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Research Article

Novel Benzimidazole Acetamide Derivatives as Antibacterial and Anti-Tubercular Agents: Synthesis and Characterization

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ABSTRACT

The current study focused on the development of novel potentially active anti-microbial agents based on the benzimidazole-acetamide system. A novel series of various substituted benzimidazole- N- phenyl acetamides was synthesized through a feasible scheme characterized by IR, Proton nuclear magnetic resonance (^{1}HNMR), and MASS spectral methods. All the synthesized compounds were screened for anti-bacterial activity against two gram-positive strains: *Staphylococcus aureus*, *Bacillus subtilis*; four gram-negative strains: *Escherichia coli*, *Salmonella typhi*, *Pseudomonas aeruginosa*, and *Klebsiella pneumonia*; and anti-tubercular activity against mycobacterial strain: *Mycobacterium tuberculosis*. Among the 15 compounds (6a–6o) tested, three compounds, 6e, 6f, 6l, and 6m demonstrated high potency with MIC values ranging from 6.25–12.5µg/mL against both gram-positive and gram-negative strains. In addition, compounds 6e, 6f, and 6l displayed the highest anti-tubercular activity with a MIC value of 25µg/mL.

Introduction

The unrestrained rise of drug-resistant microbes to the clinically employed antibiotics poses a perilous threat to the efficacy of the marketed antibiotics for treating numerous infectious diseases globally. The major concern is the emergence of multidrug-resistant *Mycobacterium* strains, methicillin-resistant strains of *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococci* (VRE), fluoroquinolone-resistant *Enterococcus. faecalis* (QREF), and fluoroquinolone-resistant *Pseudomonas aeruginosa* (FQRP), increase mortality rate. This drug resistance phenomenon creates a major concern to discover the novel targets and drugs in chemotherapy. Designing and developing chemical entities that are structurally distinct from the clinically established drugs represents an effective strategy to deliver novel biomolecular targets for anti-microbial action.[1-3]

The contribution of fused heterocycles is exceptional, concerning their intrinsic potential as therapeutics agents for various ailments from simple bacterial infections to life-threatening diseases. This vital role is attributed to the fused heterocycles' structural diversity, which imparts numerous biological activities for a single molecule. The majority of the drugs in the current market possess these fused heterocycles as a central part of drug action. Many research groups around the globe are making efforts to explore the novel applications of the fused heterocycles in the treatment of different diseases.[4] A recent literature review revealed that many effective anti-microbial agents show a heterocyclic moiety within their structure; in particular, substituted benzimidazole derivatives received special attention as they belong to a class of compounds with proven utility in clinical medicine. In this scenario, benzimidazole moiety represents an important bicyclic

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scaffold among the family of fused heterocyclic molecules. Benzimidazole represents a class of nitrogen-containing heterocycle and involves a benzene ring fused to a 1,3-diazole ring system. Numerous marine and terrestrial natural compounds possess benzimidazole moiety widely premediated as anti-oxidants, plant growth regulators, enzyme inhibitors, imaging reagents, fluorescence materials, and vulcanization accelerators.[5,6]

Especially in the field of therapeutics and medicinal chemistry, benzimidazoles plays a vital role and renders an extensive range of biological activities, including anti-cancer,[7-9] anti-bacterial,[10-12] anti-tuberculosis,[13,14] anti-diabetic,[15] anthelmintic,[16] anti-cancer,[17-19] anti-viral,[20] anti-oxidant,[21] anti-inflammatory,[22,23] and inhibitors of several enzymes.

Amides, RCONHR's moiety is acknowledged to play an essential role in molecular recognition, an important constituent in supramolecular chemical anion sensor technology.[24-26] Additionally, positional alignment of the amide hydrogen bonds is a key player in the selective binding with anion substrates such as DNA. Numerous researches specified that the incidence of hydrogen bonding domain, e.g., amide (–CONH–), appears to be valuable in the structures of anti-microbials.[27] Hence, our attention was focused on synthesizing benzimidazole-acetamide systems containing chemical entities due to their significant biological activities and great pharmaceutical value.

Experimental

Synthesis

All chemicals and solvents used in this work were synthetic grades purchased from Sigma-Aldrich, local vendors, and used without purification. Merck-precoated aluminum TLC plates of silica gel 60 F254 were employed for the reaction monitoring, and the spots were visualized with iodine vapors and in UV chamber. Melting points were determined by Remi electronic melting point apparatus. IR spectra were recorded on Agilent FTIR by the KBr pellet method. ^{1}H-NMR recorded on BRUKER DRX-400 MHz. Chemical shift values (δ) articulated in ppm regarding internal standard tetramethylsilane (TMS). The splitting patterns are designated as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet. MASS recorded on BRUKER ESI-IT MS. The bacterial strains were obtained from the Department of Microbiology, Osmania University, Hyderabad. The samples were sub-cultured and preserved at 4°C.

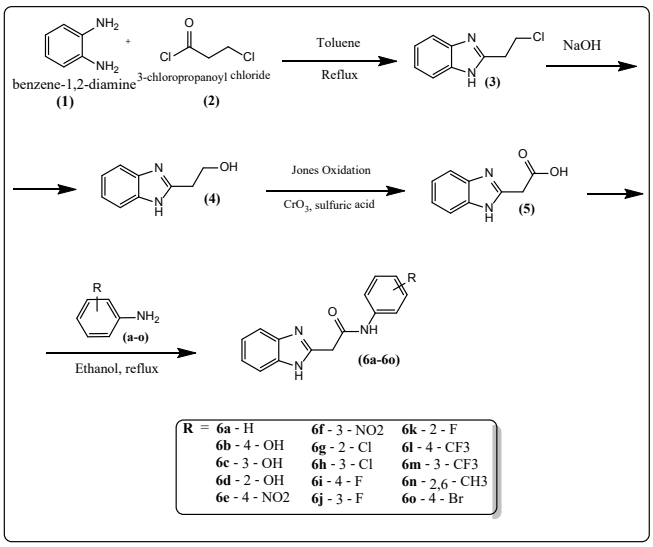

Fig. 1: Scheme of synthesis for the proposed benzimidazole acetamide derivatives.

General procedure for the synthesis of benzimidazole - acetamide derivatives

Synthesis protocol for various substituted benzimidazole – acetamides is illustrated in Fig. 1.

Procedure for the synthesis of 2-(2-chloroethyl)-1H-benzo[d]imidazole (3)

To the solution of o-phenylenediamine **(1)** (6.25 g, 50 mmol) in toluene (30 mL), 3-chloropropanoyl chloride **(2)** (6.34g, 50mmol) was added dropwise with continuous stirring over 15 minutes resulting in the formation of an off-white precipitate. Then the mixture was stirred at room temperature overnight. Reaction progress monitored using TLC with ethyl acetate/hexane (1:9). After reaction completion, the reaction mixture was partitioned twice between water (100 mL) and ethyl acetate (200 mL). Two parts of organic layers were collected, combined, washed with a brine solution, dried over sodium sulfate, and concentrated in a vacuum. The crude product was purified by column chromatography using EtOAc/Hexanes (1:9) to produce 2-(2-chloroethyl)-1H-benzo[d]imidazole **(3)** as an oily liquid (5 g, 55.5%).[28]

Procedure for the synthesis of 2-(1H-benzo[d]imidazol-2-yl) ethan-1-ol (4)

3.96g (20mmol) of 2-(2-chloroethyl)-1H-benzo[d] imidazole dissolved in 5 mL of ethanol, and to this solution 10% NaOH solution (15 mL) was added dropwise, and the reaction mixture was stirred at room temperature. Reaction progress monitored using TLC with ethyl acetate/hexane (1:9v/v). After reaction completion, the unreacted base was quenched by the acid workup with an acetic acid solution. Then the crude reaction mass was a partition in water (20 mL) and ethyl acetate (40 mL). The organic layer was washed with brine solution and dried over Na_2SO_4 and concentrated in vacuum. The crude product was purified by column chromatography using EtOAc/Hexanes (1:9) to produce 2-(1H-benzo[d]imidazol-2-yl) ethan-1-ol **(4)** as off-white solid.

Procedure for the synthesis of 2-(1H-benzo[d]imidazol-2-yl) acetic acid (5)

1.8 g (10 mmol) of 2-(1H-benzo[d]imidazol-2-yl) ethan-1-ol (4) was dissolved in DMF (10 mL) and treated with

3.76 g (10 mmol) Pyridinium Dichromate (PDC) in DMF at room temperature. Reaction progress monitored using TLC with ethyl acetate/hexane (2:8v/v). The reaction mixture was worked up twice by pouring into 7–10 vols of water and subsequent extraction with ether. The ether layers were combined, washed with brine solution, dried over anhydrous silica gel, and concentrated under vacuum. The crude product was purified by column chromatography using EtOAc/Hexanes (2:8v/v) to produce 2-(1H-benzo[d]imidazol-2-yl) acetic acid (5) as an off-white solid.

Procedure for the Synthesis of Benzimidazole-N-phenyl Acetamide Derivatives (6a-6o)

A mixture of 2-(1H-benzo[d]imidazol-2-yl) acetic acid **(5)** (0.96g, 5mmol, 1eqv), the aryl amines **(a-o)** (5 mmol, 1eqv), DIEA (2.8 mL, 15 mmol, 3eqv), and HATU (1.9 g, 5 mmol, 1eqv) in 30 mL DMF was stirred at RT overnight, monitoring the progress with TLC.[29] The reaction mixture was partitioned between dichloromethane (DCM) and water. The organic layer was dried over sodium sulphate and concentrated in vacuo. Then resulting crude material was purified by column chromatography with ethyl acetate/hexane (1:9v/v) to provide the final product **6a-6o.**

Pharmacological Evaluation

In vitro Anti-bacterial Assay

Agar disc diffusion method was employed to evaluate anti-bacterial activity of the synthesized compounds (6a–6o) by following standard guidelines of Clinical and Laboratory Standards Institute (CLSI).[30] The following bacterial strains were used in this study: Two, gram-positive strains, *S. aureus* (ATCC 25323), *B. subtilis* (ATCC 6051) and four, gram-negative strains, *Escherichia coli* (ATCC 35218) *S. typhi* (MTCC 3216), *P. aeruginosa* (ATCC 27893), and *K. pneumonia* (ATCC 31488).

A stock solution of 20 mg of each synthetic compound dissolved in 1 mL of dimethyl sulfoxide (DMSO) as solvent was prepared. Bacterial cultures collected from the source were sub-cultured to isolate pure colonies, and the pure isolates were transferred into the sterile normal saline solution and vortexed to form homogenous bacterial suspensions. The turbidity was then adjusted to 0.5 McFarland standard units, and the suspensions were poured over Mueller–Hinton agar (MHA) plates. Sterile filter paper disks (Whatman No-3 chromatography paper) with a diameter of 6 mm were placed over these plates. The sterile disks were impregnated with 20 µL (400µg in strength) of the tested compounds (20 mg/mL dissolved in DMSO). Ciprofloxacin was used as a reference standard (positive control), and sterile distilled water was selected as a negative control. Finally, the plates were incubated at 37°C for 24 hours. The inhibition zones were measured in millimeters.

The broth dilution method determined the minimum inhibitory concentration (MIC) of the synthesized compounds against the tested bacterial strains. The pure bacterial culture of each microorganism was adjusted to 0.5 McFarland standards in Mueller–Hinton broth (MHB). A two-fold serial dilution method was followed according to the guidelines of Clinical and Laboratory Standards Institute.[31] The stock solutions of the tested compounds were prepared in DMSO and diluted in sterile water. Concentrations of the tested compounds ranged from 0.8 to 400µg/mL. The minimal inhibitory concentrations (MICs) were defined as the lowest concentration of the compound that prevents visible growth of the microorganism. Ciprofloxacin was used as positive control, and sterile distilled water was selected as a negative control.

In vitro Anti-tubercular Assay

Test Organisms and Preparation of Inoculum

M. tuberculosis MTB H37Rv (ATCC 27294) strains, which are susceptible to Isoniazid, were employed to evaluate the anti-tubercular activity of the synthesized compounds. The bacterial strains were sub-cultured to have a fresh batch for the study, supplied with Muller Hinton broth at 37°C for two weeks. Bacterial suspensions with 0.5 McFarland standard turbidity, equivalents to 108 CFU was prepared by diluting it with normal saline solution. The mixture was vortexed for 30 seconds in a glass vessel, and the particles were allowed to settle.[32] 100µL of the microbial suspension was used for the inoculation.

Preparation of Test Samples and Determination of MIC

The stock solutions of 400 µg/mL of synthesized compounds were prepared in DMSO. To determine the minimum inhibitory concentration of title compounds, serial dilution of compounds with varying strengths from 200 µg/mL to 0.8 µg/mL were prepared from the respective stock solutions.

Middlebrook 7H11 agar medium was used for growing the mycobacterium, supplemented with Oleic Albumin Dextrose Catalase (OADC), after sterilization under moist heat using autoclave at 121°C for 15 minutes. Then medium was diluted with various strengths (200, 100, 50, 25, 12.5, 6.25, 3.12, 1.6, and 0.8 µg/mL) of synthesized **(6a–6o)** compounds in appropriate volumes. Using aseptic technique, 5mL of middle brook 7H11 agar medium was dispensed into each labeled quadrant of sterile quad-plates and allowed to solidify under laminar airflow with lids slightly opened. After solidification, bacterial suspension from the culture broth was inoculated aseptically through a loop (3 mm internal diameter) and incubated for 21 days at 37°C. The minimum inhibitory concentration (MIC) was determined by counting the colonies formed on the medium by comparing them with the controls. DMSO and isoniazid were served as negative and positive controls, respectively.[33]

Results and Discussion

Chemistry

As shown in the synthetic scheme (Fig. 1), all the compounds were rooted from o-phenylene diamine **(1)** and a dihalide linker, 3-chloropropanoyl chloride **(2)** in a condensation reaction to 2-(2-chloroethyl) benzimidazole **(3)**, which then easily converted to an alcohol derivative, 2-(1H-benzo[d]imidazol-2-yl) ethan-1-ol **(4)** by base hydrolysis employing 10% NaOH solution. Subsequently, the intermediate (**4)** was oxidized to a carboxylic acid, 2-(1H-benzo[d]imidazol-2-yl) acetic acid **(5)** using PDC in DMF under mild conditions. Ultimately the targeted benzimidazole acetamide derivatives were synthesized in an amide coupling reaction between the intermediate, 2-(1H-benzo[d]imidazol-2-yl) acetic acid (**5),** and various substituted anilines **(a-o)** in the presence of amide coupling catalysts HATU and DIEA in DMF at room temperature.

All the synthesized compounds **6a–6o** resulted in competitive yield, as reported in Table 1. The synthesized compounds were confirmed by ^{1}H NMR and MASS spectral data, following the structure compounds. The C–H peak of the acetamide methylene group's chemical shift value (δ) was observed around 3.30 to 3.45 as a singlet, and the N–H peak of the amide group was observed around 5.6 singlet in all the compounds. Further, the chemical shift values of the aromatic protons and their splitting patterns confirmed the structures of synthesized compounds.

In the final amide coupling reaction, the unsubstituted, trifluoromethyl, and chloro-group substituted anilines resulted in highest yields of respective benzimidazole acetamide derivatives. The highest yield (81%) was obtained with simple unsubstituted aniline, followed by -CF_3, 4-OH, and 2/3-Cl group substituted anilines.

Spectral data of the synthesized compounds was enumerated below:

Compound 6a: *2-(1H-benzo[d]imidazol-2-yl)-N-phenylacetamide*

Off white crystals, **IR (KBr):** ν_{max} in cm^{-1}: 1605.5 (C=N), 1645.5 (C=O), 3280.1 (NH), 3060.3 (=C–H), 1290.5 (C-N),1540.3 (C=C); **^{1}H NMR** (400 MHz, Chloroform-*d*) δ: 4.29 (2H, s), 6.94 (1H, ddd, J = 7.7, 7.6, 1.2 Hz), 7.07 (1H, tt, J = 7.8, 1.2 Hz), 7.14-7.32 (3H, 7.27 (dddd, J = 8.2, 7.8, 1.4, 0.5 Hz), 7.19 (ddd, J = 8.1, 7.6, 1.4 Hz)), 7.47 (2H, dddd, J = 8.2, 1.5, 1.2, 0.5 Hz), 7.63-7.72 (2H, 7.69 (ddd, J = 7.7, 1.4, 0.5 Hz), 7.66 (ddd, J = 8.1, 1.2, 0.5 Hz)). **ESI-MS:** m/z Anal. Calcd. For $C_{15}H_{13}N_3O$ ([M + H]$^+$): 251.3, found 252.2.

Compound 6b: *2-(1H-benzo[d]imidazol-2-yl)-N-(4-hydroxyphenyl) acetamide*

Off white crystals, **IR (KBr):** ν_{max} in cm^{-1}: 1607.5 (C=N), 1646.5 (C=O), 3282.1 (NH), 3049.5 (=C–H), 1294.5 (C-N),1531.3 (C=C), 3627.3 (O - H); **^{1}H NMR** (400 MHz, Chloroform-*d*) δ: 4.27 (2H, s), 6.67 (2H, ddd, J = 8.8, 2.3, 0.5 Hz), 6.94 (1H, ddd, J = 7.7, 7.6, 1.2 Hz), 7.14-7.30 (3H, 7.27 (ddd, J = 8.8, 1.7, 0.5 Hz), 7.19 (ddd, J = 8.1, 7.6, 1.4 Hz)), 7.63-7.72 (2H, 7.69 (ddd, J = 7.7, 1.4, 0.5 Hz), 7.66 (ddd, J = 8.1, 1.2, 0.5 Hz)). **ESI-MS:** m/z Anal. Calcd. For $C_{15}H_{13}N_3O_2$ ([M + H]$^+$): 267.3, found 268.3.

Compound 6c: *2-(1H-benzo[d]imidazol-2-yl)-N-(3-hydroxyphenyl) acetamide*

Off white crystals, **IR (KBr):** ν_{max} in cm^{-1}: 1607.5 (C=N), 1646.5 (C=O), 3282.1 (NH), 3049.5 (=C–H), 1294.5 (C-N),1531.3 (C=C), 3629.4 (O - H); **^{1}H NMR** (400 MHz, Chloroform-*d*) δ: 4.27 (2H, s), 6.74 (1H, ddd, J = 8.1, 2.2, 1.6 Hz), 6.94 (1H, ddd, J = 7.7, 7.6, 1.2 Hz), 7.14-7.28 (2H, 7.23 (ddd, J = 8.2, 8.1, 0.5 Hz), 7.19 (ddd, J = 8.1, 7.6, 1.4 Hz)), 7.37 (1H, ddd, J = 2.2, 1.4, 0.5 Hz), 7.46 (1H, ddd, J = 8.2, 1.6, 1.4 Hz), 7.63-7.72 (2H, 7.69 (ddd, J = 7.7, 1.4, 0.5 Hz), 7.66 (ddd, J = 8.1, 1.2, 0.5 Hz)). **ESI-MS:** m/z Anal. Calcd. For $C_{15}H_{13}N_3O_2$ ([M + H]$^+$): 267.3, found 268.3.

Table 1. Molecular formula, melting point and yield of compounds

Comp. No	*R*	*Mol. Form.*	*Melting point (°C)*	*% Yield*
6a	H	$C_{15}H_{13}N_3O$	201-203	81
6b	4-OH	$C_{15}H_{13}N_3O_2$	166-168	74
6c	3-OH	$C_{15}H_{13}N_3O_2$	163-165	68
6d	2-OH	$C_{15}H_{13}N_3O_2$	164-166	63
6e	4-NO_2	$C_{15}H_{12}N_4O_3$	171-173	70
6f	3-NO_2	$C_{15}H_{12}N_4O_3$	176-178	69
6g	2-Cl	$C_{15}H_{12}ClN_3O$	158-160	71
6h	3-Cl	$C_{15}H_{12}ClN_3O$	154-156	71
6i	4-F	$C_{15}H_{12}FN_3O$	162-164	74
6j	3-F	$C_{15}H_{12}FN_3O$	155-157	66
6k	2-F	$C_{15}H_{12}FN_3O$	149-151	68
6l	4-CF3	$C_{16}H_{12}F_3N_3O$	181-183	77
6m	3-CF3	$C_{16}H_{12}F_3N_3O$	184-186	74
6n	2,6-CH_3	$C_{17}H_{17}N_3O$	177-179	67
6o	4-Br	$C_{15}H_{12}BrN_3O$	195-197	70

Compound 6d: *2-(1H-benzo[d]imidazol-2-yl)-N-(2-hydroxyphenyl) acetamide*

Off white crystals, **IR (KBr):** ν_{max} in cm^{-1}: 1607.5 (C=N), 1646.5 (C=O), 3282.1 (NH), 3049.5 (=C–H), 1294.5 (C-N),1531.3 (C=C), 3628.9 (O - H); **^{1}H NMR** (400 MHz, Chloroform-*d*) δ: 4.27 (2H, s), 6.68 (1H, ddd, J = 8.5, 1.3, 0.5 Hz), 6.89-7.07 (2H, 7.03 (ddd, J = 8.5, 7.5, 1.2 Hz), 6.94 (ddd, J = 7.7, 7.6, 1.2 Hz)), 7.09-7.24 (2H, 7.19 (ddd, J = 8.1, 7.6, 1.4 Hz), 7.13 (ddd, J = 8.3, 7.5, 1.3 Hz)), 7.35 (1H, ddd, J = 8.3, 1.2, 0.5 Hz), 7.63-7.72 (2H, 7.69 (ddd, J = 7.7, 1.4, 0.5 Hz), 7.66 (ddd, J = 8.1, 1.2, 0.5 Hz)). **ESI-MS:** m/z Anal. Calcd. For $C_{15}H_{13}N_3O_2$ ([M + H]$^+$): 267.3, found 268.25.

Compound 6e: *2-(1H-benzo[d]imidazol-2-yl)-N-(4-nitrophenyl) acetamide*

Light brown crystals, **IR (KBr):** ν_{max} in cm^{-1}: 1606.5 (C=N), 1644.5 (C=O), 3284.2 (NH), 3051.6 (=C–H), 1284.6 (C-N), 1511.7 (-NO2); **^{1}H NMR** (400 MHz, Chloroform-*d*) δ: 4.28 (2H, s), 6.94 (1H, ddd, J = 7.7, 7.6, 1.2 Hz), 7.19 (1H, ddd, J = 8.1, 7.6, 1.4 Hz), 7.35 (2H, ddd, J = 8.6, 2.3, 0.4 Hz), 7.63-7.72 (2H, 7.69 (ddd, J = 7.7, 1.4, 0.5 Hz), 7.66 (ddd, J = 8.1, 1.2, 0.5 Hz)), 8.13 (2H, ddd, J = 8.6, 1.8, 0.4 Hz). **ESI-MS:** m/z Anal. Calcd. For $C_{15}H_{12}N_4O_3$ ([M + H]$^+$): 296.3, found 297.15.

Compound 6f: *2-(1H-benzo[d]imidazol-2-yl)-N-(3-nitrophenyl) acetamide*

Light brown crystals, **IR (KBr):** ν_{max} in cm^{-1}: 1606.5 (C=N), 1646.1 (C=O), 3287.7 (NH), 3048.2 (=C–H), 1287.8 (C-N), 1513.1 (-NO2); **^{1}H NMR** (400 MHz, Chloroform-*d*) δ: 4.27 (2H, s), 6.94 (1H, ddd, J = 7.7, 7.6, 1.2 Hz), 7.19 (1H, ddd, J = 8.1, 7.6, 1.4 Hz), 7.38-7.49 (3H, 7.41 (ddd, J = 8.2, 1.6, 1.5 Hz), 7.45 (ddd, J = 8.4, 8.2, 0.5 Hz), 7.44 (ddd, J = 8.4, 1.7, 1.6 Hz)), 7.63-7.72 (2H, 7.69 (ddd, J = 7.7, 1.4, 0.5 Hz), 7.66 (ddd, J = 8.1, 1.2, 0.5 Hz)), 7.99 (1H, ddd, J = 1.7, 1.5, 0.5 Hz). **ESI-MS:** m/z Anal. Calcd. For $C_{15}H_{12}N_4O_3$ ([M + H]$^+$): 296.3, found 297.2.

Compound 6g: *2-(1H-benzo[d]imidazol-2-yl)-N-(2-chlorophenyl) acetamide*

Off white crystals, **IR (KBr):** ν_{max} in cm^{-1}: 1607.4 (C=N), 1646.5 (c=o), 3287.5 (-NH), 3050.5 (=C–H), 1285.5 (C-N),671.5 (C-Cl); **^{1}H NMR** (400 MHz, Chloroform-*d*) δ: 4.27 (2H, s), 6.94 (1H, ddd, J = 7.7, 7.6, 1.2 Hz), 7.19 (1H, ddd, J = 8.1, 7.6, 1.4 Hz), 7.38-7.49 (3H, 7.41 (ddd, J = 8.2, 1.6, 1.5 Hz), 7.45 (ddd, J = 8.4, 8.2, 0.5 Hz), 7.44 (ddd, J = 8.4, 1.7, 1.6 Hz)), 7.63-7.72 (2H, 7.69 (ddd, J = 7.7, 1.4, 0.5 Hz), 7.66 (ddd, J = 8.1, 1.2, 0.5 Hz)), 7.99 (1H, ddd, J = 1.7, 1.5, 0.5 Hz). **ESI-MS:** m/z Anal. Calcd. For $C_{15}H_{12}ClN_3O$ ([M + H]$^+$): 285.7, found 286.65.

Compound 6h: *2-(1H-benzo[d]imidazol-2-yl)-N-(3-chlorophenyl) acetamide*

Off white crystals, **IR (KBr):** ν_{max} in cm^{-1}: 1607.4 (C=N), 1646.5 (c=o), 3287.5 (-NH), 3050.5 (=C–H), 1285.5 (C-N),671.5 (C-Cl); **^{1}H NMR** (400 MHz, Chloroform-*d*) δ: 4.29 (2H, s), 6.94 (1H, ddd, J = 7.7, 7.6, 1.2 Hz), 7.11-7.24 (2H, 7.15 (ddd, J = 8.1, 1.7, 1.6 Hz), 7.19 (ddd, J = 8.1, 7.6, 1.4 Hz)), 7.35 (1H, ddd, J = 8.2, 8.1, 0.5 Hz), 7.58 (1H, dt, J = 8.2, 1.6 Hz), 7.63-7.72 (2H, 7.69 (ddd, J = 7.7, 1.4, 0.5 Hz), 7.66 (ddd, J = 8.1, 1.2, 0.5 Hz)), 7.77 (1H, td, J = 1.7, 0.5 Hz). **ESI-MS:** m/z Anal. Calcd. For $C_{15}H_{12}ClN_3O$ ([M + H]$^+$): 285.7, found 286.6.

Compound 6i: *2-(1H-benzo[d]imidazol-2-yl)-N-(4-fluorophenyl) acetamide*

Off white crystals, **IR (KBr):** ν_{max} in cm^{-1}: 1602.4 (C=N), 1644.8 (C=O), 3283.3 (-NH), 3052.9 (=C–H), 1288.2 (C-N),1308.1 (C-F); **^{1}H NMR** (400 MHz, Chloroform-*d*) δ: 4.27 (2H, s), 6.94 (1H, ddd, J = 7.7, 7.6, 1.2 Hz), 7.02 (2H, ddd, J = 8.6, 1.5, 0.6 Hz), 7.19 (1H, ddd, J = 8.1, 7.6, 1.4 Hz), 7.63-7.76 (4H, 7.69 (ddd, J = 7.7, 1.4, 0.5 Hz), 7.73 (ddd, J = 8.6, 1.9, 0.6 Hz), 7.66 (ddd, J = 8.1, 1.2, 0.5 Hz)). **ESI-MS:** m/z Anal. Calcd. For $C_{15}H_{12}FN_3O$ ([M + H]$^+$): 269.3, found 270.25.

Compound 6j: *2-(1H-benzo[d]imidazol-2-yl)-N-(3-fluorophenyl) acetamide*

Off white crystals, **IR (KBr):** ν_{max} in cm^{-1}: 1601.9 (C=N), 1644.1 (C=O), 3282.8 (-NH), 3052.9 (=C–H), 1284.8 (C-N),1303.2 (C-F); **^{1}H NMR** (400 MHz, Chloroform-*d*) δ: 4.27 (2H, s), 6.89-7.04 (2H, 7.01 (ddd, J = 8.4, 1.7, 1.4 Hz), 6.94 (ddd, J = 7.7, 7.6, 1.2 Hz)), 7.19 (1H, ddd, J = 8.1, 7.6, 1.4 Hz), 7.31 (1H, ddd, J = 8.4, 8.2, 0.5 Hz), 7.53 (1H, ddd, J = 8.2, 1.7, 1.4 Hz), 7.63-7.73 (3H, 7.69 (ddd, J = 7.7, 1.4, 0.5 Hz), 7.71 (td, J = 1.7, 0.5 Hz), 7.66 (ddd, J = 8.1, 1.2, 0.5 Hz)). **ESI-MS:** m/z Anal. Calcd. For $C_{15}H_{12}FN_3O$ ([M + H]$^+$): 269.3, found 270.2.

Compound 6k: *2-(1H-benzo[d]imidazol-2-yl)-N-(2-fluorophenyl) acetamide*

Off white crystals, **IR (KBr):** ν_{max} in cm^{-1}: 1602.9 (C=N), 1645.9 (C=O), 3287.1 (-NH), 3051.4 (=C–H), 1289.3 (C-N),1308.1 (C-F); **^{1}H NMR** (400 MHz, Chloroform-*d*) δ: 4.27 (2H, s), 6.94 (1H, ddd, J = 7.7, 7.6, 1.2 Hz), 6.99-7.09 (2H, 7.04 (ddd, J = 8.3, 7.7, 1.4 Hz), 7.02 (ddd, J = 8.3, 1.6, 0.5 Hz)), 7.14-7.28 (2H, 7.19 (ddd, J = 8.1, 7.6, 1.4 Hz), 7.24 (ddd, J = 7.9, 7.7, 1.6 Hz)), 7.63-7.72 (2H, 7.69 (ddd, J = 7.7, 1.4, 0.5 Hz), 7.66 (ddd, J = 8.1, 1.2, 0.5 Hz)), 8.33 (1H, ddd, J = 7.9, 1.4, 0.5 Hz). **ESI-MS:** m/z Anal. Calcd. For $C_{15}H_{12}FN_3O$ ([M + H]$^+$): 269.3, found 270.25.

Compound 6l: *2-(1H-benzo[d]imidazol-2-yl)-N-(4-(trifluoromethyl)phenyl) acetamide*

Off white crystals, **IR (KBr):** ν_{max} in cm^{-1}: 1608.2 (C=N), 1649.1 (C=O), 3280.1 (-NH), 3052.5 (=C–H), 1284.5 (C-N),1310.5 (C-F); **^{1}H NMR** (400 MHz, Chloroform-*d*) δ: 4.27 (2H, s), 6.94 (1H, ddd, J = 7.7, 7.6, 1.2 Hz), 7.14-7.28 (3H, 7.25 (ddd, J = 8.2, 1.4, 0.5 Hz), 7.19 (ddd, J = 8.1, 7.6, 1.4 Hz)), 7.57 (2H, ddd, J = 8.2, 1.8, 0.5 Hz), 7.63-7.72 (2H, 7.69 (ddd, J = 7.7, 1.4, 0.5 Hz), 7.66 (ddd, J = 8.1, 1.2, 0.5 Hz)). **ESI-MS:** m/z Anal. Calcd. For $C_{16}H_{12}F_3N_3O$ ([M + H]$^+$): 319.3, found 320.3.

Compound 6m: *2-(1H-benzo[d]imidazol-2-yl)-N-(3-(trifluoromethyl)phenyl) acetamide*

Off white crystals, **IR (KBr):** ν_{max} in cm^{-1}: 1603.4 (C=N), 164939 (C=O), 3285.2 (-NH), 3050.8 (=C–H), 1285.8 (C-N),1309.5 (C-F); **^{1}H NMR** (400 MHz, Chloroform-*d*) δ: 4.27 (2H, s), 6.94 (1H, ddd, J = 7.7, 7.6, 1.2 Hz), 7.14-7.28 (3H, 7.25 (ddd, J = 8.2, 1.4, 0.5 Hz), 7.19 (ddd, J = 8.1, 7.6, 1.4 Hz)), 7.57 (2H, ddd, J = 8.2, 1.8, 0.5 Hz), 7.63-7.72 (2H, 7.69 (ddd, J = 7.7, 1.4, 0.5 Hz), 7.66 (ddd, J = 8.1, 1.2, 0.5 Hz)). **ESI-MS:** m/z Anal. Calcd. For $C_{16}H_{12}F_3N_3O$ ([M + H]$^+$): 319.3, found 320.3.

Compound 6n: *2-(1H-benzo[d]imidazol-2-yl)-N-(2,6-dimethylphenyl) acetamide*

Off white crystals, **IR (KBr):** ν_{max} in cm^{-1}: 1604.5 (C=N), 1644.5 (C=O), 3282.2 (-NH), 3052.5 (=C–H), 1293.0 (C-N),1531.2 (C=C); **^{1}H NMR** (400 MHz, Chloroform-*d*) δ: 2.27 (6H, s), 4.29 (2H, s), 6.84 (1H, t, J = 7.9 Hz), 6.89-7.02 (3H, 6.94 (ddd, J = 7.7, 7.6, 1.2 Hz), 6.99 (dd, J = 7.9, 2.6 Hz)), 7.19 (1H, ddd, J = 8.1, 7.6, 1.4 Hz), 7.63-7.72 (2H, 7.69 (ddd, J = 7.7, 1.4, 0.5 Hz), 7.66 (ddd, J = 8.1, 1.2, 0.5 Hz)). **ESI-MS:** m/z Anal. Calcd. For $C_{17}H_{17}N_3O$ ([M + H]$^+$): 279.35, found 280.25.

Compound 6o: *2-(1H-benzo[d]imidazol-2-yl)-N-(4-bromophenyl) acetamide*

Pale yellow crystals, **IR (KBr):** ν_{max} in cm^{-1}: 1605.8 (C=N), 1642.7 (C=O), 3284.2 (-NH), 3051.5 (=C–H), 1286.5 (C-N),695.6 (C-Br); **^{1}H NMR** (400 MHz, Chloroform-*d*) δ: 4.30 (2H, s), 6.94 (1H, ddd, J = 7.7, 7.6, 1.2 Hz), 7.19 (1H, ddd, J = 8.1, 7.6, 1.4 Hz), 7.37 (2H, ddd, J = 8.4, 1.5, 0.6 Hz), 7.63-7.75 (4H, 7.69 (ddd, J = 7.7, 1.4, 0.5 Hz), 7.72 (ddd, J = 8.4, 1.6, 0.6 Hz), 7.66 (ddd, J = 8.1, 1.2, 0.5 Hz)). **ESI-MS:** m/z Anal. Calcd. For $C_{15}H_{12}BrN_3O$ ([M + H]$^+$): 330.2, found 331.15.

Anti-bacterial Activity

The anti-bacterial activity of the synthesized compounds (6a–6o) was evaluated against two-gram positive strains: *S. aureus* (ATCC 25323), *B. subtilis* (ATCC 6051); four, gram-negative strains: *Escherichia coli* (ATCC 35218), *S. typhi* (MTCC 3216), *P. aeruginosa* (ATCC 27893), and *K. pneumonia* (ATCC 31488); and one mycobacterial strain: *Mycobacterium tuberculosis* MTB H37Rv (ATCC 27294). The methods include the agar disk diffusion method and broth dilution method for MIC determination following CLSI. The results of the anti-bacterial assay and anti-tubercular assay were reported in Tables 2 and 3.

The agar disc diffusion method results show that all the synthesized possess good to moderate anti-bacterial activity against the tested bacterial strains regarding the standard drug ciprofloxacin. Among the tested compounds, the compounds **6e, 6f, 6i, 6l,** and **6m** have displayed a higher zone of inhibition against all bacterial strains. In addition, compound **6e** displayed the highest inhibition capacity (22–31 mm) against all the bacterial strains. Moreover, all the benzimidazole acetamide derivatives tended to be highly active against gram-positive strains than gram-negative strains.

The results of MIC study revealed that most of the compounds possess good to moderate anti-bacterial activity, with MIC values ranging between 6.25 µg/mL and 200 µg/mL. Out of the 15 compounds tested, compounds **6e, 6f, 6l, and 6m** displayed broad-spectrum anti-

Table 2: Results of agar disk diffusion study

		Gram positive		*Gram negative*			
Comp. No	*R*	*S. aureus*	*B. subtilis*	*E. coli*	*S. typhi*	*P. aeruginosa*	*K. pneumonia*
6a	H	19	20	18	21	16	20
6b	4-OH	21	21	19	20	21	19
6c	3-OH	20	21	16	16	15	21
6d	2-OH	21	19	18	15	17	19
6e	4-NO_2	31	28	31	22	23	29
6f	3-NO_2	30	26	29	18	24	20
6g	2-Cl	21	19	20	21	23	20
6h	3-Cl	19	20	21	20	24	21
6i	4-F	28	25	26	19	21	23
6j	3-F	23	20	21	20	24	23
6k	2-F	22	21	20	20	19	21
6l	4-CF3	30	25	29	24	27	27
6m	3-CF3	28	26	22	24	23	26
6n	2,6-CH_3	15	17	16	14	19	15
6o	4-Br	21	20	18	19	17	19
Ciprofloxacin		32	30	34	33	29	35

The value of each compound consisted of 'zone of inhibition range' of 03 replicates.

Table 3: Results of MIC determination of compounds in broth dilution study

		Minimum inhibitory concentration in µg/mL						
		Gram positive		*Gram negative*				
Comp. No	*R*	*S. aureus*	*B. subtilis*	*E. coli*	*S. typhi*	*P. aeruginosa*	*K. pneumonia*	*M. tuberculosis*
6a	H	≥100	≥100	≥100	≥100	≥100	≥100	≥200
6b	4-OH	≥50	≥12.5	≥25	≥25	≥25	≥25	≥200
6c	3-OH	≥25	≥50	≥50	≥50	≥50	≥50	≥200
6d	2-OH	≥100	≥100	≥100	≥100	≥100	≥100	≥200
6e	4-NO_2	≥6.25	≥6.25	≥6.25	≥6.25	≥6.25	≥6.25	≥25
6f	3-NO_2	≥6.25	≥6.25	≥6.25	≥6.25	≥6.25	≥6.25	≥25
6g	2-Cl	≥25	≥25	≥50	≥50	≥50	≥50	≥100
6h	3-Cl	≥25	≥25	≥50	≥50	≥50	≥12.5	≥100
6i	4-F	≥6.25	≥6.25	≥6.25	≥12.5	≥6.25	≥6.25	≥50
6j	3-F	≥6.25	≥6.25	≥25	≥25	≥12.5	≥25	≥100
6k	2-F	≥12.5	≥12.5	≥25	≥25	≥25	≥25	≥200
6l	4-CF3	≥6.25	≥6.25	≥6.25	≥6.25	≥6.25	≥6.25	≥25
6m	3-CF3	≥6.25	≥6.25	≥12.5	≥6.25	≥12.5	≥12.5	≥50
6n	2,6-CH_3	≥100	≥100	≥100	≥100	≥100	≥100	≥200
6o	4-Br	≥25	≥25	≥50	≥50	≥50	≥50	≥200
Ciprofloxacin		≥3.12	≥3.12	≥3.12	≥3.12	≥3.12	≥3.12	NA
Isoniazid		NA	NA	NA	NA	NA	NA	≥6.25

The value of each compound consisted of MIC of 03 replicates.
Level of significance $p < 0.05$

microbial activity and high potency against all the tested bacterial strains with MIC values of 6.25–12.5 µg/mL. The compounds **6e** and **6f** exhibited a potent MIC of 6.25 µg/mL against all gram-positive and gram-negative strains.

From the observation of MIC data, it is apparent that the electron-withdrawing groups containing compounds displayed high potency over the electron-donating group containing compounds. The potent compounds **6e, 6f, 6l,** and **6m** possess nitro (-NO_2), and trifluoromethyl (-CF_3) group substituents on the phenyl ring that participating in the amide bond formation. The compound **6n** that contains 2,6-dimethyl group on the phenyl ring displayed the lowest anti-bacterial activity against all the bacterial strains. This further supports the importance of electron-withdrawing substituents on the synthesized benzimidazole acetamide derivatives.

Anti-tubercular activity of the synthesized compounds was tested against the *Mycobacterium tuberculosis* MTB H37Rv (ATCC 27294) strain. The MIC results of the anti-tubercular assay enumerated in Table 3. The activity of the synthesized derivatives was measured with the reference compound isoniazid. Among the all derivatives tested, the compounds **6e, 6f,** and **6l** exhibited MIC values of 25 µg/mL, followed by the compounds **6i** and **6m** that displayed 50µg/mL. Concerning the MIC value of standard isoniazid (6.25 µg/mL), compounds **6e, 6f,** and **6l** displayed good potency, and **6i** and **6m** exhibited moderate potency against the MTB H37Rv strain.

Hence, the developed benzimidazole-acetamide derivatives possess good invitro anti-microbial activity and electron-withdrawing groups on the benzimidazole acetamide derivatives are crucial from the results for the anti-bacterial spectrum as well as potency.

Conclusion

The targeted benzimidazole scaffold and various substituted aryl-amines tethered through the acetamide linkage were synthesized via a multistep synthetic scheme. The detailed spectral analysis characterized the synthesized benzimidazole acetamide derivatives, and the compounds were evaluated for their anti-bacterial activity against gram-negative and gram-positive bacterial strains. The further anti-tubercular activity of these synthesized derivatives was also evaluated against MTB H37Rv strain. The compounds **6e, 6f,** and **6l** displayed broad-spectrum anti-bacterial activity and potency among the tested benzimidazole acetamide derivatives. From the results, further investigation is needed to understand the exact mechanism of the anti-microbial property at the molecular level to develop potent anti-microbial agents to eradicate the pathogenic diseases caused by the bacteria.

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