Synthesis and Evaluation of New 1,3,4-Thiadiazole-Benzimidazole Hybrids as Potent Antibacterial Agents

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Abstract: Owing to the rapid rise in antibiotic resistance, infectious diseases have become a serious threat to public health. There is an urgent need to develop new antimicrobial agents with diverse chemical structures and novel mechanisms of action to overcome resistance. In recent years 1,3,4-Thiadiazole benzimidazole hybrids have emerged as a new class of antimicrobial agents. In the current study, we designed and synthesized six new 1,3,4-Thiadiazole-benzimidazole hybrids using nucleophilic substitution reaction between 2-mercapto-1H-benzimidazole (A) with Ethyl chloroacetate to produce compound (B), which then reacted with various 2-Amino-5-(substituted phenyl)-1,3,4-thiadiazole(C1-C6) to yield target compounds (D1-D6) and evaluated them for their antimicrobial activity. Synthesized compounds were characterized and elucidated by IR, ^{1}H and ^{13}C-NMR, spectra. Subsequently, the antibacterial activity of the final target compounds (D1-D6) was examined in vitro against four types of bacterial isolates: two gram-positive (*Staphylococcus aureus* and *Bacillus subtilis*) and two gram-negative (*Pseudomonas aeruginosa* and *Escherichia coli*). Compounds (D4 and D6) demonstrated promising pharmacological activity in vitro when compared to the standard antibacterial agent Tetracycline.

1 INTRODUCTION

The alarming rise of difficult-to-treat multidrug-resistant bacterial infections has emerged as one of the formidable challenges of the 21st century [1]–[5]. The World Health Organization (WHO) has categorised 12 pathogens as high-risk priority II pathogens that require immediate attention [6]. In particular, S. aureus is known to cause a wide range of diseases, from skin infections to serious illnesses like pneumonia, meningitis, bacteraemia, and sepsis [7]–[9]. Among the various resistant strains, methicillin-resistant (MRSA) and vancomycin-resistant (VRSA) strains are known to be sources of the spread of community infections.

Most benzimidazole derivatives prepared have a wide range of applications as medicinal agents in drug discovery [10]. Thus, benzimidazoles and their condensed systems have attracted much attention as bioconjugated heterocyclic nitrogen systems, which exhibit significant activity against some viruses, such as influenza, [11] herpes (HSV-1), [12] HIV, [13] and human cytomegalovirus (HCMV) [10]. Additionally, they are used as antitumor agents, [14] antimicrobials, [15] strong antiproliferative and DNA binding agents, [16] as well as DNA gyrase inhibitory activities, [17] and antitubercular agents [18]. Recently, most of the heterobicyclic nitrogen systems bearing or containing benzimidazole derivatives have exhibited a wide range of biological and medical properties such as antifungal, [19] antimicrobial, [20] cytotoxicity, [21] antiproliferative, [22] antituberculosis, [23] sulfonamide analogs, [24] and anticancer and antimycobacterial activities [25].

1,3,4-Thiadiazoles represent a prevalent and important class of compounds that exhibit diverse pharmacological activities such as antibacterial, anticancer, antitubercular, and anti-inflammatory properties [26]–[28]. For example, acetazolamide, [29] which contains the 1,3,4-thiadiazole structure motif, has been approved as a diuretic, whereas cefazedone has shown a broad spectrum of antibacterial activity against various pathogens [30]. In addition, another 1,3,4-thiadiazole derivative, BAS0338872, has also been reported as a potent Src/Abl tyrosine kinase inhibitor for the potential treatment of chronic myeloid leukemia [31].

Based on these observations and the search for new highly bioactive compounds, this work reports on the synthesis of new 1,3,4-thiadiazole-benzimidazole hybrids as potent antibacterial agents.

2 EXPERIMENTAL

All starting materials and solvents were purchased from Aldrich and used without purification .Melting points were recorded by Stuart smp3 electronic apparatus and are uncorrected, The FT-IR spectra were recorded on SHIMADZU model FT-IR -8400S, 1H and 13C -NMR spectra were recorded on BRUKER model Ultra shield 500 MHz spectrophotometer using DMSO-d6 as a solvent and TMS as an internal reference. The compounds were evaluated for their purity on silica gel TLC plates and the visualization of spots achieved by UV light:

A: Synthesis of 2-mercaptobenzimidazole.

A mixture of (20 mmol) of *O*-phenylendiamine, (20 mmol) of carbon disulphide, (20 mmol) of potassium hydroxide, (25 mL) of absolute ethanol and (5 mL) of water heated under reflux in 100 ml round bottom flask for 3 hours, then added cautiously (0.5 g) of charcoal and the reflux continued for 10 minutes, then charcoal was removed by filtration and (25 mL) of warm water added after heating the filtrate to 60-70 °C, and then acidified with dilute acidic acid with vigorous stirring. The mixture obtained placed in ice path for 3 hours to complete the crystallization, the product obtained was filtrated, dried and recrystallized from ethanol, the completion of the reaction and the purity of the compounds were checked by TLC (mobile phase: hexane: ethyl acetate (1:1)).

Light beige powder, yield (85%), m.p 305-307 °C, Rf = 0.56; FT-IR (KBr disk, cm-1) 3387 (N-H), 3153 (C-H, aromatic), 2572 (S-H), 1652 (C=N), 1513 and 1467 (C=C, aromatic). 1H NMR (DMSO-d6, 500 MHz, δ)12.58 (s, 1H, benzimidazole-NH),12 .15-12.42 (s, 1H, SH),7.13-7.45 (d,4H, aromatic ring). 13C-NMR (DMSO-d6, 125 MHz, δ). 168.2 (C=N). 115.2-123.0 (aromatic rings):

B: Synthesis of Ethyl-1H-benzo[d]imidazol-2 yl)thio) acetate.

A mixture of (0.03 mole) of 2-mercapto-1Hbenzimidazole A, ((70 ml) of ethanol, and (0.03 mole) of potassium hydroxide was stirred and heated at 78-80 °C for 20 min. Ethyl chloroacetate (0.03 mole) was then added, the reaction mixture was then refluxed for 4 hrs, then the solution was poured into ice-water and stirred for 30 min. The precipitate obtained was collected by filtration, washed with water until free of chloride and dried at 50 °C and recrystallized with water white powder, yield (85%), m.p 95-97 °C, Rf = 0.56; FT-IR (KBr disk, cm-1) 3456 (N-H), 3020 (C-H, aromatic), 1620 (C=N),1741(C=O, ester)1592 and 1405 (C=C, aromatic). 1H NMR (DMSO-d6, 500 MHz, δ) 12.54 (s,1H, benzimidazole-NH), 1.27 (S, 3H, CH3),4.21(q, 2H, CH2), 7.13,7.44(m ,4H, aromatic ring). 13C-NMR (DMSO-d6, 125 MHz, δ). 147.1 (C=N). 123.0-138.9 (aromatic rings). 167.9 (C=O):

C1-C6: *General procedure for the Synthesis of 2-Amino-5-(substituted phenyl)-1,3,4-thiadiazole.*

A mixture of the corresponding carboxylic acid (20 mmol), thiosemicarbazide (1.82 g, 20 mmol) and phosphorous oxychloride (10 mL) was gently refluxed for 3 h. After cooling, water (50 mL) was added slowly and the reaction mixture was refluxed for 4 h and filtered. The solution was neutralized with concentrated potassium hydroxide solution and the precipitate was filtered and recrystallized from ethanol, the completion of the reaction and the purity of the compounds were checked by TLC (mobile phase: hexane: ethyl acetate (1:3)):

C1: 5-phenyl-1,3,4-thiadiazol-2-amine.

Pale yellow powder, yield (95%), m.p 223-225 °C, Rf = 0.46; FT-IR (KBr disk, cm-1) 3421 and 3280 (NH2), 3091 (C-H, aromatic), 1633 (C=N), 1513 and 1468 (C=C, aromatic). 1H NMR (DMSO-d6, 500 MHz, δ) 7.53–8.03 (m, 5H, Ar–H), 7.22-7.52 (s, 2H, NH2). 13C-NMR (DMSO-d6, 125 MHz, δ) 161.6, 174.1 (C=N). 128.7-130.9 (aromatic ring):

C2: *5-(2-chlorophenyl)-1,3,4-thiadiazol-2-amine.*

Pale yellow powder, yield (85%), m.p 213-215 °C, Rf = 0.51; FT-IR (KBr disk, cm-1) 3218 and 3199 (NH2), 3099 (C-H, aromatic), 1666 (C=N), 1514 and 1424 (C=C, aromatic)766 (C-Cl). 1H NMR (DMSO-d6, 500 MHz, δ) 7.38–7.71 (m, 4H, Ar–H), 6.98 (s, 2H, NH2). 13C-NMR (DMSO-d6, 125 MHz, δ) 173.1, 162.6 (C=N). 127.3-130.1 (aromatic ring):

C3: 5-(3-chlorophenyl)-1,3,4-thiadiazol-2-amine.

Pale yellow powder, yield (80%), m.p 204-206 °C, Rf = 0.58; FT-IR (KBr disk, cm-1) 3372 and 3245 (NH2), 3024 (C-H, aromatic), 1639 (C=N), 1514 and 1424 (C=C, aromatic), 714(C-Cl) . 1H NMR (DMSO-d6, 500 MHz, δ) 7.48–7.97 (m, 4H, Ar–H), 7.32-7.63 (s, 2H, NH2). 13C-NMR (DMSO-d6, 125

MHz, δ) 175.1, 162.6 (C=N). 128.8-134.9 (aromatic ring):

C4: *5-(4-chlorophenyl)-1,3,4-thiadiazol-2-amine.*

Pale yellow powder, yield (86%), m.p 226-228 °C, Rf = 0.45; FT-IR (KBr disk, cm-1) 3270 and 3157 (NH2), 3053 (C-H, aromatic), 1630 (C=N), 1593 and 1486 (C=C, aromatic), 827 (C-Cl). 1H NMR (DMSO-d6, 500 MHz, δ) 7.53–8.02 (m, 4H, Ar–H), 7.32-7.62 (s, 2H, NH2). 13C-NMR (DMSO-d6, 125 MHz, δ) 173.2, 165.6 (C=N). 128.9-134.3 (aromatic ring):

C5: *5-(4-nitrophenyl)-1,3,4-thiadiazol-2-amine.*

Yellow powder, yield (82%), m.p 244-246 °C, Rf = 0.62; FT-IR (KBr disk, cm-1) 3426 and 3284 (NH2), 3106 (C-H, aromatic), 1630 (C=N), 1506 and 1456 (C=C, aromatic), 1596 and 1343 (NO2). 1H NMR (DMSO-d6, 500 MHz, δ) 8.11–8.47 (m, 3H, Ar–H), 7.35-7.72 (s, 2H, NH2). 13C-NMR (DMSO-d6, 125 MHz, δ) 176.1, 163.6 (C=N). 124.4-147.9 (aromatic ring):

C6: *5-(p-tolyl)-1,3,4-thiadiazol-2-amine.*

White powder, yield (98%), m.p 265-267 °C, Rf = 0.62; FT-IR (KBr disk, cm-1) 3286 and 3121 (NH2), 3014 (C-H, aromatic), 1631 (C=N), 1510 and 1475 (C=C, aromatic), 1341 and 1425 (CH3). 1H NMR (DMSO-d6, 500 MHz, δ) 7.26–7.72 (m, 3H, Ar–H), 7.27-7.65 (s, 2H, NH2),2.34 (s,3H, CH3). 13C-NMR (DMSO-d6, 125 MHz, δ) 174.1, 161.6 (C=N). 127.4-131.7 (aromatic ring). 21.3 (CH3):

D1-D6: General procedure for the synthesis of the compounds.

The mixture of Ethyl-1H-benzo[d]imidazol-2 yl)thio)acetate [B] 1.05g (5mmol) and 5-(substituted phenyl)-1,3,4-thiadiazol-2-amine (5mmol) are mixed in 60 ml ethanol well in a RBF and heated on water bath for 10 min, the reaction mixture is heated with reflux condenser for 10 hours, cooled to room temperature and the reaction mixture was added to 100gm of ice-water, and kept aside for the crystallization. The colorless crystals are collected by filtration, and recrystallized from water, Melting point is 148-212 oC; the yield is 80 to 90%.

D1: 2-((1H-benzo[d]imidazol-2-yl)thio)-N-(5-phenyl-1,3,4- thiadiazol-2-yl)acetamide.

Pale yellow powder, yield (89%), m.p 148-150 °C, Rf = 0.38; FT-IR (KBr disk, cm-1) 3279 (N-H, amide), 3196 (N-H, benzimidazole),3010 (C-H, aromatic), 2924, 2796 (C-H, aliphatic), 1741 (C=O), , 1635 (C=N), 1557 and 1405 (C=C, aromatic); 1H-NMR (DMSO-d6, 500 MHz, δ) 1.25 (s, 2H, CH2), 7.12-8.31 (m, 8H, Ar-H), 4.16 (s, 1H, NH, amide), 12.78 (s, 1H, benzimidazole-NH); 13C-NMR (DMSO-d6, 125 MHz, δ)) 33.73 (CH2), 126.78-133.57 (aromatic ring),149.62(C=N),156.82(C=O) 169.00-169.10(C=N of 1,3,4-thiadiazole).

D2: *2-((1H-benzo[d]imidazol-2-yl) thio)-N-(5-(2-chlorophenyl)-1,3,4-thiadiazol-2-yl) acetamide.*

Pale yellow powder, yield (88%), m.p 196-198 °C, Rf = 0.43; FT-IR (KBr disk, cm-1) 3222 (N-H, amide), 3101 (N-H, benzimidazole),3086 (C-H, aromatic), 2961, 2925 (C-H, aliphatic), 1741 (C=O), 1633 (C=N), 1512 and 1439 (C=C, aromatic),755 (C-Cl); 1H-NMR (DMSO-d6, 500 MHz, δ) 1.25 (s, 2H, CH2), 7.44-7.61 (m, 8H, Ar-H), 8.04 (s, 1H, NH, amide), 12.88 (s, 1H, benzimidazole-NH); 13C-NMR (DMSO-d6, 125 MHz, δ) 33.72 (CH2), 128.17-131.42 (aromatic ring), 152.07(C=N), 156.92(C=O) 169.08-170.61(C=N of 1,3,4-thiadiazole).

D3: *2-((1H-benzo[d]imidazol-2-yl) thio)-N-(5-(3-chlorophenyl)-1,3,4-thiadiazol-2-yl) acetamide.*

Pale yellow powder, yield (80%), m.p 188-190 °C, Rf = 0.41; FT-IR (KBr disk, cm-1) 3307 (N-H, amide), 3131 (N-H, benzimidazole),3064 (C-H, aromatic), 2972, 2853 (C-H, aliphatic), 1740 (C=O), 1616(C=N), 1565 and 1404 (C=C, aromatic) 763, (C-Cl); 1H-NMR (DMSO-d6, 500 MHz, δ) 1.52 (s, 2H, CH2), 7.03-7.89 (m, 8H, Ar-H), 8.51 (s, 1H, NH, amide), 12.36 (s, 1H, benzimidazole-NH); 13C-NMR (DMSO-d6, 125 MHz, δ) 33.73 (CH2), 121.92-134.29 (aromatic ring), 149.62(C=N), 157.92(C=O) 169.09-169.57(C=N of 1,3,4-thiadiazole):

D4: *2-((1H-benzo[d]imidazol-2-yl) thio)-N-(5-(4-chlorophenyl)-1,3,4-thiadiazol-2-yl)acetamide.*

Light beige powder, yield (90%), m.p 210-212 °C, Rf = 0.43; FT-IR (KBr disk, cm-1) 3278 (N-H, amide), 3104 (N-H, benzimidazole),3068 (C-H, aromatic), 2968, 2852 (C-H, aliphatic), 1740 (C=O), 1632 (C=N), 1513 and 1465 (C=C, aromatic), 831 (C-Cl); 1H-NMR (DMSO-d6, 500 MHz, δ) 1.12 (s, 2H, CH2), 7.51-7.80 (m, 8H, Ar-H), 8.18 (s, 1H, NH, amide), 12.62 (s, 1H, benzimidazole-NH); 13C-NMR (DMSO-d6, 125 MHz, δ) 14.46 (CH2), 121.94-134.44 (aromatic ring), 155.58(C=N), 153.92(C=O) 168.32-169.32(C=N of 1,3,4-thiadiazole).

D5: *2-((1H-benzo[d]imidazol-2-yl)thio)-N-(5-(4-nitrophenyl)-1,3,4-thiadiazol-2-yl)acetamide.*

Yellow powder, yield (89%), m.p 200-202 °C, Rf = 0.42; FT-IR (KBr disk, cm-1) 3427 (N-H, amide), 3289 (N-H, benzimidazole),3109 (C-H, aromatic), 2924, 2852 (C-H, aliphatic), 1740 (C=O), 1627 (C=N), 1503 and 1440 (C=C, aromatic), 1597 and 1343 (NO2); 1H-NMR (DMSO-d6, 500 MHz, δ) 1.18 (s, 2H, CH2), 7.10-7.89 (m, 8H, Ar-H), 8.50 (s, 1H, NH, amide), 12.12 (s, 1H, benzimidazole-NH); 13C-NMR (DMSO-d6, 125 MHz, δ) 33.74 (CH2), 121.93-137.27 (aromatic ring), 149.60(C=N), 151.82(C=O) 169.08-170.50(C=N of 1,3,4-thiadiazole).

D6: *2-((1H-benzo[d]imidazol-2-yl)thio)-N-(5-(p-tolyl)-1,3,4-thiadiazol-2-yl)acetamide.*

White powder, yield (92%), m.p 192-194 °C, Rf = 0.42; FT-IR (KBr disk, cm-1) 3287 (N-H, amide), 3125 (N-H, benzimidazole),3039 (C-H, aromatic), 2924, 2852 (C-H, aliphatic), 1740 (C=O), 1632 (C=N), 1511 and 1475 (C=C, aromatic); 1H-NMR (DMSO-d6, 500 MHz, δ) 1.49-1.72 (s, 3H, CH3),2.49 (s, 2H, CH2), 7.27-7.50 (m, 11H, Ar-H), 8.50 (s, 1H, NH, amide), 12.12 (s, 1H, benzimidazole-NH); 13C-NMR (DMSO-d6, 125 MHz, δ) 21.72 (CH2), 126.75-136.34 (aromatic ring), 156.143(C=N), 157.82(C=O) 169.08-169.27(C=N of 1,3,4-thiadiazole).

3 RESULT AND DISCUSSION

The synthetic pathway of compounds (D1-D6) is outlined in Figure 1. Compound (A) was obtained by cyclizing *O*-phenylendiamine with CS2 in the presence of potassium hydroxide using ethanol as the medium. This synthesized compound was then characterized using FT-IR and 1H and 13C- NMR. In the IR spectrum of compound (A), stretching bands corresponding to (N-H), (S-H), and (C=N) were observed at 3387 cm-1, 2572 cm-1, and 16152 cm-1, respectively. The 1H NMR spectrum of compound (A) displayed a singlet NH signal of benzimidazole at δ (12.58) ppm, Additionally, a singlet SH signal of (S-H) appeared at δ (12.15-12.42) ppm. The 13C NMR spectrum of compound (A) displayed 168.2(C=N), 115.2-123.0(aromatic ring).

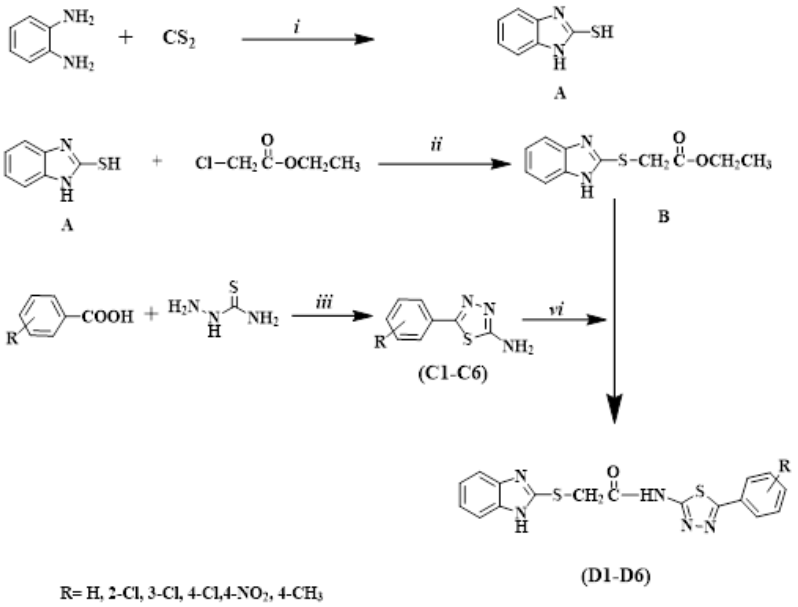

Figure 1: Synthetic route for preparation of compounds (D1–D6).

Compounds (B) was synthesized through neocleophilic substitution reactions of compounds (A) with ethyl chloroacetate in the presence of KOH in EtOH medium, yielding compounds (B). The chemical structures of this compounds were elucidated via FT-IR and 1H and 13C-NMR. In the IR spectrum of compound, the presence of amide group (N-H) bonds was confirmed by bands in the region of (3456cm-1). The presence of a carbonyl group (C=O) in the structure was proven by the presence of a sharp peak at (1741.1cm-1). Additionally, the presence of C=N in the imidazole ring was confirmed by a sharp absorption band at (1620 cm-1). The 1H NMR spectra of these compounds displayed a singlet at δ (6.9) ppm due to the NH (amide) protons and singlet at (12.54) ppm due to the NH(benzimidazole) Additionally, the methylene protons had a downfield value of 4.21 ppm, this can be attributed to the carbonyl group's inductive effect and the chlorine atom's. In the 13C NMR spectrum of the methylene carbon between the sulfur atom and the carbonyl group, a peak was observed at (33.24) ppm. Two carbon atoms in the thiadiazole ring were recorded at (163.85,157.45) ppm. The carbonyl carbon of the ester bond was observed at δ (167.2) ppm.

2-amino-5-(substituted)-1,3,4-thiadiazole (C1-C6) was synthesized by reacting various derivatives of carboxylic acid with thiosemicarbazide in the presence of phosphorousoxy chloride. The FT-IR spectra of compounds (C1-C6) showed the presence of a C=N group at (1630-1666 cm-1) and two bands at (3218-3426 cm-1) and (3121-3284 cm-1), which could be attributed to the asymmetric and symmetric stretching vibrations of the NH2 group. The 1H NMR spectra of these compounds displayed a singlet at δ (6.58-7.63) ppm due to the NH2 protons. In the 13C NMR spectra, two carbons on the thiadiazole ring were observed at δ (173.1, 162.6) ppm.

In the final step, Compound (B) was reacted with compounds (C1-C6) using Ethanol medium and reflux for 10 hrs to yield the target compounds (D1-D6). The chemical structures of all target compounds were established by FT-IR, 1H and 13C-NMR spectra. The IR data obtained for the final compounds (D1-D6) were instrumental in confirming their formation. Upon observing the data for all synthesized compounds, absorption peaks at (3222-3427 cm-1), (3101-3289 cm-1), and (1616-1633 cm-1), confirmed the presence of N-H amide, N-H benzimidazole, and (C=O, amide) groups, respectively. A sharp peak at (29243-2852 cm-1) helped to confirm the presence of C-H bonds in the final products. Additionally, the presence of C=N in the benzimidazole and 1,3,4-thiadazole nucleus was confirmed by a sharp absorption band at (1616-1632 cm-1).

In the 1H NMR spectra of all compounds, the methylene protons resonated at δ (4.14-4.16) ppm, and this downfield shift can be attributed to the inductive effect of the carbonyl group. The singlet NH signal of benzimidazole appeared at δ (12.63-12.64) in all compounds, while the singlet NH signal of (N-H amide) appeared at δ (8.46-8.04) in all compounds. The signals belonging to the aromatic region were observed at δ (7.10-8.30) ppm. In the 13C NMR spectra, the methylene carbon between the sulfur atom and carbonyl group was observed at δ (21.72-33.74) ppm. Two carbons on the thiadiazole ring were observed at δ (149.62-156.13) ppm. The carbonyl carbon of the amide group was observed at δ (168.32-170.61) ppm, all other aromatic carbons were recorded between δ (121.94) and δ (137.27). The physical properties of the synthesized compounds are listed in Table 1.

Table 1: Physical properties of the synthesized compounds.

Comp. No.	R	M.wt	Color	M.P (°C)	Yield %	Rf
A	-	150.20	Light beige	305-307	80	0.46
B	-	236.29	Wight	95-97	93	0.51
C1	H	177.23	Pale yellow	223-225	95	0.58
C2	2-Cl	211.68	Pale yellow	213-215	85	0.45
C3	3-Cl	211.68	Pale yellow	204-206	80	0.62
C4	4-Cl	211.68	Pale yellow	226-228	86	0.48
C5	4-NO_2	222.23	Yellow	244-246	82	0.54
C6	4-CH_3	189.23	White	265-267	98	0.48
D1	H	367.06	Pale yellow	148-150	89	0.47
D2	2-Cl	401.89	Pale yellow	196-198	88	0.53
D3	3-Cl	401.89	Pale yellow	188-190	80	0.38
D4	4-Cl	401.89	Light beige	210-212	90	0.43
D5	4-NO_2	412.45	Yellow	200-202	89	0.41
D6	4-CH_3	381.48	White	192-194	92	0.39

4 BIOLOGICAL EVALUATION

The newly synthesized title compounds (D1, D4, D5 and D6) were evaluated for their antibacterial activity against four types of bacterial isolates: two Gram-positive (Staphylococcus aureus and Bacillus subtilis) and two Gram-negative (Escherichia coli and Pseudomonas aeruginosa). The antibacterial activity of the tested compounds was assessed by zone of inhibition using well diffusion method. The inhibition zones (in millimeters) were measured at three different concentrations (50, 100, and 250 mg/10 mL) using dimethyl sulfoxide (DMSO) as the solvent. The results were compared with the standard antibiotic tetracycline at a concentration of 250 mg/10 mL. The results of antibacterial screening (Table 2) reveal the following information:

A) Compound D1:
   1) 50 mg/10 mL: compound D1 showed no antibacterial activity (n.s = not sensitive) against all tested bacterial strains;
   2) 100 mg/10 mL: the compound exhibited moderate activity against all bacterial strains, with inhibition zones ranging from 9 to 10 mm;
   3) 250 mg/10 mL: a significant increase in antibacterial activity was observed, with inhibition zones ranging from 13 to 16 mm. The highest activity was against B. subtilis (16 mm), followed by S. aureus and P. aeruginosa (14 mm each), and then E. coli (13 mm).

B) Compound D4:
   1) 50 mg/10 mL: compound D4 showed no antibacterial activity (n.s) against all bacterial strains;
   2) 100 mg/10 mL: the compound demonstrated moderate to good activity, with inhibition zones ranging from 8 to 13 mm. The highest activity was against B. subtilis (13 mm), followed by E. coli (11 mm), P. aeruginosa (9 mm), and S. aureus (8 mm);
   3) 250 mg/10 mL: a substantial increase in activity was observed, with inhibition zones ranging from 18 to 22 mm. The highest activity was against B. subtilis (22 mm), followed by P. aeruginosa and E. coli (20 mm each), and then S. aureus (18 mm).

C) Compound D5:
   1) 50 mg/10 mL: compound D5 showed no antibacterial activity (n.s) against all bacterial strains;
   2) 100 mg/10 mL: the compound exhibited good activity, with inhibition zones ranging from 11 to 14 mm. The highest activity was against P. aeruginosa (14 mm), followed by E. coli (12 mm), and then S. aureus and B. subtilis (11 mm each);
   3) 250 mg/10 mL: a notable increase in activity was observed, with inhibition zones ranging from 17 to 21 mm. The highest activity was against P. aeruginosa (21 mm), followed by S. aureus (20 mm), E. coli (20 mm), and B. subtilis (17 mm).

D) Compound D6:
   1) 50 mg/10 mL: compound D6 showed weak activity only against P. aeruginosa (9 mm), with no activity against the other bacterial strains;
   2) 100 mg/10 mL: the compound demonstrated good activity, with inhibition zones ranging from 10 to 14 mm. The highest activity was against P. aeruginosa (14 mm), followed by E. coli (13 mm), and then S. aureus and B. subtilis (10 mm each);
   3) 250 mg/10 mL: a significant increase in activity was observed, with inhibition zones ranging from 18 to 23 mm. The highest activity was against S. aureus (23 mm), followed by E. coli (19 mm), P. aeruginosa (18 mm), and B. subtilis (19 mm).

- Concentration-Activity Relationship: all compounds (D1, D4, D5, D6) exhibited increased antibacterial activity with higher concentrations, indicating a concentration-dependent effect;
- Differences Between Bacterial Strains: in general, Gram-positive bacteria (S. aureus and B. subtilis) were more sensitive to the compounds compared to Gram-negative bacteria (P. aeruginosa and E. coli), particularly at higher concentrations;
- Most Effective Compounds: compound D6 showed the highest antibacterial activity, particularly against S. aureus at 250 mg/10 mL (23 mm), followed by Compound D4, which exhibited strong activity against B. subtilis (22 mm).

Table 2: Diameter inhibition zone (in mm) of the compounds (D1, D4, D5, and D6).

Com-pounds	Conc. (mg/10mL)	Gram-positive		Gram-negative	
		Staphylococcus aureus	Bacillus subtilis	Pseudomonas aeruginosa	E. Coli
D1	50	n.s	n.s	n.s	n.s
	100	9	10	10	9
	250	14	16	14	13
D4	50	n.s	n.s	n.s	n.s
	100	8	13	9	11
	250	18	22	20	20
D5	50	n.s	n.s	n.s	n.s
	100	11	11	14	12
	250	20	17	21	20
D6	50	n.s	n.s	9	n.s
	100	10	10	14	13
	250	23	19	18	19
Tetra-cycline	250	29	27	26	23
n.s = no sensitive					

5 CONCLUSIONS

In this study, a new series of hybrid compounds combining 1,3,4-thiadiazole and benzimidazole scaffolds (D1–D6) were successfully synthesized and characterized using IR, ^{1}H-NMR, and ^{13}C-NMR spectroscopic techniques. The synthetic strategy employed a straightforward approach involving nucleophilic substitution followed by condensation reactions, yielding products in high purity and good yields. The antibacterial activity of the synthesized compounds was evaluated against both Gram-positive and Gram-negative bacterial strains. Among the tested derivatives, compounds D4 and D6 exhibited the most significant antibacterial activity, particularly at higher concentrations, suggesting a clear structure–activity relationship influenced by the nature of the substituents on the phenyl ring. Specifically, the presence of electron-withdrawing groups (such as Cl and NO_2) and electron-donating groups (like CH_3) enhanced the biological efficacy of the hybrids.

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