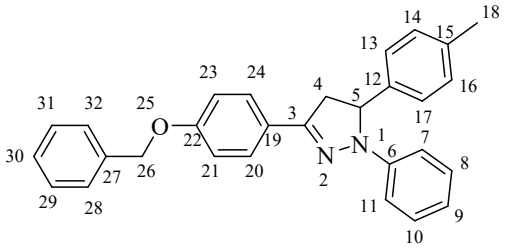



*Synthesis of new series of pyrazoline derivatives with phenylhydrazine* ***(3a-i)***

*Synthesis of 3-(4-(benzyloxy)phenyl)-4,5-dihydro-1,5-diphenyl-1H-pyrazole*

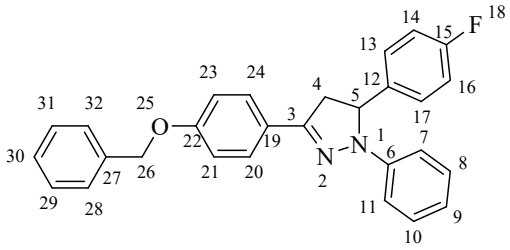
To a mixture of chalcone (0.0025 mol) and phenyl hydrazine (0.002 mol) in (20 mL) ethanol sodium hydroxide 0.5 mL NaOH (6%) was added. The reaction mixture was then refluxed for 4-5 h. After completion of reaction (monitored by TLC) the reaction mixture was distilled to remove the excess solvent, then it is poured into crushed ice. The solid obtained washed with water and recrystalised from ethanol [36, 37].

*3-(4-(Benzyloxy)phenyl)-5-(4-methylphenyl)-4,5-dihydro-1-phenyl-1H-pyrazole* ***(3a)***. $C_{29}H_{26}N_2O$; Mol. Wt.: 419: (95%), m.p. 192-193°C. $^1$H-NMR (400 MHz, chloroform-*d*) δ 7.46 – 7.29 (m, 8H Ar-H), 7.29 – 7.16 (m, 5H Ar-H), 7.05 (d, *J* = 7.8 Hz, 2H Ar-H), 7.01 (d, *J* = 8.0 Hz, 1H Ar-H), 6.90 (t, *J* = 7.5 Hz, 1H Ar-H), 6.79 (t, *J* = 7.3 Hz, 1H Ar-H), 5.64 (dd, *J* = 12.4, 6.9 Hz, 1H CH-Hx-C5), 5.18 (s, 2H $OCH_2$), 3.84 (dd, *J* = 17.1, 12.4 Hz, 1H $CH_2$-Hb-C4), 3.06 (dd, *J* = 17.1, 6.9 Hz, 1H $CH_2$-Ha-C4). $^{13}$C-NMR (101 MHz, $CDCl_3$) δ 154.90, 147.33, 144.85, 135.37, 133.94, 132.89, 130.39, 128.92, 128.90, 128.72, 128.66, 128.52, 128.50, 128.48, 127.08, 125.70, 121.61, 118.85, 113.13, 112.04, 69.44:C5, 42.08: C4.

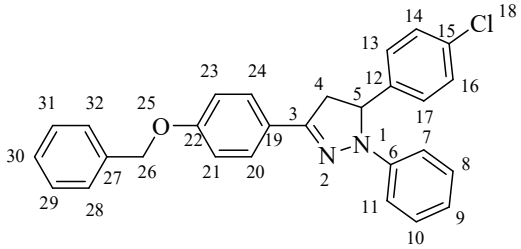



*3-(4-(Benzyloxy)phenyl)-5-(4-fluorophenyl)-4,5-dihydro-1-phenyl-1H-pyrazole* ***(3b)***. $C_{28}H_{23}FN_2O$; Mol. Wt.: 422: (90%), m.p. 168-169°C. $^1$H-NMR (400 MHz, chloroform-*d*) δ 7.88 (d, *J* = 8.6 Hz, 1H Ar-H), 7.65 (d, *J* = 8.6 Hz, 1H Ar-H), 7.48 – 7.35 (m, 6H Ar-H), 7.29 (s, 1H Ar-H), 7.23 – 7.16 (m, 3H Ar-H), 7.10 – 6.98 (m, 4H Ar-H), 6.98 – 6.72 (m, 2H Ar-H), 5.63 (dd,

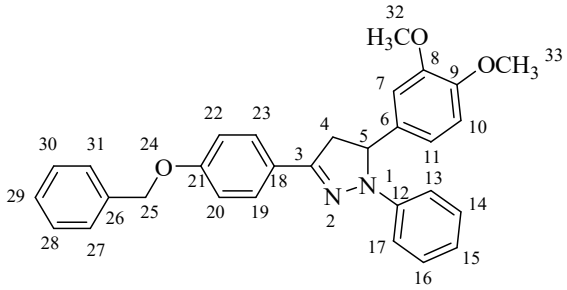
*J* = 12.5, 6.9 Hz, 1H CH-Hx-C5), 5.15 (s, 2H $OCH_2$), 3.86 – 3.78 (m, 1H $CH_2$-Hb-C4), 3.03 (dd, *J* = 17.1, 7.0 Hz, 1H $CH_2$-Ha-C4).



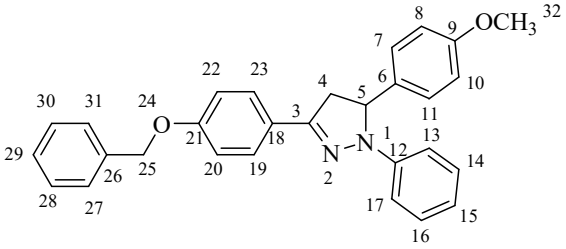
*3-(4-(Benzyloxy)phenyl)-5-(4-chlorophenyl)-4,5-dihydro-1-phenyl-1H-pyrazole* ***(3c)***. $C_{28}H_{23}ClN_2O$; Mol. Wt.: 439: (92%), m.p. 190-191°C. $^1$H-NMR (400 MHz, chloroform-*d*) δ 7.61 (d, *J* = 8.6 Hz, 2H Ar-H), 7.41 – 7.31 (m, 6H Ar-H), 7.23 – 7.16 (m, 5H Ar-H), 7.03 – 6.98 (m, 3H Ar-H), 6.88 (t, 1H Ar-H), 6.79 (t, 1H Ar-H), 5.62 (dd, *J* = 12.5, 7 Hz, 1H CH-Hx-C5), 5.15 (s, 2H $OCH_2$), 3.78 (dd, *J* = 12.5, 17 Hz, 1H $CH_2$-Hb-C4), 3.01 (dd, *J* = 17.1, 7.0 Hz, 1H $CH_2$-Ha-C4). $^{13}$C-NMR (101 MHz, $CDCl_3$) δ 154.91, 146.16, 144.58, 135.28, 134.12, 133.98, 131.42, 130.11, 128.96, 128.90, 128.76, 128.68, 128.64, 126.84, 121.59, 119.07, 113.15, 112.07, 69.45 :C5, 41.89: C4. $^{13}$C-DEPT NMR (101 MHz, $CDCl_3$) δ 128.96, 128.90, 128.76, 128.68, 128.64, 126.84, 121.59, 119.07, 113.15, 112.07, 77.25.



*3-(4-(Benzyloxy)phenyl)-4,5-dihydro-5-(3,4-dimethoxyphenyl)-1-phenyl-1H-pyrazole* ***(3e)***. $C_{30}H_{28}N_2O_3$; Mol. Wt.: 465: (87%), m.p. 178-180°C. $^{13}$C-NMR (101 MHz, $CDCl_3$) δ 154.91, 146.16, 144.58, 135.28, 134.12, 133.98, 131.42, 130.11, 128.96, 128.90, 128.76, 128.68, 128.64, 126.84, 121.59, 119.07, 113.15, 112.07, 69.45:C5, 41.89:C4. $^{13}$C–DEPT-NMR (101 MHz, $CDCl_3$) δ 128.92, 128.89, 128.70, 128.49, 127.03, 121.63, 119.03, 118.67, 113.02, 112.01, 110.50, 107.99, 77.25, 55.94.



*3-(4-(Benzyloxy)phenyl)-4,5-dihydro-5-(4-methoxyphenyl)-1-phenyl-1H-pyrazole* ***(3i)***. $C_{29}H_{26}N_2O_2$; Mol. Wt.: 435: (86%), m.p. 198-200 °C. $^1$H-NMR (400 MHz, chloroform-*d*) δ 7.87 – 7.76 (m, 2H, Ar-H), 7.47-7.59 (m, 2H, Ar-H), 7.38 (t, 2H, Ar-H), 7.32 – 7.28 (m, 4H, Ar-H), 7.14 – 6.77(m, 8H, Ar-H), 5.15 (dd, *J* = 12.8, 7 Hz, 1H CH-Hx-C5), 4.73 (s, 2H $OCH_2$), 3.89 (dd, 1H $CH_2$-Hb-C4),3.86 (s, 3H $OCH_3$), 3.3 (dd 1H $CH_2$-Ha-C4). $^{13}$C-NMR (101 MHz, $CDCl_3$) 159.47, 155.55, 151.52, 141.08, 133.05, 131.53, 130.40, 128.55, 128.09, 127.78, 127.48, 127.03, 126.53, 123.41, 121.18, 114.01, 113.06, 106.14, 82.54, 69.31, 55.34. $^{13}$C-dept NMR (101 MHz, $CDCl_3$) δ 130.40, 128.94, 128.09, 127.78, 127.48, 127.02, 126.53, 123.41, 121.18, 114.01, 113.06, 106.13, 82.54, 77.25, 69.31, 55.34.




*Antibacterial activity*

The anti-bacterial activity of some synthesized pyrazolines was conducted against *Escherichia coli (E. coli)* ATCC (11229) as a Gram-negative bacterium and *Staphylococcus aureus* ATCC (25923) as a Gram-positive bacterium. The well diffusion method was utilized to evaluate the antimicrobial activity. To prepare the bacterial inoculum, it was adjusted to a concentration of 1.5 × $10^8$ colony-forming units per millilitre (CFU/mL) using a standard McFarland (0.5) turbidity standard [38, 39].

A mother solution (2000 µg/mL) of synthesized compounds were prepared in dimethyl sulfoxide (DMSO). Serial dilutions of this solution were then made to achieve concentrations of (1000, 800, 600, 400, and 200 µg/mL). Using a sterilized metal cork borer, wells with an 8 mm diameter were created in agar plates. Following this, 300 µL of each dilution was added to the wells. The plates were incubated aerobically at 37 °C for 24 hours. After incubation, antimicrobial activity was assessed by measuring the diameter of the inhibition zones around the wells, recorded in millimetres [40].

*Docking study*

The docking study of synthesized pyrazoline derivatives (**3a-i**) was performed using the molecular operating environment (MOE-Dock 2019.0102) software. The chemDraw Professional (2021) was used to draw the structure of synthesized compounds and saved as .mol files. To prepare these compounds for docking, their structures underwent energy minimization using the MOE program with the Amber10: EHT force field. For the docking studies, the 3D crystallographic structure of DNA gyrase subunit B (PDB ID: 1KZN) was obtained from the Protein Data Bank and used as the target model. The active site was identified using MOE's Site Finder based on a coumarin-based inhibitor, and MOE Dock was employed to dock the ligands into this site [41]. The docking results were assessed by calculating the interaction scores between the ligands and the active site, and the top five ligand conformers with the highest scores were selected. Validation of the docking procedure was accomplished by re-docking the ligand into the active site, which showed identical binding interactions with the co-crystallized ligand, with a root mean square deviation (RMSD) of less than 2 [42].

## CONCLUSIONS

The Claisen-Schmidt condensation reaction between *p*-benzyloxy acetophenone (**1**) and substituted benzaldehydes was successfully conducted to synthesize chalcones, which were subsequently transformed into pyrazolines. Moreover, the antibacterial potential of newly developed compounds was studied, using both in silico and in *vitro* approaches. The integrated results from these studies suggest that these novel compounds exhibit significant antibacterial activity. Computational models indicate that the synthesized compounds can effectively interact with bacterial enzymes or receptors, disrupting their function and thereby inhibiting bacterial growth. Further studies, including in *vivo* evaluations and toxicity assessments, are needed to fully understand their efficacy, safety, and potential for therapeutic.

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*Conflict of interests*

The authors declare that they have no conflicts of interest.

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