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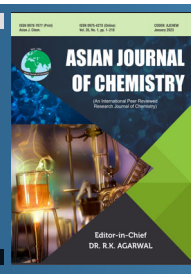

Synthesis, Antimicrobial and Antitubercular Activity of Novel Imidazole Carboxamides

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A series of novel imidazole carboxamide derivatives (**6a-j**) were synthesized in moderate to good yields *via* the oxidative esterification of 1*H*-imidazole-4-cabaldehyde (**1**) with phenol (**4**) followed by the aminolysis of various substituted anilines (**5a-j**) in the presence of 1,3-*bis*(2,6-diisopropylphenyl)imidazol-2-ylidene (**2**) (IPr, 10 mol%) and TEMPO (**3**) in toluene. All the compounds were characterized by IR, ^{1}H NMR, ^{13}C NMR and mass spectral data. The synthesized compounds were assessed *in vitro* for their antimicrobial and antitubercular potential. Among the synthesized compounds **6a**, **6f** and **6g** had significant antibacterial and antifungal activity. Synthesized N-phenyl-1*H*-imidazole-4-carboxamide (**6a**) possessed a broad activity spectrum towards *S. aureus*, *B. subtilis*, *P. aeruginosa*, *E. coli*, *A. niger* and *C. albicans* strains employed in the study. The synthesized compounds N-(4-nitrophenyl)-1*H*-imidazole-4-carboxamide (**6e**) and N-(3,4-dichlorophenyl)-1*H*-imidazole-4-carboxamide (**6j**) demonstrated the maximum antitubercular activity.

Keywords: Imidazole carboxamide derivatives, Antibacterial activity, Antifungal activity, Antitubercular activity.

INTRODUCTION

One of the primary factors in the mortality of patients with infectious diseases is the ineffectiveness of antibiotic therapy. For instance, infections spurred on by antimicrobial-resistant microorganisms result in approximately 50,000 deaths annually in Europe and the United States alone, as well as hundreds of thousands of deaths in other nations [1]. As a result of their resistance to the majority of current antimicrobial medications, microorganisms including vancomycin-resistant enterococci (VRE), methicillin-resistant *Staphylococcus aureus* (MRSA) and ESBL (extended spectrum β-lactamase) producing *Enterobacteriaceae* are particularly dangerous nowadays. The level of resistance to third-generation fluoroquinolones and cephalosporins is noteworthy and varies between 48-89% and 68-95%, respectively, depending on the region, according to a study that assessed the sensitivity of *Escherichia coli* to antibiotics [2,3].

Mycoses, driven by micromycetes, dermatomycetes and yeasts, have been rising in recent years. There are also strains of these infections that are resistant to modern antifungal medications [4]. As a result, ketoconazole and fluconazole have no effect on 82.2% and 76.9% of *Candida albicans* strains, respectively [5]. Additionally, fluconazole-resistant strains of *Cryptococcus neoformans* are being isolated more frequently [6]. The weakening of human immunity as a result of harmful environmental variables, unrestrained use of antimicrobial medications and usage of cytostatics, to mention a few, all contribute to the rise in infectious and infectious-inflammatory ailments [7]. The development of antibiotic-resistant pathogens and the rapid spread of multidrug-resistant strains both reduce the effectiveness of antibiotics, demonstrating the continual need for the identification of novel active substances and the development of highly potent and safer antimicrobial medications [8-10].

Tuberculosis (TB) is the ninth leading cause of death globally, caused by a single infectious agent, ranking ahead of HIV/AIDS. *Mycobacterium tuberculosis* (Mtb), which infects the lung and can infect other parts of the body, is the source of this lethal airborne disease. According to the WHO 2019 global tuberculosis report, TB is the main cause of death owing to antimicrobial resistance and among people who have HIV. National authorities reported more than 500,000 new TB cases to the WHO. Thus, the development of novel therapeutic agents to treat tuberculosis is currently in critical demand [11].

Imidazole, a five-membered aromatic heterocycle with two nitrogen atoms, is a structural component of numerous

biologically significant compounds and intermediates, such as histidine, numerous cofactors, purines and synthetic bioactive substances [12]. The imidazole ring may easily interact with the biopolymers of biological systems due to its highly polar and amphoteric nature, ability to readily donate or accept protons, abundance of binding sites and ability to readily form various weak interactions. The distinctive bioactivity patterns of imidazole-based small compounds, which range from anti-leishmanial, antiviral, anticancer, antidepressant, anti-HIV, antitubercular, analgesic, anti-inflammatory, antibacterial and antifungal, antioxidant and neuro-protective to chemotherapeutic properties, have been regarded as prospective pharmacological agents based on multiple literature surveys [13-17].

Due to not only their beneficial characteristics, such as good tissue permeability and penetrability and great bioavailability, but also a comparatively low incidence of toxic and adverse effects, imidazoles and in particular their salts offer a boundless and emerging sector [18]. Numerous imidazole-based compounds (Fig. 1) have so far been successfully developed and used as clinical drugs. Furthermore, it has been acknowledged [19] that many imidazole-containing ligands may display significant chemical-shift modifications when attached to a molecular target, such as protein, providing important information regarding alterations in the local structure of the target or ligand. Various β-lactamase, hemeoxygenase and carboxypeptidase inhibitors, as well as substances with antiviral, antitubercular, antifungal, antibacterial, anticancer and anti-inflammatory activity, have imidazole core structures [20,21].

EXPERIMENTAL

All the chemicals and reagents utilized in the study were provided by S.D. Fine Chemicals (India) and E. Merck. The open tube capillary method was used to determine the melting points and the results are uncorrected. The purity of the compounds was examined using appropriate solvent solutions on thin-layer chromatography (TLC) plates (silica gel G), with the spots being detected using UV light and iodine vapours. FT-IR spectrometer model Perkin-Elmer 1720 was used to obtain IR spectra (KBr pellets). Mass spectra under electron impact conditions (EI) were acquired at 70 ev ionizing voltage with a VG Prospec instrument. ^{1}H NMR spectra were obtained using TMS as an internal standard in DMSO-d_6/$CDCl_3$.

Synthesis of imidazole carboxamides: In 50 mL round-bottomed flask, 10 mL of toluene, 1,3-*bis*(2,6-diisopropylphenyl)-imidazol-2-ylidene (**2**) (IPr, 10 mol%) and TEMPO (**3**) (0.5 mmol, 0.156 grams, 2 equiv.) were dissolved. 1*H*-imidazole-4-carbaldehyde (**1**) (0.5 mmol, 0.048 g, 1 equiv.) was treated with phenol (**4**) (0.047 g, 0.5 mmol, 1 equiv.) for 4 h at 100 ºC in the presence of the above solution [20]. Then, to a reaction vessel, various substituted anilines (**5a-j**) (0.5 mmol) were added and the reaction mixture was agitated for 18 h at 40 ºC. Thin-layer chromatography was used to monitor the progress of the reaction using *n*-hexane/ethyl acetate (2:8) mobile phase system. After reaching room temperature, a methanol-water mixture was added to the reaction mixture in order to quench it. A rotary evaporator was used to evaporate the crude reaction mass under vacuum after it had been extracted using three equal portions (3 × 10 mL) of dichloromethane. The final product was purified using column chromatography with a mobile phase of *n*-hexane and ethyl acetate (**Scheme-I**).

***N*-Phenyl-1*H*-imidazole-4-carboxamide (6a):** Pale yellow solid, yield: 74.5%; m.p.: 138-139 ºC; IR (KBr, ν_{max}, cm^{-1}): 1620 (C=N), 1690 (C=O), 3352 (NH), 1310 (C-N), 1495 (C=C); ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.61 (dd, *J* = 6.0,

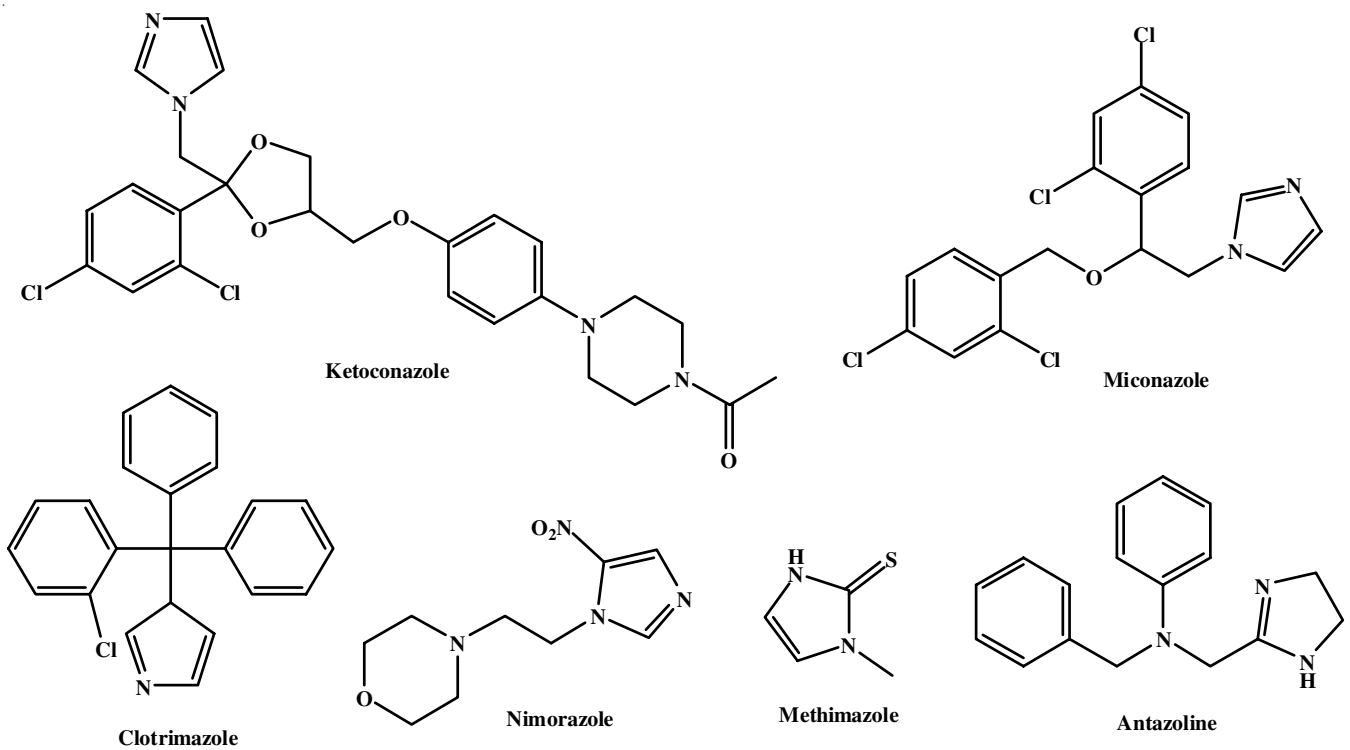
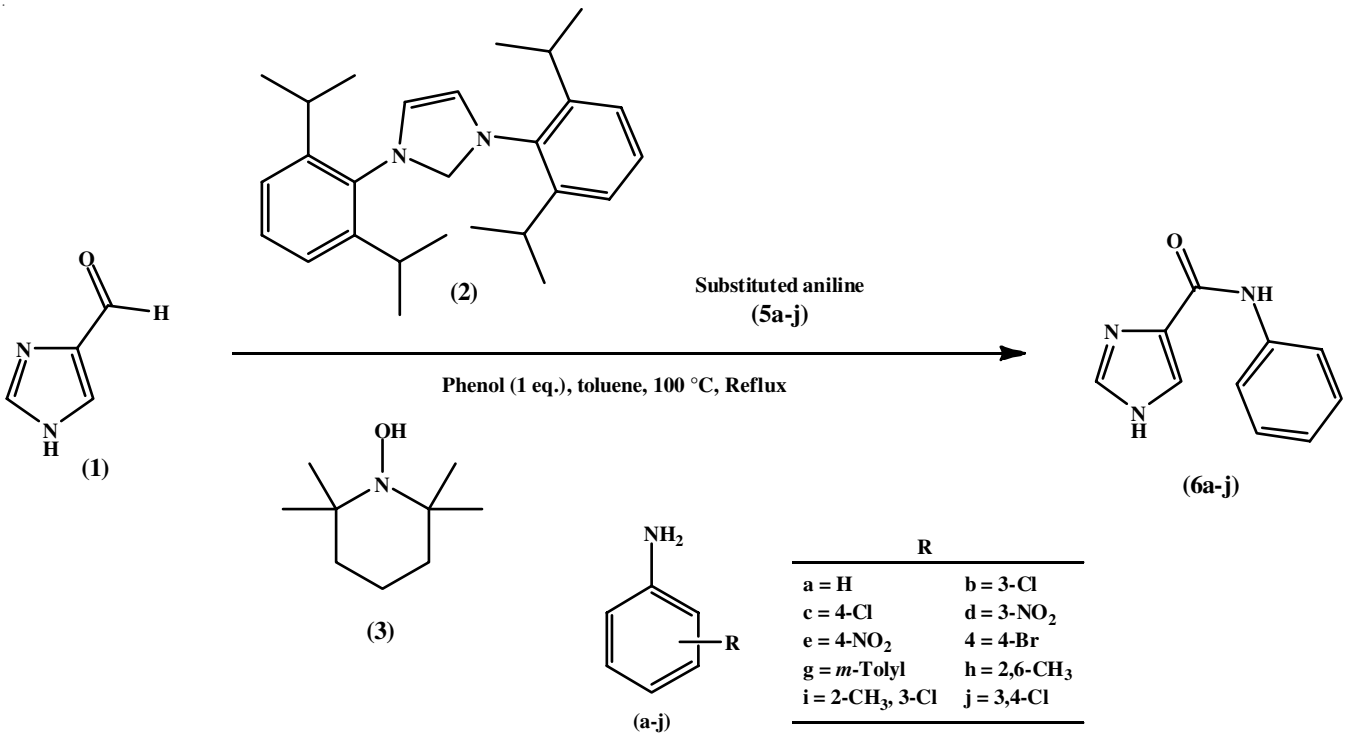


Fig. 1. Structures of some pharmaceuticals bearing imidazole skeleton



Scheme-I: Synthesis of imidazole-4-carboxamides

4.9 Hz, 1H), 10.29 (s, 1H), 8.14 (dd, *J* = 6.0, 1.8 Hz, 1H), 7.89 (dd, *J* = 5.1, 1.7 Hz, 1H), 7.72-7.67 (m, 2H), 7.36-7.29 (m, 2H), 7.09 (tt, *J* = 6.8, 1.2 Hz, 1H); ^{13}C NMR δ 117.8 (1C, s), 119.9 (2C, s), 127.8 (1C, s), 128.2 (2C, s), 133.7 (1C, s), 135.3 (1C, s), 137.4 (1C, s), 159.9 (1C, s). ESI-MS: *m/z* Anal. calcd. for $C_{10}H_9N_3O$ ([M + H]$^+$): 187.20, found 188.05.

***N*-(3-Chlorophenyl)-1*H*-imidazole-4-carboxamide (6b):** Pale yellow solid, yield: 71.7%; m.p.: 156-158 ºC; IR (KBr, ν_{max}, cm^{-1}): 1638 (C=N), 1697 (C=O), 3381 (NH), 1242 (C-N), 1471 (C=C); ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.61 (dd, *J* = 6.0, 4.9 Hz, 1H), 10.26 (s, 1H), 8.14 (dd, *J* = 6.0, 1.8 Hz, 1H), 7.89 (dd, *J* = 5.1, 1.7 Hz, 1H), 7.84 (t, *J* = 2.1 Hz, 1H), 7.55 (ddd, *J* = 8.0, 2.2, 1.2 Hz, 1H), 7.38 (t, *J* = 8.0 Hz, 1H), 7.17 (ddd, *J* = 7.8, 2.2, 1.2 Hz, 1H); ^{13}C NMR δ 117.8 (1C, s), 119.9 (1C, s), 120.2 (1C, s), 127.0 (1C, s), 130.0 (1C, s), 132.3 (1C, s), 133.7 (1C, s), 135.3 (1C, s), 138.2 (1C, s), 159.9 (1C, s). ESI-MS: *m/z* Anal. calcd. for $C_{10}H_8ClN_3O$ ([M + H]$^+$): 221.64, found 222.45.

***N*-(4-Chlorophenyl)-1*H*-imidazole-4-carboxamide (6c):** Pale yellow solid, yield: 78.2%; m.p.: 180-181 ºC; IR (KBr, ν_{max}, cm^{-1}): 1618 (C=N), 1702 (C=O), 3367 (NH), 1243 (C-N), 1486 (C=C); ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.61 (dd, *J* = 6.0, 4.9 Hz, 1H), 10.40 (s, 1H), 8.14 (dd, *J* = 6.0, 1.8 Hz, 1H), 7.89 (dd, *J* = 5.1, 1.7 Hz, 1H), 7.65-7.59 (m, 2H), 7.42-7.36 (m, 2H); ^{13}C NMR δ 117.8 (1C, s), 120.5 (2C, s), 128.9 (2C, s), 133.6-133.8 (2C, 133.7 (s), 133.7 (s), 135.3 (1C, s), 137.4 (1C, s), 159.9 (1C, s). ESI-MS: *m/z* Anal. calcd. for $C_{10}H_8ClN_3O$ ([M + H]$^+$): 221.64, found.

***N*-(3-Nitrophenyl)-1*H*-imidazole-4-carboxamide (6d):** Pale yellow solid, yield: 67.1%; m.p.: 166-167 ºC; IR (KBr, ν_{max}, cm^{-1}): 1600 (C=N), 1699 (C=O), 3366 (NH), 1242 (C-N), 1485 (C=C); ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.94 (s, 1H), 10.61 (dd, *J* = 6.0, 4.9 Hz, 1H), 8.61 (t, *J* = 2.3 Hz, 1H), 8.14 (dd, *J* = 6.0, 1.8 Hz, 1H), 8.08-8.02 (m, 1H), 7.89 (dd, *J* = 5.1, 1.7 Hz, 1H), 7.81 (ddd, *J* = 8.0, 2.5, 1.0 Hz, 1H), 7.62 (t, *J* = 8.0 Hz, 1H); ^{13}C NMR δ 112.0 (1C, s), 117.8 (1C, s), 119.9 (1C, s), 123.3 (1C, s), 129.6 (1C, s), 133.7 (1C, s), 135.3 (1C, s), 137.5 (1C, s), 143.9 (1C, s), 159.9 (1C, s). ESI-MS: *m/z* Anal. calcd. for $C_{10}H_8N_4O_3$ ([M + H]$^+$): 232.20, found.

***N*-(4-Nitrophenyl)-1*H*-imidazole-4-carboxamide (6e):** Pale yellow solid, yield: 64.4%; m.p.: 161-163 ºC; IR (KBr, ν_{max}, cm^{-1}): 1622 (C=N), 1662 (C=O), 3066 (NH), 1276 (C-N), 1458 (C=C); ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.01 (s, 1H), 10.61 (dd, *J* = 6.0, 4.9 Hz, 1H), 8.29-8.23 (m, 2H), 8.14 (dd, *J* = 6.0, 1.8 Hz, 1H), 7.92-7.85 (m, 3H); ^{13}C NMR δ 116.6 (2C, s), 117.8 (1C, s), 125.0 (2C, s), 133.7 (1C, s), 135.3 (1C, s), 137.4 (1C, s), 147.3 (1C, s), 159.9 (1C, s). ESI-MS: *m/z* Anal. calcd. for $C_{10}H_8N_4O_3$ ([M + H] +): 232.06, found.

***N*-(4-Bromophenyl)-1*H*-imidazole-4-carboxamide (6f):** Pale yellow solid, yield: 74.5%; m.p.: 189-190 ºC; IR (KBr, ν_{max}, cm^{-1}): 1623 (C=N), 1669 (C=O), 3083 (NH), 1306 (C-N), 1458 (C=C); ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.61 (dd, *J* = 6.0, 4.9 Hz, 1H), 10.53 (s, 1H), 8.14 (dd, *J* = 6.0, 1.8 Hz, 1H), 7.89 (dd, *J* = 5.1, 1.7 Hz, 1H), 7.68-7.62 (m, 2H), 7.52-7.46 (m, 2H); ^{13}C NMR δ 117.8 (1C, s), 121.9 (2C, s), 122.3 (1C, s), 131.7 (2C, s), 133.7 (1C, s), 135.3 (1C, s), 137.4 (1C, s), 159.9 (1C, s). ESI-MS: *m/z* Anal. calcd. for $C_{10}H_8BrN_3O$ ([M + H]$^+$): 266.09, found.

***N*-(*m*-Tolyl)-1*H*-imidazole-4-carboxamide (6g):** Pale yellow solid, yield: 62.5%; m.p.: 133-134 ºC; IR (KBr, ν_{max}, cm^{-1}): 1633 (C=N), 1681 (C=O), 3311 (NH), 1313 (C-N), 1537 (C=C); ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.61 (dd, *J* = 6.0, 4.9 Hz, 1H), 10.27 (s, 1H), 8.14 (dd, *J* = 6.0, 1.8 Hz, 1H), 7.89 (dd, *J* = 5.1, 1.7 Hz, 1H), 7.46-7.38 (m, 2H), 7.18 (t, *J* = 7.7

Hz, 1H), 6.97-6.91 (m, 1H), 2.29 (s, 3H); ^{13}C NMR δ 21.3 (1C, s), 117.8 (1C, s), 118.6 (1C, s), 119.9 (1C, s), 128.1 (1C, s), 129.0 (1C, s), 133.6-133.8 (2C, 133.7 (s), 133.7 (s)), 135.3 (1C, s), 138.4 (1C, s), 159.9 (1C, s). ESI-MS: *m/z* Anal. calcd. for $C_{11}H_{11}N_3O$ ([M + H]$^+$): 201.22, found.

***N*-(2,6-Dimethylphenyl)-1*H*-imidazole-4-carboxamide (6h):** Pale yellow solid, yield: 57.9%; m.p.: 141-142 ºC; IR (KBr, ν_{max}, cm^{-1}): 1632 (C=N), 1681 (C=O), 3310 (NH), 1313 (C-N), 1537 (C=C); ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.61 (dd, *J* = 6.0, 4.9 Hz, 1H), 9.33 (s, 1H), 8.14 (dd, *J* = 6.0, 1.8 Hz, 1H), 7.89 (dd, *J* = 5.1, 1.7 Hz, 1H), 7.09 (s, 3H), 2.19 (s, 6h); ^{13}C NMR δ 17.7 (2C, s), 117.8 (1C, s), 128.0 (1C, s), 129.0 (2C, s), 129.2 (2C, s), 133.7 (1C, s), 134.1 (1C, s), 135.3 (1C, s), 159.9 (1C, s). ESI-MS: *m/z* Anal. calcd. for $C_{12}H_{13}N_3O$ ([M + H]$^+$): 215.25, found.

***N*-(3-Chloro-2-methylphenyl)-1*H*-imidazole-4-carboxamide (6i):** Pale yellow solid, yield: 66.2%; m.p.: 176-178 ºC; IR (KBr, ν_{max}, cm^{-1}): 1629 (C=N), 1690 (C=O), 3197 (NH), 1301 (C-N), 1559 (C=C); ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.61 (dd, *J* = 6.0, 4.9 Hz, 1H), 10.00 (s, 1H), 8.14 (dd, *J* = 6.0, 1.8 Hz, 1H), 7.89 (dd, *J* = 5.1, 1.7 Hz, 1H), 7.38 (dd, *J* = 8.2, 1.2 Hz, 1H), 7.31 (dd, *J* = 8.0, 1.3 Hz, 1H), 7.18 (t, *J* = 8.0 Hz, 1H), 2.24 (s, 3H); ^{13}C NMR δ 14.6 (1C, s), 117.3 (1C, s), 117.8 (1C, s), 128.7 (1C, s), 129.8 (1C, s), 130.0 (1C, s), 133.7 (1C, s), 134.8 (1C, s), 135.3 (1C, s), 140.2 (1C, s), 159.9 (1C, s). ESI-MS: *m/z* Anal. calcd. for $C_{11}H_{10}ClN_3O$ ([M + H]$^+$): 235.67, found.

***N*-(3,4-Dichlorophenyl)-1*H*-imidazole-4-carboxamide (6j):** Pale yellow solid, yield: 63.4%; m.p.: 184-185 ºC; IR (KBr, ν_{max}, cm^{-1}): 1628 (C=N), 1639 (C=O), 3196 (NH), 1300 (C-N), 1557 (C=C); ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.61 (dd, *J* = 6.0, 4.9 Hz, 1H), 10.51 (s, 1H), 8.14 (dd, *J* = 6.0, 1.8 Hz, 1H), 7.95-7.87 (m, 2H), 7.55 (dd, *J* = 7.9, 2.2 Hz, 1H), 7.41 (d, *J* = 8.0 Hz, 1H); ^{13}C NMR δ 117.8 (1C, s), 120.5 (1C, s), 121.7 (1C, s), 129.8 (1C, s), 130.5 (1C, s), 130.9 (1C, s), 133.7 (1C, s), 135.3 (1C, s), 138.2 (1C, s), 159.9 (1C, s). ESI-MS: *m/z* Anal. calcd. for $C_{10}H_7Cl_2N_3O$ ([M + H]$^+$): 256.09, found.

Antimicrobial activity: The titled compounds (**6a-j**) were examined for antimicrobial activity using the agar plate method at doses of 30 µg/mL and 60 µg/mL. In this work, Gram-positive (*Bacillus subtilis* and *Staphylococcus aureus*), Gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa*) bacterial and fungal (*Aspergillus niger* and *Candida albicans*) strains were chosen. The bacterial and fungal cultures were procured from Department of Microbiology, Osmania University, Hyderabad, India. Standards ampicillin or nystatin was used as the positive control, while DMSO was used as the negative control. The zone of inhibition, which was measured in millimetres, was used to assess the antibacterial activity of the synthesized compounds in triplicates.

Peptone and meat extract included in the nutrient medium, which contains (g L^{-1} distilled water) for all bacteria. The pH was adjusted to 7.0. Agar was added at 2% for solid media. At 121 ºC for 20 min, all media were sterilized. The Agar Diffusion Method was used to carry out the antimicrobial screening. A concentration of 100 mg/mL^{-1} was achieved by dissolving 1 mg of each of the newly synthesized compounds in 1 mL of DMSO, which was then made up to 10 mL with sterile water [22].

Antitubercular activity: The Ogawa-Kudoh medium was utilized to culture the *M. tuberculosis* H37Rv strains (ATCC 27294) for 10 days at 37 ºC. A colony was taken out for testing and grown in Middlebrook 7H9 broth that had been supplemented with catalase, dextrose, bovine serum albumin and oleic acid (OADC). It also had 0.5% glycerol as a 'C' source and 0.5% Tween 80 to prevent lump formation. At 37 ºC, the broth was maintained for 15 days. In accordance with the No. 1 McFarland scale, the bacterial suspensions were made. Stock solutions of the test compounds were diluted in Middlebrook 7H9 broth that had been enriched with OADC and solubilized in DMSO. As positive control medications, pyrazinamide and isoniazid were solubilized as per the manufacturer's instructions (Sigma-Aldrich, India).

The resazurin microtiter approach was employed to determine the antitubercular activity. In brief, 100 µL of Middlebrook 7H9 broth supplements were poured into each well of a 96-well plate and then solutions were serially diluted to acquire various concentrations of the examined compounds (0.8, 1.6, 3.12, 6.25, 12.5, 25, 50 and 100 µg mL^{-1}). Each well received 100 µL of bacterial solution (5×10^5 CFU mL^{-1}) following dilutions. The samples were then incubated for 24 h at 37 ºC after the plates had been cultured for 7 days at this temperature with 30 µL of resazurin solution diluted in 0.01% sterile water added to each well. On a microplate reader known as the TP-Reader at a wavelength of 492 nm, the reading was done based on the colour change and the absorbance. On alternate days, each substance was examined in triplicate. The MIC was established as the lowest concentration that inhibits *M. tuberculosis* growth by 90% [23].

RESULTS AND DISCUSSION

The designed scheme achieved the synthesis of imidazole carboxamide derivatives (**6a-j**) *via* the oxidative esterification of 1*H*-imidazole-4-cabaldehyde (**1**) with phenol (**4**), followed by the aminolysis of various substituted anilines (**5a-j**) in the presence of 1,3-*bis*(2,6-diisopropylphenyl)imidazol-2-ylidene (**2**) (IPr, 10 mol%) and TEMPO (**3**) in toluene.

All the synthesized compounds (**6a-j**) were readily obtained in moderate to good yields. The structures of the synthesized compounds were confirmed by using ^{1}H NMR, ^{13}C NMR, IR and MASS spectral studies. The spectral data matches with respect to synthesized compounds.

The synthesized derivatives (**6a-j**) have characteristic IR peaks at 1638-1600 cm^{-1}, indicating the existence of a C=N group on the aromatic ring and at 3381-3066 cm^{-1}, indicating the existence of an NH group on the aromatic ring. Peaks at 1702-1639 cm^{-1} revealed the presence of the -C=O group in the synthesized derivatives, while distinctive peaks at 1313-1242 cm^{-1} and 1559-1458 cm^{-1} indicated the existence of the C-N and C=C groups in the aromatic ring, respectively. Proton NMR spectra showed multiplet signals at 6.91–8.08 δ ppm, which are indicative of synthesized derivatives aromatic protons. Due to the presence of -CH_3 on phenyl ring, derivatives

6g, **6h** and **6i** exhibited singlet at 2.29-2.19 ppm. The C-H peak of the imidazole ring chemical shift value (δ) was observed around 7.62 to 7.85 ppm as a singlet and the N-H peak of the amide group was observed around 10.29 to 10.61 ppm as a singlet in all the compounds. The ^{13}C NMR spectra data matches with the synthesized compounds.

Antimicrobial activity

Antibacterial activity: In this study, two Gram-positive (*Staphylococcus aureus* and *Bacillus subtilis*) and two Gram-negative (*Pseudomonas aeruginosa* and *Escherichia coli*) strains were used to test the antibacterial activity of the synthesized imidazole-4-carboxamides **(6a-j)**. The biological activity data of all compounds tested for antibacterial activity against Gram-negative and Gram-positive pathogens are shown in Table-1 and are indicated as the zone of inhibition in mm. An *in vitro* antibacterial assay was used to determine titled derivatives antibacterial potential. The comparison of the ability of synthesized compounds to suppress the microbial growth was done using the reference standard (ampicillin at 10 µg/mL) zone of inhibition.

At concentrations of 30 and 60 µg/mL, compounds **6a**, **6f** and **6g** significantly inhibited Gram-positive bacterial growth more than the other compounds. Among the two Gram-positive bacterial strains, compound **6a** had more inhibitory efficacy against *B. subtilis* strain, whereas compounds **6f** and **6g** showed more resistance towards *S. aureus*. The most effective substance against *B. subtilis* is compound **6a** (33.6 mm), while compound **6f** (32.4 mm) is highly effective against *S. aureus*, which demonstrated higher antibacterial activity at a dose of 60 µg/mL when compared to the standard ampicillin (33 and 32 mm) at a level of 10 µg/mL (Table-1).

The growth of Gram-negative bacteria *E.coli* was significantly inhibited at a concentration of 30 µg/mL by derivatives **6a** and **6f**; while at a concentration of 60 µg/mL compounds **6a**, **6e**, **6f**, **6h** and **6i** showed significant an antibacterial activity. Compounds **6a**, **6f** and **6i** displayed the highest antibacterial activity at a dose of 30 µg/mL, whereas compounds **6a**, **6f** and **6g** exhibited significant activity at a dose of 60 µg/mL against *P. aeruginosa.* Between the two Gram-negative bacterial strains, *E. coli* had better inhibitory potential against all the compounds than *P. aeruginosa*. Compound **6a**, which is the most potent compound, demonstrated the equipotent antibacterial potency at a level of 60 µg/mL when compared to reference standard ampicillin activity at a dose of 10 µg/mL. The zones of inhibition of compound **6a** against *E. coli* and *P. aeruginosa* were 26.45 and 23 mm, making **6a** the most potent compound of all the derivatives (Table-1).

The results of the *in vitro* antibacterial assay demonstrated that the basic imidazole carboxamide nucleus is essential for the antibacterial activity. The substituents on the phenyl ring have the ability to differentiate between Gram-positive and Gram-negative bacteria. Gram-positive bacterial strains are susceptible to the activity of compounds with strong electron-withdrawing groups, such as **6f** (4-Br) and the unsubstituted compound **6a** (-H).

In contrast to Gram-positive bacterial strains, Gram-negative strains are susceptible to the activity of compounds with strong electron-withdrawing groups, such as **6f** (4-Br), **6e** (4-NO_2) and **6i** (3-Cl, 2-CH_3) and compounds with electron-donating groups, such as **6g** (*m*-tolyl) and **6h** (2,6(-CH_3)$_2$. In contrast to Gram-negative bacteria, imidazole-4-carboxamide derivative showed good potential to inhibit Gram-positive bacteria. The relative selectivity of imidazole-4-carboxamide may be due to the compound's stronger interactions with the Gram-positive macromolecular network compared to that of the more permissive Gram-negative bacteria.

Antifungal activity: Interestingly, derivatives **6a** and **6g** at a concentration of 30 µg/mL and derivatives **6a, 6f, 6g** and **6h** at a concentration of 60 µg/mL, considerably inhibited the growth of *A. niger*. Compounds **6a**, **6f** and **6g** at both concentrations displayed a significant antifungal profile towards *C. albicans*. Compounds **6f** and **6g** demonstrated the highest antifungal activity against *C. albicans* compared to standard

TABLE-1
ZONE OF INHIBITION (mm) OF THE COMPOUNDS AGAINST DIFFERENT PATHOGENS

Compound	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	21.6	33.6	20.4	30.0	21.85	26.45	16.10	23.00	15.6	22.8	18.0	21.6
6b	14.4	16.8	12.0	15.6	13.80	17.25	11.50	14.95	10.8	15.6	13.2	16.8
6c	13.2	18.0	13.2	18.0	12.65	16.10	11.50	17.25	12.0	16.8	10.8	15.6
6d	14.4	18.0	12.0	18.0	11.50	16.10	10.35	17.25	13.2	15.6	9.6	14.4
6e	12.0	15.6	10.8	14.4	11.50	18.40	8.05	13.80	10.8	14.4	10.8	15.6
6f	20.4	28.8	22.8	32.4	14.95	20.70	13.80	18.40	18.0	24.0	19.2	26.4
6g	18.0	25.2	20.4	27.6	13.80	17.25	11.50	18.40	20.4	25.2	20.4	27.6
6h	13.2	15.6	13.2	16.8	12.65	18.40	12.65	16.10	14.4	20.4	12.0	18.0
6i	12.0	16.8	13.2	15.6	13.80	18.40	14.95	14.95	12.0	16.8	13.2	16.8
6j	13.2	16.8	12.0	18.0	11.50	16.10	12.65	17.25	9.6	13.2	12.0	15.6
DMSO	3		3		3		2		2		2	
Ampicillin (10 µg/mL)	33		32		29		31		–		–	
Nystatin (10 µg/mL)	–		–		–		–		30		26	

nystatin at a concentration of 10 µg/mL. Compound **6g** displayed identical inhibitory activity at a concentration of 60 µg/mL against *A. niger* compared to standard Nystatin at a concentration of 10 µg/mL. The zones of inhibition of **6g** against *A. niger* and *C. albicans* were 25.2 and 27.6 mm, respectively, making **6g** the most potent antifungal of all the derivatives. Compounds **6a** and **6f** were the next most effective antifungals. All the synthesized compounds were more susceptible to *C. albicans* than *A. niger*. These results suggest that imidazole-carboxamide derivatives might have antifungal properties (Table-1).

The examined fungal strains can be more effectively combated by compounds with electron-donating **6g** (*m*-tolyl) groups than by those with electron-withdrawing **6f** (4-Br) groups. It is concluded that the antimicrobial activity can be modulated by the presence of imidazole moiety, amide linkage and phenolic substrate. Thus, the basic imidazole carboxamide moiety is essential for the activity.

Antitubercular activity: The synthesized imidazole-carboxamide derivatives (**6a-j**) were screened against H37Rv (ATCC 27294) strain for exploring their antitubercular efficacy. Eight (compounds **6b-h** and **6j**) out of ten imidazole carboxamide derivatives examined had MIC values between 3.12-12.5 µg mL^{-1}, indicating that they were active. Compounds **6e** and **6j** were identified to be the most active of all the compounds examined, with MIC values 3.12 µg mL^{-1}, which are equivalent to the standard drug pyrazinamide (MIC, 3.12 µg mL^{-1}). Least activity was exhibited by compounds **6i** and **6a**, with MIC, 25 µg mL^{-1} (Table-2).

TABLE-2
MIC OF SYNTHESIZED DERIVATIVES AGAINST *Mycobacterium tuberculosis* (MTB) STRAINS

Compd.	MTB (H37Rv)	Compd.	MTB (H37Rv)
6a	25	**6g**	6.25
6b	6.25	**6h**	6.25
6c	12.5	**6i**	25
6d	6.25	**6j**	3.12
6e	3.12	Isoniazid	0.75
6f	12.5	Pyrazinamide	3.12

The SAR of imidazole carboxamide derivatives disclosed that the compounds bearing nitro group (**6e**) at the 4-position and chloro groups at 3 and 4-positions (**6j**) showed significant activity (MIC, 3.12 µg mL^{-1}). It was observed that electron withdrawing substitutions on the phenyl ring enhanced the activity against Mtb. Compounds **6b**, bearing a chloro substituent and **6d**, bearing a nitro substituent at 3 position, as well as compound **6g**, bearing a tolyl group at *meta* position and compound **6h**, bearing a methyl group at 2,6-position, exhibited better activity (MIC, 6.25 µg mL^{-1}, respectively). The least active compounds in the series are 3-chloro-2-methyl (**6i**) and phenyl group (**6a**) (MIC, 25 µg mL^{-1}).

Addition of another chloro group at the 4-position of **6b** or the 3-position of **6c** leads to most active compound, **6j** (MIC, 3.12 µg mL^{-1}). Among the chloro substituted compounds **6b** (3-Cl) and **6c** (4-Cl), substitution at the 3-position (MIC, 6.25 µg mL-1) favours activity over substitution at the 4-position (MIC, 12.5 µg mL^{-1}). However, in the case of compounds **6e** (4-NO_2) and **6d** (3-NO_2), nitro substitution at the 4-position (MIC, 6.25 µg mL^{-1}) is found to be more favourable for the activity than the 3-position (MIC, 6.25 µg mL^{-1}). There is no change in the activity observed with changing the halogen substitution at the 4-position of the phenyl ring from chloro (**6c**) to bromo (**6f**). The MIC values were similar (12.5 µg mL^{-1}) for the halogen substituted compounds **6c** (Cl) and **6f** (Br). Further, addition of an electron releasing methyl group at the 2-position of **6b** (MIC, 6.25 µg mL^{-1}) resulted in a substantial decrease in activity, *i.e.*, **6i** (MIC, 25 µg mL^{-1}). Methyl group substitution at *meta*-position (**6g**) and at 2,6-position (**6h**) demonstrated the identical activity with MIC, 6.25 µg mL^{-1}.

Overall, the electron-withdrawing and electron-releasing groups on the phenyl rings were found to be favourable for activity. However, the strong electron-withdrawing nitro group possessing compound **6e** and the weak electron-withdrawing group possessing compound **6j** displayed the maximum activity.

Conclusion

In this study, a facile approach for the synthesis of novel imidazole carboxamide analogues (**6a-j**) is reported. Most of the synthesized analogues displayed the promising antimicrobial activities against various strains of fungi and bacteria. At concentrations of 30 and 60 µg/mL, compounds **6a**, **6f** and **6g** significantly inhibited Gram-positive bacterial growth more than the other compounds. Compound **6a** was found to be the most potent compound among all the derivatives, inhibited the growth of Gram-negative bacteria at both concentration of 30 and 60 µg/mL. Compounds **6a**, **6f** and **6g** showed significant antifungal activity against the fungal strains employed in the study at both concentrations of 30 and 60 µg/mL. In *in vitro* antituibercular activity, compounds **6e** and **6j** were identified to be the most active of all the compounds examined, with MIC 3.12 µg mL^{-1}. As a result, this proposed method could be used to synthesize molecules with important medical and pharmacological applications.

CONFLICT OF INTEREST

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

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