Synthesis of novel benzimidazole clubbed pyrazole heterocycles derivatives as potentially antibacterial agents

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Abstract

Some new derivatives of benzimidazole bearing pyrazole heterocycles 4a–o (2-(1H-benzo[d] imidazol-1-yl)-1- (3, 5-diphenyl-4,5-dihydro-1H- pyrazol-1-yl) ethan-1-one) were synthesized by simple synthetic protocol. The title compounds (4a-o) have been characterized by infrared spectroscopy, ^{1}H and ^{13}C NMR spectroscopy and mass spectroscopy. Anti-bacterial activity of synthesised compounds was carried out in which 3-nitro derivatives 4e and 4o showed good activity against both Gram positive and Gram negative bacterial strain in vitro.

Compound 4e was most potent against Gram negative bacteria (E. coli, MIZ= 21mm and E. aerogenes, MIZ = 24mm) in comparison to amoxycilin (E. coli, MIZ= 16mm and E. aerogenes, MIZ = 18mm) while compounds 4i (S. aureus, MIZ = 34mm;) and 4o (S. aureus MIZ = 36mm) were most active against Gram-positive bacteria of the series.

Keywords: Benzimidazole, Pyrazole, Antibacterial, Gram positive and Gram negative.

Introduction

Many anti-bacterial drugs are available to treat bacterial diseases. However, due to side effects and the development of drug resistance in bacterial strain, there is need to design, synthesize and develop more potent and safer anti-bacterial agents. The major goals in current biomedical research are to find new effective compounds against multi-resistant pathogens[17]. Heterocyclic compounds known in the literature for their antimicrobial activities are those with benzimidazole ring and those with pyrazole ring. Aromatic compounds have a very wide range of medicinal properties.

It is well-known that benzimidazoles are structural isosteres to purines that are essential substrates for the biosynthesis of nucleic acids and proteins inside the bacterial cell wall. The purine-like structure enables benzimidazole derivatives to obstruct the biosynthesis of nucleic acids and proteins by competing with purines, ultimately leading to the death of the bacterial cell[32].

Benzimidazole derivatives developed a considerable interest in medical domain due to their therapeutic action as antitumor[8,20,27], antimicrobial[1,2,18,22,23,31], antihelmintic[24], antihistamine[5,6], proton pump inhibitors[15], anti-inflammatory[4,13] and anti-hypertensive[33] drugs. Additionally, pyrazole is five membered heterocyclic compounds and its derivative possesses a diversity of biological activities as analgesic[11,19,26], anticonvulsivant[14], antitumor[12,16,21,28], antidiabetic[10] and antimicrobial[3,7,11,25,30].

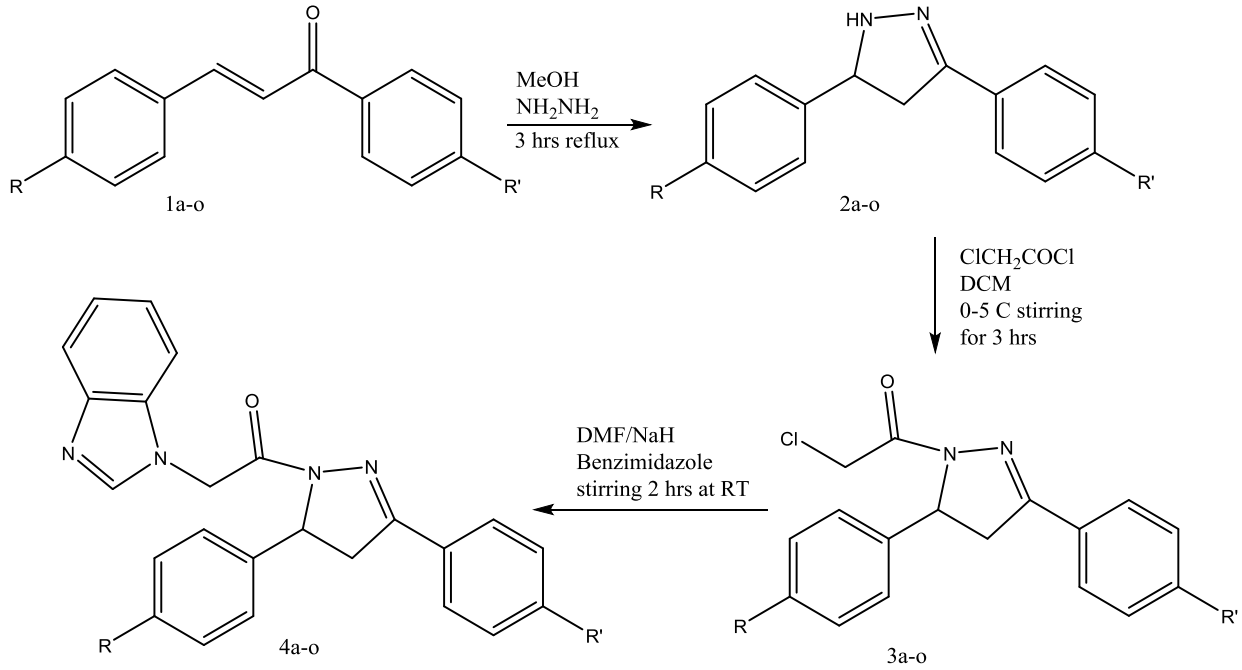
In modern medicinal chemistry, synthesis of new bioactive compounds is obtained by hybridization of two different bioactive molecules with opposite pharmacophoric functions[9]. In this work, we present and discuss results on the synthesis of benzimidazoles capped 3,5 disubstituted pyrazole derivatives and antimicrobial activity screening. The antimicrobial activity of the compounds was evaluated against two Gram-positive bacterial strains, namely, *Staphylococcus aureus* (ATCC-25923) and *Bacillus subtilis* (ATCC 6633) and two Gram-negative bacterial strains, namely, *Escherichia coli* (ATCC-25922) and *Enterobacter aerogenes* (ATCC-27853).

Material and Methods

All melting points were determined in open capillary tube and are uncorrected. Infrared spectra were recorded in KBr on Perkin– Elmer RX1 spectrophotometer. The ^{1}H NMR and ^{13}C NMR spectra were measured in dimethyl sulfoxide-d_6 or $CDCl_3$ solutions on a Brucker 500 MHz spectrometer using TMS as an internal reference (chemical shift in δppm). The mass spectra were recorded on a QP2010 Shimadzu instrument. All the synthesized compounds were microanalyzed satisfactorily for C, H and N by Elementar Vario EL III elemental analyzer.

Synthesis of 3,5-diphenyl-4,5-dihydro-1H-pyrazole (2): A mixture of different chalcone (0.01 mol) and hydrazine hydrate (0.014 mol) was refluxed in 25ml methanol for 3 hours. After completion of reaction, excess methanol was removed by distillation and reaction mass was poured into cold water, white solid obtained filtered and washed with water. Yield: 76%, M.P.: 78^{0}C,

Synthesis of 2-chloro-1-(3,5-diphenyl-4,5-dihydro-1H-pyrazol-1-yl)ethan-1-one (3): A solution of compound (2) 0.01 mole in dichloromethane 30ml, stirred for 10 minutes at 0-5^{0}C, then add drop wise chloroacetylchloride 0.012 mole and stirring for 3 hours at room temperature. After completion of reaction, excess DCM was distilled out and then concentrated mass was poured into water, white solid was observed, filter and wash with water: Yield: 80%, M.P.: 124^{0}C



4a: R=H, R'= H	4f: R=H, R'=2-Cl	4k: R=H, R'=4-Br
4b: R=2-Cl, R'=H	4g: R=2-Cl, R'=2-Cl	4l: R=2-Cl, R'=4-Br
4c: R=4-CH_3, R'=H	4h: R=4-CH_3, R'=2-Cl	4m: R=4-CH_3, R'=4-Br
4d: R=4-OCH_3, R'=H	4i: R=4-OCH_3, R'=2-Cl	4n: R=4-OCH_3, R'=4-Br
4E: R=3-NO_2, R'=H	4j: R=3-NO_2, R'=2-Cl	4o: R=3-NO_2, R'=4-Br

Scheme 1: Synthesis pathway of the targeted compounds 4a-o

Synthesis of 4a–o (2-(1H-benzo[d] imidazol-1-yl)-1- (3,5 substituted-diphenyl-4,5-dihydro-1H- pyrazol-1-yl) ethan-1-one): Stir a solution of NaH 0.01 mole in DMF 10ml for 5 minutes at room temperature, then add slowly benzimidazole 0.01 mole with further stirring for 30 minutes at room temperature. Add drop wise solution of compound (3a-o) 0.01 mole in 10ml DMF. Reaction mass was stirred for 2 hours and after completion of reaction, mass was poured into water, precipitate was observed, filter and wash with water, purify further by column chromatography.

(4a) 2-(1H-benzo[d]imidazol-1-yl)-1-(3,5-diphenyl-4,5-dihydro-1H-pyrazol-1-yl)ethan-1-one. White solid; Yield : 45%, M.P. : 160^{0}C; ^{1}H NMR (500 MHz, $CDCl_3$) δ(ppm): 3.25 (dd, 1H, 7.8, pyrazoline- CH_2), 5.39(s, 2H, -CH_2-CO), 3.78 (dd, 1H, 10.8 pyrazoline- CH_2), 5.56(dd, 1H, 7.2, pyrazoline-CH), 7.15-7.78(m, 14H, Ar-H), 8.03 (s, 1H, benzimidazole). ^{13}C NMR (125 MHz, $CDCl_3$) δ(ppm): 163.67, 156.11, 143.99, 143.38, 140.69, 134.22, 131.05, 130.63, 129.03, 128.97, 128.11, 126.86, 125.68, 123.13, 122.22, 120.25, 109.83, 60.54, 46.98, 42.26. IR (KBr, cm^{-1}): 3048 (Aromatic C-H), 2928 (Aliphatic C-H str), 1682 (C=O str), 1500, 1442 (C=N str); ESI MS (m/z): 380 (M^+); Anal. Calcd. For: $C_{24}H_{20}N_4O$; C, 75.77; H, 5.30; N, 14.73; O, 4.21; Found: C, 75.51; H, 5.37; N, 14.53; O, 4.42.

(4b) 2-(1H-benzo[d]imidazol-1-yl)-1-(5-(2-chlorophenyl)-3-phenyl-4,5-dihydro-1H-pyrazol-1-yl)ethan-1-one; White solid; Yield: 82%; M.P. : 172^{0}C; ^{1}H NMR (500 MHz, $CDCl_3$) δ(ppm): 3.15 (dd, 1H, 7.8, pyrazoline- CH_2), 5.27(s, 2H, -CH_2-CO), 3.56 (dd, 1H, 10.8 pyrazoline- CH_2), 5.50(dd, 1H, 7.2, pyrazoline-CH), 7.11-7.75(m,13H,Ar-H), 8.03 (s, 1H, benzimidazole). ^{13}C NMR (125 MHz, CDCl3) δ(ppm): 164.25, 156.64, 144.23, 143.56, 140.78, 134.28, 131.27, 129.88, 129.26, 129.14, 128.56, 127.55, 125.73, 123.22, 122.43, 120.41, 110.22, 60.12, 47.21, 42.18. IR (KBr, cm^{-1}): 3076 (Aromatic C-H), 2989 (Aliphatic C-H str), 1677 (C=O str), 1527, 1465 (C=N str); ESI MS (m/z): 412 (M^+), 414 (M + 2); Anal. Calcd. For: $C_{24}H_{19}ClN_4O$; C, 69.48; H, 4.62; Cl, 8.54; N, 13.50; O, 3.86; Found: C, 69.54; H, 4.68; Cl, 7.53; N, 13.01; O, 3.90.

(4c) 2-(1H-benzo[d]imidazol-1-yl)-1-(3-phenyl-5-(p-tolyl)-4,5-dihydro-1H-pyrazol-1-yl)ethan-1-one,; M.P. : 174^{0}C;^{1}H NMR (500 MHz, $CDCl_3$) δ(ppm): 3.22 (dd, 1H, 7.8, pyrazoline- CH_2), 5.42(s, 2H, -CH_2-CO), 3.36 (dd, 1H, 10.8 pyrazoline- CH_2), 5.63(dd, 1H, 7.2, pyrazoline-CH), 7.18-7.84 (m,13H,Ar-H), 8.14 (s, 1H, benzimidazole). ^{13}C NMR (125 MHz, $CDCl_3$) δ(ppm): 163.72, 156.11, 144.22, 142.14, 140.26, 134.62, 130.24, 131.13, 129.72, 129.32, 128.54, 126.14, 125.67, 123.23, 122.27, 120.32, 110.56, 64.28, 47.91, 40.20. IR (KBr, cm^{-1}): 3062 (Aromatic C-H), 2954 (Aliphatic C-H str), 1692 (C=O str), 1512, 1450 (C=N str); ESI MS (m/z): 394 (M^+); Anal. Calcd. for: $C_{25}H_{22}N_4O$; C, 76.12; H, 5.62; N, 14.20; O, 4.06; Found: C, 76.56; H, 5.32; N, 14.28; O, 4.14.

(4d) 2-(1H-benzo[d]imidazol-1-yl)-1-(5-(4-methoxy phenyl)-3-phenyl-4,5-dihydro-1H-pyrazol-1-yl)ethan-1-one; White solid; M.P. : 184^{0}C; ^{1}H NMR (500 MHz, $CDCl_3$) δ(ppm): 3.32 (dd, 1H, 7.8, pyrazoline- CH_2), 5.42(s, 2H, -CH_2-CO), 3.82 (dd, 1H, 10.8 pyrazoline- CH_2), 5.62(dd, 1H, 7.2, pyrazoline-CH), 7.12-7.82(m,13H,Ar-H), 8.12 (s, 1H, benzimidazole). ^{13}C NMR (125 MHz, $CDCl_3$) δ(ppm): 166.72, 157.27, 142.56, 143.66, 140.12, 133.68, 130.92, 130.36, 129.30, 128.54, 128.10, 126.78, 125.86, 123.47, 122.23, 120.26, 109.88, 61.54, 46.65, 42.62; IR (KBr, cm^{-1}): 3062 (Aromatic C-H), 2993 (Aliphatic C-H str), 1690 (C=O str), 1547, 1462 (C=N str); ESI MS (m/z): 410 (M^+); Anal. Calcd. For: $C_{25}H_{22}N_4O_2$: C, 73.15; H, 5.40; N, 13.65; O, 7.80; Found: C, 72.41; H, 5.49; N, 13.66; O, 7.97.

(4e) 2-(1H-benzo[d]imidazol-1-yl)-1-(5-(3-nitrophenyl)-3-phenyl-4,5-dihydro-1H-pyrazol-1-yl)ethan-1-one; Yellow solid; M.P. : 220^{0}C; ^{1}H NMR (500 MHz, $CDCl_3$) δ(ppm): 3.21 (dd, 1H, 7.8, pyrazoline- CH_2), 5.43(s, 2H, -CH_2-CO), 3.80 (dd, 1H, 10.8 pyrazoline- CH_2), 5.54(dd, 1H, pyrazoline-CH), 7.18-8.14(m,13H, Ar-H), 7.98 (s, 1H, benzimidazole). ^{13}C NMR (125 MHz, $CDCl_3$) δ(ppm): 166.54, 157.29, 144.37, 143.47, 140.97, 135.68, 131.22, 130.91, 129.23, 129.62, 128.47, 126.52, 125.21, 123.47, 122.46, 120.41, 110.54, 60.48, 46.52, 42.32; IR (KBr, cm^{-1}): 3056 (Aromatic C-H), 2987 (Aliphatic C-H str), 1690 (C=O str), 1574 (-NO_2), 1547, 1462 (C=N str); ESI MS (m/z): 425 (M^+); Anal. Calcd. For: $C_{24}H_{19}N_5O_3$; C, 67.76; H, 4.50; N, 16.46; O, 11.28; Found: C, 66.52; H, 4.50; N, 16.13; O, 12.42.

(4f) 2-(1H-benzo[d]imidazol-1-yl)-1-(3-(4-methoxyphenyl) -5-phenyl-4,5-dihydro-1H-pyrazol-1-yl)ethan-1-one; white solid; M.P. : 196^{0}C; ^{1}H NMR (500 MHz, $CDCl_3$) δ(ppm): 3.47 (dd, 1H, pyrazoline- CH_2), 3.92 (Ar-OCH_3), 5.11(s, 2H, -CH_2-CO), 3.66 (dd, 1H, 10.8 pyrazoline- CH_2), 5.60(dd, 1H, pyrazoline-CH), 7.03-7.85(m,13H,Ar-H), 8.20 (s, 1H, benzimidazole). ^{13}C NMR (125 MHz, $CDCl_3$) δ(ppm): 164.52, 156.87, 144.56, 143.42, 141.85, 134.80, 131.52, 130.98, 129.85, 129.12, 128.17, 126.88, 125.72, 123.08, 122.14, 120.31, 109.84, 61.42, 46.47, 42.31. IR (KBr, cm^{-1}): 3097 (Aromatic C-H), 2998 (Aliphatic C-H str), 1666(C=O str), 1523, 1456 (C=N str); ESI MS (m/z): 410 (M^+); Anal. Calcd. For: $C_{25}H_{22}N_4O_2$: C, 73.15; H, 5.40; N, 13.65; O, 7.80 Found: C, 73.00; H, 5.63; N, 13.45; O, 7.60

(4g) 2-(1H-benzo[d]imidazol-1-yl)-1-(5-(2-chlorophenyl)-3-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)ethan-1-one; White solid; M.P.: 156^{0}C; ^{1}H NMR (500 MHz, $CDCl_3$) δ(ppm): 3.33 (dd, 1H, pyrazoline- CH_2), 5.43(s, 2H, -CH_2-CO), 3.78 (dd, 1H, pyrazoline- CH_2), 3.88 (s, 6H, Ar-OCH_3), 5.62(dd, 1H, 7.2, pyrazoline-CH), 7.14-7.82 (m, 12H, Ar-H), 8.03 (s, 1H, benzimidazole). ^{13}C NMR (125 MHz, $CDCl_3$) δ(ppm): 164.74, 157.18, 144.52, 143.42, 140.71, 134.28, 132.23, 130.84, 129.18, 128.42, 128.27, 127.54, 126.11, 123.48, 122.46, 120.88, 109.92, 64.12, 47.01, 42.56. IR (KBr, cm^{-1}): 3047 (Aromatic C-H), 2956 (Aliphatic C-H str), 1672(C=O str), 1550, 1445 (C=N str); ESI MS (m/z): 444 (M^+); Anal. Calcd. for: $C_{25}H_{21}ClN_4O_2$; C, 67.49; H, 4.76; Cl, 7.97; N, 12.59; O, 7.19; Found: C, 67.54; H, 4.84; Cl, 7.13; N, 12.74; O, 7.27.

(4h) 2-(1H-benzo[d]imidazol-1-yl)-1-(3-(4-methoxypheny l)-5-(p-tolyl)-4,5-dihydro-1H-pyrazol-1-yl)ethan-1-one; White solid; M.P. : 214^{0}C; ^{1}H NMR (500 MHz, $CDCl_3$) δ(ppm): 3.22 (dd, 1H, pyrazoline- CH_2), 5.32(s, 2H, -CH_2-CO), 3.62 (dd, 1H, 10.8 pyrazoline- CH_2), 3.82 (s, 3H, Ar-OCH_3), 5.56(dd, 1H, 7.2, pyrazoline-CH), 7.10-7.72 (m, 12H, Ar-H), 8.06 (s, 1H, benzimidazole). ^{13}C NMR (125 MHz, $CDCl_3$) δ(ppm): 163.72, 156.17, 143.88, 143.54, 140.78, 134.43, 131.36, 130.69, 129.22, 128.44, 128.26, 126.65, 125.72, 123.26, 122.26, 120.15, 109.88, 61.48, 47.23, 42.78. IR (KBr, cm^{-1}): 3054 (Aromatic C-H), 2962 (Aliphatic C-H str), 1647(C=O str), 1552, 1454 (C=N str); ESI MS (m/z): 440(M^+); Anal. Calcd. For $C_{26}H_{24}N_4O_2$: C, 73.56; H, 5.70; N, 13.20; Found: C, 73.56; H, 5.70; N, 13.20.

(4i) 2-(1H-benzo[d]imidazol-1-yl)-1-(3,5-bis(4-methoxy phenyl)-4,5-dihydro-1H-pyrazol-1-yl)ethan-1-one; White solid; M.P. : 194^{0}C; ^{1}H NMR (500 MHz, $CDCl_3$) δ(ppm): 3.18 (dd, 1H, pyrazoline- CH_2), 5.33 (s, 2H, -CH_2-CO), 3.62 (dd, 1H, pyrazoline- CH_2), 5.56 (dd, 1H, pyrazoline-CH), 7.16-7.77(m, 12H, Ar-H), 8.03 (s, 1H, benzimidazole). ^{13}C NMR (125 MHz, $CDCl_3$) δ(ppm): 163.64, 156.17, 143.98, 143.41, 140.57, 134.21, 131.12, 130.75, 129.17, 129.00, 128.14, 126.80, 125.71, 123.23, 122.14, 120.23, 109.87, 62.06, 46.95, 42.27. IR (KBr) (cm_1): 3009 (CH_2 str), 1690 (C=O str), 1682, 1675 (C=N str); ESI MS (m/z): 440 (M+); Anal. Calcd. for: $C_{26}H_{24}N_4O_2$; C, 70.89; H, 5.49; N, 12.72; Found: C, 70.89; H, 5.49; N, 12.72.

(4j) 2-(1H-benzo[d]imidazol-1-yl)-1-(3-(4-methoxy phen yl)-5-(3-nitrophenyl)-4,5-dihydro-1H-pyrazol-1-yl)ethan-1-one.); Dark yellow solid; M.P. : 196^{0}C; ^{1}H NMR (500 MHz, $CDCl_3$) δ(ppm): 3.16 (dd, 1H, pyrazoline -CH_2), 5.42(s, 2H, -CH_2-CO), 3.79 (dd, 1H, pyrazoline- CH_2), 5.57(dd, 1H, pyrazoline-CH), 7.17-7.87(m, 12H, Ar-H), 7.99 (s, 1H, benzimidazole). ^{13}C NMR (125 MHz, $CDCl_3$) δ(ppm): 163.71, 156.24, 144.28, 143.39, 140.64, 134.21, 131.09, 130.72, 129.10, 129.11, 128.17, 126.94, 125.64, 123.11, 122.08, 120.21, 109.88, 61.49, 46.99, 42.27. IR (KBr, cm^{-1}): 3051 (Aromatic C-H), 2986 (Aliphatic C-H str), 1689 (C=O str), 1575 (-NO_2), 1544, 1460 (C=N str.); ESI MS (m/z): 455 (M^+); Anal. Calcd. For: $C_{25}H_{21}N_5O_4$: C, 65.93; H, 4.65; N, 15.38; Found: C, 65.98; H, 4.61; N, 15.34.

(4k) 2-(1H-benzo[d]imidazol-1-yl)-1-(3-(4-bromophenyl)-5-phenyl-4,5-dihydro-1H-pyrazol-1-yl)ethan-1-one; Yellow solid; M.P. : 184^{0}C; ^{1}H NMR (500 MHz, $CDCl_3$) δ(ppm): 3.14 (dd, 1H, pyrazoline -CH_2), 5.32(s, 2H, -CH_2-CO), 3.82 (dd, 1H, 10.8 pyrazoline- CH_2), 5.60(dd, 1H, 7.2, pyrazoline-CH), 7.14-7.79(m, 12H, Ar-H), 8.02 (s, 1H, benzimidazole). ^{13}C NMR (125 MHz, $CDCl_3$) δ(ppm): 163.64, 156.12, 143.98, 143.44, 140.70, 134.27, 131.01, 130.32, 129.27, 128.93, 128.07, 126.56, 125.74, 123.26, 122.21, 120.24, 109.80, 62.05, 46.23, 42.33. IR (KBr, cm^{-1}):

3054 (Aromatic C-H), 2957 (Aliphatic C-H str), 1665 (C=O str), 1527, 1436 (C=N str), 808 (C-Br); ESI MS (m/z): 458 (M^+); Anal. Calcd. For: $C_{24}H_{19}BrN_4O$: C, 58.38; H, 3.67; N, 11.35; Found: C, 58.42; H, 3.72; N, 11.27.

(4l) 2-(1H-benzo[d]imidazol-1-yl)-1-(3-(4-bromophenyl)-5-(2-chlorophenyl)-4,5-dihydro-1H-pyrazol-1-yl)ethan-1-one; Dark yellow solid; M.P. : 188^{0}C; ^{1}H NMR (500 MHz, $CDCl_3$) δ(ppm): 3.24 (dd, 1H, pyrazoline -CH_2), 5.44(s, 2H, -CH_2-CO), 3.79 (dd, 1H, pyrazoline -CH_2), 5.53(dd, 1H, pyrazoline-CH), 7.10-7.75(m, 12H, Ar-H), 8.04 (s, 1H, benzimidazole). ^{13}C NMR (125 MHz, $CDCl_3$) δ(ppm): 163.68, 156.18, 144.08, 143.42, 140.63, 134.24, 131.11, 130.69, 129.08, 128.91, 128.14, 126.80, 125.72, 123.18, 122.20, 120.23, 109.84, 60.56, 47.17, 42.27. IR (KBr, cm^{-1}): 3047 (Aromatic C-H), 2991 (Aliphatic C-H str), 1675 (C=O str), 1560, 1457 (C=N str), 807 (C-Br);ESI MS (m/z):492 (M+); Anal. Calcd. for: $C_{24}H_{18}BrClN_4O$: C, 58.38; H, 3.67; N, 11.35; Found: C, 58.38; H, 3.67; N, 11.35.

(4m) 2-(1H-benzo[d]imidazol-1-yl)-1-(3-(4-bromophenyl)-5-(p-tolyl)-4,5-dihydro-1H-pyrazol-1-yl)ethan-1-one.); Light yellow solid; M.P.: 174^{0}C; ^{1}H NMR (500 MHz, $CDCl_3$) δ(ppm): 2.27 (s,3H, Ar-CH_3), 3.12 (dd, 1H, J = 19.2, 11.1, pyrazoline-CH_2), 5.47(s, 2H, -CH_2-CO), 3.62 (dd, 1H, 11.0, pyrazoline-CH_2), 5.50 (dd, 1H, 11.4, pyrazoline-CH), 7.01-7.76(m, 12H, Ar-H), 7.98 (s, 1H, benzimidazole). ^{13}C NMR (125 MHz, $CDCl_3$) δ(ppm): 163.64, 155.08, 143.94, 143.30, 137.91, 137.59, 134.17, 132.15, 129.66, 129.61 128.22, 125.57, 125.36, 123.07, 122.17, 120.18, 109.76, 60.49, 46.86, 42.03, 21.07. IR (KBr, cm^{-1}): 3036 (Aromatic C-H), 2943 (Aliphatic C-H str), 1674 (C=O str), 1593, 1450 (C=N str), 821 (C-Br); ESI MS (m/z): 472 (M^+); Anal. Calcd.for: $C_{25}H_{21}BrN_4O$: C, 63.43; H, 4.47; N, 11.84; Found: C, 69.58; H, 4.67; N, 13.54.

(4n) 2-(1H-benzo[d]imidazol-1-yl)-1-(3-(4-bromophenyl)-5-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)ethan-1-one.; Light yellow solid; M.P. : 186^{0}C; ^{1}H NMR (500 MHz, $CDCl_3$) δ(ppm): 3.27 (dd, 1H, 7.8, pyrazoline- CH_2), 3.87 (s, 3H, OCH_3), 5.41(s, 2H, -CH_2-CO), 3.80 (dd, 1H, 10.8 pyrazoline- CH_2), 5.54(dd, 1H, 7.2, pyrazoline-CH), 7.14-7.65(m,12H,Ar-H), 8.07 (s, 1H, benzimidazole). ^{13}C NMR (125 MHz, $CDCl_3$) δ(ppm): 163.72, 156.17, 143.91, 143.45, 140.94, 134.28, 131.20, 130.85, 129.25, 128.42, 128.08, 126.80, 125.12, 123.34, 122.05, 120.54, 109.81, 60.57, 46.77, 42.24. IR (KBr, cm^{-1}): 3042 (Aromatic C-H), 2956 (Aliphatic C-H str), 1664 (C=O str), 1543, 1442 (C=N str), 819 (C-Br); ESI MS (m/z): 488 (M^+); Anal. Calcd. For: $C_{25}H_{21}BrN_4O_2$: C, 61.36; H, 4.33; N, 11.45; O, 6.54; Found: C, 61.62; H, 4.56; N, 11.14.

(4o) 2-(1H-benzo[d]imidazol-1-yl)-1-(3-(4-bromophenyl)-5-(3-nitrophenyl)-4,5-dihydro-1H-pyrazol-1-yl)ethan-1-one; Yellow solid; M.P. : 228^{0}C; ^{1}H NMR (500 MHz, $CDCl_3$) δ(ppm): 3.24 (dd, 1H, 7.8, pyrazoline- CH_2), 5.37(s, 2H, -CH_2-CO), 3.56 (dd, 1H, 10.8 pyrazoline- CH_2), 5.54(dd, 1H, 7.2, pyrazoline-CH), 7.20-7.82 (m,12H,Ar-H), 7.99 (s, 1H, benzimidazole). ^{13}C NMR (125 MHz, $CDCl_3$) δ(ppm): 163.66, 156.13, 143.90, 143.38, 140.71, 134.82, 131.44, 130.96, 129.28, 129.45, 128.17, 126.91, 125.64, 123.62, 122.23, 120.41, 110.21, 60.54, 46.98, 42.26; IR (KBr, cm^{-1}): 3097 (Aromatic C-H), 2967 (Aliphatic C-H str), 1677 (C=O str), 1554, 1462 (C=N str), 812 (C-Br); ESI MS (m/z): 503 (M+); Anal. Calcd. For: $C_{24}H_{18}BrN_5O_3$: C, 57.16; H, 3.60; N, 13.89; Found: C, 56.22; H, 3.67; N, 13.70.

Biological assay: Novel derivatives 4a-o were evaluated for their *in vitro* antibacterial activity against two Gram-positive bacterial strains, namely, *Staphylococcus aureus* (ATCC-25923) and *Bacillus subtilis* (ATCC 6633) and two Gram-negative bacterial strains, namely, *Escherichia coli* (ATCC-25922) and *Enterobacter aerogenes* (ATCC-27853) using the well diffusion method.

Results and Discussion

Chemistry: The synthesis of new and potentially useful compounds 4a-o in a 3,5 disubstituted pyrazole ring was linked to the benzimidazole moiety through an amide bond link and was carried out by a simple and efficient synthetic procedure. Different substituted chalcones (1a-o) on treatment with hydrazine hydrate in presence of ethanol gave 3,5-substituted-diphenyl-4,5-dihydro-1H-pyrazole (2a-o) which on further treatment with 2-chloroacetyl chloride in presence of methelendichloride formed 2-chloro-1-(3,5-substituted-diphenyl-4,5-dihydro-1H-pyrazol-1-yl)ethan-1-one (3a–o). Benzimidazole reacts with sodium hydride to form benzimidazole anion, treated with compounds 3a-o in presence of DMF resulting in the formation of 2-(1H-benzo[d]imidazol-1-yl)-1-(3,5-substituted-diphenyl-4,5-dihydro-1H-pyrazol-1-yl)ethan-1-one (4a–o).

All obtained compounds 4a-o were fully characterized using elemental analysis, Mass, FT-IR and NMR spectroscopic techniques. Both ^{1}H- and ^{13}C-NMR data were consistent with the presence of pyrazole and benzimidazole moieties. The ^{1}H-NMR data of all derivatives revealed the absence of -NH proton of benzimidazol ring which was assigned to be the formation of 4a-o. Mass spectrum of compound 3a revealed the molecular ion peak M^+ at m/z= 298 corresponding to molecular mass of this compound.

Common functional groups like aromatic C-H, aliphatic C-H, C=O, C=N, C=C that are presented in all synthesized compounds 4a–o, had shown their IR signals at 3047, 2948, 1682, 1500 and 1442 cm^{-1} respectively which indicated the formation of the desired product.

Furthermore, ^{1}H-NMR spectrum recorded a double dublate peak at δ = 3.12, 3.62 ppm attributable to the pyrazole ring protons as well as to absence of the Benzimidazole ring -NH- proton. It was confirmed that different chloroacetylatedamines are attached with benzimidazole ring by amide formation and it is also confirmed by the ^{1}H NMR spectrum which give a singlet peak at δ = 4.55 ppm of methylene group =N-CH_2- proton.

Mass spectra of compound 4a revealed the molecular ion peak M^+ at m/z = 380, it is corresponding to the molecular mass of this compound. In the ^{13}C NMR spectrum, synthetic product 4a revealed a signal at δ=163.64 ppm arising from the C=O group and the signal obtained in the region of δ = 42.26 ppm confirmed the presence of carbon of methyl group.

Biological Studies: Biological activities of compounds containing pyrazole capped with benzimidazole rings have encouraged considerable interest in discovering the synthesis of new and potentially useful compounds. Novel derivatives 4a-o were evaluated for their *in vitro* antibacterial activity against two Gram-positive bacterial strains, namely, *Staphylococcus aureus* (ATCC-25923) and *Bacillus subtilis* (ATCC 6633); and two Gram-negative bacterial strains, namely, *Escherichia coli* (ATCC-25922) and *Enterobacter aerogenes* (ATCC-27853) using the well diffusion method. Amoxicillin and ciprofloxacin were used as the reference standard. The results of screening derivatives 4a-o are summarized in table 1.

Among the series, compound 4e exhibited excellent antibacterial activity against Gram-negative *Escherichia coli* and *Enterobacter aerogenes* bacteria with a MIZ value 21 and 24 mm respectively. Compound 4o showed good antibacterial activity against Gram-negative *Escherichia coli* and *Enterobacter aerogenes* bacteria with a MIZ value 19 and 26 mm respectively. These both compounds exhibited slightly higher antibacterial activity than the control (amoxicillin, MIZ = 16mm), Compounds 4i and 4o were potent against *Staphylococcus aureus* with MIZ value 34 and 36mm respectively. However, all other compounds in the series were found to have good activity against Gram-negative bacteria and moderate active against Gram-positive bacteria as compared to amoxicillin.

Therefore, the biological results obtained for the new benzimidazole analogues 4o showed promising antibacterial activity against Gram-positive and Gram-negative bacteria (Table 1). A brief investigation of the structure-activity relationship (SAR) revealed that 3-NO_2 and 4-OCH_3 substitution at the benzene ring contributed to better antibacterial activity.

In addition, the linkage of benzimidazole to pyrazole with tertiary amide linkage was found to enhance the antibacterial activities of the final products. Results presented in table 1 indicate that all the tested compounds exhibited greater activity against Gram-negative bacteria strains than amoxicillin.

Table 1
In-vitro antibacterial activity of compounds 4a-o

Compound Code	Zone of Inhibition (mm)			
	Gram Positive		Gram negative	
	Staphylococcus aureus	***Bacillus subtilis***	***Escherichia coli***	***Enterobacter aerogenes***
4a	16	14	16	12
4b	17	13	15	14
4c	16	13	18	15
4d	15	14	18	19
4e	27	19	21	24
4f	24	18	17	21
4g	15	20	14	13
4h	25	22	15	19
4i	34	26	19	17
4j	28	22	17	17
4k	16	13	11	14
4l	24	20	17	22
4m	20	21	16	15
4n	19	16	17	17
4o	36	25	19	26
Amoxicilin	40	26	16	18
Ciprofloxacin	40	36	25	34

Conclusion

Some new derivatives of benzimidazole bearing pyrazole heterocycles 4a–o (2-(1H-benzo[d]imidazol-1-yl)-1- (3,5-diphenyl-4,5- dihydro-1H-pyrazol-1-yl) ethan-1-one) were synthesized and characterized by infrared spectroscopy, ^{1}H and ^{13}C NMR spectroscopy and mass spectroscopy. Among these, derivatives 4e and 4o showed good activity against both Gram positive and Gram negative bacterial strain *in vitro*.

Compound 4e was most potent against Gram negative bacteria (*E. coli*, MIZ= 21mm and *E. aerogenes*, MIZ = 24mm) in comparison to amoxycilin (E. coli, MIZ= 16mm and *E. aerogenes*, MIZ = 18mm). while compounds 4i (*S. aureus*, MIZ = 34mm) and 4o (S. aureus MIZ = 36mm) were most active against Gram positive bacteria of the series.

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