

# Synthesis, DFT, Molecular docking Analysis and Antibacterial, Antioxidant Activities of tri-substituted pyrazoles

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Pyrazole and its derivatives are contemplated crucial compounds in heterocyclic chemistry which are also used extensively in organic synthesis. These cycles are known for their biological and pharmacological activities. The present investigation is in the interest of some synthesized derivatives containing the pyrazole moieties. (5-Hydroxy-3,5-dimethyl-4,5-dihydro-pyrazol-1-yl)-pyridin-4-yl-methanone (**1**) and Furan-2-yl-(5-hydroxy-3,5-dimethyl-4,5-dihydro-pyrazol-1-yl)-methanone (**2**) were synthesized by cyclocondensation of the 1,3-dicarbonyl with the hydrazine derivative with a simple and rapid approach to obtain substituted pyrazole. All structures of these compounds were elucidated by spectral (<sup>1</sup>H NMR and <sup>13</sup>C NMR) analysis. The antibacterial activity of the synthesized compounds was screened against two Gram-positive and Gram-negative bacteria, and all of them displayed moderate activity. The radical scavenging activity of these compounds were evaluated using 1,1-diphenyl-2-picrylhydrazyl (DPPH), the synthetic compounds showed moderate antioxidant activities. In addition, the results obtained from antibacterial activity were further explained with the help of DFT and molecular orbital calculations with a basis set 6-311+G (d, p). The synthesized compounds were docked with 6RKV enzymes with the use molecular docking tools and the docking results are explained all interactions amino acid residue of enzyme and compounds.

**Keywords:** Pyrazole; biological activities; DFT; NMR; molecular docking

## I. INTRODUCTION

Pharmaceutical research on the natural products represents a major strategy for the discovery and the development of new substances with therapeutic interest. The pyrazole or pyrazoline nucleus has many biological activities and medical properties such as antibacterial (Nimbalkar & Hote, 2015), sedative-hypnotic, antifungal (Karrouchi *et al.*, 2018), anticonvulsant, antiviral, antiparasitic (Shah *et al.*, 2011) anti-tubercular and insecticidal (Flores *et al.*, 2014). 1,3,5-

Trisubstituted pyrazoles constitute a number of drugs including sildenafil, celecoxib, rimonabant and difenamizole (Khan *et al.*, 2016). In view of the interest in the activity of pyrazole and its derivatives, and in continuation of our research on the synthesis of new compounds of biological and pharmacological interest, we interested herein the preparation, biological activity, DFT and molecular docking studies of tri-substituted pyrazoline. It is also known in the literature that the synthesis of 1,3,5-trisubstituted pyrazoles are synthesized by cyclocondensation of hydrazines with

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diketones in acidic medium (Venkateswarlu *et al.*, 2018). here we use the method described in reference (Arfan *et al.*, 2009) we use silica gel and to synthesize our products (**1**) and (**2**). all synthesized compounds have studied for antimicrobial activities against selected bacterial as strains by the agar well diffusion method. Free radical scavenging activity has been investigated by using DPPH method. all the synthesized compounds, (**1**) and (**2**) exhibited moderate antibacterial and antioxidant activities.

## II. MATERIALS AND METHODS

### A. Solvents and Materials

All solvents and chemicals were used as received and without any purification, then FT-IR spectra were performed on JASCO FT/IR-4100. <sup>1</sup>H-NMR and <sup>13</sup>C-NMR spectra were recorded in deuteron MD<sub>3</sub>OD or CDCl<sub>3</sub> on a Fourier ARX 400 spectrometer at 400 MHz for proton and 100 MHz for Carbon 13. Chemical shifts (δ) are given in ppm and *J* values in Hertz (Hz). Thin-layer chromatography (TLC) was carried out on precoated Merck silica gel 60 F<sub>254</sub>. Melting points were determined on an Electro thermal capillary fine control apparatus which are uncorrected.

## III. RESULT AND DISCUSSION

### B. Chemistry

The synthesis of method is depicted in (Figure 1). Title compounds are synthesized using the previously reported method (Arfan *et al.*, 2009). It is known in the work of the literature that the condensation reaction of acetylacetone and hydrazine derivatives in ethanol and catalyzed by acetic acid directly give the corresponding pyrazole ring, but when the reaction medium is changed by the addition of silica gel, the majority products (**1**) and (**2**) are obtained; we can explain that this method is stopped at the alcohol function and does not pass to the stage of elimination of water. Here examining the pharmacological profile of the hydroxyl group, we synthesize the pyrazole substituted by the hydroxyl function and also study their biological properties, named antibacterial and antioxidant activities.

### A.1. Synthesis of (5-Hydroxy-3,5-dimethyl-4,5-dihydro-pyrazol-1-yl)-pyridin-4-yl-methanone (**1**)

White powder, 70% yield, m.p. 128 –130°C, IR (KBr): ν 3229 (OH), 2928 (CH), 1626 (C=O), 1546 (C=N) cm<sup>-1</sup>. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ = 1.96 (s, 3H, CH<sub>3</sub>), 2.02 (s, 3H, CH<sub>3</sub>), 3.04 (dd, 2H, CH<sub>2</sub>), 7.87 (s, 2H, pyridine), 8.81 (s, 2H, pyridine). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): δ = 166.04, 156.51, 149.70, 141.74, 123.35, 92.81, 51.12, 26.71, 16.18. Anal. Calcd for C<sub>11</sub>H<sub>13</sub>N<sub>3</sub>O<sub>2</sub>: C, 70.26; H, 5.98; N, 19.17; O, 14.60.

### A.2. Synthesis of Furan-2-yl-(5-hydroxy-3,5-dimethyl-4,5-dihydro-pyrazol-1-yl)-methanone (**2**)

Yellow powder, 68% yield, m. p. 112 –115°C, IR (KBr): ν 3340 (OH), 2989 (CH), 1636 (C=O), 1545 (C=N) cm<sup>-1</sup>. <sup>1</sup>H NMR (400 MHz, MD<sub>3</sub>OD): δ = 2.09 (s, 3H, CH<sub>3</sub>), 2.24 (s, 3H, CH<sub>3</sub>), 3.00-3.04 (dd, 2H, CH<sub>2</sub>), 6.78 (s, 1H, furan), 7.89 (s, 1H, furan), 8.12 (s, 1H, furan). <sup>13</sup>C NMR (100 MHz, MD<sub>3</sub>OD): δ = 156.31, 152.63, 149.19, 145.44, 124.63, 113.11, 90.80, 51.10, 24.01, 14.60. Anal. Calcd for C<sub>10</sub>H<sub>12</sub>N<sub>2</sub>O<sub>3</sub>: C, 57.68; H, 5.81; N, 13.45; O, 23.05.

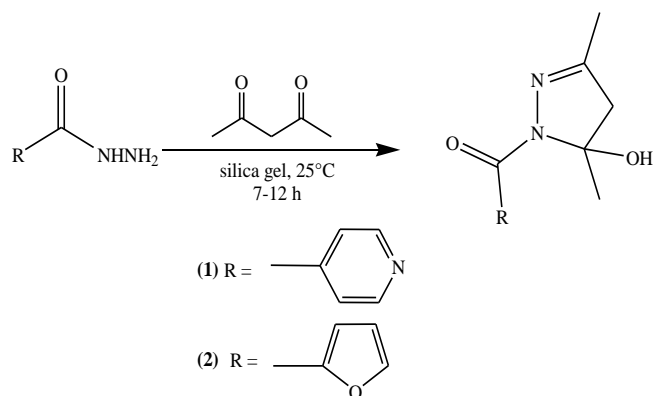


Figure 1. Synthesis of compounds (**1**) and (**2**)

### C. Antibacterial activity

The title compounds were tested against three standard bacterial strains for the determination of their antibacterial activity, against a variety of bacterial strain such as *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923 and *Pseudomonas aeruginosa* ATCC 27853 using dimethyl sulfoxide (DMSO) as a solvent. The agar well diffusion method was used to determine antimicrobial activity (Celikel & Kavas, 2009). Similarly, the impregnated disks were placed on the medium suitably spaced apart and the plates were then incubated at 5°C for 1 hour to permit good diffusion. After that, they were transferred to an

incubator at 37°C for 24 hours for bacteria. The antibacterial activity was evaluated by measuring the inhibition zone diameter observed. Standard drugs like Ampicillin, Gentamycin were also used for comparison purpose.

Compounds (1) and (2) reported here were evaluated for their antibacterial activities against *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Zone inhibition values were measured in millimeters. data on the antibacterial activities of the title compounds are given in (Table 1). The values of these inhibitory zones reveal that the compounds exhibit moderate antibacterial activities against all microbial strains at concentrations of 50 and 100 µg/mL, respectively. The biological activity of these compounds results from the presence of the pyrazole, pyridine and furan rings which play a very important role in elucidating the mechanism of transformation reaction in biological systems. The DMSO control showed no antimicrobial activity against the bacterial strains tested, while the test compound was found to be active.

Table 1. Antibacterial activity of compound (1) in an agar diffusion test

	Compound (1)		Compound (2)		AMP 10 µg/disc
	100 µg/ml	50 µg/ml	100 µg/ml	50 µg/ml	
<i>E.coli</i>	11,42 ± 0,64	9,00 ± 1,12	13,01 ± 0,98	12,25 ± 1,22	28
<i>S.aureus</i>	10,78 ± 1,58	8,57 ± 1,08	12,28 ± 1,52	11,33 ± 2,08	14
<i>P.aurengenosa</i>	10,69 ± 1,02	8,33 ± 1,52	12,66 ± 1,52	11,48 ± 1,52	19

Zone of inhibition in mm: < 10: weak; > 10: moderate; >16: Significant  
 AMP: Ampicillin  
 GEN: Gentamycin  
 Values are the average of three replicates.

#### D. Antioxidant activity

The antioxidant potential of any compound can be determined on the basis of its scavenging activity of the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical as described by (Leitao *et al.*, 2002). Stock solutions of samples were prepared by dissolving 10 mg of synthesized samples in 10 ml of methanol to give a concentration of 1mg/ml. Then prepared sample concentrations of 5, 15, 25, 30, 40 and 50 µg/ml. The methanol solution of DPPH (20 mg/l) was prepared daily. The mixtures were made by adding 100 µl of test sample to 900 µl of DPPH solution. Ascorbic acid was used as standard (0-30 µg/ml). These solution mixtures were

kept in dark for 30 min and optical density was measured at 517 nm using the spectrophotometer. The optical density was recorded and % of inhibition was calculated using the formula given below:

$$\text{Percent (\%)} \text{ inhibition of DPPH activity} = 100 \times (A - B)/A$$

Where A = optical density of the blank and B = optical density of the sample

The IC<sub>50</sub> value was calculated by linear regression of plots where the abscissa represented the concentration of the title compounds and compared with the standard reference compound ascorbic acid. The statistical analyze were performed by a Microsoft Excel 2007 and all the numerical results were expressed graphically.

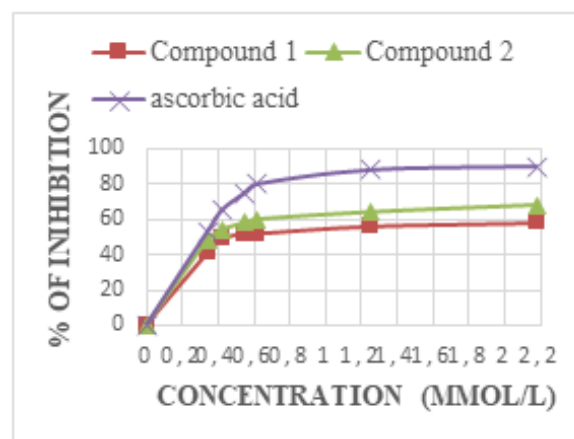


Figure 2. Antioxidant activity results using DPPH

The obtained results indicated that synthesized compounds (1) and (2) showed a good antioxidant activity with value of IC<sub>50</sub> 56.10 and 59.16 µg/ml. These values were nearly close to standard ascorbic acid with IC<sub>50</sub> value 37.10 µg/ml from regression equation in (Figures 2). all compounds indicating the excellent scavenging activity because the presence of the hydroxyl group in the structures may be responsible for trapping the free radical DPPH.

#### E. Computational study

All quantum chemical studies were performed with the Gaussian package 09 (Frisch *et al.*, 2003). Gauss View 5.08 (Dennington *et al.*, 2008) has been used for the visualization of the structure and The optimized geometry and electronic structure of the title compounds in the gas phase were carried out using the Density Functional Theory (DFT) with hybrid functional B3LYP, based on Becke's three-parameter

functional, including Hartree-Fock exchange contribution with a nonlocal correction for the exchange potential proposed by Becke together with the nonlocal correction for the correlation energy provided by (Lee *et al.*, 1988). with the basic set 6-311+G (p, d) (Gonzalez *et al.*, 1990). The values of HOMO, LUMO energies and global reactivity such as chemical potential ( $\mu$ ), hardness ( $\eta$ ), were calculated using Koopman's theorem was determinate (Zhan *et al.*, 2003) to relate the HOMO and LUMO energies to the IP and EA, respectively:

$$IP = -E_{\text{HOMO}}, \text{ and } EA = -E_{\text{LUMO}}$$

The electronegativity ( $\chi$ ), defined by (Mulliken *et al.*, 1934) as the average of (IP) and (EA):

$$\chi = (IP + EA) / 2$$

Chemical hardness is a useful concept to understand the reaction of chemical systems and is associated with the stability and reactivity of a chemical system (Pearson *et al.*, 1985). Chemical hardness can be calculated as follows:  $\eta = (IP - EA) / 2$ . The softness of a molecule is calculated by:  $S = 1/2\eta$ . Electronic energy calculation values of title compounds are summarized in (Table 2).

Table 2. Theoretical results of compounds (1) and (2)

Compound	HOMO (eV)	LUMO (eV)	Energy gap (eV)	$\mu$ (D)	$\chi$ (eV)	$\eta$ (eV)	$S$ (eV)
(1)	-6.35	-1.96	4.39	6.51	4.15	2.19	0.23
(2)	-6.40	-2.95	3.45	4.24	4.76	1.73	0.29

According to the electrostatic results illustrated in (Table 2) have been explained with respect to the experimental biological activity of the compounds synthesized. The compound (2) shown a LUMO energy of -2.95 eV is biologically active compared to the compound (1) of -1.96 eV. The values of biological activity between the two compounds can be explained by the difference in LUMO energy levels. LUMO energy represents the ability to accept an electron that is related to electron affinity (Gece, 2008). The difference energy between HOMO and LUMO characterizes their chemical stability. Farther, energy is used to estimate the limiting electron density by predicting the most reactive position in  $\pi$ -electron systems. It is also used to explain several types of reactions in conjugate systems (Choi & Kertesz, 1997). It has been observed that compounds with

higher LUMO orbitals have remarkable biological activity. This difference in energy compared to the LUMO orbitals can be explained by the furan and pyridine heterocycles. the oxygen atom is more electronegativity than the nitrogen atom therefore the furan ring reactivates than pyridine which clearly indicates that the electronegativity plays an important role in the stability of LUMO orbitals which has an influence on the biological activity. In addition, quantum studies have shown that the energy values of HOMO can be an indicator of antioxidant activity, of which we note in (Table 2) the values of the HOMO orbitals between the two compounds (1) and (2) very close to one of the other which shows that they have good antioxidant activity with  $IC_{50}$  between 56.10 and 59.16  $\mu\text{g/ml}$ . which also proves the influence of the substituents on the pyrazole ring mainly the hydroxyl group.

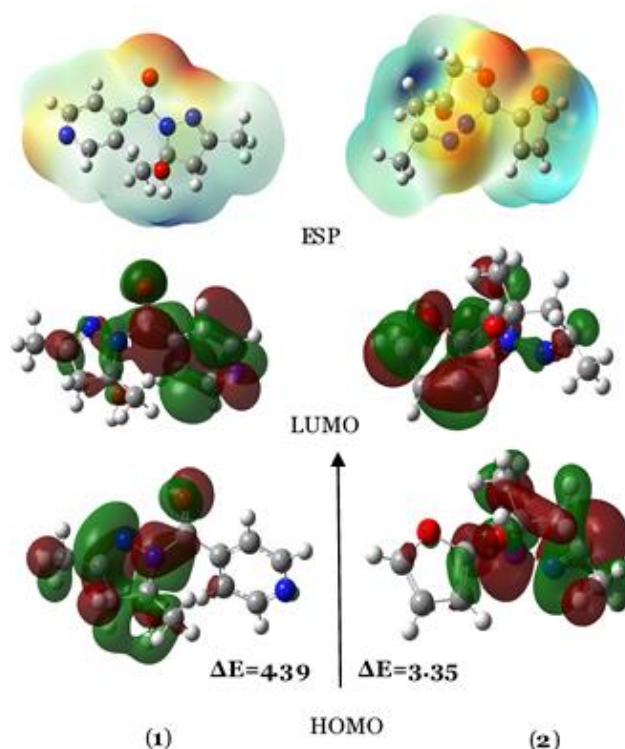


Figure 3. Band gap energy and Molecular electrostatic potential for compounds (1) and (2)

### F. Molecular docking

The three-dimensional (3D) structure of DNA gyrase from *E. Coli* is a special enzyme used in homeostatic control of DNA and the target for antibacterial compounds was obtained from the Protein Data Bank (PDB ID: 6RKV) (Vande Broeck *et al.*, 2019). All molecular structures were sketched in Chem-Draw ultra-version 17.1 (2017) and were submitted to

Chem3D Ultra Visualizing program to obtain standard 3D structure in (pdb) format. The receptor and ligand files were docked using AutoDock 4.2 along with Auto Dock Tools was used to perform docking simulation. Discovery Studio Visualizer 2017 was employed to analyze the interactions of the studied compounds and to the enzyme.

Docking studies are computational techniques to explore possible modes of ligand and receptor (enzyme) binding, the results of the analysis suggest that the compounds we used for docking exhibited interactions between the hydrogen bonds with target protein. Among these compounds, compound (**1**) exhibited good inhibition activity against the protein 6RKV is indicated by its lower binding energy value -5.29 kcal/mol and hi forms one hydrogen bond, the residue involved in the interaction with compound is with Met 111. we also notice that the oxygen of furan ring in the compound (**2**) is produced hydrogen bonds interaction with Val 268 and Hhe 96, respectively, but the N atom of the pyridine fraction did not make any hydrogen bond which shows that our explanation by the theoretical DFT method is really correct, the oxygen atom more electronegative than nitrogen therefore the antibacterial activity increases in molecules that contain several electronegative atoms. The docking conformation of more active compound (**2**) with furan ring and pyrazole substituted by hydroxyl showed a good interaction as well as a good docking score (-6.07 kcal/mol). However, in the present study, the molecular docking of the synthesized compounds was carried out to study their binding pattern with DNA gyrase and compare them with a standard inhibitor (Ampicillin) which exhibited moderate inhibitory activity against the 6RKV protein is indicated by its lower binding energy value of 4.82 kcal/mol. The docking results are shown in (Table 3).

Table 3. Calculated binding affinity values between proteins 6RKV and ligands

Compound	Free Energy of Binding Kcal/mol	Inhibition constant at 298.15 K ( $\mu\text{M}$ )	H-bonds	Interaction residues of 6RKV	Hydrophobic interactions
( <b>1</b> )	-5.29	132.02	1	Met 101	Phe 513, Tyr 100, Ile130, Ala 128,
( <b>2</b> )	-6.07	35.32	3	Val 268, Phe 96, Gln 276	Gln 276, Try 266, Phe 96, Ile 112, Ile 264
Ampicillin	-4.82	290.62	4	Asp 36, Arg 46, Asp157	Pro 43, Arg 47, Asp 157

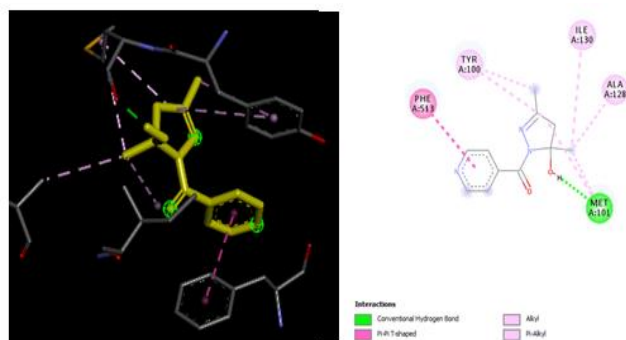


Figure 4. 3D and 2D docking pose showing interaction for compound (**1**) in the binding site of DNA gyrase (PDB ID: 6RKV)

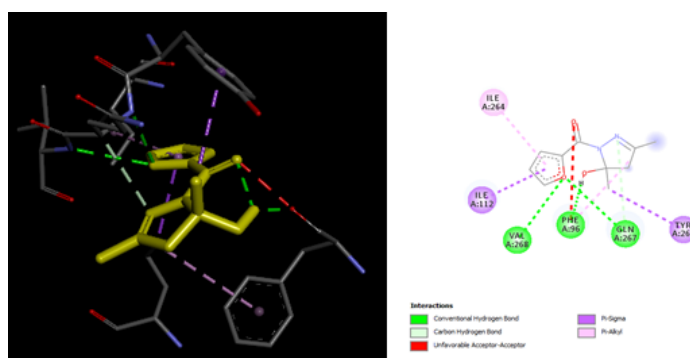


Figure 5. 3D and 2D docking pose showing interaction for compound (**2**) in the binding site of DNA gyrase (PDB ID: 6RKV)

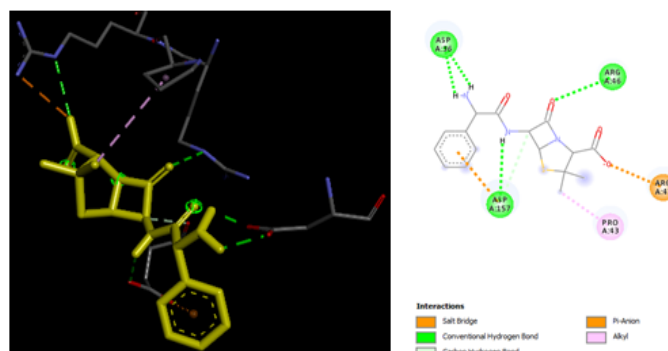


Figure 6. 3D and 2D docking pose showing interaction for Ampicillin in the binding site of DNA gyrase (PDB ID: 6RKV)

#### IV. CONCLUSION

In this study, the synthesis of two derivatives pyrazole trisubstituted was performed and all these compounds synthesized showed promising moderate antibacterial and antioxidant activity. Density functional theory DFT/ B<sub>3</sub>LYP with the basic set 6-311+ G (p, d). Calculations were carried out to study molecular structures, vibrational spectra, HOMO-LUMO and photophysical properties, and we tried to find a relation between the biological activities results and the theoretical data. Docking results revealed that compound (**2**) had least binding energy against the 6RKV receptor with a

binding energy value of – 6.07 kcal/mol. in silico, the results indicate that molecular docking studies were related with experimental results of antibacterial activity.

#### V. ACKNOWLEDGEMENT

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