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Synthesis and docking studies of 4-aryl-1-phenyl-*1H*-pyrazol-5-ylimino-2-methoxy phenyl acetate as antibacterial agents

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ABSTRACT

*4-Aryl-1-phenyl-1H-Pyrazol-5-ylimino-2-methoxyPhenol has been synthesized by condensation of 4-Aryl-5-amino pyrazol and Vanillin. 4-Aryl-1-phenyl-1H-Pyrazol-5-ylimino-2-methoxyPhenol further acylated using different acylating agent viz acetyl chloride, benzoyl chloride, p-nitro benzoyl chloride, p-tolyl benzoyl chloride. Structure elucidation of the synthesized compounds was done based on analytical and spectral data. Synthesized compounds were evaluated for their antimicrobial activity against Pseudomonas, E. coli, S. aureus and Bacillus subtilis. Compounds **5c**, **5i** and **5k** showed the equipotent activity compared with Chloramphenicol and Cephalothin as reference drugs. Docking studies of the promising compounds was done on MurB using Hex 6.3 docking program to study their observed activity.*

Key words: O-acylation, Pyrazole, Antibacterial activity, docking studies.

INTRODUCTION

One of the most interesting areas of research is the search for compounds that combat resistant bacteria. Rapidly developing resistance of pathogenic bacteria to available antibiotics is becoming a serious public health threat.

Pyrazole moiety is great importance to chemists as well as biologists as it is found in a large variety of biological and pharmacological activities, including effects as anti-inflammatory antipyretic and analgesic [1-4], anticancer [5], anticonvulsant [6] , antidepressant [7], antitubercular [8], antidepressants [9] , herbicidal [10] etc. Similarly, the compounds containing pyrazole moieties have been shown to exhibit Antibacterial activities [11, 12]. Furthermore, pyrazole derivatives have also emerged as a group of compounds possessing a broad spectrum of antimicrobial agents [13-16]. (Fig. 1)

In view of these findings and in continuation of our work on the synthesis of antimicrobial agents of pharmaceutical interest, we thought it valuable to synthesize a new series of compounds having pyrazole moieties along with Schiff bases pharmacophore with the hope that molecules will have synergistic biological effect giving potent antimicrobial agents. Docking study of pyrazole derivatives were carried out using *MurB* enzyme. *MurB* enzyme is essential for the viability of bacterial cells [17,18]. *MurB* is an attractive target for inhibitors with the potential to have broad antibacterial activity. The enzymes involved in peptidoglycan biosynthesis are among the best known targets in the search for new antibiotics. Bacterial peptidoglycan is an extensively cross linked polymer unique to

prokaryotic cells, and the enzymes involved in peptidoglycan biosynthesis are essential for bacterial cell survival. More than ten synthetic transformations required for biosynthesis of Peptidoglycan for this a specific enzyme require [19].

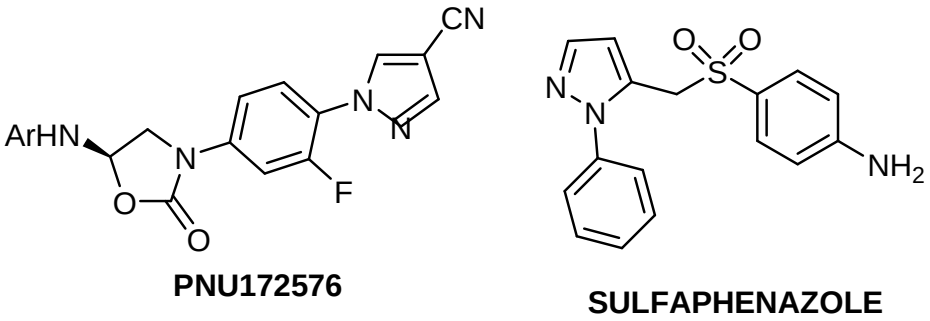


Fig. 1 Representative structures having pyrazole moieties as antimicrobial agents

Aminopyrazole compounds can be easily obtained by the reaction of nitriles with hydrazine hydrate [20-22]. Amino pyrazole derivatives used in synthesis of different biological active heterocyclic compound such as pyrazolopyrrolopyridines [23] and Pyrazolo[3,4- b]pyridines [24]. In view of these above, an attempt has been undertaken for the synthesis of heterocyclic derivatives of pyrazole possessing potent biological activities. We have successfully synthesized of 4-Aryl-1-phenyl-1H-Pyrazol-5-ylimino-2-methoxy Phenyl acetate using 5-aminopyrazole and vanillin as starting compound and finally O-acylation of Schiff bases were carried out by different acylating reagents. The synthesized compounds were tested for their possible antibacterial activity.

MATERIALS AND METHODS

Synthesis

Melting points of all the synthesized compounds were determined by open capillary method and are uncorrected. The monitoring of reaction and checking of purity of the product were done using pre-coated silica gel plates and visualized using iodine chamber/UV lamp. IR spectra were recorded on a Perkin-Elmer Spectrum One FT-IR spectrometer. 1H-NMR spectra were scanned on a (Varian Mercury) on YH-400 MHz FT NMR in $CDCl_3$ and $CDCl_3$ using tetramethylsilane as an internal standard. Mass spectra were recorded from an HP 1100 LC/MSD mass spectral instrument (positive and negative APCI ion source, 50-200 V, nitrogen). All the chemicals and solvents used were of synthetic grade (Sd. Fine, chemicals, Mumbai, India).

Synthesis of 4-Aryl-1-phenyl-1H-Pyrazol-5-ylimino-2-methoxyPhenol **(3a-c)**

To the clear solution of 5-amino pyrazole **1a** (2.6 g, 0.01 mole) and vanillin (1.5 g, 0.01 mole) in DMF (250 ml), acetic acid (1 ml) was added and the reaction mixture was refluxed for three hours (TLC check). Then the solution was allowed to attain room temperature. Then it was drop wise poured in to crush ice with constant stirring. Solid obtained on cooling was filtered, dried and crystallized from ethanol afforded compounds.

4-(3-(4-chlorophenyl)-1-phenyl-1H-pyrazol-5-ylimino)methyl)-2-methoxyphenol ***3a***

Yield 83 %; m.p. 200^0C; IR (cm-1) (KBr): 1049(C-O-C), 1610(C=C), 3330(Ar-OH). ^{1}H-NMR (δ, ppm)($CDCl_3$): 3.97(3H, s, OCH3), 6.01(1H,s, H-Ar), 6.15(1H, bs, OH), 7.1 (1H, d, Ar-H), 7.19(1H, d,Ar-H), 7.22(1H, dd, Ar-H), 7.42(5H, m, Ar-H), 7.57(2H, d, Ar-H), 7.71 (2H, d, Ar-H). 8.71(1H,s, H-C=N).

4-(3-(4-Bromophenyl)-1-phenyl-1H-pyrazol-5-ylimino)methyl)-2-methoxyphenol ***3b***

Yield 84 %; m.p. 201^0C; IR (cm-1) (KBr): 1059(C-O-C), 1600(C=C), 1630(N=CH), 3350 (Ar-OH). ^{1}H-NMR (δ, ppm)($CDCl_3$): 3.90(3H, s, OCH_3), 6.10(1H, bs, OH), 6.56 (1H, d, H-Ar), 7.01(1H, d, Ar-H), 7.12(1H, d, Ar-H), 7.21(1H, dd,Ar-H), 7.41(5H, m, Ar-H), 7.57(2H, d, Ar-H), 7.69(2H, d, Ar-H). 8.67(1H,s, H-C=N).

4-((1-phenyl-3-p-tolyl-1H-pyrazol-5-ylimino)methyl)-2-methoxyphenol ***3c***

Yield 89 %; m.p. 186^0C; IR (cm-1) (KBr): 1050(C-O-C), 1615(C=C), 1635(N=CH), 3300(Ar-OH). ^{1}H-NMR (δ, ppm)($CDCl_3$): 1.94(3H, s, Ar-CH_3), 3.98(3H, s, OCH_3), 6.21(1H, bs, OH), 6.50(1H, d, Ar-H), 7.03(1H, d, Ar-H),

7.16(1H, d, Ar-H), 7.31(1H, d, Ar-H),7.47(5H, m, Ar-H), 7.61(2H, d, Ar-H), 7.71(2H ,d, Ar-H), 8.77(1H, s, H-C=N).

4- Aryl-1-phenyl-1H-Pyrazol-5-ylimino-2-methoxy phenyl acetate ***(5a-l)***
Acyl Chloride **4** (0.01 mole) and of 4-Aryl-1-phenyl-1H-Pyrazol-5-ylimino-2-methoxyPhenol **3** (0.01 mole) were added in Ethanol (25ml) containing and 50% NaOH (2 ml) solution. The mixture was stirred for 30 min at 0 ^{0}C and then refluxed for 9 hr. After the completion of reaction, content was poured in ice, filtered and dried to obtained crude product **5.**

4-(3-(4-chlorophenyl)-1-phenyl-1H-pyrazol-5-ylimino)methyl)-2-methoxyphenylacetate ***5a.***
Yield 78 %; m.p. 195^{0}C; IR (cm-1) (KBr): 1060 (C-O-C), 1615(C=C), 1636(N=CH), 1712(C=O**). ^{1}H-NMR (δ, ppm)(CDCl$_3$):** 2.21(3H, s, CH_3), 3.91(3H, s, OCH_3), 6.81(1H, s, Ar-H), 7.23(1H, dd, Ar-H), 7.37(1H, dd, Ar-H), 7.53(1H, dd, Ar-H), 7.61(2H, d, Ar-H), 7.67(2H, d, Ar-H), 7.87(5H, m, Ar-H), 8.81(1H, s, H-C=N**). Mass (M/Z):** 403, 388.

4-(3-(4-chlorophenyl)-1-phenyl-1H-pyrazol-5-ylimino)methyl)-2-methoxyphenylbenzoate ***5b.***
Yield 79 %; m.p. 203^{0}C; IR (cm-1) (KBr): 1052 (C-O-C), 1605(C=C), 1630(N=CH), 1731 (C=O). **^{1}H-NMR (δ, ppm) (CDCl$_3$):** 3.82(3H, s, OCH_3), 5.93(1H, s, Ar-H), 6.90(1H, d, Ar-H), 7.01(1H, s, Ar-H),7.21(1H, s, Ar-H),7.43(5H, m, Ar-H), 7.52(2H, d, Ar-H), 7.59(2H, d, Ar-H), 8.10(5H, m, Ar-H), 8.42(1H, s, H-C=N). **Mass (M/Z):** 507, 492, 401.

4-(3-(4-chlorophenyl)-1-phenyl-1H-pyrazol-5-ylimino)methyl)-2-methoxyphenyl4-methylbenzoate ***5c.***
Yield 91 %; m.p. 193^{0}C; IR (cm-1) (KBr): 1058(C-O-C), 1618(C=C), 1589(N=CH), 1735(C=O). **^{1}H-NMR (δ, ppm) (CDCl$_3$):** 2.24(3H, s Ar-CH_3), 3.94(3H, s, OCH_3), 6.68(1H, s, Ar-H), 7.25(1H, d, Ar-H), 7.29(1H, d, Ar-H), 7.33(1H, dd, Ar-H), 7.50(5H, m, Ar-H), 7.63(2H, d, Ar-H), 7.68(2H, d, Ar-H), 7.82(2H, d, Ar-H), 8.24(2H, d, Ar-H), 8.72(1H, s, H-C=N).

4-(3-(4-chlorophenyl)-1-phenyl-1H-pyrazol-5-ylimino)methyl)-2-methoxyphenyl4-nitrobenzoate ***5d.***
Yield 90 %; m.p. 187^{0}C; IR (cm-1) (KBr): 1050(C-O-C), 1585(C=C),1 636(N=CH), 1742(C=O). **^{1}H-NMR (δ, ppm) (CDCl$_3$):** 3.87(3H, s, OCH_3), 5.93(1H, s, Ar-H), 7.39(5H, m, Ar-H), 7.53(2H, d, Ar-H), 7.55(1H, d, Ar-H), 7.61(2H, d, Ar-H), 7.73(2H, d, Ar-H), 7.86(1H, s, H-C=N), 7.96(2H, d, Ar-H), 8.28(1H, d, Ar-H), 8.30(1H, d, Ar-H). Mass **(M/Z): 552.**

4-(3-(4-Bromophenyl)-1-phenyl-1H-pyrazol-5-ylimino)methyl)-2-methoxyphenylacetate ***5e.***
Yield 85 %; m.p. 206^{0}C; IR (cm-1) (KBr): 1065(C-O-C), 1590(C=C),1620(N=CH), 1720 (C=O). **^{1}H-NMR (δ, ppm) (CDCl$_3$):** 2.20(3H, s, CH_3), 3.93(3H, s, OCH_3), 6.85(1H, s, Ar-H), 7.33(1H, dd, Ar-H), 7.37(1H, dd, Ar-H), 7.57(1H, dd, Ar-H), 7.66(2H, d, Ar-H), 7.77(2H, d, Ar-H), 7.87(5H, m, Ar-H), 8.80(1H, s, H-C=N). **Mass (M/Z)**: 489, 313, 151.

4-(3-(4-Bromophenyl)-1-phenyl-1H-pyrazol-5-ylimino)methyl)-2-methoxyphenylbenzoate ***5f.***
Yield 79 %; m.p. 174 ^{0}C; IR (cm-1) (KBr): 1055(C-O-C), 1605(C=C), 1620(N=CH), 1727(C=O). **^{1}H-NMR (δ, ppm) (CDCl$_3$):** 3.85(3H, s, OCH3), 5.98(1H, s, Ar-H), 6.94(1H, d, Ar-H), 7.11(1H,s,Ar-H),7.21(1H,s,Ar-H),7.45(5H,m,Ar-H), 7.52(2H, d, Ar-H), 7.59(2H, d, Ar-H), 8.17(5H, m, Ar-H), 8.42(1H, s, H-C=N).

4-(3-(4-Bromophenyl)-1-phenyl-1H-pyrazol-5-ylimino)methyl)-2-methoxyphenyl 4-methylbenzoate ***5g.***
Yield 90 %; m.p. 170^{0}C; IR (cm-1) (KBr): 1060(C-O-C), 1605(C=C), 1636(N=CH), 1736(C=O). **^{1}H-NMR (δ, ppm) (CDCl$_3$):** 1.80(3H, s Ar-CH_3), 3.95(3H, s, OCH3), 6.69(1H, s, Ar-H), 7.20(1H, d, Ar-H), 7.27(1H, d, Ar-H), 7.35(1H, dd, Ar-H), 7.50(5H, m, Ar-H), 7.66(2H, d, Ar-H), 7.70(2H, d, Ar-H), 7.80(2H, d, Ar-H), 8.20(2H, d, Ar-H), 8.77(1H, s, H-C=N). **Mass (M/Z):** 565.

4-(3-(4-Bromophenyl)-1-phenyl-1H-pyrazol-5-ylimino)methyl)-2-methoxyphenyl 4-nitrobenzoate ***5h.***
Yield 92 %; m.p. 188^{0}C; IR (cm-1) (KBr): 1065(C-O-C), 1610(C=C), 1620(N=CH), 1742(C=O). **^{1}H-NMR (δ, ppm) (CDCl$_3$):** 3.85(3H, s, OCH_3), 5.98(1H, s, Ar-H), 7.34(5H, m, Ar-H), 7.56(2H, d, Ar-H),7.58 (1H, s, Ar-H),7.62(2H, d, Ar-H), 7.76(2H, s, Ar-H), 7.88(2H, s, Ar-H), 8.24(1H, d, Ar-H), 8.29(1H, d, Ar-H), 8.31(1H, s, H-C=N). **Mass (M/Z):** 595.

4-((1-phenyl-3-p-tolyl-1H-pyrazol-5-ylimino)methyl)-2-methoxyphenyl acetate ***5i***
Yield 87 %; m.p. 203^{0}C; IR (cm-1) (KBr): 1060(C-O-C), 1615(C=C), 1636(N=CH), 1720(C=O). ^{1}H-NMR (δ, ppm) ($CDCl_3$): 2.20(3H, s, CH_3), 2.31(3H, s, Ar-CH_3), 3.96(3H, s, OCH_3), 6.91(1H, s, Ar-H), 7.21(1H, dd, Ar-H), 7.37(1H, dd, Ar-H), 7.53(1H, dd, Ar-H), 7.61(2H, d, Ar-H), 7.67(2H, d, Ar-H), 7.87(5H, m, Ar-H), 8.85(1H, s, H-C=N).

4-((1-phenyl-3-p-tolyl-1H-pyrazol-5-ylimino)methyl)-2-methoxyphenyl4-methylbenzoate ***5j***
Yield 75 %; m.p. 169^{0}C; IR (cm-1) (KBr): 1045 (C-O-C), 1600(C=C), 1630(N=CH), 1670(C=O). **^{1}H-NMR (δ, ppm) ($CDCl_3$):** 2.32(3H, s, Ar-CH_3), 3.92(3H, s, OCH_3), 5.92(1H, s, Ar-H), 6.94(1H, d, Ar-H), 7.11(1H,s,Ar-H), 7.23(1H, s, Ar-H),7.42(5H,m, Ar-H), 7.52(2H, d, Ar-H), 7.59(2H, d, Ar-H), 8.15(5H, m, Ar-H), 8.39(1H, s, H-C=N). **Mass (M/Z):** 487.

4-((1-phenyl-3-p-tolyl-1H-pyrazol-5-ylimino)methyl)-2-methoxyphenyl 4-methylbenzoate ***5k***.
Yield 73 %; m.p. 221^{0}C; IR (cm-1) (KBr): 1060(C-O-C), 1611(C=C), 1646(N=CH), 1736(C=O). **^{1}H-NMR (δ, ppm) ($CDCl_3$):** 1.71(3H, s Ar-CH_3), 1.96(3H, s, Ar-CH_3), 3.94(3H, s, OCH3), 6.68(1H, s, Ar-H), 7.25(1H, d, Ar-H), 7.30(1H, d, Ar-H), 7.39(1H, dd, Ar-H), 7.51(5H, m, Ar-H), 7.63(2H, d, Ar-H), 7.68(2H, d, Ar-H), 7.80(2H, d, Ar-H), 8.22(2H, d, Ar-H), 8.69(1H, s, H-C=N). **Mass (M/Z):** 501.

4-((1-phenyl-3-p-tolyl-1H-pyrazol-5-ylimino)methyl)-2-methoxyphenyl 4-nitrobenzoate ***5l***
Yield 91 %; m.p. 201^{0}C; IR (cm-1) (KBr): 1060(C-O-C), 1615(C=C), 1624(N=CH), 1730(C=O). **^{1}H-NMR (δ, ppm) ($CDCl_3$):** 2.74(3H, s, Ar-CH_3), 3.87(3H, s, OCH_3), 5.93(1H, s, Ar-H), 7.08(2H, d, Ar-H), 7.39(5H, s, Ar-H), 7.55(2H, d, Ar-H), 7.86(1H, d, Ar-H),7.93(2H, d, Ar-H), 7.99(1H, s, H-C=N), 8.01(2H, d, Ar-H), 8.28(1H, d, Ar-H), 8.30(1H, d, Ar-H).

Antibacterial activity
All the synthesized compounds were tested for antibacterial and antifungal activity by following method. Method: Agar-well diffusion method

Gram +ve bacteria: *Staphylococcus Aureus* and *Bacillus subtilis*
Gram –ve bacteria: *Pseudomonas* and *Escherichia coli*
Concentration: 100 ppm, 200 ppm and 300 ppm
Solvent: DMSO, Standard drug: *Cephalothin* for Gram positive Bacteria and *Chloramphenicol* for Gram negative Bacteria.

Preparation of culture medium and inoculation
For antibacterial activity, 35 gm nutrient agar and 10 gm agar-agar were suspended in distilled water (1000 ml) and suspended by boiling. Media and Petri dishes were sterilized in autoclave at pressure 15 lbs for 20 minutes. Under aseptic condition, 20 ml of media was dispended into sterilized Petri dishes to yield a uniform depth of 6 mm. The media was allowed to solidify at room temperature for 0.5 h. After solidification of the medium; the bacterial cultures were inoculated by spread plating technique.

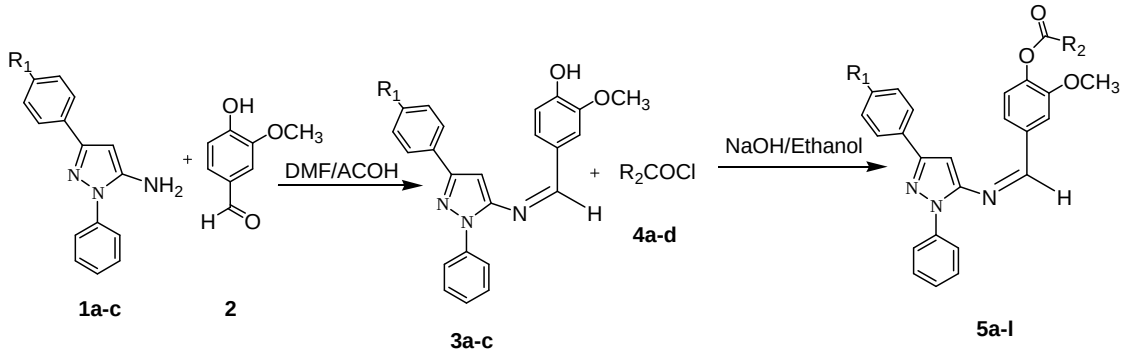
Disc application and incubation
Discs of 6 mm diameter were prepared from Whatman No. 1 filter paper, sterilized by autoclaving and subsequently dried at 80^{o}C for an hour. The sterilized discs were immersed in respective formulations of compounds. The discs were placed on nutrient agar surface with flamed forceps and gently pressed down to ensure contact with the agar surface. The discs were spaced for enough to avoid both reflection waves from the edges of Petri dishes and overlapping rings of inhibition and finally, the Petri dishes were incubated for 24 h at 37^{o}C in an inverted position. After 24 h the diameter (mm) of the inhibition zone around each spot were measured. Antibacterial activities were indicated by clear zone of growth inhibition.

Docking procedure
Molecular docking work was performed with the Hex molecular modeling package version 6.3. The three dimensional crystal structure of *E. coli MurB* enzyme (PDB code 2MBR) was used throughout the work. The ligands were converted to 2D and 3D energy-minimized conformations using Hex 3D Ultra 6.0. respectively and visualize the conformation by using Acceryl Discovery Studio 3.1 Client.

RESULTS AND DISCUSSION

Chemistry

Pyrazole derivatives were prepared by a series of reactions as illustrated in Scheme 1. Reaction of 5-amino pyrazole **1a-c** with vanillin **2** in DMF and acetic acid was added and the reaction mixture was refluxed for three hours gave Schiff bases **3a-c** as the sole separable product. The purity of the compounds was ascertained by TLC and the structures of the synthesized compounds were assigned by IR, ^{1}H NMR, and Mass spectra. The assignments of the structures of products **3a-c** were based on their correct spectroscopic data. The most differentiating bands in IR spectra are shown at rang of two characteristic absorption bands at 1610-1650 cm^{-1} and 3393-3450 cm^{-1}, assignable to a conjugated CH=N group and OH group respectively. Furthermore, aromatic C=C stretching around 11690-1620 cm^{-1} and C-O-C stretching around 1040-1060 cm^{-1}. ^{1}H-NMR (400 MHz) spectra of **3a-c** indicate the presence of a singlet at δ 8.60-8.75 ppm which could be assigned to CH=N. Compound **3a-c** show characteristic signal at δ 6.10-6.25 ppm and δ 3.90-3.98 ppm for OH and OCH_3 respectively.



1a-c **2** **3a-c** **5a-l**

1,3	R_1
a	4-Cl
b	4-Br
c	4-CH_3

5	R_1	R_2
a	4-Cl	CH_3
b	4-Cl	C_6H_5
c	4-Cl	4-CH_3-C_6H_4
d	4-Cl	4-NO_2-C_6H_4
e	4-Br	CH_3
f	4-Br	C_6H_5
g	4-Br	4-CH_3-C_6H_4
h	4-Br	4-NO_2-C_6H_4
i	4-CH_3	CH_3
j	4-CH_3	C_6H_5
k	4-CH_3	4-CH_3-C_6H_4
l	4-CH_3	4 NO_2-C_6H_4

(Scheme 1)

In addition, treatment of **3a-c** with substituted acyl chloride **4a-d** in the presence of sodium hydroxide afforded the O-acylated Schiff bases **5a-l**. The structural assignments of all ester **5a-l** derivatives can be easily seen from their IR spectra. Absorption bands of ester group at about 1710 – 1750 cm^{-1} is structural characteristics. Thus, appearance of a IR peak for ester and disappearance of peak of OH group also confirms the formation of **5a-l** derivatives. ^{1}H-NMR (400 MHz) spectra of compound **7e** show three singlets for at δ 2.20, 6.85 and 3.93 ppm indicating presence of one CH_3, one OCH_3 and 3rd proton of pyrazole ring. A multiplate were observed in the aromatic region at δ7.33-7.87 ppm indicating presence of aromatic hydrogen for two phenyl ring and one aromatic vanillin moiety. A singlet at δ 8.80 ppm indicating the presence of CH=N group. The structure of compound **7e** was further confirmed by the mass spectral data which showed molecular ion peak M^+ at *m/z* 489 corresponding to molecular formula $C_{25}H_{20}BrN_3O_3$.

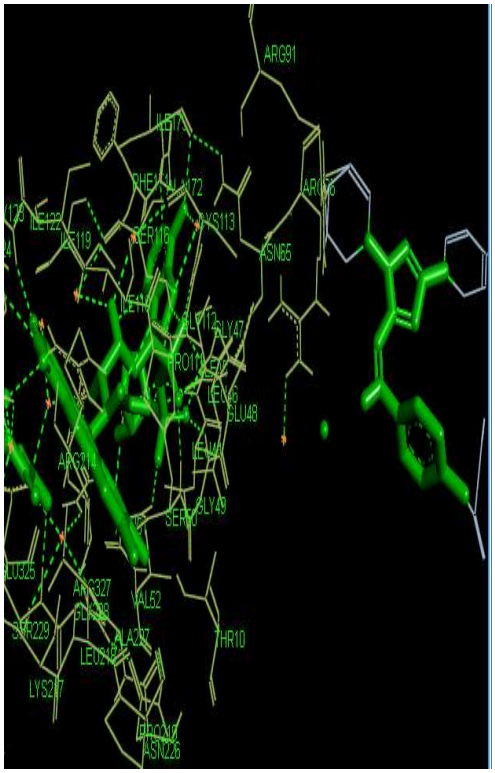
Antibacterial activity

Synthesized Compound 5(a-l) were evaluated for their in vitro antimicrobial activity against two Gram-positive (*S. aureus* and *B. subtilis*) and two Gram negative bacteria (*Pseudomonas* and *Escherichia coli*) by the agar diffusion method as per the guidelines of the National Committee for Clinical Laboratory Standards [25]. The antibacterial activities of the compounds were dose dependent and remarkable at 100 ppm, 200 ppm and 300 ppm in DMF. The antimicrobial screening results were measured by the average diameter of the inhibition zones, expressed in mm, and presented in **Table 1.** Since, Schiff bases derivatives were already reported to be antibacterial and antifungal activity [26] the corresponding Schiff bases derivatives of the 1H-pyrazole discussed herein are also expected to exhibit similar activities. This finding may promote the synthesis of more active 1H-pyrazolein the future.

Table1: Quantitative Antimicrobial Activity of compounds Parent (5a-l) Standard (Zone of inhibition in mm)

Entry	*Pseudomonas*			*E. coli*			*S. aureus*			*Bacillus subtilis*			ΔG (Kcal/mol) With *MurB*
5	Conc. in ppm			Conc. in ppm			Conc. in ppm			Conc. in ppm			
	100	200	300	100	200	300	100	200	300	100	200	300	
A	01	14	18	10	13	15	05	09	11	10	11	13	-248
B	08	10	16	05	10	10	02	10	12	07	11	12	**-198**
C	**13**	**17**	**25**	**19**	**15**	**17**	**21**	**23**	**02**	**16**	**15**	**18**	-255
D	02	11	11	02	10	11	14	16	25	15	17	27	-277
E	01	05	03	13	14	14	01	23	19	03	03	10	-298
F	10	11	16	15	11	11	03	10	12	06	10	12	-271
g	13	10	25	11	05	13	02	04	06	12	15	18	-228
h	18	10	06	10	06	10	10	06	10	07	07	18	**-215**
i	**19**	**18**	**25**	**18**	**20**	**15**	**17**	**18**	**17**	**15**	**19**	**19**	-233
j	06	05	06	13	05	06	20	14	05	06	05	06	-256
k	**14**	**17**	**20**	**27**	**21**	**25**	**20**	**16**	**17**	**11**	**16**	**17**	-258
l	05	11	09	10	07	06	14	13	14	12	09	05	-256
Cephalothin	25			31			--	--	--	--	--	--	
Chloramphenicol	--	--	--	-	--	--	28			30			

A) **B)**



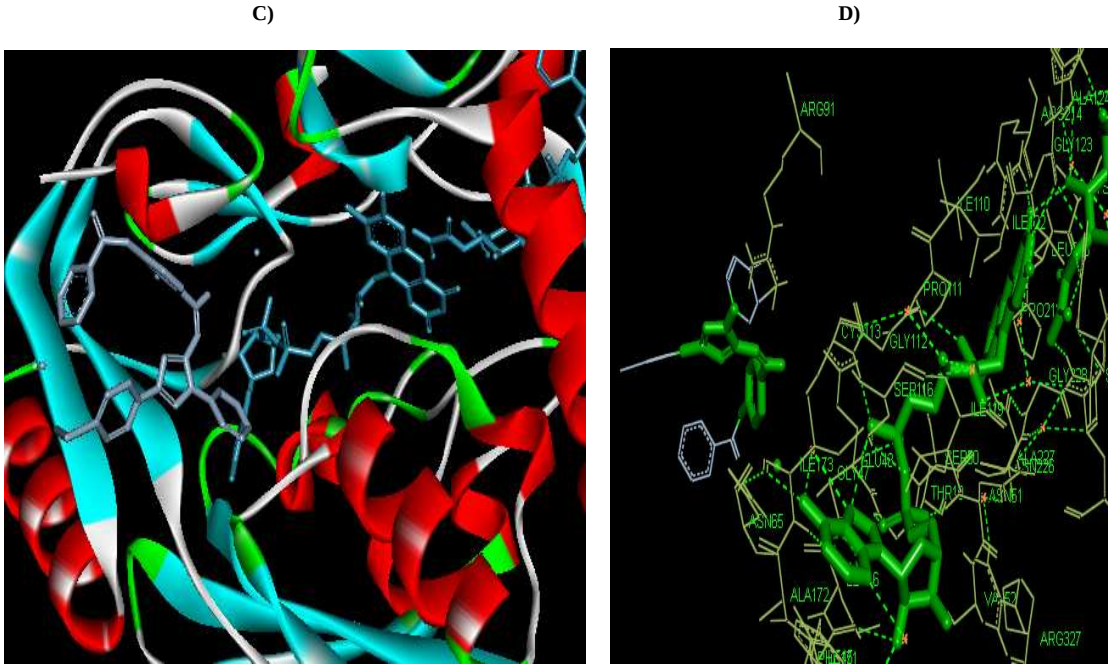


Fig. 2 A) Interaction of 5b with ARG91. B). Binding of 5b with amino acid of ARG91 C) Interaction of 5h with ARG91. D). Binding of 5h with amino acid of ARG91

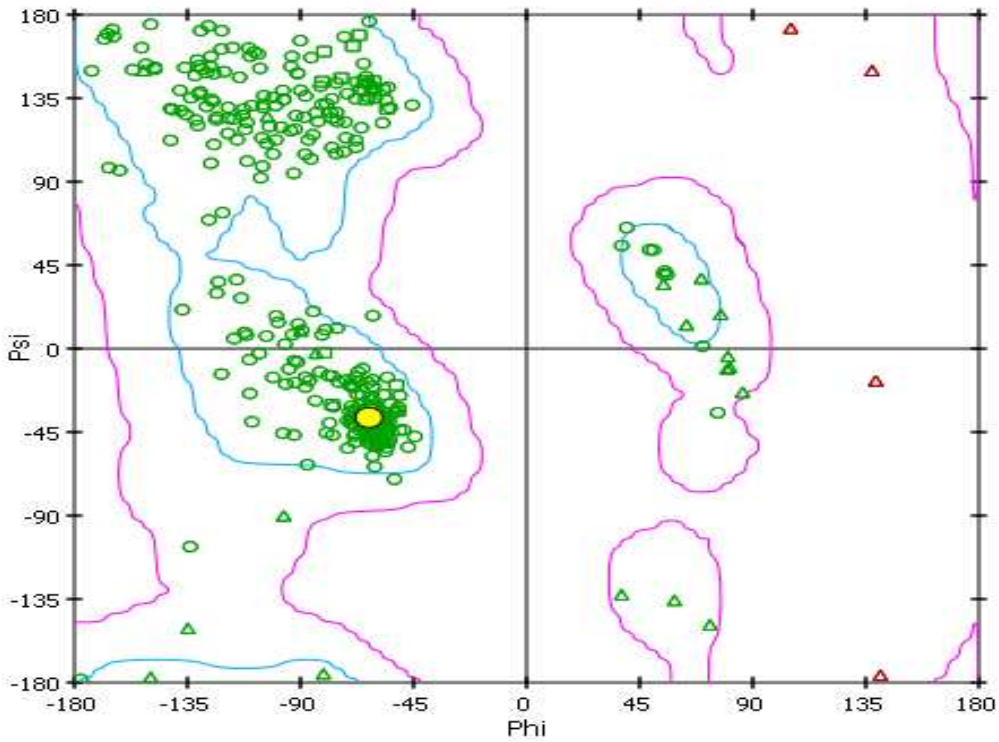


Fig. 3 Ramachandran *plot*: The figure shows the pairs of (psi, phi) angles active binding ARG91 residues with compound 5b

Docking procedure

In order to study the possible interactions between our synthesized compounds and the active site of the *MurB* enzyme, a docking process were undertaken using Hex 6.3. The three dimensional crystal structure of *E. coli MurB* enzyme (PDB code 2MBR) was used throughout the work. The docking of ligand molecules with *MurB* enzyme

revealed that all the compounds exhibited the bonding with amino acids in the active pockets. Molecular docking study and Ramachandran plot of the enzyme active site of *MurB* enzyme (PDB code 2MBR) were depicted in respectively (Fig.2, 3). Among the pyrazole derivatives **5a-l** only **5b** and **5h** moiety is stabilized by the hydrophobic interaction between the side chains of ARG91.

CONCLUSION

Series of 4-Aryl-1-phenyl-1H-Pyrazol-5-ylimino-2-methoxyPhenyl acetate **5a-l** have been synthesized. The docking analysis performed on a series of 4- Aryl-1-phenyl-1H-Pyrazol-5-ylimino-2-methoxy phenyl acetate (5a-l). On the basis of experimental finding by docking studies, possible binding modes of the pyrazole derivatives have been thoroughly explored. One major observation includes almost all of the compounds docked with *E. coli MurB* enzyme but only **5b** and **5h** these moiety show interaction with ARG91display a general binding mode. The anti-bacterial study of the synthesized compounds showed good to excellent activity against tested Gram-positive and Gram-negative bacteria**.** Among these, particularly, 4-((E)-(3-(4-chlorophenyl)-1-phenyl-1H-pyrazol-5-ylimino)methyl)-2-methoxyphenyl 4-methylbenzoate **5c,** 4-((E)-(1-phenyl-3-p-tolyl-1H-pyrazol-5-ylimino)methyl)-2-methoxyphenyl acetate **5j** and 4-((E)-(1-phenyl-3-p-tolyl-1H-pyrazol-5-ylimino)methyl)-2-methoxyphenyl 4-methylbenzoate **5k** found to have better potency against Gram-positive bacteria and Gram-negative Bacteria.

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