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Synthesis and Antimicrobial Evaluation of Pyrazole-4-carboxamide Derivatives

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Microbial resistance to the clinically employed antibiotics becomes a threat to the treatment protocols of microbial infectious diseases. Current work aims to synthesize a novel series of pyrazole-4-carboxamide derivatives **(6a-j)** in a multicomponent reaction between 1*H*-pyrazole-4-cabaldehyde and various substituted anilines in the presence of 1,3-*bis*(2,6-diisopropylphenyl)imidazol-2-ylidene (iPr)/TEMPO/phenol under oxidative amidation condition. All the synthesized derivatives **(6a-j)** were examined for their antifungal and antibacterial activities. All compounds (**6a-j**) showed significant antimicrobial potential against Gram-positive bacteria (*B. subtilis* and *S. aureus*), Gram-negative bacteria (*E. coli* and *P. seruginosa*) and fungal strains (*A. niger* and *C. albicans*). Compounds **6a, 6f** and **6g** exhibited better antimicrobial activities.

Keywords: Pyrazoles-4-carboxamides, Multicomponent synthesis, Oxidative amidation, Antimicrobial activity.

INTRODUCTION

Unrestrained rise of drug resistant microbes to the clinically employed antibiotics, pose a perilous threat to the efficacy of the marketed antibiotics for treatment of numerous infectious diseases, globally. Occurrence of drug resistance creates a major concern to discover the novel drugs and targets in the area of chemotherapy. From the clinically established drugs structurally distinct chemical entities designing and developing is an effective approach as they may transfer the novel biomolecular targets for antimicrobial action [1-3].

The contribution of heterocycles is exceptional, concerning with their intrinsic probability as therapeutics agents for the variety of malady from manageable bacterial infections to life threatening diseases. This vital role is attributed to the structural diversity of the heterocycles, which imparts the numerous biological activities for a single molecule. In the current market majority of the drugs have these heterocycles as a core scaffold for drug action [4].

Pyrazole scaffold build a crucial place in pharmaceutical industry as they constitute the fundamental structure for many commercial drugs such as celecoxib, sildenafil and rimonabant (Fig. 1) [5]. Pyrazole derivatives have diverse biological properties including anti-TB activities [6], antimicrobial [7], anti-HIV [8,9], antiproliferative [10], antifungal [11] and anti-inflammatory [12]. Recent literature describes the potential of pyrazole tethered molecular hybrids as antitubercular agents especially quinoline pyrazole derivatives [13,14], azetidine-pyrazoles [15,16], pyrimidine pyrazole derivatives [17], pyrazole derivatives [18], coumarin-pyrazoles [19], pyridine and azole-pyrazoles [20,21]. Thus in view of the importance of pyrazole-based compounds we wish to describe a simple and environmentally benign approach towards the synthesis of pyrazole-4-carboxamides and their antimicrobial activity against the selected fungal and bacterial strains.

EXPERIMENTAL

All chemicals and solvents used were of synthetic grade purchased from Sigma-Aldrich and employed with no further purification. Merck-precoated aluminium TLC plates of silica gel 60 F_{254} were used for the reaction monitoring and the spots were viewed through iodine vapours and in UV chamber. Column chromatography was used for the purification and isolation of the pure compounds. Melting points were determined by Remi electronic melting point apparatus. Using KBr pellet method

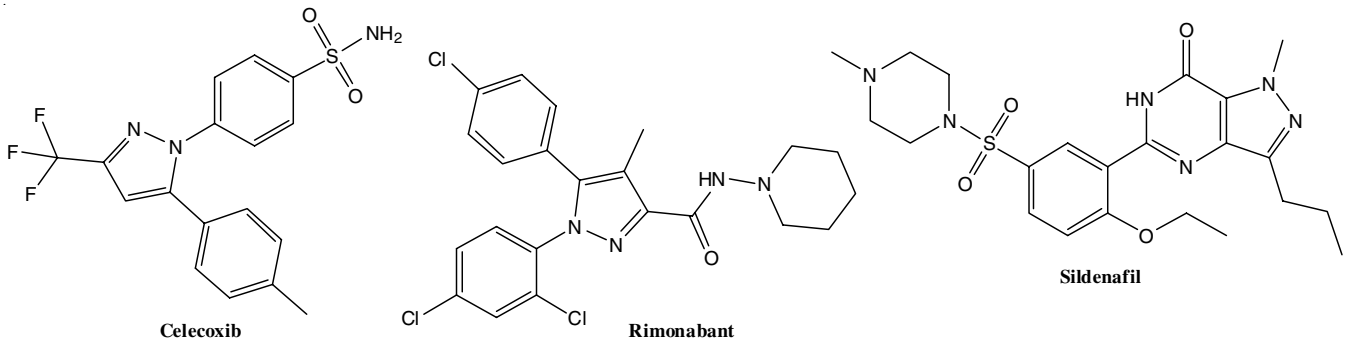


Fig. 1. Structures of important pharmaceuticals containing pyrazole scaffold

on Agilent FTIR recorded IR spectra. BRUKER DRX-500 MHz were used to record ^{1}H NMR. BRUKER ESI-IT MS was employed to record MASS. Bacterial and fungal cultures employed were procured from Department of Microbiology, Osmania University, Hyderabad, India.

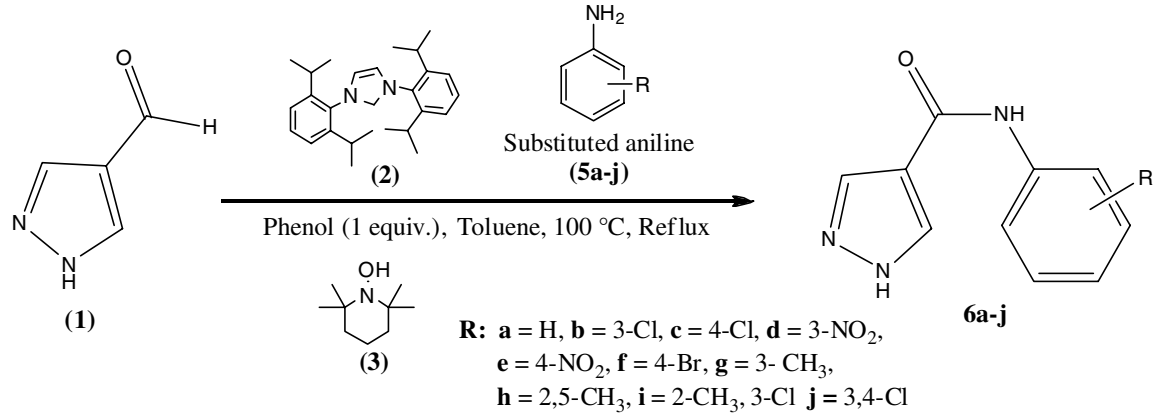
General procedure for the synthesis of pyrazole-4-carboxamides (6a-j): In a 50 mL round bottom flask, 1,3-*bis*-(2,6-diisopropylphenyl)imidazol-2-ylidene (**2**) (iPr, 10 mol%), TEMPO (**3**) (0.5 mmol, 0.156 g, 2 equiv.) were dissolved in toluene (10 mL). In presence of above solution, 1*H*-pyrazole-4-cabaldehyde (**1**) (0.5 mmol, 0.048 g, 1 equiv.) was reacted with phenol (**4**) (0.047 g, 0.5 mmol, 1 equiv.) at 100 ºC for 4 h [22]. Then, various substituted anilines (**5a-j**, 0.5 mmol) were supplemented to the reaction vessel and the reaction mixture was stirred at 40 ºC for 18 h. Reaction progress was monitored by TLC with *n*-hexane/ethyl acetate (2:8) mobile phase system. The reaction mixture was then cooled to room temperature and quenched by the adding up of methanol-water mixture. The crude reaction mass was extracted with three equal portions (3 × 10 mL) of dichloromethane, washed with brine solution and evaporated under vacuum with rotary evaporator. Final products (**6a-j**) were purified *via* column chromatography *n*-hexane/ethyl acetate mobile phase (**Scheme-I**).

***N*-Phenyl-1*H*-pyrazole-4-carboxamide (6a):** Light brown crystals, m.p.: 118-119 °C; yield: 81%. IR (KBr, ν_{max}, cm^{-1}): 1540.3 (C=C), 1290.5 (C-N), 3060.3 (=C–H), 3280.1 (NH), 1632.5 (C=O), 1603.2 (C=N); ^{1}H NMR (500 MHz, $CHCl_3$-d_6): δ 7.09 (tt, *J* = 6.8, 1.2 Hz, 1H), 7.36-7.29 (m, 2H), 7.71-7.65 (m, 2H), 8.06 (dd, *J* = 3.4, 1.8 Hz, 1H), 8.13 (d, *J* = 1.8 Hz, 1H), 10.45 (s, 1H), 11.04 (d, *J* = 3.4 Hz, 1H). ^{13}C NMR (125 MHz, $CHCl_3$-d_6): δ 124.15, 131.87, 132.97, 139.15, 142.67, 146.23, 169.42. ESI-MS: *m/z* Anal. calculated for $C_{10}H_9N_3O$ ([M + H]$^+$): 187.20, observed: 188.15.

***N*-(3-Chlorophenyl)-1*H*-pyrazole-4-carboxamide (6b):** Light brown crystals, m.p.: 123-124 °C; yield: 78%. IR (KBr, ν_{max}, cm^{-1}): 3628.4 (O-H), 1528.3 (C=C), 1288.0 (C-N), 3051.5 (=C–H), 3281.1 (NH), 1642.5 (C=O), 1606.5 (C=N); ^{1}H NMR (500 MHz, $CHCl_3$-d_6): δ 7.17 (ddd, *J* = 7.8, 2.2, 1.2 Hz, 1H), 7.38 (t, *J* = 8.0 Hz, 1H), 7.69 (ddd, *J* = 8.3, 2.2, 1.2 Hz, 1H), 7.96 (t, *J* = 2.2 Hz, 1H), 8.06 (dd, *J* = 3.4, 1.8 Hz, 1H), 8.13 (d, *J* = 1.8 Hz, 1H), 10.40 (s, 1H), 11.04 (d, *J* = 3.4 Hz, 1H); ^{13}C NMR (125 MHz, $CHCl_3$-d_6): δ 124.15, 131.87, 132.97, 139.15, 142.67, 146.23, 169.42. ESI-MS: *m/z* Anal. calculated for $C_{10}H_8ClN_3O$ ([M + H]$^+$): 221.65, observed: 222.50.

***N*-(4-Chlorophenyl)-1*H*-pyrazole-4-carboxamide (6c):** Light brown crystals, m.p.: 128-129 °C; yield: 85%. IR (KBr, ν_{max}, cm^{-1}): 1605.5 (C=N), 1645.5 (C=O), 3285.1 (NH), 3040.5 (=C–H), 1296.0 (C-N), 1525.3 (C=C), 1525.3 (C=C), 2895.4 (C-H); ^{1}H NMR (500 MHz, chloroform-d_6): δ 7.42-7.36 (m, 2H), 7.75-7.69 (m, 2H), 8.06 (dd, *J* = 3.4, 1.8 Hz, 1H), 8.13 (d, *J* = 1.8 Hz, 1H), 10.63 (s, 1H), 11.04 (d, *J* = 3.4 Hz, 1H). ^{13}C NMR (125 MHz, $CHCl_3$-d_6): δ 124.79, 131.88, 133.61, 138.65, 139.18, 142.67, 146.23, 169.42. ESI-MS: *m/z* Anal. calculated for $C_{10}H_8ClN_3O$ ([M + H]$^+$): 221.65, observed: 222.50.

***N*-(3-Nitrophenyl)-1*H*-pyrazole-4-carboxamide (6d):** Light brown crystals, m.p.: 120-121 °C; yield: 73%. IR (KBr, ν_{max}, cm^{-1}): 1512.8 (NO_2), 1090.5 (C-O), 1530.3 (C=C), 1292.0 (C-N), 3050.5 (=C–H), 3282.1 (NH), 1645.5 (C=O), 1603.5



R: **a** = H, **b** = 3-Cl, **c** = 4-Cl, **d** = 3-NO_2, **e** = 4-NO_2, **f** = 4-Br, **g** = 3- CH_3, **h** = 2,5-CH_3, **i** = 2-CH_3, 3-Cl **j** = 3,4-Cl

Scheme-I: Synthesis of pyrazole-4-carboxamide derivatives (**6a-j**)

(C=N); ^{1}H NMR (500 MHz, $CHCl_3$-d_6): δ 7.62 (t, *J* = 8.0 Hz, 1H), 7.83 (ddd, *J* = 8.1, 2.5, 1.0 Hz, 1H), 8.08-8.02 (m, 2H), 8.13 (d, *J* = 1.8 Hz, 1H), 8.61 (t, *J* = 2.3 Hz, 1H), 11.04 (d, *J* = 3.4 Hz, 1H), 11.14 (s, 1H). ^{13}C NMR (125 MHz, $CHCl_3$-d_6): δ 124.76, 131.87, 133.57, 138.64, 139.13, 142.66, 146.22, 169.41. ESI-MS *m/z* Anal. calculated for $C_{10}H_8N_4O_3$ ([M + H]$^+$): 232.20, observed: 233.20.

***N*-(4-Nitrophenyl)-1*H*-pyrazole-4-carboxamide (6e):** Light brown crystals, m.p.: 130-131 °C; yield: 70%. IR (KBr, ν_{max}, cm^{-1}): 1512.8 (NO_2), 1090.5 (C-O), 1535.3 (C=C), 1295.0 (C-N), 3050.5 (=C–H), 3285.1 (NH), 1645.5 (C=O), 1645.5 (C=O). ^{1}H NMR (500 MHz, $CHCl_3$-d_6): δ 8.04-7.98 (m, 2H), 8.06 (dd, *J* = 3.4, 1.8 Hz, 1H), 8.13 (d, *J* = 1.8 Hz, 1H), 8.29-8.23 (m, 2H), 11.04 (d, *J* = 3.4 Hz, 1H), 11.42 (s, 1H). ^{13}C NMR (125 MHz, $CHCl_3$-d_6): δ 124.76, 131.87, 133.57, 138.64, 139.13, 142.66, 146.22, 169.41. ESI-MS: *m/z* Anal. calculated for $C_{10}H_8N_4O_3$ ([M + H]$^+$): 232.20, observed: 233.20.

***N*-(4-Bromophenyl)-1*H*-pyrazole-4-carboxamide (6f):** Light brown crystals, m.p.: 141-142 °C; yield: 81%. IR (KBr, ν_{max}, cm^{-1}): 1530.3 (C=C), 1292.0 (C-N), 3050.5 (=C–H), 3282.1 (NH), 1645.5 (C=O), 1606.5 (C=N). ^{1}H NMR (500 MHz, $CHCl_3$-d_6): δ 7.52-7.46 (m, 2H), 7.67-7.62 (m, 2H), 8.06 (dd, *J* = 3.4, 1.8 Hz, 1H), 8.13 (d, *J* = 1.8 Hz, 1H), 10.78 (s, 1H), 11.04 (d, *J* = 3.4 Hz, 1H). ^{13}C NMR (125 MHz, $CHCl_3$-d_6): δ 126.69, 129.18, 131.94, 136.55, 139.13, 142.66, 146.22, 169.41.ESI-MS: *m/z* Anal. calculated for $C_{10}H_8BrN_3O$ ([M + H]$^+$): 266.10, observed: 267.05.

***N*-(*m*-Tolyl)-1*H*-pyrazole-4-carboxamide (6g):** Light brown crystals, m.p.: 113-114 °C; yield: 68%. IR (KBr, ν_{max}, cm^{-1}): 1530.3 (C=C), 1293.0 (C-N), 3050.5 (=C–H), 3283.1 (NH), 1646.5 (C=O), 1606.5 (C=N); ^{1}H NMR (500 MHz, $CHCl_3$-d_6): δ 2.23 (s, 3H), 6.94 (ddd, *J* = 7.2, 2.6, 1.5 Hz, 1H), 7.18 (t, *J* = 7.6 Hz, 1H), 7.48-7.42 (m, 1H), 7.53 (t, *J* = 2.2 Hz, 1H), 8.06 (dd, *J* = 3.4, 1.8 Hz, 1H), 8.13 (d, *J* = 1.8 Hz, 1H), 10.52 (s, 1H), 11.04 (d, *J* = 3.4 Hz, 1H). ^{13}C NMR (125 MHz, $CHCl_3$-d_6): δ 22.38, 122.88, 124.33, 132.08, 132.97, 133.98, 138.40, 143.93, 146.49, 169.69. ESI-MS: *m/z* Anal. Calculated for $C_{11}H_{11}N_3O$ ([M + H]$^+$): 201.23, observed 202.20.

***N*-(2,6-Dimethylphenyl)-1*H*-pyrazole-4-carboxamide (6h):** Light brown crystals, m.p.: 107-108 °C; yield: 63%. IR (KBr, ν_{max}, cm^{-1}): 1300.6 (C-F), 1285.5 (C-N), 3053.5 (=C–H), 3285.5 (NH), 1645.5 (C=O), 1606.5 (C=N); ^{1}H NMR (500 MHz, $CHCl_3$-d_6): δ 2.19 (s, 6H), 7.09 (s, 3H), 8.06 (dd, *J* = 3.4, 1.8 Hz, 1H), 8.13 (d, *J* = 1.8 Hz, 1H), 10.34 (s, 1H), 11.04 (d, *J* = 3.4 Hz, 1H). ^{13}C NMR (125 MHz, $CHCl_3$-d_6): δ 18.67, 131.85, 132.67, 133.77, 138.16, 146.18, 169.37.ESI-MS: *m/z* Anal. calculated for $C_{12}H_{13}N_3O$ ([M + H]$^+$): 213.25, observed: 214.20.

***N*-(3-Chloro-2-methylphenyl)-1*H*-pyrazole-4-carboxamide (6i):** Light brown crystals, m.p.: 146-147 °C; yield: 72%. IR (KBr, ν_{max}, cm^{-1}): 670.5 (C-Cl), 1285.5 (C-N), 3050.5 (=C–H), 3285.5 (NH), 1645.5 (C=O), 1606.5 (C=N); ^{1}H NMR (500 MHz, $CHCl_3$-d_6): δ 2.24 (s, 3H), 7.18 (t, *J* = 8.0 Hz, 1H), 7.31 (dd, *J* = 7.9, 1.2 Hz, 1H), 7.48 (dd, *J* = 8.2, 1.2 Hz, 1H), 8.06 (dd, *J* = 3.4, 1.8 Hz, 1H), 8.13 (d, *J* = 1.8 Hz, 1H), 10.60 (s, 1H), 11.04 (d, *J* = 3.4 Hz, 1H). ^{13}C NMR (125 MHz, $CHCl_3$-d_6): δ 15.45, 121.54, 131.96, 133.50, 134.70, 139.27, 140.07, 145.73, 146.44, 169.63. ESI-MS: *m/z* Anal. calculated for $C_{11}H_{10}ClN_3O$ ([M + H]$^+$): 235.67, found 236.60.

***N*-(3,4-Dichlorophenyl)-1*H*-pyrazole-4-carboxamide (6j):** Light brown crystals, m.p.: 159-160 °C; yield: 69%. IR (KBr, ν_{max}, cm^{-1}): 1285.5 (C-N), 3050.5 (=C–H), 3285.5 (NH), 1645.5 (C=O), 1606.5 (C=N); ^{1}H NMR (500 MHz, $CHCl_3$-d_6): δ 7.41 (d, *J* = 8.0 Hz, 1H), 7.58 (dd, *J* = 8.0, 2.3 Hz, 1H), 8.00 (d, *J* = 2.2 Hz, 1H), 8.06 (dd, *J* = 3.4, 1.8 Hz, 1H), 8.13 (d, *J* = 1.8 Hz, 1H), 10.63 (s, 1H), 11.04 (d, *J* = 3.4 Hz, 1H). ^{13}C NMR (125 MHz, $CHCl_3$-d_6): δ 124.76, 126.06, 131.92, 134.59, 135.40, 139.28, 143.63, 146.63, 169.56. ESI-MS: *m/z* Anal. calcd. for $C_{10}H_7Cl_2N_3O$ ([M + H]$^+$): 256.09, observed: 257.05.

Antibacterial activity: Antibacterial activity was screened for the titled compounds (**6a-j**) at the doses of 30 and 60 μg/mL by agar plate method by using standard protocols [23]. Various Gram-negative bacteria (*P. aeruginosa* and *E. coli*) and Gram-positive (*S. aureus* and *B. subtilis*) were selected for the antibacterial activity screening. Standard DMSO and ampicillin were used as negative and positive control, respectively. To evaluate the antibacterial activity of the test compounds, the zone of inhibition measured in mm and the experiment was carried out in triplicates. In distilled water, sodium chloride, meat extract and peptone were dissolved and adjusted the pH of medium to 7.2. Into 100 mL flask, agar was dissolved and distributed in 40 mL quantities and by using autoclave sterilized all the contents at 121 ºC (15 lbs/sq.in) for 20 min. Above-mentioned 18 h of old cultures of test organism were inoculated in to the medium at 1% level with and transferred into sterile 15 cm diameter petri plates. The plates were set aside at room temperature for 30 min to allow the setting of medium. With the help of a sterile borer 6 mm diameter holes were made at the corner of the plate at equal distance for the preparation of cup agar plates. Sterile pipettes were employed to place the solution of test compounds in the cups. One cup was used as control in each plate with ampicillin in 10 μg/mL concentration 2 drops (0.05 mL) of DMSO was used as standard. The plates were incubated for 1 h at room temperature. Subsequently, the plates were incubated at 37 ºC for 24 h and recorded zone of inhibition. The experiments were carried out in duplicate and the average diameter of the zones of inhibition was recorded and noted.

Antifungal activity: The synthesized compounds were also tested for their antifungal activity at 60 and 30 μg/mL doses by agar plate method using the above protocols. The experimentation was carried out in triplicates and the derivatives were tested for their antifungal activity on *A. niger* and *C. albicans*. Standard DMSO and Nystatin were supplied as negative and positive controls, respectively. Antifungal activity was evaluated by measuring the zone of inhibition in mm. small chips of peeled potatoes were boiled with 200 mL of water for 30 min. During boiling the chips were crushed and pulp was removed after cooling by filtration through muslin cloth. Agar and dextrose were added slowly by stirring and the volume was made up to 1000 mL. DMSO and nystatin in 10 μg/mL concentration was taken as standard and control correspondingly. All the plates were incubated at room temperature (30 ºC) for 48 h after that the plates were inspected and the diameter of the zones were noted.

RESULTS AND DISCUSSION

The synthesis of pyrazole-4-carboxamide derivatives (**6a-j**) were carried out *via* one pot oxidative amidation as reported in the literature by using 1,3-*bis*(2,6-diisopropylphenyl) imidazol-2-ylidene/TEMPO/phenol and then reaction with aniline [22]. In first step, the reaction of pyrazole-4-carboxaldehyde (**1**) with 1,3-*bis*(2,6-diisopropylphenyl)imidazol-2-ylidene/TEMPO/phenol under oxidative esterification to give the pyrazole-4-carboxylate phenol ester intermediate which was not isolated and immediately reaction with various substituted anilines (**5a-j**) to effort the final products (**6a-j**) in moderate to good yields. The synthesized compounds were confirmed by using ^{1}H NMR, IR and mass spectral analysis. The ^{1}H NMR spectra shows the characteristic C-H peak of pyrazole ring was observed as a singlet around 7.6 to 7.85 ppm and the N-H peak of the amide group was observed as a singlet in all the compounds around 10-10.5 ppm.

The IR spectra of pyrazole-4-carboxamide derivatives (**6a-j**) shows the following characteristic peaks. The peak between 1608.5-1063.5 cm^{-1} corresponds to (C=N), the peaks between 1645.5-1638.5 cm^{-1} related to (C=O), the peak at 3280.1 cm^{-1} is due to (NH), the peak between 3060.3-3058.5 cm^{-1} is due to (=C–H), the peak at 1290.5 cm^{-1} is due to (C-N), the peak at 1540.3 cm^{-1} represent the (C=C), the peak around 3630.4 cm^{-1} is due to (O-H), the peak at 670.5 cm^{-1} is due to (C-X) and the peak at 1512.8 cm^{-1} represent the (NO_2) group.

Antimicrobial activity

Antibacterial activity: The antibacterial assay of the pyrazole-4-carboxamide derivatives (**6a-j**) was carried out against two Gram-negative bacteria namely, *P. aaeruginosa*, *E. coli* and two Gram-positive bacteria namely *S. aureus* and *B. subtilis* by employing agar plate method. The results are depicted in Table-1. The pyrazole-4-carboxamide derivatives (**6a-j**) display considerable antibacterial activity. Interestingly, it is observed that pyrazole-4-carboxamide derivatives (**6a-j**) were more active against the Gram-positive strains with compare to the Gram-negative strains. Among tested the compounds, **6a**, **6f** and **6g** were found active against all the Gram-negative and Gram-positive strains. Furthermore, it was observed that pyrazole-4-carboxamide derivatives (**6a-j**) displayed a dose dependent inhibition of bacterial growth in the investigation. The results shows that the unsubstituted phenyl ring (**6a**) and bromine substituted phenyl ring (**6f**) compounds possess the better antibacterial activity with compare to others.

Antifungal activity: Pyrazole-4-carboxamide derivatives (**6a-j**) were also screened for their potency to inhibit the growth of fungi *Candida albicans* and *Aspergillus niger*. The activity results are enumerated in Table-1. The antifungal activity results revealed that compounds **6a**, **6f** and **6g** showed a significant antifungal activity with compare to other tested compounds. It is also observed that *A. niger* is more sensitive to pyrazole-4-carboxamide derivatives (**6a-j**) as compared to *Candida albicans*. Compound **6g** was found to be most potent antifungal agent among all the derivatives and showing the maximum growth inhibition with 23 mm of average zone of inhibition against *Candida albicans* followed bycompounds **6f** and **6a**.

Conclusion

A series of pyrazole-4-carboxamide derivatives (**6a-j**) were synthesized through a suitable multicomponent one pot synthetic method using commercially available starting materials. These synthesized compounds were screened for the antibacterial and antifungal properties. The results revealed that all the compounds demonstrated moderate to good antibacterial and antifungal properties. Compounds **6a**, **6f** and **6g** displayed potent antimicrobial action than the remaining compounds. Further studies in molecular level are needed to clearly understand the detailed mechanism of the antimicrobial action of the synthesized derivatives. Establishment of the molecular level mechanism may serve as tool to develop these classes of compounds into a lead molecule that can be developed into potent antimicrobial agents to annihilate the pathogenic ailments caused by the fungi and bacteria.

TABLE-1
ANTIMICROBIAL ACTIVITY OF THE PYRAZOLE-4-CARBOXAMIDE DERIVATIVES (**6a-j**)

Compd.	Zone of inhibition (mm)											
	Gram-positive bacteria				Gram-negative bacteria				Fungal strains			
	B. subtilis		*S. aureus*		*E. coli*		*P. aeruginosa*		*A. niger*		*C. albicans*	
	30 µg/mL	60 µg/mL	30 µg/mL	60 µg/mL	30 µg/mL	60 µg/mL	30 µg/mL	60 µg/mL	30 µg/mL	60 µg/mL	30 µg/mL	60 µg/mL
6a	18	28	17	25	19	23	14	20	13	19	15	18
6b	12	14	10	13	12	15	10	13	09	13	11	14
6c	11	15	11	15	11	14	10	15	10	14	09	13
6d	12	15	10	15	10	14	09	15	11	13	08	12
6e	10	13	09	12	10	16	07	12	09	12	09	13
6f	17	24	19	27	13	18	12	16	15	20	16	22
6g	15	21	17	23	12	15	10	16	17	21	17	23
6h	11	13	11	14	11	16	11	14	12	17	10	15
6i	10	14	11	13	12	16	13	13	10	14	11	14
6j	11	14	10	15	10	14	11	15	08	11	10	13
DMSO	3		2		3		2		3		2	
Ampicillin (10 µg/mL)	32		34		30		31		–		–	
Nystatin (10 µg/mL)	–		–		–		–		28		25	

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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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