ORIGINAL ARTICLE

Synthesis, characterization and antimicrobial activity of some novel benzimidazole derivatives

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J. Adv. Pharm. Technol. Res.

ABSTRACT

A series of novel *N*-((1*H*-benzoimidazol-1-yl) methyl)-4-(1-phenyl-5-substituted-4, 5-dihydro-1*H*-pyrazol-3-yl) benzenamine were synthesized by treating various 1-(4-((1*H*-benzoimidazol-1-yl) methylamino) phenyl)-3-substitutedprop-2-en-1-one with phenyl hydrazine in the presence of sodium acetate through a simple ring closure reaction. The starting material, 1-(4-((1*H*-benzoimidazol-1-yl) methylamino) phenyl)-3-substitutedprop-2-en-1-one, was synthesized from *o*-phenylenediamine by a multistep synthesis. All the synthesized compounds were characterized by spectroscopic means and elemental analyses. The title compounds were investigated for *in vitro* antibacterial and antifungal properties against some human pathogenic microorganisms by employing the agar streak dilution method using Ciprofloxacin and Ketoconazole as standard drugs. All title compounds showed activity against the entire strains of microorganism. Structural activity relationship studies reveal that compounds possessing an electron-withdrawing group display better activity than the compounds containing electron-donating groups, whereas the unsubstituted derivatives display moderate activity. Based on the results obtained, *N*-((1*H*-benzoimidazol-1-yl) methyl)-4-(1-phenyl-5-(4-(trifluoromethyl) phenyl)-4,5-dihydro-1*H*-pyrazol-3-yl) benzenamine 5i was found to be very active compared with the rest of the compounds and standard drugs that were subjected to antimicrobial assay.

Key words: Antibacterial, antifungal, chalcone, mannich base, pyrazole

INTRODUCTION

The diverse parasitic bacteria such as *Staphylococcus aureus*, *S. pyogenes*, *Salmonella typhimurium* and *Escherichia coli* have significant impact on the mucosal health of humans. Infection with *S. aureus*, *S. pyogenes*, *Salmonella typhimurium* and *E. coli* may have resulted in massive destruction of host tissue and life-threatening diseases. These bacterial parasites cause food poisoning, rheumatic fever and diarrhea, which affect millions of individuals in developing countries.[1] More than 50 million people worldwide are infected and up to 1,10,000 of these die every year. Amoxicillin, Norfloxacin and Ciprofloxacin are the most commonly used drugs for this bacterial infection but are associated with severe side-effects.[2]

A continuous increase in the number of infections caused by bacteria resistant to one or multiple antibiotic classes poses a significant threat and may lead to treatment failures and complications.[3,4] Therefore, significant efforts have been made by many research groups to find out new antimicrobial agents.

Benzimidazoles and their analogs are well-known biologically active *N*-containing heterocycles reported to possess various biological activities.[5-12] On the other hand, pharmacologically, pyrazole and its derivatives represent one of the most important classes of organic heterocyclic compounds possessing antibacterial, antifungal, herbicidal and antiviral activities.[13-16] Some of its derivatives have been reported to exhibit significant anti-arrhythmic, sedative, hypoglycemic and anti-inflammatory activities.[17-20]

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Access this article online

Quick Response Code:

Website: www.japtr.org

DOI: 10.4103/2231-4040.126983

Based on these findings, and in continuation of our drug research program concerning synthesis of new, safer and more biologically active compounds, it was of interest to synthesize a new series of benzimidazole-coupled pyrazole derivatives with the hope to obtain more active and less-toxic antimicrobial agents.

MATERIALS AND METHODS

Chemistry

All solvents used were of laboratory grade and were obtained from SD Fine Chemicals (Mumbai, India) and Merck (Mumbai, India). Ciprofloxacin and Ketoconazole were received as gift samples from Dr. Reddys Laboratories, Hyderabad, India. Melting points were determined in open glass capillary tubes and were uncorrected. Compounds were routinely checked for their purity on Silica gel G (Merck) Thin layer chromatography (TLC) plates; iodine chamber and UV lamp were used for visualization of TLC spots. The IR spectra were recorded in KBr pellets on a BIO-RAD FTS FT-IR spectrophotometer. ^{1}H-NMR spectra were recorded on a Bruker DPX-300 NMR spectrometer in $CDCl_3$ using tetramethylsilane (TMS) as an internal standard. The chemical shifts are reported on a ppm scale. Mass spectra were obtained on a JEOL-SX-102 instrument using electron impact ionization. Elemental analyses were performed on a Perkin Elmer model 2400 CHN analyzer and were within ±0.4% of the theoretical values.

General procedure for the synthesis of title compounds

Synthesis of benzimidazole (2)

Benzimidazole was prepared according to the reported literature.[21] Briefly, a mixture of *o*-phenylenediamine 1 (2.7 g, 0.025 mol) and 90% formic acid (1.56 g, 0.034 mol) was refluxed at 100°C for 2 h. The resulting solution was cooled and made alkaline to litmus with 10% sodium hydroxide solution. The product 2 obtained was filtered, washed with water and dried at 100°C. Yield 79%, m.p. 169-172°C. IR (KBr, cm^{-1}): 3285 (NH), 3062 (Ar–CH). ^{1}H-NMR ($CDCl_3$, 300 MHz) δ ppm: 5.24 (s, 1H, NH), 7.18-7.89 (m, 5H, Ar–CH). ESI-MS: *m/z* 118 [M$^+$]. Anal. Cald for $C_7H_6N_2$: C, 71.17; H, 5.12; N, 23.71. Found: C, 71.35; H, 5.11; N, 23.65.

Synthesis of 1-(4-((1H-benzimidazol-1-yl) methylamino) phenyl) ethanone (3)

Benzimidazole 2 (1.18 g, 0.01 mol) and *p*-aminoacetophenone (1.35 g, 0.01 mol) was dissolved in 40 mL of ethanol. To the above solution, formaldehyde (0.3 g, 0.01 mol) was added and stirred magnetically at room temperature for 3 h. Then, the resulting solution was refluxed on a water bath for 1 h and cooled in an ice bath. The product thus separated **3** was filtered, dried and crystallized from ethanol. Yield 72%, m.p. 145-147°C. IR (KBr, cm^{-1}): 3270 (NH), 3079 (Ar–CH), 2953 (CH_3–CH), 1697 (C = O). ^{1}H-NMR ($CDCl_3$, 300 MHz) δ ppm: 2.91 (s, 3H, CH_3), 4.50 (s, 2H, CH_2), 5.06 (s, 1H, NH), 7.05-8.14 (m, 9H, Ar–CH). ESI-MS: *m/z* 265 [M$^+$]. Anal. Cald for $C_{16}H_{15}N_3O$: C, 72.43; H, 5.70; N, 15.84. Found: C, 72.70; H, 5.68; N, 15.79.

Synthesis of 1-(4-((1H-benzimidazol-1-yl) methylamino)phenyl)-3-substitutedprop- 2-en-1-one (4a–4l)

A mixture of 1-(4-((1*H*-benzimidazol-1-yl) methylamino) phenyl) ethanone 3 (2.65 g, 0.01 mol) and different aromatic aldehydes (0.01 mol) was dissolved in a minimum quantity of ethanol. To this, a few drops of 10% sodium hydroxide solution was added and stirred for 5 h and kept in a refrigerator for 24 h. Then, the reaction mixture was poured in crushed ice and stirred well. The product separated out 4a-4l was filtered, dried and recrystallized from ethanol.

1-(4-((1H-benzimidazol-1-yl) methylamino) phenyl)-3-phenylprop-2-en-1-one (4a)

Yield 75%, m.p. 231-233°C. IR (KBr, cm^{-1}): 3309 (NH), 3065 (Ar–CH), 2852 (CH_2–CH), 1753 (C = O). ^{1}H-NMR ($CDCl_3$, 300 MHz) δ ppm: 4.15 (s, 2H, CH_2), 5.21 (s, 1H, NH), 7.24-7.99 (m, 14H, Ar–CH), 8.32-8.50 (m, 2H, CH = CH). ESI-MS: *m/z* 353 [M$^+$]. Anal. Cald for $C_{23}H_{19}N_3O$: C, 78.16; H, 5.42; N, 11.89. Found: C, 78.01; H, 5.44; N, 11.92.

1-(4-((1H-benzimidazol-1-yl) methylamino) phenyl)-3-(4-methoxyphenyl) prop-2-en-1-one (4b)

Yield 79%, m.p. 184-186°C. IR (KBr, cm^{-1}): 3272 (NH), 3077 (Ar–CH), 2878 (CH_2–CH), 1745 (C = O). ^{1}H-NMR ($CDCl_3$, 300 MHz) δ ppm: 2.95 (s, 3H, OCH_3), 3.82 (s, 2H, CH_2), 5.57 (s, 1H, NH), 7.08-8.14 (m, 13H, Ar–CH), 8.29-8.46 (m, 2H, CH = CH). ESI-MS: *m/z* 383 [M$^+$]. Anal. Cald for $C_{24}H_{21}N_3O_2$: C, 75.18; H, 5.52; N, 10.96. Found: C, 75.38; H, 5.50; N, 10.93.

1-(4-((1H-benzimidazol-1-yl) methylamino) phenyl)-3-p-tolylprop-2-en-1-one (4c)

Yield 72%, m.p. 217-220°C. IR (KBr, cm^{-1}): 3348 (NH), 3102 (Ar–CH), 2855 (CH_2–CH), 1750 (C = O). ^{1}H-NMR ($CDCl_3$, 300 MHz) δ ppm: 3.22 (s, 3H, CH_3), 4.38 (s, 2H, CH_2), 5.33 (s, 1H, NH), 7.10-8.31 (m, 13H, Ar–CH), 8.37-8.64 (m, 2H, CH = CH). ESI-MS: *m/z* 367 [M$^+$]. Anal. Cald for $C_{24}H_{21}N_3O$: C, 78.45; H, 5.76; N, 11.44. Found: C, 78.71; H, 5.74; N, 11.47.

1-(4-((1H-benzimidazol-1-yl) methylamino) phenyl)-3-(4-aminophenyl) prop-2-en-1-one (4d)

Yield 74%, m.p. 152-154°C. IR (KBr, cm^{-1}): 3321 (NH), 3067 (Ar–CH), 2864 (CH_2–CH), 1739 (C = O). ^{1}H-NMR ($CDCl_3$, 300 MHz) δ ppm: 3.67 (s, 2H, CH_2), 4.49 (s, 2H, NH_2), 5.15 (s, 1H, NH), 6.82-7.98 (m, 13H, Ar–CH), 8.20-8.43 (m, 2H, CH = CH). ESI-MS: *m/z* 368 [M$^+$]. Anal. Cald for $C_{23}H_{20}N_4O$: C, 74.98; H, 5.47; N, 15.21. Found: C, 74.75; H, 5.49; N, 15.26.

1-(4-((1H-benzimidazol-1-yl) methylamino) phenyl)-3-(4-chlorophenyl) prop-2-en-1-one (4e)

Yield 70%, m.p. 222-224°C. IR (KBr, cm^{-1}): 3263 (NH), 3050 (Ar–CH), 2859 (CH_2–CH), 1741 (C = O), 756 (C–Cl). ^{1}H-NMR ($CDCl_3$, 300 MHz) δ ppm: 3.90 (s, 2H, CH_2), 5.79 (s, 1H, NH), 7.05–8.01 (m, 13H, Ar–CH), 8.17-8.32 (m, 2H,

CH = CH). ESI-MS: *m/z* 389 [M^{+2}]. Anal. Cald for $C_{23}H_{18}ClN_3O$: C, 71.22; H, 4.68; N, 10.83. Found: C, 71.47; H, 4.67; N, 10.79.

1-(4-((1H-benzimidazol-1-yl) methylamino) phenyl)-3-(4-hydroxyphenyl) prop-2-en-1-one (4f)

Yield 77%, m.p. 208-211°C. IR (KBr, cm^{-1}): 3585 (OH), 3290 (NH), 3128 (Ar–CH), 2873 (CH_2–CH), 1746 (C = O). ^{1}H-NMR ($CDCl_3$, 300 MHz) δ ppm: 4.24 (s, 2H, CH_2), 5.57 (s, 1H, NH), 5.94 (s, 1H, OH), 7.20–8.16 (m, 13H, Ar–CH), 8.22-8.49 (m, 2H, CH = CH). ESI-MS: *m/z* 369 [M^+]. Anal. Cald for $C_{23}H_{19}N_3O_2$: C, 74.78; H, 5.18; N, 11.37. Found: C, 75.02; H, 5.19; N, 11.33.

1-(4-((1H-benzimidazol-1-yl) methylamino) phenyl)-3-(4-fluorophenyl) prop-2-en-1-one (4g)

Yield 75%, m.p. 195–197°C. IR (KBr, cm^{-1}): 3256 (NH), 3043 (Ar–CH), 2880 (CH_2–CH), 1757 (C = O), 1152 (C–F). ^{1}H-NMR ($CDCl_3$, 300 MHz) δ ppm: 4.05 (s, 2H, CH_2), 5.88 (s, 1H, NH), 6.91–8.02 (m, 13H, Ar–CH), 8.15–8.36 (m, 2H, CH = CH). ESI-MS: *m/z* 371 [M^+]. Anal. Cald for $C_{23}H_{18}FN_3O$: C, 74.38; H, 4.88; N, 11.31. Found: C, 74.10; H, 4.89; N, 11.34.

1-(4-((1H-benzimidazol-1-yl) methylamino) phenyl)-3-(4-nitrophenyl) prop-2-en-1-one (4h)

Yield 73%, m.p. 227-229°C. IR (KBr, cm^{-1}): 3285 (NH), 3062 (Ar–CH), 2866 (CH_2–CH), 1748 (C = O), 1520 and 1342 (NO_2). ^{1}H-NMR ($CDCl_3$, 300 MHz) δ ppm: 4.58 (s, 2H, CH_2), 5.20 (s, 1H, NH), 7.19-8.25 (m, 13H, Ar–CH), 8.31–8.57 (m, 2H, CH = CH). ESI-MS: *m/z* 398 [M^+]. Anal. Cald for $C_{23}H_{18}N_4O_3$: C, 69.34; H, 4.55; N, 14.06. Found: C, 69.57; H, 4.54; N, 14.02.

1-(4-((1H-benzimidazol-1-yl) methylamino) phenyl)-3-(4-(trifluoromethyl) phenyl) prop-2-en-1-one (4i)

Yield 77%, m.p. 173-175°C. IR (KBr, cm^{-1}): 3292 (NH), 3086 (Ar–CH), 2861 (CH_2–CH), 1734 (C = O), 1145 (C–F). ^{1}H-NMR ($CDCl_3$, 300 MHz) δ ppm: 4.13 (s, 2H, CH_2), 5.36 (s, 1H, NH), 7.00–8.03 (m, 13H, Ar–CH), 8.14-8.45 (m, 2H, CH = CH). ESI-MS: *m/z* 421 [M^+]. Anal. Cald for $C_{24}H_{18}F_3N_3O$: C, 68.40; H, 4.31; N, 9.97. Found: C, 68.61; H, 4.32; N, 9.94.

1-(4-((1H-benzimidazol-1-yl) methylamino) phenyl)- 5-phenylpenta-2,4-dien-1-one (4j)

Yield 81%, m.p. 201-203°C. IR (KBr, cm^{-1}): 3314 (NH), 3059 (Ar–CH), 2876 (CH_2–CH), 1738 (C = O). ^{1}H-NMR ($CDCl_3$, 300 MHz) δ ppm: 3.79 (s, 2H, CH_2), 5.65 (s, 1H, NH), 7.26–8.27 (m, 14H, Ar–CH), 8.39–8.81 (m, 4H, CH = CH). ESI-MS: *m/z* 379 [M^+]. Anal. Cald for $C_{25}H_{21}N_3O$: C, 79.13; H, 5.58; N, 11.07. Found: C, 79.40; H, 5.56; N, 11.05.

1-(4-((1H-benzimidazol-1-yl) methylamino) phenyl)-3-(4-hydroxy-3-methoxyphenyl) prop-2-en-1-one (4k)

Yield 72%, m.p. 179-181°C. IR (KBr, cm^{-1}): 3551 (OH), 3277 (NH), 3114 (Ar–CH), 2889 (CH_2–CH), 1752 (C = O). ^{1}H-NMR ($CDCl_3$, 300 MHz) δ ppm: 3.38 (s, 3H, OCH_3), 4.26 (s, 2H, CH_2), 5.12 (s, 1H, NH), 5.73 (s, 1H, OH), 6.74-7.80 (m, 12H, Ar–CH), 8.03–8.38 (m, 2H, CH = CH). ESI-MS: *m/z* 399 [M^+]. Anal. Cald for $C_{24}H_{21}N_3O_3$: C, 72.16; H, 5.30; N, 10.52. Found: C, 71.95; H, 5.31; N, 10.55.

1-(4-((1H-benzimidazol-1-yl) methylamino) phenyl)-3-(3-nitrophenyl) prop- 2-en-1-one (4l)

Yield 71%, m.p. 213-215°C. IR (KBr, cm^{-1}): 3338 (NH), 3095 (Ar–CH), 2870 (CH_2–CH), 1743 (C = O), 1539 and 1336 (NO_2). ^{1}H-NMR ($CDCl_3$, 300 MHz) δ ppm: 4.41 (s, 2H, CH_2), 5.44 (s, 1H, NH), 6.95–8.12 (m, 13H, Ar–CH), 8.29–8.46 (m, 2H, CH = CH). ESI-MS: *m/z* 398 [M^+]. Anal. Cald for $C_{23}H_{18}N_4O_3$: C, 69.34; H, 4.55; N, 14.06. Found: C, 69.56; H, 4.56; N, 14.08.

Synthesis of N-((1H-benzimidazol-1-yl) methyl)- 4-(1-phenyl-5-substituted-4,5-dihydro-1H-pyrazol-3-yl) benzenamine (5a–5l)

Title compound 5a-5l was synthesized by adding phenyl hydrazine (1.08 g, 0.01 mol) in fraction with the well-stirred mixture of 1-(4-((1H-benzimidazol-1-yl) methylamino) phenyl)- 3-substitutedprop-2-en-1-one 4a-4l (0.01 mol) in ethanol (25 mL). To this, a catalytic quantity of sodium acetate was added. The reaction mixture was then refluxed for a period of 10 h. Then, the reaction mixture was kept in a refrigerator for 24 h and poured in ice cold water with vigorous stirring. The products obtained 5a–5l were separated by filtration, washed with water, dried and recrystallized using ethanol to get the pure form.

N-((1H-benzimidazol-1-yl) methyl)- 4- (1,5- diphenyl- 4,5- dihydro-1H-pyrazol- 3-yl) benzenamine(5a)

Yield 73%, m.p. 214-216°C. IR (KBr, cm^{-1}): 3292 (NH), 3087 (Ar–CH), 2865 (CH_2–CH). ^{1}H-NMR ($CDCl_3$, 300 MHz) δ ppm: 2.64-2.86 (d, 2H, CH_2 of pyrazole), 3.41–3.75 (t, 1H, CH of pyrazole), 4.62 (s, 2H, CH_2), 5.47 (s, 1H, NH), 7.03–7.89 (m, 19H, Ar–CH). ESI-MS: *m/z* 443 [M^+]. Anal. Cald for $C_{29}H_{25}N_5$: C, 78.53; H, 5.68; N, 15.79. Found: C, 78.76; H, 5.66; N, 15.74.

N-((1H-benzimidazol-1-yl) methyl)-4- (5- (4-methoxyphenyl)-1-phenyl- 4,5-dihydro-1H-pyrazol -3-yl) benzenamine (5b)

Yield 70%, m.p. 202-204°C. IR (KBr, cm^{-1}): 3278 (NH), 3050 (Ar–CH), 2883 (CH_2–CH). ^{1}H-NMR ($CDCl_3$, 300 MHz) δ ppm: 2.80 (s, 3H, OCH_3), 2.99-3.31 (d, 2H, CH_2 of pyrazole), 3.65–3.84 (t, 1H, CH of pyrazole), 4.82 (s, 2H, CH_2), 5.23 (s, 1H, NH), 7.14-8.06 (m, 18H, Ar–CH). ESI-MS: *m/z* 473 [M^+]. Anal. Cald for $C_{30}H_{27}N_5O$: C, 76.09; H, 5.75; N, 14.79. Found: C, 76.34; H, 5.73; N, 14.75.

N-((1H-benzimidazol-1-yl) methyl)-4- (1-phenyl-5-p-tolyl-4, 5-dihydro-1H-pyrazol-3-yl) benzenamine (5c)

Yield 77%, m.p. 188–191°C. IR (KBr, cm^{-1}): 3303 (NH), 3079 (Ar–CH), 2860 (CH_2–CH). ^{1}H-NMR ($CDCl_3$, 300 MHz) δ ppm: 2.97 (s, 3H, CH_3), 3.02–3.25 (d, 2H, CH_2 of pyrazole), 3.40–3.69 (t, 1H, CH of pyrazole), 4.56 (s, 2H, CH_2), 5.51 (s, 1H, NH), 6.93–8.13 (m, 18H, Ar–CH). ESI-MS: *m/z* 457 [M^+]. Anal. Cald for $C_{30}H_{27}N_5$: C, 78.75; H, 5.95; N, 15.31. Found: C, 78.49; H, 5.96; N, 15.35.

N-((1H-benzimidazol-1-yl) methyl)-4- (5-(4-aminophenyl)- 1-phenyl-4, 5-dihydro-1H-pyrazol-3-yl) benzenamine (5d)

Yield 72%, m.p. 176–178°C. IR (KBr, cm^{-1}): 3285 (NH), 3043 (Ar–CH), 2851 (CH_2–CH). ^{1}H-NMR ($CDCl_3$, 300 MHz) δ ppm: 2.76–3.01 (d, 2H, CH_2 of pyrazole), 3.14–3.36 (t, 1H, CH of pyrazole), 4.62 (s, 2H, CH_2), 4.97 (s, 2H, NH_2), 5.35 (s, 1H, NH), 7.23–8.20 (m, 18H, Ar–CH). ESI-MS: *m/z* 458 [M^+]. Anal. Cald for $C_{29}H_{26}N_6$: C, 75.96; H, 5.71; N, 18.33. Found: C, 76.15; H, 5.70; N, 18.28.

N-((1H-benzimidazol-1-yl) methyl)-4- (5-(4-chlorophenyl)-1-phenyl-4, 5-dihydro-1H-pyrazol-3-yl) benzenamine (5e)

Yield 80%, m.p. 236–238°C. IR (KBr, cm^{-1}): 3321 (NH), 3096 (Ar–CH), 2875 (CH_2–CH), 779 (C–Cl). ^{1}H-NMR ($CDCl_3$, 300 MHz) δ ppm: 3.09–3.37 (d, 2H, CH_2 of pyrazole), 3.58–3.82 (t, 1H, CH of pyrazole), 4.74 (s, 2H, CH_2), 5.48 (s, 1H, NH), 7.06–8.31 (m, 18H, Ar–CH). ESI-MS: *m/z* 479 [M + ^{2}]. Anal. Cald for $C_{29}H_{24}ClN_5$: C, 72.87; H, 5.06; N, 14.65. Found: C, 72.69; H, 5.08; N, 14.69.

4-(3-(4-((1H-benzimidazol-1-yl) methylamino) phenyl)-1- phenyl-4, 5-dihydro- 1H-pyrazol-5-yl) phenol (5f)

Yield 71%, m.p. 225–227°C. IR (KBr, cm^{-1}): 3547 (OH), 3316 (NH), 3062 (Ar–CH), 2889 (CH_2–CH). ^{1}H-NMR ($CDCl_3$, 300 MHz) δ ppm: 2.81–3.04 (d, 2H, CH_2 of pyrazole), 3.43–3.70 (t, 1H, CH of pyrazole), 4.58 (s, 2H, CH_2), 5.39 (s, 1H, NH), 5.85 (s, 1H, OH), 6.72–7.85 (m, 18H, Ar–CH). ESI-MS: *m/z* 459 [M^+]. Anal. Cald for $C_{29}H_{25}N_5O$: C, 75.80; H, 5.48; N, 15.24. Found: C, 75.97; H, 5.49; N, 15.21.

N-((1H-benzimidazol-1-yl) methyl)-4- (5-(4-fluorophenyl)-1-phenyl-4, 5-dihydro- 1H-pyrazol- 3-yl) benzenamine (5g)

Yield 75%, m.p. 209–211°C. IR (KBr, cm^{-1}): 3265 (NH), 3041 (Ar–CH), 2868 (CH_2–CH), 1132 (C–Cl). ^{1}H-NMR ($CDCl_3$, 300 MHz) δ ppm: 2.56–2.90 (d, 2H, CH_2 of pyrazole), 3.39–3.67 (t, 1H, CH of pyrazole), 4.78 (s, 2H, CH_2), 5.22 (s, 1H, NH), 7.15–8.14 (m, 18H, Ar–CH). ESI-MS: *m/z* 461 [M^+]. Anal. Cald for $C_{29}H_{24}FN_5$: C, 75.47; H, 5.24; N, 15.17. Found: C, 75.71; H, 5.23; N, 15.12.

N-((1H-benzimidazol-1-yl) methyl)-4- (5-(4-nitrophenyl)-1-phenyl-4, 5-dihydro-1H-pyrazol-3-yl) benzenamine (5h)

Yield 79%, m.p. 194–196°C. IR (KBr, cm^{-1}): 3290 (NH), 3074 (Ar–CH), 2887 (CH_2–CH), 1541 and 1316 (NO_2). ^{1}H-NMR ($CDCl_3$, 300 MHz) δ ppm: 2.78–3.02 (d, 2H, CH_2 of pyrazole), 3.66–3.91 (t, 1H, CH of pyrazole), 4.65 (s, 2H, CH_2), 5.57 (s, 1H, NH), 6.99–8.29 (m, 18H, Ar–CH). ESI-MS: *m/z* 488 [M^+]. Anal. Cald for $C_{29}H_{24}N_6O_2$: C, 71.30; H, 4.95; N, 17.20. Found: C, 71.57; H, 4.94; N, 17.16.

N-((1H-benzimidazol-1-yl) methyl)- 4- (1-phenyl-5-(4-(trifluoromethyl) phenyl)-4 , 5-dihydro-1H-pyrazol-3-yl) benzenamine (5i)

Yield 74%, m.p. 230-233°C. IR (KBr, cm^{-1}): 3302 (NH), 3058 (Ar–CH), 2850 (CH_2–CH), 1124 (C–F). ^{1}H-NMR ($CDCl_3$, 300 MHz) δ ppm: 2.60-2.93 (d, 2H, CH_2 of pyrazole), 3.37–3.65 (t, 1H, CH of pyrazole), 4.81 (s, 2H, CH_2), 5.44 (s, 1H, NH), 7.28–8.32 (m, 18H, Ar–CH). ESI-MS: *m/z* 511 [M^+]. Anal. Cald for $C_{30}H_{24}F_3N_5$: C, 70.44; H, 4.73; N, 13.69. Found: C, 70.21; H, 4.74; N, 13.72.

N-((1H-benzimidazol-1-yl) methyl)-4- (1-phenyl-5-styryl-4, 5-dihydro-1H-pyrazol-3-yl) benzenamine (5j)

Yield 73%, m.p. 243–245°C. IR (KBr, cm^{-1}): 3297 (NH), 3080 (Ar–CH), 2876 (CH_2–CH). ^{1}H-NMR ($CDCl_3$, 300 MHz) δ ppm: 3.15–3.48 (d, 2H, CH_2 of pyrazole), 3.52–3.73 (t, 1H, CH of pyrazole), 4.67 (s, 2H, CH_2), 5.36 (s, 1H, NH), 6.81–7.96 (m, 19H, Ar–CH), 8.19–8.46 (m, 2H, -CH = CH-). ESI-MS: *m/z* 469 [M^+]. Anal. Cald for $C_{31}H_{27}N_5$: C, 79.29; H, 5.80; N, 14.91. Found: C, 79.10; H, 5.82; N, 14.96.

4-(3-(4-((1H-benzimidazol-1-yl) methylamino) phenyl)-1 -phenyl-4, 5-dihydro-1H-pyrazol-5-yl) -2-methoxyphenol (5k)

Yield 75%, m.p. 151–153°C. IR (KBr, cm^{-1}): 3591 (OH), 3279 (NH), 3095 (Ar–CH), 2856 (CH_2–CH). ^{1}H-NMR ($CDCl_3$, 300 MHz) δ ppm: 2.53 (s, 3H, OCH_3), 2.86–3.19 (d, 2H, CH_2 of pyrazole), 3.40–3.71 (t, 1H, CH of pyrazole), 4.74 (s, 2H, CH_2), 5.25 (s, 1H, NH), 5.60 (s, 1H, OH), 7.07–8.09 (m, 17H, Ar–CH). ESI-MS: *m/z* 489 [M^+]. Anal. Cald for $C_{30}H_{27}N_5O_2$: C, 73.60; H, 5.56; N, 14.31. Found: C, 73.81; H, 5.55; N, 14.27.

N-((1H-benzimidazol-1-yl) methyl)-4- (5-(3-nitrophenyl)-1-phenyl-4, 5-dihydro-1H-pyrazol-3-yl) benzenamine (5l)

Yield 72%, m.p. 250–252°C. IR (KBr, cm^{-1}): 3314 (NH), 3060 (Ar–CH), 2872 (CH_2–CH), 1508 and 1325 (NO_2). ^{1}H-NMR ($CDCl_3$, 300 MHz) δ ppm: 2.57–2.96 (d, 2H, CH_2 of pyrazole), 3.35–3.59 (t, 1H, CH of pyrazole), 4.53 (s, 2H, CH_2), 5.50 (s, 1H, NH), 7.12–8.18 (m, 18H, Ar–CH). ESI-MS: *m/z* 488 [M^+]. Anal. Cald for $C_{29}H_{24}N_6O_2$: C, 71.30; H, 4.95; N, 17.20. Found: C, 71.54; H, 4.96; N, 17.15.

Antimicrobial activity

In this study, all the synthesized compounds were screened for antimicrobial activity by the agar streak dilution method. The antibacterial activity of the compounds was evaluated against four Gram-positive bacteria: *Staphylococcus aureus* ATCC 9144, *Staphylococcus epidermidis* ATCC 155, *Micrococcus luteus* ATCC 4698 and *Bacillus cereus* ATCC 11778, and three Gram-negative bacteria: *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 2853 and *Klebsiella pneumoniae* ATCC 11298. The antifungal activity of the synthesized compounds were evaluated against two fungi, *Aspergillus niger* ATCC 9029 and *Aspergillus fumigatus* ATCC 46645. Bacterial strains were cultured overnight at 37°C in Mueller Hinton broth, and the yeast was cultured overnight at 30°C in YEPDE agar for antibacterial and antifungal activity tests. Test strains were suspended in nutrient agar to give a final density of 5×10^{-5} cfu/mL.

Minimum inhibitory concentration (MIC)

The MIC of the compound was determined by the agar streak dilution method.[22] A stock solution of the synthesized

compound in dimethyl formamide was prepared and graded quantities of the test compounds were incorporated in specified quantity of molten sterile agar (nutrient agar for antibacterial activity and Sabouraud's dextrose agar medium for antifungal activity). A specified quantity of the medium (40–50ºC) containing the compound was poured into a Petri dish to give a depth of 3–4 mm and allowed to solidify. A suspension of the microorganism was prepared to contain approximately 5×10^{-5} cfu/mL and applied to plates with serially diluted compounds in dimethyl formamide to be tested and incubated at 37°C for 24 h and 48 h for bacteria and fungi, respectively. The MIC was considered to be the lowest concentration of the test substance exhibiting no visible growth of bacteria or fungi on the plate. The observed MIC is presented in Table 1.

RESULTS AND DISCUSSION

Chemistry

In the present work, a series of new *N*-((1*H*-benzimidazol-1-yl) methyl)-4-(1-phenyl-5-substituted-4,5-dihydro-1*H*-pyrazol-3-yl) benzenamine analogs 5a-5l were synthesized and characterized. In the first step, benzimidazole 2 was synthesized from *o*-phenylenediamine and formic acid by the ring closure reaction with a loss of two water molecules. In the next step, compound 2 was treated with *p*-aminoacetophenone and formaldehyde results in the corresponding Mannich base 3 by the Mannich reaction. Further treatment of compound 3 with different aromatic aldehydes in the presence of catalytic quantity of sodium hydroxide gave 1-(4-((1*H*-benzimidazol-1-yl) methylamino) phenyl)-3-substitutedprop-2-en-1-one 4a–4l (a chalcone derivative) with elimination of water. Finally, the title compounds 5a–5l were synthesized by treating various chalcones 4a–4l with phenyl hydrazine in the presence of sodium acetate through a simple ring closure reaction. The chemical reactions involved in the synthesis of the title compounds are summarized in synthetic Scheme 1. The method used for the preparation and isolation of the compounds gave materials of good purity, as evidenced by their spectral analyses and by TLC.

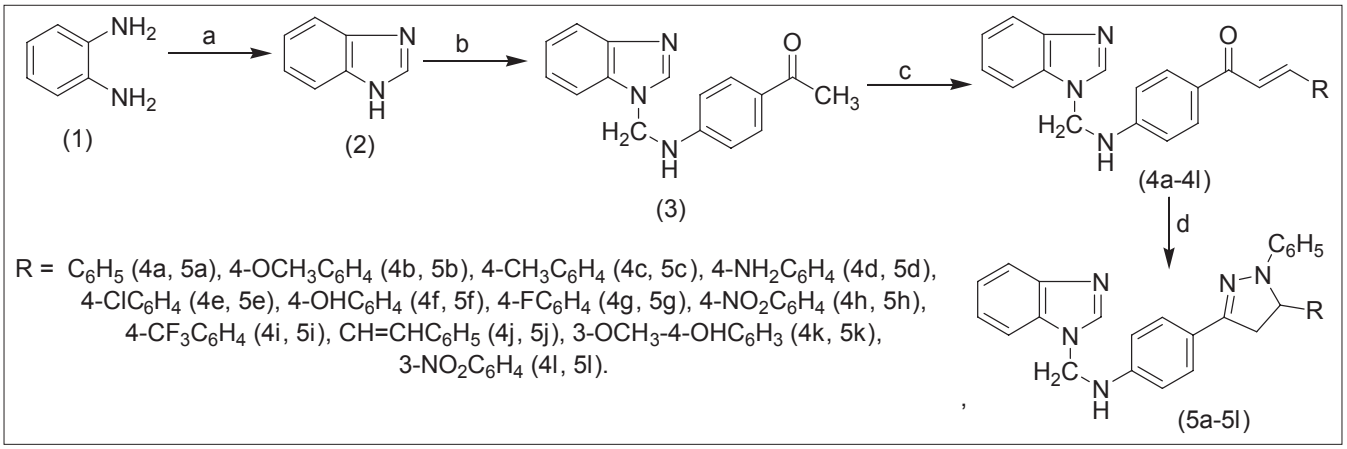
IR, ^{1}H-NMR, mass spectra and elemental analyses of the synthesized compounds are in accordance with the assigned structures. The IR spectra of all the synthesized compounds showed some characteristic peaks, indicating the presence of particular groups. Formation of benzimidazole 2 was confirmed by the appearance of a singlet at δ 5.24 ppm for one proton of benzimidazole NH. The presence of an absorption band in IR at 1697 cm^{-1} corresponding to C = O stretching confirms the formation of compound **3**. This was further confirmed by the appearance of a singlet at δ 2.91 ppm for three protons and a singlet at δ 4.50 ppm for two protons in its ^{1}H-NMR spectra, which might be assigned to CH_3 and CH_2 groups, respectively. Formation of compound 4a–4l was confirmed by the appearance of multiplet (double doublet) for two protons around δ 8.03-8.81 ppm in its ^{1}H-NMR spectra, which might be assigned to the CH = CH group of chalcones. ^{1}H-NMR spectra of compound 5a–5l showed a doublet peak at δ 2.56-3.48 ppm and triplet peak at δ 3.14-3.91 ppm corresponding to two protons of CH_2 (C–4) and one proton of CH (C–5) of pyrazole, confirming its formation.

Antimicrobial screening

All the title compounds were screened for their *in vitro* antimicrobial activity by the agar streak dilution method. To control the sensitivity of the test organisms, the MICs of Ciprofloxacin and Ketoconazole were determined in parallel experiments. The MIC values were determined as the lowest concentration that totally inhibited visible growth of the microorganisms. The MICs of the test compounds 5a–5l and standard drugs is efficiently presented in Table 1. From the results, it was found that compound 5e, 5g and 5i (MIC: 15.62 µg/mL) displayed comparable activity like Ciprofloxacin against *S. aureus*. Compounds 5g and 5i displayed an equivalent activity (MIC: 7.81 µg/mL) against

Table 1: Minimum inhibitory concentration in µg/mL of the synthesized compounds 5a-5l

Compounds	*S. aureus*	*S. epidermidis*	*M. luteus*	*B. cereus*	*E. coli*	*P. aeruginosa*	*K. pneumoniae*	*A. niger*	*A. fumigatus*
5a	31.25	31.25	15.62	62.5	31.25	31.25	15.62	62.5	31.25
5b	125	62.5	31.25	125	62.5	125	31.25	125	125
5c	125	62.5	125	125	62.5	62.5	31.25	125	125
5d	62.5	31.25	31.25	31.25	62.5	62.5	31.25	125	62.5
5e	15.62	15.62	7.81	15.62	15.62	7.81	7.81	31.25	15.62
5f	62.5	31.25	31.25	31.25	62.5	125	31.25	125	62.5
5g	15.62	7.81	7.81	7.81	15.62	7.81	7.81	31.25	7.81
5h	31.25	15.62	15.62	15.62	15.62	15.62	7.81	31.25	15.62
5i	15.62	7.81	3.9	7.81	7.81	15.62	7.81	15.62	7.81
5j	31.25	31.25	15.62	31.25	31.25	62.5	15.62	125	31.25
5k	62.5	62.5	31.25	31.25	62.5	125	31.25	125	125
5l	31.25	15.62	15.62	15.62	31.25	15.62	7.81	31.25	15.62
Ciprofloxacin	15.62	7.81	7.81	7.81	15.62	7.81	3.9	-	-
Ketoconazole	-	-	-	-	-	-	-	15.62	7.81



Scheme 1: Reagents and conditions: (a) HCOOH, 2 h; (b) *p*- $NH_2C_6H_4COCH$, HCHO, 4 h; (c) RCHO, C_2H_5OH, NaOH, 5 h; (d)C_6H_5NHNH, C_2H_5OH, CH_3COONa, 10 h

S. epidermidis, whereas the rest of the sequence displayed lesser activity (MIC: 15.62–62.5 µg/mL). Against *M. luteus*, compound 5i showed superior activity (MIC: 3.9 µg/mL) than standard drug, whereas compounds 5e and 5g exhibited comparable activity (MIC: 7.81 µg/mL) as Ciprofloxacin, while the others demonstrated trivial activity than the standard. Compounds 5g and 5i demonstrated equal activity (MIC: 7.81 µg/mL) as Ciprofloxacin, whereas the rest of the series exhibited shoddier activities than the standard against *B. cereus*. Compound 5i displayed potent activity (MIC: 7.81 µg/mL) than the standard, while the rest of the series exhibited lower activity against *E. coli* (MIC: 31.25–62.5 µg/mL) except 5e, 5g and 5h. Compounds 5e and 5g displayed an equivalent activity (MIC: 7.81 µg/mL) as standard against *P. aeruginosa*. None of the synthesized compounds displayed the same activity (MIC: 3.9 µg/mL) as Ciprofloxacin against *K. pneumoniae*. Against *A. niger*, except compound 5i, the rest of the compounds showed weaker activity (MIC: 31.25–125 µg/mL) than Ketoconazole. Compounds 5g and 5i showed comparable activity (MIC: 7.81 µg/mL) against *A. fumigates*, while the others had lower activity (MIC: 15.62–125 µg/mL) than the standard. Of the various tested derivatives, *N*-((1*H*-benzimidazol-1-yl) methyl)-4-(1-phenyl-5-(4-(trifluoromethyl) phenyl)-4,5-dihydro-1*H*-pyrazol-3-yl) benzenamine 5i was found to be the more potent compound. This compound exhibited better activity against *M. leutus* and *E. coli*, while it displayed equal activity as standard against *S. aureus*, *S. epidermidis*, *B. cereus*, *A. niger* and *A. fumigatus*.

Structural activity relationship (SAR)

From the antimicrobial studies, it was found that compounds 5e, 5g, 5h, 5i and 5l showed potent antimicrobial activity, which might be due to the presence of electron-withdrawing substituents like chloro, fluoro, nitro and trifluoromethyl groups on phenyl ring attached at the C-5 of the pyrazole nucleus. Compounds possessing electron-donating substituents like methoxy, methyl, amino and hydroxyl groups (5b, 5c, 5d, 5f and 5k) demonstrate less *in vitro* antimicrobial activity. SAR studies reveal that compounds possessing an electron-withdrawing group displayed better activity than the compounds containing electron-donating groups, whereas the unsubstituted derivatives displayed moderate activity. The SAR of the title compounds are schematically represented in Figure 1.

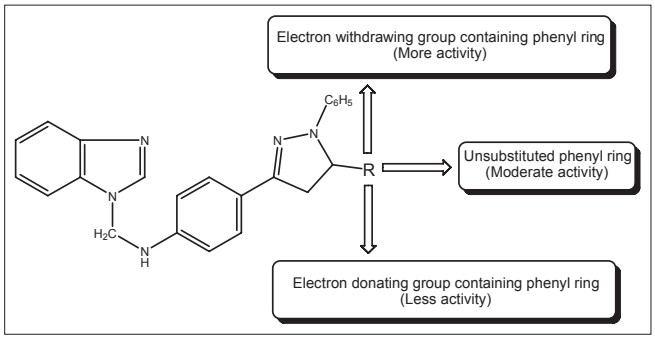


Figure 1: Schematic representation of SAR of title componds (5a-5l)

CONCLUSION

With an aim of developing potent antimicrobial agent, a series of novel pyrazole-attached benzimidazoles were synthesized from *o*-phenylenediamine by the multistep reaction synthesis and characterized by FT-IR, ^{1}H-NMR, mass spectroscopy and elemental analysis. All the title compounds were screened for their *in vitro* antimicrobial activity by the agar streak dilution method, and its MIC was determined against various strains of microorganisms. Results revealed that compounds containing an electron-withdrawing group at the phenyl group attached to C-5 of pyrazole displayed superior antimicrobial activities than compounds possessing an electron-releasing group. Moreover, the unsubstituted derivatives displayed moderate activity. Among several tested compounds, *N*-((1*H*-benzimidazol-1-yl) methyl)-4-(1-phenyl-5-(4-(trifluoromethyl) phenyl)-4, 5-dihydro-1*H*-pyrazol-3-yl) benzenamine 5i showed

better activity. Hence, this compound may serves as a lead molecule to obtain clinically useful antimicrobial agent.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the management of Bapatla College of Pharmacy, Bapatla, for providing infrastructure facilities to carry out this research work.

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How to cite this article: Krishnanjaneyulu IS, Saravanan G, Vamsi J, Supriya P, Bhavana JU, Sunil Kumar MV. Synthesis, characterization and antimicrobial activity of some novel benzimidazole derivatives. J Adv Pharm Technol Res 2014;5:21-7.

Source of Support: Nil, **Conflict of Interest:** Nil.