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ORIGINAL ARTICLE

Facile synthesis of 4-aryl-*N*-(5-methyl-1*H*-pyrazol-3-yl)benzamides *via* Suzuki Miyaura reaction: Antibacterial activity against clinically isolated NDM-1-positive bacteria and their Docking Studies

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Abstract The production of new pyrazole amide derivatives (**6a-h**) and their potential against New Delhi metallo-β-lactamase-1 (NDM-1) producing bacteria was described in the present manuscript. The 4-bromo-*N*-(5-methyl-1*H*-pyrazol-3-yl)benzamide (**5**) was synthesized *via* direct amidation of protected 5-methyl-1*H*-pyrazol-3-amine (**3**). The target pyrazole amide derivatives (6a-h) were synthesized in moderate to excellent yield *via* Palladium catalyzed Suzuki cross-coupling of intermediate molecule (5) with different aryl and heteroaryl boronic acids. NMR and Mass Spectrometry were used to characterize the derivatives. The *in vitro* antibacterial effect against NDM-1-positive *Acinetobacter baumannii* and *Klebsiella pneumoniae* of newly synthesized analogues (6a-h) were determined by Agar well diffusion method. Moreover, MIC and MBC values were also evaluated against the tested bacteria. In addition, the Molecular Docking study of pyrazole amide derivatives (6a-h) against the NDM producing *A. baumannii* was performed to investigate the intermolecular interaction. The binding affinity and their values were compared with L-captopril. The **6b** had greatest potential value and was appeared as a promising antibacterial agent.

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1. Introduction

The production of novel antimicrobial drugs still has its demand because the infections and decreasing effect of currently available antibiotic drugs against the resistive microorganisms are the key factors of morbidity and mortality in developing and developed countries (Gabriel et al., 2007; Raghavan et al., 2012; Phillips et al., 2017; Tuchilus et al., 2017). Recently, the researchers show more interest in nitrogen containing heterocycles, particularly pyrazoles and its derivatives (Gurunanjappa et al., 2015). Nowadays, pyrazoles have developed more attention because of their remarkable pharmacological properties (Tsutomu and Toshitaka, 1978; García-Lozano et al., 1997; Steinbach et al., 2000; Uslaner et al., 2009). Pyrazole is a fundamental heterocyclic moiety in numerous bioactive molecules due to various active sites (Jacob and Ganguly, 2016). The pyrazole skeleton is a leading molecule in pharmaceutical industry with a lot of biological activities (Goda et al., 2003; Mansour et al., 2003; El-Emary, 2006). The literature survey exposes that pyrazoles exhibited antibacterial (Sangapure et al., 2001; Mert et al., 2014; Li et al., 2015), antifungal (Gupta et al., 2005; Ashish et al., 2006), anti-inflammatory (Makhsumov et al., 1986; Udupi et al., 1998), antitubercular (Ashish et al., 2006), anticancer (Chetan et al., 2004; Balbi et al., 2011; Kamal et al., 2015), antipyretic (Souza et al., 2002), anticonvulsant (Ashok and Sharma, 2001), analgesic (Prokopp et al., 2006; Szabó et al., 2007; Chowdhury et al., 2009; Hwang et al., 2011), antimicrobial (Pimerova and Voronina, 2001; Bekhit et al., 2008) and anti-tumor activities (Park et al., 2005). These activities have motivated the researchers to develop the pyrazole template systems to demonstrate its efficacy.

New-Delhi metallo-β-lactamase (NDM-1) was first identified in the clinical isolates of *K. pneumoniae* and *E. coli* in 2009 from a Swedish patient who had been hospitalized in New Delhi, India (Qamar et al., 2018) and reported in *K. pneumoniae* in 2011 from Colombia and Guatemala (Latin America) (Pasteran et al., 2012; Pérez et al., 2013). In Brazil, it was recognized in *P. rettgeri* (Carvalho-Assef et al., 2013) and *E. hormaechei* in 2013 (Carvalho-Assef et al., 2014). Majority of cases concerned to India, Bangladesh, Pakistan and the Balkans region (Nordmann et al., 2011; Bonnin et al., 2012; Johnson and Woodford, 2013). NDM-1-positive *A. baumannii* of sequence type (ST) 25 was initially removed from the urine sample of seventy one year male. The gene of NDM-1 was distinguished by quantitative polymerase chain reaction, the sequence of which was confirmed by its existence in almost 100 kb plasmid (Pillonetto et al., 2014). Carbapenem and all other β-lactam medication are inactive for NDM-1 as clinically no beneficial NDM-1 inhibitor is available (Ning et al., 2018). Hence, our research group planned to synthesize some pyrazole derivatives and subsequently screened for their antibacterial activity against NDM-1-positive *A. baumannii* and *K. pneumonia*e.

The recent literature revealed the synthesis of pyrazole derivatives by Suzuki coupling reaction (Channar et al., 2018a) and their relevant biological activities on the bases of molecular docking studies (Channar et al., 2018b). The hybrid compounds bearing pyrazole and benzamide groups were recently prepared and their structural properties were determined (Channar et al., 2019; Saeed et al., 2020). So, in present work, 4-bromo-*N*-(5-methyl-1*H*-pyrazol-3-yl)benzamide **(5)** by one pot condensation of N-protected 3-amino-5-methyl-1*H*-pyrazole **(3)** with 4-bromobenzoic acid **(4)** in the presence of titanium tetrachloride and its derivatives **(6a-h)** *via* Suzuki Miyaura cross coupling reactions were synthesized. All the target molecules were screened for antibacterial activity by agar well diffusion method against NDM producing bacteria. Subsequently, MIC and MBC values were evaluated and molecular docking was also performed.

2. Experimental section

2.1. General information

All chemicals were bought from Sigma Aldrich USA and commercial grade solvents were used. Melting points of final compounds were checked with Buchi melting point apparatus (B-540, New Castle). Bruker NMR spectrophotometer, Billercia (USA) was used to achieve NMR spectra by using $CDCl_3$ and DMSO d_6 solvents. Mass spectra were recorded on JEOL spectrometer (JMS-HX-110, USA). Silica gel (70–230 mesh) was utilized in column chromatography for compound's purification. The progress of reactions was tested by TLC on silica gel 60 PF_{254} cards (Merck). Visualization of product on TLC was observed with UV lamp (254–365 nm).

2.2. General protocol for the production of compounds

2.2.1. Procedure for the synthesis of tert-butyl 3-amino-5-methyl-1H-pyrazole-1-carboxylate (3)

5-methyl-1*H*-pyrazol-3-amine **(1)** (1.0 eq., 30.89 mmol) was taken in round bottom flask and dissolved in 180 ml of 1,4-dioxane Triethylamine (1.5 eq., 46.33 mmol) and di-*tert*-butyl pyrocarbonate **(2)** (1.5 eq., 46.33 mmol) were added in the flask and whole mixture was refluxed for 1–1.5 h. After that, the solvent was extracted via rotary evaporator and residue was diluted with ethyl acetate. Then, column chromatography was used to pure the crude product by using n-hexane: ethyl acetate (70:30) and a colorless powder resulted (Kusakiewicz-Dawid et al., 2009).

2.2.2. Procedure for the production of 4-bromo-N-(5-methyl-1H-pyrazol-3-yl)benzamide (5)

$TiCl_4$ (3.0 eq., 44.77 mmol) and the *tert*-butyl 3-amino-5-methyl-1*H*-pyrazole-1-carboxylate **(3)** (1.0 eq., 14.92 mmol) were mixed with solution of 4-bromoobenzoic acid **(4)** (1.0 eq., 14.92 mmol) in pyridine (150 ml) in a schlenk flask. The reaction mixture was continued on stirring for 2 h at 85 °C in the sealed schlenk flask. After cooling the reaction mixture, pyridine was removed by co-evaporation of toluene and residue was added in aq. solution of 1 N HCl (150 ml) and extracted by dichloromethane (3 × 150 ml). The saturated aqueous solution of sodium hydrogen carbonate (3 × 150 ml) was used to wash the mixed organic layers. Then, solution was dehydrated by anhydrous sodium sulphate and dried by evaporation. The resulted residue was refined by flash column with ethyl acetate and n-hexane (20:80) and final product resulted with yield 75% which were then analyzed by ^1H *NMR* and ^{13}C *NMR* to determine the structures (Leggio et al., 2017).

2.2.3. Procedure for the production of 4-aryl-N-(5-methyl-1H-pyrazol-3-yl)benzamide (**6a-h**)

In an oven dried schlenk flask, 1,4-dioxane (8 ml), 4-bromo-*N*-(5-methyl-1*H*-pyrazol-3-yl)benzamide **(5)** (1.0 eq., 0.526 mmol), with tetrakis(triphenylphos phine)palladium (7 mol%) at room temperature were added and under inert atmosphere. After stirring the reaction for half an hour, boronic acid (1.1 eq., 0.579 mmol), potassium phosphate (2.0 eq., 1.052 mmol) and water (2 ml) were inserted (Dang et al., 2007; Ahmad et al., 2017; Malik et al., 2020). Then, mixture was continued on stirring at reflux for 15–30 h. After cooling, the mixture was filtered using ethyl acetate. Then, solvent was vaporized on rotary evaporator and to get the pure product, the resulted residue was passed through column by using *n*-hexane and ethyl acetate (70:30). Further, the final product was dried, recrystallized and characterized with mass spectrometry and NMR (Miyaura and Suzuki, 1995; Ikram et al., 2015; Ahmad et al., 2019a; Ahmad et al., 2019b). The NMR spectra of synthesized compounds are presented within Figs. S1–S20 (supplementary data).

2.3. Characterization data

2.3.1. Tert-butyl 3-amino-5-methyl-1H-pyrazole-1-carboxylate (**3**)

White solid, MP = 99–101 °C, (70% yield, 2.35 mg). ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.31 (s, 2H, NH_2), 5.21 (s, 1H, H-C, pyrazole), 2.14 (s, 3H, CH_3), 1.62 (s, 9H, O-C(CH_3)$_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 153.20 (pyrazole-C_3), 150.79 (C═O), 150.59 (pyrazole-C_5), 89.27 (pyrazole-C_4), 84.94 (O—C), 28.03 (3C, 3CH_3), 14.40 (CH_3). EI/MS *m/z* (%): 198.2 (54) $[M+H]^+$; $[M-CH_3]$ = 182.1 (71); $[M-NH_2]$ = 181.1 (21). HRMS for $C_9H_{15}N_3O_2$ calculated: $[M]^+$; 197.1164. Found: $[M]^+$; 197.1047.

2.3.2. 4-bromo-N-(5-methyl-1H-pyrazol-3-yl)benzamide (**5**)

Off white solid, MP = 227–229 °C, (75% yield, 3.5 g). ^{1}H NMR (600 MHz, DMSO d_6): δ 12.12 (s, 1H, H-N), 10.75 (s, 1H, H—N—C═O), 7.93 (d, *J* = 8.4 Hz, 2H, Ar), 7.69 (d, *J* = 9.0 Hz, 2H, Ar), 6.39 (s, 1H, H-C, pyrazole), 2.23 (s, 3H, CH_3); ^{13}C NMR (151 MHz, DMSO d_6): 163.31 (C═O), 140.43 (pyrazole-C_3), 133.31 (Ar-C_1), 131.17 (2C, Ar-C_3, C_5), 129.74 (2C, Ar-C_2, C_6), 125.10 (Ar-C_4), 96.31 (pyrazole-C_4), 10.70 (CH_3). EI/MS *m/z* (%): 282.2 (47) $[M+H]^+$; $[M-CH_3]$ = 264.0 (79); [M−Br] = 200.1 (24). HRMS for $C_{11}H_{10}BrN_3O$ calculated: $[M]^+$; 279.0007. Found: $[M]^+$; 278.9896.

2.3.3. 4′-chloro-N-(5-methyl-1H-pyrazol-3-yl)biphenyl-4-carboxamide (**6a**)

Off white solid, MP = 184–186 °C, (81% yield, 180 mg). ^{1}H NMR (600 MHz, DMSO d_6): δ 12.13 (s, 1H, H-N), 10.74 (s, 1H, H—N—C═O), 8.10 (d, *J* = 7.8 Hz, 2H, Ar), 7.77 (t, *J* = 8.7 Hz, 4H, Ar), 7.62 (dd, *J* = 12.6, 7.2 Hz, 2H, Ar), 6.43 (s, 1H, H—C, pyrazole), 2.24 (s, 3H, CH_3); ^{13}C NMR (151 MHz, DMSO d_6): 163.85 (C═O), 141.53 (pyrazole-C_5), 137.91 (Ar), 133.34 (Ar), 132.98 (pyrazole-C_3), 131.98 (Ar), 131.48 (Ar), 128.95 (2C, Ar), 128.61 (2C, Ar), 128.44 (2C, Ar), 126.41 (2C, Ar), 96.41 (pyrazole-C_4), 10.82 (CH_3). EI/MS *m/z* (%): 312.9 (64) $[M+H]^+$; $[M-CH_3]$ = 296.1 (89); [M−Cl] = 276.2 (18). HRMS for $C_{17}H_{14}ClN_3O$ calculated: $[M]^+$; 311.0825. Found: $[M]^+$; 311.0714.

2.3.4. 3′-chloro-N-(5-methyl-1H-pyrazol-3-yl)biphenyl-4-carboxamide (**6b**)

Off white solid, MP = 227–229 °C, (90% yield, 200 mg). ^{1}H NMR (600 MHz, DMSO d_6): δ 12.15 (s, 1H, H-N), 10.77 (s, 1H, H—N—C═O), 8.12 (d, *J* = 8.4 Hz, 2H, Ar), 7.82–7.80 (m, 3H, Ar), 7.70 (d, *J* = 7.8 Hz, 1H, Ar), 7.64–7.62 (m, 2H, Ar), 6.45 (s, 1H, H-C, pyrazole), 2.24 (s, 3H, CH_3); ^{13}C NMR (151 MHz, DMSO d_6): 163.83 (C═O), 141.28 (pyrazole-C_5), 133.86 (pyrazole-C_3), 133.06 (Ar), 131.98 (Ar), 131.49 (Ar), 131.42 (Ar), 130.78 (Ar), 128.74 (Ar), 128.66 (2C, Ar), 128.44 (Ar), 126.65 (2C, Ar), 125.54 (Ar), 96.43 (pyrazole-C_4), 10.83 (CH_3). EI/MS *m/z* (%): 312.9 (49) $[M+H]^+$; $[M-CH_3]$ = 296.1 (78); [M−Cl] = 276.1 (23). HRMS for $C_{17}H_{14}ClN_3O$ calculated: $[M]^+$; 311.0825. Found: $[M]^+$; 311.0702.

2.3.5. 3′-chloro-4′-fluoro-N-(5-methyl-1H-pyrazol-3-yl) biphenyl-4-carboxamide (**6c**)

Off white solid, MP = 233–235 °C, (77% yield, 181 mg). ^{1}H NMR (600 MHz, DMSO d_6): δ 12.11 (s, 1H, H-N), 10.74 (s, 1H, H—N—C═O), 8.10 (d, *J* = 8.4 Hz, 2H, Ar), 7.97 (dd, *J* = 7.2, 2.4 Hz, 1H, Ar), 7.81 (d, *J* = 8.4 Hz, 2H, Ar), 7.77–7.75 (m, 1H, Ar), 7.51 (t, *J* = 9.0 Hz, 1H, Ar), 6.43 (s, 1H, H-C, pyrazole), 2.24 (s, 3H, CH_3); ^{13}C NMR (151 MHz, DMSO d_6): 163.77 (C═O), 157.99 (Ar), 156.35 (Ar), 140.45 (pyrazole-C_5), 136.95 (Ar), 133.50 (pyrazole-C_3), 128.86 (Ar), 128.40 (2C, Ar), 127.54 (Ar), 126.58 (2C, Ar), 120.26 (Ar), 117.40 (Ar), 117.26 (Ar), 96.42 (pyrazole-C_4), 10.82 (CH_3). EI/MS *m/z* (%): 330.9 (66) $[M+H]^+$; $[M-CH_3]$ = 314.1 (85); [M−Cl] = 294.2 (31). HRMS for $C_{17}H_{13}ClFN_3O$ calculated: $[M]^+$; 329.0731. Found: $[M]^+$; 329.0747.

2.3.6. 3′-acetyl-N-(5-methyl-1H-pyrazol-3-yl)biphenyl-4-carboxamide (**6d**)

Off white solid, MP = 209–211 °C, (57% yield, 130 mg). ^{1}H NMR (600 MHz, DMSO d_6): δ 12.12 (s, 1H, H-N), 10.75 (s, 1H, H—N—C═O), 8.26 (s, 1H, Ar), 8.14 (d, *J* = 8.4 Hz, 2H, Ar), 7.99 (t, *J* = 8.6 Hz, 2H, Ar), 7.86 (d, *J* = 7.8 Hz, 2H, Ar), 7.64 (t, *J* = 7.8 Hz, 1H, Ar), 6.44 (s, 1H, H—C, pyrazole), 2.67 (s, 3H, CH_3—C═O), 2.25 (s, 3H, CH_3); ^{13}C NMR (151 MHz, DMSO d_6): 197.90 (NH—C═O), 163.88 (C═O), 147.06 (Ar), 142.03 (pyrazole-C_5), 139.57 (Ar), 138.62 (Ar), 137.57 (Ar), 133.45 (pyrazole-C_3), 131.44 (Ar), 129.44 (Ar), 128.46 (2C, Ar), 127.61 (Ar), 126.68 (2C, Ar), 126.49 (Ar), 96.43 (pyrazole-C_4), 26.85 (CH_3—C═O), 10.83 (CH_3). EI/MS *m/z* (%): 320.5 (51) $[M+H]^+$; $[M-CH_3]$ = 304.2 (72); $[M-COCH_3]$ = 276.2 (16). HRMS for $C_{19}H_{17}N_3O_2$ calculated: $[M]^+$; 319.1321. Found: $[M]^+$; 319.1257.

2.3.7. Methyl 4′-(5-methyl-1H-pyrazol-3-ylcarbamoyl) biphenyl-4-carboxylate (**6e**)

Off white solid, MP = 261–263 °C, (38% yield, 91 mg). ^{1}H NMR (600 MHz, DMSO d_6): δ 12.14 (s, 1H, H-N), 10.79 (s, 1H, H—N—C═O), 8.13 (d, *J* = 8.4 Hz, 2H, Ar), 8.06 (d, *J* = 8.4 Hz, 2H, Ar), 7.91 (d, *J* = 7.8 Hz, 2H, Ar), 7.86 (d, *J* = 8.4 Hz, 2H, Ar), 6.41 (s, 1H, H—C, pyrazole), 3.86 (s, 3H, O—CH_3), 2.24 (s, 3H, CH_3); ^{13}C NMR (151 MHz,

DMSO d_6): 165.92 (O—C═O), 163.71 (C═O), 143.53 (Ar), 141.44 (pyrazole-C_5), 138.66 (Ar), 133.79 (pyrazole-C_3), 129.76 (2C, Ar), 128.92 (Ar), 128.42 (2C, Ar), 127.11 (2C, Ar), 126.75 (2C, Ar), 126.47 (Ar), 96.29 (pyrazole-C_4), 52.10 (O—CH_3), 10.80 (CH_3). EI/MS *m/z* (%): 336.5 (36) $[M+H]^+$; $[M-CH_3]$ = 320.2 (89); $[M-COOCH_3]$ = 276.2 (32). HRMS for $C_{19}H_{17}N_3O_3$ calculated: $[M]^+$; 335.1270. Found: $[M]^+$; 335.1144.

2.3.8. N-(5-methyl-1H-pyrazol-3-yl)-4-(thiophen-2-yl) benzamide (**6f**)

Off white solid, MP = 279–281 °C, (84% yield, 170 mg). ^{1}H NMR (600 MHz, DMSO d_6): δ 12.11 (s, 1H, H-N), 10.67 (s, 1H, H—N—C═O), 8.06–8.02 (m, 3H, Ar, thiophene), 7.84 (d, J = 7.8 Hz, 2H, Ar), 7.66–7.65 (m, 2H, Thiophene), 6.42 (s, 1H, H-C, pyrazole), 2.24 (s, 3H, CH_3); ^{13}C NMR (151 MHz, DMSO d_6): δ 163.88 (C═O), 147.04 (thiophene), 140.47 (pyrazole-C_5), 138.60 (Ar), 137.83 (pyrazole-C_3), 132.51 (2C, Ar), 128.37 (Ar), 127.32 (2C, Ar), 126.17 (thiophene), 125.74 (thiophene), 122.34 (thiophene), 96.40 (pyrazole-C_4), 10.84 (CH_3). EI/MS *m/z* (%): 284.5 (43) $[M+H]^+$; $[M-CH_3]$ = 268.2 (71). HRMS for $C_{15}H_{13}N_3OS$ calculated: $[M]^+$; 283.0779. Found: $[M]^+$; 283.0823.

2.3.9. N-(5-methyl-1H-pyrazol-3-yl)-4-(pyridin-3-yl) benzamide (**6g**)

Off white solid, MP = 203–205 °C, (86% yield, 171 mg). ^{1}H NMR (600 MHz, DMSO d_6): δ 12.10 (s, 1H, H-N), 10.78 (s, 1H, H—N—C═O), 8.97 (d, J = 1.8 Hz, 1H, Pyridine), 8.61 (d, J = 4.2 Hz, 1H, Pyridine), 8.14 (d, J = 7.2 Hz, 3H, Ar, Pyridine), 7.85 (d, J = 8.4 Hz, 2H, Ar), 7.54–7.47 (m, 1H, Pyridine), 6.43 (s, 1H, H-C, pyrazole), 2.24 (s, 3H, CH_3); ^{13}C NMR (151 MHz, DMSO d_6): δ 172.23, 163.82 (C═O), 148.98 (Ar), 147.75 (pyridine), 146.78 (Ar), 139.85 (pyrazole-C_5), 138.68 (pyridine), 134.61 (pyridine), 134.29 (pyrazole-C_5), 133.68 (2C, Ar), 128.50 (2C, Ar), 126.68 (pyridine), 123.88 (pyridine), 96.40 (pyrazole-C_4), 10.83 (CH_3). EI/MS *m/z* (%): 279.4 (58) $[M+H]^+$; $[M-CH_3]$ = 263.2 (76). HRMS for $C_{16}H_{14}N_4O$ calculated: $[M]^+$; 278.1168. Found: $[M]^+$; 278.1078.

2.3.10. 4′-methoxy-N-(5-methyl-1H-pyrazol-3-yl)biphenyl-4-carboxamide (**6h**)

Light brown solid, MP = 247–249 °C, (69% yield, 152 mg). ^{1}H NMR (600 MHz, DMSO d_6): δ 12.10 (s, 1H, H-N), 10.67 (s, 1H, H—N—C═O), 8.06 (d, J = 8.4 Hz, 2H, Ar), 7.71 (dd, J = 20.0 Hz, 8.7 Hz, 4H, Ar), 7.05 (d, J = 9.0 Hz, 2H, Ar), 6.41 (s, 1H, H—C, pyrazole), 3.81 (s, 3H, O—CH_3), 2.24 (s, 3H, CH_3); ^{13}C NMR (151 MHz, DMSO d_6): δ 163.97 (C═O), 159.40 (Ar), 142.58 (pyrazole-C_5), 132.24 (pyrazole-C_3), 131.37 (Ar), 131.24 (Ar), 129.81 (Ar), 128.33 (2C, Ar), 127.99 (2C, Ar), 125.78 (2C, Ar), 114.46 (2C, Ar), 96.38 (pyrazole-C_4), 55.20 (O-CH_3), 10.85 (CH_3). EI/MS *m/z* (%): 308.5 (34) $[M+H]^+$; $[M-CH_3]$ = 292.2 (72); $[M-OCH_3]$ = 276.2 (30). HRMS for $C_{18}H_{17}N_3O_2$ calculated: $[M]^+$; 307.1321. Found: $[M]^+$; 307.1373.

2.4. Antibacterial activity

2.4.1. Agar well diffusion assay of compounds (**6a-h**) against NDM producing bacteria

In vitro antibacterial activity of the novel compounds **(6a-h)** was determined by agar well diffusion method (Zone inhibition) against NDM-1-positive *K. pneumoniae* and *A. baumannii* (Spooner and Sykes, 1972; Chung et al., 1990; Taye et al., 2011; Molla et al., 2016). In short, 0.5 McFarland bacterial suspension was prepared and streaked on Mueller Hinton Agar plate and sterile 6 mm cork borer was utilized to form wells on each plate. Various concentrations (10, 20, 30, 40 and 50 mg) of compounds (**6a-h**) in each 100 µl DMSO solvent were inserted separately in the wells with sterile pipettes. Simultaneously, Meropenem (10 µg) disc was used against the pathogens as antibiotic controls for comparison with the synthesized compounds (**6a-h**). Then, the plates were kept for incubation at 37 °C overnight aerobically. After proper incubation, the zone of inhibition of every well was measured with vernier caliper in millimeters and the following criteria was applied; no activity (< 1 mm), low activity (1–7 mm), moderate activity (7–10 mm), good activity (11–15 mm) and excellent activity (> 15 mm) (Zaidan et al., 2005). The assay was carried out three time and mean values were calculated for final antibacterial activity.

2.4.2. Minimum inhibitory concentration (MIC) of molecules (**6a-h**) against NDM positive bacteria

Microbroth dilution test was used to measure the MIC (% w/v) of the above mentioned samples **(6a-6h)** (Janovska et al., 2003; Joshi et al., 2009; Jeong et al., 2014). In 50 ml falcon tube, two to three isolated colonies were added in 20 ml of double-strength lysogeny broth (LB) medium and incubated overnight aerobically at 37 °C. Bacterial suspension was diluted to get 0.5 McFarland turbidity at optical density (OD) of 0.07 at 600 nm. In short, serial dilutions of compounds 6a-6 h (5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%) were prepared in DMSO and 100 µl of each compound dilution was inserted in 96 wells of flat-bottom micro titer plate. After that, 100 µl of bacterial suspension was inserted into each well. Negative-control wells had 100 µl of LB while positive-control wells had 100 µl of LB with bacterial suspension. Micro titer plate was incubated at 37 °C overnight at shaking incubator (MaxQTM Mini 4450, Thermo Fisher Scientific). MIC was calculated by comparison of each well with negative and positive-control wells and all procedures were performed in triplicate.

2.4.3. Minimum bactericidal concentration (MBC) of compounds (**6a-h**)

Minimum bactericidal concentration (% w/v) is the first dilution with no growth on agar plate. To determine the MBC, a 10 µl sample was taken from no-visible-growth wells of micro titer plate and was injected on the nutrient agar plates (Oxoid, Hampshire, UK) and then incubation was done aerobically at 37 °C for day and night. The plates were detected for cell

viability and any colonies established were recorded as bacterial or no bacterial evolution. The lowest concentration was considered as MBC at which no visible growth was seen (Wikler, 2006; Jeong et al., 2014). All procedures were repeated in triplicate.

2.4.4. Isolates confirmation

A. baumannii and *K. pneumoniae* were clinically separated from blood samples. These isolates were identified by modified UTI chrome agar (Aldrich Sigma, UK), VITEK® 2 compact (BioMérieux, France) and MALDI-TOF mass spectrometer (Bruker Daltonics, Germany).

2.4.5. Molecular characterization of NDM

Bacterial DNA was extracted by a commercially available bacterial DNA kit (Qiagen, UK). Molecular identification of NDM was determined by analyzing the sequence of primers of the gene organized downstream and upstream (Senda et al., 1996; Qamar et al., 2019). Amplified DNA was extracted using QIAquick Gel extraction kit (Germany). DNA was shipped to Macrogen (Korea) for Sanger sequencing and data were analyzed using bioinformatics tools NCBI and BLAST. Both of the isolates were confirmed as NDM-1 producers.

2.4.6. Minimum inhibitory concentration of NDM producing isolates

MIC of *A. baumannii* and *K. pneumoniae* was determined using VITEK® 2 compact device. The antibiotics tested were ticarcillin/clavulanic acid, ampicillin/sulbactam, piperacillin, cefuroxime axetil, cefuroxime, cefixime, cefepime, ceftriaxone, meropenem, trimethoprim, levofloxacin, minocycline, moxifloxacin, tigecycline, tetracycline, colistin, chloramphenicol and aztreonam.

Both of these isolates were 100% resistant to commonly used antibiotics such as β-lactams, meropenem, β-lactam inhibitors, chloramphenicol and levofloxacin however most effective drug was colistin (Table 1).

2.5. Molecular docking study

The intermolecular interaction between the pyrazole derivatives (**6a-6h**) and NDM positive *A. baumannii* protein were analyzed through AutoDock Vina software (Trott and Olson, 2010). The binding energies of generated ligand with protein receptor were calculated by Lamarckian genetic algorithm. A lot of binding conformations of NDM-1 active site to embelin ligand were estimated and ten embelin binding conformations with maximum hypothetical values were superpose in the active site of NDM-1. For this purpose, high resolution crystal structure of NDM-1 bonded to L-captopril was selected as a model to execute the overall docking study (Huttenhower et al., 2012). The configuration was obtained from PDB record under the PDB code of 4EXS (Berman et al., 2000). The directly contacted residues of amino acid with L-captopril were defined as the binding spot for the docking calculations. The

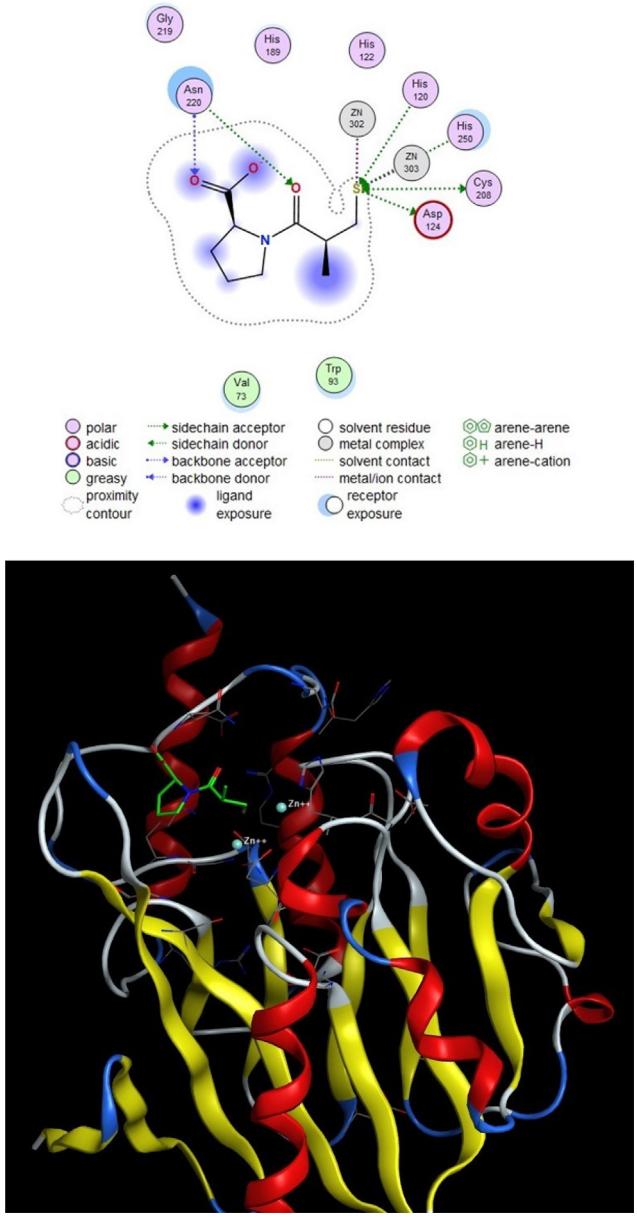


Fig. 1 2D (top) and 3D (bottom) structures of L-captopril with NDM positive *A. baumannii*.

Table 1 MIC (μg/ml) of different antibiotics against NDM-1 positive bacteria.

Isolates	SAM ≥32	PIP ≥128	CFM ≥4	CRO ≥64	CXA ≥64	CXM ≥64	FEP ≥64	LEV ≥8	ATM ≥64	MEM ≥16	MNO ≥16	MXF ≥8	TGC ≥8	CS ≥16	TE ≥16	C ≥64	TMP ≥16
A. baumannii	≥32	≥128	≥4	≥64	≥64	≥64	≥64	≥8	≥64	≥16	≥16	≥8	1	≤0.5	≥16	≥32	≥16
K. pneumoniae	≥32	≥128	≥4	≥64	≥64	≥64	≥64	≥8	≥64	≥16	≥16	≥4	2	≤0.5	> 16	≥16	≥16

SAM: Ampicillin/sulbactam, PIP: Piperacillin, CFM: Cefixime; CRO: Ceftriaxone; CXA: Cefuroxime axetil; CXM: Cefuroxime; FEP: Cefepime; LEV: Levofloxacin; ATM: Aztreonam; MEM: Meropenem; MNO: Minocycline; MXF: Moxifloxacin; TGC: Tigecycline; CS: Colistin; TE: Tetracycline; C: Chloramphenicol; TMP: Trimethoprim.

binding site having eleven amino acid residues (His250, Asn220, Gly219, Cys208, His189, Asp124, His122, His120, Trp93, Val73, Met67) with two ions (Zn^{+2}). To establish the position for docking, the L-captopril as well as water molecules were detached from crystal structure system. While, the synchronized zinc ion was retained in NDM-1′s exciting site because of its major character to enhance the NDM-1-ligand/substrate collaboration. Most part of the L-captopril was covered up with active site of NDM-1 and its thiosulphur group interact with Zn^{2+} directly as presented by molecular dynamic simulation (Fig. 1).

3. Results and discussion

3.1. Chemistry

The route for the production of pyrazole hybrids **(6a-h)** is illustrated in Scheme 1. First step involves the protection of 5-methyl-1*H*-pyrazol-3-amine **(1)** by di-*tert*-butyl tri-carbonate **(2)** and triethylamine to produce *tert*-butyl 3-amino-5-methyl-1*H*-pyrazole-1-carboxylate **(3)** in 70% yield. Further, the intermediate compound 4-bromo-*N*-(5-methyl-1*H*-pyrazol-3-yl)benzamide **(5)** in 75% yield was obtained by one pot condensation of **3** with 4-bromo benzoic acid **(4)** in pyridine and mediated by $TiCl_4$. In this step, the deprotection of target molecule was carried out automatically because the acidic water was used in the work up process. In the final step, the Suzuki cross coupling reactions of 4-bromo-*N*-(5-methyl-1*H*-pyrazol-3-yl)benzamide (**5**) were carried out by using different aryl/ hetero aryl boronic acids and target molecules **(6a-h)** were produced with moderate to good yield. Structural representation of these molecules and their yields are given in Fig. 2. All desired compounds were characterized by ^{1}HNMR, ^{13}CNMR and mass spectrometry. The compound **6b**, having 3-chloro substituent, was obtained in excellent yield of 90% and other molecules incorporating 4-chloro (**6a**), 3-chloro-4-flouro (**6c**), 3-acetyl **(6d)** 2-thiophene (**6f**), 3- pyridine (**6g**) and 4-methoxy (**6h**) also showed good yield. While, the compound **6e** produced in low yield of 38% and it might be due to steric effect of bulky group of ester attached with boronic acid.

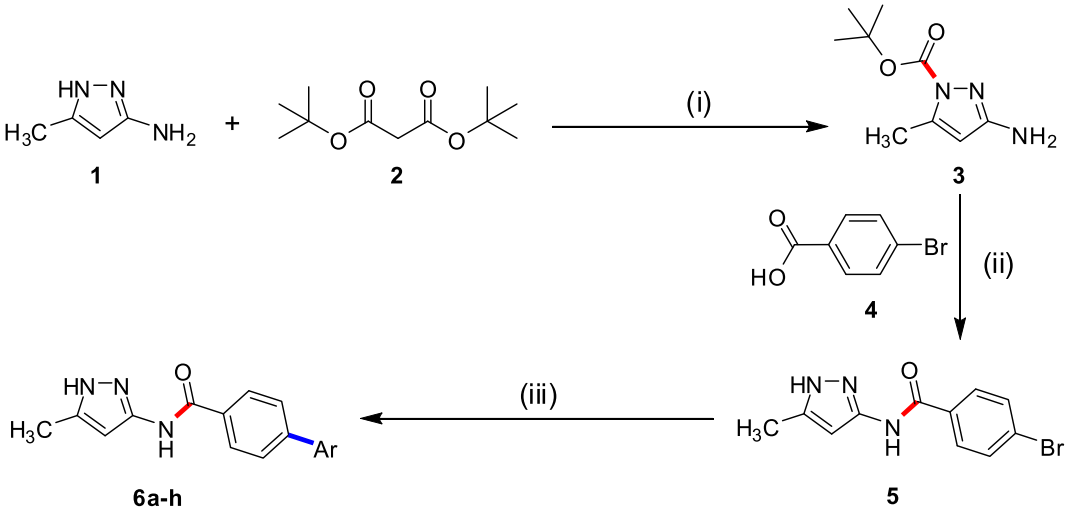
3.2. Antibacterial activity

The molecules **6a-h** were screened for antibacterial activity at five concentrations (10, 20, 30 40 and 50 mg/well) against NDM-1-positive *A.* and *K. pneumoniae* by agar well diffusion method. MIC and MBC were calculated by broth dilution methods. The results presented in Table 2 and Figs. 3, 4 regarding the activity exhibited that *A. baumannii* bacteria is more susceptible against the tested molecules. The zone of inhibition (mm) increases as the concentration of compounds increases. Compound **6b** and **6d** showed highest zone of inhibition (25 ± 1) at 50 mg concentration of compound as compared to other compounds against NDM positive *A. baumannii* while meropenem antibiotic was inhibited at 5 mm zone of inhibition (Table 2, Fig. 3 and Fig. S21 in supplementary data). The compounds **6h** and **6f** displayed low activity as compared to other compounds. However, the other molecules presented good activity. The values of MIC and MBC of the tested molecules were calculated against *A. baumannii* and results displayed that only compound **6b** exhibited MIC and MBC of 50 mg while all other compounds did not show any remarkable value (Fig. S22, S23 in supplementary data). NDM producing *K. pneumoniae* was inhibited by compound **6b** at a dose of 50 mg (15 ± 1), 40 mg (14 ± 2) and 30 mg (13 ± 1 mm) but remaining compounds failed to exhibit inhibition (Fig. S23, S24 in supplementary data).

3.3. Docking study

3.3.1. Molecular docking study of the NDM-1 *A. baumannii*/*6a-6h* compounds

Molecular docking study was performed to examine the vast conformation zone of **6a-6h** compounds surrounded by active



Scheme 1 Synthesis of 4-bromo-*N*-(5-methyl-1*H*-pyrazol-3-yl)benzamide (**5**) and 4-aryl-N-(5-methyl-1H-pyrazol-3-yl)benzamide (**6a-h**). Reagents and conditions: (i) **1** (30.89 mmol, 3 g), **2** (46.33 mmol, 10.11 g), Triethyl amine (46.33 mmol, 6.46 ml), solvent 1,4-dioxane (180 ml), Reflux, Time 1 h; (ii) **3** (14.92 mmol, 2.94 g), **4** (14.92 mmol, 3 g), $TiCl_4$ (44.77 mmol, 4.92 ml), Temperature 85 °C, Time 2 h, solvent pyridine 150 ml; (iii) **5** (0.526 mmol, 0.2 g), $Pd(PPh_3)_4$ (7 mol%, 0.03682 mmol, 0.042 g), K_3PO_4 (1.052 mmol, 0.22 g), aryl boronic acids/pinacol esters (0.579 mmol), 1,4-dioxane/H_2O (4:1), reflux, Time 20–30 h.

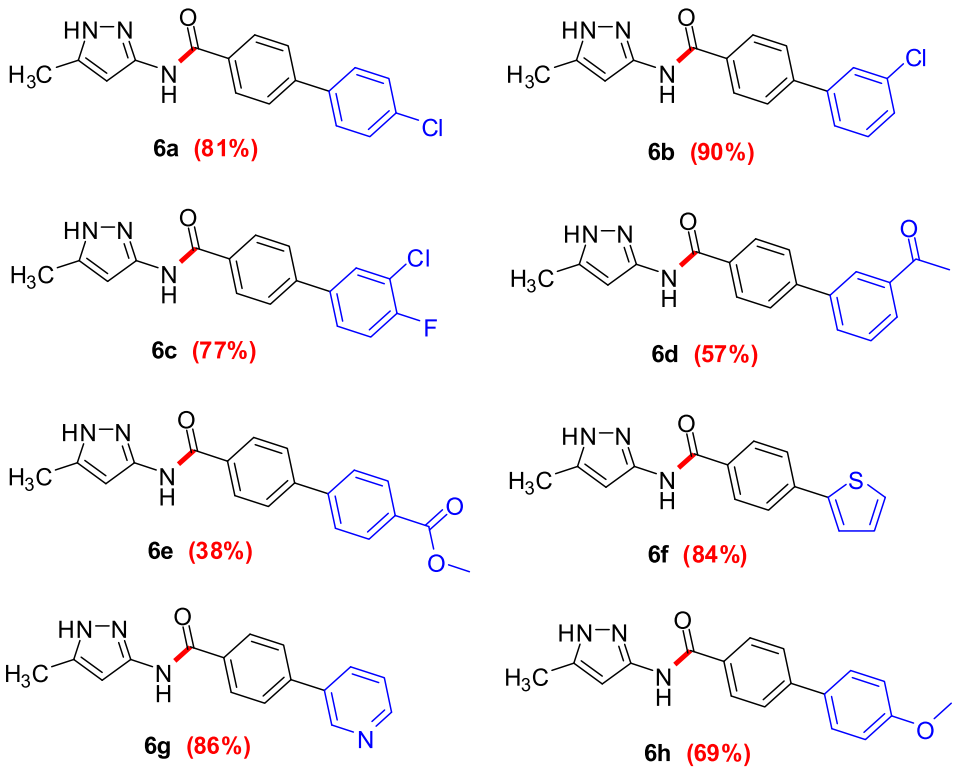


Fig. 2 An overview of 4-aryl-*N*-(5-methyl-1*H*-pyrazol-3-yl)benzamide (6a-h) *via* Suzuki coupling reactions.

Table 2 Zone of inhibition (mm) of compounds against clinically isolated *A. baumannii*.

Comp. no.	Zone (mm) (50 mg)	Zone (mm) (40 mg)	Zone (mm) (30 mg)	Zone (mm) (20 mg)	Zone (mm) (10 mg)	Zone (mm) DMSO	Zone (mm) Meropenem
6a	20 ± 1	17 ± 1	15 ± 1	15 ± 1	12 ± 1	15 ± 2	6
6b	25 ± 1	22 ± 1	18 ± 1	17 ± 1	14 ± 1	15 ± 1	5
6c	20 ± 2	18 ± 1	17 ± 1	15 ± 1	13 ± 1	14 ± 2	6
6d	25 ± 1	23 ± 2	22 ± 1	21 ± 1	20 ± 1	19 ± 2	5
6f	18 ± 2	16 ± 1	15 ± 1	14 ± 1	13 ± 2	13 ± 1	5
6g	20 ± 2	16 ± 2	14 ± 1	13 ± 1	12 ± 1	12 ± 2	7
6h	14 ± 1	13 ± 1	12 ± 1	12 ± 2	10 ± 1	9 ± 1	7

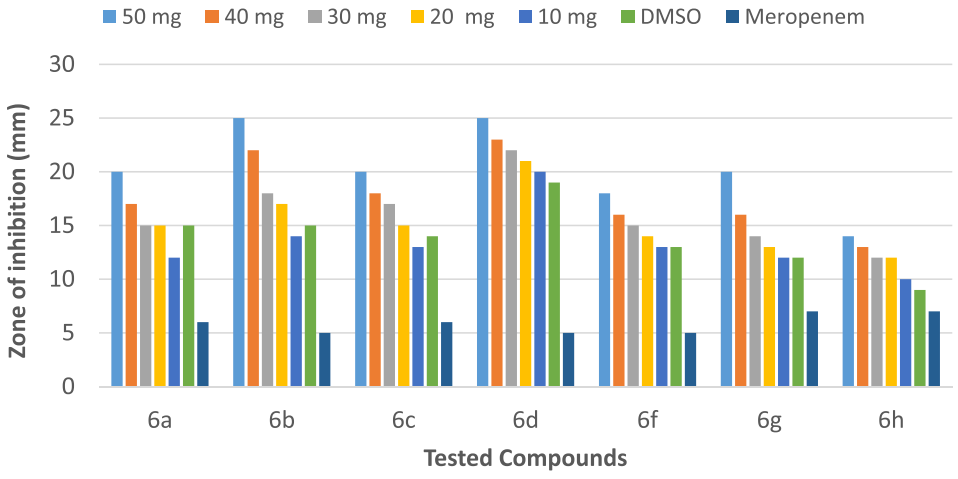
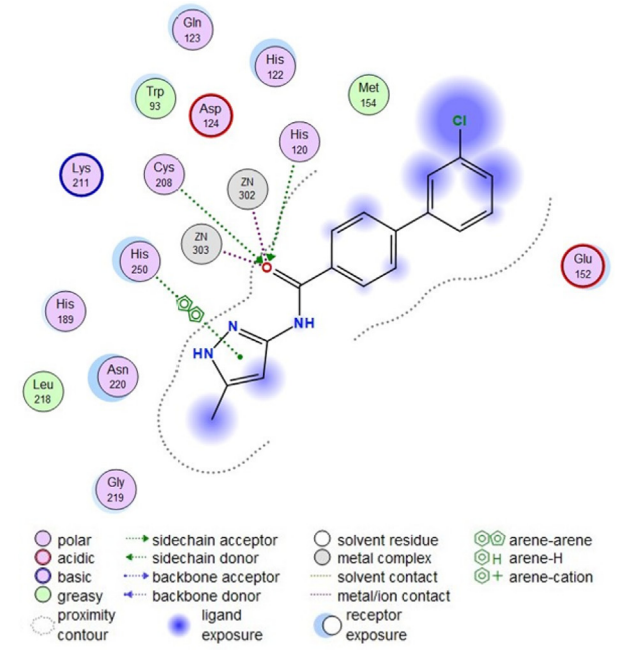


Fig. 3 Inhibition zone of each fraction of compounds (6a-6h) against *A. baumannii*.



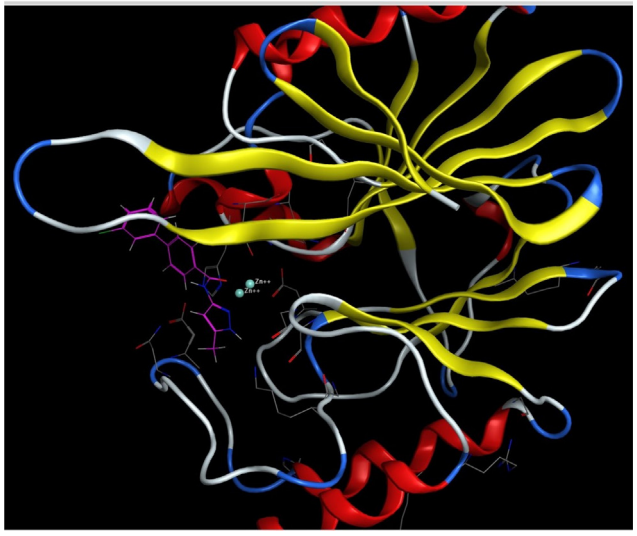


Fig. 4 Two-dimensional (2D, top) and three-dimensional (3D, bottom) diagram of the **6b** compound with NDM1 protein.

site of NDM-1. The 10 binding conformations for each compound (**6a-6h**) were superposed with NDM-1 active site. Except a few fluctuations at compounds moieties and groups, all conformations displayed alike binding approaches toward the NDM-1 protein. The aromatic chains of the **6a-6h** compounds were also very persistent in numerous docking sorts, however this chain was extremely supple and having significant swings (Table S1 in supplementary data).

To explain the molecular strategy of NDM-1 binding with compound (**6b**), the non-bonded collaborations at interface of NDM-1-embelin were determined. Most of the zones of embelin hybrid were embedded into the active site of NDM-1, indicating a broad Van der Waals interaction between embelin and NDM-1. Amide group (combination of amine and carbonyl groups) of **6a-6h** compounds interconnect by the catalytic corner of enzyme while the other part pointed out of this corner. Moreover, all the compounds (**6a-6h**) were found close to di-Zn^{2+}, thus establishing a solid coordination bond. The most stable geometry was observed for **6b** (-8.29 kcal mol^{-1}), **6d** (-8.42 kcal mol^{-1}) and **6e** (-8.73 kcal mol^{-1}) with minimum entropic energies. The 2D and 3D structures of compound **6b** with NDM1 protein are shown in Fig. 4 and the rest are given in supplementary data (Fig. S25, S26 and S27). The carbonyl group of amides and nitrogen of the pyrazole ring formed coordinate bond with di-Zn^{2+} ions. A hydrogen bonding between the Cys208 and oxygen of the carbonyl group was also observed which constitute recognition specificity for the NDM1-**6b** binding. Histidine amino acid (His122 and His250) groups interact with pyrazole ring and benzene rings through arenes interactions. Similar interactions behavior is observed for 6d and 6e compounds (Fig. S28). These types of binding interaction founded in previous docking study of the embelin with NDM-1, representing the significance of coordination bond in detection and collaboration of NDM-1-ligand with suitable drug. Based on the strong interaction between embelin and NDM-1, it was suggested that embelin restored meropenem activity against a panel of NDM positive pathogens, *i.e. E. coli*, *K. pneumoniae*, and *A. baumannii* (Ning et al., 2018).

The docking study reinforced the antibacterial activities of newly designed pyrazole derivatives against NDM-1 *A. baumannii* and demonstrated that compound **6b** renovated meropenem activity against NDM positive bacteria as comparable results of embelin were reproduced by our compounds **6b**, 6d and 6e. In preview of results, compound **6b** was found to be a potent antibacterial antibiotic hybrid against NDM-1- *A-baumannii*. Thus, we believed that **6b** could be used as promising carbapenem adjuvant candidates against NDM-1-producing bacterial strains.

4. Conclusion

In this study, we have demonstrated the efficient preparation of 4-bromo-*N*-(5-methyl-1*H*-pyrazol-3-yl)benzamide **(5)** and its novel derivatives **(6a-h)** *via* Suzuki cross coupling reactions in moderate to good yield. The synthesized pyrazole hybrids **(6a-h)** exhibited remarkable antibacterial activity by agar well diffusion method against NDM-1-positive *A. baumannii* and *K. pneumoniae*. Microbroth dilution test was used to measure the MICs and MBCs. Though all eight compounds were capable of inhibiting bacterial growth at different concentrations, the compound **6b** exhibited better antibacterial properties with MIC value of 50 mg against *A. baumannii*. Molecular docking study was also performed by AutoDock Vina software to examine the vast conformation zone of all compounds as a result of binding interaction against NDM-1 *A. baumannii* protein. The most stable geometry was observed for compounds **6b**, 6d and 6e with minimum entropic energies and their results are comparable to already reported inhibitor embelin. Hence, *in vitro* activity and molecular docking elaborated the antibacterial potential of **6b** and revealed that these potential antibacterial pyrazole amide hybrids would be processed in future to develop newer antibacterial analogs with markedly improved metabolic stability.

CRediT authorship contribution statement

Gulraiz Ahmad: Methodology, Visualization, Investigation, Writing - review & editing. **Nasir Rasool:** Methodology,

Visualization, Investigation. **Muhammad Usman Qamar:** Conceptualization. **Mohammad Mujahid Alam:** Conceptualization, Supervision. **Naveen Kosar:** Methodology, Visualization, Investigation. **Tariq Mahmood:** Writing - review & editing. **Muhammad Imran:** Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.arabjc.2021.103270.

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