Synthesis, biological evaluation and molecular docking studies of novel pyrazole, pyrazoline clubbed pyridine as potential antimicrobial agents

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Abstract

We have prepared fifteen hybrid pyrazole, pyrazoline clubbed pyridine containing compounds **(5a-o)** and tested for their antibacterial and antifungal activities for the development of potential antimicrobial agents. The structures of this novel series were characterized by various spectral techniques like IR, ^{1}H NMR, ^{13}C NMR, LC-MS, and elemental analysis. The synthesized compounds **5d, 5e, 5i, 5k, 5m** and **5o** exhibited significant antimicrobial activity in the comparison of standard drugs. Molecular docking studies that have been carried out to emphasize the binding orientations of these molecules were in good compliance with crystal structure interactions. The predicted drug-likeness (ADME) properties were found to be in the acceptable range.

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Graphical Abstract

KEYWORDS

antimicrobial activity, molecular docking, pyrazole, pyrazoline, pyridine

1 | INTRODUCTION

An increase in antimicrobial resistance including antibiotic resistance is one of the major global threats.[1] The development of methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Staphylococcus aureus* (VRSA) have spread the resistance to the available antibiotics.[2] The development of multidrug-resistant (MDR) superbugs, extensively drug-resistant (XDR) and totally drug-resistant (TDR) strains have caused the need of developing new effective antimicrobials. Pyrazole, pyrazoline and pyridine heterocycles themselves possessed different biological activities and are parent heterocycles of various commercially available drugs. Pyrazole, first isolated from watermelon seeds is an active heterocycle used as an antimicrobial,[3] antiviral,[4] anti-tubercular,[5] anticancer,[6] analgesic and anti-inflammatory[7] agents. Pyrazoline is dihydropyrazole possessed anticancer,[8] anti-inflammatory[9] and antimalarial[10] activity, etc. Pyridine is also an important heterocycle in medicinal chemistry and is associated with several pharmaceutical activities.[11-18] Figure 1 represents the concept of drug design based on commercially available drugs isoniazid, fezolamine and dipyrone heterocycles.

It is well-known that the activity of a drug is attained through its interaction with the active site of the target protein. Molecular docking is one of the best methods to study intermolecular interactions of a protein-ligand complex. The preferred geometry and orientation of a ligand that interacts with protein were assessed employing the docking score in Schrodinger. The binding free energies were the ΔG for an ideal hydrogen bond, ionic, aromatic, or lipophilic interactions were calculated by using the following equation.

$$\Delta G_{bindMM\text{-}GB/SA} = \Delta E_{intramolecular} + \Delta G_{solvation} - T\Delta S_{conf} + E_{vdW} + E_{electrostatic} + E_{protein}$$

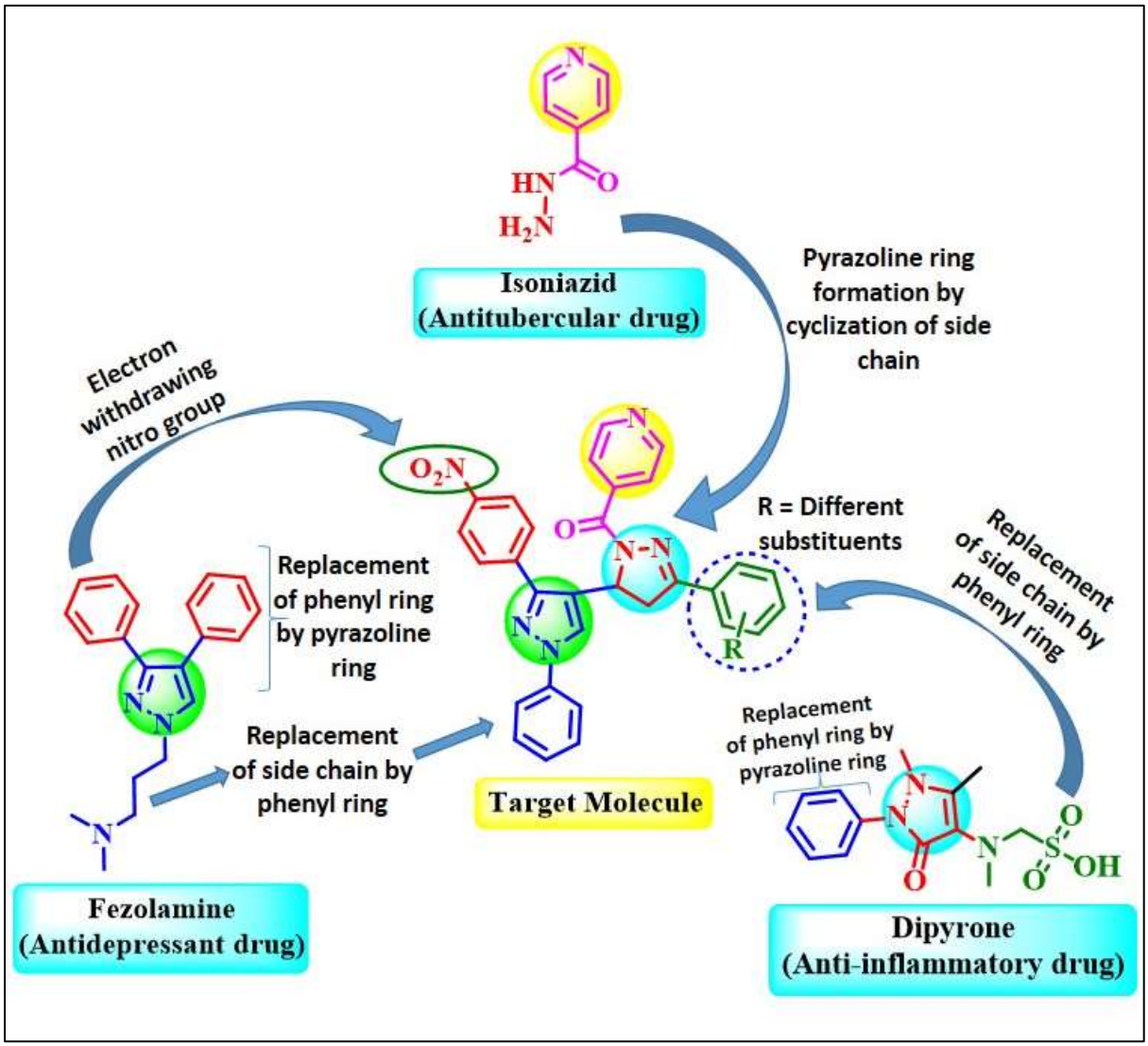
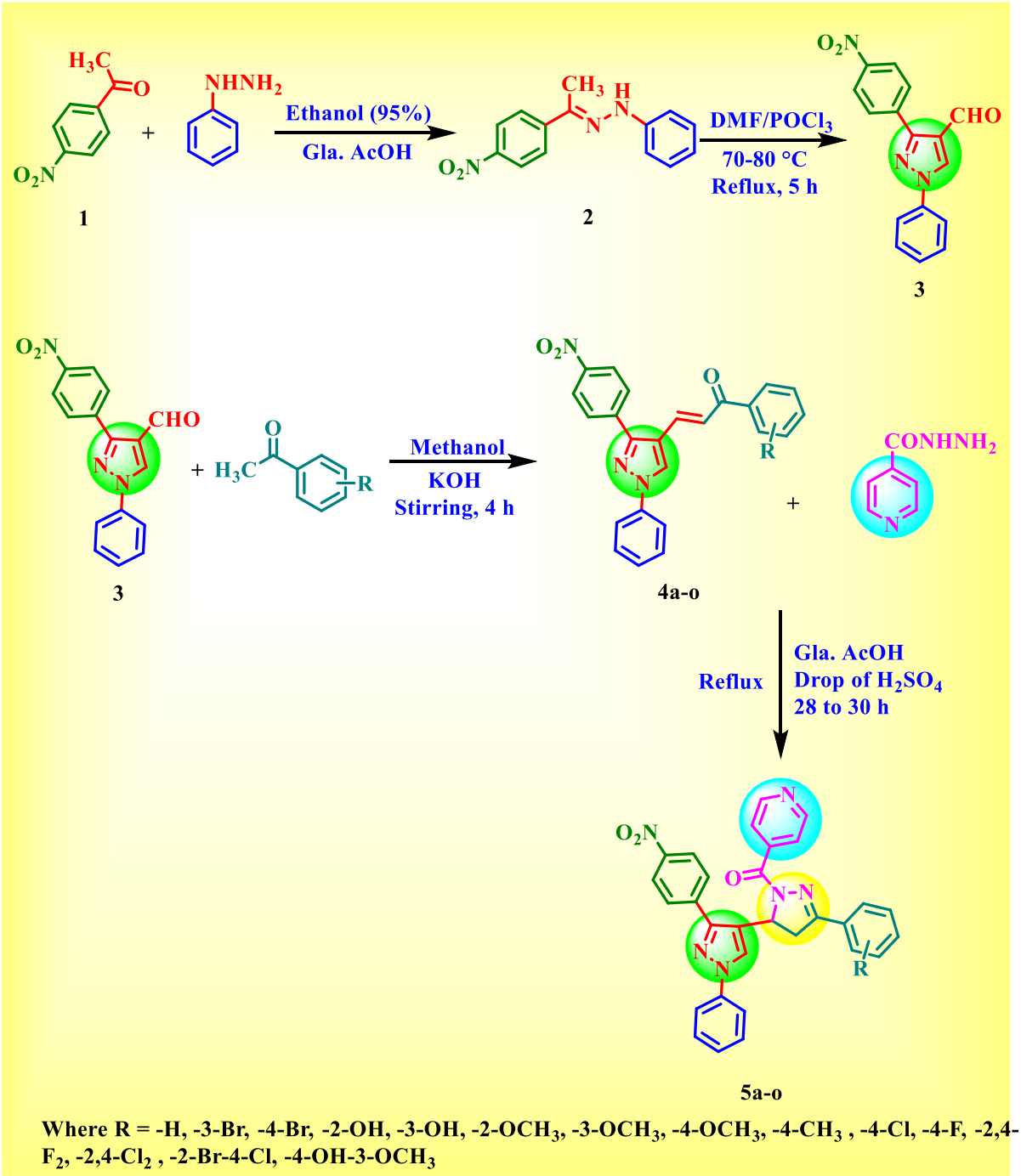


FIGURE 1 Concept of design based on the commercially available drugs containing pyrazole, pyrazoline and pyridine heterocycles



SCHEME 1 Synthetic pathway for compounds 5a-o

2 | RESULTS AND DISCUSSION

2.1 | Chemistry

The synthetic route for the titled compounds (3'-(4-nitrophenyl)-1'-phenyl-5-(aryl)-3,4-dihydro-1'*H*,2*H*-[3,4'-bipyrazol]-2-yl)(pyridin-4-yl)methanones **(5a-o)** was achieved as described in Scheme 1. The formation of chalcones **(4a-o)** was achieved by Aldol condensation of 3-(4-nitrophenyl)-1-phenyl-1*H*-pyrazole-4-carbaldehyde **(3)** and substituted

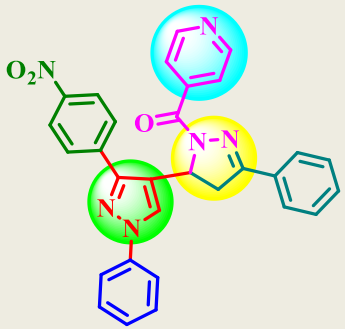
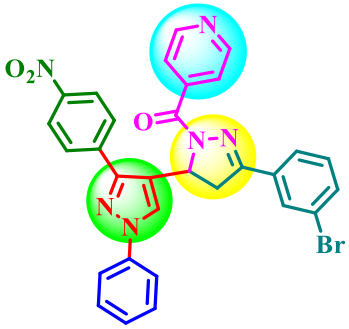
acetophenones. The reaction of these chalcones **(4a-o)** with isoniazid forms titled compounds (3'-(4-nitrophenyl)-1'-phenyl-5-(aryl)-3,4-dihydro-1'*H*,2*H*-[3,4'-bipyrazol]-2-yl)(pyridine-4-yl)methanones **(5a-o)**.

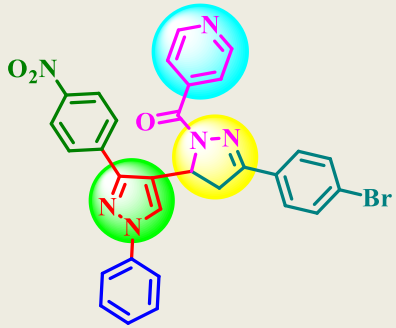
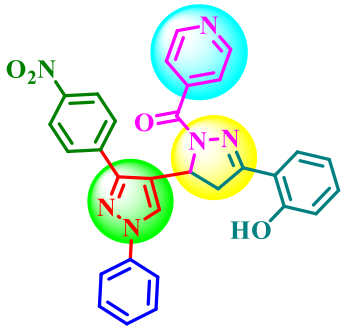
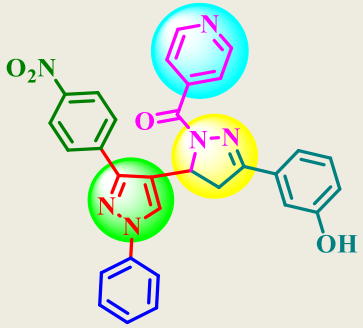
A series of (3'-(4-nitrophenyl)-1'-phenyl-5-(aryl)-3,4-dihydro-1'*H*,2*H*-[3,4'-bipyrazol]-2-yl)(pyridine-4-yl)methanones **(5a-o)** were synthesized and structures of these products were confirmed based on various spectroscopy analysis. In the IR spectra of compound **5k**, a characteristic absorption band at 1647 cm^{-1} represent the presence of >C=O group in the structure from isoniazid ring. Different vibrations at 3142, 3047 and 3244 cm^{-1} confirm the presence of aromatic ring by -C-H- stretching. Different vibrations at 1504 and 1598 cm^{-1} confirm the presence of heterocyclic rings by >C=C< and >C=N- stretching respectively. An absorption band appeared at 1448 cm^{-1} correspond to -C-N< stretching vibrations. The presence of fluoro and nitro groups were confirmed by -C-F- and –N=O stretching at 1072 and 1465 cm^{-1} respectively. The ^{1}H NMR spectra of compound **5k** represent the presence of twenty-one protons in the structure by signals at different values. Multiple signals at δ =7.40-8.90 ppm confirm the presence of a total of seventeen aromatic hydrogens of phenyl ring while a singlet peak observed at δ = 7.70 ppm was due to one proton of pyrazole ring. Two doublets at δ = 3.55 and δ = 3.90 ppm confirm the presence of two hydrogens at C_3 of pyrazoline ring while a singlet at δ = 6.12 ppm correspond to hydrogen at C_4 of pyrazoline ring. The ^{13}C NMR spectra of compound **5k** confirm structure by different peaks. A signal that appeared at δ = 167.2 ppm was due to carbon of the carbonyl group. Presence of pyrazole ring confirmed by different signals at δ = 117.3, 123.1 and 149.5 ppm while different chemical shifts at δ = 149.8, 140.4 and 121.7 ppm found due to carbons of the pyridine ring. The presence of pyrazoline ring was confirmed by signals at δ = 39.1, 61.2 and 151.2 ppm. In mass spectra of compound **5k**, *m/z* = 533.60 showed propinquity with proposed molecular weight. The characteristic spectra were given as supporting information.

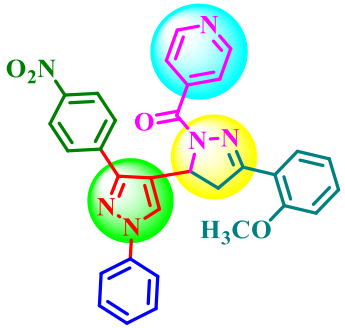
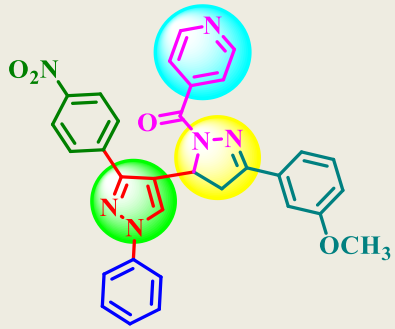
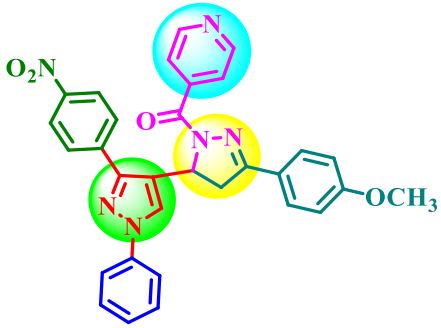
2.2 | Biological evaluation

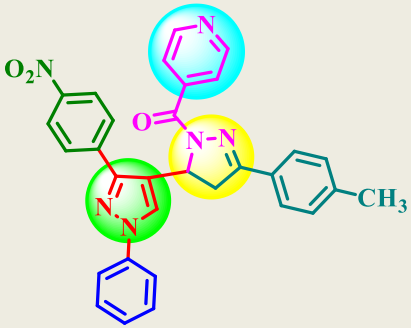
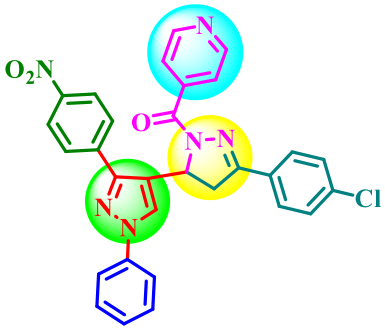
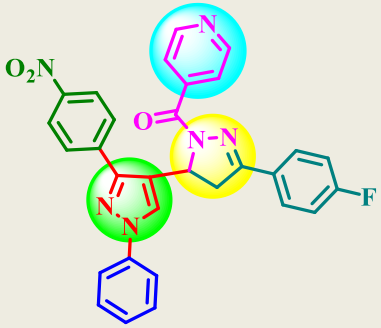
2.2.1 | Discussion on antimicrobial activity

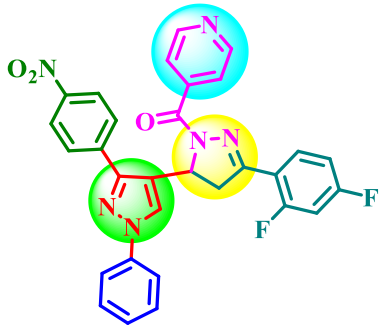
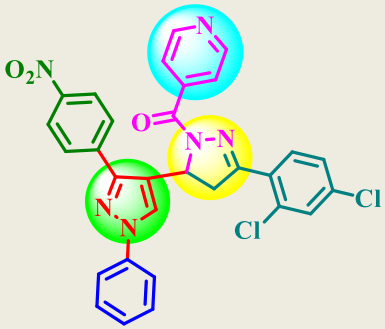
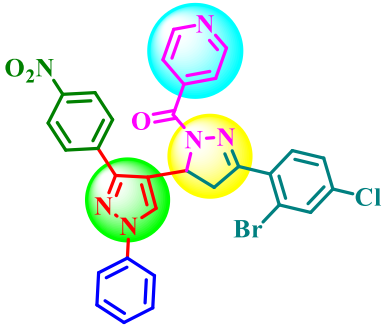
The results of the antimicrobial activity of synthesized compounds **(5a-o)** were represented in Table 1. Compound **5m** (2,4-dichloro) showed significant antibacterial activity against different bacterial strains. Compound **5m** (2,4-dichloro) showed excellent activity (MIC= 12.5 μg/mL) against *P. aeruginosa* and very good activity (MIC = 25 μg/mL) against *S. pyogenes.* Compounds **5e** (3-OH) and **5k** (4-F) displayed good activity (MIC = 50 μg/mL) against *S. aureus* and *E. coli* respectively. Compounds **5c** (4-Br) and **5n** (2-Br-4-Cl) were also active against *S. aureus* and *E. coli*, respectively with MIC = 62.5 μg/mL. The remaining derivatives of this series possess moderate antibacterial activity against tested Gram-positive and Gram-negative strains of bacteria. Ampicillin and chloramphenicol were used as standard drugs for antibacterial screening. Compounds **5i** (4-CH_3) and **5o** (4-OH-3-OCH_3) showed significant antifungal activity against different fungal strains. Compound **5i** (4-CH_3) showed excellent activity (MIC = 12.5 μg/mL) against *A. niger* while compound **5o** (4-OH-3-OCH_3) showed excellent activity (MIC = 12.5 μg/mL) against *C. albicans* and *A. clavatus*. Compound **5d** (2-OH) displayed good activity (MIC = 50 μg/mL) against *A. niger*. The remaining derivatives of this series possess moderate antifungal activity against tested fungal strains. Nystatin and griseofulvin were used as standard drugs for antifungal screening.

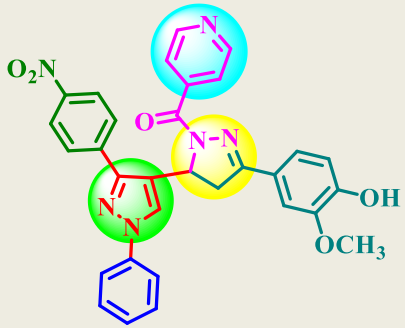
Entry	Pyrazole, pyrazoline clubbed pyridine derivatives-structural formula	Molecular Formula	Yield (%)	M. P. (ºC)	Minimum bactericidal concentrations (MBC) in µg/mL				Minimum fungicidal concentrations (MFC) in µg/mL		
					E. c.	*P. a.*	*S. a.*	*S. p.*	*C. a.*	*A. n.*	*A. c.*
5a		$C_{30}H_{22}N_6O_3$	68	159-161	100	500	500	500	250	>1000	>1000
5b		$C_{30}H_{21}BrN_6O_3$	75	145-148	125	100	100	250	1000	500	500

5c		$C_{30}H_{21}BrN_6O_3$	70	210-212	100	250	62.5	100	1000	>1000	>1000
5d		$C_{30}H_{22}N_6O_4$	68	170-172	250	500	100	125	500	50	>1000
5e		$C_{30}H_{22}N_6O_4$	65	202-204	100	500	50	500	100	>1000	>1000

5f		$C_{31}H_{24}N_6O_4$	65	191-193	125	250	125	500	100	1000	1000
5g		$C_{31}H_{24}N_6O_4$	66	177-179	100	250	500	500	>1000	>1000	100
5h		$C_{31}H_{24}N_6O_4$	69	152-154	100	500	500	250	500	1000	1000

5i		$C_{31}H_{24}N_6O_3$	71	207-209	100	500	250	250	>1000	12.5	>1000
5j		$C_{30}H_{21}ClN_6O_3$	75	124-126	500	500	100	200	500	500	500
5k		$C_{30}H_{21}FN_6O_3$	73	130-132	50	500	200	200	500	500	500

5l		$C_{30}H_{20}F_2N_6O_3$	68	191-193	100	200	250	250	200	1000	500
5m		$C_{30}H_{20}Cl_2N_6O_3$	69	204-206	250	12.5	500	25	250	500	500
5n		$C_{30}H_{20}BrClN_6O_3$	70	212-214	62.5	100	500	500	1000	1000	1000

5o		$C_{31}H_{24}N_6O_5$	71	198-200	100	500	125	500	12.5	>1000	12.5
Ampicillin					100	100	250	100	-	-	-
Chloramphenicol					50	50	50	50	-	-	-
Nystatin					-	-	-	-	100	100	100
Griseofulvin					-	-	-	-	500	100	100

Abbreviations: *E. c. - Escherichia coli, P. a. - Pseudomonas aeruginosa, S. a. - Staphylococcus aureus, S. p. - Streptococcus pyogenes; C.a. - Candida albicans, A. n.- Aspergillus niger, A. c. – Aspergillus clavatus.*

TABLE 1 Antimicrobial screening results of compounds 5a-o with structural and molecular formula

2.3 | Molecular docking studies

The standard drugs selected for antimicrobial activity preferably ampicillin is an anti-staphylococcus agent that can withstand acid attack acts by inhibition of cell wall synthesis, while chloramphenicol is used in treating streptococcal infections acts by inhibiting the production of proteins.[19] The best-preferred orientation of the standard drugs has been characterized by molecular docking studies. Further validation of our docking has been carried out and their root mean square deviation (RMSD) values were found to be 0.648 Å (ampicillin) and 0.243 Å (chloramphenicol). The carboxylic acid group attached to β-lactam of standard ampicillin has formed two H-bond interactions with the amine group of Ser 41 and Arg 110 in the protein 3KP3 with a dock score of -8.253 kcal/mol, while the benzyl amine group has interacted with carboxy terminal of Glu 39 with single H- bond as depicted in Figure 2. Similarly, the nitro group of chloramphenicol has formed two hydrogen bond interactions with the amine functionality of Lys 74 and Arg 70 with a dock score of -5.554 kcal/mol, in the protein 4EJV as shown in Figure 3. Their binding energies were -87.984 kcal/mol and -67.278 kcal/mol for ampicillin and chloramphenicol respectively. All the synthesized compounds were docked into the active site of ampicillin and chloramphenicol their dock scores with binding free energies in both proteins were tabulated in Table 2.

The molecular docking studies reveal that all the synthesized molecules were suitably oriented in the active site of proteins displaying H-bond and π-π stacking interactions with amino acids Glu 39, Arg 110, Ser 41 in protein 3KP3, and Lys 74, Arg 70 in 4EV protein which were identical to that of standard ampicillin and chloramphenicol as depicted in Figure 4 and Figure 5. Among the synthesized compounds, the compounds **5k** (50 μg/mL), **5n** (62.5 μg/mL) were effective against *E. coli*, while **5e** (50 μg/mL), **5m** (25 μg/mL) were effective against *P. aeruginosa* and **5o** (12.5 μg/mL) the only compound that is effective against fungal strains such as *C. albicans* and *A. clavatus*. The minimum inhibitory bacterial concentrations for the above-mentioned compounds were found to be lesser than the standard drugs inhibitory concentration. As far as activity is concerned high electronegative group such as

fluorine at para position as in compound **5k** (4-F) aid in retaining hydrophobic nature contributing for antimicrobial activity, while electron donor group (-OH group) that is meta directing in **5e** (3-OH) compound is found to be essential feature for treating pseudomonol infections. Both electron donating groups at meta (-OCH_3 group) and para (-OH group) positions in compound **5o** (4-OH-3-OCH_3) aid in exhibiting antifungal activity.

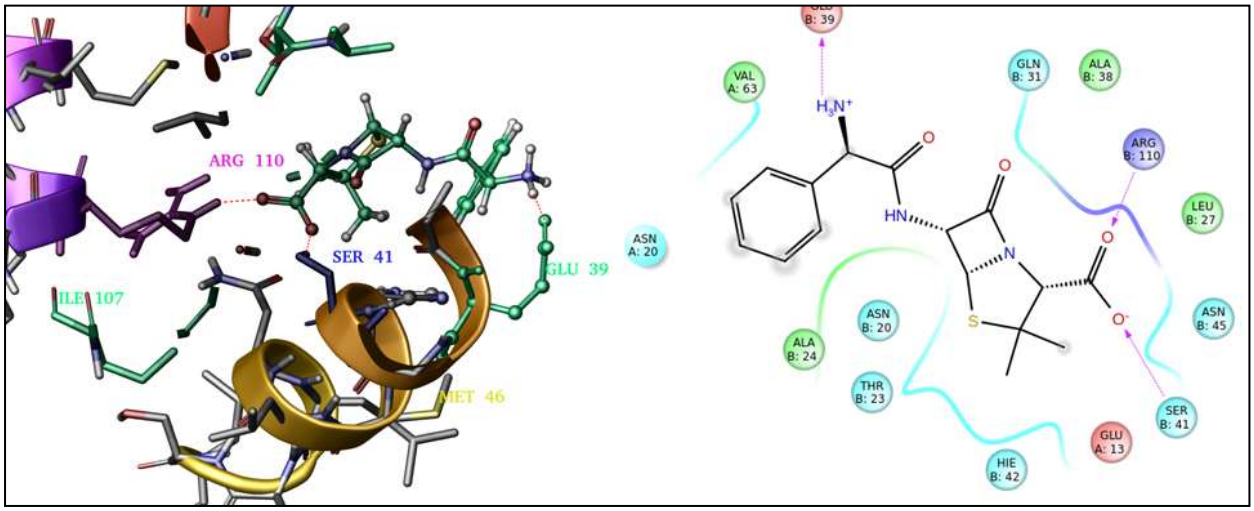


FIGURE 2 Dock pose image of the standard drug ampicillin with its ligand interaction diagram displaying hydrogen bond interactions with Serine 41, Glu 39, Arg 110 in the 3KP3 protein

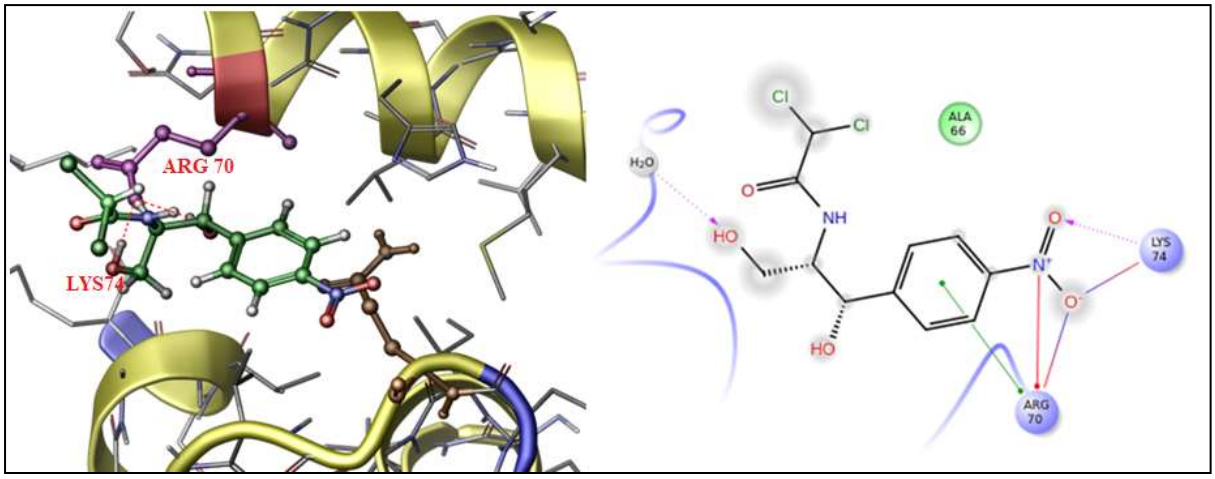


FIGURE 3 The dock pose image of standard chloramphenicol showing hydrogen bond interaction with Lys 74 and one π-π stacking interaction with Arg 70 in the protein 4EJV

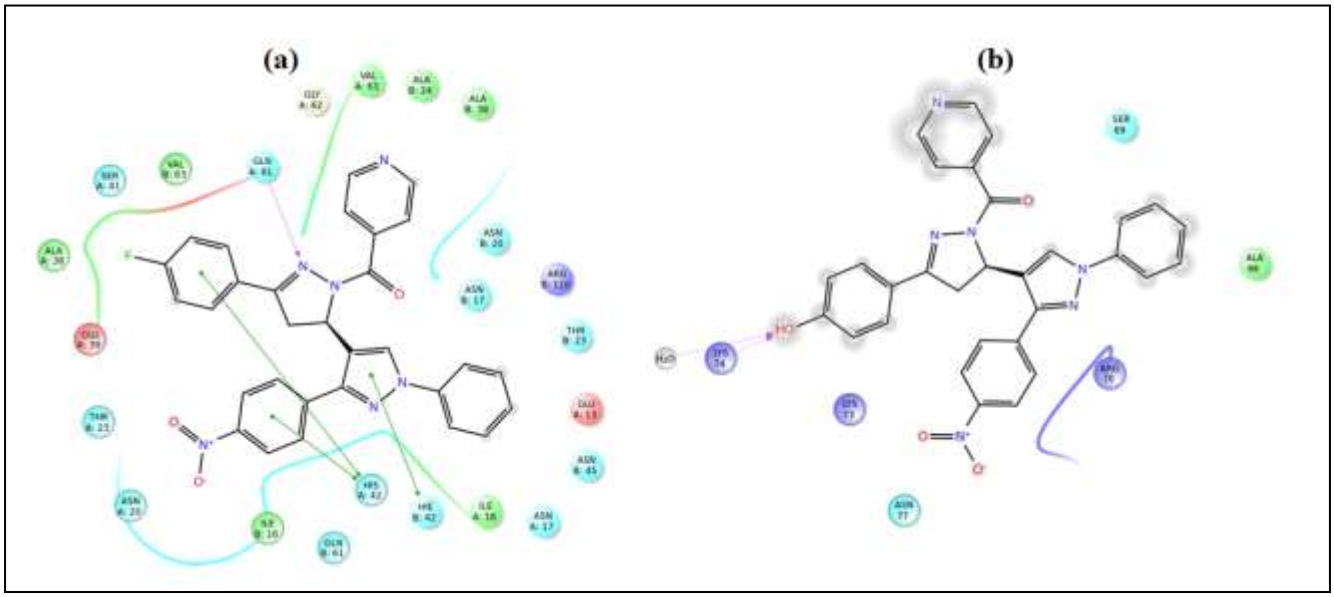


FIGURE 4 Dock pose image of compound 5k in the proteins 3KP3 (a) displaying H-Bond interaction with Gln 61 and two π-π stacking interaction with His 42, while 4EJV (b) forming H-bond with Lys 74 and water

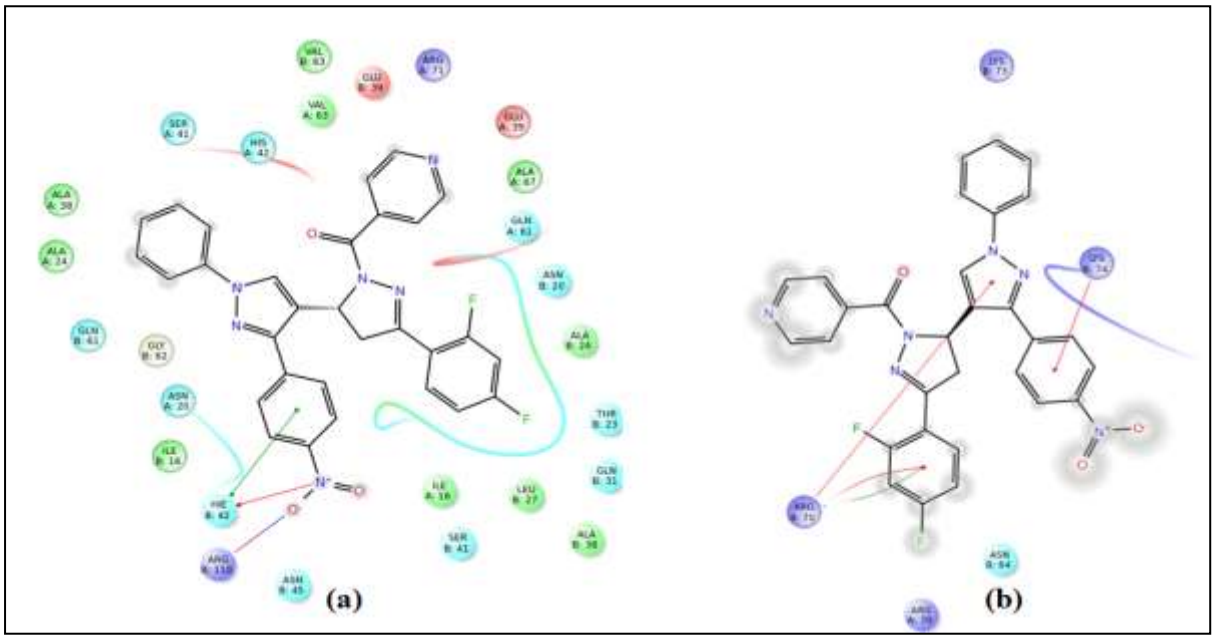


FIGURE 5 Dock pose image of compound 5l in the proteins 3KP3 (a) displaying H-Bond interaction with His 42 and Arg 110, while 4EJV (b) forming H-bond with Lys 74 and Arg 70

TABLE 2 The molecular docking scores with binding energies for the synthesized compounds 5a-o in both proteins 3KP3 and 4EJV

Sr. No.	Compounds	Antimicrobial Dock scores		Antimicrobial binding energies	
		3HUN (kcal/mol)	4EJV (kcal/mol)	3HUN (kcal/mol)	4EJV (kcal/mol)
1	5a	-6.444	-6.935	-56.832	-51.111
2	5b	-7.838	-7.801	-85.728	-51.958
3	5c	-6.294	-5.249	-55.726	-52.106
4	5d	-7.565	-4.539	-71.540	-58.862
5	5e	-6.574	-7.462	-49.423	-55.606
6	5f	-7.844	-6.260	-78.894	-62.456
7	5g	-5.909	-5.701	-61.146	-63.483
8	5h	-6.133	-8.304	-76.290	-54.691
9	5i	-7.771	-5.391	-74.117	-57.374
10	5j	-7.176	-7.743	-63.541	-58.363
11	5k	-6.072	-6.924	-54.642	-58.097
12	5l	-6.454	-4.194	-60.383	-49.184
13	5m	-8.555	-2.377	-89.343	-51.135
14	5n	-7.658	-3.809	-57.3296	-64.120
15	5o	-8.427	-3.482	-88.0809	-62.073
16	Ampicillin	-8.253	-	-87.984	-
17	Chloramphenicol	-	-5.554	-	-67.278

3 | CONCLUSION

A novel series of titled compounds based on pyrazole, pyrazoline clubbed-pyridine **(5a-o)** were prepared and tested for their antimicrobial potency. The antimicrobial screening results revealed the potency of synthesized compounds **5d, 5e, 5i, 5k, 5m** and **5o** as antimicrobial agents against various bacterial and fungal species. Further, it is observed that the presence of electron-donating groups in compounds such as 2-OH, 3-OH, 4-CH_3 and 4-OH-3-OCH_3 augmented antifungal and antibacterial potency while the presence of electron-withdrawing groups in compounds such as 4-F and 2,4-dichloro showed an increment in the antibacterial potency. Molecular docking studies and ADME properties calculations were effective in

compliance with the antimicrobial activity that supports our explanation for antibacterial activity.

4 | EXPERIMENTAL SECTION

4.1 | Synthesis

4.1.1 | Synthesis of 3-(4-nitrophenyl)-1-phenyl-1*H*-pyrazole-4-carbaldehyde (3)

3-(4-Nitrophenyl)-1-phenyl-1*H*-pyrazole-4-carbaldehyde **(3)** was prepared in two steps as described under:

4.1.1.1 | Synthesis of 1-(1-(4-nitrophenyl)ethylidene)-2-phenylhydrazine (2)

To a solution of p-nitroacetophenone **(1)** in ethanol (95 %, 30 mL), glacial acetic acid (1 mL), and phenylhydrazine (0.01 mol) were added and the reaction mixture was warmed for an hour. The precipitates formed were filtered and washed with 95 % ethanol and recrystallized from methanol.

4.1.1.2 | Synthesis of 3-(4-nitrophenyl)-1-phenyl-1*H*-pyrazole-4-carbaldehyde (3) from compound (2)

A solution of compound **(2)** (0.11 mol) in dimethylformamide (5 mL) was added dropwise to previously cooled mixture of dimethylformamide (0.35 mol) and phosphorus oxychloride (0.35 mol) which was then allowed to attain room temperature and refluxed at 70–80 °C for 5 h. After cooling at room temperature, the mixture was treated with a cold saturated K_2CO_3 solution. The precipitate was filtered, washed with water and recrystallized from ethanol (95%).

Yield: 65 %; m.p.:173-175 ºC; Anal. calcd. for $C_{16}H_{11}N_3O_3$: C, 65.53; H, 3.78; N, 14.33; Found: C, 65.55; H, 3.80; N, 14.37 %.

4.1.2 | Synthesis of 3-(3-(4-nitrophenyl)-1-aryl-1H-pyrazol-4-yl)-1-phenylprop-2-en-1-ones (4a-o)

A mixture of 3-(4-nitrophenyl)-1-phenyl-1*H*-pyrazole-4-carbaldehyde **(3)** (0.01 mol) and substituted acetophenones (0.01 mol) were stirred in methanolic potassium hydroxide at room temperature for 4 hours. The product generated was then filtered, washed with water and recrystallized from 95 % ethanol.

Yield **(4a)**: 71%; m.p.: 190-192 °C; Anal. calcd. for $C_{24}H_{17}N_3O_3$: C, 72.90; H, 4.33; N, 10.63; Found: C, 72.95; H, 4.36; N, 10.68 %.

4.1.3 | General preparation of of (3'-(4-nitrophenyl)-1'-phenyl-5-(aryl)-3,4-dihydro-1'*H*,2*H*-[3,4'-bipyrazol]-2-yl)(pyridine-4-yl)methanones (5a-o)

3-(3-(4-Nitrophenyl)-1-aryl-1H-pyrazol-4-yl)-1-phenylprop-2-en-1-ones **(4a-o)** (0.001 mol) and isoniazid (0.002 mol) were taken in a round bottom flask containing 20 mL glacial acetic acid and refluxed for 28 hours above 140 ºC using a drop of H_2SO_4 as a catalyst. The reaction mixture was then poured onto crushed ice. The separated product was filtered, washed with water and recrystallized from 95 % ethanol. All compounds of series were prepared using the same method.

Characterization of (3'-(4-nitrophenyl)-1',5-diphenyl-3,4-dihydro-1'*H*,2*H*-[3,4'-bipyrazol]-2-yl)(pyridin-4-yl)methanone (5a)

Yellow; Yield: 68 %; m.p.: 159-161 ºC; IR (KBr)ν_{max}**:** 3245 (C-H stretching, aromatic ring), 3144, 3049 (C-H stretching, -CH_2-), 1649 (>C=O stretching), 1592 (>C=N- stretching, aromatic ring), 1500 (>C=C< stretching, aromatic ring), 1450 (C-N< stretching), 1465 (-NO_2 stretching); ^{1}H NMR (500 MHz, DMSO-d_6, δppm): 7.72 (s, 1H, =C<u>H</u>-N), 7.44-8.92 (m, 18H, Ar-<u>H</u>), 6.16 (s, 1H, >C<u>H</u>-), 3.93 (dd, 1H, <u>H</u>b-C-Ha-C), 3.50 (dd, 1H, Hb-C-<u>H</u>a-C); ^{13}C NMR (125 MHz, DMSO-d_6, δppm): 39.2, 61.8, 103.7, 104.7, 119.5 (2), 123.4 (2), 124.2 (2), 126.0, 127.1, 128.3 (2), 129.4, 129.6, 129.7, 129.9 (2), 131.0, 133.2, 139.4, 141.3, 143.4, 143.7, 145.3, 147.6, 151.3 (2), 165.1; LCMS: *m/z* = 515.10 [M^+] (36 %); Anal. calcd. for $C_{30}H_{22}N_6O_3$: C, 70.03; H, 4.31; N, 16.33; Found: C, 70.10; H, 4.39; N, 16.39 %.

Characterization of (5-(3-bromophenyl)-3'-(4-nitrophenyl)-1'-phenyl-3,4-dihydro-1'*H*,2*H*-[3,4'-bipyrazol]-2-yl)(pyridin-4-yl)methanone (5b)

Cremes yellow; Yield: 75 %; m.p.: 145-148 ºC; IR (KBr)ν_{max}**:** 3250 (C-H stretching, aromatic ring), 3148, 3059 (C-H stretching, -CH_2-), 1665 (>C=O stretching), 1580 (>C=N- stretching, aromatic ring), 1505 (>C=C< stretching, aromatic ring), 1453 (C-N< stretching), 1425 (-NO_2 stretching), 540 (C-Br stretching); ^{1}H NMR (500 MHz, DMSO-d_6, δppm): 7.65 (s, 1H, =C<u>H</u>-N), 7.40-8.96 (m, 17H, Ar-<u>H</u>), 6.14 (s, 1H, >C<u>H</u>-), 3.96 (dd, 1H, <u>H</u>b-C-Ha-C), 3.57 (dd, 1H, Hb-C-<u>H</u>a-C); ^{13}C NMR (125 MHz, DMSO-d_6, δppm): 39.4, 61.6, 117.2, 119.1 (2), 121.6 (2), 123.7 (2), 124.6 (2), 126.3 (3), 126.9, 127.5, 129.7 (2), 129.9, 133.4, 136.2, 139.7, 139.9, 140.5, 147.8, 149.6 (2), 149.4, 151.0, 167.6; LCMS: *m/z* =595.03 [M^+] (39 %); Anal. calcd. for $C_{30}H_{21}BrN_6O_3$: C, 60.72; H, 3.57; N, 14.16; Found: C, 60.78; H, 3.59; N, 14.20 %.

Characterization of (5-(4-bromophenyl)-3'-(4-nitrophenyl)-1'-phenyl-3,4-dihydro-1'*H*,2*H*-[3,4'-bipyrazol]-2-yl)(pyridin-4-yl)methanone (5c)

Cremes yellow; Yield: 70 %; m.p.: 210-212 ºC; IR (KBr)ν_{max}**:** 3255 (C-H stretching, aromatic ring), 3141, 3051 (C-H stretching, -CH_2-), 1661 (>C=O stretching), 1578 (>C=N- stretching, aromatic ring), 1505 (>C=C< stretching, aromatic ring), 1456 (C-N< stretching), 1421 (-NO_2 stretching), 545 (C-Br stretching); ^{1}H NMR (500 MHz, DMSO-d_6, δppm): 7.64 (s, 1H, =C<u>H</u>-N), 7.38-8.91 (m, 17H, Ar-<u>H</u>), 6.10 (s, 1H, >C<u>H</u>-), 3.91 (dd, 1H, <u>H</u>b-C-Ha-C), 3.50 (dd, 1H, Hb-C-<u>H</u>a-C);^{13}C NMR (125 MHz, DMSO-d_6, δppm): 39.5, 61.4, 117.4, 119.7, 121.0 (2), 123.7 (2), 124.8 (2), 125.7, 126.1 (3), 128.5 (2), 129.6 (2), 131.9 (2), 135.7, 139.4, 139.8, 140.0, 147.5, 149.6, 149.8 (2), 151.4, 167.2; LCMS: *m/z* =595.01 [M^+] (41 %); Anal. calcd. For $C_{30}H_{21}BrN_6O_3$: C, 60.72; H, 3.57; N, 14.16; Found: C, 60.78; H, 3.66; N, 14.23 %.

Characterization of (5-(2-hydroxyphenyl)-3'-(4-nitrophenyl)-1'-phenyl-3,4-dihydro-1'*H*,2*H*-[3,4'-bipyrazol]-2-yl)(pyridin-4-yl)methanone (5d)

Yellow; Yield: 68 %; m.p.: 170-172 ºC; IR (KBr)ν_{max}: 3545 (-OH stretching, aromatic ring), 3240 (C-H stretching, aromatic ring), 3135, 3032 (C-H stretching, -CH_2-), 1645 (>C=O stretching), 1599 (>C=N- stretching, aromatic ring), 1500 (>C=C< stretching, aromatic ring), 1457 (C-N< stretching), 1464 (-NO_2 stretching); ^{1}H NMR (500 MHz, DMSO-d_6, δppm): 7.63 (s, 1H, =C<u>H</u>-N), 7.40-8.92 (m, 17H, Ar-<u>H</u>), 6.13 (s, 1H, >C<u>H</u>-), 5.37 (s, 1H, -OH), 3.92 (dd, 1H, <u>H</u>b-C-Ha-C), 3.54 (dd, 1H, Hb-C-<u>H</u>a-C); ^{13}C NMR (125 MHz, DMSO-d_6, δppm): 40.7, 61.7, 117.8, 117.9, 118.4, 119.6 (2), 121.4, 121.8 (2), 123.5 (2), 124.9 (2), 126.3 (2), 129.5 (2), 132.6, 132.7, 139.4, 139.7, 140.5, 147.8, 149.6 (2), 149.5, 151.4, 162.7, 167.9; LCMS: *m/z* = 531.20 [M^+] (61 %); Anal. calcd. for $C_{30}H_{22}N_6O_4$: C, 67.92; H, 4.18; N, 15.84; Found: C, 67.96; H, 4.22; N, 15.89 %.

Characterization of (5-(3-hydroxyphenyl)-3'-(4-nitrophenyl)-1'-phenyl-3,4-dihydro-1'*H*,2*H*-[3,4'-bipyrazol]-2-yl)(pyridin-4-yl)methanone (5e)

Pale Yellow; Yield: 65 %; m.p.: 202-204 ºC; IR (KBr)ν_{max}: 3545 (-OH stretching, aromatic ring), 3245 (C-H stretching, aromatic ring), 3130, 3020 (C-H stretching, -CH_2-), 1647 (>C=O stretching), 1600 (>C=N- stretching, aromatic ring), 1502 (>C=C< stretching, aromatic ring), 1460 (C-N< stretching), 1460 (-NO_2 stretching); ^{1}H NMR (500 MHz, DMSO-d_6, δppm): 7.59 (s, 1H, =C<u>H</u>-N), 7.38-8.94 (m, 17H, Ar-<u>H</u>), 6.15 (s, 1H, >C<u>H</u>-), 5.30 (s, 1H, -OH), 3.91 (dd, 1H, <u>H</u>b-C-Ha-C), 3.57 (dd, 1H, Hb-C-<u>H</u>a-C); ^{13}C NMR (125 MHz, DMSO-d_6, δppm): 39.5, 61.7, 114.8, 117.8, 118.7, 119.5 (2), 120.0, 121.4 (2), 123.0 (2), 124.9 (2), 126.5 (3), 129.7 (2), 130.7, 135.2, 139.7, 139.9, 140.8, 147.6, 149.8 (2), 149.4, 151.7, 158.6, 167.3; LCMS: *m/z* = 531.15 [M^+] (47 %); Anal. calcd. for $C_{30}H_{22}N_6O_4$: C, 67.92; H, 4.18; N, 15.84; Found: C, 67.99; H, 4.22; N, 15.89 %.

Characterization of (5-(2-methoxyphenyl)-3'-(4-nitrophenyl)-1'-phenyl-3,4-dihydro-1'*H*,2*H*-[3,4'-bipyrazol]-2-yl)(pyridin-4-yl)methanone (5f)

Pale yellow; Yield: 65%; m.p.: 191-193 ºC; IR (KBr)ν_{max}: 2830 (C-H stretching, -OCH_3), 3232 (C-H stretching, aromatic ring), 3130, 3020 (C-H stretching, -CH_2-), 1636 (>C=O

stretching), 1610 (>C=N- stretching, aromatic ring), 1502 (>C=C< stretching, aromatic ring), 1475 (C-N< stretching), 1455 (-NO_2 stretching); ^{1}H NMR (500 MHz, DMSO-d_6, δppm): 7.55 (s, 1H, =C<u>H</u>-N), 7.34-8.90 (m, 17H, Ar-<u>H</u>), 6.10 (s, 1H, >C<u>H</u>-), 3.82 (s, 3H, -OCH_3), 3.91 (dd, 1H, <u>H</u>b-C-Ha-C), 3.51 (dd, 1H, Hb-C-<u>H</u>a-C); ^{13}C NMR (125 MHz, DMSO-d_6, δppm): 40.6, 55.4, 61.8, 111.6, 117.2 (2), 119.6 (2), 121.5, 121.6 (2), 123.6, 124.6 (2), 126.6 (3), 129.6 (2), 131.4, 132.1, 139.5, 139.7, 140.6, 147.8, 149.6 (2), 149.4, 151.6, 160.4, 167.3; LCMS: *m/z* = 545.20 [M^+] (32%); Anal. calcd. for $C_{31}H_{24}N_6O_4$: C, 68.37; H, 4.44; N, 15.43; Found: C, 68.45; H, 4.49; N, 15.48 %.

Characterization of (5-(3-methoxyphenyl)-3'-(4-nitrophenyl)-1'-phenyl-3,4-dihydro-1'*H,2H*-[3,4'-bipyrazol]-2-yl)(pyridin-4-yl)methanone (5g)

Pale yellow; Yield: 66 %; m.p.: 177-179 ºC; IR (KBr)ν_{max}: 2833 (C-H stretching, -OCH_3), 3232 (C-H stretching, aromatic ring), 3129, 3022 (C-H stretching, -CH_2-), 1633 (>C=O stretching), 1600 (>C=N- stretching, aromatic ring), 1509 (>C=C< stretching, aromatic ring), 1470 (C-N< stretching), 1455 (-NO_2 stretching); ^{1}H NMR (500 MHz, DMSO-d_6, δppm): 7.52 (s, 1H, =C<u>H</u>-N), 7.28-8.88 (m, 17H, Ar-<u>H</u>), 6.12 (s, 1H, >C<u>H</u>-), 3.87 (s, 3H, -OCH_3), 3.94 (dd, 1H, <u>H</u>b-C-Ha-C), 3.57(dd, 1H, Hb-C-<u>H</u>a-C); ^{13}C NMR (125 MHz, DMSO-d_6, δppm): 39.4, 55.1, 61.6, 113.4, 116.3, 117.6, 119.4 (2), 120.6, 121.6 (2), 123.4, 124.9 (2), 126.6 (3), 129.4 (2), 129.1, 135.7, 139.4, 139.8, 140.7, 147.8, 149.4 (2), 149.9, 151.8, 160.7, 167.6; LCMS: *m/z* = 545.18 [M^+] (32%); Anal. calcd. for $C_{31}H_{24}N_6O_4$: C, 68.37; H, 4.44; N, 15.43; Found: C, 68.42; H, 4.50; N, 15.49 %.

Characterization of (5-(4-methoxyphenyl)-3'-(4-nitrophenyl)-1'-phenyl-3,4-dihydro-1'*H*,2*H*-[3,4'-bipyrazol]-2-yl)(pyridin-4-yl)methanone (5h)

Yellow; Yield: 69 %; m.p.: 152-154 ºC; IR (KBr)ν_{max}: 2835 (C-H stretching, -OCH_3), 3232 (C-H stretching, aromatic ring), 3125, 3022 (C-H stretching, -CH_2-), 1629 (>C=O stretching), 1601 (>C=N- stretching, aromatic ring), 1504 (>C=C< stretching, aromatic ring), 1464 (C-N< stretching), 1451 (-NO_2 stretching); ^{1}H NMR (500 MHz, DMSO-d_6, ppm): 7.45 (s, 1H,

=C<u>H</u>-N), 7.21-8.86 (m, 17H, Ar-<u>H</u>), 6.10 (s, 1H, >C<u>H</u>-), 3.84 (s, 3H, -OCH_3), 3.85 (dd, 1H, <u>H</u>b-C-Ha-C), 3.52 (dd, 1H, Hb-C-<u>H</u>a-C); ^{13}C NMR (125 MHz, DMSO-d_6, ppm): 39.4, 55.1, 61.7, 114.7 (2), 117.3, 119.7 (2), 121.8 (2), 123.7, 124.8 (2), 126.9 (3), 128.1 (3), 129.4 (2), 139.1, 139.4, 140.6, 147.3, 149.8 (2), 149.0, 151.2, 162.7, 167.8; LCMS: *m/z* =545.15 [M^+] (34 %); Anal. calcd. for $C_{31}H_{24}N_6O_4$: C, 68.37; H, 4.44; N, 15.43; Found: C, 68.45; H, 4.52; N, 15.56 %.

Characterization of (3'-(4-nitrophenyl)-1'-phenyl-5-(*p*-tolyl)-3,4-dihydro-1'*H*,2*H*-[3,4'-bipyrazol]-2-yl)(pyridin-4-yl)methanone (5i)

Yellow; Yield: 71 %; m.p.: 207-209 ºC; IR (KBr)ν_{max}: 3220 (C-H stretching, aromatic ring), 3115, 3017 (C-H stretching, -CH_2-and -CH_3), 1614 (>C=O stretching), 1595 (>C=N- stretching, aromatic ring), 1500 (>C=C< stretching, aromatic ring), 1445 (C-N< stretching), 1447 (-NO_2 stretching), 1373 (-C-H stretching, -C-CH_3); ^{1}H NMR (500 MHz, DMSO-d_6, δppm): 7.35 (s, 1H, =C<u>H</u>-N), 7.18-8.80 (m, 17H, Ar-<u>H</u>), 6.05 (s, 1H, >C<u>H</u>-), 3.74 (dd, 1H, <u>H</u>b-C-Ha-C), 3.50 (dd, 1H, Hb-C-<u>H</u>a-C), 2.35 (s, 3H, -CH_3); ^{13}C NMR (125 MHz, DMSO-d_6, δppm): 21.4, 39.7, 61.4, 117.5, 119.2 (2), 121.5 (2), 123.0, 124.6 (2), 126.2 (3), 127.4 (2), 129.2 (2), 129.9 (2), 133.3, 139.4, 139.5, 140.6, 140.9, 147.0, 149.3 (2), 149.4, 151.3, 167.9; LCMS: *m/z* = 529.20 [M^+] (38 %); Anal. calcd. for $C_{31}H_{24}N_6O_3$: C, 70.44; H, 4.58; N, 15.90; Found: C, 70.49; H, 4.63; N, 15.96 %.

Characterization of (5-(4-chlorophenyl)-3'-(4-nitrophenyl)-1'-phenyl-3,4-dihydro-1'*H*,2*H*-[3,4'-bipyrazol]-2-yl)(pyridin-4-yl)methanone (5j)

Pale yellow; Yield: 75 %; m.p.: 124-126 ºC; IR (KBr)ν_{max}: 3214 (C-H stretching, aromatic ring), 3115, 3020 (C-H stretching, -CH_2-), 1620 (>C=O stretching), 1590 (>C=N- stretching, aromatic ring), 1499 (>C=C< stretching, aromatic ring), 1440 (C-N< stretching), 1435 (-NO_2 stretching), 762 (C-Cl stretching); ^{1}H NMR (500 MHz, DMSO-d_6, δppm): 7.20 (s, 1H, =C<u>H</u>-N), 7.12-8.72 (m, 17H, Ar-<u>H</u>), 6.15 (s, 1H, >C<u>H</u>-), 3.72 (dd, 1H, <u>H</u>b-C-Ha-C), 3.49 (dd, 1H, Hb-C-<u>H</u>a-C); ^{13}C NMR (125 MHz, DMSO-d_6, δppm): 39.4, 61.5, 117.2, 119.5 (2), 121.3 (2),

123.0, 124.7 (2), 126.6 (3), 128.2 (2), 128.3 (2), 129.7 (2), 134.0, 136.1, 139.1, 139.7, 140.0, 147.2, 149.5 (2), 149.6, 151.3, 167.0; LCMS: *m/z* =549.10 [M^+] (70 %); Anal. calcd. for $C_{30}H_{21}ClN_6O_3$: C, 65.63; H, 3.86; N, 15.31; Found: C, 65.70; H, 3.93; N, 15.36 %.

Characterization of (5-(4-fluorophenyl)-3'-(4-nitrophenyl)-1'-phenyl-3,4-dihydro-1'*H*,2*H*-[3,4'-bipyrazol]-2-yl)(pyridin-4-yl)methanone (5k)

Light Yellow; Yield: 73 %; m.p.: 130-132 ºC; IR (KBr)ν_{max}**:** 3224 (C-H stretching, aromatic ring), 3142, 3047 (C-H stretching, -CH_2-), 1647 (>C=O stretching), 1598 (>C=N- stretching, aromatic ring), 1504 (>C=C< stretching, aromatic ring), 1448 (C-N< stretching), 1465 (-NO_2 stretching), 1072 (C-F stretching); ^{1}H NMR (500 MHz, DMSO-d_6, δppm): 7.70 (s, 1H, =C<u>H</u>-N), 7.40-8.90 (m, 17H, Ar-<u>H</u>), 6.12 (s, 1H, >C<u>H</u>-), 3.90 (dd, 1H, <u>H</u>b-C-Ha-C), 3.55(dd, 1H, Hb-C-<u>H</u>a-C); ^{13}C NMR (125 MHz, DMSO-d_6, δppm): 39.1, 61.2, 115.2 (2), 117.3, 119.4 (2), 121.7 (2), 123.1, 124.3 (2), 126.3 (3), 129.3 (2),129.5 (2), 132.1, 139.3, 139.5, 140.4, 147.6, 149.5, 149.8 (2), 151.2, 165.3, 167.2; LCMS: *m/z* = 533.60 [M^+] (37 %); Anal. calcd. for $C_{30}H_{21}FN_6O_3$: C, 67.66; H, 3.97; N, 15.78; Found: C, 67.72; H, 4.05; N, 15.83 %.

Characterization of (5-(2,4-difluorophenyl)-3'-(4-nitrophenyl)-1'-phenyl-3,4-dihydro-1'*H*,2*H*-[3,4'-bipyrazol]-2-yl)(pyridin-4-yl)methanone (5l)

Light Yellow; Yield: 68 %; m.p.: 191-193 ºC; IR (KBr)ν_{max}**:** 3229 (C-H stretching, aromatic ring), 3143, 3045 (C-H stretching, -CH_2-), 1642 (>C=O stretching), 1596 (>C=N- stretching, aromatic ring), 1504 (>C=C< stretching, aromatic ring), 1450 (C-N< stretching), 1468 (-NO_2 stretching), 1075 (C-F stretching); ^{1}H NMR (500 MHz, DMSO-d_6, δppm): 7.74 (s, 1H, =C<u>H</u>-N), 7.41-8.85 (m, 16H, Ar-<u>H</u>), 6.09 (s, 1H, >C<u>H</u>-), 3.95 (dd, 1H, <u>H</u>b-C-Ha-C), 3.57 (dd, 1H, Hb-C-<u>H</u>a-C); ^{13}C NMR (125 MHz, DMSO-d_6, δppm): 39.4, 61.3, 111.2, 112.0, 113.0, 117.0, 119.5 (2), 121.7 (2), 123.1, 124.7 (2), 126.3 (3), 129.6 (2), 132.1, 139.2, 139.7, 140.1, 147.8, 149.3 (2), 149.2, 151.3, 161.5, 163.8, 167.7; LCMS: *m/z* =551.20 [M^+] (52 %); Anal. calcd. for $C_{30}H_{20}F_2N_6O_3$: C, 65.45; H, 3.66; N, 15.27; Found: C, 65.52; H, 3.70; N, 15.32 %.

Characterization of (5-(2,4-dichlorophenyl)-3'-(4-nitrophenyl)-1'-phenyl-3,4-dihydro-1'*H*,2*H*-[3,4'-bipyrazol]-2-yl)(pyridin-4-yl)methanone (5m)

Pale yellow; Yield: 69 %; m.p.: 204-206 ºC; IR (KBr)ν_{max}: 3223 (C-H stretching, aromatic ring), 3130, 3030 (C-H stretching, -CH_2-), 1625 (>C=O stretching), 1509 (>C=N- stretching, aromatic ring), 1460 (>C=C< stretching, aromatic ring), 1450 (C-N< stretching), 1435 (-NO_2 stretching), 755 (C-Cl stretching); ^{1}H NMR (500 MHz, DMSO-d_6, δppm): 7.77 (s, 1H, =C<u>H</u>-N), 7.33-8.75 (m, 16H, Ar-<u>H</u>), 6.12 (s, 1H, >C<u>H</u>-), 3.99 (dd, 1H, <u>H</u>b-C-Ha-C), 3.54 (dd, 1H, Hb-C-<u>H</u>a-C); ^{13}C NMR (125 MHz, DMSO-d_6, δppm): 39.1, 61.5, 117.2, 119.2 (2), 121.1 (2), 123.0, 124.2 (2), 126.3 (3), 126.6, 128.1, 129.6, 129.9 (2), 133.5, 134.6, 135.8, 139.4, 139.9, 140.7, 147.9, 149.6 (2), 149.6, 151.3, 167.8; LCMS: *m/z* = 583.07 [M^+] (72 %); Anal. calcd. for $C_{30}H_{20}Cl_2N_6O_3$: C, 61.76; H, 3.46; N, 14.40; Found: C, 61.83; H, 3.52; N, 14.50 %.

Characterization of (5-(2-bromo-4-chlorophenyl)-3'-(4-nitrophenyl)-1'-phenyl-3,4-dihydro-1'*H*,2*H*-[3,4'-bipyrazol]-2-yl)(pyridin-4-yl)methanone (5n)

Pale yellow; Yield: 70 %; m.p.:212-214 ºC; IR (KBr)ν_{max}: 3230 (C-H stretching, aromatic ring), 3121, 3018 (C-H stretching, -CH_2-), 1620 (>C=O stretching), 1505 (>C=N- stretching, aromatic ring), 1452 (>C=C< stretching, aromatic ring), 1440 (C-N< stretching), 1425 (-NO_2 stretching), 765 (C-Cl stretching), 545 (C-Br stretching); ^{1}H NMR (500 MHz, DMSO-d_6, δppm): 7.64 (s, 1H, =C<u>H</u>-N), 7.27-8.50 (m, 16H, Ar-<u>H</u>), 6.11 (s, 1H, >C<u>H</u>-), 3.94 (dd,1H, <u>H</u>b-C-Ha-C), 3.51 (dd,1H, Hb-C-<u>H</u>a-C); ^{13}C NMR (125 MHz, DMSO-d_6, δppm): 39.5, 61.2, 117.6, 119.8 (2), 121.9 (2), 123.6 (2), 124.9 (2), 126.9 (3), 127.5, 129.3 (2), 130.7, 132.1, 133.8, 137.5, 139.5, 139.6, 140.8, 147.6, 149.6 (2), 149.8, 151.0, 167.3; LCMS: *m/z* = 626.80 [M^+] (32%); Anal. calcd. for $C_{30}H_{20}BrClN_6O_3$: C, 57.39; H, 3.21; N, 13.38; Found: C, 57.45; H, 3.25; N, 14.49 %.

Characterization of (5-(4-hydroxy-3-methoxyphenyl)-3'-(4-nitrophenyl)-1'-phenyl-3,4-dihydro-1'*H*,2*H*-[3,4'-bipyrazol]-2-yl)(pyridin-4-yl)methanone (5o)

Light yellow; Yield: 71 %; m.p.: 198-200 ºC; IR (KBr)ν_{max}: 3541, (-OH stretching, aromatic ring), 3234 (C-H stretching, aromatic ring), 3123, 3019 (C-H stretching, -CH_2-), 2825 (C-H stretching, -OCH_3), 1623 (>C=O stretching), 1508 (>C=N- stretching, aromatic ring), 1454 (>C=C< stretching, aromatic ring), 1444 (C-N< stretching), 1428 (-NO_2 stretching); ^{1}H NMR (500 MHz, DMSO-d_6, δppm): 7.52 (s, 1H, =C<u>H</u>-N), 7.27-8.52 (m, 16H, Ar-<u>H</u>), 6.15 (s, 1H, >C<u>H</u>-), 5.32 (s, 1H, -OH), 3.91 (dd,1H, <u>H</u>b-C-Ha-C), 3.89 (s, 3H, -OCH_3), 3.54 (dd,1H, Hb-C-<u>H</u>a-C); ^{13}C NMR (125 MHz, DMSO-d_6, δppm): 39.5, 56.3, 61.2, 117.5 (2), 119.6 (2), 121.8 (2), 123.3 (2), 124.5 (2), 126.6 (2), 127.4 (2), 129.5 (2), 130.6, 132.4, 133.8, 137.6, 139.6, 140.5, 147.0, 149.5, 149.6 (2), 151.0, 167.6; LCMS: *m/z* =561.20 [M^+] (53 %); Anal. calcd. for $C_{31}H_{24}N_6O_5$: C, 66.42; H, 4.32; N, 14.99; Found: C, 66.49; H, 4.36; N, 14.93 %.

5 | MATERIALS AND METHODS

5.1 | Antimicrobial screening

For the antimicrobial screening of the compounds **(5a-o)**, we have used standard MTCC strains of bacteria and fungi. The newly synthesized product was tested according to the Broth dilution method.[20] In the present protocol, various bacteria like *Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli* and *Pseudomonas aeruginosa* were used. Ampicillin and chloramphenicol used as standard drugs for antibacterial activity. The fungal strains like *Aspergillus niger*, *Candida albicans* and *Aspergillus clavatus* used for the antifungal activity. The details were given in supporting information.

5.2 | Methodology for molecular docking

The tentative drug molecular binding interactions into the preferred active site of the receptor were assessed by means of molecular docking studies. The proteins in which the standard drugs used for activity studies that have been crystallized were selected for molecular docking. Since there were no available antifungal proteins with Nystatin and Griseofulvin as co-crystal structures, molecular docking studies were therefore carried on antimicrobial

proteins. Therefore the proteins, *Staphylococcus epidermidis* in complex with ampicillin (PDB ID:3KP3)[21] and *Staphylococcus epidermidis* TcaR in complex with chloramphenicol (PDB ID:4EJV)[22] has been retrieved from the protein data bank to assess essential binding free energies along with its interaction into the antimicrobial proteins. Grid was centered on the respective crystal structures and all the synthesized molecules were docked into the active site using Glide docking in Schrodinger.[23] The essential drugs like properties were evaluated for the synthesized molecules using QikProp.[24]

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SUPPORTING INFORMATION

Supporting information associated with this article can be found, in the online version.

CONFLICT OF INTEREST

The authors confirm that there is no conflict of interest.

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