Synthesis, Molecular docking study and *in-vitro* microbial evaluation of some novel substituted pyrazole derivatives

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Abstract

The alarming rise of bacterial resistance is occurring worldwide and endangering the efficacy of antibiotics. Therefore, development of new and efficient antibacterial agents remains paramount. A series of novel pyrazole moiety was synthesized and characterized by means of 1H NMR and MS spectra. All the designed compounds subjected for docking studies, among the docked compounds, compound 18d possesses significant docking score **-8** K/cal when compared to standard drug ciprofloxacin. The compound 3d and 4d shows a significant docking score of -7.7 K/cal along. The remaining docked compound shows a docking score range from 6 to 9 K/cal along with one or two hydrogen bond interactions.The MIC value of all the synthesized compounds was evaluated by broth microdilution method using Mueller Hinton medium. Tested compounds showed variable activity against the tested Gram-positive and Gram-negative bacterial strains. Compounds 6d, 7d, 11d, 15d and 16d showed high activity against S. aureus and S. epidermidis and also exhibited bactericidal activity against this strain in MBC determination. The whole study was compared with Ciprofloxacin. Based on above binding this study may be concluded as the substitution in the pyrazalone molecules blocks the activity of DNA Gyrase B enzyme in bacterial organism.

Key word: Pyrazole, docking studies, Synthesis, Ciprofloxacin. MIC

1. Introduction

Antibacterial drugs are chemical compounds with the capability of preventing the growth and survival of bacteria in a selective manner while exhibiting minimal toxicities to host organisms [1]. There are two types of antibacterial drugs: antibiotics and antimicrobial agents. Antibiotics are natural compounds or synthetic analogues that are isolated from microorganisms [2]. Purely synthetic compounds with antibacterial properties are known as antimicrobial agents. Drug design methodologies are employed to guide structure activity relationships (SAR) of chemical structure and biological function towards the optimization of potency, biological efficacy, reduced toxicity, and clinically applicative pharmacological (ADME) properties [3]. Although bacteria contain upwards of 200 conserved essential proteins, the number of currently exploited biological targets remain limited12. Thus far, there have been several classes of compounds discovered with diverse mechanisms of action against pathogenic bacteria (**Figure 1**) [4,5]. Interestingly, the starting molecular platforms for most clinically useful antimicrobial drugs were discovered serendipitously. Medicinal chemistry programs were developed to rationally optimize these molecular platforms into clinically useful drugs [6].

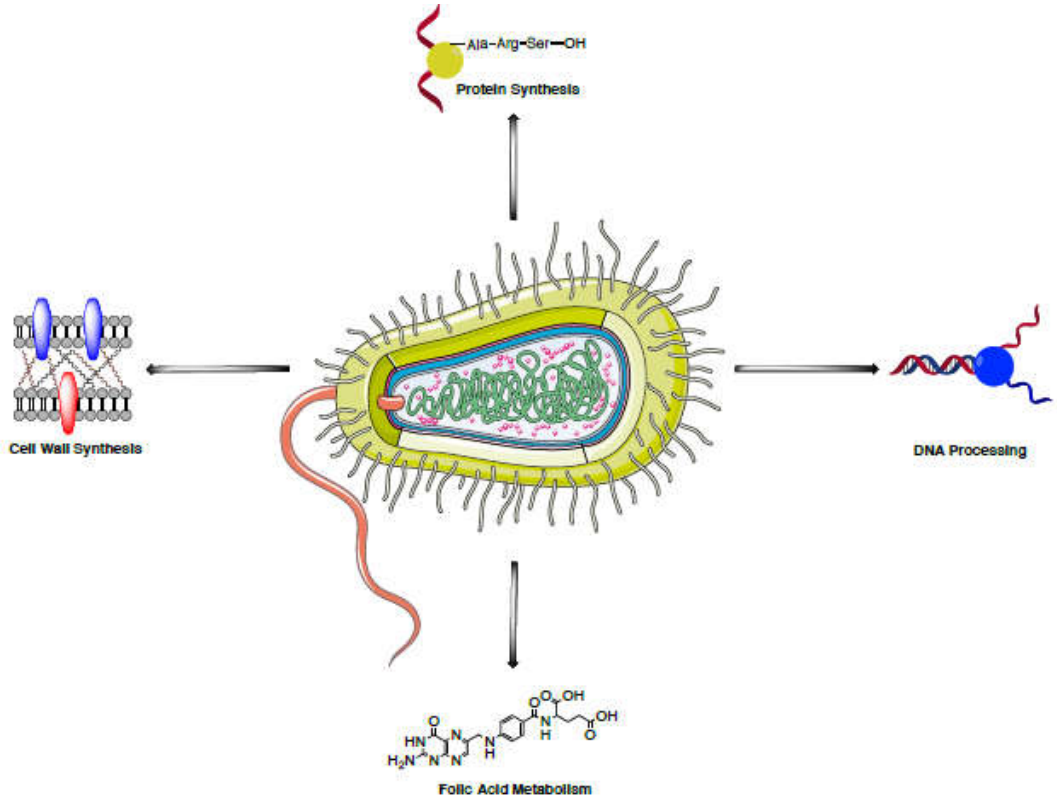


Figure 1. Biological pathways targeted by the major classes of antibacterial drugs.

Recently, researcher's attention has been paid to heterocycles containingnitrogen atom, particularly pyrazoles and their derivatives [7].They have been found to denote versatile biological activities suchas monoamine oxidase inhibitor [8,9], antihepatotoxicity [10,11],antileishmanial[12,13], anti-inflammatory[14], antiproliferative[15],tissue non-specific alkaline phosphatase inhibitor [16], cyclin-dependentkinase inhibitor [17], anticancer [18], antimicrobial [19], andantioxidant [20]. Development of novel chemical structures ofpyrazole derivatives is currently a trending topic due to their wonderfulbiological actions [21]. In this paper, we will focus on the synthesis of some novel pyrazole derivatives and related compounds andtheir performance as potent antimicrobial candidates.

2. Materials and Method

The Melting point were determined using open capillary tube method. The completion of the reaction was checked by thin layer chromatography using the solvent system Chloroform:Methanol(9:1). IR (cm-1) spectra (in KBr pellets) were recorded on a Shimadzu FT-IR spectrophotometer. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker Advance II using TMS as an internal standard. The Mass spectra were recorded on a LC-MS system.

2.1.In-silico molecular docking studies

2.1.1. Devices and materials

In the molecular scenario in the modern drug design, the docking is commonly used to understand the interaction between the target ligand-receptor and the target lead molecule's binding orientation with its protein receptor and is quite frequently used to detect the associations between the target components. The research work was done in-silico by utilizing bioinformatics tools. Also, we utilize some of the offline programming's like protein data bank (PDB) www.rcsb.org/pdb, PubChem database, Marvin sketch. The molecular docking studies were carried out through Discovery studio [22].

2.1.2. Preparation of protein

By utilizing the offline program protein data bank (PDB), we take the Topoisomerase II (PDB ID: 4FM7) was obtained from PDB website. From the protein (4FM7) we removed the crystal water, followed by the addition of missing hydrogens, protonation, ionization, energy minimization. The SPDBV (swiss protein data bank viewer) force field was applied for energy minimization. Prepared protein is validated by utilizing the Ramachandran plot [23].

2.1.3. Identification of active sites

Identification of active amino acid present in the protein is detected by using Protein-ligand interaction profile (PLIP) https://plip-tool.biotec.tu-dresden.de/plipweb/plip/index offline tool in google. From this, I found the active amino acid present in the protein [24].

2.1.4. Preparation of Ligands

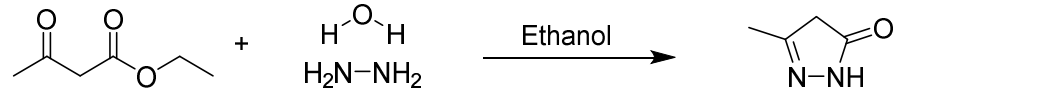
By utilizing the Marvin sketch tool, the molecules are designed in two and three-dimensional structures. After designed molecule, the structure was optimized in 3D optimization in Marvin sketch and saved as a pdb format [25].

2.2.Synthesis of designed compounds (Scheme 1)

2.2.1. Step-1: Synthesis of 5-methyl-2,4-dihydro-3H-pyrazol-5-one (A)

Ethyl acetoacetate (0.5 mol) was taken in a 250 mL conical flask and stirred magnetically during slow drop wise of a solution of (0.5 mol) hydrazine hydrate in 40 mL absolute ethanol. The temperature of the reaction mixture was increased during reaction so that temperature was regulated around 60^{O}C. A crystalline deposit was separated after stirring for 1h at 60^{O}C. The reaction mixture was cooled in an ice bath to complete the crystallization. After standing for completion of crystallization, it was filtered and the residue was recrystallized from cold alcohol.

Step 1

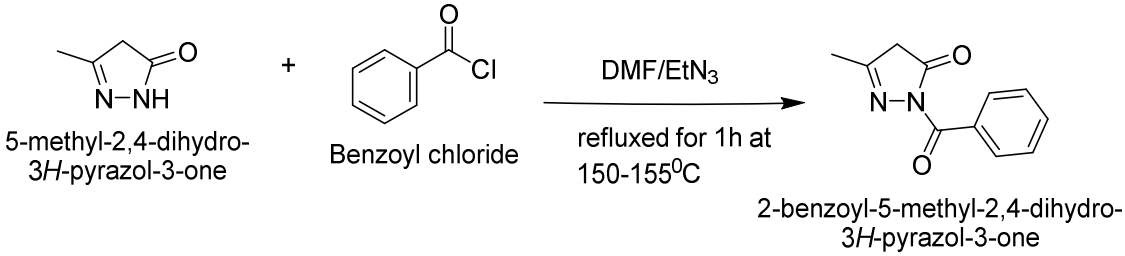


Ethyl acetoacetate Hydrazine hydrate 5-methyl-2,4-dihydro-3*H*-pyrazol-3-one

2.2.2. Step-2: Synthesis of 2-benzoyl-5-methyl-2,4-dihydro-3H-pyrazol-3-one (B)

A mixture of 3-methyl pyrazol-5-one (0.01mol) in 40 ml DMF was added to the mixture of substituted acid chloride (0.012 mol) in triethylamine (0.012mol). Reaction mixtures were refluxed for 1h at 150-155^{0}C until the starting material disappeared by TLC. After the reaction was completed, the precipitate formed upon cooling and it was filtered and recrystallized from ethanol to achieve the final compounds (B).

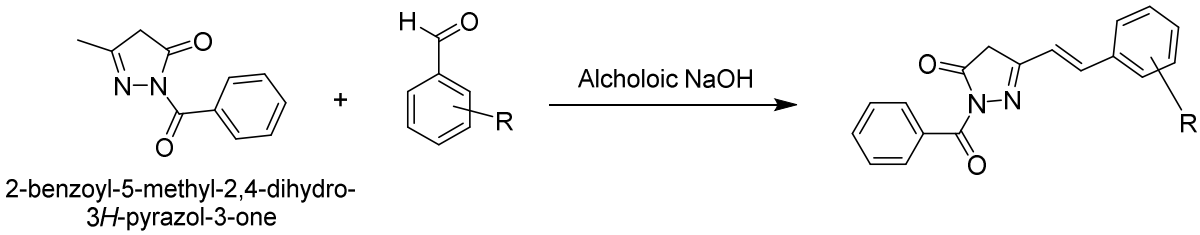
Step 2



2.2.3. Step-3: Synthesis of 3-methyl pyrazol-5-one derivatives (C)

2-benzoyl-5-methyl-2,4-dihydro-3H-pyrazol-3-one (0.01 mol) was taken in a 100 mL round bottomed flask, and then 50 mL of freshly prepared 20% sodium hydroxide alcoholic solution was poured into it. The mixture was stirred with magnetic stirrer for 30 min. Substituted aromatic and aliphatic aldehyde (0.01 mol) was added to the reaction mixture and kept under stirring for 8 h. The reaction mixture was transferred into crushed ice and neutralized with dilute hydrochloric acid to precipitate the product. Residue was filtered, dried and recrystallized from ethanol. Similarly, other compounds were prepared with some change in refluxing time and reaction workup.

Step 3



2.2.3.1.(E)-2-benzoyl-5-styryl-2,4-dihydro-3H-pyrazol-3-one (C1)

$C_{18}H_{14}N_2O_2$; White solid; 186 – 191^{O}C; IR KBr pellet (cm^{-1}): 3073 (CH stretching aromatic); 2732 (CH stretching alkane); 1615 (C=0 stretching ketone); 1407 (C - N bending); 850 (aromatic ring); ^{1}H NMR (500 MHz, DMSO) δ: 7.89 (m, 1H), 7.63 (m, 2H), 7.44 (m, 1H), 7.39 (m, 1H), 7.53 (m, 1H), 7.21 (s, 2H), 7.09 (dd, J = 8.7, 2.4 Hz, 2H), 3.36 (s, 2H); ^{13}C NMR (126 MHz, DMSO) δ: 170.31, 157.06, 155.82, 154.34, 134.70, 134.50, 129.99, 129.36, 129.30, 128.79, 128.02, 127.88, 127.26, 127.02, 126.63, 124.45, 123.71, 119.40, 119.01, 108.40, 107.37, 30.60. Elemental Analysis: C, 74.47; H, 4.86; N, 9.65; O, 11.02; Actual mass: 290, Found mass: 290.

2.2.3.2.(E)-2-benzoyl-5-(4-nitrostyryl)-2,4-dihydro-3H-pyrazol-3-one (2d)

$C_{18}H_{13}N_3O_4$; White solid; 153 – 156^{O}C; IR KBr pellet (cm^{-1}): 3073 (CH stretching aromatic); 2732 (CH stretching alkane); 1580 (C=0 stretching ketone); 1407 (C - N bending); 918 (aromatic ring); ^{1}H NMR (500 MHz, DMSO) δ: 7.66 (d, J = 25.9 Hz, 3H), 7.61 (m, 2H), 7.42 (s, 4H), 7.21 (s, 1H), 7.01 (s, 2H), 6.10 (s, 1H), 3.34 (m, 2H).; ^{13}C NMR (126 MHz, DMSO) δ: 170.31, 134.70, 134.50, 129.99, 129.36, 129.30, 128.79, 128.02, 127.88, 127.26, 127.02, 126.63, 124.45, 123.71, 119.40, 119.01, 30.60. Elemental Analysis: C, 64.48; H, 3.91; N, 12.53; O, 19.09.; Actual mass: 335, Found mass: 335.

2.2.3.3.(E)-2-benzoyl-5-(4-chlorostyryl)-2,4-dihydro-3H-pyrazol-3-one (3d)

$C_{18}H_{13}ClN_2O_2$; White solid; 176 – 179^{O}C; IR KBr pellet (cm^{-1}): 3073 (CH stretching aromatic); 2732 (CH stretching alkane); 1615 (C=0 stretching ketone); 1482 (C - N bending); 850 (aromatic ring); ^{1}H NMR (500 MHz, DMSO) δ: 7.66 (d, J = 25.9 Hz, 3H), 7.61 (m, 1H), 7.42 (s, 1H), 7.21 (s, 1H), 7.01 (s, 2H), 6.10 (s, 1H), 3.29 (m, 3H); ^{13}C NMR (126 MHz, DMSO) δ: 170.31, 134.70, 134.50, 129.99, 129.36, 129.30, 128.79, 128.02, 127.88, 127.26, 127.02, 126.63, 124.45, 123.71, 119.40, 119.01, 30.60; Elemental Analysis: C, 66.57; H, 4.03; Cl, 10.92; N, 8.63; O, 9.85; Actual mass: 324, Found mass: 326 (M+2).

2.2.3.4.(E)-2-benzoyl-5-(2-hydroxystyryl)-2,4-dihydro-3H-pyrazol-3-one (4d)

$C_{18}H_{14}N_2O_3$; White solid; 157 – 161^{O}C; IR KBr pellet (cm^{-1}): 3073 (CH stretching aromatic); 2732 (CH stretching alkane); 1615 (C=0 stretching ketone); 1407 (C - N bending); 850 (aromatic ring); ^{1}H NMR (500 MHz, DMSO) δ: 10.83 (s, 1H), 7.66 (d, J = 25.9 Hz, 1H), 7.61 (m, 1H), 7.42 (s, 2H), 7.21 (s, 1H), 7.01 (s, 1H), 6.10 (s, 1H), 3.34 (m, 2H); ^{13}C NMR (126 MHz, DMSO) δ: 170.31, 134.70, 134.50, 129.99, 129.36, 129.30, 128.79, 128.02, 127.88, 127.26, 127.02, 126.63, 124.45, 123.71, 119.40, 119.01, 30.60; Elemental Analysis: C, 70.58; H, 4.61; N, 9.15; O, 15.67.; Actual mass: 306, Found mass: 306.

2.2.3.5.(E)-2-benzoyl-5-(2-bromo-6-hydroxystyryl)-2,4-dihydro-3H-pyrazol-3-one (6d)

$C_{18}H_{13}BrN_2O_3$; White solid; 158 – 163^{O}C; IR KBr pellet (cm^{-1}): 3073 (CH stretching aromatic); 2732 (CH stretching alkane); 1615 (C=0 stretching ketone); 1407 (C - N bending); 850 (aromatic ring); ^{1}H NMR (500 MHz, DMSO) δ: 9.67 (s, 2H), 8.04 (m, 2H), 7.70 (s, 1H), 7.56 (s, 2H), 7.27 (d, J = 24.7 Hz, 105H), 7.08 (m, 2H), 3.33 (m, 1H); ^{13}C NMR (126 MHz, DMSO) δ: 172.53, 164.76, 144.14, 143.88, 135.76, 134.68, 132.09, 131.58, 125.02, 120.92,

120.90, 120.34, 40.88; Elemental Analysis: C, 60.19; H, 4.38; N, 14.04; O, 10.69; S, 10.71; Actual mass: 384, Found mass: 386 (M+2).

2.2.3.6.(E)-2-benzoyl-5-(2-(furan-2-yl)vinyl)-2,4-dihydro-3H-pyrazol-3-one (7d)

$C_{16}H_{12}N_2O_3$; White solid; 172 – 174^{O}C; IR KBr pellet (cm^{-1}): 3073 (CH stretching aromatic); 2732 (CH stretching alkane); 1615 (C=0 stretching ketone); 1407 (C - N bending); 850 (aromatic ring); ^{1}H NMR (500 MHz, DMSO) δ: 8.47 (s, 1H), 8.42 (m, 2H), 8.26 (d, J = 13.2 Hz, 1H), 8.13 (s, 2H), 7.98 (d, J = 23.9 Hz, 1H), 7.60 (s, 1H), 3.29 (s, 1H); ^{13}C NMR (126 MHz, DMSO) δ: 170.30, 155.81, 154.37, 134.50, 130.00, 129.31, 128.03, 127.26, 127.03, 124.45, 119.01, 32.23.; Elemental Analysis: C, 68.56; H, 4.32; N, 9.99; O, 17.12.; Actual mass: 280, Found mass: 281 (M+1).

2.2.3.7.(E)-2-benzoyl-5-(2-(pyridin-2-yl)vinyl)-2,4-dihydro-3H-pyrazol-3-one (11d)

$C_{17}H_{13}N_3O_2$; White solid; 166 – 170^{O}C; IR KBr pellet (cm^{-1}): 3073 (CH stretching aromatic); 2732 (CH stretching alkane); 1615 (C=0 stretching ketone); 1407 (C - N bending); 850 (aromatic ring); ^{1}H NMR (500 MHz, DMSO) δ: 8.47 (s, 1H), 8.35 (m, 2H), 8.26 (d, J = 13.2 Hz, 1H), 8.13 (s, 1H), 7.98 (d, J = 23.9 Hz, 1H), 7.60 (s, 1H), 3.29 (s, 1H).; ^{13}C NMR (126 MHz, DMSO) δ: 170.30, 155.81, 154.37, 134.50, 129.99, 129.31, 128.03, 127.26, 127.03, 124.45, 119.01, 108.41, 33.13.; Elemental Analysis: C, 70.09; H, 4.50; N, 14.42; O, 10.98; Actual mass: 291, Found mass: 291.

2.2.3.8. (E)-2-benzoyl-5-(4-methoxystyryl)-2,4-dihydro-3H-pyrazol-3-one (15d)

$C_{19}H_{16}N_2O_3$; White solid; 148 – 152^{O}C; IR KBr pellet (cm^{-1}): 3073 (CH stretching aromatic); 2732 (CH stretching alkane); 1615 (C=0 stretching ketone); 1407 (C - N bending); 850 (aromatic ring); ^{1}H NMR (500 MHz, DMSO) δ: δ 7.85 (s, 2H), 7.73 (s, 1H), 7.66 (s, 1H), 7.57 (s, 1H), 7.35 (s, 1H), 7.08 (m, 1H), 6.94 (d, J = 8.0 Hz, 1H), 5.41 (s, 2H), 3.12 (d, J = 6.2 Hz, 1H); ^{13}C NMR (126 MHz, DMSO) δ: δ 155.10, 154.28, 144.59, 139.51, 131.99, 129.79, 127.66, 127.05, 126.64, 124.26, 118.52, 111.05, 107.83, 65.03, 40.07; Elemental Analysis: C, 71.24; H, 5.03; N, 8.74; O, 14.98; Actual mass: 320, Found mass: 320.

2.2.3.9.Phenyl (E)-4-(2-(1-benzoyl-5-oxo-4,5-dihydro-1H-pyrazol-3-yl)vinyl)benzoate (16d)

$C_{25}H_{18}N_2O_4$; White solid; 148 – 152^{O}C; IR KBr pellet (cm^{-1}): 3073 (CH stretching aromatic); 2732 (CH stretching alkane); 1615 (C=0 stretching ketone); 1407 (C - N bending); 850 (aromatic ring); ^{1}H NMR (500 MHz, DMSO) δ: 7.87 (s, 1H), 7.66 (s, 2H), 7.49 (s, 2H), 7.32 (s, 1H), 7.10 (s, 1H), 6.95 (d, J = 33.0 Hz, 1H), 6.17 (s, 2H), 6.09 (s, 1H), 3.33 (s, 1H); ^{13}C

NMR (126 MHz, DMSO) δ: 170.30, 134.50, 129.99, 129.31, 128.03, 127.26, 127.03, 125.84, 125.54, 124.45, 119.01, 108.41, 33.13; Elemental Analysis: C, 73.16; H, 4.42; N, 6.83; O, 15.59; Actual mass: 410, Found mass: 413 (M+3).

2.2.3.10. (E)-2-benzoyl-5-(4-((phenylamino)methyl)styryl)-2,4-dihydro-3H-pyrazol-3-one (18d)

$C_{25}H_{21}N_{23}O_2$; White solid; 172 – 176^{O}C; IR KBr pellet (cm^{-1}): 3073 (CH stretching aromatic); 2732 (CH stretching alkane); 1615 (C=0 stretching ketone); 1407 (C - N bending); 850 (aromatic ring); ^{1}H NMR (500 MHz, DMSO) δ: 7.87 (s, 1H), 7.66 (s, 2H), 7.49 (s, 2H), 7.32 (s, 1H), 7.10 (s, 1H), 6.95 (d, J = 33.0 Hz, 1H), 6.17 (s, 2H), 6.09 (s, 1H), 3.87 (s, 2H), 3.33 (s, 2H).; ^{13}C NMR (126 MHz, DMSO) δ: 168.50, 156.63, 148.92, 148.40, 125.65, 122.36, 121.60, 109.59, 109.19, 107.64, 106.93, 106.34, 103.91, 102.56, 102.06, 64.39, 26.57.; Elemental Analysis: C, 75.93; H, 5.35; N, 10.63; O, 8.09; Actual mass: 395, Found mass: 395.

2.3.*In vitro* antibacterial screening of synthesized compounds

2.3.1. Determination of minimum inhibitory concentration (MIC)

The newly synthesized compounds were screened against two bacterial strains, *Staphyloccocus aureus* NCIM 5021 and *Staphylococcus epidermidis* NCIM 2493 following the guidelines of Clinical Laboratories Standard Institute (CLSI 2007) [26]. The tests were performed in Mueller Hinton medium (Hi-media) by broth micro dilution method, in 96-well micro titer plates. All the compoundswere dissolved in sterile dimethyl sulfoxide (DMSO) to screen their antibacterial activity. Ciprofloxacin was dissolved in sterile DMSO and used as positive control, while sterile DMSO served as a negative control. The final volume for MIC protocols was 100 µL, whereas DMSO concentration in assays well was less than 1%. Synthesized compounds were tested in the concentration range of 19.74 to 221.98 µM. The bacterial suspensions at 105 Colony Forming Unit/mL (CFU/mL) concentrations were inoculated to the corresponding wells. Following inoculation 96- well microtitre plates was incubation at 37 °C for 24 h. Plates were then agitated and read for the absorbance at a wavelength of 600 nm. All tests were performed in triplicate and the results were taken as a mean. After MIC determination, 50 µL aliquot from each well was sub-cultured on Mueller-Hinton agar plates and were further incubated at 37 °C for 24 h. All experiments were performed in triplicate. The wells are visually inspected for turbidity under black background to determine the growth of the organism. The wells containing the antimicrobial agent in concentration sufficient to inhibit the growth remain clear. In experimental terms the MIC is

the concentration of the drug present in the last clear well, i.e. the well having the lowest antibiotic concentration in which growth is not observed.

2.3.2. Determination of Minimum bactericidal concentration (MBC)

The Minimum Bactericidal Concentration (MBC) is the lowest concentration of an antibacterial agent required to kill a bacterium over a fixed, somewhat extended period, such as 18 hours or 24 hours, under a specific set of conditions. It can be determined from the broth dilution of MIC tests by sub-culturing to agar plates that do not contain the test agent. The MBC is identified by determining the lowest concentration of antibacterial agent that reduces the viability of the initial bacterial inoculum by a pre-determined reduction such as ≥99.9%. The MBCs were determined by sub-culturing aliquots (20 µl) from the bacterial suspension wells with no visible bacterial growth at concentrations greater than the defined MlC and from control wells onto nutrient agar plates. The plates were incubated aerobically at 37 °C for 24 hours and colonies were comparatively counted with the starting inoculum. MBC was determined as the concentration at which a ≥99.9% decrease in bacterial counts (i.e., 3 log l0 reduction in cfu/ml) was achieved or the lowest concentrations that did not produce any bacterial growth in the agar plates were regarded as MBC values. All experiments were repeated in triplicate for each strain. The ratio MBC/MIC ≤2 indicates bactericidal activity, while MBC/MIC ratio ≥4 shows bacteriostatic activity of test compound.

3. RESULTS AND DISCUSSION

3.1. Molecular docking

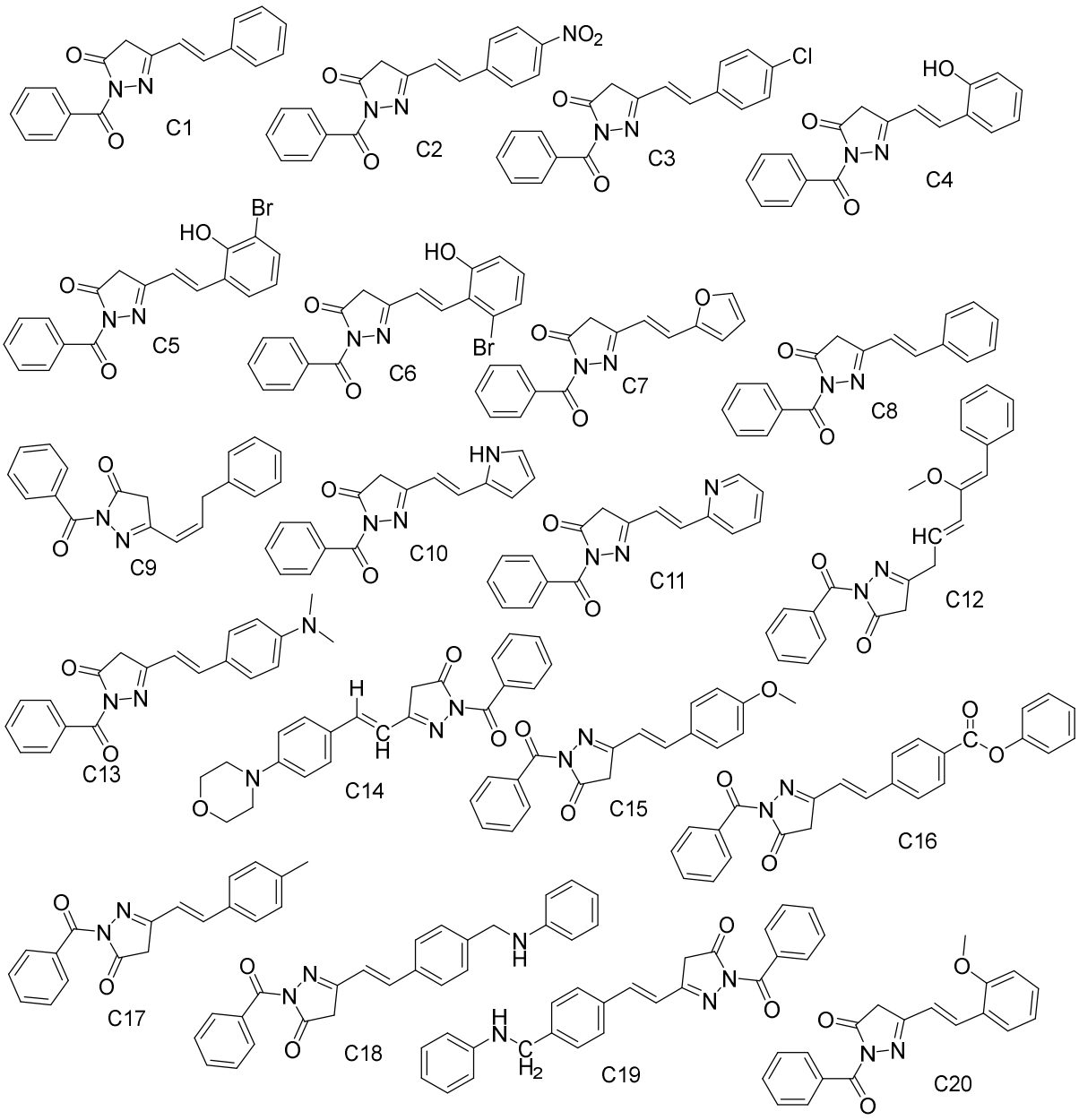


Figure 2. Chemical structure of newly designed compounds

The *in-silico* docking study of the 20 designed molecules **(Figure 2)** to the enzyme's active sites was performed by the C dock module of Discovery studio to determine the binding affinities of the ligands. The designed compounds were docked towards DNA gyraseB (PDB ID: 3U2D) in order to ascertain their DNA gyraseB inhibition activity against inflammation. All the compounds were exhibited good affinity for the receptor when compared with ciprofloxacin with MurD (PDB ID: 3U2D) inhibitory activity as an anti-microbial agent. The Docking scores of docking studies against DNA gyraseB (PDB ID:

3U2D) are shown in **Table 1**. From the in-silico docking results, it is evident that the interactions are mainly lipophilic factors due to the presence of aromatic heterocyclic rings.

Among the docked compounds, compound 18d possesses significant docking score **-8** K/cal when compared to standard drug ciprofloxacin. The compound 18d shows 2 hydrogen bonds between the amino acids ALA 47 and GLU 45. The compound 3d and 4d shows a significant docking score of -7.7 K/cal along with 2 hydrogen bonds with amino acids ALA 47 and GLU 45. The remaining docked compound shows a docking score range from 6 to 9 K/cal along with one or two hydrogen bond interactions. **Figures 3 - 12** shows the docking pose of the designed compounds. Based on the docking score the derivatives 1d, 2d, 3d, 4d, 6d, 7d, 11d, 15d, 16d and 18d are selected for the synthesis by conventional method.

Table 1. Docking score of designed compounds

Compound code	**Binding Affinity**
1d	**-7.1**
2d	**-7.6**
3d	**-7.7**
4d	**-7.7**
5d	-5.1
6d	**-7**
7d	**-7.4**
8d	-6
9d	-6.3
10d	-6.3
11d	**-7.8**
12d	-6.4
13d	-6.5
14d	-6.8
15d	**-7.6**
16d	**-7.7**
17d	-6.2
18d	**-8**
19d	-5.9
20d	-5.6

Standard (Ciprofloxacin)	**-9.58**

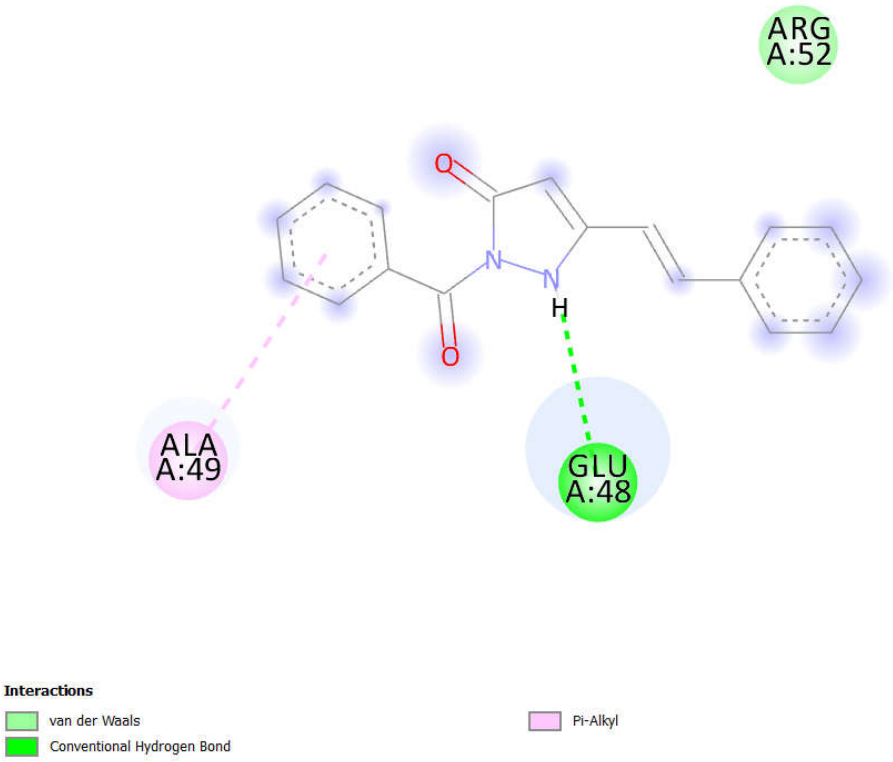


Figure 3. 2D docking pose of compound C1

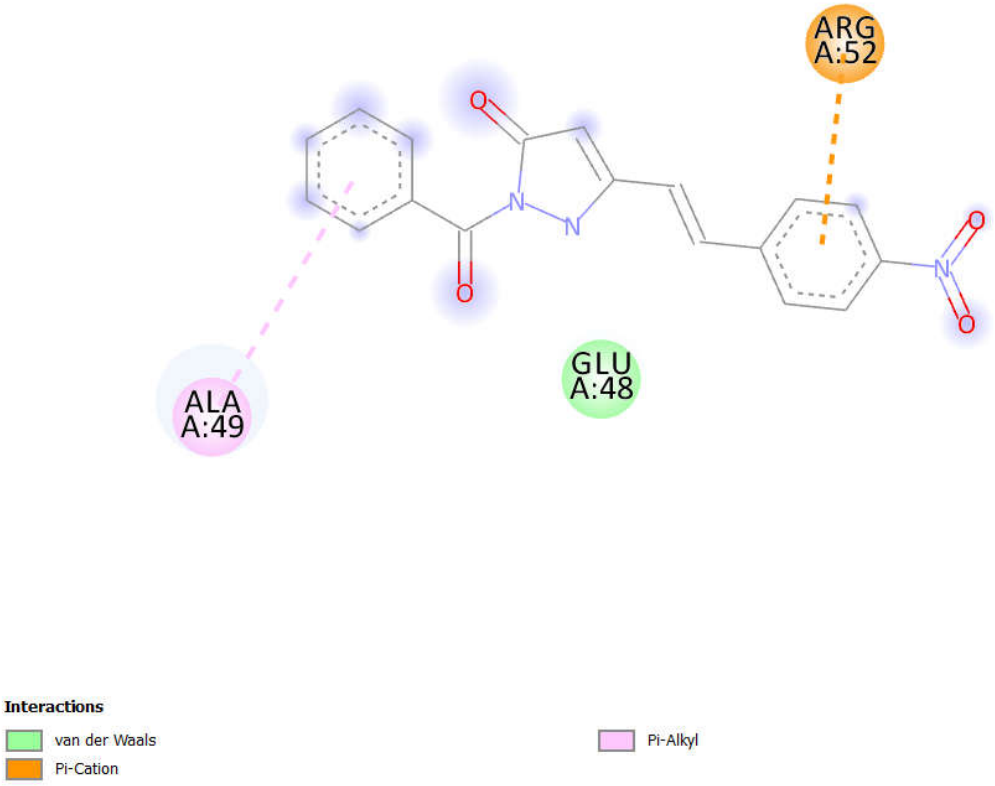


Figure 4. 2D docking pose of compound C2

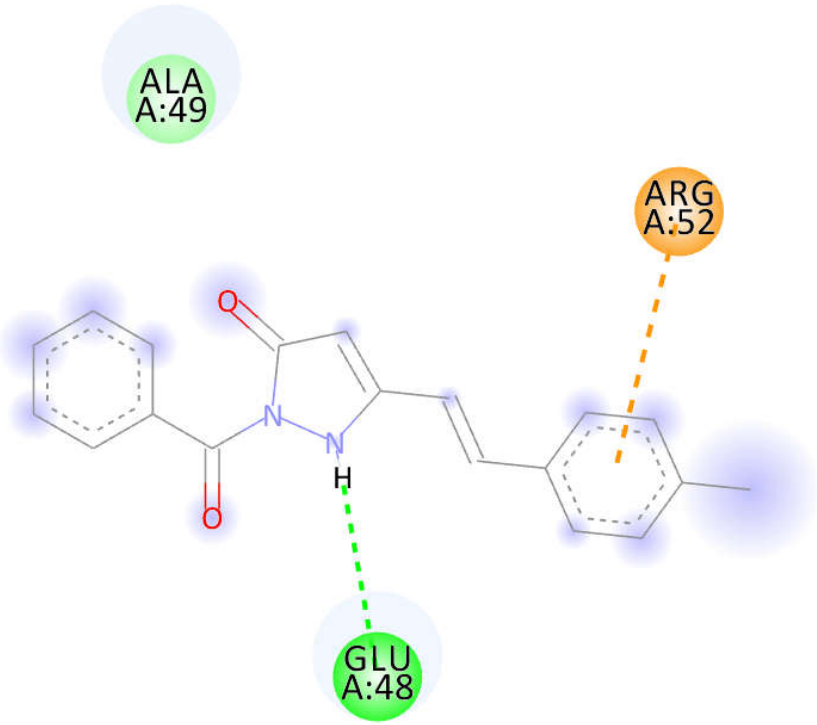


Figure 5. 2D docking pose of compound C3

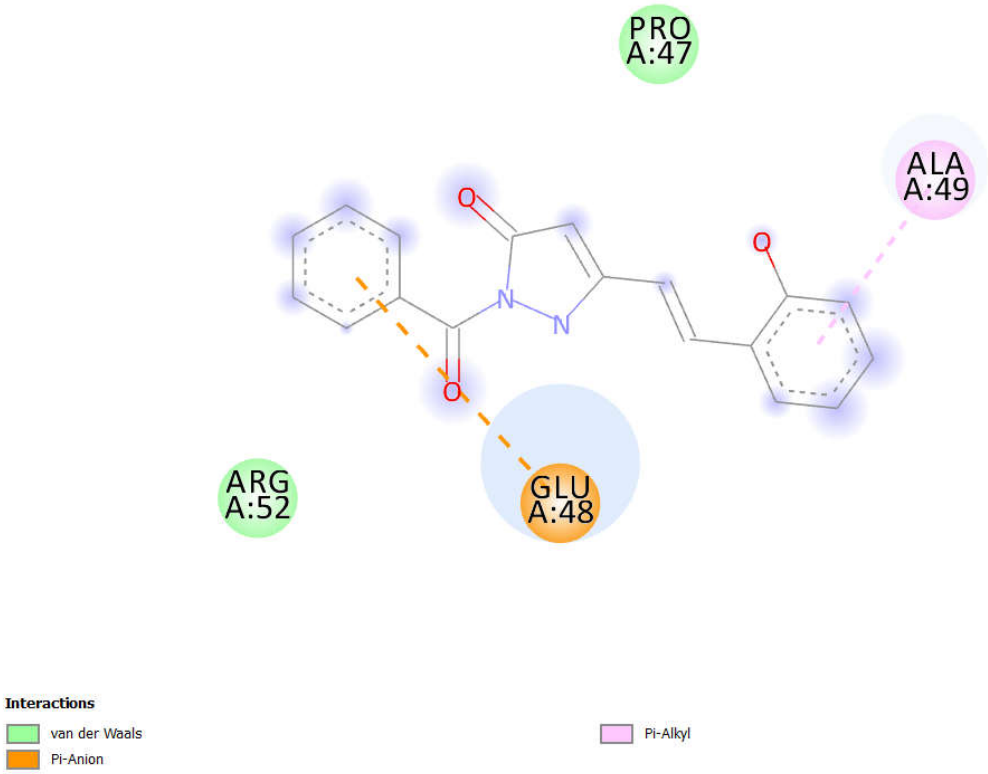


Figure 6. 2D docking pose of compound C4

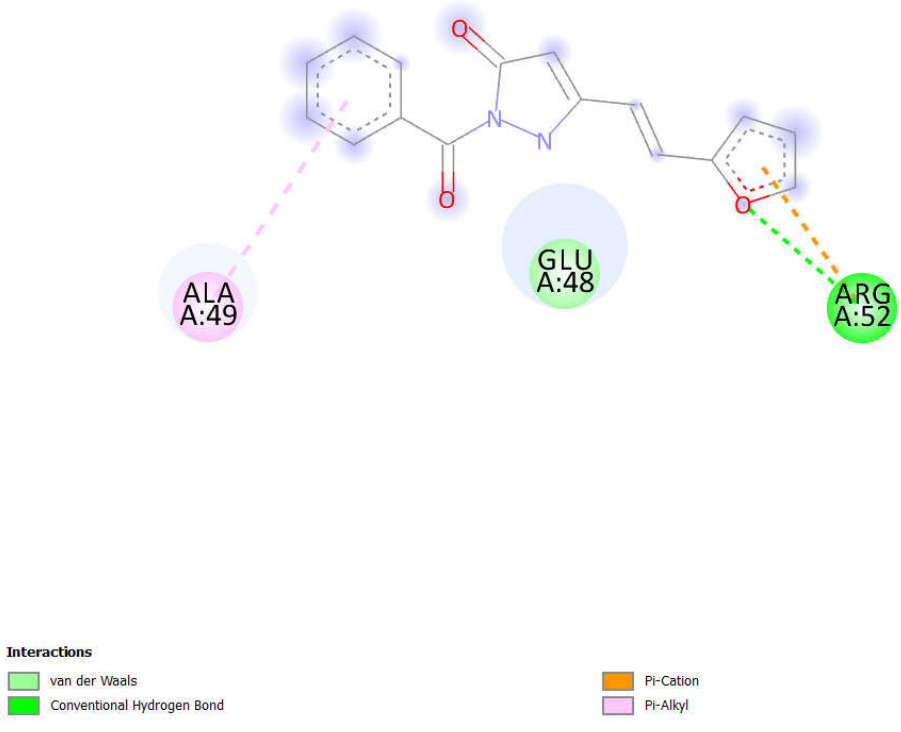


Figure 7. 2D docking pose of compound C7

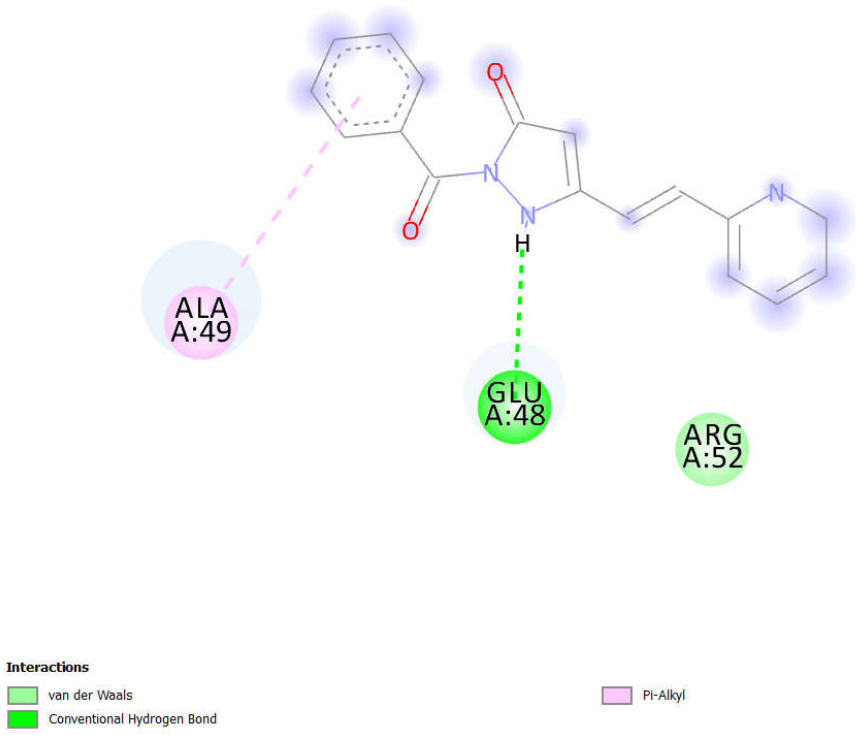


Figure 8. 2D docking pose of compound C11

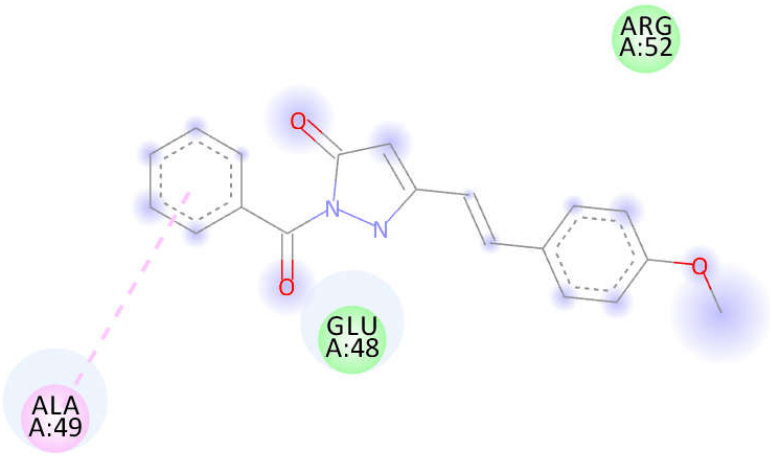


Figure 9. 2D docking pose of compound C15

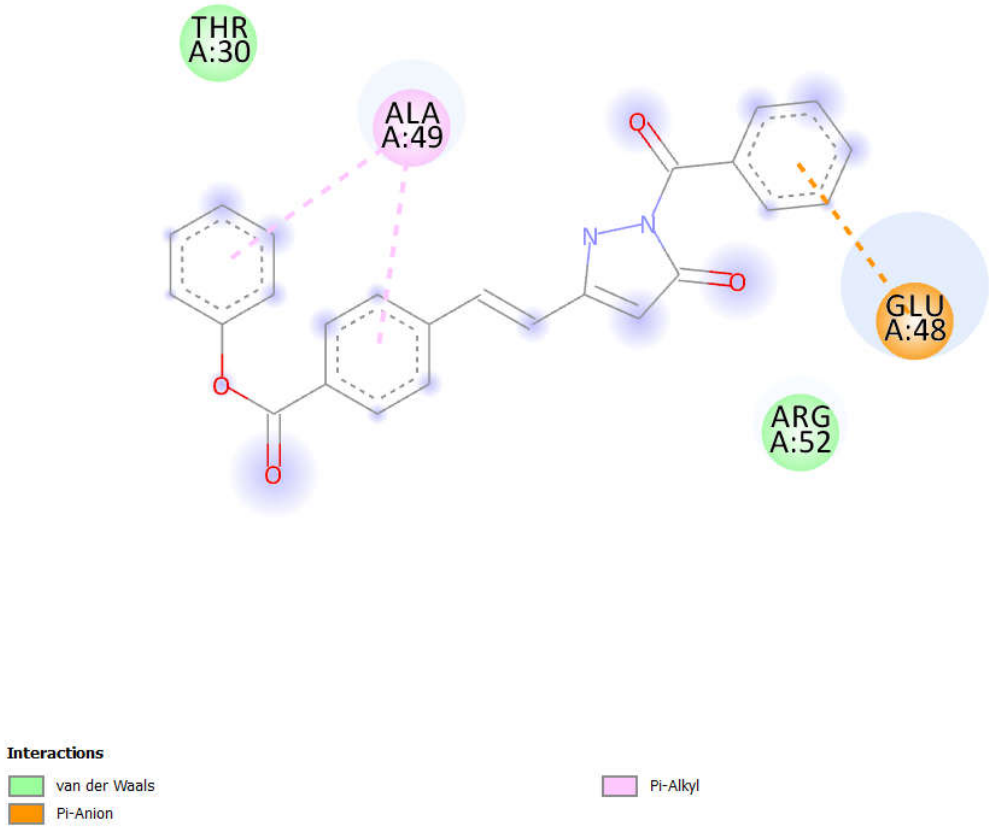


Figure 10. 2D docking pose of compound C16

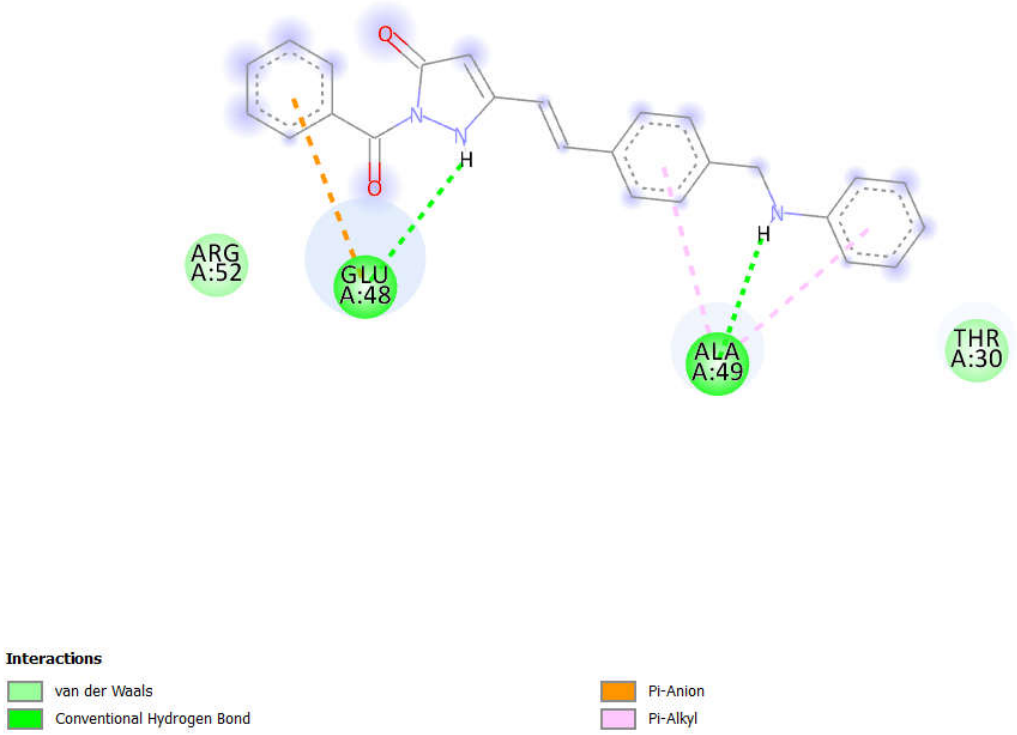


Figure 11. 2D docking pose of compound C18

3.2. Synthetic work

5-methyl-2,4-dihydro-3H-pyrazol-5-one (A) was prepared byethyl acetoacetate was taken in a 250 mL conical flask and stirred magnetically during slow drop wise of a solution of hydrazine hydrate in absolute ethanol. A crystalline deposit was separated after stirring for 1h at 60^{O}C. The reaction mixture was cooled in an ice bath to complete the crystallization. Further the compound A is dissolved in DMF and this mixture was added to substituted acid chloride in triethylamine. Reaction mixtures were refluxed until the starting material disappeared by TLC. After the reaction was completed, the precipitate formed upon cooling and it was filtered and recrystallized from ethanol to achieve the 2-benzoyl-5-methyl-2,4-dihydro-3H-pyrazol-3-one (B). further the compound B react with freshly prepared 20% sodium hydroxide alcoholic solution was poured into it. The mixture was stirred with magnetic stirrer for 30 min. Substituted aromatic and aliphatic aldehyde (0.01 mol) was added to the reaction mixture and kept under stirring for 8 h. The reaction mixture was transferred into crushed ice and neutralized with dilute hydrochloric acid to precipitate the product. Residue was filtered, dried and recrystallized from ethanol.The IR spectrum of the final synthesized compounds showed absorption bands around 2850 – 2950 shows the presence of CH stretching alkane group, the peak 2030 shows presence of CH stretching aromatic and the peak at 1489–1464 cm^{-1} for CH_2, 1379–1344 cm^{-1} for CH_3, and 800–700 cm^{-1} for aromatic rings. These compounds also exhibited appropriate peaks at corresponding ppm in their ^{1}H NMR spectra. The ^{1}H NMR spectra of the synthesized compounds revealed a singlet signal a signal at 7.5-8.5 for H of aromatic ring. The ^{13}C NMR spectra of synthesized compounds revealed a signal at 160 – 175 for carbonyl carbon, and a signal at 120 – 145 for aromatic carbon. The corresponding molecular ion peaks in the LC–MS spectra were in conformity with the assigned structures. All the synthesized compounds were subjected to in vitro anti-bacterial studies.

3.3. DeterminationofMinimuminhibitoryconcentration(MIC)

Antibacterial activity of synthesized compoundswasevaluated by broth microdilution method (CLSI 2007) using Mueller Hinton medium(Hi-media). The MIC value of tested compounds is presented, respectively in **Table 2**andcomparedwiththestandarddrugsciprofloxacin. All the tested compoundsexhibited variable activity against the tested Gram-positive andGram-negative bacterial strains. Among all tested compounds **6d, 7d, 11d, 15d and 16d**exhibited maximum activity against *S. aureus* NCIM 5021 and *S. epidermidis NCIM 2493* with MIC of 45 – 50 µM.

against*S.aureus*NCIM5022comparedtothestandarddrugsciprofloxacin(MIC6.3µM). Based on the study the compound 17 and 18 which is substituted with a bulky groups produce a significant MIC value compared with standard drug.

Table 2. Minimuminhibitoryconcentration of tested compounds

Compound	**Minimuminhibitoryconcentration(µM)***	
	Staphyloccocus aureus NCIM 5021	***Staphylococcus epidermidis NCIM 2493***
1d	78.2±12.5	60.64±51.0
2d	161.32±3.4	133.44±6.4
3d	119.32±3.6	122.64±5.2
4d	122.18±4.2	138.31±8.3
6d	81.21±4.1	98.65±6.7
7d	45.34±2.8	39.57±8.9
11d	74.28±5.3	70.65±7.1
15d	60.64±51.0	35.64±5.4
16d	33.44±6.4	22.18±4.2
18d	122.64±5.2	191.32±3.6
Ciprofloxacin	6.18±0.82	6.33±0.52

*Valuesare mean± SEM(*n*=3).

3.4. Determination of Minimum Bactericidal Concentration (MBC)

MBC of tested compounds is reported in **Table 3**. It is evident that all the tested compounds expect compound 1d are bactericidal (MBC/MIC ≥2) against S. aureus NCIM 5021. In the case of S. epidermidis, the compounds 1d, 2d, 4d, and 11d possess the bacteriostatic activity and remaining compound have bactericidal activity on selected strain. This study was compared with a reference drug ciprofloxacin.

Table 3. Represents minimum bactericidal concentration (MBC) and MBC/MIC ratio of title molecules against selected bacterial strains

Compound	**MBC & MBC: MIC**	***Staphyloccocus aureus NCIM 5021***	***Staphylococcus epidermidis* NCIM 2493**

1d	MBC	103.23±1.4	109.45±0.54
	MBC: MIC	4	4
2d	MBC	97.49±0.25	92.68±0.54
	MBC: MIC	2	4
3d	MBC	99.33±1.25	106.81±0.2
	MBC: MIC	2	2
4d	MBC	60.25 ± 4.7	69.35±0.25
	MBC: MIC	2	4
6d	**MBC**	**65.51±0.54**	**69.35±0.97**
	MBC: MIC	**2**	**2**
7d	**MBC**	**52.06±0.54**	**67.53±0.25**
	MBC: MIC	**2**	**2**
11d	MBC	67.36±0.54	81.22±0.25
	MBC: MIC	2	4
15d	MBC	93.77±0.54	191.8±0.2
	MBC: MIC	2	2
16d	MBC	87.68±0.97	93.77±0.25
	MBC: MIC	2	2
18d	MBC	127.43±0.54	154.43±1.4
	MBC: MIC	2	2
Ciprofloxacin	MBC	2.1±0.45	1.9±0.78
	MBC: MIC	1	<1

4. SUMMARY AND CONCLUSION

In the present work we designed the novel set of pyrazole molecule as GyraseD inhibitors. All the designed compounds were subjected to molecular docking studies using discovery studio software.Based on docking score all the compounds show significant docking score among them compound 1d, 2d, 3d, 4d, 6d, 7d, 11d, 15d, 16, and 18d shows top ten compound. Further we selected these compounds for synthesis in conventional method. Based on the scheme presented in material method section, the designed compounds were synthesized and purified by the recrystallization techniques. High yields (80-95%) were obtained under relatively milder reaction conditions using N,N- dimethylformamide and

acetone as solvent.All our synthesized compounds were purified by column chromatography and characterized by FT-IR, ^{1}H-NMR, ^{13}C-NMR and LC-MS spectral data. In the ^{1}H-NMR spectra splitting patterns for the aromatic protons were observed to be in agreement with the substitution pattern of respective compounds. In the ^{13}C-NMR spectrum of synthesized compounds the carbonyl carbon of benzamide, azomethine carbon of benzothiazole and aromatic SP^2 hybridized carbon signals appeared in the expected region.The MIC value of all the synthesized compounds was evaluated by broth microdilution method using Mueller Hinton medium. Tested compounds showed variable activity against the tested Gram-positive and Gram-negative bacterial strains. Compounds 6d, 7d, 11d, 15d and 16d showed high activity against S. aureus and S. epidermidis and also exhibited bactericidal activity against this strain in MBC determination. The whole study was compared with Ciprofloxacin.Based on above binding this study may be concluded as the substitution in the pyrazalone molecules blocks the activity of DNA Gyrase B enzyme in bacterial organism.

Disclosure statement

The authors declare that there is no conflict of interest.

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