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Copper Catalyzed Chan-Lam Coupling Reaction of Pyrazoles at Ambient Temperature and their Antimicrobial Evaluation

KALPESH V. VILAPARA[1,*], SAGAR P. GAMI[2], SHOBHNA A. GADARA[3] and YOGESH T. NALIAPARA[1]

[1]Department of Chemistry, Saurashtra University, Rajkot-360005, India
[2]Department of Chemistry, Bhavan's Sheth R.A. College of Science, Ahmedabad-380001, India
[3]Sheth P.T. Arts & Science College, Godhra-389001, India

*Corresponding author: E-mail: kalpeshvilapara@yahoo.com

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An efficient protocol to synthesize a series of N-aryl pyrazoles *via* C-N bond Chan-Lam coupling reaction using diverse boronic acid at room temperature using $Cu(OAc)_2$/TEA as a catalytic system without utilization of any ligand or additive and exposed to air to afford moderate to excellent yield. The characterizations of newly synthesized compounds were confirmed by FTIR, MS, ^1H NMR, ^{13}C NMR, and elemental analysis. All the synthesized compounds were assayed for their antibacterial activity against *Staphylococcus aureus* MTCC-96, *Escherichia coli* MTCC-443, *B. subtilis* MTCC-441, *S. typhi* MTCC-98, and antifungal activity against *Aspergillus niger* MTCC-282 and *Aspergillus clavatus* MTCC-1323 at different concentration and compared with standards drugs. The minimum inhibition concentration (MIC) of the compounds was studied by micro broth dilution method.

Keywords: Chan-Lam coupling, *N*-Aryl pyrazoles, Boronic acids, Microbial assay.

INTRODUCTION

Pyrazole ring skeleton are ubiquitous motif in pharmaceuticals, pesticides as well as in chemical industries [1]. Among aromatic heterocycles, pyrazole derivatives especially celecoxib [1] is targeted many times for design and synthesis of structurally related pyrazole analogues. Some other biologically relevant molecules like deracoxib [2], SC-236 [3] and lead 10 [4] described for their potential antimigratory activity, neovascular inhibition, control of VEGF production and apoptosis growth of tumer cells. Pyrazole derivatives exhibit a wide range of biological activities such as antiviral [2], anticancer [3], anti-inflammatory [4], anticonvulsant [5], antitubercular [6], antimicrobial activities [7-9]. Modification in the structural profile by different functionality at different position in pyrazole motif affect few pharmacological properties [10,11]. The organic molecules, including pyrazole derivatives which possess trifluoromethyl functional group, have a potential to modify the pharmacological activities [12,13]. Also, the incorporation of aryl functionally into the pyrazole ring enhances the pharmacological activities to a great extent. The existence of different functional group, on the pyrazole ring as well as phenyl ring, can severely modify the pharmacological properties of such molecules [14]. In present article, we report the synthesis of novel pyrazole motif by Chan-Lam coupling reaction.

Recent literatures revealed that an infection of microbial increased rapidly because of antibacterial resistance due to excessive use of antimicrobial drugs [15]. Resistance to antimicrobial drug has resulted in morbidity and mortality from treatment failures and high cost for the treatments [16]. Many antimicrobial agents have been applied for treatment but still the medical field needs extensive efforts for the development of new antimicrobial agents to overcome the highly resistant species of microbes.

Development of carbon-heteroatom bond formation has always been a center of attraction among chemists. Therefore the development of new methods for their synthesis is still interesting area for the researchers. Transition metal-catalysts are well known for cross-coupling reactions for C-N bond construction [17-20]. In last decade, Buchwald and Hartwig have done pioneering work on palladium-catalyzed cross-coupling methodology for C-N bond formation of amine with

aryl halides, triflates, and tosylates [21-26]. For last few years, copper catalysts have been emerged as a new era for C-N and C-O bond linkages to overcome palladium chemistry, due to the high stability, low toxicity and low cost of copper reagents. Initial reports have been made by Chan *et al*. [27,28], they introduced boronic acids to add carbon partner of carbon heteroatom bonds because of easy availability and high reactivity in cross coupling chemistry. In comparison to Ullmann coupling reaction and other Pd-mediated cross coupling transformations, Cu-metal chemistry has numerous benefits *e.g.*, mild operating conditions, simple bases and open flask chemistry (air as an oxidant). Since last few years, this methodology has been explored for variety of NH containing nucleophiles such as amines, amides, imides, ureas, hydrazines, carbamates, sulfonamide, sulfonyl azide and different classes of aromatic heterocycles (imidazole, pyrazole, indole, *etc*.). In last decade, several catalytic version of Chan-Lam coupling reaction have also been reported [29-32].

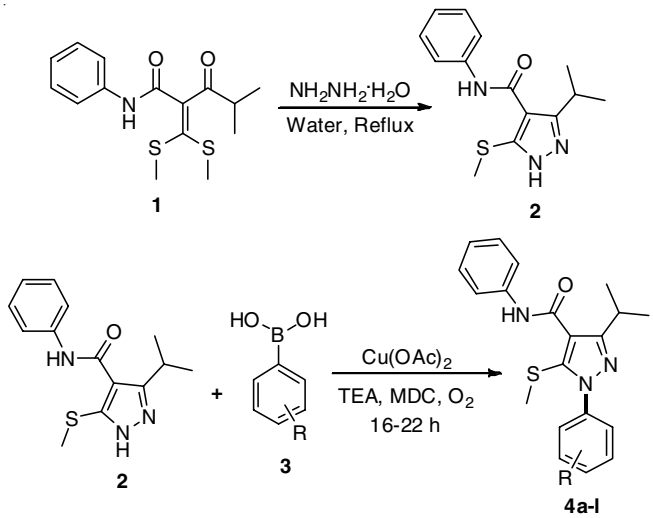
Encouraged by the above reports and promising biological profile, some novel pyrazole motifs have been synthesized using α-acyl ketene dithioacetals (α-AKDTAs) as a starting material for the construction of pyrazole cores which were subject to Chan-Lam cross coupling to get target molecules.

EXPERIMENTAL

All the chemicals were purchased from Sigma-Aldrich Chemicals and used as received. Reactions were monitored by thin layer chromatography (TLC) on pre-coated silica gel GF_{254} plates (E-Merck Co.) by using appropriate solvent systems. Melting points were determined in open capillaries and are uncorrected. Infrared (IR) spectra (KBr pellets) were recorded on a Shimadzu-FTIR-8400 spectrophotometer over frequencies ranging from 4000-400 cm^{-1}. The NMR spectra (^1H & ^{13}C NMR) were recorded on a Bruker Avance Spectrospin 400 MHz spectrometer using $CDCl_3$ as solvents and TMS as an internal standard. Mass spectra were recorded on a Shimadzu GC-MS-QP-2010 spectrometer by using Electron Impact (EI) (0.7 kV) ionization source. The ion source temperature was 220 ºC and the interface temperature was 240 ºC.

Synthetic procedure for 3-isopropyl-5-(methylthio)-*N*-phenyl-1*H*-pyrazole-4-carboxamide (2): Compound 5-isopropyl-3-(methylthio)-*N*-phenyl-1*H*-pyrazole-4-carboxamide was synthesized according to previous report [32].

General procedure for the synthesis of 3-isopropyl-5-(methylthio)-*N*,1-aryl-1*H*-pyrazole-4-carboxamide (4a-l): A 50 mL round bottomed flask charged with dry dichloromethane (10 vol) and dry molecular sieves. 5-Isopropyl-3-(methylthio)-*N*-phenyl-1*H*-pyrazole-4-carboxamide (**2**) (5 mmol), triethyl amine (20 mmol), boronic acid (**3**) (6 mmol), and copper(II) acetate (6 mmol) were added to this solution. The suspension then stirred for 2 days under air. The calcium chloride guard tube was used to protect the reaction from moisture. The reaction was monitored by TLC using ethyl acetate:hexane as a mobile phase. The suspension was diluted with dichloromethane, filtered and washed with water and brine. The organic phase was dried over Na_2SO_4 and the solvent removed under reduced pressure to afford desired product **4a-l** (**Scheme-I**). All the synthesized compounds were purified by column chromatography using ethyl acetate:hexane as solvent system.



Scheme-I: Synthesis of N-substituted pyrazoles (**4a-l**)

Spectral data

3-Isopropyl-5-(methylthio)-*N*-phenyl-1*H*-pyrazole-4-carboxamide (2): Yield, 88 %; m.p. 136 ºC; IR (KBr, ν_{max}, cm^{-1}): 3250, 3142, 3032, 2926, 2863, 1647, 1548, 1446, 763, 748. ^1H NMR (400 MHz, $CDCl_3$, δ ppm): 13.00 (s, 1H), 9.68 (s, 1H), 7.64 (d, *J* = 8.0 Hz, 2H), 7.31 (t, 2H), 7.05 (t, 1H), 3.40-3.33 (m, 1H), 2.50 (s, 3H), 1.24 (d, *J* = 6.8 Hz, 6H); MS (*m/z*): 275 (M^+); Elemental analysis for $C_{14}H_{14}N_3OS$ calcd. (found) %: C, 61.06 (61.48); H, 6.22 (6.72); N, 15.26 (14.86).

3-Isopropyl-5-(methylthio)-*N*,1-diphenyl-1*H*-pyrazole-4-carboxamide (4a): Time: 18 h; yield, 84 %; m.p. 106 ºC; IR (KBr, ν_{max}, cm^{-1}): 3228, 3131, 3064, 2970, 2863, 1641, 1596, 1443, 755, 696. ^1H NMR (400 MHz, $CDCl_3$, δ ppm): 9.49 (s, 1H), 7.68 (d, *J* = 7.6 Hz, 2H), 7.57-7.47 (m, 6H), 7.37 (t, 3H), 7.14 (t, 1H), 3.89-3.78 (m, 1H), 2.25 (s, 3H), 1.37 (d, *J* = 6.8 Hz, 6H); ^{13}C NMR (400 MHz, $CDCl_3$, δ ppm): 161.86, 161.26, 139.17, 138.49, 133.41, 129.28, 129.19, 128.97, 126.50, 124.37, 120.09, 117.47, 27.58, 22.20, 20.01; MS (*m/z*): 351 (M^+); Elemental analysis for $C_{20}H_{21}N_3OS$ calcd. (found) %: C, 68.35 (67.87); H, 6.02 (6.34); N, 11.96 (12.21).

1-(4-Cyanophenyl)-3-isopropyl-5-(methylthio)-*N*-phenyl-1*H*-pyrazole-4-carboxamide (4b): Time: 17 h; yield, 87 %; m.p. 116 ºC; IR (KBr, ν_{max}, cm^{-1}): 3288, 3135, 3064, 2958, 2926, 2866, 2222, 1648, 1444, 750, 693. ^1H NMR (400 MHz, $CDCl_3$, δ ppm): 9.28 (s, 1H), 7.82 (d, *J* = 9.6 Hz, 2H), 7.79 (d, *J* = 8 Hz, 2H), 7.67 (d, *J* = 7.2 Hz, 2H), 7.38 (t, 2H), 7.16 (t, 1H), 3.82-3.74 (m, 1H), 2.30 (s, 3H), 1.36 (d, *J* = 6.4 Hz, 6H). ^{13}C NMR (400 MHz, $CDCl_3$, δ ppm): 162.69, 160.77, 142.49, 138.16, 133.71, 133.12, 129.34, 126.52, 124.67, 120.10, 119.22, 118.21, 112.21, 27.58, 22.07, 20.26. MS (m/z): 376 (M^+); Elemental analysis for $C_{21}H_{20}N_4OS$ calcd. (found) %: C, 67.00 (67.38); H, 5.35 (5.67); N, 14.88 (14.42).

1-(4-Fluorophenyl)-3-isopropyl-5-(methylthio)-*N*-phenyl-1*H*-pyrazole-4-carboxamide (4c): Time: 16 h; yield, 83 %; m.p. 128 ºC; IR (KBr, ν_{max}, cm^{-1}): 3305, 3282, 3067, 3053, 2983, 2959, 1644, 1478, 1221, 742. ^1H NMR (400 MHz, $CDCl_3$, δ ppm): 9.43 (s, 1H), 7.68 (d, *J* = 7.6 Hz, 2H), 7.55-7.51 (m, 2H), 7.37 (t, 2H), 7.20 (t, 2H), 7.14 (t, 1H), 3.88-3.77 (m, 1H), 2.25 (s, 3H), 1.36 (d, *J* = 6.8 Hz, 6H). ^{13}C NMR (400 MHz, $CDCl_3$, δ ppm): 163.85, 161.89, 161.09, 138.36, 135.16, 133.56,

129.26, 128.27, 124.41, 120.06, 116.24, 116.02, 27.51, 22.14, 19.96. MS (*m/z*): 369 (M^+); Elemental analysis for $C_{20}H_{20}N_3OSF$ calcd. (found) %: C, 65.02 (65.41); H, 5.46 (5.11); N, 11.37 (11.88).

1-(4-Bromophenyl)-3-isopropyl-5-(methylthio)-*N*-phenyl-1*H*-pyrazole-4-carboxamide (4d): Time: 18 h; yield, 85 %; m.p. 118 ºC; IR (KBr, ν_{max}, cm^{-1}): 3295, 3270, 3128, 3062, 2959, 2866, 1645, 1494, 752, 575. ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 9.40 (s, 1H), 7.67 (d, *J* = 8.4 Hz, 2H), 7.64 (d, *J* = 8.4 Hz, 2H), 7.45 (d, *J* = 8.6 Hz, 2H), 7.37 (t, 2H), 7.14 (t, 1H), 3.86-3.77 (m, 1H), 2.26 (s, 3H), 1.36 (d, *J* = 6.8 Hz, 6H). ^{13}C NMR (400 MHz, $CDCl_3$, δ ppm): 161.10, 161.00, 138.31, 138.05, 133.40, 132.31, 129.27, 127.85, 124.44, 122.76, 120.05, 117.95, 27.51, 22.10, 20.07. MS (*m/z*): 429 (M^+); Elemental analysis for $C_{20}H_{20}N_3OSBr$ calcd. (found) %: C, 55.82 (55.26); H, 4.68 (4.24); N, 9.76 (10.14).

1-(4-Chlorophenyl)-3-isopropyl-5-(methylthio)-*N*-phenyl-1*H*-pyrazole-4-carboxamide (4e): Time: 16 h; yield, 79 %; m.p. 110 ºC; IR (KBr, ν_{max}, cm^{-1}): 3281, 3263, 3129, 3056, 2959, 2863, 1640, 1443, 1411, 833, 752, 694. ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 9.40(s, 1H), 7.68 (d, *J* = 8.0 Hz, 2H), 7.52 (d, *J* = 8.8 Hz, 2H), 7.48 (d, *J* = 8.8 Hz, 2H), 7.37 (t, 2H), 7.14 (t, 1H), 3.85-3.77 (m, 1H,), 2.26 (s, 3H), 1.36 (d, *J* = 6.8 Hz, 6H). ^{13}C NMR (400 MHz, $CDCl_3$, δ ppm): 162.06, 161.03, 138.33, 137.56, 134.77, 133.45, 129.34, 129.27, 127.59, 124.44, 120.07, 117.91, 27.54, 22.13, 20.04. MS (*m/z*): 385 (M^+); Elemental analysis for $C_{20}H_{20}N_3OSCl$ calcd. (found) %: C, 62.25 (62.72); H, 5.22 (5.56); N, 10.89 (10.28).

1-(4-Ethylphenyl)-3-isopropyl-5-(methylthio)-N-phenyl-1H-pyrazole-4-carboxamide (4f): Time: 19 h; yield, 77%; m.p. 114 ºC; IR (KBr, ν_{max}, cm^{-1}): 3301, 3059, 2963, 2928, 2868, 1644, 1465, 1435, 1378, 752. ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 9.50 (s, 1H), 7.69 (d, *J* = 7.6 Hz, 2H), 7.43 (d, *J* = 8.8 Hz, 2H), 7.40-7.32 (m, 4H), 7.13 (t, 1H), 3.87-3.79 (m, 1H), 2.77-2.70 (q, 2H), 2.25 (s, 3H), 1.37 (d, *J* = 7.2 Hz, 6H), 1.28 (t, 3H). ^{13}C NMR (400 MHz, $CDCl_3$, δ ppm): 161.68, 161.29, 145.35, 138.51, 136.83, 133.33, 129.25, 128.59, 126.37, 124.29, 120.05, 117.17, 28.78, 27.55, 22.19, 20.02, 15.68. MS (*m/z*): 379 (M^+); Elemental analysis for $C_{22}H_{25}N_3OS$ calcd. (found) %: C, 69.62 (70.08); H, 6.64 (6.48); N, 11.07 (11.52).

3-Isopropyl-1-(4-methoxyphenyl)-5-(methylthio)-*N*-phenyl-1*H*-pyrazole-4-carboxamide (4g): Time: 21 h; yield, 73 %; m.p. 120 ºC; IR (KBr, ν_{max}, cm^{-1}): 3288, 3142, 2953, 2914, 1648, 1468, 763, 654. ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 9.36 (s, 1H), 7.62-7.57 (m, 2H), 7.43-7.32 (m, 4H), 7.27 (t, 2H), 7.11 (t, 1H), 3.89 (s, 3H), 3.78-3.67 (m, 1H), 2.28 (s, 3H), 1.32 (d, *J* = 6.4 Hz, 6H). ^{13}C NMR (400 MHz, $CDCl_3$, δ ppm): 162.24, 161.37, 142.87, 137.54, 134.88, 134.33, 133.40, 129.29, 127.59, 121.90, 116.03, 115.81, 49.65, 27.58, 22.13, 20.03. MS (*m/z*): 381 (M^+); Elemental analysis for $C_{21}H_{23}N_3O_2S$ calcd. (found) %: C, 66.12 (66.78); H, 6.08 (6.62); N, 11.01 (11.46).

1-(3-Chlorophenyl)-3-isopropyl-5-(methylthio)-*N*-phenyl-1*H*-pyrazole-4-carboxamide (4h): Time: 17 h; yield, 74 %; m.p. 102 ºC; IR (KBr, ν_{max}, cm^{-1}): 3252, 3172, 3035, 2972, 2830, 1653, 1406, 829, 746, 669. ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 9.37 (s, 1H), 7.54 (d, *J* = 7.9 Hz), 7.41-7.31 (m, 4H), 7.21 (d, *J* = 8.4 Hz, 2H), 7.11 (t, 1H), 3.86-3.73 (m, 1H), 2.31 (s, 3H), 1.32 (d, *J* = 6.8 Hz, 6H). ^{13}C NMR (400 MHz, $CDCl_3$, δ ppm): 162.70, 161.36, 140.32, 139.63, 134.30, 131.47, 129.62, 122.69, 121.93, 117.86, 116.04, 115.82, 114.12, 27.59, 22.13, 20.05. MS (*m/z*): 385 (M^+); Elemental analysis for $C_{20}H_{20}N_3OSCl$ calcd. (found) %: C, 62.25 (65.92); H, 5.22 (5.64); N, 10.89 (10.38).

1-(4-(Dimethylamino)phenyl)-3-isopropyl-5-(methylthio)-*N*-phenyl-1*H*-pyrazole-4-carboxamide (4i): Time: 20 h; yield, 67 %; m.p. 128 ºC; IR (KBr, ν_{max}, cm^{-1}): 3304, 3178, 2968, 2920, 1644, 1488, 776. ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 9.31 (s, 1H), 8.16-7.83 (m, 2H), 7.81-7.57 (m, 4H), 7.38 (t, 2H), 7.16 (t, 1H), 3.87-3.70 (m, 1H), 3.29 (s, 6H), 2.39 (s, 3H), 1.37 (d, *J* = 4.8 Hz, 6H). ^{13}C NMR (400 MHz, $CDCl_3$, δ ppm): 162.39, 160.78, 143.59, 138.18, 134.14, 131.45, 129.31, 126.74, 124.59, 120.66, 120.18, 118.88, 48.05, 27.52, 22.15, 21.11. MS (*m/z*): 394 (M^+); Elemental analysis for $C_{22}H_{26}N_4OS$ calcd. (found) %: C, 66.97 (66.48); H, 6.64 (6.92); N, 14.20 (14.58).

3-Isopropyl-5-(methylthio)-*N*-phenyl-1-(4-(trifluoromethyl)phenyl)-1*H*-pyrazole-4-carboxamide (4j): Time: 18 h; yield, 81 %; m.p. 109 ºC; IR (KBr, ν_{max}, cm^{-1}): 3300, 3283, 3267, 2969, 2929, 2872, 1663, 1496, 1247, 780, 755. ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 9.27 (s,1H), 7.71 (d, *J* = 8.4 Hz, 2H), 7.67 (d, *J* = 8.8 Hz, 2H), 7.60 (d, *J* = 7.6 Hz, 2H), 7.30 (t, 2H), 7.07 (t, 1H), 3.79-3.70 (m, 1H), 2.21 (s, 3H), 1.29 (d, *J* = 6.8 Hz, 6H). ^{13}C NMR (400 MHz, $CDCl_3$, δ ppm): 162.43, 160.95, 141.89, 138.29, 133.60, 129.34, 127.23, 126.50, 126.39, 126.36, 124.57, 120.12, 118.59, 27.59, 22.13, 20.19. MS (*m/z*): 419 (M^+); Elemental analysis for $C_{21}H_{20}N_3OSF_3$ calcd. (found) %: C, 60.13 (60.52); H, 4.81 (4.34); N, 10.02 (10.44).

3-Isopropyl-5-(methylthio)-*N*-phenyl-1-(*m*-tolyl)-1*H*-pyrazole-4-carboxamide (4k): Time: 22 h; yield, 69 %; m.p. 98 ºC; IR (KBr, ν_{max}, cm^{-1}): 3265, 3176, 3028, 2970, 2856, 1668, 1473, 763. ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 9.40 (s,1H), 7.60 (d, *J* = 8.0 Hz, 2H), 7.33-7.22 (m, 4H), 7.17 (d, *J* = 8.0 Hz, 2H), 7.04 (t, 1H), 3.80-3.70 (m, 1H), 2.35 (s, 3H), 2.16 (s, 3H), 1.29 (d, *J* = 7.2 Hz, 6H). ^{13}C NMR (400 MHz, $CDCl_3$, δ ppm): 161.70, 161.25, 139.35, 138.47, 133.37, 129.77, 129.23, 128.85, 127.11, 124.29, 123.55, 120.03, 117.23, 27.53, 22.18, 21.54, 20.02. MS (*m/z*): 365 (M^+); Elemental analysis for $C_{21}H_{23}N_3OS$ calcd. (found) %: C, 69.01 (68.74); H, 6.34 (6.68); N, 11.50 (11.16).

1-(3-Bromophenyl)-3-isopropyl-5-(methylthio)-*N*-phenyl-1*H*-pyrazole-4-carboxamide (4l): Time: 19 h; yield, 68 %; m.p. 100 ºC; IR (KBr, ν_{max}, cm^{-1}): 3308, 3238, 3128, 3049, 2970, 2830, 1670, 1484, 763, 669. ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 9.38 (s, 1H), 7.63 (d, *J* = 8.0 Hz, 2H), 7.43-7.35 (m, 4H), 7.19 (d, *J* = 8.2, 2H), 7.07 (t, 1H), 3.88-3.73 (m, 1H), 2.35 (s, 3H), 1.28 (d, *J* = 6.6 Hz, 6H). ^{13}C NMR (400 MHz, $CDCl_3$, δ ppm): 162.48, 161.57, 139.28, 137.71, 133.78, 131.69, 128.62, 126.92, 124.48, 123.12, 120.36, 115.76, 27.59, 22.18, 20.12. MS (*m/z*): 430 (M^+); Elemental analysis for $C_{20}H_{20}N_3OSBr$; C, 55.82 (55.38); H, 4.68 (4.24); N, 9.76 (9.42).

Biological assay: Pyrazole derivatives were screened for their *in vitro* antibacterial and antifungal activities following micro broth dilution method [33-35]. Antibacterial activity was screened aginst Gram-positive [*Bacillus subtillis* (MTCC 441), *Staphylococcus aureus* (MTCC 96)] and Gram-negative [*E. coli* (MTCC 443), *Salmonella typhi* (MTCC 98)] microorganisms. Antifungal activity was screened against *Aspergillus*

niger (MTCC 282) and *Aspergillus clavatus* (MTCC 1323) microorganisms. The standard drugs used for this study were gentamycin, ampicillin, chloramphenicol, ciprofloxacin and norfloxacin, while nystatin and griseofulvin were used for antifungal screening. The standard strains used for screening of antibacterial and antifungal activities were procured from the Culture collection and gene bank (MTCC), Chandigarh, India. Mueller Hinton Broth was used as a nutrient medium for bacteria and Sabouraud dextrose Broth for fungal growth. Inoculums size for test strain was adjusted to 10^8 CFU/mL by comparing the turbidity. The results were recorded in the form of primary and secondary screening. The stock solution (2000 µg/mL) of the compounds under investigation and standard drugs were prepared by successive twofold dilution. In primary screening, 1000, 500 and 250 µg/mL concentrations of the compounds were used. The compounds found to be active in this primary screening were further screened. In secondary screening, 200, 100, 50, 25, 12.5 and 6.25 µg/mL concentrations were used. The inoculated wells were incubated overnight at 37 ºC in a humid atmosphere. The highest dilution showing complete inhibition was considered as a minimum inhibition concentration (MIC).

RESULTS AND DISCUSSION

The present work reports an efficient, mild and green approach for the synthesis of *N*-aryl pyrazoles (**4a-l**). The 2-(*bis*(methylthio)methylene)-4-methyl-3-oxo-N-phenylpentanamide (**1**) synthesized by some modification in previously reported procedure [32]. 5-Isopropyl-3-(methylthio)-*N*-phenyl-1*H*-pyrazole-4-carboxamide (**2**) was obtained by condensation of compound **1** with hydrazine hydrate in water at reflux temperature. The reaction of **2** with various arylboronic acids (**3**) in presence of copper(II) acetate and triethylamine in dichloromethane at room temperature for appropriate time to afford targeted compounds **4a-l** in moderate to excellent yield (67 to 87 %).

Antibacterial activity: All the synthesized compound screened for their antibacterial activity against *Bacillus subtillis* (MTCC 441), *Staphylococcus aureus* (MTCC 96), *Escherichia coli* (MTCC 443) and *Salmonella typhi* (MTCC 98). The MIC value of newly synthesized compounds **4a-l** and standard drugs against microbes are presented in Table-1. It was observed that all the synthesized compounds had comparable antibacterial activity against tested bacteria. Compounds **4c**, **4e** and **4j** were the most active compounds against all of the evaluating bacterial strains. Compound **4j** shows comparable activity against *E. coli* with respect to chloramphenicol (50 µg/mL). Compounds **4c** and **4e** showed comparable inhibitory activity against *E. coli* with respect to ampicillin(100 µg/mL) and compound **4a** (200 µg/mL) moderately active against *E. coli* as compared with ampicillin (100 µg/mL). Compounds **4c**, **4e** and **4j** (100 µg/mL) showed comparative activity against *S. typhi* with respect to ampicillin (100 µg/mL). Compound **4j** (100 µg/mL) showed more activity than ampicillin (250 µg/mL) against *B. subtillis* but mildly active with respect to chloramphenicol and ciprofloxacin while compounds **4c** and **4g** (100 µg/mL) showed comparative activity. Compounds **4d**, **4j** and **4l** (250 µg/mL) showed activity as comparable to ampicillin (250 µg/mL) tested against *S. aureus*. From the activity data, it was observed that halogen substituent especially fluorine group enhanced the antibacterial activity against *E. coli*, *S.typhi* and *B. subtillis* pathogens.

Antifungal activity: Compound **4j** (100 µg/mL) showed comparable activity against *A. niger* with respect to nystatin and griseofulvin (100 µg/mL) while compounds **4e** and **4h** showed mild as compared to standard drugs. Compounds **4e** and **4j** showed moderately activity against *A. clavatus* as compared to standard drugs. Most of the compound found inactive against the tested microbes.

From the activity data, we tried to correlate structure activity relationship (SAR) on the basis of different functional group substituent on aromatic ring (*N*-aryl aromatic ring). In the present paper, it is observed that halogen group at 4th position

TABLE-1
ANTIMICROBIAL ACTIVITY OF SYNTHESIZED COMPOUNDS (**4a-l**)

Compound	R	Antibacterial activity				Antifungal activity	
		E. coli (MTCC 443)	*S. typhi* (MTCC 98)	*B. subtillis* (MTCC 441)	*S. aureus* (MTCC 96)	*A. niger* (MTCC 282)	*A. clavatus* (MTCC 1323)
4a	H	200	250	500	500	500	500
4b	4-CN	250	200	500	500	500	>1000
4c	4-F	100	100	250	500	500	250
4d	4-Br	250	250	500	250	250	500
4e	4-Cl	100	100	500	500	200	200
4f	4-CH_2CH_3	250	250	500	500	500	500
4g	4-OCH_3	250	250	250	500	500	500
4h	3-Cl	250	250	500	500	200	500
4i	3-$N(CH_3)_2$	250	200	500	500	500	> 1000
4j	4-CF_3	50	100	100	250	100	200
4k	3-CH_3	250	250	500	500	> 1000	500
4l	3-Br	250	250	500	250	500	> 1000
Gentamycin	–	≤ 6.25	≤ 6.25	≤ 6.25	≤ 6.25	–	–
Ampicillin	–	100	100	250	250	–	–
Chloramphenicol	–	50	50	50	50	–	–
Ciprofloxacin	–	25	25	50	50	–	–
Norfloxacin	–	≤ 6.25	≤ 6.25	≤ 6.25	≤ 6.25	–	–
Nystatin	–	–	–	–	–	100	100
Griseofulvin	–	–	–	–	–	100	100

on aromatic ring affected antibacterial activity and antifungal activity considerably against selected pathogens and standard drugs.

Conclusion

In summary, we report a mild and green synthetic strategy for the tetrasubstituted pyrazole derivatives (**4a-l**) using Chan-Lam cross coupling reaction. Structure of all the synthesized compounds confirmed by spectroscopic data and elemental analysis. Additionally, all the synthesized compounds were screened for their antimicrobial activity against the selected pathogens and compared with standard drugs. Compound **4e** having 4-fluoro and compound **4j** with 4-trifluro substituent were observed as most active against tested bacterial and fungal strains. Except compounds **4e**, **4h** and **4j** all the compounds found inactive against fungal strains.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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