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Synthesis, Structural Elucidation and Pharmacological Screening of 2-Substituted Benzimidazoles as Dual Anti-Fungal, Antioxidants and Anti-TB Candidates

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ABSTRACT
The present research describes the design, synthesis, spectral characterization, and in-vitro biological evaluation of a new library of 2-(5-substituted-1H-benzimidazol-2-ylthio)-N-arylacetamide derivatives (SS1–SS21). These compounds were developed to investigate their antibacterial, antifungal, antitubercular, and antioxidant potential. The synthetic pathway involved S-alkylation of 5-methoxy, 5-ethoxy, and 5-chloro benzimidazole-2-thiols with suitably substituted N-(chloroacetyl) aryl amines, yielding the target molecules in good to excellent yields. Structural confirmation was achieved through FT-IR, ^{1}H-NMR, mass spectrometry, melting point determination, and TLC, all of which supported the successful formation of the designed thioether-linked N-aryl acetamides. Antibacterial screening against Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Staphylococcus aureus, and Bacillus subtilis showed that several nitro- and chloro-substituted derivatives displayed MIC values comparable to or better than ampicillin. Antifungal studies against Candida albicans, Aspergillus niger, Epidermophyton floccosum, Trichophyton rubrum, and wild Penicillium species revealed that methoxy- and ethoxy-substituted compounds exhibited strong fungicidal activity, in some cases approaching or surpassing Griseofulvin and nearing the effectiveness of Nystatin. Antitubercular evaluation against Mycobacterium tuberculosis H37Rv identified SS5 and SS15 as moderately active, supported by molecular docking results showing favorable binding energies and key interactions with cyclopropane-fatty-acyl-phospholipid synthase enzymes. Antioxidant activity assessed using the DPPH assay indicated potent radical-scavenging effects, particularly for electron-donating methoxy and ethoxy derivatives such as SS9 and SS10. Structure–activity relationship (SAR) analysis revealed that electron-withdrawing groups tend to improve antibacterial activity, whereas electron-donating substituents enhance antifungal and antioxidant potential. Overall, this study highlights benzimidazole-2-thiol–based N-aryl acetamides as promising multifunctional scaffolds with noteworthy antimicrobial and antioxidant activities, making them valuable candidates for further optimization in heterocyclic drug design.
Keywords: *Benzimidazole-2-thiol derivatives; N-aryl acetamides; S-alkylation; Antibacterial activity; Antifungal activity; Antitubercular screening; Antioxidant activity; Molecular docking; SAR*

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INTRODUCTION

Benzimidazole-based systemic fungicides represent one of the most important therapeutic and agricultural interventions for combating fungal infections due to their exceptional potency, broad-spectrum activity, and structural versatility. Among these, classic agents such as benzimidazole, 2-methyl benzimidazole, thiabendazole, and carbendazim have established themselves as gold-standard treatments, demonstrating significant activity particularly against *Aspergillus fumigatus*, *Candida albicans*, and several pathogenic dermatophytes [1]. Their effectiveness is closely tied to their mechanism of action, which involves selective inhibition of fungal microtubule assembly and mitotic spindle formation, thereby disrupting cell division. The benzimidazole ring, due to its planar aromatic structure and strong interaction with tubulin, makes these molecules effective inhibitors of fungal growth at both superficial and systemic levels [2].

Fungal infections remain a persistent and often life-threatening global health challenge. Human fungal diseases arise from allergic responses, mycotoxin exposure, or direct infection (mycoses) [3]. They range from mild, superficial conditions—such as athlete's foot, ringworm, and onychomycosis—to severe systemic infections including histoplasmosis, cryptococcosis, candidiasis, and invasive aspergillosis [4]. Even in healthy individuals, fungi can occasionally cause disease, but the greatest burden of morbidity and mortality occurs in immunocompromised populations. Opportunistic pathogens like *Candida*,

Cryptococcus, and *Aspergillus* readily exploit weakened immune defenses, making individuals susceptible to disseminated and invasive fungal disease [5]. Immunocompromised groups at high risk include newborns, patients receiving chemotherapy, individuals undergoing organ or bone marrow transplantation, sufferers of extensive burns, and those subjected to prolonged corticosteroid or antibiotic therapy [6]. While patients with AIDS are particularly vulnerable, others such as diabetics, individuals with chronic skin lesions, hospitalized surgical patients, and individuals with malnutrition or neutropenia are also predisposed to severe fungal infections [7]. The rise of invasive fungal diseases coincides with modern medical practices that prolong life but impair immune function, such as immunosuppressive therapies, prosthetic implants, and intensive care support [8]. In recent decades, the incidence, severity, and complexity of fungal infections have increased dramatically. Sepsis caused by fungal pathogens is rising worldwide and now represents a significant proportion of hospital-acquired bloodstream infections [9]. Dermatophyte infections such as candidiasis and tinea pedis remain among the most common superficial fungal diseases globally; although rarely fatal, they contribute substantially to morbidity and healthcare burden [10]. In contrast, pathogens including *Aspergillus fumigatus*, *Candida albicans*, *Cryptococcus neoformans*, and *Pneumocystis carinii* are capable of causing rapid, aggressive, and often fatal systemic infections in immunocompromised individuals. Epidemiological studies consistently show that candidiasis and aspergillosis account for nearly 80–90% of systemic fungal infections in such populations [11].

Despite the availability of several antifungal classes—such as azoles, polyenes, echinocandins, and allylamines—treatment failure remains common. Several fungi, including non-albicans *Candida* species, *Aspergillus* variants, and rare molds, are showing reduced susceptibility to current antifungals, complicating clinical management [12]. Resistance mechanisms include altered drug efflux pumps, mutations in target enzymes, and biofilm-associated tolerance. These trends underscore the urgent need for new antifungal agents, especially those capable of overcoming emerging resistance patterns [13]. Benzimidazole derivatives, with their proven antifungal potency and modifiable chemical scaffold, offer a promising foundation for next-generation antifungal development. However, their limitations—including poor activity against certain taxa like *Alternaria solani*—highlight the need for structural optimization and the development of hybrid or multi-target benzimidazole derivatives [14]. Given the escalating threat of fungal pathogens and the limited efficacy of current antifungal therapies in severely ill or immunocompromised patients, the continuous discovery and development of stronger, safer, and more selective benzimidazole-based antifungals remains a critical priority [15]. As fungi and microbes continue to evolve resistance to existing agents, medicinal chemistry efforts must focus on designing novel benzimidazole analogues with enhanced bioavailability, improved tubulin-binding affinity, and reduced toxicity. Benzimidazole-based compounds are drawing attention due to their broad spectrum of biological activities, including antibacterial, antifungal, antiviral, and antiparasitic effects. The benzimidazole nucleus resembles purine bases, allowing it to interact with microbial proteins, DNA, enzymes, and structural components [16]. A compound named Antibiofilm Compound-1 (ABC-1), a benzimidazole derivative identified through small-molecule screening, demonstrates remarkable potency against biofilm formation in both Gram-positive and Gram-negative bacteria—including *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Biofilms contribute significantly to antimicrobial resistance by shielding bacteria from drugs and immune responses. Therefore, compounds capable of disrupting biofilm development represent an important therapeutic innovation [17]. One of the most promising antibacterial applications of benzimidazole analogues lies in their ability to disrupt bacterial cell division, specifically by targeting the protein FtsZ, a critical cytoskeletal element responsible for forming the Z-ring during cytokinesis [18].

The Southern Research Institute identified several FtsZ inhibitors—including pteridine-based and pyridopyrazine compounds—following a systematic screening of known tubulin inhibitors. Interestingly, the classical tubulin inhibitors thiabendazole and albendazole, both benzimidazole derivatives, significantly inhibited Mycobacterium tuberculosis (Mtb) cell division [19]. Because of the structural similarities between tubulin inhibitors and these FtsZ-targeting compounds, the benzimidazole scaffold was considered an excellent template for designing novel anti-tubercular agents. Rational drug design strategies allowed researchers to synthesize new benzimidazole libraries, many of which displayed promising MIC values against the Mtb H37Rv strain [20].

MATERIAL AND METHODS

Chemicals and Reagents

Analytical grade chemicals and solvents were used throughout the study. Substituted anilines, chloroacetyl chloride, o-phenylenediamine derivatives, methoxy and ethoxy precursors, and other reagents were procured from standard commercial suppliers. All solvents were distilled prior to use where necessary.

Reaction progress was monitored by TLC on silica gel plates using appropriate solvent systems, and spots were visualized under UV light or by iodine vapor.

Synthesis of 5-Methoxy-2-Mercaptobenzimidazole

5-Methoxy-2-mercaptobenzimidazole was synthesized via a multi-step process starting from *p-anisidine* (4-methoxyaniline). In a typical procedure, *p-anisidine* was converted to its corresponding dithiocarbamate (intermediate) by reaction with carbon disulfide in the presence of an alkaline medium (e.g., potassium hydroxide) in ethanol. Cyclisation of this intermediate in the presence of an oxidizing agent afforded the benzimidazole ring system bearing a methoxy group at position 5 and a mercapto group at position 2. The crude product was filtered, washed with water and recrystallised from a suitable solvent (e.g., ethanol) to afford pure 5-methoxy-2-mercaptobenzimidazole as a crystalline solid.

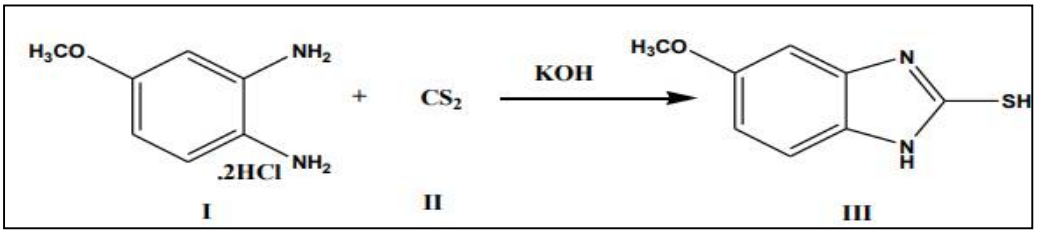


Figure 1: Synthesis of 5-Methoxy-2-Mercaptobenzimidazole

Synthesis of 5-Ethoxy-2-Mercaptobenzimidazole

An analogous method was applied to obtain 5-ethoxy-2-mercaptobenzimidazole, using 4-ethoxyaniline as the starting material. Formation of the corresponding dithiocarbamate followed by intramolecular cyclisation produced the desired 5-ethoxy-substituted benzimidazole-2-thiol. The product was isolated, washed and recrystallised to analytical purity.

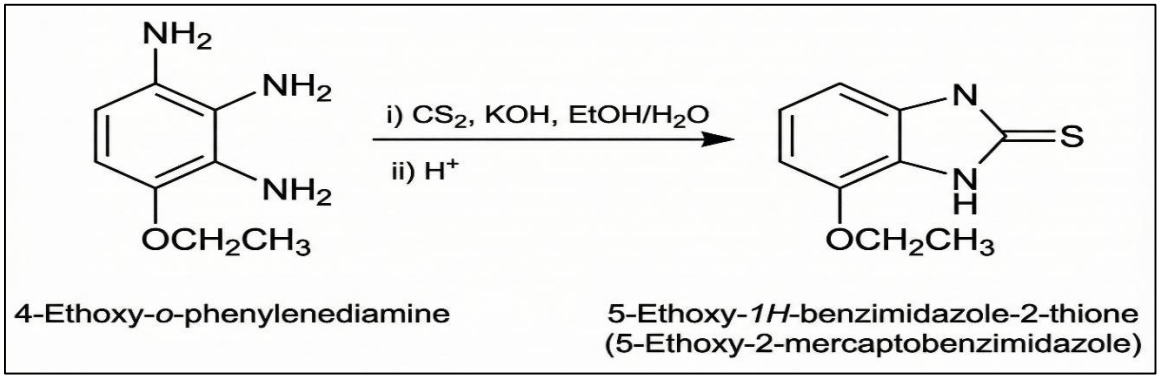


Figure 2: Synthesis of 5-Ethoxy-2-Mercaptobenzimidazole

Synthesis of N-(Chloroacetyl) Substituted Aryl Amines

A series of N-(chloroacetyl) aryl amines were prepared by acylation of substituted anilines. Substituted aniline (1 mol) was dissolved in an appropriate inert solvent such as dioxane or dichloromethane, cooled in an ice bath, and treated dropwise with chloroacetyl chloride (1.1–1.2 mol) in the presence of a base (e.g., potassium carbonate or triethylamine) to neutralise the generated hydrochloric acid. The mixture was stirred at room temperature or under gentle reflux until completion (typically 4–6 h), as monitored by TLC. After completion, the reaction mixture was washed with water, the organic layer separated and dried over anhydrous sodium sulfate, and the solvent evaporated. The crude product was recrystallised or triturated to yield pure N-(chloroacetyl) aryl amines. Various electron-donating (e.g., methoxy) and electron-withdrawing (e.g., nitro, fluoro, chloro) substituents at different positions on the aromatic ring were incorporated to generate structural diversity. (Their percentage yield as per the TLC-UV Spectral data is given in Table 2 in result segment)

Synthesis of 2-(5-Substituted Benzimidazol-2-ylthio)-N-Arylacetamides (SS1–SS21)

To a stirred solution of 5-methoxy- or 5-ethoxy-2-mercaptobenzimidazole in ethanol, equimolar potassium hydroxide was added to generate the thiolate anion in situ. The mixture was stirred for a short period to ensure complete deprotonation of the thiol.To this solution, the corresponding N-(chloroacetyl) aryl amine was added slowly, and the reaction mixture was heated under reflux for 12–16 hours. The nucleophilic sulphur attacked the electrophilic carbon of the chloroacetyl moiety, displacing chloride and forming the thioether-linked N-aryl acetamide.

After completion (confirmed by disappearance of starting materials on TLC), the reaction mixture was cooled to room temperature and poured into ice-cold water to precipitate the product.The solid was filtered, washed thoroughly with water to remove inorganic salts, and recrystallised from ethanol or ethanol-water mixtures to afford the final derivatives SS1–SS19. Two 5-chloro benzimidazole analogues (SS20, SS21) were prepared similarly.

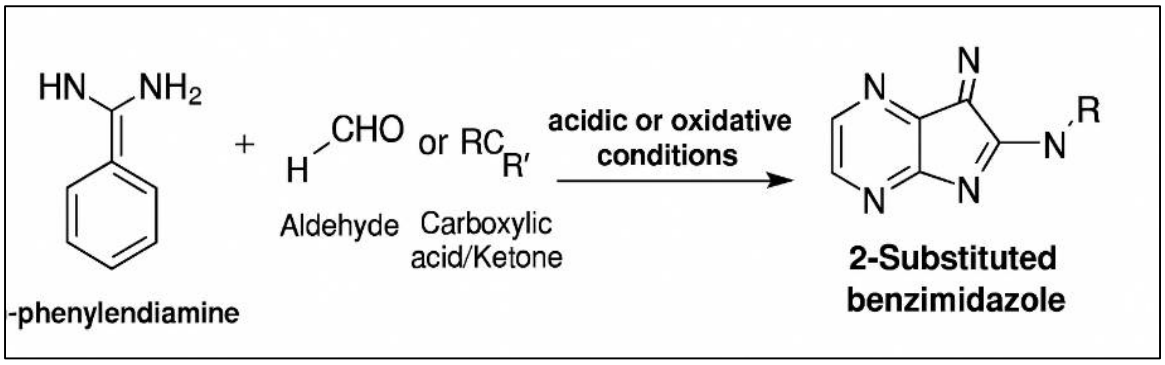


Figure 3: General synthetic scheme for the preparation of SS1–SS21 from 5-substituted benzimidazole-2-thiols and N-(chloroacetyl) aryl amines

Spectral Characterization

The synthesized benzimidazole derivatives have been characterized by the researchers using double-beam Fourier Transform Infrared (FT-IR) spectrophotometry. Using the double-beam model made it possible to discern various characteristic absorption bands such as the N-H stretching (around 3100-3400 cm^{-1}), the C=N stretching (about 1600 cm^{-1}) and the C-N stretching. The presence of various substituents such as the hydroxyl, nitro, and halos was confirmed by the bands positional of the shifts and the intensity changes. There were also shifts along with the metal ion bondage in the metal-benzimidazole complex. The changes in the spectra confirmed the synthesized benzimidazole analogues and the tautomeric preferences, its pictorial outgrowths is mentioned in Figure 5.

^{1}H-NMR Spectroscopy

^{1}H-NMR spectra were recorded in a suitable deuterated solvent (e.g., DMSO-d_6 or $CDCl_3$). Characteristic signals included:

- Singlets for methoxy protons (–OCH_3) around δ 3.7–3.9 ppm;
- Triplets and quartets for ethoxy protons (–OCH_2CH_3), with the methylene adjacent to oxygen (–OCH_2–) resonating near δ 3.9–4.2 ppm and the terminal methyl (–CH_3) appearing as a triplet around δ 1.2–1.4 ppm;
- Methylene protons (–S–CH_2–CO–) as singlets or AB-type patterns near δ 3.8–4.5 ppm;
- Aromatic protons of the benzimidazole and aryl rings distributed between δ 6.5–8.5 ppm, with splitting patterns depending on substitution patterns;
- Amide N–H protons typically as downfield singlets or broad signals around δ 9–11 ppm;
- Benzimidazole N–H proton appearing as a distinct downfield signal, often slightly more deshielded than the amide N–H.

Mass Spectrometry

Mass spectra (e.g., ESI-MS) of selected derivatives displayed molecular ion peaks $[M]^+$ or $[M+H]^+$ consistent with their calculated molecular masses. Fragmentation patterns supported the presence of the benzimidazole core, thioether linkage and substituents. Typical fragments included cleavage at the thioether or amide link, loss of methoxy/ethoxy groups, and characteristic isotopic patterns for chloro-substituted derivatives.

In-Vitro Biological Evaluation

Antibacterial Activity

Antibacterial activity of the synthesised derivatives was evaluated by the broth microdilution method. Serial two-fold dilutions of each compound were prepared in sterile Mueller–Hinton broth to obtain a range of concentrations. Standardized inocula of *E. coli*, *P. aeruginosa*, *K. pneumoniae*, *S. aureus* and *B. subtilis* were added to each well. After incubation at 37 °C for 18–24 hours, the MIC was recorded as the lowest concentration with no visible growth. Ampicillin served as the standard drug for comparison.

Antifungal Activity

Fungicidal activity was determined against *C. albicans*, *A. niger*, *E. floccosum*, *T. rubrum* and wild *Penicillium* spp. Serial dilutions of compounds in Sabouraud's dextrose broth were inoculated with the test organisms and incubated under appropriate conditions. The minimum fungicidal concentration (MFC) was defined

as the lowest concentration that prevented growth upon subculture onto drug-free agar. Griseofulvin and Nystatin were used as reference antifungal agents.

Antitubercular Activity

Selected derivatives were screened against *M. tuberculosis* H37Rv using the microdilution method. Serial dilutions of each compound were prepared in Middlebrook medium, inoculated with a standard mycobacterial suspension and incubated for an extended period at 37 °C. MIC was determined as the lowest concentration that prevented visible growth. Isoniazid and Rifampicin served as positive controls.

Antioxidant Activity (DPPH Assay)

Antioxidant activity was assessed using the DPPH radical scavenging assay. Different concentrations (e.g., 10, 50, 100 µg/mL) of each compound were mixed with a fixed concentration of DPPH solution in methanol. After incubation in the dark for a set time (e.g., 30 min), the decrease in absorbance was measured spectrophotometrically at 517 nm. Radical scavenging (%) was calculated relative to a control, and a standard antioxidant (e.g., ascorbic acid) was used for comparison.

RESULT

Chemistry and Spectral Analysis

Table 1 summarizes the synthesis of eleven N-(chloroacetyl) aryl amines (A1–A11) derived from differently substituted anilines. All compounds were obtained as crystalline solids with good yields (74–85%), showed characteristic melting points, and exhibited consistent Rf values in EtOAc:hexane (3:7), confirming successful synthesis across a range of electron-donating and electron-withdrawing substituents.

Table 1. List of synthesized N-(Chloroacetic) aryl amines (A1–A11) with substituents and yields

Code	Aryl Amine Used	Aryl Substituent (s)	Position	Physical Appearance	Melting Point (°C)	Rf (EtOAc: Hexane 3:7)	% Yield
A1	Aniline	–H	—	White crystalline solid	108–110	0.48	82%
A2	*p*-Anisidine	$-OCH_3$	Para	Off-white crystals	114–116	0.52	79%
A3	*p*-Nitroaniline	$-NO_2$	Para	Yellow crystalline solid	156–158	0.43	85%
A4	*o*-Nitroaniline	$-NO_2$	Ortho	Yellow powder	150–152	0.41	83%
A5	*p*-Fluoroaniline	–F	Para	White crystals	98–100	0.46	80%
A6	*p*-Chloroaniline	–Cl	Para	Light beige solid	118–120	0.50	82%
A7	*m*-Chloroaniline	–Cl	Meta	White crystalline	110–112	0.49	78%
A8	*p*-Toluidine	$-CH_3$	Para	White to off-white crystals	105–107	0.54	76%
A9	*m*-Anisidine	$-OCH_3$	Meta	Off-white powder	118–120	0.53	74%
A10	*o*-Chloroaniline	–Cl	Ortho	Slightly yellow crystals	121–123	0.45	81%
A11	*p*-Aminobenzoic acid methyl ester	$-COOCH_3$	Para	White powder	125–127	0.55	77%

The synthetic methodology furnished the target benzimidazole derivatives SS1–SS21 in good yields (Table 3), generally with straightforward work-up and purification. TLC confirmed the formation of single major products, and melting points were in relatively narrow ranges, indicating good purity. The overall synthetic route is operationally simple and scalable, relying on readily available starting materials and standard laboratory techniques.

Table 2: Physical data (melting point, yield, Rf values) of SS1–SS21.

Code	5-Substitution	Aryl Substituent (Ar)	Appearance	Melting Point (°C)	Rf (EtOAc: Hexane 4:6)	Yield (%)
SS1	5-OCH_3	4-OCH_3	Off-white crystals	198–200	0.41	78
SS2	5-OCH_3	4-H	White solid	192–194	0.43	76
SS3	5-OCH_3	4-NO_2	Yellow crystals	212–214	0.38	82
SS4	5-OCH_3	2-NO_2	Pale yellow	205–207	0.37	80
SS5	5-OCH_3	4-Cl	White crystalline	199–202	0.40	75
SS6	5-OCH_3	3-Cl	Beige solid	196–198	0.42	74
SS7	5-OCH_3	4-F	White crystals	188–190	0.44	72
SS8	5-OCH_3	4-CH_3	White crystals	185–188	0.45	73
SS9	5-OCH_3	3-OCH_3	Off-white	194–197	0.46	79
SS10	5-OCH_3	4-$COOCH_3$	White solid	207–210	0.39	80
SS11	5-OCH_3	2-Cl	Slight yellow	202–204	0.40	77
SS12	5-OC_2H_5	4-OCH_3	White solid	172–175	0.48	74
SS13	5-OC_2H_5	4-H	Off-white	170–172	0.49	72
SS14	5-OC_2H_5	4-NO_2	Yellow crystals	193–196	0.40	78
SS15	5-OC_2H_5	2-NO_2	Pale yellow	188–190	0.39	76
SS16	5-OC_2H_5	4-Cl	White solid	178–180	0.45	71
SS17	5-OC_2H_5	3-Cl	Off-white	175–178	0.46	70
SS18	5-OC_2H_5	4-CH_3	White crystals	168–170	0.50	73
SS19	5-OC_2H_5	3-OCH_3	White powder	174–177	0.47	75
SS20	5-Cl	4-OCH_3	White solid	208–211	0.38	80
SS21	5-Cl	4-NO_2	Yellow crystals	220–223	0.34	82

FT-IR spectra of the final derivatives showed strong amide C=O stretching bands in the range 1640–1685 cm^{-1} and N–H stretching bands between 3200–3400 cm^{-1}, confirming successful acylation and formation of the amide linkage. The presence of methoxy or ethoxy groups was indicated by characteristic C–O stretching. The disappearance of the thiol S–H stretching band and appearance of C–S related bands supported conversion of the thiol into a thioether.

^{1}H-NMR spectra confirmed substitution patterns and the presence of methoxy/ethoxy, methylene, aromatic and N–H protons. The benzimidazole N–H signal, typically at δ 11–13 ppm (depending on solvent and substitution), and the amide N–H around δ 9–11 ppm, were clearly observed and were diagnostic of the intact benzimidazole and amide functionalities. Integration values corresponded well with the number of protons in each environment, confirming the proposed structures.

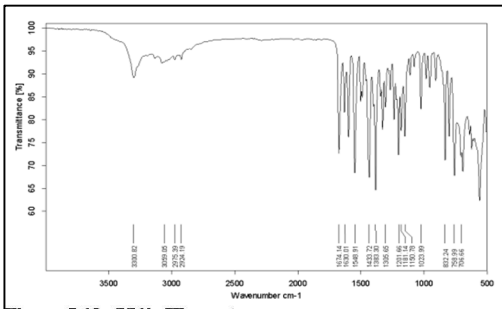


Figure 5.12: SS1's IR spectrum

Table 5.12: SS1's IR interpretation

I.R. frequency (cm-1)	Remarks
1674.14	C=O (-NHCO) stretching
3059.05	C-H stretching
3300.82	N-H stretching

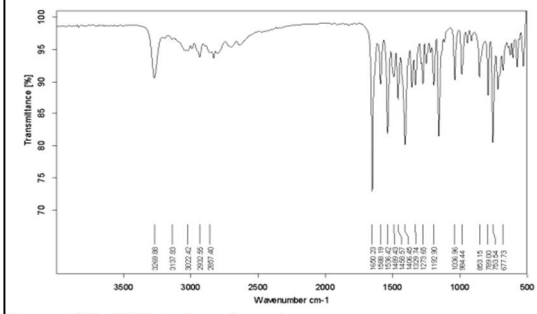


Figure 5.13: SS2's infrared spectrum

Table 5.13: SS2's IR interpretation

I.R. frequency (cm-1)	Remarks
1650.23	C=O (-NHCO) stretching
3022.42	C-H stretching
3269.88	N-H stretching

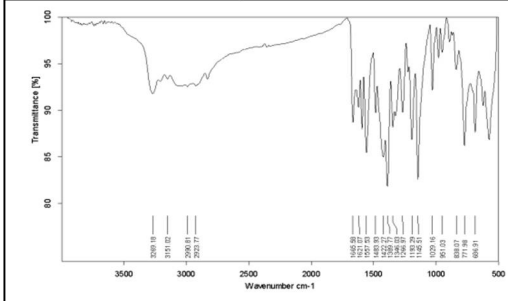


Figure 5.14: SS3's infrared spectrum

Table 5.14: SS3's IR interpretation

I.R. frequency (cm^{-1})	Remarks
1665.58	C=O (-NHCO) stretching
2990.81	C-H stretching
3269.18	N-H stretching

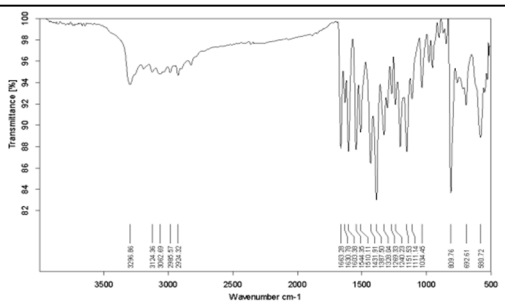


Figure 5.15: SS4's infrared spectrum

Table 5.15: SS4's IR interpretation

I.R. frequency (cm-1)	Remarks
1663.28	C=O (-NHCO) stretching
3062.69	C-H stretching
3296.86	N-H stretching

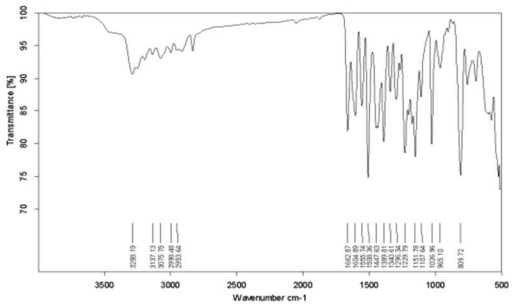


Figure 5.16: SS5's infrared spectrum

Table 5.16: SS5's IR interpretation

I.R. frequency (cm-1)	Remarks
1662.87	C=O (-NHCO) stretching
3075.75	C-H stretching
3288.19	N-H stretching

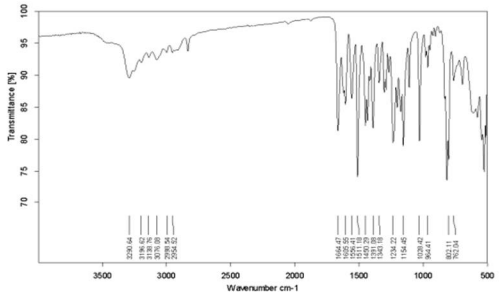
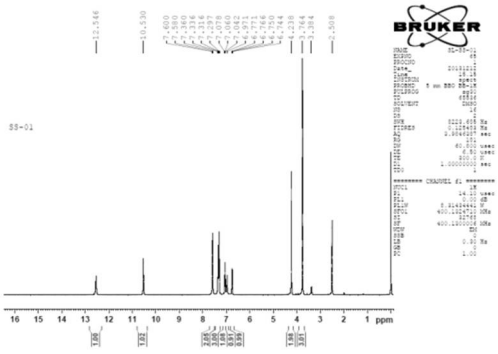


Figure 5.17: SS6's infrared spectrum

Table 5.17: SS6's IR interpretation

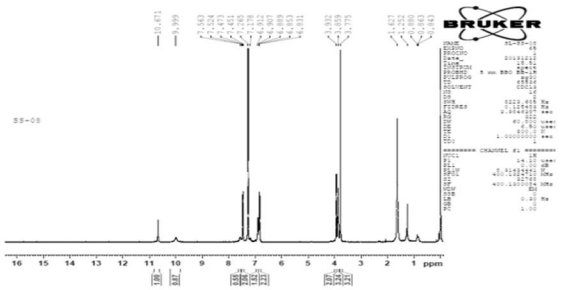
I.R. frequency (cm-1)	Remarks
1664.47	C=O (-NHCO) stretching
3076.08	C-H stretching
3290.64	N-H stretching

Figure 4: Representative FT-IR spectrum of a methoxy-substituted derivative (e.g., SS1-SS6)



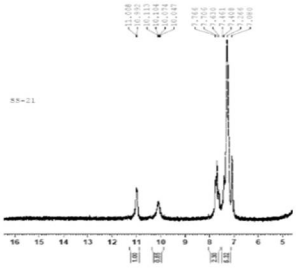
¹HNMR (DMSO, ppm): 3.764 (s, 3H, OCH3), 4.238 (s, 2H, CH_2), 10.530 (s, 1H, NH), 12.546 (s, 1H, Ring NH), 6.744-7.600 (m, 8H, ArH)

Figure 5.41: SS1's NMR spectra



1HNMR (CDCl3, ppm): 3.775 (s, 3H, OCH3), 3.859 (s, 3H, OCH3), 3.932 (s, 2H, CH2), 9.999 (s, 1H, NH), 10.677 (s, 1H, Ring NH), 6.831-7.563 (m, 7H, ArH)

Figure 5.42: SS8's NMR spectra



¹HNMR ($CDCl_3$, ppm): 3.965 (s, 2H, CH_2) 10.047 (s, 7.080-7.766 (m,7H, ArH)

Figure 5.46: SS21's NMR spectra

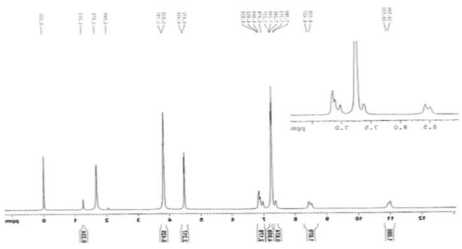
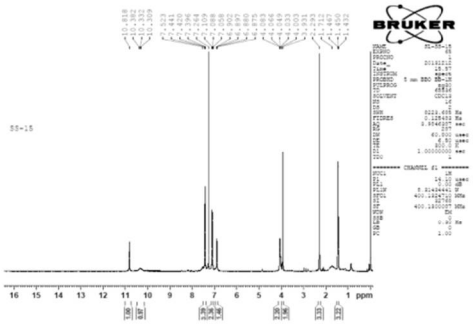


Figure 5.43: SS11's NMR spectra

Figure 5.44: SS14's NMR spectra



¹HNMR ($CDCl_3$, ppm): 1.251 (s, 3H, CH3), 2.293 (t, 3H, OCH3), 3.931 (q, 2H, OCH_2), 4.083 (s, 2H, CH_2) 10.309 (s, 1H, NH), 10.818 (s, 1H, Ring NH), 6.875-7.523 (m,7H, ArH)

Figure 5.45: SS15's NMR spectra

Figure 5: ^{1}H-NMR spectrum of SS1 (5-methoxy series)

Mass spectra further supported the identity of the synthesized compounds. Observed molecular ion peaks matched calculated molecular weights, including isotopic patterns consistent with chloro-substituted analogues. Fragmentation patterns were consistent with cleavage at the thioether and amide bonds, yielding fragments that could be rationalized in terms of the benzimidazole core and the substituted aryl acetamide moieties.

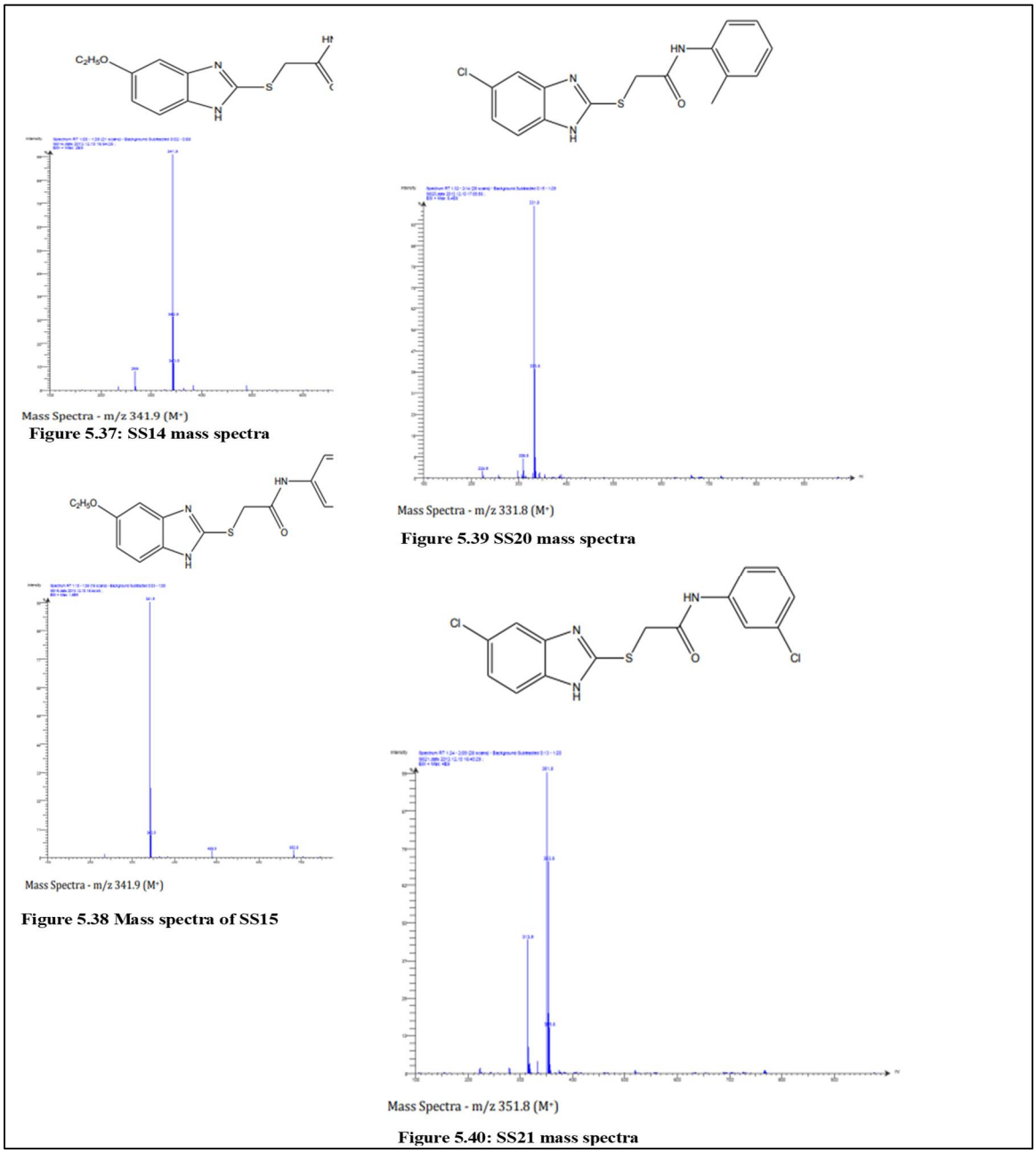


Figure 6: Mass spectrum of a representative compound (e.g., SS9)

Antibacterial Activity

Methoxy-substituted derivatives tended to show pronounced activity against Gram-negative strains such as *E. coli* and *K. pneumoniae*. Nitro-substituted aryl acetamide moieties, particularly those bearing para-nitro groups, produced lower MIC values, suggesting the beneficial influence of electron-withdrawing groups on binding to bacterial targets. Ethoxy analogues exhibited appreciable activity against *P. aeruginosa* and *B. subtilis*, indicating that enhanced lipophilicity may improve penetration through bacterial membranes, particularly in the case of Gram-negative organisms with complex outer membranes.

Table 3: MIC values (µg/mL) of SS1–SS19 against tested bacterial strains.

CODE NO.	*E. coli*	*P. aeruginosa*	*K. pneumoniae*	*S. aureus*	*B. subtillis*
	MTCC 443	MTCC 1688	MTCC 109	MTCC 96	MTCC 441
SS-1	100	125	100	250	125
SS-2	200	250	100	200	100
SS-3	100	100	125	250	250
SS-4	250	250	250	125	200
SS-5	500	500	200	125	100
SS-6	250	200	250	500	100
SS-7	100	100	200	200	250
SS-8	100	100	100	250	200
SS-9	125	125	100	250	125
SS-10	62.5	100	125	100	100
SS-11	125	125	62.5	125	250
SS-12	100	125	250	250	125
SS-13	250	250	250	500	250
SS-14	100	200	62.5	500	200
SS-15	250	250	100	250	250
SS-16	250	200	250	200	500
SS-17	250	20	200	250	100
SS-18	250	125	125	250	100
SS-19	250	250	100	500	100
Ampicillin	100	100	250	100	250

The MIC data clearly demonstrates the relative potency of the various derivatives compared to ampicillin. Certain compounds showed broad-spectrum activity, inhibiting both Gram-positive and Gram-negative bacteria, while others were more selective, suggesting that subtle variations in substitution pattern can modulate target specificity (Table 3).

Antifungal Activity

The antifungal screening data indicated that a subset of the benzimidazole derivatives possesses potent activity against both yeasts and filamentous fungi. Several compounds demonstrated MFC values lower than or comparable to those of Griseofulvin against *C. albicans* and *A. niger*, while a few approached the fungicidal efficacy of Nystatin.

Methoxy and ethoxy substitution at the benzimidazole 5-position appeared to favor antifungal potency, possibly due to increased lipophilicity and enhanced interaction with fungal cell wall or membrane components. Compounds bearing electron-donating substituents on the aryl ring, such as additional methoxy groups, also showed enhanced antifungal activity in some cases, which might reflect their ability to interact with specific fungal enzymes or receptors.

Table: 4 From SS1 to SS19 antifungal activity

CODE NO.	*C.Albicans*	*A. niger*	*T.rubrum*	*E.floccosum*	*Penicillium spp.*	
	MTCC 227	MTCC 282	MTCC296	MTCC 7880	WILD STRAIN	
SS-1	250	500	500	500		1000
SS-2	1000	250	250	1000		1000
SS-3	1000	1000	250	500		1000
SS-4	500	1000	500	1000		250
SS-5	1000	500	250	1000		500
SS-6	1000	250	500	500		1000
SS-7	1000	1000	1000	500		1000
SS-8	500	1000	1000	1000		1000
SS-9	250	1000	1000	200		500
SS-10	1000	500	500	250		500
SS-11	1000	1000	1000	250		1000
SS-12	1000	250	250	1000		1000
SS-13	250	1000	1000	1000		250
SS-14	500	1000	1000	1000		1000
SS-15	1000	500	1000	500		1000
SS-16	1000	500	1000	1000		1000
SS-17	1000	1000	1000	1000		250
SS-18	500	200	250	1000		250
SS-19	500	250	250	500		1000
Nystatin	100	100	---	---		---
Griseofulvin	500	100	---	---		---

The dermatophytes *E. floccosum* and *T. rubrum* were moderately to highly susceptible to a number of derivatives, suggesting potential application in the treatment of superficial fungal infections such as ringworm and athlete's foot. Activity against wild *Penicillium* strains indicated a broader antifungal spectrum, although further species-specific testing would be required for clinical relevance.

Antitubercular Activity

Two selected derivatives exhibited moderate inhibition of *M. tuberculosis* H37Rv with MIC values in the tens of micrograms per milliliter range. While these values are higher than those of standard antitubercular drugs (Isoniazid, Rifampicin), they demonstrate that the benzimidazole-2-thiol scaffold, when coupled with suitable N-aryl acetamide side chains, can interfere with mycobacterial growth.

Table: 5 Antitubercular activity

Compound code	Minimum inhibitory concentration (µg / ml)
SS5	50
SS15	25
Isoniazid	0.2
Rifampicin	0.4

The antitubercular activity observed is especially significant when considered alongside the docking data, which revealed favorable binding of these compounds to enzymes involved in the biosynthesis of mycolic acids. Since mycolic acids are essential components of the mycobacterial cell wall, enzyme inhibition in this pathway can lead to decreased cell wall integrity and compromised survival of the bacilli.

Antioxidant Activity

Some compounds, especially those with multiple methoxy/ethoxy groups, achieved high percentage inhibition (>80–90%) at 100 µg/mL, approaching the activity of standard antioxidants at similar concentrations. The presence of heteroatoms (oxygen, nitrogen, sulphur) within a conjugated system may also facilitate delocalization of unpaired electrons and stabilization of the resulting radical species, further enhancing antioxidant potential. The overall results demonstrate that introducing N-aryl acetamide fragments onto the 2-position of 5-methoxy- and 5-ethoxy-substituted benzimidazole-2-thiols produces a series of molecules with diversified biological activity profiles. The synthetic approach is straightforward and exploits simple nucleophilic substitution chemistry, making it amenable to the generation of larger libraries for further SAR studies.

Table 6: Antioxidant activity

		%			%			%
Code	**Conc**	**Scavengin**	**Code**	**Conc**	**Scavenging**	**Code**	**Conc**	**Scavenging**
	(µg/ml)	**g Activity**		**(µg/ml)**	**Activity**		**(µg/ml)**	**Activity**
	100	33.94		100	20.81		100	23.30
SS1	50	30.32	SS8	50	18.44	SS15	50	20.25
	10	21.95		10	11.76		10	11.76
	100	29.86		100	92.19		100	20.81
SS2	50	23.76	SS9	50	85.29	SS16	50	11.76
	10	23.08		10	70.59		10	8.54
	100	33.60		100	86.88		100	23.98
SS3	50	26.47	SS10	50	75.45	SS17	50	21.61
	10	19.91		10	54.98		10	12.44
	100	25.00		100	16.86		100	19.46
SS4	50	20.48	SS11	50	6.90	SS18	50	16.29
	10	10.29		10	5.77		10	8.82
	100	30.66		100	37.78		100	16.97
SS5	50	26.58	SS12	50	29.86	SS19	50	13.35
	10	14.48		10	23.87		10	7.81
	100	22.85		100	17.42		100	18.44
SS6	50	21.61	SS13	50	11.76	SS20	50	9.28
	10	10.63		10	7.24		10	4.07
	100	26.36		100	23.87		100	13.80
SS7	50	19.57	SS14	50	17.19	SS21	50	10.07
	10	8.26		10	8.37		10	7.58

DISCUSSION

The antibacterial screening revealed that the majority of synthesized derivatives displayed moderate to good inhibitory activity against the tested bacterial strains. Several derivatives exhibited MIC values comparable to ampicillin against at least one organism, and in some cases were more active. In the study conducted by Saxena et al., (2020) these benzimidazole derivatives are versatile class of heterocyclic compounds [21] which exhibits excellent antimicrobial activities in broad spectrum but in our findings mostly against one organism.

In the DPPH assay, most compounds exhibited concentration-dependent radical scavenging activity. Derivatives bearing electron-donating substituents such as methoxy and ethoxy, particularly on the benzimidazole ring and/or on the pendant aryl ring, tended to be stronger antioxidants. This is consistent with the mechanism of DPPH scavenging, in which hydrogen or electron donation from the compound to the DPPH radical leads to its reduction.

From an SAR perspective, electron-withdrawing nitro substituents on the aryl ring consistently enhanced antibacterial activity, especially against Gram-negative organisms. Nitro groups may increase the overall polarity of the molecule while simultaneously influencing electron density distribution across the aromatic and benzimidazole rings, thereby strengthening interactions with bacterial enzymes or receptors. Additionally, nitro groups can participate in specific hydrogen bonding patterns or act as acceptors in weak non-covalent interactions.

Electron-donating groups such as methoxy and ethoxy, particularly when present both on the benzimidazole ring and the aryl acetamide moiety, appeared to enhance antifungal and antioxidant activities. These groups increase electron density, facilitate radical stabilization and can improve the ability of the molecule to partition into lipid-rich environments such as fungal membranes. The increased lipophilicity may help the compound accumulate at sites where it can inhibit key fungal targets or disturb membrane integrity.

The comparison between 5-methoxy and 5-ethoxy series suggests that slight changes in the length and bulk of the alkoxy substituent can lead to differences in biological profiles. While both types of derivatives showed antibacterial and antifungal activity, ethoxy-substituted compounds often displayed stronger effects against certain fungal strains, possibly reflecting improved membrane affinity and altered binding orientation within fungal enzymes whereas in the illustrative SAR study[22] by Poyraz M et al., (2008) referred that alkoxy group substitution with more than 3 -C ameliorate antibacterial activities but significantly reduce its spectrum because of compromised selectivity of molecule size for cell membrane penetration by microbial cell structure but in our study we confined with molecular size up to 2 c alkoxy substitution and got optimum arena of antimicrobial spectrum.

Moderate antitubercular activity in selected derivatives, combined with docking evidence for binding to mycolic acid biosynthetic enzymes, provides a promising starting point for optimization. Rational modifications such as introducing additional hydrogen bond donors/acceptors, tuning lipophilicity, or incorporating heterocycles known to interact with mycobacterial proteins could yield more potent derivatives. Strategies like bio isosteric replacement of the amide, variation in linker length, and exploration of different substituent patterns on the aromatic ring may further refine activity.

The antioxidant data are particularly interesting when taken together with the antimicrobial results. Compounds with dual antimicrobial and antioxidant profiles may be advantageous in clinical scenarios where oxidative stress contributes to disease progression or tissue damage. In infections, reactive oxygen species (ROS) may be generated as part of the host immune response, leading to collateral damage. Having agents that can reduce microbial load and simultaneously scavenge excess radicals can be beneficial.

Despite these encouraging findings, there are limitations to the current study. Only in-vitro models were employed, and the behavior of these compounds in biological systems in vivo remains unknown. Pharmacokinetic properties such as absorption, distribution, metabolism and excretion (ADME) could significantly influence efficacy and safety. Additionally, cytotoxicity towards mammalian cells was not assessed; some benzimidazole derivatives can exhibit cytostatic or cytotoxic effects, which may be desirable in anticancer contexts but undesirable for antimicrobial use. Therefore, further studies evaluating safety profiles, selectivity indices and in-vivo efficacy models are essential.

Furthermore, the mechanism of antimicrobial action was not experimentally elucidated. While docking suggests potential enzyme targets in *M. tuberculosis*, direct enzymatic assays, target validation and resistance profiling would be required to confirm the proposed mode of action and identify off-target effects. Future studies could also explore synergistic effects with existing antibiotics, especially for derivatives that show moderate activity alone.

Overall, the present work enriches the benzimidazole medicinal chemistry portfolio by offering new derivatives with a balance of antimicrobial and antioxidant properties and provides a rationale for further optimization guided by SAR and docking insights.

CONCLUSION

A series of novel 2-(5-methoxy/5-ethoxy-1H-benzo[d]imidazol-2-ylthio)-N-arylacetamides have been successfully synthesised and characterised using standard physicochemical and spectroscopic methods. The synthetic scheme is practical and adaptable, relying on accessible starting materials and straightforward nucleophilic substitution reactions. Biological evaluation revealed that many of the derivatives possess significant antibacterial and antifungal activity. Certain compounds displayed MIC or MFC values comparable to or better than standard drugs against particular strains, highlighting their potential as leads for further development. Selected derivatives exhibited moderate antitubercular activity against *M. tuberculosis* H37Rv, which was supported by docking studies indicating favorable binding to mycolic acid biosynthetic enzymes. In addition, several compounds showed strong antioxidant activity in the DPPH assay, suggesting potential for dual antimicrobial and antioxidant therapeutic applications.

In conclusion, the present study identifies benzimidazole-2-thiol-based N-aryl acetamide derivatives as promising multifunctional molecules with antibacterial, antifungal, antitubercular and antioxidant potential. These compounds warrant further optimization through systematic SAR exploration, detailed mechanistic investigations and comprehensive in-vivo evaluation. With appropriate refinement, they may contribute to the development of new therapeutic agents to address the pressing challenges of antimicrobial resistance and oxidative stress-mediated pathology.

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CONFLICT OF INTEREST

There is no conflict of Interest

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