Synthesis, Molecular Docking Studies and Evaluation of Antimicrobial Activities of Pyrazoline Derivatives

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ABSTRACT

The novel compounds substituted phenyl -3-methyl -1,4,5- triphenyl -1, 3a, 4, 5 tetrahydrapyrazolo [3-4c] pyrazole (IVa-IVe)have been prepared substituted benzylidene pyrazolone (IIIa-IIIe) reacts with phenyl hydrazine substituted 3(furan-2-yl)-1,5 diphenyl-1H-pyrazol *(Va-Ve)* have been prepared by reaction of 3-(furan-2-yl)-1,5-diphenyl-4,5-dihydro-1H-pyrazole and DDQ in DMF. The structural identification of the compounds was based on IR, 1H NMR and Mass spectral data. All the synthesized compounds have been screened for their antimicrobial activity and antifungal activities. The antimicrobial activities of the synthesized compounds have been compared with the standard drug, Streptomycin. Purity of synthesized compounds have been checked by TLC. A molecular docking study of the synthesized derivatives was also examined. The in-silico interaction results aligned with the in vitro analysis of the synthesized compounds and demonstrated well against Ciprofloxacin *(*1KZN 2XCT 6A09*)*. Synthesized compounds Vd with – 8.3 kcal/mol exhibited good binding scores.

Key Word: Chalcone, Pyrazoline, DDQ, Antimicrobial activity, Molecular Docking.

INTRODUCTION

Heterocyclic compounds play a vital role in the advancement of organic synthesis and find a wide range of applications in the field of medicinal chemistry. The ring system containing nitrogen as a hetero atom has an important role in many biological processes and therapeutic agents. Heterocyclic compounds play an important role in designing new classes of structural entities of medicinal importance with potential new mechanisms of action. Heterocyclic compounds play significant roles in the drug discovery process[1-3,] and analysis of drugs in late development or on the market shows that 68% of them are heterocycles. Recent research works in synthetic chemistry have suggested that pyrazoline derivatives possess significant biological activity such anticonvulsant[4], antidepressant[5], anti-hypertensive[6], anti-inflammatory[7-8], analgesic [9], anticancer [10-14], antimicrobial[15-19], antimycobacterial[20], antiamoebic[21-22], antimalarial[23] activities. Nitrogen-containing heterocyclic compounds[24] like pyrazolines have received considerable attention in recent years due to their biological activity like anti-inflammatory[25] , anticonvulsant[26]. The synthesised 2-pyrazoline-based derivatives directly through the reaction of phenylhydrazines and substituted chalcones, and the proposed reaction has been accepted as a simple and convenient procedure for the preparation of 2-pyrazolines[27]. In the early decades of the 20th century, they made major contributions to expand this field and prepared different substituted 2-pyrazolines. They investigated the stability of hydrazone intermediates and pyrazoline formation in reactions composed of various aromatic and aliphatic α,β-unsaturated aldehydes, and ketones with hydrazine derivatives[28].On the other hand, microwave-assisted organic reactions have emerged as a new Lead in organic synthesis with important advantages like a highly accelerated rate of reaction along with improvement in yield and quality of product. Thus, keeping in view the advantages of these techniques and the immense biological importance of pyrazolines, it was felt worthwhile to study the reaction under microwave irradiation and to screen the target compounds for antimicrobial activity.

The presence of two nitrogen atoms within their structure, along with their varied chemical configurations, leads to a wide range of potential biological activities, allowing for significant diversification and pharmacological activities. Pyrazoline is a stable molecule with immense importance and is well known for its various biological

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activities. Electrophilic substitution reaction occurs preferentially at carbon-4 due to the higher electron density gained from the two nitrogen atoms [29]. The pyrazoline nucleus occurs in several natural compounds and pharmacologically active substances displaying a broad range of biological activity.

MATERIALS AND METHODS:

Melting points were determined in open capillary tubes and are reported uncorrected. All reagents and solvents used were of laboratory grade. Reaction progress was monitored on pre-coated TLC plates, and spots were visualised under a UV lamp. The IR spectra were recorded on a PERKIN ELMER spectrometer in the frequency range 4000 – 400 cm-1 in Nujol mull and as KBr pellets. 1H NMR spectra were recorded on a Bruker 400 MHz NMR spectrometer using TMS as an internal standard. Reactions were carried out in a domestic microwave oven at 180 watts.

Experimental protocols:

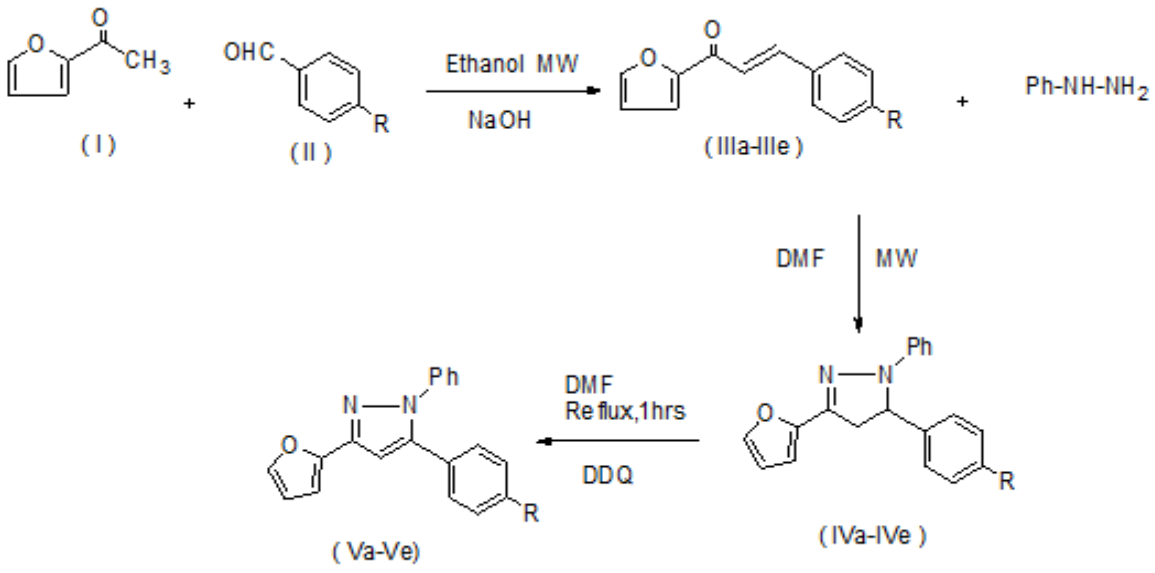
Firstly, 1-phenyl-3-methyl-5-pyrazolone. Then this 1-phenyl-3-methyl-5-pyrazolone (0.05 mole) and substituted benzaldehyde (0.05 mole) were taken in a conical flask with glacial acetic acid, and sodium acetate was added to the reaction mixture. The reaction mixture was placed in a microwave oven for 1-2 min at 180 W, then cooled in a refrigerator overnight. The product obtained was filtered, washed with water and recrystallized from ethanol. Then these substituted benzylidene pyrazolone (IIIa-IIIe) react with phenyl hydrazine in a microwave oven for 3 min at 180 watt gives different substituted fused pyrazoline.

Synthesis of substituted phenyl -3-methyl -1,4,5- triphenyl -1, 3a, 4, 5 tetrahydrapyrazolo [3-4c] pyrazole (IVa-IVe).

A mixture of substituted benzylidene pyrazalone (0.025 mole) (IIIa-IIIe) reacts with phenyl hydrazine (0.025 mole) in a microwave oven for 1 to 3 min at 180 watts. After cooling, the solution was poured into crushed ice, and the product obtained was filtered & recrystallized using ethanol, yielding the pure compound (IVa-IVe).

Synthesis of substituted 3(furan-2-yl)-1,5 diphenyl-1H-pyrazol (Va-Ve).

A mixture of substituted benzylidene pyrazolone (0. 025) (IIIa-IIIe) reacts with phenyl hydrazine (0. 025) in microwave oven for 1 to 3 min at 180 watts. After cooling the solution was poured in to crushed ice and the product obtained was filtered & recrystallized using ethanol.



Scheme 1. Synthesis of pyrazoline derivatives IVa-IVe and Pyrazol derivatives Va-Ve.

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Table 1 : Physical data of synthesized Pyrazoline compounds

Sr. No.	Compound	R	Reaction time (min)	Molecular Formula	Molecular weight	Yield	M. Pt.
1	IV a	-H	1.51	$C_{19}H_{16}N_2O$	288	60	175°C
2	IV b	-Cl	2.35	$C_{18}H_{15}N_2OCl$	322.5	61.24	190°C
3	IV c	-Br	2.56	$C_{19}H_{15}N_2OBr$	366	65	185°C
4	IV d	$-NO_2$	1.35	$C_{18}H_{15}N_3O_3$	333	53	210°C
5	IV e	$-CH_3$	1.56	$C_{20}H_{18}N_2O$	302	50	180°C

Table 2: Physical data of synthesized Pyrazole compounds

Sr. No.	Compound	R	Molecular Formula	Molecular weight	HRMS (*m/z*) $[M]^{+\bullet}$	HRMS (m/z) Calculated $[M+H]^+$	Yield (%)	M. Pt. (°C)
1	Va	-H	$C_{24}H_{17}N_3SO$	395	395	396	54	150
2	Vb	-Cl	$C_{24}H_{16}N_3OCl$	397.5	397	398	72	170
3	Vc	-Br	$C_{24}H_{16}N_3OBr$	473.9	441	442	68	175
4	Vd	$-NO_2$	$C_{24}H_{16}N_4SO_3$	339	440	441	71	180
5	Ve	$-CH_3$	$C_{25}H_{19}N_3SO_2$	413	425	426	62	170

Spectral characterization :

3-(furan-2yl)-1,5-diphenyl-4,5-dihydro-1H-pyrazole (IVa):

m.pt : 175°C ; δ (ppm); δ 7.75(CH,S,of 2-furan),5.19(CH,t ,of methine) 3.90 (CH2,d,methylene), 6.83(CH,S,of 1-benzene ,1-N-C),7.40(CH,S,of 1-benzene 1-C) ;C-H str. 3029, C=C str. 1563, -C-O-C Str. 1251, C-O-C Str. 1035, C=N Str. 1599, C-N Str. 1163; 288 Molecular ion , 77$C_6H_5^+$, 211 ($C_{13}H_{11}ON_2^{\cdot+}$)

5(4-chlorophenyl)-3-(furan-2-yl)-1-phenyl-4,5-dihydro-1H-pyrazol (IVb).

m.pt : 190°C ; δ (ppm); δ 7.75(CH,S,of 2-furan),5.19(CH,t ,of methine) 3.90 (CH2,d,methylene), 7.44(CH,of-C-Cl),6.83(CH,S,1-benzene,1-N-C);C-H str. 3113, C=C str. 1510, -C-O-C Str. (asym) 1224, C-O-C Str. (sym) 1023, C=N Str. 1590, C-N Str. 1168, C-Cl 759;322 Molecular ion peak, 111 $C_6H_5Cl^{\cdot+}$, 255 $C_{15}H_{12}N_2Cl^{\cdot+}$

5(4-bromophenyl)-3-(furan-2-yl)-1-phenyl-4,5-dihydro-1H-pyrazol (IVc).

m.pt : 185°C ; δ (ppm); δ 7.75(CH,S,of 2-furan),5.19(CH,t ,of methine),3.90(CH_2, d, methylene), 7.92(CH,of –C-Br), 6.83(CH,S,1-benzene,1-N-C) ;C-H str. 3050, C=C str 1563, -C-O-C Str. (asym) 1254 , C-O-C Str. (sym) 1071, C=N Str. 1596, C-N Str 1154, C-Br 751;366 Molecular ion peak, 77 $C_6H_5^{\cdot+}$, 299 $C_{15}H_{12}N_2Br^{\cdot+}$

3-(furan-2-yl)-5-(4-nitrophenyl)-1-phenyl-4,5-dihydro-1H-pyrazol (IVd).

m.pt : 210°C; δ (ppm); δ 7.75(CH,S,of 2-furan),5.19(CH,t ,of methine) , 3.90 (CH2,d,methylene), 6.83(CH,S,1-benzene,1-NC),8.21(CH,of 1-benzene ,1-N=O);C-H str. 3060, C=C str 1499, -C-O-C Str. (asym) 1253 , C-O-

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C Str. (sym) 1057, C=N Str.1596 ,C-N Str 1165, NO2 1499 (asym), 1336 (sym);333 Molecular ion peak, 211 $C_{13}H_{11}N_2O^{\cdot+}$

3-(furan-2-yl)-1-phenyl-5-*p*-tolyl-4,5-dihydro-1H-pyrazole (IVe).

m.pt : 180°C; δ (ppm); δ 7.75(CH,S,of 2-furan),5.19(CH,t ,of methine),3.90(C H2, d, methylene), 7.12(CH,of –C-O), 6.83(CH,of 1-benzene,1-NC).2.35(for –CH3); C-H str. 3051, C=C str 1563, -C-O-C Str. (asym) 1253 , C-O-C Str. (sym) 1068, C=N Str. 1597,C-N Str 1153;316 molecular ion peak, 77 $C_6H_5^{\cdot+}$,299 $C_{15}H_{12}N_2^{\cdot+}$.

3(furan-2-yl)-1,5 diphenyl-1H-pyrazol (Va).

δ (ppm); δ 6.44 – 6.42 (CH,S,of 2-furyl), 5.16 (CH,t ,of methine), 3.92 (CH2,d,methylene), 7.88 – 6.85 (CH,of 1-benzene);C-H str. 3056, C=C str 1499, -C-O-C Str. (asym)1223, C-O-C Str. (sym) 1072, C=N Str. 1597 ,C-N Str 1167;316 molecular ion peak, 77 $C_6H_5^{\cdot+}$,299 $C_{15}H_{12}N_2^{\cdot+}$.

5(4-chlorophenyl)-3-(furan-2-yl)-1-phenyl-1H-pyrazol (Vb).

δ (ppm); δ 6.92 (CH,S,of 2-furyl), 3.11 (CH,t ,of methine), 3.92 (CH2,d,methylene), , 8.34 – 7.72 (CH,of 1-benzene);C-H str. 3077, C=C str 1513, -C-O-C Str. (asym) 1223, C-O-C Str. (sym) 1024, C=N Str. 1597 ,C-N Str 1167 , C-Cl str 759;320 molecular ion peak, 243 $C_{13}H_8N_2OCl^{\cdot+}$.

5(4-bromophenyl)-3-(furan-2-yl)-1-phenyl-1H-pyrazol(Vc).

δ (ppm); δ 7.95 – 7.00 (CH,S,of 2-furyl), 2.96 (CH,t ,of methine), 6.13 (_-H,S, 1-pyrazole), 8.34 – 7.72 (CH,of 1-benzene);C-H str. 3061, C=C str 1462, -C-O-C Str. (asym) 1073, C-O-C Str. (sym) 1226, C=N Str. 1596 ,C-N Str 1156 , C-Br str 763;364 molecular ion peak, 77 $C_6H_5^{\cdot+}$,287 $C_{13}H_8N_2OBr^{\cdot+}$.

5(4-Nitrophenyl)-3-(furan-2-yl)-1-phenyl-1H-pyrazol (Vd).

δ (ppm); δ 6.99 (CH,S,of 2-furyl), 4.22 (CH,t ,of methine), 6.73 (_-H,S, 1-pyrazole), 8.30 – 7.01 (CH,of 1-benzene);C-H str. 3062, C=C str 1516, -C-O-C Str. (asym) 1080, C-O-C Str. (sym) 1289, C=N Str. 1597 ,C-N Str 1179 , $-NO_2$ (asym) 1515, $-NO_2$ (sym) 1342;331molecular ion peak, 122 $C_6H_4NO_2^{\cdot+}$,209 $C_{13}H_9N_2O^{\cdot+}$.

3-(furan-2-yl)-1-phenyl-5-*p*-tolyl=1H-pyrazol (Ve).

δ (ppm); δ 6.99 (CH,S,of 2-furyl), 4.22 (CH,t ,of methine), 6.73 (_-H,S, 1-pyrazole), 8.30 – 7.01 (CH,of 1-benzene);C-H str. 3056, C=C str 1499, -C-O-C Str. (asym) 1073, C-O-C Str. (sym) 1229, C=N Str. 1597 ,C-N Str 1183 , 2865($-CH_3$, q,Methyl);331molecular ion peak, 122 $C_6H_4NO_2^{\cdot+}$,209 $C_{13}H_9N_2O^{\cdot+}$.

Synthesis and Computational Analysis of Ligands

The 2D structures of the synthesized compounds IVa and IVe were generated in .mol format using ChemDraw 16.0 and then analyzed using the Gaussian 09 software suite. Density Functional Theory (DFT) calculations were performed at the B3LYP level of theory with the 6-31G(d,p) basis set. Full geometry optimizations were carried out to obtain the most stable, energy-minimized structures, and the corresponding 3D coordinates were used to determine the minimum-energy conformations.

Preparation of Macromolecular Structures

The atomic arrangement of receptor proteins complexed with ciprofloxacin (PDB IDs: 1KZN, 2XCT, and 6A09) exhibits a crystal-like configuration. Following standard global protocols, the protein structures were prepared by removing water molecules and cofactors. Prior to docking, any bound ligands were deleted to ensure accurate analysis. Polar hydrogens were then added using the automated protein preparation function in AutoDock 4.2.6, facilitated through MGL Tools 1.5.6, to generate properly formatted target protein files for subsequent molecular docking studies.

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Molecular Docking Analysis Using AutoDock Vina

Molecular docking was performed using AutoDock Vina, with setup facilitated through the graphical user interface of AutoDock Tools (ADT 1.5.6). The docking grid box was configured using AutoDock 4.2.6. Multiple docking pockets and ligand orientations were explored to identify the optimal grid configuration yielding the most reliable docking results. The AutoDock Vina algorithm was then used to predict the most favourable binding conformations between the target proteins and ligands.

Docking simulations were performed with ciprofloxacin-bound receptor proteins (1KZN, 2XCT, and 6A09) and the synthesised ligands IVa and IVe. A standard docking protocol was employed, generating up to nine distinct conformations per ligand. The conformers with the lowest binding free energies were selected for further analysis.

The docking grid was defined with dimensions of 20 × 20 × 20 points in the x-, y-, and z-directions, using a grid spacing of 0.375 Å. The grid box centres were set to 62 × 30 × 62 Å for 2XCT and 65 × 40 × 65 Å for 1KZN. The protein–ligand interactions of the selected docking poses were analyzed using Discovery Studio Visualizer and PyMOL. The results highlighted key binding pockets, hydrogen bonds, hydrophobic interactions, and electrostatic forces, represented through colour-coded ribbons, sticks, and lines for enhanced visualisation.

RESULTS AND DISCUSSION

Synthesis of pyrazoline derivatives IVa and IVe

To synthesise novel pyrazoline derivatives with antimicrobial properties by synthesizing pyrazoline derivatives using different reaction conditions. In the process, Chalcone (IIIa-IIIe) was synthesised from 1-phenyl-3-methyl-5-pyrazolone and substituted benzaldehyde through the Claisen-Schmidt condensation in glacial acetic acid. Sodium acetate was added to the reaction mixture. The reaction mixture was heated in the microwave oven for 1 min to 2 min at 180 watts and then allowed to cool in the refrigerator overnight. Immediately following the formation of *substituted* chalcone, Substituted Pyrazoline was synthesised from phenyl hydrazine and substituted benzylidene pyrazolone in a microwave oven for 1 to 3 min at 180 watts. After cooling the solution, it was poured over crushed ice, and the product was filtered and recrystallised using ethanol. The step-by-step process of creating pyrazoline derivatives is depicted in Figure 1. The process of determining the structure of the synthesised compounds was achieved through the use of NMR, IR and Mass spectroscopic techniques.

Table 3: Antimicrobial activity of 5-(4-substituted phenyl)-3-(furan-2-yl)-1-phenyl-4,5-dihydro-1H-pyrazole derivatives

Compound	**Zone of inhibition in mm**				
	Antibacterial			**Antifungal**	
	S. aureus	***E.coli***	***S.typhi***	***R. oryzae***	***P. notatum***
IVa	**Resistant**	**12 ± SD**	**12 ± SD**	**12 ± SD**	**10 ± SD**
IVb	**10 ± SD**	**12 ± SD**	**18 ± SD**	**20 ± SD**	**15 ± SD**
IVc	**Resistant**	**Resistant**	**Resistant**	**20 ± SD**	**10 ± SD**
IVd	**12 ± SD**	**15 ± SD**	**15 ± SD**	**Resistant**	**20 ± SD**
IVe	**12 ± SD**	**Resistant**	**10 ± SD**	**15 ± SD**	**Resistant**
Streptomycin	**24 ± SD**	**22 ± SD**	**18 ± SD**	**16 ± SD**	**18 ± SD**

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Antimicrobial activity

The compounds (IVa – IVe) synthesised were screened for their antimicrobial activity(Table 2). Compounds IVa, IVb and IVd were found to be slightly sensitive against the organism *S. aureus,* whereas compounds IVc and IVe were found to be resistant. Compounds IVa, IVb, and IVc were found to be slightly sensitive, while compounds IVd and IVe were found to be resistant against the organism *E. coli.* Compounds IVb and IVc were found to be resistant compounds. IVa, IVc, and IVd were found to be slightly sensitive, while compound IVb was found to be highly sensitive, and compound IVe was found to be resistant against the organism *S. typhi*. Compounds IVb and IVe were found to be highly sensitive towards *R. oryze.* Compounds IVc and IVd were found to be slightly sensitive against *R. oryzae,* and compound IVa was found to be resistant against the organism *Rhizopus oryzae.* Compound IVa was found to be highly sensitive against organism *P. notatum,* whereas compounds IVb, IVc, IVd, and IVe were found to be slightly sensitive against organism *P. notatum*. All synthesized compounds were compared with a standard drug (Streptomycin) (Table 2).The synthesised compounds have stronger activities when compared with the compounds reported in the literature, which differ in substituents and functional groups. These activities might be due to substituted functional groups.

In silico molecular docking

The most active compound Vd exhibited a docking best score of -8.3 kcal/mol with the target protein 1KZN, revealing multiple binding interactions (Figure 1). Residue ARG A:136 forms a vital hydrogen bond (6.28 Å) with hydrogen atoms (NH) of the carbonothioyl group of 5u. Additionally, GLU A:391 formed an attractive charge (4.31Å) interaction with the N-atom of the hydrazono group. Residue GLN A:392 was found connected with oxygen atom of the sulfonate group via H-bond (2.65Å). Further, residue TYR A:393 was found linked to S-atom of the sulfonate group and the NH of hydrazono group through pi-sulfur (5.52Å) and unfavourable donor-donor (2.64Å) interactions. Another residue, LEU A:250 developed crucial pi-linkages via pi-sigma (3.65Å) and pi-alkyl (7.88Å) interactions with pi-electrons of the naphthalene ring. Similarly, PRO A:234 induced two pi-alkyl (5.04 and 5.44Å) interactions with pi-electron density of the naphthalene ring. Further two interactions, including pi-alkyl (4.31Å) and unfavourable donor-donor (1.96Å) were produced by ARG A:36 with NH of the carbonothioyl group and the pi-electrons of the dichlorophenyl ring. And residue PRO A:277 shaped pi-alkyl (5.29Å) interaction with pi-electrons of the diethylamino-phenyl ring.

Table 4: Molecular docking results of novel synthesized compounds against *Ciprofloxacin* (1KZN 2XCT 6A09)

	Docking score (kcal/mol)			
	Bacterial targets			**Fungal target**
Comp ID	**1KZN (*E. Coli*)**	**2XCT (*S. aureus*)**	**6A09 (*S. typhi*)**	**4M8B (common fungal target)**
IVa	-7.3	-7.7	-6.3	-6.3
IVb	-7.5	-7.7	-6.6	-6.6
IVc	-6.6	-7.6	-6.5	-6.6
IVd	-7.8	-8.3	-7.0	-6.6
IVe	-6.7	-7.8	-6.5	-6.7
Va	-7.9	-9.0	-6.6	-7.3
Vb	-7.6	-8.2	-6.7	-7.1
Vc	-7.6	-8.3	-6.6	-7.1

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Vd	**-8.3**	**-9.1**	**-7.3**	-7.4
Ve	-7.8	-8.4	-6.7	**-7.5**
Ciprofloxacin	-7.3	-8.2	-6.4	-6.5

Table 5: Molecular docking results of novel synthesized compounds against *Ciprofloxacin* (1KZN 2XCT 6A09)

Target protein	**Most docked compds. ID**	**Docking score (kcal/mol)**	**Interactions: interacting residue (interaction type)**
1KZN	**IVd**	-7.8	ARG A:136 (hydrogen bond, 6.28 Å); GLU A:50 (pi-anion); ILE A:90, ILE A:78 (pi-alkyl, alkyl); THR A:165 (pi-sigma)
2XCT	**IVd**	-8.3	DG X:18 (conventional hydrogen bonding); DG X:17 (conventional hydrogen bonding); VAL:U 1268(pi-sigma)
6A09	**IVd**	-7.0	LYS B:79(conventional hydrogen bonding,2.15 Å);GLU C:138(pi-anion, 3.64 Å)
4M8B	**IV**	-6.7	GLY R:65(conventional hydrogen bonding,2.21 Å);ASP(conventional hydrogen bonding,2.27 Å); TYR R:81(pi-stacked,5.11 Å)

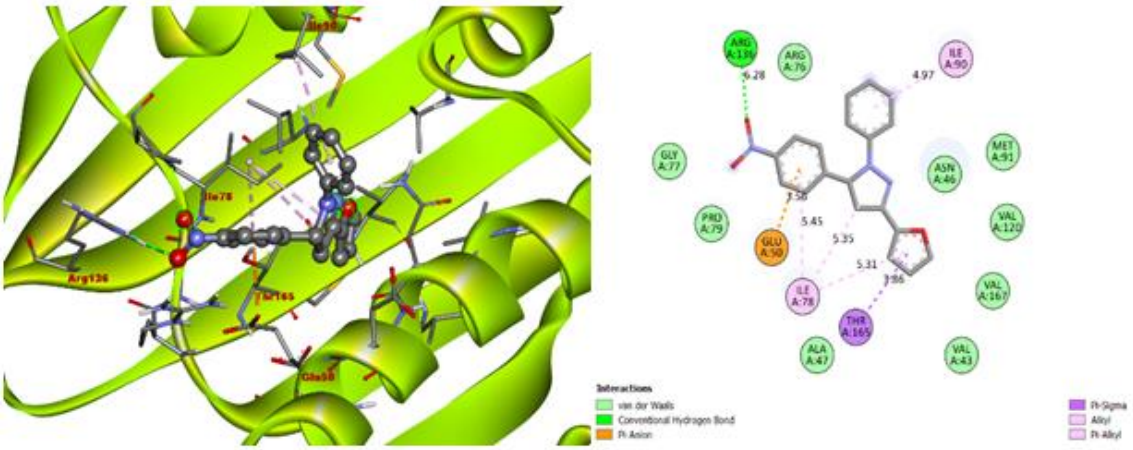

Fig. 1. The 3D and 2D binding interactions of compound IVd against *Ciprofloxacin (*1KZN 2XCT 6A09*)*.

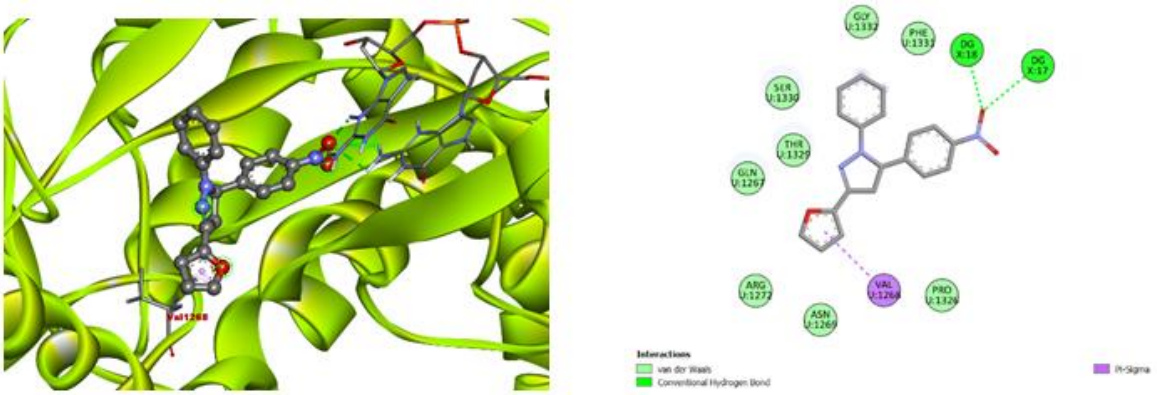

Fig. 2. The 3D and 2D binding interactions of compound IVd against *Ciprofloxacin (*1KZN 2XCT 6A09*)*.

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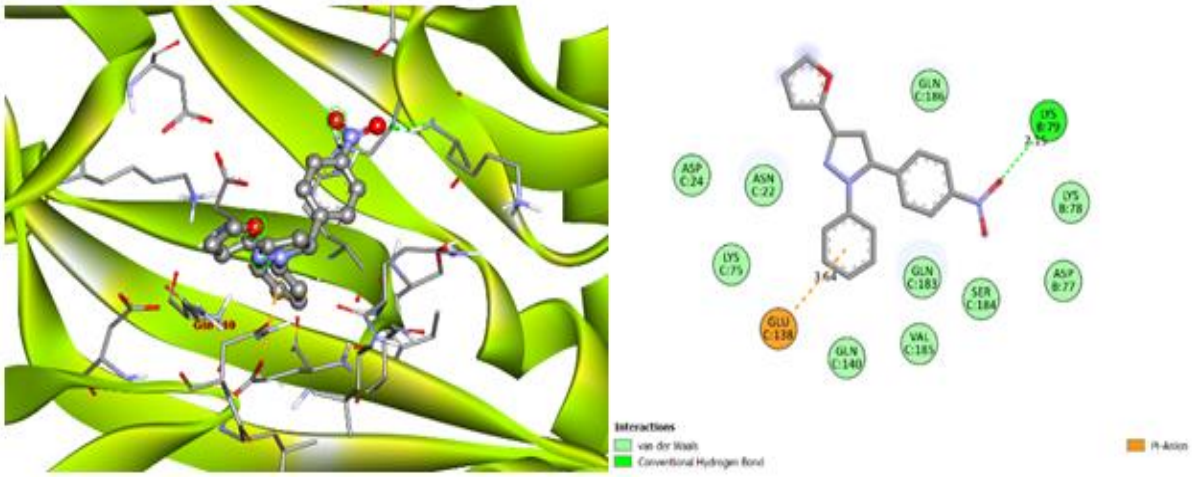

Fig. 3. The 3D and 2D binding interactions of compound IVd against *Ciprofloxacin (*1KZN 2XCT 6A09*)*.

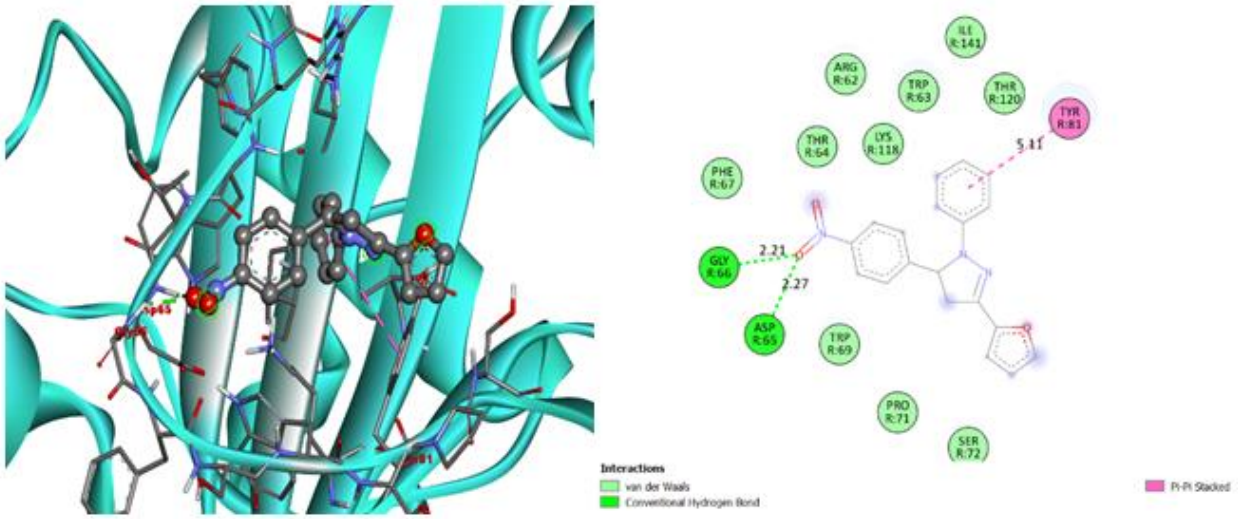

Fig. 4. The 3D and 2D binding interactions of compound IVe against *Ciprofloxacin (*1KZN 2XCT 6A09*)*.

Table 6: Molecular docking results of novel synthesized compounds against *Ciprofloxacin* (1KZN 2XCT 6A09)

Target protein	**Most docked comp. Id**	**Docking score (kcal/mol)**	**Interactions: interacting residue (interaction type)**
1KZN	**Vd**	-8.3	ARG A:136 (**hydrogen bond, 2.21 Å**); ASN:46 (**hydrogen bond, 2.48 Å**); GLU A:50 (pi-anion); ILE A:90, ILE A:78 (pi-alkyl); PRO A:79, ALA A:47 (pi- alkyl)
2XCT	**Vd**	-9.1	ASP U:437(**hydrogen bond, 2.18 Å**); SER U:438(**hydrogen bond, 2.04 Å**); ARG U:458 (pi-cation); DG X:9, DA Y: 13 (pi-pi Stacked)
6A09	**Vd**	-7.3	GLN B:140 (**hydrogen bond,2.41,2.55Å**); LYS B:75 (**hydrogen bond, 2.24 Å**); LYS C:78; LYS C:79, (pi- alkyl)
4M8B	**Ve**	-7.5	TYR R :81(**hydrogen bond, 2.76 Å**); HIS R:139 (pi-cation); THR R:120, LEU R:79 (pi-Sigma)

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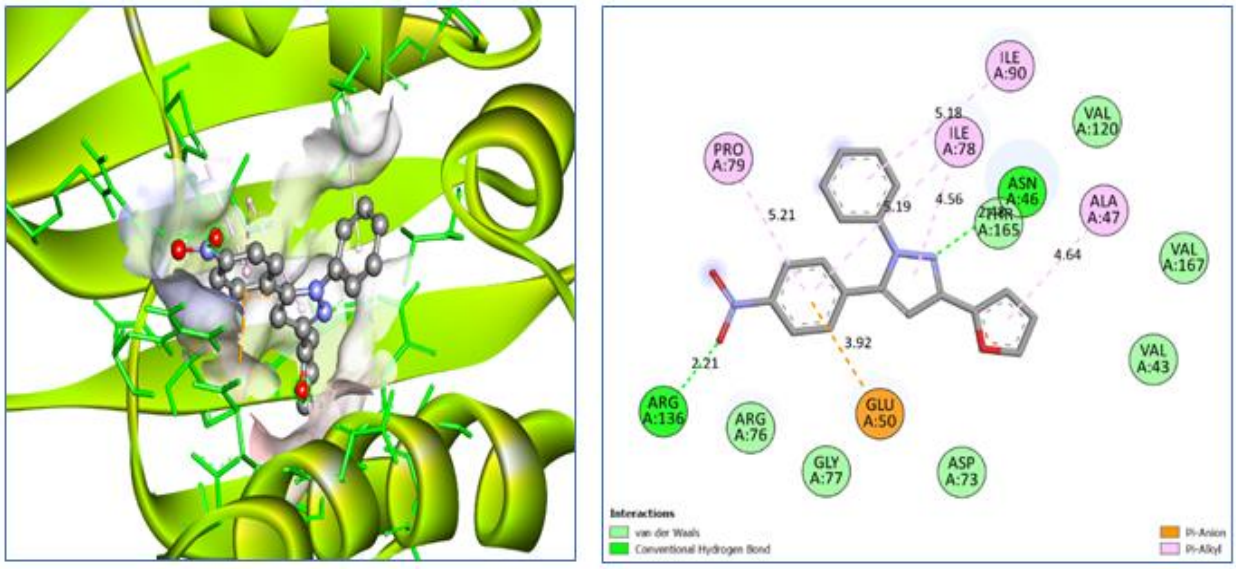

Fig. 5. The 3D and 2D binding interactions of compound Vd against *Ciprofloxacin (*1KZN 2XCT 6A09*)*.

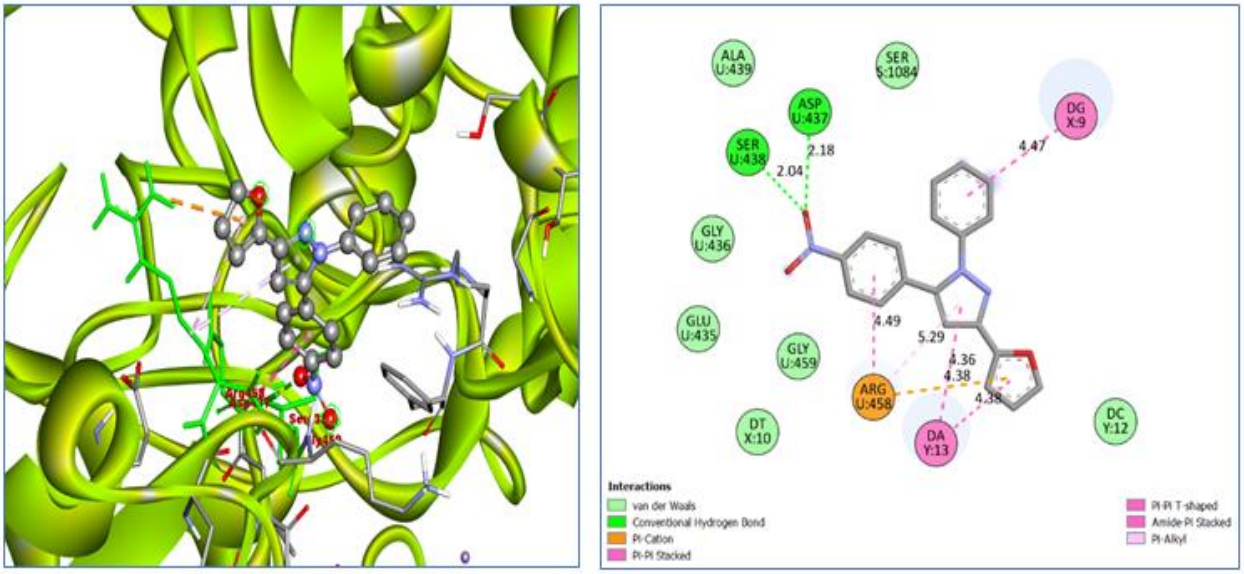

Fig. 6. The 3D and 2D binding interactions of compound Vd against *Ciprofloxacin (*1KZN 2XCT 6A09*)*.

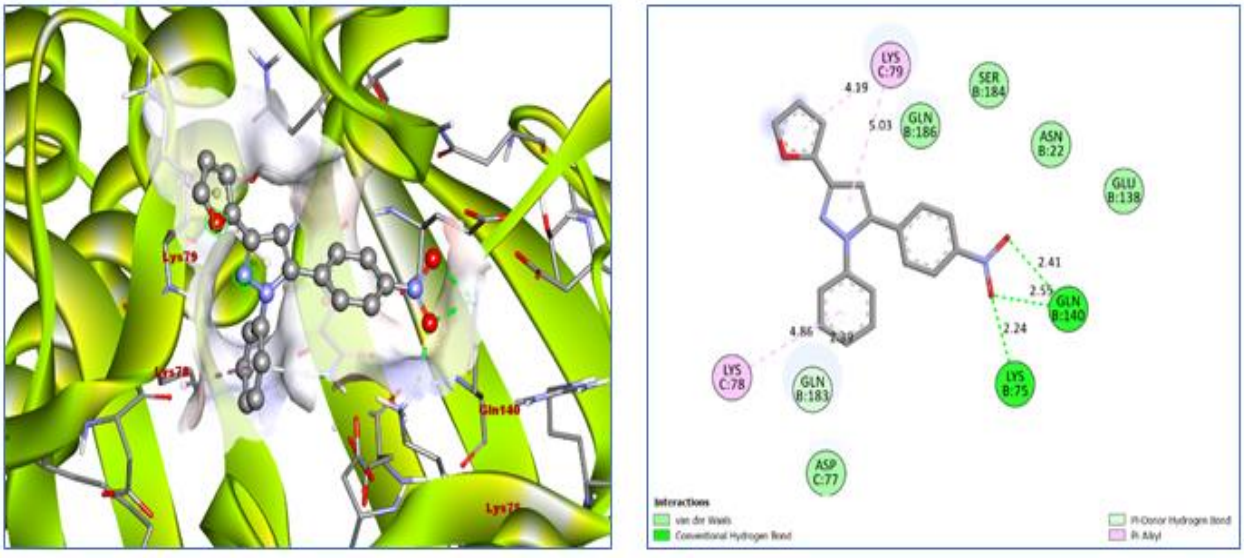

Fig. 7. The 3D and 2D binding interactions of compound Vd against *Ciprofloxacin (*1KZN 2XCT 6A09*)*.

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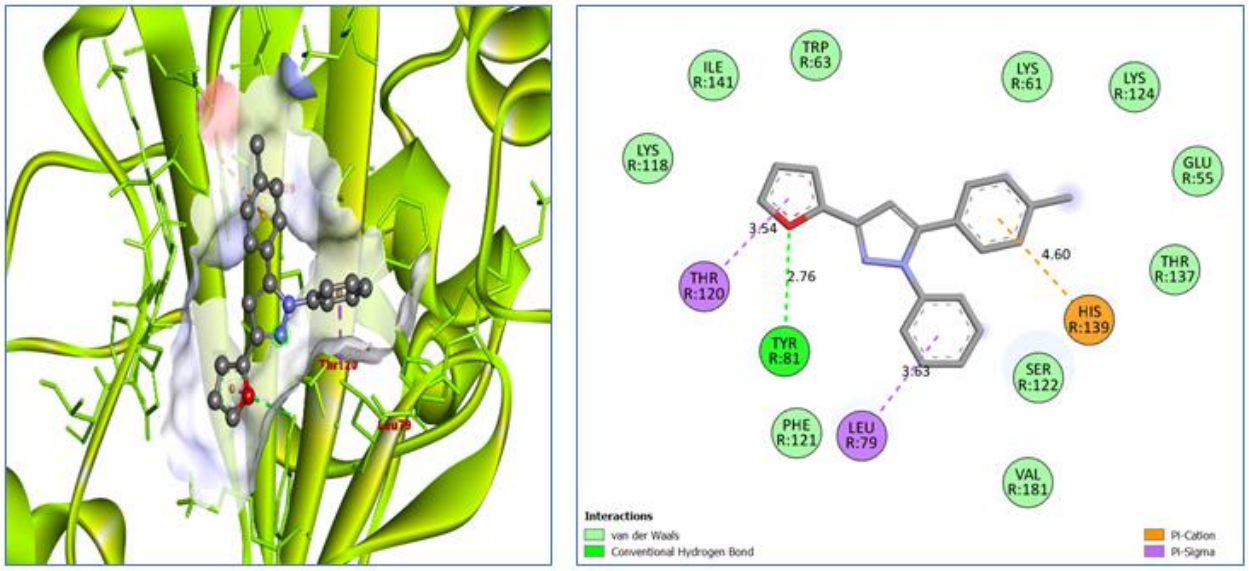

Fig. 8. The 3D and 2D binding interactions of compound Vd against *Ciprofloxacin (*1KZN 2XCT 6A09*)*.

***In silico* molecular docking study of novel synthesized compounds (IVa and IVe) against *Ciprofloxacin (*1KZN 2XCT 6A09*)*.**

The DNA gyrase, an enzyme belonging to the bacterial topoisomerase family, regulates the topology of DNA during transcription, replication, and recombination by inducing transient breaks in both DNA strands. In this regard, bacterial DNA gyrase is essential for bacterial survival and therefore necessary to be exploited as an antibacterial drug target. Therefore, in this study, the molecular docking analysis of the synthesised compounds was conducted to investigate their binding pattern with DNA gyrase and compare them with a standard inhibitor (ciprofloxacin) (Figs. 1-4). The synthesised compounds were found to have minimum binding energy ranging from − 7.4 to − 9.1 kcal/mol (Table 3), with a good result achieved with compound **IVd**(− 7.8 kcal/ mol). The binding affinity, H-bond, and residual interaction of two compounds and ciprofloxacin are summarised in Table 3. *In silico* molecular docking study of novel synthesised compounds against ***Ciprofloxacin*** (1KZN 2XCT 6A09). Therefore, in this study, the molecular docking analysis of the synthesised compounds was conducted to investigate their binding pattern with DNA myeloperoxidase II-α and compare them with a standard inhibitor (Figs. 1-4).

CONCLUSIONS

Synthesized compounds were characterized using melting point and spectroscopic techniques (1 H and 13C NMR). The *in vitro* antibacterial activities of synthesized compounds were evaluated against three bacterial strains: *S. aureus, S. typhi* and *E. coli* with the best inhibitory activity displayed by compounds IVd against *S. aureus.*Compound also showed promising antibacterial activity against *E. coli,* and *S. typhi* as well as compound IVb showed good activity against *S. typhi*. The synthesized compounds were evaluated for their *in-silico* molecular docking analysis using ciprofloxacin and found to have a minimum binding energy ranging from – 6.3 kcal/mol to – 8.3kcal/mol. Compound Vd showed very good binding score –8.3 kcal/mol with both of the proteins and had perfect alignment with *invitro* results. Binding scores against ciprofloxacin (1KZN 2XCT 6A09) are also very impressive for both synthesized compounds. The results of *in-silico* molecular docking study of the synthesized compounds have shown comparable residual interactions and better docking scores than scores compared to ciprofloxacin as well as all the docking results are in good agreement.

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