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SYNTHESIS, *IN VITRO* ANTIBACTERIAL ACTIVITY AND DOCKING STUDIES OF NEWER PYRAZOLE DERIVATIVES

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ABSTRACT

A series of pyrazole derivatives have been synthesized. The structures of novel compounds were characterized by ^{13}CNMR, ^{1}HNMR, IR and Mass. The purpose of this study was to investigate the antibacterial activity of newly developed pyrazole derivatives against two types of gram-positive bacteria and three types of gram-negative bacteria. The antibacterial activity of quantitatively prepared novel compounds is evaluated by the disc-diffusion method against *Staphylococcus aureus (S.a), Bacillus subtilis (B.s), Escherichia coli (E.c), Klebsiella pneumoniae (K.p)* and *Proteus vulgaris (P.v)*. Most of the compounds showed crucial antibacterial behavior against gram-positive and gram-negative bacteria. Furthermore, molecular docking of compounds A2 and B2 compounds into the active binding site of crystal structure of *Escherichia coli* MurB enzyme (PDB Id: 1MBT). A key enzyme in the peptidoglycan biosynthesis was performed to gain a comprehensive understanding into plausible binding modes and docking interactions of the ligands. Docking results indicated that the compounds A2 and B2 have considerable binding energies towards the active site of *Escherichia coli* MurB.

Keywords: Pyrazole, Antibiotic, Antibacterial activity, Molecular docking study.

INTRODUCTION

Pyrazole derivatives are well established in the literature as important biologically active heterocyclic compounds. These derivatives are the subject of many research studies due to their wide spread potential biological activities such as anti-inflammatory[1], anti-microbial[2], anti-viral[3], anti-tumour[4,5], anti-convulsant[6], anti-depressant.[7] Some of 4,5-dihydro-1H-pyrazoles possess important pharmacological activities such as anti-tumor[8] and anti-inflammatory activities.[9] Few of oxime containing pyrazole derivatives exhibit regulators for apoptosis and autophagy in A549 Lung cancer cells.[10] In view of the above mentioned facts, it was envisaged that this active pharmacophore would generate novel molecular templets which are likely to exhibit interesting biological properties. In continuation of our interest in the synthesis of biologically active heterocycles, we report herein the synthesis and anti bacterial activity of some newer pyrazoles. This combination was suggested in an attempt to investigate the influence of such structure variation on the anticipated biological activities, hoping to add some synergistic biological significance to the target molecules.

MATERIALS AND METHODS

General Procedures and Materials

Melting points were recorded in open capillary and were uncorrected. IR spectra (KBr) were obtained using a Bruker WM-4(X) spectrometer (577 Model). Mass spectral data were acquired by using a commercial LCQ ion trap mass spectrometer (Thermo Finnigan, San Jose, CA, USA) equipped with an ESI source. ^{1}H NMR (300 MHz, DMSO-d_6) and ^{13}C NMR (75 MHz, DMSO-d_6) spectra were recorded on a Varian FT 300 MHz NMR Spectrometer. All the starting materials were obtained from Aldrich or Fluka used as received. The progress of the reaction was monitored by thin-layer chromatography using 0.25 mm silica gel plates. Column chromatography was performed using silica gel (Acme Synthetic Chemicals, India; finer than 200 and 60–120 mesh).

Antibacterial Activity

The antibacterial cell susceptibility testing was performed by agar disc-diffusion technique[11] against two gram-positive bacteria, *Staphylococcus aureus, (S.a)* (MTCC-96) and *Bacillus subtilis, (B.s)* (MTCC-619) and three gram-negative bacteria, *Escherichia coli*, *(E.c)* (MTCC-722), *Klebsiella pneumoniae, (K.p)* (MTCC-109) and *Proteus vulgaris, (P.v)* (MTCC-1771). Pure cultures of the organisms were obtained from Department of Biotechnology, Kakatiya University, India. A standard inoculum, 1-2 x 107 cfu/ml 0.5 Mc Farland standards[12] was introduced onto the surface of sterile Nutrient agar plate and evenly distributed by using a sterile glass spreader. Sterilized antibiotic discs (6 mm in diameter, prepared using Whatmann No. 1 paper) were placed over the medium. To find out antibacterial activity 75 µg of the compounds (initially dissolved in chloroform) were transferred to each disc with the help of a micropipette, simultaneously maintaining chloramphenicol (30 µg) disc as a standard. After overnight incubation at 37 ºC the diameters of zones of growth inhibition in millimeters (mm) were measured with a ruler and compared with standard antibiotic. Control measurements were carried out with chloroform. Compounds exhibiting antibacterial activity by the above disc diffusion method were screened for Minimum inhibitory concentration (MIC) using method described by Villanova, 1982. Diluted compounds ranging from 200-5 µg/ml were mixed in nutrient broth and 0.1 ml of active inoculums was added to each tube. The tubes were incubated aerobically at 37^{o} C for 24 hr. The lowest concentration of the compound that completely inhibited bacterial growth (no turbidity) in comparison to control was regarded as MIC.

Molecular Docking

The co-crystallized structure of target enzyme MurB (PDB id: 1MBT) was obtained from Protein Data Bank (RCSB) (http://www.rcsb.org/pdb). To carry out in silico studies, the 2D structures of the compound A2 and B2 were drawn in ChemBioOffice 2010 and converted to energy minimized 3D structures in pdb file format using MarvinSketch (ChemAxon). The target protein file was prepared by removing the structural water molecule, hetero atoms and co-factors by leaving only the residues associated with protein by using Discovery Studio 4.0 Visualizer (DSV). AutoDock 1.5.6 (MGL tools-1.5.6) tool was used to prepare target protein file. The active binding site of protein identification was carried out using CastP (serversts-fw.bioengr.uic.edu/castp/calculation.php).
Docking simulations for the compounds A2 and B2 were performed against the active site of MurB enzyme. Then, finally docking results were visualized using Maestro elements tutorial 1.8.

Chemical Methods

General procedure for the synthesis of 3,5-dimethyl-1H-pyrazole

A mixture of 20 mmol of acetyl acetone and 20 mmol of hydrazine hydrate taken in 20mL of methanol cool to 0 ^{o}C, then stirred it for half an hour. A white crystalline solid separated. Filter off the solid and wash with water to get pure compound.

General procedure for the synthesis of Pyrazole Derivatives (A_1-A_8)

A mixture of 3, 5-dimethyl-1H-pyrazole (10 mmol) and Alkyl Halide (10 mmol) were added to a stirring solution of K_2CO_3 (catalytic amount) and the reaction mixture was stirred at room temp for 24 h. The reaction mixture was washed with water (2 X 50 mL) and the product is extracted in chloroform (2 X 20 mL). The organic layer was dried on anhydrous sodium sulphate, the solvent was evaporated, the progress of the reaction was monitored by TLC and the sample was purified by column chromatography using 4-5% methanol in chloroform mobile phase to obtain the afforded pure title compounds in good yields. The IR spectra of (A_1-A_8) showed the characteristic band for C=N at 1673–1680 cm^{-1} and C=C at 1598–1600 cm^{-1}.

1-Hexadecyl-3,5-Dimethyl-1-Tetradecyl-1H-Pyrazol-1-Ium (A_1)

Yield 43%; it was obtained as light green solid, mp: 110-112°C. ^{1}H NMR (300 MHz, $CDCl_3$) δ/ppm 0.8-0.9 [t, 6H, N-[CH_2-CH_2-$(CH_2)_{13}$-C$\underline{H}_3$]$_2$, 1.2-1.3 [m, 48H, N-[CH_2-CH_2-(C$\underline{H}_2$)$_{12}$-CH_3]$_2$, 2.1 [m, 4H, N-[CH_2-C$\underline{H}_2$-$(CH_2)_{13}$-CH_3]$_2$, 2.3 [s, 6H, Ar-(C$\underline{H}_3$)$_2$], 3.4 [t, 4H, N-[C$\underline{H}_2$-CH_2-$(CH_2)_{13}$-CH_3]$_2$, 5.8 [s, 1H, aromatic].

^{13}C NMR (75 MHz, DMSO-d_6) δ/ppm: 11.8(Ar-CH_3), 14.1(-CH_3), 20.1 (CH_2), 22.1 (Ar-CH_3), 28.1 (CH_2), 29.2 (CH_2), 29.8 (CH_2), 31.9 (CH_2), 59.8 (CH_2), 105.1 (Ar), 146(Ar). Mass spectrum (LCM): m/z 518 $[M]^+$ for $C_{35}H_{69}N_2^+$. Elemental Analysis: Calculated : %N: 5.41, %C: 81.16, %H: 13.43. Observed : %N: 5.42, %C: 81.11, %H13.47.

1,3,5-Trimethyl-1-Tetradecyl-1H-Pyrazol-1-Ium (A_2)

Yield 50%; it was obtained as black solid, mp: 120-122°C. ^{1}H NMR (300 MHz, $CDCl_3$) δ/ppm 0.9 [t, 3H, N-[$(CH_2)_{13}$-C$\underline{H}_3$], 1.2-1.3 [m, 22H, N-[CH_2-CH_2-(C$\underline{H}_2$)$_{11}$-CH_3], 2.1 [m, 2H, N-[CH_2-C$\underline{H}_2$-$(CH_2)_{11}$-CH_3], 2.3 [s, 6H, Ar-(C$\underline{H}_3$)$_2$], 2.9[s, 3H, Ar-(C$\underline{H}_3$)], 3.4 [t, 2H, N-[C$\underline{H}_2$-CH_2-$(CH_2)_{11}$-CH_3], 5.8 [s, 1H, aromatic].

^{13}C NMR (75 MHz, DMSO-d_6) δ/ppm: 11.2(Ar-CH_3), 14.4(-CH_3), 19.8. (-CH_2), 22.1 (Ar-CH_3), 28.2 (-CH_2), 29.1 (-CH_2), 29.6 (-CH_2), 31.7 (-CH_2), 61.8 (-CH_2), 104.1 (Ar), 145.2(Ar). Mass spectrum (LCM): m/z 307 $[M]^+$ for $C_{20}H_{39}N_2^+$. Elemental Analysis: Calculated : %N: 9,11, %C: 78.11, %H: 12.78. Observed : %N: 9,12, %C: 78.14, %H: 12.74.

1-Hexadecyl-1,3,5-Trimethyl-1H-Pyrazol-1-Ium (A_3)

Yield 40%; it was obtained as blue solid, mp: 124-126°C. ^{1}H NMR (300 MHz, $CDCl_3$) δ/ppm 1.0 [t, 3H, N-[$(CH_2)_{15}$-C$\underline{H}_3$], 1.2-1.4 [m, 26H, N-[CH_2-CH_2-(C$\underline{H}_2$)$_{13}$-CH_3], 2.0 [m, 2H, N-[CH_2-C$\underline{H}_2$-$(CH_2)_{13}$-CH_3], 2.3 [s, 6H, Ar-(C$\underline{H}_3$)$_2$], 3.0[s, 3H, Ar-(C$\underline{H}_3$)], 3.5 [t, 2H, N-[C$\underline{H}_2$-CH_2-$(CH_2)_{13}$-CH_3], 5.9[s, 1H, aromatic]. ^{13}C NMR (75 MHz, DMSO-d_6) δ/ppm: 11.3(Ar-CH_3), 14.3(-CH_3), 19.9. (-CH_2), 22.0 (Ar-CH_3), 28.4 (-CH_2), 29.3 (-CH_2), 29.7 (-CH_2), 31.8 (-CH_2), 61.9 (-CH_2), 104.2 (Ar), 145.4(Ar). Mass spectrum (LCM): m/z 336 $[M]^+$ for $C_{22}H_{43}N_2^+$. Elemental Analysis: Calculated : %N: 8.34, %C: 78.74, %H: 12.92. Observed : %N: 8.37, %C: 78.73, %H: 12.90.

3,5-Dimethyl-1,1-Ditetradecyl-1H-Pyrazol-1-Ium (A_4)

Yield 43%; it was obtained as light yellow solid, mp: 112-114 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ/ppm 0.9 [t, 6H, N-[CH_2-CH_2-$(CH_2)_{11}$-C$\underline{H}_3$]$_2$, 1.2-1.3 [m, 44H, N-[CH_2-CH_2-(C$\underline{H}_2$)$_{11}$-CH_3]$_2$, 2.0 [m, 4H, N-[CH_2-C$\underline{H}_2$-$(CH_2)_{11}$-CH_3]$_2$, 2.2 [s, 6H, Ar-(C$\underline{H}_3$)$_2$], 3.3 [t, 4H, N-[C$\underline{H}_2$-CH_2-$(CH_2)_{11}$-CH_3]$_2$, 5.7 [s, 1H, aromatic]. ^{13}C NMR (75 MHz, DMSO-d_6) δ/ppm: 11.2(Ar-CH_3), 14.2(-CH_3), 19.6. (-CH_2), 22.5 (Ar-CH_3), 28.3 (-CH_2), 29.4 (-CH_2), 29.8 (-CH_2), 31.6 (-CH_2), 60.9 (-CH_2), 104.3 (Ar), 145.3(Ar). Mass spectrum (LCM): m/z 490 $[M]^+$ for $C_{33}H_{65}N_2^+$. Elemental Analysis: Calculated : %N: 5.72, %C: 80.91, %H: 13.37. Observed : %N: 5,75, %C: 80.87, %H: 13.38.

1,3,5-Trimethyl-1-Octadecyl-1H-Pyrazol-1-Ium(A_5)

Yield 45%; it was obtained as blue solid, mp: 126-128°C. ^{1}H NMR (300 MHz, $CDCl_3$) δ/ppm 1.0 [t, 3H, N-[$(CH_2)_{17}$-C$\underline{H}_3$], 1.2-1.4 [m, 30H, N-[CH_2-CH_2-(C$\underline{H}_2$)$_{15}$-CH_3], 2.0 [m, 2H, N-[CH_2-C$\underline{H}_2$-$(CH_2)_{15}$-CH_3], 2.3 [s, 6H, Ar-(C$\underline{H}_3$)$_2$], 3.0[s, 3H, Ar-(C$\underline{H}_3$)], 3.5 [t, 2H, N-[C$\underline{H}_2$-CH_2-$(CH_2)_{15}$-CH_3], 5.9[s, 1H, aromatic]. ^{13}C NMR (75 MHz, DMSO-d_6) δ/ppm: 11.2(Ar-CH_3), 14.2(-CH_3),

19.5. (-CH_2), 21.5 (Ar-CH_3), 28.0 (-CH_2), 29.2 (-CH_2), 29.4 (-CH_2), 31.6 (-CH_2), 62.1 (-CH_2), 104.0 (Ar), 145.2(Ar). Mass spectrum (LCM): m/z 363 $[M]^+$ for $C_{24}H_{47}N_2^+$. Elemental Analysis: Calculated : %N: 7.70, %C: 79.27, %H: 13.03. Observed : %N: 7.76, %C: 79.22, %H: 13.02.

3,5-Dimethyl-1-Octadecyl-1-Tetradecyl-1H-Pyrazol-1-Ium(A_6)

Yield 48%; it was obtained as light green solid, mp: 112-114 ^oC. ^1H NMR (300 MHz, $CDCl_3$) δ/ppm 0.9 [t, 6H, N-[CH_2-CH_2-$(CH_2)_{13}$-C$\underline{H}_3$]$_2$, 1.2-1.3 [m, 52H, N-[CH_2-CH_2-(C$\underline{H}_2$)$_{13}$-CH_3]$_2$, 2.0 [m, 4H, N-[CH_2-C$\underline{H}_2$-$(CH_2)_{13}$-CH_3]$_2$, 2.4 [s, 6H, Ar-(C$\underline{H}_3$) $_2$], 3.4 [t, 4H, N-[C$\underline{H}_2$-CH_2-$(CH_2)_{13}$-CH_3]$_2$, 5.8 [s, 1H, aromatic]. ^{13}C NMR (75 MHz, DMSO-d_6) δ/ppm: 11.6(Ar-CH_3), 14.2(-CH_3), 20.4 (-CH_2), 22.3 (Ar-CH_3), 28.3 (-CH_2), 29.4 (-CH_2), 29.9 (-CH_2), 31.8 (-CH_2), 59.6 (-CH_2), 105.2 (Ar), 145.0 Ar). Mass spectrum (LCM): m/z 546 $[M]^+$ for $C_{37}H_{73}N_2^+$. Elemental Analysis: Calculated : %N: 5.13, %C: 81.39, %H: 13.48. Observed : %N: 5.12, %C: 81.37 %H: 13.51.

3,5-Dimethyl-1,1-Dioctadecyl-1H-Pyrazol-1-Ium(A_7)

Yield 46%; it was obtained as light yellow solid, mp: 114-116 ^oC. ^1H NMR (300 MHz, $CDCl_3$) δ/ppm O.8-0.9 [t, 6H, N-[CH_2-CH_2-$(CH_2)_{15}$-C$\underline{H}_3$]$_2$, 1.2-1.3 [m, 60H, N-[CH_2-CH_2-(C$\underline{H}_2$)$_{15}$-CH_3]$_2$, 2.1 [m, 4H, N-[CH_2-C$\underline{H}_2$-$(CH_2)_{15}$-CH_3]$_2$, 2.3 [s, 6H, Ar-(C$\underline{H}_3$) $_2$], 3.4 [t, 4H, N-[C$\underline{H}_2$-CH_2-$(CH_2)_{15}$-CH_3]$_2$, 5.6 [s, 1H, aromatic]. ^{13}C NMR (75 MHz, DMSO-d_6) δ/ppm: 11.2(Ar-CH_3), 14.2(-CH_3), 19.6. (-CH_2), 22.5 (Ar-CH_3), 28.3 (-CH_2), 29.4 (-CH_2), 29.8 (-CH_2), 31.6 (-CH_2), 60.9 (-CH_2), 104.3 (Ar), 145.3(Ar). Mass spectrum (LCM): m/z 602 $[M]^+$ for $C_{41}H_{81}N_2^+$. Elemental Analysis: Calculated : %N: 4.65, %C: 81.79, %H: 13.56. Observed : %N: 4.64, %C: 81.77, %H: 13.59.

1,1-Dihexadecyl-3,5-Diphenyl-4,5-Dihydro-1H-Pyrazol-1-Ium(B_1)

Yield 49%; it was obtained as light red solid, mp: 122-124 ^oC. ^1H NMR (300 MHz, $CDCl_3$) δ/ppm 1.0 [t, 6H, N-[CH_2-CH_2-$(CH_2)_{13}$-C$\underline{H}_3$]$_2$, 1.2-1.3 [m, 52H, N-[CH_2-CH_2-(C$\underline{H}_2$)$_{13}$-CH_3]$_2$, 1.8 [m, 4H, N-[CH_2-C$\underline{H}_2$-$(CH_2)_{13}$-CH_3]$_2$, 2.6 [d, 2H, -C$\underline{H}_2$-CH-Ar], 2.7 [t, 1H, Ar-C$\underline{H}$-], 3.3 [t, 4H, N-[C$\underline{H}_2$-CH_2-$(CH_2)_{13}$-CH_3]$_2$, 7.1-7.2 [m, 10H, aromatic]. ^{13}C NMR (75 MHz, DMSO-d_6) δ/ppm: 14.2(-CH_3), 19.6. (-CH_2), 22.5 (-CH_2), 27.3 (-CH_2), 29.4 (-CH_2), 29.6 (-CH_2), 31.6 (-CH_2), 34.6 (-CH_2), 60.5 (-CH_2), 63.5 (-CH), 126.0, 127.5, 128.2, 129.5, 131.4, 134.2, 135.4 (Ar), 164.3(-C=C-). Mass spectrum (LCM): m/z 672 $[M]^+$ for $C_{47}H_{79}N_2^+$. Elemental Analysis: Calculated : %N: 4.17, %C: 83.99, %H: 11.85. Observed : %N: 4.19, %C: 83.96, %H: 11.85.

1-Methyl-3,5-Diphenyl-1-Tetradecyl-4,5-Dihydro-1H-Pyrazol-1-Ium(B_2)

Yield 50%; it was obtained as white solid, mp: 130-132^oC. ^1H NMR (300 MHz, $CDCl_3$) δ/ppm 1.0 [t, 3H, N-[$(CH_2)_{13}$-C$\underline{H}_3$], 1.2-1.3 [m, 22H, N-[CH_2-CH_2-(C$\underline{H}_2$)$_{11}$-CH_3], 1.74 [m, 2H, N-[CH_2-C$\underline{H}_2$-$(CH_2)_{11}$-CH_3], 2.6 [d, 2H, -C$\underline{H}_2$-CH-Ar], 2.7 [t, 1H, Ar-C$\underline{H}$-], 3.0 [s, 3H, N-(C$\underline{H}_3$)], 3.3 [t, 2H, N-[C$\underline{H}_2$-CH_2-$(CH_2)_{11}$-CH_3], 7.1-7.2 [m, 10H, aromatic]. ^{13}C NMR (75 MHz, DMSO-d_6) δ/ppm: 14.1(-CH_3), 19.5. (-CH_2), 22.3 (-CH_2), 27.8 (-CH_2), 29.3 (-CH_2), 29.8 (-CH_2), 31.6 (-CH_2), 34.4 (-CH_2), 62.1 (-CH_2), 65.5 (-CH), 126.2, 127.5, 128.2, 129.5, 131.4, 134.2, 135.4 (Ar), 164.3(-C=C-). Mass spectrum (LCM): m/z 434 $[M]^+$ for $C_{30}H_{45}N_2^+$. Elemental Analysis: Calculated : %N: 6.46, %C: 83.08, %H: 10.46. Observed : %N: 6.48, %C: 83.05, %H: 10.47.

1-Methyl-1-Octadecyl-3,5-Diphenyl-4,5-Dihydro-1H-Pyrazol-1-Ium(B_3)

Yield 44%; it was obtained as green solid, mp: 124-126^oC. ^1H NMR (300 MHz, $CDCl_3$) δ/ppm 1.0 [t, 3H, N-[$(CH_2)_{17}$-C$\underline{H}_3$], 1.2-1.3 [m, 30H, N-[CH_2-CH_2-(C$\underline{H}_2$)$_{15}$-CH_3], 1.74 [m, 2H, N-[CH_2-C$\underline{H}_2$-$(CH_2)_{15}$-CH_3], 2.6 [d, 2H, -C$\underline{H}_2$-CH-Ar], 2.7 [t, 1H, Ar-C$\underline{H}$-], 3.0 [s, 3H, N-(C$\underline{H}_3$)], 3.3 [t, 2H, N-[C$\underline{H}_2$-CH_2-$(CH_2)_{15}$-CH_3], 7.1-7.2 [m, 10H, aromatic]. ^{13}C NMR (75 MHz, DMSO-d_6) δ/ppm: 14.2(-CH_3), 19.6. (-CH_2), 22.5 (-CH_2), 27.3 (-CH_2), 29.4 (-CH_2), 29.6 (-CH_2), 31.6 (-CH_2), 34.6 (-CH_2), 62.1 (-CH_2), 65.5 (-CH), 126.0, 127.5, 128.2, 129.5, 131.4, 134.2, 135.4 (Ar), 164.3(-C=C-). Mass spectrum (LCM): m/z 489 $[M]^+$ for $C_{34}H_{53}N_2^+$. Elemental Analysis: Calculated : %N:

5.72, %C: 83.37, %H: 10.91. Observed : %N: 5.70, %C: 83.38, %H: 10.92.

1-Octadecyl-3,5-Diphenyl-1-Tetradecyl-4,5-Dihydro-1H-Pyrazol-1-Ium(B_4)

Yield 42%; it was obtained as light yellow solid, mp: 110-112 ^{o}C. ^{1}H NMR (300 MHz, $CDCl_3$) δ/ppm 1.0 [t, 6H, N-[CH_2-CH_2-$(CH_2)_{13}$-C$\underline{H_3}$]$_2$, 1.2-1.3 [m, 52H, N-[CH_2-CH_2-(C$\underline{H_2}$)$_{13}$-CH_3]$_2$, 1.7 [m, 4H, N-[CH_2-C$\underline{H_2}$-$(CH_2)_{13}$-CH_3]$_2$, 2.6 [d, 2H, -C$\underline{H_2}$-CH-Ar], 2.7 [t, 1H, Ar-C$\underline{H}$-], 3.3 [t, 4H, N-[C$\underline{H_2}$-CH_2-$(CH_2)_{13}$-CH_3]$_2$, 7.1-7.3 [m, 10H, aromatic]. ^{13}C NMR (75 MHz, DMSO-d_6) δ/ppm: 14.3(-CH_3), 19.4. (-CH_2), 22.6 (-CH_2), 27.6 (CH_2), 29.5 (-CH_2), 29.6 (-CH_2), 31.8 (-CH_2), 34.8 (-CH_2), 61.5 (CH_2), 64.8 (CH), 126.2, 127.4, 128.5, 129.8, 131.3, 135.3 (Ar), 164.5(-C=C-). Mass spectrum (LCM): m/z 672 $[M]^+$ for $C_{47}H_{79}N_2^+$. Elemental Analysis: Calculated : %N: 4.17, %C: 83.98, %H: 11.85. Observed : %N: 4.16, %C: 83.97, %H: 11.87.

RESULTS AND DISCUSSION

Chemistry

The synthetic strategies adopted for the synthesis of the target compounds are depicted in Scheme-1&2. In scheme -1, Prepared 3,5-dimethylpyrazole, treating acetyl acetone with hydrazine hydrate in presence of methanol and catalytic amount of pyridine. Synthesized novel compounds (A_1-A_8) using 3,5-di- methylpyrazole and alkyl halides. In scheme-2 i.e., prepared dihydropyrazole treating the chalcone with hydrazine hydrate in presence of ethanol. Synthesized novel compounds (B_1-B_4) using dihydropyrazole and alkyl halides. The structures of all the newly synthesized compounds were elucidated on the basis of their spectral (IR, NMR and Mass) and elemental analysis data. The synthesized compounds were also assayed for their anti bacterial activity.

Antibacterial Activity

It is well known that pyrazoles possess significant anti-bacterial activity. The essential attributes for an antibiotic to have efficient antibacterial activity are:

i) Possess significant antibacterial activity and;
ii) Uptake by the bacterial cell.

To incorporate these attributes in a single molecule we developed pyrazole based compounds containing two units:

i) Pyrazoles as the head group which is responsible for the antibacterial activity and;
ii) Anchoring groups to the molecule enhance the uptake by the bacterial cell.

Here, we are reporting the antibacterial activity of (A_1-A_8 and B_1-B_4) compounds compared with Chloramphenicol against gram-positive bacteria *Staphylococcus aureus*, Sa (MTCC-96) and *Bacillus subtilis*, BS (MTCC-619) and gram-negative bacteria, *Escherichia collie*., E.C(MTCC-722), *Klebsiella pneumonia*, K.P(MTCC-109) and *Proteus vulgaris*, P.V(MTCC-1771). The results are shown in Table 1. The compounds A_5, B_1, B_2, B_3 and B_4 are not showed antibacterial behavior against *Escherichia collie*. The compounds A_5, A_7, A_8 and B1 not showed antibacterial behavior against *Staphylococcus aureus*. The compounds A_5, A_7, A_8, B_1 and B3 not showed antibacterial behavior against *Proteus vulgaris* and *Klebsiella pneumonia*. The compounds A_5, A_7, A_8, B_1, B_2 and B3 not showed antibacterial behavior against *Bacillus subtilis*. The Compounds A_7 and A_8 showed moderate antibacterial behavior against *Escherichia collie*. The Compounds B_2, B_3 and B_4 showed moderate antibacterial behavior against *Staphylococcus aureus*. The Compounds B_2 and B_4 showed moderate antibacterial behavior against *Proteus vulgaris* and Klebsiella *pneumonia*. The Compound B_4 showed moderate antibacterial behavior against *Bacillus subtilis*. The Compounds A_2, A_3 and A_4 showed more activity, whereas compound A_1 showed higher antibacterial activity against *Escherichia collie*, *Staphylococcus aureus, Proteus vulgaris, Klebsiella pneumonia* and *Bacillus subtilis*. Based on the above evidences we can conclude that the compounds having pyrazole as head group seems to be useful as antibacterial drug, but there is still

a need of further structure activity relation studies on these compounds.

Molecular Docking

Molecular docking study was performed to evaluate the free energy of binding, the binding modes and key protein-ligand interactions. A2 and B2 compounds were docked into the active sites of *E.coli* MurB enzyme (PDB Id: 1MBT). The free energies of binding observed in docking results are -6.94 (A2) and 6.35 (B2) K. cal/mol were evident for good affinity between the protein and ligand due to salt bridge the active sites of the receptor.

CONCLUSION

In summary, we have developed and synthesized efficient newer pyrazole derivatives for use in antibacterial activity. The present synthetic protocols should, in principle, be applicable in synthesizing various pyrazole derivatives for use in antibacterial activity. The antibacterial activity of these newer compounds were studied and found that these compounds are active against both gram-positive bacteria and gram-negative bacteria. Antimicrobial activity and molecular docking studies suggested that the present series of A_1-A_8 and B_1-B_4exhibited promising activities against microbial pathogens. The binding energies well supported the antibacterial inhibiting activity of A2 and B2 further helped to investigate the binding orientations of ligands with active pockets of an enzyme. All these results could be useful to evaluate novel antibacterial inhibitors and can be consider as a lead compounds for the development of antibacterial agents for the treatment of bacterial infection.

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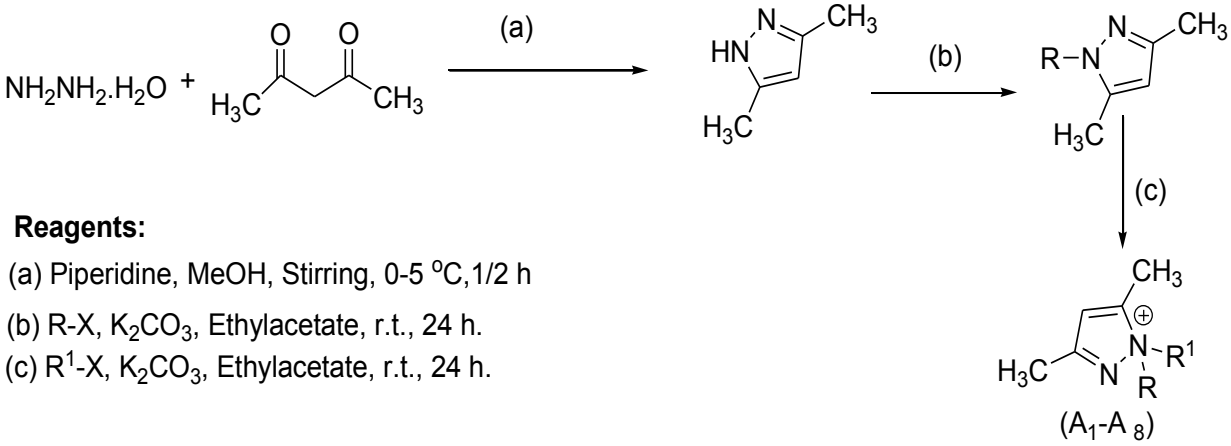
Scheme 1:



Reagents:

(a) Piperidine, MeOH, Stirring, 0-5 ^{o}C,1/2 h

(b) R-X, K_2CO_3, Ethylacetate, r.t., 24 h.

(c) R^1-X, K_2CO_3, Ethylacetate, r.t., 24 h.

S. No.	Compound	R-X	R^1-X
1	A_1	$C_{14}H_{29}Br$	$C_{16}H_{33}Br$
2	A_2	$C_{14}H_{29}Br$	CH_3Br
3	A_3	$C_{16}H_{33}Br$	CH_3Br
4	A_4	$C_{14}H_{29}Br$	$C_{14}H_{29}Br$
5	A_5	$C_{18}H_{37}Br$	CH_3Br
6	A_6	$C_{18}H_{37}Br$	$C_{14}H_{29}Br$
7	A_7	$C_{18}H_{37}Br$	$C_{18}H_{37}Br$
8	A_8	$C_{18}H_{37}Br$	$C_{16}H_{33}Br$

Table I: Determination of Antibiotic susceptibility using disc-diffusion method (numbers present the diameters of zones of growth inhibition in mm)

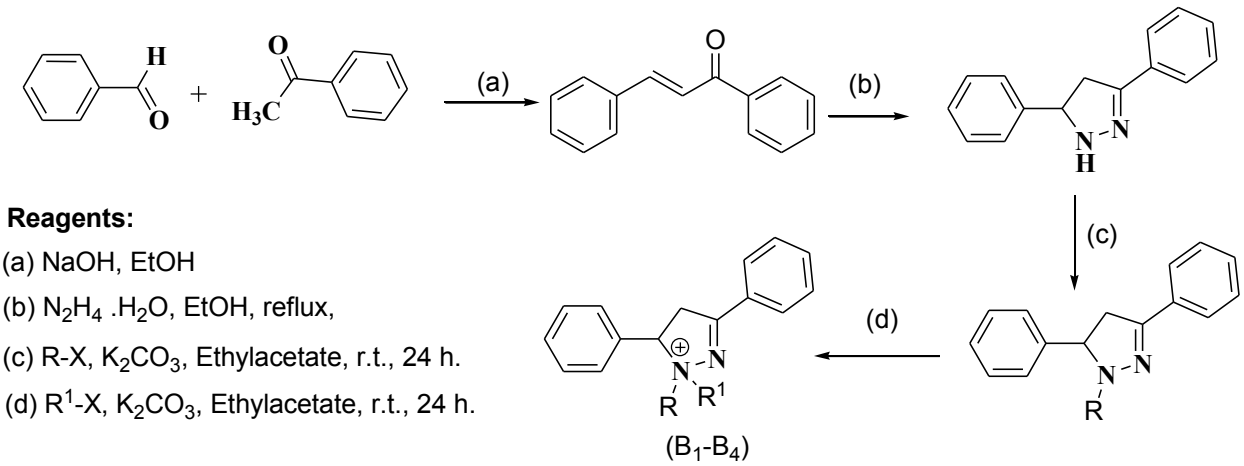
Bacterial strain	CH	A_1	A_2	A_3	A_4	A_5	A_6	A_7	A_8	B_1	B_2	B_3	B_4
E.c	31	25	18	13	15	-	10	10	10	-	-	-	-
P.v	14	25	18	18	15	-	12	-	-	-	10	-	10
K.p	24	20	15	10	13	-	10	-	-	-	10	-	10
B.s	23	20	15	11	14	-	10	-	-	-	-	-	10
S.a	23	25	14	12	14	-	12	-	-	-	10	10	10

For abbreviations used see Experimental Section. The concentration of compounds is 75 µg/mL and the concentration of Chloramphenicol(CH) is 30 µg/mL.

Table II: MIC values in µg/mL

Bacterial strain	CH	A_1	A_2	A_3	A_4	A_5	A_6	A_7	A_8	B_1	B_2	B_3	B_4
E.c	8	10	15	40	35	-	60	60	60	-	-	-	-
P.v	20	10	15	20	35	-	50	-	-	-	60	-	60
K.p	10	15	30	70	40	-	60	-	-	-	70	-	70
B.s	10	15	30	70	35	-	60	-	-	-	-	-	70
S.a	10	10	20	45	35	-	50	-	-	-	60	60	60

Scheme 2:



Reagents:

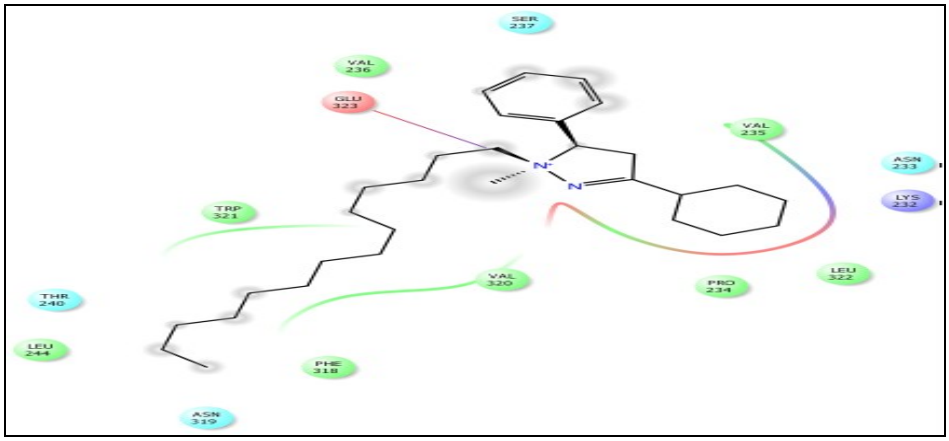
(a) NaOH, EtOH

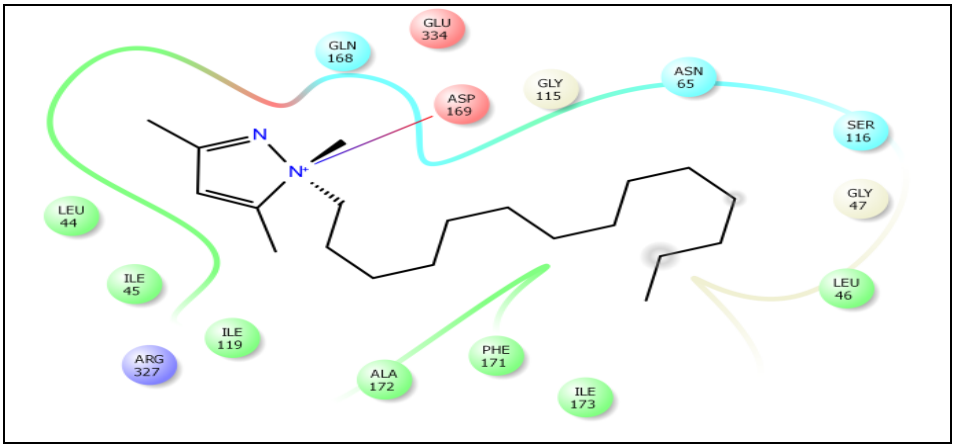
(b) N_2H_4 .H_2O, EtOH, reflux,

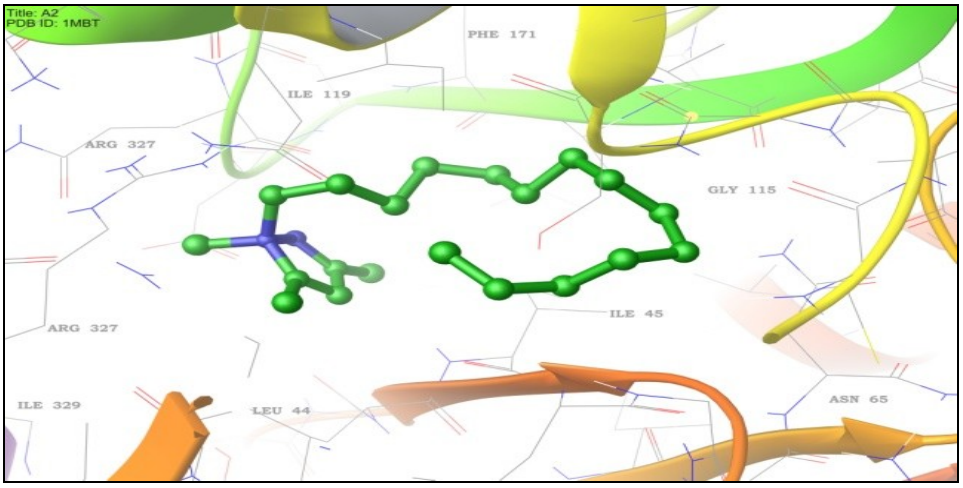
(c) R-X, K_2CO_3, Ethylacetate, r.t., 24 h.

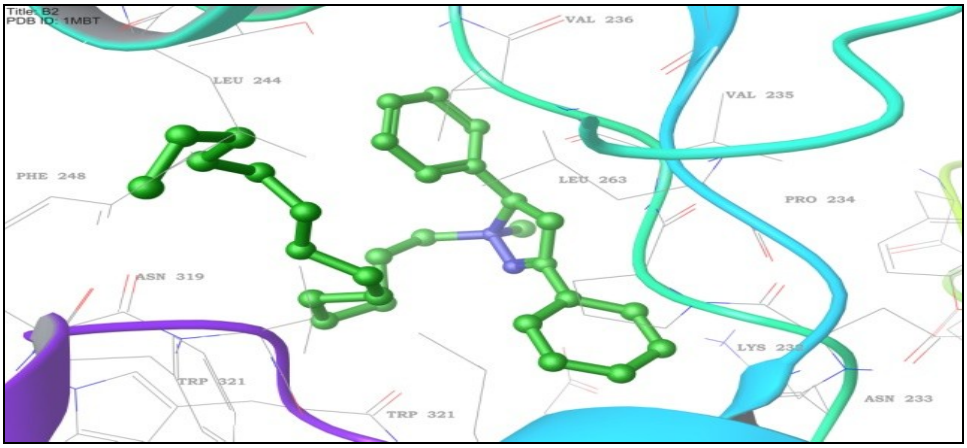
(d) R^1-X, K_2CO_3, Ethylacetate, r.t., 24 h.

S. No.	Compound	R-X	R^1-X
1	B_1	$C_{16}H_{33}Br$	$C_{16}H_{33}Br$
2	B_2	$C_{14}H_{29}Br$	CH_3Br
3	B_3	$C_{18}H_{37}Br$	CH_3Br
4	B_4	$C_{14}H_{29}Br$	$C_{18}H_{37}Br$









Figurre 1-4: 2D and 3D Representation of A2 ,B2

Table III: Autodock binding energies, no. of hydrogen bonds and residues involved in hydrogen bonding interaction of ligands for E. Coli (PDB id: 1MBT)

Compound	Binding Energy (kcal/mol)	Inhibition Constant Ki (nM)	Run
A1	-4.03	1.12 mM	7
A2	-6.94	8.19 uM	2
A3	-6.64	13.53 uM	6
A4	-1.98	35.59 mM	4
A5	-5.46	99.38 uM	9
A6	-2.92	7.18 mM	6
A7	-3.53	5.47 mM	3
A8	-1.69	58.04 mM	4

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